



# Latin America

Biennial Meeting

## Abstract Book

*“Bridging the gap between science and governance”*



## Society for Environmental Toxicology and Chemistry



SETAC Latin America 12<sup>th</sup> Biennial Meeting

**ABSTRACT BOOK**

*“Bridging the Gap between Science and Governance”*

SANTOS - SP

2017

## *Welcome Letter*

The Society of Toxicology and Environmental Chemistry (SETAC), the Universidade Federal de São Paulo (UNIFESP), the Universidade Estadual Paulista (UNESP) and the Universidade Santa Cecília (UNISANTA) have the pleasure to welcome you to the **SETAC Latin America 12th Biennial Meeting**, from September 7<sup>th</sup> through 10<sup>th</sup>, 2017, here in Santos, São Paulo, Brazil. This year, under the theme “Bridging the Gap between Science and Governance”, we prompt participants to ponder and explore the translation of findings bred within academic research into the development of new technologies, generation of wealth and establishment of suitable policies, especially concerning environmental matters. Most importantly, we hope to inspire boundless scientific thinking, regardless of the grave political scenario.

Santos is a historic city located 75 km from the State capital, São Paulo, and one of Brazil’s oldest towns. The city is home to the largest and busiest harbor in Latin America and also to a record breaking beachfront garden! We are confident that this view, along with the pleasant seaside breeze, will motivate all of those who have been conducting cutting edge studies to participate and engage in fruitful discussions, with the common goal of advancing the field of toxicology and environmental chemistry. To assure the high quality of this meeting, the following technical and scientific program is offered, which includes lectures, special sessions, round tables and oral and poster presentations.

We are honored to have organized an event of such importance for the advancement of science and governance in Latin America. Mostly, we hope this meeting will provide a setting for stimulating dialogues, productive connections and professional growth that subsidizes, ultimately, the quality and sustainability of our environments.

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We especially acknowledge the support of our sponsors, without which the accomplishment of this event, in the format as it was technically and scientifically envisioned, would not have been possible.

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## Lectures

### L1: Questioning Conventions Will Improve Our Science and Decision Making

Speaker: **Michael C. Newman, Virginia Institute of Marine Science, USA**

**Description:** The science of ecotoxicology and practice of ecological risk assessment have progressed remarkably since the environmental awakening of the 1960s. To support the pressing regulatory needs of the 1970s, scientific concepts, techniques and evidence were drawn together to create a new science of ecotoxicology. A loose assortment of regulatory approaches was systematized based on this new science in order to address the blatant pollution issues of that time. These same concepts and practices are now applied to make decisions about increasingly global and subtle contaminant effects. And regulatory demands on them are becoming increasingly more complex, as in the cases of predicting ecological impacts of endocrine disruptors and interpreting probabilistic risk assessments. There is a growing need for conceptual and technical conventions to evolve to better meet modern needs. Ecotoxicology is transitioning from a young science emphasizing description and quantification to a mature science that also requires meticulous inferences and rigorous testing of explanations. Conventional methods and concepts require closer scrutiny and possible replacement. In this talk, examples of compromised inference and decision making will be identified using the tools of modern statistics, cognitive psychology and sociology. Common cognitive mistakes will be highlighted and recommendations made for avoiding them. Next common team decision-making errors and ways of minimizing them will be illustrated. How new concepts and methods enter and then move through environmental science and regulatory communities will be illustrated with eighteen ecotoxicology innovations. Qualities of successful and unsuccessful innovations will be highlighted and related to the need to incorporate best current science into regulatory decision making.

### L2: Soil Ecotoxicology – An Integrated Part of the Risk Assessment of Contaminated Terrestrial Environments

Speaker: **John Jensen, Aarhus University – Department of Bioscience, Denmark**

**Description:** The talk will initially give a brief introduction on the history of soil ecotoxicology testing, a scientific and regulatory discipline that originated in Europe but is now a part of the testing regime in many other regions, including the USA and Canada. Current status and need for future development is outlined. Finally, the talk aims to evaluate the challenges that arise when using soil ecotoxicology in predictive (prognosis) and descriptive (diagnosis) risk assessment procedures of hazardous chemicals and contaminated land.

### L3: Evaluación de Riesgos de contaminantes químicos en el ambiente en Latinoamérica, desde los POPs a los emergentes: desafíos y oportunidades

Speaker: **Ricardo Barra Rios, University de Concepción, Chile**

**Description:** La evaluación de riesgos de contaminantes es una actividad relativamente reciente en Chile y en la región de América Latina, que se estructura en dos componentes fundamentales, la evaluación de la exposición y de los efectos de contaminantes en el ambiente. Lamentablemente, estos dos componentes no necesariamente dialogan porque son desarrollados por dos comunidades diferentes de científicos, por una parte los químicos ambientales/análisis y por otra los (eco)toxicólogos. Por lo que se requiere una mirada multi e interdisciplinaria del problema. La componente de exposición puede ser abordada a través de modelos de simulación y de la medición de contaminantes en el ambiente, esto último es siempre complejo, por lo que hemos desarrollado nuevas estrategias de abordaje como lo son los muestreadores pasivos de contaminantes ambientales, una técnica innovadora que permite varias características de selectividad y de muestreo integrado temporal, que técnicas tradicionales de muestreo no logran. Por otra parte, por el lado de los efectos se requiere una serie de herramientas desde los estudios de biomarcadores bioquímicos y moleculares hasta los estudios poblacionales para entender los impactos que, por ejemplo, tienen las descargas industriales (mezclas complejas de contaminantes) sobre la fauna nativa en nuestros ecosistemas fluviales, donde es difícil identificar el o los contaminantes causantes de los efectos observados. La integración entre estos dos componentes es necesario hacerla en un contexto donde siempre hay faltas de información y no existe apoyo en las regulaciones. De lo que se trata, es que dicha falta de información no impida que avancemos hacia un esquema que nos permita tener procedimientos cada vez más robustos para evaluar los impactos negativos de los contaminantes químicos en el medio ambiente. En esta presentación ejemplificaremos algunos casos realizados por nuestro grupo de investigación que incluyen la investigación de contaminantes tradicionales y emergentes en zonas remotas y altamente contaminadas, enfatizando en las lecciones aprendidas y los desafíos para las futuras generaciones de ecotoxicólogos. Agradecimiento a FONDAF CRHIAM 1513 0015, FONDECYT 1140466, FIP 2014-42, MUSELS NC 1200.

### L4: Per/Polyfluoroalkyl substances (PFAS) and emerging contaminants in water – how to drive and overcome the challenges on these analyses

Speaker: **Maurício Marques, Agilent Technologies**

**Description:** Chemicals are being discovered in water that previously had not been detected or are being detected at levels that may be significantly different than expected. These are generally referred to as contaminants of emerging concern (CECs) where we may also include pharmaceuticals and personal care products (PPCP). CECs and PPCPs are increasingly being detected at low levels in surface water, and there is a concern that these compounds may be a risk to the human health and environment. So, it's becoming important to know their presence, frequency of occurrence or source. There are many CECs and PPCPs that act as so-called endocrine disruptors (EDCs). EDCs are compounds that alter the normal functions of hormones resulting in a variety of health effects. EDCs can alter hormone levels leading to reproductive effects in aquatic organisms, and evaluating these effects may require testing

methodologies not typically available along with endpoints not previously evaluated using current guidelines. In the same way, Per/Polyfluoroalkyl substances (PFASs) are widely used in manufacturing and industry due to their highly desirable properties. They are used as surfactants, fire-retardants, nonstick cookware coatings, and other applications. Because of their unique properties, they have been detected almost ubiquitously in the environment. In summary, this talk will present some results and suggest how to address and overcome these challenge analyses.

### L5: The Science in Science-Based Policies: A Personal Perspective on the Challenges of Collaboration to Protect the Environment and Human Health

**Speaker:** Mary C. Reiley, SETAC North America, USA

**Description:** As scientists, one of the most satisfying parts of our jobs is when our work is used to solve environmental problems. Yes, we came to be scientists because we were curious, found the world we live in fascinating, and we were full of questions about how it worked; how it all connected. But as much as the breakthrough findings and new understandings are exciting, it's when all of our efforts pay off in the evidence and insights needed to solve a problem; show the way; correct, improve or confirm our local or global choices that we feel a true sense of accomplishment and that our work was not only recognized for its quality, but it helped remedy yesterday's environmental problems, today's environmental challenges, or avoid tomorrow's environmental catastrophe. But to get from environmental problem to scientific question to science to science-based policy in order to formulate quality science-based policies is not done in a scientific vacuum nor by a single investigator or single discipline. It's not even done by a single sector (business, government or academia) running solo. To investigate an environmental problem, propose alternative solutions and forecast the outcomes of each to make and implement an informed, optimized and successful solution while avoiding or minimizing unintended consequences requires multiple disciplines and all three sectors to integrate their needs, science, resources and solutions. So, with all of the disciplines to integrate, the sectors to involve, the decision makers to educate and the stakeholders to engage – how do we get the science into science-based policy?

### L6: Implications of Xenoestrogens on the Physiology of the Neotropical Fish *Cnesterodon*

#### *decemmaculatus* – Reproduction and Sex Differentiation

**Speaker:** Gustavo Manuel Somoza, IIB-INTECH – CONICET-UNSAM, Argentina

**Description:** It is known that teleost fish have a hormone-dependent sexual dimorphism, and the process of sex differentiation is also dependent on steroid hormones. In this context, they can be used as model organisms to study the adverse effects of steroid-derived anthropogenic chemicals. Nevertheless, several fish model species are being used to study these effects, because of the diversity of fish species, there is a need to look for local models. Wastewaters are important sources of estrogenic compounds like the natural estradiol (E2) and 17 $\alpha$ -ethynylestradiol (EE2), and they have been reported in surface waters of Brazil and Argentina, in some case at high

concentrations. Therefore, it is important to look for potential effects of these substances on the physiology of local species. Under this conceptual framework, a series of studies was conducted with the ten spotted live-bearer, *Cnesterodon decemmaculatus*, a Poeciliid fish (the same family of *Gambusia*, *Xiphophorus* and *Poecilia* spp.) widely distributed in Argentina, Chile, Uruguay and the South of Brazil. This is a gonochoric, sexually dimorphic and ovoviviparous species. Females are larger than males, and the male anal fin is modified into a gonopodium. First, the responses of the gonopodium morphology and the gonadal and liver histology were assessed in adult males exposed long-term to sublethal concentrations of EE2. Two experiments were conducted exposing fish to concentrations of EE2 ranging from 20 to 200 ng/L during 8, 12 and 16 weeks. Intersex gonads (testis-ova) were observed after 8 and 16 weeks in fish exposed to 200 ng EE2/L and 100 ng EE2/L, respectively. Oocytes' development from testis germ cells and replacement of the efferent duct periodic acid-Schiff–positive secretion surrounding spermatozeugmata by parenchymal tissue and duct structure alterations were the major observed changes in the gonads. However, no response was observed in the gonopodium morphology. Liver histology was also altered, showing increasing steatosis, single-cell necrosis to generalized necrosis, and disruption of acinar organization from 100 ng EE2/L to 200 ng EE2/L. Also, in order to know the effects of xenoestrogens on the sex differentiation process, a series of studies was conducted to characterize the morphology of secondary sexual characteristics and gonads using sexually undifferentiated fish. The exposure of sexually undifferentiated fries to EE2 was also evaluated on mortality, sex ratio and the occurrence of pigmentation in the perianal area. This pigmentation presented a clear relationship with water borne EE2. An occurrence frequency of 0, 38, 85 and 100% was observed in fish exposed to 0, 20, 100 y 200 ng/L of EE2 respectively. The percentage of phenotypic males decreased from 49 (control) to <17 % in exposed fish (20 to 200 ng/L), independently of the concentration. Also, a concentration-dependent increase was observed in the number of sexually undifferentiated fish. In summary, the present results showed that although EE2 was not able to alter the morphology of a fully developed gonopodium, it was capable of inducing development of testicular oocytes in adult males at environmentally relevant concentrations. Thus, externally normal but intersex *C. decemmaculatus* males would be expected in the wastewater-receiving streams that the species inhabits. Exposure of fries to EE2 alters sex ratio reducing male proportion. In sum, this study shows that EE2 exposure effects vary according to the life stage at environmentally relevant concentrations and show that *C. decemmaculatus* can be used as a test organism to evaluate the impact of endocrine disruptors.

### L7: Fate and Transfer of Toxic Compounds Through the Aquatic Food Web – Looking for Markers of Aquatic Pollution in Human Food and Drinking Water

**Speaker:** Daniel A. Wunderlin, Universidad Nacional de Córdoba, Argentina

**Description:** Aquatic ecosystems are directly or indirectly affected by pollutants, which may have deleterious effects for the aquatic biota. Heavy metals are among main pollutants, which are easily

transported and accumulated in the aquatic web, being one important pathway by which humans can be exposed to environmental toxics. The use of stable isotope nitrogen ( $\delta^{15}\text{N}$ ) constitutes a valuable tool to evaluate biomagnification of contaminants in a food chain. The trophic transfer of elements can be estimated using relationships between the element concentration and  $\delta^{15}\text{N}$  of studied species. On the other hand,  $\delta^{15}\text{N}$  has also been used to trace the impact of anthropic activities, mainly untreated sewage discharges, among others, on the environment allowing distinguishing regions with different types of contamination as well as areas of greater or lesser risk for food production. Our current research is focused on the evaluation of the trophic transfer behavior of metals and metalloids within a limited aquatic food web (plankton, shrimp (*Palaemonetes argentinus*) and fish (*Odontesthes bonariensis*), along three reservoirs from the Province of Córdoba (Argentina), with different eutrophication conditions: San Roque (SR), Los Molinos (LM) and Río Tercero (RT). Additionally, we look to verify the feasibility of using  $\delta^{15}\text{N}$  as chemical marker of anthropic pollution in different compartments of these reservoirs, namely water and different levels of the food web (plankton, shrimps and fish). Multi-elemental analysis was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Stable isotope ratios ( $\delta^{15}\text{N}$ ) was measured by IRMS. The range of  $\delta^{15}\text{N}$  values in studied species suggested a food chain with increasing trophic levels as follows: plankton–shrimp–fish, which is consistent with what is already known about the dietary habits of fish (Silverside). Positive slopes were found for P, Ga, Cu, Zn, As, Sr, Ba and Hg in SR reservoir; but only Ag, Hg and P showed positive slope in LM reservoir, while P and Hg had positive slope in RT reservoir. Only the regression for Hg and P in LM, Hg in RT and P in SR showed a significant positive slope ( $P < 0.0001$ ), demonstrating the biomagnification of these elements through the trophic chains studied. Conversely, significant negative slopes ( $P < 0.0001$ ) were found for the rest of the elements in three reservoir studied, indicating biodilution of these elements. Results also show distinctive patterns in  $\delta^{15}\text{N}$  between studied reservoirs, allowing the identification of a reservoir (SR) highly influenced by sewage, which was evident in water and biota living in this reservoir. Further research studies are being conducted to confirm this result, looking for an interesting alternative to link sewage discharges with levels of stable isotopes in biota, including edible fish; thus, pointing out risk for people drinking water or eating fish from such impacted reservoirs.

### L8: Evaluación de riesgo (WOE) en sedimentos acidificados por enriquecimiento de CO<sub>2</sub>

**Speaker:** Angel del Valls, Espanha

**Description:** Durante los próximos años se espera una acidificación generalizada en la mayoría de los sistemas acuáticos asociada con el enriquecimiento de CO<sub>2</sub> en la atmósfera provocado por el uso de combustibles fósiles. Una de las tecnologías más recientes que se ha propuesto para la mitigación de este enriquecimiento es la captura y almacenamiento de CO<sub>2</sub> en estructuras geológicas estables en el medio marino (CAC). Esta tecnología consiste en la captura, purificación, y subsecuente almacenamiento del CO<sub>2</sub> en formaciones geológicas (terrestres o marinas) estables a lo largo del tiempo. Las formaciones geológicas más comúnmente utilizadas para este propósito debido a su abundancia y capacidad para el almacenamiento de CO<sub>2</sub> son: los

formaciones profundas salinas, los pozos agotados de petróleo y gas y las zonas mineras de carbón entre otras. Este tipo de tecnología ha sido propuesta y aprobada por diversos convenios internacionales como una de las posibles tecnologías para mitigar el aumento de CO<sub>2</sub> en la atmósfera: Convenio de Londres, 2006, 2007; Convenio OSPAR, 2007, Directiva del Parlamento Europeo, 2008, etc. Sin embargo, en estas mismas instancias internacionales se recomienda evaluar los posibles riesgos asociados con esta tecnología. Existen diferentes escenarios que se pueden producir asociados con fugas potenciales durante el proceso de inyección de CO<sub>2</sub> o durante su almacenamiento. Estas fugas tendrán un impacto directo en el medio acuático acidificando el mismo. Esta acidificación está asociada con el aumento de la concentración de protones relacionada con el aumento de CO<sub>2</sub> y conlleva la aparición de efectos físicoquímicos y biológicos que deben ser evaluados siguiendo las pautas propuestas en las guías de los convenios internacionales. En este ámbito, se aplican los métodos integrados basados en el peso de la evidencia (weight-of-evidence) que determinan la evaluación integrada de los riesgos asociados con la acidificación del medio. El método implica el uso de diferentes líneas de evidencia (concentración de contaminantes y su especiación en sedimentos, toxicidad bajo condiciones de laboratorio, efectos evaluados bajo condiciones de campo y estudios de bioacumulación/biomagnificación). La integración de los resultados de estas líneas de evidencia permitirá la cuantificación de la polución asociada con estos escenarios de enriquecimiento en CO<sub>2</sub>. Durante el desarrollo del trabajo se muestran diferentes resultados obtenidos en cada una de las líneas de evidencia que ponen de manifiesto el riesgo asociado con el uso de esta tecnología y la necesidad de utilizar este tipo de metodologías integradas para llevar a cabo una correcta evaluación del riesgo ambiental.

### L9: Metals in Coastal Ecosystems – Old Problems and New Challenges

**Speaker:** Julián Blasco Moreno, Instituto de Ciencias Marinas de Andalucía (CSIC) ICMAN

**Description:** Metals occur in the rocks of the earth and soil, resulting from erosion processes, and its presence in the environment is related to natural cycles. However, other metal sources are related to anthropogenic origin, such as mining, smelters or industrial activities and commercial applications, among others. Metals are transported by different pathways, depending on their physicochemical properties to seawater. Coastal and estuaries are one of the most valuable ecosystems on earth because they provide a wide number of ecosystem services. Nearly half of the world's population lives near of the coast areas, provoking high pressures in these ecosystems. One of the problems associated with the population increase in coastal areas is pollution. Metal contamination is not a new problem, and episodes of metal pollution have been reported in the scientific literature since the beginning of the industrial revolution. In the last century, many instances of contamination and pollution in coastal areas have been recorded. However, societal changes and the increase of ecological consciousness have generated a tendency to improve industrial processes, lessen residues in production, and focus on a healthy environment. However, in emergent countries, the residues management is a pending task. New technological



developments, such as nanotechnology, can represent benefits to human beings, but the release of these engineered nanomaterials and specifically metallic and oxide metal nanoparticles in coastal areas can represent a new source of risks and challenges. Due to the nature of these nanomaterials, new approaches for assessing their toxicity should be implemented. Additionally, the effect of rising temperature and ocean acidification, associated to global climate change, can affect to bioavailability and toxic effects. In this talk, we are going to summarize the problems related to the occurrence of metals (legacy pollutants) in coastal ecosystem and the new challenges associated with the presence of metallic (MeNPs) and metallic oxide (MeOxNPs) nanoparticles in marine ecosystems, focusing on two key trophic levels (phytoplankton and molluscs). The main findings regarding the behavior of nanoparticles and ionic strength (AgNPs), homo and heteroagglomeration processes, effect of UV-A in the toxicity of TiO<sub>2</sub> NPs on phytoplankton species and species sensitivity to nanoparticles, among others, will be presented. We will also show the accumulation and elimination strategies (CuONPs, AuNPs), intracellular location and toxicity regarding the mollusc, which was pointed out as a target species for nanotoxicity.

### L10: Microplastic Pollution: A Real Threat to the Environment?

**Speaker:** Brian Quinn, University of the West of Scotland, Institute of Biomedical and Environmental Health Research, Scotland

**Description:** Recently, there have been many publications highlighting the ubiquitous presence of microplastics in the marine environment, but fewer papers are reporting the potential impact of these novel contaminants. This talk aims to summarize the extent of microplastics pollution internationally and the issues involved in microplastic research, and it will focus on the known and suspected impacts of microplastics on marine and freshwater organisms. Brian Quinn has co-authored the only book dedicated to microplastics (*Microplastic Pollutants* (2016) Elsevier) and has authored several published articles on various aspects of microplastic contamination, ranging from method development for sample (biological and sediment) separation and quantification to the uptake and impact of microplastics on aquatic organisms.

### L11: Advances in -omics Technologies in Ecotoxicology

**Speaker:** Nancy Denslow, University of Florida, USA

**Description:** Technological advances over the past decade have revolutionized medical sciences and now have opened up the same promise for environmental sciences. It is now possible to obtain good-quality sequence information for non-model species relatively quickly and inexpensively. The main step forward came with the development of massively parallel DNA sequencers, which are now able to sequence more than 800 million cDNAs in parallel. The price of obtaining sequence information keeps dropping on a logarithmic scale, much faster than anyone predicted. Information obtained for the first human genomes have enabled annotations of many non-model organisms by homology and have opened these huge leaps forward in technology for ecotoxicology. These approaches have enabled scientists to study whole microbe populations in the environment and in the gut, through metagenomics. They have also

allowed better understanding of molecular pathways that are altered when organisms, from microbes to humans, are exposed to contaminants in their environments that cause disease. Changes in gene transcription can be measured using microarrays developed for organisms of interest or through direct RNA sequencing. We have learned that the genome is not mostly junk, as originally thought, but that there are multiple non-coding RNAs that are produced to control gene expression. In addition, we have made technological advances in mass spectrometers that are used for proteomics, metabolomics and, more recently, for lipidomics. These emerging molecular techniques are being complemented by a large array of cell-based assays that are also being developed and that can serve as specific measurable endpoints that link back to the *in vivo* studies. The hope is that in the future, toxicology will use fewer animal models and be able to screen more chemicals at lower cost. Development and understanding of adverse outcome pathways may be the answer as we map out what appears to be a finite number of biochemical perturbations that lead to higher order endpoints, such as effects on individual organisms, populations and ecosystems. The field of bioinformatics has developed in tandem with the -omics technologies that enable investigators to analyze large datasets with appropriate statistical methods and to integrate information across all the technologies into a more holistic interpretation of the actual effects of contaminant exposures. Several applications of the existing technology for ecotoxicology, population genetics and general biology of aquatic species have recently been published. However, these technologies are not full proof and there are still some pitfalls and drawbacks that should be understood. The main constraint for non-model species is in correct annotation and developing appropriate bioinformatics tools to deal with the overabundance of data, data storage and accessibility. Following in these initial footsteps, newer, more sophisticated technologies are on the horizon, responding to the challenge in medical sciences to advance to personalized medicine and to help understand how exposures relate to disease.

## Round-Table Discussions

### RT 1 Title: Air pollution: the actual environmental health crisis we face

Participant: Dr. Hebe Carreras – Universidade de Cordoba, Argentina

Participant: Dr. Paulo Saldiva – Universidade de São Paulo – USP

Participant: Dra. Deborah Tasat – Universidade San Martín, Argentina

**Description:** We may not always see it, but air pollution is the cause of some of our most common illnesses. The last report of the World Health Organization (WHO) confirms that 92% of the world's population lives in places where air quality levels do not meet WHO standards. As result, a significant deterioration of human health has been observed, particularly in urban areas. In fact, in 2012, an estimated 6.5 million deaths (11.6% of all global deaths) were associated with indoor and outdoor air pollution together. Still more worrying is that almost 90% of the air pollution-related deaths occur in low and middle-income countries and in poor neighbourhoods of high-income cities. Therefore, reduction in air pollutants can have

particularly large health benefits for lower income groups as well as for susceptible populations such as children, elderly, individual with previous respiratory and cardiovascular diseases and pregnant women among others. The main sources of air pollution in urban environments comprise vehicle emissions, biomass and waste burning, power plants and industrial activities. However, human activity is not the only source of air pollution. Pollution from natural sources like forest fires, volcanic eruption and sandstorms can also influence air quality. Urgent measures to tackle air pollution are needed. The purpose of this session is to understand how exposure to air pollutants (particles and gases) affect different biological processes (inflammation, oxidative stress, vascular dysfunction, infections, etc.) and up to what extent these effects are directly associated to air pollutant composition and physicochemical characteristics. Thus, the results of the present proposal will provide scientific evidence for national and regional governments to build environmental policies to further prevent, protect and intensify the surveillance on air pollution. In addition, this session could help raise awareness on air pollution as an important risk factor to health and climate.

#### RT 2 Title: Molecular biomarkers in environmental toxicology

Participant: Dr. Afonso Bainy – Universidade Federal de Santa Catarina – UFSC

Participant: Dra. Nancy Denslow – University of Florida, USA

Participant: Dr. Igor Dias Medeiros – Univeridade Federal de São Paulo – UNIFESP

Participant: Dr. João Carlos Setubal -Universidade de São Paulo – USP

**Description:** Molecular biomarkers and omics technologies are improving environmental analysis and helping to elucidate molecular effects and mechanisms of xenobiotic biotransformation in model and non-model organisms. Besides methodological implications in the molecular protocols, an overview of computational methods for the analysis of genomic and transcriptomic data from Next Generation Sequencing will be addressed. The aim will be to show how important are good molecular and bioinformatics practices so that satisfactory results can be obtained with respect to the biological goals of such research projects

#### RT 3 Title: Latin America ecotoxicology effluents regulation: status, equalization and progressive goals

Participant: Dr. Ricardo O. Barra – Universidad de Concepción – Chile

Participant: Dr. Fernando Aquinoga – APLYZIA soluções ambientais

Participant: Dr. Alexandre Arenzon – Universidade Federal do Rio Grande do Sul – UFRGS

Participant: Dra. Helena de Assis – Universidade Federal do Paraná – UFPR

**Description:** In LA, only Brazil has federal environmental legislation that includes the assessment of acute and chronic toxicity potential of effluent discharges to watersheds (CONAMA 2011). However, just monitoring the effluent quality is not sufficient for

protecting the biota of the natural aquatic ecosystem and maintaining the ecosystem services; continuously improvement targets to reduce the potential for toxic releases should be implemented. In Brazil, only the Rio Grande do Sul State legislation (CONSEMA 2006) includes progressive targets for reducing the toxicity of industrial and domestic effluents, but unfortunately, after ten years in force, this law was revoked. Efforts should be made to learn from these and other examples and expand the effluent control based on ecotoxicological tools to other parts of LA

#### RT 4 Title: Contaminantes de preocupação emergente para América Latina: Ocorrência, efeitos e riscos em ecossistemas aquáticos

Participant: Dr. Bruno Nunes – Universidade de Aveiro – Portugal

Participant: Dr. Camilo D. Seabra Pereira – Universidade Federal de São Paulo – UNIFESP

Participant: Dr. Ítalo Braga Castro – Universidade Federal de São Paulo – UNIFESP

**Description:** A introdução de novas substâncias no mercado leva invariavelmente a seu despejo no ambiente. Portanto avaliações do potencial ecotoxicológico desses compostos, empregando múltiplas ferramentas analíticas que salvaguardam os ecossistemas dos seus efeitos deletérios são fundamentais. No entanto, fármacos e biocidas usados em tintas anti-incrustantes não foram sujeitas a estes processos sistemáticos de avaliação de risco ambiental. Essas classes de compostos foram apenas recentemente investigadas no continente Latino Americano, tendo revelado ocorrência e atividade tóxica considerável. Adicionalmente, à luz do alto consumo de cocaína e subprodutos nas regiões costeiras metropolitanas e sua consequente detecção em águas marinhas e estuarinas ao redor do mundo, alertam para inclusão dessas moléculas no grupo de contaminantes de preocupação emergente. Além disso, efeitos biológicos adversos decorrentes da exposição a essas drogas ilícitas em organismos marinhos também foram evidenciados por estudo recentes. Nesse contexto, a mesa-redonda tem como objetivo apresentar e debater conhecimento recente sobre a ocorrência e o risco ambiental de três classes de contaminantes emergentes em regiões costeiras da América Latina apresentando estudos de caso para o fármaco paracetamol, a droga ilícita Crack e biocidas anti-incrustantes de terceira geração.

#### RT 5 Title: Global Horizon Scanning Project – Priority Research Questions for Latin America

Participant: Dr. Pedro Carriquiriborde – Universidad Nacional de la Plata – CONICET

Participant: Dr. Julie Brodeur – Instituto de Recursos Biológicos, Argentina

Participant: Dra. Tatiana Heid Furley – APLYZIA Soluções Ambientais

Participant: Karina S.B. Miglioranza – IMyC, CONICET-UNMDP, Argentina

Participant: Dra. Helena Assis – Universidade Federal do Paraná

**Description:** SETAC Latin America (LA) is one of the five Geographic Units of SETAC, a global professional organisation with the mission of achieving Environmental Quality Through Science®

and ensuring a balanced participation from government, business and academia. The unique tri-partite forum which SETAC provides, facilitates a multidisciplinary and multi-stakeholder approach to the identification and resolution of environmental quality problems. The Global Horizon Scanning (GSH) Project was established to identify and prioritize the most critical environmental research needs. One hundred research questions were submitted by SETAC LA members and the 20 priority research questions were selected in the LA GSH Workshop held during the 11th SLA Biennial Meeting which took place in Buenos Aires in 2015. The aims of the current Round Table, are: i) to present the results of the 2015 GSH Workshop, ii) to evaluate the whole exercise and identify strength and weakness of the process, iii) to have feedback from stakeholders on SETAC's identified priority research areas for LA, as well as to discuss how the selected questions could help decision-makers design environmental strategic plans.

### RT 6 Title: Coastal Governance and Sustainable Urban Planning

Participant: Dra. Alessandra Aloise de Seabra – Universidade Santa Cecília

Participant: Dr. Marcus Polette – Universidade Vale do Itajaí

Participant: Dr. Mohamed Ezz El-Din Mostafa Habib – Universidade de Campinas/Universidade Santa Cecília

**Description:** Economic and territorial models of development throughout the twentieth century were not very respectful with the environment, substantially modifying the natural, rural and urban areas. With more than half of the world's population living in cities and predicting that number doubles by 2050, we live in the Urban Age, where economic activities, social and cultural interactions, as well as environmental and humanitarian impacts are concentrated in the cities, which represents significant sustainability challenges in housing, infrastructure, health, education, jobs, natural resources, etc. It is in the cities that the 2030 Agenda for Sustainable Development finds an ideal position to transform the broad and abstract Agenda into something concrete and efficient. Cities can approach objectives and goals in a practical way, adapting them to their specific contexts and helping their citizens to understand how actions contribute to their fulfilment. Well planned cities can be sources of solutions to the current global problems. The New Urban Agenda presents a paradigm shift based on city science, establishing norms and principles for urban planning, construction, development, management and improvement in five main pillars of application: national urban policies, urban legislation, urban planning, local economy, and municipal finance. In this new scenario, the city can be a powerful instrument to achieve sustainable development.

## Short Courses

### SC1: Estratégias químico-analíticas em estudos ambientais e ecotoxicológicos

**Instructor:** Mary Rodrigues de Marchi, Universidade Estadual Paulista (UNESP – Araraquara)

**Description:** Serão discutidos diversos aspectos a considerar no estabelecimento da estratégia analítica, como parte integrante do estudo ambiental e/ou ecotoxicológico, incluindo o planejamento do estudo, o número de amostras, o tamanho das amostras, os contaminantes potencialmente presentes na matriz (biótica ou abiótica), as características físico-químicas e de dispersão ambiental dos analitos de interesse, e os cuidados na amostragem e sua conservação. Também serão abordados aspectos relacionados a confiabilidade analítica dos resultados para determinação de contaminantes orgânicos.

### SC2: Uso de bioensaios in vitro na avaliação da qualidade de águas costeiras

**Instructor:** Lucas Buruaem, Universidade Estadual Paulista (UNESP – Litoral Paulista)

**Description:** Os ecossistemas aquáticos em especial aqueles localizados em zonas costeiras recebem uma alta carga de substâncias químicas originadas de atividades antrópicas, potencializadas pela crescente ocupação humana, desenvolvimento e uso de novos produtos. Além disso, há a necessidade constante da expansão e otimização dos sistemas de coleta e tratamento dos efluentes domésticos, que em muitos casos são introduzidos no ambiente em condições inadequadas. Como resultado, regiões sob influência desses lançamentos recebem uma mistura complexa de contaminantes. No sentido de estimar a toxicidade desses efluentes, os métodos ecotoxicológicos considerados como convencionais utilizando bioensaios com organismo-teste estimam os efeitos em níveis de organismo e população como, por exemplo, mortalidade, reprodução, desenvolvimento e comportamento, sem estabelecer uma relação com o evento iniciador da toxicidade observada. Recentemente as técnicas bioanalíticas têm sido empregadas em estudos de qualidade águas e efluentes como linha de evidencia adicional para predição da toxicidade através da quantificação da bioatividade baseada no mecanismo de ação de diferentes grupos de contaminantes. No presente minicurso serão apresentados alguns dos principais bioensaios in vitro comerciais envolvendo linhagens de células e bactérias modificadas geneticamente, e também será discutida a utilização dessa abordagem dentro de um programa de monitoramento da qualidade ambiental em zonas costeiras.

### SC3: The Endocrine System: Global Perspectives on Testing Methods and Evaluation of Endocrine Activity

**Instructor:** Ellen Mihaich, Environmental and Regulatory Resources, LLC

**Description:** In response to concerns that certain environmental chemicals might interfere with the endocrine system of humans and wildlife, regulations have been promulgated in various regulatory bodies around the world targeting the evaluation of these types of effects. The purpose of this short course is to address key topics related to endocrine system evaluation and regulatory requirements around the world. The course will provide basic information on the vertebrate endocrine system, mechanisms of control and adverse effects. The focus will be the estrogen, androgen and thyroid systems, although new endocrine system targets will be discussed. The requirements of the USEPA's Endocrine Disruptor Screening



Program as well as those for REACH and other regulatory initiatives around the world, including the development of definitions and criteria in the EU, will be reviewed. Specific screens and tests used in these programs will be reviewed, including plans for the evolution of the USEPA program, such as EDSP21 and the development of adverse outcome pathways. Use of weight-of-evidence evaluations in interpreting the data will be covered. Finally, an interactive simulation will be staged where small groups of participants can engage in a transparent and quantitative weight-of-evidence evaluation of data.

#### SC4: Introduction to Methods in Community Ecotoxicology

**Instructors:** Luis F. Gusmão and Fabiane Gallucci, Universidade Federal de São Paulo (UNIFESP)

**Description:** Bench ecotoxicological tests have been an important tool to assess the risk of contaminants to the biota. These tests are easy to conduct, quick to generate responses and highly replicable, having been used, for instance, to determine the relative sensitivity of different species to a certain contaminant. However, bench tests fail to replicate the complex exposure scenarios organisms experience in situ. These scenarios are a result of interactions of the chemical, physical and biological domains, which can alter significantly any assessment of risk. For instance, the toxicity of contaminants can be directly affected by environmental factors such as temperature, salinity, pH and organic matter. In addition, the response of a single individual in situ is not an isolated result of its exposure to contaminants and the environment but also a result of changes in the population and community which it is inserted. For this reason, studies trying to understand how higher levels of biological organization respond to contaminants in natural conditions are essential to generate more “realistic” risk assessments. The objective of this course is to give a general introduction to the various approaches used in the assessment of risk in natural biological communities. Several approaches will be explored, from experimentation with microcosms and mesocosms to the application of modelling to simulate biological response to contaminants.

#### SC5: Biomarcadores de contaminação ambiental: respostas moleculares, bioquímicas e histológicas.

**Instructors:** Luciane Alves Maranhão, Universidade Federal de São Paulo (UNIFESP – Baixada Santista), and Paloma Gusso Choueri, Universidade Estadual Paulista (UNESP – Litoral Paulista)

**Description:** Este minicurso apresenta os conceitos de poluição marinha em áreas costeiras e informa sobre práticas do uso de bioindicadores e biomarcadores para diagnóstico de ambientes impactados e seus efeitos subletais na fauna. Respostas moleculares, bioquímicas e histológicas serão elucidadas ao longo do curso, assim como os critérios para a seleção de biomarcadores, principais classes de biomarcadores de contaminação ambiental, técnicas utilizadas e estudos de caso utilizando biomarcadores para avaliação da qualidade ambiental e aplicados à análise de risco ecológico de substâncias emergentes. **Objetivos:** – Apresentar a importância de respostas subletais em estudos de contaminação ambiental. – Discutir

as classes de biomarcadores, assim como suas vantagens e desvantagens comparados a outros métodos. – Elucidar sobre a inclusão de tais respostas na legislação ambiental. Capacitar o aluno com conhecimentos básicos sobre biomarcadores e suas aplicações.

#### SC6: Soil Ecotoxicology – Standard Tests Using Soil Invertebrates and Plants

**Instructors:** Vanessa Bezerra de Menezes Oliveira and Maria Edna Tenório Nunes, Universidade de São Paulo (Escola de Engenharia de São Carlos – EESC-USP)

**Description:** Although interest in soil ecotoxicological tests is increasing in Latin America, research groups are still in doubt about what is the best way to test soil organisms and which test is the most appropriated to respond to a given issue. The main purpose of this short course is to share knowledge on the various standardized test possibilities available in soil ecotoxicology and to discuss their applications. Presented examples will include laboratory (e.g., earthworms, collembola, mites and enquitreidae), semi-field (e.g. multispecies, mesocosms, microcosms), and field (e.g., soil diversity assessment) tests.

#### SC07: Bridging the gap between metal speciation and toxicity in aquatic environments: improvements, challenges and perspectives

**Instructors:** Dr. Maikel Rosabal, Université du Québec à Montréal (UQAM)

**Description:** The environmental behavior of inorganic contaminants in aquatic environments is markedly determined by physicochemical factors (e.g., pH, hardness) and biological ones (e.g., microorganisms) occurring in specific studied sites. The distribution of these contaminants among various chemical species will strongly influence their potential to be bioaccumulated and then to exert their toxicity. In this mini-course, we will review the current state of the field of metal speciation in different environmental compartments, primarily water and biota. The free ion activity (FIA) model and the biotic ligand model (BLM) as equilibrium models to predict metal toxicity will be reviewed for water samples. On the biological level, we will consider the subcellular metal partitioning approach, which represents an important improvement in linking metal bioaccumulation with toxicity once these contaminants enter cells. We will be discussing the challenges, limitations and future perspectives of all these models and approaches, which represent essential tools in performing realistic environmental risk assessments of trace elements in aquatic ecosystems.

## Symposium

#### Chemicals Risk Assessment and Management in Latin America

**Description:** The goal of the one-day symposium is to review the available chemical risk assessment and management regulations and their implementation in Latin America. We look forward to an exchange of knowledge and ideas among the leading scientists,



engineers and regulators in Latin America, and the lessons learned will benefit all of us.

## Platform Abstracts

### A marriage of convenience: Interactions of trace nutrients and contaminants with microbial communities

#### 1 Co-selection of antibiotic and heavy metal resistance bacteria from sediments of a port area

B. Zampieri, UNESP- Campus do Litoral Paulista / Laboratory of Marine Microbiology; V. da Costa Andrade, A. Bartelochi Pinto, UNESP São Paulo State University / Laboratory of Marine Microbiology; A.F. Oliveira, São Paulo State University UNESP Campus Experimental do Litoral Paulista / Laboratory of Marine Microbiology

The use and disposal of antibiotics in the environment has been highly regulated to prevent the selection and spread of antibiotic resistance bacteria. However, in addition to antibiotic agents, heavy metals contaminations might promote the spread of antibiotic resistance via co-selection. In this way, the objective of the present study was to identify co-selection of antibiotic and heavy metals in bacteria isolated from Araçá Bay, São Sebastião- SP, Brazil. For this, it was determined concentrations of Zn, Cd, Cr and Cu in 37 stations distributed along Araçá Bay. It was isolated strains for resistance test for heavy metal and antibiotics. After the tests, the resistant strains were identified through biomolecular methods. All metal concentrations tested were significantly higher in the sublittoral zone, near the port activity. The bacteria were more resistant to the metals that present higher concentrations in the study region (Cr and Zn). One hundred of bacteria strains was isolated and tested. Few strains tolerated Cd concentration levels greater than 200 mg L<sup>-1</sup>. In the case of Cr, 36% of the strains grew even at a concentration of 3,200 mg L<sup>-1</sup>. In the analyses using Zn and Cu, few strains (4% and 2%, respectively) tolerated 1,600 mg L<sup>-1</sup>. The strains with higher MIC (minimum inhibitory concentration) for metals were tested through Mc Farland method for antibiotics resistance tests. 4.1% of strains were resistant to Imipenem, 45.8% were resistant to Oxacilin (OXA), 62.5% were resistant to Ceftriaxon (CRX), 79.1% were resistant to Cefepime (CPM), 1% were resistant to Tetracyclin (TET) and all strains were sensible to Eritromicin (ERI). This shows that higher concentrations of Zn and Cr in the sediment led to a greater number of strains resistant to Cr, Zn, OXA, CPM and CRX. This relationship could be observed through Principal Components Analysis (PCA). The most resistant strains to metals and antibiotics were the species belonging to the genera *Staphylococcus*, *Bacillus* and *Vibrio*. Many of these bacteria are pathogenic and can cause serious problems to humans. This reinforces the problem of antibiotic-resistant strains in the environment, bringing a public health problem.

#### 2 An Update on the Derivation of a Marine Arsenic Water Quality Guideline Value

M. Valdivia, Universidad Católica del Norte / Facultad de Ciencias del Mar; L.A. Golding, G. Batley, S. Apte, CSIRO Land and Water

Arsenic is a widespread environmental concern in South America. Mobilisation of arsenic from mineral deposits in the Andes is accelerated in the presence of mining activity, ultimately resulting in elevated arsenic concentrations in coastal waters, however, the risks to marine biota are uncertain. Existing international marine water quality guideline values (GVs) for arsenic are unreliable since they were derived using an assessment factor applied to a limited set of toxicity data, often with no distinction between arsenic oxidation states. The Australian GV for marine arsenic are an interim environmental concern level (ECL) of 2.3 µg/L for arsenite (AsIII) and a low reliability GV of 4.5 µg/L for arsenate (AsV) (ANZECC/ARMCANZ, 2000). Since they were derived, new toxicity data have become available but additional reliable data were still required. As a collaborative effort between Australia and Chile, an update was undertaken of the AsV GV, given its dominance in oxidized seawaters due to the rapid oxidation of AsIII to AsV in surface waters. Progress to date was recently reported (Golding et al. SETAC-AU 2016). Our latest results, including data from a newly developed chronic marine copepod bioassay, will be presented here. A database for AsV toxicity was created from chronic toxicity tests conducted on 9 Australasian marine species representing 5 taxonomic groups (1 crustacea, 2 bivalves, 1 sea urchin, 3 green microalgae, 2 diatoms). Chronic effect concentrations (IC/EC10s) based on measured dissolved arsenic ranged from 13 to 18,000 µg/L with the green microalga

*Chlorella salina* and the copepod *Acartia sinjiensis* being the most sensitive species tested. The BurrliOz software (version 2.0) was used to model a species sensitivity distribution and derive a draft marine AsV GV. A novel aspect of our approach was the investigation of the ameliorative effects of nutrients on AsV toxicity to green algae, diatoms, mussels and sea urchins. Increasing nutrient concentrations decreased AsV toxicity in most, but not all of the tested species, suggesting that different mechanisms of toxicity were involved. The development of a reliable marine AsV GV will be a powerful tool for industry and resource managers to conduct robust ecological risk assessments, and will be particularly useful for tackling the challenges of managing arsenic contamination in coastal regions of Latin America.

### Addressing complexity in ecotoxicology: from multiple stressors to ecosystem level impact assessment

#### 3 The microcosm approach using meiofauna communities as a tool in ecotoxicology

F. Gallucci, Federal University of Sao Paulo / Marine Sciences

Ecological risk assessment has been based on individual and population level standard procedures. Although these have been important to assess adverse effects on ecosystems, they are generally simplified and lack ecological realism. Microcosm studies using natural meiobenthic communities offer a good compromise between the complexity of the ecosystem and the often highly artificial settings of laboratory experiments. The aim of this study is to demonstrate the usefulness of microcosms with meiofauna as a tool in ecotoxicology. To do so, I will present the results from two case studies that have evaluated the impacts of different contaminants on meiofauna. The first case study investigated the effects of exposure to sewage-impacted pore water simultaneously at the community level using meiofauna microcosms and at the population level using laboratory fecundity tests with the copepod *Nitocra* sp. Both approaches were efficient in detecting differences in toxicity between the cleaner and most polluted sites, but only community data detected differences within the environmental contamination gradient. The second case study was divided in two experimental setups. The first assessed the effects of the antifouling biocides Irgarol and Diuron on meiofauna, and the second examined the influence of sediment organic carbon content on Irgarol toxicity. Results have shown high mortality, changes in community structure and species loss at biocide levels frequently encountered in the field. The severe effects observed contrast to other studies that have detected lower impacts. Considering the relatively high tolerance of meiofauna to pollution when compared to other taxa, it is unlikely that the disparities in results are due to a higher sensitivity of these organisms. It is more plausible to expect that severe effects are due to the microcosm approach used, which considers the entire community and its physical and chemical environment, preserving species interactions and different exposure routes. In addition to information about toxicity, the experiments gave indications about sensitive and tolerant species, indirect ecological effects and variation in contaminant bioavailability. Considering the importance of meiofauna for benthic ecosystems, the microcosm approach using natural communities might be a valuable addition as a higher tier approach in ecotoxicology.

#### 4 Calcification biomarkers in the coral MUSSISMILIA HARTTII: combined effects of seawater acidification and copper exposure

L. Marangoni, FURG- Universidade Federal do Rio Grande / Instituto Ciências Biológicas; M. Novazzi, FURG Universidade Federal do Rio Grande; J. Marques, Universidade Federal do Rio Grande; A. Bianchini, Universidade Federal do Rio Grande - FURG / Instituto de Ciências Biológicas

Seawater acidification results in reduced calcification and increased dissolution rates of major marine calcifiers, such as corals. In turn, seawater enrichment with copper (Cu) is a notable local impact threatening coral reefs. Metals forming strong complexes with carbonate will be more strongly affected by ocean acidification (OA), increasing their free ionic form, which is known to be toxic to organisms. In fact, inorganic speciation of metals in seawater as a function of pH and time show that the largest percentage (30%) increase for carbonate-dominated metals is for Cu<sup>2+</sup>. In this context, the present study aimed to evaluate the combined effects of OA and Cu exposure in the calcification process of the scleractinian coral *Mussismilia hartii*, one of the main framework builders in Southern Atlantic reefs. Polyps from 5 colonies were collected in the Recife de Fora Municipal Park (Porto Seguro, BA, Brazil), and kept in aquaria at a marine mesocosm (Projeto Coral Vivo, Porto Seguro, BA, Brazil). After acclimation, organisms were kept under control condition (pH 8.1; 1.0 µg/L Cu) or exposed to sub-lethal concentrations of Cu (1.6, 2.0 and 3.2 µg/L) combined with different pH treatments (pH 7.8; 7.6 and 7.2). Treatments were selected according to IPCC (2014) predictions and applied in isolation and combined (16 treatments; n=3

per treatment). After 35 days of exposure, corals were evaluated regarding the activity of enzymes associated with the calcification process (Ca-ATPase and carbonic anhydrase). Data were analyzed using factorial ANOVA followed by the Dunnett test. Carbonic anhydrase activity was reduced in all treatments tested, except to the isolated treatments of Cu ( $1.6 \mu\text{g L}^{-1}$ ) and pH (7.8) when compared to control condition. Ca-ATPase activity was also reduced in the combined treatments of Cu ( $2.0$  and  $3.2 \mu\text{g/L}$ ) and pH (7.5 and 7.2) with respect to control condition. Once the experiment was performed in a mesocosm system, it incorporates more ecological complexity, thus providing more ecologically relevant data. In summary, our results indicate that the calcification process in the coral *M. herttii* may be affected by exposure to environmentally relevant Cu concentrations and OA. The interaction between these stressors enhances the deleterious effects on *M. herttii* physiology. Future scenarios implicating the combination of these stressors may lead to more severe impacts to coral reef ecosystems.

#### 5 Inter-specific variability in the antioxidant defense system in *Aegla* (Crustacea, Decapoda): is it phylogenetically and environmentally driven?

S.C. Faria, Universidade Federal do Rio Grande FURG / Instituto de Ciências Biológicas; R.D. Klein, Universidade Federal do Rio Grande FURG / ICB; P.G. Costa, FURG / Escola de química e alimentos; M. Lauer, Universidade Estadual de Londrina / DCIF; M.S. Crivellaro, S. Santos, Universidade Federal de Santa Maria / Departamento De Biologia; S.d. Bueno, Universidade de Sao Paulo / Instituto de Biociências; A. Bianchini, Universidade Federal do Rio Grande - FURG / Instituto de Ciências Biológicas

The antioxidant defense system (ADS) protects organisms against the potential oxidative stress induced by environmental stressors, underlying processes of habitat diversification. The anomurans *Aegla* constitute the most threatened freshwater decapod taxon of South America, occupying pristine habitats with narrow distribution: almost 60% of species are under threat, with 72% of the Brazilian representatives. It is not known whether the concerning with aeglid conservation is only consequence of ecological-geographical constraints as historically proposed, or also due to a reduced capacity of ADS. Using phylogenetic comparative methods, we addressed three main questions: Is the variability of freshwater physicochemical parameters and tissue ADS phylogenetically structured? How do environmental features correlate with tissue ADS? How do they vary among species? Physicochemical parameters [temperature, pH, conductivity, dissolved oxygen content, alkalinity, ion composition (sulfate,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ) and concentrations of total organic carbon and metals (Ag, Cu, Cd, Cr, Fe, Mn, Pb and Zn)] of water collected at the sampling sites of ten aeglid species were measured. Additionally, metal accumulation and ADS parameters [metallothionein-like proteins (MTLP), antioxidant capacity against peroxyl radicals (ACAP), and glutathione system (GSH-GSSG)] were evaluated in hepatopancreas. Metal accumulation and water conductivity and pH were phylogenetically correlated, while all other water physicochemical traits demonstrated plastic variability. All metals were at natural concentrations, and were not associated with bioaccumulation levels. MTLP and ACAP correlated to the eigenvector 1 (39.2% of variance): ACAP is driven by conductivity and concentration of most metals; MTLP, interestingly, was not related to metal concentration, apparently underpinned by the relative stable GSH/GSSG ratio. In this context, metal accumulation and ACAP appear to be interesting biomarkers in aeglids: metal tissue accumulation under a phylogenetic perspective enable predictions about metal tolerance among closely related species, while small environmental disorders can be detected by changes in ACAP. Thus, the incorporation of phylogenetics to evaluate the environmental correlates of ADS parameters retrieves a still cryptic evolutionary history of aeglid physiology, unraveling a potential framework for future monitoring strategies of this conspicuous freshwater group.

#### 6 Habitat fragmentation and avoidance caused by the herbicide atrazine: The chemical barrier effect isolating the fish population

C. Araujo, Consejo Superior de Investigaciones Científicas / Department Ecology and Coastal Management; D. Silva, University of Sao Paulo - USP / Biotechnology - DEBIQ; L.E. Gomes, Escola de Engenharia de Lorena-USP / DEBIQ; R. Acayaba, Chemistry Institute - University of Campinas; M. Moreira dos Santos, CFE - Centre for Functional Ecology / Centro de Ecologia Funcional; R. Ribeiro, Universidade de Coimbra / Life Sciences; C.C. Raimundo, Chemistry Institute - University of Campinas / Química Analítica; M. Pompêo, University of São Paulo  
The herbicide atrazine has been recognized as a potential toxicant due to effects caused on organisms. However, information on how atrazine can affect the spatial distribution of the organisms is inexistent. As this effect has been observed for some contaminants, in the present study we hypothesized that atrazine discharges could trigger spatial avoidance in the fish *Poecilia reticulata* and provoke a chemical barrier isolating the populations. Firstly, fish were exposed to an atrazine gradient in a non-forced exposure

system, in which organisms moved freely among the concentrations, to assess their ability to avoid atrazine. Secondly, a chemical barrier formed by atrazine, separating two clean habitats (extremity of the non-forced system), was simulated to assess if the presence of the contaminant could prevent organisms to migrate to the other side of the system. Initially, it was observed that fish were able to detect and avoid atrazine contamination to environmentally relevant concentrations ( $0.001 \mu\text{g}\cdot\text{L}^{-1}$ ) below those described to cause sub-lethal effects. The  $\text{AC}_{50}$  (concentration causing an avoidance to 50% of the exposed population) was  $0.0146 \mu\text{g}\cdot\text{L}^{-1}$ . Atrazine chemical barrier at  $100 \mu\text{g}\cdot\text{L}^{-1}$  (concentration causing an avoidance of 80%) and  $1000 \mu\text{g}\cdot\text{L}^{-1}$  (concentration causing torpidity in avoidance tests) caused a reduction in the migratory potential of the fish of 47 and 91%, respectively. Contamination by atrazine, besides driving the spatial distribution of fish populations, can act as a chemical barrier by isolating fish populations. This study includes a novel approach to be integrated to environmental risk assessment schemes to assess the contamination effects such as habitat fragmentation and population displacement and isolation.

#### 7 Potential effects of triclosan on spatial displacement and local population decline of the fish *Poecilia reticulata*

D. Silva, University of Sao Paulo - USP / Biotechnology - DEBIQ; C. Araujo, Consejo Superior de Investigaciones Científicas / Department Ecology and Coastal Management; J. López-Doval, Catalan Institute for Water Research / Depto de Ecología Laboratorio de Limnología; M.B. Neto, University of São Paulo; F. Silva, Engineering School of Lorena - University of São Paulo / Biotechnology; T. Paiva, University of Sao Paulo - USP / Biotechnology; M. Pompêo, University of São Paulo  
Triclosan (TCS) is an emerging contaminant of concern in environmental studies due to its potential adverse effects on fish behavior. Since spatial avoidance has been shown to be a sensitive, immediate, and relevant behavioral endpoint, the aims of the present study were as follows: (i) to determine if TCS has the capacity to trigger a spatial avoidance response in *Poecilia reticulata*, inducing its displacement to less contaminated areas; (ii) to predict the population immediate decline (PID) at the local scale caused by exposure to TCS, by integrating both lethality and avoidance responses; and (iii) to determine whether mortality is overestimated when forced and continuous exposure is used to assess toxic effects. Fish were exposed to TCS in a traditional forced exposure system, in order to assess mortality, and to a TCS gradient in a non-forced exposure system in which the fish could move freely among the concentrations. Two different non-forced exposure scenarios were simulated: (#1) a spatially permanent and wider gradient, including low and high concentrations; and (#2) a scenario with high concentrations, simulating a local discharge event. The fish were able to avoid TCS concentrations as low as  $0.2 \mu\text{g}\cdot\text{L}^{-1}$ . The intensity of avoidance was concentration-dependent, but was not dependent on the pattern (scenario) of the gradient. In general, up to the highest concentration tested ( $2,000 \mu\text{g}\cdot\text{L}^{-1}$ ), the local scale PID was essentially determined by the avoidance response. Mortality determined from the forced exposure systems was overestimated, relative to the non-forced exposure system, since the organisms were continuously and mandatorily exposed to the contaminant. The reduced mortality in a non-forced environment does not imply a lower effect at the ecosystem level, because part of the population (avoiders) is expected to disappear at the local scale by moving towards environments that are more favorable. TCS can be considered a potential environmental disturber, since at environmentally relevant concentrations ( $< 2 \mu\text{g}\cdot\text{L}^{-1}$ ) it could cause an immediate decline in the fish population by triggering an avoidance response.

#### 8 Contact time influences Cr (VI) solution adsorption by chitosan beads: An ecotoxicity assay with *Allonais inaequalis*

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A contaminant very common in aquatic environments in Brazil is the Cr(VI) which concentration is due to tannery effluents disposal, electrochemical plating discharge and runoff of agrochemicals used in agricultural activities. Chitosan has been applied as metal ions adsorbent, consisting in an interesting alternative to use in aquatic environments due its biocompatibility and biodegradability. Thus, this work aims to evaluate the mortality of organisms exposed to residual concentrations of Cr (VI) with different contact time between chitosan-metal ion. *Allonais inaequalis* was the specie studied due its peculiarity as benthic organisms that make incursions in the water column. Ecotoxicological bioassays were conducted in triplicates, in flasks of 500mL, with 30g of sterile sand, 240mL of Cr(VI) solution in the following concentrations: 0.5, 1.0 and 1.5 for chronic tests and, 1.0, 1.5 and 2.0  $\text{mg L}^{-1}$  for acute tests. Then, were added 5g of chitosan beads with 6 organisms in each sample, fed with 5 mL of Tetramin solution ( $5 \text{mg L}^{-1}$ ). Bioassays conditions were: photoperiod of 12h light/12h dark, pH 7, conductivity of  $16\text{-}56 \mu\text{S cm}^{-1}$ , temperature of  $24^\circ\text{C} \pm 2$  and

eration only for chronic tests (4 mL s<sup>-1</sup>). Chitosan from Chengyue Planting Co. Ltda – China was characterized by degree of acetylation (5.5%) and molecular weight ( $M_n \approx 85 \text{ kg mol}^{-1}$ ). The chitosan beads with average diameter of  $2.8 \pm 0.4 \text{ mm}$  were crosslinked with epichlorohydrin. The organisms were exposed to Cr(VI) in absence and presence of chitosan beads, with different contact time between chitosan-metal ions, as follow: at (T=0) beads were added at the same time of organisms; at (T=24) beads were added 24h before organisms and at (T=48) beads were added 48h before organisms. Mortality rate of *A. inaequalis* reduced in presence of chitosan compared to tests without chitosan. With increasing contact time between chitosan and metal ions solution, a reduction in the mortality rate of exposed organisms was observed, indicating that high metals removal and low impacts on tested organisms were observed, as long as chitosan remains in contact with the liquid. Nevertheless, the ecotoxicological bioassays will be assessed with RAPD (random amplified polymorphic DNA) technique, which will focus on *A. inaequalis* genotoxicity evaluation of Cr(VI) with different contact time between chitosan-metal ion. Toxicity evaluation with RAPD technique is novel and can elucidate the DNA alteration with the Cr.

### 9 Effects of fragmentation and mercury contamination on marsh periwinkle (*Littoraria irrorata*) movement

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Habitat fragmentation is currently one of the primary causes of global biodiversity loss. However, not much is known about the interaction effects of fragmentation and contamination. This study performed different laboratory experiments to evaluate how fragmentation and mercury contamination can interact and affect the movement behavior of the marsh periwinkle *Littoraria irrorata*. Random microlandscapes were created using 10x15 inch trays and fragmented with copper tape that repulses the snail. *Spartina alterniflora* blades were set in one side of the tray as to attract the across the microlandscapes. Control and mercury-exposed snail movements were recorded with a digital camera. In the first experiment four different microlandscape patterns were created for three levels of habitat cover (100, 85 and 70 percent of cover). Organisms were exposed to two concentrations of mercury (400 µg/L and 450 µg/L) in the water for 96h before the experiment. Twelve organisms were used for each combination of level of cover and mercury concentration. The position of each organism was digitized as x/y coordinates and the mean speed, total distance travelled, and time to start moving were calculated. In the second experiment, one microlandscape pattern was used for six levels of habitat cover (100, 85, 70, 55, 40, and 25 percent of cover). Snails were exposed to two different concentrations of mercury (250 µg/L and 350 µg/L). Fifteen organisms were used for each combination of level of cover and mercury concentration. The length and weight of organisms were recorded in both experiments. For each experiment, a generalized liner model with the logit link function was used to evaluate the effects of fragmentation and mercury exposure on the probability of crossing the microlandscapes. In both experiments, fragmentation and mercury had significant effects in the probability of the organism to cross the landscape. The mean speed of organisms was also affected by the mercury exposure but not by fragmentation. These findings suggest that contamination might enhance the effects of fragmentation by affecting the navigation and motion capacity of organisms. Future *in situ* studies will consider more realistic and long term scenarios.

### 10 Biological tolerance and sublethal effects in *Ucides cordatus* (Linnaeus, 1763) fed by contaminated leaves

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Crustaceans may develop biological tolerance when exposed to contamination over time, resulting from an adaptation achieved during their life or by genetic selection. Aquatic toxicity occurs through direct absorption of chemicals present in water, sediment or by feeding. In the contaminated mangroves of Cubatão (SP), there are records that the leaves of *Rhizophora mangle* have high rates of pollutants. These leaves are the main source of food to the Uçá-Crab (*Ucides cordatus*), a species considered sentinel of this ecosystem. There are some evidences that *U. cordatus* has developed biological resistance to the main contaminants present in the mangroves of Cubatão. The objective of the study was to assess if the crabs that live in this contaminated mangrove have a higher biological tolerance comparatively to animals living in a pristine area (Jureia-Itatins). Ninety specimens from Cubatão (CUB) and ESEC Jureia-Itatins (JUR: pristine mangrove) were fed with leaves of *R. mangle* for

43 days (15 of acclimation: animals fed with uncontaminated leaves). The *ex-situ* experiment was divided in three treatments: Control (crabs from Jureia fed with uncontaminated leaves), CUB and JUR (crabs from Cubatão and Jureia fed with contaminated leaves, respectively). At seven-day intervals, the genotoxicity (Micronucleus Assay) and cytotoxicity (Neutral Red Assay) were evaluated (T-0, T-7, T-14, T-21 and T-28). The genetic and physiological effects observed in animals from CUB and JUR treatments were statistically different from those belonging to the Control right after the first week of experiment (T-7) and remained significant throughout the period of 21 days. The physiological effects observed in the animals from CUB and JUR showed no differences during the whole experiment. In relation to genotoxicity, crabs from JUR showed higher effect recorded after 14 days of feeding, remaining stable after this period. However, the animals from CUB showed a significant temporal decrease in the frequency of micronucleated cells, possibly due to the biological genetic adaptation to contaminants found in the leaves. In conclusion, the tolerance and adaptation capacity may vary according to the level of organization analyzed and, possibly, the animals resident in the mangroves of Cubatão have developed genetic mechanisms of tolerance to the main contaminants of this region.

### 11 Probability of genocytotoxic damages in *Ucides cordatus* (Linnaeus, 1763) from mangroves of São Paulo (Brazil) contaminated with metals

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The Uçá-crab (*Ucides cordatus*) can be considered a sentinel of mangroves due to its close contact with environmental compartments (water and sediment) and for revealing disturbances in its biological and population parameters when exposed to contamination. Metals have the highest toxicity and environmental persistence, although information about the contamination of the mangroves of the State of São Paulo is still limited. Six mangroves areas were studied (Bertioga, Cubatão, São Vicente, Jureia, Iguape and Cananéia), each of them represented by three subareas. Tree samples of water (from the burrows of the crab) and sediment were collected from these 18 locations for the determination of a total of six metals (Cd, Cr, Cu, Hg, Mn and Pb) by atomic absorption spectrophotometry. Ten adult male *U. cordatus* specimens (carapace width > 60 mm, in intermolt stage and free of parasites) were captured from these same locations in order to analyze the frequency of micronucleated cells (MN%: genotoxic) and the neutral red retention time in lysosomes (NRRT: cytotoxic). A Spearman's rank correlation was performed to relate the means of metals concentration in each abiotic compartment and the results of the biomarkers (MN% and NRRT). Only the significant relations were selected and the probabilities of damages were calculated for geno (CG<sub>50%</sub>) and cytotoxic (CC<sub>50%</sub>) concentration, using a nonparametric sigmoid model, by least squares estimation. Guidelines values of metals were compared to those concentrations recorded in mangroves in São Paulo. The results have showed that only two metals (Pb: water and sediment, and Hg: sediment) had a significant correlation with the biomarkers responses. All estimates of the curve slope and the probability of 50% (CG and CC) were significant (p < 0.05). For Pb the CG<sub>50%</sub> occurred with 0.171 µg/g in water and CC<sub>50%</sub> was 6.49 µg/g in sediment, while for mercury the CG<sub>50%</sub> succeeded with 162.8 ng/g in sediment. Genetic (MN%) and physiological (NRRT) damages may have pathological consequences that determine populations impacts, which already have been noticed for this species in the mangroves of São Paulo. The results and other available data may encourage environmental institutions to prioritize effective actions directed toward environmental for public policies aimed at environmental recovery, as well as the possible use of these two biomarkers of this crustacean in evaluations of contamination by metals in the mangrove ecosystems.

### 12 Relationships between trace metals and *Anomalocardia brasiliana* bioaccumulation after exposure to superficial sediments from Sepetiba bay, Brazil

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Bivalves are species frequently used in marine and coastal ecosystem monitoring programs, mainly due to their food behavior and low mobility; these characteristics expose organisms to both water column and sediments. Mollusks are commonly consumed by local people along the coastal area of Sepetiba bay. The purpose of this work was to evaluate the bioaccumulation of trace metals in *Anomalocardia brasiliana* soft tissues after an exposure experiment with sediments from a contaminated area, in



Setetiba bay. Sediment samples were collected at five stations in Madeira Island coast and grain size, organic carbon content, major and trace metals were analyzed. Individuals of *A. brasiliensis*, collected from a reference area at São Paulo coast (Canaã), were exposed during 21 days to sediments from the study area. After this exposure, soft tissues were digested with HNO<sub>3</sub> concentrated in a microwave oven. Cd, Cr, Cu, Ni, Pb and Zn were quantified in ICP-MS for tissues extracts and ICP-OES was used to HCl 6 mol L<sup>-1</sup> extracts from sediments. An equilibrium-partitioning model to predict pore water metal availability was applied based on acid-volatile sulfide (AVS) normalization. The bioaccumulation assay exhibited the following decreasing order: Zn>Ni>Pb>Cd>Cr, while the mean concentration of metals in sediment showed the following trend Zn>Pb>Cu>Cd>Cr>Ni. The mean concentration of Cd, Cr, Pb and Zn in tissues exceeded the permissible limit of Brazilian legislation; mostly near to Saco do Engenho inlet. Normalization approach to AVS suggested that metals are likely unavailable in the sediment pore water, except for sediments at Guarda Channel Mouth. Metals concentrations were, in general, lower in organisms exposed to the control sediments and sediments sampled at larger distances from the major metal source. Relationship between SEM/AVS ratios and metal bioaccumulation in laboratory assays was not conclusive. In our study, bioaccumulation increased linearly with the sediment metal concentrations, independently of AVS. Metal bioaccumulation appeared to occur at SEM/AVS ratios less than 1, as observed near to the major source and Itaguaí Harbor. Our results demonstrate that, if metal accumulation from ingested sediments contributes to the total body metal contents, the use of AVS models only for assessing metal bioavailability will underestimate bioaccumulation and potential effects related to trace metal contamination of filter-feeding benthic organisms.

## Discussing our physiological, biochemical or molecular studies on aquatic organisms exposed to environmental pollutants to improve the quality and the ecotoxicological sound of our research - Part 1

### 13 How necessary is to include the principles of sound ecotoxicology in our studies?

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An interesting manuscript was published by Harris et al in 2013 postulating a series of "principles of sound ecotoxicology". However, still today many published studies lack many of those principles. From the twelve principles, in this presentation three of them are contrasted against experiences gathered in our own lab: i) Define de exposure: measurements of the actual concentrations in bioassays conducted with the organochlorine endosulfan showed not only that concentrations can drastically dropped during the experiment, but also that the isomer composition can change. Similarly, measurement of EE2 before and after media renewal demonstrated this compound virtually disappear from test chambers in 48 hs, ii) Consider dose response: several times studies testing only one concentration are presented, but studies evaluating several biomarkers have shown in our lab that usually random significant responses are observed under interspecific exposures, but clear dose-response are obtained when model toxicants are used, iii) Repeat the experiment: we have obtained very different results among experiments for both, lethal and sublethal responses against the same toxicant under equivalent conditions, especially when the substances are slightly toxic. In addition, a thirteen principle could be added to Harris, principles, "Use positive controls": Several times we obtain responses that you only realize they are irrelevant when you compare them against a positive control. In conclusion, several examples are given supporting the need of applying principles of sound ecotoxicology in our studies.

### 14 Nanoeotoxicology of carbon nanomaterials: facts and caveats

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Nanotechnology has brought impressive advances to many fields of modern science. In this sense, carbon nanomaterials (CNM) are nanomaterials considered for several applications including biomedicine and bioremediation because of their flexible structure and possibilities for chemical functionalization. Ecotoxicological data for aquatic organisms show a clear prevalence of studies employing freshwater organisms. However, and considering that agglomeration and deposition seems to be a common fate for several nanomaterials, data coming from estuarine and marine organisms are urgently needed, once ionic force agglomerates CNM as fullerenes (C60). Studies with shrimp *Litopenaeus vannamei* showed in gills, hepatopancreas and gut the characteristic D and G band of graphene oxide (GO) using Raman spectroscopy. Also, and because of their high relative surface area, some CNM can adsorb toxic molecules

favoring its cellular entry. Co-exposure of GO and arsenic (As) induced higher accumulation of this metalloid in shrimp gut. Moreover, As metabolism was different in shrimp organs only exposed to As when compared with those co-exposed to GO+As. As caveats it must be mentioned the potential artifacts that several CNM (fullerene and carbon nanotubes, for example) induce in several techniques employed to measure toxic endpoints as comet assay, micronuclei test or thiobarbituric acid reactive substances. A previous verification of potential interferences in the selected variables should be mandatory. New strategies have been considered in recent years to cope with the increasing number of nanomaterials that must be evaluated about their potential toxicity. For example, implementation of *in silico* methods based in Docking Simulation (DS) appears to be an efficient alternative for the prediction nanomaterials hazard. Studies performed with mitochondria proteins (ADP/ATP carrier- ANT-1 and Voltage-Dependent Anion Channels- VDAC) showed they probably are targets for VDAC with single-walled carbon nanotubes (SWCNT).

### 15 Toxicity and intracellular fate of graphene-related nanomaterials in fish cell lines

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NanoReg2 project aims to develop and implement grouping and Safe by Design strategies for nanomaterials (NMs). For this purpose, toxicity of selected carbon-based NMs have been assessed in order to refine Safe by Design approaches considering three pillars: safer product, safer use, and safer production process. Graphene-related NMs are one of the newest and most important NMs due to their unique physicochemical properties. Despite this, to date, only a few toxicological studies have focused on these materials and derivatives. The objectives of the present study were to investigate the underlying mechanism of toxicity of carbon nanofibers and graphene oxide on two different cell models including fish hepatocytes (topminnow fish hepatoma cell line, PLHC-1) and macrophages (carp leukocyte cell line, CLC). Dynamic light scattering and transmission electron microscopy (TEM) were used to monitor particle size evolution in cell culture medium and results showed stable suspensions for all NMs under experimental conditions. Cells were exposed to a concentration range of 0-200 µg ml<sup>-1</sup> of NMs for 24 and 72 h and cell viability was assessed by applying on the same set of cells three different cytotoxicity assays (alamarblue, 5-carboxyfluorescein diacetate-acetoxymethyl ester, and neutral red uptake that provide information on the mitochondrial activity, plasma membrane integrity and lysosomal function, respectively). Moreover, interference of NMs with the assay reagents has been tested as well. Some differences in cellular responses to the different types of NMs were observed, however, in most cases toxicity was pronounced only at the highest exposure concentration. In general, both cell lines exhibited time-dependent toxic responses. TEM was used to investigate possible internalization and intracellular fate of these NMs in hepatocytes and macrophages. Some NMs were visualized in the cells even at the lowest exposure concentrations. This is the first study to examine the cytotoxic effects of these graphene-related NMs that are used in a variety of intermediate industrial products. This research is supported by the EU's Horizon 2020 research and innovation programme (NanoReg2, Grant Agreement n° 646221).

### 16 Subcellular metal partitioning measurements in two groups of aquatic organisms (insects, fish): contributions to the understanding of metal toxicity

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Knowledge of subcellular partitioning of trace metals in the cells of aquatic organisms is useful for understanding and predicting metal toxicity. Such subcellular measurements can reveal the metal-detoxification strategies used by aquatic organisms to cope with potentially toxic metals. In addition to these detoxification responses, the subcellular metal concentrations that are likely to be responsible for adverse effects in living cells can be estimated by measuring metal accumulation in physiologically important potentially sensitive sites such as heat-denatured proteins, mitochondria and microsomes. In order to determine the metal-handling strategies used by aquatic organisms, two animals with differing tolerances to trace elements were collected from metal-contaminated environments: final-instar larvae of the insect *Chaoborus* (whole body) and North Atlantic yellow eels (*Anguilla anguilla* and *A. rostrata*; liver). A subcellular partitioning procedure using differential centrifugation, NaOH digestion and thermal shock steps was applied to distinguish between metal (Ag, As, Cd, Ni, Pb and Tl) accumulation in detoxified metal fractions and accumulation in putative metal-sensitive fractions. Our results showed that metal sequestration by metallothioneins or



metallothionein-like proteins played an important role in metal detoxification (e.g., for Cd, Ag), preventing the binding of nonessential metals at physiologically important sites. Incorporation of metals such as Ni, Pb and Tl into mineral inclusions was another important and complementary metal-detoxification strategy. However, these strategies were not completely successful since significant accumulations in metal-sensitive fractions were observed for all the metals studied, even for animals exposed at the lower end of the metal contamination gradient. These inappropriate bindings of nonessential metals could induce deleterious effects. Among the metal-sensitive fractions, mitochondria appear to be a major binding compartment (for As, Cd, Pb and Tl). The percentage of accumulated trace metals stored in detoxified form varied greatly among metals and animals. Such information should help the development of improved models for predicting metal toxicity, based on the biologically active pools that contribute to toxic responses and not on the total accumulated metal concentrations.

#### 17 Local morphometry and biogeochemistry affects methylmercury bioaccumulation and biological responses in fish from an Amazon floodplain lake

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Mercury in the Amazon River system is derived from a variable mixture of natural and anthropogenic sources. Anoxic environments in floodplain lakes have been identified as the main sites of mercury methylation, a critical step in the bioaccumulation of mercury in the aquatic biota of this system. Lago Janauacá is located along the southern floodplain of Solimões River, 40 km upstream from Manaus. The northern part of this lake is shallower than the southern region, which results in different patterns of thermal and oxygen stratification. We hypothesized that this would result in different patterns of mercury dynamics and ecotoxicological responses in these regions. To test this hypothesis we collected *Hoplias malabaricus*, a sedentary predatory fish, in the northern and southern regions of the lake during the dry season of 2015. Samples of water and sediments and in-situ measurements of relevant physicochemical parameters were also collected at both sites. Mercury analyses were performed following EPA 1630 and Pichet et al (1999). Metallothionein and histopathological lesions in fish were determined according to Viarengo et al (1997) and Bernet et al (1999). MeHg concentrations in water were significantly higher in the southern region (t-test,  $p = 0.0114$ ) due to greater stratification of temperature and oxygen which favored mercury methylation. Fish from the southern region presented significantly higher MeHg bioaccumulation (t-test,  $p=0.017$ ), higher metallothionein content (t-test,  $p=0.036$ ) and higher occurrence of hepatic histopathologic lesions (t-test,  $p=0.036$ ) than fish from the northern region. Our results demonstrated the importance of local variations in morphometry and stratification in controlling the methylation and bioaccumulation of mercury and its ecotoxicological consequences in this Amazon floodplain lake.

#### 18 Metal Bioaccumulation and Metallothioneins induction in blue crabs *Callinectes danae* exposed to Lead (Pb)

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The blue crab *Callinectes danae* is distributed throughout the Atlantic Ocean from Florida (USA) to the Southern Coast of Brazil. This species has been used as a biomonitor model for metal contamination in estuarine environments. Different metals are expected to bioaccumulate differently according to the type of tissue. An effort to understand the processes of bioaccumulation due to pathways such as feeding and water contact may improve the use of crabs such as *C. danae* as biomonitors. The presence of lead (Pb) in the environment has been recognized as a serious public health problem. In Brazil, contamination events caused by the inappropriate disposal of slags, ore and residues from Pb mining and refining activities at the Santo Amaro da Purificação (BA), Adrianópolis (PR) and Ribeira (SP) are some examples that have still motivate studies on the biological effects of Pb. The aim of this study is to assess bioaccumulation of Pb and metallothioneins induction through dietary and waterborne exposures in *Callinectes danae*. Adult individuals of *C. danae* were collected in the south area of the Cananéia-Iguape-Peruíbe Protected Area (APA-CIP). After the

acclimatization and depuration period, exposure assays were performed during 2 periods (7 and 14 days), considering two test-concentrations of Pb ( $0.5$  e  $2.0 \mu\text{g g}^{-1}$ ) in 4 treatments: 1) control, using non-contaminated artificial salinized water and non-contaminated artificial food; 2) contaminated water and non-contaminated food; 3) non-contaminated water and contaminated food; 4) combined treatment using contaminated water and food). The partial results for the exposure tests suggest that absorption through the gills is the most effective pathway for accumulation of Pb in the evaluated periods at the concentration of  $2 \mu\text{g g}^{-1}$  Pb in water. For the other treatments, possibly the evaluated periods were apparently too short to verify an effective accumulation of Pb in the tissues, which does not mean that it could not occur in longer periods of time. Other hypotheses would be that the depuration systems were effective or that the absorption simply did not occur, regardless of the time it was evaluated. For hepatopancreas, two-way ANOVA did not detect any significant statistical differences in results of metallothioneins. It was observed that the combined treatments were more effective in inducing the activity of metallothioneins in gills, which suggests that the pathways act simultaneously.

#### 19 Genetic biomarkers of Chilean northern scallop *A. purpuratus* in San Jorge Bay. Addressing potential risk of metals and hypersaline discharges

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Coastal marine areas are among the most important places for human settlements however, the rapid urbanization and industrialization have continuously pressure the coastal ecosystems and frequently, simultaneously and synergistically contribute its degradation. In Chile, recent scientific research in the northern region, particularly San Jorge Bay (Antofagasta), indicate multiple stressors including a high degree of metal contamination along the bay's coast line as evidences of notorious effects of the mining industrial port activity and increasing hypersaline discharges as result of sea water desalination for both domestic and industrial purpose. Our research approach included an iterative process focused in temporal and spatial trends assessment of key-stressors mass balance and its specific causal-effects relationship models using genetic molecular specific biomarkers in a key-bioindicator organism (*A. purpuratus*), in order to differentiate it from other recurrent natural events in this bay (e.g upwelling periods). Starting from RT-PCR primers design and validation under field semi-controlled and lab condition, our specific scallop biomarkers for metal (metallothioneins, MTs) and hypersaline water exposure (osmotic regulation kinase, OSRK) measured in several scallop tissues, have proven to be reliable specific biomarkers indicating a zonal restricted impact and time-sensitive variation of stressors in San Jorge Bay. Furthermore, differential hypersaline effluent effects were observed. It is expected that these real-time genetic/molecular responses when coupled with environmental physicochemical and available long-term ecological monitoring data sets (currently under analysis), will provide a useful starting point for developing high throughput models and foundation for ecological risk assessment, focus in development, compatibility, long-term sustainability of this essential human activities in northern Chile, ensuring the protection of marine aquatic receiving environments. Keywords: Genetic biomarkers, metals, water desalination, *A. purpuratus*.

#### 20 DNA damage in the freshwater fish *Australoheros facetus* acutely exposed to imidacloprid: possible explanation through an oxidative mechanism.

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The presence of neonicotinoid insecticides in a wide type of environments around the world and its possible negative effects in non-target organisms are important topics in environmental risk assessment. Particularly, there is a large amount of reports about imidacloprid (IMI) presence in freshwater bodies, and its effects on aquatic invertebrates. Nevertheless, negative effects in freshwater fish are usually limited to mortality endpoints, and there is a lack of information about sublethal effects in this

taxon. Hence, the objective of this work was to evaluate genotoxic effects and oxidative damage of the Southamerican fish *Australoheros facetus* exposed to environmental concentrations of IMI. Fish were exposed in an acute experiment for 48 h to 1, 10, 100 and 1000  $\mu\text{g/L}$  IMI. DNA damage through the Micronucleus (MN) and the Comet assays were performed in blood cells, the last with ENDO III enzyme modification to detect pyrimidine bases oxidation. Lipid and protein oxidation were quantified in liver, brain and gills. DNA damage through the MN frequency was evidenced at 1000  $\mu\text{g/L}$ , while with the comet assay- damage index this effect was observed at 100 and 1000  $\mu\text{g/L}$ . Moreover, an increase of ENDO III sites at all IMI concentrations tested was observed. Lipid peroxidation was detected at 100  $\mu\text{g/L}$  IMI in gills as well as at 1000  $\mu\text{g/L}$  IMI in brain. These results show that a process of DNA bases oxidation occurs from low IMI concentrations, and at high IMI concentration there is DNA fragmentation too. Therefore, these results suggest that the possible mechanism of DNA damage could start with an oxidation process, leading to DNA fragmentation. Comparatively, comet assay was a more sensitive tool than MN frequency to evaluate DNA damage. In addition, lipid peroxidation in gills and brain alert about possible sublethal effects like oxidative damage induced by IMI in different key tissues of fish. These negative effects must be taken into account as possible endpoints in ecological risk assessment of IMI in freshwater bodies, mainly because DNA damage could trigger other effects at organism or population levels.

## 21 Determination of Aflatoxin B<sub>1</sub> in Muscles, Organs, Plasma and Water of *Rhamdia quelen* through LC/MS-MS

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**Introduction:** The presence of aflatoxins in the raw materials used in animal feed may result in residues in the final product. The ingestion of this contaminated products may present a risk to human health. **Objective:** This study aimed to investigate the possible accumulation of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in muscle organs, intestinal content, plasma and water of *Rhamdia quelen* that ingested animal feed contaminated with AFB<sub>1</sub>. **Methods:** Six hundred and twenty four *Rhamdia quelen* fingerlings were divided in 4 different feeding groups (G1, G2, G3 and G4 containing 0, 45, 90 and 180  $\mu\text{g.kg}^{-1}$  of AFB<sub>1</sub>, respectively) and randomly divided into 24 aquariums (n=26 fingerlings by aquarium) and fed for 56 consecutive days with pelleted feedstuffs. In order to quantify AFB<sub>1</sub> in water, 2 mL samples were collected from each aquarium on day 0 and then weekly onwards. Each week, all samples from each group were composed into a single sample per group per week. At the end of the trial period, there were a total of 36 samples. In order to measure the concentration of AFB<sub>1</sub> in muscle, organs (liver, kidneys, gills and intestine), intestinal content and plasma, 12 animals per group were sampled and samples were pooled at days +28, +42 and +56, totaling 12 pooled samples at the end of the trial period. The samples were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). The chromatographic determination was performed with reverse phase column, Gemini NX C<sub>18</sub> column (150 x 2.0 mm, 5 m). **Results:** The presence of AFB<sub>1</sub> was not detected in organs, muscle or water analyzed. AFB<sub>1</sub> was detected only in plasma samples from G3 and G4. AFB<sub>1</sub> levels were 0.87, 0.61, 3.79 and 6.22, 5.81 and 7.08  $\mu\text{g.kg}^{-1}$  of in G3 and G4, on day +28, +42 and +56, respectively. AFB<sub>1</sub> was absorbed and distributed throughout the organism since it was detected in plasma, but there was no accumulation in the viscera or muscles. **Conclusion:** The different levels and times of consumption do not present a linear or increasing trend in the quantities of AFB<sub>1</sub> absorbed and accumulated by the body of *Rhamdia quelen*.

## 22 Effect of crude extract of cyanobacteria *Radiocystis fernandoi* on the osmoregulatory processes of the neotropical fish, *Piaractus mesopotamicus*.

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The proliferation of cyanobacteria in rivers and lakes can increase the release of toxic substances, the cyanotoxins. The species *Radiocystis fernandoi* produces toxic compounds such as microcystins and peptides which may interfere with the activities of the osmoregulatory enzymes being potentially toxic. Juveniles pacu (*Piaractus mesopotamicus*) were divided into 3 experimental groups (CE3h, CE6h and CE24h) which received intraperitoneal (i.p.) injections of crude extract of *R. fernandoi* containing 100  $\mu\text{g.kg}^{-1}$  of MC-LR equivalent diluted in saline solution (NaCl 0.9%) and their respective controls (C3h, C6h and C24h) which received only saline solution. After 3, 6 and 24 h, blood and gills samples were collected for determination of plasma ions, activity of endogenous PP1 e PP2A phosphatases, Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA), H<sup>+</sup>-ATPase (HA) and carbonic anhydrase (CA) and histology of gills. The activities of

PP1 e PP2A did not change in any groups. The concentration of Na<sup>+</sup> decreased in CE3h and increased in CE6h. The concentration of K<sup>+</sup> decreased in all experimental groups. The activity of NKA increased in the first 3 h exposure and may be a mechanism to control ionic imbalance as the number of labeled chloride cells (CC) decreased after 3 h exposure in both the filaments and the lamellae. After 24 hours to restore ionic homeostasis. The HA activity did not change and the AC activity decreased 3 h after the i.p. injection with the reduction in the number of chloride cells that decreased after 3 h in both the filaments and the lamellae. The CC decreased in lamellae in CE6h groups and, after 24 h, the number of these cells increase in the lamellae. Histopathology was characterized by hyperplasia in the gills of fish exposed for 3 and 24 h; fish from CE24h groups presented lamellar atrophy and fusion and hyperplasia and rupture of lamellar epithelium. The results suggest that the cyanotoxins and other components present in the crude extract of *R. fernandoi* causes cellular changes in the gills which affect the osmoregulatory processes and plasma ion concentrations. Financial support: CAPES, CEMIG (Proc. GT 346), FAPESP (Proc. 2012/00728-1).

## 23 Depuration and toxic effects of Microcystins in a freshwater fish from water supply reservoir

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Reservoirs are artificial lakes used for water supply, power energy and recreation. These activities have caused environmental impacts such as eutrophication. The eutrophication is a phenomenon that results in algae and cyanobacterial blooms. Cyanobacteria can produce cyanotoxins that cause toxic effects to the organisms and human health. Iraí reservoir (Paraná, south Brazil) is used to water supply and other studies have reported Microcystins (MCs) in this water body. The aim of this study was to determinate the concentration of MC-LR, YR and RR in water and fish (*Geophagus brasiliensis*) samples from Iraí reservoir, and evaluate MCs depuration and toxic effects in *G. brasiliensis*. In August 2015, water and fish sample were collected in Iraí reservoir. Water sample was used to qualitative and quantitative analyses of phytoplankton and quantification of MCs. Fish were divided in two groups, the first one was called immediate group (IMM) and tissues were collected on the same day. The liver was collected in order to evaluate biotransformation and hepatotoxicity effects, gills were used to measure the activity of carbonic anhydrase, muscle and feces were collected in order to determinate MCs concentration. The second group, we called depuration group which were maintained in clean water for 90 days. Fish were maintained in controlled conditions, water and feces samples from aquaria were collected during the experiment. At day 7, 15, 30 and 90 fish samples were collected. Fish were subjected to the same procedure performed in the IMM. Phytoplankton samples showed high dominance and density of *Microcystis aeruginosa* (104.075 cells/mL). MCs concentrations were found in water and in fish from reservoir and depuration experiment. After 90 days it was possible identify MCs in the samples. Carbonic anhydrase activity decreased in 30 and 90 days. Hepatic GST activity and DNA damage increased in 15 and 30 days, at the same period that MCs concentrations increased in the water and feces sample. Our results suggest MCs and possible others composites in Iraí reservoir cause toxic effects in fish. Fish are accumulating MCs and the depuration starts in 15 days, but 90 days was not enough for the total toxin elimination. It is essential the development of monitoring strategies in this area in order to minimize the toxic effects to the aquatic organisms.

## 24 Combining biomarker responses and active biomonitoring for assessing diffuse sources of contamination in the reservoirs of the Iguçu River

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along four cascading reservoirs in the Iguaçú River, using caged tilapia (*Oreochromis niloticus*) as the animal model. Chemical analysis in the muscle showed high levels of metals in fish from the reservoir closest to an industrialized and environmentally degraded area. Persistent organic pollutants, as Polychlorinated biphenyls and organochlorine pesticides were detected in the water of two reservoirs in concentrations above the limits permitted by the Brazilian Legislation. Fish from all reservoirs had hepatic alterations, such as necrosis and inflammatory processes. Significant variations of biochemical biomarkers were also observed in all reservoirs, with no clear evidence of a contamination gradient. Fish from an intermediate reservoir showed indicative responses of higher environmental impact, such as increased levels of lipid peroxidation (LPO), increased CYP3A transcript levels and activity in liver, higher incidence of eosinophils in gonads and higher levels of polycyclic aromatic hydrocarbons (PAHs) metabolites in bile, than fish from others reservoirs. Thus, the alteration of these biological responses associated to the occurrence of different chemicals both in water and tissue of caged animals suggested the bioavailability of contaminants to biota. Although the current study provided relevant data that can be used for assessment of the risk of environmental chemicals to biota, additional studies with feral or caged animals of longer duration may be necessary to evaluate the risks of the waterways to humans and wildlife.

## Neotropical Ecotoxicology and Conservation: Legacy and Emerging Pollutants in the Neotropics

### 25 An analysis of the health effects of Red Tide on Florida Manatees (*Trichechus manatus latirostris*) using an OMICS approach

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Persistent and recurrent red tide (*K. brevis*) algal blooms are one of the major health threats to manatee (*Trichechus manatus latirostris*) populations in the southwest region of Florida. We compared serum from control and animals ill from red tide toxicity in a proteomics experiment and white blood cells in a transcriptomics experiment. Serum from red tide affected manatees indicated impacts to the immune system, including proteins involved in inflammation, wounds and injuries. In the manatees recovering from red tide, several proteins were elevated including, ceruloplasmin-like (2.32-fold increase compared to animals not exposed to red tide), complement pyruvate kinase isozymes M1/M2 isoform 3 (2.29-fold increase), angiotensinogen (2.08-fold increase), complement C4-A isoform 1 (1.83-fold increase), and C3 (1.42 fold increase). These proteins are associated with acute-phase response, amyloid formation and accumulation, copper and iron homeostasis, the complement cascade pathway, and other important cellular functions and may serve as good biomarker proteins for manatees. RNAseq was performed on total RNA isolated from manatee white blood cells to compare the red tide group to other manatees. The genes with the highest fold changes and lowest p-values (< 0.05) were the OSCAR gene (3.68 fold increase, p value of 0.0485), transmembrane protein 56-like (3.53-fold increase, p value of 0.000036), myotubularin related protein 2, transcript variant 2 (MTMR2) (3.53-fold increase, p value of 0.0015), thymocyte selection associated family member 2 (THEMIS2) (3.38-fold increase, p value of 0.0005), matrix metalloproteinase 9 (MMP9) (3.32-fold increase, p value of 0.0450) and haptoglobin (HP) (3.28-fold increase, p value of 0.0013). Several genes were down regulated including a number involved in immune response. This work indicated that the immune system, leukocyte production and differentiation, neuronal activity and synapse and tumor invasiveness were major pathways affected by red tide exposure.

### 26 Acute and chronic effects of aluminum oxide nanoparticles on *Daphnia magna*

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Aluminum oxide nanoparticles ( $Al_2O_3$  NP) has a wide range of applications as coatings and abrasives and as additives in the fields of composites and heat transfer-enhancing nanofluids. Owing to their commercial applications, have received considerable attention regarding possible environmental impacts. Thus, the aim of this study was to assess toxicity of  $Al_2O_3$  NP and micron-sized  $Al_2O_3$  ( $Al_2O_3$  Bulk) to

*Daphnia magna*. The influence of size, crystalline structure and large surface area was investigated. After a detailed identification of nanomaterial physico-chemical characteristics, *D. magna* was investigated with both acute and chronic toxicity tests. Were examined with 48-h acute toxicity tests and 21-day chronic tests. We investigated the effects of  $Al_2O_3$  NP and  $Al_2O_3$  Bulk on survival, reproduction and longevity. In addition, evaluated the production of reactive oxygen species (ROS), a key factor in response to oxidative stress. Acute toxicity tests show an increase in toxicity and ROS level was observed with increase in the concentration,  $Al_2O_3$  Bulk tested are less toxic than the  $Al_2O_3$  NP, effective concentration ( $EC_{50}$ ) values of  $Al_2O_3$  NP (1490 mg/L) were lower than that of  $Al_2O_3$  Bulk (4280 mg/L). Chronic exposure tests show effects on reproduction, length, and longevity of *D. magna*, these effects occurred generally at concentrations, which were one order of magnitude below the acute toxic levels. The NOEC (No Observed Effect concentration) and LOEC (lowest observed effect concentration) were calculated as 5 mg/L and 15 mg/L for  $Al_2O_3$  NP and 125 mg/L and 250 mg/L for  $Al_2O_3$  Bulk respectively. The presented results may help to understand potential impacts of NP and may assist in terms of use and safe disposal of NP as the reduction of its detrimental effects in environmental.

### 27 Impact of microplastics in marine sediments: effects on reproduction of epibenthic copepod *Nitocra* sp

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Pollution due to marine waste has gathered attention in the last years, and plastic residues have been held to account for more than half of its composition. Among all these residues there's the pellets, plastic granules from different polymers derived from the industries and used as raw material to build different objects. Nowadays, there's an increasing occurrence in the marine environment and due to its porosity, pellets tend to be highly associative with contaminants, working as chemical vectors transporting mostly organic pollutants. In this context, our research project aimed to evaluate the plastic pellets toxicity in the sediment using an ecotoxicological approach. Were evaluated chronic effects on epibenthic copepod *Nitocra* sp. exposed to the treatments elutriate and whole-sediment. Three experiments were performed for each treatment, using spiked sediment with virgin and beach-stranded pellets. Environmentally relevant concentrations were assessed during a period of ten days, and posteriorly the organisms were fixed and dyed to assess the number of offspring/female. Toxicity was observed on the whole-sediment treatment spiked with virgin and beach-stranded pellets, which can be directly associated with additives used to manufacture the virgin polypropylene pellets. Some of these additives are recognized as endocrine disrupters, and could become available to epibenthic organisms, which would cause decrease on reproductive capability. For the beach pellets, its toxicity may be associated with capacity of sorption for a variety of substances by interchanging them with different environmental matrices. In summary, it was concluded that environmental relevant concentrations of microplastics in sediment are toxic, representing a risk to marine ecosystems.

### 28 Accumulation of pesticides, cholinesterase inhibition and reduced body condition in juvenile *Jenynsia multidentata* from the Pampa Region of Argentina

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The aim of this study was to characterize the level and nature of the pesticide contamination reaching one-sided livebearer fish (*Jenynsia multidentata*) in a watercourse situated within the main agricultural region of Argentina, and to assess the effects of this contamination on fish health. Juvenile one-sided livebearer fish (*Jenynsia multidentata*) were collected in December 2011 and March 2012 from three sites along the Pergamino River. Pesticide contamination was characterized by extracting whole fish and analytically determining thirty different pesticide molecules. The biomarkers catalase, glutathion-S-transferase, and cholinesterases were assessed. Body condition was calculated as an estimate of the amount of energy reserves possessed by the fish. Seventeen different pesticides were detected in fish tissues with 81% of captured animals containing at least one pesticide molecule. The pyrethroid insecticides fenvalerate and bifenthrin were most frequently detected, being respectively found in 41.8 and 36.4% of samples tested. Highly toxic dichlorvos and pirimiphos-methyl were detected in the fish. Differential levels of contamination could not be established amongst sites but were observed within sites amongst the two



sampling dates. The months when pesticide residues were most abundant in Site A and B corresponded to the months when body condition was lowest in the two sites. The inhibition of cholinesterases activity in March when body condition was reduced in Site B and C also points out to a role of insecticide contamination in the reduction of body condition. These findings provide strong new evidence that current-used agricultural pesticides can accumulate in wild fish and impact their health and energetics

### 29 Biochemical markers of oxidative stress and neurotoxicity in the neotropical fish *Phalloceros harpagos* exposed to paracetamol and propranolol

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The contamination of aquatic environments is a consequence of human activities, leading to the release of a large number of distinct xenobiotics that can drastically affect the ecosystem, by causing deleterious effects to aquatic species. Production, consumption and inappropriate disposal of pharmaceuticals have important contributions for such contamination. Studies about the effects of these substances on non-target species from neotropical regions are however still scarce. In this scenario, the present study aimed to assess the effects of two pharmaceuticals, the anti-inflammatory and antipyretic paracetamol and the non-selective  $\beta$ -blocker propranolol, on the neotropical fish species *Phalloceros harpagos*, through the analysis of biochemical markers that indicate oxidative stress and neurotoxicity, namely catalase (CAT) in the liver, glutathione-S-transferases (GSTs) in the gills and cholinesterases (ChEs) in the head. The test organism was exposed to environmentally relevant concentrations of these drugs under acute (96h) and chronic (28d) exposures. For the acute exposure, the concentrations used were 0 (control), 8, 80, 800, 8000, 80000  $\mu\text{g.L}^{-1}$  for paracetamol, and 0 (control), 0.1, 1, 10, 100, 1000  $\mu\text{g.L}^{-1}$  for propranolol. For the chronic exposure, the concentrations were 0 (control), 5, 10, 20, 40, 80  $\mu\text{g.L}^{-1}$  for paracetamol, and 0 (control), 0.0625, 0.125, 0.25, 0.5, 1  $\mu\text{g.L}^{-1}$  for propranolol. Acute exposure results showed increased GSTs activity for higher concentrations of paracetamol (80, 800, 8000 and 80000  $\mu\text{g.L}^{-1}$ ) and propranolol (10 and 1000  $\mu\text{g.L}^{-1}$ ). CAT was not significantly changed for the exposed groups in relation to control group after exposure to paracetamol. ChEs activity was significantly increased following acute exposure to paracetamol. Differently, propranolol acute exposure did not result in any alteration of ChEs activity. Following the chronic exposures to both paracetamol and propranolol, CAT and ChEs activities were not altered. However GSTs activity was significantly increased for paracetamol (20  $\mu\text{g.L}^{-1}$ ) and propranolol (0.25 and 0.5  $\mu\text{g.L}^{-1}$ ). This set of results demonstrates that chronic exposures were causative of the activation of the phase II conjugation pathway for both drugs, even at environmentally relevant concentrations. Acute effects of paracetamol in terms of cholinesterasic activity are paradoxical and suggest the establishment of unknown physiological underlying mechanisms of regulation of its expression.

### 30 Effects of metals, metalloids and organochlorine compounds on haemosporidian blood parasites and physiological stress on birds in Central Mexico.

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Mining, which dumps large amounts of metals and metalloids in the environment, and agriculture, which exposes natural systems to diverse types of agrochemicals, are among the most important economic activities in Central Mexico. Despite the importance of these activities for economic development, they are important sources of pollution. The current study was located at the transition between the neotropical

and the nearctic regions in Central Mexico. We evaluated the potential effects of exposure to metals (As, Pb, and Sb) and organochlorine compounds on prevalence of haemosporidian parasites that are transmitted by dipteran insects to birds and cause bird malaria and haemoprotoeosis. We also evaluated effects on physiological stress measured through the heterophil/lymphocyte (H:L) ratio. Within bird families, percent haemosporidian prevalence was in general higher in sites exposed to arsenic, lead, and antimony than in unexposed sites. Contrastingly, within families, percent prevalence was lower in sites exposed to organochlorine compounds than background prevalence in unexposed sites. Regarding H:L, the effects of pollutants were inconsistent among species and pollutant types. These results suggest that very complex effects at the level of communities and ecosystems may occur. In the future, investigations of the effects of different types of contaminants on several compartments of ecological systems, as well as the interactions among these compartments will likely yield very exciting results.

### 31 Evaluación de la concentración de mercurio en especies estuarina de interés comercial en el Golfo de Guayaquil de Guayaquil - Ecuador

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El Golfo de Guayaquil es el estuario más grande en la costa Este del Pacífico en América del Sur, con una superficie de área de 13.701 km<sup>2</sup>. Dos áreas protegidas dentro del Golfo; la Reserva de Producción Faunística Manglares El Salado (RPFMS) y la Reserva Ecológica Manglares Churute (REMCH) fueron escogidas para este estudio. Ambas reservas difieren en el grado de uso de la tierra, número de habitantes y desarrollo urbano. Debido a esto la RPFMS es considerada como sector suburbano mientras que la REMCH es considerada como sector boscoso-semipristino además de ser considerado como sitio protegido RAMSAR. El objetivo de este estudio fue evaluar la concentración de mercurio en el sedimento y en diferentes especies acuáticas (i.e. *Mytella spp.*, *Crassostrea columbiensis*, *Ucides occidentalis*, *Litopenaeus vannamei* y *Diapterus peruvianus*) de interés comercial en ambas reservas ecológicas. La concentración promedio de mercurio en el sedimento en la RPFMS fue de 1,027 mg/kg (ps), que excede las directrices de la calidad de los sedimentos (0,71 mg/kg) y de 0,05 mg/kg (ps) en la REMCH. Resultados preliminares en las especies acuáticas muestran una concentración promedio de mercurio  $\pm$  DS en camarones de 0,029  $\pm$  0,01 mg/kg (ps) (REMCH) y 0,014  $\pm$  0,090 mg/kg (ps) (RPFMS); 0,125  $\pm$  0,035 mg/kg (ps) (REMCH) en ostiones y 4,896  $\pm$  0,76 mg/kg (ps) en mejillones colectados en la RPFMS, este último excediendo el valor máximo permitido (0,5 mg/kg) considerado por varios países. El cálculo de factores de acumulación de mercurio biota-sedimento (BSAF, por sus siglas en inglés) resultó de 1,9 a 6,7 y 0,3 para mejillones y camarones en la RPFMS, respectivamente y de 2,0 y 0,4 en ostras y camarones en la REMCH, respectivamente. Estos resultados indican que el mercurio está siendo bioacumulado en los bivalvos analizados en ambas reservas (BSAF >1) y que la RPFMS por su cercanía a zonas industriales y/o urbanizadas presenta mayor contaminación por mercurio. Finalmente, los mejillones pueden ser utilizados como una especie centinela para monitorear la contaminación de mercurio en zonas estuarinas del Ecuador. Además, se debería considerar otras especies estuarinas como bioindicadores de contaminación por mercurio, ya que estas son de interés comercial y poseen una gran demanda, por lo cual la acumulación de mercurio en ellas podría tener un efecto negativo en la salud humana.

### 32 Spatial variation in PAH, OCP and PCB profiles in egg yolks: Conservation implications for hawksbill turtles in the Yucatan Peninsula Mexico

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The Yucatan Peninsula, Mexico hosts one of the most valuable nesting populations of the critically endangered hawksbill turtle in the Neotropics. However, baseline data on persistent organic pollutants and polycyclic aromatic hydrocarbons within hawksbill eggs are limited or lacking worldwide. To support conservation of hawksbills, and improve their use in bio-monitoring we aimed to increase understanding of the presence and spatial variation in polycyclic aromatic hydrocarbon (PAH), organochlorine pesticide (OCP) and polychlorinated biphenyl (PCB) compounds in eggs among three nesting beaches within the Yucatan Peninsula. We confirmed maternal transfer of a large number of compounds from each group. The composition of PAHs showed greater spatial variation among the beaches than the composition of OCPs and PCBs as detected by multivariate ordination (nMDS). This was confirmed by analysis of individual compounds using Peto-Prentice test and Kaplan-Meier estimation for values below detection limits. Human activities including petroleum



industries and agricultural land use at local and distant locations, and movement of POPs and PAHs by atmosphere and oceanic currents were likely reflected in the pollution profiles observed. We argue that conservation of hawksbills in the context of POPs and PAHs requires cooperation across geographical and political boundaries. Similarly, spatial planning of protected areas and the use of turtle eggs as bio-monitoring tools for POPs and PAHs needs to account for the spatial variation in pollution profiles of eggs among nesting beaches.

### 33 Ecotoxicology of Lake Atitlán, Guatemala

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Lake Atitlán, the deepest lake in Central America, is home to a diverse group of Mayan cultures. More than 250,000 people live within the Lake Atitlán watershed and a large proportion of the population depends upon fish, invertebrates, and agricultural production from it. Cultural eutrophication of the lake has accelerated in recent decades. The majority of wastewater produced by the basin's population is discharged into the lake, mostly untreated. Wastewater nutrients combined with fertilizer runoff are resulting in the formation of potentially toxic cyanobacterial blooms, while fecal pathogens and pesticide residues in the lake are a direct threat to public health. We examined concentrations of neurotoxic metals in important subsistence foods (fish, crabs, snails) and found higher concentrations of arsenic, copper and manganese (all used in fungicides) in crabs and snails than in fish. We deployed a Polar Organic Chemical Integrative Sampler (POCIS) to monitor hydrophilic pesticides (10 herbicides and 20 carbamate insecticides) and a Semi-Permeable Membrane Device (SPMD) to monitor 76 lipophilic pesticides in the lake. Concentrations of hydrophilic pesticides were not notably elevated above lab blanks. However, concentrations of 65 of the 76 (86%) measured lipophilic pesticides were notably elevated above lab blanks. Pesticides with especially high concentrations in the SPMD membrane were organochlorines: the fungicides hexachlorobenzene and chlorothalonil, HCH alpha (a byproduct of the insecticide lindane), members of the cyclodiene family of insecticides (heptachlor epoxide, chlordane gamma (trans), chlordane alpha (cis), nonachlor (cis), dieldrin, endrin), endosulphan insecticides (alpha-endosulphan, endosulphan sulphate), the insecticides permethrin and cypermethrin, and DDT and its metabolites. Concentration of the organophosphate insecticide chlorpyrifos was also elevated. These results indicate that intensive use of agrochemicals in the watershed has resulted in elevated concentrations of endocrine-disrupting pesticides that have a high capacity for bioaccumulation and biomagnification in wildlife and humans, as well as elevated concentrations of certain neurotoxic metals.

### 34 A Simple Model for Monitoring Chemical Pollution in Protected Areas

I. Rodríguez-Jorquera, Rio Cruces Wetland Center / Wildlife Ecology and Conservation; W.E. Espejo, Universidad de Concepción / Facultad de Ciencias Ambientales Eula; M. Quiroz, Universidad de Concepción, Faculty of Environmental Sciences and EULA-Chile Centre, / Department of Aquatic Systems; J. Celis, Universidad de Concepción / Department of Animal Science, Faculty of veterinary science; R. Barra, Universidad de Concepción / Facultad de Ciencias Ambientales Eula Protected areas (PAs) correspond to a clearly defined geographical area, managed through legal instruments or other effective means to achieve long-term conservation of nature, ecosystem services and associated cultural values. These environmental policy instruments have become a nature conservation strategy helping to achieve conservation goals in most nations. However, in the biodiversity conservation plans typically formulated to manage PAs the impact of chemical pollutants is not considered. Recently, scientists have raised attention to this widespread issue highlighting the necessity to build human capacity and establish monitoring plans that include the detection, fate and transport of chemical pollutants and their effects on biota inside PAs. Moreover, since there is no model for monitoring chemical contaminants in PAs, it is vital to propose a model that can be included in PAs management plans. A good protected area management program should consider among its objectives the monitoring of different types of pollutants, including the basal levels of these chemicals, in the case of contaminants that may have natural origin (trace metals) as well as less obvious sources such as the contribution, via biotransformation, of high number individuals of migratory species, either migratory birds that spend part of their cycle within the protected area (feeding or nesting) or fish (diadromes, anadromes or catadromes) as contaminant input into a PA. Further, it is important to consider all possible input of chemical compounds that could enter to PAs, whether through the water cycle (precipitation, surface runoff, or groundwater),

atmospheric deposition, and potential downstream contamination inside PAs. But the determination of just the occurrence will never achieve the main goal of the monitoring which should be to understand the impact of contaminant on organism populations or the ecosystem interactions. For instance, low concentrations of highly bioaccumulative compounds may have long term consequences vs. high concentration of highly toxic compound with more evident short-term consequences.

### Is Everything an Endocrine Disruptor? Challenges and Strategies for Assessing Chemicals for Endocrine Activity

#### 35 Recommended Approaches for the Assessment of Chemicals for Endocrine Disruption

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Regulatory initiatives for identifying and managing chemical endocrine disruptors (EDs) are being developed/implemented in several jurisdictions in response to concerns that exogenous chemicals may interfere with the endocrine systems of humans and environmental organisms. The intention of this presentation is to highlight some 'best practices' for assessment of chemicals for their ED potential. According to the World Health Organization's International Programme on Chemical Safety, an ED must (1) alter the function of the endocrine system; and (2) as a consequence of that alteration, cause an adverse health effect in humans or wildlife. Based on this definition, the identification of a chemical as an ED should rely on a systematic weight-of-evidence (WoE) approach that evaluates information from different sources (scientific publications, industry and government studies, etc.) for consistent evidence of adversity linked to an endocrine mode of action. A WoE approach to ED evaluations has been supported by regulatory authorities in many geographies, and exemplar WoE assessments for ED have been published in the peer reviewed literature. Potency, which is the relationship between the effect of an agent and the dose necessary to achieve that effect, is an important consideration in WoE. Potency thresholds (i.e., the minimum dose that produces a detectable effect via a specific mode of action) can be estimated for endocrine-active chemicals to determine if adverse effects are plausible. This provides a more realistic context for endocrine assessments to avoid identifying, for example, common foods like soy and coffee as endocrine disruptors. Furthermore, the use of pre-existing lists of EDs is one practice that is **not** recommended for regulatory initiatives aimed at the identification and assessment of EDs. Often, ED lists are not transparent in their listing methodologies and/or omit critical procedures in list creation (e.g., weight of evidence, standard approach to evaluate data quality and reliability, criteria required for designation as an ED or potential ED, etc.). Furthermore, some lists do not consider important exposure and risk components. Best practices to assess whether a chemical has endocrine activity or is an ED should not be determined by an examination of lists, but rather through a systematic WoE evaluation of the relevant data that includes consideration of potency.

#### 36 Just Liver - Proteomic approaches to discriminate hepatotoxicity from endocrine disruption

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There is increasing evidence that wildlife populations, especially in the aquatic environment, have been affected by endocrine-related impairments. The involvement of chemical exposure is deemed to be one cause. These chemicals became known to the public in the 1990s as endocrine disruptors. In order to be defined as an endocrine disruptor, a substance should demonstrate an adverse effect and an endocrine disruption mode-of-action, and the causal connection of both. Consideration of the specificity of endocrine effects is of special importance in a regulatory context, meaning that an adverse endocrine effect should occur at dose levels lower than other forms of toxicity, e.g. hepatotoxicity, and hazard-based regulation for endocrine disruptors should only be triggered if endocrine effects are the most sensitive endpoint in (eco)toxicological tests. Falsely identifying chemicals as potential endocrine disruptors would cause inappropriate regulatory action. In some cases, discrimination between endocrine and especially hepatotoxic effects is not easily made. The adverse outcome could likely be the same, while the chemical exerts its toxic potential at completely different sites in the organism. As liver toxicity would be acceptable, the same adverse effect based on endocrine action would lead to a rejection of acceptance. The above described limitation in defining the cause of toxicity effects emphasizes the need for more mechanistically-based alternative approaches for hazard assessment,

e.g. the adverse outcome pathway (AOP) approach. It intends to provide the causal link between a molecular initiating event (MIE) to an adverse outcome (AO) at organism or population level, integrating all existing information on mechanistic data at different biological levels, the key events (KE), from the molecular to the organ level. Thus, our idea is to combine data obtained during standard Fish Short Term Reproduction Tests (FSTRAs) with zebrafish (*Danio rerio*), with molecular data, i.e. proteomics, as a functional component connecting the molecular mode of action (MoA) of a substance to an effect phenotype. Test substances include endocrine disruptors and hepatotoxic substances, with ideally identical AO for fish. This -omics approaches will thus allow discrimination of endocrine disruptors from substances with hepatotoxic properties in order to avoid triggering regulation due to a suspected endocrine MoA.

### 37 Challenges in Assigning Endocrine Specific Modes of Action: Recommendations for Researchers and Regulators

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Extensive discussion is occurring in the European Union and internationally on how to identify and potentially categorize endocrine disruptors (ED). There is general agreement that the evaluation of ED should be based on the WHO/IPCS (2002) definition. According to WHO/IPCS: "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations." This definition embodies the key elements of adversity, endocrine mode of action (MoA), and a causal link between endocrine activity and adverse effect. The mere existence of an endocrine mode of action (i.e., endocrine activity) is not a harmful property, a type of toxic effect, or a hazard. Rather, endocrine activity, if causally linked to an adverse outcome, can potentially lead to a hazard to health or to the environment, particularly after long-term exposure. Defining that there is a causal link between an indication of activity and an adverse effect, particularly one that would be the lead or primary toxic effect most relevant for risk assessment is important, yet can be difficult in studies with many apical endpoints. For instance, stress is a non-specific, neuro-endocrine response that can lead to adverse outcomes. In addition, non-endocrine toxic mechanisms (e.g., hepatotoxicity, acetylcholinesterase inhibition) may operate secondarily or in parallel to impact the endocrine system and apical endpoints downstream. Furthermore, endocrine responses may be adaptive in nature, designed to maintain homeostasis rather than induce an irreversible adverse effect. The likelihood of indirect effects is increased in (eco)toxicological studies requiring the use of maximum tolerated dose levels, which must produce some adverse effect. The misidentification of indirect effects as truly ED has serious consequences in terms of triggering animal and resource intensive testing and potentially severe regulatory consequences. A hypothesis-testing, weight of evidence framework and case study examples from a recent SETAC Pellston Workshop® will be used to help clarify the differences between substances that do have chronic hormonal toxicities and those that might be responding to general, systemic toxicity.

### 38 Tributyltin (TBT) - A non-conventional endocrine disrupter

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A Pellston Workshop™ "Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances(EHRA)" was held in 2016. The aim was to provide objective advice on environmental hazard- or risk-based approaches for regulating endocrine-disrupting substance (EDS). The workshop also considered the importance of data quality criteria in the framework. Case studies used empirical data to evaluate different assessment methods. Tributyltin (TBT), long recognized as an EDS was selected as one of these case studies. Many TBT published papers have reported effects on several taxa, especially gastropods. Searches of open literature, regulatory datasets and ToxCast data including 160 references were selected, in an attempt to capture relevant data. Open literature studies were first assessed for relevance, and then for reliability using the ToxR tool and/or Klimisch criteria. Only studies ranked as Klimisch 1 and 2 were used for subsequent analysis. Klimisch scores do not

necessarily apply to field studies, therefore expert judgment was used to evaluate these studies. The levels of the OECD Conceptual Framework for the Testing and Assessment of EDS were used as a guide however, only a few TBT studies were performed according to standard test guidelines. These non-standard TBT studies were thus organized according to their test design, exposure duration, portion of the life-cycle exposed, and type of biological responses. Assessments revealed that TBT's primary endocrine mechanism of action was with the nuclear receptors RXR and PPAR $\gamma$ . This molecular initiating event alters a range of reproductive, developmental and metabolic pathways at the organism level. The information gathered on biological effects relevant for populations of aquatic animals was used to construct Species Sensitivity Distributions (SSDs) based on NOECs and LOECs. Fish appear at the lower end of these distributions, showing that they are as sensitive or more sensitive as molluscs. Concentrations in the range of 1 ng/L for water exposure and 10 ng/g for whole-body burden have been shown to elicit endocrine-type responses. Current screening and risk assessment methodologies were able to identify TBT as a potent endocrine disruptor with high environmental risk. If those approaches were available when TBT was first introduced, it is likely that its use would have been regulated sooner, thus avoiding the well documented detrimental effects on marine gastropod populations over several decades.

### 39 Probabilistically risk assessment via breast milk in infants to polybrominated diphenyl ethers (PBDEs)

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Polybrominated diphenyl ethers (PBDEs) are synthetic compounds ubiquitous in the environment. PBDEs were used as fire retardants in household products including mobile phones, computers, televisions, upholstery textiles, curtains, carpets, mattresses, furniture, etc. PBDEs induced endocrine, reproductive, and behavior toxicological effects. PBDEs are detected in tissues and breast milk. Therefore, it is important to study the risk assessment to infants fed the first year of life with breast milk. Probability distributions of PBDEs concentrations and fat content in breast milk consumed on a body weight basis by infants were obtained from the literature of a human milk study previously published (Johnson-Restrepo et al., 2007). The probabilistic calculations of the exposure were using the Monte Carlo simulations. For each simulation, 25,000 iterations were chosen. The daily intake (DI) of the PBDEs in breast milk were calculated using the following equation:  $DI = C \times F \times I$ . Where DI is the intake dose (ng/Kg.bw/day), C is the concentration of the contaminant in milk on a lipid basis (ng/g-lipid), F is the fat content in milk (g-lipid/g-milk) and I is the dairy milk consumption (g/day). Hazard risk ratio (HRR) was calculated to non-carcinogenic health effects. Therefore HRR values greater than one represent a potential risk for infant fed with milk as the most important food. In this work was calculated the potential risk and its uncertainty by modeling with Monte Carlo that newborn children have exposure to PBDEs. The overall concentration of daily intake PBDE (mean  $\pm$  SD) was  $76.3 \pm 308$  and ranged from 0.06 to 1910 ng/g-lipid wt. The average dose to infants were 0.512, 0.278, 0.081, and 0.035  $\mu$ g/Kg body weight per day, for sum of PBDEs, BDE-47, BDE-99, and BDE-153, respectively. In relation to risk analysis, calculated hazard quotients for sum of PBDEs, BDE-99, and BDE-153 do not show a health concern, however the individual congener BDE-47 was predicted that exposure dose to breast-feeding infant exceed 2.78 of reference of dose (RfD) reported by USEPA. Sensibility analysis determined that the uncertain of variables were PBDE concentrations(73%) in breast milk, fat content(24.6%), and dairy milk consumption(1.8%). **Acknowledgment:** Thanks to Colciencias and Universidad de Cartagena for supporting the project No. 110759634967. **References** B. Johnson-Restrepo, R. Addink, C. Wong, K. Arcaro, K. Kannan. (2007). J. Environ. Monitor. 9 (11), 1205-1212.

### 40 Tier 1 EDSP and Regulatory Safety Studies Provide a Weight of Evidence that Glyphosate is not an Endocrine Disruptor

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The EDSP includes 11 validated Tier 1 *in vitro* and *in vivo* screening assays that evaluate the potential for a chemical to interact with endocrine pathways. The endocrine screening system was designed to have redundancy between the mammalian and aquatic systems. Glyphosate was in the initial group of compounds that received test orders for screening under the EDSP. The compounds in this initial group were selected based on exposure potential and not on known or suspected interaction with the endocrine system. Results from *in vitro* assays determined that glyphosate is not estrogenic or androgenic and does not impact steroidogenesis. These findings are consistent with peer-reviewed studies using mechanistically and functionally equivalent assays and predictions from structure activity relationships. Additionally, the *in vitro* results from the Tier 1 assays are consistent with the *in vivo* results from

existing regulatory safety studies. These higher tier regulatory studies can provide definitive evidence of endocrine disruption or lack thereof, and are the most powerful tool for hazard identification. Consistent with the results from several multigeneration rat studies, glyphosate exposure had no impact on estrogenic, androgenic, steroidogenic and thyrogenic endpoints in *in vivo* mammalian assays, including the Uterotrophic, Hershberger, and male and female pubertal assays. Consistent with the pubertal assays, the amphibian metamorphosis assay found no impact on thyroid-driven developmental endpoints. Results from the short-term fish reproduction study were consistent with the results of the EDSP battery and an existing fish full life cycle study that showed no impact on all measured endpoints. From the weight of evidence provided by the Tier 1 EDSP assays along with the higher tier regulatory safety studies, it can be concluded with a high level of confidence that glyphosate is not an endocrine disruptor. This conclusion is consistent with the recent weight of evidence assessment by the U.S. EPA and evaluators in the European Union that also determined glyphosate does not impact estrogenic, androgenic, thyrogenic and steroidogenic pathways.

#### 41 Remediation of 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethinylestradiol (EE2) in water: emphasis on the biological reversibility of Zebrafish gonadal tissues

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The removal from the aquatic environment of compounds that influence the hormonal dynamics of biological systems is a global concern. The aim of this research was to investigate the remediation of artificially enriched 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethinylestradiol (EE2) in water by three materials (powdered activated carbon - PAC; powdered natural zeolites - ZP; and aquatic humic substances - AHS) through chemical analyses and ecotoxicological *in vivo* assays. Adult zebrafish were chronically exposed (21 days) to a relevant concentration (30 ng.L<sup>-1</sup>) of each hormone in the laboratory. Water enriched with estrogens was treated with PAC, ZP or AHS for 2 h, promoting clearance of the available medium over a 7-day interval. Morphological, reproductive and histopathological effects were monitored after each experiment. PAC demonstrated adequate chemical efficiency for the removal of both hormones. Decreased embryo production and hatching rate and F1 sex ratio alterations (favoring females) were related to female exposure to E2. Spermatogenesis and oogenesis were delayed in animals exposed to EE2 in association with impaired reproduction. None of the treatments reversed the sub-lethal effects after the clearance periods. Therefore, the clearance test interval should be extended in future research.

#### 42 Polymorphisms of Glutathione-S-Transferase, Cytochrome-P450, and Metallothionein as Modifiers of Risk for Chronic Arsenic Exposed

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This study assessed the association between genetic polymorphisms, anthropometric factors, demographic factors, and lifestyle as risk factors for developing chronic health effects in individuals exposed to arsenic from drinking groundwater. Groundwater samples (n=19) for human consumption from the municipalities of Margarita and San Fernando (Bolívar Department) were analyzed for arsenic by Hydride Generation-Atomic Absorption Spectrometry. Moreover, the lifetime average daily dose of arsenic (LADD) was assessed. A surveys of demographic and exposure factors was applied of a population (n=99) from the same municipalities aged between 18 and 75 years. The health status of those surveyed was confirmed with individual records from the health services. In addition, *GSTT1* and *GSTM1* deletions and *GSTP1*-rs1695, *MT-2A*-rs28366003, *CYP1A1*-rs4646903, were evaluated either by direct PCR or Restriction Fragment Length Polymorphism (PCR-RFLP). The fragments of each gene were amplified through PCR with the primers described previously by some papers. The digestion products were separated by electrophoresis in 18% polyacrylamide gel (*GSTP1* and *MT-2A*) and on a 2% agarose gel (*CYP1A1*). As an internal positive control was used one sample by each gene, identified as heterozygous variant, previously confirmed by sequencing. A multivariate analysis was performed to evaluate the association between chronic health effects, SNPs and other contributing factors, using an estimate of the relative risk "Odds Ratio" (OR) and 95% confidence intervals (CIs). The Single Nucleotide Polymorphism SNPs and the LADD of arsenic

were incorporated in the regression analysis as independent variables and presence of at least one chronic health effect was incorporated as dependent variable, adjusted by potential confounders identified in the literature. The arsenic concentrations in groundwater were on average 34.6 $\pm$ 24.7  $\mu$ g/L. The prevalence of chronic health effects was 24.2%, and *GSTP1* ( $p=0.03$ ), *CYP1A1* ( $p=0.005$ ), shellfish ( $p=0.01$ ), alcohol consumption ( $p=0.02$ ) and LADD ( $p=0.000$ ) were associated with a significant risk for chronic health effects. We conclude that *GSTP1* and *CYP1A1* together with seafood consumption and alcohol are associated with the risk of chronic health effects in individuals exposed to arsenic from drinking groundwater.

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#### 43 Inorganic arsenic at low dose impairs spermatogenesis and sperm maturation in prepubertal male rats

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Arsenic (As) is a metalloid found in organic and inorganic form. The most toxic form is the inorganic. Contaminated water and agricultural products, as well as contaminated sea food, are the main prospectors of intoxication. Exposure to As is correlated to human health damage, including the incidence of different types of cancer, such as skin, bladder, kidney and lung cancer. Besides, As can impair developmental and reproductive processes. The aim of this study was to investigate if the exposure to inorganic arsenic during male prepubertal development interferes with morphometric parameters of reproductive organs, as well as the dynamics of the spermatogenic process, which could characterize future impairment on production and maturation of male gametes. For this purpose, Wistar rats were used as experimental model since they are one of the most closely models related to humans physiologically, besides having a quick life cycle. Wistar male rats (21 days old) received water and rodent chow *ad libitum* and were distributed into 3 groups (n = 10/experimental group): negative control (drinking water), As1 (AsNaO<sub>2</sub> at 0,01mg/L of drinking water), As2 (AsNaO<sub>2</sub> at 10mg/L of drinking water). Consumption of drinking water was measured on alternate days from PND 23 to PND 53. On PND 53 the animals were euthanized. Morphometric analysis of testis showed a statistically significant reduction in the diameter of seminiferous tubule in As2 compared to As1 ( $p < 0.05$ ). The number of abnormal tubules of treated animals (As1 and As2) increased compared to control ( $p < 0,001$ ) (i.e. presence of vacuolization, acidophilic cells and epithelium degeneration). Seminiferous tubular interstitial of As1 and As2 groups presented a greater amount of fluid and intense inflammatory infiltration points. In the epididymis there was a reduction in the amount of spermatozoa in the ductular lumen, besides epithelium areas presenting cribriform aspect in As1 and As2, exfoliation of cells in the light (in As1) and vacuoles (in As2). In the interstitial compartment of the epididymis, inflammatory infiltrates were observed in the region of initial segment of As1 and As2 groups. Our results show the effects of arsenic on reproductive system of juvenile male rats, altering the pattern of histological organization of the testis and epididymis, demonstrating altered spermatogenic activity, which can cause long term impacts on the reproductive health of exposed animals, even at low doses of As.

#### 44 Impairment on sperm number and quality of rats exposed to manganese during adulthood

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Manganese is one of the most common chemical elements found on Earth. It is essential for the development of bones and cartilage, as well as for protein, lipids and carbohydrates metabolism and also acts as enzyme cofactor. Supraphysiological doses of manganese can cause chronic intoxication, called "Manganism". The main sources of intoxication are through mining and the steel industry. In November 2015, after the Samarco's dam collapse, high levels of manganese were found in the mud and water of the Rio Doce River, reaching even the marine environment. Faced with this, studies on the toxicological risks of this metal on human health are of high relevance. The objective of this study is to evaluate the impact of exposure to high levels of manganese during adult life on the production and quality of sperm. Among the experimental models used in research, wistar rat is one of the most closely related to humans physiologically, as well as having a quick life cycle and short gestation period. Wistar adults male rats were distributed in three experimental groups: control (saline solution), Mn1 (MnCl<sub>2</sub> at 5mg/kg), and Mn2 (MnCl<sub>2</sub> at 15 mg / kg) (n = 8 /group). The treatment was applied daily by intraperitoneal route, from the postnatal day (PND) 90 to 120. The rats were euthanized on DPN 120. Besides the organs of the



reproductive system, liver, kidneys and adrenal, were weighed in a precision analytical balance. The testicles and epididymis were used for sperm counts and the content of the vas deferens was used for analysis of sperm morphology and motility. In the Mn2 group, there was a reduction in body weight. It was also observed a reduction in androgen-dependent reproductive organs weight in the Mn2 and Mn1 groups, such as the epididymis and prostate. Treatment with Mn induced an increase in the number of spermatozoa with morphological abnormalities and impaired sperm motility. In addition, there was a decrease in the sperm number in the epididymis of animals exposed to Mn when compared to the control. Our results show that Manganese exerted reproductive toxicity through direct action on the germ cells, since an increase in the percentage of spermatozoa with morphological abnormalities was found in the treated groups. In addition, manganese altered the process of

## Soil ecotoxicology in Latin America: joining efforts between science and governance

### 45 State of the art of Soil Ecotoxicology in Latin America

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Soils from some Latin America (LA) countries face some of the most serious environmental impacts attributed to both historical and current agricultural practices and industrial activities and, simultaneously, support the highest biodiversity levels on the planet. Despite this, soil ecotoxicology in LA is a relatively new research field and the majority of scientific contributions come from Brazil. The present work aimed to analyze the available published studies on soil ecotoxicology conducted in LA by (or in collaboration with) Latin American researchers, using available databases (Scopus Database, Web of Science and Science Direct). It presents an overview of the main research topics, summarizes the recent advances but also highlights the research needs. Studies where the ecotoxicity of pesticides was evaluated using earthworms as test-species were the most abundant. Further improvements include the development of methods and procedures for: 1) identifying natural soils to be used as reference test-substrates in ecotoxicological tests; 2) identifying and discerning the range of sensitivities of native test species to soil contaminants; 3) adapting environmental guidelines to be applicable to tropical/sub-tropical conditions; 4) adopting higher tier-testing for full development and implementation of ERA schemes. The protection of LA soils, that safeguard the provision of goods and services, is currently framed in legislation and other regulations but for its full implementation significant improvement and additional training programs are required.

