



18th International Symposium on Toxicity Assessment

Conference Proceedings

July 16-21, 2017

Limeira, SP – Brazil

18th International Symposium on Toxicity Assessment

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July 16-21, 2017
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FINAL PROGRAM

July 16, 2017	Sunday
14:00-18:00	Registration
18:00-19:00	Opening Lecture: Global Developments in Environmental Toxicology: Protecting Complex Ecosystem Functions and Provisioning of Ecosystem Services, Tracy K. Collier, USA
19:00-21:00	Reception
July 17, 2017	Monday
08:00-08:30	Registration
08:30-09:00	Opening remarks
09:00-10:00	Keynote: Critical Appraisal and Perspectives on Gastropod Imposex Studies, Toshihiro Horiguchi, Japan
10:00-10:20	Break
10:20-12:00	Oral Session 1: Emerging Contaminants
12:00-13:30	Lunch
13:30-15:30	Oral Session 2: Dyes, Health and Environmental Impacts
15:30-17:00	Poster Session 1
17:00-18:00	Keynote: Emerging Unregulated DBPS: New Discoveries, New Sources, And Insights into Toxicity, Susan D. Richardson, USA
July 18, 2017	Tuesday
08:00-08:30	Registration
08:30-09:00	Remarks
09:00-10:00	Keynote: Pesticide Impacts on Pacific Salmon – A Commercial, Ecological, and Cultural Resource in Western North America, Nathaniel L. Scholz, USA
10:00-10:20	Break
10:20-12:00	Oral Session 3: Pesticides and Environmental Impacts
12:00-13:30	Lunch
13:30-15:30	Oral Session 4: Nanomaterials and Nanotoxicology
15:30-17:00	Poster Session 2
19:30-21:30	Wine and Cheese Party
July 19, 2017	Wednesday
11:00-17:00	Field Day - Quilombo Farm
July 20, 2017	Thursday
08:00-08:30	Registration
08:30-09:00	Remarks
09:00-10:00	Keynote: Solutions for Present and Future Emerging Pollutants in Land and Water Resources Management, Werner Brack, Germany
10:00-10:20	Break
10:20-12:00	Oral Session 5: Soil and Wastewater
12:00-13:30	Lunch
13:30-15:30	Oral Session 6: Ecotoxicology and Fish
15:30-17:00	Poster Session 3
19:30-23:00	Folklore party

July 21, 2017	Friday
08:00-08:30	Registration
08:30-09:00	Remarks
09:00-10:40	Oral Session 7: Alternative testing
10:40-10:50	Break
10:50-12:00	Oral Session 8: Sediment toxicity assesment
12:00-13:30	Lunch
13:30-15:00	Student awards, closing ceremony and announcement of ISTA 19

KEYNOTES

ISTA18-007906 - GLOBAL DEVELOPMENTS IN ENVIRONMENTAL TOXICOLOGY: PROTECTING COMPLEX ECOSYSTEM FUNCTIONS AND PROVISIONING OF ECOSYSTEM SERVICES

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Increasingly, the environmental toxicologists of our planet are reporting that chemical contaminants are having serious effects on ecosystems, and affecting the ability of humans to derive services from many contaminant-affected ecosystems. Coming to this recognition has been a slow and arduous process, because for the past several decades, environmental toxicology has been based on single species toxicity testing. This reliance on narrowly focused toxicity testing was initially necessary, but led to the development of environmental protection policies that now are resistant to better management practices that can be developed from our global understanding of the problems that chemical contaminants pose to the fabric of ecosystems. Problems such as the worldwide loss of pollinating insects, highly toxic cyanobacterial blooms in freshwater systems, loss of fisheries and decreased ability to consume the fish that do remain, and stormwater impacts to receiving waters, are happening now, and will likely be exacerbated by climate change. To address this “wicked problem”, we require more comprehensive management strategies than are generally afforded by our “one chemical at a time” approach. In this presentation I will discuss these issues, and how we as humans are now seeing the loss of ecosystem services that support our existence on the planet. Examples of better management approaches will be presented, as a catalyst for further discussion among meeting participants.

ISTA18-004537 - CRITICAL APPRAISAL AND PERSPECTIVES ON GASTROPOD IMPOSEX STUDIES

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The term, “imposex” was defined by Smith 1), meaning imposed sexual organs, to describe the syndrome of a superimposition of male genital tracts (penis and vas deferens) on female prosobranch gastropods, although the first report of masculinized females was made by Blaber 2). Imposex is thought to be an irreversible syndrome 3). Reproductive failure may be brought about in severely affected stages, resulting in population decline and/or mass extinction 4-6). Imposex is known to be induced in many species by tributyltin (TBT), and also by triphenyltin (TPhT), from antifouling paints on ships and fishing nets 7-11). Approximately 200 species of Caenogastropoda had been reported to be affected by imposex worldwide 12-14), including 39 species among 69 Japanese species examined 13,15).

Regarding the mechanism by which organotins, such as TBT and TPhT, induce the development of imposex in gastropods, six hypotheses have been proposed: (1) an increase in androgen (e.g., testosterone) levels as a result of TBT-mediated inhibition of aromatase 16); (2) an increase in testosterone levels owing to the inhibition of acyl CoA-steroid acyltransferase 17,18); (3) TBT-mediated inhibition of the excretion of androgen sulfate conjugates, with a consequent increase in androgen levels 19); (4) TBT interference with the release of penis morphogenetic/retrogressive factor from the pedal/cerebropleural ganglia 20); (5) an increase in the level of an alanine-proline-glycine-tryptophan amide (APGWamide) neuropeptide in response to TBT 21); and (6) activation of the retinoid X receptor (RXR) 22). Although scientific debate is still continuing, there are several papers in which a hypothesis of activation of RXR is supported 23-29). Here, perspectives on gastropod imposex studies, including basic ones on physiology and endocrinology of gastropod molluscs, will be also discussed.

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ISTA18-008065 - EMERGING UNREGULATED DBPS: NEW DISCOVERIES, NEW SOURCES, AND INSIGHTS INTO TOXICITY

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Drinking water disinfection by-products (DBPs) are an unintended consequence of using chemical disinfectants to kill harmful pathogens in water. DBPs are formed by the reaction of disinfectants with naturally occurring organic matter, bromide, and iodide, as well as from anthropogenic pollutants, such as pharmaceuticals and pesticides. Potential health risks of DBPs from drinking water include bladder cancer, early-term miscarriage, and birth defects. Risks from swimming pool DBP exposures include asthma and other respiratory effects. Major efforts have been made toward uncovering the DBPs responsible for these effects. However, >50% of halogenated DBPs formed during chlorination are still unaccounted for. Even less is known for alternative disinfectants like chloramines and ozone, and DBPs from anthropogenic contaminants, like pharmaceuticals, are only recently being explored. DBPs from desalination are also being identified, along with new DBPs formed by impacts of hydraulic fracturing produced waters. Mass spectrometry (MS) has been a primary tool of choice for identifying these unknown compounds, with high resolution-MS particularly important for determining empirical formulas. This presentation will provide the state-of-the-science for non-target identification of new DBPs, along with information on new sources of DBPs and important new toxicological information, including potential mechanisms of toxicity.

ISTA18-009846 - PESTICIDE IMPACTS ON PACIFIC SALMON – A COMMERCIAL, ECOLOGICAL, AND CULTURAL RESOURCE IN WESTERN NORTH AMERICA

N L SCHOLZI

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Modern agricultural pesticides, including herbicides, insecticides, and fungicides, pose a challenge to natural resource conservation in freshwater habitats worldwide. Spray drift, surface runoff, and similar transport processes deliver complex mixtures of agrochemicals to streams, lakes, and rivers that support indigenous, recreational, and commercial fisheries. Moreover, in the United States, intensive monitoring by the U.S. Geological Service and other agencies has shown that pesticides commonly occur in critical habitats for fish and other aquatic species that are listed for protection under the U.S. Endangered Species Act. These widespread chemical detections have raised concerns over whether pesticides are limiting the recovery of some endangered species. In response, the National Academies of Science recently reviewed the state of the underlying science, culminating in the 2013 report “Assessing Risks to Endangered and Threatened Species from Pesticides”. Over a similar timeframe, Brazil has surpassed the United States and other countries as the world’s largest consumer of pesticides. This increase reflects an expanding use of agrochemicals on sugarcane, corn, cotton, soybeans, rice, fruits, and many other crops. As a case example, this presentation will review NOAA research on pesticide threats to migratory Pacific salmon and their habitats in western North America, with an emphasis on anticholinesterase insecticides – e.g., organophosphates and carbamates. Key information gaps will be considered, including sublethal toxicity, the combinatorial impacts of pesticide mixtures, interactions between chemical and non-chemical stressors, and biological scaling from individual-based effects to wild populations. New research to reduce these types of uncertainty is needed to more accurately assess ecological risks to aquatic resources in the U.S., Brazil, or elsewhere in the world.

ISTA18-003840 - SOLUTIONS FOR PRESENT AND FUTURE EMERGING POLLUTANTS IN LAND AND WATER RESOURCES MANAGEMENT

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Water is a vital resource for natural ecosystems and human life, and therefore, as stated by the European Union (EU) Water Framework Directive (WFD), a “heritage which must be protected, defended and treated as such”. However, the achievement of the good ecological status required by WFD is still a challenge in many European surface waters. Emerging pollutants such as pharmaceuticals, biocides, personal care products and many others are hypothesised to play a role for water quality and risks for ecosystems and human health. The 5-year Collaborative Project SOLUTIONS addresses complex mixtures of potentially toxic compounds in the water cycle including legacy, presently used and future chemicals with monitoring-, modelling- and scenario-based approaches focusing on the assessment and prediction of the impact of these mixtures, on the identification of drivers of mixture toxicity focusing on deriving River Basin Specific Pollutants (RBSPs) and on the identification of priority mixtures. Priority mixtures with respect to specific targets following different criteria are used to establish chemical fingerprints based on common sources, modes of action, fate etc.. The impact of mixtures in the environment is addressed by the development and demonstration of a set of effect-based tools following the philosophy of adverse outcome pathways from key events on a molecular level via cellular and organism responses up to the community. These tools are evaluated and demonstrated in large scale case studies such as Rivers Danube, Rhine and Ebro as well as in small streams. Results from the River Danube indicate low to moderate effects on a large range of endpoints, most of them explained only to a minor extent by target analytes. More severe effects and higher contamination can be observed in smaller streams with stronger impact of municipal and industrial wastewater as well as from agriculture. Prospective assessment using an integrated system of models and databases provides extensive predictions of concentrations and risks in the Danube river. First evaluations for individual chemicals such as PFOS and PFOA indicate good agreement between monitoring and modelling. Both approaches together are used to propose RBSPs.

ORAL PLATFORM PRESENTATION

ISTA18-329846 - A COMPREHENSIVE REVIEW OF THE EVIDENCE ABOUT HIGH NATURAL BACKGROUND RADIOACTIVITY AS AN ENVIRONMENTAL HEALTH CONCERN IN THE BRAZILIAN NORTHEAST THROUGHOUT MUTAGENIC TESTS USING DIFFERENT EXPERIMENTAL MODELS.

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One of the most important reservoirs of uranium worldwide is placed in the Brazilian semi-arid. Nevertheless, the information about natural radioactivity as an environmental concern is still scarce. This work aimed to review in a comprehensive way the available information related to the environmental mutagenic potential and its association with natural background radioactivity present in the region by using micronuclei tests in a battery of different experimental models. Water, soil and air samples from five cities of Rio Grande do Norte State were analyzed to quantify the levels of radon, uranium, thorium and potassium. The ecotoxicological assessment involved the analysis of water samples using Micronuclei Test in *Oreochromis niloticus*, *Tradescantia pallida* and human lymphocytes. Moreover, the mutagenic effect on the settled population was assessed by the Micronuclei and other nuclear abnormalities frequency found in exfoliated mucosa cells. High levels of radon in soil and water samples (range 427.8 Bq/L) was found. The indoor air radon levels also covered a wide range of concentration (range: 4563.1 Bq/m³). The annual effective dose was 6.5 mSv/y with a range of 80.5 mSv/y. Sixty seven percent of the houses presented a radon level in hazardous concentrations for human exposure. Regardless the experimental model applied, a high genomic instability was observed.

The results summarized in this work revealed a clear environmental mutagenic potential due to the presence of high natural background radioactivity in the study region and the need of further epidemiological studies aimed to define the resulting impact on human health.

ISTA18-890792 - ADVANCED OXIDATION PROCESSES ON DOXYCYCLINE DEGRADATION: MONITORING OF ANTIMICROBIAL ACTIVITY AND ACUTE TOXICITY

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Contaminants of emerging concern (CEC), such as antimicrobials for veterinary use, are persistent compounds. Their presence in the environment may cause adverse reactions in human beings and contribute to the development of resistant bacteria. Doxycycline (DOX), an antimicrobial of tetracycline's class, is widely used in prophylaxis and in the treatment of diseases in animals. However, it is rare the total metabolization of this drug. The original drug and their metabolites reach the environment by feces and urine of animals. Advanced oxidation processes (AOPs) have been highly efficient in degrading CEC. This study evaluates the efficiency of photolysis (UV), peroxidation (H₂O₂), photoperoxidation (UV/H₂O₂) and ozonation (O₃) at different pH levels to degrade DOX in aqueous solution (500 µg L⁻¹). More than 99.6% of DOX degradation resulted from the AOPs (UV/H₂O₂ and ozonation in basic medium (O₃/OH⁻)). In addition, to evaluate the toxicity of the original solution and throughout the degradation time, antimicrobial activity tests were conducted using the *Escherichia coli* (Gram negative) and *Bacillus subtilis* (Gram positive) bacteria. Antimicrobial activity reduced as the drug's degradation increased in both AOPs. An acute toxicity test using the bioluminescent marine bacterium *Vibrio fischeri* (Microtox®) was also carried out. When applied the UV/H₂O₂ process, an increase in the toxicity as the high levels of degradation were achieved, suggesting that toxic byproducts were formed and/or happened synergistic effects between of compounds. When applying O₃/OH⁻ process, the opposite effect was observed, probably less toxic byproducts were formed.

Acknowledgements: FAPESP and CNPq

ISTA18-966028 - ADVERSE EFFECTS OF COCAINE BY-PRODUCT IN THE MARINE MUSSEL Perna perna: REPRODUCTION, HEALTH STATUS AND NEUROTOXICITY.

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In light of the high consume of cocaine and by-products in metropolitan coastal regions and their occurrence already reported in seawater, the potential toxicity for marine organisms is of environmental concern. Our study aimed to assess adverse effects on reproduction, health status and neurotoxicity of crack cocaine in different life stages of the marine mussel *Perna perna*. For this purpose, gametes and embryos were exposed to crack cocaine from 1 to 48 hours, and reproduction endpoints were assessed (fertilization rate and embryo-larval development). Adults were exposed for 7 days to control (water), solvent control (DMSO 0.001%) and concentrations of crack cocaine: 500 ng/L (environmental concentration), 5,000 ng/L and 50,000 ng/L. Lysosomal membrane stability (LMS) and cholinesterase activity (ChE) were determined at 48h, 96h and 168h of exposure. Concentration that affects 50% of the fertilization rate was determined ($EC_{50} = 2.95 \text{ mg/L} \pm 0.16$). For the embryolarval development test, it was determined $NOEC = 1.125 \text{ mg/L}$ and $LOEC = 2.25 \text{ mg/L}$. LMS was reduced after 96h of exposure to all crack cocaine concentrations ($p < 0.05$). Neurotoxicity was not observed in digestive glands. In gills, ChE activity was significant after 48h and 168h of exposure to 50,000 ng/L compared to the solvent control ($p < 0.05$) and to 500 ng/L compared to T0 ($p < 0.05$), respectively. After one week of exposure, ChE activity was observed in adductor muscle of organisms exposed to 50,000 ng/L compared to the control ($p < 0.05$). Our study provided new evidences on ecological risks of cocaine and its by-product for marine ecosystems.

ISTA18-115206 - ANALYTICAL METHOD AND OCCURRENCE OF 9 PESTICIDES REPRESENTATIVE FROM SUGARCANE PLANTATION IN SURFACE WATER OF THE STATE OF SÃO PAULO, BRAZIL

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São Paulo State is the biggest sugarcane producer in Brazil and the second at pesticide consumption. The aim of this project was to develop a method to determine the presence of 9 pesticides, 7 herbicides (simazine, atrazine, ametryn, clomazone, diuron, hexazinone and tebuthiuron) and 2 insecticides (carbofuran and imidacloprid) in surface water using liquid chromatography tandem-mass spectrometry and solid phase extraction as sample preparation. Limits of detection (LOD) and quantification (LOQ) were ranged from 1.5 to 2.1 ng L⁻¹ and from 4.6 to 6.5 ng L⁻¹, respectively, and mean recovery was 66%, which allowed obtaining a sensitive and accurate method for the determination in trace levels. Eight rivers (Jacaré-Guaçu, Do Ouro, Córrego Rico, Mogi-Guaçu, São Domingos, Turvo, Pardo and Sapucaí) located in the main sugarcane area from São Paulo were sampled five times, between October/2015 to October/2016, totaling 38 analyzed samples. The most frequently detected pesticides were diuron (100%), tebuthiuron (95%), hexazinone (95%), ametryn (76%) and imidacloprid (76%). The pesticide that presented the highest concentrations were tebuthiuron, reaching 241 ng L⁻¹. The risk to aquatic life was evaluated dividing the concentration range of each pesticide by the lowest predicted no-effect concentration (PNEC) retrieved from peer-reviewed literature. Although, with a small data set, a potential risk for aquatic life was observed for ametryn, atrazine, diuron, hexazinone and imidacloprid

ISTA18-459613 - ASSESSMENT OF MULTIGENERATIONAL DELTAMETHRIN EXPOSURE EFFECTS ON DAPHNIA POPULATION WITH DATA MINING TOOLS

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Pesticides and fungicide widely used in modern agriculture may reach surface waters at concentrations possibly hazardous to aquatic communities. This study focus on long-term toxic effects of deltamethrin on daphnia magna population. For this purpose, three generations of daphnia magna were exposed at five different concentrations of deltamethrin during 21 days. We used random forest and neural network method to produce a comparative predictive analysis of growth and survival data collected of our experiment. Our analysis revealed significant deltamethrin effects on daphnia survival from the second generation, when exposure concentrations exceeded 10 ng / L. Daphnia growth was also affected from the second generation. Thus our analysis allowed estimating time at which the process of bioaccumulation prevailed of deltamethrin elimination. Furthermore, this analysis can be extrapolated for long-term population dynamics in contaminated environment.

ISTA18-834177 - CHAROPHYTE CELL TOXICITY RESPONSE TO NCUO AND CUSO4: DOES IT DEPEND ON AMBIENT CALCIUM?

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Metal nanoparticles (NPs) may interact with the cell directly or induce toxicity through the release of metal ions. Algae cells are surrounded by the cellulose cell wall that serves as a mechanical barrier and ion exchange phase between cell membrane surface and external solution. The carboxyl groups of polyuronic acids are the main binding sites of calcium and other cations. Recently, it has been found that charophyte cell of *Nitellopsis obtusa* exposed to 100 mg/L nCuO suspension or 3.2 mg Cu/L CuSO₄ accumulates major part of Cu in the cell wall, at least, 63 % or 74 %, respectively, of the Cu measured in the whole cell. Also, the presence of CuO NPs in the cell wall has been visualized by scanning electron microscope images as well as confirmed by energy dispersive X-ray spectrum data. In the SEM images, the deposits of insoluble CaCO₃ and nCuO aggregates are clearly seen on the surface of the charophyte cell. Since the calcification of algal cells depends on hardness of aquatic media, the modification of external medium in model experiments may shed lights on the behaviour of nCuO in natural waters. In this report, we present and discuss the data on possible influence of exogenous/cell wall-bound calcium on susceptibility of *N. obtusa* cells to different chemical forms of Cu.

ISTA18-923972 - COLOR YES, CANCER NO

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Although end-use properties of synthetic dyes are critical to their commercial success, the need to demonstrate safety to human health and the environment is an important requirement in the commercial use of technically viable organic dyes and their auxiliary chemicals. With regard to dyes for textiles, safety is often demonstrated by conducting a battery of tests designed to assess genotoxicity (especially mutagenicity and carcinogenicity) and ecotoxicity. The genotoxicity of dyes for textile and related applications came to the forefront when it was found that well-known azo dye precursors such as benzidine and beta-naphthylamine were human carcinogens. This outcome led to extensive testing of azo dyes and their aromatic amine precursors for mutagenic and/or carcinogenic potential. While those studies were aimed mainly at risk assessment and product regulation, the utility of the resultant data for the design of alternatives to environmentally unfriendly compounds was also examined.

The present paper provides an overview of studies pertaining to the environmental chemistry of azo dyes, by far the major class of textile dyes. Specifically, a summary of studies conducted largely in our laboratory since the inception of the dye chemistry program more than 35 years ago is presented. Covered are studies pertaining to the design of textile dyes that pose low risk to human health and the environment, with emphasis on identifying potential alternatives to dyes and dye intermediates that raised genotoxicity or aquatic toxicity concerns. A comparison with natural dyes is also presented.

ISTA18-147091 - DESIGN OF SUSTAINABLE PERMANENT COLORANTS FOR HAIR

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Hair coloration is a prevalent practice among individuals dissatisfied with the natural, and in many cases, graying appearance of their hair. Dominating the global market are permanent (long-lasting) hair dyes, desired for their superior resistance to removal by multiple shampoos. Permanent hair coloration involves the oxidation of PPD-based precursors in the presence of couplers like resorcinol. Produced within the hair fiber are oligomers that become too large and too hydrophobic to be easily desorbed. Despite toxicity concerns with certain precursors and couplers required for coloration, permanent hair dyes have employed the same concept for decades. The present study pertains to the design of environmentally-benign alternatives that can compete with the economy and efficacy of conventional permanent hair dyes. In this regard, two types of colorants were explored as potential alternatives. The first type were super-hydrophobic acid dyes and are of commercial importance for applications requiring excellent wash resistance for wool. Six dyes with varying alkyl chain length (C4 - C12) belonging to this family were synthesized, and four dyes of the second type which contain nonionic sulfonamide groups and ligands that facilitate dimerization through metal-complexation, were synthesized. Dye purity was confirmed by chromatographic analysis, and both dye types were found to have affinity towards human hair keratin fibers and films.

ISTA18-132352 - ECOTOXICOLOGICAL IMPACT OF RE-MOBILIZED SEDIMENTS AND FLOOD EVENTS FOR LOCK REGULATED RIVERS AND WETLANDS

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In recent years, sediments have become a central topic of scientific and public discussion as an important factor for determining water quality. While the quality of surface waters in Germany has significantly improved during the past years, highly contaminated sediments still create a considerable threat to the quality of several European catchment areas. For several European river basins, including Neckar, Rhine and Elbe, highly contaminated old sediments can be described as “potential chemical time bombs”. An important process which may remobilize such sediments and which is still of increasing importance in relationship to the global climate change is more often occurrence of stronger floods in Europe as well as in other parts of the world. To understand and predict possible toxicological and ecotoxicological consequences of contaminants mobilized from sediments by flood events it is necessary to develop scientific approaches for the assessment of regularly flooded rivers. The combination of hydrodynamics and ecotoxicological investigations is devolving to an emerging field of research. Recently, it was shown that hydrodynamic aspects can be involved as additional line-of-evidence in Weight-of-evidence studies assessing the impact of sediments. In the last decade several studies were published addressing the ecotoxicological impact of flood events or using combined approaches for evaluating flood events and the risk of erosion. Here, we summarize different concepts and case studies for the assessment of sediment quality and report on some novel integrative test methods for assessing sediment toxicity including contaminant re-mobilization during simulated re-suspension

ISTA18-176750 - ENVIRONMENTAL BENZO[A]PYRENE IS A TRANSGENERATIONAL SKELETAL TOXICANT – A NOVEL DISCOVERY USING THE MEDAKA BONE MODEL

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Benzo[a]pyrene (BaP) is ubiquitous in the environment and found in high concentrations in cigarette smoke. Ancestral exposure of medaka to environmentally relevant levels of BaP resulted in the increased occurrence of dorsal-ventral compressed vertebra segments in the unexposed F3 larvae. Such impairment is persistent to adulthood, leading to decreased bone thickness in F3 male. Histopathological analyses revealed changes in osteoblast abundance (larvae) and activity (adult male), which were further corroborated by the deregulation of key osteoblast genes expression. Attempt was also made to link the antagonistic expression of conserved bone miRNAs with their target bone gene expression in F3 vertebrae. Five functional pairs of mRNA/miRNA were identified (Osx/miR-214, Col2a1b/miR-29b, Runx2/miR-204, Sox9b/miR-199a-3p, APC/miR-27b), which are potentially responsible for the deregulation of osteoblast differentiation and activity in F3 offspring. Significant changes in the cellular levels of histones (H3K4me2, H3K27me2) and global DNA methylation patterns were detected in F3 larvae (BaP vs CTL). The findings support the potential of epigenetic mechanism(s) mediating BaP-induced transgenerational toxicity. From an ecotoxicological perspective, abnormal skeletal integrity renders the fish more susceptible to bone fractures and exerts a far-reaching impact on individual fitness and survival. Re-assessment of the ecological risk of BaP is urgently needed. Moreover, the mechanisms of bone formation and metabolism are conserved between medaka and mammals, the results of medaka may shed light on the potential transgenerational effect of BaP on skeletal disorders in mammals.

