CHARACTERISATION AND BIOTRANSFORMATION OF HEAVY OILS IN THE CONTAMINATED SOIL ENVIRONMENT

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For my wife

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ABSTRACT

The effective assessment and management of heavy oil-contaminated sites presents a major technical challenge requiring, in part, chemical characterisation of the waste-soil matrix. Heavy petroleum wastes are amongst the most problematic organic matrices to define and present a recurrent problem for environmental analytical chemists. A detailed assessment of refractory oil wastes is rarely possible since many conventional analytical techniques are subject to substantial constraints when applied in isolation to the extremely complex, high-boiling analytes encountered at such sites. However, characterisation of the residual oil content of contaminated soils is desirable because these extracts harbour information on contaminant source terms and potential biotreatability. This work details the development of an integrated analytical approach to the characterisation of heavy oil source terms and biotransformation using a combination of conventional and novel analytical techniques.

Rapid alumina-column chromatography provided initial compositional analysis of the oils and isolated the individual class fractions for subsequent analysis. Analysis by thin layer chromatography with flame ionisation detection (TLC-FID) provided a fingerprint of the class fraction distribution within each oil that closely matched those obtained by the column fractionation process. Stable carbon isotope mass spectrometry (IRMS) was also developed as a potential screening tool for distinguishing between oils. Whole oil $\delta^{13}C$ showed a small but significant decrease with decreasing oil asphaltene content, from -26.8 $^{o}/_{oo}$ for the heaviest oils to -28.8 $^{o}/_{oo}$ for the lighter, predominantly paraffinic oils. In agreement with the conventionally observed trend, $\delta^{13}C$ values of isolated class fractions were found to increase in the order: $\delta^{13}C_{sat}$ (~ $\delta^{13}C_{oil}$) < $\delta^{13}C_{aro}$ < $\delta^{13}C_{pol}$ < $\delta^{13}C_{asp}$, with $\delta^{13}C_{sat}$ up to 2.5 $^{o}/_{oo}$ more negative than $\delta^{13}C_{pol}$ or $\delta^{13}C_{asp}$. However, this variation was much

less pronounced for the heavier oil samples. Therefore, plots of isotope type curves exhibited a clear distinction between heavier oils, which produced much flatter type curves, and lighter oils, for which type curves were characteristically sloping.

Extended analysis of isolated oil saturate fractions by GC-FID demonstrated the spread of *n*-alkanes within each oil and highlighted the dominance of unresolved complex material (UCM) in the heavier oils. GC-EI MS (SIM) analysis of individual biomarker compounds allowed a variety of source term indices to be evaluated. The values of [*n*-alkanes:17α(H),21β(H)-hopane], [phytane: 17α(H),21β(H)-hopane] and [tricyclic terpanes:hopanes] indices decreased most significantly as the combined total of polar and asphaltene compounds within oil samples increased, indicating that these ratios may be the most useful in oil source term characterisation. Finally, GC-IRMS analysis of selected *n*-alkanes and phytane provided an isotopic fingerprint that may be of use in identification of contaminant source terms.

The biotransformation of heavy oils in soil microcosms was studied over a period of 9 months. Over this time, a medium-to-heavy boiling ballast oil and a crude oil exhibited a total reduction in solvent extractable material (SEM) of approximately 80 $\%^{w}/_{w}$ and 60 $\%^{w}/_{w}$, respectively. No.6 Fuel Oil was found to be largely recalcitrant to biotransformation. Abiotic controls indicated that up to 60 $\%^{w}/_{w}$ of the ballast oil was lost due to microbial activity, and that its half-life was approximately 120 days. Over the 9 months, the $\%^{w}/_{w}$ of saturates within the ballast oil SEM decreased from 74.6 to 23.2; the $\%^{w}/_{w}$ of aromatics increased from 12.5 to 23.0, the $\%^{w}/_{w}$ of polars increased from 8.6 to 31.3 and the $\%^{w}/_{w}$ of asphaltenes increased from 4.3 to 22.5. Similar changes were observed for the crude oil.

The reliability of several oil biomarker source correlation indices was determined. The most reliable were those comprising individual hopane isomer pairs, which remained almost constant in both crude and ballast oils even after extensive biodegradation. Of these, the [17α(H),21β(H)-norhopane:17α(H),21β(H)-hopane] was the preferred index, staying

constant at 0.7 for both oils over the 9 month biotransformation period. Methods of monitoring oil biotransformation through biomarker analysis were also assessed. The most sensitive indicator of oil biotransformation was the ratio of total *n*-alkanes to 17α(H),21β(H)-hopane. For the ballast oil, this ratio decreased from 748.0 to 8.5 over 9 months. The carbon isotopic composition of individual *n*-alkanes and norpristane isoprenoid did not vary significantly with oil biotransformation and so may be used as source correlation parameters. Further analysis of standard physically-weathered diesel range organics provided additional evidence in support of these conclusions.

These results are of considerable use to researchers and practitioners in the field of contaminated land assessment elucidating the source terms, weathering characteristics and bioremediation potential of complex heavy oil waste matrices.

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GLOSSARY

Acid Tar
: A highly sulphonated, high-boiling acidic waste product of the re-refining of used motor oil.

Asphaltenes
: Chemical components of oil exhibiting extended, cross-linked structures that are insoluble in low-boiling, non-polar solvents (e.g., hexane, pentane).

Bioavailability
: The capacity of a particular compound or group of compounds to participate in biological reactions.

Biodegradation
: The conversion of an organic compound, ultimately to carbon dioxide and water, by microbiological catalysis.

Biomarkers
: Compounds in oil whose skeleton is preserved throughout most of oil diagenesis and catagenesis.

Bioremediation
: A managed process for removing soil contaminants through the enhancement of natural microbiological processes.

Bioremediation Potential
: The capacity of a contaminants in soil to be successfully treated with bioremediation technologies.

Biotransformation
: A broad term signifying the alteration of a compound's chemical structure through microbial catalysis.

Bulk Oil
: Term referring to a petroleum product in its entirety.

Characterisation
: The process through which the physical, chemical and/or biological properties of a particular compound or product are distinguished.

Class Components of an Oil
: Individual compounds contained within a particular class fraction.

Class Fraction
: A group of compounds within an oil, possessing similar chemical properties, that is defined by the solvent with which the compounds are eluted during chromatographic analysis.

Co-metabolism
: The fortuitous microbial transformation of one compound by an enzyme that is produced for the transformation of another molecule (the primary substrate).

Column Chromatographic Fractionation
: Column chromatographic-isolation of individual class fractions within an oil.

Conserved Internal Markers
: Compounds within oils that are more resistant to weathering than the majority of constituents.

Detailed Component Analysis
: Identification and evaluation of individual compounds in a particular oil.

Extended Analysis
Synonymous with Detailed Component Analysis.

Heavy (Fuel) Oils
Petroleum products that consist mainly of high-boiling compounds.

Heterocompounds
Chemical compounds containing one or more atoms other than carbon or hydrogen.

Heterotrophic Bacteria
Bacteria that utilise external organic sources of carbon as their source of energy.

Hopanes
Pentacyclic alkane biomarkers ubiquitous in crude oils.

Hydrocarbons
Compounds consisting solely of hydrogen and carbon.

Intrinsic Bioremediation
The management of natural microbial processes to reduce the amount of contaminant in soil or groundwater.

Ion Chromatogram
In GC-EI MS, the set of peaks produced by ions possessing one particular mass-to-charge ratio.

Isotope Ratio
In this study, a representation of the ratio of C_{13} to C_{12} within a particular compound or mixture of compounds.

Isotopic Composition
In this study, the relative amounts of C_{13} and C_{12}, as shown by the Isotope Ratio.

Microcosm
Pertains in this study to the experimental apparatus through which microbial activity was studied in the laboratory

Mineralisation
The complete microbially-induced conversion of an organic compound to carbon dioxide and water.

Natural Attenuation
Synonymous in this case with Intrinsic Bioremediation

Natural Organic Matter
The portion of a soil derived from the decay of biogenic material.

Oil and Grease
A collective term used to describe the total amount of organic material extracted by solvent from a particular contaminated soil.

Oily Wastes
Collective term referring to the petroleum contaminants contained within a soil.

Petroleum Products
Individual fossil fuel products obtained from the atmospheric and vacuum distillation of crude oil.

Quality Control
The measures taken during sample analysis to ensure that errors are identified, quantified and minimised.

Remediation

The process through which contaminants in soil are removed or transformed to a less harmful form.

Residual Contamination

The recalcitrant portion of a petroleum contaminant that remains in the subsurface.

Risk Assessment

The process that identifies specific hazards, assesses potential routes and levels of exposure, relates dose to potential effects (where required) and characterises risks according to their relative importance.

Risk Management

The process through which identified risks to human health and the wider environment are controlled or reduced

Saturated Zone

The area of the soil subsurface below the permanent water table mark (i.e., below the groundwater level).

Screening Techniques

Analytical techniques that convey information on the bulk composition of an oil.

Selective Ion Monitoring

A mode of operation for GC-EI MS in which analysis can be focused on individual target compounds within an oil sample.

Source (Correlation) Indices

Ratios of compounds or groups of compounds that are resistant to weathering and specific to particular oil types.

Source Diagnostic Parameters

Criteria by which commonality between two or more oils can be established.

Source Term

In the context of this thesis, is an alternative term for the contaminant(s) contained within the soil.

Tiered Analytical Strategy

The term given to the collection of analytical techniques used to characterise a particular environmental contaminant.

Unresolved Complex Material

The portion of an oil that cannot be resolved by gas chromatographic analysis.

Unsaturated Zone

The area of the soil subsurface above the highest permanent water table mark (i.e., above the level of soil groundwater)

Waste-Soil Matrix

A collective term referring to the arrangement of soil and oil in the contaminated environment.

Weathered State

The extent to which a particular oil has been altered by weathering.

Weathering

Collective term referring to the natural physical, chemical and biological processes that alter the composition of an oil following spillage.

Weathering Index

The ratio of a compound easily lost during weathering to a more persistant used to evaluate the extent of weathering undergone by a particular oil.

LIST OF ACRONYMS

ANOVA -	Analysis of Variance
API -	American Petroleum Institute
BSI -	British Standards Institute
BTEX -	Benzene, Toluene, Ethylbenzene, Xylene
CDU -	Crude Distillation Unit
CRPB -	Clyde River Purification Board
CSIA -	Compound Specific Isotope Analysis
DCM -	Dichloromethane
DNAPL -	Dense Non-Aqueous Phase Liquid
DRO -	Diesel Range Organics
EA -	Environment Act (1995)
EEA -	European Environment Agency
EPA -	Environmental Protection Act (1990)
EU -	European Union
GC-CI MS -	Gas Chromatography-Chemical Ionisation Mass Spectrometry
GC-EI MS -	Gas Chromatography-Electron Impact Mass Spectrometry
GC-FID -	Gas Chromatography-Flame Ionisation Detection
GC-IRMS -	Gas Chromatography-Isotope Ratio Mass Spectrometry
GC-SIMDIS -	Gas Chromatography-Simulated Distillation
HPLC -	High Performance Liquid Chromatography
ICRCL -	Interdepartmental Committee on the Redevelopment of Contaminated Land
IRMS -	Isotope Ratio Mass Spectrometry
LGC -	Laboratory of the Government Chemist
LNAPL -	Light Non-Aqueous Phase Liquid
LSD -	Least Significant Difference
NAPL -	Non-Aqueous Phase Liquid

NATO-CCMS -	North Atlantic Treaty Organisation-Committee on Challenges to Modern Society
NOM -	Natural Organic Matter
PAH -	Polynuclear Aromatic Hydrocarbon
PDB -	Pee Dee Belemnite
QC -	Quality Control
RCEP -	Royal Commission on Environmental Pollution
SAC -	Scottish Agricultural Centre
SAPA -	Saturates, Aromatics, Polars, Asphaltenes
SD -	Standard Deviation
SEM -	Solvent Extractable Material
SFE -	Supercritical Fluid Extraction
SIM -	Selective Ion Monitoring
TIC -	Total Ion Chromatogram
TLC-FID -	Thin Layer Chromatography-Flame Ionisation Detection
UCM -	Unresolved Complex Material
UNEP -	United Nations Environment Program
US EPA -	United States Environmental Protection Agency

CHAPTER 1. INTRODUCTION

1.1 OVERVIEW OF SOIL ENVIRONMENTAL ISSUES

Contamination of the soil environment by past and present human activities is now recognised as an issue of major international concern. Its emergence as such is due to a number of factors (European Environment Agency (EEA), 1995; Royal Commission on Environmental Pollution (RCEP), 1996), including:

(a) the increasing demands being placed on the natural environment by expanding populations, and the concomitant shortage of 'usable' land for development and housing;

(b) a general increase in public concern over the impact of industrial activities on the environment;

(c) the prevalence of contaminated land, particularly in Western developed countries, as a result of historical and present-day industrial activities;

(d) the recognition of soil as a living, non-renewable resource of crucial importance to the biogeochemistry of global ecosystems;

(e) the adoption of sustainable development as a central tenet of future human endeavour, particularly regarding the use of natural resources, and;

(f) the emergence of environmental liability issues as a key driver in corporate financial risk management.

For industrialised countries in particular, the problem of contaminated land carries significant environmental and economic implications. In the UK alone, there are an estimated 100,000 contaminated sites carrying a potential cleanup cost of almost £600 m (Tadesse *et al.*, 1994). A recent report by the UK Groundwater Forum specifies contaminated land as one of the highest priority issues, and one which is increasing in scale (Water Quality International, 1996). Throughout the European Union, estimates of the total number of contaminated sites

range from at least 55,000 (EEA, 1995) to over 500,000 (Pollution Prevention, 1993), with an estimated remediation market value upwards of £ 1 bn. In 1993 in the United States, over 30,000 abandoned or uncontrolled sites had been identified, some 1,200 of which were placed on the U.S. National Priority List for immediate attention (Tadesse *et al.*, 1994). In Canada, over 10,000 contaminated sites have been identified, 1,000 of which are categorised as presenting 'high risk' to human health (Smith, 1991). Though these figures are arguably a reflection of how contaminated land is defined rather than the actual number of sites, they nevertheless provide a general indication of the scale of the contaminated land problem with which the industrialised world is now faced.

As a result of these pressures, considerable efforts to raise awareness of soil contamination issues and thus identify and develop the central themes of contaminated land management are being made at the international level (for example by United Nations Environment Programme (UNEP) (Tadesse *et al.*, 1994), the European Environment Agency (EEA) (EEA, 1995) and the North Atlantic Treaty Organisation's Committee on Challenges to Modern Society (NATO CCMS) (Bardos, 1994), the national governmental level (Denner, 1994), and independently, through collaborative efforts within and between academic and business circles. Many countries now have in place or are in the process of putting into place national regulatory frameworks and policies designed to protect the soil environment, promote the investigation, remediation and restoration of contaminated land and resolve the liability issues associated with soil and groundwater contamination (Harris, 1994).

Yet despite these advances, contaminated land remains perhaps the least well understood forms of environmental contamination. There are several reasons for this:

(i) the heterogeneity of soil;

(ii) the vast chemical complexity of many industrial contaminants, particularly those derived from fossil fuels (Altgelt & Boduszynski, 1994);

(iii) the capacity of soil to receive and hold hidden from view large amounts of contaminant (EEA, 1995);

(iv) the site-specific nature of contaminated land (Sims, 1990; Hrudey and Pollard, 1993), and:

(v) poor communication between relevant scientific disciplines (Rowley, 1993).

As a result, the underlying complexities of what constitutes a contaminated site, how should historical contamination be treated, who should pay for contaminated site remediation and what level of remediation should be sought remain heavily debated and largely unresolved.

The application of analytical methods to evaluate the presence, source and distribution of contaminants in soil is fundamental to resolution of these issues (Douglas *et al.*, 1992; Fan *et al.*, 1994; Hrudey & Pollard, 1993). Much effort is currently being directed towards improving existing analytical methodologies and developing innovative approaches, with a view to improving our understanding of the composition of complex wastes in soil and enhancing the overall quality of data obtained during investigation of contaminated sites (Mattney Cole, 1994; Pollard *et al.*, 1994b). The demand for reliable, accurate information on soil contaminants has become especially intense in recent years, due to the financial and legal implications of contaminated land transactions (Harris, 1994; ICI Engineering Report, 1994), and the emergence of risk assessment as a tool for managing contaminated sites, which itself requires a more detailed picture of contaminant behaviour (Paustenbach, 1989; Petts, 1994).

As a result of their widespread use since the 1920's, the single largest source of chemical contamination of terrestrial and groundwater environments are petroleum products (Douglas & Uhler, 1993). Unfortunately, these wastes are highly problematic because they contain a wide variety of potentially toxic compounds of variable solubility, are often persistent in soils and are often not easily characterised or treated by conventional technologies. A key issue is the almost ubiquitous presence of residual petroleum contamination at former industrial sites (RCEP, 1996; Rowley, 1993), caused by spillage of heavy oils, which are relatively dense, exceptionally diverse mixtures of high-boiling,

structurally complex compounds. As waste products, these are often amongst the most complex organic matrices encountered at contaminated sites. Conventional analytical techniques are normally incapable of providing the level of chemical insight required to fully assess the source, fate and partitioning behaviour of contaminants and estimate the potential environmental health risks posed by their presence at a site (Birnbaum *et al.*, 1996; Pollard *et al.*, 1994).

The success of treatment technologies for remediating contaminated land depends fundamentally on a reduction in toxicity and chemical mass on the part of the contaminants. Selection of appropriate remedial technologies requires an understanding of how the unit process or combination of processes (the 'treatment train') achieves these reductions. Bioremediation is a key process for the treatment of organic wastes and can be defined as 'a managed or spontaneous process in which biological, especially microbiological, catalysis acts on pollutant compounds, thereby remedying or eliminating environmental contamination' (Madsen, 1991).

Bioremediation can be achieved through management of the natural assimilation process in such a way as to limit adverse effects of a contaminant plume, called natural attenuation or 'intrinsic bioremediation', or through an engineered bioremediation system, which could be *in-situ* or *ex-situ* (e.g. biopile treatment), and which seeks to create conditions favourable for accelerated microbial mineralisation of contaminants (Madsen, 1991).

Evaluating the bioremedial potential of petroleum waste prior to field application (i.e., its susceptibility to treatment by bioremediation techniques) is particularly important, since heavy oil residues can be highly recalcitrant to the microbial degradation processes that remove many subsurface contaminants (Rifai *et al.*, 1995; Pollard *et al.*, 1994). Furthermore, current methods for quantitatively determining the biodegradation of organic contaminants are not well established. The development of techniques able to accurately verify the *in situ* biodegradation

of wastes in the field is clearly crucial to the continued development and validation of bioremediation technologies (Morris *et al.*, 1996; Herbert *et al.*, 1996; Madsen, 1991).

At present, much of the essential information on the nature, origin, distribution, potential toxicity and biotreatability of residual petroleum contamination is not being accessed by analytical approaches. This is severely restricting the effectiveness of management programmes at sites featuring this type of contamination. The research detailed here addresses this problem through the development of a novel approach to the characterisation of heavy, residual oils in the contaminated soil environment, in which a combination of non-conventional analytical techniques, more suited to the overall complexity of the waste, are used to improve the chemical description of residual petroleum contamination. A range of disciplines and topics are represented in this work, and these are shown in Figure 1.1.

Specifically, a number of screening techniques have been utilised that provide insight into the chemical composition of heavy oil wastes, and so give a provisional indication of waste bioremediation potential. Furthermore, using conserved biomarkers as indicators of source characteristics and chemical mass balance, detailed chemical information on these problematic oils is used to identify their source and predict their weathered state. This information is also essential to the success of their treatability by microbial processes, whether natural or engineered. In brief, the research reveals the fact that heavy oils harbour information critical to their successful identification and treatment providing appropriate analytical techniques are adopted to access this information. To the knowledge of the author, such an exhaustive account of these wastes in soil is absent from the literature.

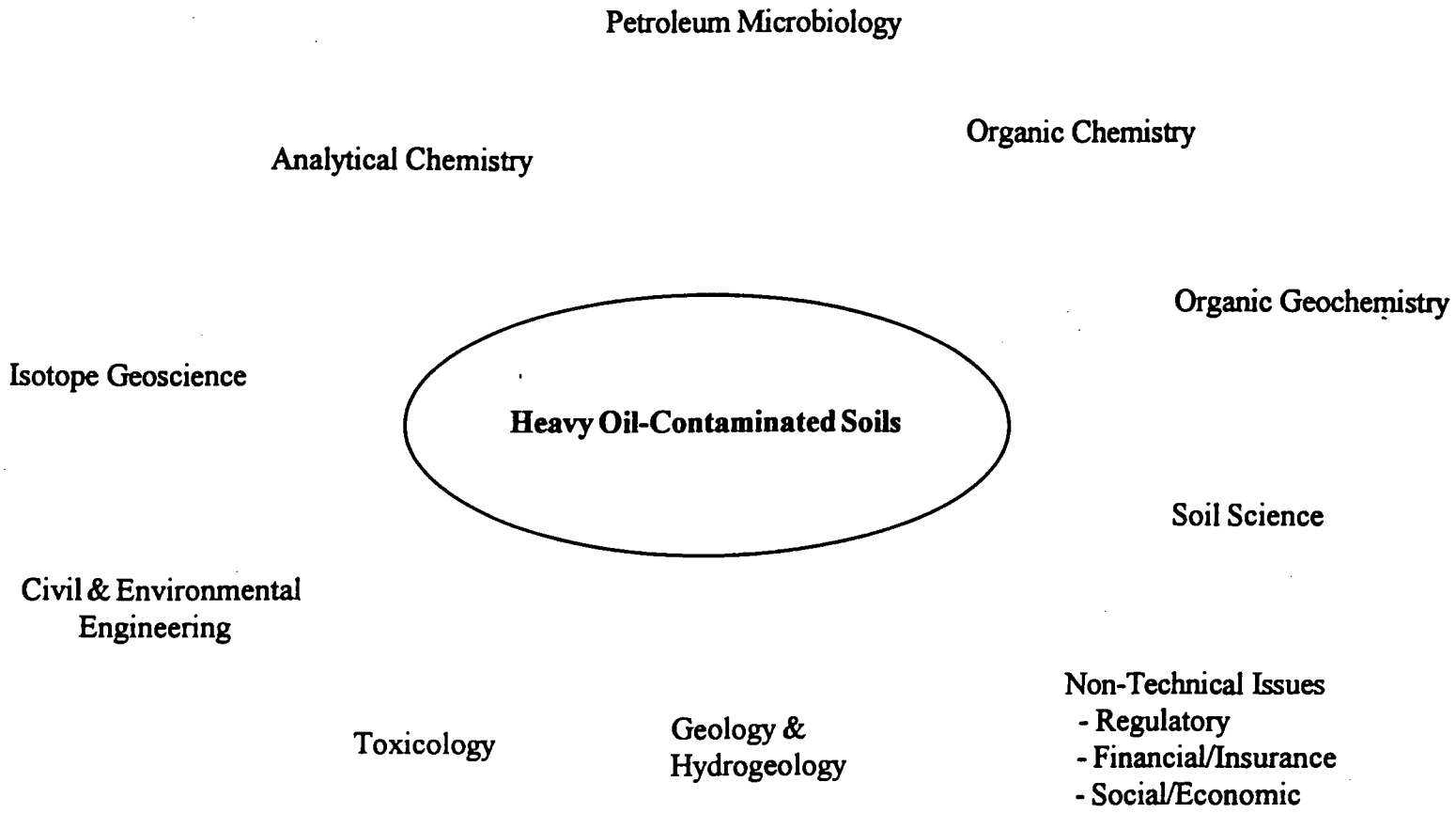


Figure 1.1 The Range of Disciplines Related to Heavy Oil-Contaminated Soil

1.2 CONTAMINATED LAND

1.2.1 An Historical Perspective

The historical contamination of soils as a consequence of past industrial activity results from the spillage, leakage, poor handling and inadequate disposal of chemicals. All industrially developed countries have accumulated an inventory of contaminated land that reflects the type and scale of their industrial activities, past and present. In the UK, which has a long history of industrial development, the contamination of soils from heavy fossil fuel-driven industries (e.g., gas works and power stations) continued largely unabated from the mid-nineteenth century until the 1970s (RCEP, 1996). The industries most closely associated with contaminated land, and the type of wastes typically produced by each are shown in Table 1.1 (Rowley, 1993).

In recent years, intensified pressure on the soil environment due to urban expansion and infrastructure requirements, waste disposal, raw material extraction and intensive agricultural practices has resulted in the discovery of an increasing number of contaminated sites. In Europe, as in the UK at present, no register of contaminated sites yet exists, and so the extent of soil contamination is difficult to assess. However, recent estimates of the number of contaminated sites in selected EU countries, together with equivalent figures from the United States and Canada, are shown in Table 1.2. Although these figures provide a useful illustration of the scale of our inherited contaminated land problems, it is important to recognise (i) that they depend to a large extent upon how contaminated land is defined (EEA, 1995), and (ii) that the number of contaminated sites identified in any particular country has shown a tendency to increase over time as new investigative techniques develop, definitions of contaminated land are modified and more contamination is discovered (Harris, 1994).

As a result of their widespread use throughout the industrialised world, crude oil and associated petroleum products have been identified as the largest single group of soil and

Table 1.1 Typical Industries Giving Rise to Contaminated Land[1]

Industry Sector	Typical Sites	Possible Contaminants Present[2]
Chemical	Wood treatment plants; pesticide and pharmaceutical handling sites; paint works;	Specialised organic compounds, phenols, hydrocarbons, PCBs, acids, alkalis, solvents and metals.
Petrochemical	Oil refineries; tank farms; fuel storage and distribution depots; tar distilleries	Crude oils and petroleum products.
Energy	Gas works; power stations	Coal, coke dust, heavy petroleum products, phenols, cyanides, sulphur compounds and asbestos.
Transport	Garages; vehicle manufacturers and maintenance workshops; railway depots	Petroleum products, lubricating oils, metals and asbestos.
Water Supply & Wastewater Treatment	Waterworks; sewage treatment plants	Sewage sludge (containing metals, refractory organic compounds, microorganisms).
Miscellaneous	Military land; tanneries; rubber manufacturers; waste disposal sites (including landfill sites)	Petroleum products, specialised organic compounds, leachates, metals and gases.

[1]Adapted from Rowley (1993)

[2]The contaminants listed are examples of contaminants typically associated with these industries.

Table 1.2 Estimated Number of Contaminated Sites in Selected Countries[1]

Country	Number of Contaminated Sites	Number of Sites Requiring Remediation
Belgium/ Luxembourg	20,000	5,000
France	100,000	20,000
Germany	200,000	50,000
Italy	30,000	10,000
The Netherlands	110,000	30,000
Spain	25,000	5,000
United Kingdom	100,000	30,000
United States	33,000	1,200
Canada	10,000	1,000

[1]Sources: Tadesse et al. (1994) and Hrudey & Pollard (1993)

groundwater contaminants (Douglas & Uhler, 1993). From a historical perspective, petroleum contamination at former industrial sites is a priority because contamination at these sites normally involves discharges of non-volatile petroleum products (e.g., heavy fuel oils, lubricating oils and tar products) into the soil environment. These wastes frequently form 'pockets' of discrete oil product (free product) that become trapped in the soil matrix and accumulate over many years following deposition in the vicinity of process operations (RCEP, 1996). In the UK, the Nineteenth Report of the Royal Commission on Environmental Pollution (RCEP, 1996) identifies non-volatile hydrocarbons, tars, and other recalcitrant organic residues as amongst the most pervasive and significant long-term problems in soil at former industrial sites.

1.2.2 Current Issues in Contaminated Land Management

The plethora of historically contaminated sites in industrialised countries has had a significant impact across a broad range of disciplines. Current issues in contaminated land management can be divided into those arising from the technical difficulties of assessing and remediating contaminated sites, and those associated with legal, financial and political (or non-technical) matters pertaining to site management. At stake is the question of how best to manage an increasing number of contaminated sites in the face of limited financial resources and considerable technical uncertainties. Increasingly, contaminated sites are being categorised according to the risks they pose to human health and the wider environment (Paustenbach, 1989; Blacker & Goodman, 1994). The process of identifying and evaluating the significance of risks is known as risk assessment and the process through which identified risks are controlled or reduced is known as risk management. These issues are discussed in more detail below.

The manner in which contaminated land is defined has significant implications for the way in which sites suspected of containing contamination can be regulated, investigated and treated. In the UK, contaminated land is defined as:

> "any land which appears to the local authority in whose area it is situated to be in such condition (by reason of substances in, on or under the land) that: (a) significant harm is being caused, or there is a significant possibility of such harm being caused; or (b) pollution of controlled waters is being, or is likely to be caused" (Environment Act, 1995).

Other countries and organisations use alternative definitions, but consistent to most jurisdictions is a need to establish the link between the presence of soil contamination, site-specific conditions and the possibility of harm to humans and/or the environment. For the purposes of identifying contaminated land it is now essential that an assessment of the contaminants extends beyond the analytical measurement of bulk oil concentration to provide information of use in the assessment of the potential risks associated with a site. This is also the case during site remediation operations, where a reduction in the risks to human health and the environment must be demonstrated, through confirmation of contaminant loss and/or toxicity reduction.

These requirements place renewed and considerable pressure on the quality of information provided by analytical techniques, and add to the already substantial technical challenges that currently surround the site investigation and remediation process.

1.2.2.1 Technical Challenges

The technical aspects of contaminated site management are concerned principally with determining the source, abundance, distribution, the direction and speed of movement, the toxicity and the potential treatability of contaminants in the subsurface. Invariably, a combination of complex wastes, heterogeneous soil subsurfaces and confounding site-specific factors (e.g., site location and topography) give rise to a host of technical challenges

that must be addressed to fully characterise the contaminant source terms (Sims, 1990). A review of the literature on contaminated land reveals a number of key themes that repeatedly arise during contaminated site assessment and remediation:

(i) the design of sampling strategies must adequately take into account the spatial variabilities in contaminant distribution (Pollard, 1994);

(ii) current techniques used for defining subsurface geology and hydrogeology of contaminated sites are often limited (Macdonald and Kavanaugh, 1994);

(iii) conventional field screening and laboratory analytical techniques are often unable to provide the fundamental data necessary for an informed assessment of sites contaminated with complex organic products (Bauman, 1991; Sims, 1990; Douglas, 1993; Pollard *et al.*, 1995).

(iv) heavy or highly weathered petroleum residues are extremely common in the contaminated soil environment at industrial sites. These wastes are particularly difficult to characterise using current chemical monitoring techniques, and present a considerable threat to soil and groundwater quality (Bauman, 1991; Pollard *et al.*, 1995);

(v) the need for reliable source diagnostic parameters capable of discriminating between different oils and identifying the source of a particular petroleum contaminant (Wang *et al.*, 1994b);

(vi) the need for a better elucidation of the influence of site-specific factors (for example, site geology and waste type) on the selection of remediation techniques (Sims, 1990);

(vii) a greater understanding of the strengths and weaknesses of respective remediation technologies is needed to optimise their application and integration, particularly as emphasis shifts away from approaches that simply remove or transfer contaminated soils to landfill towards sustainable techniques that require their remediation and ultimate destruction from the environment (RCEP, 1996; Bardos, 1994);

(viii) the need for clarification of where and when bioremediation technologies, including natural attenuation (or 'intrinsic bioremediation'), are likely to be effective (RCEP, 1996);

(ix) the confirmation and quantitative determination of *in-situ* biodegradation of petroleum contaminants in soil (Madsen, 1991; Voos, 1996; Douglas *et al.*, 1993);

(x) remediation of residual non-aqueous phase liquids (NAPL) in the subsurface (Macdonald & Kavanaugh, 1994);

(xi) the development of innovative, cost-effective technologies for routine and non-routine remediation scenarios (Long, 1993), and;

(xii) finally, there is growing recognition that the management of contaminated land requires effective communication between disciplines. Key technical areas are chemistry (for the development of analytical methodologies, and for understanding the fate, partitioning and potential toxicity of contaminants), geology and hydrogeology (for site characterisation, including soil types and flow of groundwater, and for remedial design), environmental microbiology (for understanding the effects of spilled contaminants on soil microbial consortia), toxicology (for definition of the ecological risk and human health effects presented by contaminants) and engineering (for risk management and remediation activities) (Rowley, 1993; Hrudey & Pollard, 1993).

1.2.2.2 Non-Technical Considerations

To gain a full understanding of the issues that influence the way in which contaminated land is handled, and the motives that lie behind many of the technical issues discussed above, it is necessary to briefly explore the legal, financial and political matters that come to bear upon contaminated land.

The principle issue lying behind the assessment and remediation of contaminated land is the commercial reality of maintaining or restoring the value of property that has become contaminated (Crowcroft and Pollard, 1995). A dynamic 'stock' of contaminated land is continually being replenished by contaminating industries and diminished by economic development and environmental regulation. In the UK, this situation is supported

by a set of statutory provisions, regulations, guiding principles and common case law aimed at controlling sites that present significant risks to human health and the environment, and establishing liability for remediation costs.

The overall stated aim of UK Government policy is to promote sustainable development and encourage the development of contaminated sites rather than greenfield sites (This Common Inheritance, 1990). The key pieces of UK legislation pertaining to contaminated land are:

(i) the 1990 Environmental Protection Act, which for operating sites provides for the control of activities having the potential to contaminate land through Integrated Pollution Control;

(ii) the 1991 Water Resources Act, which contains powers to prevent and remedy the pollution of groundwater, and;

(iii) the recent 1995 Environment Act, the provisions in which are not yet in force but in which a risk-based approach to historically contaminated land identification and mechanism for remediation is introduced.

UK Common Law also provides a mechanism for dealing with situations where contamination from one party's activities adversely affects the concerns of another (Harris, 1994; Denner, 1994).

The overall guiding principle for establishing liability in the UK, introduced in the Environment Act 1995, is that remediation costs should be born by the polluter (the 'polluter pays' principle). Where this is not possible, the owner or occupier may become the liable party, subject to various exclusions. During land transactions, however, the responsibility for identifying land contamination is the buyer's, in accordance with the established principle of 'caveat emptor' ('let the buyer beware'). Thus, the assessment and remediation of contaminated land may be required:

(i) by the owner of the land in response to regulatory pressure;

(ii) by the owner to remove liabilities prior to divestiture of land;

(iii) by the business owner, in an effort to rationalise company assets and internal liabilities, and to realise inefficient and wasteful aspects of business operations, or;

(iv) by a potential buyer wishing to protect against exposure to future environmental liabilities.

Furthermore, site investigations may also be carried out by land developers wishing to upgrade or improve land in support of planning applications, for industrial use or residential use, and for future sale. Between 1976 and 1990 the UK Department of the Environment, through the Interdepartmental Committee on the Redevelopment of Contaminated Land (ICRCL), issued guidance notes on the redevelopment of contaminated land and put forward soil quality criteria for a limited range of individual contaminants. These guidelines are soon to be superseded by revised risk-based soil assessment criteria.

1.2.2.3 Risk Assessment and Management

Risk assessment at contaminated sites entails characterisation of the source of a hazard (i.e., monitoring the abundance, distribution, toxicity and chemical form of the contaminants), identification of exposure routes via which a contaminant may come into contact with a sensitive receptor (including, for example, determination of the subsurface partitioning of contaminants), assessment of the relationship between the contaminants and the adverse effect produced (analysis of the toxicological properties of the contaminants) and, finally, estimation of the risk (Petts, 1994). Its primary function is as a technical framework in which to examine contaminated land, derive target remediation levels and protect environmental health (RCEP, 1996).

The technical uncertainties and limitations of chemical monitoring techniques described above in Section 1.2.2.1 have particular relevance to the assessment of hazards and exposure routes, and to the verification of successful control or attenuation of contaminants. This is particularly the case for petroleum contamination.

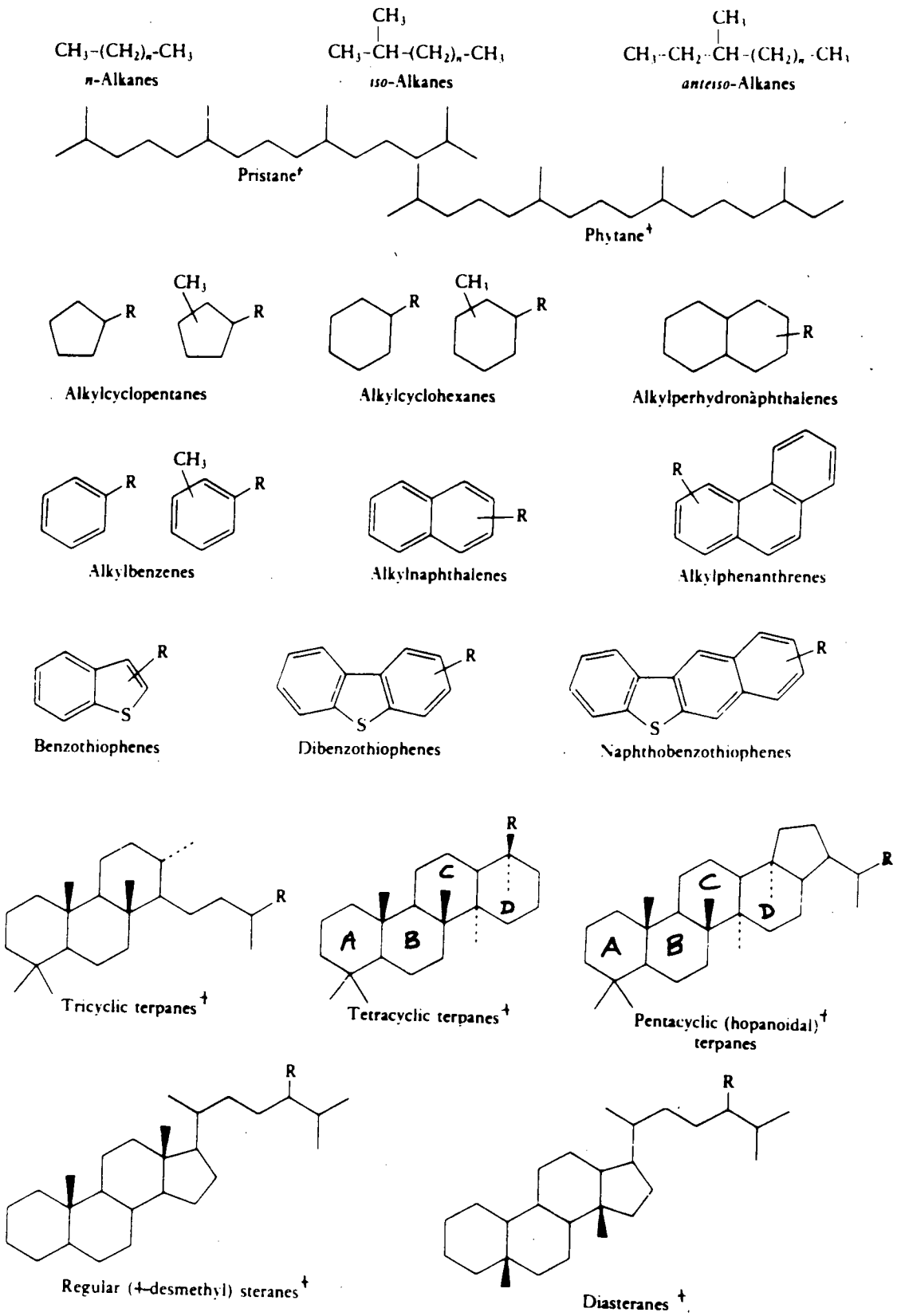


Figure 1.2 Chemical Structures of Compounds in Petroleum
(† = Common Biomarkers)

(iii) Olefins, or alkenes, which are generally almost non-existent in crude oils but which may occur in cracked refinery streams, particularly light and middle distillates (Altgelt & Boduszynski, 1995). Olefins contain at least one non-aromatic carbon-carbon double bond in their chemical structure.

(iv) Aromatics, which generally take the form of one or more benzene rings bearing attached alkyl side chains. Monoaromatic compounds comprise of only one aromatic ring (e.g., benzene, toluene); diaromatic compounds possess two aromatic rings (e.g., naphthalene), and so on. Aromatic compounds may also contain cyclic alkanes as part of their structure, giving rise to many different individual components. Increasing numbers of aromatic rings result in compounds of increasing structural complexity, as does the arrangement of the rings and the way in which the rings are fused (e.g., catacondensed as in chrysene, or pericondensed as in fluoranthene and pyrene), and the nature and position of alkyl side chains. The most abundant aromatic hydrocarbons are the low molecular weight alkylbenzenes and alkyl naphthalenes, in which the alkyl chains are usually of length comparable to the *n*-alkane aliphatic hydrocarbons. On polynuclear aromatic hydrocarbons (PAHs), alkyl substituents are usually shorter, taking the form of methyl and ethyl groups. Some examples of aromatic hydrocarbons described above are shown in Figure 1.2.

(v) Biomarkers, which are specific constituents of oil and oil-derived products that can be unambiguously linked to the specific biological compounds from which they derive. Typically present in low abundance (< 1 % w/w in most crude oils), biomarkers are considered to be 'fossil' compounds, because they possess the same basic inert macromolecular structure as their biological precursor compounds present in the original deposits. The commonest biomarker compounds are acyclic isoprenoids, steranes and terpanes, formed by the same reaction pathways as other biogenic organic compounds, i.e., defunctionalisation of oxygen-

containing groups within the original biological species, (for example, pentacyclic triterpenoid for formation of hopanes) followed by hydrogenation to yield the final saturated alkane (Killops and Killops, 1993). Structures of some important hydrocarbon biomarkers are shown, along with other compounds typically found in oil, in Figure 1.2.

Of the terpane biomarkers, tricyclic terpanes usually occur in the C_{20} - C_{26} carbon number range, tetracyclic terpanes occur in the C_{24} to C_{27} range and pentacyclic terpanes, or hopanes, in the range C_{29} - C_{35}. Further variations in structure occur in the position and nature of any methyl ring substituents, the length of the alkyl chain and as a result of isomerisation at chiral acyclic and cyclic centres. The latter phenomenon is caused by the loss and re-addition of hydrogen at chiral centres during petroleum generation and results in the formation of epimer pairs, the ratio of which is considered to be unique to individual oils (Killops and Killops, 1993).

Heterocompounds are present within oil as a result of the occurrence of oxygen, nitrogen and sulphur within the original oil-producing biological detritus. Heterocompounds are found in varying amounts in oils, depending upon the depositional environment, the maturity of the oil formation and the extent of degradation undergone by the oil in the reservoir. Compounds containing sulphur, such as benzothiophene, dibenzothiophene and their alkyl derivatives, are often the most abundant heterocompounds in oil deposits, with the analogous oxygen-containing (e.g., benzofurans and dibenzofurans) and nitrogen-containing (e.g., pyrrole and pyridine derivatives) compounds in lower amounts, although for many oils the relative amounts may differ. The range of possible heteroatom substitutions and functionalities adds considerable complexity to the composition of oils.

Oil constituents have, by convention, been categorised (according to their solubility characteristics) into four solubility classes, or class fractions: the saturate fraction, typically consisting of *n*-alkanes, isoprenoids and alicyclic alkanes, and soluble in alkane solvents such

as heptane and pentane; the aromatic fraction, comprising mono-, di- and polynuclear aromatic hydrocarbons, soluble in aromatic solvents, e.g., benzene; the polar fraction (sometimes referred to as resins), containing sulphur- (benzothiophenes), nitrogen- (quinolines, carbazoles), and oxygen-substituted (carboxylic acids) heterocompounds, soluble in polar solvents such as methanol; and the asphaltene fraction, a highly complex, low solubility class comprising naphthenic acids, metalloporphyrins, extended carboxylic acids and other high molecular weight hetero-substituted compounds (Leahy & Colwell, 1990).

The class fraction distribution of an oil is a surrogate means of conveying information on its chemical composition and also assists with the classification of petroleum products as 'light', 'medium' or 'heavy'. Light oils, such as gasoline, diesel and kerosene, are generally comprised of low boiling, less dense compounds, which usually report in the saturate class fraction. Light oils are therefore commonly characterised by large saturate class fraction abundances. Conversely, so-called 'heavy' oils, such as heavy fuel oil, some lubricating oils and tars, usually contain significant amounts of high boiling compounds. Since the majority of polyaromatic, polar and asphaltenic class compounds exhibit high boiling points, oils termed 'heavy' are generally associated with large polar and asphaltene contents and lower saturate class fraction abundances. However, it should be recognised that this may not always be the case, since some highly paraffinic oils rich in *n*-alkanes may have significantly higher boiling points than oils with less saturates (Altgelt & Boduszynski, 1994).

Whereas the composition of crude oils is determined by the geochemical environment of the reservoir in which it forms, the relative abundance of chemical components within industrial contaminant source terms depends principally upon the degree of processing the oil has undergone. Petroleum products are in essence different boiling fractions of crude oil obtained from distillation columns operated either at atmospheric pressure (for lighter boiling cuts) or under vacuum (for higher boiling cuts). The respective products are, therefore,

commonly characterised according to distillation temperature and carbon number range. Gasoline, for example, is characterised as distilling between approximately 40 and 200 °C and comprising C_5 to C_{10} alkanes and monoaromatics (particularly benzene, toluene, ethyl benzene and xylene (BTEX) compounds); diesel fuels generally distill up to *ca.* 325 °C and contain higher boiling straight chain alkanes of carbon number C_{10} to C_{25}; heavier fuel oils comprise compounds of C_{14} and over and are generally found to distill above 275 °C. Properties and description of various petroleum products are shown in Table 1.3.

Although these and other similar parameters (such as density and viscosity) are useful means of describing generic bulk properties of different oil types, they do not convey the extreme chemical complexity of these products and the fact that even the simplest petroleum products such as gasoline may contain many hundreds of individual, and closely related, compounds. Heavier products, are even more chemically complex, typically consisting of several thousand individual chemical species (Altgelt & Boduszynski, 1994). It is partly because of this vast chemical complexity, and partly because of the limitations of analytical measuring techniques, that it is not possible to complete a full analysis of every compound contained within an oil.

1.3.2 Contaminant-Soil Interactions

A variety of interactions take place between the spilled petroleum product and the soil environment that affect the chemical composition of the contaminants and their partitioning throughout the subsurface. These processes have significant bearing upon how a particular contaminant may be analysed, the risks that it may pose and the manner in which it should be treated.

1.3.2.1 Subsurface Partitioning Characteristics

The subsurface partitioning of petroleum contaminants is important to the characterisation of petroleum-contaminated land in three main respects.

Table 1.3 Characteristics of Petroleum Products[1]

Fraction	Distillation Temperature (°C)	Carbon Number Range	No. of Paraffin Isomers
Gases	Below 20	C_1 - C_4	4
Gasoline	40 - 200	C_4 - C_{10}	*ca*. 400
Middle Distillates (e.g., Diesel, Kerosene)	175 - 325	C_{12} - C_{25}	*ca*. 3.7×10^7
Gas Oil	275 - 550	C_{15} - C_{45}	*ca*. 8.2×10^{15}
Atmospheric Residues	> 450	> C_{20}	> 8.2×10^{15}
Vacuum Residues, Asphaltenic Material	> 550	> C_{60}	> 2.0×10^{22}

[1]Adapted from Nyer and Skladany (1989), and Altgelt and Boduszynski (1994)

Firstly, sampling and analytical programmes must take into account the full range of phases into which contaminants may have partitioned in order to realise the true extent of contamination. Methods which focus only on one or two phases risk underestimating the amount of contaminant actually present (Hrudey & Pollard, 1993).

Secondly, knowledge of subsurface partitioning allows appropriate remediation technologies to be selected and targeted accordingly (Sims, 1990). This has particular relevance for heavy oil contaminants, where the free product trapped in the subsurface (residual contamination) acts as a secondary source from which contaminants can gradually leach into local groundwater. The application of groundwater pump and treat technologies which focus only on removing contaminants from extracted groundwater fail to target the residual contamination and, therefore, often result in an unnecessarily prolonged, expensive and ineffective site cleanup operation.

Thirdly, the partitioning of contaminants throughout the possible subsurface phases is an important factor in site risk assessment and management, since phase-partitioning governs the routes by which receptors (human or otherwise) may become exposed to the contaminants and the subsequent bioavailability of the exposed contaminant (i.e., its propensity to take part in biological reactions). At sites featuring highly volatile contaminants that partition readily into the vapour phase, for example, inhalation exposure will be a primary concern, whereas when polar contaminants are present, and groundwater becomes contaminated, ingestion will be an important exposure route.

In short, the partitioning of contaminants between the available air, water, soil natural organic matter (NOM), soil mineral and free product phases during their passage through the subsurface is governed by the fundamental physico-chemical properties of the contaminants and the geology and hydrogeology of the subsurface (Mattney Cole, 1994).

Immediately upon entering the subsurface, petroleum contaminants move downwards due to gravity at a rate governed principally by the permeability of the subsurface and the

density of the contaminant. In general, soils possessing higher organic matter or clay contents tend to impede the flow of contaminants, because of their higher adsorption capacity and lower permeability. Sandy soils, on the other hand, are much more permeable and exhibit lower adsorption capacities, and so convey contaminants much more readily.

Fluctuations in the amounts of water and air contained within pore spaces at the various soil depth horizons are a key factor in the behaviour of contaminants entering the subsurface. In general, an increase in depth is characterised by an increase in soil water content and a reduction in soil oxygen content. In the unsaturated zone, the soil above the highest permanent groundwater level, interstitial spaces are partially filled with water and partially with air. Below this zone soil becomes increasingly saturated and oxygen-deficient, until 100 % saturation is reached below the permanent water table (the saturated zone).

The area immediately below the unsaturated zone is termed the capillary zone. Here, water from below is drawn up by capillary forces into normally unsaturated interstitial spaces thereby increasing the degree of saturation. In many cases, the depth of each zone may vary as the water table fluctuates periodically according to seasonal variations or on an occasional basis (e.g., due to heavy rainfall) (Morgan & Watkinson, 1989). As a general rule, the bulk contaminant plume migrates freely through the unsaturated zone until it reaches the intermediate saturated zone, whereupon some lateral spreading of the plume will take place (usually in the direction of groundwater flow). As the free product flows, discrete 'pockets' of residual contamination become immobilised in the unsaturated zone.

It is generally possible to identify six phases into which petroleum wastes may partition (Figure 1.3) (Hrudey & Pollard, 1993):

(i) immobilised pockets of residual contamination in the unsaturated zone;

(ii) individual contaminant constituents adsorbed to soil NOM or mineral matter;

(iii) the vapour phase, through evaporation;

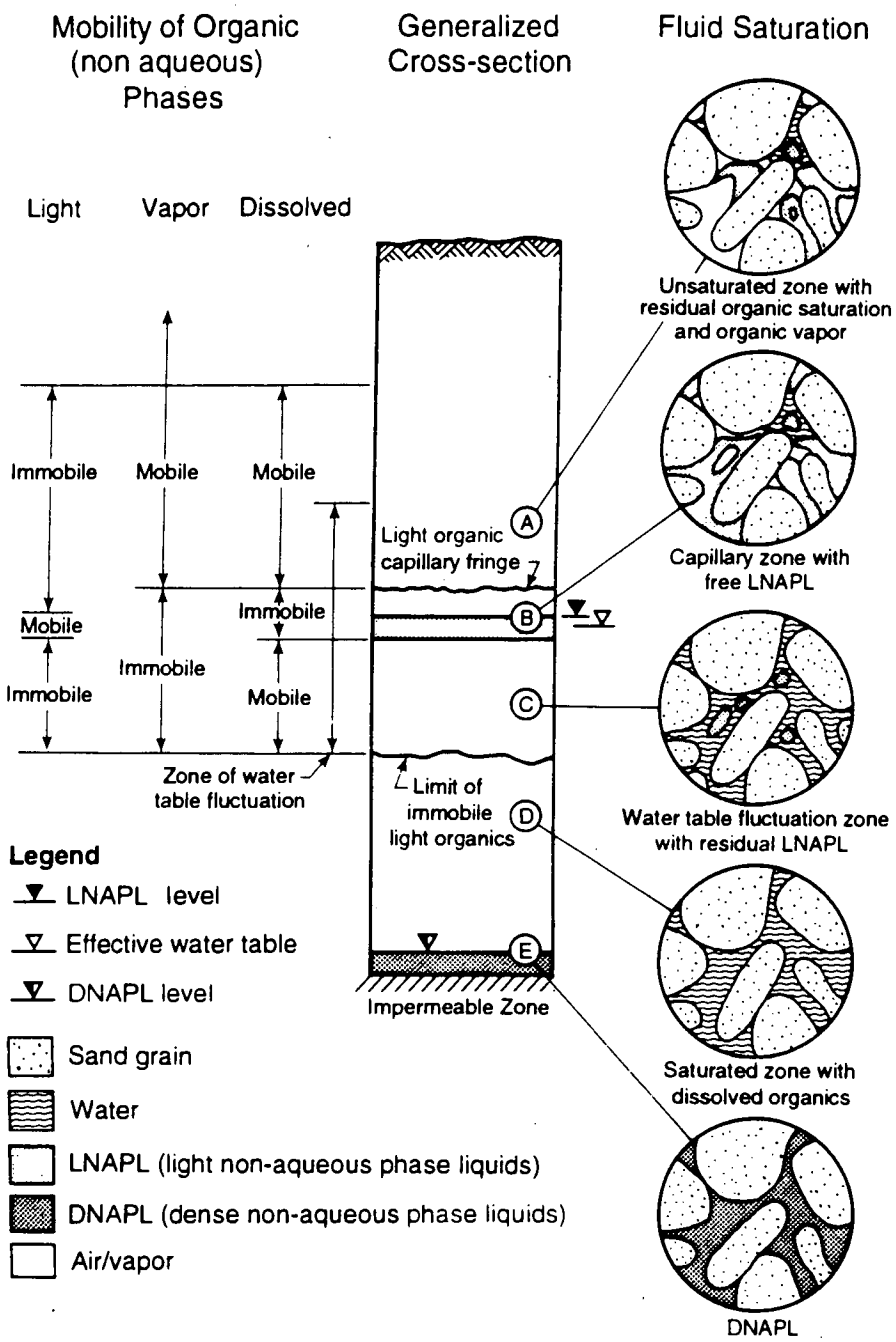


Figure 1.3 Subsurface Phase Distribution of Petroleum Contaminants (Hrudey & Pollard, 1993)

(iv) the aqueous phase, dissolved in groundwater in the saturated zone or interstitial water in the unsaturated zone;

(v) mobile contaminant, termed non-aqueous phase liquid (NAPL), which is either less dense (LNAPL) or more dense (DNAPL) than water, and;

(vi) a contaminant-water emulsion.

At heavy oil contaminated soils, the majority of waste components partition between the free product and water, according to their aqueous solubility, with the more polar waste components tending to partition into the aqueous phase and nonpolar components partitioning to the NOM or bulk organic nonaqueous phase liquid (NAPL), the free product. The mobility of NAPL tends to decrease with increasing saturation, since water is much more mobile through pore spaces than petroleum. Thus, as contaminant penetration increases (and saturation increases), aqueous phase contaminants tend to migrate much more rapidly through soils, depending on their volatility, rate of biodegradation and flow of soil water, whereas NAPL tends to become trapped in pore spaces by surrounding water.

Trapped NAPL in the saturated and intermediate (capillary) zones can result in the continual leaching of contaminants into the aqueous phase over time, particularly as water levels rise and fall, and the smearing of bulk NAPL occurs across soil particles (Johnson & Laidler, 1994). If water levels rise sufficiently high, or there is considerable flow of water down the soil horizon (e.g., as a result of heavy rain or rapid snow melt), contaminants may also be drawn into the aqueous phase from the residual contamination in the unsaturated zone (Mattney Cole, 1994). Heavy or residual oil contamination, therefore, represents a significant long-term threat to both soil and groundwater quality.

1.3.2.2 Contaminant Weathering and Biotransformation

Immediately upon entering the soil subsurface, petroleum contaminants are subjected to a number of physical, chemical and biological reactions that remove the more labile

components of the waste to alter its composition (Morgan & Watkinson, 1989; Atlas, 1991). These processes are collectively referred to as 'weathering'. Specifically, weathering can be divided into four main processes: (i) volatilisation, (ii) dissolution, (iii) chemical alteration, and (iv) biotransformation.

The extent to which any particular contaminant is weathered, and the manner in which this occurs, depends principally upon the chemical composition of the fresh product and the specific properties of the receiving soil. External environmental factors such as temperature, humidity and precipitation may also affect contaminant weathering, although to a lesser degree (Morgan & Watkinson, 1989). Because weathering involves the transformation and transport of individual contaminants away from the initial bulk waste, it has significant influence on the nature of the hazard presented by particular waste, the possible routes via which exposure might occur and the treatability of the contaminants. Analysis of contaminant weathering through chemical monitoring techniques is, therefore, of great importance to the overall characterisation of a particular contaminated site.

(i) Abiotic Weathering.

The principle physical processes influencing the post-depositional transformation of spilled petroleum products are volatilisation, chemical alteration and dissolution. Volatilisation refers to the partitioning of compounds into the vapour phase and their subsequent migration to and loss through the soil surface. Volatilisation is most prevalent in the immediate aftermath of a petroleum release, and is enhanced when lateral spreading of the contaminant occurs (e.g., when vertical penetration is restricted) and in hotter climates (Bossert & Bartha, 1984). Following removal of low-boiling components to volatilisation, spilled petroleum products tend to become much more viscous and recalcitrant in the subsurface. However, in many cases volatilisation is not considered to be a significant attenuation mechanism, due to the movement of petroleum products down the soil column, where evaporation is minimised (Morgan &

Watkinson, 1989). Clearly, for heavy oils containing few low-boiling components, volatilisation is very limited.

Chemical alteration processes such as hydrolysis, photolysis, oxidation and reduction tend to be a factor only for specific chemicals, such as chlorinated solvents, and in specific environments (e.g., predominantly aquatic environments) . As such, these processes are of little importance to petroleum products in soil (Morgan & Watkinson, 1989).

Dissolution is the process whereby contaminants partition into the aqueous phase and are subsequently attenuated by the flow of groundwater (Mattney Cole, 1994). This is considered to be by far the most significant physical process affecting spilled petroleum products in soil. The rate at which different compounds are lost to dissolution, therefore, depends on their solubilities in water. The most susceptible components in most petroleum products are the heterocompounds (e.g., phenols and benzothiophenes), followed by the aromatics (particularly BTEX compounds) and lastly the saturated hydrocarbons, which are essentially unaffected by dissolution.

In addition to the short-term removal of readily water-soluble compounds, dissolution may also have a longer-term effect on any residual contamination left in the unsaturated zone, whereby compounds from pockets of NAPL are slowly but steadily leached into the subsurface aqueous phase. As discussed above (Section 1.3.2.1), this is a common occurrence at sites where residual petroleum contamination is a feature, and can present a major long-term threat to groundwater quality.

(ii) Biotic Weathering.

Under most conditions, the biotransformation of petroleum products in soil due to the action of soil microbial communities is the predominant route of weathering (Morgan & Watkinson, 1989; Leahy & Colwell, 1990). This is a natural process, similar to that carried out by many other living organisms (including humans), in which soil microorganisms use organic compounds as external energy sources to obtain carbon, for cellular growth

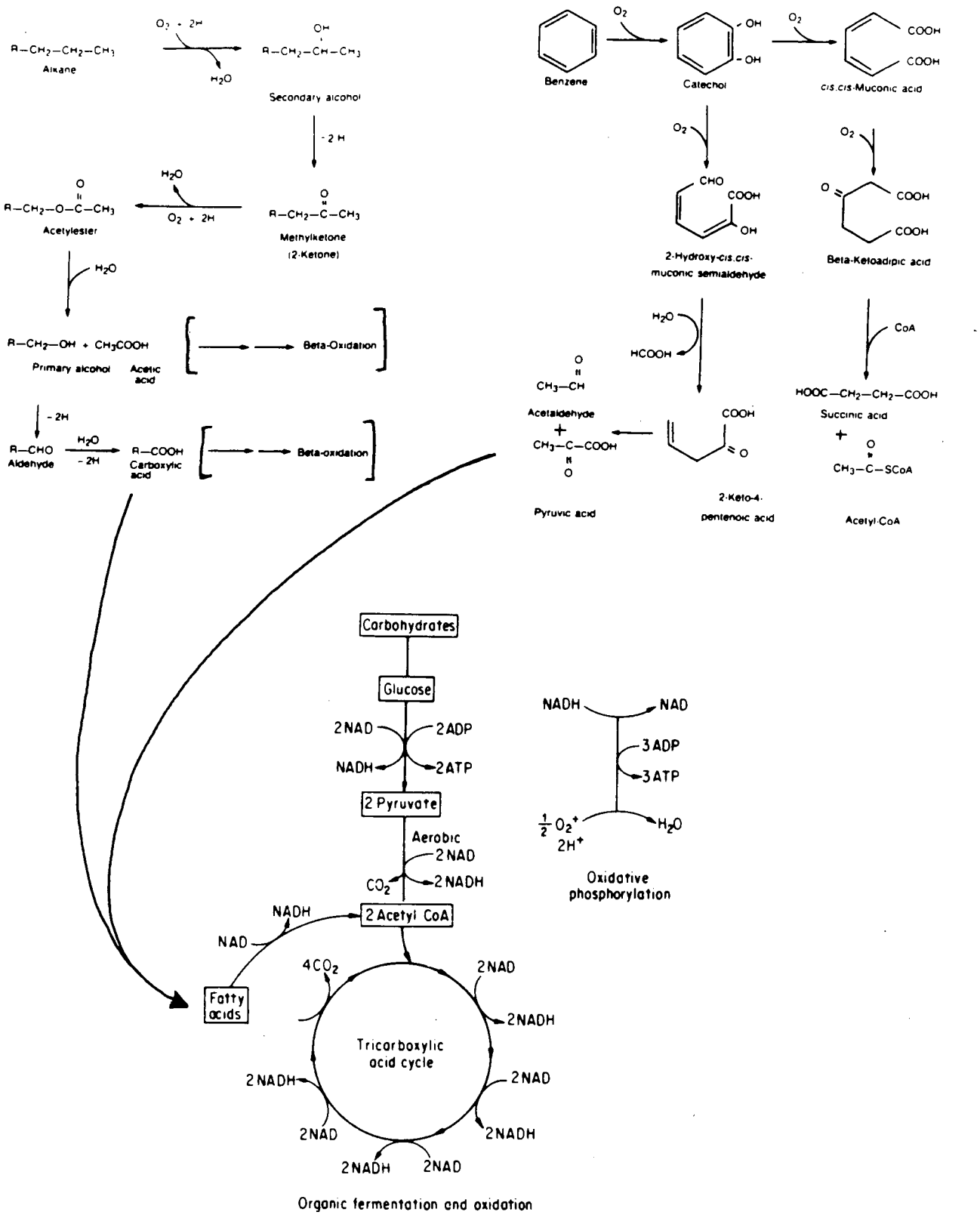


Figure 1.4 Selected Aerobic Oil Biotransformation Pathways

(iv), the length of time contaminants have been in the soil (microbial recalcitrance has been shown to increase with increasing contaminant soil residence times (Hatzinger & Alexander, 1995)).

Factors (ii) and (iv) are particularly relevant to this study, as residual petroleum contaminants frequently exist as discrete pockets of NAPL in the subsurface at contaminated sites, are poorly bioavailable and have usually spent considerable time in the subsurface.

In summary, elucidation of the respective weathering processes is crucial to the effective management of contaminated land because: (a) weathering has a significant effect on the nature of the hazard posed by a particular waste and the routes via which exposure might occur, and is, therefore, an important consideration in risk assessment; (b) weathering can thwart attempts to identify potentially responsible parties by altering contaminant source terms to such an extent that chemical fingerprinting techniques fail to match contaminants with suspected source products; and (c) it allows remediation efforts to be focused on enhancing the preferred natural degradation routes.

In particular, characterisation of biotic weathering is crucial to the appropriate application of bioremedial techniques, particularly for residual petroleum contaminants that display limited bioavailability and that have been in the subsurface for a considerable period of time. Whereas much has been published on the microorganisms responsible for biotransformation, the metabolic products of biotransformation and the classification of metabolic pathways, experimental techniques for measuring biotransformation rates have not been established (Englert *et al.*, 1993). One of the greatest challenges for the successful development of bioremediation technologies is the development of chemical monitoring techniques capable of verifying *in situ* biotransformation (Madsen, 1991; Voos, 1996; Herbert, 1996). Elucidation of contaminant weathering through chemical monitoring is, therefore, a fundamental aim of this work.

1.3.3 Remediation of Petroleum-Contaminated Land

Where site investigation data reveal a need to remediate a piece of contaminated land, a number of factors are usually taken into account before remediation operations commence. The key question is how best to achieve pre-determined remediation goals using the range of remediation techniques available. For petroleum products in the soil environment, a wide range of remediation techniques are available, each exhibiting particular strengths and weaknesses (Long, 1993; Tadesse *et al.*, 1994; Ellison, 1992; Bardos, 1994; Sims, 1990). Feasibility tests to determine the efficacy of candidate techniques increases the possibility of successful remediation. The most efficient approach of remediation techniques is usually a 'treatment train' approach, in which a number of remediation techniques are applied to effect the complete removal of the range of contaminants represented (Sims, 1990).

In the UK, the predominant means of controlling a contaminated site to date has been to either remove the contaminated soil, usually to landfill, or to cap the contaminated area using a low-permeability barrier (RCEP, 1996). Both of these approaches contain inherent disadvantages. Neither ensures the removal of contaminants from the environment. Removal is quick and cheap, but is disadvantaged by rising landfill costs, soil transportation difficulties, the possibility of adverse health and safety effects created by contaminated soil disturbance, and because it does not present a viable long-term solution. Capping is also cheap and fairly quick, but uncertainties over subsurface geology and hydrogeology and the possibility of barrier degradation, as well as the lack of long-term viability create questions over its continued use (RCEP, 1996).

Increasing emphasis is, therefore, being placed on the development of long-term, sustainable methods for the treatment of petroleum-contaminated soils. A broad range of methods exist, which can generally be categorised as follows (RCEP, 1996; Gilbert, 1992):
(i) Physical processes, e.g., soil vapour extractions, pump and treat systems and air stripping. These techniques isolate or concentrate contaminants in a form that allows their destruction or

recycling by some other process. Their advantages are their relatively low cost and their high level of user control. However, they do not destroy the contaminants themselves, and often tackle only the symptoms of contamination, and not the actual source;

(ii) Chemical processes, such as solvent extraction, chemical dehalogenation, oxidation and surface amendments, which exploit the chemical reactivity of contaminants to facilitate their destruction, immobilisation, extraction, conversion or neutralisation. These techniques have the advantage of actually attempting the destruction of contaminants (or facilitating their straightforward destruction), although this is not always successful, and the process may even add chemicals to the subsurface;

(iii) Thermal treatment, e.g., high-temperature incineration and techniques that enhance contaminant mobility. These techniques also lead to the ultimate destruction of contaminants, but often involve high-costs and the use of secondary treatment processes;

(iv) Solidification, e.g., vitrification, or cement-based solidification. Used predominantly for metal-contaminated soils, these techniques can immobilise contaminants and remove environmental risks for many years, but can involve high cost and do not actually involve the destruction of contaminants, and;

(v) Biological methods, such as *in-situ* bioremediation, natural attenuation, land farming, *ex-situ* bioslurry and biopile techniques, and bioventing.

Biological techniques are emerging as the preferred method of treating petroleum-contaminated soils because they are relatively cheap, they facilitate the complete or partial degradation of the contaminant to a less harmful or less mobile form, and they can be applied to large areas of land. Moreover, unlike other treatment approaches, they do not damage the integrity of the soil infrastructure and so are considered to offer a 'sustainable' approach to contaminated land treatment. In essence, bioremediation techniques harness the natural microbial transformation of petroleum products in soil to reduce concentrations of a particular product to acceptable levels. This may be achieved through management of the natural

assimilation process in such a way as to limit adverse effects of a contaminant plume, called intrinsic bioremediation, or through an engineered bioremediation system, which could be *in-situ* or *ex-situ*, and which seek to create conditions favourable for accelerated microbial mineralisation of contaminants (see Table 1.4).

Although a wide variety of bioremediation techniques have been developed, they have the common aim of contaminant reduction through microbial action and are, therefore, governed by the same general principles of petroleum microbiology described in Section 1.3.2.2. Despite the advantages of these techniques, they can be subject to several potential disadvantages at the present time, such as variable treatment times, the possible generation of new, more toxic biotransformation intermediates, and the poor degradation of microbially recalcitrant or non-bioavailable compounds (Pollard *et al.*, 1994).

A prime requirement at the present time, therefore, are methods for demonstrating the effectiveness of bioremediation, particularly intrinsic bioremediation, in the field. However, no standard, universally-approved analytical techniques have been established for:
(i) monitoring the actual loss of contaminants due to microbial activity, and;
(ii) assessing the bioremediation potential of petroleum contaminants, through detection of microbially resistant compounds or evaluation of contaminant bioavailability.

This has led to a gradual erosion of confidence in the effectiveness of the technique. Recently, with the application of bioremediation methods in a risk management framework, it has become important to demonstrate through exposure and risk assessment analysis that managed bioattenuation results in the reduction or control of risks to humans and the environment. Developing an understanding of the fate, partitioning and transport properties of petroleum contaminants in soil through chemical analysis is an essential element of this demonstration.

Table 1.4 Biological Treatment Techniques for Petroleum-Contaminated Soil[1]

Technology	Mode of Operation	Advantages	Limitations
In-Situ Engineered Bioremediation	Enhancement of natural biotransformation processes through engineered introduction of nutrients, water and oxygen into the contaminated soil subsurface	- Low cost - Does not destroy soil structure and biology - Little or no residual wastes generated - Highly effective in certain conditions and for certain petroleum wastes	- Low cleanup levels may not be possible - Uncertain remediation timescales - Confirmation of cleanup difficult - Limited in low-permeability soils - Limited efficiency in colder climates
Natural Attenuation	Remediation of contaminated area through management of biotransformation processes and containment	As above, and - Can be applied to larger areas - Even lower costs involved - Completely 'natural' approach not involving exogenous materials	As above, and - Effectiveness even more influenced by site conditions - Long-term monitoring required
Land Farming	Contaminated soil removed and spread over treatment area. Natural microbial activity enhanced by periodic addition of nutrients and water, and aerated by tilling	- Low cost - Can be used as 'polishing' treatment at end of treatment train - Can achieve low contaminant levels - Established technology	- May take up large treatment area - Possibility of emissions due to evaporation - Requires removal and transport of contaminated soil
Bioventing	Engineered removal of volatile compounds from soil matrix, *in-situ* or *ex-situ*, with subsequent treatment of extracted vapour.	- Effective removal of low-boiling contaminants - Little or no residual waste to dispose - Moderate treatment times	- Treatment of extracted vapour (e.g., with activated carbon) may be expensive - Limited for medium and high boiling contaminants - Limited for low-permeability soils
Bioreactor/ Bioslurry	Engineered *ex-situ* treatment involving optimisation of conditions for microbial degradation of individual portions of contaminated soil	- Complete mineralisation of contaminants possible - Fast treatment times possible	- Most expensive of biological technologies - Technology not fully developed

[1]Sources: Jespersen et al. (1993) and Ellison (1992)

1.4 THE CHALLENGE OF HEAVY OIL-CONTAMINATED SOILS

Some of the most pressing technical concerns that arise whenever contamination of soil environment occurs, or is discovered to have occurred, relate to:

(i) the type, amount and distribution of contamination present;

(ii) the risk to human health and the environment presented by these contaminants;

(iii) the source of the contamination;

(iv) the selection of appropriate site management methods, and how best to control the contamination or effect its treatment, and;

(v) the effectiveness of remediation treatment techniques in removing the contaminants from the soil environment, particularly bioremediation techniques.

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 microbial recalcitrance of individual components are such that resolution of these issues is fraught with uncertainties (Song *et al.*, 1990).

For the purposes of this thesis, the particular challenges that such sites present have been categorised into two broad themes that reflect the concerns cited above:

(1) The analytical challenges associated with the chemical characterisation of these wastes (termed the 'contaminant source terms'), and the constraints these impose upon assessment of risks, waste remediation potential, and general contaminant behaviour in the subsurface, and;

(2) The challenges associated with the assessment of biotransformation of heavy oil contaminants and their potential sources, particularly following prolonged weathering.

These themes form the two central areas of research in this thesis, and are discussed in detail below.

1.4.1 Characterisation of Heavy Oil Source Terms

Techniques used in the analysis of petroleum hydrocarbons in soils and water can be divided into two categories, depending on their point of application and the level of analytical detail which they are capable of producing: screening techniques, including field monitoring and conventional non-specific methods, and techniques for detailed component analysis. In formulating an effective, well-balanced, financially viable analytical strategy, an accurate understanding of the capabilities and limitations of individual techniques is of utmost importance.

Conventional analytical methods are subject to substantial constraints when applied in isolation to heavy or residual petroleum wastes. Specifically, the number, diversity, polarity, high molecular weight and low volatility of the contaminants are such that:

(i) Rapid spectrophotometric field screening techniques are unable to accurately determine the amount of contaminant present, or resolve individual groups of components (Douglas & Uhler, 1993). The central constraint concerns the insensitivity of the technique to extracted waste components not exhibiting detectable absorption bands at the monitoring wavenumber, i.e., non-saturate class components. Heavy or highly weathered oily wastes often contain reduced saturate class fraction abundances. In addition, samples are quantitated against a standard hydrocarbon mixture and not the actual spilt material. Since different hydrocarbons exhibit different responses to the spectrophotometer, such a comparison cannot be relied upon to produce true contaminant concentrations (Fan *et al.*, 1994).

(ii) GC-FID, the most widely used tool for analysing crude oils and associated products (Fan *et al.*, 1994) is sometimes unable to achieve sufficient analytical recovery or adequate resolution of individual compounds (non-volatile, refractory residues, for example, invariably bond tightly to gas chromatographic columns and are generally only removed following prolonged heating at elevated temperatures).

Methods based on GC-FID are, therefore, unable to facilitate an accurate quantitative analysis of heavy oil contaminants, and often feature; high recoveries, caused by carry over (or memory effects) from high boiling residual components on the column, column bleed and mass discrimination of high molecular weight hydrocarbons in the injection port and/or syringe; low recoveries, which can be caused by the use of overly diverse hydrocarbon component standards; and, most significantly, the presence of a large unresolved complex mixture (UCM) (Figure 1.5), which impedes component detection and may cause high or low recoveries, through individual peak and baseline interference, respectively (Gough & Rowland, 1990).

(iii) The use of conventional GC-CI MS and GC-EI MS can sometimes be seriously impeded, not only by poor chromatographic resolution of high boiling components, but also by lengthy sample preparation and analysis procedures, and resultant high costs. Furthermore, in their standard form, these methods are used to detect only a few specified priority PAH pollutants, and critical information on the composition and behaviour of the bulk of the petroleum contaminants is missed (Douglas & Uhler, 1993).

Faced with limited analytical capabilities, many site assessors have turned instead to generic, 'catch-all' methods for characterising heavy oil-contaminated sites, such as measurement of 'total oil and grease' parameter (Martin Jr. *et al.*, 1990). This approach involves the collective measurement of all hydrocarbon constituents over a particular detection range (specific to the analytical instrumentation used), which may or may not be defined. Whilst this method is useful for indicating the amount of contamination present, it provides no information on the chemical composition of the contaminants. In addition, poor extraction efficiencies (Douglas *et al.*, 1992) and superfluous analysis of co-extracted natural organic matter (NOM) (White & Irvine, 1994) can result in considerable inaccuracies in reported contaminant loads. Use of this surrogate approach in isolation, therefore, precludes a meaningful assessment of risks and waste remediation potential.

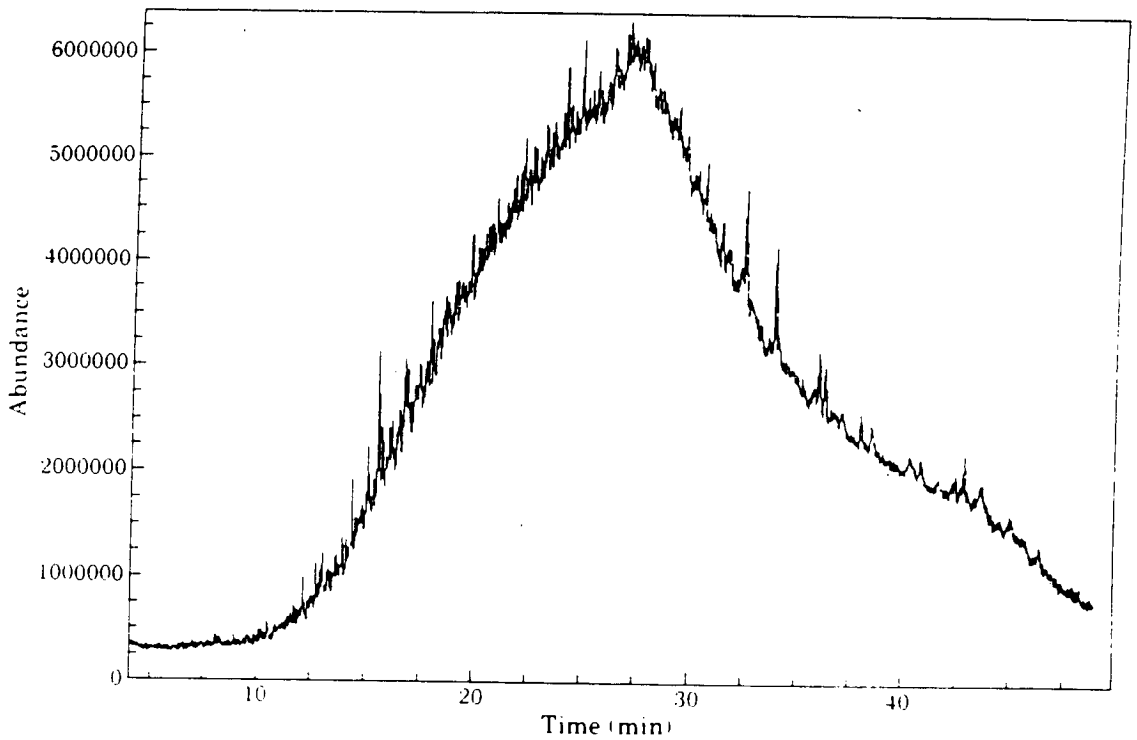


Figure 1.5 Example of Unresolved Complex Material (UCM) in GC Profiles of Heavy Oils

For heavy oil-contaminated sites, the need to assess bioremediation potential is particularly acute because of their known resistance to microbial degradation in some cases. As previously discussed (Section 1.3.2.2), this is due to the chemical composition of the waste product, which may contain a predominance of high-molecular weight, structurally complex compounds inherently resistant to biotransformation, and the length of time it has been resident in the subsurface, which influences contaminant weathering, bioavailability and phase-partitioning.

At many heavy oil-contaminated sites, residual contamination has usually been in the subsurface for an unknown length of time, and will have undergone an unspecified period of natural biodegradation. As biodegradation proceeds, the more labile components of the waste, usually the saturate class fraction components, are removed, leaving behind a residual fraction increasingly concentrated in recalcitrant petroleum components (mainly polar and asphaltenic). Thus, the degree of natural biodegradation already undergone by a particular petroleum waste, as indicated by its proportion of recalcitrant constituents, has a significant bearing upon its future amenability to managed natural attenuation or enhanced bioremediation.

Analytical screening of petroleum wastes to assess the type and relative abundance of waste class fractions (particularly the saturate and asphaltene class fractions, the most and least responsive to microbial activity, respectively), therefore, makes it possible to gain insight into the propensity of a particular waste to undergo further biotransformation, either intrinsic or engineered. As discussed above, at heavy oil-contaminated sites information of this type is not being provided by current techniques.

1.4.2 Characterisation of Heavy Oil Weathering

Two important issues pertaining to the characterisation and treatment of petroleum products in the environment are how to unambiguously distinguish contaminant losses due to

microbial activity from those due to other processes, and how to fingerprint heavy oils that may have undergone extensive weathering for source identification purposes.

In many cases, invariably involving the characterisation of crude oil contamination of the marine environment, these questions have been addressed through the use of biomarker-based weathering and source indices (e.g., Volkman *et al.*, 1992). The derivation of these indices is based on accepted principles of petroleum microbiology and, in particular, on the differential rates at which oil components are biotransformed (Section 1.3.2.2).

1.4.2.1 Assessment of Biotransformation

The most convincing documented approach to the assessment of oil biotransformation in field and laboratory studies involves measuring the loss of petroleum components relative to internal conservative compounds, usually oil biomarkers (particularly 17α(H),21β(H)-hopane) resistant to microbial attack, in the form of a weathering (or biotransformation) index (Butler *et al.*, 1991). As discussed in Section 1.3.1, chemical biomarkers are compounds that can be unambiguously traced to biological precursor compounds (Killops & Killops, 1993). The most commonly used biomarkers are pristane, phytane, hopanes and steranes (Figure 1.2), and these are ubiquitous in crude oils and petroleum products.

A range of weathering indices have been used, largely within the marine environment. Most commonly, the depletion in *n*-alkanes is monitored through evaluation of the [*n*-alkanes:17α(H), 21β(H)-hopane] ratio (Prince *et al.*, 1994; Croft *et al.*, 1995). Alternatively, single compounds may be used as surrogate parameters for conveying information on the behaviour of the bulk contaminant. For example, [C_{17}:pristane] and [C_{18}:phytane] have often been used as indicators of oil weathering (Senn & Johnson, 1985; Christensen & Larsen, 1993). Another approach has been to monitor the loss of more amenable alkanes to less amenable alkanes, e.g., [$C_{14+16+18}$:$C_{24+26+28}$] (Wang *et al.*, 1994).

A decrease in the value of weathering indices over time is used as evidence of oil biodegradation. Indices may be evaluated using the measured concentrations of these components (Butler *et al.*, 1992), or quantified peak areas (or heights) following GC-MS analysis (Nordtest Method, 1991). The latter approach is more convenient, but does not convey information on the absolute concentration of oil samples in the environment, and can only be used for determining trends between spatially or temporally altered oil samples.

Examples of the use of weathering indices in oil degradation studies include:

(i) monitoring of spilled crude oil depletion on beaches of Prince William Sound following a bioremedial response to the Exxon Valdez oil spill (Prince *et al.*, 1994);

(ii) evaluation of the relative degrees of biodegradation experienced by marine crude oils (Pande *et al.*, 1994);

(iii) identification of the flow patterns of dissolved hydrocarbons in groundwater (Senn & Johnson, 1985), and;

(iv) monitoring the transformation of oily wastes during controlled bioremedial activities (Croft *et al.*, 1995).

A fuller description of the rationale and previous application of the weathering indices used in this study is provided in Chapter 5 (Section 5.1.4.2).

A number of concerns exist regarding the use of biomarkers as conserved internal markers for monitoring petroleum degradation. These include:

(i) the true microbial recalcitrance of supposed conserved internal markers has not yet been fully assessed (for example, pristane and phytane have both been shown to biodegrade in more weathered petroleum samples, and recently 17α(H),21β(H)-hopane was shown to degrade under optimised conditions (Morris *et al.*, 1996));

(ii) the biotransformation indices that most accurately track the biotic loss of petroleum contaminants have yet to be identified;

(iii) the uncertainty associated with comparing microbially facile waste components with persistent biomarkers, since individual compounds have different solubilities, transport and volatility characteristics (Madsen, 1991);

(iv) uncertainty over the relative sensitivities to biotic loss of the respective biomarker indices, and;

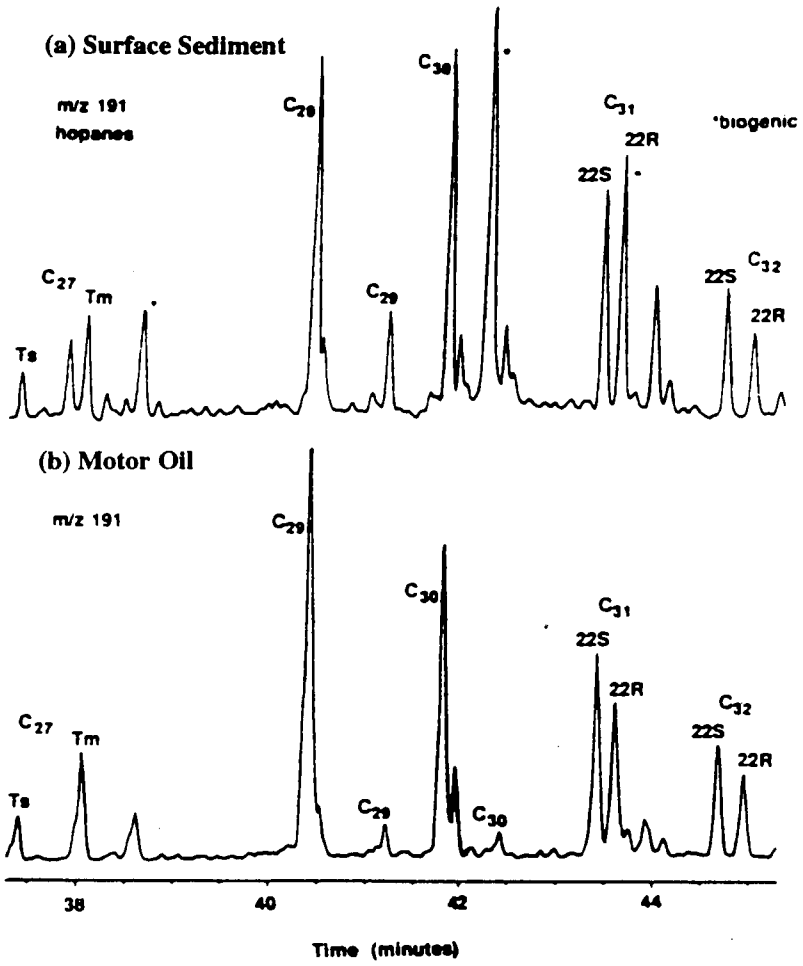
(v) the validity of the analytical methodologies used to identify and determine the compounds of interest, particularly with regards to peak assignment, compound resolution and detector specificity (Redican *et al.*, 1996).