### 46 The TRIAD - a new ISO standard for retrospective risk assessment in soil (ISO 19204)

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Within the last years the important role of soil organism (microbes, plants and invertebrates) in providing functions and services (e.g. food production, nutrient cycling etc.) became more and more apparent. However, this growing evidence is not (yet) part of the regulatory practice in most parts of the world – despite some notable exceptions (e.g. The Netherlands or to a lesser extent, Germany). Even in these countries data gained in ecotoxicological standard tests are mainly used for the derivation of soil values (usually different ones with, if exceeded, different consequences for the usage of the site to be evaluated). However, in general the assessment of potentially contaminated sites is based on chemical parameters (e.g. the concentration of a specific metal in soil). However, usually it is impossible to evaluate the biological quality of a soil using solely chemical parameters, because of interactions between individual chemicals, their different bioavailability (depending on various soil properties) and the complexity of soil organism communities. Therefore, an approach combining chemical, ecotoxicological and ecological data is needed for a robust and practical risk assessment. Actually, the TRIAD, originally developed decades ago in North America for the evaluation of sediments, is such an approach. The basic idea of the TRIAD is the combined assessment of data coming from three lines of evidence (LoE):  
- Environmental chemistry: data about concentrations of toxic substances;  
- Ecotoxicology: results from testing soil samples with microbes, plants, invertebrates;  
- Ecology: monitoring observations of the ecosystem at the test site.  
Despite not (yet) regularly required in European regulations various examples of using the TRIAD are available from the scientific literature (e.g. the EU project LIBERATION). Based on these experiences ISO was able to prepare an international standard (ISO 19204; 2016). In our contribution we will present the performance of an “ideal” TRIAD, consisting of five steps (the testing efforts belong to the third step). In order to perform the testing as efficient as possible the practical

work of all three LoEs start with simple and quick methods. Depending on the respective results more complex tests can be required on the second and third tier. In the majority of all cases standard ISO methods are available, but at the highest tier and here mainly in the LoE Ecology, non-standard tests are still possible.

### 48 Chronic exposure of *Lactuca sativa* to a mine-contaminated soil from La Planta-Marayes (Argentina): II. Toxicity and bioaccumulation of Zn, Cu and Pb.

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Mine residues-associated metals may alter the survival and growth of organisms. The aim of this study was to evaluate the chronic toxicity and bioaccumulation of Zn, Cu and Pb in lettuce (*Lactuca sativa*) exposed to contaminated soil with residues from an abandoned gold mine in La Planta-Marayes. Two samples were taken from the first 20 cm of soil in La Planta town: A contaminated soil with mine residues (Site 1) and one located to 2 km which was used as reference (Site 2). A completely random experimental design consisted of 8 treatments: mixtures of soil from Site 1 and 2 (dw/dw) in the range of 0.1 to 7.5% ( $n=6$ ), and 2 control groups (an artificial soil recommended by an OECD guideline, and soil sample from Site 2). Sixteen replicates for each treatment were used. Experimental unit consisted of 1 kg of soil and a 3-week-old seedling into a flowerpot. Exposure was conducted in a greenhouse for 28 days. The hotplate *aqua regia* method was used to extract the pseudo total metal fraction from soil samples. Endpoints were: number of plants with completely necrotic aerial biomass, number of green leaves, plant's height and width, chlorophyll content, stem diameter, leaf area and fresh aerial biomass. NOEC, LOEC and  $IC_{50}$  or  $EC_{50}$  were estimated. After the exposure period, a microwave acid assisted extraction from aerial and root biomass was performed. Metal concentrations were measured with an atomic absorption spectrophotometer. Bioaccumulation and Translocation Factors (BAF and TF) were determined. Metal concentrations in aerial biomass were compared with permissible levels established by the European Union and FAO/WHO. Comparison between the control groups indicated the usefulness of Site 2 as a reference soil for toxicity studies. Time- and concentration-dependent toxicity effects were observed. Plant's width and height, fresh aerial biomass, leaf area and number of green leaves were the most sensitive endpoints (LOEC=0.1%). High concentrations of Zn ( $301.16 \pm 14.56$ ;  $1453.33 \pm 381.60 \mu\text{g g}^{-1}$ ) were quantified in plant tissue. BAF and TF showed that metals were accumulated predominantly in root. A negative correlation was observed between metal concentrations in plant tissue and toxicity endpoints. Concentrations of Zn, Cu, and Pb in aerial biomass exceeded the permissible levels in all treatments. These results indicated a high toxicity and metal bioaccumulation in lettuce, and a potential risk to the human consumption even in the reference soil.

### 49 Ecotoxicological assessment of the mining waste from Mariana (MG) using *Folsomia candida* (Collembola) and *Enchytraeus crypticus* (Enchytraeidae)

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In November 2015, the city of Mariana (MG) has faced the worst environmental disaster related to a rupture of “Fundão” Dam, which was built to accumulate the mining waste from Samarco Mineradora. The rupture caused severe impacts to the terrestrial and aquatic environments, where tons of the waste has been placed. In relation to terrestrial system, the effects of the waste deposition in soil to edaphic organisms need to be evaluated in order to understand the real consequences to the environment and so be able to propose actions for restoration and management of the affected area. Enchytraeids and collembola are terrestrial invertebrates, which directly interact with soil particles and solution and are very sensitive to environmental contaminations (OWOJORI et al., 2009) being good indicators of pollution in terrestrial environments. To evaluate the acute and chronic toxicity of the mining waste, the species *Folsomia candida* and *Enchytraeus crypticus* were used in this study. The ecotoxicological assays were done using five treatments, which consisted in the mixture of a natural soil (NS) from Mariana city (uncontaminated) and the waste (W). The combinations of mixture were: P1= 75% NS and 25% W; P2= 50% NS and 50% W; P3= 25% of NT and 75% of the W and P4= 100% W. The tested organisms



originated from laboratory cultures (NEEA/SHS/EESC/UPS) and the ecotoxicological tests for *E. crypticus* were based on the ISO 16387 (2004) guideline and the effects on collembolan *F. candida* according to OECD 232 (2009). Effect concentrations (EC<sub>x</sub>) were calculated using logistic nonlinear regressions. No effect was observed at the survival level of both species for all treatments tested. However, reproduction of the two species was significantly affected by the 75% W treatment. The proportions responsible by 20 and 10% inhibition of the juvenile production of *E. crypticus* was 49.2% and 27.3%, respectively for and 50, 20 and 10% inhibition of the juvenile production and to *F. candida* the proportions responsible by 50, 20 and 10% inhibition of the reproduction was 87.0%, 69.68% and 61.2%, respectively. Results showed that, although the survival has not been affected by the mining waste, low concentrations of this residue caused significant effects at the reproduction level of the *E. crypticus* and *F. candida*, which may compromise certain soil functions. More studies need to be done using other edaphic organisms to an appropriate ecological risk assessment of the area.

#### 50 Mitotic activity biomarkers in *Deschampsia antarctica* from different polluted and unpolluted sites

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The scientific stations generate local sources of contamination as, for example, occasionally oil spills. Adverse effects on the ecosystem can be early detected through the use of sentinel organisms and/or its biomarkers. *Deschampsia antarctica* is the only native grass described so far in Antarctica. The lack of knowledge about the sensitivity of this species to xenobiotics could be an innovative tool in risk assessment. Plant samples were taken in areas near and far from the Argentinean Scientific Station Carlini (Potter peninsula, 25 de Mayo Island). Entire plants were fixed in 70% ethanol. The meristems of roots and leaves were stained to evidence the effect biomarkers: presence of mitotic abnormalities, mitotic index (MI) and phases index (PI). Mitotic division was not found in the pristine area samples. Stranger Point and Elephant Hut samples presented very low MI and absence of mitotic abnormalities. The biggest MI corresponds to the sites near the electric generator, fuel tanks and the "Chapel". In addition, these samples presented mitotic abnormalities as disorganized metaphases, outside plate chromosomes, anaphases with bridges, diagonal anaphases and telophases. The IF showed differences according to the proximity of the pollution source: metaphase index value was high near to fuel tanks while the anaphase index was high near to the electric generator. The site called "Chapel" presented very low percentage of abnormalities and no evidence of a pollution source near it. Thus, alterations could have originated spontaneously at this zone. Micronuclei were not observed in all the studied areas. In the same areas, the presence of pollutants is evidenced by spontaneous aneugenic effects (disorganized anaphases) and clastogenic effects (presence of fragmented chromosomes, chromosomes out of metaphase plate or bridges at anaphase). The results reported herein, encourage us to postulate *D. antarctica* as a useful organism for the *in situ* biomonitoring of the Antarctic terrestrial ecosystem.

#### 51 Sensitivity distribution profiles using organisms exposed to contaminated soil from an abandoned gold mine in La Planta-Marayes (Argentina)

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Adverse effects on human and wildlife health by exposure to mining residues have generated public and scientific concern. The lack of a Closure Plan in abandoned mines causes environmental pollution due to changes in the landscape and the persistence of metals. La Planta was a small mining town until the gold mine was abandoned five decades ago. The aims of this study were to carry out sensitivity distribution profiles using acute and chronic toxicity data of aquatic and terrestrial organisms exposed to surface water and soil contaminated with mine residues, and to correlate physicochemical parameters with toxicity endpoints. Three water samples were taken on the riverbed of the Papagayo River: at La Planta, 3 km upstream and 3

km downstream. Six soil (0-20 cm depth) samples were taken: one in the site with mining residues (CP1), three in the contamination plume at 700, 1400 and 2100 m from CP1 (CP2, CP3 and CP4 respectively), and two in reference sites (2 km upstream and 2 km downstream from CP1). Routine physicochemical parameters and metal concentrations (ICP-MS) were determined for surface water, soil and elutriate matrices. Toxicity tests were carried out on *Desmodesmus spinosus*, *Daphnia magna*, *Cnesterodon decemmaculatus*, *Eisenia andrei*, *Lactuca sativa*, and *Raphanus sativus*, according to OECD and USEPA guidelines. Experimental design consisted of 6-10 treatments by triplicate, with sample concentrations ranging from 0.0001 to 100%, depending on each concentration-response curve, and a control group. Toxicity endpoints were reported as NOEC, LOEC and LC<sub>50</sub>, EC<sub>50</sub> or IC<sub>50</sub>, and used to calculate toxic units (TU). Regarding all endpoints, no toxicity was observed in the water samples (pH: 8.2; EC: 6.3 mS/cm) nor in soil and elutriates of the reference sites (pH: 8.4; EC: 558 mS/cm). Sensitivity distribution profile showed that 52% of the endpoints in CP1 (pH: 2.3; EC: 41.3 mS/cm) have values of EC<sub>50</sub> less than 1%. Reproduction inhibition of *D. magna* was the most sensitive endpoint (IC<sub>50</sub>-21d = 0.003%). Hazardous concentration for 5% (HC<sub>5</sub>) was 0.009% in CP1. TU showed positive correlations with EC and concentration of As, Bi, Cu, Zn and Pb, whereas showed negative correlations with pH. This study allowed to assess multispecies sensitivity to contaminated soil and to identify physicochemical parameters related to toxicity. However, surface water quality was not affected at this sampling time. These data will allow conducting an ecological risk assessment.

#### 52 The role of ecotoxicology in ecological risk assessment (ERA) in contaminated sites: Brazilian experience

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The Ecological Risk Assessment is foreseen in Brazil by Federal Regulation, published in 2009 by National Council of the Environment - CONAMA (Resolution 420), to the management of contaminated sites that present risk to organisms or ecosystems. However, this resolution describes only procedures to human risk assessment. Nevertheless, the ecotoxicological data base was considered in the establishment of values to identify contaminated sites. The state of São Paulo is one of the few states in Brazil that has specific policies and legislation to carry out ERA. The 2013 State Legislation established that one of the criteria to confirm the risk of a contaminated site is through ERA. The technical procedures to the identification and rehabilitation stages of contaminated sites was established in 2017 by the Environmental Agency of the State of São Paulo (CETESB) Among these, an ecological risk assessment is required in situations where there is a natural ecosystem under influence, or that may be under the influence, of a contaminated site under investigation. ERA aims to verify the occurrence of risk to a species, community or ecosystem. To do so, it is recommended that at least three lines of evidence should be considered: chemical, ecotoxicological and ecological. The use of standardized methods for ecological assays, national (Brazilian Association of Technical Standards - ABNT) or international (as International Organization Standardization - ISO) and laboratories accredited by the National Institute of Metrology, Quality, and Technology (INMETRO), are recommended. Ecotoxicological toxicity tests with aquatic organisms (as *Daphnia*, *Ceriodaphnia*, fish, *Hyalella*, *Chironomus*), terrestrial organisms (as *Eisenia*, *Enchytraeus*) and plants, are usually asked for these studies. These tests are carried out in order to measure the actual toxicity present in environmental samples from the site in investigation. Regarding the guidelines in the elaboration of an ERA, there is a multidisciplinary working group, to the elaboration of a Brazilian standard. The ecological risk approach started to have a real and important role in contaminated sites risk evaluation after new regulation by CETESB. An increase in the specialized staff, in the number of accredited laboratories, mainly for terrestrial tests, and an advance in the development of ecotoxicological tests that can identify, not only, acute but also sublethal effects for aquatic and terrestrial organisms, is expected

#### 53 Toxic effects of a binary mixture of chlorpyrifos and goethite (α-FeOOH) nanoparticles in *Eisenia andrei*

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*Eisenia andrei* earthworms are one of the most important bioindicator organisms to evaluate the impact of environmental contaminants in soils. The aim of this work was to investigate the interactions of a binary mixture of the organophosphate pesticide chlorpyrifos ( $0.75 \mu\text{g cm}^{-2}$ ) and goethite nanoparticles (NPs) ( $50 \mu\text{g cm}^{-2}$ ). Organisms were exposed to embedded filter papers for 72 h to evaluate contact effects. The selected chlorpyrifos concentration was close to the value that induces a 50% inhibition on the acetylcholinesterase enzyme (AChE) using acetylthiocholine as substrate. The activity of carboxyl esterases (CES) was also determined using p-nitrophenyl butyrate as substrate. Animals exposed to NPs alone presented similar AChE and CES activities than control ones. Those exposed to the binary mixture showed a similar inhibition of CES (53%) when compared to animals exposed to CPF alone (49%), but the AChE activity was not inhibited (91%). Spectroscopic UV/Vis studies revealed that in aqueous phase, the pesticide is strongly removed from the solution in the presence of NPs. This result could explain the lack of inhibition of AChE. The uptake of NPs by *E. andrei* was verified by measuring the Fe content using atomic absorption spectroscopy. Compared to controls, the iron content progressively increased after 24 and 48 h of exposure in the presence of NPs alone. However, values were lower than controls after 72 h ( $26 \pm 3$  and  $54 \pm 9 \mu\text{g Fe g}^{-1}$  ww respectively) suggesting that by contact exposure, a systemic effect occurred. At this time, earthworms exposed to the binary mixture containing NPs plus chlorpyrifos presented higher levels of iron than control organisms ( $45 \pm 10$  and  $27 \pm 8 \mu\text{g Fe g}^{-1}$  ww respectively) while no significant changes were observed in those exposed to chlorpyrifos alone ( $27 \pm 5 \mu\text{g Fe g}^{-1}$  ww). On the other hand, a suspension of bulk goethite did not modify the Fe basal levels at any time. Therefore, by contact exposure goethite NPs exhibited a different behavior than bulk goethite in *E. andrei*. In addition, these NPs would be able to retain chlorpyrifos since the AChE enzyme was not inhibited when earthworms were exposed to the binary mixture. However, the inhibition of CES could not be prevented, although NPs alone did not exert any effect on this enzyme activity. Altogether, these results show the complex interactions that may be found in binary mixtures of NPs and other contaminants.

#### 54 Effects of the pesticides KRAFT 36 EC (a.i. abamectin) and SCORE 250 EC (a.i. difenoconazole) on terrestrial invertebrates

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Pesticides are widely used all over the world. The effects of the indiscriminate use of pesticides to the environment are numerous and they have been reported at both aquatic and terrestrial environmental compartments. However, much more information is available for aquatic than for terrestrial systems in the world. In Brazil (and Latin America, in general), this gap on terrestrial studies is even more pronounced and so, researchers are being strongly encouraged to run their experiments in tropical conditions and use natural soils, in order to promote an appropriated risk assessment of pesticides in tropical regions. Both abamectin (insecticide and acaricide) and difenoconazole (fungicide) are widely used to prevent diseases in several cultures such as, potato, beans and strawberry. Abamectin may reach the soils via application in the agricultural fields but also through animal feces since it is used to control endo and ecto parasites. The use of the active ingredient abamectin has already been banned in the European community due to its acute toxicity and the suspect of the reproductive toxicity to human being. The aim of this study was to determine the direct effect of the insecticide KRAFT 36 EC, which has abamectin as active ingredient (a.i.), and the fungicide SCORE® 250 EC, a.i. difenoconazole, on non-target terrestrial invertebrates. Ecotoxicological tests were used to evaluate chronic and acute toxicity in potworms (*Enchytraeus crypticus*), collembolan (*Folsomia candida*) and acari (*Hypoaspis aculeifer*). Results showed that, for both products, the collembolan *F. candida* was the most sensitive, followed by the enchytraeid *E. crypticus* and the mite *H. aculeifer* was the least sensitive. All organisms tested showed highest sensitivity to the insecticide in comparison to the fungicide. For the *F. candida* species, 10% effect was observed for the organism reproduction at the recommended dose (strawberry crops) of the commercial product and 50% effect was found at 3 times the recommended dose (RD). Being three applications the recommended by the commercial product manufactures, and considering the degradation time of the substances, the 50% effect concentration will be easily found in the environment and may be harmful to important organisms, which plays an important role on the maintenance of soil quality.

#### 55 Effects of pesticides on soil microorganisms and its consequences to *Enchytraeus crypticus* populations

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Agriculture is one of the main economic sectors in Brazil and it is very dependent on the use of pesticides, which may be toxic to non-target organisms. Enchytraeids play an important role in the organic matter degradation and its diet is largely based on microbial communities. Hence, this work aimed at analyzing the effects of the insecticide Kraft® 36 EC (a.i. abamectin) and the fungicide Score® 250 EC (a.i. difenoconazole), isolated and in mixture (recommended doses for strawberry cultures), to: i) soil microbial communities; ii) three different densities of *Enchytraeus crypticus* population; and iii) the trophic relationship between microorganisms and *E. crypticus*; over 84 days after the contamination. After 0, 7, 14, 28, 56 and 84 days from the contamination, the soil microbial biomass carbon (MCB), the soil basal respiration (SBR), the microbial metabolic quotient ( $q\text{CO}_2$ ) and the  $\beta$ -glucosidase enzyme (BG) were analyzed. After 0, 28, 56 and 84 days from contamination, reproduction tests with *E. crypticus* in initial densities of 5, 10 and 50 adults were performed. The pesticides may have served as substrate for the MCB increasing over the time, despite of the microbial stabilization in all treatments after 28 days. Oppositely, the metabolic indicators (SBR and  $q\text{CO}_2$ ) decreased over time showing the highest levels immediately after the contamination, which indicates the stress of communities caused by the pesticides. The BG was inhibited by the fungicide in the first analysis, which demonstrates the hazard potential of this kind of pesticide in affecting the carbon cycle and, consequently, soil quality. Although those variations on microbial communities, their parameters and functions were not widely affected, which express the concept of functional redundancy of the edaphic system. In relation to *E. crypticus* tests, the importance of microorganisms on their diet was ratified, since positive correlations between the size of microbial communities and the enchytraeids rate of increase were observed. The different densities of *E. crypticus* were not significantly influenced by the effects of pesticides on the microorganisms. Considering these results and the critical Brazilian agricultural circumstances, we emphasize the importance of compliance of the recommendations on pesticides application and reinforce the initiative of decreasing their use.

#### Antifouling paints: the Brazilian viewpoint about a global environmental problem.

##### 56 Áreas Marinhas Protegidas do Litoral Paulista sob Ameaça do TBT

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Áreas Marinhas Protegidas (AMP) são fundamentais para proteção e preservação dos ecossistemas costeiros e oceânicos tendo como finalidade proteger zonas estratégicas de ameaças ambientais incluindo a contaminação por substâncias químicas perigosas. Entre os químicos perigosos que podem afetar a vida marinha, o Tributilestanho (TBT), utilizado por muitos anos como ingrediente de tintas anti-incrustantes, é conhecido como o mais perigoso produto já lançado deliberadamente no meio ambiente pelos seres humanos. Sua ação sobre organismos não-alvo, pode levar a alteração no sistema sexual de diversos gastrópodes marinhos, promovendo o surgimento de características masculinas em fêmeas (*imposex*). Essa alteração se converteu em um excelente biomarcador específico da contaminação marinha por TBT. Nesse sentido, diversos estudos têm mostrado a incidência de imposex em diversas regiões do mundo. Na América do Sul, a Rede Nacional de Estudos em Anti-incrustantes (RNEA) levantou dados referentes a presença de *imposex*, demonstrando que seus efeitos estão presentes, inclusive dentro de AMPs. Considerando o exposto, e o fato do litoral do Estado de São Paulo possuir diversas áreas de proteção ambiental marinhas (APAs), a ocorrência de imposex em *Stramonita brasiliensis*, foi avaliada no de duas APAs do litoral paulista. Para isso, foram selecionados 12 pontos, distribuídos no interior das Áreas de Proteção Ambiental Marinhas Central e do Litoral Norte Paulista, considerando as fontes potenciais de TBT para o ambiente, tais como marinas e atracadouros. Em cada ponto, foram coletados 30 indivíduos adultos os quais foram anestesiados e posteriormente avaliados com relação a presença de imposex com base nos índices: % imposex, FPLI, RPLI e VDSI. Todos os locais amostrados, com exceção da praia de Boicunganga, no município de São Sebastião, indicaram a ocorrência de imposex que variou de 11 a 100%. De forma geral, os níveis de imposex foram mais elevados em pontos amostrados nas proximidades de marinas e portos pesqueiros e decresceram com o distanciamento dessas fontes potenciais. Essa observação denota que apesar do banimento global das tintas à base de TBT, esse composto continua a representar um grave problema ambiental para o litoral sul-

americano. Além disso, medidas devem ser tomadas para efetivamente proteger unidades de conservação dos impactos produzidos por substâncias químicas perigosas.

#### 57 Butyltin contamination in sediments from Pará state, Amazon Brazil

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TBT-based antifouling paints were banned by the International Maritime Organization in 2008 due to very low concentrations cause gastropod imposex, oyster malformation, and mussel larvae mortality. Given the relatively high logKow of TBT suggests a strong affinity this compound with sediments. The distribution of BT compounds in the sediments samples obtained at river outfalls, fishing ports, shipyards, and industrial zone docks of Pará Harbors (north Brazil) was investigated. Nineteen sediment samples were collected from various locations in 2016 and analyzed for monobutyltin (MBT), dibutyltin (DBT), and tributyltin (TBT) by gas chromatography coupled mass spectrometry (GC-MS). This study is the first report on BT contamination in harbors from Amazon. The Pearson Correlation showed no significant correlation of grain size with TBT, DBT and MBT accumulation. Silt and clay were prevalent in the sediment from Pará state. Higher levels of fine particles (silt) occurred in the saline areas, while clay occurred in sediment samples collected in freshwater. Very fine (clay) to silt were found only in the marine area. The percentage of sediment samples containing coarse fraction were low (< 10%). TBT was present in eight of the nineteen sediment samples collected from the Pará. In this areas TBT concentrations ranged from < LOQ (below limit of quantification) to 27.13 ng Sn/g (harbor). DBT was detected in seven of the stations and varied from 1.89 (P13, beach) to 12.25 ng Sn/g (P4, harbor). MBT was detected only in four sampled sites and ranged from < LOQ to 14.2 ng Sn/g (harbor). The high level of TBT was found in mangrove harbor, while DBT and MBT was found in freshwater harbor. Maximum BTs levels were found in the station freshwater harbor. The results showed that the ratio of TBT to BTs in sediment was generally low, suggests historical inputs organotins, because TBT degradation in aquatic systems may occur way debutylation of TBT to DBT and MBT.

#### 58 Toxicity of common contaminants on aquatic invertebrates in a port area

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Port activities are among the main sources of aquatic contamination, including the active substances of antifouling paints. These booster biocides can be detected in regions close to the marinas, ports and areas where there is intense circulation of vessels. In addition, urban areas and industrial activities are often associated with port activities resulting in the release of several contaminants in the environment, including the ammonia, which has been found in the composition of these discharges. The objective of this study is to evaluate the toxicity of two contaminants, the biocide Irgarol and ammonia, on aquatic invertebrates, namely: embryonic development of the sea urchin *Echinometra lucunter*, reproduction of the copepod *Nitocra* sp., and mortality of the nauplii of *Artemia salina*. *E. lucunter* embryos were exposed to the concentrations 0.001; 0.01; 0.1; 1.0; and 10.0 mg/L of ammonia and 0.1; 1.0; 10.0; 100.0; and 1000.0 µg/L of Irgarol. Ovigerous females of *Nitocra* sp. and nauplii of *A. salina* were exposed to the concentrations 0.01; 0.1; 1.0; 10.0; and 100.0 mg/L of ammonia and 1.0; 10.0; 100.0; 1000.0; and 10000.0 µg/L of Irgarol. The assays with *E. lucunter* showed LC<sub>50</sub>-42h of 0.03 mg/L, LOEC of 0.01 mg/L and NOEC of 0.001 mg/L of total ammonia; and no toxicity was observed to Irgarol at the concentrations tested. For the test with *Nitocra* sp., the observed EC<sub>50</sub>-7d was 27.89 mg/L, LOEC of 100.0 mg/L, and NOEC of 10.0 mg/L of total ammonia. As for the *A. salina* nauplii, the value found for the LC<sub>50</sub>-48h was 368.47 mg/L, LOEC of 1000.0 mg/L, NOEC of 100.0 mg/L of total ammonia; the data relative to the exposure of the nauplii and *Nitocra* sp. to Irgarol are being analyzed and will be presented. The nauplii of *A. salina* were more resistant to ammonia when compared to the copepods, which had intermediate sensitivity, and sea urchin embryos, which presented higher sensitivity. These results demonstrate that different species may respond differently to different contaminants, requiring a battery of assays with various organisms to assess the ecological risks of chemicals and effluents.

#### 59 Imposex in *Thaisella coronata*: a study case in Amazon

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Imposex (female masculinization) is induced especially in gastropods exposed to tributyltin (TBT). The purpose of this study was to evaluate the occurrence of imposex in *Thaisella coronata* collected in port and beach areas from the coast of Pará state (north Brazil – Amazon region). In addition, butyltin levels (TBT, DBT, MBT) in tissue and sediment samples from the same studied areas were also measured. BT levels were determined after derivatization by Grignard reaction using gas chromatography coupled mass spectrometry (GC-MS). Imposex status was determined by RPLI, VDSI and % imposex. The PERMANOVA and T test do not showed differences between port and beach areas considering the butyltin levels in sediments and biota. TBT in sediment was found in two of the three port areas (1.52 ng.g<sup>-1</sup> and 2.79 ng.g<sup>-1</sup>). DBT concentrations ranged from -1 and was found in both regions, port and beach. This finding indicate the TBT degradation in sediment samples probable induced by solar radiation or microbial activities. In the biota samples TBT concentrations ranged from -, DBT ranged from < LOQ to 94.76 ng.g<sup>-1</sup> and MBT ranged from -1. The imposex incidence was observed only in a port area (100%, RPLI = 1.77). Linear Regression analysis did not show relation of TBT accumulation with imposex indexes. On the other hand, DBT accumulation strongly relationship with FPL<sub>Stand</sub> and RPLI<sub>Stand</sub> (R<sup>2</sup> = 0.919, p = 0.0025; R<sup>2</sup> = 0.868, p = 0.0068, respectively). This data confirm that imposex is a valid tool to monitor bioavailable BTs, by fact of DBT is a degradation product of TBT. This study reported for the first time the imposex occurrence in *Thaisella coronata* and suggested this organism as a suitable tool to monitor TBT contamination. Despite statistical analysis do not showed differences between port and beach, the results showed that TBT was generally the most abundant in port sediment. Among six sampled sites, five presented imposex incidence, attributed to ship/boat traffic (probably using TBT-based antifouling in paints) in association to local hydrodynamics conditions.

#### 60 Occurrence of Imposex in *Strombus pugilis* and *Melongena melongena* gastropods and their relation to contamination sites in the Colombian Caribbean

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Organotin compounds (OTC) are widely used in antifouling paints for boats and surfaces exposed to seawater such as buoys, aquaculture nets, marine ducts and offshore constructions. These paints are used to avoid an undesirable growing of marine organisms (fouling). OTC are also endocrine disruptors that leach from those paints and affect aquatic life causing imposex in gastropods, the growth of a pseudopenis that eventually blocks the female reproductive apparatus, that ends in infertility. Colombian Caribbean have identified several sites contaminated with OTC. The aim of study was to identifies gastropods (n=173) and relation to imposex from Cartagena city and its surroundings (Cartagena Bay, Baru Island, and Tesca marsh) and the Gulf of Morrosquillo (Caimanera marsh, Covenas beach, and Tolu port). We did not find imposex in *Melongena melongena* (Linnaeus, 1758) from Tesca marsh (n=26) and *Strombus pugilis* (Linnaeus, 1758) from Baru Island (n=20) and Covenas beach (n=26) probably due to the small boat traffic in those areas. *S. pugilis* specimens from Cartagena Bay (n=39) indicated imposex; females showed polifurcated penises up to 15 mm with a standard relative penis size index (RPSI) of 24 and 81% of affected females (%I). *M. melongena* from Caimanera marsh (n=40) and Tolu port (n=22) showed less imposex than those from Cartagena Bay, RPSI 8.4 and 3.2, and 39% and 7%I, respectively; However, *M. melongena* does not seem a good indicator of imposex because a higher RPSI was expected from this port. We found correlation of imposex in gastropods with boat traffic and expected contamination in the Colombian Caribbean probably due to organotin compounds leaching from hull paints. Imposex is an important index to monitor the contamination with endocrine disruptors such as organotin compounds used as antifouling agents in paints for boat hulls.

#### 61 Effects of tributyltin on the eyes, swimming, feeding and growth of newborn guppies *Poecilia vivipara*

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Tributyltin (TBT) is an antifouling biocide highly toxic to non-target aquatic organisms. Newborns of *Poecilia vivipara* obtained from breeding stock kept in the laboratory were exposed for 96 h to tributyltin (TBT) in treatments 0 (control), 0 (solvent control - dimethylsulfoxide); 0.01; 1.0; 5.0; 7.5 and 10 µg L<sup>-1</sup> of TBT. Ten fish with six days of life, 7 mg of weight and 8,9 mm of total length were used at the beginning of exposures. After exposure, we evaluated the swimming speed of the individual fish by tracking videos, swimming resistance by standardized counter-current swimming, quantitative measures of the ability to capture *Artemia* nauplii, growth in weight and length, and histology of the eyes and retina. Macroscopic



analysis of the eyes showed a darkening of the iris region after exposure to 5.0; 7.5 and 10.0  $\mu\text{g TBT L}^{-1}$ . Histopathological analyzes revealed a pigment compaction in the pigment epithelium layer of the retina and in the iris, with degeneration of retinal epithelial cells. In addition, changes in the photoreceptor layer were also observed in fish exposed to 5.0; 7.5 and 10.0  $\mu\text{g TBT L}^{-1}$ . Significant reductions relative to controls of 85% in the mean swimming speed, 60% in the swimming resistance index, and of 33.6% in the daily catch of *Artemia nauplii* throughout the exposure were detected at 7.5  $\mu\text{g TBT L}^{-1}$ . In agreement with the reduction in prey catch, a reduction in the growth of fish exposed to 7.5  $\mu\text{g L}^{-1}$  of 56.3% in biomass and 64.3% in standard length was detected. The histopathological changes detected in the retina and iris at 5.0; 7.5 and 10.0  $\mu\text{g TBT L}^{-1}$  may have reduced the fish visual exploration and prey detection capacity, which together with the detected reduction in swimming speed, might have led to a deficiency in prey capture. This was corroborated by the reduction in prey capture observed, culminating in the growth reduction of *P. vivipara* newborns. These changes favor the possibility of higher predation risk of the exposed fish, and reduce the chances of these fish recruiting to the adult population.

## 62 Does Sediment Organic Carbon Influence the Toxicity of the Biocide Chlorothalonil?

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Chemical factors such as sediment redox potential, salinity, pH and organic carbon content interfere in the stability of apolar organic compounds, making them more or less available to the biota. Contaminants with low solubility in particular, as the case of the antifouling biocide Chlorothalonil, exhibit a sortive affinity to the organic carbon present in sediments. As such, is expected that biocide levels available in the interstitial water are inversely related to organic carbon amounts in sediment. In this study, a microcosm experiment using a natural meiobenthic community was performed to investigate the influence of sediment organic carbon content in the toxicity of Chlorothalonil. Meiofauna communities were exposed for 7 days to three environmental relevant concentration of Chlorothalonil (0.01  $\mu\text{g.g}^{-1}$ , 0.1  $\mu\text{g.g}^{-1}$  and 0.2  $\mu\text{g.g}^{-1}$ ) in sediments with different organic carbon content (0.1% and 0.4%) and were compared to non-contaminated treatments. In sediments presenting low levels of organic carbon, the densities of total meiofauna, nematodes and ostracodes were significantly lower in treatments contaminated with moderate and high concentrations comparing to the controls. Multivariate structure of nematode assemblages was also significantly different in contaminated treatments. On the other hand, sediments with lower organic carbon amounts showed no differences in meiofauna univariate and multivariate descriptors between contaminated and control treatments. The differential response of the community to contamination by Chlorothalonil in the two different sediments clearly demonstrates the influence of organic carbon on toxicity. It further suggests a higher bioavailability of the contaminant for meiofauna in sediments slightly enriched. Since Chlorothalonil has a tendency to partition onto organic matter, it is possible that contamination of meiofauna was mainly through the ingestion of contaminated particles, rather than through the ingestion and/or absorption of interstitial water, as it has been previously suggested.

## 63 Efeitos do clorotalonil sobre o sistema imune de mexilhões *Perna perna*

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As tintas anti-incrustantes têm sido amplamente utilizadas para fins de proteção de embarcações contra incrustações marinhas. Muitos biocidas que compõem essas tintas surgiram como alternativas ao uso do TBT, um composto que exerce exacerbada toxicidade para diversos organismos não alvo, tais como ostras *Crassostrea gigas* e mexilhões *Perna perna*. O biocida clorotalonil, por exemplo, começou a ser utilizado na composição de tintas anti-incrustantes depois do banimento do TBT e seus efeitos sobre a fauna marinha ainda são parcialmente desconhecidos. Nesse sentido, o presente trabalho teve como objetivo avaliar os efeitos do clorotalonil sobre parâmetros do sistema imunológico de mexilhões *P. perna*. Para isto, animais foram expostos a 0 (controle, com 0,016 % do solvente dimetil sulfóxido), 0,1  $\mu\text{g/L}$  e 10  $\mu\text{g/L}$  de clorotalonil por 96 h. Após 24 h e 96 h, a hemolinfa foi retirada e os seguintes parâmetros imunológicos foram analisados nos hemócitos: número total de hemócitos, geração de espécies reativas de oxigênio, viabilidade celular (MTT e vermelho neutro), atividade fagocítica, adesão celular e dano de DNA. Tanto após 24 h quanto

96 h, pode-se observar que a viabilidade celular foi diminuída, enquanto que a adesão celular foi aumentada. Além disto, após 96 h de exposição ao contaminante, um aumento na atividade fagocítica também pode ser observado em ambas concentrações testadas. O número de células presentes na hemolinfa, o nível de espécies reativas de oxigênio e o dano de DNA, entretanto, não foram afetados. Sendo assim, os resultados encontrados no presente trabalho sugerem que o clorotalonil é um biocida que afeta o sistema imune de organismos não alvo, o que pode, inclusive, afetar a capacidade destes animais em responder a outros estressores ambientais de maneira eficiente.

## 64 Exposure to chlorothalonil causes change in biomarkers of contamination in the estuarine polychaete *Laeonereis acuta*

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Chlorothalonil is a broad spectrum fungicide applied in agricultural activities with the purpose to fight against foliar and crop diseases, in addition to currently being widely used as an active biocide in antifouling paints. Thus, the aim of this study was to evaluate the effects of chlorothalonil exposure on biochemical biomarkers of oxidative metabolism as well as cholinesterases in the estuarine polychaete *Laeonereis acuta*. For this, the activity of the enzymes catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutamate cysteine ligase (GCL), acetylcholinesterase (AChE) and propionylcholinesterase (PChE) were evaluated as well as the levels of reduced glutathione (GSH), antioxidant capacity and levels of lipid peroxidation. After acclimatization the animals were exposed for 24 and 96 h at the following nominal concentrations of chlorothalonil: 0.1, 10.0 and 100.0  $\mu\text{g/L}$ , originated from stock solutions prepared on DMSO. No difference was observed in the levels of reactive oxygen species in animals exposed to chlorothalonil. However, there was a reduction of the antioxidant capacity against peroxy radicals of the animals exposed to the highest concentration after 96 h concomitantly with an increase in the levels of lipid peroxidation. Considering the components of the antioxidant defense system, no differences were observed in the activity of CAT and SOD enzymes at both experimental times. However, the activity of the GCL enzyme was increased after 24 h of exposure to the highest concentration, as well as an increase in GST activity after 96 h in this same treatment. GSH levels were increased in the animals exposed to the lowest concentration, and reduced in the highest concentration after 96 h. Regarding the intermediate metabolism, a reduction was observed in lactate levels at 10  $\mu\text{g/L}$  after 24 h, while the higher concentration induced an increase in lactate levels after 96 h. There were no differences in glycogen levels at any of the exposure times. AChE activity was inhibited in the animals exposed to the highest concentration after 96 h. These results demonstrate that chlorothalonil was toxic to *L. acuta*, altering its metabolism and causing a situation of oxidative stress in high concentrations after 96 h of exposure. In addition, it has been shown its capacity to alter the activity of enzymes of the cholinergic system, which are widely used in biomarkers studies.

## Uncharted Environment and International Cooperation

### 65 Sharing international experiences on the Environmental Effects on aquatic ecosystems, lessons learned and outlook: The EULA Chile Experience

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The issue of environmental pollution have attracted a series of instances for international cooperation, in this paper a long term relationship between the EULA Chile Environmental Sciences Centre at the University of Concepcion in Chile, will be described. Starting in 1989, as a result of the Italian International cooperation, the center was built to address environmental issues in the Biobio River basin and the adjacent coastal zone, with an important initial funding the center was dedicated initially to study the Biobio river (a large freshwater ecosystem in Chile) through different sub projects addressing different components of the ecosystem that at time were practically unknown. One important feature of this starting project was that it considered also training of postgraduate students, since an associated PhD program on environmental sciences were created to support the further Center development after the international support ended in 1993. The environmental sciences at that time had no the reputation that have nowadays, and after the initial three years, there were a strategic decision that the center should continue to develop their activities in three major areas: Aquatic Systems (marine and estuarine), Environmental Engineering and Land use planning. The seed for the ecotoxicological approach in the Biobio River were first used in the early 90's, with the support of colleagues of the University of Milan (Maro Vighi) and Siena (Ma Cristina Fossi, Silvano Focardi and Claudio



Leonzio) and then developed through a strong team dedicated to understand the effects of pollution in the Biobio system. The development of summer schools started in 1997, attracted students Chile and Latin America to learn from these starting ecotoxicological experiences in the Biobio river. Teaching courses always considered field and laboratory work. The SETAC support was instrumental in to develop a wider network of scientist interested in to collaborate with our small team located in Chile. In 2003 Canadian Colleagues in a SETAC LA meeting held in Argentina, expressed strong interest in cooperate in the development of a environmental effects monitoring based on fish in the Biobio and other freshwater ecosystems in Chile. This fruitful experience of cooperation was accompanied from the training of PhD students, scientific papers, international courses, and research collaborations that continued up today. Thanks to FONDAP CRHIAM 15 13 0015 and EULA Chile Centre.

## Pharmaceuticals in the environment: Single and mixture toxicity of pharmaceuticals

### 66 Regulatory requirements for veterinary medical products (VMP) in the European Union, especially for the protection of dung and soil organisms

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Veterinary medical products (VMPs), in particular avermectins, are widely used in agriculture, especially in cattle, horse and sheep farming. For about 20 years, ecotoxicological data are required within the registration process of VMPs in the European Union. In the past, the environmental risk assessment (ERA) of VMPs in the terrestrial compartment was mainly based on the results of laboratory tests with a small number of dung (i.e. beetles, flies) and soil (e.g. earthworms, springtails) species. Standardized OECD methods for soil organisms were already developed for other chemicals, especially pesticides, but no test with dung organisms was available. In order to improve this situation members of the SETAC Interest Group DOTS (= Dung Organism Toxicity Test Standardization), consisting of experts from agencies, universities and companies, was founded to develop such methods - and within a few years OECD tests for two dung and one beetle species became standardized (one more beetle test is still under development). If risks are identified in these laboratory tests with invertebrates, a high-tier field study could be conducted according to European regulatory requirements. However, without a standardized method such tests were rarely performed. Based on an extensive literature review DOTS members discussed the different options. Afterwards, and with support from the German Environmental Agency (UBA), an international research project was undertaken to develop and validate a test method under different field conditions (e.g. climate) at four sites in Canada, France, Zurich and The Netherlands. Using the avermectin Ivermectin as a model substance, the effects of this VMP on dung beetles, dung flies, earthworms, springtails and dung degradation were investigated for up to one year. Clear and consistent effects of ivermectin residues were detected on dung organisms (especially sepsid flies), while there were no or few effects on earthworms and springtails, respectively. Dung degradation at the four study sites was also not affected. This lack of effects is probably caused by the fact that dung organism activity is only one of many factors (e.g. climate) that affect dung degradation. In summary, this contribution will present the current stage of regulatory and scientific discussions in the European Union regarding the ERA of VMPs in the terrestrial environment, especially the performance of higher-tier field studies and the evaluation of their results.

### 67 Chronic exposure to carbamazepine affects feeding and biochemical markers in zebrafish.

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Carbamazepine (CBZ) is used to treat epilepsy, bipolar disorder, trigeminal neuralgia, being one of the most prescribed pharmaceuticals worldwide. As a consequence of its high usage CBZ reaches aquatic environment via hospital and domestic wastewater. Thus, CBZ is often detected in surface water and sediments becoming a marker of anthropogenic pollution. Few studies are found regarding the chronic effects of CBZ on aquatic organisms, especially with fish. In the present study a chronic test was performed (nine weeks). Three treatments were used: a control group in water, an environmentally relevant concentration ( $10 \mu\text{g.L}^{-1}$ ) and a second concentration corresponding to 5% of the  $\text{LC}_{50}$  value ( $10000 \mu\text{g.L}^{-1}$ ). This study aimed to assess the chronic toxicity of CBZ to adults of *Danio rerio* using the following parameters: growth rate, reproduction (total number of eggs and viability), feeding behavior (first strike and total feeding time) and biomarkers (antioxidant defenses (Catalase (CAT) and Glutathione-S-Transferase (GST)), neuromarker (acetylcholinesterase activity

(AChE)), and metabolism biomarker (dehydrogenase lactate - LDH)). Our results showed no effects in reproduction and growth. On the other hand, CBZ affected behavior and enzymatic activity of exposed organisms. Regarding behavioral effects, an increase in total time for food intake were observed at 10 and  $10000 \mu\text{g.L}^{-1}$ . Enzymatic activity was also affected at environmentally relevant concentration ( $10 \mu\text{g.L}^{-1}$ ) showing to be a very sensitive parameter: CAT activity decreased in the liver and gills, while GST in the gills and AChE activity in the head increased. In the highest concentration ( $10000 \mu\text{g.L}^{-1}$ ) the CAT activity remains inhibited in liver and gills; GST presents induction in the gills and liver, and an inhibition in the intestine; LDH activity showed an increased activity in liver and decreased in muscle and gills; AChE was induced in the head and muscle. In summary, fish chronic exposure to low concentration of CBZ affects feeding behaviour, reproduction and biochemical pathways. Altogether, these results showed that the CBZ may have a direct effect on the population fitness, since they provide information from a realistic environmental scenario.

### 68 Does low concentrations of fluoxetine affect the neurobehavioural development of zebrafish?

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The occurrence of psychoactive compounds in natural waters has drawn attention of researchers worldwide. Fluoxetine (FLX) residues are found in environmental samples, including surface water, sediments, and groundwater. For these reasons, there is an immediate concern to understand how this compound may induce risks to natural ecosystems acting as a toxic agent. The aim of this study was to evaluate the acute toxic effects of FLX using zebrafish embryos. To assess the lethal and sub lethal effects of this psychoactive compound, an extended version of the OECD Protocol nº 236 "Fish Embryos Toxicity" (FET) test were performed during 168 h. Seven concentrations of FLX (0; 0.01; 0.27; 0.74; 2.02; 5.51; 15.0 mg/L) were tested. In order to evaluate a subtle effect of FLX in cholinesterase activity, embryos were also exposed to lower concentrations ranging from 0.001 – 1.053 mg/L, and the activity of acetylcholinesterase (AChE) was measured using two substrates, propionylcholine (PCh) and acetylcholine (ACh). We observed that FLX was toxic with a  $168 \text{ h-LC}_{50}$  value of 1.18 mg/L, and caused behavioural changes, as loss of equilibrium ( $168 \text{ h-EC}_{50} = 1.02 \text{ mg/L}$ ). Cholinesterases seems to be inhibited by FLX with a dose-response inhibition of AChE that were obtained at concentrations as low as 0.001 mg/L. Adverse effects on developmental and biochemical parameters caused by FLX was detected at low concentrations levels (0.006 mg/L). Thus, data from the present study indicate a potential ecological risk for non-target organisms.

### 69 Fluoxetina: Estudo ecotoxicológico e avaliação do risco ambiental em ecossistema costeiro

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Na última década evidenciou-se o crescente uso de medicamentos da classe dos antidepressivos. Nesse contexto, a Fluoxetina (FLX), um inibidor seletivo da recaptação da serotonina, está entre os fármacos mais prescritos em âmbito global. Como consequência, diversos estudos tem reportado a ocorrência desse fármaco em ambientes aquáticos em um range de concentrações que variam de -1. Apesar da ocorrência comprovada da FLX no ambiente marinho e estuarino, há poucos estudos que abordaram uma avaliação de risco nesses ecossistemas. Nesse sentido, o presente estudo avaliou os efeitos biológicos adversos da FLX em diferentes níveis de organização biológica, bem como alterações metabólicas empregando como organismo modelo o bivalve marinho *Perna perna*. Para isso foram delineados os seguintes métodos: (i) ensaios de toxicidade normalizados para avaliação de efeitos

agudo (taxa de fertilização) e crônico (desenvolvimento embriolarval); (ii) atividade de CYP 450 like proteins, glutationa-S-transferase (GST) e Glutationa peroxidase (GPx); (iii) efeitos subletais, tais como lipoperoxidação (LPO), danos em DNA, alteração da atividade de colinesterase (Che) e diminuição na estabilidade da membrana lisossômica. Efeitos sobre a fertilização e desenvolvimento embriolarval foram evidenciados a partir da exposição a 0,25 mg.L<sup>-1</sup> de FLX. A investigação dos processos metabólicos envolvidos na biotransformação desse fármaco demonstrou alteração na atividade de CYP 450 like proteins e glutationa-S-transferase. Efeitos citogenotóxicos foram evidenciados a partir de 48 horas de exposição através da avaliação dos níveis de peroxidação lipídica, estabilidade da membrana lisossômica, danos em DNA e colinesterase, em concentrações a partir de 3 ng.L<sup>-1</sup>. As avaliações de risco realizadas com as concentrações ambientais de FLX demonstraram um baixo risco para os ensaios agudo e crônico normalizados. No entanto, quando respostas subletais foram empregadas, efeitos adversos foram observados em concentrações ambientalmente relevantes, denotando o risco ambiental desse fármaco em ecossistemas costeiros.

#### 70 Endocrine disrupting effect of Ibuprofen on fish (*Rhamdia quelen*)

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Non-steroidal anti-inflammatory drugs (NSAIDs) have been found frequently in effluents and in surface waters, due to high prescribing and consumption by the population. These compounds can act as endocrine disrupters in the aquatic environment, which in low doses can mimic, block or interfere with normal hormonal activity. The objective of this study was to evaluate endocrine disrupting effect of ibuprofen, a NSAIDs, in male fish (*Rhamdia quelen*). The fish were divided into 4 groups, one control group (acetone, the ibuprofen solvent) and three groups exposed to 0,1, 1,0 and 10,0 µg/L of ibuprofen. During the exposure (14 days), the 1/2 of the water was replaced with the drug concentrations every 24 hours. At the end of the exposure, the fish were anesthetized and euthanized, blood, brain and gonad were collected. The quantification of hormones testosterone and estradiol were measured in plasma. Biochemical biomarkers as activities of catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST), superoxide dismutase (SOD), reduced glutathione (GSH), lipoperoxidation (LPO) levels, genotoxic biomarkers (comet assay) and gonadosomatic index were measured in gonads. The cyp19a1b (brain aromatase) gene expression was measured in the hypothalamus. The hormone concentrations were not statistically different compared to the control group. The results of the biochemical biomarkers showed only a reduction in the GST activity in group exposed to 10µg/L of ibuprofen compared to the control group. It was not observed genotoxicity and there were no changes in gonadosomatic index in groups exposed to ibuprofen. Although ibuprofen did not directly interfere with the testosterone and estradiol concentration, it decreased the brain aromatase expression in all the exposed groups compared to control group. Since this enzyme is responsible for converting testosterone to estradiol a long term exposure in the environment can result in hormonal changes as already reported in the literature and consequently in the fish reproduction.

#### 71 Toxic effects of Paracetamol in fish *Rhamdia quelen* (Jundiá)

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The presence of human-use drugs in environmental compartments has become increasingly common throughout the world, since in addition to the incorrect disposal of drugs, the treatment system often does not eliminate them. The present work aimed to evaluate possible toxic effects of paracetamol in *Rhamdia quelen* fish using biochemical and genetic biomarkers. The fish were divided in four groups and exposed to 0.25, 2.5 and 25 µg.L<sup>-1</sup> of paracetamol concentrations and a control group exposed to acetone (paracetamol solvent) via hidric. After 14 days of exposure, the fish were anesthetized with 1% benzocaine, and blood was collected for leukocyte count. The fish were then euthanized by medullary section and the posterior kidney was collected for evaluation of biomarkers. Water for paracetamol analysis was sampled at 0, 24 and 48 hours, 7 and 14 days after exposure. For the biochemical biomarkers the antioxidant system was performed, including enzymes such as glutathione S-transferase (GST), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) and other non-enzymatic factors such as reduced glutathione (GSH) and lipoperoxidation. For genotoxicity, comet assay was carried out. The concentrations of paracetamol were 0.65 ± 0.33, 2.58 ± 0.97 and 12.79 ± 5.85 µg.L<sup>-1</sup> for the 0.25, 2.5 and 25 µg.L<sup>-1</sup> groups,

respectively. In comparison to the control group, a reduction in the total number of leukocytes in the 25 µg.L<sup>-1</sup> group was observed. The GST and SOD activities didn't present significant statistical differences. The catalase activity was increased in the 2.5 and 25 µg.L<sup>-1</sup> groups when compared to control. GPx activity increased in all tested groups when compared to control group. The concentration of GSH was increased in the 0.25 and 2.5 µg.L<sup>-1</sup> groups. Lipoperoxidation wasn't observed. DNA damage was observed at concentrations of 2.5 and 25 µg.L<sup>-1</sup> when compared to control group. Since the posterior or excretory kidney is the primary site for hematopoiesis in the fish and the hematopoietic tissue forms a support matrix for the nephrons, the results suggested nephrotoxicity of low concentrations of paracetamol due to the leukocytopenia, a disbalance in the antioxidant system and damage to the DNA of *R. quelen*.

#### 72 Environmental risk assessment of sediments contaminated with ibuprofen and triclosan employing *Callinectes* sp.

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Population increase in coastal zones has generated environmental impacts, among which we can highlight the inadequate disposal of industrial and domestic effluents in marine and estuarine waters. Domestic effluents transport a wide variety of contaminants, such as pharmaceuticals and personal care products, which have recently been classified as emerging pollutants. Thereby, the European and North American environmental agencies began to regulate the approval, commercialization and disposal of these emerging pollutants through directives. It has been shown that an important tool in the environmental risk assessment is the responses of biological changes to the exposure and to the effect of xenobiotics that have provided relevant information on bioavailability and toxicity. Thus, the present study aims to evaluate the environmental risk of triclosan (TCS) and ibuprofen (IBU) in marine sediments using the crabs *Callinectes* sp. To achieve this purpose, the identification and quantification of the environmental concentrations of the main pharmaceutical compounds presents in marine sediments was carried out and the changes in the biological responses in gill, hepatopancrea and hemolymph tissues of *Callinectes* sp. exposed to sediment contaminated with TCS and IBU. Samples of the Santos submarine sewage outfall (Bay of Santos, São Paulo, Brazil) were collected so that environmental concentrations could be quantified. These samples were analyzed by HPLC-ESI-MS / MS in negative mode. The concentrations found for TCS and IBU were 15.14 and 49.0 ng.g<sup>-1</sup>, respectively. The crabs used in the assays were exposed to the spiked sediment with TCS and IBU for a period of 15 days. At the end of this period the assay was performed to evaluate the stability of the lysosomal membrane by the neutral red retention time, which showed effect at the concentration of 1 ng.g<sup>-1</sup> in both compounds. Considering the international protocols of environmental risk assessment the chemical data and ecotoxicological were integrated and the risk quotients estimated for TCS and IBU presented values higher than 1.0 indicating a high environmental risk of these compounds in marine sediments.

#### 73 Muscle concentrations of human pharmaceuticals and hepatic EROD and BROD activity in fish from the inner La Plata River and Lower Uruguay River

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The aim of these study was to assess the occurrence and concentrations of six pharmaceuticals: atenolol (ATE), caffeine (CAF), carbamazepine (CBZ), enalapril (ENA), metformin (MET), sildenafil (SIL) in four fish species: *Prochilodus lineatus*, *Salminus brasiliensis*, *Leporinus obtusidens* and *Pimelodus maculatus*. Fish were collected from two areas, one located in the southern sector of the riverine La Plata River (RLP) and other at the Lower Uruguay River (URU) during 2016 fall. Chemical analysis in muscle was carried out using the rotary extractor method followed by HPLC-MS/MS. Additionally, EROD, BROD and GST activities were fluorometrically and spectrophotometrically measured in *P. lineatus* and *P. maculatus*. The limit of detection (LOD) and quantification (LOQ) of assessed pharmaceuticals were between 0.5-3.3 µg/kg and 5.1-11.2 µg/kg, respectively. Recoveries were between 33% and 117%. Overall occurrence showed that CBZ, ENA and SIL were ubiquitous, followed by ATE (75% RLP-100% URU), then CAF (25% RLP-50%

URU), and finally MET, varying highly among sites (0% RLP-100% URU). Usually frequencies were higher at URU. Maximum concentrations were higher for SIL (529 µg/kg, RLP), followed by ATE (282 µg/kg URU), then ENA (106 µg/kg, RLP), CBZ (100 µg/kg, URU) and CAF (90 µg/kg, RLP), and lower for MET (29 µg/kg, URU). Average total pharmaceutical concentration was higher in fish from RLP (234.4 µg/kg) than in URU (156.8 µg/kg). The species with higher total concentration was *L. obtusidens* (438 µg/kg), followed by *S. brasiliensis* (240 µg/kg), then *P. lineatus* (221 µg/kg) and *P. maculatus* (109 µg/kg). BROD and GST activities were significantly different among sites only for *P. lineatus*, with 79 and 57% higher in RLP than in URU, respectively. Some of the measured concentrations were above the maximum residues limits (MRL) established by the European Union for diclofenac in bovine muscle (5 µg/kg). In addition, higher activities of the biotransformation enzymes were observed in fish collected in the site with greater pharmaceutical concentrations. Therefore, including these compounds in monitoring programs is advised. This was the first report on bioaccumulation of pharmaceuticals in fish of the “del Plata” basin.

#### 74 Effects on fertilization rate and embryolarval development of *Perna perna* mussels exposed to crack cocaine in different pHs

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After administration, a significant proportion of illicit drugs are excreted and reaches the wastewater treatment plants where they may not be totally eliminated by conventional treatment technologies. Recent research demonstrates they can influence and alter structure as well as behavior of aquatic organisms. The amounts of illicit drugs consumed worldwide are comparable with those of therapeutic drugs, as millions of individuals are current users of cocaine, heroin, amphetamine like stimulants, marijuana and others. Since 1970, cumulative carbon dioxide (CO<sub>2</sub>) emissions from fossil fuel combustion, cement production and flaring have tripled, and cumulative CO<sub>2</sub> emissions from forestry and other land have increased by about 40%. Rising atmospheric CO<sub>2</sub> concentration is causing global warming and ocean acidification. The inorganic carbon system is one of the most important chemical equilibria in the ocean and is largely responsible for controlling seawater's pH. Based on that, the hypothesis of this work is that the acidification in the marine environment will provoke a difference in toxicity of the illicit drug cocaine and its byproducts. The main objective of this work was to assess the acute and chronic toxicity of crack cocaine combined to ocean acidification to *Perna perna* mussel. In a first experiment fertilization rate and embryo-larval development were assessed at five different concentrations (6,25 mg.L<sup>-1</sup>; 12,5 mg.L<sup>-1</sup>; 25 mg.L<sup>-1</sup>; 50 mg.L<sup>-1</sup> and 100 mg.L<sup>-1</sup>) in four different pHs (8.5; 8.0; 7.5; 7.0). The exposure of the spermatozoid for 1h to crack-cocaine didn't show toxicity in different pHs. Results of embryolarval assays showed inhibitory concentrations (IC50) of crack-cocaine in 9.07 mg/L<sup>-1</sup> and 8.71 mg/L<sup>-1</sup> in pH 8.5 and 8.0 respectively. In pHs 7.5 and 7.0 the effects observed in embryo development were associated to the low pH level, since effects were found in the control samples. Our results showed differences in effect concentrations due to decrease in pH, suggesting alterations on cocaine toxicokinetic in acidification scenarios

#### 75 Health risk due to intensive livestock contamination. antimicrobial resistance in aquatic environments

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Livestock contributes to water pollution through the discharge of sediments, antibiotics, pesticides, fertilizers. There is an outstanding risk of biological contamination associated with this kind of production because animal feces and urine containing pathogens can be transported to surface water through runoff. Antibiotics are extensively used in livestock to prevent and treat disease, as well as to growth promoters, can reach the aquatic environment by contaminating water courses with feedlot residual liquids. The aim of this work was to assess biological contamination and to estimate health risk from watercourse which connects a feedlot with. A quantitative microbial risk assessment (QMRA) of *Salmonella* infection was performed to estimate the human health risks associated with the use of Burgos's creek for recreational purposes. Seasonal samples were taken between 2012 and 2016 at the feedlot outlet (site 1), 5 km from the feedlot (site 2), 0.4 km from Burgos's creek the mouth of the canal (site 5) and Burgos's creek, 3.6 km upstream of the mouth of the watercourse (site 6). In these samples, concentrations of coliform, *E. coli*, enterococci

(indicators of fecal contamination) and *Salmonella* spp (enteropathogen) organisms were measured. At sampling, site 1, mean values of 1.50 x 10<sup>5</sup> for *E. coli* and 2.70 x 10<sup>4</sup> for enterococci were observed in 100 mL. At site 2, a significant decrease in enterococci was detected. No significant differences were found between site 2 and site 5 in the counts of the indicator bacteria. The regression analysis showed a high correlation between the number of indicator bacteria at both sampling sites. The estimated average annual risk of infection, with *Salmonella* was 1.74 x 10<sup>-4</sup> and 2.37 x 10<sup>-3</sup> at sites 1 and 2 respectively. In site 6, an annual risk of 5.77 x 10<sup>-4</sup> was detected, reaching a value of 6.73 x 10<sup>-4</sup> in site 5. A high degree of resistance to tetracycline was observed in the isolates of *Salmonella* spp. The contamination produced by the feedlot gives rise to a high health risk because Burgos's creek used for recreational activities. The health risk is increased by the fact that *Salmonella* isolates showed resistance to the antibiotics such as tetracycline, gentamicin and cefepime used. This resistance spreads to other animals and humans either directly by contact or indirectly via the food chain, water, air, and manure and sludge-fertilized soils compromising seriously the antibiotic therapeutics efficacy. . .

#### 76 Synergic effects of Triclosan and Triclocarban by acute, genotoxic and phytotoxic approaches with *D. magna* and *L. Sativa*

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Priority contaminants such as pharmaceutical and personal care products are found in aquatic environments at µg L<sup>-1</sup> concentration levels in all world. These compounds are not completely withdrawn by wastewater treatment processes and can be harmful to aquatic organisms causing adverse effects that still not known. Triclosan (TCS) and Triclocarban (TCC) are substances commonly used in Brazil as antiseptic in personal care products such as soaps, deodorants and toothpastes. This preliminary study evaluated the ecotoxicity and genotoxicity of TCS, TCC and their mixtures in *Daphnia magna* acute bioassay besides phytotoxicity experiment by test of germination and growth inhibition of lettuce seeds (*Lactuca sativa*). The effective concentrations to *D. magna* (EC<sub>50d</sub>) were 446.77 and 67.37 µg.L<sup>-1</sup> to TCS and TCC, respectively. The tests with binary mixtures were based on EC<sub>50d</sub> from TCS and TCC respectively (450+70, 225+35, 125+17, 62.5+8.5, 31.2+4.2 and 15.6+2.1 µg.L<sup>-1</sup>). The EC<sub>50d</sub> of the mixture was 126 + 19.6 µg L<sup>-1</sup> which indicates a synergic effect between TCS and TCC, it being more toxic than the compounds by themselves. Based on acute toxicity results the genotoxicity of TCS and TCC was evaluated by comet bioassay in *D. magna* with 48 h of exposition. The tests were carried out initially with separated compounds at 300 and 25 µg L<sup>-1</sup> for TCS and TCC, respectively and for binary mixing the concentrations of 31.25 + 4.25 µg.L<sup>-1</sup> of TCS and TCC. These concentrations were chosen because were had no acute effect on the organisms. The results showed significant genotoxicity (p < 0.05) to the TCS and the mixture compared to controls again indicating the synergistic effect between TCS and TCC. The triclocarban genotoxicity showed no statistical difference however the average of score values to TCC is above that upper limit of the standard deviation of the control group therefore the damage caused by TCC couldn't be disregarded. Phytotoxicity tests are still on going, but preliminary studies with *Lactuca sativa* seeds, both substances caused radicle growth inhibition with EC<sub>50</sub> of 860.70 and 574.00 µg L<sup>-1</sup> for TCS and TCC, respectively.

#### 77 Global distribution of erythromycin in the environment: review and analysis of its occurrence, bioaccumulation and resistance hazard assessment

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Over the past decades, large amounts of antibiotics have been globally consumed against antimicrobial agents and their presence in the environment have become a major public health concern due to their potential to develop antimicrobial resistance (AMR). Through a comprehensive literature review, information of measured environmental concentrations (MECs) of the common antibiotic erythromycin (ERY) were collected from peer-reviewed papers reporting ERY analyses in: 1. water/liquid samples of wastewater treatment plant (WWTP) effluent, surface water, tap/drinking water, groundwater, and estuarine and coastal systems; 2. solid samples of sewage sludge, soils and sediments; and 3. tissues of aquatic organisms. These MECs were grouped in a database, which led to elucidation of global patterns of ERY distributions



in these environmental matrices. Further, a probabilistic environmental hazard assessment (PEHA) was performed with the maximum reported MEC in order to identify the probabilities of exceeding a predicted no-effect concentration (PNEC) of  $1.0 \mu\text{g}\cdot\text{L}^{-1}$  for promoting AMR in water/liquid samples from different geographic regions. More information was available (312 publications) for water analysis than solid samples (97 publications). Across environmental compartments we examined, ERY has mainly been detected in Asian, North American and European continents with the majority of studies performed in China, USA, Spain and the United Kingdom. For surface water, 72.4% of the Asian studies have been performed in China, while 85.4% of the observations from North America were from the USA, and Spain represented 41.9% of the European surface water studies. Results from the PEHA indicated that the likelihood of exceeding the ERY PNEC for AMR was higher in Asia (33.3%) than in Europe (20%) or North America (17.8%) WWTP effluents for example. We further identified a higher likelihood of encountering ERY in estuarine and marine sediments compared to samples from freshwater systems. Unfortunately, ERY occurrence data is comparatively limited in these coastal and marine systems, as well as in large geographic regions including Southwest Asia, Eastern Europe, Africa, and Central and South America. Future studies are needed to understand risks of ERY and other antibiotics to human health and the environment in rapidly developing regions where waste management systems and infrastructure is being implemented slower than access to pharmaceuticals is occurring. \n

### Contaminants of emerging concern (CECs) in the environment: current knowledge and future challenges

#### 78 ARE TATTOO INKS SAFE? - IN VITRO AND IN VIVO TOXICOLOGICAL EVALUATION

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In the last decade, the number of people who have adhered to global trend of tattooing the body has increased significantly. This has increased the production and development of technologies applied to tattoo inks. However, regulatory agency requirements for these inks are poorly enforced and some components can be harmful to both humans and the environment. In this sense, tattoo inks were studied to identify the composition and its toxicological effects. In the description of the composition of the indicated inks, it contains water, additives and pigments. The pigments are represented by organic dyes and nanostructures. In this work four commercial inks were selected: blue, green red and black. To determine the composition and size of the particles contained in the inks, analyzes of XRD, SEM-EDS, FTIR, PZ, DH and TEM were performed. To evaluate the toxicity of the inks, *in vitro* and *in vivo* tests, different parameters and trophic levels organisms were analyzed, such as: the growth of plants *Lactuca sativa*, immobility and mortality of *Daphnia magna* crustacean and cell viability of HaCat cells (immortalized human keratinocytes). The characterization results indicated that tattoo inks have nanoscale diameters, and confirmed the presence of functional groups of the vehicles contained in ink as well as the presence of some metals. The toxicological results of the exposure of *Lactuca sativa* seeds to the inks led to growth inhibition (5 and 10%) and also morphological changes. Acute tests, performed according to ISO 12713 standard, with *Daphnia magna* showed that the color with lower toxicity is black, followed EC50 is red, followed by green, blue and red respectively. In HaCat cells the loss of mitochondrial viability was confirmed more significantly than lysosomal viability, which was only inhibited at the highest concentration tested. The MTT assay also showed that the red ink has a higher toxicity and in general for all inks increases viability in 12 hours, decreasing significantly in the other times tested. This study provides an overview of the composition and toxicological effects of tattoo inks in epidermis and in the environment after the disposal, since the nanomaterials contained in the inks can become an environmental problem.

#### 80 REDUCED GRAPHENE OXIDE (RGO) INDUCES DNA DAMAGE IN MELANOMA CULTURE CELLS (B16F10)

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Reduced graphene oxide (RGO) is a nanomaterial of generation after carbon nanotubes. The appearance of the RGO is similar to that of a sheet with nanometric dimensions, about 350 nanometers long and 5 nanometers thick. Besides their electrical and physical properties, graphene materials have applications in the biomedical sector for therapy, diagnosis and drug delivery, and no other material has comparable properties. Although some progress has been made in diagnosis and drug delivery, the therapeutic applications of graphene remain in their early stages. Caring for the biologic and nonbiological applications of graphene is owed to the potential toxicity of reduced graphene oxide. Hence, there is an urgent need to identify environmentally friendly and simple approaches to prepare biocompatible graphene materials for biomedical applications. We aimed to evaluate the possible genotoxic effects of exposure to the RGO. Melanoma culture cells, B16F10 were exposed to 10, 20 and 40ng/mL of RGO for 24, 48 e 72 hours. The alkaline comet assay was carried according to Singh (1988) with adaptations. It was observed chromosomal damage at all of the concentrations used and there was not difference between them. However, the DNA injury was proportional to the time of exposure to the RGO. The data suggest that caution should be exercised regarding the biomedical applications of RGO.

#### 81 Titanium dioxide in neotropical mangrove: transfer through food web and subcellular internalization in top trophic chain fish

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Among the varieties of nanoparticles (NP) produced, metal oxides have been increasingly applied due to their photocatalytic nature, particularly, the titanium dioxide NP ( $\text{TiO}_2\text{-NP}$ ). Considering the presence of  $\text{TiO}_2\text{-NP}$  in the environment due to industrial and sewage releasing, especially in aquatic ecosystems, *in-situ* studies are required to evaluate the behaviour of these NP in the environmental compartments and biota. Therefore, the purpose of this study was to quantify if Ti is present in abiotic samples (surface water, sediment, atmospheric particulate matter) and in six trophic levels (plankton, plants, oyster, crab, shrimp, fish) of neotropical estuarine mangrove ecosystems and evaluate its potential for biomagnification or biodilution in the food web. Moreover, the nanocrystallographic structure of Ti in abiotic matrixes and in fish organ/tissues was analysed to identify the  $\text{TiO}_2\text{-NP}$  oxidation state. Ti was identified and quantified in almost all matrix analysed, excepting for surface water in all sites. Linear regression showed biodilution through trophic chain based in plankton as primary producer and biomagnification when plants are the primary producers. Nanocrystallographic analyses showed that  $\text{TiO}_2\text{-NP}$  in atmospheric particulate matter samples were similar to those found in each fish organ, the top trophic chain organism, independent of their localization: inside cytoplasm and in the cell nucleus suggesting that atmospheric particulate matter may be the source of  $\text{TiO}_2\text{-NP}$  in these mangrove ecosystems.

#### 82 Toxicity studies applied to evaluate the modified zeolites adsorbent for treating copper-complex dyes from simulated dyehouse wastewater

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Textile dyes occur in wastewaters in different concentrations due to their poor fixation to fabrics. Even at low concentrations, textile dyes can cause waste streams to become highly colored. Aside from their negative aesthetic effects, certain textile dyes have been shown to be toxic, and in some cases, these compounds are carcinogenic and mutagenic. Zeolite synthesized from fly and bottom ashes and modified with hexadecyltrimethylammonium (ZMF and ZMB, respectively) were used as adsorbent to remove dyes - Solophenyl Navy and Solophenyl Turquoise and their hydrolyzed forms Solophenyl Navy Hydrolyzed and Solophenyl Turquoise Hydrolyzed, respectively, from simulated textile wastewater. The purpose of the research was to use bioassays with *Lemna minor*, *Ceriodaphnia dubia* and *Chironomus tepperi* to determine the acute and chronic toxicity of these dyes before and after adsorption process. The acute results indicated *C. tepperi* showed to be less sensitive when compared with other test organisms, showing values of  $48\text{hLC}_{50}$  70.8 and  $211 \text{mg}\cdot\text{L}^{-1}$  for SN and ST. On the other hand, *C. dubia* showed to be very sensitive to the dyes tested ( $48\text{hLC}_{50}$  1.25;



54.5; 0.78 and 2.56 mgL<sup>-1</sup> for SN, ST, SNH and STH), while *L. minor* presented response for higher concentration of the dyes, as chlorosis, loss of roots, reduction of the size and etc., showing values of EC<sub>50</sub> of 18.9; 69.4; 10.9 and 70.9 mgL<sup>-1</sup> for SN, ST, SNH and STH). Chronic tests with *C. tepperi* showed changes in survival, growth, development and gender differences for SN and ST dyes. After adsorption treatment with ZMF the acute effects were substantially reduced for both dyes and their hydrolyzed forms, showing absence of toxicity after treatment. However, the treatment with ZMB showed no difference and/or increased toxicity in some cases. Toxicity Identification Evaluation testes were realized and showed the baseline acute effects were substantially reduced after treatment with Ethylenediaminetetraacetic acid, showing the most of the toxicants in this study are cations metals. These experimental results suggest both dyes, raw and hydrolyzed, are toxic and their removal are necessary.

### 83 Non-invasive human biomonitoring techniques for per- and polyfluoroalkyl substances

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Non-invasive biomonitoring techniques present many ethical and practical benefits for investigating human exposure to hazardous contaminants, but are inherently difficult to validate for chemicals which are ubiquitous in the indoor environment. For per- and polyfluoroalkyl substances (PFASs) there only a limited number of studies using non-invasive human samples typically including a small number of PFASs. Here we investigated the validity and potential applications of using hair, nail and urine samples for characterizing internal exposure to range perfluoroalkane sulfonic acids, perfluoroalkyl carboxylic acids and chlorinated polyfluoroalkyl sulfonic acids (Cl-PFESAs). Human samples were obtained from two different populations: i) highly exposed fishery employees from Tangxun Lake, Hubei province (n=8) and ii) a background exposed population from Shijiazhuang, Hebei province (n=41). Passive uptake experiments for hair and nails were performed using both spiked standard solutions and naturally contaminated house dust and air. Nail and serum samples from the highly exposed fishery employees showed that perfluorohexane sulfonic acid, perfluorooctane sulfonic acid (PFOS), the eight carbon chlorinated polyfluoroalkylether sulfonic acid (C8 Cl-PFESA) and perfluoroundecanoic acid were transferred from serum to nails in a dose dependent manner (p>0.65; p< 0.05). Measurements of PFOS and C8 Cl-PFESA in hair and nail samples in a background exposed population displayed a plausible link to internal exposure by evaluation of statistical correlations and transfer ratios in urine compared to the highly exposed population. In contrast, the occasional detection of short-chain PFASs in the background population was more likely a consequence of uptake from the external environment. Passive uptake experiments confirmed that all studied PFASs can be absorbed in hair and nail matrices from standard solutions with low efficiency. No significant uptake of PFASs in hair was observed over 56 days of contact with naturally contaminated indoor dust and air. In conclusion, the current study supports the use of nail and urine for long-chain PFASs as a measure of internal exposure. However, further studies are needed to better understand homologue specific transfer rates and the potential for passive uptake under realistic conditions. For short-chain PFASs, urine is the preferred sampling matrix with respect to both detection capability and reliable interpretation of internal exposure.

### 84 Occurrence of flame retardants in soil, dust, well water and leachate from a Brazilian landfill site

J. Cristale, UNICAMP - University of Campinas / School of Technology; T.G. Belé, UNESP - UNIV ESTADUAL PAULISTA; S. Lacorte, IDAEA-CSIC / Environmental Chemistry; M. Marchi, Unesp- Institute of Chemistry / Analytical Chemistry Landfills can behave as punctual sources of flame retardants (FRs) if the solid residues containing FRs are not properly managed. In this study, ten organophosphorus flame retardants (OPFRs), eight polybrominated diphenyl ethers (PBDEs) and four new brominated flame retardants (NBFRs) were determined in soil, dust, well water and leachate samples from a landfill site in Brazil. The landfill reached its maximum capacity in 2009 and a recycling cooperative is operating in the place nowadays. Soil, dust, well water and leachate samples were collected in different points of the landfill site, including an area for electronic waste storage, an area for disposal of mattresses and furniture, a place where materials were separated for recycling, offices, concierge, etc. OPFRs were detected in all soil samples at levels ranging from 0.2 to 137 ng g<sup>-1</sup>. The highest levels of TCIPP and TDCIPP were observed in soil samples collected in a place where mattresses and upholstered furniture is stored in the open air, which is in accordance to the usage of these FRs in polyurethane foam. BDE-99, BDE-209,

DBDPE and BTBPE were the most abundant brominated compounds in soil, and the highest levels were observed in the samples collected next to a storage place of electronic waste. Concerning dust samples, FRs were detected at levels ranging from 8.7 ng g<sup>-1</sup> to 28800 ng g<sup>-1</sup>. The highest levels of brominated FRs were observed in dust samples collected in the place where electronic waste is stored and in the local where the recycling cooperative operates. The highest levels of OPFRs in dust were observed in an office located in the landfill area. The occurrence of OPFRs in water from monitoring wells and leachate was studied. TCEP, TCIPP and TDCIPP were detected in the well water sample collected downstream the area of storage of mattresses and upholstered furniture, which indicated that the inadequate disposal of polyurethane foam residues on soil can generate groundwater pollution by OPFRs. Finally, TCEP, TCIPP, TDCIPP, TNBP, TIBP and TBOEP were detected in leachate, which demonstrated that these compounds are still released even after five years of the end of the landfill operation. In conclusion, this case study demonstrates that an inappropriate management of waste material containing flame retardants in landfill sites can result in uncontrolled emission of these contaminants to environmental matrices.

### 85 Toxicity of commercial aqueous film foam formers (AFFF) to marine organisms

M. Ueda de Carvalho, São Paulo State University - UNESP Campus Experimental do Litoral Paulista / NEPEA- Núcleo de Estudos em Poluição e Ecotoxicologia Aquática; L.A. Maranhão, UNIFESP Instituto do Mar / Departamento de Ciências do Mar; D.M. Abessa, Universidade Estadual Paulista - UNESP/CLP / Marine Biology and Coastal Management; L. Buruaem, São Paulo State University - UNESP Campus Experimental do Litoral Paulista / Instituto de Biociências Câmpus do Litoral Paulista The second largest fire in fuel tanks ever registered in the world has occurred in Brazil, specifically in the Alemoa region, within the biggest Latin American port, the Port of Santos. This fire was followed by the release of chemical residues (fuels and waters contaminated with firefighting residues) into the Santos Estuarine System (SES). After the spill, massive mortalities of fauna and flora were observed, including eight tons of dead fish along the estuary. This event has drawn attention to the lack of toxicity studies on aqueous film foam formers (AFFF) which are commonly used in firefighting. Such studies are scarce, and there are no environmental policies regulating such products in Brazil. This study aimed to investigate the toxicity levels of eight AFFFs used to combat the fire in the 2015's emergency, by using worldwide standard toxicity tests. The commercial AFFFs were provided by the Brazilian Environmental Agency – IBAMA. Acute toxicity was determined using *Artemia sp* individuals, while chronic toxicity was tested on *Lytechinus variegatus* embryolaval stages. For the analysis of data and the comparison of the AFFFs toxicities, the EC<sub>50</sub>, NOEC and LOEC were calculated. Preliminary trials following the recommended AFFF manufacturers' dilutions showed absolute lethality of organisms. *Artemia sp* trials showed EC<sub>50</sub> in dilutions ranging from 0.00016% to 0.32100% up to 48 hours after exposure. LOECs ranged from 1% to 0.01% dilution. NOECs varied from 0.1% to 0.001%. To the *L. variegatus* embryos, the EC<sub>50</sub> values ranged from 0.00027% to 0.02569% at 24h exposure; the LOEC was estimated at 0.001% and the NOEC was 0.0001%. Some AFFFs presented even inferior values for LOEC and NOEC, respectively. Chemical analyses of the AFFFs tested revealed the presence of substances that are currently restricted by the Stockholm Convention (as perfluorooctane sulfonic acid – PFOS), emergent compounds (as perfluorooctanoic acid – PFOA), among others. All AFFFs analyzed demonstrated high toxic potential in marine organisms, and this result constitutes important data for scientific and general knowledge, environmental regulation and usage of such products.

### 86 Hormones and environmental estrogens in conventional sewage treatment plants and receiving waters in Minas Gerais, Brazil

D.N. Magalhaes, Universidade Federal de Vicosa / Engenharia Civil; A.H. Mounteer, Universidade Federal de Vicosa Increasing concern combined with lack of data on the role of Brazilian domestic sewage treatment plants (STPs) as sources of microcontaminants in aquatic ecosystems motivated this investigation into the presence of estrogenic activity and endocrine disrupting compounds (EDCs) in conventional municipal sewage treatment plants (STPs) in Brazil. Composite samples of raw and treated sewage and grab samples of receiving waters and sediments upstream and downstream of STP discharges were collected at three plants operating with different, commonly used, biological treatment processes over the course of eighteen months. Estrogenicity (YES assay) and the presence (qualitative GC/MS) of seven estrogenic compounds commonly found in domestic sewage: estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethynlestradiol (EE2), bisphenol-A (BPA), 4-nonylphenol (4-NP) and 4-octylphenol (4-OP) were analyzed. The activated sludge system (STP1) presented the

highest estrogenicity removal efficiencies, with average raw and treated sewage loads of 9.2 and 0.3 mg E<sub>2</sub>-Eq/d (96.7% removal). The systems operating anaerobic biological processes, STP2 (UASB reactor) and STP3 (septic tank followed by anaerobic filter), were less efficient in removing estrogenicity, with STP2 presenting average raw and treated sewage loads of 10.6 and 6.0 ng E<sub>2</sub>-Eq/L (43.4% removal) and STP3 presenting an average increase in estrogenicity (raw sewage with 2.3 mg E<sub>2</sub>-Eq/d and treated sewage with 2.3 mg E<sub>2</sub>-Eq/d) during the monitoring period. Some raw sewage samples were toxic to *Saccharomyces cerevisiae*, possibly leading to underestimation of estrogenicity in the YES assay. Increased estrogenicity downstream of treated sewage discharge was only detected at STP2, where treated sewage was diluted only five-fold after mixing with the receiving water, whereas at the other two STPs treated sewage was diluted 500 to 2000-fold after mixing. Natural and synthetic hormones (E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, EE<sub>2</sub>) were detected sporadically in samples from the three STPs, while the alkylphenols (4-OP and 4-NP) were rarely detected. BPA was found most frequently in untreated and treated sewage, receiving waters and sediments, both upstream and downstream of all STPs, since it is still commonly used in Brazil in many plastic products. Hormones, especially E<sub>1</sub>, not detected in upstream samples were detected in sediments downstream from STPs 1 and 2, evidence of the contribution of treated sewage discharge towards sediment contamination.

### 87 Electrochemistry removal of abuse drugs

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The consumption illicit drugs is increasing, among them, the cocaine is one of the most wide spread due to its high addicting level. In fact, abuse drugs and their metabolites are every time more frequently and have been detected in a several systems around the world. Therefore, they have become a novel group of environmental emerging pollutants. Hence, the detection and degradation of such drugs have been subject of study; and to remove them several advanced oxidation processes (AOPs) was proposed. Among AOPs the Electro-oxidation (EO) technic presents itself as a low cost and eco-friendly and can have got high efficiency using nano-structural materials. In this sense, the aim of this work is use a low cost anodes (graphite and graphite nanostructured titanium oxide) at electrochemical technics to oxidation of cocaine. The synthesis of cylindrical electrodes was performed by microwave deposition, and two diameters (5 and 2 mm) were proved. Different samples solutions (1 mg.mL<sup>-1</sup> of cocaine in NaCl 50 mM; 1 mg.mL<sup>-1</sup> of cocaine in Na<sub>2</sub>SO<sub>4</sub> 50 mM; and 10 mg.mL<sup>-1</sup> of cocaine in Na<sub>2</sub>SO<sub>4</sub> 100 mM) were tested in order to evaluate the efficiency of electrochemistry remediation. Degradation of cocaine was carried out in a batch system using an undivided cell of 5 mL capacity, and was applied a voltage of 10.0 V for 20 minutes. Cocaine degradation was monitored by UV-Vis spectrometry through characteristic peak at 233 nm and differential Pulse Voltammetry (DPV) and Square Wave Voltammetry (SWV) measurements were performed. The electrochemical essay let to estimate a decay of cocaine levels reached ca. 90% after 10 minutes for all electrodes. Nevertheless, the device presenting high cylindrical diameter and the NaCl salt medium exhibited higher efficiency.

### 88 Illicit drugs as new environmental pollutants: monitoring of cocaine and its main metabolite benzoilecgonine in marine ecosystems

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Urban sewage is the main source of marine and estuarine pollution in Brazil, due mainly to the population growth in coastal areas, and inefficiency of Sewage Treatment Plants (STPs) in collecting, treating and disposing effluents appropriately. In coastal cities, this sewage disposal occurs through structures known as Submarine Sewage Outfall (SSO). Many substances present in sewage can lead to contamination of marine ecosystems, including contaminants of emerging concern as pharmaceuticals and illicit drugs. The increasing use and, often, abuse of these substances worldwide has begun to represent a threat to non-target organisms, mainly in the aquatic environment. Thus, it is extremely important to understand their occurrence and fate in the environment. In this study we report data on occurrence of cocaine (COC) and its main human metabolite benzoilecgonine (BE) in Santos Bay (São Paulo, Brazil). The water samples (surface and bottom) were collected in 6 stations around the Submarine Sewage Outfall (SSOS), and subsequently Solid Phase Extraction (SPE) was performed. The determination of COC and its metabolites BE

was performed by Liquid Chromatography–ion trap tandem Mass Spectrometry (LC-MS/MS). Our results indicated the presence of CO and BE in all water samples at concentrations ranging from 27.5 to 81.7 ng.mL<sup>-1</sup> (for COC) and 0.59 to 2.89 ng.mL<sup>-1</sup> (for BE). These data showed that the region of the SSOS suffers from the continuous discharge of effluents contaminated with illicit drugs, which can lead to contamination of seafood, as well as adverse effects on non-target organisms. Thus, monitoring the occurrence and effects of illicit drugs in the marine ecosystems may provide important information about the sources, behavior and fate of these substances in aquatic ecosystems, as well as providing subsidies for environmental legislation.

### 89 Toxicological evaluation of cocaine by-product (crack cocaine): energy status and neuroendocrine effects in marine mussels *Perna perna*.

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The increasing use of illicit drugs not only produces public health problems, but also elucidates the potential for environmental impacts. Brazil is the main crack market and the second for cocaine in the world. The main destination of these substances and metabolites in coastal zones is the marine environment through wastewater and sewage outfalls. There is a lack of studies about the ecotoxicology of illicit drugs on marine environment. Mussels play an important role in biomonitoring programs for the water quality worldwide. To predict possible adverse effects on energy and neuroendocrine status on marine biota, mussels *Perna perna* (n=160) were exposed for 7-days to cocaine by-product (crack cocaine)-spiked water under laboratory conditions. Two controls (marine water – control, and solvent control - DMSO 0.001% v/v) and three crack cocaine concentrations were tested, included the underlined environmental concentrations found in the Santos Bay (São Paulo, Brazil): 0.5, 5 and 50 µg.L<sup>-1</sup>. Biochemical markers were observed in mussels' gonads after 48, 96 and 168 hours of exposure: cellular energy status (total lipids content – TLP; and mitochondrial electron transport activity - MET), metabolism of monoamines (monoamine oxidase activity - MAO) and inflammation properties (cyclooxygenase activity - COX). After 168 hours of exposure, mussels exposed to the concentration of 50 µg.L<sup>-1</sup> showed a significant increase of total lipids (TLP) and the activity of MET in the gonads compared to the solvent control (p < 0.05). Cyclooxygenase activity (COX) was significantly lower than the control in mussels exposed to 96 hours at the concentration of 5 µg.L<sup>-1</sup> (p < 0.05). Cocaine by-product affected *P. perna* physiology and health. As filter feeding bivalves of great economic and ecological relevance, adverse effects on mussels' *P. perna* can culminate in ecosystem perturbations. This study emphasizes the importance of selecting a battery of biomarkers that are potential monitors for stress on marine organisms, related to mode of action of drugs with possible profound effects on organism survival, inflammation process and reproduction.

### Discussing our physiological, biochemical or molecular studies on aquatic organisms exposed to environmental pollutants to improve the quality and the ecotoxicological sound of our research - Part 2

#### 90 Analysis of the Antioxidant System and Neurotoxic Effects in *Rana catesbeiana* exposed to water from an urbanized stream in southern Brazil

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The aquatic natural resources receive constant discharge of several chemical compounds derived from the agricultural, industrial and pharmaceutical activities. Biomarkers can be used as instruments to understand how these compounds affect the environment and aquatic organisms. The frog *Rana catesbeiana* is a specie that lives for a long period of time and is quite susceptible to chemical substances present in the water. The aim of this work was to evaluate neurotoxic and antioxidant system alterations in *Rana catesbeiana* tadpoles exposed to waters of the Rio Cascavel, Paraná. A total of 190 L of water was collected in three different river sites, at the source (24°56'924 "S and 53°26" 215 "W), urbanized area (24 ° 59'167" S and 53°26'137 "W) and rural area (24°59'50.16 "S and 53°26'8.22" W). The tadpoles spent 7 days at acclimatation, then, 48 tadpoles were distributed in 4 aquariaums, 3 of them filled with collected water and 1 was the control group, filled with non-chlorinated water. Passed 7 days of treatment the liver was removed and homogenized with Tris-HCl buffer pH 7.4, centrifuged at 12,000g, the supernatant was removed and frozen at -20 ° C. The samples were used for analysis of Cholinesterase (ChE), Superoxide

Dismutase (SOD), Catalase (CAT) and Lipoperoxidation (LPO). The data were analyzed by ANOVA one-way, followed by the Tukey-HSD post-hoc test ( $\alpha = 0.05$ ). It was not observed the neurotoxic effect of ChE activity, however, a slight increase in SOD activity and elevation of CAT activity in urban and rural environments. There was a decrease in the LPO reaction, especially among tadpoles exposed to rural water. The increase of CAT and decrease in LPO points to the possible presence of xenobiotic agents present in the urban and rural regions. Xenobiotics can cause tissue, cellular and even genetic damage, lead by the activity of the oxygen reactive species, the animals affected by them can suffer an array of metabolic malfunctions, interfering in their survival capacity, which may impair their growth, development and reproduction, reducing individual and population fitness.

#### 91 Effects of 17 $\alpha$ -ethynylestradiol on the testicular structure of two neotropical fish species.

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The effects of estrogenic compounds in surface waters originated from human activities can affect not only human health but also aquatic wildlife through different mechanisms such as sex inversion, induction of intersex individuals, prevention of gonadal maturation and alterations in sexual behavior. In addition, relatively high concentrations some estrogenic compounds like estradiol ( $E_2$ ) and 17 $\alpha$ -ethynylestradiol ( $EE_2$ ), have been reported in surface waters of Brazil and Argentina. Therefore, the objective of this work was to evaluate the effects of environmentally relevant concentrations of  $EE_2$  on differentiated testicles of two native species, *Cnesterodon decemmaculatus* (Cyprinodontiformes), and *Odontesthes bonariensis* (Atheriniformes), with a similar distribution in Argentina, Uruguay and the South of Brazil. In the case of *C. decemmaculatus*, adult males were exposed to concentrations of  $EE_2$  ranging from 20 to 200 ng/L during 8, 12 and 16 weeks and intersex gonads (testis-ova) were observed after 8 and 16 weeks of fish exposed to fish exposed to 200 ng  $EE_2$ /L and 100 ng  $EE_2$ /L, respectively, with no alterations in the morphology of the gonopodium. In the case of *O. bonariensis*, juvenile male pejerrey were exposed to 15 ng  $EE_2$ /L for 20 days using a static exposition system. Gene expression of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (*hsd11b2*), the enzyme involved in testicular differentiation, was significantly down regulated. On the other hand, the expression of gonadal aromatase (*cyp19a1a*), the enzyme involved in ovarian differentiation, was significantly up regulated in testes of exposed fish. The histological analysis of the testes showed that, in the treated group, the caudal portion of the gonad exhibited typical characteristics of the ovarian differentiation process of pejerrey larvae. Even though sex inversion or intersex occurrence has not been reported yet in pejerrey, it is likely that the caudal portion remains susceptible to the action of xenoestrogens in sexually immature pejerrey and could impair the reproductive capacity of those individuals. In summary, the present results showed that  $EE_2$  exposure effects vary according to the species and the life stage at environmentally relevant concentrations of different local fish species. Then it would be feasible that presence of estrogens in south American surface waters would be able to disrupt sexual development or impair the reproductive capacity of fish populations in different ways.

#### 92 Biological responses of *Colossoma macropomum* (Cuvier, 1818) contaminated by naphthalene and subsequently exposed to hypoxia

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Variations in dissolved oxygen concentration in the water occur daily and seasonally in Amazonian freshwater ecosystems and, can magnify the effects of aquatic contamination. In this context, the main goal of the present study was to evaluate the physiological, biochemical, genotoxic, histopathological and genetic responses of *Colossoma macropomum* after naphthalene contamination and subsequent hypoxia exposure. Firstly, we observed the effect of naphthalene on the metabolic rate at different oxygen tension to determine the critical oxygen tension ( $PO_2$  crit). Thus, tambaqui was intraperitoneally injected with 50 mg/kg of naphthalene, using corn oil as the vehicle solution. Fish were kept for 96 hours in glass tanks and then transferred to respirometry chambers, and then submitted to progressive hypoxia, and the  $PO_2$  crit was determined. In the second experiment, fish received an intraperitoneal injection of naphthalene (50 mg/kg) and were maintained for 96 hours under constant aeration ( $6.0 \text{ mg O}_2 \cdot \text{L}^{-1} \pm 0.25$ ), subsequently subjected to 6 hours of hypoxia ( $1.08 \text{ mg O}_2 \cdot \text{L}^{-1} \pm 0.10$ ). The results revealed that the injection of naphthalene did not affect oxygen

consumption and  $PO_2$  crit in *C. macropomum*. Moreover, only hypoxia modulated hematological parameters and plasma glucose. Instead, naphthalene contamination reduced the activity of biotransformation (GST) and antioxidant (CAT) enzymes in normoxic conditions, but in contaminated fish exposed to hypoxia, there was an increase in both enzymes activities. There was no lipid peroxidation (LPO). As regard as genotoxicity, the naphthalene injected animals presented genotoxic damages with no differences between hypoxia and normoxia groups. Instead, most liver lesions were moderate, but some were accentuated by further exposure to hypoxia, mainly focal necrosis. Naphthalene and hypoxia presented opposite effects on the transcription of the tumor suppressor gene TP53. We concluded that the responses of *C. macropomum* to naphthalene were magnified by hypoxia exposure mainly regarding liver damage; also, hypoxia impaired the transcription of TP53 in fish contaminated with naphthalene, demonstrating the importance of hypoxia as a risk factor for aquatic contamination, especially at Amazon region.

#### 93 Molecular Pathways Altered in Male Tilapia After Subchronic Low Benzo(a)pyrene Exposure

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Benzo[a]pyrene (BaP) is an important lipophilic environmental contaminant and is widely dispersed in the environment. BaP contamination in aquatic systems is of great concern particularly in organisms of economic impact due to its carcinogenicity and adverse effects on the survival, development and reproduction in fish. The objective of this study was to gain a better insight into the mechanism of BaP toxicity in a non-model organism of economic impact, i.e., tilapia. We used high-throughput RNA-Seq to analyze whole genome gene expression in the liver and testis of male tilapia following repeated intraperitoneal (i.p.) injection of a low dose of BaP (3 mg/kg every 6 days) for 26 days. In addition, the morphometric endpoints were examined as determinants of general health. After 26 days, the tilapia were sacrificed, and the liver and testis samples were collected for RNA-Seq analysis. The morphometric endpoints including factor condition, hepatosomatic index and gonadosomatic index were significantly decreased after BaP treatment. BaP exposure affected the expression of 1444 genes (607 up and 837 down-regulated) and 309 genes (167 up and 142 down-regulated) in the liver and testis, respectively ( $p$ -value  $< 0.05$ ; fold change  $> \pm 1.5$ ). These genes were involved in many biological processes such as detoxification, enzymes/oxidative stress, checkpoint pathways, and metabolism in the liver. In testis, genes regarding signaling transduction, immune system, and metabolism were altered. Pathway analysis using Gene Set Enrichment Analysis (GSEA) indicated that in both tissues, the metabolic pathways and cell signaling pathways were altered, i.e., heme oxidation and insulin action. In the liver, specific pathways were affected related to diseases, i.e., thyroid hormones in adipose tissue metabolism. The Gene ontology (GO) analysis revealed potential effects on lipid hydrolysis and phospholipid metabolism process in the liver. In testis, GSEA determined that Intermediate filament polymerization and glycolysis were affected. The GO categories were related to signal transduction and inflammatory response. Recently, it has been reported that disruption in metabolic pathways has a role in carcinogenesis. Therefore, it is suggested that activation of these pathways and the others related to signaling pathways, together with depleted morphometric endpoints, provide new insights to the mechanism of action of chronic exposure to low dose BaP in male tilapia.

#### 94 Molecular and biochemical responses of biotransformation systems in oysters *Crassostrea brasiliana* (Lamarck, 1819) exposed to pyrene and fluorine

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Polycyclic aromatic hydrocarbons (PAHs) comprise a class of highly distributed organic pollutants in aquatic environments. Exposure to PAHs pose risks to animal



health risks, since can be carcinogenic, mutagenic and/or teratogenic. Upon entering the cell, its metabolism occurs through the activation of biotransformation reactions. In order to evaluate the molecular responses and biotransformation enzymes in Oysters *Crassostrea brasiliana* were exposed to two concentrations of pyrene (50 and 100 mg. L<sup>-1</sup>) and fluorine (100 and 200 mg. L<sup>-1</sup>), for 12 and 96 h. Half-life of these compounds in water were quantified by fluorescence and chemical analyses were carried out to check the concentration of the PAHs in the soft tissue of oysters. Transcription levels of genes of biotransformation of phase I (*CYP1-like*; *CYP2-like*; *CYP2A1* and *CYP356A1-like*) and phase II (*GST W-like*; *Gstm-like* and *SULT-like*) and EROD activity, GST and GSTm were evaluated in the gills. Both PAHs were bioaccumulated by oysters. The half-life of the pyrene in water was (100 mg.L<sup>-1</sup> = 2 h and 12 min), lower than that of fluorine (100 m g.L<sup>-1</sup> = 5h and 54 min). This may be related to greater lipophilicity of pyrene, facilitating your entry in the intracellular medium through the plasma membrane. After exposure to fluorine, there was only an increase in the level of gene transcripts in *CYP2A1* (200 mg.L<sup>-1</sup>, 96 h). In oysters exposed to pyrene, there were increased levels of transcripts of *CYP2A1* (24 and 96 h); *GST W-like* (24 and 96 h) and *SULT-like* (24h) in 50 mg.L<sup>-1</sup> and in all genes assessed in 100 mg.L<sup>-1</sup> 24h exposed group. In addition there was an increase of EROD activity and GSTm (96 h), suggesting a significant participation of enzymes and genes related to metabolism of biotransformation of phases I and II of the pyrene. The results contribute in the search for biomarkers of contamination by PAHs in *C. brasiliana* and show a possible participation of these genes and enzymes in the metabolism of biotransformation of pyrene. In addition, suggest the participation of the *CYP2A1* gene in the PAHs Biotransformation in gills of *C. brasiliana*.

#### 95 The transcriptome of the brown mussel *Perna perna* when exposed to anthracene

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The brown mussel *Perna perna* (Linnaeus, 1758) helps the monitoring of chemical compounds in marine ecosystems. However its molecular mechanisms of detoxification and stress response remain unclear. Elucidating these mechanisms is crucial to understand the toxic effects of chemical pollutants and to develop biomarkers to assess marine ecosystems. In this study, *P. perna* individuals were exposed to anthracene (ANT) and its mRNA complement was sampled sequenced with Illumina technology. Chemical analysis of the soft tissue identified ANT concentrations 268 - 715 fold higher in the exposed group compared to controls, demonstrating that the exposure procedure was successfully accomplished. Transcriptome sequencing of *P. perna* generated 273.152.390 paired reads that were assembled in 231.728 contigs of average length 720 bp and N50 1083 bp, which 66.541 contigs (28,7%) could be annotated using GenBank genes, Pfam domains, Gene Ontology (GO) terms and KEGG pathways. The terms "oxidation-reduction process" and "binding" were the most abundant terms in biological process and molecular functions GO categories, respectively. In KEGG pathways, "Signal transduction" in "Environmental Information Processing" was the pathway with the most number of predicted proteins assigned. It was possible to identify transcripts similar to genes related with biotransformation reactions of phases I, II and III, including CYPs and GSTs. Transcripts similar to CYPs and GSTs isoforms were highly expressed in the group exposed to ANT, however no CYP, GST, or even other genes related with biotransformation reactions were classified as differentially expressed. On the other hand, several hypothetical genes were differentially expressed, which suggests that *P. perna* uses unknown mechanisms of biotransformation to deal with ANT stress contamination. Immune related-genes were both up and down-regulated, as was also observed for *Perna viridis* exposed to benzo(a)pyrene, suggesting that ANT promotes alteration in the immune response of *P. perna*. A qPCR validation is being carried out to verify results here described. <sup>1</sup>

#### 96 Cytogenotoxic effects of a cocaine byproduct (crack cocaine) to marine mussel *Perna perna*.

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The cocaine and its byproducts have grown into epidemics in metropolitan regions, becoming a social and environmental problem in Brazil. Their occurrences have been reported in domestic effluents, rivers and coastal waters. Our study aims to evaluate the cytogenotoxicity of the crack cocaine in the marine mussel *Perna perna*, considered a sentinel organism in programs of environmental monitoring. Adult organisms (n=160) were exposed to two controls (marine water and DMSO 0.001% v/v) and to three concentrations of crack cocaine: 0.5 µg.L<sup>-1</sup> (environmental concentration), 5 µg.L<sup>-1</sup> and 50 µg.L<sup>-1</sup>, for seven days. Gills and digestive glands were collected after 48, 96 and 168 hours of exposure. Biochemical responses related to the xenobiotics metabolism were determined through biomarkers, including Phase I and II, antioxidant defenses and subcellular effects. After 96h of exposure, the lysosomal membrane stability decreased to all crack cocaine concentrations (p<0.05). In gills, GST activity raised after 48h of exposure to 5 and 50 µg.L<sup>-1</sup>, and after 168h this activity was significantly higher in organisms exposed to 5 and 50 µg.L<sup>-1</sup> when compared to controls (p<0.05). In digestive glands, GST activity decreased after 96h of exposure to 50 µg.L<sup>-1</sup> when compared to controls (p<0.05). GPX activity increased in gills after 48h of exposure to 5 µg.L<sup>-1</sup> when compared to controls (p<0.05). It was also observed significant increase of lipid peroxidation and DNA damage in gills after 96h exposed to 5 µg.L<sup>-1</sup>. In digestive glands, it was detected a significant increase in DNA damage after 96h in organisms exposed to 5 µg.L<sup>-1</sup> when compared to DMSO control (p<0.05). The results bring evidences that exposure to crack cocaine is able to generate oxidative stress and cytogenotoxic effects in gills of *Perna perna*, which proved to be a reliable model to assess environmental risk of cocaine in marine ecosystems.

#### 97 Lysosomal stability in oysters *Crassostrea* sp. from three different populations from the coast of São Paulo, Brazil

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Coastal zones continuously receive inputs of contaminants, which greatly affect its quality. Biological effects resulting from environmental pollution are complex and have not been properly estimated by chemical analysis and toxicity tests. A reliable form to assess the environmental quality consists of analyzing organisms exposed to constant, complex and diffuse sources of contamination. The appropriate use of biomarkers in sentinel organisms may provide an estimate of the potential risk associated with contamination. This study aims to evaluate if different levels of potential contamination affect differently the stress responses in oysters from three sites along the coast of São Paulo state. The physiological condition of oysters *Crassostrea* sp. from Cananéia (reference site), Santos and Bertioga was studied by analyzing the lysosomal membrane stability in haemocytes, measured using the neutral red retention time (NRRT) assay. Adult organisms were collected in spring (August, September, and October/2016) and autumn (April and May/2017). During the spring, the times of retention of the neutral red dye were similar in oysters from different sites and the reference area (p > 0.05). Interestingly, an uncommon red tide occurred along the coast of the state mainly affecting Cananéia, which may have influenced the reduction of the NRRT for oysters from this region. Such biological events (algal blooms) are more likely to occur at this time of year due to the typical climatic conditions, and may have the potential to even the adverse effects of pollution on distinct bivalve populations subject to different levels of contamination. During the autumn campaign, organisms from both contaminated sites (Santos and Bertioga) showed significant reduction in the mean NRRT relative to the reference site (p < 0.05). A previous study in the same region (Catharino *et al.*, 2015) observed that in both seasons the oysters from Cananéia had a higher NRRT than the oysters from the other two sites. Overall, the NRRT were lower in the present study. These results may be due to the exposure of the organisms to pollutants, since lysosomes are organelles that absorb a wide variety of organic and inorganic substances. However, further investigations are required and being carried out to confirm this hypothesis.

#### Reliable analytical data in environmental studies: sample treatment and analytical determination issues

#### 98 Innovative methods for the determination of PPCPs in drinking water treatment sludge

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In recent years, sample preparation techniques have been modified with the aim of developing miniaturized, fast and easy methods with lower solvent consumption. Alternative and/or renewable materials have been used in order to obtain cheaper and greener techniques. Once pesticides, pharmaceuticals and personal care products (PPCPs) are usually at trace levels in complex matrices, sample preparation is a crucial step. Extraction of organic compounds from complex matrices, as environmental samples, usually require a clean up step during the sample preparation, since at this stage many interferences are eliminated, providing better selectivity during the analysis and reducing the effects from the matrix co-extractives. Thus, the aim of this work is to present the use of innovative materials employing the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe), matrix solid phase dispersion (MSPD) and Liquid Chromatography coupled tandem Mass Spectrometry (LC-MS/MS) for the determination of pesticides and PPCPs from drinking water treatment sludge (DWTS). Acknowledgment: CNPq, CAPES, FINEP e FAPERGS. References: Cerqueira, M. B., Caldas, S. S., & Primel, E. G. 2014. New sorbent in the dispersive solid phase extraction step of quick, easy, cheap, effective, rugged, and safe for the extraction of organic contaminants in drinking water treatment sludge. *J. Chromatogr. A*, 1336, 10-22. Cerqueira, M. B. R., Guilherme, J. R., Caldas, S. S., et al. 2014. Evaluation of the QuEChERS method for the extraction of pharmaceuticals and personal care products from drinking-water treatment sludge with determination by UPLC-ESI-MS/MS. *Chemosphere*, 10, 74-82. Soares, K.L., Cerqueira, M.B.R., Caldas, S.S., Primel, E.G., 2017. Evaluation of alternative environmentally friendly matrix solid phase dispersion solid supports for the simultaneous extraction of 15 pesticides of different chemical classes from drinking water treatment sludge. *Chemosphere*, 182, 547-554.

#### 99 Obtaining certified reference material: organic substances in biota

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Use of certified reference material (CRM) to assess performance of analytical methods is one of the requirements of the Water Framework Directive among the member states of the European Community. Although, the number of CRMs available is limited in relation to the number of organic substances that should be prioritized in monitoring programs of water and biota quality. Considering the complexity of the process to obtain a CRM and the lack of knowledge by the majority of professionals about it, mainly researchers and students involved in environmental analyses, this work aims to discuss the steps for obtaining a CRMs regarding matrix biota, from the project proposal to the post-certification stability study. The limited number of CRMs for biota is mainly due to the laborious process of obtaining a material naturally contaminated with the analytes of interest. Contaminated parts of fish or other organism are mixed with other less or uncontaminated to achieve the desired levels. Once the base material is obtained, a study is required to obtain a homogeneous and stable material, as well as conditions of storage and transport that will guarantee the stability of the matrix and the analytes. The main objective is to obtain a homogeneous material with characteristics of a fresh material so that it will be as close as possible to the samples to be analyzed. Studies evaluating short- and long-term stability are performed considering reference conditions, which shall not result in any modification of the matrix or analytes, and critical conditions that provide information about transport and storage conditions at extreme temperatures. After certification, obtained by an interlaboratory campaign applying different methods of analysis and careful quality control, the materials are submitted to post-certification stability studies to ensure that the matrix and analytes are stable and the levels and uncertainties are acceptable.

#### 100 Determination of organic contaminants in marine organisms: quality control and bioavailability prediction

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The aquatic environment is continuously exposed to the input of organic contaminants originating from anthropogenic sources, such as urban and industrial effluents, which can impact biological organisms even at very low concentrations (e.g. part per trillion). The potential risks of these contaminants can be strongly influenced by bioavailability as well as contamination level and exposure way and time. Marine organisms can be exposed to these contaminants through sediment, the water column and their diet and the accumulation/uptake can occur through active and/or passive mechanisms. The major issue in determination of these contaminants in biological tissues involves mainly the complexity of matrix. A great variety of techniques are applied and the determination must to consider the correct selection of target tissue directly related with the contaminant exposure and bioavailability. In terms of bioaccessability

prediction, chemical methods have been applied simultaneously to bioassays and can provide the rate of exposure depending on the contaminant source. Quality control in analytical determination must also to be considered once pronounced matrix effect mainly in recurrence of high lipid content in samples can be observed. The use of analytical control in measurements as well as the development of robust methods for analysis and monitoring can help to understand the impact of these chemicals on organisms and the risks to aquatic communities.

#### 101 Determinação de HPAs em *Spartina alterniflora*: aspectos analíticos ambientais

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*Spartina alterniflora* é uma espécie extensamente estudada por possuir conhecida ação fíto remediadora de contaminantes orgânicos e metais, além de ser uma espécie bastante resistente a variações sazonais sendo largamente encontradas nos mangues, não somente no Brasil como em outros países. Amostras de *Spartina alterniflora* foram utilizadas para a otimização e validação do método de extração com a fortificação em 12 concentrações em duplicata ou triplicata, onde as concentrações variavam de 15 a 1300 ng/mL. O método validado apresentou valores de coeficiente de variação menor de 20% e recuperações dentro da faixa de 70 a 120%. Os valores de limites do método são LD de 2,9 a 14,8 ng/g, LQ de 15 a 20 ng/g e faixa de trabalho de 15/20 à 1300 ng/g. O método consiste em 1,0 g de amostra liofilizada, extração por duas vezes com 20 mL de n-hexano em banho de ultrassom com a temperatura monitorada entre 35-40 °C por 30 minutos. Foram utilizados também uma mistura de compostos deuterados como surrogate contendo Acenafene-D10, Criseno-D12, Naftaleno-D8, Perileno-D10 e Fenantreno D10 e 1,1-bifenil-2-fluor como padrão interno nas respectivas concentrações, 40 e 3 ng/mL. O método otimizado foi aplicado em amostras de *Spartina* na parte aérea de folhas e caule e na parte em contato com o sedimento, raízes e foi observado que há uma grande diferença nas concentrações sendo muito maior nas raízes o que já foi reportado por WANG et al., 2012, que afirmou que as concentrações dos HPAs na espécie *Spartina alterniflora*, quando analisadas individualmente as partes, pode-se observar que há uma diminuição da concentração no sentido raiz, caule e folhas, o inverso do que foi encontrado para outros gêneros de espécies de gramíneas, *phragmites* e *scirpus*. As concentrações encontradas para as folhas e caule foram de 22 a 53 ng/g e nas raízes de 38 a 123 ng/g. No estudo em desenvolvimento pretende-se efetuar testes biológicos em sedimento associado a *Spartina alterniflora*, para avaliar sua eficiência na detoxificação deste compartimento.

Wang, Z. et al. Distribution of PAHs in tissues of wetland plants and surrounding sediments in the Chongming wetland, Shangai, China. *Chemosphere*. V. 89., p. 221-227. 2012.

#### 102 Developing and validation of a quantification method to analgesic compounds in surface waters from Paraíba do Sul River

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In the last three decades there has been an increasing concern about the environmental occurrence and impact of pharmaceuticals which are represented by a large group of emerging organic micro - pollutants. In addition, they are often poorly removed by conventional wastewater treatment techniques, and behave as (pseudo-) persistent contaminants in the environment. The goal of this work is to develop and validate a SPE-LC-MS/MS based on a method to analyse five analgesic substances more caffeine on the surface waters, more specifically at Paraíba River, one of the most important rivers from Brazil. The selected drugs were diclofenac, ibuprofen, naproxen and salicylic acid which are analysed at negative mode, and paracetamol, which was analysed at positive mode as such as caffeine. The first step was to fit the ionization source parameters - voltage, temperature, nebulizer pressure and drying gas flow - with the better mobile phase additive/organic modifier. The better conditions ionization source were 3000 V, 300°C, 50 psi and 13 L.min<sup>-1</sup> with acetic acid 0.04% / acetonitrile for negative mode and ammonium hydroxide 0.01% / metanol for positive mode. All analytes showed linearity (R<sup>2</sup>>0,99) within interval of 50 -500 ng.L<sup>-1</sup>. The SPE conditions were optimized testing two different cartridge: Oasis HLB (which show hydrophobic - lipophilic interactions) and Strata X-A (based on anion exchange mechanisms) and evaluating recovery rate of a ultrapure water sample spiked with the analytes. Ibuprofen and naproxen had a good recovery at Strata X-A (> 80%), a

similar value for caffeine and paracetamol at OASIS HLB. Ibuprofen and Naproxen showed good recovery at Oasis HLB, when a 5mg/L EDTA solution was added in the sample. Salicylic acid showed a poor recovery at Oasis HLB (40%)

### 103 Fast sample preparation method for PBDEs, PCBs, and PAHs in human breast milk for analysis by GC-MS/MS

A.C. Torres-Moreno, L. Puente-de la Cruz, University of Cartagena / Environmental chemistry group; B. Johnson-Restrepo, University of Cartagena / Environmental Chemistry Research Group

Persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) are ubiquitous in the environment. Therefore, humans are exposed to these chemicals generating a risk to toxic effects including endocrine disruption (PCBs and PBDEs) and carcinogenicity (PAHs). The aim of the project was to develop and validate an analytical methodology to determine in human breast milk: PCBs (33 congeners), PBDEs (26 congeners), and PAHs (22 compounds) by using a Gas Chromatography couple to Tandem Mass Spectrometry (GC-MS/MS). The sample treatment procedure was based on a modified version of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach in addition to two cleanup steps, one with KOH in methanol to remove lipids and the other with Solid Phase Extraction (SPE). Homogenized breast milk samples (20 ml) was placed into 50 µl of a polypropylene tubes, after the addition of Ethyl Acetate, and spiked with internal standard of <sup>13</sup>C-isotope label standard (<sup>13</sup>C-PBDEs and <sup>13</sup>C-PCBs) and deuterated label standard (<sup>2</sup>H-PAHs), were vortex for 1 min. HPLC grade water was used instead of sample (20 ml) for the blank procedures. Organic phase separation was induced by addition of NaCl (2 g) and MgSO<sub>4</sub> (6 g), the samples were vortex for 1 min, and centrifuged (4500 rpm at 5 min). Extracts were transferred to two vials, 1 ml for gravimetric lipid content and the other 15 ml for cleanup. For the cleanup steps, the extracts were treated with KOH in methanol (saponification for 30 min at 60 °C), centrifuged (2500 rpm at 5 min), and the organic phase was extracted with toluene (3 times with 5 ml). Toluene extracts were concentrated under N<sub>2</sub> gentle stream to 2 ml, passed through Solid Phase Extraction (SPE) column packed with Florisil (1 g), Na<sub>2</sub>SO<sub>4</sub> (1 g), and eluted with 10 ml of dichloromethane/n-hexane (1:3). Fractions were concentrated under N<sub>2</sub> gentle stream to 100 µl and transfer to a vial containing inserts for analysis on GC-MS/MS, TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS – Thermo Scientific). The matrix-spiked absolute recoveries of the entire analytical procedure: extraction, cleanup, and SPE steps were > 90% in all cases. Validation of the method included limit of detection (< 1 ppb), limit of quantification (< 2ppb), linear range (5 – 500 ppb), and linearity of calibration curve (R > 0.995).

### 104 Pesticide metabolites in fish by Clean-up application, solid-liquid extraction and GC-MS analysis for human dietary risk at Ceará coast, Brazil

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The risk to human health and environment due to overuse of pesticides has been increasingly recognized, together with the interest in food quality. Fish are an important food source on which the bioaccumulation of pesticides and their metabolites threatens all consumers. This study investigated the occurrence of pesticide metabolites in catfish from the estuaries of Ceará (ECR), Pacoti (EPR) and Jaguaribe (EJR) rivers, Ceará, Brazil, using gas chromatography-mass spectrometry (GC-MS), and calculated Estimated Daily Intake (EDI) and Risk Quotient (RQ) to assess human health risk due to these fish consumption by local population. Catfish were collected on April/May 2016 at ECR (n=11), EPR (n=10) and EJR (n=7) by fishermen, under ICMBio license 51308-1. Metabolites analyzed were: Chlorpyrifos methyl (Chlorp-M), S-metolachlor (S-Metola), 1-Hydroxypyrene (1-OHP), Malaoxon (Malaox), 2-Chloro-4,6-diamino-1,3,5-triazine (2-CAAT), Carbofuran phenol (CarbPhenol) and 3-Phenoxybenzoic acid (3-PBA). Muscle samples were lyophilized, homogenized and submitted to solid-liquid extraction and *clean up* processes. Method performance was validated by linearity (R<sup>2</sup>: 0.9815 - 0.9957), sensibility, limits of detection (LOD: 0.33 – 35.99 mg mL<sup>-1</sup>) and quantification (LOQ: 1.10 – 119.95 mg mL<sup>-1</sup>), precision (0.20 – 13.47 %) and recovery (45.47 – 60 %), and was satisfactory for detection of all compounds except 1-OHP. Only three were detected in catfish: 3-PBA [3.06 ± 3.88 (EJR) - 7.43 ± 3.38 ngg<sup>-1</sup> (ECR)], CarbPhenol [6.04 ± 0.0 (ECR, EJR) - 6.68 ± 0.9 ngg<sup>-1</sup> (EPR)] and Malaox [20.67 ± 0.04 (EJR) - 24.29 ± 3.47 ngg<sup>-1</sup> (ECR)] and didn't differ between estuaries, despite the diversified pollution, and Malaox contents were significantly higher (p < 0.001) for the three estuaries. EDI (mg day person<sup>-1</sup>) calculated were 0.002 – 0.004 (3-PBA), 0.003 (CarbPhenol) and 0.010 – 0.012 (Malaox), but RQ, based on the parental compounds values, was low for 3-PBA (0.03 – 0.07) and Malaox (0.03 – 0.04), and high for CarbPhenol (1.49 – 1.65).

Results represent an alert to the high possibility of risks to human health due to the use of pesticides in Brazil, mainly Carbofuran. Besides, the specie *Sciades parkeri*, found in ECR and EJR, is currently classified as Vulnerable. Legal actions for protection and management of tropical estuaries must be performed with strict force, since the conservation of these environments is the fundamental way of ensuring species preservation and ecological relationships balance.

### 105 Photocatalytic degradation of triclosan: toxicity of byproducts on microalgae *Kirchneriella lunaris* and *Scenedesmus acuminatus*

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Triclosan (TCS) is an antimicrobial widely used in various consumer healthcare products, soaps, and some plastic formulations. Due to the broad use a small portion of TCS has been found in various environmental matrices such as water and sediments. Several studies have shown that the sunlight irradiation of TCS via direct or indirect photoreactions produce dichlorodibenzo-*p*-dioxins which represented the major degradation products in aquatic environment. Therefore, understanding of potential effects on aquatic organisms is so important. The algal toxicity tests are relatively simple and inexpensive method to evaluate the toxicity of chemicals. In this work the toxicity of TCS under heterogeneous photocatalysis using UV/TiO<sub>2</sub> was assessed by inhibition of cell growth of microalgae *Kirchneriella lunaris* and *Scenedesmus acuminatus*. The bioassays were conducted in three different groups. The experiment 1 comprised the Conway medium (control), and the experiment 2 carried out in the Conway + TCS, and the test 3 in the Conway + TCS + UV/TiO<sub>2</sub>. All tests contained initial inoculum concentration of 20,000 cells/mL in the test tubes and nominal test concentrations of TCS were 0 to 5.0 mg/L. Algae cells were counted daily and the inhibitory concentration could be estimated after 21 days by measuring the direct fluorescence. The results in the experiment 2 showed significant reduction of 53-62% on both microalgae cell growth from 0.5 mg/L whereas 100% exhibited significant inhibition at 1.0 mg/L. In the test 3 after 60 min of UV/TiO<sub>2</sub> irradiation was not found to have significant effects on any of the algae and the average inhibition was 1.75%. These results indicate that the process applied has not generates toxic byproducts to the microalgae *Kirchneriella lunaris* and *Scenedesmus acuminatus*.

### 106 The expression of uncertainty of measurement in toxicity tests results

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Acute and chronic toxicity testing with effluents carried by Aquatic Ecotoxicology laboratory of CETESB (Environmental Agency of São Paulo State) are accredited to ABNT-NBR ISO/IEC 17025 (equivalent national standard for ISO/IEC 17025:2005), which requires the estimative of the uncertainty of measurement associated with numerical results. As these analyses include living organisms, with variable individual sensitivity, the application of rigorous statistical procedures to estimate this uncertainty is troublesome and questionable. To compare some alternative approaches currently proposed for the evaluation of uncertainty related to ecotoxicological assays, an exercise was carried out with *Daphnia similis* data. These additional methods comply with EURACHEM/CITAC principles and include proficiency studies and interlaboratory reproducibility. Each of these two procedures resulted in distinct uncertainty values and confirmed that the total variance of biological response is controlled by the sample characteristics and concentrations analysed, as mentioned by other authors. Beside that, it is required the uncertainty of toxicity tests should be established for each sample through duplicate analyses, which would be an expensive and hard work approach. In view of such limitations, the laboratory decided to follow the CAEAL procedure (Canada, that meet the requirements of ISO/IEC 17025) and report the 95% confidence limits generated by specific softwares for each endpoint and particular test. Such confidence interval has the probability of containing the true value around the point estimate (LC, EC or IC), describing how reliable is the estimative as its width reflects the biological variability, the experimental design and the test performance.

### Current situation and research needs for ecotoxicological assessment of pesticides in aquatic and terrestrial ecosystems

#### 107 Research needs to improve ecological risk assessment in Brazil

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OESTE; A. Waichman, UFAM; R. Oliveira, University of Brasilia / Department of Genetics and Morphology; O. Malaspina, UNESP Universidade Estadual Paulista Júlio de Mesquita Filho / Departamento De Biologia, Centro de Estudos de Insetos Sociais; R. Nocelli, Ciências Biológicas / Departamento de Ciências da Natureza Matemática e Educação; A. Tornisielo, BASF SA / GENCS - E-Fate; A.P. Cione, Syngenta Crop Protection, Inc. / Environmental Safety; A. Shiwa, DuPont Co; L. Murakami, Bayer AG Crop Science Division; X. Patino, Bayer CropScience; A. Ferraz, G. Guimarães, ANDEF

Since 2010 there is an effort to implement the risk evaluation of pesticides at Brazilian Institute for the Environment and Renewable Natural Resources – Ibama. Currently, only new active ingredients and active ingredients submitted to the process of reevaluation are evaluated considering procedures of risk assessment of pesticides. In 2015 Ibama created two Working Groups to discuss and develop clear and scientifically-based schemes of risk assessment to pollinators and aquatic organisms to be adopted in Brazil. These groups are coordinated by Ibama and are composed of members from government, academia and industry. At first, general and specific protection goals for maintenance of ecosystem services related to insect pollinators and to aquatic organisms were established. However, there are gaps in knowledge and research needs to ensure that procedures related to pesticides registry will be able to provide appropriate protection of crops without unacceptable risks to those non-target organisms. Some of the main gaps are: how native species are sensitive to pesticides, in what extension standard species can be used as good surrogates to protect native species, what are the levels of exposure in Brazilian scenarios and how the exposure routes can be different for the native species. Some of the information that could fill in these gaps could be provided by construction of SSD's curves for native species and pesticides of different modes of action, residue studies in relevant matrices to the non-target organisms, monitoring of water bodies located near agricultural crops, geo-referenced data regarding monitoring of Brazilian soils with description of soil characterization and validation of spray drift models. To provide these information and may be able to improve the process and reduce the uncertainty, it is necessary the generation of data, which only can be provided by basic research. Even facing these uncertainties, Ibama published in february the Normative Instruction 02/2017 that establishes procedures to risk assessment of pesticides to pollinators. The risk assessment process, that is dynamic and based on science, was developed on available information so far. The main intention of disclosure these gaps is to stimulate scientific research related to these topics, considering that Brazilian scientific community could fill and generate data under Brazilian conditions which will subsidizes environmental risk assessment and regulatory decision making.

#### 108 Ecotoxicological impacts of pesticides to soil organisms

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Brazil is the largest consumer of pesticides in the world since 2008. The use of pesticides has increased 288% from 2000 to 2012. According to the Food and Agriculture Organization (FAO), by 2050 the world's population will increase 34% from today reaching 9.1 billion people. About 70% of the world's population will be urban (compared to 49 percent today). In order to feed this larger and urban population, food production (i.e. food used for biofuel, human and animal nutritions – cereals and meat) will have to be a lot improved. Hence, the growing demand for the increase of food production has led producers to seek alternatives to improve productivity using, many times, excessive amounts of pesticides and fertilizers. The effects caused to terrestrial ecosystem by the excessive pesticides and fertilizers applied into soil are the most neglected, especially in tropical and or subtropical countries. Information used to assess the risk of the pesticides use in tropical terrestrial ecosystem are usually taken from data obtained in temperate regions. European data do not always express the reality in tropical regions (i.e different climate conditions and soil properties) and so the results may not be always trusted. To solve this problem, specialists in soil ecotoxicology and ecology in Brazil are strongly influencing Brazilian researchers to run experiments in tropical conditions and use natural soils to evaluate ecotoxicological effects of pesticides to terrestrial organisms. Thus, the main goal of this study is to review data generated in Brazil regarding pesticides effects on terrestrial ecosystems, show some important results and propose further investigations on the theme.

#### 109 Feeding activity of soil fauna after herbicide application: effects of glyphosate formulations measured by bait-lamina test

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Soil fauna plays an important role in regulating nutrient cycling through predation and fragmentation of organic residues. The bait-lamina test was proposed as a practical

method to assess soil faunal feeding activity. This work aimed to evaluate the feeding activity of soil fauna after the application of four commercial glyphosate herbicides, Roundup Original(R), Trop(R), Zapp QI 620(R) e Crucial(R), to desiccate black oat. The recommended dose (720 g ha<sup>-1</sup> acid equivalent, for each product evaluated) was applied for this purpose at the Experimental Farm of Federal University of Santa Catarina (UFSC), in Curitiba, SC, Brazil, in three plots per treatment. As Controls were used three plots without application of herbicides. The bait lamina test (ISO 18311, 2016) consisted of vertically inserting 16-hole-bearing plastic strips filled with 70% cellulose, 25% wheat flour and 5% activated charcoal. The baits were exposed in situ at the 5th day after the herbicide applications, in November 2016, being three groups of eight baits per plot (2 x 2 m), inserted in three plots per treatment. After 40 days of exposure, the percentage of consumption was determined by recording the number of empty holes. Results from treatments were compared to Control using ANOVA followed by Dunnett's test (p< 0.05). The mean consumption in Control was 63.4% (?10.7), Roundup Original(R) was 69.2% (?25.9), Trop(R) was 57.7% (?2.2), Zapp(R) was 68.2% (?13.8), and Crucial(R) was 37.9% (?7.0), being this last one significant lower than the Control (p< 0.05). Among these herbicides, Crucial(R) is the only one belonging to toxicological level I, red label, considered extremely toxic according to Brazil National Health Surveillance Agency (ANVISA). The other belong to level III, blue label, considered moderately toxic. Result indicated significant reduction of feeding activity in plots where Crucial(R) was applied, which can indicate impairment to ecosystem functions such as nutrient cycling. Further studies should be carried out to identify which groups of soil fauna are affected by this product. Funding: AGRISUS and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brasil, Projeto Universal CNPq 454842/2014-7.

#### 110 Do agrochemicals pose a significant threat to biodiversity in sugarcane-producing landscapes?

L.C. Schiesari, University of São Paulo

The environmental benefits of a global adoption of biofuels is critically contingent on which, where and how biofuel feedstocks are produced. Sugarcane is the most energetically efficient first-generation source of ethanol, yielding over eight units of biofuel energy output per-unit energy input when compared with two for beet, wheat, or corn. Brazil responds to 35% of the global production of sugarcane with 9.7 million hectares of cropland expanding over two of the world's biodiversity hotspots – the Atlantic Forest and Cerrados. Nevertheless, there is surprisingly little knowledge on the biodiversity consequences of sugarcane production in general, and of agrochemical management in particular. Sugarcane as a crop is the third largest consumer of pesticides in Brazil, in turn the largest pesticide consumer in the world. By 2010 there were 62 active ingredients registered for the management of sugarcane pests in the country, of which 5 cholinesterase inhibitors, 8 reprotoxic, 37 known, potential or suspected endocrine disruptors and 9 known or probable carcinogens. Of further potential ecotoxicological relevance are inorganic fertilizers and vinasse, a byproduct of sugarcane molasses distillation that is sprayed in sugarcane fields as an organic fertilizer. Pattern analysis indicates large increases in freshwater productivity and moderate levels of freshwater biodiversity (including amphibians and predatory aquatic insects) in sugarcane fields. However, occasional tadpole dieoffs in sugarcane drainage ponds have been witnessed and ammonia concentrations measured in the field, and herbicides applied at doses recommended by the manufacturers, are sufficient to elicit lethal and/or sublethal effects in native amphibian species in prolonged exposure in the lab. Considering that a significant share of pesticide applications occur outside of the rainy season and most published research efforts are biased towards freshwater systems, impacts of land management on terrestrial communities, and on the terrestrial phases of semiaquatic species with complex life cycles may be currently underestimated.

