Sorption of PAHs to humic acid- and iron(III)carbonate particles by using passive dosing vials for investigating the transport of organic contamination in stormwater runoff

Nielsen, Katrine; Mikkelsen, Peter Steen; Baun, Anders; Eriksson, Eva

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3RD YOUNG ENVIRONMENTAL SCIENTISTS MEETING KRAKÓW POLAND 11-13 FEBRUARY 2013

"INTERDISCIPLINARY DISCOURSE ON CURRENT ENVIRONMENTAL CHALLENGES"

11 - 13 February 2013

Students Advisory Council
Speaking tube of SETAC Europe students
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<th>Monday, 11 February 2013</th>
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<td>09:00-11:00</td>
<td>Registration (Next to Aula)</td>
</tr>
</tbody>
</table>
| 11:00-13:00 | Workshop Part I (Room P.01/2/3)  
               Valery Forbes - The Essentials of Scientific Networking |
| 13:00-14:00 | Lunch (Level -1) |
| 14:00-16:30 | Workshop Part II (Room P.01/2/3)  
               Valery Forbes - The Essentials of Scientific Networking |
| 16:30-17:00 | Coffee Break (Level -1) |
| 17:00-18:00 | Career Talk I (Room P.1.1)  
               Sue-Martina Starke (German Federal Environment Agency) |
| 18:00-19:00 | Opening Ceremony (Aula) |
| 20:00 | Get Together |

<table>
<thead>
<tr>
<th>Day 2</th>
<th>Tuesday, 12 February 2013</th>
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</table>
| 09:00-10:40 | Aquatic Ecotoxicology (P.1.1)  
              J. Zubrod, T. Floehr, V. Knezevic, D. Englert  
              Environmental Chemistry (P.1.2)  
              M. Brinkmann, C. Schür |
| 10:40-11:10 | Coffee Break (Level -1) |
| 11:10-12:50 | Aquatic Ecotoxicology (P.1.1)  
              J. Zubrod, T. Floehr, V. Knezevic, D. Englert  
              Environmental Chemistry (P.1.2)  
              M. Brinkmann, C. Schür |
| 12:50-13:50 | Lunch (Level -1) |
| 13:50-15:30 | Aquatic Ecotoxicology (P.1.1)  
              J. Zubrod, T. Floehr, V. Knezevic, D. Englert  
              Life Cycle Assessment  
              SESSION CANCELED |
| 15:30-16:00 | Coffee Break (Level -1) |
| 16:00-17:00 | Career Talk II (Room P.1.1)  
              Matthias Bergtold (BASF) |
| 17:00-18:30 | Poster Social (Room P.01/2/3) |
| 20:00 | Conference Dinner |
### Day by day schedule (cont.)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 1</th>
<th>Session 2</th>
</tr>
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</table>
| 09:00-10:40 | Nanoparticles (P.1.1)  
D. Kaiser, M. Weil | Terrestrial Ecotoxicology (P.1.2)  
D. Chmolowska, H. Azarbad |
| 10:40-11:10 | Coffee Break (Level -1) |                                                 |
| 11:10-12:50 | Nanoparticles (P.1.1)  
D. Kaiser, M. Weil | Effects and Exposure Modelling (P.1.2)  
D. Jevtić |
| 12:50-13:50 | Lunch (Level -1) |                                                 |
| 13:50-14:50 | Career Talk III (Room P.1.1)  
Alistair Boxall (University of York) |                                                 |
| 14:50-16:30 | Omics and Biomarkers (P.1.1)  
M. Brinkmann, E. Brockmeier | Ecological Risk Assessment (P.1.2)  
M. Melato, J. Zubrod |
| 16:30-17:30 | Closing Ceremony (Aula) |                                                 |

Meet our sponsors during the meeting at the Job Corner
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Room P.1.1</th>
<th>Room P.1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>Schmidt et al., Galway-Mayo Institute of Technology, Ireland</td>
<td>Evaluation of effects of the pharmaceuticals diclofenac and gemfibrozil on marine mussels (Mytilus spp.). Evidence for chronic sublethal effects on stress-response proteins</td>
<td>Poma et al., IRSA-CNR Water Research Institute, Italy</td>
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<td></td>
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<td></td>
<td>Novel brominated flame retardants (NBFRS) contamination in sediments from Lake Maggiore basin</td>
</tr>
<tr>
<td>09:25</td>
<td>Englert et al., University of Koblenz-Landau, Germany</td>
<td>Varying wastewater dilution in receiving streams - implications for stream ecosystem structure and function</td>
<td>Mueting &amp; Lydy, Southern Illinois University, USA</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Fate of a transgenic insecticidal protein, a pyrethroid insecticide, and neonicotinoid insecticides within a maize agricultural ecosystem</td>
</tr>
<tr>
<td>09:50</td>
<td>Dimitrov et al., Wageningen University, The Netherlands</td>
<td>Effects of the fungicide tebuconazole on fungal and bacterial communities in the sediment of outdoor freshwater microcosms</td>
<td>Schür et al., RWTH Aachen University, Germany</td>
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<tr>
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<td>Kinetics for membrane dialysis extraction of pyrene, phenanthrene and chrysene from n-hexane and cow milk</td>
</tr>
<tr>
<td>10:15</td>
<td>Rico et al., Wageningen University, The Netherlands</td>
<td>Direct and indirect effects of the antibiotic enrofloxacin on tropical freshwater microcosms</td>
<td>Vierke et al., German Federal Environment Agency, Germany</td>
</tr>
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<td>Fate of short chain perfluorinated carboxylic and sulfonic acids in a water-saturated sediment column investigated under near-natural conditions</td>
</tr>
<tr>
<td>10:40</td>
<td>Coffee break</td>
<td></td>
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</tr>
<tr>
<td>11:10</td>
<td>Peric et al., University of Barcelona, Spain</td>
<td>Aquatic and cellular toxicity of ionic liquids and their potential biodegradability in water</td>
<td>Ochiai et al., Ehime University, Japan</td>
</tr>
<tr>
<td></td>
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<td>Transfer and distribution of hydroxylated polychlorinated biphenyls (OH-PCBs) in the brain of finless porpoises (Neophocaena phocaenoides)</td>
</tr>
<tr>
<td>11:35</td>
<td>Biermans et al., Belgian Centre for Nuclear Research, Belgium</td>
<td>Biological effects induced in Arabidopsis thaliana after aquatic exposure to radioactive contaminants</td>
<td>Kurtz et al., University of Koblenz-Landau, Germany</td>
</tr>
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<td>Effects of olive oil production wastewater on soil arthropods in two different cultivation scenarios in Israel and Palestine</td>
</tr>
<tr>
<td>12:00</td>
<td>Knežević et al., University of Novi Sad, Serbia</td>
<td>Sensitivity and recovery potential of Lemna minor after exposure to herbicide mixtures</td>
<td>Cesar et al., Fluminense Federal University Niterói, Brazil</td>
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<td></td>
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<td>Distribution of mercury, copper and zinc in soils and fluvial sediments from an abandoned gold mining area in southern Minas Gerais state, Brazil</td>
</tr>
<tr>
<td>12:25</td>
<td>Wolf et al., Goethe University Frankfurt, Germany</td>
<td>When predictions go wrong: mixture toxicity of a repellent and a pyrethroid on aquatic invertebrates</td>
<td>Torres, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Switzerland</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Simplify your sediment pore water analysis</td>
</tr>
<tr>
<td>12:50</td>
<td>Lunch</td>
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<table>
<thead>
<tr>
<th>Session</th>
<th>Room P.1.1</th>
<th>Room P.1.2</th>
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<tbody>
<tr>
<td><strong>Aquatic Ecotoxicology</strong>&lt;br&gt;J. Zubrod, T. Floehr, V. Knezevic, D. Englert</td>
<td><strong>Life Cycle Assessment</strong>&lt;br&gt;SESSION CANCELED</td>
<td></td>
</tr>
<tr>
<td><strong>13:50</strong></td>
<td>Tassou &amp; Schulz, University of Koblenz-Landau, Germany</td>
<td>Riechkof &amp; Günther, TU Dresden, Germany</td>
</tr>
<tr>
<td></td>
<td>Environmentally-relevant tebufenozide concentrations affect reproduction in the freshwater midge <em>Chironomus riparius</em> in a chronic toxicity test</td>
<td>Carbon footprint for the joint production of a wood-based product and its by-product - A case study</td>
</tr>
<tr>
<td><strong>14:15</strong></td>
<td>Di Paolo et al., Swiss Centre for Applied Ecotoxicology Eawag-EPFL, Switzerland</td>
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<tr>
<td></td>
<td>Can the sensitivity and predictive potential of zebrafish early life stage (ELS) tests be improved by additional endpoints and chemical analysis?</td>
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<tr>
<td><strong>14:40</strong></td>
<td>Vignet et al., IFREMER, France</td>
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<tr>
<td></td>
<td>Long term effects of an early and continuous exposure to PAHs on zebrafish behavioral responses</td>
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<tr>
<td><strong>15:05</strong></td>
<td>Le Bihanic et al., University of Bordeaux, France</td>
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<tr>
<td></td>
<td>Comparative effects of three PAH fractions from light and heavy crude oils and from a PAH-contaminated sediment on Oryzias latipes Japanese medaka early life stages</td>
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### Platforms, Wednesday, 13 February 2013

<table>
<thead>
<tr>
<th>Session</th>
<th>Room P.1.1</th>
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<tbody>
<tr>
<td><strong>Nanoparticles</strong>&lt;br&gt;D. Kaiser, M. Weil</td>
<td><strong>Terrestrial ecotoxicology</strong>&lt;br&gt;D. Chmolowska, H. Azarbard</td>
<td></td>
</tr>
<tr>
<td><strong>09:00</strong></td>
<td>Stevenson et al., Ecology, Evolution and Marine Biology, University of California, USA</td>
<td>Musso et al., University of Aveiro, Portugal</td>
</tr>
<tr>
<td></td>
<td>The effect of titanium dioxide nanoparticles on freshwater organisms</td>
<td>Invasive vs. native grasses in Cerrado (Brazilian savanna): physiological and morphological responses to a mosaic of environmental conditions</td>
</tr>
<tr>
<td><strong>09:25</strong></td>
<td>Ribas et al., Federal University of Paraná, Brazil</td>
<td>Pariyar &amp; Burkhardt, University of Bonn, Germany</td>
</tr>
<tr>
<td></td>
<td>Toxic effects of lead and nanoparticles mixed in anterior kidney cell cultures from freshwater fish</td>
<td>Effects of aerosol particles on crop plants</td>
</tr>
<tr>
<td><strong>09:50</strong></td>
<td>Seitz et al., University of Koblenz-Landau</td>
<td>Srećković et al., University of Novi Sad</td>
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<tr>
<td></td>
<td>Product and size specific ecotoxicity of titanium dioxide nanoparticles to <em>Daphnia magna</em></td>
<td>Extremely low frequency (50 Hz) electromagnetic field exposure alters nutritive stress response in <em>Eisenia fetida</em> (Lumbricidae)</td>
</tr>
<tr>
<td><strong>10:15</strong></td>
<td>Ramskov et al., Roskilde University, Denmark</td>
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<td>Nanoparticle shape affects bioaccumulation and toxicity in a deposit-feeding snail</td>
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<tr>
<td><strong>10:40</strong></td>
<td>Coffee break</td>
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<tr>
<td>Session</td>
<td>Room P.1.1</td>
<td>Room P.1.2</td>
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</tbody>
</table>
| 11:10   | **Nanoparticles**  
D. Kaiser, M. Weil | **Effects and exposure modeling**  
D. Jevtić, A. Agatz |
| Walter et al., ECT Oekotoxikologie GmbH, Germany | Daniels et al., RWTH Aachen University, Germany |
| Acute and chronic effects of magnetite-based nanocomposites on invertebrates (*Hyalella azteca* and *Chironomus riparius*) and zebrafish embryos (*Danio rerio*) | Comparison of mechanistic models and standardized regression analyses to describe toxic effects in ecotoxicology |
| 11:35   | **Burkart et al., TU Dresden, Germany**  
A novel method for the determination of effects of nanomaterials on organisms related to waste water treatment plants | Qiu et al., Leiden University, The Netherlands  
Predicting copper toxicity in different ecotypes of earthworms based on biotic ligand model concept |
| 12:00   | **Waailewijn-Kool et al., VU University, The Netherlands**  
The effect of pH on the toxicity of ZnO nanoparticles to *Folsomia candida* in amended field soil | Zimmer et al., Vrije Universiteit Amsterdam, The Netherlands  
Interaction between food and toxicant leads to hormesis in the pond snail *Lymnaea stagnalis* |
| 12:25   | **Sovová et al., Institute of Chemical Technology, Prague, Czech Republic**  
Natural and artificial organic substances alter algal toxicity of nano CeO2 | Bui et al., RWTH Aachen University, Germany  
Food dependent life cycle parameters of *Nitocra spinipes* – implications to extrapolate effects to population level |

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<td><strong>Lunch</strong></td>
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<thead>
<tr>
<th>Session</th>
<th>Room P.1.1</th>
<th>Room P.1.2</th>
</tr>
</thead>
</table>
| 14:50   | **Brockmeier et al., University of Florida, USA**  
Evaluating the impacts of androgen exposure on Eastern Mosquitofish (*Gambusia holbrooki*) global hepatic gene expression patterns using a custom microarray | Nybom et al., University of Eastern Finland, Finland  
Responses of *Lumbriculus variegatus* to activated carbon amendments |
| 15:15   | **Rock et al., Helmholtz Zentrum München, Germany**  
Catecholamines and other biomarkers in stressed and non-stressed amphipods | Bluhm et al., RWTH Aachen University  
Potential biofuels in an ecotoxicological investigation |
| 15:40   | **Ogunkeyede et al, University of Nottingham, UK**  
The characterisation of crude oil and oil contaminated soil from the Niger Delta by catalytic hydropyrolysis | Diepens et al., Wageningen University, The Netherlands  
Optimizing sediment conditions for macrophyte testing in the context of prospective risk assessment |
| 16:05   | **Mikowska & Świergosz-Kowalewska, Jagiellonian University, Poland**  
Molecular biomarkers as indicators of bank vole populations’ response to metal pollution | Peters et al., University of Koblenz-Landau, Germany  
Effects of anthropogenic pollutants on ecosystem functions in freshwater bodies – A Review |
Floor plan

Ground floor

First floor
Dear participants and guests,

We are very pleased to welcome you to the 3rd Young Environmental Scientists (YES) Meeting, organized by the SETAC Europe Student Advisory Council (SAC).

Taking into account that the promotion of young scientists has to be the first priority in a sustainably operating scientific community, the SAC was created in 2006 as the speaking tube of SETAC Europe’s students. Since then we consider it our responsibility to support young researchers in the fields of ecotoxicology, environmental chemistry, and life cycle assessment to become well-networked and self-confident scientists.

Besides many other activities aimed to achieve this goal, the SAC started a series of student-only congresses, i.e. the YES Meetings. These conferences allow the most promising SETAC students to meet in a relaxed atmosphere, expand their personal networks, and further develop scientific and soft skills crucial to effectively serve SETAC’s goal “Environmental Quality through Science”. The first two meetings of this kind were organized at the University of Koblenz-Landau (2009) and RWTH Aachen University (2011) in Germany and were overwhelmingly positively received. We thus consider it our obligation to follow this path, and are glad to welcome you this time at one of the oldest universities in Europe, the Jagiellonian University in Krakow.

Traditionally, on the first day of the meeting the participants are provided with a soft skills training, and this year it is our pleasure to welcome Valery Forbes (University of Nebraska-Lincoln), giving a workshop on the essentials of scientific networking. Furthermore, on each of the three meeting days another senior scientist will provide insights into her/his personal career, as well as information on crucial skills required for careers in science. In this regard, we are also pleased to welcome Sue-Martina Starke (German Federal Environment Agency), Matthias Bergtold (BASF) and Alistair Boxall (University of York).

The primary aim of this meeting’s scientific program is – like it was for the precursor meetings – to discuss highly topical relevant emerging environmental issues (along with those traditionally covered by SETAC) in eight sessions. After each platform presentation (lasting 15 minutes) the auditorium will have enough time to both discuss the results and suggest improvements concerning presentation layout and speaker’s performance. During a poster social similar interactions are intended.

Last but not least, we would like to draw your attention to our numerous sponsors. This meeting – with no conference fees and travel grants for all participants – would not have been possible without the financial support of SETAC, universities, companies and private persons. We highly acknowledge this promotion of the next generation of researchers and encourage participants to meet some of our sponsors at the Job Corner.

Hoping that we have once again prepared an interesting scientific and social programme, we wish you a successful and productive meeting. Have a good time!

Dragan Jevtić     Jochen Zubrod
Chair of the Local Organization Committee   Chair of the Student Advisory Council
Welcome
by heads of Institute of Environmental Sciences and Faculty of Biology and Earth Sciences of the Jagiellonian University

Dear Participants of the 3rd YES Meeting,

It is our pleasure to welcome you at the Institute of Environmental Sciences and the Third Campus of the Jagiellonian University. The campus is still under construction, and the whole area undergoes massive infrastructure development. We believe that, on a longer run, this will bring us one of the most modern university campuses in Europe, accompanied with a large “Technology Park”, growing just outside our windows. To make use of these developments, we are constantly seeking for new partners in the area of scientific research and expanding our network. Our international PhD programme has grown significantly in the past few years, with many young scientists from all over the world coming to our Institute to conduct their research. We believe that supporting and encouraging young researchers should be one of the priorities of the modern-day scientific community, and we are proud to host such a unique event as the YES meeting, enabling our young colleagues to meet in a relaxed atmosphere, learn from experienced scientists - and all that without being limited by ever-present financial constraints.

The theme of the meeting, “Interdisciplinary discourse on current environmental challenges”, reflects SETAC’s global policy by bridging the gaps between different scientific disciplines, and all for the benefit of protecting the environment. Additionally, we welcome unique soft-skills training provided to the participants, and strongly believe that it will help in creating a generation of self-confident and successful scientists.

Apart from science, we would like to stress that Kraków is a city with a very rich history. It has been the capital of Poland for centuries, and today is one of the most visited cities in the region. Its historical sites, museums, and art galleries are definitely worth a visit. On top of that, with 200.000 students and over 8 million tourists a year, Kraków is a city that never sleeps. Whatever you are after in your free time - history, art, restaurants, clubs - you will not be disappointed.

Finally, we would like to thank the European Student Advisory Council and Local Organising Committee from our University for investing a lot of time and effort to make this possible.

We wish you all a successful and interesting meeting!

Maria Niklińska
Małgorzata Kruczek
Director of the Institute of Environmental Sciences
Dean of the Faculty of Biology and Earth Sciences of the Jagiellonian University

10
Welcome by the SETAC Europe President

Dear SETAC Young Environmental Scientists (YES),

Welcome to the YES meeting in Krakow, Poland. This is the third meeting organized by the SETAC Europe Student Advisory Council (SAC) and the first outside Germany, under the motto “Interdisciplinary Discourse on Current Environmental Challenges.”

As President of SETAC Europe, I am very proud of what the SAC is doing to support SETAC Europe’s student members with networking and becoming self-confident scientists.

The first two YES meetings were both a great success, with very high quality science and many opportunities for attendees to learn from pairs and mentors and to establish fruitful contacts. I am pretty sure that the Krakow meeting will be a success as well, with 110 presentations (talks and posters) in 8 sessions. Thanks to the generous sponsors and the contribution of SETAC Europe, the meeting is not only free of charge but travel grants are also available for all the participants.

This type of meetings does not happen without dedicated people and a lot of effort. SETAC Europe is particularly grateful to Jochen Zubrod, Chair of the Student Advisory Council, and to the Scientific Committee: Michael Melato (chair), Markus Brinkmann (co-chair), Dragan Jevtić, Jochen Zubrod, Tilman Floehr, Annika Agatz, Varja Knežević, Grazia Barberio, Dominic Englert, Mirco Weil and, especially to the Local Organizing Committee from the Jagiellonian University: Dragan Jevtić (chair), Agata Tarasek, Natnael Tesfaye Hamda, Katarzyna Stępień, Magdalena Mikowska, Michel Asselman, Zmnako Awrahman.

I am really confident that you all participants will have a very positive meeting and you will come back home richest than when you left, richest of knowledge, contacts, experience and, last but not least, friends.

Looking forward to meeting most of you in Glasgow at the SETAC Europe Annual meeting,

Best wishes,

Paolo Masoni
President SETAC Europe
Welcome
by the Scientific Committee

Indeed it is a wonderful honour and privilege to welcome you all at this occasion arranged by the SAC (Student Advisory Council) under the umbrella of SETAC Europe (Society of Environmental Toxicology and Chemistry). This meeting is organized specifically for young environmental scientist coming from every part of the world. No registration fee is charged and travelling expenses are reimbursed for every participant.

The YES-Meeting grants the young scientists with an opportunity to pause for a moment and to reflect back on their journey within the research community. The environment provides young scientists the platform to present their own work being followed by extensive discussions. A communication workshop will be held, equipping the participants with soft skills necessary for a successful career in science. Furthermore, established senior scientists representing academia, business and government will give career talks. These talks provide insights in opportunities as well as challenges associated with different career paths. Additionally, a job corner is provided for our scientists to get in contact with potential future employers. The atmosphere offers ample opportunities, to exchange the ideas, set a path for research collaboration and further investigations in science with others and to share experiences. Eventually, you can learn to understand others customs and cultures on a social level.

In the first YES initiative meeting in 2009 we said: “New challenges in environmental science” in Landau, Germany. In 2011: “Environmental challenges in a changing world”, in Aachen, Germany. On the same note, we now proudly announce the 3rd Young Environmental Scientists (YES) meeting under the motto “Interdisciplinary Discourse on Current Environmental Challenges” in Krakow, Poland.

The successes of these meetings are honoured by numerous sponsors. It would have been an impossible mission to have all our participants worldwide to this meeting with full support of travelling fees. We acknowledge every helping hand, time and effort.

The scientific committee wishes you all an excellent and productive meeting. You are very welcome. Take the advantage of this special event.

Aupaki Michael Melato
Chair of the Scientific Committee
Cape Town, South Africa

Markus Brinkmann
Co-chair of the Scientific Committee
Aachen, Germany
Acknowledgements
Sponsors of the 3rd Young Environmental Scientists Meeting

Private sponsoring: Mirco Bundschuh
Acknowledgements

Local organizing committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dragan Jevtić (chair)</td>
<td>Dragan Jevtić</td>
</tr>
<tr>
<td>Agata Tarasek</td>
<td>Agata Tarasek</td>
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<td>Katarzyna Stępień</td>
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<td>Magdalena Mikowska</td>
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<td>Michel Asselman</td>
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<td>Zmnako Awrahman</td>
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<td>Terézia Horváthová</td>
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Scientific committee

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<tr>
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<tr>
<td>Michael Melato (chair)</td>
<td>Cape Peninsula University of Technology, South Africa</td>
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<tr>
<td>Markus Brinkmann (co-chair)</td>
<td>RWTH Aachen University, Germany</td>
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<td>Dragan Jevtić</td>
<td>Jagiellonian University, Poland</td>
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<td>Jochen Zubrod</td>
<td>University of Koblenz-Landau, Germany</td>
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<td>Tilman Floehr</td>
<td>RWTH Aachen University, Germany</td>
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<td>Annika Agatz</td>
<td>University of York, United Kingdom</td>
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<td>Varja Knežević</td>
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<td>Grazia Barberio</td>
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<td>Dominic Englert</td>
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<td>Mirco Weil</td>
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<td>C. Schür</td>
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<td>B. Salieri</td>
<td>University of Bologna, Italy</td>
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<td>Dominic Kaiser</td>
<td>BASF, Germany</td>
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<td>Erica Brockmeier</td>
<td>University of Florida, USA</td>
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<td>Hamed Azarbad</td>
<td>Jagiellonian University, Poland</td>
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<td>Dominika Chmolowska</td>
<td>Jagiellonian University, Poland</td>
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About the venue…

Krakow

Kraków, Krakow, Cracow - the second largest and one of the oldest cities in Poland. Situated on the Vistula River (Polish: Wisła) in the Lesser Poland region, the city dates back to the 7th century. Krakow has traditionally been one of the leading centres of Polish academic, cultural, and artistic life and is one of Poland's most important economic hubs. It was the capital of Poland from 1038 to 1596, it is now the capital of the Lesser Poland Voivodeship.

Krakow's historic center, which includes the Old Town, Kazimierz and the Wawel Castle, was included as the first of its kind on the list of UNESCO World Heritage Sites in 1978. The Old Town district of Krakow is home to about six thousand historic sites and more than two million works of art. Its rich variety of historic architecture includes Renaissance, Baroque and Gothic buildings. Krakow's palaces, churches, theatres and mansions display great variety of color, architectural details, stained glass, paintings, sculptures, and furnishings. Points of interest outside the city include the Wieliczka salt mine, the Tatra Mountains, the former Nazi concentration camp at Auschwitz, and Ojcowski National Park.

Krakow is home to approximately 800,000 people, while the Krakow metropolitan area totals up to one and a half million people. It is also home to about 170,000 students. There are five nature reserves in Krakow, with a combined area of ca. 48.6 hectares. Due to their ecological value, these areas are legally protected. The western part of the city, along its northern and north-western side, borders an area of international significance known as the Jurassic Bielany-Tyniec refuge.
About the venue…

Jagiellonian University

The Jagiellonian University (Polish: Uniwersytet Jagielloński, often shortened to UJ) was established in 1364 by Casimir III the Great. It is the oldest university in Poland, the second oldest university in Central Europe and one of the oldest universities in the world. Programmes of study are offered in 48 disciplines and 93 specialisations. The university’s Jagiellonian Library (Biblioteka Jagiellońska) is one of Poland’s largest, with almost 6.5 million volumes. It has a large collection of medieval manuscripts, including Copernicus’ De Revolutionibus and the Balthasar Behem Codex.

The 3rd campus

The Campus of the 600th Anniversary of the Jagiellonian University Revival, known as the Third Campus and located in Pychowice, in the eastern part of Krakow, consists of modern buildings housing departments of science, natural science and Faculty of Management and Social Communication. The enlargement of the campus ground by more than 100,000 m² will help to make a proper use of the JU research and educational potential.

The Institute of Environmental Sciences

The main fields of studies at the Institute of Environmental Sciences are: evolutionary biology, physiological ecology, biodiversity in aquatic and terrestrial ecosystems, ethology, plant-microorganisms interactions, ecotoxicology and stress ecology. We are also active in environmental monitoring, nature conservation and ecological education. There are 47 academic staff working at the Institute, including 11 professors, 9 associate professors and 18 assistant professors and research assistants. Besides, 36 technical employees help us in research. Our 60 PhD students participate actively in research and teaching.
Social programme

Get Together

The Get Together will take place in the Klub Muzyczny MASADA, in Kraków’s Kazimierz district. This district is a special place, because it has been shaped by the close neighbourhood of Christianity and Judaism lasting for several centuries.

Snacks and sandwiches will be served. Each meeting participant receives 4 coupons worth 2 PLN and 4 coupons worth 4 PLN for alcoholic or non-alcoholic drinks. Further drinks are available for cash (no credit cards accepted).

