Effect of weathering and biodegradation on markers used in oil fingerprinting

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Abstract

The chemical fingerprinting of crude oils and petroleum products is an important technique in environmental spill investigations. Due to the environmental impact of petroleum spills, there is a need to understand the fate of crude oil hydrocarbons in the environment as well as identifying the source for forensic purposes for blame apportionment litigation and, for clean-up and restoration planning. Once petroleum hydrocarbons are spilled in the environment, they are gradually subjected to increased dispersion and degradation processes, collectively termed weathering. This work investigated the effects of the main weathering processes including evaporation, water washing, aerobic and anaerobic biodegradation, on petroleum hydrocarbon molecular markers. The study focused on aliphatic and aromatic compound composition and concentration changes including biomarkers and non-biomarker geochemical markers, in two crude oils (light and medium) from the Niger Delta region of Nigeria and one from the North Sea. Marine sediment from Whitley Bay, UK and river sediment from the Niger Delta, were used as inocula for the aerobic biodegradation microcosms, while anoxic sediment from the River Tyne was used for the anaerobic microcosms. Gravimetric and chromatographic data was obtained from crude oil evaporation experiments in accordance with the Nordtest methodology, where Nigerian light oil was the most affected by evaporation (63% loss by weight), followed by the North Sea oil (49%), and Nigerian Medium oil (36%) after 30 days evaporation. Only relatively low-molecular weight components (including *n*-alkanes $<C_{20}$) and adamantanes were observed to be affected by evaporation while the terpanes, steranes, triaromatic steranes, bicyclic sesquiterpanes, PAHs and their alkylated homologues, were unaffected. Similarly, only relatively low-molecular weight components were observed to be affected by water washing experiments and they were less affected when compared with the evaporation experiments. The aerobic biodegradation experiments with Whitley Bay sediment showed the most effects where *n*-alkanes were completely degraded for the North Sea oil after

100 days incubation. The degradation extent was ranked using the Peters and Moldowan (PM) scale which progressed from level 0 (intact) for the starting oil, level 1 (very slight) for 5, 10, and 20 days incubated samples, level 2-3 (slight to moderate) for 50 and 100 days periods, and level 4 (moderate to heavy) after 300 days. Many of the weathering parameters unaffected by the evaporation and water washing experiments as shown on the Nordtest diagnostic ratio plots, however biomarkers were observed to be affected by biodegradation. Nordtest diagnostic ratio plots of the Whitley Bay sediment experiments show alteration of the parameters such as those based on the dibenzothiophenes and phenanthrenes from 50 days incubation at PM levels 4-5 (heavy) to PM levels 6-10 after 300 days incubation. The C_{21} pregnanes and some C_{15} bicyclic sesquiterpanes were affected after 100 days, whereas the terpanes including novel tricyclic terpanes and tetracyclic terpanes identified in the two Nigerian oils together with the steranes and triaromatic steroids were generally unaffected, indicating the potential usefulness of the diagnostic ratio based on these compounds, even in heavily biodegraded oils. Anaerobic biodegradation experiments for 300 days incubation resulted in much less hydrocarbon removal than aerobic experiments, but *n*-alkanes were depleted resulting in methane production. Novel bicyclic sesquiterpanes, and unidentified suspected pentamethylnaphthalene peaks were identified in the two Nigerian crudes and their resistance to weathering and their potential for use as markers for forensic and correlation purposes were found to be similar to other bicyclic sesquiterpanes. The correlation efficiency of the Nordtest methodology for oils in sediment is affected by high sediment background levels of the geochemical markers considered. Therefore, the determination of their background concentration is necessary in those cases, and certain parameters with high background levels should be ignored to avoid false negatives in the correlation.

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Chapter 1 INTRODUCTION

1.1 General Introduction

Crude oil (petroleum) plays an inescapable role in modern society today and consequently, the global demand for it grows as the world population rises with the corresponding quest for industrialisation in both developed and developing countries (Albers, 2002; NRC, 2003). Crude oil hydrocarbons remain the dominant source of energy worldwide and are expected to remain for decades to come (Wang *et al.*, 2006; Dargay and Gately, 2010; OPEC, 2019). Because economic substitutes for crude oil and its products are not foreseeable in the near future, crude oil is being used worldwide which lead to its widespread presence in the environment from spills and their attendant environmental impact (Mudge *et al.*, 2008). The widespread global usage of crude oil and refined products unavoidably results in both deliberate and accidental releases to the environment, and the associated environmental degradation has raised legislative and regulatory concerns over liability (Urdal *et al.*, 1986). Hence, environmental forensics which entails tracing the sources of pollutants in the environment plays a very important role in resolving unambiguously the issues of oil spill source identification responsible for environmental damage and for blame apportioning (Urdal *et al.*, 1986; Wait, 2000).

Most major oil spills recorded are waterborne and consequently most of the reports and publications concerning petroleum in the environment are maritime (NRC, 1975; GESAMP, 1977; NRC, 1985; NRC, 2003) although a significant portion of spills reaching the marine ecosystem are land based, as well as atmospheric hydrocarbon deposition (Wang *et al.*, 2006; Stout and Wang, 2007b). Some past records of world annual petroleum input into the marine environment are as follows; 6.1 million tonnes/year in 1975 (NRC, 1975; NRC, 1985), 4.67 million tonnes/year in 1981 (Kornberg, 1981; NRC, 2003), 3.27 million tonnes/year in 1983

(Baker, 1983; NRC, 1985; NRC, 2003), 3.2 million tonnes/year in 1985 (NRC, 1985), 1.3 million tonnes/year (NRC, 2003; Farrington, 2013), 1.25 million tonnes/ year in 2007 (GESAMP, 2007) . Statistical records from International Tanker Owners Pollution Federation Limited (ITOPF) also showed the reduction trend of marine tanker spills of large spills (greater than 700 tonnes) and medium spills (7-700 tonnes) from an annual average number of 78.8 in the 1970s to 6.4 in 2018 with corresponding quantities from 287,000 tonnes to 113,000 tonnes (ITOPF, 2018b). Although tanker accidental spills may not be the largest contribution to marine pollution, they are significant due their recorded impacts on the marine ecosystem. According to the National Research Council (2003), tanker spills constitute 8% of the annual global estimates whereas 47% is due to natural seepage, 33% due to consumption activities such as land-based run-offs and non-tanker operational releases and spills, 4% due to oil transportation, 4% due to exploration activities, and 5% due to other sources like atmospheric deposition and jettisoned aircraft fuel.

1.2 Crude Oil Composition

Crude oil or petroleum is a complex mixture of organic compounds (Ehrhardt and Blumer, 1972; Peters *et al.*, 2005; Speight, 2005). It consists mostly of hydrocarbons in the form of non-polar saturated and aromatic compounds with smaller quantities of polar nitrogen, sulphur, and oxygen (NSO) containing compounds found in the resin and asphaltenes fractions, as well as trace metals which are mainly in porphyrin complexes (Speers and Whitehead, 1969; Posthuma, 1977; Tissot and Welte, 1984; Peters and Moldowan, 1993b; Filby, 1994; Peters *et al.*, 2005; Speight, 2005; Wang *et al.*, 2006). Composition varies amongst crude oils depending on the contribution of organic matter or biological precursors, depositional palaeoenvironmental conditions of sedimentation and geochemical processes that lead to the formation of the crude oils or its origin (Didyk *et al.*, 1978; Tissot and Welte, 1984; Volkman, 1986; Hedges and Keil, 1995; Koopmans *et al.*, 1996; Peters *et al.*, 2005).

Elemental analysis of crude oils show that they contain the following limits by percentage weight: 80-87 carbon, 10-15 hydrogen, 0-10 sulphur, 0-1 nitrogen, 0-5 oxygen, and a wide range of trace metals such as vanadium, nickel, iron, sodium, calcium, copper and uranium (Posthuma, 1977).

1.2.1 Saturated Hydrocarbons

Saturated hydrocarbon compounds can be categorised into acyclic (normal and branched alkanes), and cycloalkanes (Tissot and Welte, 1984; NRC, 1985; Killops and Killops, 1993; Heath *et al.*, 1997; Widdel and Rabus, 2001). Alkanes (also known as paraffins) include the straight-chain compounds known as *n*-alkanes (*n*-paraffins), which occur as a homologous series with general formula C_nH_{2n+2} (Clark and Blumer, 1967). The *n*-alkanes have been reported to occur in most crude oil as the most dominant hydrocarbon constituents, often with maximum carbon atom numbers of around 40, although carbon atoms of greater than 60 have been reported (Posthuma, 1977; Killops and Killops, 1993). The branched-chain alkanes (sometimes referred to as branched paraffins, e.g. 2-methylpropane and 2-methylheptane), include the isoprenoids (e.g. pristane and phytane) as most important hydrocarbons of this group usually within C_{10} to C_{20} carbon range, which is believed to be mostly derived from plants pigments notably from phytol side chain of chlorophyll, though some are thought to be from archaebacterial lipids (Brooks *et al.*, 1969; Powell and McKirdy, 1973; Posthuma, 1977; Tissot and Welte, 1984; Ten Haven *et al.*, 1987; Peters *et al.*, 2005). Steroids and terpenoids which can have specific known animal or plant precursors can also exist as saturated hydrocarbons known as biological markers or biomarkers (Posthuma, 1977; Tissot and Welte, 1984; NRC, 1985; Wang *et al.*, 2004; Wang *et al.*, 2006; Yang *et al.*, 2006; Song *et al.*, 2016).

Olefins do not usually occur naturally in crude oils but are formed during processing (Meinschein, 1969; Buchanan, 2000; Gary and Handwerk, 2001).

1.2.2 Aromatic Hydrocarbons

The next most abundant hydrocarbon constituent in crude oil after saturated hydrocarbons are the aromatic hydrocarbons including their alkylated derivatives which are usually less abundant than saturated hydrocarbons, though the two fractions have been reported to comprise on average 80% (by mass) of oil constituents (Widdel and Rabus, 2001). Killops and Killops (1993), reported the average oil hydrocarbon composition as follows; normal and branched alkanes 33%; cycloalkanes 32%; aromatic hydrocarbons 35%. Aromatics are compound with one (mono) or more (poly) aromatic (benzene) rings (Stogiannidis and Laane, 2015), and include those that contain both aromatic and naphthenic rings (sometimes termed naphthenoaromatics) such tetralin and also many other quantitatively important compounds in this category that contain saturated hydrocarbon chains (e.g. alkylbenzenes) (Posthuma, 1977; Widdel and Rabus, 2001). The content of aromatic compounds increases with the specific gravity of the crude oil, and the PAHs are known to be less soluble in water, less volatile, high boiling and melting points, and low vapour pressure (McElroy *et al.*, 1989; Albers, 2002; Ou *et al.*, 2004). Whilst boiling and melting points increase with aromatic rings, solubility and vapour pressure decrease with increase in aromatic rings respectively (Albers, 2002). PAHs could be of natural sources such as the seeps from oil deposits, natural fires, volcanoes, and weathering of ancient sediment, or anthropogenic such as thermal conversion of organic matter or its incomplete combustion (Stogiannidis and Laane, 2015).

Aromatics have not been widely used for pollution source identification as compared to saturated hydrocarbons, but attention has been drawn to them recently due to their increased toxicity to biota in the ecosystem and their well-established carcinogenic properties

(Youngblood and Blumer, 1975; Jones *et al.*, 1983; Boehm *et al.*, 2007; Guo *et al.*, 2007; Stogiannidis and Laane, 2015). Characterisation and differentiation of aromatic compounds is important for diagnosing sources of hydrocarbons, and higher degree of alkylation favoured by low temperatures indicate petroleum (crude oil) derived or diagenetic hydrocarbons whereas lesser degree of alkylation suggests combustion derived hydrocarbon compounds (Tissot and Welte, 1984; NRC, 1985; Wang *et al.*, 1999; Peters *et al.*, 2005; Stogiannidis and Laane, 2015).

1.2.3 Non-Hydrocarbons in crude oil

The nonhydrocarbon constituents in petroleum comprised of: sulphur compounds, oxygen compounds, nitrogen compounds, porphyrins, asphaltenes, and trace metals (Peters *et al.*, 2005; Speight, 2005). Nonhydrocarbons are observed to be in minor concentrations in crude oil, but however predominate in heavy oils (Posthuma, 1977; Moldowan and Peters, 1993; Filby, 1994; Speight, 2005; Wang *et al.*, 2006; Stout and Wang, 2007a). Sulphur compounds constitute the most important constituents of the nonhydrocarbons in terms of their amounts and associated problems caused during refining. Sulphur contents gives details about the origin of crude oil and they range from 0% in sweet crudes from the far East to 5% in heavy Western Venezuelan crudes, and 15% in asphalt seeps (Posthuma, 1977; Speight, 2005). Most of the sulphur in crude oil is organically bound and originates from source rock. Oxygen compounds have also been identified in oils in minor amounts (0-2%) in fractions distilled above 400°C and comprise of phenols, naphthenic acids, carboxylic acids, ketones, esters, lactones, fluorenones, dibenzofurans and ethers (Tissot and Welte, 1984; NRC, 1985; Peters *et al.*, 2005; Speight, 2005). Nitrogen compounds have been identified in all crude oils and are generally categorised into basic compounds such as pyridines, quinolones, benzoquinolines, and acridines whereas the non-basic compounds are derivatives of pyrroles, indoles, carbazoles and benzocarbazoles (Clark and Brown, 1977; Bakel, 1990; Gerasimova *et al.*, 2005). The amount of nitrogen in crude oils is about 1% maximum, but that of nitrogen compounds vary between

0.5 and 15% most of which are concentrated in the residues of higher boiling point fractional distillation (Posthuma, 1977).

Porphyrins are nitrogen containing compounds consisting of four condensed pyrrole rings and they provide evidences of the biological origin of petroleum as they are mostly derived from chlorophyll (NRC, 1985; Popp *et al.*, 1989; Bakel and Philp, 1990; Killops and Killops, 1993; Lash, 1993; Ramírez-Pradilla *et al.*, 2019). Porphyrins are found in petroleum and occur as organometallic complexes of vanadium and nickel, they are the most stable organometallic porphyrins in the environment and, have also been reported to occur as a result of metal exchange, decarboxylation, and hydrogenation (Tissot and Welte, 1984; Lash, 1993; Ramírez-Pradilla *et al.*, 2019). Asphaltenes are another important fraction (0-20%) of petroleum with high molecular weight (1000-10,000) that contain nitrogen, sulphur, oxygen and metals (Posthuma, 1977). Asphaltenes are reported to consist of 10-20 rings with aliphatic and naphthenic side chains which to a large extent are influenced by the geochemical properties of petroleum and their origins as well as emulsification behaviour in spill situation (NRC, 1985; ITOPF, 2014).

Crude oils contain traces of metals such as vanadium, nickel, iron, sodium, calcium, copper, and uranium according to (Bakel and Philp, 1990; Killops and Killops, 1993; Ramírez-Pradilla *et al.*, 2019). However, vanadium and nickel are said to be the most abundant metallic constituents of petroleum present in porphyrin complexes (Posthuma, 1977; NRC, 1985).

1.3 Oil Spill Management

Oil spills have been a global issue of interest for many decades now and numerous oil spill incidents have been recorded that had negative consequences on both land, atmospheric and marine environment (IPIECA, 1991; Farrington, 2013; Stout and Wang, 2016b; ITOPF, 2018b) and also on the socio-economic livelihood of the people affected (ITOPF, 2004). Owing to the high demand for fossil fuel energy, oil pollution often occur as a result of interdependent activities, this prompted the need for stakeholders to come together to deal with oil pollution nationally and in collaborations with other countries through conventions. The most prominent convention is the International Convention on Oil Pollution Preparedness, Response and Co-operation, 1990 (OPRC 90) by International Maritime Organisation (IMO) (IPIECA, 2000). The convention was adopted in 1990 and entered into force in 1995 to strengthen a legal framework to protect and preserve the human environment generally and the marine environment from oil pollution. Other international organisations involved in the preparedness and response to oil and other harmful substance pollution into the environment include the International Oil Pollution Compensation Fund (IOPCF), the United Nations Environment Programme (UNEP), the World Bank Oil, Gas, Mining and Chemicals, and the UNEP World Conservation Monitoring Centre. Some regional organisations involved are the Bonn Agreement by European Commission (North Sea), the Helsinki Commission (Baltic Sea), the Oslo-Paris Commission (OSPAR) etc., within the Europe. The South Pacific Regional Environment Programme (SPREP) within the South Pacific, and Northwest Pacific Action Plan Marine Environment Emergency Preparedness and Response Regional Activity Centre (NOWRAP MERRAC) in the Northwest Pacific (IMO, 2019), the Global Initiative for West, Central and Southern Africa (GIWACAF) within the West, Central and Southern African regions(GIWACAF, 2020). There are also several oil industry organisations involved, with the most prominent among them is the International Petroleum Industry Environmental Conservation Association (IPIECA) and the Conservation of Clean Air and Water in Europe (CONCAWE). A number of response organisations also exist, such as Oil Spill Response Limited (OSRL) in the United Kingdom, the International Tankers Owners Pollution Federation (ITOPF), the Norwegian Clean Seas Association for Operating Companies

(NOFO), and the Marine Spill Response Corporation (MSRC) in the United States (IMO, 2019).

1.4 Research Justification

Petroleum (crude oil, crude oil products, natural gas, and related bitumen) has been known and used (as sealants, illuminants, lubricants, and products for medicinal purpose) in different forms since 3800 BC (Rossini, 1960). Published literature on petroleum chemistry dates back to late nineteenth century, though most developments in petroleum geochemistry (practical combination of chemistry and geology of petroleum) began in the late 20th century (Hunt *et al.*, 2002). The evolution of petroleum geochemistry revealed the complex nature of the petroleum with respect to its origin and occurrence, and to its application in exploration, migration, accumulation and alteration (Hunt *et al.*, 2002). Development of the chemical technique of distillation of petroleum into kerosine in 1860 resulted in replacement of whale oil as an illuminant, Kerosine from petroleum usage as illuminant was short-lived and overtaken by usage of gasoline and diesel fractions for fuel with the invention of the internal combustion engine (Hunt *et al.*, 2002). The first successful source rock- crude oil correlation carried out in Uinta Basin of Utah in 1954 using fractionation, column chromatography, infrared and elemental analyses, while in 1955 was the application of mass spectrometric analyses led to the discovery of *n*-alkanes distribution in crude oil and sediment extracts (Hunt *et al.*, 2002). In 1960 gas chromatography with thermal conductivity and flame ionisation (later coupled with mass spectrometry) became commercially available which was very useful for separation, identification and determination of distribution different organic compounds in crude oils and sediments. Other coupled analytical technique followed such as the liquid chromatography/mass spectrometry (LC/MS), gas chromatography/infrared spectrometry (GCIR) and nuclear magnetic resonance (NMR) spectroscopy (Hunt *et al.*, 2002). The late

1960s and 1970s saw the development of the use of biomarkers, also referred to as biological markers, molecular markers, chemical fossils, and geochemical fossils which were used to compare structurally similar organic compounds in crude oils and sediments to their probable precursors in living organisms as well as oil-rock and oil-oil correlations (Peters *et al.*, 2005). The applications of these discoveries led to many reports over the years on spill occurrences as well as investigations into the impact on terrestrial, atmospheric and aquatic environments, human health and socio-economic wellbeing (NRC, 1975; GESAMP, 1977; Hardy *et al.*, 1977; Baker, 1983; NRC, 1985; IPIECA, 1991; NRC, 2003; Couillard *et al.*, 2005; Wang *et al.*, 2006; Farrington, 2013; Faksness *et al.*, 2015; Beyer *et al.*, 2016; GESAMP, 2018). In particular, the quantitative analysis of biomarkers and other molecular markers used for oil spill fingerprinting and assessment the fate of oils in the environment, helped to reduced global tanker oil spillage from the 1970 to 2018 (ITOPF, 2021). A number of studies have also been conducted both in the laboratory and in the field on the effect of weathering on molecular markers or biomarkers in different crude oils using these various specialised techniques of oil fingerprinting for correlation purposes (e.g. Daling *et al.*, 2003) (See Table 0.1 and

Table 0.2) and the work in this thesis extends the knowledge in this area.

Table 0.1: Some oil spill forensic investigations conducted on crude and products from sites around the world

Investigation	Sample Type	Sample Source	Analytical Technique	Biomarker (s)	Experiment	Reference (s)
Revision of Nordtest Methodology	Crude	Visund & Statfjord (North Sea)	GC-FID, GC/MS	23(Hopanes & Steranes), 6(PAHs)	Waterborne Spill (field)	Daling et al., 2003
Classification of weathered Crudes	Crude	Norway (13), UK (6), Denmark (1), Soviet (3), Nigeria (3), Libya (1), Iran (2), Abu-Dhabi (1)	HRCG-MS	Triterpanes & Steranes	Waterborne (lab)	Urdal et al., 1986
Forensic oil Spill Identification	Spilled oil, sludge tank, centrifuge outlet	Great Belt, Denmark	GC-FID, GC/MS	PAHs, Hopanes, Steranes	Waterborne Spill (field)	Hansen, 2002
Application of Bicyclic sesquiterpanes in forensic identification light petroleum products in water	Gasoline, Diesel	Northbay, Ontario, Canada	GC-FID, GC/MS	BTEX, PAHs, Hopanes, Steranes	Waterborne Spill (field)	Yang et al., 2008

Forensic Investigation for dispute settlement between two neighbours	Diesel, kerosene, bunker C	Fuel storage facility and handling, US	GC-FID, GC/MS	Isoprenoids, sesquiterpanes, PAHs	Soil samples (field)	Stout et al., 2005
A study of 22-year-old Arrow oil samples	Bunker C	Chedabucto Bay, Canada	GC-FID, GC/MS	Hopanes, Steranes, PAHs	Sediment samples (field)	Wang et al., 1994
Application of sesquiterpanes for spill source identification	Diesel	Lachine canal, Quebec, Canada	GC-FID, GC/MS	sesquiterpanes	Sewer outlet pipe (field)	Wang et al., 2005
Forensic comparison and identification of Round Robin samples using multicriterion approach	Diesel	Harbor spill, Netherlands	GC-FID, GC/MS	Sesquiterpanes, PAHs	Waterborne Spill (field)	Wang ae al., 2005
Source identification of marine hydrocarbons	Tar ball, crude oil	South Shore, Devonshire Parish, Bermuda, South Louisiana	CG-FID	Pr/ph, nC_{17}/pr, N-C_{17}/backgr.	Tar ball on shore	Ehrhardt and Blumer, 1972

Table 0.2: Some oil weathering studies carried out on crude oil and products from site around the world.

