A preliminary study on fate of oil in marine sediments inhabited by the polychaete *Nereis diversicolor*

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Abstract

In this study the fate of oil in marine sediment inhabited by the polychaete *Nereis diversicolor* is investigated. Data from two preliminary biodegradation experiments have been analysed using univariate plots as well as multivariate data analysis (Principal Component Analysis). Results show that when *Nereis diversicolor* is not present in the sediment the degradation of oil is very slow and weathering processes, e.g. evaporation and dissolution, are the only processes changing the composition of oil. When *Nereis diversicolor* is present in the sediment marked changes in the oil composition occur as indicated by changes in several biodegradation indices. The results indicate that multivariate data analysis is potentially a strong tool in the evaluation of changes in oil composition due to weathering and biodegradation processes, and that the isomeric patterns of C1-Fluorenes could be applied as indices for infaunal degradation. The present work is considered a pilot study indicating the need for further investigations of interactions between bioturbating infaunal organisms and persistent sediment pollutants.

1 Introduction

Pollution of the marine and terrestrial environment due to oil spills occurs frequently, and major spills may constitute great environmental risks. The risk is associated with the toxicity and persistency in the environment of fractions of the
spilled oil. Oil released into the environment is subject to biodegradation and a range of weathering processes that removes the oil and changes its composition [1-2]. When studying biodegradation and weathering it is important to be able to distinguish between these two kinds of loss, so that loss due to weathering is not interpreted as loss due to biodegradation and vice versa, neither in the laboratory nor in field studies [2].

Numerous studies have investigated the fate of petroleum hydrocarbons in marine sediments, especially the effects of biodegradation [1,3], and various bacterial strains have been found to be capable of degrading petroleum related compounds under different experimental conditions [4]. Especially PAHs (polycyclic aromatic hydrocarbons) have received much attention in biodegradation studies due to their observed toxicity and persistency in the environment [4].

Biodegradation processes in aquatic and terrestrial ecosystems are complex, and strongly influenced by a variety of factors. In this study, two preliminary experiments were performed using homogeneous oil/sediment mixtures. The study focuses on the effect of bioturbating macroinfauna on the degradation of petroleum hydrocarbons in sediment.

Bioturbation plays an important role in organic matter decomposition by increasing the water/sediment interface and by irrigating the sediment. The tube-borrowing polychaete Nereis diversicolor is found at very high densities (≈ 4000 individuals/m²) mainly in organic-rich estuarine ecosystems along the European coast. Most burrow constructors maintain contact with the overlying water by ventilating water through their burrow systems and thus increase the transport of ions and gases, e.g. oxygen, over the water/sediment interface [5 and references herein]. In the same way, burrowing activity may also alter the concentrations of pollutants in the sediment as deposited pollutants become buried and/or released. This may either result in long-lasting negative effects on the marine ecosystem or in an increased removal of pollutants by biodegradation, as they become bioavailable by dissolution or by increasing the oxygenated part of the sediment. Nereis diversicolor is therefore important in relation to potential biodegradation of oil spilled in eustarine ecosystems. Gilbert et al (1994) observed in a laboratory experiment that the building of burrows by Nereis diversicolor was inhibited by the presence of crude oil. On the other hand, the presence of Nereis diversicolor also removed oil by increasing the release of petroleum hydrocarbons by physical processes as well as by microbial degradation of linear alkanes in the burrows [5].

Experimental data from the two experiments are analysed by univariate plots as well as multivariate techniques, e.g. Principal Component Analysis (PCA). Multivariate data analyses have previously been applied in a study of the early fate of oil in the marine environment following the major (2700 tonnes) MS Baltic Carrier oil spill in Denmark, 2001 [6].
2 Experimental

2.1 Artificial weathering of oil

A North Sea light crude oil, supplied by Statoil, Denmark, was used for the biodegradation experiments. Prior to the experiments, the crude oil was weathered by evaporation in a glass tube by passing air through 50 ml of oil for 72 hours. The evaporative mass loss for the North Sea crude was 35%.