Address:

Klub Muzyczny MASADA
Ulica Skawińska 2
31-216 Kraków
Phone: 0 504 384 612
web: www.masadaklub.pl

Meeting Dinner

The Dinner will be held in the Pod Wawelem, a restaurant close to the Royal Wawel Castle. We will have a choice of starters, soup, main dish, and desert. Each participant will receive one free drink (non-alcoholic or beer), and free coffee or tea at the end. Additional drinks are available, e.g. mineral water (0.3 L), soft drinks (0.2 L) or beer (0.4 L) starting at 5 PLN.

Address:

Restauracja "Pod Wawelem" Kompania Kuflowa
Ulica Św. Gertrudy 26-29
31-069 Kraków
Tel.: +48 12 421 23 36
Public transportation

Transport in Krakow is based around a fairly dense network of tramway and bus lines operated by a municipal company. It is punctual, efficient, safe and cheap. Tickets can be bought at kiosks, shops, and special vending machines (Polish: *biletomat*) are located at many bus and tram stops. There are two types of *biletomats*: those with touch-screens (at bus stops only) accept coins, notes, and sometimes credit cards, while the smaller ones (e.g. on board of the bus) accept coins. The driver is allowed to sell only 60 minutes tickets, and only if you have the exact change prepared.

There are several types of tickets - see table on the right for prices. We suggest to all participants to buy a 7-day ticket when they arrive in Krakow - that will easily cover the duration of your stay, and the only thing to think about would be to have your ticket with you. For students younger than 26 years, a special discount of 50% applies (student proof needed).

When you board the bus or tram, validate your ticket immediately. Tickets are valid for the period stated, starting with the validation. Punch your ticket only once during the time of its validity. During this time you may change buses and trams as many times as you like.

<table>
<thead>
<tr>
<th>Tickets</th>
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<tr>
<td>One way ticket</td>
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<tr>
<td>20 Minutes</td>
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<tr>
<td>60 Minutes</td>
<td>5.00</td>
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<tr>
<td>24 hrs</td>
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<td>48 hrs</td>
<td>24.00</td>
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<td>72 hrs</td>
<td>36.00</td>
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<tr>
<td>7 days</td>
<td>48.00</td>
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</table>

Taxis

There are over a dozen of licensed taxi companies in Kraków. Please check the table on the right for respective phone numbers. Phone numbers starting with “+48 800” are toll free number when calling from a local phone.

<table>
<thead>
<tr>
<th>Provider</th>
<th>Phone</th>
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<tbody>
<tr>
<td>Radio Taxi Wawel</td>
<td>+48 800 666 666</td>
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<tr>
<td>MPT Radio Taxi</td>
<td>+48 800 444 444</td>
</tr>
<tr>
<td>Barbakan Radio-Taxi</td>
<td>+48 800 400 400</td>
</tr>
<tr>
<td>City-Taxi</td>
<td>+48 12 266 64 44</td>
</tr>
<tr>
<td>Euro-Taxi</td>
<td>+48 12 266 61 11</td>
</tr>
<tr>
<td>Express Rotunda Taxi</td>
<td>+48 800 111 111</td>
</tr>
<tr>
<td>Mila Cars</td>
<td>+48 800 446 66 68</td>
</tr>
<tr>
<td>iCar</td>
<td>+48 800 653 55 55</td>
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</table>

Taxi fares

Fares depend on the length of your journey and the rates your driver uses. Most charge 2.30 PLN per kilometre within the city boundaries, Mon-Sat between 6am and 10pm. On Sundays and public holidays as well as at night the rate in the city is 3.50 PLN per kilometre. Generally 60 PLN is enough to get from one end of the city to the other.
From the airport

For traveling from and to the airport, three means of transportation are available (see figure below for respective stations at the airport). For students younger than 26 years, a special discount of 50% applies (student proof needed).

Bus

Krakow Airport is served by two regular bus lines (208 and 292) and one night line (902). Note that there are two types of bus tickets - for Krakow City Area and for Krakow Metropolitan area (Polish: Aglomeracja). If you take the bus at the airport you will need to buy a ticket for Krakow Metropolitan area. Thus we recommend buying a single ride ticket, and for using public transportation in the city itself we recommend a 7-day ticket (please see page 18 for further information). A single trip from airport to city center for 1 Person costs 4.00 PLN.

Taxi

A ride from the airport to Krako city center is 70-100 PLN (see page 18). If you are heading directly to Ruczaj (the part of the city where the UJ Third Campus is located) some companies offer quick transport (Mila cars; only 15 min to Ruczaj, via high-way, 49 PLN).

Train

The train service is the fastest link between the centre of Krakow and the airport. Travel time is approximately 18 minutes. One-way ticket price is 12 PLN, roundtrip 20 PLN (valid for 30 days). The train station is located 200 m from the T1 international terminal. Ticket can be purchased at the train, from train attendant or at the ticket machines close to main exit in terminal T1 and at Krakow Central Station located at platform #1.
Transport:

12, 18

Routes of direct trams to/from the Third Campus

194

Routes of direct buses to/from the Third Campus

609

Routes of direct night buses to/from the Third Campus

Approximate location of tram/bus stops
Access to the meeting venue

Tram and bus stops:
1. “Kampus UJ” tram stop (11,18,23,52) - Coming from the centre
2. “Kampus UJ” tram stop (11,18,23,52) - Going to the centre
3. “Kampus UJ” bus stop (194,609) - Coming from the centre
4. “Kampus UJ” bus stop (194,609) - Going to the centre
5. “Ruczaj” tram stop (11,18,23,52) - Coming from the centre
6. “Ruczaj” tram stop (11,18,23,52) - Going to the centre
7. “Ruczaj” bus stop (194,609) - Coming from the centre
8. “Ruczaj” bus stop (194,609) - Going to the centre

Wifi access

Free internet access is provided by the Jagiellonian University:
Network: UJ_Open;
Username: YES
Password: SETACYES2013
Workshop:  
Valery Forbes - The Essentials of Scientific Networking

Most graduate students and postdocs receive formal training and practice in giving oral and poster presentations and in writing scientific papers and communicating science in a formal setting. But what about all of those other, potentially tricky or even disastrous, encounters that you are likely to have as scientists for which it just seems that you’re supposed to know how to conduct yourself. This workshop will focus on exactly those types of situations. For example, you’ve prepared your conference presentation, practiced a million times, you are now at the conference and suddenly find yourself face-to-face with that big-name professor whose papers have had a major influence on your work. How do you introduce yourself? What do you say to strike up conversation and not appear like a complete jerk? Are there tricks for being remembered – in a good way? In this workshop we will use interactive methods and group problem solving to find solutions to some of the most common networking situations that you will find yourselves in as young environmental scientists.

About the author

Valery E. Forbes is the Director of the School of Biological Sciences at the University of Nebraska-Lincoln. She has her Ph.D. in Coastal Oceanography from the State University of New York at Stony Brook. From 1989-2010 she lived in Denmark where her latest position was Founding Chair of the Department of Environmental, Social and Spatial Change and Professor of Aquatic Ecology and Ecotoxicology at Roskilde University. Specific research topics include population ecology and modelling, fate and effects of toxic chemicals in sediments, and ecological risk assessment. Valery Forbes has published over 100 internationally peer-reviewed articles and two books on these topics. She is on the editorial board of several international journals and provides scientific advice to the private and public sectors.
Evaluation of effects of the pharmaceuticals diclofenac and gemfibrozil on marine mussels (*Mytilus* spp.). Evidence for chronic sublethal effects on stress-response proteins

W. Schmidt¹, L.-C. Rainville², G. McEneff¹, L. Sheehan² and B. Quinn¹

¹Irish Centre for Environmental Toxicology, Galway-Mayo Institute of Technology, Dublin Road, Galway, Ireland
²Proteomics Research Group, Department of Biochemistry, University College Cork, Cork, Ireland
³Irish Separation Science Cluster (ISSC), Dublin City University, Dublin, Ireland

Correspondence: schmidt.wiebke@gmail.com

Over recent years the presence of human pharmaceuticals in the aquatic environment, such as the non-steroidal anti-inflammatory drug diclofenac and the lipid regulator gemfibrozil, has become an environmental issue. A major source of these novel pollutants is through the release of waste water effluents, both treated and untreated, into the aquatic environment. Diclofenac and gemfibrozil are commonly found throughout the western world, including in the Irish aquatic environment, in concentrations ranging from high ng/L to low µg/L. The potential toxicity of these compounds on non-target animals, like shellfish, remains largely unknown. In this study, the marine mussel (*Mytilus* spp.) was exposed to environmentally relevant and elevated concentrations (1 & 1000µg/L, respectively) for 14 days. Chronic sublethal effects were investigated by looking at the protein expression signatures (PES) using two-dimensional gel electrophoresis (2DE) and a suite of biomarkers comprising glutathione S-transferase, lipid peroxidation and DNA damage. Additionally, mussels were left to recover for an additional seven days after the treatment to examine whether blue mussels have the potential to recover from an exposure.

The expression of twelve proteins was significantly altered by gemfibrozil and/or diclofenac, seven of which were successfully identified by LC-MS/MS analysis. These proteins were involved in energy metabolism, oxidative stress response, protein folding and immune response. An oxidative stress response was also confirmed by the biomarker response. Changes in the PES over time revealed that mussels were still facing stress for up to seven days post exposure.

The successful application of environmental proteomic approach demonstrates its potential use in pollution biomonitoring and contributes to the understanding of the biological effects of pharmaceuticals in non-target organisms, such as the widespread blue mussels.

**Keywords:** diclofenac - gemfibrozil – *Mytilus* spp. - proteomics – biomarkers
Varying wastewater dilution in receiving streams - implications for stream ecosystem structure and function

D. Englert\textsuperscript{1}, J. P. Zubrod\textsuperscript{1}, R. Schulz\textsuperscript{1}, M. Bundschuh\textsuperscript{1,2}

\textsuperscript{1}Institute for Environmental Sciences, University of Koblenz-Landau, Landau Campus Landau, Germany
\textsuperscript{2}Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence: englert@uni-landau.de

In the past years, increasing summer droughts – likely driven by climate change – reduced the water flow of low-order streams also in regions of temperate Europe and hence their dilution potential for secondary treated wastewater. Despite the potentially involved risks for ecosystem integrity, there is a lack of information about the effects of different dilution potentials of wastewater on receiving streams and finally ecosystem functions. Therefore, the present study investigated adverse effects of secondary treated wastewater released into a third-order stream (Queich, southwest Germany) during seasons with low (summer; \textasciitilde 90\% wastewater) compared to high (winter; \textasciitilde 35\% wastewater) dilution potential on the ecosystem function of leaf litter decomposition. Besides sampling of the macroinvertebrate communities, coarse- and fine-mesh leaf litter bags as well as \textit{in situ} bioassays with the leaf shredding amphipod, \textit{Gammarus fossarum} were conducted to assess implications in leaf litter decomposition. Adverse effects in macroinvertebrate mediated leaf mass loss (\textasciitilde 65\%; \textit{n}=5), gammarids’ \textit{in situ}-measured feeding rate (\textasciitilde 80\%; \textit{n}=3-6), leaf associated fungal biomass (\textasciitilde 40\%; \textit{n}=5) and shifts in macroinvertebrate community structure (\textit{n}=3-6) were apparent (partially up to 500 m) downstream of the wastewater treatment plant effluent during both seasons. However, effects were more pronounced during low flow conditions occurring in summer. Moreover, a \textit{G. fossarum} laboratory feeding trial (\textit{n}=25) demonstrated the potential of powdered activated carbon to reduce the ecotoxicity of released wastewater for this key species in leaf litter decomposition. The present study urges the development and evaluation of adequate management strategies, e.g. the upgrade of wastewater treatment plants, to protect the integrity of freshwater ecosystems, which is required by the European Water Framework Directive – especially under projected climate change scenarios.

\textbf{Keywords:} ecosystem function – leaf decomposition – \textit{in situ} – wastewater – \textit{Gammarus}
Presentations: Tuesday, February 12th
Session: Aquatic ecotoxicology

Effects of the fungicide tebuconazole on fungal and bacterial communities in the sediment of outdoor freshwater microcosms

M. R. Dimitrov1,2, P. J. Van den Brink2,3, T. C. M. Brock3, S. Kosol4, L. Maltby4, R. van Wijngaarden3, H. Smidt1

1Laboratory of Microbiology, Wageningen University, The Netherlands
2Aquatic Ecology and Water Management Group, Wageningen University, The Netherlands
3Alterra, Wageningen UR, The Netherlands
4Department of Animal and Plant Sciences, The University of Sheffield, United Kingdom

Correspondence: mauricio.rochadimitrov@wur.nl

Only a few studies have been published on the non-target effects of fungicides on aquatic microbial communities. One reason for the lack of information regarding effects of fungicides on non-target micro-organisms may be the basic dossier requirements for EU registration. So far, only information from ecotoxicity studies performed on vertebrates, invertebrates and primary producers are asked for the aquatic effect assessment. Aquatic fungi and bacteria, however, play an important role in freshwater ecosystems, contributing significantly to decomposition processes. Hence, aims of the present work were(1) to evaluate the impact of tebuconazole on sediment microbial community structure of an experimental freshwater ecosystem, and (2) to evaluate whether the current effect assessment procedure (based on standard tests with fish, aquatic invertebrates and algae) is protective for aquatic fungi and bacteria. To this end, outdoor microcosms were set up in an experimental ditch and four test systems served as controls while tebuconazole was applied to four other microcosms at an intended concentration of 238 µg a.i./L. This treatment level is in line with the HC5 (based on acute toxicity tests with aquatic organisms) for tebuconazole, as described by Maltby et al. (2009). Throughout the experiment sediment samples were taken from each microcosm and used for total DNA isolation. The isolated DNA was used to access bacterial and fungal community structure by Denaturing Gradient Gel Electrophoresis (DGGE) and bacterial community structure and composition was also accessed by 454-pyrosequencing of PCR-amplified ribosomal RNA gene fragments. Significant treatment-related effects of tebuconazole on the sediment bacterial and fungal community structure could not be demonstrated and only minor effects on relative abundance of bacterial populations were observed.

Keywords: aquatic ecotoxicology – tebuconazol – microorganisms – DGGE – pyrosequencing
**Presentations: Tuesday, February 12th**

**Session: Aquatic ecotoxicology**

**Direct and indirect effects of the antibiotic enrofloxacin on tropical freshwater microcosms**

A. Rico¹, R. P. A. van Wijngaarden², M. R. Dimitrov¹, K. Satapornvanit³, P.J. Van den Brink¹,²

¹Aquatic Ecology and Water Quality Management Group, Wageningen University and Research Centre, The Netherlands

²Alterra, Centre for Water and Climate, Wageningen University and Research Centre, The Netherlands

³Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Thailand

Correspondence: andreu.rico@wur.nl

Antibiotics used in human medicine, livestock and aquaculture production are released into the environment by untreated waste water effluents or by the leaching/runoff of agricultural fields, posing a potential risk for aquatic ecosystems. The objective of the present study was to assess the potential toxic effects of the fluoroquinolone antibiotic enrofloxacin on the structure and functioning of tropical aquatic ecosystems. Enrofloxacin was applied at a concentration of 1, 10, 100 and 1000 µg/L for 7 consecutive days in 600L outdoor plankton-dominated microcosms in Thailand. The experiment was run in duplicate with two untreated controls and had a duration of 4 weeks after the first antibiotic application. The effects of enrofloxacin were assessed on seven ecosystem structural (i.e., macroinvertebrates, zooplankton, phytoplankton, periphyton and bacterial community) and functional (i.e., organic matter decomposition, nutrient cycling) endpoints. The results of the chemical analysis showed that enrofloxacin had a very low persistence in the water column (calculated first-order half-dissipation rate = 15-16h), and about 10% of the applied dose was transformed into its main by-product ciprofloxacin. The evaluation of the dynamics of the invertebrate and primary producer communities did not reveal significant differences between the treated and untreated microcosms. However, antibiotic-related effects were demonstrated in the structure and abundance of bacterial communities inhabiting the water column (NOEC = 1 µg/L) and in the ammonia (NOEC = 100 µg/L) and nitrate (NOEC = 1 µg/L) concentrations during the treatment period, indicating a clear dose-response effect on the abundance and structure of the microbial community and a potential impairment of the related (de-)nitrification processes. The results of this study suggest that enrofloxacin is not likely to result in direct or indirect toxic effects on the invertebrate and primary producer communities at environmentally relevant concentrations. However, ecosystem functional endpoints such as nutrient cycling should be taken into account in the risk assessment of antibiotics.

**Keywords:** antibiotics – aquatic ecotoxicology – microcosms – microorganisms – risk assessment
Aquatic and cellular toxicity of ionic liquids and their potential biodegradability in water

B. Peric, J. Sierra, E. Martí, R. Cruañas, M.A. Garau

Soil Science Unit, Faculty of Pharmacy, University of Barcelona, Spain

Correspondence: brezana@hotmail.com

There has been a rapidly growing interest in ionic liquids (ILs) in the last decades because of the fascinating set of physical, chemical, and biological properties that these materials possess, and which allow them to be used in many applications, such as: separations, synthesis, catalysis, biotechnology, pharmaceuticals, etc. The ILs are considered to be “green” alternatives to conventional solvents and should lead to novel environmentally friendly processes. The analyzed ILs belong to the two major groups of ILs - aprotic and protic ones. The evaluation of aquatic toxicity was done by performing bioassays with bacteria (*Vibrio fischeri*), algae (*Pseudokirchneriella subcapitata*) and aquatic plant (*Lemna minor*). Ready biodegradability in an aerobic aqueous medium was also studied. Test systems with enzyme (acetylcholinesterase) and leukaemia rat cells (IPC-81) were also used in order to perform a more in-depth evaluation of toxicity. The obtained EC50 indicate that the presumed “greenness” of the ILs can be questioned in some cases of the aprotic ILs, as they can be classified as harmful, toxic or very toxic for aquatic organisms. No adverse effects on the acetylcholinesterase activity and no elevated toxicity towards IPC-81 cell line were found for the investigated ILs. There is a difference in levels of toxicity between the analyzed ILs, which can be attributed to the differences between groups and also to differences in structure and complexity of the molecules, with more complex molecules with elongated carbon chains being predominately more toxic. Effective concentrations in these test systems were generally some orders of magnitude lower than the EC50 of the conventional solvents like acetone, acetonitrile, methanol, etc. As far as biodegradability in water is concerned, aprotic ILs proved to be resistant to biodegradation, whilst most of the protic ILs fitted into “readily biodegradable” criteria set by the OECD.

**Keywords:** ionic liquids – “greenness” – aquatic toxicity – cytotoxicity – biodegradability
Biological effects induced in *Arabidopsis thaliana* after aquatic exposure to radioactive contaminants

G. Biermans\(^1,2\), N. Horemans\(^1\), N. Vanhoudt\(^1\), H. Vandenhove\(^1\), E. Saenen\(^1,2\),
J. Wannijn\(^1\), M. Van Hees\(^1\), R. Nauts\(^1\), A. Cuypers\(^2\)

\(^1\)Belgian Centre for Nuclear Research (SCK•CEN), Boeretang 200, 2400 Mol, Belgium
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Despite significant efforts in research, the biological effects of radiation in non-human biota and plants in particular, remain poorly characterized. However, this knowledge is essential in assessing the effects of radioactive exposure in release scenarios, such as routine discharge or accidents (such as the events in Chernobyl and Fukushima). In order to unravel the underlying biological mechanisms involved in plant response to radiation exposure, this study looks at the dose-dependent effects of α- and γ-radiation on *Arabidopsis thaliana* at a morphological, physiological and molecular level. 14-day old seedlings were exposed for 4 and 7 days in a hydroponic setup to a broad activity concentration range of either the β emitter \(^{90}\)Sr or the α-emitter \(^{241}\)Am. Leaf area, photosynthesis and biomass were measured. In addition, we quantified ROS-dependent DNA base modification (8-OHdG) and the expression of genes involved in repair of single and double DNA strand breaks, the cell cycle and the lipid pool. Our results show a transient increase in leaf area of 30 to 40% during \(^{90}\)Sr exposure, an effect which reverses after 96 hours of exposure. After 4 days, \(^{90}\)Sr-exposed roots showed increased in fresh weight, while their dry weight percentage declined with increasing activity concentration, effects which had disappeared after 7 days. Shoots showed a dose-dependent increase in base modification at both time points and a similar response pattern in expression of genes involved in DNA damage repair. Results of \(^{241}\)Am exposure show root biomass is affected 4 and 7 days, while shoot biomass remains constant. Combination of these data will enable us to compare the biological mechanisms involved in the plant response to β- and α-radiation.

**Keywords:** *Arabidopsis thaliana* – ionizing radiation – DNA damage – biological mechanisms – radionuclide release
Sensitivity and recovery potential of \textit{Lemna minor} after exposure to herbicide mixtures

V.Ž. Knežević\textsuperscript{1}, T.O. Tunić\textsuperscript{1}, D.V. Kerkez\textsuperscript{2}, A.M. Tubić\textsuperscript{2}, P.S. Gajić\textsuperscript{1}, D.D. Savić\textsuperscript{1}, I.S. Teodorović\textsuperscript{1}

\textsuperscript{1} Laboratory for Ecotoxicology (LECOTOX), University of Novi Sad, Novi Sad, Serbia
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As duckweed species are the only aquatic macrophytes used in risk assessment of plant protection products under the European Regulation, there is a vast amount of published data concerning the effects of single herbicides and their mixtures on \textit{Lemna} sp. However, the laboratory tests seem to be under-used; they can provide more ecologically relevant information which is currently completely overlooked. Population recovery after exposure to single toxicants and cocktails is an important element in vulnerability assessment which could be easily studied under laboratory conditions using \textit{Lemna} species. Therefore, the objectives of this study were a) to assess whether cocktails of herbicides with similar and dissimilar modes of action (MoAs) have antagonistic, synergistic or additive effect on \textit{L. minor} as a representative photosynthetic non-target organism in aquatic ecosystems and b) whether recovery patterns after exposure to cocktails differ from those after single chemical exposure. \textit{L. minor} was exposed for 7 days to three binary herbicide mixtures: i) atrazine + trifluralin, ii) atrazine + isoproturon, iii) isoproturon + trifluralin and cocktail of atrazine + isoproturon + trifluralin. Three exposure concentrations were selected based on individual IC\textsubscript{10}, IC\textsubscript{25} and IC\textsubscript{50} values of single substance sensitivity and recovery tests conducted previously. After 7 day exposure to herbicide mixtures, the plants recovery potential has been assessed. The Concentration addition (CA) model has shown good predictability in the estimation of mixture toxicity for both substances with similar and dissimilar MoA, especially in equipotent mixtures having overall toxicity close to EC50 values. Toxicity data from tests with environmentally realistic concentrations were relevant input for the assessment of herbicide risk to aquatic life. Recovery patterns have proved to be practical tool for distinguishing between phytostatic and phytotoxic effect of herbicides, underlining the necessity of inclusion of recovery potential assessments in standard laboratory practice.

Keywords: mixture toxicity – herbicides – recovery potential – \textit{Lemna minor}
When predictions go wrong: mixture toxicity of a repellent and a pyrethroid on aquatic invertebrates
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The use of repellents and insecticides for protection from diseases is increasing globally. The most common and effective repellent, N,N-diethyl-m-toluamide (DEET), is often combined with pyrethroid (e.g. permethrin) impregnated cloth to maximize efficiency. DEET’s mode of action is a competitive inhibition of acetylcholine esterase, whereas permethrin irreversibly opens pre-synaptic, voltage dependent sodium channels. Both result in elevated levels of acetylcholine and hence increase nervous excitation, which ultimately kills target organisms, like mosquitos. Due to their widespread usage, both substances are frequently found in the aquatic environment. Therein, invertebrates are classic examples of non-target organisms affected by such substances. The water flea Daphnia magna and the European fingernailclam Sphaerium corneum were chosen as regional pelagic and benthic model organisms, respectively, to evaluate the effects of a combined exposure to DEET and permethrin. Predictions on mixture toxicity were made using single substance data and applying the models of concentration addition and independent action. To further investigate a possible unspecific toxicity, a novel test to quantify oxidative stress in daphnids was established, and ultrastructural anomalies in both species were recorded. The outcome of these experiments, as well as a critical analysis of current test designs, prediction models, and the interpretation of mixture toxicity data will be presented and discussed.

Keywords: DEET – permethrin – mixture toxicity – Daphnia magna – Sphaerium corneum
Environmentally-relevant tebufenozide concentrations affect reproduction in the freshwater midge *Chironomus riparius* in a chronic toxicity test

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Studies reporting the effects of tebufenozide, a non-steroidal ecdysone agonist that mimics natural moulting hormones in Chironomids exist in the literature. However, nothing is known about its multigenerational or chronic effects on the reproduction of aquatic insects, although tebufenozide was reported to persist in aquatic ecosystems. Here, we investigated the chronic toxicity of tebufenozide in two consecutive generations of *Chironomus riparius* using environmentally-relevant concentrations that ranged from 4 to 26.2 µg/L. We began the test with the first instar larvae in the parental (P) generation, quantifying life cycle parameters (emergence, sex ratio, development rate, fecundity and fertility) until the emergence in the subsequent F1 generation. A reduction in reproduction was observed in addition to a significant decrease in male developmental rate of midges for all treatments, in the F1 generation compared to the P generation (paired t-test; p < 0.001). Furthermore, two-way analysis of variance revealed a significant exposure × generation effect on male fraction with male fraction increasing (P generation) or decreasing (F1 generation) with increasing treatment. These effects on *C. riparius* indicate the importance of conducting chronic studies with environmentally-relevant concentrations to investigate population-level endpoints for endocrine disrupting chemicals.

Keywords: Tebufenozide – *Chironomus riparius* – full life-cycle test – population-level effects – endocrine disruption
Can the sensitivity and predictive potential of zebrafish early life stage (ELS) tests be improved by additional endpoints and chemical analysis?

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Zebrafish ELS tests usually consider morphology, mortality, and nominal chemical concentrations for toxicity assessment, evaluating limited selection of acute endpoints and underestimating toxic effect concentration. Therefore we investigated how additional endpoints and chemical analysis would improve the sensitivity and predictive potential of ELS tests. Gene expression (qPCR), behavioral and morphological endpoints were evaluated in zebrafish embryos exposed to PCB126 3-3000ng/L between 3h-5dpf, following two test setups: eFET, extended fish embryo test until 5dpf; and pELS, prolonged ELS test until 14-28dpf. Morphological and behavioral endpoints were scored daily. Additional chemical analysis test had solution and fish sampling at 0/48hpf/120hpf. After exposure, eFET fish were preserved for qPCR; and pELS fish were raised in clean water to study delayed effects. SybrGreen qPCR on whole-fish RNA had target genes for mode-of-action (\textit{cyp1a}) and observed effects in eFET (\textit{sox9b}, \textit{col11a2}: cartilage), \textit{bactin2} as reference. Sample preparation and sample extraction were performed by adaptation of existing protocol. PCB126 quantitative determination was done by GC/HRMS. Toxicity results are discussed for nominal concentrations. Until 3dpf no significant effect occurred. At 5dpf reduced swim-bladder inflation presented dose-dependent response in \textit{≥30ng/L}. Reduced swim-up, increased equilibrium-lost, and reduced swimming were observed in 300-3000ng/L. \textit{cyp1a} was overexpressed in \textit{≥100ng/L}, while \textit{sox9b} and \textit{col11a2} overexpressed in 30ng/L and underexpressed in 100-3000ng/L. Delayed mortality achieved 100% in 300-3000ng/L, while surviving fish from 100ng/L presented higher sublethal effects occurrence. Free concentrations were ca. 50% of nominal at test start, dropping to 20% of initial values 24h after partial change (24hpf/48hpf). Internal concentrations were close to free values at 48hpf, increasing by factors of 4-5 until 120hpf. Delayed effects agree with pELS in other species, and molecular and behavioral endpoints contributed to predict them. Linking free or even better internal concentrations of chemicals with toxic effects is important.