ISTA18-485402 - GAMMARUS FOSSARUM AS A SENSITIVE TOOL TO REVEAL RESIDUAL TOXICITY OF TREATED WASTEWATER EFFLUENTS

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Wastewater treatment plant effluents have to be non-toxic to the aquatic environment, therefore not only chemical abatement but also ecotoxicological evaluation through relevant bioassays are required. Standardized bioassays currently used are often not sensitive enough to reveal a residual toxicity in treated effluents. Therefore, attention must be paid to the development of well-adapted approaches implementing sensitive organisms and relevant endpoints. In this study, the toxicity of two differently treated effluents towards the ecologically relevant species *Gammarus fossarum*, was evaluated. Organism fitness traits such as reproduction and sperm DNA integrity were followed in exposed organisms. In complement, enzymatic biomarkers were measured to indicate the presence of neurotoxic compounds (acetylcholinesterase activity), the presence of pathogens likely to increase the toxic effects of chemical compounds (phenol-oxidase activity), and the presence of toxic compounds inducing detoxification mechanisms (glutathione-S-transferase activity). After an in situ 2-week exposure to each treated effluent, enzymatic activities were not modified, but females showed a retarded molt cycle, a reduced fecundity and fertility, and more than 90% of developed embryos exhibited developmental malformations. In addition, a slight but significant genotoxic effect was measured in gammarid sperm. The assessment of reproduction impairment and genotoxicity in *Gammarus fossarum* seems to be a valuable and sensitive tool to reveal residual toxicity in effluents containing a mixture of micropollutants at very low concentrations.

ISTA18-047752 - IN VITRO FUNCTION OF THE ARYL HYDROCARBON RECEPTOR PREDICTS IN VIVO SENSITIVITY TO DIOXINS AMONG ALL OVIPAROUS VERTEBRATESM HECKER¹; J DOERING¹¹University of Saskatchewan

Adverse effects of exposure to dioxin-like compounds (DLCs) in vertebrates are primarily driven by activation of the aryl hydrocarbon receptor (AHR). However, mechanisms for the great differences in sensitivity to these effects among species of fish were unknown. Therefore, this study 1) investigated sensitivities to activation by the model DLC, 2,3,7,8-TCDD, of AHR1s and AHR2s among seven species of fish known to greatly differ in sensitivity to TCDD, and 2) characterized the relationship between in vitro sensitivity to activation of AHRs and in vivo sensitivity of embryos to TCDD. All AHR1s and AHR2s were activated in a concentration-dependent manner by exposure to TCDD. There was no significant linear relationship between EC50 of AHR1 and LD50 of embryos. However, a highly significant positive linear relationship ($R^2 = 0.96$) was observed between EC50s of AHR2s and LD50s of embryos. The slope and y-intercept for this linear relationship for AHR2 of fishes is not statistically different from those of the previously determined significant linear relationship among EC50 of AHR1 and LD50 of embryos of birds to TCDD. Results of this study suggest that sensitivity to activation of AHR2, but not AHR1, mediates adverse effects of and sensitivity to TCDD among phylogenetically diverse species of fish, which is comparable to that previously demonstrated for AHR1 of birds, resulted in a single equation for predicting sensitivity to TCDD across species of oviparous vertebrates. This biological model has the potential to guide more objective ecological risk assessment of DLCs for species of fish that are not easily studied, including threatened or endangered species.

ISTA18-400696 - INVERTEBRATE IMMUNITY: POTENTIAL USES IN UNDERSTANDING TOXICANT EFFECTS IN NOVEL MODELS

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Many toxicants have immuno- modulatory effects which threaten the survival of an individual through impairment of host defences. While immunity is well-understood in many vertebrate models and some commercially important species, such as shrimp and oysters, little is known of immunity in many ecologically important species commonly exposed to environmental stressors. While invertebrates lack antibody-mediated immunity and the long-term memory component of the vertebrate adaptive immune repertoire, they possess potent and diverse immune defences. Given that different taxa can vary widely in which immune parameters may be dominant in defence strategies, it is important to understand their 'baseline' processes when considering how xenobiotics may impact on this. The amphipod crustacean *Parhyale* is a widely used model organism which has great potential to be used for research in environmental immunotoxicology. Aspects of the crustacean immune system, such as the recent demonstration of extracellular chromatin traps from the haemocytes, are highly probably to occur in the animals' response to xenobiotics such as microplastics. Here we review the immune cells and proteins of the crustacean immune system and suggest how these could be assessed in *Parhyale* to provide vital information in furthering the use of this amphipod as a model organism for judging environmental impacts.

ISTA18-688271 - INVESTIGATION OF TOXICANT-PARTICLE SORPTION-DESORPTION PROCESSES BY ASSESSMENT TOXICANT BIOAVAILABILITY IN THE AQUEOUS PHASE

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Toxicants are frequently associated with natural and/or anthropogenic particles and these complex associations influence toxicant bioavailability, organism exposure, and toxicity. Sorption-desorption of toxicants with particulates depends largely on the physicochemistry of the particles and toxicants, and these processes are difficult to investigate because methods of analysis can disrupt particle-toxicant interactions. Our objective was to use changes in zebrafish gene expression to evaluate toxicant bioavailability and thereby inform on sorption-desorption processes of toxicants with particles in the aqueous phase. Aqueous dispersions of TiO₂-nanoparticles (NPs) were prepared, and toxicants (Hg²⁺, Cu²⁺, anthracene, benzo(a)pyrene) with different physicochemistry were allowed sorb to the NPs under various conditions. Expression profiles of gene transcripts responsive to each toxicant [metallothionein (mt2), Hg²⁺, Cu²⁺; and cytochrome P4501A (cyp1a), anthracene, benzo(a)pyrene] were determined according to toxicant concentration, and expression of these genes in exposed zebrafish (age < 120 hours postfertilization) used as analytical tools to assess changes in toxicant bioavailability during sorption-desorption processes with NPs. Results indicate that toxicant physicochemistry, NP type, size, surface area, and crystalline structure can influence aqueous-phase sorption-desorption processes. Understanding sorption desorption processes of toxicants with particles is important for risk assessment and use of toxicant bioavailability measurements can provide unique information on these processes in the environmentally relevant aqueous phase.

ISTA18-650068 - NANOTOXICOLOGY OF CARBON NANOTUBES IN VIVO (MICE C57BL/6N) UNDER NANOREG-REGULATORY PROTOCOL (EUROPEAN COMMUNITY-BRAZIL AGREEMENT)

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The objective of this project was to evaluate the histopathological effects of all samples of carbon nanotubes on the mice: Printex 90 (carbon black), and pristine NCTs: MN-400, MN-401, MN-402, MN-403, NRCWE-006; NRCWE-040, NRCWE-046; functionalized CNTs: NRCWE-041, NRCWE-047, NRCWE -042 and NRCWE-048 (54 µg/ animal/ instillation) divided on 3 big groups which were applied by intratracheal instillation. The response on kidneys of mice C57BL/6N Tac was analysed between 3-90 days instillation. The results show that in standard group, the worse inflammation response was at MN-402 with 100% and 33% of sections presented precipitated of nanotubes, hemosiderin and glomerular atrophy as also in MN-400 (80% and 80%, respectively). On the other hand at MN-401, showed levels of inflammation, hemosiderin (the same of Printex 90 Control group). In the Group I the response were with 77% of inflammation in NRCWE-040. Best results were observed in the samples for the group II in NRCWE-047 where the worse inflammation and hemosiderin response in both was 57%. In general, considering the chemical physical parameters of the nanostructures can be said that the data with carbon black, which is a positive example toxic effect on amines, clearly shows that high level of inflammation would be a positive effect of carbon nanomaterials. Additionally, it is noted that occurs nanotoxicity of the order of CNT- Pristine> CNT-OH> CNT-COOH. A parameter that could be important is the length (by TEM) and sizes (Z average) and apparently not dependent on the diameter of the CNTs. The smallest BET apparently was more toxic in NCT-Pristine. Acknowledgments: CNPq/MCTI/INMETRO-NANOBIOS- INOMAT.

ISTA18-360825 - NEXT STEPS FOR REDUCING ANIMAL USE IN THE AGROCHEMICAL INDUSTRY

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Recent successes in the 3Rs, for example removal of the redundant 1-year dog study from Canadian and draft Brazilian legislation for agrochemicals, have been long journeys but illustrate that analysis of available databases for chemicals can be very valuable for informing global policy changes. Agrochemicals remain the most highly regulated industry when considering human health assessment, which unfortunately means large numbers of animals are often used in the required toxicology testing programmes. Which studies are ripe as the next opportunities to give 3R benefits? Which tests are the “low-hanging fruits”? In this presentation, three case studies will be shared as proposals for the next obvious choices for refining the testing requirements for agrochemicals. Firstly, replacement of in vivo acute toxicity tests with in vitro alternatives will be discussed. Secondly, the opportunity to integrate multiple endpoints into single studies, which can give significant 3Rs benefits, will be presented. Finally, approaches to impact the 3Rs through incorporating sound science (for example, toxicokinetic measurements and human exposure-based toxicity testing) will be discussed. Through cross-sector and cross-industry collaborations, it should be possible to take great strides in the march towards animal-free safety assessment. Importantly, global harmonisation will be a key factor in this journey.

ISTA18-975779 - PREPARATION OF GRAPHENE OXIDE DERIVATIVES AND ITS APPLICATION FOR REMOVAL CONTAMINANTS IN WATERP. PREDIGER¹, T. CHEMINSKI¹, T. F. NEVES¹, W. B. NUNES¹, L. R. SABINO¹ AND C. R. D. CORREIA²¹School of Technology, University of Campinas-UNICAMP, Limeira, SP, 13484-332, Brazil (patriciap@ft.unicamp.br) #
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This research deals with the synthesis of graphene oxide (GO), reduced graphene oxide (rGO) and derivatives containing immobilized surfactants and their application in contaminants removal from water. These nanomaterials were evaluated in batch experiments as adsorbent to surfactants Triton X-100® (TX100) and benzalkonium chloride (BZC), and dyes, as phenolphthalein and acridine yellow (AY). The GO, rGO, GO containing polyethyleneglycol (PEG) and tetradecyl trimethylammonium salt (TTA) were synthesized and fully characterized. In order to optimize the TX-100® removal process, the influence of several key parameters was investigated, including contact time under different solid/liquid ratios, ultrasound assistance, pH and temperature. Under the optimized conditions, the maximum removal capacity (MRC) of TX-100® by GO, GO-PEG and rGO were 1.200 mg/g; 1160 mg/g, 1600 mg/g, respectively. The obtained results for TX-100® are higher than those found in others studies, reaching 100% removal for rGO at high concentration of TX-100® (400 mg/L). Up to now, BZC surfactant and dyes adsorption process are not under optimized conditions. Initial data regarding BZC removal revealed the MRC of 430 mg/g; 410 mg/g 320 mg/g by GO, rGO and GO-TTA, respectively. Phenolphthalein adsorption showed the MRC around 110 mg/g for rGO and GO-TTA; and 66 mg/g for GO. Whereas acridine yellow was fully adsorbed, reaching 230 mg/g of MRC for GO and rGO. The very high removal capacities observed at neutral pH indicate that GO and its derivatives are potential filtering agents to remove contaminants in environmental samples. Acknowledgements: FAPESP (2015/07033-7); CNPQ, FAEPEX.

ISTA18-560939 - PRODUCTION AND CHARACTERIZATION OF FLAVONOID-LOADED NANOSTRUCTURED LIPID CARRIER: EVALUATION OF ITS ACTIVITY AGAINST SKIN CANCERA F COSTA¹, P A LIMA¹, D STANISIC², S B DOS REIS³, M B DE JESUS³, L TASIC², N DURÁN^{1 4}¹Institute of Chemistry, Department of Physical Chemistry, State University of Campinas, Campinas, SP, Brazil # ²Institute of Chemistry, Department of Organic Chemistry, State University of Campinas, Campinas, SP, Brazil # ³Institute of Biology, Department of Biochemistry and Tissue Biology, State University of Campinas, Campinas, SP, Brazil # ⁴NanoBioss Laboratory, Institute of Chemistry, University of Campinas, Campinas, SP, Brazil

The skin cancer represents around 25% of the new cases of cancer in Brazil, where melanoma is the most dangerous type of the disease due to its high mortality index. Melanoma cells can suffer metastasis and quickly spread to other organs of the body. Occasionally, drugs and treatments used for cancer therapy have too severe side-effects such as vomit, nausea and loss of hair, making cancer treatment more difficult and painful. Nowadays, some researches in cancer have shown that flavonoids could be a new strategy as active compound or complementary therapy. Flavonoids are known to be antioxidant, anti-inflammatory and antiproliferative; however some of them are poorly water soluble and low biocompatible. In order to overcome these problems, this research aims to produce nanostructured lipid carriers (NLC - nanoparticles made of a mix of solid and liquid lipids) loaded with flavonoid for topical application in skin cancer treatment. Therefore, combining the use of a natural flavonoid with the characteristics of NLC it's expected to have a powerful drug delivery system, which could increase the action of flavonoid against cancer cells for skin cancer therapy. The experimental stability of the NLC (time and temperature) is very good. The measures from DLS showed that the average of size was around 200 nm and zeta potential from -25 to -35 mV. The characterization of morphology, in vitro release of the drug, skin permeation, encapsulation efficiency and encapsulation loading have shown promising results. Finally, the in vitro cytotoxicity and cell uptake studies were done with human melanoma cell lines and are good previous tests before in vivo experiments.

ISTA18-971496 - RADON THE INVISIBLE ENEMY

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Radon has been an element of concern for many years. This gas can accumulate indoors depending on the type of house and its location. Radon as a radioactive element is not in and of itself that dangerous. However, the gas decays to radioactive elements polonium and lead which are both metals. Thus, when radon is inhaled some of it is transformed to radioactive metals that remain in the lungs producing radioactive alpha and beta particles. The exposure of people to radon has been correlated with lung cancer in many studies.

This talk will discuss radon and potential high concentration of indoor radon in measurements in Texas. There has also been some indoor radon measurements in Brasil that will be discussed.

ISTA18-956502 - SOIL ENVIRONMENTAL GENOTOXICITYS LEMIERE¹ AND P VASSEUR²

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It is now established that risk assessment and management strategies for contaminated environments should consider biological responses, including environmental genotoxicity. The characterization of terrestrial ecosystem contamination by genotoxicants requires to assess their environmental and toxicological bioavailabilities and to identify adverse effects at the individual and population levels.

Genotoxicity in field species is assessed by means of markers focusing on genomic alterations and DNA damage, like DNA adducts (less used lately), micronuclei and nuclear abnormalities and DNA breaks. Epigenetic changes are not investigated so far. The comet assay (with the use or not of endonucleases) has nowadays largely supplanted pre-existing techniques for DNA damage assessment. All these tools/techniques have first been used, optimized and validated with aquatic species. Their application to soil species has emerged in a second step and is now widespread. We will present briefly some results illustrating interests of the comet assay to approach the “genotoxic bioavailability” of terrestrial contaminants and their effects on earthworms.

In both aquatic and terrestrial environments, genotoxicity is generally measured in somatic cells. In some cases, it could be related to mutations, embryo mortality or reproduction impairment. However, there is a need for works linking (1) genotoxicity in individuals and (2) population dynamics and genetics. Studies with gametes, early life stages and juveniles, and in environmentally relevant conditions should be conducted to reach such objectives.

ISTA18-137274 - THE IMPACTS OF ESTROGENIC EDCs ON FISH POPULATION SUSTAINABILITY: A DEFINITIVE NEED FOR MULTIGENERATIONAL RISK ASSESSMENT

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Increasing evidence suggests that estrogenic EDCs (EEDCs) induce multigenerational impacts on fish reproduction and survival of offspring. While the direct impact of EEDCs on fish reproduction has been thoroughly investigated, a concomitant assessment of both reproduction and immune impairments is lacking, particularly on a multigenerational level. To enable comprehensive re-assessment of EEDCs risk, a holistic approach was undertaken to assess the impacts of 17 α -Ethinylestradiol (EE2) on reproduction and immune function of adult *Oryzias melastigma* (F0) and their offspring (F1-F4), using three different environmentally relevant exposure scenarios. Reproductive fitness of fish was evaluated by an array of phenotypic endpoints including: fecundity, fertilization rate, hatching success and sex ratio. Immune competence was assessed through host-resistance assays against bacteria challenge and monitoring overall mortality. To elucidate potential multigenerational impacts of EE2, the same battery of reproductive and immune endpoints was employed for F1-F4 progeny. Our results indicate that EE2 can reduce reproductive success and immune competence of the directly exposed F0 parents, as well as reproductive success of F3-F4 offspring, which had never been exposed to EE2. Currently, the fish are being assessed at the tissue, cellular, genetic, and epigenetic levels to elucidate the mechanism(s) through which these transgenerational impacts transpire. The results highlight the importance of the combined assessment of reproductive and immune competence for EEDCs, and further support the need for taking a multigenerational approach for proper risk assessment of the impacts of EEDCs.

ISTA18-662133 - THE TOXICITY OF SILVER NANOPARTICLES (AGNPS) TO THREE FRESHWATER INVERTEBRATES: HYDRA VULGARIS, DAPHNIA CARINATA AND PARATYA AUSTRALIENSIS

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The toxicity of manufactured nanoparticles varies greatly depending on the test species in consideration. For a better toxicity, assessment species in different trophic levels or species with different life strategies should be considered. In this study, we examined whether organic (Tyrosine)-coated silver nanoparticles (Ty-AgNP) were toxic to three Australian freshwater invertebrates: *Hydra vulgaris*, *Daphnia carinata* and *Paratya australiensis*, representatives of three different life strategies. Additionally, their sensitivity to Ty-AgNPs was compared to ionic silver (Ag⁺). Our results show that, *D. carinata* is 7 times more sensitive to Ty-AgNP than *H. vulgaris* when 48-h LC50 values are compared. When assessing the effects of Ag⁺ this difference increased to 40 times.

When comparing *Daphnia* with shrimps, the 96-h LC50 for *P. australiensis* was higher than 48-h LC50 of *D. carinata* indicating that *D. carinata* is more sensitive. Similarly, *D. carinata* was 5 times more sensitive than *P. australiensis* after 24-h exposure to Ag⁺. Based on the LC50 values of both Ty-AgNP and Ag⁺, *D. carinata* was the most sensitive species followed by *P. australiensis* and *H. vulgaris* though the latter is in a much lower taxonomic grouping, has a permeable body and lacks an exoskeleton. Our results highlight the importance of using a multispecies approach when assessing the effects of toxic substances including manufactured nanoparticles.

ISTA18-768731 - THE USE OF ENVIRONMENTAL POLICIES IN DREDGING OF SEDIMENTS AND THE PRESENCE OF MARINE ANTIFOULINGS IN BRAZILIAN PORT AREAS.

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Marine antifouling paints were developed to avoid biofouling growth on boats hulls and underwater structures. Many different formulations are employed in these paints to achieve this goal. After the organotins ban by IMO in 2008 other biocides were employed. The use of biocides or combinations of biocides still raises concern for antifouling toxicity on non-target organisms. After the release from the coating, the biocides may to a lesser or greater extent, complex and/or partition in the marine environment. Biocide loaded suspended matter may accumulate in sediments, depending in the environmental half-lives of each biocide compound. These biocide-loaded sediments are a common characteristic of the areas where activities such as shipyards, ports and marinas, making them “hot spots” of pollution as well as revealed in numerous scientific studies all around the world. In these areas, regular dredging is in many instances required, raising the question of safety. In Brazil, the Resolution 454 of the CONAMA, in force since 2012, regulates the dredging activities. This study was aimed to evaluate the current status of antifouling pollution in coastal sediments in Brazil, considering the application of this policy, and comparing the Brazilian regulations with that is being applied in other countries. Considering that in can be easily shown that using conservative environmental parameters and the legal limits for TBT in marine sediments in Brazil, the dispersion of a single cubic meter of dredged material could make more than five thousand cubic meters of seawater reach the legal limits for dissolved TBT, the magnitude of the problem could be easily understood.

ISTA18-728812 - TOWARDS CLOSED LOOP RECYCLING OF POLYESTER FABRIC: DEVELOPMENT OF AN EFFICIENT DECOLORIZATION METHOD

XIUZHU FEI, DAVID HINKS, HAROLD S. FREEMAN

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Polyester (PET) fibers now exceed cotton in total consumption, making PET the number one fiber in the textile industry. It is a synthetic material derived from non-renewable petroleum sources. Disposing PET textiles in land field sites causes a potential environmental problem, due to their resistance to biodegradation. Further, disposal of PET following a single lifetime of use is a waste of valuable resources. With environmental stewardship in mind, an efficient approach to recycling colored PET textiles has drawn wide attention. Dye removal from colored products is a crucial step in the recycling process. Bearing in mind that disperse dyes are used to dye polyesters, this work involved the selection of a group of structurally diverse dyes to develop an efficient PET decolorization method. Dyes chosen included azo, anthraquinone, quinoline and nitro disperse dyes, and sodium formaldehyde sulfoxylate (SFS) was used as the decolorizing agent. A medium containing an organic solvent combined with water facilitated simultaneous dye extraction and decolorization. An optimized combination of treatment time (30 min), SFS concentration (10g/L), temperature (100 °C), and liquor ratio (1:50) was found effective for decolorizing disperse dyes with various chromophores. It was also found after decolorization that the organic solvent could be successfully recovered and reused, therefore significantly reducing treatment wastes. As judged by physical tests, it was found that SFS decolorization has no adverse influence on fabric strength. It was also found that the decolorized fabrics could be dyed the original or a different color, and that this process could be repeated multiple times.

ISTA18-003492 - TOX-BOX: HAZARD-BASED RISK MANAGEMENT OF ANTHROPOGENIC TRACE SUBSTANCES FOR PROTECTION OF THE DRINKING WATER SUPPLY

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Given the increasing number of drinking water relevant but toxicologically unknown anthropogenic micropollutants, the HRIV concept (health-related indicator values) is used for their regulation. So far, the HRIV concept is rather theoretical and defines biological end points like genotoxicity, neurotoxicity, and subchronic and chronic toxicity, but it does not devise the experimental approach to obtain toxicological data. The aim of the joint research project “Tox-Box” was therefore, to identify and establish in vitro assays that allow a fast and reliable toxicological evaluation of known and emerging trace substances. It is part of the funding scheme “Risk Management of Emerging Compounds and Pathogens in the Water Cycle” (RiSKWa). A guideline for defining in vitro test strategies to assess the hazard potential for the relevant endpoints genotoxicity and neurotoxicity as well as endocrine effects was developed and health-oriented indicator values were derived. To assess the relevance for humans, metabolism-competent cell lines were used that have proven to be highly specific regarding the human metabolism or S9 was added for the metabolic activation of substances. An extension of the HRIV concept by experimental modules allows for a timely collection of data that increase the scientific basis for deriving the HRIVs. With the extended HRIV concept, the substances are prioritized and further actions regarding the toxicological assessment are defined. Sponsored by the Federal Ministry of Education and Research (BMBF) Support Code: 02WRS1271

ISTA18-164186 - TOXICITY OF CHLORINATED NATURAL DYES TO DAPHNIA SIMILIS

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In the past few years, there has been increased interest in the use of natural dyes for textile coloration as alternatives to synthetic ones that are toxic or mutagenic to a variety of organisms. Among the natural dyes, Madder (CI Natural Red 8), Cochineal (CI Natural Red 4), Weld (Natural Yellow 2), Fustic and Logwood (Natural Black 1), have been used since ancient times to dye cotton, silk and wool. Natural dyes have poor affinity for textiles, which can lead to a high dye amount in the resultant wastewater and the need for treatment to remove color. Conventional biological treatment is inefficient for color removal and consequently dyes can remain in the environment. A chemical method such as chlorination has proven efficient and economical for wastewater decolorization. For synthetic dyes, this process can lead to the formation of toxic products. However, little information is available concerning the chlorination of wastewater containing natural dyes. The aim of this study is to decolorize these well-known dyes and evaluate their chlorinated products toxicity to *D. similis*. A solution of the 5 dyes in water was treated with chlorine gas until total decolorization, observed by UV-Vis spectrophotometry, was achieved. Dyes and the corresponding chlorinated samples were tested for acute toxicity to *Daphnia similis* (48 h) (OECD, 2004). For all dyes, except Madder, chlorination increased toxicity to *Daphnia*. For Madder, there was a 10-fold decrease in toxicity upon chlorination. The nature of the chlorination products and their individual toxicities are under investigation.

