Accepted Manuscript

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

Odd G. Brakstad, Trond R. Størseth, Marianne U. Rønsberg, Bjørn Henrik Hansen

Please cite this article as: Brakstad, O.G., Størseth, T.R., Rønsberg, M.U., Hansen, Bjø.Henrik., Biodegradation-mediated alterations in acute toxicity of water-accommodated fraction and single crude oil components in cold seawater, *Chemosphere* (2018), doi: 10.1016/j.chemosphere.2018.04.032.

1   Short communication

2   **Biodegradation-mediated alterations in acute toxicity of water-**

3   **accommodated fraction and single crude oil components in cold seawater**

4

5   **Authors:** Odd G. Brakstad[1*], Trond R. Størseth[1], Marianne U. Rønsberg[1] and Bjørn Henrik

6   Hansen[1]

7   [1]SINTEF Ocean, Dept. of Environment and New Resources, N-7465 Trondheim, Norway

8

9   *Corresponding author: Odd G. Brakstad; e-mail odd.brakstad@sintef.no; Phone +47

10   98243447; Fax no. +47 73597043

11

12   Key words: Biodegradation; seawater; acute toxicity; Microtox assay; water-soluble fractions;

13   PAH

14

15 **ABSTRACT**

16 Hydrocarbon biodegradation may be slower in cold Arctic than in temperate seawater, and

17 this will affect the toxicity time window of the hydrocarbons. In this study, the acute toxicities

18 of water-soluble phases of 1,3-dimethylnaphthalene, phenanthrene, fluoranthene, and low

19 energy water-accommodated fractions (LE-WAFs) of an evaporated (200°C+) crude oil, were

20 screened by a Microtox bioassay during biodegradation in cold seawater (4-5°C). The water-

21 solubility of fluoranthene was too low to provoke a toxic response at any time, whereas the

22 toxicity of 1,3-dimethylnaphthalene and phenanthrene decreased over time in relation to

23 biotransformation of these compounds. In LE-WAFs, the Microtox EC_{50} was associated with

24 biodegradation of the predominant hydrocarbons (naphthalenes, 2- to 3-ring PAH), as well as

25 with phenol degradation products. The acute toxicities of single hydrocarbons and LE-WAFs

26 persisted for a longer period in the cold seawater than previously shown at higher seawater

27 temperatures. These results suggest implications for fate and effects assessment of

28 hydrocarbons after oil spills in cold environments, like the Arctic. However, further

29 biodegradation studies using Arctic seawater and relevant species for toxicity testing are

30 needed for confirmation.

31

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

The objective of this study was to determine the relation between biodegradation and acute toxicity 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, 2011). Single HCs were dissolved in dichloromethane (DCM; 5.5 mg/ml), and were carefully spotted on 2.25 cm^2 Fluortex^{TM} (Sefar AG, Heiden, Switzerland) hydrophobic adsorbents surfaces (100 µL), and adsorbent air-dried (30 min) to evaporate solvent. Adsorbents were then submerged in acclimated, aerated and amended SW in completely filled (no air bubbles) 275 ml flasks (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.

79   Low-energy water-accommodated fractions (LE-WAFs) of a crude naphthenic oil

80   (Troll 2007-0287), evaporated at 200°C to simulate 0.5-1 day on the sea (Daling et al., 1990),

81   were prepared at an oil:SW ratio of 1:100 in acclimated, aerated and nutrient-amended SW,

82   or in the same SW with $HgCl_2$ (sterilized controls), at 4-5°C for 96 hours as previously

83   described (Singer et al., 2000). LE-WAFs were distributed on 275 ml flasks as described

84   above.

85   Flasks with single HCs and LE-WAFs were incubated at 4-5°C in the dark for up to 63

86   days, with triplicate sampling after 0, 10, 14, 21, 28, 42 and 63 days of incubation.