Furthermore, the use of biomarkers as conserved internal compounds must be validated in more environments and for a wider variety of petroleum products. The application of biotransformation indices to heavy or residual oil-contaminated sites is particularly attractive, given their prevalance in the environment, although the difficulties associated with the chemical analysis of these contaminants may exacerbate these already very formidable challenges.

1.4.2.2 Diagnostic Source Identification

Fingerprinting of petroleum products in the environment is commonly undertaken to assess the source of a particular contamination event. It is most commonly achieved through a qualitative comparison of individual biomarker mass chromatogram patterns (obtained by GC-EI MS) (see Figure 1.6), but can also be achieved quantitatively, from a calculation of the ratio of one biomarker isomer to another.

Ratios of certain biomarkers, referred to in this thesis as oil (or source) correlation indices, are sensitive to the geological source of an oil, remaining consistent between related oils. Moreover, because of the environmental persistence of biomarker compounds, their values remain unaltered by oil weathering. A positive or negative identification rests upon a correlation of the contaminant with a candidate source product. Fingerprinting is important for differentiating between potential



;ure 1.6 GC-EI MS Ion Chromatograms for m/z 191 showing the Similarity in Hopane Distribution between (a) an Estuarine Sediment Sample, and (b) a Common Motor Lubricating Oil (Volkman *et al.*, 1992)

contaminant sources, and for establishing potential liability for a spillage, for example when contaminants migrate between land properties (Douglas *et al.*, 1992).

The most commonly used source correlation indices are [pristane:phytane] and [17α(H),21β(H)–hopane:17α(H),21β(H)–norhopane]. As is the case for the weathering indices, these ratios may be evaluated using either the concentration of the individual index components, or their peak areas or heights.

Recently, oil correlation indices have been used to:

(i) demonstrate that certain Alaskan shoreline oil residues originated from pre-1970 industrial oil usage rather than as a result of the *Exxon Valdez* oil spill (Kvenvolden *et al.*, 1995);

(ii) confirm the source of highly weathered No. 6 fuel oil released over 22 years ago from the oil tanker *Arrow* (Wang *et al.*, 1994a), and;

(iii) monitor chemical compositional changes and the fate of weathered crude oil residues from an arctic beach (Wang *et al.*, 1995).

In contaminated land, source correlation indices may be particularly useful for resolving multi-party liability issues that might arise from cross-boundary migration of spilled petroleum products (Douglas & Uhler, 1993). Previous applications of the biomarker indices examined in this study, and the grounds for their utilization, are summarised in Table 1.5.

For heavy or residual petroleum contaminants, diagnostic fingerprinting creates uncertainties, not just because of the problems associated with GC-FID and GC-MS analysis, but also because of the high degree of weathering experienced by the contaminants. Recently, diagnostic parameters that compare the relative abundance of biomarker isomers and PAHs in crude oils have been shown to be reliable source indices in cases of severely weathered crude oils (Douglas *et al.*, 1992; Wang *et al.*, 1994a). However, there is little knowledge of the effectiveness of these source indices when used to establish the sources of heavy oils in the contaminated soil environment, which may have undergone extensive weathering.

Table 1.5 Some Previous Uses of Source and Weathering Indices

Index	Use	Matrix	Rationale	Reference
C_{17}:pristane C_{18}:phytane	Weathering indices	Distillate-contaminated groundwater Residual diesel oil contamination in subsoil	Preferential biotransformation of straight chain alkanes relative to isoprenoid pristane and phytane. Low index values related to increased maturity of contaminant sample	Senn and Johnson (1985) Christensen and Larsen (1993)
Pristane:phytane	Source index	Crude oil in intertidal sediment samples	Ratio constant for light weathering due to initial microbial recalcitrance of branched alkanes	Wang *et al*. (1994)
C_{18}:17α(H),21β(H)-hopane Phytane:17α(H),21β(H)-hopane *n*-Alkanes:17α(H),21β(H)-hopane	Weathering Indices	Crude oil in sandy sediment Crude oil in intertidal sand zone(simulated)	Quantified loss of degradable aliphatic alkanes relative to recalicitrant pentacyclic hopanes to determine microbial degradation of crude oil	Butler *et al*. (1991) Croft *et al.* (1995)
17α(H),21β(H)-hopane: 17α(H),21β(H)-norhopane	Source index	No.6 Fuel Oil in intertidal sediment samples Tar balls and crude oil residues on rocky shoreline	Both cyclic biomarkers demonstrated to be conserved in highly weathered samples. Index value used to distinguish between possible oil residue sources.	Wang *et al.* (1994) Kvenvolden *et al.* (1995)
Tricyclic terpanes: pentacyclic terpanes	Source or weathering index	No.6 Fuel Oil in intertidal sediment samples	Both cyclic terpane groups resistant to general weathering. Ratio may change for highly weathered oils due to ultimate biotransformation of lower carbon/ring number terpanes	Wang *et al.* (1994)

Concerns over the reliability of source and weathering indices are valid because information gleaned this way may be used as a means of establishing liabilities for petroleum contamination, or in screening risk management decisions at contaminated land sites, both of which could involve considerable financial outlay. There is, therefore, a clear need to establish the reliability of source correlation indices and investigate the relative sensitivities and reliability of weathering indices in heavy oils, where uncertainties over the analytical problems are most acute.

In particular, the capacity to monitor contaminant loss due to biotransformation has been identified as one of the most important research needs in the biological treatment of contaminated soils (Voos *et al.*, 1996; Herbert *et al.*, 1996). At the present time no standard experimental procedure exists for quantitatively estimating biotransformation rates, although the need for such a methodology has been recognised by many authors (Douglas and McMillen, 1996; Herbert *et al.*, 1996; Voos *et al.*, 1996).

The work detailed in this thesis addresses the challenges in both of the themes discussed in Sections 1.4.1 and 1.4.2.

In the first section of the study, an approach for addressing the analytical challenges detailed in (1) (Section 1.4) was developed. Details of the results of the successive application of the selected screening and extended analytical techniques to a range of carefully selected heavy oil samples are provided, with the overall aim of improving the description of heavy oil-contaminant source terms.

In the second part of the study, the difficulties described in (2) (Section 1.4) are addressed. Details of the microcosm biotransformation of selected heavy and crude oils are provided. This allowed a full investigation of the performance of established and novel oil biotransformation and diagnostic source indices. Furthermore, it provided an opportunity to contribute to the limited literature pertaining to the laboratory-based (i.e., non-case study) biotransformation of heavy oils, specifically, the experimental design, manipulation of

microcosm conditions, biotransformation kinetics and class fraction variations that are of considerable value in the field-scale design of bioremedial operations.

Finally, a study of the influence of physical weathering on the value of selected source and weathering indices was performed, to shed further light on the ability of biomarker indices to discriminate between biotic and abiotic weathering.

The capacity of the approach detailed above to provide the requisite information is tested using a suite of heavy oils representative of the type of heavy and residual oil contamination encountered at former industrial sites.

The focus of the study is the development of an analytical framework through which the reliable and accurate characterisation of heavy oils can be achieved. The model for this framework is the tiered analytical approach that has found increasing application recently to the chemical analysis of complex petroleum wastes.

1.4.3 The Tiered Analytical Approach

Two general strategies have been developed over recent years for characterisation of petroleum wastes in the environment. The first addresses the waste matrix as a whole and seeks to provide information relating to the full range of compounds encountered in such samples. These have mainly been developed into screening techniques (Section 1.4.1). The second focusses on improved methods of analytical clean-up and specialised chromatographic separation and detection procedures. These generally facilitate the extended characterisation of individual contaminants (Section 1.4.1).

The optimum approach is one that integrates both these strategies into a single tiered framework of chemical analysis (Miller & Stainken, 1990; Pollard *et al.*, 1994; Douglas *et al.*, 1992). The tiered approach is particularly well-suited to the chemical characterisation of heavy and residual petroleum wastes because it allows the constraints associated with individual analytical techniques to be offset against one another.

This thesis details a tiered analytical strategy that utilises a suite of non-conventional analytical techniques, more suited to the overall complexity of the waste than those in conventional use, to attempt a fuller description of heavy oil contaminants through both screening and extended analysis (Figure 1.7).

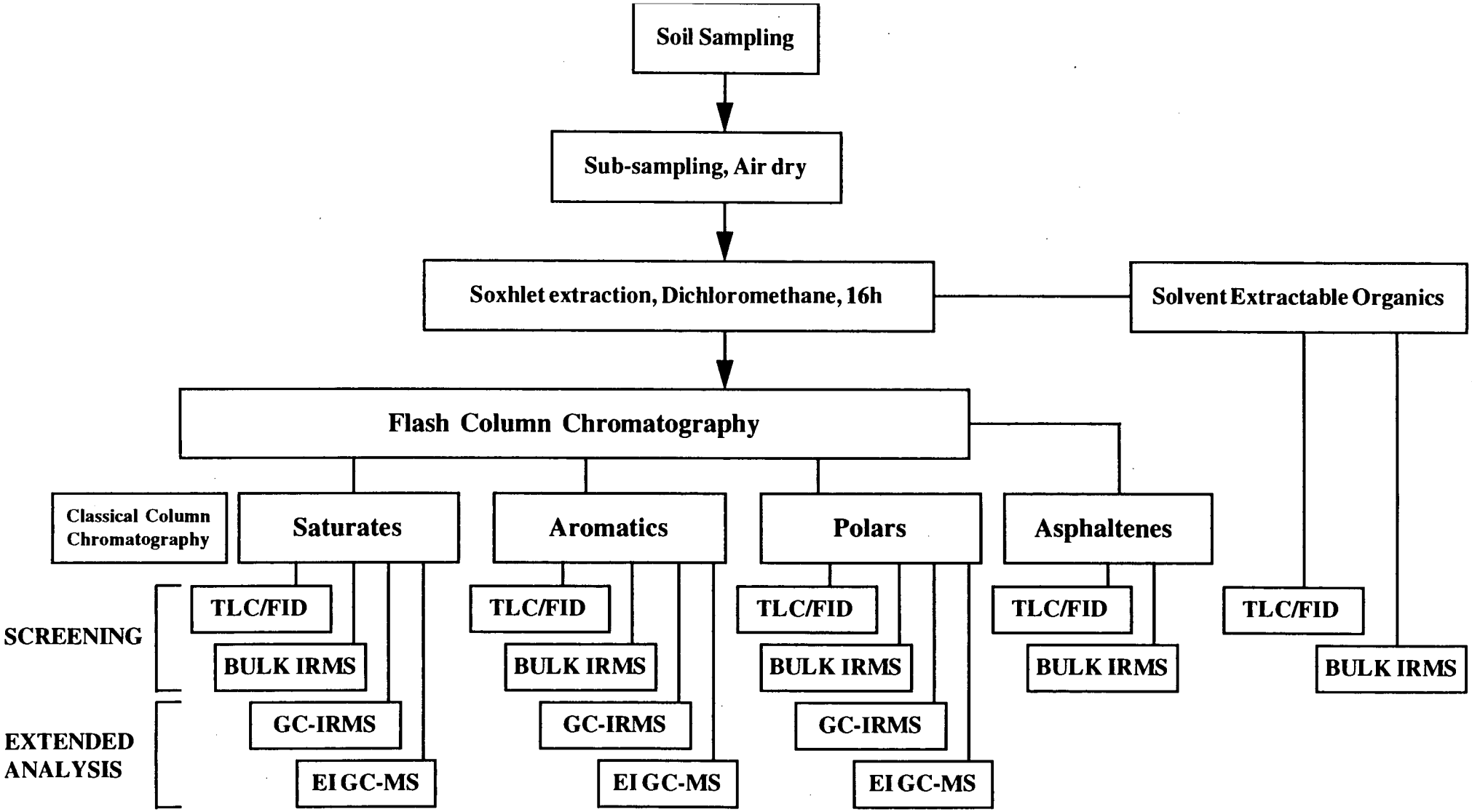
1.4.3.1 Elements of Screening Approach

(i) Soxhlet extraction of soil organics: This is an established extraction technique suitable for non-volatile and semi-volatile organics (Fan *et al.*, 1994). It is used here for extraction of contaminants from the waste-soil matrix for subsequent analysis and for evaluation of soil contaminant loads. As discussed above, a key factor is the extraction efficiency of the technique, i.e., the proportion of the actual contaminant load extracted and the need to determine the amount of soil NOM extracted.

The use of alternative extraction techniques, such as supercritical fluid extraction (SFE) and sonication was also considered. Despite the documented advantages of SFE in many cases (Fan *et al.*, 1994), its application to heavy oil-contaminated soils has not yet been fully characterised. Sonication, though especially effective in certain cases, has been shown to produce lower recoveries in creosote-contaminated soils than Soxhlet (Brilis & Marsden, 1990). Overall, therefore, it was felt that use of the more established Soxhlet method would provide a more reliable means of extraction in this case.

In this work, soil extraction was carried out to allow the characterisation of contaminant source terms in authentic heavy oil-contaminated soils, and to allow the reduction of solvent-extractable material (SEM) to be tracked during the oil biotransformation in the microcosm study.

(ii) Rapid column fractionation: Column fractionation is an established method for separation of saturate, aromatic, polar and asphaltene class fractions in petroleum, carried out mainly to



F igure 1.7. Schematic of Tiered Analytical Strategy used in this Work

isolate portions of a particular waste prior to GC-FID or GC-EI MS analysis (Wang *et al.*, 1994b).

A number of procedures for the fractionation of petroleum products have been reported, including high-performance liquid chromatography (Akhlag, 1993) and liquid-liquid extraction (Lucke *et al.*, 1985). However, classical column adsorption chromatography remains the preferred method of fractionation because of its low cost, low maintenance and ease of operation (Wang *et al.*, 1994b).

The column utilised in this work has been developed for the rapid fractionation of oil samples, and is tested here for its ability to provide suitably resolved heavy oil class fractions.

(iii) TLC-FID: A screening technique finding increasing application in the characterisation of petroleum products is Iatroscan™ thin layer chromatography with flame ionisation detection (TLC-FID). TLC-FID has been used for the analysis of a diverse range of chemical species in a variety of environments. Volkman and Nichols (1991) and Karlsen and Larter (1991) provided a detailed description of the key technical elements of TLC-FID analysis. They also described the applications of the technique to lipid class detection in marine environments, and the spatial distribution of saturated, aromatic and combined asphaltene and polar fractions in petroleum reservoirs, respectively.

In the context of contaminated land characterisation, Pollard *et al.* (1994) used TLC-FID to fingerprint a variety of residual hydrocarbon contaminants, taken from petroleum- and creosote-contaminated sites, according to their class fraction distribution. By using variations of TLC solvent schemes to focus on the aromatic and polar components of waste samples, the authors were able to correlate observed waste class composition with the extent of weathering experienced by the wastes. As discussed (Section 1.3.2), characterisation of contaminants in this way is of great utility in the management of petroleum-contaminated land.

TLC-FID has also been used extensively in the component class characterisation of various heavy whole oils and oil fractions in a non-contaminative context (e.g., Poirier and George, 1983). In particular, Fuhr *et al.* (1986) has developed the use of TLC-FID in the chemical characterisation of heavy oil and bitumen processing residues resulting from the extraction and refining of heavy oil sands in Alberta, Canada. This technique would, therefore, seem to be particularly suited to the screening of heavy oil contaminants because it provides an account of the class fraction distribution within the entire waste extract, even for the heaviest oil samples. Furthermore, the major drawback of the method, loss of volatile components from the flame ionisation detector (Karlsen & Larter, 1991), is clearly of limited concern for these samples.

In this study, method development of TLC-FID in the characterisation of heavy oil-contaminant source terms is continued, using a range of heavy oils of varying composition. Class fraction fingerprints of each oil are obtained, and these are compared with the results obtained by column fractionation to provide evidence of a robust analytical methodology.

(iv) Bulk IRMS: An approach which has proved particularly useful in the characterisation of marine oils in terms of oil source, maturity, migration and depositional environment involves bulk fraction and compound specific isotope analysis (Sofer, 1984; Schoell *et al.*, 1992; Schoell, 1984; Stahl, 1980; BjorØy *et al.*, 1992).

The stable carbon isotopic composition of crude oils and their related products is essentially a manifestation of the myriad physical and biogeochemical processes that influence oil formation and refinery. The complex isotopic fractionation patterns induced by these processes result in characteristic $^{13}C/^{12}C$ ratios that can be interpreted to elucidate the depositional environment, maturity, migration and biodegradation of the crude oil (e.g., Bowler *et al.*, 1993). In practical terms, the technique involves combustion of samples to CO_2 followed by analysis by mass spectrometry to determine the relative amounts of $^{44}CO_2$, $^{45}CO_2$ and $^{46}CO_2$

Aggarwal and Hinchee (1991) assessed the aerobic *in-situ* biodegradation of hydrocarbons at three jet fuel-contaminated sites by monitoring the preferential fractionation of the heavier isotope into soil gas CO_2. Stahl (1978) and Suchomel *et al.* (1990) also provide evidence on the utility of bulk isotope mass spectrometry in the determination of petroleum behaviour in soil through isotopic analysis of CO_2.

However, to the author's knowledge, no studies have yet evaluated the utility of the technique in the direct analysis of terrestrial petroleum contamination. Given the range of constraints that currently afflict this area of environmental analytical chemistry, it seems appropriate in the first instance to investigate the potential utility of the technique as a means of screening heavy or weathered oils of the type that exist as residual contamination in the soil environment.

This approach has potential use for the contaminated soils because there is evidence that recalcitrant polar and asphaltene class fractions become prevalent in heavy oil-contaminated soils (Westlake *et al.*, 1974; Bossert & Bartha, 1984) and that the dominance of such fractions in oils may be evident from the oil isotopic composition (Silverman, 1971; Schoell, 1984). The basic aim of this part of the study is to determine the nature of the relationship between the bulk isotopic composition of an oil and its isolated class fractions and the chemical composition of the oil.

Furthermore, since bulk isotope analysis can be applied to the entire oil extract, regardless of complexity or chemical composition and without analytical clean-up, and is restricted only by loss of volatile organic compounds, it may be of particular use in the characterisation of heavy oil contaminants.

(v) GC-FID: Use of this technique here is limited to the screening of heavy oil saturate class fraction extracts prior to extended analysis, to ascertain appropriate contaminant

concentrations. In doing so, GC-FID profiles also provide useful qualitative information on the approximate range of *n*-alkanes and the amount of UCM present within a particular oil.

The initial screening stage of the strategy is focused primarily on the provision of information on the abundance and bulk composition of heavy oils, although some elements (column chromatographic fractionation and GC-FID) are used in preparation for extended analysis. It is intended that this information will assist in the assessment of heavy oil bioremediation potential and in the initial screening of risks associated with a particular site.

1.4.3.2 Elements of Extended Analytical Approach

In the second stage of analysis, attention is directed towards the detection of individual compounds within the saturate class fraction of heavy oils that can assist in characterisation of the contaminant source term, as represented by the reference oils and acid tars, and allow diagnostic source fingerprinting and the evaluation of oil weathering to be undertaken.

(i) GC-EI MS operated in Selected Ion Monitoring mode (SIM): The use of target analysis by GC-EI MS in combination with rapid column chromatographic separation of the saturate fraction may be attractive for analysis of complex heavy oil contaminants because it focuses only upon the saturate fraction without interference from other oil constituents. This class fraction is of interest because it is the most easily depleted during oil biodegradation, and so is particularly relevant to bioremediation techniques, and because it contains important biomarkers used in oil weathering and source studies.

In this approach, molecules enter the ionisation region of the mass spectrometer in the order in which they elute from the end of the GC column. Here, they are bombarded with fast electrons at very low pressure to cause them to fragment into smaller, relatively stable ions in a manner that is characteristic of the parent molecule. In selective ion monitoring, the detector of

the GC-EI MS can be focused on only those ions displaying the specified mass to charge ratio (m/z).

The utility of this technique relies on the fragmentation of a particular parent molecule into a characteristic pattern of fragment ions. For any given molecule, some fragment ions are more abundant than others, and some may be shared by related compounds similar in structure. As discussed above, analysis of selected ion peaks produced by characteristic, environmentally-persistent biomarker compounds generates information of particular importance in determining contaminant source terms, weathered state and potential treatability.

Upon EI fragmentation, most tri-, tetra- and pentacyclic terpanes yield fragment ions at m/z 191, caused by cleavage and detection of the AB-ring ion (see Figure 1.2) (Killops & Killops, 1993). Few other compounds produce ions at this mass:charge ratio in comparable abundance, and so the response of the MS at m/z 191 over time reveals the distribution of triyclic terpanes, tetracyclic terpanes and hopanes within the original oil sample. It should be noted, however, that the peak area response in the ion chromatograms does not correspond to the absolute abundance of the target compound in the original oil sample and so should not be equated directly with the concentration of the component in question. When relative changes in peak areas within a sample are observed, however, this should not be a matter of concern.

Specific component analysis of isolated saturate class fraction by GC-EI MS is applied in four ways in this research.

Firstly, the use of biomarker indices in the forecasting of bioremediation potential of heavy oils is examined. For this to be possible, there must be a relationship between the values of the indices and the composition of the oil; this research represents a preliminary examination of this relationship by determining the values of six selected source and weathering indices for five unweathered heavy oils of known class composition.

Secondly, a variety of source correlation indices are assessed according to their ability to discriminate between different contaminant source terms, empirically represented by

unrelated crude oils. Effective source indices should vary significantly in value between oil samples from different backgrounds whilst remaining relatively consistent between oils from the same family.

Thirdly, performance studies on the sensitivity and reliability of various weathering indices in monitoring oil biotransformation is assessed, using soil microcosm extracts that have been subjected to increasing degrees of microbial transformation and, for empirical comparison, standard physically-weathered diesel range organics.

Fourthly, the reliability of selected diagnostic source indices is assessed for oil samples that have been subjected to increasing degrees of microbial transformation and physical weathering.

(ii) GC-IRMS: The advent of compound specific isotope analysis (CSIA) for carbon in crude oils and crude oil fractions by gas chromatography-coupled isotope ratio mass spectroscopy (GC-IRMS), first developed by Sano *et al.* (1976) and Matthews and Heyes (1978), and technically examined recently by Eakin *et al.* (1992), Ricci *et al.* (1994) and Merrit *et al.* (1994), has elevated characterisation of petroleum hydrocarbons to a level of sensitivity and detail unobtainable by conventional IRMS. The extensive, time-consuming and often unreproducible fractionation procedures required for reliable IRMS analysis of individual class components are removed, facilitating the fractionation and examination of samples in less than two hours.

Since its development, 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) and the determination of the source terms of oils and other organic matter (Rieley *et al.*, 1991). In many cases, CSIA of oil biomarkers, *n*-alkanes and isoprenoids, 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 biodegradation undergone by crude oils (Killops and Killops, 1993).

The application of CSIA to the characterisation of heavy oils contaminants, like bulk IRMS, is a novel one. Its potential utility is based on isotopic characterisation of components from within the oil saturate fraction, which, as previously described, plays a crucial role in determining the biotransformation characterisics of an oil.

In the first part of the study, method development of GC-IRMS in the characterisation of heavy oils is detailed. This is, in effect, a baseline study to determine whether this technique can reliably provide the requisite isotopic information from such complex oils. This is important, because the limitations imposed by GC coupling, particularly those associated with the presence of UCM, represent a possible drawback of this method. Here, $\delta^{13}C$ values are obtained for all detected *n*-alkanes and phytane, to form an isotopic fingerprint that may be used for oil characterisation purposes.

Based on these results, an isotopic fingerprint of five *n*-alkanes and norpristane in oils at successive stages of microbial transformation are analysed. Norpristane was detected here in place of phytane which could not be sufficiently resolved in all cases. From these results, it may be possible to establish the utility of CSIA either as a means of evaluating the extent of biotransformation undergone by oils (through monitoring of microbially-induced isotopic changes within the saturate fraction) or as a possible means of source correlation (through an assessment of unchanging isotopic fingerprint).

It is clear that, at present, there is a sound technical understanding of many of the issues that surround assessment and remediation of petroleum contaminated soils, although much still needs to be done to optimise site characterisation methods. One of the greatest challenges to the well-being of the soil environment is the plethora of heavy oil-contaminated sites, which present many technical uncertainties. As contaminated land becomes more important, so the need to resolve these uncertainties will grow.

CHAPTER 2. STUDY RATIONALE AND OBJECTIVES

2.1 STATEMENT OF PROBLEM

The legacy of contaminated sites in the industrialised world is a major problem and constrains sustainable economic development. Remediation of these sites, reduction or control of the risks they pose to human health and the wider environment, and the apportioning of liabilities between responsible parties are three key issues relating to this problem that require sound scientific solutions.

Chemical characterisation of the waste matrix supplies vital information on each of these issues. Information on the composition of oily waste allows the subsurface partitioning, potential toxicity and potential treatability of the waste to be assessed; the evaluation of defined component ratios provides evidence of microbial transformation of value to the success of bioremediation technologies; and chemical fingerprinting allows different oils to be correlated, even after extensive weathering, for source identification purposes.

At heavy oil-contaminated sites conventional analytical techniques are unable to cope with the complexity of the contaminants, and so this information goes undetected. As a result, many heavy oil-contaminated sites are left poorly characterised (in terms of risk and treatability) and are dealt with inappropriately, inefficiently, or more often, not at all.

2.2 STATEMENT OF HYPOTHESIS AND RESEARCH OBJECTIVES

The hypothesis throughout this work has been that heavy oil-contaminated soils harbour characteristic chemical information essential to their identification and remediation. Current techniques do not solicit this information, but if appropriate analytical parameters

were identified and developed, improved knowledge could result in the more informed selection of technologies for treatment.

To test this hypothesis, four clear objectives were identified:

(i) to identify the key issues and requirements relating to heavy oil contamination of the soil environment that can be resolved through greater analytical capability (specifically, the characterisation of heavy oil waste source terms, the screening of oil bioremediation potential, the assessment of source diagnostic parameters and the characterisation of oil biotransformation). These issues and requirements are detailed in Chapter 1;

(ii) to develop a novel approach to the characterisation of heavy-oil contaminated soil through the application and development of previously untried methods;

(iii) to consolidate these techniques within a tiered analytical strategy applicable to a variety of heavy oil wastes, and;

(iv) to determine the capability of each technique to provide information of relevance to the issues identified in (i) above.

2.3 EXPERIMENTAL DESIGN

Following on from the research objectives listed above, a number of analytical techniques and procedures were identified as being necessary for the characterisation of heavy oils. They are detailed in Section 1.4.3. The techniques were categorised according to the level of detail they could potentially provide and in light of current information needs, and arranged in a tiered analytical strategy (depicted in Figure 1.7). This strategy embodies the overall experimental design of this thesis.

The work was performed in two distinct stages:

(i) analytical method development of techniques and formation of tiered analytical strategy with which heavy oils in the soil environment may be characterised; and,

(ii) comprehensive characterisation of heavy oil biotransformation and performance assessment of source and weathering (or biotransformation) indices, accomplished through a 9-month soil microcosm study.

2.4 STRUCTURE OF THESIS

In Chapter 1, the rationale underpinning the research is explored. Details are provided of the causes and effects of petroleum contamination and the pressures that have a bearing upon the manner in which contaminated sites are treated. Most importantly, the particular challenges posed by heavy oils in the contaminated soil environment are detailed in accordance with objective (i) above. Chapter 2 provides a brief but precise account of the overall aim of the research, the hypothesis that is being tested, the experimental design and the structure of the thesis. Chapter 3 covers all experimental details pertaining to the analytical instrumentation used in the study and provides details on the method development studies undertaken to validate the final analytical protocol. In Chapter 4, the results obtained over the course of the study are presented. In Chapter 5, the results for each particular section of the work are discussed. Chapter 6 lists the conclusions that can be drawn from this work, including those that contribute to original knowledge. Chapter 7 presents suggestions for future work. The references cited throughout the thesis are listed in Chapter 8, and finally, a number of appendices are included, featuring critical raw data, published papers, training and conference presentations. Key chapters include a summary of the main points arising from that section.

CHAPTER 3. EXPERIMENTAL

In line with the stated objectives of this work (Section 2.2), development of the analytical methods used to characterise heavy oils in terms of composition and treatability was one of the primary goals of this research. Here, details are first provided of the procedures carried out to develop the analytical methods that comprise the tiered analytical scheme and the results of the quality control procedures adopted.

Secondly, details of the work undertaken in relation to the soil microcosm study are provided, in which several of the methods developed over the first part of the study are used to characterise the microbial transformation of heavy oils.

3.1 ANALYTICAL METHOD DEVELOPMENT

In this section, details are provided of the heavy oils selected as primary analytical standards (hereafter termed 'reference oils') and the experimental procedures and operating conditions pertaining to each of the techniques that comprise the tiered analytical strategy (Figure 1.7): Soxhlet extraction (through the extraction of spiked soil samples), rapid column chromatography fractionation, TLC-FID, bulk IRMS, GC-FID, GC-EI MS and GC-IRMS.

Method validation of the bulk IRMS and GC-EI MS stages of the strategy necessitated the analysis of additional heavy oil samples (acid tars). For these techniques, analysis of the reference oils alone did not provide sufficient evidence of their suitability for the intended purpose, i.e., the characterisation of heavy oils. Hence, the acid tar samples provided valuable information on the validity of each of these techniques. Because column fractionation and GC-FID are integral preparatory steps for GC-EI MS characterisation, the acid tars were analysed by these techniques also. The acid tar samples are described below. Sections have also been included on glassware preparation and the overall approach to quality control.

3.1.1 Selection of Primary Standards

The principal factor influencing the choice of primary standards for analytical method development was the need to obtain samples that were representative of the type of residual contamination encountered at heavy-oil contaminated sites. A further requirement was that the standards were well-characterised, in terms of original source and chemical composition. As no such oil standards are commercially available, six heavy oils were obtained from the British Petroleum oil refinery at Grangemouth, Scotland. These oils were taken from various stages of the refining process and shown through analytical characterisation to possess physical and chemical properties (provided in Table 3.1) associated with the type of heavy oil contaminants described above. Representative samples (1 kg) were removed as grab samples from key refinery processes. Homogenisation of each sample container prior to subsampling was achieved by vigorous shaking and mixing with a glass rod.

The six reference oils used to verify each of the analytical methods used in this study were:

(i) 'API (American Petroleum Institute) separator oil'. An arbitrarily-defined, complex mixture of process plant run-off collected from a waste oil separator unit. One of the lowest boiling of the six reference oils, the oil was golden in colour and could be poured easily at room temperature. A useful descriptive indication of the texture of the oil is provided by the rate at which a standard glass rod moves unaided through the oil. In this case, the rod fell rapidly through the sample, demonstrating a low oil viscosity. The oil displayed a boiling range of room temperature to *ca.* 400 ^{o}C and a carbon number range of *ca.* C_8 to C_{30}, as determined by simulated distillation gas chromatography (GC-SIMDIS) (profile provided in Figure 3.1).

(ii) Ballast oil. A mixture of oils originating from the storage tanks of oil transportation vessels. This oil was similar in appearance to API separator oil, displaying a relatively low viscosity, a boiling range of room temperature to *ca.* 400 ^{o}C and a carbon number range of *ca.*

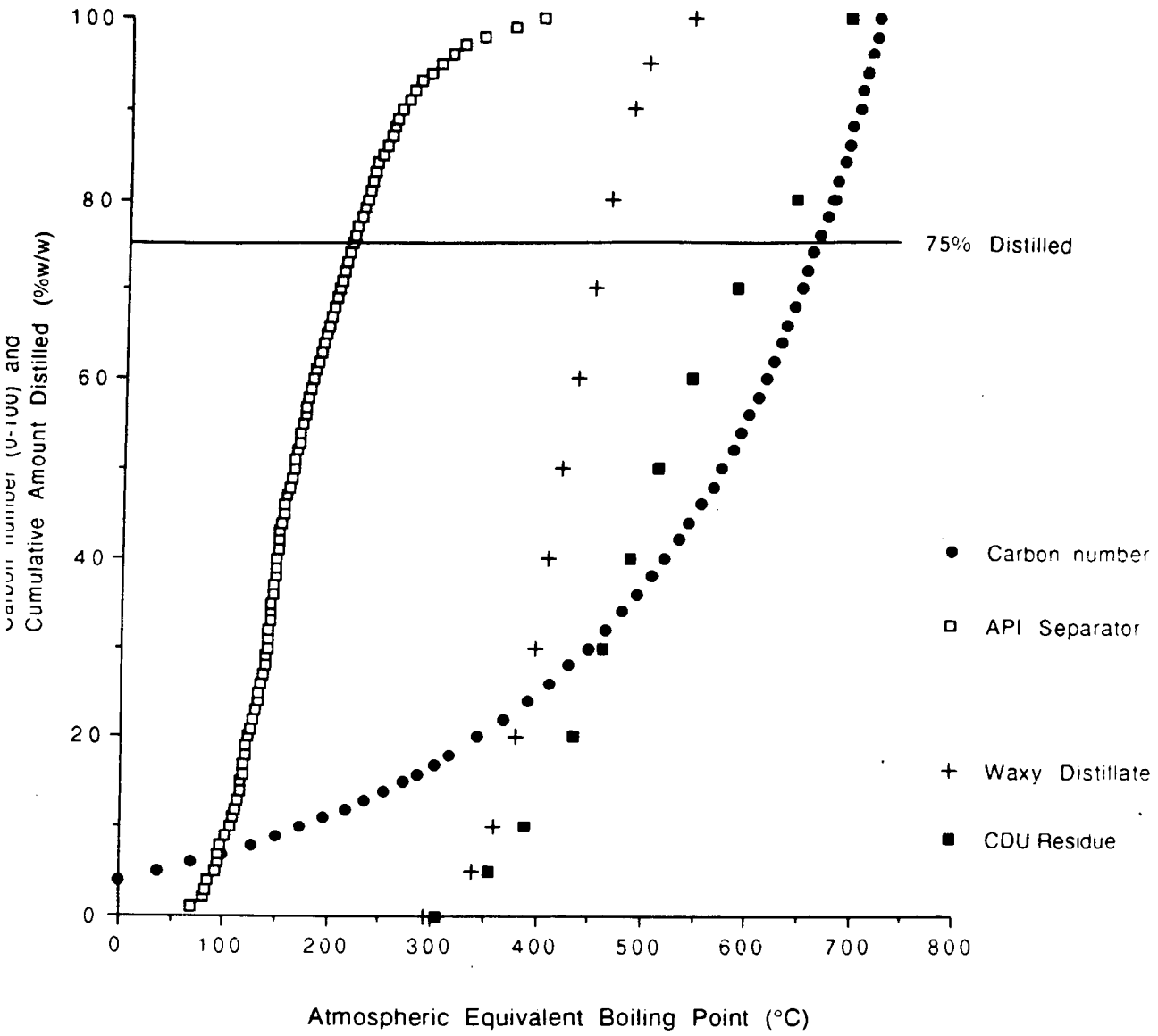


Figure 3.1 GC-SIMDIS Profiles of Selected Reference Oils

- The plot of boiling point vs. carbon number for *n*-alkanes C_4 - C_{100} is included for interpretive purposes;
- The relative steepness of the curves indicates the boiling point range over which different oils are distilled, thus the API separator oil has a bulk molecular weight distribution of *ca.* C_8 to C_{30}, the waxy distillate has a range of *ca.* C_{16} to C_{50}, and the residue oil has a range of *ca.* C_{16} to C_{90};
- The point at which the intercept at the SIMDIS profile (obtained from interpolating horizontally from 25 % cumulative weight distilled) crosses the carbon number plot during vertical interpolation to the x-axis provides a relative description of the molecular weight of > 75 $\%^{w}/_{w}$ of the sample, in terms of carbon number;
- Thus, over 75 $\%^{w}/_{w}$ of the API separator oil has a molecular weight of below *ca.* C_{15}, over 75 $\%^{w}/_{w}$ of the waxy distillate has a molecular weight of below *ca.* C_{32}, and over 75 $\%^{w}/_{w}$ of the residue oil has a molecular weight of below *ca.* C_{62}.

Table 3.1 General Characteristics of Reference Oils

Reference Oil	Origin/Crude Source	Generic Chemical Groups Present	GC-SIMDIS Boiling Range (°C)	GC-SIMDIS Approximate Carbon Number Range
API Separator Oil	Skimmed from surface process run-off streams. North Sea oil blend.	High paraffinic and naphthenic content, but may contain appreciable aromatic and heavier compounds	R.T. to ca. 400	6 to 30
Ballast Oil	Discharge from oil tanker storage facilities. Wide variation in product type. Mainly North Sea oil blend	Predominantly alkanes, with some aromatic groups	R.T. to ca. 400	6 to 30
Waxy Distillate	Taken from crude oil distillation unit. North Sea oil blend.	Mainly high-boiling (long-chain) paraffinic compounds, with significant naphthenic content.	ca. 250 to 550	15 to 46
No.6 Fuel Oil	Commercially-defined refinery product, sampled from heavy fuel oil stock tank. North Sea oil blend.	Mixture of straight-run alkanes, some olefins, heavier aromatics and polar compounds.	ca. 60 to 600	10 to 60
Residue	Residual oil drawn from bottom of crude oil distillation unit. North Sea oil blend.	Mainly high-boiling compounds, mixture of alkanes, heavier aromatics and polars.	ca. 250 to 700	16 to >75
Bitumen	Tarry residue drawn from bottom of vacuum distillation unit. North Sea oil blend.	Predominantly heavy aromatic, polar and asphaltenic groups.	ca. 500 to > 700	N/A

(R.T. = Room Temp)

C_6 to C_{35}. As with the API separator oil, the glass rod was observed to fall rapidly through the sample.

(iii) Waxy distillate. Drawn from the refinery vacuum distillation line, this paraffinic oil had the appearance of a solidified waxy paste, golden in colour, which could not be poured at room temperature. In this oil, the glass rod remained suspended in its original position. GC-SIMDIS analysis indicated a boiling range of *ca.* 150 °C to 550 °C and a carbon number range of *ca.* C_{15} to C_{50} (Figure 3.1).

(iv) Residue oil. A blend of residues drawn from the crude oil distillation unit. The oil had a dark, waxy texture that just supported a glass rod at room temperature, and was shown by GC-SIMDIS to have a boiling range of *ca.* 200 °C to 600 °C and a carbon number distribution of *ca.* C_{15} to > C_{60} (Figure 3.1).

(v) No. 6 Fuel Oil (Bunker C). A defined product of crude oil refining, this sample was drawn from the refinery heavy fuel oil stock tank. A black, viscous oil that poured very slowly at room temperature, the sample exhibited a boiling range of *ca.* 150 °C to 700 °C and a carbon number distribution of *ca.* C_{15} to > C_{60}. The glass rod was observed to sink extremely slowly through the oil, with more rapid movement requiring the application of force.

(vi) Bituminous extract. A highly asphaltic sample removed from the vacuum distillation unit. The heaviest of the reference oils, the sample was black in colour and formed a solid mass that could only be penetrated by the glass rod with considerable force. GC-SIMDIS tests (Figure 3.1) identified a boiling range of *ca.* 300 °C to > 800 °C and a carbon number range of *ca.* C_{25} to > C_{100}.

3.1.2 Acid Tars

The analytical strategy described in this thesis was designed for application to genuine heavy oil contaminants of varying chemical complexities. To develop the bulk IRMS and GC-EI MS stages of the tiered analytical strategy, a set of three authentic heavy oil wastes

(acid tars), labelled AT1, AT2 and AT3, were obtained from a contaminated lubricating oil recovery plant in the Midlands. A waste product of the re-refining of used motor oils, acid tars are some of the most complex and problematical organic products (RCEP, 1996) and are likely to require special treatment under the new regulatory regime for contaminated land introduced in the Environment Act, 1995. Analysis of these samples, therefore, provided a measure of the limits of complexity to which the established analytical methods could be applied.

Samples of acid tar-contaminated soil were supplied in labelled 2 kg plastic tubs. Portions of contaminated soil were first extracted using the established Soxhlet extraction technique and then analysed by rapid column chromatography class fractionation, bulk IRMS screening, GC-FID screening and GC-EI MS. GC-IRMS was not applied to these samples, as it was not possible to resolve individual compound peaks to the extent that defensible compound specific isotope ratios could be determined.

3.1.3 Analytical Methods Used

Analytical characterisation of heavy oil samples was accomplished using a suite of techniques, covering initial solvent extraction and chromatographic cleanup of oils, screening techniques and more sophisticated analytical methods. A schematic of the tiered analytical strategy is shown in Figure 1.7. The study incorporated a variety of techniques, most of which were highly specialised in nature.

Analytical data were obtained using instrumentation at a variety of sites. In each case, method development, calibration and quality control regimes were established. Table 3.2 summarises the range of techniques used, the site of the work, the capacity in which each technique was employed and analytical quality control parameters undertaken. In brief, the TLC-FID work was completed at the laboratories of Geochem Ltd., in Chester; all bulk IRMS, GC-FID and GC-IRMS analyses were carried out at the Scottish Universities Research and Reactor Centre, East Kilbride; the GC-EI MS (SIM) investigation of contaminant source terms

Table 3.2 Description of Methods Used

Method	Purpose Served	Equipment Specification	Location	Method Development Procedures/Issues	QC Regime
TLC-FID	Class compositional fingerprinting of reference oils	Iatroscan TH-10 Mk III TLC-FID system	Geochem Ltd. Chester	- Solvent scheme - Comparison with column chrom. results - Analysis of SAPA fractions	- Triplicate analysis; - evaluation of σ; - comparison with standard oils.
IRMS	Screening of reference oils and acid tars	SIRA 10 MS, VG Micromass 602D	SURRC	- Analysis of standard graphite - Analysis of ref.oils - Analysis of acid tars	- Triplicate analysis; - evaluation of σ; - ANOVA of bulk $\delta^{13}C$ values
GC-FID	Screening samples prior to GC-EI MS and GC-IRMS	AI Model 93 GC, (Section 3.1.7.1)	SURRC	- Sample concentration - GC temperature ramp - Detector parameters	- Solvent blank analysis - Standard solution analysis
GC-EI MS	- Biomarker indices for heavy oil characterisation - Source indices for crude oils - Assessment of biotransformation and source correlation indices	HP 5890 GC VG Trio 1 MS HP 5890 GC HP 5972 MSD	CRPB Geochem Ltd.	- Sample concentration - GC temperature ramp - MS parameters - Baseline resolution of *n*-alkane peaks	- Solvent blank analysis - Standard mixture analysis - Use of internal standard - Reproducibility of peak areas
GC-IRMS	- Compound-specific isotope analysis of reference oil - Analysis of isotopic changes with oil biotransformation	HP 5890A GC VG ISOCHROM II	SURRC	- Sample concentration - GC temp. ramp - Charac'n of UCM	- Solvent blank analysis - Standard mixture analysis - Reproducibility of $\delta^{13}C$

was carried out at the former Clyde River Purification Board; and the GC-EI MS (SIM) analysis of heavy oils during the biotransformation study was completed at Geochem Ltd.. All analyses were conducted by the author, although technician assistance with GC-FID, GC-EI MS and GC-IRMS was provided, in the form of initial training in instrumental software handling, routine maintenance and autosampler preparation (for GC-EI MS analyses).

3.1.4 Preparation of Glassware

Particular attention was paid to ensuring that all glassware used in the extraction, collection and storage of the oil samples, and subsequent analysis was adequately clean (Nordtest, 1991). Prior to use, all glassware required for experimental work was acid-washed in a 10 % HNO_3 solution, washed again in a 10 $\%^{v}/_{v}$ mixture of detergent and de-ionised water, rinsed with de-ionised water and solvent, and finally oven-dried at 105 ^{o}C.

3.1.5 Analytical Quality Control Procedures

Heavy petroleum products are exceptionally complex analytical matrices and it was necessary to adopt stringent quality control (QC) procedures to ensure successful analyses and valid interpretation. The quantitative analysis of the oils in this study is fundamental to the examination of the stated hypothesis and validity of the conclusions made. This relies on quantification of the errors inherent to the analytical procedures used (Laboratory of the Government Chemist, 1995).

Throughout this work, efforts were made to characterise experimental errors and reduce the level of uncertainty associated with the results through careful experimental design and the use of standard experimental procedures, appropriate calibrated standards, reference materials, statistical tests and error calculations. Unless otherwise stated, results are presented in terms of the 'sample mean ± the standard deviation of the sample' (as the estimate of error). Where required to demonstrate the significance of the variation of results between samples, such as in the bulk isotopic measurements, analysis of variance (ANOVA)

and significance tests were adopted. Details and results of the statistical calculations carried out are provided in the text.

3.1.6 Screening Techniques

The screening stage of the analytical strategy comprised extraction of organic contaminants from soil matrix by Soxhlet extraction, saturate/aromatic/polar/asphaltene (SAPA) class fractionation of extracted organics, thin layer chromatography with flame ionisation detection (TLC-FID) and bulk fraction isotope ratio mass spectrometry (IRMS) (Section 1.4.3). Method development of each of these was carried out using the reference oils. Further analysis of the acid tars was required to verify the bulk IRMS method as a potentially useful screening technique.

3.1.6.1 Determination of Solvent Extractable Material (SEM)

Soxhlet extraction has been shown to be a reliable method for removal of heavy oils from contaminated soil samples (Fan *et al.*, 1994). In this study, an established Soxhlet extraction procedure (Pollard *et al.*, 1992) was tested using a variety of soil samples spiked by adding a known amount of ballast oil and No.6 Fuel Oil to soil portions. The reproducibility of the recoveries for each oil was determined through triplicate extraction after overnight equilibration.

Spiked soil samples were first air-dried under a forced draught in a fume cupboard at ambient temperature. Once dry, samples were crushed, sieved (< 2 mm) and an accurately weighed (± 1 mg) amount added to a dry Soxhlet thimble. A known amount (1 - 2 g) of oven dried, anhydrous Na_2SO_4 (dried at 400 °C for 4 hours (EPA Method 3611A) was also added to each thimble, to ensure that extracted organics were free of moisture. Organic extracts were recovered from dry contaminated soil subsamples (*ca.* 40 g) by conventional Soxhlet extraction for 16 hours using HPLC grade dichloromethane (DCM) (150 ml). Following solvent extraction, DCM extracts containing the extracted organics were reduced overnight at ambient

temperatures under forced draught to remove the DCM. Solvent extractable material (SEM) was gravimetrically determined using a Mettler AJ150 balance until a consistent value was obtained (i.e., until all of the DCM solvent had evaporated).

Minimum Soxhlet extraction efficiencies for ballast oil-soil and No.6 Fuel Oil-soil matrices were determined by processing a series of control samples comprising known amounts of soil and fresh oil. These recoveries were considered to be the minimum possible obtainable for each oil in this study since they were determined from freshly treated soils from which some loss of lower boiling compounds during extraction is likely to occur. This imbalance in the mass balance equation will be less significant for samples extracted after a period of weathering, since these will have already lost any lower molecular weight compounds through evaporation or biotic degradation.

Minimum Soxhlet extraction efficiencies were 75.3 % ± 1.8 (1σ) for the ballast oil treated samples and 98.3 % ± 2.2 (1σ) for the No. 6 Fuel Oil treated samples, based on the percent of oil recovered from spiked soil samples (e.g., for initial amounts of ballast oil of 0.821 g, 0.835 g and 0.807 g, the amount recovered was 0.619 g, 0.643 g and 0.592 g). Recoveries were lower for the ballast oil because it contained more of the low boiling compounds that can be lost to evaporation during the Soxhlet extraction procedure than the crude oil or No.6 Fuel Oil.

3.1.6.2 Sample Preparation and Chromatographic Cleanup

The asphaltene fraction of the extracted acid tars and reference oils was gravimetrically determined by an *n*-pentane precipitation method adapted from Speight *et al.* (1984). An accurately known amount of sample (0.3 - 0.5 g) from an homogenised reference oil was dissolved in toluene (1 ml g^{-1} of sample) and *n*-pentane (40 ml ml^{-1} of toluene) in a pre-weighed, acid-washed, 250 ml borosilicate Erlenmeyer flask and warmed (ca. 40 °C) and stirred for 2 hours. After brief cooling, the precipitated asphaltenes were filtered (Millipore,

cellulose acetate, 0.8 mm), oven-dried (100 ^{o}C, 20 mins.) and gravimetrically determined. The *n*-pentane soluble fraction (maltenes) of each oil sample was determined gravimetrically using a Mettler AJ150 balance following evaporation of the solvent by air-drying under a forced draught in a fume cupboard (16 h).

Component class fractionation of all the oil samples analysed in this study was achieved using an adaptation of a classical chromatographic cleanup procedure (EPA Method 3611A, 1990). In the approach developed here, a Quick-Sep™ lateral reservoir flash chromatography apparatus, in which solvent can be introduced into the column without disassembly of the pump inlet adapter, was used to provide a more rapid separation and with greater resolution than that achievable by conventional gravity column fractionation. 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 ^{o}C), followed by anhydrous Na_2SO_4 (*ca.* 10 mm, dehydrated by heating at 400 ^{o}C for 4 hours). The column was prepared by pouring an equilibrated slurry of alumina stationary phase and *n*-pentane solvent down a glass rod into the chromatography apparatus. A single 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 % of pump capacity).

All solvents used were HPLC grade. The column was pre-eluted with 50 ml *n*-pentane prior to the loading of the maltene fraction of each reference oil onto the column by pipette in 2 ml of *n*-pentane. Care was taken to ensure that the column load did not exceed 0.300 g, so that difficulties associated with column overloading (e.g., incomplete class fraction recoveries) were avoided (EPA Method 3611A). Component classes were obtained using an elution scheme shown by Pollard *et al.* (1992): 150 ml of *n*-pentane (for elution of saturates), 150 ml of toluene (for mono-, di- and polyaromatics), and 150 ml of a dichloromethane/methanol mixture (50/50) (for polar compounds; *e.g.* benzofurans and

fluorenones, highly polar aromatics, benzothiophenes and carbazoles, and any remaining strongly polar compounds) (Fuhr *et al.*, 1984). Fractions were collected in pre-weighed, acid-washed borosilicate flasks and reduced under a forced draught at ambient temperature to allow evaporation of the eluting solvent and subsequent calculation of gravimetric recovery.

Further cleanup of the pentane fraction containing the *n*-, branched and cyclic alkanes was carried out by classical column chromatography using a method described by Rawluk (1991). A champagne column (Supelco, 120 mm, 30 ml reservoir) was packed with neutral Aldrich STD Grade alumina (100mm, *ca.* 150 mesh, activated overnight at 130 ^{o}C) and anhydrous Na_2SO_4 (*ca.* 5 mm, dehydrated by heating at 400 ^{o}C for 4 hours), and pre-eluted with 25 ml of *n*-pentane. The *n*-pentane fraction isolated by flash column separation was introduced onto the column in 2 ml of *n*-pentane. Eluting solvents were introduced into the column reservoir in the order: 20 ml *n*-pentane (for saturates), 10 ml of toluene (for aromatics), 10 ml of DCM-methanol (50:50) (for polar compounds). Each class fraction was collected in a pre-weighed, acid-washed borosilicate flask and gravimetrically determined following fume cupboard-evaporation of the eluting solvent.

The reproducibility of the asphaltene precipitation was determined by triplicate analysis of selected ballast oil and No.6 Fuel Oil samples. Mass balance calculations showed that the total recoveries of samples (i.e., the sum of the recovered maltenes and asphaltenes) for both samples were found to be between 90 % and 110 % of the total amount of oil originally analysed. Standard deviations of asphaltene measurements were approximately 15 % of the value of the sample mean.

Column chromatography reliability was established through triplicate analysis of API separator oil, ballast oil and Nigerian crude oil (see Section 3.1.7). For the saturate, aromatic and polar class fractions, the relative standard deviations were calculated to be under 5 %. Column recoveries (i.e., the sum of the saturate, aromatic and polar fraction gravimetric recoveries) ranged between 84.2 % and 93.6 % of the original weight of sample applied, indicating that the

reported mean weight percentages of class fractions may be slightly lower than the actual amounts contained within the extracts. However, because column recovery values were consistently between these two values, this was not thought to unduly influence the results.

To further assess the reproducibility of the method, saturate, aromatic and polar fractions isolated from each of the oils were re-combined and fractionated once more. In each case, the percent weight of the respective class fractions isolated for the second time were within 3 $\%^{w}/_{w}$ of the values obtained in the first fractionation. Recoveries for the repeated samples varied from those obtained initially by up to 7.5 %, although there was no apparent trend in this variation.

Since procedural reproducibility was of key importance to the successful application of the analytical scheme, every effort was made to regulate potentially variable elements of the methodology, *e.g.*, the flow rate of the mobile phases through the column, the addition of the samples to the column and the final gravimetric analysis (samples were weighed periodically throughout the forced draught evaporation of the eluting solvent until a consistent reading was observed). For gravimetric recoveries using the Mettler AJ150 balance, the limit of detection was estimated to be approximately 0.1 mg (taken from instrument specifications).

3.1.6.3 Thin Layer Chromatography-Flame Ionisation Detection (TLC-FID)

The component class distribution (above carbon number C_{10}) within each of the reference oils was determined by Iatroscan™ TLC-FID analysis (Pollard *et al.*, 1992). In this system, individual oil class fractions are separated by thin layer chromatography on silica gel-coated quartz rods from which they can be directly measured using a sensitive flame ionisation detector. Samples (2 µl) of each reference oil and each isolated fraction were spotted onto Iatron S-III silica-coated quartz 'chromarods' using a 50 µl syringe. Ten chromarods were developed simultaneously, with the standard on 1 rod and three samples analyzed in triplicate on the remaining 9 rods. Care was taken during the sample spotting to

apply reproducible spot sizes and minimize band spreading. The saturate, aromatic and combined polar fractions of each sample were then separated by successively placing the rods in three chromatography paper-lined (Whatman No.6) TLC developing tanks, containing 150 ml *n*-hexane (saturates), 150 ml pentane/DCM (45/55) (aromatics) and 150 ml DCM/methanol (98/2) (polars), for 30, 15 and 3 minutes, respectively.

Two other potential solvent schemes were considered, *n*-pentane-toluene-DCM/methanol (in accordance with column fractionation solvent scheme) and *n*-hexane-DCM-DCM/methanol (in accordance with Pollard *et al.* (1992)). In the former, the volatility of *n*-pentane led to very rapid elution, which may be detrimental to the reproducibility of the technique. None of the TLC-FID studies of petroleum characterisation reported in the literature were found to use *n*-pentane in this way. The latter solvent scheme was chosen by Pollard *et al.* (1992) to separate oils into four class fractions (including two 'polars' fractions), which was not necessary in this work. The selected solvent scheme provided the clear, reproducible resolution of class fractions and was therefore considered to be satisfactory for application to the reference oils.

Typical chromatograms of neat reference oils are shown in Figure 3.2, in which the saturate, aromatic and polar peaks can be clearly discerned. The observed elution times, which were strictly controlled by stop-watch to maximise reproducibility (Karlsen & Larter, 1991), resulted in development of each fraction to approximately 90 %, 50 % and 25 %, respectively, of the rod length. The rods were air-dried for 30 seconds between each developing tank and before Iatroscan analysis to remove the solvent and produce a stable baseline. Scanning the rods through the H_2 flame of the FID immediately after analysis demonstrated that high boiling samples had been completely combusted and cleaned the rods for re-use.

The Iatroscan TH-10 Mk III detector was operated at scanning speed 4 (30 sec rod^{-1}), a H_2 pressure of 0.8 kg cm^{-1} and an air flow of 2000 ml min^{-1} (Volkman & Nichols, 1991). The detector operates by sequentially burning off the separated compound groups on the quartz

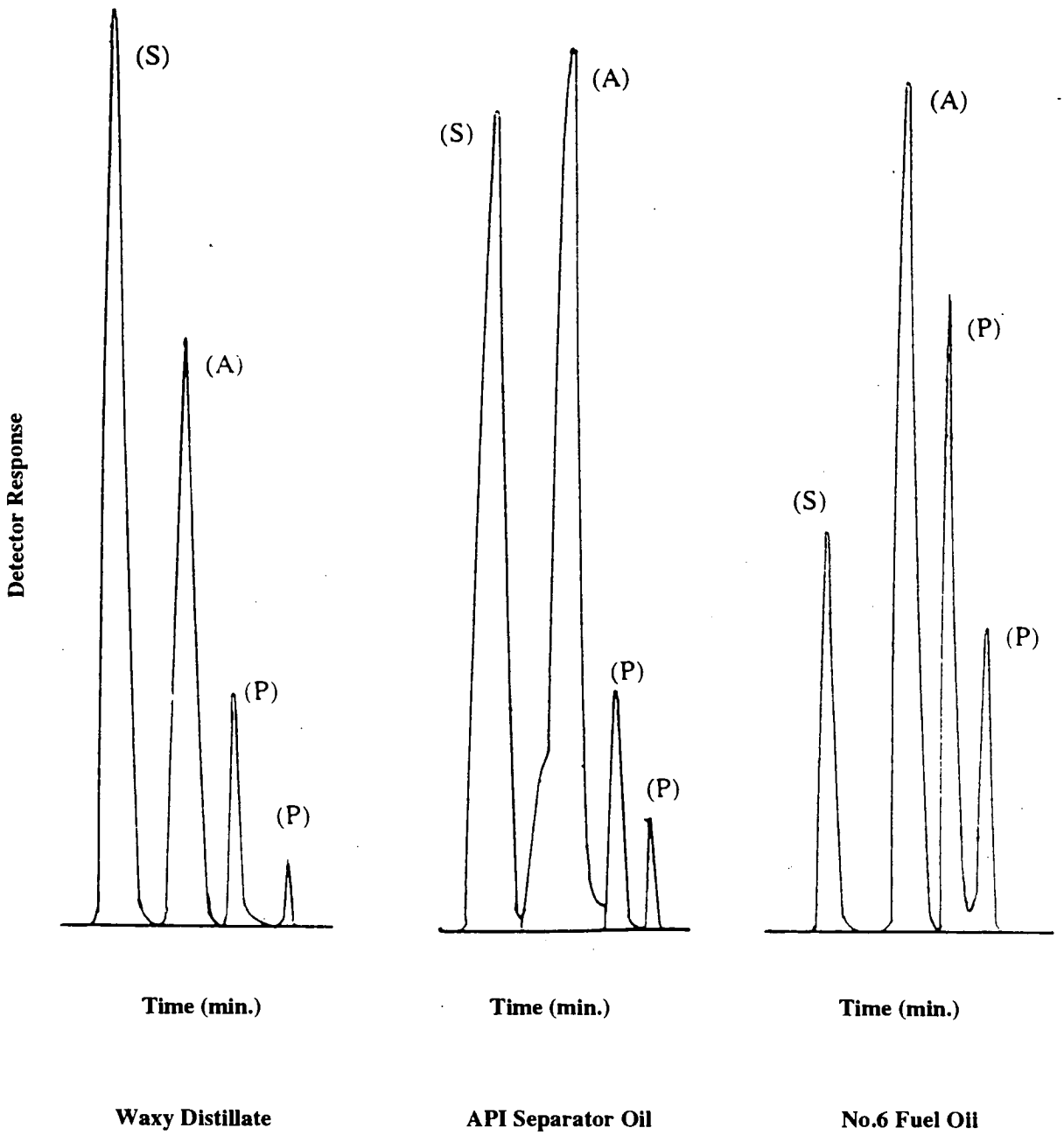


Figure 3.2 TLC-FID Chromatograms for Waxy Distillate, API Separator Oil and No.6 Fuel Oil [(S) - Saturates, (A) - Aromatics, (P) - Polars]

rods in an air/hydrogen atmosphere. Direct FID output was recorded on a BBC Goerz Metrawatt SE 120 chart recorder followed by integration by a Trilab computing integrator equipped with Sekonic S-2000 GP chart recorder and Trivector PC.

Class fractions were quantified by expressing the peak area of each fraction as a percentage of the total peak area and then corrected by a calibration factor obtained from simultaneous analysis of a standard oil. The standard oil used was a well-characterised heavy oil from the Geochem sample bank with a class composition (gravimetrically determined to be 66.9 $\%^{w}/_{w}$ saturates; 19.1 $\%^{w}/_{w}$ aromatics; 13.6 $\%^{w}/_{w}$ combined polars) chosen to reflect the composition of the sample oils. Repeated analysis of the standard using the scheme chosen here produced a class fraction distribution of 65.2 $\%^{w}/_{w}$ (± 1.6) saturates, 22.8 $\%^{w}/_{w}$ (± 1.5) aromatics and 11.7 $\%^{w}/_{w}$ (± 1.0) combined polars. Individual oil samples were analysed in triplicate to facilitate evaluation of the standard deviations (SDs) of individual class fractions values.

A measure of the validity of this method can be obtained from an analysis of the individual SAPA class fractions isolated using the column chromatography method described in Section 3.1.6.2. Results of the TLC-FID analysis of the individual SAPA class fractions isolated by the column chromatography process are shown in Figure 3.3 (a)-(f). For all the oils except the No.6 Fuel Oil, the column-defined saturates class fractions (labelled in Figure 3.3 as 'pentane') were measured by TLC-FID to consist of over 80 % saturates and up to 20 % aromatic compounds. Similarly, the polars fraction (labelled 'DCM/Meth') eluted from the column chromatography was found to contain less than 20 % aromatics and saturates when measured by TLC-FID. The largest disparity between the two methods occurred in the column aromatic (or 'toluene') class fraction, which, in the TLC-FID analysis, was assessed to consist of up to 45 % polar class components.

Figure 3.4 (a) to (f) compares the class fraction distribution of each oil obtained by TLC-FID analysis with that obtained by column chromatographic fractionation. With two

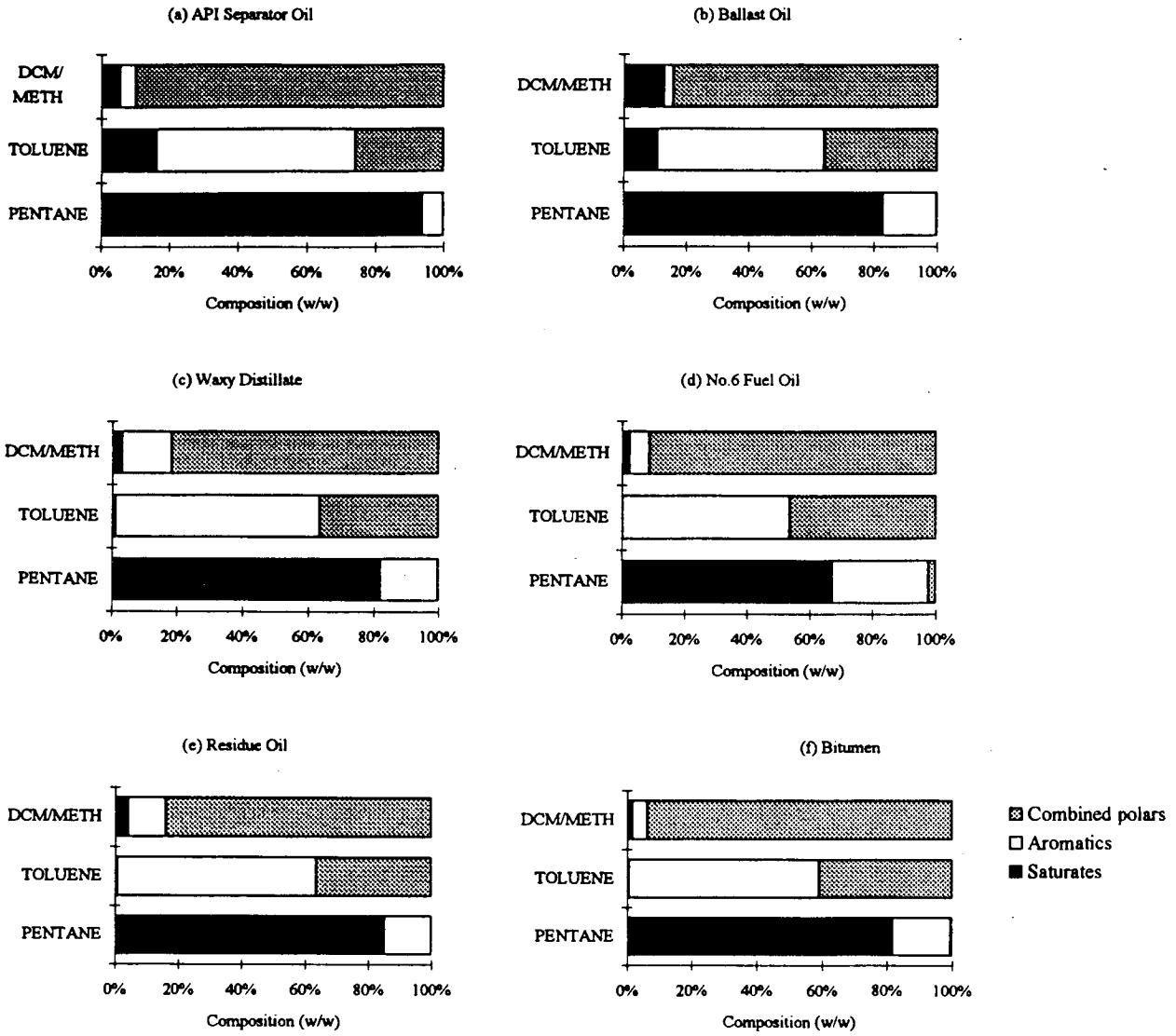


Figure 3.3 TLC-FID Class Fraction Fingerprint of Column Chromatography Class Fractions

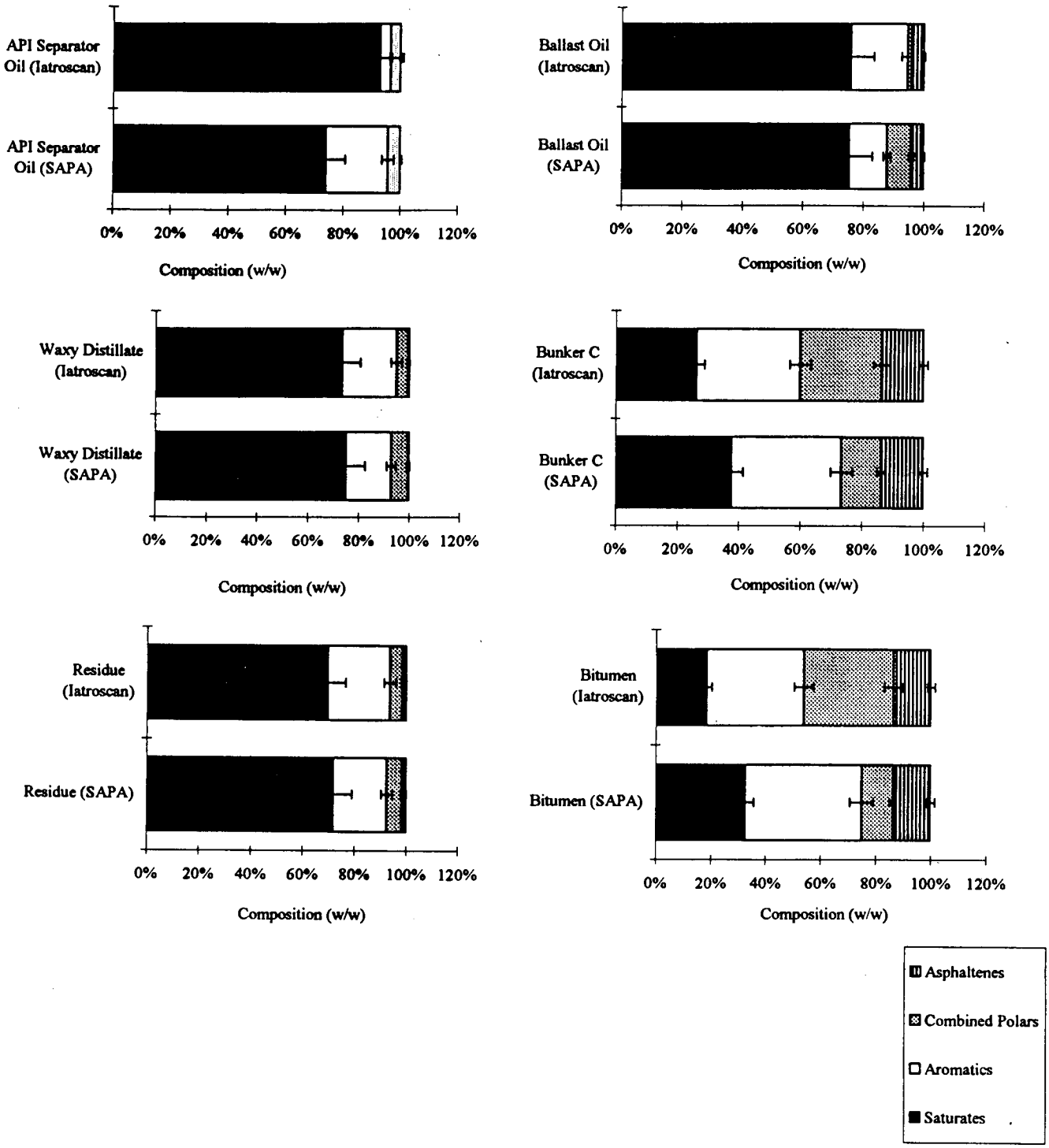


Figure 3.4 Comparison of SAPA Column Fractionation and Iatroscan TLC-FID Results for API Separator Oil, Ballast Oil, Waxy Distillate, No.6 Fuel Oil (Bunker C), Residue Oil and Bitumen

exceptions, the corresponding percentages of oil class fractions for each oil are within 15 % (and often within 5 %). The most conspicuous disparities between the two screening methods occur for the saturate content of the API separator oil and the polar content of the bitumen, which differ by approximately 25 % in both cases.

In most cases, the standard deviations associated with the reported component class values lay between 0.5 and 4.0 (n = 3), in approximate agreement with those obtained by Pollard *et al.* (1992). The most notable exceptions were the ballast oil saturates and aromatics, which yield SDs of 10.0 and 15.5, respectively. Error bars are shown in the TLC-FID fingerprints depicted in the respective graphics. These data suggest that TLC-FID provides sound compositional information on the reference oils and is an integral part of the overall tiered analytical strategy.

3.1.6.4 Stable Carbon Isotope Ratio Mass Spectrometry (IRMS)

Isotope ratios are expressed in terms of $\delta^{13}C$ values, which are reported in per mil ($^{o}/_{oo}$), and calculated relative to the standard PeeDee belemnite (PDB), according to the relationship:

$$\delta^{13}C = (R_S/R_R - 1)1000\ ^{o}/_{oo} \qquad (3.1)$$

(where R is the ratio of $^{13}C/^{12}C$ in the sample (R_S) and the reference (R_R)). Whole oil and oil class fraction subsamples (*ca.* 5 mg) are converted to CO_2 in preparation for isotope ratio mass spectrometry analysis by dry furnace combustion (850 °C, 6 hrs) in sealed, evacuated quartz tubes (15 - 20 cm in length, 9 mm in diameter) containing excess fired cupric oxide as an oxygen source. Combustion tubes containing the samples and CuO were sealed under vacuum by a standard glassblowing torch. Sample CO_2 isolation and purification was accomplished on a vacuum system by vacuum line manipulation and cryogenic distillation.

Isotope ratio data were obtained on a duel inlet SIRA 10 isotope ratio mass spectrometer (VG Micromass 602D). The work was carried out at the Scottish Universities Research and Reactor Centre, East Kilbride. Sample isotope ratios are evaluated initially relative to a reference CO_2 stream calibrated relative to the standard PDB carbonate formation. Absolute calibration of the reference CO_2 relative to PDB is effected through the use of the NBS Standard 19 carbonate. Final sample $\delta^{13}C$ values are reported relative to the standard PDB with corrections made for ^{17}O contributions. A more detailed technical description of this technique is supplied by Boutton (1991).

Stable carbon isotopic measurements were obtained for the six reference oils and three acid tars. For each sample, random errors were evaluated by determining the isotopic composition in triplicate. Standard deviations of bulk oil and class fraction $\delta^{13}C$ values were generally found to be less than 0.3 $^{o}/_{oo}$, although the asphaltene class fractions of the No.6 Fuel Oil and the waxy distillate displayed SDs of 0.5 and 0.4, respectively. $\delta^{13}C$ values for each whole oil ($\delta^{13}C_{oil}$) and class fraction (e.g., $\delta^{13}C_{sat}$) are given in Table 3.3. Individual $\delta^{13}C_{oil}$ values were found to increase (i.e., become less negative) with increasing combined polar and asphaltene contents and an elevation in the upper end of oil boiling range, ranging between -26.8 $^{o}/_{oo}$ for AT1 and AT2 to -28.8 $^{o}/_{oo}$ for the residue oil. Calculated values of $\delta^{13}C_{oil}$, determined from the weighted isotopic contribution of each class fraction, were in close agreement with the measured $\delta^{13}C_{oil}$ values. The statistical validity of the observed trend in $\delta^{13}C_{oil}$ values was determined through extensive repeat analysis of samples and a thorough statistical examination of results through analysis of variance (ANOVA) (Sincich, 1989).

The ANOVA of bulk isotope ratios of whole oils facilitates a statistically valid comparison of the variance between the $\delta^{13}C_{oil}$ of the different oils and the variance of each $\delta^{13}C_{oil}$ value. Averaging the $\delta^{13}C_{oil}$ these values produced an average within-sample variance (σ^2_1) of 0.07. The between sample variance (σ^2_2), calculated from the standard deviation of the

Table 3.3 Bulk Isotope Ratio Measurements for Reference Oils and Acid Tars ($\delta^{13}C$, $^{o}/_{oo}$)

	AT1					AT2					AT3				
	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD
Whole Oil	-26.89	-26.77		**-26.83**	0.08	-26.83	-26.84		**-26.84**	0.01	-26.99	-27.05		**-27.02**	0.04
Saturates	-26.85	-26.88		**-26.87**	0.02	-26.75	-26.81		**-26.78**	0.04	-27.02	-27.09		**-27.06**	0.05
Aromatics	-26.42	-26.36		**-26.39**	0.04	-27.16	-26.11		**-26.64**	0.74	-25.96	-26.21		**-26.09**	0.18
Polars	-26.77	-27.04		**-26.91**	0.19	-27.01	-26.66		**-26.84**	0.25	-26.26	-26.95		**-26.61**	0.49
Asphaltenes	-26.63	-26.67		**-26.65**	0.03	-26.21	-26.86		**-26.54**	0.46	-27.01	-26.99		**-27.00**	0.01

	Bitumen					No.6 Fuel Oil					API Separator Oil				
	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD
Whole Oil	-27.44	-27.60	-27.21	**-27.42**	0.20	-27.61	-27.40	-27.32	**-27.44**	0.15	-28.46	-28.76	-28.26	**-28.49**	0.25
Saturates	-27.32	-27.44	-27.48	**-27.41**	0.08	-27.35	-27.49	-27.44	**-27.43**	0.07	-29.15	-28.86	-28.92	**-28.98**	0.15
Aromatics	-27.68	-27.15	-27.09	**-27.31**	0.32	-27.51	-27.33	-27.07	**-27.30**	0.22	-27.97	-27.59	-27.88	**-27.81**	0.20
Polars	-27.10	-27.13	-27.19	**-27.14**	0.05	-27.11	-27.14	-27.03	**-27.09**	0.06	-27.69	-27.76	-27.67	**-27.71**	0.05
Asphaltenes	-27.29	-27.39		**-27.34**	0.07	-27.70	-26.98		**-27.34**	0.51	-26.66	-26.57		**-26.62**	0.06

	Residue Oil					Waxy Distillate					Ballast Oil				
	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD	n1	n2	n3	Mean	SD
Whole Oil	-28.96	-28.72	-28.71	**-28.80**	0.14	-28.97	-28.67	-27.86	**-28.50**	0.57	-28.72	-28.53		**-28.63**	0.13
Saturates	-29.07	-28.88		**-28.98**	0.13	-28.71	-28.93	-28.60	**-28.75**	0.17	-28.83	-28.87	-28.83	**-28.84**	0.02
Aromatics	-28.33	-28.09	-28.11	**-28.18**	0.13	-27.90	-27.89	-27.93	**-27.91**	0.02	-27.75	-27.85	-27.93	**-27.84**	0.09
Polars	-27.98	-27.42	-27.55	**-27.65**	0.29	-28.14	-27.65	-27.78	**-27.86**	0.25	-27.65	-27.12	-27.74	**-27.50**	0.34
Asphaltenes	-27.72			**-27.72**	N/D	-27.72	-27.09		**-27.41**	0.45	-27.14			**-27.14**	N/D

The above data indicates that the Soxhlet extraction, rapid column fractionation, TLC-FID and bulk IRMS methods were able to provide valuable information on the bulk composition of heavy oils and serve as useful screening tools in the tiered analytical strategy.

3.1.7 EXTENDED ANALYSIS

In accordance with the final tier of the tiered analytical strategy, three methods were used to obtain a more detailed insight into the composition of the reference oils and acid tars; GC-FID, GC-EI MS and GC-IRMS. GC-FID was essentially carried out to screen samples prior to analysis by GC-EI MS and GC-IRMS. Through GC-EI MS analysis of the reference oil and acid tar samples, the capacity of established biomarker-based source and weathering indices to provide information on heavy oil composition was assessed.

An additional empirical assessment of selected source indices was carried out using a set of crude oil samples. Biomarker source indices are commonly used to discriminate between crude oils from different sources, as the distribution of biomarkers within an oil is dependent on the geochemical environment in which the oil was formed (Section 1.3.1). These samples were used to evaluate the capacity of selected source indices to distinguish between four crude oils. Four crude oils were obtained from the former Clyde River Purification Board (CRPB) sample bank; a Nigerian export crude oil, an Iraqi export crude oil, a North Sea oil from the Ninian South field and a North Sea crude from the Forties field.

To assess the suitability of GC-IRMS in the characterisation of heavy oils, a method for the compound-specific isotope analysis of the reference oils was developed. Acid tars were not analysed by GC-IRMS because the GC-FID results demonstrated that resolution of individual compound peaks could not be achieved. In addition, triplicate characterisation of the Nigerian crude oil by GC-IRMS was useful for method development purposes, as this technique has been routinely applied to crude oils in the past, and provided a baseline

evaluation of the compound-specific isotopic composition of this oil prior to microbial transformation

3.1.7.1 Gas Chromatography-Flame Ionisation Detection (GC-FID)

Analysis by gas chromatography was performed on an AI Model 93 gas chromatograph equipped with a 25 m, high temperature aluminium-clad diphenyl:disiloxane carborane (5 %:95 %) column (SGE HT5) with a 0.53 mm i.d. and 0.15 micron film thickness. This column is the high temperature equivalent of the more commonly used DB5 column, which is commonly used for analysis of petroleum hydrocarbons (e.g., Gough & Rowland, 1991). Oil saturate fractions were made up to a concentration of approximately 10 mg ml^{-1} in DCM, which was found through trial-and-error to be the optimum oil concentration for the operating conditions. 2 µl aliquots were injected in splitless mode. A linear temperature gradient was employed, the column temperature being held at 75 °C for 2 minutes following injection, ramped at 10 °C min^{-1} to 350 °C, then held at this temperature for a further 10 minutes. This temperature gradient was found to produce a satisfactory resolution for screening purposes. The injector and detector temperatures were set at 400 °C. A helium carrier gas was used at a flow rate of 7.5 ml min^{-1}.

n-Alkane peaks were identified by comparing sample GC-FID retention data with that obtained for a standard solution of 5 *n*-alkanes containing C_{15}, C_{20}, C_{25}, C_{30} and C_{40} in DCM. The standard solution was run daily and elution times for the standard components were found to remain consistent. Neat DCM was also routinely run to check for carry over between samples, particularly those containing a considerable high boiling fraction. Solvent blanks did not detect any 'carry-over' between runs, maintaining totally flat baselines each time. Routine analysis of the standard *n*-alkane solution allowed the *n*-alkane peaks within each oil sample to be identified; identification of other peaks (e.g., isoprenoids) was accomplished through the use of a C_{17}/pristane and C_{18}/phytane standard mixture and comparison with GC-

profiles reported in the literature. Poor baseline resolution was a feature of all the chromatograms, although this is often the case for heavy oils.

3.1.7.2 Gas Chromatography-Electron Impact Mass Spectrometry (GC-EI MS)

Oil saturates fractions were made up to a concentration of approximately 10 mg ml^{-1} in DCM. All GC-EI MS analyses, except those associated with the reference oil biotransformation study (Section 3.2.3), were carried out at the former Clyde River Purification Board, East Kilbride. Analyses were conducted on an HP model 5890 gas chromatograph equipped with a 60 m x 0.25 mm i.d. DB5 column (with a diphenyl:disiloxane (5 %:95 %) stationary phase) and a VG Trio 1 mass spectrometer detector and operated in splitless mode. 1 µl aliquots were injected through a 1m x 0.53 mm deactivated fused silica retention gap using an HP model 7673A autoinjector. A linear temperature gradient was employed, the column temperature being held at 30 °C for 1 minute following injection, ramped at 6 °C min^{-1} to 300 °C, then held at 300 °C for a further 30 minutes. Helium carrier gas was used at a flow rate of 7.5 ml min^{-1}. The GC-EI MS interface was maintained at 250 °C.

Electron impact ion chromatograms were obtained in selective ion monitoring mode (SIM) under the following conditions: 0.06 seconds dwell time, 0.5 a.m.u. span, 150 µA electron current, 70 eV electron energy. Two target ions were selected on the basis of previous studies (Butler *et al.*, 1991; Wang *et al.*, 1994b). These were *m/z* 85 (for detection of *n*- and isoprenoid alkanes) and *m/z* 191 (for tri-, tetra- and pentacyclic terpanes). All *n*-alkanes in a particular oil were used for index evaluation. Since only the tricyclic, tetracyclic and pentacyclic terpanes produce fragment ions in abundance at m/z 191 (Killops & Killops, 1993), this ion is particularly suited to monitoring the distribution of these compounds. Mass chromatograms for *m/z* 191 (tri-, tetra- and pentacyclic terpanes) and 85 (saturated hydrocarbons) ions for the Nigerian crude oil are shown in Figure 3.5 with the peaks identified described in Table 3.4.