ISTA18-949862 - XENOPUS OOCYTES IN ENVIRONMENTAL TOXICOLOGY: A PROMISING TOOL?

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Amphibians stand for organisms of choice to assess toxicity. For example, the FETAX test (Frog Embryo Teratogenesis Assay – Xenopus) allows quick testing of chemicals with endpoints such as survival, growth and malformations of tadpoles. We develop complementary methods in amphibian toxicology, using *Xenopus laevis* oocytes and young tadpoles as biological models.

Indeed the xenopus oocytes present many advantages: they are easy to handle and could be obtained in large numbers. Their life cycle is well known and all the cells were synchronous. So different types of toxicity endpoints can be assessed with these gametes, regarding key moments of their cycle when conducted in contaminated environments. In a same way, contaminant exposures on fertilization and the precocious embryonic development can be performed.

We will present the different kinds of data that can be obtained from maturation and survival ratios, analysis of meiotic spindle formation, to physiological data or automatic biometric measurements on tadpoles. Several exposures have been conducted using environmental contaminants as metals (lead, cadmium) and pesticides (glyphosate, deltamethrin) and obtained results will be presented.

Analyzing early stages procured complementary information to preexisting methods. Moreover oocytes seem to be particularly impacted in case of some exposures and suggest that they could be considered as a pertinent model to assess environmental contaminants effects.

FLASH PRESENTATIONS

ISTA18-262115 - ADVERSE EFFECTS OF COCAINE BYPRODUCT IN THE REPRODUCTION OF SEA URCHIN IN AN OCEAN ACIDIFICATION SCENARIO

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Fate and effects of illicit drugs in aquatic ecosystems are also of environmental concern. In fact, the amounts of illicit drugs consumed worldwide are comparable with those of therapeutic drugs. Ocean acidification is a predictable consequence of rising atmospheric CO₂. Considering the alkaline pH of marine waters and the pK_a of illicit drugs detected in coastal zones, some compounds could have bioavailability and toxicity to the marine biota altered by pH variations. 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 toxicity of crack cocaine combined with ocean acidification. Ecotoxicity was assessed to sea urchin (*Echinometra lucunter*) fertilization rate and embryo-larval development. It was tested five different concentrations (6,25 mg.L⁻¹; 12,5 mg.L⁻¹; 25 mg.L⁻¹; 50 mg.L⁻¹ and 100 mg.L⁻¹) in four different pHs (8.5; 8.0; 7.5; 7.0). The exposure of the spermatozoid for 1h with the increasing concentrations of crack-cocaine didn't show significant difference with control sample in pHs 8.5; 8.0 and 7.5. Results of embriolarval assays showed inhibitory concentrations (IC₅₀) of crack-cocaine in 9.82 mg/L-1, 10.71 mg/L-1 and 12.37 mg/L-1 in pHs 8.5; 8.0 and 7.5 respectively. In pH 7.0 the effects observed in fertilization rate and embryo development were associated to the low pH level, since effects were found in the control sample. To the best of our knowledge, this study is the first ecotoxicological assessment of an illicit drug in different ocean acidification scenarios.

ISTA18-342360 - AQUATIC RISK ASSESSMENT SCHEME FOR PESTICIDES IN BRAZIL

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Since 2010 the Brazilian Institute for the Environment and Renewable Natural Resources – Ibama have been working to implement the risk evaluation of pesticides. Thus, in 2015 a working group (government, academia and industry) was created to discuss and develop a clear and scientifically-based scheme of aquatic risk evaluation for pesticides in Brazil. The first discussions were about the definition of a General Protection Goal (GPG) that would guide us in the whole process. For this, Brazilian legislation about protection of aquatic environment was surveyed and the GPG was defined: “to protect the water body to guarantee the aquatic ecosystem sustainability and also to maintain the aquatic organisms, the water supply for the animals and the economical, recreational and subsistence activities”. An effort has been done to establish Specific Protection Goals (SPGs) and to define which ecotoxicological tests will be required in each phase of the risk assessment to reach these goals. In addition, the working group is starting discussions on safety factors to be used in initial phases of this evaluation gathering results of acute tests conducted with native species exposed to registered pesticides - which will be compared with results of the same tests conducted with standard species -, for calculating a ratio that helps us to decide on safety factors. Moreover, the group is constructing vulnerability maps of surface and groundwater using geospatialized information, such as: slope, soil organic carbon, clay, sand and run off. This discussion is still ongoing and future actions include the validation of these maps and the definition of exposure scenarios.

ISTA18-177336 - BIOLOGICAL EFFECTS OF OCEAN ACIDIFICATION AND SEDIMENT TOXICITY ON THE HEALTH OF MYTELLA CHARRUANA

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In coastal ecosystems, sediments act as a sink for contaminants, such as metals. However, acidification processes caused by CO₂ leakage associated with Carbon Capture and Storage activities may enhance the mobility of metals from the sediments to the water column resulting in toxic effects on marine biota. Considering the relevance of this topic, there is an urgent need to delineate risks and potential synergy between pollution and other environmental stressors such as acidification. This study aims to investigate the biological effects on mussels caused by CO₂ induced acidification and sediment toxicity. Experiments involving direct release of CO₂ through marine sediment was conducted using *Mytella charruana*. Organisms were exposed to contaminated and non-contaminated sediments during 14 days at different pH, ranging from 8.1 to 6.0. The end points measured were cytotoxicity and mortality. The Neutral Red Retention test shows an average improvement of 24% in general health status of this organisms at the non-contaminated site. This result could indicate a phenotypic plasticity to the physiological stress of acidification. On the other hand, the contaminated sediment assay shows an average of 22% drop in the retention time, thus reflecting a progressive reduction in the health of organisms. Mortality data reveals that the lowest pH analyzed (6.0), presented the high mortality in both experiments, 47% at non-polluted in contrast to 76% at the polluted site. Therefore, results have demonstrated a consistent pattern of lethal and sublethal effects that corroborate with the critical current environmental harm caused by increasing sediment toxicity and acidification processes.

ISTA18-386259 - CHRONIC TOXICITY ASSESSMENT OF FLUOXETINE USING ZEBRAFISH: A LINK BETWEEN GROWTH, FEEDING AND BIOCHEMICAL EFFECTS

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Pharmaceuticals are considered emerging pollutants being found worldwide in rivers, lakes, and estuaries in concentrations raging from ng to µg per liter. Fluoxetine (FLX) is among the top 100 drugs prescribed annually worldwide. This selective serotonin reuptake inhibitor is highly detected in environmental samples and has the potential to modulate levels of serotonin, a neurotransmitter that regulates many important physiological and behavioural processes in vertebrates and invertebrates. Despite that fact, ecotoxicological effects of this psychiatric drug still poorly studied. The present study aims to evaluate the effects of chronic exposure to FLX on fish. Zebrafish adults were exposed to FLX during 30 days following the OECD protocol (no. 215). The assays were performed in triplicate with six treatments of FLX, 0; 0.01; 0.1; 1; 10; 100 µg/L. A total of 60 fish per treatment (20 per replicate) were used. At the 15th and 30th days of exposure were analysed growth, behavior (feeding and swimming), and acetylcholinesterase in muscle and head tissues (only at 30th day of exposure). Our results showed severe effect of FLX at the highest concentration, namely a decrease in feeding activity (first strike time, 15th and 30th day $p < 0.001$) followed by a decrease in the total feeding time and growth ($p < 0.001$). Additionally, chronic exposure affects acetylcholinesterase activity at lower doses. In summary, the present study demonstrated that chronic exposure of zebrafish to FLX can affect the feeding behaviour, growth and neurological markers suggesting a potential ecological risk for fish population.

ISTA18-379986 - CLASSIFICATION & LABELLING OF HUMAN HEALTH AND ENVIRONMENTAL HAZARDS FOR CHEMICALS: KEEPING A GLOBAL PERSPECTIVE

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Introduction: There are different classification & labeling (C&L) systems and responsible bodies for chemicals across the globe, but many jurisdictions have adopted the United Nations Globally Harmonized System (GHS), either fully, partially or with amendments.

Methods: An analysis was undertaken of the chemicals whose harmonised C&L has been agreed by the European Chemicals Agency (ECHA) Risk Assessment Committee (RAC). RAC classifications were compared to initial proposals from the dossier submitter (DS), covering >900 individual classifications. Statistical analysis was conducted. This 'base set' was compared to relevant opinions from other bodies (European Food Safety Authority (EFSA), International Agency for Research on Cancer (IARC), US Environmental Protection Agency (US EPA), & Joint FAO/WHO Meeting on Pesticide Residues (JMPR)) & conclusions were compared.

Results: Of the 'base set' of RAC conclusions (n=215), 80 had been concluded by EFSA, 27 by IARC, & 33 by JMPR. RAC agreed with the majority (79%) of the C&L proposed by EU Member State or DS. In 10% of cases, RAC proposed a more severe C&L compared to DS & less severe in 7% of cases. The highest concordance (99%) of opinions from RAC & DS was for Aquatic Acute. These conclusions were contrasted with other bodies, showing discordance in reviewer opinion, despite the same dataset; particularly when expert judgment is relied upon.

Conclusion: When considering global safety profile and hazard classifications of chemicals, greater alignment, robust & predictable classifications are fundamental for chemicals & would provide a more consistent assessment globally, providing better public protection.

ISTA18-038670 - COMPARISON OF THE REMOVAL OF BIOLOGICALLY ACTIVE COMPOUNDS BY AEROBIC AND ANAEROBIC PROCESSES

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The release of sewage into water bodies can cause adverse effects on aquatic communities because of its toxic potential. In recent years, research on micropollutants, especially endocrine disrupters (ED) in aquatic environments, has greatly increased. Many toxic substances, including most of the potentially present in the sewage, are resistant to biological degradation and therefore the treatment of sewage by conventional processes. Various DE removal efficiencies have been reported for conventional sewage treatment, but it is not known whether these differences are due to non-optimized process or to factors inherent to the process. Two sequential batch reactors were operated in parallel, one in aerobic and one anaerobic condition. The raw sewage showed an acute effect, causing mortality of the organisms within 48 hours of the *Ceriodaphnia dubia* chronic toxicity test with and 25% IC of 10.04%. In treated effluents, a reduction in toxicity was observed, with a mean removal of 78% and 69% for the aerobic and anaerobic systems, respectively. Regarding estrogenic activity, raw sewage samples presented values of 0.3 and 0.75 ng / L. The samples from the aerobic reactor did not present estrogenic activity, whereas the anaerobic reactor showed estrogenic activity removal in only one of the campaigns carried out.

ISTA18-272595 - CONTAMINATION OF SOIL AND MEDICINAL PLANT PHYLLANTHUS NIRURI LINN. WITH CADMIUM IN CERAMIC INDUSTRIAL AREA: A HEALTH AND ENVIRONMENTAL SAFETY CASE

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Phyllanthus niruri is a plant widely used due to natural extracts that possess medicinal properties. In a complex phenomenon, the specie response to high levels of heavy metals on environment, including cadmium (Cd), a potential toxin. Consequently, consuming population is affected by cadmium intake, which can result in acute or chronic toxicity. The kidney is the critical organ affected by this exposure, once it accumulates 50% of the metal storage. The first signal of cadmium-induced renal damage is the increased excretion of low-molecular weight proteins and urinary specific enzymes, which has been recognised as sensitive biomarkers of impairment on proximal tubular cell function. Permanent exposure may progress to glomerular proteinuria, causing albuminuria and decline of glomerular filtration rate, which may result in renal failure. In this sense, this study aimed to assess the contamination in the soil and medicinal plant *P. niruri* by Cd in ceramic industrial area, in Monte Carmelo, Brazil. Soil and plant (divided in root, shoot and leaves) samples were collected from contaminated monitoring site and from a rural area, utilized as a reference site, and analyzed with atomic absorption spectrometer for Cd concentrations. *P. niruri* was found to be sensitive to soil contamination by Cd attributed to ceramic industrial emissions. Results revealed that Cd bioaccumulation in roots (3.02 mg/kg) and shoots (1.35 mg/kg) was associated with significant increase ($P < 0.05$) in the concentration of active lignans compounds phyllanthin and hypophyllanthin at leaves. The high concentrations of Cd and active compounds offer risk of contamination and super dosage.

ISTA18-040942 - DETERMINATION OF POTENTIALLY TOXIC ELEMENTS IN SURFACE SOIL LOCATED ON THE MEDIANS OF THE MAIN ACCESS HIGHWAYS IN CAMPINAS CITY (SP) USING X RAY FLUORESCENCE

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The presence of metals in vehicle emissions is due to several factors, such as the composition of fuels and lubricating oils, the wear of engine components, and their use in catalytic converters. These pollutants disposed in the soil can contaminate other matrices, such as groundwater and air, or be re-integrated into the food chain, through absorption by plants and ingestion of the animals. The surface soils are the most affected, due to the depositions of pollutants through dry and humid channels. With the hypothesis that high concentrations of vehicular traffic's related elements would be detected, the main objective of this study was to monitor the concentration of potentially toxic elements (heavy metals of environmental interest) in surface soil located on the medians of the main access highways in Campinas City. To achieve these results 60 composed samples were collected per highway. Using Energy Dispersion X-Ray Fluorescence (ED-XRF), seven elements of toxic-environmental interest (V, Cr, Ni, Cu, Zn, and Pb) were detected and quantified and nine natural soil composition elements (Al, Si, Ca, Fe, Mg, K, Ti, Mn and Rb) were considered. The concentrations obtained were compared to national and international reference values, and some elements related to vehicular traffic exceeded the prevention values established by CETESB for the state of São Paulo. In order to evaluate the influence of highway distance on elementary concentrations ANOVA and Tukey statistical tests were used. The Cr, Cu and Zn elements showed a decrease in their concentrations as the collections moved away from the highway, indicating their relation with the vehicular emissions.

ISTA18-066729 - DINITROPHENYLAZO DYE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND MUTAGENICITY EVALUATION

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Dinitrophenylazo is a common class dye used in textile-dyeing factories and mutagenic compounds can be formed by their reduction and subsequent chlorination. Some of those dyes can be transformed in chlorinated compounds with a three-ring nitrogen group called phenylbenzotriazole (PBTA) already found in the environmental samples. The objective of this work is to characterize the compound formed from reduction and chlorination reactions of the dye C.I. Disperse Violet 93 (DV93) previously found in rivers in Brazil. The reductive reaction was done using metallic iron under controlled temperature. The reaction were analyzed by Thin Layer Chromatography, the target compound were developed with ninhydrin and isolated by flash chromatography in silica gel. (yield about 7%). Nuclear Magnetic Resonance (NMR) and High Resolution Mass Spectrometry analyses (HRMS) confirmed the non Cl-PBTA product. The microplate agar, MPA, a modified version of the Salmonella/microsome mutagenicity assay using YG1041 strain in dose response experiment in the presence of metabolic activation (S9) was carried out and the non Cl-PBTA product resulted less mutagenic than the original dye. The chlorination reaction of the compound is also now being conducted and further mutagenicity testing will be performed.

ISTA18-668651 - DISPERSE RED 1 PROMOTES GENETIC INSTABILITY IN LIVER CELLS AFTER IN VIVO EXPOSURE

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Several azo dyes have shown to be mutagenic for in vitro assays but in vivo mutagenic studies are still scarce. Previously, we identified the capability of Disperse Red 1 (DR1) to induce DNA breaks in bone marrow and germ cells after in vivo treatment with concentrations from 0.5 to 500 mg/kg body weight (bwt). Herein, we investigated the effect of Disperse Red 1 (purity >95%) at concentrations (0.5 and 5 µg/kg bwt.) equivalent to 10 to 100 times higher than those found in river water. Gene expression profile of 84 target genes (Mouse Stress & Toxicity PathwayFinder PCR Array – SABiosciences) was evaluated in liver cells of Swiss mice orally treated (gavage) with the dye. Twenty-four hours after a single treatment, liver was collected and RNA extracted to assess gene expression. Our data showed upregulation of CDKN1A (cyclin-dependent kinase inhibitor 1A-P21) for 0.5 µg/kg bwt and IL1B (interleukin-beta 1) after treatment with both doses. In conclusion, DR1 was able to modulated genes associated to cell cycle block at G1-S and to inflammatory process. These findings will add important information for the understanding of the mode of action of this substance.

ISTA18-910445 - ECOTOXICITY EVALUATION OF DYES FOR ATMOSPHERIC PLASMA INDUCED DYEING OF TEXTILES

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The textile wet processing industry produces significant amounts of wastewater which, if discharged into the environment, could contribute to the contamination of rivers worldwide. Thus, there is renewed interest in dyeing processes that circumvent wastewater production. In this regard, atmospheric plasma treatment of textile fabric offers an approach to drastically reduce effluent generation, since no water bath is needed during the dyeing process. The objective of this study was to evaluate the acute ecotoxicity of seven dyes developed to be used in a plasma dyeing process. The dyes evaluated were synthesized from the textile dye Disperse Red 1 (N-Ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline (DR1), which have been largely study and showed high toxicity to aquatic organisms. Dyes SZ-1, SZ-2, SZ-3, SZ-4, SZ-5, SZ-6 and SZ-7 were tested at a maximum concentration of 1,000 µg/L, the limit of solubility, and stock solutions were prepared in deionized water containing 1% of dimethyl sulfoxide (DMSO) to facilitate dissolution. Acute toxicity tests with *Daphnia similis* were performed according to OECD guideline and the results were compared to DR1 dye. Negative controls and solvent controls were tested accordingly. SZ-1 (EC50 100 µg/L) was more toxic than DR1 (EC50 180 µg/L). SZ-2, SZ-3, SZ-6 and SZ-7 were all less toxic than SZ-1 and DR1 and neither one was able to immobilize 100% of organisms at the highest concentration tested (1,000 µg/L). Dyes SZ-4 and SZ-5 showed no toxicity until 1,000 µg/L. These results show the importance of synthesis of new dyes, with economic and ecological concerns, producing dyes less toxic than the prototype ones.

ISTA18-395322 - ETHANOL AND ALCOHOL ETHOXYLATE USED AS CO-SUBSTRATE IN LINEAR ALKYL BENZENE SULFONATED DEGRADATION IN COMMERCIAL LAUNDRY WASTEWATER UNDER ANAEROBIC REACTOR

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Anionic surfactants are used in greater volume than any other surfactants because of their highly potent detergency. Linear Alkylbenzene Sulfonated (LAS) is an anionic surfactant derived from petroleum that accounts for about 40% of all surfactant used in the world. The present study evaluated the degradation of LAS in commercial laundry wastewater using an anaerobic fluidized bed reactor (FBR) filled with expanded clay (support material) on a pilot scale (18.8 L) with hydraulic retention time of 18 hours, as well as to evaluate the ethanol and a nonionic surfactant linear alcohol ethoxylated (LAE) effect as co-substrates. The FBR was operated in six different operational phases that differed according to the LAS concentration influent and the presence of co-substrates. LAS influent concentration varied between 7.9±4.7 mg/L and 43.9±18 mg/L. Co-substrate concentrations were 20 mg/L of LAE and 205 mg/L of ethanol. LAS monitoring (influent and effluent) and LAE were performed by high performance liquid chromatography coupled to mass spectrometry. The highest average LAS removal efficiency was found in Phase III (50%) for 25.9 ± 11 mg/L and Phase IV (56%) for 43.9±18 mg/L. Without LAE as co-substrates the LAS removal efficiency dropped to 41.2% (21.9±10 mg/L) in Phase V, and without ethanol, the LAS removal efficiency was reduced to 18.8% (20.2±16.4 mg/L). Thus, the operational stages that had co-substrates (LAE and ethanol) led to high LAS removal efficiency, proving that the use of readily biodegradable carbon sources as co-substrates help the linear sulfonated alkylbenzene removal in commercial laundry wastewater.

ISTA18-990169 - FOUR YEARS OF ESTROGENIC ACTIVITY ASSESSMENT IN SURFACE WATERS OF SÃO PAULO STATE, BRAZIL

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Endocrine disrupting chemicals (EDCs) have the ability to alter the endocrine system of organisms. Such compounds are present in several industrial and domestic activities and reach the aquatic environment via wastewater discharges or agricultural surface runoff. The aim of this study was to determine the overall estrogenic activity of surface waters of São Paulo State, Brazil. For this purpose, 10 sites from the routine Surface Water Quality Program of CETESB were selected that were already surveyed for physical, chemical and biological variables from 2013 to 2016. Samples were extracted in solid phase and tested for estrogenic activity using bioluminescent yeast estrogen assay (BLYES). Results were expressed in 17-estradiol equivalent quotient (EEQ). Estrogenic activity was below the limit of quantification (0.1 EEQ) in 31.7% of 227 samples. All sites presented estrogenic activity at least once. The results have shown the presence of estrogenic activity in a range from 0.11 to 26.6 EEQ in the sampling sites in four years of study. Statistical analysis showed a positive correlation between estrogenic activity and the biochemical oxygen demand performed in the samples, indicating that this activity detected in most sites is related to wastewater discharges. Due to the presence of estrogenicity higher than 5.0 EEQ in a few sampling sites, further chemical analyses and other bioassays are recommended in order to provide a better understanding of the estrogenic activity present at these sites.

ISTA18-990170 - REMOVAL OF AN AZO DYE USING FENTON AND FENTON-LIKE PROCESSES: EVALUATION OF THE PROCESS FACTORS BY BOX-BENHKEN DESIGN AND ECOTOXICITY TESTS

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Gisele Augusto Rodrigues de Oliveira

The conventional treatment of textile effluents is usually not effective to remove azo dyes and can still generate products more toxic than the original dyes. The present study evaluated the effects of the process factors on the removal efficiency of Disperse Red 343 (DR343) azo dye by Fenton processes. The effects of iron and H₂O₂ concentrations, pH, and time on dye removal and residual H₂O₂ were evaluated using a Box-Benhken Design. Additionally, the ecotoxicity of the treated samples was analyzed on different organisms (lettuce seed, *Artemia salina*, and zebrafish early-life stages). Dye removal was assessed by spectrophotometry and it was only affected by iron concentration in both Fenton and Fenton-like processes with positive linear and quadratic effects (removal: 5.8% - 100%). Similarly, residual H₂O₂ was decreased with an increase in iron concentration, whereas the initial H₂O₂ concentration had the opposite effect. At the end of the process residual H₂O₂ was in the range from 0% to 7.4%. No significant phytotoxic effect was observed after the treatments. In turn, the treatment time had significant effect on *A. salina* mortality in Fenton process, showing a quadratic effect, which suggests the formation of toxic oxidation by-products at the beginning of the process followed probably by total mineralization. The samples obtained using the best treatment conditions did not induce lethal or sublethal effects to zebrafish early-life stage. Therefore, Fenton and Fenton-like processes can be successfully used to remove DR 343 dye from water, but the effects of the different process factors on toxicity should be analyzed.

ISTA18-245788 - THE EFFECTS OF AYAHUASCA INFUSION (BANISTERIOPSIS CAAPI AND PSYCHOTRIA VIRIDIS) IN EARLY LIFE STAGES OF ZEBRAFISH (DANIO RERIO).