#### 111 A first approach for the prioritization of pesticides for aquatic life protection in Brazil

G. Umbuzeiro, SCHOOL OF TECHNOLOGY -UNICAMP / LEAL; A.F. Albuquerque, University of Campinas / LEAL Laboratory of Ecotoxicology and Environmental Microbiology School of Technology

The process of approval of pesticides in Brazil is still based on hazard evaluation, but there is an effort going on for the implementation of risk assessment in this process. But until we do not have this process fully implemented it is important to provide information on the risk of the pesticides already in use. For that it is necessary to have data on occurrence of pesticides in national waters. But before that, we need to establish which compounds should be included in the monitoring programs. It is not possible to monitor 380 active ingredients approved for use in the country crops. This work presents a preliminary prioritization process based on tonnage of commercialized pesticides, followed by the comparison with the regulated pesticides

in the surface water law (CONAMA N° 357/2005), which are historically the ones that are included in the monitoring program performed by the national/state environmental agencies. At this preliminary step, we selected national TOP 40 active ingredients, based on the commercialization report from IBAMA in the last 5 years. We observed that only 6 active ingredients of the national TOP 40 are regulated in the CONAMA N° 357/2005. This fact suggests the need of a revision of the CONAMA norm. The next steps of prioritization process will be the critical selection of information on hazard (ecotoxicological data) and occurrence of each active ingredient in Brazilian waters applying minimum credibility parameters to obtain a list of the national priority compounds to be recommended for monitoring studies and regulation.

### 112 Consequences of agroindustrial land management on native freshwater communities

L.C. Schiesari, University of São Paulo

The reconciliation of large-scale agricultural production with biodiversity conservation necessarily depends on better understanding the pathways by which land use and land management influence native biota. Our research programme employs a tiered approach to tease apart the various drivers of degradation that together comprise what we perceive as the environmental impacts of industrial agriculture. We conducted habitat and biodiversity surveys in lentic water bodies distributed across gradients in land use intensity in rural state of São Paulo (seasonal Atlantic Forest/cerrado forest < pastures < sugarcane plantations) and in the upper Xingu Basin (transitional Amazonian broadleaf/cerrado forest < pastures < soybean plantations), Brazil. The biological model system included amphibian larvae, their resources (algae, detritus) and predators (fishes, dragonfly naiads, adult and nymphal heteropterans, adult and larval beetles). Hypotheses of drivers of community reorganization were tested in laboratory, mesocosms, and/or field experiments. We found that land use had a strong signal on freshwater biodiversity, but this signal was strikingly different in lands converted to sugarcane and soybean. Sugarcane fields were impoverished relative to cerrados but supported several species of tadpoles and insects, therefore being apparently permeable and functioning as habitat to at least some freshwater fauna. By contrast, relative to forest soybean fields were enriched in amphibians but devoid of insects. This occurred because land conversion effectively creates lentic habitats by increasing soil compaction, but heavy pesticide application affects invertebrate predators more than amphibians. Soybean fields thus act as population sinks for insects and possibly also for amphibians, which may repeatedly recolonize fields from adjacent streams. Decreasing stream buffer zones coupled with land use intensification increases the total 'pesticide footprint' over time with potential threat to stream communities. By attacking some of the same research questions at multiple scales of experimentation we draw a line of extrapolation between highly controlled field experiments and realistic field settings.

### 113 Occurrence of pesticides in aquatic ecosystems in Southern Brazil and bioaccumulation in fish sub-chronically exposed

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Currently, Brazil is the world leader in the use of pesticides. Chronic exposure to multiple contaminants in the environment, even at low levels, affects the resident biota, including the fish species. Contaminant accumulation in the fish tissues can induce several changes in the organism. Thus, the aim of this work was to investigate the occurrence of organochlorine pesticides (OCs) and current use pesticides (CUPs) in water, sediment and the relation with bioaccumulation in a Neotropical fish. Juveniles of *Prochilodus lineatus* were submitted to sub-chronic *in situ* tests during 120 days, confined in large cages (6000 L) simultaneously at two lentic environments: an agricultural area that receives inputs of pesticides (experimental site: EXP) and fish farming station of the State University of Londrina (reference site: REF), both in Northern Paraná, Southern Brazil. Throughout the exposure period, water and sediment samples and fish were collected from both sites and 23 OCs compounds and 33 CUPs were analyzed. Organochlorine compounds were quantified using a gas chromatograph equipped with a <sup>63</sup>Ni electron capture detector (ECD) and an Elite-5MS silica capillary column. A multiresidue quantitative method, based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed for the simultaneous analysis of 33 CUPs in waters samples. Compared to the REF site, the waters samples of the EXP showed higher concentrations of fipronil, atrazine, 2,4D, carbendazim and malathion. Concentrations of OCs above the limit of quantification were detected in a few water samples. However, in the sediments of the EXP site

higher concentrations of  $\gamma$ -HCH,  $\alpha$ -HCH, heptachlor, aldrin,  $\alpha$ -endosulfan and op'DDE were found. The OCs that more bioaccumulated in liver fish along the 120 days of exposure in EXP site were (% increase compare to REF): endosulfan sulfate (815%), chlorotalonil (230%), op'DDE (177%),  $\alpha$ -HCH (170%),  $\gamma$ -HCH lindane (136%),  $\alpha$ -endosulfan (97%), heptachlor (72%),  $\beta$ -HCH (51%), 4,4- DDD (36%) and  $\beta$ -endosulfan (32%). Thus, these results show a direct relation between pesticides bioaccumulation in *P. lineatus* and the presence of these contaminants in environment, mainly in the sediment, and point to the great contribution of trophic uptake to pesticide accumulation in this detritivorous fish species.

### 114 Toxicity of abamectin and difenoconazole mixtures to a Neotropical cladoceran after simulated run-off and spray drift exposure

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Often risk assessments of pesticides in tropical countries have often been disputed for being largely based on risk evaluations conducted in temperate regions. Although pesticide sensitivity comparisons between temperate and tropical freshwater organisms have indeed not revealed consistent differences, risk assessments are currently still based on a relatively small tropical toxicity dataset. In addition, greater levels of runoff and spray drift may be expected in tropical than in temperate agroecosystems, indicating that aquatic life in edge-of-field water bodies is likely to be subjected to higher concentrations of pesticides and their mixtures. The aim of the present study was to evaluate the toxicity of Kraft® 36 EC (a.i. abamectin), Score® 250 EC (a.i. difenoconazole) and their mixture to the Neotropical cladoceran *Macrothrix flabelligera*. Laboratory toxicity tests with the individual formulated products indicated EC50-48 h values of 3.1 and 659  $\mu\text{g a.i./L}$  given as nominal test concentrations, respectively. Mixtures of the two pesticides revealed a concentration-dependent deviation of the independent action model with antagonism at low and synergism at high pesticide mixture concentrations. Laboratory toxicity tests were also conducted with microcosm water that was treated with the individual or mixtures through runoff or direct overspray. Microcosm tanks receiving runoff water from experimental soil plots applied with recommended doses of the individual pesticides did not show toxicity to the test organism. Microcosms that received runoff water containing the pesticide mixture, however, did cause a short-term effect on immobility. The microcosms that were treated by direct overspray of both pesticide formulations showed the most pronounced toxic effects. Study findings suggest a potential risk of these pesticides at environmentally relevant concentrations, especially when they are both present.

### 115 Exposure to sublethal concentrations of carbaryl affects the development, biomarkers and locomotor activity of zebrafish early life stages

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The widespread use of pesticides in agriculture all over the world has dramatically increased in the last years. The impact of these in non-target wildlife with serious consequences to aquatic ecosystems remains a huge concern presently, although environmental concentrations are far from lethal doses. Therefore, a more effective risk assessment of these chemicals is achieved if a battery of sublethal endpoints is used. Within this context, the main goals of this work was to evaluate the effects of Carbaryl – a carbamate insecticide – in zebrafish (*Danio rerio*) embryo development, biomarker response and behaviour. Developmental endpoints included delay, hatching success, edema incidence, malformations, reduction of size and alterations in heartbeat rate. Biomarkers included Acetylcholinesterase (AChE), Glutathione-S-transferase (GST), Lactate dehydrogenase (LDH) and Catalase (CAT). Behaviour was assessed through a locomotion assay at 96 hpf (hours post fertilization) using a Zebrafish (Viewpoint, France) video tracking system. Behaviour has demonstrated to be a very sensitive endpoint in detecting acute toxicity effects of neurotoxic compounds such as carbaryl. The effects of Carbaryl on zebrafish embryos development was time and dose dependent. At the sublethal level, carbaryl significantly affected heartbeat rate,

decreased body length and increased incidence of edema and malformations. Moreover, carbaryl exposure resulted in a significant decrease in enzymatic activity (AChE, GST and LDH) and impairment of the locomotor behaviour of zebrafish embryos reflected by a decrease in the total distance moved. When compared to the developmental endpoints, biomarkers and locomotor behaviour were the most sensitive parameters showing effects at very low concentrations. This study highlights the importance of considering sublethal effects, such as those denoted at the behaviour level, of environmental contaminants in risk assessment in order to better and more accurately estimate its effects in aquatic ecosystems. **Keywords:** Danio rerio, locomotion, biochemical markers, embryos, carbamate

#### 116 Plaguicidas, planes posconsumo insuficientes y afectaciones ecotoxicológicas no monitoreadas Caso Soracá, Boyacá COLOMBIA

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El departamento de Boyacá es una de las despensas agrícolas en Colombia, suministra alimentos como papa, cebolla, arveja con usos convencionales de agroquímicos. La población campesina sostiene una clara dependencia de pesticidas y no reciben capacitación para su manipulación y correcta disposición final, lo cual conlleva a que los envases y empaques (EE) sean quemados, reutilizados, enterrado o depositados en sistemas acuáticos a pesar que en Colombia los importadores tienen la obligación de adelantar campañas de recolección y disposición final de estos elementos dentro de los planes de gestión de residuos peligrosos (RESPEL). El objetivo fue evaluar la implementación de plan postconsumo en Soracá dado que es un municipio netamente agrícola, cuenta con centro de acopio de envases y desarrolla campañas de recolección. La metodología consistió en encuestar a los agricultores y vendedores (n=238) sobre qué ingredientes activos aplicaban/vendían, si conocían o no plan postconsumo y si conocían los riesgos a la salud por el uso de plaguicidas, se contrastó esta información con EE depositados en el centro de acopio (n=1534 empaques segregados e identificados), así se obtuvo listado de ingredientes activos que se utilizan en el territorio y por revisión bibliográfica se determinaron riesgos ecotoxicológicos que podrían generar, para alertar a las autoridades de salud ambiental sobre esta situación. Los resultados indican que en general lo campesinos desconocen la gestión adecuada de EE, no reciben capacitación sobre protección a la salud humana, ni sobre la necesidad de proteger patrimonio natural de la región que garantice que la actividad agrícola sea sostenible. Las campañas de recolección fueron insuficientes frente a la gran cantidad de plaguicidas usados en la zona ya que fue necesario hacer dos campañas adicionales, los ingredientes activos que usan son 88, la mayoría de categoría toxicológica III- Medianamente tóxico, entre los insecticidas se contabilizaron 33 productos diferentes usados principalmente en papa, este exceso podría estar afectando a especies de fauna y flora que cumplen diversas funciones en los ecosistemas receptores fenómeno no evidenciado por la falta de investigaciones en ecotoxicología. Se adelantaron campañas de educación con los actores de la zona en adecuado manejo y recolección de estos empaques y se pretende empezar a realizar evaluaciones ecotoxicológicas en el corto plazo.

### What can mammalian & ecological toxicologists learn from each other? Leveraging approaches to advance risk assessment

#### 117 Leveraging approaches across mammalian and environmental risk assessments

C. Terry, DAS; J. Wheeler, Dow Agrosciences

The fields of toxicology and ecotoxicology are evolving to meet ever increasing demands to assess more substances. This inevitably requires approaches that are faster, more cost effective and utilize fewer vertebrate animals. The current paradigm shift in human health and ecological risk assessment, aiming for animal-free testing and utilisation of innovative approaches, provides a prime opportunity to look across risk assessment approaches and identify opportunities for collaboration. In addition, refined hazard and risk assessments are more often required in both human and ecological risk assessment, driven by new regulatory requirements. This has also highlighted the potential for greater collaboration across human and environmental health assessments. For agrochemicals, there are already examples of leveraging information across these two requirements. These include, wild mammal risk assessments, assessments for potential endocrine disruption properties and the use of toxicokinetic/toxicodynamic data. Case-studies will be shared to illustrate these opportunities. However, such approaches are challenging ecotoxicology approaches that have historically focused on adverse effects of concern (growth, development and reproduction) and less so on a mechanistic understanding. As such key differences and

potential areas for further collaboration will be discussed.

#### 118 Human Health Risk Assessment: new challenges for the future

C. Correa, Planitox

A new paradigm for human risk assessment is needed, although currently used human risk assessment methodology has served us well to date. A number of imperatives demand a reappraisal of the methodology itself and of its applications. It includes: the deep evaluation of the current methodology making use of animals for toxicity testing; the transparency and objectivity of various aspects of the risk assessment process and the high cost of meeting the regulatory requirements maintaining a high level of protection for exposed populations which is a disincentive to the development of potentially useful, innovative products. It is essential to take account of the future contexts in which risk assessments may be applied, when identifying a suitable conceptual framework for future risk assessment procedures. The proposed paradigm must include the development of “an exposure-driven, flexible, tiered approach, drawing continually on advances in technology and scientific understanding of biology, which meets the needs of stakeholders”. Development and application of the paradigm will involve input from a new range of methods and tools. This will require a much greater dependence on scientific judgement in order to better assess the weight of evidence. This has major implications for the training and range of research experience of future risk assessors.

#### 119 Ecological Risk assessment process for pesticides and Brazilian legislation

E. Nascimento, University of São Paulo USP

Ecological risk assessments are intended to evaluate the possible occurrence of adverse ecological effects resulting from exposure to one or more stressors. Pesticides are a class of compounds that raises many concerns due to their notorious toxicity. The ecological risk assessment process consists of 3 phases: a problem formulation (Phase 1: identification of endpoints and how to assess them), analysis (Phase 2: exposure profile), and risk characterization (Phase 3: risk estimation and risk evaluation). This generic process can be used to evaluate situations where the use of pesticide is of concern to the environment as a whole but also to specific species. Government establishes criteria to develop such risk assessments. The purpose of this presentation is to overview this process in the light of Brazilian legislation, in particular to the new Regulatory Instruction no. 2, February 9, 2017, related to the pesticide risk assessment for insect pollinators.

#### 120 Endocrine Disrupting Chemicals: effects on mammals' health.

A. Lourenço, University of Paraná

Several environmental chemicals are classified as endocrine disrupting chemicals (EDCs). According to the U.S. Environmental Protection Agency (EPA), an EDC is defined as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process”. EDCs can be found in polyvinyl chloride (PVC) plastics, medical devices, pharmaceuticals, consumer products, children’s toys, children products, printing inks, paints, household products, construction materials, lubricating oils, stabilizing additives, textiles, pesticides, flame retardants and, most importantly, in food (packaging materials and in the inner lining of food cans). Due to the great amount of these substances in our everyday environment it is of extreme importance to know and understand how they can affect mammals’ development, not only humans, but also wildlife mammals. EDCs have been suspected to be associated with altered reproductive function in males and females; increased incidence of breast cancer, abnormal growth patterns and neurodevelopmental delays, as well as changes in immune function. In this lecture we are going to summarize some of the most well known and abundant EDCs, such as phthalates and bisphenol A (BPA), and how these environmental toxicants affect mammal’s development. Otherwise, it is important to discuss what are the alternatives to animal testing in this area of research.

#### 121 A Sustainable Integrated Approach to Human Health and Environmental Protection for Crop Protection Chemicals

D. Wolf, Syngenta; J. Mehta, Dow Agro Sciences LLC; M.R. Embry, ILSI Health & Environmental Sciences Institute (HE

Environmental risk assessment and management in the 21st century would benefit from a holistic approach that addresses the complex interrelationship between humans and ecosystems through the integration of human health and ecological issue resolution. This integration should occur during problem formulation and will ultimately inform risk management decisions. The knowledge to implement such an integrated approach already exists but needs to be aligned and operationalized. To accomplish this, all disciplines should be brought together at the beginning of a risk



assessment or management effort, at the problem formulation stage. Available methods allow better integration across human health and ecological impacts and most importantly, driven by incorporation of exposure assessment. An exposure-driven assessment of Crop Protection Chemicals proposes a paradigm shift in support of global, harmonized, risk assessment-based regulatory decision-making. The application of best available science, via integration of new and traditional data streams, will create tailored exposure-driven risk assessments. A Crop Protection RoadMap and Regulatory Outcome Pathway encourages direct communication between the regulatory community and the regulated industry. This communication is essential for rapid incorporation of new technologies and advancing science, and would increase the speed, efficiency and accuracy of regulatory decision-making. A risk-based approach should be the basic operating principle for regulatory decision-making and is essential to scientifically-defensible pesticide stewardship. A common framework of an integrated approach that enhances efficiency and improves risk management is available. This framework, called RISK21, is an applicable, accurate, and resource appropriate approach to the evolving world of risk assessment that is scientific, transparent, and efficient. It is a problem formulation-based, exposure-driven, tiered data acquisition approach that allows a decision on safety to be made when sufficient evidence is available and maximizes the ability to inform the process and optimize resource usage. The framework enables the assessment of the value of available information and deciding what, if any, additional evidence is needed to reach a decision. This presentation will describe the use of the RoadMap and RISK21 approach for Crop Protection Chemicals, focusing on the integration of human and ecological health.

## The dirty dozen and a few more: status and trends of POPs in wildlife in the Americas

### 122 A comprehensive analysis of hotspots and patterns of p,p'-DDE accumulation in migrant Birds in North America

M.A. Mora, Texas A&M University / Wildlife and Fisheries Sciences

The Stockholm Convention on Persistent Organic Pollutants was adopted in 2001 in Stockholm, Sweden, and entered into force on May 2004. One of the main aims of the convention was to eliminate or restrict the production and use of persistent organic pollutants (POPs). As a result of the agreement a global monitoring plan was established to identify changes in POPs concentration over time at the regional and global level. Monitoring studies with birds have been conducted in North America since pesticides begun to be used in agriculture in the early 1940s. After more than 40 years since the DDT ban in the U.S., Canada, and Mexico, it is of great interest to examine the fate and persistence of DDT and metabolites (i.e. p,p'-DDE) on birds. To assess how DDE impacted bird populations in North America since the DDT ban, we analyzed the results of nearly 300 studies published in the peer reviewed literature from 1980-2009. The objectives of this study were to determine if DDE concentrations had decreased in North American birds over a 30 year period; to examine if there were any longitudinal or latitudinal patterns in the distributions of DDE; and if DDE concentrations in birds could be linked to global distillation of POPs or to the grasshopper effect. Our analysis suggests no strong overall linear trends of decreasing or increasing DDE concentrations with latitude. However, DDE concentrations were the highest in the central United States, and lower near northern Canada and southern Mexico. The test of a linear year effect was significant ( $p < 0.0001$ ) indicating that as year increased, DDE concentrations decreased. When the data were divided by regions, tests for a difference in both longitudinal and latitudinal trends were significant ( $p < 0.001$ ), suggesting that concentration of DDE were decreasing at the same rate in all regions of North America. As expected, our results suggested that DDE residues are decreasing over time in birds from the low to mid-latitude regions. The majority of supported models suggested that DDE concentrations in birds were highest in the upper mid-latitudes (40°- 50°) than in other portions of North America.

### 123 Trends in POPs on the Pacific Coast of Canada using seabird indicator species

J.E. Elliott, Environment Canada / Science Technology Branch; K. Elliott, McGill University / Department of Natural Resource Sciences; S. Lee, Environment and Climate Change Canada

There is a continuing need to monitor ecosystem contamination by xenobiotic compounds, particularly those, such as POPs, which are persistent and accumulate in food chains. Eggs of aquatic and predatory birds have proven to be an efficient and effective means of measuring and tracking POPs as they are transferred from the female bird to the egg via yolk lipid. Here we report and discuss data from long term monitoring of seabird eggs from the northeast Pacific. For this program, the marine

system was divided, and representative species selected. The nearshore subsurface is monitored using two cormorant, *Phalacrocorax*, species, *auritus* and *pelagicus*, both of which feed on a variety of benthic and pelagic fish. The offshore subsurface is monitored using the rhinoceros auklet, *Cerorhinca monocerata*, a feeder mainly on small pelagic fishes, with the offshore surface species, the Leach's storm-petrel, *Oceanodroma leucorhoa*, which feeds mainly on surface plankton and larval fishes. At three breeding colonies each along the Pacific coast of Canada and at four year intervals 15 eggs are collected and analyzed as five pools of 3 eggs each. Data will be presented on long term temporal trends and spatial variation for representative legacy and more recent POPs. For example, a recent retrospective study using archived samples, shows, as reported for more polluted environments, that brominated flame retardants, such as polybrominated diphenyl ethers and hexabromocyclododecane increased by an order of magnitude between 1990 and 2011 in eggs of Leach's storm petrel from the Gulf of Alaska. Use of stable isotopes will be discussed in relation to the role of dietary variation, possibly related to marine regime shifts, in explaining variation in contaminant levels in seabirds.

### 124 POPs in a wildlife in Brazil: A Review

J.d. Padilha, Federal University of Rio de Janeiro / Biofisica; F.B. Torres, Biophysics Institute Federal University of Rio de Janeiro Brazil / Biophysics

There is growing concern about the possible negative effects that Persistent Organic Pollutants (POPs) can cause to the environment and humans. Some of these chemicals, such as DDT, PCBs and PBDEs, are studied for their toxicity, persistence, bioaccumulative and biomagnification processes. It is important for Brazil to develop ecotoxicological studies in its territory to gather information on the environmental dynamics of several POPs. However, there are few studies about POPs in wild animals in Brazil. To identify studies related to POPs in vertebrates, a systematic study was conducted, evaluating the existing database in Brazilian literature. We researched available information on Scencedirect, Springer and Web of Science. As a result we found 31 publications (10-fish, 17-delphinids, 4-seabird) between 2005-2016. No publications on terrestrial mammals were found. The works were concentrated in the southeastern and southern regions of Brazil. Data were divided in two groups, from 2005 to 2010 and 2011-2016. All results are in ng/g. PCBs and ΣDDT were the main POPs measured (22 publications), followed by PBDEs (6 publications). From 2005 to 2010 the values found on available database for fish muscle (in wet weight - w.w) for ΣPCBs ranged from 3.15 to 6.50 and for ΣDDT from 1.27 to 3.03. In delphinids the concentrations (lipid weight - l.w) found were ΣPCBs: 1,055-1,135 and ΣDDT: 219-2,125. More recently, from 2011-2016, the values found in seabirds liver for ΣPCBs ranged from < 1.67 to 24,687 and for ΣDDT from < 1.89 to 6,611 (w.w), in relation to fat ΣDDT: < 0.87-24.37 and ΣPCBs: < 1.55-127.7 (w.w). In eggs ΣPCBs ranged from 0.02 to 12.56 and ΣDDTs from 0.01 to 2.73 (w.w). For delphinids the values (l.w) in blubber were ΣPCBs: 909-119,300; ΣDDT: 264-27,500; ΣPBDEs: 3-1,327. In liver, ΣPCBs: 1.52-26,199. For the same period the values in fish muscle were ΣPCBs: 8.57-1,454; ΣDDT: 1.42-249.5 and ΣPBDEs < 4.00-18.1 (w.w). POPs concentrations are higher in the second group, pointing to an increase over the years. In addition, the delphinids presented the highest values of the POPs in relation to the other studied animals. Brazil have a huge territory and high biodiversity and POPs may pose a threat to species and ecosystems. The lack of information on those chemicals is a challenge to conservation and to better regulation by the government. More studies should be carried out in Brazil, especially in terrestrial biota.

### 125 Spatial and temporal distribution of POPs levels in Chilean wildlife species: A Review

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The toxicity and the world distribution of Persistent Organic Pollutants (POPs) is well known for more than three decades. This compounds are harmful for human and wildlife. In the northern hemisphere there are several studies about the status, characterization and distribution of POPs in the environment and its effect on humans as on wildlife health. In Latin America the concern about the effects of this substance in wildlife species health is increasing although in Chile there is still a lack of information. Chilean researches are mainly centered on marine resources, river fish and seabirds. It has been described very high levels of PCBs and DDTs in seabirds with concentration above 1000ng/g dry weight in liver and muscle, followed by some species of fish with POPs levels between 500-1000 ng/g dry weight also found in liver and muscle. However there is no possible comparison between regions or time because of the fragmentation of the data, as we can observe, studies in Chile are centered on

wildlife from the south part of the country, a few researches are from the north and there is very poor information of the species from the middle area of Chile. Also we could find information since 1993 to 2002 but they are developed in different regions and on different species. In conclusion, in order to improve the lack of information, it is necessary to carry out monitoring programs to know the actual status under which wildlife in Chile is. Likewise, to obtain a wider view, this studies must include more locations and aiming for a better comprehension of the mechanism that this compounds have to transport themselves through the atmosphere and transferee to terrestrial and aquatic complex food webs, also should include several species from different trophic levels. Acknowledgements: E. Vergara and W. Espejo are scholarship USACH-Chile for MSc and CONICYT-Chile for PhD studies, respectively. This study was financially supported by the project INACH RG\_09-14 granted to J. Celis and by the project 214.074.051-1.0 and 216.153.025-1. of the Dirección de Investigación de la Universidad de Concepción.

#### 126 Latitudinal and temporal tendency of persistent organic pollutants on Antarctic seabirds A review

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Persistent organic pollutants (POPs) are considered highly dangerous substances, due to their high prevalence in the environment and the effects caused by different organisms within the trophic webs. Until recently the Antarctic was considered pristine areas. However, it has changed since studies began in the sixties have shown the presence of POPs in Antarctic aquatic ecosystems. High levels of toxic substances in marine organisms are explained by the processes of bioaccumulation and biomagnification. Marine birds are key species in the aquatic coastal ecosystem and have physiological traits being considered susceptible to anthropogenic pollutants. POP transportation mechanisms resulted as a consequence of global fractioning, implying that under low latitudes, warm temperatures favor evaporation processes of these compounds from off the ground; while at the same time colder climate conditions at higher latitudes the opposite atmospheric sinking mechanisms would benefit. Although there is data that backs this hypothesis, knowledge of POP behavior in the Antarctic is still scarce. We reviewed the POPs levels reported from different species of seabirds that inhabit Antarctic. We observed that they have only been reported ΣDDT, DDE, HCB, Σchlordanes, ΣHCHs, Dieldrin, ΣMirex, ΣPCBs, ΣPBDEs and PFOS in Antarctic seabirds. All POPs reported a tendency to decrease in the time scale; however ΣDDT and DDE showed a slight decrease. When observed on a latitudinal scale it was observed that these increase at a higher latitude (colder areas). However, no significant P-value was observed in any of the cases. Acknowledgements: W. Espejo is scholarship CONICYT-Chile for PhD studies. Many thanks to INACH for the financing of projects RG\_09-14 (J Celis), T18-09 (R Barra), T-12-13 (D. González-Acuña) and T 31-11 (G. Chiang) and projects as well as the chance to do research on Antarctic birds.

#### 127 Bioaccumulation of organochlorine pesticides and PCBs by the narrownose smooth-hound shark in a nursery area of the Southwestern Atlantic

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Coastal marine areas function as sinks for diverse anthropogenic contaminants, such as Persistent Organic Pollutants (POPs) which included organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). POPs are lipophilic compounds that biomagnify in the food web. In this sense, chondrichthyans are able to accumulate significant concentrations of POPs due to their relative longevity, moderate to large size, lipid-rich livers and high trophic position. The narrownose smooth-hound shark *Mustelus schmitti* is the most abundant and the most frequently landed sharks in Argentina. An important nursery area for this species is located in coastal waters of

Southwestern Atlantic between 39° and 41° S (El Rincón Area, RA), where it develops an intensive agriculture production, commercial harbors and industries. For that reason, levels and distribution of POPs were analyzed in liver and gonads of both sexes at different maturity stages and embryos of *M. schmitti*, captured in coastal waters of RA. Concentrations were determined by GC-ECD and reported as ng/g wet weight. Livers of both sexes showed a predominance of PCBs, whereas gonads presented similar proportions of OCPs and PCBs. In addition, concentrations were higher in livers (OCPs=7.49±6.52, PCBs=12.38±11.48) than in gonads (OCPs=6.85±8.88, PCBs=5.25±4.98). In mature individuals, ovaries (14.29±11.12) presented higher concentrations of POPs than testicles (2.31±2.01), but there were no differences comparing immature gonads of females (14.03±7.39) and males (18.61±17.04). The embryos in early stage of development, presented the same POPs pattern but a lower level (8.94±6.76) than mature females. In all samples, endosulfan were the most frequent OCPs, 63 % of them shows a-b-isomers ratios ≥1, suggesting the use of the technical mixture application for agricultural proposes before their ban in Argentina. The most abundant PCBs were tetra- (PCB-44) and hexa- (PCB-149, PCB-153) congeners, which could have stemmed from historical usage of Arochlor 1254 and 1260. Our results revealed that land use in surrounding areas represents current sources of POPs to coastal marine waters. Chondrichthyans biaccumulate high levels of POPs since early stages of development. Therefore due to the low reproductive potential, long-term pollutants monitoring programs in coastal areas are necessary for fishery management strategies and species conservation.

#### 128 Non-destructive biomarkers in giant toad (*Rhinella marina*) of the industrial and agriculture zone of the lower basin of the Coatzacoalcos River

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In this study, the activity of nondestructive biomarkers (glutathione s-transferase -GST-, butyrylcholinesterase -BChE-, aminolevulinic acid dehydratase -δ-ALAD-, condition factor -FC- and apoptosis) was determined in blood of giant toads (*R. marina*) in two areas of the lower basin of the Coatzacoalcos River (one of the sites with highest pollution in Mexico: POPs, BPCs, heavy metals) to compare with reference organisms (toads maintained in the laboratory). Of each sampling station areas were collected from 11 to 20 giant toads, to which they were taken weight, height and blood samples (plasma, erythrocyte package). Subsequently, in these biological samples most of non-destructive biomarkers were determined by methods of UV-Visible spectrophotometry in microplate reader, only apoptosis was determined by flow cytometry in the erythrocyte package. Subsequently, the toads were released at the place where they were collected. Residents giant toads of industrial zone of Coatzacoalcos River basin showed an increase of GST activity and BChE activity of 1.4 times compared with laboratory organisms; this is possibly an effect of chronic exposure of existing organic pollutants on the site. A percentage of 60 to 65% inhibition of δ-ALAD found in Coatzacoalcos amphibians compared with laboratory organisms, this could be due to chronic lead exposure on both the industrial and agricultural sites. A decrease of 1.4 times in the FC of toads residents found in the industrial zone, which could be an effect of exposure to contaminants, where organisms have an energetic imbalance, possibly for expend more energy for the detoxification of pollutants. Apoptosis in erythrocytes showed 10.7 times and 33.3 times more in toads of Coatzacoalcos river basin (agriculture and industrial zones, respectively), compared to the laboratory; this result is one of the earliest studies of the use of apoptosis as a biomarker in field with amphibians, and possibly could be evidence of damage to cells by exposure to pollutants, mainly of the organochlorine type. Statistical correlations showed that some biomarkers may be related to each other, and, in turn, may be performing functions at the same time to regulate or balance the homeostasis of exposed organisms. The results in this research demonstrated that non-destructive biomarkers can be very useful in biological monitoring of an ecological risk assessment without affecting populations of organisms in ecosystems.

#### Ecological Risk Assessment and Site Remediation in Latin America: The state of the practice

##### 129 Ecological Risk Assessment and Site Remediation in Latin America: The state of the practice

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Aires, Argentina.

Regulatory and scientific developments are exerting ever increasing influence over the role of ecological risk assessment (ERA) in site remediation in Latin America. Whereas ERA methods and approaches had previously been relegated to the realm of pesticide and other product registration as well as specialized applications such as dredged material management, recent regulation and guidance is bound to change the panorama significantly. For example, The Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT of Mexico) has published a guide introducing ERA, and the Ministerio de Ambiente (MINAM) of Peru has published a guide for assessing environmental risks. This in conjunction with recent legislation on Environmental damages (e.g., in Mexico and Chile) are likely to prompt application of ecological risk concepts and assessment frameworks in remediation decision-making. The organizers intend to solicit presentations from representatives of government agencies, industry, scientific investigation organizations, and environmental consulting. The session will provide an overview of regulatory and scientific developments, an introduction to the risk assessment framework developed by U.S. Environmental Protection Agency for site investigation and remediation, illustrations of case studies making the case for ERA as a means of enhancing sustainability, and technical and institutional obstacles to the adoption of ERA practice. The session will be designed to be of value to experienced practitioners as well as those unfamiliar with ERA concepts.

### 130 Guidelines for ecological risk assessment (era) in contaminated sites - Cetesb, São Paulo, Brazil

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In Brazil, Ecological Risk Assessment (ERA) application in site remediation is still rare, although its use is foreseen by national (CONAMA Resolution 420/2009) and state legislations (e.g. Decree 59263/2013 of São Paulo State), partly because a National Standard is still under discussion in the Brazilian Technical Standards Association (ABNT). In São Paulo, the State Environmental Agency (CETESB) reviewed its "Procedures for the Management of Contaminated Sites" in February 2017, establishing guidelines for the execution of an ERA for the first time in the country. The responsible parties will present an ERA when there is a natural ecosystem under the influence of a Contaminated Site under Investigation. The ERA methodology should be previously approved by CETESB and the main objective of the study is to verify the occurrence of risk to a species, community or ecosystem due to exposure to chemical contaminants of concern (COCs). It should involve different environmental compartments, considering exposure, direct and indirect effects on ecological receptors, structural and functional measurement endpoints, in a spatial and a temporal scale. The study should follow the weight-of-evidence approach, considering, at least, chemical, ecotoxicological and ecological lines of evidence. The bioavailability potential of COCs, the occurrence of functional and/or structural effects, with causal relationships, and the levels of risk that receptors, communities or ecosystems are exposed to, are expected to be investigated. The study should define background concentrations/reference locations and propose both risk calculation methodology and the level of acceptable risk. The final report should include a risk map and discuss the need for remediation in specific locations. Some issues have already been identified in ERAs that are underway, such as the use of models for the initial risk prediction to wildlife receptors that rely on previous knowledge of receptors characteristics, such as habitat, home-range and diet, to establish exposure and intake of chemicals. This data is mostly unavailable for local wildlife, consequently the use of default toxicity references in models generate higher uncertainty in the risks predictions. Nevertheless, since these requirements took effect in São Paulo as of this year, it is expected that some aspects should still evolve towards the definition of appropriate methodologies to local problems, involving terrestrial and aquatic ecosystems.

### 131 CETESB Ecological Risk Assessment legal framework: Regulatory background and implications for the regulated community

A.C. Chirmici, Ramboll Environ; L.P. Garcia, Mattos Filho, Veiga Filho, Marrey Jr e Quiroga Advogados

On February 10, 2017, the environmental agency of the state of São Paulo (CETESB) published Board of Directors Decision No. 038/2017/C. By ratifying and detailing

further the previous procedures formerly issued in 2007 (Board of Director Decision No. 103/2007/C/E), CETESB has established guidelines that comprise procedures for ecological risk assessment at 4.1.6.2 of Annex 2: "Procedures for the Management of Contaminated Sites". The procedures apply at any contaminated site where a "natural ecosystem" is under the influence or may be under the influence of a contaminated site under investigation. The objective of the risk assessment is to verify the existence of risk to one species, a community, or an ecosystem and requires the risk assessment to be conducted according to "Unit of Exposure" or "Environmental Compartment". Respondents are required to submit a detailed work plan that must specify a variety of technical tasks and approaches for the risk assessment including development of a Conceptual Site Model and proposal of unacceptable risk levels. The Procedures present a number of uncertainties that have important legal and technical implications. The Board Decision is not objective about the circumstances in which an ecological risk assessment should be applied. Brazil does not yet have an approach set out to ecological risk assessments and, therefore, the area has a vast technical field to be explored. This presentation will provide attendees with 1) a foundational background on the development of these procedures, the development and implementation of human health risk assessment procedure 2) comparison between the human health risk assessment and the ecological risk assessment guidelines 3) primary sources of legal and technical uncertainty on the application of the Procedures, and 4) our conclusions and recommendations to the regulated community on how to manage legal and technical uncertainties so as to have more predictable outcomes. The conclusions around the proper enforcement of the ecological risk assessment will also consider how this mechanism is applied by other countries.

### 132 Ecotoxicity tests in risk assessment and risk management: experiences gained from contaminated sites and their implementation by regulatory agencies

J. Niemeier, UFSC; J.F. Sousa, University of Coimbra / Department of Life Sciences of University of Coimbra

Soil contamination requiring remediation is a major challenge of concern around the world. The protection of soils, its diversity and ecological functions, has become an important goal of environmental agencies. The majority of the ecological risk assessment (ERA) studies carried out in Latin America are based just on chemical characterization, focused on limit values of contaminants in soils and water, usually not including biological/ecological and ecotoxicological parameters. However, chemical analysis *per se* are not sufficient to indicate ecological risk for some reasons: effects resulting from interaction between contaminants in complex mixtures, contaminants or metabolites not analysed in the main scope of the study, different bioavailability depending of the soil type and aging. The aim of this work is to present how ecotoxicity tests and ecological assessments can be used in supporting decisions made when assessing risk to the terrestrial environment. Ecotoxicological and ecological assessments includes a plethora of laboratory and field methods, encompassing both structural and functional parameters, that should be used to complement the chemical evaluations and thus allowing a more robust and less uncertain characterization of the potential ecological risk at a certain area. Moreover, they can help prioritizing sites for remediation measures, or even being used in post-remediation monitoring programs aiming to evaluate the efficiency of the remediation actions. The feasibility and usefulness of different assessment tools to be used in different tiers of triad approach, as well as their direct link to protection goals, will be discussed.

### 133 Aplicação do Método de Avaliação de Risco Ecológico da EPA em Área Contaminada por Pesticidas, Compostos Orgânicos e Metais-Estudo de Caso

F. Gimenes, A. Yoshinari, A. Canale, A. Rodrigues Junior, R. Coelho, GEOKLOCK. A Avaliação de Risco Ecológico (ARE) é uma ferramenta que permite a tomada de decisões visando a proteção da biota no âmbito do gerenciamento das áreas contaminadas. Apesar de não haver metodologia nacional estabelecida, existem diversas internacionais com diferentes abordagens. Metodologias desenvolvidas pela USEPA preconizam o desenvolvimento do estudo em etapas, partindo de premissas conservadoras, as quais são refinadas com dados mais realísticos ao final de cada etapa. Neste estudo, é apresentada a ARE conduzida no entorno de uma área industrial contaminada por pesticidas, compostos orgânicos e metais, onde existem habitats de interesse (um corpo hídrico e fragmentos florestais). O estudo compreendeu as Etapas 1 a 3A da metodologia estabelecida no documento *Ecological Risk Assessment Guidance for Superfund* (USEPA, 1997). Na Etapa 1 (formulação do problema), foi realizada a caracterização ecológica, coletadas amostras dos compartimentos ambientais de interesse (solo superficial, sedimento e água superficial) e selecionados os padrões de referência ecológica. A integração dos resultados desta etapa subsidiou a elaboração do modelo conceitual de exposição ecológica, com a definição dos habitats e receptores ecológicos e a seleção das substâncias químicas de interesse



ecológico (COPEC – *contaminants of potential ecological concern*). Na Etapa 2 (cálculo dos potenciais riscos), foram calculadas as doses de exposição para os receptores por COPEC, selecionada as doses de referência ecológica e calculados os quocientes de risco, cujos resultados indicaram potencial risco para hexaclorobenzeno, pesticidas e Ba, Cu, Hg e Sb. Na Etapa 3A (refinamento dos cálculos de risco), foram assumidas premissas de exposição (*background*, 95%UCL e composição da dieta) e toxicidade (NOAEL, LOAEL e MATC) mais realísticas para estes COPEC, resultando potencial risco para as aves herbívoras e invertívoras associados aos teores de hexaclorobenzeno e Hg no solo superficial. Para os demais COPEC e compartimentos considerados no estudo, os potenciais riscos se mostraram aceitáveis. A aplicação da referida metodologia se mostrou adequada para o gerenciamento de uma área contaminada, uma vez que permitiu refinar o modelo conceitual de exposição, de forma a direcionar a etapa subsequente de ensaios toxicológicos (plantas e minhocas) e químicos (biodisponibilidade e especiação do Hg), norteando as ações necessárias à proteção dos receptores ecológicos.

#### 134 Assessing the ecological risks of metals in sediments from the Guanabara bay (Brazil) through a tiered approach based on multiple lines of evidence

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The Guanabara Bay (GB) comprises approximately 70% of the industries of Rio de Janeiro State. The wastes produced by these sources, combined with the domestic sewage loads, have transformed the region into one of the most polluted areas of the Brazilian coast. The present study evaluated the ecological risk of metals in GB's sediments through a tiered approach. The chosen method combines multiple lines of evidence: geochemistry, comparison of environmental concentrations with sediment quality guides, chronic toxicity tests using the organisms *Anomalocardia brasiliensis* and *Nitocra sp.*; and acute toxicity tests using the organisms *Tiburionella viscana*, *Kalliapseudes schubartii*, and *Anomalocardia brasiliensis*. To verify the existence of cause and effect relationships between metals and toxicity, the *Toxicity Identification Evaluation* (TIE) technique, multivariate analysis (PCA) and qualitative matrices were used. The sediments showed high to moderate levels of metals (Zn, Pb, Cu, Cr) associated with high toxicity. In addition, the TIE has revealed that metals, ammonia and organic compounds contribute to cause sediment toxicity. Metals are an important class of contaminants for the GB, as they are present in high concentrations and are responsible for negative biological effects, as indicated by TIE and integrative analyzes (PCA and qualitative analysis by decision matrix). The Guanabara Bay sediments present high levels of Cr, Cu, Zn and Pb contamination, especially near the harbor areas, in the inner bay, and in BG's NW portion. The Guapimirim environmental protected area (NE portion of BG) presented a better environmental condition, presenting the lowest concentrations of toxic metals and ammonia, indicating the importance of the protected areas. It can still be concluded that, in addition to the metals, Guanabara Bay suffers from ammonia contamination (mainly in its innermost regions) and organic contaminants in a more general way.

#### 135 In Situ Pore Water Quality Evaluation in a creek located in a chemical production facility in Rio de Janeiro State, Brazil

A.C. Chirmici, P.S. Yoshikawa, R.A. Campos, Ramboll Environ; P.N. Booth, Ramboll Environ / Ecological Sciences

Among in situ samplers methods, the modified Hesslein In-Situ Pore Water Sampler (Peeper Sampler) can be applied to the requirements of monitoring in-situ sediment and allow temporal sampling of pore water from sediments for understanding the path followed by discharged pollutants within the hyporheic zone. This presentation will provide the results of pore water quality evaluation using Peepers samplers in a creek located at an operating facility located in Rio de Janeiro State, Brazil. The operations in this area began in 1978. Previous studies of groundwater quality identified plumes of carbon tetrachloride and chloroform in the shallow aquifer. Both plumes were not delineated on its northern portion, due to the absence of sufficient data to exclude the possibility of discharge in the creek. The objective of this study was verify whether the known Carbon Tetrachloride and Chloroform groundwater plumes are reaching the subject surface water body. Considering that the Carbon Tetrachloride and Chloroform plumes presents a similar configuration (lateral extent), a total of 8 (eight) samplers at the right margin of the creek were proposed: one sampler upgradient to the plume predicted discharge zones, five samplers on the discharge zone and

two samplers installed downgradient the discharge zone. Measurement of pore water concentrations of potentially toxic, hydrophobic organic compounds is important to the assessment of ecological risk in water bodies because this measurement more accurately represents exposure concentrations to ecological receptors (e.g., benthic invertebrates) than simple equilibrium partitioning and transport modeling.

#### Other

#### 136 Circular economy: environmental lessons learned in Brazil-Colombia higher education institution relationship

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In the new economy and consumer culture, industries and services employ a variety of product decision making based on customization strategies. Great manufacturing and distribution strives to present policies of differentiation and segmentation, but all these changes only extend the commodification of non-existent needs leading to hyperconsumption (Lipovetsky, 2007). Industrial capitalism, based on continuous and cumulative development of new products and services has generated several environmental impacts. As a result, Earth's ability to provide resources and absorb waste produced by civilization is rapidly reaching a critical and irreversible level (Wagner et al., 2002, Lena et al., 2005). On the other hand, global discussion on environmental sustainability has increased its relevance in recent years. A resilience threshold of global ecosystem should be considered. Understanding the dynamics of ecosystems requires effort in mapping the so-called ecosystem functions. These functions translate into ecosystem services as they benefit human societies (May, 2010). In nature itself, waste is never discarded: one system residue is food by the other. Thus, the establishment of rational and ecologically sustainable use of nature for local populations benefits, leads them to incorporate concern for biodiversity conservation to their own interests, as a component of development strategy (SACHS, 2009). A model that has been presented as an alternative to reduce environmental impacts and use natural resources in a more viable way is circular economy, which is concerned with a more efficient generation of products including disposal material as a productive input (Gansky, 2011). The challenge, though, is changing the actual production mindset. In addition, using LCA (life cycle assessment) factors should be the guideline to innovation towards a more sustainable economy. In order to mitigate environmental damages impact it is necessary to consider strategies to minimize energy consumption, raw materials, and also production processes optimization, reducing the amount of packaging material. This study presents two Latin American case studies in which concepts of environmental sustainability were applied in higher education institutions. Brazilian and Colombian approaches present different perspectives of practical applications focused on sustainability.

#### 137 Social Life Cycle Assessment in supplier selection: an application of SAM in the supply chain of cellulose nanocrystals

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The need to assess the social performance of suppliers throughout the life cycle has become increasingly relevant. In this sense, Social Life Cycle Assessment (SLCA) can play an important role in identifying social hotspots in the life cycle of products and point out possible improvements in the social performance of the organizations involved. Due to the recent interest in the reuse of coconut shells in other processes, cellulose nanocrystals from the coconut shell fiber to be incorporated into edible and inedible biofilms has been developed at laboratory stage. Therefore, the objective of this study is to evaluate the potential social performance of the main suppliers of the production of cellulose nanocrystals obtained from the coconut fiber through SLCA. The system of the evaluated product is formed by the processes of the main raw material processing, not including the phases of manufacture, use and final disposal of biofilms as no matter the supplier, the quality is considered the same as well as the use and final disposal. The selected potential companies that are part of the life cycle of the product are: a company that extracts coconut water and three coconut shell processing plants, all installed in the state of Ceará. The Subcategory Evaluation Method (SAM) was used to evaluate the subcategories of four stakeholders (workers, local community, society and actors in the value chain). Consumers were not considered because the product is not yet produced. SAM allows to score organizations at four levels, from A to D. Level A is given to proactive organizations in relation to the subcategory and levels C and D are distinguished by the context in which the organization is installed. Level B implies that the organization meets the

basic requirements defined for each subcategory. The results showed that there is great improvement potential for most of the subcategories evaluated, especially by coconut husk companies. Actions concerning the formalization of work, as well as informing workers about their rights should be a priority. As for the local community and society, the actions involve their involvement in the decisions and strategies of the company. Finally, as for SAM, it is suggested that other sub-levels are added so that the differences evidenced by the data collection are translated into different levels of evaluation.

### 138 Avaliação da espécie *Oreochromis niloticus* suplementadas com duas biomassas residuais de algas utilizando biomarcadores bioquímicos

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As microalgas são grandes produtoras de compostos antioxidantes, também são uma das principais fontes de ácidos graxos poli-insaturados, suprimindo toda uma cadeia trófica com esse composto. A utilização de microalgas para a produção de biocombustíveis é uma alternativa à utilização de combustíveis fósseis. A biomassa de microalgas é produzida com diferentes meios de cultivo em fotobiorreatores compactos e é submetida à extração lipídica, esse processo gera uma biomassa residual desengordurada (*Residual Algae Biomass* – RAB) que pode ser utilizada como uma fonte alternativa na suplementação de rações para peixes. Este trabalho utilizou duas biomassas de microalgas da espécie *Acutodesmus obliquus* crescidas em diferentes meios de cultivo; meio sintético Chu (A) e meio composto da biodigestão do efluente oriundo de suinocultura (B); para produzir rações contendo 1%, 2% e 3% da RAB (A) e rações contendo 1%, 2% e 3% da RAB (B). Também foram produzidas rações sem a adição de RAB. Utilizaram-se peixes da espécie *Oreochromis niloticus* (Tilápia do Nilo), sendo cada grupo contendo n=15. Durante o período de experimentação (60 dias) a alimentação, com as diferentes rações produzidas, foi disponibilizada três vezes ao dia (*ad libitum*). Após o experimento, os peixes foram anestesiados e eutanasiados por secção medular. A avaliação foi feita por biomarcadores bioquímicos: acetilcolinesterase (AChE) em cérebro e músculo; catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), superóxido dismutase (SOD) e lipoperoxidação (LPO) em fígado. Os resultados foram submetidos à Análise de Variância (ANOVA), seguida do teste de Newman-Keuls; teste de Kruskal-Wallis, seguido do teste de Dunn, sendo a normalidade verificada pelo teste Kolmogorov-Smirnov. Os resultados para as rações produzidas com a RAB (A) indicaram aumento da atividade da AChE cerebral nos grupos contendo 2% e 3% dessa RAB. Para a AChE muscular, SOD, CAT, GPx e GST não houveram alterações entre os grupos. Foi observada uma redução da LPO nos três grupos (1%, 2% e 3%) contendo a RAB (A). Os resultados para as rações produzidas com a RAB (B) não demonstraram alteração da atividade para a AChE cerebral e muscular. Houve redução da SOD e CAT no grupo contendo 3% dessa RAB. Para a GPx e GST não foram observadas alterações entre os grupos. Foi observada uma redução da LPO para o grupo contendo 3% da RAB (B).

### 139 Efeitos ecotoxicológicos de biochar de sabugo de milho sobre "*Eisenia andrei*"

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Diferentes matérias-primas têm sido utilizadas para a obtenção de biochar, um produto com ampla aplicação no meio agrícola. Para garantir seu uso seguro ao ecossistema terrestre, são necessários estudos ecotoxicológicos envolvendo organismos-teste. O presente estudo objetivou a avaliação ecotoxicológica de biochar de sabugo de milho sobre "*Eisenia andrei*" (*Oligochaeta*). Foram realizados ensaios com produtos de pirólises a 350, 450 e 550 °C, conforme as normas ABNT NBR 15537 (2014), ISO 11268-1 (2012) e ISO 11268-2 (2012). Os organismos-teste foram expostos às concentrações de 5, 10 e 20 ton.ha<sup>-1</sup>, para avaliação do comportamento de fuga e de variáveis relativas à reprodução (biomassa de adultos, número de casulos e juvenis em 28 e 56 dias), em substrato artificial tropical (SAT). As variáveis foram analisadas quanto aos fatores concentrações e temperatura, por meio de Análise da Variância fator duplo, seguido do post-hoc test Tukey-HSD ( $\alpha=0,05$ ). Foi possível

observar que as variáveis fuga, redução de massa dos adultos, número de juvenis e de casulos aos 28 dias, assim como número de juvenis, e número de casulos aos 56 dias não apresentaram diferenças estatísticas significativas entre as concentrações e as diferentes temperaturas de pirólise do biocarvão ( $p>0,05$ ). Conclui-se, então, que o biochar de sabugo de milho originado nas temperaturas 350, 450 e 550°C não implica em efeitos ecotoxicológicos sobre "*E. andrei*", quanto a aspectos comportamentais de fuga e características do processo reprodutivo, apresentando potencial para ser empregado no condicionamento de solos.

### 140 Efeitos neurotóxicos e sobre o sistema antioxidante em "*Eisenia andrei*" exposta ao ácido aminometilfosfônico (AMPA) e ao biochar de casca de arroz

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A fim de fundamentar o uso da casca de arroz, na forma de biochar, para aplicação segura em solos contaminados com ácido aminometilfosfônico (AMPA), principal metabólito do glifosato, o presente trabalho tem como objetivo identificar os efeitos de AMPA e de biochar de casca de arroz (obtido a 700 °C), de forma isolada e em misturas, sobre o sistema enzimático antioxidante e a atividade colinérgica de "*Eisenia andrei*", após exposição por 30 dias. Os ensaios foram baseados na norma ISO 11268-2 (2012), de acordo com seguintes tratamentos: controle negativo, biocarvão 20 ton.ha<sup>-1</sup> (BC20), AMPA 100 e 2500 µg.kg<sup>-1</sup> (AMPA100 e AMPA2500, respectivamente) e as misturas AMPA100+BC20 e AMPA2500+BC20. Foram realizados ensaios de lipoperoxidação (LPO), determinação da atividade da catalase (CAT), superóxido dismutase (SOD), glutathione S-transferase (GST) e colinesterases (ChE's). Não houve alteração na atividade das ChE's pela exposição aos xenobióticos isolados e em mistura, o que sugere a possibilidade de manutenção de aspectos de transmissão de impulso nervoso dos organismos-teste, mesmo quando em solos contaminados com AMPA100, AMPA2500 e BC20. Já quanto ao sistema antioxidante de "*Eisenia andrei*", a mistura AMPA2500+BC20 induziu um aumento na atividade da CAT ( $p<0,05$ ), diferindo estatisticamente de todos os tratamentos. Os organismos do grupo AMPA2500+BC20 apresentaram, ainda, atividade aumentada da GST, quando comparados aos expostos à mistura AMPA100+BC20 ( $p<0,05$ ), indicando a influência dos níveis de AMPA sobre processos de detoxificação de xenobióticos, quando em mistura com BC20. A presença de BC20 resultou também em inibição na atividade da SOD, quando em mistura com AMPA100 (tratamento AMPA100+BC20), em comparação à mesma concentração de AMPA, porém sem biochar ( $p<0,05$ ), sugerindo que o biochar pode influenciar no potencial ecotoxicológico do AMPA100 em afetar a enzima SOD. Adicionalmente, a resposta dos indivíduos a AMPA100+BC20 diferiu do controle negativo ( $p<0,05$ ), quanto à atividade da SOD, o que demonstra a importância dos ensaios em misturas destes xenobióticos, para se avaliar possíveis impactos ecotoxicológicos da utilização de biochar de casca de arroz em solos com concentrações residuais de AMPA, especialmente tendo em vista o panorama de uso mundial de glifosato na agricultura. O presente trabalho destaca a necessidade de que tecnologias de disposição de biochar em áreas agrícolas sejam melhor estudadas quanto a aspectos ecotoxicológicos.

### 141 Estudos ecotoxicológicos com Biocarvão e AMPA isolados e em mistura, sobre a reprodução de "*Eisenia Andrei*"

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A fim de fundamentar o uso do biocarvão de casca de arroz (BCA) como proposta para aplicação segura em solos naturais contaminados com ácido aminometilfosfônico (AMPA), principal metabólito do herbicida glifosato, o presente trabalho tem como objetivo identificar em condições laboratoriais os efeitos do AMPA e do BCA, isolados e em misturas, sobre a reprodução das minhocas. Foram realizados ensaios de reprodução com BCA (pirólise 700° C), a partir de exposição dos organismos às concentrações 20 ton.ha<sup>-1</sup> isolado e em mistura com AMPA na proporção de 100 e 2500 µg.kg<sup>-1</sup> SAT, seguindo a norma ISO 11268-2 (2012). Foram avaliados os parâmetros biomassa dos adultos, número de casulos e juvenis. Após 14 dias de exposição, as misturas de biocarvão, nas duas concentrações de AMPA, reduziram significativamente a biomassa dos adultos, quando comparado com o tratamento

AMPA100 ( $p < 0,05$ ). Aos 28 dias, as misturas apresentaram diferenças significativas em relação à redução de biomassa do controle, assim como o tratamento AMPA100 isolado ( $p < 0,05$ ). AMPA isolado, ou em mistura, induziu uma redução de biomassa em relação aos organismos expostos apenas ao BCA ( $p < 0,05$ ). AMPA2500 isolado influenciou na redução de biomassa em relação a mistura com AMPA100 ( $p < 0,05$ ). A mistura de BCA com AMPA2500 reduziu a biomassa das minhocas em relação a presença isolada do AMPA2500. É possível fazer inferências quanto à baixa toxicidade do biocarvão. Porém, quando em mistura, o mesmo parece influenciar na biomassa dos adultos, o que pode estar relacionado com processos de sorção do AMPA, aumentando assim o potencial ecotoxicológico. Em relação à reprodução, a mistura com AMPA100 resultou em uma produção maior de casulos em relação ao AMPA100 isolado e AMPA2500 isolados ( $p < 0,05$ ). No grupo AMPA2500 isolado foi encontrado um número de juvenis menor que AMPA100 isolado e em mistura ( $p < 0,05$ ). A presença de biocarvão com AMPA2500 apresentou um redução no número de juvenis quando comparado com a mesma concentração de AMPA, porém sem biocarvão.

#### 142 Estudos ecotoxicológicos de biochar de casca de arroz sobre "Eisenia andrei"

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O uso de resíduos agrícolas, como a casca de arroz carbonizada, para aplicações na agricultura está associado à alta disponibilidade da matéria-prima no país. Para agregar valor a este resíduo, a segurança em sua disposição em áreas agrícolas precisa ser garantida por meio de ensaios ecotoxicológicos. Neste contexto, tendo em vista as possibilidades de aplicação de biochar de casca de arroz em sistemas agrícolas, faz-se necessário caracterizar sua toxicidade ao ecossistema solo. O presente estudo objetivou avaliar os efeitos ecotoxicológicos da aplicação de biochar de casca de arroz (BCA), utilizando como modelo a espécie "Eisenia andrei". Foram realizados ensaios de reprodução com BCA produzido por pirólise, em três temperaturas, 350, 450 e 550 °C, a partir de exposição dos organismos às concentrações de 2,5, 5 e 10 ton.ha<sup>-1</sup>, seguindo a norma ISO 11268-2 (2012). Foram avaliados as variáveis de biomassa dos adultos, número e massa de casulos e juvenis, em 28 e 56 dias. As variáveis foram analisadas quanto aos fatores concentração e temperatura, por meio da Análise da Variância fator duplo, seguido do post-hoc test Tukey-HSD ( $\alpha = 0,05$ ). Foi possível observar que as variáveis fuga, número e massa dos juvenis e decasulos aos 28 e 56 dias não apresentaram diferenças estatísticas significativas entre as concentrações e as diferentes temperaturas de pirólise do biocarvão ( $p > 0,05$ ). Contudo, o % de redução de massa de adultos expostos a concentração de 2,5 ton.ha<sup>-1</sup> de biocarvão de casca de arroz foi significativamente menor do que a massa do respectivo grupo controle e demais concentrações ( $p < 0,05$ ) em todas as temperaturas. Conclui-se que o biochar de casca de arroz produzido a partir de pirólise a 350, 450 e 550°C pode ser considerado como tendo impactos ecotoxicológicos reduzidos, em avaliação com a espécie "Eisenia andrei".

#### 143 CO<sub>2</sub> - induced acidification on sediment affects the fecundity of the estuarine copepod Nitokra sp.

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The use of Carbon Capture and Storage technology (CCS) is increasingly being considered as a viable alternative to reduce the troublesome level of CO<sub>2</sub> that is emitted to the atmosphere. This technology basically consists in capturing the CO<sub>2</sub> at the main pollution sources and transporting to safe geological storage. Despite this technology is considered safe, leakages of CO<sub>2</sub> to water column can occur causing an increase of the ocean acidification process which can result in damages on marine biota such as benthic copepods. Apart from the direct effects of acidification, protons can interact with contaminants in sediments such as metals, making them more bioavailable. Considering these aspects this study investigates the effects of acidification caused by CO<sub>2</sub> leakage on the fecundity of fertilized females of copepod *Nitokra* sp, in three different areas at Baixada Santista estuarine system (polluted and unpolluted), under different pH values (8.2, 7.0 and 6.0). Benthic copepods as *Nitokra* sp, have an essential role as the principal link between primary producers and the other trophic level of the marine ecosystem. The sediment collected on the first polluted area is near the ferryboat that connects the city of Santos with Guarujá; the second polluted area is in Piaçaguera channel (close to petrochemical and siderurgy plants); and the sediment

chosen as control, due to the little anthropic activity is located in Itaguapé beach. The bioassays were performed over a 96-hour period, with 10 fertilized females per treatment. The two - way ANOVA showed that fecundity of *Nitokra* sp tends to decrease as the pH values fall in both areas, due to acidification caused by CO<sub>2</sub> leakage from the marine substrate. Supplementary to that, a smaller number of descendants was detected in both polluted area when compared to the unpolluted one. This difference in progeny number can be related to the contaminants, such as metals that can be found in the sediment of polluted areas. The presence of metals in polluted substrate could be connected to an intensive traffic of ships that leads to the port of Santos especially near the ferryboat area, and the presence of industries of the Industrial Pole of Cubatão may also explain the presence of metals in the area of Piaçaguera channel. Therefore, this work contributes to a better understanding about the implications of acidification caused by CO<sub>2</sub> leakages deriving from Carbon Capture and Storage technology in marine biota and their risks.

#### 144 Effects of ocean acidification on estuarine bivalve molluscs: cytotoxic and lethality responses

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Rising CO<sub>2</sub> concentrations and contamination in coastal ecosystems are among the main problems concerned this century, once it causes serious environmental perturbations. As a mitigation proposal to reduce the CO<sub>2</sub> levels, Carbon Capture and Storage has been used. However, leakage of the stored CO<sub>2</sub> may occur leading to acidification processes that could enhance the mobility of contaminants present in the sediments and consequently cause toxic effects on marine biota. To understand the biological impacts of these global trends, the aim of this work was to evaluate lethal and sublethal effects of two marine bivalve molluscs, *Crassostrea brasiliana* and *Mytella charruana*, under estuarine sediment toxicity testing in ocean acidification scenarios. To do so, the bivalves were exposed to contaminated or control sediments during 10 days for *C. brasiliana* and 14 days for *M. charruana* at different pH, ranging from 8.1 to 6.5. The endpoints measured were cytotoxicity (Neutral Red Retention Time - NRRT) and survival, analyzed through ANOVA. Cytotoxic test presented significant average drop (48%;  $p < 0,05$ ) for the oysters over the exposure period at the control sediment assay, while mussels showed a subtle but significant improvement (17%;  $p < 0,05$ ), indicating the ability of acclimatization of the healthy individuals of *M. charruana* to the physiological stress of acidification. In the sediment-contaminated assay, a similar pattern was observed, once both bivalves decreased NRRT, oysters (8,5%;  $p < 0,05$ ) and mussels (16%;  $p > 0,05$ ), showing a drop in health status of the organisms according to increase in mobilization of contaminants. In both tests, oysters showed less NRRT than mussels. At control sediment assay 100% survival of oysters was observed in contrast to an average of 75% of mussels survival. Mussels also presented lower survival values in all pH treatments in contaminated sediment, indicating a higher resistance of the oysters to acidification. However, there is a convergence of lower survival rates for both species at more acidic pH in contaminated sediments (oysters, 28%; mussels, 79%), indicating lethal effects on bivalves when there is synergy between contamination and acidification. The results evidence that mussels are more susceptible to lethal effects under short-term exposure whereas oysters had more pronounced sublethal effects in acidification and mainly with contamination conditions, thus indicating ecological impacts of this scenarios in marine bivalve.

#### 145 Histopathological evaluation as a biomarker of health status of Mytella charruana exposed to CO<sub>2</sub> induced seawater acidification

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Ocean acidification is a rising concern due to its deleterious effects on aquatic organisms. Besides that, combined effects of acidification and other local stressors like metal pollution are largely unknown. Acidification changes the seawater chemistry promoting mobility of many contaminants from the sediments to the water column increasing its availability. Considering that gills are the first target of toxic water contaminants, the present study aims to use histopathological evaluation of gills as a



biomarker for health status in adult mussels *Mytella charruana* exposed to CO<sub>2</sub> sediment acidification and metal toxicity. For that, mussels were exposed for 14 days to experiments involving direct release of CO<sub>2</sub> simulating different pH (7.6, 7.0, 6.5 and 6.0) and a control seawater pH (8.1) in estuarine sediments from a reference or a polluted site. After the euthanasia, tissues were paraffinized and stained with Hematoxylin-eosin for a semi-quantitative histopathological analysis of the gills. Ten parameters of histopathological alterations in gills with different weight and intensities were used to be statistically analyzed through two-way ANOVA. The results from the reference site showed significant differences ( $p < 0.05$ ) between pH 6.0 and control pH 8.1. In contrast, the polluted site presented progressive damage according to pH reduction since significant differences ( $p < 0.05$ ) were evidenced between control pH 8.1 and pH 7.0, 6.5 and 6.0, with exception of pH 7.6. Our results indicate that acidification alone does not appear to affect individuals within 14 days, except at the lowest pH exposed (6.0). However, when occur synergic effects between acidification and contamination, histopathological changes in gills can be observed at almost all pH, probably due to the increasing in mobilization of contaminants. The absence of response at pH 7.6 indicates that *M. charruana* has physiological mechanisms that protect body tissues against short-term exposure and low pH changes but seems to be not sufficient in highly acidified seawater condition. As *M. charruana* is an important marine species for commercialization and ecologically, the investigation of the health status through histopathological evaluation is relevant for the monitoring of water quality and environmental assessment.

#### 146 Sediment Toxicity Identification Evaluation: the importance of the test species

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The Toxicity Identification and Evaluation approach (TIE) has the ability to identify the substance responsible by the toxicity observed in complex mixtures, such as sediments. Such approach involves the combination of toxicity tests and physical and chemical manipulations with the tested samples, which changes selectively the toxicity of specific classes of contaminants and provide evidences on the culprit for the toxicity. However, sediment TIE does not have a standardized protocol, presenting methodological variations both in relation to the toxicity tests and the chemical manipulation. It is pertinent to evaluate not only the adequacy of the standardized toxicity tests, but mainly to investigate other toxicity tests that provide greater reliability in the TIE results. In the current study we aimed to assess the importance of the test organism in a TIE framework through the development and application of a TIE using the mortality test with *Nitokra* sp. nauplii and comparing to a TIE using the standardized sea urchin embryolarval development test. Sediment samples were collected at sites under the influence of different contamination sources in the Santos Estuarine System, plus a reference area to assess the contribution of natural confounding factors to the toxicity. The discrepancy of responses among the TIE frameworks suggest that metals at most sampling sites, are available to the organism that feed on the sediment (*Nitokra* sp. nauplii) but not to organisms exposed to contaminants via water (sea urchin embryos). On the other hand, sea urchin embryolarval development was more sensitive to acid-volatile substances present in the interstitial water, probably sulfides. This may be because *Nitokra* sp. is probably more tolerant to substances commonly found in estuarine sediments. In addition, in cases when the exposure route does not influence the difference in responses, the sea urchin embryolarval development test, which is based on a sublethal response, tends to be more sensitive than the mortality test with *Nitokra* sp. nauplii which consists in evaluating a lethal response. Therefore, the comparison between the tests of *Nitokra* sp. mortality and sea urchin embryolarval development showed the importance of choosing different test organisms to cope with the complexity of the mixture of contaminants in the sediments, different sensitivities to different contaminants and to the naturally occurring substances and different routes of exposure each organism.

### Challenges and new developments in environmental risk assessment of pesticides in Latin America

#### 147 Ecotoxicological Studies in Laboratory to Determine Pesticide Effects on Bees

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Demand for food driven by population growth will require an increase of agricultural production by at least 70% by 2050. With the increase in concerns about protection of bee populations and the ecosystems services they provide, correct management of pesticides is essential to conserve pollinators. There have been extensive discussion on methodologies for new studies and risk assessment procedures in Europe and North America and more recently in Brazil. A publication from IBAMA of a proposal for risk assessment to bees was released in 2017, representing an important step on this direction for LATAM. A tiered approach is fundamental to risk assessment with laboratory studies in first step of the effects characterization. Such studies are routinely performed by the agrochemical industry under harmonized guidelines and GLP. Publications of data from the academic sector are also common and can provide valuable additional information to aid the risk assessment. Review of recent publications has highlighted a number of issues which limits their applicability in a regulatory scenario, e.g. relationship of offered concentrations to those encountered by bees in the field and reporting of offered concentrations rather than actual consumed doses. This paper will define a process for defining appropriate concentrations for ecotoxicological studies with bees in two approaches. 1) to determine endpoints for the risk assessment: the range of concentrations chosen should be based on previous knowledge from a range-finding test, allowing the results to provide a dose-response that can meet the statistical determination of such endpoints 2) to test the effect of concentrations in the field: it is important to reflect the level of exposure that bees will likely experience in the field. For oral toxicity studies the relevant concentration can be determined by considering the dilutions of the active ingredient from the applied product (formulation) to the tank mixture until it reaches nectar and pollen residues. After these dilutions the amount of product bees will ingest is related to the intake of pollen and nectar, data that is available for honeybees. The representativeness of the honeybee toxicity data to other non-*Apis* species also will be reviewed. Data from the literature will be presented to highlight the importance of taking into account the size of the species in defining toxicity to define extrapolation factors.

#### 148 Measurement of residues in bee relevant matrices of crops in Brazil

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The honey bee is an important beneficial insect. Due to the specific use of honey bees for crop pollination, honey bee colonies can be exposed to pesticides when foraging for nectar and pollen from treated crops. The traces of pesticides present in pollen and nectar (bee relevant matrices) represents a realistic potential exposure level of what bees could find in the environment, therefore a robust and reliable method to sample and measure these residues is part of risk assessment schemes in several parts of the world, also in the recently developed Normative Instruction 2-2017 published by IBAMA. Exposure studies that measure the level of residues in bee relevant matrices are considered higher tier study designs. Since field agronomic conditions should be re-created in these studies and at the same time highly accurate analytical measurements need to be done from the samples that are collected in the field there is a series of challenges to overcome. Here, we describe different methods that were used to measure trace level residues in pollen and nectar of representative crops of tropical areas and in particular of the Brazilian agriculture. We describe general methods developed to sample pollen and nectar, focus on some common issues encountered during the conduct of these studies and place the measurements derived from these studies into a risk assessment context. Exposure of africanized bees can be derived from residue studies on surrogate treated or target crops through residue analysis of pesticides in bee relevant matrices.

#### 149 Side-effects evaluation of pesticides on africanized honey bee colonies in higher tier studies in Brazil

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The honey bee is an important beneficial insect. Due to the specific use of honey bees for crop pollination, honey bee colonies can be exposed to multiple and prolonged pesticides when foraging on treated crops for nectar and pollen. In agriculture, practices to control crop pests including pesticides, should not pose unacceptable risk to honey bee health at the colony level. As part of a risk assessment studies to evaluate the potential side-effects on colonies can be used to simulate realistic field worst case exposure scenario. The design of such studies set a series of challenges since field exposure conditions should be re-created but and at the same time be influenced minimally by external other factors different from the evaluated test item. Here, we

compare different methods which were designed to explore this goal, focusing on strengths and weaknesses of the most common study designs and identifying some common issues encountered during the conduct of these study types. The relevant endpoints derived from these studies are discussed. In semi-field studies based on EPPO 1/170 (4), colonies are set up in individual tunnels erected over a highly attractive flowering crop or the target crop applied with the test item. The bees are usually exposed to the treated crop for 7-10 days. In a field test according to EPPO 1/170 (4), multiple colonies are set up at the edge of at least one field treated with the test substance. Potential effects can be detected by comparing data to colonies set up at an untreated field.

**150 Estimating Environmental Quality Standards of antifouling booster biocides**  
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Many antifouling biocides (AFB) have been developed and applied to different types of embarkation to avoid biofouling. Currently, copper and zinc oxides are usually employed as biocides in paint formulations. In addition, organic biocides are also incorporated to boost their toxicity, resulting in paint formulations containing up to four AFB. Because booster biocides may pose toxicity to non-target organisms, there have been effort to understand the toxicity and effects of such compounds to wildlife. In order to investigate if and how these AFB disturb coastal ecosystems, the main goal of this work was to estimate and refine their Environmental Quality Standards (EQSs), which are tools used for assessing the chemical status of water bodies, discussing the findings towards hazard and risk assessment in coastal waters. Published data on the ecotoxicity of the booster biocides dichloro-octylisothiazolin (DCOIT), Irgarol 1051, diuron, 2-(thiocyanomethylthio)benzothiazole (TCMTB), chlorothalonil, dichlofluanid, thiram, zinc pyrithione, triphenylborane pyridine (TPBP), capsaicin, nonivamide, tralopyril and medetomidine to coastal organisms were collated, and used to derive EQSs following the European Technical Guidance. EQSs were estimated based on the calculated Predicted No Effect Concentrations (PNECs), using either the deterministic (single toxicity values) or the probabilistic (species sensitivity distribution (SSD)) approaches. Resulting EQSs ( $\mu\text{g.l}^{-1}$ ) were: Irgarol 1051:  $1.4 \times 10^{-3}$ ; diuron:  $2.2 \times 10^{-2}$ ; chlorothalonil:  $8.5 \times 10^{-2}$ ; dichlofluanid:  $2 \times 10^{-1}$ ; ZnPT:  $1.4 \times 10^{-2}$ ; DCOIT:  $6.7 \times 10^{-4}$ ; TPBP:  $6.2 \times 10^{-3}$ ; TCMTB:  $8.6 \times 10^{-2}$ ; and thiram:  $1.8 \times 10^{-1}$ . EQSs for capsaicin, nonivamide, tralopyril and medetomidine could not be estimated due to a lack of toxicity data. Comparing the calculated EQSs to the published data on the occurrence of these AFB in coastal waters, it was found that Irgarol 1051, diuron and thiram have been detected above their EQSs in Northeast Brazil, indicating that such AFB may pose high risk to Brazilian coastal ecosystems. Worldwide, Irgarol 1051, diuron and DCOIT were detected in levels above their EQSs. The present study has contributed to estimate reliable EQSs, which can be used in the "effects assessment" tier in environmental risk assessments of antifouling biocides to Brazilian and world coastal ecosystems.

## Environmental issues related to the failure of the Fundão iron mining Dam

**151 Ecotoxicity assessment of the iron mining waste to cladocerans by different routes of exposure**

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In 2015, the rupture of the "Fundão" Dam, in Minas Gerais state, was considered of the worst environmental disasters in Brazil, and implications to the aquatic and terrestrial environment are being reported in short and long terms as well as in temporal and spatial scale. The impacts to the aquatic system included reduction of primary production and dissolved oxygen, increase of suspended material, conductivity and metal concentration, with several consequences to the biological communities. In this scenario, the organisms were exposed to different routes of exposure, including metals dissolved in the water and associated to the particles dissolved or in suspension, or their release from the sediment. Hence, the aim of this study was to evaluate the toxicity of the mining waste to the cladocerans *Daphnia similis* and *Ceriodaphnia silvestrii*. Test conditions were based in the procedures described by ABNT NBR 12713/2016 guideline and were performed with combinations of natural soil (NS) and waste (W) as follow: P1= 75% NS and 25% W;

P2= 50% NS and 50% W; P3= 25% of NT and 75% of the W and P4= 100% W. For each combination natural soil - waste, two experimental conditions were tested. In the first condition, both species were exposed to the natural soil or waste combination + water from the organisms cultures in the 1:4 proportion (weight:volume). In the second condition, tests were performed using the elutriate, as described by SETAC (1993). Results obtained in the different conditions were compared to the control treatments by One way ANOVA and no statistical differences were found to both species tested at the acute toxicity tests performed. Because the organisms tested are inhabitant of the water column, they will only be affected if the contaminants are available in the water fraction. The metals present in the eluate were not enough to cause acute toxicity to the organisms tested. Results described here does not suggest that the waste cause no effect to the cladocerans at all, but that further studies must be performed in order to evaluate long term toxicity. Furthermore, metals present in the sediment may be released to the environment by different ways, which were not assessed in this study, i.e. changes on physical and chemical properties of the water and sediment, adsorption or biomagnification at the food chain.

**152 Acute and chronic toxicity of the iron mining waste to Chironomus sancticaroli (Diptera)**

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The deposit of mining wastes implies, between other problems, in risk of dam rupture. Once the dam is broken, the entire hydrographic basin is affected with severe impacts to the aquatic and terrestrial environment. In the aquatic system, part of the waste will settle down in the sediment affecting the benthonic organisms, such as the macroinvertebrates as the Chironomidae larvae (Diptera), beside other aquatic organisms. In this context, the present study aimed to evaluate the acute and chronic toxicity of the iron mining waste, from the rupture of the "Fundão" Dam (Minas Gerais, Brazil), to *Chironomus sancticaroli*. Organisms has been maintained in the laboratory (NEEA/SHS/EESC/USP) according to Fonseca (1997) and EPA (2000), modified by Viveiros (2012). The toxicity was evaluated using five treatments, which consisted in the mixture of a natural soil (NS) from Mariana city (uncontaminated) and the waste (W). The combinations of mixture were: P1 = 75% NS and 25% W; P2 = 50% NS and 50% W; P3 = 25% of NT and 75% of the W and P4 = 100% W. No observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) were determined by the one way ANOVA, followed by the Dunnet test. Lethal doses (LDx) were calculated by the Probit nonlinear regression. For the acute tests, no significant differences were found between the control and the waste treatments. However, effects on the survival of the organisms were found at the chronic test. There was a reduction in 50, 20 and 10% of the organisms at the proportions of 65.0; 19.7 and 10.5% of the waste, respectively. Because the *Chironomus sancticaroli* are organisms that inhabit the sediment, they are important bioindicators of pollution on this compartment, which receives a large amount of the waste due to its dynamic in the aquatic environment.

**153 Analysis of element contamination in farmers' water living throughout Rio Doce after dam collapse**

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The worst environmental disaster in Brazilian history, Samarco 'Fundão dam' collapse in Mariana, state of Minas Gerais, caused a massive flood of mud composed of mining waste. The flood ran throughout more than 600km of the Doce River, resulting in direct destruction of ecosystems, impacts on fauna and flora, as well as serious socioeconomic losses that caused problems regarding clean water supply for human consumption and agriculture. This study aimed to evaluate the water suitability for agriculture and human consumption used by family farmers in Minas Gerais and Espírito Santo after the tragedy by the quantification of chemical elements. In July 2016, 8 months after the event, samples of water - well, river or public service - used for human and animal consumption and irrigation were collected at 48 points in 3 cities affected by the rupture of the dam: Belo Oriente (n=16), Governador Valadares (n=16) and Colatina (n=16). The analysis focused on: Cr, Cu, Ni and Zn determined by FAAS; Fe, As, Cd, Pb, Sn and Mn on ICP-MS, and Hg-total on CVAFS. The concentrations of As, Cd, Cr, Cu, Hg, Ni, Pb, Sn and Zn were within levels considered as safe for human consumption and agriculture use. The highest concentrations were found for Fe and Mg. Belo Oriente, Governador Valadares and Colatina had respectively 5, 12 and 10 points with values above the stipulated as safe for human consumption and/or

irrigation by the National Environment Council (Brazil). Our study revealed that water used by many families through the Doce River are unsuitable for agriculture or consumption. There are no pre-disaster studies in the region, so we can not affirm the disaster is responsible for these high levels. Nevertheless, it is very important to note that the disaster caused families shifting the source of water they rely on, from river to well or neighbour donation, due to lack of public and private support. We can highlight that the use of this water in the long term can be associated with health risks.

#### 154 Ecotoxicological assessment of the Doce river surface waters after the Fundão dam collapse

F. Aquinoga de Mello, L.B. Mendes, APLYSIA Environmental Research and Solutions; R.P. Campelo, L.C. Medeiros, B.V. Pimenta, RENOVA Foundation; R. Smith, Hydrobiology; T. Furley, APLYSIA Environmental Research and Solutions

Due to the disruption of the tailings dam in the municipality of Mariana-MG, on 5 November 2015, monitoring to assess the ecotoxicological effects in water samples collected from different sites affected by the turbidity plume was performed. For this study, 1048 ecotoxicological assays were evaluated, collected from 23 sampling points along the Doce river, between the 7<sup>th</sup> November 2015 and the 28<sup>th</sup> January 2016. Within this period it was possible to evaluate two scenarios, one in the Estate of Espírito Santo, with results from before and after the passage of the plume, and other in Minas Gerais with only data from after the passage of the plume. The assays were conducted with three different trophic levels, including the fish *Danio rerio* (247 acute assays), the microcrustaceans *Daphnia similis* (371 acute assays) and *Ceriodaphnia dubia* (190 chronic assays), and the bacterium *Vibrio fischeri* (371 assays). In addition, an evaluation with *C. dubia* of 29 samples that were tested filtered and unfiltered from after the arrival of the plume was conducted, aiming evaluate the influence of suspended material on the toxicity. The results showed, in general, the absence of acute ecotoxicity in the water samples. From 858 assays, 98.72% showed no toxicity. On the other hand, 77.89% (148/190) of the chronic assays, demonstrated chronic toxicity to *C. dubia*. The results from before and after the passage of the plume pointed to an increase in toxicity frequency after the arrival of the plume. In addition, it was observed that after 2.5 weeks after the arrival of the plume toxicity levels returned either to pre-plume conditions or decreased in toxicity. These results suggested that the chronic toxicity observed in the samples was related to the suspended material present in the water, since the reduction of the toxicity over time matched the decrease in the amount of material suspended in the water column. This was confirmed by the non-toxicity observed in the filtered samples. Furthermore, the comparison of the results from the filtered and non-filtered samples showed a reduction of the toxicity, since 96.55% (28/29) of the filtered assays did not show toxicity. These results demonstrated that the toxic effects observed were related to the passage of the plume and primarily associated with the physical effects of the presence of suspended particles, affecting the filter-feeding species in long-term exposures.

#### 155 THE BENTO RODRIGUES (MARIANA, MG) MINING DISASTER: ECOTOXICOLOGICAL EVALUATION AND MONITORING OF BIOLOGICAL IMPACTS IN ESTUARINE AND COASTAL AREAS

A. Bianchini, Universidade Federal do Rio Grande - FURG / Instituto de Ciências Biológicas

The Bento Rodrigues mining disaster occurred on 5 November 2015, when an iron ore tailings dam in Bento Rodrigues (Mariana, MG, Southeastern Brazil) collapsed. The burst dam released about 60 million cubic meters of toxic mud. The iron mining waste flowed into the Doce River and caused destruction across ~500 km along the Minas Gerais and Espírito Santo states. The toxic brown mudflows reached the "Mar de Regência" (Atlantic Ocean) 17 days later since the dam has collapsed. Evidences from 1.5-year evaluation and monitoring program developed in cooperation with the Brazilian "Instituto Chico Mendes da Biodiversidade - ICMBio" (Ministry of Environment) have indicated that the incident has significantly impacted the physicochemical characteristics of estuarine and marine waters and sediments, as well as wildlife's health. However, the total impact and environmental consequences to the river and the beaches near the Rio Doce's mouth, as well as to the wildlife are still being evaluated and monitored. A long-term (5-years plan) environmental program is currently in progress aiming to monitor the ecotoxicological impacts on aquatic ecosystems (estuary, beaches, mangroves, and marine areas) associated with the dispersion of the mining tailings that reached the Doce River estuary and the adjacent (coastal and marine) regions. Evaluation and monitoring actions are especially focused on Brazilian Marine Conservation Units (UC), such as APA Costa das Algas, RVS Santa Cruz, REBIO Comboios, RESEX Cassurubá and PARNA Marinho dos Abrolhos at the Espírito Santo and South Bahia coasts. The environmental monitoring program in course includes: (1) evaluation of metal concentrations in water and aquatic (estuarine and marine) organisms from different trophic levels (zooplankton, coral,

shrimp and fish species); (2) biomarkers responses to metal contamination in aquatic (estuarine and marine) organisms; and (3) evaluation of microbiota and detection of bioindicators of environmental impacts in sediments, water and coral reefs. Data generated in the scope of the evaluation and monitoring program in course will be presented and discussed.

#### 156 Ecotoxicological assessment of the continuous use of flocculants and coagulants after Fundão dam collapse

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The disruption of the Fundão tailings dam generated a turbidity plume that flowed through the Doce River. As a mitigation mechanism, the use of flocculants and coagulants in the Santarém dam was authorized, aiming to retain the waste, by settling the fine solids coming from the dam and reduce the suspended solids concentration in the water column. In this context, four products, being two coagulants and two flocculants were evaluated in three stages. The first being the evaluation of each isolated product, using 3 species of different trophic levels. The second, an evaluation of combinations of coagulants and flocculants proposed to be used in Santarém, through ecotoxicological assays with 8 species (4 trophic levels), to produce 4 species sensitivity distribution curves (SSD), aiming to identify the combination that presented less risk to the environment. The third phase was to evaluate samples from upstream and downstream of the dosing area, assessing the effects of the application and the removal of suspended particles. The first phase demonstrated that the coagulants had higher toxicity (0.032 to 0.33 mg/L) compared with the flocculants (0.36 to 166.56 mg/L), with *D. rerio* being the most sensitive species for coagulants and *C. dubia* for flocculants. The second stage the sensitivity of the species ranged from 0.7 to 187.14% of the proposed doses. In general, *C. sancti-caroli* was the most sensitive species, while the species *D. similis*, *D. magna*, *D. rerio* and *P. promelas* were not sensitive to the concentrations evaluated (the maximum tested was double of the proposal for use). From the SSD it was observed that the concentrations of hazardous effects to 5% of the species were between 1.42 to 2.94% of the proposed dose, and the fraction of affected species if 100% of the proposed dose directly reached the environment would be from 53.16 to 62.74%. It should be highlighted that these concentrations are related to the residual fraction of the combination and not to the proposed dose. The third phase confirmed that there was no increase in toxicity with the application, since there was no difference between upstream and downstream applications to the organisms evaluated. It was also observed that the filtration of the samples from upstream showed a reduction of chronic toxicity to *C. dubia* indicating that the toxic effects were related with the physical effects of suspended particles.

#### Environmental hazard and risk assessment for the oil and gas industry

##### 157 Development of Regulations Governing Offshore Drilling Fluids

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Offshore drilling regulations govern many technical and environmental aspects of the drilling process. In this presentation various global regulations governing the environmental impact of drilling fluids and associated cuttings to fauna living in the water column and seafloor are reviewed and assessed. Regulations determine whether drill fluids and associated cuttings generated offshore can be discharged into the ocean or must be returned to shore for disposal. Their technical and procedural requirements range along a spectrum of prescriptive and performance-based approaches. Prescriptive regulations set specific standards that operators must meet. Performance-based regulations identify outcomes for operators to meet and allow flexibility in determining how they are achieved. Governing agencies generally produce regulations that include a blend of prescriptive and performance-based approaches to determine acceptable components of drilling fluids and whether they and associated cuttings can be discharged offshore. Each of these approaches has strengths and weaknesses. Prescriptive regulations provide clear compulsory standards, ensures how these standards are met and facilitates monitoring and enforcement of standards. Performance-based regulations set environmental goals and provide for greater flexibility to meet the goals but may be more difficult to interpret and enforce. Two examples highlighting these types of regulatory approaches, that are often studied during regulatory development, are the prescriptive approach followed the United States Environmental Protection Agency in the western Gulf of Mexico and the performance-based approach followed by nations drilling in the North Sea. Examples



of how these approaches are applied to the development of regulations in Latin America and other regions of the world are offered.

#### 158 Applying Risk-based approaches when Performing Environmental Assessments for Deep-Water Offshore Disposal of Drill Cuttings

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As the global population continues to expand, with projections reaching 9-billion people by mid-century, oil and gas will continue to provide the majority of energy needs. Challenges exist however in that older fields are no longer as productive as they have been historically, resulting in new fields being tapped. These can often be in logistically challenging locations, such as deep-water (e.g. >1000 meters). Such depths present difficulties too for scientists tasked with assessing environmental risk. A primary concern is ensuring cuttings disposal does not cause undo harm to benthic communities. Deep-water environments are not easily observed and/or prohibitively expensive to conduct numerous wide-ranging, long-term field studies. As such scientists rely on laboratory testing, the limited available data from field observations globally and computer modeling to create a weight-of-evidence approach for determining risk. A risk assessment requires information on both hazard and exposure of a substance. While hazard can be determined from controlled laboratory experiments, assessing exposure scenarios in deep-water environments can be more challenging. A number of modeling platforms exist that can predict spatial and temporal exposure conditions; however, there have been a select number of comprehensive field studies from around the world (Malaysia, Ghana, Brazil, and US Gulf of Mexico), from which data on exposure conditions, and subsequent impacts on the benthic community, may be extracted. Additionally, proactive operational measures such as monitoring during drilling and post-drilling can be conducted by rig operators to ensure minimal environmental damage and provide data for future analysis. Thus, hazard data from the laboratory combined with both modeled and field observed exposure conditions will allow for a robust weight-of-evidence risk determination. Here we present the various tools and options that can be utilized in the risk assessment process related to offshore disposal of drill cuttings along with challenges and opportunities moving forward.

#### 159 Sampling Effort of Ecotoxicity Tests in Drilling Fluids Environmental Management

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Drilling fluids are complex mixtures of chemicals elaborated to support drilling activities such as: well cleaning, cuttings suspension, cooling and lubricating of the bit, minimize friction along the well column, stabilization of the well and pressure balance. Drilling fluids are pumped into the wells, descending through the column and, once expelled by the bit, they return to the surface through the space between the column and the wall of a well. When water based fluids are used for drilling in sections with riser, this fluid is discharged to the sea after some environmental tests to verify the quality of the effluent. In 2015 CGPEG/IBAMA[1] has required a comprehensive Fluids Monitoring Program under the licensing processes for the Oil & Gas industry which states several tests to be performed in the fluids that are discharged to sea by the drilling platforms. The main purpose of the fluids monitoring program is to assure minimal quality of the effluents to be discharged to the sea to avoid harmful effects in the environment. The ecotoxicity test is one of the required parameters from the program. Samples shall be taken both prior (pre-use) to its use and its discharge to sea (post-use) and sent to an onshore lab to be analyzed. A total of 136 tests have been performed in Peregrino Field Drilling fluids since the new directives became valid, with 97.8% of the results within the 30.000 ppm limit established and 100% of the 'pre-use' tests approved. The results demonstrate that testing the fluids prior to its use would not be necessary when a good management of the formulations is handled. Furthermore, the real impact might come from what is being in fact discharged. Hence, it is suggested that testing the fluids only before its discharge would be enough for the effectiveness of the program. [1] Brazil Environmental Authority for the Oil & Gas Industry.

#### 160 Optimizing Regulatory Test Methods - A Review of Sediment Toxicity Testing of Synthetic Drilling and Base Fluids used in the Gulf of Mexico.

J. Griffith, Environmental Enterprises USA, Inc. / Drilling Fluids; V. McNew, Environmental Enterprises USA, Inc. / Marketing Manager

Test methods mandated for environmental regulatory testing are not always optimized for the application in which they are applied. The test organism selected will play a major role in the significance, success, and interpretation of test results. It is important to select test organism species based on environmental relevance as well as practical

concerns such as availability of inter laboratory standard reference toxicant data, wide particle size distribution, total organic carbon and total volatile solids tolerance ranges, commercial availability, established laboratory culture methods, and compatibility with the selected test material substrate, exposure methods, and endpoints. It is also important to optimize specific test procedures which are not always clearly or fully defined within test methods. This presentation will highlight the challenges of reducing variables and variability in the 10-day and 96-hour Sediment Toxicity Test, EPA Method 1644. This test method was first implemented in EPA Region 6 in 2001 for synthetic base fluids and synthetic base drilling fluids which would be used for drilling in the Gulf of Mexico. Collaboration between commercial and private labs has helped to reduce intra and inter laboratory variability by developing a unified Standard Operating Procedure. Since bioassays involve live organisms, it is impossible to eliminate all variability but independent research at Environmental Enterprises USA, Inc. has helped minimize extraneous sources of variability within the method so that meaningful test results can be achieved with greater repeatability and less variability.

#### 161 ALTERNATIVE BIOLOGICAL MODELS AND TOOLS FOR ASSESSING THE FATE AND EFFECTS OF HYDROCARBONS ASSOCIATED WITH OIL AND GAS ACTIVITIES IN AQUATIC SYSTEMS

A. Bianchini, Universidade Federal do Rio Grande - FURG / Instituto de Ciências Biológicas

Brazil has been poised for the adoption of native species as biological models, as well as the development of robust tools to ensure that environmental risks associated with chemical contamination of aquatic ecosystems are correctly understood. Oil and gas activities have been significantly raised in Brazil, with exploitation being especially based on offshore wells and facilities. Therefore, scientifically validated approaches to develop risk assessment strategies for new and existing oil and gas activities are urgently needed, especially in deep sea areas. In the last two decades, our research team has developed several studies aiming to identify adequate biological models and to develop new and alternative tools for assessing the fate and effects of aquatic contaminants, including hydrocarbons. Research performed has included several species of planktonic, nektonic and benthic organisms from freshwater, estuarine and marine ecosystems. In turn, biological tools to assess the fate and effects of chemical contaminants, including hydrocarbons, have comprised a suite of molecular, biochemical, physiological and genetic biomarkers. Main findings and conclusions arising from our studies will be presented and discussed. Focus will be done on Brazilian native species as suitable biological models, as well as on selected and useful biomarkers for assessing the fate and effects of hydrocarbons in aquatic systems, especially deep sea areas.

#### 162 Meta-Barcoding accelerates species discovery and unravel great biodiversity of benthic invertebrates in marine sediments in Campos basin, Brazil

M. Rebelo, UFRJ

Biodiversity is currently assessed for environmental characterizations and monitoring through a laborious and time-consuming process of morphological taxonomy. We used rRNA 18S, rRNA 28S and COI, together with NGS and Bioinformatics to identify benthic invertebrate organisms from sediment samples collected in five stations in the Campos Basin in southeast Brazil, an important oil extraction area and one of the best-studied marine biota in Brazil. A total of 3.3 million sequences were clustered in Operational Taxonomic Units and more than 1.6 million sequences (about 50% of all reads) were assigned to 957 prokaryotes and 577 eukaryotes. BLAST identified 23 phyla, 60 classes, 62 orders, 70 families, 67 genus and 46 species of eukaryotes. By meta-barcoding we identified phyla that are traditionally found in samples of marine benthos, such as Annelida, Arthropoda, Mollusca and Chordata, as well as rare phyla like Entoprocta and Gastrotricha. Taxa identified with meta-barcoding were compared to morphology data from previous studies in the area (REVIZEE, Habitats Project) and geo-validated with the database Global Biodiversity Information Facility. For several taxa, this is the first evidence of occurrence in Campos the area and the number of OTU identified suggests an enormous unveiled benthic biodiversity in Campos Basin. Our study supports the application of Meta-Barcoding for environmental characterization and monitoring programs, reducing from years to few months the time currently required for species identification and biodiversity determination.

#### 163 Laboratory-scale Bioremediation of Drill Cuttings Containing a Synthetic Drilling Fluid

G. Rosales, E. Cacchione, Shell Cia Argentina de Petroleo SA

The process of drilling oil and gas exploration and production wells requires the use of a fluid, termed drilling mud, to lubricate, cool, help move material out of the well bore, and maintain well bore integrity. The drilling mud can either be made with water

or a non-aqueous drilling fluid (NADF) as the lubricating agent. In this study, a synthetic Shell drilling fluid, termed Neoflo 4633, was used to drill wells in the Neuquén area in Argentina. During drilling, the drilling mud mixes with the material from the well bore, generating what is called drill cuttings as a waste stream. The standard method for disposing of drill cuttings in this area is incineration. However, due to the more environmentally friendly profile of Neoflo 4633, the possibility of using bioremediation to treat the cuttings was raised. This presentation describes a laboratory experiment exploring bioremediation as a method for treating drill cuttings in Argentina. Both composting and landfarming were assessed using local soils, fertilizers, and bulking agents. Drill cuttings were mixed with soil from Sierras Blancas to obtain initial total petroleum hydrocarbon (TPH) concentrations less than 10%. Locally-sourced poplar wood chips were added as a bulking agent, to improve soil aeration and handling. Two types of fertilizers were assessed as a source of nitrogen, phosphorus, and potassium: an inorganic fertilizer (diammonium phosphate and potassium nitrate) as well as organic fertilizers made from pig manure pellets. The TPH degradation process was monitored for 81 days, during which total heterotrophic colonies (CFUs) were regularly counted. Also, the composition of the residual hydrocarbon was also analyzed using gas chromatography at varying time points. After 50 days of treatment, TPH degradation in the intermediate zones with an added inorganic fertilizer reached 89% in biopiles and 81% in landfarming. In the case of the production zone, TPH degradation in biopiles with inorganic fertilizers reached 68% at the end of the test (81 days), while the landfarming percentage was slightly above that value. Total bacterial count throughout the test period showed increased bacterial activity levels during the process.

#### 164 Distribution and sources of Polycyclic Aromatic Hydrocarbons (PAHs) in surficial sediments of Todos os Santos Bay (Bahia, Brazil)

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Coastal environments contaminated by Polycyclic Aromatic Hydrocarbons (PAHs) have been a constant concern due to the mutagenic, carcinogenic, and toxic properties of these compounds. Todos os Santos Bay (TSB), one of the largest bays in the world, presents anthropogenic activities which are potential contamination sources. The present study aimed to evaluate the PAH distribution and origin in TSB sedimentary matrix. Forty sediment samples were collected throughout the study area. The analysis of the 16 PAHs, listed as the priority by the United States Environmental Protection Agency (USEPA), was performed using gas chromatography–mass spectrometry (GC-MS). The sample sedimentary fractions were evaluated through a laser diffraction particle size analyzer. The total organic carbon content was performed through an elementary analyzer. The total concentrations of the 16 HPAs in the sediments ranged from below the detection limit of the method (< LDM) to 533 ng g<sup>-1</sup> dry weight. The highest PAH concentrations were from areas close to the coast, associated with anthropogenic activities developed along the bay. The lowest concentrations were associated with the furthest area off the coast. The contamination sources and continental drainage associated with sediment geochemistry and bay hydrodynamics influenced the PAHs distribution of throughout the study area. Diagnostic ratios indicated that pyrogenic processes as pyrolysis of fossil fuel, biomass, and coal are the main PAH sources for sediments. The PAH total levels, expressed as the toxicity equivalent (TEQ<sub>carc</sub>), are relatively low when compared to contaminated sediments from several bays in the world.

### The evolution of Life Cycle Sustainability: Approaches and environmental labeling in Latin America

#### 165 Identification of most sensitive characterization factors in the Life Cycle Assessment of main Brazilian products

C. Lie Ugaya, UTFPR / DAMEC; F. Cuzinsky, Federal University of Technology Paraná / PPGEM

Characterization factors (CFs) are used in Life Cycle Assessment (LCA) to obtain the potential environmental impacts of products and organizations. Many impact categories, as acidification, eutrophication, smog and water scarcity, vary according to regional conditions, demanding regionalized CFs. Currently there are several methods used for each impact category. In a former study, it was noticed that there is a slight change in the number of elementary processes that mostly contribute depending on the method. In fact, regionalization of CFs has been identified as an important topic by the Brazilian Life Cycle Impact Assessment Research Network, the Brazilian LCA Programme and by the attendees of the Brazilian LCA Forum aiming to improve the results obtained in LCA studies. As regionalizing may rely upon huge amount of data, that are not always available, and data processing and assessment, the

aim of this study is to identify the elementary flows in which the CFs should be prioritized. With this purpose, the most sensitive CFs in LCA of main Brazilian products was obtained. Sensitivity analysis (SA) has been used in several LCA studies, but very few based on perturbation analysis. Besides, the application of SA is mostly in the inventory phase. The list of products was based on the list published by IBGE (2011) and selected according to a previous study. Then, the relative perturbation of the CFs to 417 impact categories (except climate change, ozone depletion and resources) available in ecoinvent v.3 was calculated in MatLab. The results showed that among the impact categories, 24 presented sensitivity higher than 1, most of them related to ecosystem quality and land use. Moreover, among the 84,686 CFs, less than 0,1% has a perturbation higher than 1 and less than 2%, higher than 0,1. All in all, the results of the study showed that very few impact categories and elementary flows are sensitive to changes and the efforts to regionalize of the CFs could be based on this list.

#### 166 Freshwater Eutrophication Fate Factor of the Brazilian sub watershed Litorânea do Ceará

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Eutrophication compromises the quality of freshwater available in water bodies and have huge adverse economic effects in many sectors. This impact category in Life Cycle Impact Assessment (LCIA) depends on the fate and effect factors. The first models did not include regionalization, however, as this impact is region dependent, the recent models turned to be more sophisticated. Even so, there is still lack of regionalized characterization factors (CFs). This paper aims to estimate the fate factors (FF) for freshwater eutrophication of six hydrographic regions inside the Brazilian sub watershed Litorânea do Ceará. The method applied was developed by Helmes et al (2012) and takes into account the geographical differences by water bodies and only consider the water use for agriculture propose. Within the sub watershed Litorânea do Ceará, the agriculture water consumption and characteristics of water bodies are different; therefore, it has seen as an advantage to divide the sub watershed in six regions. The FFs varied from 5 to 160 (days) for each region and 54 (days) for the entire sub watershed, which is higher than the ones obtained in previous study. Although some improvements are suggested, this paper enables future developments of regionalized fate factors for freshwater eutrophication in Brazil.

#### 167 PROPOSTA DE AJUSTE NA CONTABILIZAÇÃO DO METANO BIOGÊNICO NO MÉTODO GREENHOUSE GAS PROTOCOL

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Pegada de Carbono é um dos índices mais usados pelo segmento empresarial para verificação do desempenho ambiental de produtos. Em termos metodológicos, tal indicador pode ser determinado por três enfoques: PAS 2050, GHG Protocol para Produtos, e ISO 14067. Em comum a todos está a obrigatoriedade de inclusão de remoções e emissões fósseis e biogênicas, e de emissões por mudança no uso da terra. No entanto, no GHG e na ISO é obrigatório relatar tais parcelas em separado. A elaboração do método GHG Protocol para Produtos envolveu diversas etapas, sendo uma delas o *road testing*, na qual algumas empresas aplicaram uma versão inicial do método para calcular a pegada de carbono de algum produto. Para auxiliar nessa etapa, a empresa PRé Consultants implementou um método de avaliação de impacto chamado Greenhouse Gas Protocol em seu software SimaPro. Nessa implementação estão indicados resultados de quatro dessas subcategorias: emissões fósseis e biogênicas, mudança no uso da terra e absorção de carbono, a qual, porém, não é classificada como fóssil ou biogênica. A confrontação dos resultados obtidos por essa abordagem com aqueles originários do IPCC 2007 GWP 100a, método padrão para análises de mudanças climáticas, denota incompatibilidades. Apoiado nessa constatação, este estudo propõe uma alteração na forma como o Greenhouse Gas Protocol contabiliza o metano biogênico (CH<sub>4,b</sub>) com o intuito de harmonizar seus resultados aos do IPCC. Esse ajuste consiste em dividir a contribuição do CH<sub>4,b</sub> em duas parcelas: CH<sub>4,b,fóssil</sub> cujo fator de impacto FI = 22,25; e CH<sub>4,b,biogênico</sub> com FI = 2,75. Além de fundamentar a proposta, a pesquisa usa exemplos de aplicação dos métodos original e com alteração do GHG para discutir efeitos de cada concepção. Um dos exemplos analisados é o produto “gado de corte”, proveniente da base de dados Ecoinvent. O resultado obtido pelo método de avaliação de impacto IPCC 2007 GWP 100a é 43% maior do que aplicando o Greenhouse Gas Protocol, levando-se em consideração apenas as parcelas condizentes. A partir dessa readequação espera-se que o método Greenhouse Gas Protocol passe a gerar dados que coadunem com a definição de cada parcela da Pegada de Carbono. A alteração também deve influenciar na contabilização de emissões de gases de efeito estufa de empresas – os inventários corporativos.

### 168 O uso da rotulagem ambiental e da pegada de carbono como fomentadores do uso da ACV no Brasil

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Apesar de largamente utilizada no mundo e de ser reconhecida como a mais completa “ferramenta ambiental”, a ACV ainda é pouco difundida no Brasil. Este fato inquietante motivou a elaboração da tese que deu origem a este artigo, no presente artigo o foco será dado a busca de formas e ações que podem colaborar com a reversão deste quadro de pouco uso da metodologia no Brasil. A pesquisa foi exploratória, composta por uma revisão bibliográfica somada a uma pesquisa de campo e teve uma finalidade aplicada. As formas pelas quais a implementação efetiva e ampla das metodologias que abordam o ciclo de vida, especificamente a ACV, no Brasil podem ser centradas, segundo este estudo, em duas vertentes, uma baseada na rotulagem ambiental e outra na pegada de carbono. Ambas mantêm vínculos estreitos com a ACV, sendo que a rotulagem ambiental pode ser entendida como resultante diretamente de estudos de ACV e a pegada de carbono pode ser entendida como uma parte integrante de um estudo de ACV. A pesquisa de campo apontou que a rotulagem ambiental pode sim ter uma contribuição válida na disseminação e uso da metodologia, o que foi reforçado no âmbito internacional e nacional, mas que poderiam haver prejuízos quanto à qualidade e amplitude do estudo, além de que ao ser implementada para este intuito, a ACV não geraria necessariamente na empresa o entendimento dos benefícios do pensar orientado ao ciclo de vida. Em relação aos consumidores, há dúvidas se iria realmente promover o entendimento esperado quanto aos impactos gerados pelos produtos certificados. Além destes pontos negativos a rotulagem ambiental, apesar do lançamento do programa do INMETRO em Março de 2016, tem perspectivas negativas quanto ao tempo necessário para seu desenvolvimento e implementação efetiva no Brasil, desta forma, não iria cumprir o objetivo de colaborar com a disseminação da ACV em tempo. Por outro lado, a pegada de carbono por ser parte da ACV, segue o mesmo padrão (quando baseada na ISO 14067) da abordagem do ciclo de vida quanto as fases e etapas a serem seguidas em sua aplicação, trazendo desta forma a prática da abordagem do ciclo de vida as empresas que a executam. Outra questão quanto ao uso da pegada de carbono como primeiro passo a implementação da ACV é que esta metodologia, em decorrência principalmente dos resultados e compromissos assumidos pelos países participantes da COP 21, tende a ter um maior uso e eventual cobrança, inclusive legal, nestes países.

### 169 Multicriteria decision analysis as a facilitator for studying sustainability in life cycle assessment: an initial reflection

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The growing awareness of the importance of environmental protection and the potential impacts associated with products and services demonstrates the interest in developing methods to better understand and reduce these impacts, such as the Life Cycle Assessment (LCA), a technique for the systematic evaluation of aspects and environmental impacts of a product or service system. However, such impacts are not restricted only to the environmental field when the objective is to analyze for sustainability. Thus, it is necessary to extend the study of LCA, including the social and economic dimensions. In this way, the Life Cycle Sustainability Assessment (LCSA), the “new” LCA, is a technique that consists of evaluating the environmental, economic and social impacts of the life cycle of a given product or service. The LCSA is composed of the integration of the Environmental Life Cycle Assessment (LCA), Life Cycle Costing (LCC) and Social Life Cycle Assessment (S-LCA) tools. However, quantifying the sustainability assessment requires an integrated methodological structure, due not only to its multidisciplinary character, but also to the complexity of the problems involved. The purpose of this article is to present an initial reflection of how Multi Criteria Decision Analysis (MCDA) can be used as an alternative to make possible the development of an integrated methodological structure for the sustainability of LCA. A systematic review of the literature was carried out to analyze the studies published in the research platforms “Science Direct”, “Scopus” and “Springer” using the keywords “life cycle sustainability assessment”, “multicriteria”, “multi-criteria”. The results of the systematic review demonstrate that MCDA is a facilitator for studying sustainability in LCA because it considers complex problems with high level of uncertainty, conflicting objectives, different types of data and multiple interests and perspectives. Besides that, in the current studies that use Multi Criteria Decision Making Methods (MCDM) in the LCSA the decision criteria for each dimension are generally based on the value attributed by the stakeholders, needing to include the technical criteria derived from sustainability indicators and environmental, social and economic impact assessment methods in decision making.

### Resolving historical gaps on microplastic pollution research:

### contributions of the Latin America scientific community

#### 170 Effect of PVC-rich diet on genotoxic parameters in erythrocytes of juvenile common snook fishes (*Centropomus undecimalis*)

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Plastic pollution in marine ecosystems represents a major global concern because of the threat to human and environmental health. Microplastics enter marine environments due to losses during the production, transportation, and tools manufacturing. Different types of plastic and additives are known to be toxic and their effects can be severe in aquatic organisms due to ingestion. The fish *Centropomus undecimalis* can be found throughout the Brazilian coast, including estuarine areas, where it is highly prized for consumption and sport fishing. Therefore, the evaluation of sub-lethal effects by ingestion of microplastics becomes of extreme importance. The objective of the present study was to evaluate the genotoxicity of PVC powder ingested as part of the diet. For this purpose, comet assay, frequency of micronuclei and nuclear anomalies in blood samples of fish exposed to the PVC enriched food were assessed. *C. undecimalis* juvenile specimens were obtained from the Marine Fisheries Laboratory of the Federal University of Santa Catarina. Thirty-six fishes, which were separated into 3 groups, were distributed in 9 aquaria of 15 liters (4 fish/aquarium) containing sea water, (3 aquaria/group): Group A (CTRL) fed with standard food; Group B, fed with PVC enriched food; Group C, fed with ration rich in PVC plus standard food. During the experiment the parameters of salinity, temperature, dissolved oxygen, ammonia and nitrite were analyzed and kept within the ideal ranges. To prepare the standard diet, the 1.5 mm diameter commercial Aquaxel food was crushed and then reconstituted in water and air dried. In order to make the PVC enriched diet, the commercial ration was crushed and mixed with PVC powder (50% w / w) and then reconstituted in water and dried in the air (PVC-rich diet). All groups were fed 3 times a day (8h, 12h and 16h) for 1 week. After one week the animals were anesthetized with clove oil and the length and weight measurements of the specimens were performed (for the calculation of the condition factor) and blood samples were obtained for assessing DNA damage. The blood smear was stained with Giemsa and subsequently 2,000 blood cells/specimen were counted for the evaluation of the presence of nuclear and micronuclei abnormalities. Blood samples were also analyzed through single cell gel comet assay. *C. undecimalis* treated with PVC powder mixed with the diet (Group B) for one week showed increased genotoxic damages to erythrocytes.

#### 171 Polycyclic aromatic hydrocarbons (PAHs) in plastic pellets from São Paulo coast, Brazil.

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Pellet in the marine environment is related to the large use of plastic generated by human activity. The raw material of the plastic is marketed in the form of granules known as pellets that are unintentionally released during the transport process, reaching the marine environment. The polycyclic aromatic hydrocarbons (PAHs) are hydrophobic contaminants that are potentially carcinogenic and can be adsorbed by plastic pellets. In this study, PAHs were analyzed in plastic pellets collected on 37 beaches of 13 cities, along the São Paulo coast, Southeast of Brazil. Before the analysis, pellets were separated by degree of discoloration and composition. The PAHs were analyzed only in polyethylene (PE) yellowing pellets for standardization with the Pellet Watch Program. The samples were Soxhlet-extracted with dichloromethane/n-hexane and the cleaned up was carried out by adsorption chromatography using a column of 5% deactivated alumina. Gas chromatograph equipped with mass spectrometer (GC/MS) was used to identification and quantification of PAH. The content of PAHs found in PE pellets ranged from < 1.0 to 1945.15 ng g<sup>-1</sup>. The highest content of PAHs as well as the highest amount of PE yellowing pellets were found in São Paulo central coast, known as Baixada Santista, and was higher than in southern and northern areas. The highest PAHs content are related mainly to the presence of the most important port of Latin America (Port of Santos) and one of the biggest industrial complex Cubatão, the most important industrial complex of Brazil. The same pattern was observed in previous studies using a mixture of polymers of different colors.



## Poster Abstracts

### Ecological Risk Assessment and Site Remediation in Latin America: The state of the practice

#### SP001 Caracterização de compostos orgânicos para serem empregados como material reativo na retenção de metais

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A Política Nacional de Resíduos Sólidos (Lei Federal nº12.305/10) discorre sobre a importância da gestão municipal integrada de resíduos orgânicos. Uma alternativa é a compostagem, um método rápido, eficaz e que demanda reduzidos recursos, tecnológicos e financeiros. Por conseguinte, a tendência é a produção, cada vez maior, dos compostos orgânicos, constituídos essencialmente de matéria orgânica humificada e biologicamente estável. Nessa perspectiva, o objetivo desta pesquisa foi promover uma caracterização geoquímica e física de dois compostos com o intuito de avaliar seu potencial de utilização na recuperação de áreas contaminadas por metais potencialmente tóxicos, visando principalmente minimizar a biodisponibilidade destes metais. Os compostos estudados foram produzidos no Campus II da USP (São Carlos), apresentando como entrada resíduos do Restaurante Universitário, e as leiras foram geridas seguindo o sistema indiano (método Indore). Com o intuito de promover uma comparação, uma leira continha apenas resíduos orgânicos de pré-preparo (composto indiano), enquanto a outra, além desta entrada, incluía alimentos cozidos (composto total). Quanto aos resultados, foram obtidos valores de pH em água e  $\rho$ H de, respectivamente, 7,4 e -1,0, para o composto indiano, e 6,5 e -0,4, para o composto total. A condutividade elétrica foi superior para o composto total ( $2200 \mu\text{S cm}^{-1}$ ) em relação ao composto indiano ( $665 \mu\text{S cm}^{-1}$ ), provavelmente devido à presença de condimentos nos resíduos. O teor de matéria orgânica foi levemente superior para o composto indiano ( $418,70 \text{ g kg}^{-1}$ ) em relação ao composto total ( $376,17 \text{ g kg}^{-1}$ ). O carbono orgânico resultante (base seca) foi de 8,32% e 10,69%, para os compostos indiano e total. A capacidade de troca catiônica por titulometria foi de  $34 \text{ cmol}_c \text{ kg}^{-1}$  (composto indiano) e  $75 \text{ cmol}_c \text{ kg}^{-1}$  (composto total). Por fim, a granulometria se mostrou similar, com uma porcentagem de partículas compreendida no intervalo de diâmetro de argila e silte inferior a 1%, e predomínio da fração areia, superior a 90%, em ambos os casos. Esses dados revelam condições em torno da neutralidade, predomínio de cargas negativas e considerável presença de MO e CTC, potencializando a imobilização metálica. Assim, estes materiais exibem características promissoras para seu emprego em barreiras reativas e/ou selantes, potencializando a adsorção de cátions metálicos e agregando valor econômico e tecnológico ao composto.

#### SP002 Caracterização de uma área contaminada pelo vazamento de combustíveis, no entorno de um posto de combustíveis.

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Em Santa Catarina, a Fundação do Meio Ambiente (FATMA) possui 2.580 processos de licenciamento de empreendimentos que lidam diretamente com combustíveis líquidos derivados de petróleo. Dentre esses, 25% apresentam áreas contaminadas em função de vazamentos de combustíveis, as quais, não raro, estão situadas próximas de locais de preservação permanente, como rios, lagoas, mangues ou até mesmo no entorno de estações ecológicas federais. Nestes casos, o principais poluentes são tolueno, benzeno e xilóis (chamados, no conjunto, de BTEX). O principal objetivo do presente estudo foi realizar uma comparação das concentrações de BTEX, PAH e TPH medidas antes e após a remediação (realizada por empresa contratada pelo empreendedor) de um caso real de contaminação, e realizar testes ecotoxicológicos com amostras de água retiradas dos solos contaminados. O plano de amostragem do solo/água da área em estudo se baseou nos mapas das plumas de contaminantes (BTEX, PAH e TPH). Os métodos analíticos utilizados foram os indicados no *Standard Methods For Examination Of Water And Wastewater* - APHA/AWWA/WEF, e nas normativas da USEPA, como a SW-846 Method 8015B (Total Petroleum hydrocarbons - TPH as Gasoline and Diesel (Revision 2, December 1996)), utilizando-se Cromatógrafo Gasoso com espectrômetro de massa, do Laboratório de Avaliação Ecotoxicológica (CCB). Resultados obtidos evidenciaram a contaminação ainda residual em solos após o processo de remediação, também

caracterizada pelos testes de toxicidade, (reprodução) com *Daphnia magna* e embriões de *Danio rerio*. As amostras de água coletadas na área contaminada promoveram a redução significativa do número de neonatos. Da mesma forma, promoveram alterações significativas no desenvolvimento dos embriões, além da perda da viabilidade.

#### SP003 Ecological risk analysis of the sediments in the linguado channel (Babitonga bay - Brazil) through weight of evidence approach

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The ecological risk assessment systematizes data in order to understand the relationships between contaminants and their adverse effects on biological systems. Although these guidelines are valuable tools for evaluating sediments, their use requires evidences due to complex mixtures of chemicals that may have interference factors in the analysis. Babitonga's Bay, located in the northeast region of Santa Catarina State is considered the last great formation of mangrove in the southern hemisphere, with a water depth of  $134 \text{ km}^2$  and a volume of  $780 \text{ million m}^3$ . It has a rich biodiversity and it attracts artisanal fishing activity, besides being the source of subsistence for a portion of the population around the Bay. Positioned among the most dynamic economies in the State, it contributes with 14.5% of PIB, with emphasis on the metalworking, textile and plastic sectors. However, little investment in domestic sewage treatment has been carried out, which conflicts with Baía's use of economic activities. Canal do Linguado, southwestern part of the Bay, was one of the links with the Atlantic Ocean until the 1930s, when it was closed for the construction of a railway network, modifying the local hydrodynamics. Its possible reopening presents the accumulated sediment volume and the presence of contaminants as a challenge, emphasizing the need to evaluate the associated ecological risks. This study aims to evaluate the ecological risk potential associated to the sediments of Canal do Linguado through a matrix of weight of evidence. Data from the chemical analyzes for the inorganic compounds had been used to generate the risk quotient and the average Sediment Quality Values. Characterizing sediments regarding their risk, a matrix of weight of evidence was used with information on chemical concentration, toxicity and biomagnification. The results point out an environment complexity, with contaminated sediment and a indicative of Hg biomagnification. The closest areas to the dam have higher content of fine sediments, organic carbon and inorganic chemical compounds with great risk potential. Interfering factors in the ecotoxicological analyzes were also observed and the lack of data on the alteration of the benthic community inaccurate the ecological risk associated with the reopening of the canal.

#### SP004 Estudo de zeólita para ser empregada na retenção de metais potencialmente tóxicos (Pb, Cd e Zn)

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Áreas de disposição de resíduos perigosos necessitam sistemas de revestimento de base, para agirem como barreiras contra a migração dos contaminantes para o ambiente, evitando assim possíveis efeitos tóxicos aos organismos e risco à saúde humana. As zeólitas, devido a sua grande habilidade de troca iônica e alta seletividade por cátions metálicos, vêm sendo considerada como material alternativo, viável e de baixo custo, para esta finalidade, bem como para seu emprego em barreiras reativas. As zeólitas constituem-se de aluminossilicatos hidratados e com estrutura cristalina microporosa. Sendo que a substituição do  $\text{Si}^{4+}$  pelo  $\text{Al}^{3+}$  na estrutura, cria uma carga negativa, permitindo a aptidão de troca iônica no material. Nesse contexto, uma zeólita proveniente de Cuba foi caracterizada visando seu futuro uso como adsorvente de metais potencialmente tóxicos (Pb, Cd e Zn) em áreas de disposição de resíduos de mineração. Para esta caracterização foram realizadas as seguintes análises: pH,  $\rho$ pH, condutividade elétrica (CE), potencial de oxirredução (Eh), granulometria, difratometria de raios x (DRX), análise térmica diferencial (ATD), fluorescência de raios x (FRX) e capacidade de troca catiônica (CTC). O pH em água obtido foi de 6,2, enquanto que o pH em KCl foi de 3,7. Resultando no  $\rho$ pH de -2,5, o que indica predominância de cargas negativas e consecutivamente possibilidade de reter cátions. A CTC de  $180 \text{ cmol}_c \cdot \text{kg}^{-1}$  foi considerada satisfatória para esse tipo de material. A CE  $67 \mu\text{S/cm}$  e o Eh de +297 mV indicaram baixa presença de sais dissolvidos e meio oxidante. A análise granulométrica indicou selecionamento do material entre 0,4 a 1,0 mm. A análise de DRX demonstrou que esse material é composto por uma mistura majoritariamente de duas zeólitas: clinoptilolita e mordernita. Também foi identificada pelo DRX a presença de quartzo e feldspato. O ATD confirmou a presença de clinoptilolita, indicando ser essa a zeólita majoritária na amostra, como também confirmado pela relação de Si/Al de 4,9. Assim, a zeólita estudada é a clinoptilolita. Essa zeólita apresentou propriedades básicas importantes para ser utilizada como

alternativa de material adsorvente em barreiras de áreas de disposição de resíduos de mineração contendo metais. Porém, ainda se faz necessário um estudo real da capacidade de retenção metálica através de ensaios de adsorção. O uso de materiais reativos na retenção de metais, minimiza a biodisponibilidade dos mesmos para a biota.

**SP005 Metals and arsenic in catfish from a Marine Protected Area under past and present human pressures: consumption risk factors to the local population**

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The toxicological risk for traditional populations living in marine polluted areas due to metals and As in seafood is seldom assessed, although eliminating the risk of human exposure to contaminants is one of the indicators associated with the socioeconomic goals of Marine Protected Areas (MPAs). The current study aimed to estimate the potential toxicological risk of metals (Cu, Mn, Zn, Cr, Cd, Pb) and arsenic (As) for human health through the ingestion of fish locally harvested in a MPA, the Cananéia-Iguape-Peruíbe Environmental Protected Area (APA-CIP). *Cathorops spixii*, a catfish widely consumed by local population, was collected across the estuary in three seasons with different rain regimes. Concentrations of metals and As in muscle tissues were quantified and compared with the national and international action levels regarding human consumption. In addition, the target hazard quotient (THQ) for metals and As, the cancer risk (CRisk) for As, and the number of eligible meals per month were estimated. Cd, Pb, and As were found at concentrations above action levels for human consumption. Depending on the level of exposure of the local population, the consumption of *C. spixii* may pose risk to human health. Highest THQs were estimated for fish collected in sites closer to the main contamination sources in the APA-CIP, i.e. the Ribeira de Iguape River (RIR) mouth (P1) and the city of Cananéia (P4, P5, and P6). Although As in *C. spixii* showed low risk of causing systemic effects, the cancer risks estimated based on these levels of As are high, especially in those sites under the influence of the city. The exposure of the local population to seafood containing metals and As cannot be disregarded in environmental studies and management of the APA-CIP.

**The dirty dozen and a few more: status and trends of POPs in wildlife in the Americas**

**SP006 Bioaccumulation of POPs in a terrestrial food-web of an avian top predator, the Cooper's Hawk (*Accipiter cooperii*)**

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Several types of persistent organic pollutants (POPs), including legacy compounds, such as PCBs and DDE, flame retardants and perfluorinated compounds, are released from multiple sources into the ambient environment and can negatively impact many physiological functions and ultimately survival and reproduction of exposed wildlife. Protocols to assess bioaccumulation of these persistent chemicals within terrestrial systems are far less developed compared to aquatic systems. Currently regulatory agencies in Canada, the USA, and the EU use only bioaccumulation information for fish to assess the bioaccumulation potential of chemicals. However, recent studies have shown that some chemicals that are not bioaccumulative in aquatic food-webs can significantly biomagnify in terrestrial food-webs. As a primary step in addressing this data gap and to better understand the bioaccumulation behavior of chemicals in terrestrial food-webs, we aim to produce a food-web model to assess the presence of and biomagnification of POPs in an apex avian predator, the Cooper's hawk (*Accipiter cooperii*). Avian predators are particularly useful indicators of environmental change and ecosystem health as greater bioaccumulation is often seen in avian species than compared to mammalian species. We focused our study sites around 17 Cooper's hawk nests within urbanized areas of Metro Vancouver. We sampled the hawk's food chain by collecting samples of common prey species, mainly American robins (*Turdus migratorius*), European starlings (*Sturnus vulgaris*), and House sparrows (*Passer domesticus*). To represent the lower trophic levels several species of ground-beetles (*Carabidae spp.*), sow and pill bugs (*Oniscidea spp.*), earthworms (*Lumbricidae spp.*), and Himalayan blackberries (*Rubus armeniacus*) were also

collected. Soil and air samples were also collected near hawk nesting sites. All samples have been analyzed for a wide range of contaminants listed as priorities for monitoring by the Chemical Management Plan of the Canadian federal government. Resulting data will be inputted into a food-web model to examine terrestrial bioaccumulation processes.

**SP007 Bird lead poisoning: Research advances in Latin American countries**

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Lead poisoning has been recognized as a toxic agent responsible for disease and death of humans and animals for at least 2,500 years. The largest sources of lead are usually related with the battery industry as well as with lead-based paints, and ingestion of shotgun pellets by birds. The first studies (1959) on this problem estimated that the annual losses of waterfowl were approximately 2 to 3% of the fall populations in North America. In 1990 it was estimated that more than one million waterfowl, especially ducks and Geese, died each year from lead poisoning. This caused the US government to ban the use of lead pellets for hunting migratory waterbirds in 1991. In 2014 in Quito, Ecuador, the Convention on the Conservation of Migratory Species of Wild Animals (CMS), tried to approve a document that sought to impose a ban on ammunition of lead at international level, but it was rejected by the European Union (EU), The United States and other countries given the lack of scientific evidence and robustness of the report presented. Following a series of negotiations, the EU supported the text on condition that the obligation to veto the lead was eliminated, leaving this decision as a simple recommendation for each country to decide whether to prohibit the use of this type of ammunition. In Latin American countries, the legislation has been lax and unable to stop the use of lead shot that mainly affects ducks, geese and birds of prey. In this study, our objective was to examine the research work carried out in the Latin American countries in the last 10 years, to assess the problem of lead contamination and its effects on wild bird populations. We consulted specialized journals on wildlife toxicology, animal health and environmental chemistry, as well as government and nongovernmental websites. Our results highlight the importance of increasing our efforts in research to generate solid arguments and help to solve this serious environmental problem.

**SP008 Effect of organochlorine compounds on bird ecological stress and body mass index in an agricultural region in Central Mexico**

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Agriculture is vital for food production. However, in Mexico and in many other parts of the world, large amounts of agrochemicals are used to increase agricultural production. Agrochemicals may have negative effects in some wild organisms. These compounds, for instance, may negatively influence the immunological system of organisms through disruption of the endocrine system. These effects increase susceptibility to diseases, as well as body condition. Studies of the effects of organochlorine compounds on wild animals are scarce. The objective of this research was to determine the potential effect of organochlorine agrochemical concentrations in wild birds from three different families: Cardinalidae, Fringillidae, and Columbidae, on the heterophile/lymphocyte ratio (H:L), a measure of physiological performance related to ecological stress, and on body condition. Birds were captured at three agricultural sites in San Luis Potosí, Mexico. Blood and feather samples were obtained to determine organochlorine concentrations, as well as the H:L ratio and body condition. Eighteen organochlorine compounds were detected in bird feathers, including some that are banned in several countries, such as Endosulfan, DDT, Aldrin, and Dieldrin. The effects of different organochlorine compounds on birds were inconsistent. Within the Cardinalidae family, for instance, the H:L ratio increased as the concentration of Endosulfan II (b) in feathers increased. This result is consistent with our original expectancy. In contrast, some other pollutants such as Cis chlordane and Endosulfan I (a) had negative effects on H:L ratios. Regarding body mass index, contrasting responses by birds were also obtained. These results may be related to a number of factors that were not studied, such as indirect effects on parasites, competitors, food, possible development of genetic resistance by birds, etc. Future studies are recommended to characterize the mechanisms through which organochlorine compounds affect bird stressors.