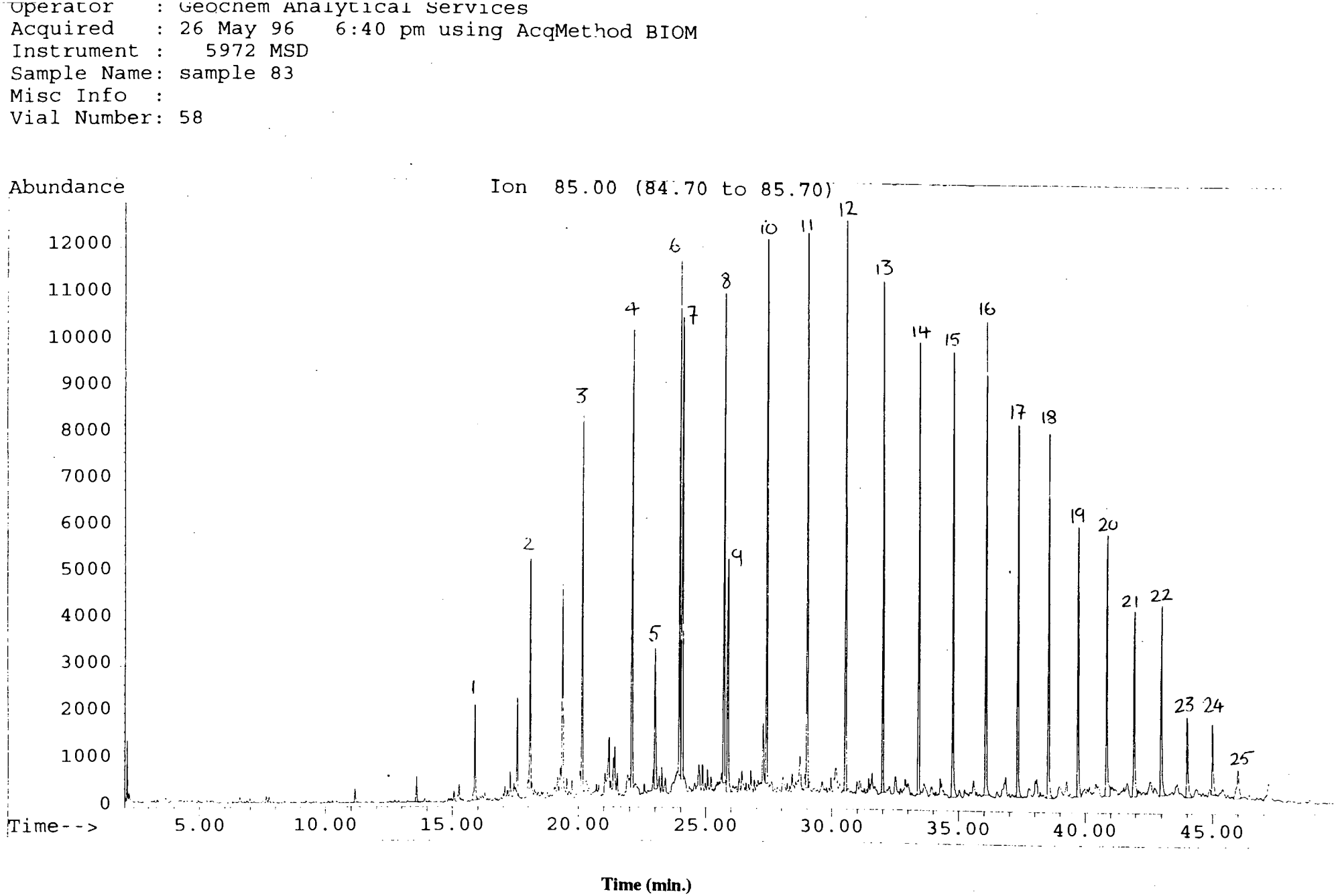


Figure 3.5 (a) Example GC-EI MS Ion Chromatogram at m/z 85 for Crude Oil

Operator : Geochem Analytical Services
Acquired : 26 May 96 6:40 pm using AcqMethod BIOM
Instrument : 5972 MSD
Sample Name: sample 83
Misc Info :
Vial Number: 58

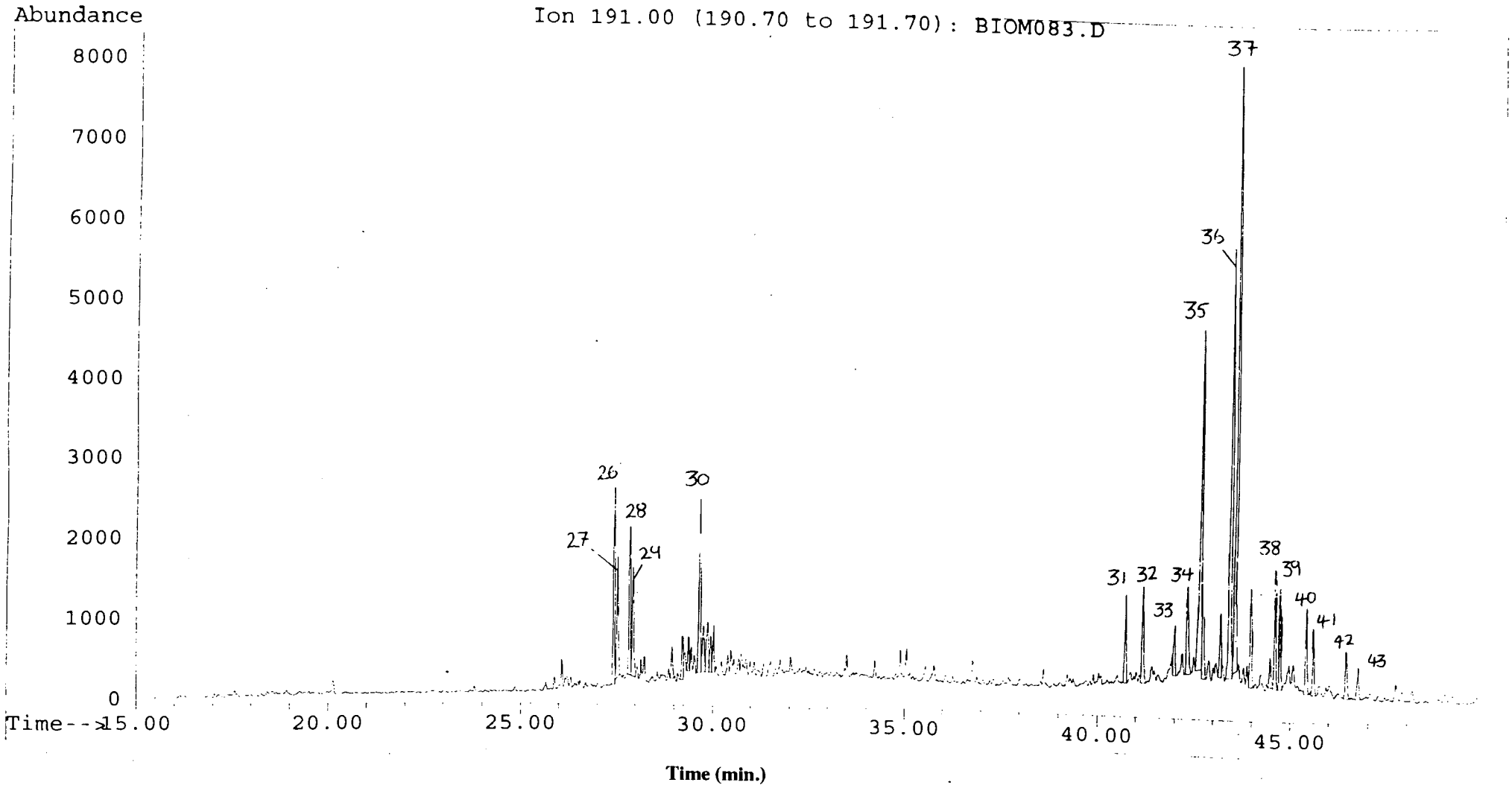


Figure 3.5 (b) Example GC-EI MS Ion Chromatogram at m/z 191 for Crude Oil

Table 3.4 Peak Identification for Crude Oil m/z 85 and 191 Fragmentograms (shown in Figure 3.4)

(a) m/z 85 fragmentogram

Peak	t_R (min)	Compound[1]	MW	Empirical Formula	Peak	t_R (min)	Compound[1]	MW	Empirical Formula
1	15.880	Tridecane	184	C13H28	14	33.429	Tricosane	324	C23H48
2	18.071	Tetradecane	198	C14H30	15	34.773	Tetracosane	338	C24H50
3	20.141	Pentadecane	212	C15H32	16	36.080	Pentacosane	352	C25H52
4	22.101	Hexadecane	226	C16H34	17	37.339	Hexacosane	366	C26H54
5	23.009	Norpristane	254	C18H38	18	38.549	Heptacosane	380	C27H56
6	23.965	Heptadecane	240	C17H36	19	39.711	Octacosane	394	C28H58
7	24.062	Pristane	268	C19H40	20	40.848	Nonacosane	408	C29H60
8	25.732	Octadecane	254	C18H38	21	41.938	Triacontane	422	C30H62
9	25.877	Phytane	282	C20H42	22	42.991	Hentriacontane	436	C31H64
10	27.402	Nonadecane	268	C19H40	23	44.007	Dotriacontane	450	C32H66
11	29.012	Eicosane	282	C20H42	24	45.000	Tritriacontane	464	C33H68
12	30.549	Heneicosane	296	C21H44	25	46.004	Tetratriacontane	478	C34H70
13	32.013	Docosane	310	C22H46					

(b) m/z 191 fragmentogram

Peak	t_R (min)	Compound[1]	MW	Empirical Formula	Peak	t_R (min)	Compound[1]	MW	Empirical Formula
26	27.414	C23 Tricyclic Terpane (S ?)	318	C23H42	36	43.414	18α(H)-or β(H)-Oleanane	412	C30H52
27	27.511	C23 Tricyclic Terpane (R ?)	318	C23H42	37	43.559	17α(H),21β(H)-Hopane	412	C30H52
28	27.826	C24 Tricyclic Terpane (S ?)	332	C24H44	38	44.600	17α(H),21β(H)-Homohopane (22S)	426	C31H54
29	27.911	C24 Tricyclic Terpane (R ?)	332	C24H44	39	44.721	17α(H),21β(H)-Homohopane (22R)	426	C31H54
30	29.641	C24 Tetracyclic Terpane	330	C24H42	40	45.411	17α(H),21β(H)-Bishomohopane (22S)	440	C32H56
31	40.715	Ts: 18α(H),21β(H)-Trisnorhopane	370	C27H46	41	45.593	17α(H),21β(H)-Bishomohopane (22R)	440	C32H56
32	41.163	Tm: 17α(H),21β(H)-Trisnorhopane	370	C27H46	42	46.440	17α(H),21β(H)-Trishomohopane (22S)	454	C33H58
33	41.998	Unidentified Hopane	N/D	N/D	43	46.743	17α(H),21β(H)-Trishomohopane (22R)	454	C33H58
34	42.325	Unidentified Hopane	N/D	N/D					
35	42.652	17α(H),21β(H)-30-Norhopane	398	C29H50					

[1]Structures of selected compounds shown in Figure 1.2

Source correlation and weathering indices were calculated to single decimal place precision by determining the ratio of the integrated peak areas on mass chromatograms of the compound(s) featured in the index (Nordtest, 1991; Pande *et al.*, 1994). Peak areas, used for evaluating the source and weathering ratios, were determined automatically, although peak baselines were set manually to avoid the erroneous incorporation of any baseline rise into the peak area calculation.

The spectrometer was tuned daily using a heptacosafluorotributylamine standard (mol. wt. 671.1). Sample blanks, containing neat solvent, and standard mixed *n*-alkane solutions were routinely analysed to guard against sample carry over and to assist with sample peak identification.

Repeat analysis of selected samples indicated relative standard deviation in mean biomarker ratio values of up to 10 %. Peaks corresponding to the *n*-alkanes and isoprenoids were determined by the use of analytical standards comprising 5 *n*-alkanes (C_{15}, C_{20}, C_{25}, C_{30} and C_{40}) and a mixture of C_{17}/pristane and C_{18}/phytane, both in DCM. Tricyclic and pentacyclic terpane (hopane) peak identification was achieved using 17β(H),21β(H)–hopane as an internal standard (since this hopane is not normally present in petroleum products it is commonly used as an internal standard) and by comparison of mass chromatograms at *m/z* 191 with corresponding reference chromatograms from the literature (Prince *et al.*, 1994; Wang *et al.*, 1994b; Seifert & Moldowen, 1979) and from CRPB library spectra.

Assessing the effect of the column chromatographic fractionation process on the values of selected source correlation indices was an important element of the quality assurance regime, since any such effects may cloud the variations in these ratios due to differences in source terms. Selected ratios were determined for the residue oil and ballast oil before and after passing through the alumina chromatography column. The results, shown in Table 3.5, indicate that the differences between the two sets of values were mostly within the reproducibility of the measurements, although in both cases, the [phytane:17α(H),21β(H)hopane] ratio is the most

Table 3.5 Selected Biomarker Indices Before and After Adsorption Column Chromatography

Biomarker Ratio	Index Values[1]							
	Ballast Oil		Residue Oil		No.6 Fuel Oil		Crude Oil	
	Before	After	Before	After	Before	After	Before	After
C18: Phytane	1.8 (0.2)	1.6 (0.2)	1.4 (0.1)	1.3 (0.1)	2.4 (0.3)	2.2 (0.2)	1.9 (0.3)	1.4 (0.1)
Pristane: Phytane	0.7 (0.1)	0.7 (0.1)	N/D	N/D	N/D	N/D	2.0 (0.2)	0.9 (0.1)
Phytane: 17α(H),21β(H)-Hopane	41.1 (4.1)	39.9 (4.0)	1.8 (0.2)	2.1 (0.2)	2.5 (0.3)	4.2 (0.4)	0.6 (0.1)	0.6 (0.1)
17α(H),21β(H)-Hopane: 17α(H),21β(H)-Norhopane	0.9 (0.1)	1.1 (0.1)	1.7 (0.2)	1.7 (0.2)	0.9 (0.1)	1.1 (0.1)	0.7 (0.1)	0.8 (0.1)

[1]Figures in brackets are standard deviations, determined throught triplicate analysis of selected samples

inconsistent. The results indicate that column chromatographic analysis did not alter the values of the indices and need not, therefore, be considered to be important in this respect.

Sample blanks run regularly during GC-EI MS analyses did not indicate the presence of any 'carry-over' between runs. Triplicate analysis of selected oil saturate extracts allowed the standard deviations (SDs) of ratio values to be determined, and these are reported accordingly. In general, the magnitude of the SD increased with increasing mean index value; relative standard deviations were found to be predominantly below 10 %, although values up to 25 % were recorded.

Further confirmation of the reliability of the GC-EI MS methodology was obtained through qualitative comparison of the m/z 85 and 191 chromatograms acquired here with those acquired previously by the CRPB. No significant differences were detected between corresponding chromatograms, thereby providing further evidence of the validity of the analytical procedures used in this work.

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

Oil samples were analysed in DCM solution at a concentration of *ca.* 10 mg ml^{-1} using a VG ISOCHROM II system. Individual compounds were isolated using a HP5890A gas chromatograph, equipped with a standard 30 m x 0.32 mm i.d. neutral DB5 column (diphenyl:disiloxane (5 %:95 %) stationary phase) and a dedicated stable carbon isotope ratio mass spectrometer. 2 µl aliquots 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. The injector and detector temperatures were set at 350 °C. A helium carrier gas was used 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 5 *n*-alkanes containing C_{15}, C_{20}, C_{25}, C_{30} and

C_{40}, and a C_{17}/pristane and C_{18}/phytane mixture. A typical heavy oil GC-IRMS profile is shown in Figure 3.6.

After passing through the silica/CuO combustion chamber, individual compounds were detected on a dedicated SIRA II Triple Collector isotope ratio mass spectrometer. Sample isotope ratios are evaluated initially relative to a reference CO_2 stream calibrated relative to the standard PDB carbonate formation. Final sample $\delta^{13}C$ values are reported relative to the standard PDB with corrections made for ^{17}O contributions.

The most important factor influencing the success of GC-IRMS in the determination of petroleum contaminants relates to the inherent limitations of all GC-coupled techniques when applied to weathered hydrocarbon wastes, namely, the presence of significant UCM. Manifested as baseline rise in GC-IRMS chromatograms, UCM can affect the signal-to-noise ratio of the individual peaks and result in uncertainty in the final value for compound $\delta^{13}C$. As the main aim in this work was to examine the feasibility of this technique in the characterisation of oily wastes under operational conditions, UCM was accepted as a necessary feature of these data, and one that it was important to determine the effect of upon individual compound $\delta^{13}C$ values. This was especially so for highly weathered oils, in which many compounds of interest are present in low abundance. Physical separation of class components prior to GC-IRMS analysis, through column chromatography, was useful for isolating the specific compound groups of interest but was unable to remove UCM material.

The effect of the UCM was studied by determining the reproducibility of the individual isotopic measurements through triplicate analysis of each sample. The minimum level of beam current detected was 2×10^{-10} amps, which meant that any 'peak' that did not produce a beam current above this level was not analysed. This figure is routinely used by the SURRC as the minimum from which a meaningful value of $\delta^{13}C$ could be determined. Routine Isochrom calibration was accomplished daily by analysis of a standard mixture of C_{10}, C_{11}, C_{12} *n*-alkanes

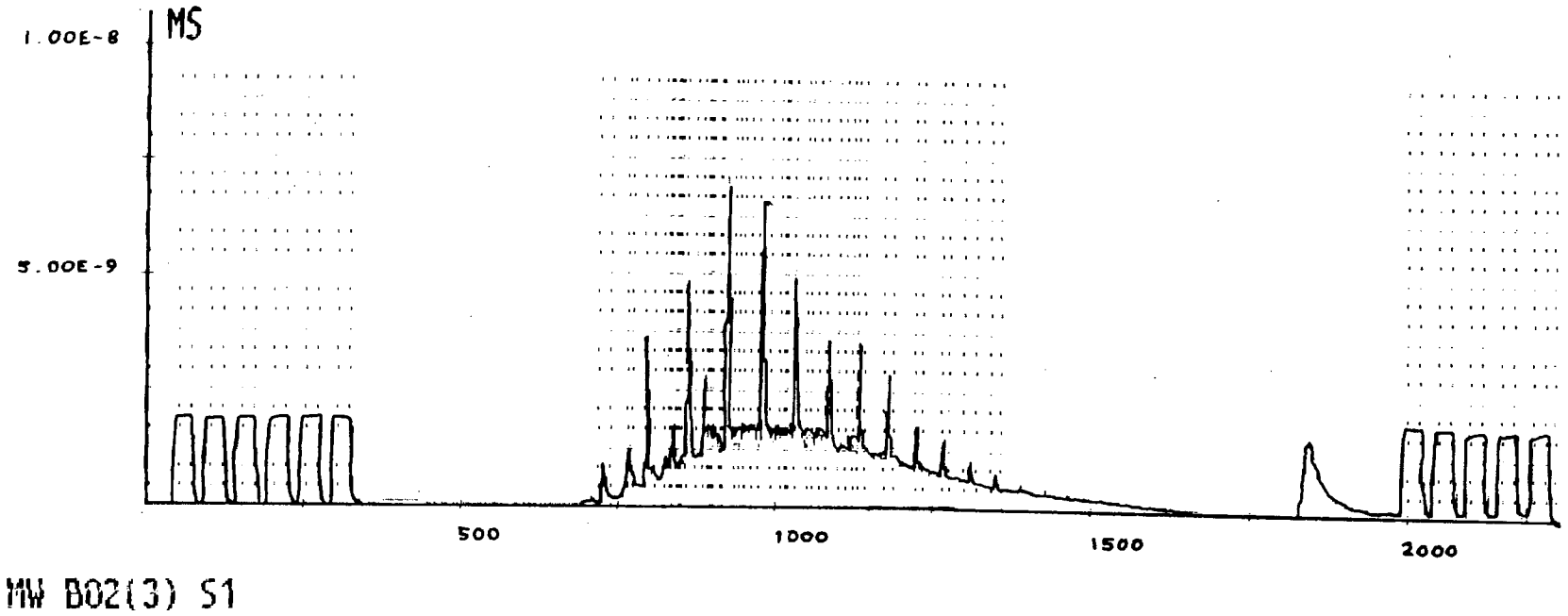


Figure 3.6 GC-IRMS Chromatogram for Ballast Oil Saturate Class Fraction

and methyldecanoate. Absolute calibration of the reference CO_2 relative to PDB is effected through the use of the NBS Standard 19 carbonate.

Standard deviations calculated from triplicate analysis of all samples were predominantly between 0.2 $^{o}/_{oo}$ and 0.6 $^{o}/_{oo}$, which compares favourably with reported ranges of precision (Bakel *et al.*, 1995). Several samples produced SDs of up to 1.2 $^{o}/_{oo}$, which, though relatively large, has often been reported in previous work (e.g., Schoell *et al.*, 1994). In this work, a possible explanation of this was the highly complex nature of these oils and, in particular, the presence of UCM in the chromatograms.

The extent to which the alumina column chromatographic fractionation of the oil samples affected the isotope ratios of individual compounds was also assessed by GC-IRMS analysis of crude oil and reference oil *n*-alkanes prior to and following column chromatography. The results, shown in Table 3.6, demonstrate that for the majority of compounds, pre- and post- column fractionation isotope ratios differed by up to 0.5 $^{o}/_{oo}$, which is within the reproducibility of the technique. These data indicate that alumina column chromatography causes little or no change in the isotope ratios of the detected alkanes, in agreement with previous studies (Bakel *et al.*, 1995).

These details support the development of a valid analytical strategy with which the source terms of heavy oil contaminants may be characterised (Sections 2.2 and 2.3). One of the key issues in this respect is the use of GC-EI MS biomarker-based indices of oil weathering and source. The performance of these indices has been assessed in this work through a 9-month soil microcosm study.

Table 3.6 Values of Individual $\delta^{13}C$ Before and After Adsorption Column Chromatography (‰)

	API Separator Oil				Ballast Oil				Residue Oil				Crude Oil			
Compound	Before[1]	After[2]	SD[3]	Diff.[4]	Before[1]	After[2]	SD[3]	Diff.[4]	Before[1]	After[2]	SD[3]	Diff.[4]	Before[1]	After[2]	SD[3]	Diff.[4]
C16	-28.59	-28.53	0.64	-0.06	-29.27	-28.97	0.46	-0.31	-27.90	-28.78	0.36	0.88	-28.57	-28.71	0.55	0.14
C17	-28.62	-28.52	0.95	-0.10	-29.84	-29.22	0.01	-0.62	-29.09	-29.13	0.10	0.04	-28.35	-29.07	0.66	0.72
C18	-29.15	-29.30	0.68	0.15	-29.23	-28.99	0.05	-0.25	-29.11	-28.70	0.22	-0.41	-28.46	-28.60	0.24	0.14
Phytane	-29.75	-29.45	0.40	-0.30	-30.29	-29.77	0.66	-0.52	-29.79	-29.37	0.36	-0.42	-29.50	-29.57	1.00	0.07
C19	-28.07	-29.48	0.14	1.41	-29.41	-29.14	0.11	-0.27	-29.25	-28.92	0.23	-0.33	-28.73	-28.79	0.64	0.05
C20	-28.74	-29.03	0.31	0.29	-29.44	-28.76	0.36	-0.68	-29.53	-29.00	0.16	-0.53	-28.76	-28.79	0.67	0.02
C21	-28.98	-28.99	0.29	0.01	-29.35	-28.95	0.34	-0.40	-29.46	-28.88	0.26	-0.58	-28.60	-28.75	0.64	0.15
C22	-28.70	-28.98	0.28	0.28	-29.26	-28.69	0.29	-0.57	-29.50	-28.91	0.27	-0.59	-28.75	-28.79	0.62	0.04
C23	-28.50	-28.88	0.20	0.38	-28.94	-28.92	0.29	-0.02	-29.49	-28.82	0.29	-0.67	-28.57	-28.60	0.66	0.02
C24	-28.16	-28.70	0.25	0.54	-28.95	-29.13	0.37	0.18	-29.45	-28.84	0.28	-0.61	-28.68	-28.76	0.71	0.07
C25	-28.07	-28.58	0.17	0.51	-28.97	-29.03	0.57	0.06	-29.37	-28.77	0.32	-0.60	-28.60	-28.69	0.73	0.08
C26		-28.41	0.31		-28.90	-29.17	0.21	0.27	-29.38	-28.92	0.26	-0.46	-28.65	-28.74	0.65	0.09
C27		-28.44	0.23						-29.32	-28.89	0.30	-0.43	-28.47	-28.72	0.66	0.25
C28		-28.45	0.27						-29.33	-28.89	0.24	-0.44	-28.62	-28.75	0.65	0.13
C29									-29.26	-28.70	0.28	-0.56	-28.38	-28.64	0.40	0.26
C30									-29.12	-28.62	0.41	-0.50	-28.16	-28.52	0.18	0.36

[1]Based on single CSIA of neat oil

[2]Based on triplicate analysis of oil saturates

[3]Standard deviations for triplicate analyses

[4]Difference between before and after isotopic measurements

3.2 OIL BIOTRANSFORMATION STUDIES

To fulfil one of the key aims of the thesis and investigate the biotransformation of heavy oils, a series of soil microcosm experiments were designed. In this section, details of the experimental materials and methods used in the soil microcosms are provided.

3.2.1 Soil Microcosms: Design and Conditions

The soil microcosm study was designed in close accordance with the British Standard 7755 Subsection 4.1.1 guidelines, 'Guidance on the selection and conduct of tests for determining the biodegradation of organic chemicals in soil under aerobic conditions' (British Standards Institute (BSI), 1995). This International Standard provides general guidelines for soil microcosm studies, an area where at present there is no agreed standard methodology. Where experimental details are not provided in this document, guidance was taken from comparable soil microcosm studies reported in the literature, in particular Chaineau *et al.* (1995), Huesemann (1995) and Hatzinger and Alexander (1995).

Three different oils were separately mixed with a well-characterised sandy-loam topsoil and left for a period of 9 months under optimal biotransformation conditions. Each oil was sampled in triplicate at the predetermined sampling points. So that losses due to biotic activity could be distinguished from those due to abiotic processes, control microcosms were also prepared in which the soil microbial community was poisoned with mercuric chloride ($HgCl_2$) (Chaineau *et al.*, 1995). Due to the extremely toxic nature of this substance, stringent safety precautions were required for this stage of experimental work, including the acquisition of detailed material safety data sheets, the use of protective clothing, masks and eyewear, and the adoption of special waste disposal arrangements. Oil losses in these microcosms could therefore be directly attributed to abiotic process. Blank microcosms, containing no oil, were prepared to assess the contribution of soil natural organic matter.

3.2.1.1 Soil Specifications and Oil Selection

The soil used in the microcosm study was a sandy clay loam topsoil purchased from the Scottish Agricultural College (SAC) in Auchincruive, Ayrshire. This soil type has a favourable combination of clay, soil and sand particles conducive to microbial activity (BSI, 1995). In particular, a high clay content in soils tend to retain a high moisture content, which restricts the flow of oxygen and limits biotransformation; high soil organic matter on the other hand tends to increase microbial activity (Sims, 1990).

The soil was of a non-carbonaceous gley type, taken from SAC conservation beds at a depth of 0 - 26 cm, and, as it had not been used for agricultural purposes for 8 years, was considered free of any previous contamination. Prior to delivery, the soil was sieved to less than 2 mm, to produce a more homogeneous soil distribution and increase oxygen dispersion throughout the soil (Chaineau *et al.*, 1995). In accordance with the BSI Guidelines, a full analysis of soil properties and field history was obtained. Soil particle size analysis indicated a clay content (particles < 0.002 mm) of 21.5 $\%^{w}/_{w}$, a silt content (particles 0.002 - 0.063 mm) of 21.2 $\%^{w}/_{w}$ and a total sand content (0.063 - 2 mm) of 57.3 $\%^{w}/_{w}$, which evidenced the suitability of the soil to oil biotransformation (Sims, 1990).

Further soil analysis indicated a pH of 6.0, an organic matter content of 5.9 %, and a gravimetric moisture content of 38.72 % (at 0.05 bar). Most heterotrophic bacteria are most active at a soil pH near neutrality (Leahy & Colwell, 1990), and monitoring pH was important to guard against a build up of acidic biotransformation intermediates which may be poisonous to soil microorganisms (Sims, 1990). Furthermore, knowledge of soil water holding capacity (WHC) was important for promoting optimum conditions for biotransformation, which have been widely reported as being at approximately 50 - 80 % of the soil WHC (Song *et al.*, 1990). Monitoring soil moisture content was also important, since a presence of water is essential to microbial catalysis but an excess of water can limit oxygen availability and reduce microbial activity. A complete list of the soil's chemical and physical properties are given in Table 3.7.

Table 3.7 Chemical and Physical Properties of Soil used in Biotransformation Study[1]

Soil Sampling Details		Particle Size Analysis (% w/w)		Soil Analysis[3]	
Soil Texture:	Sandy Clay Loam	**Clay (< 0.002 mm)**	21.5	**pH (water)**	6.0
Sampling Date:	17-Jun-95	**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 - 0.125 mm)**	16.0	**CEC[2] (me of CEC in 100 g soil)**	13.1
Soil Type:	Non-calcereous gley	**Fine Sand (0.125 - 0.250 mm)**	23.6	**Nitrogen ($mg\ g^{-1}$)**	4.0
Previous Agricultural Use:	None for 8 years	**Medium Sand (0.250 - 0.500 mm)**	12.3	**Field Capacity Moisture Content (% at 0.05 bar)**	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		

[1]Information provided by Scottish Agricultural College

[2]Cation Exchange Capacity (Quantity of exchangeable ions per unit weight of soil, expressed in milliequivalents (me) per 100 g)

[3]For significance to microbial activity refer to Section 1.3.2.2, Section 1.3.3 and Section 3.1.1

With reference to soil biological properties, BSI Guidelines suggest that the presence of an active microbial population within the soil may be determined through the use of a biodegradable reference mixture. It is also stated that it may be useful to determine the microbial activity of the soil prior to the biodegradation test. In this study, the use of a crude oil (the Nigerian crude oil - see Section 3.1.7.2) as a biodegradation reference was considered to be a satisfactory means of confirming the biological activity of the soil, as the microbial susceptibility of crude oils under controlled conditions is well documented (Atlas, 1981; Leahy &Colwell, 1990; Bartha, 1986). Moreover, since we are concerned solely with the chemical changes experienced by the oil substrate, a full speciation of the soil's microbial consortia and balance of bacteria, actinomycetes and fungi was considered unnecessary.

As previously described, the biotransformation of three oils was studied; the Nigerian crude oil, the ballast oil and the No.6 Fuel Oil. The latter two heavy oils were chosen because they represent different types of heavy oil contaminant encountered at many sites, their theoretically disparate responses to microbial attack and the relative ease with which they could be chemically analysed following chromatographic cleanup (compared to, for example, the acid tar and bitumen samples) using the analytical strategy developed. Because heavy oil biotransformation is not well documented and cannot easily be predicted, the Nigerian crude oil was included in the microcosm study. Crude oil can be expected to undergo steady microbial transformation under optimal environmental conditions (e.g., Swannel *et al.*, 1995). Evaluation of the crude oil during biotransformation, therefore, provides a useful reference study for comparisons with previously reported studies and a context for monitoring the loss of heavy oils. The origins of the three oils and details of their analytical characterisation are described in previous sections.

3.2.1.2 Microcosm Preparation and Monitoring

Microcosms were prepared in wide-necked, acid-washed 500 ml erlenmeyer flasks. A total of 124 individual microcosm flasks were prepared: 27 flasks for soils treated with each oil (to allow triplicate analysis at the 9 scheduled sample points), 9 sterilised control flasks for each oil (one for each sample point) and a total of 16 blank microcosm flasks.

For the treated and sterilised microcosms, an initial concentration of oil in soil of 2.00 ± 0.05 $\%^{w}/_{w}$ by weight was prepared by adding 4.0 (± 0.1) g each oil to 200 (± 0.1) g soil portions via a glass dropping pipette. A similar contaminant loading has been used in several previous microcosm studies (Sims, 1990; Chaineau *et al.*, 1995; Aprill *et al.*, 1990) and was judged to be low enough to avoid inhibition of soil microbial activity and high enough to allow changes to oil composition to be clearly discerned through chemical analysis. This mixture was then homogenised using a blender prior to addition into the flasks. The 27 soils intended for use in the sterilised control flasks were first autoclaved at 121 °C under a pressure of 103.4 Pa for 15 minutes to destroy soil microbes. Care was taken to ensure that all equipment brought into contact with the oils was cleaned thoroughly following use with concentrated (10M) nitric acid, a detergent solution and solvent (Pollard *et al.*, 1992).

Treated, sterilised and blank soils were amended with a nutrient solution of 1.07 g NH_4NO_3 and 0.42 g K_2HPO_4 dissolved in 38 ml of distilled, de-ionised water, enough to bring the soil to 50 % of its maximum WHC. Maximum microbial activity is reportedly found between 40 % and 60 % of the WHC (BSI Guidelines). This provided a nitrogen and phosphorus input of 1.75 mg g^{-1} soil and 0.35 mg g^{-1} soil, respectively, and an equivalent molar C:N:P ratio of 100:8.75:1.75, comparable to that of previous similar studies (Huesemann, 1995; Chaineau *et al.*, 1995). This is believed to be close to the optimum C:N:P ratio for oil biotransformation in soil, although recently it has been suggested that a ratio of 100:1:0.1 is enough to support enhanced microbial activity (Lethbridge *et al.*, 1995).

The 27 control flasks were also amended with a 1 $\%^{w}/_{w}$ solution of $HgCl_2$, to ensure continued suppression of the soil microbial community.

The water content of the microcosm flasks was monitored throughout the period of study by weighing. Any water lost was replaced by an appropriate amount of distilled, de-ionised water, so that the original water content was maintained to within ± 5 %. A commensurate amount of $HgCl_2$ was added to the control flasks whenever their water content needed to be restored.

pH measurements of a 1:5 (v/v) suspension of soil in water (with KCl buffer) taken at regular intervals using a standard laboratory pH meter indicated that soil pH remained at approximately 6.5 throughout the study. Amendment of pH was not, therefore, required.

Microcosms were incubated in a temperature-programmable, fan-assisted oven at 30 °C, maximum mesophilic activity generally being found between 25 °C and 35 °C (Bartha, 1986). The range of temperatures to which the flasks were exposed during incubation was recorded at regular intervals and found to be within the 2 °C drift specified by the BSI Guidelines.

3.2.1.3 Sampling

The sampling regime adopted was that recommended in the BSI Guidelines, i.e., 0 days, 2 days, 4 days, 8 days, 16 days, 32 days, 64 days, 128 days and 256 days after initial application of the oil. This sampling frequency is intended to reflect the more rapid degradation of oil during the initial stages of incubation, and is sufficiently long to allow significant oil biodegradation. Destructive sampling was accomplished by emptying the entire contents of each flask onto clean, labelled aluminium foil. Following sampling, soils were left to air dry under forced-draught in a fume cupboard in preparation for extraction and analysis.

Under the conditions described above, ballast oil, crude oil and No.6 Fuel Oil microcosms were sampled in triplicate at the pre-determined sampling stages, i.e., after 0, 2,

4, 8, 16, 32, 64, 128 and 256 days. The extraction and subsequent analysis of oil extracts were carried out using methods described in Sections 3.1.6 and 3.1.7.

3.2.2 Soil Microcosms: Analysis

Analytical characterisation of the oils during biotransformation was accomplished using the Soxhlet extraction, column fractionation, GC-EI MS and GC-IRMS methods developed over the first part of the research.

3.2.2.1 Determination of Solvent Extractable Material

Experimental details of the Soxhlet extraction method used to determine soil solvent extractable material (SEM) from the respective microcosm flasks are provided in Section 3.1.6.1. The reproducibility of individual SEM values obtained for each microcosm flask was determined by triplicate extraction of randomly selected flasks. In these samples, treated soils were air-dried, sieved, mixed and divided into three portions for simultaneous extraction.

Triplicate extraction of selected microcosm flasks indicated that SEM precision within each individual microcosm flask was ± 3.8 % for the ballast oil, ± 1.8 % for the crude oil and ± 1.3 % for the No.6 Fuel Oil. Extraction of blank microcosms (i.e., those left untreated) indicated a mean solvent-extractable natural organic matter content of 0.23 ± 0.04 mg g^{-1}. The values of SEM for the treated and control soils have been corrected for this contribution.

3.2.2.2 Determination of Individual Class Fraction Variations

Experimental details of the de-asphaltening procedure and column fractionation method used to determine the class fraction distribution of extracted organic matter are provided in Section 3.1.6.2. For each oil sample, the individual standard deviations of the mean class fractions percentages for each oil sample analysed are provided alongside the results, to assist convenient comparison (Tables 4.7, 4.8 and 4.9 for ballast oil, crude oil and

No.6 Fuel Oil, respectively). Results show that the precision of the class fraction evaluations is much greater over the initial stages of biotransformation, with typical relative standard deviations of up to approximately 10 % and frequently below this. At the later stages of biotransformation, however, the precision of the SAPA measurements decreases, with relative standard deviations recorded up to 25 % and very occasionally higher than this. This is probably due to the unpredictability of oil biotransformation, which causes increasingly disparate amounts of oil to become depleted in microcosms as microbial activity proceeds.

3.2.2.3 GC-EI MS Analysis

Analysis of the biomarkers and *n*-alkanes contained within the purified saturate class fraction extracts (approximately 10 mg ml^{-1} in DCM) from each microcosm flask was accomplished using a Hewlett Packard 5890 series II gas chromatograph combined with a HP 5972 mass selective detector. The GC was equipped with a Restek XT1-5 column (equivalent to the standard diphenyl:disiloxane (5 %:95 %) stationary phase DB5 column) of length 30 m, diameter 0.25 mm and film thickness 0.25 μm. 2 μl aliquots were injected in splitless mode. A linear temperature gradient was employed, the column temperature being held at 35 °C for 1 minute following injection, ramped at 6 °C min^{-1} to 300 °C, then held at this temperature for a further 15 minutes. The injector and detector temperatures were set at 300 °C and 280 °C , respectively. A helium carrier gas was used at a flow rate of 7.5 ml min^{-1}.

Electron impact ion chromatograms were obtained in selective ion monitoring mode (SIM), at a dwell time per ion of 0.1 seconds and an ion span width of 0.5 a.m.u.. As in the source term analysis, the two target ions selected were m/z 85 (for detection of *n*- and isoprenoid alkanes) and m/z 191 (for tri-, tetra- and pentacyclic terpanes).

Source correlation and weathering indices were calculated to single decimal place precision by determining the ratio of the automatically-determined integrated peak areas on

mass chromatograms of the compound(s) featured in the index in question (Nordtest, 1991; Pande *et al.*, 1994). The rationale for their selection is provided in Section 5.1.4.2.

Sample blanks, containing neat solvent, and standard mixed *n*-alkane solutions were routinely analysed to guard against sample carry over and to assist with sample peak identification. Repeat analysis of selected samples indicated that standard deviations in biomarker ratio values were once again up to 10 %. Peaks corresponding to the *n*-alkanes and isoprenoids were determined by the use of an analytical standard comprising 5 *n*-alkanes (C_{15}, C_{20}, C_{25}, C_{30} and C_{40}) and a C_{17}/pristane and C_{18}/phytane mixture. Tricyclic and pentacyclic terpane (hopane) peak identification was possible from the relative retention times of major peaks relative to 17α(H),21β(H)–hopane and by comparison of mass chromatograms at *m/z* 191 with corresponding reference chromatograms from well-characterised standard crude oils and from the literature (Prince *et al.*, 1994; Wang *et al.*, 1994).

Routine analysis of solvent blanks indicated no measurable carry-over between GC-EI MS runs. For convenience, values for the precision of each index values at each sampling point for the ballast oil, crude oil and No.6 Fuel Oil, determined through triplicate analysis of soil microcosms are provided with the index values obtained during the study (Tables 4.10, 4.11 and 4.12, respectively). In general, indices showing the greatest changes in value with oil weathering were found to have the largest relative standard deviations, particularly towards the latter stages of the study (generally between 5 % and 15 %, but occasionally up to 28 %). For the source correlation indices that changed very little in value with oil biotransformation, relative standard deviations were on the whole much smaller (< 10 %). The errors associated with each of the measurements are represented as error bars in each of the charts in this Section.

Comparing the values of several source correlation and weathering indices for oils extracted after 0 days (i.e. undegraded) (see Tables 4.10, 4.11, 4.12) with those obtained previously for the fresh oils (during the source term investigation - see Tables 4.3 and 4.4)

was a useful additional way of checking for systematic errors. Although the two sets of values were obtained using different mass selective detectors, the values were generally in close agreement (e.g., [phytane:17α(H)21β(H)-hopane] = 39.9 and 41.9 for the ballast oil, and 0.6 in both cases for the crude oil; [C_{18}:phytane] = 1.6 and 2.0 for the ballast oil, 1.4 and 1.9 for the crude oil and 2.2 and 2.7 for the No.6 Fuel Oil; and [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane] = 0.9 and 0.7 for the ballast oil, 0.8 and 0.7 for the crude oil and 0.9 and 0.7 for the No.6 Fuel Oil). The largest shift in value was for the No.6 Fuel Oil [phytane:17α(H)21β(H)-hopane] ratio, which had a value of 4.2 in the source term study and 2.5 in the microcosm extract, although the reason for this difference is not clear.

3.2.2.4 GC-IRMS Analysis

Experimental details of the compound specific isotope analysis performed on the purified saturate class fraction extracts from each microcosm flask are the same as those described in Section 3.1.7.3.

For the treated soils, the $\delta^{13}C$ values presented are the means of the three extracts of each oil obtained at each sampling point. Standard deviations for these values are provided with the results of this analysis, in Tables 4.14, 4.15 and 4.16, and shown as error bars in associated graphics. The standard deviations of the individual means at each sampling point were found to be mostly between 0.2 and 0.8 $^{o}/_{oo}$, which compares favourably with precisions recorded in the literature, although in some instances the reproducibility lowers to 1.5 $^{o}/_{oo}$.

The effect of the isotopic composition of the soil natural organic matter (NOM) was also identified as being a potential source of error in the characterisation of contaminant isotopic changes. However, because the amount of NOM extracted from blank (untreated) soil microcosms was substantially less than the amounts of oil extracted from treated soils, the contribution of NOM isotopic composition was considered to be minimal.

3.2.3 Weathered Diesel Range Organics (DRO) Standards

The principle route of weathering for petroleum contaminants in the soil environment is microbial degradation (Section 1.3.2.2). However, in certain circumstances, particularly for petroleum contaminants containing some low-boiling compounds, physical weathering of oil may occur (Morgan & Watkinson, 1989). To investigate the physical effects of oil weathering, a set of progressively weathered diesel range organic (DRO) standards were obtained from Restek. These oils represent middle distillate petroleum products (typical boiling range 150 - 300 °C) (Nyer & Skladany, 1989), consisting predominantly of alkane hydrocarbons in the carbon number range of C_{10} - C_{28}, as characterised by gas chromatography with flame ionisation detection (GC-FID) analysis.

DRO samples at three stages of weathering were obtained: fresh (unweathered), 25 $\%^{w}/_{w}$ weathered and 50 $\%^{w}/_{w}$ weathered. The percent of weathering was determined by gravimetric weight loss as a percentage of the initial amount. In addition to providing useful information on physical weathering, the DRO samples also served as standards with which to engender greater confidence in the GC-EI MS and GC-IRMS analytical regime.

3.2.3.1 Analysis of DRO Standards

Weathering was effected by the manufacturers, using a combination of gentle heating of the DRO in a water bath with nitrogen blowdown, to prevent oxidation. The percent weathering indicated with each sample (i.e., 25 $\%^{w}/_{w}$, 50 $\%^{w}/_{w}$) was determined by monitoring the gravimetric weight loss from the original unweathered substrate. Experimental details of the GC-EI MS and GC-IRMS analyses performed on the DRO standards are given in Sections 3.1.7.2 and 3.1.7.3, respectively.

For the GC-EI MS, the relative standard deviation of the source correlation and weathering indices were taken to be approximately 10 %, as determined through triplicate analyses of reference and crude oils. The standard deviations of the index values, provided

in Table 4.17, indicate that the trends between weathered samples are not unduly affected by imprecision in the measurements.

The standard deviations of the isotope ratios, determined from triplicate CSIA analysis of DRO samples, are again provided with the results, in Table 4.18. The precision was comparable to that obtained in previous CSIA studies, at between 0.2 and 0.8 $^{o}/_{oo}$ in most cases. For the *n*-alkanes, the shift in isotopic composition between the fresh and weathered samples was not significantly above the reproducibility of the individual $\delta^{13}C$ measurements. Where appreciable shifts occurred for the 50 % w/w weathered DRO sample, low abundance of compounds in this sample was such that the reproducibilities of the $\delta^{13}C$ values could only be determined for half the compounds.

In the preceding discussion, method development of the techniques comprising the tiered analytical strategy was detailed, in accordance with the first stage of experimental design (Section 2.3). This work was critical to the fulfilment of two of the principle aims of this thesis (development of novel analytical methodologies and construction of tiered analytical strategy - see Section 2.2), and for developing the analytical approach with which a third, characterisation of oil biotransformation, could be effected. In the next chapter, the results of the analysis of contaminant source terms and of the soil microcosm study are presented, obtained using the methods described above.

CHAPTER 4. RESULTS

4.1 METHOD DEVELOPMENT FOR HEAVY OIL CHARACTERISATION

In accordance with the structure of the tiered analytical strategy, the results of the screening techniques and the detailed component, extended analysis are considered separately.

4.1.1 Screening Techniques

Results are presented here for the screening stage of the tiered analytical scheme (Figure 1.7), which includes Soxhlet extraction (for acid tar-contaminated soils only), column chromatography class fractionation, TLC-FID and bulk IRMS.

4.1.1.1 Extraction of Acid Tar Wastes

Extraction of the acid tar-contaminated soil samples was carried out to provide authentic samples for the investigation of heavy oil contaminant source terms and did not necessitate an accurate determination of acid tar recoveries. However, in order to check the integrity of the sample handling and Soxhlet extraction scheme, gravimetric recoveries were quantified and compared with those recorded by the suppliers. The results, shown in Table 4.1, demonstrate the high solvent extractable contents (SEM) of these samples, with, in our study, values obtained up to 286 mg g^{-1} dry soil (28.6 % $^{w}/_{w}$). SEM values were found to be very similar to those reported by the suppliers, although since these values were obtained using cyclohexane as the extraction solvent (compared with dichloromethane in our analysis), some differences in contaminant recoveries were expected.

Based on the triplicate extraction of each acid tar-contaminated soil, standard deviations (SDs) were calculated to be 15.7 mg g^{-1} for AT1 (mean recovery 159.7 mg g^{-1}), 35.2 mg g^{-1} for AT2 (mean recovery 255.1 mg g^{-1}) and 26.6 mg g^{-1} for AT3 (mean recovery 224.8 mg g^{-1}).

Table 4.1 Solvent Extractable Material (SEM) Recoveries for Acid Tar-Contaminated Samples

Sample	Amount Soil Extracted (g)	Amount SEM Recovered (g)	SEM mg g^{-1} dry soil	Mean SEM mg g^{-1} dry soil	SD	CEM[1] mg g^{-1} dry soil
AT1	32.64	5.40	165.44	159.70	15.69	145.00
	23.67	3.36	141.95			
	33.02	5.67	171.71			
AT2	24.98	5.41	216.57	226.51	9.99	260.00
	26.76	6.33	236.55			
	22.04	4.99	226.40			
AT3	35.61	6.93	194.61	224.51	27.13	N/D
	27.88	6.45	231.35			
	26.74	6.62	247.57			

[1] Cyclohexane Extractable Material (CEM), figures provided by suppliers

4.1.1.2 Column Fractionation

The gravimetrically-determined distribution of component classes, and the corresponding standard deviations, within the reference oils and acid tar wastes are shown in Table 4.2.

The ballast oil and waxy distillate were found to contain a very high proportion of saturate class compounds in this analysis (both > 70 $\%^{w}/_{w}$), consistent with their initial classification as the lightest of the reference oils. The aromatic, polar and asphaltene contents of these oils were correspondingly low (< 5 $\%^{w}/_{w}$). The API separator oil showed a slightly lower saturate class content (51.3 ± 2.6 $\%^{w}/_{w}$) and slightly greater aromatic and polar class contents (30.7 ± 1.5 $\%^{w}/_{w}$ and 14.9 ± 0.8 $\%^{w}/_{w}$, respectively) than these oils, despite displaying a physical similarity to the ballast oil. The asphaltene content (3.1 ± 0.4 $\%^{w}/_{w}$) of this oil was, however, close to that of the ballast oil. The residue oil, despite displaying a high viscosity and a relatively high boiling range, was found to consist 71.8 ± 3.6 $\%^{w}/_{w}$ saturates and 1.6 ± 0.2 $\%^{w}/_{w}$ asphaltenes. The No. 6 Fuel Oil (36.7 ± 1.8 $\%^{w}/_{w}$ saturates, 34.9 ± 1.7 $\%^{w}/_{w}$ aromatics, 12.6 ± 0.6 $\%^{w}/_{w}$ polars and 15.8 ± 2.3 $\%^{w}/_{w}$ asphaltenes) and bitumen (31.7 ± 1.6 $\%^{w}/_{w}$ saturates, 41.3 ± 2.1 $\%^{w}/_{w}$ aromatics, 11.0 ± 0.6 $\%^{w}/_{w}$ polars and 16.0 ± 2.4 $\%^{w}/_{w}$ asphaltenes) exhibited sequentially higher polar and asphaltene contents and lower saturate contents, characteristic of heavier oils (Altgelt & Boduszynski, 1994).

Column fractionation results for the acid tar samples demonstrate that these samples contained the greatest proportion of heavy-end polar and asphaltene class compounds of all the oil samples analysed. The heaviest of these was AT1, which exhibited a polar content of 12.0 ± 0.6 $\%^{w}/_{w}$ and an asphaltene content of 33.2 ± 4.9 $\%^{w}/_{w}$. Polar and asphaltene contents for AT2 and AT3 were 8.7 ± 0.4 $\%^{w}/_{w}$ and 26.5 ± 4.0 $\%^{w}/_{w}$, and 4.6 ± 0.2 $\%^{w}/_{w}$ and 29.6 ± 4.4 $\%^{w}/_{w}$, respectively. The saturate fraction within all three acid tar samples was also significant, however, at 43.3 ± 2.2 $\%^{w}/_{w}$ for AT1, 60.2 ± 3.0 $\%^{w}/_{w}$ for AT2 and 61.1 ± 3.1 $\%^{w}/_{w}$ for AT3.

Table 4.2 Normalised Class Fraction Distribution of Reference Oils and Acid Tars[1]

Oil	Saturates		Aromatics		Polars		Asphaltenes	
	%w/w	SD[2]	%w/w	SD[2]	%w/w	SD[2]	%w/w	SD[2]
AT1	60.2	3.0	4.6	0.2	8.7	0.4	26.5	4.0
AT2	61.1	3.1	4.6	0.2	4.6	0.2	29.7	4.5
AT3	43.3	2.2	11.6	0.6	12.0	0.6	33.2	5.0
Bitumen	31.7	1.6	41.3	2.1	11.0	0.6	16.0	2.4
No.6 Fuel Oil	36.7	1.8	34.9	1.7	12.6	0.6	15.8	2.4
Residue Oil	71.8	3.6	20.7	1.0	5.9	0.3	1.6	0.2
Waxy Distillate	75.1	3.8	17.9	0.9	6.7	0.3	0.3	0.0
API Separator Oil	51.3	2.6	30.7	1.5	14.9	0.7	3.1	0.5
Ballast Oil	75.3	3.8	12.3	0.6	8.1	0.4	4.3	0.6

[1]Determined by adsorption column chromatography

[2]Standard deviations, based on relative standard deviations obtained from selected triplicate analyses (RSD = 5 % for Sats, Aros, Pols; 15 % for Asps)

4.1.1.3 Thin Layer Chromatography-Flame Ionisation Detection (TLC-FID)

TLC-FID analysis was completed on the six reference oils. Figure 4.1 shows the TLC-FID class compositional fingerprint of each of the six reference oil. The four sections within each horizontal bar are the normalised proportions (by weight percentage) of component classes, as defined by the eluting solvent, within each reference oil. The ballast oil, the waxy distillate and, to a lesser extent, the API separator oil were found to display high saturate contents (> 70 %) and low polar and asphaltene contents. The residue oil was also found to display a predominant saturate fraction and aromatic class fraction. Class fraction fingerprints for the heavier bitumen and No.6 oil samples demonstrated that these oils have a much more evenly distributed chemical composition, with comparable amounts of saturates, aromatics, polars and asphaltenes.

4.1.1.4 Isotope Ratio Mass Spectrometry (IRMS)

Individual whole oil and class fraction $\delta^{13}C$ values for each of the oils, presented in the form of characteristic type curves are shown in Figure 4.2. Isotope type curves were first used by Stahl (1978) as a means of correlating the isotope ratios of individual class fractions with that of the kerogen of the source rock from whence the crude oil originated. Type curves are generated by a plot of the class fraction isotope ratios aligned according to their increasing polarity, and include the whole oil isotope ratio in this case in place of the kerogen. The horizontal spacing of the fractions is arbitrary, but is justified through its widespread previous application (Stahl, 1978; Chung *et al.*, 1981; Schoell, 1984; Stahl, 1977).

For the lighter oil samples the results are in general agreement with the conventionally observed trend, increasing in the order; $\delta^{13}C_{sat}$ ($\cong \delta^{13}C_{oil}$) < $\delta^{13}C_{aro}$ < $\delta^{13}C_{pol}$ < $\delta^{13}C_{asp}$, with $\delta^{13}C_{sat}$ up to 2.5 $^{o}/_{oo}$ more negative than $\delta^{13}C_{pol}$ and $\delta^{13}C_{asp}$. Thus the API separator oil, ballast oil, residue oil and waxy distillate type curves are very similar, displaying saturate class isotope ratios that are significantly different from those of the remaining aromatic, polar and

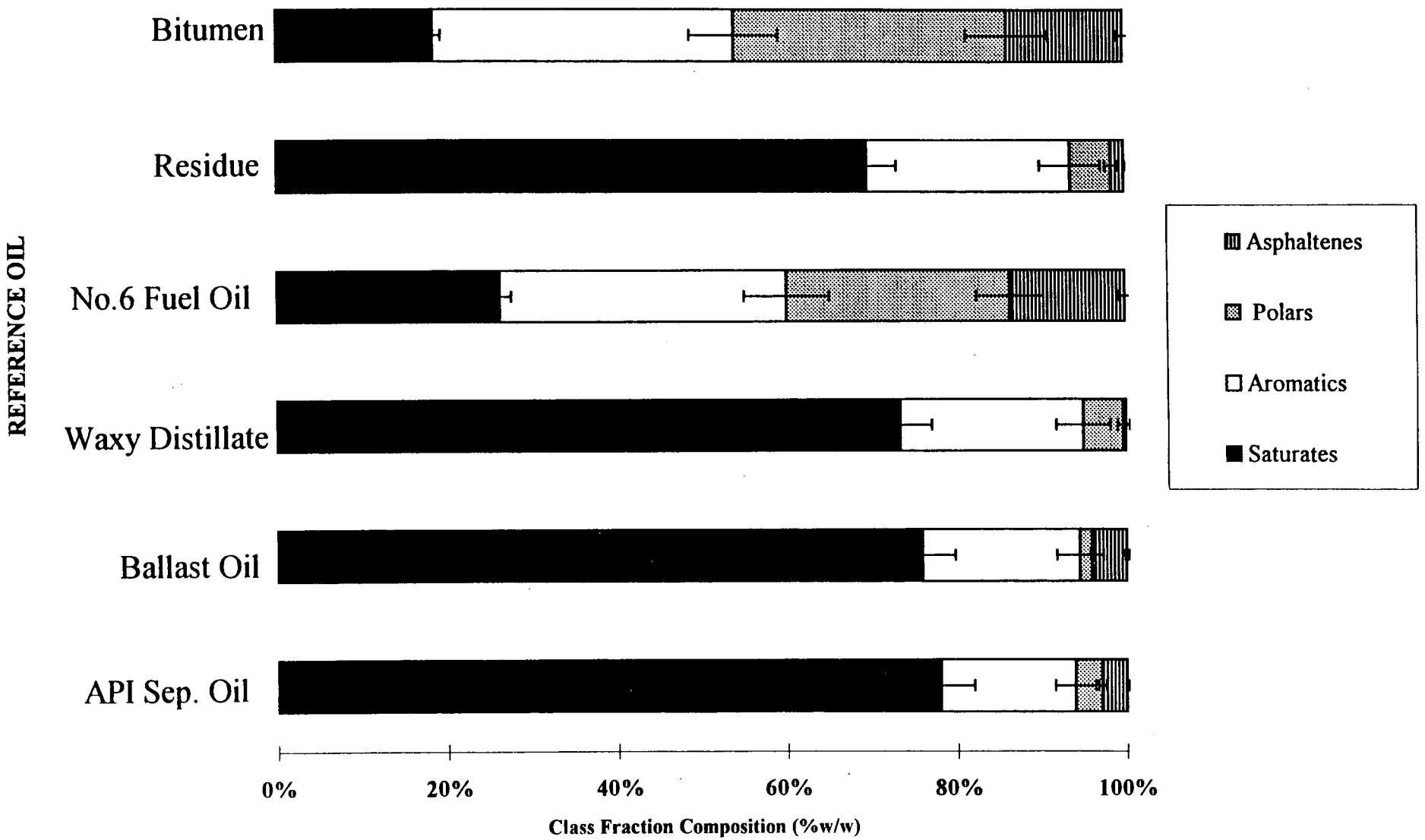


Figure 4.1 Class Fraction Fingerprint of Reference Oils determined by Iatroscan TLC-FID

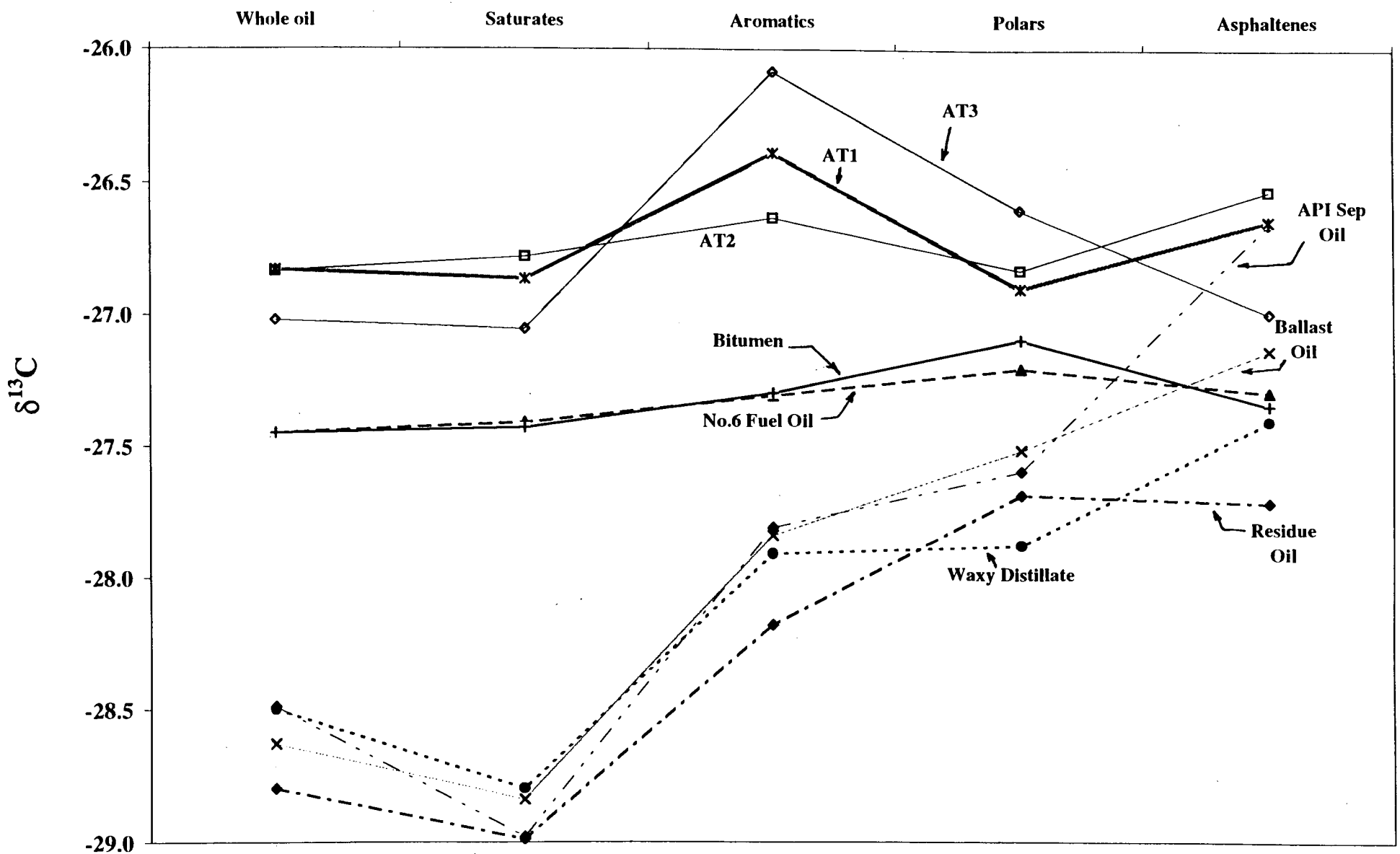


Figure 4.2 Isotope Type Curves for Reference Oils and Acid Tars

asphaltene class fractions. In contrast, isotope type curves for the heavier acid tar, bitumen and No.6 fuel oils show no conspicuous variation in class fraction isotopic composition, resulting in zero gradient, or flat, type curves. For the acid tar sample, all class fraction $\delta^{13}C$ values lay between -26.4 and -26.9 $^{o}/_{oo}$; for the bitumen and No.6 Fuel Oil, the range was -27.4 to -27.2 $^{o}/_{oo}$. Error bars are omitted for reasons of clarity but SDs are provided in Table 3.3.

4.1.2 Extended Analysis

4.1.2.1 Gas Chromatography-Flame Ionisation Detection (GC-FID)

Figures 4.3 and 4.4 show the GC-FID chromatograms of the six reference oils and the three acid tars, respectively. The API separator oil, ballast oil and No.6 Fuel Oil GC profiles display a range of *n*-alkanes centred on C_{18} - C_{22} with asymmetric tails of *n*-alkane peaks up to *ca.* C_{30}. The residue oil profile is similar to these, although higher *n*-alkane peaks are more prominent and there is a slight baseline rise signifying the presence of unresolved complex material (UCM). The UCM is the dominant feature of the waxy distillate chromatogram, which bears a close resemblance to that of typical lubricating oil profiles and includes a significant amount of high boiling components (> C_{30}). For the bitumen oil, GC-FID failed to detect or resolve any saturate class components, despite lengthy and repeated sample cleanup.

GC-FID chromatograms for the acid tar samples are characterised by a dominant UCM 'hump' upon which sit sets of much smaller, poorly resolved peaks. At lower retention times, all three chromatograms exhibit a clearly discernible, though not very prominent, series of peaks corresponding to the lower carbon number *n*-alkanes (from approximately C_{15} to C_{22}). However, as the UCM becomes more pronounced the *n*-alkane peaks are much harder to identify, and become mixed with other sample constituent peaks such that individual peaks cannot be assigned with any degree of confidence.

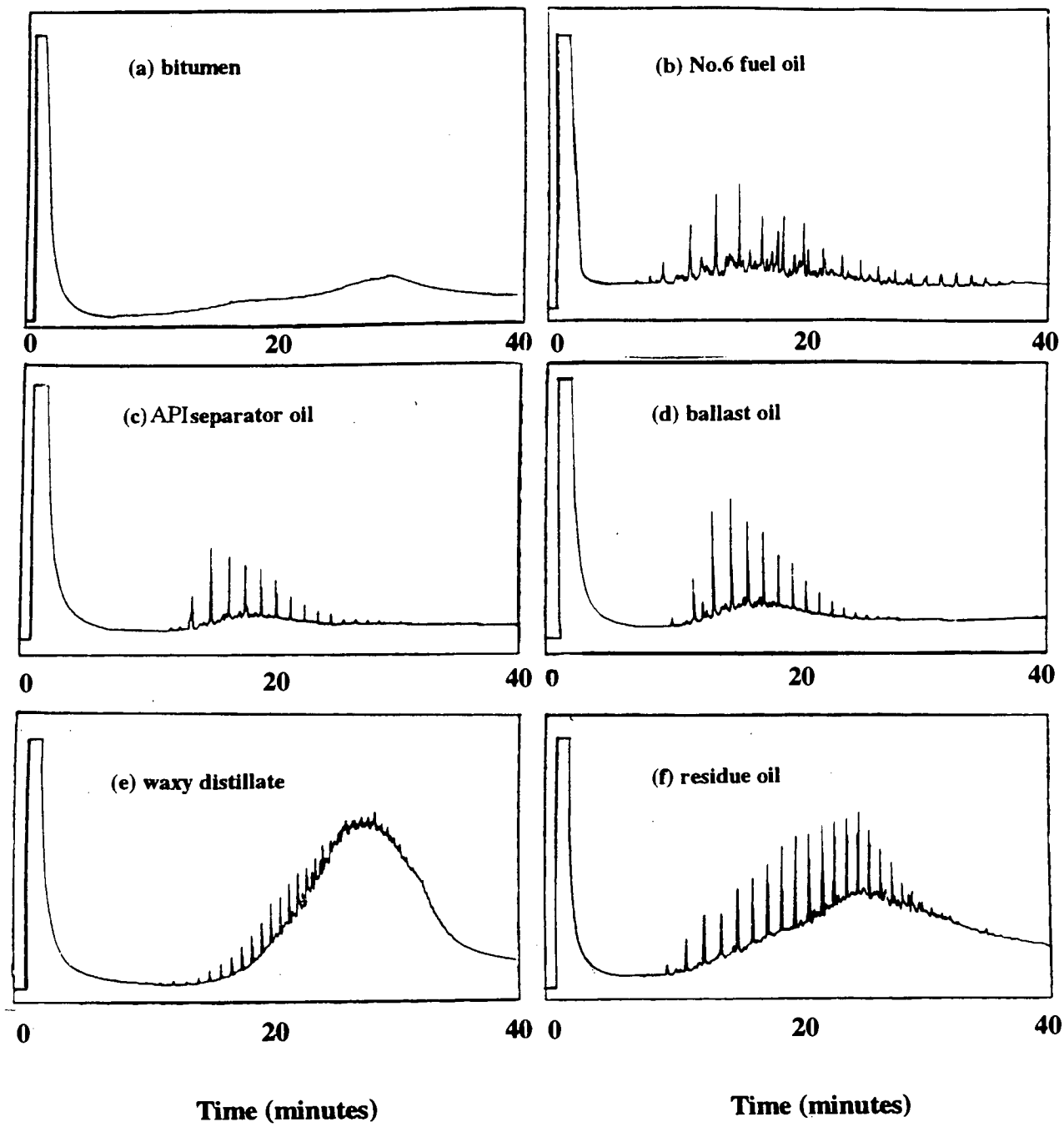


Figure 4.3 Gas Chromatography-Flame Ionisation Detection Profiles of Reference Oils

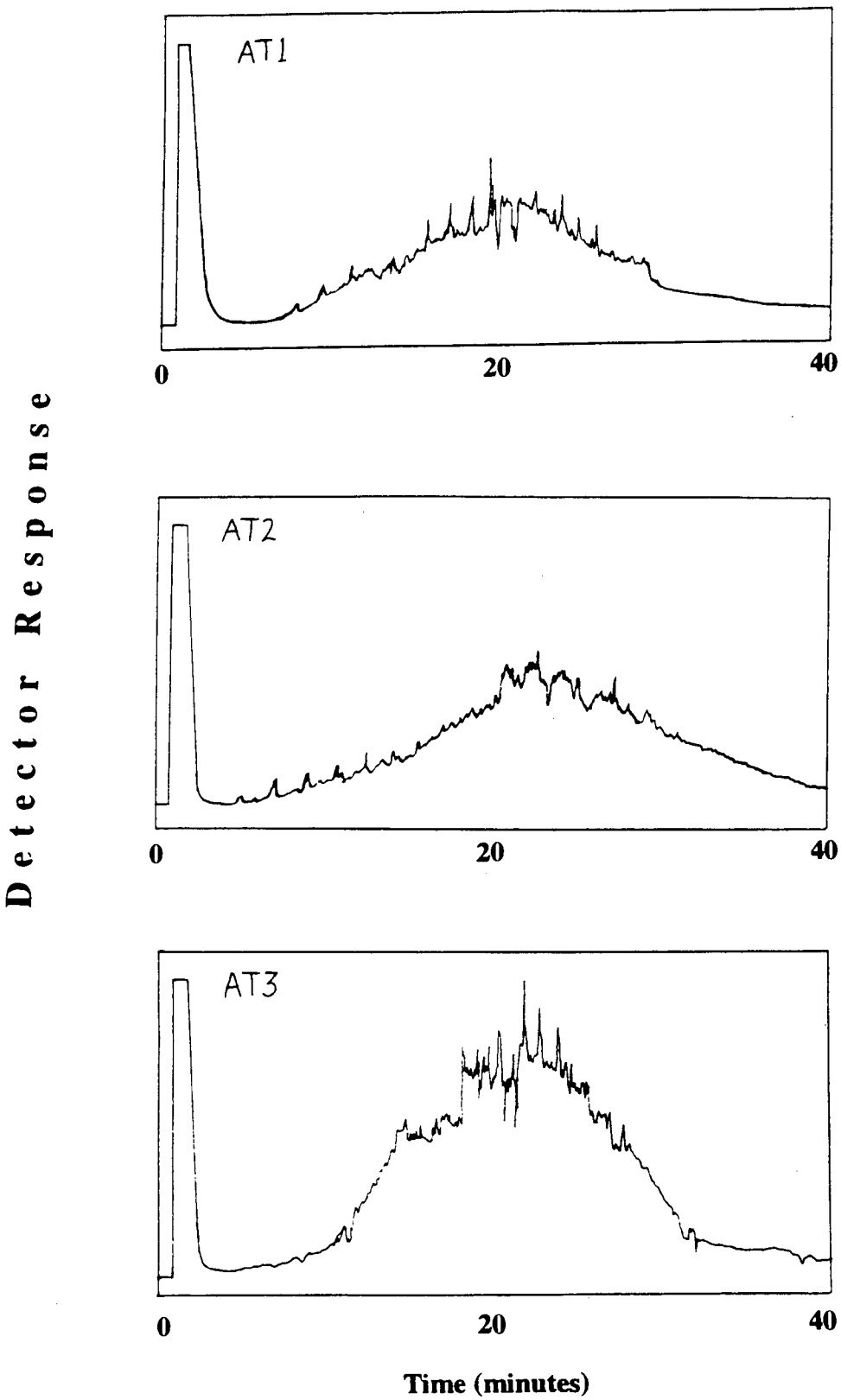


Figure 4.4 Gas Chromatography-Flame Ionisation Detection Profiles of Acid Tar Samples

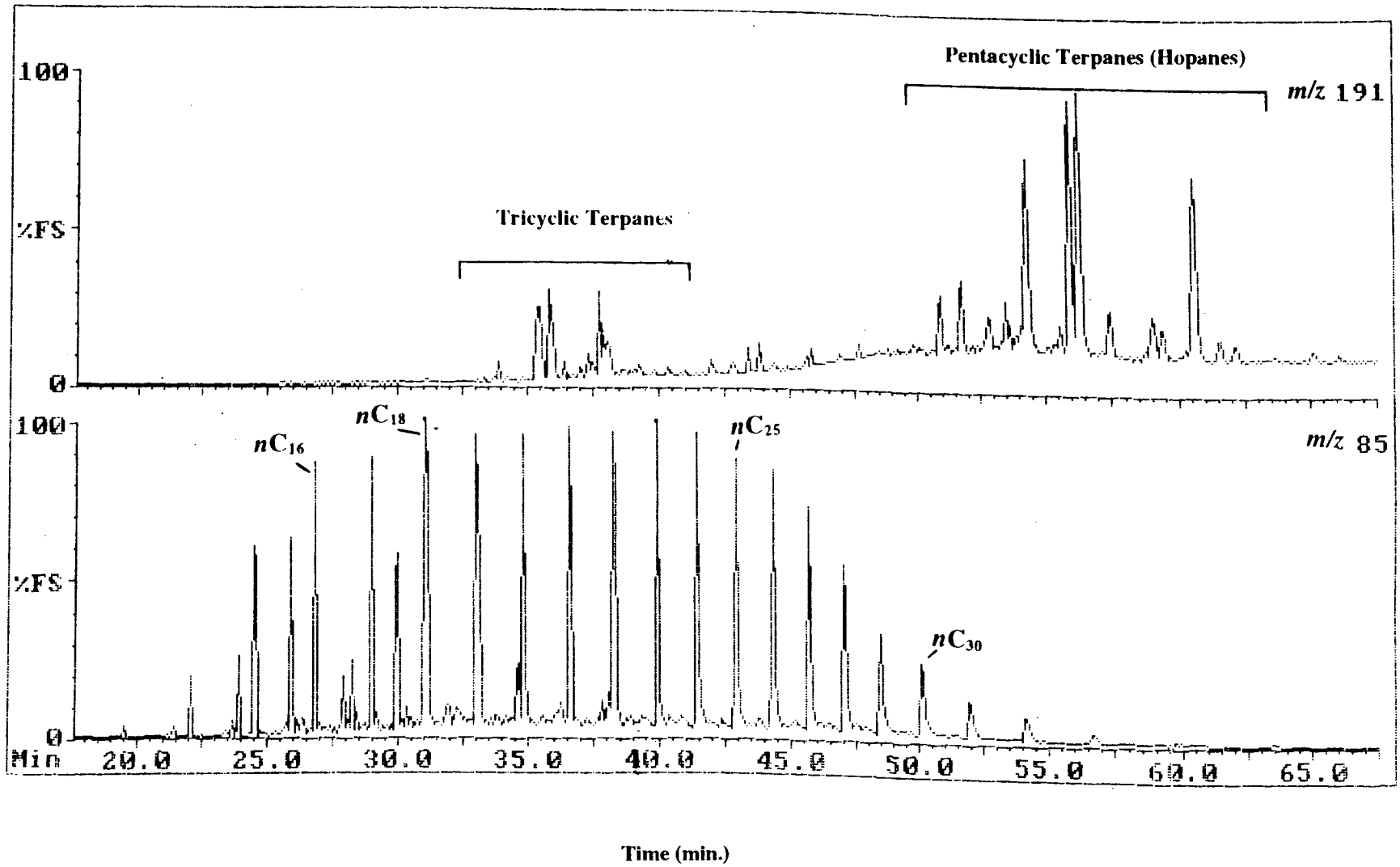


Figure 4.5 GC-MS Chromatograms of Saturated Hydrocarbons (m/z 85) and Terpanes (m/z 191) for Residue Oil. Peaks were identified by several means (see Section 3.1)

terpanes, or hopanes. For the API separator oil, ballast oil and No.6 Fuel Oil the former group were in far greater abundance, whereas for the residue oil the peaks in each group were of comparable size.

The following ratio of compounds were evaluated for each samples: [C_{18}:phytane], [pristane:phytane], [17α(H),21β(H)–hopane:17α(H)21β(H)–norhopane], [phytane:17α(H),21β(H)–hopane], [*n*-alkanes:17α(H),21β(H)–hopane] and [tricyclic terpanes:hopanes]. The rationale for their selection is discussed in Chapter 5, Section 5.1.4.2.

Values for the six selected source and weathering indices for each oil are given in Table 4.3. Two indices, [17α(H),21β(H)–hopane:17α(H),21β(H)–norhopane] and [C_{18}:phytane], changed very little between the oils, both showing an overall spread in value of only 0.4 and 0.9, respectively. The remaining indices showed a marked dependence on oil composition. The indices found to be most sensitive to oil composition were [*n*-alkanes: 17α(H),21β(H)–hopane] and [phytane:17α(H),21β(H)–hopane]. The former ratio showed the largest variation in value between the oils, decreasing from 575.2 in the lighter ballast oil, to 406.4 in the API separator oil, 85.6 in the residue oil, 81.0 in the No.6 Fuel Oil and as low as 1.3 in the acid tar sample AT1. A similar, though less prodigious change is shown by the [phytane:17α(H),21β(H)–hopane] ratio which was found to be 39.9 in the ballast oil, 27.4 in the API separator oil, 2.1 in the residue oil, 4.2 in the No.6 Fuel Oil and 0.2 in AT1. The [tricyclic terpanes:hopanes] index also decreased with increasing oil boiling range, with values of 12.1 for the ballast oil, 8.2 for the API separator oil, 0.5 for the residue oil and 0.2 for AT2, but produced an unexpectedly high value of 11.6 for the No.6 Fuel Oil.

(ii) Crude Oils

TICs and ion 85 and 191 chromatograms for the four crude oils were also obtained. Those for the Nigerian crude oil are shown in Figure 3.5. As anticipated, each oil produced the characteristic spread of clearly resolved *n*-alkane peaks, from approximately C_{14} to C_{30}, in the

Table 4.3 Selected GC-MS Biomarker Indices for Reference Oils and Acid Tars

HEAVY OIL SAMPLE

Biomarker Ratio	Ballast Oil		API Sep. Oil		Residue Oil		No.6 Fuel Oil		AT1		AT2		AT3	
	Peak Area	Index[1] Value	Peak Area	Index[1] Value	Peak Area	Index[1] Value	Peak Area	Index[1] Value	Peak Area	Index[1] Value	Peak Area	Index[1] Value	Peak Area	Index[1] Value
C18:	370487	1.6	965065	1.6	6E+05	1.3	8E+05	2.2	848779	1.5	1999160	2.2	411573	1.3
Phytane	228890		597497		5E+05		4E+05		557075		916253		310140	
Pristane:	160445	0.7	262505	0.4	N/D		N/D		N/D		N/D		N/D	
Phytane	228890		597497											
n-Alkanes:	3E+06	575.2	9E+06	406.4	2E+07	85.6	7E+06	81.0	5E+06	1.4	1E+07	3.4	3E+06	2.0
17α(H),21β(H)-Hopane	5736		21824		2E+05		91395		4E+06		3044280		1E+06	
Phytane:	228890	39.9	597497	27.4	5E+05	2.0	4E+05	4.2	557075	0.2	916253	0.3	310140	0.2
17α(H),21β(H)-Hopane	5736		21824		2E+05		91395		4E+06		3044280		1E+06	
17α(H),21β(H)-Hopane:	5736	1.1	21824	1.0	2E+05	1.7	91395	1.1	4E+06	0.8	3044280	0.8	1E+06	0.7
17α(H),21β(H)-Norhopane	5293		20910		1E+05		82767		5E+06		3690830		2E+06	
Tricyclic Terpanes:	244830	12.1	713074	8.2	6E+05	0.5	4E+06	11.6	8E+06	0.5	1904771	0.2	3E+06	0.7
Hopanes	20301		87385		1E+06		4E+05		1E+07		1.2E+07		5E+06	

[1]Relative standard deviation, based on selected triplicate analyses, ± 10 %

m/z 85 chromatogram and the distinct grouping of peaks in the m/z 191 chromatogram corresponding to the tricyclic and pentacyclic terpanes.

The same source correlation indices described above were evaluated for the crude oils. As previously indicated, the rationale for their selection is discussed in Section 5.1.4.2. The results are given in Table 4.4. The greatest numerical variation in value between the three crude oil families was observed for the [*n*-alkanes:17α(H),21β(H)–hopane] index, which was evaluated to be 10.4 for the Nigerian crude oil, 184.7 and 166.5 for the two North Sea crude oils and 277.5 for the Iraqi crude oil. Other indices that varied in such a way as to reflect the respective crude oil families were [pristane:phytane], [phytane:17α(H),21β(H)–hopane] and [tricyclic terpanes:hopanes]. The value of the [17α(H),21β(H)–hopane:17α(H)21β(H)–norhopane] source correlation index also varied between the oils, albeit in a pattern that did not reflect the differences in crude oil geochemical background. The [C_{18}:phytane] ratio varied very little between the oils, yielding a value of 1.4 for the Nigerian crude oil, 2.3 and 1.2 for the two North Sea crude oils and 1.4 for the Iraqi crude oil.

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

Baseline compound specific isotope measurements of the saturate fractions of the four reference oils selected for extended analysis (API separator oil, ballast oil, residue oil and No.6 fuel oil) and the Nigerian crude oil are presented in Table 4.5. The acid tars were not analysed by GC-IRMS, because it was not possible to obtain a sufficient level of GC resolution to reliably determine the isotope ratio of individual compounds. This problem is discussed in Section 5.1.4.3. GC-IRMS chromatograms for each of the oils are shown in Appendix A. Within each sample, $\delta^{13}C$ values were determined for the range of individual *n*-alkanes and phytane.

The narrow range of isotope ratio values obtained for the compounds contained within each reference oil sample was found to be in close agreement with the bulk isotope values obtained for the saturate fractions as a whole (Table 3.3). UCM is present in varying amounts in

Table 4.4 Source Correlation Index Values for Crude Oil Samples

Biomarker Ratio	m/z ratio	Nigerian crude oil		North Sea crude oil 1		North Sea crude oil 2		Iraqi crude oil	
		Integrated Peak Areas	Index[1] Value	Integrated Peak Areas	Index[1] Value	Integrated Peak Areas	Index[1] Value	Integrated Peak Areas	Index[1] Value
C_{18}:phytane	85, 85	$^{547718}/_{392658}$	1.4	$^{1080190}/_{470507}$	2.3	$^{1217090}/_{994841}$	1.2	$^{827991}/_{591110}$	1.4
C_{18}:17α(H),21β(H)–hopane*	85, 191	$^{547718}/_{643292}$	0.9	$^{1080190}/_{64456}$	16.8	$^{1217090}/_{92972}$	13.1	$^{827991}/_{36723}$	22.6
n-Alkanes:17α(H),21β(H)–hopane*	85, 191	$^{6722148}/_{643292}$	10.4	$^{11902853}/_{64456}$	184.7	$^{15475568}/_{92972}$	166.5	$^{10189770}/_{36723}$	277.5
Pristane:phytane*	85, 85	$^{679901}/_{392658}$	1.7	$^{301221}/_{470507}$	0.6	$^{732941}/_{994841}$	0.7	$^{575899}/_{591110}$	1.0
Tricyclic terpanes:total hopanes*	191, 191	$^{416355}/_{2875231}$	0.2	$^{193585}/_{388602}$	1.0	$^{400842}/_{711858}$	1.3	$^{444433}/_{523083}$	5.6
Phytane:17α(H),21β(H)–hopane*	85, 191	$^{392658}/_{643292}$	0.6	$^{470507}/_{64456}$	7.3	$^{994841}/_{92972}$	10.7	$^{591110}/_{36723}$	16.1
17α(H),21β(H)–hopane: 17α(H),21β(H)–norhopane	191, 191	$^{643292}/_{765122}$	0.8	$^{64456}/_{86303}$	0.7	$^{92972}/_{53612}$	1.7	$^{36723}/_{17195}$	2.1

*Proposed source correlation indices

[1] Confidence limits: ± 10 %

Table 4.5 Results of Compound Specific Isotope Analysis of Reference Oils ($\delta^{13}C$, $^{o}/_{oo}$)

	API Separator Oil					Ballast Oil					Residue Oil				
Compound	n1	n2	n3	Mean $\delta^{13}C$	SD	n1	n2	n3	Mean $\delta^{13}C$	SD	n1	n2	n3	Mean $\delta^{13}C$	SD
C16	-27.9	-29.21	-28.4	**-28.53**	0.64	-29.3	-28.6		**-28.97**	0.46	-29	-28.4	-29	**-28.78**	0.36
C17	-28.62	-29.42	-27.53	**-28.52**	0.95	-29.23	-29.21		**-29.22**	0.01	-29.18	-29.02	-29.19	**-29.13**	0.10
C18	-28.64	-29.27	-30	**-29.30**	0.68	-29.02	-28.95		**-28.99**	0.05	-28.85	-28.45	-28.8	**-28.70**	0.22
Phytane	-29.17	-29.27	-29.91	**-29.45**	0.40	-29.30	-30.24		**-29.77**	0.66	-29.77	-29.25	-29.09	**-29.37**	0.36
C19	-29.64	-29.36	-29.45	**-29.48**	0.14	-29.06	-29.22		**-29.14**	0.11	-28.7	-29.17	-28.87	**-28.92**	0.23
C20	-28.83	-28.87	-29.38	**-29.03**	0.31	-28.69	-28.45	-29.15	**-28.76**	0.36	-29.18	-28.87	-28.94	**-29.00**	0.16
C21	-28.76	-28.89	-29.31	**-28.99**	0.29	-28.57	-29.22	-29.05	**-28.95**	0.34	-29.17	-28.68	-28.78	**-28.88**	0.26
C22	-28.80	-28.85	-29.30	**-28.98**	0.28	-28.38	-28.76	-28.94	**-28.69**	0.29	-28.70	-29.21	-28.82	**-28.91**	0.27
C23	-28.70	-28.84	-29.09	**-28.88**	0.20	-29.25	-28.81	-28.69	**-28.92**	0.29	-29.10	-28.52	-28.84	**-28.82**	0.29
C24	-28.43	-28.74	-28.93	**-28.70**	0.25	-29.13	-28.76	-29.49	**-29.13**	0.37	-29.12	-28.57	-28.84	**-28.84**	0.28
C25	-28.40	-28.61	-28.74	**-28.58**	0.17	-29.21	-29.48	-28.39	**-29.03**	0.57	-29.10	-28.47	-28.74	**-28.77**	0.32
C26	-28.12	-28.37	-28.73	**-28.41**	0.31	-29.37	-28.96	-29.17	**-29.17**	0.21	-28.90	-29.18	-28.67	**-28.92**	0.26
C27	-28.18	-28.61	-28.54	**-28.44**	0.23						-28.90	-28.59	-29.19	**-28.89**	0.30
C28	-28.2	-28.76	-28.4	**-28.45**	0.27						-28.67	-29.15	-28.84	**-28.89**	0.24
C29											-28.65	-28.45	-29.00	**-28.70**	0.28
C30											-28.24	-29.06	-28.55	**-28.62**	0.41

Table 4.5 (cont.) Results of Compound Specific Isotope Analysis of Reference Oils ($\delta^{13}C$, $^{o}/_{oo}$)

	No.6 Fuel Oil					Crude Oil				
Compound	n1	n2	n3	Mean $\delta^{13}C$	SD	n1	n2	n3	Mean $\delta^{13}C$	SD
C16	-27.15	-27.25		-27.20	0.07	-28.32	-29.10		-28.71	0.55
C17	-27.46	-26.30		-26.88	0.82	-28.60	-29.53		-29.07	0.66
C18	-28.61	-27.79		-28.20	0.58	-28.43	-28.77		-28.60	0.24
Phytane	N/D	N/D		N/D	N/D	-28.86	-30.28		-29.57	1.00
C19	-27.70	-27.37		-27.54	0.23	-28.33	-29.24		-28.79	0.64
C20	-27.77	-27.51		-27.64	0.18	-28.31	-29.26		-28.79	0.67
C21	-27.93	-27.49		-27.71	0.31	-29.20	-28.30		-28.75	0.64
C22	-27.93	-27.43		-27.68	0.35	-29.23	-28.35		-28.79	0.62
C23	-27.88	-27.45		-27.67	0.30	-28.13	-29.06		-28.60	0.66
C24	-27.75	-27.38		-27.57	0.26	-29.26	-28.25		-28.76	0.71
C25	-27.75	-27.41		-27.58	0.24	-28.17	-29.20		-28.69	0.73
C26	-27.74	-27.54		-27.64	0.14	-29.20	-28.28		-28.74	0.65
C27	-27.83	-27.46		-27.65	0.26	-28.25	-29.18		-28.72	0.66
C28	-27.76	-27.48		-27.62	0.20	-28.29	-29.21		-28.75	0.65
C29	-27.85	-27.67		-27.76	0.13	-28.35	-28.92		-28.64	0.40
C30						-28.39	-28.64		-28.52	0.18

each of the chromatograms, with the residue oil and No.6 Fuel Oil samples being most affected. The effect of the UCM upon the isotope ratios of the resolved compounds is discussed in Section 3.1.7.3.

In summary, the results presented above, together with those presented in the method development section (Section 3.1) provide evidence of the utility of the techniques in the characterisation of heavy oil contaminant source terms and of the overall capabilities of the tiered analytical strategy. The implications of these results in light of the overall hypothesis under examination are discussed in Chapter 5 (Section 5.1). In the next section, the application of the solvent extraction, column fractionation, GC-EI MS and GC-IRMS methods to the characterisation of oils at varying stages of microbial transformation are presented.

4.2 OIL BIOTRANSFORMATION STUDIES

The comprehensive characterisation of oil biotransformation is one of the key objectives of this thesis. This includes an evaluation of the bulk compositional changes that occur during oil biotransformation, as well as an assessment of source and weathering indices. The results of the soil microcosm study undertaken to facilitate this investigation are presented below.

4.2.1 Soil Microcosm Results

Characterisation of the reference oil microcosms was carried out in four stages: determination of soil solvent extractable material (SEM), class fraction analysis by rapid column chromatography, GC-EI MS analysis of oil biomarkers, and subsequently, source and weathering indices, and GC-IRMS analysis of oil saturate fractions.