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The psychoactive brew known as ayahuasca (*Banisteriopsis caapi* and *Psychotria viridis*) is a sacramental beverage incorporated by non-indigenous religions originated from the Amazon region. This sacred beverage has touted healing properties in shamanic rituals and an increasing body of evidence indicates that its use is safe in ritualistic context. The prospect of use of ayahuasca in a therapeutic context or in a recreational context still lacks of preclinical data about its safety. Zebrafish has become a popular model for the study of nervous system and behaviour because of its many advantages for high-throughput screening. In addition, locomotor assays using zebrafish larvae are also gaining popularity for safety assessment of common drugs. The present study aimed to evaluate the effects of ayahuasca on fish embryos behaviour. The locomotor activity of zebrafish larvae was assessed using the tracking system Zebrabox-ZEB 478 a system that monitors movements by automated video recording. Locomotion was evaluated for 24 embryos per treatment group including the control in 96 well plates at 120, 144 and 168 hours post fertilization. Data showed that ayahuasca seems to impair locomotor activity of zebrafish larvae. Ayahuasca treated groups exhibited a significant decrease in the total distance moved at all evaluated periods when compared to the control. Further studies should focus on unravelling the associated properties of ayahuasca on the zebrafish nervous system. Future perspective of our work team is to compare these results with previous data obtained with mammalian model in order to validate zebrafish embryos test as a tool to ayahuasca toxicological assessment.

ISTA18-386260 - THE TRICYCLIC ANTIDEPRESSANT AMITRIPTYLINE INDUCES LETHAL AND SUBLETHAL EFFECTS ON ZEBRAFISH EARLY LIFE-STAGES AT LOW DOSES OF EXPOSURE

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Amitriptyline is a tricyclic antidepressant which has an inhibitory effect on the serotonin and norepinephrine uptake in the presynaptic nerve endings. In this study, the Fish Embryo Toxicity (FET) Test (OECD 2013) is proposed as a tool to evaluate the effects of an antidepressant drug – Amitriptyline. The FET test started with newly fertilized eggs exposed to amitriptyline (AMT) concentrations of 0; 0.1; 0.28; 0.79; 2.23; 6.3; 17.5 and 50 mg/L followed by a second battery toxicity tests using sublethal concentrations of AMT (0, 0.006, 0.088, 1.58 and 28.12 µg/L). Enzymatic activity of cholinesterases, glutathione-S-transferase, catalase and lactate dehydrogenase were analyzed. From the FET test, a dose response inhibition for the hatching time was observed for AMT at 72 h of exposure with EC50 values of 5.72 ± 4.18 mg/L. Regarding mortality, at 168 h of exposure a LC50 of 3.10 ± 0.99 mg/L was obtained, so amitriptyline was acutely toxic to zebrafish embryos. Besides mortality, AMT induced behavioural alterations and teratologies, such as tail deformations and oedemas, in the exposed embryos. The biochemical tests showed an increase in the enzymatic activity of cholinesterases tested in the highest concentration of AMT, 28.12 µg/L. In summary, the present study aimed to evaluate the usefulness of zebrafish embryos for the embryotoxicity assessment of antidepressants, namely amitriptyline. Effects on behavior might be explained by indirect effects on cholinergic pathways. Considering the high sensitivity of embryos and the wide range of responses triggered by amitriptyline exposure FET test seems to be a promising tool for the toxicity assessment of psychiatric drugs.

ISTA18-568783 - THE USE OF ECOTOXICITY TESTS WITH DAPHNIA SIMILIS TO EVALUATE THE EFFICIENCY OF GRAPHENE NANOMATERIAL IN THE REMOVAL OF LAMBDA-CYHALOTHRIN

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Graphene derivatives have been used as potential adsorbents agents of many pollutants in aqueous solutions. In this work Graphene Oxide (GO) and reduced Graphene Oxide (rGO) were tested for a pesticide removal, Lambda-Cyhalothrin (LC). LC is an insecticide from the pyrethroid class and widely used in agriculture for pest control. It is very toxic to non target species such as microcrustacea with EC50 for *Daphnia similis* of 0.50 µg L⁻¹ (0.22 – 1.17). The objective of this work was to verify the efficiency of removal of LC using two different graphene based nanomaterials: GO and reduced rGO using acute toxicity tests with *D. similis*. Acute toxicity tests were performed according to OECD protocol for 48h with phoperiod using 20 organisms per concentration. The LC at 30 µg L⁻¹ was mixed with nanomaterial for 60 min in three different experiments using GO (100 mg L⁻¹) and rGO (40 mg L⁻¹ and 8 mg L⁻¹) and then serial dilutions were prepared in culture medium. The water solubility limit of LC was the maximum concentration used in the acute toxicity tests (5 µg L⁻¹). Also, acute toxicity tests were carried out with GO and rGO and no toxicity was observed. The EC50 was 0.32 µg L⁻¹ (0.23 – 0.43) to the mixture GO + LC therefore no removal of the insecticide was observed. However, when treated with rGO, at two independent concentrations of the nanomaterial no toxicity was observed. The high removal capacities observed for rGO probably is due to the π -stacking interactions between the nanomaterial and LC.

ISTA18-218457 - THE USE OF THE OYSTER, CRASSOSTREA BRASILIANA, AS A MODEL ORGANISM FOR EVALUATING THE EFFECTS OF MARINE CO₂-DRIVEN ACIDIFICATION

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While carbon capture and storage in sub-seabed geological structures is being used as a technological alternative to reduce the anthropogenic CO₂ emissions, the environmental effects of potential CO₂ leaks during the operational phases are still poorly understood. The Brazilian native oyster, *Crassostrea brasiliana*, was used as a model organism to assess the effects of CO₂-associated acidification under laboratory. The bivalves were exposed to sediment-seawater acidified to various pre-established pH (ranging from 8.0 to 6.5) by directly bubbling CO₂ into the test chambers. To identify if the CO₂-related acidification could influence metal toxicity the sediment samples were collected in two different coastal areas, with relatively low (Σ metals 2,121.14 mg Kg⁻¹) and high (Σ metals 46,275.2 mg Kg⁻¹) metal contamination. Oysters mortality and the stability of the lysosomal membrane, verified through the neutral red retention time (NRRT) was employed as endpoints. After 10 days of exposure, no significant mortality was observed for the organisms exposed to both sediments tested for the entire pH treatments, with the exception of high metal-contaminated sediments at pH 6.5, which indicates lower sensibility of these organisms to acidification compared to other bivalve species. Although no significant variations were observed for the NRRT for the day 5 of the exposure time, a significant increase in hemolymph cytotoxicity was observed in relation to the control groups for the pH 6.5 for both sediments tested. In conclusion, potential drop in pH in coastal areas would lead to lethal and sub-lethal effect on oysters enhanced by the presence of contaminants such metals.

ISTA18-715546 - TOXICITY AND INFLAMMATORY RESPONSE OF MELAMINE CYANURATE NANOPARTICLES ON RENAL CELLS

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Subsequent to the episode of melamine tainted-milk in China, we have attempted to investigate the early cellular events of kidney cells in response to melamine and related lithogenic ions. A two-compartment transwell culture system allowed us to investigate the instant contact of the apical surface of the human kidney cortical WT 9-12 cell line with the melamine cyanurate crystals formed by mixing different concentrations of melamine and cyanuric acid, under orbital shaking. In response to the clinically relevant ratio of melamine and cyanurate, i.e. 99:1 (v/v), 25% of the cells were destroyed to demonstrate significant disturbance to the tight junction monolayer of cortical epithelium. In this connection, secretion of T-helper 1(Th1)/T-helper 2(Th2) cytokines including IL-5, IL-6, IL-8, MCP-1 were stimulated, in both the apical and basolateral sides of the culture. Such cytokine expression was also correlated with the profile of gene expression microarray and numerous cytokine and receptor genes were up-regulated, in addition to the down-regulation of genes for voltage-dependent calcium channels. Current results indicated that, apart from cytotoxicity, melamine cyanurate induced inflammatory response at protein and gene levels. In particular, IL-5 is regarded as a Th2 cytokine that stimulates B cell growth and increases immunoglobulin secretion. This suggests the shift of the cell microenvironment towards a Th2 type immune response which may favour humoral response. Furthermore, migration of immunocompetent cells, such as neutrophils and monocytes, could be facilitated by IL-8 and MCP-1 to the microenvironment of renal cortex.

ISTA18-110782 - TOXICOLOGICAL EVALUATION OF NAIL POLISHES WASTE DISCARDED IN THE ENVIRONMENT

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Disposal of nail polish represents a great risk to human and environmental health due to their chemical composition, large consumption and inadequate discarding. We investigated the toxicogenetic effects of solubilized and leached extracts from nail polish simulating their disposal on water and landfill, respectively, and identified their constituents. Leached and solubilized extracts were obtained according to Brazilian Association of Technical Norms and evaluated by Salmonella/microsome mutagenicity assay (TA98 and TA100); LDH, WST and cytokinesis-block micronucleus tests with HepG2 cell; comet assay in RTG-2 fish cell and fish embryo acute toxicity test with zebrafish. Gas chromatography-mass spectrometry and spectroscopy of plasma mass inductively coupled were used to identify the constituents. Leached and solubilized extracts only induced effects in TA98 with and without S9 from 25% and 6.25% to 25%, respectively. Both extracts did not significantly increase the frequency of micronucleated; however, the cell viability was affected after 24h exposure. Solubilized showed a slight increase of DNA damage in RTG-2. For zebrafish, solubilized and leached induced mortality from 50% and 100%, respectively, after 24h exposure. Moreover, both extracts caused yolk sac and pericardial edemas, blood accumulation and absorption of the yolk sac from 48h exposure. Organic compounds and metals were identified. In summary, both extracts induced frameshift mutation, but solubilized was more mutagenic than leached. Solubilized is also more toxic than leached for both zebrafish and RTG-2 cells. So, the improper disposal of nail polish may pose risks to human and environmental health.

POSTER PRESENTATION

ISTA18-073062 - A MULTITROPHIC EVALUATION OF CARBON NANOSPHERES TOXICITY

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Lately many new carbon structures have been discovered and produced, among them there are the carbon nanospheres (CNS) defined as 50 to 1000 nm diameter spheres. Despite of their excellent performance in various fields as drug delivery, heterogeneous catalysis, encapsulation of support and electrode materials, few data are available about their toxicity. Thus, the present work intended to investigate effects of CNS on aquatic organisms using a multitrophic test battery. A CNS sample was produced by CVD method from heavy oil feedstock derived from petroleum (US8722007B2 patent). It presented 165nm mean diameter, size range: 50- 600nm and 26 m²/g surface area. The test battery consisted on the following assays: *Vibrio fischeri* 30min luminescence, *Daphnia similis* 48h immobility, *Ceriodaphnia dubia* 48h immobility and 8d reproduction, *Danio rerio* embryo 96h survival and *Grandierella bonnieroides* 10d survival. Also, hidrodinamic diameter and zeta potencial of suspended particles in cladocerans and fish test-water was analised through DLS. The results obtained for acute exposures were EC50 38 mg/L to *Vibrio fischeri*, 25 mg/L to *D.similis*, 8 mg/L, *C. dubia* and >10g/Kg dry weight sediment to *G.bonnieroides*. Subchronic exposure of zebrafish embryos showed LC50 >50 mg/L and nonsignificant development alterations. Lastly, the reproduction decreasing of *C.dubia* presented NOEC 5mg/L. The particles on CNS suspensions in cladocerans test-water presented 157- 288nm diameter and remained stable (Potencial zeta ≈ -20mV) for 48h, while on fish test-water were bigger, 312- 470 nm, and instable after 24h, showing a sharp drop of potencial zeta from ≈ -18 mV to values around zero after 48h.

ISTA18-153555 - ACUTE COPPER EXPOSURE ABOLISHES RHYTHMICITY OF CLOCK GENES IN DANIO RERIO LIVER AND BRAIN

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The circadian clock is a key cellular timing system that coordinates physiology and behavior. Light is a key regulator of the clock mechanism via its activation of per and cry clock gene expression. Evidences point to a key role for reactive oxygen species (ROS) in resetting this process. The objective was to explore the copper as a ROS generator, using an innovative approach that investigated its effects on the circadian timing. Liver and brain from the *Danio rerio* exposed to 0, 3, 20 and 43 µg/L copper concentrations were collected. Daily oscillations of superoxide dismutase (SOD) and catalase (CAT) enzymes and their correlations both with clock genes (*per1*, *per2*, and *cry1a*) and with the organism's energy cost were determined. CAT expression were found to correlate with *per2* and *cry1a* and thus provide data to support the hypothesis of hydrogen peroxide production by a phototransducing flavin-containing oxidase. Higher SOD activity was found to be correlated with higher intracellular ATP levels. Copper, by its turn, disturbed the daily oscillation of the antioxidant enzymes and the clock genes. Rhythmicity of *per1* in both the brain and liver was disturbed and that of *cry1a* was abolished in the liver on the intermediate copper concentration. Coordination between the SOD and the CAT enzymes was lost when the copper concentrations exceeded the limit established by law. It is possible to conclude that the synchronization of the organism with the environment may be impaired due to the acute copper exposure. Furthermore, the knowledge of the oscillatory patterns of the evaluated parameters should be observed in order to produce better toxicological research works.

ISTA18-221085 - ACUTE TOXICITY ASSESSMENT OF SEA WATER AND SEDIMENT IN AN AREA UNDER THE INFLUENCE OF A CHLORINATED SEWAGE OUTFALL USING *PARHYALE HAWAIENSIS*

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One solution for sewage disposal in several countries is primary treatment followed by chlorination and its discharge in the sea. In Santos city, SP, Brazil, 1 million of cubic meters of urban effluent are discharged into the Santos bay every day, 4.5 km from the beach. To assess the toxicity of environmental samples it is important to use species that are representative of the ecosystem we want to protect. So, the aim of this work was to use the native organism *Parhyale hawaiiensis* to evaluate the acute toxicity of Santos water and sediment samples. Samples of water and sediment of 3 collection points were evaluated, being 40, 20 and 10 meters far from the sewage outfall pipe. Acute toxicity tests were performed in water, pore water and elutriates using 96-wells microplates, and in sediment using 12 wells-microplates containing dried sediment and water at salinity 30 in a ratio of 1:4 (w/v). No significant mortality of *P. hawaiiensis* neonates exposed to the water, pore water and elutriates of sediments samples from all sites were observed in relation to their respective controls. The dried sediment samples were toxic, with 42, 92 and 50% mortality. The observed toxicity is probably related to contaminants adsorbed to the sediment particles. The sediment of the area seems to be adversely affected by the influence the outfall discharge but to confirm these results more analysis are under way. Acknowledgements: FAPESP 2015/24758-5 and CNPq 400362/2014-7.

ISTA18-492519 - ACUTE TOXICITY IN *CAMPOMANESIA XANTHOCARPA*

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Introduction: Plant extracts are traditionally used with therapeutic potential acting against diseases and being difficult to purify and synthesize. *Campomanesia xanthocarpa*, a common species from the central-southern region of Brazil, was used for the research carried out. This species has an effect on the treatment of ulcers, antithrombotic activities, glucose control and weight reduction in preclinical tests. Objective: This study aimed to evaluate the anti-inflammatory activity of the hydroethanolic extract of *Campomanesia xanthocarpa* in experimental models of inflammation of carrageenan-induced pleurisy in rats. Methods: In this model, Wistar rats were treated orally with extract of the leaves of *C. xanthocarpa* at doses of 30, 100, 300 mg / kg, and with the 2 compounds isolated at a dose of 1mg / kg. In the positive control group, dexamethasone was given at 1 mg / kg subcutaneously, the so-called naive group was treated with saline alone and did not receive intrapleural carrageenan. Pleurisy was induced by injection of carrageenan (50 µL) into the intrapleural cavity after one hour of the treatments. Four hours after induction of pleurisy the inflammatory exudate was collected, leukocytes were counted in the Newbauer chamber. The statistical tests used were ANOVA (one-way) followed by the Newman-Keuls test. RESULTS AND DISCUSSION: The extract and compounds showed significant anti-inflammatory action, $P > 0.001$ in relation to the control, the crude extract at the concentrations of 100 mg / kg were very similar to the effects of dexamethasone, whereas the pure compounds presented percentage inhibition But both were significant.

ISTA18-047751 - AN INTEGRATED ASSESSMENT OF REPRODUCTIVE AND GENERAL HEALTH OF FATHEAD MINNOW (PIMEPHALES PROMELAS) POPULATIONS INHABITING AN EFFLUENT-DOMINATED STREAM, WASCANA CREEK, SK, CANADA

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There is increasing global concern regarding the impact of emerging contaminants released by municipal wastewater effluents (MWWEs) into surface waters. In particular, there are concerns regarding the effects that endocrine disrupting compounds (EDCs) have on resident wildlife as conventional wastewater treatment technologies are often inefficient at removing such compounds. Waterbodies in semi-arid prairie regions may be at particular risk of exposure to EDCs due to the uniqueness of prairie surface water systems as during dry periods small streams or creeks receiving MWWEs are often dominated by effluents. This study aimed to characterize the impacts of MWWEs on wild fathead minnow (FHM) populations in an effluent dominated prairie stream, Wascana Creek, located in the Canadian prairies. FHMs were collected up- and downstream of the MWWWE outfall to assess effects on overall health (condition factor, somatic indices) and reproduction (secondary sex characteristics, plasma sex steroids, histopathology, gene expression). Fish collected downstream of the MWWWE fallout had lower gonadosomatic indices and significantly greater hepatosomatic indices compared to fish from upstream populations. There was significant disruption of regulation of key genes along the hypothalamus-pituitary-gonad-liver axis associated with reproductive processes. Also, gonadal degradation and delayed maturation occurred in both males and females. Exposed males displayed lower scores of secondary sexual characteristics. This study highlights the potential ecological risks of EDCs associated with MWWEs, and the need for implementing more effective measures to remove them at wastewater treatment plants.

ISTA18-843851 - ANALYSIS OF OXIDATIVE STRESS AND COELOMIC FLUID OF ANNELIDS EXPOSED TO IMAZALIL FUNGICIDE

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Imazalil is a fungicide that induce teratogenic effects on vertebrates, genetic damage and adverse effects on reproductive and neurobehavioral systems. The purpose of this work is to analyze possible alterations in the biomarkers of oxidative stress and in the coelomic fluid of annelids after acute exposure to the contaminant. The test was carried out according to ISO 11268-1 with some adaptations in Red-Yellow Latosol (50% WHC). Four concentrations (0.01, 0.1, 1 and 10 mg/kg) and the control were used, each with 6 replicates and in each replicate 10 organisms. Mortality and biomass variation were observed during the test; and quantified the activities of GST (Glutathione S-transferases) and Catalase. The coelomic fluid collection was also performed. After the tests no mortality was observed, however, the loss of biomass was significant at all concentrations, justified by the adaptation of the organisms to the environment. The GST enzyme presented no significant difference between the control and the other concentrations. The increase in activity of both enzymes studied was observed at the concentration of 0.1 mg/kg. This may indicate that up to 0.1 mg/kg the system produces and fights the contaminant, but from 1 mg/kg the compound begins to adversely affect the enzymes production system. In the coelomic fluid analysis, no significant differences were observed in cell density, however, there was a decrease in viability from the concentration of 0.1 mg/kg. The results of catalase, GST and cell viability indicate a possible toxicity of Imazalil, but further tests with longer exposure times will be performed.

ISTA18-634719 - ANTIOXIDANT METABOLISM AND ECOTOXICOLOGICAL INDEXES IN PLANTS CULTIVATED IN AN OXISOL CONTAMINATED WITH DIFFERENT DOSES OF CADMIUM

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Plants present various defense mechanisms against phytotoxicity, with diverse physiological responses, making it necessary to better elucidate soil-plant interaction under such circumstances. This work aimed to evaluate the sensitivity of plant species to cadmium (Cd) contamination through biochemical analyses and ecotoxicological indexes. For this purpose the tested plants (corn, rice, bean and radish) was cultivated in representative soil in Minas Gerais. The experiment was conducted in a greenhouse at Federal University of Lavras-Brazil, according to ISO 11269-2. The four sensitivity species were cultivated with Oxisol (clay 24%; sand 64%; pH 4.8; P 1.13 mg dm⁻³; K 32 mg dm⁻³; Ca 0.3 cmolc dm⁻³; Mg 0.1 cmolc dm⁻³; Al 0.6 cmolc dm⁻³; CEC in natural pH 1.1 cmolc dm⁻³; V 9.6%; SOM 1.6%), in different Cd doses (0.4; 0.72; 1.29; 2.3; 4.1; 13.6; 24.4 mg kg⁻¹). After 21 days it was evaluated the activity of enzymes of the antioxidant system; the concentration of no observable effects (NOEC) and minimum effective concentration in which there were observable effects (LOEC). The change in superoxide dismutase (SOD) activity occurred in greater proportions in the bean (38%). For catalase (CAT), there was an increase in the four species, being higher in rice (146%). Only the monocotyledons presented significant increases in the activity of the ascorbate peroxidase (APX) after exposure to Cd. The NOEC and LOEC values followed these patterns of variations. Thus, we conclude that plants change their antioxidant metabolism differently as a response to stress. For the ecotoxicity indexes, the species presented sensitivity variations and differed in regard to NOEC and LOEC values.

ISTA18-596668 - APPLICATION OF PHOTOCATALYTIC TREATMENT IN TEXTILES EFFLUENTS AND ITS TOXICOLOGICAL EVALUATION WITH THE BIOINDICATORS ALLIUM CEPA L. AND ARTEMIA SALINA L.

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The textile industry produces large amounts of effluent that needs appropriate standards to be released in the environment, without risking the public health or the recipient river. The objective of the work was to apply a treatment for the raw wastewater from a textile industry by means of heterogeneous photocatalysis and assessing its toxicity, comparing it with the effluent treated by the industry. They were evaluated four textile effluents, the raw and the treated effluent of the industry and the effluents submitted to the phototreatment, in the presence and in the absence of titanium dioxide. Toxicological analysis were carried out by cytotoxicity tests with *Allium cepa* L. and immobility/mortality tests with *Artemia salina* L. microcrustacean, it was also measured the dissolved oxygen (DO) and the chemical oxygen demand (COD). The values of COD from all treated effluents didn't fit the standards (reduction of 60% and <200 mg O₂/L), but all treated effluents increased the DO content compared to the raw effluent. The toxicity test of *Allium cepa* L. showed no cytotoxicity of the effluents, however the immobility/mortality test of *Artemia salina* L. revealed a rise of the mortality and toxicity of the raw and the treated effluent of the industry in concentrations of 100% and 50%. In the case of phototreated effluents, only in the concentration of 100% of the samples with and without TiO₂ were toxic. The photocatalytic treatments were more efficient than the treatment applied by the industry, though, all those treatments require further reduce the COD and an increase the DO content to reach the required by law.

ISTA18-658161 - ASSESSMENT OF THE EFFECTS OF SOME BIOTIC AND ABIOTIC FACTORS ON THE DNA METHYLATION STATUS IN GAMMARUS FOSSARUM

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Studying genotoxicity in gametes offers some prospects for understanding delayed effects on the offspring and on the population dynamics. However, the modification of the DNA sequence by mutations is not the only mode of action of toxic substances on the genome. Epigenetic effects may add up to effects mediated by genetic pathways and their study allows to improve the understanding of changes in life history traits (reproduction, growth and development) known to be partly driven by epigenetic regulations in many species. As such, it seems of value to examine the role of contaminants on epigenetic changes in complement to their effects on the primary structure of the genome for the assessment of the trans-generational consequences of contaminant exposure. Among epigenetic marks, DNA methylation is rather prevalent in mammalian species and thus often examined. However, the methylation status in invertebrates is still rather scarce. In the present study, the global DNA methylation was assessed in the ecologically relevant species *Gammarus fossarum*, a detritivorous freshwater invertebrate. DNA methylation was measured by assessing the percentage of 5 methyl cytosine (5meCYT/ total CYT) of the whole genome by HPLC-MS/MS determination. The influence of abiotic/biotic factors on global DNA methylation was evaluated. Effect of sex, of the stage of development, and of the genetic strain was studied. Influence of starvation and temperature (8°C, 12°C and 18°C) in one month-exposed organisms was studied. Finally, the effects of contaminants on the global DNA methylation were assessed through laboratory (cadmium exposure) and field experiments (caging at polluted sites).

ISTA18-613997 - ATRAZINE INDUCES CELL DEATH ON ROOT TIP CELLS OF ALLIUM CEPA L.

