Bioavailability and mixture effects of metals in different European mussel populations

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More than 100 million tons of chemicals that have the potential to pose a risk to the environment are produced in Europe each year. A subset of these chemicals may, intentionally or not, enter and affect the environment. To protect the environment and the diverse services it provides, it is important to know what the impact and risk of a chemical release may be. Underestimating the risk can have harmful effects on the environment and on human health. Overestimating the risks may, unnecessarily, increase the costs of preventing or ameliorating pollution. Hence, accurate knowledge of the effects and the associated risks is essential.

Predicting the effect of a chemical is, currently, primarily based on the results of single-species experiments with freshwater organisms that are exposed to a single stressor in a standardized (laboratory) environment. However, in reality organisms are not exposed to these standardized conditions, but live in and are exposed to a variable environment. Furthermore, inter-population differences in sensitivity may exist due to differences in local adaptation and even a single organism's sensitivity may change during its lifetime. Finally, organisms may be exposed to multiple stressors, natural or anthropogenic, simultaneously. Hence, it is suggested that it might not be possible to accurately predict the adverse effects using the currently prescribed methods.

The main objective of this research was to examine the effect of these potential sources of variation on the toxicity of chemicals on marine organisms in order to increase the realism of current environmental risk assessment procedures. This was accomplished by assessing the influence of environmental variation, mixture toxicity, population variability and life-stage variation on the accumulation and toxicity of Cu on a Cu sensitive marine test species, the mussel.

In part one of this research, the combined influence of the two main marine environmental variables, salinity and dissolved organic carbon (DOC), on the distribution, accumulation and toxicity of Cu in mussel larvae (*Mytilus galloprovincialis*) was assessed. By using synchrotron radiation X-ray fluorescence, the distribution and accumulation of Cu in mussel larvae were determined. Cu body burden concentrations varied between 1.1 and 27.6 µg/g DW larvae across all treatments and Cu was homogeneously distributed at a spatial resolution level of 10x10 µm. The 48 h Cu EC10 varied between 2.8 and 11.2 µg/L, confirming that mussels are very sensitive to Cu. Cu accumulation and toxicity decreased with increasing DOC concentrations which can be explained by an increase in Cu complexation. In contrast, an increase in salinity increased the Cu toxicity. This change could not be explained by Cu speciation or competition processes and suggests a salinity-induced alteration in physiology, resulting in a changed Cu sensitivity.

In part two a similar experiment was performed with two populations of settled mussels (North Sea and Baltic Sea). Baltic Sea mussels were chosen because previous research had indicated that the mussel population in that region is already stressed, due to the low salinity of this marine system. It was hypothesized that environmentally stressed populations would be more sensitive to anthropogenic pollution as they have to allocate more energy towards basic maintenance. The Baltic Sea population did accumulate more Cu compared to the North Sea population (both in the gills and in the total soft tissue). However, both populations exhibited an equal sensitivity to copper. This suggests that environmentally stressed populations are not necessarily more sensitive to anthropogenic pollution and that different populations may have a different way to cope with excess Cu. The influence of salinity and DOC on the accumulation and toxicity of Cu to settled mussels was very limited in both populations. Hence, it is concluded that DOC-Cu complexes are bioavailable to settled mussels. Due to the absence of a protective effect by DOC in settled mussels, implementing a DOC correction factor to determine a Cu environmental quality standard for Cu – as is done for freshwater environments – cannot be proposed for marine environments.

Organisms are not only exposed to a changing environment, but are also frequently exposed to multiple metals simultaneously. In the North Sea, for example, high Cu concentrations frequently coincide with high concentrations of other metals like Ni and Zn. Nevertheless, little information is available on the effect of metal mixtures, certainly of environmentally realistic concentrations, in the marine environment. Therefore, in part three of this research, the effect of metal mixtures was assessed. The effect of the Cu-Ni binary mixture on *Mytilus edulis* larvae was assessed using a full factorial design. The reproducibility of the results was assessed by repeating this experiment 5 times during a 3-year period and having them being performed by different researchers. The data were analyzed using a Markov Chain Monte-Carlo algorithm (MCMC). The use, for the first time for mixture toxicity analysis, of this statistical tool enabled the estimation of both the mixture toxicity deviation from the reference models and the uncertainty on the deviation. The results demonstrated that mussel larvae were about 100 times less sensitive to Ni than to Cu (average Cu EC50: 4.1 µg/L vs Ni EC50: 414.7 µg/L). When mussel larvae were exposed to a mixture of these metals, a reproducible ratio-dependent deviation from the concentration addition reference model was observed. Antagonism was observed at high nickel concentrations (> 200 µg/L) but, more importantly, low concentrations of Ni (as low as 4.9 µg/L) resulted in a synergistic interaction with Cu. To our knowledge this is the first time that synergism (according to the concentration addition reference model) was observed at low, environmentally relevant, metal concentrations. This highlights the need to consider mixture effects in marine environmental risk assessment procedures.

In part 4 mussel larvae from two populations (North Sea and Baltic Sea) were exposed to Cu, Zn, Ni and a Cu-Zn mixture to assess both the influence of the mixture and determine possible inter-population differences in metal (mixture) sensitivity. The Baltic Sea mussel larvae were approximately 20 % smaller and grew slower than North Sea larvae. This agrees with previous research that suggested that settled Baltic Sea mussels are stressed by the low salinity and therefore grow slower. Mussel larvae from the Baltic Sea were three times more sensitive to Zn (as single substance) and Ni, as expected based on the proposed but untested hypothesis that the Baltic Sea mussel population would be more sensitive (due to the environmental stress) to metal exposure. However, both populations had an equal sensitivity to Cu and the effect of the Cu-Zn mixture was also similar in both populations. This indicates that inter-population variability in sensitivity is metal-dependent. It can be concluded that: all variables investigated in this study changed the accumulation and/or the toxicity of Cu in mussels. The assessed environmental variables, i.e. salinity and DOC, had a strong influence on the accumulation and toxicity of Cu in mussel larvae but not in settled mussels. Furthermore, the influence of salinity on the Cu toxicity in mussel larvae could not be explained based on complexation and competition. Therefore, using the current knowledge, the development of a universal marine BLM based only on the water chemistry is currently not possible. Next to the influence of environmental factors, we have provided evidence that synergistic metal mixture interactions can occur at concentrations currently measured in marine environments. To adequately protect marine organisms, metal mixture interactions should be included in future environmental risk assessment procedures. Finally, the two assessed populations were equally sensitive to Cu. This suggests that naturally stressed populations are not 'by default' more sensitive to pollution than unstressed populations. However, population differences in organism sensitivity to other metals (Zn and Ni) were observed, indicating that inter-population variability is pollutant-dependent and that this knowledge may need to be included in future ERA procedures.