2.2 Biodegradation experiment A

6.0 g of weathered North Sea crude was mixed with 3 kg of wet sediment from Roskilde Fjord, Denmark (< 4 mm grain size) in a mechanical mixer (Workmatic, Malavasi Robots Da Cucina) resulting in an oil/sediment mixture with a nominal oil concentration of 2000 ppm.

12 PVC buckets with internal diameters of 7 cm were filled with 100 g of the homogenised oil-contaminated sediment. 100 ml of seawater (Roskilde Fjord, Denmark) with a salinity of 15% was then cautiously poured on top of the sediment. The water was thoroughly aerated during the experiment using an air pump and air stones. The experimental set-up is summarised in Table 1.

Table 1: Summary of experimental setup (Biodegradation experiment A).

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>0 days</th>
<th>14 days</th>
<th>40 days</th>
<th>64 Days</th>
<th>124 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Overlying water</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>- Overlying water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

After 40 days the overlying water was removed in three of the buckets leaving the sediment to dry out and creating approximately 100% oxic conditions. 10 ml of water was poured onto the dried sediment once every 14 days to re-establish moisturous conditions.

2.3 Biodegradation experiment B

Weathered North Sea crude was mixed with wet sediment from Roskilde Fjord, Denmark (< 1 mm. grain size, pre-frozen). 13.0 g of oil was mixed carefully with 1 kg of sediment, then another 64 kg were slowly added over 2 hours during mixing in a concrete mixer. Mixing was continued for another hour resulting in an oil/sediment mixture with a nominal oil concentration of 200 ppm.

12 PVC buckets with internal diameters of 14 cm were filled with 1,5 kg of the homogenised oil-contaminated sediment. The sediment was cleaned at the top with 2-3 cm of seawater (Roskilde Fjord, Denmark) three times to remove sulphides and other toxic compounds. 800 ml of seawater was then cautiously poured on top of the sediment surface, and the water was aerated for 2 hours. The experimental set-up is summarised in Table 2.
Table 2: Summary of the experimental set-up (Biodegradation experiment Bi)

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>0 days</th>
<th>14 days</th>
<th>30 days</th>
<th>60 days</th>
<th>132 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nereis diversicolor</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nereis diversicolor</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1/1</td>
<td>0</td>
</tr>
</tbody>
</table>

Eight individuals of *Nereis diversicolor*, between 0.5 – 4.0 g, wet weight were supplied to half of the cores. If the animals did not burrow within 15 min. they were removed and replaced with fresh ones. The individuals were categorised into five different weight classes and divided equally between buckets. The total weight of the eight *Nereis diversicolor* in each bucket was approximately the same.

The water was thoroughly aerated through the entire incubation period using an air pump and air stones. After the experimental cores had been set up they were checked regularly during the incubation period. When an individual was found dead a new one of approximately the same weight replaced it. Water was changed four times during the period of 132 days, and the number of individuals were estimated every month by counting holes and by feeding individuals with crushed fish food. These counts were considered estimates of activity and the number of individuals alive in the core, respectively. New individuals were supplied to the cores, if necessary, so that each core contained a total of eight active individuals. After 0, 14, 30, 60, and 132 days 1-5 sediment samples were extracted and analysed.

### 2.4 Chemical analysis

Sediment extractions were performed using a microwave-assisted extraction procedure [7]. Briefly, 5-10 g of wet sediment was weighed into microwave extraction vessels (Teflon). Then 30 ml solvent (1:1 hexane:acetone mixture) spiked with squalane (internal standard), in appropriate amounts, was added and the sample was extracted for 30 min. (at 160 psi) in an MES-1000 microwave solvent extraction system (CEM Corp., Matthews, NC). After the extraction, water was removed using Na₂SO₄ (water free) and the extracts were concentrated by evaporation and transferred to 10-ml vials. Extracts were analysed by high resolution GC/MS in Selected Ion Recording mode (SIR) using a HP 5890 chromatography (Hewlett-Packard) combined with a VG 70-250S high-resolution (VG Analytical Ltd., Manchester, UK) mass spectrometer (double-focusing) equipped with a Restek Rtx – 5SIL MS w/Integra Guard (30m × 0.25mm, 0.25 μm film thickness) capillary column and an on-column injector.