**SP009 Paradoxal effects of extreme opposite concentrations of mercury on the**

#### hepato-nephrotoxic system of the bumblebee *Bombus atratus*

P. Balsamo, Federal University of Sao Carlos UFSCar Sorocaba; F.L. Lissoni, Universidade Federal de São Carlos / Biology; V.H. Thomé, M. Provase, C.R. Lima, Federal University of Sao Carlos UFSCar Sorocaba; S. Irazusta, CEETEPS / Coordenadoria de Pós-graduação - CEETEPS; M.C. Jones, Universidade Federal de São Carlos UFSCar Sorocaba / Department of Biology; F. Abdalla, UFSCar / Biology. Bees are valuable bioindicators, providing precise information regarding environmental conditions through different kinds of analyses, ranging from studies of residual contaminants present in their nests to physiological, morphological and molecular approaches using organs, tissues and cells. Therefore, we investigated the effect of 0.2 ppb and 110 ppb mercury (Hg) on the hepato-nephrotoxic system (HNS) in bumble bee *Bombus atratus*. The exposures were performed by offering separately solutions of 0.2 µg L<sup>-1</sup> and 110 µg L<sup>-1</sup> to experimental groups (n = 11 for each). The bees were kept in incubator under controlled conditions of temperature and humidity for 48h, kept individually in special boxes with food *ad libitum*. We analyzed the morphology of trophocytes, oenocytes and pericardial cells associated with the myogenic region of the dorsal vessel and used immunohistochemical assays to investigate the expressions of Hsp70 and Hsp90 proteins chaperones, and also DAPI + F-Actin (Alexa Fluor 488 phalloidin) and acridine orange (AO) staining techniques to evaluate DNA alterations. The results indicated a paradoxical effect between 0.2 ppb and 110 ppb Hg on the HNS of bees. At 110 ppb Hg, trophocytes presented round nucleus, indicating loss of regular function, oenocytes with picnotic nuclei and perinuclear vacuoles and pericardial cells at stage IV with high pinocytosis activity. The expression of the Hsp70 protein presented drastic reduction in relationship to the control group in contrast to Hsp90 and the acridine orange and DAPI-F-Actin confirmed the cell nuclear collapse. On the other hand, 0.2 ppb of Hg indicated the total collapse of trophocytes, oenocytes and pericardial cells, being much lethal than 110 ppb. Therefore a 500-folds lower dose can have a higher action on disruption of internal organs of bees. The same drastic disruption of the fat body was observed in previous studies using 2 ppb cadmium in *B. morio* and the pesticide (thiamethoxam) with a dose 10-folds lower than the sublethal dose for Africanized *Apis mellifera*. The pericardial cells present an different action from the fat body, seeming to be associated with the type of xenobiotic. Based in our studies we hypothesized that (1) the HSN cells present different levels of sensibility for “detection” of toxicants, causing (2) different “trigger strategies” against the xenobiotics and (3) somehow there is a signaling pathway among the HSN, which still should be deeply studied and corroborated.

#### SP010 PCBs, DDTs, and chlordanes in *Scomberomorus cavalla*: risk assessment for human intake at Recife metropolitan area, Pernambuco, northeastern Brazil

D.d. Miranda, Instituto de Biologia da UFBA / Biology; G.T. Yogui, Universidade Federal de Pernambuco / Oceanography. Persistent organic pollutants (POPs) have the potential for causing damage to biota even at low concentrations. Teratogenicity, endocrine disruption, and carcinogenicity are some of the health risks potentially triggered by POPs. These compounds are still frequently detected in the environment even after decades of banishment. Consumption of contaminated food such as fish is the main route of exposure of POPs to human populations. This study aimed to: (1) estimate exposure of the human population at Recife metropolitan area (RMA) to PCBs, DDTs and chlordanes through consumption of king mackerel (*Scomberomorus cavalla*); and (2) check whether the estimated human intake exceeds the limits recommended by Brazilian and international regulations. A total of 20 specimens were purchased from artisanal fishermen at RMA. Muscle tissue (i.e. fillet without skin) was sampled and extracted for analysis. On a lipid weight (lw) basis, mean concentrations of PCBs and DDTs were 262 ng g<sup>-1</sup> (range: 60.9 to 867 ng g<sup>-1</sup>) and 43.6 ng g<sup>-1</sup> (range: 10.5 to 171 ng g<sup>-1</sup>), respectively. These values are below the tolerance levels of tolerance established by Brazilian regulation for edible fish: 3000 ng g<sup>-1</sup> lw for PCBs and 1000 ng g<sup>-1</sup> lw for DDTs. On a wet weight (ww) basis, average concentrations were 8.57 ng g<sup>-1</sup> (range: 0.71 to 20.9 ng g<sup>-1</sup>) for PCBs, 1.42 ng g<sup>-1</sup> (range: 0.22 to 3.67 ng g<sup>-1</sup>) for DDTs and 0.07 ng g<sup>-1</sup> (range: nd to 0.59 ng g<sup>-1</sup>) for chlordanes. These values are at least three orders of magnitude lower than the regulatory levels established by the US Food and Drug Administration (FDA) for edible fish tissues: 2000 ng g<sup>-1</sup> ww for PCBs, 5000 ng g<sup>-1</sup> ww for DDTs and 300 ng g<sup>-1</sup> ww for chlordanes. PCB toxic equivalents for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TEQ-PCB) were calculated for the specimens investigated. The mean concentration was 0.011 pg g<sup>-1</sup> ww (range: 0.001 to 0.048 pg g<sup>-1</sup>). Considering the daily average fish consumption of the Brazilian population (39.7 g d<sup>-1</sup>) and the average body weight of the adult population in Pernambuco State (65 kg), the mean daily intake was estimated to be 0.007 pg kg<sup>-1</sup> d<sup>-1</sup> (pg of TEQ-PCB per kg body weight per day). This value is more than 500 times lower than the maximum tolerable dose (4 pg kg<sup>-1</sup> d<sup>-1</sup>) proposed by the World Health

Organization (WHO). In conclusion, according to several approaches, regular consumption of king mackerel at RMA does not pose a health risk for human populations.

#### SP011 Penguins as a bioindicator of marine pollution, temporal and latitudinal analysis of lead concentrations

W.E. Espejo, Universidad de Concepcion / Facultad de Ciencias Ambientales Eula; J. Celis, Universidad de Concepcion / Department of Animal Science, Faculty of veterinary science; D. González-Acuña, University of Concepcion / Department of Animal Science, Faculty of Veterinary Sciences; R. Barra, Universidad de Concepcion / Facultad de Ciencias Ambientales Eula; G. Chiang, MERI Foundation / Biology. Penguins are seabirds that inhabit in the Southern Hemisphere. They are animals that are at the top of the food web of marine ecosystems, and have a crucial role in maintaining the environment's health. Even though penguins live in remote areas of the world, humans have a big impact on them. Actually, penguins are considered among the endangered seabirds of the world, since about two-thirds of penguin species are on the IUCN Red List of Threatened Species. Lead is toxic for being a non-essential trace element and because this metal can be biomagnified through the trophic chain. We reviewed the Lead levels (dry weight) reported from different species of penguins. On a time scale, lead levels have been reported in penguins between 1988 and 2014, in which we observed a slight increase in lead concentrations in penguins as the years increased. In the latitudinal scale, we observed that there is a decrease in lead concentrations reported in penguins at higher latitudes (colder areas and less anthropic activity). These data would indicate that the lead concentrations would be increasing slightly with the increase of the anthropic activity in the present times. While the latitudinal distribution could be explained more by the local anthropic activity than by a global transport such as the POPs. Acknowledgements: W. Espejo is scholarship CONICYT-Chile for PhD studies. Many thanks to INACH for the financing of projects RG\_09-14 (J Celis), T18-09 (R Barra), T-12-13 (D. González-Acuña) and T 31-11 (G. Chiang) and projects as well as the chance to do research on Antarctic birds.

#### What can mammalian & ecological toxicologists learn from each other? Leveraging approaches to advance risk assessment

##### SP012 Crossing frontiers - using zebrafish and nematode models for both ecological and mammalian toxicity screening purposes

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Under the principles of the 3Rs (reduction, replacement and refinement) there is a need for ‘animal’ alternative toxicity screening assays to assess potential risks to man and the environment. For human health hazard assessment these screening assays need to be translational to humans, have high throughput capability, and from an animal welfare perspective be harmonized with the principles of the 3Rs. To avoid some of the limitations of cell culture systems (i.e. lack of biotransformation capacity, multicellular or multi organ complexity) we have used *Caenorhabditis elegans* (nematode) and *Danio rerio* (zebrafish) larvae models as alternative assays for developmental and reproductive toxicology (DART) hazard assessment of some candidate chemicals. Both models follow the guidelines on the protection of experimental animals (Council of Europe, Directive 2010/63/EU) and are counted as non-animal tests. In our studies the results of the zebrafish and nematode studies were in alignment with data obtained from conventional mammalian toxicity studies indicating that these have potential as developmental toxicity screens. Although their applicability domain has yet to be established it is apparent that there are a number of conserved genetic pathways across the species. In addition to providing mammalian toxicity screening data we believe that, with careful selection of dosing methods and consideration of exposure mechanisms, these models can also provide relevant information for environmental hazard and risk assessment to both aquatic and terrestrial organisms. By combining both mammalian and ecotoxicity considerations into the test designs these models could provide a unique opportunity for environmental and mammalian toxicologists to interact to develop powerful diagnostic tools for a range of applications. For example, such models could have significant potential in screening product categories (e.g. to assess which products should be selected for higher tiered testing) and in screening of water and groundwater samples. In the latter examples the models could be used to provide a more holistic indication of both health and environmental risks from contaminated waters. The models and



potential applications will be discussed in the presentation but we recognise that the true potential of such screens will only be realised if we are not 'shackled' by the boundaries of our classical specialisms and embrace an interdisciplinary approach from the outset.

**SP013 Total Mercury Concentration in Jaguar (*Panthera onca*), Ecological Station and Taiamã, Pantanal of Mato Grosso, Brazil**

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Mercury is one of the pollutants most resistant to degradation and its bioaccumulation can be highly harmful to aquatic biota, transposing trophic chain levels and reaching top food chain predatory mammals. The Taiamã Ecological Station is a Federal / ICMBIO reserve. The jaguars of Taiamã have as preferential diet the fish and alligator, already evaluated in other studies as bioindicators of mercurial contamination in the Pantanal of Mato Grosso, Brazil. The objective of this study was to evaluate the concentrations of total mercury in jaguar (*Panthera onca*), using a non - invasive method of barbed wire and adhesive tape and collection of hairs samples obtained in collar / ICMBIO / CENAP. For the composite samples, seven sample points were monitored for six months with a Trap camera, with the objective of individualizing them. A total of 15 samples were collected, of which 13 were individual and 02 were composites (03 and 04 individuals per sample). For the quantification of the concentrations of total mercury was used atomic absorption spectrophotometer, Perkin Elmer (FIMS - system). The average concentration of mercury ( $\mu\text{g}/\text{Kg}$ ) was 4,265,448 (2,130,531 min and 7,259,444 max). There was no significant difference in the concentrations of total mercury between the sexes (T test = 1,571, df = 10,8, p = 0.143), although the females presented the highest concentrations, which surely can cause over the years to Biodiversity. Age also did not explain the variations in concentrations ( $R^2 = 0.1603$ , p = 0.1753). However, there was a significant negative difference between the concentrations of total mercury and the animals' weight ( $R^2 = 0.3516$ ; p = 0.03269). Thus, lighter animals had the highest concentrations of mercury in the body, suggesting that a mercury demethylation mechanism may be occurring over the life of these animals or individual factors of metal absorption in the body. The results are unprecedented and demonstrate bioaccumulation through trophic chain levels, reaching the largest predatory cat in the Pantanal, Mato Grosso, Brazil through its diet almost exclusively of fish and alligators.

**The evolution of Life Cycle Sustainability: Approaches and environmental labeling in Latin America**

**SP014 Análisis de ciclo de vida: Reutilización de residuos de procesos productivos en la industria cerámica como medio para reducir impacto ambiental**

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Se realizó un LCA de todos los procesos productivos de una industria cerámica sanitaria que está ubicada en el sur del Área Metropolitana de Buenos Aires y se encuentra en un área residencia mixta. El objeto de este análisis fue disminuir el consumo de materia prima, agua potable y la generación de residuos que va a disposición final a fin de disminuir el impacto ambiental de dichos procesos y abaratar costos. Se investigaron las entradas y salidas en elaboración de la pasta, moldes, esmalte, colado de la pieza, secado, esmaltación, horneado, control de calidad y despacho. La metodología utilizada involucró dos etapas una cualitativa y una cuantitativa, en las que se analizaron todos los consumos de agua y todos los residuos generados, en todos los sectores de establecimiento para poder identificar la viabilidad de cada una de las propuestas que surgieron del LCA. Del análisis realizado, surge que las posibilidades de reúso, recuperación, reciclado o reincorporación hacia otros procesos productivos son varias. Las propuestas que resultaron viables y representaron una mejora significativa del proceso fueron: 1. El reúso del agua proveniente de la plata de tratamiento de efluentes para el proceso de humectación de los moldes de yeso, el lavado de moldes de prensas automáticas y limpieza de equipamiento. 2. La utilización del residuo de barbotina debajo las bancas de colado manual, el aprovechamiento del polvillo de pulido como materia prima para su utilización en la industria ladrillera. 3. El aprovechamiento del yeso de los moldes como materia prima para la industria cementera. 4. Optimizar el secado de material proveniente de la plata de tratamientos para ser aprovechado por industria ladrillera. 5. La optimización de la inclusión como materia prima de piezas mal coladas. 6. Utilización de las piezas con fallas de horneado para el rubro de la construcción como material inerte para el relleno

de suelos.

**SP015 Condicionantes ao uso da ACV no Brasil**

W. Motta, IBICT / Information Science Post-Graduation Program

Apesar de largamente utilizada no mundo e de ser reconhecida como a mais completa "ferramenta ambiental", a metodologia da avaliação do ciclo de vida -ACV ainda é pouco difundida no Brasil. Este fato inquietante motivou a elaboração da tese que deu origem a este artigo, no presente artigo o foco será dado aos fatores condicionantes para a implementação de uma metodologia complexa como a ACV no Brasil. A pesquisa foi exploratória, composta por uma revisão bibliográfica somada a uma pesquisa de campo e teve uma finalidade aplicada. Tais fatores condicionantes puderam ser divididos em motivadores ao uso da ACV, complexidades da metodologia e impactos ambientais mais relevantes. Os condicionantes de seu uso por parte das empresas foram relacionados ao atendimento dos anseios por seus consumidores, principalmente quanto a **imagem percebida da empresa** por estes. Aqui houve uma forte associação desta imagem com questões como rotulagem ambiental e declarações ambientais de produtos (EPD). Outro ponto foi referente ao vínculo dos resultados provenientes de estudos de ACV a tomada de decisões estratégicas nas empresas, ratificando assim sua importância na sustentação e **suporte aos gestores nas questões decisórias**. Outro aspecto que foi identificado como determinante (neste caso relacionada principalmente a realidade brasileira) para a disseminação de seu uso é o referente as **leis e incentivos**. Estes determinantes se tornam fatores cruciais na busca por aproximação das empresas ao uso da metodologia, pois trazem um maior entendimento da forma pela qual estas visam o uso da mesma. Outro importante resultado foi quanto aos impactos ambientais mais contemplados e avaliados nos estudos de ACV, pontuando a **pegada de carbono**, o **consumo de energia** e o **consumo de água**. Esta informação pode vir a colaborar para o direcionamento de estudos e elaboração de treinamentos para que as empresas possam ter maior familiaridade com os benefícios do uso da metodologia frente a estes impactos. As complexidades apontadas endossam as informações encontradas na literatura, pontuando principalmente as questões relacionadas a **ICV, Modelagem, AICV e Trade-offs / interpretação / incertezas**. Assuntos estes que já estão endereçados com projetos nacionais e internacionais, que se encontram em andamento, como os conduzidos pelo IBICT, *Life Cycle Initiative* e outros.

**SP016 LCA del proceso productivo de discos ópticos: Disminuyendo impactos ambientales**

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Se analizó el ciclo de vida del disco óptico, 1-Adquisición de materiales vírgenes; 2-Procesamiento de materias primas; 3-Fabricación y terminación de producto; 4-Embalaje-Packaging; 5-Transportación y Distribución; 6- Usos y Vida Útil; 7- Desecho y Disposición final Se analizó el caso de una planta industrial ubicada en la Ciudad de Lanús, municipio al sur del Área Metropolitana de Buenos Aires. Argentina. En la producción del disco se utilizan cantidades que fueron determinadas de policarbonato grado óptico de alta calidad técnica. Entre sus elementos, dispuestos en capas, se encuentra un 90 % de policarbonato, unos 16 gramos por unidad, y el 10 % restante se distribuye en los otros componentes metálicos, en algunos casos se utiliza plata u oro. Este LCA se enfocó especialmente en los descartes y el manejo del disco como residuo sólido, con el objeto de conocer su valoración como material reciclable y las posibilidades de reutilización en cadenas productivas. Para ello se utilizaron las estadísticas del consumo, la generación de residuos y la disposición final. Se observó que existe descarte y eliminación de una cantidad importante de discos obsoletos. Estos residuos provienen de dos fuentes principales clasificables como: mercado legal y mercado ilegal. En ambos casos, el material es desechado en la corriente de residuos sólidos urbanos y son eliminados por medio del sistema de relleno sanitario y una cantidad observable se introducen en ambientes con escaso control, por ejemplo: basurales, espacios públicos, desagües y otros. Por lo que se pudo determinar que existe un grado de desaprovechamiento de materias primas valorables. Los datos obtenidos relacionados al desecho de los discos, indicaron que estos residuos no son segregados al reciclaje como un material reciclable o de utilidad para diversos usos. Se consideró que este residuo puede ser recuperado para sustitución de materias primas vírgenes, de modo que con el retorno a la cadena productiva se puede favorecer la extensión de la vida útil de sus componentes. Esto requiere la incorporación de etapas de separación de los discos y procesos tecnológicos de recuperación de los componentes. Para optimizar el ciclo de vida es necesario contar con el desarrollo de tecnologías específicas para los procesos de tratamiento del reciclaje de este material.

La incorporación de estas tecnologías resulta económicamente viable para disminuir los costos de producción y los impactos ambientales de la introducción de los mismos al ambiente.

**SP017 operational evaluation of processes and socioeconomic environment of recycling units and composting of domiciliar solid waste in Brazil**

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The composting of the urban solid residue (USR) is an alternative of recycling of the organic substance to the ground, contributing in the reduction of the environmental impacts and the improvement of the public health. The quality of the final product solid waste compost quality (SWCQ), for agricultural use, is related with the efficiency of the composting process. In this work, an the relative self-sufficiency of processes of composting in units of recycling and composting (URC)'s in operation in the State of São Paulo. Starting from three case studies, the contribution to public policies was demonstrated through an integrated view of urban solid waste management and proximity to agriculture using modeling and expert system; the second addresses is a composting and recycling evaluation system considering the quality of compost and local infrastructure of treatment of urban household waste and the latter case, it is the selection of indicators. Multicriteria analysis was applied, making it possible to use of social, economic and ecological scores. A final score was calculated combining those three dimensions of the self-sufficiency, indicating which the process "more adjusted" among the alternatives evaluated.

**The role of eco-innovations in the transition to the circular economy**

**SP018 Life Cycle Assessment as a facilitator for the transition from the Linear Model to the Circular Model**

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As government and population become more critical of the environmental situation of the companies and their current production model, various instruments, techniques and methods have been developed and improved to understand the relationship between industry and the environment. One of these techniques is the Life Cycle Assessment (LCA), a technique for the systematic evaluation of the environmental aspects and impacts of a product or service system. Even with all evolution about sustainable thinking, the linear model of production and consumption is still dominant in the economy, where the products are manufactures by virgin raw materials and then used and discarded. As the current model has proved unsustainable, a new economic model has been highlighting, known as Circular Economy (CE), this model proposed closed loops of production and consumption, in this way using the products, materials and components at their highest level of usefulness and value. Therefore, the present paper aims to identify, describe and analyze the relationship between LCA and CE. Besides that, show if the relationship between these two themes has already been discussed in academic circles. In this sense a systematic review of the literature was performed to analyze the studies published in the research platform "web of knowledge" using the strings "circular economy" and "life cycle assessment". The articles were analyzed based on the year of publication, journal where they were published, methodology used and relationship presented between the themes. With this search a total of 54 articles were found, of this total it was possible to verify that most was published in 2016 (53,7%) in the Journal of Cleaner Production. The results achieved by the systematic review prove like a theme behaves in relation to the other, moreover t made it clear that despite the existence of some articles that relate LCA to CE, the number of publications at this point is still very scarce, evidencing the need for further research. The LCA is a technique that is within the scope of the CE, proving to be a facilitator for the transition from the linear model to the circular model, which could be verified through the proposed systematic review.

**Pharmaceuticals in the environment: Single and mixture toxicity of pharmaceuticals**

**SP019 Acute and chronic effects of florfenicol and oxytetracycline on the tropical freshwater cladoceran *Ceriodaphnia silvestrii***

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In order to contribute to the increase of the knowledge on the sensitivity of tropical native species to antimicrobials, acute and chronic toxicity tests were performed with the tropical freshwater cladoceran *Ceriodaphnia silvestrii*. Tests were carried out with the two most commonly used antimicrobials in intensive fish farming in both Brazil and worldwide – the florfenicol (FLO) and oxytetracycline (OTC). In acute tests, 48-h EC<sub>50</sub> values ranged from 20.15 to 30.32 mg L<sup>-1</sup> for FLO and from 17.85 to 27.54 mg L<sup>-1</sup> for OTC, showing that the sensitivity of *C. silvestrii* to two studied antimicrobials was similar. Chronic tests also revealed that these antimicrobials can cause impairments on the reproduction and population growth rate of *C. silvestrii* at concentrations lower than those found for other cladoceran species. The 7-day EC<sub>50</sub> values obtained for reproduction of *C. silvestrii* ranged from 0.18 to 0.37 mg L<sup>-1</sup> for FLO and from 0.24 to 0.35 mg L<sup>-1</sup> for OTC, whereas the concentrations of both FLO and OTC above 1 mg L<sup>-1</sup> decreased the population growth rate of *C. silvestrii* by 25.08% and 31.82%, respectively. Based on the highest environmental concentrations measured in Brazilian water bodies, OTC was the only antimicrobial that may pose a high ecological risk (Risk Quotient, RQ > 1) for this cladoceran species, whereas the FLO had a low ecological risk (RQ < 1). Our results support previous claims on the advantages of using native species to better lead the ecological risk assessment of chemicals in tropical ecosystems.

**SP020 Assessment of the growth inhibition induced by three beta-blockers of environmental concern in the macrophyte *Lemna minor* L. (1753)**

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The beta-blockers are among the most frequently reported antihypertensive pharmaceuticals in freshwater and saltwater environments. However, data on the ecotoxicity induced by these compounds are scarce and frequently limited to results from acute tests, while toxicity posed by pharmaceuticals should be mainly of chronic concern. Therefore, this study aimed to enlarge the knowledge regarding the sub-lethal toxicity caused by beta-blockers included in the list of emerging substances of the NORMAN (network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances), by focusing on the first trophic level. The beta-blockers propranolol hydrochloride, sotalol hydrochloride and bisoprolol fumarate, all of them with ≥ 98.8 % purity, were evaluated using the Lemna sp. growth inhibition test, according to the OECD (Organisation for Economic Co-Operation and Development) guideline n. 221 (2006), using the species *Lemna minor* as test-organism. The Steinberg medium was used as dilution water. The endpoint evaluated was the frond number. Results were expressed in terms of the pharmacological base content present in each salt. The EC<sub>10</sub> calculated from the tests were of 15.2, 118.2 and > 1000 mg L<sup>-1</sup> for propranolol, bisoprolol and sotalol, respectively. These results revealed that propranolol was the most toxic beta-blocker to the macrophyte. The level of toxicity of the three selected beta-blockers towards the *L. minor* decreased in the order of propranolol > bisoprolol > sotalol, that correlates well to their hydrophobicity. Since macrophytes are not described to possess beta-adrenergic receptors, it is not expected a specific mode of action related to the harmful effects observed in *L. minor* exposed to beta-blockers. Therefore, these compounds are probable to act via narcosis, also called baseline toxicity, in the macrophytes. In this case, the tested pharmaceuticals are expected to be as toxic as their log Kow (octanol-water partition coefficient) indicate, which was confirmed in our experiments. The results obtained in this study contributes to fill data gaps regarding the potential toxic effects to non-target organisms for environmental risk assessment purposes. Additional tests to investigate the sub-lethal effects caused by these pharmaceuticals should be performed specially in fish and amphibians, which have been described to possess beta-adrenergic receptors.

**SP021 Avaliação ecotoxicológica de anti-histamínicos para organismos aquáticos**

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O consumo de fármacos vem aumentando ao longo das últimas décadas em função de diversos fatores, mas principalmente, devido ao crescimento populacional. O aumento do consumo, por sua vez, tem contribuído para uma distribuição generalizada desses metabólitos no meio ambiente, gerando uma grande preocupação quanto aos riscos ambientais causados por tais contaminantes, tanto na qualidade das águas superficiais, como na saúde pública. Os anti-histamínicos foram introduzidos no mercado mundial por volta de 1940 para o tratamento de alergias e, atualmente dispõe de mais de 40

tipos de substâncias, dentre elas a cetirizina e a loratadina, que embora sejam comercializadas há décadas, ainda pouco se sabe sobre o comportamento e os efeitos de tais fármacos e/ou seus metabólitos nos ecossistemas aquáticos. Diante disso, o presente trabalho teve como objetivo analisar os efeitos letais e subletais da cetirizina e loratadina para microcrustáceos e peixes. Para tanto, foram realizados testes de toxicidade aguda com os organismos-teste *Daphnia similis* e *Poecilia reticulata* (Comitê de Ética em Pesquisa com Animais, nº 22/2016), seguindo as metodologias descritas na ABNT (NBR 12713/2004 e NBR 15088/2004), respectivamente. E testes de toxicidade crônica com *Ceriodaphnia silvestrii* (ABNT NBR 13373/2005). Os resultados dos valores médios da CE<sub>50,48h</sub> decetirizina e loratadina para *D. similis* foram de 35,36 e 0,83 mg/L, respectivamente, já para *P. reticulata* os valores da CL<sub>50,48h</sub> foram de 145,62 mg/L e 0,74 mg/L, respectivamente. Em relação aos efeitos subletais para *C. silvestrii*, de acordo com os resultados preliminares, foi observada uma significativa alteração na reprodução dos organismos em baixas concentrações, estando na faixa de 2 µg/L (cetirizina) e 10 µg/L (loratadina). Apesar de ter sido registrado toxicidade aguda em faixas da ordem de mg/L, os resultados dos testes de toxicidade crônica demonstraram que o efeito tóxico ocorre em concentrações ainda mais baixas, da ordem de µg/L. Tais concentrações estão próximas daquelas registradas nos corpos hídricos, visto que tais fármacos já foram detectados com valores da ordem de ng/L a µg/L (Gros *et al.*, 2006; Bahlmann *et al.*, 2012). Diante dos resultados obtidos, é importante destacar que se busque mais informações sobre os efeitos subletais, bem como os da mistura desses contaminantes, em organismos de diferentes níveis de organização biológica, uma vez que a entrada desses compostos no ambiente aquático é intermitente.

#### SP022 Bioaccumulation of pharmaceutical compounds and illicit drugs in fish from Argentina

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Pharmaceutical compounds (PhC) and illicit drugs (ID) are Contaminants of Emerging Concern, which are continuously released into waters with potential adverse effects on biota. Therefore, the presence of PhC and ID receives more attention from scientific and government areas. Bioaccumulation of PhC and ID has been observed around the world, however data from Argentina remains poorly studied. This research assessed the bioaccumulation of PhC, ID and their metabolites in muscle, liver and gills of four fish species (*Rhamdia quelen*, *Hypostomus fuliginosus*, *Hoplias lacerdae* and *Prochilodus lineatus*) from two different areas: 1-Paraná river, an urban stream which receives the discharge of two wastewater treatment plants (WWTP) from Posadas city, and 2-Acaraguá river, inside of the natural reserve "Antonia Ramos Research Center" which does not receive WWTP effluents. Twenty-Seven PhC were analyzed by LC-MS/MS Agilent 6420 Triple Quad with ESI. Fish from the natural reserve presented higher levels of PhC than the Paraná river (12.8 and 6.6 ng/g ww, respectively). It could be associated with a dilution effect due to the Parana river flow (17,300 m<sup>3</sup>/s) that is several times higher than the WWTP discharge (0.25 m<sup>3</sup>/s). Levels of PhC, ID and their metabolites in liver of all species were markedly higher than those observed in gills and muscle. Antibiotics such as sulfamethoxazole (SUL) and trimethoprim (TRI) presented the highest levels (10.7 ng/g ww TRI+SUL), however erythromycin was found in 100% of samples (range 0.7-5.6 ng/g ww). TRI+SUL are broad-spectrum antibiotics commonly used for treating bacterial infections in human and animals. Caffeine levels ranged from 0.6-13 ng/g ww in both areas, which is an indicator of untreated sanitary wastewater inputs. Norfluoxetine, a metabolite of fluoxetine, was the main antidepressant found (2.4-3.8 ng/g ww) showing the increase consumption of this group of PhC in Argentina. Benzoylcegonine, a primary metabolite of cocaine, was found in both areas with the highest levels in fish from the reserve (1.6 ng/g ww). These findings reveal the occurrence of PhC in natural areas in Argentina, which represent biodiversity reserves of the Atlantic forest. Moreover, these results also show that further studies of PhC and their metabolites within different environmental compartments are needed. The potential effects of PhC, which are bioactive even at low concentrations, on the non-target biota should be addressed in future studies.

#### SP023 Carbamazepine induces the follicular development in zebrafish (*Danio rerio*) female gonads

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Carbamazepine is an anticonvulsant drug used in the treatment of epilepsy, trigeminal neuralgia, manic-depressive illnesses and generalized tonic-clonic convulsive crises. Due to its high prescription and relative stability carbamazepine has been often found in domestic effluents and surface waters. Once in the aquatic ecosystems carbamazepine can cause adverse effects on reproduction, growth, survivor, gene expression and behavior of non-target organisms. In the present study, the animal model zebrafish (*Danio rerio*) was used to assess the effects of carbamazepine on fish female gonads. A total of 20 adult organisms per replicate (n=3) were exposed for a period of nine weeks to the following concentrations of carbamazepine: 0, 10 and 10000 µg/L. For histologic analysis, the animals were entirely fixed with Davidson's fixative during 24 hours. After this period, the material passed through the standard process of histology, which includes alcoholic dehydration, diaphonization by xylol and paraffin embedment. Posteriorly, longitudinal cuts were performed on organisms with 05 µm of thickness. The slides were stained with H&E and analyzed with optical microscope (Zeiss Akioskop 2). All females had their entirely gonads photographed through 20X magnification. The photomicrographs were analyzed in the Image J software. At the software, the total number of follicular cells was counted; each follicle maturation stage was quantified per area. The maturation stages were defined based on Menke *et al.* (2011) following the classification of oögonia, pre-vitellogenic follicle, vitellogenic follicle and pre-ovulatory follicle. The control group showed a mean value of pre-ovulatory follicles of 8.11 per µ<sup>2</sup>, while for exposed groups the means values were 10.55 per µ<sup>2</sup> for 10 µg/L and 19.80 per µ<sup>2</sup> for 10 mg/L. These results showed that exposed fish presented a higher number of pre-ovulatory follicles, suggesting that carbamazepine may have estrogenic effect, inducing the follicles maturation in *Danio rerio*

#### SP024 Chronic and acute sediment toxicities in the region affected by a major fire in a petrochemical terminal (Alemoa, Santos, SP, Brazil)

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The Santos Estuarine System (SES) is critically impacted due to the presence of multiple sources of contamination. From April 2<sup>nd</sup> to 9<sup>th</sup> 2015, the SES was seriously affected by a major fire in a petrochemical terminal, and six fuel tanks with 34 million L-capacity were burnt. The fire combat involved the use of 61.670 L of aqueous film-forming foams (AFFFs), containing complex and persistent mixtures of perfluorinated and hydrogenated surfactants (PFCs). It is estimated that a billion liters of water mixed with fuel per day were released into the estuary, dragging together large amounts of polycyclic aromatic hydrocarbons (PAH) and PFCs. This study aimed to evaluate the sediment quality in the region affected by the chemicals' leakage. Five field surveys were conducted across time (*i.e.* 15 days, 1 month, 3 months, 6 months and 1 year after the fire), in which sediments were collected at seven sites in the SES from upstream (station 1) to downstream (station 7). Control sediments were collected at the reference site (Ilhabela, SP, Brazil). Chronic and acute toxicities of whole sediments were assessed respectively by measuring the fecundity of the copepod *Nitocra* sp. exposed over 7 days, and the mortality of the marine amphipods *Tiburonellaviscanain* 10-days tests. Results indicated that significantly toxic sediments to *T. viscana* (T-test;  $p < 0.05$ ) occurred along the whole area; the first campaigns exhibited higher numbers of toxic samples, especially in the vicinity of the terminal. The later campaigns showed toxicities down stream. Chronic toxicity to *Nitocra* sp. was detected in the samples 1, 2, 3, 4 e 7, collected 15 days after the fire ( $p < 0.05$ ). Despite not statistically significant, fecundities of copepods exposed to sediments from stations 5 and 6 presented 50 % of reduction, compared to the control. In the other surveys, all sediment samples were considered significant toxic. In conclusion, sediments sampled nearby the leakage area presented a poor quality. The effects of the leakage apparently were more evident just before the fire and were more related to the acute toxicity to *T. viscana*. The occurrence of chronic toxicity in almost all the samples suggests that these effects are not only related with the fire episode, but also to the chemicals from other multiple sources located nearby.

#### SP025 Contaminación de aguas por fármacos: Evaluación de factores de riesgo y propuestas de minimización de la contaminación

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En el presente trabajo se desarrolló la problemática emergente que representa la presencia de fármacos en aguas superficiales del mundo entero. El objetivo principal del mismo fue realizar propuestas de gestión, con el fin de disminuir los riesgos ambientales generados por la falta de control, manejo y disposición final de los residuos farmacéuticos que se liberan al ambiente y que llegan al Río de la Plata, a través de aguas residuales o por percolación desde los rellenos sanitarios y basurales del Área Metropolitana de Buenos Aires, con el fin de disminuir los riesgos ambientales. La presencia de medicamentos en los cuerpos de agua superficial es una problemática emergente que se presenta en los ríos de todo el mundo, especialmente en aquellos donde son volcadas las aguas residuales de las grandes ciudades, las cuales carecen de métodos de tratamiento adecuados para éstos nuevos contaminantes. Se han desarrollado estudios que demuestran consecuencias en los organismos vivos presentes en los ecosistemas de río y ambientes terrestres, entre los que se distinguen efectos ecotoxicológicos subletales como la feminización, inhibición de los procesos reproductivos, de crecimiento y la movilidad de distintas especies animales. Basada en esos estudios científicos, la presente investigación se propuso con el fin de disminuir la carga de fármacos presentes en las aguas superficiales. Se propusieron tres programas para enfrentar el problema: el primero se diseñó con el fin de generar un sistema de recolección diferenciada de los fármacos en desuso o vencidos y sus envases con el fin de ser reutilizados o reciclados. El segundo programa propuesto busca implementar un sistema de tratamiento de aguas residuales adecuado en el AMBA para tratar los principales medicamentos más consumidos en la Región y encontrados en el Río de la Plata. Por último se diseñó un programa de sensibilización y capacitación para la correcta aplicación de los otros programas. Se concluyó que la aplicación de dichos programas permitirá dar respuesta al Principio Precautorio, para evitar las consecuencias de la contaminación por fármacos. Además, se consideró que es necesario mejorar la calidad ambiental de las poblaciones con el fin de evitar enfermedades asociadas al ambiente y por ende el consumo de medicamentos.

#### SP026 Ecotoxicological evaluation of acetylsalicylic acid in the echinoderm *Equinometra lucunter*

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The occurrence of pharmaceuticals released into the aquatic environment has generated major concerns, because recent studies have demonstrated that these emerging pollutants can significantly compromise the physiology, metabolism and behavior of local biota species. In previous studies performed by group, different pharmaceuticals were found in seawater samples collected from the area of influence of Santos (São Paulo, Brazil) submarine outfall, at concentrations that ranged from  $\text{ng}\cdot\text{L}^{-1}$  to  $\mu\text{g}\cdot\text{L}^{-1}$ . Among the different pharmaceuticals found in these seawater samples, the present study focused on ecotoxicological analyzes with acetylsalicylic acid (ASA), a nonsteroidal drug that provides analgesic, antipyretic and anti-inflammatory effects in humans through inhibition of both types of cyclooxygenase (COX-1 and COX-2). Ecotoxicological assays were performed with the echinoderm *Equinometra lucunter*, considering the water bodies that receive this pharmaceutical. Acute toxicity assays were conducted according to U.S. Environmental Protection Agency protocol (USEPA), and the results were classified according to Directive 93/67 EEC, which classifies different substances according to specific toxicity tests results. Chronic toxicity tests were performed according to the protocol of the National Organization of Brazilian Standards. The results for acute and chronic toxicity showed an IC50 above 100  $\text{mg}\cdot\text{L}^{-1}$  and this value classified ASA as "non-toxic" according to Directive 93/67 EEC. Chronic toxicity tests with ASA exhibited a NOEC (No Observed Effect Concentration) of 25  $\text{mg}\cdot\text{L}^{-1}$  and LOEC (Lowest Observed Effect Concentration) of 50  $\text{mg}\cdot\text{L}^{-1}$ . Despite these results, adverse biological effects in aquatic environment with ASA are unlikely to occur, considering the environmental concentrations previously detected for this pharmaceutical in seawater samples.

#### SP027 Effects of lithium at a concentration considered safe on the cardiac physiology of bullfrog tadpoles, *Lithobates catesbeianus*

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The current society is dependent on several synthetic substances used in our daily life. Due to the rapid expansion of technologies, the population has been employing several substances (e.g., cosmetics, medicines, nourishment) whose biochemical properties have not been studied yet. As a consequence, these compounds eventually reach water bodies, where they can exert unknown effects in aquatic organisms. Individuals of different species can suffer the effects of these exogenous substances, among them the anuran amphibians that have been suffering a drastic decline in the number of their populations due to anthropic actions, among them the contamination by xenobiotics. According to the Brazilian Environmental Agency (CONAMA) the lithium concentration that is considered environmentally safe to classes 1 and 2 waters is 2.5  $\text{mg}\cdot\text{L}^{-1}$ . Therefore, bullfrog tadpoles were exposed to this concentration in triplicate during 96 h. After euthanasia, the histological analysis of the ventricle was performed in order to verify a possible cardiac hypertrophy. The data was analyzed by the T bi caudal test to compare the two means (control and exposed groups), generating the following result: 3.48 with the respective error of 0.42, indicating that the results obtained to the exposed animals were similar to those observed to the control. Therefore, despite it is known that lithium competes with calcium in the sarcolemma, resulting in a drastic decrease of the contraction force, this negative inotropic effect is not promoted by an alteration in the size of myofibrils. We are developing further studies in order to verify whether calcium-transporting mechanisms are affected by lithium.

#### SP028 Effects of the acute exposure of aquatic organisms to the beta-blocker bisoprolol, including a behavioral evaluation

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The beta-blocker bisoprolol (BIS) has been detected in surface waters at concentrations exceeding the threshold values established by regulatory agencies, requiring that risk assessment be performed. Nonetheless, this task is hampered by the lack of ecotoxicity data. Thus, we aimed to evaluate the acute effects of BIS on aquatic organisms, employing lethal and sub-lethal endpoints. BIS fumarate, with 98.8 % purity, was evaluated in the *Lemma minor* growth inhibition test (OECD 221, 2006), *Daphnia similis* acute test (OECD 202, 2004 and ABNT 12713, 2016), *Danio rerio* fish embryo acute test (FET) (OECD, 236), as well as in the *Hydra attenuata* test (according to Trotter et al., 1997). The endpoints evaluated were frond number, fresh weight and total frond area for *L. minor*; immobilization for *D. similis*; morphological alterations for *H. attenuata*; and survival, pericardial edema, deformities, heart beat rate (beats per 20 s), tail detachment and hatching success for *D. rerio*. In addition, the effects of BIS on the locomotor activity of *D. rerio* larvae were assessed at 120 h post-fertilization, using the tracking system Zebrabox-ZEB 478 (v. 3.22, Viewpoint Life Sciences, Lyon, France). Only locomotion in the dark period was used to calculate differences between control and treatments. The EC<sub>50</sub> values obtained for *L. minor* were of 338.7 (316.4 - 361.0)  $\text{mg}\cdot\text{L}^{-1}$  for frond number, 313.1 (286.4 - 339.7)  $\text{mg}\cdot\text{L}^{-1}$  for frond area and 345.1 (319.3 - 370.9)  $\text{mg}\cdot\text{L}^{-1}$  for fresh weight. The EC<sub>50</sub> obtained for *D. similis* was of 93.1 (86.9 - 99.3)  $\text{mg}\cdot\text{L}^{-1}$ . For *H. attenuata*, the EC<sub>50</sub> obtained was of 119.6 (114.2 - 124.7)  $\text{mg}\cdot\text{L}^{-1}$ . Regarding the FET with *D. rerio*, lethal effects were not observed until the highest concentration tested of 100  $\text{mg}\cdot\text{L}^{-1}$ . However, the behavioral assessment showed that there was a statistically significant difference ( $\alpha = 0.05$ ) between this highest test concentration and the control, for the total measured time that the larvae displayed movement. However, at environmentally relevant concentrations of 0.01 and 0.1  $\text{mg}\cdot\text{L}^{-1}$ , those differences were not observed. Considering that BIS have been quantified in surface waters at concentrations in the  $\mu\text{g}\cdot\text{L}^{-1}$  order, an acute risk is not expected to occur for this pharmaceutical. However, the EC<sub>50</sub> value obtained for *D. similis* allows to classify BIS as hazardous to the environment. Therefore, additional chronic tests must be performed in order to evaluate the long-term risk posed by this pharmaceutical.

#### SP029 Evaluation of losartan ecotoxicity in *Astyanax altiparanae* by comet assay and biochemical biomarkers

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Antihypertensives are among the most medicines consumed in all world and for this reason frequently found in environmental matrices. However the effects in non-target

organisms are still unknown. In this therapeutic class the losartan is the main drug described in Brazil. There are studies reporting considerable concentrations of losartan in aquatic environment ( $\mu\text{g L}^{-1}$ ) and in effluents it was already found in  $\text{mg L}^{-1}$  scale. Therefore losartan is a relevant example of emerging contaminant that needs to be analyzed under ecotoxicological aspects. Whereas those studies on adverse effects in different aquatic organisms are still insufficient the investigations on early parameters of ecotoxicity are of great biological importance. This study evaluated the influence of the losartan in biochemical and genetic biomarkers in species *Astyanax altiparanae*. The experiments were divided into two stages. First the organisms were exposed to unique losartan concentration (2,5 mg L<sup>-1</sup>) at different exposition times 6, 12, 24 and 48 h. After that were tested two concentrations (0.25 and 2.5 mg L<sup>-1</sup>) in exposure times of 24 and 96 h. In comet assay, reduced DNA damage scores were verified when compared to the control groups, especially at the exposure time of 12 h. This may suggest formation of crosslinks, which must be confirmed through specific analysis. The relation between time and observed effects couldn't be fully defined, however the results show that biomarkers tended to be affected from 12 hours of exposition. Losartan showed no change in acetylcholinesterase activity but the results indicated potential to induce oxidative stress on fish.

**SP030 High efficient low cost carbon anodes on the electrochemical remediation of amoxicillin**

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Among the emerging pollutants thrown off at the sewage systems, owing to the inherent persistence and lethality, the antibiotics deserves special attention. The amoxicillin (AMX) is a widely consumed antibiotic for treatment of bacterial infections in human and animals. Therefore, many studies have focused on the AMX environmental removal. Nevertheless, few treatment systems have proved to be efficient applicable in real scale. Thus, the aim of this work is the evaluation of  $\text{TiO}_2/\text{C}$  nanostructured anodes on the electrochemical remediation (ER) of AMX. The anodic efficiency of  $\text{TiO}_2/\text{C}$  was compared with unmodified conventional Titanium ( $\text{Ti}^0$ ) and Carbon graphite (C) electrodes. The experimental parameters included AMX concentration of 12 mg/L, current density of 50 mA and treatment regimen from 10 to 40 minutes under constant agitation. The electrolytic system consisted in tap water (TW),  $\text{Na}_2\text{SO}_4$  or NaCl solutions. The AMX decay was monitored by UV and mass spectrometry and differential pulse voltammetry, whereas the resulting toxicity of the treated assay solutions was checked by zebrafish (*Danio rerio*) embryo assay. As result the  $\text{TiO}_2/\text{C}$  anode presented the high efficiency, leading to the complete AMX removal in less than 10 minutes, when the medium was 0.1 M NaCl. Yet in tap water, in which the electrical conductivity is very low, the removal fell to 40%. The efficiency of the supported electrolytes was in the order of  $\text{NaCl} > \text{Na}_2\text{SO}_4 > \text{tap water}$ . In turn, the zebrafish assays showed toxicity decreasing for all treated assay samples, in comparison to AMX start solution. Therefore, it can conclude that the electro-oxidation of AMX at  $\text{TiO}_2/\text{C}$  anode may be a low cost and efficient treatment alternative for human consuming water.

**SP031 Marine sediment contamination in a subtropical zone (Santos Bay, Brazil): Pharmaceuticals, personal care products and cocaine**

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Although the continuous input of domestic sewage in coastal areas has been reported, few data are available about the presence of pharmaceuticals and personal care products (PPCPs) in coastal environments of South America. The aim of this study was to determine the environmental concentration of PPCPs and cocaine in sediments from Santos Bay (Brazil), a degraded coastal area due to urban and industrial pollution. A sediment sample, collected at five points around the Submarine Sewage Outfall of Santos, was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and 35 compounds were investigated. The presence of five pharmaceuticals (caffeine, carbamazepine, diclofenac, fluoxetine and ibuprofen), one personal care product (triclosan), and one illicit drug (cocaine) was evidenced. Considering the absence of information about the occurrence of PPCPs and illicit drugs in marine sediments of tropical and subtropical areas of South America, the data reported here

subsidize the assessment of environmental risks of such substances in coastal zones.

**SP032 Modeling the dispersion of endocrine disruptors in the Santos Estuarine System (Sao Paulo State, Brazil)**

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Estrogens are hormones responsible for growth and reproduction. They are naturally synthesized by animals and humans alike. Xenoestrogens are identical to natural hormones, but they are manmade and used as oral contraceptives. Xenoestrogens are a specific group of drugs found in domestic wastewater and some environmental matrices. These compounds remain after conventional sewage treatment and, consequently, affect both the environment and non-target aquatic organisms. In this study, we used the Delft3D hydrodynamic model to estimate the amount of both natural and synthetic estrogens that have been released in the Estuarine System of Santos and São Vicente and the Santos Bay. The data on flow from the sewage treatment plants and on average concentrations of natural and synthetic estrogens released in aquatic environments were obtained from the literature. The results of the modeling showed higher concentrations of estrogens in the estuarine waters of the Largo Pompeba region, the São Vicente Canal, and the Santos Bay, which are regions that receive greater inflows of domestic sewage. The results also suggest that higher concentrations of estrogenic compounds are expected to be found in areas with higher levels of salinity.

**SP033 Multirresistência de E. coli a antibióticos: Avaliação da qualidade da água de reservatórios urbanos da Região Metropolitana de Curitiba Brasil**

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O desenvolvimento urbano em conjunto com a falta de esgotamento sanitário geram riscos potenciais ao abastecimento de água da população em países em vias de desenvolvimento, sendo o mais crítico, a ocupação das áreas de contribuição de reservatórios de abastecimento urbano que, eutrofizados podem produzir riscos à saúde da população. Contribuições oriundas de criações de animais e também de esgoto doméstico hospedam micro-organismos diversificados, podendo apresentar resistência a diferentes antibióticos e atuar como disseminadores de genes de resistência. Neste contexto, o presente trabalho teve como objetivo diagnosticar a qualidade microbiológica da água de reservatórios urbanos da Região Metropolitana de Curitiba-Paraná, Brasil (reservatórios Passaúna e Piraquara II) quanto à presença de coliformes totais e termotolerantes, pela técnica do número mais provável (NMP/100 mL), bem como perfil de resistência de *Escherichia coli* a antibióticos. A pesquisa experimental envolveu a coleta de amostras de água superficial para análise de coliformes totais e termotolerantes, isolamento de *E. coli* e determinação de resistência a antibióticos, por meio do antibiograma e cálculo do índice de resistência (IRA). Os resultados mostraram valores de coliformes termotolerantes que variaram de < 1,8 até 540.000 NMP/ 100 mL em ambos os reservatórios investigados. Com relação ao perfil de resistência, amostras coletadas nos dois reservatórios apresentaram *E. coli* multirresistente. No Passaúna, a multirresistência foi observada em 3 pontos de coleta, aos antibióticos polimixina B, ampicilina + sulbactam, cefotaxima, piperacilina + tazobactam, ampicilina, cefazolina, gentamicina, tetraciclina, imipenem e sulfazotrim. No Piraquara II, foi observada multirresistência em 2 pontos de amostragem, de cujas cepas isoladas apresentaram resistência a polimixina B, imipenem, tetraciclina, ceftazidima, cefepima e cafazolina. Os resultados do IRA total para os dois reservatórios apresentaram valores que variaram de 0 a 0,066, que apesar de baixos, sugerem que as áreas analisadas precisam estar em constante observação, tendo em vista a possibilidade de expansão populacional causando maior probabilidade das cepas se tornarem multirresistentes.

**SP034 Poultry litter as a source of fluoroquinolone antibiotics in agricultural soils from Rio de Janeiro**

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The use of poultry litter as fertilizer in agricultural areas is an important source of veterinary antibiotics to environment. The presence of antibiotics in soils can affect biogeochemical cycles such as C and N mineralization, and still affect the dynamics and fate of pesticides. In addition, antibiotics can be harmful to non-target organisms, affecting them directly or through alterations between ecological relations. Enrofloxacin (ENR), a fluoroquinolone, is employed extensively in Brazilian poultry farming for prophylaxis and therapeutics and ciprofloxacin (CIP), its main metabolite,

is widely prescribed for human bacterial infections. The aim of this study was to evaluate the presence of both antibiotics in thirty poultry litter samples from seventeen poultry farms and thirty soil samples from six agricultural areas. Samples were collected at the main poultry center in Rio de Janeiro state, where, according to our estimates, in 2016, about 30,000 tons of poultry litter were produced and 560 kg of ENR were administered at the farms. The analyses were performed using high-performance liquid chromatography (HPLC) with a fluorescence detector. Quantification was based on linear calibration curves by matrix-matched standard solution. Samples were analyzed in duplicate. According to the results, ENR occurred in 60% of litter samples with concentrations ranging from 51 to 84,164 ng.g<sup>-1</sup>. The extreme value found was almost three fold higher than reported extreme concentration in a previous study from São Paulo poultry farms. CIP was present in 100% of poultry litter samples, with concentrations ranging from 399 to 16,247 ng.g<sup>-1</sup>. For soil samples, ENR was detected in 60%, with concentrations varying from 27 to 1,391 ng.g<sup>-1</sup>. CIP occurred in 57% of the samples with concentrations varying from 25 to 976 ng.g<sup>-1</sup>. A high persistence of both antibiotics was observed in soils, confirming previous studies, where residues (> 200 ng.g<sup>-1</sup>) were still present after eight months of application. The results confirm the need for more studies about the possible impacts derived from the constant burden of veterinary antibiotics on tropical agricultural soils, as well as the risks to food safety and human health.

#### SP035 Presence of Hormones in Antarctic Wastewater Effluents

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Many countries have established scientific stations in Antarctica. There are about 65 summer and 30 winter stations. This situation, coupled with the intensification in tourism to Antarctica in recent years, leading to an increase in anthropic pressure. Human activities generate domestic wastewaters. This type of wastes can generate impacts on the environment, especially in Antarctica, characterized by its fragility and ecosystem services that have global implications. One of these impacts can be given by the presence of hormones in wastewaters that can act as endocrine disruptors in the native biota. This work aims to measure the presence of hormones in waste water in Deception Island (DI) and King George Island (KGI), belonging both to the South Shetland Archipelago. Samples of 1 liter were taken in duplicate at the outflow of the wastewater treatment plants from the bases (one in DI and three in KGI). The samples were concentrated by solid phase extraction columns (SPE) HLB of 500 mg. The SPEs were dried and then were sent to the laboratory of Pesticides and Water in Spain. The SPEs were eluted and samples were analyzed by QTOF, determining 13 hormones. The results indicate presence of both female and male hormones in all bases sampled. The concentrations of hormones were found in the order of ngL<sup>-1</sup>. The presence of Progesterone, cortisol, 4 Androstenedione, testosterone, Dihydrotestosterone, 11 hydroxyandrostenedione, 11 Ketoandrostenedione, 11 ketotestosterone and estrone were determined. This work marks baseline and guidelines for further improving the sustainability of the Antarctic environment. **Acknowledgments** This study was supported by the project INACH RT-09-15. The authors acknowledge logistic and scientific support of INACH during the Antarctic expeditions.

#### SP036 Psychoactive drugs in aquatic environment and ecological risk assessment

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This study focused on seven psychoactive drugs, being six benzodiazepines (alprazolam, bromazepam, clonazepam, diazepam, lorazepam and oxazepam) and one antidepressant (citalopram) widely consumed by the modern society and detected in different aqueous matrices, i.e. surface water (SW), wastewater effluent (WWE), and hospital wastewater effluent (H-WWE). Ecological risk assessment is presented for freshwater ecosystems. In total, 883 entries of measured environmental concentrations (MEC) were found, corresponding to one target compound in each aqueous matrix: 414 for WWE (121 scientific papers), 93 for H-WWE (8 scientific papers), and 376 for SW (100 scientific papers). Concentrations of all investigated psychoactive drugs in all aqueous matrices ranged from 0.14 to 840000 ng L<sup>-1</sup>. Maximum MEC for each compound in all aqueous matrices were compared to the predicted no-effect concentrations (PNEC) to estimate a risk quotient (RQ) as a MEC/PNEC ratio. PNEC values for citalopram and diazepam were calculated from acute experimental toxicity data available for aquatic organisms (algae, crustaceans and fish). In the lack of data for the other compounds (alprazolam, bromazepam, clonazepam, lorazepam and oxazepam), acute values were estimated using USEPA ECOSAR v1.11. Results indicated that citalopram presented the highest MEC values detected in SW and WWE

(76000 and 840000 ng L<sup>-1</sup>, respectively). Bromazepam exhibited the lowest MEC (19 ng L<sup>-1</sup>) detected in SW. Green algae were found to be the most sensitive group to almost all psychoactive drugs, except for diazepam (*Daphnia magna*), which exhibited the lowest calculated and derived effect concentrations and were, therefore, used to derive PNEC values. Due to the high MEC values in SW, an extreme level of risk was assigned to citalopram (47.5) and alprazolam (5.5); the others psychoactive drugs showed RQ < 1. Risk based on WWE concentrations was considered extreme for citalopram (525.0), followed by bromazepam (4.7), and oxazepam (2.3). MEC values for H-WWE indicates that only Oxazepam (4.4) may pose a threat to receiving water bodies. Uncertainties regarding compounds solubility in water could alter the predicted effects by ECOSAR and therefore further evaluation through ecotoxicological assays is strongly recommended. Results found in the study corroborate with the consolidated observation that wastewaters effluents are the major source of pharmaceuticals detected in surface waters.

#### SP037 Resultados preliminares da avaliação do risco ambiental dos fármacos loratadina e cetirizina para *Chironomus xanthus*

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A loratadina e cetirizina são anti-histamínicos muito utilizados pela população para o tratamento de doenças respiratórias como asma, resfriados e congestão nasal. A incompleta eliminação dos resíduos farmacêuticos e seus metabólitos nas estações de tratamento de esgotos (ETE's) podem ser considerados um dos principais contribuintes do lançamento desses poluentes nos corpos hídricos. Dessa forma, os organismos aquáticos estão continuamente expostos a tais compostos. Dados encontrados na literatura demonstram a ocorrência da cetirizina e da loratadina em estações de tratamento de efluentes, bem como em corpos hídricos receptores em concentrações da ordem de ng/L a µg/L (Gros *et al*, 2006; Bahlmann *et al*, 2012). Nesse contexto, o objetivo do presente estudo foi determinar o valor da CL<sub>50</sub> da cetirizina e loratadina para o organismo-teste *Chironomus xanthus*. Para tanto, foram realizados ensaios de toxicidade aguda com 96 horas de duração, seguindo a metodologia descrita por Fonseca & Rocha (2004). Para o cálculo da CL<sub>50</sub>, 96h utilizou-se o programa Trimmed Spearman-Kärber (Hamilton *et al*, 1977). Os resultados preliminares determinaram os valores médios da CL<sub>50</sub>, 96h para *C. xanthus* exposta a cetirizina e loratadina de 198,07 e 2,40 mg/L, respectivamente. Muito embora os valores encontrados no presente estudo estejam acima das concentrações comumente detectadas no ambiente, estes valores podem representar um potencial risco ecológico nos ecossistemas aquáticos, uma vez que a entrada desses compostos no ambiente é intermitente e a exposição contínua a doses subletais, bem como os efeitos combinados desses fármacos podem provocar alterações fisiológicas e comportamentais não previstas nos testes de toxicidade aguda. Dessa forma, este trabalho revela a necessidade de se ampliar os estudos com tais fármacos, avaliando-se os efeitos da exposição prolongada, bem como os da mistura, a fim de se obter uma melhor compreensão dos riscos ambientais da sua presença nos ecossistemas aquáticos.

#### SP038 Sensitivity of *Nitokra sp* copepod exposed to UVA and UVB filters used in commercial formulations

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Large discharges of pollutants released in the marine environment have as final destination the sediment, affecting the quality and biodiversity. Pharmaceuticals and personal care products (PPCPs) are lipophilic and poorly biodegradable, with potential for bioaccumulation and persistence in the environment. Compounds from commercial sunscreens are found in the oceans, because they are often used by people. This study evaluated the acute effect of UVA and UVB filters - Benzophenone-3 (BZ-3), Octyl Salicylate (OS) and Octyl Methoxycinnamate (OMC), detected in commercial sunscreens, performing the test both in a mixture as isolated - using *Nitokra sp* copepod and the method described in ISO14669:1999. When exposed to the water soluble fraction of the UV filters mixture, the mean of LC50-96h was 11.47 %, corresponding to 190 mg.L<sup>-1</sup> of BZ-3, 150 mg.L<sup>-1</sup> of OS and 230 mg.L<sup>-1</sup> of OMC, while the isolated UV filter showed a tendency to increased lethality. The tests were performed in parallel with the reference substance DSS, and were within the acceptability limits of the LECOTOX LC50-96h = 7.76 (3.98-11.55) mg.L<sup>-1</sup> DSS. It was concluded that the test organism and the method used were suitable for the tests



with the commercial UV filters mixture and that, although the filters used are fat-soluble, there are bioavailable concentrations in the environment. The tested mixture caused deleterious effects to *Nitokra* sp. in concentrations lower than ANVISA (2006) allows, therefore more research is needed.

**SP039 Toxicity of 17 $\alpha$ -ethynylestradiol in estuarine sediments for the fecundity of the copepod *Nitokra* sp.**

R.B. Choueri, Unifesp Universidade Federal de São Paulo / Departamento de Ciências do Mar; R. Antunes, Universidade Federal de São Paulo - UNIFESP / Instituto do Mar; F.H. Pusceddu, Santa Cecília University / Biologia Marinha; A. Alves, Other The increasing use of pharmaceutical compounds, both human and veterinary, has raised concern about their discards. In coastal zones, the discharge of sanitary effluents is done by sewage outfalls and also directly by homes not served by basic sanitation, which causes risk to marine biota. Therefore, assessments of the effect of potentially toxic compounds from sanitary effluents on marine organisms are required. One of the compounds found is 17 $\alpha$ -ethynylestradiol (EE2), a synthetic hormone found in contraceptive drugs. Due to its relatively high Kow, EE2 may adsorb to the sediments in marine or estuarine environments. The current study aims to evaluate the effect of 17 $\alpha$ -ethynylestradiol in estuarine sediments to a benthic copepod. Harpacticoid copepods are ecologically relevant to estuarine ecosystems because it is the basis of the trophic chain. Gravid *Nitokra* sp. females were exposed to clean natural sediments spiked with different concentrations of 17 $\alpha$ -ethynylestradiol (0.1 to 100  $\mu$ g/kg plus sediment and co-solvent negative control treatments and after 7 days the production of nauplii and copepodites was recorded. The assays were performed under static conditions, 25 $\pm$ 2  $^{\circ}$ C, and 12:12h light:dark cycle. 4 replicates were done for each treatment. A fertility index was estimated based on the number of descendants produced by each adult female. The data were evaluated for their normality (Shapiro-Wilk test), and homoscedasticity (Levene test) The treatments (different concentrations) were compared to the respective controls (water, sediment or sediment only and co-solvent) through one-way ANOVA (with Dunnett post test). For all tests,  $\alpha$ =5%. The results show an inhibition of the reproduction on the highest concentrations of EE2 in the sediments. In the 100  $\mu$ g/kg of EE2, the fertility of *Nitokra* sp. was significantly reduced in relation to the sediment and co-solvent control treatments. Previous studies have reported that this concentration is environmentally relevant, which reveals a high environmental risk of this pollutant of emerging concern to the *Nitokra* sp.

**SP040 Ecotoxicological study and environmental risk assessment of the antihypertensive Losartan in a Brazilian coastal zone**

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The antihypertensive losartan (LOS) is among the most commonly consumed drugs in many countries. Although LOS has been detected in the marine environments, there is a lack of ecotoxicological studies assessing metabolism and effects on invertebrates. In the present study an environmental risk assessment was performed employing the measured environmental concentrations (MEC) in surface water from Santos Bay (Brazil), and a battery of biomarkers to evaluate sublethal responses. It was found occurrence of LOS at the maximum concentration of 32 ng.L<sup>-1</sup>. Biomarkers results demonstrated induction of CYP like, GST and GPx activities, as such as DNA and lysosomal membrane damages. Considering the integration between MEC and PNEC, a risk quotient higher than "1" (RQ> 1) was established, which indicates significant environmental risk of LOS in this coastal zone.

**The Future of Pesticides in Sugarcane - Environmental and Management Perspectives**

**SP041 Cow bone char as an adsorbent to decrease hexazinone leaching in a Brazilian soil**

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Hexazinone (3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione) herbicide is used extensively in sugarcane throughout the Brazil, and can be easily leached to groundwater sources. Bone char (BC), as well as biochar have been shown to strongly sorb organic compounds and could be used as an adsorbent to increase herbicide sorption and decrease leaching, although its application form is still little known. The aim of this research was to assess the absorbent effect of a cow BC on the leaching of hexazinone applied to the topsoil and incorporated surface layer (0-10 cm) in a sandy loam Brazilian soil under laboratory conditions. The BC was added to soil at 0 (control – unamended) and 5% (w w<sup>-1</sup>) ratio, corresponding 60 t ha<sup>-1</sup>. A glass column (0-30 cm) was prepared for each treatment and analyses of <sup>14</sup>C-herbicide were performed by liquid scintillation counter. The leached was collected after 200 mm rainfall simulation over 48 h. Hexazinone residues were detected throughout the soil profile, reaching the deepest layers (25-30 cm) around 28%, and the highest amount of this herbicide was found in the leached (~41%) in the unamended soil. However, when the soil was amended with BC in the topsoil the herbicide was fully retained in the BC and when this material was incorporated in the topsoil, the hexazinone remained retained 0-5 cm deep not reaching the other depths and leached. We conclude that the cow bone char is an excellent adsorbent to reduce leaching of hexazinone in a Brazilian soil through the high sorption of this herbicide on BC, regardless of application form of the carbonaceous material. **Acknowledgements:** The authors would like to thank the São Paulo Research Foundation (FAPESP) process 2016/17683-1, for the financial support.

**SP042 Hexazinone and diuron runoff in green cane systems**

I.R. Vaz, Federal University of Viçosa; R. Barizon, S.C. Queiroz, Embrapa Brazilian Agricultural Research Corporation; J.B. Regitano, University of São Paulo USP Sugarcane is a major crop in Brazil and of great importance to the world. Higher yields implicate in higher use of pesticides, such as diuron and hexazinone. The adoption of green cane system, in which the straw is kept in the soil surface after mechanical harvesting, has changed the environmental behavior of these herbicides. Therefore, the goal of this research was to evaluate runoff losses of diuron and hexazinone in green cane systems. The 3x2x2 (12 treatments) factorial experiment was performed in a randomized block with 4 replicates. The factors were i) 3 levels of sugarcane straw (0, 50% and 100%, based on a dose of 14 t ha<sup>-1</sup>); ii) 2 levels of initial soil moisture (10 and 18% VWC), and iii) 2 rainfall periods (0 and 3 d after herbicides application). A rainfall simulator was adjusted to simulate an 80 mm h<sup>-1</sup> rainfall event for one and a half hour (120 mm) over plots of 1 m<sup>2</sup>. A commercial product containing diuron and hexazinone was used at rate of 3 kg ha<sup>-1</sup> dissolved in 700 L ha<sup>-1</sup>, according to label recommendations. The amounts of water and sediments were registered and herbicides concentrations analyzed by UPLC. Herbicides attached to the sediments were estimated according to sorption data from the literature. The results were evaluated by ANOVA and means compared by Tukey test (p< 0.05). Sugarcane straw decreased water, sediments, and diuron losses by runoff, but did not affect hexazinone losses. In other words, crop residues cannot prevent losses of highly soluble molecules, such as hexazinone. Greater herbicides losses were observed in the aqueous phase, even for the control treatment (without straw), since straw reduces the amounts of detached sediments. However, no difference was observed between the two levels of straw (50 and 100%), meaning that 7 t ha<sup>-1</sup> is sufficient for mitigating water, sediments, and diuron losses by runoff. Higher soil moisture (18 versus 10%) resulted in higher herbicides runoff. Yet, rainfall period did not affect herbicide losses, indicating that 3 days were not long enough for enhancing these herbicides dissipation or sorption.

**SP043 Highly effective removal of azoxystrobin from drinking water using a bone char**

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Azoxystrobin (methyl (E)-2-2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl-3-methoxyacrylate) is a post-emergence broad spectrum strobilurin fungicide of the class of synthetic compounds called  $\beta$ -methoxyacrylates and used mainly for cereals. Bonechar has been used extensively as an adsorbent. However, bonechar as an adsorbent to remove fungicide in water has been not reported. The aim of this research was evaluating cow bone char added as an adsorbent for removing azoxystrobin from drinking water samples. Drinking water samples (10 mL) were collected from a cold water faucet, which are used regularly for human consumption. Concentration at 5  $\mu$ g mL<sup>-1</sup> of azoxystrobin was prepared and added directly in drinking water contaminating with fungicide. This drinking water was amended at 0 (control – unamended), 0.01, 0.1, and 1 g of cow bonechar, and supernatants were analyzed at 1 and 7 d by high-

performance liquid chromatography (HPLC), equipped with a UV-Vis detector. The removal of azoxystrobin was ~96, 100, and 100%, with bone char addition of 0.01, 0.1, and 1 g, respectively. Azoxystrobin concentration for all treatments was lower than the control (without the bone char). At 7 d after application of bone char, azoxystrobin not desorbed this carbonaceous material, remaining strongly retained. This demonstrated the immense potential of bone char as an alternative adsorbent for azoxystrobin remediation in polluted waters and wastewaters.

**SP044 Toxicity of two residues derived from agrarian activity: genotoxicity and mutagenicity of sugarcane and Orange Vinasse**

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Vinasse is a by-product obtained through the fermentation of different vegetal sources, such as orange, sugarcane, corn and beet. Since its introduction in Brazil, sugarcane has gained prominence in agricultural scenario and, consequently, the waste generated by it, such as vinasse, has also increased. Like the sugar and alcohol industry, the orange juice processing industries also produce a large volume of liquid waste. There are different possible alternatives for reuse of these rich in organic matter effluents. Fertilization is the most reuse for sugarcane vinasse. However, this alternative has generated great concern due to possible contamination of soil and water. Orange vinasse, because it has the same characteristics as sugarcane vinasse, also has potential for this purpose. Thus, this study aimed to verify the vinasse toxic potential of two different sources: sugarcane and orange. For this, *Allium cepa* was used as test organism, where the chromosomal aberration and micronucleus tests were applied in seeds exposed directly to dilutions of 2.5 and 5% of both effluents. Genotoxicity was verified by the micronuclei frequency and the quantification of cells carrying chromosomal aberrations in the meristematic cells, while the mutagenic potential was verified through the occurrence of micronucleated cells in the F<sub>1</sub> region. The results obtained showed that both types of vinasse are genotoxic and potentially mutagenic. Therefore, the use of these residues in the environment needs caution. **Financial Support:** FAPESP (processes 2014/17998-7, 2012/50197-2).

**Environmental hazard and risk assessment for the oil and gas industry**

**SP045 Ecotoxicological evaluation of non-aqueous drilling fluids using a Brazilian native versus an exotic amphipod.**

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The Brazilian Federal Environmental Agency (IBAMA) has adopted some EPA rules for discharging drill cuttings coated with non-aqueous based drilling fluid from oil and gas operations in Brazilian open sea. The EPA method 1644 describes the procedures to evaluate the toxicity of non-aqueous base fluids (NAF) and synthetic based drilling mud (SBM) using *Leptocheirus plumulosus* as test species. Despite being an exotic species, Brazilian laboratories keep *L. plumulosus* cultures for many years. Otherwise, *Grandidierella bonnieroides* is widely distributed in Brazilian coastal estuaries, has also been successfully cultured and demonstrated to be sensitive to many toxicants in water-only toxicity tests as well as in field-collected and spiked sediment toxicity tests. The present study was carried out to evaluate the effects of three NAF (olefin, ester and paraffin) and two SBM, SBM1 (11,6 ppg) and SBM2 (9,9 ppg) spiked to natural sediment on the amphipods. Reference toxicant experiments were also performed using C16-C18 internal olefin or a C16-C18 internal olefin SBM of similar density, in order to obtain the Sediment Toxicity Ratio (STR) between the references and samples. Sediment was collected in Jabaquara beach, RJ, and transferred to the laboratory in chilly bins. After press-sieving, it was kept at 4 ± 2 °C until spiking for no longer than 60 days. Spiking procedures and whole-sediment tests were set up following the methods described in EPA 1644 and Brazilian Standards Association (ABNT, 2015). The amphipods were exposed to 5 concentrations of SBM and NAF spiked sediment plus controls for 4 and 10 days, respectively, in static testing system. In NAF tests *L. plumulosus* was up to three times more sensitive than *G. bonnieroides*. The average LC50 obtained in SBM 1 and SBM 2 tests using *G. bonnieroides* was 139.78 mL/ kg<sub>dw</sub> (n= 3; SD=69.47; CV= 49.70%) and 132.97 mL/ kg<sub>dw</sub>, while *L. plumulosus* showed a LC50 of 160.07 mL/ kg<sub>dw</sub> and 34.82 mL/ kg<sub>dw</sub>, respectively. In this study, although *G. bonnieroides* has shown to be less sensitive than *L. plumulosus*

to the NAF and SBM, the test method presented lower variability. In olefin tests using *L. plumulosus*, SBM 2 test using *G. bonnieroides* and SBM 1 tests using both species, STRs ≤ 1 were obtained, meeting the EPA criteria for drilling cuttings discharge. The method using *G. bonnieroides* as test species demonstrated to be suitable for this purpose.

**SP046 Fate and behavior of oil sands naphthenic acids in treatment wetland - negative-ion electrospray ionization Orbitrap mass spectrometry**

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Large volumes of oil sands process-affected water (OSPW) are generated during the extraction of bitumen from oil sands in the Athabasca region of northeastern Alberta, Canada. As part of the development of treatment technologies, molecular characterization of naphthenic acids in wetlands is a topic of research to better understand their fate and behaviour in aquatic environments. The naphthenic acid fraction in OSPW is described by a diverse suite of heteroatom compounds with general formula C<sub>n</sub>H<sub>(2n+z)</sub>O<sub>x</sub>N<sub>β</sub>S<sub>γ</sub>, where n is the carbon number, z is a negative, even integer that indicates hydrogen displacement due to formation of rings or double bonds, x, β and γ are the oxygen, nitrogen, and sulfur numbers respectively. Reported here is the application of high-resolution negative-ion electrospray Orbitrap- mass spectrometry for molecular characterization of naphthenic acids in laboratory treatment wetlands. The effectiveness of the wetlands to remove OSPW-NAAs was evaluated by monitoring the changes in distributions of pre- and post-treated NA compounds. After correction for measured evapotranspiration, the removal rate of the naphthenic acids (NAs) followed first-order kinetics, with increased rates observed for structures with relatively low number of rings or double bonds. The degree of alkylation affected the rates to a lesser extent. These findings are expected to help in the optimization of conditions for toxicity reduction in OSPW affected wetlands.

**SP047 Polycyclic aromatic hydrocarbons concentrations in vegetation leaves in Serra do Mar vegetation near a petroleum refinery**

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Plants have been used as biomonitors to confirm phytotoxic impacts caused by air pollutants and to demonstrate the release of toxic compounds into the environment and food chain. The leaves are used to evaluate the potential for contamination of vegetation due to atmospheric deposition. In 2015, two sampling campaigns (rainy season-1st and dry season-2nd) were carried out in Serra do Mar in Cubatão (São Paulo, Brazil). Leaves from five plant species were collected for the quantification of 16 polycyclic aromatic hydrocarbons (PAHs) in two points on “Caminho do Mar” (PCM), a site under the influence of a refinery, and from a reference point in the “Núcleo Itutinga-Pilões” (PI). The samples were processed without washing in a knife mill. The preparation method was based on QuEChERS AOEC-2007 (modified). The determination was performed by GC-MS-MS. The highest results of 16 PAHs occurred in the dry season 2nd, mainly due to the greater accumulation of naphthalene. The mean values of PAHs were 24,060 µg kg<sup>-1</sup> in PCM and 1,014 µg kg<sup>-1</sup> in PI. The first campaign results showed a distinct profile of PAHs quantified between the PCM and PI points, with a significant enrichment of low molecular weight PAHs with 2 and 3 rings in PCM, notably naphthalene. On the other hand, the compounds detected in highest concentrations in the first campaign PI were benzo(a)anthracene and benzo(a)pyrene, which have 4 and 5 rings. In the second campaign, only naphthalene was determined in PI. This different profile in the PI between the campaigns can be explained by the reversible nature of the PAHs accumulation showing that the detected compounds in the first campaign did not migrate into the plant tissues. The PAHs profiles in *Cecropia* sp, *Hedychium coronarium*, *Philodendron* sp and *Psidium guajava* were similar, whereas the one found for *Tibouchina* sp was different from the others, accumulating exclusively congeners of 3 rings. In this study it was not possible to distinguish the accumulated PAH from the fraction deposited on the leaves surface. Further studies on the accumulated fraction are needed to understand the PAHs dynamics in vegetation.

**SP048 Risk assessment of difficult-to-test substances: a case study on novel surfactants**

D. Lyon, Shell Oil Co. / Shell Health Risk Science Team; S.A. Hughes, Shell Health - Americas / Shell Health Risk Science Team; J. Dawick, Shell Health / Risk Science Team

Chemical ecological risk assessment is based on understanding the fate and hazard of a substance, often with a focus on aquatic toxicity for regulatory purposes. However, difficult-to-test substances, like substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), pose significant challenges for conventional chemical risk assessment. This is exacerbated by the large number of chemicals in commerce classified as UVCBs, which include petroleum substances, fragrances/essential oils, pine resins, and surfactants. For example, of 11,998 registered substances in REACH, 10% are multi-constituent and 21% are UVCBs. The primary challenge with UVCBs is that each component of a UVCB may have a different fate and hazard. If the substance has other difficult-to-test characteristics, such as being volatile, poorly soluble, etc., it becomes very difficult to accurately assess hazard using conventional testing approaches. Shell is developing a suite of novel surfactants and, as part of responsible product development, has designed and commissioned a range of environmental fate and ecotoxicological studies. These novel surfactants are a good example of difficult-to-test substances, as they are poorly soluble, highly biodegradable, and UVCBs. This poster discusses the issues encountered with testing these products, the test method development for these difficult-to-test substances, and the justification for the approaches taken in the testing strategy. The testing results are compared with modelled outcomes and results from analogous classes of anionic surfactants (e.g. detergent range surfactants). The overall approach can be generalized to other difficult-to-test substances/UVCBs.

**SP049 Screening methods for assessing toxicity and fate of produced waters**

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Due to their location there are significant logistical challenges with undertaking environmental risk assessments posed by offshore discharges. It is therefore important to be able to prioritise discharges where further investigations are warranted. As part of this process a 'toolbox' of simple screening tools have been developed and applied for the assessment of offshore produced water discharges. These require small, easily transported sample volumes providing quick and cost effective reliable information on the inherent hazard properties of the effluent (toxicity, hydrocarbon content, bioaccumulation potential) without extensive laboratory testing. These tools are typically applied to the first tier of a discharge assessment and include Microtox<sup>TM</sup> testing, solid phase micro extraction with gas chromatographic analysis (SPME-GC) and Quantitative Structure Activity Modelling (QSAR) analysis. The obtained information, together with a dilution assessment, will be used to screen at an early stage and thereby decide whether any subsequent tier assessment is required. To date, 22 produced water samples from the North Sea, South-East Asia and Africa have shown that toxicity increases in line with bioavailable hydrocarbons, suggesting hydrocarbon contamination is a major contributor to effluent toxicity amongst other factors, and bioaccumulation potential is of low concern (BCF < 2000) even before taking into account biodegradation and volatilisation of BTEX components. Where higher tier assessments are necessary, a more detailed analytical characterisation of the produced water or a whole effluent approach, or both processes combined, will be required. The place of these tools within the tiered risk assessment framework is presented.

**SP050 Toxicity tests using *Grandidierella bonnieroides* and *Leptocheirus plumulosus* exposed to olefin spiked sediment: preliminary results.**

L. Kraus, LabTox; B. Lee, Burnell Lee / Higher Olefins and Derivatives

The Brazilian Federal Environmental Agency (IBAMA) has adopted EPA Method 1644 to evaluate the toxicity of non-aqueous base fluids (NAF) and synthetic-based fluids (SBM) using amphipods. The method describes procedures for spiking base fluid into marine sediment and using *Leptocheirus plumulosus*, as test species. *L. plumulosus* is an exotic species but cultured in many Brazilian laboratories. In this study, *Grandidierella bonnieroides*, a native Brazilian amphipod also cultured in the laboratories, has been used in EPA Method 1644 and shown to be sensitive to NAF toxicity in natural field-collected sediment. The study was carried out to evaluate the effects of a SBM (olefin) spiked in natural sediment on the *L. plumulosus* and *G. bonnieroides* amphipods. Per IBAMA and EPA regulatory criteria, experiments were also simultaneously performed using C1618 internal olefin (IO) as a reference

substance. Sediment, was collected in Jabaquara beach (Paraty-RJ) and transferred to the laboratory in chilled bins. The sediment was press-sieved to remove large debris and indigenous organisms, kept at  $4 \pm 2$  °C and used within 60 days. The tests were set up following the methods described in EPA method 1644 and Brazilian Standards Association (ABNT, 2015). Sixty amphipods (six replicates of 10) were exposed to 5 concentrations of NAF spiked sediment and a blank control for 10 days, in a static testing system. Each replicate contained approximately 150ml sediment and 600ml 20 ppt salinity overlying natural sea water. Test organisms were not fed during the tests. In tests using *G. bonnieroides* the 10-d LC<sub>50</sub> obtained were between 1.01 g/kg<sub>dw</sub> and 7.46 g/kg<sub>dw</sub> for olefin and between 2.27 g/kg<sub>dw</sub> and 3.49 g/kg<sub>dw</sub> for C1618 IO reference. In tests using *L. plumulosus* the 10-d LC<sub>50</sub> were between 0.67 g/kg<sub>dw</sub>, and 3.09 g/kg<sub>dw</sub>, for olefin and between 0.53 g/kg<sub>dw</sub>, and 1.45 g/kg<sub>dw</sub> for C1618 IO reference. Although *G. bonnieroides* is less sensitive than *L. plumulosus*, in this study, the species was shown to be suitable for determining the sediment toxicity ratio (STR = LC<sub>50</sub> C1618 IO/LC<sub>50</sub> organic base fluid) using this method. To pass regulatory criteria the STR ≤ 1. The STR results were similar for both species. In one test the STR was > 1 for both species, while in the other tests the STRs were ≤ 1, meeting IBAMA and EPA criteria for discharging of drill cuttings coated with non-aqueous base fluid.

**Challenges and new developments in environmental risk assessment of pesticides in Latin America**

**SP051 Developing Realistic Exposure Scenarios for Environmental Risk Assessment of Pesticides in Brazilian Surface Water**

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Standard scenarios increase the consistency of the regulatory evaluation process by minimizing the subjective influence when performing the pesticide environmental concentration - PEC calculation, also make interpretation much easier and enable the adoption of a consistent scientific process for a Tier 1 evaluation (FOCUS, 2000). The Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) adopts the United States Environmental Protection Agency (USEPA) methodology (IBAMA, 2016), using the "standard farm pond" scenario (Parker et al., 1995) for surface water in the Tier 1 evaluation and, therefore, it may lead to the registration of pesticides without considering Brazilian agricultural scenarios in environmental risk assessment - ERA. Thus, the aim of this paper is to present a methodology for mapping landscapes with reservoirs and crops and calculating the relation between the watershed (with soybean crop and reservoirs) area and the reservoirs area to support the establishment of scenarios for ERA of pesticides in Brazil. A case study to soybean crop in Goiás (GO) state and in Brasília/DF was conducted. The selection of municipalities was performed using soybean planted area. Visual interpretation of satellite images was carried out in the selected municipalities to identify landscapes with soybean crop and reservoirs, using as the basic unit of mapping watersheds generated from SRTM (3 arc-seconds) DEM with ArcSWAT in ArcGIS 10.3. The area of each selected watershed with reservoirs and soybean crop and the area of the reservoirs were estimated and some statistics of the relation between the watershed area and the reservoirs area were calculated. The municipalities selected were Rio Verde/GO, Jataí/GO, and Cristalina/GO, besides Brasília/DF. The presence of reservoirs was observed in more than 5.3% of watersheds with soybean crop. In the mapped reservoirs, with the exception of only two, values of the relation between the watershed area and the reservoir area were greater than the reference value, which is 10 m<sup>2</sup>.m<sup>-2</sup> (Parker et al., 1995; FOCUS, 2001). The presented methodology was effective for generating initial information necessary to establish scenarios at first tier of ERA for pesticides in surface water, supporting modelling applications in the Brazilian pesticide registration process. The existing model *ARAQUÁ* (SPADOTTO and MINGOTI, 2014) can be modified and parameterized for the scenarios mapped.

**SP052 Effects of fungicide pyraclostrobin on the mortality and behavior of the stingless bee, *Trigona spinipes***

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The ecosystem services of pollination carried out by bees are of great value for the maintenance of agroecosystems, and they play an important role in the economy due to the honey commercialization. The stingless bees *Trigona spinipes* are considered a rescue pollinator because they may compensate the decline of some native pollinators in degraded tropical landscapes. Application of agrochemicals as fungicides on crops can impact bee at individual and colony levels. Our objective was to evaluate the



mortality and behavior of *Trigona spinipes* foragers, exposed to low concentrations (25ppb, 125ppb) of the fungicide pyraclostrobin by ingestion. About 100 forager bees were collected in flight activity, using trap net, in a green urban area. In laboratory, bees were conditioned in plastic pots (cages) and acclimatized in B.O.D. (Temperature: 34±2°C; Humidity: 90±5%) for one day before bioassay, and they were fed with syrup (water and sugar, 1: 1) during this time. After, cages containing bees (N= 10 individuals per pot) were organized in the following groups, in triplicate: (G-I) control (with 5% acetone solvent), (G-II) 25ppb i.a., and (G-III) 125ppb i.a. of pyraclostrobin, which were added to syrup offered *ad libitum* to bees. Behavioral and mortality observations were performed 24, 48 and 72 hours after the onset of exposure. After 24h, exposed-bees intensified food consumption in relation to those from G-I. After 48 h, exposed-bees decreased food intake in relation to G-I, but increased the level of locomotor activity, although with signs of lack of equilibrium, in both exposed groups. After 72h, the bee behavioral pattern clearly changes in G-II and G-III, in comparison to G-I. Behavioral changes in G-II and G-III, included slowness and lack of balance in locomotor activity, spasms and agglomeration of individuals, which move together inside cages. The concentration of 25ppb doubled the mortality of bees in relation to G-I. On the contrary, greater survival was observed in bees exposed to 125ppb, although this concentration induced more evident behavioral effects that indicated decrease of metabolic activity in organism. These mortality data suggest a hormetic effect. Behavioral data indicate that this fungicide may be harmful to *Trigona* at the colony level, and may negatively influence the dynamics of the population, since they collect pollen with agrochemical residues and transport to the hive.

#### SP053 Pesticides leaching: alternative approaches for classification

M. Meli, K. Coelho, S. Roulier, ADAMA

Stricter environmental regulations dealing with the authorization of plant protection products have been approved or are under development in many South American countries and elsewhere. Data on the environmental behaviour of pesticides are therefore requested by regulatory authorities to assess the potential of pesticides to enter surface water or groundwater bodies, and, consequently, their potential impacts on human health and non-target species. The same active substances are often applied for plant protection in more than one region of the world, and it is common to use the same data to support registration requests in different countries. These data, while robust and scientifically sound may have been produced following different procedures, or reported in a different format than what the competent authorities in a specific country might be accustomed to. For example, the leaching behaviour of pesticides can be evaluated from a number of standardized laboratory studies. Both the OECD and the US EPA, among others, have produced guidelines for such studies, which include column leaching, thin layer chromatography, batch equilibrium adsorption/desorption and others, and can be conducted on fresh or aged residues, and include or not a reference substance. Leaching behaviour can also be estimated from semi-field and field studies, such as lysimeter studies and field dissipation studies. Using data from an active substance currently registered in a number of Latin American countries we will show how the results of some of these studies can be used in different ways to classify the leaching behaviour of a chemical, either by direct comparison with a reference substance, or through the calculation of indexes which allow to put the specific substance on a predetermined scale. Furthermore, we will attempt to clarify some of the confusion that often surrounds the conservatism and applicability of different classification methods to be applied in the regulatory classification of plant protection products.

#### SP054 Statistically based approach to select worst-case groundwater scenarios for environmental risk assessment of pesticides in Brazil.

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Exposure assessment of groundwater is an important step within the framework of environmental risk assessment of pesticides. To do so, predicted environmental concentrations (PEC) are obtained using simulation models associated to exposure scenarios. Ideally, these scenarios should represent worst-case conditions to guarantee a safe use of pesticides and a high degree of groundwater protectiveness. In this work, we demonstrate a statistically based approach to select worst-case scenarios for groundwater using spatial variability of soil-climate data. To illustrate the approach, a case study was considered in Mato Grosso do Sul State, Brazil, for different agricultural scenarios. A total of 3780 scenarios was considered in the analysis (i.e. combinations of soil properties, weather time series, pesticide properties, application periods and crops) and simulations were carried out using the mechanistic PEARL model for PECs at 3 and 5 m depth. 34-years weather time series and 15 generic pesticides (i.e. combinations of three half-lives values and five organic carbon sorption

coefficients) were used in the simulations. As a result, the cumulative distribution functions of the statistical population of PECs in groundwater were obtained taking into account the spatial (i.e. area subject to exposure assessment) and temporal (i.e. variability of weather within the area) components for the area of intended pesticide use. By doing so, contour diagrams were plotted where an exposure endpoint PEC can be easily deduced for any given percentile from the overall statistical population of PECs, that defines the percentiles of the spatial and temporal distributions (e.g. 90<sup>th</sup> percentile overall that would mean the combination of 85<sup>th</sup> spatial and 95<sup>th</sup> temporal). Thereafter, some specific worst-case scenarios can be easily identified and used for the exposure assessment. It is important to point out that for this approach to be used in Brazil in the selection of worst-case exposure scenarios for groundwater, it is necessary the definition of an exposure endpoint for risk assessment by the authorities. This approach can be an important procedure in the sense that precondition for pesticide authorization should be different as a function, for example, due to a specific soil-climate conditions and defined area of regulatory interest.

#### Reliable analytical data in environmental studies: sample treatment and analytical determination issues

##### SP055 Acute Toxicity Identification Evaluations with the bacteria *Allivibrio fischeri* in the Surface Waters of São Paulo city

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Toxicity Identification Evaluations (TIE) studies comprise various methodologies where physicochemical methods and ecotoxicological tests are used to identify toxic substances and/or groups of toxic substances that effectively contribute to the total toxicity of a sample. The routinely used approach is based on fractionation of sample according to its physicochemical properties: volatility, solubility and chemical properties such as the ability to form complexes, to be reduced or oxidized, or to be ionizable. In parallel with the chemical tests, toxicological tests are also carried out to assess the loss of toxicity. Therefore it applies methods described by the United States Environmental Protection Agency (USEPA) in three documents, each one focusing on a TIE phase: Characterization, Identification and Confirmation. The TIE in this way becomes a very powerful tool to determine toxic substances present in water, and thus, it can direct government agencies to more effective actions in controlling river pollution in metropolitan areas. This work uses TIE USEPA protocols, especially the characterization and identification phases, applying them to the MICROTOX<sup>®</sup> system, as well as its execution to determine the classes of compounds responsible for the observed toxicity in river waters from São Paulo state, such as Tamanduateí, Tietê and Pinheiros. The tests with addition of EDTA (Ethylenediamine tetraacetic acid) and sodium thiosulphate in the sample did not present significant results, indicating that metals is not the cause of the sample toxicity. In the solid phase extraction, a complete reduction of toxicity was observed for the three Brazilian rivers, which presented mean EC20 in 15 min. of respectively, 13.87%, 4.80% and 7.17%. Such reduction is independent of pH range employed (acid, neutral and alkaline), and the column used C18, HLB (Hydrophilic Lipophilic Balanced). Aeration has also shown reduction of the toxicity to the mean EC20 values in 15 min. 28.11%, 15.67% and 48.96% respectively for the Tamanduateí, Tietê and Pinheiros rivers. The reduction of toxicity in SPE and aeration methods indicated a presence of organic compounds as the major class of xenobiotics, in particular, surfactants and volatile compounds that may originated from sewage treatment plant effluents, or by irregular discharge of them into the river.

##### SP056 Effects of BmNPV Virus in *Bombyx mori*: assessment of oxidative stress neurotoxicity

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Sericulture is the management of the Silkworm (*Bombyx mori* L.) with the mulberry, that the leaves are used as food source. Production losses occur due to contamination of these animals by bacteria, fungi, protozoa and viruses. The present study evaluated the antioxidant and cholinergic effects caused by the BmNPV virus on the of *Bombyx mori*. Were sampled 120 animals of 5th instar separated in two different groups: control (A) (leaves sprayed with distilled water) and treatment (B) (leaves sprayed with aqueous solution containing the virus concentration of 7.8x10<sup>7</sup> caps / mL). The sampling of biological material occurred at times 4, 8, 12, 24, 48, 72, 96, 120, 144 and

192 hours after virus inoculation, and subsequently performed the evaluation of the antioxidant system (superoxide dismutase - SOD, catalase - CAT, lipoperoxidation - LPO) and neurotoxic evaluations (Cholinesterase - ChE). In 8 hours of infection, there was elevation of LPO, increased activity of SOD and CAT enzymes, which may be related to the response of the widespread installation of viruses into cells. The first six hours of infection are called the initial phase, moment when the virions are released. Between 6 and 12 hours, occurs early viral replication and systemic infection. In 24h of experiment, there was an increase in SOD activity. In the period between 18 and 36 hours post infection, the release of virus from the cells occurs and the late phase of infection occurs between 24 and 96 hours, with the onset of symptoms. In 48 hours and 96 hours of infection, there was a reduction of the LPO, coinciding with the late phase of infection in which the rupture occurs of infected cells. In 48h and 72h, there was also a decrease in CAT activity and an increase in SOD activity, which may be associated with decreased caterpillar feeding. At 192 hours, the activities of CAT and SOD enzymes were elevated, which probably responds to the need of the animal to use the enzymatic machinery for pupal encapsulation. Regarding the neurotoxic effects, it was possible to observe that there were no significant statistical differences in the comparison between the groups. Thus, the effect of viral infection on the *B. mori* antioxidant system becomes evident. Therefore, prevention is important to minimize losses throughout the crop.

**SP057 Environmental distribution of pesticides associated with horticultural production in a small watercourse**

T.M. Mac Loughlin, Centro de Investigaciones de Medio Ambiente; D. Marino, Centro de Investigaciones del Medio Ambiente UNLP CONICET / Centro de Investigaciones del Medio Ambiente; L. Peluso, Centro de Investigaciones del Medio Ambiente UNLP CONICET / Centro de Investigaciones del Medio Ambiente CIMA. In recent years, the horticultural belt in the outskirts of La Plata, Buenos Aires, Argentina (Cinturón Horticola Platense, CHP), has grown in terms of size and productivity as well as in importance in the country. Vegetable production systems are characterized by intense pesticide use, yet the impact on the surrounding environment are largely unknown. The occurrence and concentrations of pesticides in water, suspended particulate matter (SPM) and sediments were studied in five sites along the Carnaval creek, a stream surrounded by horticulture production. Four sampling campaigns (C1-C4) were conducted during seasons of low and high pesticide application. Water samples were filtered in situ through 0.45  $\mu\text{m}$ . Surface water samples were extracted by liquid-liquid extraction; SPM by solid-liquid extraction and sonication; and sediments by modified QuEChERS. The different extracts were analyzed by gas or liquid chromatography, depending on the chemical nature of the analyte, both coupled with mass spectrometry in selective ion mode. For glyphosate and its environmental metabolite, the aminomethylphosphonic acid-AMPA, precolumn derivatization with FMOC-Cl was performed. In all, 20 samples for each matrix were analyzed. In water samples, glyphosate and AMPA were detected in 50% and 75% of the samples, respectively. Other herbicides were present in 25% of the samples. Insecticides (5) in water had an average 25% and azoxystrobin had a 30% detection frequency. In C2, all samples had concentrations above the guideline for aquatic life protection. In SPM, glyphosate and AMPA were detected in 75% of the samples. In sediments, C4 presented the highest total pesticide mass, 8675  $\mu\text{g}/\text{kg}$ ; 90.5% due to herbicides. In C1, 72.0% of the 5163  $\mu\text{g}/\text{kg}$  found were due to insecticides, and 20.9% due to herbicides. The highest concentrations found were 4634  $\mu\text{g}/\text{kg}$  of glyphosate + AMPA (C4), 2258  $\mu\text{g}/\text{kg}$  of chlorpyrifos (C1), and 652  $\mu\text{g}/\text{kg}$  of epoxiconazole (C4). Along the Carnaval creek, surface water concentrations reflected on the use of pesticides in the area: from the headwater, herbicide concentrations declined as insecticide concentrations increased, a consequence of the growing horticultural activity. One of the most relevant findings is the presence of herbicides, such as glyphosate and trifluralin, in horticultural production, where the use of this types of compounds is not expected. This is the first study in Argentina to analyze the presence of fungicides in sediments.

**SP058 Evaluation of matrix effect on booster biocides in sediments: first results**  
F. Abreu, FURG / Oceanography Institute; I.B. de Castro, Universidade Federal de São Paulo / Instituto do Mar; G. Fillmann, FURG Universidade Federal do Rio Grande. Several analytical studies on environmental samples evaluate the occurrence and behavior of booster biocides used as active ingredients in antifouling paint formulations. However, potential matrix effects (ME) are not always considered. The ME is a signal of suppression or enhancement in chromatography analysis and can underestimate or overestimate the quantified analyte. This study aimed to evaluate the ME associated to Irgarol 1051, diuron, DCOIT and TCMTB on estuarine sediment samples from Rio Grande (RG) and Santos (ST), Brazil. One sample from each estuary was spiked with 500ng of each biocide (triplicate analyze) and left over night for

equilibration. Extraction was performed using sonification and purification by solid phase extraction. Each final extract was then used to construct calibration curves with at least 10 points ( $r^2 > 0.99$ ). All curves were injected in Liquid Chromatography–Tandem Mass spectrometry (LC-MS/MS). The ME was assessed by comparing the slopes of matrices (or sediment; Sm) and pure solvent (Ss) curves ( $ME = 100 * (1 - Sm/Ss)$ ). Results showed that ME is higher to Santos samples since an enhancement in the quantified analytes was registered for all biocides. In this estuary, moderate ME for diuron (33% $\pm$ 8) and high ME for irgarol (59.3% $\pm$ 22.6), DCOIT (62% $\pm$ 34) and TCMTB (85%; N=1) were observed. For Rio Grande estuary, lower ME for diuron (16.6% $\pm$ 4.6), a moderate ME for irgarol (27.3% $\pm$ 8.7) and TCMTB (22.7% $\pm$ 6.9) and a high ME for DCOIT (53.0% $\pm$ 6.3) were observed. The high organic carbon levels in Santos samples can affected biocides quantification. Besides, these biocides belong to different chemicals groups and their proprieties could cause interact differently with sediment matrices. To illustrate, DCOIT has the highest Koc among the studied biocides and apparently have a stronger interaction with sediments. On the other hand, diuron and irgarol with lower organic carbon/water partition coefficient (*Koc*) seems to suffer less matrix effect. The relevant conclusion of this study is that the ME is dependent on sediment characteristics (organic or inorganic matter contents) and sources. Future works should consider ME minimization as an aspect to generate reliable results. For organic pollutants, one calibration curve for sediments with similar characteristics is suggested, as well as use internal standards before samples injection to ME compensation.

**SP059 Evaluation of the solubility of metallic NP in the presence of *Chlorella vulgaris* exudates**

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The importance of nanotechnology to society is evidenced by the wide range of applications of nanoparticles (NPs). Inevitably such particles find their destination in aquatic ecosystems where toxic or non-toxic effects but interactions with environmental constituents begin to be revealed. In this research we evaluated the solubility of Cu and CuO NPs in the presence of *Chlorella vulgaris* exudates. For this, the cultures of *Chlorella* were filtered and exudates with molecular weight higher than 10 kDa were isolated. In addition, we performed toxicity tests exposing the microalgae to 7 NPs concentrations, ranging from 2.2 to 1100  $\mu\text{g L}^{-1}$ . The metal (Cu) was quantified by graphite furnace atomic absorption spectrometry after ultrafiltration in 3 kDa membranes. From the results of the solubility experiments, variations in the percent dissolved Cu for the initial additions of Cu-NPs was around 70%, reaching in the last additions values of 20%. In the additions of the order of 3  $\mu\text{g L}^{-1}$  CuO-NPs, the percentage of Cu dissolved varied around 80 to 60%, decreasing to 20% in higher concentrations Cu and CuO NPs presented low toxicity to *Chlorella*. The values of chlorophyll-a concentration were similar between the control and the treatments with NP-Cu, however in concentrations of the order of 3 to 60  $\mu\text{g L}^{-1}$ , there was a reduction in chlorophyll-a concentrations. In the tests using NP-CuO, as the NP concentration increased, there was an increase in chlorophyll synthesis. From the results we conclude that evaluation of the environmental risks of NPs is essential for the safe use of these nanomaterials, since organic matter can influence the bioavailability of the metals present in the nanoparticles, making such substances available in a form considered toxic to the environment.

**SP060 PCB distribution in air and soils of contrasting areas of Buenos Aires Province, Argentina**

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Polychlorinated biphenyls (PCBs) have been banned for more than 20 years in the region; however, due to their high chemical stability, great transport capacity and recurrent emissions, they are still detected in the environment at relatively high concentrations. Buenos Aires Province is the most populated state of Argentina; it has the prime agricultural land of the country as well as heavy industrial development (i.e. vehicles, textiles, chemicals, petrochemicals, steel). In order to evaluate spatial gradients and sources of PCB in Buenos Aires state, 13 sites distributed in areas with contrasted human impact were visited to deploy (4 months) polyurethane foam passive air samplers (PUFs) and collect surface soil samples (3-4 subsamples in the first 5 cm). Samples were extracted with organic solvents (PUFs: 24 h with petroleum ether in Soxhlet; lyophilized soils: 2/1/2 petroleum ether: acetone :dichloromethane in

ultrasonic bath), purified on a silica column and analyzed in HRGC-ECD. In soils,  $\Sigma 53$  PCBs range from non-detectable (46% of samples) to 3141 pg g<sup>-1</sup> (dry weight; below international and national guidelines) showing an industrial > rural gradient with a clear predominance of high molecular weight congeners: hexa-CBs (66±24%) > hepta-CBs (17±14%) > penta-CBs (13±13%). In contrast,  $\Sigma 53$  PCB concentrations in air which range from non-detectable (15% of samples) to 364 pg m<sup>-3</sup> show a different spatial gradient: urban > industrial > suburban > rural (with a high rural outlier, probably related to a nearby old waste incinerator) and a lighter compositional pattern: penta-CBs (40±15%) > hexa-CBs (29±11%) > di+tri-CBs (14±14%) > tetra-CBs (13±9%) more pronounced in more polluted sites. These results confirm that industrial and populated sites are the principal sources of PCBs with a fresher airborne signal enriched in more volatile congeners (actual emission or reemission) contrasted with the aged composition of soils, with a prevailing accumulation of more persistent, higher molecular weight PCBs.

**SP061 Plumas de pinguino como bioindicadores de riesgo a elementos tóxicos en ambientes marinos costeros de la isla 25 de Mayo, Antártida.**

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Las plumas de aves marinas son eficientes biomonitores del contenido de elementos tóxicos, como por ejemplo el Hg, en ambientes marinos costeros. Además, constituyen un alerta temprana para el seguimiento de ciertos elementos como Ni y Cu, permitiendo un muestreo conservativo y el estudio retrospectivo del contenido de contaminantes. Este trabajo presenta el análisis de las concentraciones elementales en plumas de pinguinos adultos (n: 9-15) y pichones (n: 10-15), de tres especies mediante el análisis por Activación Neutrónica Instrumental. Las colonias monitoreadas están situadas en la Península Barton: pinguino barbijo (*Pygoscelis antarctica*), y en Península Potter: pinguinos adelia (*Pygoscelis adeliae*) y papúa (*Pygoscelis Papua*), isla 25 de Mayo, Shetlands del Sur, Antártida. Tendiendo en cuenta el desvío estándar, las concentraciones no muestran diferencias significativas. Sin embargo, la tendencia de los datos indica que las concentraciones de Ag, Hg, Cr y As, difieren entre especies. En las muestras de plumas de pinguino adulto el valor medio de Cr fue de 0,503 ± 0,279 mg/kg para la especie barbijo, 0,788 ± 1,44 mg/kg para adelia y 4,87 ± 0,447 mg/kg para papúa. También se encontraron diferencias entre adultos y pichones, los valores medios de Hg (0,906 ± 0,466 mg/kg) y Ag (0,192 ± 0,063 mg/kg) de los individuos adultos de la especie papúa fueron mayores que en los pichones (0,398 ± 0,264 mg/kg) y (0,139 ± 0,091 mg/kg) respectivamente; esta relación se invierte para Cr y As. Si bien los resultados estarían reflejando los hábitos alimentarios de las especies, un incremento en el número de réplicas permitirá confirmar las tendencias observadas.

**SP062 Use of Raman spectroscopy for monitoring organic compounds in the area of influence of Santos submarine outfall, São Paulo, Brazil**

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Although the analytical methods employed in monitoring water quality indicators for inorganic compounds are well developed, efforts are concentrated to address the problem of monitoring organic compounds in environmental samples, since the analytical methods most commonly used for organic compounds (e.g. chromatographic methods) are time consuming and expensive. This study aimed the development of a methodology for the monitoring of organic compounds in seawater samples collected from the area of the submarine sewage outfall of Santos using Raman spectroscopy. Water column sampling was conducted in 6 stations surrounding submarine sewage outfall in Santos Bay. Samples were also collected from a reference station, outside of the influence of the sewage outfall. At each sampling station, 2 liters of seawater were collected from surface water (1 m) and bottom water (8 m) using a Van Dorn bottle. Next, the samples were packaged into amber glass bottles and transported to the laboratory in an insulated box with ice until processing. Samples were then filtered in a 0.45 µm filter and analyzed in a portable near-infrared, dispersive Raman system (Dimension P-1 Raman, Lambda Solutions, Inc., MA, USA), with an 830 nm and 250 mW laser excitation and spectral resolution of about 2

cm<sup>-1</sup>. The spectrometer uses a Raman probe (Vector Probe, Lambda Solutions, Inc. MA, USA) of 3 m long. The deep-depleted, deep-cooled CCD detector is shot-noise limited. For spectral collection, water samples were placed in a sample holder with 100 µL capacity and the probe was placed at a 10 mm distance perpendicular to the sample surface. The signal scattered by each sample was then collected by the probe and coupled to the Raman spectrometer for dispersion and detection. The Raman signal was collected in 5 s and 10 scans for all samples. The gross spectra were calibrated, pre-processed and stored for further analysis. On the Raman spectra from surface and bottom water samples, we found peaks at 1557 and 1565 cm<sup>-1</sup>, which are characteristics of ring stretches, probably of benzene derivatives. Peaks were also found at 1470 and 889 cm<sup>-1</sup>, characteristics of CH<sub>3</sub> and CH<sub>2</sub> deformations and CC skeletal stretch, respectively, possibly from n-alkanes in these samples. Based on the results obtained in this study, improvements in the environmental monitoring systems for organic compounds can be developed through Raman spectroscopy, reducing the discharge of these compounds into aquatic ecosystems.

**Soil ecotoxicology in Latin America: joining efforts between science and governance**

**SP063 A interação entre *Enchytraeus crypticus* e ácaros altera a sensibilidade dos organismos?**

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Os enquitreídeos são organismos de importante papel nos serviços do ecossistema, realizando processos terrestres essenciais, como degradação de matéria orgânica e ciclagem de nutrientes, agindo como regulador biológico. Eles têm sido amplamente utilizados em testes ecotoxicológicos, com métodos padronizados internacionalmente. Entretanto, no Brasil, a presença de ácaros invasores (não predadores) na cultura de *Enchytraeus crypticus* tem sido frequentemente relatada por pesquisadores que cultivam o organismo em diferentes laboratórios. Apesar de estes ácaros não serem predadores, eles competem com os enquitreídeos por alimento e espaço. Erradica-los das culturas têm sido uma tarefa árdua e muitos laboratórios optam por não o fazer. No entanto, para o sucesso da cultura em laboratório, é correta avaliação dos resultados dos testes ecotoxicológicos com diferentes contaminantes, faz-se necessário rigor das condições físico químicas e biológicas de manutenção e cultivo destes organismos, visto que pequenas alterações nestes fatores, podem alterar a sensibilidade dos mesmos. O objetivo desse estudo foi avaliar se a presença de ácaros na cultura de *E. crypticus* pode causar alteração da sensibilidade do organismo-teste. Para tanto, a sensibilidade dos organismos foi avaliada utilizando o ácido bórico como substância de referência. A metodologia utilizada foi adaptada do protocolo ISO 16387 (2012). O solo padrão utilizado foi SAT (Solo Artificial Tropical) com 5% de matéria orgânica (fibra de coco). Os testes foram realizados com culturas contaminadas com ácaros e culturas sem contaminação. O tempo de exposição foi de 21 dias a 20 ± 1°C e as concentrações de ácido bórico utilizadas foram: 12,5; 25; 50; 75; 100 e 125 mg/ Kg de solo seco. Os resultados obtidos demonstraram que houve diferença na sensibilidade dos enquitreídeos *Enchytraeus crypticus* exposto à substância de referência quando provenientes das diferentes culturas.

**SP064 Ácido bórico como substância de referência para ensaio com *Enchytraeus crypticus* em solo artificial tropical**

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O ácido bórico vem sendo proposto como substituto de substâncias de referência que atualmente são recomendadas nas normas ISO, mas que causam grande risco de exposição ao pesquisador e ao meio ambiente, além de algumas já terem sido banidas. O objetivo foi avaliar a resposta do *E. crypticus* ao ácido bórico verificando sua aplicabilidade para futura normatização como substância de referência para o ensaio de reprodução com esta espécie. Foi realizado o ensaio de reprodução de *E. crypticus* seguindo as recomendações da norma ABNT NBR ISO 16387. O ensaio foi realizado em SAT com teor de 5% de material orgânico, onde foram aplicadas as concentrações 25, 50, 100, 200 e 400 mg por Kg<sup>-1</sup> de ácido bórico. No solo controle foi adicionada somente água destilada. A umidade do solo nos tratamentos foi ajustada até 50% da capacidade de retenção de água. O ensaio foi incubado a 20°C ± 1°C, em fotoperíodo 16:8 h luz:escuro, durante 28 dias. O número médio de juvenis para cada concentração e para o controle foi contabilizado à lupa e registrado. A concentração de efeito para 50% (CE50) foi calculada em 165,2 mg.kg<sup>-1</sup> (IC 95% = 135,8-194,6 mg.kg<sup>-1</sup>) seguindo o modelo de regressão linear. O ensaio foi repetido e valores próximos foram obtidos.



Além disso, estes valores foram próximos a valores já relatados para esta espécie em solo artificial OECD. Assim, o ácido bórico tem apresentado resultados satisfatórios para ser usado como uma substância de referência a ser recomendada pelas normas ISO e ABNT para o ensaio de reprodução de *E. crypticus*. \n Apoio: Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq – Brasil, Projeto Universal CNPq 454842/2014-7.

#### SP065 Acute toxicity of acetamiprid to earthworm, *eisenia andrei*

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In this study, we used two different types of bioassay, a contact filter paper toxicity bioassay and an avoidance toxicity bioassay, to analyze the acute toxicity of Acetamiprid on earthworm species, *Eisenia andrei*. Acetamiprid, a neonicotinoid insecticide, has been used widely in agriculture worldwide. Due to structural and physiological similarities between pest and non- pest species, most insecticides are toxic not only to the target species, but also to a range of non-target organisms, works by disrupting the insect nervous system. In the filter paper contact test was held by protocol OECD 207 (OECD, 1984) in concentrations 0.00016; 0.0016; 0.016; 0.16  $\mu\text{g}\cdot\text{cm}^{-2}$ . All organisms were weighed over the 24, 48 and 72 h periods. In the avoidance test was held according to the proposed procedure ISO 17512-1 250 (2011) in concentrations 0.01; 0.1; 1  $\text{mg}\cdot\text{kg}^{-1}$  with use of Oxisol. After 48 hours, a count of the organisms in each of the sections was performed to verify attraction and/or repulsion to acetamiprid. The results of the contact assay determined the  $\text{LC}_{50}$  after 72h of exposure (0.0526  $\mu\text{g}\cdot\text{cm}^{-2}$ ) due to the number of deaths. Acetamiprid has a time and concentration-dependent toxicity. It was observed that even the organisms exposed to the lowest concentrations presented morphological alterations such as: strangulation, swelling and deformities in the clitellum, besides lethargy and sensitivity. In the avoidance test, 60 and 90% of the earthworms were in the control soil for the concentrations of 0.1 e 1  $\text{mg}\cdot\text{kg}^{-1}$ , respectively. Some organisms of the concentration 1  $\text{mg}\cdot\text{kg}^{-1}$  presented nodules and partitions. In the lowest concentration the organisms did not show repulsion to the substance. It is possible that the acetamiprid affects the long-term survival of organisms. The conclusions: the use of acetamiprid may compromise the survival of the annelids of the *Eisenia andrei* species and this may affect other edaphic organisms.

#### SP066 Analysis of biomarkers of *eisenia andrei* after chronic exposure to imazalil fungicide

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Imazalil is a systemic fungicide commonly used in seed treatment and post-harvest. Since it is an emerging contaminant, its ecotoxicological effects have been little studied. However, research indicates that it can cause morphological abnormalities, DNA damage, central nervous system effects and can act as teratogenic agent in vertebrates. The study aims to evaluate possible alterations in oxidative stress enzymes and cells of the immune system of annelids *Eisenia andrei* species after chronic exposure to the compound. The test was performed according to ISO 11268-2 standards in Red-Yellow Latosol (50% WHC). Four concentrations of the compound (0.01, 0.1, 1 and 10  $\text{mg}\cdot\text{kg}^{-1}$ ) and the control were used, each containing 6 replicates and 10 organisms. During the test, mortality and biomass variation were observed; activity of the enzymes GST (Glutathione S-transferases) and Catalase were quantified; as well as analysis of the coelomic fluid. The results showed that the biomass changes were not significant when compared to the control, the same occurred with mortality. The changes in catalase were not significant, however, GST presented an increase in concentration of 0.1  $\text{mg}\cdot\text{kg}^{-1}$  at the 30<sup>th</sup> day, indicating an increase in the detoxification capacity of the organism in this period. There was a significant increase in the percentage of Eletrocytes at the 30<sup>th</sup> day while proportional to a decrease in Hyaline Amebocytes, which may be justified by the function of these cells. While amebocytes act on coagulation, inflammation and wound healing, the electrocytes are responsible for encapsulation, storage of endogenous materials and tissue detoxification. Although, it should be noted that at the 45<sup>th</sup> day of exposure the slides of concentrations 0.1; 1; 10  $\text{mg}\cdot\text{kg}^{-1}$  presented extremely degraded cells. There was a significant increase in the cell density of the organisms exposed to the concentration

of 10  $\text{mg}\cdot\text{kg}^{-1}$  at the 30<sup>th</sup> day. However, concentrations of 1 and 10  $\text{mg}\cdot\text{kg}^{-1}$  showed significantly lower viability over the control throughout the experiment. That is, the organism identified the compound as a toxic agent late, when it already had a negative effect on the system. At 45<sup>o</sup>, the lowest concentrations (0.01 and 0.1  $\text{mg}\cdot\text{kg}^{-1}$ ) also showed a decrease in viability. Therefore, can be concluded that the compound has a sub-lethal toxicity to *Eisenia andrei*. Especially to the cells of your immune system, where it acts in a time and concentration-dependent manner.

#### SP067 Arsenic (As) and Lead (Pb) occurrence in soil samples near to scientific stations in King George Island and Deception Island, Antarctica.

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Antarctica is not free from deleterious human impacts. Anthropogenic pollution can reach the polar regions through atmospheric circulation and sea currents, e.g., organochlorines, heavy metals and plastic. Increasing human activities in Antarctica after the International Geophysical Year (1957-58) have caused several pollution problems, mainly near scientific stations. King George Island (KGI) is a part of the archipelago of the South Shetland Islands, considered one of the areas with high anthropogenic presence. Today KGI is one of the favorite tourist Antarctic destinations and it is considered one of the areas with the highest concentration of international scientific stations in the world. Deception Island (DI), a volcano island and part of the archipelago, is today one of the most visited areas by tourist cruises. Heavy metals occur naturally in the Earth's crust. However, human activities have introduced high loads of these elements into the environment, making difficult the differentiation of natural and anthropogenic contributions. Although sediments usually accumulate heavy metals, soils along with rocks, are the terrigenous sources of elements to adjacent sediments and can indicate local hot spots. The aim of this work is to evaluate the occurrence of As and Pb within soil samples of Fildes Bay in KGI and in DI, Antarctica. Soil samples (top 10cm) were collected during the summer of 2016, in the 53st Chilean Antarctic Expedition (ECA-53). Surface soil samples (n = 9) were collected in the marine terrace in front of the scientific stations of Fildes Bay, KGI, and in the stations of DI. Organic matter (OM) was determined by calcination (450 C, 24h) and clay minerals were determined by X-ray diffraction (XRD). Pb and As were determined by ICP-MS. The results show low OM and high clay content for all the samples. In relation to Pb and As, results are similar in magnitude to the reported previously in soil and sediments in Aldmiralty Bay (KGI) and in penguin feathers from KGI and DI. Compared with other industrialized sectors, Pb and As content are low. Continuous environmental monitoring, determination of local background through soil and sediments, transference rates to biota, and further ecotoxicological studies are essential for identifying and preventing pollution in Antarctica. **Acknowledgments** This study was supported by the project INACH RT-09-15. The authors acknowledge logistic and scientific support of INACH during the Antarctic expeditions.

#### SP068 Arsenic and heavy metals in soils affected by coal mining in Samacá (Colombia)

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Coal mining is one of the most usual economic activities in the Andes region in Colombia. We sampled soils in the proximity of four coal underground mines in the municipality of Samacá and analyzed by ICP-AES the concentrations of the heavy metals aluminum (Al), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn) and the metalloids arsenic (As). We employed a sequential extraction to separate the organic and exchangeable fractions of each element and single extractions to characterize the different phases of arsenic in soil. In addition, we traced 50m transects from the coal residuals toward the more undisturbed zone, in opposite direction to the mines, to describe the plant composition and to evaluate the change of diversity in intervals of 12.5m. The most frequent elements in the soils were Al (365-927  $\text{mg}\cdot\text{kg}^{-1}$ ), Fe (366-1080  $\text{mg}\cdot\text{kg}^{-1}$ ) Mn (13.83-45.34  $\text{mg}\cdot\text{kg}^{-1}$ ) and Zn (13.46-45.24  $\text{mg}\cdot\text{kg}^{-1}$ ), but the exchangeable fraction of As, more available for uptake by plants, had concentrations potentially toxic (6.98 $\pm$ 0.35  $\text{mg}\cdot\text{kg}^{-1}$ ). The specific determinations showed that around 80% of As is bonded as arsenate to oxides of Al and Fe. Overall, we found 68 species of plants distributed differentially in relation with the site and distance to the coal source. In three sites, the indices of diversity Margaleff and alfa-Fisher near the mines were lower than at higher distances from the coal source. In the clusters of these three sites, the general composition of the distance 0-12.5m diverged from the rest of the intervals of distance. We found eight species that could have high tolerance to As because to be unique for the nearest zone of coal mines: *Alnus*

*acuminata*, *Baccharis angustifolia*, *Baccharis latifolia*, *Camomila matricaria*, *Lolium multiflorum*, *Myrica parvifolia*, *Solanum lycioides* and *Trifolium repens*. The abundance of the generalist species *Penisetum clandestinum*, that was found at all intervals of distance from the source, was positive correlated with the concentration of As. We concluded that underground coal mining in Samacá is related with potentially toxic concentrations of As, that must be immobilized to avoid its transport toward crop fields. For further studies we propose a deeper research on the tolerance of *Alnus acuminata* and *Myrica parvifolia* to As, since they are the woody species that could be used for reforestation programs of post-mining soils.

**SP069 Atenuação do efeito mutagênico do lodo de esgoto após bioestimulação, visando seu uso agrícola**

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O tratamento de efluentes realizado em estações de tratamento de esgoto (ETE) gera um resíduo denominado lodo de esgoto (LE). Devido ao aumento populacional dos centros urbanos e à cobrança por saneamento básico, esse resíduo tem sido foco de atenção pela sua produção crescente. Por apresentar uma alta concentração de matéria orgânica e macronutrientes, tem sido proposta a sua utilização como aditivo agrícola, visando a substituição de fertilizantes sintéticos, a reciclagem de nutrientes e uma redução nos custos com o descarte deste resíduo em aterros sanitários. Contudo, a presença de contaminantes no LE, incluindo alguns metais e substâncias orgânicas tóxicas, pode inviabilizar sua aplicação agrícola, por ser altamente detrimental ao ambiente. Portanto, este trabalho propôs a redução da toxicidade do LE aeróbio, de origem sanitária, proveniente da ETE da cidade de Americana/SP, por meio do processo de bioestimulação, utilizando bagaço de cana-de-açúcar (BAG) e borra de café (BO). Para aplicação do estudo foram realizadas diluições do LE nas seguintes proporções volumétricas: LE+SOLO(S) (3:3), LE+S+BO (3:3:1) e LE+S+BAG (3:3:1). Além disso, no tempo inicial, foi avaliado também o LE puro. A avaliação da efetividade do processo foi realizada caracterizando o potencial mutagênico das amostras, por meio do teste do micronúcleo em *Allium cepa*, em células meristemáticas e F1, no período inicial (T0) e após dois meses (T1) do processo de bioestimulação. Em T0, todas as amostras apresentaram resultados significativos para a presença de micronúcleos nas células meristemáticas. No entanto, não houve correspondência desses resultados para as células F1, com exceção da amostras de LE puro. Para esta amostra, houve uma fixação dos danos observados para as células meristemáticas nas células filhas (F1), indicando uma alta potencialidade mutagênica deste resíduo. Após dois meses de bioestimulação, o potencial mutagênico foi significativamente reduzido para as amostras LE+S e LE+S+BAG. Porém, um efeito mutagênico significativo ainda foi observado para a amostra LE+S+BO. Assim, para o período estudado, o BAG parece ser caracterizar como um agente bioestimulante bastante efetivo na detoxificação do LE, já que após dois meses de processo, o efeito mutagênico da amostra contendo BAG foi atenuado. Assim, a bioestimulação com este material (BAG) parece ser uma tecnologia promissora para transformar o LE em um material seguro para uso agrícola.

**SP070 Avaliação da ecotoxicidade de herbicidas à base de glifosato: ensaios de fuga convencionais e multiespécies com minhocas, colêmbolos e isópodos**

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O glifosato é um dos princípios ativo mais empregados na agricultura mundial. Porém, há poucos estudos sobre seus possíveis efeitos de ecotoxicidade para a fauna do solo. Ensaios de comportamento de fuga podem indicar se os organismos deixam um local após a aplicação de um agroquímico, o que pode indicar impacto sobre as funções ecossistêmicas exercidas pela fauna edáfica. O objetivo deste trabalho foi avaliar o efeito de aplicações de quatro herbicidas à base de glifosato, na dessecação de aveia preta, para a macro e mesofauna edáfica usando ensaios de fuga. Os produtos Roundup Original®, Trop®, Zapp QI 620® e Crucial®, foram aplicados na dose recomendada (720 g ha<sup>-1</sup> do equivalente ácido glifosato, independentemente do produto comercial) para dessecar aveia preta (*Avena strigosa*) na Fazenda Experimental da Universidade Federal de Santa Catarina (UFSC) em Curitiba, SC, Brasil. O solo foi classificado como Cambissolo háplico de textura argilosa, com 2,2% de matéria orgânica. Onze dias após a aplicação, o solo foi coletado, levado ao laboratório e peneirado. A umidade foi ajustada para cerca de 50% da capacidade de retenção de água antes de iniciar os ensaios. Ensaios de fuga convencionais foram realizados com minhocas

*Eisenia andrei*, de acordo com a norma ABNT NBR ISO 17512-1, e com colêmbolos *Folsomia candida*, de acordo com a norma ISO 17512-2, combinando o solo coletado em parcelas após a aplicação dos herbicidas com o solo coletado nas parcelas controle (sem aplicação). Um ensaio multiespécies com *E. andrei* e isópodos *Porcellio dilatatus* foi realizado com base no ensaio de fuga para minhocas, porém usando uma base de substrato húmico, contendo de um lado a palha da aveia que recebeu a aplicação de herbicidas, e de outro a palha da aveia das parcelas sem aplicação. Os resultados foram analisados usando o teste exato de Fisher (p < 0,05), avaliando se houve fuga dos solos ou palha com o herbicida. Não houve fuga em nenhum dos tratamentos avaliados, tanto para o solo quanto para a palha, para nenhuma das espécies avaliadas. Como os organismos parecem não evitar o solo e palha com as aplicações, recomenda-se a realização de estudos que visem verificar possíveis efeitos crônicos (p.ex., reprodução) para as populações de macro e mesofauna expostas. Fonte de recursos: AGRISUS, Programa Iniciação Científica UFSC, e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brasil, Projeto Universal CNPq 454842/2014-7.

**SP071 Avaliação da ecotoxicidade de quatro formulações de herbicidas à base de glifosato para colêmbolos *Folsomia candida***

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No sul do Brasil é comum o uso de glifosato para dessecação da cultura da aveia preta (*Avena strigosa* Schreb.) cultivada como cobertura na época de inverno. Porém, a presença de resíduos de agrotóxicos no solo pode impactar negativamente na sua qualidade. Os colêmbolos são organismos da mesofauna edáfica fundamentais para a decomposição e ciclagem de nutrientes, sendo reconhecidamente sensíveis a presença de resíduos de agrotóxicos. O presente trabalho teve como objetivo realizar a avaliação preliminar de efeitos ecotoxicológicos do uso de herbicidas à base de glifosato, na dessecação da aveia preta, sobre a fauna do solo. Para os ensaios, amostras de solo não contaminado foram coletadas na Área Experimental Agropecuária da Universidade Federal de Santa Catarina, em Curitiba, SC, no Planalto Catarinense. O solo foi classificado como Cambissolo háplico de textura argilosa, com 2,2% de matéria orgânica e pH de 4,74. O solo foi contaminado em laboratório com quatro herbicidas à base de glifosato, em quatro doses: Crucial® (8,73; 17,45; 34 e 69,8 mg/Kg); Roundup Original® e Trop® (6; 12; 24 e 48 mg/Kg); Zapp QI 620® (7,75; 15,5; 31 e 62 mg/Kg). As doses mais altas selecionadas são consideradas superestimativas de concentração de glifosato em solo imediatamente após a aplicação de herbicidas. Nos controles, o solo recebeu apenas água destilada. Foi realizado o ensaio de reprodução com colêmbolos *Folsomia candida*, seguindo a norma ABNT NBR ISO 11267, avaliando possíveis efeitos sobre as populações expostas destes organismos, além de comparar a ecotoxicidade das diferentes formulações. Os ensaios foram realizados a 20°C em fotoperíodo 16:8 h luz:escuro, com duração de 28 dias. Ao final do ensaio, o número médio de juvenis obtidos nos tratamentos foi comparado com o número médio obtido no controle, usando ANOVA seguida do teste de comparações múltiplas de Dunnet (p < 0,05). Não houve diferenças estatisticamente significativas entre o número médio de juvenis nos solos com aplicação dos produtos e no solo controle. Os resultados indicam ausência de efeitos destas formulações sobre a reprodução de colêmbolos em todas as doses avaliadas. Outros autores também não verificaram efeitos de herbicidas à base de glifosato na reprodução de colêmbolos. Recomenda-se estudos com outros grupos de organismos e a verificação de efeitos *in situ* para as comunidades autóctones destas áreas. Fonte de recursos: AGRISUS e Projeto Universal CNPq número 449731/2014-6.

