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IDENTIFYING SOURCE CORRELATION PARAMETERS FOR HYDROCARBON WASTES USING COMPOUND-SPECIFIC ISOTOPE ANALYSIS

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Capsule

Compound-specific isotope analysis (CSIA) is a promising method for identifying source correlation compounds in soils contaminated with heavy or weathered petroleum wastes.

Abstract

A preliminary evaluation of compound-specific isotope analysis (CSIA) as a novel, alternative method for identifying source correlation compounds in soils contaminated with residual heavy or weathered petroleum wastes is presented. Oil-contaminated soil microcosms were established using soil (sandy-loam, non-carbonaceous gley) amended with ballast-, crude- or N$^{o.}$ 6 fuel oil. Microcosms were periodically sampled over 256 days and $\delta^{13}C$ values (which express the ratio of ^{13}C to ^{12}C) determined at each time point for five *n*-alkanes and the isoprenoid norpristane using gas chromatography-isotope ratio mass spectrometry (GC-IRMS). Although some temporal variation was observed, no significant temporal shifts in the $\delta^{13}C$ values for the five *n*-alkanes

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were measured in all three oils. Isoprenoid isotope ratios ($\delta^{13}C$) appeared to be least affected by biotransformation, especially in the N$^{o.}$ 6 fuel oil. The research suggests that the $\delta^{13}C$ of isoprenoids such as norpristane, may be of use as source correlation parameters.

Keywords: stable isotopes, heavy oils, environmental diagnostics, contaminated land.

INTRODUCTION

At sites featuring contamination of the soil environment by heavy oils or residual petroleum wastes, the vast complexity of the waste-soil-groundwater matrix and the hydrophobicity, inaccessibility and recalcitrance of individual waste components are such that resolution of these issues is fraught with uncertainties (Potter & Simmons, 1998; Pollard *et al*., 2004). Particular challenges that such sites present may be categorised into two broad themes; (i) analytical challenges associated with the chemical characterisation of these wastes (Pollard *et al*, 1994; Whittaker *et al*, 1996), and (ii) challenges associated with the assessment of biotransformation of heavy oil contaminants and their potential sources, particularly following prolonged weathering (Whittaker, 1996).

Fingerprinting of petroleum products in the environment is commonly undertaken to assess the source of a particular contamination event. It is commonly achieved through a qualitative comparison of mass chromatograms, but can also be achieved by quantifying the ratio of one biomarker to another. Fingerprinting is important for differentiating between potential contaminant sources and for establishing potential liability for a spillage; for example when contaminants migrate across boundaries of ownership (Douglas *et al*., 1992; Douglas & Uhler, 1993).

Ratios of certain biomarkers, referred to as source correlation indices, are sensitive to the geological source of an oil, but remain consistent between related oils. Moreover, because of the environmental persistence of biomarker compounds, their values remain unaltered by oil weathering and biotransformation processes. The most commonly used source correlation indices are [pristane:phytane] and [17α(H),21β(H)-hopane:17α(H),21β(H)-norhopane] (Whittaker *et al*., 1999). For heavy or residual petroleum contaminants, diagnostic fingerprinting creates uncertainties, especially because of the high degree of weathering experienced by the contaminants. Instead, diagnostic parameters that compare relative abundance of biomarker isomers and polyaromatic hydrocarbons (PAHs) in crude oils have been shown to be reliable source indices for marine spills (Douglas *et al*., 1992; Wang *et al*., 1994). However, there is little knowledge of the effectiveness of these indices for heavy oils in the contaminated soil, as opposed to the marine, environment.

Our own work, on the characterisation of heavy oils, has focussed on improved analytical strategies and methods (Pollard *et al.,* 1994; Whittaker *et al.,* 1996; 1999; Pollard *et al.*, 2004) for these problematic environmental matrices. Screening whole reference oils by stable carbon isotope fingerprinting without GC separation (Whittaker *et al.,* 1996) demonstrated the utility of the technique for source term characterisation and highlighted the need for compound-specific studies. The advent of compound-specific isotope analysis (CSIA) for assessing the isotopic signature of carbon in crude oils and crude oil fractions by gas chromatography-coupled isotope ratio mass spectroscopy (GC-IRMS) was first developed by Barrie *et al.* (1984). Since then, further technical examination and development of the method in crude oils and crude oil fractions has been undertaken (Reiley *et al*., 1991; Eakin *et al*., 1992; Merrit *et al*., 1994; Sessions *et al*., 2001). These refinements have elevated the characterisation of petroleum hydrocarbons to a level of sensitivity and detail unobtainable by conventional IRMS. CSIA yields data of the

isotopic composition of a single compound relative to an international standard that are usually expressed as δ values in units of parts per thousand (‰). Because of this, CSIA is able to quantify isotopic composition and provide an additional (and often unique) means to (i) allocate and distinguish sources of organic compounds, and (ii) identify and quantify transformation reactions (Schmidt *et al*., 2004). CSIA is a continuous flow technique, which utilises the linking of a separation method (until now solely gas chromatography) via an on-line combustion/pyrolysis unit with a multicollector mass spectrometer. A review of CSIA principles and technical aspects has been published by Meier-Augenstein (1999).

CSIA has become a mainstay of petroleum geochemistry research, providing key information on the factors that influence the composition of oil components (Bjorøy *et al*., 1991; Sofer *et al*., 1991; Lichtfouse, 2000) and the determination of the source terms of oils and other organic matter (Mansuy *et al*., 1997; Rogers & Savard, 1999; Mazeas & Budzinski, 2002). In many cases, the CSIA of *n*-alkane and isoprenoid biomarkers has also provided a valuable set of correlation parameters for the identification of oil sources and depositional environments (Bowler *et al*., 1993) and the extent of biotransformation observed in crude oils (Killops & Killops, 1993). Chemical fingerprinting of the *n*-alkane fraction of crude oils and refined products in combination with isotopic characterisation of carbon in the individual homologues has been successfully used to allocate sources of sediment contamination and bird-feather oiling (Mansuy *et al*., 1997; Rogers & Savard, 1999; Mazeas & Budzinski, 2002).

