Addressing emerging issues in marine metal ecotoxicology with novel analytical techniques

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Two emerging issues in marine metal ecotoxicology are (1) the relative importance of animal physiology (elemental homeostasis) towards metal toxicity in transitional waters with intermediate salinities and (2) metal mixture toxicity. For both issues a mechanistic understanding of metal and element homeostasis upon mixture exposure is needed to enhance fundamental knowledge and applied risk assessment procedures. This study aimed at assessing the physiological impact of metal (mixture) toxicity on the homeostasis of essential elements. We used micro X-ray fluorescence (XRF) techniques to analyse mussel gills.

Gills of mussels (*Mytilus edulis*) of approximately 1.5cm, exposed to a control or copper contaminated artificial seawater, were dissected and (a) immediately cryogenically frozen on-site or (b) dehydrated in a graded aceton:water series and fixed with hexamethyldisilazane (HMDS). Elemental distributions were analysed with synchrotron based micro-XRF. In a second series of experiments, mussels were exposed to different salinities and Cu concentrations, upon which HMDS-embedded gills were analysed with laboratory source based micro-XRF. In a proof-of-principle experiment to study metal mixture toxicity, small *M. edulis* were exposed for 48h to one of the following treatments in artificial seawater (i) control, (ii) 100µg.l^{-1} added Cu, (iii) 1mg.l^{-1} added Zn or (iv) a mixture of 100µg.l^{-1} Cu and 1mg.l^{-1} Zn. Again, HMDS-embedded gills were analysed with laboratory source micro-XRF.

Preliminary results indicate that the internal distribution of metals in mussel gills at the micro scale is not significantly affected by the dehydration and HMDS fixation and enhanced tissue preservation, compared to the cryogenically prepared samples. Gills of mussels exposed to 40µg.l^{-1} Cu at a salinity of 32psu exhibited a decrease in K content compared to the control. Analyses of mussel gills exposed to salinity and combined Cu/salinity stress are ongoing. As an example of differences in elemental homeostasis between single metal (Cu or Zn) and mixture exposed mussels, this K depletion upon Cu exposure was present to a lesser extent upon Zn exposure, but was absent upon exposure to the mixture.

Micro-XRF can provide fundamental mechanistic knowledge about physiological effects of metal mixtures or metal toxicity in transitional waters. It revealed different physiological consequences of metal mixture exposures compared to single metal exposures.