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Short Communication

Biodegradation-mediated alterations in acute toxicity of water-accommodated fraction and single crude oil components in cold seawater

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HIGHLIGHTS

- Acute toxicity of oil compounds were reduced during biodegradation in cold seawater.
- Two PAH compounds showed toxicity reductions in relation to their biotransformation.
- The toxicity reduction of a crude oil LE-WAF followed depletion of the predominant PAH.
- Acute toxicities persisted for longer periods than in previous tests with warmer seawater.

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ABSTRACT

Hydrocarbon biodegradation may be slower in cold Arctic than in temperate seawater, and this will affect the toxicity time window of the hydrocarbons. In this study, the acute toxicities of water-soluble phases of 1,3-dimethylnaphthalene, phenanthrene, fluoranthene, and low energy water-accommodated fractions (LE-WAFs) of an evaporated (200 °C+) crude oil, were screened by a Microtox bioassay during biodegradation in cold seawater (4–5 °C). The water-solubility of fluoranthene was too low to provoke a toxic response at any time, whereas the toxicity of 1,3-dimethylnaphthalene and phenanthrene decreased over time in relation to biotransformation of these compounds. In LE-WAFs, the Microtox EC_{50} was associated with biodegradation of the predominant hydrocarbons (naphthalenes, 2- to 3-ring PAH), as well as with phenol degradation products. The acute toxicities of single hydrocarbons and LE-WAFs persisted for a longer period in the cold seawater than previously shown at higher seawater temperatures. These results suggest implications for fate and effects assessment of hydrocarbons after oil spills in cold environments, like the Arctic. However, further biodegradation studies using Arctic seawater and relevant species for toxicity testing are needed for confirmation.

1. Introduction

Biodegradation of hydrocarbons (HCs) in seawater (SW) after oil spills is associated with oxidative processes. Aerobic *n*-alkane degradation is associated with monooxygenases, in which the alkane is converted to alcohols and further to acetyl-coA (Harayama et al., 1999), while aromatic HCs are degraded primarily by dioxygenases (e.g. Van Hamme et al., 2003). Resulting metabolites are more water-soluble and thus associated with lower octanol-water partitioning coefficient (Kow). Acute effect concentrations (e.g LC_{50}) of HCs, predicted as the relations between LogKow and $LogLC_{50}$ (French-McCay, 2002), is expected to result in reduced acute toxicity after oxidative processes like biodegradation. Relations between biodegradation and acute toxicity have been investigated in several studies, mainly in soil or groundwater (Wang et al., 1990; Belkin et al., 1994; Tiehm et al., 1997; Renoux et al., 1999; Juhasz et al., 2000; Ruberto et al., 2006), or with bacterial cultures (Pagnout et al., 2006; Fernando Bautista et al., 2009). However, only few have studied these relationships in oil-polluted SW (Brakstad and Faksness, 2000). In cold SW environments like the Arctic, dissolution of oil, as well as biodegradation processes, are expected to be slower than in temperate environments (Faksness et al., 2008; Bagi et al., 2013). This may be compensated by the presence of cold-adapted (psychrophilic/psychrotrophic)

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bacterial communities (Yakimov et al., 2003, 2004).

The objective of this study was to determine the relation between biodegradation and acute toxicity of HCs in SW, using a rapid screening bioassay. Three aromatic HCs and the water-accommodated fraction (WAF) of a crude oil were used at low temperatures, relevant for Arctic conditions.

2. Materials and Methods

2.1. Biodegradation experiments

SW was collected from a depth of 80 m (below thermocline) in a non-polluted Norwegian fjord (Trondheimsfjord; 63°26′N, 10°23′E). The SW was supplied by a pipeline system from the source to our laboratories (salinity of 34‰, temperature of 6–8 °C, and dissolved oxygen (DO) of 8.5 mg/L when reaching our laboratory), passing a sand filter for removal of coarse particles. Nutrient analyses of the SW (Eurofins Environment Testing, Bergen, Norway) showed 23 μg/L total phosphorus, 20 μg/L PO_4-P, 940 μg/L total nitrogen, 160 μg/L NO_2+NO_3-N, 500 μg/L NH_4-N, 2.0 mg/L total organic carbon (TOC/NPOC), and <0.05 mg/L Fe. The SW was acclimated to 5 °C (7 days before start of the experiments), aerated by bubbling with sterile-filtered air, and amended with mineral nutrients as described in OECD Guideline 306 (OECD, 1992).

Single HCs included 1,3-dimethylnaphthalene (1,3-DMN; CAS no. 575-41-7; 96% purity), phenanthrene (Phe; CAS no. 85-01-8; 98% purity) and fluoranthene (Fluor; CAS no. 206-44-0; 98% purity), all purchased from Sigma-Aldrich. HC properties are described in Table S1 (Supplementary Information), including physical data and predictions of biodegradabilitiy and acute ecotoxicity (Episuite, vs. 4.1, US EPA). Single HCs were dissolved in dichloromethane (DCM; 5.5 mg/ml), and were carefully spotted on 2.25 cm^2 Fluortex™ (Sefar AG, Heiden, Switzerland) hydrophobic adsorbent surfaces (100 μL), and the adsorbents air-dried (30 min) to evaporate the solvent. Adsorbents were then submerged in acclimated, aerated and amended SW in completely filled (no air bubbles) 275 ml flasks at nominal HC concentrations of 2 mg/L. Sterilized SW controls were poisoned with $HgCl_2$ (100 mg/L). Negative controls of DCM without HCs were also included.