In this paper, application of CSIA for the characterisation of heavy oil contaminants in soil is investigated. Source correlation index reliability was assessed according to the constancy of the $\delta^{13}C$ value during oil weathering. Indices that remain constant are useful as they allow the source of an oil to be determined (providing a sample of the fresh oil is available). Oil-amended soil microcosms were established and incubated at conditions optimum for oil biotransformation.

An isotopic fingerprint of five *n*-alkanes and norpristane (phytane could not be sufficiently resolved in all samples) in oils at successive stages of microbial transformation was obtained. The aim of the study was to establish the utility of CSIA to determine source correlation parameters by assessing temporal variations in the isotopic fingerprint.

MATERIALS AND METHODS

Soil microcosms

Soil microcosms amended with one of three oils (Nigerian crude oil, ballast oil and $N^{o.}$ 6 fuel oil) were established in close accordance with British Standard 7755 (British Standards Institute, 1995). Microcosms were prepared in wide-necked, acid-washed 500 ml Erlenmeyer flasks. A total of 124 individual flasks were prepared: three oils sampled at nine different points in a time series, nine sterilised control flasks (one for each sampling point) and a total of 16 blank microcosm flasks. A sandy-loam (non-carbonaceous gley) soil (Table 1) which had not been used for agriculture for eight years was sieved (<2.0 mm). 200 g soil and 4.0 g of oil (homogenised) were added to 108 flasks. Control microcosms were autoclaved (121 °C; 103.4 Pa) for 15 minutes and treated with a 1 % $^{w}/_{w}$ solution of mercuric chloride, $HgCl_2$, to suppress microbial activity (Chaineau *et al.*, 1995). Blank microcosms containing no oil were prepared to assess contributions from soil organic matter (SOM). All microcosms were amended with a nutrient solution providing an equivalent molar C:N:P ratio of 100:8.75:1.75, believed optimum for oil biotransformation in soil (Huesemann, 1995; Chaineau *et al.*, 1995).

Microcosms were arranged in a randomised block design and incubated at 30 °C for 256 days. Water content (weight) of microcosms was maintained to within ± 5 % with distilled de-

ionised water. Control flask weight was maintained with $HgCl_2$ solution. Soil pH was monitored throughout the study. Sacrificial soil samples were taken at 0, 2, 4, 8, 16, 32, 64, 128 and 256 days. All samples were air dried under forced-draught in a fume cupboard prior to solvent extraction and analysis.

Solvent-extractable matter (SEM) was Soxhlet-extracted from soil subsamples (*ca*. 30 g) for 16 h using 150 ml HPLC-grade dichloromethane (DCM). DCM extracts were reduced overnight at ambient temperatures in a forced draught fume cupboard. Full experimental details of the column fractionation have been reported previously (Whittaker & Pollard, 1994). Component class fractionation was achieved using an adaptation of a classical chromatographic cleanup procedure (EPA Method 3611A, 1990). A Quick-Sep™ lateral reservoir flash chromatography apparatus was used to provide more rapid separation. The column (90 mm x 30 mm) was packed with neutral Aldrich STD grade alumina (80 mm, *ca*. 150 mesh, activated for 12 h at 130 °C), followed by anhydrous Na_2SO_4 (*ca*. 10 mm, dehydrated by heating at 400 °C for 4 hours). The column was prepared by pouring equilibrated slurry of alumina stationary phase and *n*-pentane solvent down a glass rod into the chromatography apparatus. A piston air pump (Fisons, 50 W) was connected to the top of the column and the air pressure adjusted to produce a down-flow elution rate of *ca*. 20 ml min^{-1} (2 % pump capacity). Component classes were obtained using an elution scheme reported by Pollard *et al*. (1992): 150 ml *n*-pentane (elution of saturates), 150 ml toluene (mono-, di- and polyaromatics), and 150 ml 50/50 dichloromethane/methanol mixture (polar compounds, highly polar aromatics, benzothiophenes and carbazoles). Further cleanup of the pentane fraction was performed using a method described by Rawluk (1991). Fractions were collected in pre-weighed acid-washed borosilicate flasks and reduced further under forced draught (ambient temperature).

Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS)

Solvent extracts were analysed in DCM solution at a concentration of *ca.* 10 mg ml^{-1} using a VG ISOCHROM II GC-IRMS system. Individual compounds were isolated using an HP5890A gas chromatograph, equipped with a standard 30 m x 0.00032 m i.d. neutral DB5 column (diphenyl:disiloxane (5 %:95 %) stationary phase) and a dedicated carbon isotope ratio mass spectrometer. Aliquots (2 μl) were injected in splitless mode. A linear temperature gradient was employed, the column temperature being held at 40 °C for 2 minutes following injection, ramped at 10 °C min^{-1} to 320 °C, then held at this temperature for a further 4 minutes. Injector and detector temperatures were set to 350 °C. Helium was used as a carrier gas at a flow rate of 7.5 ml min^{-1}. *n*-alkane peaks were identified by comparing sample GC retention data with that obtained for a standard solution of five *n*-alkanes containing C_{15}, C_{20}, C_{25}, C_{30} and C_{40}. Conversion of oil class fraction subsamples to CO_2 for isotopic analysis was accomplished by dry combustion (850 °C; 6 h) in sealed, evacuated quartz tubes containing excess fired cupric oxide as an oxygen source. Isotope ratio data were obtained on a SIRA 10 isotope ratio mass spectrometer (VG Micromass 602D) and are reported relative to the international standard 'Vienna Pee Dee Belemnite' (VPDB; ^{13}C:^{12}C = 0.01118 (Werner & Brand, 2001)) with corrections made for ^{17}O contributions. Isotope ratios are expressed in terms of $\delta^{13}C$ values (‰) calculated relative to the standard VPDB, according to the following relationship:

