Ecotoxicity testing of environmentally realistic contaminant mixtures using passive samplers: what can we learn from repeating toxicity tests over an extended period of time?

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Marine ecosystems are nowadays exposed to a multitude of pressures e.g. an increasing input of contaminants (Ghekiere, Verdonck et al. 2013, Gustavsson, Magner et al. 2017). Although there exist many approaches to assess the impact of contaminants on a broad range of aquatic organisms, it remains a challenge to expose aquatic test organisms to **environmentally realistic contaminant mixtures** (ERCMs). The use of passive sampling opens new possibilities to work with such complex mixtures and to transfer them into biotest systems by either applying passive dosing (for equilibrium based samplers) or extract spiking (for integrative samplers). The advantages and disadvantages of both methods have been described in detail elsewhere (Jahnke, Witt et al. 2016).

Our research objective was to investigate whether or not ERCMs have **effects on marine phytoplankton** and if these effects can be explained by measured contaminant concentrations and the use of multivariate statistics. In addition, we looked at the **repeatability of** our **test results over an extended time period** of 16 months.

We used extracts of divinylbenzene Speedisk™ passive samplers deployed in and outside of the harbour of Zeebrugge (Belgium) to spike several 72 h growth inhibition tests with the marine diatom *Phaeodactylum tricornutum* following ISO 10253 (2006). The different growth inhibition tests were performed over a period of 16 months: i.e. 0, 8 and 16 months after extraction.

We observed **statistically significant** ($p < 0.05$, ANOVA followed by Dunnett's multiple comparison test) **growth stimulation** of up to $6.4 \pm 0.5\%$ and $11 \pm 2\%$ (in the harbour) and $7.0 \pm 0.5\%$ and $14 \pm 3\%$ (outside of the harbour) after an extract storage time of 0 and 8 months, respectively. After 16 months the previously observed effects disappeared completely. In order to explain the differing ecotoxicological responses a targeted chemical analysis (UHPLC-Q-Exactive™) was performed for the quantification of 89 personal care products, pesticides and pharmaceuticals. We identified 36 ± 5 and 29 ± 15 compounds in triplicate speedisk™ extracts from samplers deployed in and outside of the harbour, respectively. Further, the analysis revealed that testing occurred at concentration levels that were very similar to those measured in water grab samples taken during sampler deployment indicating that our **tests were performed at realistic environmental contaminant concentration levels**.

The disappearance of the observed stimulation effects after an extract storage time of 16 months led to the hypothesis that the main contributing contaminants causing stimulation must have degraded over time. The application of **multivariate analysis** (i.e. principal component analysis) allowed us to discriminate samples causing stimulation and non-stimulatory samples based on few contaminants such as the β-blocker atenolol or the antidepressant venlafaxine. Currently we are performing additional multivariate analysis on non-targeted compounds to be able to fully explain the observed stimulation effects and link them to specific mixture components.

Keywords: realistic contaminant mixtures; mixture toxicity; growth stimulation; passive sampling; multivariate statistics