87

88   *2.2   Analyses and calculations*

89   Primary biodegradation was determined using GC-MS analyses of DCM extracts of

90   single HCs, or the LE-WAFs (SIM mode). In samples with single HCs, adsorbents were

91   placed in 30 ml DCM with Na_2SO_4 and stored at 4°C until extracted, while SW phases were

92   solvent-solvent extracted with DCM (see below). Semi-volatile organic compounds (SVOC)

93   in LE-WAFs included 60 targeted compounds or compound groups of C0- to C4-alkylated

94   naphthalenes, 2- to 6-ring PAH, C0- to C5-alkylated phenols, and C0-C4-alkylated decalines

95   (Brakstad et al., 2014; Brakstad et al., 2015a). The SVOC analytes were quantified in a gas

96   chromatograph coupled to a mass spectrometer (GC-MS; Agilent 6890 plus GC coupled with

97   an Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] modus; Agilent

98   Technologies). Deuterated SIS-PAH standards (naphthalene, phenanthrene, chrysene,

99   perylene; 50-250 µg/ml) and RIS-PAH standards (acenaphthene, fluorene; 100 µg/ml) were

100   used for the SVOC compound quantification. The response values for individual target

101   analytes were determined, with a signal-to-noise ratio of 10 as the lower detection limit, and a

102   lower limit of detection (LOD) of 0.01 µg/L was defined for individual oil compounds. Total

103   extractable material (TEM) in DCM extracts was quantified by GC-FID analyses (Agilent

104   6890N with 30 mDB1 column; Agilent Technologies), using *o*-terphenyl as surrogate internal

105   standard (SIS), and 5α-androstane as recovery internal standard (RIS),and a LOD of 0.1 µg/L

106   (Brakstad et al., 2015).

107   Dissolved oxygen (DO) was measured in the flasks with an oxygen meter (YSI Inc.,

108   Yellow Springs, OH, USA), biochemical oxygen demand (BOD) determined, and theoretical

109   oxygen demand (ThOD) calculated as a measure of ultimate biodegradation (OECD, 1992).

110   A closed vial Microtox^{TM} bioassay was used to determine EC_{50} concentrations in

111   soluble fractions of single HCs or LE-WAFs (Hokstad et al., 1999), using the marine

112   luminescent bacterium *Aliivibrio fischeri*.

113   Biotransformation (primary biodegradation) was determined by calculating the

114   percentage concentrations of the compounds (1,3- dimethylnaphthalene and phenanthrene,

115   and compound groups in the LE-WAF) in natural SW, compared to the concentrations of the

116   same compounds in the sterilized SW at each sampling date. Mineralization (ultimate

117   biodegradation) of single HCs and LE-WAFs was determined by comparison of BOD and

118   ThOD as follows: $y = 100 - (\frac{100}{ThOD} \, x \, \frac{BOD_n}{C_0})$, where BOD_n is BOD at day *n*, and C_0 is the

119   measured concentration of HCs or LE-WAF (TEM) at the start of the experiment (day 0). The

120   calculated ThOD values of the single compounds are shown in Table S1, while a ThOD of 3.0

121   was used as a ThOD of the LE-WAF, being quantitatively predominated by aromatic HCs

122   with ThOD values close to 3.0. Biotransformation kinetics were determined as first-order rate

123   coefficients and half-lives (GraphPad Prism version 6.01; GraphPad Software, La Jolla CA,

124   U.S.A).

125

126   **3.   Results and Discussions**

127   *3.1 Biodegradation and toxicity of 1,3-dimethylnaphthalene and phenanthrene*

6

128   Initial Microtox studies showed no detectable EC_{10} (Table 1) of the soluble

129   fluoranthene fraction (EC_{50} outside range), combined with concentrations in the SW phase

130   below LOD of 0.01 µg/L (Table 1), and further analyses of this compound were therefore not

131   performed in the study.