Investigation	Sample Type	Sample Source	Analytical Technique	Weathering process (es)	Degree of weathering	Reference (s)

Characteristics of bicyclic sesquiterpanes in crude oils and petroleum products	Crude/Diesel	Prudhoe Bay, Alaska/Ottawa, Canada	GC-FID, GC/MS	Evaporation	6.3 – 22.0%	Yang et al., 2009
Surface weathering and dispersibility of MC252 crude oil	Crude oil	Mississippi Canyon, US	ND	Evaporation, photo-oxidation, water-in-oil emulsion	Evaporation: 26.9-54(wt. %) Emulsion: 65%	Daling et al., 2014
Weathering of petroleum in the marine environment	Crude oil	Prudhoe Bay, Alaska	ND	Spreading & drift, evaporation, dissolution, dispersion, ice interactions, shoreline interactions, photochemical oxidation, water-in-oil emulsion, adsorption onto suspended particles	Drifting: 2-3% Evap.: 15-20% Mouse thickness: 0.5-10cm	Payne and McNabb, 1984
Fate of hydrocarbons discharged at Sea	Crude	Prudhoe Bay, Alaska,	ND	Drifting, evaporation, spreading. dissolution,	Drifting: 2-3% Evap.: 50-70%	Mackay and McAuliffe, 1988

				mouse formation, photolysis, submergence, biodegradation	Spreading mean thickness:0.1mm dissolution: 1%	
Methods and factor affecting petroleum fingerprints in the environment	Crude	St. Bernard Parish, Louisiana, US	ND	Spreading, evaporation, dissolution, biodegradation,	Evap.: 10-50 wt.% Dissolution: 1-3 wt.%	Stout and Wang, 2007
Source identification of marine hydrocarbons	Tar balls, crude oil	South Shore, Devonshire Parish, Bermuda, South Louisiana, Gay Head on Martha's Vineyard, Massachusetts, US	GC-FID	Evaporation, dissolution, microbial degradation, chemical degradation	ND	Ehrhardt and Blumer, 1972
Chevron main pass block 41 oil spill	Crude	11 Miles East of Mississippi River, Delta		Evaporation, emulsion,	25 – 30%, 4 – 65%	McAuliffe et al., 1975

Note: ND - No Data

1.5 Impact of Petroleum Pollution in the Niger Delta Region of Nigeria

Nigeria has been immensely affected by oil pollution due to exploration and production of crude oil within the Niger Delta region since the first discovery in 1956 followed by the commercial production in 1958 by Shell D'Arcy and later Shell British Petroleum (now Royal Dutch Shell) at a village called Oloibiri in Bayelsa State, Nigeria (FMEnv, 2006; Onuoha, 2008; UNEP, 2011; Kadafa, 2012; Ite *et al.*, 2013; Anifowose *et al.*, 2016). The Nigerian Federal Ministry of Environment (FMEnv, 2006) reports that studies have shown that an estimate of 9-13 million barrels is said to have been spilled over the 50 years of oil exploration and exploitation in Nigeria mostly in the Niger Delta region. Many incidents of oil spill have been recorded over the years however, the most notable ones include; GOCON's Escravos spill in 1978 of about 300,000 barrels, Shell Petroleum Development Company's (SPDC) Forcados tank 6 terminal in Delta State in July, 1978 where 580,000 barrels was spilled into the environment, Well 5 Funiwa blow-out from 17th to 30th January, 1980 with an estimated spill of 421,000 barrels, Opoi North oil spillage in 1980 with 280,000 barrels spilled into the environment, Oyakama oil spillage on the 10th May, 1980 with 30,000 barrels spilled into the environment, the Idoho spill of January 1998, of about 40,000 barrels, Bonga Oilfield Spillage by the Shell Nigeria Exploration and Production Company (SNEPCO) on the 20th December, 2011with 40,000 barrels of crude spilled into the Atlantic Ocean (FMEnv, 2006; Nwilo and Badejo, 2006; Kadafa, 2012; Okafor-Yarwood, 2018). Nigerian oil spill real-time data can be accessed through a web-based geographic information system (GIS) platform called the 'Oil Spill Monitor' could be assessed on https://oilspillmonitor.ng/ managed by the National Oil Spill Detection and Response Agency (NOSDRA), the lead agency in oil spill management in Nigeria (TheOilSpillMonitor, 2019). **Figure 1** shows an example indicating the profiles of the spill monitor enlarged as shown by red arrows. It could be on satellite mode or map mode which includes OpenStreetMap, MapBox Reg and so on, but the mode in the figure is the

National Geographic map. The spills are categorised using coloured circles as shown on the legend. The black-coloured circles on the map are location of spills caused by oil company’s action, the red-coloured continuous circles are spill locations caused by third party interference which could either be a mistake by another company doing its own business, oil theft or sabotage. The purple-coloured circles are spills locations that were not visited for investigation and clean up response due to lack of logistics or the sea would have quickly taken care of things. The red-coloured dotted circles represent locations of spills where the quantity spilled was not ascertained, and the ash-coloured circles represent the spill locations that were cleaned up. The status of spills is by default ‘not invalid’, but a drop down will show different status as ‘confirmed’ i.e., spill confirmed, ‘invalid’ i.e., mistakenly reported spill, ‘new’ i.e., newly reported, and ‘reviewed’ i.e., awaiting confirmation. The contaminant has its default setting as ‘not gas’, the drop down will show other contaminants such as ‘ch’ for chemicals or drilling mud, ‘co’ for condensate, ‘cr’ for crude oil, ‘ga’ for gas, ‘no’ for no spill, and ‘re’ for refined products. The incident date could be used to locate a spill by date or a range of period, and the filter could be used to filter further information either in tabular or graphical form. There are no spill data before 2006 as NOSDRA was established in 2006. However, a data summary between January, 2010 to January, 2020 from the Oil Spill Monitor shows a total of 9609 oil spill incidents with a total of 452,616.215 barrels recorded, no joint investigation visits were conducted for 1916 of these spill sites due to logistics problem as most of the sites were located off-shore and NOSDRA does not have the capacity to access these locations without the help of the oil companies, and 3159 of these spill incidents were without estimated quantities provided by the oil companies (TheOilSpillMonitor, 2019). Hence, these figures are subject to reviews and studies have shown that the causes of these spills in Nigeria are; as a result of corrosion (50%), due to sabotage (28%), due to oil production operations (21%), and as a result of other reasons such as engineering drills, machine failures, ineffective well controls, errors during oil vessels loading and unloading operations (1%) (Nwilo and Badejo, 2006; Aroh *et al.*, 2010).

Nigeria has a coastline which is contiguous to the Atlantic Ocean, and it is approximately 853 km in length. This coastline lies between the geographical locations of latitude 4° 10’ to 6° 20’ N and longitude 2° 45’ to 8° 35’ E. The terrestrial demarcation of this area is about 28,000 km^2 and a continental shelf with surface area of 46,300 km2. The coastline is a low lying area of not more than 3 m above the sea level and is generally characterised by fresh water swamps, mangrove swamp, tidal channels, sand bars and lagoon marshes (Dublin-Green *et al.*, 1999). The climate of the Nigerian coastal area is tropical in nature, consisting of rainy season (April to November) and dry season (December to March). It is also characterised by high temperatures and humidity with an annual rainfall ranging between 1,500 and 4000 mm (Kuruk, 2004). The region also experiences mainly an onshore south westerly wind confined to azimuths of 215° to 266° with wind speed of 2-5 m/s which increases to about 10 m/s during the rainy seasons with heavy rainfall and thunderstorms. Such knowledge provides useful information for trajectory modelling used during oil spill clean-up and restoration assessment.

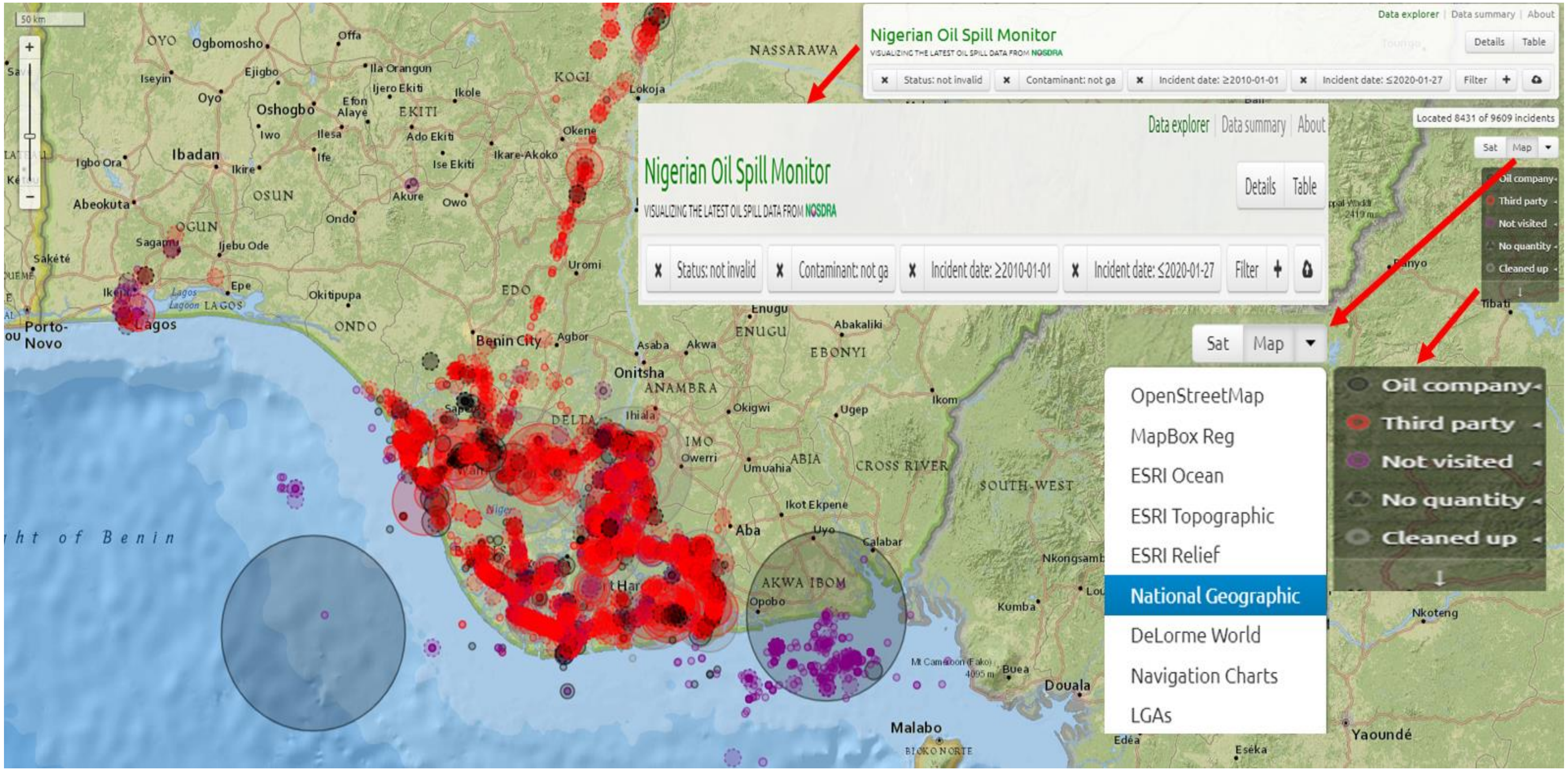


Figure 1: Data Summary from 01-01-2010 to 27-01-2020 from NOSDRA Oil Spill Monitor

1.5.1 Legal Framework

The National Oil Spill Detection and Response Agency (NOSDRA) is the lead government agency in oil spill management in Nigeria. NOSDRA was established by Act No. 15 of 2006 as a parastatal under Federal Ministry of Environment (FMEnv) and is the institutional framework for the coordination and implementation of the National Oil Spill Contingency Plan (NOSCP) for Nigeria. The NOSCP is an elaborate government document that contains the modus operandi produced by NOSDRA in collaboration with other relevant government and non-governmental bodies and implemented by NOSDRA in accordance with the OPRC 90 international conventions as Nigeria has ratified. It also keys into the West, Central and Southern Africa oil spill management system oil spill management in Nigeria prior to the establishment of NOSDRA

The institutional framework before the establishment of NOSDRA was drawn from the Nigerian constitution as the sole owner of mineral oil and gas, which meant that individuals and companies engaging in oil activities must obtain licenses from the Federal Ministry of Petroleum Resources. Consequently, the legal framework for the control and mitigation of oil pollution in Nigeria was enacted as follows; the Mineral Oil Safety Regulation 1963, Petroleum Regulations 1967, Petroleum Refining Regulation 1974 amongst many others and their subsequent amendments (Nwilo and Badejo, 2006; Kadafa, 2012). Establishment of the Federal Environment Protection Agency (FEPA) Act in 1988 and the Oil Pollution Act (OPA 1990) of 1990 were breakthrough that brought about improvement in oil pollution management in Nigeria. The FEPA 1988 provided the government framework for issuing standards for water, air, land quality and oil companies followed the Environmental Impact Assessment (EIA) decree No 86 of 1992 for oil company’s proposed projects likely impacts and mitigation (Kadafa, 2012). The OPA 1990 provided the legal framework upon which Department of

Petroleum Resources (DPR) issued the Environmental Guidelines for the Petroleum Industry in Nigeria (EGASPIN) in 1991.

Crude oil undergoes a natural alteration process in the environment termed ‘weathering’. When crude oil is released to the environment, a wide variety of changes occur to it for the time it spends in the environment. These changes could be through physical, chemical or biological processes. This process is the focus of this research and further details will be discussed in chapter 2.

1.6 Aims

The overall aims of this project are to investigate the effects of the main weathering processes including evaporation, water washing, aerobic and anaerobic biodegradation, on molecular markers in crude oils that are used in fingerprinting oils for oil-source and oil-oil correlation for environmental forensic purposes in comparison with past work.

1.7 Research Questions

The research attempts to provide answers to the following questions:

1. What are the effects of different weathering processes including evaporation, water washing and biodegradation on marker compounds used in oil-source correlation?
2. What is the influence of an environmental background of indigenous (non-oil derived) and other oil-derived hydrocarbons, on the reliability of fingerprinting results?
3. Are there novel marker compounds in the crude oils apart from the conventional markers such as steranes, terpanes and PAH that could be used for correlation purposes which could add to the existing body of knowledge discussed in section 1.4?

4. Identification of the limitations in the existing fingerprinting parameters.

1.8 Objectives

1. To determine the effect of evaporation on the marker compounds in the crude samples after 1, 3, 10 and 30 days evaporation using GC-FID and GC-MS.
2. To determine the effect of water washing on the marker compounds in crude samples by washing with artificial 1.7% salinity typical for brackish water 5, 10, 15, and 20 times using GC-FID and GC-MS.
3. To determine the effect of optimised aerobic biodegradation on the marker compounds in crude oils using sediment from Whitley Bay, UK and Bodo, Nigeria, as inocula incubated for 5, 10, 20, 50, 100 and 300 days respectively using GC-FID and GC-MS.
4. To determine the effect of optimised anaerobic biodegradation on the marker compounds in crude oils using sediment from the River Tyne, UK, as inoculum and, incubated for 100, and 300 days respectively using GC-FID and GC-MS.
5. To determine the influence of microbial community in the sediments on degradation of compounds in the crude oils.

Chapter 2. Literature Review

2.1 Demand for Fossil Fuel Energy

Global demand for crude oil has been increasing since the 1920s to date to meet the industrialisation needs of both developed and developing nations as human population continue to rise (Wang *et al.*, 2006; Dargay and Gately, 2010). Despite the hunt for alternative sources of energy lower CO_2 emissions, to limit the effects of climate change due to greenhouse gas induced global warming, the demand for crude oil is forecast to increase for decades to come with an estimated long-term demand growth of 11.9 mb/d and with a corresponding supply growth of 11.8 bm/d for 2018 to 2040 (OPEC, 2019). A shorter-term forecast reported demand growth of 4.4 mb/d from 2019 to 2026 was however affected by supply shortfall of 6.3 mb/d in 2020 likely due to the Covid-19 pandemic (IEA, 2021). With these forecasts for increased worldwide usage of petroleum, it will continue to find its way into the environment (as spills) and with the attendant environmental impacts (Mudge *et al.*, 2008).

2.2 Crude in the environment, sources, fate, impact and sink

Owing to the increasing demand for crude oil and its refined products, the widespread usage of petroleum has resulted in unintentional and also sometimes in deliberate releases into the environment with often serious ecotoxicological effects, some of which are irreversible, from about a century ago (NRC, 2003), which necessitated the need to proactively take steps to prevent the releases or respond appropriately when prevention fails, to minimise the impact.

2.2.1 Crude oil and hydrocarbons in the marine environment

The amounts of petroleum released into the marine by different means from 1970s has been reported in number of publications since then. A significant annual contribution of 47% has been reported to come from natural marine seeps, which are the biggest single source going back millions of years, 33% from consumption activities (land-based run-off, non-tanker operational releases and spills), 8% from tanker spills, 5% from other (atmospheric deposition, jettison from aircraft fuel), 4% from transportations (cargo washings, coastal facility and pipeline spills), and 3% from extraction processes (platforms and produce water) (NRC, 2003; Prince, 2005). Depending on the frequency of accidents and war situation that may contribute to marine input, the total annual oil input into the marine fluctuates such as 3.2 million tonnes/year in 1981 (GESAMP, 1993), 2 – 8 million tonnes/year in 1985 (NRC, 1985), 2.35 million tonnes/year in 1990 (GESAMP, 1993). Better policymaking and mitigative actions for the last half a century have resulted in over 90% reduction of discharges in the global spill trend from the 1970s to 2010s (see Figure 0.1) with the locations of the some of the major spills shown in Figure 0.2.

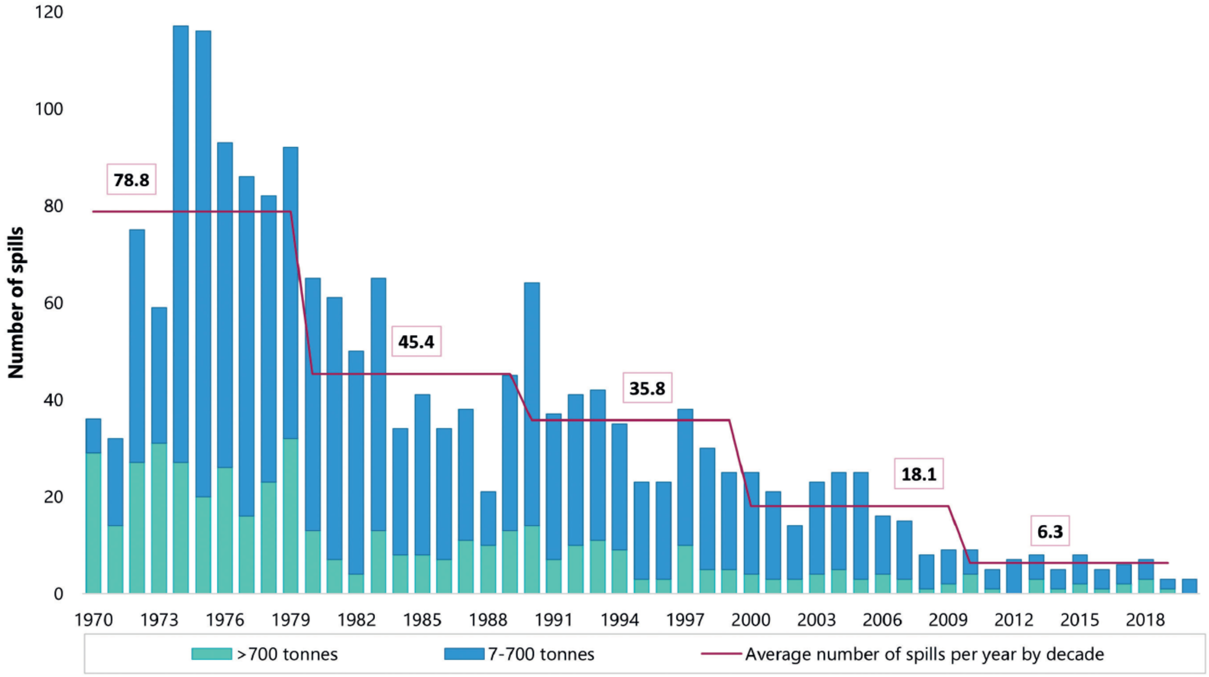


Figure 0.1: Number of medium (7-700 tonnes) and large (>700 tonnes) tanker spills from 1970-2020. (ITOPF, 2021)

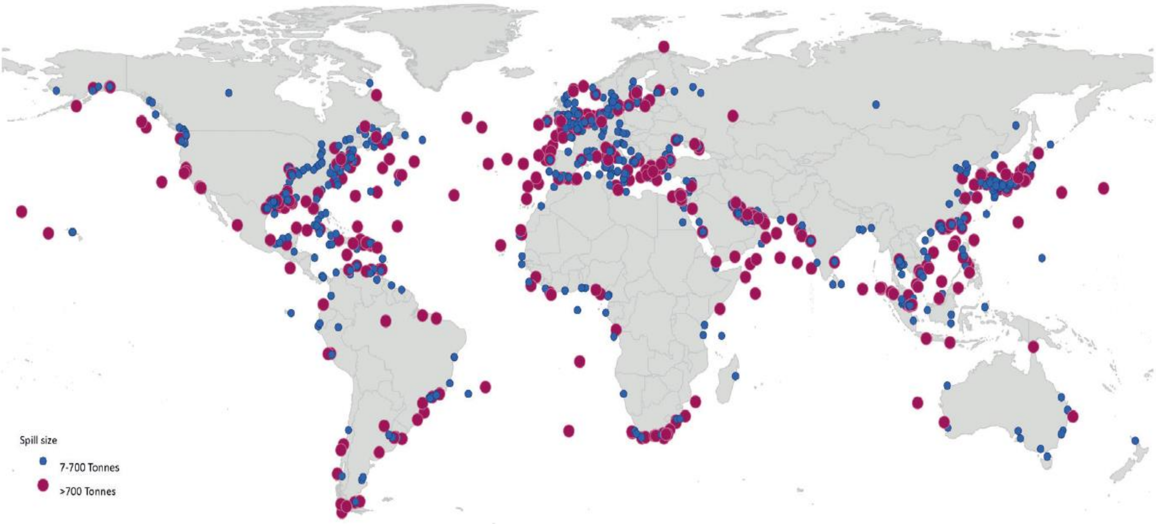


Figure 0.2: Map of large spills (>700 tonnes) from 1970-1920. (ITOPF, 2021)

2.3 Crude oil weathering

Crude oil and its products when spilled into the marine, undergo natural alteration processes collectively termed as ‘weathering’ processes, which occur at different rates depending on factors such as the type and quantity of the oil released, initial properties (viscosity, density, solubility etc.) of the oils and prevalent climatic and Sea conditions (temperature, winds, currents etc.) (Payne and Jr, 1984; NRC, 2003; Daling *et al.*, 2014). The weathering processes determine whether the oil remains at sea or is washed ashore and their relative significance and occurrence is time dependent and can control the extent of impact of the spilled oil (NRC, 2003; ITOPF, 2014).

