COMBINED EXPOSURE TO METAL MIXTURES AND PREDATOR STRESS: SUBLETHAL EFFECTS ON *ASELLUS AQUATICUS*

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Introduction

Current environmental quality standards (EQS) are mainly based on toxicity tests in which test organisms are exposed to single compounds for a limited period of time. However, this does not represent the real impact of metal exposure in the natural environment. In aquatic ecosystems, trace metals most often occur in mixtures which could result in antagonistic, synergistic or additional toxic effects. Besides the presence of these pollutants, natural stressors such as predators are present as well, which could also influence the accumulation and toxicity of metals. The aim of this study is to investigate sublethal effects resulting from combined exposure to predation pressure and metal mixtures of Cu, Cd and Pb on the freshwater isopod *Asellus aquaticus* L.

Methods

A 10-day laboratory experiment was conducted in which individuals of *A. aquaticus* were exposed to metal concentrations equal to, tenfold and hundredfold of the EQS: 7, 70 and 700 $\mu g.L^{-1}$ for Cu; 0.15, 1.5 and 15 $\mu g.L^{-1}$ for Cd; and 7.2, 72 and 720 $\mu g.L^{-1}$ for Pb. Additionally, we added four control treatments without metals. Animals were exposed to the different metals separately as well as to binary and tertiary mixtures. These metal treatments were combined with two predator treatments in which cues of predators caught from the same river as the used *A. aquaticus,* were absent or present.

Metal concentrations were prepared in 100 mL EPA medium-hard water with the following stable isotopes: ^{65}Cu, ^{116}Cd and ^{204}Pb (CortecNet, Voisins-Le-Bretonneux, France). The exposures occurred in acid-washed (1% HCl) polypropylene containers (125 mL) under controlled light conditions (16:8 h light:dark photoperiod) and temperature (15 ± 1 °C) in a climate chamber type WT15'/+5DU-WB (Weiss Technik, Reiskirchen-Lindenstruth, Germany). One individual of *A. aquaticus* was placed in each container together with two alder leaf disks (*Alnus glutinosa*, d = 16 mm) that were dried, weighed and 'conditioned' for six days (Bloor & Banks, 2006). Ten replicates per treatment were used. General water characteristics and mortality were monitored daily.

At the start of the experiment we determined the length of each isopod with ImageJ and calculated the dry weight (dw) using the following formula: $\log_e(dw) = 2.71\log_e(length) - 4.58$ (Graça et al., 1993). At day 10, the animals were filmed for 30 minutes after which the videos were processed with the tracking program Lolitrack v.4, that calculated the average velocity ($mm.s^{-1}$), the average acceleration ($mm.s^{-2}$), the active time (%) and the moved distance (mm). Next, to determine the respiration rates, we placed the isopods in glass chambers with oxygen mini sensors in which oxygen concentrations ($mg.L^{-1}$) were measured for 4 h using the programs WitroxView and Fibsoft. Lastly, the leaves and isopods were dried for min. 72 h, weighed and digested. Trace metals and major ions were determined for the filtered water samples, isopods and leaves with an HR-ICP-MS (Element XR, Thermo Scientific, Finnigan element 2, Bremen, Germany). Dissolved organic carbon of the medium was quantified using a TOC-analyzer (TOC-VCPH, Shimadzu Corporation, Kyoto, Japan). Furthermore, we also determined the growth and feeding rates (Bloor and Banks, 2006). Statistical analyses (two-way ANOVAs) were performed in R. If a significant difference was found ($\alpha = 0.05$), a Tukey HSD test was conducted. Animals that died before the end of the experiment were not included in the analyses.

Results

The maximum recorded mortality was 40% in one of the control treatments with predator cues, 30% mortality was found in the mixture of the EQS of Cd and Pb with predator cues. However, mortality in the other treatments did not exceed 20%.

Metal exposure ($F = 17.1$, $p < 0.001$), predator stress ($F = 17.6$, $p < 0.001$) and the interaction between metals and predator stress ($F = 1.81$, $p = 0.02$) had a significant effect on the feeding rates of the isopods. Higher feeding rates were found in the EQS and 10xEQS treatments, while lower rates were found in 100xEQS treatments. Isopods of treatments without predator stress showed higher feeding rates than those of treatments with predator cues. We also found a significant effect of metal exposure on the growth rate ($F = 7.68$, $p < 0.001$). However, no significant difference was found between the predator treatments nor were any significant interactions between the predator and metal treatments observed.

Conclusion

Exposure to metals and/or predator stress resulted in several significant effects and even important interactions between the stressors. Studying the effects of exposure to multiple stressors will eventually lead to the construction of more ecologically-relevant monitoring strategies and EQS for trace metals.

References

Bloor, M. C.; Banks, C. J. (2006). An evaluation of mixed species in-situ and ex-situ feeding assays: The altered response of *Asellus aquaticus* and *Gammarus pulex*. *Environment international*, *32*, 22-27.

Graça, M.A.S.; Maltby, L.; Calow, P. (1993). Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus*. *Oecologia, 96*, 304–309.