132   The concentrations of 1,3-dimethylnaphthalene and phenanthrene were separately

133   measured on the adsorbents and in the water phase. Most of the compounds rapidly dissolved

134   to the water phase, although measurements in sterilized SW also showed moderate dissolution

135   of 1,3-dimethylnaphthalene from the adsorbents later in the experimental period. Results in

136   natural SW showed faster depletion of both compounds in the water than on the adsorbents,

137   and comparison to sterilized SW demonstrated that depletion was caused by

138   biotransformation (Fig. S1, Supplementary Information). Biotransformation rates and half-

139   lives were determined for the adsorbed fractions of 1,3-dimethylnaphthalene and

140   phenanthrene, and for the total concentrations of the compounds (sum of the adsorbed and

141   solubilized HCs). Since the relative depletion of 1,3-dimethylnaphthalene and phenanthrene

142   was faster in the water than the adsorbed phase, faster degradation of the total concentrations

143   was determined (Fig. 1). Biotransformation rates for the of sum of adsorbed and solubilized

144   compounds resulted in half-lives of 9 days for 1,3- dimethylnaphthalene, and 15 days for

145   phenanthrene, while corresponding data for adsorbed HCs were 16 and 35 days, respectively

146   (Fig. 1; Table S2). Mineralization of 1,3- dimethylnaphthalene and phenanthrene was

147   determined from their ThODs (see Table S1). Mineralization half-lives of the HCs were 32

148   (1,3- dimethylnaphthalene) and 63 (phenanthrene) days (Table S2), resulting in

149   mineralization:biotransformation ratios for 1,3- dimethylnaphthalene and phenanthrene of 3.5

150   and 4.2, respectively.

151   Higher Microtox EC_{10} (%) of 1,3- dimethylnaphthalene and phenanthrene (lower

152   toxicity) at day 0 than days 10 and 14 were related to the lower dissolved HC concentrations

7

153 at day 0, since dissolution continued during the biodegradaton period. Comparison of EC_{10}

154 values as function of dissolved concentrations showed comparable results for

155 1,3-dimethylnaphthalene and phenanthrene (Table 1). After 21 days of incubation, no toxicity

156 (EC_{10}>100%) of 1,3-dimethylnaphthalene was measured, while no phenanthrene toxicity was

157 measured after 28 days, both results in accordance with reduced HC concentrations as the

158 result of biodegradation (Table 1; Fig. 1). The Microtox data are mainly in agreement with

159 previous results, showing EC_{50} values of 0.79 mg/L for 1,3-DMN and 0.14 mg/L for Phe

160 (Renoux et al., 1999; Parvez et al., 2008). The toxicity reductions were in agreement with

161 reductions in chemical concentration (biotransformation).

162

163 *3.2 Biodegradation and toxicity of LE-WAFs*

164   The LE-WAF contained 0.3 mg/L SVOC ($\sum$ decalines, naphthalenes, 2- to 6-ring

165 PAH and C0- to C5-alkylated phenols). Biotransformation and mineralization half-lives of 11

166 and 33 days, respectively (Fig. 1; Table S2), resulted in a mineralization:biotransformation

167 ratio of 3.0. Biotransformation of LE-WAF groups showed rapid naphthalene transformation,

168 while PAH was first biotransformed after a lag-period of at least 10 days.

169 Phenols/alkylphenols showed a rapid increase in WAFs after 10-14 days, with 4.5 times

170 higher concentrations than in the sterilized controls, followed by a rapid decline after days 21

171 and 28 (Fig. S2). These increased phenol concentrations are assumed to be the result of

172 degradation products from other aromatic compounds like naphthalenes and phenanthrenes

173 (Haritash and Kaushik, 2009). However, also degradation of monoaromatic hydrocarbons

174 (BTEX; benzene, toluene, ethylbenzene, xylenes) may have contributed to the phenol

175 concentrations (Brakstad and Faksness, 2000), although not measured here. In a separate

176 experiment, using the same setup for preparation and degradation of LE-WAFs, BTEX

8

177 concentrations at T0 was 487 µg/L decreasing rapidly to 138 (day 10), 44.1 (day 14), 0.14

178 (day 21) and 0.05 (day 28) (Hansen et al., 2018).