The following m/z ions were measured and applied in the univariate and/or multivariate data-analysis: \( n + 1 \)-branched alkanes (m/z 113.1), Alkyltoluenes (m/z 106.1), C1-2-Naphthalenes (m/z 141.1), C3-Naphthalenes (m/z 170.1), C1-2-Fluorenes (m/z 179.1), Biphenyl (m/z 154.1), Dibenzothiophene (m/z 184.0), and Tri/Pentacyclic triterpanes (m/z 191.2).
2.5 Data analysis

GC/MS fragmentograms were pre-processed using a pre-processing procedure developed by the author, calculating peak areas and diagnostic ratios automatically [7]. A limited number of diagnostic weathering and biodegradation ratios were then analysed by univariate plots and multivariate data analyses, i.e. Principal Component Analyses (PCA).

In the data analysis compound names are abbreviated as follows: Dibenzothiophene (DBT), (Squalane Sq(IS), C_{30}-17\alpha(H),21\beta(H)-hopane (Hop), Pristane (Pr), Phytane (Ph), methyl-fluorene (MF), 2-methyl-fluorene (2MF), 1-methyl-fluorene (1MF), sum of the three C1-Fluorene isomers (sumF)), Biphenyl (B), Ethyl-naphthalenes (EN), meta-Alkyltoluenes – alkylchain with 7-15 carbons (mAT), ortho-Alkyltoluenes – alkylchain with 7-15 carbons (oAT), and n-alkanes with x carbons (nCx).

Principal Component Analysis (PLS-Toolbox 2.1 Eigenvector Research Inc. implemented in Matlab 5.3) was conducted on data from the biodegradation experiments. PCA is a factor analysis method that generates new independent variables that are linear combinations of the original input variables (e.g. diagnostic ratios). This method reduces the dimensionality of the data to a few important “principal components” (axes) that best describe variations in the data. The first axis (PC1) demonstrates the most prominent trend and successive axes (PC2, PC3 etc.) demonstrate additional trends in decreasing order of importance. The projection of samples onto the principal components give their scores and is defined as the co-ordinates in the new system of co-ordinates spanned by the PCs. The loadings describe the cohesion between the variables and the PCs, and are defined as cosine to the angles between the variables and the respective PC.

3 Results and discussion

3.1 Univariate data analysis

Changes in nC17/pristane and nC18/phytane ratios have long been recognised and used as indicators of biodegradation [2,8-9]. During the course of experiment A, the decrease in nC17/pristane and nC18/phytane (data not shown) is not significant.

Figure 1 show the values of nC17/pristane and nC18/phytane from day 0-132 in biodegradation experiment B. In samples not containing individuals of *Nereis diversicolor* no changes in the ratios are observed, except after 132 days where a decrease in the ratio of nC17/pristane was seen.

The decrease after 132 days is probably due to an artefact, as the ratio of nC18/phytane did not decrease to the same extent. Even though no statistical validation can be made, the decrease in both nC17/pristane and nC18/phytane ratios in samples containing *Nereis diversicolor* are indisputable. Especially in samples from the oxygenated part of the sediment inside borrows of *Nereis diversicolor* (60 days NDB), a decrease to 87% and 75%, respectively, of the values at day 0 is observed.
Figure 1: Values of nC17/pristane (left axis) and nC18/phytane (right axis) ratios during biodegradation experiment A. ND indicate that the sediment contained *Nereis diversicolor*, whereas NDB indicate that sediment from borrows of *Nereis diversicolor* was analysed. Bars denote ± 1 standard deviation. When bars are not present (0-132 days), no duplicate samples were analysed. Arrows indicate samples containing *Nereis diversicolor*.