$$\delta^{13}C = \left(\frac{R_S}{R_R} - 1 \right) 1000 \quad (1)$$

where R_S is the ratio of ^{13}C to ^{12}C in the sample, and R_R is the ratio of ^{13}C to ^{12}C in the Vienna Pee Dee Belemnite standard (0.01118). The reproducibility of the individual isotopic

measurements, determined through repeated analysis of a laboratory standard graphite sample, were ±0.05 ‰ (1σ); for each sample, the isotopic composition was determined in triplicate, with standard deviations generally less than ±0.3 ‰.

RESULTS

Confirmation of oil biotransformation

A pre-requisite to investigating the performance of source correlation parameters was to verify that subject oils had undergone significant biotransformation over the 256-day microcosm study. This was verified by monitoring the bulk oil loadings and the changes in oil composition with time. It was confirmed that, for both ballast and crude oils, biotransformation reduced the amounts of oil in the microcosms by ~60 % w/w (overall microbial degradation rate of 0.05 g kg^{-1} d^{-1}). Full details and results of these experiments have been reported elsewhere (Pollard *et al.*, 1999).

Variation in Solvent Extractable Material (SEM)

Solvent Extractable Material (SEM, mg g^{-1} of air-dried soil) recovered from the treated and control soils was corrected for natural organic matter. Average recovery for the ballast oil-treated soils decreased most dramatically ($P<0.05$), from a maximum of 14.54 ± 0.25 mg m^{-1} at t=0 to 2.96 ± 0.54 mg g^{-1} after 256 days. Recoveries from the ballast oil control microcosms decreased much less sharply, from an initial 14.75 mg g^{-1} to 11.93 mg g^{-1} after 256 days. Average crude oil recoveries also decreased significantly ($P<0.05$) over the course of the study, from 17.70 ± 0.44 mg g^{-1} to 6.47 ± 1.47 mg g^{-1}. Recoveries from the corresponding control microcosms were again much higher, decreasing from 17.96 mg g^{-1} to 16.94 mg g^{-1}. For the No.6 fuel oil microcosms,

there was very little decrease in SEM over time (20.71 ± 0.90 mg g^{-1} to 19.85 ± 0.84 mg g^{-1} in the treated soils; 20.03 mg g^{-1} to 19.71 mg g^{-1} in the controls).

Using this information, the percentage loss of each oil due to abiotic and biotic processes at each sampling point was determined (Figure 1). In the case of the ballast oil microcosms, abiotic weathering processes are significant in the early stages of the experiment, but this influence decreases over time. For the crude and No.6 fuel oil, abiotic weathering plays little role in the overall weathering process, apart from day 64 for the crude oil microcosms. Here, the role of abiotic weathering is highly significant ($P<0.01$).

GC-IRMS analysis

Compound specific isotope analysis of microcosm extracts provided the $\delta^{13}C$ of the *n*-alkanes C_{14} (for ballast oil and crude oil only), C_{16} (for No.6 Fuel Oil only), C_{17}, C_{18}, C_{24} and C_{26}, and the isoprenoid alkane norpristane (iC_{18}) over the 256-day microcosm study. Mean isotope ratios, associated standard deviations and corresponding results from control microcosms for each compound at each sampling point for the ballast oil-, crude oil- and No.6 fuel oil-treated soils are provided in Tables 2, 3 and 4, respectively.

For ballast oil (Table 2), the $\delta^{13}C$ values for the *n*-alkanes did not, in general, vary according to any readily identifiable trend. The only obvious shift in isotopic composition was for C_{14}, which had a $\delta^{13}C$ of -30.8±1.5 ‰ after 2 days and -28.4 (n = 1) ‰ after 128 days (the compound was not detected at 256 days). Isotope ratios for C_{17}, C_{18}, C_{24}, C_{26} and norpristane did not vary by any significant amount from their initial values. The fluctuations in $\delta^{13}C$ for the compounds are shown in Figure 2a. Similar results were obtained for the ballast oil control flasks with no significant variations in isotopic composition detected (Table 2).

For the crude oil extracts (Table 3), the isotopic composition of the five *n*-alkanes (C_{14}, C_{17}, C_{18}, C_{24}, C_{26}) and norpristane appeared to shift slightly in favour of the heavier C_{13} isotope and become less negative with increased oil weathering. However a shift to more negative $\delta^{13}C$ values was observed at 64 days for all compounds (Figure 2b). Overall, the shift was greatest for the C_{14}, C_{17}, C_{18}, C_{24} alkanes, which experienced statistically significant ($P<0.05$) increases in $\delta^{13}C$ of between 1.5 ‰ and 2 ‰ over the 256 days. The isotope ratios of C_{26} and norpristane also increased with increased oil weathering, but the magnitude of the shift was not significant. Further, the $\delta^{13}C$ of these compounds could not be determined at 256 days, when the greatest isotopic shifts would have been expected.

For the No.6 fuel oil, the individual compounds within the extracts exhibited no significant shifts in $\delta^{13}C$ over the course of the study. All isotope ratios were found to lie between -27.0 ‰ and -28.2 ‰. A plot of compound $\delta^{13}C$ variation with time (Figure 2c) indicates that the isotope ratios of the *n*-alkanes oscillated within the specified range over the course of the study. The isoprenoid $\delta^{13}C$ did not appear to undergo these fluctuations. The No.6 fuel oil microcosms, the isotope ratios of the *n*-alkanes and isoprenoid did not alter significantly over the course of the study.