179   The initial Microtox EC_{50} of 25 % for the LE-WAF was maintained for 14 days,

180 declined after 21 days, and was out of range (EC_{50}>100% WAF) after 28 days (Table 1). We

181 were able to estimate EC_{10}-up until day 42, however, for the last two time points (days 28 and

182 42), the confidence intervals were very wide. The toxicity was associated with the combined

183 effect of naphthalenes and phenol/alkylphenols and was reduced with the decline of

184 phenol/alkylphenol concentrations after 21 days (Fig. S2). As mentioned above, also BTEX

185 may have contributed to the phenol accumulation and toxicity (Brakstad and Faksness, 2000).

186 Also in the LE-WAFs, the acute toxicity was associated with depletion measured by the

187 chemical analyses (biotransformation) rather than with the oxygen consumption

188 (mineralization).

189   The results from these studies show slower biotransformation and longer persistence

190 of acute toxicity expressed by Microtox EC_{50}s than a previous biodegradation study of LE-

191 WAFs (DOR 1:10000) in natural SW at 13°C, which resulted in naphthalene and PAH

192 biotransformation half-lives of 1-2 days and a removal of Microtox toxicity after 7 days of

193 incubation (Brakstad and Faksness, 2000).

194   The Microtox assay measures the toxicity of truly soluble compounds, and if

195 compounds are attached to particles in the SW, or to the glass walls of flasks used for the

196 biodegradation experiment, these may be unavailable for the bacteria in the Microtox assay.

197 However, the TOC concentrations in the SW was low (2 mg/L), while sterilized controls of

198 single compounds and LE-WAFs showed negligible depletion of soluble compounds,

199 demonstrating negligible glass wall attachment (Fig. S1).

200

201 **4.   Conclusions**

202   This biodegradation study of 1,3-dimethylnaphthalene, phenanthrene and and LE-

203   WAF of a crude oil in cold SW, showed that the acute toxicities of single compounds,

204   determined by a Microtox screening bioassay, were mainly associated with the

205   biotransformation of the compounds, rather than the slower mineralization process, which

206   involves the complete mineralization of the original compounds and its degradation products.

207   In the LE-WAF, phenol/alkylphenols as degradation products, may have contribute to the

208   toxicity, together with the measured aromatic HCs and BTEX. The cold SW used in these

209   studies resulted in slower biodegradation and longer periods of acute toxicity compared to

210   previous studies with higher SW temperatures. SW temperature may therefore be expected to

211   affect the time window of toxicity after an oil spill, for instance in the Arctic. However, these

212   results should be confirmed through studies with Arctic SW and relevant species for toxicity

213   testing.

214

Acknowledgements

219

References

221   Bagi, A., Pampanin, D.M., Brakstad, O.G., Kommedal, R., 2013. Estimation of hydrocarbon
222   biodegradation rates in marine environments: A critical review of the Q10 approach. Mar.
223   Environ. Res. 89, 83-90.

224   Belkin, S., Stieber, M., Tiehm, A., Frimmel, F.H., Abeliovich, A., Werner, P., Ulitzur, S.,
225   1994. Toxicity and genotoxicity enhancement during polycyclic aromatic hydrocarbons'
226   biodegradation. Environ. Toxicol. Water Qual. 9, 303-309.

227 Brakstad, O., Faksness, L.-G., 2000. Biodegradation of water-accommodated fractions and
228 dispersed oil in the seawater column. SPE International Conference on Health, Safety and
229 Environment in Oil and Gas Exploration and Production. Society of Petroleum Engineers,
230 Paper 61466.

231 Brakstad, O.G., Almås, I.K., Krause, D.F., 2017. Biotransformation of natural gas and oil
232 compounds associated with marine oil discharges. Chemosphere 182, 555-558.

233 Brakstad, O.G., Nordtug, T., Throne-Holst, M., 2015. Biodegradation of dispersed Macondo
234 oil in seawater at low temperature and different oil droplet sizes. Mar. Pollut. Bull. 93, 144-
235 152.

236 Daling, P.S., Brandvik, P.J., Mackay, D., Johansen, O., 1990. Characterization of crude oils
237 for environmental purposes. Oil and Chem. Pollut. 7, 199-224.

