Using no observed effects to identify main contributing micropollutants in mixture toxicity assessment

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1. Introduction

In current environmental risk assessment, researchers assess effects caused by single substances towards single species and extrapolate for realistic conditions where organisms are usually exposed to complex micropollutant mixtures. The use of passive sampling opens new possibilities to work with such mixtures and 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 were described in detail elsewhere [1].

Only few ecotoxicological studies tested environmentally realistic contaminant mixtures e.g. using either passive dosing [2] or extract spiking [3]. Interestingly **exposure of marine diatoms to realistic mixture concentration levels ranged from 50 % growth stimulation to 100 % growth inhibition** [2, 3] and the authors concluded that *"exposure to low levels of persistent organic pollutants may threaten sensitive genotypes and benefit healthy populations"* [3]. This argument could be adapted to stimulation effects stating that *"stimulation effects favouring the growth of one species may limit the growth of concurring species"*.

Our initial research objective was to investigate whether or not environmentally realistic contaminant mixtures have effects on marine phytoplankton and how eventual effects could be explained. Based on our findings we further formulated the hypothesis that **no observed effects can be used to identify main contributing micropollutants to observed growth stimulation effects caused by environmentally realistic contaminant mixtures**.

2. Materials and methods

In the presented research we used extracts of Speedisk™ passive samplers deployed in and outside of the harbour of Zeebrugge (Belgium) for 8 weeks to spike 72 h growth inhibition tests with the marine diatom *Phaeodactylum tricornutum* following ISO 10253. The different growth inhibition tests were performed over a time of 16 months with tests 0, 8 and 16 months after extraction and thus considerably different extract storage time.

In detail, Speedisks™ passive samplers were rinsed with seawater on site after retrieval and further rinsed in the lab by applying 10 mL HPLC grade water. The samplers were eluted with 10 mL of a mix of methanol: acetonitrile (50:50 v/v) and the elution solvent fully evaporated under nitrogen. The precipitate was re-dissolved in 1 mL of a methanol: HPLC water mix (10:90 v/v) + 0.1 % formic acid + 0.01 % EDTA. Re-dissolved extract were stored at -20 °C in the dark until chemical analysis or biotesting. For each location 3 replicate Speedisk™ extracts were analysed and tested.

For biotesting a concentration series with 10 concentration treatments following dilution factor 5 was prepared. *P. tricornutum* was cultured following ISO 10253 and 4 days prior to the actual test, a pre-culture with a cell density of 10,000 cells/mL was inoculated in 100 mL erlenmeyer flasks. At test start 100 µL of each concentration treatment were added to 50 mL of fresh algae test medium in triplicates. Further a dilution series of extracts of non-deployed Speedisks™ (procedural blanks, beside test 1) as well as 6 flasks with pure test medium (controls) and another 6 flasks with test medium spiked with the pure solvent used for extraction (solvent controls) were included. All flasks were inoculated with 10,000 cells/mL from the pre-culture and kept randomly under continuous white light (7,500 – 9,000 lux) at 22 ± 1 °C for 72 h. Algae cells were randomly counted every 24 h using a particle counter (Beckman Z™ series Coulter Counter®).

3. Results and discussion

3.1. Algae growth inhibition experiments

The algae growth inhibition experiments resulted in growth stimulation of *P. tricornutum* for both sampling locations for the extracts stored for 0 (test 1) and 8 (test 2) months. The extracts stored for 16 (test 3) months did not show any effects on the growth of *P. tricornutum* as seen in Figure 1. Whenever stimulation effects were observed, these were constantly significant over the 3 to 5 highest concentration treatments for test 2 and 1, respectively.