DISCUSSION

We sought to see whether significant changes in $\delta^{13}C$ occur during the weathering of heavy oil wastes. A previous study looking at the biotransformation of the same heavy oil wastes has reported significant losses in solvent extractable matter over time (Pollard *et al.*, 1999). Significant changes in class fraction distribution were also reported. These changes demonstrated that significant biotransformation of the heavy oily wastes had occurred during the 256-day microcosm experiment. From an environmental forensics viewpoint, it is often necessary to

allocate a contamination to a particular source in order to implement suitable risk reduction measures or to identify responsible parties in litigation (Morrison, 2000).

Results of the GC-IRMS analysis of ballast oil, crude oil and No.6 fuel oil extracts at successive stages of biotransformation (Tables 2 – 4) suggest that norpristane (the isoprenoid alkane biomarker) is isotopically heavier than in the *n*-alkanes in the fresh samples. This is in contrast to the results for pristane, which had previously been found to be isotopically lighter than *n*-alkanes in the same oils (Whittaker *et al*., 1996). A plausible explanation is the documented unpredictability of the nature of isotopic variations between these two compounds (Bjorøy *et al*., 1990; 1991; Sofer *et al*., 1991; Bowler *et al*., 1993).

For each oil, plots of the mean $\delta^{13}C$ variations of the five *n*-alkanes and norpristane over time (Figure 2a, b, c) indicate that microbial degradation induces fluctuations in isotopic composition of these compounds, but generally the overall shifts in compound $\delta^{13}C$ over the 256 days were not substantial. For the ballast oil (Figure 2a):

(i) The isotope ratios of the ballast oil *n*-alkanes and norpristane at 0 days of biotransformation were effectively the same (within *ca.* 0.5 ‰, the established reproducibility of the technique) as the values after 256 days of weathering, although for the *n*-alkanes some marked fluctuations in isotopic composition were observed.

(ii) The $\delta^{13}C$ of norpristane did not vary substantially during the study, experiencing less sizeable fluctuations in isotope ratio than the *n*-alkanes, and is therefore proposed as a possible oil diagnostic parameter.

(iii) All ballast oil compounds detected experienced a sharp decrease in $\delta^{13}C$ (1.0 – 1.5 ‰) over the first four days of the study. This may be due to preferential loss of ^{13}C to abiotic weathering processes, which were shown to be most influential over the initial stages of the study (figure 1). Following this, the C_{14}, C_{24} and C_{26} $\delta^{13}C$ gradually

increased over the course of the study to return to their original values. The $\delta^{13}C$ of C_{17} and C_{18} varied to a much lesser degree.

(iv) This latter variation, although small, may be the result of microbial action on the C_{14}, C_{24} and C_{26} alkanes, causing the heavier isotope to gradually accumulate in these compounds. Previous studies document that a shift in the order of 1 – 2 ‰ might be expected to result from microbial activity (e.g. Bowler *et al*., 1993). The reason that this effect is diminished in C_{17} and C_{18} is because of co-elution of pristane and phytane with these compounds.

For the crude oil samples (Fig 2b):

(i) Isotope ratios in the crude oil samples appeared to shift in favour of the heavier isotope over the course of the study, typically by 1.5 – 2.0 ‰. This suggests that biotransformation of this oil may be monitored through the detection of compound isotope ratios, which increase as biotransformation proceeds and the lighter isotope is preferentially removed during microbial catabolism.

(ii) The trend observed in (i) is reversed by a decrease in all isotope ratios by day 64. This may be due to a sudden shift in the rate of oil biotransformation, causing a marked rearrangement in compound isotopic fractionation. This is corroborated by the evidence presented in Figure 1, which shows a highly significant increase ($P<0.01$) in the proportion of weathering due to abiotic processes.

(iii) There is no clear difference between the isotopic variations of the *n*-alkanes and those of norpristane. Thus in this case, the use of isoprenoid $\delta^{13}C$ would not be a reliable source diagnostic parameter.

For the No.6 fuel oil (Fig 2c):

(i) no overall shift in isotope ratio was observed for any of the compounds over the course of the study.

(ii) Individual values did appear to fluctuate in value between 0 and 256 days, but by less than ± 0.5 ‰. This is within the reproducibility of the technique and cannot be interpreted as a genuine manifestation of microbial activity.

The influence of microbial activity on oil isotopic composition has been studied by several authors (Schmidt *et al*., 2004; Griebler *et al*., 2004). Stahl (1980) examined the nutrient-enhanced degradation of crude oil over 42 days by determining compositional and stable carbon isotope variations within different class fractions. Although the aromatic and polar fraction $\delta^{13}C$ values remained constant (-27.6 ‰ and -27.1 ‰, respectively), the saturate fraction became isotopically heavier by 0.7 ‰ and the asphaltene fraction isotopically lighter by 1.1 ‰. Other, compound-specific, studies using GC-IRMS have found no change in the isotopic composition of individual *n*-alkanes of a variety of crude oils with biotransformation (Sofer *et al*., 1991; Mazeas *et al*., 2002).

The results presented here for ballast and crude oil reveal shifts in *n*-alkane $\delta^{13}C$ values of up to 2.5 times those observed by Stahl (1980). Although no studies were carried out on the effect of microbial activity on the whole oil isotope ratio, it would seem logical that an oil consisting predominantly of saturate class fraction components would also undergo shifts in isotope ratio. The use of oil $\delta^{13}C$ as source correlation parameters would, in such cases, be undermined. However, the results presented in this paper do suggest that the isoprenoid $\delta^{13}C$ is a more reliable source correlation index.