4.2.1.1 Variation in Solvent Extractable Material (SEM)

SEM for the soil microcosms at the various stages of biotransformation were determined using the Soxhlet extraction method described in Section 3.1.6.1. Variations in SEM (in mg g^{-1} of air-dried soil) recovered from the treated and control soils, in both cases corrected for the amounts of natural organic matter (NOM) in the soil, at each sample point are shown in Table 4.6. These show that the average recovery for the ballast oil-treated soils decreased most dramatically, from a maximum of 14.54 ± 0.25 mg g^{-1} at 0 days 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 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 than these, decreasing by just over 1 mg g^{-1}, 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. Average recoveries for this set of samples decreased only marginally, from 20.71 ± 0.9 mg g^{-1} to 19.85 ± 0.84 mg g^{-1} in the treated soils, and from 20.03 mg g^{-1} to 19.71 mg g^{-1} in the controls.

In Figures 4.6 (a) - (c), the decline in SEM (expressed as a percentage of the initial recovery) in treated and control microcosms for the ballast oil, crude oil and No.6 Fuel Oil are shown. The charts demonstrate much more clearly the differences in recoveries between the treated and control flasks. For the ballast oil, this difference becomes most obvious after approximately 16 days, and the two recoveries diverge much more rapidly thereafter; for the crude oil microcosms, the point of divergence is later, after approximately 32 days. No such differences were observed for the No.6 fuel oil, for which recoveries remained almost constant throughout the study.

Using this information, it was possible to determine the amounts of each oil lost due to abiotic and biotic processes at each sampling point (Figure 4.7 (a) - (c)). It should be noted that the losses due to abiotic processes determined by the control microcosms are likely to be

Table 4.6 Solvent Extractable Material (SEM) Recoveries for Each Soil/Oil Microcosm[1]

TIME (days)	Ballast Oil: Individual SEM values ($mg\ g^{-1}$)			Mean SEM	SD	Control SEM[2]	Crude Oil: Individual SEM values ($mg\ g^{-1}$)			Mean SEM[2]	SD	Control SEM[2]	No.6 Fuel Oil: Individual SEM values ($mg\ g^{-1}$)			Mean SEM	SD	Control SEM[2]
0	*14.82*	*14.99*	*14.50*	**14.77**	0.25	**14.98**	*18.04*	*17.45*	*18.30*	**17.93**	0.44	**18.19**	*19.98*	*21.75*	*20.10*	**20.61**	0.99	**20.26**
2	*14.67*		*11.63*	**13.15**	2.15	**13.94**	*17.97*	*17.98*	*16.90*	**17.62**	0.62	**18.21**	*21.23*	*21.09*	*22.19*	**21.50**	0.60	**20.91**
4	*12.68*	*12.20*	*10.08*	**11.65**	1.38	**13.52**	*16.64*	*17.60*	*15.37*	**16.53**	1.12	**18.02**	*22.15*	*20.00*	*22.34*	**21.49**	1.30	**20.35**
8	*11.84*	*10.36*	*11.22*	**11.14**	0.74	**13.29**	*15.54*	*14.09*	*13.66*	**14.43**	0.99	**18.09**	*20.66*	*19.74*	*20.79*	**20.40**	0.57	**19.99**
16	*11.35*	*9.34*	*8.66*	**9.78**	1.40	**13.06**	*15.31*	*15.26*	*16.32*	**15.63**	0.60	**17.94**	*17.52*	*22.72*	*15.57*	**18.80**	3.70	**20.07**
32	*7.14*	*8.31*	*8.65*	**8.03**	0.79	**13.05**	*13.16*	*14.73*	*13.31*	**13.73**	0.87	**17.48**	*21.75*	*19.86*	*20.23*	**20.61**	1.00	**21.18**
64	*5.37*	*7.12*	*6.74*	**6.09**	0.92	**12.99**	*11.64*	*12.18*	*13.50*	**12.44**	0.96	**17.44**	*18.64*	*22.96*	*20.13*	**20.57**	2.19	**19.92**
128	*4.37*	*6.11*	*5.25*	**5.24**	0.87	**12.50**	*7.23*	*9.14*	*10.47*	**8.95**	1.63	**17.30**	*19.92*	*21.23*	*19.40*	**20.15**	0.94	**20.14**
256	*2.56*	*3.51*	*3.49*	**3.08**	0.54	**12.16**	*8.35*	*5.54*	*6.21*	**6.70**	1.47	**17.17**	*19.14*	*20.38*	*20.73*	**20.08**	0.84	**19.94**

[1]SEM determined using dichloromethane as solvent

[2]Result of single extraction only

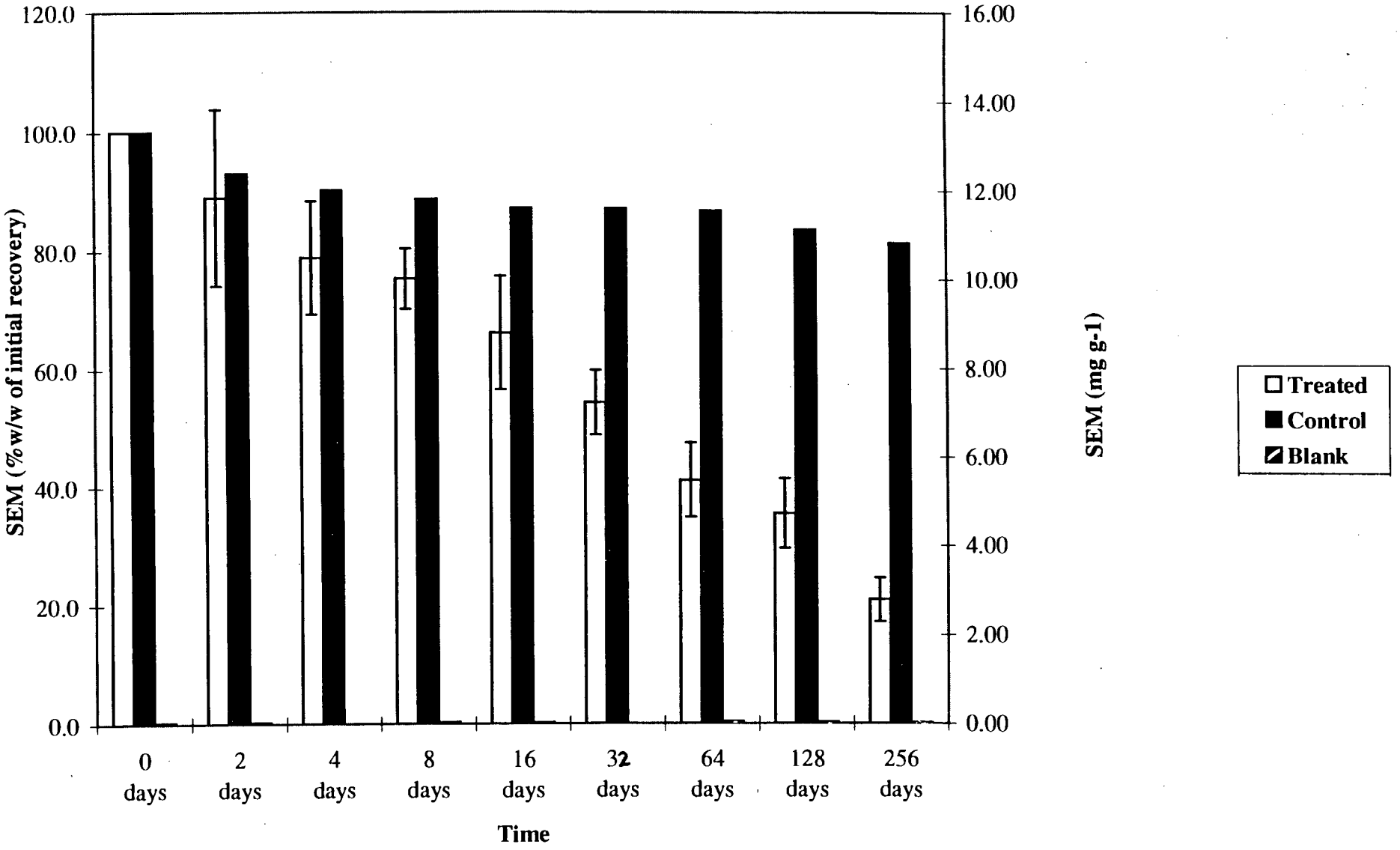


Figure 4.6 (a) Solvent Extractable Material (SEM) Variations in Ballast Oil Treated and Control Soils, and Blank Soil Microcosms

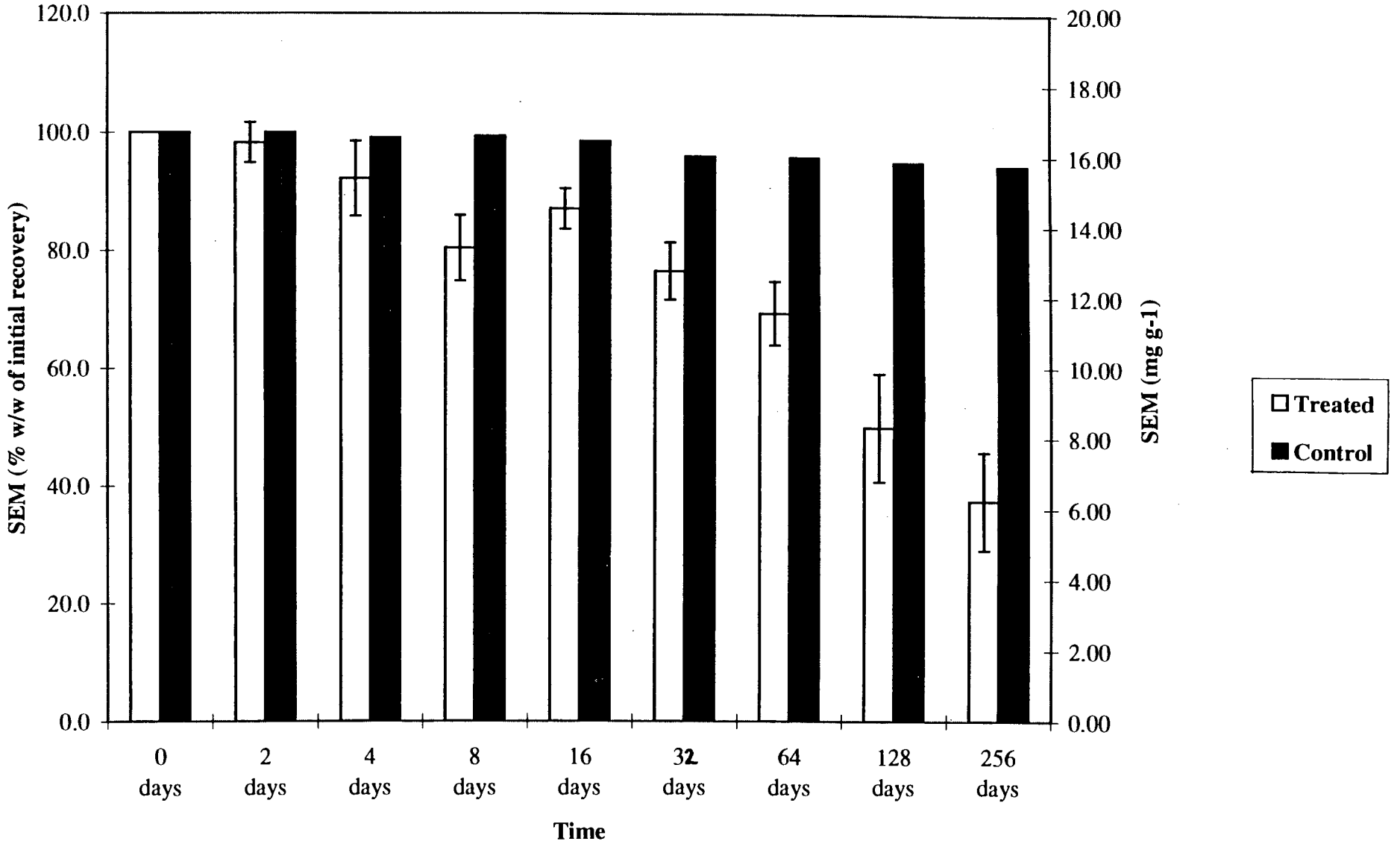


Figure 4.6 (b) Solvent Extractable Material (SEM) Variations in Crude Oil Treated and Control Soils

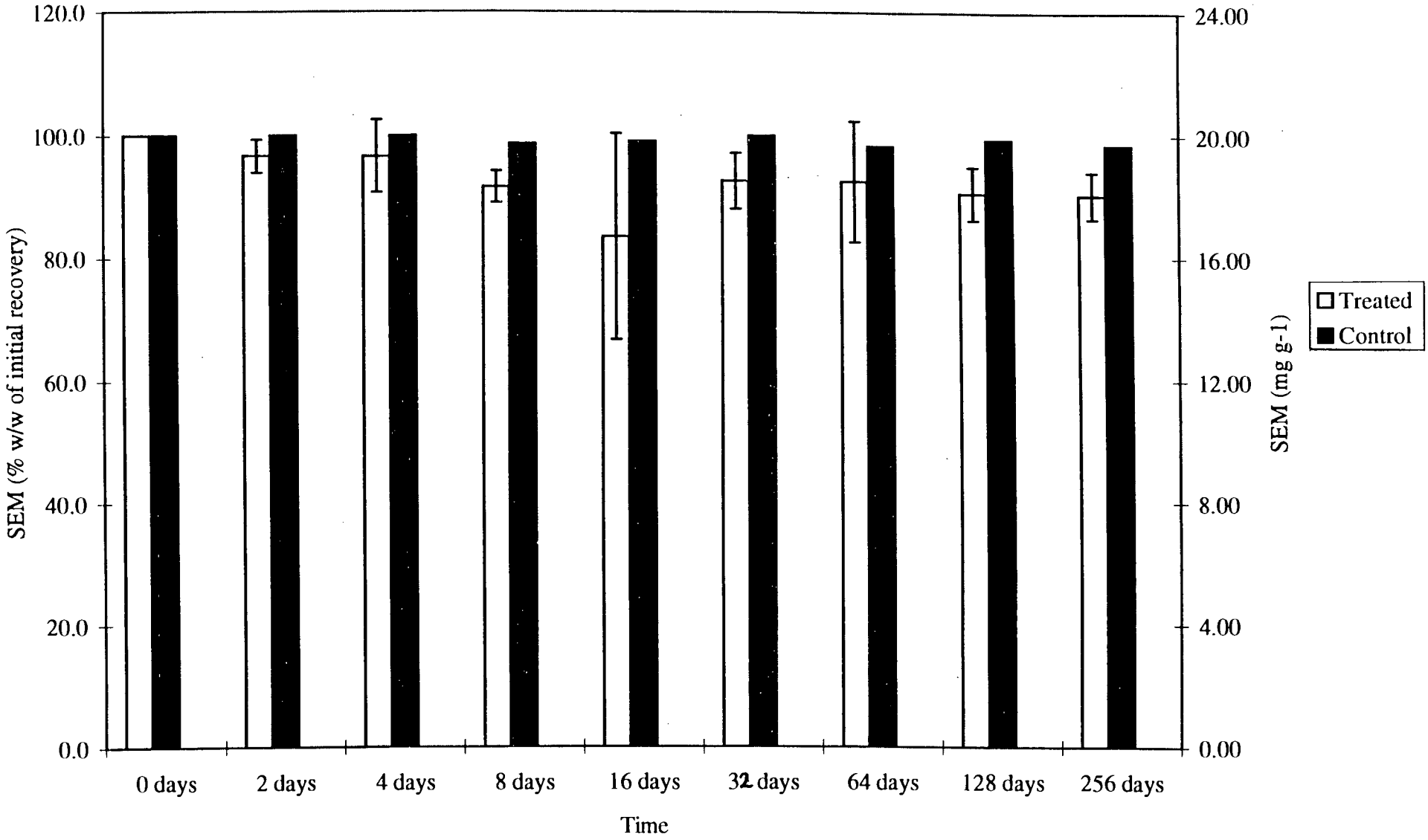


Figure 4.6 (c) Solvent Extractable Material (SEM) Variations in No.6 Fuel Oil Treated and Control Soils

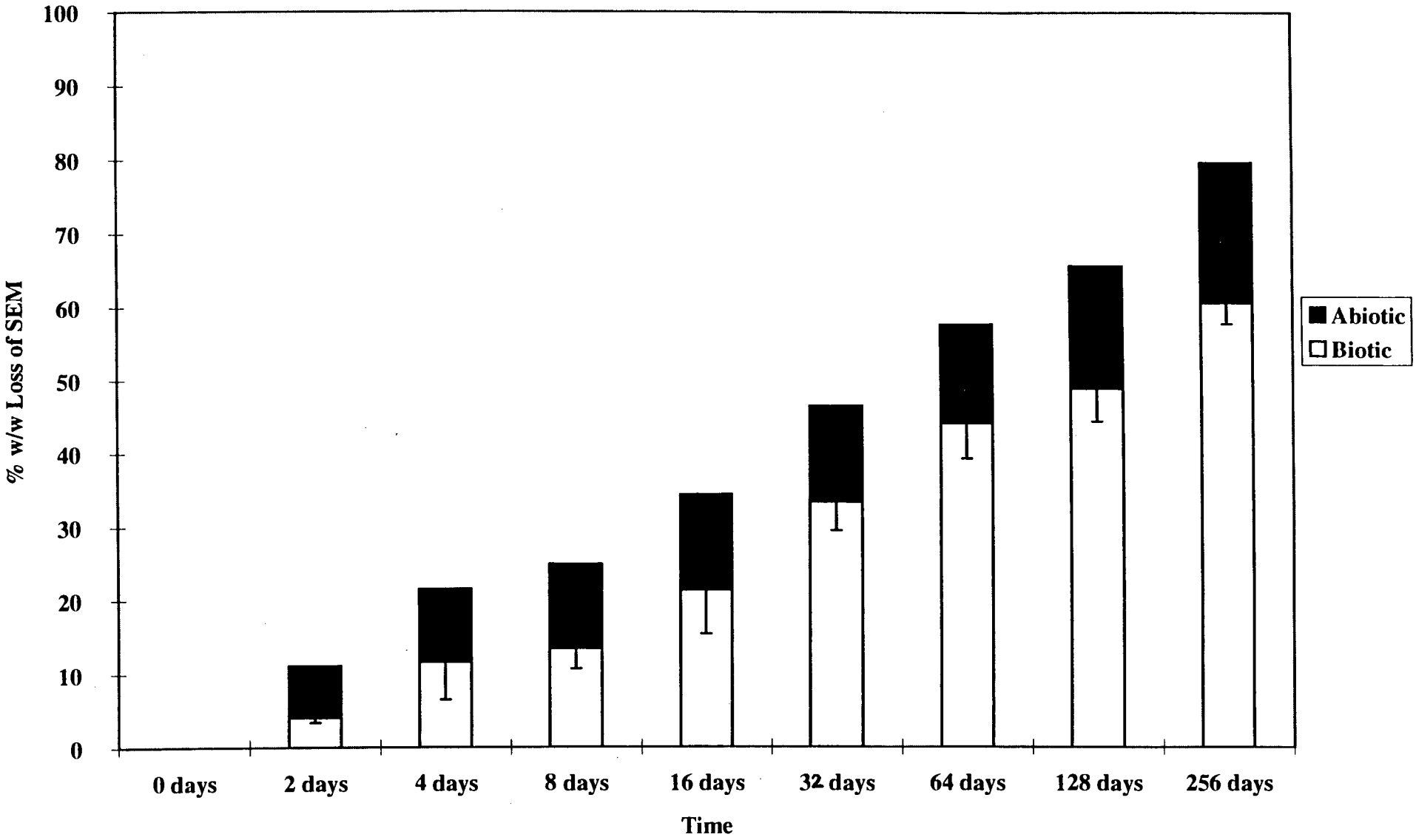


Figure 4.7 (a) Comparison of Abiotic (Control) and Biotic (Treated - Control) Contributions to SEM Variations for Ballast Oil Microcosms

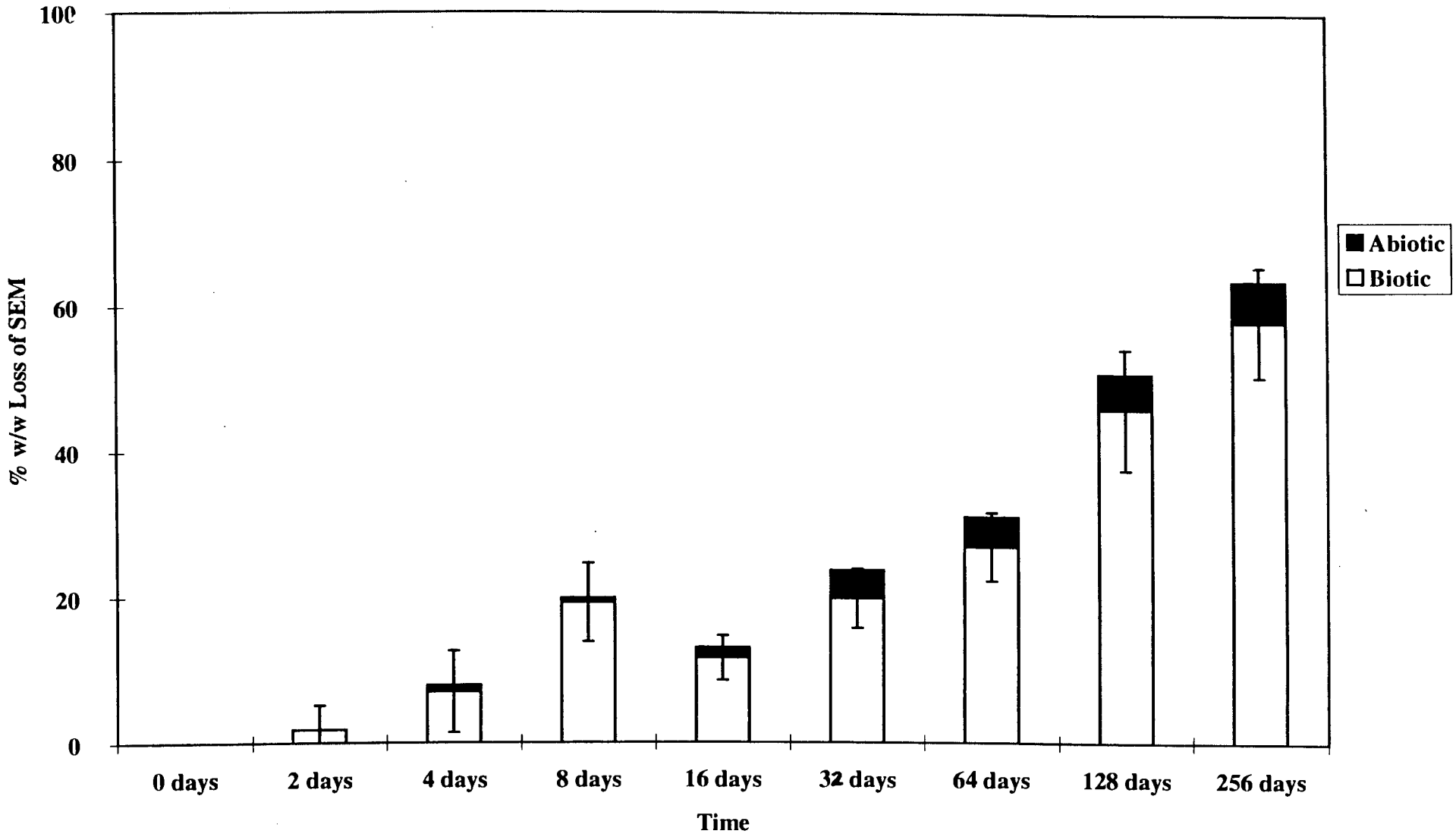


Figure 4.7 (b) Comparison of Abiotic (Control) and Biotic (Treated - Control) Contributions to SEM Variations for Crude Oil Microcosms

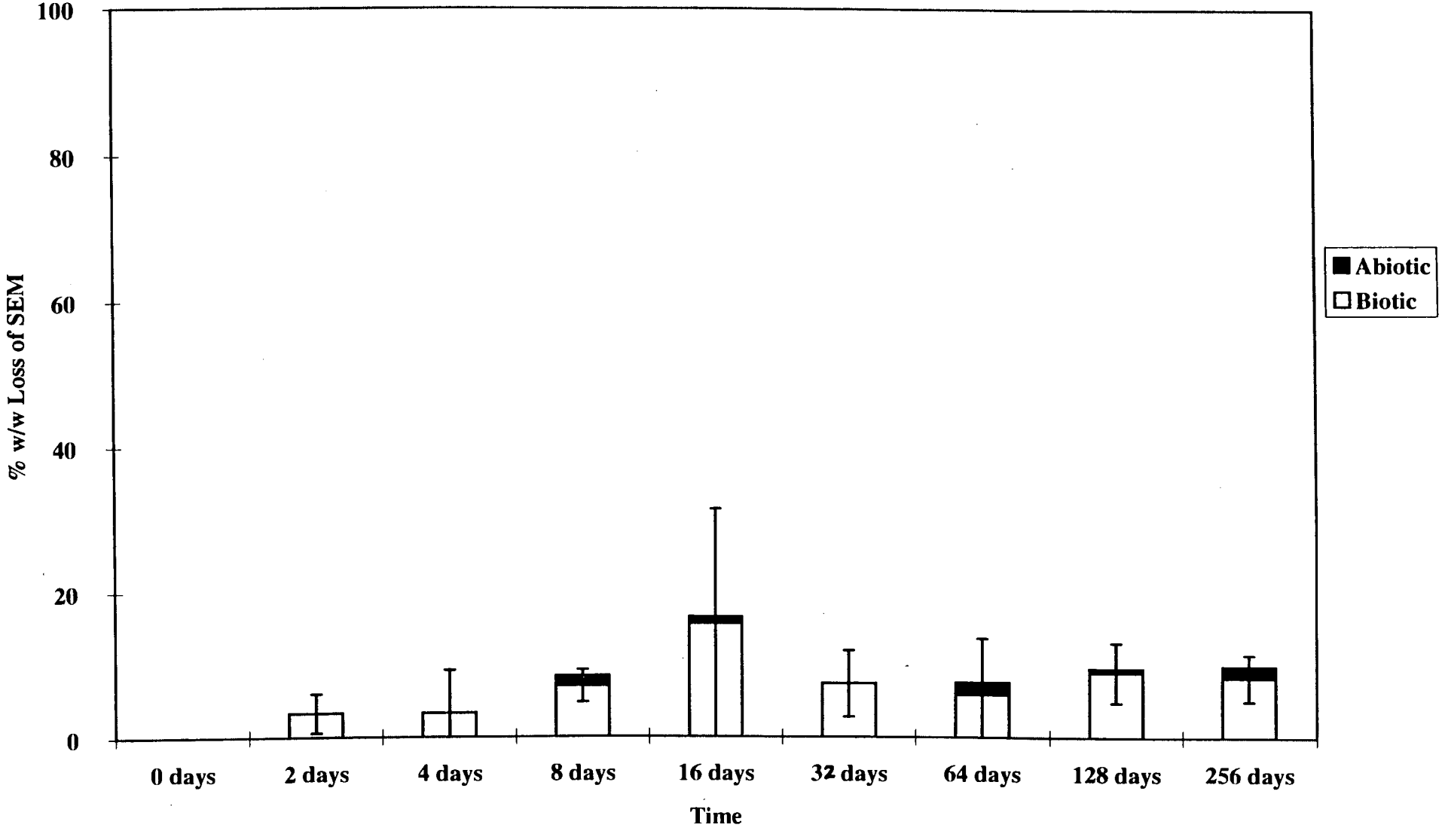


Figure 4.7 (c) Comparison of Abiotic (Control) and Biotic (Treated - Control) Contributions to SEM Variations for No.6 Fuel Oil Microcosms

overestimates of the abiotic losses occurring in the treated soils, particularly for those samples taken in the second half of the study, since in the control microcosms there is no competition between abiotic and biotic weathering processes. For both ballast and crude oils the abiotic and biotic losses are comparable over the initial period of study; after the 16 days sample point for the ballast oil, and 32 days sample point for the crude oil, the control microcosm recoveries become almost constant, and the bulk of the losses observed are due to microbial activity. For the No.6 Fuel Oil, no such trend is observed. The loss of each oil due to biotransformation alone, using a linear time axis, is shown in Figure 4.8. A full discussion of these results and their implications is presented in Section 5.2.1.1, including a quantitative assessment of the kinetics of the observed biotransformation reactions.

4.2.1.2 Variation in Individual Class Fraction Distributions

The amounts of saturates, aromatics, polars and asphaltenes (SAPA) recovered in each of the ballast oil, crude oil and No.6 fuel oil microcosm extracts (expressed as a percentage by weight of the SEM) are shown in Tables 4.7, 4.8 and 4.9, respectively. The major compositional change in all three oils is the decrease in $\%\,^{w}/_{w}$ of the saturate fraction. This decrease is particularly interesting in the No.6 Fuel Oil extracts, given the lack of variation in the SEM values for these microcosms. The class fraction variations are more clearly illustrated in Figures 4.9 (a), (b) and (c), in which the average amounts of the SAPA class fractions within each extract (again expressed as a $\%^{w}/_{w}$ of the SEM) are plotted against time for the ballast oil, crude oil and No.6 Fuel Oil, respectively. Results show that the average $\%\,^{w}/_{w}$ of saturates decreased for each of the extracts (from $74.6 \pm 0.9\ \%^{w}/_{w}$ to $23.2 \pm 3.4\ \%^{w}/_{w}$ for the ballast oil, from $91.7 \pm 0.8\ \%^{w}/_{w}$ to $70.1 \pm 2.9\ \%^{w}/_{w}$ for the crude oil and from $36.9 \pm 1.2\ \%^{w}/_{w}$ to $22.0 \pm 2.3\ \%^{w}/_{w}$ for the No.6 Fuel Oil), whilst the average proportions of polars and asphaltenes concomitantly increased.

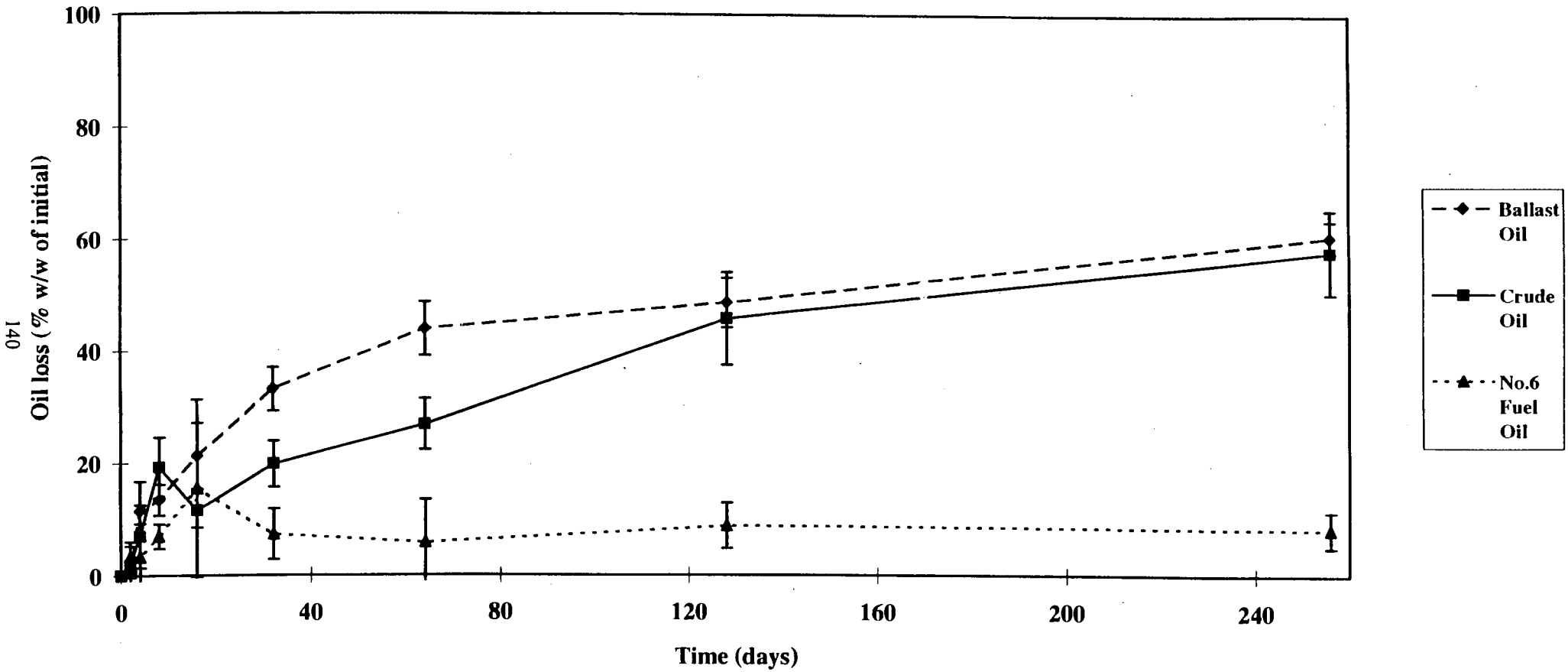


Figure 4.8 Microbial Losses (% w/w) of SEM with Time for Ballast Oil, Crude Oil and No.6 Fuel Oil Microcosms

Table 4.7 Variations of Class Fraction Distribution in Ballast Oil-Treated and Control Microcosms (%$^{w}/_{w}$)

TIME (days)	Saturates (%$^{w}/_{w}$ of SEM)						Aromatics (%$^{w}/_{w}$ of SEM)					
	Treated					Control	Treated					Control
	n1	n2	n3	Mean	SD		n1	n2	n3	Mean	SD	
0	74.2	75.6	73.9	**74.6**	0.9	**75.2**	12.7	11.6	13.3	**12.5**	0.9	**11.8**
2	74.8		71.2	**73.0**	2.5	**74.9**	12.9		13.1	**13.0**	0.1	**11.7**
4	73.0	72.4	71.0	**72.1**	1.0	**73.4**	13.3	14.7	12.3	**13.4**	1.2	**12.5**
8	71.8	69.0	70.5	**70.4**	1.4	**73.0**	12.6	13.1	12.5	**12.7**	0.3	**12.5**
16	65.7	64.2	62.3	**64.1**	1.7	**72.8**	12.9	13.6	13.8	**13.4**	0.5	**12.7**
32	62.7	60.9	64.5	**62.7**	1.8	**72.5**	15.6	12.8	14.3	**14.2**	1.4	**12.2**
64	48.2	60.5	48.1	**52.3**	7.1	**71.9**	12.9	16.5	15.3	**14.9**	1.8	**12.5**
128	45.1	55.7	51.5	**50.8**	5.3	**71.8**	16.1	13.7	15.6	**15.1**	1.3	**13.2**
256	24.4	26.0	19.2	**23.2**	3.6	**69.1**	20.6	24.8	23.5	**23.0**	2.2	**13.2**

TIME (days)	Polars (%$^{w}/_{w}$ of SEM)						Asphaltenes (%$^{w}/_{w}$ of SEM)					
	Treated					Control	Treated					Control
	n1	n2	n3	Mean	SD		n1	n2	n3	Mean	SD	
0	8.8	8.2	8.8	**8.6**	0.3	**8.9**	4.3	4.6	4.0	**4.3**	0.3	**4.1**
2	8.6		11.2	**9.9**	1.8	**9.0**	3.7		4.5	**4.1**	0.6	**4.4**
4	9.9	9.6	9.0	**9.5**	0.5	**9.3**	3.8	3.4	7.7	**5.0**	2.4	**4.7**
8	11.3	11.9	10.1	**11.1**	0.9	**9.8**	4.3	5.9	6.9	**5.7**	1.3	**4.6**
16	12.9	12.5	14.2	**13.2**	0.9	**9.8**	8.5	9.7	9.7	**9.3**	0.7	**4.7**
32	14.4	14.5	13.4	**14.1**	0.6	**10.4**	7.2	11.9	7.9	**9.0**	2.5	**4.8**
64	22.4	16.1	16.6	**18.4**	3.5	**10.4**	16.6	6.9	20.0	**14.5**	6.8	**4.8**
128	20.7	13.3	20.2	**18.1**	4.1	**10.2**	18.1	17.2	12.6	**16.0**	3.0	**4.8**
256	36.6	26.8	30.5	**31.3**	4.9	**10.6**	18.4	22.2	26.9	**22.5**	4.3	**5.1**

Table 4.8 Variations of Class Fraction Distribution in Crude Oil-Treated and Control Microcosms

TIME (days)	Saturates (% $^{w}/_{w}$ of SEM)						Aromatics (% $^{w}/_{w}$ of SEM)					
	Treated					Control	Treated					Control
	n1	n2	n3	Mean	SD		n1	n2	n3	Mean	SD	
0	92.6	91.4	91.1	**91.7**	0.8	**92.0**	1.9	2.4	1.5	**1.9**	0.5	**2.0**
2	92.5		90.9	**91.7**	1.1	**91.2**	1.8		1.7	**1.8**	0.1	**2.4**
4	88.1	89.8	85.6	**87.8**	2.1	**91.3**	1.7	1.9	1.9	**1.8**	0.1	**2.2**
8	78.3	90.2	84.4	**84.3**	6.0	**91.1**	2.5	2.0	2.0	**2.2**	0.3	**2.4**
16	88.2	82.4	86.6	**85.7**	3.0	**89.9**	2.2	1.5	1.9	**1.9**	0.4	**1.9**
32	86.9	78.7	85.2	**83.6**	4.3	**88.9**	2.2	2.0	2.2	**2.1**	0.1	**2.2**
64	82.2	80.4	82.4	**81.7**	1.1	**88.9**	2.2	2.8	2.4	**2.5**	0.3	**2.3**
128	73.3	74.0	75.6	**74.3**	1.2	**88.4**	3.4	2.6	2.6	**2.9**	0.5	**2.9**
256	71.3	66.8	72.2	**70.1**	2.9	**89.1**	2.2	3.1	2.8	**2.7**	0.5	**2.9**

TIME (days)	Polars (% $^{w}/_{w}$ of SEM)						Asphaltenes (% $^{w}/_{w}$ of SEM)					
	Treated					Control	Treated					Control
	n1	n2	n3	Mean	SD		n1	n2	n3	Mean	SD	
0	3.1	3.4	3.9	**3.5**	0.4	**3.0**	2.4	2.8	3.5	**2.9**	0.6	**3.0**
2	3.3		4.0	**3.7**	0.5	**2.8**	2.4		3.4	**2.9**	0.7	**3.6**
4	3.5	3.9	3.4	**3.6**	0.3	**3.2**	6.7	4.4	9.1	**6.7**	2.4	**3.3**
8	4.7	4.4	4.9	**4.7**	0.3	**2.4**	14.5	3.3	8.8	**8.9**	5.6	**4.2**
16	5.0	3.8	4.5	**4.4**	0.6	**2.9**	4.6	12.3	7.1	**8.0**	3.9	**5.3**
32	4.4	4.8	4.9	**4.7**	0.3	**3.1**	6.1	14.5	7.7	**9.4**	4.5	**5.8**
64	4.9	7.9	6.3	**6.4**	1.5	**3.3**	10.7	8.9	8.8	**9.5**	1.1	**5.6**
128	10.5	10.1	7.8	**9.5**	1.5	**3.2**	12.9	13.3	14.0	**13.4**	0.6	**5.5**
256	6.5	9.4	11.2	**9.0**	2.4	**3.0**	20.0	20.7	13.8	**18.2**	3.8	**5.0**

Table 4.9 Variations of Class Fraction Distribution in No.6 Fuel Oil-Treated and Control Microcosms

TIME (days)	Saturates (% $^{w}/_{w}$ of SEM)						Aromatics (% $^{w}/_{w}$ of SEM)					
	Treated					Control	Treated					Control
	n1	n2	n3	Mean	SD		n1	n2	n3	Mean	SD	
0	37.9	35.5	37.2	**36.9**	1.2	**37.2**	36.0	36.2	35.2	**35.8**	0.5	**34.7**
2	37.4	37.0	38.7	**37.7**	0.9	**37.8**	34.7	38.7	36.8	**36.7**	2.0	**34.3**
4	37.1	38.5	35.6	**37.1**	1.5	**36.1**	35.3	36.6	33.5	**35.1**	1.6	**35.6**
8	35.3	39.8	38.7	**37.9**	2.3	**38.2**	32.9	35.3	36.4	**34.9**	1.8	**33.7**
16	31.1	34.9	38.1	**34.7**	3.5	**37.1**	34.5	34.6	34.7	**34.6**	0.1	**35.2**
32	29.7	32.7	33.5	**32.0**	2.0	**36.4**	36.4	31.5	34.2	**34.0**	2.5	**35.9**
64	33.0	32.5	34.4	**33.3**	1.0	**37.0**	32.2	34.8	35.8	**34.3**	1.9	**36.0**
128	19.1	24.2	27.9	**23.7**	4.4	**36.8**	37.5	38.8	36.7	**37.7**	1.1	**34.8**
256	24.6	20.5	20.9	**22.0**	2.3	**36.9**	32.1	34.1	24.9	**30.4**	4.8	**35.7**

TIME (days)	Polars (% $^{w}/_{w}$ of SEM)						Asphaltenes (% $^{w}/_{w}$ of SEM)					
	Treated					Control	Treated					Control
	n1	n2	n3	Mean	SD		n1	n2	n3	Mean	SD	
0	13.0	13.5	11.7	**12.7**	0.9	**12.9**	13.1	14.8	15.9	**14.6**	1.4	**15.2**
2	12.6	11.9	10.7	**11.7**	1.0	**13.0**	15.3	14.4	13.9	**14.5**	0.7	**14.8**
4	14.0	12.4	12.0	**12.8**	1.1	**14.0**	13.6	12.4	18.8	**14.9**	3.4	**14.3**
8	14.5	12.9	11.1	**12.8**	1.7	**13.3**	17.3	12.0	13.8	**14.4**	2.7	**14.8**
16	12.2	14.4	13.1	**13.2**	1.1	**12.3**	22.2	16.1	14.1	**17.5**	4.2	**15.5**
32	10.9	11.4	13.1	**11.8**	1.2	**11.8**	23.0	24.4	19.2	**22.2**	2.7	**16.0**
64	14.8	11.4	15.4	**13.9**	2.2	**11.7**	20.0	21.3	14.4	**18.6**	3.7	**15.3**
128	15.0	12.3	12.2	**13.2**	1.6	**12.0**	28.4	24.7	23.2	**25.4**	2.7	**16.4**
256	18.9	20.9	24.9	**21.6**	3.1	**11.5**	24.4	24.5	29.4	**26.1**	2.9	**15.9**

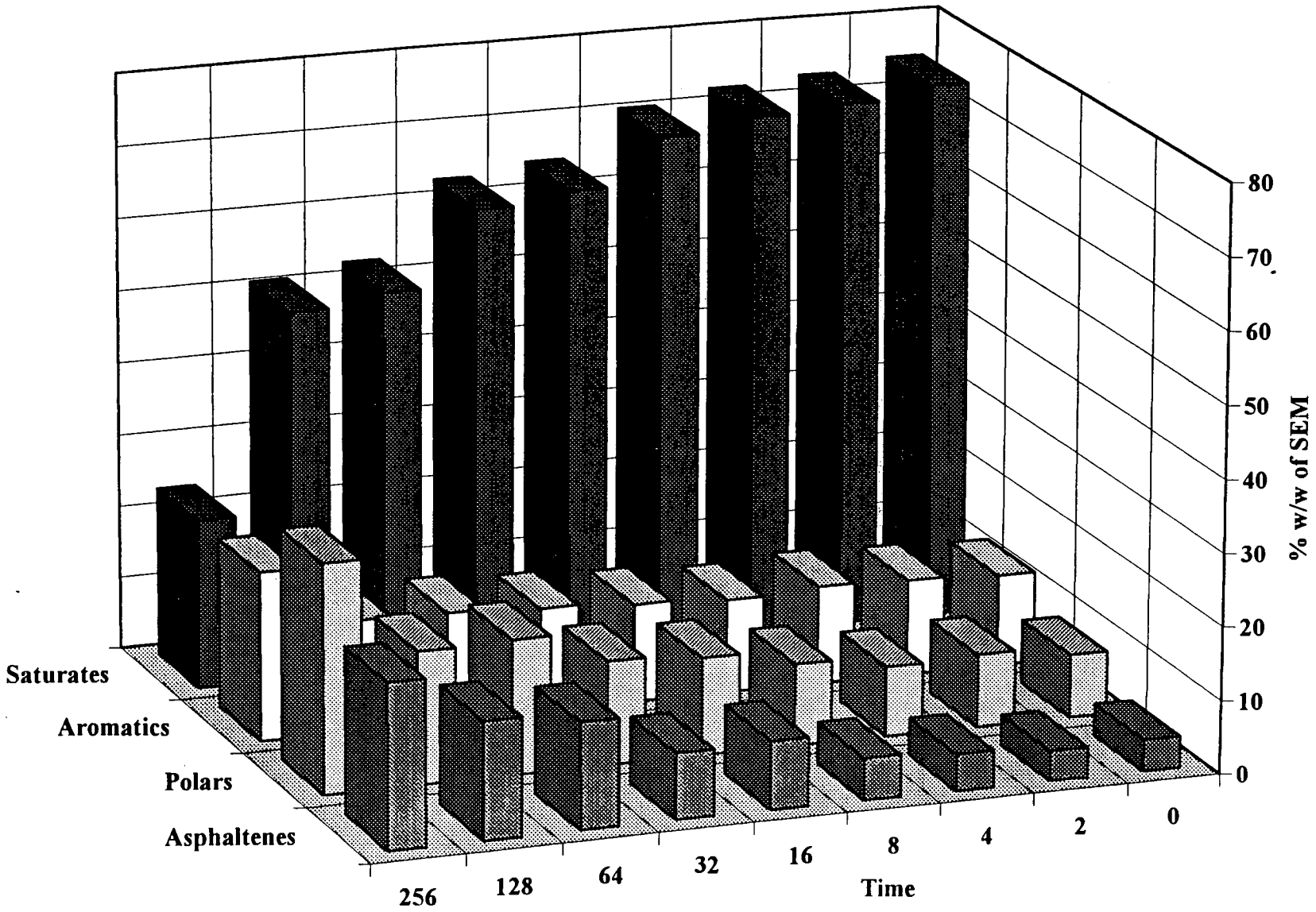


Figure 4.9 (a) Class Fraction Variations (as % w/w of SEM) in Ballast Oil-Treated Soils

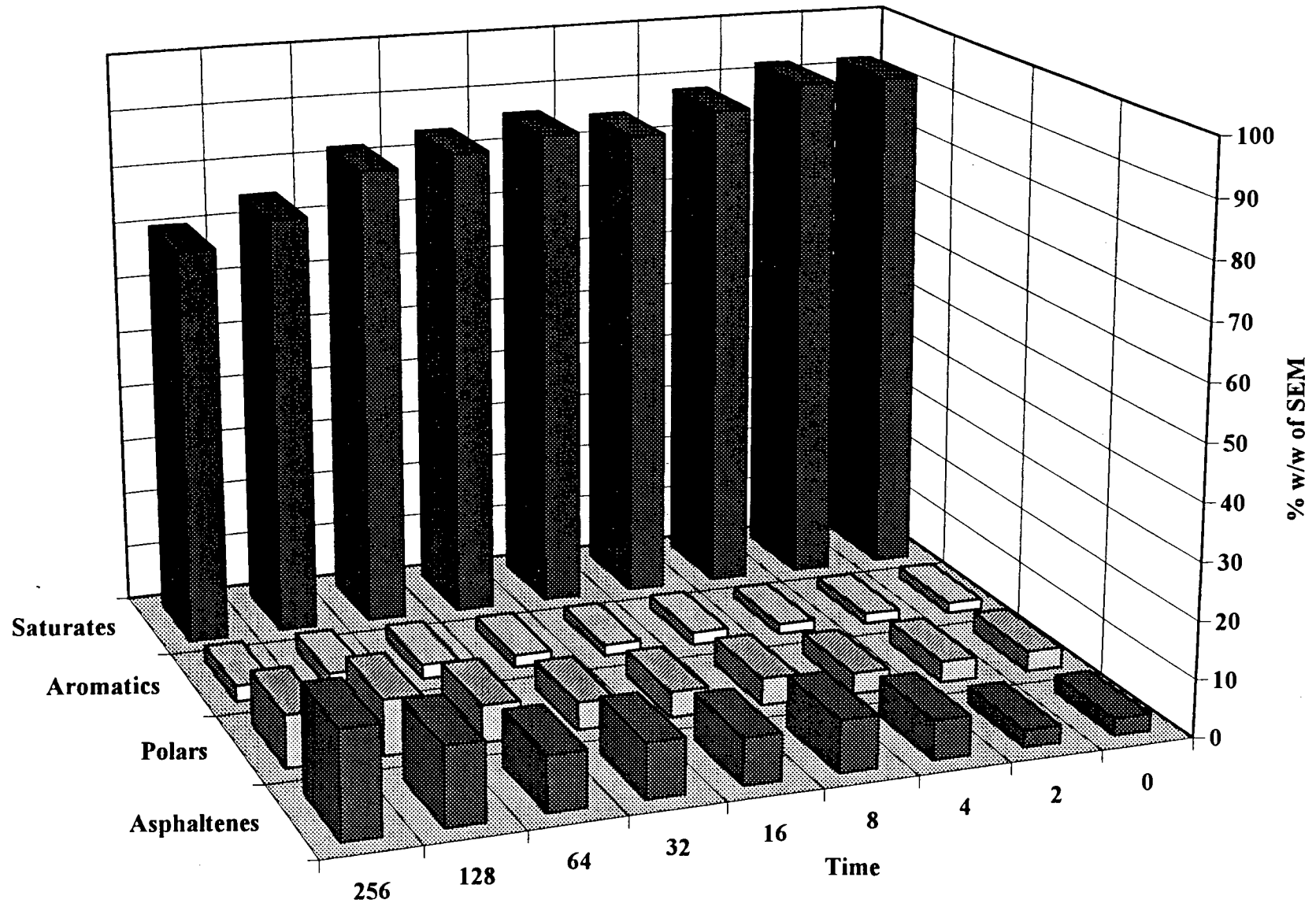


Figure 4.9 (b) Class Fraction Variations (as % w/w of SEM) in Crude Oil-Treated Soils

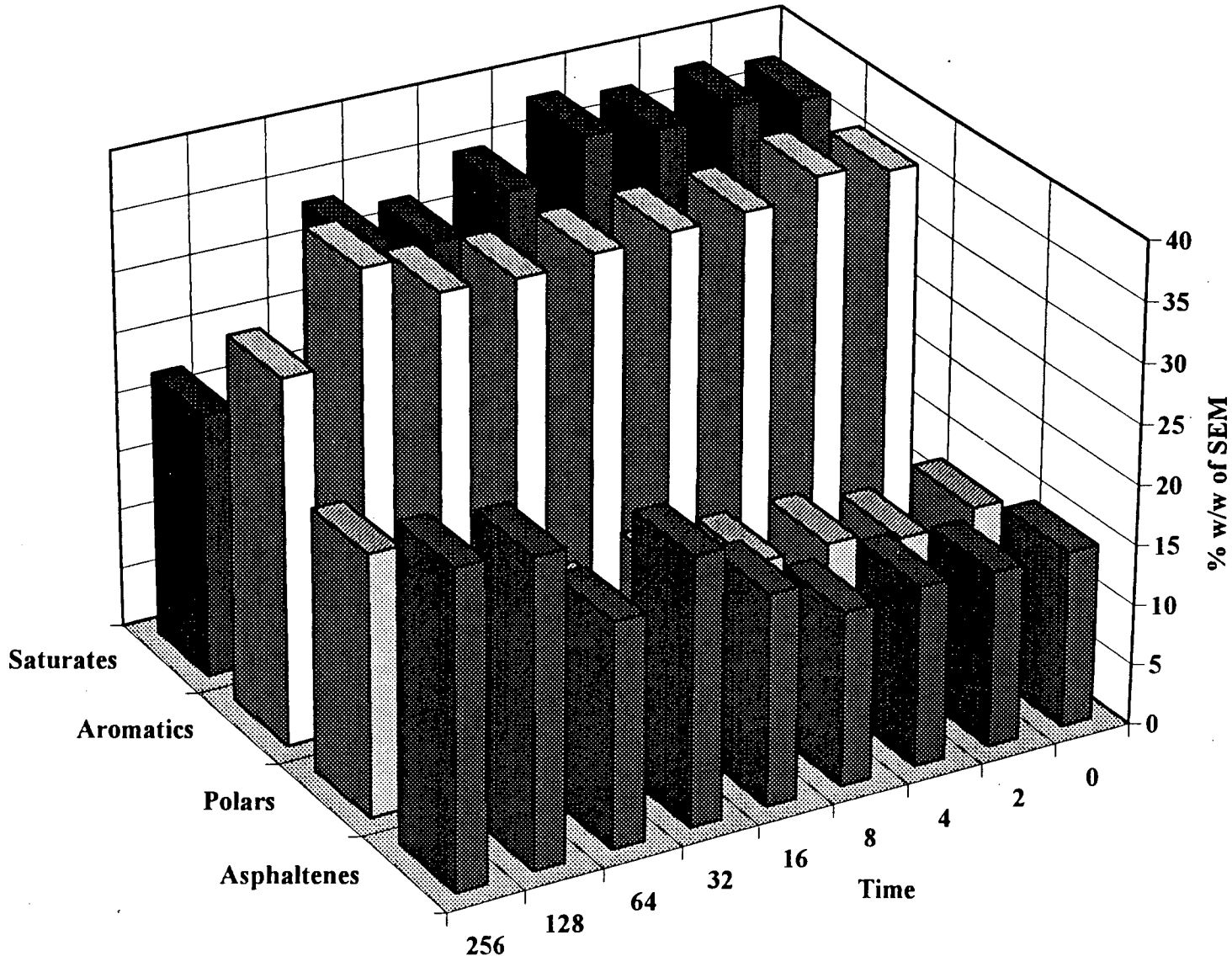


Figure 4.9 (c) Class Fraction Variations (as % w/w) in No.6 Fuel Oil-Treated Soils

With the exception of the ballast oil-treated soils, aromatic contents of extracts did not vary substantially. For this oil, the $\%^{w}/_{w}$ of aromatics, polars and asphaltenes in treated soil extracts increased from 12.5 ± 0.9, 8.6 ± 0.4 and 4.3 ± 0.3 at 0 days to 23.0 ± 2.2, 31.3 ± 5.0 and 22.5 ± 4.3 after 256 days, respectively. For the crude oil-treated soils, polar and asphaltene class fractions increased from an initial content of 3.5 ± 0.4 $\%^{w}/_{w}$ and 2.9 ± 0.6 $\%^{w}/_{w}$, to 9.0 ± 2.4 $\%^{w}/_{w}$ and 18.2 ± 3.8 $\%^{w}/_{w}$, respectively. Similarly, for the No.6 Fuel Oil-treated soils, the $\%^{w}/_{w}$ of polar and asphaltene class fractions increased from 12.7 ± 0.9 and 14.6 ± 1.4 to 21.6 ± 3.1 and 26.1 ± 2.9, respectively.

By comparison, SAPA class fraction distributions in extracts from the control microcosms, shown in Figures 4.10 (a), (b) and (c), show very little variation, with the proportion of class fractions fluctuating by less than 5.0 $\%^{w}/_{w}$ over the course of the study. The overall changes in abundance of the four class fractions within the ballast oil, crude oil and No.6 Fuel Oil-treated soils and control soils over the entire 256 days are summarised in Figure 4.11.

From the average $\%^{w}/_{w}$ SAPA recoveries, the average recoveries in mg g^{-1} were determined for each oil, and these are shown in Figures 4.12 (a) - (c). These data demonstrate the stark changes in the actual amounts of each class fraction recovered from the respective microcosms. As previously indicated, the loss of saturates from each oil is a feature of all three charts. For the ballast oil extracts, the amount of saturates recovered decreased by almost 94 %; for the crude and No.6 Fuel Oil extracts, the decreases are *ca.* 70 % and 40 %, respectively. Ballast oil extracts also decreased slightly in aromatic class fraction content with time, but did not alter in their polar and asphaltene contents. The amount of aromatics and polars recovered from crude oil extracts stayed almost constant throughout the study, at 0.3 ± 0.0 mg g^{-1} and 0.6 ± 0.1 mg g^{-1}, respectively, whilst the asphaltene content increased from 0.5 ± 0.1 mg g^{-1} to 1.2 ± 0.4 mg g^{-1}. For the No.6 Fuel Oil extracts, recoveries of aromatics decreased, from 7.4 ± 0.1 mg g^{-1} to 6.1 ± 0.9 mg g^{-1},

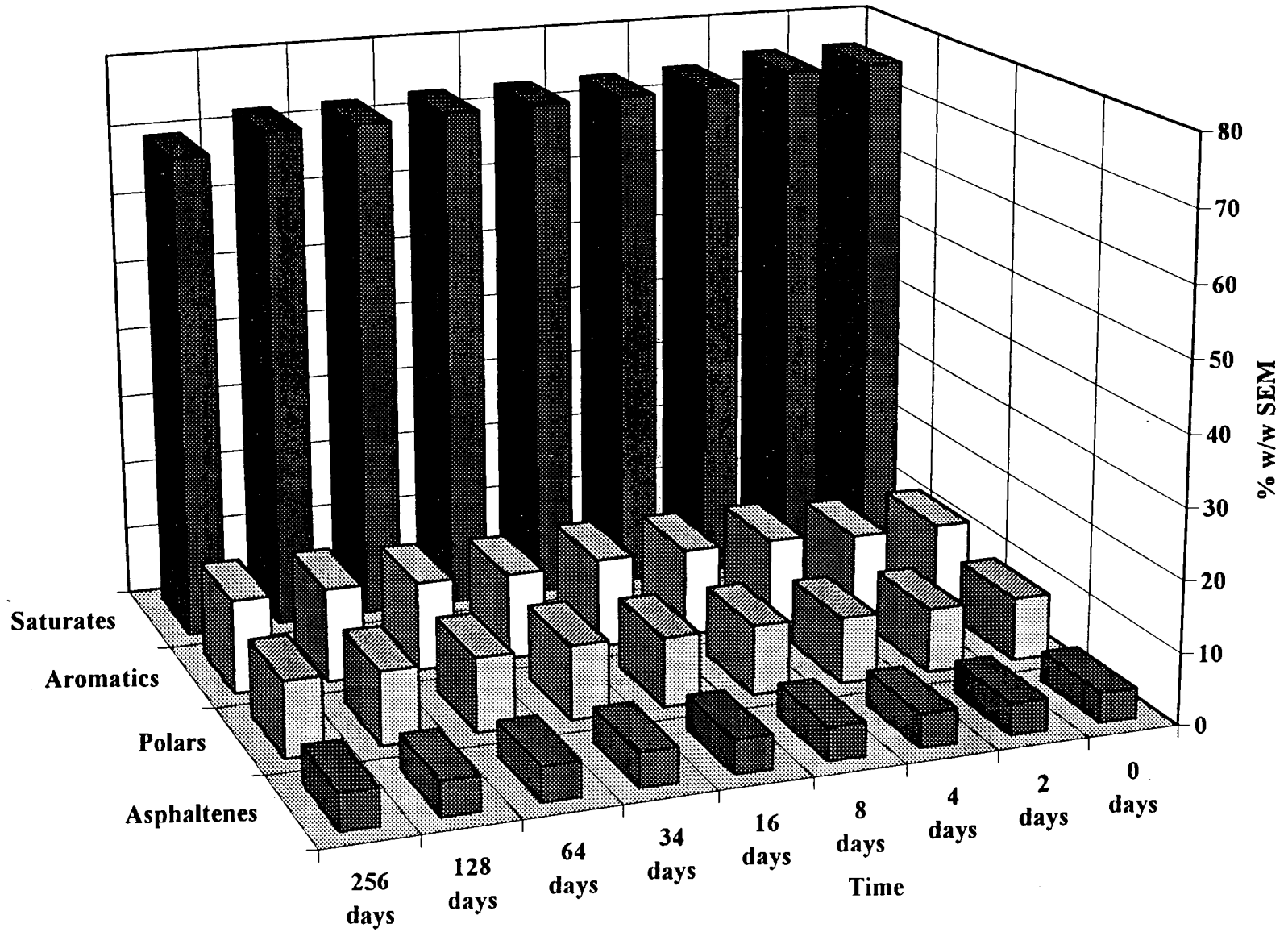


Figure 4.10 (a) Class Fraction Variations (% w/w of SEM) for Ballast Oil Control Microcosms

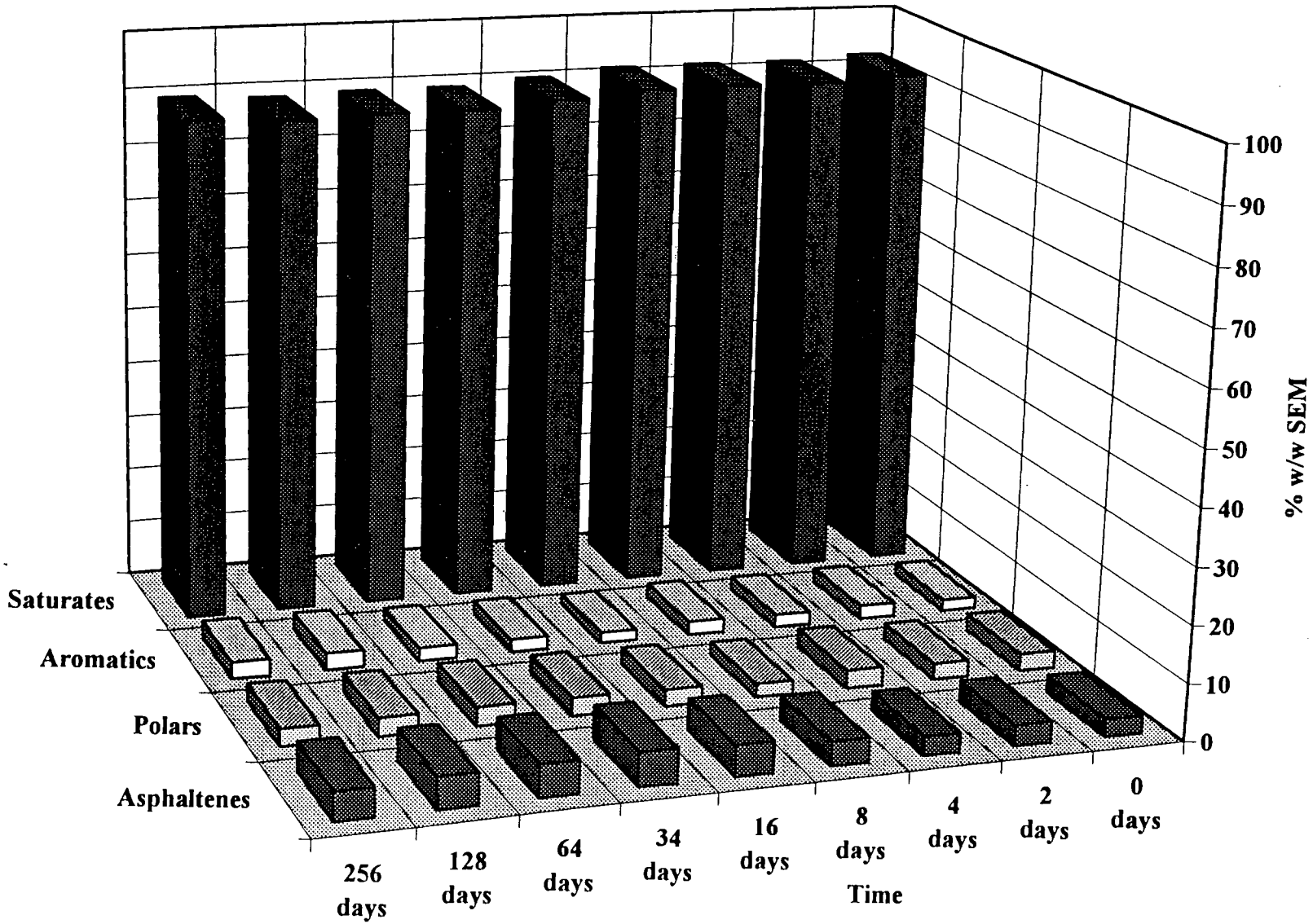


Figure 4.10 (b) Class Fraction Variations (as % w/w of SEM) for Crude Oil Control Microcosms

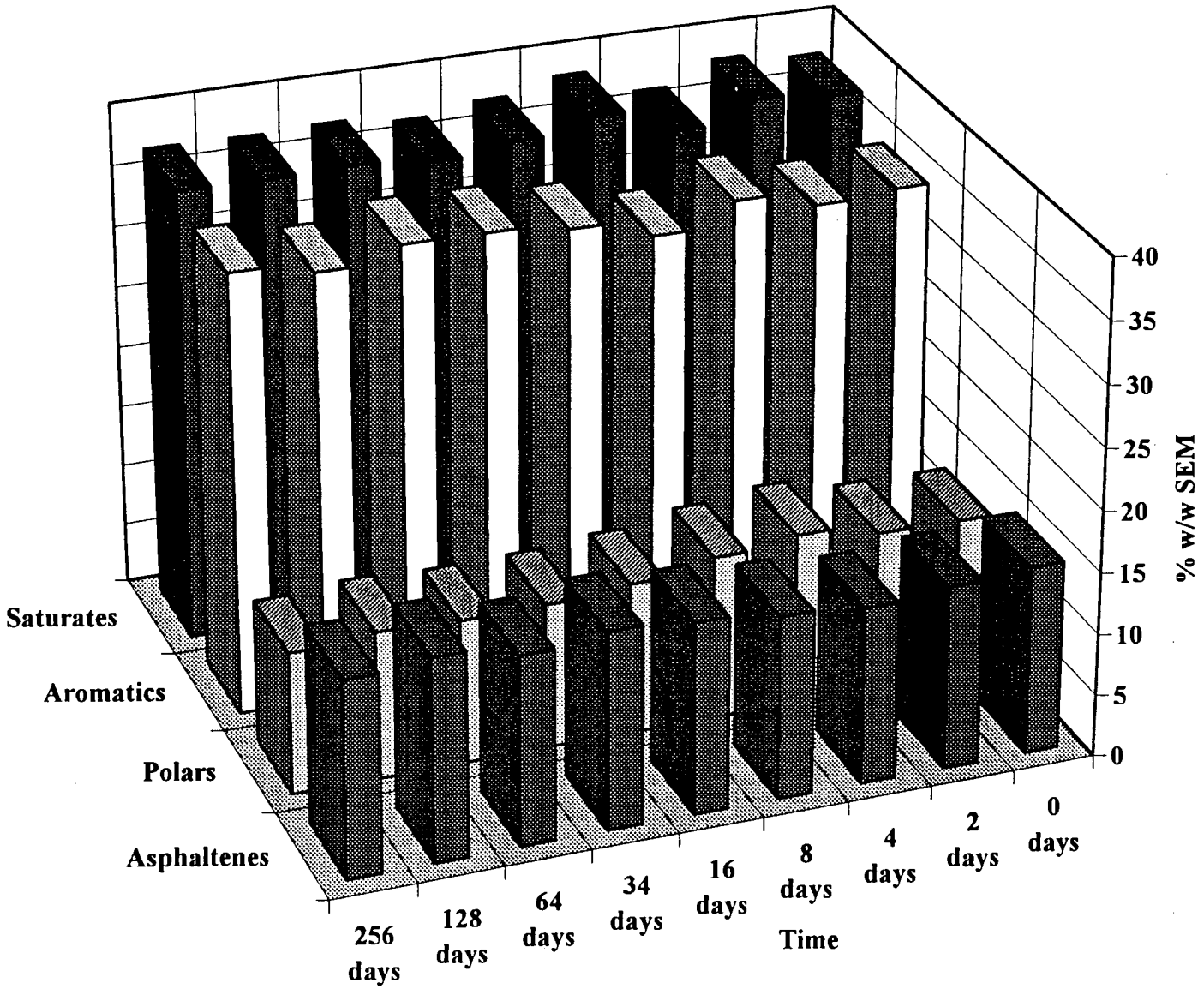


Figure 4.10 (c) Class Fraction Variations (as % w/w of SEM) for No.6 Fuel Oil Control Microcosms

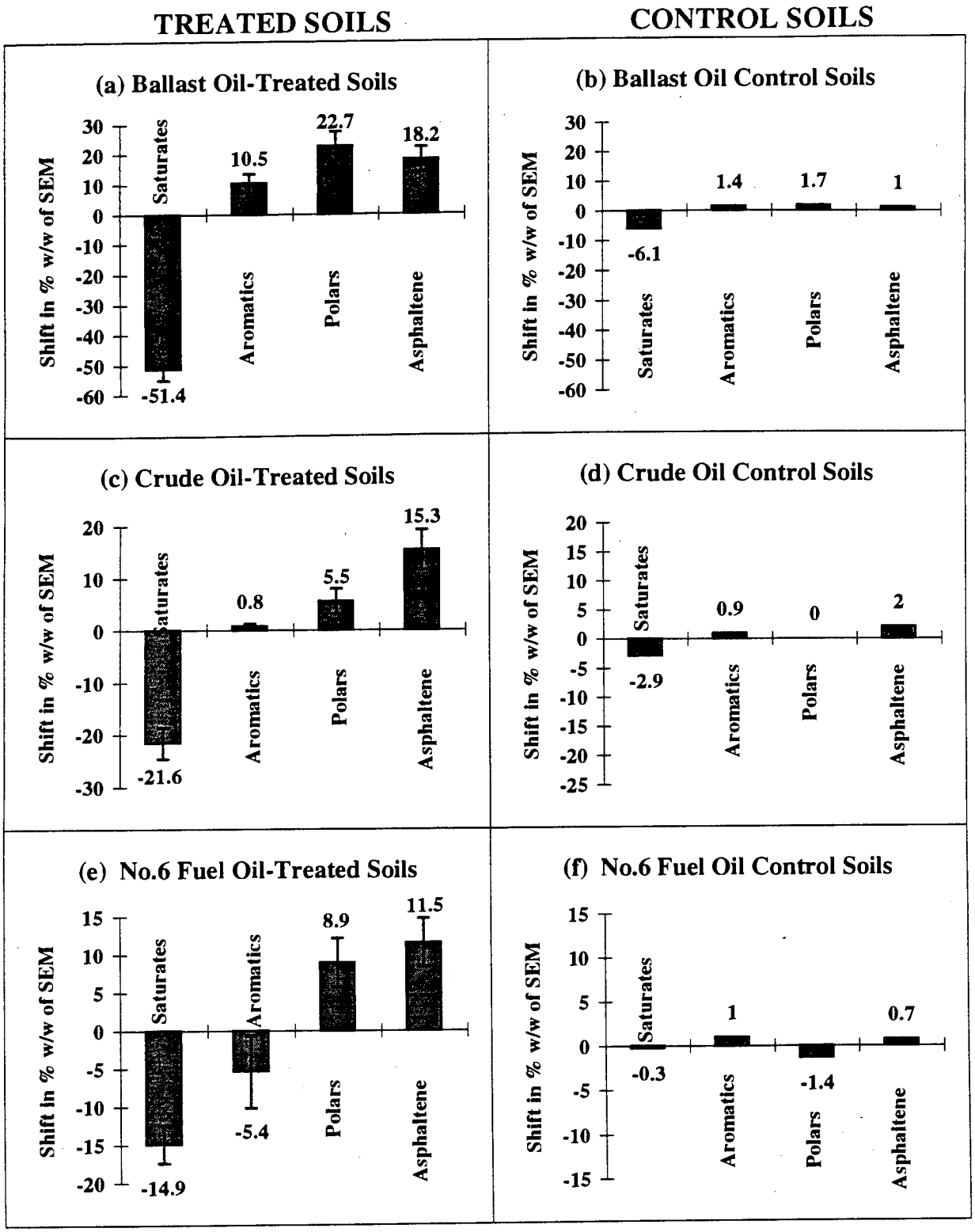


Figure 4.11 Overall Shift in Relative Class Fraction Recoveries (expressed as % w/w of SEM) between 0 and 256 Days for Oil Microcosms

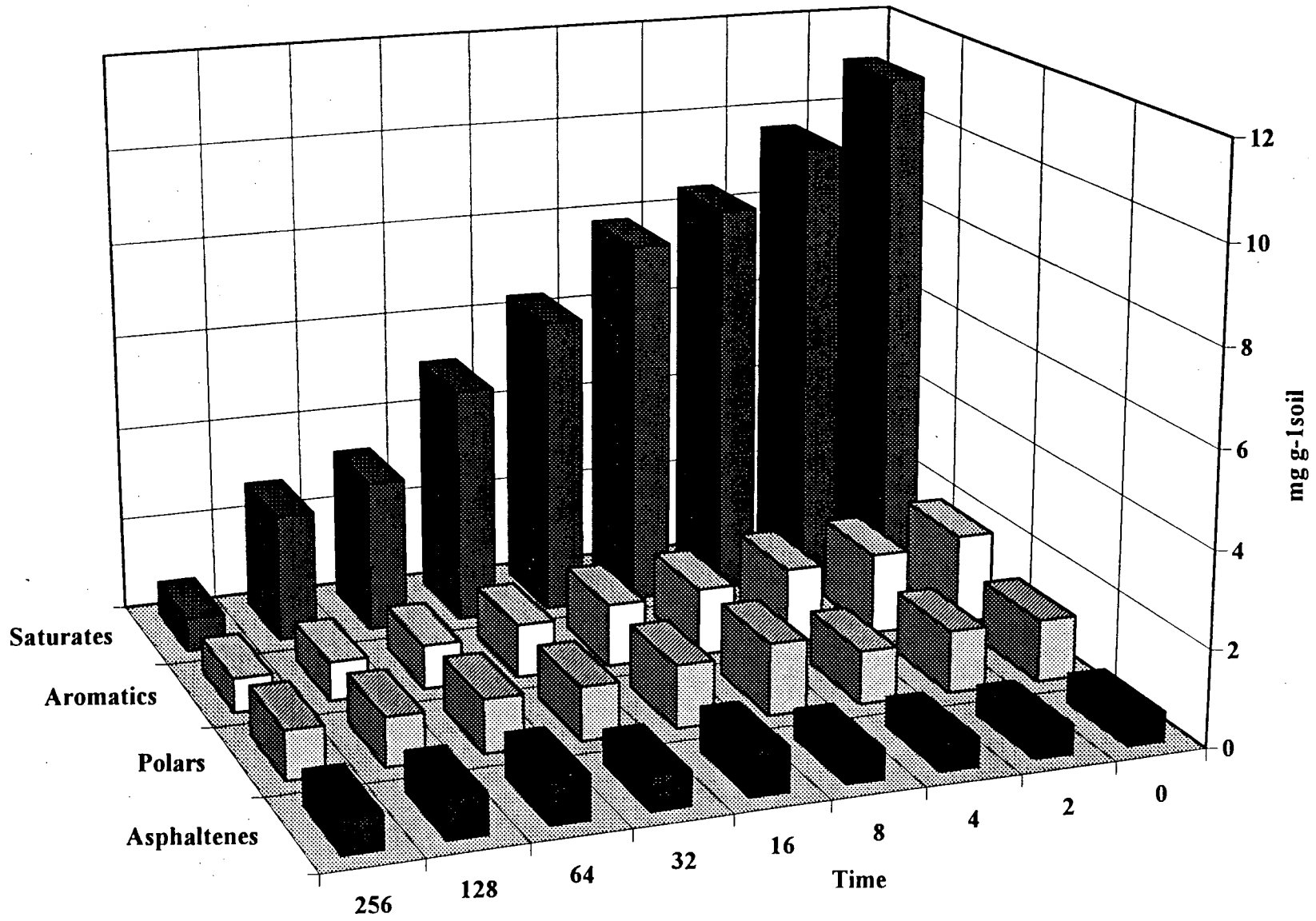


Figure 4.12 (a) Class Fraction Recoveries (in mg g-1 of dry soil) for Ballast Oil-Treated Soils

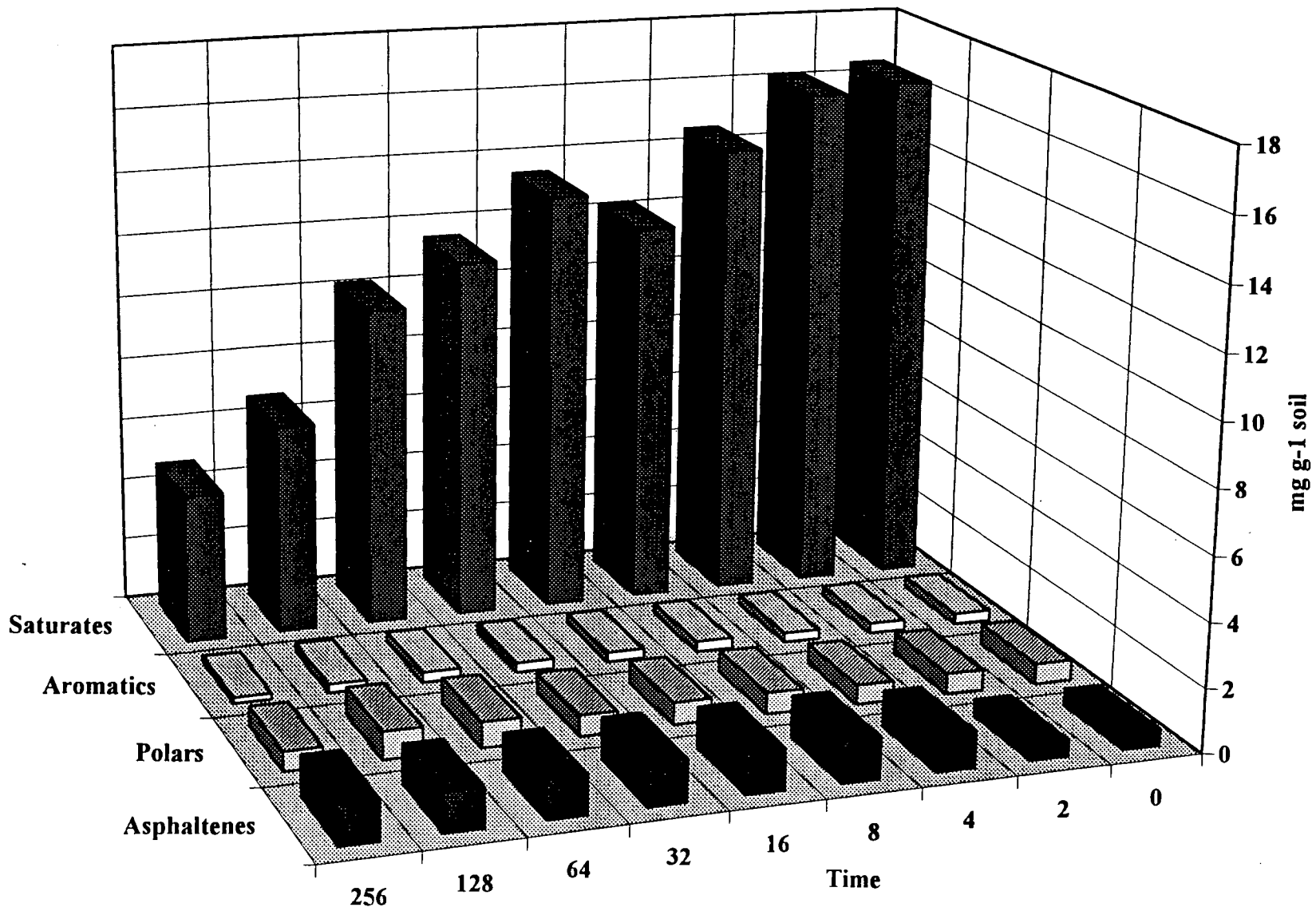


Figure 4.12 (b) Class Fraction Recoveries (in mg g-1 of dry soil) for Crude Oil-Treated Soils

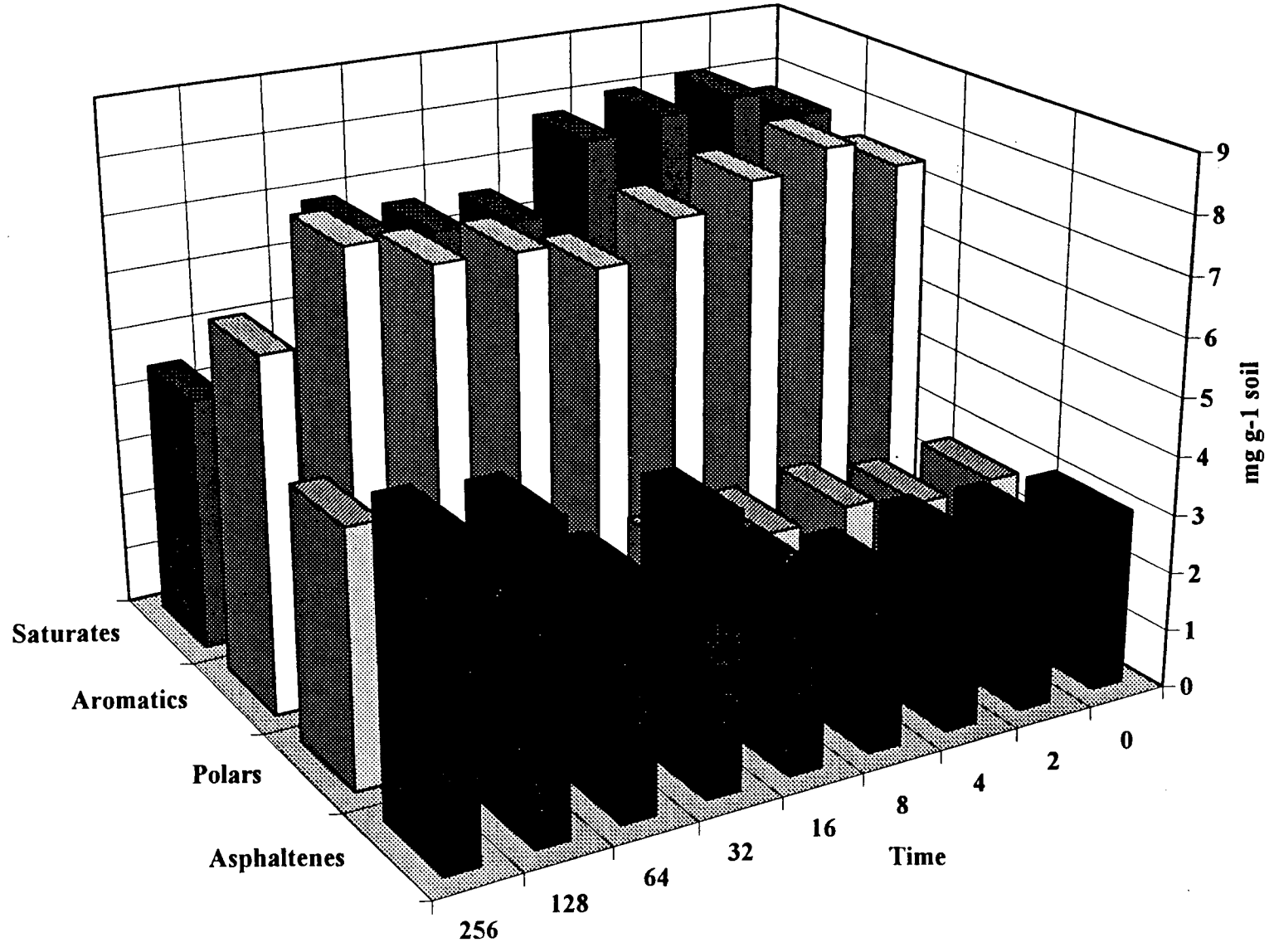


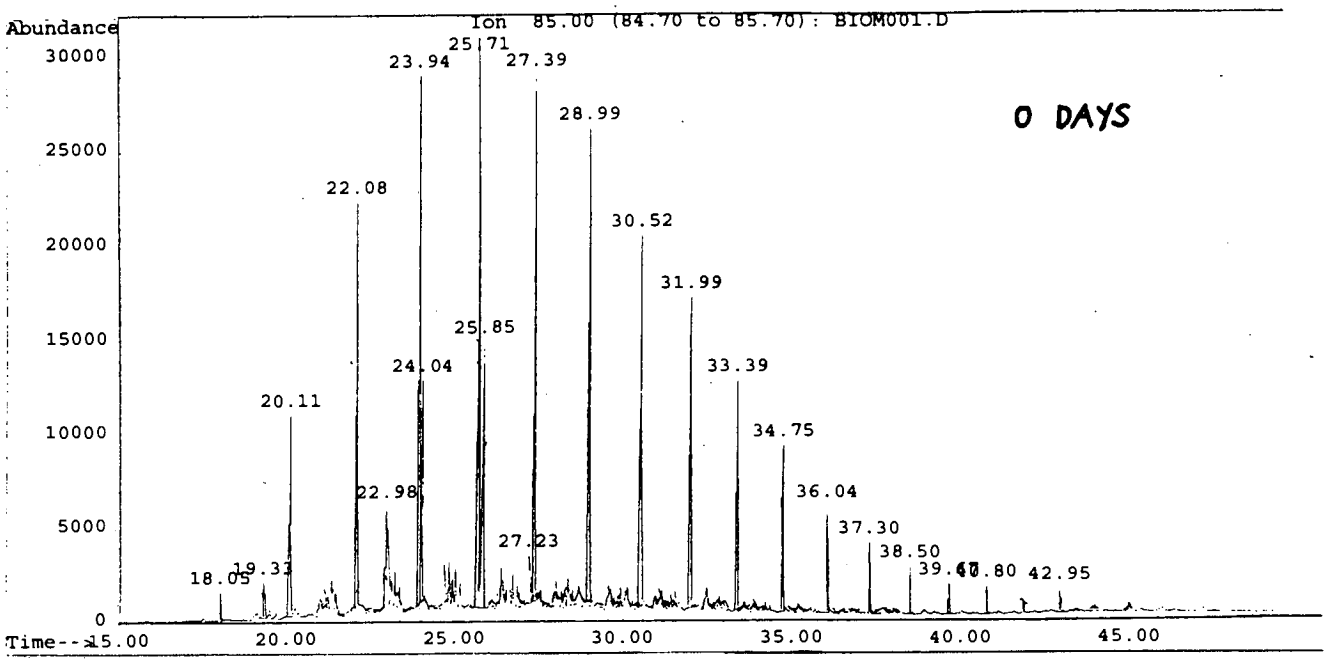
Figure 4.12 (c) Class Fraction Recoveries (in mg g-1 dry soil) for No.6 Fuel Oil-Treated Soils

whereas polar and asphaltene recoveries increased, from 2.6 ± 0.2 mg g^{-1} and 3.0 ± 0.4 mg g^{-1} to 4.3 ± 0.4 mg g^{-1} and 5.2 ± 0.6 mg g^{-1}, respectively.