2.3.1 Physical weathering processes

The physical weathering of spilled crude oil has been described in several reviews (Jordan and Payne, 1980; NRC, 2003; Stout and Wang, 2007a; ITOPF, 2014). It includes evaporation, which is a process where the volatile components of oil evaporate into the atmosphere. The rate of evaporation largely depends on the ambient temperature, wind speed, proportion of low-molecular components which are more volatile and initial spreading which enhances evaporation by increasing the surface area of the spilled oil. Spreading is a horizontal movement of oil that depends on its viscosity, where low viscous oils spread and weather faster than more viscous oil. Dispersion depends on the nature of the oil and the sea conditions as low viscous oil breaks faster from the slick into droplets down the water column in the presence of waves and turbulence. Emulsification occurs when oils take up water and form stable emulsions called 'mouse' of between 60 and 85% water, expanding by five times the initial volume. The rate of biodegradation in the mousse is reduced as oxygen and nutrients supplies are cut-off by the thick layer formed at the surface (Floodgate, 1984). Dissolution is also a process with the rate and extent dependent on oil composition, spreading, water temperature, turbulence, and extent of dispersion of the oil components generally, similar to evaporation (Jordan and Payne, 1980). Dissolution contributes very little in these processes because the greater percentage of the more volatile components such as benzene and toluene would likely evaporate before being dissolved. Sedimentation and sinking are when dispersed oil droplets may sink in combination with suspended particles down the water column, and with further favourable conditions these particles laden oil droplets will settle to the seabed as sediment. Some of the sinking droplets could be washed to the shore where shoreline interaction occurs within the inter tidal zone (Ehrhardt and Blumer, 1972; NRC, 2003; Stout and Wang, 2007a; ITOPF, 2014).

2.3.2 Chemical weathering processes

Photo-oxidation is a process where oil components are removed in the presence of light. It is an oxidation stimulated by light which occurs for quite a long period where especially aromatic components are oxidised to more polar compounds such as carboxylic acids and ketones etc. Although its overall effect is quite minimal, outer layers of viscous water-in-oil emulsions can be oxidised to form protective layers and are later washed ashore in form of persistent tarballs (Hardy *et al.*, 1977; NRC, 2003; ITOPF, 2014).

2.3.3 Biological weathering processes

A range of micro-organisms found in the marine environments such as bacteria, fungi, algae, moulds, yeasts etc, can metabolise petroleum hydrocarbon compounds as sources of carbon and energy which is referred to as biodegradation. It usually involves aerobic respiration as the most favourable energy making process of the two metabolic pathways and then a detoxification process whereby water-soluble metabolites are egested from the organism (NRC, 2003). Biodegradation is the predominant hydrocarbon mineralisation process in the aquatic environment and the rate depends on the oil properties, presence of hydrocarbon-degrading microorganisms, oxygen, nutrients and temperature. Whilst complete microbial degradation produces CO_2 and water as end, incomplete degradation produces intermediate compounds (Ehrhardt and Blumer, 1972; Hardy *et al.*, 1977; NRC, 2003; Stout, 2007; ITOPF, 2014). While simple or low-molecular hydrocarbon compounds are degraded directly by micro-organisms, more complex hydrocarbon compounds may require a succession of different microbial species to get mineralised completely or they may be too complex to degrade (Whittle *et al.*, 1982; NRC, 2003). The processes explained in sections 2.3.1, 2.3.2, and 2.3.3 are shown in Figure 0.3.

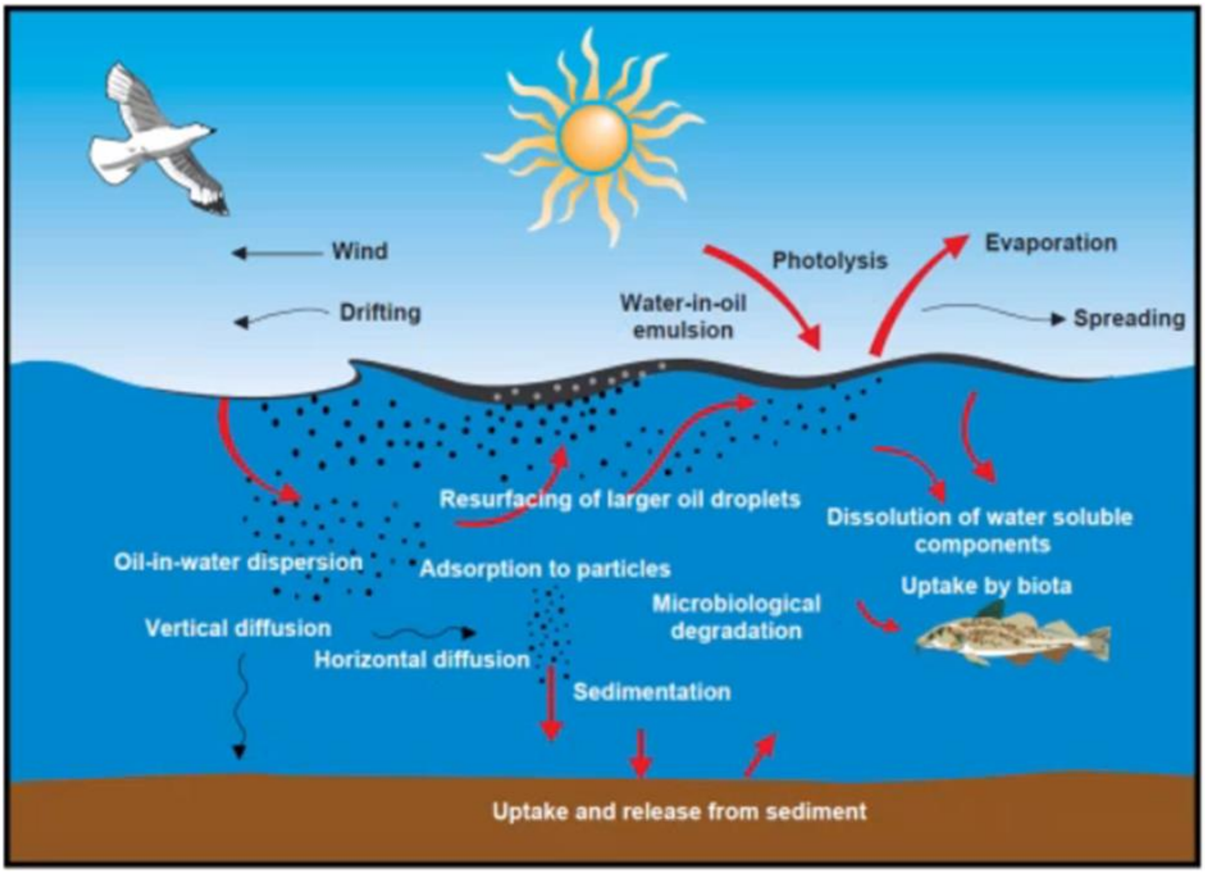


Figure 0.3: A conceptual process model of the weathering and fate processes of spilled oil in the marine environment (Source: SINTEF 2014)

The evaporation concept shown in Figure 0.3 will be discussed in more detail in chapter 3, while water-in-oil emulsion, dispersion, dissolution and phase fractionation (in deep reservoirs) are mostly influenced by water washing discussed in chapter 4. Microbial degradation effects are discussed in chapters 4 and 5 respectively.

2.4 Oil Spill Response

When an oil spill occurs, response is often necessary to curtail its impact on the receiving environment. Effective spill response greatly depends on a well laid down plan contingency plan and preparedness to be executed during spill event, where roles and responsibilities are assigned under a chain of command (NOSCP-REVISED, 2013; ITOPF, 2018a). Contingency planning generally includes risk assessment, strategy policy, operational procedures, and information directory. Adequate information about the location of the spill, type of oil spilled, time or date of spill, environmental and weather conditions etc. are important for quick decision-making on the response strategy to take to either allow the natural condition to eventually remove the spilled oil by natural attenuation if no imminent risks are assessed or to respond and protect any resources or ecosystem at risk. Dispersants could be applied for spills in the marine environment, but the application is time-dependent within a window of opportunity, or the spill could be recovered using the appropriate response equipment during clean-up operation (NOSCP-REVISED, 2013; ITOPF, 2018a). Post clean-up remediation action may also be necessary to restore the impacted environment closer to its initial state. When required, there are different methods of remediation techniques to decide on depending on the risk assessed (Protection, 2006; Prince and Douglas, 2010; Mercer and Trevors, 2011; Michel *et al.*, 2013; ITOPF, 2014; Singh *et al.*, 2014; O'Brien *et al.*, 2017).

2.5 Biodegradation mechanisms and limiting factors

The mechanism of biodegradation in natural ecosystems, as discussed in section 2.3.3, are very complex where microbes selectively remove compounds in oils and in sequence (Atlas, 1981; Head *et al.*, 2003). The sequence of relative susceptibility to biodegradation amongst the saturated hydrocarbon components in oil starting with the more easily attacked compound classes have been reported as *n*-alkanes > alkylcyclohexanes > acyclic isoprenoid alkanes > C14-C16 bicyclic alkanes > steranes > hopanes > diasteranes, and that of the aromatic hydrocarbon compounds as monoaromatic > diaromatics > triaromatics (Connan, 1984a; Volkman *et al.*, 1984; Rowland *et al.*, 1986; Atlas and Bartha, 1992). Several studies have been carried out on biodegradation of oil hydrocarbons as carbon sources to yield energy needed by the microbes both in the laboratory and in the field (Connan, 1984a; Volkman *et al.*, 1984; Wardroper *et al.*, 1984; Lee and Levy, 1989; Prince and Walters, 2016). For aromatic compounds, the rate of biodegradation decreases with increase in number of aromatic rings and alkylation (Jones *et al.*, 1983; Rowland *et al.*, 1986; Cassani and Eglinton, 1991; Wang and Fingas, 1995). Biodegradation rates of petroleum hydrocarbons in the environment is dependent on limiting factors such as chemical and physical compositions of the oil, petroleum-degrading microorganisms, oxygen availability, temperature, and nutrient availability (Atlas, 1981; Lee and Levy, 1989).

2.5.1 Oil Chemistry

Crude oil is a complex mixture different hydrocarbon compounds and biodegradation is known to significantly alter its chemical composition and physical properties in surface and subsurface environments thereby reducing the concentrations of low-molecular weight components of aliphatic and aromatic fraction while polar fractions, viscosity, density, sulfur content, and acidity tend to increased (Connan, 1984a; Moldowan and Peters, 1993; Peters *et al.*, 2005; Speight, 2005; Larter *et al.*, 2006a; Wang *et al.*, 2006). The physical state of the oil is also important because hydrocarbon degrading microorganisms are known to act mainly at the oil-water interface (Atlas and Bartha, 1992). Oil compound classes possess varied susceptibility to biodegradation some of which have been utilised for a classification scheme developed by Peters and Moldowan (1993) commonly known as PM Scale level 1-10 and its modifications where components such as *n*-alkanes are degraded first and heavy aromatic components take much longer time to degrade (Moldowan and Peters, 1993; Head *et al.*, 2003; Larter *et al.*, 2012). The polar components asphaltenes and resins are known to increase in relative abundance during biodegradation due to their relative recalcitrance (Tissot and Welte, 1984; Bonnamy *et al.*, 1987). Although the asphaltic fraction of petroleum are known to be affected by microbial attacked, but the biodegradation pathways are not as well-known as that of the aliphatic and aromatic fractions (Atlas, 1981; Connan, 1984a; Atlas and Hazen, 2011).

2.5.2 Petroleum-degrading microorganisms

Microorganisms are known to have the ability to utilise hydrocarbons as their sole carbon sources of energy at a rate dependent on the chemical composition of the petroleum and the prevailing environmental factors (Atlas, 1981; Atlas and Bartha, 1992; Atlas and Hazen, 2011). The earliest studies where aerobic hydrocarbon-degrading microcosms were first isolated were conducted by Söhngen (1906) and Kaserer (1906) (Bushnell and Haas, 1941) and it is known that hundreds of species and genera of bacteria, archaea, yeast and fungi can degrade petroleum hydrocarbons (Atlas, 1981; Bossert and Bartha, 1984; Floodgate, 1984; Prince, 2005; Atlas and Hazen, 2011). Although hydrocarbon-degrading microorganisms are also found in the soil, sediments, and freshwater ecosystems, more attention has been focused on the marine environment because it the largest receptor of hydrocarbon pollutants and also has the greatest variety of hydrocarbon-degrading bacteria which are ubiquitous in marine systems (Atlas, 1981; Lee and Levy, 1989). Several studies have also reported elevated levels of oil-degrading microorganisms after oil spills in different environments (Atlas, 1981; Floodgate, 1995; Prince, 2005; Atlas and Hazen, 2011; Mercer and Trevors, 2011; Hazen *et al.*, 2016).

2.5.3 Oxygen availability

Substantial amount of dissolved oxygen (3 to 4mg) is required to oxidise 1mg of hydrocarbon to CO_2 and H_2O, but oxygen limitation for the degradation of hydrocarbons is however quite rare in the open ocean since the hydrocarbons are often found at the near surface of the water column and well oxygenated and also due to the low oil concentration as result of natural and chemical dispersion (Lee and Levy, 1989; Hazen *et al.*, 2016). The presence of oxygenase enzymes responsible for biodegradation of oil will still continue the degradation at low dissolved oxygen concentration due to their high affinity for oxygen (Lee and Levy, 1989). While oxygen concentrations in beaches, sediments and peaty mangrove soils are usually low and also the large stable mousse and tarballs formed, which are usually moved to the shorelines by tidal energy thereby creating oxygen limiting environmental condition (Ward *et al.*, 1980; Lee and Levy, 1989; NRC, 2003).

2.5.4 Nutrient availability

The availability of inorganic nutrients in the marine environment especially nitrogen and phosphorus, are known to be limiting factors for petroleum hydrocarbons microbial degradation (Atlas, 1981; Lee and Levy, 1989; Floodgate, 1995; Atlas and Hazen, 2011; Singh *et al.*, 2014; Hazen *et al.*, 2016). These nutrients are required to increase biodegradation rates which are low in seawater due to competition from other species such as phytoplanktons (Lee and Levy, 1989). Several studies have reported different nitrogen and phosphorus concentrations under different oils, seawater, sediment, and shoreline conditions at which P/N, C/N, and C/P ratios were related to microbial degradation rates, while some have been used as data for nutrients kinetic parameters modelling (Atlas, 1981; Swannell and Head, 1994; Floodgate, 1995; Singh *et al.*, 2014).

2.5.5 Temperature

Hydrocarbon-degrading microorganisms have been isolated from different environments with biodegradation observed from sub-zero temperatures to above 70°C, but with slower degradation taking place at low and high temperatures (Atlas, 1981; Lee and Levy, 1989; Head *et al.*, 2003). Different hydrocarbon degraders grow at low (psychrotrophic), medium (mesophilic), and high (thermophilic) temperatures respectively (Lee and Levy, 1989). Temperature also affects the physical and chemical state of the oil, which differ depending on the oil composition. At low temperatures volatilisation of low-molecular-weight hydrocarbons which are toxic oil degraders is slowed down thereby delaying the initial oil biodegradation (Atlas, 1981; Lee and Levy, 1989).

2.6 Biodegradation Scales

Different schemes used to describe the extent of biodegradation of petroleum have been developed. The relative amounts or abundance of compound classes based on their differential resistance to biodegradation are compared and used to rank the extent of biodegradation (Peters *et al.*, 2005). The following are some of these schemes.

2.6.1 The Peters and Moldowan Scale

The Peters and Moldowan scale (PM Scale) is the most widely used ranking scheme which shows the effects of various levels of biodegradation (from 1 to 10) on the composition of a typical mature oil (Peters and Moldowan, 1993b). The relative levels of biodegradation scale is mostly from field empirical observations, and is based partially on changes in bulk chemical and physical properties and partially on the principles of sequential microbial metabolic breakdown (Peters *et al.*, 2005). **Figure 0.4** shows the PM scale modified after Wenger et al. (2002), with a range description from “slight” to “severe”, like the earlier Volkman scheme (**see Figure 0.5**) which is based on alteration of biomarker compounds.

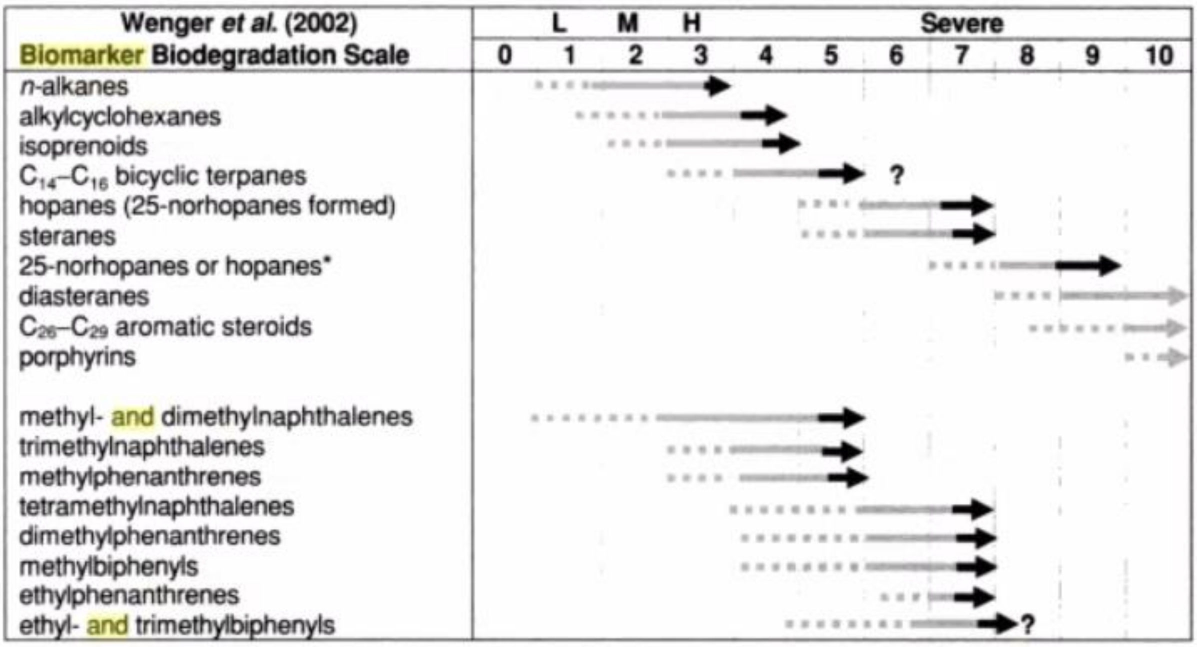
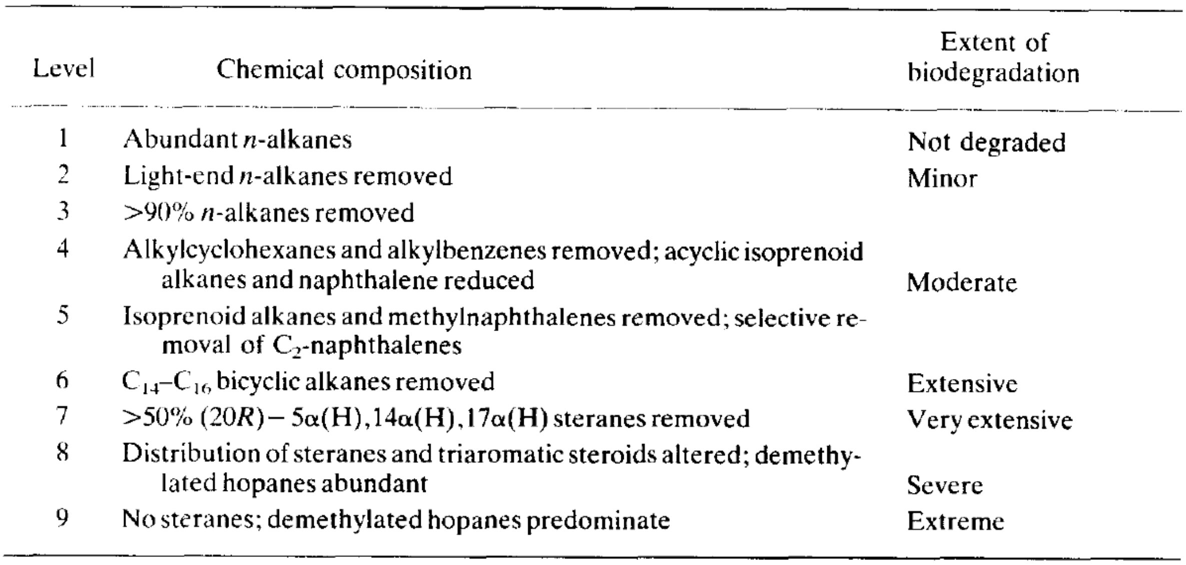


Figure 0.4: Wenger et al. (2002) biodegradation scale in comparison with the Peters and Moldowan (1993). (Source: Peters et al., 2005). The arrows indicate dashed lines (compound classes are first altered), solid grey lines (substantially depleted compound class), and black (Compounds class eliminated completely). In Peters and Moldowan (1993), the ranges on the scale were described as follows; “light” = 1-3, “moderate” = 4-5, “heavy” = 6-7, “very heavy” = 8-9, and “severe” = 10.



Level	Chemical composition	Extent of biodegradation
1	Abundant *n*-alkanes	Not degraded
2	Light-end *n*-alkanes removed	Minor
3	>90% *n*-alkanes removed	
4	Alkylcyclohexanes and alkylbenzenes removed; acyclic isoprenoid alkanes and naphthalene reduced	Moderate
5	Isoprenoid alkanes and methylnaphthalenes removed; selective removal of C_2-naphthalenes	
6	C_{14}–C_{16} bicyclic alkanes removed	Extensive
7	>50% (20*R*)– 5α(H),14α(H),17α(H) steranes removed	Very extensive
8	Distribution of steranes and triaromatic steroids altered; demethylated hopanes abundant	Severe
9	No steranes; demethylated hopanes predominate	Extreme

Figure 0.5: The Volkman biodegradation index (Source: Volkman et al., 1984)

2.6.2 Wenger scheme

The Wenger et al. (2002) scheme is a more pragmatic biodegradation scale because it contains a more comprehensive list of compounds, compound classes and carbon number range (see Figure 0.6). The scale defines biodegradation broadly from very slight to severe and is essentially based on the presence or absence of single key compound classes and a consideration of the extent changes within a compound class, indicated by using a dotted or lightly shaded areas in the presence/absence plot.