CONCLUSIONS

The whole oil isotope ratio of oils containing significant saturate class fraction content may become more positive following extensive microbial transformation. Previous work (Stahl, 1980; Killops & Killops, 1983) has shown that extensive biotransformation caused the $^{13}C/^{12}C$ ratio to shift by a small but possibly significant amount in favour of ^{13}C in some *n*-alkanes. This may undermine the use of whole oil $\delta^{13}C$ values as source correlation parameters in some cases (e.g. for heavily mineralised oils). However this work has shown that the compound-specific isotope ratio may indeed be a useful source term parameter. The isotopic shift of specific compounds has a very low sensitivity to biotransformation and hence is very resistant to weathering. Although preliminary, this study has shown that isoprenoid isotope ratios appear to be less affected by microbial degredation than *n*-alkanes, and may be of use as source correlation parameters. However, abiotic degradation may still cause significant variation in isoprenoid isotope ratios. The factors affecting abiotic degradation within the oil-weathering process need to be understood further if $\delta^{13}C$ values are to be implemented as source correlation parameters.

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GLOSSARY OF TERMS

Equation 1

R_S = ratio of ^{13}C to ^{12}C in the sample

R_R = ratio of ^{13}C to ^{12}C in the Vienna Pee Dee Belemnite standard (0.01118)

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Table 1. Chemical and physical properties of soil used in biotransformation study.

Table 2. Variation in isotopic composition of individual compounds in ballast oil-treated and control soils (‰).

Table 3. Variation in isotopic composition of individual compounds in crude oil-treated and control soils (‰).

Table 4. Variation in isotopic composition of individual compounds in No.6 Fuel Oil-treated and control soils (‰).

Fig. 1. Ratios of abiotic (control) to biotic (treated – control) % contributions toSEM variations for (a) ballast oil microcosms, (b) crude oil microcosms, (c) No.6 fuel oil microcosms.

Fig. 2. Variation in *n*-alkane and norpristane isotope ratios ($\delta^{13}C$) for (a) ballast oil microcosms, (b) crude oil microcosms, (c) No.6 fuel oil microcosms; where ◇ represents C_{14} (or C_{16} in the case of No6 fuel oil); □ represents C_{17}; △ represents C_{18}; ■ represents C_{24}; ○ represents C_{26}; and ● represents norpristane.

Table 1

Soil sampling details		Particle size analysis (% w/w)		Soil analysis	
Soil texture	Sandy clay loam	**Clay (<0.002 mm)**	21.5	**pH (H_20)**	6.0
Sampling date	17th June 1995	**Silt (0.002 – 0.063 mm)**	21.2	**Available P ($\mu g\ g^{-1}$)**	10.0
Land use	Conservation beds in arboretum	**Total sand (0.063 – 2.000 mm)**	57.2	**Available K ($\mu g\ g^{-1}$)**	60.0
Sampling depth	0 – 26 cm	**Very fine sand (0.063 – 0.250 mm)**	16.0	**CEC**	13.1
Soil type	Non-calcareous gley	**Fine sand (0.125 – 0.500 mm)**	23.6	**N ($mg\ g^{-1}$)**	4.0
Previous agricultural use	None for 8 years	**Medium sand (0.250 – 0.500)**	12.3	**Field capacity moisture content (%)**	38.7
		Coarse sand (0.500 – 1.000 mm)	4.7	**Organic Matter (%)**	5.9
		Very coarse sand (1.000 – 2.000 mm)	0.7		

C14							C17						
Time	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**
0	-29.16			-29.16			**0**	-29.23	-29.21	-29.84	-29.43	0.36	
2	-29.14	-32.05	-31.26	-30.82	1.50	-28.75	**2**	-29.84	-29.47	-30.27	-29.86	0.40	-29.65
4	-29.09		-28.83	-28.96	0.18	-28.98	**4**		-29.69	-29.83	-29.76	0.10	-29.69
8	-27.35			-27.35		-29.55	**8**		-29.46	-29.96	-29.71	0.35	-29.74
16	-29.26	-28.77		-29.02	0.35		**16**	-29.68	-29.59	-30.21	-29.83	0.34	
32		-28.37	-28.45	-28.41	0.06		**32**	-29.76	-29.32	-29.21	-29.43	0.29	
64							**64**						-30.04
128			-28.44	-28.44			**128**		-29.30	-30.01	-29.66	0.50	-30.15
256						-29.14	**256**	-30.14	-29.83	-29.30	-29.76	0.42	-29.14

C18							C24						
Time	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**
0	-29.02	-28.95	-29.23	-29.07	0.15		**0**	-29.13	-28.76	-29.49	-29.13	0.37	
2	-29.84	-29.59	-29.51	-29.65	0.17	-29.55	**2**	-29.98	-30.30	-29.97	-30.08	0.19	-28.82
4	-31.26	-29.69	-29.72	-30.22	0.90	-29.82	**4**	-32.49	-29.40	-29.30	-30.40	1.81	-29.21
8		-29.64	-29.99	-29.82	0.25	-29.72	**8**	-29.22		-29.37	-29.30	0.11	-29.42
16	-29.65	-29.54	-30.07	-29.75	0.28		**16**	-29.25	-28.86	-29.79	-29.30	0.47	
32	-29.74	-29.17	-29.17	-29.36	0.33		**32**	-29.51	-28.88	-28.64	-29.01	0.45	
64		-27.73		-27.73		-29.78	**64**			-28.87	-28.87		-29.11
128	-29.01	-29.98		-29.50	0.69	-29.96	**128**	-28.07	-29.43	-28.98	-28.83	0.69	-29.07
256	-29.92	-29.61	-29.08	-29.54	0.42	-29.28	**256**	-29.24	-29.35		-29.30	0.08	-28.7