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Atrazine (ATZ) is an herbicide, class triazines, widely used in weed control. It is considered an emerging pollutant, potentially carcinogenic to humans and persistent in the environment. To better understanding the toxicity of emerging pollutants in the environment, assays with plant models are applied. Thus, the objective of the present work was to evaluate the effect of ATZ on *Allium cepa* L. (onion), through cell cycle analysis, in association with DNA fragmentation detected by TUNEL assay. Cells were exposed for 48 h to ATZ, Methyl Methanesulfonate (MMS - Positive Control) and distilled water (Negative Control). For the cytogenetic analysis, the parameters condensed nuclei (CN) and sticky chromosomes (SC) were considered as a comparison with the results obtained in TUNEL assay. In 82% of the cells treated with ATZ the nuclei was TUNEL-positive. In addition, a high frequency of NC (5.70) and SC (93.90) were observed. In MMS-treated cells, 95% of the nuclei were TUNEL-positive, and the frequency of CN and SC was 0.40 and 10.80, respectively. For the negative control only 5% of the nuclei were TUNEL positive, NC was not observed and the frequency of SC was low (0.30). The results obtained demonstrate that ATZ is a genotoxic compound that causes DNA breaks similar to the alkylating action of MMS. Such damages can trigger the cell death machinery, which initially induces the formation of CN, as observed in the present work. In addition, it is evident that the application of different tests (i.e. TUNEL and cytogenetic analysis) help to better understand toxic effects of environmental pollutants.

ISTA18-41699 - BDE-47 CAUSES LETHALITY AND TERATOGENICITY EFFECTS ON ZEBRAFISH

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Polybrominated diphenyl ethers (PBDE) are widely used as flame retardants. They have been detected in samples of blood and adipose tissue of humans and wildlife animals, mainly due to their physical and chemical properties which favours their bioaccumulation and provides high resistance to environmental degradation. The congener BDE-47 is one of the most used and that presents high toxic potential, such as induction of endocrine disruption, neurotoxicity and hepatotoxicity. It is known that BDE-47 can be accumulated in the environment, especially in aquatic organisms, thus we evaluated the toxicity of this compound in an ecotoxicological model. Zebrafish (*D. rerio*) was used to evaluate the toxicity of this environmental contaminant on their embryonic and larval stages. The tests were performed according to the recommendations of the Fish Embryo Acute Toxicity (FET) Test, OECD No. 236. Endpoints, described in this OECD protocol, such as lethality (e.g. coagulation, hatching), sub-lethality (e.g. eye development, pericardium and yolk oedema) and teratogenicity (e.g. delayed growth, inflated swim bladder) were assessed at concentrations of 0.1, 1, 5, 10 and 25 μM and the statistical analysis performed using ANOVA and Dunnett post-hoc test. We observed that the highest tested concentration of BDE-47 (25 μM) caused effects, such as pericardium and yolk oedema which culminates as a lethal effect on zebrafish embryos/larvae, while 10 μM caused impaired swim bladder inflation, indicating also a teratogenic effect. We can conclude that the exposure of zebrafish to BDE-47 can impair their development and may even lead to death.

ISTA18-395411 - BIOCHEMICAL AND GENOTOXIC EFFECTS OF THE INSECTICIDE IMIDACLOPRID ON THE NEOTROPICAL FRESHWATER FISH ASTYANAX ALTIPIRANAE (TELEOSTEI: CHARACIDAE)ÉRYKA C. ALMEIDA^{1,2}; CARLOS E. D. VIEIRA³; RAPHAEL D. ACAYABA⁴; CASSIANA C. M. RAIMUNDO⁴; ERNANI PINTO¹; CLÁUDIA B. R. MARTINEZ³; ANA L. FONSECA²

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Imidacloprid has been detected in freshwater habitats around the world at concentrations above the guideline established by some countries. This neonicotinoid insecticide interferes in the functioning of arthropod's nervous system and has the potential to cause cytotoxic and genotoxic damage in vertebrates. In the present study, specimens of the Neotropical fish lambari (*Astyanax altiparanae*) were exposed to four concentrations (3, 30, 300 and 3000 $\mu\text{g.L}^{-1}$) of a imidacloprid commercial formulation, and to a control, for 96 hours. To evaluate its genotoxicity, the frequency of micronuclei (MN) and other nuclear abnormalities (ENA) were estimated in erythrocytes. Glutathione S-transferase (GST) activity was determined in liver, gill, muscle and brain as a biomarker of biotransformation. Glutathione (GSH) concentration and the levels of lipid peroxidation (LPO) and protein carbonylation (PCO) were quantified in the same tissues to verify the occurrence of oxidative stress. To determine imidacloprid's neurotoxicity, acetylcholinesterase (AChE) activity was measured in brain and muscle tissues. This insecticide led to a significant increase in the contents of GSH in muscle and LPO in gills, and in ENA frequency on animals exposed to the highest concentration tested. The results show for the first time that the acute exposure to imidacloprid, at a concentration of 3000 $\mu\text{g.L}^{-1}$, cause changes in the oxidative status leading to damages in *A. altiparanae*. Also, genotoxic and oxidative stress parameters are effective to evaluate the toxic effects of this insecticide in lambari, acting as an alert before the impairment of the entire population.

ISTA18-902661 - BLYES AND FET: A PRELIMINARY UNDERSTANDING BETWEEN EFFECTS AND TOTAL ESTROGENIC ACTIVITY ON SURFACE WATERS

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Endocrine disrupting chemicals (EDCs) are included in a range of substances and are present in practically all environmental matrices, generally at low concentrations. For the occurrence evaluation of these substances, several in vitro tests are employed, among of them Bioluminescent Yeast Estrogen Screening (BLYES), which measures the total estrogenic activity in EEQ. However, this assay is not able to provide information about adverse effects to aquatic organisms. In order to observe aquatic communities effects, organic extracts of surface water samples from rivers and reservoirs of São Paulo state with results above the detection limit in BLYES (> 0.1 EEQ) were performed with embryonic assays with *Danio rerio*. The methodology was conducted according OECD 236, to verify acute effects such as: absence of heart beats, absence of somites, no tail detachment and coagulated embryo. Embryonic malformations were evaluated, such as: reduction of organism size, cardiac and vitelline edema, spine curvature and reduction of heart beating, which are characteristics of chronic effects. Among the 21 tested samples, 24% showed acute effects for *Danio rerio* and 14%, chronic effects. The effects observed probably would be associated with EDCs and/or other contaminants that may bind to the receptor in BLYES assay. The information obtained by embryonic assay with *Danio rerio* was suitable to show the effects of these groups of contaminants of the organic extracts and would complement the BLYES response. Accomplishing tests to evaluate effects on embryo reproductive system to establish a correlation between estrogenic activity and acute and chronic effects observed on FET is necessary.

ISTA18-923494 - CASE STUDY: APPLICATION OF THE TIE (TOXICITY IDENTIFICATION EVALUATIONS) METHODOLOGY IN THE ENVIRONMENTAL DIAGNOSIS OF GROUNDWATER CONTAMINATED BY PESTICIDES

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The TIE methodology (USEPA 1991, 1993), developed for liquid effluents, can be applied to other types of aqueous samples. In this study it was used to assist in the environmental diagnosis of a former industrial area contaminated by a mixture of compounds, mainly pesticides, in order to identify the chemical substances that cause toxicity to the groundwater and to give direction to the analytical scope of the next steps. The study was conducted according to the procedures defined in Phases I and II of TIE. In Phase I, groundwater samples were submitted to acute toxicity tests for *Ceriodaphnia dubia*, with an exposure of 5 organisms per concentration and duration of 48 hours. New tests were conducted in the toxic samples, preceded by treatments aiming the fractionation and removal of non-polar organic substances, metals, ammonia, etc. In Phase II, the treatments with better results to reduce toxicity were repeated and the extracts were analyzed with gas and liquid chromatography. From a total of 30 samples submitted to Phase I, 11 presented some degree of toxicity and were selected for toxicity reduction treatments. The solid phase extraction by the C18 column showed the best results. Repeating this Phase II treatment, with methanol and water, allowed the distinction of metals from non-polar organic compounds. The chemical analyses of the methanolic extracts revealed the presence of pesticides associated to the processes of the former industrial unit. The application of this methodology was efficient, since it allowed to delineate the main zones of contamination and to guide the direct investigations and analytical scope.

ISTA18-600083 - CHARACTERIZATION OF PISTIA STRATIOTES L. RESIDUE FROM PHYTOREMEDIATION OF CONTAMINATED SOLUTION WITH CD FOR BIOGAS PRODUCTION: PRELIMINARY RESULTS.

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The use of aquatic macrophytes in the phytoremediation process in contaminated environments with heavy metals has presented promising results. However, waste disposal still makes this process not very attractive. In this study, the objective was to characterize the chemistry of *Pistia stratiotes* used in Cd-phytoremediation, which will be tested for biogas production. The residue of *P. stratiotes* used in this study were collected from nutrient solution Hoagland and Arnon (1942), contaminated with Cd at the concentration of 0.8 mg.L⁻¹, maintained for 20 days. The sludge used to start the reactors was collected from an anaerobic reactor at the Effluent Treatment Station (ETE) of a slaughterhouse. For this purpose, the following reactors were assembled in triplicate: 1) Only anaerobic sludge (100%), 2) The residue of *P. stratiotes* (60% and 40% distilled water); 3) Residue of *P. stratiotes* + sludge and 4) Residue of *P. stratiotes* + sludge + Cd (after phytoremediation), 60% of *P. stratiotes*, 6% of sludge and 33% of distilled water respectively 3 and 4. In all these the following parameters were analyzed: Total Solids (ST), Total Fixed and Volatile Solids (STF and STV), pH and Chemical Oxygen Demand (COD). For the mean STV / ST ratio, values of 0.76; 0.85; 0.72; 0.76, respectively, for treatments 1, 2, 3 and 4. Manera Neto (1992) estimates an approximate value of 0.8 for the STV/ST ratio, which induces that all treatments have potential for biogas production. It is intended to evaluate the quality of the biogas produced as an alternative in the remediation of contaminated aquatic environments with metals.

ISTA18-042634 - COMPARISON OF THE MUTAGENICITY OF SURFACE WATER AND SUSPENDED PARTICULATE MATTER FROM DANUBE RIVER USING DIAGNOSTIC STRAINS FOR THE AMES TEST

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The use of diagnostic strains in the Salmonella/microsome assay with sensitivities to different classes of compounds can provide interesting information on the mutagenicity of complex environmental mixtures. This work is part of the Solution Project and the research was performed with organic extracts of filtered surface waters and their respective suspended particulate matters (SPM) collected at 13 different sites along the Danube River. We used four specific strains: TA98, TA1538, YG1041 and YG5185 to test the samples according to the Salmonella/microsome microsuspension protocol in absence and presence of metabolic activation (S9). Maximum doses tested were 80 ml equivalent for water and 100 mg equivalent for SPM per tube. A total of 70% of the water samples and 92% of the SPM exhibited mutagenicity, in at least one strain. Strains sensitivity were YG1041>TA1538>TA98>YG5185 for both samples. The agreement between the positive responses for filtered water and SPM was of 61% in absence and 69% in presence of metabolic activation but the mutagenicity profiles varied among the samples and we identified different patterns of mutagenicity in relation to the tested strains depending of the collection sites. Mutagenic potencies of filtered water varied from 120 to 2100 revertants per liter equivalent and 60 to 8900 revertants per g equivalent of SPM. Acknowledgement the SOLUTIONS project has received funding from the European Unions Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 603437.

ISTA18-287857 - DELAYED FLUORESCENCE IN ALGAE TEST: BIOPHOTONIC BEHAVIOR AND TOXICITY PERFORMANCE

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The delayed fluorescence (DF) has been applied in biological tests as an indicator of metabolic activities. This phenomenon is related to the interactions and photonic response after excitation with external light source. Different groups have demonstrated good relation of DF with metabolic conditions and chronic toxicity tests in *Acetabularia acetabulum* and *Desmodesmus subspicatus*. The DF profiles are connected to good metabolic maintenance of photosynthesis plastiquinone pools on the PSI and the PSII systems. This work describes the DF behavior in chronic tests using *Raphidocellis subcapitata*, adapted to ABNT12648 and OECD201. The DF measurements were done in algae growth inhibition tests using photon-counting IR sensitive system after 1h, 24h, 48h and 72h, for the “control” and the “chronic” samples for NaCl (1; 2; 3; 5 and 8 g/L) and Ametryn (1; 2; 3; 6 and 10 µg/L). Statistical data presented correlations among toxicity tests, DF measurements and cell growth, showing that the DF technique is faster than the traditional standard protocols, with EC50 results done after less than 24h for Ametryn and 48h for NaCl. The dose-response curves performed by DF analysis in 24h tests are correlated with ABNT12648-72h tests in a faster and cheaper procedure with reduced samples' volume.

ISTA18-777661 - DERIVATION OF WATER QUALITY CRITERIA AND ENVIRONMENTAL RISK ASSESSMENT FOR THE PHARMACEUTICAL METFORMIN

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Metformin is an oral anti-diabetic frequently detected in aquatic environments, but its ecotoxicological data are scarce, making it difficult establishing environmental risk assessment (ERA) and water quality criteria (WQC). Metformin was tested using acute and chronic tests with organisms from three different trophic levels, *Lemna minor*, *Daphnia similis* and *Hydra attenuata*. Data from the literature were also used for deriving WQC and evaluating risk quotient (RQ). WQC values were deriving based on the European Guidance document for deriving environmental quality standard under the Water Framework Directive (TGD). The methodology used for evaluating RQ (MEC/Predicted non-effect concentration) is in accordance with the European guideline on the ERA of medicinal products for human use. The maximum environmental concentration (MEC) value of 20.015 µg L⁻¹ used was retrieved from the literature. Both the acute (14400 µg L⁻¹) and chronic (5600 µg L⁻¹) lowest values were obtained from *D. similis* tests. The short-term WQC (MAC-WQC) derived was 144 µg L⁻¹ and the long-term WQC (AA-WQC) was 560 µg L⁻¹. In this case, according to the TGD, the MAC-WQC is set equal to the AA-WQC for direct ecotoxicity. The RQ obtained was 0.04, indicating that a risk is not expected for metformin. However, metformin has been pointed out as an endocrine disruptor. The WQC and ERA guidelines allow to using only those endpoints that have clear established consequences at the population level of the test species. Therefore, metformin endocrine disrupting effects cannot be used to derive WQC and to evaluate ERA yet. Thus, a potential risk for metformin cannot be ruled out.

ISTA18-693619 - DEVELOPMENT OF A PROCEDURE FOR GST EXPRESSION IN THE AMPHIPOD PARHYALE HAWAIENSIS AND ITS APPLICATION IN PAHS AND AGNP EXPOSURE

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Glutathione S-transferases enzymes (GST) are well recognized to be part of the detoxification processes of different contaminants and changes in its expression can provide early information regarding toxicity effects. *Parhyale hawaiiensis* is of great interesting because it is marine, epibenthic and representative from circumtropical regions. Although molecular tools have been explored in *P. hawaiiensis* for genetics and evolutionary purposes no information regarding ecotoxicology has been provided. The aim of this work is to establish procedures for GST expression and investigate its changes in *P. hawaiiensis* exposed to benzo(a)pyrene and silver nanoparticles. Primers for two sequences of GST and a reference gene (18S) were designed and efficiencies were between 90 and 110%. The cDNA was cloned, sequenced and target sequences were confirmed. The expression of target genes was evaluated by qPCR. Adults (8 months) provided from our culture were individually exposed to benzo[a]pyrene at 0.1 and 1 µg L⁻¹ for 8, 24 and 48h. Also, animals were exposed via feeding every other day with control and contaminated pellet food containing either AgCl or AgNP (500 mg kg⁻¹) for 4,7 and 14 days. In both experiments, no significant differences were observed in the expression of the GST genes. More experiments are underway using different classes of chemicals to investigate possible changes in the expression of the target genes. Acknowledgements: FAPESP 2014/08829-7 and CNPq 400362/2014-7

ISTA18-813005 - DNA AND CELLULAR DAMAGE INDUCED BY EXTRACTS OF TWO SOLANUM SPECIES MEASURED BY THE TUNEL ASSAY

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In the last years, it is of great importance studies that identify natural compounds in plants, which presents allelopathic potential for pest control. It represents a natural alternative to synthetic herbicides, being more sustainable and less impacting to environment. In the genus *Solanum* several species with allelopathic action have been identified. In this sense, the present study aimed to access the allelopathic mechanism of action of the extracts of *Solanum muricatum* e *Solanum betaceum* trough TUNEL assay. For this end, seeds of *Lactuca sativa* L. var. Verônica were used as plant model. Seeds was directly exposed for 96 h to the extracts at concentration previously determine as IC₅₀ (concentration that inhibit 50% the plantlet growth) for each species: 2.06 g/L for *S. muricatum* and 1.93 g/L for *S. betaceum*. Distilled water was used as negative control. Three slides were prepared for each treatment following the squashing method. The 3'-OH DNA-strands breaks were marked with the kit "DeadEnd™ Fluorometric TUNEL System (Promega®)". We evaluated 300 cells per treatment. The frequency of TUNEL-positive cells was 12% of the total cells evaluated for *S. muricatum* extract and 7.33% for the extract of *S. betaceum*. Both extracts led to the appearance of cells that, in addition to having TUNEL-positive nuclei, presented cytoplasm shrinkage, totalizing 26% of the evaluated cells for *S. muricatum* extract and 30.67% for *S. betaceum* extract. These results suggest the potential of the extracts of these *Solanum* species to lead the cells to death, which is evidenced, by the DNA fragmentation and vacuolization of the cytoplasm. These are possible mechanism of their herbicidal action.

ISTA18-779808 - DNA DAMAGE IN MERISTEMATIC CELLS OF ALLIUM CEPA L. INDUCED BY ATRAZINE

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The pesticides or agrochemicals are substances that possess biocidal action, used in agricultural activities to control pests. They are toxic to living beings and have genotoxic, mutagenic and carcinogenic properties. Atrazine (ATZ) is an herbicide, of the class triazine. It is considered as emerging pollutant and is widely used in the pre-emerging control of weeds. In this sense, the present study had as objective to detect DNA damage caused by ATZ through the Comet assay and cytogenetic analysis in root tip cells of *Allium cepa* L. Cells were exposed for 48 h to the treatments: ATZ (0.003 g L⁻¹), Methyl Methanesulfonate (MMS - 4x10⁻⁴ M), as Positive Control and distilled water as negative control (NC). The Comet assay was performed in triplicate (three slides per treatment). The nucleoids obtained were evaluated as visual scores (0 to 4) and the DNA damage calculated through Arbitrary Units (AU). For cytogenetic analysis, were considered the chromosome and nucleus alterations observed in the mitotic cycle in 10,000 cells per treatment. The ATZ caused DNA damage significantly equal to those induced by the alkylating agent, MMS, Presenting high values of AU (ATZ - 292; MMS - 327). In cells treated with ATZ we also observed a high frequency of chromosomal losses (ATZ - 48.40; MMS - 111.30), compared to the NC (0.00). Therefore, the results showed that ATZ is a genotoxic compound which induces DNA damage, and that the use of techniques such as the Comet assay in association with cytogenetic analysis is of great value for access to the toxic risk of environmental pollutants.

ISTA18-048091 - ECOTOXICITY ASSESSMENT OF TWO AURAMINE DYE FORMULATIONS

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Dyes are being considered emerging contaminants, since literature have been reporting the occurrence of several dyes in surface water. Auramine is an industrial diarylmethane dye prohibited in some countries due to its carcinogenic properties. However, in Brazil and other countries auramine dyes are still manufactured and sold. No literature data is available regarding the toxicity of auramine to aquatic organisms. Auramine is found in two chemical formulation: non-salted (trade name: auramine base) and mono-hydrochloride salt (trade name: auramine O). The objective of this study was to compare the toxicity of the two dyes to freshwater aquatic organisms of three trophic levels following the Criteria for Reporting and Evaluating ecotoxicity Data (CRED) recommendations. EC5048 h were, 11 and 14 nmol L⁻¹ for *Daphnia similis*; IC1072h 0.059 and 0.87 nmol L⁻¹ for *Raphidocelis subcapitata*; and LC5096h 7 and 18 nmol L⁻¹ for *Danio rerio*, for auramine base and auramine O, respectively. In general, ionized form (auramine O) is less toxic than the base for all tests, especially when comparing algae results because the dye in its ionized form was 14 times more toxic than auramine base. This difference could be explained because ionized dye is more hydrophilic with a Kow of 0.58 in comparison to auramine base that has a Kow of 2.98. Therefore, auramine O tends to stay in water and consequently will less absorbed by living organisms. Chronic tests with *D. similis* and *D. rerio* will be performed to complement the toxicity evaluation and to provide data to derive a predicted no effect concentration (PNEC).

ISTA18-341884 - ECOTOXICITY EVALUATION OF COAL FLY ASH TO DAPHNIA SIMILIS AND DANIO RERIO

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Coal Fly ash is a major solid waste from coal-fired power stations. In Brazil, more than 4 million tons per year of fly ash are generated with a tendency to increase every year. Only 30% of fly ash is applied as raw material for cement and concrete production. The remaining is disposed in on-site ponds, nearby abandoned or active mine sites, or landfills. The inadequate disposal of fly ash may pose a significant risk to the environment due to the possible leaching of hazardous pollutants into the surrounding soil and groundwater. A combination of leaching tests and ecotoxicological analyses were used in this work for the evaluation of the adverse effects of coal fly in non-target organisms. Ashes were collected from coal-fired power plant located in South of Brazil. Acute toxicity tests were performed with *Danio rerio* embryos and *Daphnia similis*, according to OECD 236 and ABNT NBR 12713, respectively. Coal fly ash sample was subjected to a leaching procedure using USEPA SW 864 Method 1311. The leachate was prepared in seven dilutions: 1.56%, 3.12%, 6.25%, 12.5%, 25%, 50%, and 100%. The assays were performed in triplicates and the results showed lethality of *Danio rerio* after 96 hours of exposure to the leachate, and the calculated LC50 was 4.39%. The ecotoxicity tests with *Daphnia similis*, observed immobility after 48 hours of exposure to the leachate, and EC50 calculated was 7.25%. The results of these tests indicate toxicity of the coal fly ash leachate toward exposed organisms.

ISTA18-754391 - ECOTOXICITY EVALUATION OF THE PESTICIDE PIRIMIPHOS-METHYL TO AQUATIC ORGANISMS

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Brazil is the largest consumer of pesticides in the world. Once in the environment pesticides can cause impact to non-target organisms and their effects must be evaluated. Pirimiphos-methyl is an organophosphate acaricide and insecticide used in different crops. Although this pesticide is approved and used worldwide, ecotoxicity data are still scarce. The objective of this study is to evaluate the toxicity of pirimiphos-methyl to aquatic organisms from different trophic levels. Ecotoxicity tests were conducted with freshwater organisms: algae *Raphidocelis subcapitata*, cladocera *Daphnia similis*, cnidarian *Hydra attenuata* and the marine amphipod *Parhyale hawaiiensis*. Culturing conditions and toxicity tests were conducted in accordance with international protocols for each organism. The growth inhibition concentration (IC10) for *R. subcapitata* was 850 µg L⁻¹, and the effect concentrations (EC50) for *D. similis* and *H.attenuata* were 0.3 and 1250 µg L⁻¹, respectively. The EC50 for the marine amphipod was 201 µg L⁻¹. *D.similis* was the most sensitive organism to the pesticide in our study. More tests are being conducted with chronic endpoints to improve hazard evaluation of pirimiphos-methyl and to provide ecotoxicity data to the literature.

ISTA18-375390 - ECOTOXICOLOGICAL ASSESSMENT AND IDENTIFICATION OF TOXICITY IN STREAM PIRES, LIMEIRA, SP, BRAZIL

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The stream Pires is the main tributary of the stream Pinhal, source used in water catchment for distribution to the population of Limeira, SP. Previous studies in micro basin stream Pinhal pointed acute toxicity only in stream Pires of water to the organism *Ceriodaphnia dubia*. Thus it is important to know the sources of contamination and its contaminants to ensure quality water for the city. In the present work the ecotoxicological evaluation was carried out using the Toxicity Identification and Evaluation (TIE). The principle of this method is based on the fractionation of the samples through a series of physical and chemical processes in order to eliminate or separate groups of compounds to check their toxic potential. To perform the TIE was used the organism *Ceriodaphnia dubia* and *Daphnia similis*, by means of tests of acute toxicity, manipulations (Phase I) indicated respectively nonpolar organic compounds, and cationic metal responsible for acute toxicity. Manipulations of Phase II using spectrometry ICP-MS permitted the identification zinc at concentrations of 270-1330 µg/L. The characterization and confirmation of organic compounds is not always necessary because in some cases only the class of toxic compounds can provide sufficient information to determine the appropriate treatment or control options of the toxicity source. According to the analysis results, it can be concluded that zinc is the primary agent responsible for the toxicity of the samples collected in stream Pires for the tested organisms.