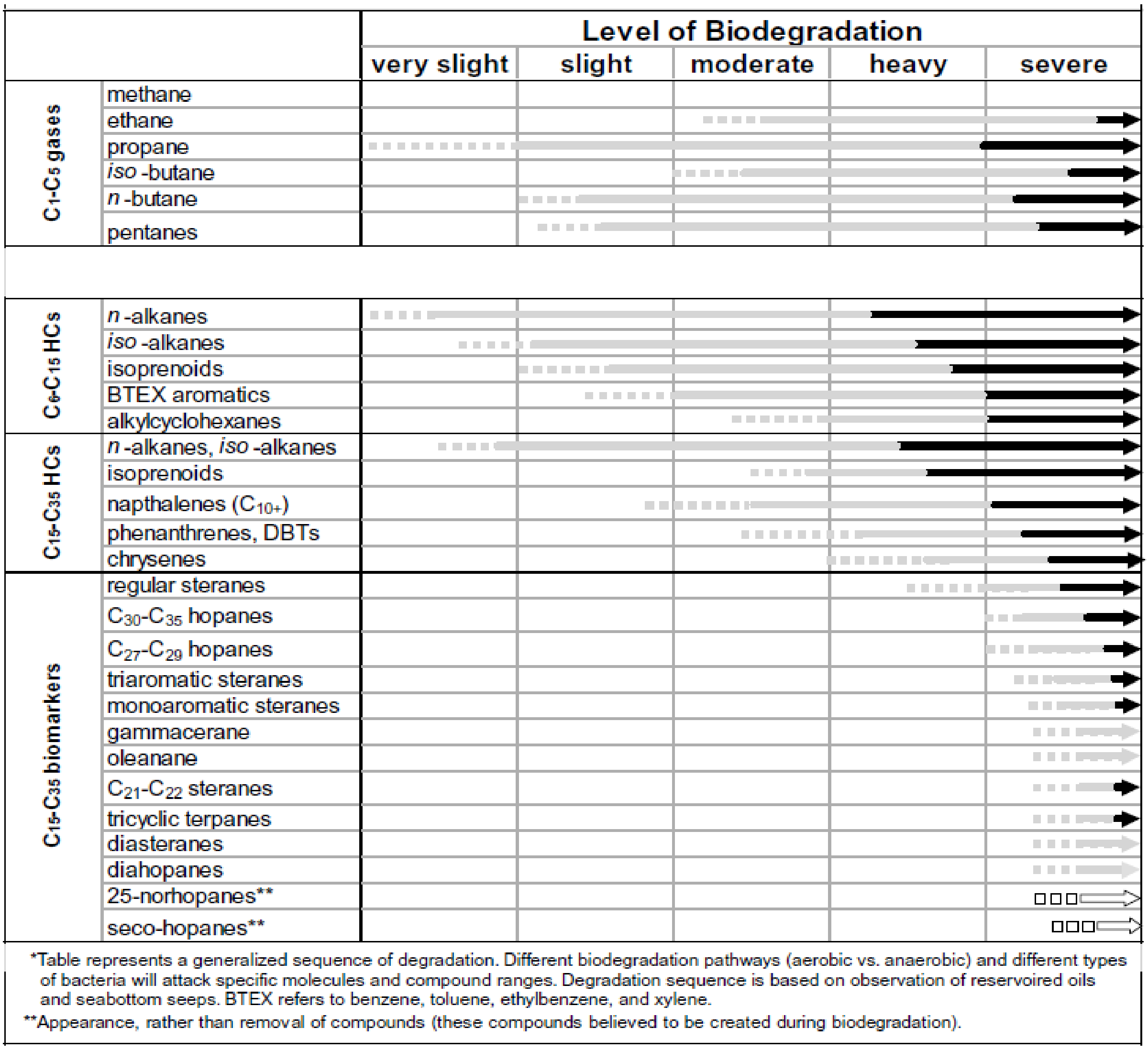


*Table represents a generalized sequence of degradation. Different biodegradation pathways (aerobic vs. anaerobic) and different types of bacteria will attack specific molecules and compound ranges. Degradation sequence is based on observation of reservoired oils and seabottom seeps. BTEX refers to benzene, toluene, ethylbenzene, and xylene.

**Appearance, rather than removal of compounds (these compounds believed to be created during biodegradation).

Figure 0.6: Wenger Scheme (Source: Wenger et al., 2002).

2.6.3 MANCO Scheme

The Modular Analysis and Numerical Classification of Oils (MANCO) scheme provides a scale with a higher resolution compared to other schemes earlier discussed, and considers their limitations in describing the extent of biodegradation in super heavy oils (Larter *et al.*, 2012). The MANCO scheme involves the use of integrated scoring of the extend of biodegradation of several compound classes which differs from the other schemes by: (i) eight compound classes of differing reactivity, that are appropriate to heavy oil and bitumen, have been identified and used; (ii) the extent of alteration within a compound class has been used in at least a semi-quantitative manner and (iii) the extent of alteration within a compound class and between compound classes has been combined in an algorithm using a linear function within a compound class and power function between classes (Larter *et al.*, 2012). Mass chromatograms of various compound classes are inspected and assign scores (Manco vector) which is then algorithmically summed to a single Manco number, which provides the overall estimate of the extent of biodegradation of heavy oil, or sand/bitumen samples (Larter *et al.*, 2012). The score ranges from 0 for pristine oils are assign a scale PM 0 up to PM 8.

2.7 Oil spill identification techniques

Chemical fingerprinting is the most frequently used in oil spill identification techniques owing to its recent development and improvements which involve pattern recognition of the relative distribution of petroleum hydrocarbons and the analysis source-specific markers (Wang *et al.*, 1999).

2.7.1 Chemical fingerprinting

This is a technique is to indicate unique patterns by specifying the presence and relative abundance of analyte of interest. It is a technique used to characterise complex hydrocarbon compound mixtures found in crude oils. It is employed by geochemists in petroleum exploration especially using biomarkers and geochemical markers, but also applied in environmental forensics for source identification, differentiation, characterisation of fugitive petroleum pollutants (Wang and Fingas, 1997; Peters *et al.*, 2005; Mudge *et al.*, 2008). It involves the use of both instrumental and non-instrumental analysis of hydrocarbon and the methods used include gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), size-exclusion HPLC, infrared spectroscopy (IR), supercritical fluid chromatography (SFC), thin layer chromatography (TLC), ultraviolet (UV), fluorescence spectroscopy, isotope ratio mass spectrometry, nuclear magnetic resonance (NMR), and gravimetric methods (Wang and Fingas, 1997; Wang *et al.*, 1999) . GC with GC-MS are the most often used.

Chemical fingerprinting is employed by environmental scientists to assess spill impact on natural resources, predict how the spill could impact on environment in the long-term, to select the best spill response strategy and clean-up measures, and dispute settlement related to liability (Mudge, 2008; Wang, 2008). Chemical fingerprinting of petroleum is dependent on factors which include (1) the genetic history of the crude oil over geological time scale, (2) the influence of any refining processes, (3) the effect of weathering on the spilled crude or refined product in the environment and (4) the extent of interferences of the spilled oil when mixed with naturally occurring hydrocarbons in the environment or isobaric influences (Mudge *et al.*, 2008) . Fingerprinting could be positive and negative or “match” and “non-match” based on correlation, and can be conducted as semi-quantitative (peak ratios), fully quantitative (total concentrations), and qualitative (visual observation), and details about the chemicals involved is often required by regulatory agencies (Mudge *et al.*, 2008).

2.7.2 Fingerprinting protocols

Different protocols are employed by different countries and regions. The most popular and widely used protocols include those by the European Committee for Standardisation (CEN), American Petroleum Institute (API), American Society for Testing and Materials (ASTM), and the Nordtest Methodology (Wang and Fingas, 1997). The Nordtest methodology is a tiered analytical approach, which was revised and later adjusted into guidelines for European Committee for Standardisation (CEN) (Daling *et al.*, 2002b). The CEN guidelines for oil spill identification was later adopted into Bonn Agreement in 2005 which resulted in Part 1 and Part 2 document in 2006, and the Part 2 was later amended in 2012 (Kienhuis *et al.*, 2016).

2.7.3 Diagnostic compounds

Diagnostic compounds are vital to the chemical fingerprinting process. They act as key indicators for correlation between oil spill samples and suspected sources by way of ratio comparisons. There is no set of universally accepted diagnostic compounds and so therefore, the choice depends on the uniqueness of the spill source identification task in focus (Wang *et al.*, 1999; Wang and Fingas, 2003; Mudge *et al.*, 2008). The Nordtest Methodology relies on the use of diagnostic ratios of biomarkers and polycyclic aromatic hydrocarbons (PAHs) (Daling *et al.*, 2003). The characteristics attributed to useful diagnostic compounds include (1) tendency for high degree of specificity when compared to different oils, (2) relative ubiquity in the petroleum, (3) being able to be measured accurately within the complex mixtures, and (4) relative resistance to degradation in the environment (Mudge *et al.*, 2008). Biomarkers such as terpanes, steranes, mono and triaromatic steroids are the most commonly used by organic geochemists (Peters and Moldowan, 1993b), whereas phenanthrenes and dibenzothiophenes are the most common diagnostic compounds within the PAHs family (Daling *et al.*, 2003).

2.7.4 Nordtest Methodology

The correlation methodology used is based on the multi-tiered system approach of the Nordtest Method adopted by European Committee for Standardisation (CEN) presented in protocol or decision chart as shown Figure 0.7. It is widely used technique in most countries of Europe, the USA and Canada (Daling *et al.*, 2002b). The methodology is a tiered "levels" protocol/ decision chart for analyses and data treatment based on oil gas chromatography (GC/FID) screening of all samples (i.e., both spilled oils and suspected sources) as Level 1 in the protocol, analysis (fingerprinting) of spill and suspected source samples using gas chromatography with mass spectrometry (GC/MS) as Level 2. Diagnostic ratios of selected PAHs and biomarkers are extracted by pattern comparison at this level, evaluated and selected based on variability due to weathering. Selected diagnostic ratios can then be compared by statistical treatment of the correlations using confidence limits, with final assessment being concluded into four operational and technical defensible identification terms: as positive match, probable match, inconclusive or non-match in level 3 (Daling *et al.*, 2002b).

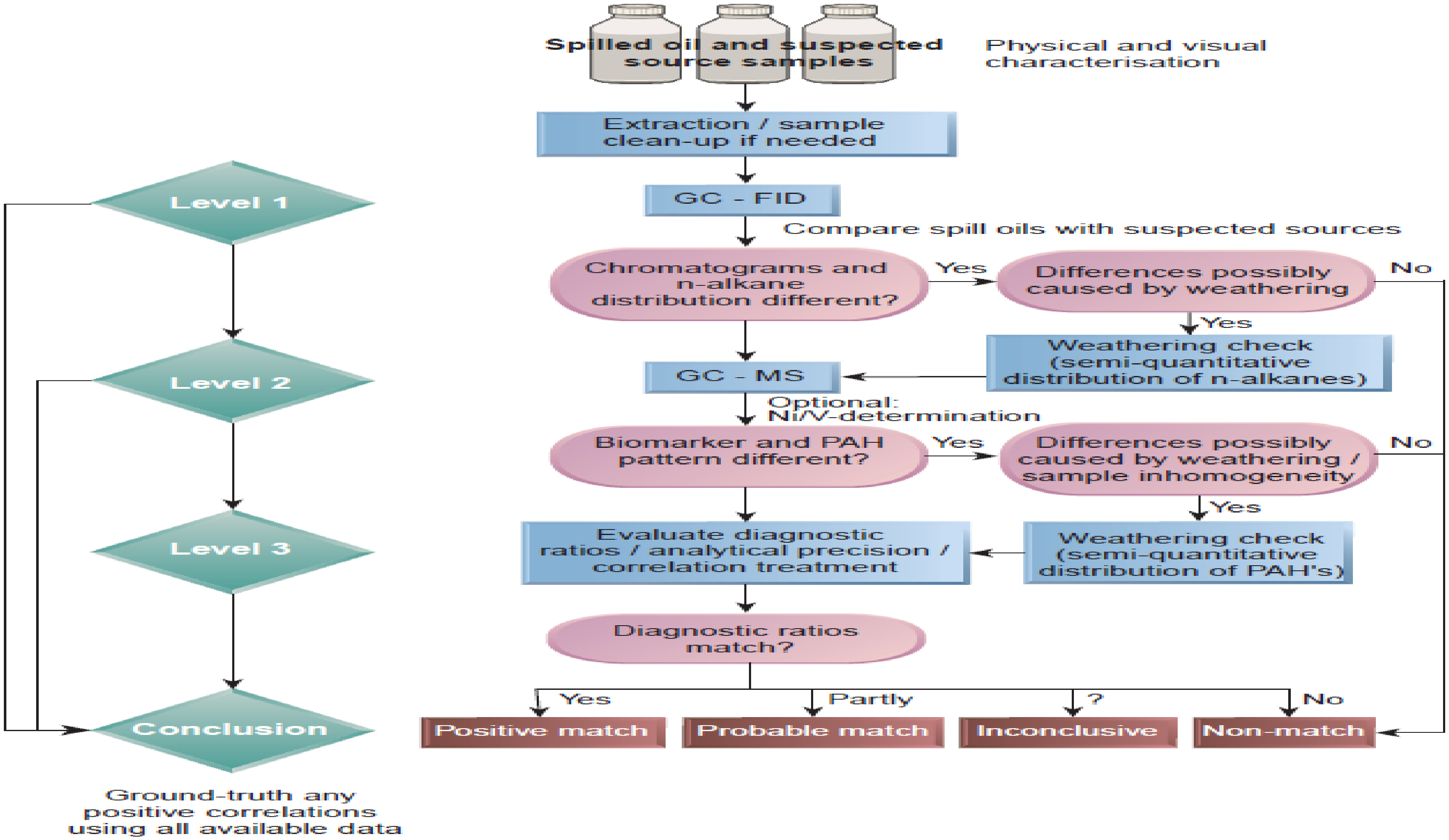


Figure 0.7: Protocol/Decision Chart for the Oil Spill Identification Methodology (Source: Daling et al., 2002).

Chapter 3: Effects of evaporation on the composition of crude oils

3.1 Introduction

Evaporation have been reported as the single most important and dominant process for weathering or mass removal from surface slicks of oil spills (Wang and Fingas, 1995; NRC, 2003; Simecek-Beatty and Lehr, 2007). Evaporation is of prime importance to understanding the fate and behaviour of oils in the environment and it is an abiotic (physical) weathering process. Evaporation of crude oil or its products principally occurs immediately it is spilled into the environment and in the hours to days afterwards, thereby resulting in the alteration of its chemical composition and physical characteristics (Wang and Fingas, 1995). Acquisition of accurate and precise quantitative information on the extent of weathering of oil provide data which may be useful in oil spill modelling for better understanding of its fate when spilled into the environment (Wang and Fingas, 1995; Fingas, 2012; Fingas, 2013). Evaporation of crude oil or its distillate products unlike water, is not air boundary-layer regulated owing to its complex composition and cannot be represented by simple mass transfer rate evaporation equation(Fingas, 2012; Fingas, 2013). Crude oil evaporation greatly depends on factors which include; time, temperature, wind speed, turbulence, thickness or increased surface area of the slick (Fingas, 2013; ITOPF, 2014). Fingas (2013) states that almost all models include evaporation. Many evaporation studies on different types of oil and distillate products have reported evaporation rates of 15-75% within first few days of spill (McAuliffe *et al.*, 1975; Payne and Jr, 1984; Mackay and McAuliffe, 1988; Wang and Fingas, 1995; Wang *et al.*, 1999; Stout and Wang, 2007b; Fingas, 2013; Daling *et al.*, 2014). Better understanding of these processes is important for quick decision-making for contingency planning for oil spill response and countermeasure, restoration of damaged resources and also how they affect molecular makers used in oil fingerprinting for oil spill source identification. This chapter will investigate and discuss the effect of evaporation on the chemical composition and especially the molecular marker composition of crude oil samples.

3.2 Aim and objective

The aim of the experiments detailed in this chapter is to investigate the effect of evaporation on the chemical compositions of the three different oil samples.

The specific objectives were;

1. To determine the rates of evaporation of the different oil samples
2. To obtain detailed compositional information of the oils samples such as *n*-alkanes, acyclic isoprenoids, biomarker compounds, polynuclear aromatic hydrocarbons (PAHs) and their alkyl homologues and any relevant less well-known compound found.
3. To quantitatively determine the concentrations of the analytes in (2) above and compare the compositional and concentrations changes with respect to different weathering degrees.
4. To quantitatively examine ratios of compounds used for weathering evaluations
5. To quantitatively relate weathering percentages by evaporation with the concentration changes of target analytes

3.3 Materials and Methods

3.3.1 Reagents and Materials

Chromatographic-grade distilled solvents used include petroleum ether (BP 40-60°C) and dichloromethane (DCM) and methanol. Silica gel 60(230-400 mesh ASTM) and aluminium oxide 90 active neutral (70-230 mesh ASTM) were purchased from Merck, Darmstadt, Germany and 500gr white non-absorbent cotton wool purchased from Fisher Scientific, UK. The silica gel, the aluminium oxide and the cotton wool were pre-extracted in a Soxtec solvent extractor using an azeotropic (DCM-methanol) solvent mixture. The silica gel and the aluminium oxide were stored in glass bottles, covered with aluminium foil and activated in an oven over night at 130°C before use (e.g. Later *et al.*, 1985; Bundt *et al.*, 1987). Aliphatic hydrocarbon standards were squalane (99% purity) and 5α-cholestane (98% purity) obtained from Aldrich, Steinem, Germany, *n*-heptadecylcyclohexane (98% purity) obtained from Lancaster Synthesis, Morecambe, UK, 5α-androstane (98% purity) obtained from Sigma-Aldrich, Steinem, Germany. PAH standards were 1, 1'-binaphthyl (98% purity) obtained from Lancaster Synthesis, White Lund, UK, and *p*-terphenyl (99% purity) obtained from Fluka Chemika, Buchs, Switzerland. Two crude (light and medium) oil samples from the Niger Delta region of Nigeria and one from the North Sea were obtained. Flint glass evaporation Petri dishes of 100mm diameter and 10mm height obtained from VWR, Leicestershire, UK. The empty dishes weighed and weighed again after 2ml of crude samples were measured into the dishes in triplicates and placed in fume cupboard (see **Error! Reference source not found.**) to evaporate at an average temperature of 20°C monitored using a mercury thermometer, and an average air flow velocity of 1m/s measured using a digital anemometer for 1 day, 3 days, 10 days and 30 days respectively. Every set of samples were weighed at the end of their respective evaporation days before fractionation.

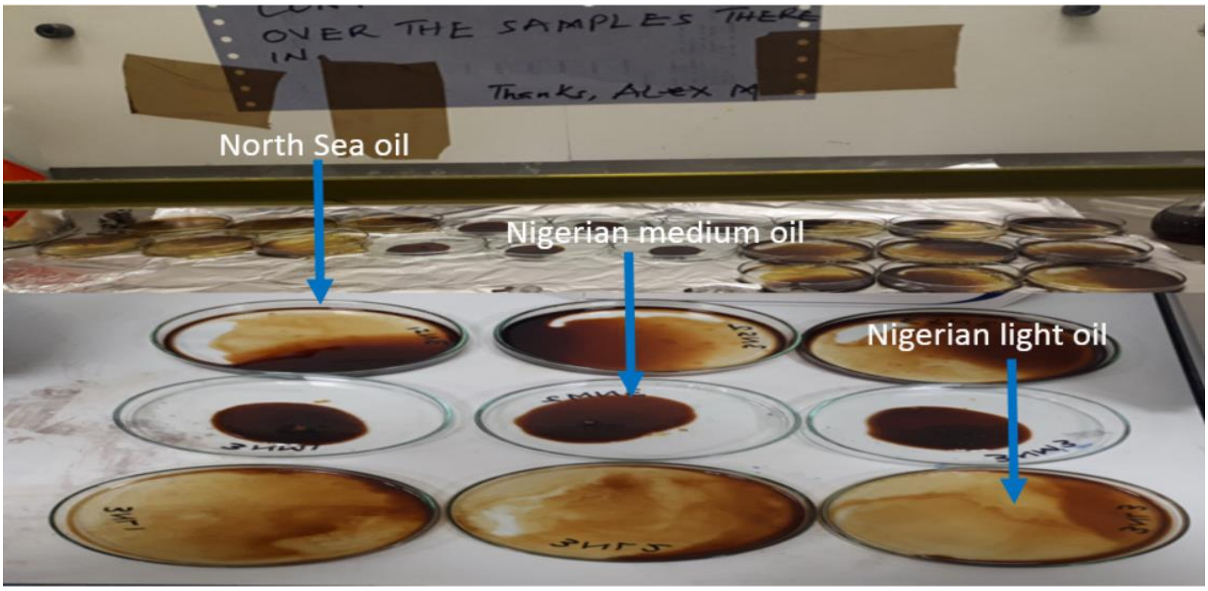


Figure 0.1: Oil evaporation experimental set up in fume cupboard

3.3.2 Column chromatographic fractionation of hydrocarbons

Glass chromatography columns (28 cm l x 10 mm i.d.) were set up on clamps and pre-extracted cotton wool was used to plug the bottom of the columns. A slurry of silica gel in petroleum ether was poured into the columns followed by slurry of alumina at the top to form alumina/silica (1:4) column. Weighed aliquots (~50mg) of the weathered crude oil samples from the evaporation Petri dishes were adsorbed onto small quantities (~1g) of alumina and then charged onto the alumina/silica column. The column were eluted with petroleum ether (70ml) as aliphatic fraction (F1) for n-alkanes and biomarker analysis, followed by elution of a PAHs-containing fraction (F2) (70ml 50% DCM in petroleum ether) for analysis of PAHs similar to that previously reported (e.g. Wang *et al.*, 1994a; Wang *et al.*, 1994b; Wang *et al.*, 2004; Wang *et al.*, 2005).