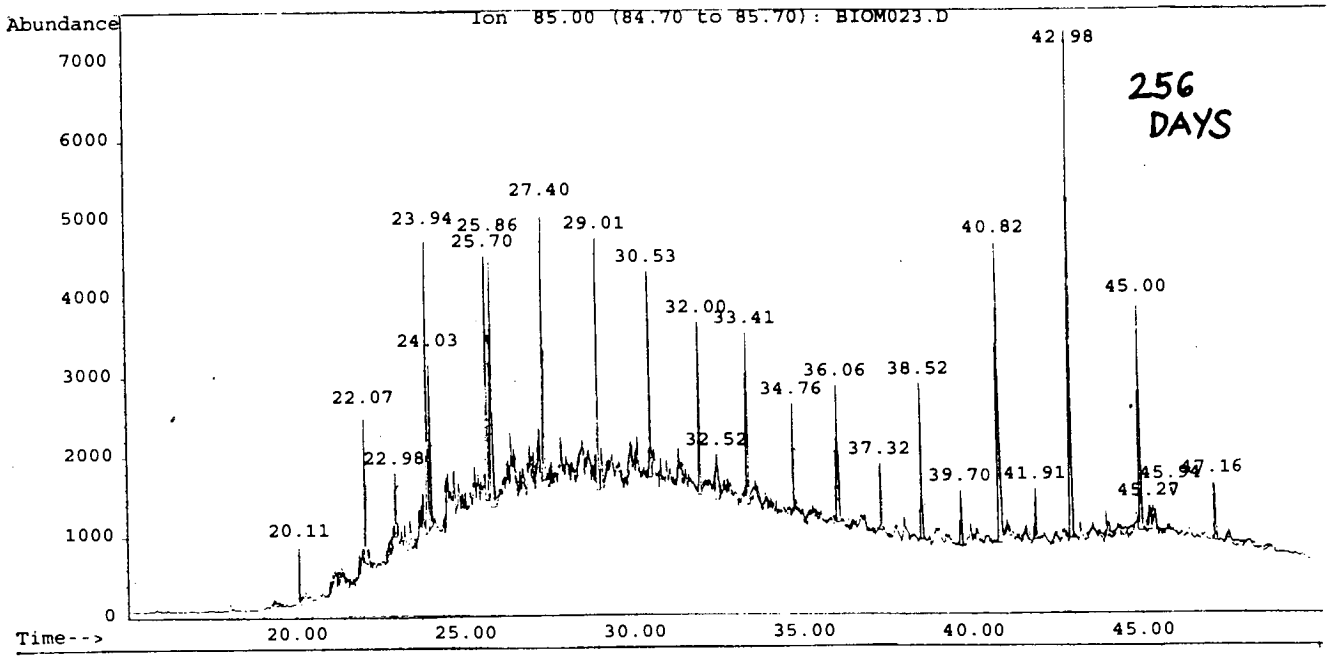
4.2.1.3 GC-EI MS Analysis

Qualitative Analysis. Ion chromatograms at m/z 85 and m/z 191 were obtained for oil saturate class fractions at each stage of the biotransformation study. Ion chromatograms shown in Figures 4.13 (a) and (b) show the m/z 85 and m/z 191 chromatograms of ballast oil alkanes at the commencement of the study and after 256 days. Equivalent chromatograms for the crude oil and No.6 Fuel Oil are shown in Figures 4.14 (a) and (b), and 4.15 (a) and (b). A qualitative examination of the data indicates that, for each oil, weathered samples displayed a much lower abundance of *n*-alkane peaks and a greater proportion of UCM than the fresh samples. The distribution of cyclic alkanes, shown in the m/z 191 chromatograms, does not change significantly over time in the majority of the oil samples, although some depletion of tricyclic alkanes is observed in the highly weathered crude oil extracts.

Quantitative Analysis. To provide more a definitive characterisation of oil biotransformation, the data were analysed quantitatively, by calculation of ratios of individual compounds and groups of compounds. For each microcosm flask, the following peak areas were determined: *n*-alkanes, the isoprenoid alkanes norpristane (iC_{18}), pristane (iC_{19}) and phytane (iC_{20}), the four major tricyclic terpanes (C_{20}-C_{24}), C_{24} tetracyclic terpane and the hopane isomers 17α(H)21β(H)-hopane and 17α(H)21β(H)-30-norhopane, 17α(H)21β(H)-homohopane (22S) and 17α(H)21β(H)-homohopane (22R), and 17α(H)21β(H)-bishomohopane and 17α(H)21β(H)-methylhopane. In some cases, peaks were not detected for these compounds and no value obtained for the associated indices, and these are denoted by the term N/D. Source and weathering parameters were determined in triplicate at each sample point and for each oil. The results are shown in Tables 4.10 (a) and (b), 4.11 (a) and (b) and

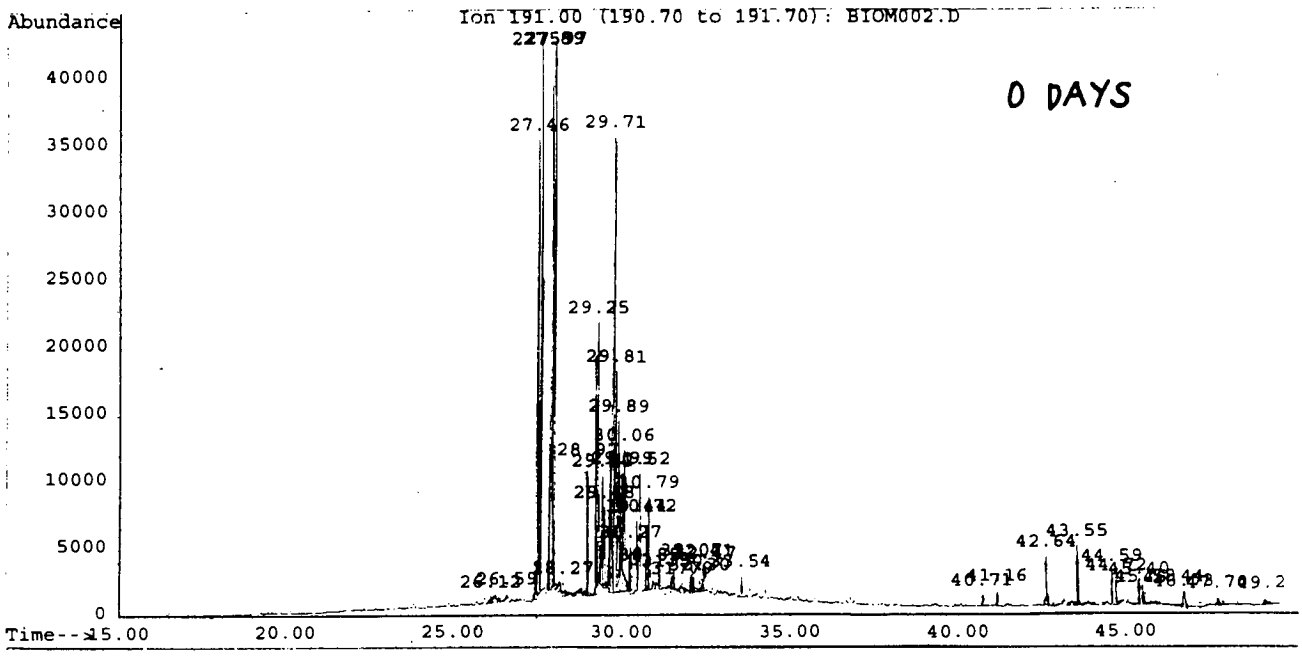


Time (min.)

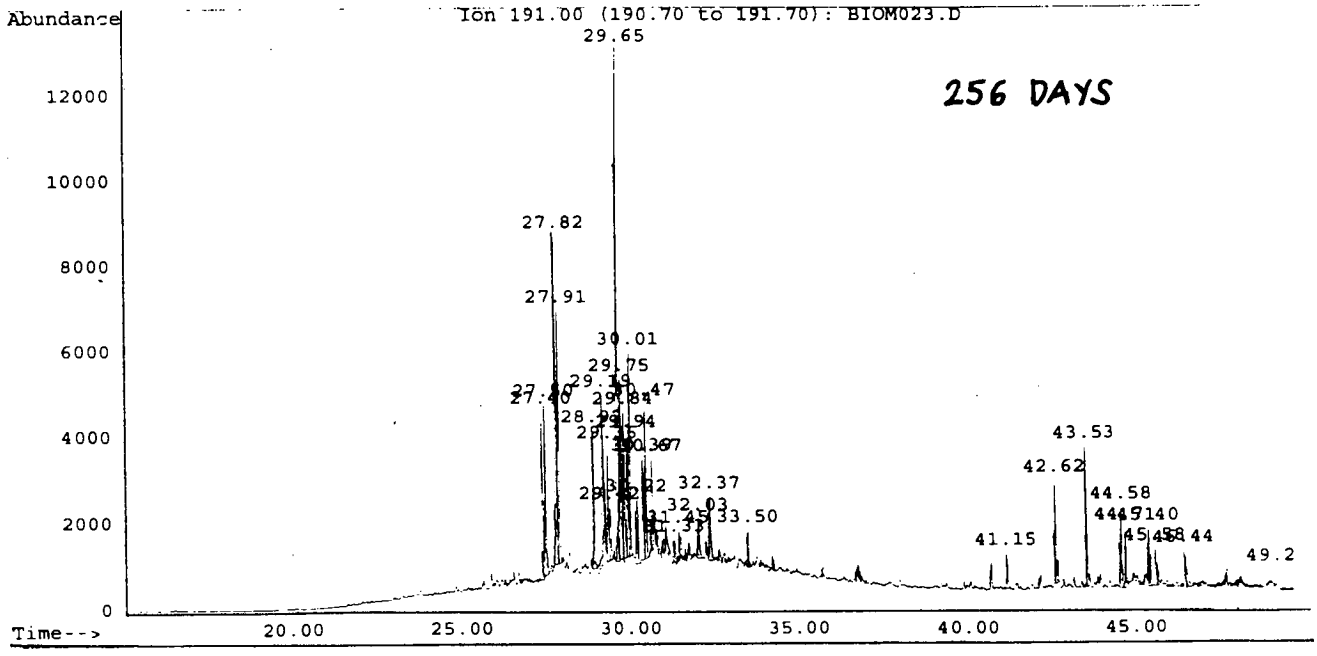


Time (min.)

Figure 4.13 (a) GC-EI MS Ion Chromatograms at m/z 85 for Ballast Oil at 0 days (top) and 256 days (below)

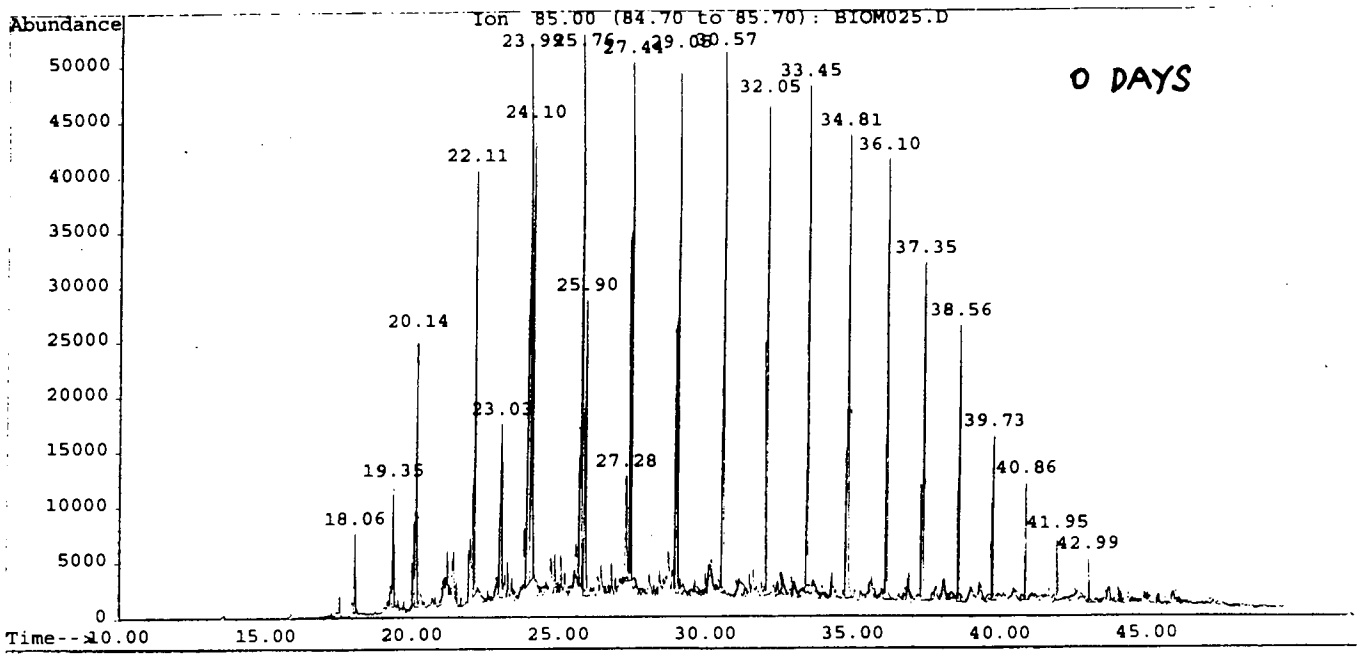


Time (min.)

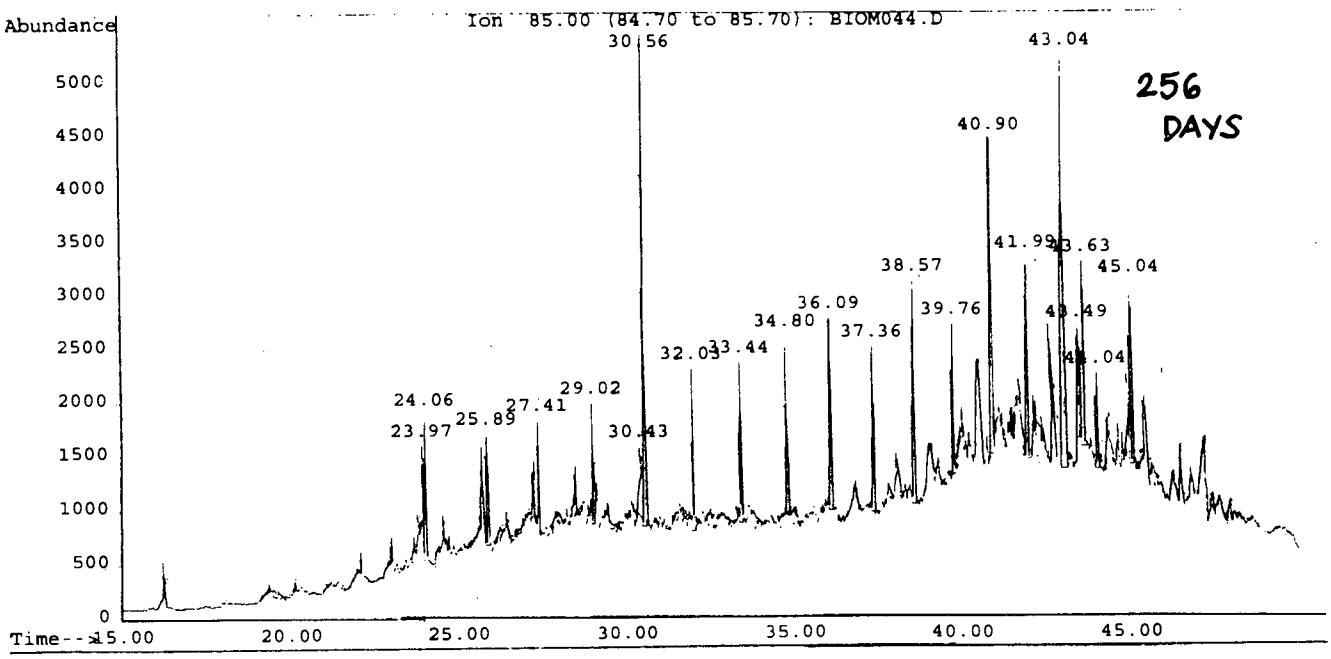


Time (min.)

Figure 4.13 (b) GC-EI MS Ion Chromatograms at m/z 191 for Ballast Oil at 0 days (top) and 256 days (below)

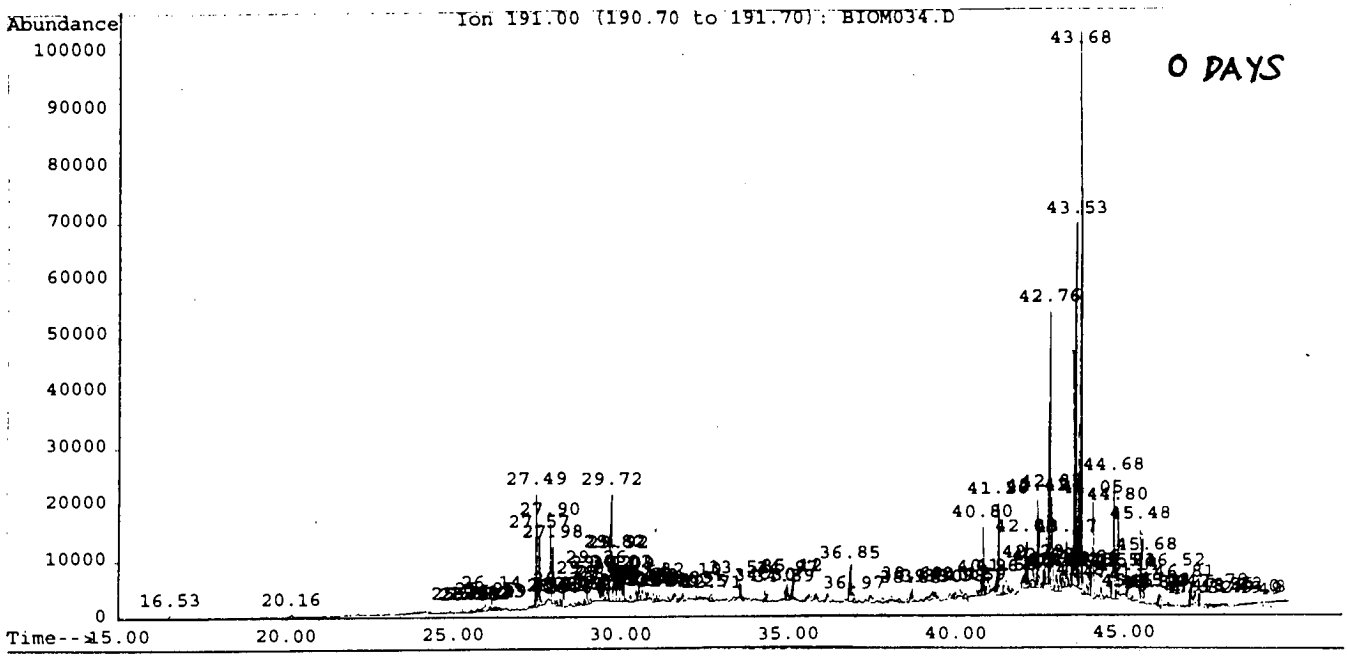


Time (min.)

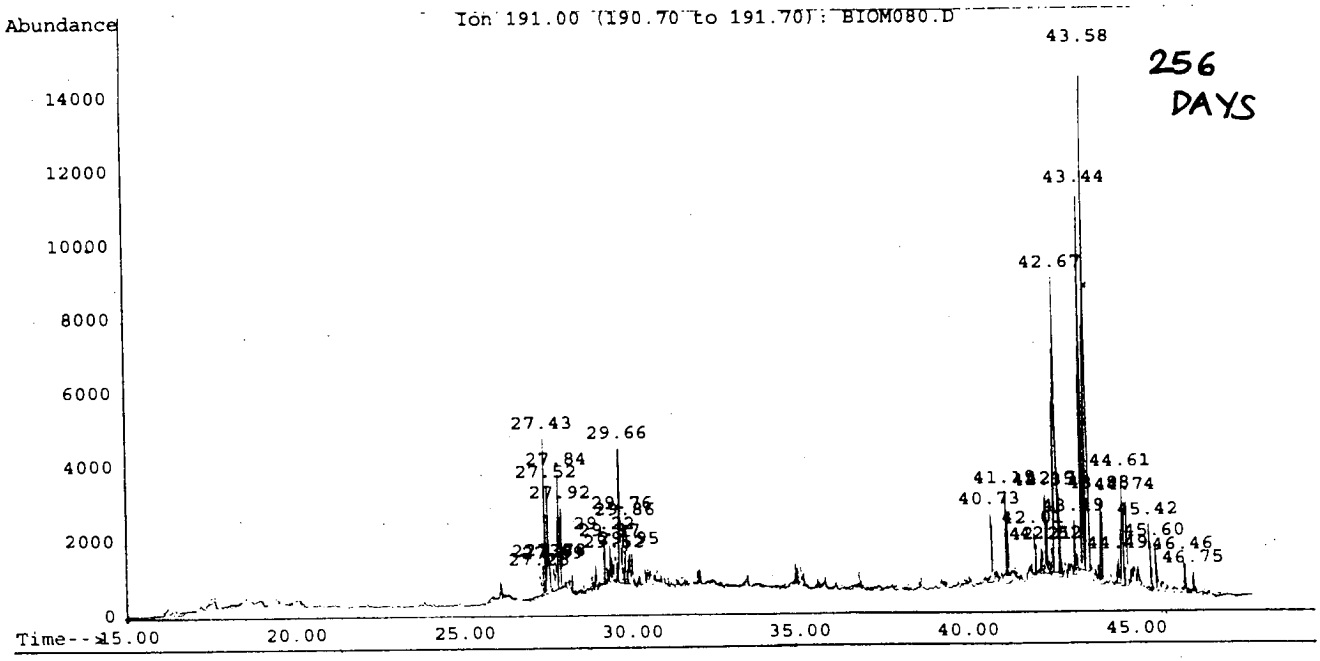


Time (min.)

Figure 4.14 (a) GC-EI MS Ion Chromatograms at m/z 85 for Crude Oil at 0 days (top) and 256 days (below)

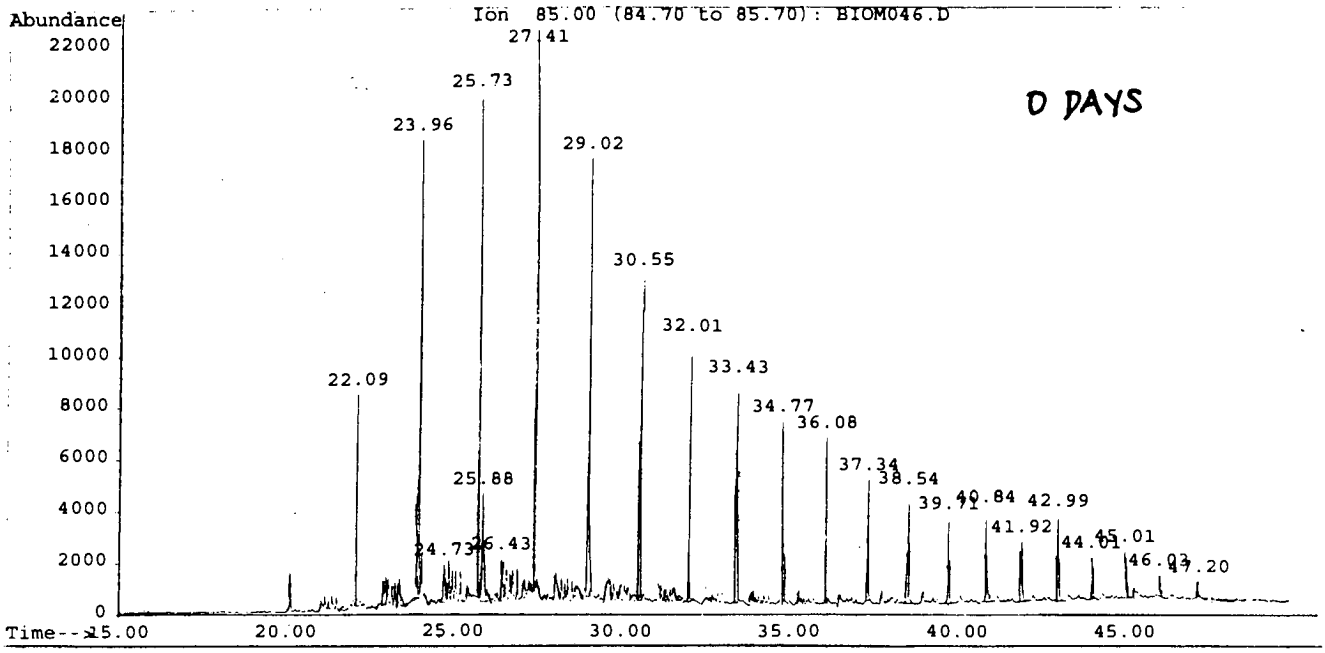


Time (min.)

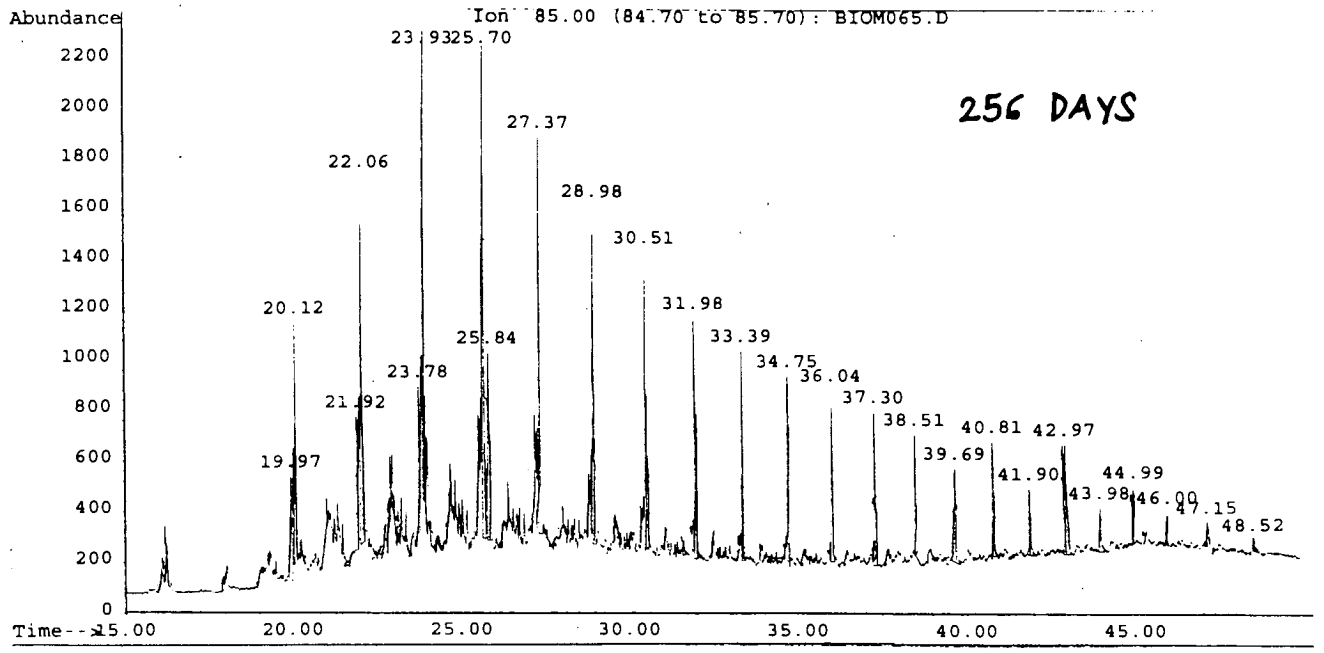


Time (min.)

Figure 4.14 (b) GC-EI MS Ion Chromatograms at m/z 191 for Crude Oil at 0 days (top) and 256 days (below)

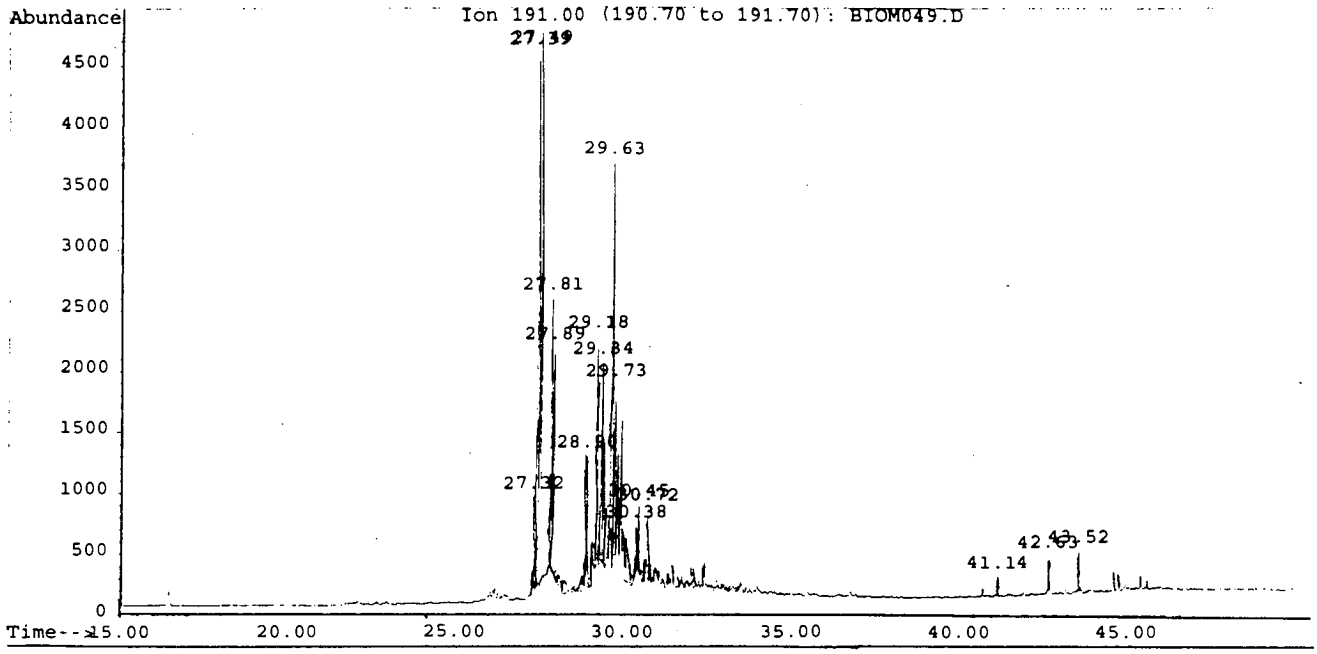


Time (min.)

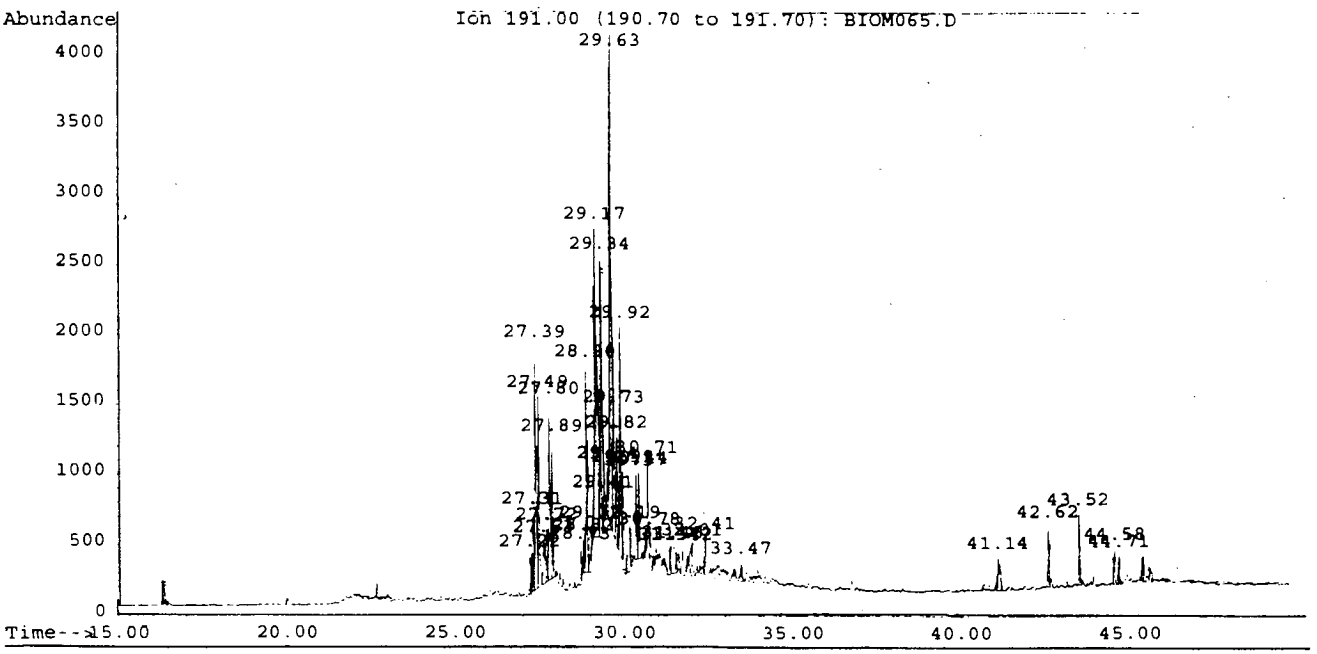


Time (min.)

Figure 4.15 (a) GC-EI MS Ion Chromatograms at m/z 85 for No.6 Fuel Oil at 0 days (top) and 256 days (below)



Time (min.)



Time (min.)

Figure 4.15 (b) GC-EI MS Ion Chromatograms at m/z 191 for No.6 Fuel Oil at 0 days (top) and 256 days (below)

4.12 (a) and (b) (for the ballast oil, crude oil and No.6 Fuel Oil, respectively; (a) for weathering indices, (b) for source indices). Mean index values for each extract at the successive sampling points are also provided, together with the calculated standard deviations of these measurements and the corresponding results for the control microcosms.

Ballast Oil Weathering Indices. For the ballast oil, all seven weathering indices recorded a decrease in value with increasing oil biotransformation. The largest variation in value was observed for [*n*-alkanes:17α(H)21β(H)-hopane], which decreased from an initial mean value of 748.9 ± 37.7 to 8.5 ± 1.0 after 256 days. Steady and numerically substantial decreases were also obtained for the [$\Sigma C_{14\text{-}28}$:C_{24}tetracyclic terpane] and [$\Sigma C_{14\text{-}28}$:Σtricyclic terpanes] ratios. The ratio of low-to-high carbon number *n*-alkanes, [$C_{14+16+18}$:$C_{24+26+28}$] also decreased overall during the study, but the variation was more erratic than was the case for the former three indices. The least sensitive of the weathering indicators tested were found to be the ratios of C_{16}, C_{17} and C_{18} to their branched-chain analogues, norpristane, pristane and phytane. The values of these indices decreased by between 1.5 and 1.9, which, though small in relation to the other indices, was nevertheless much greater than the standard deviation of the mean values at each sampling point.

In the control microcosms, the weathering indices were evaluated after 2 days, 4 days, 32 days, 64 days and 256 days. For these samples, the [*n*-alkanes:17a(H)21b(H)-hopane] ratio decreased only marginally with time, producing a final value of 419.6. A similar trend was observed for the [$C_{14+16+18}$:$C_{24+26+28}$] index, which decreased slightly from 10.9 (single measurement only) after 2 days to 7.3 after 256 days. Values for the [$\Sigma C_{14\text{-}28}$:C_{24}tetracyclic terpane] and [$\Sigma C_{14\text{-}28}$:Σtricyclic terpanes] indices did not show any decrease during the study. Similarly, the ratios of [C_{16}:norpristane], [C_{17}:pristane] and [C_{18}:phytane] did not register any appreciable variation over the course of the study. These figures were obtained from the analysis of a single microcosm flask at each sampling point, and so it was not possible to determine the variation of these values.

Table 4.10 (a) Variation of Weathering Index Values with Time for Ballast Oil-Treated and Control Soils

WEATHERING INDEX		0 days	2 days	4 days	8 days	16 days	32 days	64 days	128 days	256 days
	n1,n2,n3	4.1 4.5 3.0	2.9 2.5 3.2	3.3 2.8 2.3	3.2 3.4 2.7	2.7 3.6 2.8	N/D 3.0 2.6	N/D N/D 2.0	N/D 2.1 1.9	2.1 1.8 2.2
C_{16}:	Mean (SD)	3.9 (0.8)	2.8 (0.4)	2.8 (0.5)	3.1 (0.3)	3.0 (0.5)	2.8 (0.3)	2.0 (N/D)	2.0 (0.1)	2.0 (0.2)
Norpristane	Control[1]		5.4	6.7			5.6	N/D		5.1
	n1,n2,n3	2.9 3.1 3.1	2.3 2.4 2.4	2.6 2.2 2.6	2.0 2.5 2.5	2.0 2.2 2.0	N/D 2.0 2.1	2.7 2.4 1.9	2.3 2.2 2.4	1.6 1.5 1.4
C_{17}:	Mean (SD)	3.0 (0.1)	2.4 (0.1)	2.4 (0.2)	2.3 (0.3)	2.1 (0.1)	2.0 (0.0)	2.3 (0.4)	2.3 (0.1)	1.5 (0.1)
Pristane	Control[1]		2.8	3.1			2.7	3.5		3.1
	n1,n2,n3	2.0 1.8 1.6	1.6 2.0 1.7	1.5 1.4 1.6	1.5 1.7 1.3	1.5 1.5 1.5	N/D 1.7 1.6	1.9 1.8 1.7	1.9 1.6 1.7	0.9 0.7 0.7
C_{18}:	Mean (SD)	1.8 (0.2)	1.8 (0.2)	1.5 (0.1)	1.5 (0.2)	1.5 (0.0)	1.6 (0.1)	1.8 (0.1)	1.7 (0.2)	0.7 (0.1)
Phytane	Control[1]		2.0	1.7			1.6	2.0		2.2
	n1,n2,n3	9.7 11.2 10.2	4.0 3.1 4.3	3.4 3.5 3.9	4.2 4.3 3.4	5.4 6.6 4.7	N/D 4.7 4.1	1.1 1.6 2.3	1.5 3.1 1.1	1.5 1.3 1.6
$C_{14+16+18}$:	Mean (SD)	10.4 (0.7)	3.8 (0.6)	3.6 (0.3)	4.0 (0.5)	5.6 (1.0)	4.4 (0.5)	1.7 (0.6)	1.9 (1.0)	1.5 (0.2)
$C_{24+26+28}$	Control[1]		10.9	7.7			9.3	5.2		7.3
	n1,n2,n3	709.7 784.8 752.4	N/D 420.9 500.6	N/D 445.9 514.9	586.1 N/D 427.1	627.9 684.9 531.0	N/D 585.8 490.3	N/D N/D 322.2	N/D 267.3 N/D	9.5 8.2 7.7
ΣC_{14-28}:	Mean (SD)	748.9 (37.7)	460.8 (56.3)	480.5 (48.8)	506.6 (112.4)	614.6 (77.8)	538.0 (67.5)	322.2 (N/D)	267.3 (N/D)	8.5 (1.0)
17α21β-Hopane	Control[1]		614.3	538.5			552.3	470.5		419.6
	n1,n2,n3	13.2 14.7 13.8	13.4 12.4 13.4	14.4 10.5 13.6	13.7 10.3 10.6	10.6 9.5 10.7	N/D 8.2 10.1	12.2 0.3 14.2	0.3 16.7 0.3	1.6 1.5 1.3
ΣC_{14-28}:	Mean (SD)	13.9 (0.8)	13.1 (0.6)	12.8 (2.1)	11.5 (1.9)	10.3 (0.7)	9.1 (1.4)	8.9 (7.5)	5.8 (9.5)	1.5 (0.2)
Tricyclic Terps	Control[1]		14.9	14.7			12.1	12.7		9.1
	n1,n2,n3	76.1 81.2 69.2	56.6 49.0 56.6	59.3 44.5 60.7	59.1 45.4 44.1	46.1 44.7 44.1	N/D 33.2 40.4	34.1 0.7 43.5	0.9 48.6 0.7	2.4 2.0 1.9
ΣC_{14-28}:	Mean	75.5 (6.0)	54.0 (4.4)	54.8 (9.0)	49.5 (8.3)	45.0 (1.1)	36.8 (5.1)	26.1 (22.5)	16.7 (27.6)	2.1 (0.3)
Tetracyclic Terp.	Control[1]		84.3	81.2			60.2	52.9		41.7

Table 4.10 (b) Variation of Source Correlation Index Values with Time for Ballast Oil-Treated and Control Soils

SOURCE INDEX		0 days	2 days	4 days	8 days	16 days	32 days	64 days	128 days	256 days
	n1,n2,n3	0.9 0.9 0.9	0.7 0.9 0.8	0.6 0.7 0.7	0.9 0.8 0.7	0.8 0.8 0.8	N/D 0.8 0.8	0.2 0.4 0.8	0.4 0.7 0.4	0.6 0.6 0.6
Pristane:	Mean (SD)	0.9 (0.0)	0.8 (0.1)	0.7 (0.1)	0.8 (0.1)	0.8 (0.0)	0.8 (0.0)	0.5 (0.3)	0.5 (0.2)	0.6 (0.0)
Phytane	Control[1]		0.9	0.7			0.9	0.7		0.9
	n1,n2,n3	41.4 43.1 41.0	N/D 26.3 39.7	N/D 37.4 41.1	48.8 N/D 37.3	51.5 54.2 47.9	N/D 49.9 39.3	N/D N/D 25.6	N/D 23.8 N/D	1.1 1.2 1.2
Phytane:	Mean (SD)	41.9 (1.1)	33.0 (9.5)	39.2 (2.6)	43.1 (8.1)	51.2 (3.2)	44.6 (7.5)	25.6 (N/D)	23.8 (N/D)	1.2 (0.1)
17α21β-Hopane	Control[1]		43.1	31.5			30.8	37.1		42.3
	n1,n2,n3	0.7 0.8 0.8	N/D 0.8 0.8	N/D 0.7 0.7	0.8 N/D 0.9	0.7 0.7 0.7	N/D 0.7 0.8	N/D N/D 0.7	N/D 0.8 N/D	0.7 0.7 0.7
17α21β-Norhopane:	Mean (SD)	0.7 (0.0)	0.8 (0.1)	0.7 (0.0)	0.9 (0.0)	0.7 (0.0)	0.8 (0.0)	0.7 (N/D)	0.8 (N/D)	0.7 (0.0)
17α21β-Hopane	Control[1]		0.8	0.7			0.7	0.8		0.8
	n1,n2,n3	1.1 1.2 1.1	1.0 0.9 1.0	1.0 0.9 1.0	1.0 1.0 1.0	1.0 1.0 0.9	N/D 0.9 0.9	0.7 0.7 0.8	0.7 0.8 0.7	0.6 0.6 0.6
C23 (S,R) Tri.Ts.:[2]	Mean (SD)	1.1 (0.0)	1.0 (0.1)	0.9 (0.1)	1.0 (0.0)	1.0 (0.0)	0.9 (0.0)	0.7 (0.1)	0.7 (0.1)	0.6 (0.0)
C24 (S,R) Tri.Ts.	Control[1]		1.0	0.8			1.0	1.2		1.2
	n1,n2,n3	1.5 1.2 1.5	N/D 1.3 N/D	N/D 1.3 1.4	N/D N/D N/D	N/D N/D N/D	N/D N/D N/D	N/D N/D 1.3	N/D 1.3 N/D	1.2 1.3 1.3
17α21β-Homohop. (22S):[3]	Mean (SD)	1.4 (0.2)	1.3 (N/D)	1.4 (0.1)				1.3 (N/D)	1.3 (N/D)	1.3 (0.0)
17α21β-Homohop. (22R)	Control[1]		1.2	N/D				1.2		1.4

[1]Control index values based on single extraction only

[2]Sum of C23-Tricyclic terpane (S) and (R) peak areas

[3]Ratio of 17α21β-homohopanes (22S) and (22R)

Ballast Oil Source Correlation Indices. Changes in the values of the ballast oil source correlation indices were very small, with the exception of those for [phytane:17α(H)21β(H)-hopane], which decreased from 41.9 ± 1.1 to 1.2 ± 0.1 as the proportion of phytane in the extracts diminished. The most reliable source indices were [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane] and the ratio of 17α(H)21β(H)-homohopane (22S) and 17α(H)21β(H)-homohopane (22R) isomers, [22S:22R], which did not vary by greater than 0.2 over the entire study period. The values for [pristane:phytane] and [C_{23}tricyclic terpanes:C_{24}tricyclic terpane] were found to remain steady over the initial period of study, at 0.9 and 1.1 (precisions up to 0.1), respectively, until the 64 day samples, whereupon their values began to decrease, by approximately 0.3 in both cases. Source correlation indices in the ballast oil control microcosms also remained largely constant, varying by between 0.2 and 0.4 in most cases. The exception to this was once again the [phytane:17α(H)21β(H)-hopane] index, which decreased in value slightly over the latter period of biotransformation to produce a final value of 27.3, from a value of 31.5 after 2 days.

Plots comparing the changes in the mean values of ballast oil source and weathering indices in the treated soils and control soils are shown in Figure 4.16 (a) (weathering indices) and (b) (source indices).

Crude Oil Weathering Indices. For the crude oil treated soils, the greatest shift in mean value was observed for the [$\Sigma C_{14\text{-}28}$:C_{24}tetracyclic terpane] ratio, which decreased from 52.7 ± 7.3 at 0 days to 7.3 ± 2.1 after 256 days. However, this decrease was only manifest over the last two sets of samples, since for the samples taken at 4, 8, 16, 32 and 64 days, the value for this ratio was found to be successively increase to a maximum of around 66. A similar phenomenon was also observed for the [$\Sigma C_{14\text{-}28}$:Σtricyclic terpanes] ratio, which overall decreased from 17.5 ± 1.5 initially to 15.6 (± n/d) after 128 days and 0 at 256 days (due to undetected tricyclic terpanes in these highly weathered samples), but was seen to increase to

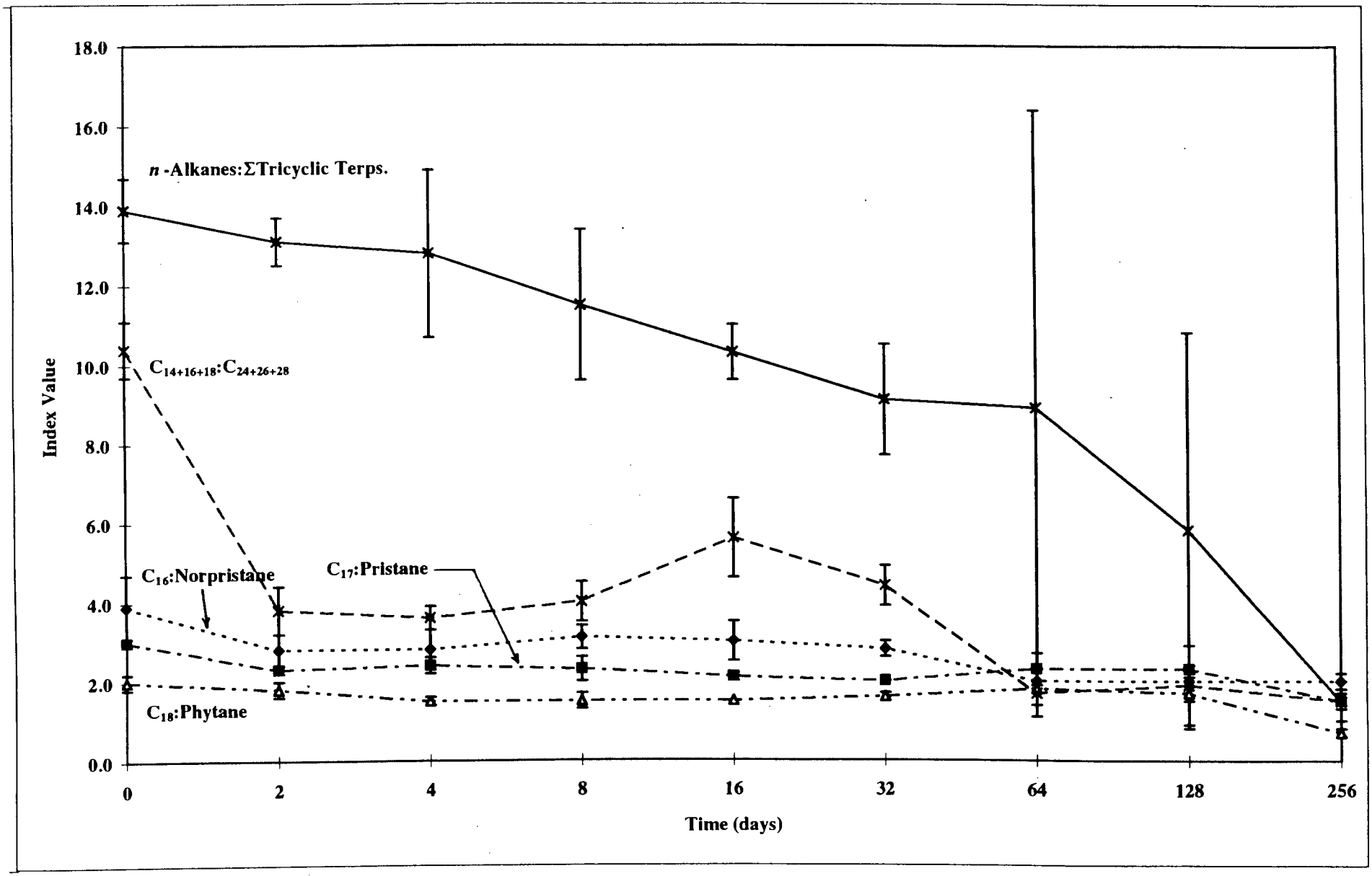


Figure 4.16 (a) Variation in Weathering Index Value for Ballast Oil-Treated Soils

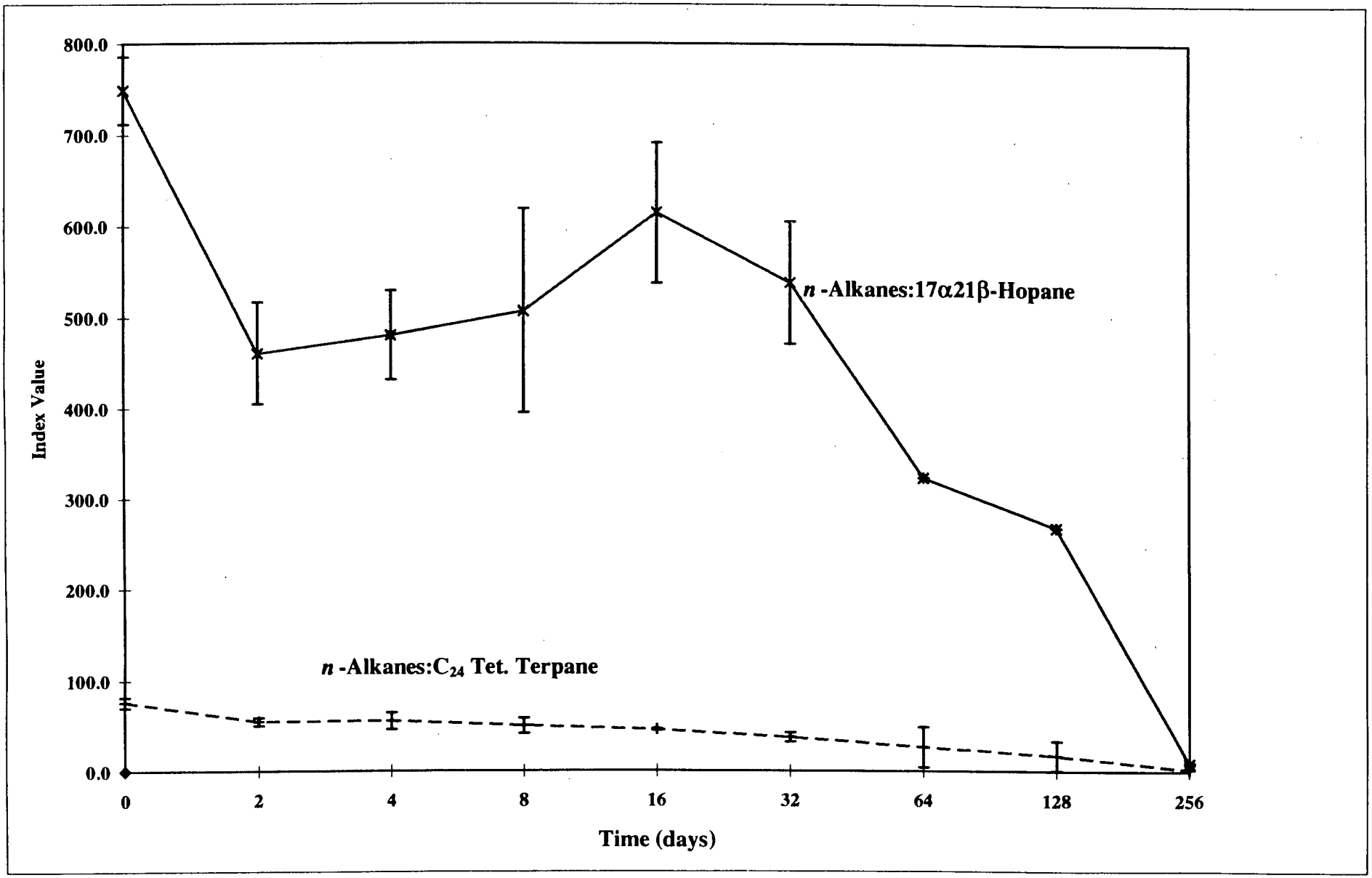


Figure 4.16 (a) Variation in Weathering Index Value for Ballast Oil-Treated Soils

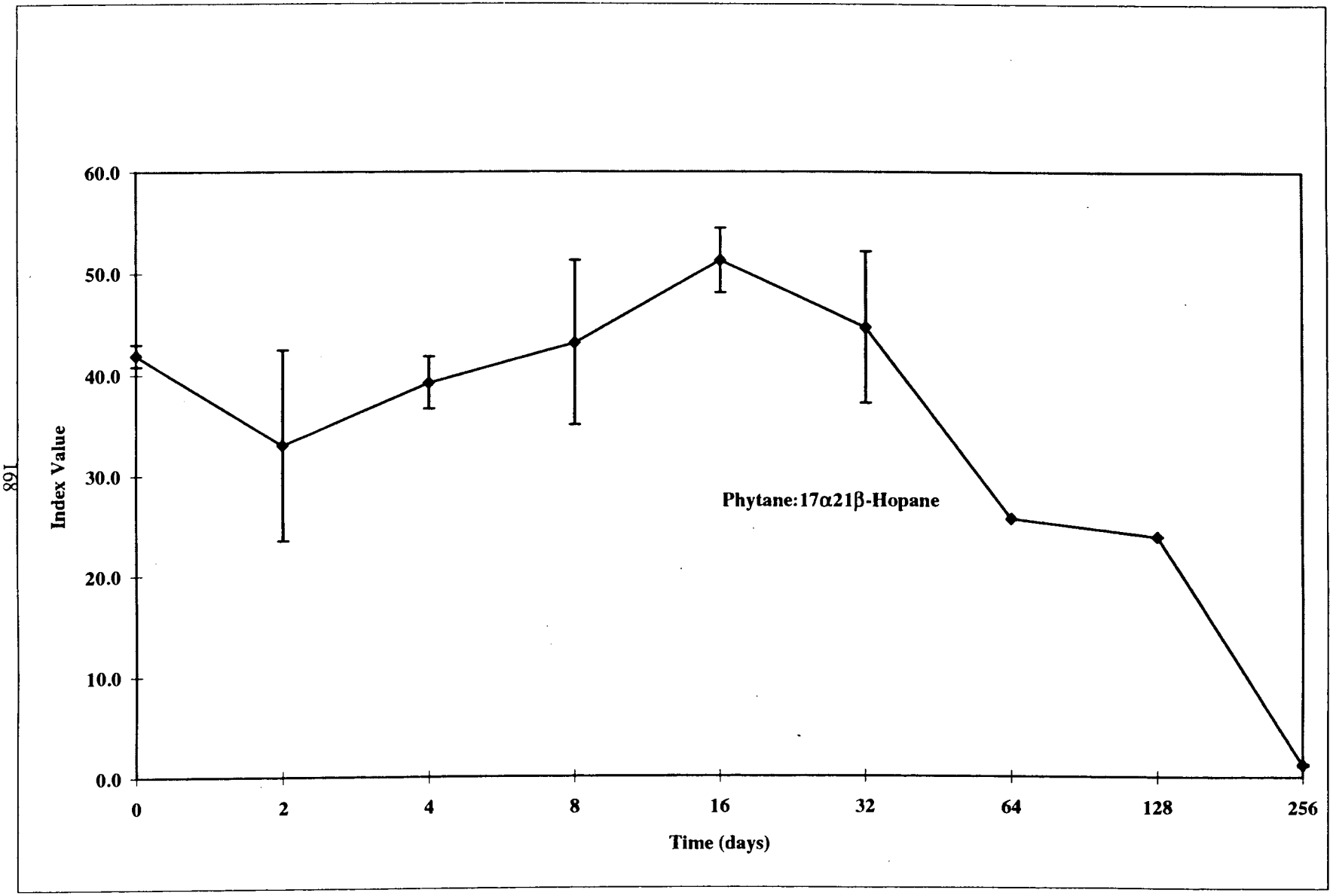


Figure 4.16 (b) Variation in Source Index Value for Ballast Oil-Treated Soils

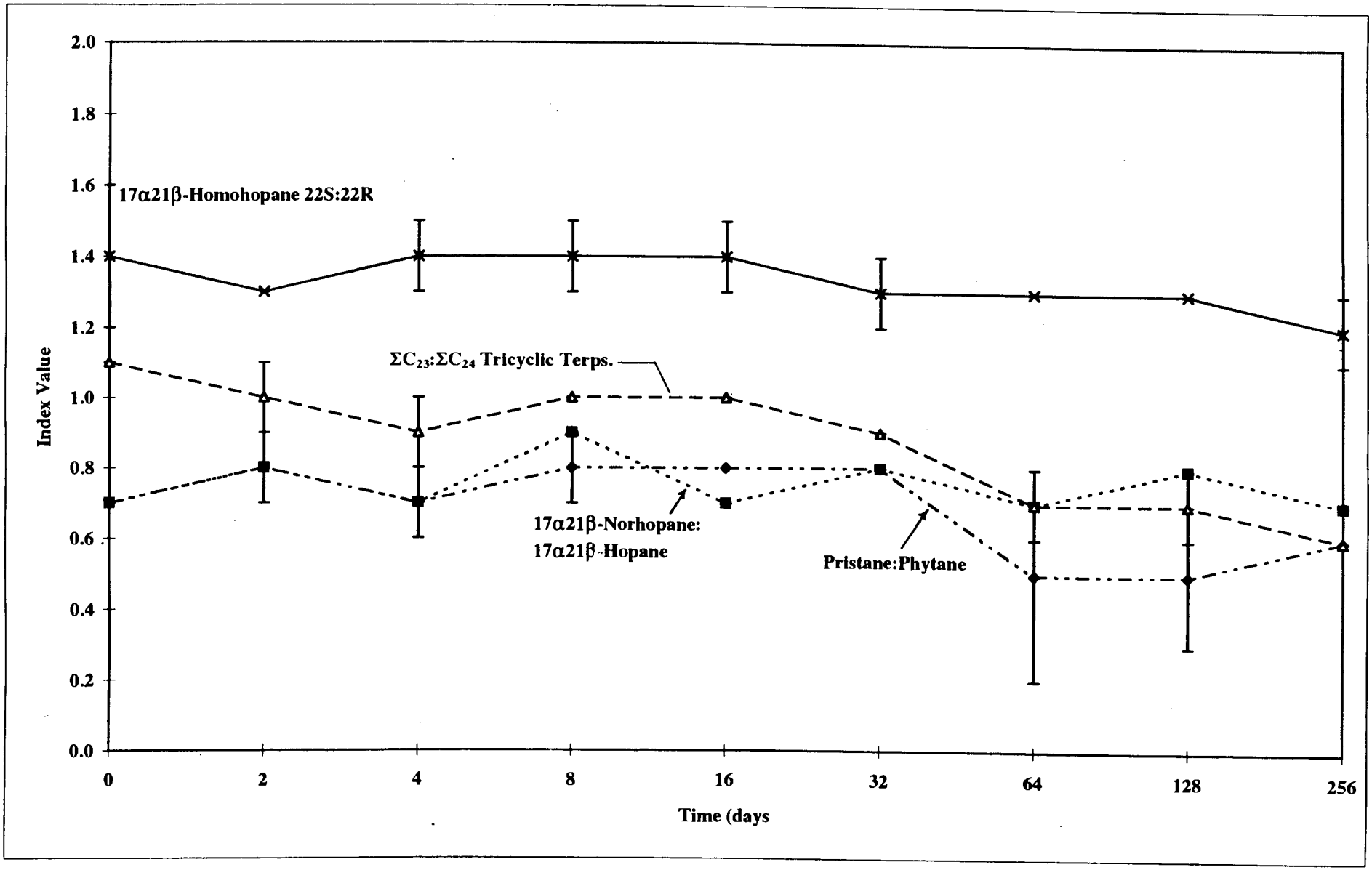


Figure 4.16 (b) Variation in Source Index Value for Ballast Oil-Treated Soils

Table 4.11 (a) Variation of Weathering Index Values with Time for Crude Oil-Treated and Control Soils

WEATHERING INDEX		0 days	2 days	4 days	8 days	16 days	32 days	64 days	128 days	256 days
	n1,n2,n3	2.3 2.2 2.1	N/D 1.7 1.8	2.0 1.8 2.0	2.0 1.8 2.0	N/D 2.2 N/D	2.6 N/D 1.9	1.5 1.8 N/D	0.5 1.5 N/D	N/D N/D N/D
C_{16}:	Mean (SD)	2.2 (0.1)	1.7 (0.1)	1.9 (0.1)	1.9 (0.1)	2.2 (N/D)	2.2 (0.5)	1.6 (0.2)	1.0 (0.7)	
Norpristane	Control[1]		2.2	2.1			2.1	2.1		2.6
	n1,n2,n3	1.8 1.6 1.3	N/D 1.1 1.2	1.3 1.9 1.5	1.1 0.9 0.9	0.9 0.9 N/D	0.9 1.3 1.1	2.1 1.6 0.9	0.9 0.7 N/D	1.3 0.5 0.9
C_{17}:	Mean (SD)	1.6 (0.2)	1.1 (0.1)	1.5 (0.3)	1.0 (0.1)	0.9 (0.0)	1.1 (0.2)	1.5 (0.6)	0.8 (0.2)	0.9 (0.4)
Pristane	Control[1]		0.9	1.2			1.3	1.6		1.3
	n1,n2,n3	2.1 1.6 2.1	N/D 1.4 1.6	2.0 2.2 2.0	2.3 1.7 1.6	1.4 1.4 N/D	1.5 1.6 1.5	2.1 2.3 1.9	0.9 0.9 N/D	1.0 0.4 1.0
C_{18}:	Mean (SD)	1.9 (0.3)	1.5 (0.1)	2.1 (0.1)	1.9 (0.4)	1.4 (0.0)	1.5 (0.1)	2.1 (0.2)	0.9 (0.0)	0.8 (0.3)
Phytane	Control[1]		1.6	1.6			1.3	1.2		2.2
	n1,n2,n3	1.0 0.9 1.0	N/D 1.1 1.0	0.6 0.7 0.6	0.5 0.6 0.6	0.6 0.5 N/D	0.5 0.4 0.4	0.3 0.4 0.3	0.5 0.5 N/D	0.2 0.5 0.3
$C_{14+16+18}$:	Mean (SD)	1.0 (0.1)	1.1 (0.0)	0.6 (0.0)	0.6 (0.0)	0.6 (0.0)	0.4 (0.1)	0.3 (0.1)	0.5 (0.0)	0.3 (0.2)
$C_{24+26+28}$	Control[1]		0.9	1.2			0.9	1.1		1.0
	n1,n2,n3	16.0 14.5 14.5	N/D 15.4 16.7	15.4 13.7 13.7	14.7 15.8 18.4	14.2 15.6 N/D	12.8 11.8 13.4	9.9 10.1 10.5	2.7 0.9 N/D	0.5 0.2 0.3
ΣC_{14-28}:	Mean (SD)	15.0 (0.9)	16.1 (0.9)	14.3 (1.0)	16.3 (1.9)	14.9 (1.0)	12.7 (0.8)	10.2 (0.3)	1.8 (1.3)	0.4 (0.1)
17α21β-Hopane	Control[1]		16.2	17.9			14.9	14.3		14.3
	n1,n2,n3	19.1 16.2 17.1	N/D 14.7 15.2	19.0 19.9 19.5	19.9 20.6 22.9	20.6 20.6 N/D	25.1 21.5 27.5	57.3 26.9 27.5	N/D 15.6 0.3	0.0 0.0 0.0
ΣC_{14-28}:	Mean (SD)	17.5 (1.5)	15.0 (0.4)	19.5 (0.4)	21.1 (1.6)	20.6 (0.0)	24.7 (3.0)	37.2 (17.4)	15.6 (N/D)	0.0
Tricyclic Terps[2]	Control[1]		18.0	18.5			15.5	15.5		17.4
	n1,n2,n3	53.6 44.9 59.5	N/D 39.9 39.8	56.8 48.4 59.4	52.4 60.1 61.4	59.7 66.1 N/D	70.4 57.2 70.2	78.3 58.0 60.5	N/D 19.7 N/D	9.4 5.3 N/D
ΣC_{14-28}:	Mean	52.7 (7.3)	39.8 (0.0)	54.9 (5.8)	57.9 (4.9)	62.9 (4.5)	65.9 (7.6)	65.6 (11.1)	19.7 (N/D)	7.3 (2.1)
Tetracyclic Terp.[3]	Control[1]		49.2	47.5			40.1	42.3		45.9

[1]Control index values determined from single extract only

[2]Sum of C23 (S and R) and C24 (S and R) tricyclic terpane peak areas

[3]C24 Tetracyclic terpane

Table 4.11 (b) Variation of Source Correlation Index Values with Time for Crude Oil-Treated and Control Soils

SOURCE INDEX		0 days	2 days	4 days	8 days	16 days	32 days	64 days	128 days	256 days
	n1,n2,n3	2.0 2.0 1.9	N/D 1.4 1.6	2.1 1.7 1.8	2.1 2.1 2.0	1.8 1.7 N/D	1.8 1.9 1.6	1.3 1.5 1.6	1.6 1.1 N/D	1.7 0.9 1.3
Pristane:	Mean (SD)	2.0 (0.0)	1.5 (0.1)	1.9 (0.2)	2.1 (0.1)	1.8 (0.1)	1.8 (0.2)	1.5 (0.1)	1.3 (0.3)	1.3 (0.4)
Phytane	Control[1]		1.8	1.8			1.9	1.8		2.3
	n1,n2,n3	0.6 0.5 0.6	N/D 0.9 1.0	0.6 0.5 0.5	0.5 0.7 0.8	0.6 0.7 N/D	0.5 0.4 0.5	0.3 0.3 0.4	0.2 0.1 N/D	0.0 0.0 0.0
Phytane:	Mean (SD)	0.6 (0.0)	0.9 (0.0)	0.6 (0.0)	0.7 (0.2)	0.6 (0.0)	0.5 (0.1)	0.3 (0.0)	0.2 (0.1)	0.0
17α21β-Hopane	Control[1]		0.8	0.8			0.6	0.6		0.6
	n1,n2,n3	0.7 0.8 0.7	N/D 0.7 0.7	0.7 0.7 0.8	0.6 0.7 0.6	0.7 0.7 N/D	0.7 0.7 0.7	0.6 0.6 0.7	0.7 0.7 N/D	0.7 0.7 0.7
17α21β-Norhopane:	Mean (SD)	0.7 (0.0)	0.7 (0.0)	0.7 (0.0)	0.6 (0.0)	0.7 (0.0)	0.7 (0.0)	0.6 (0.0)	0.7 (0.0)	0.7 (0.0)
17α21β-Hopane	Control[1]		0.7	0.7			0.7	0.8		0.6
	n1,n2,n3	1.3 1.2 1.3	N/D 1.2 1.2	1.3 1.4 1.4	1.3 1.2 1.3	1.6 1.4 N/D	1.7 1.4 1.5	1.1 1.3 1.3	N/D 2.5 N/D	0.0 0.0 0.0
C23 (S,R) Tri.Ts.:[2]	Mean (SD)	1.3 (0.1)	1.2 (0.0)	1.4 (0.1)	1.3 (0.1)	1.5 (0.2)	1.5 (0.2)	1.2 (0.1)	2.5 (N/D)	0.0
C24 (S,R) Tri.Ts.	Control[1]		1.3	1.1			1.3	1.8		1.4
	n1,n2,n3	1.3 1.3 1.3	N/D 1.3 1.3	1.3 1.4 1.3	1.3 1.3 1.2	1.3 1.3 N/D	1.3 1.3 1.2	1.3 1.3 1.3	1.3 1.3 N/D	1.3 1.3 1.2
17α21β-Homohop. (22S):[3]	Mean (SD)	1.3 (0.0)	1.3 (0.0)	1.3 (0.0)	1.3 (0.0)	1.3 (0.0)	1.3 (0.0)	1.3 (0.0)	1.3 (0.0)	1.3 (0.0)
17α21β-Homohop. (22R)	Control[1]		1.2	1.3			1.3	1.3		1.3
	n1,n2,n3	1.3 1.2 1.3	N/D 1.4 1.3	1.5 1.4 1.3	1.5 1.6 1.4	1.3 1.7 N/D	1.6 1.3 1.7	1.4 1.5 1.5	1.4 1.3 N/D	1.4 1.4 1.3
17α21β-Bishomohop. (22S):[4]	Mean (SD)	1.3 (0.0)	1.3 (0.1)	1.4 (0.1)	1.5 (0.1)	1.5 (0.3)	1.6 (0.2)	1.5 (0.1)	1.3 (0.0)	1.3 (0.0)
17α21β-Bishomohop. (22R)	Control[1]		1.3	1.3			1.1	1.4		1.4

[1]Control index values based on single extraction only

[2]Sum of C23-Tricyclic terpane (S) and (R) peak areas

[3]Ratio of 17α21β-homohopanes (22S) and (22R)

[4]Ratio of 17α21β-bishomohopanes (22S) and (22R)

a maximum of 37.2 ± 17.4 at 64 days. Note, however, the magnitude of the standard deviations of these results, which were elevated in comparison to those obtained for other indices. The [*n*-alkanes:17α(H)21β(H)-hopane] was found to decrease steadily for the successively weathered samples, from 14.9 ± 0.9 at 0 days to 0.4 ± 0.1 at 256 days, after an initial slight rise at 2 days (to 16.1 ± 0.9). Similarly, the [$C_{14+16+18}$:$C_{24+26+28}$] index recorded a steady decrease in value from 1.0 ± 0.1 initially to 0.3 ± 0.16 at 256 days. The values of [C_{16}:norpristane], [C_{17}:pristane] and [C_{18}:phytane] varied fairly erratically for the 32 days of the study, generally increasing and decreasing significantly (i.e. by amounts much greater than the mean standard deviations) between successive sample points, but decreased over the latter period for the more weathered samples. In the control crude oil microcosms, none of the weathering indices were found to vary substantially over the 256 days. Small but genuine overall decreases were found for the [*n*-alkanes:17α(H)21β(H)-hopane], [ΣC_{14-28}:C_{24} tetracyclic terpane] and [C_{18}:phytane] indices, with the remaining indices showing unpredictable variations between consecutive samples.

Crude Oil Source Correlation Indices. The source correlation indices for the crude oil treated soils displaying the highest level of consistency throughout the study were the hopane isomer pairs [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane], with mean values that varied between 0.6 and 0.7 (precisions less than 0.05), the ratio of 17α(H)21β(H)-homohopane (22S) and 17α(H)21β(H)-homohopane (22R) isomers, [22S:22R], which gave a mean value of 1.3 (precisions less than 0.05) at every sampling point, and [17α(H)21β(H)-bishomohopane:17α(H)21β(H)-methylhopane], which varied between 1.3 and 1.6 (precision up to 0.1). The [pristane:phytane] and [phytane:17α(H)21β(H)-hopane] ratios showed moderate variations over the first 5 sampling points (i.e., until day 32), but significantly decreased in value for the samples taken after 64, 128 and 256 days. Similarly, the values of [C_{23}tricyclic terpanes: C_{24}tetracyclic terpane] were consistently between 1.2 and 1.5 between 0 and 64 days (precision up to 0.1), but increased to 2.5 (single measurement) after 128 days

and finally dropped to 0 for the final set of samples at 256 days. In the control microcosms, the source correlation indices were found to remain almost constant over the study, with the notable exception of [pristane:phytane] value at 256 days, which was elevated by 0.3 - 0.4 in relation to previous values.

Plots comparing the changes in the mean values of crude oil source and weathering indices in the treated soils and control soils are shown in Figure 4.17 (a) (weathering indices) and (b) (source indices).

No.6 Fuel Oil Weathering Indices. For the No.6 Fuel Oil treated soils, the magnitude of the changes in the respective weathering indices were in general much smaller than those determined for the crude and ballast oil microcosms. The greatest overall change in value was again the [*n*-alkanes:17α(H)21β(H)-hopane], which decreased markedly over the latter part of the study, from 81.9 ± n/d initially to 49.2 ± 10.4 after 128 days and 18.0 ± 14.2 after 256 days. A more gradual decline was observed for both the [ΣC_{14-28}:C_{24}tetracyclic terpane] index, from 12.2 ± n/d initially to 2.4 ± 1.9 at 256 days, and the [ΣC_{14-30}:Σtricyclic terpanes] index, from 2.9 ± n/d to 1.8 ± 1.5. The [C_{17}:pristane] ratio also decreased in a more regular fashion of the study, from 10.6 ± n/d initially, to 2.6 ± 1.4 at the end. Of the remaining weathering indices, [C_{16}:norpristane] decreased to 0 by the 16 day sampling point, and [C_{18}:phytane] and [$C_{16+18+20}$:$C_{26+28+30}$] both showed small but unpredictable variations throughout the study. In the control microcosms, the [*n*-alkanes:17α(H)21β(H)-hopane] index show a much smaller overall decrease, despite showing elevated values after 2 and 4 days. Similar fluctuations were evident for the [ΣC_{14-28}:C_{24}tetracyclic terpane] and [ΣC_{14-30}:Σtricyclic terpanes] indices, which, despite producing similar values for the first and last sets of control microcosms, were found to drop and then rise in value during the study. The opposite was observed for the [C_{18}:phytane] and [$C_{16+18+20}$:$C_{26+28+30}$] ratios, which appeared

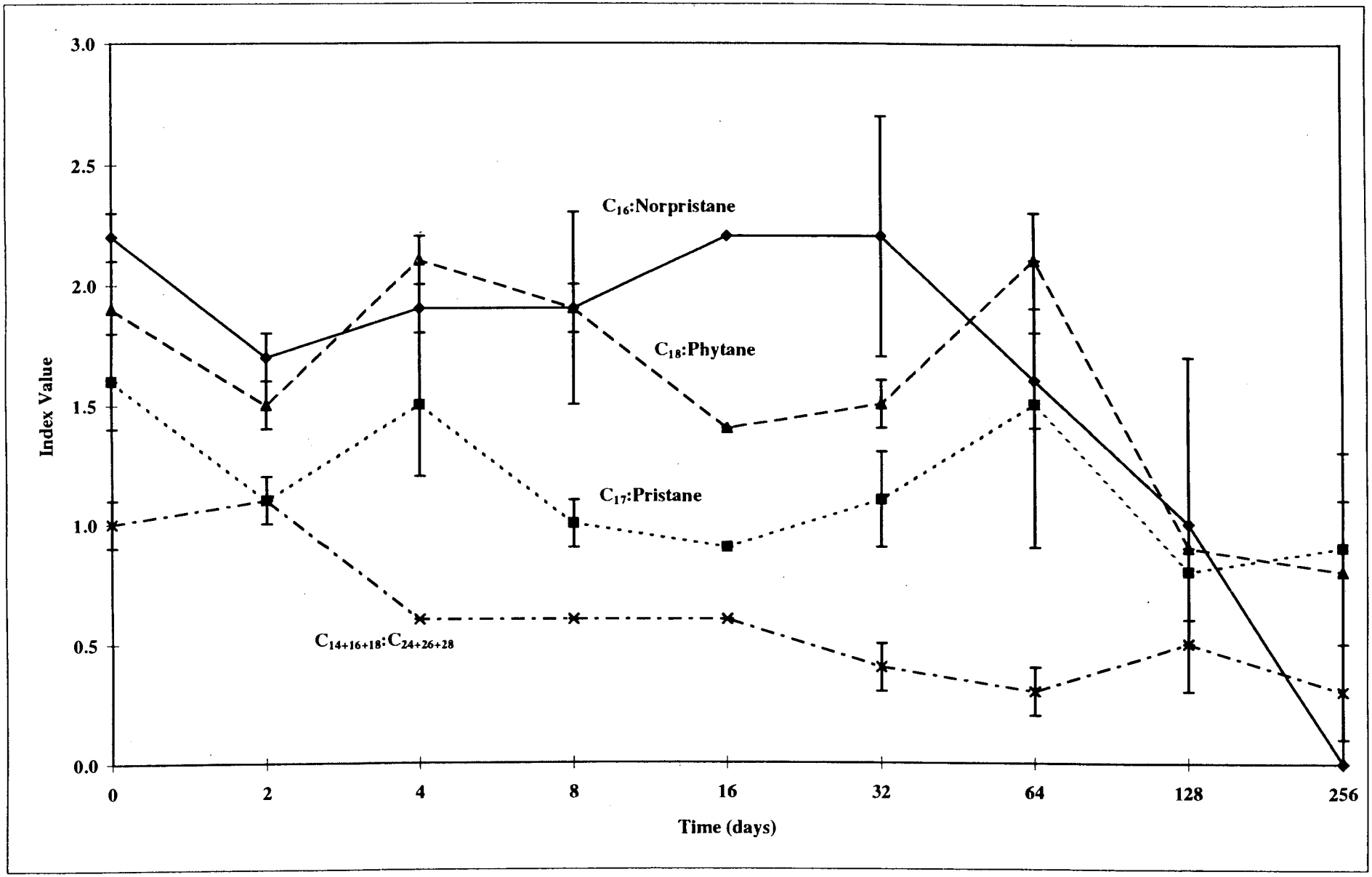


Figure 4.17 (a) Variation in Weathering Index Values for Crude Oil-Treated Soils

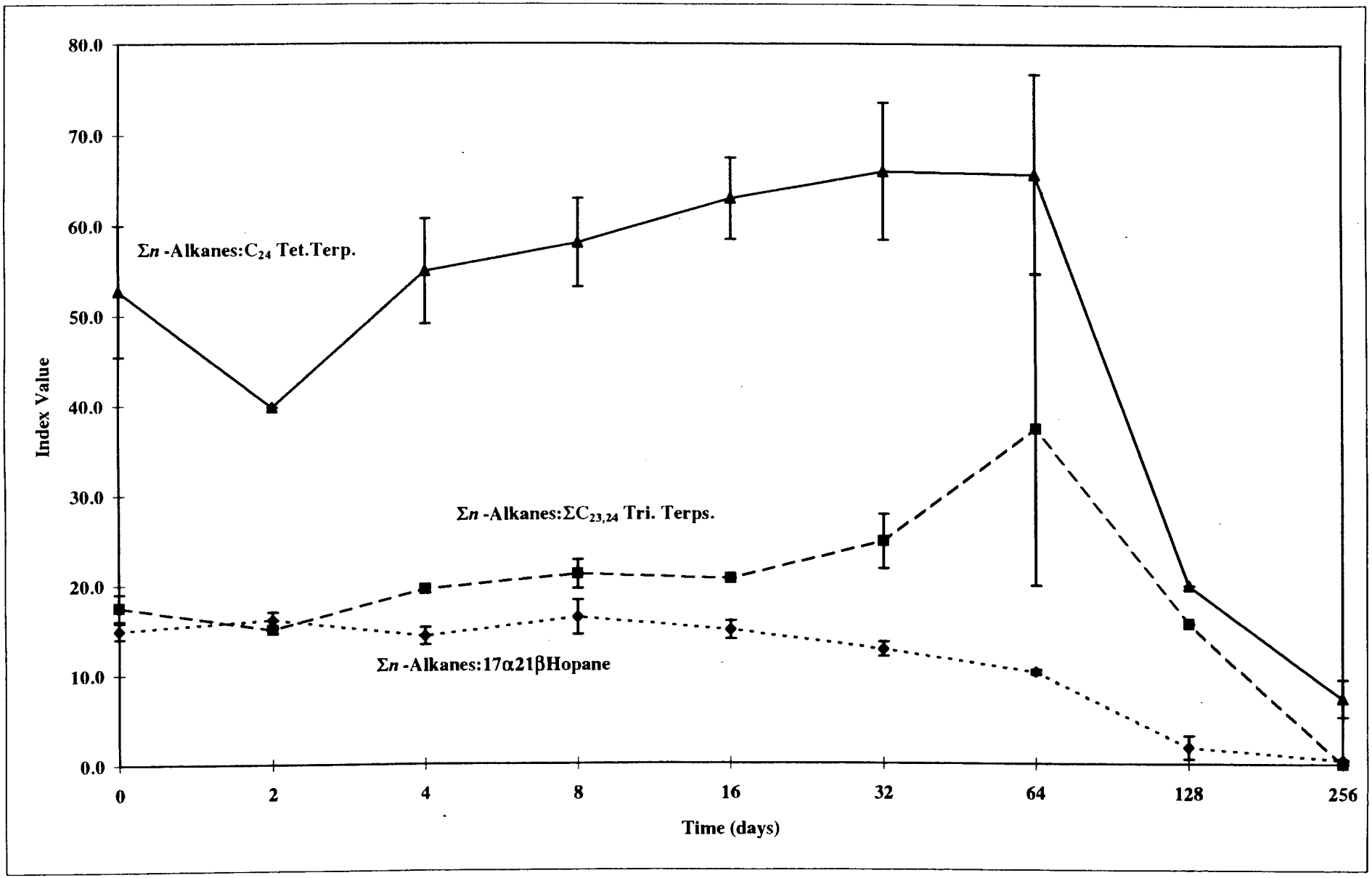
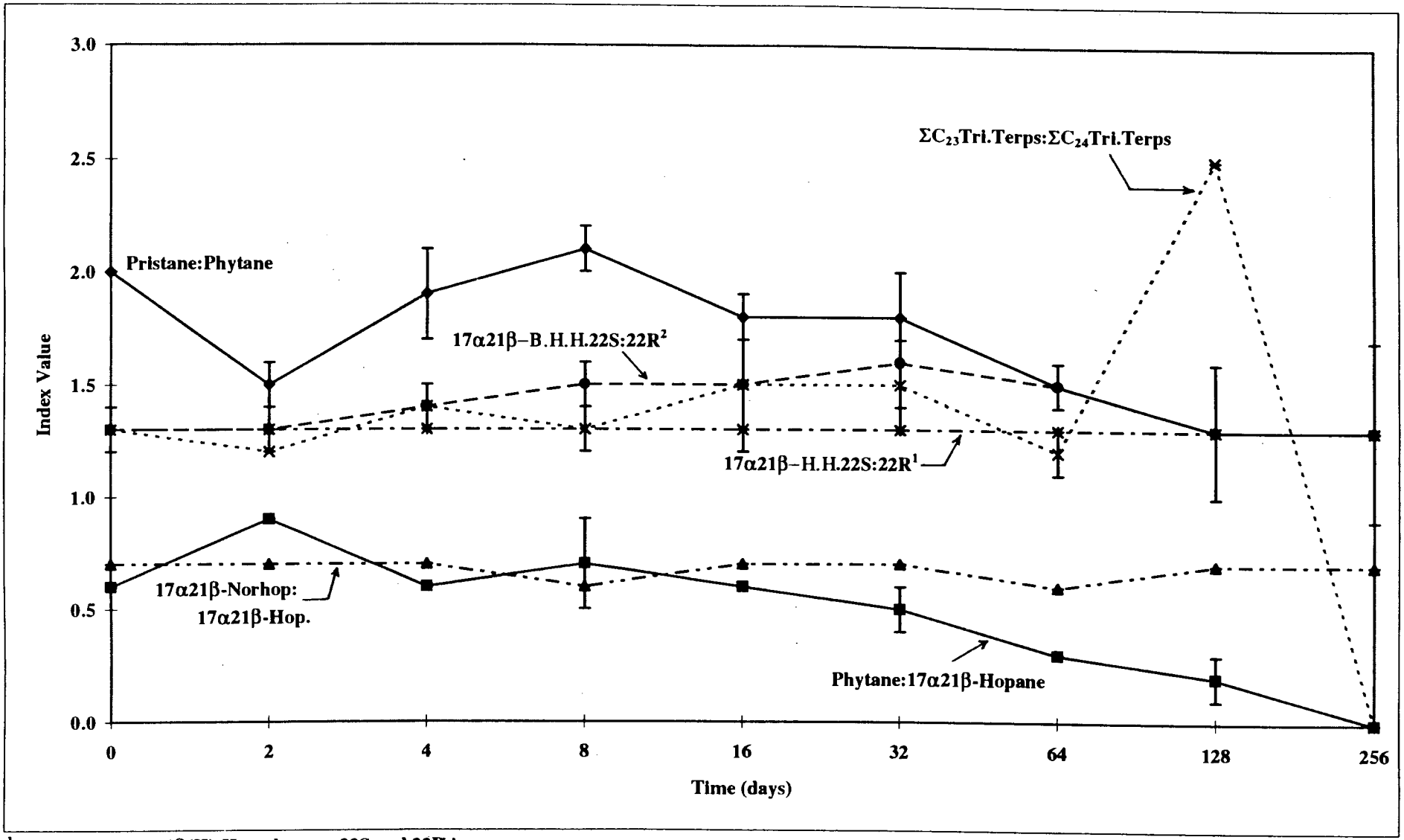


Figure 4.17 (a) Variation in Weathering Index Values for Crude Oil-Treated Soils



[1]Ratio of 17α(H),21β(H)-Homohopane 22S and 22R isomers

[1]Ratio of 17α(H),21β(H)-Bishomohopane 22S and 22R isomers

Figure 4.17 (b) Variation of Source Correlation Index Values for Crude Oil-Treated Soils

Table 4.12 (a) Variation of Weathering Index Values with Time for No.6 Fuel Oil-Treated and Control Soils

WEATHERING INDEX		0 days	2 days	4 days	8 days	16 days	32 days	64 days	128 days	256 days
C_{16}: Norpristane	n1,n2,n3	N/D N/D N/D	6.0 N/D N/D	N/D 2.7 2.9	N/D 2.7 1.9	N/D N/D N/D	N/D N/D N/D	N/D N/D N/D	N/D N/D N/D	N/D 6.6 N/D
	Mean (SD)	N/D	6.0 (N/D)	2.8 (0.1)	1.5 (1.4)					6.6 (N/D)
	Control[1]		N/D	N/D						N/D
C_{17}: Pristane	n1,n2,n3	10.6 N/D N/D	10.8 6.9 N/D	N/D 7.8 7.0	N/D N/D 6.7	9.7 7.3 N/D	9.8 6.2 N/D	10.0 N/D 6.7	4.9 N/D N/D	1.6 4.2 2.1
	Mean (SD)	10.6 (N/D)	8.9 (2.8)	7.4 (0.6)	6.7 (N/D)	8.5 (1.7)	8.0 (2.5)	8.4 (2.4)	4.9 (N/D)	2.6 (1.4)
	Control[1]		11.6	11.0			N/D	6.2		N/D
C_{18}: Phytane	n1,n2,n3	2.7 N/D N/D	3.1 3.4 3.0	N/D 2.9 2.4	3.7 2.0 2.3	3.5 3.4 N/D	3.2 5.8 N/D	2.9 N/D 2.4	1.8 2.7 3.4	0.6 2.3 2.2
	Mean (SD)	2.7 (N/D)	3.2 (0.2)	2.7 (0.3)	2.7 (0.9)	3.4 (0.0)	4.5 (1.9)	2.6 (0.3)	2.6 (0.8)	1.7 (1.0)
	Control[1]		2.5	3.9			N/D	3.6		5.0
$C_{16+18+20}$: $C_{26+28+30}$	n1,n2,n3	2.0 N/D N/D	1.9 1.8 1.9	N/D 1.7 1.7	1.7 1.8 1.8	2.1 2.3 N/D	1.8 2.0 4.0	2.3 N/D 1.4	1.3 1.9 1.7	1.0 2.2 1.8
	Mean (SD)	2.0 (N/D)	1.9 (0.1)	1.7 (0.0)	1.8 (0.0)	2.2 (0.2)	2.6 (1.2)	1.8 (0.6)	1.6 (0.3)	1.7 (0.6)
	Control[1]		3.4	2.2			1.7	3.1		1.4
ΣC_{14-30}: 17α21β-Hopane	n1,n2,n3	81.9 N/D N/D	86.6 92.9 N/D	N/D 82.9 85.2	70.4 N/D 80.6	85.2 N/D N/D	83.6 83.3 N/D	82.5 N/D N/D	37.3 54.3 56.2	1.7 25.2 27.2
	Mean (SD)	81.9 (N/D)	89.8 (4.5)	84.1 (1.7)	75.5 (7.2)	85.2 (N/D)	83.5 (0.2)	82.5 (N/D)	49.3 (10.4)	18.1 (14.2)
	Control[1]		108.6	94.4			93.4	106.8		125.1
ΣC_{14-30}: Tricyclic Terps[2]	n1,n2,n3	2.9 N/D N/D	2.3 2.4 2.4	N/D 2.1 2.1	2.2 2.4 1.8	1.3 1.7 N/D	1.7 1.6 0.1	2.4 N/D 0.2	1.5 2.3 2.5	0.1 2.8 2.6
	Mean (SD)	2.9 (N/D)	2.4 (0.0)	2.1 (0.1)	2.1 (0.3)	1.5 (0.3)	1.1 (0.9)	1.3 (1.5)	2.1 (0.6)	1.8 (1.5)
	Control[1]		2.6	2.1			2.1	3.0		3.7
ΣC_{14-30}: Tetracyclic Terp.[3]	n1,n2,n3	12.2 N/D N/D	8.3 8.4 8.4	N/D 8.0 8.7	7.4 6.7 7.1	5.8 4.9 N/D	5.5 5.9 0.1	8.0 N/D 0.5	3.6 6.6 5.0	0.2 3.2 3.8
	Mean	12.9 (N/D)	8.4 (0.1)	8.3 (0.5)	7.1 (0.4)	5.4 (0.7)	3.8 (3.2)	4.2 (5.3)	5.1 (1.5)	2.4 (1.9)
	Control[1]		9.3	7.4			7.8	10.9		9.5

[1]Control index values determined from single extract only

[2]Sum of C23 (S and R) and C24 (S and R) tricyclic terpane peak areas

[3]C24 Tetracyclic terpane

to rise in value initially and then fall again to just above and just below the start value, respectively.

