Toxicity of coastal waters: use of a quick algal bioassay

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TU 081

Optimization of the SPE step in the analysis of β-blockers and β-adenonomintes in natural water samples by SPE-GC technique

MF Caban1, A Michalk, M Ciezkowska, M Kwiatkowski, P Stepnowski, J Kumiska

Environmental sampling methods, especially sewage and marine-water samples are complex and often contain interfering elements that can mask or interfere with the analysed pharmacueticals. In this study various direct analysis methods are presented and discussed. The results of the SPE analysis will be discussed.

TU 082

Mustard fractionation based on normal phase slope and reverse phase HPLC (RP-HPLC) for isolation of endemic disrupting chemicals in environmental extracts

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Lithium-21 Fractionated Direct Analysis (EDA) approach aims to identify adverse pollutants by reducing the complexity of environmental matrices. Single hyperfractionation combined to biosay may fulfill to isolated active chemicals and to direct chemical analyses to these "classical" pollutants. However, although the promising emergence of chemist tools (e.g. Orbitrap), identification of unknown active chemicals is still time and cost consuming due to the complexity of each active fraction (e.g. mixture effect). Hence, further fractionation steps are often needed. The aim of this study was to develop and to test the use of a first pre-fractionation step on SPE that will be followed by a RP-HPLC fractionation. First the separation of 12 EDCs have been evaluated with several elution conditions. Silica cartridges with 4-step elution - heptane-heptane/dichloromethane (50/50, v/v), ethyl-acetate and methanol/water (50/50, v/v) and methanol have been chosen for further investigations. For these conditions, recoveries were assessed for the mixture alone and for a blank sediment extract spiked with this mixture. Finally, a natural sediment known to exert eestoxic, PXR and/or CAR activity in the in vivo conditions. Good mixture recoveries (74-110 %), were obtained. The fractionation F1 contained only the PCBs and the PAHs, while 4-tert-octylphenol, triphenyl phosphate and fenofibrate were detected only in F2. Finally, steroids, benzo(a)fluoranthene were detected in F3 and F4 contained more polar chemicals.

Fractionation on natural sediment allows isolation of TCDD-like activity in F1 and F2 while PAH like activity was detected in F1, F2 and F3. Then enzymogenic compounds were only detected in F2 and F3. Interestingly, the sum of the enzymogenic activity found in the 3 fractions is higher than the activity determined in the crude extract, which suggests an occurrence of anti-estrogentic chemicals. Finally, PXR-like activity was mainly detected in F3. This pre-fractionation protocol allows, in the present case study, the isolation of several biological activities. Based on this first isolation directed hyperfractionation has then been undergone, RP-HPLC is used for the isolation of the target compound. So far, this approach has been chosen for further investigations. The concentrated extracts are tested in an algal bioassay with the potential to reveal the existence of unknown active compounds in the sediment.

TU 083

Towards a common mass spectra database for the identification of unknowns in environmental samples


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The identification of unknown compounds in environmental samples isolated during non-target screening or effect-directed analysis (EDA) is often a challenge on the way to the successful outcome. The identification of the compounds is a crucial step in the food chain. It remains however difficult to quantify the toxic effects of these chemicals: the relative contribution of anthropogenic and natural chemicals on the total chemical pressure is unknown. Also insight in the potential synergistic action of toxicants and toxicants is lacking, while in the field many confounding factors (e.g. changing nutrient and light regimes) may influence their effects.

The first step to unravel the complex interaction between algae and toxic substances is to provide knowledge on chemical compounds causing phytotoxic effects. In this study we use passive samplers which extract the freely dissolved concentration in the water during a period of 6 weeks to test the toxic potential of the extracted compounds. The extracts are tested in an algal bioassay with different marine algal species (e.g. Dunaliella tertiolecta, Phaeodactylum tricornutum) to include differences in algal sensitivity. Use of Pulse Amplified Modulation (PAM) fluorometry provides a quick (4 s) method to determine toxicity of algae based on changes in photosynthetic ef-ficiency. In an Effect Directed Analysis (EDA) protocol will be performed to unravel which chemical compounds are responsible for the toxic effect on the algae. In 2010-2011 passive samplers are exposed at Hanswert (Westerchelde, The Netherlands) and collected every 6 weeks to include the seasonal dynamics of both anthropogenic as well as natural compounds. Here, first results of this sampling campaign are presented and discussed. The results of the EDA analysis will be used in experiments where mixture toxicity, multi stress and community effects are taken into account to describe the overall toxic effect under relevant field conditions.