238 Faksness, L.-G., Brandvik, P.J., Sydnes, L.K., 2008. Composition of the water accommodated
239 fractions as a function of exposure times and temperatures. Mar. Pollut. Bull. 56, 1746-1754.

240 Fernando Bautista, L., Sanz, R., Carmen Molina, M., González, N., Sánchez, D., 2009. Effect
241 of different non-ionic surfactants on the biodegradation of PAHs by diverse aerobic bacteria.
242 Int. Biodeter. Biodeg. 63, 913-922.

243 French-McCay, D.P., 2002. Development and application of an oil toxicity and exposure
244 model, OilToxEx. Environ. Toxicol. Chem. 21, 2080-2094.

245 Gibbs, C.F., Davis, S.J., 1976. The rate of microbial degradation of oil in a beach gravel
246 column. Microb. Ecol. 3, 55-64.

247 Hansen, B. H., Farkas, J., Nordtug, T., Altin, D., Brakstad, O. G., 2018. Does microbial
248 biodegradation of water-soluble components of oil reduce the toxicity to early life stages of
249 fish? Env. Sci. Technol. DOI: 10.1021/acs.est.7b06408.

250 Harayama, S., Kishira, H., Kasai, Y., Shutsubo, K., 1999. Petroleum biodegradation in marine
251 environments. J. Mol. Microbiol.Biotechnol. 1, 63-70.

252 Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of Polycyclic Aromatic
253 Hydrocarbons (PAHs): A review. J. Hazard. Mater. 169, 1-15.

254 Hokstad, J.N., Daling, P.S., Buffagni, M., Johnsen, S., 1999. Chemical and Ecotoxicological
255 Characterisation of Oil–Water Systems. Spill Sci. Technol. Bull. 5, 75-80.

256 Juhasz, A.L., Stanley, G.A., Britz, M.L., 2000. Degradation of High Molecular Weight PAHs
257 in Contaminated Soil by a Bacterial Consortium: Effects on Microtox and Mutagenicity
258 Bioassays. Biorem. J. 4, 271-283.

259 Lofthus, S., Almås, I.K., Evans, P., Pelz, O., Brakstad, O.G., 2016. Biotransformation of
260 potentially persistent alkylphenols in natural seawater. Chemosphere 156, 191-194.

261 OECD, 1992. Biodegradability in seawater. OECD Guidelines for the Testing of Chemicals,
262 Section 3.

263 Pagnout, C., Rast, C., Veber, A.-M., Poupin, P., Férard, J.-F., 2006. Ecotoxicological
264 assessment of PAHs and their dead-end metabolites after degradation by Mycobacterium sp.
265 strain SNP11. Ecotoxicol. Environ. Saf. 65, 151-158.

266 Parvez, S., Venkataraman, C., Mukherji, S., 2008. Toxicity assessment of organic pollutants:
267 Reliability of bioluminescence inhibition assay and univariate QSAR models using freshly
268 prepared Vibrio fischeri. Toxicol. In Vitro 22, 1806-1813.

269 Renoux, A.Y., Millette, D., Tyagi, R.D., Samson, R., 1999. Detoxification of fluorene,
270 phenanthrene, carbazole and p-cresol in columns of aquifer sand as studied by the Microtox®
271 assay. Water Res. 33, 2045-2052.

272 Ruberto, L.A.M., Vazquez, S.C., Curtosi, A., Mestre, M.C., Pelletier, E., Mac Cormack, W.P.,
273 2006. Phenanthrene Biodegradation in Soils Using an Antarctic Bacterial Consortium.
274 Biorem. J. 10, 191-201.

275 Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., Tjeerdema,
276 R.S., 2000. Standardization of the Preparation and Quantitation of Water-accommodated
277 Fractions of Petroleum for Toxicity Testing. Mar. Pollut. Bull. 40, 1007-1016.

278 Tiehm, A., Stieber, M., Werner, P., Frimmel, F.H., 1997. Surfactant-Enhanced Mobilization
279 and Biodegradation of Polycyclic Aromatic Hydrocarbons in Manufactured Gas Plant Soil.
280 Environ. Sci. Technol. 31, 2570-2576.

