Can we track the possible influence of chemical warfare agents on microbial community in the marine environment?

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All across the globe, several locations are found where the seafloor is covered with conventional munition compounds and chemical warfare agents. These are the results of official and unofficial dumping events that took place right after World War I and II (Beck *et al.*, 2018). One of these dumpsites is the *Paardenmarkt* site which is located 500 m off the coast of Belgium. Here, approximately 35.000 tons of munitions were dumped in the sea. One of these compounds is mustard gas (a chemical warfare agent), which hydrolyses quickly to ThioDiGglycol (TDG) in seawater. This compound can interact with the microbial ecosystem in marine environment. So far, most of the studies on the microbial interaction with TDG were performed in soil environments (Medvedeva *et al.*, 2009). The knowledge on how the marine microbiome interacts with TDG is still limited. When properly understood, microbial interaction with TDG can become valuable for biomonitoring and targeted bio-engineering for bioremediation.

Here, we studied the effects of TDG at different concentrations (mg/L - µg/L) on a marine microbiome. Marine sediment was collected nearby the *Paardenmarkt* site in the Belgian part of the North Sea. Two types of microcosms were set up in triplicate, containing filter-sterile seawater supplemented with nutrients and 2 mg/L acetate, sediment with microorganisms, and different concentrations of TDG (300 µg/L and 2 mg/L). Two controls were taken into account. The first one contained only seawater with sediment (without TDG), and the second one contained seawater, TDG (2 mg/L) and autoclaved sediment. The latter served to assess the interaction between TDG and the sediment. Additional incubations were set-up in the presence of high concentration of TDG, 80 mg/L both in aerobic and anoxic conditions to enrich for microbes able to use TDG as a C source. Microbial growth was monitored through flow cytometry. The phenotypic diversity was assessed over time as described in Props *et al.*, (2016). Chemical analysis of TDG concentration was performed using LC-MS.

Our first results suggest that TDG, when present at low concentrations (300 µg/L and 2 mg/L), cannot support microbial growth. However, the live cell count was the same as the two control experiments. This indicates minimal toxic influence of TDG on marine microbiome. As a second result, the concentration of TDG in the microcosms rapidly decreased after spiking (within 2 days). The TDG concentration in the control experiments decreased correspondingly, thus leaving the distinction between adsorption and biodegradation difficult. Nevertheless, during enrichment with TDG as a sole C source, microbial growth was observed in line with the reduction of TDG. The phenotypic diversity assessment of the enriched samples revealed a shift in community structure over time. This provides us with a strong indication that TDG biodegradation occurred. Further analysis on biotransformation metabolites and adsorption kinetics together with Illumina sequencing is needed to draw the complete picture of TDG degradation in the marine environment.

References

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