ISTA18-388856 - ECOTOXICOLOGICAL MONITORING AND ASSESSMENT OF ESTUARINE SURFACE WATER OF BAIXADA SANTISTA (SÃO PAULO, BRAZIL)

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This work presents an evaluation of relationship between toxicity and water quality chemical parameters of estuarine surface water samples collected in the Baixada Santista, as well as the influence of the tidal condition (syzygy and quadrature) and temperature on the ecotoxicological results. This evaluation was supported by statistical analysis, which was based on the results obtained along 08 sampling campaigns carried out every four months in 05 locations and during both tidal periods, totalizing 80 samples between 2014-2016. Samples were collected in Meio and Icanhema Rivers and Santos Channel. Pearson correlation analysis ($p < 0.05$) did not indicate correlation to most of the analyzed parameters, except for phosphorus ($r = 0.330$), pH ($r = -0.331$) and dissolved oxygen ($r = -0.329$). ANOVA did not show significant statistical difference between toxic effects and tidal regime; on the other hand, the statistical test indicated reduction of toxic effect on tested organisms with warmer temperatures. It is worthy to mention that higher temperatures decrease oxygen solubility in water and favor displacement of ammoniacal N equilibrium to the non-ionizable form (ammonia). Additionally the warmer temperatures reflect periods in which there is a substantial input of domestic load in surface waters (being phosphorus an important tracer), by the increase of tourism activities in the region. On the other hand, pH reduction influences mobilization of substances to water column. Such facts may explain partially the toxic results observed and positively correlated with phosphorus and negatively with pH and dissolved oxygen, in addition to the influence of the higher temperatures on the observed toxicity

ISTA18-370652 - EFFECT OF COPPER ON TOXICITY TEST VALIDATION WITH ENCHYTRAEUS CRYPTICUS

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Different groups of organisms have been widely used in toxic compounds evaluation in the environment. *Enchytraeus crypticus*, family *Enchytraeidae* and *Oligochaeta*, presents its biology dependent on soil quality, feed 80% of microorganisms and 20% decomposing organic matter, being ecologically relevant organisms, due to its decomposition activity in many types of soil around the world. As starting point for the activities of the Laboratory of Soil Ecotoxicology (LAECOS), the culture was implanted at 20 ° C in nutrient agar plates and the soil toxicity test with the organism *Enchytraeus crypticus* according to ABNT 16387 (2012). In order to validate the test implementation, copper (Cu) was chosen, considered essential micronutrient in the soil, however, when in high amounts this metal can trigger toxic effects both plants and soil biota. The test was performed in artificial soil (SAT) and copper chloride at 0 (control), 35, 70, 120 and 300% concentrations. After soil contamination with copper, ten organisms with visible clitellum were added in five replicates in photoperiod 16h light / 8h dark, during the period of 21 days, temperature between 18°C and 22°C. There was no apparent effect of copper concentration on the organisms tested, but the test fulfilled the validity criteria in terms of survival ($\geq 80\%$) and reproduction (≥ 50 juveniles) in the controls. New concentrations will be tested to evaluate the effect of copper on organisms.

ISTA18-427278 - EFFECT OF PREINCUBATION IN THE SENSITIVITY OF THE MICROPLATE SALMONELLA MUTAGENICITY ASSAY (MPA)

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The Salmonella/microsome assay is the most used mutagenicity test to evaluate chemicals and environmental samples. The assay is also used for regulatory purposes, water quality monitoring programs and effect-directed analysis. Some researchers have modified the testing conditions and developed new protocols especially with the aim of reducing reagents and quantity of sample. An example is a miniaturized version using liquid media called Microplate Fluctuation Protocol (MPF), which is already in use. We are developing an agar miniaturized test based on the microsuspension protocol. Different conditions were assayed with the objective to find the best version that would allow the use of strains with different spontaneous revertants rate. The protocol, Microplate Agar (MPA), is performed in microplates with 12 wells and uses 4 times less sample, S9 mixture, and reagents than the microsuspension version. The objective of this study was to verify the best conditions for the top agar S9 Incubation step. For that, we tested the addition of the biotin and histidine in the agar plates and in the top agar as well as the effect of the pre incubation with and without shaking in the mutagenicity of 2-aminoanthracene. Tests were performed with 5% rat liver S9 and TA98 strain. We concluded that the shaking during pre incubation provided higher sensitivity and there was no difference where the biotin and histidine is added.

ISTA18-612032 - EFFECT OF THE SILVER NANOPARTICLES STABILIZED WITH POLYVINYL ALCOHOL OR CARBOXYMETHYL CELLULOSE IN THE ACUTE TOXICITY IN ARTEMIA SALINA

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This research has the aim to rate the stability of silver nanoparticles (nAg) obtained from different chemical synthesis and rate the toxicity in the organism assayed *Artemia salina*. nAg were prepared by the chemical reduction of the precursor AgNO₃ 5,45 x 10⁻³ mol L⁻¹ with the reducer NaBH₄ 3,03x10⁻³ mol L⁻¹, stabilized using the stabilizer polyvinyl alcohol (PVA) and carboxymethylcellulose sodic (CMC) both at 1%. The formation of the nAg was monitored by spectrophotometry Uv-Vis, the average hydrodynamic radii were determined by Dynamic Light Scattering and the morphological appearance by transmission electron microscopy. Stability of the nAg solutions were rated through spectrophotometry Uv-Vis, in different time of aging of the solution. Acute toxicity was rated by the influence of the solutions of nAg newly synthesized at the mortality of *Artemia salina* obtaining the values of LC₅₀. Solutions of nAg showed Uv-Vis band around 400nm Nanoparticles have shown itself spherical with sizes between 3 to 55 nm. Solution of nAg stabilized by PVA have shown from 30 days the surging of a shoulder starting at 440 nm, indicating a large distribution of sizes or shape differences of the nanoparticles. CMC plasmonic bands obtained after 30, 90, 120, 150 and 180 days synthesis showed indicating stabilization of the average size of the nanoparticles over time. The LC₅₀ values obtained with *Artemia salina* were 68.81 and 62.91% using PVA or CMC, respectively. Solutions of nAg synthesized and rated in this research offers risk to the environment independent of the stabilizer used.

ISTA18-959224 - EFFECTS OF DICHLOFLUANID ON MARINE ORGANISMS

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Marine structures are exposed to the biofouling process that damage them, and improve costs of maintenance, repair and fuel consumption on vessels. Several substances have been used to avoid these consequences, as the antifouling paints. The third generation of antifoulants is characterized by low specificity and rapid degradation, as an example of dichlofluanid, commonly used for this purpose despite the lack of data about its effects on marine biota. Microalgae and microcrustaceans are a common model used in ecotoxicology, by this ease of culture and getting.

Our study sought to observe the potentially toxic effects of dichlofluanid on *Tetraselmis* sp. (Microalga, a target group of the biocide) and *Artemia* sp. (Branchiopoda, a non-target group of the biocide) in bioassays under laboratorial conditions, determining the growth (GR) and inhibition rates (IR) of the microalga, and NOEC, LOEC and LC₅₀ effects on *Artemia* sp.

As results, we observed a wide range of effect concentration, observed in similar studies. *Artemia* sp. showed a different pattern of response to dichlofluanid in one of the assays, ranging the LC₅₀ of 33.20 (NOEC=0.1µg/L, LOEC=1µg/L)-838.55µg/L (NOEC=100µg/L, LOEC=1000µg/L). *Tetraselmis* sp. showed GR toxicity at 100µg/L in 96h of exposure, but the greatest IR are observed in the 24h of exposure (40% at 100µg/L), corroborating with the half-life of dichlofluanid.

The data presented showed the capacity of the biocide affect target and non-target groups, leading to a potential risk in the use of the compound, since its presence is constantly renewed in the environment, especially in port areas due to its usage as an antifoulant on vessel paints.

ISTA18-115623 - EFFECTS OF GLYPHOSATE COMBINATION WITH 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON PHOTOSYNTHETIC RESPONSE OF THREE GREEN ALGAE SPECIES.

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In the last decades, the occurrence of glyphosate-resistant weeds has stimulated the joint application of herbicides with different mechanisms of action. An alternative is a combination of 2,4-Dichlorophenoxyacetic acid (2,4-D) herbicides, which, like glyphosate, is one of the herbicides most commonly used in Brazil. Considering this scenario and the possible contamination of streams adjacent to agriculture fields, the present study measured the impact of commercial formulations Roundup® (glyphosate) and Bratt® (2,4-D) on the photosynthetic response of an important primary producers group in streams, the macroalgae. Three green algae were studied: *Oedogonium* sp., *Spirogyra* sp., and *Nitella subglomerata* A. Braun. The data were analyzed through the techniques of chlorophyll a fluorescence, oxygen evolution and chlorophyll a content after one (T1) and seven days (T7) of exposure. The concentrations used were 3.5 mg.L⁻¹ and 2 mg.L⁻¹ to Roundup® and Bratt®, respectively, which represent the recommended concentration to weed control. Comparing the results, the herbicide mixture generated in *Oedogonium* sp. and in *Nitella subglomerata* a significant reduction in the parameters related to the proportion of open reaction center associated (22,8% and 29,5%, respectively) with the increase of energy dissipation by the reaction center (27,5 and 44,8%) in T1. On the other hand, *Spirogyra* sp. had a remarkable increase of photosynthetic yield parameter (198%) in T7. These responses suggest a relative dependence on the time of exposure and that macroalgae analyzed may undergo productivity fluctuations when exposed to these herbicides.

ISTA18-784431 - EFFECTS OF TEBUCONAZOLE ON THE PHOSPHORYLATION OF HISTONE H3 AT SERINE 10 IN MITOTIC CHROMOSOMES OF LACTUCA SATIVA L.

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The active principles of commercial fungicides from the triazole class – Tebuconazole (TBZ) was detected in irregular amounts in food samples analyzed for the ANVISA on PARA 2011. These substances are toxic for living beings, showing genotoxic, mutagenic and carcinogenic properties, even at low concentrations. In this context, it is important to elucidate the effects of these compounds as well as their toxicity mechanisms. Thus, this study aimed to understand the mechanism of action of TBZ by evaluating the distribution of phosphorylation of Histone H3 at serine 10 (H3S10ph) on cell cycle of the plant model *Lactuca sativa*. The immunosinal was detected with primary antibody Rabbit Polyclonal IgG against H3S10ph, followed by a secondary antibody Goat anti-rabbit IgG-FITC. The alterations on cell cycle due to the TBZ treatment (31.25 µg L⁻¹) were compared with negative control (Ca Cl₂ – 0.01 M) cells. TBZ induces alterations in all phases of the cell cycle as demonstrated by distribution and number of abnormal signals of the H3S10ph. The H3S10ph is a (peri) centromeric marker and we observed scattered marks throughout the chromosomes or no presence of such marks in normal prophase, metaphase and anaphases. In addition, c-metaphase without the immunosinal were also observed in exposed-cells. Sticky metaphase, multipolar anaphases and anaphases with bridges presented the signal of H3S10ph weaker than expected or scattered by the cell. So far, the present research contributes to the understanding of the mechanisms of action of TBZ on plant cell cycle. It was demonstrated that the some alterations could be related to the inactivation of the centromeres.

ISTA18-535928 - EFFECTS OF THE YEAR'S SEASONS AT THE GERMINATION AND INITIAL GROWTH OF ONIONS SEEDS EXPOSED TO THE WATERS OF THE RIVER MARMELEIRO-PR.

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Water contamination is one of the biggest impasses nowadays. Referring to the River Marmeleiro, located at the city of Marmeleiro-PR, a lot of activities can be polluting its water, as the occupation of the people who live riverside, the plantations, the animals, the slaughterhouses and the municipal cemetery. Thus, this work was aimed to evaluate the effects of the river's water over the germination and initial growth of the onion's seed. The water samples were collected along the four year's seasons (spring, summer, autumn and winter) in six points of the river: in the source (P1), in the beginning of the urban zone (P2), in the point of water captation to the water treatment station (P3), in the urban central region of the city (P4), in the point of release of the treated effluent at the treatment station of the drain (P5) and in the river's mouth (P6). It was calculated the number of germinated seeds per day, the medium size of the germinated roots per day, the percentage and speed of the germination. The statistical analysis were done by Tukey test ($\alpha=0.05$; $n=4$). In the summer, P3 showed the biggest medium number of germinated seeds on the second, third and fourth day, and also the biggest percentage and speed of germination, being it different and bigger than the positive control. In all stations evaluated there were no statistical differences in the analyzed variables in relation to the negative control and between the points. Therefore, regardless of the year's station, the collection points did not interfere at the germination or initial growth of the roots. Thus, these waters may not cause adverse effects to plantations and may be used for irrigation.

ISTA18-408839 - ENVIRONMENTAL ACTIONS AND QUALITY MANAGEMENT - THE ALLIES OF ACADEMIC RESEARCH IN THE EVALUATION OF ENVIRONMENTAL TOXICITY

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CETESB - Companhia Ambiental do Estado de São Paulo

It is unnecessary to draw a time line to portray the first manifestations indicating the anthropic effects on the planet and the change of social paradigm that guided us to the present environmental context. Emerging pollutants, dyes and pesticides are increasingly present in the environment and their effects are perceived in the contamination of air, water, sediments and soils. As a result of academic research, risk situations are identified, which require environmental agencies, legislation, monitoring and control actions. To properly meet these demands, the agencies need to strengthen their structure, applying quality management systems, seeking standardization, functionality and continuous improvement, which should be extended to academic research due to the importance of knowledge generation. Standards and regulations are created and revised to generate consistent and reliable data, and laboratories, regardless of their structure and vocation, can use management systems to demonstrate their competence. Environmental protection agencies in different countries have focused on the design and implementation of diagnostic and regulatory systems, which form the basis for the generation of strategies to protect human and environmental health. São Paulo State issued the Resolution SMA 100/2013, which requires analytical results, including sampling, to be performed and issued by accredited laboratories according to ISO / IEC 17025, thus reinforcing the consistency of the data generated. The implementation of quality systems in research laboratories results in gains such as increased credibility in results, organization of work practices, evidence of technical competence, ease of management, effectiveness and efficiency. It is in this context that the reliability of the information generated acquires a high degree of importance given its role in environmental and control actions as allies of academic research in the evaluation of environmental toxicity.

ISTA18-477160 - EVALUATION OF GENOTOXICITY DAMAGE IN COBIA REARED IN TWO DIFFERENT SYSTEMS AND INJECT WITH B-NAPHTHOFLAVONE

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Genotoxicity damage can be associated to reduced growth rate and decrease survival of many aquatic organisms. Although genotoxicity biomarkers are commonly used on environmental monitoring, they can be also very useful to monitoring aquaculture systems in order to improve techniques. In this work, cobia has been cultivated for 11 weeks in two different tanks, the first one was an indoor tank with open water circulation system and the other one was a near shore cage tank. Comet assay and the micronuclei and others nuclear abnormalities assay detected higher DNA damage and nuclear abnormalities frequency on the fish reared in the indoor tank. These results show that both techniques are complementary. In another experiment, cobia were injected with β -naphthoflavone (BNF) at concentrations of 2mg/kg and 10mg/kg on laboratory controlled conditions, and maintained for 7 days in a rearing system tanks to better understand the response mechanisms of this species to a toxicology substance. The comet assay didn't detect any significant differences between fish injected with BNF and the controlled groups, however the nuclear abnormalities assay showed significant differences between fish injected and the control. The DNA damage identified by the comet assay are repairable breaks on the DNA strands, meanwhile, nuclear abnormalities are permanent damages. Therefore, it's possible that the period of maintenance had been enough to purify the organisms and to repair the breaks on DNA. Looking at both experiments, cobia seems to respond very well to genotoxic elements, consequently the comet assay and nuclear abnormalities assay seems to be sensitive techniques to monitoring purposes.

ISTA18-285974 - EVALUATION OF THE CYTOTOXICITY OF ESSENTIAL OILS PREVIOUSLY ANALYZED AS ANTIFUNGAL POTENTIALS.

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Cryptococcosis is a fungal infection caused by the species *Cryptococcus neoformans*. The main population susceptible to cryptococci is that of immunocompromised patients, especially patients with acquired immunodeficiency syndrome, causing meningitis. Once the treatment has important toxic effects, the search for new antifungal compounds is necessary. After evaluating and proving the antifungal activity of the essential oils *A. balsamifera*, *V. zizanioides*, *C. cassia* and cinnamaldehyde, the objective of the study was to evaluate the safety of these compounds using in vitro methods.

The cytotoxicity was performed on cell line BALB c / 3T3 of accordance with neutral red uptake (NRU) assay recommended by ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods)

The results found for the cytotoxicity of the essential oils of *A. balsamifera*, *V. zizanioides*, *C. cassia* and cinnamaldehyde were, respectively, 82.7, 112.4, 5.5 and 4.4 $\mu\text{g/mL}$.

ISTA18-981088 - EVALUATION OF THE PRODUCTION PROCESS OF PECTIN AND VINASSE BIODEGRADABLE FILMS FOR SOIL APPLICATIONS

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The irrigation with vinasse can improve soil fertility, and represents a good alternative to its disposal. However, in case of excessive dosages the polluting potential of vinasse can be problematic considering negative impacts on the soil structure and water resources. So the search for alternative uses for this wastewater is a challenge to the sector. On the other hand, the interest in alternative materials intended for agriculture applications has grown over the last years. Biopolymers, such as proteins and polysaccharides, have emerged as alternative matrices to synthetic polymers applied in agriculture. Pectin is a natural polysaccharide derived from citrus processing, well known for its gel and film forming properties. This study aimed the use of vinasse as the solvent in the production of pectin films. Vinasse had its pH lowered and then high methoxyl pectin and glycerol were added. The effect of small amounts of starch addition was also studied. The solution was poured into frames and dried at 40°C/18h. The films were characterized according to its visual aspects, moisture content, water vapor permeability (WVP) and mechanical properties. Pectin/vinasse films were flexible, homogenous and showed a continuous structure. It was observed that starch enhances film elongation and reduces WVP, moisture and tensile strength compared to films without starch. However, in ratios higher than 70:30 (pectin: starch), films could not be properly removed from frames. Pectin/vinasse films produced show potential to be used in agriculture applications such as seedling bags, reducing the impact of conventional plastics and allowing recycling of nutrients from vinasse to soil.

ISTA18-707428 - EVALUATION OF THE THREAT OF OCEAN ACIDIFICATION RELATED TO CO₂ LEAKAGE FROM CARBON CAPTURE AND STORAGE ACTIVITY USING NITOKRA SP.

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Carbon Capture and Storage Technology (CCS) comes as an alternative to reduce alarming levels of CO₂ that is discharged in the atmosphere. However, leakages of CO₂ to the water column can occur causing an increase of the ocean acidification process, which cause damages on biota and the marine environment. Furthermore, the decreasing of pH values changes the mobility of metals at the sediment, increasing their bioavailability and toxicity to marine organisms such as benthic copepods. Considering these aspects, this study investigates the effects of acidification caused by CO₂ leakage on the fecundity of fertilized females of copepod *Nitokra* sp, in two different areas at Santos Estuarine System (polluted and unpolluted), under different pH values (8.0, 7.6, 7.0, 6.5 and 6.0). This assay was performed over a 96 hour period. 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 CO₂ leakage from the marine substrate. Besides that, smaller progeny number was observed in the 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 area. The presence of metals in polluted substrate could be related to an intensive traffic of ships that leads to the port of Santos. Therefore, this work contributes to a better understanding about the implications of acidification caused by CO₂ leakages deriving from Carbon Capture and Storage technology in marine biota.

ISTA18-705454 - EXPLORING THE MECHANISMS OF GRAPHENE OXIDE TOXICITY TO ZEBRAFISH EMBRYO.

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Many applications of graphene oxide (GO) are still in the research phase, but its promising uses have raised concerns about their risks to human health and the environment. Environmental factors, as the presence of humic substances in aquatic environment, can affect nanomaterials behavior and its toxicological effects. A previous study of our group revealed that the exposure of zebrafish embryos to GO is related to a reduction in total body length and in acetylcholinesterase activity. Thus, the aim of this study was to investigate the implications of GO exposure in muscular development and acetylcholinesterase expression. Zebrafish embryos (n=48/group) were exposed to GO (100 mg/L) in the presence or absence of humic acid (HA, 20 mg/L) during 5 days. Larvae were not feed during the experiment and were exposed under a 14/10 h light/dark cycle, 26 ± 1°C. The mortality in all groups was below 10% and no malformation was observed. At the end of the experiment, alive larvae were fixed with PFA 4% to evaluate its muscular morphology (confocal microscopy, SHG technique) or frozen in liquid nitrogen to perform RT-PCR analysis (AChE expression). Measurements of sarcomere and somite length were performed. No change in muscular tissue was observed, neither differences in AChE expression among groups were detected. So, further experiments have to be performed to clarify the mechanisms of graphene toxicity. FAPESP grants 2014/01995-9, 2014/12891-0, 2014/15640-8.

ISTA18-011576 - GENOTOXICITY AND MUTAGENICITY OF WATER SAMPLES FROM A RURAL AREA OF THE PAMPEAN REGION (ARGENTINA)

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The Pampa Region (Argentina) is a large plain of fertile land suitable for agriculture and livestock. Fertilizers and animal manure, which are both rich in nitrogen and phosphorus, are the primary sources of nutrient pollution in the aquatic ecosystems. Rural activities also produce a large variety of pollutants, such as herbicides, veterinary pharmaceuticals, hormones and heavy metals, causing environmental concern due to the harmful effects on non-target organisms. The aim of this work was to assess the genotoxicity and mutagenicity of water samples from a rural area of Buenos Aires (Argentina) using the *Allium cepa* test. Water samples were collected in a drainage channel in March, June and December. Quantitative analysis of nutrients (P and N), metals (Cu, Pb and Zn) and glyphosate was performed. The *A. cepa* test was performed using onion seeds. Distilled water was used as negative control. It was measured the mitotic index (MI), and frequencies of chromosome aberrations (CA) and micronucleus (MN) by counting a minimum of 5 slides and 5000 cells. The maximum concentrations of N-NH₄⁺, N-NO₂⁺ and P-PO₄³⁻ were 13.5, 0.14 and 10.66 mg L⁻¹, respectively, showing eutrophic characteristics. The maximum concentrations of metals (Cu=0.012, Pb=0.041, Zn=0.044 mg L⁻¹) and glyphosate (0,014 mg L⁻¹) were not genotoxic to *A. cepa*. However, all sampling sites showed statistically high MN frequencies in June, when the minimum rainfall occurred. The results showed that the complex mixture of xenobiotics in these surface waters could produce synergistic effects in living organisms. *A. cepa* showed to be a useful tool for the detection of mutagenicity in water samples from rural areas.

ISTA18-484429 - HEAVY METALS PRESENCE IN LIPSTICKS: INVESTIGATION OF HEALTH IMPACTS AND THEIR DISPOSAL IN THE ENVIRONMENTMAEHATA P¹, SEO E S M¹, COTRIM M E B²

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Lip cosmetics are involved in two major discussions: the public health and environmental problems. From this information, we intend to analyze the impact on human health, study the relationship among price, duration and color and investigate what are the possible impacts on the environment, assuming some waste disposal routes. The cosmetics analyzed were lipsticks, divided by brand, price and color. The first analysis was by x-ray fluorescence (XRF). Then, the raw material went through digestion with nitric and hydrochloric acids under heating, and diluted with distilled water, then filtered. The following readings were performed by optical emission spectrometry with inductively coupled plasma (ICP-OES). From the results of the analysis by XRF (found 18 metal elements), the matrix for analysis by ICP-OES is composed of: aluminum, calcium, cadmium, cobalt, chromium, copper, iron, potassium, manganese, nickel, lead, silicon and titanium. Toxic metals have been identified (Ni, Mn, Cd and Cr) in the samples. Although the examined lipsticks showed positive for toxic metals (Pb, Cd, Ni, Cr and Mn), their levels comply with national legislation, regard to limits for cosmetics and foods. However, it is important to note that the cosmetic and food laws have great differences in the limits for heavy metals. The study of disposal for lipsticks showed that even lipsticks that are most used, there is a waste of nearly 1/3 of the product due to the inner package. This information may help in a conscious consumption of lipsticks, including the risk associated with the use of a set of makeups (foundation, eyeshadows, mascara, blush and lipstick) with other cosmetics.