C26							Norpristane						
Time	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**
0	-29.37	-28.86	-29.17	-29.13	0.26		**0**	-29.01	-28.57		-28.79	0.31	
2	-30.00	-30.58	-30.51	-30.36	0.32	-28.92	**2**	-28.92	-28.65	-28.90	-28.82	0.15	-28.73
4	-30.60	-29.51	-29.49	-29.87	0.64	-28.96	**4**	-28.57	-28.68		-28.63	0.08	-28.96
8	-29.32			-29.32			**8**	-28.42	-28.39	-28.87	-28.56	0.27	-28.81
16	-29.52			-29.52			**16**	-28.57	-28.48	-29.10	-28.72	0.34	
32							**32**	-28.45	-27.89		-28.17	0.40	
64			-28.89	-28.89		-28.76	**64**			-28.44	-28.44		
128	-28.19	-29.35	-29.06	-28.87	0.60	-29.10	**128**		-28.48	-28.48	-28.48	0.00	-28.72
256	-28.97	-29.21		-29.09	0.17	-28.95	**256**	-29.21	-28.92		-29.07	0.21	-28.22

Table 2

C14							C17						
Time	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**
0	-28.32	-28.57		-28.45	0.18		**0**	-28.35	-28.60		-28.48	0.18	
2	-27.96	-28.88		-28.42	0.65	-27.39	**2**	-28.29			-28.29		-27.35
4	-28.27	-28.58	-28.63	-28.49	0.20	-27.11	**4**	-27.94	-28.36	-28.36	-28.22	0.24	-27.44
8	-27.05	-27.56	-27.63	-27.41	0.32	-26.87	**8**		-27.18	-27.33	-27.26	0.11	-27.12
16	-27.21	-27.40	-27.79	-27.47	0.30		**16**	-27.26	-27.40	-27.45	-27.37	0.10	
32	-27.71	-28.20	-29.34	-28.42	0.84		**32**	-27.22	-28.04	-28.53	-27.93	0.66	
64	-29.72	-30.34	-31.04	-30.37	0.66		**64**	-29.92	-29.99	-30.58	-31.16	0.36	
128	-28.75	-29.07	-26.36	-28.06	1.48		**128**	-28.55	-29.10	-27.32	-28.32	0.91	
256		-27.27	-26.72	-27.00	0.39	-26.91	**256**	-27.05	-26.84	-26.48	-26.79	0.29	-27.08

C18							C24						
Time	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**
0	-28.46	-28.43		-28.45	0.02		**0**	-28.68	-28.25		-28.47	0.30	
2	-27.47	-28.27		-27.87	0.57	-27.08	**2**	-28.67	-28.88		-28.78	0.15	-27.32
4	-27.88	-28.08	-28.43	-28.13	0.28	-27.28	**4**	-28.15	-28.50	-29.33	-28.66	0.61	-27.57
8	-26.15	-27.12	-27.06	-26.78	0.54	-26.87	**8**	-27.11	-27.65	-27.41	-27.39	0.27	-26.96
16	-26.98	-27.55	-27.56	-27.36	0.33		**16**	-27.32	-27.73	-27.68	-27.58	0.22	
32	-27.05	-27.78	-28.38	-27.74	0.67		**32**	-27.73	-28.07	-28.90	-28.23	0.60	
64	-28.97	-29.93	-30.52	-29.81	0.78		**64**	-29.04	-29.90	-30.77	-29.90	0.87	
128	-28.11	-28.93	-27.03	-28.02	0.95		**128**	-28.45	-28.97	-26.85	-28.09	1.10	
256	-26.90	-26.54	-26.27	-26.57	0.32	-27.11	**256**		-26.78	-26.29	-26.54	0.35	-27.21

C26							Norpristane						
Time	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean $\delta^{13}C$**	**SD**	**Control**
0	-28.65	-28.74		-28.70	0.06		**0**	-27.80	-27.90		-27.85	0.07	
2	-29.53	-29.54		-29.54	0.01	-27.59	**2**	-28.13	-28.94		-28.54	0.57	-27.04
4	-28.62	-28.52	-30.02	-29.05	0.84	-27.65	**4**	-28.42	-28.63	-28.80	-28.62	0.19	-27.09
8	-27.82	-28.11	-27.83	-27.92	0.16	-27.49	**8**	-27.09	-27.73	-27.61	-27.48	0.34	-26.61
16	-27.69	-28.21	-28.00	-27.97	0.26		**16**	-27.41	-27.41	-27.92	-27.58	0.29	
32	-28.34	-28.41	-29.11	-28.62	0.43		**32**	-27.72	-28.21	-29.02	-28.32	0.66	
64	-29.29	-29.81	-30.19	-29.76	0.45		**64**	-29.92	-30.39	-31.27	-30.53	0.69	
128	-28.69	-29.06	-27.08	-28.28	1.05		**128**	-28.61		-26.46	-27.54	1.52	
256						-27.44	**256**						-27.24