No.6 Fuel Oil Source Correlation Indices. Because of the low abundance of the higher carbon number hopanes (i.e., the homohopanes, bishomohopanes and methylhopanes) in these samples, only four source correlation indices were determined for the extracts from the No.6 Fuel Oil microcosms. Of these, [pristane:phytane] and [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane] changed least over time, the former varying between 0.3 and 0.6, and the latter between 0.7 and 0.8. The [C_{23}tricyclic terpanes:C_{24}tricyclic terpane] also remained largely the same between 0 and 32 days, at approximately 2.3, but then decreased over the final three sample points to 1.5 ± 0.4 after 256 days. The [phytane:17α(H)21β(H)-hopane] index varied more erratically during the study, increasing from 2.5 ± n/d initially to a maximum of 4.0 ± n/d before decreasing to 1.2 ± 0.9 after 256 days. Results from the control microcosms were very similar to these, in that none of the indices registered significant changes with time. The most consistent indices were [C_{23}tricyclic terpanes:C_{24}tricyclic terpane] and [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane], which varied between 2.4 and 1.8, and 0.7 and 1.2, respectively. The [phytane:17α(H)21β(H)-hopane] again varied most erratically during the study, between 4.6 (2 days) and 1.9 (256 days). Only two values of [pristane:phytane] could be determined, at 2 and 4 days, and these were 0.3 and 0.4, respectively, in close agreement with the values obtained for this index in the treated soils.

Plots comparing the changes in the mean values of No.6 Fuel Oil source and weathering indices in the treated soils and control soils are shown in Figure 4.18 (a) (weathering indices) and (b) (source indices).

The relative sensitivities of the weathering and source indices are shown in summarised form in Figure 4.19 (a) and (b), Figure 4.20 (a) and (b), and Figure 4.21 (a) and (b), which depict, respectively, the overall change in value of each weathering (a) and source

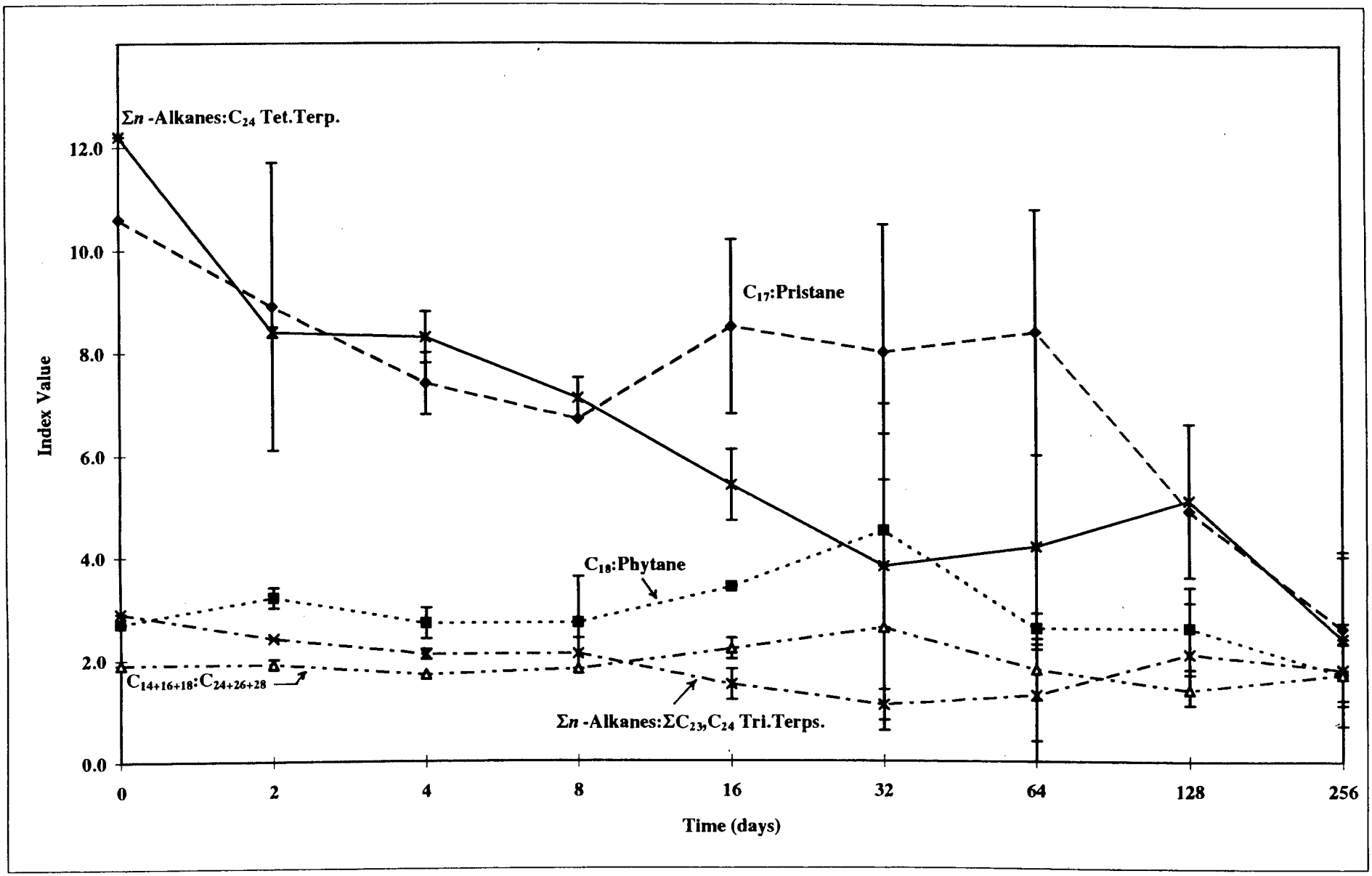


Figure 4.18 (a) Variation in Weathering Index Values for No.6 Fuel Oil-Treated Soils

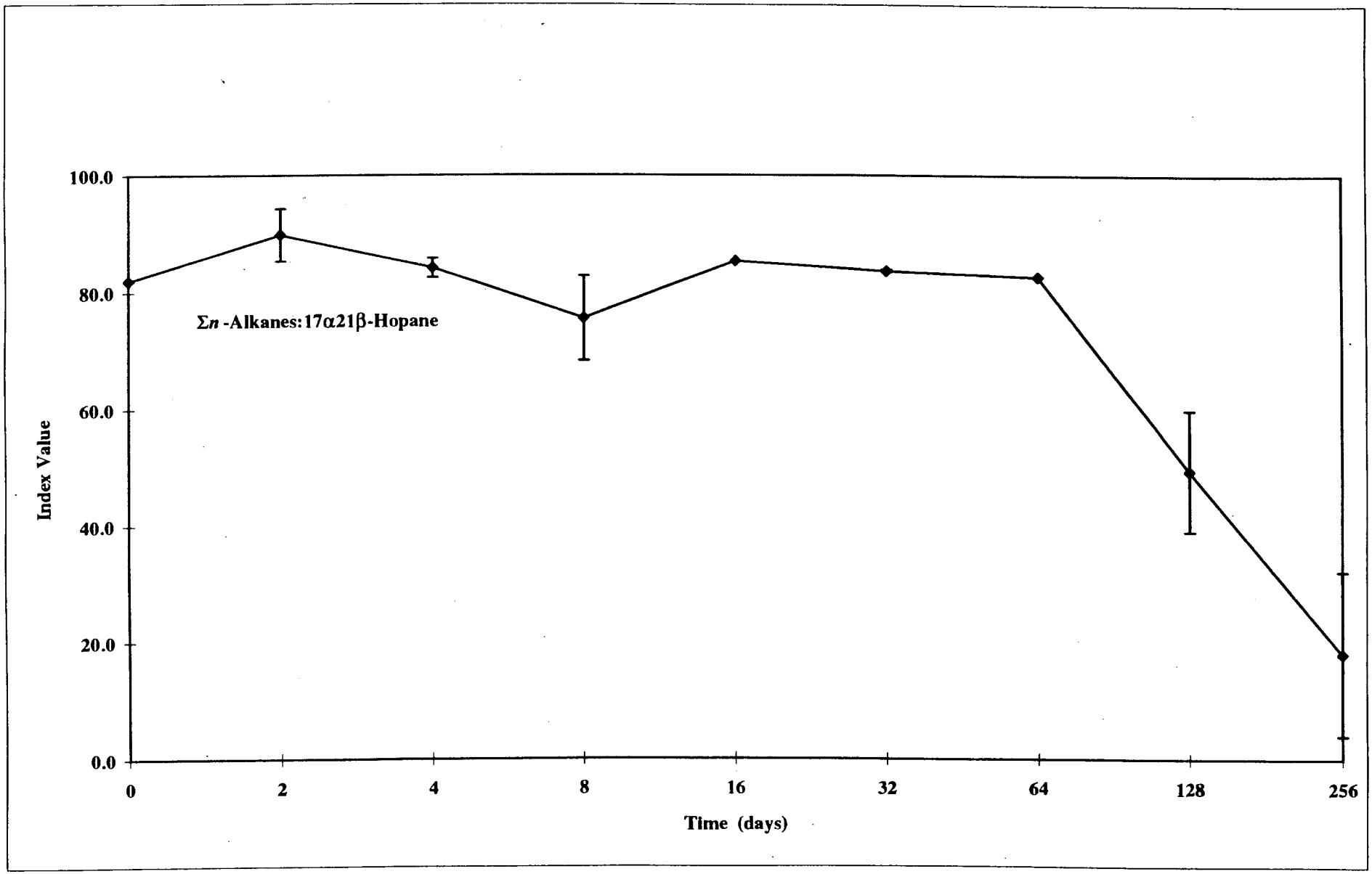


Figure 4.18 (a) Variation in Weathering Index Values for No.6 Fuel Oil-Treated Soils

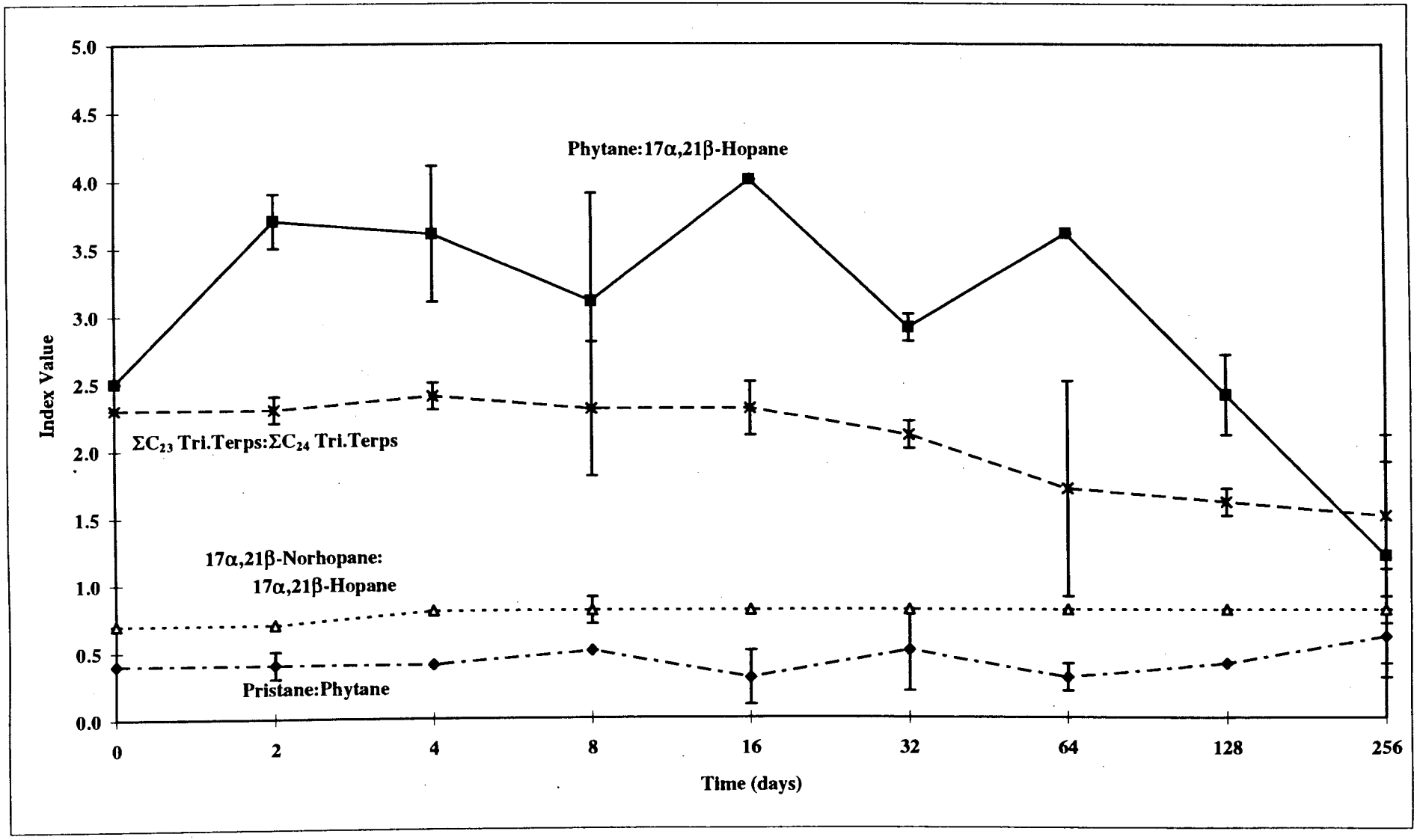


Figure 4.18 (b) Variation of Source Correlation Index Values for No.6 Fuel Oil-Treated Soils

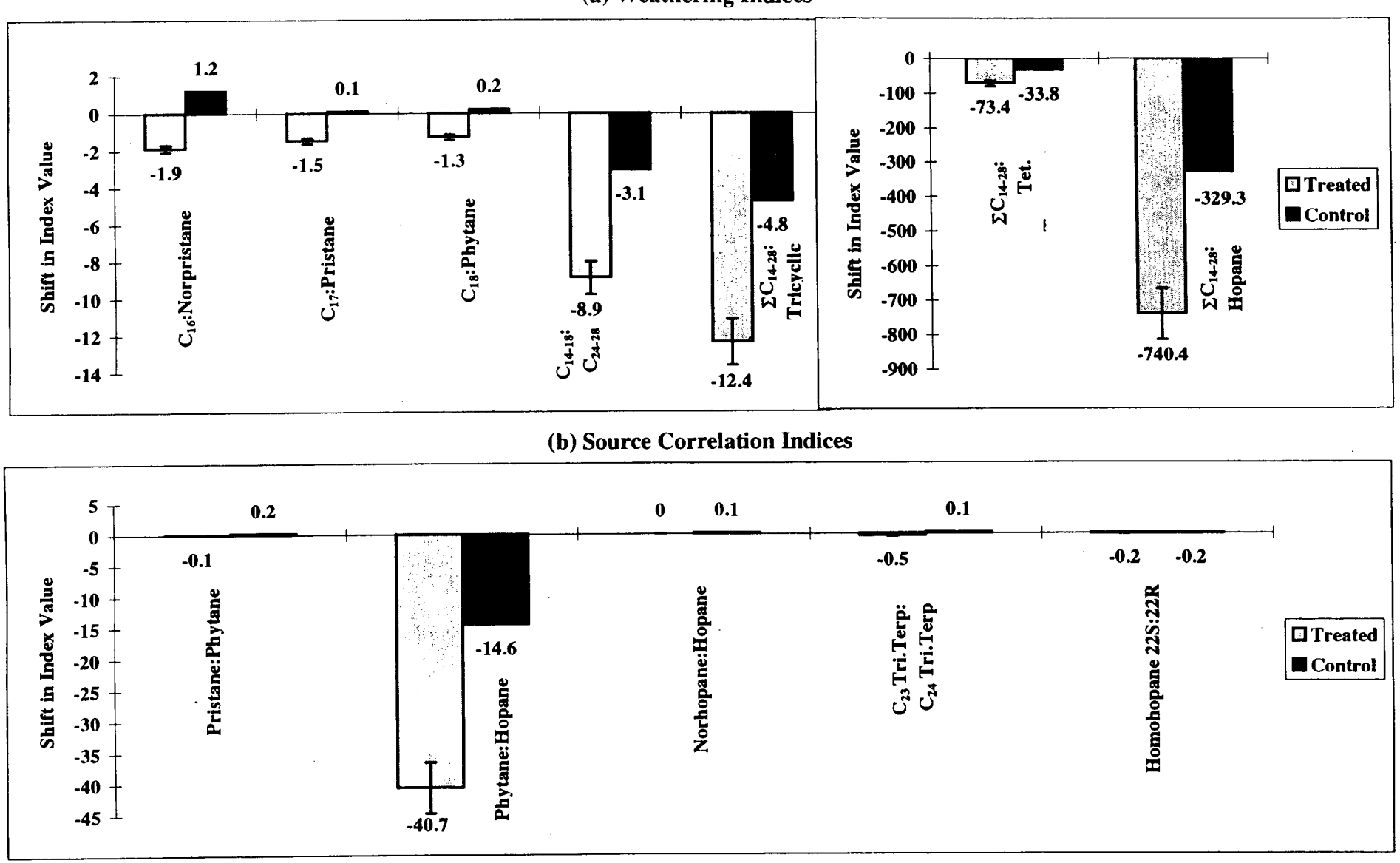


Figure 4.19 Overall Shifts in Value of (a) Weathering Indices and (b) Source Correlation Indices for Ballast Oil Microcosms over 256 Days

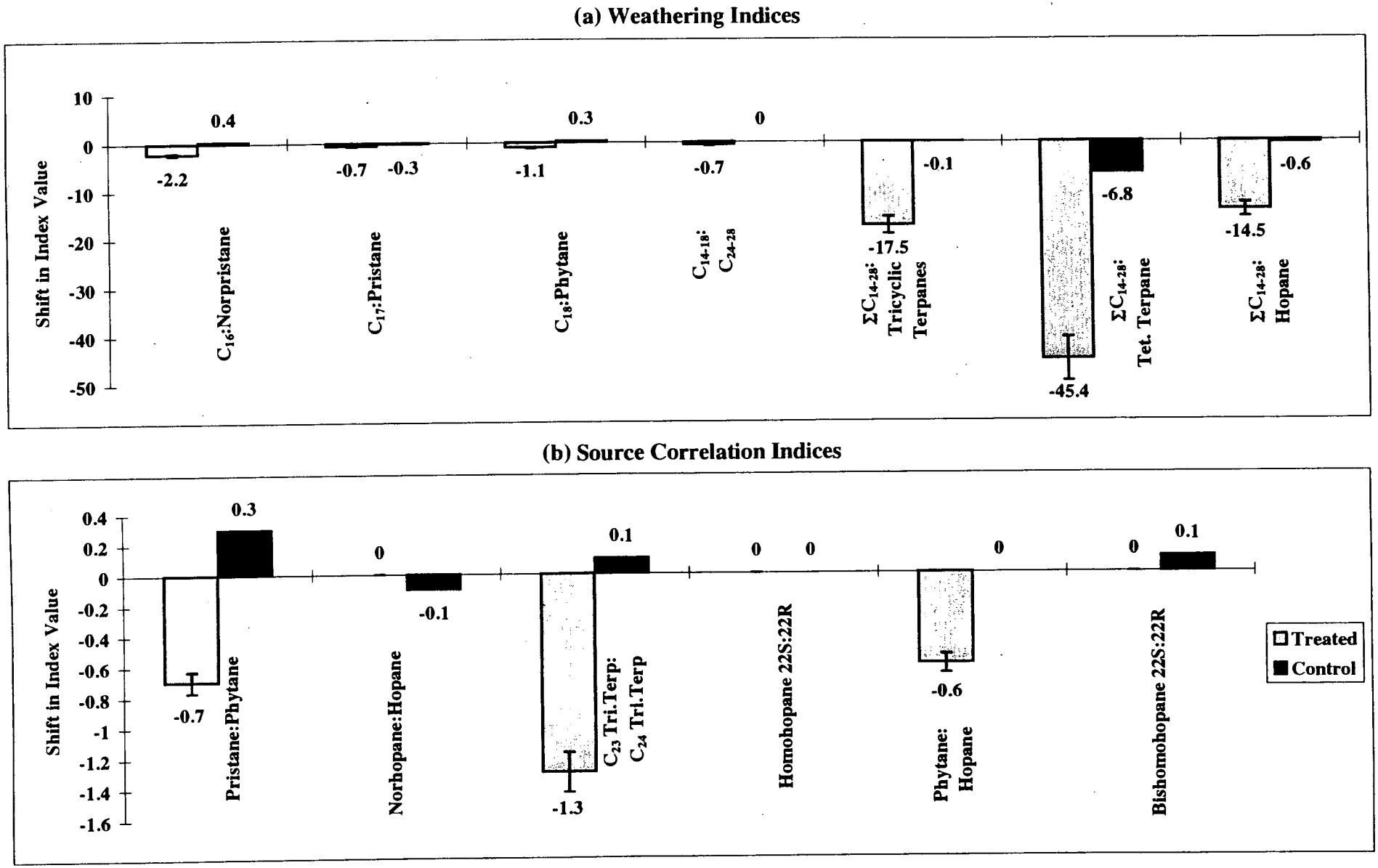


Figure 4.20 Overall Shifts in Value of (a) Weathering Indices and (b) Source Correlation Indices for Crude Oil Microcosms over 256 Days

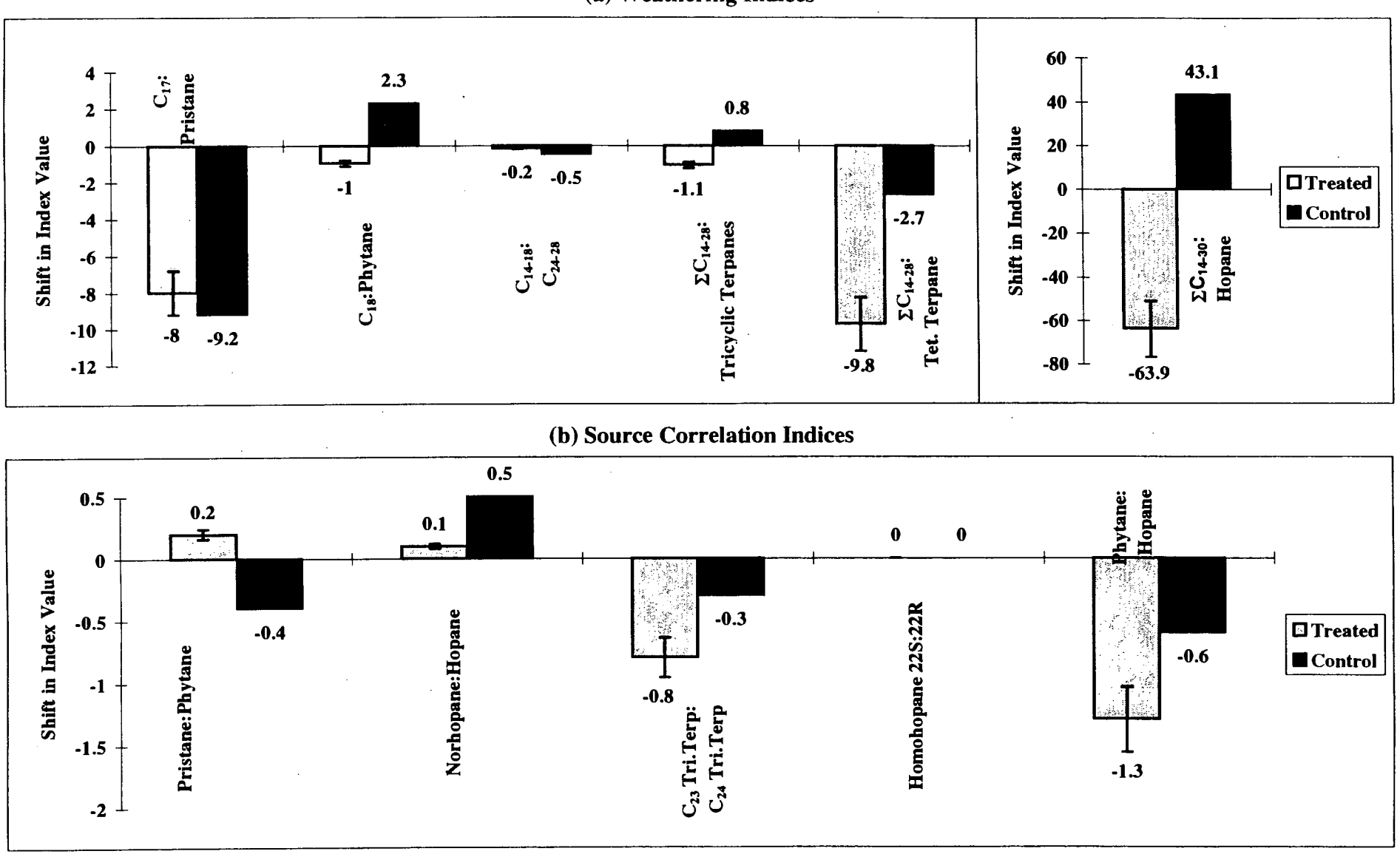


Figure 4.21 Overall Shifts in Value of (a) Weathering Indices and (b) Source Correlation Indices for No.6 Fuel Oil Microcosms over 256 Days

(b) index over the entire 256 days for the ballast oil, crude oil and No.6 Fuel Oil treated and control microcosms.

To test the significance of the variations in the source correlation and weathering indices for each oil over the entire biotransformation study, an analysis of variance (ANOVA) was conducted. For each index, the average of the within-sample variances of the index values at each of the nine sampling points was compared to the overall between-sample variance of the nine mean index values, calculated from the standard deviation of the mean index values from the overall mean index value (i.e., the average of all nine mean values). If the null hypothesis is valid, i.e., if the nine mean values do not differ significantly, then the ratio of the between-sample variance to the within-sample variance (F_{calc}) is less than or equal to the critical value of F. If the mean values do differ significantly (the alternative hypothesis), F_{calc} is larger than the critical value of F and the null hypothesis can be rejected. The critical value of F is determined with reference to the appropriate statistical table, and is dependent upon the number of degrees of freedom of the numerator and denominator in the F_{calc} ratio. To identify the reason for a significant ANOVA result in which the null hypothesis is rejected, the value of the least significant difference (LSD) was determined. The ANOVA test is particularly useful in this case, since it provides a clear statistical argument for distinguishing between the source correlation indices, for which the null hypothesis should be true, and weathering indices, for which the alternative hypothesis should hold.

The results of the ANOVA for each oil are presented in standard format in Table 4.13. For each source and weathering index, the within-sample and between-sample variance, F_{calc} value, critical F value and LSD are provided. Based on the results obtained, the null hypothesis is either rejected or accepted for each index, depending on whether the mean values did or did not vary significantly over the course of the study. The significance of these results is discussed in the next chapter (Section 5.2.1.3), and includes a correlation of the values of selected weathering indices and the amounts of saturates recovered from the respective soil

Table 4.13 Analysis of Variance (ANOVA) of Weathering and Source Correlation Indices from Soil Microcosms

Weathering Indices	BALLAST OIL					CRUDE OIL					No.6 FUEL OIL				
	Mean Individ. Variance[1] (σ^2_1) / Overall Variance[2] (σ^2_2) (F_{calc})	D of F[3]	F_{test}[4]	Significant Variation ?	LSD[5]	Mean Individ. Variance[1] (σ^2_1) / Overall Variance[2] (σ^2_2) (F_{calc})	D of F[3]	F_{test}[4]	Significant Variation ?	LSD[5]	Mean Individ. Variance[1] (σ^2_1) / Overall Variance[2] (σ^2_2) (F_{calc})	D of F[3]	F_{test}[4]	Significant Variation ?	LSD[5]
C_{16}: Norpristane	2.51	8,14	2.69	NO	0.49	1.80	7,13	2.83	NO	0.37	0.95	2,3	9.55	NO	2.46
C_{17}: Pristane	4.18	8,17	2.55	YES	0.24	1.22	8,15	2.64	NO	0.33	2.04	8,7	3.73	NO	2.01
C_{18}: Phytane	5.40	8,17	2.55	YES	0.17	5.34	8,15	2.64	YES	0.25	0.86	8,12	2.85	NO	1.00
$C_{14+16+18}$: $C_{24+26+28}$	17.30	8,17	2.55	YES	0.80	15.26	8,15	2.64	YES	0.08	0.33	7,14	2.76	NO	0.63
ΣC_{14-28}: 17α21β-Hopane	13.84	8,10	3.07	YES	70.60	34.72	8,15	2.64	YES	1.26	12.88	8,8	3.44	YES	7.98
ΣC_{14-28}: Tricyclic Terps	0.91	8,17	2.55	NO	5.09	2.24	7,16	2.66	NO	8.49	0.50	8,13	2.77	NO	0.99
ΣC_{14-28}: Tetracyclic Terp.	2.98	8,17	2.55	YES	15.72	9.30	8,14	2.70	YES	9.33	1.76	8,13	2.77	NO	2.74
Source Indices															
Pristane: Phytane	1.18	8,17	2.55	NO	0.15	2.07	8,15	2.64	NO	0.24	0.47	8,7	3.73	NO	0.15
Phytane: 17α21β-Hopane	8.53	8,10	3.07	YES	6.88	15.91	8,15	2.64	YES	0.08	2.47	8,8	3.44	NO	0.68
17α21β-Norhopane: 17α21β-Hopane	3.86	8,10	3.07	YES	0.03	2.07	8,15	2.64	NO	0.03	0.78	7,7	3.77	NO	0.05
C23 (S,R) Tri.Ts.:[2] C24 (S,R) Tri.Ts.	18.91	8,17	2.55	YES	0.05	20.11	7,15	2.70	YES	0.12	0.95	8,14	2.70	NO	0.44
17α21β-Homohop. (22S):[3] 17α21β-Homohop. (22R)	0.65	5,5	5.05	N0	0.10	0.62	8,15	2.64	NO	0.04	-	-	-	-	-
17α21β-Bishomohop. (22S):[4] 17α21β-Bishomohop. (22R)	-	-	-	-	-	0.54	8,15	2.64	NO	0.16					

[1]Mean variance of index values at each sampling point
[2]Overall variance between sampling points
[3]Number of degrees of freedom σ^2_1 and σ^2_2, respectively
[4]Critical F value from statistical tables
[5]Least significant difference between index values

microcosms (i.e., a comparative assessment of the capacity of different weathering indices to "track" the depletion of saturates from the different oils), and a quantitative assessment of the effectiveness of the respective source correlation indices.

4.2.1.4 GC-IRMS Analysis

Compound specific isotope analysis of the microcosm extracts provided the $\delta^{13}C$ of the *n*-alkanes C_{14} (for the ballast oil only), C_{16}, C_{17}, C_{18}, C_{24} and C_{26}, and the isoprenoid alkane norpristane (iC_{18}) over the 256-day study. The pristane and phytane peaks were not sufficiently resolved to facilitate reliable evaluation of their respective isotope ratios. 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 given in Tables 4.14, 4.15 and 4.16, respectively.

Ballast Oil. In general, the $\delta^{13}C$ values for the *n*-alkanes did not 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 $^{o}/_{oo}$ after 2 days and -28.4 (n = 1) $^{o}/_{oo}$ 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 more clearly in Figure 4.22. In the ballast oil control flasks, the results were much the same, with no recognisable variations in isotopic composition detected in any of the compounds during the study.

Crude Oil. In the crude oil extracts, the isotopic composition of the five *n*-alkanes (C_{16}, 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. The shift was greatest for the C_{16}, C_{17}, C_{18}, C_{24} alkanes, which experienced falls in their $\delta^{13}C$ of between 2 $^{o}/_{oo}$ and 3 $^{o}/_{oo}$ over the 256 days. However, as demonstrated in Figure 4.23, which shows the variations of the mean isotope ratios of each of the compounds with time, this increase is not a smooth one, but

Table 4.14 Variation in Isotopic Composition of Individual Compounds in Ballast Oil-Treated and Control Soils (‰)

TIME	C_{14}						C_{17}					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-29.16			-29.16	N/D		-29.23	-29.21	-29.84	-29.43	0.36	
2	-29.14	-32.05	-31.26	-30.82	1.50	-28.75	-29.84	-29.47	-30.27	-29.86	0.40	-29.65
4	-29.09		-28.83	-28.96	0.18	-28.98		-29.69	-29.83	-29.76	0.10	-29.69
8	-27.35			-27.35	N/D	-29.55		-29.46	-29.96	-29.71	0.00	-29.74
16	-29.26	-28.77		-29.02	0.35		-29.68	-29.59	-30.21	-29.83	0.34	
32		-28.37	-28.45	-28.41	0.06		-29.76	-29.32	-29.21	-29.43	0.29	
64						N/D						-30.04
128			-28.44	-28.44	N/D	N/D		-29.30	-30.01	-29.66	0.50	-30.15
256						-29.14	-30.14	-29.83	-29.30	-29.76	0.42	-29.14

TIME	C_{18}						C_{24}					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-29.02	-28.95	-29.23	-29.07	0.15		-29.13	-28.76	-29.49	-29.13	0.37	
2	-29.84	-29.59	-29.51	-29.65	0.17	-29.55	-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	-32.49	-29.40	-29.30	-30.40	1.81	-29.21
8		-29.64	-29.99	-29.82	0.25	-29.72	-29.22		-29.37	-29.30	0.11	-29.42
16	-29.65	-29.54	-30.07	-29.75	0.28		-29.25	-28.86	-29.79	-29.30	0.47	
32	-29.74	-29.17	-29.17	-29.36	0.33		-29.51	-28.88	-28.64	-29.01	0.45	
64		-27.73		-27.73	N/D	-29.78			-28.87	-28.87	N/D	-29.11
128	-29.01	-29.98		-29.50	0.69	-29.96	-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	-29.24	-29.35		-29.30	0.08	-28.70

TIME	C_{26}						Norpristane					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-29.37	-28.86	-29.17	-29.13	0.26		-29.01	-28.57		-28.79	0.31	
2	-30.00	-30.58	-30.51	-30.36	0.32	-28.92	-28.92	-28.65	-28.90	-28.82	0.15	-28.73
4	-32.60	-29.51	-29.49	-30.53	1.79	-28.96	-28.57	-28.68		-28.63	0.08	-28.96
8	-29.32			-29.32	N/D	N/D	-28.42	-28.39	-28.87	-28.56	0.27	-28.81
16	-29.52			-29.52	N/D		-28.57	-28.48	-29.10	-28.72	0.34	
32							-28.45	-27.89		-28.17	0.40	
64			-28.89	-28.89	N/D	-28.76			-28.44	-28.44	N/D	N/D
128	-28.19	-29.35	-29.06	-28.87	0.60	-29.10		-28.48	-28.48	-28.48	0.00	-28.72
256	-28.97	-29.21		-29.09	0.17	-28.95	-29.21	-28.92		-29.07	0.21	-28.22

Table 4.15 Variation in Isotopic Composition of Individual Compounds in Crude Oil-Treated and Control Soils (‰)

TIME	C_{16}						C_{17}					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-28.32	-28.57		-28.45	0.18		-28.35	-28.60		-28.48	0.18	
2	-27.96	-28.88		-28.42	0.65	-27.39	-28.29			-28.29	N/D	-27.35
4	-28.27	-28.58	-28.63	-28.49	0.20	-27.11	-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		-27.18	-27.33	-27.26	0.11	-27.12
16	-27.21	-27.40	-27.79	-27.47	0.30		-27.26	-27.40	-27.45	-27.37	0.10	
32	-27.71	-28.20	-29.34	-28.42	0.84		-27.22	-28.04	-28.53	-27.93	0.66	
64	-29.72	-30.34	-31.04	-30.37	0.66	N/D	-29.92	-29.99	-30.58	-30.16	0.36	N/D
128	-28.75	-29.07	-26.36	-28.06	1.48	N/D	-28.55	-29.10	-27.32	-28.32	0.91	N/D
256		-27.27	-26.72	-27.00	0.39	-26.91	-27.05	-26.84	-26.48	-26.79	0.29	-27.08

TIME	C_{18}						C_{24}					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-28.46	-28.43		-28.45	0.02		-28.68	-28.25		-28.47	0.30	
2	-27.47	-28.27		-27.87	0.57	-27.08	-28.67	-28.88		-28.78	0.15	-27.32
4	-27.88	-28.08	-28.43	-28.13	0.28	-27.28	-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	-27.11	-27.65	-27.41	-27.39	0.27	-26.96
16	-26.98	-27.55	-27.56	-27.36	0.33		-27.32	-27.73	-27.68	-27.58	0.22	
32	-27.05	-27.78	-28.38	-27.74	0.67		-27.73	-28.07	-28.90	-28.23	0.60	
64	-28.97	-29.93	-30.52	-29.81	0.78	N/D	-29.04	-29.90	-30.77	-29.90	0.87	N/D
128	-28.11	-28.93	-27.03	-28.02	0.95	N/D	-28.45	-28.97	-26.85	-28.09	1.10	N/D
256	-26.90	-26.54	-26.27	-26.57	0.32	-27.11		-26.78	-26.29	-26.54	0.35	-27.21

TIME	C_{26}						Norpristane					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-28.65	-28.74		-28.70	0.06		-27.80	-27.90		-27.85	0.07	
2	-29.53	-29.54		-29.54	0.01	-27.59	-28.13	-28.94		-28.54	0.57	-27.04
4	-28.62	-28.52	-30.02	-29.05	0.84	-27.65	-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	-27.09	-27.73	-27.61	-27.48	0.34	-26.61
16	-27.69	-28.21	-28.00	-27.97	0.26		-27.41	-27.41	-27.92	-27.58	0.29	
32	-28.34	-28.41	-29.11	-28.62	0.43		-27.72	-28.21	-29.02	-28.32	0.66	
64	-29.29	-29.81	-30.19	-29.76	0.45	N/D	-29.92	-30.39	-31.27	-30.53	0.69	N/D
128	-28.69	-29.06	-27.08	-28.28	1.05	N/D	-28.61		-26.46	-27.54	1.52	N/D
256						-27.44						-27.24

Table 4.16 Variation in Isotopic Composition of Individual Compounds in No.6 Fuel Oil-Treated and Control Soils (‰)

TIME	C_{16}						C_{17}					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-27.15	-27.25	-26.60	-27.00	0.35		-27.46	-26.3		-26.88	0.82	
2	-28.25		-27.29	-27.77	0.68	N/D	-28.17	-26.97	-27.51	-27.55	0.60	-28.37
4		-27.48		-27.48	0.00	N/D	-27.88	-27.67		-27.78	0.15	-27.68
8	-26.97	-27.14		-27.06	0.12	-27.39	-27.27	-27.45		-27.36	0.13	-27.59
16	-27.91	-27.92		-27.92	0.01		-27.99	-28.17		-28.08	0.13	
32	-26.89	-28.26		-27.58	0.97		-27.57	-27.89		-27.73	0.23	
64	-27.80	-28.47		-28.14	0.47	N/D	-28.07	-28.28		-28.18	0.15	N/D
128	-27.04	-27.25	-27.13	-27.14	0.11	N/D	-27.32	-27.49	-27.54	-27.45	0.12	N/D
256			-27.16	-27.16	N/D	-26.91		-28.31	-27.52	-27.92	0.56	-27.64

TIME	C_{18}						C_{24}					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-28.61	-27.33	-27.79	-27.91	0.50		-27.75	-27.38		-27.57	0.26	
2	-27.89	-26.89	-27.32	-27.37	0.50	-27.80		-27.24	-27.90	-27.57	0.47	N/D
4	-27.07	-27.40		-27.24	0.23	-27.48	-28.19	-27.97		-28.08	0.16	N/D
8	-27.05	-27.44		-27.25	0.28	-27.44		-27.61	-27.51	-27.56	0.07	-27.82
16	-27.97	-28.06		-28.02	0.06		-28.06	-28.13		-28.10	0.05	
32	-27.24	-27.84	-27.33	-27.47	0.32		-27.41	-27.85	-27.35	-27.54	0.27	
64	-28.06	-28.12		-28.09	0.04	N/D	-27.72	-27.85		-27.79	0.09	N/D
128	-27.32	-27.93	-27.56	-27.60	0.31	N/D	-27.48	-27.75	-27.82	-27.68	0.18	N/D
256		-28.31	-27.43	-27.87	0.62	-27.60			-27.60	-27.60	N/D	-27.37

TIME	C_{26}						Norpristane					
	n1	n2	n3	Mean $\delta^{13}C$	SD	Control	n1	n2	n3	Mean $\delta^{13}C$	SD	Control
0	-27.74	-27.54		-27.64	0.14		-28.01	-27.35	-27.53	-27.63	0.34	
2		-27.28	-27.63	-27.46	0.25	N/D	-28.00	-26.97	-27.31	-27.43	0.52	N/D
4		-27.83		-27.83	N/D	N/D	-27.41	-27.52		-27.47	0.08	N/D
8	-27.51	-27.27		-27.39	0.17	-27.71	-27.06	-27.15		-27.11	0.06	-27.20
16	-28.24	-26.69		-27.47	1.10		-27.41	-27.65		-27.53	0.17	
32	-27.34	-28.11	-27.58	-27.68	0.39		-27.17	-27.53	-27.71	-27.47	0.27	
64	-27.76	-27.93		-27.85	0.12	N/D	-27.56	-27.38		-27.47	0.13	N/D
128	-27.24	-27.65	-27.60	-27.50	0.22	N/D	-27.24	-27.26	-27.19	-27.23	0.04	N/D
256			-27.73	-27.73	N/D	-27.53	-26.87		-27.12	-27.00	0.18	-26.96

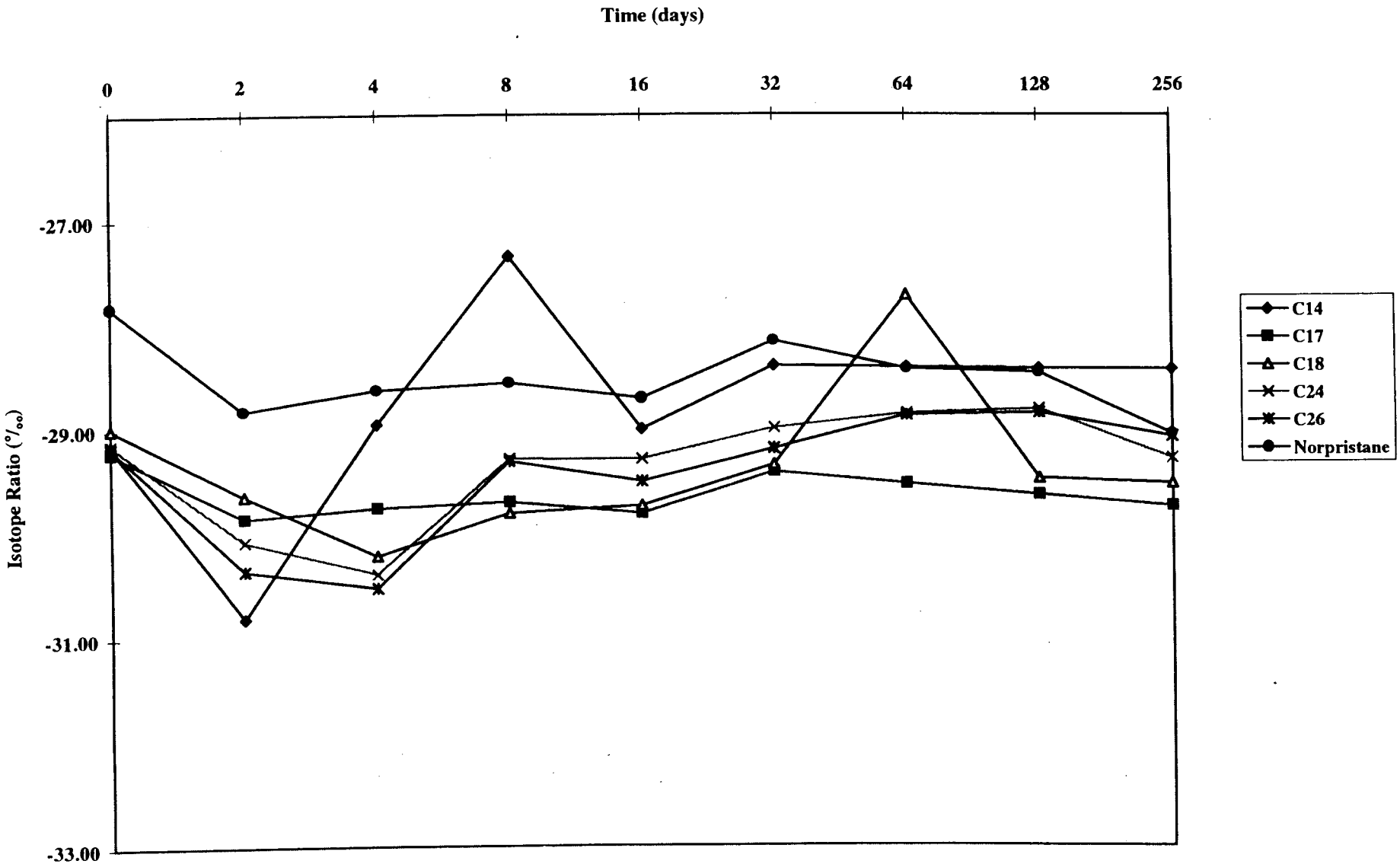


Figure 4.22 Variation in *n*-Alkane and Norpristane Isotope Ratios for Ballast Oil Microcosms (error bars omitted for reasons of clarity, but given in Table 4.14)

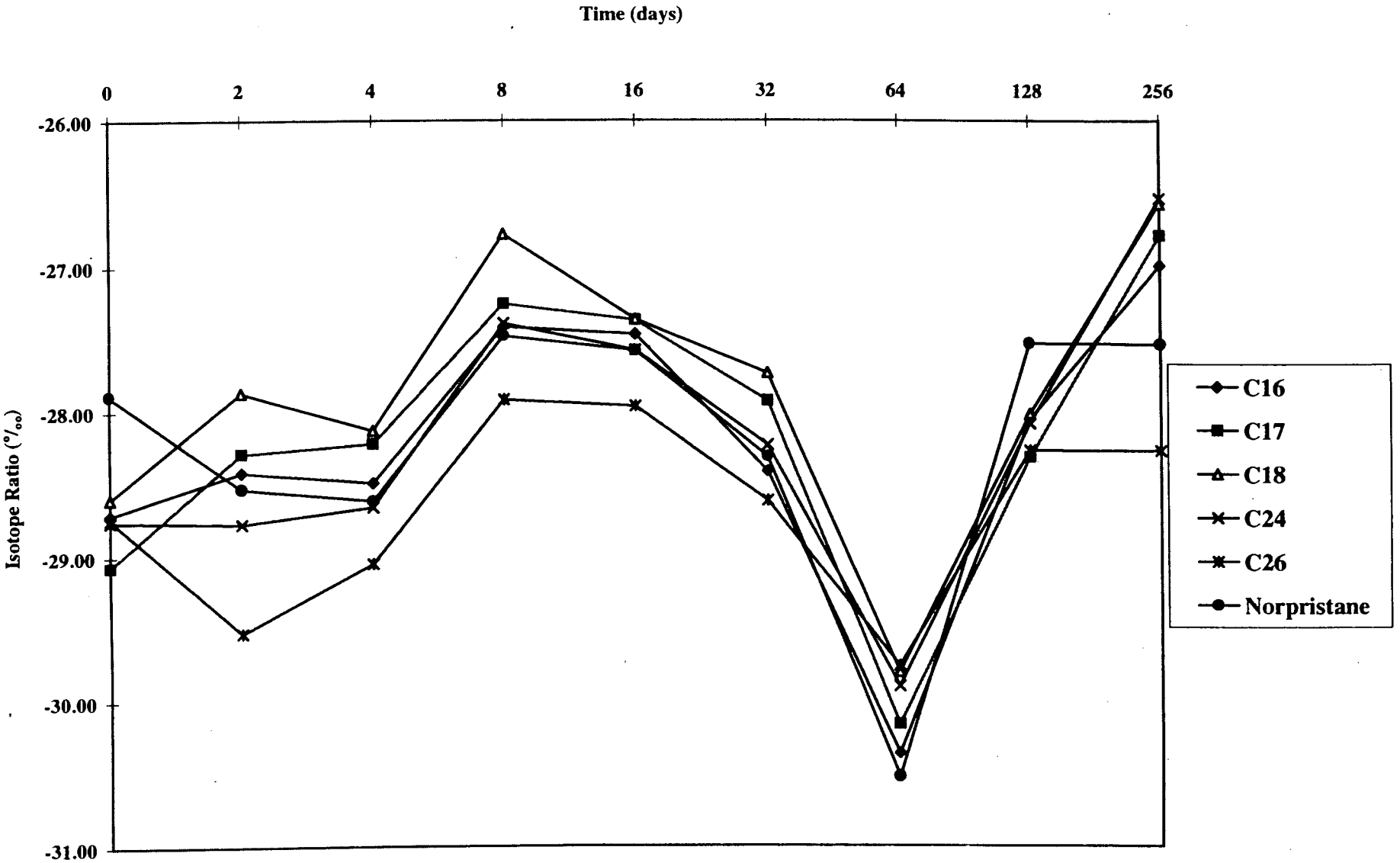


Figure 4.23 Variation in *n*-Alkane and Norpristane Isotope Ratios for Crude Oil Microcosms (error bars omitted for reasons of clarity, but given in Table 4.15)

the overall result of a series of increases and decreases between the sample points. The isotope ratios of C_{26} and norpristane also increased with increased oil weathering, but the magnitude of the shift was not outwith analytical imprecision. However, the $\delta^{13}C$ of these compounds could not be determined at 256 days, when the greatest isotopic shifts would be expected. Isotope ratios of the compounds extracted from the control microcosms did not vary significantly during the study.

No.6 Fuel Oil. The individual compounds from within the No.6 Fuel Oil 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 $^{o}/_{oo}$ and -28.2 $^{o}/_{oo}$. A plot of compound $\delta^{13}C$ variation with time, shown in Figure 4.24, indicates that the isotope ratios of the *n*-alkanes oscillated within the specified range, alternately increasing and decreasing between successive sampling points. The isoprenoid $\delta^{13}C$ did not appear to undergo these fluctuations. As expected, in the No.6 Fuel Oil control microcosms, the isotope ratios of the *n*-alkanes and isoprenoid did not alter significantly over the course of the study.

4.2.2 Weathered Diesel Range Organics (DRO) Standards

The diesel standards used in the investigation of physical oil weathering were supplied with details both of the procedure adopted by the manufacturers to effect DRO weathering (described in Section 3.2.3) and the results of GC-FID analysis of the fresh, 25 % $^{w}/_{w}$ and 50 % $^{w}/_{w}$ weathered samples. These demonstrated the loss of lower boiling *n*-alkanes and the increase in UCM in the more weathered samples. Analysis of these samples in this study comprised GC-EI MS (SIM) and GC-IRMS.

4.2.2.1 GC-EI MS Analysis

Qualitative Analysis. Inspection of the TICs and m/z 85 and 191 chromatograms for the fresh, 25 % $^{w}/_{w}$ and 50 % $^{w}/_{w}$ weathered DRO samples (TICs shown in Appendix A) reveal the compositional differences between the samples: in the fresh DRO samples, a spread of

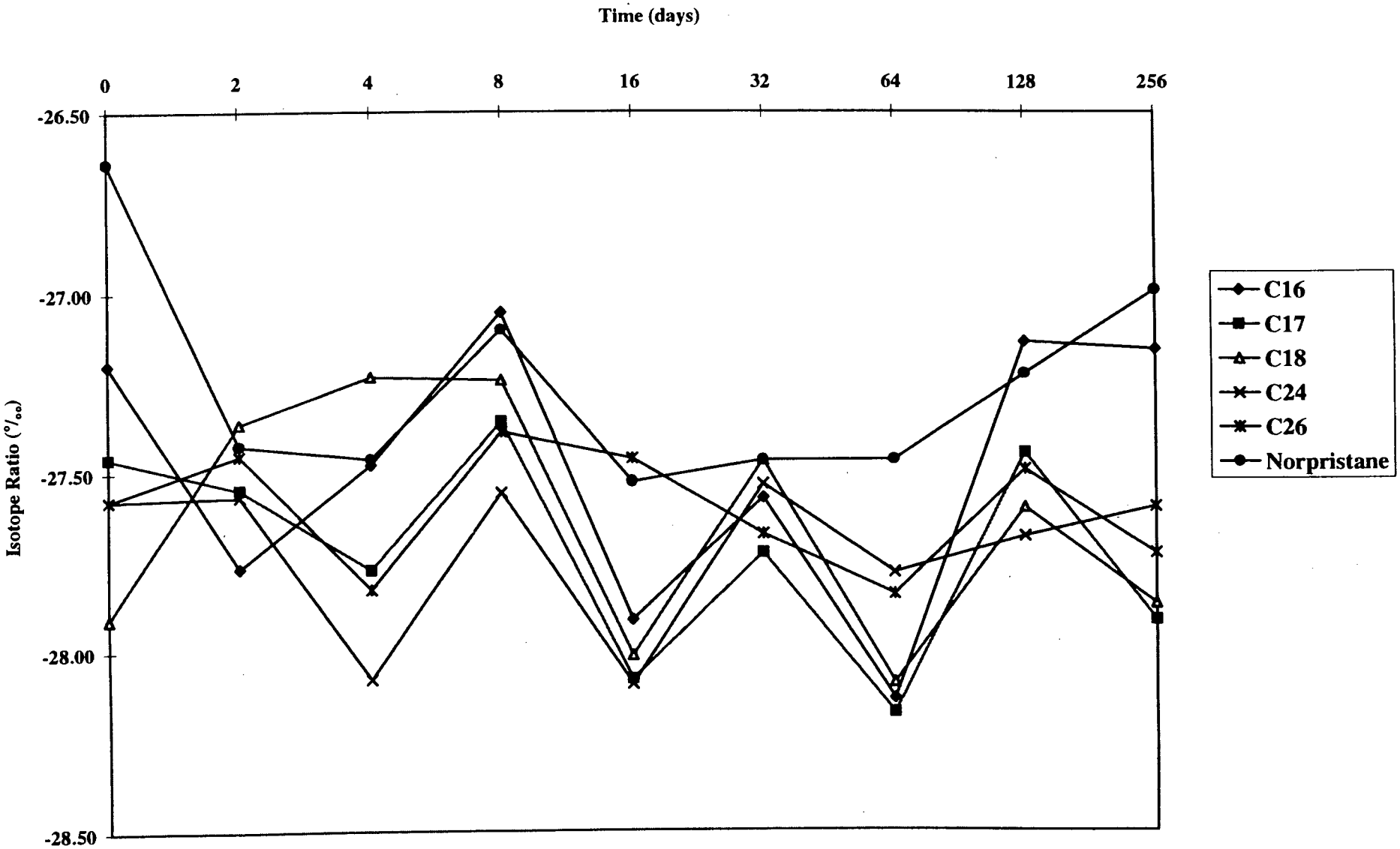


Figure 4.24 Variation in *n*-Alkane and Norpristane Isotope Ratios for No.6 Fuel Oil Microcosms (error bars omitted for reasons of clarity, but given in Table 4.16)

peaks corresponding to the *n*-alkanes from C_9 to C_{26} and centred on approximately C_{16} can be clearly seen, with isoprenoid peaks also prominent. The 25 % $^{w}/_{w}$ weathered DRO sample produced a narrower total ion chromatogram, centred upon the C_{16} and C_{17} peaks, displaying prominent peaks for *n*-alkanes from C_{13} to C_{22}, with a series of smaller peaks corresponding to *n*-alkanes with carbon number C_{23} and higher at the tail end of the chromatogram. Isoprenoid peaks in this sample were less pronounced than those in the fresh sample. In the 50 % $^{w}/_{w}$ weathered sample, TIC peaks were obtained for the range of *n*-alkanes between C_{15} and C_{26}, although the distribution of the peaks did not display the same characteristic shape as those obtained for the fresh and 25 % $^{w}/_{w}$ weathered samples, due to the low abundance of the alkanes in this sample. The m/z 191 chromatograms of the three samples were almost identical, consisting of two tightly grouped clusters of peaks corresponding to the tricyclic terpanes in the sample. Magnification of these groups, through MS software manipulation, showed that the distribution of peaks was the same in each sample.

Quantitative Analysis. Four weathering indices and three source correlation indices were evaluated for the standard weathered diesel range organics (DRO) samples. Results are shown in Table 4.17.

The weathering indices found to be least sensitive to physical weathering, i.e., those that varied least in value between the respective DRO samples, were [C_{18}:phytane] and [C_{17}:pristane]. In this study, results indicate that both indices remained unaltered between the fresh and the 25 % $^{w}/_{w}$ weathered DRO samples, at 2.1 ± 0.2 and 1.9 ± 0.2, respectively. For the 50 % $^{w}/_{w}$ weathered DRO, the [C_{17}:pristane] index was found to increase in value to 2.5 ± 0.3; [C_{18}:phytane] for the 50 % $^{w}/_{w}$ weathered DRO decreased to 1.4 ± 0.1. For the weathering indices in which tricyclic terpanes are used as the conserved marker species, the [*n*-alkanes:tricyclic terpanes] ratio was found to be the most sensitive, decreasing from 20.4 in the fresh DRO sample, to 11.4 ± 1.1 in the 25 % $^{w}/_{w}$ weathered sample and 4.0 ± 0.4 in the 50 % $^{w}/_{w}$ weathered sample. The [C_{18}:tricyclic terpanes] weathering index also changed significantly

Biomarker Ratio[1]	m/z ratio	Empirical formula and MW	DRO Samples					
			Unweathered		25 % Weathered		50 % Weathered	
			Integrated Peak Areas	Index[2] Value	Integrated Peak Areas	Index[2] Value	Integrated Peak Areas	Index[2] Value
Weathering Indices								
C_{17}:pristane	85,85	$C_{17}H_{36}$ (240),$C_{19}H_{40}$ (268)	$^{1324320}/_{712924}$	1.9	$^{1639220}/_{868378}$	1.9	$^{1194220}/_{476244}$	2.5
C_{18}:phytane	85,85	$C_{18}H_{38}$ (294),$C_{20}H_{42}$ (282)	$^{1092420}/_{528977}$	2.1	$^{1392010}/_{676104}$	2.1	$^{1081590}/_{778478}$	1.4
C_{18}:tricyclic terpanes	85,191	$C_{18}H_{38}$ (294),$C_{20\text{-}26}H_{36\text{-}48}$ (276-360)	$^{1092420}/_{526813}$	2.1	$^{1392010}/_{947682}$	1.5	$^{1081590}/_{2060559}$	0.5
n-alkanes:tricyclic terpanes	85,191	$C_{10\text{-}22}H_{22\text{-}46}$ (142-310), $C_{20\text{-}26}H_{36\text{-}48}$ (276-360)	$^{10744878}/_{526813}$	20.4	$^{10841482}/_{947682}$	11.4	$^{8036180}/_{2060559}$	3.9
Source Indices								
pristane:phytane	85,85	$C_{19}H_{40}$ (268),$C_{20}H_{42}$ (282)	$^{712924}/_{528977}$	1.4	$^{868378}/_{676104}$	1.3	$^{476244}/_{778478}$	0.6
pristane:tricyclic terpanes	85,191	$C_{19}H_{40}$ (268),$C_{20\text{-}26}H_{36\text{-}48}$ (276-360)	$^{712924}/_{526813}$	1.4	$^{868378}/_{947682}$	0.9	$^{476244}/_{2060559}$	0.2
phytane:tricylic terpanes	85,191	$C_{20}H_{42}$ (282),$C_{20\text{-}26}H_{36\text{-}48}$ (276-360)	$^{528977}/_{526813}$	1.0	$^{676104}/_{947682}$	0.7	$^{778478}/_{2060559}$	0.4

[1] Indices are categorised according to previous usage
[2] Confidence limits: ± 10 %

with DRO weathering, decreasing from 2.1 ± 0.2 in the fresh sample, to 1.5 ± 0.2 in the 25 % $^{w}/_{w}$ weathered sample and 0.5 ± 0.1 in the 50 % $^{w}/_{w}$ weathered sample.

The values of the three proposed source correlation indices, [pristane:phytane], [pristane:tricyclic terpanes] and [phytane:tricyclic terpanes], for DRO samples at each stage of weathering are given in Table 4.17. The former index was found to remain almost unchanged between the fresh and the 25 % $^{w}/_{w}$ weathered samples, at 1.4 ± 0.1 and 1.3 ± 0.1. For the 50 % $^{w}/_{w}$ weathered sample, its value decreased to 0.6 ± 0.1. The [pristane:tricyclic terpanes] and [phytane:tricyclic terpanes] indices both decreased with increased DRO weathering: values of [pristane:tricyclic terpanes] for the fresh, 25 % $^{w}/_{w}$ weathered and 50 % $^{w}/_{w}$ weathered DRO samples were 1.0 ± 0.1, 0.7 ± 0.1 and 0.4 ± 0.0, respectively, whereas those for [phytane:tricyclic terpanes] changed from 1.4 ± 0.1 to 0.9 ± 0.1 to 0.2 ± 0.0 with increased DRO weathering.

4.2.2.2 GC-IRMS Analysis

Stable carbon isotope ratios for the individual *n*-alkanes and isoprenoids norpristane, pristane and phytane in the three DRO samples are presented in Table 4.18. The $\delta^{13}C$ values presented are the means of up to three repeats, and the standard deviation of each mean is also provided, where >1 value was obtained. Within the fresh DRO sample, $\delta^{13}C$ values appeared to decrease (i.e., become more negative) with increasing carbon number. This can be seen more clearly in Figure 4.25, which shows the isotopic fingerprint of the fresh DRO *n*-alkanes and isoprenoids. The C_{12-18} *n*-alkanes displayed isotope ratios that varied between 24 $^{o}/_{oo}$ and 26 $^{o}/_{oo}$. The high $\delta^{13}C$ of C_{17} is more than likely an anomaly, as the standard deviation of this measurement is relatively high. For the higher carbon number alkanes, the $\delta^{13}C$ values are very similar, and consistently between -26 $^{o}/_{oo}$ and -27 $^{o}/_{oo}$. The pristane and phytane isoprenoids were both isotopically lighter than the *n*-alkanes, with $\delta^{13}C$ of -28.2 ± 0.7 $^{o}/_{oo}$ and

Table 4.18 Variation in Isotopic Composition of Individual Compounds in DRO Weathered Standards

Compound	DRO Sample					
	Fresh		25 % Weathered		50 % Weathered	
	Mean $\delta^{13}C^1$ (‰)	SD	Mean $\delta^{13}C^1$ (‰)	SD	Mean $\delta^{13}C^1$ (‰)	SD
C_{14}	-25.50	0.88	-24.94	0.37	-	-
C_{15}	-25.89	0.58	-26.10	0.83	-	-
C_{16}	-25.28	0.91	-26.00	0.08	-28.40	N/D
Norpristane	-24.75	0.82	-25.45	0.38	-27.50	N/D
C_{17}	-22.84	1.06	-23.69	0.84	-27.11	0.45
Pristane	-28.17	0.70	-28.53	0.26	-30.62	N/D
C_{18}	-24.03	0.83	-24.97	0.54	-27.11	0.35
Phytane	-27.80	0.33	-28.37	0.00	-30.04	N/D
C_{19}	-26.78	0.76	-26.16	0.02	-26.81	0.89
C_{20}	-26.80	0.55	-26.42	0.06	-26.98	0.83
C_{21}	-26.84	0.61	-26.63	0.19	-27.06	0.77
C_{22}	-26.78	0.57	-26.14	0.53	-26.83	0.87
C_{23}	-26.69	0.57	-26.86	0.53	-27.80	N/D
C_{24}	-26.31	0.68	-27.06	N/D	-27.39	N/D

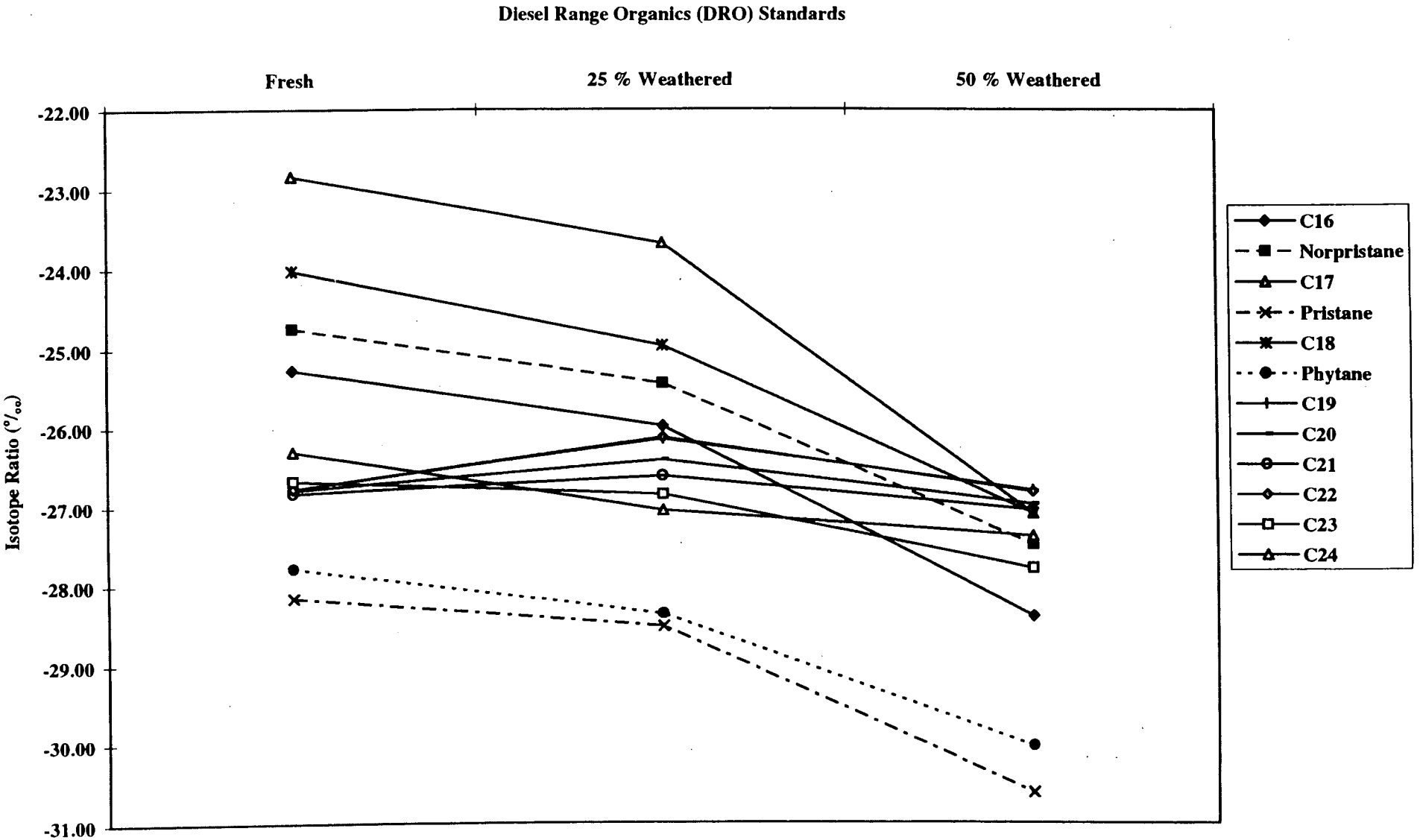


Figure 4.25 Variation in n-Alkane and Isoprenoid Isotopic Composition for Fresh, 25 % and 50 % Weathered DRO Standards (error bars omitted for reasons of clarity, but given in Table 4.18)

-27.8 ± 0.3 $^{o}/_{oo}$, respectively, although the C_{18} isoprenoid, norpristane, displayed a similar isotope ratio to its corresponding *n*-alkanes.

The isotopic fingerprint of the 25 $\%\ ^{w}/_{w}$ weathered DRO was similar to that obtained for the fresh DRO (Figure 4.25). In this sample, the lower carbon number *n*-alkanes (< C_{19}) were again slightly heavier isotopically than the high carbon number alkanes, although the difference was not as pronounced as in the fresh sample and C_{12} and C_{13} were too low in abundance to produce measurable peaks. In particular, $\delta^{13}C$ for C_{15} and C_{16} were both more negative, at -26.1 ± 0.8 $^{o}/_{oo}$ and -26.0 ± 0.1 $^{o}/_{oo}$, respectively. The higher carbon number alkanes once again displayed isotope ratios of between -26 $^{o}/_{oo}$ and -27 $^{o}/_{oo}$. However, the relationship between alkane and isoprenoid $\delta^{13}C$ were again apparent, with norpristane, pristane and phytane having isotope ratios of -25.5 ± 0.4 $^{o}/_{oo}$, -28.53 ± 0.3 $^{o}/_{oo}$ and -28.37 ± 0.0 $^{o}/_{oo}$, respectively.

Isotopically, all the *n*-alkanes in this sample were very similar, with $\delta^{13}C$ values of approximately -27 $^{o}/_{oo}$ and no distinction in $\delta^{13}C$ between high and low carbon number alkanes. No *n*-alkanes below C_{16} were detected. The C_{16} $\delta^{13}C$ is the one exception to this, at -28.4 (n=1) $^{o}/_{oo}$, but this may be due to systemic error due to the low abundance of this compound in the detector. Once again, however, the isotope ratios of pristane and phytane are significantly different from the *n*-alkanes, at -30.6 (n=1) $^{o}/_{oo}$ and -30.0 (n=1) $^{o}/_{oo}$, respectively, whilst that of norpristane did not (-27.5 (n=1) $^{o}/_{oo}$).

CHAPTER 5. DISCUSSION

The implications of these results for the objectives identified in Chapter 1 (Section 1.4.2) and summarised in Chapter 2 (Sections 2.2 and 2.3), are discussed with reference to the two principle themes of the working hypothesis, namely; the characterisation of heavy oil contaminant source terms, and the biotransformation potential of heavy oils in the soil environment.

5.1 METHOD DEVELOPMENT FOR HEAVY OIL CHARACTERISATION

5.1.1 Overall Objectives

The objectives of this research were (Section 2.2):

(i) to identify the key issues and requirements relating to heavy oil contamination of the soil environment that can be resolved through greater analytical capability (specifically, characterisation of heavy oil waste source terms, screening of oil bioremediation potential, assessment of source diagnostic parameters and characterisation of oil biotransformation);

(ii) to develop a novel approach to the characterisation of heavy-oil contaminated soil through the application and development of previously untried methods;

(iii) to consolidate these techniques within a tiered analytical strategy applicable to a variety of heavy oil wastes; and,

(iv) to determine the capability of each technique to provide information of relevance to the issues identified in (i) above.

Here, those results relating to the characterisation of heavy oil contaminant source terms are discussed in light of these objectives.

5.1.2 Construction of Tiered Analytical Strategy

Previous workers have described the use of a staged approach to the characterisation of petroleum products in the environment (Sauer *et al.*, 1993; Douglas *et al.*, 1992; Butler *et al.*, 1991; Pollard *et al.*, 1994). The majority of these cases have been concerned with crude oil contamination. In devising an analytical protocol for the characterisation of heavy oil-contaminated soils, Pollard *et al.* (1994) incorporated TLC-FID and conventional GC CI MS into a tiered analytical approach, and this was shown to produce a greater all-round description of heavy oil waste extracts than that previously obtained.

This work develops the approach further to incorporate a wider set of tools especially suited to the characterisation of heavy oils in the contaminated soil environment. Specifically, the analytical scheme used in this work:

(i) features screening techniques that address the waste extract as a whole (including polar and asphaltene class components) without relying upon GC-based analysis, or C-H bond recognition;

(ii) uses target GC-EI MS to facilitate detailed component analysis of heavy oils to provide potentially useful compositional information without interference from unresolved complex material, or unrecoverable high-boiling waste constituents;

(v) investigates the novel utility of stable isotopes, through bulk fraction isotope ratio mass spectrometry screening (IRMS) and compound specific isotope analysis through gas chromatography-linked IRMS, in the characterisation of heavy oils. This represents an entirely novel application of these methods.

The results of each stage of analysis are discussed below in terms of their historical relevance and their relevance in the context of the overall objectives of this thesis.

5.1.3 Screening Techniques

The screening of waste-soil matrices is a vital first stage of rational site investigation programmes, often severely restricted for heavy oils in terms of scope and accuracy. The results discussed below indicate that screening of heavy oils can yield important information from the waste matrix if tailored to suit the complexity of the oils.

5.1.3.1 Solvent Extraction

Soxhlet extraction of oils from contaminated soils is an established technique, developed in this work principally to allow the solvent extractable material (SEM) from the soil microcosms to be evaluated accurately (Section 4.2.1.1), but also applied to the acid tar-contaminated soils (Section 4.1.1.1).

The quality control procedures described in Chapter 3, Section 3.1.6.1, demonstrate that in this work good extraction efficiencies of over 75 % ± 1.8 were obtained for the ballast oil and over 90 % for the No.6 Fuel Oil, and that the proportion of soil natural organic matter (NOM) extracted along with the contaminants in the microcosm study (Figure 4.6) was negligible (mean NOM extracted = 0.2 mg g^{-1} soil). These results indicate that past concerns over the superfluous extraction of NOM (White & Irvine, 1994) and inefficient extraction of organics (Pollard *et al.*, 1994b) are not an issue for the samples evaluated in this study.

The results support the view (Fan *et al.*, 1994) that this method is of definite utility in the extraction of semi-volatile and non-volatile organics from soil matices, provided quality control studies on NOM extraction, reproducibility and spiked sample-recoveries (including the length of contact between oil and soil) are carried out, and indicate that the SEM variations in the microcosms study are reliable evidence of oil biodegradation.

5.1.3.2 Column Fractionation

The use of column chromatographic fractionation of oil samples has been used principally to isolate fractions in preparation for further analysis (e.g., Douglas *et al.*, 1992;

Pollard *et al.*, 1994). Results of the column fractionation procedure used here, shown in Table 4.2, reveal the relative abundance of each class fraction within each of the reference oils and acid tars. This information allowed the oil samples to be compared in terms of their class compositions. Thus, acid tars were shown to contain the highest proportion of asphaltenes, with the bitumen, No.6 Fuel Oil, API separator oil, ballast oil, residue and waxy distillate comprising successively lower asphaltene contents and, in general, greater saturate class contents. However, elevated saturate class contents in the acid tars indicates that the proportion of this class fraction is not necessarily indicative of lighter oils. These data support the view expressed by Altgelt & Boduszynski (1994) that the classification of an oil as light or heavy based solely upon saturate class fraction abundance may sometimes be misleading, and indicate that, where possible, information of other types should be sought (e.g., type of other oil constituents present, boiling range and polarity) before the true nature of an oil can be determined.

Key factors in the use of column fractionation are the length of time taken for fractionation, the resolution of the fractions isolated and the recovery of oil from the column (Wang *et al.*, 1994b). In light of this, the following points can be made from work described here:

(i) the lateral reservoir system facilitates a more rapid fractionation of samples than that achieved through conventional apparatus;

(ii) subsequent analysis of fractions isolated here by TLC-FID (Figure 3.3) demonstrates that this speed of analysis has not resulted in any substantial reduction in class fraction resolution, and;

(iii) column recoveries of between 90 and 95 % compared favourably with previously reported values of 96.1 % (Later *et al.*, 1981) and 95 - 103 % (Wang *et al.*, 1994).

These results provide evidence that the fractionation of oil samples by rapid column chromatography can be used:

(i) to facilitate the class fraction fingerprinting of heavy oils, in order to obtain insight into the abundance of saturates, aromatics, polars and asphaltenes within an oil. Such information may be of use in assessing the bioremediation potential of a particular waste, since, as discussed in Section 1.3.2.2, oils with a predominance of asphaltenic material may not be susceptible to further biotransformation due to the microbial recalcitrance of these compounds (Bartha, 1986), and;

(ii) to provide a reliable means of isolating the saturate class fraction from interference in preparation for detailed component analysis, which is of key importance to the rapid identification of diagnostic source ratios and weathering/biotransformation indices.

5.1.3.3 Thin Layer Chromatography-Flame Ionisation Detection (TLC-FID)

Class fraction fingerprints for the heavy oils analysed in this study, shown in Figure 4.1 and discussed in Section 1.3.1, provide additional evidence of the capacity of TLC-FID to supply information on the abundance of saturate-, aromatic-, polar- and asphaltene-class compounds within heavy oils. In particular, the results demonstrate the wide variations in composition between the six reference oils. The predominance of the saturate class fraction in the waxy distillate, residue oil, ballast oil and API separator oil is revealed, together with the growing influence of polar and asphaltene class compounds in the bitumen and No.6 Fuel Oil.

The full class fraction fingerprint provided by TLC-FID provides a much greater insight into the composition of an oil, and, therefore, its subsurface partitioning (Section 1.3.2.1) and bioremediation potential (Section 1.3.2.2) than conventional screening techniques that focus only on oil 'Total Petroleum Hydrocarbons' (TPH) (Douglas *et al.*, 1993) or measurement of the 'total oil and grease parameter' (Martin Jr. *et al.*, 1990).

TLC-FID has been applied to a wide variety of crude oils, heavy petroleum products, bitumens and oil process residues, as a means of obtaining information on the bulk composition of oil (Fuhr *et al.*, 1986; Poirier & George, 1983; Volkman & Nichols, 1991; Karlsen & Larter, 1991). For example, Fuhr *et al.* (1986) determined the normalised saturate, aromatic, polar and asphaltene content of a bitumen sample to be 20.7 %, 43.1 %, 18.1 % and 18.1 %, respectively. The standard deviations (SDs) of these measurements were between 1.4 and 1.9, which compares favourably with the SDs obtained in this work, which lay between 0.5 and 4.0. A documented drawback of the technique is its inability to detect compounds of carbon number < *ca.* C_{10} (Karlsen & Larter, 1991). For heavy oil samples, this is not a major problem. Its application to heavy oil contaminants in the soil environment was investigated by Pollard *et al.* (1992), who determined the class fraction fingerprints of extracts from petroleum- and creosote-contaminated sites in terms of SAPA (saturates, aromatics, polars, asphaltenes) and $SA_1A_2A_3P+A_4$ distribution (saturates, aromatics of increasing ring number (A_1 - A_3), polars and asphaltenes (A_4)). These results extend the utility of this methodology to a wider range of heavy oil waste types, represented by the reference oils, and demonstrate that the class compositional screening of heavy oil wastes in soil is an effective screening tool within the tiered analytical scheme.

Method development studies provided valuable insight into the integrity of the column fractionation methodology. Corresponding class fraction abundance for each reference oil for both column and TLC-FID methods (Figure 3.4) were in agreement generally to within 15 %. This compares favourably with previous comparisons reported by Pollard *et al.* (1992), who found agreement to within *ca.* 15 %, but is greater than the differences reported by Fuhr *et al.* (1986), who found class fraction abundances from the two methods to be within *ca.* 5 %. Furthermore, TLC-FID analysis of the pentane, toluene and DCM/methanol fractions isolated through column chromatography (Figure 3.3) indicates that there is little overlap of compounds between the class fractions. This is useful because it indicates that the saturate

fraction is sufficiently well resolved to undergo further analysis by GC-EI MS and GC-IRMS, and provides additional evidence of the validity of the column fractionation procedure.

The differences between the TLC-FID and SAPA column fractionation results (Figure 3.4) are possibly due to the differences in solvent schemes between the two methods. Because the respective class fractions are in essence solubility classes (Section 1.3.1), variations in extraction solvents will result in variations in the composition (and, therefore, the abundance) of each class fraction. For example, alkyl benzenes which contain commensurate elements of aromaticity and saturate characteristics may report in either the saturate or aromatic class fraction, depending on the polarities of the mobile phases used during the extraction process. Pollard *et al.* (1994) proposed that a 35-50 % lower aromatic fraction reported by column separation was the result of high molecular weight PAHs eluting in the polar fraction. Other studies (Karlsen & Larter, 1991; Fuhr *et al.*, 1986) have also demonstrated this phenomenon. Karlsen & Larter (1991), for example, suggested that differences in the amounts of saturates within a crude oil determined by TLC-FID and medium-pressure liquid chromatography may be caused by alkylbenzene compounds reporting in the aromatics class fraction in the former method and the saturate class fraction in the latter. These results, therefore, contribute to current understanding of the limits of class fraction resolution obtainable by TLC-FID and suggest that it may be very useful as a means of obtaining an approximate guide to the composition of a heavy oil waste.