3.3.3 Gas chromatography and gas chromatography - mass spectrometry

Gas chromatography with flame ionisation detection (GC-FID) analysis of aliphatic samples was performed on an Agilent 5890 GC in split less mode, with the injector at 280°C, FID at 300°C. The sample (1ul) in hexane was injected by an HP7673 auto sampler and the split opened after 1 minute. After the injection the GC temperature programme and data acquisition commenced. Separation was performed on a fused silica capillary column (30m x 0.25mm i.d) coated with 0.25um dimethyl poly-siloxane (HP-5 phase). The GC was temperature programmed from 50°C-310°C at 5°C min and held at final temperature for 20 minutes with hydrogen as the carrier gas (flow 1ml/min, pressure of 50kPa, split at 30 mls/min). The GC data was acquired on a Thermo-Atlas laboratory data system on a dedicated channel.

Gas chromatography-mass spectrometry (GC-MS) analysis of the aliphatic and PAH fractions was performed on an Agilent 7890B GC with a split/split less injector (280°C) linked to Agilent 5977B MSD (electron voltage 70eV, source temperature 230°C, quad temperature 150°C multiplier voltage 1200V, interface temperature 310°C). The acquisition was controlled by a HP Z240 computer using Chemstation software, initially in full scan mode (50-600 amu/sec) or in selected ion mode (20ions 3.0 cps 20ms dwell) for greater sensitivity. The sample (1ul) in hexane was injected by an Agilent7683B auto sampler and the split opened after 1 minute. After the solvent peak had passed the GC temperature programme and data acquisition commenced. Separation was performed on an Agilent fused silica capillary column (30m x 0.25mm i.d) coated with 0.25um dimethyl polysiloxane (HP-5) phase. The GC was temperature programmed from 50-310°C at 5°C min and held at final temperature for 10 minutes with Helium as the carrier gas (flow rate of 1ml/min, initial pressure of 50kPa, split at 20 mls/min). The acquired data was stored for later data processing, integration and printing. Peaks were identified and labelled after comparison of their elution order with known standard mixtures or from geochemistry literature and also for the PAH by comparison of their mass spectra with those of the NIST05 library. The NIST Mass Spectral Library is a software and peer-reviewed databases that are developed and supported by the National Institute for Standards and Technology USA.

3.4 Results and Discussion

3.4.1 Gravimetric determination of percentage evaporation

The results of the evaporation by weight loss of the three oil samples are shown in

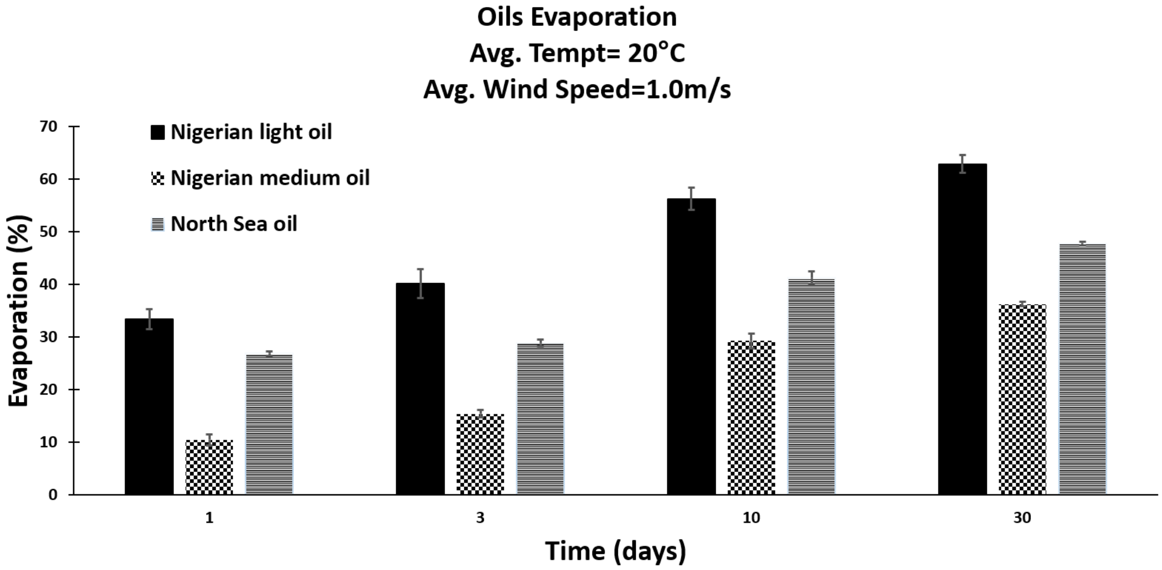


Figure 0.2 and were calculated as follows;

$$\%\boldsymbol{weathering} = \frac{(\boldsymbol{m_i} - \boldsymbol{m_f})}{(\boldsymbol{m_i} - \boldsymbol{m_e})} \times \mathbf{100\%} \text{..} \textbf{Equation 0.1}$$

Where, %weathering is the percentage evaporative mass-loss over the period of 30 days, m_i is the initial mass of Petri dish and oil, m_f is the final mass of Petri dish and oil, m_e is the mass of empty Petri dish respectively.

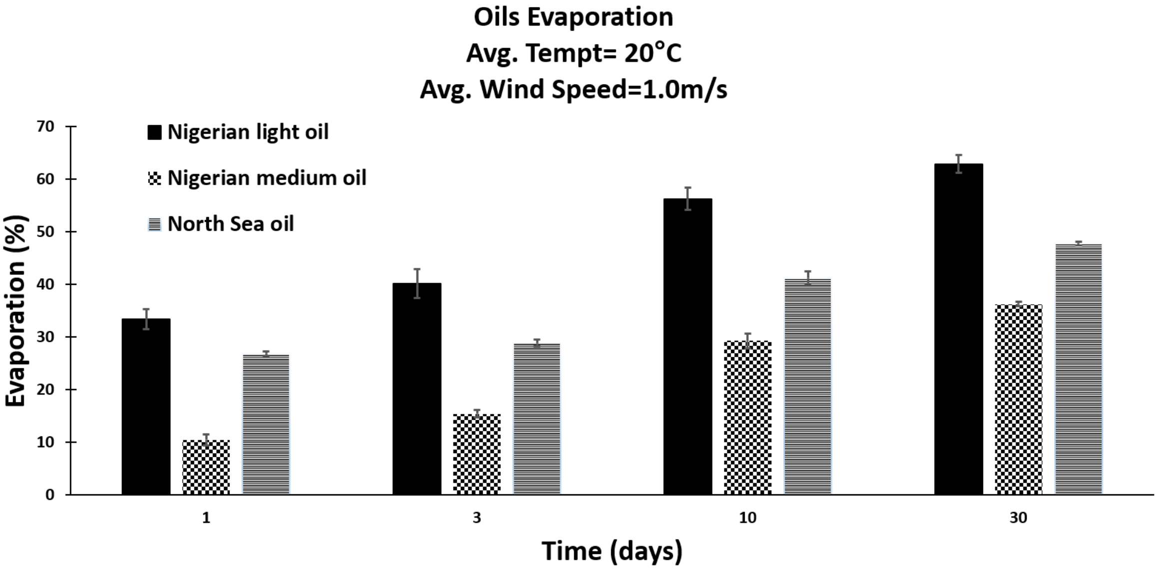


Figure 0.2: Oil evaporation percentages by weight loss. **The error bars show ±1 standard error of replicate microcosms (n = 3)**

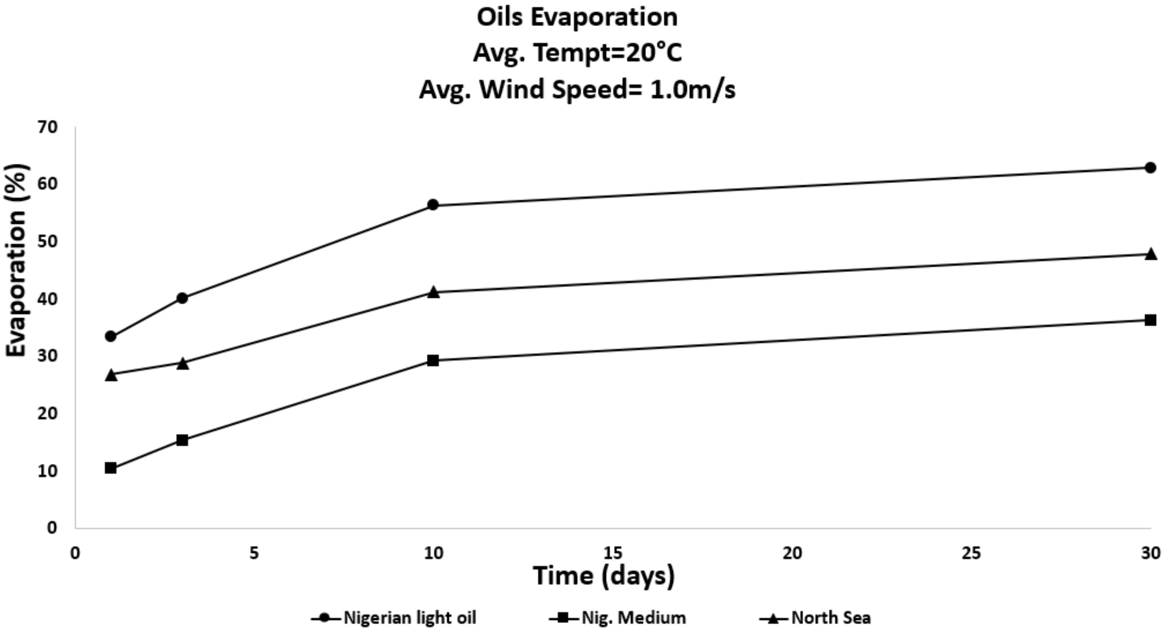


Figure 0.3: Plot of evaporation weight loss versus time for the three oil samples.

The graph of evaporation weight loss plotted against time (Figure 0.3) for the three oils remained almost horizontal from 10 to 30 days of evaporation which can be related to little or no further compositional change as a result of evaporative weathering. The Nigerian Light oil was the most evaporated with evaporation percentages of 33%, 40%, 56%, and 63% after 1, 3, 10, and 30 days respectively. Followed by the North Sea oil with 27%, 29%, 41%, and 49%

respectively for the same corresponding days of the experiment. The Nigerian medium oil was the least evaporated owing to its viscous and waxy nature with 10%, 15%, 29%, and 36% for the corresponding days. This sample could have been affected by film formation which is known to slow evaporation in these type of experiments (Fingas, 2011). It was the least spread out on the Petri dishes, and hence, it would have resulted in reduced surface area exposure to the air flow.

3.4.2 *n*-Alkanes and acyclic isoprenoids composition and concentration changes

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, **Error! Reference source not found.**, and **Error! Reference source not found.** below are GC-FID chromatograms of the aliphatic (F1) fractions of the Nigerian Light and Medium oils, and the North Sea oil respectively, showing the effects of weathering due to evaporation on the *n*-alkanes in the oils. Although *n*-alkanes are not particularly useful in ascertaining the source of a spill, they do provide useful information on the extent of weathering as well as freshness of oil samples (Wang and Fingas, 1995). The main observation from the chromatograms is the complete loss of the relatively volatile low-molecular weight components from *n*-C_8 to *n*-C_{13} for all the three samples after 30 days.

Another observation is the relative size increase of unresolved complex mixture (UCM), or "hump", signifying GC unresolved hydrocarbons present in weathered oils (Volkman *et al.*, 1992; Dembicki, 2017). Based on this, the non-weathered Nigerian medium oil is observed not to be as fresh compared with the other two samples (see Figure 0.5). The relative increase in less volatile higher-molecular weight components of the saturated fractions which are a bit more resistant to evaporative weathering is also observed. Table 0.1,Table 0.2, and Table 0.3 show the summary of *n*-alkanes (*n*-C_8 to *n*-C_{39}), pristanes and phytanes and their respective concentrations for the weathered Nigerian light, Nigerian medium, and North Sea oils respectively, obtained by integrating peak areas against the peak areas of known amounts of internal standard (*n*-heptadecylcyclohexane) using GC-FID.

Recovery or surrogate standard (squalene) was also used to compute the percentage recoveries which was over 90% for all the samples. These are mean values from triplicates with relative standard deviation (RSD) of mostly 5% or less in all the samples results. Computed using the equation (1) below given as:

$$\boldsymbol{X_{wt_{Corr}}} = \frac{\boldsymbol{X_{area}}}{\boldsymbol{IS_{area}}} \times \frac{\boldsymbol{IS_{wt}}}{\boldsymbol{Fraction}} \times \frac{\mathbf{100}}{\%\boldsymbol{recovery}} \text{...} \textbf{Equation 0.1}$$

Where; X_{wtCorr} is the corrected of the target analyte computed, X_{area} and IS_{area} are the response area of the target analyte and the internal standard in the solution to be measured respectively, IS_{wt} is weight of internal standard in the solution to be measured, Fraction is the aliquot fraction of the total solvent extractible material (TSEM) analysed by GC-FID, and %recovery is the surrogate recovery of the extraction process given as:

$$\%\boldsymbol{recovery} = \frac{\boldsymbol{SS_{area}}}{\boldsymbol{IS_{area}}} \times \frac{\boldsymbol{IS_{wt}}}{\boldsymbol{RRF}} \times \frac{\mathbf{1}}{\boldsymbol{SS_{wt}}} \times \frac{\mathbf{1}}{\boldsymbol{fraction}} \times \mathbf{100} \text{..................} \textbf{Equation 0.2}$$

Where; SS_{area} and SS_{wt} are response area of surrogate(recovery) standard in the solution to be measured and weight of the surrogate standard respectively, other parameters as defined in equation (1) above, and RRF is relative response factor for each hydrocarbon component in the solution computed relative to internal standard, and it is given as:

$$\boldsymbol{RRF} = \frac{\boldsymbol{SS_{area}}}{\boldsymbol{IS_{area}}} \times \frac{\boldsymbol{IS_{wt}}}{\boldsymbol{SS_{wt}}} \text{..} \textbf{ Equation 0.3}$$

Where all parameters are as defined in equations (1) and (2) above respectively.

Equation (2) above is corrected from equation (5) given as:

$$\boldsymbol{X_{wtAnalyte}} = \frac{\boldsymbol{X_{area}}}{\boldsymbol{IS_{area}}} \times \frac{\boldsymbol{IS_{wt}}}{\boldsymbol{fraction}} \text{...} \textbf{Equation 0.4}$$

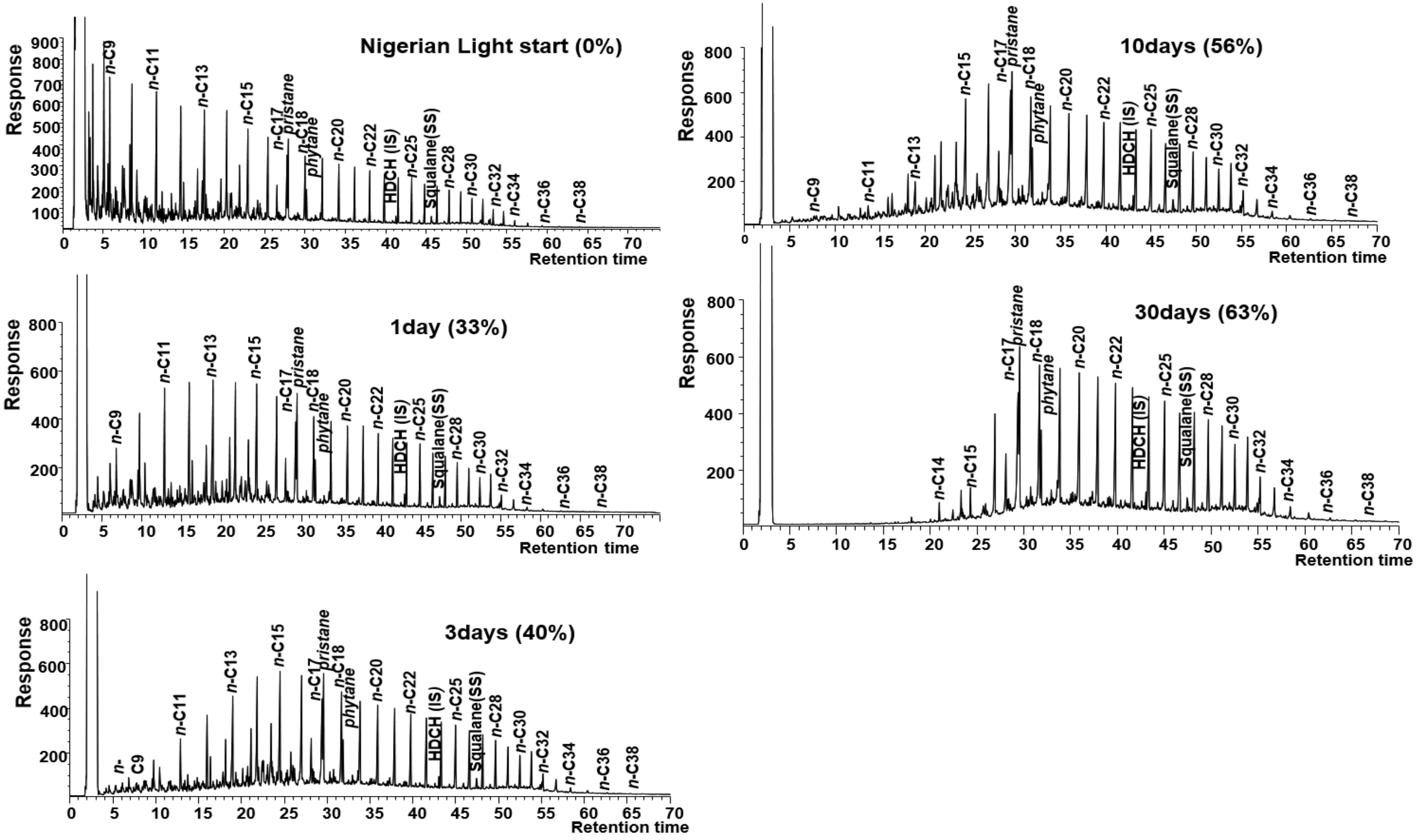


Figure 0.4: GC-FID chromatograms of aliphatic fractions of the Nigerian Light oil showing the days of evaporation and corresponding weight loss.

These results indicate a general reduction of volatile low-molecular weight components (*n*-C_8 to *n*-C_{13}) with increasing evaporative weathering, whereas significant build-up of the less volatile high-molecular weight components (*n*-C_{14} to *n*-C_{23}) were observed, while the larger molecular weight components (*n*-C_{24} to *n*-C_{39}) were relatively unaffected by the evaporative weathering (Wang and Fingas, 1995; Stout and Wang, 2017). Figure 0.7, Figure 0.8, and Figure 0.9 are graphical representation of *n*-alkanes distribution with varying degrees of evaporative weathering for Nigerian light, Nigerian medium, and North Sea oils respectively. These changes could be explained using the Nigerian light oil, the source oil has its highest *n*-alkanes abundances between *n*-C_9 (7.31 mg/g oil) and *n*-C_{16} (5.32 mg/g oil), the highest abundances shifted to between *n*-C_{11} (5.70 mg/g oil) and C_{17} (6.76 mg/g oil) at 33% evaporation in 1 day. The highest abundances further shifted to between *n*-C_{12} (5.68 mg/g oil) and *n*-C_{18} (6.08 mg/g oil) at 40% evaporation in 3 days, this shifts continuous to between *n*-C_{14} (5.40 mg/g oil) and *n*-C_{23} (5.90 mg/g oil) at 56% evaporation in 10 days, and finally to between *n*-C_{16} (9.64 mg/g oil) and *n*-C_{26} (6.04 mg/g oil) at 63% evaporation for 30 days respectively, and similarly, same goes for Nigerian medium and North Sea oils. Generally, most abundant aliphatic components shift to the higher carbon *n*-alkanes range as weathering increased. The sum range of *n*-alkanes concentrations crossed the weathered oil (0% - 63%) for Nigerian light oil is 115.12 mg/g oil – 133.93 mg/g oil, and that of the Nigerian medium is 58.16 mg/g oil – 84.15 mg/g oil, whereas that of North Sea oil is 72.32 mg/g oil – 56.55 mg/g. This is as a result of thermodynamics of loss of low-molecular weight components to weathering and build-up of high-molecular weight components correspondingly, and it is an indication of dominance of high-molecular weight components build-up over low-molecular weight component loss in Nigerian Light and Medium oils and reversed is the case for North Sea oil.