These results indicate that the presence of *Nereis diversicolor* increases the rate of degradation. Another interesting feature, observed in Figure 1, is that nC18/phytane apparently is a more sensitive biodegradation index than nC17/pristane.

### 3.2 Multivariate data analysis

In biodegradation experiment A, a total of 12 samples (0, 40, 64, and 124 days of incubation) were investigated by PCA. Three duplicate samples were analysed after 124 days of incubation using two different treatments; one duplicate sample (17a/b) was dried out during the last 84 days of incubation. 10 diagnostic ratios (weathering and biodegradation) were applied in the analysis; sample data were mean-centred and variables scaled prior to analysis (variables were not mean-centred). The two first principal components (PCs) describe 85.8% of the variation in the 10 original variables (67.9 and 17.9%, respectively). The score and loading plots of PC1 versus PC2 are shown in Figure 2a and 2b, respectively. In the score plot (Figure 2a), samples from different incubation times and treatments are distributed in a distinct pattern.
Figure 2: a) Score plot of data from biodegradation experiment A. Solid circles denote groupings related to the time of incubation, whereas punctured circles indicate double determinations of three samples incubated for 124 days. b) Loading plot of data from biodegradation experiment A. Circles indicate groupings of highly correlated diagnostic ratios.
PC 1 describes the time of incubation, and samples with low incubation time are observed at high values of PC 1. PC 2, to some extent, describe the different treatments that the three duplicate samples (14a/b, 15a/b, and 17a/b) have been exposed to. These samples are located at similar PC 1 values, but at different PC 2 values.

The loading plot (Figure 2b) shows some interesting features. The ratios DBT/nCx and DBT/(IS or Hop) have high PC 1 loadings, whereas the biodegradation index nC18/Ph and the ratio C3N/nC16-17 have high PC 2 loadings. The ratio nC18/Hop describes evaporation, but it has also been applied as a biodegradation index in the literature [10].

Samples located at high values of PC1 have, generally, higher values of the weathering ratios DBT/(Sq or Hop) and DBT/nCx. Dibenzothiophene has a high water solubility (1.47 mg/L) compared to n-alkanes with 19-35 carbons (ca. 0.001 mg/L) and C30-17α(H),21β(H)-hopane. These ratios are therefore good indicators of dissolution. During the entire incubation period, physical processes, mainly evaporation and dissolution, affects the oil composition. This is seen as a marked decrease of Dibenzothiophene and n-alkanes compared to the internal standard Squalane and C30-17α(H),21β(H)-hopane, a highly conserved endogenous marker. Furthermore, Dibenzothiophene decreases faster than n-alkanes (nC19-35) indicating that dissolution is an important process changing the oil composition during the incubation period.

At high values of PC2 samples have low values of nC18/Ph and C3N/nC16-17 compared to an average sample (close to 0 on PC2 axis) indicating a high degree of biodegradation. The changes in oil composition observed between day 64-124 depend on the treatment of samples. In the dried sample, ratios of nC18/Ph decrease indicating some minor degree of biodegradation. The sample 15a/15b is located at low PC2 values indicating an increased evaporation (decrease in the ratio nC18/Hop.

Evaluated on basis of this limited dataset, the ratio nC18/Hop cannot be recommended as a biodegradation index since its effect is opposite compared to the ratio nC18/Ph.

In biodegradation experiment B, 10 samples (0, 14, and 60 days of incubation) are investigated by PCA. 9 diagnostic ratios (biodegradation) were applied in the analysis. Data were centred and variables scaled to variance prior to the analysis. The first two principal components describe 71.1% of the variation in the 9 original variables (53.8 and 17.3%, respectively). The score and loading plots of PC1 versus PC2 are shown in Figure 3a and 3b.