The total time taken from the initial spotting of the 10 samples to completion of the FID analysis is approximately 75 minutes. This results in an analysis rate of up to 70 samples per day. It has been reported that, if properly maintained, the rods can be used for more than 100 analyses without reduction in performance (Fuhr *et al.*, 1986). This level of operational reliability, coupled with a capacity for multi-sample analysis in a relatively short space of time, has established TLC-FID as a robust, cost-effective screening tool. The class fraction fingerprints obtainable by this method are likely to be of great value at weathered, heavy oil-

contaminated areas, where the relative abundance of component types provides important clues to the treatability and weathered state of the waste stream. In particular, the class fingerprints allow the subsurface partitioning of heavy oil contaminants to be investigated (Section 1.3.2.1). Wastes containing high polar and asphaltenic class fraction abundances, for example, are prone to partition into the soil organic phase, exhibit poor overall bioavailability and gradually leach polar contaminants into surrounding groundwater. Thus, TLC-FID can provide information relevant to the selection of appropriate remediation technologies and the identification of possible human exposure routes.

5.1.3.4 Isotope Ratio Mass Spectrometry (IRMS)

Variations in the whole oil and class fraction isotope ratios of the heavy oil samples (Table 3.3) provides evidence that the overall isotopic composition of an oil is influenced by its chemical composition and that, therefore, IRMS may be used as a predictive tool for conferring compositional information on unknown contaminant source terms.

The results shown in Table 3.3 demonstrate that $\delta^{13}C_{oil}$ (whole oil isotope ratios) decreased in value (i.e., became more negative) between the acid tars and the bitumen and No.6 Fuel Oil samples, and again between the bitumen and No.6 Fuel Oil samples and the API separator, waxy distillate, residue and ballast oils. This trend correlates most closely with that of the asphaltene class fraction abundance of the oils and suggests that lower oil isotope ratios are associated with oils containing lower amounts of asphaltenic material.

The method development work described in Section 3.1.6.4 indicates that the variations in $\delta^{13}C_{oil}$ values found in this work, which ranged between -26.8 $^{o}/_{oo}$ for AT1 and AT2 to -28.8 $^{o}/_{oo}$ for the residue oil, though narrow, is significant enough to justify this discrimination between the various petroleum fuel samples analysed. A survey of previous studies involving the use of isotope ratios in the characterisation of oil samples, including those by Philp and

Engel (1987), Fuex (1977), Stahl (1978) and Sofer (1984), indicated that changes of as low as 0.6 $^{o}/_{oo}$ have previously been considered significant to distinguish between oil samples.

Previous work indicates that asphaltenes are commonly found to be isotopically heavier than other oil components because of the underlying isotope-selective processes that affect the formation of the respective SAPA class fractions and, in particular, to the congregation of ^{13}C in compounds that retain stronger inter- and intramolecular linkages (Silverman & Epstein, 1958). As the majority of these compounds report in the polar and asphaltene class fractions, any oil rich in these components would, therefore, be expected to display a relatively high (less negative) isotope ratio.

The differences in isotope ratio between the SAPA class fractions are illustrated more clearly by the isotope type curves presented in Figure 4.2. These results show a clear distinction between the 'flat' shape of isotope type curves for the heavier oil samples, i.e., the acid tar, bitumen and No.6 fuel oil, and the 'angled' shape of the lighter oil samples (API separator oil, ballast oil, waxy distillate and residue oil).

Many studies have demonstrated the use of isotope type curves to elucidate the isotopic variations between respective class fractions (Stahl, 1978; Stahl, 1980; Schoell, 1984). The majority of studies feature angled type curves in which isotope ratios increase in the order;

$$\delta^{13}C_{sat} \ (\cong \delta^{13}C_{oil}) < \delta^{13}C_{aro} < \delta^{13}C_{pol} < \delta^{13}C_{asp} .$$

This is in agreement with the type curves obtained for the lighter reference oils (API separator oil, ballast oil waxy distillate and residue oil). In some cases, a similarity in $\delta^{13}C$ between either aromatic and polar, or polar and asphaltene class fractions has been found (Stahl, 1979). This characteristic is also seen in these results (Figure 4.2) and may be due to the chemical similarity of compounds from within the three fractions. Previous work also indicates that bulk $\delta^{13}C_{oil}$ values do not often differ significantly from the saturate class $\delta^{13}C$ values (Stahl, 1980; Schoell, 1984). This is also observed in the work presented here, although this may be expected

Furthermore, these results also demonstrate that for extremely complex petroleum residues, in this case the bitumen and acid tar samples, even extensive column chromatographic fractionation and high temperature GC conditions cannot produce any more than a broad qualitative indication of sample complexity. A more detailed interpretation of these chromatograms may be achieved through chemical oxidation of the UCM, as described by Gough and Rowland (1990).

For the purposes of this thesis, the GC-FID results established the complexity of the samples prior to detailed component analysis and provided a benchmark against which the subsequent results could be compared.

5.1.4.2 Gas Chromatography-Electron Impact Mass Spectrometry (GC-EI MS)

Analysis of selected ion peaks produced by characteristic, environmentally-persistent biomarker compounds may be a possible source of information on heavy oil-contaminant source terms, represented by the reference oils and acid tars, particularly in relation to potential treatability. Further target analysis of geochemically unrelated crude oils (i.e., oils taken from different formation basins) was also carried out, as an empirical study of the capacity of selected source correlation indices to distinguish between discrete oil samples.

To achieve this, a variety of biomarker indices adapted from the literature were evaluated for reference oils, acid tars and crude oils. The indices selected are described below.

(i) [C_{18}:phytane]. Senn and Johnson (1985) used this index (and the closely related [C_{17}:pristane] index) to rank samples of spilled diesel oil in terms of their weathered state. In their study, from the freshest diesel sample to the most weathered, [C_{18}:phytane] decreased from 2.7 to 0.3. More recently, values were used to estimate the age of diesel oil contamination, ranging between 2.2 for fresh oil to 0.2 for samples approximately 20 years old (Christensen & Larsen, 1993). This ratio has also been used to illustrate weathering of crude

oils spilled in the marine environment. Wang *et al.* (1995), for example, found that extensive weathering of oily residues on an arctic beach resulted in a decrease in the value of both [C_{17}:pristane] and [C_{18}:phytane].

(ii) [pristane:phytane]. This source correlation index has been recently used in studies of weathered crude oil residues in the marine environment. In one study, this ratio was found to remain almost constant, at approximately 0.5, for a range of highly weathered crude oil beach residues and the original crude oil source (Wang *et al.*, 1994). In a related study, a slight decrease between fresh and weathered samples was observed, from 0.87 to 0.75 (Wang *et al.*, 1994). However, because both pristane and phytane have been shown to be susceptible to biotransformation (Section 1.4.2.1), the reliability of this source correlation index is believed to be questionable in highly weathered oil samples. Evidence of this was presented by Hostettler and Kvenvolden (1994), who found [pristane:phytane] values of between 1.2 and 'not detected' for beach oil residues that originally were known to display a value of 1.4 for the same index.

(iii) [C_{18}:17α(H),21β(H)–hopane], [phytane:17α(H),21β(H)–hopane], [*n*-alkanes:17α(H),21β(H)–hopane]. These ratios have been used widely to demonstrate the loss of crude oil from beaches undergoing active bioremedial treatment (Butler *et al.*, 1991), and in more general studies of oil degradation and maturity (e.g., Croft *et al.*, 1995; Pande *et al.*, 1994). All three indices were shown in these studies to record the loss of oil components relative to the pentacyclic terpane found to be most resistant to oil weathering processes, 17α(H),21β(H)–hopane. Most recently, Swannell *et al.* (1995) found [phytane:17α(H), 21β(H)–hopane] decreased from 0.45 to 0.25 during enhanced oil biodegradation over 600 h for oil-contaminated fine-grained sediments in laboratory micrcosms.

(iv) [tricyclic terpanes:hopanes]. Literature evidence suggests that lower molecular weight tricyclic terpanes degrade more rapidly than the more conserved pentacyclic terpanes (hopanes) in very weathered samples (Wang *et al.*, 1994a). As a result, this index has been proposed as a possible means of quantifying oil weathering in highly degraded samples and for distinguishing between highly weathered oils.

(v) [17α(H),21β(H)–hopane:17α(H)21β(H)–norhopane]. These hopane biomarkers have been shown to be both the most conserved and abundant biomarkers within oils (Wang *et al.*, 1994b). In general, oil samples with [17α(H),21β(H)–hopane:17α(H)21β(H)–norhopane] values within approximately 0.5 of one another have been previously documented as being from the same original source (e.g., Sauer *et al.*, 1993), although related oils often display a variation of much less than this. Few comparisons have been made for unrelated oils.

Reference Oils and Acid tars

The values of the selected biomarker indices for the reference oils and acid tars are shown in Table 4.3. All the oil samples analysed were produced from North Sea crude oils. This was important, as it ensured that variations in source and weathering indices were caused by differences in oil composition and not genetic background.

The size of variation in index value between the heavy oils decreased in the order: [*n*-alkanes:17α(H),21β(H)–hopane] >> [phytane:17α(H),21β(H)–hopane] > [tricyclic terpanes:hopanes] > [C_{18}:phytane] ≈ [17α(H),21β(H)–hopane:17α(H),21β(H)–norhopane]. The latter two indices were effectively constant between the respective oil samples; the [pristane:phytane] index could only be determined for two of the oils as pristane was not always detected, and so could not support assessment in this respect.

The consistency of the [17α(H),21β(H)–hopane:17α(H),21β(H)–norhopane] index between the seven heavy oils can be explained by the recalcitrance of these biomarkers, which

would not be expected to fluctuate in abundance during oil production. As all the oil samples ultimately originate from the same crude oil source, the ratio of these biomarkers would be expected to remain broadly constant.

The reason for the lack of variation in the value of the [C_{18}:phytane] index is not clear. It may be possible that because the physical properties of C_{18} and phytane are so similar, they behave similarly during oil production and do not become depleted or enhanced relative to one another. Nonetheless, based on these results, these indices appear to be insensitive to variations in oil composition and would not, therefore, make useful indicators of oil degradation state or bioremediation potential.

The variations of the [*n*-alkanes:17α(H),21β(H)–hopane] and [phytane:17α(H), 21β(H)–hopane] ratios are useful in that they exhibit very low values for oils containing a sizeable combined polar and asphaltene fraction (the acid tars) and ones which may not, therefore, be suitable for treatment by bioremedial technologies. As these indices would also be expected to produce low values for oils that have undergone extensive weathering, due to losses of *n*-alkanes and phytane during biotransformation, these indices may also be useful in assessing the degradation state of oily wastes.

The [tricyclic terpanes:hopanes] also produced low values for the acid tars, but was found to be low also for the residue oil, a sample rich in saturates and low in asphaltenic material. This index may not, therefore, be as robust an indicator of composition as the preceeding two.

The application of target component indices to the assessment of heavy oils in this way represents a novel extension of their utility. Previously, target analysis has principally concentrated on the characterisation of crude oils, either for source correlation purposes or for determining oil biodegradation (Wang *et al.*, 1994; Kvenvolden *et al.*, 1994; Butler *et al.*, 1991; Douglas *et al.*, 1992). This work indicates that target analysis can also be used to extract useful information from heavy oil source terms that previously had gone undetected.

It should be noted that the specific numerical values obtained for the respective indices in this study should not be used as general values in the characterisation of other oil samples, since other geochemical factors are also known to influence the value of biomarker indices (Killops & Killops, 1993). The importance of these results lies in the trend between the various heavy oil samples, which, in this case, provides evidence that target component analysis by GC-EI MS can shed important light on the composition of contaminant source terms, and by extension, their treatability, and so address some of the concerns detailed in Section 1.4.

Crude Oils

Index values for the four crude oils, given in Table 4.4, show that five of the seven indices adequately discriminated between the respective crude oil types; [C_{18}:17α(H),21β(H)–hopane], [*n*-alkanes:17α(H),21β(H)–hopane], [pristane:phytane], [tricyclic terpanes:hopanes] and [phytane:17α(H),21β(H)–hopane]. All of these indices show discrete values for each of the oil samples to allow clear discrimination between unrelated oils and yet support a positive correlation of the two North Sea crude oils.

Of these indices, [C_{18}:17α(H),21β(H)–hopane] and [*n*-alkanes:17α(H),21β(H)–hopane] would be expected to change significantly in value with oil weathering because of the degradability of *n*-alkanes and C_{18} components in relation to the recalcitrant 17α(H),21β(H)–hopane. Therefore, for fresh oils that have not been exposed to environmental weathering, such as the samples analysed in this study, these ratios may indeed make useful oil correlation parameters. However, they may be unsuitable for correlations involving weathered oil samples.

The other indices able to discriminate between the respective crude oil samples are [tri- and tetracyclic hopanes:pentacyclic hopanes], [pristane:phytane] and [phytane:17α(H),21β(H)–hopane]. The values of all these indices would be expected to remain more or less constant during light to moderate oil weathering. For highly weathered samples, however, the latter two

indices may become unreliable due to the susceptibility of both pristane and phytane to prolonged microbial activity (Madsen, 1992). The tri- and tetracyclic alkanes have also been shown to degrade in heavily weathered samples (Wang *et al.*, 1994), but they would be expected to be persistent in the environment for longer than pristane and phytane due to their cyclical molecular structures (Section 1.3.2.2).

Based on these results, the [tri- and tetracyclic hopanes:pentacyclic hopanes] index is, therefore, proposed as the oil correlation index best able to differentiate between both fresh and weathered crude oils.

Of the remaining biomarker indices, [17α(H),21β(H)-hopane:17α(H),21β(H)–norhopane] fails to discriminate between the Nigerian and one of the North Sea crudes (0.8 and 0.7), and [C_{18}:phytane] fails to discriminate between the Nigerian and Iraqi crude oils (both 1.4). The former result is surprising, given the accepted recalcitrance of the hopane (Prince *et al.*, 1994) and suggests that oil correlation carried out through isolated use of this index could lead to erroneous diagnosis of oil source commonality. Work by Morris *et al.* (1996), who found that 17α(H),21β(H)-hopane became depleted in a petroleum fuel waste by up to 75 % over a 30-day incubation period suggests also that this index may be an unreliable source correlation parameter for weathered oils.

In the majority of studies where a correlation between two or more oils is made, a fresh oil is correlated with an oil contaminant (e.g., Pande *et al.*, 1994; Wang *et al.*, 1994; Kvenvolden *et al.*, 1995). Hence, some discrepancy in the extent of weathering experienced by the two samples will be inevitable. Therefore, of the indices tested in this section of the thesis, only [pristane:phytane] and [17α(H),21β(H)-hopane:17α(H),21β(H)–norhopane] indices (or similar hopane pairs) are determined for correlation purposes. Usually, the two oils under analysis are known (or suspected) source oil, and contaminant. As yet, no standard criterion exists for establishing positive or negative commonality between different oil samples, and so numerous values have been used. In most cases, however, variations in source index

value of less than 0.3 (and occasionally less than 0.5) (*Sauer et al.*, 1993) are interpreted as evidence of commonality.

Results for the [pristane:phytane] index suggest that an upper limit of variation between positively correlated oils of 0.3 is insufficient in this case, as this would lead to a false positive correlation between the North Sea crude oil 2 and the Iraqi crude oil. For these oils, a value of between 0.1 or 0.2 would be a more appropriate criterion for establishing commonality.

The remainder of the indices evaluated in this study are generally used to indicate the extent of oil weathering undergone by contaminants, through comparison with fresh samples. These results indicate that for fresh or moderately weathered crude oils (based on mass balance calculations, for example), these indices can also be used as source correlation indices.

The optimum index in this respect (i.e., that which may be used most reliably) would appear to be [tricyclic terpanes:hopanes], which produced markedly different values for each crude oil family that could be predicted to remain fairly constant in light to moderately weathered oils. However, for crude oils that have undergone no weathering, the greatest sensitivity to source variations was shown to be [*n*-alkanes:17α(H),21β(H)–hopane].

These results demonstrate the wider utility of component-specific analysis in the source characterisation of oil.

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

Adaptation of GC-IRMS to the characterisation of heavy oils in the contaminated soil environment requires an understanding of how observed isotopic changes are linked to changes at a molecular level. Primarily, this entails an examination of the degree of isotopic fractionation that occurs as a result of microbial transformation, although an understanding of the way in which individual compound $\delta^{13}C$ vary within oils is also important. The initial stage of this involves method development of the technique as applied to authentic heavy oils.

Results of the GC-IRMS analysis of four of the reference oils and one crude oil are presented in Table 4.5. The most striking feature of these isotopic fingerprints is the small variation in $\delta^{13}C$ between the individual compounds in each oil. In all five cases, the range of *n*-alkane isotope ratios is less than 1 °/oo.

The most conspicuous variation in component $\delta^{13}C$ is for the isoprenoid biomarker, phytane. When detected, this compound appears to be isotopically lighter than most of the *n*-alkanes (as indicated by its slightly more negative isotope ratio), albeit by a maximum of approximately 1 °/oo . For No.6 Fuel Oil, phytane was not detected. However, the lower $\delta^{13}C$ of C_{18} in this case may be interpreted as being a result of the influence of phytane, from which it could not be analytically resolved by GC.

The lower value of C_{17} $\delta^{13}C$ in ballast oil, residue oil and crude oil may also be due to inadequate resolution of this peak from the pristane peak. This observation is not apparent in the API separator oil and the No.6 Fuel Oil (in which the isotope ratio of C_{17} is actually higher than all other compounds). However, the standard deviations of these two measurements are also unusually high, and this may have shrouded any small isotopic variation.

Previous studies have shown that the isotope ratios of crude oil *n*-alkanes and isoprenoids are influenced by a variety of known and unknown factors, including anoxicity of depositional environment, whether the crude oils are marine-derived, terrestrially-derived or lacustrine (Sofer *et al.*, 1991), and type of source rock organic matter (Bowler *et al.*, 1993), and do not follow easily predictable trends (BjorØy *et al.*, 1991). Isoprenoids have been shown to be either isotopically heavier or lighter than the corresponding *n*-alkanes, depending on the oil source environment and genetic make-up (BjorØy *et al.*, 1990).

For example, in one particular oil, BjorØy *et al.* (1990) observed sequential shifting of $\delta^{13}C$ values from -29.6 °/oo to -28.8 °/oo and back to -29.4 °/oo as *n*-alkane carbon number increased from C_6 to C_{11} and then to C_{19}, whereas in another geochemically unrelated oil, the $\delta^{13}C$ values remained uniform over a similar range of carbon numbers and boiling ranges.

Moreover, the authors observed that in one of the oils isoprenoids were isotopically lighter than their corresponding *n*-alkanes, whereas in the other they were isotopically heavier.

Bowler *et al.* (1993) observed a depletion of ^{13}C with increasing carbon number, suggested to be due to a contribution from ^{13}C depleted longer chain components of biogenic origin, of up to *ca.* 2 °/oo and provided further evidence that the disparity between isoprenoid and *n*-alkane isotopic composition varies amongst oils.

Results presented here, therefore, are necessarily specific to these oils only. They demonstrate that for these oils, there is no observable variation in *n*-alkane isotopic composition with carbon number, and that the isoprenoid alkanes are isotopically lighter than their corresponding *n*-alkanes. The results also suggest that this approach is of limited use in differentiating between discrete heavy oil samples in the contaminated soil environment (over and above the degree of specificity obtainable by bulk fraction IRMS). The utility of these isotopic fingerprints (Figure 4.2) as either source or weathering parameters has been investigated as part of the oil microcosm study, the results of which are presented in Section 4.2.1.4 and discussed in Section 5.2.1.4.

Perhaps the most important factor influencing the success of CSIA in the determination of petroleum contaminants relates to the inherent limitations of all GC-coupled techniques when applied to weathered hydrocarbon wastes featuring significant UCM. Adequate resolution of waste components is a primary necessity if accurate, reproducible compound $\delta^{13}C$ values are to be obtained. This is especially so for alicyclic saturate biomarkers, which are usually present in low abundance. In many cases, CSIA of oil biomarkers, as well as *n*-alkanes and isoprenoids, has provided a valuable additional set of correlation parameters (Schoell *et al.*, 1994)

In some cases, physical separation of class components prior to GC-IRMS analysis is useful for isolating specific analytes of interest. For example, several authors have made use of molecular sieves for separating *n*-alkanes from branched and cyclic alkanes in the determination of biomarker isotope ratios (Schoell *et al.*, 1992). Unfortunately, however, these methods are

unable to remove UCM material and therefore are of limited usage for improving the resolution of heavy or weathered hydrocarbon wastes.

In this work, method development results (described in Section 3.1.7.3) indicate that GC-IRMS can be legitimately used to obtain information on the isotopic composition of *n*-alkanes and abundant isoprenoid alkanes in heavy oils, provided appropriate cautions to minimise or estimate the effect of the UCM on the measured isotope ratios are taken, but cannot detect hopane or sterane biomarkers. Furthermore, for extremely heavy oil, in this case the acid tars and bitumen samples, GC-IRMS analysis is not recommended, since resolution of individual peaks prior to isotopic analysis is not possible (as demonstrated by the GC-FID results).

It is clear that if GC-IRMS is to become of genuine use as a tool for analysing petroleum hydrocarbons in the soil environment, emphasis must be placed on fortifying the technical aspects of CSIA alluded to above. A recent development that may facilitate the routine use of CSIA in this context is that of GC-MS-IRMS, which combines GC separation of analytes with simultaneous analysis by IRMS and EI- or CI-MS. (Meier-Augenstein *et al.*, 1994; Douthitt, 1994). This technique holds considerable promise for the many areas of science that currently utilise isotopic analysis as a means of investigation. If these requirements can be fulfilled, it may be that isotopic fingerprinting of bulk organic extracts, component classes and biomarkers will become of considerable use in elucidating the source terms and weathered state of oily wastes in the soil environment.

In their totality, the results of all the techniques used in the tiered analytical strategy demonstrate that current approaches to the characterisation of heavy oil contaminants in soil poorly represent these wastes, and that information pertaining to oil composition and treatability is available through collective application of the screening and detailed component techniques detailed in this analytical scheme. In particular, analytical screening of heavy oil

wastes at contaminated industrial sites through rapid column chromatography and TLC-FID has been shown to provide a class fraction fingerprint that makes it possible to gain insight into the propensity of a particular waste to undergo further biotransformation, whether intrinsic or engineered, and to estimate the subsurface partitioning of these wastes, which assists in risk assessment studies. Screening of contaminants by bulk IRMS has also been shown to be of potential utility in discriminating heavily asphaltenic oils, and, therefore, of potential value in assessing refractory oily wastes.

Furthermore, these results show that extended analysis of heavy oil contaminant source terms is possible, providing that sufficient chromatographic cleanup of the waste is carried out, that through target analysis of biomarker compounds partial characterisation of heavy oils in terms of potential biotreatability is possible, and that isotopic fingerprinting of heavy oils is possible except in the most heavily asphaltenic samples.

In summary, it is clear that heavy oil wastes possess unique chemical and physical properties, not found in other petroleum products, that necessitate the application of alternative approaches for their characterisation. The cause of these differences, and, therefore, of the constraints that affect heavy oil characterisation, is the chemical composition of the waste matrix. The presence of high boiling asphaltenic material, in particular, is a major hinderance to conventional analytical methods, which are designed principally for lighter oils containing mainly low-boiling, non-polar components. These studies have shown that oily wastes containing significant high-boiling and polar compounds can be characterised in such a way as to gain a greater understanding of the way these oils behave in the subsurface, as governed by their chemical characteristics.

5.2 OIL WEATHERING STUDIES

Two of the most important issues pertaining to the characterisation and treatment of petroleum products in the environment (Section 1.4.2), i.e., the unambiguous and accurate measurement of oil biotransformation and the performance assessment of diagnostic source indices, are investigated here through a full characterisation of the microcosm biotransformation of three oils (two of the reference oils and one crude oil), and the analysis of physically-weathered diesel oil standards.

5.2.1 Soil Microcosm Studies

The microcosm study discussed below allows a performance assessment of weathering and source indices for heavy oil, and makes possible a full investigation of the compositional changes that occur during heavy and crude oil biotransformation, and the conditions and kinetics of biotransformation. Such a complete single account of heavy oil biotransformation in soil is not available in the current literature.

The four stages of characterisation undergone by oil samples taken at each stage of the microcosm study (0 to 256 days) are:

(i) evaluation of solvent extractable material (SEM), to allow the reduction in contaminant loads to be monitored;

(ii) column chromatographic fractionation, to allow the class fraction variations throughout the study to be determined;

(iii) detailed component analysis of isolated saturate fractions by GC-EI MS, to allow the performance of weathering and source indices to be evaluated and assessed over the course of biotransformation, and;

(iv) GC-IRMS analysis of saturates, to assess the merit of stable carbon isotopic analysis as a means of characterising petroleum contaminants in soil.

5.2.1.1 Variations in Solvent Extractable Material

The primary aim of the solvent extraction procedure was the provision of oil extracts for subsequent characterisation. However, in this work it was vital to confirm that degradation of oil was taking place, and in particular to distinguish between biotic and abiotic degradation, and so a full quantitative analysis of solvent extractable material at each stage of the microcosm study was carried out.

The results, presented in Section 4.2.1.1, provide clear evidence that the ballast oil and crude oil were significantly degraded over the course of the microcosm experiment, and confirm that the bulk of the losses were due to microbial activity.

For the ballast oil (Figure 4.6 (a)), the amount of SEM decreased steadily over the first 8 to 16 days of the study by approximately 30 $\%^{w}/_{w}$. Between days 16 and 256, SEM recoveries decreased considerably such that by the end of the study over 80 $\%^{w}/_{w}$ of the oil had been lost. In comparison, SEM in the control soils (which demonstrated abiotic losses) decreased by approximately 15 $\%^{w}/_{w}$ over the first 16 days of the study, remaining fairly constant after this point.

A similar pattern is seen in the crude oil microcosms (Figure 4.6 (b)). Here, however, reductions in SEM became significant between 16 and 32 days, slightly later than in the ballast oil microcosms. SEM reductions in the crude oil control soils were much smaller in comparison (*ca.* 5 $\%^{w}/_{w}$).

These results indicate that although microbial transformation of these oils begins almost immediately upon contact with the soil, there appears to be a 'lag' period (of approximately 16 days for the ballast oil, and 32 days for the crude oil) before biotransformation reaches its optimum steady rate. This has been reported by other workers, most commonly in field bioremedial operations (Rifai *et al.*, 1995) and may be due to the time taken for soil biomass to develop, become accustomed to the change in conditions caused by the introduction of the contaminant to the soil, and overcome any initial toxicity.

A comparison of the abiotic and biotic losses in the ballast oil (Figure 4.7 (a)) and crude oil (Figure 4.7 (b)) microcosms supports this observation. For the ballast oil, abiotic and biotic losses are comparable between days 0 and 16. After this point, there is a marked increase in the biotic losses whilst the abiotic losses remain fairly constant. For the crude oil, biotic losses were found to rise steadily before day 32, after which point a sharp increase was observed. The results also indicate that abiotic losses are not of great significance for crude oil in this case.

Results in Figure 4.6 (c) indicate the recalcitrance of No.6 Fuel Oil in this study. No significant reductions in SEM were observed in either the treated or control soils, although the former did produce some losses (up to 10 $\%^{w}/_{w}$) due to biotic activity (Figure 4.7 (c)). The sharp reduction in SEM at day 16 of the study may be indicative of some biotransformation, but the reproducibility of this data point is high, and no other flasks were found to produce such a result. Microbial toxicity may play a role in this oil's lack of biotransformation.

The kinetics of petroleum biotransformation are recognised as being highly complex and extremely difficult to quantify (Song *et al.*, 1990). In general, the degradation of organic chemicals in soil systems is often defined using zero and first order rate models (Sims, 1991), although the complexity of the reaction is such that biotransformation is thought to be intermediate between zero and first order (Bossert & Bartha, 1984).

In zero order reactions, the rate of transformation is independent of concentration as the reaction proceeds and can be characterised by the equation $C_t = C_0 - kt$, where C_t is the concentration of a particular component at time t, C_0 is the initial concentration of that component and k is the zero order rate constant. The concentration of oil as the reaction proceeds does not influence the rate of reaction.

In a first order biotransformation reaction, the concentration of the oil decreases exponentially with time, with the rate determined by k, the first order rate constant. First order kinetics generally prevail when the biological activity of the soil is much greater than the

concentrations of the compound(s) being degraded (Sims, 1990). The first order reaction is defined by the equation

$$\ln(C_t/C_0) = -kt, \qquad (5.1),$$

where C_t and C_0 are defined as described above. Thus, a plot of $\ln(C_t/C_0)$ versus t will produce a straight line if the reaction is first order, with the slope of the line equal to the rate constant k. The half-life of a first order biotransformation is independent of initial concentration and can be determined by substituting $C_0/2$ for Ct in the rate equation, which then becomes equal to

$$t_{1/2} = 0.693/k \qquad (5.2).$$

In this work, the kinetics of biotransformation was investigated through plots of $\ln(C_t/C_0)$ vs. t for ballast oil and crude oil (shown in Figure 5.1). Results suggest that in both cases, the early portions of the plot are not linear, possibly due to oil volatilisation, with initial SEM losses less likely to be the result of first order behaviour. However, from approximately 32 days, the ballast oil plot appears to become more linear in appearance. For the crude oil plot, the results are less conclusive, and without more initial data cannot be confidently interpreted as being indicative of first order behaviour. It is interesting to note that the points at which the plots become more linear coincide with the points at which, for the respective oils, the abiotic and biotic losses of SEM diverge, i.e., when biotransformation of each oil begins to be the dominant route of contaminant loss. The conditions within each microcosm at these points would probably be most conducive to first order reaction, i.e., lower contaminant concentrations relative to the microbial activity of the soil.

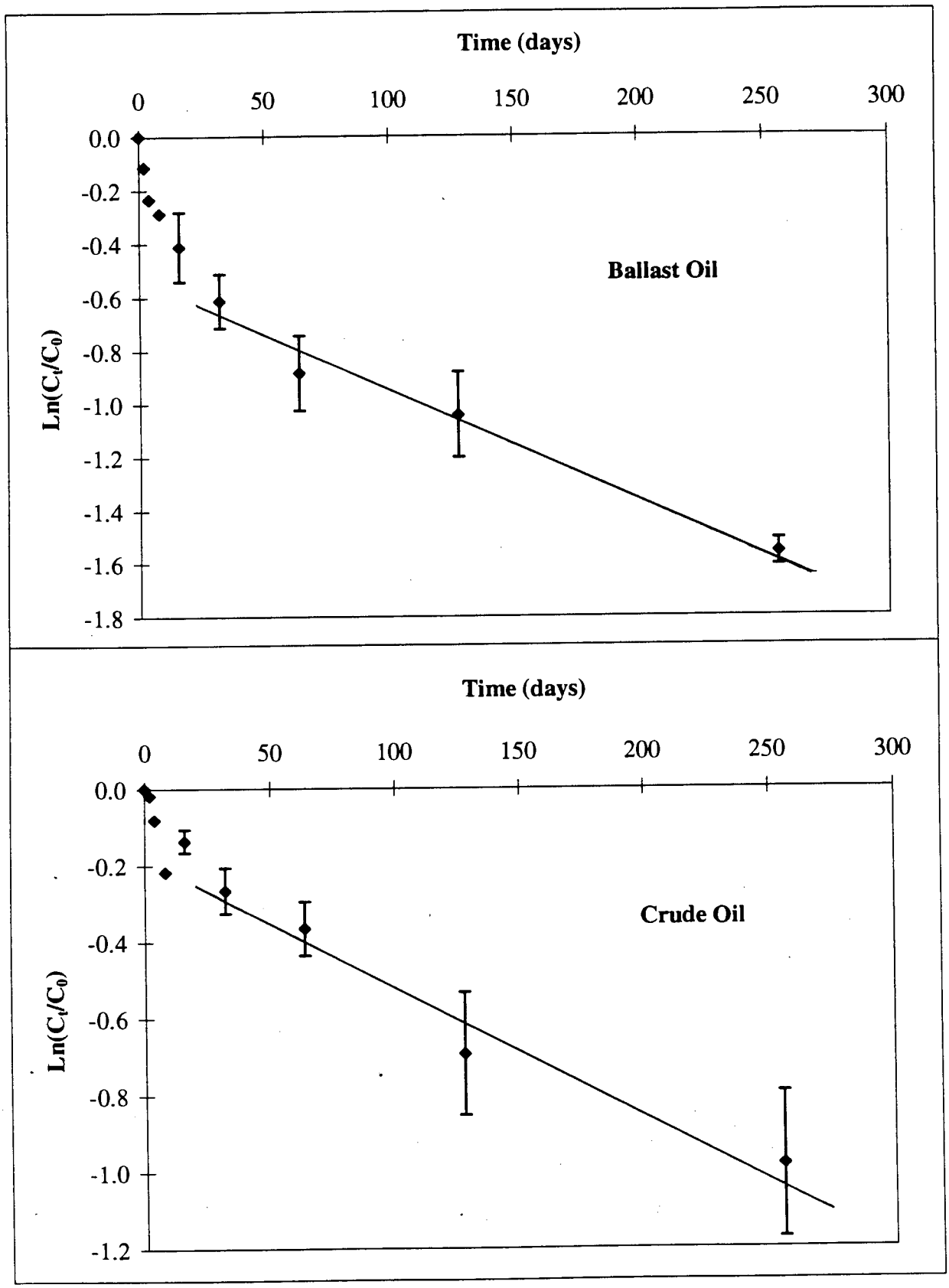


Figure 5.1 Plot of Ln(Ct/C0) vs Time (days) for the Soil Microcosm Study of Ballast Oil and Crude Oil from 32 to 256 days

To estimate rate constants for the biotransformation of the oils, the slopes of the best fit straight line through latter linear portions of each plot were determined (see Figure 5.1). These value correspond approximately to the rate constant for the first order reaction, k.

For the ballast oil and crude oil biotransformation, k was estimated to be -0.0042 and -0.0033, respectively. Using these rate constants, the $t_{1/2}$ values for the steady-state biotransformation of ballast oil and crude oil (i.e., over the period 32 to 256 days) were approximated to be 165 days and 210 days, respectively. Inspection of the % $^{w}/_{w}$ loss of SEM due to microbial activity for each oil over the entire 256 days (Figure 4.8), indicates that a 50 % reduction in SEM for the ballast oil and crude oil occurs after *ca.* 160 days and *ca.* 200 days, respectively.

Few studies have attempted a complete quantification of petroleum biotransformation kinetics, with authors usually preferring to either assume first order behaviour (e.g., Herbert *et al.*, 1996; Rifai *et al.*, 1996), or interpret results without reference to the reaction kinetics (e.g., Swannell *et al.*, 1995). Song *et al.* (1990) encountered difficulties in the microcosm biotransformation of a range of oils, including home heating oil, diesel oil and No.6 Fuel Oil. In place of a quantitative kinetic analysis, the authors determined an empirical half-life of each oil, this being the time taken for the total fuel concentration in soil to be reduced by half. For the heating oil and diesel oil, $t_{1/2}$ was found to be approximately 12 to 15 days. However, no half-life could be determined for the No.6 Fuel Oil, due to the length of time taken for this oil to biodegrade at the chosen conditions (27 °C, unamended).

The results presented here contribute to current knowledge by suggesting that first order kinetics may prevail over the latter stages of oil biotransformation, when oil concentrations are low enough (or microorganism populations are large enough) to produce uninhibited biotic activity. Over the initial stages of biotransformation, particularly when oil concentrations are elevated, the reaction is more complex and so first order kinetics should not be presumed. A plot of $\ln(C_t/C_0)$ vs. t can be used to estimate when first order kinetics

are followed, and from this it may be possible to estimate how long a particular bioremediation operation may be required to act upon a particular contaminated site to reduce contaminant levels to the required amount. This would clearly be of substantial benefit to practitioners in the field of contaminated land, particularly if experimentation times could be reduced.

However, the results also indicate that the kinetics of *in-situ* petroleum biotransformation are extremely complicated and may only adhere to first order behaviour under certain, ideal conditions, particularly when contaminant loads in soil are low relative to active microbial populations. This may be due to a number of factors, including:

(i) the variable biotransformation and volatilisation rates of individual petroleum components;

(ii) the variation in metabolic capability of the soil microbial consortia;

(iii) the initial microbial toxicity of the oil and the increasing microbial recalcitrance of the remaining contaminant as biotransformation proceeds, and;

(iii) the growth in microbial numbers during biotransformation (Bartha, 1986).

The overriding conclusion to be drawn from these results is that microbial transformation reduced the amounts of ballast oil and crude oil in contaminated soils by approximately 60 % $^{w}/_{w}$ in both cases, and that the aim of effecting oil biotransformation had been met. From an initial soil loading of 20 g of oil per kg dry soil, this represents an overall microbial degradation rate of 0.05 g oil per kg soil per day. This compares favourably with published data on degradation rates for heavy oils, sludges and crude oils, which varied between 0.02 and 0.6 g oil per kg soil per day (Herbes & Schwall, 1978). Overall, total reductions in SEM for these oils were 70 % $^{w}/_{w}$ and 65 % $^{w}/_{w}$, respectively. This indicates that subsequent analysis based on the premise of microbial transformation of oils are valid.

With regards to the undegraded portions of the oils, studies have shown that the principle reason for the persistence of oil residues recalcitrant to further biotransformation is

poor bioavailability, and that removal and incubation of these residues can reduce their abundance by up to 70 % (Lethbridge *et al.*, 1995). Future studies may, therefore, be directed towards investigating this possibility for heavy oil residues.

5.2.1.2 Variations in Individual Class Fraction Distributions

Characterisation of the bulk compositional changes that take place within the three oils, as represented by the four 'class fraction' solubility classes (saturates, aromatics, polars and asphaltenes) was a crucial aspect of the microcosm study, allowing a complete picture of the compositional changes that occurred in each oil as a result of biotransformation to be formed.

This information was important in confirming the microbial degradation of oils in support of both the weathering and source index study, but particularly the former, where it was important to be able to link the changes in weathering index value with the depletion of the whole oil, and not just the specific components that comprise the index. Chromatographic isolation of the saturate fraction was also required to facilitate the subsequent detailed component analysis by GC-EI MS and GC-IRMS.

Results are presented in Section 4.2.1.2. In the ballast oil and crude oil microcosms, the overall $\%\ ^{w}/_{w}$ depletion of saturate class fraction were 51.4 $\%\ ^{w}/_{w}$ and 21.6 $\%\ ^{w}/_{w}$, respectively (Figure 4.11). Like the SEM recoveries discussed above, saturate class fraction amounts were found to decrease slowly over the first 16 to 32 days of the study, and then more rapidly, with the greatest overall decrease in both oils occurring between 128 days and 256 days (Figures 4.9 (a) and (b)). Concomitantly, percent weight increases in the aromatic, polar and asphaltene class fractions were most evident over the latter period of the study. Class fraction variations from the control microcosms (Figure 4.10 (a), (b) and (c)) indicate that no significant changes occurred in these samples, and that, therefore, the variations observed in the treated soils were the result of microbial activity.

That the pattern of biodegradation of the saturate class fraction in the ballast and crude oil samples over the course of the study mirrored the depletion in SEM is not surprising, given their predominance in these oils and their amenability to microbial attack. These results provide firm additional evidence that substantial microbial degradation of these oils took place over the course of the microcosm study and provide support for later conclusions regarding biomarker indices.

The overall class fraction changes in the No.6 Fuel Oil samples were unexpectedly sizeable (Figure 4.11), given the expected recalcitrance of this oil to biotransformation. Despite an overall reduction in SEM of only *ca.* 10 $\%^{w}/_{w}$, saturate class fraction abundances dropped by 14.9 $\%^{w}/_{w}$, and polar and asphaltene abundances increased, by 8.9 $\%^{w}/_{w}$ and 11.5 $\%^{w}/_{w}$, respectively. Evidence that these changes were microbially induced was provided by the control microcosms for No.6 Fuel Oil, which exhibited little change in composition over time. These results can be explained more clearly with reference to the actual amounts of class fraction obtained from each sample (Figures 4.12 (a), (b) and (c)).

Figures 4.12 (a) and (b) demonstrate the steady depletion of saturates in the ballast oil and crude oil, respectively. A compared to the class fraction variations in the control microcosms (Figures 4.10 (a), (b) and (c)), suggests that microbial degradation of the oils had taken place. In both these oils, there is also a slight depletion in aromatic class fraction abundance. Polar and asphaltene abundances remained steady over the course of the study, which provides further evidence of the microbial recalcitrance of these compounds in soils.

Variations in the abundance of the respective class fraction in the No.6 Fuel Oil (Figure 4.12 (c)) indicate that despite showing a small overall drop in SEM over the 256 days, the saturate fraction abundance declined steadily whilst the the amount of asphaltenes recovered almost doubled. This phenomenon has been previously documented (Westlake *et al.*, 1974; Gibson, 1984), and may be due to production of cross-linked, high molecular weight polymers during biotransformation of lower molecular weight components (Bossert

& Bartha, 1984). This result is important, because these molecular species are not generally regarded to be amenable to microbial transformation, and could undermine the success of bioremedial technologies at heavy oil-contaminated sites.

Other studies have also characterised the microbial recalcitrance of high molecular weight compounds within oil. For example, Heusemann (1995) found that saturates and aromatics of carbon number > C_{44} were less degraded than saturates and aromatics of carbon number < C_{44}. For the aromatics, the amounts of >C_{44} compounds remaining after a 52-week period of amended mesocosm biotransformation was up to 30 % greater than the low carbon number equivalents. For the saturates, the difference in the amounts remaining over the period of study were much lower (< 10 %), suggesting that the recalcitrance to biotransformation of high carbon number saturate compounds is not substantially lower than the low carbon number saturates. The results presented in this study corroborate this suggestion by demonstrating the preferential biodegradation of saturate class fraction components before those of other class fraction types.

Furthermore, Chaineau *et al.* (1995) noted a reduction in concentration of saturated hydrocarbons and aromatics of approximately 70 % and 65 %, respectively, during the 250-day soil microcosm biodegradation of fuel oil, whereas the polar class fraction remained almost constant over the period of study. Huddleston & Cresswell (1976) also reported that the 22-month land treatment of an oil initially containing 22 $\%^{w}/_{w}$ saturates, 28 $\%^{w}/_{w}$ aromatics and 50 $\%^{w}/_{w}$ combined polars and asphaltenes, resulted in an 82 $\%^{w}/_{w}$ reduction in the saturate fraction, a 60 $\%^{w}/_{w}$ reduction in the aromatic fraction but only a 1 $\%^{w}/_{w}$ loss of polars and asphaltenes. These patterns of change in oil composition during biotransformation are very similar to the changes observed in this study, indicating that the desired microbial transformation had been achieved, although depletions in aromatic class fraction abundance in the oils degraded in this study were not as large as previously observed. This is partially due to the low aromatic content of the oils under study, but may also be due to the nature of the

heavy oil aromatics, which though not analysed by detailed component analysis, may be expected to contain a significant portion of polynuclear aromatic hydrocarbons (PAHs) and heterocompounds not normally susceptible to microbial degradation.

In summary, based on the results presented in this thesis, the following conclusions were drawn:

(i) successful biotransformation of ballast oil and crude oil was achieved;

(ii) in the ballast oil and crude oil microcosms, saturate class fraction compounds were depleted by *ca.* 90 % and 75 %, respectively, of their original values. This provides further evidence that these oils have been heavily mineralised by soil microbial populations;

(iii) asphaltene and polar compounds remained at constant levels throughout the period of study, thereby demonstrating their recalcitrance to microbial transformation;

(iv) in the No.6 Fuel Oil microcosms, a slight depletion in saturate class fraction abundance was accompanied by an increase in asphaltene abundance. This has important implications for the bioremediation potential of an oil, where the application of bioremediation technologies may result in an increase in the amount of recalcitrant compounds in a waste, which could result in the production of a residual waste source term highly resistant to microbial treatment.

Furthermore, these results also support the observation of Hatzinger & Alexander (1995), who found that the susceptibility of oils to microbial transformation decreased over time. The authors suggest that this is because of diminishing bioavailability of contaminants with time. Lethbridge *et al.* (1995) also report that decreasing bioavailability of oil contaminants can result in poor biodegradation levels. The results presented here suggest that a contribution to this reduction in bioavailability may be the production (or at least the accumulation) of recalcitrant, non-aqueous soluble asphaltenic material as biotransformation proceeds. If a class fraction characterisation of the oily waste in question is not carried out such information could go unnoticed.

5.2.1.3 GC-EI MS Analysis

Obtaining information on the source of oily wastes in the soil environment is of vital importance to the apportionment of liability in incidents of cross-boundary contamination (Section 1.4.2.2). Evaluating the extent of weathering or biotransformation undergone by the waste is also significant to the informed application of risk management strategies, where it is crucial to be able to demonstrate loss of organic contaminants to microbial catalysis during bioremedial activity (Section 1.4.2.1). An analytical approach that has been used to characterise both the source and weathered state of spilled petroleum contaminants involves the measurement of the abundance and distribution of biomarker compounds within the contaminant matrix (Sections 1.3.1 and 1.4.3.2).

In this study, the sensitivity and accuracy of a number of weathering indices has been assessed through the analysis of oil samples taken from the soil microcosm studies at various stages of biotransformation. A selection of source correlation indices have also been determined with a view to establishing the most reliable (i.e., the most consistent in value) over the course of the microcosm study. The results provide important new evidence that will assist in addressing the uncertainties that surround the monitoring of oil biotransformation (Section 1.4.2.1) and the identification of contaminant sources (Section 1.4.2.2).

(i) Weathering Indices

The reduction in SEM and the variations in oil class fraction over the course of the microcosm study provide strong evidence that substantial biotransformation of the ballast oil and crude oil had taken place. Evaluation of weathering indices for these oils, therefore, allows the sensitivity (i.e., the size of the change in index value) and accuracy (i.e. the extent to which the changes in index value match the changes in oil SEM) of each index to be assessed, with the overall aim of identifying those indices best able to convey information on oil biotransformation characteristics. The indices evaluated in this study

are commonly used to monitor oil losses during microbial transformation or oil weathering in the marine environment. The rationale for their selection is provided in Section 5.1.4.2.

For the ballast oil and crude oil, results indicate (Tables 4.10 (a) and 4.11 (a)) that the most sensitive weathering index was [*n*-alkanes:17α(H)21β(H)-hopane], which for the ballast oil decreased from an initial mean value of 748.9 (37.7) to 8.5 (1.0) and for the crude oil decreased from 81.9 (n/d) to 18.1 (14.2) after 256 days. The magnitude of these changes is probably due to the significant discrepancy between the susceptibility of the *n*-alkanes to biotransformation and the microbial recalcitrance of the hopane (Prince *et al.*, 1994). ANOVA of the values of [*n*-alkanes:17α(H)21β(H)-hopane] over the 256 days (Table 4.16) indicated that the variation was a statistically significant one. The use of this index in a similar context was reported by Butler *et al.* (1991), who used this ratio to demonstrate the 80 % depletion in crude oil in contaminated beaches over a 6-month period, although Croft *et al.* (1995) observed that the value of this index decreased by only a small margin, from 551 to 518, during the 45-day biodegradation of oil-contaminated sand. These results provide evidence that the [*n*-alkanes:17α(H)21β(H)-hopane] index is a highly sensitive means of determining oil depletion during biotransformation activities.

Of the other weathering indices examined, the [C_{16}:norpristane], [C_{17}:pristane] and [C_{18}:phytane] indices also reduced in value over the period of study, but to relatively smaller degrees and in a manner that did not follow an easily predictable trend (Tables 4.10 (a) and 4.11 (a)). For the ballast oil, the overall changes in these indices were 1.9, 1.5 and 1.1, respectively, which is very similar to the changes observed by previous authors (Croft *et al.*, 1995; Davies & Tibbetts, 1987; Christensen & Larsen, 1993; Swannell *et al.*, 1995). Davies & Tibbetts (1987), for example, found that crude oil in submerged sediments [C_{18}:phytane] decreased slightly from 1.3 to 1.1 after 106 days and to 0.4 after 437 days, whereas Christensen & Larsen (1993) observed a decrease in [C_{17}:pristane] of 2.0, from 2.2 to 0.2, between fresh diesel oil and diesel that had been in the soil subsurface for an estimated 20

years. In this study, ANOVA of these indices indicated that only the variations of [C_{17}:pristane] and [C_{18}:phytane] were significant for the ballast oil, and only [C_{18}:phytane] produced significant variation between crude oil microcosm samples.

Based on these results, it would appear that [C_{18}:phytane] may be used as an indicator of oil weathering. However, many authors have documented that phytane is susceptible to concerted microbial attack (Prince *et al.*, 1994; Butler *et al.*, 1991; Madsen, 1991), and in this case this is demonstrated by the decrease observed for the ratio [phytane:17α(H)21β(H)-hopane], shown in Tables 4.10 (b) and 4.11 (b) (for the ballast oil and crude oil, respectively). For the ballast oil, the value of this index remains at 41.9 ± 10 over the first 32 days of soil microcosm study, and then drops to 1.2 at day 256. For the crude oil, the index remained at 0.6 ± 0.3 over the first 64 days of study, and then lowered to a final value of 0 after 256 days. Both of these results provide evidence that phytane becomes depleted in heavily biotransformed oils, and indicates that the weathering index [C_{18}:phytane] may be used to monitor oil biotransformation reliably only in light-to-moderately weathered samples.

The [$\Sigma C_{14\text{-}28}$:C_{24}tetracyclic terpane] and [$\Sigma C_{14\text{-}28}$:Σtricyclic terpanes] ratios decreased more steadily in value during the study, suggesting that these indices may be useful for monitoring oil biotransformation. However, only the latter index was found by ANOVA to produce significant variations between the respective ballast oil and crude oil samples. This was the most sensitive of the two indices in both oil microcosms, decreasing from 75.5 ± 6.0 to 2.1 ± 0.3 in the ballast oil and from 12.9 ± N/D to 2.4 ±1.9 in the crude oil. The reason for the negative ANOVA result for the [$\Sigma C_{14\text{-}28}$:Σtricyclic terpanes] index may be the disparities in variance of the values at each sampling point, which in ANOVA tests are usually assumed to be similar. These indices have not been previously reported, and the results presented here provide evidence that they may be of use as indicators of oil weathering. This is important because in some crude oil distillation cuts, particularly middle distillate products such as diesel

oil or lighter fuel oils (home heating oil, for example) hopanes may not be present in sufficient abundance to be used as conserved internal markers as they are too high-boiling. These results indicate that in such cases, the tetra-and tricyclic terpanes may be used in place of hopanes to provide a more sensitive indicator of oil weathering than an [isoprenoid:*n*-alkane] index.

The ratio of low-to-high carbon number *n*-alkanes, [$C_{14+16+18}$:$C_{24+26+28}$], also produced lower values as the microcosm study proceeded, decreasing from 10.4 ± 0.7 to 1.5 ± 0.2 in the ballast oil and from 2.0 ± N/D to 1.7 ± 1.0 in the crude oil. ANOVA of these indices found that, in both cases, the variation was significant. Wang *et al.* (1995) evaluated a similar index ([C_{8-12}:C_{16-20}]) to determine the weathered state of fuel oil spilled on an Arctic beach, and found that from a value in fresh oil 2.3, the index lowered to between 0.2 and 0.6 for 'less weathered' samples and to between zero and 0.007 for 'highly weathered' samples. Based on these criteria, the crude oil would not be adjudged to be weathered significantly whilst the ballast oil would weathered only moderately. The results obtained in this study suggests that this index is sensitive oil weathering. However, the use of this index may not be applicable in circumstances where substantial oil biotransformation has taken place, because recent results by Hatzinger & Alexander (1995) suggest that higher number ($> C_{44}$) *n*-alkanes are not significantly more resistant to microbial activity than *n*-alkanes of carbon number $< C_{44}$. Thus for confirmation of oil biotransformation, this index should not be relied upon to provide accurate information as both index numerator and denominator would be expected to decrease in value.

In terms of sensitivity, therefore, and taking into account the ANOVA results and the concerns over the [$C_{14+16+18}$:$C_{24+26+28}$] index, the weathering indices suggested by these results to be most sensitive to oil biotransformation are (in order of decreasing sensitivity):

[*n*-alkanes:17α(H)21β(H)-hopane] >> [ΣC_{14-28}:C_{24}tetracyclic terpane] > [C_{18}:phytane].

To further characterise these indices in terms of their capacity to accurately reflect changes in oil composition, the relationship between the actual decrease in oil saturate class fraction abundance (mg g^{-1} soil) and the decrease in saturate class abundance as predicted by

each index (using the actual initial abundance as a starting point). This was calculated by determining the equivalent decrease in mg g^{-1} abundance that would produce the observed decrease in index value. The results of this calculation, shown in Figures 5.2 (a) (for the ballast oil) and (b) (for the crude oil), indicate that [ΣC_{14-28}:C_{24} tetracyclic terpane] and, to a slightly lesser degree, [*n*-alkanes:17α(H)21β(H)-hopane] produced ballast oil saturate class fraction concentrations that most closely matched the actual abundances in the ballast oil samples determined by gravimetric column fractionation. For the [ΣC_{14-28}:C_{24}tetracyclic terpane] index, the calculated and actual abundancies were generally within 0.5 mg g^{-1}, whereas for [*n*-alkanes:17α(H)21β(H)-hopane], the difference between the two values was found to be up to *ca.* 3 mg g^{-1}.

For the crude oil, the index that most closely matched the actual loss in saturates was the [*n*-alkanes:17α(H)21β(H)-hopane] index, although discrepancies in value of up to *ca.* 5 mg g^{-1} were observed. Here, the [ΣC_{14-28}:C_{24}tetracyclic terpane] index produced calculated saturate abundances of up to 10 mg g^{-1} apart from the actual amounts determined.

In this study, the [*n*-alkanes:17α(H)21β(H)-hopane] index was, therefore, identified as the index most sensitive to oil biotransformation and the index that most accurately reflected the actual depletion of oil from the contaminated soil. This index was also found to be the most reliable for the No.6 Fuel Oil saturates, which though not as depleted over the microcosm study as either the ballast oil or crude oil saturates, caused the [*n*-alkanes:17α(H)21β(H)-hopane] to fall from 81.9 to 18.1 over 256 days. Some authors have noted that there may be limitations to the utility of this index. For example, Morris *et al.* (1996) found that hopane concentration in fuel oil dropped over a 30-day period from 436 ppm to 96 ppm in one incubated oil-soil sample. As the actual abundance of the hopane in the soil microcosms was not determined in this study, it is not possible to state whether this compound was or was not completely conserved over the course of the experiment. If hopane is slightly depleted during biotransformation, then the estimates of oil depletion produced by the

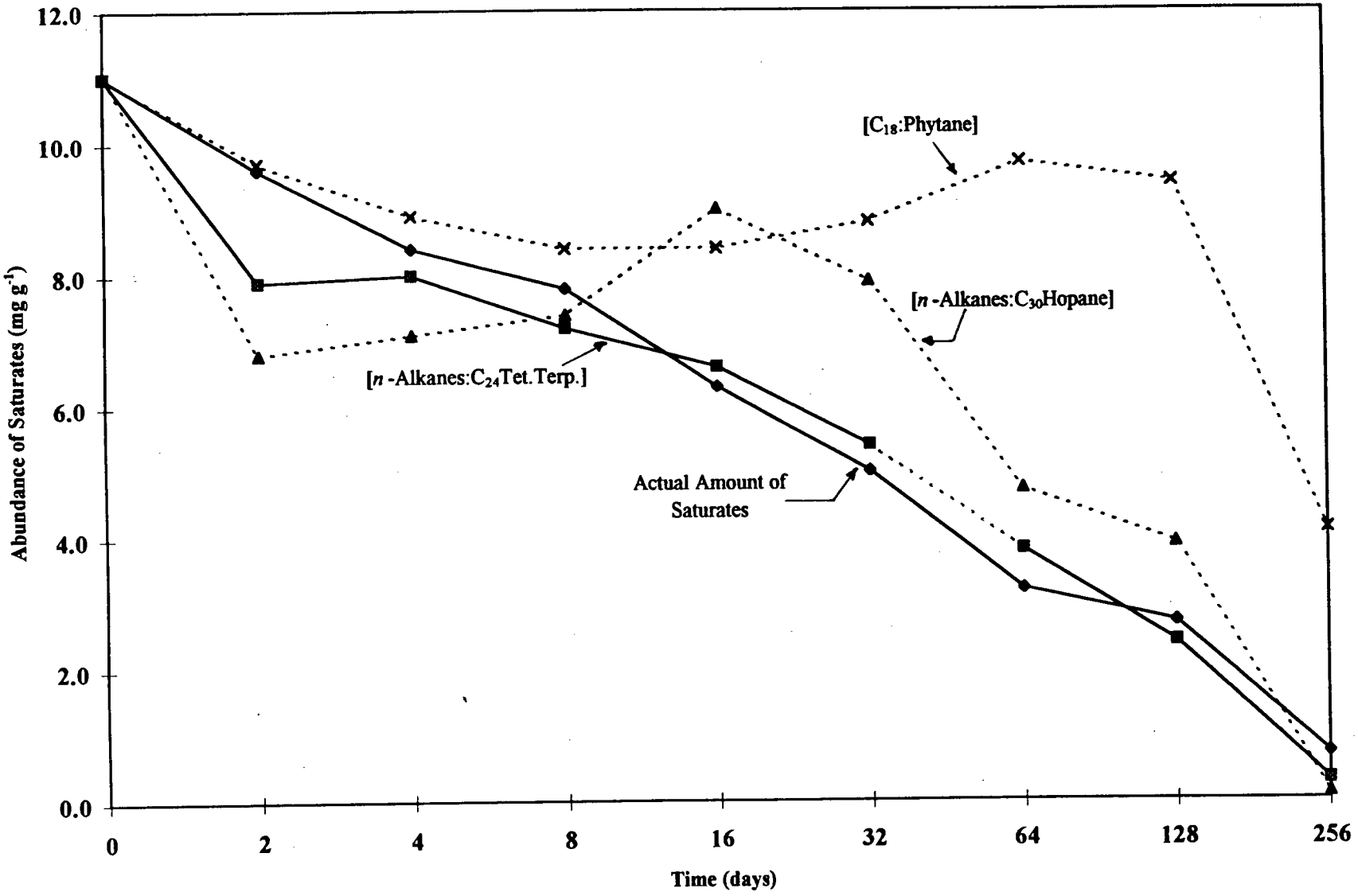


Figure 5.2 (a) Comparison of Actual Abundance of Saturates in Ballast Oil (mg g^{-1}) with Amounts Predicted by Selected Weathering Indices

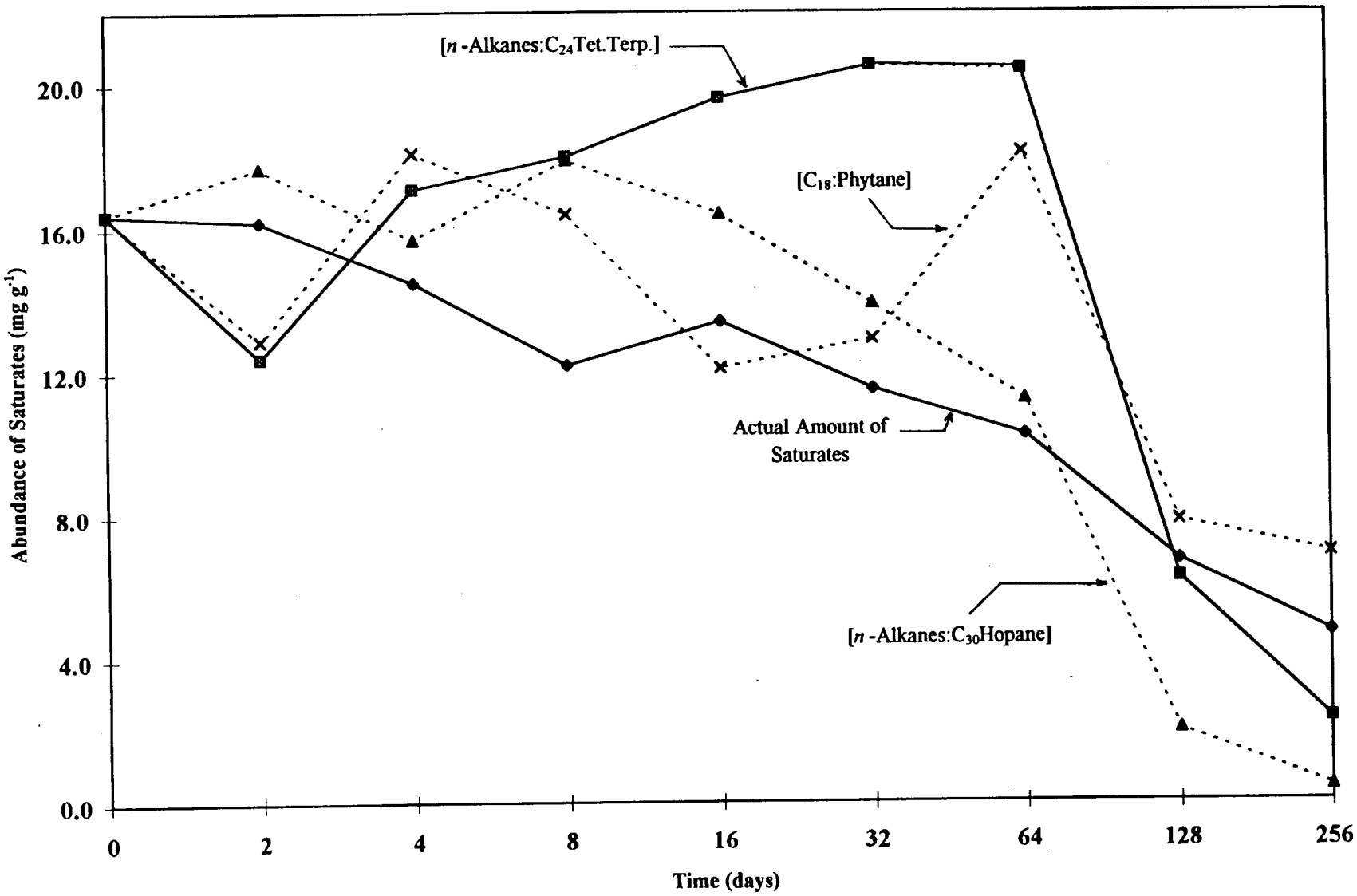


Figure 5.2 (b) Comparison of Actual Abundance of Saturates in Crude Oil (mg g^{-1}) with Amounts Predicted by Selected Weathering Indices

[*n*-alkanes:17α(H)21β(H)-hopane] index will be underestimates of the actual amount of oil depleted.

(ii) Source Correlation Indices

Source correlation index reliability is based upon the constancy of the value of the index during oil weathering. Indices that remain constant are useful because they allow the source of an oil to be determined, providing a sample of the fresh source candidate is available for comparison.

Source correlation index values were evaluated in this study for each of the oils under examination at each sampling point over the 256 days. Clearly, the most useful results are those obtained for the ballast oil and crude oil, because of the extent to which these oils were depleted over the study. Results, provided in Tables 4.10 (b) and 4.11 (b) (for the ballast oil and crude oil, respectively), indicate that the most reliable source indices are those comprising ratios of hopane isomers, specifically:

(i) the [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane] for the ballast oil and crude oil, which exhibited mean values of 0.7 ± 0.1 for the ballast oil, and mean values that varied between 0.6 and 0.7 (precisions less than 0.05) for the crude oil;

(ii) the ratio of 17α(H)21β(H)-homohopane (22S) and 17α(H)21β(H)-homohopane (22R) isomers, [22S:22R], which gave a mean value of 1.3 (precisions less than 0.05) at every sampling point for the crude oil microcosms, and;

(iii) [17α(H)21β(H)-bishomohopane:17α(H)21β(H)-methylhopane], which varied between 1.3 and 1.6 (precision up to 0.1) for the crude oil microcosms.

The results also show that the [pristane:phytane], [phytane:17α(H)21β(H)-hopane] and [C_{23}tricyclic terpanes:C_{24}tricyclic terpane] indices remained fairly constant over the initial stages of oil biotransformation (up to *ca.*, day 32), but then decreased significantly in value. ANOVA calculations for each of the source correlation indices suggest that [phytane:17α(H)21β(H)-hopane] and [C_{23}tricyclic terpanes:C_{24}tricyclic terpane] varied

significantly between samples over the course of the study, which indicates that these indices are not useful for source correlation purposes. Swannell *et al.* (1995) also noted a decrease in the former of these indices of 0.19 (from 0.44 to 0.25) during the nutrient-enhanced biodegradation of crude oil in contaminated sediments over 600 hours. Pande *et al.* (1994) also noted that [phytane:17α(H)21β(H)-hopane] decreased in increasingly biodegraded crude oil samples, in this instance from 200 in fresh crude oil to 4.3 in the most degraded sample. These results suggest that these indices may be useful as source diagnostic parameters in light-to-moderately degraded oils, but are unreliable when prolonged weathering has been in effect.

These results provide clear evidence of the utility of the hopane isomer indicators in discriminating between heavily biotransformed oil samples. Hostettler & Kvenvolden (1994), for example, found that [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane] varied by 1.4 ± 0.2 between fresh crude oil spilled from the *Exxon Valdez* oil tanker and oily residues taken from beaches in the vicinity of the spill site several years after the incident. Similarly, Pande *et al.* (1994) found that this index ranged from 1.2 to 1.7 for five crude oils from the same geochemical family but at different stages of natural biodegradation, and Davies *et al.* (1987) observed that this index varied by only 0.04 from an initial value of 0.6 over the 437 day biodegradation of crude oil in submerged marine sediments. Sauer *et al.* (1993) calculated the ratio of [18α(H)21β(H)-trisnorhopane:17α (H)21β(H)-trisnorhopane], two C_{27} hopanes, to be within the range 2.6 to 3.3 for beached oil in the Arabian Gulf to establish a match with a Kuwait crude oil (index value = 2.7) believed to be the source of the contamination, although Wang *et al.* (1995) have suggested that this index becomes altered in heavily weathered samples and may be unreliable in such circumstances as a source correlation index. In their study, Wang *et al.* found that [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane] was the most reliable of the paired hopane source indices, as this maintained a value of 0.95 ± 0.2 between fresh crude oil and 13-year-old residues of the oil spilled on an Arctic Beach in a controlled oil spill environment.

In summary, the results obtained in this study provide firm evidence that hopane pair indices, specifically [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane], [17α(H)21β(H)-homohopane (22S):17α(H)21β(H)-homohopane (22R)] and [17α(H)21β(H)-bishomohopane:17α(H)21β(H)-methylhopane], may be used to support a correlation between fresh and weathered oil samples for source identification purposes not just in the crude oil-contaminated marine environment, but also for heavy and crude oil contamination of the terrestrial environment. This will be of major utility in helping to locate the source of petroleum products that have spread in the subsurface, either through lateral soil migration or carried by groundwater, to contaminate areas away from the original spill site. In particular, where liability for a contaminant site is disputed, e.g., when the source of residual contamination cannot be identified, GC-EI MS fingerprinting may be used to establish a match between the contaminant and a possible source candidate, even if it is highly weathered. This has significant legal implications, as well as assisting in a more general sense with contaminant treatment and risk assessment.

In light of the concerns detailed in Sections 1.4.2.1 and 1.4.2.2, and the research objectives detailed in Section 2.2, the following conclusions can be drawn from this study of biomarker indices:

(i) the [*n*-alkanes:17α(H)21β(H)-hopane] index was identified as the index most sensitive to oil biotransformation and the index that most accurately reflected the actual depletion of oil from the contaminated soil;

(ii) [C_{17}:pristane] and [C_{18}:phytane] may be used as indicators of oil weathering for lightly degraded samples;

(iii) [$\Sigma C_{14\text{-}28}$:C_{24}tetracyclic terpane] is a more accurate and sensitive weathering index than the isoprenoid:alkane indices, and this may be of particular use for middle distillate petroleum products;

(iv) the hopane pair indices [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane], [17α(H)21β(H)-homohopane (22S):17α(H)21β(H)-homohopane (22R)] and [17α(H)21β(H)-bishomohopane:17α(H)21β(H)-methylhopane] may be reliably used to support a correlation between fresh and weathered oil samples for source identification purposes in the contaminated soil environment, and;

(v) phytane appears to become depleted whenever significant biotransformation takes place, as demonstrated by the decrease in [phytane:17α(H)21β(H)-hopane] in the latter stages of the soil microcosm study. This index may, therefore, be used as a source correlation index for light-to-moderately weathered oil samples, but not for highly weathered samples. For the same reason, [C_{18}:phytane] may underestimate the extent of weathering experienced by an oil sample if significant weathering has taken place.

5.2.1.4 GC-IRMS Analysis

Compound specific isotope analysis of heavy and crude oil samples during the contaminant source term investigation demonstrated the validity of GC-IRMS in heavy oil analysis and provided bench-mark isotopic fingerprints of each oil (Table 4.5). The isotope ratios of individual *n*-alkanes and isoprenoids are useful oil correlation parameters (Section 5.1.4.3), but a more valuable insight into the utility of this technique in the characterisation of heavy oil-contaminated soils is provided by a study of the variations of compound isotope ratios over a period of oil weathering.

Results of the GC-IRMS analysis of ballast oil, crude oil and No.6 Fuel Oil extracts at successive stages of biotransformation, obtained through the microcosm study, are presented in Section 4.2.1.4 (Figure 4.22, 4.23, 4.24, respectively). An interesting feature of all three sets of results is that norpristane, the isoprenoid alkane biomarker, is isotopically heavier than the *n*-alkanes in the fresh samples. This is in contrast to the results for pristane, which had been previously found to be isotopically lighter than *n*-alkanes in the same oils (Section 5.1.4.3). A

plausible explanation for this is the documented unpredictability of the nature of isotopic variations between these two types of compounds, as discussed in Section 5.1.4.3.

For each oil, plots of the mean $\delta^{13}C$ variations of the five *n*-alkanes and norpristane over time (illustrated in Figure 4.22, 4.23 and 4.24) indicate that microbial degradation induces fluctuations in isotopic composition of these compounds, although the overall shifts in compound $\delta^{13}C$ over the entire 256 days are not substantial.

A number of observations on the patterns of isotopic fluctuation within the three oil can be made, based on the plots shown in Figures 4.22, 4.23 and 4.24. The GC-IRMS results for the ballast oil samples (Figure 4.22) were interpreted as follows:

(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 °/oo, 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 over the course of the study;

(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 $\delta^{13}C$ (of between 1.0 and 1.5 °/oo) over the first 4 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, or to soil matrix effects . 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} also varied in this way, but to a much lesser degree;

(iv) these shifts, though 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 $^{o}/_{oo}$ might be expected to result from microbial activity (e.g., Bowler *et al.*, 1993), which is approximately the shift observed here. 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. The microbial recalcitrance of these compounds limits isotopic fractionation, and so the combined alkane-isoprenoid peak will show a smaller overall shift than the fully resolved alkane peak.

For the crude oil samples (Figure 4.23), the following observations were made:

(i) isotope ratios in the crude oil samples appeared to shift in favour of the heavier isotope over the entire course of the study, typically by 1.5 - 2.0 $^{o}/_{oo}$, particularly over the latter stages. This suggests that the 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) this trend is reversed, however, by a decrease in all isotope ratios at the 64 day stage of the study. This may be due to a sudden shift in the rate of oil biotransformation, causing a marked rearrangement in compound isotopic fractionation, although no evidence for such a significant shift is seen in other results, or analytical effects, although similar changes were noted in controls and no marked changes in standard mixture $\delta^{13}C$ were detected. However, this does not appear to mark the beginning of a significant trend in compound isotopic variation, as the ratios continue to increase again after this point;

(iii) for these samples, 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 samples (Figure 4.24), no overall shift in isotope ratio was observed for any of the compounds over the course of the study. Individual values did appear to fluctuate in value between 0 and 256 days, but usually by no more than *ca.* 0.5 $^{o}/_{oo}$. This is within the reproducibility of the technique and cannot, therefore, be interpreted as a genuine manifestation of microbial activity. This is not surprising, however, given the poor biotransformation of the No.6 Fuel Oil over the course of the study.

The influence of microbial activity on oil isotopic composition has been studied by several authors. Stahl (1980) examined the nutrient-enhanced bacterial degradation of crude oil over 42 days by determining resultant compositional and stable carbon isotope variations within component class fractions. Although the aromatic and heterocompound (polar) fraction $\delta^{13}C$ values once again remained constant (at -27.6 °/oo and - 27.1 °/oo, respectively) throughout the experiment, the saturate fraction became isotopically heavier by 0.7 °/oo and the asphaltene fraction isotopically lighter by 1.1 °/oo.

Other studies using GC-IRMS have found no change in the isotopic composition of individual *n*-alkanes of a variety of crude oils with oil maturity or biodegradation (Sofer *et al.*, 1991). Many authors have also recognised the fact that whole oil $\delta^{13}C$ values often remain effectively constant during oil weathering and so can be used as source correlation parameters for weathered oils in the environment (Fan *et al.*, 1994; Madsen, 1992; Teschner & Wehner, 1985; Hostettler and Kvenvolden, 1994).

The results presented here for the ballast oil and crude oil reveal shifts in *n*-alkane $\delta^{13}C$ values of up to two-and-a-half times those observed by Stahl for the bulk saturate fraction. 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. These results suggest that the isoprenoid $\delta^{13}C$ is a more reliable source correlation index, since in this case it did not appear to shift in value.

Based on these results, it is possible to draw the following conclusions:

(i) it is conjectured, based on previous work (Stahl, 1980; Killops & Killops, 1983), that extensive biodegradation 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, although based on these results it is not possible to

unambiguously find this to be the case. Nevertheless, the observed shifts may undermine the use of oil $\delta^{13}C$ as source correlation parameters in some cases (e.g., for heavily mineralised oils);

(ii) the whole oil isotope ratio of oils containing a significant saturate class fraction content may become more positive following extensive microbial transformation, and so may not be completely reliable as a source correlation parameter;

(iii) use of this isotopic shift as an indicator of oil weathering is not recommended, however, without further work, due to its very low sensitivity to oil biotransformation, and the existance of other, more reliable indicators (e.g., biomarker weathering indices);

(iv) isoprenoid isotope ratios appear to be less affected by microbial degradation than *n*-alkanes, and may be of use as source correlation parameters in some cases.

5.2.2 Weathered Diesel Range Organics (DRO) Standards

In light of the complexity of the biotransformation discussed above, it was considered appropriate to support the results through the characterisation of a less complex, purely abiotically weathered system. The effect of physical weathering on weathering indices is likely to be greatest in the period immediately following spillage of lighter petroleum products, such as diesel oil, into relatively impervious receiving media (Section 1.3.2.2). To investigate this, four weathering indices and three source correlation indices were evaluated for diesel range organics (DRO) samples at three stages of physical weathering; fresh, 25 % $^{w}/_{w}$ weathered and 50 % $^{w}/_{w}$ weathered. Just as biotic losses are measured according to the relative biotransformation rates of index compounds, so physical weathering should produce variations in index values related to the difference in the volatility of the index compounds. Where this difference is large, physical weathering might be expected to induce significant variations in the value of the index.

GC-FID chromatograms were supplied by the manufacturer as evidence of weathering, and, as discussed in Section 1.3.2.2, these demonstrated the increasing loss of

lower boiling *n*-alkanes and growing amount of UCM associated with weathering. The primary purpose of these standards was to provide supplementary insight into the trends and behaviour of the weathering and source indices characterised in the microcosm study.

5.2.2.1 GC-EI MS Analysis

(i) Performance of Weathering Indices

In this study, results indicate that abiotic loss mechanisms could have a significant effect on weathering index values. As expected, physical weathering exerts the greatest influence on those indices in which the tricyclic terpanes are measured ([*n*-alkanes:tricyclic terpanes] and [C_{18}:tricyclic terpanes]) (Table 4.17). This is presumably due to the high boiling points of these compounds relative to other diesel components.

Both [C_{18}:phytane] and [C_{17}:pristane] indices appear to be largely unaffected, at 2.1 and 1.9, respectively, by light physical weathering (up to 25 $\%^{w}/_{w}$ in the weathered DRO samples). However, for DRO samples that were 50 $\%^{w}/_{w}$ weathered, these indices varied in value by up to 0.7 (increasing for [C_{17}:pristane] and decreasing [C_{18}:phytane]). The implications of these results are that physical weathering of oil contaminants does have an effect on the value of weathering indices, in largely the same manner as microbial weathering, and that, therefore, where significant physical weathering is suspected of having taken place, reductions in the values of weathering indices described in this section and in Section 5.2.1.3 are not necessarily due to microbial activity. In such cases, laboratory studies can be used to distinguish abiotic from biotic weathering (such as the soil microcosms described in this thesis) and unambiguously interpret variations in oil weathering indices.

(ii) Performance of Source Correlation Indices

These results provide further evidence that both pristane and phytane were ultimately susceptible to depletion through physical weathering and, therefore, their use in source correlation indices is inappropriate in significantly weathered samples. This is evidenced by the

steady decrease in the values of [pristane:phytane], [pristane:tricyclic terpanes] and [phytane:tricyclic terpanes] between fresh and 50 $\%\ ^{w}/_{w}$ weathered DRO.

More generally, it would appear that in the absence of a source correlation index that maintains its value throughout the weathering process, defining source commonality between mid-distillate petroleum products such as the DRO samples analysed here may require a more careful consideration of oil characteristics rather than a straightforward calculation of pristane:phytane or isoprenoid:tricyclic terpane ratios, particularly for highly weathered samples. Alternative oil fingerprinting techniques were demonstrated by Baugh and Lovegreen (1990), who were able to define different refined petroleum products using representative GC-FID profiles, and differentiate between crude and refined petroleum products using the C_{20}-C_{30} hydrocarbon GC peaks.

5.2.2.2 GC-IRMS Analysis

The range of compound specific isotope value presented in Figure 4.25 for the respective DRO standards indicates that, in this case, physical weathering has caused the preferential removal of the heavier isotope from the DRO mixture. This is manifested by decreases in compound isotope ratios of up to 2 $^{o}/_{oo}$. Previous work by Aggarwal & Hinchee (1991) on the isotopic composition of CO_2 produced from the biotransformation of fuel oil in the soil environment indicated that the heavier isotope may be partitioned into the atmosphere, although no measurements of the actual residual petroleum compounds were made.

In this purely empirical study, therefore, results suggest that isotopic changes in individual alkanes, norpristane, pristane and phytane within DRO standards indicated that significant physical weathering of oils caused preferential depletion of the heavier isotope, leaving residual compounds isotopically lighter by up to 2 $^{o}/_{oo}$. This may be a possible explanation for the shifts in isotope ratio observed for the individual compounds within the ballast oil and crude oil microcosms between 0 and 2 days, when it was speculated (and indicated

by control microcosms) that physical weathering was most significant. If so, a possible interpretation of the results is that the initial physical weathering of oil causes preferential partitioning of the heavier isotope into the atmosphere, leaving the residual oil isotopically lighter (although no mechanism for this can be put forward based on these results). Following this, microbial transformation of the oil becomes the dominant route of contaminant depletion, which causes the isotope ratios of residual oil compounds to increase, as the soil microorganisms preferentially catabolise C_{12}-C_{12} bonds (which possess a lower bond energy than C_{12}-C_{13} bonds). The extent of isotope discrimination in this respect has yet to be established in the literature.

If these isotopic shifts are genuine, and not an artefact of the physical weathering process itself, this may shroud the isotopic effects of biotransformation (which causes residual oils to become isotopically heavier) in circumstances where physical weathering is a major route of contaminant loss. If possible, therefore, it would be desirable to assess the relative significance of possible weathering routes through additional means (e.g., laboratory microcosms, assessment of prevailing environmental conditions).

In summary, the information produced from the soil microcosm study demonstrated that the weathering of petroleum contaminants in the soil environment can be elucidated to a greater extent than that currently achieved through the application of appropriate analytical methods. Specifically, evaluation of the [*n*-alkanes:17α(H)21β(H)-hopane] index was identified as the most sensitive to oil biotransformation and the index that most accurately reflected the actual depletion of oil from the contaminated soil. The commonly used indices [C_{17}:pristane] and [C_{18}:phytane] were found to be of use as an indicator of oil weathering only for lightly degraded samples. Furthermore, hopane pair indices were shown to be a reliable means of supporting a correlation between fresh and weathered oil samples for source identification purposes in the contaminated soil environment.

The benefits of this enhanced understanding are greatest during the remediation of contaminated land, where a profile of the contaminant beyond a measurement of the total abundance is desirable for estimated on-going risks and further remediation requirements, and also in the identification of contaminants sources, particularly following incidents of cross-boundary migration of contaminants. An insight into the subsurface partitioning of heavy oils may also be gained through the application of the analytical approach detailed in this study, which allows possible exposure routes to be assessed. For the oils analysed in this study, for example, the No.6 Fuel Oil with its high polar and asphaltene content would exhibit a considerable residual NAPL phase, that was poorly bioavailable but which, however, would tend to leach polar contaminants into surrounding groundwater. Major exposure routes for this contaminant would, therefore, be ingestion of free contaminant with soil and consumption of contaminated groundwater. Moreover, because of its poor bioavailability, soil contaminated with this oil would not be expected to be readily cleaned up using bioremediation techniques. This exposure/remediation scenario is typical for heavy oils, given their preponderance of polar/asphaltenic components. Other heavy oils containing significant high-molecular weight alkanes, such as the waxy distillate, might be expected to be amenable to bioremediation techniques, and groundwater contamination would be less likely, since the polar fraction of these oils is very small. This information may be of considerable use to researchers and practitioners in the field of contaminated land assessment and facilitates a unique account of heavy oil characterisation and biotransformation.

CHAPTER 6. CONCLUSIONS

The conclusions to this thesis can be considered in five distinct groups that reflect the objectives of the research.

6.1 Overall Strategy

- The construction of a tiered analytical strategy provides a means by which the chemical analysis of reference heavy oils, hitherto characterised primarily according to total amount only, can be achieved.
- The tiered analytical strategy detailed in this work develops existing schemes so as to incorporate a wider set of tools suited to the characterisation of heavy oils in the contaminated soil environment.
- The application of the selected techniques to heavy oil contaminants in the soil environment in this work supports the hypothesis that such wastes harbour important characteristic chemical information essential to their assessment and remediation, and that through the application of appropriate analytical methods, this information can be obtained.

6.2 Contaminant Source Term Screening

- Soxhlet extraction results support the view that this method is of definite utility in the extraction of semi-volatile and non-volatile organics from soil matices, provided the appropriate quality control checks are carried out, and indicate that the SEM variations in the microcosms study are reliable.
- Column fractionation results provide evidence that the fractionation of oil samples by rapid column chromatography can be used: (i) to provide an important benchmark against which the utility of subsequent screening techniques could be established; (ii)

to facilitate the reliable, rapid class fraction screening of heavy oils; (iii) to provide a reliable means of isolating the saturate class fraction from interference in preparation for detailed component analysis, which is of key importance to the identification of diagnostic source ratios and weathering indices.

- TLC-FID is a reliable, robust and cost-effective screening technique capable of providing a class fraction fingerprint of heavy oils and residual petroleum wastes that may be used to assess waste weathered state and bioremediation potential.
- IRMS analysis of oils appears to be a potentially useful method for identifying a predominance of polar and asphaltenic material in heavy oils, and for providing an additional source correlation parameter for undegraded oily contaminants.
- Isotope type curves may be used to distinguish between petroleum fuels containing markedly different class fraction distributions and to indicate the presence of a large polar plus asphaltene content. However, they appear unsuitable for discriminating between oils of similar composition.

6.3 Contaminant Source Term Detailed Analysis

- GC-FID results re-confirm that for extremely complex petroleum residues, in this case the bitumen and acid tar samples, even extensive column chromatographic fractionation and high temperature GC conditions cannot produce any more than a broad qualitative indication of sample complexity.
- The application of GC-EI MS target component indices to the assessment of heavy oils in this way represents a novel extension of their utility. Results indicate that target analysis can be used to estimate the approximate 'heaviness' of oil samples, as determined by their composition, and so convey information on the bioremediation potential of the waste matrix (which diminishes for heavier oil wastes).

- Based on the GC-EI MS results, the [tri- and tetracyclic hopanes:pentacyclic hopanes] index is proposed as the oil correlation index best able to differentiate between both fresh and weathered crude oils.
- GC-EI MS results for the [pristane:phytane] index suggest that an upper limit of variation between positively correlated oils of 0.3 is insufficient to discriminate between different crude oils in this study, and that, therefore, a lower limit of variation (of *ca.* 0.15) may be more reliable.
- GC-IRMS can be legitimately used to obtain information on the isotopic composition of *n*-alkanes and abundant isoprenoid alkanes in heavy oils, provided appropriate cautions to minimise or estimate the effect of the UCM on the measured isotope ratios are taken, but cannot detect hopane or sterane biomarkers.
- Furthermore, for extremely heavy oil (in this case the acid tars and bitumen samples), GC-IRMS analysis is not recommended, since resolution of individual peaks prior to isotopic analysis is not possible (as demonstrated by the GC-FID results).
- GC-IRMS results demonstrate that for the heavy oils analysed in this study, there is no observable variation in *n*-alkane isotopic composition with carbon number, and that the isoprenoid alkanes are isotopically lighter than their corresponding *n*-alkanes.

6.4 Oil Microcosm Study

- Variations in solvent extractable material (SEM) from successively weathered oil samples suggest that the biotransformation of the oils in this study followed approximate first order kinetics, particularly over the later stages of the study when oil concentrations are low relative to the active soil microbial population.
- SEM results also show that microbial transformation reduced the amounts of ballast oil and crude oil in contaminated soils by approximately 60 $\%\ ^{w}/_{w}$ in both cases. From an

initial soil loading of 20 g of oil per kg dry soil, this represents an overall microbial degradation rate of 0.05 g oil per kg soil per day.