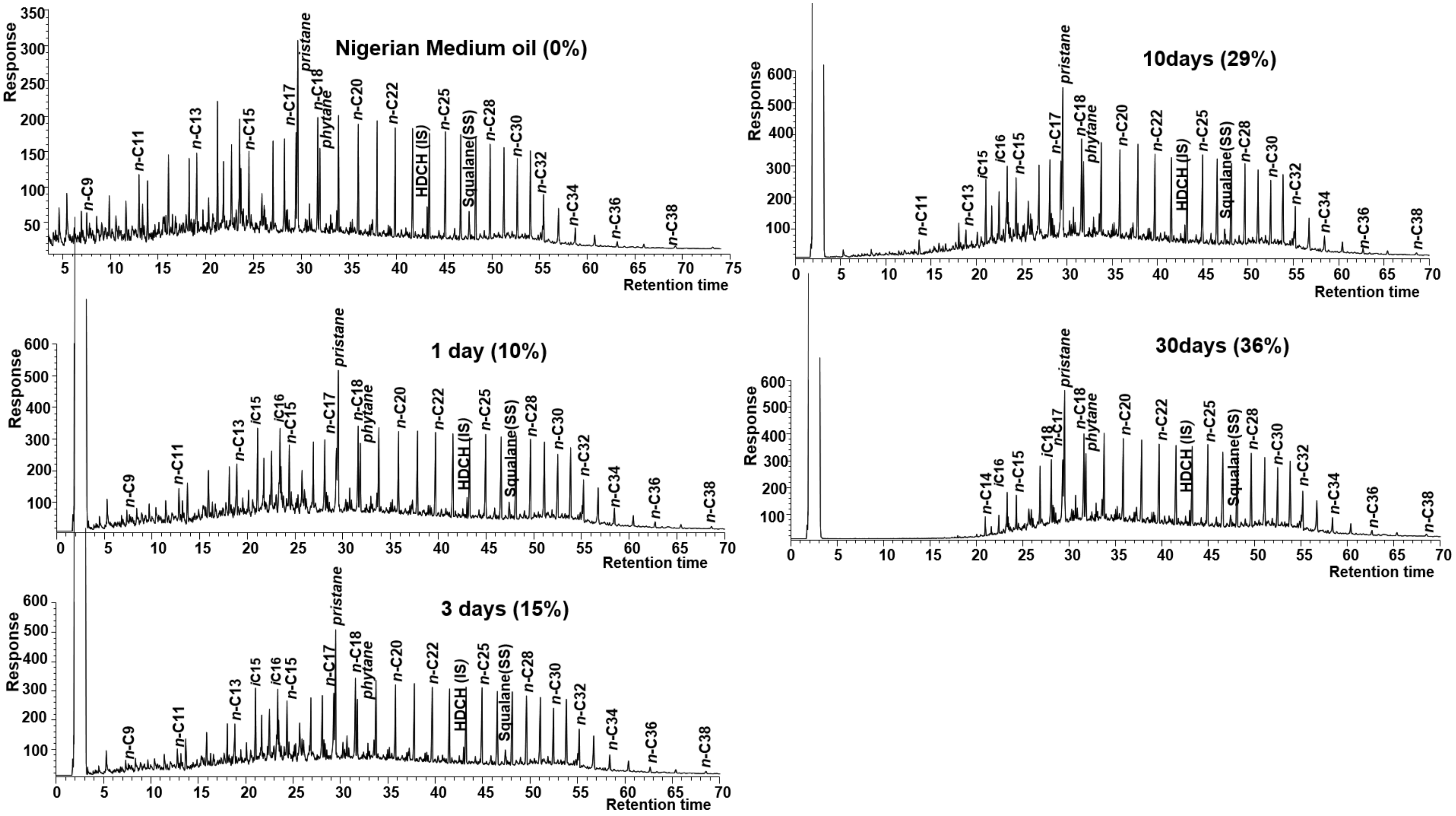


Figure 0.5: GC-FID chromatograms of aliphatic fractions of the Nigerian Medium oil showing the evaporation days and corresponding percentage weight loss

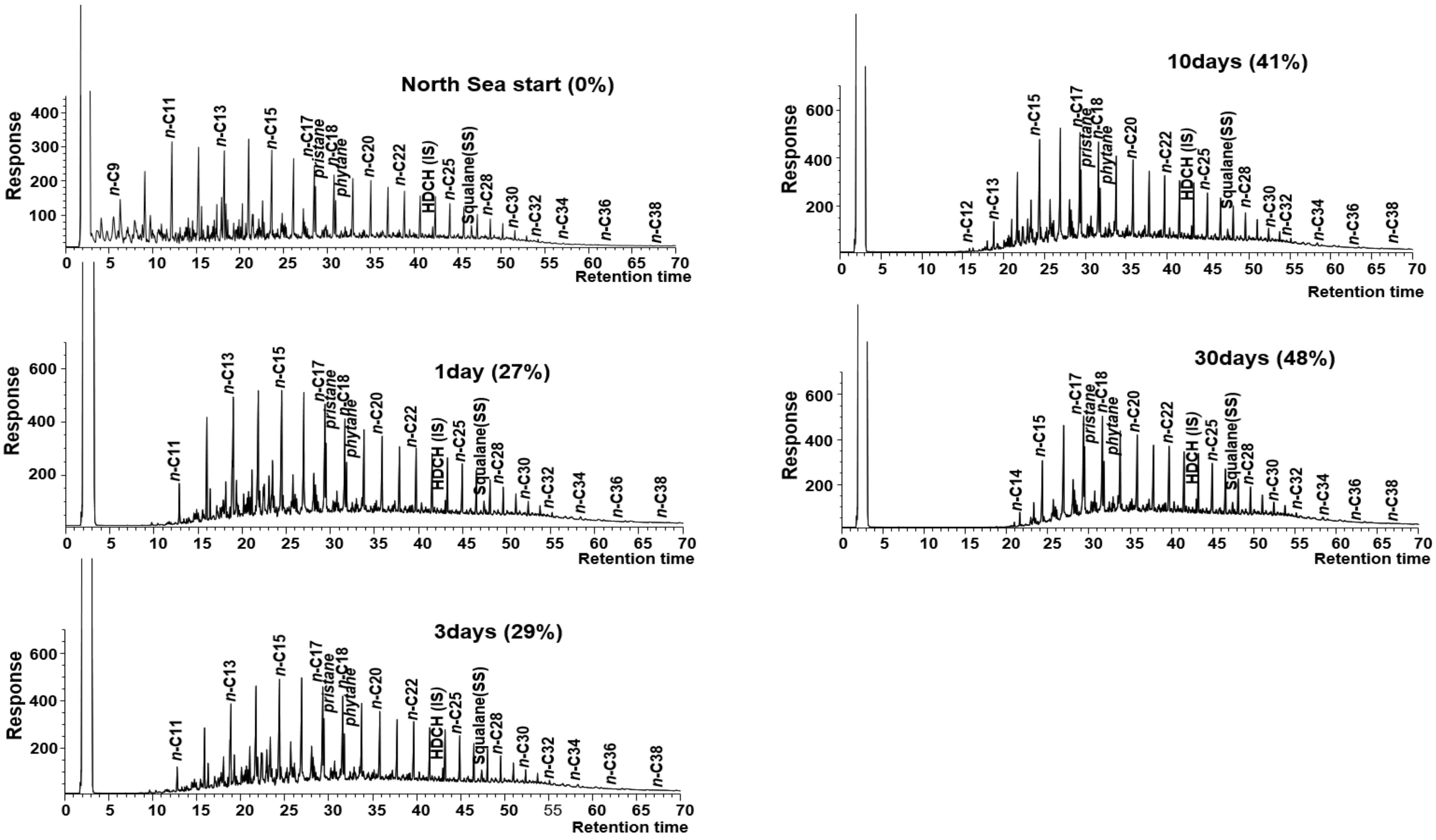


Figure 0.6: GC-FID chromatograms of aliphatic fractions of the North Sea oil showing evaporation days and corresponding percentage weight loss

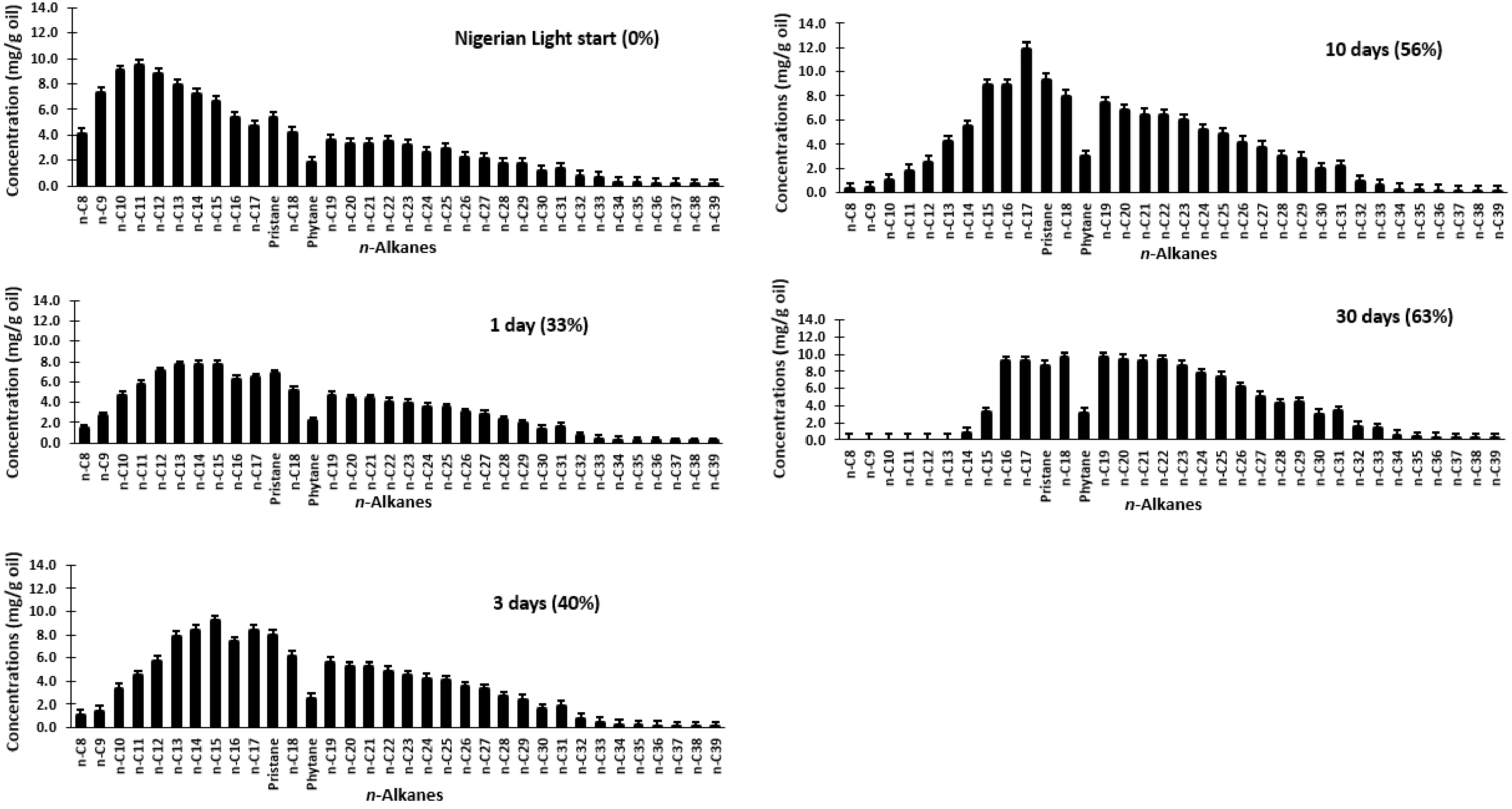


Figure 0.7 Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane for the Nigerian Light oil. **The error bars show ±1 standard error of replicate samples (n = 3).**

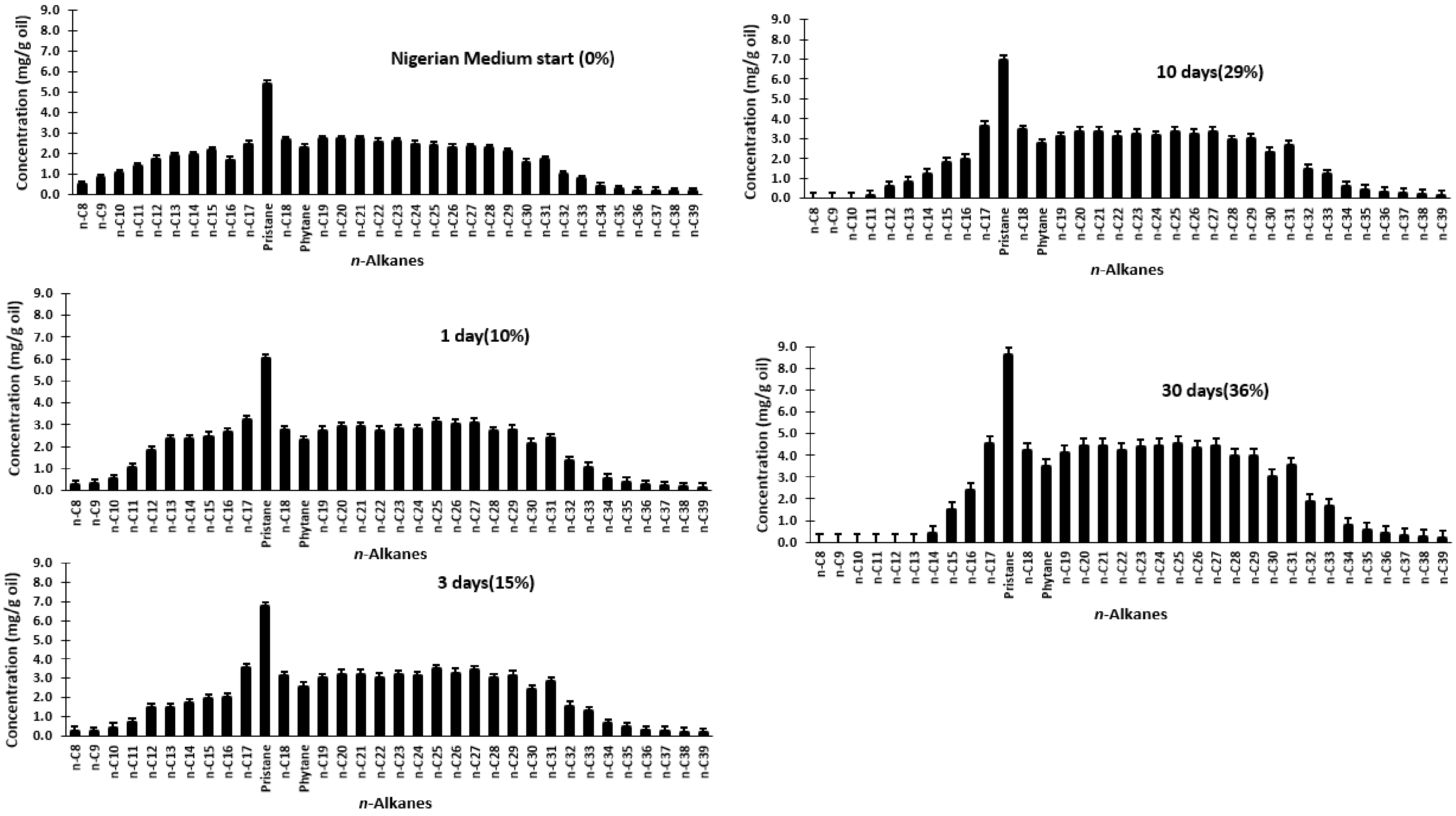


Figure 0.8: Graphical representation of concentration changes of *n*-alkanes, pristane and phytane for the Nigerian Medium oil. **The error bars show ±1 standard error of replicate samples (n = 3).**

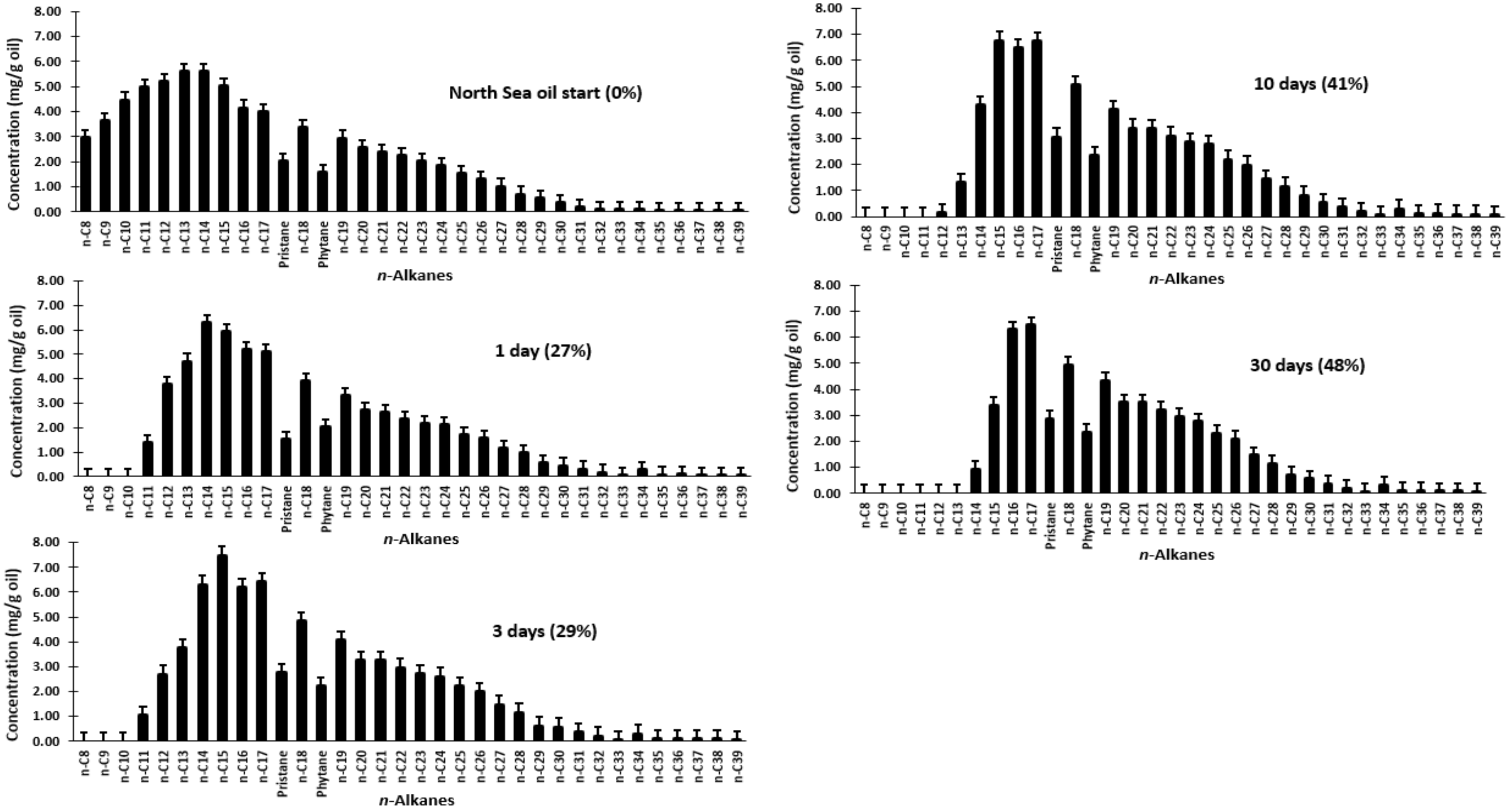


Figure 0.9: Graphical representation of concentration changes of *n*-alkanes, pristane and phytane for the North Sea oil. **The error bars show ±1 standard error of replicate samples (n = 3).**

The concentration of the total C_8 to C_{39} *n*-alkanes for the non-weathered oils were 115.12 mg/g, 58.16 mg/g, and 72.32 mg/g for Nigerian Light, Nigerian Medium, and North Sea oils respectively, which are within the ranges reported by Fingas (2015) for other crude oils, e.g. 172 mg/g (Scotia Light, Canada), 73.8 mg/g (South Louisiana, USA), 79.0 mg/g (Cook Inlet, Alaska), 35.6 mg/g (Troll, North Sea), 21.5 mg/g (Platform Elly, US West Coast), and non-detectable (Alberta oil/sands bitumen, Canada)(Fingas, 2015).

The ratios C_{17}/pristane, C_{18}/phytane, and pristane/phytane remained unaffected by the evaporative weathering for all the oil samples clearly owing to the fact that they are compounds with about the same volatility. The values of the carbon preference index (CPI) which is the ratio of distribution of odd number n-alkanes over the even number n-alkanes, was found to be approximately 1.0 which is typical for petroleum (Bray and Evans, 1961; Wang and Fingas, 1995; Wang *et al.*, 1999). The CPI values for Nigerian oil samples are slightly higher than that of the North Sea oil, which arises from a higher odd n-alkanes dominance and this could be due to the build-up of high-molecular weight n-alkanes with increase in weathered percentages in the Nigerian oils as compared to North Sea oil probably due to their origin, with Pr/nC_{17} and Ph/nC_{18} ratios of 1.2 and 0.4 for the Nigerian Light and 2.2 and 0.9 for the Nigerian Medium oils which are within the terrigenous sources on a Pr/nC_{17} versus Ph/nC_{18} cross plot, whereas the ratios for the North Sea oil were 0.5 and 0.5 which of marine origin (Zhang *et al.*, 2017).

3.4.3 Quantitative comparison of *n*-alkanes ratios and evaluation

The ratios of the *n*-alkanes concentrations in Table 0.1, Table 0.2, and Table 0.3 were plotted relative to *n*-C_{30} to compare these concentrations on the same level. The choice of *n*-C_{30} as reference is due to its resolved nature and also highly resistant to evaporative weathering(Wang and Fingas, 1995).

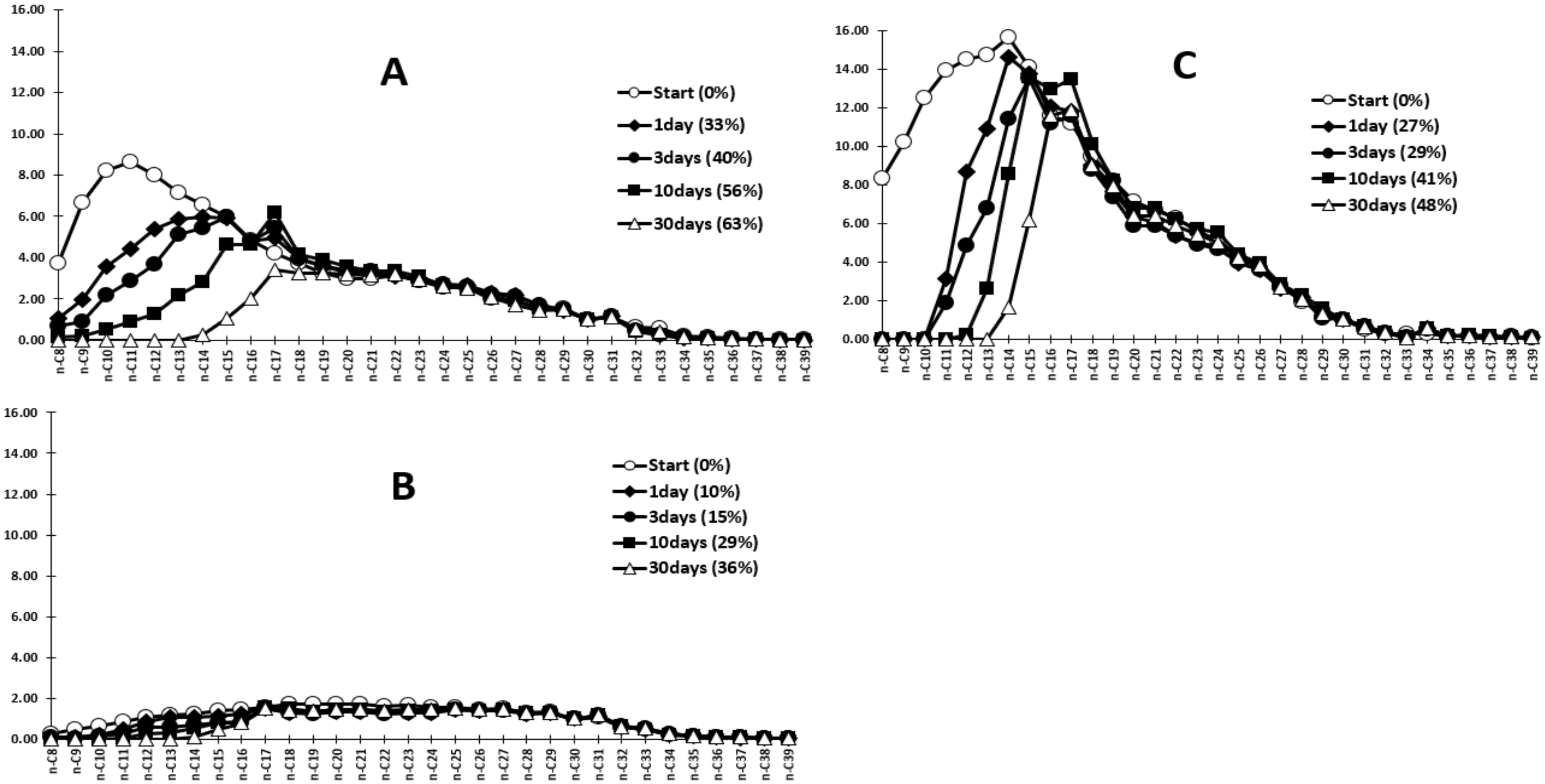


Figure 0.10: Plots of abundances of *n*-alkane in the weathered oils relative to *n*-C_{30} for Nigerian Light (A), Nigerian Medium (B), and North Sea (C) oils respectively.