ISTA18-139903 - HISTOPATHOLOGICAL CHANGES CAUSED BY FIPRONIL AND THYMOL IN THE THYROID OF MICE (MUS MUSCULUS) (NON-TARGET ORGANISMS OF ACARICIDAL PRODUCTS)

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The indiscriminate use of tickcides has caused damage to the environment and to the nontarget organisms. Among those currently available in the market, fipronil, a highly toxic synthetic chemical has already proven its action in some species. Thymol, a product of plant origin, has a tickcide action and has been considered less harmful to the environment and to non-target organisms. The application of the acaricides in the cattle is done by aspersion bath, which causes the death of the ectoparasites. However, the effects caused by these products on nontarget organisms are unknown. Assessing the toxicity of a chemical through thyroid changes becomes an alternative that provides quick and accurate response. Thus, this study evaluated the morphology of the thyroid of females of *Mus musculus* mice exposed to the acaricides fipronil (2%) and thymol (2 mg/mL) through aspersion baths. After 14 days, the animals were euthanized, the thyroid removed and processed per histological routine (Committee on Animal Research and Ethics - UNESP Rio Claro, protocol 4243). The results showed that the exposures to both, fipronil and thymol, caused morphological alterations in the thyroid of the mice exposed. Were observed structural disorganization of the whole gland, fusion between thyroid follicles, vacuolization in the cells of the follicular epithelium and Integrity loss of the colloid present within the thyroid follicles. Along with blood analysis and body weight measurement, the results suggest that both chemicals are potentially toxic to the thyroid and consequently to the body, since this endocrine gland, the thyroid, is responsible for regulating the entire metabolism of vertebrates.

ISTA18-064116 - IL-1B PRODUCTION EVALUATION IN HACAT KERATINOCYTES AS A NON-ANIMAL MODEL FOR EYE IRRITATION PREDICTION OF CHEMICALS

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Eye irritation is a mandatory parameter in human risk and safety evaluation for products including chemicals, pesticides, medicines and cosmetics. Historically, this endpoint has been evaluated using the rabbit Draize eye test, an *in vivo* model that was never formally validated. Due to advances in scientific knowledge, economic and ethical issues, non-animal methods based on mechanisms of toxicity are being developed and validated for increasing the capability of these models to predict eye toxicity. In this study, the Cytometric Bead Array (CBA) assay was used to evaluate the inflammatory cytokine profile produced by HaCaT human keratinocytes after exposure to chemicals with different UN GHS eye irritation classifications. As a first step, cytotoxic profile of the chemicals, including 3 non-irritants and 7 eye irritants (GHS Category 1, 2A and 2B), was evaluated after 24 h exposure using MTT assay and 80% cell viability (CV80) was calculated for each chemical. Then, the cells were exposed to the chemicals at CV80 for 24 h and supernatants and cell lysates were analyzed by CBA assay for quantification of the following cytokines: IL-6, IL-8, IL-10, IL-1 β , TNF and IL-12p70. Regarding cytotoxicity evaluation, chemicals showed different cytotoxicity profiles and data demonstrated no correlation with their UN GHS classification. Among the cytokines evaluated, the IL-1 β production has changed after exposure. The higher levels of IL-1 β intracellular were found in GHS Category 1 chemicals, followed by Category 2. Thus, these findings show that IL-1 β evaluation using HaCaT model can be a good endpoint to classify chemicals according to the potential to promote eye irritation.

ISTA18-795946 - INFLUENCE OF SEDIMENT ACIDIFICATION AND CONTAMINATION ON MUSSEL BURROWING BEHAVIOR

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Environmental pollutants such as metals cause serious risks to many aquatic organisms. In this sense, previous research has characterized that future scenarios of pH reduction enhances the mobility of metals retained in marine sediment, thus increasing the infauna toxicity. Once the organism's health is directly associated to sediment conditions, infaunal behavior appears ideal for assessing the effects of global climate stressors. The present study addresses the interactive effects of metal toxicity and sediment acidification on *Mytella charruna* burrowing activity. The mussels were exposed to contaminated and control sediments at future pH scenarios conditions (8.0–6.1) and the response was observed daily up to 120 hours. In both sediment, the burrowing rate decreased gradually with the pH reductions and presents a drop after 96h of exposure, which may represent the critical time spent in adaptive strategies of this specie to environmental stress. Significant differences on infaunal mussel behavior were observed among temporal variation pattern, with a different trend in pH 6.5 related to other treatments in the control sediment. In contrast, for the contaminated site clear differences in pH 6.5 and 6.0 compared to the others were observed. Thus, the lower burrowing rates associated with the lower pH proves that these stressors in synergy act negatively to organism's health. Given that for the entire pH treatments undertaken the control sediment systematically presented higher burrowing ratios in comparison to the contaminated sediment, it can be concluded that the sediment toxicity strongly influences the behavior and consequently the health of this bivalves.

ISTA18-450444 - INFLUENCE OF UV/H₂O₂ TREATMENT ON TOXICITY OF URBAN WASTEWATER METAL-CONTAINING

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The objective of the present work was to evaluate the toxicity of secondary wastewater, spiked with metals, by means of the germination and growth of rocket seeds, before and after the treatment with the advanced oxidative process, UV/H₂O₂. The applied methodology was based on standard methodology of Analysis of Seeds (RAS), 2009. The tests were carried out in a set of disposable Petri dishes with qualitative filter. The effluents were fortified, separately and in combination, with different concentrations of Cu, Zn, Pb, Ni, Cr, Fe and Al. In the Petri dishes were added 4 mL of the effluents before and after treatment with UV/H₂O₂ and, twenty seeds, arranged evenly and placed in germination chamber with controlled temperature at 20°C and photoperiod of eight hours. The experiment was carried out in triplicate and the control was carried out through the use of distilled water. After 5 days of experiment the number of germinated seeds at each concentration were counted and the size of the seedling measured (radicle and hypocotyl). Initial results demonstrate that germination and seed development are significantly affected after treatment of sanitary sewage with UV/H₂O₂ at high concentrations of metals. Preliminary tests allow us to conclude that, at the concentration of 10 ppm of metals, the treatment with UV/H₂O₂ promoted a decrease in the toxicity of wastewater, while in the concentrations of 100 and 300 ppm of metals, treatment with UV/H₂O₂ promoted an increase in toxicity of secondary wastewater.

ISTA18-278799 - INTOXICATION BY PESTICIDES IN SÃO PAULO CITY, SP-BRAZIL

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Brazil is the world leader in pesticide consumption and this chemical pose a risk for accidental intoxication. Most of the intoxication has occurred in workers, who manipulate these products, but others groups are also at risk. São Paulo city is one of the greatest urbanized cities in the world and the vast majority of intoxications occur due to drug addiction (~50%) followed by medicine ingestion (~25%); although, around 15% are due to pesticides. It is believed that pesticide poisonings occur mainly by products used at home. In order to establish the main type of toxic agent, the age group and route of exposure, this work was done. Data were obtained in the Notification System, SINAN, from 2008 to 2016. The most affected groups are between 1 to 4 and 30 to 39 years old, and 4,472 people were poisoned by pesticides. Usually children intoxications are accidental by inappropriate storage and use of the household products. Although 40% of adult's intoxications are due to suicide attempt through rodenticide use, it is believed to be products used illegally as rodenticides, since in Brazil the registered rodenticides are solid formulations with low concentrations of active ingredient of indandione or coumarin groups. More than 70% of intoxications occurred due to ingestion, predominating this route of exposition. The divulgation that many household products are actually pesticides is important, and therefore poses a health risk, especially to vulnerable groups like children. In order to minimize the impacts of these products in intoxications another point to highlight is the lack of inspection in the irregular pesticides commercialization, especially rodenticides.

ISTA18-285691 - MICROPLASTICS IN WATERS OF GUANABARA BAY (RJ, BRAZIL): A RELEVANT VECTOR OF CONTAMINATION?

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Microplastics, particles of 300 μm to 5 mm, are contaminants of increasing environmental concern. Its presence in the water column is worrisome due to its persistence, adsorption capacity and dispersion of organic pollutants and potential of ingestion by organisms at the base of the trophic chain. In this context, the main objective of this study was to evaluate the occurrence of these residues in surface waters of Guanabara Bay, a place that, despite the degradation, still integrates a wide biodiversity. Water samples were collected in the summer of 2016, through horizontal drag with 330 μm net. Particles <5 mm were isolated, morphologically classified, quantified and qualitatively analyzed. The potential of the microplastic as a transportation vector of contaminants for the biota was raised in this study through the techniques used, which indicated the presence of interferents in the plastic identification, which may be contaminants adsorbed and/or chemical additives. The spectra obtained by infrared spectroscopy FT-IR-ATR indicated the main polymers identified, polyethylene (71.5%) and polypropylene (23.8%), and atypical bands. The determination of the chemical composition by elemental analysis indicated the presence of other elements in the material. Optical microscopy images showed incrustations in the plastic and the predominance of colored microplastics (above 40%), that is indicative of the pigment chemical additive. The results of this study confirm the occurrence of microplastics in bay waters, indicating that their ingestion by marine organisms may cause toxic results due to the possible transport of other anthropogenic contaminants to these organisms.

ISTA18-462293 - MINIMUM PERFORMANCE AND ADEQUACY CRITERIA FOR EVALUATION OF WATER OCCURRENCE DATA ON PESTICIDES - A PROPOSAL

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The credibility of the data used to quantify hazard and exposure is key in risk assessment processes. For aquatic life protection, criteria to evaluate the quality of hazard information have been established since 1997 and recently improved in Criteria for Reporting and Evaluating ecotoxicity Data (CRED). Scientific literature on criteria evaluation for water exposure data still scarce. In a recent review on pesticides in Brazilian freshwaters, a cut off criteria was applied and from 29 papers only 17 provided data with the defined acceptability criteria. The objective of this work was to propose a minimum set of criteria to evaluate water occurrence data on pesticides from monitoring studies. The proposal is to use a decision tree with two main criteria, performance and adequacy. The performance criteria are related to reliability of the data including sampling, sample pretreatment, analytical method and its parameters of quality assurance (selectivity, recovery, sensitivity, matrix effects, ruggedness, measurement uncertainty). The adequacy criteria are related to the method sensitivity to ensure reliable checking with levels of interest. A score system is proposed for each criterion based on amount of information presented, in four categories good, moderate, poor and insufficient. The application of this credibility evaluation will lead to standardization and transparency in the evaluation of exposure data in water monitoring studies leading to a better-quality risk assessment process.

ISTA18-963376 - MONITORING OF AREA PREVIOUSLY USED FOR OCEANIC DISPOSAL OF DREDGED SEDIMENTS IN SANTOS HARBOR REGION, SP – BRAZIL, AIMING TO ACCESS SYSTEM RESILIENCE

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In the Santos Harbor region, an area managed by the Port Authority and supervised by Brazilian environmental authorities was used for oceanic disposal of dredged sediment from November 2004 to July 2013.

After discontinuation of sediment disposal, four monitoring campaigns were carried out between July 2013 and March 2015 in the disposal site and adjacent areas. Monitoring included sediment and surface water quality, bioaccumulation and rocky shore communities in order to access system resilience.

Sediment and surface water results indicated tendency to quality improvement, being in compliance with regulatory guidelines. In sediment, ecotoxicological and mutagenic tests were also carried out indicating no adverse effect or ecological risk to biota.

Regarding to contaminants bioaccumulation, no significant change in concentration was observed, as expected, since it is not possible to establish a direct correlation between these results and dredging activities.

The most recent campaigns for monitoring rocky shore communities showed considerable diversity, with high percentage of coverings in the monitored areas and low proportion of empty spaces or presence of sediment/sand. It is worth to mention that the main effects of sediment disposal on rocky shore communities are sedimentation, burial and increase of turbidity, processes which occur during disposal activities. Since this activity is discontinued, these impacts no longer exist.

The results obtained in the four monitoring campaigns indicated adequate management and marine environment recovery in the studied area which was once used for disposal of dredged material in Santos Harbor region.

ISTA18-273353 - MORPHOLOGICAL CHANGES IN LARVAE OF ASTYANAX ALTIPARANAE (LAMBARI) INDUCED BY EXTRACTS OF MICROCISTINS PRODUCERS AND NON-PRODUCERS STRAINS OF CYANOBACTERIA

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The species *Astyanax altiparanae* inhabits freshwater Neotropical environments. Larvae of this species were examined as standard test organisms for the evaluation of acute toxicity of cyanobacterial samples. The cyanobacterial extracts were obtained from the MC-LR-producer strain MIRS-4 (*Microcystis panniformis*) and from the non-producer microcystin NPCD-1 strain (*Microcystis aeruginosa*). Extracts were analyzed by LC-MS. Solutions of 0.1, 1.0 and 10.0 µg/mL of pure MC-LR and 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL of MIRS-4 and NPCD-1 extracts were assayed using water as control. Pure MC-LR exhibited no acute toxicity in larvae. For the extracts, larval mortality occurred at all concentrations tested. Larval lethality was higher at concentrations of 0.4 and 0.5 mg/mL for both strains of cyanobacteria, with >90% larval mortality in 96h (p<0.05). At the end of the experiment (96h), the surviving larvae at concentrations of 0.1, 0.2 and 0.3 mg/mL of both cyanobacterial extracts were analyzed in order to verify morphological aspects. Edema, tail and vertebral spine curvature and body weight changes were qualitatively evaluated. These changes were observed for MIRS-4 and NPCD-1 strains. Since no toxicity was observed for pure MC-LR, further mass spectrometric analyses were carried out to determine other compounds produced by cyanobacteria. LC-MS results showed that the MIRS-4 strain also produces MC-YR and MC-RR, however, in small amounts when compared to MC-LR. Strain NPCD-1 is able to produce a series of peptides, including microginins. The analogue 742, containing Tyr and Leu/ILe, was identified (m/z 742). Other secondary metabolites may contribute with toxicity.

ISTA18-752448 - MUTAGENIC PROFILE OF TOTAL ATMOSPHERIC PARTICULATE MATTER (PM) SAMPLES FROM LIMEIRA, STOCKHOLM AND KYOTO USING A LARGE SET OF SALMONELLA STRAINS

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PM mutagenicity is related to both primary and secondary pollutants. Environmental conditions such as temperature and period of solar radiation have great influence both in the degradation of pollutants and the formation of secondary pollutants. Different strains used in Salmonella/microsome assay present different sensitivities to specific mutagens. We aimed to verify if cities with great differences in environmental conditions would have different mutagenicity profiles for a large set of strains. To make direct comparisons possible, all samples were collected, processed, and tested using the same protocols. Organic extracts from pooled total PM samples collected during winter in Limeira, Stockholm and Kyoto were tested using the Salmonella/microsome microsuspension assay with TA1538, TA98, YG5185, YG1041, TA102, TA104, TA100, TA97a and YG7108 strains, without and with metabolic activation system (S9). Despite the differences in the mutagenic potencies observed for the extracts from the evaluated samples, their mutagenic profiles were quite similar. Compounds that induce frameshifts, base-pair substitution, and transitions/transversions mutations are present in all samples. All extracts were mutagenic for all used strains, except for TA102, and with higher potencies without S9. The highest mutagenicity was observed with YG1041 indicating an important contribution of nitro-compounds for the mutagenic responses of all samples. The differences in the environmental conditions among the cities do not seem to affect the PM mutagenic profile. FAPESP (2015/23364-3)

ISTA18-770759 - MUTAGENICITY ASSESSMENT OF SULFONATED NATURAL PHENOLIC LIPIDS USING ASTYANAX LACUSTRIS

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The (re)emergence of various insect-borne diseases causes a great demand for insecticides, mainly synthetic products, that end up contaminating water and soils and causing adverse effects on living beings. Surfactants of petrochemical origin are also extremely used, impacting the environment and human health too. The technical cashew nut shell liquid (tCNSL), an underestimated byproduct released on industrial preparation of cashew nut, and its phenolic lipids has been studied and presented insecticidal and antibacterial activities. In addition, these products have a very similar structure of most common petrochemical surfactants. In this context, in order to control populations of insect vectors in breeding sites and to meet the needs of the population in a sustainable way, the phenolic lipids were sulfonated, obtaining surfactants with larvicidal and antimicrobial activity. The present work aims to evaluate the mutagenic potential of the new compounds in different concentrations through micronucleus assay in *Astyanax lacustris*. Preliminary tests with sulfonated tCNSL were carried out. The fishes were exposed at concentrations of 10 and 100 mg L⁻¹ for 72h at 25±3°C. The results showed no statistical difference between the negative control and the tested concentrations and between them, indicating that sulfonated tCNSL is not mutagenic for *A. lacustris* in these concentrations (P>0.05), a very promising results. However, the tests are being repeated to confirm them and sulfonated phenolic lipids isolated from the tCNSL are also being tested for effect comparison and to assess the viability of their use.

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ISTA18-752449 - NITRO-PAHS PROFILE OF ORGANIC EXTRACTS FROM TOTAL ATMOSPHERIC PARTICULATE MATTER (PM) SAMPLES COLLECTED IN LIMEIRA, STOCKHOLM, AND KYOTO

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Nitro-PAHs can be emitted from combustion sources or formed by photochemical conversion of PAHs. Temperature and the solar radiation govern formation, degradation, and concentrations of nitro-PAHs in PM. Some nitro-PAHs are predominantly formed by atmospheric reactions (e.g. 2-nitrofluoranthene: 2-NF) and others are originated from combustion sources (e.g. 1-nitropyrene: 1-NP). A ratio of 2-NF/1-NP <5 indicates that combustion sources are dominant while a ratio >5 indicates the predominance of the photochemical products. Moreover, 2-NF can be formed by both daytime and nighttime reaction, while 2-nitropyrene (2-NP) is only formed during daytime. A 2-NF/2-NP ratio of 5-10 and >100 suggests that nitro-PAHs are formed in presence and absence of solar radiation, respectively. We aimed to characterize the nitro-PAHs profile in extracts of pooled total PM samples collected during the winter in Limeira, Stockholm and Kyoto. 7 nitro-PAHs including 1-NP, 2-NP, and 2-NF were analyzed by two-dimensional HPLC. The \sum 7 nitro-PAHs (ng/m³) in Limeira was ~ 15 and 13 times higher than in Stockholm and Kyoto. The 2-NF/1-NP ratios obtained for Limeira, Stockholm and Kyoto were 3.8, 33.3 and 10, and the 2-NF/2-NP ratios were 6.6, 33.3 and 20. Those ratios indicate that the nitro-PAHs present in the PM sample from Limeira are mostly originated from combustion and/or formed by daytime reactions. While the ratios obtained for Stockholm and Kyoto indicate that the nitro-PAHs are mostly formed in the atmosphere and by reactions that occur during the nighttime. The results are consistent with the sources of pollution and the environmental conditions of each studied city. FAPESP (2015/23364-3)

ISTA18-896814 - NON-ANIMAL PLATFORM FOR OCULAR TOXICITY CATEGORIZATION OF PESTICIDES

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Non-animal methods for assessing ocular toxicity have been adopted and recommended that companies can use in vitro assays to classify ocular toxicity of chemical mixtures instead of the Draize test in rabbits. However, most of the results obtained with such in vitro methodologies refer to pure substances or to mixtures whose results do not necessarily resemble each other between the methods and therefore do not allow comparisons.

In this context, ocular toxicity tests are required to evaluate the dangers associated with eye exposure to several materials and chemicals. This study used a layered approach combining methods of Short Time Exposure (STE), Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) and Bovine Corneal Opacity and Permeability (BCOP) to suggest a better characterization of the potential for ocular irritation related to the depth of corneal damage of 20 pesticides of different categories as to their potential for eye irritation.

For STE test, SIRC rabbit corneal cells were exposure to pesticides (0.05 or 5%) for 5 min and the cytotoxicity evaluated by MTT reduction. BCOP and HET-CAM methods were performed according to OECD TG 437 (OECD, 2013) and ICCVAM (2010), respectively. For all assays, positive and negative controls were used. Our preliminary results showed that this non-animals platform is able to correctly classify pesticides mixtures, in comparison previous categorization using animals.

ISTA18-212186 - PARHYALE HAWAIENSIS (DANA,1853) (AMPHIPODA: HYALIDAE) POPULATION DYNAMICS: MODELING AND PERFORMANCE ANALYSIS VIA MATLAB® AND SIMULINK®

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Demographic parameters, such as size class structure, abundance trends, sex ratio, net reproductive rate (R_0), generation time (T) and population per capita population growth rate (r) can be used to estimate the increase or decrease in the abundance (N) of populations through mathematical models. Software as MATLAB® and Simulink® allow us to create models that via simple input insertions can evaluate the population abundance behavior integrated in time. Changes in abundance vary according to a species growth rate provided by the model at a given time. For the model validation, published data of demographic parameters of the species *Parhyale hawaiiensis* were used: generation time (T) of 3.51 ± 1.31 months and population growth rate of 0.06 ± 0.17 per capita per month. The logistic growth model obtained indicated that an initial population of 10 individuals would reach the support capacity for the species in a natural environment in 35 to 45 generations, an equivalent period of 130 to 150 months, close to the value found by Alegretti et al. (2016) with field data. Modelling estimated parameters are an important tool in evaluating the efficiency of conservation measures, in estimating the extinction probability of species living under high pressure, as well as in evaluating effects of intrinsic growth rate variations over time due to temporary stresses.

ISTA18-327741 - PERFORMANCE, HISTOLOGICAL PARAMETERS AND SOMATICS INDEXES OF ANTARCTIC FISH (TREMATOMUS NEWNESI, NOTOTHENIDAE) EXPOSED TO WATER-SOLUBLE FRACTION (WSF) OF DIESEL FUEL AND LOW SALINITY

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The elevation of Planet temperature has as consequence the increase of the oceanic evaporation, that will cause substantial changes in the salinity of its waters. Thus, in the polar regions, together with the constant melting of the glaciers, there will be an decreased in salinity. Another problem is the increased risk of diesel fuel spills due to the leakage during the refueling activities of the research stations, as well as the transit of tourist. Therefore, the present study aims to evaluate the impact of diesel fuel contamination associated with the decrease of water salinity in *Trematomus newnesi*, for 15 days. Four experimental groups were established: control group (CTR)- salinity 35‰; Diesel group (35D)- salinity 35‰ and exposure to 1000ppm water-soluble fraction (WSF) of diesel fuel; Low salinity group (25)- salinity 25‰; And Low salinity and diesel group (25D)- salinity 25‰ and 1000ppm WSF exposure. At the end of the experiment the gills, liver and digestive tract samples were collected for histopathological evaluation as well as, biomass data for the evaluation of somatic indices and blood smears. Preliminary results showed that, all groups lost mass during the experiment, and 35D had a greater mass loss than groups 25 ($p < 0.05$) and 25D ($p < 0.01$). The carcass yield and the condition factor of the four experimental groups presented similar values. The viscero-somatic index of the 35D was higher than CTR ($p < 0.05$) and 25 ($p < 0.05$), Moreover, the intestinal coefficient of 35D was higher than 25 ($p < 0.01$). The histopathological evaluations are under analysis, and are necessary to better elucidate the changes observed in these indices.

ISTA18-634720 - PHYSIOLOGICAL ASSESSMENTS OF TOXICITY SENSITIVE SPECIES GROWN IN CAMBISOL WITH INCREASING CONCENTRATIONS OF CADMIUM

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Cadmium (Cd) may present a distinct behavior in soils, which is associated to its characteristics and interactions with other elements. If absorbed and accumulated, it may cause many toxic effects on plants. The photosynthetic apparatus is the most affected by this trace element. In order to better understand the physiological responses of the species submitted to stress caused by Cd, this work aimed to evaluating α chlorophyll fluorescence of species sensitive to this trace element. The experiment was conducted in a greenhouse at Federal University of Lavras-Brazil, according to ISO 11269-2. The four sensitivity species (corn, rice, bean and radish) were cultivated with Cambisol (clay 31%; sand 47%; pH 5.3; P 2.6 mg dm⁻³; K 34 mg dm⁻³; Ca 1.6 cmolc dm⁻³; Mg 0.4 cmolc dm⁻³; Al 0.5 cmolc dm⁻³; CEC in natural pH 2.6 cmolc dm⁻³; V 34 %; SOM 2.9%) in different Cd doses (0.4; 0.72; 1.29; 2.3; 4.1; 13.6; 24.4 mg kg⁻¹). After 21 days it was evaluated the photochemical potential efficiency value of photosystem II (PSII) and the electron transport rate (ETR). Although the visible symptoms were not very evident, due to its attributes, in most of species tested at the concentration considered as a prevention value (0.4 mg kg⁻¹) there were already significant changes. Beans and corn were the most sensitive species and radish, at the highest dose did not differ from control. Thus, we conclude that plants presented metabolic responses specific to each species. Even in low Cd concentrations, the plants presented alterations due to phytotoxicity caused by Cd. The photosynthetic apparatus were altered, leading to negative effects.