\textbf{Keywords:} fish early life stage – internal concentrations – gene expression – behavior – delayed effects
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Session: Aquatic ecotoxicology

Long term effects of an early and continuous exposure to PAHs on zebrafish behavioral responses

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Polycyclic aromatic hydrocarbons (PAH) emission in the environment is constantly increasing with human activity. The goal of this study was to assess long term consequences of fish lifecycle exposure to PAH mixtures on behaviour and reproduction. PAHs were extracted from sediment collected in the Seine Estuary (France), a site representative of high level pyrolytic PAH contamination. The extract was used to spike zebrafish food (adapted to fish size from larvae to adult) targeting environmental concentrations. Zebrafish larvae were exposed to PAH mixtures through diet from their first meal (5 day post fertilisation) onward and reared during 1 year. These fish were monitored for several physiological processes including growth, reproduction abilities as well as several behavioural traits. Behavioural assays such as locomotion, sudden dark change challenge, T-maze and novel tank exploration were performed at adult stage (F0). F0 reproduction was also monitored (including spawn frequency, size, and fertilisation rates). These fish progeny (F1) was also monitored for in particular larvae survival, locomotion during 72-hrs and sudden dark change. The two last behavioural tests were also performed at adult stage on F1 at 2 months post fertilisation.

In F0 adults, in many cases, trends to deviate from control fish could be observed for exposed fish behaviour but with no significant differences but significant reduction of growth and reproduction was observed for exposed fish. F1 larvae displayed disruption of several behavioural traits.

This study indicates that long-term exposure of zebrafish to environmentally relevant mixtures of PAHs through diet induced reproduction and growth disruption as well as progeny behavioural defects measured at the larval stage. Consequences on fish abilities (e.g. predator escape, food search, courtship) will be discussed from an ecological point of view.

This work was supported by the French ANR CES in the frame of the ConPhyPoP (2009-002) research project and a doctoral fellowship (Région Poitou-Charentes and Ifremer).

Keywords: zebrafish – contaminant – PAH – behaviour – reproduction
Comparative effects of three PAH fractions from light and heavy crude oils and from a PAH-contaminated sediment on *Oryzias latipes* Japanese medaka early life stages

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in aquatic environment and accumulate in sediments. A novel approach was recently proposed to evaluate the toxicity of the bioavailable fraction of hydrophobic pollutants with the Japanese medaka embryo-larval assay and sediment-contact exposure (MELA\(_{sed}\)). Fish early life stages are suitable models for toxicity testing since they are sensitive to a wide range of chemicals and are considered as an *in vitro* assay by the EU animal experimentation legislation. The assay consists in a direct contact exposure of embryos with chemical-spiked artificial sediment during their whole development until hatching. Several endpoints are recorded at different developmental stages.

This work aimed to compare the developmental toxicity of three PAH complex mixtures extracted from a PAH-contaminated sediment (Seine estuary, France) and two crude oils (Arabian Light and Erika) at three different environmental concentrations roughly equivalent to 0.5, 4 and 10 µg total PAH.g\(^{-1}\) d.w. sediment. These extracts are characterized by different PAH compositions and proportions. Pyrolytic extract from the Seine estuary (France), mainly composed of non-substituted molecules, delayed hatching, induced deformities, disrupted larvae swimming activity, and damaged DNA notably at the highest concentration tested. Besides, light and heavy petrogenic extracts composed of 65 and 40% respectively of methyl-naphthalene and methyl-phenanthrene molecules, induced acute effects. Hatching rate of exposed individuals was significantly reduced by 70% at the intermediary concentration tested and no individual hatch at the highest concentration for both petrogenic extracts. PAH fraction from Arabian Light oil induced more teratogenicity and larvae swimming activity failure than PAH fraction from Erika oil.

This study highlighted the high sensitivity of medaka embryos to PAHs and the differential toxicity of pyrogenic and petrogenic PAHs. It also demonstrated the MELA\(_{sed}\) relevance to evaluate toxicity of particulate-bound pollutants. Authors would like to thank the French ANR CES for financial support and the CEDRE for providing crude oils.

**Keywords:** developmental abnormalities – DNA damages – larvae behaviour – crude oil extract – pyrolytic extract
Novel brominated flame retardants (NBFRs) contamination in sediments from Lake Maggiore basin

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Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are currently the most studied groups of Brominated Flame Retardants (BFRs). Due to their toxicological risk and persistence, the production and use of Penta- and Octa-BDE technical mixtures has been banned in Europe since 2004. Despite the use of Deca-BDE in electrical and electronic equipment has been recently banned in the EU since 1 July 2008, there are no restrictions on the production or use of Deca-BDE in most other countries or for other industrial uses. Consequently to increasing international regulations on BFR formulations, alternatives to Penta-, Octa-, and Deca-BDE technical mixtures are being developed and become commercially used. Some of these “Novel BFRs” (NBFRs) are: decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), hexabromobenzene (HBB), and pentabromoethylbenzene (PBEB). Lake Maggiore basin, a heavily industrialized and densely populated area, was selected for studying BFR contamination in Italy. In particular, BFRs were quantified in the basin by analysing six sediment cores collected in 2011 from the lake, and some grab samples collected from three tributaries (Bardello, Boesio, and Toce). Lake sediments covered a period from 2004 to 2011, whereas the core sampled at station LM_28 was cut into slices in order to reconstruct BFR contamination of the lake since 1970 with the aim of understanding pollution dynamics. Analyses of PBDEs, HBCD and NBFRs were undertaken by GC-MS/MS, while DBDPE was analysed by GC-ECD. Among the considered PBDEs, results showed a dominant presence of BDE-209 (>95%), while the concentration levels of PBE, HBB, HBCD, and BTBPE in lake sediments were very low and in a few cases reached detection limits. The DBDPE presence in the Pallanza Bay sediments was not negligible, and could be ascribed to a possible contamination of Toce river sediments. The tributaries Bardello and Boesio were greatly contaminated with BDE-209, HBCD, and DBDPE.

Keywords: PBDEs – HBCD – Novel BFRs – Lake Maggiore – sediments
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Session: Environmental chemistry

Fate of a transgenic insecticidal protein, a pyrethroid insecticide, and neonicotinoid insecticides within a maize agricultural ecosystem

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With the increasing prevalence of transgenic crops used in combination with insecticides, the ultimate fate of these pesticides is critical to understanding potential non-target species effects. Concentrations of Cry1Ab, a transgenic insecticidal Bt protein used in maize, tefluthrin, a pyrethroid insecticide, and thiamethoxam and clothianidin, neonicotinoid insecticides used as a seed coating were measured in soil, runoff water, groundwater, and soil pore water before, during, and after maize planting for three years. A research farm in Christian County, Illinois was divided into separate non-Bt and Bt fields with three different rates of tefluthrin applied in replicate strips in both fields. Samples were analyzed for Cry1Ab using enzyme linked immunosorbent assays; for tefluthrin, samples were analyzed using GC-ECD; and for neonicotinoids, samples were analyzed using HPLC-DAD and HPLC-UV. Cry1Ab proteins were found in soil at low concentrations throughout the growing season in soils collected from the Bt field. Only a few soil samples collected from the non-Bt field contained Cry1Ab. Runoff water samples from both fields frequently contained Cry1Ab and were the highest concentrations of any water samples. Groundwater and soil pore water often did not have detectable concentrations of Cry1Ab. Tefluthrin was detected at the highest concentrations in soil samples, and was, in general, not found to be transported by water. Neonicotinoid seed coating compounds were detected in water and soil samples throughout the growing season with the highest concentrations found in soil pore water samples. Concentrations of pesticides were low or near the reporting limit before planting indicating that most of the chemicals and proteins have been metabolized or broken down in the environment before the next growing season. While Cry1Ab proteins were not found in environmental matrices at ecologically relevant concentrations, tefluthrin and neonicotinoids often were detected at or above documented LC50 literature values during the growing season.

Keywords: pesticides – fate and transport – pyrethroids – genetically modified organisms
Membrane devices have gained importance in many different scientific fields. The applications in passive sampling and extraction are of particular interest for ecotoxicology and especially for the assessment of toxic potentials.

Membrane dialysis extraction (MDE) uses semi-permeable membrane “layflat” tubing (low-density polyethylene) into which the sample is applied. The tubing is placed inside an acceptor solvent (hexane), hence utilizing a concentration gradient as the driving force for extraction. The compounds investigated were three polycyclic aromatic hydrocarbons (PAH): pyrene, chrysene and phenanthrene.

The focus was to better understand the substance properties influencing the timely manner of diffusion. Extractions were performed on 2 different matrices, hexane and milk. Half-times and recoveries were derived from the resulting data. MDE achieved extraction efficiencies for PAH ranging between 83.9 % and 96.3 %. No distinct correlation was found between analyte's molecular weight, molecular volume or log(K_{ow}) and the half-times. Molecular structure (aromatic ringsystems, sterical extensiveness) appeared to be the most influential property on extraction kinetics.

Extractions with milk as a matrix gave increased recoveries, likely as the result of altered volume ratios between n-hexane inside/outside the tubing and therefore shifted equilibrium concentrations. The data pointed towards lipophilicity as a major influence besides the structure regarding the half-time-increasing effect of milk.

MDE showed good results regarding close-to exhaustive extraction for PAH. Results indicate that extraction kinetics likely depends on the analyte's molecular structure.

Further investigations have gone into researching more different PAHs, environmental relevant concentrations and alterations in the extraction process. Data has yet to be analyzed and should be available at the meeting. They could reveal a clearer pattern of the influential properties and therefore enable predictions of extraction behaviour. This might render MDE applicable for fractionated extraction. Other matrix materials (tissue, sediment) should be investigated to prove applicability in other areas of ecotoxicology.

**Keywords:** PAH – extraction – milk – LDPE – MDE
Fate of short chain perfluorinated carboxylic and sulfonic acids in a water-saturated sediment column investigated under near-natural conditions

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Perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) have been used in numerous consumer and industrial products in the last 50 years. Perfluorooctanoic acid (PFOA) and perfluorooctansulfonic acid (PFOS) are the best investigated representatives out of these groups. Nowadays they are known to be toxic, bioaccumulative and persistent and industry has started to shift to a shorter chain chemistry i.e. C\textsubscript{4-6} PFCAs and PFSAs. These chemicals have already been found in surface waters which may be a potential source for drinking water, for example after sediment filtration. Up to now, data on the transport of PFCAs and PFSAs in sediments is scarce. The aim of this study was to gain an understanding on the fate of C\textsubscript{4-10} PFCAs and C\textsubscript{4,6,8} PFSAs in a water-saturated sediment column representing a riverbank filtration scenario under near-natural conditions. The column was filled with coarse-grained medium sand and fed with surface water (pH 7.4 – 7.9). Analytes (< 15 ng L\textsuperscript{-1}) and a tracer were spiked in the supernatant, water samples were collected from the supernatant and after 40 and 80 cm of sediment passage in regular intervals and analysed after solid phase extraction using LC-ESI-MS/MS. Short chain PFCAs and PFSAs with up to six C-atoms showed complete and tracer-like breakthrough. Longer chain ones were retarded either due to sorption to the sediment or due to a gradual release from insoluble complexes and suspended matter in the aqueous phase. The study reports the first column-derived sediment-water partition coefficients ranging from 0.01 cm\textsuperscript{3} g\textsuperscript{-1} to 0.41 cm\textsuperscript{3} g\textsuperscript{-1} for C\textsubscript{4,6} PFSAs and from 0.004 cm\textsuperscript{3} g\textsuperscript{-1} to 6.5 cm\textsuperscript{3} g\textsuperscript{-1} for C\textsubscript{4,5,6,8,9} PFCAs. The results clearly indicate that if contaminated surface waters are used as a resource for drinking water production via sediment passage short-chain PFCAs and PFSAs might poses a threat for drinking water.

Keywords: riverbank filtration – PFCAs – PFSAs – sand-water partition coefficient – column study
Transfer and distribution of hydroxylated polychlorinated biphenyls (OH-PCBs) in the brain of finless porpoises (*Neophocaena phocaenoides*)

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Recent studies reported that several hydroxylated polychlorinated biphenyls (OH-PCBs) could competitively bind to thyroid hormone (TH) transport protein transthyretin (TTR), and inhibit TH transport. THs are essential for neuronal development, and *in vitro* studies have shown that the low doses of OH-PCBs suppressed the TH-dependent gene transcription and development of mouse Purkinje cells. It is reported that cetaceans accumulate higher levels of persistent organic pollutants such as PCBs, and their hydroxylated metabolic products, OH-PCBs, which have also been detected in their plasma. However, there is a few reports on the accumulation features of OH-PCBs in cetacean brains. In this study, levels and accumulation patterns of OH-PCBs in the brain of finless porpoises (*Neophocaena phocaenoides*) stranded or bycaught along Japanese coasts were determined (*n*=13). In addition, the present study investigated the levels and patterns of OH-PCBs in the eight regions of the brain: frontal lobe, occipital lobe, limbic system including hippocampus, hypothalamus, pituitary gland, cerebellum, pons and medulla oblongata of finless porpoises to examine the link between the distribution in the brain and its toxicological implications. The median OH-PCBs level in the brain was 33 pg/g wet wt, comparable with the level found in blood (31 pg/g wet wt). There was a positive correlation between the levels in the blood and brain (*p*<0.01), indicating OH-PCBs transfer into the brain in a concentration dependent manner. OH-PCBs homolog profiles were different in the brain and blood samples, *penta*-chlorinated homologs being dominant (52%) in the brain, suggesting the specific transfer and accumulation of these homologs in this organ. Variation in the levels and accumulation features of OH-PCBs was found in the brain regions, with localized accumulation in the pituitary gland (150 pg/g wet wt). This result indicates potential localization and brain region-specific effects of OH-PCBs in the finless porpoises.

**Keywords**: OH-PCBs – thyroid hormone – brain – cetacean – finless porpoise
Effects of Olive Oil Production Wastewater on Soil Arthropods in Two Different Cultivation Scenarios in Israel and Palestine

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Olive oil production wastewater (OPWW) is a challenging effluent which accrues during the olive oil extraction process. Considering the edaphic mesofauna, no ecotoxicological evaluation was performed until now although OPWW is applied controlled and uncontrolled in all olive oil producing countries directly onto the soil. The main purpose of this study was to close this knowledge gap and investigate the difference of effects of a controlled OPWW application on Oribatida and Collembola in two contrasting cultivation scenarios in Israel (intensive, irrigation, semi-arid, loess) and Palestine (extensive, plowing, hot-summer Mediterranean, clay loam). In order to distinguish effects of moistening from other OPWW effects control plots were treated with tap water with the same amount of 14.7 L/m\textsuperscript{2} in both fields. Additionally to the extraction of arthropods by custom-build modified Berlese-Tullgren funnels before and after the treatment, soil chemistry (pH, Cations, Humidity, Water drop penetration time, elemental composition) was determined. Collembola and Oribatid mites responded differently to the treatments. In Beit Rima, the abundance of Collembola increased in OPWW and water treated plots. In contrast emergence of Oribatida was suppressed in OPWW treated plots only. Significant relationships between the community changes and pH as well as magnesium were found. In Gilat, Oribatid abundance increased at the end of the study. In terms of soil chemistry, cation and organic carbon content as well as water drop penetration time increased after OPWW application while pH decreased. The daily irrigation in Gilat attenuated soil acidification and hydophobicity probably through elution of compounds into deeper layers. The found effects of OPWW on soil chemistry and soil biology in extensive as well as intensive managed olive orchards were a first step to monitor future changes and underlined the need for further research on the ecotoxicological profile of OPWW.

Keywords: olive oil – waste water – Oribatida – Collembola – soil chemistry
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Session: Environmental chemistry

Distribution of mercury, copper and zinc in soils and fluvial sediments from an abandoned gold mining area in southern Minas Gerais state, Brazil

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In the 19th century, rural areas of the Descoberto Municipality (southern Minas Gerais State, Brazil) were submitted to gold extraction, using rudimentary techniques of mineral processing. In 2006, local population detected metallic mercury on soil surface and a critical perimeter (8000m²) of contamination was delimited by public research institutions. This work proposes the study of the potential mobility of mercury, zinc and copper in soils and bottom fluvial sediments into this critical zone of pollution at the Descoberto Municipality. Metals bioavailability and potential mobility were studied through granulometric determination, mineralogical characterization (by X-Ray diffratometry, in the total samples), determination of pH and organic matter (using LECO Equipment). The quantification of total and potentially bioavailable concentrations of aluminum, iron, mercury, copper and zinc was performed by Atomic Absorption. Geoaccumulation Indexes (IGEO) were calculated for evaluating the intensity of metals pollution in sediments. Soil and sediments were extremely sandy. Values of pH were in the neutral range (between 5.0 and 7.5 units), while organic matter concentrations were very low (<3%). Mineralogical characterization indicated the presence of quartz, kaolinite and gibbsite for all samples. Silimanite and microcline were detected for some samples and are associated with the weathering of gnaissic rocks from the regional geology. Total mercury, zinc and cooper concentrations were higher than the limits recommended by Brazilian documents. Geoaccumulations indexes revealed that most of the sediment samples were "low to moderately polluted" by zinc and copper, while just one sample was classified as "very strongly polluted" by mercury. Mercury speciation revealed the predominance of its elementary form for all samples, and low concentrations for exchangeable, strongly bound and residual fractions. Zinc and copper behavior was strongly controlled by iron and aluminum concentrations (p<0.05), while their bioavailable contents were very low compared to the total concentrations (<2%).

Keywords: contamination – metals – sediments – soils – gold mining
Simplify your sediment pore water analysis
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The extraction of pore water from sediment cores is one of the most important techniques for the investigation of small-scale biogeochemical processes. Sampling can be problematic because of small sample volumes, high spatial resolution, and high sensitivity of the pore water to ambient changes. Extraction techniques are often laborious, time consuming and prone to contaminations. The analytical method of choice must be able to cope with small sampling volumes and should include all desired chemical parameters. Here we present a combination of pore-water extraction using Rhizon samplers and subsequent analysis with a portable electrophoresis (CE) instrument. The major inorganic anions and cations of the sediment porewater, including manganese(II) and ferrous iron, can be analyzed in less than 15 minutes. The time between sampling and analysis is less than 20 seconds allowing to comprise the oxygen-sensitive reduced iron (Fe(II)) within the set of cations (including Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, NH\textsubscript{4}\textsuperscript{+}, Mn(II)) without splitting, acidification or dilution of the sample. The CE instrument requires less than 20 µL sample volume, which increases the spatial resolution of the sediment profile and guarantees a very fast sampling procedure even in sediments with low water content. The equipment is inexpensive, easy to handle and fully portable and therefore feasible for on-site applications.

Keywords: pore water – sampling – capillary electrophoresis – rhizons – iron(II)
Presentations: Tuesday, February 12th
Session: Life cycle assessment

Carbon footprint for the joint production of a wood-based product and its by-product – A case study
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The scarcity of resources increasingly motivates the shift to resource efficient manufacturing. For this purpose, a product carbon footprint is conducted to identify significant environmental impacts of the manufacture of a wood-based product and its by-product (wood chips which are further used in heat production) in the European Union. The carbon footprint quantifies the material flows and the corresponding 100-year global warming potential impacts of processes, production lines or services throughout their life cycles in physical units. The most important inputs to the life cycle inventory are steel, natural gas and diesel (by mass), water, recycled waste water and wood (by volume) as well as heat and electricity (by energy). The emissions include mainly carbon dioxide, followed by nitrous oxide and methane and result mainly from drying, cooking and processing the wood. The main contributors are the heat from the wood chips and the disposal of the wood chips ashes, the electricity consumed as well as the manufactured and recycled steel. When differentiating the emissions for the wood-based product and its by-product, the results of this case study indicate that the material loss is the main contributor to the global warming potential. The results can change significantly depending e.g. on the moisture content of the wood chips used for heat production or the steel recycling quota. Key parameters and their variability are discussed to provide corporate compartments, such as accounting, product development or process optimization with metrics to align their decisions with improving resource efficiency. On the basis of this approach, organizations can identify environmental optimization potentials and support more resource efficient decisions.

Keywords: LCA – carbon footprint – wood-based product – resource efficiency – decision
The importance of uncertainty analysis in life cycle assessment studies

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Uncertainty analysis is defined by the International Organization for Standardization as “a systematic procedure to quantify the uncertainty introduced in the results of a life cycle inventory analysis due to the cumulative effects of model imprecision, input uncertainty and data variability” (ISO 14044, 2006). It is common in LCA studies, however, to ignore uncertainty analysis due to time and budget constraints. It is argued here that uncertainty analysis is as important as the overall LCA results themselves, and should always be reported along with the results of the study. LCA studies are often used to support decision-making and policy development. Therefore, the lack of uncertainty analysis may lead to incorrect decisions being made, especially when a comparative study is being conducted.

This study uses a simplified example for the life cycle of an electric kettle to conduct Process, Input-Output, and Integrated Hybrid LCAs for the system, and to determine where the uncertainties arise in each LCA. These uncertainties are categorized as parameter, model and scenario. Parameter uncertainty is quantified using Monte Carlo Analysis, and model and scenario uncertainties are quantified by finding the maximum variation due to the alteration of decisions made during the modelling process and the application of different scenarios. It is further demonstrated from assessing the uncertainties in the Process and Input-Output LCAs, how the two methods can best be combined in a hybrid analysis in order to reduce the overall uncertainty for the study.

Keywords: life cycle assessment – LCI methodologies – uncertainty analysis
The effect of titanium dioxide nanoparticles on freshwater organisms

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The population-level effect of nanoparticles has not been widely studied but is of crucial importance for predicting the consequences of nanomaterial exposure in natural environments. We conducted a two month long experiment investigating the effect of nanoparticle titanium dioxide (TiO₂) on the water flea, *Daphnia pulicaria*, and the influence of UV light on nanoparticle toxicity. We exposed daphnid populations to three concentrations of TiO₂ (2, 10 and 50 μg/L) in three different light regimes (constant UVA light, UVA light on a 12:12 cycle, and no UV light) for 60 days. We counted and identified the size classes of all individuals in these treatments to monitor the population dynamics of the *Daphnia*. At the end of two months, we collected a subset of the daphnid populations and exposed them to higher concentrations of TiO₂ (50, 100 and 500 μg/L) for a week to investigate whether the organisms’ past exposure to TiO₂ increased their ability to tolerate the nanoparticle. We collected *Daphnia* from both the long and short-term experiments to analyze uptake of titanium into the organisms themselves. Overall, we found no effect of these environmentally relevant concentrations of TiO₂ on the structure, size, and persistence of *Daphnia* populations. Higher concentrations of TiO₂ did induce production of resting eggs in the *Daphnia* exposed to the nanoparticles in the presence of UVA light, a stress response in this species, but none of these concentrations compromised the survival of these daphnid populations. Further, past exposure to TiO₂ had no effect on the tolerance of the *Daphnia* to higher concentrations of TiO₂, since these higher concentrations also did not have a significant effect on daphnid survival. To our knowledge, this work represents the longest experiment investigating the effect of nanoparticles on *Daphnia* and one of the few focusing on population-level consequences of nanoparticle exposure. We also investigated the effect of TiO₂ on the nutrient dynamics and survival of the freshwater green algae, *Chlamydomonas reinhardii*, and will present preliminary results from this work.
Toxic effects of lead and nanoparticles mixed in anterior kidney cell cultures from freshwater fish

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Heavy metals as lead and nanoparticles are providing a growing concern for environmental health. They can be found together in the environmental and the hypothesis is that the nanoparticles increase the metal uptake. This study aimed to evaluate the cytotoxicity effects and nitric oxid (NO) production of the lead and nanoparticles titanium dioxide (TiO2) alone and the mixture of them, in anterior kidney cell cultures from the freshwater fish Hoplias malabaricus. Cells from monocytic lineage were separated from the anterior kidney and placed (106 cells.well-1). After 24h non-adherent cells were removed and the remaining cells were treated with lead at 10, 1, 0.1 and 0.01ug.mL-1 or TiO2 at 15, 1.5, 0.15, 0.015 and 0.0015ug.mL-1 or the lead plus all concentration used of TiO2, in the presence or absence of 1ng.mL-1 lipopolysaccharide (LPS). Cytotoxicity and NO concentration were evaluated after 24h. The viability of the cells decreased at the all concentration of lead (29±2%, 36±3%, 31±1.5% and 40±2% respectively). The TiO2 alone didn’t reveal differences of cell viability (13±0,7%) in all tested concentration. However, all lead concentrations increased significantly the cell viability when TiO2 was present in 15 and 1.5ug.mL-1 (90±1.3% and 95±2.1%). In the lowest tested TiO2 plus lead concentrations, an intensification in the reduction of cell viability occurred (20±3%). The NO production showed a decrease in all concentrations of lead, differently of TiO2 that presented NO reduction only at 15 and 1.5ug.mL-1. NO production decreased in both lead and nanoparticles groups after LPS induction. The lead plus TiO2 showed a recovery of NO production as well as in the presence or absence of LPS. These results suggest that in vitro some concentrations the TiO2 can protect the lead effect in culture cells. However for this effect is necessary one balance between the concentrations of heavy metal and nanoparticles.

Keywords: titanium dioxide nanoparticles – lead – fish – cytotoxicity – culture cells
Presentations: Wednesday, February 13th
Session: Nanoparticles

Product and size specific ecotoxicity of titanium dioxide nanoparticles to
Daphnia magna

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Titanium dioxide nanoparticles (nTiO2) are increasingly applied in numerous products, which eventually results in their unintended release into aquatic environments. Once there, such particles may provoke adverse implications in wildlife. To assess their potential effects, various ecotoxicological experiments were performed using inter alia the standard test organism Daphnia magna. Interestingly, these bioassays uncovered an inconsistent ecotoxicity of nTiO2, which varied several orders of magnitude. This discrepancy may potentially be driven by differing characteristics of the particles used in such studies. In order to evaluate this uncertainty we conducted a series of 96-h acute toxicity tests with Daphnia especially focusing on two nTiO2 characteristics, namely the crystalline structure (nTiO2 products A-100 and P25 consisting of anatase and a mixture of anatase as well as rutile, respectively) and the initial particle size (65nm; 100nm; 140nm). Each experiment was conducted in the absence and presence of seaweed extract to account for the ubiquitous presence of dissolved organic carbon (DOC) in natural surface water bodies. The experiments without DOC showed an approximately 3-fold increased toxicity of A-100 compared to P25. Furthermore, the same experiments displayed – irrespective of the product used – that the smallest particle size class resulted with an effect size of about 85% in significantly lower 96-h EC50 values compared to the respective biggest size class. These findings were finally supported by a 2x3 factorial test uncovering product and size specific effects of nTiO2 on the test species. In contrast, no acute effects occurred in the presence of DOC, regardless which product and particle size class was used. Our results highlight the necessity to differentiate between apparently similar products, but also the urgent need to consider environmental relevant parameters (e.g. DOC) during environmental risk evaluations of nanoparticles.