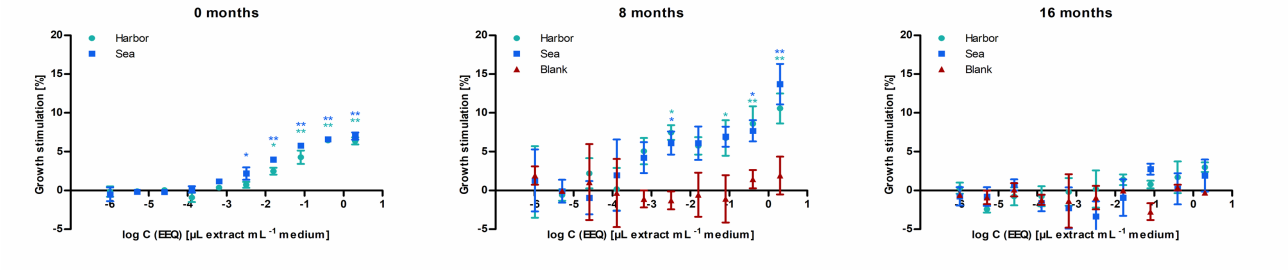

Figure 1 Concentration-response testing of the Speedisk™ extract after different extract storage times (0, 8 and 16 months). Test concentrations range from 0.001 µL to 2 µL extract per mL medium. Shown is the % of growth stimulation vs. the log extract equivalent concentration (log C EEQ). Blank (red triangles) Speedisks™ were tested in duplicates, harbour (green circles) and sea (blue squares) samples in triplicates. Error bars represent the standard deviation (SD) and the significance levels indicate significant differences of the growth rates as compared to the controls (ANOVA followed by Dunnett's Multiple Comparison Test; significance levels: * = P ≤ 0.05 and ** = P ≤ 0.01).

3.2. Chemical analysis

Chemical analysis of the Speedisk™ extracts revealed the presence of 36 ± 5 out of a list of 89 pesticides, pharmaceuticals and personal care products (PPPCPs) in the harbor of Zeebrugge and 29 ± 15 PPPCPs outside of the harbor. Table 1 shows the concentrations of part of the analysed compounds.

The tested summed PPPCP concentrations were 125 ± 16 ng/L and 116 ± 42 ng/L for the harbor and the sea extracts, respectively. These concentrations correlate well with summed PPPCP concentration levels analysed in grab samples taken at the moment of passive sampler deployment at both sampling locations.

Table 1 Example of 8 compounds analysed in the Speedisk extracts. Shown are the concentrations in 1 mL re-dissolved extract in ng/L for the Speedisk™ triplicates as well as their mean and standard deviation (SD). Empty cells indicate no detection of the compound in the respective extract. PE = Pesticides, PH = Pharmaceuticals

Compound	Compound group	Concentration Harbour Zeebrugge [ng/L]					Concentration Sea Zeebrugge [ng/L]				
		Harbor 1	Harbor 2	Harbor 3	Mean	SD	Sea 1	Sea 2	Sea 3	Mean	SD
Acetamiprid	PE		97	104							
Acyclovir	PH	1659		915			5451	1139	508	2366	2690
Alachlor	PE										
Alprazolam	PH							129			
Amantadine	PH	3490	3618	3655	3588	87	13809	11432	12074	12438	1230
Amitriptyline	PH										
Atenolol	PH	3080	2973	2965	3006	64	12908	6403	6218	8510	3810
Atrazine	PE	1239		1183				2517			

4. Conclusions

At this moment we are analyzing both Speedisk™ extracts and test media to compare the contaminant concentrations in the different tests. Once the results are available (december 2017) we will perform principal component analysis to identify main contributing compounds to the observed effects for both targeted and non-targeted analysis.

Our results showed growth stimulation effects on *P. tricornutum* for Speedisk™ ecxtracts stored 0 and 8 months while the effects disappeared after 16 months. We therefore hypothesised that the main contributing micropollutants to the observed effects must have degraded during the extract storage. Stimulation effects favouring specific phytoplankton species might lead to shifts in community compositions and might have impacts on marine ecosystems. We therefore emphazise the implementation of environmentally realistic contaminant mixture toxicity assessment into environmental risk assessment.

5. References

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