ISTA18-419875 - PM LEVELS AND ELEMENTAL COMPOSITION IN THE GREATER AREA OF VOLOS, GREECE DURING THE LIFE+ACCEPT-AIR PROJECT.

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Over the last decades, the city of Volos (central Greece) has been facing air quality degradation, due to urbanization and industrialization. In this context, daily and seasonal variations of PM₁₀ and PM_{2.5}, organic (OC) and elemental carbon (EC) and trace elements concentrations were measured in the urban area of Volos during two month-long campaigns covering warm and cold season of 2011-2012. It was found that both PM₁₀ and PM_{2.5} were quite high, with significant seasonal variation while maximum levels were observed during cold season. Trace elements such as Ca, K, Fe, S and Si exhibited the highest concentrations in both PM fractions. Zn, Mn, Fe and Pb exhibited relatively high concentrations compared to other urban-traffic sites in Greece, but similar concentrations to urban-industrial environments. A large increase in both OC and EC during cold season was observed and OC/EC ratio exhibited very high values, close to 10, suggesting biomass burning emissions. These results point towards contribution of residential heating and biomass burning stoves and/or fireplaces. The project LIFE+ "ACCEPT-AIR" (ENV/GR/000289) is an Environment Policy & Governance project which is funded by the European Commission within the framework of the LIFE Environment programme.

ISTA18-961486 - POTENCIAL EFFECTS OF GLYPHOSATE MIXTURE ON ZEBRAFISH EARLY-LIFE STAGE

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Glyphosate-based herbicides are the most commonly used worldwide because they are effective and relatively nontoxic to nontarget species. Unlimited and uncontrolled use of such pesticides can have serious consequences for human health and ecological balance. Some scientists and regulators assumed that glyphosate is safe for mammals, including humans. However, some of the substances added are occasionally more toxic than the active ingredient itself. We herein investigated the difference between individual and joint embryotoxicity of the constituents of glyphosate-based formulation: active ingredient glyphosate (GLY), surfactant polyethoxylated tallow amine (POEA) and GLY + POEA using early life-stage of zebrafish as a model to justify the interactive effects of these ingredients, comparing with the commercial formulation Glyphosate Atanor 48 (ATN). ATN formulation (LC50-96h 29.91 mg/L glyphosate acid equivalent) was more toxic than GLY alone. Moreover, ATN inhibited embryo hatching (EC50-72h 33.38 mg/L) and caused yolk sac (EC50-72 h 25.40 mg/L) and tail (EC50-72 h 26.71 mg/L) deformities. POEA alone was the most toxic (LC50-96h 3.02 mg/L) of the formulation ingredients. GLY + POEA was more embryotoxic than individual constituents with LC50-96h 0.16 mg/L and EC50-72 h 0.02 mg/L and 0.34 mg/L for hatching inhibition and delay in larval yolk sac absorption, respectively. Therefore, it is essential to clarify combined effects for pesticide regulators and bioassay with zebrafish early life-stage could be used to expedite hazard identification of pesticide formulations and reduce ethical concerns related to animal experimentation.

ISTA18-260658 - PROCESS WATER FROM HYDROTHERMAL CARBONIZATION: TOXICOLOGICAL EVALUATION AND ITS POTENTIAL STIMULATION ON GERMINATION OF MAIZE AND LETTUCE.

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Hydrothermal carbonization (HTC) is a process used to convert biomass in a material with higher carbon content, generating solid material (hydrochar), gaseous phase and aqueous phase called process water, which little is researched about. Studies conducted by the group showed the viability of the use of vinasse and sugarcane bagasse in HTC process. Therefore, was made the characterization of process water, and its toxic effect was evaluated in studies with *Artemia salina* and on germination of seeds. Volatile, semi-volatile and non-volatile compounds, total organic carbon (TOC) and process water nutrients were characterized using GC/MS, TOC analyzer and FAAS, respectively. Toxicity studies of process water were conducted at the concentrations of 50, 100, 250 and 500 mg L⁻¹ of TOC present in process water. Germination experiments were made using 5 replicates for each treatment, with seeds of maize (*Zea mays*) and lettuce (*Lactuca sativa*) using agarose as a growth medium, for 7 days. In these experiments the germination index, percentage of germination, shoot and root length, and biomass yield, were evaluated. Fatty acids (C10, C18), acetic and propanoic acids, furans, phenols, K, Mg, Ca, Mn, P, Fe, Cu and TOC were found in the process water. Acetic acid and phenols in process water probably affected negatively the development of lettuce. However, for maize, the concentration of 250 mg L⁻¹ of TOC in process water stimulated its development. The LC50 for experiments with *Artemia* was 185 mg L⁻¹, using the test of acute aquatic toxicity, classifying the concentrations used as practically non-toxic for *Artemia*. Acknowledgments: FAPESP, CAPES, CNPq.

ISTA18-059893 - RELATION BETWEEN GASTRIC CANCER AND AGRICULTURAL ACTIVITIES: A SYSTEMATIC REVIEW

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Global estimates point stomach cancer as the fifth most common cause of cancer. It contributed with 8.8% of all cancer-related deaths in 2012 worldwide. Pesticide contamination is a problem all over the world. In 2014, Brazil was the first country in global consumption of pesticides, with São Paulo, Paraná, and Minas Gerais accounting for 50% of Brazil's total consumption. The literature on the relationship between gastric cancer and occupational exposure to pesticides presents controversial results. The objective of this study was to conduct a systematic review of the literature, on the relationship between the incidence of gastric cancer and agricultural occupational activity. The methodology consisted in the search for articles in the databases PubMed, Scielo, Cochrane Library, Scopus. The keywords for the search were searched mainly in MeSH (PubMed) and DeCS (Virtual Health Library). The systematic review was based on PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). Inclusion criteria were: human studies on the relationship between gastric cancer or gastric neoplasias and pesticides, agrochemicals and organophosphates. The initial search resulted in 836 articles, from which 99 studies were included. Some articles evidenced a high rate of exposure to pesticides classified as probable carcinogens, as herbicides (2,4-D and trifluralin), insecticides (chlordane and malathion), fungicides (mancozeb and maneb) and acaricides (methyl bromide and propargite) associated with gastric cancer. The results of the present study point to the association between gastric cancer and agricultural occupational activity or the use of agrochemicals and pesticide

ISTA18-911469 - REMOVAL OF EMERGING CONTAMINANTS IN WASTEWATER RECLAMATION PLANT

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The wastewater reclamation plant (WWRP) studied employs a biological treatment followed by Membrane Bio Reactor (MBR) system with ultrafiltration membranes for treated domestic sewage from about 175,000 inhabitants, and produces 70 L s⁻¹ of reclaimed water quality to meet urban purposes, non-potable. The aims of this study was to determine the concentration of atrazine, caffeine, bisphenol A, triclosan, estrone, 17 β -estradiol and estriol in raw sewage, pre-MBR system effluent and the final effluent for obtained the removal efficiency of these compounds in WWRP by monthly sampling held between March 2015 and February 2016 in order to complete a seasonal period. The removal efficiency in WWRP was compared with other conventional wastewater treatment plants (WWTP) located in the same city. The determination of this contaminants was performed using Solid Phase Extraction (SPE) with OASIS HLB (Waters) cartridges and Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) with an electrospray ionization source from Agilent Technologies. The limits of detection (LOD) ranged between 2,8 and 6,5 ng L⁻¹. The results indicated that caffeine removal efficiency was above 99.99 %. The determination of others target compounds in raw sewage was hampered due the strongly matrix effect and these samples are being re-analyzed in order to obtain an estimate of removal efficiencies for all target compounds. Atrazine and bisphenol A also were determine in treated effluents with concentrations between 3.0 and 97 ng L⁻¹. The hormones and triclosan were not found in concentration above LOD in treated effluents.

ISTA18-674488 - SACCHAROMYCES CEREVISIAE IMMOBILIZED ONTO CROSS-LINKED CHITOSAN BEADS: APPLICATION OF A NOVEL MATERIAL FOR REMOVAL OF DYE TOXICITY

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Waste from industrial activities causes serious damage to the environment, among these, we can mention waste effluents discharged by the textile industries. Dyes are the main residues of these effluents, being highly toxic compounds for the environment. The aim of the present study was to improve the adsorption of dyes by *Saccharomyces cerevisiae* by immobilizing their cells on cross-linked chitosan beads, forming a novel material for adsorption of dyes. Toxicity tests were performed before and after treatments with the microcrustacean *Daphnia similis*. Mathematical models of kinetics, isotherm, and thermodynamics were applied to a better interpretation of the obtained data. The results showed that the immobilization of yeast cells made the adsorption more efficient. The kinetic studies can prove that intraparticle diffusion was occurring, where the dye bound internally and externally to the synthesized material. The thermodynamic studies confirmed a spontaneous and endothermic adsorption, being influenced by the increase in temperature. The studies of the isotherms respected the Freundlich model, indicating lateral interactions, the formation of multiple layers, and chemisorption. These results were confirmed by Fourier transfer infrared (FT-IR) spectroscopy. Toxicity studies have confirmed that the treatment is effective in removing almost all toxicity from the solution, reducing the mortality of *D. similis* by more than 90%. Thus, the efficiency of the material synthesized in removing the dye and its toxicity from the aqueous solution can be proven, being a possible solution for the treatment of industrial textile effluents.

ISTA18-784432 - SPENT POT LINER INDUCED DNA DAMAGE ON HUMAN LEUKOCYTES

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Spent Pot Liner (SPL), a toxic solid waste generated in industry from the aluminum mining and processing. It is regarded as an environmental pollution agent when is dumped on environment. Thus, it is important to access the toxicological risk of SPL for exposed organisms. Comet assay is an efficient test to detect genotoxic compounds by DNA damage observation. So, in the present study, the genotoxic potential of SPL was evaluated through comet assay on human leukocytes. After ethics committee approval (COEP – UFLA n°. CAAE 11355312.8.0000.5060), blood aliquots collected from healthy volunteers were exposed to increasing concentrations of SPL (from 0.1 to 80 g L⁻¹). The frequency of DNA damage was determined by visual scores (0 from 4) and the results were expressed on percentage of damage and arbitrary units (AU). CaCl₂ (0.01 M) was applied as negative control (NC) and doxorubicin (10µg mL⁻¹) as positive control (PC). It was observed a dose-dependency between SPL treatments: as SPL concentration for cell incubation increases, the frequency of damage on DNA also increases. Cells incubated on the NC presented nucleoids class 0 to 2, while those exposed to SPL presents nucleoids class 0 to 4. SPL-incubated cells increasing significantly the frequency of nucleoids class 4. For the PC the UA of damage was 267.74, lower than that observed for treatments with highest doses of SPL (40 g L⁻¹ – 287.40 and 80 L⁻¹ – 315.30). In this sense, it was demonstrated the SPL is a genotoxic agent that induces DNA damage on exposed organisms.

ISTA18-679801 - STUDY ON TOXICITY AND BIODEGRADABILITY OF CLEANING EFFLUENTS FROM AN AGRO-INDUSTRIAL COMPLEXS M PALÁCIO¹, S C FABRISI AND D R MANENTI²

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Most of the products used for cleaning in food industry are considered biodegradable. However, the interactions between these products and the residual organic matter from production processes are insufficiently researched. Due to many difficulties faced in biological wastewater treatment systems operation, the aim of the research was to analyze the toxicity and biodegradability of effluents from an agro-industrial complex; as well as the effect of the addition of sanitizers and detergents in these wastewaters. Toxicity was evaluated using the bacteria *Vibrio fischeri*, while the Zahn-Wellens test was used to measure the biodegradability. The equalized effluent generated in slaughter production time, named general production effluent, showed a toxicity factor (FT) equal to 4. The additions of 50.00 mg L⁻¹ of sodium hypochlorite and 1.14 mg L⁻¹ of quaternary ammonium raised the FT of this effluent to 8. The effluent from poultry slaughterhouse cleaning reached the lowest average biodegradability. The addition of products with the active ingredients peracetic acid, linear alkylbenzene sulphonate, sodium lauryl ether sulfate and nonylphenol ethoxylate interfered at different stages of the general production effluent biodegradability test. The results show that the addition of cleaning products has a negative effect on the toxicity and biodegradability of effluents with similar characteristics to those studied.

ISTA18-987401 - THE REGULATION OF NANOPESTICIDES IN BRAZIL, US AND UE: A COMPARATIVE CASE STUDY OF NANOCID®

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Currently there is no specific nanomaterials legislation in Brazil. The House of Representatives is debating a bill nº 6741/2013 (Regulatory Framework for Nanotechnology), but it is widely criticized by the scientific community, which fears the stagnation of researches and commercial development. EU legal acts have incorporated a nanomaterial definition: nº 1169/2011 and nº 10/2011 on food information to consumers and plastic food contact materials; nº 1223/2009 and nº 528/2013 on cosmetic and biocidal products. In the US, the Environmental Protection Agency (EPA) is responsible for regulating pesticides under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). No specific provisions for nanomaterials are available now under this regulation. However, as the regulatory debate continues, new products have been launched on the world market, including nanopesticides, such as Nanocid®, and Nanosilva (NSPW-L30SS) with silver nanoparticles, both marketed in the US. Technological developments grow fast and reports on new and potential applications of nanomaterials are not always accompanied by thorough/uniform physico-chemical characterization of the nanomaterial, nor by a complete risk assessment including exposure and hazard assessment. However, such information is vital to develop safe applications. While pesticides can be tightly regulated, it might be required some modifications to address the special considerations about nanotechnology. Governments and agencies need to make without delay a complex decision about the suitability of existing regulatory systems and nanotechnology regarding human health and environmental protection.

ISTA18-112948 - THE USE OF SALMONELLA/MICROSSOME DIAGNOSTIC STRAINS IN THE EVALUATION OF MUTAGENICITY OF ESTUARINE AND MARINE SEDIMENTS

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Port activities can pose risk to marine ecosystems because of industrial discharges, accidental spills and especially during sediment dredging activities of the channel to allow boats traffic. Another source of contamination of marine coasts is submarine outfalls. For example, in Santos bay 12 m³/s of chlorinated pre treated sewage is daily released. Persistent contaminants tend to accumulate in the sediments and can be available to the resident biota. As complementary to chemical analysis the Salmonella/microsome assay (Ames test) has been used to evaluate the presence of mutagenic compounds. We selected Santos, SP, Brazil collect sediment samples, both in port under dredging activities as in the bay under the influence of the outfall. The aim of this study was to verify the applicability of diagnostic strains in the characterization of the mutagenicity of selected sediment samples. They were dried, extracted by ultrasonication, with methanol and dichloromethane (2.5:1) and cleaned up with cooper for sulfur removal. The protocol of the Ames test used was the MPA (Microplate Agar). Different strains, TA98, YG1041, YG5185, TA100, TA1535 and YG7108 that are sensitive to specific classes of mutagens were used. Sediments under the influence of the emissary of Santos presented negative responses for the 6 strains both, with and without of metabolic activation (S9) but the higher concentrations testes presented toxicity. Fractionation of those samples are under way to allow the detection of the mutagens. Sediment amples from the dredging activities of Santos port were collected and prepared as described above and mutagenicity tests are being conducted.

ISTA18-011577 - TOXICITY AND GENOTOXICITY ASSESSMENT OF COASTLINE SEDIMENTS OF THE RÍO DE LA PLATA ESTUARY (ARGENTINA) UNDER THE INFLUENCE OF HEAVY METALS

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The Río de La Plata estuary represents the most important estuarine environments of the America continent. The coastline is deeply modified by urban and industrial settlements and is heavily polluted by organic compounds and heavy metals. In this work, the toxicity and genotoxicity of sediments and sediment pore water from the Argentina coastline Río de La Plata estuary were assessed. Samples from 14 sites along 190 km river coastline were tested directly (pore water) or subjected to acidic extraction. The extracts (pH 4.93±0.05) were obtained by shaking for 24 h using a buffered solution. Quantitative analysis of metals (Cd, Cr, Cu, Pb and Zn) in sediments and extracts was performed. Toxicity was assessed using the green alga *Pseudokirchneriella subcapitata*, and genotoxicity was assessed through using the *Salmonella typhimurium* Ames test. The heavy metal concentrations were: Cd (1.120-4.965), Cr (9.150-1614.260), Cu (6.775-205.700), Pb (15.670-399.320) and Zn (34.190-222.475) in sediments (mg Kg⁻¹), and Cd (0.001-0.023), Cr (0.010-0.100), Cu (0.010-0.146), Pb (0.024-0.304), Zn (0.035-0.994) in the extracts (mg L⁻¹). The pore water samples showed stimulation of algal growth, probably due to the high nutrient concentrations. However, the acidic extracts were highly toxic to the algae, showing EC50 values between 10% and 30% extract concentration. The pore water samples and the extracts were not genotoxic to *S. typhimurium*, neither for TA98 nor TA100 strains. The results show that the analysis of sediment pore water samples and sediment extracts together with bioassays may be suitable tools to characterize the toxic potential sites in the Rio de la Plata coastline.

ISTA18-574976 - TOXICOLOGICAL TESTING RATIONALE FOR ACID AND/OR ESTER FORMS OF AN AGROCHEMICAL: ARYLEX™ AS A CASE STUDY

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Many agrochemicals exist in multiple forms of acid, esters or salts e.g., 2,4-D, triclopyr, etc. In such cases, a scientific rationale following the principles of 3Rs (Replacement, Reduction and Refinement) in animal research has been applied for toxicity testing.

A common scenario is the ester/salt form of a chemical is hydrolysed in mammals into the acid form so the systemic exposure is to the acid form, that ester/salt/acid forms are bio-equivalent. In that case, the toxicological package can be developed on acid form, while the ester/salt form can be tested in select (e.g., subacute) studies to determine toxic equivalence. As a result, each ester/salt does not need animal-intensive like carcinogenicity or reproductive studies. Appropriate Reference Doses (RfDs) can be determined from the package for human risk assessments.

Halauxifen-methyl (Arylex™), a new herbicide, is rapidly and completely metabolised into halauxifen acid in rats, therefore, they were bio-equivalent. Consequently, acid form was tested for all toxicity studies. Ester and acid forms were equivalent for acute-, geno- and developmental-toxicity but not for target organ toxicity. Ester form caused liver while acid form (at relatively high doses) caused kidney toxicity.

Tox-non-equivalence was addressed by a series of molecular studies elucidating liver Mode-of-Action (MoA) caused by halauxifen-methyl. It was found that ester form at high doses activates nuclear receptors but acid form does not. A weight-of-evidence and MoA-based RfDs was determined for human risk assessments. Importantly, this approach has been ratified by regulatory agencies globally (US, Canada, EU, China, Australia and New Zealand).

ISTA18-396921 - USE OF NEUTRAL RED RETENTION TIME ASSAY FOR THE RAPID ASSESSMENT OF CELLULAR STRESS INDUCED BY DICHLOFLUANID ON THE HAEMOCYTES OF BROWN MUSSELS (PERNA PERNA)

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In order to protect submerged structures from biofouling, biocides, such as dichlofluanid, have been applied as active principles of anti-fouling paints. These substances may be released to the aquatic environment and cause toxic effects and risks to the biota. There are few systematic studies on the occurrence and effects of these biocides for the Brazilian coast. Therefore, studies are needed to evaluate the potential effects of such substances on non-target aquatic organisms. Biomarkers are capable to evaluate the toxic and metabolic effects of contaminants at lower levels of organization and they can be used as an early warning of further toxic effects. The neutral red retention time (NRRT) relies on the fact that healthy lysosomes retain the dye for longer than stressed ones. This study aimed to evaluate the cellular stress caused by different concentrations of dichlofluanid on the haemocytes of mussel *Perna perna*. Dichlofluanid solutions were prepared at different concentrations (0.01; 0.1; 1; 10; 100 µg/L) and mussels were exposed in laboratory for 96 hours; controls consisted on not contaminated water and a solvent control (0.05% acetone). Five animals of each concentration were analyzed to the NRRT assay after 48 and 96 hours of exposure. The NRRT at the end of the bioassay was significantly lower ($p < 0.05$) for the animals exposed at 10 µg/L (retention time mean = 33 min) and 100 µg/L (retention time mean = 15 min) when compared to the solvent control (retention time mean = 97.5 min) after 96 hours. The dichlofluanid is able to destabilize lysosomal membrane and could cause damage in sub-cellular levels at concentrations higher than those found in the environment (<0.6 µg/L).

ISTA18-515190 - USE OF VINASSE IN THE PRODUCTION OF BIODEGRADABLE PECTIN BEADS AS AN ALTERNATIVE SOIL FERTILIZER

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The use of vinasse, the main wastewater from ethanol industry, in fertigation is common in the areas of sugar cane production. This practice aims to enhance soil fertility through recycling of vinasse nutrients reducing the amounts of chemical fertilizers. However, its indiscriminate use can cause several environmental problems such as soil salinization and groundwater contamination. The direct application of large volumes of vinasse to the soil may lead to deficiencies in the absorption of nutrients by the plants, since excess nutrients can become toxic to soil and groundwater. As an alternative to fertigation and as a mean of extending the use of vinasse in crops far from the ethanol industry areas, this study proposes the use of vinasse, as the solvent, in the production of pectin beads. Pectin is a natural polysaccharide, derived mainly from citrus processing. It is soluble in acidic solution, and due to its good gel forming properties, biodegradability and non-toxicity is a promising biopolymer to controlled/slow release applications. The physical-chemical properties of the obtained pectin/vinasse beads was evaluated. Vinasse had its pH lowered and pectin (3-7% m/v) was added. The solution was dropped into ethanol with calcium chloride (1-1.5% m/v) and the formed beads were stirred for 30 min and dried at 30°C/24h. Beads were characterized by their size, moisture content, solubility in water and chemical composition. Pectin/vinasse beads (5% pectin/ ethanol + CaCl₂ 1%) were spherical with average diameter of 2.6 mm, 15% moisture and 25% solubility in water. These beads are an interesting alternative to the use of vinasse as fertilizer.

ISTA18-033401 - VERMIREMEDIATION OF CONTAMINATED SOIL FROM A COMPLEX OF CERAMIC INDUSTRIES USING EARTHWORM EISENIA FETIDA

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Among bioprocessing technologies, vermiremediation has been utilized as an alternative choice to stabilize various kinds of sediments, including solid wastes and contaminated soils. This work, therefore, aims to determine better conditions for use of earthworm *Eisenia fetida* for bioremediation of soil from a ceramic industrial area contaminated with Cadmium (Cd) and Chromium (Cr). First, laboratory tests were conducted to compare the effects on earthworm *E. fetida* growth, survival, morphology, behavior and reproduction rates of six concentrations (0, 6.25, 12.5, 25.0, 50.0 and 100%) of contaminated soil mixed in artificial soil and cow dung during 28 days of incubation. Second experiment consisted of employing earthworm *E. fetida* in a predetermined LOEC concentration to removing heavy metals from superficial soil collected from a ceramic industrial area during 56 days of vermistabilization. According laboratory tests, earthworms maintained at 6.25% of contaminated soil showing increase in mean weight (27%) and bioaccumulation of Cd (2.32 mg/Kg) and Cr (25.37 mg/Kg), resulting in a significant decrease in the level of Cd (25%) and Cr (31%) from soil. At field simulated condition, vermiremediation confirms same results obtained from laboratory incubation and its results indicated increasing in mean weight (41%), high load of Cd (2.33 mg/Kg) and Cr (26.66 mg/Kg) on tissues of earthworms, causing the significant decrease in soil levels of Cd (33%) and Cr (38%). In conclusion, at relevant environmental tested concentration, vermiremediation of contaminated soil from ceramic industrial area using earthworm *E. fetida* is an effective technology for bioremediation.

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