Effects of global change on the emission, fate, effects, and risks of chemicals in aquatic ecosystems

Marie Curie Actions

SHORT TITLE:	ECORISK2020
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ORGANISATION:	Wageningen University
TOPIC:	H2020-MSC_ITN 2018
PROJECT NUMBER:	813124

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813124

Deliverable:

New framework to assess resilience of microbial communities to chemical stress

Microbial communities play an essential role in ecosystems and control the transformation and mineralization of natural compounds in sediments (Tyson et al., 2004). Microbiota exhibit high levels of diversity and abundance and can rapidly modify their energetic performance and activity to respond to changing environmental conditions, including chemical pollution (Acosta-González and Marqués, 2016).

While studies focussing on single pollutants' effects on microbial communities have been published recently, knowledge about an overall impact of a combination of chemicals on microbial ecosystems is scarce (Milan et al., 2018; Yan et al., 2018). The two main reasons are: 1) measuring concentrations of all potential contaminants in the environment is unrealistic due to the technical challenges and analytical costs, and 2) the sum of the measured concentrations of different chemicals does not inform us about the exposure levels, because toxic concentrations are compound-specific. This makes the exposure, hazard, and risk assessments of chemical mixtures in the environment extremely challenging (Gobas et al., 2018).

The concept of 'chemical activity' offers a way to overcome these constraints for chemical mixtures of neutral chemicals at concentrations below their specific toxicity concentration, by enabling the conversion of concentrations of various chemicals into a common, unitless, currency (Gobas et al., 2018). In such cases, when many neutral organic chemicals are present at low concentrations, additivity of toxicity is often observed (Escher et al., 2002). This holds true even when the substances are not related chemically, or exhibit different modes of action when acting alone at acute levels (Escher and Hermens, 2002). The phenomenon is defined as baseline toxicity, or narcosis (Escher et al., 2002) and is related to disturbances in cell membrane functioning. Chemical activity relates a chemical's

concentration to its maximum solubility in the environmental media (Gobas et al., 2018; Schwarzenbach et al., 2003), it is additive, and correlates to baseline toxicity, thereby offering an integrative tool to quantify the biological potency of chemical mixtures in background areas addressing the following research questions and aims of this study:

Specific research questions

i. Can chemical activity of different complex low-dose chemical mixtures be used as a measure to understand their biological effects?
ii. Does resilience towards chemical activity show different responses caused by chemical or pulsed stressors and do they depend on the microbial community?

Aims of this study

a) Developing concepts and tools for using chemical activity as a measure to understand the effects of chemical mixtures on microbial communities and their functions
b) To understand the effects of chemical activity on microbial community functions

In experiments, microbial communities will be exposed to various chemical mixtures (combinations of various hydrophobic hazardous chemicals such as PCBs and PAHs) with different chemical compositions but with the same chemical activities. The concentration of each chemical will be below the threshold level of its specific toxicity (Fig. 1A). The latter will ensure that the observed effects are not related to the specific mode of action of the test chemicals present in the mixture. To test the concept, experiments will be conducted first using *Daphnia magna* as test organisms, whereas immobilisation will be used as toxicity endpoint (Castro et al., 2018). Once this approach has been successfully proven on *D. magna*, it will be applied to bacterial communities.

To assess the resilience of microbial communities to chemical stress, bacterial communities in aquatic sediments from freshwater systems will be used. The bacteria will be exposed to the same chemical mixtures that were tested with *D. magna* beforehand. To ensure a constant exposure level towards the chemicals during the time of the experiments, passive dosing via silicone rods will be performed (Fig. 1B). To do so, saturated chemical mixtures will be prepared in methanol from pure compound crystals. Those saturated methanol solutions render the maximum chemical activity possible for the specific chemical compounds. The saturated methanol solutions can be diluted to the desired chemical activity, which must be still below baseline toxicity. Those diluted chemical solutions can thus be used to load silicone rods with the intended chemical activity of the mixture. Via passive dosing, *D. magna* and the bacterial communities can be exposed to the needed chemical activity. At a later stage, additional pulsed stressors (e.g., temperature increase) can be added to the bacterial community to mimic chemical exposure in a changing environment in the scope of climate change. To identify any changes within the bacterial community, thorough testing of the bacterial community must be performed before and after the exposure to the chemical mixtures, to enable a comparison of the communities.

To analyse the effects of chemical stress on the sediment microbial communities we will use amplicon-based sequencing of marker genes with 16S rRNA. This technique is a cost-effective and powerful tool to assess and compare the structure of microbial communities at a high phylogenetic resolution (Aßhauer et al., 2015). The metagenomic 16S rRNA data will also serve to predict the functional profiles of the microbial communities with the support of statistical programs (e.g., using the R package Tax4Fun). Further, effects of chemical mixtures

on microbial gene functions (e.g., denitrification, ammonium oxidation) will be analysed. Functions will be selected depending on the community composition after exposure. Some gene functions, e.g., those needed to regulate the carbon, nitrogen, sulphur, and phosphorus cycles, are of particular interest as they are the metabolic pathways driving a variety of ecosystem processes (Allison and Martiny, 2008). The concentration of the used chemical compounds in the experiments will be analysed via extraction and GC-MS analysis.

These experiments will help to understand and assess the resilience of sediment communities towards chemical pollution as well as to develop useful community-level proxies.

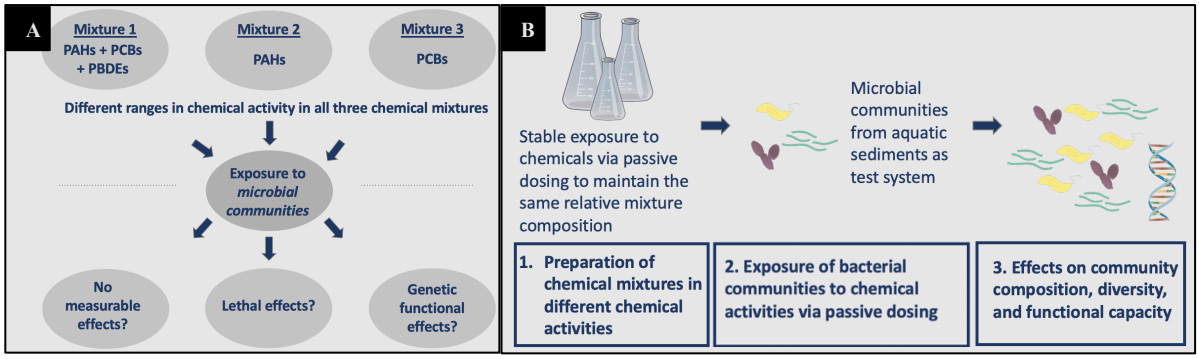

Figure 1. A Experimental concept using chemical activity. Bacterial communities from aquatic sediments will be exposed to three chemical mixtures, different in chemical composition and chemical activities. Exposure of the bacteria is expected to lead to either no measurable effects, lethal effects, genetic functional effects, or a combination of all. **B** Workflow illustration of the experimental design.

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