**SP072 Avaliação de Risco Ecológico do ecossistema terrestre com base no modelo agrícola convencional brasileiro: Estudo de caso em Bom Repouso (MG)**

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O método de avaliação do Risco Ecológico (ARE) proposto pela Agência Americana de Proteção Ambiental (USEPA), pode ser usado para calcular os danos e impactos causados ao meio ambiente pela ação antrópica. Esse método consiste em três etapas: formulação do problema, avaliação da exposição e caracterização do risco. No Brasil existe poucas pesquisas na área e, portanto, verifica-se uma necessidade de novos estudos e aplicações da Avaliação de Risco Ecológico no país, demonstrando uma análise de viabilidade para os ecossistemas localizados em regiões tropicais e subtropicais. Buscando ampliar a aplicação da ARE em ecossistemas terrestres tropicais, e levando em consideração uma problemática do município de Bom Repouso - MG, Sua principal fonte econômica é o cultivo convencional (com uso de agrotóxicos e fertilizantes) de morango e batata inglesa. Desta forma, o principal objetivo deste

trabalho foi o de Avaliar o Risco Ecológico causado pela utilização de agrotóxicos em cultivos convencionais de morango sobre o ambiente terrestre, ainda muito negligenciado no país. KRAFT 36 EC (ia abamectina) e SCORE® 250 CE (ia difenoconazol). *Testes com invertebrados terrestres (Folsomia candida, Enchytraeus crypticus e Hypoaspis aculeifer)* foram realizados para aferir a toxicidade dos compostos de organismos do solo. A concentração ambiental foi calculada com a ajuda do programa matemático FOCUS, o cálculo da distribuição ambiental prevista,

**SP073 Avoidance test with earthworm *Eisenia andrei* in soil amended with poultry litter**

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Poultry litter is a by-product of poultry meat farming consisting of a vegetable substrate and waste (feces, urine, feed and veterinary products). It is continuously used as an agricultural fertilizer, which solves the problem of waste disposal for production as well as providing the required nutrients for agriculture. However, it is also a potential source of environmental contamination. The aim of the present study was to assess behavior effects of soil amended with poultry litter on *Eisenia andrei*. In addition, were determined in the litter sample the fluoroquinolone enrofloxacin (ENR), an antibiotic commonly used in Brazilian poultry farming and ciprofloxacin (CIP), its main metabolite. Fluoroquinolones were determined using high-performance liquid chromatography (HPLC) with a fluorescence detector. The litter was sampled from São José do Vale do Rio Preto, the largest poultry meat pole of Rio de Janeiro state. The avoidance test was performed according to ISO 17512-1 (2011) in soil (Red-Yellow Ultisol) with poultry litter in percentages of 5, 10, 15, 20, 25, 50, 75 and 100. In each container, there were two sections, separated by a partition, one of which housed a 200g soil control while the other was a mix of soil and poultry litter. The sections were moistened and the partitions between the control and the spiked soil were removed. In this empty space, 10 earthworms were introduced, allowing their free movement. After 48 hours of exposure, it was determined whether the worms were in the control or spiked soil. The mortality rate was verified. A completely randomized experimental design was employed, with five replicates for each group. Loss of soil habitat function is considered if more than 80% of the total organisms exposed to the control soil sample are found. The avoidance test revealed that poultry litter strongly repels the worms from their habitat. All live worms recovered were in the soil controls. In addition, a strong morphology alteration was observed, such as swelling, partition and bottlenecks. For the proportions of 25, 50, 75 and 100% worm mortality rates were 22.8, 36.3, 11.3, 51.6%, respectively. Besides that, 5 µg.g<sup>-1</sup> of ENR and 23 µg.g<sup>-1</sup> of CIP were measured from litter sample. In view of these results, we intend to follow up the studies, assessing the possible effects in acute and chronic tests, as well as to evaluate if antibiotic residues contributed to the observed effects.

**SP074 Chronic exposure of *Lactuca sativa* to a mine-contaminated soil from La Planta-Marayes (Argentina): I. Bioavailability of Zn, Cu and Pb.**

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The unplanned disposal of raw mine residues causes environmental pollution due to the release of metals, rocks and chemicals. The bioavailability of metals causes the adverse effects on organisms. The aim of this study was to determine the bioavailable concentrations of Zn, Cu and Pb in a contaminated soil with residues from an abandoned gold mine in La Planta-Marayes. Two samples were taken from the first 20 cm of soil in La Planta town: A contaminated soil with mine residues (Site 1), and one located to 2 Km which was used as reference (Site 2). A completely random experimental design consisted of 7 treatments: mixtures of soil from Site 1 and 2 (dw/dw) in the range of 0 to 100%. Three replicates per treatment were used. The hotplate *aqua regia* method was used to extract the pseudo total fraction (PF). Mobilizable and soluble fractions (MF and SF) were extracted using DTPA and deionized water, respectively. Metal concentrations were quantified using an atomic

absorption spectrophotometer. Also pH and electrical conductivity (EC) were measured in the PF and SF. Concentrations of metals in PF were compared with pre-anthropogenic concentrations and soil quality guidelines to determine pollution. Also Geoaccumulation Index, Contamination Factor and the Contamination Degree were determined. Site 2 was characterized by a basic pH (8.80 ± 0.09) and low salinity (EC = 0.85 ± 0.04 mS cm<sup>-1</sup>), whereas Site 1 was characterized by an acid pH (2.57 ± 0.03) and high salinity (EC = 7.26 ± 1.50 mS cm<sup>-1</sup>). Results showed the highest metal concentration in PF and lowest in the bioavailable fractions (MF and SF). High metal concentrations and low values of pH in PF were associated with an increase of mine residues, while no differences in EC were observed. The percentage of metal extracted in MF showed a concentration-dependent decrease of Zn and Pb, whereas an increase of Cu. A concentration-dependent increase was observed in SF of Zn and Pb (*p* < 0.05). The comparison with soil quality guidelines and pre-anthropogenic concentrations showed the usefulness of Site 2 as a reference site. The results indicated that Site 1 is heavily contaminated at least with Zn (9975 ± 696.87 µg g<sup>-1</sup>), Cu (457.17 ± 3.21 µg g<sup>-1</sup>) and Pb (1608.33 ± 50.52 µg g<sup>-1</sup>). A remediation proposal for this site is suggested.

**SP075 Combination of fractionation procedure and bioassay to determine cytotoxic potential of sewage sludge samples**

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Due to the constantly increased production of sewage sludge (SS) and to its high agronomic potential, the use of this waste as soil conditioner has been recommended. However, the application of SS in agricultural soils requires some attention since SS may contain potentially hazardous substances. The aim of the present study was to establish a correlation between the chemical fractions of the SSs studied and their biological activities regarding the presence of cytotoxic compounds, in order to allow a better comprehension of the effects induced by these contaminants in complex environmental matrices such as SS. The SSs assessed in this study were obtained from two WWTPs located in the municipality of Americana, SP, Brazil. These WWTPs differ in the composition of the sewage received and also in the process used to treat the sewage, which leads to the production of SS with quite distinct characteristics. To better determine the presence of cytotoxic compounds in SS, a toxicity identification fractionation (TIF) procedure were performed, dividing the target fractions into several fractions according to their polarity. Two different extractions were carried out following a combination of ultrasonic extraction and solid phase extraction (SPE). Fractions from extraction 1 were eluted from the HLB cartridge by using diethyl ether (FI), ethyl acetate (FII), and methanol (FIII) as solvents. Fractions from extraction 2 were eluted from the silica SPE cartridge by using n-hexane (FIV) and n-butyl chloride (FV) as solvents. Cytotoxicity was determined after 24 h of exposure of HepG2 (human hepatoma) cells to the different fractions using the MTT assay, a simple and quantitative method for determining cell viability based on the mitochondrial metabolism of a tetrazolium salt to a blue formazan. A dose-dependent cytotoxic effect was observed for all tested fractions. Regarding the anaerobic sludge, FI, FII and FIII presented significant cytotoxic effects in concentrations above 12.5 mL/mL, while for FIV and FV no effect was observed in concentrations of 37,5 mL/mL. However, for the aerobic sludge, FII and FIII was the most cytotoxic, followed by FV (effects in above 25 mL/mL). Due to the adverse biological effect observed, chemical analyses should be performed to identify the cytotoxic compounds present in the fractions. These results also warn that SS may contain contaminants with high cytotoxic activity, which implies restrictions on its use in agricultural soils.

**SP076 Early ecotoxicity screening of metsulfuron-methyl herbicide to soil fauna: avoidance behavior tests with earthworms and collembolans**

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Herbicides correspond to more than 50% of the agrochemical's sales in Brazil. The active ingredient metsulfuron-methyl belongs to the sulphonylurea chemical group, presenting acetolactate synthase (ALS) enzyme inhibiting mode-of-action. This is a common herbicide formulation, and such products show high levels of activity in low doses. Metsulfuron-methyl formulated products are recommended for pre-emergence application in sugar cane and post-emergence in other crops, e.g. oats and wheat for broadleaf weed control. However, there are few studies regarding its ecotoxicological effects to soil fauna. This study aimed to evaluate the ecotoxicity of a metsulfuron-methyl herbicide to soil fauna in avoidance behavior tests, using the earthworm species *Eisenia andrei* Bouché 1972 and the collembola species *Folsomia candida* Willem 1902, when exposed to contaminated soil with Allyl®. The tests followed the ABNT



NBR ISO 17512-1 (2011) and ISO 17512-2 (2011) guidelines. Six concentrations of the commercial product (15.62; 31.25; 62.50; 125; 250 e 500 mg.kg<sup>-1</sup>) were applied in tropical artificial soil (TAS; 5% organic matter) in the laboratory. TAS with distilled water was used as control treatment. After 48 h exposure, the average number of organisms was recorded for each trial. The results were analyzed by the Fisher's exact test ( $p < 0.05$ ). Avoidance behavior of earthworms was observed at 250 and 500 mg.kg<sup>-1</sup>, while non-avoidance behavior was observed for collembolan at this concentration range. Results showed that earthworms are more sensitive than collembolans for the tested formulation. Further studies must embrace acute and chronic tests in order to evaluate effects on survival and reproduction of these groups of organisms.

**SP077 Ecotoxicological effects of the insecticide fipronil to the collembolan *Folsomia candida* in natural tropical soil**

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The pesticide fipronil is one of the most used insecticide in Brazil. Fipronil belongs to the phenyl pyrazole group, a compound with a hydrophobic characteristic and persistence in soil. It acts as an inhibitor of nerve signals in insects, making it a risk for non-target terrestrial and aquatic organisms. Fipronil has been used to control pests in agriculture, being the second pesticide most used in sugarcane cultures, and to control diseases in domestic animals. Because it has low solubility in water, and affinity for organic matrices such as lipids, the compound may be harmful to soil edaphic organisms, which play an important role in the maintenance of soil quality through processes such as nutrient cycling. The main objective of this study was to evaluate the effects on survival and reproduction of *Folsomia candida* when exposed to the insecticide Regent 800WG®, which contains 80% of fipronil in the formulation, using a natural tropical soil. The organisms used in the test were of synchronized age (10 and 12 days) and the test followed the guideline ISO 11267. The chosen concentrations of fipronil were based on the recommended doses for the control of the pest *Migdolus fryanus* in sugarcane cultures (1.3 mg of the commercial product (c.p) kg<sup>-1</sup>). Concentrations of 0.26, 0.52, 1.04 and 2.08 mg of fipronil kg<sup>-1</sup> of dry soil were used. According to the obtained results, fipronil had a severe effect on *Folsomia candida* reproduction and survival. The organisms reproduction was inhibited in approx. 50% at the first concentration (0.26 mg of fipronil), which represent just one-fourth of the recommended dose. The organisms survival was affected at concentrations LC20 and LC50 of 0.1 and 0.2 mg respectively. Further studies are being performed in order to evaluate the effects of the insecticide in smaller doses and at the subsequent generations of *Folsomia candida* born and grown in the test conditions.

**SP078 Ecotoxicological evaluation of sewage sludge applied in soil as organic fertilizer**

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The sewage sludge is a sub product of wastewater treatment plant, which is rich in nitrogen, phosphorus, micronutrients and organic matter, and therefore, its use as fertilizer for the soil's improvement and recovery is indicated. However, there are some substances, as metals and sodium, that could be toxic to the edaphic fauna. Therefore, the present work aimed to evaluate the toxicity of the sewage sludge applied in the soil as an organic fertilizer. The experiment was based on simulation of the application of sewage sludge in a degraded soil (negative control) at the doses of 15t/ha, 30t/ha, 60t/ha, 120t/ha and 240t/ha. Beyond these dosages, there was a treatment with only sludge sewage. It was made an acute toxicity test with the earthworm *Eisenia andrei* and a germination and growth test with the radish *Raphanus sativus*, following the specific protocols. The earthworm test was carried out in plastic containers (2,5L) filled with 500g dw of soil. At each receipt, 10 individuals were placed. The test was run at 23.3±1.8 °C and a 12L:12D photoperiod. For the germination and growth test, it was used as a positive control a fertile soil. The soil and the sludge sewage/soil mixtures were placed in 9.5cm-diameter-pots, and 10 seeds were planted at each pot. They were maintained at 23.0±1.8 °C and a 16L:8D photoperiod. No toxic effects were observed at the dosages tested. At the acute test with the earthworm, the mortality rate was below the 10% in all the treatments, and no significant difference was found between them ( $p > 0.05$  - Kruskal-Wallis test). Significant difference ( $p > 0.05$ ) for the radish germination rate between the treatments was not observed either, this rate was above 95% for all dosages. Concerning the initial growth, evaluated as dry weight of the aerial part, it was verified an increasing of the biomass of the seedlings with the increase of the sludge sewage dosages in the degraded soil (positive control: 59.3±10.2mg; negative control: 40.8±10.0mg; 15t/ha: 36.2±2.2mg; 30 t/ha: 38.5±6.7mg; 60t/ha: 40.5±5.1mg; 120t/ha: 52.3±6.5mg; 240t/ha: 51.3±11.2mg; pure sludge sewage:

74.8±7.9mg), that could be related with the soil fertility improvement from the sludge sewage application. Analysing the results, it was possible to conclude that the sewage sludge, at the dosages applied in the soil in this research, were not toxic for the species tested.

**SP079 Effect of pyrethrum biocide on midgut of Africanized honey bees**

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*Apis mellifera* honey bee is a eusocial insect that is an important pollinator in agroecosystems, because this bee species maintains natural flora and increasing the productivity of many crops. However, the habitat fragmentation for urbanization or agriculture, as well as pollution and agrochemicals, contribute to the decline of bee populations because these multiple stressors have weakened the colonies. Insecticides used for control pest-insect in agriculture are very dangerous for bees. As biocides are considered safer and less toxic to non-target organisms in comparison to synthetic chemical compounds, it is important to perform ecotoxicological tests with honeybees exposed to systemic biocides that are translocated to pollen and nectar of cultivated plants. The pyrethrins are used as active ingredient in many commercial formulations, and the biocide pyrethrum is highly toxic and has neurotoxic effects in insects. In this context, our purpose was to analyze comparatively the morphological changes in the midgut of honey bee (*Apis mellifera*) induced by two concentrations of pyrethrum biocide: 0.1 ng.µL<sup>-1</sup> (100 ppb) and 0.01 ng.µL<sup>-1</sup> (10 ppb) of pyrethrum on syrup. For ecotoxicological assays by acute oral exposure (48 h), newly-emerged bees from three different colonies were randomly divided into cages, which comprises the pyrethrum-exposed, acetone (solvent) and control groups (n=20 individuals per cage), assayed in quadruplicate. Syrup was provided *ad libitum* for 48 h. After exposure, the animals were euthanized, their midgut was collected and routinely processed for morphological analysis by optical microscopy. The results showed a concentration-dependent intensity of the following changes: increasing in the release of apocrine secretion from epithelium, increasing in the release of cells into the lumen, and thickening of peritrophic matrix in the biocide exposed-groups. These results indicated that the midgut epithelium is responsive to chemical exposure, and increase their secretion production such as enzymes and compounds of the matrix peritrophic in order to protect the cells against toxic xenobiotics as pyrethrum, which probably is cytotoxic because increased the release of digestive cells into the lumen. By the way, low concentrations of biocide pyrethrum in the environment can induce sublethal effects in honey bee, which could compromise the food digestion and absorption by midgut and decrease the bee performance over the time.

**SP080 Effects of the use of biosolid of the ETE/Ilha/RJ on seeds germination tests in *Lactuca sativa*, on avoidance and lethality tests in *Eisenia andrei*.**

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Mud from wastewater treatment plants can be turned into biosolid, its application in the form of fertilizer and as substrate for reforestation being seen as a sustainable and economically viable practice in dealing with final destination. An evaluation of this effect on the biota is needed though, before spreading this practice. The biosolid used in the essays has an exclusively domestic origin at Ilha do Governador ETE/CEDAE (RJ) where 525 l/s of sludge are treated. Heavy metal residues and pathogens values are lower than those established by CONAMA 375 regulation, therefore qualifying the biosolid as Class A. Different concentrations of the residue were applied to two types of natural soils: ultisol and planosol, at EMBRAPA/Seropédica Experimental Field. Mixtures were sifted (2mm-pore screen), used as substrates and rehydrated to 60% of its field capacity. Physical and physico-chemical parameters, germination percentage and lettuce (*Lactuca sativa*) plantule growing, escape and lethality in *Eisenia andrei* annelids, were evaluated. In seed germination essays, 6 plates (10-seed each) were used for each treatment. After 96 hours the numbers of the germinated ones were registered and, after 6 days, plantules measurement was performed. 12, 25, 37, 50 e 62% concentrations of biosolid, besides the control, were used in the treatments. Escape essays and lethality of annelids concentrations were 15, 30, 45, 60 e 75% of biosolid (besides the control). Five replicas from the escape essay and 4 replicas from the lethality essay were used, both with 10 adult clitellates per replica. The escape essay was 48 hours long and the lethality one 14 days long. All tests temperature stayed

at 25°C±2°C and photoperiod was 12 hours long. Toxicity varies according to the doses and soil type. For seeds germination, values of LC50 have a 27% to 31% range. Plantule size reduction is directly proportional to the biosolid concentrations increase. In the escape essay, AC50 have a 39% to 42% range. There were no deaths in any treatment of the lethality essays. The conductivity high values and the salinity presence in higher concentrations of the biosolid may be related to the residue toxicity.

**SP081 Elution pattern of lysimeters treated with Gamma Alumina based Nanoparticles containing Ni**

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Nanomaterials are widely used for different purposes all around the world. Different researches have begun to study deleterious effects of these materials on living organisms. However, scarce evidence of their fate in the environment is available and it is mostly based on modelling studies. The great variety of industrial applications of metal based nanomaterials allows predicting final deposition in soils of both degradation products and intermediate products formed during their synthesis. Gamma alumina based nanoparticles containing Ni are used in the fabrication of catalysers as well as other commercial products. We have developed a preliminary three months assay to evaluate Al and Ni elution from lysimeters consisting in PVC pipes filled with soil from the Patagonia, irrigated with deionized water simulating rain regimen and maintained under laboratory conditions after different initial treatments: a) none (Control, n° 1), b) AlCl<sub>3</sub>·6H<sub>2</sub>O and NiCl<sub>2</sub>·6H<sub>2</sub>O solutions prepared to add 100 mg of Al and Ni /soil kg in one application (n°2), c) AlCl<sub>3</sub>·6H<sub>2</sub>O and NiCl<sub>2</sub>·6H<sub>2</sub>O salts in solid state (100 mg of each/soil kg) (n°3) and d) gamma alumina based + Ni nanoparticles suspension, added during several days to reach 200 mg of nanoparticles /soil kg (n°4). Samples from lysimeter eluates were pooled monthly and metals content was analysed by ICP-MS spectrometry. As eluates from lysimeters 1 and 4 had a more intense orange colour than those obtained from lysimeters 2 and 3, we decided to quantify also Fe in all the eluates, which concentration varied among lysimeters and was in accordance with colour. Al concentration reached 892,00 ± 28,28 ug/L in lysimeter 4 and was higher in this one than in the rest of them, in first month sample. Ni only eluted considerably from lysimeter 3 at this time. Interestingly Al and Ni concentrations in lysimeters 4 and 3 respectively decreased from month one to month three, indicating rapid percolation of them through this soil material under the irrigation protocol established. In order to characterize the soil material and lysimeter conditions, moisture and granulometry were also evaluated after disassembling them. Present results demonstrate that Al can be detached from the studied nanoparticles when they are deposited on soil surface and irrigated. Further analyses of metals concentration remaining in the soil after the established treatments and irrigation periods should be useful to perform future environmental risk evaluations.

**SP082 Ensaios de fuga da minhoca *Eisenia andrei* em solo tratado com o inseticida imidacloprido**

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Minhocas ajudam a degradar matéria em decomposição, fertilizar e aerar os solos, dentre outros importantes serviços ecossistêmicos. Elas podem ser utilizadas como bioindicadores da presença de agrotóxicos e também para remediar solos contaminados. Como a aplicação de agrotóxicos pode diminuir a quantidade de minhocas presentes no solo, são feitos testes de rejeição que utilizam um solo controle e uma única concentração de agrotóxico como metodologia, entretanto no campo a situação pode ser diferente. Assim, este trabalho observou o comportamento de minhocas *Eisenia andrei* em solos contaminados com diferentes concentrações do inseticida imidacloprido. O solo foi coletado em área não exposta a agrotóxicos, seco, peneirado em malha de 2 mm e reumedecido a 60% da capacidade de campo uma semana antes do teste. Foram preparadas três caixas plásticas dividida por divisórias de papelão em 12 parcelas (de 14,5 cm x 12 cm cada) de forma aleatória e cada três parcelas foram preenchidas com 1 kg de solo não tratado com imidacloprido (controle), solo contaminado com 0,47 (C1) , 0,9 (C2) e 1,4 (C3) µg.g<sup>-1</sup> de imidacloprido. Em cada parcela foram colocadas 3 minhocas *Eisenia andrei* adultas cliteladas com peso superior a 300 g, removendo-se, então, as divisórias. Depois de alguns minutos, as minhocas se enterraram e um filme plástico foi colocado sobre cada caixa para evitar a fuga dos animais durante o ensaio. Após 48 horas da colocação dos animais, as divisórias foram colocadas novamente em cada caixa, foi retirada cada porção do solo e as minhocas presentes removidas e contadas. As análises mostraram

que o número de minhocas foi significativamente maior nos solos controles do que em cada uma das concentrações testadas (controle e C1, p=0,008; controle e C2, p=0,009; controle e C3, p=0,02; Teste T, α=0,05)). Como foram utilizadas três concentrações, os animais puderam optar entre solo não tratado e solo com concentrações diferentes de imidacloprido durante seu deslocamento pela caixa por 48h. As análises estatísticas mostraram que os animais escolheram preferencialmente permanecer no solo não tratado com imidacloprido (solo controle), embora nas três caixas tenham sido encontradas minhocas nas três concentrações testadas. Possivelmente, a presença de minhocas vivas nas diferentes concentrações testadas está relacionada com o fato dessas concentrações (recomendadas para prática agrícola) estarem abaixo da concentração letal. Agradecimentos: FAO/IAEA (Projeto RLA 7019)

**SP083 Enzymatic biomarkers of earthworms *Eisenia andrei* exposed to imazalil**

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The fungicide Imazalil is registered for use in postharvest application and storage. This substance has potential for contamination, genetic damage in the reproductive systems, and teratogenic effect in vertebrates, and possible neurobehavioral effects. However, knowledge about the effects of Imazalil on antioxidant defense systems in the earthworm is vague and not comprehensive. In the present study, the changes in the activity of catalase (CAT) and glutathione-s-transferase (GST) in the earthworm *Eisenia andrei* exposed to Imazalil were examined in a contact filter paper toxicity bioassay. The assay was performed at 4 concentrations (0.01; 0.1; 1; 10 µL.cm<sup>2</sup>) plus the controls, each containing 20 replicates and 1 organism. The assay was done in a beaker with filter paper moistened with 1 mL of the tested solutions. During the period of 24, 48 and 72 h were evaluated catalase and GST activities and compared with the control sample. Mortality rate, biomass and morphological changes were also observed in the same periods. During the experiment there were not enough deaths to calculate the LC<sub>50</sub> and the biomass did not have significant change when compared to the control. However, it was possible to observe morphological alterations, such as swelling and color changes, in all concentrations. Such effects may have triggered a response from the organism defense mechanisms. The GST enzyme showed a significant decrease after 48 h of exposure to concentrations of 0.01 µL.cm<sup>-2</sup>; 0.1 µL.cm<sup>-2</sup>; and 10 µL.cm<sup>-2</sup> concentrations. Catalase activities showed increase after 72 h at the same concentrations. This indicates that the contaminant began affecting the enzymes from 48 h of exposure, but only after 72 h the organism identified the same as a potential toxic agent. Therefore, can be concluded that although it did not cause mortality, the contaminant presented a sub-lethal toxicity, this is, has the capacity to impair the survival of the organism in the long term.

**SP084 Teste de Toxicidade com semente *Lactuca sativa* para avaliar efeito de cobre no solo**

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A contaminação do solo ocorre devido à disposição incorreta de substâncias tóxicas. Essas substâncias, como por exemplo o cobre, em concentrações elevadas podem causar efeitos tóxicos em plantas. Testes de toxicidade utilizando sementes tem sido muito utilizados, devido ser sensíveis a agentes químicos, rápida germinação e baixo custo. O teste foi realizado seguindo a Norma U.S. EPA "Ecological Effects Test Guidelines" OPPTS 850.4200 – Seed Germination/Root Elongation Toxicity Test/1996. Foi preparado um solo controle com água deionizada e um solo contaminado com cinco concentrações de cobre (50, 100, 250, 350 e 450 mg/Kg Cu) sendo que cada uma foi montada em triplicata. Foi colocado em cada placa de Petri um papel de filtro e 4 mL da amostra correspondente. Com o auxílio da pinça foram colocadas 20 sementes em cada placa. As placas foram incubadas por 120 horas a temperatura de 20°C. Após isso, foi medido o comprimento da radícula de cada plântula correspondente a cada concentração e também o número de sementes não germinadas. Os resultados obtidos no teste de toxicidade, tanto na germinação quanto no comprimento médio da radícula de cada plântula, mostraram que não foi possível calcular os valores de EC50 quando comparados com o controle, posteriormente será realizado novos testes com concentrações maiores. Isso pode ter ocorrido devido aos valores das concentrações serem baixas, e o cobre em concentrações baixas serve de micronutriente para as plantas.

**SP085 Trace metals concentration in marine mammals from southern Brazil: the bottlenose dolphin *Tursiops truncatus* and the sea lion *Otaria flavescens***

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Contamination of marine environments by trace metals has been increasing over the years due to the growth of human population, industries and harbor activities in coastal areas. Once introduced into the environment, trace metals can be incorporated into the organisms and, through the trophic chain, reach top-predators such as the bottlenose dolphin *Tursiops truncatus* and the sea lion *Otaria flavescens*. In the estuarine region of Patos Lagoon, in Rio Grande do Sul state, these species coexist, sharing the same food resources and possibly the same sources of contamination. The aim of the present work was to evaluate the levels of trace metal contamination in different tissues (liver, kidney and muscle) in both species. Samples were obtained from dead animals that washed ashore in southern Brazil. Aliquots of approximately 2 g of wet weight of each tissue were oven dried at 60 °C, then digested in 2 ml of nitric acid (65% HNO<sub>3</sub>) and diluted in MilliQ water to analyze the concentration of Pb, Cu, Cd, Ag and Fe by atomic absorption spectrophotometer (AAS - flame or furnace). Results were expressed as µg/g of dry weight. Pb was not found in most of the samples. The muscle was the tissue that presented the lowest metals concentrations. For *T. truncatus* specimens, the kidney was the tissue with the highest concentrations of Cd (3.74 ± 6.12) and Cu (17.72 ± 9.84), while the liver had the highest concentrations of Ag (0.10 ± 0.08) and Fe (1239.18 ± 599.34). In *O. flavescens* individuals, the kidney had a higher concentration of Cd (7.09 ± 4.88), while the liver presented higher concentrations of Cu (31.08 ± 26.52), Ag (0.61 ± 0.41) and Fe (1598.66 ± 2678.06) than the other species ones. The high concentrations of Fe and Cu are probably related to the fact that they are essential metals. However, the Fe content was greater, proving that it is required in higher amounts than Cu. The presence of Cd and Ag indicates a contamination of these animals by anthropic action. Although there were differences in trace metals concentrations between species, it is clear that kidney is the main organ for Cd accumulation while for Ag the main organ is the liver. Finally, kidney and liver can be considered as more suitable organs for metal environmental biomonitoring than muscle, for both species included in this study.

## Contaminants of emerging concern (CECs) in the environment: current knowledge and future challenges

**SP086 Assessment of Hg and Cd levels at Baixada Santista - Southeastern Brazil**  
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Mercury and Cadmium are highly toxic elements and have a long residence time in the environment. Both elements are occasionally found in industrial waste since they are normally used on multiples activities like ore, metal casting and fungicides. Many of those could be found at Baixada Santista due to the intense urbanization and industrialization, especially at Cubatão, started in the 20<sup>th</sup> century. Thus, the aim of this study is to assess and locate spatially high concentrations of Hg and Cd. A total of 44 surface sediment samples were collected and the elements were quantified by ICP-OES for Cd, and VGA-ICP-OES, for Hg. The results of Hg showed an average of 0.48 mg kg<sup>-1</sup> with a minimum of 0.015 mg kg<sup>-1</sup> and a maximum of 7.69 mg.kg<sup>-1</sup> in which was found on only one sample. Its spatial distribution presented higher levels at the upper estuary, moreover 21 samples were above Brazilian regulations (CONAMA 454/2012), which means that those levels represent a probable adverse effect in the biological community. The levels of Cd, in most of the samples, were below the method quantification limit (0.38 mg kg<sup>-1</sup>) and it was found only in two samples ([Cd] = 0.44 and 0.84 mg kg<sup>-1</sup>) located inside the industrial complex channel. Both samples were under Brazilian regulation but its presence may not be neglected. Since Cd has a geochemical feature of being bound to the exchangeable fraction, its concentration normally reflects an instant deposition. Once there are multiple sources of these heavy metals in the study area, it is difficult to investigate which is the exact source, but since both elements presented similar spatial distribution and Cd reflects an instant deposition, it is a strong evidence that these elements came from industrial effluents from Industrial Complex of Cubatão activities. From the levels of Hg and Cd, it is possible to conclude that there is not an embraced contamination at Baixada Santista but a small contamination constrained inside the industrial complex channel.

**SP087 Avaliação da toxicidade do Di-n-butil ftalato (DBP) por meio do FEET Test (Fish Embryo Extend Toxicity Test)**

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Os ftalatos são empregados na produção de plásticos com a finalidade de aumentar a flexibilidade desse material. No entanto, por não serem covalentemente ligados à matriz do plástico, os ftalatos podem ter como destino final o ambiente aquático, sendo categorizado atualmente como contaminante emergente. Dentre os ftalatos, o Di-n-butil ftalato (DBP) é tido como uma substância de elevada preocupação, devido sua ampla utilização em produtos comercializados e a seu potencial tóxico à mamíferos, porém, pouco se sabe sobre seus efeitos deletérios à organismos aquáticos. Desta forma, o presente trabalho teve como objetivo avaliar a toxicidade do DBP (0,007; 0,015; 0,031; 0,062; 0,125 mg/L) utilizando embriões de *zebrafish* por 96 h (efeito agudo) e 168 h (efeito subcrônico). Metanol 1%-v/v foi utilizado como controle solvente. Os resultados mostraram que DBP causou mortalidade significativa nos embriões de *zebrafish*, sendo a CL<sub>50</sub> de 0,026 mg/L. Diversas alterações no desenvolvimento dos embriões foram observadas após 168 h de exposição ao DBP, como segue: atrasos na eclosão das larvas, pigmentação anormal, má absorção do saco vitelino, edemas pericárdico e no saco vitelino; acúmulo de hemácias no pericárdio, nanismo e ausência de equilíbrio. Com base nos resultados obtidos conclui-se que DBP pode induzir efeitos deletérios em peixes, como a letalidade e malformações durante o desenvolvimento embrionário e larval, requerendo, assim, uma regulamentação dos limites aceitáveis para assegurar a qualidade do ecossistema aquático.

**SP088 Bio-electrochemical remediation by a laccase extract immobilized in microporous activated carbon fiber felt.**

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The electro-oxidation (EO) technologies are promising remediation processes, specially on the removal of organic pollutants. In order to achieve the complete mineralisation of target compounds, different anodes have been applied in EO system. Besides the high efficiency, it is necessary to develop efficient, low cost and eco-friendly electrodes that provide sustainable EO. Therefore, the aim of this work was to promote the bio-EO of indigo carmine dye (widely common in textil industry), using new anode based on a microporous activated carbon fiber felt (ACFFs) and ACFFs with immobilized Laccase enzyme (Lcc) from *Pycnoporus sanguineus*. The ACFFs anodes (pores < 3.2 nm, concentrated at 1.2 nm and surface area of about 1300 m<sup>2</sup>.g<sup>-1</sup>) were prepared with a size of 2.0 x 1.0 x 0.1 cm (specific surface area of 29.63 cm<sup>2</sup>). These anodes had its surface modified by amination using monoaminoethyl-N-aminoethyl (MANAE) activation protocol in order to allow the Lcc immobilization by ion exchange directly on the anode. The assays of discoloration were realized with a Tensimeter, wherein was applied a voltage of 10 V in an electrochemical cell (effective volume = 10 mL) with magnetic stirring in at room temperature (28 ± 2 °C). Finally, the different electrolytes (NaCl, Na<sub>2</sub>SO<sub>4</sub> and tap water - TW) and treatment times (2-60 min) were established. The results were discolorations of 62.7% with ACFF anode and 83.60% with ACFF-MANAE-Lcc anode, both for 60 min in TW. This remediation rates show that this new anode has low cost and efficiency in the degradation of indigo dye and can be applied for other organic pollutant.

**SP089 Chronic toxicity of Fluoxetine to the embryo-larval development of *Echinometra lucunter***

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Fluoxetine is a commonly pharmaceutical compound found in marine environments affected by domestic sewage. The presence of this selective serotonin reuptake inhibitor (SSRI) in aquatic environments may cause toxicity to non-target organisms, possibly leading to harmful effects and bioaccumulation. The biological effects caused



by these substances have been scarcely studied in marine organisms and coastal ecosystems. This study aimed to evaluate the sublethal effects of fluoxetine on the embryo-larval development of the sea urchin *Echinometra lucunter*. Fertilized eggs were exposed to different concentrations of the substance (30, 300, 3000 e 30000 ng.L<sup>-1</sup>) and to a control (clean seawater without fluoxetine). After exposure, we verified significant reduction of the embryos development to echinopluteus larvae, starting from 3000 ng.L<sup>-1</sup> (54.0±10.9%), in comparison to the control (83.5±3.1%). The non-observed effect concentration (NOEC) was estimated as 300 ng.L<sup>-1</sup> and the lowest observed effect concentration (LOEC) was 3000 ng.L<sup>-1</sup>. The inhibition concentration to 50% exposed embryos (IC<sub>50</sub>-42 h) was estimated as 25,000.88 ng.L<sup>-1</sup> (12,884.9 – 48,745.59). Fluoxetine can be an important stressor to aquatic organisms, because it may induce behavior, grow or development impairment. Our results indicate that bioactive substances such as fluoxetine might affect *Echinometra lucunter* embryo-larval development in concentrations above 3000 ng.L<sup>-1</sup>.

**SP090 Cinética y equilibrio de adsorción de sildenafil y carbamazepina sobre carbón activado para su potencial uso en plantas de tratamiento de efluentes**

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El ingreso de contaminantes emergentes en aguas superficiales ha aumentado considerablemente durante los últimos años debido al incremento en el empleo de diferentes compuestos químicos destinados a mejorar la calidad de vida, entre ellos compuestos farmacéuticos. Este tipo de compuestos han sido detectados en las aguas superficiales gracias a nuevas y más avanzadas técnicas analíticas y a un interés creciente en la comunidad científica respecto a su dinámica ambiental. Muchos de estos compuestos no son removidos de las aguas residuales mediante plantas de tratamiento convencional, llegando a los causes de los ríos y causando alteraciones en la biota acuática y hasta en los seres humanos. Así, el sildenafil se ha encontrado en los músculos de peces autóctonos de Argentina. En estudios previos, de capacidad de remoción de contaminantes emergentes, realizados con carbón activado granular en batch, se determinó una capacidad de adsorción de hasta 300 mg/g y tiempos de equilibrio superiores a 14 d en las condiciones de ensayo. A los efectos de diseñar columnas que puedan ser empleadas en plantas de tratamiento a escala real, por lo tanto, eficientes y rápidas, se hace necesario analizar la cinética de adsorción para otro tipo de adsorbente. Por esta razón, en el presente trabajo se estudia la capacidad de adsorción y eficiencia de remoción del carbón activado en polvo (mesh < 36 µm, área superficial de 1328 m<sup>2</sup>/g, tamaño de poro de 0.66 nm) empleando dos fármacos modelos, carbamazepina y sildenafil. Los ensayos se realizaron en condiciones batch a 25 °C y 90 rpm. Las concentraciones analizadas estuvieron entre 5 y 40 mg/L en contacto con 100 mg de adsorbente por litro de solución. Los resultados se analizaron mediante las isotermas de Langmuir y Freundlich y las cinéticas de pseudo primer y pseudo segundo orden. Porcentajes de remoción superiores al 90 % fueron obtenidos antes de 10 horas para carbamazepina y sildenafil, con tiempos de equilibrio inferiores a 48h y capacidad máxima de adsorción de hasta 500 mg/g. Los datos obtenidos se ajustaron a cinéticas de pseudo segundo orden, con constantes de velocidad (g/mg<sup>2</sup>h) entre 0.010-0.001 para carbamazepina y 0.011-0.0005 para sildenafil. Se concluye que el carbón en polvo presenta mejor capacidad y velocidad de adsorción que el carbón granular, indicando la conveniencia de este material para estudios futuros en columnas a escala piloto.

**SP091 Detection of current- use pesticides in Argentinian surface waters using polar organic chemical integrative samplers (POCIS)**

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There have been few studies on the occurrence of current-use pesticides (CUP) in Argentina. Therefore, the objective of this work was to evaluate whether pesticide contamination of the freshwater resources in Argentina is currently occurring by monitoring two selected water bodies impacted by agriculture using passive sampling techniques. Polar Organic Chemical Integrative Samplers (POCIS) were deployed in the Ctalamochita River (Córdoba province) and in Brava Lake (Buenos Aires province) during December, 2014 and February, 2015. POCIS provide an estimate of

the time weighted average (TWA) concentration of target chemicals in water. The results revealed that the fungicides, tebuconazole, carbendazim and azoxystrobin, and the herbicides, atrazine, dicamba and 2,4-D were detected at one or more sampling locations in each watershed. In Brava Lake, the highest estimated TWA concentration was for atrazine in the effluent stream (Tajamar creek) monitored during December 2014. It was not unexpected to find atrazine, since this herbicide is a widely detected CUP in surface waters. The estimated TWA concentration of atrazine was significantly lower in the influent stream at El Peligro and this compound was not detected at the same location during February, 2015. The herbicides, dicamba and 2, 4-D showed a similar trend with atrazine. The occurrence of the fungicides generally followed the same trend as the herbicides, with higher concentrations in the effluent stream compared to the influent stream. In POCIS deployed in Ctalamochita river, the herbicides dicamba and 2,4-D were present at the highest estimated TWA concentrations, mainly at Puente los Potreros station, while the levels of atrazine remained fairly constant across all locations. The estimated TWA concentrations for the fungicides azoxystrobin, carbendazim and tebuconazole were relatively low in comparison to the herbicides. Other fungicides used in pharmaceuticals and personal care products (i.e. ketoconazole, climbazole) were not detected in POCIS from La Brava, indicating that there were no nearby sources of municipal wastewater. However, traces of the pharmaceutical, fluconazole were detected in POCIS in amounts < LOQ in Ctalamochita river, therefore indicating a low degree of wastewater contamination. The presence of atrazine and fungicides deserves more attention because they have the potential to induce endocrine disrupting effects on aquatic organisms.

**SP092 Efficient electrochemical remediation of cyanotoxins in tap water using designer TiO<sub>2</sub>@carbon electrodes**

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Microcystin-leucine arginine (MC-LR) and nodularin are relevant toxic secondary metabolite produced by freshwater cyanobacteria. This toxin has a high potential hazard health due to potential interactions with liver, kidney and the nervous system. The aim of this work was the design of a simple and environmentally friendly electrochemical system based on highly efficient nanostructured electrodes for the removal of MC-LR in tap water. Titania nanoparticles were deposited on carbon (graphite) under a simple and efficient microwave assisted approach for the design of the electrode, further utilized in the electrochemical remediation assays. Parameters including the applied voltage, time of removal and pH (natural tap water or alkaline condition) were investigated in the process, with results pointing to a high removal efficiency for cyanotoxins (60% in tap water and 90% in alkaline media experiments, under optimized conditions for MC-LR).

**SP093 Fitotoxicidade e Mutagenicidade do Óxido de Grafeno Reduzido em *Allium cepa***

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During recent decades, semiconductor nanoparticles have attracted enormous research attention. In particular, the effect of particle size has been widely studied. Graphene[S1], a two-dimensional sheet of sp<sup>2</sup>-hybridized carbon atoms packed into a honeycomb lattice, has recently garnered much attention for its excellent physical and chemical properties. Reduced graphene oxide (rGO) is a nanomaterial of generation after carbon nanotubes. The appearance of the rGO is similar to that of a sheet with nanometric dimensions, about 350 nanometers long and 5 nanometers thick. Graphene materials also have applications in the biomedical sector for therapy, diagnosis and drug delivery, and no other material has comparable properties. Although some progress has been made in diagnosis and drug delivery, the therapeutic applications of graphene remain in their infancy. The difference in the application of graphene in the biological and nonbiological sectors is owed to the toxicity of chemically reduced graphene oxide. Hence, there is an urgent need to identify environmentally friendly, and simple approaches to prepare biocompatible graphene materials for biomedical applications. In the present work the phytotoxicity and the geno / mutagenicity of rGO were evaluated in the *Allium cepa* bioindicator. One hundred seeds were exposed to distilled water (negative control), to 3% formaldehyde (positive control) and to five concentrations of rGO (0.01- 0.1- 1.0- 10.0- 100.0 ppm) in triplicate. When they reached 2 cm they were collected and the germinated seeds were quantified, after which they were stored in the Carnoy fixative. Then, they were

submitted to 1N HCl hydrolysis for 10 minutes at 60 °C and stained by the Schiff reagent. For slide preparation an intact meristem root was placed on it and covered with a drop of acetic carmin and with a coverslip. The root is then crushed and fixed. At 400X magnification chromosomal aberrations and micronuclei are enumerated at 500 cells per slide (5 slides per treatment). The results showed absence of phytotoxic effect of rGO, but genotoxic and mutagenic effects were observed at concentrations of 10 and 100 ppm. It was observed that the chronic toxicity of rGO occurred in the highest concentrations tested, which in a very unlikely way will be found in the environment, but may represent an occupational risk, in the sector of production of these nanomaterials. Similar results were found by this group for multiwalled carbon nanotubes (MWCNT). [S1]

#### SP094 History of Anthropogenic Activity in the Estuarine System of Santos and São Vicente

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Baixada Santista is one of the most populated and exploited regions in Brazil. In the past few decades, it has undergone a large process of environmental degradation due to intense industrialization. Metals have great importance in such context, as they can reflect the influence of mankind on the environment. This paper ultimately aims at the environmental reconstruction of the Estuary of Santos and São Vicente regarding local pollution, evaluated through metal analysis in sediments. For this purpose, nine sediment cores were analyzed, thereby allowing a historical perspective on the impact of anthropization over this coastal environment. Partial sediment digestion was performed using the following methods: SW 846 US EPA 3050B (USEPA, 1996), and steam generation, method SW 846 US EPA 7471 A (USEPA, 1994). The results led to two, different environments: the Bertioga Canal and the Upper Santista Estuary. The sediment cores from Bertioga comprised late metal accumulation, especially of As, Cu, Pb, Zn, and Hg. When confronting regional historical data to the gathered figures on metal concentration, one can attest the recent increase in their amount since the 1980s. Even though the data does not indicate contamination in the sediment, the higher values present in the upper layers of each sample bear witness to the anthropic influence over the region. The likely sources of these metals are located in the Upper Santista Estuary, but also in an oil spill dating back to 1980s. The sediment cores collected in this Upper Estuary revealed high metal concentration, including Cu, Pb, Zn, and Hg. The Hg values stood out, as pollution parameters signaled high contamination levels of it. The metal concentration in this region is coherent with the local industrial and urban growth. Such impacts on this estuarine area preceded those observed in Bertioga. Indeed, metal concentration have increased since the 1950s, but growing industrial activity in the region has sharply multiplied such values from the 1970s on. The metal pollution peaked in the 1990s, when the environmental programs designed for that ecosystem in the previous decade start effecting change.

#### SP095 Initial assessment of the sorption properties of emerging contaminants on marine sediments

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Emerging contaminants (ECs) refers to a large group of chemicals that are not necessarily new, but are not or are only partly regulated and are not yet included in monitoring programs. Although these compounds have been increasingly studied in the aquatic environment in the last decades, the knowledge on the environmental behavior and fate of ECs under marine conditions is still limited. The environmental behavior of organic compounds in coastal and marine systems is influenced by their sorption properties, which play an important role in controlling their fate, transport, bioavailability, and toxicity. Thus, the aim of this work is to investigate the sorption of selected emerging contaminants in marine sediments. Briefly, sorption experiments were performed in replicates placing the sediments in glass tubes and adding 50mL of sterile seawater with known concentrations of target compounds (octocrylene, benzophenone-3 (BP3), OTNE, tonalide, galaxolide, and triclosan). The analytes were extracted and analyzed using the analytical protocol previously developed by Pintado-Herrera et al. (2013), where water samples were extracted using stir bar sorptive extraction and separation, identification and quantification of target compounds were performed using gas chromatography coupled to a triple quadrupole mass spectrometer (GC-MS/MS). According to the results of preliminary experiments, the sediment/solution ratio was set to 1:500 and the equilibrium was achieved within 48 hours. Loss of compounds was tested by adding standard solution to glass vials without sediments. Therefore, volatilization and adsorption by the glass could be considered

negligible. Distribution coefficients and isotherms have been assessed with different sediments (sand and clay) at different concentrations (from 1 to 100 µg/L) and temperatures (8, 18, and 28 °C). Preliminary results regarding the distribution coefficient at one concentration level (10 µg/L) and for one type of sediment (50.4% sand, 3.8% silt, 45.8% clay, and total organic carbon content of 2.7%) were calculated to compare the sorption affinities between the compounds. The  $K_d$  values were 0.8 mL g<sup>-1</sup> for OTNE, 1.5 mL g<sup>-1</sup> for BP3 and galaxolide, 1.9 mL g<sup>-1</sup> for tonalide, 3.5 mL g<sup>-1</sup> for triclosan, and 50.0 mL g<sup>-1</sup> for octocrylene.

#### SP096 Lethal and sublethal effects of cobalt ferrite nanoparticles on aquatic organisms

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Cobalt ferrite nanoparticles (CoFe<sub>2</sub>O<sub>4</sub>) are superparamagnetic materials with several applications, among them, ceramic materials and biomedicine. Different usage have been investigated in CoFe<sub>2</sub>O<sub>4</sub>, studies have shown potential to remove oil stains in water, due to its large surface area available for pollutants adsorption. However, few studies about the toxicity of this material and its impact on ecosystems are available, except for effects on unicellular algae population. Considering that environmental remediation intends to reverse harmful effects of pollutants, studying possible deleterious effects of this material to aquatic organisms become relevant to ensure the security of nanoparticles to application of cobalt ferrite to oil removal. Several components of fauna and flora are affected by oil spills. In fact, organisms of water column, such as zooplankton and phytoplankton, are most affected by environmental remediation processes. So, the evaluation of adsorbent material toxicity to freshwater and marine organisms becomes indispensable. In this context, freshwater organisms *Daphnia similis* (acute exposure), *Ceriodaphnia dubia* (chronic exposure) and the saltwater *Mysidopsis juniae* (acute exposure) were exposed to raw CoFe<sub>2</sub>O<sub>4</sub> in order to evaluate material's biocompatibility. According to OECD's (2012) suggestions, particles agglomeration and suspensions stability during the exposition period were also analyzed. The results showed a decrease on *C. dubia* reproduction at concentrations higher than 1.25mg/L (NOEC), whereas for other species, *M. juniae* and *D. similis*, we obtained EC50 >50mg/L. The sample was polydispersed in culture medium, presenting large size variation that exceeded the nanoscale, forming micrometer's agglomerates for example, on the highest concentration, 50mg/L, for 72h the average diameter size value was 1.562 µm. The present study suggests no acute toxicity of cobalt ferrite, but some hazard of chronic exposure. On the other hand, other assessments are necessary, including other organisms and endpoints on *in vivo* and *in vitro* assays in order to understand the interactions among material and biota.

#### SP097 Mercury in fish as a tool to evaluate the degree of anthropization of estuaries in Southeast Brazil.

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The coastal region of Brazil has been historically impacted by anthropic, industrial, agricultural and harbor activities, which affect the environment by the release of contaminants such as heavy metals, prominently by Mercury (Hg). This element has high mobility, affinity with organic matter and biomagnification capacity, being toxic to the biota mainly in its methylated form. In the estuaries of: Paranaguá (Paraná), Cananéia and Santos (São Paulo), the presence of potentially polluting activities at different levels were noticed. Thus, an analysis of this contaminant and its influence on the biota of these sites is extremely important. In this study, the investigations are on the concentrations of Hg in the dorsal muscular tissue of three species of benthivorous fish *Stellifer rastrifer*, *Paralichthys brasiliensis* and *Isopisthus parvipinnis*, sampled from the three estuaries in the summer and winter of 2015. The Hg extraction methodology followed the method 7471A (USEPA, 1994) and the samples were analyzed by Optical Spectrometry Emission with Plasma Inductively Coupled to a Steam Generator. The concentrations of Hg obtained in the fish samples ranged from 19.2 to 269.2 µg kg<sup>-1</sup> in Paranaguá; 18.1 to 111.1 µg kg<sup>-1</sup> in Cananéia and 18.1 to 229.4 µg kg<sup>-1</sup> in Santos. Analysis of variance was performed to verify which factors have influenced the bioaccumulation of Hg in fish, including location, season, gender, and fish species. Only the local factor significantly influenced the concentrations of Hg in the fish analyzed. The highest average concentration of Hg was obtained in Santos fish (79.2 µg kg<sup>-1</sup>), while the average concentration in Paranaguá (60.3 µg kg<sup>-1</sup>) was similar. This behavior is expected since these estuaries have presented potential sources of contaminants and suffered greater pressure by anthropic activities. The concentrations of Hg in Cananéia fish (39.7 µg kg<sup>-1</sup>) differed significantly from the other sites, with lower average concentrations.

This estuary is considered to be less polluted, and it is a protected area, recognized as a Natural Patrimony of Humanity by UNESCO. The results obtained allowed to present a current panorama of the contamination and conditions of the addressed biota, which now might serve as a basis for comparisons with different estuarine systems. In addition to it, this study corroborates to the use of Hg in studies of environmental monitoring and analysis of the degree of anthropization of coastal regions.

#### SP098 Occurrence and fate of fluorinated siloxanes in wastewater treatment processes

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Although used for more than six decades, siloxanes are considered emerging environmental contaminants. Fluorinated polysiloxanes, consist of a backbone of alternating silicon-oxygen [Si-O] units with fluorinated groups attached to each silicon atom, had vast usage in cosmetics and other skin use formulations where long-lasting oil as well as water repellency. The first member and still the most common commercial fluorinated polysiloxanes is poly[methyl(trifluoropropyl)siloxane] (PMTFPS). Tris(trifluoropropyl)trimethyl-cyclotrisiloxane (D3F) and tetrakis(trifluoropropyl)tetramethyl-cyclotetrasiloxane (D4F) are important raw materials for poly[methyl(trifluoropropyl)siloxane] (PMTFPS). Hence, D3F and D4F, as impurities in PMTFPS, might be released into the environment through different pathways during the production and application of PMTFPS. To the best of our knowledge, scientific studies about fluorinated siloxanes on the emission, environmental occurrence and potential environmental impacts are still very limited. We investigated the occurrence and fate of D3F and D4F in the wastewater treatment plant, with a capacity of 1000 m<sup>3</sup>/d wastewater, including bar screens, primary clarifier, regulating tank, anaerobic tank, oxic tank, and secondary clarifier. The hydraulic retention times in the primary clarifier, anaerobic tank, oxic tank, and secondary clarifier were 5.0, 2.5, 10, and 3.5 h, respectively. The total solid retention time was about 15 d. Three sampling events were carried out and 24 h composite wastewater samples were collected continually into 4L glass tubes using automatic samplers at flow proportion mode, while the sludge samples were collected at the same time. Overall, D3F and D4F were detected in all water (0.455-3.35µg/L) as well as in solid samples (0.736-7.36µg/g), with solid-water partition values at 942-1456L/Kg for D3F and 1355-2417L/Kg for D4F. The mean removing efficiencies of D3F and D4F in the whole wastewater treatment processes were 93.5 and 95.6%, respectively. Sorption to sludge (76-85%) had dominant contributions to their total removal, followed by removal in primary clarifier (10.5- 18%) and oxic tank (6.5-8.3%). Elimination experiments showed that: 1) hydrolysis half-lives of D3F and D4F in primary clarifier (pH=2.5-4.6) were 8.4-15.6 h and 17.5-36.8 h; 2) D3F and D4F were hardly degraded in oxic sludge treatment units, and their volatilization rates in oxic unit were also limited ( mean: 85.5 and 189 h for D3F and D4F).

#### SP099 Occurrence of Pesticides, Hormones, Bisphenol A and Triclosan in surface waters of Sao Paulo State

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Over the last few decades, the occurrence of emerging contaminants in the aquatic environment has become a worldwide issue of increasing environmental concern. The emerging contaminants include pharmaceuticals, personal care products, steroid hormones, industrial chemicals, pesticides and many other compounds. On this study the focus was the presence of some of the most used pesticides in Brazilian agriculture, estrogen hormones, bisphenol-A and triclosan in surface waters of São Paulo State. All assessed substances are considered endocrine disrupting chemicals. Ten surface waters sites were assessed: Guarapiranga Reservoir, Ribeirão Pires River, Araras River, Ribeirão Grande River, Sapucaí-Guaçu River, Piracicaba River, São Miguel Arcanjo River, Jaguari Reservoir, Jaguari River and Cascata Reservoir. Samples were prepared for analysis by solid-phase extraction and analyzed by liquid chromatography-mass spectrometry. The analyzed substances were: Pesticides (Fipronil, Carbendazim, Imidacloprid, Simazine, Carbofuran, Hexazinone, Tebutiuron, Amethrin, Atrazine, Diuron, Clomazone, Azoxystrobin, Malathion, Tebuconazole), Hormones (Estrone, Estradiol, Estriol, Ethinylestradiol, Progesterone, Testosterone, Diethylstilbestrol, Mestranol, Levonorgestrel), Triclosan (TCS), Bisphenol A (BPA), Octylphenol and Nonylphenol. The occurrence of pesticides was observed in all sampled sites. Worldwide pesticide use has increased dramatically,

including in Brazil. The use of pesticides has resulted in the presence of their residues in various environmental matrices, and in surface waters pesticides represent a considerable hazard for the aquatic environment. The most recurrent pesticides in analyzed samples were: carbendazim (3.8 – 68.0 ng L<sup>-1</sup>), malathion (0.9 - 54.5 ng L<sup>-1</sup>), diuron (4.5 – 92.0 ng L<sup>-1</sup>) and atrazine (2.25 – 43.0 ng L<sup>-1</sup>). The BPA presence was observed in all sampled sites at least once for each site, with concentrations from 1.0 to 377.5 ng L<sup>-1</sup>. Hormones were observed in 70% of the sampled sites at least once, but in lower concentrations than reported in the literature with effects on aquatic biota. The widespread presence of these chemicals highlights the need for further studies in order to understand the harmfulness of these contaminants to aquatic species. Also the need for studying if the found levels of these chemicals are able to affect the aquatic life. Key words: Pesticides, hormones, surface waters, emerging contaminants

#### SP100 Prospecting of fungi in mangrove sediment from the port area of the North Coast of the State of São Paulo, Brazil.

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The Araçá Bay mangrove is impacted by the accidental spills of oil and by-products and by the waste resulting from the activities of the Port of São Sebastião and the Almirante Barroso Maritime Terminal (TEBAR). The high toxicity of hydrocarbons derived from petroleum has the potential to affect living beings, because it has character carcinogenic and mutagenic. Among the microorganisms, fungi species are being studied in the process of bioremediation for their ability to metabolize the compounds present in the residues derived from petroleum. This work aimed to identify the main genera of fungi found in the mangrove sediments of Araçá Bay, São Sebastião (SP), impacted by petroleum products. Four sediment samples were collected in the intertidal region, two in summer and two in winter. Sediment samples were processed and inoculated in growth medium Potato Dextrose Agar (PDA) using the "Spread Plate" technique, subsequently the isolates was identified through the macro and micromorphological characteristics of the filamentous fungi. Yeasts were isolated in selective chromogenic medium HiChrome Agar (Difco). It was obtained 73 isolates, being 26 molds of the genus *Aspergillus sp* and 23 of the genus *Penicillium sp*. The two genera found are known to be capable of degrading petroleum hydrocarbons and some their derivatives. Among the yeasts, 18 isolates of *Candida tropicalis* and 8 of *C. albicans* were identified. Both species are considered pathogenic and also demonstrate preference in degrading hydrocarbons from linear chains. The degradation capacity of fungi allows the removal of these compounds from the environment, transforming them into substances such as carbon dioxide and water, remedying the affected areas and reducing the negative impacts caused by these contaminants. In this way, the research about the bacterial biodiversity of sediments and their potential to degrade hydrocarbons and derivatives are fundamental to the knowledge of the biotechnological potential of the microbiota and the possibility of their sustainable use.

#### SP101 Steroidal estrogens in surface water: Why safe exposure limits are not yet defined?

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The presence of emerging contaminants in aquatic systems has raised the interest of the scientific community, particularly with regard to endocrine disruptors like estrogenic steroids. Known steroid estrogens (estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol) have been most investigated for endocrine disruption, but no safe limits have been legislated for them. The objective of this study was to determine whether the data available in the literature are adequate to establish safe estrogenic steroid exposure concentrations for aquatic biota, such as predicted no effect concentration (PNEC). A wide range of estrogenic steroids concentrations could be observed in several countries, with some values of concern (e.g., China and Brazil), indicative of actual problems in certain surface waters. Based on experimental surveys examining estrogenic steroids, the measured effects in ecotoxicological tests showed a frequency of 26% in relation to events of biological relevance (such as populations, reproduction and development), which are essential to extrapolate results to maintenance of aquatic populations. By assessing these features in this review, sufficient, even rudimentary, information was found to establish PNECs for estrogenic steroids. The lack of consensus among researchers has postponed the choice of two PNECs established in recent years. Considering the unquestionable impact of estrogenic steroids on aquatic environments, the adoption of any available PNECs as



standards in national/international legislation is an urgent need for the protection of aquatic life.

### Null results are also results

#### SP102 A AVALIAÇÃO SOCIAL DO CICLO DE VIDA E A RESPONSABILIDADE SOCIAL

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As empresas conscientizam-se cada vez mais da necessidade e benefícios do comportamento socialmente responsável que visa a governança organizacional com melhor desempenho junto à sociedade. O conceito de responsabilidade social (RS) tem assumido papel central frente ao desafio. Assim, a Avaliação Social do Ciclo de Vida (ACV-S) tem sido apresentada como uma metodologia sistêmica e integrada aos aspectos econômicos e ambientais que auxilia na tomada de decisões e comunicação com as partes interessadas, evidenciando impactos sociais de produtos/serviços numa perspectiva de ciclo de vida. O problema central deste estudo avalia se o conjunto de indicadores definidos na ACV-S contempla a demanda por práticas de RS das empresas focando o mercado de celulose e papel, a correlação com a normatização contábil, indicadores sociais do segmento e necessidades das partes interessadas. A comunicação empresarial tem sido realizada especialmente, por meio da publicação dos relatórios de sustentabilidade. Em análise preliminar, constatou-se que tais publicações apresentam linguagem genérica e subjetiva. As informações não possibilitam a aplicação dos indicadores propostos pela ACV-S, nem dos padrões normativos nacionais e internacionais, além de não atender objetivamente as diretrizes para RS indicadas na norma ISO 26000. O objetivo foi analisar a compatibilidade entre os indicadores sociais da ACV, da RS e normas contábeis para o mercado de celulose e papel no Brasil. Foi estabelecido como metas: descrever os indicadores de ACV-S e das diretrizes de RS para duas empresas representativas do setor; apresentar as formas de comunicação empresarial às partes interessadas; relacionar (in)compatibilidades entre os indicadores sociais da ACV, de RS do mercado e da normatização contábil; e, avaliar a base de indicadores sociais utilizados pelas empresas na comunicação às partes interessadas. A pesquisa foi bibliográfica e documental com dados secundários. O segmento foi escolhido pela relevância econômica, social e ambiental e as empresas a partir do porte, inserção no mercado e afinidade com a ACV. Um banco de dados foi criado para as informações dos relatórios empresariais (2010-2015). O conjunto de indicadores da ACV-S foi 90% compatível com os indicadores GRI. A análise preliminar das correlações dos indicadores aponta favoravelmente à ACV-S, indicando conexão com as exigências atuais. Foi detectada elevada subjetividade na comunicação com as partes interessadas.

#### SP103 Citizen Perceptions and Attitudes Regarding Water Quality and Sources of Pollution

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Two random sample surveys were issued to evaluate citizen awareness regarding water issues. The first survey was conducted in 2008 during a relatively mild drought in the southern USA, which intensified through 2009-2012 when much the region endured exceptional drought. The original survey was re-issued to another random sample in 2014 and represents an opportunity to investigate changes in public attitudes following extended exposure to water restrictions and reduced water availability. Citizens were asked to indicate the level of importance of 16 water resource issues. The three issues considered important or very important were clean drinking water, clean rivers and lakes, and clean groundwater. There were no significant differences between responses for 2008 and 2014, except for respondents' indications of the importance of water for municipal use and within state transfer/sale of water rights (both  $p < 0.02$ ). Between 2008 and 2014, there was a significant difference in citizens' evaluation of surface water quality with more respondents indicating they believe surface water quality is good, but deteriorating; poor; or they have no opinion/don't know ( $p < 0.05$ ). Respondents in 2014 indicated that they knew or suspected that nitrate and phosphate fertilizers and pesticides affect either surface or groundwater quality in their area. However, 2014 respondents also indicated that industry, oil wells and mining, and new suburban development were most responsible for existing pollution problems in rivers and lakes. Finally, there was no difference between 2008 and 2014 responses regarding the most important actions that should be taken to protect water resources with improving water quality monitoring to detect pollution (92%),

residential water conservation (91%) and educating municipal officials (90%) considered very important or important by respondents.

### Current situation and research needs for ecotoxicological assessment of pesticides in aquatic and terrestrial ecosystems

#### SP104 Azinphos-Methyl vs Carbaryl: Effects on B-Esterases of a Freshwater Snail

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Organophosphate and carbamate compounds are widely used in agriculture for pest management control. The main mechanism of action of these kinds of insecticides consists in the inhibition of the enzyme acetylcholinesterase. In Argentina, Loewy et al. (1999, 2011), through surface and ground water monitoring detected a maximum concentration of the organophosphate azinphos-methyl (AZM) of  $79.3 \mu\text{g L}^{-1}$  and of the carbamate carbaryl (CAR) of  $45.7 \mu\text{g L}^{-1}$ . We aimed to compare the effect of AZM and CAR on the activity of cholinesterases (ChEs) and carboxylesterases (CEs) of *Biomphalaria straminea*, a freshwater gastropod native to Argentina. Two bioassays were conducted in which four treatments were designed for each: dechlorinated tap water, acetone in dechlorinated tap water (solvent control), and AZM/CAR in dechlorinated tap water at 20/12.68 and 200/126.8  $\mu\text{g L}^{-1}$ , respectively. The concentrations of CAR were chosen so as to have the same molarity as AZM. Also, a fifth treatment was added for CAR, the equivalent to 126.8  $\mu\text{g L}^{-1}$  of a formulation with 85 % of the active compound. As AZM has been prohibited (resolution 149/2016) we couldn't get a formulation. Eight glass vessels were used per treatment with 200 mL of the corresponding solution. Based on previous stability studies, AZM and CAR solutions were renewed every 96 and 48 h, respectively. After 14 days of exposure homogenates were made with the organisms' soft tissues. In the supernatant fraction, the activity of ChEs and CEs, using as substrates p-nitrophenyl acetate (p-NPA) and p-nitrophenyl butyrate (p-NPB), were measured. The activity of ChEs was significantly inhibited (22 %) with 200  $\mu\text{g L}^{-1}$  of AZM with respect to the water control. Regarding CEs, the highest concentration of AZM also significantly inhibited the enzyme by 24 % with p-NPA and 44 % with p-NPB, in relation to the water control. Instead, CAR did not elicit an effect on the activity of ChEs and CEs. Our results show that CE activity, measured with p-NPB, could be considered a sensitive biomarker in *B. straminea* after a subchronic exposure to AZM. Neither of the biomarkers evaluated in this study resulted sensitive after a subchronic exposure to CAR. The formulation of CAR did not show to be more toxic than the active compound itself.

#### SP105 Biomarkers of exposure to pesticides in aquatic macroinvertebrates of RAMSAR wetland "Ciénega de Cabezas" of San Luis Potosí, México

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In this research the objective was to determine the activity of enzymes acetylcholinesterase (AChE), glutathione s-transferase (GST) and malondialdehyde (MDA), considered biomarkers of pesticide exposure, for two seasons (dried and rainy) in aquatic macroinvertebrate tissue (freshwater snails -*Pachychilus* sp-) residents of sites impacted by agriculture in Ciénega de Cabezas, located in Tamasopo, San Luis Potosí, Mexico (designated RAMSAR wetland in 2008). Sampling was carried out in March (dry) and November (rains) in 2016. Three sampling stations were selected along the wetland with agricultural influence (cane cultivation). Snails were collected by linear transects of 200 meters; then 30 organisms were randomly selected. After selection, the organisms were dissected and stored in liquid nitrogen ( $-186^\circ\text{C}$ ) for final transport. In the laboratory, the samples were homogenized with buffer to obtain the post-mitochondrial fraction (s12). In this fraction the enzymes AChE and GST, and the lipid peroxidation product (MDA) were determined by

methods of UV-Visible spectrophotometry in microplate reader. The activity of AChE in *Pachychilus* sp obtain a statistically significant decrease in dry season ( $p < 0.05$ , MWU,  $N_1 = 30$ ,  $N_2 = 30$ ). GST activity in snails captured during the dry season obtain a statistically significant decrease ( $p < 0.001$ , MWU,  $N_1=30$ ,  $N_2=30$ ). The concentration of MDA for the dry season obtain a statistically significant increase in the rainy season ( $p < 0.05$ , MWU). Decrease of AChE is related to exposure to pesticides, so it may be possible for snails to be under exposure, this could be confirmed by the activity found in GST, which has been attributed to its increase, as a possible route of detoxification of pesticides in invertebrate organisms. With MDA, it could be assumed that organisms are under effects of lipid peroxidation by exposure. The response of AChE, GST and MDA in *Pachychilus* spp. in different seasons in the Ciénega de Cabezas, could be related to the exposure to pesticides used in agricultural areas near of this wetland. This is the first study of this type that is carried out in biota, which could serve as a basis for future research or to carry out environmental or biological monitoring, in this wetland.

#### SP106 Cell biomarkers of stress and cytotoxicity in midgut and Malpighi tubules of honey bee exposed to realistic concentrations of picroxystrobin.

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The exposure of bees to agrochemicals has been appointed as one of the factors responsible for the worldwide decline of populations of *Apis mellifera* africanized that is a specie with great relevance for keeping the food maintenance. Thus, this study aimed to analyze the morphology of organs from digestive and excretory systems, under cytotoxic conditions induced by the exposure to xenobiotics, specifically the contamination by the picroxystrobin fungicide. Several stressors affect bees, which present debilitated immunological system, decrease of midgut microbioma diversity, pathogen infestations and performance losses, threatening the worldwide food production. Fungicides of the strobilurine group have potential lethal and sub-lethal effects on honeybees. However, their action mechanism on the bees remain unknown. Toxicological bioassays by continuous oral exposures were performed with newly-emerged workers that were divided in groups: control, solvent-control, and the exposed to picroxystrobin at 18 ppb (PXT) or to picroxystrobin at 9 ppb (PXT). Mortality was recorded daily and survival rates were performed at the end of bioassays. The organs of the bees collected at different moments after the beginning of the exposure (24 h, 48 h, 72 h and 96 h) were submitted to histological and immunohistochemical analyzes for labelling of HSP70 and DNA fragmentation. The picroxystrobin reduced the survival of the bees in both concentrations. After 24 h exposure to the fungicide, the morphology of the midgut and the Malpighi tubules was altered, with an attempt to reconstitute the damaged intestinal epithelium and increase the excretory activity of the tubules. Exposure to the fungicide increased the intensity of HSP70 immunolabelling in the midgut and Malpighi tubules, protecting epithelial cells against cell death, along 24 h and 48 h of continuous exposure. After 72 h and 96 h of continuous exposure, only in the midgut presented positive cells to TUNEL reaction in the basal region of epithelium, when there was a decrease in the immunostaining for HSP70. The data demonstrate that the fungicide is cytotoxic to the midgut of *A. mellifera* at realistic concentrations, which can lead the bee to death by starvation.

#### SP107 Differential Sensitivities of Neurotoxic and Enzymatic Responses in the Endemic Snail *Chilina gibbosa* to Carbaryl and Acetamiprid Used in Argentina

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*Chilina gibbosa* is a freshwater snail endemic to southern Argentina and Chile, vulnerable to the presence of toxic contaminants in surface water. In Argentina, it is commonly found in rivers, lakes and reservoirs of the Río Negro and Neuquén provinces, where fruit production is one of the main economic activities and large amounts of pesticides of different chemical nature have been found in the surrounding water. Our aim was to characterize the acute effect of carbaryl (CAR, carbamate) and acetamiprid (ACP, neonicotinoid) on *C. gibbosa* neurotoxic response and enzymatic activities. We define neurotoxic response as the protrusion of the entire head-foot region of the snails. Measured enzymes were cholinesterases (ChE), carboxylesterases (CE) using p-nitrophenyl acetate (p-NPA) and p-nitrophenyl butyrate (p-NPB) as substrates, and glutathione S-transferase (GST). We exposed snails for 48 h to a range of 0.05 to 500  $\mu\text{g L}^{-1}$  CAR. Separately, we exposed snails for 48 h to a range of 10 to 1000  $\mu\text{g L}^{-1}$  ACP. These ranges include maximum concentrations reported as found in the environment and national guidelines for the protection of the aquatic biota. In the case of CAR, we obtained similar sensitivities between the activities of ChE (NOEC 5  $\mu\text{g L}^{-1}$ ; LOEC 13  $\mu\text{g L}^{-1}$ ; IC<sub>50</sub> 37  $\mu\text{g L}^{-1}$ ; 75% inhibition at 500  $\mu\text{g L}^{-1}$ ) and CE measured using p-NPB (IC<sub>50</sub> 11  $\mu\text{g L}^{-1}$ ; 88% inhibition at 500  $\mu\text{g L}^{-1}$ ; no dose response relationship found  $\leq 5 \mu\text{g L}^{-1}$ ). These values are above the national guidelines for aquatic biota protection (0.5  $\mu\text{g L}^{-1}$ ). We did not find any effects on the activities of CE using p-NPA and GST. For ACP, we found an increase in CE activity using p-NPA and no effect on the activities of ChE, CE using p-NPB and GST. Neurotoxic response was not observed for either pesticide. A previous study from our laboratory reported *C. gibbosa* neurotoxic response and ChE activity as very sensitive biomarkers for acute toxicity of azinphos-methyl (AZM, organophosphate) with an IC<sub>50</sub> value of 0.02  $\mu\text{g L}^{-1}$ , equal to national guidelines, and NOEC 0.001  $\mu\text{g L}^{-1}$ . CE activity was only inhibited at much higher concentrations (IC<sub>50</sub> 1000  $\mu\text{g L}^{-1}$ ; NOEC 500  $\mu\text{g L}^{-1}$  p-NPB; NOEC p-NPA 1000  $\mu\text{g L}^{-1}$ ). Contrastingly, ChE activity is less sensitive for CAR and is not altered by ACP. Our results provide further information on the adverse effects of two different pesticides on an endemic species of South America and aid towards a better ecological risk assessment of pesticides on non-standard organisms.

#### SP108 Early ecotoxicity screening of isoxaflutole herbicide to soil fauna: avoidance behavior tests with earthworms and collembolans

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Isoxaflutole is a pro-herbicide that belongs to carotenoid biosynthesis inhibitors mode-of-action and isoxazole group. Isoxaflutole is quickly degraded into diketonitrile active form when applied to the soil. It is a common product for pre-emergence use in crops like corn and potato, and as post-emergence use in sugarcane and forest species, acting in the weed control of monocotyledons and some dicotyledons. Despite being discovered in the early 90's, there are few studies about this active ingredient. This study aimed to evaluate the ecotoxicity of an isoxaflutole herbicide to soil fauna in avoidance behavior tests, using the earthworm species *Eisenia andrei* Bouché 1972 and the collembolan species *Folsomia candida* Willem 1902, when exposed to contaminated soil with Provence® 750 WG. The tests followed the ABNT NBR ISO 17512-1 (2011) and ISO 17512-2 (2011) guidelines. Six concentrations of the commercial product (15.62; 31.25; 62.50; 125; 250 e 500  $\text{mg.kg}^{-1}$ ) were applied in tropical artificial soil (TAS; 5% organic matter) in the laboratory. TAS with distilled water was used as control treatment. After 48 h exposure, the average number of organisms was recorded for each trial. The results were analyzed by the Fisher's exact test ( $p < 0.05$ ). Avoidance behavior of earthworms was observed at 250 and 500  $\text{mg.kg}^{-1}$ , while non-avoidance behavior was observed for collembolan at this concentration range. Results showed that earthworms are more sensitive than collembolans for the tested formulation. Further studies must embrace acute and chronic tests in order to evaluate effects on survival and reproduction of these groups of organisms.

#### SP109 Efeitos in vitro do Roundup® na viabilidade e funcionalidade mitocondrial de hepatócitos de Danio rerio

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Roundup® é o herbicida mais utilizado no mundo, contaminando rios e bacias, podendo causar diversos efeitos em organismos aquáticos. Alguns estudos tem mostrado que o Roundup® leva a alterações em parâmetros de funcionalidade mitocondrial em hepatócitos de ratos. Uma vez que o ambiente aquático é severamente afetado pelo aporte destes contaminantes, o objetivo do presente estudo foi avaliar o

efeito do Roundup® na viabilidade e funcionalidade mitocondrial em uma linhagem estabelecida de hepatócitos de *Danio rerio* (linhagem ZFL). Células foram expostas ao Roundup® (65µg.L<sup>-1</sup>; 650µg.L<sup>-1</sup>; 6500 µg.L<sup>-1</sup> e 65000µg.L<sup>-1</sup>) e após 24 e 48 h os seguintes parâmetros mitocondriais foram medidos: consumo de oxigênio, níveis de espécies reativas de oxigênio e viabilidade mitocondrial monitorada através do ensaio de MTT. No tempo de 24h foi observada uma redução (29,65±3,68%) da viabilidade mitocondrial nas células expostas à maior concentração de Roundup® quando comparadas com o grupo controle (100±14,75%). Os demais parâmetros não apresentaram variação significativa. No tempo de 48h, foi observado uma redução da viabilidade mitocondrial nas concentrações de 6500 µg.L<sup>-1</sup> e 65000µg.L<sup>-1</sup> de Roundup® (61,69±9,21% e 56,44±12,18%) quando comparadas ao grupo controle (100±6,71%). Além disso, também foi observado um aumento nos níveis de espécies reativas de oxigênio nas células expostas à maior concentração de Roundup® (227,17±25,01%) quando comparadas ao grupo controle (100±31,88%). O fato da maior concentração afetar a viabilidade mitocondrial indica que possivelmente o Roundup® esteja alterando a funcionalidade mitocondrial em termos de funcionamento da cadeia transportadora de elétrons, o que pode estar levando ao aumento dos níveis de espécies ativas de oxigênio nas células expostas ao contaminante. As células de zebrafish demonstraram ser mais sensíveis uma vez que no presente estudo foram observados efeitos em concentrações menores do que aquelas observadas em estudos com hepatócitos de ratos. Desta forma, pode-se inferir que o Roundup® altera a funcionalidade mitocondrial e consequentemente pode afetar a capacidade das mitocôndrias em produzir ATP.

**SP110 Effect of the fungicide Pyraclostrobin on the development on larvae of Africanized Apis mellifera in environmentally relevant concentrations**

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Currently, honey bees have suffered a great population decline triggers to the interaction of biotic and abiotic factors, including pesticides applied on crops, which reach agroecosystems in sublethal concentrations. However, toxicity tests of pesticides on honeybee workers carried out by regulatory agencies may not show the effects caused by sublethal doses/concentrations arriving in the hive, specifically on larval development. The fungicide pyraclostrobin is applied in several agricultural crops in Brazil and worldwide. It is known that it has an inhibitory action on mitochondrial function that affects a synthesis of ATP in adult bees, but there is no information about its effects on the ontogenetic development of these non-target insects. This study aimed to evaluate the effect of larval exposure to two environmentally relevant concentrations of pyraclostrobin along the development of Africanized *Apis mellifera*. Bioassay was conducted with larvae from three different colonies and the larvae were individually contaminated by acute oral exposure, at the day 4, according to experimental groups (N = 16 larvae per group in triplicate): I) control (contain the acetone as solvent of fungicide); II) Pyraclostrobin 5 ppb; III) Pyraclostrobin 25 ppb; and Dimethoate 8.8 µg/larvae (standard insecticide for the test validation at concentration suggested by OECD). Cumulative mortality was observed in 72 hours after exposure (D5 to D7), and the statistical analyses were performed by generalized linear models (GLM). Mortality of honeybee larvae at 72 hours had no significant difference,  $p > 0.05$  (0.68), among control group and pyraclostrobin-exposed groups in both concentrations present in diets (5 ppb and 25 ppb). The mortality of the dimethoate was higher than expected by the LD50 of 72 hours, which indicate that LD50 for Africanized honeybee is different that described for European honeybee. However, although the tested concentrations have no effect on the mortality tax during larval phase, it is necessary to observe their effects on the pupa stages, as well as the emergence and its longevity of the imago.

**SP111 Effects of different glyphosate formulations on the morphology of the livebearing *Jenynsia multidentata*.**

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Roundup® is the most widely glyphosate-based herbicides used in the world. The mode of action of glyphosate as herbicide is the inhibition of EPSPS, a key enzyme involved in the biosynthesis of essential amino acids in plants. However, recent studies have shown that glyphosate can affect animal species, considered as non-target. In this

context, the objective of this study was to analyze and compare the effects of different glyphosate-based formulations, Roundup Original® (RO), Roundup Transorb® (RT) and Roundup WG® (RWG) on morphological aspects of gill, liver and brain of the livebearing *Jenynsia multidentata*. Fish (males and females) were exposed (24 and 96 h) to 0.5 mg.L<sup>-1</sup> of glyphosate in the three formulations, which were calculated based on the concentration of glyphosate present in each formulation. Previous results showed that 0.5 mg.L<sup>-1</sup> of glyphosate do not cause death in *J. multidentata*, regardless of whether the formulation is RO, RT or RWG. Among the histopathological damages, fatty degeneration, glial proliferation and lamellar hyperplasia were the most prominent alterations observed in the liver, brain and gills, respectively. These lesions were quantified using a histopathological index to assess aquatic pollution. In general, the tissues from fish exposed to the herbicides (RO, RT and RWG) showed significantly higher lesions in relation to the control group (non-exposed to glyphosate). The histopathological severity and the affinity for a particular organ (gills, liver and brain) vary between RO, RT and RWG formulations and between males and females. The RWG and RT were worst than RO. Also, the degree of lesions increased over the time of exposure (24 to 96 h). In the case of the brain, RT caused more severe lesions. In the liver, lesions were more evident in fish exposed to RWG and females were more affected than males; and finally, in the gills, RT and RWG formulations were more harmful than RO for both genders. Considering results presented here, RT and RWG are of greater risk. Moreover, mechanisms of toxicity also seem to be different between formulations and possibly it is linked to their compounds other than glyphosate. *J. multidentata* supported as a good bio-monitor species that inhabits regions of irrigated rice plantations in Rio Grande do Sul / Brazil, where tons of Roundup® herbicides are released every year. The experiments were approved by CEUA / FURG (23116.005051/2013-59). CNPq Proc. 449695/2014-0

**SP112 Fish Embryo Toxicity Test (FET) to evaluate atrazine effects**

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Surface water samples from São Paulo state were collected to perform Bioluminescent Yeast Estrogen Screen (BLYES) and chemical analyses (LC-MS/MS). Results showed environmental concentrations of atrazine from 2 to 43 ng L<sup>-1</sup> on chemical analyses. Some studies have been performed to evaluate toxic effects on non-target organisms (fish) using herbicides such as Atrazine, a moderately toxic compound classified as an endocrine disrupting chemical (EDC) that can affect reproduction of several aquatic organisms with a compromise of vitellogenin production. To determine toxicity on embryonic stages of fish to different environmental chemicals and waste water, Fish Embryo Toxicity Test (FET) was designed using *Danio rerio* as model specie on this test, according to OECD 236 or ISO 15088 protocols, however these protocols observe only acute toxicity based on endpoints such as coagulated eggs, non-detachment of the tail, lack of heart beating and lack of somite formation. Some abnormalities can be recorded after the exposure on FET test but they are not considered as endpoint, neither any other compromised biomarker by EDC action. In order to evaluate the possibility of using these chronic endpoints and to verify if those environmental concentrations of atrazine are ecologically relevant, compromising reproductive aspects, FET test using *Danio rerio* were performed to assess lethal concentrations, sublethal concentrations and vitellogenin quantification after atrazine exposure. Occurrence of morphological abnormalities (microcephaly, spine curvature, edema, reduced size) and mortality of the embryos were determined exposing 20 fertilized eggs to atrazine concentrations from 2 to 64 mg L<sup>-1</sup>. The LC50 and EC50 were obtained after 96 hours of exposure. Organisms that survived each concentration were frozen to further vitellogenin quantification. Preliminary average concentrations obtained (LC 50; 96h= 48.15 mg L<sup>-1</sup> and EC 50; 96h= 27 mg L<sup>-1</sup>) were considerably higher than concentrations observed on environmental samples. Therefore, surface water concentrations would not cause mortality or deformity in fish emphasizing the necessity to observe possible effect on vitellogenin concentration. Data will be analyzed and compared with the environmental concentration of atrazine to establish the potential application of vitellogenin as endpoint on FET test. Keywords: Fish Embryo Toxicity Test, Atrazine, BLYES, Vitellogenin, *Danio rerio*

**SP113 Hexachlorobenzene biodegradation by *P. castanella* during soil growth**

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Hexachlorobenzene (HCB) has been banned in many countries, because it has been considered one of the forms of persistent organic pollutants (POPs), presenting mutagenic, carcinogenic and teratogenic characteristics. In spite of this, the industrial activities of direct and / or indirect generation of this compound led to the inadequate disposition of this pollutant in the environment. The HCB accumulation in the biosphere implies the need to develop technologies capable of degrading this compound, as well as those derived from its degradation. Biological treatments using basidiomycetes present advantages over traditional treatments, since these fungi produce an extracellular and non-specific ligninolytic enzyme system capable of catalyzing the degradation of organochlorines and also have mycelial growth by hyphae expansion, which extend for long distances and penetrate all layers of soil to be treated. Thus, this work aimed to evaluate the ability of a basidiomycete to degrade HCB during soil growth. Cultures of *Psilocybe castanella* CCIBT2781 were prepared in MEA 2%, from which mycelial growth fragments (31.79 cm<sup>2</sup>) were removed to inoculate each Kg of solid substrate. This substrate was composed of sugarcane bagasse and soybean meal (C: N 80 ratio), with 70% moisture content. The inocula were incubated at 28 ° C, for 21 days, and after that period 3 g of this colonized substrate were introduced into a culture system containing 30 g of HCB-soil (1104 mg HCB. Kg<sup>-1</sup>), 2.5% commercial gypsum, 5% soybean oil and Tween 20 emulsion (1: 9, w / w), with humidity adjusted to 70% of the maximum water retention capacity. The systems were incubated for 70 days, at 28 ° C. Soil without HCB was used as control culture systems. The fungal biomass was estimated by the amount of ergosterol extracted (Silva et al., 2010), and the chlorinated residues were extracted from the systems after fungal cultivation, according to Andrea et al. (2001), the determination of chlorides was performed by potentiometry, and the quantification was done by gas chromatography. It was observed a 32.40% degradation of HCB, confirmed by the 53.3% increase in chloride concentration. The presence of pentachlorobenzene and pentachlorophenol in the extracts proven the biodegradation. In addition, the growth of the fungus in the presence of HCB did not differ significantly from the growth in control soil, thus confirming the tolerance of this basidiomycete to the presence of the chlorinated compounds.

#### SP114 Human Risk Assessment of Pesticide-Contaminated Vegetables

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According to FAO, healthy food intake is the one that has to have quality and quantity of the needed nutrients and they also ought to be safe. However, many foods such as vegetables often contain pesticide residues which could represent a potential risk of intoxications for chronic-exposed populations. Pesticide residues in food and crops are a direct result of pesticide application for pest control and, to a lesser extent, from pesticide-contaminated soil. The city of Centenario is part of the Green Belt that supplies to the Concentration Market of Neuquén an average of 15% of the revenue from fresh vegetables (leafy vegetables, tomatoes, peppers, eggplant, etc.) and other crops (squash, potatoes, onions, etc.). The objective of the study was to evaluate the risk of Centenario's population by the intake of vegetables with pesticide residues. Data from pesticide residues in vegetables obtained from Concentration Market of Neuquén were provided by the INTI (Instituto Nacional de Tecnología Industrial) of Neuquén and the "Regional Program for the Rational Use of Agrochemicals in Horticulture". Data of amount and frequency of intake of each vegetable, among other factors were obtained from the Centenario's population by a standardized questionnaire. While the maximum residue levels (MRLs) of pesticides registered for each crop were not overcome in most samples, in some cases unregistered pesticide residues for certain vegetables or the presence of various pesticide residues in the same crop were determined. Among unregistered pesticides for these crops are some highly toxic insecticides such as chlorpyrifos and endosulfan and carcinogens such as DDE (DDT metabolites). The results showed that consumption of vegetables, throughout the period of life with chlorpyrifos residues, endosulfan and DDE did not produce chronic effects under the assumptions of the study. The estimated cancer risk by the presence of DDE residues in vegetables was within the acceptable distribution range (10<sup>-6</sup>-10<sup>-4</sup>). It is worth to mention that there were several uncertainties in the risk assessment that may underestimate the actual risk of the study population.

#### SP115 Pentachlorophenol in soil biodegradation by *Lentinus crinitus*

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Pentachlorophenol is a pesticide that is banned in many countries, including Brazil. However, the uncontrolled disposal of industrial waste, containing organochlorines, resulted in contamination of the soil, causing environmental liabilities, which still require strategies for its decontamination. Treatments employing basidiomycetes present advantages, since such fungi produce an extracellular and nonspecific ligninolytic enzyme system, capable to catalyze the degradation of different chemical compounds. In addition, these fungi present mycelial growth by hyphae expansion, which extend for long distances and penetrate all layers of soil to be treated. Thus, in an attempt to assess the *Lentinus crinitus* CCIBT2611 in bioremediation *in vivo* systems, for pentachlorophenol (PCP) soil were studied. The fungal inoculum was prepared with solid substrate (sugarcane bagasse, soy flour and starch), with 70 % of humidity. The capacity of the fungus to degrade PCP was evaluated using flasks containing the culture system: sandy soil (30g) with PCP PA (200 mg kg<sup>-1</sup>), commercial gypsum (2.5%), emulsion of vegetable oil and Tween 20 (9:1, p.p.) (5.0%), inoculum (10%, dry mass) and 15.0% distilled water. The flasks were held at 28 ° C for 10 days. The following parameters were determined: enzymatic activities of laccase and manganese-dependent peroxidase (ABTS and phenol red as substrates, respectively) and PCP (High Performance Liquid Chromatography, HPLC). The initial laccase activity (inoculum) was 17.6 U/L, with an increase of about 4 times after incubation in the treatment systems (59.3 U/L on the 5<sup>th</sup> day and 87 U/L on the 10<sup>th</sup> day), evidencing the stimulation of laccase production by the presence of PCP. The initial activity of the peroxidase was 53.1 U/L, with a decrease in the value after incubation of the treatments (5.7 U / L on the 5th day and 8.0 U / L on the 10th day), evidencing inhibition of the peroxidase production in the presence of PCP. The fungus was able to degrade 98.7% of PCP, until the 5<sup>th</sup> day of growth, reaching 100% between 5-10 days of incubation. The results obtained demonstrate the ability of *L. crinitus* to degrade PCP soil, reinforcing the potential of basidiomycetes for the bioremediation of soils contaminated with organochlorines. An advantage of the use of this fungus is to reduce the time of conduction of the bioremediation systems to 10 days, in relation to results reported in the literature, for other treatments using basidiomycetes.

#### SP116 Pesticide Risk Assessment in the Pampa Region of Argentina: Framework for exposure characterization

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The estimation of the Predicted Environmental Concentrations (PEC) of a pesticide is an essential first step when performing an ecotoxicological risk assessment for aquatic environments. In the past, the lack of readily available databases, scenarios and tools to define PEC have limited the development and use of pesticide risk assessment practices in the Pampa region of Argentina. The Pesticide Water Concentration (PWC) is a free computer model developed by the U.S. Environmental Protection Agency, which allows to estimate probable concentrations of pesticide in surface water for use in risk assessment. The objectives of the current project are (1) to develop climate and soil databases necessary for using PWC in the Pampa region, (2) to realize a sensitivity analysis of PWC to detect the sensitive parameters of the model, and (3) to define exposure scenarios describing the various subregions of the Pampa region. For soil data, a soil map with a resolution of 1:2.500.000 and which includes values for the various soil horizons was selected. In this soil database, the Pampa region is divided in 82 cartographic units. On this basis, we proceeded to generate one weather file per soil cartographic unit for the period 1984-2014 by ordinary kriging using data from 30 meteorological stations. To detect the presence of temporal variations in the climatic variables used, trend analysis was performed using the Mann-Kendall Test. This test demonstrated the presence of significant upward trends in maximum temperatures over the region that were sometimes, but not always, accompanied by upward trends in evapotranspiration. As pan evaporation data were not continuous for the entire time period, these values were calculated from pan coefficients obtained by relating evapotranspiration to pan evaporation data available in each meteorological stations. Calculated pan coefficients ranged between 0.55 and 0.68 over the territory. The sensitivity analysis of the PWC model was realized using the SENSAN section of the PEST model. The most sensitive parameters were, in decreasing order, (1) water body dimensions, (2) chemical, (3) run off, (4) erosion, (5) applications, (6) benthos, (7) water column, (8) crop. The ongoing final step of the project consists in building and comparing exposure scenarios associated to the different cartographic units so as to define a framework for pesticide exposure characterization and risk assessment in the Pampa region.

#### SP117 Potential of water resources contamination by the use of vinasse as fertilizer over the years using mathematical modeling

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Brazil is the largest producer of ethanol in the world and São Paulo state stands out with about 50% of this production. Vinasse, the main residue generated in the sugarcane processing to ethanol, has acid pH, great organic load and a great metals quantity, which makes it an excellent fertilizer, but with great potential pollutant against the high quantity produced. The high quantities released in the fields can promote physical changes in the soil, favoring the vinasse components percolation with toxic potential reaching the ground water or leaching to water resources close to the application sites. Extensive use of vinasse as a soil fertilizer will complete 40 years and there is no data of its effects over the years. With this in view, mathematical modeling was performed using a hypothetical area as a model; vinasse application was simulated for three months per year for 30 years; The concentrations that would reach the ground water that would be 20m and 10m deep and the concentrations that would reach a surface water that would be 400m and 250m respectively from the fertigation site were calculated. With the water table at 20m, percolated vinasse reaches ground water level in less than 3 years, reaching a maximum concentration of 42% approximately in 30 years. The leachate from vinasse begins to reach the surface water located at 400m in 16 years approximately, reaching a concentration of 4.8% in 30 years. At ground water at 10m, percolated vinasse would reach the ground water about 1.64 years, reaching a maximum concentration of approximately 17% in 20 years. Taking into account the vinasse toxic potential in previous studies using *Oreochromis niloticus* exposed to a similar dilutions to those found by modeling, an alert is raised about the impacts and damages caused by the intense use of this residue over the years, in contrast to its benefits for the the harvest period. Financial Support: FAPESP 2012/50197-2, 2015/26157-9

#### SP118 Pyrethroid residues and diastereomeric selectivity pattern on chicken eggs from commercial farms and home production in Rio de Janeiro

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In this study, pyrethroid insecticides were determined on chicken eggs from commercial farm (n = 60) and home egg production (n = 30). These pyrethroids were investigated: bifenthrin, phenothrin, permethrin, cyfluthrin, cypermethrin and fenvalerate, including most diastereomers. Residues in home samples could be due to a greater diversification of exposure sources, as these chickens live in direct contact with soil, being fed with a mix of feed, food remains, forage grasses and terrestrial invertebrates. On the other hand, on commercial farms, chickens are raised in suspended cages and fed with industrialized feed, which limits the sources of exposure to water supply, feed, or direct application for ectoparasite treatment. Pyrethroid residues were found in 79% of the analyzed samples. Cypermethrin presented the highest occurrence, being quantified in 62 samples (69%) in concentrations (lipid weight – l.w.) varying between 0.29 and 6,408 ng.g<sup>-1</sup>, followed by phenothrin (24%), 21 - 3,910 ng.g<sup>-1</sup>, permethrin (14%), 2.96 - 328 ng.g<sup>-1</sup>, and bifenthrin (11%), 3.77 - 16.7 ng.g<sup>-1</sup>. Cyfluthrin and fenvalerate were not detected. Home-produced eggs had a higher occurrence of pyrethroids (97%). In commercial production, 70% of the samples presented pyrethroid residues. Moreover, clear diastereomeric selectivity in Type I (permethrin and phenothrin) and Type II (cypermethrin) pyrethroids was observed. This is the first report about the presence of pyrethroids in home-produced eggs and the first description of a selectivity pattern with the predominance of cis diastereomers in chicken eggs. Further studies about transfer dynamics, bioaccumulation and metabolic degradation of stereoisomers are required, as well as determining if this selectivity pattern in food can increase consumer's health risk.

#### SP119 The employ of *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces, Poeciliidae) as target organism in pesticide genotoxic evaluation

S. Soloneski, National University of La Plata / PB, Depto 17; M. Larramendy, National University of La Plata / Faculty of Natural Sciences and Museum  
The increasing attention in the use of nontraditional fish species for ecotoxicology and genotoxicology research, not only lies in the ability to reveal genotoxic and cytotoxic effects induced by many potential environmental xenobiotics, but also helps researchers understand the real performance of such ecosystems. Small fish are increasingly used as organism toxicity models, and one of these, the ten spotted livebearer fish (*Cnesterodon decemmaculatus*), has recently been employed in geno- and cytotoxicity testing. *C. decemmaculatus* is proving itself as a useful animal model system for studying environmental toxicity, developmental biology, genetics, and genotoxicology. As a versatile tool *in vivo* and *in situ*, *C. decemmaculatus* has a

number of features that make it attractive as a laboratory model organism for toxicity studies. In the present study, the genotoxic effects of the herbicides glyphosate, dicamba and 2,4-D were evaluated using the comet assay methodology and the micronuclei detection in circulating blood erythrocytes from *C. decemmaculatus* as a laboratory-exposed target fish. Our results indicate that *C. decemmaculatus* may be considered as a sentinel organism due to its role in the trophic chain.

#### SP120 Toxicological effects of the pyrethroid lambda-cyhalothrin for a Neotropical teleost

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The lambda-cyhalothrin (λ-CL), a α-cyano-pyrethroid, has been used against a wide range of insects in agriculture and for public health purposes, standing out among the most used pesticides in Brazil, both alone as well as in combination with other compounds. As a result of inputs from agricultural areas and urban sprays, this compound tends to accumulate in water bodies and its potential effects on non-target organisms are still poorly investigated in Neotropical species. Thus, the aim of the present study was to identify the potential hazard of the commercial formulation Karate Zeon CS® (5% encapsulated λ-CL, Syngenta S.A. Brazil) in juveniles of *Prochilodus lineatus* (14.1 ± 1.14 g, 10.77 ± 0.6 cm [mean ± SD, n=40]). Fish were exposed to λ-CL at nominal concentrations of 5 (CL5), 50 (CL50), 250 (CL250) and 500 (CL500) ng.L<sup>-1</sup> of a.i or only to clean water (CTR) for 96 h, under semi-static condition with daily water renewal. Biochemical parameters such as glutathione content (GSH), activity of the glutathione S-transferase (GST), superoxide-dismutase (SOD), catalase (CAT), glutathione-peroxidase (GPx), α and β-esterases (α/β-EST), acetylcholinesterase (AChE), calcium and magnesium ATPase (Ca<sup>2+</sup>/Mg<sup>2+</sup>ATPase) and lipoperoxidation (LPO) were evaluated in various organs. When compared to CTR fish, significant increases (p < 0.05) of LPO were observed in the gill and kidney of fish exposed to CL500. Increases of GST activity were observed in the liver, gill, brain and white muscle of fish from CL500. In the gill, decrease of SOD activity was observed in CL250 and CL500 groups. Increases of CAT activity were observed in the brain of fish from all experimental groups while in the liver a decline occurred in all concentrations. Fish exposed to all λ-CL concentrations showed significant increases of α-EST and β-EST in the liver and of plasmatic β-EST. Lambda-cyhalothrin also promoted decreases in branchial Ca<sup>2+</sup>ATPase of fish exposed to CL50, 250, 500, and Mg<sup>2+</sup>ATPase in CL250 and CL500 groups. Also, there was a significant decrease in muscle AChE in fish exposed to all concentrations. Taking together, these results suggest that λ-CL has the potential to promote several effects in *P. lineatus*, such as oxidative stress and disturbances in osmoregulatory and neurotransmission processes at concentrations environmentally relevant.

#### SP121 Triad approach for ecological risk assessment of pesticides: effects on ecosystems in contamination scenarios

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Ecological risk assessment (ERA) studies are important to assess environmental changes that have been caused by anthropogenic activities. This integration models show the estimation of adverse risk effects across the biological organization levels potentially exposed to perturbation. Our study adopts a four-tiered risk scenarios (tier-0: conceptual model; tier-1: simple screening; tier-2: refined screening; tier-3: detailed screening; tier-4: final assessment), integrating terrestrial and aquatic responses to the fungicide Pyrimethanil. The risk characterization was based on the triad of the chemical, ecotoxicological and ecological line of evidences (Loe). The exposure scenarios (immediate, middle and short-term exposure) represented the integration of aquatic, terrestrial and ecosystem risk. The risk indicators score, between zero (no risk) to one (high risk), indicate limits of accepted risk values for different soil uses (nature, agricultural, residential and industrial land-use), according to the risk indicators (0.00 < IR < 0.25 no risk; 0.26 < IR < 0.50 low risk; 0.51 < IR < 0.75 moderate risk; 0.76 < IR < 1.00 high risk). The holistic fungicide ERA approach, reporting the integration of trophic levels with multitrophic interactions in ecosystem models and supplementary with individuals' responses. To attempt it, the experiments evaluated the exposure effects on ecosystems with terrestrial and aquatic compartments analysis with the effects on terrestrial plants, earthworms, collembolan, mites, zooplankton, phytoplankton, macrophyte, aquatic macroinvertebrates, fish, and tadpoles. The results obtained suggest that the integrated risk values followed the exposure gradient from immediate, middle and short-term exposure. For the aquatic compartment, integrated risk showed low risk to short-term exposure to moderate risk for the others exposures. For the terrestrial compartment, the integrated risk showed moderate risk increase followed the exposure gradient. Finally, the ecosystem integrated risk showed

moderate risk for the short and middle-term exposure and high risk for the immediate-term exposure. We concluded that the integrated ERA for the fungicide pyrimethanil is an important register for the deleterious effects and responses to impacts of pesticides, prompting the possible environmental losses and changes of the ecosystems functions and services.

**SP122 The employ of *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces, Poeciliidae) as target organism in pesticide genotoxic evaluation**

S. Soloneski, National University of La Plata / PB, Depto 17; M. Larramendy, National University of La Plata / Faculty of Natural Sciences and Museum

The increasing attention in the use of nontraditional fish species for ecotoxicology and genotoxicology research, not only lies in the ability to reveal genotoxic and cytotoxic effects induced by many potential environmental xenobiotics, but also helps researchers understand the real performance of such ecosystems. Small fish are increasingly used as organism toxicity models, and one of these, the ten spotted live-bearer fish (*Cnesterodon decemmaculatus*), has recently been employed in geno- and cytotoxicity testing. *C. decemmaculatus* is proving itself as a useful animal model system for studying environmental toxicity, developmental biology, genetics, and genotoxicology. As a versatile tool *in vivo* and *in situ*, *C. decemmaculatus* has a number of features that make it attractive as a laboratory model organism for toxicity studies. In the present study, the genotoxic effects of the herbicides glyphosate, dicamba and 2,4-D were evaluated using the comet assay methodology and the micronuclei detection in circulating blood erythrocytes from *C. decemmaculatus* as a laboratory-exposed target fish. Our results indicate that *C. decemmaculatus* may be considered as a sentinel organism due to its role in the trophic chain.

**Other**

**SP123 Avaliação de Potencial de Efeito Tóxico na Água e no Sedimento em uma Área de Preservação Ambiental, litoral norte do estado do ES/Brazil**

T. Furley, APLYSIA Environmental Research and Solutions; K. Chagas, Aplysia Soluções ambientais / TÉCNICO; J. Entringer, M. Simonelli, Instituto Federal do Espírito Santo; A. Peruchi, R. Reis, APLYSIA Environmental Research and Solutions. Existe uma tendência mundial de avaliação se as Áreas de Proteção Ambiental, realmente, cumprem seu valor de proteger e preservar a biodiversidade. A compreensão da resposta do ecossistema a diferentes perturbações é essencial para aprimorar o sistema de manejo em regiões costeiras (LECARI et al., 2002). Deste modo, este estudo teve como objetivo caracterizar o potencial de efeito tóxico da água e do sedimento coletado em regiões marinhas especialmente protegidas. A área de estudo compreende a Unidade de Conservação de Proteção Integral denominada “Refúgio da Vida Silvestre de Santa Cruz” e a Unidade de Conservação de Uso Sustentável denominada de “Área de Proteção Ambiental Costa das Algas”, localizadas no litoral norte do estado do ES, abrangendo três municípios: Serra, Fundão e Aracruz. Foram realizadas três campanhas de coleta nos meses de maio/2016, julho/2016 e janeiro/2017 e definidos quinze pontos amostrais para as matrizes água e sedimento. Os ensaios ecotoxicológicos aplicados para as amostras de água abrangeram dois níveis tróficos, sendo do tipo crônico e qualitativo tanto para a diatomácea marinha *Skeletonema costatum* quanto para o equinodermata *Echinometra lucunter*. O sedimento total foi avaliado por meio do ensaio agudo com o organismo anfípoda *Grandidierella bonnieroides*. Todos os ensaios seguiram os métodos preconizados pelas normas técnicas da ABNT. Os resultados, após uma avaliação não só meramente estatística, indicaram para a matriz água a não existência de efeitos tóxicos para *E. lucunter* em nenhum ponto amostral das três campanhas, ou seja, as águas das áreas protegidas não desencadearam interferências nas percentagens de desenvolvimento embrio-larval dos organismos. Para as algas, houve inibição superior a 10 % da taxa de crescimento apenas nos pontos 01 e 05 da terceira campanha, estes pontos se localizam em uma região mais intensamente edificada de acordo com o mapa de uso e ocupação do solo elaborado pela equipe do Instituto Federal de Educação de Vitória. Para o sedimento, na primeira campanha não houve mortalidade para os anfípodas, porém na segunda (6 pontos) e terceira (5 pontos) campanhas ocorreram mortalidades, principalmente acentuadas na 3ª campanha. De modo geral, pode-se inferir que para os organismos avaliados, a matriz sedimento é a que se encontra em piores condições principalmente para os pontos próximos a fronteira norte da APA.

**SP124 Avaliação por biomarcadores bioquímicos e genéticos de duas dietas compostas por diferentes biomassas de alga em peixes da espécie *Rhamdia quelen***

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Engineering; H.C. Silva de Assis, Universidade Federal do Paraná / Departamento de Farmacologia; M.M. Cestari, Universidade Federal do Paraná / Departamento de Genética

Estudos têm sido desenvolvidos visando a aplicabilidade de microalgas cultivadas em fotobiorreatores compactos para a produção de biocombustíveis, dados os impactos provocados pela extração e uso de combustíveis fósseis. Neste processo, a biomassa proveniente das microalgas é submetida à extração lipídica, gerando uma biomassa residual desengordurada (*Residual Algae Biomass* – RAB). A RAB, por sua vez, possui potencial para enriquecimento na nutrição animal (apontando uso alternativo para este resíduo). Neste cenário, objetivou-se avaliar o potencial genotóxico da RAB quando utilizada como fonte de alimento em peixes. Testou-se a biomassa residual da microalga *Acutodesmus obliquus*, cultivada em meio sintético (CHU) e em meio composto por efluente de suinocultura biodigerido (ESB). Para os ensaios (96h), utilizou-se peixes da espécie *Rhamdia quelen* (Siluriformes: Heptapteridae), alimentados duas vezes ao dia *ad libitum* e separados em 3 grupos: 1 - Alimentação convencional, sem RAB; 2 - Alimentação com RAB cultivada em meio CHU; e 3 - Alimentação com RAB cultivada em meio ESB. Ao final dos experimentos, os peixes foram anestesiados e eutanasiados por secção medular. Analisou-se os biomarcadores: ensaio de difusão do DNA e ensaio cometa em eritrócitos; acetilcolinesterase (AChE) em cérebro e músculo; catalase (CAT), glutatona peroxidase (GPx), glutatona S-transferase (GST), superóxido dismutase (SOD) e lipoperoxidação (LPO) em fígado. Os resultados foram submetidos à Análise de Variância (ANOVA), seguida do teste de Newman-Keull's; e ao teste de Kruskal-Wallis, seguido do teste de Dunn, sendo a normalidade verificada pelo teste Kolmogorov-Smirnov. Os resultados do ensaio de difusão do DNA e ensaio cometa em eritrócitos indicaram que não houve diferença entre os três grupos analisados. Quanto à atividade da AChE no cérebro, observou-se aumento apenas no grupo 3. Não foram observadas diferenças entre os grupos nas atividades de AChE em músculo, assim como para GST (enzima de biotransformação de fase II), LPO (indicador de integridade da membrana) e GPx (atuante na degradação de H<sub>2</sub>O<sub>2</sub>) em fígado. Foi observada redução na atividade da CAT (responsável pela degradação do H<sub>2</sub>O<sub>2</sub> intracelular no grupo 3), e para a metaloenzima SOD (responsável pela degradação do radical superóxido - O<sub>2</sub><sup>-</sup>), foi observada redução nos grupos 2 e 3.

**SP125 Biodegradação de hidrocarbonetos derivados de petróleo**

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Vazamentos e derramamentos de produtos oriundos do petróleo representam uma grande ameaça ao meio no qual entram em contato, pelo fato de conterem substâncias com alto grau de toxicidade, tais como, os hidrocarbonetos monoaromáticos: tolueno, etilbenzeno, xileno e benzeno. Estes hidrocarbonetos monoaromáticos (BTEX) ao entrarem em contato com o solo, podem ser adsorvidos nos sólidos do mesmo ou dissolvidos na solução do solo, e por apresentarem grande mobilidade podem atingir rapidamente o lençol freático. Diante desta perspectiva, o trabalho visou a utilização de duas espécies de fungos para biodegradar os compostos BTEX de solos contaminados do entorno de locais como postos de combustíveis, com o intuito de verificar como os mesmos se comportam, o crescimento radial, a faixa de tolerância ao contaminante, a atividade enzimática e a capacidade de biodegradação. As espécies de fungos utilizadas foram *Lentinus* e *Pleurotus ostreatus*. Para verificação da eficácia dos fungos na degradação dos hidrocarbonetos monoaromáticos foi efetuada a produção de três tipos de meio de cultura em placas de Petri, sendo o primeiro em Ágar-batata, o segundo em Ágar-batata com presença de solo contaminado e o terceiro em Ágar-batata com presença de solo sem contaminação. Notou-se que o desenvolvimento dos fungos nos três tipos de meio de cultura foram semelhantes, ou seja, a presença dos HC não inibiu o crescimento fúngico, obtendo-se um crescimento radial completo em 4 dias. A avaliação da atividade enzimática no meio de cultivo revelou a presença de atividades de lacase e Mn-peroxidase. Estudos da taxa de biodegradação estão sendo realizados.

**SP126 Comparing experimental designs and statistical analyses to estimate thresholds in ecotoxicology**

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Different methods are used in ecotoxicology to estimate thresholds in survival data. This work uses Monte Carlo simulations to evaluate the accuracy of four methods (maximum likelihood (MLE) and MCMC estimates (Bayesian) of the NEC model, Piecewise regression and ROC curves) in estimating true and apparent thresholds in survival experiments with datasets having different slopes, background mortalities, and designs. Datasets were generated with models that include a threshold parameter (NEC) or not (log-logistic). Accuracy was estimated using the RMSEs, and RMSE ratios were used to estimate the relative improvement in accuracy by each design and



method. All methods had poor performances in shallow and intermediate curves, and accuracy increased with the slope of the curve. The EC<sub>5</sub> was generally the most accurate method to estimate true and apparent thresholds, except for steep curves with a true threshold. In that case, the EC<sub>5</sub> underestimated the threshold, and MLE and Bayesian estimates were more accurate. In most cases, weighted information criteria did not provide strong evidence in support of the true model, suggesting that identifying the true model is a difficult task. Piecewise regression was the only method where the weighted criteria had high support for the threshold model; however, the rate of spurious threshold model selection was also high. Threshold estimates, under the experimental conditions evaluated in this work, should be carefully used in survival analysis.

**SP127 Desarrollo de biomarcador de salinidad a partir del Ostión del Norte (*Argopecten purpuratus*) para la evaluación de descargas de plantas desaladoras**

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**SP128 Effect of pyraclostrobin fungicide on the survival rate in workers of Africanized *Apis mellifera* (Hymenoptera: Apidae)**

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Bees through pollination are essential in many agricultural crops and also in the maintenance and conservation of native vegetation diversity. Nevertheless, bees are being affected by different environmental stressors such like as fungicides, although they are considered to be poorly toxic to insects, they are increasingly present in many crops and their continuous exposure may cause potential toxic effects on bees. This study aimed to analyze the effect of pyraclostrobin fungicide on the survival rate of Africanized honeybee. Toxicological bioassays by continuous oral exposures were performed with newly-emerged workers (20 individuals in triplicate per group) that were divided in control, solvent-control and the exposed groups: pyraclostrobin

125ppb; pyraclostrobin 25 ppb; and pyraclostrobin 5 ppb. During oral exposures, food with fungicide was offered ad libitum during the whole time of bioassay, i.e. eighteen days. Mortality was recorded daily and survival rates were performed at the end of experiments by means of Kaplan-Meier Survival Analysis: Log-Rank. After eighteen days of exposure, the results showed decreased longevity of bees in the groups exposed to pyraclostrobin 5ppb compared to the control group and solvent-control. In the other concentrations of the fungicide no differences were observed in relation to the control group. The oral continuous exposure to sublethal concentrations of pyraclostrobin for the period of eighteen-five days induced the potencial negative impact on the survival rate of honeybees even in low concentrations. Concluding, the results showed the risk of the continuous exposure at sublethal concentrations of pyraclostrobin fungicide, indicating that the intra-colonial exposure through food can affect the performance of individuals and, consequently, the homeostasis of the colony.

**SP129 Environmental exposure of legacy and current use pesticides during prenatal life in women from North Patagonia, Argentina**

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The occurrence of organochlorine (OCPs) and current use pesticides (CUPs) in environmental compartments has received attention due to their persistence, ubiquity and toxicity. Because of their lipophilic nature, they can bioaccumulate in fatty tissues and can cross through the placenta to fetus. Therefore, the contamination of the placenta with environmental pollutants provides an indicator of exposures for both mother and fetus. Argentina is one of the major producers of cereals (soybean), oilseed and fruits. Rio Negro basin provides 4% of the worldwide production of apples and pears concomitantly with an intensive use of pesticides from 50 years ago. This study focusses on the bioaccumulation of OCPs and CUPs in placenta of women from North Patagonia, Argentina. The samples were taken from two populations: -urban: Neuquén city (38°57'S-68°02'W) and -rural: Upper Valley of the Rio Negro (39°02'S-67°35'W), which is characterized by the intensive fruit production. Furthermore, socio-demographic characteristics of the women recruited, the neonate anthropometric parameters and CUPs exposure biomarkers were considered. OCPs (endosulfans, DDTs, HCHs, heptachlor, chlordane) and CUPs (chlorothalonil, chlorpyrifos, trifluralin) were quantified by GC-ECD/GC-MS. Total pesticides levels in rural population were 5 times higher than those in urban (87.8 and 20.5 ng/g lipid weight, respectively). Pesticides pattern distribution was different in both areas, -rural: endosulfan (39.4 ng/g lw) > DDTs (9.7 ng/g lw), and -urban: DDTs (13.7 ng/g lw) > endosulfan (6.1 ng/g lw). All samples presented a-b/endosulfan ratio >1 (4-14), showing a recent exposure to technical mixture which was banned in 2013. The prevalence of pp'-DDE in both areas (>96%, range 9.6-13.1 ng/g lw), indicates that DDT is still relevant even though it has been banned in Argentina since 1998. Among CUPs, only chlorothalonil was found at low levels (rural=1.1 ng/g lw, urban=0.4 ng/g lw). This report about OCPs and CUPs occurrence in placenta shows the relevance of the environmental exposure to DDT and endosulfan on the mother of highly vulnerable populations. Efforts should be made to improve the environmental health and the safety conditions of general population and, particularly of families living in rural areas

**SP130 ESTIMACION POR ALTIMETRIA SATELITAL DEL AUMENTO DEL NIVEL DEL MAR: RIESGO PARA ZONAS URBANAS DE BRASIL Y URUGUAY**

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Los satélites altimétricos determinan la altura de la superficie oceánica respecto de un punto de referencia, el nivel medio mundial del mar, que se denomina "geoido terrestre". Desde sus órbitas, los altímetros miden la topografía de la superficie del océano con gran exactitud para derivar la velocidad y dirección de las corrientes y los remolinos oceánicos, y observar las mareas y otros fenómenos. Entre las aplicaciones de la altimetría se puede mencionar: medición de la topografía de la superficie oceánica (para para describir con exactitud las variaciones climáticas estacionales, interanuales, etc.), mapeo de la distribución espacial y estacional de la altura de las

olas y la velocidad del viento, medición de las capas de hielo, los glaciares y el hielo marino, etc. En este Trabajo se aplicaron los datos altimétricos con el objetivo de evaluar temporalmente la elevación del nivel del mar en 11 puntos o nodos de control entre Brasil y Uruguay (nodos de OSTM - Ocean Surface Topography Mission) que corresponden a la ubicaciones con información sobre el nivel medio del mar. La base de datos altimétricos corresponde a OSTM permitió trabajar con datos desde el 1/3/1950 hasta el 27/6/09. Se ha comprobado un aumento significativo del nivel del mar en todos los nodos analizados, resultando especialmente afectado el punto 9 correspondiente al punto Lat-26° 0' 0" Long-47° 0' 0". El aumento del nivel del mar. Se concluye que para el periodo 1950-2009 el  $\Delta h$  en los nodos cercanos las costas de Brasil han sufrido un incremento promedio que oscila entre ente 9 y 13 cm aumentando el riesgo de las poblaciones costeras ante eventos extremos.

**SP131 Percolado da vinhaça de cana-de-açúcar: histopatologia e imunomarcção de proteínas de estresse celular em peixes (*Oreochromis niloticus*)**

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A vinhaça de cana-de-açúcar é um resíduo gerado em uma proporção quinze vezes maior que à produção de etanol. Esse resíduo é utilizado como fertilizante da própria monocultura de cana devido a sua grande carga orgânica e presença de metais utilizados pelas plantas como micronutrientes. Entretanto, as elevadas quantidades lançadas podem encharcar o solo e chegar aos corpos hídricos por percolação e lixiviação. Dado o comprovado potencial tóxico da vinhaça bruta foi objetivo desse estudo avaliar o potencial tóxico do percolado da vinhaça, utilizando tilápias (*Oreochromis niloticus*) como organismos teste. Após percolação em colunas de solo, realizou-se um bioensaio, em réplica, constituído por um grupo controle, com exposição dos peixes à água limpa; um grupo com exposição ao percolado da vinhaça a 2,5%; e outro com exposição à vinhaça bruta também a 2,5%. Após exposição, as brânquias e os fígados foram utilizados para análise histopatológica e marcação de proteínas HSP70 nos fígados. Não houve alterações significativas nas brânquias dos peixes expostos. Já no fígado, tanto a vinhaça bruta como o percolado causaram alterações histopatológicas estatisticamente significativas como degeneração hidrópica, perda de limite celular, núcleo picnótico e desorganização tecidual. Houve também um aumento significativo na imunomarcção de proteínas de estresse celular HSP70 nos fígados tanto no grupo exposto à vinha bruta quanto no percolado, sendo maior para a vinhaça bruta. Os resultados sugerem que o percolado da vinhaça é menos tóxico do que a vinhaça bruta, porém ainda assim é capaz de causar importantes alterações nos fígados dos peixes expostos, assim como apresentar ação proteotóxica. Tendo isso em vista é necessária muita cautela na disposição desse resíduo no ambiente, visando a redução de seu impacto ambiental. Apoio Financeiro: FAPESP processos: 2012/50197-2; 2015/15624-5; 2015/15623-9; 2015/26157-9.

**SP132 Polycyclic aromatic hydrocarbons in surface sediments of a tropical estuary surrounded by sugarcane monoculture**

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Estuarine environments continuously receive input of several chemical contaminants, including polycyclic aromatic hydrocarbons (PAHs). These chemicals have been widely investigated in environmental matrices because they present high toxicity to organisms, also affecting ecosystems. Due to their hydrophobic nature, these compounds are usually adsorbed onto suspended particulate matter in the water column and are eventually accumulated in sediments. This study assessed concentrations and distribution of PAHs in sediments of a marine protected area (MPA) located in the Goiana River estuary, northeastern Brazil. Main sources of these compounds to the local estuary were also identified. Samples were collected at 12 stations along the estuary with a stainless steel van Veen grab sampler. Sedimentary organic matter (OM) was determined gravimetrically by combustion at 450 °C for 6 h. PAHs were extracted with a mixture of n-hexane:dichloromethane (1:1, v/v) in a Soxhlet apparatus. Extracts were cleaned up by column adsorption chromatography (silica and alumina). Identification and quantification of PAHs was done in a gas chromatography-mass spectrometry system (GC/MS). Concentration of PAHs ranged from < 0.06 to 156 ng g<sup>-1</sup> dry weight (dw). Average levels of total PAHs (26.8 ± 49.7 ng g<sup>-1</sup> dw) classify the Goiana estuary as low contaminated. The most abundant

individual PAHs were fluoranthene, pyrene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene that accounted for approximately 50% of total PAHs. Fluoranthene and pyrene have been pointed out as good proxies for burning of sugarcane prior to harvesting, a common practice in crops of the Goiana River watershed. PAHs in sediments were positively correlated with organic matter and fine sediments. Diagnostic ratios indicated a prevalence of PAHs from pyrolytic sources, probably due to sugarcane burning, vehicle combustion engines and/or small vessel engines. According to sediment quality guidelines, there is a low probability of adverse effects to the local biota. A continuous monitoring is recommended in order to track temporal trends of contaminants and to ensure conservation of the Goiana estuary MPA.

**SP133 Preliminary results from geochemical fractionation of Cd and Zn in sediment profiles from Sepetiba Bay, Rio de Janeiro, Brazil.**

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The sediment quality of Sepetiba Bay is affected by trace metals from wastes of industrial activities. High concentrations are found at the northeastern part of the bay, where Zn and Cd have their major inputs coming from the Ingá industrial landfill leaching. Studies about metal's mobility are scarce. Two sediment cores from different areas within the bay were studied (Marambaia Bay and Delta of São Francisco Channel). Cadmium and Zn concentrations, total organic carbon and sediment grain size were analysed. A sequential extraction procedure was applied to understand the trace metals partitioning among the different fractions of the sediment. Trace metals were classified into six fractions: (1) carbonates, (2) reducible oxyhydroxides, (3) amorphous oxyhydroxides, (4) organic matter, (5) silicates, and (6) sulfides. Organic carbon contents were high at SFC Delta (1.4%) and lower in Marambaia Bay (0.4%). The grain size dominance was muddy in SFC Delta (67% silt and 17% clay) and sandy in Marambaia Bay (61% sand). The total concentration of metals in the inner part of the bay (SFC delta) was higher than the previously established geochemical background values (Cd: 0.34 and Zn: 54 mg kg<sup>-1</sup>) and the mean concentrations from Marambaia bay (Cd: 0.32 and Zn: 62 mg kg<sup>-1</sup>). There is a peak in Zn reaching 1870 mg kg<sup>-1</sup> at 90 cm depth of the SFC delta core. The majority of Cd and Zn concentrations were available in geoavailable forms (1<sup>st</sup>-4<sup>th</sup> fraction) for both cores. The main phase for Cd was carbonate fraction for the SFC delta core. Meanwhile, the Marambaia Bay contains reducible oxides as a secondary phase for Cd and Zn. Zinc concentrations prevailed as reducible oxyhydroxides down to 40 cm depth at the SFC Delta core, below this depth towards the core's base the carbonate phase is the main phase for this element. These selective extraction results suggest that Cd and Zn are present in environmentally available forms in both cores.

**SP134 Relevamiento de pasivos ambientales de estaciones de servicio abandonadas en el Partido de Lanús.**

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Durante el transcurso de la última década, se ha producido el cese de actividad de 3.000 estaciones de servicio en Argentina, siendo el Área Metropolitana de Buenos Aires la zona más afectada. Dentro de los 49,35 km<sup>2</sup> que abarca el Partido Lanús, se estima que existen 60 estaciones de servicio que han sido abandonadas, reconvertidas para otro uso sin recibir previamente el tratamiento adecuado o que están actualmente en funcionamiento pero que presentan situaciones de fuga o filtraciones. En general estas instalaciones tienen entre 6 y 10 tanques soterrados de combustibles, los que pueden tener un volumen variable entre 10000 y 30000 litros cada uno. La falta de implementación de las medidas de control y seguimiento que requieren estas áreas una vez que dejan de estar operativas implica un riesgo para la población; la cual se encuentra expuesta a los peligros de explosividad y toxicidad, generados por los compuestos orgánicos volátiles y los combustibles almacenados. Las posibles fugas además constituyen una amenaza para la población por tratarse de NAPLs, dado que el nivel piezométrico se encuentra a 3,5 m, y varía después de lluvias copiosas produciendo anegamientos que favorece la fugacidad de los mismos. La carencia de información respecto al estado actual de las estaciones comprendidas en el área bajo estudio, y la ausencia de la implementación del tratamiento pertinente motivaron que el objetivo de este trabajo fuera localizar los posibles pasivos ambientales de las

estaciones de servicio cerradas, abandonadas o inactivas en el territorio de Lanús. A tal fin, se llevó a cabo el relevamiento espacial de las estaciones existentes, la verificación de pérdidas e identificación del grado de mitigación o remediación que presentan las áreas en las que se desarrolló la actividad. Como resultado, se confeccionó el mapa del estado de las estaciones de servicio en el Partido de Lanús; categorizando a las mismas en función del grado de mitigación o remediación. A partir de la información elaborada se procedió al reconocimiento de los pasivos ambientales para su posterior análisis con el objeto de definir cuales constituyen sitios peligrosos y definir los mecanismos de monitoreo adecuado. Se espera establecer nuevos lineamientos de procedimientos adecuados para garantizar una correcta gestión de los mismos. Key Words: Tanques soterrados-Mapas de riesgos-Sitios peligrosos-Gestión Ambiental Urbana.

#### SP135 Respostas Toxicológicas com Amostras de Sedimentos de um Complexo Portuário no Brasil, Costa Sudeste. A Escolha do Organismo Bentônico

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A demanda de ensaios ecotoxicológicos com sedimento tem aumentado devido a obras de regiões costeiras e o descarte de efluentes no meio marinho, que podem acumular contaminantes nos sedimentos. A incerteza quanto ao significado ecológico da contaminação de sedimentos em comunidades bentônicas levou ao desenvolvimento de métodos para testar a toxicidade dos sedimentos de ambientes marinhos ou estuarinos e para atender as exigências da Resolução CONAMA 454, 2012. Ao redor do mundo, vários organismos aquáticos têm sido utilizados em ensaios ecotoxicológicos com sedimento, promovendo uma grande variabilidade experimental, sem que os laboratórios façam uma análise crítica da representabilidade ambiental do organismo a ser utilizado no ensaio. O objetivo deste estudo foi comparar a resposta ecotoxicológica de um copépodo e dois anfípodos em amostras de sedimento total coletadas em uma área de descarte de material dragado. No total foram realizadas três campanhas de amostragem, uma no ano de 2014 e outras duas no ano de 2015, onde foram estudados quatro pontos amostrais (P1, P2, P3 e P4), vale ressaltar que o ponto P01 fica em uma Área de Preservação Ambiental. Foi realizada a classificação granulométrica, análises físicas e químicas e ensaios ecotoxicológicos qualitativos crônicos e agudos com o organismo *Nitokra* sp. (copépodo), *Leptocheirus plumulosus* e *Grandidierella bonnieroides* (anfípodas). Os resultados físicos e químicos apresentaram valores abaixo do nível 1 e 2 da CONAMA 454, exceto para o arsênio, nas três campanhas, enquanto que a classificação granulométrica apresentou predominância da fração silte arenoso. Os resultados das análises ecotoxicológicas para o copépodo *Nitokra* sp. apresentaram ecotoxicidade para todos os pontos amostrais em todas as campanhas realizadas, enquanto que para os anfípodas *Leptocheirus plumulosus* e *Grandidierella bonnieroides* não apresentaram efeito tóxico, exceto para o ponto P2 para maioria dos pontos estudados. A partir do resultado coloca-se como hipótese que a resposta ecotoxicológica do organismo *Nitokra* sp. pode estar relacionada às características intrínsecas do sedimento e não a um indicativo de contaminação ambiental. Com base nestes resultados é possível ressaltar a importância de levar em consideração as particularidades experimentais exigidas pelos organismos teste, assim como uma análise crítica da área de estudo para que a escolha do organismo teste seja adequada a avaliação pretendida.

#### SP136 Sedimentary hydrocarbons as a tool for investigating colonization in Latin America: a case study of Recife, northeastern Brazil

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The Capibaribe River estuary flows through the city of Recife, Pernambuco State, northeastern Brazil. Prior to colonization (ca. 500 years ago), the area was composed of dense vegetation (Atlantic forest and mangrove) that was gradually replaced by sugarcane crops. In the late 19th century, sugarcane mills were transferred to the backlands of Pernambuco State and manufacturing plants (mainly textile) were settled in the vicinity of Capibaribe estuary. The economic development of Recife led to urban expansion and an exponential population growth in the 20th century. Currently, Capibaribe estuary harbors the Port of Recife, several marinas and tourism activities in addition to receiving wastewaters from the metropolitan area. In order to investigate the input of hydrocarbons to the Capibaribe estuary over the past centuries, a sediment core (150 cm deep) was collected in the lower estuary. The core was sliced every 2 cm for determination of aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs). The top 7 cm were discarded due to contamination during core subsampling. Local sedimentation rate was estimated to be 0.45 cm year<sup>-1</sup> using lead-210 dating. The sediment core used for determination of hydrocarbons covered a period from 1687 to 1994 AD. Total AHs ranged from 101 to 635 µg g<sup>-1</sup> dry weight

(dw) while total PAHs (sum of 16 priority PAHs) ranged from 162 to 690 ng g<sup>-1</sup> dw. AHs indicated a prevalence of natural, terrestrial sources (mangrove and Atlantic forest). PAHs were predominantly from pyrolytic sources. Combustion of biomass was the dominant source of PAHs to the estuary from the 17th to the 19th century. Local specific sources likely include sugarcane burning, deforestation, and wood/coal combusted as fuel in railroad transportation. In the 20th century, upon the onset of oil industry, there is also contribution from incomplete combustion of petroleum and its derivatives (kerosene, gasoline and diesel) associated mainly with vehicular emissions. The presence of unresolved complex mixtures (UCM) in the upper core (20th century) confirmed oil input to the local sediments. In the late 20th century, governmental programs for controlling carbon dioxide emissions from vehicle engines have reduced PAH concentrations in the estuarine sediments, highlighting the importance of government regulations issued for controlling pollution.

