Spying bacteria for dumped World War I munition at the coast of Knokke-Heist

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In the years after World War I and II, several countries were confronted with a surplus of both chemical and conventional warfare agents. A way to deal with these bombshells was to dump them at sea since storage on land was too dangerous. Those munition dumpsites can be found worldwide, with major hotspots at the coast of Japan and in the Baltic and Adriatic sea. Due to the sensitive nature of the dumped materials, proper records are often missing. However, it is estimated that at least a staggering 1.6 million tons of munition are dumped worldwide (Wilkinson, 2017). After World War I, Belgian leftover munition was dumped on the sandbank *Paardenmarkt* at the coast of Knokke-heist. This is only a mere 500 m off the Belgian coast and located next to one of the biggest harbors in Northwest Europe, Zeebrugge. This sandbank was chosen with the assumption that the bombshells would sink in the sand layers over time, which also happened. The dumpsite is three km² in size, and an estimated 35.000 tons of munition were dumped (Missiaen, 2013). Multiple types of conventional and chemical warfare agents were used during World War I, with all of them one thing in common: tri-nitro-toluene (TNT). This compound poses ecotoxic threats to the immediate surroundings of the buried shells and poses explosion danger.

Monitoring the leakage of the bombs is difficult as analytical tests face issues with the high degree of dilution at sea and the low detection limits. Therefore, a microbial approach could offer a solution. Small concentrations of TNT could influence the microbiome, inducing a community change which could be used as a biomonitoring tool.

We conducted experiments with marine sediment sampled close to the *Paardenmarkt* site. Filter-sterilized seawater supplemented with nutrients and acetate (1 mg/L) as C source was added. We tested out two TNT concentrations (300 µg/L and 2 mg/L) under aerobic conditions to see the microbial shift caused by TNT exposure. Secondly, a degradation experiment with a TNT range of 40-80 mg/L under different conditions (aerobic, anoxic) was also performed. The microbial community was monitored with flow cytometry. GC-MS was used to quantify TNT and TNT degradation products.

Our results suggest that bacteria can degrade TNT when concentrations of 2 mg/L, 40 mg/L, or even 80 mg/L were used. TNT concentration dropped to zero fast, in 7 days for aerobic flasks and 15 days for the anoxic flasks. In the experiment where 2 mg/L TNT was added, total cell count remained unaltered with the reduction in TNT concentration. However, the total cell concentration and live-cell count were the same as in control without TNT. Nevertheless, TNT metabolites were detected, which shows some bacteria can bio-transform TNT.

For the experiments with 40-80 mg/L TNT, the biodegradation of TNT was correlated with a 2 log unit growth. This suggests the bacteria could grow using this toxic product as C source. The decrease in TNT was also correlated with an increase in TNT degradation products. Degradation products found in the experimental set-ups were different. Combined with the condition-specific phenotypic fingerprinting, the role of different microorganisms/phenotypes for the degradation of TNT in different environmental conditions is confirmed.

In the future, the degradation of TNT at a constant low concentration (<1 µg/L) and assessment of degraders by Illumina sequencing will be performed.

References
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