TU 084

Construction of a water toxicity sensor based on luminescent bacteria

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Maximisation of bioluminescence is a key component for the successful design of a microorganism that can be used as a water monitor. An important aspect of this is to construct a reporter signal that can be quantified and to create a sensor that is robust under real-world conditions.

A promising route towards a robust sensor is the use of microorganisms that can produce luminescent signals in response to specific compounds. The objective of this work was to test the sensitivity of bioluminescent bacteria towards water congeners and to develop a water toxicity sensor. A novelty in the development of bioluminescent water toxicity sensors is that the growth of the luminescent bacteria is not triggered by toxicants, but by specific groups of toxicants (e.g. different categories of pharmaceuticals and pollutants).

TU 085

Toxicity of coastal waters: use of a quick algal bioassay

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Primary production by microalgae embodies the carrying capacity of marine ecosystems and is therefore tightly linked to nutrient limitation. However, this relation has changed in recent years with increasing industrial chemical inputs may have a direct impact on coastal plankton communities and hence on the carrying capacity of estuaries and marine ecosystems. At the same time the frequency and intensity of algal blooms in coastal ecosystems are increasing leading to observed changes in environmental conditions. Good mixture recoveries (74-110 %), were obtained. The fractionation F1 contained only the PCBs and the PAHs, while 4-tert-octylphenol, triphenyl phosphate and fenofibrate were detected only in F2. Finally, steroids, benzo(a)fluoranthene were detected in F3 and F4 contained more polar chemicals.

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Improvements in the analytical technology and tools such as accurate and multidimensional mass spectrometry (e.g. Fourier-transform infrared (FTIR), Orbitrap) in combination with liquid chroma- tography and soft ionisation techniques such as electron spray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) allow the analysis of a broad range of compounds including polar one (or polar substances) and the restriction of the elemental composition in many cases to one or few functional groups. However, all these databases containing accurate mass spectra generally contain relatively few spectra and are not yet widely used, as many compounds relevant in environmental samples are still absent from these databases. One obstacle is the comparability of mass spectra generated with different settings, ionisation and spectrometric techniques due to increased instrument specificity, compared with the relatively reproducible El-MS spectra.

Our aim is to improve the identification of unknowns in environmental samples using a common open access mass spectra database including MS data from all instrument types and with so- phisticated data evaluation tools. The web-based database MassBank [1] was developed within a metabolomics consortium [2] and is a possible tool to achieve this target. The database is free and allows the storage of a wide variety of spectra including El-MS, ESI-QToF-MSMS and ESI-FTI- CR-MS. Different tools are available to process the raw data and upload the data to MassBank including a spreadsheet based record editor for the addition of metadata.

References:


TU 086

Dissolved and intracellular microcystins in lake waters during a Planktothrix rubescens algal bloom: HPLC quantification and crustacean acute toxicity test

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Microcystins, highly toxic cyclic peptides, are a group of hepatotoxins produced by a number of aquatic species of cyanobacteria, such as Microcystis, Anabaena and Planktothrix. Worldwide, concentrations in water have been associated with human and animal intoxication. As predictable, endocellular toxin was 90-95% of the total microcystin content; the endocellular toxin was detected and quantified using high performance liquid chromatography (HPLC-DAD). The only microcystin detected was [d-Asp3] microcystin-LR. Maximum level as dissolved microcystin was 0.7 µg/L on April 2009 sample. In the same sample, the highest endocellular concentration (30.8 µg/L) of [d-Asp3] microcystin-LR was measured. As predictable, endocellular toxin was 99.9% of the total microcystin content; the endocellular

TU 087

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