#### SP137 The Monitoring of the traffic vessels in the Rio de Janeiro Port area

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Today we live in a time of great global interdependency. National frontiers are losing their meaning, at least when considering commercial exchanges between nations. According to IMO, about 90% of world trade is seaborne. This intense ship traffic has produced a considerable amount of impacts on the marine environment. These impacts may be due to the introduction of invasive species, such as the so-called "sun coral" (*Tubastrea coccinea* and *T. tagusensis*), or pathogens such as the *Vibrio cholerae*. Besides, the use of biocides in antifouling paints is also damaging for marine communities. Every ship of more than 100 ton is registered in IMO, and should be compliant with the international regulations for the safety of life in the seas and of the marine environment. The study here presented is aimed at developing a simple, non-expensive methodology for monitoring the vessels in the Rio de Janeiro port. The presence of the vessels from non-signatory countries of the IMO convention A928 (Antifouling Convention 2001) was registered as well as the presence of vessels coming from risk areas for the occurrence of *V. cholera*, according to the WHO data. Ship data were provided by Marine Traffic, a free version of which was used. Shipping was monitored at the port from December 2015 to April 2017. Information such as IMO number register, origin Port, arrival date, and flag was compiled for a total of 2088 vessels. Those from non-signatory countries of the AFS Convention were 11,9% of the total ship movement. This observation is higher than expected, as only 6,45% of the global tonnage is registered in non-compliant countries. However, as Brazil, Uruguay and Chile are the only South American countries that follow the organotin ban, this observation could be explained. Some 468 ships (22,4%) came from possible risk areas in respect of *V. cholerae* introduction. The proposed method, besides being time-consuming to be applied, has showed to be able to indicate the possibility of environmental problems related to marine traffic even before the ships arrival.

#### SP138 Trace elements in feathers and blood of Antarctic seabirds

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Heavy metals cause great concern mainly due to their toxicity and potential for anthropogenic increase in bioavailability. Seabirds are used as sentinels of environmental contamination because they have great longevity and occupy high trophic positions. Antarctic penguins are present in the southernmost continent all year round. Skuas are migratory, breeding during austral summer in Antarctica and migrating away from that continent in winter. Blood and feathers were used for evaluating the exposure of *Pygoscelis papua* (Ppa, n=25), *P. antarcticus* (Pan, n=34), *P. adeliae* (Pad, n=10) and *Catharacta maccormicki* (Cma, n=23) to Fe, Mn, Zn, Cu, Cd, Sn, Se and Pb. Samples were collected from Admiralty Bay, King George Island, Antarctic Peninsula, in 2012-2013 and 2013-2014 summers. Analyses were performed using an ICP-MS, Perkin Elmer Elan 9000. Regarding blood samples, a significant year-to-year increase in Fe, Mn and Se concentrations was found for Cma, as well as in Pb, Sn and Cd for Pan. Concerning Cd, Zn and Cu, concentrations were apparently higher in penguins in the present study than in previous investigations on the same island. For feathers, Fe, Mn, Zn and Cd concentrations were significantly higher in Cma than in penguins. Significant positive correlations between metal concentrations and morphometric measurements (related to bird growth) were found for Cd in Ppa



and Pan, as well as for Cu and Fe in Cma. Cma migrates to a more polluted area (Northern Hemisphere) during austral winter, which may explain the highest concentrations in this species. The positive correlation between metal levels and morphometric data seems to be a reflection of the bioaccumulation process. The year-to-year increase in trace-element (TE) concentrations may be related to an intensification of touristic and scientific activities in Antarctica, in addition to a global scale increase in TE availability; however, studies presenting a sampling design appropriate to the evaluation of possible temporal variations should shed further light on these findings.

**SP139 Urea as indicator of urban sewage input in coastal waters considering salinity variation in drainage channels to the beach, Santos, São Paulo-Brazil**

H. Dias, Instituto Oceanográfico - Universidade de São Paulo / Oceanografia Física, Química e Geológica; B. Sutti, Instituto Oceanográfico da Universidade de São Paulo; V.G. Chiozzini, E.d. Braga, Instituto Oceanográfico Universidade de São Paulo / DOF Urea is the main excretion product of the nitrogen metabolism in mammals and the human being is an effective introducer of this compound in urban aquatic systems by sewages. Urea decomposition in ammonium contributes to the relation between them in waters with different salinities, what takes importance on the evaluation of the urea as indicator of water quality parameter in the coastal environment. The Santos city historically presented problems of inundation associated to health cases, to solve this problem seven rain drainage channels (from city to beaches) were constructed. In function of the demographic increase in the last decades, unlawful wastewater links reach the channels. Nowadays, contamination in the rain drainage channels is continued compromising the beaches and coastal water quality. To evaluating the presence of urea as sewage marker in water body with different salinities, water samples were obtained from internal and external part in three of the seven drainage channels (C1, C3 and C6) floodgates and in the sea near the beaches. To a complete understanding of urea role in this aquatic system, the N-forms of dissolved inorganic nitrogen (ammonium, nitrite, and nitrate) associated to basic hydrological parameters (oxygen, temperature, salinity and pH) were measured. The salinity results showed that freshwater (sal. < 0,5) prevailed in the C1, both in the internal (0.15) and in the external (0.15) part, whereas in the C3 (14.01) and C6 (30.08) occurred a distinction between water bodies, confirming marine influence mainly in the C6 and in the internal ones (0.22 and 5.09, respectively), there were higher predominance of freshwater demonstrating the efficiency of the floodgates in water bodies isolation. Under these conditions, the higher urea values in the internal parts (9.1 to 14.2  $\mu\text{M}$  N-urea) in comparison to observed in the external parts (1.9 - 6,8  $\mu\text{M}$  N-urea) evidenced the continued sewage inputs in the internal parts of rain drainage channel and the potential coastal pollution source. In addition, the ammonium concentration in the same locals reached values considerably higher than those of urea, ranging from 7.6  $\mu\text{M}$  N-NH<sub>4</sub><sup>+</sup> (C3-external) to 49.9  $\mu\text{M}$  N-NH<sub>4</sub><sup>+</sup> (C1-internal), confirming an origin associated to both urea and organic matter degradation. The urea-ammonium relation is a potential sewage markers of contamination in urban and coastal waters.

**SP140 Vulnerability and environmental risk associated with flooding in coastal cities of La Plata River, Buenos Aires, Argentina.**

V. Arias, NATIONAL UNIVERSITY OF LANUS; M. Di Stefano Fernández, A. Fonseca, NATIONAL UNIVERSITY OF LANUS / Department of Productive Development and Technology; m.j. garcia, ISA / UMR Equipe Traces rue de la Doua Villeurbanne France; S.O. Demichelis, NATIONAL UNIVERSITY OF LANUS / Productive Development and Technology

There is a significant proportion of the population living in flooding areas of the La Plata river coast. The risk of flooding is exacerbated by the vulnerability of the population as these sites lack of basic facilities such as tap water supply, sewage and waste collection. Floods in coastal cities are mainly caused by "sudestadas", which frequency and severity are increasing due to climate change. The aim of the project is to develop management tools that tend to reduce risks during and after extreme events that generate floods and thus wastewater spill over. In this work the cities of Quilmes, Berazategui, Ensenada, Berisso, Magdalena and Punta Indio were studied. In which coastal urban areas in risk were delimited based on census radius, from the 2010 Census (INDEC-REDATAM). The delimited areas were characterized by territorial, population and extreme events data from statistical sources, background studies and newspaper articles. Health, climate, and socioeconomic variables were analyzed from variables such as NBI; Water courses; Level quotas; Origin of tap water; Quality of connections to basic services; Domestic drains. In the preliminary analysis, it has been observed that the areas analyzed show great differences in the degree of urbanization of flooded areas, access to basic services, employment and educational level. The results shows that the coastal area of Quilmes is the most vulnerable area, due to its geomorphologic characteristics that exacerbate and make more frequent the

phenomenon of chronic inundations post floods, fact that determines a greater number of evacuees. Finally, there were selected two riverside sites of Quilmes, where currently it has been carrying out post-event waterlogged water analysis, surveys of neighbors and officials in order to evaluate the health risk and estimate the damages and economic losses by floods. As a result of this research, it is desired to develop tools such as protocols for action before, during and post-events, risk assessments, and land-use planning guidelines to achieve comprehensive flood management in coastal areas. \n

**Late Poster Only Session**

**SP141 Effects of flow rate on Ethylene Vinyl Acetate (EVA) passive sampler performance**

F. Tucca, Universidad de concepcion / Facultad de Ciencias Ambientales Eula; D. Cardenas, Universidad de concepcion / Facultad de Farmacia; E. Osorio, Universidad de concepcion / Facultad de Ingeniería Agrícola; C. Pérez, Universidad de concepcion / Facultad de Ciencias Ambientales Eula; J.L. Arumí, Universidad de concepcion / Facultad de Ingeniería Agrícola; R. Barra, Universidad de concepcion / Facultad de Ciencias Ambientales Eula

Passive sampler devices (PSDs) in water play a key role as monitoring tool to assess the exposure of contaminants. The spatial-temporal integrative information with PSDs deployed in the field, may give a useful method for the detection of water bioavailable priority contaminants, as well as contaminants of emerging concern. However, PSDs in kinetic phase are often affected for diverse environmental factors during its exposure in water, acting on the sampling parameters. Ethylene vinyl acetate (EVA) polymer was used for determining the occurrence of agrochemicals in the Central Valley of Biobío region, Chile (36°S; 72°W). A calibration system for EVA devices ( $V_{EVA} = 0.17 \pm 0.03$  mL; thickness =  $7.0 \pm 1.0$   $\mu\text{m}$ ) was designed to determine the sampling rate ( $R_s$ ) and sampler-water partition coefficient ( $K_{sw}$ ) through both low (0.15 - 0.30  $\text{m s}^{-1}$ ) and high water flow (0.50 - 0.70  $\text{m s}^{-1}$ ). EVA devices were spiked with twenty organochlorine pesticides (OCPs). Later, PSDs were deployed in the field (Chillan river, Central Valley of Biobío Region) to determinate the bioavailability of OCPs in freshwater. The Central Valley of Biobío region is characterized for an intensive agricultural activity. During calibration, EVA showed a higher sampling rate when it was exposed to higher water flows. A plot constructed between log  $K_{sw}$  and log  $K_{ow}$  reported a good correlation to define log  $K_{EVA-water}$  ( $R^2 = 0.8 - 0.9$ ). PSDs deployed in Chillan River detected thirteen OCPs, which ranged between 1.0 and 22.0  $\text{ng L}^{-1}$ . Here the higher detected concentrations were endosulfan sulfate and endosulfan II with 22.1 ( $\pm 2.7$ ) and 16.6 ( $\pm 0.9$ )  $\text{ng L}^{-1}$ , respectively. OCPs concentrations were in agreement to detected levels in the Central Valley of Biobío region through grab water sampling. In conclusion, the EVA passive sampler can be a powerful tool to detect trace concentrations of hydrophobic chemicals in fluvial systems released from non point sources. Acknowledgements: CONICYT/FONDAP N° 15130015 (CRHIAM). \n \n

**SP142 Persistent organic pollutants and molecular biomarkers in cetaceans from south Pacific and antarctic peninsula.**

R. Sanchez, Universidad de Concepcion; G. Chiang, MERI Fundation / Biology; F. Tucca, Universidad de concepcion / Facultad de Ciencias Ambientales Eula; S. Jara, Universidad de concepcion; H. Moya, Facultad de Ciencias Ambientales/Centro EULA, Universidad de Concepción / Sistemas Acuáticos; C. Aguayo, R. Barra, Universidad de concepcion

The Persistent Organic Pollutants (POPs) are a global environmental problem because of their extraordinary toxicity, persistence and mobility in the environment. They tend to accumulate in fatty tissues due to their usually high  $K_{ow}$ . These compounds not only bioaccumulate in organisms but also biomagnify through the food web, reaching high concentrations in top predators, so it's possible find them in high concentrations in marine mammals populations. Additionally, POPs acts as endocrine disruptors by changing hormonal signals in organisms that can alter their physiological behavior. Cetaceans have a long life besides having large lipid reserves in relation to their size and occupy the higher level in the food web. Therefore the aim of this study was to evaluate a set of biomarkers aimed to assess the level of toxicological stress caused by the accumulation of POPs in cetaceans throughout non lethal methods. Biopsia skin samples showed high concentrations of polychlorinated biphenyls (PCBs) that were detected in *Grampus griseus* and *Balaenoptera bonaerensis*, being the most frequent congener in these Antarctic species PCB-28, while on the samples taken in patagonia the heavier congeners PCB-138, PCB-153 and PCB-180, were more often observed. The specie that accumulated the highest concentrations of organochlorine Pesticides (OPs) was *Megaptera novaeangliae*, being  $\beta$ -HCH the isomer that presented a greater accumulation. High concentrations of Endosulfan sulfare were detected in Antarctic

species. Toxicological stress evidence was observed by overexpression of biomarkers HSP70 and E2F-1. No clear evidence was found that cetaceans are suffering endocrine disruption, because the expression of ER $\alpha$  and AhR was stable. *Megaptera novaeangliae* presented a negative correlation in the expression of CYP1A with  $\Sigma$ log organochlorine. This work was supported by INACH T31-11 and FONDAP CRHIAM 1513005.

#### SP143 Aquatic Risk Assessment Scheme for Pesticides in Brazil

D.M. Máximo, IBAMA / COASP; R. Rebelo, IBAMA / CCONP; F. Aviz, IBAMA; R. Souza, IBAMA / CSR; A. Waichman, UFAM; R.P. SCORZA JUNIOR, Embrapa / EMBRAPA AGROPECUARIA OESTE; G. Umbuzeiro, SCHOOL OF TECHNOLOGY - UNICAMP / LEAL; R. Oliveira, University of Brasília / Department of Genetics and Morphology; A. Tornisiolo, BASF; L. Murakami, Bayer AG Crop Science Division; X. Patino, Bayer CropScience  
Since 2010, the Brazilian Institute for the Environment and Renewable Natural Resources – IBAMA has been working to implement an environmental risk assessment framework for pesticide registrations. In 2014 IBAMA officialised the intention to initiate a cooperation project with the formation of tripartite Technical Working Groups (government, academia and industry). In 2015 the Aquatic Technical working Group engaged in activities to develop a risk assessment scheme for aquatic organisms. The first step of the process involved the definition of the General Protection Goals (GPG). To do so, Brazilian legislation related to the protection of aquatic environments was surveyed and the GPG was defined as: “to protect the water bodies to guarantee the aquatic ecosystem sustainability and to maintain the aquatic organisms, the water supply for the animals and the economical, recreational and subsistence activities”. Additional work has been made to establish Specific Protection Goals (SPGs), and the definition of the ecotoxicological tests that will be required at each phase of the risk assessment. In addition, the working group is discussing whether there is the need of additional safety factors based on a comparison of available acute toxicity studies of pesticides in local species versus results obtained with standard species. Moreover, on the exposure side, the working group has proposed a statistically and geographical approach to assess the vulnerability of surface water to pesticides and therefore support the definition of the exposure scenarios. As a conclusion, with this presentation we intend to show the main outcomes and deliveries of the working group for aquatic risk assessment of pesticides in Brazil.

#### SP144 Population trends and neonicotinoid insecticide concentrations in hummingbirds

C.A. Bishop, Environment and Climate Change Canada / Wildlife Research Division Science and Technology Branch; A.J. Moran, Rocky Point Bird Observatory / The Hummingbird Project; M. Toshack, Simon Fraser University / Biological Science; S. WILSON, Environment and Climate Change Canada; E. ELLE, Simon Fraser University / Biological Sciences Department; A. SMITH, Environment and Climate Change Canada / Canadian Wildlife Service; J.E. Elliott, Environment Canada / Science Technology Branch

There are 14 species of hummingbirds occurring in North America, and among the seven with reliable data, three species are showing significant declines in populations as indicated by analysis of the USGS North American Breeding Bird Survey BBS. The steepest negative trends annually are for pacific coastal Rufous Hummingbirds and Allen’s Hummingbirds although Broad-tailed Hummingbirds are also declining. There may be multiple factors associated with those declines and stressors may act at different life stages and locales throughout the year as hummingbirds migrate, breed and overwinter. We examined neonicotinoid pesticide exposure in hummingbirds in the core of Rufous Hummingbird range. In the Fraser Valley of southern British Columbia, we quantified hummingbird presence and use of blueberry fields that were conventionally sprayed or organic and compared them to natural areas and found hummingbirds were commonly present in hedgerows of farms. Neonicotinoid insecticide concentrations were detected in blueberry flowers a year post spray and detected at 3 ng/g in pooled samples of urine of Rufous and Anna’s hummingbirds collected within 0.5 to 1 km of conventionally- sprayed blueberry fields.

#### SP145 Níveis de contaminantes orgânicos e inorgânicos que impactam o ambiente aquático nas três regiões hidrográficas do Rio Grande do Sul

P.G. Costa, FURG / Escola de química e alimentos; G.M. Souza, I.S. Anni, FURG / ICB; R.D. Klein, Universidade Federal do Rio Grande FURG / ICB; S.C. Faria, Universidade Federal do Rio Grande FURG / Instituto de Ciências Biológicas; C.S. de Oliveira, J.L. Dal Pizzol, T.G. Zocke, J.B. Machado, B.D. Righi, FURG / ICB; A. Venquiaruti Escarrone, FURG- Universidade Federal do Rio Grande / ICB; A. Bianchini, Universidade Federal do Rio Grande - FURG / Instituto de Ciências Biológicas

Bacias hidrográficas são importantes zonas de reprodução e crescimento para muitas espécies de peixes e invertebrados. Desta forma, a poluição dos recursos hídricos traz sérias consequências tanto econômicas (redução da produção pesqueira) quanto ecológicas (diminuição da densidade e diversidade biológicas). Além disso, muitos contaminantes são transferidos e acumulados ao longo das cadeias alimentares, ameaçando também de forma indireta a saúde de seus consumidores, que podem ser tanto organismos aquáticos quanto seres humanos. Com o intuito de avaliar a qualidade dos recursos hídricos nas três regiões hidrográficas do Rio Grande do Sul (Guaíba, Litoral e Uruguai), foram realizadas coletas sazonais (inverno 2015 e verão de 2016) de amostras de água, plâncton e biota (invertebrados e vertebrados) em 9 municípios (Santa Isabel, Santa Vitória do Palmar, Cristal, Lavras do Sul, Caraá, São Leopoldo, São Pedro do Sul, Alegrete e Uruguaiana), que contemplam estas regiões hidrográficas. Em campo, foram determinados vários parâmetros físico-químicos da água (pH, temperatura, concentração de O<sub>2</sub> dissolvido e salinidade). Em laboratório, foram determinadas as concentrações de metais (Ag, Cu, Zn, Cd, Fe, Ni e Pb), íons (K, Na, Mg e Ca) e carbono orgânico nas amostras de água, visando a aplicação do modelo do ligante biótico para avaliar a biodisponibilidade e toxicidade dos metais. Nas amostras de biota, foram determinadas as concentrações de metais, HPAs (16 prioritários) e praguicidas. Os níveis de metais nas amostras de água de alguns corpos d’água avaliados estavam próximos aos limites estabelecidos pela Resolução CONAMA 357/2005. No entanto, a aplicação do BLM indicou que os ambientes avaliados estavam aptos a suportar o aporte de metais recebido, sem apresentarem risco à biota aquática local. Nas amostras de biota, Cd, Cu e Pb foram os metais mais representativos, em termos de efeitos biológicos. Por sua vez, endossulfan, HCHs e HPA de baixo peso molecular (2-3 anéis aromáticos) foram os contaminantes orgânicos mais representativos, em termos de efeitos biológicos. Quanto aos pesticidas fosforados, estes não foram detectados nas amostras de biota (< LQ<sub>m</sub>). Palavras-chave: Metais, BLM, Pesticidas clorados, Pesticidas fosforados, HPA.

#### SP146 Ecotoxicological comparison between industrial waste recovered and commercial iron oxide nanoparticles

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Iron oxides nanoparticles (NPs) recovered from acid mine drainage (AMD) represent a potential raw material for the low cost production of iron oxides with a high degree of purity to be used as catalysts for organic pollutant degradation processes through ozonation. Thus, the toxicity of iron oxides NPs needs to be determined in order to predict their impact on the environment. In this context, the objective of this study was to evaluate the ecotoxicity of iron oxides produced from AMD and commercial hematite. Four bioassays widely used in NPs toxicity tests were applied in this study: *Vibrio fischeri*, *Artemia* sp., *Lactuca sativa* L. and *Allium cepa* L.. The materials were characterized by XRD, BET surface area, porosity and TEM. Microtox data showed no significant differences in toxicity, indicating some stability of the NPs. For all samples the GL values were < 10, indicating low or no toxicity. And, like the toxicity data with *vibrio fischeri*, the others tests corroborated with this result, showing low or no toxicity of these nanoparticles in the evaluated trophic levels. These results provide that the iron oxides NPs recovered from AMD are cheaper, environmentally friendly and its use as catalysts is no more toxic when compared with the commercial hematite.

#### SP147 PERSISTENT ORGANIC POLLUTANTS AND DNA DAMAGE IN EARTHWORMS IN LOW BASIN OF COATZACOALCOS RIVER, MEXICO

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The low basin of the Coatzacoalcos River is located south of the State of Veracruz, Mexico. Is one of the largest industrial zones in the country and Latin America, is a malaria endemic area, and a natural flood plain. All these activities have generated various types of pollutants in this area. These include Persistent Organic Pollutants (POPs) that represent potential risk to the ecosystems due to their high toxicity, persistence and biomagnification through food chains. Effects can be evaluated in wild biomonitors by using biomarkers of exposure and effect. The main objective of this study was to evaluate the exposure of POPs and genotoxicity in wild earthworms captured in different scenarios of the Lower Rio Coatzacoalcos (industrial, urban and rural). Analysis of persistent organic pollutants in soil and tissue was performed by Gas-Mass Chromatography and DNA damage was evaluated by the comet assay. The results showed that there is a larger concentration of persistent organic pollutants in

soil and earthworms of the industrial zone, followed by the urban and rural areas. The highest damage to DNA was registered in the industrial area, followed by urban and rural areas. This evidenced the presence of soil contamination in the region and that they are bioavailable and generate potential risk effects for health of the organisms that inhabit the surface soil (soil life). This work provides evidence of the potential ecological risk that exists in the Lower Basin of the Coatzacoalcos River.

**SP148 Avoidance behavior tests showing the joint effects of pyraclostrobin (fungicide), fomesafen (herbicide) and adjuvant in natural soil**

J. Schmitt, V. Milcheski, F. Bratti, D. Furlan, G.F. Tomio, F.B. de Santo, UFSC Curitiba; N. Guerra, Federal University of Santa Catarina; J. Niemeyer, UFSC Brazilian's use of agrochemicals reached more than half million tons and is growing up, evolving rapidly as far as agricultural frontiers enlarge and new areas become part of this scenario. Pyraclostrobin is an active ingredient with a large spectrum of antifungal action belonging to the strobilurin group. Acting as a post emergence herbicide for early control in broad-leaved weeds in bean and soybean crops, the active ingredient fomesafen is included on diphenyl-ether chemical group which inhibits the action of protoporphyrinogen oxidase (PROTOX) enzyme. To increase agrochemical's contact with plant surface it is necessary adjuvant use, such as mineral oil. In Brazil it is common to use mixtures of these products in an only application. Ecotoxicological studies regarding their effects to soil fauna are nearly nonexistent, especially when mixtures of these products are used. Thus, this study aimed to evaluate the ecotoxicity of pyraclostrobin (P), fomesafen (F), an adjuvant (A) isolated or associated and their effects to soil fauna in avoidance behavior tests, using the earthworm specie *Eisenia andrei* Bouché 1972. The formulated products Comet® (pyraclostrobin 200g L<sup>-1</sup>), Flex® (fomesafen 250g L<sup>-1</sup>), and Nimbus® (adjuvant, type mineral oil 50mL L<sup>-1</sup>). Natural soil without application was used as control (Co). Tests followed ISO 17512-1 (2011) guideline. Seven combinations were evaluated (Co x P; Co x F; Co x A; Co x F+A; Co x P+F; Co x P+F+A; Co x Co) and applied doses followed products' field recommendations to bean. Agrochemical applications were made using costal sprayer pressurized at CO<sub>2</sub>, on a field soil classified as haplic cambisol (2.2% of organic matter and pH 5.4), placed on boxes. After 48 h exposure, the mean number of organisms was recorded for each trial. Results were analyzed by Fisher's exact test (p<0.05). Significant avoidance behavior was observed only for Co x P combination. Antagonistic effects were observed in Co x P+F+A combination when herbicide, fungicide and adjuvant were associated but non-avoidance was observed. Results showed that earthworms are more sensitive to fungicide than to herbicide with non-interference of adjuvant. Further studies must embrace chronic tests in order to evaluate effects on reproduction of this organism.

**SP149 Ensaios ecotoxicológicos com fauna edáfica para avaliação da contaminação com resíduos de indústria de papelão**

M. Silva, Federal University of Santa Catarina; G. Fockink, Universidade Federal de Santa Catarina (UFSC) / Campus de Curitiba; Grosskopf, C. Novicki, E. Schabatoski, E. kuhlsler Junior, G. Dalla Costa, V.L. Fischer, UFSC Curitiba; J. Niemeyer, UFSC Alguns organismos edáficos tem grande importância na avaliação da qualidade dos solos. São fundamentais quando se trata de decomposição da matéria orgânica, ciclagem de nutrientes e microporosidade do solo, sendo saprófagos comuns na maioria dos solos brasileiros. Entre esses organismos encontram-se as minhocas e enquitreídeos (Oligochaeta), e colêmbolos (Collembola). A disposição de resíduos de indústrias de papelão em áreas florestais é uma prática comum no Brasil, mesmo não sabendo os seus efeitos adversos sobre a biota. O objetivo deste trabalho foi avaliar o efeito da disposição de resíduo de duas indústrias de papelão em solo de plantio de *Pinus* sp. sobre o comportamento de fuga da macro e mesofauna edáfica. Os resíduos foram originados da produção de papelão utilizando aparas de papel, oriundos do processo final da ETE (lodo). Os ensaios foram baseados nas normas ISO 17512-1 e ISO 17512-2 para minhocas *Eisenia andrei* e colêmbolos *Folsomia candida*, e o ensaio de fuga com enquitreídeos *Enchytraeus crypticus* foi baseado na norma ISO 17512-1, porém realizado em placa de petri. O tempo de leitura foi após 72 h. Como controle, foi usado o solo artificial tropical (70% areia, 20% caulim, 10% pó de casca de coco). As combinações testadas foram Controle x Resíduo 1 (R1) e Controle x Resíduo 2 (R2), com cinco réplicas para cada combinação. Os dados foram avaliados usando o teste exato de Fisher (p<0,05). Ao final do tempo de exposição, a maioria dos organismos encontrava-se nos resíduos, não apresentando comportamento de fuga. Porém, no ensaio com enquitreídeos, houve letalidade de organismos acima do aceito para o ensaio (>10%), o que pode indicar letalidade destes organismos em contato com os resíduos. Estes resultados indicam a importância de seguir-se com as avaliações de exposição crônica, uma vez que as populações de macro e mesofauna buscam alimentar-se destes materiais.

**SP150 Endocrine disruption: Collaboration between mammalian toxicology and ecotoxicology needed!**

J. Wheeler, R.M. Green, Dow Agrosciences Endocrine disrupting properties require specific evaluation under several legislations globally. The development of specific criteria to "identify endocrine disrupting properties" is underway to enable hazard-based regulation in the European Union. Whilst in the United States and Japan, scientific, risk-based approaches are being developed. Both approaches require assessments for human health and the environment (wildlife). Regardless of the regulatory process, most geographies use the World Health Organisation International Programme on Chemical Safety definition of an endocrine disrupter or variants thereof. This requires the identification of an adverse effect, underlying endocrine mechanism and establishing a plausible link between the two. The environmental and human health approaches differ according their respective protection goals. Either at the individual or population level. Ecotoxicologists accept some individual level effects as long as population stability and recruitment are not impacted. Such determinations rely on biological, statistical, design and potentially ecological modelling for sound interpretation. This can often mean the same data may be interpreted very differently for the protection of individual human health compared to wild populations (e.g. wild mammals). It has also led to the development of many new screening and definitive tests in multiple taxa. These traditionally are outside of the focus human health being in non-mammalian models (fish, amphibians and birds). However, for endocrine disruption they have been shown to be good general models for specific activities (e.g. amphibian metamorphosis and thyroid activity) or particularly sensitive indicators in screening batteries (e.g. fish for steroidogenesis). Ecotoxicology has historically focused on adverse effects of concern (growth, development and reproduction) and less so on a mechanistic understanding. Consequently, the regulation of endocrine disruption based partly on establishing mechanisms requires a better understanding more in line with toxicological approaches. Considering the need to screen so many substances, the drive to higher throughput approaches and the conserved nature of the vertebrate endocrine system the need for collaboration between ecotoxicology and toxicology has never been greater.

**SP151 Are all chemicals endocrine disruptors?**

J. Wheeler, Dow Agrosciences; S. Marty, Dow Chemical Company; E.M. Mihaich, ER2; L.S. Ortego, Bayer CropScience / Environmental Toxicology and Risk Assessment; K.K. Coody, The Dow Chemical Company / Toxicology Environmental Research and Consulting; L. Zorrilla, Bayer CropScience LP Endocrine disrupting (ED) properties require evaluation under the European REACH regulation, as well as for regulation of plant protection and biocidal products. Specific criteria is under development in the EU to identify ED properties to enable hazard-based regulation, while in the US and Japan, risk-based approaches are being developed. Irrespective of the regulatory process, most geographies use the WHO IPCS definition, or variants thereof, requiring that a substance is demonstrated to cause a change in endocrine function that consequently leads to an adverse effect in an intact organism to identify it as an endocrine disruptor. Such a definition is broad and at its most cautious might capture many mechanisms that in general would not specifically be considered ED. For instance, stress is a non-specific, neuro-endocrine response that can lead to adverse outcomes. In addition, other toxic mechanisms (e.g. liver toxicity) may also secondarily impact the endocrine system and tissues. Furthermore, endocrine responses may be adaptive in nature, designed to maintain homeostasis, rather than inducing an irreversible adverse effect. Such factors should be considered when screening and testing substances for potential endocrine activity or disruption. Following the large scale screening of pesticides and pesticide inerts under the USEPA's Endocrine Disruptor Screening Program, practical experience with screening assays has highlighted some of these factors as important to data interpretation and study design. Clearly, the misidentification of indirect effects as truly ED has serious consequences in terms of triggering unnecessary higher testing (animal and resource intensive) and potentially severe regulatory consequences in the EU (removal from the market). A review of effects that could be misinterpreted as ED was undertaken using both practical experience with the assays and relevant, peer-reviewed, scientific literature. Focus was on the ecotoxicology screens (fish short term reproduction and amphibian metamorphosis) while drawing parallels to mammalian toxicology. This work demonstrates that without a suitable framework for interpreting study results, potentially all chemicals could be considered ED at some exposure level, if too broad a definition of endocrine disruption is used. This presentation highlights the problem of distinguishing non-endocrine from endocrine mechanisms of action when operating in a purely hazard-based regulatory environment.



#### SP152 Efecto de 5 mezclas de herbicidas en el crecimiento y la reproducción de *Eisenia foetida*

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El objetivo fue estudiar el efecto de glifosato, metsulfuron, atrazina, metolaclor, 2,4D, clopiralid, diclosulam, paraquat, pinoxaden +cloquintocet mexil a dosis recomendada a campo, y 10 veces esta dosis, así como las mezclas a las mayores dosis de glifosato y metsulfuron, atrazina y metolaclor, 2,4D y clopiralid, diclosulam y paraquat, y, pinoxaden +cloquintocet mexil y metsulfuron, sobre el crecimiento y la reproducción de *Eisenia foetida*. El experimento y las determinaciones se realizaron siguiendo la metodología propuesta en la norma OECD 2004. Para el análisis de las mezclas se utilizó la metodología propuesta por Colby (1967). El análisis de la varianza para la variable tasa de crecimiento relativo mostró efecto muy significativo de los tratamientos, siendo el control positivo menor a los demás tratamientos, como era esperable. Ningún tratamiento se diferenció del testigo sin tratar, excepto el 2.4 D a dosis alta, lo que resultó llamativo puesto que hace referencia a un efecto de promoción en el crecimiento. Respecto a las variables reproductivas, sólo se encontraron efectos significativos en el número de juveniles. El tratamiento pinoxaden+ cloquintocet mexil a dosis recomendada fue el único que determinó menor número de juveniles que el testigo (43% del valor registrado en el tratamiento sin herbicidas). Los restantes tratamientos resultaron con un comportamiento intermedio. El número de cocones eclosionados y totales no tuvieron efecto significativo de los tratamientos. Considerando la magnitud del impacto biológico de las diferencias, las 2 mezclas destacables fueron atrazina-metolaclor, en la que el efecto antagónico determinó menores reducciones a las esperadas y, diclosulam-paraquat, que resultando sinérgica presentó mayor impacto negativo en las lombrices que el esperado. El análisis indicó además ausencia de correlación entre la tasa de crecimiento relativa y las variables reproductivas enfatizando, tal como proponen otros autores, la importancia y necesidad de contemplar distintos biomarcadores en estudios de ecotoxicidad.

#### SP153 Efeitos reprodutivos do glifosato na mosca-da-fruta *Drosophila melanogaster*

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Pesticidas são substâncias químicas desenvolvidas para prevenir ou combater organismos indesejáveis, principalmente na agricultura. O herbicida que ocupa o topo do ranking de uso, no Brasil e no mundo, é o glifosato, que apesar de possuir um mecanismo de ação definidos, é capaz de causar efeitos em organismos não-alvo, como alterações reprodutivas e no balanço redox de diferentes animais. Com base nisso, foi investigado o efeito do glifosato sobre a taxa de mortalidade, dano lipídico e perímetro dos ovários em *Drosophila melanogaster*. As concentrações de glifosato utilizadas foram de 1,0, 2,0, 5,0 mg/L para o ensaio da mortalidade, dano lipídico (LPO) e perímetro dos ovários, onde nesse último teste houve a adição da concentração de 10,0 mg/L, nos tempos de 24 e 96h. O herbicida foi diluído em água Milli-Q e a exposição foi feita via alimentação. Foram utilizados 30 animais por frasco (15 fêmeas e 15 machos), n≥4. Para a mortalidade os animais foram observados diariamente. Para a LPO, os animais foram separados por sexo em pools de 15 animais e o protocolo empregado foi o método FOX. Com relação ao perímetro dos ovários, os mesmos foram retirados com o auxílio de pinças, fotografados em microscópio estereoscópico e analisados posteriormente no software ImageJ. A análise estatística foi feita por ANOVA (mortalidade 72 e 96h, LPO e perímetro dos ovários) e por teste não-paramétrico (mortalidade 24 e 48h). Os resultados mostraram que o glifosato não causou aumento significativo na mortalidade nem na peroxidação lipídica dos animais expostos ao glifosato. Dados prévios na literatura mostram que moscas expostas às mesmas concentrações de glifosato, só que através da formulação comercial Roundup Original, não é capaz de causar lipoperoxidação, tanto em 24 quanto em 96h. Porém com relação à análise reprodutiva, no presente estudo foi visto que o glifosato foi capaz de causar uma diminuição no perímetro dos ovários de fêmeas expostas às concentrações de 2,0 e 5,0 mg/L para o tempo de 24h. Resultados similares já foram vistos para outros animais, como peixes e mamíferos que apresentaram distúrbios no sistema reprodutivo quando expostos ao glifosato, a concentrações similares ou inferiores às utilizadas no presente estudo. Em moscas, a diminuição vista no perímetro dos ovários em 22% para a concentração de 2,0 mg/L pode representar um déficit reprodutivo, já que menos ovos conseguiriam ser produzidos e armazenados, podendo causar problemas na manutenção da espécie.

#### SP154 Swimming behavior as an early endpoint in sub-lethal metal exposures

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Contaminants considerably affect behavioral aspects of aquatic and terrestrial organisms. Copepod fitness may decrease when exposed to metals. Disturbances in copepod swimming can be used as an endpoint to evaluate environmental impacts caused by metal exposures. In this context, the aim of this study was to investigate behavioral effects of zinc sulfate (at concentration 3,50mg/L<sup>-1</sup>) on swimming pattern of female copepods (*Temora stylifera*) after 6-hour exposures to zinc. Swimming behavior was recorded using a tridimensional video-tracking system. Copepod trajectories provided the basis to evaluate the swimming behavior through the video analysis in software (Fiji Is just Image). Changes in velocities swimming were observed. Trajectories of exposed copepods had changes in complexity. The swimming state frequency of was compared in controls and exposed copepods (such as break, slow and fast swimming). These results suggest that swimming observations were a sensitive endpoint of sub-lethal effects on microcrustaceans. \n

#### SP155 Transcriptional responses of biotransformation enzymes coding genes in mantle of oysters *Crassostrea gasar* exposed to phenanthrene

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Polycyclic aromatic hydrocarbons (PAHs) are among the main contaminants in aquatic environments. Among the PAHs, phenanthrene (PHE) is one of the most abundant in marine environments. Besides damaging the ecosystem, PAHs can be bioaccumulated by organisms changing its homeostasis and inducing several defense systems to its elimination. Although the metabolic mechanisms are not elucidated, it is known that marine invertebrates are capable of to biotransform PHE. Bivalves such as oysters are filtering and sessile organisms, well distributed and frequently used as sentinel organisms in studies of aquatic contamination. In this context, this study evaluated the transcriptional responses of the biotransformation enzymes coding genes in the mantle of *Crassostrea gasar* (= *Crassostrea brasiliana*) exposed to PHE. Oyster *C. gasar* were exposed to two sublethal PHE concentrations (100 µg.L<sup>-1</sup> and 1000 µg.L<sup>-1</sup> PHE), during three periods of exposure (1, 5 and 10 days) and the mantle was collected for the quantification of transcript levels by qPCR. Upregulation were observed in the transcription of phase I and II biotransformation genes (*CYP17A1-like*, *CYP3A-like*, *CYP2A1* and *GSTπ-like*) especially in animals exposed to the concentration 1000 µg.L<sup>-1</sup> PHE. Gene-coding cytochrome P450 isoforms analyzed showed an increasing levels of transcription up to 5 days of exposure. After 10 days of exposure, only *CYP2A1* was upregulated approximately 1.5-fold in the group exposed to 1000 µg.L<sup>-1</sup>. The *GSTmicrosome-like* from animals exposed for 1 day to 100 µg.L<sup>-1</sup> PHE showed decreased transcript levels in relation to the control group. The results showed that even animals considered resistant to exposure to hydrocarbons, such as bivalves, when exposed to these contaminants, showed molecular responses highlighting the potential toxic effect of these compounds on the homeostasis of the animal. Interestingly, the data show that the mantle presents late responses to PHE exposure, when compared to other tissues analyzed in previous studies, such as in the gill and the digestive gland in this species. The mantle is a susceptible and responsive tissue to PHE in *C. gasar* oysters and may be proposed as a complementary organ in the evaluation of the toxic effects caused by exposure to hydrocarbons.

#### SP156 Detecção de staphylococcus multirresistentes em água e sedimentos do rio Itanhaém

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A ocupação desordenada de áreas costeiras gera impactos ambientais, dentre eles, o descarte de efluentes, bem como a presença de bactérias patogênicas resistentes. A resistência bacteriana é uma preocupação mundial, exigindo um controle sobre o uso e descarte de antibióticos, bem como monitoramento de cepas resistentes. Recentemente, houve um aumento de *Staphylococcus* sp. multirresistente, não apenas em amostras clínicas, mas também em amostras ambientais, sendo um grave problema de saúde pública. O presente estudo teve por objetivo avaliar a qualidade da água e sedimentos em amostras do Rio Itanhaém, verificar a presença de *Staphylococcus* sp. e o perfil de resistência das cepas isoladas. Amostras de água e sedimento foram coletadas em 6 pontos localizados ao longo do Rio Itanhaém. Amostras de água foram acondicionadas em frascos plásticos estéreis, e as de sedimento em sacos plásticos estéreis, mantidos sob refrigeração, até seu processamento no Laboratório de

Microbiologia Marinha (UNESP CLP). Para a remoção de microrganismos aderidos ao sedimento, 20g de sedimento foram adicionadas à 180 mL de água destilada estéril, e agitadas em agitador Kline por 10 minutos duas vezes. Foi empregada a técnica da Membrana Filtrante, utilizando membranas de 0,45  $\mu\text{m}$  de porosidade. Os volumes filtrados para amostra de água foram 10, 25 e 50 mL, e de 1 e 5 mL para amostras de sedimento. O meio Ágar mTec foi empregado para a enumeração de *E. coli*, e Ágar mEnterococcus para a enumeração de *Enterococcus* sp. A detecção e isolamento de *Staphylococcus* deu-se por Spread Plate em Ágar Manitol. Cepas características foram isoladas e confirmadas por bioquímica. O antibiograma deu-se por meio do Método de Disco-Difusão (Kirby-Bauer) e os antibióticos testados foram: Amoxicilina + Ácido Clavulânico (AMC), Cefoxitina (CFX), Cefalotina (CFL), Clindamicina (CLI), Ciprofloxacina (CIP), Eritromicina (ERI), Gentamicina (GEN), Sulfazotrim (SUT) e Vancomicina (VAN). As análises microbiológicas mostraram alterações nos padrões de qualidade da água (CONAMA 274/2000), especialmente para *E. coli*, onde temos nos pontos 2: 8000 UFC mL<sup>-1</sup>; 3:2200 UFC mL<sup>-1</sup> e 4:3300 UFC mL<sup>-1</sup>. O ponto P2 apresentou valores em desacordo com a CONAMA 357/2005, e presença de cepas resistentes à GEN, SUT, CIP, CLI e VAN, influenciado, provavelmente, pela disposição de efluente pela ETE. A deposição do efluente da ETE pode alterar a qualidade da água, podendo ser um ponto de dispersão de organismos resistentes.

**SP157 Phytotoxicity and ecotoxicity of crude extract of *Microcystis aeruginosa* V.d. de Paula**, Federal Technological University of Paraná / Academic Department of Chemistry and Biology (DAQBi); J. Micheletto, R.D. Carneiro, M. Soares, L. Martins, A. Freitas, Federal Technological University of Paraná UTFPR / Academic Department of Chemistry and Biology DAQBi

Cyanobacterial blooms caused by the species *Microcystis aeruginosa*, producer of microcystin-LR toxin, occur frequently in eutrophic water bodies, can cause damage to aquatic organisms and terrestrial plants. The objective of this work was to evaluate acute phytotoxicity in *Lactuca sativa* (lettuce) and *Sinapis alba* (mustard), acute ecotoxicity in *Daphnia magna* and chronic in *Desmodemus subspicatus* of crude extract of *M. aeruginosa*, obtained from laboratory culture. In phytotoxicity assays, germination and root growth were evaluated, and CO<sub>2</sub> production was measured by the respirometric method. Acute toxicity tests with *D. magna* and chronic with *D. subspicatus* were performed according to ABNT standards 12713:2016 and 12648:2011, respectively. The crude extract of *M. aeruginosa* significantly inhibited mustard rootlets growth with  $p < 0.001$  in the Student's T-Test, this response may be related to inhibition of plant hormones that regulate initial development. While for lettuce seeds, there was no inhibition in root elongation, possibly linked to oxidation of microcystin-LR or other secondary metabolites by detoxifying enzymes. In respirometry, the inhibition for initial development in mustard was confirmed with lower CO<sub>2</sub> production compared to negative control. In lettuce it was observed a change in the respiratory rate, evidencing physiological effects in its development. In *D. magna* the crude extract of *M. aeruginosa* was toxic only in its pure form, causing 76% immobility. For *D. subspicatus* there was inhibition of growth in dilutions of 50% and 25% of extract.

**SP158 Efeito do efluente contaminado com compostos nitroaromáticos no teor de pigmentos de *Lemna aequinoctialis* Welw.**

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Sabe-se que desde o século XIX os explosivos fundamentados quimicamente em compostos nitroaromáticos são produzidos, testados e usados de maneira inadequada, causando assim, a contaminação de solos e corpos d'água. No Brasil a produção de explosivos concentra-se nos estados de São Paulo, Paraná e Minas Gerais e até o presente momento, não há limites restritivos na Legislação Brasileira para estes compostos. Seguindo essa premissa, o presente trabalho objetivou avaliar o efeito do efluente de indústria de explosivos (água vermelha) no teor de pigmentos (clorofila e carotenoides) da planta aquática *Lemna aequinoctialis* Welw. (lentilha d'água). Foram realizados ensaios ecotoxicológicos com a lentilha d'água submetidas às concentrações de 0,05; 0,10; 0,15; 0,20 e 0,25% do efluente Água Vermelha (AV) contaminado com compostos nitroaromáticos, além do controle. A análise estatística foi realizada por meio de Modelos Lineares Generalizados (MLG). Observou-se que houve diminuição no teor de clorofila *a* de forma dose-dependente, cujas diferenças foram significativas ( $p < 0,05$ ). Por outro lado, a clorofila *b* aumentou seu teor até a concentração de 0,20%, com uma redução significativa na concentração de 0,25%, refletindo em altos valores na relação clorofila *a/b* nesta concentração, com diferenças significativas ao nível de 5% de probabilidade. No teor de carotenoides observou-se oscilação entre as concentrações testadas, porém o controle diferiu da concentração

mais elevada (0,25%) ao nível de 5% de significância. Concluiu-se que o efluente contaminado com compostos nitroaromáticos afeta de forma diferenciada a síntese de pigmentos clorofilianos e carotenoides. A biossíntese de pigmentos envolve via bioquímicas alternativas, muitas vezes independente e competitiva, e desta forma, afeta a produção de pigmentos, revelando certa plasticidade deste metabólito frente ao estresse.

**SP159 Fluorescência da clorofila *a* em resposta ao efeito da acetoneitrila em *Salvinia* sp.**

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A análise da fluorescência da clorofila *a* pode ser mensurada por estímulos de pulsos luminosos no tecido vegetal adaptado ao escuro por meio de avaliação da cinética rápida (curva OJIP). A curva transitória OJIP é um método rápido, preciso e não destrutivo que fornece indicativos da funcionalidade do aparato fotossintético. Nesse contexto, o objetivo deste trabalho foi avaliar possíveis efeitos tóxicos da acetoneitrila na macrófita *Salvinia* sp. por meio da emissão da fluorescência transitória da clorofila *a*. A desinfecção das plantas de *Salvinia* sp. cultivadas no Laboratório de Ecotoxicologia foi realizada com solução de hipoclorito de sódio 2% por 5 minutos. As plantas foram aclimatadas por 24 horas em solução nutritiva de Hoagland 25%, pH 7,0 e mantidas sob fotoperíodo 12:12 (luz/escuro) e luminosidade constante de 3000 lux. Após aclimação, cerca de 1,5 g de plantas de *Salvinia* sp. foram colocadas em potes plásticos com 200mL de solução nutritiva de Hoagland 25%, as quais foram submetidas aos seguintes tratamentos: 0; 0,05; 0,10; 0,25; 0,50 e 0,75% de acetoneitrila recuperada. A fluorescência da clorofila *a* através da cinética de indução rápida (OJIP) foi avaliada por meio do fluorômetro PAM-2500 (Heinz Walz, GmbH). Os resultados revelaram uma curva OJIP típica, onde a maior concentração de acetoneitrila (0,75 %) não diferiu estatisticamente do tratamento controle ( $p < 0,05$ ). As doses mais baixas de acetoneitrila (0,05; 0,10; 0,25 e 0,50%) apresentaram redução na emissão de fluorescência em relação ao controle e à maior dose (0,75 %) nas folhas de *Salvinia* sp., cujas diferenças foram significativas ( $p < 0,05$ ). Estes resultados demonstram reduções no pool de plastoquinonas, afetando o lado doador e receptor de elétrons do PSII. Desta forma, a fluorescência transitória OJIP indicou maior toxicidade nas diluições intermediárias de acetoneitrila em plantas de *Salvinia* sp. Os resultados observados na dose mais elevada podem ser devido ao aumento da atividade fotossintética para suportar o estresse causado pelo tóxico.

**SP160 Fluorescência da clorofila *a* (OJIP) em macrófitas expostas a efluente de indústria de explosivos**

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Compostos nitroaromáticos como o TNT (2,4,6 trinitrotolueno), são frequentemente liberados na água e no solo por atividades industriais, se tornando um problema ambiental. Muitas macrófitas aquáticas são conhecidas por seu uso na fitorremediação de efluentes contaminados. A fluorescência da clorofila *a* (Fchl *a*) é um indicador sensível da presença de danos no aparato fotossintético e já tem sido utilizada para detectar danos por compostos nitroaromáticos. O objetivo deste trabalho foi avaliar o efeito tóxico do efluente de indústria de explosivos contaminado com compostos nitroaromáticos na cinética rápida da Fchl *a* em macrófitas aquáticas. As macrófitas *Azolla* sp., *Salvinia biloba* e *Pistia stratiotes*, cultivadas no Laboratório de Ecotoxicologia Aquática da Universidade Estadual do Centro - Oeste (UNICENTRO), Guarapuava, PR, foram aclimatadas por 48h em solução nutritiva de Hoagland (25%) antes dos bioensaios. Os experimentos foram realizados em recipientes de 200 mL contendo solução de Hoagland 25%, no pH 5,5, com 3 repetições de cada tratamento, sendo o controle e as diluições de 0 e de 0,25% do efluente, durante 7 dias, com fotoperíodo de 12h de luz e temperatura média de 24°C. Foram realizadas análises da cinética rápida da fluorescência da clorofila *a* (teste OJIP) com o fluorômetro PAM 2500 (Heinz Walz, GmbH). O tratamento com efluente afetou a cinética rápida da clorofila, principalmente na fase J-I-P. As três macrófitas quando expostas a 0,25% de efluente apresentam valores de F<sub>0</sub> (O) elevados em relação aos seus controles, assim como os valores da fase J. Aumentos de F<sub>o</sub> podem ocorrer, quando há danos no centro de reação do fotossistema II. Nas fases I a P houve redução em *Azolla* sp. e *Salvinia biloba*, onde os valores de I-P se assemelham a J. O transporte de elétrons parece ser afetado pelo efluente contaminado, pois as fases da O-J-I-P são distinguíveis quando se compara o controle com os tratamentos. Porém, na fase I-P em *P. stratiotes* observou-se decréscimo mais pronunciado, do que nas pteridófitas. A amplitude da fase de I-P pode ser um indicador aproximado do conteúdo PSI. Com este trabalho é possível

observar que o efluente de indústria de explosivos a 0,25% causa danos ao aparato fotossintético em nível do PSI, demonstrado pela extinção da fase I-P em *Azolla* sp. e *S. biloba*, bem como decréscimo do nível I em *P. stratiotes*. As pteridófitas foram mais sensíveis ao efluente do que a alface d'água.

## Addressing complexity in ecotoxicology: from multiple stressors to ecosystem level impact assessment

### FP001 Altered Activity Level and Heart Frequency of Bullfrog Tadpoles Exposed to the Commercial Herbicide Gamit® and Its Active Principle Clomazon

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Herbicides are one of the most used types of pesticide worldwide. Through effluents and soil leaching, these xenobiotics reach waterbodies and may have an impact on non-target populations. Amphibians particularly represent a group of non-target organisms strongly affected by these xenobiotics, since much of the life cycle happen inside the water, in addition to many anatomical and physiological characteristics that make them even more sensitive to any environmental changes. Among the most used herbicides in Brazil, the commercial formulation Gamit®, whose active principle is Clomazon, stands out. Thus, this study aimed to analyze the effects of an environmentally relevant concentration (0.5 mg/L) of the Gamit® formulation and its active principle Clomazon on the activity level (AL - % of active individuals) and on the *in loco* heart rate (fH - bpm) of bullfrog tadpoles (*Lithobates catesbeianus*). The exposure to Clomazon led to a decrease in the AL ( $38.0 \pm 7\%$ ) of the animals, which was even more expressive in the Formulation Gamit® exposed group ( $33.0 \pm 3\%$ ), when compared to the control group ( $70.0 \pm 5\%$ ). These results were accompanied by a decrease in the cardiac chronotropism, as corroborated by the bradycardia occurred in both Clomazon ( $23 \pm 1$  bpm) and Gamit® groups ( $19.0 \pm 1$  bpm), when compared to control ( $36.0 \pm 1$  bpm). The reduction in tadpoles' activity level in response to Clomazon and Gamit® is probably due to the fact that this xenobiotic inhibits the cholinesterase's activity in the motor end-plate of the skeletal muscle fiber, impairing tadpoles' swimming performance. This effect of the overstimulation of cholinergic system was also observed in the heart, as corroborated by the bradycardia occurred *in loco*. In sum, our results demonstrate that these herbicides in fact exert a negative impact on the cardiac function of bullfrog tadpoles. Therefore, more effective conservation strategies should be performed, as well as environmental alternatives for treatment and / or reduction of these xenobiotics.

### FP002 Análise da toxicidade de sedimento contaminado com cromo em *Chironomus xanthus*

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O cromo é utilizado em diferentes setores industriais, inclusive aplicado em curtumes como curte de couros, cujo processamento gera resíduos sólidos e líquidos contendo concentrações deste metal, que se lançados no meio aquático sem o devido tratamento, pode atingir os sedimentos e afetar a biota aquática. É nesse contexto que em 2001, cerca de 150 toneladas de aparas de couro foram lançadas no solo e a partir do runoff, lixiviadas para o córrego Monte Alegre, localizado no município de Bueno de Andrada (SP), contaminando o sedimento aquático e ocasionando alterações na comunidade de macroinvertebrados aquáticos do córrego. Baseado nessas informações, o presente trabalho visou avaliar a toxicidade de sedimento contaminado com cromo por meio de testes de toxicidade aguda e crônica em *Chironomus xanthus*. Foram coletadas amostras de sedimento com draga Ekman-Birge em três pontos distintos do córrego, sendo um ponto à nascente, um intermediário localizado em áreas agrícolas e outro na área contaminada. Os organismos foram expostos ao sedimento contaminado para avaliação da mortalidade, em testes agudos com duração de 96 h, preparados em frascos de 500 mL onde foram adicionados 240 mL de água deionizada, 60 g de sedimento, 5 mL de alimento (solução de Tetramin 5g L<sup>-1</sup>) e 6 larvas de III instar. O teste crônico foi desenvolvido sob as mesmas condições do agudo, exceto pela adição de 6 larvas de *C. xanthus* de I instar, aeração (4 mL s<sup>-1</sup>) e duração de 8 dias. Ambos os testes foram desenvolvidos em temperatura de 24±2 °C, pH 7, condutividade entre 15-100 µS cm<sup>-1</sup> e fotoperíodo de 12h luz/12h escuro. Os resultados do teste crônico indicaram toxicidade para o ponto intermediário, com aproximadamente 95% de mortalidade dos organismos expostos, indícios de

toxicidade para a área contaminada e não toxicidade para a nascente. O teste agudo, da mesma forma, indicou não toxicidade para a nascente, elevada toxicidade (100% de mortalidade) para o ponto intermediário e indícios de toxicidade (50% de mortalidade) para a área contaminada. Com isso, percebe-se que no terceiro ponto não há contaminação significativa de cromo como o esperado, apenas indícios, sendo provável que esse metal tenha se convertido a outras formas insolúveis ou tenha sido adsorvido à matéria orgânica presente no sedimento. Em relação ao ponto intermediário, é preciso que haja uma melhor análise, pois a contaminação pode ser proveniente da plantação de cana-de-açúcar presente no local.

### FP003 Assessing the genotoxic effects and enzymatic response of the *Oreochromis niloticus* to aluminum exposure

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Aluminum (Al) is the most abundant metallic element in the earth's crust. For this reason, Al is an element frequently present in different environmental compartments. Despite of its natural occurrence, Al may become toxic depending to its environmental concentration, for example in water where Al can access the different trophic levels by the bioaccumulation and biomagnification. After the breakup of the two dams in Minas Gerais, Brazil, containing mining rejects, high levels of dissolved Al in water bodies were measured in Rio Doce. In this context, genotoxic effects and enzymatic response to the Al exposure were studied in juveniles of *Oreochromis niloticus*. The genotoxic effects were evaluated by applying the micronucleus test. The enzymatic responses were evaluated by the determination of the catalase (CAT) and glutathione S-transferase (GST) activity in the liver and gills. The fishes were arranged in individual aquariums (5 L) during an exposure of 96 hours (n = 6 per treatment). The treatments tested were: control (without addition of Al), T<sub>1</sub>: 0.05 mg/L Al, T<sub>2</sub>: 0.1 mg/L Al, T<sub>3</sub>: 0.2 mg/L Al. The concentration 0.1 mg/L corresponds to the limit established by the Brazilian federal regulation (CONAMA Resolution 357/2005). The aluminum was added to the water as a solution of aluminum chloride hexahydrate (AlCl<sub>3</sub>·6H<sub>2</sub>O) whose pH was corrected by the addition of sodium hydroxide (NaOH) until it reached neutral pH corresponding the control. After the experimental period, the animals were anesthetized and the blood was collected to perform the micronucleus test, after that fish were euthanized and liver and gills were collected to perform the CAT and GST activity. Linear regression analysis was applied to evaluate the results. In micronucleus test, the analyzes indicated that exposure to the aluminum concentrations tested were not significantly associated to the genotoxic effects ( $F_{1,21} = 0.038$ ,  $r^2 = 0.001$ ,  $p = 0.847$ ). CAT and GST analyzes were also not significantly associated to changes in the enzymatic activity when fish were exposed to these aluminum concentrations tested ( $F_{1,19} = 3.151$ ,  $r^2 = 0.142$ ,  $p = 0.092$  for CAT and  $F_{1,16} = 0.079$ ,  $r^2 = 0.005$ ,  $p = 0.781$  for GST). The results indicate that Al in these concentrations tested does not cause genotoxic damage and significant alterations of the enzymatic activity of CAT and GST in *Oreochromis niloticus*. Probably, this lack of significantly results is related with the time of exposure, only 96 h, for low concentrations of Al.

### FP004 Assessing the influence of confounding biological factors when estimating bioaccumulation of PCBs with passive samplers in aquatic ecosystems

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Ecosystem-level biomonitoring and risk assessment are challenging tasks requiring knowledge of the factors that control bioaccumulation in order to define the key receptors. Risk assessment can be done by exploiting easily sampled compartments (sediment, water) and utilizing predictive models and passive samplers, which have been proven powerful tools in determining concentrations of lipid soluble organic compounds at different trophic levels. Passive samplers are promising surrogates for organisms, mimicking bioaccumulation. However, several biological (physical, biochemical) characteristics disturb the passive partitioning process in organisms by accelerating or restraining bioaccumulation, resulting in species-specific body residues of hydrophobic organic contaminants (HOCs). In addition to site-specific



characteristics and HOC concentrations, age, sex, diet, biotransformation capability and habitat-specific characteristics may affect body residues. In this study, two passive sampler types, PE and PDMS were deployed in a PCB-contaminated freshwater lake water and sediment, to assess their bioaccumulation prediction capacity. In order to understand the importance of biological characteristics in the bioaccumulation process, we explored bioaccumulation in biota from plants and plankton to mussels and fish. Passive samplers were good predictors of PCB concentrations in fish, whereas concentrations in algae and invertebrates were overestimated. The best model performance was achieved for fish in trophic levels 3–4. Bioaccumulation was species-specific and dependent on the trophic level and diet. Closer examination revealed that metabolic capability changes during the life span, and source of nutrition determined the biomagnification of HOCs, which differed between the fish species. Thus, species composition and available prey selection compose a unique bioaccumulation scenario and the resulting body residues. Due to the existing variation in body residues derived from passive samplers, extrapolating the results from one to another ecosystem must be done with caution. Conformation of the molecule has a significant impact on its bioaccumulation, potential to cause toxicity and species-specific metabolic pathway, thus making metabolism a question of great interest also in this research paper, by focusing the monitoring on relevant species and PCB congeners that differ in their metabolic capacity

#### FP005 Assessment of sediment quality in a tropical estuary using an integrated approach

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Estuaries are suitable for settling cities, ports and industrial plants. As a consequence, this environment receives continuously a range of contaminants that will be eventually deposited in sediments. This study investigated sediment quality in the Capibaribe Estuarine System (CES), an urbanized estuary in northeastern Brazil. The CES is formed by several streams that flow through Recife metropolitan area, receiving discharges of both domestic and industrial wastewaters. It also harbors the Port of Recife. Sediments were sampled at five sites in the estuary. Chemical analyses included aliphatic (AHs) and polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (DDTs), polychlorinated biphenyls (PCBs) and metals. Benthic copepods (*Tisbe biminiensis*) were exposed to sediments and elutriates obtained from mixing sediment with seawater. Ecological structure of the local benthic community was also investigated. The “Sediment Quality Triad” (SQT) approach was used together with Principal component analysis (PCA) as a simultaneous evaluation of chemical contamination and biological assessments. Concentration of AHs ranged from 21.9 to 474  $\mu\text{g g}^{-1}$  dry weight (dw). Total PAH concentrations ranged from 539 to 16217  $\text{ng g}^{-1}$  dw. Total DDTs ranged from 3.15 to 11.4  $\text{ng g}^{-1}$  dw while total PCBs ranged from 6.27 to 24.0  $\text{ng g}^{-1}$  dw. Total concentration of metals (sum of Al, Cd, Cr, Cu, Fe, Hg, Ni, Pb and Zn) ranged from 185 to 489  $\mu\text{g g}^{-1}$  dw. The highest contaminant concentrations were recorded close to the port in the lower estuary and at the confluence of several streams in the upper estuary. At these sites, sediments and elutriate showed both acute (mortality) and chronic (fecundity and development inhibition) toxicity to *T. biminiensis*. The lowest benthic diversity was observed at the confluence of streams in the upper estuary. The SQT approach and the PCA indicated that the upper estuary is the most critical area of the CES since chemical contamination affected structure of the local benthic community. In addition, results indicated that sediment quality increases toward the head of the estuary although pollution in the vicinity of the port cannot be neglected (toxicity was observed in both bioassays carried out with these sediments). The triad approach proved to be a good tool for investigating local environmental degradation and may be used in the estuarine management.

#### FP007 Avaliação de potenciais contaminantes em *Callinectes danae* (siri-azul), *Mugil curema* (parati) e *Mytella spp* (sururu) no estuário de Santos

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Diversos organismos estuarinos são utilizados na alimentação humana, e podem ser considerados bioindicadores da qualidade ambiental, ressaltando a necessidade de se estudar a presença de potenciais contaminantes em seus tecidos. Este trabalho teve por

objetivo avaliar a qualidade de três diferentes espécies: *Callinectes danae* (siri-azul), *Mugil curema* (parati) e *Mytella spp* (sururu), amplamente distribuídos no estuário de Santos. Os resultados foram obtidos a partir de duas campanhas de monitoramento, realizadas em março de 2012 e janeiro de 2013. Para a escolha das espécies considerou-se sua ocorrência durante o ano todo na região, o interesse no consumo pela população local e a associação destas espécies com o sedimento. A amostragem ocorreu em três áreas para cada organismo, localizadas no estuário de Santos. Em laboratório, foi realizada a biometria e extração do tecido muscular para análise; as amostras foram armazenadas sob congelamento até a realização das análises químicas. Foram analisados os seguintes parâmetros: arsênio, cádmio, cromo, cobre, chumbo, manganês, níquel, zinco, mercúrio, bifenilas policloradas (PCB), compostos orgânicos semivoláteis (SVOC), hidrocarbonetos policíclicos aromáticos (HPA) e pesticidas organoclorados (POC). Em ambas as campanhas nenhum dos PCB, HPA e POC analisados foram quantificados, considerando todas as amostras para as três espécies estudadas. Dentre os metais, na primeira campanha (março de 2012) foram quantificados cobre, manganês níquel e zinco nas amostras das três espécies estudadas. As concentrações de zinco em *Callinectes danae* foram 51,8 e 52,7  $\text{mg/kg}$ . Já na segunda campanha (janeiro de 2013) foram quantificados os mesmos parâmetros (cobre, manganês níquel e zinco) nas três espécies estudadas, e apenas em uma amostra de *Mytella spp* foi quantificado arsênio. Com base nos resultados obtidos, não foram observadas diferenças entre os resultados obtidos nas duas campanhas. Os organismos analisados apresentam-se com boa qualidade e aptos para consumo, uma vez que apenas metais, que tratam-se de micronutrientes essenciais aos organismos, foram quantificados.

#### FP008 Avaliação do potencial de toxicidade dos pesticidas Kraft e Score (isolados e mistura) em *Daphnia similis* por meio de experimentos de percolação

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No século passado, a agricultura brasileira sofreu diversas transformações tecnológicas, sendo determinante para a ascensão do país na exportação de matérias-primas agrícolas. Dentre as ferramentas que impulsionaram a produtividade, os agrotóxicos foram essenciais no auxílio do controle de pragas, doenças e plantas daninhas, porém, o desconhecimento de efeitos a organismos não alvos, aliados a práticas agrícolas incorretas, tem contribuído para alterações nos ecossistemas. Por meio das chuvas ou da irrigação, os agrotóxicos podem atingir águas subterrâneas, cujos impactos são ainda pouco estudados. Assim, o presente estudo avaliou o potencial de contaminação de águas subterrâneas pelos agrotóxicos Kraft (acaricida) e Score (fungicida), isoladamente ou em mistura, por meio de teste de toxicidade aguda com o cladóceros *Daphnia similis*. Para os experimentos foram utilizados simuladores (unidades experimentais com 0,1  $\text{m}^3$  de solo), os quais receberam as doses recomendadas dos agrotóxicos (isolados e em mistura), sendo submetidos a ciclos de aplicação e de chuva em dias alternados. Os experimentos foram realizados a temperatura de 23°C, sendo utilizada a água percolada para os testes de toxicidade. Os resultados foram analisados por ANOVA seguida de Turkey ou ANOVA seguida de Dunnett. Os resultados indicam que a maior toxicidade ocorreu na presença do Kraft (isolado e em mistura), no dia da aplicação do agrotóxico, com valores de mortalidade entre 73,3 e 100% para a adição isolada do Kraft e de 96,6 a 100% quando em mistura com o Score. Na ausência de aplicação do pesticida, e somente na simulação da chuva, a mortalidade foi mais expressiva apenas em um período de chuva (Kraft: 100% e mistura com Score: 93,33  $\pm$  13,03%), indicando a rápida degradação dos ingredientes ativos. Os resultados demonstram o potencial tóxico do produto Kraft, indicando a suscetibilidade também dos efeitos em águas subterrâneas e a necessidade de uma maior controle/fiscalização na sua utilização. \n

#### FP009 Avaliação ecotoxicológica de efluentes domésticos tratados por alagados construídos

L.B. Barszcz, F. Bellato, Federal University of ABC; D.R. Matheus, Federal University of ABC / Center for Engineering, Modeling and Applied Social Sciences. A água é um bem essencial para a sobrevivência de todos os seres vivos. Atualmente, tem-se observado o despejo de efluentes domésticos e industriais causando a degradação dos recursos hídricos. Estudos de ecotecnologias para tratamento destes efluentes se fazem necessários, em especial em pequenas comunidades, que acabam gerando poluição difusa nos corpos d'água, realidade da grande maioria dos municípios brasileiros. Aliado a outros indicadores de qualidade, ensaios ecotoxicológicos são muito importantes para monitorar a qualidade dos efluentes e dos corpos receptores. O objetivo deste trabalho foi avaliar o desempenho de alagados construídos com fluxo subsuperficial horizontal cultivados com as macrófitas

aquáticas *Eleocharis* sp. (junco); e *Typha* sp. (taboa), na redução da toxicidade de efluentes domésticos. Para isso, foram utilizados ensaios ecotoxicológicos com o microcrustáceo *Daphnia similis*, com a dicotiledônea *Lactuca sativa* (alface), a monocotiledônea *Sorghum vulgare* (sorgo) e com as macrófitas aquáticas *Lemna* sp. (lentilha d'água) e *Azolla* sp. O efluente doméstico se mostrou tóxico à *Daphnia similis* com média de EC<sub>50</sub> de 1,3%. Os tratamentos com macrófitas reduziram significativamente a toxicidade do efluente, com médias de EC<sub>50</sub> 73% para o tratamento com junco, EC<sub>50</sub> 53,17% para o tratamento com taboa, EC<sub>50</sub> 78,25% para o tratamento com taboa+junco. O controle (sem macrófitas) também reduziu significativamente a toxicidade, com média de EC<sub>50</sub> 63,13%. Nos ensaios de fitotoxicidade, a alface e o sorgo não se mostraram sensíveis ao efluente analisado, embora sejam para outros tipos de efluentes. A macrófita *Lemna* sp. também não foi sensível ao efluente estudado, no entanto observou-se a capacidade de identificar, com esta espécie, o potencial eutrofizante do efluente, já que a mesma se desenvolveu melhor no efluente bruto do que nos efluentes tratados. Com a macrófita *Azolla* sp. foi possível verificar que o efluente bruto possui maior potencial eutrofizante se comparado com os tratamentos com junco, taboa e controle, onde houve remoção de N e P. No entanto, evidenciou-se efeito tóxico para *Azolla* sp. nos efluentes tratados.

#### FP010 Bioaccumulation and oxidative stress in *Geophagus brasiliensis* (Quoy & Gaimard, 1824) exposed to lead contaminated food in different temperatures

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The deleterious effects of lead can be aggravated by environmental variants such as water temperature. In Brazil, water temperature has great variation. Species like *Geophagus brasiliensis* has a resistance to such variations, since it is present throughout the country. Despite resistance to temperature variation, the increase in temperature can cause some physiological imbalances in fish. Thirty two juveniles of *G. brasiliensis* were exposed to lead, at nominal concentrations of 0 and 60 mg.kg<sup>-1</sup> of lead at two temperature conditions (25 and 28 °C), totaling 4 treatments (25/0, 25/60, 28/0, 28/60). The animals were fed daily with commercial food containing the specific concentrations of lead at the rate of 1.5% of live weight. Analyses of oxidative stress and bioaccumulation of lead were carried out. The treatment 25/60 presented the highest change between analyzes. The liver increased 8,3 mg.kg<sup>-1</sup> of lead in treatment 25/60 and 4,61 mg.kg<sup>-1</sup> between treatments 28/0 and 28/60. Despite the food contamination, there was an increase of 11,70 mg.kg<sup>-1</sup> of lead between 25/0 and 25/60 treatments. There was a significative difference between organisms contaminated with 60 mg.kg<sup>-1</sup> of lead in CAT and GST analyses, the difference between the same treatments was of 1,74 U.mg<sup>-1</sup> of protein. The higher temperatures could be a benefic factor to lead contaminated fishes because it can interfere in metal absorption and metabolism in addition to facilitate excretion, decreasing damage in the organisms tissues.

#### FP011 Biochemical and genotoxic effects in oysters (*Crassostrea rhizophorae*) exposed to iron and manganese.

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The estuarine ecosystem receives a large amount of domestic and industrial waste, suffering direct and indirect impacts of environmental contamination. Oysters are considered excellent bioindicators for studies of pollutants effects in the aquatic environment. The present study was conducted to evaluate the toxicity of Fe and Mn in *Crassostrea rhizophorae*, exposed to different concentrations of these metals, using biochemical and genotoxic biomarkers. The work was divided into two protocols: in the first one, the oysters were exposed to Mn (0,049; 0.1 and 0.5 mg.L-1) and Fe (0.3; 0.440 and 1.5 mg.L-1) separately and, in the second one, the oysters were exposed to Fe and Mn in association (Fe + Mn 0.049 0.3; Fe + Mn 0.440 0.1; 1.5 Fe + Mn 0.5 mg.L-1). Gill tissue was used for biochemical analysis (activity of the enzymes Catalase (CAT) and Glutathione S-transferase (GST)) and hemolymph to genotoxic analysis (Comet Assay). CAT activity increased significantly in oysters exposed to the second concentrations of Fe and Mn isolated and when associated with each other. However, there was an inhibition of the enzyme into the last concentrations for all protocols. GST enzyme activity increased significantly in the first and second concentration of Mn and Fe isolated, and when associated with each other this increase was observed only in the first concentration. And again, there was a significant

reduction when oysters were exposed in the last concentration of the protocols. In relation to the genotoxic damage, it was observed that in all experimental protocols tested there was significant damage in the DNA in all groups exposed to metals. This increase was gradual and significant as the Fe and Mn concentrations increased. The data presented in this paper demonstrates the significant toxic potential of these metals in causing DNA damage, oxidative stress systems activation and detoxification.

#### FP012 Biomarkers responses in *Rhamdia quelen* exposed in situ on a brazilian river located near agricultural areas

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Aquatic environments are impacted by the use of agrochemicals in agricultural areas near rivers. To evaluate its impact on wildlife, *in situ* exposure has become an alternative to evaluate these environments. In this study the use of the *Rhamdia quelen* as a bioindicator was investigated through in situ exposition. Were placed 10 fishes per cage in each site of a total of three sites in Vacacaí river. The first site (S1) is located in a region with a large riparian forest (30°27'14.7"S 54°22'25.5"W). The second site (S2) is located close to rice and soybean crops (30°22'40.7"S 54°20'54.8"W). The third site (S3) is an urbanized region (30°20'30.5"S 54°18'19.9"W). A group of fishes (N=10) were kept in laboratory conditions as control group. Samples of water were collected at the sites in the first and the last day of *in situ* exposure, than were evaluated qualitatively and quantitatively for agrochemicals. After 96-hours of exposure, the fishes were euthanized and brain, gill, liver and muscle were removed for biochemical assay. Biomarkers of oxidative damage (thiobarbituric acid-reactive substance - TBARS, carbonylated protein - CP), neurotoxicity (acetylcholinesterase - AChE) and antioxidant parameters (glutathione S-transferase - GST, superoxide dismutase - SOD, catalase - CAT, antioxidant capacity against peroxy radicals - ACAP and non-protein thiols - NPSH) were analyzed. The integrated biomarker response (IBR) calculation was used to aid in the interpretation of the results. Was found in water the herbicide clomazone at all sites and the fungicide tebuconazole at S2. IBR results was a great tool in this work as it helped to summarize all the biomarkers in a single result. IBR showed a higher value in S2, followed by S3 and S1. As expected, the sites considered more contaminated resulted in significant alterations in relation to the control group and the animals exposed in S1. This result makes sense, since riparian forest is thought to be effective at intercepting and controlling chemical loads from diffuse agricultural sources to entering water bodies. In conclusion, this study has the potential to serve as a reference for future studies in the field of ecotoxicology, making possible the use of *Rhamdia quelen* in *in situ* experiments in regions where environmental risks are assessed. Even, our study helps to corroborate the importance of the preservation of riparian forests and their role in the preservation of aquatic fauna.

#### FP013 Biomonitoramento do Rio Perequê, Porto Belo - SC através de biomarcadores de contaminação ambiental.

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O uso indiscriminado de agrotóxicos, em culturas como a do arroz, frequentemente localizadas em regiões de várzeas úmidas ou irrigadas, tem resultado na contaminação dos recursos hídricos e consequente dano à biota. Na rizicultura do Rio Perequê, em Porto Belo, Santa Catarina – Brasil (foco deste trabalho), o glifosato é o herbicida mais utilizado. O objetivo deste trabalho foi monitorar a saúde dos peixes do Rio Perequê através da espécie de peixe nativo cará (*Geophagus brasiliensis*), utilizando biomarcadores bioquímicos e genotóxicos. Os espécimes foram coletados em dois diferentes pontos, um localizado a montante da barragem (P1), onde se localiza o ponto de captação de água para abastecimento da ETA de Porto Belo, e o outro localizado a jusante da barragem (P2), em duas épocas diferentes da safra de arroz, pós-semeadura (primavera) e pós-colheita (outono). A análise química do glifosato foi realizada na água e sedimento dos dois pontos. Biomarcadores bioquímicos foram analisados no cérebro e músculo, como a atividade da acetilcolinesterase (AChE), e no fígado a glutatona reduzida (GSH) e lipoperoxidação (LPO). Ensaio cometa foi realizado no fígado. A normalidade e homogeneidade foram testadas, sendo utilizado o teste t pareado para amostras independentes, e o teste não paramétrico Mann-Whitney U-test. Os resultados demonstraram reduções significativas da AChE muscular e cerebral no ponto P1, na primavera e outono, respectivamente. Também

houve aumento significativo da LPO no ponto P1 na primavera. Glifosato foi encontrado apenas no sedimento do P1 (11,7 µg/Kg). O ensaio cometa de fígado apresentou número superior de danos genéticos em P1 na primavera. No ponto P2 foi observado um aumento na atividade da AChE muscular e cerebral e redução da GSH no outono em comparação com a primavera, sugerindo uma alteração sazonal neste ponto. A redução da AChE muscular, aumento da LPO, e maior número de danos genéticos de fígado em P1 na primavera quando comparado ao P2, podem estar relacionados à recente e maior exposição dos peixes ao glifosato utilizado nos períodos pré e pós-semeadura. Em se tratando de captação para tratamento de água potável, os resultados encontrados no P1 na primavera, evidenciam não só danos a biota, mas também um potencial problema de saúde pública.

**FP014 Contaminants present in the Santos-São Vicente estuary have genotoxic and cytotoxic effects on *Centropomus undecimalis* blood cells**

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Estuaries are among the most pressured ecosystems due to contamination resulting from anthropogenic activities. The Santos-São Vicente estuary is located in the Baixada Santista Metropolitan Region in São Paulo coastal zone (Brazil). This estuarine area is highly contaminated with detergents, PCBs, hydrocarbons aromatic polycyclic, metals, etc. Our study aims to verify the genotoxic and cytotoxic effects of the estuary waters on the blood of fishes *Centropomus undecimalis*. Trace metal concentration in water was analyzed by atomic absorption spectrometry. Fish collected *in situ* and controls (purchased from the Marine Farming Laboratory of the Federal University of Santa Catarina) were euthanized and their blood collected for the micronuclei and comet assay. For micronuclei, blood smears were stained with Giemsa. A total of 2,000 cell for each specimen were counted. Single cell gel comet assay was also held with the blood to assess the DNA damage and a total of 100 cells were counted. The analyses of the water for metal-trace indicate the presence of concentrations of arsenic, lead and mercury above those permitted by Brazilian legislation, and the presence of cadmium. Further analyses are being held to elucidate which organic compounds are present in the water. The results with the blood of fishes show a higher number of cell abnormalities and DNA damage in fish collected in the estuary compared to control fish, indicating that the inorganic and organic contaminants in estuary have genotoxic and cytotoxic effect, which could lead to ecological disturbances over time.

**FP015 Ecotoxicological assay in *Raphidocelis subcapitata* exposed to 17-beta-estradiol**

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The concentrations of homonyms estrogens in aquatic environments in Brazil are surplus when compared to the same class of pollutants in the world. Such a pollutant is considered an endocrine interferent and may alter reproductive functions, among others, in biological systems. Research relates effects from microinvertebrates to vertebrates, however, there are few publications with algae, which has bioaccumulation potential for the food chain. The present study aimed to analyze the toxicity of 17β-estradiol (E2) estrogenic hormone, on the micro alga *R.subcapitata*, which presents a rapid physiological response in trials. The tests were performed according to standard NR12648 (ABNT, 2011), with nominal concentrations of 10, 3000 and 6000 ng / L, based on literature for Brazilian surface water bodies. The results of the counts were analyzed statistically. It was observed that, unlike that found in the control, the algae exposed to the three concentrations of the hormone EE2 produced excessive mucilage around their cells, probably due to a mechanism of defense the presence of the hormone. At the highest concentration tested there was inhibition of algal biomass growth, believed to be by hormone interference. Considering the above, it is believed that the hormone EE2 in the concentration detected in surface waters in Brazil has the potential to cause a chronic effect in *R.subcapitata*. In the aquatic environment, it causes a decline in the food chain, thus damaging the entire biota.

**FP016 Ecotoxicological effects of Kraft® 36EC (a.i. abamectin) on macroinvertebrates communities in freshwater mesocosms.**

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The continuous growth of the human population has created an increasing demand for food production. To meet such demand, a greater quantity of a variety of pesticides is being used to produce larger volumes of food. Consequently, the contamination of soil, sediments and water bodies with pesticides is increasing, constituting a threat to the biodiversity and the functioning of ecosystems. Understanding the impacts of these pesticides on ecosystem structure and functioning is key to make better risk assessments and to guarantee both the ecosystem conservation and agriculture viability and sustainability. In this study, we conducted a risk assessment of Kraft® 36EC (a.i. abamectin), an acaricide widely used in strawberry and potato crops, using mesocosms to simulate complex aquatic ecosystems. We evaluated the changes in the macroinvertebrate community of these mesocosms over time following the direct overspray of Kraft on the water surface, and the indirect contamination following a simulated rain runoff event from Kraft-contaminated experimental field plots. Mesocosms consisted of 500-L tanks with macroinvertebrate colonization structures made of stones, leaves and wood. After eight months of stabilization to allow establishing a biocoenosis in the mesocosms, the macroinvertebrate community was monitored before and up to 35 days after contamination. The macroinvertebrate communities from all mesocosms just before contamination showed no significant differences. After contamination, the macroinvertebrate community in all mesocosms treated with Kraft were significantly different from the controls, but both spray drift and runoff treatments showed similar communities. The taxa most associated with the Kraft treatments were Gastropods and Oligochaeta, while arthropods (such as Ephemeroptera and Diptera) and Hirudinea were associated with controls. The macroinvertebrate communities in Kraft treatments were consistently different from the controls until the end of the experiment. These results suggest that arthropods are the most vulnerable taxa to Kraft, and that the pesticide entry route (spray drift or runoff) does not alter its toxicity to macroinvertebrate communities at the recommended dose evaluated. Also, the changes in macroinvertebrate communities induced by Kraft are long-lasting, and aquatic systems thus appear to show low resilience following its contamination. CNPQ: 402392/2013-2.

**FP017 Ecotoxicological sediment evaluation at Monitoring Program of Sao Paulo State - 10 years of History**

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The sediment quality monitoring program of São Paulo state began in 2000 with sediment ecotoxicological evaluation using *Hyalella azteca*, associated with the evaluation of the benthic community and chemical analyses to elaborate an environmental diagnosis as proposed by the Sediment Quality Triad. Since 2006, parallel tests were performed with *Chironomus sancti-caroli*, a native chironomid, in order to complement and compare the results obtained with the amphipod, according to the guidelines proposed by International Environmental Agencies (USEPA, Environment Canada), mainly due to differences in the sensitivity of organisms. A study was conducted to verify if the results of the chironomid test would be applicable in an integrated way for the improvement of the diagnosis in the sediment ecotoxicological evaluation. In this study, sediment samples from rivers and reservoirs were collected from 2006 to 2016 for tests with *Hyalella azteca* (166 samples), according to ABNT NBR 15470 and with *Chironomus sancti-caroli* (169 samples) based on USEPA 600/R-99/064. For each organism samples were classified as optimal (non-toxic), regular (sublethal effect – growth or mentum deformity), toxic (lethality < 50%) or very toxic (lethality ≥ 50%). This same classification was applied in the integrated results. Categories were quantified and the analyzed samples showed to *Hyalella*: 72% optimal, 7% regular; 8% toxic and 12% very toxic while to *Chironomus*, 87% optimal, 2% regular; 3% toxic and 8% very toxic. Integrating the results of *Chironomus* to those of *Hyalella* 66% of the samples were classified as optimal, 7% regular; 11% toxic and 17% very toxic. There was an increase in a percentage of toxic and very toxic categories and a decrease of optimal. Analysing the 22 toxic samples to *Chironomus*, 50% were toxic only for this chironomid. Another important fact is the change of quality in 33% of samples where there were toxic effects for both organisms, since the most severe category is adopted when they were compared. The percentages of effect observed only for *Chironomus* emphasized the difference in sensitivity when compared to the amphipod, indicating a different response, measuring effects otherwise neglected. Thus, it is important to continue to use both organisms in the sediment evaluation integrating results to improve the



sediment quality classification in the ecotoxicological assessment.  
Keywords: *Chironomus sanctificarioli*, *Hyaella azteca*, Sediment Quality Classification

#### FP018 Efectos del fungicida carbenzimidazol sobre las enzimas glutatí

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El carbenzimidazol (CBZ) es un bencimidazol ampliamente utilizado en el control de enfermedades fúngicas de uso agrícola pre y post cosecha. Actúa inhibiendo la polimerización de la B-tubulina y el ensamble de microtúbulos afectando el proceso de mitosis. Se sabe que puede alterar el proceso apoptótico de células germinales y de tejidos reproductivos habiéndose reportado también como disruptor endócrino en humanos e inductor de estrés oxidativo afectando el sistema de defensas antioxidantes. Entre las enzimas que conforman este sistema antioxidante se encuentran la Glutatión S-transferasa (GST) que cataliza la conjugación de productos de peroxidación lipídica con glutatión estimulando su eliminación, y la Catalasa (CAT), enzima que facilita la remoción de H<sub>2</sub>O<sub>2</sub>, el cual es metabolizado a oxígeno molecular y agua. El objetivo de este trabajo fue evaluar los efectos del fungicida CBZ sobre las enzimas antioxidantes GST y CAT en el pez dulceacuicícola *Jenynsia multidentata*. Para ello se realizó un ensayo estático donde se sometieron hembras adultas a concentraciones de 0; 0,05; 0,5; 5; 10 y 100 µg/L durante 24 h (n=6). Se realizaron homogenatos de branquias e hígado y se observó la dinámica de las enzimas en una curva concentración-respuesta. Los resultados demuestran que no existen diferencias significativas en la actividad enzimática entre el grupo control y las diferentes concentraciones de exposición, tanto en hígado como en branquias para GST y CAT. Considerando estos resultados, en la especie estudiada y las concentraciones expuestas, el CBZ no generaría estrés oxidativo. Sin embargo es importante seguir investigando sus efectos con otras baterías de biomarcadores como complemento de los resultados obtenidos.

#### FP019 Efeito de efluente industrial decompostos nitroaromáticos no teor de pigmentos e na fluorescência de *Scenedesmus* sp. e *Pseudokirchneriella subcapitata*

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Durante os processos de fabricação de explosivos são consumidos grandes quantidades de água, os quais geram efluentes que descartam de forma inadequada. O efluente gerado na segunda lavagem do processo de fabrico do denominado água vermelha (AV). O Presente Trabalho avaliou o Efeito do efluente AV oriundo de Indústria de explosivos contaminado com Compostos nitroaromáticos não Teor de Pigmentos e na fluorescência da clorofila *a* de clorofíceas. Foram realizados ensaios ecotoxicológicos com *Scenedesmus* sp. e *Pseudokirchneriella subcapitata*, as quais foram submetidas a diluições crescentes (0,05 0,10 0,15, 0,20 e 0,25%) do efluente AV. Ocorreram reduções dose-dependentes no teor de pigmentos clorofílicos (*Chl a* e *b*) em espécies como espécies com coeficientes de determinação de 97,3 e 94,1% para *Scenedesmus* sp. e 65,7 e 65,1% para *P. subcapitata*. Os parâmetros de fluorescência inicial (F<sub>0</sub>) e máxima (F<sub>M</sub>) também reduziram de forma dose-dependente com coeficientes de determinação de 84,0 e 91,8% para *Scenedesmus* sp. E cerca de 94% para ambos os parâmetros em *P. subcapitata*. Resposta diferencial para observação na relação F<sub>v</sub> / F<sub>0</sub> que evidencia uma atividade potencial do fotossistema II. Os resultados revelaram uma parábola voltada para baixo com ajuste polinomial (R<sup>2</sup> = 91,8%) em *Scenedesmus* sp. Por outro lado, em *P. subcapitata* a relação F<sub>v</sub> / F<sub>0</sub> reduziu de forma dose-dependente com R<sup>2</sup> = 85,3%, de forma ao teor de pigmentos. Estes dados foram utilizados na avaliação de toxicidade de efluentes contaminados com compostos nitroaromáticos. Poder-se-ia dizer que o descartá-lo pode resultar em um crescimento e desenvolvimento de clorofíceas em ambientes aquáticos. Subcapitata a relação F<sub>v</sub> / F<sub>0</sub> reduziu de forma dose-dependente com R<sup>2</sup> = 85,3%, de forma ao teor de pigmentos. Estes dados foram utilizados na avaliação de toxicidade de efluentes contaminados com compostos nitroaromáticos. Poder-se-ia dizer que o descartá-lo pode resultar em um crescimento e desenvolvimento de clorofíceas em ambientes aquáticos. Subcapitata a relação F<sub>v</sub> / F<sub>0</sub> reduziu de forma dose-dependente com R<sup>2</sup>

= 85,3%, de forma ao teor de pigmentos. Estes dados foram utilizados na avaliação de toxicidade de efluentes contaminados com compostos nitroaromáticos. Poder-se-ia dizer que o descartá-lo pode resultar em um crescimento e desenvolvimento de clorofíceas em ambientes aquáticos.

#### FP020 Efeito do hormônio 17α-etinilestradiol sobre a biologia de *Chironomus sanctificarioli* (Diptera, Chironomidae)

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A água constitui um dos compostos de maior distribuição e importância na crosta terrestre. Entre os vários compostos contaminantes dos ambientes aquáticos, os hormônios, estrógenos naturais e sintéticos, devem ser vistos com uma maior importância, devido a sua persistência no ambiente. O 17α-etinilestradiol (EE2) é um hormônio sintético utilizado na formulação de contraceptivos orais, transdérmico e injetáveis. O presente estudo apresentou como objetivo analisar o efeito do hormônio sintético 17α-etinilestradiol sobre a biologia do invertebrado aquático *Chironomus sanctificarioli* analisando três gerações do ciclo de vida da espécie. Nos experimentos foram utilizados areia fina comum (lavada em água corrente, seca em estufa a 60°C e levada a mufla por 4 horas a 560°C) e água deionizada (condutividade entre 25-55µS.cm<sup>-1</sup>, dureza entre 12 e 16 mg.L<sup>-1</sup> para CaCO<sub>3</sub>, pH entre 6,5 a 7,5). Foram realizadas 4 réplicas utilizando-se potes de 2 litros para cada concentração do hormônio e para o controle. Para os testes, foram colocadas 15 larvas, contendo 60 g de areia de cultivo e 240 ml de solução contendo 17α-etinilestradiol (na proporção 1:4, sedimento-solução) nas concentrações de 50; 100; 200; 400; 800 e 1600 µg.L<sup>-1</sup>, e fotoperíodo de 12h luz/ 12 h escuro e com temperatura de 22 a 26°C. Os testes agudos foram realizados utilizando-se larvas de IV instar sem aeração e os testes crônicos foram realizados utilizando-se larvas de I instar com aeração. Os organismos foram alimentados com 5 ml de uma solução contendo 1 litro de água deionizada e 5,0 g de ração para peixe triturada (TetraMin®). O teste realizado foi estático, ou seja, a solução não era trocada do início ao fim do teste de cada geração F<sub>1</sub>, F<sub>2</sub> e F<sub>3</sub>. Em testes agudos (96h) os resultados mostraram que o composto em estudo não apresenta toxicidade. Nos testes crônicos apenas a terceira geração mostrou uma baixa sobrevivência (43,3 a 56,6%) indicando toxicidade do composto ao organismo-teste, o que foi confirmado pelo teste ANOVA one-way com p<0,05. Apesar de não haver deformidades bucais nas larvas nas três gerações analisadas, o tempo de emergência do organismo na terceira geração foi de 22 dias, havendo um atraso de cinco dias em comparação aos demais tratamentos, indicando que o composto testado pode influenciar no ciclo de vida da espécie. Na análise da fecundidade potencial, os testes estatísticos indicaram haver diferença significativa entre os tratamentos e o controle na terceira geração analisada.

#### FP021 Efeitos do resíduo do beneficiamento de rochas ornamentais em *Oreochromis niloticus* submetidos à hipóxia

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Para avaliar os efeitos negativos do resíduo do beneficiamento de rochas ornamentais (RBRO) foi realizado um experimento de exposição de indivíduos de *Oreochromis niloticus* por 96 horas a este resíduo, utilizando concentrações de 250 e 1000 mg/L. Para avaliar o impacto sobre o metabolismo respiratório dos animais, após o período de exposição a aeração foi removida, foi medida a concentração de Oxigênio Dissolvido (OD) e os aquários foram vedados, sendo realizado o monitoramento comportamental, a contagem de batimentos operculares e a concentração de OD a cada 30 minutos, até constatação de tombamento dos animais, quando foi feita a medição de OD final. Foram observados comportamentos distintos entre os animais, em resposta à hipóxia, tendo-se notado, em indivíduos dos grupos controle e expostos, aumento do número de batimentos operculares por minuto em alguns casos, aumento do volume de água captado/liberado a cada batimento, busca constante à superfície coberta e redução das atividades natatórias, culminando em comportamentos limitantes ao experimento, como natação em zig-zag, paralisação de nadadeiras e ausência de resposta a incentivos externos. Os resultados demonstraram que a exposição ao RBRO, mesmo em curtos espaços de tempo, pode afetar a capacidade respiratória dos animais expostos, que apresentaram comportamentos limitantes em concentrações de OD superiores a 3,0 mg/L, ainda não consideradas críticas.

#### FP022 Efeitos tóxicos do resíduo do beneficiamento de rochas ornamentais em *Oreochromis niloticus*

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Para avaliar os efeitos tóxicos dos resíduos do processo de beneficiamento de rochas ornamentais (RBRO) indivíduos de *Oreochromis niloticus* foram expostos à contaminação aguda (96 horas) em diferentes concentrações (250, 500, 750 e 1000 mg/L). Foram avaliados os efeitos genotóxicos do resíduo sobre os animais, com análise das enzimas glutathione S-transferase (GST) e catalase (CAT) a partir do fígado, e danos ao DNA, com a análise de micronúcleos e teste cometa. Foram também identificados os danos morfológicos nas brânquias e quantificada a concentração de íons  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^{+}$  e  $\text{K}^{+}$  nas brânquias e na água. A análise das alterações morfológicas das brânquias indicou a ocorrência de lesões leves (até 750 mg/L) e moderadas (1000 mg/L) distribuídas pontualmente no tecido, apresentando diferença estatística significativa a partir de 750 mg/L. Foi evidenciado, com maior frequência, rompimento do epitélio lamelar, dilatação do seio sanguíneo, fusão incompleta de várias lamelas e proliferação de células de cloreto. A frequência de micronúcleos e o índice de danos ao DNA evidenciaram alterações significativas a partir das concentrações de 1000 e 750 mg/L, respectivamente. Não foi observada diferença significativa entre os tratamentos para a atividade da CAT, mas, para GST, esta foi registrada em 1000 mg/L, tendo sido observada a redução da atividade das duas enzimas na medida em que a concentração aumentou. Os resultados demonstraram que a exposição ao RBRO, mesmo em curtos espaços de tempo, pode causar prejuízos à espécie a partir da concentração de 750 mg/L.

**FP023 Effect of STX concentration variation in a natural freshwater system dominated by *Cylindrospermopsis raciborskii* as factor affecting cladoceran**

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*Cylindrospermopsis raciborskii* influences the zooplankton community structure. The cyanobacterial density and toxin concentration are possible driving factors. We investigated the relationship between STX concentration of natural samples dominated by *C. raciborskii* STX producer to an observable effect (immobility) on a lab-cultured population of Cladocera. Secondly, we verified the potential to use standard ecotoxicological tests to evaluate STX concentration in the natural environment. Peri Coastal Lake (27°84'40 S and 48°83'10 W), Brazil, was sampled monthly over a 15-month period (July 2013 to September 2014). The STX concentration (analysed by LC – MS/MS) was related to the effect on a lab-cultured population of *Daphnia magna*. We performed acute ecotoxicological tests using the whole-, sonicated- and filtrated-lake water samples. The percentage of immobility in undiluted sample (100%) and the Toxicity Factor 48h (TF48h) for each treatment were the endpoints used. We developed a Generalized Linear Model (GLM) with gamma distribution, assessing the relation between immobilized organisms and STX concentration. The STX concentration was related to the immobility of zooplankton subjected to the whole water samples (marginal significant relation,  $p=0.09$ , pseudo -  $R^2=0.18$ ) and sonicated water samples (significant relation,  $p<0.05$ , pseudo -  $R^2=0.16$ ) over the months. There was no effect on the zooplankton when the *C. raciborskii* cells were removed (filtered sample) from the water. The immobilization was irreversible in all tests, indicating mortality. The effect on the zooplankton was the same when the *C. raciborskii* cells were lysed (sonicated sample) to when the cladoceran population was subjected to the intact *C. raciborskii* cells (whole water) ( $t(20)=0.93$ ,  $p=0.36$ ). The acute intoxication effect of grazing upon STX-containing *C. raciborskii* seems to be a feature in the zooplankton community structure, as this can select for zooplankton that are able to coexist with a toxic *C. raciborskii* in the ecosystem. The TF48h (sonicated sample) showed a good linear relation (84%) with STX concentration in the lake. Hence, these tests may serve as a means to investigate STX concentration in the natural environment. However, standard protocols need to be established to enable the application of ecotoxicological tests as a preliminary screen for the presence of STX in water bodies.

**FP024 Effect of water temperature on the oxidizing parameters of *Aegla longirostri* (Crustacea, Anomura, Aeglidae).**

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According to the Intergovernmental Panel on Climate Change (IPCC) (2014), global air temperature is expected to rise by as much as 4.8 °C by 2100. Small-scale streams with riparian vegetation have a distinct microclimate, so they are particularly sensitive

to temperature changes. Considering a possible increase in the world average temperature and the deforestation of the margins with marked negative consequences for limnic organisms, the responses of the antioxidant defense system in the freshwater crustacean *Aegla longirostri* were analyzed. The animals were exposed, under laboratory conditions, to temperatures of 18 °C, 21 °C, 24 °C and 26 °C for 48 hours. The increase in temperature significantly affected the levels of all antioxidants tested in *A. longirostri* in different tissues, with the exception of the level of non-protein thiols in hepatopancreas. There were increased levels of catalase (CAT) activity in hepatopancreas and acetylcholinesterase (AChE) in muscle, oxidative damage in lipids (TBARS) in muscle and gills, increase in carbonyl protein (CP) levels in hepatopancreas and non-thiols Proteins in gills. However, glutathione-S-transferase (GST) activity decreased with increasing temperature. The results point to a clear relationship between temperature and lipid peroxidation. Damage to proteins and lipids shows that cell damage has been caused by increased temperature. The increase of *Aegla's* NPSH levels exposed to the temperature of 26°C in gills may be a protective response. At 24°C the CAT activity in the hepatopancreas was increased, being able to be consequence of the formation of ROS caused by the higher temperatures. However at 26°C the inhibition of enzyme activity was observed, which may mean that at this temperature CAT activity may have been inhibited by the fluctuation of the superoxide radicals. Despite being considered a neurotoxicity biomarker, temperature influenced AChE activity. We have shown that temperature variation, even in a short-term experiment, is capable of inducing oxidative stress. These results allow a prediction of the consequences that the increase of the global temperature will exert in ectothermic organisms like *A. longirostri*, causing alterations in its system of antioxidant defense and can affect the survival of the organisms.

**FP025 Effects of metals on the acetylcholinesterase activity of the bullfrog tadpole's brain and muscle**

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Amphibians are susceptible to exposure to a wide variety of contaminants such as metals/metalloides. Metals are pollutants that have received considerable environmental and toxicological study because do not degrade in the environment and have a variety of toxicological effects in many physiological systems. We investigated the acetylcholinesterase (AChE) activity in the brain and the muscle of bullfrog tadpoles, *Lithobates catesbeianus*, following exposures to 1 µg L<sup>-1</sup> of zinc (Zn), copper (Cu) and cadmium (Cd), alone or in combination (1:1 and 1:1:1) for 2 and 16 days. Most of the studies on inhibition of AChE involve different aquatic or terrestrial organisms. However, such studies are scarce in amphibians, despite their high vulnerability to environmental stressors such as metal combinations. The concentrations of metals tested did not cause mortality in bullfrog of tadpoles, but the AChE activity was significantly affected by metals, both alone and combined. The exposure to metal alone and combined caused a significant increase (20-220%) in AChE activity of the brain after 2 days when compared to its respective control. One of the possibilities for the increase of AChE activity by metals may be due to de novo synthesis of this enzyme as a response to an initial inhibition. On the other hand, AChE activity was inhibited (20-40%) in brain (16 days) and muscle (2 and 16 days) tissues with the exposure to metal alone and combined. Moreover, the combination Cu+Cd and Zn+Cu+Cd caused an inhibition of AChE activity from 36 to 43 times when compared to its respective control. The decrease in AChE activity in tadpoles may reflect the presence of cholinesterase inhibitors at harmful levels. Additionally, the AChE activities in groups at 16 days were higher than that presented by the groups exposed to metals at 2 days, excepting in brain when tadpoles were exposed to Cu+Cd and Zn+Cu+Cd, at which the activities were lower. The use of AChE as a biomarker provides evidences for ecotoxicological risk assessment of tadpoles populations at sites contaminated with mixtures of metals. Additionally, the bullfrog tadpole's AChE enzyme can be as useful indicator of amphibian exposure to metals and a biomarker of susceptibility in this amphibian species. The results obtained in the present study indicates that the toxicity of metals, alone and combined, to tadpoles of *L. catesbeianus* must also be considered high. Financial support: FAPESP Proc. 507523/2011.

**FP026 Effects of soluble fraction of oil diesel in gametes and pluteus larvae of *Echinometra lucunther***

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Considering the high number of accidents with diesel oil spills currently occurring in the marine ecosystem, toxicity tests aimed at assessing the effects of these pollutants on resident organisms. The aim of the present study was evaluate the toxicity of the diesel oil soluble fraction (SFD) in gametes and embryo of *E. lucinther*. The sea urchins gametes were exposed to concentrations of 0.0, 0.5, 1.5 and 2.5 % of the SFD. The viability of the gametes was significantly reduced by the exposure to SFD. The fertilization rate was significantly reduced in the exposed groups (49% for the 0.5 %, SFD 37% for the 1.5 % SFD e 29% 2.5 % for the SFD) when compared to the control group (84%). The development of the pluteus larvae was also significantly affected by exposure to SFD concentrations. The rate of larvae development was significantly reduced by the exposure to SFD (50 % for the 0.5 % SFD, 27 % for the 1.5 % SFD and 12 % for the 2.5 % SFD, respectively) when compared to the control group (80 %). The results of the present study suggest that hydrocarbons constituents of SFD have toxic properties that affect reproductive cells and also reduce larval growth rates. Therefore, it is evident that diesel oil can promote significant changes in the initial life stages of an organism and one of the ways to evaluate this toxicity would be the evaluation of this contaminant throughout the reproductive cycle of the species. \n

#### FP027 Evaluation of Cr (VI) removal by fixed-bed column filled with chitosan beads

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The presence of metals in the environment can lead to toxic effects because of biomagnification and bioaccumulation in aquatic biota. The chromium contamination in aquatic environments caused specially by tannery and electroplating effluents discharge and agrochemicals uses. An alternative to reduce the contamination in aquatic environments is the metals adsorption by biopolymers, such as chitosan-based materials. Chitosan presents high metal adsorption potential and its application in fixed-bed column has increased recently. Chemical and physical analyses are not sufficient to assess the effectiveness of the treatment system and the toxicity test to verify the harmful effects on the microbiota. A fixed-bed column filled with chitosan hydrogel beads (CHB) were evaluated in ecotoxicological bioassays with hexavalent chromium ions spiked. The feeding consisted in an aqueous solution of 5.6 mg L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (2 mg L<sup>-1</sup> of Cr (VI)), with downflow rate was 0.2 L h<sup>-1</sup>, pH of 6.5 and conductivity of 26  $\mu$ S cm<sup>-1</sup>. The column volume was 70 mL and the CHB mass added was 70.45 g. Chitosan presented DA = 75-85% (degree of acetylation), Mw= 50,000-190,000 Da (molecular weight), without pre-treatment and CHB with mean diameter of 3.135  $\pm$  0.436 mm and percentage of chitosan of 3% (m/m). The effluent was sampled in each 30 min of operation, then used in acute ecotoxicological bioassays during 96h. The *Chironomus xanthus* mortality, exposed to remaining concentrations of Cr (VI), was evaluate in each sampling time. Toxicity tests were conducted in 80 mL of liquid, 20 g of sterile sand and 5 mL of feed (Tetramin solution of 5 g L<sup>-1</sup>), where were added 6 larvae of IV instar. As expected, the concentration of chromium ion in liquid effluent raised with the time of filtration, it increased gradually from the time 0 min to 180 min T1=0-30 min (0.71 ppm); T2=30-60 min (0.70 ppm); T3=60-90 min (0.80 ppm); T4=90-120 min (1.3 ppm); T5=120-150 min (1.6 ppm); T6=150-180 min (1.8 ppm). As result, there have been no larvae deaths in bioassays with affluent and effluent. The same test was performed with the concentration of 10 mg L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and bioassays indicates potential of reducing mortality effects by fixed-bed column with chitosan hydrogel beads (CHB). To the affluent bioassays, 30% of larvae was death and the other organisms were color changes, almost dead in end of test. In effluent bioassays, there was no mortality at any time.

#### FP028 Evaluation of neurotoxicity in *Atherinella brasiliensis* of the island of Cananéia, Southeast of Brazil.

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The Cananéia Island is part of the Estuarine-lagoon Complex of Iguape-Cananéia, in southeastern Brazil. The region is among the most productive and well preserved areas in the South Atlantic, however, it suffers with the presence of contaminants, as heavy metals and persistent organic pollutants, that can affect the central nervous system of aquatic organisms. Biochemical biomarkers can be used to elucidate such effects, and previous studies already shown that these contaminants can induce different biological responses on local fish fauna. The analysis of the acetylcholinesterase activity (AChE) is used to assess neurotoxicity, since the enzyme serves to terminate synaptic transmission, preventing continuous nerve firings at nerve endings. Therefore, it is essential for the normal functioning of the central and peripheral nervous system. This study aimed to observe possible neurotoxic effects in the estuarine-resident fish species *Atherinella brasiliensis*. Two points near Cananéia Island were sampled, one in Cananéia City (south) and other in Pedrinhas Village (north), in the summer and

winter of 2016. About 20 specimens were collected per site, which were anesthetized, euthanized and had the brain and muscle collected. The data of AChE activity were tested by Shapiro-Wilk for normality. One-Way ANOVA and Kruskal-Wallis test were used for parametric and non parametric data respectively. The error considered was of type I (< 5%). Cerebral AChE activity was similar between points and seasons (p>0.05). The activity of muscle AChE was similar between the points in both seasons (p>0.05), however, it was lower in winter in both points (p< 0.001), with a reduction in Pedrinhas Village. The inhibition of this enzyme is referred as specific for organophosphates and carbamates, but also responds to metals. The marked seasonality of the region, which consequently influences the temperature, the rainfall regime and the bioavailability of contaminants, may also interfere in these responses. Therefore, for spatial variations, a higher presence of pollutants in Pedrinhas Village is reported, from sources such as effluent discharge, agricultural activities and ancient mining in nearby areas, which may affect the ichthyofauna. Chemical analyzes are also being performed to verify the presence of xenobiotics in the region that may be interfering with the health conditions of organisms.

#### FP029 Evolução da Qualidade da Água no Estuário de Santos (Santos, Brasil) ao longo de 5 anos de monitoramento

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O estuário de Santos abriga diversos terminais portuários, além de receber influência da ocupação urbana e industrial de suas adjacências. O objetivo deste trabalho foi realizar um monitoramento de longo termo acerca da qualidade das águas superficiais no estuário de Santos. Neste trabalho são apresentados os resultados obtidos das campanhas de amostragem de água superficial realizadas entre novembro/2012 e abril/2017. Neste período foram realizadas 18 campanhas de amostragem, sendo analisadas 346 amostras, em 13 pontos monitorados. Os resultados obtidos em todas estas campanhas foram comparados com as condições e padrões de qualidade estabelecidos pelo artigo 21 da Resolução CONAMA n° 357/05 (águas salobras – Classe 1). Durante as amostragens foram medidos *in situ* os parâmetros: oxigênio dissolvido (OD), pH, E<sub>H</sub>, condutividade, salinidade e temperatura. Além destes, foram coletadas amostras para análise em laboratório dos parâmetros: turbidez, sólidos suspensos totais (STS) e óleos & graxas; arsênio, boro, cádmio, cromo, chumbo, cobre, manganês, mercúrio, níquel e zinco totais e alumínio dissolvido; fósforo total e dissolvido, nitrogênio amoniacal, nitrogênio kjeldahl total (TKN), nitrato, nitrito e nitrogênio total; Parâmetros orgânicos: compostos de hidrocarbonetos policíclicos aromáticos (HPA). Os resultados obtidos indicaram amostras em desacordo à resolução supracitada, para os seguintes parâmetros: OD, pH, boro total, manganês total, alumínio dissolvido, fósforo total, nitrogênio amoniacal, nitrato e nitrito. Ressalta-se que alguns parâmetros foram quantificados, porém a respectiva resolução não estabelece padrão ou condição de qualidade como: turbidez, STS, fósforo dissolvido, TKN, nitrogênio total e, ainda, os parâmetros (óleos & graxas, arsênio total, cádmio total, chumbo total, cobre total, cromo total, mercurio total, níquel total e zinco total, além dos compostos de HPA) não foram quantificados em nenhuma das amostras analisadas. Dessa forma, com base nos resultados deste estudo, é possível concluir que as principais não conformidades observadas ao longo desse monitoramento são oriundas de contribuições orgânicas de diversas fontes, uma vez que foram identificadas baixas concentrações de OD na água e a maioria dos parâmetros (nitrogênio amoniacal, nitrato, nitrito e fósforo total) que ocorreram em maior frequência (acima de 50%) em concentrações superiores aos valores máximos estabelecidos pela Resolução CONAMA n° 357/05, são relacionados à efluentes domésticos.

#### FP030 Exposure to waterborne copper reduces thermal tolerance of guppy *Poecilia vivipara* acclimated to high temperature

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Since fish are ectothermic, ambient temperature is a major driver for physiological traits in these animals. However, they can tolerate different temperature ranges, but deviance from these ranges may lead to poor performance. Temperature tolerance ranges are evolutionarily structured and are modulated by acclimation. It is known that acclimation to elevated temperature is positively correlated with increases in upper limit of thermal tolerance. This process is related to adjustments in some physiological



traits, such as antioxidant system and energy metabolism. Interestingly, many of these traits are also involved in metal toxicity. Considering that temperature acclimation and metal toxicity are linked by physiological traits, interactions between these environmental parameters on fish thermal tolerance are expected. Thus, the main objective of the present study was to evaluate the interaction between two acclimation temperatures and two ecologically relevant concentrations of waterborne copper (Cu) in the upper limit of thermal tolerance of guppies *Poecilia vivipara* using the CTMax method. Fish were acclimated to 22°C or 28°C for 3 weeks and then exposed for 96 h to 9 µg/L or 20 µg/L Cu at the respective acclimation temperature. Twelve fish were tested at each combination of temperature and Cu exposure. Following Cu exposure, CTMax was determined based on blinded experiments using 12-L glass aquaria with heater and digital temperature data logger. The initial water temperature for CTMax determination was the respective acclimation temperature. Water temperature was increased at a rate of 0.3°C/min. Water was heated until fish displayed loss of balance. CTMax values were validated when animals survived following 24h of determination. Data was analyzed through factorial ANCOVA using weigh as a covariate. CTMax was significantly higher in fish acclimated to 28°C (41.07 ± 0.50°C) than in those acclimated to 22°C (39.51 ± 0.68°C). Also, a significant and negative interaction between acclimation temperature and Cu exposure was observed. However, exposure to Cu concentrations (9 and 20 µg/L) reduced CTMax only in fish acclimated to 28°C (Control: 41.49 ± 0.18°C; 9 µg/L: 40.73 ± 0.43°C; 20 µg/L: 40.96 ± 0.51°C). In light of these findings, we can conclude that acclimation to elevated temperature enhances Cu toxicity, leading to a reduced thermal tolerance in *P. vivipara*.

**FP031 Genomic damage assessment on Uçá Crabs (*Ucides cordatus*) from a tropical estuary exposed to polycyclic aromatic hydrocarbons-contaminated sediments**

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Polycyclic aromatic hydrocarbons (PAHs) are priority organic compounds in environmental studies due to their toxic potential to biological systems. They are known carcinogenic and mutagenic precursors to animals and humans. The Potengi estuary (Natal / Rio Grande do Norte State) is very important because besides working as a nursery for various organisms, it houses the Port of Natal, fishing terminals, industries, among other activities that may be threatening the health of this ecosystem. This study aimed to investigate genomic damage on Uçá crabs (*Ucides cordatus*) from Potengi estuary exposed to contaminated sediments by PAHs. *U. cordatus* and their respective burrows' sediments were collected in 4 stations. The PAHs concentrations were determined by gas chromatograph coupled to a mass spectrometer, and the genetic alterations were evaluated by micronucleus test (macrolesions) and comet assay (microlesions). Total PAHs ranged from 3.25 ng g<sup>-1</sup> to 1065 ng g<sup>-1</sup>. One station was classified as low contaminated, and the others as moderately contaminated. Some compounds showed concentrations above threshold effects level, suggesting that occasionally they may cause toxic effects to local organisms. Micronuclei frequency results in Uçá crabs was significantly higher than the control, and a positive correlation with PAHs was observed. High molecular weight compounds were the most correlated with DNA damage, pointing out these compounds as important genotoxic stressors. There was no correlation between comet assay and PAH or micronuclei frequency, indicating that some other environmental variables and/or compounds are also causing microlesions. PAHs have been chronically introduced into Potengi estuary and they may be threaten the conservation of *U. cordatus*, which is already listed as an overexploited species or under threat of overexploitation. The methodology used was efficient in the evaluation of genomic damage associated with contamination, but complementary studies investigating other biologic parameters and classes of contaminants are necessary.

**FP032 Genotoxic effects of the association between iron and manganese in *Oreochromis niloticus*.**

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In November 2015, the dams of Fundão and Santarém broke down, resulting in the leakage of about 34 million cubic meters of tailings sludge from iron mining. The mud reached two states, Minas Gerais and Espírito Santo (Brazil). In a preliminary analysis, several contaminants were found above the maximum permitted limit, according to CONAMA Resolution 357/05. Iron (Fe) and manganese (Mn) were found in high concentrations and in this way the fish of the Rio Doce are exposed to the high levels of the two metals. The exposure of aquatic organisms to these metals may cause

genotoxic damage and induce the generation of reactive oxygen species, causing oxidative damage to the biomolecules. The objective of the present study was to evaluate the toxicity of the association between Fe and Mn in *Oreochromis niloticus* by a genotoxic analysis (micronucleus test). The fish were arranged in individual aquariums (6 L) during an acute exposure of 96 hours (n=9 per treatment). The treatments tested were: control (without addition of metal), T1 - 3.81 mg/L Fe + 0.5 mg/L Mn and T2 - 7,62 mg/L Fe + 5,23 mg/L Mn. After the experimental period, the animals were anesthetized and the blood was collected to perform the micronucleus test, according to Grisolia et al. (2005). All animals exposed to the association of Fe and Mn showed a significant increase in the number of erythrocyte micronuclei (0.69 and 1.22 %, respectively) when compared to control (0.06%). There was no substantial difference between the treatments. The results of the present study indicate that Fe and Mn in association cause genotoxic damage during cell division when the nuclear membrane is constituted around the chromosomes of the daughter cells, through the chromosomal fragments that were not included in the main nucleus.

**FP033 Glyphosate herbicide disrupts spermatogenic cysts in males of zebrafish *Danio rerio***

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Glyphosate-based herbicide (GBH) is a broad-spectrum organophosphate, soluble in water, used to inhibit the growth of weeds. When applied in terrestrial systems, GBH penetrates into soil, reaching the aquatic ecosystems and affect non target organisms, as fishes. In these organisms, glyphosate (GLY) may acts as an endocrine-disruptive agent, inducing disturbances of the reproductive system. In this study, we investigated the reproductive toxicity of GBH in males of zebrafish *Danio rerio*, by the cell responses of the somatic and germ lines. Adult males were exposed for 15 days at three different concentrations of GLY from Roundup WG herbicide (0.065, 0.65 and 6.5 mg GLY/L aquarium water). The 0.065 mg GLY/L concentration was based on the maximum permissible concentration of GLY in Brazilian waters for human drinking (357/2005/CONAMA). Non-exposed males were used as controls. After treatments, males were decapitated, the testicles were dissected and immediately fixed in Karnovsky solution for electron transmission microscopy analysis. All the procedures adopted in this work were approved by Ethic Committee (n.0746/CEUA/UFSC). Ultrastructural analysis allowed the identification of subcellular changes induced by the GLY. In general, exposed males presented anomalous organization of testicles. In these animals, most of the spermatogenic cysts were not recognized and spermatogonium and spermatids were observed in the lumen. Sertoli cells showed non-electrondense cytoplasm with large vesicles, non-apparent nucleus and disrupted cell membrane. Also, intercellular bridges between the germ cells were not observed in the disrupted cysts. Our results show the impact of GLY in the testicles organization and suggest the endocrine-disruptive effect of this herbicide on male reproduction.

**FP034 Heavy metals and organic contaminants in superficial sediments of the highly polluted Riachuelo River, Argentina**

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The Riachuelo River, a tributary of the Río de la Plata estuary, has been historically polluted by the discharge of crude urban-industrial effluents. In order to determine potential toxic effects, surface sediments were collected with a "Van Veen" grab sampler in four sampling sites during 2014 in the lower Riachuelo River. Sediment samples were homogenized and splitted for the determination of the grain size composition (sieve and pipette method), total organic carbon (TOC) contents (Thermo Finnigan elemental analyzer), the determination of metals (Thermo Elemental atomic absorption spectrophotometer), total petroleum hydrocarbons (TPH) by Fourier transform infrared spectroscopy (Thermo Nicolet IR100) and for the determination of polychlorinated biphenyls (PCBs) by HRGS-ECD (Agilent 7890). Grain size composition was dominated by fine sediments (silt + clay= ~60%) and TOC contents ranged between 4.2% and 6.1%, with an average value of 5.1±0.8%. Anthropogenic trace metals were significantly enriched (2-16 times) relative to the average earth's crust abundance (Zn: 698±54; Cr: 575±341; Cu: 178±13; Pb: 161±51; Ni: 28±2.6 µg g<sup>-1</sup>) and exceeded 5-15 times Canadian sediment quality threshold effect levels (TEL), above which adverse biological effects are expected to occur (Zn: 123; Cr: 37.3; Cu: 35.7; Pb: 35.0 µg g<sup>-1</sup>). In contrast, major metals were depleted (Fe: 27626±2791; Mn:

266±41 µg g<sup>-1</sup>) compared with the crustal composition possibly reflecting evasion to the water column through reductive dissolution of Fe and Mn-oxides under anoxic conditions. TPHs ranged from 4.7 to 6.9 mg g<sup>-1</sup> (5.4±1.0 mg g<sup>-1</sup>) comparable to highly polluted areas affected by oil discharges in La Plata harbor (6.1-10.7 mg g<sup>-1</sup>) whereas total PCBs ranged from 148 to 267 ng g<sup>-1</sup> (219±50 ng g<sup>-1</sup>), much higher than reported values for coastal Río de la Plata sediments (< 0.1-100 ng g<sup>-1</sup>) and exceeding 6 times Canadian TEL values (34.1 ng g<sup>-1</sup>). In order to estimate the environmental risk of these sediments, the ratios between the average metal and PCBs concentrations and TELs were calculated. Estimated risks decreased from Cr (15) >> PCBs (6.4) > Zn (5.7) > Cu (5.0) > Pb (4.6). Average Cr concentrations even exceeded for more than six times the probable effect level (PEL<sub>Cr</sub> = 90 µg g<sup>-1</sup>) while PCBs were below (PEL<sub>PCBs</sub> = 277 ng g<sup>-1</sup>) indicating that Cr has the highest potential toxicity and environmental risk regardless a potential combined effects in this multiple-contaminant stressed environment.

**FP035 How does manganese affect tilapia (*Oreochromis niloticus*)? A genotoxic and biochemical bioassay.**

G. Carvalho Coppo, L. Souza Passos, T. Miura Pereira, University of Vila Velha; J. Merçon, University of Vila Velha / Programa de Pós Graduação em Ecologia de Ecossistemas; D. Cabral, T. Onesorge Miranda Lopes, B.V. Barbosa, University of Vila Velha; A. Chippari Gomes, Universidade Vila velha / Applied Ichthyology Laboratory In 2015, two dams in Minas Gerais containing mining tailings were broken, culminating into an accident that affected the states of Minas Gerais and Espírito Santo (Brazil), and decreased the quality of water and life as a whole in the affected rivers. Analyzes carried out in the affected rivers, one year after the event, verified the presence of many metals in concentrations higher than those found soon after the arrival of the tailings mud. Manganese was one of the highest concentrations, and although essential to living things, when in high concentrations it is detrimental to the proper functioning of the organism. In this context, the present work aimed to evaluate the toxic effects of manganese in *Oreochromis niloticus* by biochemical analyzes (activity of glutathione S-transferase (GST) and catalase (CAT)), genotoxicity (comet assay) and quantification of metals in the tissues of the animals (gills, liver and muscle). Thirty-two animals were exposed to four different treatments: control group (T1 - 0.0 mg/L); 0.5 mg/L (T2); 2.5 mg/L (T3) and 5.23 mg/L (T4) of manganese, during the 96 hours experimental period. After the 96-h period, blood was collected from all animals via the caudal vein puncture for the comet assay, and the hepatic and gill tissues for the enzymatic assays and quantification of the accumulated metal in the liver, gills and skeletal muscle. Exposure to the contaminant caused a significant increase in DNA damage index in erythrocytes of organisms exposed to T3 and T4 treatments. The enzyme GST showed a significant increase in its activity only in the liver of the organisms exposed to T4 treatment. In relation to CAT, a significant increase was observed in the gills of the exposed animals at all manganese concentrations tested. Manganese was accumulated in greater amounts in the liver, followed by gills and muscle. It is concluded that manganese causes significant damage to genetic material and generates nuclear abnormalities, activates the body's detoxification system, and is liable to accumulate in animal tissue.

**FP036 Individual and combined effects of Zn, Mn and Fe on the Neotropical teleost *Prochilodus lineatus*: bioaccumulation and oxidative stress biomarkers**

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Metal bioaccumulation and oxidative stress biomarkers were determined in the Neotropical teleost, *Prochilodus lineatus*, in order to understand the effects of short-term exposure to essential metals Zn, Mn and Fe, both individually and combined in a mixture. Three independent tests were carried out. In the Zn test, fish were exposed to three concentrations (0.18, 1.0 and 5.0 mg L<sup>-1</sup>) or to water only (CTR); in the Mn test, fish were exposed to three concentrations (0.1, 0.5 and 5.0 mg L<sup>-1</sup>) or to Mn CTR; and in the Mix test fish were exposed to Fe (5.0 mg L<sup>-1</sup>) and a mixture of Zn (1.0 mg L<sup>-1</sup>) + Mn (0.5 mg L<sup>-1</sup>), with and without Fe, or to Mix CTR. After exposure for 96 h, tissues were removed for metal bioaccumulation analysis. Oxidative stress biomarkers, such as lipid peroxidation (LPO), protein carbonylation (PCO), reduced glutathione (GSH), metallothionein (MT) and the activity of superoxide dismutase (SOD) and glutathione S-transferase (GST) were determined in liver tissue. DNA damage was analyzed in blood cells using the comet assay. In control groups, metal distribution varied from one tissue to another, depending on the metal analyzed, as follows: for Zn: <em>muscle< blood cells< brain=gills< bile< liver< kidney

**FP037 Influência da variação pluviométrica sobre a toxicidade da água e do sedimento no trecho paulista do rio paraíba do sul**

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O objetivo deste trabalho foi avaliar como a precipitação pluviométrica pode influenciar a toxicidade da água e do sedimento de um trecho paulista do Rio Paraíba do Sul. Amostras de água e sedimento foram coletadas trimestralmente no Rio Paraíba do Sul, entre os meses de setembro de 2013 a agosto de 2014, em pontos a montante e a jusante do perímetro urbano de quatro municípios paulistas: Aparecida, Guaratinguetá, Lorena e Cachoeira Paulista. A determinação do efeito tóxico agudo (ETA) da água e do sedimento foi realizada utilizando o crustáceo *Daphnia similis*. O efeito tóxico crônico (ETC) da água foi determinado utilizando a alga *Raphidocelis subcapitata* e do sedimento utilizando o díptero *Chironomus xanthus*. Os resultados foram expressos como tóxico e não-tóxico a partir do software Toxstat versão 3.3. Com o intuito de determinar como a precipitação influencia sobre a toxicidade em diferentes compartimentos do corpo hídrico, os resultados dos bioensaios e as médias mensais de precipitação da região foram submetidos à análise dos componentes principais (ACP) através do software Past. No presente estudo, das 32 amostras de água coletadas, 28,1% apresentaram ETC e 6,25% apresentaram ETA. A toxicidade na água foi observada somente no período de maior precipitação, novembro de 2013 e fevereiro de 2014. Quanto às amostras de sedimento, o ETC foi observado em 21,9% das amostras enquanto o ETA foi observado em 15,6%. Embora o sedimento tenha apresentado toxicidade em todos os períodos avaliados, o período com menor precipitação obteve maior número de pontos que apresentaram toxicidade. O componente principal formado pela ACP explicou 75,48% de variância entre as variáveis avaliadas neste estudo. O ETA (0,785) e ETC (0,556) observados na água mostraram-se mais intimamente relacionados à precipitação (0,654) quando comparado ao ETA (-0,162) e ETC (-0,338) do sedimento. Os resultados obtidos sugerem que a precipitação pluviométrica atua de forma distinta sobre os diferentes compartimentos de um corpo hídrico. A toxicidade da água é influenciada pelo aumento das chuvas, uma vez que podem promover o arraste de compostos tóxicos para a coluna d'água. Diferentemente, a toxicidade no sedimento mostrou-se mais relacionada a períodos de seca. A redução da vazão do rio, devido à escassez de chuvas, pode propiciar a deposição de compostos tóxicos no leito do rio.

**FP038 Interactive effects of ocean acidification, increasing temperature and copper enrichment in the zooxanthellated coral MUSSISMILIA HARTTII**

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Symbiosis between corals and zooxanthellae (microalgae of the genus *Symbiodinium*) is one of the main features of reef building corals. This relationship is a major determinant of individual's coral health and, ultimately, reef function. Disruption of coral-zooxanthellae symbiosis (known as bleaching) due to rising seawater temperature, ocean acidification and chemical pollutants is a major threat to coral reefs. Considering that these stressors are expected to occur concomitantly, the present study aimed to evaluate the combined effects of climate change (seawater acidification and warming) and local stressors (copper enrichment) in the stability of the relationship between the scleractinian coral *Mussismilia harttii* and their endosymbionts. The photosynthetic efficiency and chlorophyll *a* content were evaluated as bleaching proxies. An experiment of acidification, increasing temperature and copper (Cu) enrichment was conducted in a marine mesocosm (Projeto Coral Vivo, Porto Seguro, BA, Brazil). Fragments of the coral *M. harttii* were collected (Porto Seguro, BA, Brazil), acclimated, and exposed (up to 28 days) to nominal Cu concentrations (0 and 5 µg/L), increasing temperature (+2°C), and acidification (-0.3 pH). Treatments were selected according to IPCC (2014) predictions and applied in isolation and combined (8 treatments; n=9 per treatment). Data were analyzed using one-way ANOVA followed by the Tukey test. After 28 days, corals exposed to the combined treatments of seawater acidification and increased temperature, as well as seawater acidification, increased temperature and Cu enrichment, showed significant reduction in chlorophyll *a*. Also, photosynthetic efficiency was significantly reduced in corals exposed to the combined treatment of seawater acidification, increased temperature and Cu enrichment. In summary, our findings indicate a higher bleaching occurrence in the treatment that combined both global (acidification and warming) and local (Cu enrichment) stressors, a likely future scenario to occur in several reefs worldwide. Considering the lack of knowledge about the ecophysiology of Southern Atlantic coral species and studies that consider the scenario of multiple stressors associated with global climate change and chemical contamination, our findings are expected to provide key information for the conservation of reefs at both national and global scales.

**FP039 Is 1+1 more than 2? Joint effects of traditional xenobiotics and**

**nanoparticles on two aquatic species, *Daphnia magna* and *Chironomus riparius***  
K. Pakarinen, University of Eastern Finland / Department of Environmental and Biological Sciences; K.M. Figueiredo, University of Eastern Finland / Department of environmental and biological sciences

Environmental exposure to chemicals usually occurs to several chemicals simultaneously. Joint effects may lead either additive, synergistic, or diminutive effects. In the current study, joint effects of carbon nanomaterials (fullerenes) were studied with two widespread traditional xenobiotics, organotin compounds (TOTs) or benzo[a]pyrene (B[a]P), on two aquatic species, *Daphnia magna* and *Chironomus riparius*. Fullerenes are a relative new group of materials with unique properties promising new applications, but also new hazards. Production and use of fullerenes are increasing rapidly, which increases the potential unintentional release to environment. An important property of fullerenes is the ability to bind other chemicals, which may lead to joint effects. Fullerenes can themselves affect reproduction on aquatic invertebrates. Also TOTs and B[a]P affect reproduction on the investigated species. Both species were exposed in all of the phases of their life cycle in several generations, thus mimicking natural situation in contaminated areas. Endpoints for *D.magna* were survival, offspring production, sex ratio, production of resting eggs, and adult size. For *C.riparius* the endpoints were survival, larval growth, emergence time, sex ratio of emerged adults and their ability to produce offspring in several generations.