Keywords: nanomaterials – metal oxide – photocatalyt – immobility – crustacea
Engineered copper oxide (CuO) nanoparticles (NPs) are used in various applications, such as catalysts, microelectronics, solar energy and paints or fabrics for antimicrobial properties. CuO NPs released into the aquatic environment are likely to agglomerate and/or aggregate upon contact with water and subsequently accumulate into sediments. As a result, deposit-feeding organisms may be at particular risk of exposure to CuO NPs. Although studies examining the ecotoxicological effects of NPs are increasing, most studies are based on bulk vs. nanosize comparisons. However, biological responses to NPs may also be governed by other parameters such as particle shape. Thus, in this study three different shapes of CuO NPs (rods, spheres, spindles) were used to examine the effect of shape on bioaccumulation and toxicity in the sediment-dwelling freshwater gastropod *Potamopyrgus antipodarum*. Snails were exposed for two weeks to clean natural sediment, or sediment amended with either aqueous Cu or rod-, sphere- or spindle-shaped CuO NPs at a nominal exposure concentration of 240 µg Cu/g dry weight of sediment. Following exposure, snail growth was found to be significantly impaired in snails exposed to the sphere- and spindle-shaped CuO NPs. However, toxicity was not directly linked to bioaccumulation of Cu into snails, as by far the highest amount of Cu taken up (into whole snails, tissue and shell) was observed for snails exposed to aqueous Cu. Overall, our results suggest that both particle form (i.e., free ion vs. particle) and shape have an important influence on the toxicity and bioaccumulation of Cu in *P. antipodarum*, and that correlations between these parameters are not straightforward.

**Keywords:** nanoparticles — sediment exposure — deposit-feeder — bioavailability — bioaccumulation
Acute and chronic effects of magnetite-based nanocomposites on invertebrates (Hyalella azteca and Chironomus riparius) and zebrafish embryos (Danio rerio)

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In the project Fe-NANOSIT, iron-based nanocomposites are developed for remediating contaminated groundwater and wastewater. For catalytic treatment of industrial wastewater, magnetite particles (Pd-magnetite, magnetite-zeolite, TiO_2-magnetite) are designed. Ecotoxicity of these nanocomposites is evaluated using standard test methods. Since magnetite composites quickly agglomerate and precipitate in the test media, polyaspartate is used as dispersant. Acute toxicity of Pd-magnetite has been determined in several test systems with fish embryos (Danio rerio), the crustacean Hyalella azteca and larvae of the midge Chironomus riparius. Chronic toxicity was tested with C. riparius. Different tissues of the test organisms were analyzed to assess the uptake of the particles. Reference tests with bulk iron and FeCl_3 were performed with H. azteca and D. rerio embryos.

Overall, toxicity of the particles that were tested so far was low. For Pd-magnetite and magnetite-zeolite, no toxic effects were detected at concentration ranging from 1.0 to 100 mg/L in the used test systems. Likewise, the results of the reference tests with bulk iron and FeCl_3 did not indicate acute toxicity to H. azteca and embryos of D. rerio at the tested concentrations (bulk iron: 100.0 mg/L, FeCl_3: 1.0 and 10.0 mg/L). In further tests, the ecotoxicity of magnetite-zeolite and TiO_2-magnetite will be analyzed.

Keywords: iron-based nanoparticles – Hyalella azteca – Chironomus riparius – Danio rerio – wastewater treatment
A novel method for the determination of effects of nanomaterials on organisms related to waste water treatment plants

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Nanomaterials have innovative properties and are increasingly manufactured for various applications. Especially nanosilver (nAg) is appreciated for its antibacterial effects and belongs to the most intensively deployed nanomaterial in housekeeping articles. An introduction of nAg released from such consumer products into sewer facilities and finally the occurrence in waste water treatment plants (WWTPs) is very likely. However, adverse effects on the activated sludge community which is responsible for the water purification is poorly understood. In situ studies so far investigated effects of nAg on single species or on the activity of the whole community, especially focusing on nitrification inhibition. However, results proved to be contradictory due to differences in experimental setups, such as exposure scenarios and boundary conditions. Furthermore, the effects on interactions within the community have been widely neglected. Only few experiments have addressed accumulation of nanomaterials in simple food chains or investigated community effects under site relevant conditions.

The overall aim of the project presented here, is to establish innovative test approaches to investigate effects of nanomaterials on communities in waste water treatment plants. It is obvious, that traditional test guidelines using algae, daphnia or fish are not appropriate. Therefore, in a first attempt methods have been established for relevant organisms in water treatment to characterize the impact of nanomaterials in single species test. In a second step, an experimental design will be established which provides the possibility to characterize the impact on a structured community taking interactions into account. This strategy should lead to a detailed understanding of the effects of nAg on communities. On the one hand this will allow a better experimental design of the final tests in model laboratory scaled treatment plants with natural inflow. On the other hand these results enable an easier interpretation of data derived from complex experiments.

Keywords: nanosilver – WWTP – bacteria – ciliate – toxicity test system
The effect of pH on the toxicity of ZnO nanoparticles to Folsomia candida in amended field soil

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Toxicity and bioavailability of manufactured nanoparticles (NPs) in soil is determined by physicochemical properties of the particles as well as by soil properties, such as pH and organic matter. This study aimed at assessing the effect of soil pH on the toxicity of ZnO-NP to Folsomia candida in Dorset field soils with pH\textsubscript{CaCl\textsubscript{2}} level adjusted to 4.5, 5.9 and 7.2. To unravel the contribution of particle size and soluble Zn to ZnO-NP toxicity, non-nano ZnO and ZnCl\textsubscript{2} were also tested.

Zn sorption increased with increasing soil pH and Freundlich $k_f$ values ranged from 98.9 to 333 l/kg for ZnO-NP and from 64.3 to 187 l/kg for ZnCl\textsubscript{2}. No effect of ZnO particle size was found on sorption and little difference was found in toxicity between 30 and 200 nm ZnO. Survival of F. candida was not affected by ZnO at concentrations up to 6400 mg Zn/kg d.w. The effect on reproduction decreased with increasing soil pH for all three Zn forms, with 28-d EC50s of 553, 1481 and 3233 mg Zn/kg d.w. for ZnO-NP and 331, 732 and 1174 mg Zn/kg d.w. for ZnCl\textsubscript{2}, at pH 4.5, 5.9 and 7.2, respectively. EC50s based on porewater Zn concentrations increased with increasing pH for ZnO-NP from 4.77 to 18.5 mg Zn/l, while for ZnCl\textsubscript{2} no consistent pH-related trend in EC50s was found (21.0-63.3 mg Zn/l). Calcium levels measured in ZnCl\textsubscript{2}-spiked soils were ten times higher than in ZnO-spiked soils. A Biotic Ligand Model (BLM) was applied to take into account the effect of calcium and proton levels in the soil pore water on the toxicity of the calculated Zn$^{2+}$ ion concentrations. Preliminary results suggest that the decreased toxicity of ZnCl\textsubscript{2} compared to ZnO-NP was due to a protective effect of calcium and not a particle effect.

Keywords: zinc oxide nanoparticles – soil – pH – Folsomia candida – bioavailability
Presentations: Wednesday, February 13th
Session: Nanoparticles, Wednesday

Natural and artificial organic substances alter algal toxicity of nano CeO$_2$
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The development and use of engineered nanomaterials (NMs) is increasing at an ever accelerating rate and even though the research in environmental impacts of nanomaterials is developing quickly, the available information about the likely inputs, fate, behaviour and effects of NMs is still insufficient. Moreover, the chemistry and toxicity of NMs is greatly affected by environmental conditions, such as pH, salinity or presence organic substances. The aim of this work was to evaluate acute aquatic toxicity of nano CeO$_2$, used as fuel additive, catalyst and UV blocking agent, of two particle size, 2.1 and 47.7 nm, towards freshwater green algae Scenedesmus subspicatus and the influence of two organic substances, the artificial surfactant sodium dodecyl sulfate (SDS) and naturally occurring humic acid (HA) on this toxicity. The 2.1 nm CeO$_2$ had a more important effect than the form with the larger particles. The presence of SDS at the concentration of 0.15 ml/l enhanced significantly the effect of the two tested substances with almost a 4-fold decrease of 72 h EC50 in the case of the smaller form. The HA at the concentration of 10 mg/l (i.e. 4.96 mg C/l) on the other hand mitigated the toxicity of both of the forms of CeO$_2$.

**Keywords**: nanomaterials – toxicity – Scenedesmus subspicatus – sodium dodecyl sulfate – humic acid
Invasive vs. native grasses in Cerrado (Brazilian savanna): physiological and morphological responses to a mosaic of environmental conditions
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Cerrado is a South-American savanna-type biome and a hotspot for conservation for its high levels of biodiversity and endemism. The region is characterized by strong rain seasonality, a vegetation/soil gradients and a fire regime, which has been occurring naturally for over 32000 years. These environmental characteristics can be altered to a greater or lesser extent by human activities and changes in land use. Alien grasses, for instance, are serious threat to Cerrado ecosystem’s integrity. They can displace the native flora and change the fire regime. This study aimed to evaluate the responses of an invasive and a native species to water stress, fertilization and clipping - simulating the field environmental conditions. Plants of both species were submitted to different soil moisture regimes, from drought to flood, with or without fertilizer addition, for three weeks at 28ºC and were then clipped. Plants were left to re-sprout in the same conditions, to account for the response to clipping. Several morphological and physiological parameters were assessed: height, biomass, number of leaves and tillers, leaf area, chlorophyll $a$ fluorescence, pigments (chlorophyll $a$, $b$, carotenoids, antocyanins), MDA (lipid peroxidation) and protein content. Results show significant differences among treatments and species as response to water and nutrient availability. Flood showed to be as stressful as drought. Clipping was more harmful to native species whereas invasive species showed to be more efficient to use resources and accumulate biomass, showing less stress levels and responding more positively to nutrient addition.

Keywords: drought – flood – fertilizer – invasion – management
Effects of aerosol particles on crop plants
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Plant surfaces are a major sink for aerosol deposition. The deposited particles are mostly hygroscopic and may become deliquescent within the humid leaf boundary layer due to plant transpiration. These highly concentrated solutions may then interact with leaf surfaces and thus create a water sink in close vicinity to the stomata, thereby influencing plant water relations. The ecological role of deposited aerosol particles has frequently been studied by applying additional particles to the foliar surface. It has been reported that the deposition of hygroscopic particles may lead to an increase in transpiration. The leaf surfaces can however, be already contaminated with significant amounts of atmospheric deposits. Therefore, the fate of particles deposition on plants can experimentally be assessed by particle exclusion experiments. Beans and sunflowers plants were grown in well watered soil or hydroponics; in ventilated greenhouses with ambient air (AA), and filtered air (FA, particle exclusion) with almost particle free condition. By excluding the particles more than 99%, stomatal conductance and transpiration of sunflowers and beans were 20 – 40% lower than for the respective AA plants. While predawn water potentials were not different, midday water potentials of AA plants were lower than for FA plants. These results for the first time show an impact of ambient aerosol particles on plant ecophysiology. The results suggest that particulate air pollution may decrease the water use efficiency (WUE) and possibly the drought tolerance of plants.

Keywords: atmospheric aerosols – ecophysiology – particle deposition – transpiration – tree drought mortality
Presentations: Wednesday, February 13th
Session: Terrestrial ecotoxicology

Extremely low frequency (50 Hz) electromagnetic field exposure alters nutritive stress response in *Eisenia foetida* (Lumbricidae)

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An *in vivo* model was set up to establish morphometric characteristics of B protocerebral neurosecretory neurons (cell and nuclei size, citoplasmatic/nuclear ratio) in the earthworm *Eisenia foetida* (L.) exposed to synergistic effect of nutritive stress and extremely low frequency electromagnetic field (ELF EMF - 50 Hz, 50 µT, 17 V/m and 50 Hz, 150 µT, 17 V/m, respectively).

For experiment, animals were divided in to 10 groups. Groups 1-9 were continuously exposed to nutritive stress (starvation). Groups 1-8, beside nutritive stress, were subjected to homogenous, vertical orientated ELF EMF (50 Hz, 50 µT, 17 V/m and 50 Hz, 150 µT, 17 V/m, respectively). For acute treatment exposure time was 2 and 4 hours, whilst, 24 hours and 4 h/day during 76 hours for chronic exposure to each specified field strength. The ninth group was sham-exposed, i.e. exposed only to starvation and natural magnetic field, and tenth group was exposed only to natural magnetic field.

Serial brain cross-sections were stained using the Alcian blue-Periodic-Acid-Schiff-Orange G technique. All morphometric parameters were analyzed using an image processing and analysis system AxioVision Rel. 4.8.1. (Carl Zeiss MicroImaging GmbH, Germany) linked to Axio Imager 1 light microscope. Measurements were performed using Digimizer 4.0.0.0. (MedCalc Software, Belgium) image analysis software.

The values of all morphometric parameters of B neurosecretory neurons were significantly changed in animals exposed to synergistic effect of ELF EMF and nutritive stress in comparison to both sham-exposed and control groups of animals. The extent and direction of changes were measured as a function of field strength which the animals were exposed to and were also depending on the length of exposure.

The extremely low frequency electromagnetic field (ELF EMF) influences characteristics of protocerebral B neurosecretory neurons in the earthworm *Eisenia foetida* (L) and indicates “windows” of intensity in which changes are manifested.

**Keywords:** Electromagnetic field, *Eisenia foetida*, Lumbricidae, neurosecretory cells, nutritive stress
Comparison of mechanistic models and standardized regression analyses to describe toxic effects in ecotoxicology

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The application of regression models is a currently well-established statistical tool towards the evaluation of concentration-response relationships and to determine effect levels of compounds in ecotoxicology. In this study, we analyzed and compared the recommended and frequently used two-parameter regression methods Probit, Logit and Weibull. As an alternative approach to these regression methods, the toxicokinetic-toxicodynamic (TKTD) model GUTS (General Unified Threshold Model of Survival) was used to evaluate acute-toxicity tests by simulating survival with various exposure concentrations at defined time points. The study was based on experiments with the cyclopoid copepod *Mesocyclops leuckarti*, exposed to triphenyltin hydroxide (TPT).

Based on different statistical evaluation techniques, the Weibull regression generally seems to be a very suitable curve-fitting method. Taking into account the biological variability in the sensitivity of considered individuals of a population, the lower 95% confidence limit of the Weibull regression appears to be a comparatively meaningful endpoint of concentration-response analyses. Nevertheless, the calculation of LCx-values is still a poor and imprecise description of toxicity, mainly because it does not include a statement about the time course of a toxic effect.

The GUTS application can contribute to solve this issue by interpreting mortality as a response of a dynamic system (the organism). This mechanistic approach provides appropriate simulation results with a good description of the dose-response relationships. Furthermore, due to the integrated threshold of survival in the model, GUTS generates comparatively small but meaningful confidence intervals at low concentration ranges, which are of particular importance in the environmental risk assessment of chemicals. In addition, the quantification of TKTD processes by GUTS also provides the opportunity to simulate survival for more realistic exposure scenarios.

This study demonstrates another potential use of TKTD models and presents an alternative to the recommended regression models to assess toxicity tests.

**Keywords:** regression models – GUTS – TKTD – *Mesocyclops leuckarti* – TPT
Predicting copper toxicity in different ecotypes of earthworms based on biotic ligand model concept

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Copper toxicity to different ecotypes of earthworms (*Lumbricus rubellus*, *Aporrectodea longa* and *Eisenia fetida*) was investigated upon 28 days exposure in a range of soils of varying properties. A modified Langmuir-Freundlich model, rather than the common biotic ligand model (BLM), was used to relate Cu toxicity to free Cu ions and protective cations (protons and Na⁺) in the soil pore water. This modified model complies with the mechanistic knowledge obtained from an existing BLM of Cu for earthworm *Aporrectodea caliginosa*, which was developed in the solution-quartz sand system. Median lethal concentrations (LC50s) of Cu based on total concentration ranged from 47.5 to 306.7 mg/kg for *L. rubellus*, from 51.9 to 765.2 mg/kg for *A. longa*, and from 87.5 to 3733.5 mg/kg for *E. fetida* in the soils tested. The relative sensitivity of the earthworms to Cu was independent of soil types and followed the order: *L. rubellus* > *A. longa* > *E. fetida*. The modified Langmuir-Freundlich model provided good predictions for Cu toxicity and explained, respectively, 89%, 91% and 94% of variations in LC50s of Cu (expressed as free ion activity) for *L. rubellus*, *A. longa*, and *E. fetida* in different soils. Predicted LC50s never differed by a factor of more than 2 from the observed LC50s. As reflected from the model parameters, protons and Na⁺ effectively alleviated Cu toxicity to earthworms. The findings in the present study revealed that the existing Cu-BLM concept for one earthworm ecotype is also applicable to other ecotypes and the interactions between Cu ions and protective cations at the biotic ligands of different earthworms are more or less the same.

**Keywords:** earthworm – toxicity – biotic ligand model – copper – soil
Presentations: Wednesday, February 13th
Session: Effects and exposure modeling

Interaction between food and toxicant leads to hormesis in the pond snail

*Lymnaea stagnalis*

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The degree of toxicity of a chemical compound depends critically on the nutritional condition of the affected organism. While limited feeding generally has an amplifying effect on the apparent sensitivity, the intrinsic sensitivity is not necessarily affected. In ecotoxicology, this distinction is not often made, which can lead to misinterpretation of test results. In many cases, researchers are ignorant about the actual nutritional status of the organisms they are testing. However, to understand the effect of a compound, we need to distinguish between apparent and intrinsic sensitivity.

Here, we investigate a previously published study on the pond snail *Lymnaea stagnalis* exposed to the herbicide diquat under environmentally relevant concentrations. The authors found a hormetic effect pattern in juvenile feeding and time to maturation. In the experiment, the pond snail was fed with lettuce. Diquat is a non-selective herbicide and acts on the lettuce with a dose-dependent degradation of the food source as a consequence.

To study the interaction between food and toxicant, we use a Dynamic Energy Budget (DEB) model. We find that the observed effect patterns can be explained by effects on assimilation only, with assimilation enhanced by low doses of diquat, and decreased by higher exposure. With an effect on assimilation, the same amount of lettuce consumed leads to differences in the amount of energy that is obtained: higher assimilation leads to faster growth. We find that there is no effect on feeding rate per se: the effect on juvenile feeding results from the different sizes of the snails at the point of observation. The differences in growth also explain the shift in time to maturation.

The effect of diquat on assimilation thus fully accounts for the observed hormetic effect pattern in the sublethal endpoints.

**Keywords:** hormesis – Dynamic Energy Budget – nutritional condition – *Lymnaea stagnalis* - herbicide
In the present study, the effects of food quantity on the development, mortality, sex-ratio and reproduction of *Nitocra spinipes* were investigated. The results were implemented into an existing individual-based population model for harpacticoid copepods used to optimize the test design of a proposed OECD test guideline. The laboratory experiments showed an inverse correlation between development time and food concentration as well as mortality and food concentration. The sex-ratio did not show significant trends although a shift towards more males on low food concentrations was indicated. The brood size was the only reproductive parameter to be clearly correlated with the food quantity. Inter-clutch period and time to first brood were mostly unaffected. Nevertheless, threshold food levels were revealed, below this thresholds inter-clutch period and time to first brood are prolonged. Model simulations demonstrated an increase in the overall population abundance, lower population fluctuations, a lower population extinction probability and a shift towards a lower percentage of nauplii within the population structure with increasing food concentrations. Further model testing showed the inhibition of the nauplius development to have a stronger adverse effect on the survival of a population than an increased overall mortality, requiring higher food levels to maintain the same chance of survival. A detailed analysis of the population dynamics and population structure revealed the reason for this to be (1) higher amplitudes within the population fluctuations and (2) a shift towards higher percentage of nauplii within the population. Furthermore, the model showed that the time to detect effects was shorter the stronger the nauplius development was inhibited. Therefore, this study successfully demonstrated the effects of food quantity on the life cycle parameters of *Nitocra spinipes*, pointed out the importance of sublethal endpoints and managed to give feasible explanations based on modeling results.

**Keywords:** population modeling – food dependence – copepoda – harpacticoida – ecology
Presentations: Wednesday, February 13th  
Session: Omics and biomarkers

**Evaluating the impacts of androgen exposure on Eastern Mosquitofish (Gambusia holbrooki) global hepatic gene expression patterns using a custom microarray**

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Microarray technologies provide ecotoxicologists the ability to evaluate changes in expression of thousands of genes at once. One of the applications of microarrays is to find new biomarkers of exposure for use in a field setting. The aim of this project is to determine potential biomarkers of chronic androgen exposure in the non-model species the Eastern Mosquitofish (*Gambusia holbrooki*), a widespread freshwater species in the US whose androgen-driven secondary sexual characteristics make it an attractive candidate for androgen biomarker development. To achieve this aim, a custom 8x15,000 gene microarray was developed by sequencing a cDNA library created from multiple tissues from *G. holbrooki* males, females, and fry using the Roche 454 sequencer. Liver samples of female *G. holbrooki* that were treated for 14 days to 1 μg/L of the androgen 17β-trenbolone (a dose that was able to induce the formation of the male secondary sexual characteristic, the gonopodium) or the vehicle control were hybridized to the custom microarrays. After signal processing and normalization, all data were analysed using JMP genomics (SAS) to determine gene transcripts that were significantly up or down-regulated using a one-way analysis of variance (ANOVA). After removing transcripts with p-values>0.05 and with expression fold changes of less than ±1.5, there were 279 genes down-regulated and 229 genes up-regulated by trenbolone exposure. A group of genes for follow-up validation using quantitative polymerase chain reaction (qPCR) with large fold changes were selected, including steroid metabolizing enzymes, transcription factors, and oocyte development proteins. Gene set enrichment analysis was also used to determine biological processes that were significantly changed; these processes include lipid metabolism, mRNA transport, and regulation of translation. This data set provides a starting point for biomarker discovery and validation in this species for future evaluation of the impacts of androgenic chemicals in US freshwater ecosystems.

**Keywords**: biomarkers – microarray – mosquitofish – androgen – gene expression
Catecholamines and other biomarkers in stressed and non-stressed amphipods

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Catecholamines (CAs) play an essential role in the physiological processes which prepare the body for physical activity in stress situations. The neuroendocrine mechanisms inducing stress in the invertebrates crustaceans are poorly understood and different from those of vertebrates, even though evidences point to CA involvement. For the first time CA concentrations in single animals and homogenates of the crustaceans Niphargus (N.) inopinatus and Gammarus (G.) pulex were measured and examined with HPLC/EcD. Temperature stress (+6 °C and +12 °C) dependent CA concentration changes were measured to investigate the stress response in these crustaceans. An extraction protocol with detailed preparation and measuring instructions for each amphipod was created. The CA concentrations found in unstressed ground water organism N. inopinatus were 1952 [pg/mg DW] for noradrenaline, 0 [pg/mg DW] for adrenaline and 64937 [pg/mg DW] for dopamine. The CA concentrations in unstressed surface water organism G. pulex were 9.8 [pg/mg DW] for noradrenaline, 6.5 [pg/mg DW] for adrenaline and 68.2 [pg/mg DW] for dopamine. The temperature treatment shows that temperature stress (+6 °C and +12 °C) causes the first appearance of adrenaline in N. inopinatus. Furthermore an increase of adrenaline and noradrenalin and decrease of dopamine could be detected at +12 °C in G. pulex. This CA pattern was detected before in the invertebrates as a response to stress. The CA levels of N. inopinatus were higher than the levels of G. pulex in all experimentes. In particular, the average dopamine levels of N. inopinatus were 1671 times higher than in G. pulex. Additionally catecholamine related compounds could be detected. The mass traces of 2-(3,4-Dihydroxyphenyl)acetic acid (DOPAC), a degradation product of dopamine, could be identified in both crustaceans using UPLC/Q-TOF-MS and HPLC/EcD.

Keywords: amphipods – Niphargus spec. – Gammarus spec. – catecholamine – biomarker
The characterisation of crude oil and oil contaminated soil from the Niger Delta by catalytic hydropyrolysis

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Soil contamination by crude oil in the Niger delta basin has been a reoccurring event since Nigerian independence. It has left many devastating effects on the well being and economic life of the people in the largest mangrove swamps in Africa (Aworawo, 1999). Often, the spread and subsequent biodegradation of the crude oil spillage makes it difficult to determine the polluter.

Catalytic hydropyrolysis (hypy), which refers to pyrolysis assisted by high hydrogen gas pressures (15 MPa) in the presence of a dispersed sulphided Mo catalyst, possesses the unique ability to release very high yields of bound biomarkers compared to mild catalytic hydrogenation or traditional pyrolysis methods, whilst minimising alteration to their isomeric distributions (Love et al., 1995). Here hypy is used to release the aliphatic biomarkers covalently bound within the asphaltene matrix of two crude oils (OB & SH) and the solvent extract from a contaminated soil from the Niger Delta (Sonibare et al., 2009). The free phase isolated from the oil SH and the soil was found to have been subject to significant biodegradation (large unresolved complex mixture with a general depletion of n-alkanes), while the bound phase released by hypy provided reliable biomarker profiles to the crude oils and soil extract.

The biomarkers profiles show that the soil extract and both crude oils were moderately mature. Unlike the OB oil where the extended hopanes (C34 & C35) were absent, the SH oil and the soil extract have C34 & C35 hopanes, and the sterane parameters appear to be closely related. Furthermore, the relative abundance of oleanane, serving as a distinguishing feature to inform that the oils originated from terrigenous organic matter source rock (Ekweozor et al., 1979a), confirmed the correlation between oil SH and the contaminated soil.
Molecular biomarkers as indicators of bank vole populations’ response to metal pollution

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Molecular biomarkers are potential tools to study exposure and effects of different contaminants on intoxicated population. Combining results obtained with different types of biomarkers on the same individuals can give exceptionally broad view on populations, which is very hard to obtain in a single biomarker research. In case of populations from metal contaminated sites, it is important to study genetic effects, because often they are prime reasons of changes at the population levels. Efficient mechanisms of detoxification may mask exposure effects on other biomarkers, whereas studying genetic structure of individuals may help to obscure potential danger of pollution.

For our research we chose various biomarkers. This gave us comprehensive view on addressed problem - bank vole, *Myodes glareolus*, populations’ response to environmental stress. There were analyzed: (i) genetic diversity studied with microsatellite markers (ii) metallothionein gene expression (iii) ALAD gene polymorphism (iv) GSH/GSSG ratio. Our investigation was applied to three animal populations from a vicinity of zinc/lead smelters located in Silesia region (Miasteczko Śląskie, Olkusz, Katowice), where a metal industry is condensed and to three control populations (located in area without metal contamination). We found that metallothionein gene expression was up regulated in polluted populations, whereas we did not observed genetic diversity reduction or effects of cadmium/lead levels on GSH/GSSG ratio. Also, we did not find ALAD gene polymorphism in those populations which seems to be adaptation to high metal exposure. Our study showed that depending on a biomarker sensitivity to intoxication, the pattern of a response may not be the same.