281 Van Hamme, J.D., Singh, A., Ward, O.P., 2003. Recent advances in petroleum microbiology.
282 Microbiol. Mol. Biol. Rev. 67, 503-549.

283 Wang, X., Yu, X., Bartha, R., 1990. Effect of bioremediation on polycyclic aromatic
284 hydrocarbon residues in soil. Environ. Sci. Technol. 24, 1086-1089.

285 Yakimov, M.M., Gentile, G., Bruni, V., Cappello, S., D'Auria, G., Golyshin, P.N., Giuliano,
286 L., 2004. Crude oil-induced structural shift of coastal bacterial communities of rod bay (Terra
287 Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted
288 hydrocarbonoclastic bacteria. FEMS Microbiol. Ecol. 49, 419-432.

289 Yakimov, M.M., Giuliano, L., Gentile, G., Crisafi, E., Chernikova, T.N., Abraham, W.-R.,
290 Lünsdorf, H., Timmis, K.N., Golyshin, P.N., 2003. Oleispira antarctica gen. nov., sp. nov., a
291 novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. Int. J.
292 System. Evolut. Microbiol. 53, 779-785.

Tables and Figures

Table 1. Microtox EC_{10} concentrations (% of undiluted solutions), concentrations of the single HCs 1,3-dimethylnaphthalene (1,3-DMN), phenanthrene (Phe), fluoranthene (Fluor) and a LE-WAF. The concentrations of dissolved single HCs in SW (µg/L) are shown, and toxicity related to concentrations (EC_{10} in µg/L) during the biodegradation period. For LE-WAF, EC_{10} and EC_{50}-values are given as % of undiluted solutions. SW controls (adsorbents with DCM) showed $EC_{10} > 100$ %. Results are shown with 95% confidence intervals (C.I.).

|	1,3-DMN			Phe			Fluor			LE-WAFs	
Inc. (days)	EC_{10} (95% C.I.) (%)	Conc. (µg/L)	EC_{10} (µg/L)[A]	EC_{10} (95% C.I.) (%)	Conc. (µg/L)	EC_{10} (µg/L)[A]	EC_{10} (95% C.I.) (%)	Conc. (µg/L)	EC_{10} (µg/L)[A]	EC_{10} (95% C.I.) (%)	EC_{50} (95% C.I.) (%)
0	28,50 (21,81-37,24)	115 ± 40	32,78	13,24 (11,27-15,55)	296 ± 107	39,19	> 100	<0.01	ND	3,10 (2,40-4,00)	24,79 (22,57-27,23)
10	10,56 (6,821-16,34)	201 ± 82	21,23	3,85 (2,912-5,090)	381 ± 70	14,67	> 100	<0.01	ND	2,32 (1,73-3,11)	21,94 (20,33-23,67)
14	13,00 (10,26-16,47)	185 ± 6	24,05	8,65 (3,375-22,14)	332 ± 66	28,7	> 100	<0.01	ND	1,64 (1,16-2,33)	23,84 (21,17-26,85)
21	>100	45 ± 31	ND	89,07 (43,28-183,3)	155 ± 25	138,06	> 100	<0.01	ND	19,79 (14,62-26,78)	>100
28	>100	8.0 ± 12	ND	>100	60 ± 76	ND	> 100	<0.01	ND	10,00 (3,37-29,64)	>100
42	>100	<0.01	ND	>100	<0.01	ND	> 100	<0.01	ND	29,94 (14,73-60,89)	>100
63	>100	<0.01	ND	>100	<0.01	ND	> 100	<0.01	ND	>100	>100

