Explosive legacies: Revealing wartime TNT pollution in the North Sea through bacterial indicators

Schütte Wyona[1], Van Landuyt Josefien[2], Brenner Matthias[3], Vedenin Andrey[4], Gundlach Maren[5], Bünning Tobias[5], Strehse Jennifer[5], Maser Edmund[5], Boon Nico[6] and De Rijcke Maarten[7]

[1] Flanders Marine Institute (VLIZ), Jacobsenstraat 1, 8400 Oostende
E-mail: wyona.schutte@vliz.be
[2] CMET, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[3] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[4] Senckenberg am Meer, Südstrand 40, 26382 Wilhelmshaven, Germany
[5] Kiel University, Institute of Toxicology and Pharmacology, Brunswiker Str. 10, 24105 Kiel, Germany
[6] Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[7] Flanders Marine Institute, Jacobsenstraat 1, 8400, Ostend, Belgium

Millions of tons of munitions from the World Wars are littering the North Sea. They were lost during battle, can be found on shipwrecks, or have been dumped after the war. Used commonly as a military explosive, 2,4,6-trinitrotoluene (TNT) is also known for its toxic, mutagenic and carcinogenic properties. In the marine environment, corrosion ultimately results in the leakage of TNT from underwater munitions, affecting the surrounding sediments and biota, and potentially accumulating in the food chain. By analysing the changes in the marine microbial community exposed to TNT and its metabolites, we aim to identify bacterial taxa that can serve as indicators for pollution of dissolved explosives.

We collected sediments from a munition dumping area and reference site in the German Bight and exposed them to TNT-spiked seawater (0, 10, 1000µg/l) during a 4-week laboratory experiment. The sediments were kept statically in the dark at 16°C, aiming to mimic natural scenarios of TNT leaking from corroding underwater munitions. Water and sediment samples are analysed for TNT and its metabolites with gas chromatography combined with mass spectrometry (GC-MS). Using full-length 16S rRNA Nanopore gene sequencing, the microbial fingerprint in the sediments is being determined. Initial water measurements have shown a rapid decrease of TNT, with the explosive being longer detectable in high than low treatments (7 days and 1 day, respectively). Metabolites (2-ADNT and 4-ADNT) increased during the first 24 hours of the experiment and were present longer than the parent compound (up to 18 days). This trend was less pronounced in control treatments with autoclaved sediments, indicating the importance of microbial activity for degradation. Currently ongoing measurements of explosives and bacterial communities in sediments are expected to confirm this observation. Based on our previous research, where we observed a shift towards several species of *Rhodobacteraceae* in sediments collected next to a World War II destroyer, we anticipate a sensitive change in the microbial fingerprint based on the experimental treatment. Our results highlight the impact of explosives leaking from lost underwater munitions, where microbial composition changes related to TNT and its metabolites will help to identify bacterial indicators for dissolved explosives and their degradation potential.

Keywords
Microbiology; 2,4,6-trinitrotoluene (TNT); Marine Pollution; 16S RRNA Sequencing