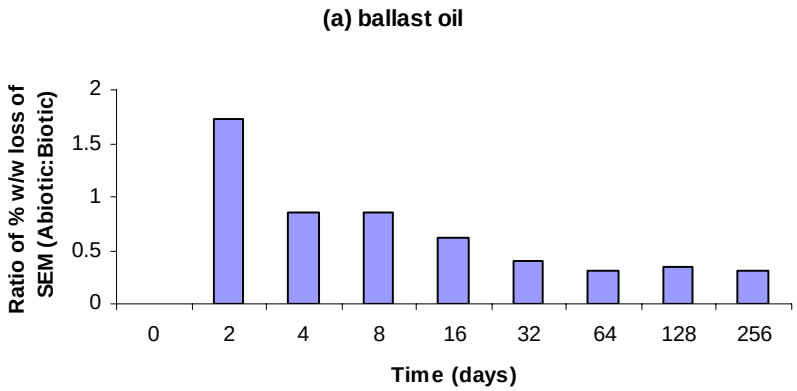
Table 3

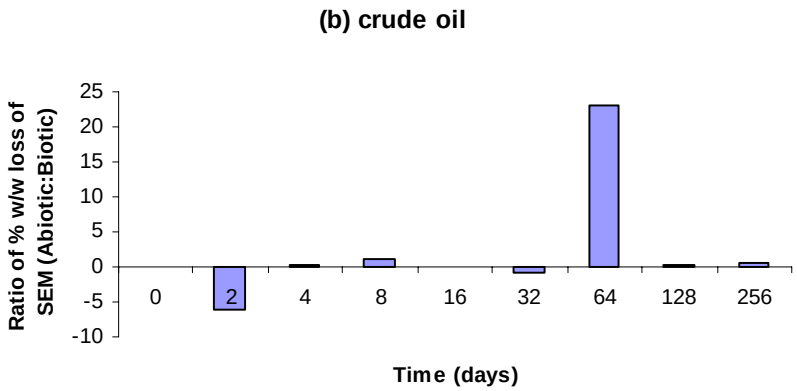
C16							C17						
Time	**n1**	**n2**	**n3**	**Mean d13C**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean d13C**	**SD**	**Control**
0	-27.15	-27.25	-26.60	-27.00	0.35		0	-27.46	-26.30		-26.88	0.82	
2	-28.25		-27.29	-27.77	0.68		2	-28.17	-26.97	-27.51	-27.55	0.60	-28.37
4		-27.48		-27.48	0.00		4	-27.88	-27.67		-27.78	0.15	-27.68
8	-26.97	-27.14		-27.06	0.12	-27.39	8	-27.27	-27.45		-27.36	0.13	-27.59
16	-27.91	-27.92		-27.92	0.01		16		-27.99	-28.17	-28.08	0.13	
32	-26.89	-28.26		-27.58	0.97		32	-27.57	-27.89		-27.73	0.23	
64	-27.80	-28.47		-28.14	0.47		64	-28.07	-28.28		-28.18	0.15	
128	-27.04	-27.25	-27.13	-27.14	0.11		128	-27.32	-27.49	-27.54	-27.45	0.12	
256			-27.16	-27.16		-26.91	256		-28.31	-27.52	-27.92	0.56	-27.64

C18							C24						
Time	**n1**	**n2**	**n3**	**Mean d13C**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean d13C**	**SD**	**Control**
0	-28.61	-27.33	-27.79	-27.91	0.50		0	-27.75	-27.38		-27.57	0.26	
2	-27.89	-26.89	-27.32	-27.37	0.50	-27.80	2		-27.24	-27.90	-27.57	0.47	
4	-27.07	-27.40		-27.24	0.23	-27.48	4	-28.19	-27.97		-28.08	0.16	
8	-27.05	-27.44		-27.25	0.28	-27.44	8		-27.61	-27.51	-27.56	0.07	-27.82
16	-27.97	-28.06		-28.02	0.06		16	-28.06	-28.13		-28.10	0.05	
32	-27.24	-27.84	-27.33	-27.47	0.32		32	-27.41	-27.85	-27.35	-27.54	0.27	
64	-28.06	-28.12		-28.09	0.04		64	-27.72	-27.85		-27.79	0.09	
128	-27.32	-27.93	-27.56	-27.60	0.31		128	-27.48	-27.75	-27.82	-27.68	0.18	
256		-28.31	-27.43	-27.87	0.62	-27.60	256			-27.60	-27.60		-27.37

C26							Norpristane						
Time	**n1**	**n2**	**n3**	**Mean d13C**	**SD**	**Control**	**Time**	**n1**	**n2**	**n3**	**Mean d13C**	**SD**	**Control**
0	-27.74	-27.54		-27.64	0.14		0	-28.01	-27.35	-27.53	-27.63	0.34	
2		-27.28	-27.63	-27.46	0.25		2	-28.00	-26.97	-27.31	-27.43	0.52	
4		-27.83		-27.83			4	-27.41	-27.52		-27.47	0.08	
8	-27.51	-27.27		-27.39	0.17	-27.71	8	-27.06	-27.15		-27.11	0.06	-27.20
16	-28.24	-26.69		-27.47	1.10		16	-27.41	-27.65		-27.53	0.17	
32	-27.34	-28.11	-27.58	-27.68	0.39		32	-27.17	-27.53	-27.71	-27.47	0.27	
64	-27.76	-27.93		-27.85	0.12		64	-27.56	-27.38		-27.47	0.13	
128	-27.24	-27.65	-27.60	-27.50	0.22		128	-27.24	-27.26	-27.19	-27.23	0.04	
256			-27.73	-27.73		-27.53	256	-26.87		-27.12	-27.00	0.18	-26.96

Table 4


(a) ballast oil
Ratio of % w/w loss of SEM (Abiotic:Biotic)
2
1.5
1
0.5
0
0
2
4
8
16
32
64
128
256
Time (days)


(b) crude oil
Ratio of % w/w loss of SEM (Abiotic:Biotic)
25
20
15
10
5
0
-5
-10
0
2
4
8
16
32
64
128
256
Time (days)

