Effects of 5 pharmaceutical substances on bryophyte *Fontinalis antipyretica* Hedw biomarkers and growth

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Potential risks associated with the release of pharmaceuticals via sewage treatment plant (STP) effluents into the environment have become an increasingly important issue for environmental regulators. This concern has been driven by widespread detection of pharmaceuticals in environmental samples as a result of improved analytical performances and the realisation of focused fields surveys. Varying levels of pharmaceuticals (less than a ng to several µg/l) have been detected in many countries in STP effluents, surface waters, seawaters, groundwaters and some drinking waters.
In order to help prioritise future research efforts within the EU, the DIADeM project suggests developing and spreading out a cross-border multidisciplinary approach to improve the diagnosis and the chemical and biological (biomarkers) monitoring of freshwaters using the river Meuse, in Belgium and France, as a case study.
Within the DIADeM project, a monitoring procedure based on the use of ecotoxicity biomarkers will be developed. The exposure of representative species of flora and fauna (a crustacean, a mollusc, a moss and a fish species) of the river Meuse will be measured by chemical analysis and the effect on the selected biomarkers will be studied. As a partner of this project, ULiège has focused on the aquatic moss *Fontinalis antipyretica*.
Five substances were chosen: diclofenac, carbamazepine, naproxen, paracetamol and irbesartan. The mosses have been exposed to a mixture of these five compounds at three different concentrations. The lowest one corresponds to the median concentrations measured for these compounds in Wallonia in a previous project (between 25 and 100 ng/l depending on the molecule). Moss specimens from an uncontaminated site were exposed to these 3 concentrations for exposure times of 4 weeks in laboratory and 5 months in the experimental rivers. The concentrations of each substance in water were monitored along with some physico-chemical parameters. The tissue concentrations of these substances in the moss specimens were then measured. The growth of the moss was evaluated and compared. The effect on selected biomarkers was studied. The results related to the monitoring of substance concentrations in water and in the tissue of moss specimens will be presented and the effect on biomarkers and morphological traits will be also discussed.

Biomarkers early-warning response of caddisfly larvae to copper and uranium

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Copper and uranium are of environmental concern because of mining. Here, we used copper and uranium as toxicants for sublethal exposure. We determined the responses of *Calamoceras marsupus* larvae to exposure of uranium and copper, using a set of biomarkers. The aim of this study was to identify potential biomarkers as early warning signals in ecotoxicological studies.We hypothesized that enzymatic responses would decrease with increasing concentration of stressors. Specimens were reared in groups of 10 in 1.5 L (3 replicates) using standard moderate hard synthetic water, and fed with alder leaf discs previously stream-conditioned. We tested two treatments of copper (35 and 70 µg L^{-1}) and uranium (25 and 50 µg L^{-1}) plus one negative control. Laboratory bioassays were performed under constant temperature 15.7 ± 0.6°C and photoperiod (12:12 h light/dark) for 35 days. We measured the activity of five enzymes in specimens exposed to all treatments: glutathione-S-transferases (GST), acetylcholinesterase (AChE), lactate dehydrogenase (LDH), catalase (CAT), and sodium-potassium adenosine triphosphatase (Na+/K+-ATPase). All enzymes' activities were expressed as nmol min^{-1}. mg^{-1} of protein,with Na+/K+-ATPase expressed as nmol of inorganic phosphate (Pi) $min^{-1}.mg^{-1}$ of protein.Catalase activity significantly increased with the increase in copper concentration ($F= 12.7$, $df=2, 8$ $p= 0.003$) from 0.20 ± 0.04 to 1.10 ± 0.21 nmol min^{-1} mg^{-1} protein (mean ± SE). Na+/K+-ATPaseactivity was affected by uranium, with lower activity (0.11 ± 0.02nM Pi min^{-1} mg^{-1} protein) at high U concentrations (50 µg U L^{-1}) and higher activity (0.15 ± 0.02nM Pi min^{-1} mg^{-1} protein) at lower concentrations (Control and 25 µg U L^{-1}; ANOVA – $F=5.49$, $df=2, 8$, $p=0.029$;).No significant differences were observed in the activities of LDH, GST and AChE among the uranium or copper treatments. Changes in enzymes' activities have been used as biomarkers to assess the stress induced by chemicals and other environmental changes. Through the present research we found that CAT and Na+/K+-ATPase seem to be promising biomarkers for use as ecotoxicological endpoints for monitoring stress conditions of copper and uranium respectively, in freshwater systems.