Figure 0.10 shows the plots of *n*-alkanes concentrations (abundances) relative to *n*-C_{30} against the n-alkanes for Nigerian light, Nigerian medium, and North Sea oils respectively. Data plots of the weathered Nigerian light oil converged at the *n*-C_{22} alkane indicating that all *n*-alkanes greater than or equal to *n*-C_{22} were not affected by evaporative weathering, similarly, that of the Nigerian medium oil converged at the *n*-C_{25} alkane whereas that of the North Sea plot converged at *n*-C_{24}.

An equation to correlate the component *n*-alkanes concentrations to percentages loss by evaporation is given as;

$$\boldsymbol{C_{conv} = C_{det}(1 - P)} \text{……………………………… } \textbf{Equation 0.5}$$

Where C_{conv} is the converted oil concentrations of oil components in weathered samples, which is equivalent to the concentrations in the source oil; C_{det} is the concentration oil components in weathered samples obtained from GC analyses; P is the weathered percentages of oil by weight (%) (Wang and Fingas, 1995).

Using equation (1) and the data in Table 0.1, Table 0.2, and Table 0.3, Figure 0.11 compares the plots of selected *n*-alkanes (*n*-C_{10}, *n*-C_{12}, *n*-C_{16}, *n*-C_{20}, *n*-C_{25}, and *n*-C_{30}) determined (C_{det}) and converted (C_{conv}) concentrations against the weathered percentages for the three oil samples. It is important to note that two contrasting factors are at play as explained earlier above, these are loss of some *n*-alkanes components due to evaporation and build-up of other *n*-alkanes components due to volume reduction by evaporation. While determined concentrations (C_{det}) include the effects of these two factors, converted concentrations (C_{conv}) eliminates the effect of volume reduction by applying the Equation (3.5)

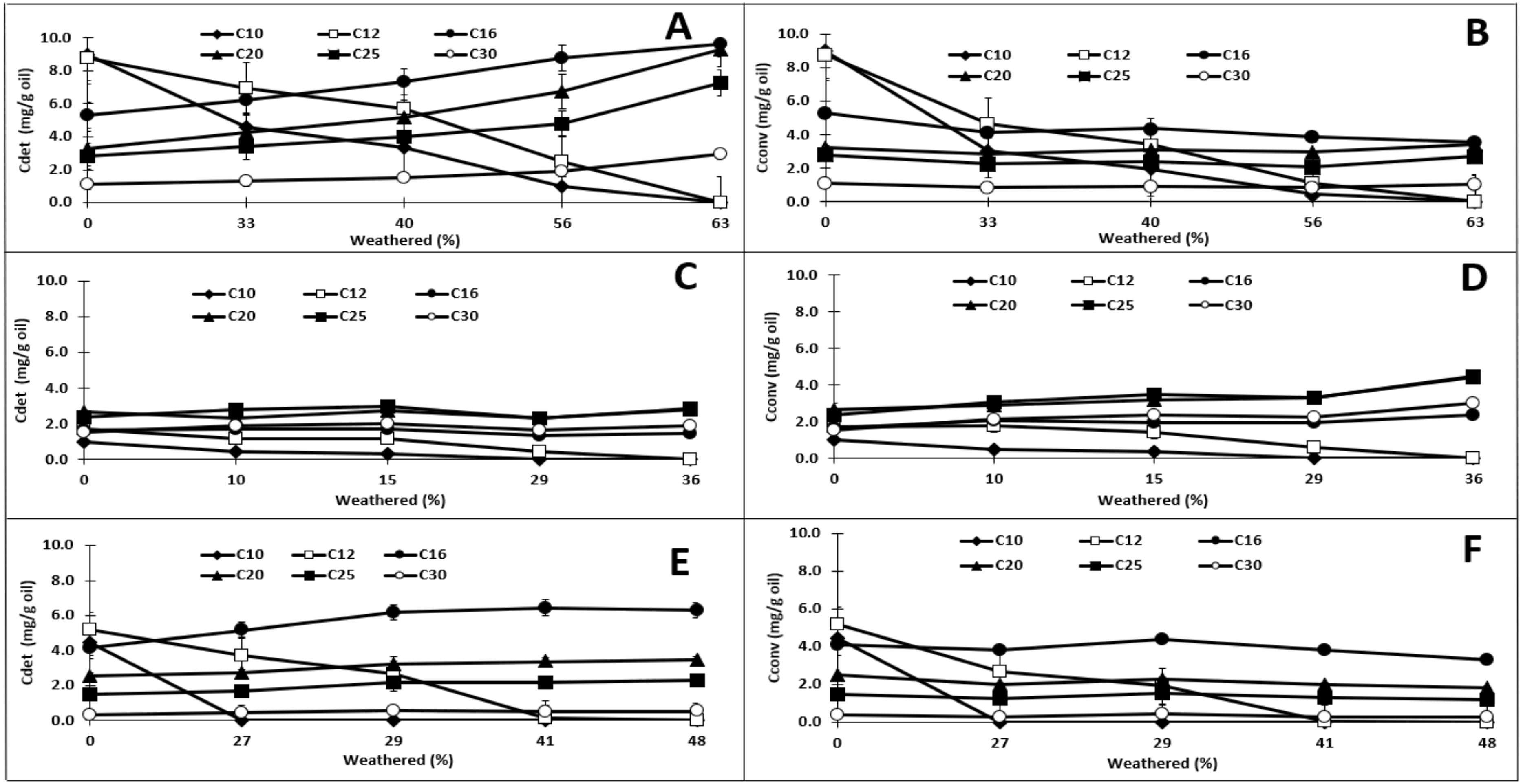


Figure 0.11: Plots of C_{det} and C_{conv} versus weathered percentages for chosen *n*-alkanes in the Nigerian light oil (A and B), the Nigerian Medium oil (C and D), and the North Sea oil (E and F) respectively.

on the assumption that volume reduction is kept constant and focused only on loss and concentration changes due to evaporation.

It can be observed above (see Figure 0.11, A and B) that there is no great change in plots of *n*-C_{10} and *n*-C_{12} n-alkanes in both C_{det} and C_{conv} plots against evaporation percentages, because they more susceptible to evaporation rather than build-up due to their low-molecular weights.

However, a noticeable reduction is observed in the plots of *n*-C_{16}, *n*-C_{20}, *n*-C_{25}, *n*-C_{30} from C_{det} to C_{conv}. The *n*-C_{16} is the most reduced because of its tendency to build-up rather than evaporate within the period of evaporation due to its molecular weight and positioning, followed by *n*-C_{20}, whereas n-C_{25} and *n*-C_{30} are the least reduced and almost unaffected by

these two opposing factors. It can be observed that from *n*-C_8 to *n*-C_{22} for this oil sample, evaporation is a function of carbon chain length where shorter chain n-alkanes evaporate faster than n-alkanes with longer chain, where *n*-C_8 to *n*-C_{13} are completely lost to evaporation after 30 days (63% oil weight loss), but little or no effect is observed on *n*-alkanes greater than the *n*-C_{22}. While pristane and phytane are reasonably unaffected by the evaporative weathering at about the same extend as observed with *n*-C_{17} and *n*-C_{18} for all the oil samples. The trend is similar for Nigerian medium oil (see Figure 0.11, C and D) except that the reduction was lower compared to that of Nigerian light oil probably as a result of the physical properties of the oil. The *n*-C_{16} was the most reduced in this case also for the reason discussed above, however the evaporation effect is observed to cease from *n*-C_{25} whereas *n*-C_8 to *n*-C_{10}, and *n*-C_{11} to *n*-C_{13} were completely lost to evaporation after 10 days (29% oil weight loss) and 30 days (36% oil weight loss) respectively. The pattern for North Sea oil (see Figure 0.11, E and F) is *n*-C_{16} was the most reduced *n*-alkane and the evaporation effect disappears from *n*-C_{24}, similarly, *n*-C_8 to *n*-C_{10}, *n*-C_{11}, and *n*-C_{12} to *n*-C_{13} were also lost completely to evaporation after 1 day (27% oil weight loss), 10 days (41% oil weight loss), and 30 days (48% oil weight loss) respectively.

3.4.4 Determination of weathering index using *n*-alkanes concentration

The susceptibility of *n*-alkanes to weathering is measured by weathering index (WI) which is used to relate the weathering tendency of oil and assess the degrees of evaporation of the actual sample (Wang and Fingas, 1995) given as;

$$\boldsymbol{WI = (C_8 + C_{10} + C_{12} + C_{14})/(C_{22} + C_{24} + C_{26} + C_{28})}$$ **..............Equation 0.6**

It is a correlation between carbon number with shorter chain length which are more susceptible to weathering as numerator and carbon number with longer chains which are less susceptible to weathering and can build-up as evaporative weathering increases make the equation 3.6 sensitive to changes of weathering degree. Whereas the numerator decreases with increase in weathered percentages thereby reducing the value of WI, the denominator tend to increase with increased weathered percentages thereby further reducing the value of WI (Wang and Fingas, 1995; Nollet, 2005; Wang and Brown, 2008). The choice of these n-alkanes comes from the fact that they are well resolved due to their relatively high abundances.

Figure 0.12 (below) are plots of values of weathering indices computed (see Table 0.1,Table 0.2, and Table 0.3) against weathered percentages for Nigerian light, Nigerian medium, and North Sea oils respectively. Straight line (best fit) are obtained with equations and R^2 values and can be used to evaluate the extent and degree of weathering of real oil samples in practice to ascertain the extent of weathering of the oil or their freshness (Wang and Fingas, 1995).

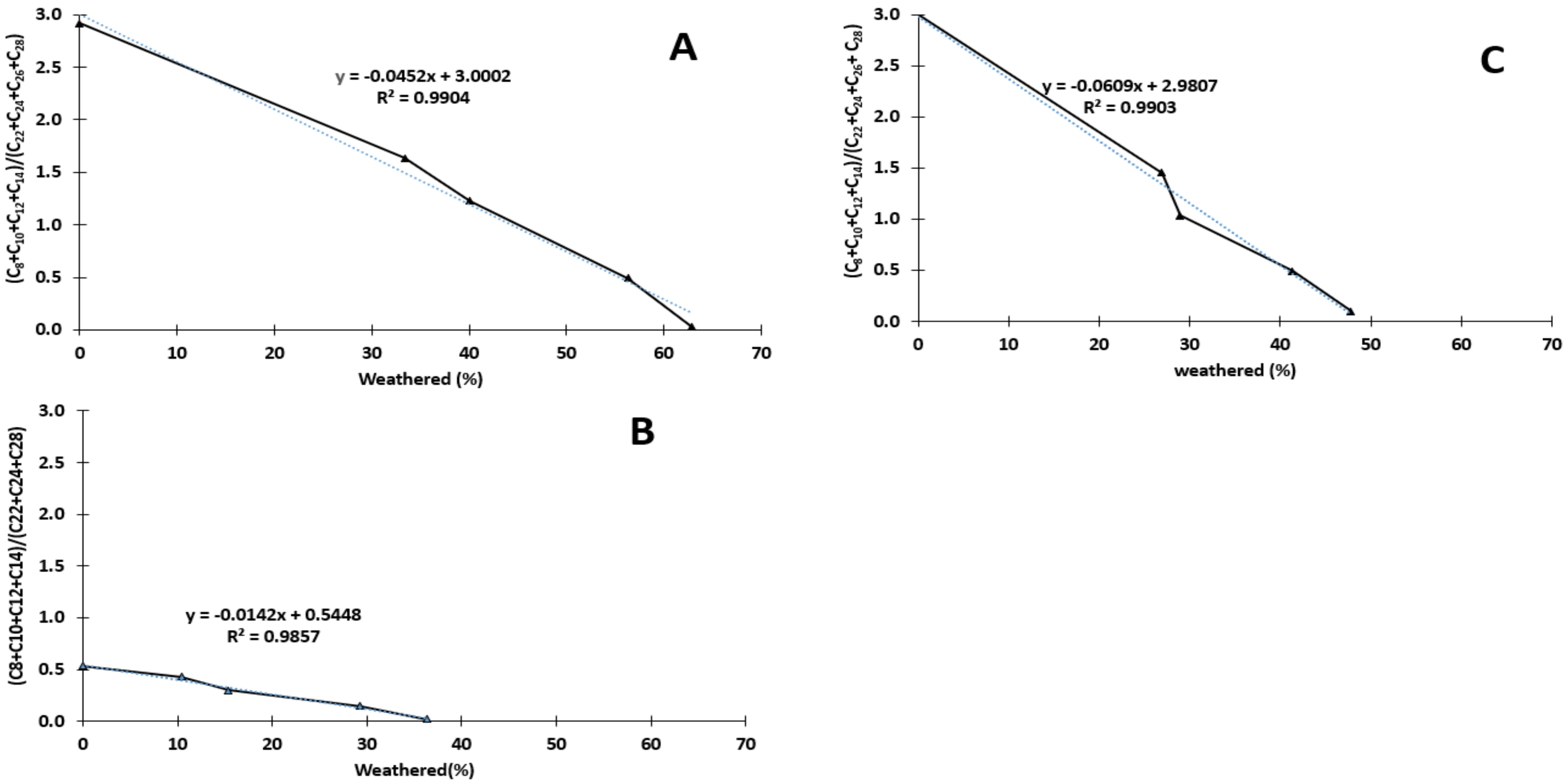
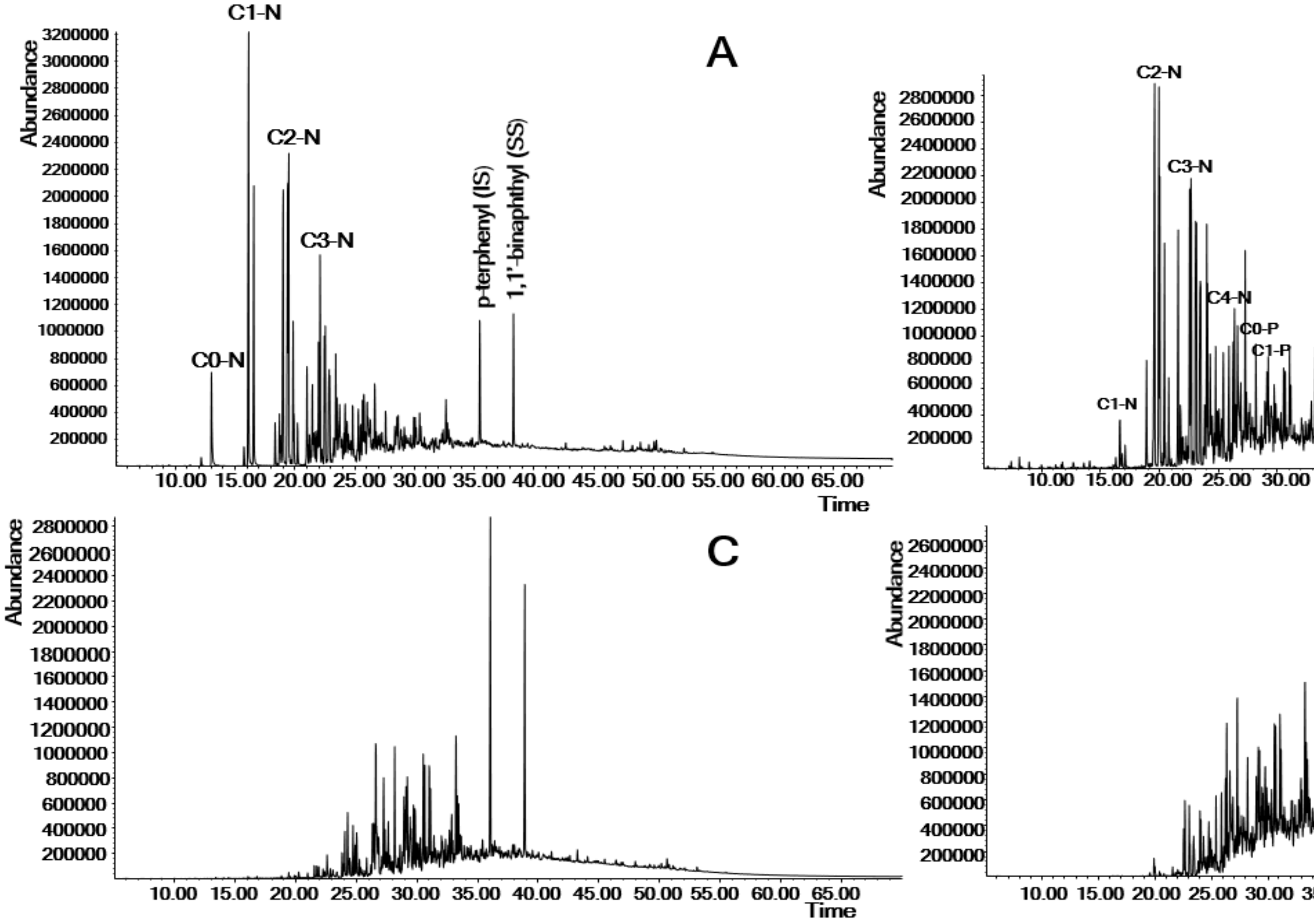


Figure 0.12: Plots of weathering index $(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})$ against weathered percentages for the Nigerian Light (A), the Nigerian Medium (B), and the North Sea (C) oils respectively.

3.4.5 Aromatic hydrocarbons composition and concentration changes



The aromatic fractions (F2) were also analysed and the GC/MS TIC chromatograms (in SIM mode) at weathered percentages of 0%, 33%, 56% and 63% (by weight) for the Nigerian Light oil is shown in Figure **0.13**, 0%, 10%, 29%, and 36% (by weight) for the Nigerian Medium oil is shown in Figure 0.14, and 0%, 27%, 41% and 48% for the North Sea oil shown in Figure 0.15 respectively. The summary of the quantitation of the measured and converted concentrations of the target PAHs are shown in **Table 0.4**, **Table 0.5**, and **Table 0.6** for the Nigerian Light, Nigerian Medium and North Sea oils respectively. The measured concentrations of the target PAHs were calculated by

$$C_A = \frac{A_{A-S} \times C_{IS-S}}{A_{IS-S} \times RRF_A \times W_S} \text{..} \textbf{Equation 0.7}$$

Where $A_{A\text{-}S}$ is the area response for the target analyte in sample solution, $A_{IS\text{-}S}$ is the area response of the internal standard spiked in the final sample solution, $C_{IS\text{-}S}$ is the concentration of internal standard spiked in the final sample solution, and W_S is the sample weight used for the analysis. RRF_A given by

$$RRA_A = \left(\frac{A_A}{C_A} \div \frac{A_{IS}}{C_{IS}}\right) \text{...} \textbf{Equation 0.8}$$

Where A_A and A_{IS} are the response area for a target analyte and internal standard in the standard solution to be measured respectively , and C_A and C_{IS} are the concentration of the target analyte and the internal standard in the standard solution respectively (Fingas, 2015), whereas the converted concentrations were calculated using equation 3.5. Homologous series of the alkylated PAHs were integrated with straight base line carefully maintained for each peak for consistency (Yang *et al.*, 2014). Relative response factors (RRFs) were calculated relative to the internal standard p-terphenyl from either unsubstituted (parent) or alkylated PAHs base on their availability in the laboratory using GC/MS (in SIM mode). Naphthalenes, fluorenes, anthracene, pyrenes, chrysenes and perylene had their RRFs computed using parent PAHs, whereas that of biphenyls and phenanthrenes from daughter PAHs, and unity was assumed as RRFs for dibenzothiophenes and dibenzofurans. **Table 0.4**, **Table 0.5** and **Table 0.6** show the measured and concerted concentrations of target alkylated PAHs computed based on mean values of triplicate samples for the Nigerian light, Nigerian medium and North Sea oils respectively.

The EPA parent and petroleum-specific alkylated (C_1 to C_4) PAHs of naphthalene, phenanthrene, dibenzothiophene, fluorene and chrysene are of great importance in oil source identification and oil spill assessment to distinguish petrogenic sources from biological and combustion derived sources of PAHs in the environment(Wang *et al.*, 1999). The detailed effects of evaporative weathering on these alkylated PAHs are analysed and discussed in this project.