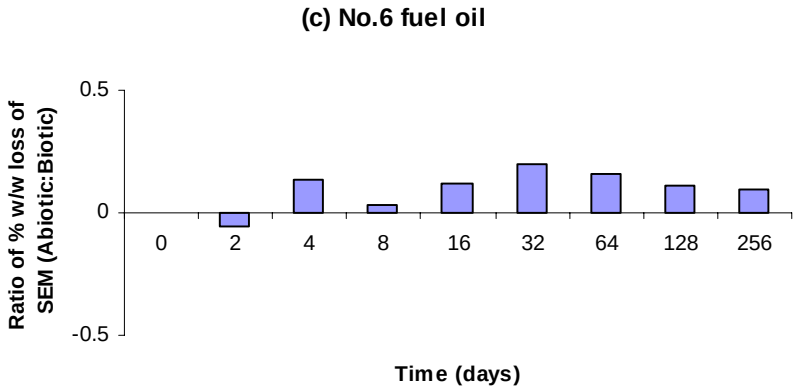
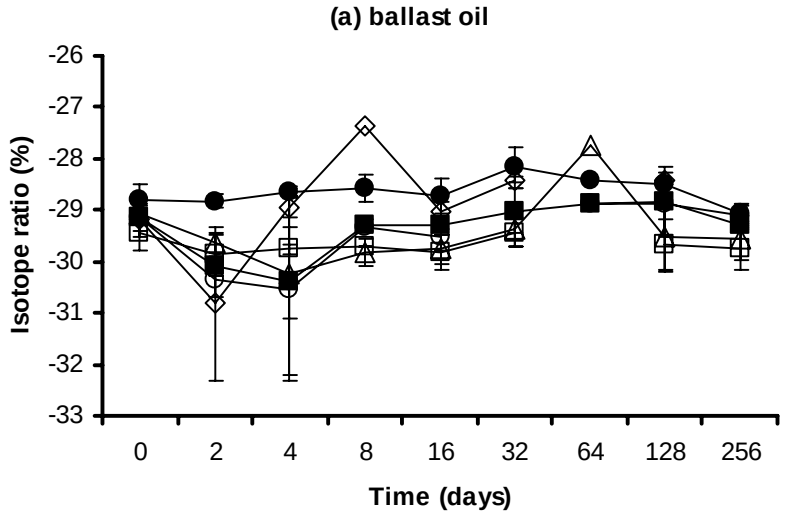
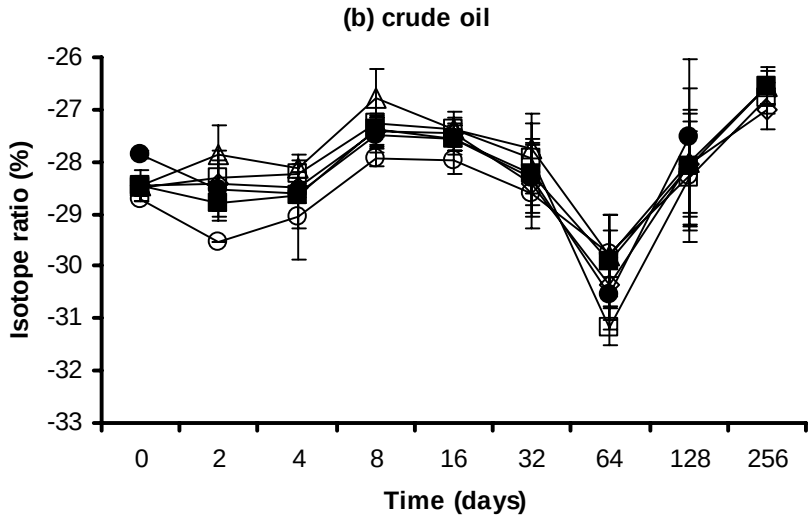

(c) No.6 fuel oil
Ratio of % w/w loss of SEM (Abiotic:Biotic)
0.5
0
-0.5
0
2
4
8
16
32
64
128
256
Time (days)

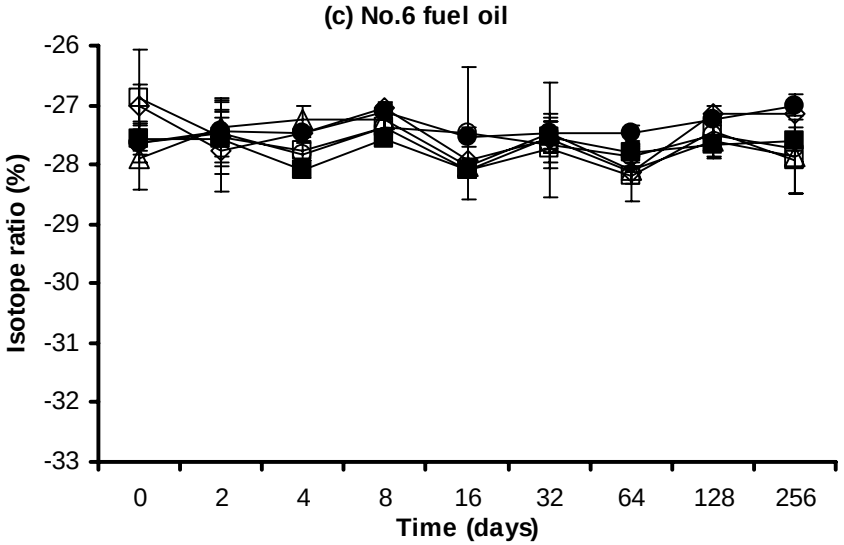
Figure 1


(a) ballast oil
Isotope ratio (%)
-26
-27
-28
-29
-30
-31
-32
-33
0
2
4
8
16
32
64
128
256
Time (days)

1


(b) crude oil
Isotope ratio (%)
-26
-27
-28
-29
-30
-31
-32
-33
0
2
4
8
16
32
64
128
256
Time (days)

2


(c) No.6 fuel oil
Isotope ratio (%)
-26
-27
-28
-29
-30
-31
-32
-33
0
2
4
8
16
32
64
128
256
Time (days)

3

Figure 2