**FP040 Liver melanomacrophages as a biomarker to lithium contamination in bullfrog tadpoles**

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Anurans can be exposed to pollutants in both aquatic and terrestrial ecosystems because they have a biphasic life cycle. The liver of anurans has a specialized cell type called melanomacrophages. These cells contain melanin, besides catabolic substances (e.g., hemosiderin and lipofuscin) in their cytoplasm. The function of these cells are related to detoxification of pollutants and neutralization of free radicals. Lithium (Li) is a metal present in aquatic environments polluted with industrial and domestic waste. Here, we tested the hypothesis that Li alters liver melanomacrophages and liver physiology. We exposed 6 bullfrog tadpoles (Gosner 25) to 2.5 mg.L<sup>-1</sup> of lithium chloride for 96 hours in a laboratory experiment. Data analysis involved an independent Student's *t* test. The area of melanomacrophages in liver of tadpoles exposed to Li (134.55±16.03 μm<sup>2</sup>) increased significantly compared to the control (91.32±11.62 μm<sup>2</sup>). Melanin is a biopolymer that neutralizes free radicals from tissue oxidation. In addition, hemosiderin increased in the treated group (1750.45±23.37 μm<sup>2</sup>) compared to the control (628.05±27.92 μm<sup>2</sup>). Hemosiderin is a catabolic from erythrocyte degradation. Therefore, we hypothesize that Li increase the degradation of erythrocytes in the liver, increasing hemosiderin production. Thus, melanomacrophages in the liver of bullfrog tadpole are good morphological biomarkers to evaluate environmental contamination by lithium.

**FP041 O uso do elutriato para análise da contaminação do solo por agrotóxicos e para a avaliação da toxicidade em organismos não-alvo**

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A agricultura é uma das principais bases da economia brasileira, sendo o Brasil um dos maiores produtores agrícolas e, ao mesmo tempo, o maior consumidor de agrotóxicos em nível mundial, o que implica em elevado potencial de contaminação de sistemas terrestres e aquáticos, com efeitos ecotoxicológicos em diferentes níveis da cadeia trófica. Uma das formas de contaminação dos ecossistemas aquáticos por agrotóxicos ocorre por processo de lixiviação do solo, o que torna essencial o uso de diferentes métodos para avaliação da toxicidade, como é o caso dos testes com elutriato. Considerando esta abordagem, avaliou-se a toxicidade aguda de amostras de elutriato provenientes de sistemas experimentais (12 simuladores) contaminados com os agrotóxicos Kraft® (inseticida/acaricida) e Score® (fungicida), aplicados isoladamente e em mistura, em *Ceriodaphnia silvestrii*. Para os experimentos foram utilizados 4 tratamentos (Kraft, Score, Kraft + Score e Controle), em simuladores preenchidos com 0,1 m<sup>3</sup> de solo (peneirado e seco em estufa a 60°C), os quais receberam a aplicação de 4,8 μL de Kraft e 1,3 μL de Score, ambos diluídos em 1 L de água Milli-Q, sendo reaplicados a cada sete dias. A cada reaplicação dos produtos,

uma parcela do solo foi retirada e congelada para posterior realização dos ensaios que seguiu a norma da ABNT NBR 13373. Sobre as unidades experimentais foram simuladas 6 chuvas (duas vezes por semana), as quais variaram em função da capacidade de infiltração do solo. Os dados foram analisados por meio de Análise de Variância (ANOVA), seguida de Dunett. Os resultados obtidos demonstraram uma imobilidade de 100% dos organismos expostos ao Kraft® e a mistura deste ao Score® (K+S), a partir do elutriato, enquanto nenhuma mortalidade foi registrada nas águas dos tratamentos Controle e Score. Pela análise das variáveis físicas e químicas (pH, oxigênio dissolvido, sólidos totais dissolvidos e condutividade), verifica-se que as mesmas se mantiveram dentro do estabelecido para a espécie-teste, exceto a turbidez, a qual variou entre 33.000 e 89.000 NTU, mesmo no Controle. Ressalta-se, porém, que no controle os organismos se mostraram ativos, evidenciando o efeito do Kraft (isolado e em mistura) na imobilidade dos organismos. Conclui-se que o inseticida/acaricida Kraft tem efeitos letais também em comunidades não alvo, o que requer maior atenção quanto a sua utilização (dose e frequência), além do potencial de contaminação das águas subterrâneas.

**FP042 Poor quality of water causes biochemical and behavioral changes in *Aegla longirostri* (Crustacea, Decapoda, Anomura)**

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Human activities introduce toxic substances into aquatic environments, generating conditions of hypoxia and poor water quality. Considering the imminent risk to aquatic organisms, the objective of this study was to analyze the effect of hypoxia and pesticide exposure on the biochemical, behavioral and survival responses of *Aegla longirostri*. Adult males were collected in a preserved lotic environment (29W 35 '38 ", 53S 33' 17"). After acclimatization under laboratory conditions, the specimens were submitted to two experiments: hypoxia and pesticide exposure. The hypoxia experiment was divided into three treatments (N = 10): 8.9 ppm OD (standard oxygenation), 2.5 ppm OD (moderate hypoxia), and 1.5 ppm OD (severe hypoxia) lasting 24 Hours. In the pesticide exposure experiment, water collected in two streams was used, one employee being a control (29W 35 '38 ", 53S 33' 17") and the other as exposure to pesticides (29W 34 '54 ", 53S 32' 02 "- containing atrazine and imidacloprid). The animals were submitted to 7 (N = 15) and 15 (N = 20) days of exposure. A behavioral analysis of the animals was performed on the 7th and 15th day of exposure. Percentage of lethality was evaluated during exposure to pesticides. After cryoextraction, the tissues (hepatopancreas, muscle and gills) of crustaceans were used in the biomarker assays catalase (CAT), acetylcholinesterase (AChE), glutathione S-transferase (GST), non-protein thiols, lipid peroxidation (PL) and carbonylation of Proteins (CP). One-way ANOVA was used to compare differences between treatments in each experiment. Hypoxia induced biochemical responses in *A. longirostri*, as evidenced by increased levels of all biomarkers tested. Exposure to pesticides altered the behavior of these crustaceans, diminishing their activity during the night, which could compromise their foraging behavior, considering that they have habit mainly nocturnal. The presence of pesticides in the water affected the antioxidant responses and the survival of *A. longirostri*, with increase of all the biomarkers tested except for GST, which was inhibited, as well as increase in the percentage of lethality. The exposure of *A. longirostri* to atrazine and imidacloprid, although at low concentrations, and moderate and severe hypoxia were able to induce oxidative damage in the short term. Therefore, it is undeniable that anthropic activities may affect the survival and integrity of aquatic organisms.

**FP043 Pyrimethanil Fungicide Reduces Genetic Diversity of *Chironomus sancticarloi* (Diptera: Chironomidae)**

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In the agricultural land use, the Pyrimethanil (4,6-dimethyl-N-phenyl-2-pyrimidinamine) fungicide is one of the most widely used in monocultures and has been detected in aquatic ecosystems. These genotoxic products increase the frequency of lesions in the DNA molecule, thereby increasing the risk of replication and transcription of altered DNA sequences. Genetic approaches can offer great tools for examining the current status of populations, understanding the history of population changes and constitute an important approach to assess contaminant-induced changes,



antedating future population directions. This study aimed to assess the loss of genetic diversity of *Chironomus sancticarloi*, exposed to Pyrimethanil by RAPD-PCR (Random Amplified Polymorphic DNA). We test the impacts of different Pyrimethanil concentrations and compare with control site. Ecotoxicological bioassays were conducted in triplicates with 10 days lasting, in flasks of 500 mL, with 60 g of sterile sand, 240 mL of Pyrimethanil solution in the following concentrations: 25 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup> and 75 mg L<sup>-1</sup>. The genetic diversity was estimated using six primers (OPB4, OPB5, OPB6, OPB7, OPB17 and OPB18) 10 bases. RAPD profiles revealed substantial differences between the control and exposed organisms. In the organisms without treatment, we found 58 bands between 2500-250pb. In the organisms treated with fungicides there was a decrease in the number of bands in all tested concentrations. We found 24 bands in the 25 mg L<sup>-1</sup> Pyrimethanil concentration, 21 bands in the 50 mg L<sup>-1</sup> concentration and 11 bands in the 75 mg L<sup>-1</sup> concentration. The results showed that there was significant loss in the genetic diversity in the organisms exposed to high Pyrimethanil concentrations when compared with control site. The use of the RAPD has proven to be an effective method for the detection of genetic diversity in *C. sancticarloi* exposed to fungicides. Although Pyrimethanil according to EFSA present a low acute toxicity, in our studies demonstrated that the organisms exposed to the fungicide, even at low concentrations, showed significant alterations in the DNA. Continuing the experiments for some additional *C. sancticarloi* larvae generations would point to additional elucidations.

**FP044 Rações comerciais para peixes podem conter esteróis, os quais são desreguladores endócrinos**

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A qualidade da alimentação utilizada para manter peixes em experimentos de laboratório, em especial, para avaliar os efeitos poluentes na fisiologia desses organismos é um fator essencial para o sucesso dos estudos. A presença de substâncias que podem alterar o metabolismo do peixe pode comprometer completamente os resultados obtidos nos experimentos. O objetivo deste estudo foi avaliar a composição de várias rações comerciais para peixes, comercializados internacionalmente, em particular a presença de esteróis. As rações de peixes em estudo foram extraídas com dois solventes diferentes - metanol e metil terc-butil éter, e os extratos obtidos foram evaporados, silanizados com MTBSA e analisados pelo GC / TOF-MS. Para as análises, foi utilizada coluna um Zebron ZB-5MSi, gás de hélio, programação de temperatura de 90 ° C, durante 1 min, de 90 a 290 ° C, utilizou-se uma rampa de 15 ° C/min. Os resultados obtidos revelaram a presença de esteróis tais como beta-sitosterol, estigmasterol em concentrações na faixa de ppm. Esses esteróis são abundante em matérias-primas como a soja, que tem sido usada amplamente para produzir alimentos para peixes. Os efeitos do beta-sitosterol e Estigmasterol descrito na literatura é bastante controverso. Particularmente em peixes, vários estudos mostraram efeitos estrogênicos. Portanto, o uso de tais rações pode comprometer significativamente os resultados obtidos na avaliação dos efeitos fisiológicos dos xenobióticos, particularmente aqueles que são desreguladores endócrinos.

**FP045 Reflexo da exposição de embriões de peixe-zebra à permetrina sobre parâmetros comportamentais de agressividade e ansiedade na fase adulta.**

M.E. Nunes, Universidade Federal de Santa Maria; L. Schimith, L.P. Leandro, D.C. Silva, I. Kemmerich, A. Lopes, N.R. Carvalho, Universidade Federal do Pampa; T. Posser, Universidade Federal do Pampa; J.L. Franco, Universidade Federal do Pampa. Inseticidas piretróides sintéticos (IPS) são considerados seguros no ponto de vista toxicológico e ambiental por apresentarem baixa toxicidade em mamíferos e menor persistência no ambiente, quando comparado a outros inseticidas, tais como organofosforados. No entanto, vários estudos têm demonstrado maior acumulação e sensibilidade a esse composto em humanos e modelos animais em estágios de desenvolvimento. Outros trabalhos também têm demonstrado uma maior prevalência do IPS permetrina (PM) no ambiente em relação a outros inseticidas. A exposição de embriões de peixe-zebra à concentrações de maiores que 0,1 mg/L levaram a alterações morfológicas, mortalidade e também alteração na expressão de genes importantes para a diferenciação do sistema nervoso durante os primeiras fases do desenvolvimento. Organismos em desenvolvimento apresentam uma alta taxa de divisão celular, o que leva a uma maior sensibilidade da persistência danos ocasionados pela exposição durante os estágios iniciais de desenvolvimento. Desse modo, este estudo tem como objetivo avaliar os efeitos da exposição de embriões de peixe-zebra à permetrina promove alteração em parâmetros comportamentais de agressividade e ansiedade durante a fase adulta. Resumidamente, embriões de peixe-zebra com até 3 horas pós fertilização (hpf) foram expostos extaticamente durante 48h às concentrações de 0,025 e 0,05 mg/L. Após a exposição, os embriões sobreviventes

foram mantidos em condições laboratoriais até atingirem a fase adulta (3 meses), onde os quais foram submetidos ao testes comportamentais de habituação ao novo tanque e de agressão induzida pelo espelho. Nós observamos um aumento significativo no comportamento agressivo dos peixes que foram expostos à PM, quando comparado ao controle, observado por um aumento no número e tempo de ataques. Também foi observado uma diminuição significativa do tempo de permanência na superfície do novo tanque e também um significativo aumento no tempo de latência a primeira entrada na superfície, comportamentos que demonstram um caráter menos ansioso do peixe-zebra. Em conclusão, foi possível observar que mesmo em concentrações subletais os efeitos da exposição a PM durante os estágios iniciais de desenvolvimento podem refletir em alterações comportamentais não-motoras relacionadas com agressão e ansiedade em peixe-zebra.

**FP046 Sediment Quality Triad in a Monitoring Network - 15 years' experience**

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Since 2002, CETESB adopted the Sediment Quality Triad, an approach proposed by Long & Chapman (1985), to evaluate sediment quality in a more robust manner, with an annual monitoring network in the State of São Paulo (Brazil). Until 2016, 146 sites were sampled, some more than once, amounting to 354 diagnoses, of which 62% were based on three lines of evidence (chemical, ecotoxicological and ecological). Besides physical characterization, analysis of heavy metals, organic contaminants and *Vibrio fischeri* assays were carried out as indicators of exposure. Ecotoxicological assays included acute and chronic assays with microcrustacean and insect. Genotoxicity assays and benthic community analysis completed the evaluation of effects. The most frequent diagnosis (58%) indicated both chemical and ecological impacts, with no ecotoxicological effect. Impairment observed in the three lines of evidence, represented 28% of the diagnoses. At 13 sites diagnosis oscillated between these two over time, indicating that to confirm the toxic potential of a site with high concentrations of contaminants, additional evaluations are necessary. Of the total sampled, 21 sites are included in the Water Quality Monitoring Network. Occasionally contaminants not observed in the water were detected in the sediment in high concentrations. In other cases, contaminants exceeding the national quality standards in the water were not observed in the sediment in critical concentrations, which suggests the existence of active sources in the basin that may have a negative effect on the aquatic community. The evaluation of effects indicated impacts to aquatic life and was complementary to the chemical diagnosis by allowing inferences about bioavailability of the contaminants and avoiding equivocal conclusions based solely in guidelines. Since high concentrations of phosphorus in sediments contribute to eutrophication, determination of total phosphorus was included in the assessment, as well as microbiological markers of sewage (*Clostridium perfringens* and *E. coli*). The application of the triad in the monitoring program improved the diagnosis obtained exclusively with water quality variables, not only determining the presence of contaminants, but their potential effect on the biota as well. The introduction of a new line of evidence to detect organic enrichment revealed the deleterious effect of domestic sewage and eutrophication on the biota, as well as the persistence of this problem.

**FP047 Teor de Pigmentos clorofilianos carotenoides em clorófitas submetidas a efluente contaminado com compostos nitroaromáticos**

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para ambos os pigmentos. *C. vulgaris* apresentou teores mais elevados e as reduções foram dose-dependentes a partir da concentração de 0,0625 em relação ao controle, com  $R^2$  de 90 e 93% para clorofilas a e b, respectivamente. Os resultados observados no teor de carotenoides (betacaroteno,  $R^2=75\%$  e astaxantina,  $R^2=76\%$ ) foram diferenciados em ambas as espécies. Houve elevação no teor de carotenoides nas diluições de 0,05 e 0,10% e redução a partir da diluição 0,15% em *P. subcapitata*. *Chlorella vulgaris* foi mais sensível ao efluente e apresentou redução a partir da concentração de 0,0625% quando comparada ao controle, com coeficientes de determinação de determinação por volta de 90%. Estes resultados permitem dizer que as algas, que representam a base das cadeias alimentares dos ecossistemas aquáticos, têm o teor de pigmentos clorofilianos e carotenoides; e consequentemente seu crescimento (dados não apresentados) afetado em presença de efluente contaminado com compostos nitroaromáticos. Pode-se dizer ainda que, as concentrações de efluente com compostos nitroaromáticos toleradas pelas espécies testadas são da ordem de 0,15%.

**FP048 Teor de Pigmentos em clorofíceas submetidas a efluente contaminado com compostos nitroaromáticos**

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Derivados do trinitrotolueno são originados nas etapas de lavagem dos processos de fabricação de explosivos. Essas indústrias geram efluentes com níveis diferenciados de toxicidade e ainda não foram estabelecidos limites restritivos para estes compostos. O presente trabalho objetivou avaliar o efeito do efluente da indústria de explosivos (água vermelha) no teor de pigmentos clorofilianos e carotenoides em *Pseudokirchneriella subcapitata* e *Chlorella vulgaris*. Foram realizados ensaios ecotoxicológicos com *P. subcapitata* e *C. vulgaris*, as quais foram submetidas a diluições crescentes (0,05 a 0,25%) e (0,0625 a 0,31), respectivamente, do efluente Água vermelha (AV) contaminado com compostos nitroaromáticos. O teor de pigmentos clorofilianos em *P. subcapitata* (clorofila a e b) foi afetado nas concentrações de 0,15 a 0,25% do efluente, em comparação ao controle. A análise de regressão confirma esta tendência com coeficientes de determinação por volta de 65% para ambos os pigmentos. *C. vulgaris* apresentou teores mais elevados e as reduções foram dose-dependentes a partir da concentração de 0,0625 em relação ao controle, com  $R^2$  de 90 e 93% para clorofilas a e b, respectivamente. Os resultados observados no teor de carotenoides (betacaroteno,  $R^2=75\%$  e astaxantina,  $R^2=76\%$ ) foram diferenciados em ambas as espécies. Houve elevação no teor de carotenoides nas diluições de 0,05 e 0,10% e redução a partir da diluição 0,15% em *P. subcapitata*. *Chlorella vulgaris* foi mais sensível ao efluente e apresentou redução a partir da concentração de 0,0625% quando comparada ao controle, com coeficientes de determinação de determinação por volta de 90%. Estes resultados permitem dizer que as algas, que representam a base das cadeias alimentares dos ecossistemas aquáticos, têm o teor de pigmentos clorofilianos e carotenoides; e consequentemente seu crescimento (dados não apresentados) afetado em presença de efluente contaminado com compostos nitroaromáticos. Pode-se dizer ainda que, as concentrações de efluente com compostos nitroaromáticos toleradas pelas espécies testadas são da ordem de 0,15%.

**FP049 The effect of acclimation on the temperature-dependent toxicity of Glyphosate to a tropical microcrustacean**

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In recent years, there has been a growing interest in understanding how organisms and ecosystems respond to multiple stressors, especially the interaction between climate change and pollution. A key aspect to understand climate change-pollution interaction is the assessment of the effect temperature on the toxicity of contaminants, known as temperature-dependent chemical toxicity (TDCT). The few TDCT studies available have shown that toxicity is heavily affected by temperature, but the shape of the response varies amongst substances and test species. Furthermore, the effect of organismal temperature-acclimation and its effect on the TDCT is still unknown. In this study, we assessed the effect of reversible and developmental acclimation on the TDCT of the tropical cladoceran *Ceriodaphnia silvestrii* to the herbicide Glyphosate, one of the most widely used herbicides in the world. We assessed the acute toxicity of the Glyphosate base herbicide AKB® herbicide to *Ceriodaphnia* in three temperatures: 20°C, 25°C and 30°C. Reversible acclimation was tested by transferring neonates (< 24 hours) borne at each of the test temperature to another test temperature before exposure to the herbicide. Developmental acclimation was tested by running the

reversible acclimation test with the second generation of organisms borne from females raised at each of the test temperatures. The results demonstrated that temperature has a significant effect on herbicide toxicity to *Ceriodaphnia silvestrii*. The reversible acclimation test showed a U-shaped pattern in the TDCT, with a lower toxicity at 25°C. The developmental acclimation test showed that organisms acclimated at 20°C were significantly more sensitive than those acclimated at 30°C. Our results suggest that temperature acclimation is a key factor in understanding the toxicity variation in *Ceriodaphnia*. Also, developmental acclimation to higher temperatures appears to offer a protective effect to *Ceriodaphnia*. Therefore, standard laboratory toxicity assessments usually made at 20°C may overestimate the toxicity of Glyphosate to tropical *Ceriodaphnia*. In order to make more realistic risk assessments, temperature must be taken into consideration by means of incorporating temperature acclimation and TDCT in standard bench protocols.

**FP050 The effect of tissue type in the assessment of bioaccumulation of heavy metals in seafood**

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Marine environments are under constant pressure from various types of anthropogenic stressors. One stressor of especial concern is the pollution by metal elements, which pose various threats to the marine ecosystem due to the persistence of these contaminants in situ and their possible toxicity. Another aspect of concern is the ability of metals to bioaccumulate in the marine food chain, increasing in concentration in higher food chain levels. Because humans occupy the highest trophic level in the planet, metal bioaccumulation poses a direct threat to human health. Several metals (known as "heavy metals") are toxic to humans, and are often found in high concentrations in seafood. Recently, it has been suggested that the effects of climate change on aquatic ecosystems are likely to contribute to an increase in bioaccumulation of contaminants in seafood, such as by the increasing of bioavailability due to the acidification of marine ecosystems. Consequently, monitoring of contaminants in seafood is becoming increasingly important. In this study, we assess multiple elements in three heavily consumed fish species from the Southeast of Brazil. Samples of muscle and swimming fins were collected from *Sphyrna guachancho* (Barracuda), *Priacanthus arenatus* (Common bigeye) and *Genidens genidens* (Guri sea catfish), all acquired from local fishermen. Multielemental quantification was done using Synchrotron light Total Reflection X-ray Fluorescence (TXRF). Results showed a significant difference of metal elements concentration between species, with *S. guachancho* showing the highest levels of heavy metals in muscle, especially Hg, Cr, Cu and Zn. The concentrations of metals were usually higher in fins than in muscle of *P. arenatus* and *G. genidens*, but not in *S. guachancho*. These results suggest *P. arenatus* and *G. genidens* are capable of allocating potentially toxic metals from muscle to fins, which is likely to reduce the bioavailability and the potential toxicity of these elements to the organism. Such ability is likely reduced in *S. guachancho* which, in addition to the fact that it occupies the highest trophic level amongst the studied species, may explain why higher concentrations of metals are found in muscle tissue. Our results suggest monitoring of heavy metals in fish should take into consideration both the type of tissue used for the assessment and the ecology of the target species.

**FP051 The use of a mesh net to separate Acartia tonsa stages on ecotoxicology essays could bias results?**

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The use of the copepod *Acartia tonsa* has been suggested in protocols of acute toxicity tests by many organizations. This copepod is a free spawning holoplanktonic organism that under favorable conditions reproduces continuously with all copepodite stages (I to VI) being found in cultures. Since they can range from 110 to 1500 µm in length, the recommendation of using a single mesh size to select copepods of a specific stage may include different stages in the experiments, changing the results of toxicity and the reliability of the test, because of the differences in sensibility between stages (e.g. early stages of *A. tonsa* are more sensitive than adults). The aim of this work was to evaluate the retention of live and fixed copepodite stages of *A. tonsa* using meshes of 180, 330 and 500 µm. For fixed organisms, 3 replicates containing 50 copepodites of each stage (i.e I to VI) were sieved on each of meshes and the number of retained organisms were recorded. For living copepods, 9 cultures were started with the same amount of adults and maintained for 3 weeks before being filtered by each net mesh (3 replicates per mesh). Those that were retained and those that passed through the nets were staged and counted. A general linear model were used to evaluate the retention. The 180 µm mesh retained all stages of living and fixed copepodites. 330 µm presented

a mean retention of the living stages of 0%, 37%, 33%, 69%, 79% and 97% for CI, CII, CIII, CIV, CV and CVI respectively, for fixed organisms it retained 5% in CI, 15% in CII, 77% in CIII, 74% in CIV, 99% in CV and 100% in CVI. The 500  $\mu\text{m}$  retained 20%, 0%, 22%, 10%, 3% and 21% of CI, CII, CIII, CIV, CV and CVI for living organisms and for fixed 1%, 0%, 2%, 1%, 25% and 22%, respectively. Retention of living organism presented significant differences ( $p < 0.05$ ) in retention of CII, CIII, CIV, CV and CVI between the meshes of 180 and 500  $\mu\text{m}$ , and CIV, CV and CVI between 330 and 500  $\mu\text{m}$ . For fixed organisms, significant differences ( $p < 0.05$ ) in retention in all the stages between 180 and 500  $\mu\text{m}$  and in CI, CII, CIII and CIV between 180 and 330  $\mu\text{m}$ . The 330 and 500  $\mu\text{m}$  also presented significant differences ( $p < 0.05$ ) between them for CII, CIII, CIV, CV and CVI. Based on the results, the separation of *A. tonsa* copepodites must be performed using a stereoscopic microscope, to ensure that the desired stage of development is the one used in the bioassay, since net meshes can not be used to separate only one stage of development.

**FP052 The use of FTIR spectroscopy as a tool to assess the effects of acidification on Amphipods *Hyale youngi***

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In recent years, several CO<sub>2</sub> mitigation technologies have been proposed to reduce damages caused by climate change. The Carbon Capture and Storage (CCS) has been highlighted as one of these technological alternatives. The process consists in the capture of gases at the source, their compaction in denser gases and their injection into geological structures. Although CCS is considered safe, few studies have assessed the risks of CO<sub>2</sub> leaking from storage. One consequence of CO<sub>2</sub> leakage is the medium acidification, which could lead to an increase in metal bioavailability, with potential toxic effects to marine organisms. The objective of this study was to assess the effects of acidification on the metabolism of amphipods *Hyale youngi* using the variation in individual macromolecular composition as an indicator of sublethal toxicity. A CO<sub>2</sub> automatic injection system was used to simulate CO<sub>2</sub> leakage in microcosms with contaminated sediment from the field. Animals were exposed for 10 days to contaminated metal sediments at different pH treatments: pH 8.1 (control), 7.6, 7.0, and 6.2. After the exposure period, Fourier Transform Infrared Spectroscopy (FTIR) was used to assess macromolecular composition of amphipods. The variation in FTIR spectra was assessed by means of multivariate analysis. In addition, an index of condition based on the ratio of the olefinic band (~3012  $\text{cm}^{-1}$  unsaturated fatty acids), CH<sub>2</sub> bands (~2924  $\text{cm}^{-1}$  saturated fatty acids) to amide I band (~1641  $\text{cm}^{-1}$ -protein content), was calculated for all treatments. Representative absorbance peaks were observed in the regions: ~ 625, 864, 1082, 1419, 1523, 1652, 2964, 3437 of all spectra. Spectra from animals exposed to various pH were significantly different. A positive correlation between pH and proportion of saturated and unsaturated fatty acids to protein was observed. Individuals exposed to pH 8.1 had higher relative contents of saturated and unsaturated lipids than those exposed to pH 7.6, 7.0 and 6.2. The results suggest animals exposed to acidic conditions show an altered metabolism, increasing the consumption of energy reserve (lipids) in comparison to animals in the control treatment. This study demonstrated that FTIR was capable to detect changes on major biochemical components of animals, and may be a useful tool to assess sublethal effects in marine organisms by means of changes in macromolecular composition associated with underlying physiological shifts in response to stress conditions.

**FP053 Toxicological assessment of technical cashew nut shell liquid combined with dimethyl sulfoxide (DMSO) using *Allium cepa* bioassay**

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The cashew plant and its derivatives have been associated with several biological effects: antioxidant, antifungal, antibacterial and insecticidal/larvicidal activities and pharmacological properties. The cashew nut is industrially prepared by submission to high temperatures (185°C) releasing technical cashew nut shell liquid (tCNSL), that is composed for phenolic lipids. This is an abundant, renewable and underestimated byproduct of cashew nut production chain. Despite the benefits, it also can occur adverse effects associated with its use, such as toxicity, cytotoxicity, genotoxicity and aneugenic and clastogenic activity. To be used for these purposes, this product, due to its viscosity and insolubility in water, needs to be emulsified using universal organic solvents. However, they also can be harmful and interfere with the action of tCNSL. So, the present study aimed to evaluate the potential toxicity and ability to induce DNA damages of tCNSL combined with dimethyl sulfoxide (DMSO) and of DMSO alone.

Four dilutions of DMSO in water were prepared (2, 2.5, 3 and 3.5%) and were added to each two concentrations of tCNSL (250.0 and 500.0  $\text{mg L}^{-1}$ ). After, *Allium cepa* seeds were exposed to dilutions for germination by 96h at 25±3°C. The analyzed parameters for the assessment were germination, mitotic, chromosomal alterations and micronuclei indexes. The results indicated that DMSO inhibits seed germination in concentrations from 2%, when applied alone. However, in combination with tCNSL these effects were partially reversed. In relation to genetic damage, the results indicated that the tCNSL in combination with DMSO didn't show statistical difference in the various tested concentrations, as well as the 2% DMSO from negative control. Nevertheless, they statistically differed from the positive control ( $P < 0.05$ ). Thus, the bioassay indicates that tCNSL combined with DMSO has no phytotoxic potential in *A. cepa*, unlike the DMSO alone, and that treatments didn't induce genetic damages in meristematic cells. **Acknowledgments:** CNPq, CAPES, FUNDECT, RESIBRAS, UFGD and UFMS.

**FP054 Use of oxidative process for the ecotoxicological reduction of cationic surfactant using freshwater microalgae.**

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Quaternary ammonium compounds (CAQs) represent the largest class of cationic surfactants widely used in domestic and industrial sectors. These compounds are toxic at low concentrations for various aquatic organisms and should be removed from the effluent before discharge into surface water. Conventional treatments, such as physical chemistry followed by biological processes, are not efficient in the complete degradation of these compounds and in the removal of toxicity. In this way, it becomes necessary to use more efficient processes. Oxidation is one of the main methods of chemical decomposition of surfactants. The objective of this work was to verify the efficiency of ozonation in the degradation of the cationic surfactant, hexadecyltrimethyl ammonium chloride, and verify the toxicity of the generated products. The surfactant solution was prepared in distilled water at a concentration of 22.8  $\text{mg L}^{-1}$ . 1000 ml solution at pH 12 was ozonized for 120 minutes in the presence and absence of antifoam (silicone). The ozone dosage was 3.64  $\text{g O}_3 \text{ h}^{-1}$ . The degradation of the surfactant was evaluated by the reduction of the total organic carbon (TOC) and the control of the generated products formed was evaluated using a chronic toxicity test with freshwater microalgae, *Pseudokirchneriella subcapitata*. In the non-silicone experiment, an excess of foam was observed in the reactor in the first minutes, as expected. But with three minutes of reaction the excess foam is gone. After 120 min of ozonation the samples were analyzed in the TOC. The maximum efficiency found was 73% removal of organic matter. No difference was observed between the experiments with and without silicone. Before treatment, the surfactant showed to be toxic to microalgae, with IC<sub>50</sub> of 0.41%. After ozonation, chronic toxicity to microalgae decreased (increased IC<sub>50</sub> values). For the test with silicone the IC<sub>50</sub> was 1.72% and without silicone it was 3.78%. As IC<sub>50</sub> values increased, no more toxic products were generated during ozonation. Comparing the IC<sub>50</sub>, it is noted that the silicone had some effect on the multiplication of the algal cells. Silicon may have impaired the exchange of gases between the medium and the atmosphere, damaging the physiological processes of microalgae. Thus, the results showed that ozonation at pH 12 without the use of the silicon and low ozone dosage is a suitable technology to reduce TOC and toxicity.

**FP055 Using ecotoxicological tests to assess the sediment quality of a tropical coastline: The case of Aracruz's coast, north of Espírito Santo, Brazil**

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The Aracruz, Espírito Santo (ES), Brazil, coastal region is subjected to different anthropogenic pressures that introduce contaminants that are adsorbed to the marine sediment. Given these influences, the present study aimed to determine the environmental quality of the Aracruz-ES coastline through toxicity tests of two invertebrate species (*Nitokra* sp. and *Echinometra lucunter*) exposed to marine sediment and elutriate, combined with principal component analysis, to identify the physicochemical variables (total organic matter - TOM, total organic carbon - TOC, total phosphorus - TP, Fe, Cd, Pb, Cr, Mn, Ni and As) that best explain the variations of the data found. Five sediment collections, during one year, were performed along the Aracruz coastline at ten sample points. The results of the sedimentary characterization for the sampling stations indicate Fe as the most abundant metallic



element present in the sediment and As as the only parameter above the limit imposed by Brazilian law. Nevertheless, most of the sediment samples were considered toxic for the sediment test with copepods (*Nitokra* sp., reproduction), and all of the sediment samples caused toxic responses in gametes and embryos of sea urchin (fertilization and development) according to the elutriate test. The toxicity results showed no significant correlation with the analyzed elements, emphasizing the influence of other factors not analyzed in this study and that can be related to the pollution sources in the region.

**FP056 Effects of the use of biosolid produced at ETE/Ilha (RJ) on aquatic organisms (*Raphidocelis subcapitata*, *Daphnia similis* and *Danio rerio*)**

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Biosolid used as nutrient and as substrate in reforestation can affect hydric resources and aquatic organisms after heavy rains or when stored incorrectly. The reduction in the population of phytoplankton algae causes damage to the biodiversity of the system and the death of zooplankton cladocerans causes changes in the trophic chain. A fish population decrease can cause changes on aquatic system as well as a shrink in ecosystemic services related to fishing. There, studying the effects of this residue use on organisms of different trophic levels is important. Tests were performed with the elutriate, being the planosol and ultisol of Embrapa Experimental Field added to biosolid concentrations of 12, 25, 37, 50 and 62%, and mixed with dilution water (proportion: 1:4). For algae, dilution was performed with culture medium; for *Daphnia*, with mineral water; and for the fish, water from their aquarium. Mixture was submitted to a Jar test stir at 150-180rpm for a 20-24h period. After 2-3h decantation, the supernatant was submitted to a 30-minute centrifugation at 2.000rpm. Essays should begin no longer than 24h after the elutriate production (NBR 15469). The essay for establishing IC<sub>50</sub> (sample concentration that causes a 50% inhibition in algae biomass in the tests recipients, when compared to the control/96h) was performed by using single-celled planktonic alga *Raphidocelis subcapitata* (NBR12648 Std). Acute essays (48h) were performed (NBR12713 Std) to establish the lethality in zooplanktonic cladocerans *Daphnia similis*; for lethality in *Danio rerio* fish (acute essays/48h) NBR15088 Std was followed. Essays for the different concentrations of biosolid show that algae are the most sensitive, followed by the cladocerans and fish with an LC/IC 50 of 16-43% range, varying according to the concentration, organism and type of soil used. Those concentrations can easily reach the aquatic environment when biosolid is used as substrate or stored in nearby areas of rivers and lakes. The application in the form of fertilizer and as substrate for reforestation being is sustainable and economically viable practice in dealing with final destination of the biosolid, but an evaluation of this effect on the biota is needed though, before spreading this practice.

**Antifouling paints: the Brazilian viewpoint about a global environmental problem.**

**FP057 Comparative environmental policies for the use of marine antifouling in Brazilian and European coastal areas**

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Antifouling paints were created with the objective of avoiding fouling accumulation of organisms on the vessels' hulls. Those paints contain powerful biocides in its formula. TBT (tributyltin - tributilestanho) is probably the most used biocide worldwide applied in almost 100% of the fleet until late 90's and also in recreational boats. Despite banned globally in 2008 by IMO (International Maritime Organization) for causing imposex on gastropods, endocrine disruption in marine mollusks and bioaccumulating on the trophic web, new formulations are available on the market. There are still many knowledge gaps about the environmental effects and impacts these new compounds including the lack of data about the potential risks compared to the banned TBT. In Sweden and England, for example, Irgarol 1051 and Diuron were also banned, whereas in Brazil the same compounds are still freely marketed. This is therefore a complex and controversial issue subjected to differential regulations and enforcement rules in different countries. Brazil does not have relevant laws, except for TBT (CONAMA Resolutions 357 and 454), or any specific definitions for biocides-based paints in legal documents placing Brazil in disadvantage compared to other countries which have restricted regulatory control tools over the entire chain from production to distribution and marketing. This study aims to develop a detailed

characterization of this problem and to establish environmental standards for antifouling paints in Brazil. Initial analysis show that new formulations do not describe compositions clearly neither provide adequate safety instructions on handling and disposal of debris. The need to develop appropriate regulatory mechanisms is imperative, both socially and environmentally, towards an adequate use and dispose of antifouling-based paints and related products.

**FP058 Efeitos letais e subletais do tributilestanho no desenvolvimento embrionário de *Danio rerio***

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O tributilestanho (TBT) tem sido amplamente utilizado pela indústria naval para formulação de tintas anti-incrustantes, e apresenta significativa toxicidade para organismos não alvo como peixes. Esse trabalho tem como objetivo analisar os efeitos letais e subletais desse contaminante para embriões e larvas do peixe *Danio rerio*. Vinte embriões de *Danio rerio* com 3 horas após a fertilização (hpF) foram expostos em cada tratamento durante um período de 96 horas às concentrações 1, 5, 25, 30, 50 e 75 µg TBT L<sup>-1</sup>, além do controle e controle do solvente (acetona). Cada indivíduo foi inspecionado em lupa num aumento de 50 a 80x diariamente para avaliação do seu desenvolvimento e documentação das patologias. A mortalidade foi atribuída quando ocorreu coagulação dos embriões, ausência de movimento e/ou batimento cardíaco das larvas, seguindo as normas da OECD 236. Efeitos no desenvolvimento embrionário e larval foram analisados a partir do índice morfológico geral (IMG), que integra informações sobre a efetiva formação de marcos morfológicos fundamentais no desenvolvimento inicial do embrião até a fase de larva, podendo atingir valor máximo de 17 numa larva que se desenvolve perfeitamente. Estes marcos morfológicos incluem características como descolamento da cauda, formação de somitos, desenvolvimento e pigmentação dos olhos, presença de batimentos cardíacos e circulação sanguínea, desenvolvimento das nadadeiras e boca, e eclosão das larvas. Larvas sobreviventes com 7 dias após a fertilização foram filmadas individualmente para avaliação de sua velocidade de natação. A concentração letal a 50% dos organismos expostos durante 96 (CL<sub>50</sub><sub>96h</sub>) foi igual a 13,9 µg L<sup>-1</sup>, indicando uma maior sensibilidade de *Danio rerio* comparado com embriões do medaka *Oryzias latipes* com CL<sub>50</sub><sub>96h</sub> igual a 46,1 µg L<sup>-1</sup>. O IMG evidenciou retardo significativo no desenvolvimento embrio-larval relativo ao controle nas concentrações 1, 5, 10 e 30 µg L<sup>-1</sup>. Foram verificadas as patologias edema pericárdico, hemorragia no coração, escurecimento da íris dos olhos, retardo na eclosão e ausência de bexiga natatória inflada. Peixes expostos ao TBT nas concentrações 1, 5, 10 e 30 µg L<sup>-1</sup> apresentaram hiperatividade natatória significativa em relação ao controle. Desta forma, o tributilestanho pode afetar o desenvolvimento embrio-larval de *D. rerio*, potencialmente afetando o recrutamento dos indivíduos expostos.

**FP059 Exposição de mexilhões *Perna perna* a biocidas anti-incrustantes e seus efeitos sobre organismos não-alvo.**

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Ecosistemas marinhos estão tipicamente sujeitos a diversos estressores ambientais, tanto de origem natural ou antropogênica. As tintas anti-incrustantes, por exemplo, vêm sendo amplamente utilizadas em ambientes aquáticos com o objetivo de proteger os cascos de embarcações contra a incrustação de organismos. Devido à alta toxicidade encontrada em alguns biocidas que compõem estas tintas, torna-se necessário investigar os seus efeitos sobre organismos não-alvo. Neste contexto, o objetivo do presente trabalho foi avaliar o efeito dos biocidas clorotalonil e DCOIT sobre aspectos fisiológicos de mexilhões *Perna perna*. Para isto, os bivalves foram expostos a 0 (controle com 0.016 mL/L de dimetil sulfoxido), 0.1 µg/L de clorotalonil, 10 µg/L de clorotalonil, 0.1 µg/L de DCOIT e 10 µg/L de DCOIT por 96 h. Após o período de exposição aos contaminantes, os organismos foram submetidos aos seguintes ensaios: avaliação do consumo de oxigênio, taxa de filtração, excreção de amônia e efluxo de rodamina b. Em uma visão geral, nossos dados demonstram que ambos os biocidas, clorotalonil e DCOIT, alteraram a maior parte dos parâmetros fisiológicos testados. Enquanto o consumo de oxigênio foi aumentado nos animais expostos, a taxa de filtração e a excreção de amônia foram diminuídas. A atividade de bombas de extrusão, avaliada pelo efluxo de rodamina, por outro lado, não foi diferente entre os indivíduos expostos e não expostos. Estes resultados indicam que os biocidas clorotalonil e DCOIT podem impactar negativamente organismos não-alvo, eventualmente reduzindo sua saúde e capacidade de sobrevivência no ecossistema marinho.

#### FP060 One more 'plus' in the International Pellet Watch: is it possible to analyse TBT?

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Microplastics, including pre-produced pellets, are an emerging class of marine pollution that is recently receiving exponential attention from the scientific community. They are ubiquitous within marine and coastal environments, and sediments are no exceptions. On coastal zones, plastic pellets co-exist with many other contaminants including biocides derived from antifouling paint formulations. These paints are essential to almost any ship or boat activity, preventing fouling and significantly reducing fuel and maintenance costs. Among several biocides used in these formulations is the synthetic tributyltin (TBT), globally considered one of the most concerning marine pollutants due to its higher environmental persistence and toxicity, when compared to other chemicals. TBT-based antifouling paints had been banned worldwide since 2008 but it is still sampled in sediments and tissues. Sediments are then the ultimate repository of microplastics and TBT on coasts. Since the 'International Pellet Watch' from Professor Hideshige Takada, a wide range of organic pollutants are being monitored when the pellet is used as a potential matrix for pollutants concentration and transport. Virgin polypropylene pellets from petrochemical facilities were fortified with TBT, followed by well-established methods of extraction and derivatization. These methods were firstly developed by Professor Takada and his team and are used worldwide until today. First results indicate that methods described for PCBs, PAHs and PBDEs are also efficient to extract and analyse organic tributyltin from pellets. Experiments now focus on the desorption of TBT from pellets initially exposed to contaminated seawater, where plastics and TBT are also in contact with estuarine organic-rich sediments. Most relevant results from these experiments are the use of pellets as a potential matrix to evaluate the transport of TBT from coasts to the ocean, effectively contributing to the International Pellet Watch; and studies on synergetic effects and availability of TBT to coastal biota when pellets are also deposited in sediments.

#### A marriage of convenience: Interactions of trace nutrients and contaminants with microbial communities

##### FP061 Concentrações ambientais de nanopartículas de cobre alteram equilíbrio bioquímico em *Chlorella sorokiniana*

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O intenso uso das nanopartículas (NPs) é decorrente de suas características peculiares, no entanto isso pode resultar em contaminação ambiental na medida em que os resíduos são carreados para os ecossistemas aquáticos. Os organismos desses ambientes são os principais afetados pelas NPs, sobretudo o fitoplâncton, que suporta os demais níveis tróficos e auxilia na manutenção do equilíbrio em ecossistemas aquáticos. Infelizmente esse equilíbrio pode ser quebrado pela mudança na composição bioquímica dos organismos, o que resulta em alimentos menos nutritivos para os próximos níveis. Este estudo objetivou investigar os efeitos de nanopartículas de cobre (NPs-Cu) na clorofíceia *Chlorella sorokiniana* exposta a concentrações ambientais que variaram de 0,95 a 5,38  $\mu\text{g L}^{-1}$ . Com foco na fisiologia e bioquímica da microalga e sob condições controladas em laboratório procedemos os experimentos, sendo que os dados de bioquímica foram obtidos em 72h de exposição, a fim de avaliar os efeitos agudos. Avaliamos a curva de crescimento, taxa de crescimento, rendimento quântico máximo do fotossistema II ( $Y_{\text{max-PSII}}$ ), proteínas, carboidratos e lipídios totais. Geralmente, o rendimento fotossintético tem sido utilizado como um "indicador" do estado fisiológico em microalgas expostas a metais, devido à sua alta sensibilidade, contudo, nesse trabalho ele se mostrou o mais conservado, indicando que os processos fotossintéticos não foram afetados. A síntese de carboidratos foi reduzida em relação ao controle e a de lipídios aumentou, sugerindo o consumo de carboidratos e acúmulo de lipídios intracelulares. Tal processo tende ser comum em situação de estresse celular que nesse caso foi desencadeada pelas NPs. Quanto à síntese de proteínas, ela foi intensificada na presença de NPs o que sugere um acúmulo dessa biomolécula resultante da impossibilidade das células se dividirem devido às NPs, uma vez que essas tendem a cessar os ciclos de divisão celular, porém os processos fotossintéticos não cessaram. De modo geral, o presente estudo mostrou que mesmo concentrações ambientalmente relevantes de NPs-Cu causaram modificações metabólicas na microalga *Chlorella sorokiniana* e esse efeito foi refletido nas gerações futuras como pode ser comprovado pela redução na taxa de crescimento nos cultivos expostos à NPs-Cu, além disso, o balanço nutricional desses organismos foi prejudicado.

#### FP062 Diversity of heterotrophic bacteria in the estuarine region of the Port of Santos, affected by the discharge of hydrocarbon derivatives and AFFF

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RMBS (Metropolitan Region of Baixada Santista) has great importance for the state of São Paulo – BR, due to its ecological aspect, with the presence of protected areas (Estuary Santos - São Vicente, mangroves and Atlantic Forest), as well as its economic aspect, with intense industrial, port and urban activities. The region is considered an example of population occupations of great environmental impact because it hosts to the Port of Santos, which is considered the largest port in Latin America, generating a great influence on the environmental quality in relation to the contamination by oil and derivatives. In 2015, the biggest fire in Brazil's industrial area occurred at Terminal Químico SA (TEQUIMAR) at the Ultracargo shipyard, which lasted nine days with the discharge of a large quantity of fuels, oil-derived hydrocarbons and aqueous film-forming foam to extinguish the fire. Degradation of hydrocarbon derivatives by microorganisms, especially bacteria, is widely known and its presence widely distributed in the oceans is often related to the concentrations present in the environment. The objective of this work was to analyze the variation of the heterotrophic bacteria present in the sediments affected by the discharge of pollutants, by comparing the data collected after the incident and 6 months later. Sediment samples were collected, processed and inoculated in Nutrient Agar using the Spread Plate technique. The colonies were isolated and characterized by the Gram staining technique. Thirty-one colonies of heterotrophic bacteria were isolated, being 39% of the genus *Bacillus* (first collection) and 34 colonies in the second collection, being 76% of the genus *Bacillus* Gram - positive. Some species of *Bacillus* are described as hydrocarbon degrading and spore forming, which facilitates their permanence and dominance in contaminated sediments for a long time. These microorganisms are ubiquitous in nature and capable of degrading various types of short chain, long chain hydrocarbons and numerous aromatic compounds, including PAH (polycyclic aromatic hydrocarbons). Due to the increase in the predominance of the *Bacillus* genus, it can be concluded that there was a bioaccumulation of the firefighting discharge, mainly hydrocarbons.

#### FP063 Ecophysiological effects of contamination by zinc on the marine microalgae *Tetraselmis gracilis* and *Dunaliella tertiolecta*

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Pollutants like trace metals entering the marine environment may have effects on aquatic organisms. Zinc represent one of the most commonly used commercially and detected pollutant in aquatic environments. This element can act as micronutrient in biochemical process and also act, in excess, as a contaminant and a negative agent in the metabolic processes. Detrimental effects on phytoplankton populations could affect nutrient cycling and food availability to higher trophic levels. This study aims to observe the toxicity effects of trace metals (Zn) on marine phytoplankton species: *Dunaliella tertiolecta* and *Tetraselmis gracilis* via population growth and photosynthetic pigments as chlorophyll a and b, carotenoids and pheopigments. The algae from culture (BMA&K-IIOUSP) with the same growth stage were inoculated in flasks (~35.000 cells  $\text{L}^{-1}$  and 70.000 cells  $\text{L}^{-1}$ , respectively by specie) and exposed to five concentrations of Zn, 5,0  $\text{mg L}^{-1}$ , 10,0  $\text{mg L}^{-1}$ , 20,0  $\text{mg L}^{-1}$ , 30,0  $\text{mg L}^{-1}$  and 50,0  $\text{mg L}^{-1}$ . A Guillard medium was used. In each group there was a control. The observation followed 24 h, 48 h, 72 h and 96 h. The growth curves of the species were evaluated, showing different behaviors. Since the first 24 hour measures, *T. gracilis* showed a growing trend for the smallest concentration (5,0  $\text{mg L}^{-1}$ , 10,0  $\text{mg L}^{-1}$ ) similar to the control, while the others ( $\geq 20,0 \text{ mg}$ ) showed a decreasing trend. The *D. tertiolecta* showed a different result, i.e. in the first 24 hours all the concentrations grew more than the control. At the end of the 96 hours essay, *T. gracilis* grew approximately 293%, 319%, 290%, 41%, 0% and 12%, in the concentrations of 0.0

mg L<sup>-1</sup>, 5.0 mg L<sup>-1</sup>, 10.0 mg L<sup>-1</sup>, 20.0 mg L<sup>-1</sup>, 30.0 mg L<sup>-1</sup> and 50.0 mg L<sup>-1</sup> respectively, comparing to the initial concentration, while *D. tertiolecta* grew approximately 337%, 300%, 215%, 203%, 196% and 232% at the same concentrations respectively. The pigments productions followed the trend of the growth curve for both species proving that the pollutants had a significant effect on the cell density but not on the pigments production. It was observed that *T. gracilis* is more sensitive to Zn contamination than *D. tertiolecta*. The zinc possibly acts in different metabolic vias and systems in each species, with population reflex when exposed to the same Zn concentration. The results of this study are an important step toward identifying the risk of trace metals to different marine organisms and understanding of their ecological effects.

**FP064 Evaluation of toxicity and color reduction of Reactive dye Yellow 160 and Red BG-3B, and textiles effluents after treatment by electron beam.**

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Textile effluents are always colored due to the amount of dyes lost during dyeing processes. The low biodegradability and high solubility makes these compounds (reactive dyes) difficult to be enough removed by conventional treatment processes. The objective of the present study is to assess toxicity of Reactive dye Yellow - 160 and Red BG-3B as well as raw effluents containing such dyes. Acute toxicity tests were carried out with reference aquatic organisms: *Vibrio fischeri* bacteria and *Daphnia similis* microcrustaceans. After this, the effluent is also submitted to EBI treatment (irradiation at electron beam accelerator) for reducing color, with 0.5 kGy to 20 kGy doses. 100% of daphnids survived at 1g.L<sup>-1</sup> of RY-160, and for Red BG-3B (C=0.564 g.L<sup>-1</sup>) EC 50% (48h) = 69.0 ± 0.66. Raw textile effluent with dye RY-160 resulted in EC 50% lower than 2% for both organisms (*D. similis* EC 50% (48h) = 1.74 ± 0.53 and 0.6 ± 0.29, *V. fischeri* (15 min). For raw textile with Red BG-3B: *D. similis* EC 50% (48h) = 6.73 ± 0.72. The EBI treatment was effective for color removal, efficiency superior to 90% at 10 kGy. The data demonstrated the importance of evaluating not only dyes but also the effluent, since it has several additives that contributed to the high toxicity.

**FP065 Resistance of Escherichia coli strains isolated from beaches, of Santos and São Sebastião (SP)**

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The disordered occupation of coastal areas generates the discharge of effluents, which can carry several pathogens, being a serious risk to public health and to the environment. Effluents from selective sites and, without appropriate treatment, can increase the distribution and the frequency of resistant bacteria. Besides, the increase of the population in coastal regions during the high season elevates the organic discharge, especially in regions of beaches, raising the risk of exposure of bathers. *Escherichia coli* are bacteria of *Enterobacteriaceae* group, and are mostly harmless. However, some can cause serious infections, being a serious risk to public health. During the last decades there was an increase in antibiotic resistance, especially in *Enterobacteriaceae* group, becoming a worldwide concern. The β-lactamic antibiotics, chiefly the carapenem group, have been used as one of the last alternative to multidrug-resistant bacteria. Thus, bacteria resistant to them are of high concern to public health. The present study aimed to isolate and identify strains of *E. coli* isolated from waters from two sites, and verify the resistance profile of the isolated strains. Water samples were collected at 5 points near to Port of Santos (S), and 5 points at Araçá Bay (A), using sterile flasks. Was performed Membrane Filter Technique to evaluate the water quality. The samples were inoculated in MacConkey Agar and strains characteristic of *E. coli* were isolated. The following antibiotics were tested by Kirby-Bauer disc diffusion method: Ertapenem, Meropenem, Imipenem, Amoxicillin + Clavulanic Acid, Cefepime, Ampicillin, Ciprofloxacin, Tetracycline, Gentamicin and Fosfomicin. The CLSI guideline was used to the susceptibility testing. Santos samples showed a higher prevalence of resistant strains, especially when compare antibiotics commonly used, such as AMP (S: 52.6%; A: 45%) and CIP (S: 47.6%; A: 0%). Araçá Bay showed lower rates of resistance, but to AMC, were 89% of the strains were resistente. Only one strain was resistente to the carapenems, while 3 strains were resistant to Fosfomicin. The strains had a higher resistance in Santos, probably due to anthropization and the presence of Port of Santos, that is the largest and the busiest

port of Latin America. Antibiotics that are commonly used in medicine worldwide, such as amoxicillin and ampicillin, showed a higher prevalence of resistance, showing a worrying risk to public health and environment

**Neotropical Ecotoxicology and Conservation: Legacy and Emerging Pollutants in the Neotropics**

**FP066 A vision of Anthropocene from the evaluation of metals and others geochemical parameters in a sediment core of Santos and São Vicente Estuary**

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The Anthropocene can be considered a scientific terminology that may be proper to designate the potential beginning of a new geological era dominated by the everlasting and growing impact of humanity on nature. After Industrial Revolution (18th century), was observed a strongly increase of trace metals in estuarine and coastal environments, mainly from industrial effluents, discharge of sewage and air pollution, which consequently interfere in the quality of sediments. The Santos and São Vicente Estuary, located in the central portion of the coast of São Paulo state, is a powerful example of water and atmospheric pollution caused by human activities in coastal environments. The aim of this work was to understand the anthropogenic modifications of the area along the 20th century from the evaluation of several geochemical parameters. To that end, the levels of metals (Al, As, Cr, Cu, Fe, Mn, Ni, Pb, Sc, V, e Zn) were measured by inductively coupled plasma optical emission spectrometry (ICP OES). Moreover, others geochemical parameters were determined, such as, sediment granulometry, total organic carbon (TOC), δ<sup>13</sup>C, δ<sup>15</sup>N, and three environmental quality indicators were obtained. The results showed that since 1950s, a majority of metals had a tendency of enrichment toward the top of the core. These highest values near the surface could be associated with the vast process of urbanization and industrialization which affects Santos and São Vicente Estuary since that decade.

**FP067 Acute toxicity of glyphosate-based herbicide on the freshwater prawn Macrobrachium potiuna (Müller 1880) (Decapoda: Palaemonidae)**

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Glyphosate-based herbicides (GBH), in especial the Roundup® formulation, are the most widely used herbicides in crops worldwide. Even though, glyphosate inhibits exclusively the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) involved with the metabolism of essential amino acids in plants, is reported that can cause cell damages and mortality in aquatic animals. GBH can enter in aquatic ecosystems by direct application to control aquatic plants, drifting, leaching and surface runoff from the agricultural areas. Acute toxicity of glyphosate has already been estimated for some aquatic invertebrate and vertebrate organisms from temperate climates, but little is known about the acute effects of GBH in tropical freshwater species. *Macrobrachium potiuna* is a small tropical palaemonid with wide distribution, occurring in Brazil, from the State of Bahia to the State of Rio Grande do Sul, and is an important component in the trophic chain of freshwater environments. Thus, the aim of this study was to determinate the acute toxicity of the Roundup® WG herbicide on the freshwater prawn *M. potiuna* by calculating the 96 hours LC<sub>50</sub>. The assays performed according to the USEPA guideline test 2003. Five concentrations of the herbicide and one control group (0.0, 26.5, 46.5, 66.5, 86.5, 106.5 mg/L) were used in aquaria (6 L) filled with 3 L of dechlorinated water. Six prawns (average size of 26 mm) were exposed at each Roundup® WG concentration in triplicate. Mortality of prawns was monitored daily for the period of 96 hours. The calculation of the LC<sub>50</sub> was performed in the PriProbit software. The value of the 96 hours LC<sub>50</sub> for *M. potiuna* was 85.54 mg/L of Roundup® WG with 95% confidence limits of 70.38-119.08 mg/L. There was no mortality in the control and in the 26.5 mg/L concentration. The mortality of the prawns was observed from the concentration 46.5 mg/L. To the best of our knowledge, this is the first report of acute toxicity of Roundup® WG in a species of the genus *Macrobrachium*. The present study reveals that specific concentrations of the herbicide Roundup® WG can cause mortality in non-target organisms. Despite the mortality of prawns in high herbicide concentrations, further studies are needed to evaluate the sub-acute toxicity of Roundup® WG in this species, which could be used as biomonitoring species.

**FP068 Adverse effects promoted by a commercial formulation of the insecticide Fipronil in gill biomarkers of sábalo (Prochilodus lineatus)**



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Fipronil (fp) is a pesticide whose toxicity in insects is well documented although limited knowledge concerning sublethal effects is available in other non-target organisms (eg, fish). Gills are the first target organ where pollutants impact in aquatic organisms, therefore gill biomarker responses elicited by sublethal concentrations of fipronil could be indicative for evaluating the immediate damage produced by this insecticide in fish. In this context, biomarker responses promoted in juveniles of *P. lineatus* were evaluated after 48h of exposure to two nominal concentrations (10 and 100 µg/L) of a commercial formulation of fipronil (Clap Bayer®) dissolved in unchlorinated tap water. Fish (34 ± 9 g, n=15) were kept in aquaria under controlled conditions and fresh media was renewed twice daily using a flow-through system. Control fish remained in aquaria with tap water. After experimental period, fish were weighed, anesthetized and gills were dissected. Condition factor (CF) and liver somatic index (LSI) were calculated, antioxidant defenses (catalase activity, CAT; glutathione-S-transferase activity, GST; antioxidant capacity against peroxy radicals, ACAP) as well as oxidative damage parameters (levels of lipid peroxidation by TBARS and oxidized protein profile, Pox). Differences between groups were analyzed using one way ANOVA and Tukey's multiple comparisons. No significant differences were detected among groups in CF, LSI and CAT. Exposure to 10 µg/L Fp elicited only GST antioxidant response (+19,6 %), however both TBARS (+29,1%) and Pox (+83,0%) levels were increased. Considering the effects promoted after 100 µg/L Fp exposure, almost all of the antioxidant defenses were activated respect to Ctrl group. In this context, GST activity activated (+21,0 %), a depletion of GSH levels (-39,5 %) was observed, besides ACAP values increased (+192,6 %) showing a decrease in the antioxidant capacity. In spite of this antioxidant response of biomarkers, oxidative damage was evident in exposed fish showing an increase of TBARS values (+11, 3 %). These results indicated that short term exposure to sublethal concentrations of formulated fipronil promoted adverse effects in gills of this native species showing that antioxidant defenses were exceeded leading to oxidative damage on lipids and proteins, these fact was evident even in ecologically relevant concentrations.

**FP069 Comparação da sensibilidade de organismos-testes frente a exposição ao inseticida abamectina e ao fungicida difenoconazol**

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Testes de toxicidade são importantes ferramentas na detecção e quantificação de contaminação ambiental, pois diferentemente das análises químicas permitem identificação do efeito em sistemas biológicos, gerando valores limiares de poluentes ambientais permissíveis que devem ser seguidos por órgãos reguladores nas tomadas de decisão. Dessa forma, a escolha de organismos-teste é de fundamental importância, uma vez que espécies mais sensíveis representam menores frações potencialmente afetadas do ecossistema, preferíveis em relação às menos sensíveis. Associado à sensibilidade da espécie, o modo de ação do contaminante, como por exemplo, a ação inseticida da abamectina e a ação fungicida do difenoconazol, deve ser considerada na definição do organismo-teste. Neste contexto, procurou-se avaliar e comparar a sensibilidade de diferentes organismos-teste em relação à abamectina e difenoconazol, indicando os mais sensíveis e apropriados para conservação ambiental. Para tanto, resultados de testes de toxicidade foram compilados de banco dados e referencial bibliográfico, além das informações obtidas nos testes de toxicidade para cinco espécies, com os compostos. Pelos resultados obtidos verifica-se que as espécies mais sensíveis a ambos os contaminantes estão entre os cladóceros, principalmente *C. dubia* para a abamectina e a *A. bahia* para difenoconazol, sendo 295 vezes e 2,65 vezes, respectivamente, mais sensíveis que *D. magna*, espécie amplamente recomendada pelas normas ABNT, ISO e OECD (o mesmo ocorreu para os peixes e as algas). Além destas espécies, os fungos também foram sensíveis ao contaminante difenoconazol, devido ao modo de ação deste composto, mostrando a relevância em se atentar as características dos poluentes testados. Muitos trabalhos ainda apresentam resultados de testes de toxicidade com peixes, mesmo considerando sua menor sensibilidade para abamectina (PAF: 63%) e difenoconazol (PAF: 67,3%). Em geral as algas foram menos sensíveis que a maioria das demais espécies, representando uma fração potencialmente afetada de até 97,8% do ecossistema. Testes de toxicidade com insetos devem ser mais desenvolvidos, principalmente com abamectina (ação inseticida), bem como se faz necessária a padronização dos mesmos nas normas vigentes. *C. riparius*, apesar de recomendado pelas normas internacionais, não é o mais apropriado para se avaliar os efeitos tóxicos de difenoconazol, sendo que, neste caso específico, a melhor escolha seria a espécie de fungo *F. fulva*.

**FP070 DNA damages in erythrocytes of a neotropical fish species as a marker for chemical co-exposure to metals and titanium dioxide nanoparticles.**

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Because of their small size and high reactivity, titanium dioxide nanoparticles (NpTiO<sub>2</sub>) have the ability to interact with other contaminants and to cause changes in these interactions. In an attempt to evaluate changes in the toxicity of two metals, lead (Pb) and aluminum (Al) when co-exposed to nanoparticles, this work performed a trophic exposure, using a Neotropical fish species, *Hoplias intermedius*. In these bioassays, fish specimens were exposed to 0.1 µg/g, 1 µg/g and 10 µg/g of titanium dioxide nanoparticles, to 40 µg/g of lead nitrate and to 50 µg/g of aluminum sulfate, as well as to the respective associations of each metal with each one of the three concentrations of nanoparticles. The exposure occurred every five days, over a period of 70 days. After this period, blood samples were processed for the following tests: piscine micronucleus test, comet assay and DNA diffusion assay. As a result, the piscine micronucleus test showed no differences in the frequency of total alterations for all the exposure groups. The comet assay in erythrocytes demonstrated that the group exposed to lead had a higher DNA damage when compared to the negative control. In relation to the response of the nanoparticles, the three groups exposed to nanoparticles only revealed an increase in DNA damage, as well as the three groups co-exposed to the contaminants. The DNA diffusion assay clarified that the group exposed to lead, as well as the co-exposure groups and the groups exposed to the two highest concentrations of NpTiO<sub>2</sub> had a higher percentage of apoptosis than the control group. For the co-exposure of aluminum with NpTiO<sub>2</sub>, the piscine micronucleus test did not show any differences in the frequency of total alterations. The comet assay in erythrocytes revealed that the group exposed only to aluminum had a lower DNA damage index when compared to the negative control. Also, the three co-exposure groups showed a decrease in DNA damage. The analysis of co-exposure groups of these contaminants also revealed that all groups, presented levels of damage similar to the aluminum exposure group, while the three groups exposed to the nanoparticles exhibited an increase in DNA damage. For this exposure, the DNA diffusion assay presented a lower frequency of apoptosis in both the group exposed to aluminum and the co-exposure groups. Only the groups exposed to the two largest nanoparticle concentrations in isolation showed an increase in the frequency of apoptosis.

**FP071 Ecotoxicidade do fenantreno no desenvolvimento embrio-larval do zebrafish Danio rerio**

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O fenantreno é um hidrocarboneto policíclico aromático (HPA) encontrado no petróleo e seus derivados em concentrações elevadas, e que apresenta significativo potencial de causar toxicidade a organismos residentes em ecossistemas aquáticos contaminados. Este trabalho avaliou os efeitos do fenantreno no desenvolvimento embrio-larval de *Danio rerio*. A exposição ao fenantreno iniciou-se no estágio de embrião 3 horas após a fertilização (hpf), e se prolongou até 96 hpf. Os tratamentos incluíram concentrações de fenantreno dissolvido na água de 10, 50, 100, 500 e 1000 µg L<sup>-1</sup>, controle e controle de solvente. Em cada tratamento 20 embriões foram mantidos individualmente em poços de placas de 24 poços, e foram feitas observações diárias de mortalidade e efeitos subletais no desenvolvimento até 96 hpf. O desenvolvimento de marcos morfológicos foi avaliado diariamente, incluindo características como descolamento da cauda, formação de somitos, desenvolvimento e pigmentação dos olhos, presença de batimentos cardíacos e circulação sanguínea, desenvolvimento das nadadeiras e boca, e eclosão das larvas. Estas avaliações são integradas no índice morfológico geral (IMG), que pode atingir valor máximo de 17 numa larva que se desenvolve perfeitamente. Foram detectadas taxas de mortalidade de 10% e 25% após 96 h de exposição nas concentrações de 500 µg L<sup>-1</sup> e 1000 µg L<sup>-1</sup>, respectivamente. Foi detectado retardo no desenvolvimento embrio-larval evidenciado pela redução significativa do IMG relativa aos controles (p < 0,05) nas concentrações de 10, 50, 100, 500 e 1000 µg L<sup>-1</sup>, com médias iguais a 16,1; 16,3; 16,0; 15,8 e 14,9, respectivamente, comparado com uma média igual a 17 no controle. O comprimento total das larvas expostas a 1000 µg L<sup>-1</sup> foi reduzido significativamente comparado aos controles. As patologias detectadas com maior frequência nos diferentes tratamentos incluíram edema pericárdico, ausência de nadadeiras peitorais, hemorragias craniofaciais e saco vitelínico não absorvido. A frequência da patologia relacionada a ausência de bexiga natatória inflada aumentou de modo concentração-dependente, e foi igual a 19%; 50%; 60%; 67% e 73% nas concentrações de 10, 50, 100, 500 e 1000

$\mu\text{g L}^{-1}$ , respectivamente. Desta forma, este estudo demonstra que o fenantreno pode afetar o desenvolvimento embrio-larval de *Danio rerio* em concentrações ambientalmente relevantes.

**FP072 Effects of the antiparasitic Ivermectin on oxidative stress biomarkers on a native fish species: an ex vivo approach**

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Thousands of active ingredients of mass-use drugs are produced commercially to improve animal and human health. These substances are considered contaminants of emerging concern and their presence in the aquatic environment -from agricultural and industrial wastewater- has raised concern regarding toxic effects on non-target aquatic organisms. Ivermectin (IVM) is one of the most widely used macrocyclic lactones and has been known as a potent, effective and safe antiparasitic drug, commonly used for treatment and prevention of internal and external parasites of livestock and humans. IVM is excreted largely unchanged in dung of treated animals. IVM residues may affect non target aquatic organisms close to cattle breeding areas such as feedlots. In fish, the pharynx is a major site through waterborne pollutants can enter the body, and gills are often among the organs most affected by such compounds. The effect of IVM was assessed on gill oxidative stress biomarkers responses of a native fish species *Cyphocharax voga* (Characiformes), after *ex vivo* exposure. Fish were sacrificed, and the branchial arches were dissected and assigned to four treatment groups: saline solution as control (C); methanol (0.05%) as vehicle control (Sv); and two nominal concentrations 50  $\mu\text{g.L}^{-1}$ (C1) and 500  $\mu\text{g.L}^{-1}$ (C2) based on environmental data. Branchial arches were kept under continuous shaking for 1h at 25°C. The biomarkers evaluated were: enzymatic activities of catalase (CAT) and superoxide dismutase (SOD), total antioxidant capacity against peroxyl radicals (ACAP), lipid peroxidation levels (thiobarbituric acid reactive substances, TBARS), and reduced glutathione levels (GSH). Statistical differences between treatments were determined using a one way analysis of variance ( $p < 0,05$ ) followed by Tukey's test. No significant differences were found between all the analyzed parameters for C and Sv groups. Both IVM concentrations showed a significant increase (20.4% for C1 and 25.4% for C2) for SOD. In addition, in case of C2 concentration, a significant increase (46.3%) for ACAP and a significant increase for TBARS levels of both treatments (102.2% for C1 and 41.3% for C2) were detected. No significant differences for CAT and GSH were found. These results suggested that an *ex vivo* exposure of *C. voga* gills to IVM would promote changes at biotransformation and oxidative stress levels, indicating that the inner response seems not to be enough to prevent oxidative damage to lipid.

**FP073 Energetic metabolism of neotropical tetra, *Astyanax altiparanae*, exposure to water-soluble fraction of gasoline (WSF), high temperature and low pH**

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Aquatic freshwater environments have undergone challenges regarding the pollution with toxic chemicals, i.e. petroleum hydrocarbons, which alter its composition to such an extent that aquatic organisms can be negatively affected. Another source of environmental challenge is related to the effects of global warming. The rising levels of atmospheric CO<sub>2</sub> have been associated with an increase in water temperature and a decrease in pH. Despite that evidence, few studies evaluated the associated effects of these environmental challenges to the ichthyofauna. To fill this gap, the aim of this work was to evaluate the effects of WSF, in normal abiotic conditions (25°C and pH 7.0) and altered abiotic conditions (30°C and pH 4.0) to the energetic metabolism of *A. altiparanae*. Three experiments were conducted separately: 1) 25°C+pH7.0; 2) 30°C+pH7.0 and 3) 25°C+pH4.0. Specimens (n=20) were exposed to WSF (0.5%) for 96h. At the same time, other 20 fishes were maintained as control. After 96h, 20 fishes (control=10; WSF-05%=10) were anaesthetized, and plasma samples were collected for glucose analyses and muscle tissue for cortisol and glycogen determinations. The remaining fishes (control=10; WSF-05%=10) were submitted to recovery (maintenance in clean freshwater) during 192h with posterior plasma and muscle sampling. The exposure to WSF in normal abiotic conditions (25°C+pH7.0) does not interfere with the concentrations of muscle cortisol ( $p > 0.05$ ). However, the exposure

associated with low pH appears to act as an endocrine disruptor inhibiting cortisol levels in all treatments ( $p < 0.05$ ). The opposite response was observed when the temperature was raised, where results suggest higher stress for the animals ( $p < 0.05$ ). The levels of glucose in all experiments present values similar to those associated in the literature with a stress condition. At 25°C+pH7.0 the exposure to WSF reduces the plasma glucose ( $p < 0.05$ ). Controls (96 and 192h) of 30°C+pH7.0 presented lower concentrations when compared to 25 °C and low pH experiments ( $p < 0.05$ ). Glycogen metabolism at the muscle tissue was higher during exposure to WSF in 25 and 30 °C experiments ( $p < 0.05$ ). Despite the same trend in low pH condition, the difference was not significant ( $p > 0.05$ ). The obtained results suggest that energetic metabolism of *A. altiparanae* was affected by temperature and pH. The availability of energetic substrates, however, was mainly influenced by WSF exposure due an increase in the animal metabolism.

**FP074 Evaluation of citotoxic, genotoxic and mutagenic effects using *Allium cepa* test in groundwater samples used for human consumption**

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The quality of water for human consumption is important, mainly when we refer human health. The contamination of groundwater by metals may be associated with human activities and natural processes. The objective of this search was to evaluate the cytotoxic, genotoxic and mutagenic effects of groundwater used for human consumption in Itaporã – MS, using *Allium cepa* test and correlate them with the results of the levels of metals (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni and Zn). Water samples were collected in underground wells that were used for consumption without previous treatment. We used parameters established by ordinance 2914/2011, for the potability standard of heavy metals. The analysis of cytotoxicity, genotoxicity and mutagenicity in *A. cepa* cells indicated that in the samples of water that aluminum and manganese showed higher values than allowed by ordinance were observed chromosomal loss and bridge, budding, cell death and micronuclei. The biological assays with *A. cepa* test using water samples correlated with chemical analysis is considered important in monitoring programs. Their use would allow to assess the risk of genetic damages that these metals contamination could cause in living organisms. These results are relevant for public health since access to drinking water is a fundamental human need and therefore a basic human right.

**FP075 High concentrations of mercury in yellowfin tuna (*Thunnus albacares*) from the Galapagos Marine Reserve and waters off Ecuador's mainland coast**

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Yellowfin tuna (*Thunnus albacares*) is one of the major large pelagic fish harvested in the Southeastern Tropical Pacific and one of the most exported and traded. The tuna fishery is both industrial and artisanal in Ecuador, being the eighth country with most metric tons of tuna caught. This species is particularly consumed in important amounts by the coastal population in Ecuador and the Galapagos Island, an insular region part of Ecuador's territory. While the consumption of tuna fish is considered a source of nutrients that brings health benefits, tuna fish meat can also impose potential risks because of contamination by methylmercury. Methylmercury is a bioaccumulative and neurotoxic pollutant causing neurological and neurodevelopmental health effects in exposed people, mainly pregnant women and children, who may consume mercury-contaminated fish harvested from the sea. Very few studies have investigated mercury in large predatory fish in this equatorial region of the Pacific. The objective of this study was to assess the concentrations of total mercury (THg) in the red muscle of yellowfin tuna caught by artisanal fisheries in the Galapagos Marine Reserve and mainland coast of Ecuador. A total of 347 tuna were sampled (243 from the Galapagos and 104 from waters offshore Ecuador's coast) Muscle samples were analyzed using a DMA 80 .The 62.3 % of the tunas has size less of 70 cm . The mean concentrations of mercury were 0.43 +/- 0.09 mg/kg . It was found that 8% of the tunas analyzed (n = 28) in this study were above the tolerable levels of Mercury established by FAO ( 1 mg/kg). The maximum value found was dry weight6.14 mg/kg, being this one of the highest values of mercury reported for yellowfin tuna in the Pacific when compared to other studies. This study contributed with new baseline data on mercury concentrations in tuna from this region of the Pacific Ocean Basin and the initial

information to establish tuna fish advisories with a limit of consumption of this species in both Ecuador and the Galapagos Islands with implications for public health in the long term

**FP076 Is Antarctica still pristine? Nototheniid fish as sentinel species of endocrine disruption**

M. Ferreira, G. Genovese, Laboratorio de Ecotoxicología Acuática, DBBE & IBBEA, CONICET - Universidad de Buenos Aires / Departamento de Biodiversidad y Biología Experimental; M. Ansaldo, Instituto Antártico Argentino / Ecophysiology and ecotoxicology; F.L. Lo Nostro, Universidad de Buenos Aires - CONICET / Laboratorio de Ecotoxicología Acuática, Instituto de Biodiversidad y Biología Experimental y Aplicada, Departamento de Biodiversidad y Biología Experimental. Antarctica has been considered one of the best-preserved environments in the world. However, in the last years, several studies highlighted the presence of endocrine disrupting chemicals (EDCs) produced by anthropic impact. We investigate potential alterations on the endocrine system by analyzing endpoints of two notothenioids, *Notothenia rossii* and *N. coriiceps*, in order to attain knowledge about potential effects of EDCs on endemic species of Antarctic ichthyofauna and to prevent future effects of pollution in the Antarctic ecosystem. Gonado- (GSI) and hepatosomatic (HSI) indices, gonadal and hepatic histology, and vitellogenin (VTG) immunodetection in plasma and mucus were assessed in females (as control) and males sampled during Antarctic summer campaigns at Potter Cove, 25 de Mayo (King George) Island. GSI and HSI showed no variation in none of the species. Histological analysis of testes showed a restricted lobular type and no histopathological alteration or intersex condition was observed. Nevertheless, liver histology showed different levels of vacuolization in both species and the presence of swelling hepatocytes in *N. coriiceps*. VTG was detected in 40% of the males studied, not only in plasma but also in skin mucus, being this fact, the first report of mucus VTG in notothenioids. This abnormal response in males would indicate the presence of EDCs in the Antarctic ecosystem. Moreover, we consider VTG detection in fish skin mucus, a noninvasive assay, a promising tool to track EDCs pollution in biomonitoring studies in Antarctica.

**FP077 Microplastics in the mangrove ecosystem of the Gulf of Guayaquil, Ecuador**

G.A. Dominguez, Escuela Superior Politécnica del Litoral (ESPOL) / Facultad de Ciencias de la Vida; M. Calle, Escuela Superior Politécnica del Litoral / Facultad de Ciencias de la Vida; J. Alava, Institute for the Oceans and Fisheries, University of British Columbia / School of Resource and Environmental Management; P.S. Ross, Vancouver Aquarium / Ocean Pollution Research Program. Global production and use of plastics have been on the rise for many decades. Recycling and reuse of plastics do not prevent that plastic trash end up in the ocean. Plastic debris are commonly found in any marine environment extending from deep sea, coastlines to inner estuaries. Large pieces of plastics are transformed to very small fractions through fragmentation and/or degradation process. These tiny plastic fragments called microplastics (MPs) that measure less than 5mm. Microplastics may be potentially harmful to the marine environments. Once MPs enter different pathways of the marine ecosystem including physical (sedimentation, accumulation), chemical (degradation and absorption) and biological (ingestion and biodegradation). The goals of our project were to evaluate the prevalence of MPs on different environmental matrices of a mangrove ecosystem and to determine the potential ingestion of MPs by aquatic organisms. MPs pellets and granules were mostly found in the intertidal and subtidal sediments. Fibers and filaments were more abundant in the water column. MPs ingestion in aquatic organisms was determined by the presence of different types of MPs in the gastrointestinal tract of different aquatic species. These initial results demonstrate the presence of MPs in estuarine environments and the possibility that MPs may have a detrimental impact in aquatic species. Our preliminary results provide evidence to better characterize the complex mixtures of MPs within the estuarine environment and the likely interactions of MPs with the estuarine aquatic species.

**FP078 Morphological analysis and immunolabelling of HSP70 in liver of the neotropical fish *Phalloceros harpagos* exposed to paracetamol**

B. Pereira, Universidade Federal de São Carlos - UFSCar - Sorocaba / Departamento De Biologia; G.N. Matus, Universidade Federal de São Carlos - UFSCar - Sorocaba / POS GRADUAÇÃO EM BIOTECNOLOGIA E MONITORAMENTO AMBIENTAL; B. Nunes, CESAM University of Aveiro; M.C. Jones, Universidade Federal de São Carlos UFSCar Sorocaba / Department of Biology; A.C. dos Santos, Universidade Federal de São Carlos UFSCar Sorocaba / Departamento De Biologia; E. Silva-Zacarin, Universidade Federal de São Carlos - UFSCar - Sorocaba / Biology. Pharmaceuticals used in human and veterinary medicine are a class of emerging environmental contaminants that are extensively and increasingly being released to

aquatic ecosystems. Paracetamol (acetaminophen) is a widely used analgesic and antipyretic drug that reaches the aquatic environment through domestic wastes. This pharmaceutical in water have potential effects on aquatic fauna, including fish. The aim of this study was to evaluate the effects induced by sublethal concentrations of paracetamol on liver of the neotropical fish *Phalloceros harpagos*, by means of cellular biomarkers (histopathology and HSP70 detection). Juvenile fishes were collected in a stream located in Cabreúva city (São Paulo state, Brazil), and submitted to acute exposure (96h) to paracetamol (0, 8, 80, 800, 8000, 80000  $\mu\text{g.L}^{-1}$ ) in controlled conditions of temperature (23°C +/- 1°C) and photoperiod (12h light/12h darkness) in a semi-static system, after acclimation period. Each group consisted of fifteen fish, individually placed inside plastic recipients previously, and filled with 250ml of water. Following the exposure time, livers were collected from fishes, fixed in 4% paraformaldehyde and processed for resin (N= 5 per group) or agarose (N= 5 per group) embeddings. Histological sections of livers were submitted to Hematoxylin-Eosin staining and analyzed by light microscope. Sections of agarose-embedded livers obtained in a vibratome were submitted to immunofluorescence procedure for HSP70 detection and analyzed by Confocal Laser Scanning Microscope. Histological features of cell death were not observed in liver tissue from paracetamol exposed-groups, probably because there were a cytoprotective effect of HSP70 in hepatocytes, as demonstrated by immunofluorescence. In all experimental groups, vacuolization was observed in hepatocytes, but their frequency and intensity degree was greater at the highest concentration of paracetamol, which have also the strongest intensity of HSP70 labelling. Vacuolization is a regressive change, but reversible when the toxic stimulus stops. Probably these alterations were induced by NAPQI (toxic metabolite) that is known produce the unfolded protein response in endoplasmic reticulum during stress, and consequently activate HSP70 to prevent cell death in hepatocytes.

**FP079 Responses of branchial biomarkers of *Prochilodus lineatus* after prolonged exposure to sublethal concentrations of fipronil**

A. Santillan, INEDES, UNLU-CONICET; P.M. Ondarza, Universidad Nacional de Mar del Plata / Laboratorio de Ecotoxicología y Contaminación Ambiental, Instituto de Investigaciones Marinas y Costeras; K.S. Miglironza, University of Mar Del Plata / Laboratorio de Ecotoxicología y Contaminación Ambiental, Instituto de Investigaciones Marinas y Costeras; F.R. de la Torre, National University of Lujan / Basic Sciences. Fipronil (Fp) is a broad-use pesticide, and exhibits severe toxicity and adverse effects to non-target organisms, including fish. In this context, the presence of biochemical responses in fish is an effective tool for detecting sublethal effects from pesticide exposure. The responses of branchial biomarkers were evaluated on juveniles of *Prochilodus lineatus* after prolonged exposure to sublethal concentrations of Fp. Fish (n=32) were exposed to two nominal concentration of Fp (0.5  $\mu\text{g/L}$ , C1 and 9  $\mu\text{g/L}$ , C2) where toxicant was dissolved in methanol. Fish were kept in controlled conditions in aquariums with daily renewal of the media. Control fish remained in unchlorinated water (Ctrl) and water with the maximum methanol concentration used (CtrlSv). Levels of Fp on water samples were also analyzed with liquid-liquid and GC-ECD extraction. After 21 days of exposure, fish were sacrificed and the branchial arches were extracted. Enzymatic activity of superoxide dismutase (SOD), antioxidant capacity against peroxy (ACAP), content of reduced glutathione (GSH), levels of lipid peroxidation by TBARS as well as determination of oxidatively modified proteins (POx) were evaluated. Statistical differences between groups were determined using ANOVA ( $p < 0.05$ ) followed by Tukey's test. No mortality was observed in either experimental group and no significant differences between the parameters of Ctrl and CtrlSv were detected. Prolonged exposition to C2 promoted an increase (20%) in enzymatic activity of SOD respect to Ctrl group. Also an increase in ACAP values (87%) was observed after exposure to C1, showing a decrease in antioxidant capacity. On the other hand, both concentrations promoted a decrease (5%) in GSH activity respect to Ctrl. In terms of oxidative damage, exposition to Fp concentrations promoted an increase in TBARS levels (207%, C1 and 134%, C2); meanwhile, only C2 group showed a significant increase (41%) in POx levels. These results suggest that exposure to Fp promoted an antioxidant response, which was not enough to prevent oxidative damage at the protein level and lipid peroxidation. Also, both concentrations proved to be an inducer of oxidative stress even at ambient relevant concentrations.

**FP080 Short-term behavioral effects of paracetamol and propranolol in the neotropical fish *Phalloceros harpagos***

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Pharmaceutical residues from human and veterinary origin have been globally detected in the aquatic environment. Drug-induced behavioral alterations in fish can modify the individual fitness and have ecological consequences. Paracetamol belongs to a group of pharmaceuticals known as analgesics, and propranolol is a beta-blocker commonly used to control high blood pressure in humans. The aim of this study was to evaluate the short-term effects induced by sublethal concentrations of paracetamol and propranolol on behavioral parameters of the neotropical fish *Phalloceros harpagos*, specifically light/dark preference (scototaxis), swimming patterns, and pigmentation. Fish were acutely exposed (96h) to paracetamol (0, 8, 80, 800, 8000, 80000  $\mu\text{g.L}^{-1}$ ) and propranolol (0, 0.1, 1, 10, 100, 1000  $\mu\text{g.L}^{-1}$ ) under controlled conditions. Scototaxis test were performed 72h after the onset of exposure, with 5 fish per exposed group. Swimming patterns in the water column and pigmentation were recorded 96h after the onset of exposure, using 15 fish per group. It was possible to observe a significant increase in the preference for the dark compartment at the highest paracetamol concentration (80000  $\mu\text{g.L}^{-1}$ ), indicating increased chemically-induced stress, at the higher tested concentration of this drug; propranolol did not cause any significant effect in terms of light/dark preference. Paracetamol did not induce significant alterations in the swimming pattern in any of the tested concentrations. On the other hand, there was a significant increase in typical swimming pattern in propranolol-exposed group (1  $\mu\text{g.L}^{-1}$ ). Although paracetamol did not induce significant changes in fish pigmentation, propranolol led to a significant decrease in the number of pigmented fishes and a significant increase in non-pigmented fishes, both for the two highest concentrations (100, 1000  $\mu\text{g.L}^{-1}$ ). The obtained results suggest that both drugs, even at ecologically relevant concentrations, can induce significant modifications in the behavior of exposed fish, with potential consequences at the ecosystem level.

#### FP081 Titanium dioxide nanoparticle induces changes in liver antioxidant system and morphology of fish after acute and subchronic exposure

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The present study investigated the toxicity of titanium dioxide nanoparticles ( $\text{TiO}_2$ -NP) on the liver of the Neotropical freshwater fish, *Prochilodus lineatus*, with emphasis on antioxidant responses and morphological damage. *P. lineatus* were exposed to 0 (control), 1, 5, 10 and 50  $\text{mg L}^{-1}$  NP- $\text{TiO}_2$  for 48 h (acute exposure) and 14 d (subchronic exposure). Titanium in liver increased after subchronic exposure to 50  $\text{mg L}^{-1}$ . After acute exposure, the reactive oxygen species (ROS) decreased, the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) as well as the metallothionein (MT) and lipoperoxidation (LPO) did not change and increased the glutathione (GSH) levels. After subchronic exposure, the ROS production, the activity of CAT and the level of MT did not change, the activity of SOD decreased and the activity of GST and the level of GSH increased. Numerous morphological degeneration changes occurred in liver after acute exposure such as absence of nucleus and nucleolus, cellular degeneration and necrosis which were the most severe changes. The histopathological index increased significantly after exposure to 5, 10 and 50  $\text{mg L}^{-1}$  NP- $\text{TiO}_2$ . GSH seems to have an essential role to maintain the redox equilibrium in the liver on both acute and subchronic exposure. However,  $\text{TiO}_2$ -NP continuous exposure may reduce liver function if cellular damages were not restored. Financial support: FAPESP Proc. 2011/10339-0 and 2014/05701-0, CNPq Proc. 301615/2015-2, CAPES.

#### FP082 Total mercury concentration in fish - pch colider, Mato Grosso, Brazil

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Mercury is a trace metal that occurs naturally in the environment released through volcanic emanations and gasification of the earth's crust, its mobilization through human activities such as gold mines, factories, pharmaceutical industry and conversions of land for various purposes, has been acting as a catalyst for a gradual process of contamination of the terrestrial and aquatic biota. In aquatic environments, Hg can take on a variety of physical and chemical forms, and among these forms methylmercury is of major interest from an ecotoxicological point of view, since it is a neurotoxin and tends to bioaccumulate and biomagnify in aquatic food chains. The

study was developed in the Small Hydroelectric Power Plant, Teles Pires river, Colider-MT. After collection the specimens were identified, sorted and taxonomically classified according to species and trophic category. Then, muscle samples were removed and submitted to acid digestion for determination of Hg by Atomic Absorption Spectrometer with Flow Injection System (FIMS - 400; Perkin Elmer). Muscle samples from 43 individuals of different species were analyzed. Concentrations of THg ranged from 20.01 ppb (*Boulengerella cuvieri*) to 614.36 ppb (*Hemisorubim platyrhynchos*), 26 individuals presented THg concentration in the muscle from 20.01 ppb to 200 ppm, 12 subjects had a THg concentration of 208, 50 ppm at 391.81 ppb, and 5 individuals (*Hemisorubim platyrhynchos*, *Serrasalmus rhombeus*, *Prochilodus nigricans*) presented a THg concentration between 400 ppp and 614.38 ppb, above the WHO limit. The results indicate that 63% of the samples are between 0 and 200 ppm. Values above 400 ppm, which represent 14% of the total, deserve special attention, monitoring the use of this fish as a primary source of protein and frequent consumption by riverine populations. The studies show an occurrence in an annual time scale of release of Hg in the environment through point and diffuse sources, in addition to metal emissions through the growth of deforestation, evolution of fires and the expansion of dams for hydroelectric and SHP. In addition, the Amazon and the Pantanal naturally present favorable environments for inorganic Hg methylation to the organic form, which may explain the high and moderate concentrations in carnivorous fish in environments devoid of gold miners.

#### FP083 Toxicity of the furniture painting booth effluent to zebrafish (*Danio rerio*) embryo before and after biological treatment

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Paint effluents are a complex mixture of organic compounds such as formaldehyde and aromatic solvents. The reuse of the water in the furniture painting booths to retain suspended paint increases the concentration of toxic substances. The objective of this work was to test the toxicity of this effluent using FET (fish embryo toxicity test) with *Danio rerio*, following the OECD 236 Guideline (2013). Samples of crude effluent were used in dilutions ranging from 6.25 to 100% in the preliminary test and from 2 to 12% in 5 definitive tests. Five concentrations were used in each test, besides the negative (reconstituted dilution water) and positive (4  $\text{mg.L}^{-1}$  of 3,4 dichloroaniline) controls. The embryos were individually placed in 24-well plates (4 wells as plate internal controls and 20 with the effluent (n=20/concentration)). The biologically treated effluent (anaerobic treatment followed by aerobic post-treatment) was tested without dilutions. In addition to the endpoints considered in the guide (coagulation, absence of heart beats, caudal detachment and formation of somites), the sublethal parameters heart rate (48 and 72h), hatch rate (96h) and swimming bladder inflation (120 h) were also analysed. All observations were made under inverted microscope. The results allowed the LC50 calculation (Trimmed Spearman-Kärber), and the analyses of the sublethal parameters (ANOVA, followed by the SNK test ( $p < 0.05$ )). The crude effluent showed high toxicity to the embryos, with LC50 (24h) of 5.54% (5.09-6.06%) and LC50 (96h) of 5.0% (4.50-5.55%), causing high embryonic death in the first 24 hours. Coagulation was the most frequent observation, and only at concentrations below 8% other lethal endpoints occurred. As a sublethal endpoint, there was a decrease in the insufflation of swimming bladder from embryos surviving the crude effluent to 4%. Considering only the surviving embryos after this period, there was no change in the hatching rate as well as in the heart rate of the hatched embryos. The treated effluent did not cause lethality, nor any sublethal alteration, indicating decrease of the toxic load after the biological treatment. In summary, the crude paint booth effluent was toxic to zebrafish embryos, causing high lethality as early as the first 24 hours of exposure, but the treated effluent showed no toxicity. Among the sublethal parameters analysed, the most sensitive was the swimming bladder inflation.

#### FP084 Toxicity of water-soluble fraction (WSF) of petroleum and five derivative products to the neotropical tetra, *Astyanax altiparanae*

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Chemical properties of petroleum derivatives vary widely across its different classes and, consequently, at the final refined product. The aromatic hydrocarbon components tend to increase the percentage of the total content during the refining process and are directly associated with toxic effects on aquatic biota. Unlike for marine environments,

the study of the toxicity of petroleum hydrocarbons for freshwater biota is scarce and restricted to few species and products. In this way, we evaluated the toxicity (LC<sub>50</sub> and LC<sub>10</sub>) of WSF of petroleum and five derivative products (gasoline, diesel, lubricant and insulating mineral oil (IMO from two commercial brands) to *A. altiparanae*. The experiments were performed independently, and acclimation to laboratory condition was performed for 48h prior exposure. For each experimental procedure 110 fishes were exposed to different concentrations (n=10/concentration) of WSF (0 [control]; 0.25; 0.5; 1.0; 2.0; 4.0; 8.0; 16.0; 32.0; 64.0 and 100.0%) for 96h. Mortality and water quality parameters (temperature, pH, NH<sub>3</sub>+NH<sub>4</sub><sup>+</sup> and NH<sub>3</sub>) were monitored every 24h. Water quality parameters did not present variation during exposure (p>0.05). LC<sub>50</sub> and LC<sub>10</sub> (95% confidence intervals) were: gasoline= 2.65 (1.02-4.46) and 0.5 (0.35-0.91); diesel= 16.22 (11.73-21.09) and 2.75 (2.31-5.06); IMO<sup>1</sup>= 17.01 (5.85-25.06) and 5.35 (3.33-8.65); petroleum= 23.59 (10.81-31.32) and 1.76 (1.39-3.86); lubricant= 27.65 (11.54-38.79) and 11.24 (6.45-16.78); IMO<sup>2</sup>= 53.58 (22.98-127.92) and 7.84 (7.50-16.73). As expected, due to higher polycyclic aromatic and monoaromatic hydrocarbons concentrations, gasoline presented the highest toxicity. Gasoline WSF is 6 times more toxic than diesel and 20 times more toxic than OMI<sup>2</sup> to *A. altiparanae*. Lubricant and IMO<sup>2</sup> presented the lowest toxicity effects to *A. altiparanae* because both present elevated concentrations of high molecular weight petroleum hydrocarbons. On the other hand, IMO<sup>1</sup> presented toxicity similar to diesel oil. This effect could be associated with the antioxidant inhibitor added to the oil for dielectric and insulating purposes, which can also present toxic effects. These results could be used as base information for subchronic studies aiming to evaluate the sublethal effects from the exposure of *A. altiparanae* to the tested petroleum products. The results could also be used for environmental risk assessment programs purposes.

### Discussing our physiological, biochemical or molecular studies on aquatic organisms exposed to environmental pollutants to improve the quality and the ecotoxicological sound of our research

#### FP085 Accessing copper metabolism through gene expression of transporting-proteins CTR1 and ATP7A in freshwater fish adapted to metal contamination

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Copper (Cu) plays a key role as cofactor of several enzymes. Therefore, organisms have developed strategies to ensure the adequate supply, distribution and regulation of intracellular Cu. Cu intake and excretion is mainly regulated by integral membrane transporters CTR1 and ATP7A respectively. Indeed, the expression dynamics of these molecules can be used as biomarker and also reveal evidences of adaptation in populations inhabiting environments chronically contaminated with metals. Field translocation experiments using the fish *Hyphessobrycon luetkenii* from non-contaminated (C) and polluted (P) areas of the João Dias creek (Minas do Camaquã, Caçapava do Sul, RS, Brazil) were performed to evaluate the expression of genes encoding for CTR1 and ATP7A in adapted fish (groups CC, CP, PP and PC). After 96 h of experiment, fish were euthanized with benzocaine and had their liver, gill and intestine dissected. Genes encoding for CTR1 and ATP7A were partially sequenced and had their expression levels quantified by qPCR in the collected samples. Fragments of the CTR1 (108 bp) and ATP7A (82 bp) were sequenced. They showed 99% and 100% identity with the same region of the gene sequences described for *A. mexicanus*, respectively. No significant changes in gill expression of genes encoding for ATP7A and CTR1 were observed after fish translocation (CP and PC groups). However, a significant higher expression of ATP7A and CTR1 was observed in fish of the PP group, thus evidencing a possible higher Cu intake in fish of PP and PC groups, as well as a possible higher Cu excretion in fish of the PP group. In liver of fish from all experimental groups, no significant changes were observed in the expression of gene encoding for ATP7A. However, an inhibition of the expression of gene encoding for CTR1 was observed in liver of fish of the CP group, which can be considered as being a mechanism to avoid an excessive intake of Cu by hepatic cells. Finally, no significant changes were observed in the expression of genes encoding for ATP7A or CTR1 in intestine of fish after translocation. However, a lower expression of the gene encoding for ATP7A was observed in intestine of fish from the PC group, thus indicating a likely lower need for Cu excretion in these fish. Additionally, fish of the CC group had higher basal levels of CTR1 gene expression than those of the PP group. This finding can be explained considering the need of fish from CC group for

incorporating Cu through the diet.

#### FP086 Alterações biométricas em conchas de *Lottia subrugosa* ao longo de um gradiente de contaminação na Baía de Paranaguá

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Estudos recentes têm indicado que alterações em conchas de moluscos podem ser utilizadas como biomarcadores alternativos para avaliar os impactos da contaminação de zonas costeiras. O molusco *Lottia subrugosa* é um pateliforme abundante na zona entre marés do litoral brasileiro. Portanto, o presente estudo avaliou alterações biométricas (altura, espessura, densidade e massa) em conchas de *L. subrugosa* coletadas ao longo de um gradiente de contaminação na Baía de Paranaguá (Paraná-Brasil). Com base em informações da literatura, foram determinados três pontos de amostragem em zonas de substratos consolidados distribuídos ao longo de um gradiente de contaminação já estabelecido para região. Sendo assim, 100 indivíduos de *L. subrugosa* foram coletados na Ponta do Poço (P1), Ilha do Mel (P2) e Ilha Galheta (P3) representando sequencialmente diferentes níveis de contaminação. Após coleta, os organismos foram conduzidos ao laboratório onde as conchas foram removidas e os parâmetros de biométricos (espessura, altura, largura e comprimento) massa e densidade das conchas foram obtidas. Os resultados indicaram, após normalização pelo comprimento das conchas, que os organismos da área mais contaminada (P1), apresentam conchas com menor altura, massa e espessura do ápice. Por outro lado, a densidade apresentou valores intermediários quando comparados aos demais pontos. Similarmente, nos pontos P2 e P3 os dados biométricos mostraram variações progressivas indicando uma coerência com o gradiente de contaminação descrito para área. Esses achados, embora preliminares, sugerem que alterações biométricas em conchas de *L. subrugosa* podem vir a ser utilizadas como biomarcadores generalistas de contaminação costeira por substâncias químicas perigosas. De fato, um estudo prévio realizado no canal de acesso a porto de Santos demonstrou alterações morfológicas e na composição elementar da concha dessa espécie, ao longo de gradiente de contaminação. Além disso, esse estudo também mostrou que biomarcadores bioquímicos tradicionais tais como danos ao DNA e correlacionaram fortemente com as alterações de concha observadas. Portanto, considerando a abundância e a ampla distribuição geográfica de *L. subrugosa*, essa espécie parece apresentar potencial para se tornar uma promissora ferramenta de baixo custo e simples aplicação para análise de impactos produzidos pela contaminação ambiental.

#### FP087 Alterações metab

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Os efluentes das fábricas de papel e celulose são reconhecidos internacionalmente por conter substâncias tóxicas com propriedades para alterar a capacidade reprodutiva dos peixes. O objetivo deste estudo foi avaliar as modificações metabólicas (usando ferramentas de metabolômica) de gônadas e fígado de peixes (*Danio rerio*) expostos a efluente de indústria de papel e celulose, bem como o desenvolvimento embrionário de embriões gerados pelos respectivos peixes. O efluente foi diluído de um fator de 1/25. Os peixes (10 machos e fêmeas) foram expostos ao efluente, sendo que machos e as fêmeas foram mantidos separados por 7 dias, a 28 oC e um ciclo claro/escuro de 12/12 h, em vidros de 3,5 L. Após a pré-exposição, machos e fêmeas foram acasalados (1 casal por Becker contendo uma rede na parte inferior) e monitorados por 6 dias com renovação diária de água/ poluente e alimentados duas vezes ao dia. Os ovos foram coletados e colocados em placas contendo o agente de teste, mantidos protegidos da luz. Os peixes foram sacrificados para retirada de fígado e gônadas, a partir das quais foi preparado um homogenato. Em seguida, foi realizada uma extração com clorofórmio/metanol/ água (3:2:1). O extrato obtido foi preparado para a realização de uma cromatografia gasosa. Para os peixes expostos, houve uma redução significativa na produção de ovos, 65%. Uma alta porcentagem dos ovos apresentou coloração escura, ou seja inviáveis. Os resultados da cromatografia revelaram significativas alterações no perfil de aminoácidos, esteróis e ácidos graxos, em ambos os tecidos, fígado e gônadas. Os resultados demonstraram um forte impacto no metabolismo, produção de ovos e desenvolvimento embrionário para os peixes em estudo, as quais apontam para a alteração da capacidade reprodutiva dos mesmos.

#### FP088 Aluminum triggers genotoxic effects in the erythrocytes of sexually mature *Astyanax altiparanae* males after 24 hours of exposure.

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Many anthropic actions in the environment, such as the introduction of metals into the water by industrial and household waste, can cause changes in physiological processes in animals, as well as genotoxic effects. Among these metals, aluminum is highlighted and some studies demonstrate this genotoxicity in fish erythrocytes after 96 h of exposure, besides oxidative stress, ionic imbalance, alteration of enzymatic activity among others. Therefore, this study aimed to evaluate the genotoxic capacity of aluminum in *Astyanax altiparanae* males during the reproductive period, after 24 hours of exposure. Sexually mature male *A. altiparanae* were subdivided into three experimental groups (n= 4 per treatment): neutral pH, acidic pH (2.5; HNO<sub>3</sub> 65%) and aluminum (0.5 mg.L<sup>-1</sup>) at acidic pH (2.5; HNO<sub>3</sub> 65%) - all groups at 25°C. After 24 hours the animals were sedated with benzocaine and blood collection was performed from the base of the caudal fin. For micronucleus (MN) and Nuclear Abnormalities (NA) tests, blood smears were fixed in methanol and stained for 8 minutes in Giemsa (10%). 1000 erythrocytes (two slides per animal) were counted and analyzed for the presence of MN and NA. No MN was found in any of the experimental groups. In relation to the presence of NA, the animals exposed to aluminum at acidic pH (2.75 ± 0.94) presented higher number of NA (P< 0.05) than the animals maintained in neutral pH (0.375 ± 0.26), and both groups did not differ in the number of NA compared to animals exposed to acidic pH (2.75 ± 1.06) (P> 0.05). Therefore, the water acidity, alone, did not trigger DNA damage in *A. altiparanae* erythrocytes, but the presence of aluminum in this acidic condition, was determinant to cause these changes in DNA for 24 hours. Studies with a wider range of biomarkers and longer exposure time must be carried out to add information about the genotoxic effect of aluminum in *A. altiparanae*. Acknowledgments: grant #2016/08770-8, São Paulo Research Foundation (FAPESP) Keywords: Ecotoxicology, Fish, Metal, Micronucleus, Nuclear Abnormalities.

**FP089 Antioxidant status, glycogen levels, and cholinesterase activity in *Nacella concinna* from Choza Cove, Hope Bay, Antarctica**

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Biological responses can be used as an early warning against exposure to naturally occurring and/or by product of anthropogenic activity pollutants entering the environment. In the present work, we determined molecular biomarkers of oxidative stress and energy reserves in the Antarctic limpet *Nacella concinna*, from different intertidal sites of Choza Cove (63°23'54" S 56°59'46" W), Hope Bay, Antarctic Peninsula. During the summer 2013-2014, specimens of *N. concinna* were collected by hand from 4 sampling sites in progression away from the waste water discharge of the Argentine Antarctic Station "Esperanza". Morphometric measurements were performed from each animal and the digestive gland, gills, gonads and central ganglion were dissected. Using spectrophotometric techniques, the activity of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione peroxidase (Gpx), as well as reduced glutathione (GSH), lipid oxidation (LPO) and protein oxidation (PO) were measured. Cholinesterase activity (CO) and glycogen levels (GL) were also registered. Through the analysis of the obtained results, we observed that *N. concinna* responses from different sites may be due to both local environmental variations (food availability, water temperature and percentage of salinity) and anthropogenic impact (xenobiotics input to the sea).

**FP090 Atividade da acetilcolinesterase e conteúdo de metalotioneínas em peixes coletados no reservat**

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A Usina Hidrelétrica (UHE) Mauá, localizada no rio Tibagi (PR), apresenta histórico de contaminação por organoclorados e metais, consequência da utilização de cloro no processo de branqueamento das pastas celulósicas de uma fábrica de papel e celulose e das antigas minas de carvão operadas na região. Portanto, o objetivo deste trabalho foi investigar os efeitos desses possíveis contaminantes em peixes neotropicais residentes do reservatório UHE Mauá com distintos hábitos alimentares, por meio de biomarcadores bioquímicos, como a atividade da acetilcolinesterase (AChE) e conteúdo de proteínas semelhante à metalotioneínas (PSMT). Os modelos biológicos utilizados foram a traíra (*Hoplias malabaricus*) e o lambari-de-rabo amarelo (*Astyanax altiparanae*), duas espécies de peixes neotropicais de hábitos alimentares distintos. *A. altiparanae* possui dieta onívora e *H. malabaricus* apresenta hábito predatório. Além dos peixes coletados no reservatório (grupos experimentais: EXP), exemplares de *A. altiparanae* e *H. malabaricus* fornecidos por pisciculturas da região, supostamente livres da entrada de contaminantes, formaram os grupos denominados controle (CTR).

Após serem anestesiados, os peixes foram amostrados nos locais de coleta para a retirada do fígado para a análise de PSMT, e do músculo e cérebro para a avaliação da atividade da AChE. Os exemplares de *A. altiparanae* do grupo EXP apresentaram conteúdo de PSMT (7,01 uM SH /mg ptn ± 3,87; n=10) significativamente menor (p=0,021) em relação ao grupo CTR (12,53 uM SH /mg ptn ± 5,69; n=10). O cérebro de *A. altiparanae* do grupo EXP apresentou atividade da AChE (33,97 nM/min/mg ptn ± 3,70 n=10) significativamente maior (p=0,045) comparado ao grupo CTR (27,73 nM/min/mg ptn ± 7,78 n=10). Os exemplares de *H. malabaricus* não apresentaram variação significativa na atividade da AChE muscular e cerebral e no conteúdo de PSMT entre os grupos EXP e CTR. A atividade da AChE pode ser reduzida na presença de alguns compostos orgânicos e íons metálicos. Por sua vez, o aumento do conteúdo hepático de PSMT também pode estar relacionado à exposição aos metais, devido à participação das metalotioneínas na homeostase de metais essenciais e não essenciais. Assim, os resultados obtidos neste trabalho sugerem que as pisciculturas nas quais os peixes foram coletados não apresentam água com a qualidade esperada, bem como, que *A. altiparanae* é aparentemente mais sensível que *H. malabaricus* considerando-se ao biomarcadores analisados.

**FP091 ATIVIDADE DA GLUTATIONA S-TRANSFERASE EM MACR**

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**Introdução** As regiões alagadas do extremo sul do país possuem alta biodiversidade de macrófitas aquáticas, portanto podem abrigar espécies ainda pouco estudadas que tenham expressiva atividade de GSTs. A cidade do Rio Grande estando inserida na planície costeira da América Latina contém a flora indicada para o estudo que se pretende. Com um papel fundamental no metabolismo de xenobióticos, as glutathione S-transferases (GSTs) são enzimas de detoxificação e podem ser induzidas ou inibidas frente à exposição a certos contaminantes. Enzimas de diferentes espécies vegetais são utilizadas como biotransformadoras de vários poluentes, diminuindo as concentrações destes no meio ambiente. Dessa forma, pretende-se relacionar a atividade das GSTs contidas nas raízes com a capacidade detoxificadora da planta. Espera-se que estas enzimas, presentes em inúmeros organismos, estejam contidas nos tecidos de vegetais estudados. As espécies vegetais estudadas foram escolhidas de acordo com sua abundância no ecossistema local e seu biótipo de ocorrência. As estruturas vegetais analisadas foram raiz, caule e folha com base em um estudo que concluiu que essas partes possuem atividade enzimática mensurável. É possível que o hábito ecológico da planta tenha influência na sua capacidade de biotransformação, sobretudo em plantas flutuantes, uma vez que suas raízes encontram-se em contato direto com a água. Portanto as plantas foram aqui agrupadas em quatro biótipos, ou seja, em flutuantes, emergentes, submersas e anfíbias Dessa forma, os objetivos fora: (1) Avaliar a atividade da GST em raízes de quinze espécies de plantas macrófitas. (2) Relacionar a atividade enzimática com o biótipo de ocorrência das macrófitas. (3) Comparar raiz, caule e folha dos vegetais entre si quanto a atividade enzimática das GSTs, para assim eleger a estrutura que mais possui atividade.

**FP092 Avaliação do potencial genotóxico e mutagênico de águas de piscicultura**

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A aqüicultura é uma atividade em expansão no Brasil quando comparada às demais atividades agropecuárias, os pesque-pagues representam uma das principais formas de comercialização do pescado no Mato Grosso do Sul, destacando-se especialmente na produção de bagres como o pintado e seus híbridos, devido ao seu filé com excelente qualidade organoléptica, características favoráveis para pesca esportiva e aceitação do público consumidor. Assim sendo, é importante que se avalie nos peixes propriedades mutagênicas e genotóxicas ocasionadas por contaminantes ambientais presentes na água, a fim de gerar informações para planos de controle da qualidade da água e da carne neste setor. O objetivo deste estudo foi avaliar a genotoxicidade e mutagenicidade em pintado amazônico provenientes de pesqueiro na cidade de Dourados-MS. As coletas ocorreram em dezembro de 2014 e março de 2015 em um pesqueiro na cidade de Dourados. Foram coletados 5 exemplares de pintado amazônico (*Pseudoplatystoma sp. x Leiarius marmoratus*) em cada período amostral. Após a captura, os peixes foram anestesiados o sangue foi coletado por punção caudal, em seguida extensões sanguíneas foram realizadas a fim de realizar a contagem de alterações metanucleares (genotoxicidade) e micronúcleo (mutagenicidade). Como resultado obtivemos que não houve diferença significativa entre as épocas para o teste de genotoxicidade e mutagenicidade. Apesar da baixa frequência de alterações metanucleares e micronúcleo, pode observar que no mês de dezembro a frequência de micronúcleos foi maior que no mês de março, já para as alterações metanucleares, a frequência foi maior em março. Com base nisso, pode-se inferir que de acordo com o



ensaio, a água não apresenta potencial genotóxico e mutagênico, contudo, faz-se necessário a realização de novos estudos a fim de avaliar de forma mais abrangente a qualidade da água.

**FP093 Binary mixture effects by monoaromatic hydrocarbons on the tropical microcrustacean *Mysidopsis juniae***

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The monoaromatic hydrocarbons benzene, toluene and xylenes (BTX) are volatile compounds present in petroleum and its derivatives. Among the most produced chemicals worldwide, they are used in the chemical industry as raw material in the manufacture of plastics, rubbers, dyes, resins, pesticides and solvents. Contamination of aquatic environments by these substances may result in to adverse toxic effects on organisms of different biological levels. The present study aimed to evaluate the toxicity of BTX isolated and in binary mixtures using the acute toxicity test (96h) with the marine microcrustacean *Mysidopsis juniae*. To achieve that, organisms were exposed to BTX concentrations in order to identify the effects on survival, as well as to study the modes of action of the substances in mixture with the application of the theoretical models of Addition Concentration (AC) and Independent Action (AI). As a result of the experiments with isolated compounds it was possible to notice that xylene was the most toxic compound ( $LC_{50}$  16,10  $\pm$  2,4mg.L<sup>-1</sup>) followed by toluene ( $LC_{50}$  38  $\pm$  5,3mg.L<sup>-1</sup>) and benzene ( $LC_{50}$  78,03  $\pm$  2,9mg.L<sup>-1</sup>) respectively. When in binary mixture deviations of the reference models for CA were observed in all experiments performed (dose ratio and antagonism). In the exposure of xylene and benzene the deviation that fit was the antagonism, this effect predicts that the mixture causes a less severe effect than the isolated substances. Where as the mixture of xylene and toluene was better explained by the deviation dose ratio where the toxicity was caused mainly by xylene. In the experiment combining toluene and benzene, the dose ratio pattern was observed as well, and the benzene was the main responsible for the toxicity. We concluded that BTX were toxic to mysids, both isolated and in binary mixtures, evidencing the importance of studies in this subject that can serve as support for evaluations and monitoring of the marine environments, considering the imminent risks of contamination in the environment and the wide scale of use of these compounds.

**FP094 Bioaccumulation kinetics of copper in *Ruditapes philippinarum* exposed to increasing, continuous and pulsed exposure: implications for growth**

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The effects of metal bioaccumulation on aquatic organisms depend on factors as magnitude, duration and frequency of exposure. The type of exposure affects the toxicokinetic processes in the organisms. In this study, 30-day toxicity tests were carried out on juveniles of the bivalve *Ruditapes philippinarum* exposed to increasing, continuous and pulsed exposure with copper-spiked estuarine sediments followed by 10-day recovery period. The aim of the study was to assess the interaction between the kinetics of subcellular copper partitioning and the growth response. Pre-exposure test concentrations after sediment spiking were 12.1 (low), 19.2 (medium) and 24.7 (high) mg Cu < 63 $\mu$ m g<sup>-1</sup> OC, representing environmentally relevant concentrations for relatively low contaminated sediments. Copper concentration in unspiked sediment (2.1 mg Cu < 63 $\mu$ m g<sup>-1</sup> OC) was adequate to prevent chronic effects to benthic invertebrates in marine environments. Bivalves were acclimated 7 days under controlled conditions. Tests were conducted exposing them to the sediments in a flow-through system during 40 days. Four treatments were set up: (1) *control*, kept in unspiked sediment; (2) *increasing exposure*, increasing copper concentrations during 10 days each; (3) *continuous exposure*, kept at the highest concentration for 30 days; and (4) *pulsed exposure*, alternating the highest concentration and the control every 10 days. Organisms were sampled at different times (T0 = initial time, T1 = 10 days, T2 = 20 days, T3 = 30 days and T4 = 40 days) and average wet weight was recorded to calculate growth rate. Tissue samples were treated to determine the net copper accumulation rates for total bioaccumulated copper, biologically detoxified (enzymes, metal-rich granules and metallothioneins-like proteins fractions) and biologically available (mitochondria, lysosomes, microsomes and heat-sensitive proteins fractions) metal pools. Results showed that the bivalves' growth rate was inversely correlated to the bioaccumulation rate and that sublethal copper concentrations stimulated the detoxification mechanisms of the organism regardless the type of exposure. However, a large stimulatory effect on growth was observed during the recovery, associated to

significant negative accumulation rate values and dependent on the type of previous exposure. This suggested that, on individual and short-term basis, pulsed exposures have a more adverse effect compared to increased or continuous exposure scenarios.

**FP095 Biochemical Effects of Water-Soluble Fraction of Oil (WSF) on the Amphipod *Hyalella curvispina***

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The Province of Neuquén is located in the northern region of the Argentinean Patagonia, where the exploitation of energy resources is the most relevant economic activity. The oil and gas fields from this area produce 48% and 23% of Argentina's total production; respectively. Therefore, this zone is exposed to oil pollution derived from drilling, production and transport of crude oil. During the last years, studies from the area have demonstrated the presence of hydrocarbons in some water courses and sediments. In addition, low concentrations of polyaromatic hydrocarbons (PAHs) such as naphthalene and pyrene were detected. Oil contamination causes a serious problem to aquatic life. Previous investigations of crude oil contamination have revealed that their toxicity is mainly due to the water-soluble fraction (WSF), rather than to the dispersed droplets of the fraction. Thus, the aim of this work was to evaluate the time-dependent effects of a sublethal dilution of WSF on the activity of detoxifying enzymes in adults of *H. curvispina*. Crude oil obtained from Chachahuen field, North Patagonia region and supplied by YPF Company was used to prepare the WSF. Amphipods were sampled from Los Barreales lake (LB) and Durán stream (DS), nearby the urban area of Neuquén City where the presence of hydrocarbons was previously determined. The enzymatic activity of glutathione-S-transferase (GST) and catalase (CAT) and reduced glutathione content (GSH), were assessed in both control and exposed (1:1600 dilution for 6, 24 and 48 hours) amphipods. Both LB and DS showed a significant increase (more than 100%) on GSH content after 6 hours of exposure compared to control groups. GST activity was significantly decreased after 24 and 48 hours of exposure in LB amphipods, while the ones from DS showed an increase on GST activity after 24 hours of exposure compared to control group. There was an increase of about 30% of CAT activity in LB organisms after 24 hours of exposure, while those from DS decreased CAT activity along whole exposure. In conclusion, control adults from the DS exhibited lower GSH content and GST activity than control adults from the LB, evidencing a differential response between the two populations in relation to the quality of the sampling site. Short-term exposure to WSF also produce oxidative stress in both populations in view of the changes observed in the components of antioxidant system.

**FP096 Biochemical responses induced by coexposure of the nanomaterial titanium dioxide (rutile and anatase) and copper in *Limnoperna fortunei*.**

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The titanium dioxide nanomaterial (nTiO<sub>2</sub>) has been produced and incorporated into various consumer products and commercial applications, due their catalytic, photocatalytic and ultraviolet (UV) light absorption properties. The wide applications of nTiO<sub>2</sub> can lead to the release of this nanomaterial into the environment endangering the organisms. In addition, nTiO<sub>2</sub> has been used in environmental remediation because of its ability to degrade or adsorb metals from water, and can coexist with metals such as Cu, for example. The nTiO<sub>2</sub> naturally exists in the crystalline forms rutile and anatase, which have different properties and can cause different effects in the organisms. This way, both nTiO<sub>2</sub> and Cu can occur together in the environment, and there is a need to study the potential combined effects of them. For this reason, this study aimed to evaluate if the coexposure to different crystalline forms of nTiO<sub>2</sub> (rutile and anatase) (1 mg/L) can influence in the toxicity and bioaccumulation of Cu (56  $\mu$ g/L) in the golden mussel *Limnoperna fortunei* after 120 h of exposure. The following parameters were analyzed in the gills, digestive gland and adductor muscle: (1) quantification of Cu, (2) determination of the activities of glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) enzymes, and (3) assessment of the levels of lipid peroxidation (LPO). The results showed that coexpositions increased Cu accumulation in the three tissues independently of crystalline form. In the gills,

rutile and coexposure rutile + Cu increased GST activity and decreased SOD activity. In the digestive gland, anatase increased GST activity and decreased SOD activity. In the adductor muscle, rutile and coexposure rutile + Cu decreased the activity of GST and SOD. All treatments inhibited CAT activity in the gills and digestive gland, while in the adductor muscle the CAT activity was inhibited by rutile and coexposure rutile + Cu. Only rutile caused lipid damage in the gills. These results suggest that both crystalline forms exhibited toxicity and that coexposure of nTiO<sub>2</sub> with Cu may be harmful to *L. fortunei*, thus more attention in use and release of nTiO<sub>2</sub> into environment are needed to avoid effects in aquatic biota.

**FP097 Biomarker responses in liver of zebrafish (*Danio rerio*) after acute exposure to sublethal concentrations of triclosan in water.**

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Triclosan (TC) is a widely used antibacterial/antifungal compound found in gels, toothpaste, deodorants, etc. The responses of hepatic biomarkers were evaluated in zebrafish (*Danio rerio*) after acute exposure to two sublethal concentrations of TC. Fish (391 ± 58 mg) were exposed to nominal concentrations of TC in water: 160 µg/L (TC1) and 340 µg/L (TC2) using a stock solution of TC dissolved in methanol, with a continuous flow system. Control fish remained in unchlorinated water (Ctrl) and water with the maximum concentration of methanol used (CtrlSv). Water samples were taken for determining TC exposure concentration by HPLC-MS. After 48 hours, fish were anesthetized, measured, weighed, sacrificed, and the liver was excised. Pooled samples were made up from 4 individuals, and 8 samples per treatment were processed (n=128). Enzymatic and non-enzymatic biomarkers were evaluated: activities of glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT), total antioxidant capacity against peroxy radicals (ACAP) and lipid peroxidation levels as thiobarbituric acid reactive substance (TBARS). Statistical differences (p<0.05) between groups were determined using analysis of variance followed by Tukey's test. No fish mortality was observed during assay period. There were no significant differences between Ctrl and CtrlSv in the different biomarkers tested. In addition no significant difference in SOD activity and ACAP level were observed. Acute exposure to TC promoted significant increase in TBARS levels by 20% in TC1 and TC2 with respect to Ctrl. However, an inhibition in CAT activity was detected in zebrafish exposed to TC1 (-24%) and TC2 (-34%) while GST activity decreased by 24% in both concentration relative to Ctrl. These results suggest that acute exposure of zebrafish to TC promoted an inhibitory response in liver at both, antioxidant activity (CAT) and biotransformation (GST). Furthermore, exposure to TC showed that lipid peroxidation in liver was promoted. Finally, both tested TC concentrations lead to similar biomarker responses after acute exposure.

**FP098 Biomarkers in native fish present in the biobio river, central Chile, convergence effects of complex chemical mixtures**

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The Biobío River is the third most important basin in Chile with a 24.369km<sup>2</sup> and supplies drinking water to about 970,000 inhabitants. Numerous companies use the water resource for their industrial processes, and as a consequence the elimination of effluents on their main channel, generating zones of punctual contamination (pulp and paper mill, oil refinery, among others), which due to the improvements in their technologies, have shown an increase in their productivity in recent years. Further, there are areas of diffuse pollution from the use of agricultural and forestry land, which together have affected the biota and water quality mainly in its lower third. To understand how the adjacent development alters the water quality and impacts the physiological state of native fish, in this work we identified sublethal responses evaluated using biomarkers strategy in native fish (EROD activity determined as pmol resorufin/mg prot./min), physiological indices (condition factor and hepatosomatic index), and environmental parameters (pH, temperature, conductivity, total dissolved solids). The obtained results exhibit an increase of the EROD activity evaluated in livers of the *Percilia irwini* species (n=106), presenting an induction gradient along the main course, with maximum values of 492±257 and 335±34 in females and males respectively, in the lower third of the river. These responses coincide to an increase in the environmental parameter values towards this zone and show a convergence effect of a complex mixture of chemical contaminants with respect to less impacted areas.

Consequently, the results of this work indicate a gradient of observable biological effects in the lower third of the Biobío River potentially attributable to the convergence of point and diffuse chemical contaminants in the study area. Mauricio Quiroz-Jara is scholarship CONICYT-Chile for PhD studies. Financial support for this study was provided by CRHIAM/Conicyt/Fondap 15130015.

**FP099 Bisfenol A não altera atividade colinérgica de *Oreochromis niloticus***

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A tilápia-do-nilo (*Oreochromis niloticus*) é uma espécie mais cultivada em piscicultura de todo o mundo. O bisfenol A (BPA) é um monômero de plástico policarbonato que está presente em uma série de produtos de consumo e é constantemente liberado no ambiente, contaminando, assim, águas e solos. Estudos utilizando BPA resultaram em alterações enzimáticas em modelos de roedores, mas quase nada se conhece os efeitos do BPA sobre os peixes. A acetilcolinesterase (AChE) é uma enzima chave que encerra a neurotransmissão entre muitos tipos de células excitáveis. O objetivo deste trabalho foi analisar os efeitos agudos do Cd sobre a atividade da AChE muscular de tilápias-do-nilo. Os peixes foram aclimatados durante 7 dias. Foram separados em duplicata, os grupos controle (CTR, n=10) que receberam injeção de intraperitoneal (IP) de veículo apenas e o grupo experimental (EXP, n= 10) no qual inoculou-se via IP, 150 mg/kg de BPA. Os peixes foram mantidos em observação durante 30 dias alimentados diariamente com ração. Após o tratamento, os animais foram anestesiados e o músculo dorsal foi excisado, homogeneizado em tampão fosfato de potássio (0,1 M) para a determinação da atividade da AChE (nmol DTNB. min<sup>-1</sup>.mg de proteína<sup>-1</sup>) por espectrofotometria (415 nm), a concentração de proteínas totais foram quantificadas e o resultado expresso em médias (M) e erro padrão (SE). Realizou-se análise de variância paramétrica (ANOVA, P < 0,05). Não houve mortalidade de peixes ao longo do período experimental e os resultados demonstraram que a atividade da AChE muscular do grupo CTR (M=2,06; SE=0,3) não foi diferente do grupo EXP (M=1,59; SE=0,22, P=0,11). Neste modelo experimental o BPA não alterou a atividade colinérgica de tilápias-do-nilo, embora persistente nos organismos, o composto avaliado parece não alterar na transmissão neuromuscular e portanto não causar efeitos no comportamento natatório dos peixes avaliados.

**FP100 Cádmio e estresse oxidativo em fígado de fêmeas de *Rhamdia quelen***

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O crescente aumento populacional atrelado às ações antrópicas acarretam a degradação do ambiente, o qual tem por sua vez a água como um dos recursos mais afetados. Devido a esse agravo, é imprescindível manter a qualidade da água para consumo e aplicações em diversas áreas, evitando a entrada de poluentes, como os metais. Dentro dessa categoria encontra-se o metal cádmio (Cd), que pode ser encontrado no ambiente por meio de resíduos de mineração, fundição, galvanoplastia e lixo tóxico descartado incorretamente. Tendo em vista que este metal é conhecido por promover efeitos tóxicos em diversos organismos, o objetivo deste trabalho foi avaliar os potenciais efeitos do cádmio em fígado de peixes fêmeas da espécie *Rhamdia quelen* através de biomarcadores bioquímicos. Estes peixes foram expostos às concentrações nominais de 0, 0,1; 1; 10 e 100 µg.L<sup>-1</sup> de Cd, por via hídrica, com condições controladas de temperatura e fotoperíodo. O bioensaio foi conduzido com a troca de 1/3 da água, a cada 24h, com reposição total do poluente. Após 15 dias de exposição, os animais foram anestesiados com benzocaína 1%, eutanasiados e o fígado coletado para análises bioquímicas. O cádmio não causou alterações na atividade das enzimas relacionadas ao sistema de biotransformação, como etoxiresorufina-O-deetilase (EROD) e glutatona S-transferase (GST). No entanto, o Cd provocou aumento na atividade da enzima superóxido dismutase (SOD) do sistema antioxidante, nas concentrações de 1, 10 e 100 µg.L<sup>-1</sup>. Contudo, a atividade das enzimas catalase e glutatona peroxidase, que são responsáveis pela degradação da espécie reativa peróxido de hidrogênio (H<sub>2</sub>O<sub>2</sub>), não foram alteradas, sugerindo uma possível superprodução de H<sub>2</sub>O<sub>2</sub> pelo aumento da atividade da SOD. Nos animais expostos a 100 µg.L<sup>-1</sup> de Cd também ocorreu aumento da lipoperoxidação, e este dano pode ter ocorrido devido às alterações observadas no sistema antioxidante. Também foi observada a redução da concentração de metalotioneínas, no grupo exposto a 10 µg.L<sup>-1</sup> de Cd, sendo esta uma molécula que atua na desintoxicação de metais e como molécula antioxidante. Desta forma, as alterações ocorridas no fígado indicam que o Cd, em concentrações semelhantes às encontradas em ambientes aquáticos, afeta o sistema antioxidante em fêmeas de *R. quelen*. Assim, a presença de Cd no ambiente pode apresentar implicações ecológicas para os organismos expostos.