**Keywords**: metals – metallothionein – ALAD – glutathione – bank vole
Presentations: Wednesday, February 13th  
Session: Ecological risk assessment

Responses of *Lumbriculus variegatus* to activated carbon amendments  
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Black carbonaceous particles have been observed to bind sediment associated hydrophobic organic compounds (HOCs) efficiently. In consequence artificial black carbonaceous materials, such as activated carbon (AC) have been studied as a potential stabilizing method for contaminated sediments and promising results have been obtained both from laboratory experiments and field trials. Yet carbon amendments may have direct adverse effects on organisms. The results from biological effects of carbon amendments are contradictory, varying from no effects to significant effects depending among other on the used organism and sediment type. Within this study clear adverse effects on oligochaete *Lumbriculus variegatus* were observed. Reduction on egestion rate, growth, reproduction and lipid content was observed with much lower dosages than generally recommended for remediation purposes. Used sediment type and AC particle size affected the responses. In sediments where *L. variegatus* thrives (feeds on the sediment and grows much) the adverse effects were milder than in sediments with lower quality for *L. variegatus*. The fine particle sized AC had stronger effects compared to the coarse AC. The proportion of ingestible particle fraction for *L. variegatus* affected the responses although the mechanism causing the effect is unknown. The strong adverse effects observed in this study shows that not everything is known about the biological effects of AC. Site specific evaluation is especially important when remediation measures are designed and further research is needed before this remediation approach can be implemented broadly on the field conditions.

**Keywords:** activated carbon – biological responses – sediment remediation – particle size – *Lumbriculus variegatus*
Presentations: Wednesday, February 13th
Session: Ecological risk assessment

Potential biofuels in an ecotoxicological investigation
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The interest in biofuels for the transport sector increased within the last years. Biofuels are considered renewable alternatives with the benefits of reduced dependence on fossil fuels and a potential to slow down the effect of global climate change due to decreased greenhouse gas (GHG) emissions. Consequently, biofuel production increased dramatically in recent years. Growing production capacities and the associated rise in biofuel consumption also increased the risk of a release into the environment. However, comparatively little knowledge on (eco)toxicological effects of biofuels are available.

In this study we investigated the potential risks of selected substances derived from biomass and with promising properties for the use in combustion engines (ethyl levulinate, 2-MTHF, 2-MF). The substances were compared to water accommodated fractions of a fossil diesel fuel using assays on the acute aquatic toxicity and biotests to reveal mechanism-specific effects. First results revealed practical problems with the investigation of these materials (e.g., due to their low pH-value and rapid corrosion of pipette tips and microplates) as well as indications for adverse impacts on the organisms or cell cultures tested. 2-MTHF revealed a lower toxic potential in the fish embryo toxicity (FET) test with zebrafish than ethyl levulinate or 2-MF. In the cytotoxicity test using RTL-W1 cells 2-MF showed the highest toxic potential. The Ames fluctuation assay gave no mutagenic potential for ethyl levulinate, 2-MTHF or 2-MF.

In conclusion, the results reveal a potential hazard of these substances and further investigations of potential biofuels or biofuel components are essential to provide a basis for identifying alternative fuels with low environmental impact.

Acknowledgement: This work was performed as part of the Cluster of Excellence "Tailor-Made Fuels from Biomass", which is funded by the Excellence Initiative by the German federal and state governments to promote science and research at German universities.
Presentations: Wednesday, February 13th
Session: Ecological risk assessment

Optimizing sediment conditions for macrophyte testing in the context of prospective risk assessment

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Aquatic macrophytes fulfil important structural and functional roles in aquatic ecosystems. Despite this, the availability of standardised methodologies to assess the environmental risks of organic chemicals to non-target aquatic macrophytes is currently limited. Test guidelines are only available as water-only tests for algae and Lemna (duckweed). The standard freshwater test species, Lemna, is a free-floating, non-sediment-rooted macrophyte and is therefore not representative of sediment-rooted emergent and submerged macrophyte species. In risk assessment, submerged sediment-rooted macrophyte species are currently suggested as test species when chemicals partition to the sediment.

The advantage of sediment tests is that nutrients can be mixed through the sediment, limiting nutrient-availability in the water layer and therefore limiting algae growth. Moreover, the addition of sediment obviates the need for axenic cultures and offers many other advantages, like increased macrophyte growth, decreased endpoint coefficients of variation and increased ecological realism. The advantages of sediment addition trigger the need for an optimal standard sediment for macrophyte testing. Although currently, OECD sediment (5% peat, 75% sand, 20% kaolinite) for invertebrate testing combined with nutrients is used, a clear rationale argument for the sediment composition in macrophyte testing is absent. Nutrients and sediment properties supporting optimum growth are still unknown and should be investigated for the development of the standardized methods. Therefore, the aim was to find the optimal organic matter (OM) content (0.5, 1, 3, 5, 10, 15%) and nutrient (nitrogen and phosphorous) concentrations (0 mg/l N:P, 75 mg/l N:P, 150 mg/l N:P, 300 mg/l N:P, 300 mg/l 3N:1P, 300 mg/l 6N:1P, 600 mg/l N:P) in OECD sediment for a 28 days sediment testing with submerged macrophytes. Test species were the dicotyledonous species Myriophyllum spicatum and the monocotyledonous species Elodea canadensis. First test results show optima for OM and nutrients, thereby underpinning standard test conditions in future macrophyte test guidelines.

Keywords: sediment – toxicity testing – macrophytes – prospective risk assessment – organic chemicals
Effects of anthropogenic pollutants on ecosystem functions in freshwater bodies
– A Review

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Anthropogenic stressors such as pesticides can seriously impair organisms in freshwater ecosystems. We reviewed 52 studies of the last 30 years on the impact of heavy metals and organic pollutants on three freshwater ecosystem functions (leaf litter breakdown, respiration and primary production). The effect concentration of each pollutant that resulted in a reduction of ≥20 % of the respective ecosystem function was derived from the studies. Moreover, to allow a comparison of the various contaminants, the effect concentrations of the contaminants were standardized using the median lethal effect concentrations of the standard test organism (Daphnia magna and Pseudokirchneriella subcapitata) as benchmark. More specifically, we computed toxic units (TU) for each contaminant concentration. For pesticides, the Uniform Principles of the European Union for the authorization of pesticides a TU < 0.01 and TU < 0.1 is assumed to have no unacceptable effects on the invertebrates and algae, respectively. Hence, these thresholds should also be protective for ecosystem functions. Approximately one quarter of the studies reported reductions in ecosystem functions below the threshold of 0.01. Furthermore, reductions in ecosystem functions had already been observed at TU’s 100 times lower than the threshold. However, no concentration-effect relationship could be established between ecosystem functions and TU.

We discuss the relevance of our results for the ecological risk assessment of the contaminants.

Keywords: freshwater – pollutants – ecosystem function – toxic unit – risk assessment
The potential of activated carbon and biochar addition for remediation of a PCB-contaminated sediment. A comparison of effects on vital functions and PCB-bioaccumulation in *Lumbriculus variegatus*.

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The *in-situ* remediation of PCB contaminated aquatic sediments via sorbent material amendment has shown promising results. The contaminant bioavailability can be decreased significantly by the application of these materials. Nevertheless there are indications of an inherited toxicity of these materials, which have to be compared to the beneficial effect of decreased contaminant bioaccumulation.

This study compared ecotoxicological effects and contaminant-binding potential of coal-based activated carbon (AC) and wood-based biochar (BC). The tested sorbents were mixed into a field-contaminated sediment (about 6.6 mg/kg PCB) in concentrations from 0.25 – 5%. Additionally the effects of AC pre-treatments were tested (boiling and solvent extraction). Evaluated parameters were the vital functions (reproduction, growth and feeding rate) and the PCB-bioaccumulation in the sediment dwelling oligochaete *Lumbriculus variegatus*.

The effect of AC on vital functions was severe. Concentrations of already 0.25% AC in the sediment led to a decrease of feeding by about 95%. Feeding was ceased almost completely at a concentration of 2.5% AC. Consequently the growth was reduced to near-zero (0.25% AC) or even turned into a net loss of biomass (2.5% AC). Reproduction was decreased, but no significant mortality was detected. Pre-treatments had no significant effects.

BC had generally milder effects on vital functions. Only at a concentration of 5% BC significant effects (slight loss of biomass, decreased feeding) were observed. Reproduction was not affected.

The adverse effects on the test organism correlated to the remediation potential of the sorbents. AC showed a significantly stronger decrease in PCB-bioaccumulation. Tissue concentrations were decreased by 56% (0.25% AC) and 98% (2.5% AC). BC showed about tenfold lower binding capabilities (Doses of 2.5% and 5% BC reduced tissue concentrations by 55% and ca. 79% respectively).

**Keywords:** sediment – activated carbon – biochar – PCB Bioaccumulation – toxicity
Metabolic changes in *Lumbriculus variegatus* exposed to contaminants and hypoxia

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Hypoxia is prevalent in the aquatic environment especially for benthic organisms living in deep waters. Levels of dissolved oxygen fluctuate because of the presence of ice cover over surface water, eutrophication, or depletion resulting from species biological demands. To understand toxic response in species exposed concurrently to contaminants under reduced oxygen, it is important to explore assessment methods that are regulated beyond the expression of genes, and have direct involvement in numerous cellular processes. In this study, we explored the influence of hypoxia on contaminant uptake and monitored the composition of endogenous metabolites of *Lumbriculus variegatus* exposed at different oxygen levels. Tissue extract analysis for benzo(a)pyrene (BaP: 3µg/L), chlorpyrifos (CPF: 100µg/L), and pentachlorophenol (PCP: 100µg/L) were performed by gas chromatography-mass spectroscopy and metabolite levels were related to treatments and exposure time. We found that chemical uptake increased in worms exposed under hypoxic condition, particularly for BaP and CPF but to a lesser extent for PCP. Succinate and glycerol-3-phosphate increased significantly (*p* < 0.0001) under hypoxic condition while sugars, cholesterol, and cysteine were effectively repressed. Individuals exposed concurrently to hypoxia and PCP were clearly affected and showed reduced levels of TCA cycle intermediates including fumarate, malate, glutamate, and hexonic acid. This suggests that energy metabolisms were perturbed in worms. Overall, hypoxia was a more dominant stressor than chemical treatment and this is apparent from the principal component analysis. Therefore, it is crucial to take into account the different external variables including oxygen deprivation, since they may influence the outcome of effect studies in ecotoxicology. It is possible that such confounding factors may exacerbate the severity of a stressor in nature and thus the toxic response in exposed organisms.

**Keywords**: Hypoxia – metabolites – *Lumbriculus variegatus* – multiple stressors – energy metabolism
Posters
Session: Aquatic ecotoxicology
AT03

A partial life-cycle toxicity test to assess the effects of organotin compounds in the freshwater snail *Lymnaea stagnalis*

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Organotin compounds tributyltin (TBT) and triphenyltin (TPT) were widely used as components of many antifouling paints. Although their use has been banned within the European Union, TBT and TPT can still be found in aquatic environments, posing a risk to aquatic non-target organisms, mainly invertebrates. A large number of studies have been performed mostly on marine mollusc species where effects of a single compound, either TBT or TPT, were assessed in independent studies. These studies pointed out similarities between TBT and TPT regarding their physico-chemical properties and biological effects. Our study aimed at assessing the effects of two structurally similar organotin compounds TBT and TPT on a freshwater gastropod species *Lymnaea stagnalis*. We exposed juvenile snails of homogeneous size and age to the same range of concentrations of TBT and TPT during 35 days in semi-static conditions. Survival, growth, and fecundity were regularly monitored. Results on survival suggest a greater toxicity of TPT than of TBT over the time course. On the other hand, TBT exhibited a much stronger impact on sub-lethal endpoints. Furthermore, we exposed snail eggs, laid by non-exposed animals and which had previously been isolated from their gelatinous matrix, to the two organotins during 21 days. Here we monitored hatching time and success, and the size of hatchlings. TPT turned out to be much more toxic to embryos than TBT. Exposures to very low concentrations of TPT caused a significant concentration-dependent decrease of hatching success and size, and an increase of hatching time, while effects of TBT on all three endpoints were observed only at high concentrations. We concluded that differences in toxicity of TBT and TPT might partly be explained not only by differences in their alkylation but also by differences in the life stages of snails.

Keywords: triphenyltin – tributyltin – *Lymnaea stagnalis* – partial life-cycle test – toxicity
**Baby teething rings exhibit endocrine activity in vitro, but not in vivo**

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Leaching of endocrine disrupting chemicals (EDCs) from plastic materials may pose a considerable health risk for both humans and wildlife.

In order to screen different plastic materials of baby teething rings for chemicals with endocrine activity, two in vitro yeast based reporter gene assays were applied. One teething ring, whose methanol extracts exhibited estrogenic and anti-androgenic activity in the Yeast Estrogen (YES) and Yeast Anti-Androgen Screen (YAAS), respectively, was further assessed in a reproduction test with the aquatic mud snail *Potamopyrgus antipodarium*. This organism has previously been shown to increase reproduction when exposed to estrogenic chemicals. Ten snails were exposed to either water containing a piece of teething ring, water alone or water spiked with ethinylestradiol (positive control). Each treatment was conducted in quadruplicates. The experiment lasted 28 days; every four days, water was extracted via solid phase extraction and replaced. Estrogenic and anti-androgenic activities of these extracts were assessed in the YES/YAAS and chemical analysis was performed via GC-MS.

Water extracts showed endocrine activity in vitro with decreasing intensity over time. However, there was no effect on the reproduction of *P. antipodarium*. The chemicals responsible for the endocrine activity were identified as commonly used preservatives. Thus relevance of EDCs in plastic materials for human and wildlife will be discussed.

**Keywords:** bioassay – effect-directed analysis – ethylene-vinyl acetate – baby toys – polymer
Concentrations of Chlorpyrifos and Cypermethrine measured in Suquía river (Córdoba-Argentina) surpassed the international water quality guidelines for pesticides in freshwaters drawing attention on the possible ecological risk. Several indications exist that many natural and synthetic chemicals could interfere with the aromatase CYP19 system and might lead to malfunctioning of the reproductive system, which is the most frequently reported symptom of endocrine disruption in fish. For teleosts fish, two cyp19 genes (cyp19a1 and cyp19a2) have been described. CYP19A1, commonly known as gonadal aromatase, is preferentially expressed in the ovary and plays an important role in sex differentiation and oocyte growth. CYP19A2, called brain aromatase, is constitutively expressed in the brain and is probably involved in the development of the central nervous system, survival, morphology, synaptogenesis, and sex behavior. The aim of the present study was to evaluate changes in cyp19a2 expression in brain of Jenynsia multidentata exposed to environmentally relevant concentrations of pesticides. For this purpose, adult female fishes were exposed over 96 hours to 40 ng/L of Cypermethrin and 400ng/L of Chlorpyrifos singly and in technical mixture. Additionally, the same mixture (40ng/L Cypermethrin plus 400ng/L Chlorpyrifos) of a commercial product was tested. The exposure solutions were daily renewed. Total RNA was extracted from brain tissue and nonspecific reverse transcription was performed with Oligo(dT)$_{15}$ primer. Quantitative polymerase chain reaction was performed using β-actin as housekeeping gen. Our results showed that Chlorpyrifos and the commercial mixture were able to inhibit significantly cyp19a2 expression while Cypermethrin and the technical mixture did not vary from the control. As a result, it could be hypothesized that Chlorpyrifos and its commercial mixture with Cypermethrin could cause or contribute to estrogen-related disorders in J. multidentata. Moreover, the differences observed between the technical and commercial mixtures reinforce the need of further studies at other pesticide concentrations and formulations.

Keywords: aromatase – pesticides – cyp19A2 expression – quantitative RT-PCR
Do otters bioaccumulate and biomagnify pharmaceuticals in the environment?

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The global consumption of pharmaceuticals is rapidly rising, with a consequent increase in the unintentional contamination of aquatic habitats with these compounds. Some pharmaceuticals are excreted from patients relatively unchanged and as these are not always effectively removed during sewage treatment processes, the residues are discharged into aquatic environments. Current understanding of the fate, behavior and effects of pharmaceuticals once they reach the environment is poor, but it is at least theoretically possible that they could affect wildlife, particularly as they are designed with the primary intention of exerting effects in biological organisms. Eurasian otters (\textit{Lutra lutra}) consume large quantities of fish, which contain detectable amounts of pharmaceuticals, so it is theoretically possible that they bioaccumulate and biomagnify these chemicals to the point where they reach sufficient concentrations to exert pharmacological effects. Here we present results from pharmacokinetic-pharmacodynamic (PBPK) modelling as well as empirical measurements of select pharmaceuticals in otter tissues and discuss the potential threat posed by these chemicals to otters and other aquatic apex predators.

\textbf{Keywords:} drugs – aquatic – pollution – Lutra lutra – PBPK model
Bioaccumulation in an estuarine trophic web: a study case from an historical contaminated location: Largo do Laranjo, Ria de Aveiro coastal lagoon in Portugal

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Largo do Laranjo (Ria de Aveiro Coastal Lagoon, Portugal) is a location with historical sediment contamination. For more than three decades was the recipient of metal discharges with particular incidence in mercury. These elements were sequestered in the sediments in high concentration. The release of sequestered metals into the aquatic medium was reported in several studies, being mainly due to tidal hydrodynamic forces and to bioturbation. The study of historical contaminated locations is of major importance for interpreting significant alterations in secondary contamination and biomagnification across trophic webs in temporal scales. Moreover, contaminants release in this particular location is of extreme importance, since surrounding populations use biota as food, therefore human health risk is present requiring monitoring.

Mercury (Hg), arsenic (As), cadmium (Cd) and lead (Pb) were analysed in Largo do Laranjo estuarine food web. Based on the variability of the trace metal concentrations communities can be distinguish by their habitat compartments and life strategies. The highest concentrations were found in sediment and in the water column communities. The high levels in the latter are explained by the re-suspension of sediment particles into the water column and with absorption and/or adsorption to the biota. However, non-essential trace metals in pelagic fishes’ muscle were much lower. From this a break in the trophic web could be infered. Several factors could explain the bioaccumulation break. Therefore, results will be explained based on the several works that had been conducted on this ecosystem: the tidal transports, residence time of particles and contaminants bio-dilution.

Besides our intention to determine bioaccumulation factors of trace metals in an estuarine food web, we aim to contribute for the periodical monitoring of this location, by following up on the work conducted in 2009, by Pastorinho and co-workers.

Keywords: bioaccumulation – estuaries – trace-metals – trophic interactions
Neuromast damage as an informative and sensitive new endpoint for the zebrafish embryo toxicity test (zFET).

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The zebrafish toxicity embryo (zFET) test is increasingly being used as a replacement test method for conventional fish tests. To extend the scope of the existing assay, the implementation of new endpoints, which allow to specify the sub-acute toxicity of a chemical, is indispensable. The analysis of neuromast damage looks very promising in this context. The neuromasts form the lateral line of adult fish, representing the main mechanosensory organ, which is essential for rheotaxis and orientation. The sensory receptors present in the neuromasts, the hair cells, are analogs to those in the inner ear of mammals. Our interest focuses on development of the posterior lateral line (PLL) zebrafish, which is used for prey detection, predator avoidance and schooling behaviour in post-hatch stages. We therefore investigated the effects of ototoxic substances on morphology and development of the neuromasts in zebrafish embryos. Additionally, we studied the behaviour by performing behavioural assays for rheotaxis and seeker response. Wild-type embryos were exposed to neomycin, cisplatin, heavy metals and other ototoxic substances for 48 to 72 hours post fertilisation (hpf). To quantitatively evaluate the neuromast development and damage after 24/48 h (or 72 h, respectively), we used in-vivo fluorescent staining with the vital dyes DASPEI and FM4-64FX, or performed immunostainings with an antibody specifically recognizing anosmin in the hair cells and an extracellular matrix protein located in the zebrafish PLL. Further, we will test the use of the cldnb:lyngfp transgenic line to visualize the PLL, which expresses GFP in the neuromasts.. In addition to the, First results presented indicate that the neuromast cells are more sensitive to exposure than the standard morphological endpoints of the FET. The assessment of neuromast damage appears very promising in terms of screening and evaluation of ototoxic substances.

Keywords: zebrafish embryo – neuromasts – ototoxicant – lateral line – neurosensory endpoint
Assessment of the mutagenic potential of sediments from the
Three Gorges Reservoir and its feeder rivers

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Sediments play a major role as a sink for many kinds of environmental pollutants. Potentially contaminated sediments in the regions of the Yangtze Three Gorges Reservoir (TGR) in China pose a risk to aquatic organisms. Additionally, annual changes in water level cause flooding events which lead to a relocation of particulate matter onto agricultural areas along the river bank. For the investigation of the sediments a German-Chinese team took samples at multiple locations along the TGR and its feeder rivers near the towns of Chongqing, Fengdu, Yunyang and Wushan in September 2011. To examine their mutagenic potential 19 of these samples were tested in the Ames fluctuation assay. All samples were tested with the Salmonella typhimurium strains TA98 and TA100, with and without S9 mix, which simulates mammalian metabolic activation system.

15 of the samples showed significant mutagenic effects when tested with TA98 and S9 mix. The mutagenic activity in the presence of TA98 and S9 mix indicates that the responsible compounds need to be activated by mammalian metabolism and then are able to cause frameshift mutations. In former research poly-aromatic hydrocarbons (PAHs) have been identified as causing similar effects. In a recent GC-MS-analysis PAHs were detected as the only hazardous group of substances in the TGR sediment samples. Therefore PAHs are considered to be responsible for the observed mutagenic effects.

The maximal induction factors of the TGR samples were twice as high as sediments from the German rivers Neckar, Rhine and Danube. Multiple sources of pollution such as flooded urban, agricultural and industrial areas as well as the release of sewage from adjacent cities into the TGR make it difficult to identify the exact source of the mutagenic compounds. Additionally complex flow behavior, especially during flooding events, seems to play an important role in the distribution of contaminated sediments.

Keywords: Yantze River – Ames test – mutagenicity – sediment toxicity – poly-aromatic hydrocarbons
Comparing individual and population level sensitivities to triphenyltin
- A tale of three models -
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Different species differ in their life-history strategies owing to which their population dynamics, responses to toxic stress and times to recovery are also different. Therefore, it is important to consider underlying processes when extrapolating effects from the individual to the population level. We used an individual-based population modelling approach to simulate the responses to triphenyltin (TPT) at the population level for three different planktonic organisms- Mesocyclops leuckarti, Daphnia magna (Preuss et al 2009) and Chaoborus crystallinus (Strauss et al 2007). Each individual-based model (IBM) uniquely describes the life cycle of the respective species. We used the General Unified Threshold model of Survival (GUTS) to simulate the toxicokinetics and toxicodynamics of TPT. Data from laboratory acute toxicity experiments showed 48 h LC$_{50}$ values to be 34 µg/l and 86 µg/l for M. leuckarti nauplii and adult females respectively, 89 µg/l for C. crystallinus L4 larvae, and 30 µg/l and 50 µg/l for D. magna neonates and adults respectively. At high food concentrations and 20°C, we simulated a single-peak scenario and a two-peak scenario of exposure to TPT with the three models. Converse results were obtained at the population level. It was observed that the C. crystallinus was the most sensitive species at the population level followed by M. leuckarti. D. magna, which was the most sensitive species at the individual level, was least sensitive at the population level. The daphnids fully recovered before the second peak while C. crystallinus did not recover at all. In conclusion, for species with similar individual level responses to toxicants, population level responses may be vastly different due the influence of different life-history strategies. It is possible to simulate these differences using the power of population modelling and make educated predictions for better risk assessment.

Keywords: individual-based model – plankton – copepod – GUTS – triphenyltin
An aquatic microcosm study on the endocrine activity of
*Bacillus thuringensis* var. *israelensis* (Bti)

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In a routine examination of ground water samples from a region that is frequently treated with Bti an estrogenic activity was detected by the use of the YES-Assay. For this reason different Bti-products, that are available on the market, were tested in the LYES-Assay. That study revealed that the detected estrogenic activity is probably associated with a component of the formulation of some of these products. Therefore it seems necessary to examine those products more closely to detect the exact origin of the estrogenic activity, and furthermore estimate a possible impact on treated areas.

In this microcosm-study an approach was made to examine a possible effect of VectoBac® TP on an aquatic community of invertebrates including an indicator-species for estrogens (*Potamopyrgus antipodarum*). The results indicated an influence on the primary production of the systems, as composition of phytoplankton- and periphyton-communities seemed to be changed. On the level of zooplankton communities no distinct effects were observed, however a slightly higher abundance of *D. magna* was detected in treatments with a concentration of 4 mg/L Bti. An endocrine activity of VectoBac® TP could not be clearly stated, as conditions in the microcosms seemed not to be sufficient for an unaffected development of *P. antipodarum* (probably due to quantity and quality of available nutrients). Even though the data of the reproduction indicated a significantly higher number of embryos in the 20 mg/L Bti-samples in the first two weeks of the test, this observation could not be verified along the rest of the study.

According to the data of this study a further observation seems reasonable as effects on the community level were observed, even though their exact nature remains unclear. Furthermore an impact on the reproduction of *P. antipodarum* should be examined more closely as clues on a possible estrogenic activity were noticed.

**Keywords:** microcosm – Bti – estrogenic activity – *P. antipodarum* – VectoBac® TP
Coral allelochemical toxicity and cytochrome P450 activity and expression relationship in butterflyfish (*Chaetodon* spp.) of differing feeding strategies

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Cytochrome P450 monooxygenase (CYP) is the primary enzyme system for detoxification of xenobiotics including dietary chemicals and pollutants. Some herbivorous insects have the ability to detoxify toxic dietary chemicals by specific CYP forms. While thorough research has uncovered certain relationships between herbivorous insects and dietary chemicals, little is known about biotransformation and detoxification of allelochemicals derived from dietary products in marine organisms. Certain species of butterflyfish of the genus *Chaetodon* have been shown to feed on several species of chemically-defended corals including the soft coral *Sinularia*. This study examined the effects of the natural product, 5-episinuleptolide (5ESL) on the expression and catalytic activities of CYP3A, CYP2 in two butterflyfish species one of which is an obligate coral feeder and a generalist feeder that can feed on coral. Fish were gavaged 1.0 mg/kg and 3.0 mg/kg of 5ESL. Initial mortality indicated that *C. multicinctus* (obligate hard coral feeder) had 100% mortality in both doses. In contrast, 80% survival was observed in *C. auriga* (generalist feeder). Testosterone hydroxylase (TOH) (6 beta, 16 alpha, 16 beta) was 130-740 times lower in *C. multicinctus* relative to *C. auriga*. These results indicate an association between CYP2 (16-alpha TOH) and CYP3A (6 beta, 16 beta TOH) catalytic activities and detoxification of 5ESL in butterflyfish with different feeding strategies which may provide a selective advantage in allowing generalists to feed on chemically defended prey. Elucidating the molecular mechanisms governing allelochemical resistance is crucial for understanding the genetic basis of adaptation in consumers that regularly feed on chemically defended prey.

