Evaluating complex mixtures in the zebrafish embryo by reconstituting field water samples: a metal pollution case-study

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Environmental risk assessment (ERA) is mainly focused on single compound analyses, resulting in limited biological information about mixture effects. The goal of this study was 1) to identify the biological effects of environmental water samples, and 2) to determine whether the observed effects could be linked to the known metal mixture pollution. First, zebrafish embryos were exposed to water samples of 5 different sites originating from 2 Flemish metal contaminated streams: 'Scheppelijke Nete' (SN) and 'Kneutersloop' (K), and a ditch (D), the pollution source of SN. Concentrations of 9 metals (Al, Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb) and 1 metalloid (As) were measured using ICP-MS. Metal ions that exceeded the surface water quality standards were used to reconstitute site-specific field water samples. We then assessed whether the effects that were observed after exposure to the environmental samples could be explained by metal mixture toxicity under standardized laboratory conditions.

Embryos exposed to "D" and "reconstituted D" water both died, which was due to the extremely high metal concentrations. Both SN and reconstituted SN water caused effects on hatching, swim bladder inflation, growth and swimming activity. Embryos from K only showed a decreased swimming activity. The field water samples of K were not reconstituted because of this limited biological response. A principal component analysis showed a high similarity between field and laboratory exposure scenarios. For example, impaired swim bladder inflation was highly correlated to impaired hatching and to the water concentrations of the metals Zn, Mn and Cd in both the field water and the reconstituted water experiments. We concluded that the toxicity that was observed after exposing to field water samples was indeed caused by the metals present in the reconstituted mixtures. The proposed workflow, which shows some similarities to the workflow of a Toxicity Identification Evaluation, could be a valuable alternative to limit both the use of animals and the cost while increasing biological relevance in environmental monitoring.