The score plot (Figure 3a) shows that samples from 60 days of incubation are located at much higher PC1 values compared to samples from day 0-14. Furthermore, samples from day 14 (both with and without Nereis diversicolor) are located at somewhat lower PC2 values compared to samples from day 0. By comparing the observations made in the score plot with the loading plot (Figure 3b) it is observed that several diagnostic ratios are highly correlated (encircled in Figure 3b). These ratios are located at low PC1 values, and samples from days 0-14 have high values of these ratios compared to samples from day 60, indicating enhanced biodegradation in samples from day 60 with Nereis diversicolor.
Figure 3: a) Score plot of data from biodegradation experiment B. Circles denote groupings related to incubation time. ND indicates samples containing *Nereis diversicolor*, and NDB denote that the sample is an extract of sediment collected from the oxygenated zone inside burrows. b) Loading plot of data from biodegradation experiment B. The circle indicate a group of highly correlated biodegradation ratios.
Furthermore, samples from day 60 have high isomer ratios (relative to the sum of C1-fluorenes) of 2MF and MF compared to 1MF indicating that the isomeric pattern of C1-fluorenes distinguish samples from day 60 containing *Nereis diversicolor* from samples that have been incubated in 0-14 days. This varies from the observation made by Wang et al (1998) using a standard freshwater inoculum in a laboratory experiment. They found that MF degraded prior to the isomers 1MF and 2MF [2]. A possible explanation could be that *Nereis diversicolor* has the capability of degrading PAHs in a way that differs from microbial degradation.

PC2, however, describe much less of the variation in the data (17.3%). The loading plot (Figure 3b) shows that the ratios B/EN and mAT/oAT are responsible for the minor variation in composition between samples from day 0 and 14, respectively. The multivariate analysis shows that samples from day 60 containing *Nereis diversicolor* have been affected by biodegradation processes. This conclusion is supported not only by a few diagnostic ratios as in the univariate analysis, but by several diagnostic ratios simultaneously, making the conclusion stronger.

### 4 Summary

The results of the two biodegradation experiments indicates that, except for samples that was dried out 84 days prior to analysis, no significant degradation occurred in biodegradation experiment A during 124 days of incubation of surface and subsurface sediments. The dried-out samples showed some degree of degradation observed as a decrease in the ratios of nC18/phytane. Indicators of weathering, especially dissolution was, however, observed during the multivariate analysis of data from experiment A.

Biodegradation experiment B provided limited results, but the tendency here was that no degradation could be observed in samples without *Nereis diversicolor*, and that evidence of biodegradation of petroleum hydrocarbons was observed in sediment samples containing *Nereis diversicolor*. Both the univariate and the multivariate data analysis showed that biodegradation has occurred; especially oil extracted from oxygenated sediment lining borrows of *Nereis diversicolor* showed enhanced biodegradation. The values of nC17/pristane and nC18/phytane decreased to 87% and 75%, respectively, of the values observed at day 0. Furthermore, the multivariate data analysis gave a distinct grouping of the two samples from day 60 with *Nereis diversicolor* present, indicating enhanced biodegradation. This conclusion was supported not only by a few diagnostic ratios as in the univariate analysis, but by several diagnostic ratios simultaneously, making this conclusion strong. Three possible explanations for the increased degradation observed in the presence of *Nereis diversicolor* may be suggested:

- *Nereis diversicolor* has the ability to degrade petroleum hydrocarbons (presence of P450-enzymes).
- The bacterial community is stimulated by the presence of mucus inside borrows of *Nereis diversicolor*. 
The bioturbation by *Nereis diversicolor* actively enhances the oxygenated part of the sediment, thereby increasing the microbial degradation. In this study, the isomeric pattern of C1-Fluorenes has turned out to be an effective indicator of biodegradation since 1MF was more susceptible towards biodegradation than MF and 2MF. This is different from the observation made by Wang *et al.* (1998) where MF was degraded faster than the isomers 1MF and 2MF [2]. A possible explanation could be that *Nereis diversicolor* has the capability of degrading PAHs, in a way that differs from microbial degradation.

5 References


[6] Christensen, J. H. Early Fate of Petrogenic Compounds in the Marine Environment Following the Baltic Carrier Oil Spill. *Polycyclic Aromatic Compounds* (Accepted for print in 22(2)).