**Keywords:** allelochemical – cytochrome P45 – biotransformation – fish metabolism
The bio-accumulation of selected metals (Cu, Fe, and Mn) in water, sediment and invertebrates (*Physa acuta*) of the Kuils River, Western Cape, South Africa

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The supply of fresh water is limited in South Africa and urban rivers are the most utilised and degraded worldwide. The demands on freshwater resources are greater than ever. Due to urban development and population increases, industries are growing and agricultural activities brought about the degradation of rivers through a deterioration of water quality. In Cape Town, Kuils river is no exception. A range of everyday sources of metal pollution exist that is entrenched in the modern way of life. This has caused an increase metals accumulation in the environment and exert toxic effects in organisms.

The study aimed to investigate the accumulation of Cu, Fe and Mn in the Kuils River water and sediment, as well as bioaccumulation in the invertebrates (*Physa Acuta*). Snails (*Physa acuta*), sediments and water were collected every 2 months for a period of one year. Samples were desiccated and digested with 55% nitric acid. The ICP-AES were used for analysis of metals.

Both metal concentrations in water for Cu and Mn, were significantly higher than the Department of Water Affairs and Forestry (DWAF) guidelines. The sediment results showed that the mean concentration of Cu and Mn, when compared to the Canadian Sediment Quality Guidelines (CSQG), were below the prescribed guidelines.

In most cases mean concentrations of Cu, Fe and Mn and in snails were much higher than in the corresponding sediment samples.

**Keywords:** bioaccumulation – contaminants – Kuils River – *Physa Acuta*
Effects of aquaculture used chemicals on *Lemna minor*


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Nowadays, the aquaculture faces a challenge of maintain both profitability and environmental sustainability. However, the increase of aquaculture has been associated to an increase in the use of chemicals. These chemicals are used in finfish and shrimp hatcheries, grow-out ponds, and cages in order to manage hygienic conditions and to control bacterial disease outbreaks but they can also reach the environment causing deleterious effects on several organisms. In this study, *Lemna minor* was used to assess the toxicity of some chemicals used in aquaculture: Benzalkonium chloride (BKC, a quaternary ammonium compound), Glutaraldehyde (GA, an aliphatic dialdehyde) and Trichlorfon (TCF, an insecticide organophosphate). In different experiments, *L. minor* was exposed to each chemical during 7 days following the OECD guideline 221. At the end of the tests, several parameters were recorded (fresh weight, dry weight, root size, number of frond and leaves, chlorosis and frond death). Our results showed BKC as the more toxic chemical tested with a 7d-EC$_{50}$growth of 17.43 mg/l, and also affecting the biomass and causing chlorosis in the organisms. For GA a higher value of 7d-EC$_{50}$growth: 73.8 mg/l was found however an induction of growth rate and biomass was observed at low doses of exposure (7d-LOEC: 8 mg/l). Furthermore, GA act on the root causing a decrease of size (7d-LOEC: 32 mg/l). The organophosphate insecticide TCF was the less toxic between compounds tested with a 7d-EC$_{50}$growth: >1000 mg/l causing effect on root size (LOEC: 612 mg/l) and fresh weight (1,224 mg/l) at the higher tested concentrations. Due the low toxicity of TCF to *L. minor* no effects are expected to other aquatic plants. However, for GA and BKC future studies are recommended to assess the chronic and sub-lethal effects of these chemicals in aquatic plants.

**Keywords:** trichlorfon – glutaraldehyde – benzalkonium chloride – growth rate – duckweed
Comparative effects of two water-accommodated fractions from light and heavy crude oils on early life stage of zebrafish *Danio rerio*.

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Aquatic environment is constantly threaten by introduction of anthropogenic pollutants. Among them, polycyclic aromatic hydrocarbons (PAHs) are widespread in these areas and can represent a threat to biological functions of the fishes. PAHs are in fact complex mixtures of large number of compounds with variable properties (incl. physical-chemical and toxicity). These properties depend on their origin, pyrolytic or petrogenic. They however share a low solubility in water making direct exposure in water impossible. The use of fish early life stage (ELS) to assess toxicity of chemicals or media is considered as *in vitro* assay and therefore allows reducing live animals tests in accordance with the European legislation. The main objective of this study is to evaluate the suitability of water-accommodated fractions (WAF) and zebrafish ELS to assess oils toxicity. For this purpose, zebrafish ELS were exposed to two WAFs, one prepared with from light (Arabian Light) and heavy (Erika) crude oils to determine at several concentrations. Toxicity assessment was performed using lethal and sublethal (e.g. DNA damage, deformations, hatching rate, heart rate, swimming activity) endpoints recorded at different developmental stages. Combination of physiological, morphological and behavioral readouts indicated that WAF prepared from heavy oil is several orders of magnitude more toxic than light oil and confirmed the suitability of this approach.

**Keywords:** water-accommodated fraction – crude oils – bioassay – zebrafish – early life stage – embryo toxicity – endpoints
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Ecotoxicity of trivalent chromium to *Daphnia magna*: importance of Cr(III) solubility
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The scientific community generally agrees in considering Cr(VI) more toxic than Cr(III) for aquatic organisms. However, this long-standing paradigm seems to be in need of some revision. First, several studies published after 2000 have shown that Cr(III) can be as toxic as Cr(VI) to freshwater algae, aquatic mosses, yeasts and pollen of terrestrial plants. Second, at circumneutral pH values, Cr(III) undergoes hydrolysis that results in the formation of Cr oxyhydroxydes. The latter are poorly soluble and their formation can markedly lower the actual exposure concentration during an ecotoxicological test.

We investigated the ecotoxicity of Cr(III) on *Daphnia magna* in two exposure media of similar hardness (about 250 mg L\textsuperscript{-1} CaCO\textsubscript{3}), but of different alkalinity (4.9 vs. 0.77 meq L\textsuperscript{-1}). Cr(III) concentrations were repeatedly measured over test duration and its chemical speciation was estimated using the software package VisualMinteq-2.61.

In high alkalinity medium, Cr(III) concentrations decreased by 90% after 6h and no Cr(III) was left in solution after 24h. No acute toxic effects were observed. Modelling of Cr speciation indicated Cr(OH)\textsubscript{3}, which is insoluble, as the dominant species. Conversely in low alkalinity test medium, where 100% toxicity could be observed, Cr(III) concentrations decreased only by 22–43% and ionic Cr(III) species were predicted as predominant. Acute (immobilization at 48h) and chronic (reproduction at 21 days) EC50s were 14.1 (13.1–14.7) and 1.9 (1.81–1.99) mg L\textsuperscript{-1} respectively.

These values alone do not justify substantial revision of current environmental quality criteria for Cr(III) (4.7 µg L\textsuperscript{-1} Probable No Effect Concentration in the EU). However, in future studies, the ecotoxicity of Cr(III) should be assessed following protocols for sparingly soluble substances to account for possible, rapid decreases in actual exposure concentrations due to Cr(III) hydrolysis.

**Keywords:** trivalent chromium – chromium speciation – chromium toxicity – *Daphnia magna* – risk assessment
Hematological and immunological effects of diclofenac in neotropical fish

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Drugs for human and veterinary use, as anti-inflammatory, frequently have been found in water bodies. In this work some effects of diclofenac in a tropical fish species were evaluated after trophic exposition. Individuals of freshwater top predator fish *Hoplias malabaricus* were exposed to 12 trophic doses of diclofenac [DIC] in 3 doses [0.2, 2 and 20µg.kg\(^{-1}\)] using fish *Astyanax* sp. as prey vehicle, for 45 days. After this time half of fishes received carrageenin [Cg] 1mg.kg\(^{-1}\) 4 hours before euthanasia. The fishes were sacrificed and hematological biomarkers [erythrocytes (ER), total and differential leukocytes counts, thrombocytes counts, hematocrit and hemoglobin concentration] and immunological biomarkers [(cell migration induce by Cg and nitric oxide (NO) basal production and induced by 1ng.mL\(^{-1}\) of lipopolysaccharide (LPS) in anterior kidney cell cultures (in fishes that didn’t receive Cg)]. The results showed an increase in ER and hemoglobin in the group treated with 0.2ug.kg\(^{-1}\) and all groups increased the thrombocytes count. The total leukocytes count showed a decrease inversely proportional to the dose in all the groups. In the differential leukocyte count, the neutrophil count showed a decrease too in all groups. The monocytes count decreased only at the highest dose. The number of resident peritoneal cells did not differ among the groups, but there was a significant reduction in the cell migration by Cg in the all DIC-treated groups due to a significant decrease in the migration of polymorphonuclear cells. Basal NO synthesis by anterior kidney cell cultures from DIC-treated animals was significant lower in the cells from the group 2 and 20ug.kg\(^{-1}\). LPS-stimulated NO production was reduced in all the groups. The results suggest that a constant trophic exposition of organisms to diclofenac can lead to potential toxic effects, including hematological and immunological changes.

**Keywords**: Diclofenac – fish – *Hoplias malabaricus* – trophic exposition – culture cells
Step to re-evaluation of risks caused by synthetic auxins to non target plants

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Synthetic auxins have been regarded as low risk pesticides for non-target plants, since assessments were dominantly based on sensitivity to *Lemna minor* - the only standard aquatic macrophyte species used ecotoxicology tests. Recently, concerns have arisen that, *L.minor*, being a floating monocotyledonous plant, would not be, in all cases, protective of all plants, namely rooted dicotile macrophytes when assessing pesticides with specific modes of action. In need of refinement of risk assessment of pesticides, protocols with new rooted macrophyte species are being developed. One of the candidate species to be used in refined risk assessment of chemicals is *M.aquaticum*. An insufficient amount of literature data on toxicity of auxin simulators (a group of herbicides with different chemical structures, but similar modes of action), showed that *L.minor* is less sensitive to auxin simulators than dicotile macrophytes. Baring that in mind, when assessing auxin simulators, it is advisable to use rooted dicotiles, since they can provide more ecologically relevant data which can be protective of all aquatic macrophytes. In order to shed light on differences in sensitivities, we conducted toxicity tests on *L.minor* and *M.aquaticum* to auxin simulators. Our results will be a contribution to re-evaluation of risk categorisation of abovementioned group of pesticides.

**Keywords**: auxin simulators – herbicides – *M.aquaticum* – *L.minor* – risk assessment
Sources and impacts of polyfluoralkyl substances (PFASs) in the Italian surface waters

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A survey of the distribution of perfluorinated carboxylates and perfluorinated sulphonates in the main Italian river basins have been carried out in 2011-‘12 in order to get a reliable picture of the polyfluoralkyl substances (PFAS) contamination and their main sources.

Monitoring campaigns on river Po (northern Italy), the major Italian river which flows into the Adriatic Sea, its tributaries, river Adige, river Tevere, river Arno and river Brenta have been carried out in different hydrological conditions. Chemical plant discharges and drinking waters in the same basins were also sampled. The monitoring campaigns allowed to identify hot spots in the main Italian basins.

A fluoropolymer factory, which discharges in the Bormida river, was confirmed as the main source of PFOA in the river Po. In order to deep into the impacts, macrobenthonic communities have been monthly sampled by artificial substrates upstream and downstream of the industrial discharge and the ecological quality has been evaluated. Organisms of specific species collected in both sites, have been processed to analyze genetic variation with the aim of identifying genetic erosion or selection due to the impact.

Another significant source of PFOA and PFBS was identified in a plant for the production of intermediate fluorochemicals in the river Brenta which discharges in the Adriatic sea, close to the lagoon of Venice.

Two important textile industrial districts in Italy (counties of Vicenza and Prato) discharge PFPeA and PFHxA in the Brenta basin and in river Arno. These shorter chain perfluorocarboxylics acids can be considered as tracers for this kind of industrial pressure.

Keywords: polyfluorinated – PFOA – surface water – macrobenthonic communities – genetic erosion
Comparing the sensitivity of *Lemna* spp. to herbicides and plant growth inhibitors with that of non-standard macrophytes.

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The lack of protection for non-standard macrophyte species in the risk assessment of plant protection products has been an increasing concern in the last few years. It has been stated that the standard species *Lemna gibba* and *Lemna minor* might not be representative for the sensitivity of other aquatic macrophytes. Differences in life history, growth strategy, exposure route or sensitivity to chemicals with specific modes of action (MoA) could be some of the reasons. Moreover, the SANCO guidance document for Aquatic Ecotoxicology also recognizes that additional testing may be required on other macrophyte species depending on the mode of action of the substance. However, it is still unclear for which MoAs additional macrophyte testing is needed and which are the most sensitive plant species or growth forms for these MoAs. Our research aimed at addressing these issues in the context of the improvement of the final SANCO guidance document. Aquatic macrophyte EC50 values were selected for synthetic herbicides and plant growth inhibitors from the (US-EPA) ECOTOX database. Due to low data availability, the relative tolerance (Trel) approach was used to enable comparing macrophyte toxicity data per MoA. Our results showed that synthetic auxins may represent a high risk for the stability of the macrophyte community if only *Lemna* spp. are tested. Nevertheless, we observed that most of the toxicity data used in macrophyte risk assessment is based on EC50 values, with a final safety factor 10 being applied. Therefore, the protection of a species population with a toxicity value over the ‘safety threshold’ may not be 100% in that case. Based on that, further research on species sensitivities to synthetic auxins but also to other slightly known MoAs is needed. Finally, it is important to determine whether a higher safety factor could also solve this lack in protection.

**Keywords:** *Lemna* – Macrophytes – Pesticides – MoA – EC50
Poorly soluble cosmetic compounds - ecotoxicological assessment and substitution (ECOSM)

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‘Personal Care Products’ include a multitude of substances designed for many different purposes, resulting in a great variability in physico-chemical properties. Substantial parts of these substances are ‘poorly soluble’, characterised by a very low water solubility of far less than 1 mg/L. The high production volume and the fact that, due to the type of application, large amounts will subsequently end up in the waste water stream, causes the need for a reliable assessment of their environmental behaviour and toxicity to aquatic organisms, e.g. as requested by the European REACH-Regulation.

At the threshold of the water solubility of ‘poorly soluble substances’, ecotoxicity tests, especially standardized tests, are very difficult to conduct. Insufficient results may lead to improper risk assessment. One promising approach addressing this problem is the so called ‘poorly solubles approach’ proposed by Tolls et al. (2009). This approach is based on a comprehensive compilation of experimental data and defines an ecotoxicological ‘threshold of no concern’ for inert substances with a narcotic mode of action. Below this threshold concentration, neither acute nor long-term adverse effects on aquatic organisms are expected to occur. The aim of the project ‘ECOSM’ is to develop the approach into a pragmatic tool for assessing the environmental risks of ‘poorly soluble substances’, not assessable with standard methods.

In this context, existing aquatic ecotoxicity tests will be adapted to passive dosing techniques and several poorly soluble compounds of ‘Personal Care Products’ shall be tested to validate the ‘poorly solubles approach’. Dodecylbenzene as the first exemplary substance has already passed the first steps in evaluation. Chemical analysis has been performed by means of gas chromatography and solid phase micro extraction. Ecotoxicity tests on organisms of different trophic levels (e.g. algae, daphnia, fish) and the adaptation to passive dosing techniques will be performed in the near future.

Keywords: personal care – poorly soluble – Dodecylbenzene – passive dosing – threshold of no concern
Distribution of metals in cytosol and physiological status of mussels as an estimation of trace metal exposure

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The estimation of a trace metal exposure of the native mussel populations under influence of maritime activities using integration of biochemical and modern analytical techniques is the main subject of this study. Areas with a frequent nautic transport are affected by the trace metal pollution caused by leaching of antifouling paints which can have a permanent effect on biochemical and physiological processes in the organism. The earliest effects of the contaminants can be noticed at a cellular level and serve as biomarkers with a final goal of an effective environmental monitoring. Combining different approaches, e.g. a possibility of an organism to deal with the stress and specific trace metal responses on the cellular level can give a better final estimation of trace metal effects on the organism in the realistic environmental conditions. Therefore, two main approaches are applied on the Mediterranean mussel (Mytilus galloprovincialis) as a bioindicator organism using the digestive gland as a target tissue. General stress is estimated using a general physiological biomarker Cellular Energy Allocation (CEA) as an alternative approach to the Scope for growth (SfG). Since the metal binding to various cytosolic biomolecules as ligands represents a way of protection against the toxicity, an investigation of cytosolic metal distribution in protein fractions of different molecular masses is of a great importance for defining possible biomarkers of the trace metal exposure. This is achieved by size exclusion (SE) high performance liquid chromatography (HPLC) connected to inductively coupled plasma mass spectrometry (ICP-MS). The results will make a step towards the characterisation of cytosolic metalloproteins responsible for metal handling in mussels under the realistic environmental exposure.

Keywords: Mytilus galloprovincialis – cytosolic metals – cytosolic metalloproteins – cellular energy allocation – SE-HPLC-ICP-MS
Effect-directed analysis and mechanism-specific bioassays to assess the toxicity of sediments in Yangtze River (China)
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The Three Gorges Dam (TGD) spanning the Yangtze River in Hubei Province, China, is regarded as one of the world’s largest hydropower projects and was recently finished in 2008, with full impoundment of its reservoir (175 m a.s.l.) in 2010. The potential benefits of the project is expected to control catastrophic flooding, produce electricity energy, increase navigability of the Yangtze River, access to drinking water and reduce greenhouse gas. As such a large project is always linked to many environmental challenges, several German research institutions work in a joint research project together with their Chinese partners on a sustainable water management (Yangtze Project-Hydro). The purpose of our “Microtox - ecotoxicity module” is to assess the toxicity of water and sediment of the Three Gorges Reservoir (TGR) and to identify the responsible organic micropollutants.

Effect-directed analysis (EDA) - based on bioassay, fractionation and chemical analysis - has shown to be a powerful tool for key toxicant identification in complex environmental samples. It involves the stepwise separation of the sample, directed by bioassays, until it is possible to identify the responsible compounds by chemical analysis. In our research, an automated fractionation procedure was performed on three coupled normal phase HPLC-columns separating compounds according to their polarity, planarity and numbers of aromatic carbon. Mutagenicity (AMES assay) and dioxin-like activity induction potency (Ethoxyresorufin-O-deethylase [EROD] assay) were measured according to previous sediment quality triad studies. Subsequently they were further assessed in the different fractions with a combination of chemical analyses. The results will help to identify the key toxicants of four ‘hot spots’ in TGR, to minimize the risk of sediments to aquatic biota and human health.

Keywords: Yangtze River – Three Gorges Dam – effect-directed analysis (EDA) – fractionation – bioassay
Leaf litter breakdown is a fundamental process in streams and mainly mediated by decomposer-detritivore-systems comprising both microorganisms and leaf-shredding macroinvertebrates. Although the former group of organisms (especially fungi) plays a crucial role by conditioning the leaf material (physical and chemical alteration), the main part of the leaf mass loss in streams can be attributed to the latter group. Shredders, however, can be affected by fungicides via two pathways: first, exposure via the water phase can result in direct adverse effects and, second, fungicide-induced changes in the leaf-associated microbial community may alter food quality for these invertebrates (= indirect effects). During the present study, both effect pathways were followed using the model shredder *Gammarus fossarum* and organic (azoxystrobin, carbendazim, cyprodinil, quinoxyfen and tebuconazole) as well as inorganic fungicides (three copper-based fungicides and sulfur). Direct effects on *Gammarus* were investigated using feeding activity assays. While exposure to all organic fungicides reduced gammarids’ feeding rates, sulfur did not result in direct toxic effects. Moreover, indirect effects were studied using food choice assays. Therefore, leaf discs conditioned in the presence or absence of a fungicide were offered simultaneously to one *Gammarus* specimen. Organic fungicides resulted in a preference for control over the fungicide-exposed leaf discs and the opposite was observed for inorganic fungicides. This observation may be triggered by a broad-spectrum antimicrobial activity of inorganic fungicides, which likely affected leaf-associated bacteria. A potentially reduced bacterial abundance could have promoted fungal growth indirectly due to a lower pressure of competition, which consequently increased the nutritional value of exposed leaf discs. Organic fungicides, in contrast, may act more specifically on fungi ultimately reducing leaves’ nutritional value. Analyses of leaf associated microbial communities will help to uncover the responsible mechanisms of toxicity. Although final conclusions cannot yet be drawn, it is obvious that organic and inorganic fungicides can act differently on the investigated decomposer-detritivore system, which should be considered in the environmental risk assessment of fungicides.

**Keywords**: ecosystem function – *Gammarus* – fungicide – aquatic hyphomycetes – food quality
Pollution pathways of pharmaceutical residues in the aquatic environment of Ghana

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This work determines the principal environmental pollution pathways of pharmaceutical in the capitol city of Ghana (Accra). The evaluation was made on the basis of the quantification of pharmaceuticals residues by liquid chromatography – tandem mass spectrometry in several environmental water samples, including sewage water, drinking water and ground water. Overall 15 pharmaceuticals were identified in the environmental samples of the 32 investigated pharmaceuticals in this study. Sewage effluents are the main source of pharmaceuticals dispersing into the aquatic environment. Several of the pharmaceuticals are also detected in the drinking water from different brands of commercial water (sachets). The data indicate that re-use of sewage water irrigation could results in contamination of groundwater and other water resources. In addition, leaching from landfills is identified as another possible source of introduction of pharmaceuticals to ground water aquifers. Finally wastewater from Accra is not treated in wastewater treatment facilities and ends directly in the Gulf of Guinea and causes pharmaceutical residues to occur in marine water bodies in an area where the population is strongly depended on the nature resources.

Keywords: pharmaceuticals – environment – sewage water – drinking water – Ghana
Biogeochemical assessment of mercury pollution in bottom sediments from the Piabanha river basin, Rio de Janeiro State, Brazil

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The Piabanha River Basin (Rio de Janeiro State, Brazil) has been strongly impacted by domestic and industrial wastes, which usually contain high concentrations of metals (including mercury). This work proposes the assessment of mercury pollution in the silt-clay fraction (< 0.075mm) of bottom sediments (15 samples) collected along the Piabanha River basin. Total mercury concentration was determined by atomic absorption. Mercury contamination levels were evaluated by comparison with the concentrations proposed by Brazilian legislation (CONAMA - 0.170 mg/kg for level one, and 0.486 for level two), as well as by calculating Geoaccumulation Indexes (IGEO). Such index allows classifying the contamination levels in comparison with the considered background (the standard shale - 0.04 mg/kg). Organic matter concentrations were quantified using the LECO Equipment. The results demonstrated that the samples were extremely sandy. The average mercury concentration was 0.281 ± 0.113 mg/kg. Although the silt-clay fraction only corresponds about to 1% of the granulometric distribution, it is able to fix about 86% of the total mercury content. In addition, organic matter also played an important role in the fixation of mercury (p<0.05). More than 90% of the samples were higher than the concentration which suggests the occurrence of adverse effects on biota (level one). The IGEOs revealed that 90% of the samples could be classified as "moderate to strongly polluted".

Hg contents in samples collected into the Petropolis urban area (RJ) were higher than the limit of high probability of ecotoxic effects (level two). In fact, a critical zone of pollution could be identified at the neighborhood of the Petrópolis Municipality. On the other hand, mercury contamination levels were substantially reduced in areas located downstream of such municipality. In future works, other metals will be quantified, in order to detail the mobility and distribution of toxic metals along the Piabanha River Basin.

Keywords: contamination – mercury – sediments – geoaccumulation index – Piabanha River
Spatial monitoring of persistent organic pollutants in surface sediments of a rural-urban drainage basin in Tanzania
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The presence of persistent organic pollutants (POPs) in Tanzanian aquatic environment is not well monitored, despite the existing pollution potential from a number of sources. In this study, we investigated for the first time, the concentration profiles of different POPs such as organochlorine pesticide (OCP) residues, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in surface sediments from the Pangani river basin (PRB). The PRB is one of the largest drainage basins in Tanzania, with its watershed exposed to multiple input sources of trace organic contaminants. Sediment samples were collected from 12 pre-selected representative stations of diverse characteristics and land-use practices in three distinct seasons; extracted by the Soxhlet method and analysed by GC/MS operated in the electron capture negative ionisation mode. The results revealed low levels of sediment contamination by eight OCP compounds, among which, the HCH isomers (120–1950 pg/g dry weight) and DDT analogues (120–9990 pg/g dry weight) were found to be the most frequently detected OCPs in the sediments profiles. Twenty eight PCB and six PBDE congeners were also detected in the samples, with their total concentrations ranging from 357 to 11000 and 38 to 2175 pg/g dry weights, respectively. The spatial distribution patterns and Hierarchical Cluster Analysis showed that historical agricultural usage in sugarcane plantations was the major source of OCP contamination, while urbanisation, transportation activities and electricity generation probably contributed to the PCBs and PBDEs contaminations. Risk assessment using sediment quality guidelines indicated no ecotoxicological risks. The results we have found provide preliminary data on levels of the organic contaminants in Pangani river basin as a new insight on the environmental quality of the area.

Keywords: organochlorine pesticides – polychlorinated biphenyls – polybrominated diphenyl ethers – sediment quality – Tanzania
Sorption of PAHs to humic acid- and iron(III)carbonate particles by using passive
dosing vials for investigating the transport of organic contamination
in stormwater runoff

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During the last decades, the growing urbanisation and increasing anthropogenic activities in urban areas have turned urban stormwater runoff into a surface water quality contamination problem. The concerns of urban stormwater runoff as a source of contamination in the receiving surface water (lakes, rivers or sea) have been raised by researchers throughout the world (e.g. Broman et. al., 1987, and Xanthopoulos et. al., 1990), and have in Europe gained increased interest in relation to the implementation of the Water Framework Directive (WFD, 2000/60/EC).

Particles (often defined as >0.45 μm) has been found to facilitate transport of organic contaminants and metals in stormwater runoff systems, but little is known about the role of the colloidal fraction including nano-sized particles (0.001-1 μm). Based on the large specific surface area of colloids and nanosized particles, their abundance, and knowledge about their facilitated transport of persistent organic pollution in natural waters, they are likely to diminish the efficiency of engineered treatment systems unless appropriately accounted for.

In this work organic and inorganic nanosized particles were investigated for their ability to sorb polycyclic aromatic hydrocarbons (PAH’s) in an aqueous solution. These particles were used as indicators for stormwater particles which are diverse in size and composition. For controlling the sorption onto the particles, passive doing vials were used (Birch et. al., 2010).

Using passive dosing vials gives the possible to control freely dissolved analyte by equilibrium partitioning from a preloaded silicone membrane. It has been found that the presence of humic acid particles (80 nm) leads to an increasing amount of two PAHs (fluoranthene and phenanthrene) in the suspension. For iron(III)carbonate particles (22 nm) sorption experiments are ongoing. Based on these results and a literature review, the importance of including particulate fractions for surface water quality assessment in relation to the WFD will be discussed.