**FP101 Cadmium effects on blood, gills and kidney of *Rhamdia quelen***

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The aquatic environment is daily affected by different contaminants, among them is the non-essential metal cadmium (Cd). The sources of this metal are natural events, as erosion of rocks, and anthropogenic activities, as industry and mining. However, it is known that even at low concentrations Cd causes alterations in different tissues of aquatic and non-aquatic organisms. In this work, we exposed adult male fish of *Rhamdia quelen* to 0 (control), 0.1, 1, 10 and 100  $\mu\text{g L}^{-1}$  of Cd, for 15 days. The 1/3 of water of each tank, was replaced daily with Cd concentrations. After exposure, the animals were anesthetized to collect blood and euthanized by medullary section to collect tissues. Blood, gills and posterior kidney cells were used in the comet assay. Cd bioconcentration was measured in posterior kidney. The concentrations of chloride ( $\text{Cl}^-$ ) and magnesium ( $\text{Mg}^{2+}$ ) ions were measured in plasma obtained by blood centrifugation. Carbonic anhydrase (CA) activity was measured in gills and posterior kidney. Also in the gills was measured the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase, and glutathione reduced (GSH), metallothioneins and lipid peroxidation (LPO) levels. Cd was bioconcentrated in posterior kidney of fish exposed to 1, 10 and 100  $\mu\text{g L}^{-1}$  of Cd. This result corroborates other studies with *R. quelen* that demonstrated nephrotoxicity of Cd. Although other studies have demonstrated effects of metals on osmoregulatory mechanisms, Cd did not alter neither CA activity, nor  $\text{Cl}^-$  and  $\text{Mg}^{2+}$  plasmatic concentrations. Also, it was not observed increased DNA damage in any of the tissues. Regarding to antioxidant system in gill the lowest Cd concentration reduced the GSH levels, while 10 and 100  $\mu\text{g L}^{-1}$  Cd reduced the GPx activity and 1 and 10  $\mu\text{g L}^{-1}$  Cd reduced LPO levels. These results showed that Cd effects in gills are not associated to a single mechanism of toxicity, since each concentration caused different response. However, these alterations did not cause genotoxicity or cell membrane damage, although the gills are in constant contact with the external environment, which make them the first target to waterborne pollutants. Thus, 15 days of Cd exposure did not cause serious toxic effects in gills, and it can be associated with the bioconcentration of Cd in other tissues, as liver and kidney, as observed in other studies, besides the action of cellular repair mechanisms in the gills.

#### FP102 Can propolis cause changes in biochemical and genotoxic biomarkers when used for fish maintenance in laboratory?

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Propolis is a complex mixture resulting from the combination of resinous substances collected by bees from flowers and modified by the addition of salivary secretions and wax. This compound may prolong the physiological functions of some aquatic organisms, once it has antioxidant and neuroprotective effect, as well as antibacterial, antifungal, anti-inflammatory action, which makes the propolis a compound of interest in the treatment of animals kept in laboratory. Therefore, the objective of this work was to evaluate the effects of propolis on biochemical (content of antioxidant glutathione – GSH; glutathione-S-transferase – GST; lipid peroxidation – LPO; acetylcholinesterase activity–AChE) and genotoxic biomarkers (DNA damage and occurrence of micronucleus) aiming the use of this product in the maintenance of fish in laboratory before ecotoxicological tests. For this purpose, we used specimens of *Hoplias malabaricus* ( $317.42 \pm 121.03\text{g}; 30.11 \pm 3.78$ ;  $n = 14$ ), divided into two groups (CTR and PROP). Fish of PROP group ( $n = 7$ ) were kept in individual aquaria containing propolis dissolved in dechlorinated water ( $13 \mu\text{g L}^{-1}$ ), and the animals of CTR group ( $n = 7$ ) were kept individually in aquaria containing only dechlorinated water. After 7 days of exposure, fish were anesthetized in benzocaine to collect blood for comet and micronucleus assays. Then, fish were killed by medullary section for the removal of the liver and gills for GSH, GST, LPO analyses and brain and muscle for AChE analyses. The results showed that the GSH concentration (in  $\mu\text{g protein mg}^{-1}$ ) was significantly higher in PROP group ( $31.93 \pm 8.72$ ) compared to CTR ( $14.31 \pm 5.00$ ) just in liver. The AChE activity (in  $\text{nmol DTNB min}^{-1}\text{mg protein}^{-1}$ ) in the brain was significantly lower in PROP group ( $14.11 \pm 1.09$ ) compared to CTR ( $16.98 \pm 1.55$ ). Fish of PROP showed no significant difference from CTR in GST activity, occurrence of LPO in liver and gills, and score of DNA damage and occurrence of micronucleus. The improvement on GSH may be related the known antioxidant activity of propolis. On the other hand, the changes in AChE activity may reflect the

neuronal influence of propolis, which may change future measurements during an experimental analysis. Thus, the propolis is a good alternative to treatment of fish before experimental analyses, but its action on antioxidant and neuronal mechanism implies in a careful choice of posterior analyses.

#### FP103 Cardiac function of bullfrog tadpoles exposed to pyrethrin, associated or not to nanoparticles.

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The uncontrolled use of agrochemicals affects the aquatic environment, reaching non-target organisms, as amphibians. Due to the concern about those chemicals in the environment, there is an effort to produce new botanical insecticides with lesser negative potential effects. Indeed, there are some techniques that reduce those effects, such as the use of carriers to decrease the amount of chemicals employed. Among those agrochemicals and carriers are the pyrethrum (PIR) and the solid lipidic nanoparticles (NLS). The aim of this study was to evaluate the effects of PIR, NLS and NLS conjugated with pyrethrum (NLS+PIR) on the cardiac function of bullfrog tadpoles (*Lithobates catesbeianus*) at a concentration of 1/5 of 400  $\mu\text{g L}^{-1}$  determined by the LC50. Effects were measured via activity level (AL-% of individuals), cardiac frequency ( $f_{\text{H}}$ -bpm) and relative cardiac mass (RVM-% of body mass). Animals were divided into 4 groups: control (CTR), pyrethrum (PIR), solid lipidic nanoparticles (NLS) and solid lipidic nanoparticles conjugated with pyrethrum (NLS+PIR) assayed in triplicate. During exposure, the AL of the animals was determined. After euthanasia, the *in situ f<sub>H</sub>* and the RVM were analysed. It was observed a tachycardia only for the NLS+PIR in relation to the CTR group. Considering that it was not observed a proportional increase in the AL, it can be suggested that increase in the  $f_{\text{H}}$  was promoted by a direct action of the associated xenobiotics on the pace-marker. Further studies will be performed to analyse the *in vitro* cardiac function.

#### FP104 Changes in biochemical parameters in sexually matured three-barbeled catfishes individuals exposed to Roundup®, Primoleo® and Facet®

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One important issue due to anthropogenic activities is the use of agrochemicals in cash crops, which can result in physiological and/or morphological disorders in both target and non-target species. In order to verify this effect, three herbicides were used: Roundup® (18 and 72  $\mu\text{g/L}$ ), Primoleo® (10 and 15  $\mu\text{g/L}$ ) and Facet® (1.75 and 14  $\mu\text{g/L}$ ) in the aquariums of the three-barbeled catfishes (*Rhamdia quelen*). The metabolism and the oxidative balance were both checked. The results showed protein depletion in the gills, an increase in hepatic glycogen storage and consumption of triacylglycerols, and no stock of muscle glycogen from the presence of all three agrochemicals checked. There were accumulation of total lipids in the liver when using Roundup® and Primoleo® and when exposed to Roundup® and Facet® the gills reserve was used. In the kidneys, Facet® caused an increase of glycogen, Roundup® and Primoleo® increased protein, and Roundup® increased total lipids levels. The most affected organ by Primoleo®, which had changes in all metabolites, was the muscle. For the oxidative balance markers analyzed here (superoxide dismutase, catalase and lipoperoxidation), we can see that the liver and kidney were the most affected, agreeing with known information about the role of these organs in the maintenance of homeostasis, specially in the functions of metabolism and excretion of xenobiotics. The results showed that Facet® was the most harmful, followed by Primoleo® and Roundup® for oxidative balance. None of the concentration used in all three herbicides caused death to the fishes, but alterations in the metabolism and oxidative balance were detected even in the smallest concentrations used.

#### FP105 Combined effects of temperature and clomazone on oxidative stress responses and B-esterase activity of two species of neotropical tadpoles

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Temperature is an important factor influencing the toxicity of chemicals in aquatic environments. Amphibians from neotropical areas experience large temperature fluctuation in their habitats, especially during the larval stage, when tadpoles are restricted to small and ephemeral ponds. When located in agricultural areas, several pesticides can contaminate these ponds; therefore, there is a great concern that combined effects of temperature and local pesticides in natural systems may be affecting tadpoles of tropical areas, such as Brazil. In this study, we evaluated the effects caused by the exposure to clomazone (Gamit®) at different temperatures (28, 32 and 36 °C) on biochemical stress responses and esterase activities in *Physalaemus nattereri* and *Rhinella schneideri* tadpoles. Results showed that temperature was an important factor inducing biochemical response in tadpoles exposed to clomazone. Antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G6PDH) had their activities increased by clomazone in tadpoles of *P. nattereri* treated at higher temperatures, with more evident responses after three days of exposure. The biotransformation enzyme glutathione-S-transferase (GST) was also induced by exposure to clomazone at 32 and 36°C. SOD was the only enzyme altered at 28 °C. In *R. schneideri*, clomazone failed to alter antioxidant enzymes at 28 °C, but SOD and GST activities were increased by clomazone at higher temperatures after three days. All enzymes had their activities returned to the control levels after eight days in *R. schneideri*. Lipid peroxidation was induced in both species of tadpoles exposed to clomazone at 32 and 36°C, but not at 28°C. Esterase analysis also showed that acetylcholinesterase (AChE) was not sensitive to clomazone and thermal stress conditions, while most treatments impaired carboxylesterase (CbE) activity. Our results evidenced that temperature modulates the effects of clomazone on biochemical response of tadpoles. This implies that tadpoles from tropical areas may present differential responses in their physiological mechanism linked to antioxidant defense to deal with temperature fluctuations and agrochemicals presence in their habitats. Thus, we highlight the need to consider the abiotic factors in further studies assessing pesticides impact in amphibian species.

**FP106 Comparación del efecto del pesticida spirotetramat en diferentes estadios de desarrollo del camarón *Macrobrachium borellii* (Crustacea: Palaemonidae)**

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La intensa utilización de pesticidas ha provocado el desarrollo de mecanismos de resistencia en los organismos blanco a diversos tipos de insecticidas como los neurotóxicos. La implementación del manejo integrado de plagas, implica el uso de programas selectivos de compuestos más eficientes y menos perjudiciales para el medio ambiente. Spirotetramat (STM) es un insecticida perteneciente al nuevo grupo de cetoenoles, el cual se destaca por su amplio espectro de acción contra insectos chupadores. Su modo de acción es a través de la inhibición de la síntesis de lípidos lo cual provoca que los estadios de desarrollo más inmaduros resulten los más susceptibles. El objetivo de este estudio fue evaluar el efecto del STM en embriones y larvas del camarón de agua dulce *Macrobrachium borellii*, una especie no blanco que fue seleccionada como modelo. Inicialmente, se realizaron diluciones seriadas del insecticida para calcular la LC<sub>50</sub>-96h. Posteriormente, se analizó la presencia de cambios histopatológicos en larvas. Así mismo se expusieron hembras ovígeras a una concentración subletal de STM (1,8 mg/L) durante 4 días con el fin de evaluar en embriones las actividades de las enzimas catalasa (CAT), glutatión-S-transferasa (GST) y superóxido dismutasa (SOD) como así también los niveles de peroxidación lipídica (LPO). El valor de la LC<sub>50</sub>-96h determinado en las larvas fue 11 (3-22) µg/L de STM, que resulta mucho más sensible a la de los adultos. Sorpresivamente, los embriones resultaron muy resistentes a la exposición con STM y no se observó efecto letal en los tratamientos con altas concentraciones de este insecticida. Este hecho probablemente se deba a que la cáscara tendría un efecto protector reduciendo el ingreso de compuestos tóxicos. Sin embargo los embriones expuestos a 1,8 mg/L de STM mostraron alteraciones en los parámetros oxidativos evaluados evidenciando un ingreso restringido del insecticida. El STM causó alteraciones histopatológicas significativas en diversas estructuras de las larvas. Los resultados de este estudio demuestran que las larvas resultaron muy sensibles al STM y que podrían estudiarse otros mecanismos que contribuyan a la resistencia presentada por los embriones.

**FP107 Comparison of the base excision repair activity in liver cell models of zebrafish (*Danio rerio*)**

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Biológicas - ICB

Fish cellular models are commonly used to study the toxic potential of environmentally relevant compounds. Several of these pollutants act on DNA and compromise its integrity. Little is known, however, about the DNA repair ability of these cellular models. Therefore, the aim of this study was to evaluate the DNA base excision repair (BER) of ZF-L (ZebraFish Liver) cell line and primary hepatocytes. We performed kinetic studies of the DNA damage levels after exposure to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 20 µM for 10 min) using the Comet Assay. As soon as 10 min after H<sub>2</sub>O<sub>2</sub> treatment, 16% and 50% of the initial damage, as measured as comet tail length, were repaired in ZF-L line and primary hepatocytes, respectively. Primary hepatocytes repaired 50% of the damages twice as fast as ZF-L cell line and showed DNA damage levels similar to control 40 min after H<sub>2</sub>O<sub>2</sub> treatment. The total recovery time for ZF-L model was of 180 min, which indicates the culture cells have a less efficient BER. In conclusion, both ZF-L cell line and primary hepatocytes exhibit BER activity, however these cellular models have different repair capacity. In addition, we demonstrated that ZF-L cell line and primary hepatocytes are useful tools for ecotoxicological studies focusing on DNA damage (as single-strand break) and BER.

**FP108 Comparison of the effects of synthetic and biological silver nanoparticles and silver nitrate on *Prochilodus lineatus* through biochemical biomarkers**

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The daily diversification of industrial development has allowed a large variety of residues to reach the aquatic environment, affecting its biota through inadequately treated industrial and domestic sewage. Among these residues, a new type of material has raised some preoccupation: metallic nanoparticles. Nanoparticles (NP) are used in large scale in the most diverse products, being silver NP (NPAg) highly used due to its antimicrobial action. The NPAg can be classified as synthetic (sNPAg), if originated from chemical reduction, or biological (bNPAg), if produced by an enzymatic reduction through microorganisms. However, very little is known about the effect of this material on live beings, both in its synthetic as well as biological forms. Thus, the objective of this work was to compare, through biochemical biomarkers, the effects of exposure to sNPAg, bNPAg and silver nitrate (AgNO<sub>3</sub>), using *Prochilodus lineatus* fish juveniles as an experimental model. Samples of *P. lineatus* were divided into a control group (CTR), exposed only to dechlorinated water and in other three experimental groups: sNPAg, exposed to 10 µg L<sup>-1</sup> of synthetic NPAg; bNPAg, exposed to 10 µg L<sup>-1</sup> of biological NPAg; and AgNO<sub>3</sub>, exposed to 10 µg L<sup>-1</sup> of silver nitrate. All groups were tested simultaneously for 4 days (96h). At the end of the exposition period, the fish were anesthetized and killed by spinal cord section for the removal of the liver and to determine the activity of the antioxidant enzymes catalase (CAT) and glutathione-S-transferase (GST), and glutathione (GSH) concentration. The groups showed no significant difference for CAT and GST enzymes activity; however, there was a significant reduction in GSH content in the groups exposed to sNPAg and to AgNO<sub>3</sub>. Thus, it was concluded that part of the hepatic cells antioxidant defenses were sensitive to sNPAg exposure similar to that observed for the AgNO<sub>3</sub> group, indicating less bNPAg toxicity.

**FP109 Desórdenes metabólicos en ejemplares de *C. decemmaculatus* expuestos a muestras del río Reconquista.**

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Se evaluó el efecto de la exposición de adultos de *C. decemmaculatus* a agua del río Reconquista sobre: ingesta (I), asimilación (A), excreción de amonio (E), tasa metabólica específica (TM), eficiencia de extracción de O<sub>2</sub> (OEE), cociente de amonio (CA) y *scope for growth* (SFG). La muestra de agua se tomó de las nacientes del cauce principal del río Reconquista, donde habita la especie. La misma fue fisicoquímicamente caracterizada, filtrada y conservada a 5°C hasta su uso. Se utilizaron ejemplares de *C. decemmaculatus* provenientes de cultivo de laboratorio. El ensayo fue por triplicado con aclimatación de 14 días en medio control (agua moderadamente dura -MHW), renovación parcial cada 96 h, alimentación *ad libitum* y carga de organismos de 0,5 g/L. las condiciones experimentales (aireación, temperatura 23±1°C, fotoperiodo 16L:8Ó, carga) permanecieron constantes todo el ensayo. La exposición fue de 12 días; diariamente se ofreció una ración de alimento del 2% de la biomasa total/réplica, luego de retiradas las heces (H) y durante 60 min. El alimento sobrante y las H producidas se secaron hasta peso constante, se pesaron y se calculó A, como I-H. A tiempo final de exposición, un grupo de animales de RR y

MHW se utilizó para determinar consumo de oxígeno, E y se calcularon OEE, TM sp, CA y el SFG. Para ello, cada ejemplar fue puesto en un respirómetro con MHW aireada (sistema de flujo abierto) por 1 h (aclimatación). La permanencia se prolongó durante otra hora (sistema de flujo cerrado). A tiempo inicial (i) y final (f) de permanencia con flujo cerrado, se midieron  $O_2$  disuelto y  $[NH_4^+]$ . Además se determinó el factor de condición (FC) y los índices hepatosomático (HSI) y gonadosomático (GSI) de los animales sobrevivientes. La evaluación estadística de los resultados se efectuó mediante ANOVA, con test "a posteriori" de Tukey o Kruskal Wallis con comparaciones de a pares. El agua de RR se clasificó como polución leve. La mortalidad fue significativamente menor en RR respecto a MHW (5 y 12% respectivamente). El FC, HSI, GSI y TMsp no dieron diferencias significativas entre grupos. En comparación con los controles, si bien los animales expuestos a RR mostraron una mayor I, la A fue menor. Además en el grupo RR la E y el CA aumentaron más de un orden de magnitud y la OEE fue un 40% menor. Los valores de SFG denotan un menor crecimiento en el grupo RR. Los resultados obtenidos sugieren un deterioro de la capacidad homeostática en los peces expuestos a RR.

#### FP110 Determination of the LC50 of bullfrog tadpoles exposed to the biocide pyrethrin during 48 hours

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Contamination of water resources is mainly due to the increasing use of xenobiotics, especially agrochemicals, eventually reaching non-target species such as anuran amphibians. Among the most employed agrochemicals are pyrethroids, insecticides responsible for mitigate pests in agricultural fields, and its active compound pyrethrum obtained from the flowers of the chrysanthemum (*Chrysanthemum cinerariaefolium*). Considering that there are no data concerning the concentration of pyrethrin that can potentially be harmful to amphibians, an ecotoxicological experiment was performed to determine the  $LC_{50}$  (48 h). For this, the following groups were tested: control, vehicle (acetone), and six concentrations of pyrethrum ( $130 \mu g.L^{-1}$ ,  $170 \mu g.L^{-1}$ ,  $192 \mu g.L^{-1}$ ,  $220 \mu g.L^{-1}$ ,  $400 \mu g.L^{-1}$ ,  $580 \mu g.L^{-1}$ ). The animals were individually in 1 L plastic bottles. The analysis of the data (Graphpad Prism 5) indicated that the  $LC_{50}$  (48 h) for the tadpoles of *L. catesbeianus* was  $400 \mu g.L^{-1}$ . This preliminar study indicate that even at very low concentrations, this biocide can actually jeopardize anuran tadpoles.

#### FP111 Diversity of Benthic Macroinvertebrates and Toxicity of Chlorpyrifos on three native species from Pesticide-Contaminated and-Uncontaminated Sites

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The production of pears and apples is one of the most important economic activities in the North Patagonian Region of Argentina with significant implications to the environment quality. Studies from the area have demonstrated the presence of pyrethroids, organophosphates and carbamates in both groundwater and drain channels. Between 2008 and 2010, azinphosmethyl and chlorpyrifos showed similar detection frequencies (more than 70% each). During the last years, the detection of both pesticides in water was significantly reduced by use' restrictions. Macroinvertebrates responded to the pesticide impairment lowering richness and enhancing the abundance of the most tolerant taxa. Thus, the first objective of this study was to compare the assemblages of benthic macroinvertebrates from the Limay River (LR) and a drain channel (DC). The second objective was to compare chlorpyrifos toxicity on three of the most abundant taxa from these two sites. Four biomonitoring were performed at LR and DC during November 2014 and December 2015. Several physical and chemical measures including nutrients were analyzed. Chlorpyrifos toxicity was evaluated on *Hyalella curvispina* (Crustacea), *Heleobia spp* (Mollusca) and *Girardia tigrina* (Platyhelminthes) in 0.2 L volume of dechlorinated water. A total of 1267 and 2749 organisms were collected throughout the study period at LR and DC; respectively. Richness at LR with a total of 24 taxa included into 7 orders and 17 families was significantly higher ( $p < 0.05$ ) than the richness at DC with 16 taxa included into 7 orders and 14 families. Organisms from the Order Ephemeroptera and Trichoptera were not found at any time at DC. The predominant

taxa at DC corresponded to the orders Annelida, Mollusca, Crustacea and Diptera, especially *H. curvispina*. Both *H. curvispina* and *G. tigrina* from LR were 6- and 2-fold more susceptible to chlorpyrifos than their counterparts from DC, and the differences were significant. On the other hand, there were no differences in the insecticide toxicity on *Heleobia spp* from both sites. Significantly higher levels of phosphorus, nitrates and nitrites were recorded at the DC in comparison to LR ( $p < 0.001$ ). In conclusion, impacts in agricultural sites were evident. Hence, contaminated conditions of aquatic environment could induce greater tolerance of the macrofauna. From all taxa found at DC, *H. curvispina* seems to be the most adapted to the site conditions, enhancing both the pesticide and nutrients tolerance.

#### FP112 Ecotoxicological analysis using *Caenorhabditis elegans* and *Lactuca sativa* to assess water quality in the Tunuyán river basin (Mendoza, Argentina)

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Water quality has long been analyzed using physicochemical and bacteriological parameters. Today scientific community agrees that to have an overall scenario of environmental impact is necessary to include ecotoxicological measurements. Both determinations are critical to establish a sustainable water management policy. In this regard, the European Water Framework Directive has established a common framework for sustainable and integrated management of water resources, in which sensitive environmental information, biological and ecological data play a leading role. In Argentina routine monitoring in general includes only standardized physicochemical and bacteriological parameters but toxicological properties are not considered. Seven sites were selected from Tunuyán River Basin (Province of Mendoza, Argentina). Monitoring were carried out from autumn 2015 to autumn 2016 to assess temporal variability. Two bioassays were used to assess toxicological characteristics of the water samples: the nematode *Caenorhabditis elegans* bioassays that use the relative growth as an end point and the *Lactuca sativa* elongation test. Jointly with these bioassays, physicochemical and bacteriological parameters were determined and a Water Quality Index based on the Canadian Council of Ministers of the Environment (CCMEWQI) according to the local normative for aquatic life protection was built. Both toxicological tests help to identify different spatio-temporal water qualities in the river basin. Multivariate statistical analyses (PCA) were developed. A toxicological ranking was established to quantify the water toxicity level using the results from *C. elegans* bioassays. Our results shown that most of the samples were in the highly toxic effect category for *C. elegans*, while *L. sativa* test seemed to exhibit no phytotoxic effect. Although a biostimulation effect on the radicle and hypocotyl elongation were observed in all samples specially in the lower basin monitoring sites. Comparison between the WQI and the toxicological ranking confirmed that traditional water quality studies do not predict potential toxic effects on living organisms. Our results verify that the bioassays show different sensibilities. *C. elegans* proved to be a more suitable tool to assess toxicity. Furthermore, they should be implemented in routine water quality monitoring to obtain a realistic scenario of water quality threats.

#### FP113 Efecto del insecticida de última generación spirotetramat en el organismo no blanco *Macrobrachium borellii* (Crustacea: Palaemonidae)

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El desarrollo de las nuevas prácticas agrícolas conlleva al desafío de la búsqueda de pesticidas que sean más benignos para el medio ambiente. Entre la clase de insecticidas de última generación, los ácidos tetrámicos, como el spirotetramat (STM), actúan inhibiendo la lipogénesis en los organismos provocando una disminución del contenido de lípidos que afecta la reproducción y el crecimiento. Debido a la escasa información disponible sobre el efecto de estos compuestos en especies no blanco, se analizó el efecto del STM sobre el camarón de agua dulce *Macrobrachium borellii*,

seleccionado como modelo de estudio. Inicialmente, se determinó la LC<sub>50</sub>-96 h en camarones adultos (machos y hembras en estado no-vitelogénico), mediante diluciones seriadas del STM, con un control negativo sin el insecticida. Posteriormente, camarones fueron expuestos a concentraciones subletales de STM (0,6 y 1,8 mg/L) durante 4 días para evaluar los niveles de peroxidación lipídica (LPO) y oxidación proteica (OP) en el hepatopáncreas, como así también la presencia de cambios histopatológicos. Adicionalmente, se evaluaron las actividades enzimáticas de colinesterasa (ChE) y transaminasas GOT y GPT en hemolinfa. El valor de la LC<sub>50</sub>-96 h fue de 4,1 (2,2-5,6) mg/L de STM. En los camarones expuestos, los parámetros antioxidantes y los niveles de LPO medidos en el hepatopáncreas fueron afectados con respecto a los controles. Asimismo, las actividades de ChE, GOT y GPT en la hemolinfa resultaron alteradas. Por otra parte, el STM causó alteraciones histopatológicas significativas en el hepatopáncreas de los camarones expuestos, entre las cuales se incluyen una atrofia en el epitelio de los túbulos digestivos, la necrosis de las células epiteliales, y la infiltración de hemocitos en el tejido conectivo entre el espacio intertubular. Los resultados obtenidos indicarían que los daños inducidos por el STM en *M. borellii* se relacionan principalmente con la alteración del metabolismo lipídico y con el efecto tóxico del insecticida. Por lo tanto, se propone intensificar los estudios respecto del efecto de este nuevo insecticida sobre otros parámetros relacionados con tales vías metabólicas.

**FP114 Efeito dos herbicidas Boral® 500 SC e Glifosato® isolados e em mistura sobre marcadores bioquímico funcionais de girinos de *Rana catesbeiana***

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Dentre os agroquímicos mais utilizados mundialmente estão os herbicidas, seu amplo uso é um dos fatores que afetam o desenvolvimento, a reprodução e a sobrevivência dos anfíbios. Contudo, seus efeitos tóxicos em animais não foram ainda suficientemente estudados; assim, estudou-se o efeito da formulação comercial de sulfentrazone (Boral® 500SC) e do glifosato (Glifosato®) nos níveis de marcadores plasmáticos (glicose, proteínas totais, ácido úrico, triglicerídeos, colesterol VLDL e corticosterona), hepáticos e musculares (superóxido dismutase, catalase, glutatona S-transferase e lipoperoxidação), além de parâmetros de condição nutricional em girinos pró-metamórficos de *Rana catesbeiana*. Os 55 animais utilizados foram pesados, medidos e distribuídos em aquários com água dechlorada, temperatura de 23 ± 2°C, fotoperíodo 12h de luz:12h de escuridão, aeração constante e alimentados com ração comercial. Os animais foram aclimatados durante 7 dias e após, divididos em cinco grupos experimentais: Controle, Boral® 500 SC [130µg/L], Glifosato® [234µg/L] e Boral® 500 SC + Glifosato® [130 + 234µg/L, respectivamente], por mais sete dias. Observamos uma mortalidade de 8,3% evidenciando o uso de concentrações subletais. Ao final do experimento, foram retiradas amostras de sangue pesados, sendo os animais medidos e pesados. Após estes foram crioeutanasiados retirados o fígado, intestino e músculo caudal, sendo estes homogeneizados e congelados para determinação a posteriori da atividade das enzimas antioxidantes e dos níveis de lipoperoxidação. Foi utilizado teste de normalidade de Kolmogorov-Smirnov, seguido do teste de Kruskal-Wallis com o complementar de Student-Newman-Keuls, para um p < 0,05. Mesmo para as baixas concentrações dos herbicidas observaram-se alterações nos parâmetros estudados, principalmente no fator de condição específico (K<sub>intestinal</sub>); nos níveis de triglicérides, de ácido úrico e de corticosterona plasmáticos; e na atividade de todas as enzimas antioxidantes e nos níveis de LPO, tanto no fígado como no músculo. A mistura de Boral® e Glifosato® parece ser mais lesiva aos animais, principalmente pela manutenção e diminuição drástica dos níveis de atividade da Glutatona S-transferase no fígado e no músculo, respectivamente. Estas alterações parecem estar ligadas a um incremento da demanda energética na tentativa de manter a homeostase e garantir a sobrevivência dos animais; diminuindo possivelmente, a energia para o crescimento e a metamorfose.

**FP115 Efeitos agudos do cádmio sobre a defesa antioxidante do peixe *Prochilodus lineatus***

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O cádmio (Cd) é um metal traço não essencial aos animais e muito tóxico aos peixes, é um contaminante frequentemente encontrado nas águas interiores devido a atividades antropogênicas. O objetivo deste trabalho foi avaliar os efeitos do Cd para espécimes juvenis do peixe neotropical *Prochilodus lineatus*. Para tanto, peixes jovens foram expostos, durante 24 e 96 h, a duas concentrações de Cd, 1 (Cd1) e 10 (Cd10) µg.L<sup>-1</sup>, que correspondem às concentrações máximas de Cd permitidas pela legislação brasileira. Os efeitos do Cd foram avaliados por meio da análise das defesas antioxidante enzimática e não enzimática da brânquia e fígado. A atividade da glutatona-S-transferase (GST) e glutatona peroxidase (GPx) tiveram suas cinéticas

lidadas em espectrofotômetro a 340 nm por 1 min. A atividade da catalase (CAT) foi determinada através do decréscimo de absorvância em espectrofotômetro a 240 nm e a concentração de GSH foi estimada leitura foi realizada em espectrofotômetro de microplacas a 412 nm. As condições experimentais impostas ao *P. lineatus* não acarretaram em alterações nas funções da GST branquial ou hepática. A atividade da CAT hepática de *P. lineatus* é muito maior do que a branquial e a exposição aguda ao Cd 10 resultou em uma marcante diminuição na atividade da CAT dos hepatócitos. Como não foi constatada nenhuma variação significativa na atividade da GPx em fígado e brânquias de *P. lineatus* é pouco plausível que a GPx tenha sido a responsável pela degradação do H<sub>2</sub>O<sub>2</sub> gerada em decorrência do Cd. Para tanto a GPx deveria degradar o H<sub>2</sub>O<sub>2</sub> oxidando a GSH. A elevação inicial no conteúdo de GSH nas brânquias, enfraquece a hipótese. A estes argumentos deve-se acrescentar o não envolvimento da GST como parâmetro sensível à exposição aguda ao Cd, pois ainda mais sem haver diminuição de GSH no fígado e na brânquia. Em conclusão, exposição aguda de curimbas juvenis às concentrações de Cd permitidas pelo CONAMA, aumentou nas brânquias, o conteúdo de GSH após 24 h e a atividade da CAT após 96 h de exposição. Houve inibição da atividade da CAT hepática dos indivíduos expostos ao Cd10 em ambos os tempos experimentais.

**FP116 Efeitos do Zn, Mn e Fe isolados e em mistura na osmorregulação do teleosteo dulcícola *Prochilodus lineatus***

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Juvenis do teleosteo dulcícola *Prochilodus lineatus* foram utilizados para avaliar os efeitos osmorregulatórios dos metais essenciais Zn, Mn e Fe, isolados e em mistura. Os peixes foram submetidos, por 96 h, a três testes independentes, cada qual com o seu respectivo grupo CTR (peixes expostos apenas à água): teste do Zn e teste do Mn, com peixes expostos ao Zn (0,18; 1,0; 5,0 mg L<sup>-1</sup>) ou ao Mn (0,1; 0,5; 5,0 mg L<sup>-1</sup>) e teste da mistura, com peixes expostos ao Fe (5 mg L<sup>-1</sup>), à mistura Zn (1,0 mg L<sup>-1</sup>) + Mn (0,5 mg L<sup>-1</sup>) e à mistura Zn+Mn+Fe, nessas mesmas concentrações. Nas brânquias foram analisadas as atividades das enzimas anidrase carbônica (AC), H<sup>+</sup>ATPase (HATP), Ca<sup>2+</sup>ATPase (CaATP), Na<sup>+</sup>K<sup>+</sup>ATPase (NKA) e Mg<sup>2+</sup>ATPase (MgATP) e no plasma as concentrações de Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup> e osmolalidade. Nos peixes expostos ao Zn observou-se uma diminuição do K<sup>+</sup>, Na<sup>+</sup> e Ca<sup>2+</sup> plasmáticos, porém nenhuma alteração foi observada na atividade das enzimas branquiais. Já a exposição ao Mn causou alterações apenas do Ca<sup>2+</sup> plasmático, a menor concentração promoveu redução do Ca<sup>2+</sup> enquanto as maiores concentrações causaram aumento do Ca<sup>2+</sup>. A maior concentração de Mn também promoveu aumento de HATP e AC indicando acidose. Nos animais expostos ao Zn+Mn verificou-se redução de Ca<sup>2+</sup> plasmático e aumento da CaATP, enquanto nos peixes expostos a Zn+Mn+Fe verificou-se aumento da CaATP e redução da HATP. Portanto, esses biomarcadores mostraram-se úteis para o monitoramento de locais contaminados por Zn, Mn e Fe em mistura, mas também Zn e Mn isoladamente, podendo ser aplicados em estudos de regiões Neotropicais no teleosteo *P. lineatus*.

**FP117 Effects of 4-nonyphenol and 17β-estradiol on synthesis of vitellogenin and steroid and thyroid hormones in immature fish *Accanthopagrus latus***

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In the study, the estrogenic effects of 4-nonyphenol (4-NP) and 17β-estradiol (E2) and synthesis of vitellogenin (VTG), level of steroid and thyroid hormones and hepatosomatic index (HIS) in immature yellowfin seabream, *Accanthopagrus latus*, were assessed. To do this, a total number of 104 fish were abdominally injected by 10, 50, 100, 150 and 200 µg/g/week and 2 µg/g/week of E2. The experiments were conducted over a period of two weeks. Induction of vitellogenin generation in plasma of treated fish was assayed indirectly by measuring of total plasma calcium and alkali-labile phosphate. The increased level of the indicators in treated fish's plasma showed the production of 4-NP in liver dose-dependently changed. Meanwhile, a significant dose-dependent increase was observed in HIS which indicated the activation of hepatic VTG production in 4-NP- and E2-injected fish. 4-NP treatment did not have any significant effect on plasma levels of testosterone. In addition, it was observed that 4-NP affect the level of thyroid hormones in fish. Plasma thyroxine levels rose in a dose-dependent manner after 7 and 14 days of the exposure. In contrast, a significant decrease in triiodothyronine levels was observed during the experiment period. Moreover, no significant change was detected for thyroid stimulating hormone levels in 4-NP-treated fish. These results demonstrated that 4-NP is so influential to induce the VTG. Based on the findings, it can be concluded that 4-NP can strongly disturb the balance of steroid and thyroid hormones with potential consequences for sexually



immature male yellowfin seabream.

**FP118 Effects of fipronil in biomarkers responses in a native species (*Cyphocharax voga*): branchial ex vivo exposure**

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Fipronil (Fp) is a current use pesticide, highly toxic with potential nocive effects on aquatic organisms. Biomarkers responses are frequently used to detect these effects in fish either on laboratory exposure or in situ assessments. In *ex vivo* experimental models, the use of gills is justified since it is the first organ in contact with environmental pollutants. Biomarkers responses promoted by two sublethal concentrations of Fp were evaluated in *Cyphocharax voga* after a branchial ex vivo exposure model. After sacrificing the fish (n=4), branchial arches were dissected and randomly assigned to four treatment groups: saline solution (Ctrl); methanol in saline solution (CtrlSv); and two nominal concentrations of Fp (9 µg/L, C1 and 100 µg/L, C2) prepared from a stock solution on methanol. Gills remained submerged in the solutions under continuous agitation for 1h at 25°C in glass containers. Levels of Fp were also analyzed with liquid-liquid extraction and GC-ECD. After incubation period, enzymatic activities of superoxide dismutase (SOD), antioxidant capacity against peroxy radicals (ACAP), lipid peroxidation levels by TBARS and reduced glutathione levels (GSH) were evaluated. Differences between groups were analyzed by one way ANOVA (p< 0.05) and Tukey's multiple comparisons. No significant differences between the parameters of Ctrl and CtrlSv were detected. Gills exposure to C1 concentration promoted an increase (15%) in SOD activity. Furthermore, a significant increase (40%) in TBARS levels was detected in C1 group respect to Ctrl. Exposition to C2 concentration promoted a decrease (38%) in GSH levels. No significant differences were observed in total antioxidant capacity (ACAP). These results indicate that the assayed concentrations of Fp proved to be an inducer of oxidative stress in gills. Enzymatic and non-enzymatic activities were not enough to prevent oxidative damage to lipid levels.

**FP119 Effects of glyphosate, a formulation, and adjuvant on phase I and II biotransformation, oxidative stress and antioxidant enzymes in *Cyprinus carpio***

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Glyphosate is the most sold herbicide worldwide since the introduction of genetically modified crops in 1996. Biomarkers are early warning sublethal responses useful for detecting exposure and adverse effects induced by toxicants before they are irreversible. In the present study, the response of phase I and II biotransformation and antioxidant systems, together with oxidative stress, in the teleost *Cyprinus carpio* after sublethal exposure to glyphosate (AI, active ingredient), a commercial formulation (FORM), and its adjuvant, polyoxyethylene amine (POEA). Two experiments were performed: in experiment 1 (EXP1) fish (62,5±6,1 g) were exposed to 0.01, 1.00 y 100.00 mg L<sup>-1</sup> of AI during 2 and 8 d., and in experiment 2 (EXP2) fish (54,4±6,1 g) were exposed to 1.00 mg L<sup>-1</sup> of IA, the 1.35 mg L<sup>-1</sup> FORM (same concentration of AI), and 0,135 mg L<sup>-1</sup> POEA (at the same concentration in FORM) during 8 d. Enzyme activities and malondialdehyde were measured by fluorometric and spectrophotometric methods adapted to microtiter plates. Neither the growth rate, the condition factor, the hepatosomatic index, or the gonadosomatic index were significantly affected at any treatment of both experiments. In the liver, benzyloxy-resorufin-O-debenzylase (BROD) and glutathione-S-transferase (GST) activities were inhibited by GLY in a dose-response way, showing statistically significant differences after 8 d at 100.00 mg L<sup>-1</sup>, EXP1. Similarly, ethoxy-resorufin-O-deethylase EROD activity was reduced in both EXP, but inhibition was only statistically significant in all treatments of EXP2 in fish exposed to GLY and FORM. CAT was significantly inhibited in fish exposed to FORM and POEA in EXP2 and also exposed to GLY in EXP1. In agreement, TBARS was significantly increased in EXP1 at the highest concentration and longest exposure time. CAT was also inhibited in gill and brain by FORM and POEA. In addition, those treatments, GPx was also significantly increased in gill. Obtained results are indicating that GLY is able to inhibit the biotransformation system and modulate the antioxidant enzymes, inducing oxidative stress in some organs, alone or in the FORM. In addition, POEA by itself, modulated the antioxidant system but without inducing significant oxidative stress.

**FP120 Effects of organophosphate Acephate on liver biomarkers of *Rhamdia quelen* (Teleostei, Heptapteridae).**

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The aim of this study was to investigate the possible neurotoxic effects and oxidative stress in different sub-lethal concentrations of organophosphorus (OP) Acephate. Juveniles of *Rhamdia quelen* were placed in 5 tanks (n=60, 12 in each) with dechlorinated water. The animals were exposed to concentrations of 0 mg.L<sup>-1</sup> (control), 1, 10, 100, 1000 mg.L<sup>-1</sup> of Acephate. After 24 hours of OP exposure, the fish were killed by medular section and liver were sampled. The samples were stored in microtubes with Tris-HCl buffer pH7.4, homogenized and centrifuged at 12000g. The supernatant was separated and stored at -80°C until the oxidative stress analysis (lipid peroxidation – LPO, superoxide dismutase – SOD, catalase – CAT) and cholinesterase activity (ChE) were done. The resulting data were analyzed by one-way ANOVA and Fisher-LSD Post-hoc test. In evaluation of the LPO reaction, it was found significant differences between the treatments groups. The group exposed to 1 mg.L<sup>-1</sup> of OP showed the highest value (13.08±2.73) and, as the concentration of OP increases the reaction decreases (100 mg.L<sup>-1</sup>= 4.18±0.10; e 1000 mg.L<sup>-1</sup>=2.36±0.35). The enzyme SOD increased in the group of animals exposed to 1000 mg.L<sup>-1</sup> of Acephate (10.70±1.22) when compared to group of control animals (6.90±0.84, p < 0.05). CAT activity from exposure of 10 mg.L<sup>-1</sup> (62.81±4.70), 100 mg.L<sup>-1</sup> (66.30±4.71) e 1000 mg.L<sup>-1</sup> of Acephate (61.32±5.07) occurs reduced values when compared to control group (88.81±4.74; p < 0,05). Due to the presence of reactive oxygen species is expected that the antioxidant system act to stabilize the oxidative stress. The activity of ChE presents a significant reduction in animals exposed to 10 e 1000 mg.L<sup>-1</sup> (17.06±2.72 and 15.20±1.44, respectively) of Acephate. In liver, ChE assists in detoxification of metabolites and, when it was inhibited, can leave the exposed individual more susceptible to diseases.

**FP121 Effects of seasonality on modulation of biochemical biomarkers in the crab *Ucides cordatus* from an Amazonian estuary**

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Japerica Bay estuary, located in Brazilian Amazon, is home to different types of aquatic organisms remaining in pristine conditions for many years. However, is currently being impacted by a cement factory's activity since 2016. The aim of this study is to characterize the modulation of oxidative stress biomarkers due to natural and seasonal physical-chemical variation before factory's activity, in extracts of gills from the crab *Ucides cordatus*. This study allows inferring the contribution of different seasons and positions along the estuary for oxidative stress showing also the viability of the specie for biomonitoring programs. The samplings were done throughout one year covering the months of June/2013 (transitional season I), September/2013 (dry season), November/2013 (transitional season II) and February/2014 (rainy season) at three sites: A) Japerica (lower estuary); B) Telha (middle estuary); C) Tapuã (upper estuary). As exposure biomarkers, GST and GCL activities as well the GSH concentration were evaluated and the lipid peroxidation was analyzed as an effect biomarker. The salinity varied greatly among sites in all the periods sampled and the peak occurred during the transitional period II for all sites. GST activity during transitional period II was lower for animals from Telha and Japerica (134.52 ± 9.72 and 129.62 ± 11.80 UGST/mg of protein respectively) occurring the same for GSH concentration (31.16 ± 3.12 and 34.27 ± 3.33 nMol of GSH respectively) and GCL activity for all the sites during the same season. Tapuã's organisms showed lower concentration of GSH (144.63 ± 24.98 and 52.35 ± 9.26 nMol of GSH respectively) during the transitional period I in relation to Japerica's and higher concentrations (70.83 ± 10.15 nMol of GSH) during rainy season. No differences were observed between points for any of the climatic periods sampled nor between periods for the crabs of Japerica and Tapuã for lipoperoxidation. Organisms from middle estuary presented higher content of peroxidized lipids (327.43 ± 310.95 nMol of CHP / g of wet tissue) at dry period when compared to other seasons. We conclude that the position along the estuary gradient influences the adaptive metabolic strategy of the animal: while organism from middle and lower estuary presented minor activation of their antioxidant system during salinity peak, animals from upper estuary showed a different approach by lowering their antioxidant defenses when exposed at low

salinity.

**FP122 Ensaio de sensibilidade in vitro do pseudofungo *Aphanomyces* sp. isolado de ovos de Danio rerio ao sulfato de cobre**

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O peixe *Danio rerio* conhecido também como “Zebrafish” ou Paulistinha vem sendo cada vez mais utilizado como modelo animal em pesquisas científicas. No Brasil ainda não dispomos de fornecedores com certificado sanitário que atestem a sua qualidade. Os peixes são adquiridos, na maioria das vezes, em lojas de aquarofilia e produtores comerciais. Há uma não conformidade evidente entre a origem do Paulistinha e a sua utilização como modelo animal em pesquisas, pois nesses locais os adultos e seus ovos são tratados com produtos de forma não padronizada. O objetivo deste trabalho foi determinar a concentração efetiva (CE50; 96h) e a concentração mínima de sulfato de cobre pentahidratado ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) responsáveis por 50 e 100% de inibição do crescimento micelial de *Aphanomyces* sp. in vitro. A cepa do oomiceto foi isolada de ovos de *D. rerio* obtidos do laboratório de criação no Instituto de Pesca em São Paulo, e identificada por PCR até gênero no laboratório de sequenciamento de DNA do Instituto de Biociências da Universidade de São Paulo. Foi realizado um ensaio preliminar com sulfato de cobre nas concentrações de 0,01; 0,1; 1; 10; 100; 1000 e 10.000  $\text{mg.L}^{-1}$  e, um definitivo nos tratamentos de 1,0; 1,75; 3,5; 5,25; 7,0; 8,75 e 10,5  $\text{mg CuSO}_4 \cdot \text{L}^{-1}$ . Após a adição e mistura do sulfato de cobre em ágar YpSs (yeast-soluble starch), este foi vertido nas placas de “Petri” em triplicatas. Após o resfriamento, um pedaço com micélio foi colocado no centro de cada placa dos tratamentos com cobre e dos controles constituídos de ágar com e sem o inóculo. O ensaio foi conduzido em câmara de temperatura controlada a 22°C no escuro durante 96 horas. Diariamente foram realizadas as medidas do diâmetro do halo de crescimento. A CE50; 96h foi estimada utilizando-se o método estatístico Trimmed Spearman Karber. No ensaio preliminar a menor concentração que inibiu 100% do crescimento micelial foi igual a 10  $\text{mg cobre.L}^{-1}$ . A CE50;96h para o sulfato de cobre foi igual a 2,62  $\text{mg.L}^{-1}$  com intervalo de confiança de 2,31 e 2,97. No ensaio definitivo a menor concentração utilizada que inibiu 100% do crescimento do micélio foi igual a 8,75  $\text{mg.L}^{-1}$ . A concentração efetiva de 2,62  $\text{mg.L}^{-1}$  e a concentração inibitória de 100% dos oomicetos equivalente a 8,75  $\text{mg.L}^{-1}$  de  $\text{CuSO}_4$ . Serão testadas in vivo nos ovos de *D. rerio* em sistema estático e sob a forma de banhos de curta duração para verificar quais condições resultam em maiores taxas de sobrevivência sem prejudicar o desenvolvimento dos organismos.

**FP123 Environmental changes magnifies the effects of different contaminants in Tambaqui (*Colossoma macropomum*, Cuvier 1818)**

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Climate change is a critical issue that raised intense scientific debates and public interest. The Intergovernmental Panel on Climate Change (IPCC, 2007), forecasts for 2100 an extreme scenario (A2) with the increase in the air temperature of 4.5 °C and 850 ppm  $\text{CO}_2$  in atmosphere. In Amazonian ecosystems, fluctuation in dissolved oxygen in the water is a natural phenomenon, especially in flooded areas, where aquatic biota must survive to hypoxia episodes. These hypoxia episodes are increasing with the changes in severe floodings and droughts that have been occurring in the last decades. In addition to all this changes, the on going increase of pollution is threatening aquatic biota, especially petroleum derivatives as the benzo[a]pyrene (BaP) and herbicides and pesticides, such as Roundup® (RD), which controls the weeds around the tanks in fish farms. Fish are sensitive to climate changes as well as to oxygen levels disturbances and, indeed, the effects of aquatic contaminants affect them too. In this context *Colossoma macropomum* (tambaqui), an Amazonian freshwater fish with great economic importance in the region, is currently used as model in monitoring programs. Here in, tambaqui fish were studied in two different experiments under two different contaminants and environmental challenges. Firstly, we injected fish with two different concentrations of BaP (8 and 16  $\mu\text{mol/kg}$ ) and, then, exposed fish during 30 days to the extreme scenario (A2) for seen by IPCC to 2100. In the second experiment, fish were acutely exposed (96h) to RD (75% of  $\text{LC}_{50}$  nominal concentration - 15  $\text{mg.L}^{-1}$ ) and subsequently submitted them to hypoxia for six hours. As a result of these challenges, fish liver was showed alterations in the expression of *ras* oncogene and the gene *hypoxia inducible factor-1a (hif-1a)* that could be related to severe histological alterations, which included necrosis. Genetic damage also was observed through Comet Assay, what suggest that environmental changes magnifies the pollutant effects in this species what may occur as well in other fish species. Work funded by CAPES (Pro-Amazônia) and INCT-ADAPTA (CNPQ and FAPEAM).

**FP124 Estado actual de la salud del ecosistema marino del sureste del Golfo de México: una perspectiva molecular.**

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**Diversas actividades antropogénicas tales como la petroquímica, industria, agricultura, turismo, entre otros, liberan al mar diversos compuestos químicos que están afectando los ecosistemas marinos. Debido a la gran necesidad de determinar la situación actual de estos ecosistemas, el objetivo principal de este estudio fue evaluar la salud ambiental del ecosistema marino del sureste del Golfo de México, mediante diferentes biomarcadores moleculares asociados con la presencia de contaminantes. Uno de los biomarcadores más utilizados para evaluar los efectos de los contaminantes en los organismos, se encuentran los genes asociados al sistema de biotransformación de xenobióticos (Citocromo P4501A y Glutación S-transferasa), genes asociados al estrés oxidativo (Catalasa, Glutación reductasa y Glutación peroxidasa) y los relacionados con el sistema reproductivo (Vitelogenina). En el mes de abril del 2016, se realizó un crucero oceanográfico en donde se colectaron 108 lenguados (familia Paralichthyidae) en 18 estaciones distribuidas a lo largo del sureste del Golfo México. La expresión génica fue determinada en el ARN extraído del hígado utilizando la metodología de cuantificación absoluta y utilizando el equipo de PCR en tiempo real. Los resultados obtenidos de la expresión génica mostraron que Citocromo P4501A, Glutación S-transferasa y los genes de estrés oxidativo (Glutación reductasa y Glutación peroxidasa), estuvieron altamente expresados en los peces colectados principalmente en la zona este y noroeste de la Península de Yucatán, mientras que la Catalasa mostró los mayores niveles de expresión en la región sureste. Por otro lado, la mayor expresión del gen de la Vitelogenina fue detectada en los peces colectados en la zona norte. Estos hallazgos sugieren la presencia de ciertos compuestos tóxicos en el sureste del Golfo de México, que pudieron haber llegado por corrientes marinas o movimientos de agua profunda provenientes del Caribe, así como también por grandes aportes de agua subterránea provenientes del anillo de cenotes (característico de la península de Yucatán), que arrastra nutrientes y contaminantes de las zonas rurales y urbanas en donde los peces se encuentran respondiendo a este tipo de contaminantes.**

**FP125 Evaluation of aluminum toxicity and acid pH on the social behavior of *Astyanax altiparanae* (Characiformes: Characidae)**

R. Honji, Universidade de Sao Paulo; D. Brito, Universidade Nove de Julho; B. Kida, Universidade de Sao Paulo; R.P. Abdalla, Universidade de Sao Paulo / Fisiologia; R.G. Moreira Whitton, Instituto de Biociencias Universidade de São Paulo / Fisiologia It is known that the exposure of teleosts fish to metals can cause physiological changes, such as, reduction or increase in the swimming behavior, which interfere in different physiological organization levels and also in the individual's fitness. Among the bioavailable metals for the aquatic biota, aluminum (Al) is considered a toxic metal and interferes with the respiratory system, restricting the respiratory scope, besides being considered as an endocrine disruptor. Previous studies indicate that the reproductive physiology of *Astyanax altiparanae* males and females is impaired when these animals are exposed to Al, suggesting that this metal can also disrupt the social reproductive behavior in this species. Therefore, the hypothesis of the present study is that exposure to Al changes in the social behavior of *A. altiparanae* and consequently decreases the social interactions between males and females, as well as, the swimming activity of these animals. For this purpose, *A. altiparanae* couples were exposed to Al (concentration of 2.0  $\text{mg.L}^{-1}$  – according previously studies) at acidic pH (group: Al), acidic pH (group: pH-ac), and at neutral pH (control), during acute exposure (1 hour). Total number of agonistic interactions between males and females, as well as, the swimming activity of these animals were quantified. Females from the control group and at pH-ac were considered more aggressive in relation to males and no attack occurred in the Al group. In addition, the swimming activity in males and females from the group exposed to Al was also lower when compared to the control ( $P < 0.006$ ) and pH-ac ( $P < 0.004$ ) groups. Based on these results, we suggest that the presence of Al in the water decreased *A. altiparanae* swimming/locomotor activity, besides influencing the social behavior of this species, acting as a deregulator of social behavior between females/males (social interactions) of this important Neotropical species. Additionally, our results suggest that the adoption of alternative reproductive tactics in *A. altiparanae* in impounded environments may inhibit its reproductive strategy.

#### FP126 Evaluation of copper effects on *Hoplias malabaricus* using biochemical biomarkers

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Copper contamination in aquatic environments can be derived from natural geochemical processes or anthropogenic activities. This metal is an essential trace element. However, when in the form of free ion, it can be toxic to aquatic organisms. In light of this, the aim of the study was to evaluate the effect of copper on the Neotropical fish *Hoplias malabaricus*. For this purpose, juveniles (N=32; 14.54 ± 0.83 cm; 34.13 ± 6.43 g) were acclimated in dechlorinated water, aerated continuously, for 10 days and fed every 3 days with prey (*Astyanax altiparanae*) until 24 hours before the exposure period. After acclimation, fish were submitted to acute toxicity tests (96 h) in individual 40 L aquaria containing only dechlorinated water (CTR; N = 8), and copper at three concentrations: 5 µg L<sup>-1</sup> (Cu 5; N = 8), 10 µg L<sup>-1</sup> (Cu 10; N = 8) and 20 µg L<sup>-1</sup> (Cu 20; N = 8). Then the animals were anesthetized with benzocaine, killed by medullary section and had gill and liver removed. The activity of the biotransformation enzyme Glutathione S-transferase (GST), the antioxidant enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) were analyzed in both organs as well as the quantification of reduced Glutathione (GSH) and lipoperoxidation (LPO). The results of each parameter, for each organ, were compared among the four groups (CTR, Cu 5, Cu 10 and Cu 20). There were no significant alterations in all parameters evaluated in liver. On the other hand, in gills, the activities of the CAT and GPx were lower in the fish exposed to the higher concentration of copper (p = 0.013 and p = 0.006 respectively), whereas SOD showed higher activity in fish exposed to Cu 5 (p < 0.001). There were no significant changes in GSH, GST and LPO. Depending on concentration, speciation and route of exposure, copper may activate or inactivate antioxidant enzymes. While activation may be linked to the ability of copper to generate reactive oxygen species, the direct interaction of copper with proteins may be the cause of enzymatic inhibition. The present results indicate that after 96 h copper is able to promote alterations on the antioxidant defenses in gills of *H. malabaricus*. Nonetheless, these changes did not led to oxidative damage.

#### FP127 Evaluation of genotoxicity and mutagenicity in *Astyanax lacustris* of fish farming waters

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The development of fish farming in the region of Grande Dourados (MS) has become an important economic sector for the population. In fish farms, water is collected from river springs or streams that may receive pollutants (agroindustrial and urban) from anthropogenic action. Then, this water passes through tanks that undergo influence of fish farming to be discarded in rivers. The objective was to associated physicochemical parameters and environmental conditions (rainfall and relative humidity) with mutagenic, cytotoxic and genotoxic effects in *Astyanax lacustris* cells, using bioassay of water of catchment and disposal sites. The water samples were collected in Rio Dourados (PRD) and Rio Brilhante (PRB) fish farms, and in each fish farm the water was collected in four sites (catchment, reservoirs (2) and disposal tanks). The mapping of the use and coverage of the soil of the fish farm was made from high resolution aerial images (2015 and 2016 years) obtained from Google Earth Pro. The water samples destined to the animal bioassay were collected in gallons of 20 liters and the fishes samples (*A. lacustris*) were purchased locally. The water samples from each sampling points were placed in properly ventilated glass aquariums at room temperature. Subsequently, 10 fishes were placed in each aquarium for 72 h (exposure). After that, the micronuclei test, nuclear morphological alterations and comet test were performed using five fishes from each sampling site. According to the images of land use and occupation it was possible to verify that the PRB presented larger occupied areas by pastures and agriculture, and this fish farm also presented a higher index of genotoxicity and mutagenicity in the summer. In the PRD was observed higher genotoxicity indexes in the winter, with no difference among water of catchment, reservoir and disposal tanks, but compared to the negative control the two sampling sites showed genotoxicity. In the summer, there was no difference between the same sites in both fish farms for the evaluated indexes. In the PRB, evaluating the genotoxicity index, there was a greater genetic change in the disposal site compared to the site of catchment of water. In the summer, in the PRD there were fewer changes in the site of catchment compared to disposal site. PRB presented a greater number of alterations than the PRD, mainly in the summer. This fact may be due to the use and occupation of the soil, as well as the management activities in the

PRB.

#### FP128 Evaluation of mutagenic activity, emergent contaminants and metals in water from fish farms

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A água usada na piscicultura em Grande Dourados vem de corpos de água e depois de passar pelos tanques de armazenamento e descartada em rios sem tratamento e pode causar danos ambientais. O objetivo foi determinar a ocorrência de metais e contaminantes emergentes, bem como uma atividade mutagênica de amostras de água. Para isso, foi coletada água de tanques de captação, reservatório e eliminação das pisciculturas Rio Brilhante (PRB) e Rio Dourados (PRD). Os metais foram determinados por espectrometria de emissão óptica de plasma indutivo acoplado (ICP-OES) e os contaminantes emergentes por cromatografia líquida acoplada à espectrometria de massa em tandem (LC-MS/MS). Uma mutagenicidade foi avaliada por *Salmonella* teste / microssoma em microssuspensão com as linhas de TA98 e TA100, na ausência e presença de S9 (- / + S9) mistura. Fe Apresentou níveis acima dos padrões estabelecidos para a Classe 2 (Conama nº 357/2005) e toxicidade crônica (EPA, 2016) nas amostras dos locais de disposição das empresas como fazendas de peixes. Apresentação de níveis acima dos mesmos padrões apenas nas amostras de PRB. Nas amostras da água do local de captação, Ni avaliado não PRB e Cu avaliado no PRD acima do padrão estabelecido. A cafeína, imidacloprid, 2-hidroxi atrazina e bisfenol A foram detectados na água dos locais de captação e eliminação das empresas como fazendas piscícolas. Sem PRB, uma atrazina também foi encontrada, Nos mesmos sites e clomazona e malação foram encontradas apenas não locais de disposição. No PRD, Hexazinona e tebutiurão foram encontrados nos locais de captação e eliminação e clausulação não encontrada local de disposição. Simazina, carbofurano, diurão, ametrina, testosterona, tebuconazol, progesterona, estriol, 17- $\alpha$ -etinilestradiol, fipronil, triclosan e octilfenol não foram detectados nas amostras de água. As amostras da captação das duas fazendas de peixe são mutagênicas para TA98 - / + S9. Para TA100-S9, uma mutagenicidade para observação apenas na água da bacia hidrográfica do PRD. Sem PRB uma amostra da água da bacia hidrográfica para tóxica para TA100-S9 e mutagênica para + S9. Uma mutagenicidade e contaminação por contaminantes de Al, Cu, Fe, In

#### FP129 Evaluation of the Toxic Effects of Fipronil for *Rhamdia quelen*: Hematologic, Biochemical, Genetic and Histopathological Analysis

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**Introduction:** The study of ecotoxicology allows the establishment of allowable limits for chemicals for the protection of aquatic life and assess the impact that these pollutants in the biota of different water bodies and aquatic organisms. Understanding the immunological abnormalities in fish is useful for the study of aquatic ecotoxicology since fish health directly reflects the health condition of any aquatic environment. **Objective:** The overall objective of this study was to determine the LC<sub>50</sub> of fipronil pesticide by water route to the silver catfish (*Rhamdia quelen*), as well as to evaluate the toxic effects of fipronil by water pathway in acute toxicity test using hematological, biochemical, histopathological and genetic biomarkers to this species. **Methods:** The silver catfish were kept in the laboratory under a static system with control of water temperature, dissolved oxygen and pH. Behavioral changes, mortality, and water quality were monitored during all experimental phases. All animals underwent biometrics and were euthanized. During the anesthetic period, blood samples were collected from the fish for the achievement of hematologic and biochemical analyzes, and the achievement of blood smears for genetic analysis. Immediately after euthanasia, the collections of gill, renal and hepatic tissue were proceeded for histopathological analysis. For data analysis we used ANOVA (ONE WAY) and to compare the means the Bonferroni's test. The normality test used was Kolmogorov-Smirnov. The level of significance was 5% ( $\alpha < 0.05$ ) using GraphPad Prism 5.0, Calif. software. **Results and Discussion:** The LC<sub>50</sub> obtained for jundiá was 0.5924 mg/L. The insecticide fipronil was able to induce hematological and biochemical changes consistent with metabolic disorders in catfish, characterized by a significant reduction in Hct and significant increase in liver enzymes AST, ALT, AP and GGT of fish treated in relation to group control. The results showed changes in flow rate compatible with conditions of poisoning by pesticides in liver, gill and kidney tissues, and morphologic changes in the nucleus of treated fish erythrocytes by fipronil. **Conclusion:** The data prove the ecotoxicological potential of fipronil,



knowing that the pathological picture generated leads to irreversible metabolic imbalance in jundiá an experimental level and possibly also in the ecological level, in cases of environmental accidents involving this pesticide.

#### FP130 Expression of the gene encoding for metallothioneins in communities of *Hypheosobrycon luetkenii* from the João Dias creek (RS, Brazil)

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Anthropogenic activities release considerable amounts of metals into aquatic ecosystems, metals are interesting because they have several implications in biological processes. Nevertheless, elevated concentrations of metals in the aquatic environment can lead to bioaccumulation and toxicity in aquatic organisms. Minas do Camaquã (Caçapava do Sul, RS, Brazil) was scenario of copper (Cu) mining for more than hundred years (1870-1996), releasing important amounts of Cu, Fe, Mn, and other metals into the João Dias creek. This contamination can have impacted the populations of local aquatic organisms for many generations. However, some biochemical and physiological mechanisms could have protected aquatic animals against the toxic effect of metals. In this context, metallothioneins (MT) are metal-binding proteins, thus acting in metal detoxification. Given the above, we performed an experiment of field translocation between communities of *Hypheosobrycon luetkenii* inhabiting non-contaminated (C) and polluted (P) areas of the João Dias creek, to evaluate the role of MT in fish inhabiting environments historically contaminated with metals mixture. After the experiment, fish tissues (liver, gill and intestine) were collected. Gene encoding for MT was partially sequenced and its level of expression in the different tissues was quantified by qPCR. As expected, water samples from P area had higher levels of total and dissolved Cu than those collected in the C area. In turn, Cd, Fe, Mn, Pb, and Zn concentrations (total or dissolved) were similar in both areas. Basal level of MT gene expression was higher (3.5-fold) in gills of fish from the P area than in from the C area (data normalized for 1 µg/µl RNA). In liver and intestine, no significant differences were observed between fish from the two areas. After 96 h of experiment, gene expression in gills of fish translocated from the P area to the C area was significantly down regulated (4.8-fold). Significant induction of MT gene expression was observed in liver (3.5-fold) and intestine (6.1-fold) of fish translocated from the C area to the P area. Our findings indicate that expression of gene encoding for MT in tissues of *H. luetkenii* is a sensitive biomarker to chronic exposure to Cu. Also, they indicate that fish communities have developed a dynamic regulation of MT gene expression to survive in aquatic environments with different historic of metal contamination associated with Cu mining.

#### FP131 Fluorescência da clorofila a (OJIP) em macrófitas expostas a efluente de indústria de explosivos

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Compostos nitroaromáticos como o TNT (2,4,6 trinitrotolueno), são frequentemente liberados na água e no solo por atividades industriais, se tornando um problema ambiental. Muitas macrófitas aquáticas são conhecidas por seu uso na fitorremediação de efluentes contaminados. A fluorescência da clorofila *a* (Fchl *a*) é um indicador sensível da presença de danos no aparato fotossintético e já tem sido utilizada para detectar danos por compostos nitroaromáticos. O objetivo deste trabalho foi avaliar o efeito tóxico do efluente de indústria de explosivos contaminado com compostos nitroaromáticos na cinética rápida da Fchl *a* em macrófitas aquáticas. As macrófitas *Azolla sp.*, *Salvinia biloba* e *Pistia stratiotes*, cultivadas no Laboratório de Ecotoxicologia Aquática da Universidade Estadual do Centro - Oeste (UNICENTRO), Guarapuava, PR, foram aclimatadas por 48h em solução nutritiva de Hoagland (25%) antes dos bioensaios. Os experimentos foram realizados em recipientes de 200 mL contendo solução de Hoagland 25%, no pH 5,5, com 3 repetições de cada tratamento, sendo o controle e as diluições de 0 e de 0,25% do efluente, durante 7 dias, com fotoperíodo de 12h de luz e temperatura média de 24°C. Foram realizadas análises da cinética rápida da fluorescência da clorofila *a* (teste OJIP) com o fluorômetro PAM 2500 (Heinz Walz, GmbH). O tratamento com efluente afetou a cinética rápida da clorofila, principalmente na fase J-I-P. As três macrófitas quando expostas a 0,25% de efluente apresentam valores de F<sub>0</sub> (O) elevados em relação aos seus controles, assim como os valores da fase J. Aumentos de F<sub>0</sub> podem ocorrer, quando há danos no centro de reação do fotossistema II. Nas fases I a P houve redução em *Azolla sp.* e *Salvinia*

*biloba*, onde os valores de I-P se assemelham a J. O transporte de elétrons parece ser afetado pelo efluente contaminado, pois as fases da O-J-I-P são distinguíveis quando se compara o controle com os tratamentos. Porém, na fase I-P em *P. stratiotes* observa-se decréscimo mais pronunciado, do que nas pteridófitas. A amplitude da fase de I-P pode ser um indicador aproximado do conteúdo do fotossistema I (PSI). Com este trabalho é possível observar que o efluente de indústria de explosivos a 0,25% causa danos ao aparato fotossintético em nível do PSI, demonstrado pela extinção da fase I-P em *Azolla sp.* e *S. biloba*, bem como decréscimo do nível I em *P. stratiotes*. As pteridófitas se mostraram mais sensíveis ao efluente testado do que a alface d'água.

#### FP132 Fluorescência da clorofila a em resposta ao efeito da acetoneitrila em *Salvinia sp.*

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A análise da fluorescência da clorofila *a* pode ser mensurada por estímulos de pulsos luminosos no tecido vegetal adaptado ao escuro por meio de avaliação da cinética rápida (curva OJIP). A curva transitória OJIP é um método rápido, preciso e não destrutivo que fornece indicativos da funcionalidade do aparato fotossintético. Desta forma, o objetivo deste trabalho foi avaliar possíveis efeitos tóxicos da acetoneitrila na macrófita *Salvinia sp.* por meio da emissão da fluorescência transitória da clorofila *a*. A desinfecção das plantas de *Salvinia sp.* cultivadas no laboratório de Ecotoxicologia da Universidade Estadual do Centro Oeste (Unicentro) foi realizada com solução de hipoclorito de sódio 2% por 5 minutos. As plantas foram aclimatadas por 24 horas em solução nutritiva de Hoagland 25%, pH 7,0 e mantidas sob fotoperíodo 12:12 (luz/escuro) e luminosidade constante de 3000 lux. Após aclimação, cerca de 1,5 g de plantas de *Salvinia sp.* foram colocadas em potes plásticos com 200mL de solução nutritiva de Hoagland 25%, as quais foram submetidas aos seguintes tratamentos: 0; 0,05; 0,10; 0,25; 0,50 e 0,75% de acetoneitrila recuperada. A fluorescência da clorofila *a* através da cinética de indução rápida (OJIP) foi avaliada por meio do fluorômetro PAM-2500 (Heinz Walz, GmB). Os resultados revelaram uma curva OJIP típica, onde a maior concentração de acetoneitrila (0,75 %) não diferiu estatisticamente do tratamento controle ( $p < 0,05$ ). As doses mais baixas de acetoneitrila (0,05; 0,10; 0,25 e 0,50%) apresentaram redução na emissão de fluorescência em relação ao controle e à maior dose (0,75 %) nas folhas de *Salvinia sp.*, cujas diferenças foram significativas ( $p < 0,05$ ). Estes resultados demonstram reduções no pool de plastoquinonas, afetando o lado doador e receptor de elétrons do PSII. Desta forma, a fluorescência transitória OJIP indicou maior toxicidade nas diluições intermediárias de acetoneitrila em plantas de *Salvinia sp.* Os resultados observados na dose mais elevada podem ser devido ao aumento da atividade fotossintética para suportar o estresse causado pelo toxicante.

#### FP133 Genotoxic potential in piscine erythrocytes and water physicochemical analysis of the Sinos River basin, southern Brazil

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Untreated domestic sewage discharges, agricultural runoffs, industrial activities (mainly leather), inadequate waste deposition sand removal and elimination of riparian vegetation are the main impacts in the Sinos River basin, southern Brazil. Although the river is heavily impacted, it supplies water for 1.6 million inhabitants. The Sinos River is 190 km long and among its main tributaries are the Ilha River and the Paranhana River. Despite its economic and social importance, there are only a few studies using bioindicator organisms in water quality assessment. In addition, the tributaries of the Sinos River are not monitored by state and federal agencies. Therefore, the present study aimed to assess the genotoxic potential of the Sinos River basin using the fish species *Cyprinus carpio* (L.), as well as analyze water physicochemical parameters. Water samples were collected at seven sites located in the middle section of the basin in January/2014: S1 and S2, located in the Ilha River; S3 and S4, located in the Paranhana River; S5, S6 and S7, located in the Sinos River. Fish (n=10 per site) were exposed to water samples for 72 h. A control group was maintained in tap water during the exposure period. Then, fish were killed and blood samples were obtained for the blood smears. Slides were fixed and stained with Giemsa. 3000 erythrocytes per animal were analyzed for the presence of micronucleated cells and other nuclear anomalies. Statistical analysis was performed using the Kruskal-wallis test. Levels of aluminum iron and lead exceeded the limits established by the Brazilian legislation for surface waters at all sites. Thermotolerant coliforms above the limits were found at S2, S4, S5 and S7, as a result of domestic sewage discharges and swine and cattle farms. Copper, chromium, nickel and zinc were within the limits. Micronucleus frequencies ranged from 0 to 0.2% (per 1000 cells), whereas nuclear abnormalities frequencies ranged from 0.2 to 1.4%. No statistical differences between sites and control group were observed. Although no

genotoxic effects were found under the conditions studied, water analyses indicated contamination by metals at all sites, including the sources of the tributaries – areas apparently preserved. Further studies are encouraged in order to better assess the water quality of this water resource, using long term exposure periods and samplings during different seasons of the year.

**FP134 Genotoxicity in *Astyanax fasciatus* (Teleostei: Characiformes) in polluted reservoirs of the Metropolitan Region of São Paulo**

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The Billings complex is the largest water reservoirs in the Metropolitan Region of São Paulo (RMSP), and it is characterized by an intense anthropic action. As a consequence, a large number of pollutants, such as heavy metals, pharmacological compounds, among others, are constantly released in the water. Some of these compounds can alter the integrity of DNA in aquatic organisms, resulting in tumor formation, growth abnormalities, as well as alterations in the fertility and longevity of organisms, thus compromising the integrity of aquatic communities. Therefore, to evaluate if the anthropic pressure to which the Billings reservoir is subjected affects the integrity of the aquatic fauna, Micronucleus (MN) and Nuclear Abnormalities (NA) tests were used as biomarkers in adult *Astyanax fasciatus*. Three different points in the Billings reservoir (Pedreira, Bororé and Taquacetuba) were selected, as well as the reference point, Ponte Nova reservoir, in the upper Reaches of Tietê River, which present a lower degree of anthropic pressure. The animals, all male, were anesthetized with benzocaine and a blood sample was collected from the caudal vein. Ten fish were collected per site, except from Pedreira, where it was possible to collect only two animals. Blood smears were fixed with methanol and stained with Giemsa (10%) for 7 min. 1000 erythrocytes with intact cell and nuclear membranes were analyzed, and the number of MN and NA were determined. No MN was found in the analyzed fish. According to the number of NA, the fish collected in Pedreira showed a greater DNA damage ( $42.0 \pm 5.6$ ) compared to Taquacetuba ( $8.9 \pm 1.5$ ) and Ponte Nova ( $11.9 \pm 2.5$ ). Animals from Bororé ( $14.2 \pm 1.5$ ) did not show significant differences in relation to the other sampling points. The results showed that the integrity of DNA was less affected in *A. fasciatus* sampled in the reference point, and the higher number of NA was found in animals sampled in Pedreira, suggesting that the Billings reservoir has different degrees of impact, providing different environmental conditions to the organisms that inhabit it. Keywords: *Astyanax fasciatus*, Genotoxicity, Micronucleus, Nuclear abnormalities, Polluted reservoirs.

**FP135 Hardness as interfering factor in *Ceriodaphnia dubia* chronic toxicity tests results with surface waters**

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In compliance with National Regulation on Water Quality (CONAMA 357/2005), chronic toxicity testing with *Ceriodaphnia dubia* are performed by CETESB (Environmental Agency of São Paulo State) to assess the quality of rivers and reservoirs in relation to the protection of aquatic life. Despite the occurrence of autochthonous daphnids (including other species of *Ceriodaphnia* as *C. cornuta* and *C. reticulata*), adapted to the characteristics of local waters, the option for the species recommended by international guidelines was due to its well established maintenance criteria and availability of a wide ecotoxicological database. This microcrustacean is cultured in the Aquatic Ecotoxicology laboratory in natural soft water, with total hardness ranging from 40 to 48 mg/L as CaCO<sub>3</sub>. This type of culture and dilution water was defined because this attribute affects bioavailability of many contaminants (particularly metals) and the median hardness of local waters is 31 mg/L. The recurrent toxic effects observed in samples from uncontaminated sites triggered Toxicity Identification Evaluations experiments, in which, all standard treatments did not remove or reduce the toxicity. On the other hand, it was observed that all of these samples exhibited extremely low hardness (less than 10 mg/L) and in a second series of tests, the chronic effects of these waters were eliminated after adjusting hardness to 20 mg/L. As noted by other authors, low concentrations of compounds responsible for water hardness (calcium and magnesium) may significantly reduce the test organism performance regarding growth and reproduction, leading to false positive results (i.e. declare a sample toxic when in fact it is not). Thus, to avoid interferences of low hardness in the tests results, samples of the São Paulo Water Quality Monitoring Network with total hardness lower than 10 mg/L as CaCO<sub>3</sub>, will have their values adjusted to 20 mg/L.

**FP136 Health status alterations in *Ruditapes philippinarum* after continuous urban effluent exposure**

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Marine environments are the main receptors of wastewater discharges since the coastal environments are the vicinity of domestic and industrial sources. Improvements in analytical methodologies have been currently perceived a wide variety of concern emergent contaminants (CECs) in wastewater discharges, which include persistent organic pollutants (POPs) and pharmaceuticals and personal care products (PPCPs) among others. Monitoring adverse effect provoked by effluent exposition is becoming a challenge in marine environment. Effluents are widely recognised as a complex matrix composed by a mixture of xenobiotics. Conventional methodologies (primary and secondary treatments) reduce the levels of nutrient, DBO, DQO and particulate matter to mandatory ranges established by the legislation (European Directive 91/271). Nevertheless, it has been demonstrated that these treatment cannot reduce CECs concentrations until harmless values. The present study designed and set a mobile pilot plant in a wastewater treatment plant (WWTP) in south west Spain, in order to address the potential adverse effects of a selected effluent, as a whole contaminant, which discharges in marine environment. *Ruditapes philippinarum* specimens were exposed to six different effluent concentrations (50%, 25%, 12.5%, 6.25%, and 3.15%) during seven days. After effluent exposure, lysosomal membrane stability alterations (LMS), changes energy status storage (total lipids content (TLP) and mitochondrial electron transport (MET), inflammatory mechanisms inhibition (cyclooxygenase activity (COX)), and neurotoxicity (acetylcholinesterase (AChE)) were registered in exposed organism. Results revealed different toxic effects in *R. philippinarum* depending on the effluent concentration. Energetic and neuroendocrine effects were determined after the effluent exposure. Alterations in clam's health status revealed stress conditions for the specimens. The present study pointed out the necessity of adds alternative treatments to conventional methodologies in WWTP to reduce the potential effluent toxicity and leads more scientific efforts to describe chronic effects after continuous exposure of discharged effluents.

**FP137 Hepatic melanomacrophages as a sensible biomarker to chromium contamination in bullfrog tadpole**

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Amphibians have peculiar characteristics such as skin permeability and a life cycle which depends on both aquatic and terrestrial environment. These characteristics make them very susceptible to the environmental changes. Their pigment containing-cells are present in the liver and have been used as morphological models for the detection of pollutants. Among the aquatic contaminants, the hexavalent chromium Cr(VI) is considered a metal potentially toxic and its presence is strongly related to the burning of fossil fuels, wood and paper as well as being used in cement, glass, steel, electroplating and rawhide industries. In this study we evaluated the effect of Cr(VI) on liver pigmentation by morphological and histochemical analysis of the melanomacrophages in larvae of *Lithobates catesbeianus* (bullfrog). The hepatic tissue was analyzed by means of the quantification of the area containing melanin and hemosiderin pigment in two treated groups (24h and 48h) using 18 mg/L of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and a control group using only dechlorinated water. Our results demonstrated a significant increase in melanin-containing area in the 48-hour treatment group, as well as a significant increase in hemosiderin in the 24-hour treatment group. The result is in agreement with the protection activity promoted by this cell, as observed in other works. The increase in pigmentation indicates a physiological response of the pigment containing-cell and, can confer to the melanomacrophages an environmental biomarker status.

**FP138 Histopatologia branquial do teleósteo *Prochilodus lineatus* submetido a testes in situ durante 120 dias em uma área agrícola no sul do Brasil**

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O objetivo deste trabalho foi avaliar a ocorrência de alterações histológicas nas brânquias do peixe neotropical *Prochilodus lineatus* submetido à exposição *in situ* durante 120 dias em uma área agrícola no Sul do Brasil. Juvenis de *P. lineatus* foram cedidos pela Estação de Piscicultura da Universidade Estadual de Londrina (EPUCEL).

Os peixes foram confinados em tanques-rede (6000 L) simultaneamente em dois locais, na própria EPUEL (CTR) e em um lago sujeito a entrada de efluentes agrícolas (EXP), e amostrados após 5, 15, 30, 60, 90 e 120 dias para a retirada das brânquias. Peixes amostrados antes do confinamento constituíram o grupo basal. Durante os testes *in situ*, os peixes tiveram contato com o sedimento e foram alimentados com ração três vezes por semana. As brânquias foram fixadas em Bouin, incluídas em paraplast e cortes de 5 µm foram corados com hematoxilina e eosina (H.E). Os danos histológicos foram avaliados semi-quantitativamente pelo índice de alteração histológica (IAH). Em ambos os grupos foram observadas as seguintes alterações histológicas branquiais: descolamento do epitélio lamelar e desarranjo lamelar, hipertrofia e hiperplasia das células epiteliais, hiperplasia de células mucosas, dilatação do canal marginal, dilatação do seio sanguíneo lamelar, congestão vascular e aneurisma lamelar. O valor de IAH variou de 6,2 a 17,5 no grupo CTR e de 8,5 a 17,3 no grupo EXP, sendo que em geral os grupos CTR apresentaram o valor de IAH entre 0-10, que indica um funcionamento normal do órgão, e os grupos EXP apresentaram IAH entre 11-20, indicando danos leves a moderados que podem ser revertidos. Contudo, essa variação não foi estatisticamente significativa ( $p > 0,05$ ), tanto entre os grupos EPUEL quanto EXP ao longo do tempo. Esses resultados indicam que a qualidade da água da área analisada não está comprometida a ponto de gerar patologias severas e irreversíveis nas brânquias dos peixes confinados. Entretanto, a análise histológica de outros órgãos e o uso de outros biomarcadores podem ser mais sensíveis e revelar alterações mais severas associadas aos efluentes agrícolas.

**FP139 Implicaciones de la metilación global del ADN de peces lenguados (familia: Paralichthyidae) procedentes del Sur del Golfo de México**

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La salud de los ecosistemas acuáticos puede ser evaluada a través de la respuesta biológica que causan diversas sustancias tóxicas en organismos como peses, los cuales son sensibles a contaminantes. En la actualidad, estudios moleculares relacionados con la epigenética toxicológica, han demostrado que la metilación del ADN presenta importantes implicaciones en cuanto a la respuesta asociada a la inducción o silenciamiento de genes, desarrollo de enfermedades cancerígenas y adaptación de los organismos expuestos a diversos xenobióticos en ambientes naturales. Se ha observado que dichos cambios en la metilación suelen presentarse de manera dosis dependiente en organismos expuestos a hidrocarburos, plaguicidas, metales pesados, etc. El Golfo de México ha experimentado un continuo aporte de sustancias tóxicas provenientes de diversas actividades antropogénicas y se han encontrado concentraciones de los contaminantes antes mencionados. Teniendo en mente lo anterior, el objetivo del trabajo consistió en describir los patrones de metilación global del ADN que pudieran estar asociados con la exposición a xenobióticos en lenguados de la familia *Paralichthyidae* provenientes del Sur del Golfo de México, donde ocurre una continua producción petrolera y en la cual desembocan diversos ríos como el Grijalva y el Usumacinta. Se extrajo ADN de casi 170 peces lenguados pertenecientes a cuatro especies distintas y se empleó la técnica de ELISA para determinar los niveles de metilación global del ADN. Se realizaron pruebas por rangos (Kruskal-Wallis) para identificar patrones de comportamiento de metilación en la zona de estudio. En general se observaron niveles de metilación muy por debajo de lo esperado en peces (hipometilación) en el genoma de las especies analizadas ( $\gamma=1.71\%$ ;  $\gamma=1.26\%$ ). Los menores niveles se encontraron en las estaciones frente a los estados de Tabasco y Campeche. Exposiciones a contaminantes como hidrocarburos y metales pesados ha demostrado disminuir los niveles de metilación global en el ADN de peces expuestos, causando problemas en la regulación génica. El comportamiento de dichos niveles difiere a lo largo de la zona evaluada, sugiriendo que los valores encontrados pueden estar relacionados al nivel de estrés ambiental que experimentan los peces en los diferentes puntos de la zona.

**FP140 Influência dos herbicidas Sulfentrazone (Boral® 500 SC) e Glifosato (Roundup® Original) em parâmetros bioquímicos de *Melanophryniscus admirabilis***

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Herbicidas são agroquímicos com aplicações específicas a determinados organismos, mas que podem ocasionar alterações metabólicas e funcionais em organismos não-alvo, sobretudo em ecossistemas aquáticos. Os anfíbios constituem um grupo amplamente ameaçado, em decorrência da sinergia de diversos fatores. *Melanophryniscus admirabilis* é um bufonídeo com descrição para apenas uma

localidade no estado do Rio Grande do Sul (Brasil), e avaliada como Criticamente em Perigo de extinção devido a diferentes ameaças, como a fragmentação e perda de qualidade do habitat, além de exposição a contaminantes utilizados em culturas agrícolas no entorno da área de ocorrência da espécie. Objetivou-se analisar alterações em parâmetros metabólicos e do balanço oxidativo em girinos de *M. admirabilis* expostos a duas concentrações do herbicida Boral® 500 SC (Sulfentrazone) – 130 e 980 µg.L<sup>-1</sup>, e outras de Roundup® Original (Glifosato) – 234 e 2340 µg.L<sup>-1</sup>. Foram analisados, em homogeneizado total de girinos, os níveis de glicogênio, proteínas e ácido úrico; lipoperoxidação (LPO) – níveis de TBARS; e atividade das enzimas Superóxido Dismutase (SOD), Catalase (CAT) e Glutationa S-Transferase (GST). Os resultados obtidos evidenciaram alterações significativas nos parâmetros mensurados, nos grupos expostos aos herbicidas. A mobilização conjunta das enzimas e metabólitos analisados possivelmente foi capaz de conter o dano oxidativo nestes animais. Entretanto, as alterações observadas podem vir a afetar estes animais ao longo do ciclo de vida, especialmente durante e após o período metamórfico. A aparente inibição enzimática observada, além de indicar grande suscetibilidade dos girinos frente à exposição a estes agentes, pode refletir um prejuízo da capacidade destes animais em lidar com situações ambientais adversas e sinérgicas. A ausência de mortalidade nos girinos de *M. admirabilis* sugere resistência às concentrações dos herbicidas testados; contudo, caberia a comparação da capacidade antioxidante desta espécie com outras espécies de bufonídeos. Este estudo demonstrou que a exposição de girinos de *M. admirabilis* a concentrações subletais dos herbicidas Boral® 500 SC e Roundup® Original conduziu à ruptura da homeostase energética e oxidativa, possivelmente comprometendo etapas futuras do ciclo de vida destes animais. Assim, pode-se sugerir que a presença de culturas agrícolas no entorno da área de ocorrência de *M. admirabilis* representa potencial risco a esta espécie.

**FP141 Interference of hypoxia on the effects of the insecticide fipronil in the expression of brain GABA-a receptor and HIF of *Oreochromis niloticus***

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Decomposition of organic matter in association with high temperatures cause depletion of dissolved oxygen (OD), leading to environmental hypoxia. Nile tilapia (*Oreochromis niloticus*) are highly resistant to hypoxia, GABA being one of the main neurotransmitter involved in the reduction of metabolism and triggering defense mechanisms against hypoxia-derived injuries, including antioxidant defenses. Metabolic changes, due to exposure to environmental pollutants, may negatively affect the adaptive responses of fish to hypoxic situations. The main mechanism of action of the insecticide fipronil is the inhibition of the normal nerve impulse of the cells by competing with  $\gamma$ -aminobutyric acid (GABA) for GABA receptors. The main objective of this study was to evaluate the expression of the GABA-a receptor and the hypoxia induction factor (HIF) in the encephalon of fish submitted to hypoxia, in order to better understand how fipronil interferes with GABA-a and HIF responses to hypoxia. *O. niloticus* were exposed for 3 and 8 hours to hypoxia and fipronil (0.1 e 0.5 g L<sup>-1</sup>), isolated or in combination, Low dissolved oxygen (< 2 mg.L<sup>-1</sup>) in the water was maintained by pumping gaseous nitrogen gas in water. After the exposure experiment, brains were collected for analyzes of gene expression of the subunits alpha, beta and gamma of the GABA-a receptor, and HIF using the quantitative real-time PCR (qPCR). Results showed a decrease in the transcription of all tested genes in all treatments after 3 hours of exposure, indicating that both hypoxia and fipronil exposure, isolated or in combination, modulates negatively the expression of GABA-a receptors and HIF. After 8 h, the transcript levels of GABA-a subunits and HIF returned to control levels, indicating a response of the organism to balance an initial negative effects of the treatments. However, the expression of HIF and of the subunit gamma of the GABA-a receptor remained low in fish exposed to the combination of hypoxia and fipronil. Our results indicates that that fipronil significantly interferes with the expression of GABA receptors and HIF under hypoxia situations, possibly impairing brain adaptations of fish to hypoxia in the environment.

**FP142 Metabolic stress induced by sub-lethal exposure to CuO nanoparticles and Cu in two Amazon fish species**

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Copper oxide nanoparticles (nCuO) are widely used in boat antifouling paints and may be released into the environment and induce toxicity to aquatic organisms. The physiological impacts of nCuO on fish are unclear and the dissolution of ionic copper may contribute to toxicity too. Thus, it is important to distinguish the effects of nCuO from the dissolved copper. Herein, we evaluated the effects of nCuO and dissolved copper on metabolic rate ( $MO_2$ ), oxidative phosphorylation capacity, and ROS generation in gill mitochondria of two Amazon fish species: *Apistogramma agassizii* and *Paracheirodon axelrodi*. For this purpose, fish were exposed to 50% of nCuO LC<sub>50-96h</sub> (*A. agassizii* 375.0  $\mu\text{g L}^{-1}$  and *P. axelrodi* 460.0  $\mu\text{g L}^{-1}$ ) and 50% of Cu LC<sub>50-96h</sub> (*A. agassizii* 20  $\mu\text{g L}^{-1}$  and *P. axelrodi* 22.9  $\mu\text{g L}^{-1}$ ) for 24, 48, 72 and 96 hours. A strong increase of  $MO_2$  occurred in *P. axelrodi* exposed to Cu, while no alteration in oxygen consumption occurred in *A. agassizii* following exposure to either Cu or nCuO. Moreover, mitochondria of *A. agassizii* presented an increased proton leak (i.e. uncoupling between respiration and ATP production) after exposure to nCuO and to Cu, resulting in decreases of Respiratory Control Rate (RCR). Interestingly, this uncoupling was directly related to an increase in ROS generation in this fish species. In contrast, the exposure to nCuO or Cu resulted in a differential response of *P. axelrodi*, where only Cu exposure promotes an increase in ROS production in gill mitochondria. Our findings revealed that nCuO and Cu had a differential toxic effect on both the Amazon fish species studied, which seems to be related to the contrasting metabolic strategies displayed by *A. agassizii* and *P. axelrodi*.

**FP143 Molecular responses of the embryonic cells of freshwater prawn *Macrobrachium olfersii* to environmental UVB radiation exposure.**

T. de Quadros, M. Jaramillo, H.S. Silva, E.C. Zeni, Y. Müller, E.M. Nazari, Universidade Federal de Santa Catarina / Biologia Celular Embriologia e Genética Ultraviolet B (UVB) radiation (280-315 nm) has received more attention as a physical environmental stressor, due to its capacity to penetrate and reach the species that live in aquatic ecosystems. In this study, using a realistic environmental dose, we investigated the effects of UVB radiation in the embryonic cells of freshwater crustacean *Macrobrachium olfersii*, focusing genes involved in mitochondrial biogenesis, such as *Tfam* and *Nrfl*. Additionally, we also investigated genes involved in fission-fusion mitochondrial dynamic, such as *Drp1* and *Mfn1*. Thus, embryos were irradiated with a UVB 6W lamp for 30 min and examined after 12 h of UVB exposure. First, the identification of homologous sequences of the genes, showed that the greater similarity in the amino acid sequences for genes of interest was obtained from the comparison to the crustacean *Hyalella azteca*. Then, the similarities obtained to mitochondrial biogenesis *Tfam* gene was 55% and to the *Nrfl* gene was 96%. The similarities obtained to mitochondrial fission *Drp1* gene was 93% and to mitochondrial fusion *Mfn1* gene was 82%. After UVB radiation exposure, the transcript levels of *Tfam* and *Nrfl* genes showed a significant increase (2.3x for *Tfam* and 1.55x for *Nrfl*) 12 hours of exposure. Also, the transcript levels of the *Drp1* gene, showed a significant increase (2.64x), in embryonic cells exposed to UVB radiation, when compared to the non-irradiated embryos. *Mfn1* transcript levels not changed after UVB radiation exposure. This study contributes for understanding of molecular responses of the embryonic cells against UVB radiation insults and reinforces the importance of this radiation as an environmental stressor in the aquatic environmental.

**FP144 Mortality evaluation and conservation oocytes of the sea urchin *Echinometra lucunter***

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Due to the great demand of gametes of sea urchin used in ecotoxicological tests and the necessity of rational use of these organisms, aiming the preservation of this species, this work had as objective to seek spawning induction techniques that reduce the mortality of the adult organisms, as well as improvements in the conservation techniques of *Echinometra lucunter* sea urchin oocytes for laboratory use. For this, the action of calcium chloride as an antidote to potassium chloride, a substance normally used as a spawning inducer and responsible for high mortality rates of reproducers used to obtain gametes used in chronic marine short - term assays, was tested. Associated with this, oocytes obtained in the induction processes were conserved in calcium and magnesium free artificial seawater enriched with two different antibiotics to evaluate the conservation and storage efficiency of these cells for posterior use in the laboratory. The solutions inducing KCl (0.5 M) and KCl + CaCl<sub>2</sub> (0.5 M and 0.25 M) proved to be efficient in the stimulation of gamete release and the normal development of embryos and larvae in the assays. The KCl inductor presented mortality rates of 60 to 100% of the organisms and the KCl + CaCl<sub>2</sub> mixture had a potential to inhibit KCl toxicity but with a high temporal variability. For oocyte conservation, antibiotic-containing media were shown to be more efficient when kept in the dark, refrigerated and under renovation, and chloramphenicol is the most effective antibiotic in storage for up to 18 days.

**FP145 Mucous cells responses in gill and metal bioaccumulation in native fish captured in the Sinos River basin, southern Brazil**

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The Sinos River basin, located in southern Brazil, supplies water for 1.6 million inhabitants. Nonetheless, its main course - the Sinos River, is heavily impacted by anthropic action, such as agricultural runoffs, domestic and industrial (mainly footwear) discharges, sand removal, elimination of riparian vegetation and so forth. Thus, this river is one of the most polluted rivers in Brazil. In addition, field studies using ecotoxicological approaches are still scarce in the region. Therefore, this study aimed to assess the Sinos River water quality using gill histopathological analyses and metal detection in fish muscle. Ten specimens of the native fish species *Bryconamericus iheringii* were captured at three sampling sites located in the middle section of the basin (S1, S2 and S3) in four sampling campaigns (January, April, July and December/2014). This section is characterized as a transitional area, since rural activities and low density are predominant upstream (S1) and more urbanized cities with higher human occupation are downstream (S3). Gills were removed, processed routinely for histological analyses and stained with Periodic Acid Schiff (PAS) for the identification of mucous cells. The concentrations of chromium (Cr), cadmium (Cd), lead (Pb), manganese (Mn) and zinc (Zn) were detected using a graphite furnace atomic absorption spectrometer. Statistical analysis was performed using the one-way analysis of variance (ANOVA). Significant differences were considered when  $p < 0.05$ . Mucous cells frequencies ranged from 24.8 to 46.9% in fish gills; however no differences between sites were found. Nonetheless, temporal variations were observed at S2 and S3, the most impacted sites. For metals, significant higher concentrations of Mn were detected at S2 in April and December, and at S1 in July. Significant higher concentrations of Zn were also detected at S2 in April. Temporal variation was found at S1 for Cd, at S2 and S3 for Mn and Zn. Although no spatial and temporal variations were observed for Cr, the concentrations of this metal exceeded the levels considered safe for human consumption in all samples. Our results evidenced more impacted at sites located downstream (S2 and S3). Furthermore, high levels of metals were found in fish captured at all sites, indicating the necessity of public policies related to environmental monitoring and management.

**FP146 Mucus formation and absence of histological changes after coexposure of the nanomaterial titanium dioxide and copper in *Limnoperna fortunei*.**

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The wide production and consequent use of nanomaterials (NM) in several areas and applications lead to the potential release of the same in environments, mainly aquatic, putting the biota at risk. The titanium dioxide (nTiO<sub>2</sub>) NM is an inorganic compound used in the industry for the manufacture of various products and has also been used for the purpose of environmental decontamination due to its ability to adsorb metals. This NM may exist as different crystalline forms (mainly as rutile and anatase) that influence its applicability, toxicity and probably its ability to adsorb metals. Copper (Cu) is a naturally occurring metal, but anthropogenic activities may increase its concentration in the environment. Although this metal is essential for many enzymes and proteins, high concentrations can cause toxic effects to organisms and consequently to ecosystems. Hardly a contaminant occurs in an isolated form in the environment, arising the need to study the possible combined effects of the same. Since NMs are emerging compounds, little is known about the effect of coexposure with other well known contaminants, such as Cu. For this reason, the objective of this work was to evaluate whether the coexposure to different crystalline forms of nTiO<sub>2</sub> (rutile and anatase) (1 mg/L) can influence on Cu (56  $\mu\text{g/L}$ ) toxicity in the golden mussel *Limnoperna fortunei* after 120 h exposure. The results showed that after 24 h of exposure to contaminants, mucus filaments from mussels were observed in the treatments with nTiO<sub>2</sub>R, nTiO<sub>2</sub>A, nTiO<sub>2</sub>R + Cu and nTiO<sub>2</sub>A + Cu, being more conspicuous in the treatment with nTiO<sub>2</sub>R. Dispersive energy spectroscopy (DES) analysis showed that the mucous filaments of the treatment with nTiO<sub>2</sub>R and nTiO<sub>2</sub>A presented Ti and O. In the control and Cu groups no mucus formation was observed. Moreover, the exposure to different contaminants did not cause histological changes

in the gills, digestive gland and adductor muscle. These results suggest that mucus formation seems to be a mechanism of protection of bivalves for elimination of contaminants and prevent the toxic effects of the same.

**FP147 Myofibrillar functional desregulation in fish: a new biomarker of damage to pesticides.**

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The myofibrils proteins are very susceptible to oxidative stress, exhibiting polymerization, insolubilization, polypeptide chain breakdown, and amino acid destruction and consequently denaturation and aggregation of biopolymers. Endosulfan (ES) modifies the ultrastructure of the skeletal muscle fibers and causes changes in the swimming behavior of fish. The objective of the present work was to explore the integrity and functionality of myofibrils (Mf) in the freshwater fish *Australoheros facetus* exposed to 0.5 and 10 µg/L ES during 24 h. Myofibrils were analyzed for contractile function by monitoring with a phase contrast microscopy. Myofibrillar Mg<sup>2+</sup> Ca<sup>2+</sup> ATPase and Mg<sup>2+</sup> (EGTA) ATPase activities, Ca<sup>2+</sup> sensitivity, SDS-PAGE profiles of Mf and lipid peroxidation were also analyzed. As expected, after addition of contraction buffer Mf from control fish contracted. On the contrary, Mf from fish exposed at 0.5 µg/L ES showed a partial contraction and not any of those from fish exposed at 10 µg/L ES contracted. As judged by its high Mg<sup>2+</sup> Ca<sup>2+</sup> ATPase activity and low Mg<sup>2+</sup> (EGTA) ATPase activity, control Mf showed good functionality. In Mf from fish exposed to 0.5 and 10 µg/L ES the activities of these enzymes were similar, suggesting denaturation or degradation of some component of tropomyosin-troponin complex. SDS-PAGE patterns of Mf from fish exposed to ES showed degradation of myosin heavy chain and of tropomyosin. Similar values of TBARS were found in both control and exposed Mf, suggesting that lipid oxidation would not be related with above mentioned changes. The effects observed expand the knowledge about the mode of action of ES in muscle and could be used as biomarkers of damage in fishes.

**FP148 OXIDATIVE AND GENOTOXIC EFFECTS OF THE INSECTICIDES IMIDACLOPRID AND λ-CYHALOTHRIN, ALONE AND MIXED, ON THE FRESHWATER FISH PROCHILODUS LINEATUS**

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Currently, Brazil is the largest consumer of pesticides in the world, among them the neonicotinoid insecticide Imidacloprid (IMI) and the pyrethroid λ-cyhalothrin (λ-CL). These insecticides have been used in agricultural areas, where their mixtures are constantly applied to improve efficacy and reduce cost. However, the use of this mixture may result in combined effects on various organisms, as both pesticides may reach aquatic ecosystems and promote impacts at different levels of biological organization. Thus, the goal of this work was to evaluate genotoxic and biochemical effects of IMI (300 µg L<sup>-1</sup>), λ-CL (150 ng L<sup>-1</sup>) and the mixture (IMI + λ-CL), for the Neotropical fish *Prochilodus lineatus*. Fish were exposed to the different treatments or only to water (CTR) during 48 h. After exposure, blood samples were taken and fish were euthanized for the removal of the gills. Blood and gill cells were used for the comet assay, and the gills were also used for the determination of lipid peroxidation (LPO) and protein carbonylation (PCO). Scores of DNA damage significantly higher were observed in blood cells of fish from the groups λ-CL+IMI (113.125 ± 8.05 n = 8) and λ-CL (94.125 ± 9.22 n = 8), in relation to CTR (49.75 ± 4.97). While in gill cells the scores of DNA damage in fish exposed to all treatments (λ-CL: 70.25 ± 1.90 n = 8; IMI: 51.625 ± 4.76 n = 8; λ-CL+IMI: 77.125 ± 4.28 n = 8) were significantly higher in relation to CTR (41.25 ± 4.79 n = 8). The gills of fish exposed to λ-CL+IMI showed a significant increase in LPO (23.18 mg / ptn ± 1.7 n = 8) compared to the λ-CL groups (6.93 mg / ptn ± 0.88 n = 7), IMI (8.46 mg / ptn ± 1.50 n = 9) and CTR (5.51 mg / ptn ± 0.8 n = 9). Also, a significant increase in PCO was observed in fish from λ-CL+IMI group (0.132 mol carbonyl / mg ptn ± 0.016 n = 8) when compared to the λ-CL (0.073 mol carbonyl / mg ptn ± 0.011 n = 8), IMI (0.086 mol carbonyl / Mg ptn ± 0.013 n = 8) and CTR (0.054 mol carbonyl / mg ptn ± 0.006 n = 9). Taking together these results show that the mixture of imidacloprid and λ-cyhalothrin is more harmful than the insecticides alone as it promotes oxidative and genotoxic damages in *P. lineatus*.

**FP149 Parental exposure to copper: DNA damage and larval impairment in *Oreochromis niloticus***

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Many xenobiotics released in nature by humans have genotoxic potential and copper is one of the most studied metals in relation to its effects on DNA. Studies suggested a strong relationship between exposure to copper and genotoxicity in somatic cells, but there is a need to understand the effects that germ cell DNA damage can produce to the future generations. Fragmentation of sperm DNA can affect the fertilization process, embryo quality and development, and induce malformations and genetic diseases in the offspring. This work aimed to evaluate the genotoxic effects of copper in *Oreochromis niloticus* males and to relate these damages with possible changes in the quality of the gametes and in the generation of offspring abnormalities. For this, the alkaline Comet assay in sperm and blood intended to evaluate DNA damage; the analysis of sperm parameters intended to evaluate the quality of the gametes and the analysis of morphological abnormalities of larvae coming from the reproduction of males exposed to copper intended to evaluate the reproductive impairment. The fish were exposed to three doses of copper sulphate by intraperitoneal injection for 96h. Copper did not interfere with the quality of gametes since sperm parameters did not presented differences between the groups exposed and control group. The three concentrations of copper used in the experiment increased DNA damage of spermatozoa. This increase followed the increased craniofacial deformities in larvae in *O. niloticus*. In conclusion, results of this work suggest copper as an inductor of DNA breakage in germ cells of *O. niloticus* males and these damages can induced larval morphological abnormalities, causing reproductive impairment to this species.

**FP150 Programa de monitoreo de hidrocarburos y metabolitos de PAHs en bilis de peces como biomarcador de exposición en el Sur del Golfo de México.**

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Definitivamente, uno de los grandes retos de la sociedad, es la detección temprana de los efectos en los organismos marinos expuestos a contaminantes, para evaluar las consecuencias biológicas y evitar grandes cambios en la biodiversidad de los ecosistemas. Una manera de establecer la conexión entre los niveles de contaminantes y sus efectos nocivos sobre los organismos, son los programas de monitoreo que han incorporado herramientas biológicas tales como biomarcadores de efecto y exposición. En este sentido, con el objeto de evaluar el impacto de los hidrocarburos del petróleo en peces del Sur del Golfo de México, se llevaron a cabo cuatro cruces oceanográficos entre 2014 y 2016, con el interés de determinar las concentraciones de los hidrocarburos del petróleo y de los metabolitos de PAHs en bilis de peces. Para ello, se colectaron 380 peces planos bentónicos como organismos centinela. Los lenguados fueron capturados en 77 sitios de muestreo a lo largo del Golfo de México (incluyendo desembocaduras de ríos y lagunas, así como áreas de explotación petrolera). Se determinó la concentración de metabolitos de PAHs en bilis (1-OH naftaleno, 4-OH fenantreno, 1-OH pireno, y benzo[a]pireno), mediante fluorescencia sincrónica (Aas et al. 2000), así como la concentración de hidrocarburos en sedimentos y organismos. Cabe mencionar que los PAHs y algunos de sus metabolitos han sido descritos como compuestos peligrosos por presentar un potencial mutagénico y carcinogénico (IARC, 1986). Los resultados obtenidos mostraron que la concentración de los cuatro metabolitos de PAHs presentan una acumulación diferencial en los organismos colectados, siendo el 1-OH naftaleno el de mayor concentración con un valor medio 286.52 µg/ml, por el contrario, los equivalentes de benzo[a]pireno fueron los de menor concentración con un promedio de 1.70 µg/ml. Al analizar con detalle la bilis de los metabolitos de bajo peso molecular (BPM) y los de alto peso molecular (APM) se observó una predominancia en la concentración de los metabolitos de BPM siendo el 1-OH naftaleno y 4-OH fenantreno, los más concentrados. Por otro lado, la concentración de hidrocarburos totales en sedimentos (min: 0.30 µg/g, max: 7497.57 µg/g) fue mayor en las zonas costeras, cerca de las desembocaduras de los ríos, confirmando que la presencia de metabolitos de BPM se encuentra asociada a compuestos derivados del petróleo, lo cual sugiere que los peces podrían estar expuestos a hidrocarburos de petróleo crudo.

**FP151 Role of CYP450 on the toxicity of azinphosmethyl and acetamiprid in pesticide-exposed and non-exposed field-populations of *Simulium* spp**

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The resistance of insects to insecticides has become a dominant problem for

contemporary agriculture. Most of resistant species are of agricultural importance, but many of them are insects of human health concern such as mosquitoes and flies. The Valley of Rio Negro and Neuquén produces a large proportion of Argentina's apples and pears. This area is irrigated by fast-flowing channels providing a favorable habitat for many non-target species including blackflies (*Simulium bonaerense*, *S. wolffhuegeli* and *S. nigristrigatum*). Therefore, these species are highly exposed to pesticides through air drift from spraying, run-off of chemicals into irrigation channels and the discharge of pesticides by the cleanup of containers, sprayer tanks, and other devices. Previously, we have observed resistance to DDT and pyrethroids in blackflies from this area. The objectives of this study were to evaluate the toxicity of azinphosmethyl (AzMe) and acetamidiprid (Acet) as well as cytochrome P450 activities in blackfly populations from pesticide-exposed (F. Oro) and non-exposed (P. del A) sites. Groups of 20 last instars were exposed for 24 h (15°C and a photoperiod of 16:8 h L:D) to different concentrations of each pesticide dissolved in acetone and applied as 0.1 mL in a final volume of 200 mL of filtered and dechlorinated water. Mortality of both treated and control larvae were determined at the end of the exposure period. Resistance ratios were calculated as the LC50 F. Oro/LC50 P del A. CYP450 activity was individually assayed from both populations. Blackflies from F. Oro was as susceptible to AzMe (LC50 = 0.015 mg/L) as the ones from P del A (LC50 = 0,011 mg/L). On the other hand, the LC50 of Acet from F. Oro (0.0066 mg/L) was 2.5-fold higher than the one from P del A (0.0026 mg/L). The average CYP450 activity of the pesticide-exposed population of blackflies was significantly higher ( $p < 0.001$ ) (65 pg 7-OHC/min/adult) than the non-exposed ones (36 pg 7-OHC/min/adult). The distribution of CYP450 activities from the pesticide-exposed population showed a shift toward higher activities (kurtosis 6.1; skewness 1.6). On the other hand, the frequency distribution of CYP450 activity in blackflies from the control site did not evidence a high departure from normality (kurtosis 0.46; skewness 0.39). Therefore, we conclude that the differences in toxicity to Acet between insecticide exposed and non-exposed populations of blackflies are at least due to enhanced CYP450 activity.

#### FP152 SILVER AND BIOLOGICAL NANOPARTICLES AND SILVER NITRATE EFFECTS ON NEOTROPICAL FISH *Prochilodus lineatus*

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Among the key aquatic ecosystem contaminants, metals stand out by their diversity, amount and behavior in relation to the environment. In addition, metals are also present as nanoparticles (NP). Among the types of NP already developed, silver nanoparticles (NPAg) are highlighted, which, due to their large antimicrobial capacity and relatively low manufacturing cost, are used in several products such as medical devices, fabrics, food packages, and pharmaceutical products in general. Thus, to evaluate toxicity of different silver forms in aquatic organisms, *Prochilodus lineatus* fish were exposed, for 96 hours, to the concentration of 10  $\mu\text{g L}^{-1}$  of total silver in the biological and synthetic forms and silver nitrate ( $\text{AgNO}_3$ ). Total silver concentration of 10  $\mu\text{g L}^{-1}$  is the maximum concentration allowed by the Brazilian legislation (CONAMA 357, 2005) for class I waters; however, there is no regulation for nanoparticles. After being exposed, the animals were anesthetized and their blood drawn. Next, the fish were killed by spinal cord section for the removal of the organs (liver, brain, muscle and gill). The following parameters were analyzed: hemoglobin content, hematocrit, number of RBCs,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  e  $\text{Mg}^{2+}$  plasmatic ionic concentration, glucose and metallothionein concentration, and  $\text{Na}^+/\text{K}^+$ -ATPase,  $\text{H}^+$ -ATPase, carbonic anhydrase and e da acetylcholinesterase activity. Animals exposed to  $\text{AgNO}_3$  showed a reduction in plasmatic  $\text{Ca}^{2+}$ , acetylcholinesterase activity inhibition, brain metallothionein reduction and an increase of these proteins in the liver, showing that this composite is neurotoxic and compromises osmoregulatory processes in these animals. Nanoparticles cause an increase in metallothionein concentration in the brain of the exposed animals, showing a detoxification reaction of this metal and presenting this organ as sensitive to silver-based composites. Based on these findings, it was possible to observe that the NPAgs were less toxic than the  $\text{AgNO}_3$  for the tested parameters. However, it is important to emphasize that the alterations found in the fish exposed to  $\text{AgNO}_3$ , at the concentration allowed by the Brazilian legislation may compromise the homeostasis of these organisms.

#### FP153 Solar and artificial UVB radiation cause cell cycle impairments in embryos of freshwater prawn *Macrobrachium olfersii*

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The shortest wavelength of solar ultraviolet B (UVB) radiation can penetrate the atmosphere and reaches the aquatic ecosystems to different depths. Many freshwater systems are shallow and consist of transparent water, and aquatic organisms that inhabit these systems might be exposed to UVB energy. The aim of this work was to verify the effect of UVB radiation on cell proliferation and cell cycle in embryos of freshwater prawn *Macrobrachium olfersii*. In order to recognize the cellular responses during the embryonic development, embryos were exposed to UVB radiation in laboratory conditions, using a 6W UVB lamp for 30 min. Non-irradiated embryos were used as controls. Additionally, we collected embryos from freshwater field, exposed to solar UVB radiation, which were used as reference for UVB environmental exposure. All the cellular analyses were performed by flow cytometry and immunohistochemistry, using antibodies for specific proteins. Our data showed that there was a significant increase in the proliferating cells labeled with anti-phosphohistone H3 (PHH3) in UVB-irradiated embryos ( $4,378.67 \pm 885.49$ ) and embryos collected in the natural environment ( $4,715.67 \pm 1,303.02$ ) in comparison to non-irradiated embryos ( $2,208.67 \pm 1,590.08$ ;  $p < 0.01$ ). When we looked for the proliferating cell nuclear antigen (PCNA), we observed a significant increase in UVB-irradiated embryos ( $4,851.0 \pm 1,020.82$ ) and embryos collected in the natural environment ( $5,266.67 \pm 1,620.54$ ) in comparison to non-irradiated embryos ( $2,330.67 \pm 1,610.61$ ;  $p < 0.01$ ). On the other hand, the expression of p53 protein did not change in embryos irradiated with UVB lamp ( $4,415.0 \pm 710.99$ ) and in embryos from the environment ( $4,339.0 \pm 2,930.34$ ), when compared to non-irradiated embryos ( $3,243.67 \pm 2,057.32$ ). The immunolocalization of proliferating cells showed that PHH3 was distributed in cells of all embryonic structures. Our results demonstrated that artificial and also solar UVB radiation induce DNA damage, which was deduced by the increased PCNA protein. However, p53 protein seems to not signaling for damage repair. Thus, considering the increase of proliferating cells is predicted that unrepaired damage can progress through the cell cycle. These findings reveal the toxicity of UVB radiation on embryonic cells of freshwater prawn *M. olfersii*. More importantly, we observed that embryos that develop in the natural environment have the same impairments induced in the laboratory conditions.

#### FP154 The effects of sediment classification pattern on a water column organism, *Ceriodaphnia dubia*

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Reservoirs are lentic environments and are consequently subject to the accumulation of contaminants. In these systems, the sediment compartment stands out because it functions as both a temporary sink of pollutants and a potential source of these elements that may become available to the water column depending on the environmental conditions. This study aimed to evaluate the concentrations of total metals in the crude sediment and interstitial water and its relationship with the ecotoxicity in the water column. The most commonly used sediment quality guidelines are the Canadian (CCME, 1995), which is based on the concentration of metals obtained in the crude sediments and on the ecotoxicological assays with benthic organisms. However, there is no protocol to evaluate how sediment quality could contribute to the water column quality, and such an interface should be investigated. Here we adapted protocols of ecotoxicological tests with benthic organisms for studies with a nectonic organism (*Ceriodaphnia dubia*). For this purpose, 6 collection points were established in the years 2013 and 2014, distributed from the beginning to the end of the system. The trials showed acute toxicities with 100% mortality for points P2 and P3 in 2013 and chronic toxicity for point P2 in 2014. This result indicates that the sediment may contribute to the toxicity in the water column and that such toxicity is possibly not related to the metals present. Based on the chemical analysis of the metals, the Canadian Sediment Quality Guidelines (SQGs) would frame the sediment as non-toxic to benthic organisms, but the SQGs have no reference standards for possible effects on nektonic organisms. It is concluded that there was no correlation between these elements and the toxicity; however the sediment showed the capacity to cause toxicity in the water column, and this fact confirms the difficulty of estimating the potential contamination of the water column by this compartment, which is fundamental to the evaluation of the interactions of the different pollutants in the system and possible effects on nektonic organisms. The performance of bioassays associated with chemical aspects is also important, since only biological systems are capable of reflecting the interactions of pollutants. CCME, Protocol for the Derivation of Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (1995). CCME EPC-98E. Prepared by Environment Canada, Guidelines Division, Ottawa.



**FP155 The hepatosomatic index of bullfrog tadpoles exposed the pyrethrum in its free form and encapsulated into the nanoparticles**

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In the last 30 years, a drastic decline in amphibian's populations have been observed worldwide. Among the causes pointed as responsible for this decline are the exposure to agrochemicals, habitat loss and pathogens. Biocides are employed in the agriculture to control target organism through a chemical or biological action. Thus, biocides are considered safer, less toxic to non-target organisms and more selective to pests. Other technology that is being developed for agricultural purposes are nanocarriers systems, since they promote a more controlled releasing of agrochemical in crops, which has been considered a safer system and less harmful to the environment. However, it is necessary to evaluate the effects of biocides and nanoparticles (alone or loaded with biocide) on living organisms, especially in the amphibian's populations that can be found in water bodies near to agroecosystems. In this context, the aim of this study was to analyze comparatively the hepatosomatic index (HSI) in the liver of bullfrog tadpoles (*Lithobates catesbeianus*) exposed to the pyrethrum biocide, either in its free form and encapsulated into the nanoparticles. In this study we have been used 20% of the LC<sub>50</sub> of pyrethrum (400 mg.L<sup>-1</sup>). For ecotoxicological assays by acute exposure (48 h), tadpoles were randomly shared into aquariums (25 L). The assays were done using the following groups (n=8 individuals per group): pyrethrum, pyrethrum encapsulated in solid lipid nanoparticles, empty solid lipid nanoparticles (SLN), acetone (vehicle), poly-vinyl alcohol (PVA, surfactant used in the SLN formulations) and control group, all experiments were performed in triplicate. After exposure time, the animals were euthanized and their livers were collected and weighted. The results *One-Way ANOVA*, post-hoc Dunnett's test, showed no significant difference of the hepatosomatic index between groups, evidencing that the exposure to these xenobiotics did not cause hyperplasia, hypertrophy or decrease of the size of the organ in acute exposure. Therefore, the morphology of this important organ that biotransforms xenobiotics probably keeps normal in the tadpoles exposed to biocide and/or solid lipid nanoparticles. Nevertheless, more studies are needed to verify the possible effects of acute and chronic exposures to biocides as well as the nanoparticles in the glycogen and lipid storages in hepatic tissue of tadpoles.

**FP156 The influence of environmental pollution in oxidative stress of *Astyanax fasciatus* (Teleostei: Characiformes: Characidae)**

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The presence of chemicals in the aquatic environment can cause alterations in biochemical and physiological processes of animals, such as oxidative stress. The aim of this study was to investigate the influence of environmental chemical contaminants in the activity of enzymes involved in oxidative stress of *Astyanax fasciatus* sampled in two reservoirs of same basin, with different degrees of eutrophication, in São Paulo State (Brazil). Adult females were sampled throughout one year in the Ponte Nova (PN, reference site) and Billings (Bil, polluted site) reservoirs. Water and sediment samples were collected in each reservoir to evaluate physical and chemical variables. The hepatosomatic index (HSI) and enzymes involved in oxidative stress were analyzed in the liver: catalase (CAT), superoxide dismutase (SOD) and reductase glutathione (GR), as well as metallothionein (MT), a protein involved in the intracellular metabolism of metals. The results showed that the concentration of metals in the water and in the sediment in Bil, during summer and winter, were higher than the recommended by Brazilian guidelines (CONAMA/357). Regarding the enzymes analyzed, females from Bil presented higher CAT activity during summer and fall than females sampled in PN but no other enzyme was altered. The results showed that the degree of pollution in Bil did not trigger the first line of antioxidative defense, normally promoted by SOD. However, the animals probably undergone oxidative stress situations that triggered the increase in CAT activity to maintain the levels of

hydrogen peroxide.

**FP157 Three spawning methods for obtaining zebrafish (*Danio rerio*) embryos for the use in toxicity tests**

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The use of fish embryos in toxicological tests has emerged as an option to the tests with adults due to sensitivity, practicality and ethical issues. Since spawning methods are quite variable in protocols, this study aimed to compare three different low cost devices for obtaining fish embryos for test purposes: mating boxes, collection trays and a funnel with a collecting cup. The mating aquarium, for just one couple, consisted of two slightly conic plastic boxes (29x18x15cm, LxWxH) fitted together. The inner box had a bottom net to prevent egg predation. The collecting tray (20x12x4 cm) had a net cover and was placed in the bottom of a 16L aquarium (with six couples). Finally, the funnel was an apparatus with a 1.5mm mesh (as the others) on the top and a "U" shaped PVC tube attached to the bottom inside of which air bubbles carried out the eggs to a collecting cup (with 60 µm mesh). This apparatus was placed in an 80L aquarium with 12 couples. Plastic plants were used for environmental enrichment in all the nets. The systems were tested for 10 times, with the interval of one week. ANOVA and SNK test (p<0,05) was used for data analyses. All methods produced embryos with high viability (more than 90% of fertilized eggs) and with one hour post fertilization, and the total number of eggs per spawning ranged from 70 to 861. Regarding the number of embryos per couple (efficacy) results showed that the mating box was the most effective, but it was the least reliable method for spawning, which occurred in only 40% of the experiments (the lowest efficiency). In the other methods, although the number of eggs per couple was low, the efficiency was high, being 70% with the funnel and 90% with the tray. Thus, the mating box, despite its effectiveness, is appropriate only when there are few animals for mating, because it is necessary consider that spawning may not occur. We observed that the presence of several couples was a stimulus for mating and spawning behaviors, as occurred in the systems with six and twelve couples. The funnel and tray methods were equivalent in efficacy, but the first was worse in efficiency. On the other hand, the funnel method was more convenient for handling the embryos with pipette, because the embryos were kept in a reduced space. Summarizing, the choice of one of the methods tested will depend on the urgency for having embryos for testing and the availability of fish for mating.

**FP158 Toxicity biomarkers in several organs of *Cyprinus carpio* exposed to sublethal concentrations of atrazine**

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In Argentina, more than 30 million hectares are annually dedicated to agriculture of cereals and oilseeds with an intensive use of pesticides usually reaching surface waters by aerial drift, runoff and leaching. In particular, atrazine (ATZ) is widely used herbicides that is frequently detected in rivers and lakes. The objective of the present study was to evaluate the response of a suit of biochemical biomarkers in different organs of *Cyprinus carpio* exposed to sublethal concentrations of the herbicide under acute and chronic exposures. Two experiments were performed: in the acute experiment (ACU), fish (45±15g) were exposed to 0, 0.2 and 2 mg L<sup>-1</sup> ATZ during 4d, in the chronic experiment (CHR) fish (43±11g) were exposed to 0, 0.002, 0.02 and 0.2 mg L<sup>-1</sup> ATZ during 28d. In both experiments fish were exposed to ATZ in 60 L stainless steel tanks by duplicated, placing 4 or 3 fish per tank in ACU and CHR, respectively. The assays were semi-static and media renewal was performed every 48 h. Phase I and II biotransformation enzymes, thiobarbituric acid reactive substances, antioxidant enzymes and acetyl-cholinesterase were measured in liver, brain gill, and muscle by fluorometric and spectrophotometric methods adapted to microtiter plates. ANOVA was used for statistical analysis, followed by the "post hoc" Tukey Honest test. Measured ATZ in test media was close to nominal concentrations. No statistically significant differences were observed in the standard growth rate, the hepatosomatic index or the gonadosomatic index of fish exposed under both conditions. On the other hand, the condition factor (K) was significantly reduced in the CHR. Under acute exposure several enzymes were modulated by ATZ. In the liver, GST activity was increased while CAT and BROD were inhibited. In the brain, GST activity was induced as well as GPx and CAT. On the contrary, GPx was inhibited in the gill. Under chronic exposure, only GST was induced in gill. According with obtained results, ATZ is able to induce the phase II biotransformation system, both under acute and chronic exposures. In addition, the herbicide was able to modulate the antioxidant system in different ways depending the tissue, but only under acute exposure and without inducing oxidative stress. The lack of response under chronic exposure could be explained as the result of compensation mechanisms. However, the reduction in K,

together with GST induction would be indicating that other adverse effects are happening.

**FP159 Variación en la expresión del CYP1A en diferentes especies de Pleuronectiformes identificadas por código de barras genético en el Golfo de México**

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La contaminación del medio marino se ha vuelto un problema de preocupación mundial debido a los efectos devastadores de los contaminantes, ya que estos aumentan de manera alarmante año con año. Lo anterior ha promovido la realización de campañas oceanográficas de monitoreo biológico y toxicológico, donde los peces planos (pleuronectiformes) han sido utilizados como organismos centinelas de los ecosistemas marino-costeros del Golfo de México (GM): esto debido a que viven asociados al sedimento, el cual generalmente funciona como depósito de contaminantes químicos y tóxicos. Durante los años 2012 al 2016 se realizaron cuatro cruces oceanográficos en las aguas mexicanas del GM, donde la salud de los pleuronectiformes colectados fue evaluada por la expresión del gen del Citocromo P450 (CYP1A) con la finalidad de determinar los valores de expresión génica asociados al proceso de transformación y eliminación de los hidrocarburos en el medio acuático. Adicionalmente, los mismos organismos utilizados para evaluar la expresión génica, fueron identificados molecularmente mediante su código de barras genético, el cual se basa en la secuenciación del gen mitocondrial Citocromo Oxidasa subunidad I (COI). Con la información obtenida de la expresión del CYP1A y la identificación de las especies nos fue posible determinar las variaciones de la expresión en los diferentes peces que se han colectado hasta ahora en el GM. De un total de 448 peces planos, se identificaron un total de 9 especies en donde de manera general, la expresión del CYP1A mostró diferencias significativas entre ellas. Los valores de expresión del CYP1A obtenidos estuvieron en un rango desde una nula expresión hasta un máximo de 9,441 copias del transcripto por  $\mu\text{g}$  de RNA, donde la especie *Citharichthys spilopterus* fue la que no se detectó expresión alguna, mientras que *Ancylopsetta dilecta* fue la especie con mayores niveles de expresión. Los resultados obtenidos muestran la importancia de identificar molecularmente a cada especie de pleuronectiformes estudiados, con la finalidad de entender las variaciones en los patrones de expresión de los genes asociados al proceso de transformación y eliminación de los contaminantes.

**FP160 ZF-L cell line exposed to titanium dioxide nanoparticles: an integrative analysis of cytotoxic, genotoxic, and mutagenic effects**

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Titanium dioxide ( $\text{TiO}_2$ -NP) nanoparticles are commonly used in many industrial products. Therefore, its daily consumption by humans is estimated at 5.4 mg day<sup>-1</sup>, which leads to an intake of 4.2 mg day<sup>-1</sup> per person, and may or may not receive appropriate treatment before being discharged. The cytotoxicity, genotoxicity, and mutagenicity of  $\text{TiO}_2$ -NP to the cell line (ZF-L) were evaluated. The characterization of  $\text{TiO}_2$ -NP was performed by measures of transmission electron microscopy (TEM) analysis; hydrodynamic diameter of the agglomerates and potential zeta (DLS, diameter light scattering). Trypan blue dye exclusion assay assessed cell viability, as well MTT assay and NRR assay. Flow cytometry evaluated the induction of apoptosis and necrosis. Genotoxicity was determined by the comet assay, while the micronucleus assay determined mutagenicity. DLS measurements showed that FBS was effective in the dispersion of the nanoparticles and prevented the formation of large agglomerates. After 24 hours of treatment, there was rupture of the cell membranes, reducing cell viability to 35.33% (1.0  $\mu\text{g mL}^{-1}$ ). Mitochondrial metabolic activity remained unchanged, but it was possible to detect the proliferation of lysosomes, which was attributed mainly to NP endocytosis. The induction of apoptosis was of 50.4%, and of necrosis of 13.9%, both in the concentration 1.0  $\mu\text{g mL}^{-1}$   $\text{TiO}_2$ -NP. In the case of necrosis, a result was 10 times greater than that presented by the negative control. Necrosis and apoptosis together indicated death of 35.7% of the cells. The comet test showed the fragmentation of the DNA, it was also possible to observe the formation of micronuclei, bridges and shoots demonstrated by the micronucleus assay. In general, this study demonstrated that  $\text{TiO}_2$ -NP, after 24 hours of exposure, significantly affect cell viability and cause DNA damage, which may become irreversible. In conclusion, this study showed the cytotoxic, genotoxic, and mutagenic

potential of  $\text{TiO}_2$ -NP for ZF-L cells. Mitochondrial and lysosome responses require further studies on the effect of  $\text{TiO}_2$ -NP on these organelles.

**FP161 Influence of Post Hatching Age on the Sensitivity of Acute Fish Test using Danio rerio for Ecotoxicological Proposes**

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Acute fish toxicity (AFT) test have been used in many countries for effluent risk assessment and/or ecotoxicological monitoring. Within it, a relevant number of countries have used OECD 203 - or some national standard protocol with minor differences like Brazilian NBR ISO 15088 - as a standard method. This protocol determines the usage of juvenile/adults fish within a specific length size ( $2 \pm 1$  cm) that may not be directly associated organism's age. Some studies have suggested a clear decrease in sensitivity to pollutants along the organism lifetime, resulting in a higher sensitivity of larvae when compared to adults. Summing up, tests with adults require a large volume of tested solution (chemicals, effluent...) when compared to larval tests, resulting in logistic costs. Due to that, the Environmental Protection Agency (US EPA) recommends that toxicity tests should be performed with organisms that are in the early stages of development, ensuring the most sensitive stage to be tested. The objective of this research is to strictly determine the optimal age for acute fish test using zebrafish (*Danio rerio*) as model. Fish were reproduced in the laboratory and nurtured with Paramecium sp. Ad libitum until 2 hours prior testing. Toxicity tests were performed in replicate for organisms aging from 2 to 24 days post hatching using Potassium Chloride (KCL) as reference solution. Until the moment, we have performed 28 tests. Organisms were exposed to the reference solution for 48 hours in a static renewal system and kept at  $25 \pm 2$  °C. LC50:48h were determined using Trimmed Spearman-Kärber 1.5 Software and their comparison were obtained by overlapping of confidence intervals method with  $\alpha=0.05$ . The usage of *D. rerio* in larval stage provides a significantly more sensible result to KCl when compared to juveniles. Nevertheless, based on the initial results is not possible to confirm the difference in sensitivity between the 2nd and 12th day of life tested. However, a clear inflection point appears on the sensitivity curve after the 14th-day post hatching. Notwithstanding, further studies aiming other reference solutions still need to be concluded.

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