[A] nd, not detected
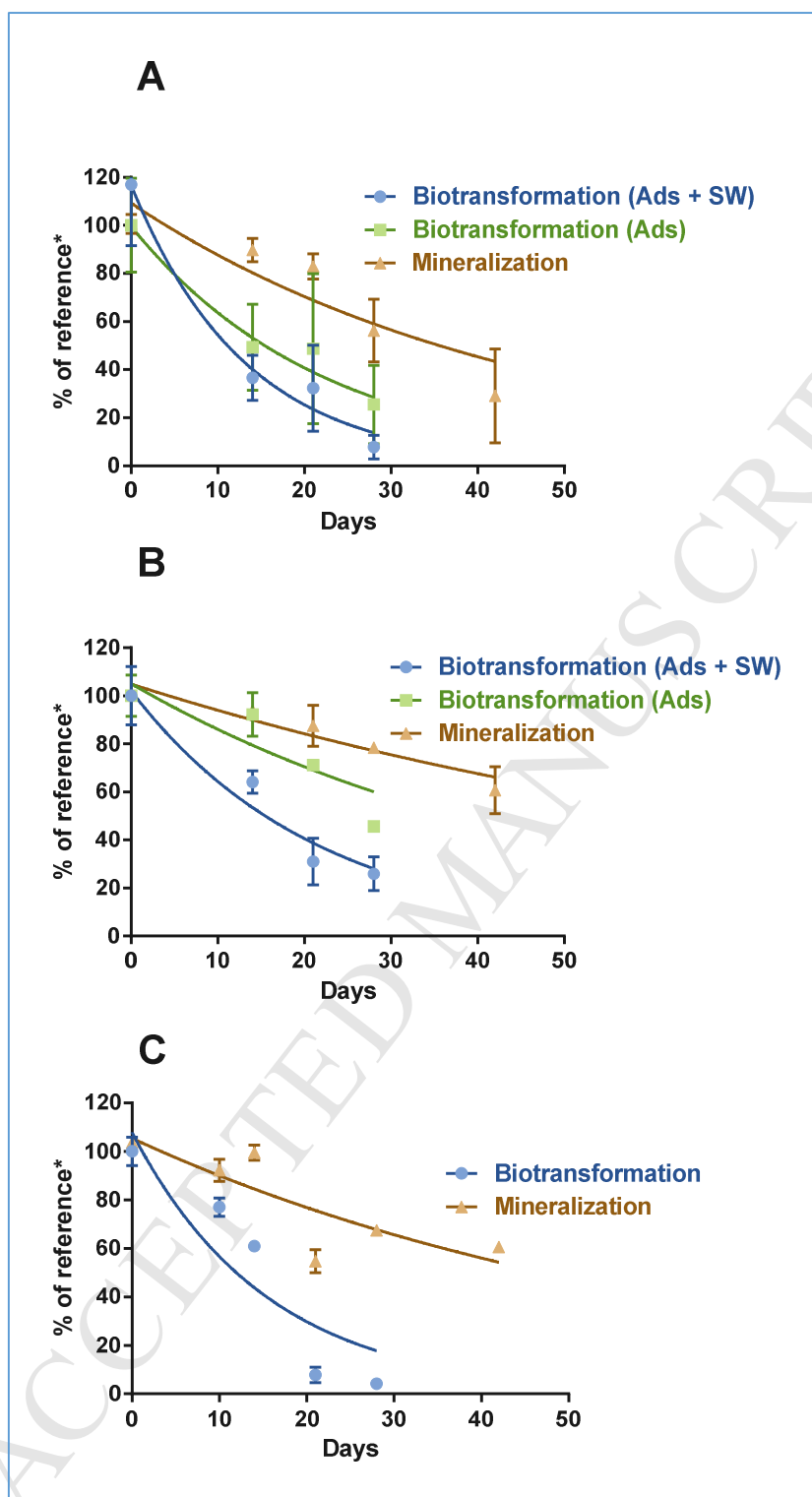
Fig. 1. Biotransformation and mineralization of 1,3-dimethylnaphthalene (A), phenanthrene (B) and LE-WAFs (C), shown as first-order rates. Calculations of % biotransformation and mineralization are described in Materials and Methods. The reference used for calculation is sterilized controls (biotransformation) or ThOD (mineralization). In samples with 1,3-dimethylnaphthalene and phenanthrene, biotransformation rates are shown for the sum of the HCs on the adsorbents and in the SW (Ads + SW) or attached to the adsorbents (Ads).

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