Keywords: nanoparticles – passive dosing – PAH – stormwater runoff – sorption
Compound lipophilicity as a descriptor to predict metabolic affinity \((1/K_m)\) in mammals

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In bioaccumulation models, biotransformation is one of the processes decreasing the concentration of chemicals in an organism. The enzymatic action of metabolism involves two processes. Firstly, the chemical needs to reach the enzyme and bind with it; secondly, a catalytic reaction must take place. The binding of the chemical and its successive catalysis are described by two enzymatic parameters: the Michaelis constant \((K_m)\) and the maximum rate of the reaction \((V_{\text{max}})\), respectively. Measured \(K_m\) and \(V_{\text{max}}\) data are lacking for many chemicals and species.

The aim of this study was to estimate the relationships between binding affinity, represented by \(1/K_m\) \((1/\mu\text{M})\), and lipophilicity, expressed by the octanol-water partitioning coefficient \((K_{ow})\), for \textit{in vitro} oxidations catalysed by alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), flavin-containing monooxygenase (FMO), and cytochrome P450 (CYP). The focus was on finding generic patterns of metabolism across enzymes. The knowledge of the underlying biochemical mechanisms is useful to further develop models for whole-body biotransformation.

For the regressions developed, \(1/K_m\) always increased with the \(K_{ow}\), as expected from the partitioning theory. The binding to FMO was not well correlated to the compound Log \(K_{ow}\), possibly because of its different reaction mechanism involving a nucleophilic attack. If strong interactions such as covalent or ion bonds are important, distribution of chemicals is expected to be weakly related to their \(K_{ow}\). Good correlations were found for the substrates of ADH, ALDH, and CYP, after excluding specific classes of outliers. These correlations suggested that the enzyme affinity of the chemicals is driven by weak, in particular hydrophobic, interactions with these enzymes. The slopes of these regressions did not statistically deviate from the typical slope (ranging from 0.46 and 0.70) that correlates protein-water distribution (Log \(K_{pw}\)) and Log \(K_{ow}\).

**Keywords:** biotransformation – enzyme – underlying mechanism – binding affinity – lipophilicity
Analysis of bottom sediments in Lake Babrukas (Trakai district, Lithuania) by x-ray fluorescence spectrometry

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Study area. Lake Babrukas, which had undergone pollution by municipal wastewater in 1964-2002 and partial mechanical restoration in 2011 was selected as an object for investigation of the effects of hydromechanical dredging on distribution of selected chemical elements in lake bottom sediments. Lake Babrukas is located in Trakai district, to the southwest of Trakai town (5357 inhabitants) and to the northeast of the Babriškės settlement (132 inhabitants).

X-ray fluorescence. Analytical method of X-ray fluorescence spectrometry and specifically Niton XL2 device for analysis of bottom sediments of lake Babrukas is analyzed. The reliability of the method of x-ray fluorescence spectrometry for analysis of heavy metals is compared with atomic absorption spectrometry. Results of this method show a very impressive correlation of atomic absorption method. Results of the analysis are overviewed and compared with concentrations of heavy metals in relevant legal documents in Lithuania. It is concluded that the X-ray method gives important and reliable information of anthropogenic impacts for lake ecosystems and human health. The assessment of heavy metals in bottom sediments with X-ray fluorescence method is much quicker and cheaper, thus it gives the opportunity to analyze more samples and more properly assess distribution of pollutants in the lake sediments.

Acquired results were used for representing horizontal distribution of metals in bottom sediments by employing Kriging interpolation method (in “Surfer 9” software), which predicts unknown values from data observed at known locations. It is concluded that Kriging interpolation is very effective for patterning spatial distribution of pollutants in the layers of sludge and facilitates understanding of geochemical processes. Results clearly illustrate anomalies in distribution of hazardous metals in the surface layer of bottom sediments.

Keywords: X-ray fluorescence spectrometry – bottom sediments – heavy metals – concentrations
Life cycle assessment (LCA) as tools for development of environmental management system

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Deterioration of the natural environment necessitated ecological activities not only on the part of consumers, but on the part of economic entities as well. It manifests itself in the strong emphasizing of environmentally friendly action and aspiration to the effective environmental management. That is why, environmental management system according to ISO 14001 requirements and EMAS decree find wide circle of supporters. One of bases of the PDCA Concept, also known as the Deminga Cycle, is improving the performance of this systems. Tool, which enables identification and quantification of environmental impacts as well setting environmental objectives is called an Life Cycle Assessment-LCA

ISO 14000 is a family of standards, which are divided to two groups, namely standards focused on an organization and standards focused on product. LCA methodology is described in the second group of standards, however it doesn't mean that it is impossible to conduct LCA-study in scope of organization activity. For the realization of such purpose, it is necessary to carry ecobalance of the enterprise. It relies on the gathering and quantitatively marking all flows of raw materials, energy, emission and streams of products and by-product, which refer to the chosen year.

The critical element in LCA study is dividing the product system in unit processes, for which will be collected appropriate data. In environmental management systems such idea also exists, because it is recommended to gather inputs and outputs of different areas of organization's activity. For facilitating of date aggregation it is necessary to distinguish the fields of activity as: material-consuming, energy-consuming, water-consuming, emissivity, waste etc. In this way, it is possible to identify hotspots, that is areas, which generate significant environmental effects and thereby require to undertake the remedial actions.

Keywords: life cycle assessment – enviromental management system – enviromental aspects – development – organization
Salinity Difference energy, also known as “Blue Energy”, is an overlooked and neglected form of renewable clean energy. With the current global aim of achieving a sustainable society, such sources of energy must be well studied and further exploited. Several techniques have been proposed over the past decades to convert into electricity the free energy available from the mixing of river and sea water, for example. More recently, a new proof-of-principle was introduced using an assembly of activated carbon nanoparticles for adsorption/desorption of salt. This new principle is called Capacitive Mixing (CAPMIX).

The cyclic CAPMIX process consists of alternating sea and river water between the nanoporous electrodes, generating ion adsorption in the sea water step and ion desorption in the river water step. A regular behaviour of this technology is found below, where constant electricity is extracted.

Due to the use of ion-exchange membranes, special attention needs to be focused on the prevention of fouling in the system, biological and chemical. A thorough research on the design and suitable materials for this technology is required. The final application requires not only an efficient physical chemistry process, but also a careful risk assessment of the biological impact in Delta regions around the globe.

**Keywords**: nanomaterials – renewable energy – environmental technology – water sciences
Study of the toxicity of dendrimers in fish embryos of Danio rerio

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Dendrimers are symmetrically branched nano molecules with a layered, spherical architecture. Due to the possibilities of designing and producing tailor-made dendrimers for very specific applications, their chemical structures and occurrence are very diverse. In recent years, dendrimers have shown to be very useful in several biomedical applications, e.g. for drug delivery and cosmetic products. However, research on these nano molecules has just begun, as numerous papers proposing new applications are documented. The benefit of dendrimers in applications for human health and care does not only rely on their specificity and efficient functionality but also on a low toxicity. Currently, toxicity assessment is primarily based on cell culture testing, whereas testing data on in-vivo is still widely lacking. There is, however, some evidence that several kinds of dendrimers show toxic effects in animals. For PAMAM G4.0, severe toxic effects on the zebrafish (Danio rerio) could be demonstrated, and our own investigations confirmed these results. Concerning the rapidly increasing applications of dendrimers in implicating also the environment, there is certainly the need for further investigations. We have therefore started to examine the effects of several dendrimers on the development of zebrafish embryos. The test approach includes the assessment of morphological as well as behavioural effects. Additionally, methods to analyse the uptake and distribution of the particles within the embryos in terms of quantification and visualisation are being explored. Here, first data on the toxicity and the uptake mechanisms in the zebrafish embryo will be presented.

Keywords: PAMAM dendrimers – zebrafish embryo – toxicity – nanoparticles
Toxicity of silver nanoparticles to microalgae: The role of ionic silver

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Among nanoparticles, silver nanoparticles (AgNPs) are the most widely used in the nanotechnology industry, mainly due to its remarkable antimicrobial capability. Their extensive production allied to their antimicrobial properties has been raising concern about their toxicity to natural ecosystems. The main issue with AgNPs toxicity is centered in their oxidation to the ionic form (Ag$^+\)). In this work we intended to answer the following questions:
- is silver more toxic to algae in the ionic form or as nanoparticles?
- can the toxicity of AgNPs to algae be attributed to Ag$^+$ release?
- can L-cysteine reduce Ag$^+$ and AgNPs toxicity to algae?

To assess the comparative toxicity of both AgNPs (uncoated, 91.25±34.93 nm) and Ag$^+$ as AgNO$_3$ we used two microalgae species: Chlorella vulgaris and Pseudokirchneriella subcapitata. Several toxicity endpoints were tested (72h exposure): growth rate, Chl $a$ autofluorescence and cell size. Ag$^+$ release from AgNPs was measured by dialysis; particle size of AgNPs was measured by DLS.

Ag$^+$ was more toxic than AgNPs to C. vulgaris and P. subcapitata (on a mass basis). Exposure of C. vulgaris to high concentrations of Ag$^+$ (18µg/L) and AgNPs (600µg/L) caused cells agglomeration, i.e., increased cell complexity. Increasing concentrations of Ag$^+$ or AgNPs caused a peak reduction in Chl $a$ autofluorescence of both algae. The presence of Ag$^+$ and AgNPs caused a decreased in cell size of both species. The decrease in cell size and the increase in cell complexity could lead to self-shading of light and enhance sedimentation rates, which can be translated in deleterious effects on aquatic systems.

Oxidation rate of AgNPs was low (0.17%); thus, toxicity of AgNPs to algae cannot be exclusively attributed to Ag$^+$ release.

Addition of L-cysteine at equimolar concentrations eliminated Ag$^+$ and AgNPs toxic effects on growth rate of both algae.

**Keywords:** AgNPs – Chlorella vulgaris – Pseudokirchneriella subcapitata – toxicity – flow cytometry
Use of an intestinal co-culture model to study the effects of Ag nanoparticles

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This study aims at establishing a more physiological intestinal co-culture model with the use of Caco-2 and HT29 cells in order to evaluate the effects of Ag particles (20 and 200 nm) on the metabolic activity (alamar blue assay), oxidative stress (DCFH-DA assay) and pro-inflammatory cytokine release (IL 8). In addition, a proteomic approach (2D-DiGE/ MALDI-ToF/Tof) was used in order to help to identify possible modes of action and explain differences in effects. The uptake and localisation of the particles and ions was assessed by Secondary Ion Mass Spectrometry (NanoSIMS 50). The contribution of Ag ions on toxicity was also evaluated (ultrafiltration and ICP-MS).

AgNO₃ induced a reduction in metabolic activity in a dose dependent manner whereas no reduction was observed in the case of Ag 20 and 200 nm. The presence of mucus showed a protective effect against oxidative stress upon exposure to H₂O₂. Ag 20 nm led to an increase in IL 8 release (5-fold). Ag was found to be distributed homogenously in the cell with aggregates observed in specific locations in the case of Ag 20 nm.

The proteomic data revealed that AgNO₃ and Ag particles induced up-regulation of oxidative stress pathways and a modulation of cytoskeleton machinery (e.g. PRDX 4 and 6, PDI, ACTB, VIL-1 or Gelsolin). Ag 200 nm and 20 nm were found to behave in different manner compared to in solution ions. Interestingly, a size dependent effect was observed: the 20 nm particles seemed to be more effective than Ag 200 nm, which were found to be close to the negative control. According to both in vitro results as well as the outcome of the proteomic study the observed differences in effects cannot be attributed solely to ions. The co-culture model seems to be more relevant in toxicological studies.

Keywords: Ag nanoparticles – Caco-2 – co-culture – NanoSIMS – proteomics
**Photostimulated synthesis and plasmonic sensitivity of silver decahedral nanoparticles**

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Practical application of synthesized nanomaterials plays an important role in the present state of the development of nanochemistry. Sensitivity is considered among the promising areas of their implementation. This study presents the sensing properties of synthesized silver nanodecahedra, namely their sensitivity to changes in the refractive index of the medium in which they are dispersed. Research has been conducted to improve methods of the experiment and the subsequent use of the sensor to more complex organic objects. Photostimulated synthesis of Ag decahedral nanoparticles under the illumination with light at wavelength 470 nm is described. Poly-acrylic acid and poly (vynil-pyrrolidon) were used as stabilizers. We have determined plasmonic sensitivity of the decahedral nanoparticles to the refractive index of the surrounding media (water-glycerol mixtures). Higher sensitivity of the particles stabilized by poly (vynil-pyrrolidon) was measured, due to the weaker bonding of PVP-molecules to the surface of silver. Plasmonic sensitivity of produced nanoparticles is determined to be within the range of 150-540 nm per refractive index units. This is the highest value for the structures with plasmon resonance at around 500 nm. The phenomenon determines the possibility and potential use of these nanoparticles as sensors to the pollutants of the environment based on the changes of the dielectric constant or the refractive index of the medium.

**Keywords:** nanoparticles – silver – sensors – refractometry – plasmonics
The influence of silver nanoparticles surface capping and size in the toxicity of different microorganisms


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In recent years, the market for nanotechnology-based products has been rising, mainly the household products segment, containing silver nanoparticles (AgNPs), which are mainly designed to release ionic silver (Ag⁺). This is the source of antibacterial activity and is highly toxic to aquatic organisms. Once in aquatic systems, the colloidal stability and, thus, toxicity of AgNPs can be affected by several parameters: surface capping agent, functional groups, background electrolyte composition, pH and ionic strength.

This work aimed to evaluate the effect of nanoparticles size and surface capping agent on the toxicity of AgNPs and compare the toxicity of AgNPs and Ag⁺ to aquatic organisms. To achieve these goals, we studied the toxicity of citrate (c)-AgNPs (60 and 100 nm), uncapped (u)-AgNPs (≤100 nm) and AgNO₃ to Vibrio fischeri, Chlorella vulgaris and Pseudokirchneriella subcapitata. Bacteria were exposed to the described toxicants following Microtox® test procedures (Bioluminescence inhibition) and algae were exposed following OECD Guideline 201 (Growth inhibition). Particle size distributions and zeta potential of AgNPs were measured using DLS (Dynamic Light Scattering), by comparing two solutions of u-AgNPs (100 mg/L), in microtox (u-AgNPs-M) and ultra-pure water (uAgNPs-UP).

The toxicity results show: V. fischeri was more sensitive to c-AgNPs than u-AgNPs; both algae species had the opposite sensitivity to AgNPs; all the organisms were more sensitive to AgNO₃. The DLS data show: u-AgNPs-M had significant higher values of z-average and conductivity and lower values of zeta potential when compared with uAgNPs-UP, leading to aggregation and instability of AgNPs.

In summary, the citrate surface capping does exert influence in the toxicity of AgNPs to the organisms in this study. The results are indicative of the toxicity being mediated by Ag⁺ release, and not so much by the effect of the particle size itself, although further studies are needed to prove it.

Keywords: AgNPs – AgNO₃ – Vibrio fischeri – Chlorella vulgaris – Pseudokirchneriella subcapitata
The influence of natural organic matter on the combined toxicity of nanosized titanium dioxide and heavy metals

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The production and utilization of titanium dioxide nanoparticles (nTiO₂) increased substantially over the past years. This provokes the risk of their unintended release into aquatic ecosystems, where they may interact with environmental (e.g. natural organic matter [NOM]) as well as anthropogenic substances (e.g. heavy metals). Their interaction and resulting ecotoxicological implications were investigated during a series of 72-h acute toxicity tests with the standard test organism Daphnia magna. Therefore, the test species was exposed to one of the nTiO₂ products A-100 and P25 at a concentration of 1 and 2 mg/L, respectively, combined with copper, which was investigated at seven concentrations. Moreover, the implications of two types of NOM, namely Suwannee river NOM and seaweed extract, were assessed at three concentration, i.e. 0, 2 and 8 mg/L total organic carbon (TOC). In presence of A-100 the copper toxicity was significantly reduced with a relative effect size of 50%; while for P25 no significant implication in toxicity was observed. However, in the presence of NOM (2 mg TOC/L Suwannee river NOM or 8 mg TOC/L seaweed extract) copper toxicity was significantly reduced by P25. Similarly, in presence of A-100 seaweed extract reduced the copper toxicity regardless of its concentration. These observations may be explained on the one hand by the adsorption of copper ions onto particles’ surfaces, limiting their bioavailability and on the other hand by the coating of nTiO₂ with NOM, stabilizing the particles in the aqueous phase. This in turn prevents the biological surface coating of test specimen, which was suggested as a potential mode of toxicity of nTiO₂. Furthermore, as a result of the stable small particle size, the active ingestion of particles and hence adsorbed copper by the filter feeder D. magna might diminish. Given the presented fundamental implications of NOM on the combined toxicity of nanoparticles and metals, environmental factors in general should be considered during risk assessment of nanoparticles.

Keywords: nanoparticles – combined toxicity – natural organic matter – environmental relevance – crustaceans
Investigating fundamental characteristics of metal toxicity in zebrafish (Danio rerio) embryos
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Metals as environmental pollutants are a serious global problem. They are released during extraction (mining), processing and manufacturing (industry) of metals, and through use (e.g. in agriculture, medicine), and disposal of metal-containing products. Once metals reach the environment, they pose a continuous hazard, as they are neither chemically nor biologically degradable. In particular aquatic organisms are affected by metal exposure since the main sources of pollution are industrial or waste water treatment discharges and land surface runoffs. Fish are exposed to metals present in bio-available form, either in ionic or elemental, at nano- to micro scale size. Especially for some heavy metals toxicity already occurs at very low concentrations. Metal exposure can lead to severe damages and impairs fish development through toxic mechanisms, which are still not clarified in detail. We therefore examine the uptake and the effects of environmentally relevant metals in various forms and modifications in zebrafish (Danio rerio) during embryonic development. For adult fish, it is known that the uptake of metals mainly occurs via the gills and the intestines, but for embryos still very little is known about the uptake, distribution and subsequent effects. In experiments with silver nanoparticles (AgNP) as an example, we could demonstrate that the AgNP are taken up by the embryos but that the toxicity is primarily mediated by the silver ions. In a next step, we are now studying the toxic impact of other metals, in ionic and elemental form, with a special emphasis on the absorption, distribution and fate of the metals in the fish embryos. An overview of the development and implementation of the tests and the analytical methods will be presented as well as first results for cadmium and new data on silver.

Keywords: metal toxicity – zebrafish embryo – nanoparticles – silver – cadmium
Effects of titanium dioxide nanoparticles on aquatic invertebrates: Analyses of mixture effects and a potential accumulation via the food chain

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Nanoscaled titanium dioxide (nTiO$_2$) is one of the most frequently used nanomaterials. Because of its photocatalytic properties, nTiO$_2$ is applied in numerous products including personal care products and wall paints. Increasing production volumes and a manifold field of applications illustrate the potential of nTiO$_2$ as an environmental contaminant. Released into the aquatic environment, nTiO$_2$ may occur in the free water column, the sediment, or adsorb onto organic particles. Pelagic invertebrates, such as the filter feeding water flea *Daphnia magna*, represent primary prey organisms in the aquatic food chain. The assimilation of particles may lead to biomagnification in aquatic food webs. Additionally, nTiO$_2$ is suspected to enhance toxicity and bioavailability of other contaminants adsorbed on the surface of the particles. Investigation of a potential biomagnification was performed by a chronic food transfer of nTiO$_2$ via the green algae *Scenedesmus acutus* var. acutus to *D. magna*. In order to analyse potential mixture toxicity of nTiO$_2$ and other contaminants acute immobilization tests were conducted using cadmium and organic contaminants. To identify interactions between nTiO$_2$ and the model organisms scanning electron microscopy pictures were taken. Additionally, residual analyses of nTiO$_2$ in medium and algae were performed using ICP-MS. The particle size distribution of nTiO$_2$ in the test media was characterised by dynamic light scattering. The results of these experiments will be presented and discussed.

**Keywords:** titanium dioxide – biomagnification – mixture toxicity – *Daphnia magna* – *Scenedesmus acutus*
Effect of Ca and pH on the toxicity of copper to the springtail *Folsomia candida* in simplified soil solutions

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This study aimed at investigating the relationship between copper toxicity and bioavailability to the soil dwelling arthropod *Folsomia candida*. Considering pore water as the main route of exposure for collembolans and to avoid the complexity of soil, simplified soil solutions were used. Different water chemistry factors affect the speciation of copper in solution and consequently influence its toxicity to the test organism. Different calcium concentrations and pH levels were therefore examined to develop a Biotic Ligand Model (BLM) for predicting toxicity of copper to *F. candida*. Test animals were exposed to copper concentrations of 0, 0.04, 0.16, 2.5, 10, 40, and 160 µM Cu for seven days, at Ca concentrations of 0.2, 0.8, 3.2, and 12.8 mM Ca and pH levels 5.0, 6.0, and 7.0. Internal copper concentrations were measured in surviving animals after finishing the exposures. Results showed a hormesis-type effect on survival rate. Toxicity decreased with increasing calcium concentrations in the solution but, due to the hormesis effect, no clear competition of calcium with copper could be observed. Toxicity of copper was higher at pH 6.0 than at pH 5.0 and 7.0. Langmuir isotherms were used to estimate copper, calcium and proton binding constants and to estimate the maximum copper level bound to the biotic ligand \( C_{Cu-BL-max} \) on the collembolans. The fraction of biotic ligands occupied by copper which causes 50% reduction in survival was calculated by plotting survival against the fraction of occupied biotic ligand estimated from measured body concentrations and \( C_{Cu-BL-max} \). Survival predicted from a BLM that used the binding constants gave a good prediction of measured data, indicating the potential of applying the BLM approach for *F. candida* using simplified soil solutions. More research is, however, needed to determine the link between copper bioavailability and its effects in real soils.

**Keywords**: copper – bioavailability – toxicity – *Folsomia candida* – BLM
Exposure of *Folsomia candida* to carbaryl and natural stressors: effects on reproduction and genotoxicity

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Terrestrial organisms can be exposed to a great variety of stressors, such as contaminants and/or other physical or biological stressors that affect their life - and genetic traits. Organisms can experience a large range of environmental fluctuations such as temperature changes, drought and flood conditions or even UV radiation increments. The aim of this work is study the effects of carbaryl to a soil-dwelling collembolan *Folsomia candida* at different abiotic conditions, evaluating their reproductive effort and genotoxicity. Single and combined exposures were carried out with carbaryl and three different natural stressors: temperature, moisture and UV radiation. The combined effects were compared to carbaryl exposures under standardized conditions: 20ºC, 60% water holding capacity. We observed that carbaryl induces changes on the survival and offspring production. DNA damage was also detected using the comet assay. Results from the combined exposure showed that fluctuations on environmental conditions such as temperature, soil moisture or radiation can induce changes on chemical toxicity.

**Keywords**: carbaryl – climatic changes – comet assay – genotoxicity – UV radiation


Ecotoxicological assessment of sewage sludge-amended tropical soils using bioassays with aquatic and edaphic organisms

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Sewage sludge (SL) is generated by Effluents Treatment Stations, contains high concentrations of organic matter and nutrients and has been widely used as a fertilizer in the agriculture. However, SL usually contains high concentrations of metals, which can cause damages on soil biota. This work proposes the ecotoxicological assessment of two tropical soils (ferralsol and chernosol) treated with SL. Test doses consisted of 6.58, 13.16, 19.74 and 32.90%. Acute toxicity tests with earthworms (Eisenia andrei) and aquatic micro-crustaceous (Daphnia similis), and reproduction tests with algae (Pseudokirchneriella subcapitata) were performed. Elutriates (1:8 - soil:water) were prepared to be tested with aquatic organisms, to simulate a scenario where soils could be leached by rainfall, thus contaminating surrounding fluvial systems. The dose of SL able to cause mortality (LC50) and immobility (EC50) on 50% of the earthworms and crustaceous, respectively, was calculated using Probit analysis. The dose recommended by agricultural application (6.58%) was not able to provoke significant effects on D. similis and E. andrei. More significant acute effects were detected for the ferralsol treatments (LC50 = 13.1%, EC50 = 26.51%) compared to the chernosol mixtures (EC50 = 31.11%). No test dose applied to chernosols was able to cause significant mortality levels on E. andrei. While all test doses were able to inhibit the reproduction of algae in the ferralsol mixtures, the lowest test dose (6.58%) did not provoke significant effects in the chernosol treatments. In conclusion, soil properties played a crucial role in the ecotoxicity of the test mixtures. The abundance of expansive clay minerals (with high capacity to adsorb metals) in the chernosol treatments might have reduced the ecotoxicity levels. It is expected that such results can subsidize the definition of "safe" ecological doses of SL to be applied to tropical soils, supporting decision-makers in actions of environmental control.

Keywords: metals – ferralsol – chernosol – sewage sludge – bioassays
Mercury ecotoxicity assessment in three tropical soil classes using acute bioassays with earthworms

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Mercury is a highly toxic metal and well-known by its capacity of causing damages on biota. This work proposes the assessment of mercury ecotoxicity and bioavailability in yellow ferralsols (YF), red ferralsols (RF) and chernosols (CH) artificially contaminated by divalent mercury, using acute bioassays with *Eisenia andrei*. Mercury lethal concentration on 50% of the organisms (LC50) was estimated using Probit analysis. Total mercury determination was performed by atomic absorption. Bioconcentration factors (BCF) were calculated through the ratio between the total mercury content in the surviving organisms and the total mercury concentration in the soil. Results revealed higher mortality levels according to the following sequence: YF (LC50 = 7mg/kg) > CH (LC50 = 15mg/kg) > RF (LC 50 = 20mg/kg). Such observation suggests that soil properties played a crucial role in the ecotoxicity and bioavailability of mercury. High levels of organic matter in the RF may have stimulated the formation stable complexes with mercury, decreasing its content in the soil solution and, consequently, its ecotoxicity. In the CH, the occurrence of expansive clay minerals suggests the existence of more effective mercury adsorption processes, decreasing its bioavailability in the soil solution. Furthermore, the CHs present high contents of nutrients, which may consist of more availability of food for the earthworms. On the other hand, YFs have a very low fertility and are predominantly composed by kaolinite (1:1 clay mineral), thus becoming mercury more available in the soil solution. Mercury BCFs were between 0.7 and 3.0 units, indicating that the organisms bioaccumulated it. The BCFs were inversely proportional to the increase of mercury content in the soil, suggesting the existence of saturation mechanisms by the metabolism. It is expected that those results can generate important data for the establishment of ecotoxicological reference values for tropical soil fauna, supporting decision-making in environmental control programs.