- In the ballast oil and crude oil microcosms, saturate class fraction compounds were depleted by *ca.* 90 % and 75 %, respectively, of their original values. This provides further evidence that these oils have been heavily mineralised by soil microbial populations.
- Asphaltene and polar compounds remained at constant levels throughout the period of study, thereby demonstrating their recalcitrance to microbial transformation;
- In the No.6 Fuel Oil microcosms, a slight depletion in saturate class fraction abundance was accompanied by an increase in asphaltene abundance. This has important implications for the bioremediation potential of an oily waste, where an increasing asphaltene abundance may prevent microbial transformation of the oil from occurring, and could go unnoticed if a class fraction characterisation of the oily waste in question is not carried out.
- The [*n*-alkanes:17α(H)21β(H)-hopane] index was identified as the index most sensitive to oil biotransformation and the index that most accurately reflected the actual depletion of oil from the contaminated soil.
- [C_{17}:pristane] and [C_{18}:phytane] may be used as an indicator of oil weathering for lightly degraded samples, and for initial verification of biotransformation.
- [$\Sigma C_{14\text{-}28}$:C_{24}tetracyclic terpane] is a more accurate and sensitive weathering index than the isoprenoid:alkane indices, and this may be of particular use for middle distillate petroleum products.
- The hopane pair indices [17α(H)21β(H)-norhopane:17α(H)21β(H)-hopane], [17α(H)21β(H)-homohopane (22S):17α(H)21β(H)-homohopane (22R)] and [17α(H)21β(H)-bishomohopane:17α(H)21β(H)-methylhopane] may be reliably used

to support a correlation between fresh and weathered oil samples for source identification purposes in the contaminated soil environment.

- Phytane appears to become depleted whenever significant biotransformation takes place, as demonstrated by the decrease in [phytane:17α(H)21β(H)-hopane] in the latter stages of the soil microcosm study. This index may, therefore, be used as a source correlation index for light-to-moderately weathered oil samples, but not for highly weathered samples. For the same reason, [C_{18}:phytane] may underestimate the extent of weathering experienced by an oil sample if significant weathering has taken place.
- Extensive biodegradation is likely to have 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, which was not observed in control studies, and this may undermine their use as source correlation parameters in some cases (e.g., for heavily mineralised oils).
- The whole oil isotope ratio of oils containing a significant saturate class fraction content may become more positive following extensive microbial transformation, and so may not be completely reliable as a source correlation parameter.
- Use of GC-IRMS-detected isotopic shifts as indicators of oil weathering is not recommended without further work, because the signal-to-noise ratio for many of the peaks was too low, and because the magnitude of the overall isotopic shifts over the course of the study was small.
- The use of isoprenoid alkane isotope ratios as source correlation parameters in biotransformed oils could not be validated, because of the conflicting results for norpristane $\delta^{13}C$ in the ballast oil and crude oil microcosms. The use of isoprenoids, or other biomarkers, in this context may be established through future study.

6.5 Physical Weathering Study of Diesel Range Organics

- In this study, physical weathering exerted the greatest influence on those indices in which the tricyclic terpanes are measured ([*n*-alkanes:tricyclic terpanes] and [C_{18}:tricyclic terpanes]) (Table 4.17), presumably as a result of the high boiling points of these compounds relative to other diesel components.
- Both [C_{18}:phytane] and [C_{17}:pristane] indices would appear to be largely unaffected by light physical weathering. However, results suggest that prolonged or intense periods of physical weathering could induce significant fluctuations in the values of these indices. The implications of these results may be profound in situations where changes in the values of these biomarker indices are used as evidence of microbial activity.
- Extensive physical (abiotic) weathering may also cause shifts in weathering indices that could be mistakenly attributed to microbial activity. Where substantial physical weathering of oil contaminants is a possibility, therefore, the use of laboratory tests (e.g., soil microcosms) to elucidate the route of weathering is recommended.
- Isotopic changes in individual alkanes, norpristane, pristane and phytane within DRO standards indicated that significant physical weathering of oils caused preferential depletion of the heavier isotope, leaving residual compounds isotopically lighter by up to 2 $^{o}/_{oo}$. If genuine, this may shroud the isotopic effects of biotransformation (which causes residual oils to become isotopically heavier) in circumstances where physical weathering is a major route of contaminant loss.

CHAPTER 7. FUTURE WORK

The work presented in this thesis is an investigation into the capacity of available analytical techniques to provide information of relevance to the assessment and remediation of heavy oil-contaminated soils. Because of the wide-ranging nature of the work, a number of possible themes for future work were identified:

(i) Firstly, method development of the analytical techniques necessitated the use of a number of carefully chosen, representative heavy oil samples. To expand on the results obtained for these oils, particularly those obtained by TLC-FID and bulk IRMS screening methods, a wider range of petroleum products should be analysed. For the TLC-FID it may be particularly useful to build up a library of class fraction fingerprints such that its use as a diagnostic screening method for determining the type of oil contaminant is enhanced (in much the same way as GC-FID fingerprinting). For the bulk IRMS, the characterisation of a wider range of samples may allow greater elucidation of the relationship between whole oil isotopic composition and oil composition and, therefore, consolidate its role as a useful screening tool;

(ii) Secondly, having used a variety of representative heavy oil wastes, it would be of value to extend the tiered analytical strategy to field application, particularly to characterise *in-situ* engineered bioremediation or natural attenuation, as the ultimate aim of the work is to promote the effective treatment of heavy oil wastes at actual contaminated sites;

(iii) Thirdly, in this work, bulk IRMS was identified as a potential screening tool for heavy oil wastes. As oils frequently undergo microbial transformation in soil, monitoring the whole oil isotopic variations within petroleum contaminants as a result of microbial transformation would also be of value as a future research endeavour. The use of bulk IRMS screening as a means of rapidly assessing the biotransformation of heavy oil wastes would be a valuable tool for practitioners, given the limitations of current screening methods in this context.

(iv) Fourthly, the ability to discriminate between multiple sources at petroleum-contaminated sites would be especially useful, since such mixtures are commonly encountered at actual sites and have serious implications for the apportionment of liability. In this study, those biomarker source indices that can most reliably be used to correlate oils and suspected sources were identified. Extension of the ability of biomarker source correlation indices to distinguish between different source terms in oil mixtures is, therefore, identified as a key topic for future work;

(v) Fifthly, further work may also be focused on the use of GC-IRMS in the analysis of heavy oils, and, in particular, on the detection and analysis of hopane biomarkers in oil samples. Isotopic fingerprints comprising *n*-alkane, norpristane, pristane, phytane and hopane isotope ratios have been shown to be of use as a source fingerprints in a geochemical context (Schoell *et al.*, 1992/3/4), and so may also be use in the characterisation of terrestrial petroleum contaminants;

(vi) Finally, the relationship between the shifts in weathering indices during oil biotransformation and other indicators of microbial activity (e.g., production of CO_2 or variations in soil microbiological communities) is another area of possible future work that would consolidate the use of weathering indices as a means of determining microbial breakdown of oily wastes.

CHAPTER 8. REFERENCES

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APPENDIX A

The information supplied in this Appendix is selected raw data obtained from the analysis of heavy oil source terms and the soil microcosm study. The data illustrates the nature of the oils, and provides examples of the type of output obtained, and the level of data interpretation required, during the study.

A1 - GC-EI MS of a standard C_{17}/pristane, C_{18}/phytane mix for peak identification.

A2, A3 - GC-EI MS m/z 85 chromatogram for ballast and crude oil control microcosms, illustrating the lack of component depletion in these oils.

A4, A5 - GC-EI MS profile of *n*-alkanes in Nigerian crude oil obtained in previous CRPB study (A4) and in this study (A5), for comparative purposes.

A6, A7 - GC-EI MS profile of terpane biomarkers in Nigerian crude oil obtained in previous CRPB study (A4) and in this study (A5), for comparative purposes.

A8 - GC-EI MS m/z 85 for AT1, illustrating the spread of *n*-alkanes in this sample.

A9 - GC-EI MS m/z 191 for AT1, illustrating the terpane distribution in this sample.

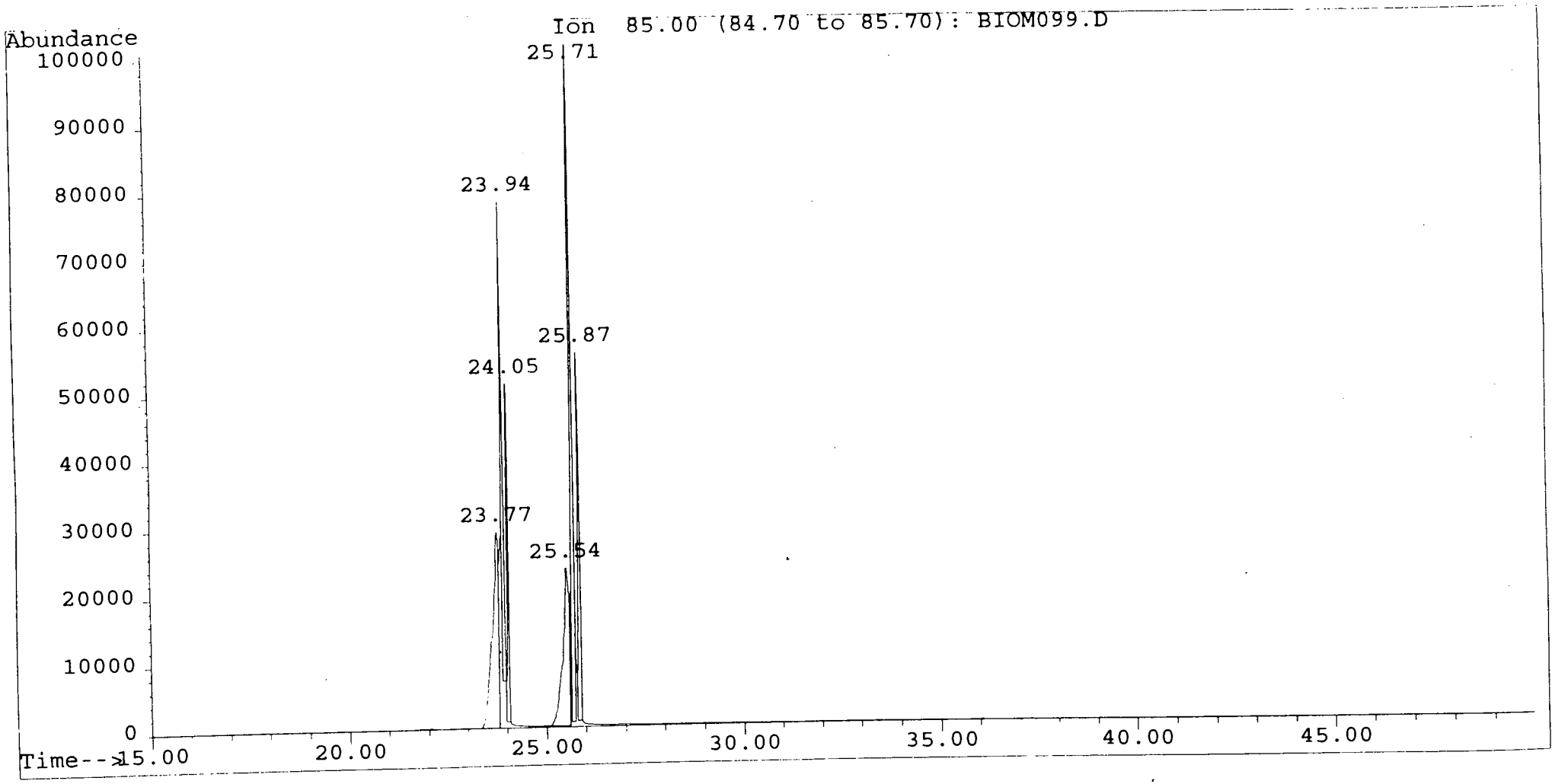
A10 - GC-IRMS chromatogram of standard *n*-alkane mix, for peak identification.

A11, A12 - GC-IRMS chromatograms of 25 $\%^{w}/_{w}$ and 50 $\%^{w}/_{w}$ weathered DRO standards, respectively.

A13-A17 - GC-IRMS chromatograms for ballast oil, API separator oil, residue oil, No.6 Fuel Oil and Nigerian crude oil saturates, illustrating the problems of UCM with heavy oils and the interpretive difficulties encountered.

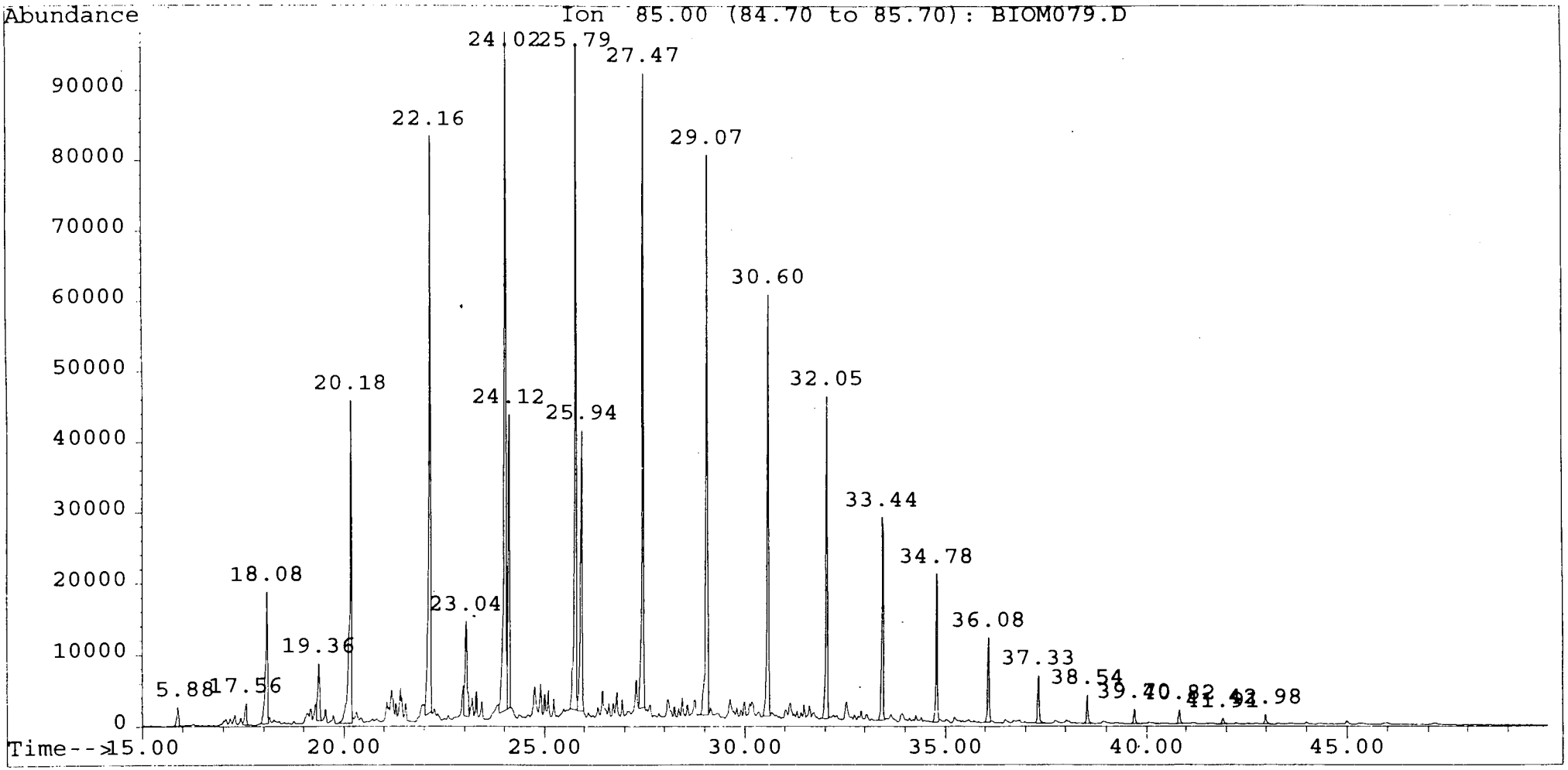
File : C:\HPCHEM\2\DATA\BIOM099.D
Operator : Geochem Analytical Services
Acquired : 28 May 96 5:13 pm using AcqMethod BIOM
Instrument : 5972 MSD
Sample Name: sample 99
Misc Info :
Vial Number: 74

A1:- GC-EI MS of a standard C_{17}/pristane, C_{18}/phytane mix for peak identification



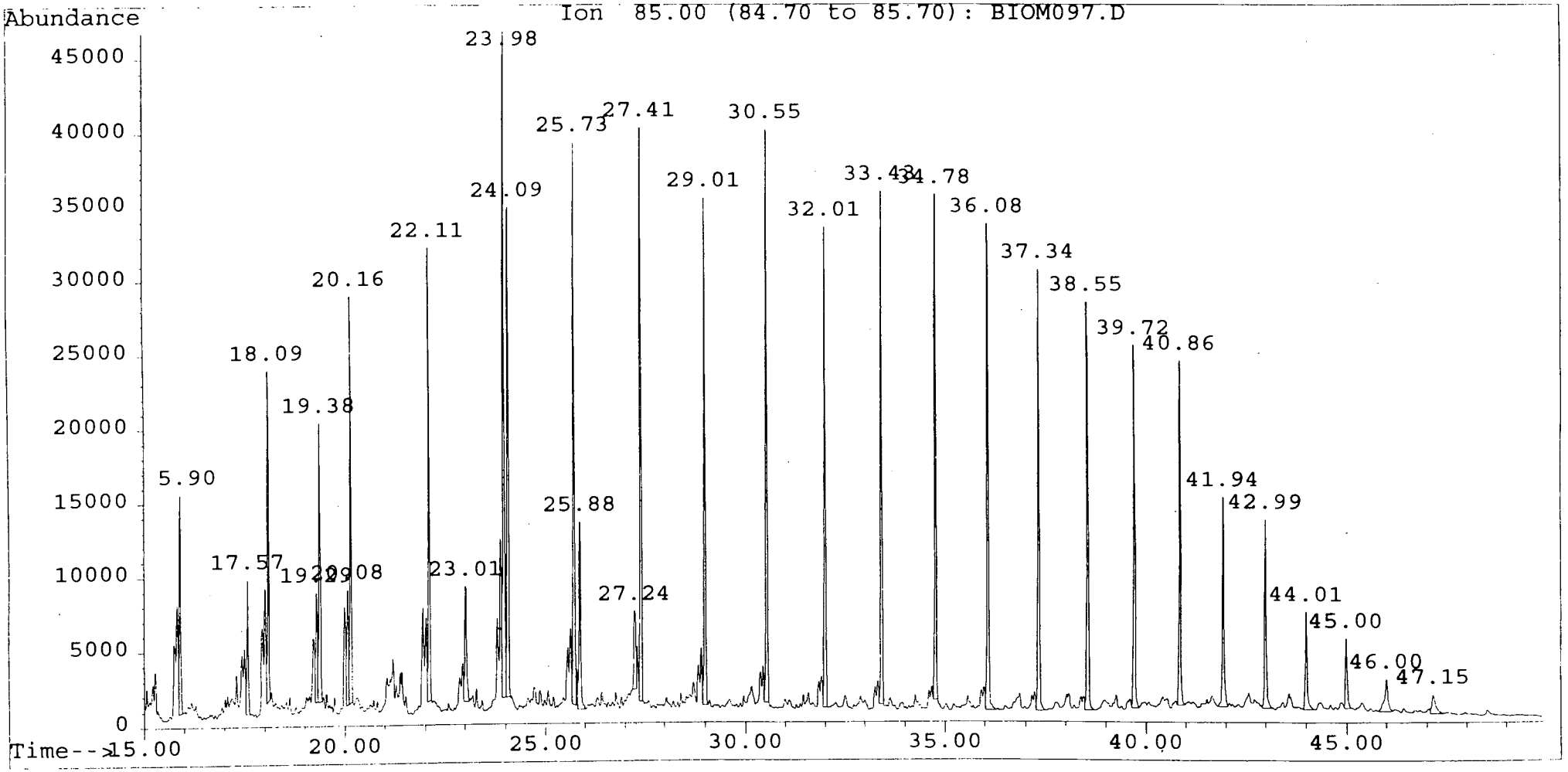
A2:- GC-EI MS m/z 85 chromatogram for ballast oil control microcosms after 256 days

File : C:\HPCHEM\2\DATA\BIOM079.D
Operator : Geochem Analytical Services
Acquired : 26 May 96 1:30 pm using AcqMethod BIOM
Instrument : 5972 MSD
Sample Name: sample 79
Misc Info :
Vial Number: 54

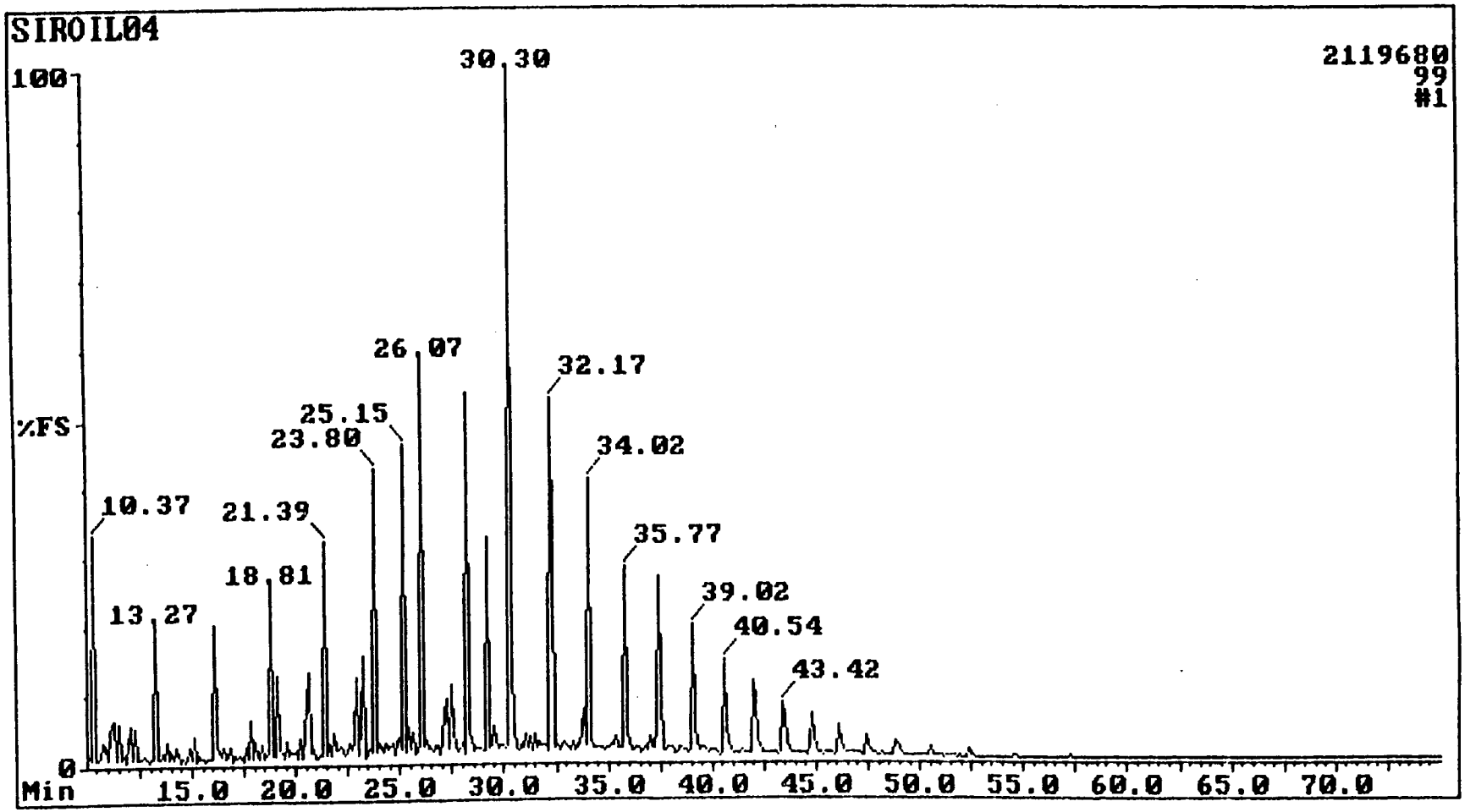


A3:- GC-EI MS m/z 85 chromatogram for crude oil control microcosms after 256 days

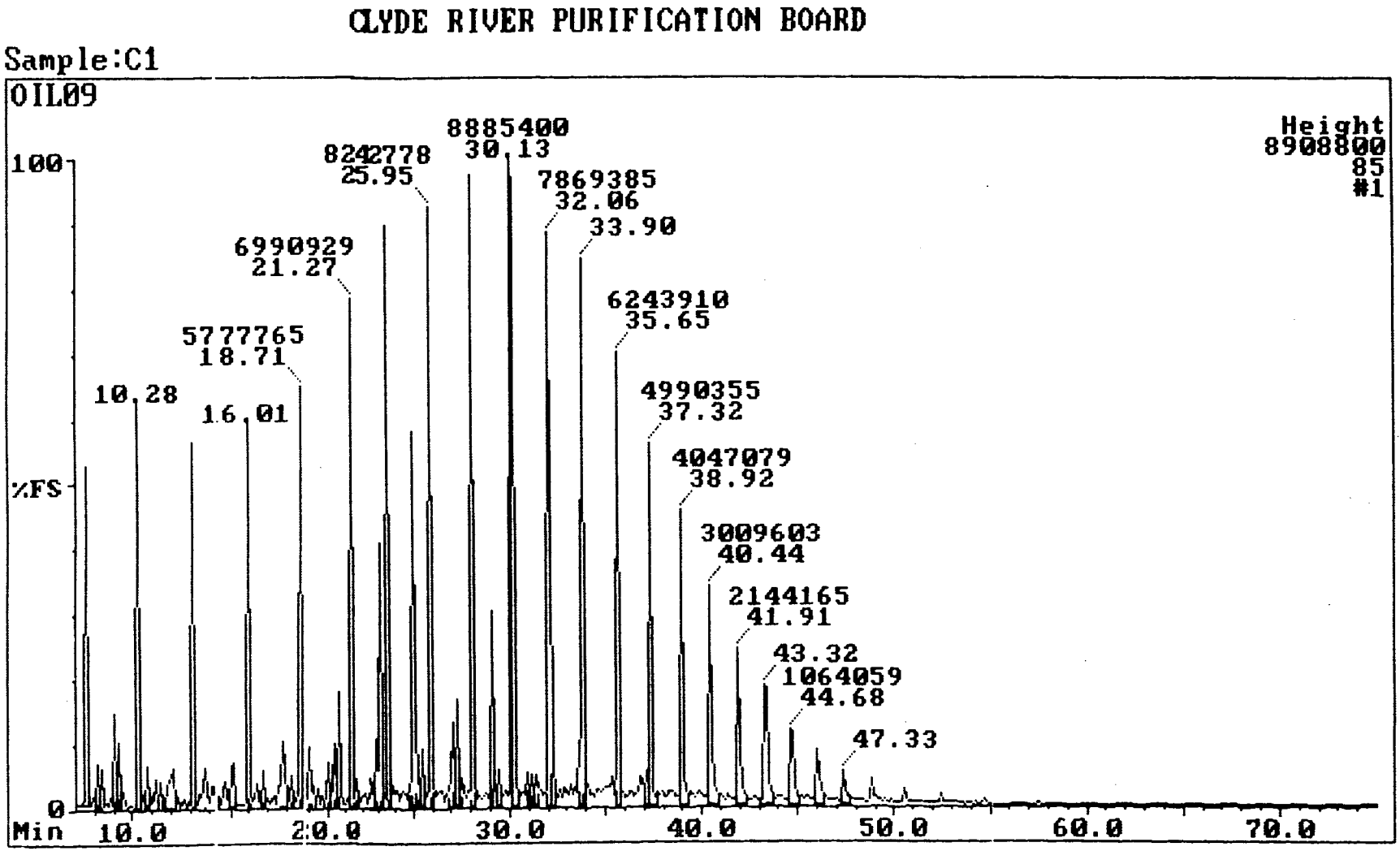
File : C:\HPCHEM\2\DATA\BIOM097.D
Operator : Geochem Analytical Services
Acquired : 27 May 96 12:42 pm using AcqMethod BIOM
Instrument : 5972 MSD
Sample Name: sample 97
Misc Info :
Vial Number: 72



A4:- GC-EI MS profile of *n*-alkanes in Nigerian crude oil obtained by Clyde River Purification Board



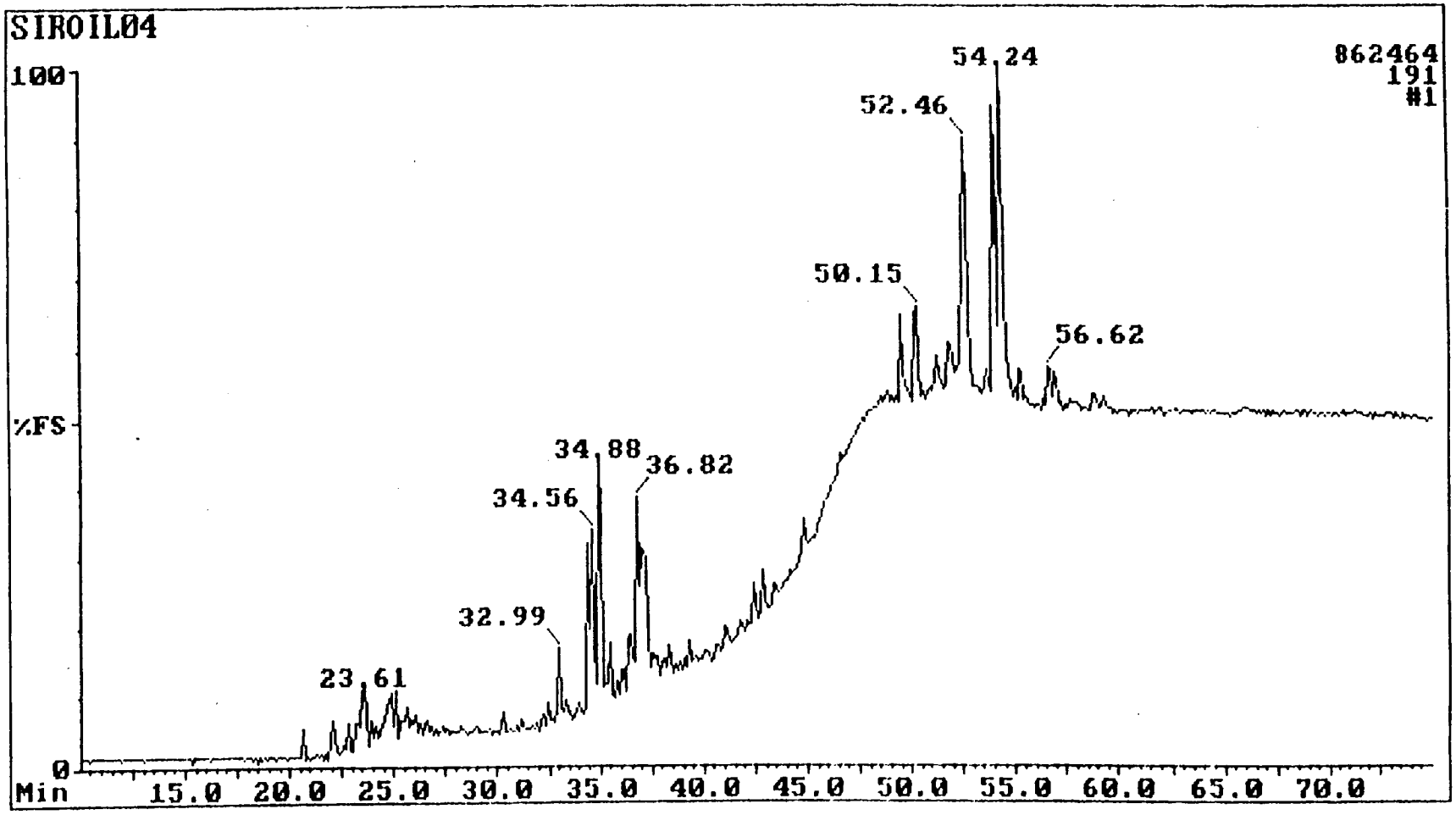
A5.- GC-EI MS profile of *n*-alkanes in Algerian crude oil obtained in this study



A5

Tricyclic Terpanes and Pentacyclic Triterpanes m/z 191
(Reference Chromatogram)

CLYDE RIVER PURIFICATION BOARD

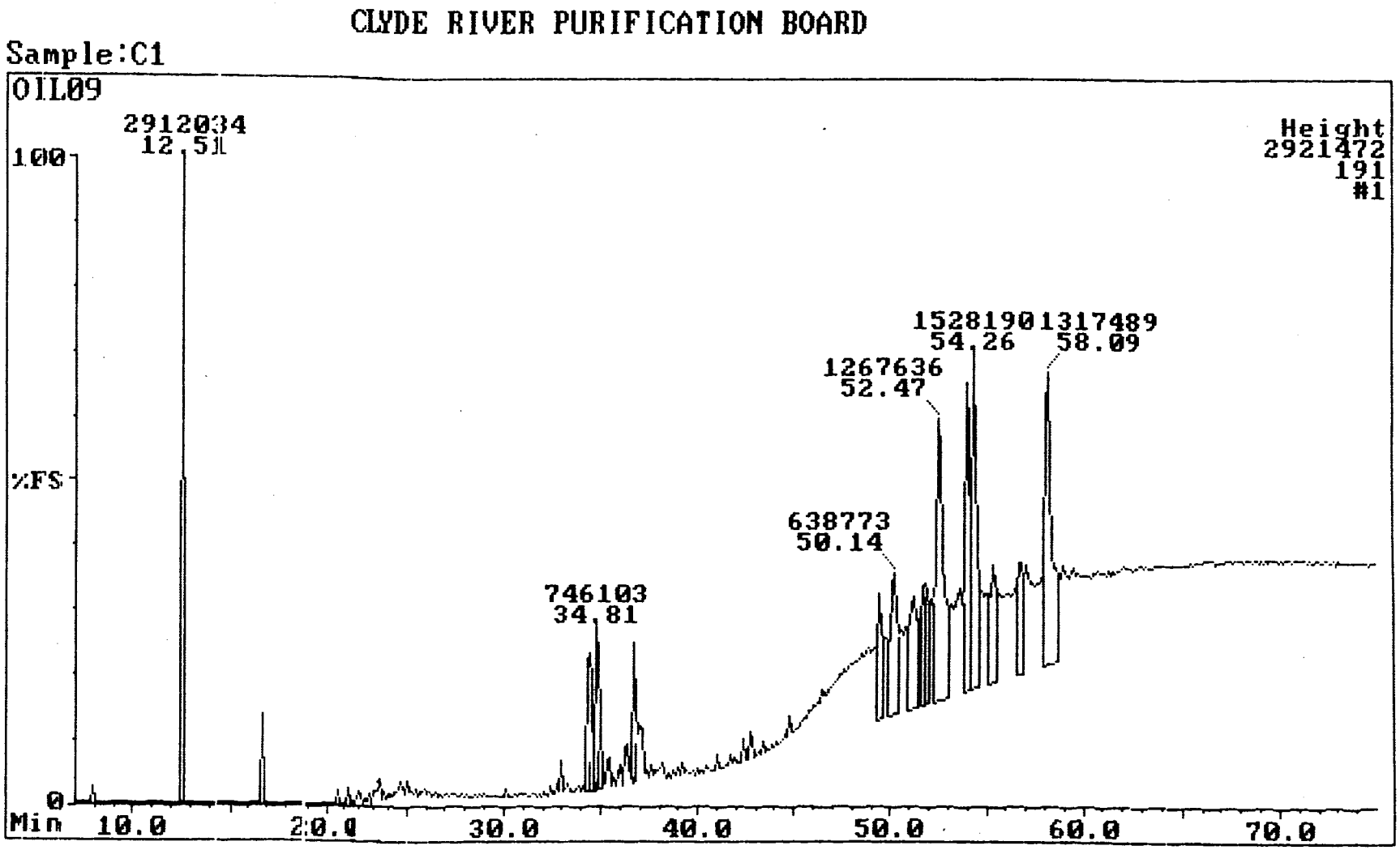


A6

A6:- GC-EI MS profile of terpane biomarkers in Nigerian crude oil obtained by Clyde River Purification Board

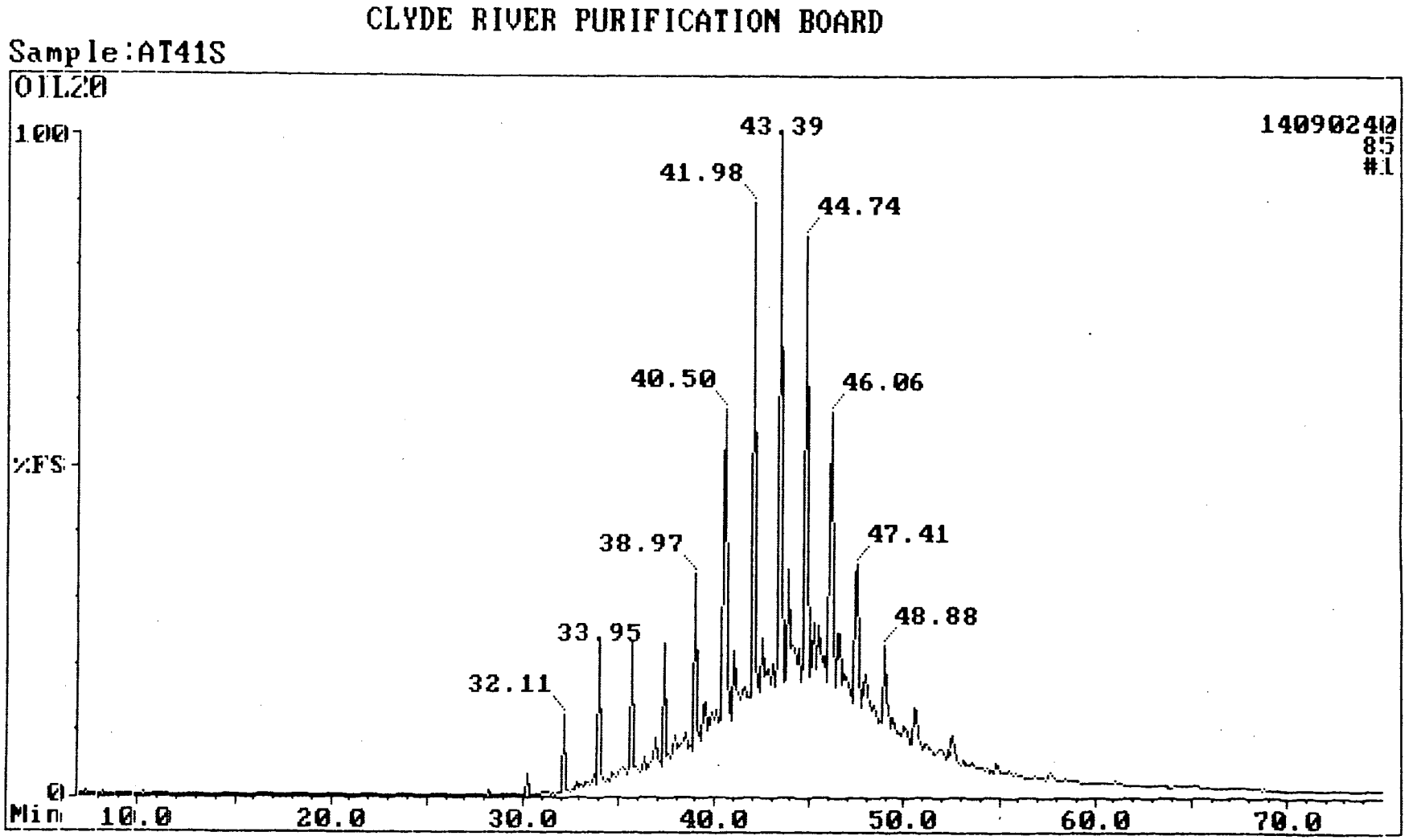
Crude oil
M/z 191

A7:- GC-EI MS profile of terpane biomarkers in Nigerian crude oil obtained in this study



A7

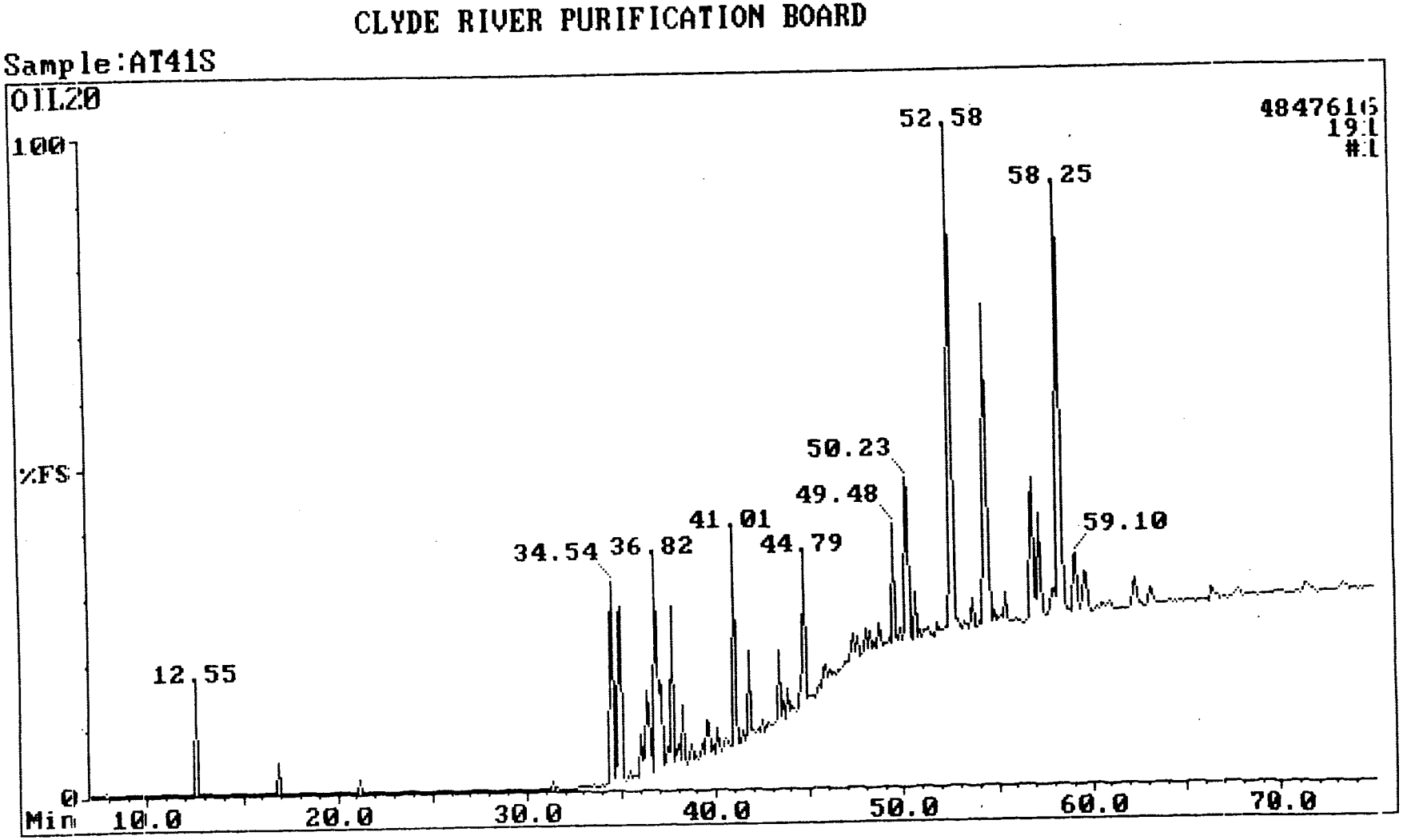
ACID TAR 1 M/Z 85
CHROMATOGRAM



A8

AT1 M/z 191 Chromatogram

A9:- GC-EI MS m/z 191 for AT1



A9

GC/IRMS of Standard Mix of n-Alkanes

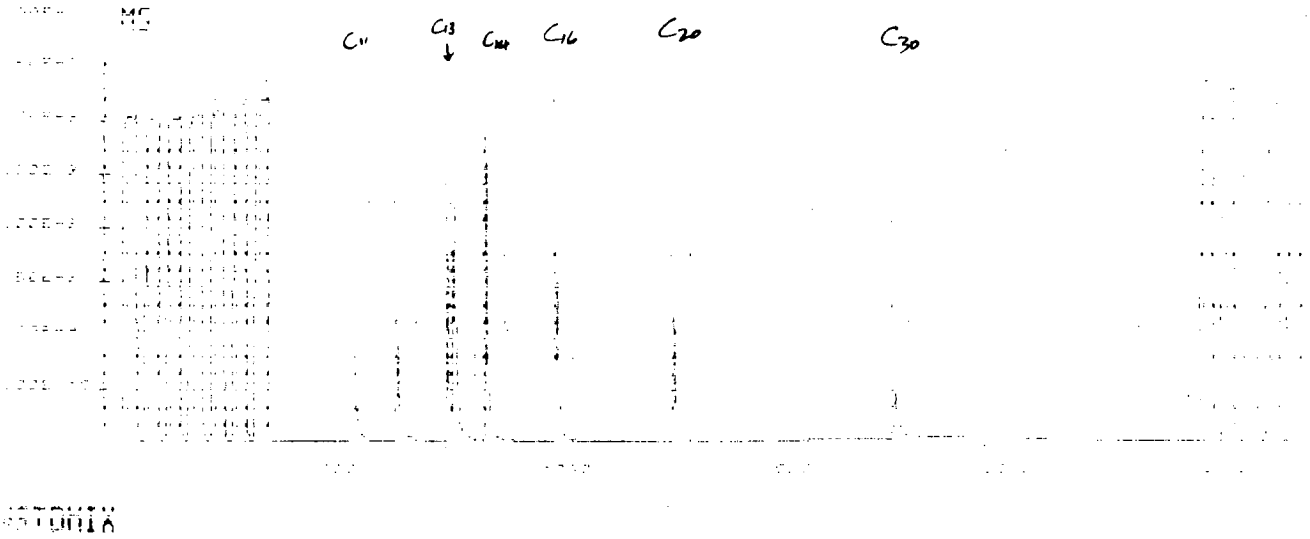
sochrom GC/IRMS
WSTDMIX run taken at 15:38:16 21/03/95

nalysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
58.9	3.817E-07	1.1582E-02	4.1600E-03
108.9	3.829E-07	1.1582E-02	4.1601E-03
158.8	3.835E-07	1.1582E-02	4.1601E-03
208.9	3.878E-07	1.1581E-02	4.1601E-03
258.8	3.950E-07	1.1581E-02	4.1599E-03
308.8	4.009E-07	1.1581E-02	4.1600E-03
358.9	4.065E-07	1.1581E-02	4.1600E-03
508.7	4.313E-07	1.1582E-02	4.1609E-03
558.6	4.209E-07	1.1583E-02	4.1610E-03
608.6	4.129E-07	1.1584E-02	4.1611E-03
658.7	4.063E-07	1.1584E-02	4.1611E-03
708.6	4.025E-07	1.1585E-02	4.1609E-03
758.7	3.993E-07	1.1586E-02	4.1610E-03
808.7	3.961E-07	1.1586E-02	4.1610E-03
td dev of fit		**1.0983E-06**	**8.6873E-08**

nalysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13
573.1	3.573E-08	1.1796E-02	4.1346E-03	1.0860	-22.96
667.3	6.290E-08	1.1791E-02	4.1330E-03	1.0856	-23.36
780.4	7.982E-08	1.1636E-02	4.1319E-03	1.0705	-37.04
796.3	1.238E-07	1.1757E-02	4.1285E-03	1.0823	-26.31
868.9	1.370E-07	1.1711E-02	4.1282E-03	1.0778	-30.45
027.4	9.010E-08	1.1726E-02	4.1301E-03	1.0792	-29.16
295.8	5.743E-08	1.1689E-02	4.1310E-03	1.0756	-32.43
797.7	3.876E-08	1.1736E-02	4.1304E-03	1.0801	-28.31



A10:- GC-IRMS chromatogram of standard *n*-alkane mix

All:- GC-IRMS chromatograms of 25 %$^{w}/_{w}$ weathered DRO standard

sochrom GC/IRMS
WSM25 (3) run taken at 16:15:39 12/04/95

nalysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
58.9	3.619E-07	1.1046E-02	4.1621E-03
108.8	3.617E-07	1.1044E-02	4.1576E-03
158.8	3.623E-07	1.1041E-02	4.1612E-03
208.9	3.677E-07	1.1039E-02	4.1620E-03
258.8	3.745E-07	1.1036E-02	4.1621E-03
308.8	3.819E-07	1.1031E-02	4.1616E-03
358.9	3.881E-07	1.1029E-02	4.1617E-03
508.7	4.043E-07	1.0820E-02	4.1625E-03
558.6	3.976E-07	1.0828E-02	4.1631E-03
608.6	3.917E-07	1.0829E-02	4.1626E-03
658.7	3.874E-07	1.0826E-02	4.1605E-03
708.6	3.833E-07	1.0829E-02	4.1602E-03
758.7	3.803E-07	1.0828E-02	4.1565E-03
808.6	3.777E-07	1.0827E-02	4.1590E-03
td dev of fit		8.0846E-06	2.0184E-06

GC-IRMS of 25% WEATHERED DRO STANDARD

nalysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13
679.6	2.622E-08	1.1129E-02	4.1592E-03	1.0834	-25.33
750.6	3.924E-08	1.1100E-02	4.1569E-03	1.0811	-27.39
775.9	7.112E-08	1.1109E-02	4.1525E-03	1.0823	-26.31
792.5	4.951E-08	1.1093E-02	4.1542E-03	1.0807	-27.77
846.1	4.982E-08	1.1092E-02	4.1509E-03	1.0811	-27.38
866.0	7.795E-08	1.1102E-02	4.1491E-03	1.0824	-26.25
876.7	3.847E-08	1.1074E-02	4.1523E-03	1.0796	-28.78
892.0	6.729E-08	1.1093E-02	4.1479E-03	1.0817	-26.84
907.6	3.594E-08	1.1098E-02	4.1500E-03	1.0823	-26.29
917.0	5.067E-08	1.1093E-02	4.1462E-03	1.0820	-26.63
948.4	9.680E-08	1.1094E-02	4.1460E-03	1.0823	-26.30
981.2	6.018E-08	1.1112E-02	4.1308E-03	1.0846	-24.20
995.7	7.621E-08	1.1107E-02	4.1434E-03	1.0842	-24.64
025.6	1.524E-07	1.1103E-02	4.1256E-03	1.0841	-24.64
061.4	7.766E-08	1.1107E-02	4.1439E-03	1.0847	-24.17
070.4	6.009E-08	1.1094E-02	4.1470E-03	1.0834	-25.29
099.2	7.083E-08	1.1125E-02	4.1408E-03	1.0870	-22.09
102.4	7.283E-08	1.1070E-02	4.1402E-03	1.0813	-27.22
167.1	7.151E-08	1.1105E-02	4.1441E-03	1.0855	-23.44
173.4	6.893E-08	1.1071E-02	4.1488E-03	1.0819	-26.69
232.4	1.197E-07	1.1085E-02	4.1460E-03	1.0840	-24.79
294.8	8.809E-08	1.1078E-02	4.1467E-03	1.0838	-24.97
353.8	6.653E-08	1.1071E-02	4.1451E-03	1.0835	-25.20
410.4	4.832E-08	1.1068E-02	4.1509E-03	1.0838	-24.98
465.0	4.653E-08	1.1058E-02	4.1542E-03	1.0832	-25.50
518.1	2.023E-08	1.1050E-02	4.1553E-03	1.0828	-25.83

A12:- GC-IRMS chromatograms of 50 %$^{w}/_{w}$
weathered DRO standard

Isochrom GC/IRMS
MWSM50 (2) run taken at 13:03:15 12/04/95

Analysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
59.1	3.868E-07	1.1569E-02	4.1660E-03
109.0	3.852E-07	1.1568E-02	4.1661E-03
159.0	3.852E-07	1.1566E-02	4.1659E-03
209.1	3.901E-07	1.1565E-02	4.1657E-03
259.1	3.969E-07	1.1565E-02	4.1658E-03
309.0	4.038E-07	1.1563E-02	4.1660E-03
359.1	4.106E-07	1.1562E-02	4.1660E-03
2508.8	4.148E-07	1.1500E-02	4.1658E-03
2558.8	4.073E-07	1.1500E-02	4.1659E-03
2608.7	4.010E-07	1.1498E-02	4.1655E-03
2658.8	3.961E-07	1.1496E-02	4.1658E-03
2708.7	3.923E-07	1.1496E-02	4.1655E-03
2758.8	3.886E-07	1.1495E-02	4.1654E-03
2808.8	3.861E-07	1.1493E-02	4.1653E-03
Std dev of fit		**7.2506E-07**	**2.0148E-07**

GC-IRMS of 50% WEATHERED DRO STANDARD

Analysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13
864.2	2.763E-08	1.1692E-02	4.1590E-03	1.0841	-24.68
891.7	2.238E-08	1.1677E-02	4.1603E-03	1.0827	-25.97
916.1	3.902E-08	1.1676E-02	4.1585E-03	1.0826	-26.01
946.6	6.170E-08	1.1680E-02	4.1566E-03	1.0832	-25.51
1024.1	1.234E-07	1.1672E-02	4.1536E-03	1.0826	-26.05
1059.9	6.176E-08	1.1681E-02	4.1539E-03	1.0836	-25.18
1069.8	4.965E-08	1.1666E-02	4.1545E-03	1.0822	-26.42
1097.9	5.091E-08	1.1703E-02	4.1509E-03	1.0859	-23.09
1101.0	7.090E-08	1.1640E-02	4.1519E-03	1.0797	-28.71
1165.9	5.894E-08	1.1684E-02	4.1513E-03	1.0842	-24.59
1172.2	6.092E-08	1.1641E-02	4.1512E-03	1.0800	-28.37
1194.0	4.771E-08	1.1671E-02	4.1540E-03	1.0830	-25.70
1231.3	9.968E-08	1.1665E-02	4.1534E-03	1.0825	-26.14
1293.1	7.491E-08	1.1660E-02	4.1544E-03	1.0821	-26.46
1352.8	5.518E-08	1.1658E-02	4.1552E-03	1.0821	-26.49
1409.5	4.261E-08	1.1656E-02	4.1563E-03	1.0821	-26.51
1464.2	2.860E-08	1.1655E-02	4.1617E-03	1.0821	-26.48

A13:- GC-IRMS chromatogram for ballast oil

sochrom GC/IRMS
W BO(3) run taken at 14:48:06 11/05/95

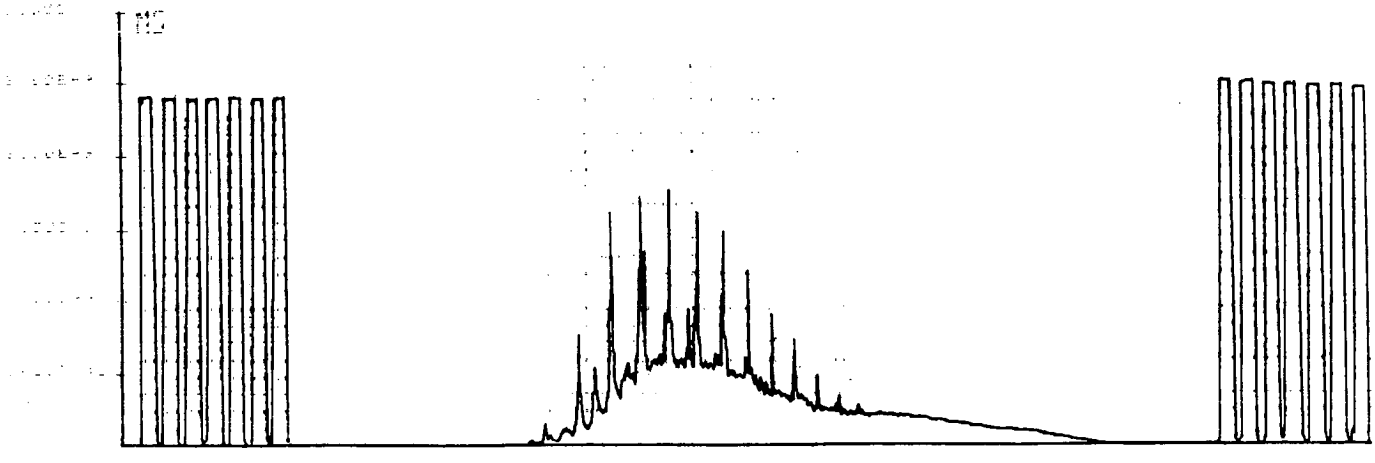
Ballast Oil
GC-IRMS

nalysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
59.2	3.065E-07	1.1521E-02	4.1903E-03
109.2	3.049E-07	1.1521E-02	4.1905E-03
159.2	3.067E-07	1.1518E-02	4.1905E-03
209.1	3.043E-07	1.1518E-02	4.1907E-03
259.2	3.045E-07	1.1516E-02	4.1903E-03
309.1	3.065E-07	1.1516E-02	4.1902E-03
359.2	3.045E-07	1.1514E-02	4.1900E-03
509.0	3.180E-07	1.1458E-02	4.1924E-03
559.0	3.160E-07	1.1457E-02	4.1923E-03
608.9	3.145E-07	1.1456E-02	4.1921E-03
659.0	3.137E-07	1.1455E-02	4.1924E-03
709.0	3.126E-07	1.1454E-02	4.1922E-03
759.0	3.112E-07	1.1454E-02	4.1920E-03
809.0	3.097E-07	1.1447E-02	4.1921E-03
td dev of fit		1.3040E-06	2.4285E-07

nalysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13
956.6	8.310E-09	1.1620E-02	4.2397E-03	1.0814	-27.18
033.8	4.877E-08	1.1592E-02	4.2106E-03	1.0790	-29.29
070.1	3.259E-08	1.1601E-02	4.2099E-03	1.0800	-28.41
078.8	3.337E-08	1.1594E-02	4.2086E-03	1.0793	-29.01
108.8	1.430E-07	1.1590E-02	4.2037E-03	1.0791	-29.23
138.5	3.431E-08	1.1597E-02	4.2071E-03	1.0798	-28.57
151.4	7.276E-08	1.1595E-02	4.2053E-03	1.0796	-28.74
177.8	1.027E-07	1.1590E-02	4.2034E-03	1.0793	-29.02
184.2	7.747E-08	1.1587E-02	4.2057E-03	1.0790	-29.30
204.9	5.503E-08	1.1596E-02	4.2050E-03	1.0799	-28.46
243.5	1.654E-07	1.1594E-02	4.2051E-03	1.0799	-28.54
269.3	5.312E-08	1.1600E-02	4.2066E-03	1.0805	-27.98
285.5	7.568E-08	1.1595E-02	4.2054E-03	1.0800	-28.42
305.6	1.341E-07	1.1591E-02	4.2051E-03	1.0797	-28.69
350.2	4.917E-08	1.1596E-02	4.2066E-03	1.0803	-28.17
365.2	1.069E-07	1.1591E-02	4.2054E-03	1.0798	-28.57
421.9	8.924E-08	1.1591E-02	4.2057E-03	1.0800	-28.38
441.7	3.533E-08	1.1592E-02	4.2066E-03	1.0801	-28.34
476.2	5.224E-08	1.1592E-02	4.2057E-03	1.0802	-28.25
528.1	5.429E-08	1.1592E-02	4.2060E-03	1.0803	-28.13
578.5	4.344E-08	1.1590E-02	4.2089E-03	1.0802	-28.21
627.7	2.960E-08	1.1587E-02	4.2096E-03	1.0800	-28.37



A14:- GC-IRMS chromatogram for API separator oil

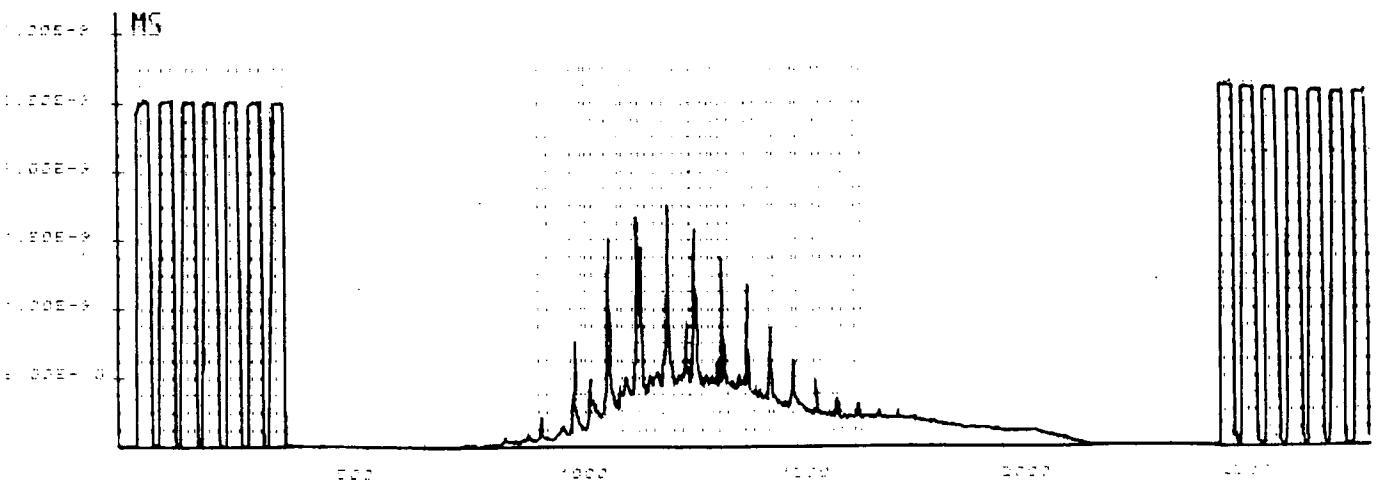
sochrom GC/IRMS
[W APIS(2) run taken at 10:13:29 11/05/95

nalysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
59.4	3.216E-07	1.1688E-02	4.1939E-03
109.3	3.205E-07	1.1688E-02	4.1940E-03
159.3	3.199E-07	1.1687E-02	4.1938E-03
209.3	3.197E-07	1.1688E-02	4.1941E-03
259.3	3.194E-07	1.1688E-02	4.1941E-03
309.2	3.196E-07	1.1688E-02	4.1939E-03
359.3	3.190E-07	1.1688E-02	4.1938E-03
509.0	3.350E-07	1.1676E-02	4.1923E-03
559.0	3.324E-07	1.1676E-02	4.1924E-03
608.9	3.306E-07	1.1676E-02	4.1927E-03
659.0	3.286E-07	1.1677E-02	4.1927E-03
709.0	3.275E-07	1.1677E-02	4.1927E-03
759.0	3.264E-07	1.1677E-02	4.1926E-03
809.0	3.254E-07	1.1677E-02	4.1924E-03
td dev of fit		6.8838E-07	1.6575E-07

nalysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13	
954.6	1.254E-08	1.1776E-02	4.1470E-03	1.0791	-29.21	
032.3	4.343E-08	1.1775E-02	4.1625E-03	1.0789	-29.42	
068.0	2.926E-08	1.1784E-02	4.1657E-03	1.0798	-28.63	
106.3	1.221E-07	1.1776E-02	4.1612E-03	1.0791	-29.27	^Ah + ?
136.6	2.259E-08	1.1783E-02	4.1633E-03	1.0797	-28.66	
149.1	6.206E-08	1.1782E-02	4.1643E-03	1.0796	-28.77	
175.5	9.129E-08	1.1777E-02	4.1587E-03	1.0792	-29.15	^Ah
181.8	7.086E-08	1.1775E-02	4.1622E-03	1.0790	-29.36	?
203.3	4.755E-08	1.1783E-02	4.1632E-03	1.0798	-28.64	
240.9	1.350E-07	1.1780E-02	4.1614E-03	1.0795	-28.87	
283.8	7.058E-08	1.1783E-02	4.1627E-03	1.0798	-28.63	
303.3	1.195E-07	1.1780E-02	4.1624E-03	1.0795	-28.89	
326.1	4.437E-08	1.1783E-02	4.1635E-03	1.0798	-28.64	
347.7	4.639E-08	1.1782E-02	4.1630E-03	1.0797	-28.70	
362.5	9.230E-08	1.1780E-02	4.1624E-03	1.0795	-28.85	
419.4	7.856E-08	1.1780E-02	4.1627E-03	1.0795	-28.84	
473.8	4.500E-08	1.1781E-02	4.1637E-03	1.0796	-28.74	
526.1	3.516E-08	1.1782E-02	4.1636E-03	1.0798	-28.61	
576.4	3.204E-08	1.1784E-02	4.1638E-03	1.0800	-28.37	
625.0	3.102E-08	1.1782E-02	4.1652E-03	1.0798	-28.61	
672.2	2.599E-08	1.1780E-02	4.1665E-03	1.0796	-28.76	



[W APIS(2)

A14

A15:- GC-IRMS chromatogram for residue oil

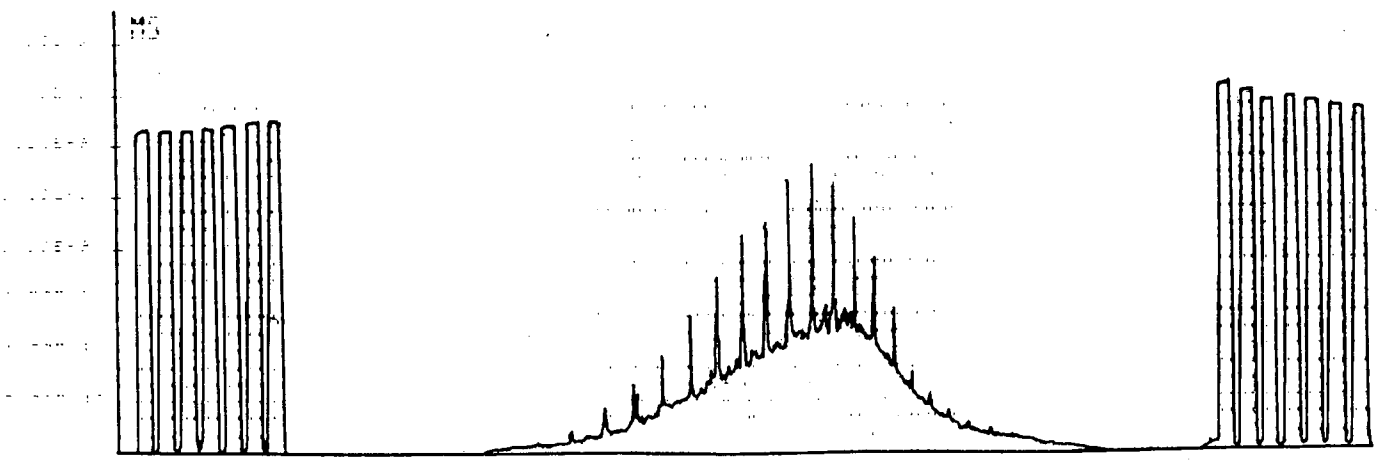
Isochrom GC/IRMS
IWROIL(1) run taken at 11:55:07 28/03/95

Analysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
59.3	4.011E-07	1.1584E-02	4.1684E-03
109.3	3.987E-07	1.1584E-02	4.1680E-03
159.3	4.017E-07	1.1583E-02	4.1683E-03
209.3	4.020E-07	1.1583E-02	4.1680E-03
259.3	4.068E-07	1.1582E-02	4.1682E-03
309.3	4.129E-07	1.1583E-02	4.1681E-03
359.3	4.142E-07	1.1582E-02	4.1682E-03
2509.1	4.512E-07	1.1586E-02	4.1687E-03
2559.1	4.405E-07	1.1586E-02	4.1689E-03
2609.0	4.319E-07	1.1586E-02	4.1690E-03
2659.1	4.246E-07	1.1587E-02	4.1691E-03
2709.0	4.240E-07	1.1587E-02	4.1689E-03
2759.2	4.202E-07	1.1587E-02	4.1693E-03
2809.1	4.175E-07	1.1587E-02	4.1690E-03
Std dev of fit		7.5005E-07	1.4872E-07

Analysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13
1026.2	1.941E-08	1.1741E-02	4.1324E-03	1.0806	-27.90
1099.1	3.966E-08	1.1728E-02	4.1342E-03	1.0793	-29.09
1167.7	2.879E-08	1.1728E-02	4.1345E-03	1.0792	-29.11
1174.2	3.878E-08	1.1720E-02	4.1347E-03	1.0785	-29.79
1233.3	7.614E-08	1.1727E-02	4.1358E-03	1.0791	-29.25
1296.7	1.064E-07	1.1723E-02	4.1355E-03	1.0788	-29.53
1341.7	6.974E-08	1.1731E-02	4.1364E-03	1.0795	-28.90
1357.0	1.271E-07	1.1724E-02	4.1352E-03	1.0788	-29.46
1378.6	6.093E-08	1.1727E-02	4.1355E-03	1.0791	-29.19
1415.0	1.160E-07	1.1724E-02	4.1354E-03	1.0788	-29.50
1434.5	5.373E-08	1.1728E-02	4.1358E-03	1.0792	-29.12
1470.1	1.312E-07	1.1724E-02	4.1348E-03	1.0788	-29.49
1523.6	1.756E-07	1.1725E-02	4.1349E-03	1.0789	-29.45
1574.3	1.904E-07	1.1726E-02	4.1346E-03	1.0789	-29.37
1590.2	9.815E-08	1.1727E-02	4.1358E-03	1.0790	-29.28
1602.6	1.420E-07	1.1727E-02	4.1361E-03	1.0791	-29.23
1623.2	2.037E-07	1.1726E-02	4.1351E-03	1.0789	-29.38
1638.2	1.142E-07	1.1727E-02	4.1358E-03	1.0791	-29.24
1650.9	1.210E-07	1.1727E-02	4.1355E-03	1.0791	-29.23
1670.1	1.770E-07	1.1726E-02	4.1357E-03	1.0790	-29.32
1684.7	1.253E-07	1.1727E-02	4.1360E-03	1.0790	-29.31
1697.1	1.130E-07	1.1727E-02	4.1360E-03	1.0791	-29.25
1714.3	1.146E-07	1.1726E-02	4.1354E-03	1.0790	-29.33
1740.3	9.336E-08	1.1729E-02	4.1363E-03	1.0792	-29.13
1756.6	9.084E-08	1.1727E-02	4.1361E-03	1.0791	-29.26
1797.9	6.316E-08	1.1729E-02	4.1362E-03	1.0792	-29.12
1838.7	4.180E-08	1.1731E-02	4.1367E-03	1.0794	-28.95
1881.0	3.750E-08	1.1733E-02	4.1359E-03	1.0796	-28.74



A16:- GC-IRMS chromatogram for No.6 Fuel oil

Isochrom GC/IRMS
MW NO6S(3) run taken at 15:42:53 11/05/95

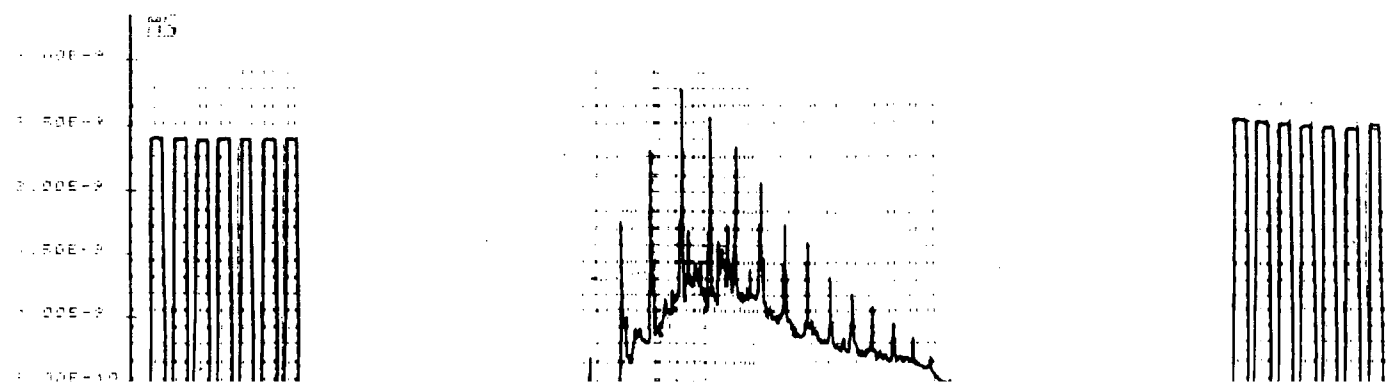
Analysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
59.2	3.045E-07	1.1436E-02	4.1907E-03
109.2	3.037E-07	1.1434E-02	4.1910E-03
159.1	3.033E-07	1.1432E-02	4.1906E-03
209.2	3.031E-07	1.1429E-02	4.1909E-03
259.2	3.033E-07	1.1428E-02	4.1911E-03
309.1	3.033E-07	1.1424E-02	4.1906E-03
359.2	3.032E-07	1.1423E-02	4.1909E-03
2509.0	3.209E-07	1.1367E-02	4.1934E-03
2558.9	3.175E-07	1.1370E-02	4.1931E-03
2608.9	3.129E-07	1.1371E-02	4.1933E-03
2659.0	3.139E-07	1.1369E-02	4.1933E-03
2708.9	3.129E-07	1.1367E-02	4.1933E-03
2759.0	3.121E-07	1.1366E-02	4.1931E-03
2808.9	3.109E-07	1.1365E-02	4.1914E-03
Std dev of fit		2.2766E-06	5.5211E-07

Analysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13
1034.6	4.501E-08	1.1532E-02	4.1997E-03	1.0820	-26.60
1070.4	3.712E-08	1.1536E-02	4.2002E-03	1.0825	-26.13
1109.4	1.038E-07	1.1515E-02	4.2016E-03	1.0804	-28.01
1117.8	4.026E-08	1.1535E-02	4.2037E-03	1.0824	-26.22
1139.9	4.835E-08	1.1528E-02	4.2027E-03	1.0818	-26.74
1179.9	1.471E-07	1.1520E-02	4.2024E-03	1.0811	-27.40
1184.8	4.500E-08	1.1520E-02	4.2040E-03	1.0812	-27.33
1207.5	1.065E-07	1.1529E-02	4.2025E-03	1.0821	-26.51
1232.4	5.178E-08	1.1525E-02	4.2023E-03	1.0817	-26.84
1245.9	2.033E-07	1.1523E-02	4.2012E-03	1.0816	-26.94
1264.0	1.363E-07	1.1526E-02	4.2007E-03	1.0819	-26.65
1279.8	1.006E-07	1.1526E-02	4.2004E-03	1.0820	-26.55
1288.5	9.132E-08	1.1526E-02	4.2005E-03	1.0820	-26.55
1309.0	2.045E-07	1.1523E-02	4.1996E-03	1.0818	-26.80
1331.3	1.228E-07	1.1527E-02	4.1991E-03	1.0822	-26.39
1338.1	9.736E-08	1.1530E-02	4.1982E-03	1.0826	-26.05
1350.2	1.519E-07	1.1527E-02	4.1982E-03	1.0823	-26.34
1368.2	1.738E-07	1.1524E-02	4.1979E-03	1.0820	-26.58
1390.4	7.633E-08	1.1528E-02	4.1974E-03	1.0825	-26.11
1401.8	9.271E-08	1.1526E-02	4.1974E-03	1.0823	-26.31
1424.3	1.510E-07	1.1523E-02	4.1953E-03	1.0821	-26.48
1445.3	8.450E-08	1.1528E-02	4.1953E-03	1.0827	-25.95
1479.1	1.480E-07	1.1525E-02	4.1946E-03	1.0825	-26.13
1530.9	1.146E-07	1.1524E-02	4.1946E-03	1.0825	-26.15
1542.8	5.213E-08	1.1525E-02	4.1947E-03	1.0827	-26.00
1581.2	1.105E-07	1.1523E-02	4.1944E-03	1.0826	-26.09
1629.9	1.120E-07	1.1521E-02	4.1939E-03	1.0825	-26.16
1676.7	8.778E-08	1.1520E-02	4.1946E-03	1.0825	-26.16
1721.5	7.880E-08	1.1519E-02	4.1943E-03	1.0825	-26.18
1765.3	6.803E-08	1.1520E-02	4.1948E-03	1.0826	-26.01
1807.2	6.233E-08	1.1520E-02	4.1954E-03	1.0828	-25.85

A16



A17:- GC-IRMS chromatogram for Nigerian crude oil

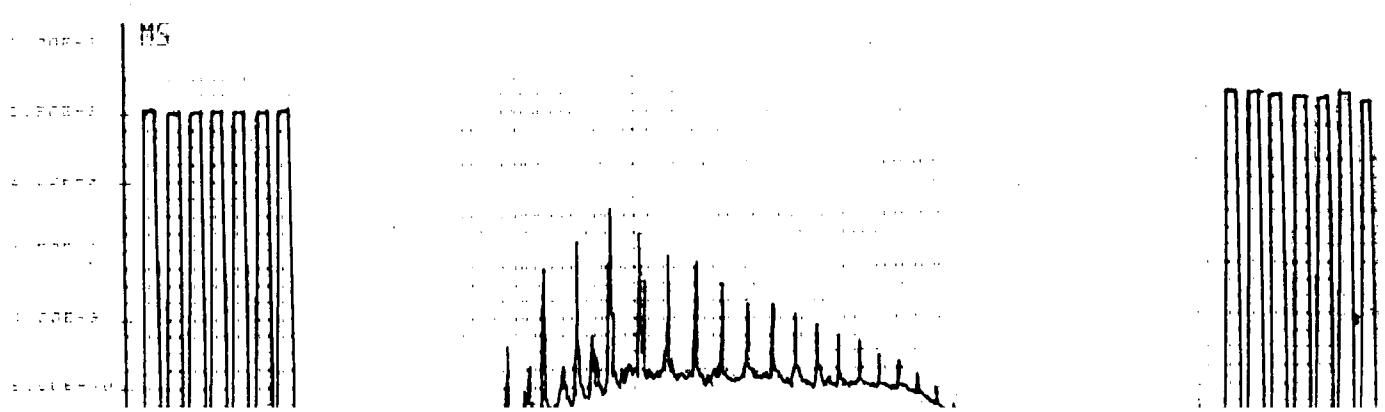
Isochrom GC/IRMS
MW C4S(2) run taken at 09:53:01 12/05/95

Analysis of reference gas data

Time	Major	ratio 2/1	ratio 3/1
59.7	3.313E-07	1.1702E-02	4.1969E-03
109.7	3.317E-07	1.1703E-02	4.1973E-03
159.6	3.311E-07	1.1703E-02	4.1972E-03
209.7	3.314E-07	1.1703E-02	4.1974E-03
259.7	3.333E-07	1.1704E-02	4.1972E-03
309.7	3.339E-07	1.1704E-02	4.1975E-03
359.7	3.316E-07	1.1704E-02	4.1973E-03
2509.2	3.446E-07	1.1705E-02	4.1969E-03
2559.3	3.417E-07	1.1705E-02	4.1974E-03
2609.3	3.392E-07	1.1705E-02	4.1974E-03
2659.4	3.380E-07	1.1705E-02	4.1975E-03
2709.3	3.361E-07	1.1705E-02	4.1975E-03
2759.4	3.346E-07	1.1704E-02	4.1972E-03
2809.4	3.361E-07	1.1705E-02	4.1973E-03
Std dev of fit		**4.4114E-07**	**2.0117E-07**

Analysis of sample peaks

Time	Major	2/1	3/1	Atom%	Delta C13
772.6	1.500E-08	1.1809E-02	4.2388E-03	1.0796	-28.76
840.4	1.901E-08	1.1813E-02	4.2647E-03	1.0798	-28.56
860.6	6.196E-08	1.1812E-02	4.2502E-03	1.0798	-28.55
887.6	2.748E-08	1.1825E-02	4.2407E-03	1.0812	-27.32
902.2	2.417E-08	1.1825E-02	4.2414E-03	1.0812	-27.36
912.2	5.165E-08	1.1817E-02	4.2289E-03	1.0805	-27.98
942.8	1.095E-07	1.1810E-02	4.2075E-03	1.0800	-28.42
985.5	7.604E-08	1.1819E-02	4.2048E-03	1.0809	-27.63
991.2	6.016E-08	1.1811E-02	4.1949E-03	1.0803	-28.18
1020.5	1.441E-07	1.1809E-02	4.1796E-03	1.0801	-28.32
1056.0	7.215E-08	1.1817E-02	4.1786E-03	1.0809	-27.59
1063.7	6.351E-08	1.1809E-02	4.1709E-03	1.0802	-28.21
1094.9	1.900E-07	1.1804E-02	4.1596E-03	1.0798	-28.60
1136.7	9.212E-08	1.1809E-02	4.1617E-03	1.0803	-28.14
1162.9	7.672E-08	1.1806E-02	4.1589E-03	1.0800	-28.43
1169.1	8.444E-08	1.1801E-02	4.1593E-03	1.0795	-28.86
1228.5	1.282E-07	1.1807E-02	4.1601E-03	1.0801	-28.33
1290.7	1.204E-07	1.1807E-02	4.1573E-03	1.0801	-28.31
1335.4	5.535E-08	1.1811E-02	4.1591E-03	1.0805	-27.96
1350.5	1.038E-07	1.1807E-02	4.1564E-03	1.0801	-28.30
1407.2	1.049E-07	1.1806E-02	4.1561E-03	1.0801	-28.35
1462.0	1.013E-07	1.1809E-02	4.1554E-03	1.0803	-28.13
1514.6	1.008E-07	1.1808E-02	4.1555E-03	1.0802	-28.25
1564.8	8.355E-08	1.1808E-02	4.1552E-03	1.0803	-28.17
1613.4	7.148E-08	1.1807E-02	4.1557E-03	1.0801	-28.28
1660.2	7.889E-08	1.1808E-02	4.1556E-03	1.0802	-28.25
1705.7	6.473E-08	1.1807E-02	4.1549E-03	1.0801	-28.29
1749.0	5.853E-08	1.1807E-02	4.1541E-03	1.0801	-28.35
1791.0	4.887E-08	1.1806E-02	4.1523E-03	1.0800	-28.39
1831.8	3.854E-08	1.1807E-02	4.1505E-03	1.0802	-28.24
2431.2	3.105E-08	1.1829E-02	4.1693E-03	1.0821	-26.51



APPENDIX B

Peer Reviewed Publications

Whittaker, M., Pollard, S.J.T. and Fallick, A.E., *Characterisation of Refractory Wastes at Heavy-Oil Contaminated Sites: A Review of Conventional and Novel Analytical Methods*, Environmental Technology, *16, 1009-1033* (1995).

Whittaker M. and Pollard S.J.T., *Characterization of Refractory Wastes at Hydrocarbon-Contaminated Sites: I. Rapid Column Fractionation and Thin-Layer Chromatography of Reference Oils*. J. Planar Chromatography, *7, 354-361* (1994).

Publications Currently Submitted for Publication and In Press

Whittaker M. and Pollard S.J.T., *A Performance Assessment of Source and Weathering Indices for Petroleum Products in the Environment*, Environmental Toxicology and Chemistry, *submitted* (1995).

Pollard S.J.T. and Whittaker M., *Heavy Oil-Contaminated Sites: Towards an Improved Analytical Strategy*, Current Research in Contaminated Land, Society of Chemical Industry, London, December (in press) (1995).

Whittaker M., Pollard S.J.T., Fallick A.E. and Preston T., *Characterization of Refractory Wastes at Hydrocarbon-Contaminated Sites: II. Stable Carbon Isotopic Fingerprinting of Reference Oils*, Environmental Pollution, *in press* (1995).

Whittaker M. and Pollard S.J.T., *Refractory Wastes at Hydrocarbon-Contaminated Sites: An Investigation of Source Terms and Extent of Weathering*, Environmental Geochemistry and Health, *in press* (1994).

Conference Platform Presentations

Whittaker M. and Pollard S.J.T., *Environmental Chemical Analysis of Heavy Oil-Contaminated Sites* RSC Young Environmental Chemists Symposium, De Montfort University, Leicester, March 1996.

Whittaker M. and Pollard S.J.T., *Stable Carbon Isotope Screening of Heavy Oil-Contaminated Sites*, SETAC World Congress: Global Environmental Protection: Science, Politics and Common Sense, Vancouver, Nov. 1995.

Whittaker M. and Pollard S.J.T., *Heavy Oil-Contaminated Sites: Towards an Improved Analytical Strategy*, Current Research in Contaminated Land, Society of Chemical Industry, London, December 1995.

Whittaker M. and Pollard S.J.T., *Characterisation of Heavy Oil-Contaminated Sites*, Society for Environmental Geochemistry and Health, 12th European Meeting, Nottingham, April 1994.

Conference Poster Presentations

Whittaker M. and Pollard S.J.T., *Characterisation of Residual Saturation at Heavy Oil-Contaminated Sites,* RSC Annual Congress, Toxicology Group, Edinburgh, April 1995.

Whittaker M. and Pollard S.J.T., *Characterisation of Residual Saturation at Heavy Oil-Contaminated Sites,* National Highlights of Chemical Science and Technology by Young Scientists from Academia, Government and Industry, London, November 1994.

Whittaker M. and Pollard S.J.T., *Characterisation of Residual Saturation at Heavy Oil-Contaminated Sites,* NATO/CCMS Pilot Study on Contaminated Land Remediation, Oxford, September 1994.

Courses Attended (University of Edinburgh, 1993 - 1996)

Sedimentary Chemistry, Dr. J.G. Farmer, Autumn 1993

Environmental Sampling and Monitoring, Dr. M. Heap, Autumn 1993

Chemical Waste Treatment, Dr. S.J.T. Pollard, Autumn 1993

Hazardous Waste Treatment, Dr. S.J.T. Pollard, Autumn 1993

Environmental Microbiology (5 Lectures), Professor I.W. Sutherland, Spring 1994

Global Change (5 Lectures), Dr. J.G. Farmer, Spring 1994

Contaminated Land: Managing Chemical Risk, Dr. S.J.T. Pollard, Spring 1994

Environmental Modelling, Dr. J.G. Farmer, Spring 1994

Toxicology and Risk Assessment, Dr. R. Agius, Spring 1994

Contaminated Land, Dr. R. Bewley, Autumn 1995