Low-energy WAFs (LE-WAFs) of a crude naphthenic oil (Troll, 2007-0287), evaporated at 200 °C to simulate 0.5–1 day on the sea (Daling et al., 1990), were prepared at an oil:SW ratio of 1:100 in acclimated, aerated and nutrient-amended SW, or in the same SW with $HgCl_2$ (sterilized controls), at 4–5 °C for 96 h as previously described (Singer et al., 2000). LE-WAFs were distributed on 275 ml flasks as described above.

Flasks with single HCs and LE-WAFs were incubated at 4–5 °C in the dark for up to 63 days, with triplicate sampling after 0, 10, 14, 21, 28, 42 and 63 days of incubation.

2.2. Analyses and calculations

Biotransformation (primary biodegradation) was determined using GC-MS analyses of DCM extracts of single HCs, or the LE-WAFs (SIM mode). In samples with single HCs, adsorbents were placed in 30 ml DCM with Na_2SO_4 and stored at 4 °C until extracted, while SW phases were solvent-solvent extracted with DCM (see below). Semi-volatile organic compounds (SVOC) in LE-WAFs included 60 targeted compounds or compound groups of C0- to C4-alkylated naphthalenes, 2- to 6-ring PAH, C0- to C5-alkylated phenols, and C0-C4-alkylated decalines (Brakstad et al., 2014, 2015). The SVOC analytes were quantified in a gas chromatograph coupled to a mass spectrometer (GC-MS; Agilent 6890 plus GC coupled with an Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] modus; Agilent Technologies). Deuterated SIS-PAH standards (naphthalene, phenanthrene, chrysene, perylene; 50–250 μg/ml) and RIS-PAH standards (acenaphthene, fluorene; 100 μg/ml) were used for the SVOC compound quantification. The response values for individual target analytes were determined, with a signal-to-noise ratio of 10 as the lower detection limit, and a lower limit of detection (LOD) of 0.01 μg/L was defined for individual oil compounds. Total extractable material (TEM) in DCM extracts was quantified by GC-FID analyses (Agilent 6890 N with 30 mDB1 column; Agilent Technologies), using *o*-terphenyl as surrogate internal standard (SIS), and 5α-androstane as recovery internal standard (RIS), and a LOD of 0.1 μg/L (Brakstad et al., 2015).

Dissolved oxygen (DO) was measured in the flasks with an oxygen meter (YSI Inc., Yellow Springs, OH, USA), biochemical oxygen demand (BOD) determined, and theoretical oxygen demand (ThOD) calculated as a measure of mineralization (ultimate biodegradation) (OECD, 1992).

A closed vial Microtox™ bioassay was used to determine EC_{50} concentrations in soluble fractions of single HCs or LE-WAFs (Hokstad et al., 1999), using the marine luminescent bacterium *Aliivibrio fischeri*.

Primary biodegradation was determined by calculating the percentage concentrations of the compounds (1,3- dimethylnaphthalene and phenanthrene, and compound groups in the LE-WAF) in natural SW, compared to the concentrations of the same compounds in the sterilized SW at each sampling date. Ultimate biodegradation of single HCs and LE-WAFs was determined by comparison of BOD and ThOD as follows: $y = 100 - \left(\frac{100}{ThOD}\ x\ \frac{BOD_n}{C_0}\right)$, where BOD_n is BOD at day n, and C_0 is the measured concentration of HCs or LE-WAF (TEM) at the start of the experiment (day 0). The calculated ThOD values of the single compounds are shown in Table S1, while a ThOD of 3.0 was used as a ThOD of the LE-WAF, being quantitatively predominated by aromatic HCs with ThOD values close to 3.0. Biotransformation kinetics were determined as first-order rate coefficients and half-lives (GraphPad Prism version 6.01; GraphPad Software, La Jolla CA, U.S.A).

3. Results and discussions

3.1. Biodegradation and toxicity of 1,3-dimethylnaphthalene and phenanthrene

Initial Microtox studies showed no detectable EC_{10} (Table 1) of the soluble fluoranthene fraction (EC_{50} outside range), combined with concentrations in the SW phase below LOD of 0.01 μg/L (Table 1), and further analyses of this compound were therefore not performed in the study.

The concentrations of 1,3-dimethylnaphthalene and phenanthrene were separately measured on the adsorbents and in the water phase. Most of the compounds rapidly dissolved to the water phase, although measurements in sterilized SW also showed moderate dissolution of 1,3-dimethylnaphthalene from the adsorbents later in the experimental period. Results in natural SW showed faster depletion of both compounds in the SW than on the adsorbents, and comparison to sterilized SW demonstrated that depletion was caused by biotransformation (Fig. S1, Supplementary Information). Biotransformation rates and half-lives were determined for the adsorbed fractions of 1,3-dimethylnaphthalene and phenanthrene, and for the total concentrations of the compounds (sum of the adsorbed and solubilized HCs). Since the relative depletion of 1,3-dimethylnaphthalene and phenanthrene was faster in the SW than the adsorbed phase, faster degradation of the