Keywords: *Eisenia andrei* – yellow ferralsol – red ferralsol – chernosol – mercury

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**Posters**

Session: Terrestrial ecotoxicology

**TE04**
Disposal of dredged sediments in tropical soils: acute ecotoxicity and bioconcentration of metals in earthworms

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The upper limit concentrations of metals established by the actual Brazilian legislation for dredged sediment disposal and soil quality do not take into consideration the properties of tropical soils and its role in the ecotoxicity. Aiming to evaluate the adequacy of these threshold values in tropical regions, the ecotoxicity of a dredged sediment (DS) from the Guanabara Bay (Rio de Janeiro, Brazil) was studied in two tropical soils. Acute and avoidance tests with *Eisenia andrei* were performed to evaluate the risk associated with its terrestrial disposal. Mixtures of the DS with a ferralsol and a chernosol (0.00, 6.58, 13.16, 19.74 and 32.90%) were used. Mercury, lead, nickel, chromium, copper and zinc concentrations were determined in soil and in tissues of surviving earthworms by atomic absorption. Earthworm median lethal concentration (LC50) was determined for the DS using Probit analysis. Bioconcentration factors (BCF) were calculated through the ratio between metal concentration in earthworm tissue and soil metal concentration. Avoidance test showed the highest toxicity to the ferralsol mixtures. The acute tests also showed higher mortality and biomass loss in ferralsol mixtures (LC50 = 10.7%) than in chernosol treatments (LC50 = 15.7%). These results suggest that the presence of expansive clay minerals (with high ability to adsorb metallic cations) might have reduced metal availability in chernosol mixtures and, consequently, the ecotoxicity of these treatments. The BCF found for zinc and copper were lower as higher the dose of DS, indicating the existence of internal regulating processes. BCFs values for the other metals were generally higher in the highest DS doses. The results obtained give a relevant contribution to the future definition of sustainable indicators to evaluate the risk associated with the disposal of DS in tropical terrestrial environments, and to support decision-makers in programs of environmental management.

**Keywords:** *Eisenia andrei* – ferralsol – chernosol – dredged sediments – metals
Ecotoxicological tests with *Folsomia candida* to evaluate toxicity of dredged sediments in tropical soils

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Avoidance and reproduction tests with *Folsomia candida* were performed to assess the risks associated with the disposal of a dredged sediment (DS - containing toxic metals and organic contaminants) from the Guanabara Bay Basin (RJ, Brazil) in two tropical soils (ferralsols and chernosols). The test doses of DS consisted of 1.25, 2.5, 5, 10 and 20%. The avoidance behavior and mortality observed in the reproduction tests showed dose-response relationships for mixtures of both soil types. In the ferralsol treatments, more than 70% of the organisms avoided the test treatments up to the second lowest test concentration (2.5%), while in the chernosol mixtures, only the highest concentration (20%) provoked significant avoidance responses. Mortality observed in the reproduction tests was significantly higher in the ferralsols mixtures than that in treatments of chernosols. However, the median lethal concentration (LC50; determined using the Priprobit analysis software) could be calculated only for ferrasol mixtures (LC50 = 8.73%). In the ferrasol treatments, the addition of DS increased the reproduction rates in the two lowest test doses (1.25 and 2.5%). Such fact may be explained by the high organic matter and nutrient contents of the DS tested that, allied to the low levels of these constituents in the ferrasols, makes its lowest test mixtures advantageous for the reproduction of the organisms. In the chernosol treatments, the inhibition of the reproduction rates showed dose-response relationships (reproduction lower as higher the test dose). In conclusion, the results obtained generally suggest that soil properties (most probably expansive clay minerals) have a very important role in the ecotoxicity of the test mixtures. It was demonstrated that ecotoxicological tests with *F. candida* may constitute relevant tools to evaluate the risk associated with the disposal of DS in terrestrial environments and, therefore, to support decision-makers in programs of environmental management.

**Keywords:** *Folsomia candida* – ferrasol – chernosol – soil – dredged sediments
Posters
Session: Terrestrial ecotoxicology
TE07

Influence of earthworms on Cd bioavailability to *Folsomia candida*

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To estimate bioavailability of metals in laboratory experiments we usually use one test species. However, in nature bioavailability can be influenced by other species living at polluted sites. Earthworms can affect soil chemical and physical properties, therefore are able to modify partitioning of metals in soil. Although numerous studies investigated the impact of earthworms activity on bioavailability of metals, only few have been done in a multi-species context and no one has used toxicokinetics approach. In this study we assessed the influence of an epigeic earthworm *Lumbricus rubellus* on bioavailability of Cd to the springtail *Folsomia candida*. In the experiment we used natural soils collected from three sites along a gradient of metal pollution. Earthworms were introduced to half of the soil replicates for four weeks. Then we determined the uptake and elimination kinetics of Cd in *F. candida* exposed for 21 days to the experimental soils. Tissue Cd concentrations were measured at days 0, 1, 2, 4, 7, 15, 21. To calculate the uptake and elimination rate constants we used the one-compartment model. We observed slightly higher values of Cd uptake rate constants in springtails exposed to the soils in which earthworms were previously kept. The differences in kinetics parameters between treatments were not significant. This experiment showed that earthworms only slightly influenced bioavailability of Cd to *F. candida*.

**Keywords:** bioavailability – cadmium – earthworms – *Folsomia candida* – toxicokinetics
Toxicokinetics and Toxicodynamics of Nickel in *Enchytraeus crypticus*

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Time-dependent accumulation and toxicity of nickel in the potworm *Enchytraeus crypticus* were investigated upon exposure in an aqueous solution embedded within an inert solid matrix (quartz sand). Internal Ni concentrations and effects on survival were determined at different time intervals over a 21-day exposure period and interpreted from the perspective of toxicokinetics and toxicodynamics. A one-compartment model was used to describe the uptake and elimination kinetics of Ni in the animals. Ni uptake in the organisms increased with the increasing exposure concentrations and exposure time. Internal Ni concentrations reached equilibrium at around 14 days, suggesting that *E. crypticus* was able to eliminate Ni. Overall Ni uptake rate constant was 3.43 L/kg/day. Median lethal concentrations (LC50) decreased with time. From the LC50-time relationship, an ultimate LC50 of 0.243 mg/L was calculated. When relating LC50 values to Ni body concentrations in the animals, they turned out to be more or less constant at 16.7 μg/g body dry weight, independent of exposure time. So, internal concentration was a better indicator for Ni toxicity than external concentration as it already took into account toxicokinetics. When relating toxicodynamics (survival in time) to toxicokinetics (accumulation in time), different elimination rate constants were found: 0.291 day\(^{-1}\) when looking at development of internal concentrations with time, and 0.028 day\(^{-1}\) when relating LC50 with time. This finding indicates that the internal concentration does not completely reflect toxicodynamics. In conclusion, toxic effect concentration of Ni decreased with exposure time until internal concentration reached equilibrium with external concentration. The present study highlights the importance of taking time into account in future toxicity testing and risk assessment practices.

**Keywords:** LC50-time relationship – bioaccumulation – elimination – lethal body concentration – uptake kinetics
Residual film method and modified residual film methods used to evaluate toxicity of three pesticides on *Tribolium castaneum*

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*Tribolium castaneum*, the red flour beetle, is one of the major pests of stored products. It is a very prolific, long-lived species quite resistant to eradication which cultures can be maintained indefinitely on a simple medium of flour and yeast. These traits make them easy to culture and manipulate in the laboratory. Therefore, it has been used largely as animal model in population ecology and toxicology. Infestation of food products with this species can be controlled with usage of insecticides. The most common method used to investigate the effects of pesticides to *Tribolium castaneum* is residual film method (RFM).

This laboratory study was undertaken to investigate effects of exposure to three widely used pesticides (dimethoate, pirimiphos-methyl and deltamethrin) using *Tribolium castaneum* adults as model organisms. The toxicity of these pesticides was investigated using RFM and modified RFM. Namely, in the original RFM organisms are in direct contact with the pesticide and have difficult to move across the glass bottom of the petri dishes. In order to make exposure conditions more suitable for these insects, in modified RFM adults were exposed to pesticides in combinations with flour.

On the basis of calculated LC$_{10}$, LC$_{50}$ and LC$_{90}$ values pirimiphos-methyl showed the highest toxicity and according to the toxicities the investigated pesticides could be arranged in the following order: pirimiphos-methyl $>$ deltamethrin $>$ dimethoate. Additionally, the results of this study showed different toxicities of pesticides depending on the exposure methods. Namely, after exposure of insects using modified RFM the mortality was significantly reduced. Survival curves estimated according to the Kaplan–Meier product-limit method showed higher survival probability for modified RFM compared to original RFM. The obtained results could be possibly explained with usage of flour which served as base on which *Tribolium castaneum* could move more easily as well as a barrier between organism and pesticide.

**Keywords:** dimethoate – pirimiphos-methyl – deltamethrin – red flour beetle – survival
Biomarker responses of earthworms exposed to organophosphate insecticide dimethoate

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Pesticides are poisons of synthetic or biological origin that are used to control and suppress the effects of organisms that are considered to be harmful. The usage of pesticides has a very broad implementation and plays a significant role in agricultural production, forestry and public health. However, despite the benefits, there are significant damages associated with the use of pesticides. Namely, many pesticides are toxic to nontarget organisms, including beneficial soil organisms, such as earthworms. Because of their natural habitats and continuous exposure to chemicals present in the soil, earthworms are a suitable indicator species for ecotoxicological assessment of pesticide soil pollution.

The aim of this study was to investigate effects of widely used organophosphate insecticide dimethoate on different earthworm species. For assessing the toxicological effects of pollutants on earthworms two most commonly used toxicity tests were applied – preliminary filter paper contact test and artificial soil test which is more representative of the natural earthworm environment. Suborganismic effects of dimethoate were examined by analyzing following biomarkers – acetylcholinesterase (AChE), carboxylesterase (CES), catalase (CAT) and efflux pump activities.

The results of this study showed that dimethoate caused significant changes in measured biomarkers and that different earthworm species display different sensitivities to dimethoate exposure. The response of measured molecular biomarkers using different toxicity tests had certain similarities; however some distinct differences were also evident. The obtained results indicate that the application of environmentally relevant doses of dimethoate, i.e. doses that are applied in agriculture, could have harmful effects on earthworms.

Keywords: soil pollution – dimethoate – earthworm – toxicity tests – biomarkers
Costs of resistance to metal pollution: multi generation experiment on small and large populations of the flour beetle *Tribolium castaneum* exposed to elevated copper level

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Habitat fragmentation caused by anthropogenic activities is a serious threat to species survival. It can cause an inbreeding depression and can lead to the extinction vortex. Parallel to fragmentation, many habitats are also affected by pollution and the two factors interact with each other. Environmental pollution can decrease population size, advancing inbreeding depression. Population size itself is an important factor that affects adaptation rate. It is assumed that smaller populations adapt faster, but they probably achieve lower “peaks” on the fitness “landscape ridge” than in case of slowly adapting large populations.

In this study we explored how population size and multi generation exposure to copper influence adaptation and resistance costs. For this purpose laboratory outbred (ca. 1000 individuals) and inbred (20 individuals) populations of *Tribolium castaneum*, selected for 20 generations for copper resistance (medium contaminated with copper at 1000 mg/kg), were compared to populations maintained in parallel in uncontaminated medium.

As a measure of resistance to copper we used fertility rate in highly contaminated medium (3000 mg Cu/kg), controlling for the fertility in medium from which a population originated. The fertility rate was significantly affected by both the population size and the multigeneration exposure to Cu: inbreeding and copper exposure lowered fertility in highly contaminated medium. Analysis of correlation between fertility in medium of origin and in highly contaminated medium showed outbred populations to have more equalized fertility in harsh conditions and no significant correlation between original fertility and fertility at 3000 mg/kg, with no effect of previous exposure to copper. The situation was different in small (inbred) populations, in which a significant correlation was found between the fertility in original medium and at 3000 mg Cu/kg, with no effect of previous exposure to copper. The situation was different in small (inbred) populations, in which a significant correlation was found between the fertility in original medium and at 3000 mg Cu/kg. Moreover, the copper pre-exposed populations differed significantly from those originating from uncontaminated medium in the slope of the relationship: the slope for populations from uncontaminated medium was close to 1:1, while Cu pre-exposed populations revealed a more shallow slope of 0.35 indicating stronger effect of high copper concentration in pre-exposed animals.

**Keywords**: metals – adaptations – multigeneration exposure – habitat fragmentation – inbreeding
Level of contamination of food by heavy metals is of global concern. In that way issues on toxicity are of interest for society and are of main priority. But contaminants migration, for example, from soil into plants is too little studied.

Some metals are vital for living organisms. For example, copper is the most important element for plant growth. Average content of Cu in soil is in range 2-100 ppm. Dose-response models are of special interest for toxicological studies, especially the part of the curve which is followed after optimum for growth.

Present study is focused on mechanistic modelling of plant growth taking into consideration contamination level of substrate. The basis of the model is above mentioned dose-response relationship, especially in the case when the plant growth is inhibited, being under chemical stress. This attitude is motivated by the necessity to obtain limit of contaminants in substrates (arable soils) which absorbed by plants. This means the limit of copper in soil which is safe for consuming these plants by human. As there was a lack of data in overview of papers for adequate composing of dose-response model, the decision to do experiments to obtain the data necessary for further kinetic modelling, for example mentioned in [1], has been taken.

Toxicological study has been done on Triticum L for range of copper in substrates (0.5; 5; 25; 75; 95; 150; 200; 250 mg/l).

As a result, the kinetic data for whole plant and it functional parts (leaves, stem, roots) has been collected and modelling has been done. Proposed technique is suitable for any contaminants as well for other plants.

**Keywords**: multicompartement model – dose-response – growth – plant
Toxicokinetic / dynamic model to explain an influence of cadmium ions on soil dehydrogenase activity

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Toxicokinetic and toxicodynamic model has been used to explain an influence of cadmium ions to soil dehydrogenase activity. The Assumption for the model were: certain concentration of cadmium ions appears in soil in single time point, cadmium ions cause irreversible deactivation of the enzyme, new active enzyme units are not produced by microorganisms during chosen short exposition and incubation time intervals. Biosorption, bioavailability, bioconcentration of cadmium ions and kinetics of enzyme deactivation has been taken into consideration. The solution of differential equations describes the changes in the activity directly expressed with the use of a dimensionless number in the range from zero to one. Model parameters were determined from research data. An experiment has been carried out for different cadmium concentration and different exposure times. Dehydrogenase activity has been measured in each sample using method with artificial proton acceptor - iodonitrotetrazolium chloride (INT). Results showed that enzyme activity decrease rapidly at the beginning of exposure to cadmium ions. Further changes were not significant. This kind of pattern can be predicted by presented model. Maximum inhibition of soil dehydrogenase activity caused by certain cadmium ion concentration can be calculated as a limit of function as time goes to infinity. Model shows how important is time of exposure in chemicals toxicity assessment and gives opportunity to plan experiment where time dependent variables like enzyme activity are taken into consideration.

Keywords: cadmium – dehydrogenase – model – soil – toxicity
Spatially explicit prioritization of human antibiotics and antineoplastics in Europe


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Pharmaceuticals have been detected around the world in a wide range of environmental media, such as urban and hospital wastewater effluents, surface waters, ground waters and drinking waters. Although reported concentrations are generally low, their specific modes of action make pharmaceuticals a group of substances of potential concern, even when present at low concentrations. European and national policy makers are struggling with the so-called ‘who-what-where?’ question: what substances can cause significant risks for what exposure groups at what locations? Methods for the prioritization of human pharmaceuticals have been developed previously. However, these focus exclusively on the aquatic environment and do not incorporate spatial variation. As a consequence, only the ‘what’-part in the ‘who-what-where?’ question is addressed. We present a screening tool for the location-specific prioritization of human pharmaceutical emissions in Europe, based on risk quotients for the aquatic environment and human health. The tool provides direction towards either monitoring activities or additional research. Its application is illustrated for a set of 11 human antibiotics and 7 antineoplastics. Aquatic risk quotients were highest for levofloxacin, doxycycline and ciprofloxacin, located in Northern Italy (Milan region; particularly levofloxacin) and other densely populated areas in Europe (e.g. London, Krakow and the Ruhr area). Risk quotients for human health not only depend on pharmaceutical and location, but also on behavioural characteristics, such as consumption patterns. Infants in eastern Spain that consume locally produced food and conventionally treated drinking water were predicted to run the highest risks. A limited comparison with measured concentrations in surface water showed that predicted and measured concentrations are approximately within one order of magnitude.

Keywords: pharmaceuticals – prioritization – spatial variation – human health – aquatic environment
Can flood events affect rainbow trout? The biomarker-cascade after exposure to PAHs in sediment suspensions

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In context of the ongoing scientific discussion about the potential ecotoxicological impacts of flood events, it is important to understand the detailed mechanisms of contaminant uptake from suspended particles and related effects on aquatic biota. As part of the interdisciplinary project Floodsearch II, rainbow trout (Oncorhynchus mykiss) were exposed to suspensions of natural sediment from the River Rhine (Ehrenbreitstein Harbour). Prior to suspension, the sediment was spiked with the polycyclic aromatic hydrocarbons (PAH) pyrene, phenanthrene, chrysene, and benzo[a]pyrene at environmentally relevant concentrations (4.1, 5.0, 3.3 or 8.3 mg kg⁻¹ dry weight, respectively). A control treatment without addition of PAHs was also included in the experimental design. The experiment was conducted first at an average temperature of 24 °C and repeated at 12 °C. The nominal concentration of suspended solids was 10 g L⁻¹ in both experiments. After 0, 1, 2, 4, 6, 8 and 12 days of exposure, physicochemical parameters, concentrations of PAHs in suspended matter, as well as biomarkers of exposure in rainbow trout (biliary PAH metabolites, hepatic 7-ethoxyresorufin O-deethylase (EROD) activity and lipid peroxidation) were measured. Instrumental chemical analyses revealed that concentrations of pyrene and phenanthrene in suspended solids decreased over time, while no significant degradation was observed for chrysene and benzo[a]pyrene. Concentrations of biotransformation products of PAHs in bile of fish increased slightly in the treatment without addition of PAHs at 24 °C, while average levels increased to 166 µg ml⁻¹ for 1-hydroxypyrene (control value 4.6 µg ml⁻¹) and 17 µg ml⁻¹ for 1-hydroxyphenanthrene (control value 0.1 µg ml⁻¹) in the spiked treatment within two days, followed by a decrease. In the 12 °C experiment, uptake of PAHs was slower. With a latency of two days, the peak of metabolism in the 24 °C experiment was followed by a peak of lipid peroxidation that indicates oxidative stress caused by PAH metabolization. EROD was not significantly induced by the treatments. Significant differences were observed between the bioavailability of freshly spiked and field-aged PAH contamination. The results of this study indicate the importance to account for the temporal variability of biomarker responses in sediment suspension experiments to comprehensively assess the biological effects caused by particle-bound pollutants.

Keywords: sediment – resuspension – flood event – biomarker
Identification of suitable reference genes for real-time RT-PCR normalization in *Bidens laevis* during pesticide and cold stress.

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Environmental monitoring demands the use of new species and sensitive methods in order to get accurate regional biomonitoring. The aquatic macrophyte *Bidens laevis* L. (fam.: Asteraceae) has desirable growing characteristics for laboratory assays, has proven to respond positively to genotoxic compounds and showed enzymatic response when exposed to the pesticide endosulfan. Nowadays, gene expression offers crucial information when analysing responses to xenobiotic compounds. It is well known that Real Time PCR (RT-qPCR) is the preferred method for studying gene expression because of its sensitivity, precision and robustness but it requires the use of reference genes (RG) with stable expression among different treatments, as well as different tissues and growth stages.

The aim of this study was to analyse the expression of six candidate genes, Elongation Factor 1-alfa (EF1a), Beta-Actin (BACT), Histone 3 (H3), Nicotinamide Adenine Dinucleotide Dehydrogenase (NADHD) and glyceraldehyde-3-phosphate-dehydrogenase (GADPH), in *B. laevis* in three tissues (root, stem and leaves) under four conditions: 1-control-: Large plants (>1500mg), temperature 22ºC, media: Hoagland solution; 2 -short-: Short plants (<250mg), temperature 22ºC, media: Hoagland solution; 3 -xenobiotic-: Large plants, temperature 22ºC, media: Hoagland solution + endosulfan 10ug/L; 4 -cold- Large plants, temperature 5ºC, media: Hoagland solution. All plants were exposed during 24hs under each condition, with 3 replicates tested for each one. The analysis of the stability of the proposed RG was performed with 4 different software methods (deltaCT, GeNorm, Bestkeeper, Normfinder) through the online software RefFinder. Primer specificity and amplification efficiency were verified for each gene.

The overall analysis showed that NADHD and H3 are the most stable genes among different treatments and tissues. However, some exceptions arose when analysing specific tissues (BACT and NADHD were the most stable in leaves) and treatments (H3, NADHD and EF-1a showed the most stable expression among different tissues under one treatment).

Keywords: Reference genes – *Bidens laevis* – RT-qPCR – xenobiotics – cold stress
Pre-validation of an experimental method for testing the transformation of $^{14}$C-labelled veterinary pharmaceuticals and biocides in liquid manure

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Manures of housed animals can be an important source for veterinary pharmaceutics and biocides found in the environment. Particularly because pig and cattle manure are applied as an organic fertilizer in agriculture. If the substances are not degraded or mineralised during the storage period before the field application on soils they enter the soil environments via this entry route. Thus, there is the need of an appropriate standardized and method on anaerobic transformation of veterinary pharmaceuticals and biocides in manure to describe the environmental fate of these compounds and their transformation products. For method pre-validation an international ringtest, organised by the German Federal Environment Agency, Fraunhofer IME and ECT Oekotoxikologie GmbH, has been performed recently. Radiolabelled salicylic acid was applied as test compound to pig and cattle manure and was incubated under anaerobic conditions over a period of 35 days. Manure samples were removed periodically, extracted and analysed for parent compound and potential transformation products. A $^{14}$C mass balance including the formation of NER, was established for each sampling point and mineralisation was determined by quantification of formed $^{14}$CO$_2$ and $^{14}$CH$_4$. The results of all participating laboratories were collected and will be evaluated statistically. The test method is going to be validated in an international ringtest in 2013. The long-term objective is a guideline in the framework of the OECD test guideline program. The poster will present the experimental test set-up and first results.

Keywords: manure – veterinary pharmaceuticals – biocides – transformation – ringtest
**Posters**

**Session:** Ecological risk assessment

**ERA02**

**Effects of insecticide resistance on environment in vineyards**

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European Grapevine Moth (EGM) is the most common pest, which reduces yields and quality in the vineyards in all European and Mediterranean countries. Insecticides are used in season to control the moth in vineyards up to 9 times from mid-season to end of the season and affect non-target organism and pose a risk in the environment. Insecticide resistance development is the most important danger of EGM due to higher application rates and use frequency of insecticides. The aim of this study is to estimate the risk factors of the pesticides in the environment, beneficials, bees etc. by using Environmental Impact Quotient (EIQ). The EIQ is a mathematical model that calculates a risk factor of pesticide in terms of environment and health. Due to the occurrence of resistance, how often pesticide applications are required and the effect of this situation on non target organisms (consumer, farm worker, non-human biota) will be estimated using EIQ.

**Keywords:** resistance – insecticide – environment – EIQ – modelling
Posters
Session: Ecological risk assessment
ERA03

Risk assessment of formaldehyde emission from engines of vehicles moving on road network
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Nowadays, the problem of air pollution is relevant in terms of the quality of the contact medium. Toxicological studies of substances, which originate from the internal combustion engines, are complicated due to some reasons. The most of existing studies are limited to obtaining of contaminants profile from network of roads source of emission by modeling of pollutant transport. There are too little papers with attempts to compare the level of pollution from roads and the incidence of various diseases, especially in Russian and Ukrainian language scientific literature.

The modeling of spatial dispersion of pollutants and the comparative analysis of the spatial distribution of disease give us possibility to use passive experiment to assess the relationship between concentrations of formaldehyde and dose-response effects. From the standpoint of health risk assessment, modeling of pollutants dispersion is powerful auxiliary tools.

The content of pollutants in the exhaust gases depends on several conditions: mode of traffic, nearest relief, condition of vehicle etc.. The main sources of ground level layer air pollution are cars, whose share in total traffic is 62 %. The share of freight transport, buses averages 7 % and 31 % respectively. These parameters are included in the model.

Spatial variability of ground level concentration of contaminants have been obtained using GIS GRASS software. The script is based on common-known technique for estimation of air pollution - ISC EPA US (Gaussian plume dispersion model). The result of simulation shows that ground level concentration of formaldehyde and risk are higher near streets with intense vehicles traffic. Risk assessment has been done also using GIS.

A correlation analysis of the incidence for the indigenous population and both of the ground level concentration and risk values have been done. Dose-response models for both cancer pathology and cardiovascular incidents, and ground level concentration of formaldehyde have been obtained.

Keywords: GIS – risk assessment – traffic – formaldehyde
Remediation of oil polluted soils by natural bacterial inoculums

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Increasing importance of oil in modern society unavoidably leads to increased numbers of accidental or intentional oil spills followed by many environmental companies introducing into market their rather expensive bioaugmentation inoculums. In this project it has been hypothesized and with the help of oil fingerprinting reflected that naturally occurring bacteria is more efficient in bioaugmentation as commercially available one and that enrichment of natural degraders is more important than commercial degrader addition.

Natural and commercially available degrader inoculums were enriched and compared in order to use them as bioaugmentation inoculums. Incubated on different carbon sources, numbers of alkylated and non-alkylated compound degraders in each inoculum was assessed by the most probable number method. Metabolic capacity was tested by changes of total petroleum hydrocarbons and alkane and aromatic biodegradation ratios in liquid cultures. Oil degradation capacity in clay soil samples was followed when incubated with different bacterial inoculums. Preliminary results show that methylated compound degraders are more present in oil adapted but non-methylated degraders in traffic impacted natural cultures, approving that alkylated PACs in the environment are present less in combustion products but more in oils. Commercial inoculum is very abundant in both categories but its efficiency is expected to decrease upon application to soil. Natural inoculums are capable to high degree of degrading alkenes and also aromatic fraction of oil that is known to be more persistent and carcinogenic. But commercial inoculums used in this experiment are very efficient to degrade only alkanes, leaving aromatic fraction only a bit or not at all altered. Therefore with no doubt reflecting that pre-exposed natural degraders are more efficient and important than commercial inoculums addition. Currently data from the last soil setup experiment is analyzed. It is expected that efficiency of commercially available bacteria inoculums decreases upon soil application due to lack of adaption to natural environmental obstacles.

Keywords: oil – bioremediation – bioaugmentation – microbiology – soil
Is it possible to use rhizosphere zoospores as a bio-mobilizer for polycyclic aromatic hydrocarbon (PAH)-degrading bacteria?

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On the basis of bioaccessibility limitation of functional bacteria toward their carbon sources like PAHs, some biological systems aiming to reduce environmental risks and to enhance bacterial transport or motility have been evaluated. We are introducing a novel bio-mobilizer approach using zoospores produced by a rhizosphere oomycete, Pythium aphanidermatum to assist the bacterial distributions of both motile Pseudomonas putida PPG7 and non-motile Mycobacterium gilvum VM552. In preliminary investigations, both bacteria did not influence the induction and production of zoospores by the oomycete, where the only highest cell density (×10^8 CFU mL\(^{-1}\)) of M gilvum VM552 showed a negative effect to reduce zoospores number. Cellular morphology and motility of the zoospores tested using a microscope connected to CellTrak 1.5 motility analysis software did not reveal negative influences by both bacteria. The transport and co-motility analyses by a modified capillary assay of both bacteria together with the zoospores evidenced that the zoospores promoted the dispersion of both bacteria. This was supported by the significant cell numbers entering to the zoospores-attractant filled capillaries compared to those other controls with and without the zoospores. This novel bio-mobilizer might be developed further as to improve co-inoculum technology for current bioremediation technologies.

Keywords: zoospores – bio-mobilizer – bacterial transport – bioremediation
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