It can be observed that the most alkylated homologues increased in relative percentage composition as the weathering increases for all the oils probably due to build-up of less volatile components. Naphthalene (C_0-N) and biphenyl (C_0-B) which decreased from 3% ($\frac{247.3}{8483.0} \times 100$) to 0% and 15% to 0% respectively, as weathering increases for the Nigerian light oil (see **Table 0.4**) followed by alkylated naphthalenes (C_1-N) from 15% to 4%. Similarly, C_0-N decreases from 6% to 0%, and C_1-N from 24% to 7%, and C0-B from 24 to 8% relative percentage composition for the Nigeria Medium oil (see **Table 0.5**). In the North Sea oil (see **Table 0.6**) C_0-N is observed to decrease from 15% to 0%, and C_1-N from 21% to 13%, however, relatively little decrease is observed in C_0-B which goes from 32% to 25%. The decrease observed in C_0-N, C_0-B and C_1-N is probably due to their low molecular weights (e.g. Stogiannidis and Laane, 2015). Other alkylated PAH groups are less affected compared to the naphthalenes and biphenyls. It is generally observed for the three oil samples that the susceptibility order of the alkyl homologues is naphthalenes > biphenyls > dibenzofurans > dibenzothiophenes > anthracene > phenanthrenes > pyrenes > chrysenes. Chrysene being almost unaffected as susceptibility reduces with increase in aromatic rings (Stogiannidis and

Laane, 2015). The distribution of target alkylated PAHs are shown in , **Error! Reference source not found.**, and **Error! Reference source not found.** for the Nigerian light, Nigerian Medium and North Sea oils respectively. The ‘bell-shape’ distributions, owing to the predominance of alkylated (mostly C_2- and C_3- PAHs) over parent (C_0-PAHs) PAHs is a characteristic of petroleum derived (petrogenic) hydrocarbons (Mudge *et al.*, 2008; Stogiannidis and Laane, 2015; Stout and Wang, 2016a). The phenanthrene /anthracene ratio > 15 in **Table 0.4**,**Table 0.5** and **Table 0.6** is another diagnostic indication of the petrogenic character of these oils (Stogiannidis and Laane, 2015). The 2-methylnaphthalane/1-mrthylnaphthalane ratios (2-MN/1-MN) for the Nigerian Medium and

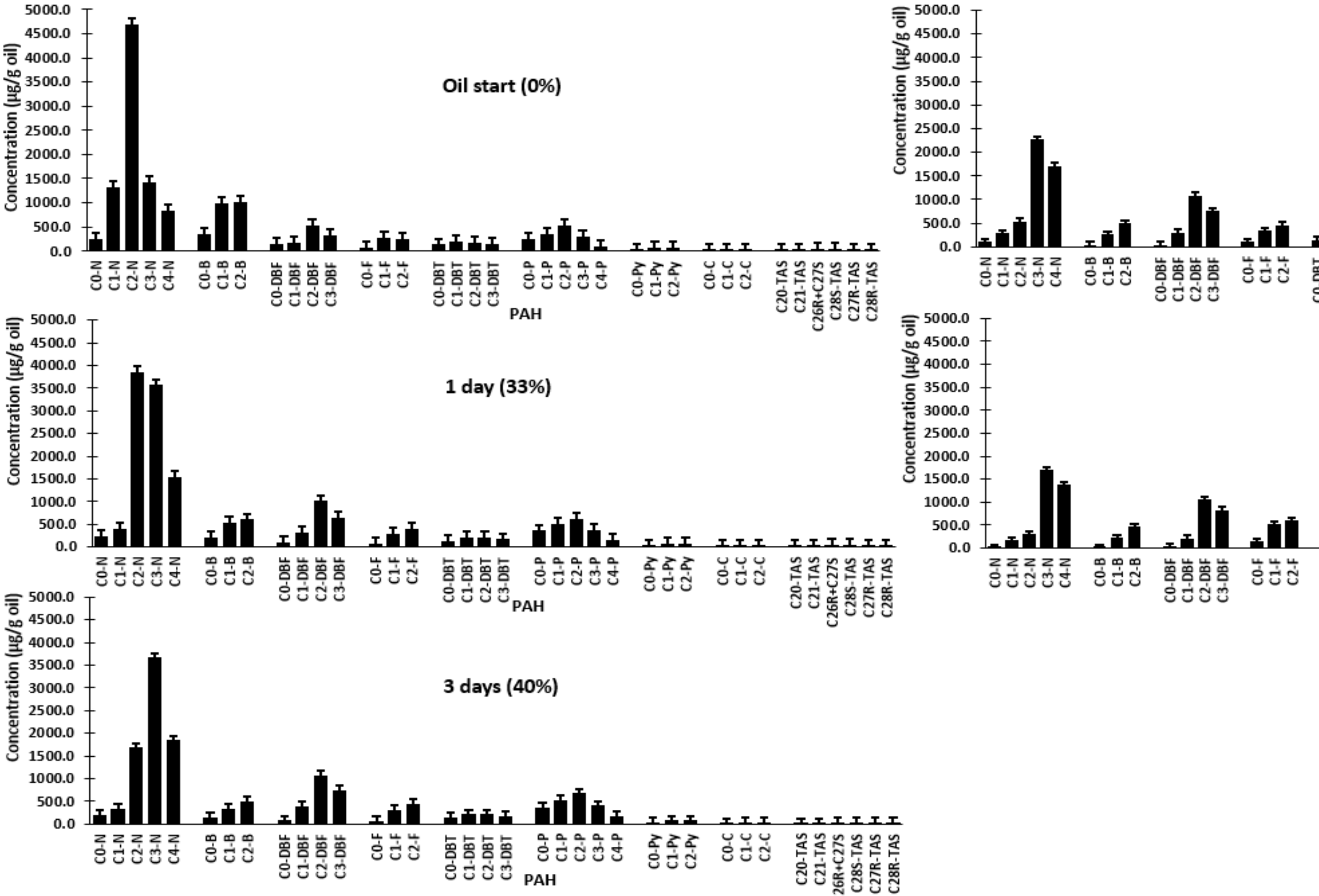


Figure 0.17: Distribution of target alkylated PAHs showing time in days and weathered percentages for Nigerian light oil. N, B, DBF, F, DBT, P, Py, C and TAS represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

North Sea oils are fairly unaffected by evaporative weathering, however that of the Nigerian Light oil is affected as the weathering reaches 56% (by weight) and beyond. The converted concentrations indicate the actual changes due to evaporation by eliminating the effect of high-molecular weight components build-up (Wang and Fingas, 1995). Two peaks were observed in the two Nigerian oils and labelled as a,b,c,d,e-pentamethylnaphthalene (Figure 0.16A) and v,w,x,y,z-pentamethylnaphthalene (Figure 0.16B) which are not quite as well resolved in the North Sea oil (Figure 0.16C) and the chromatograms are as shown in Figure 0.16. Their potential as diagnostic ratios for oil-oil correlation and differentiation were tested by normalising each with respect to the most well resolved 4-methyldibenzothiophene (4-MDBT) and these ratios appeared to be consistent which could be applied for diagnostic purposes. The normalised ratios for Nigerian light oil were 1.00:0.43:1.10, 1.00:0.45:1.14, 1.00:0.46:1.08, 1.00:0.49:1.08, 1.00:0.44:1.08 for 0%, 33%, 40%, 56% and 63% weathered percentages respectively (see **Table 0.4**). Whereas that of Nigerian medium oil was 1.00:0.51:1.39, 1.00:0.53:1.36, 1.00:0.54.1.37, 1.00:0.55:1.47, 1.00:0.54:1.40 for 0%, 10%, 15%, 29% and 36% weathered percentages respectively (see **Table 0.5**).

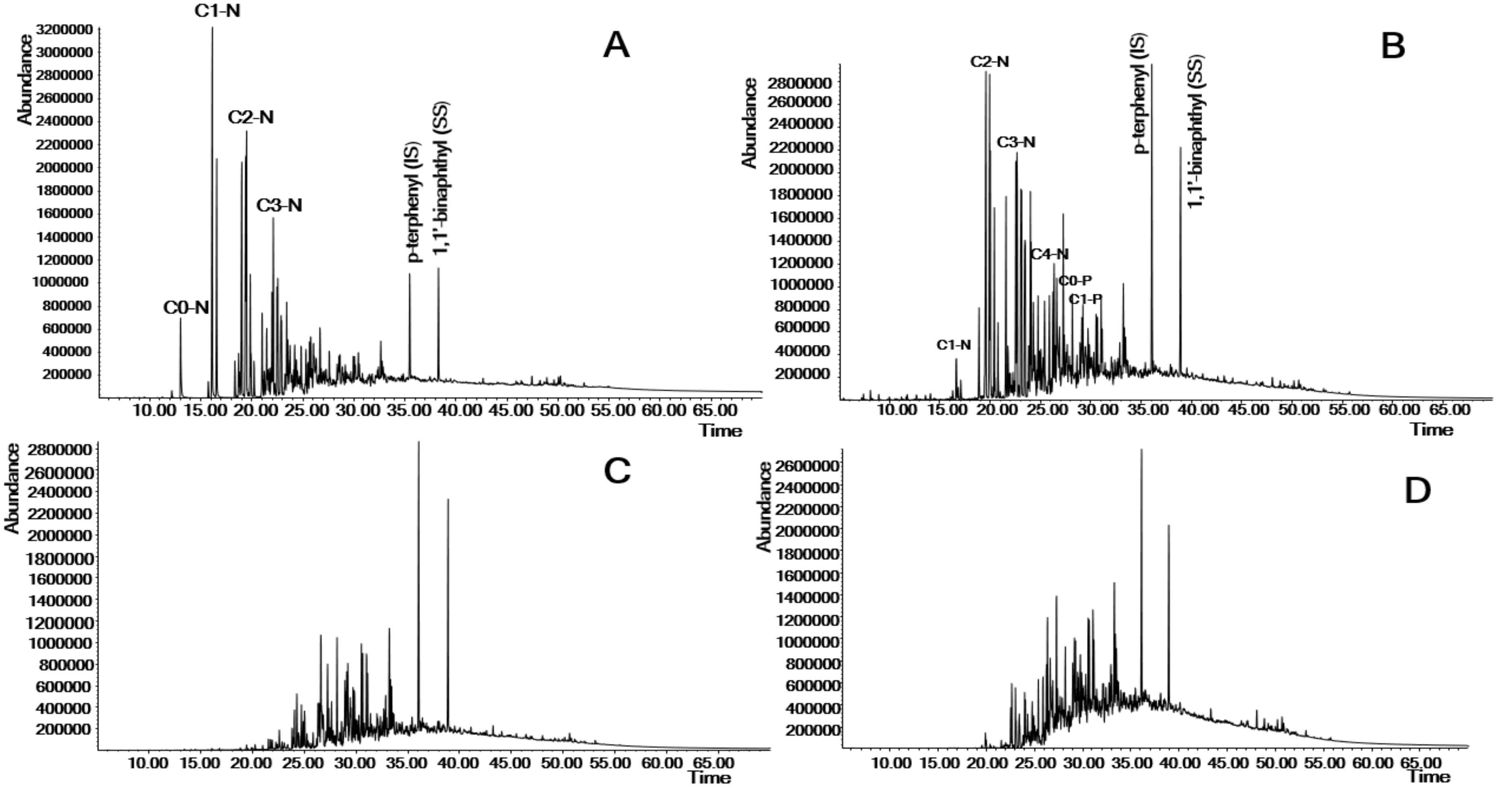


Figure 0.13: GC/MS TIC chromatograms of aromatic compounds in Nigerian light oil at weathered percentages; (A) 0%, (B) 33% (1 day), (C) 56% (10 days), (D) 63% (30 days) respectively

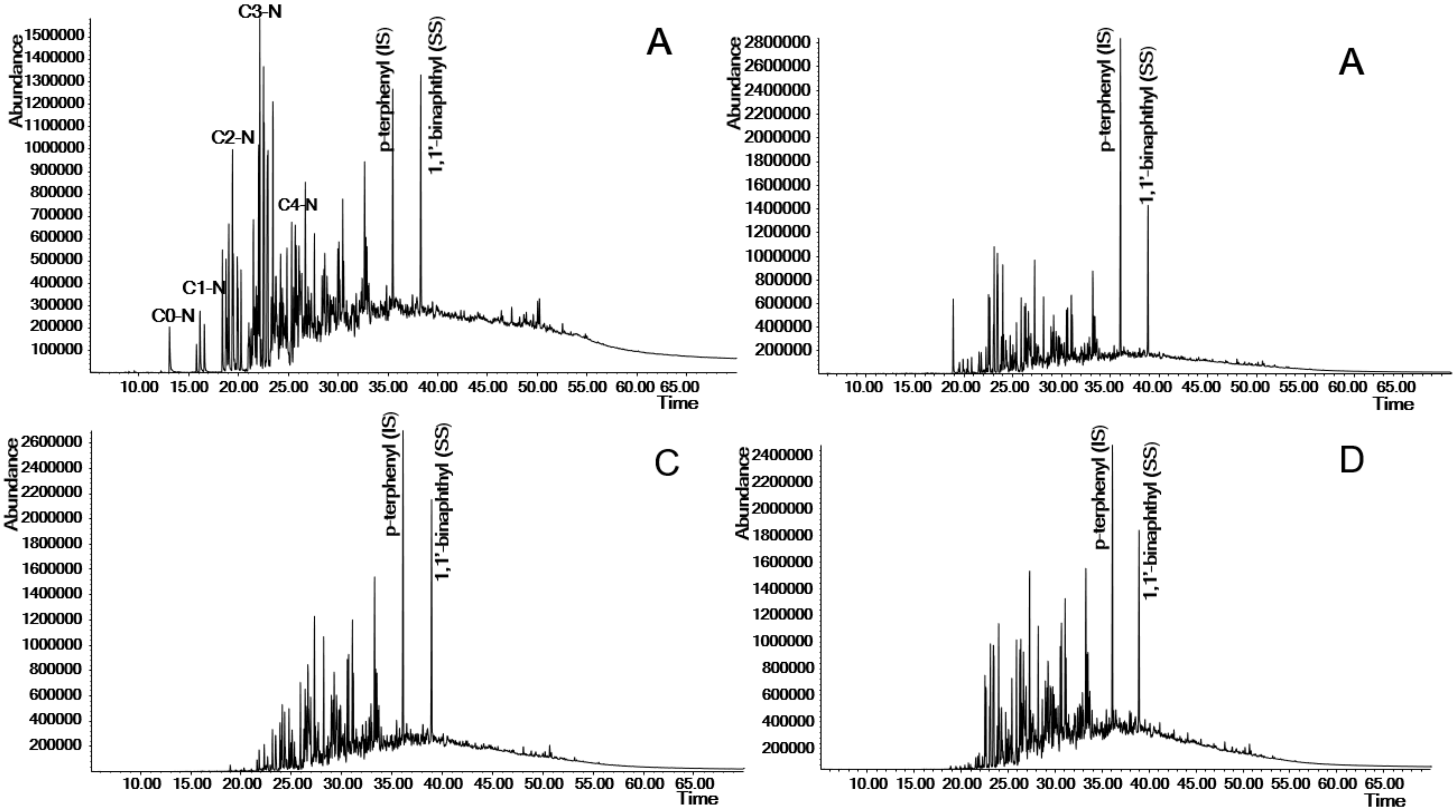


Figure 0.14: GC/MS TIC chromatograms of aromatic compounds in Nigerian medium oil at weathered percentages; (A) 0%, (B) 10% (1 day), (C) 29% (10 days), (D) 36% (30 days)

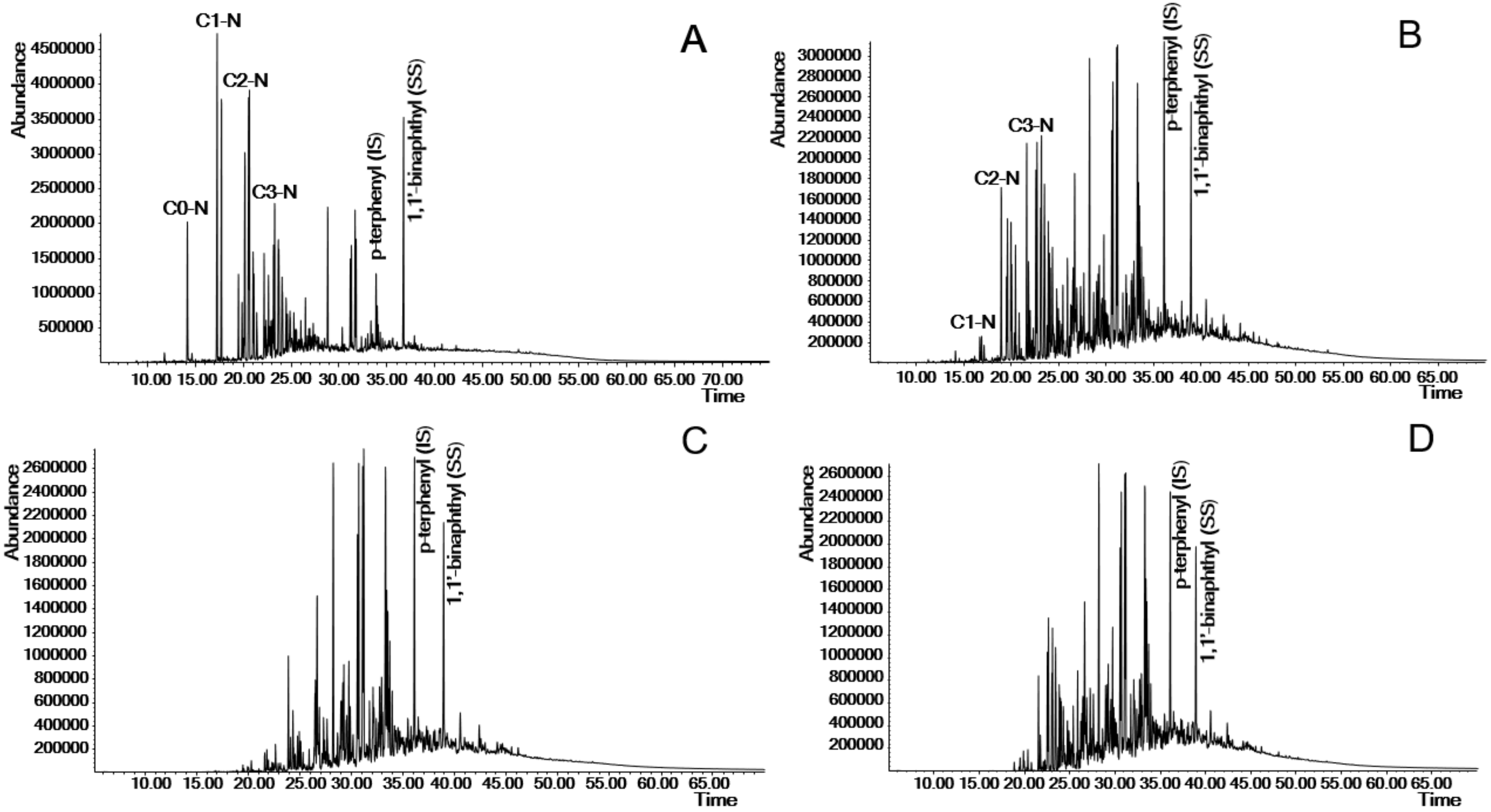


Figure 0.15: GC/MS TIC chromatograms of aromatic compounds in North Sea oil at weathered percentages; (A) 0%, (B) 27% (1 day), (C) 41% (10 days), (D) 48% (30 days)

Table 0.7 shows the changes in measured concentrations and percentage compositions of the pentamethylnaphthalenes as the degree of weathering increases. It could be noted that the compositions of a,b,c,d,e-PMN and v,w,x,y,z-PMN are fairly consistent and stable which could be applied as diagnostic ratios.

Figure 0.20, Figure 0.21 and Figure 0.22 show the plots of measured concentrations and converted concentrations versus weathered percentages for selected alkylated PAHs of naphthalenes, dibenzothiophenes and phenanthrenes for the three oil samples analysed. As stated earlier (see section 3.4.3), the converted concentrations eliminate effect of molecular build-up due to volume reduction and only losses due to evaporation is accounted for. It can be observed that naphthalenes alkylated PAHs are the most affected by evaporative weathering followed by dibenzothiophenes and phenanthrenes are the least affected, however the triaromatic steranes are the most stable aromatic components as shown in Figure 0.17Figure 0.18Figure 0.19 (e.g. Stogiannidis and Laane, 2015).

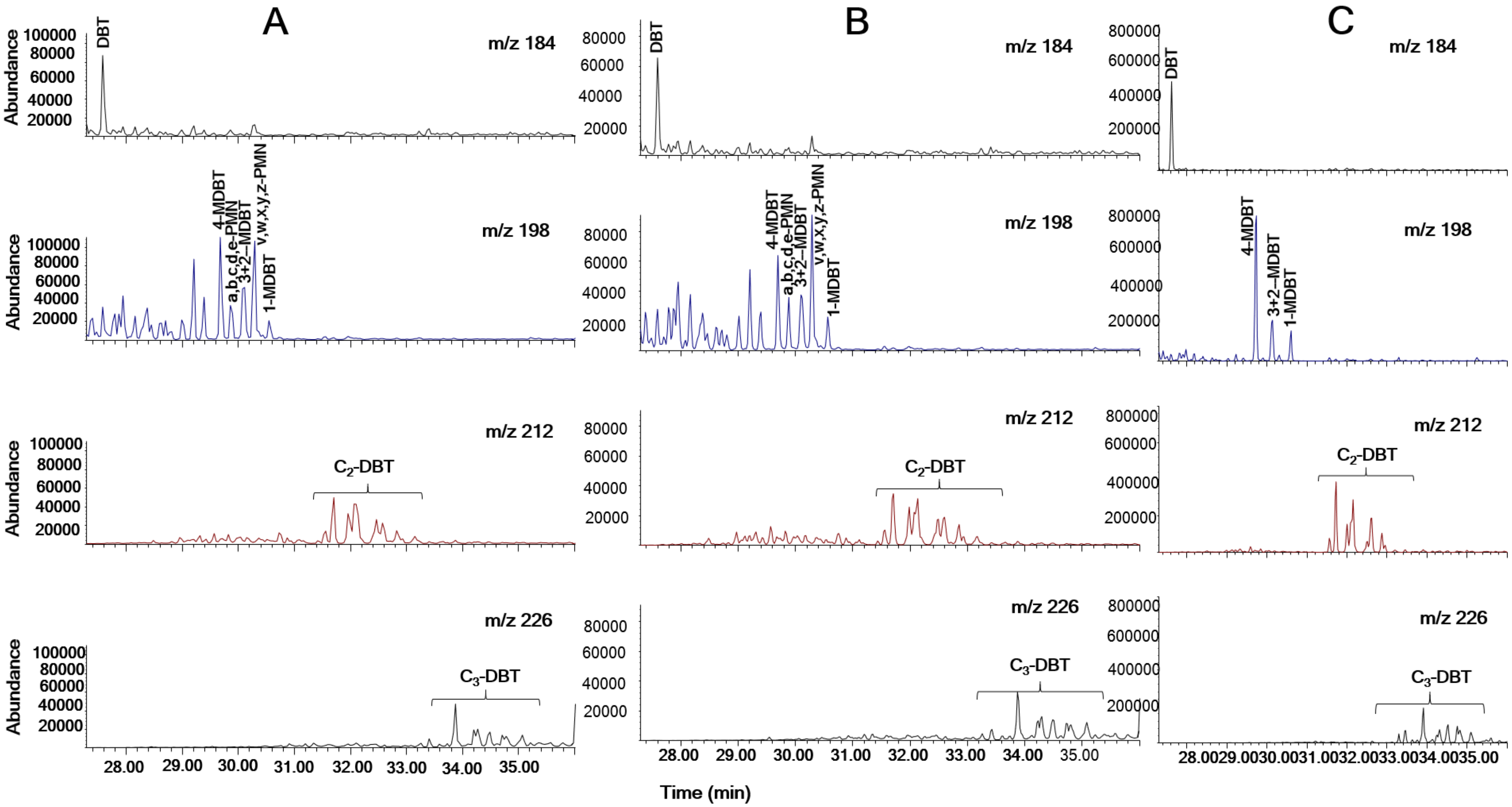


Figure 0.16: Chromatograms showing two identified alkylated PAHs (a,b,c,d,e-PMN and v,w,x,y,z-PMN) observed in Nigerian Light oil (A) and Nigerian Medium oil (B) which is not quite noticeable in North Sea oil (C)

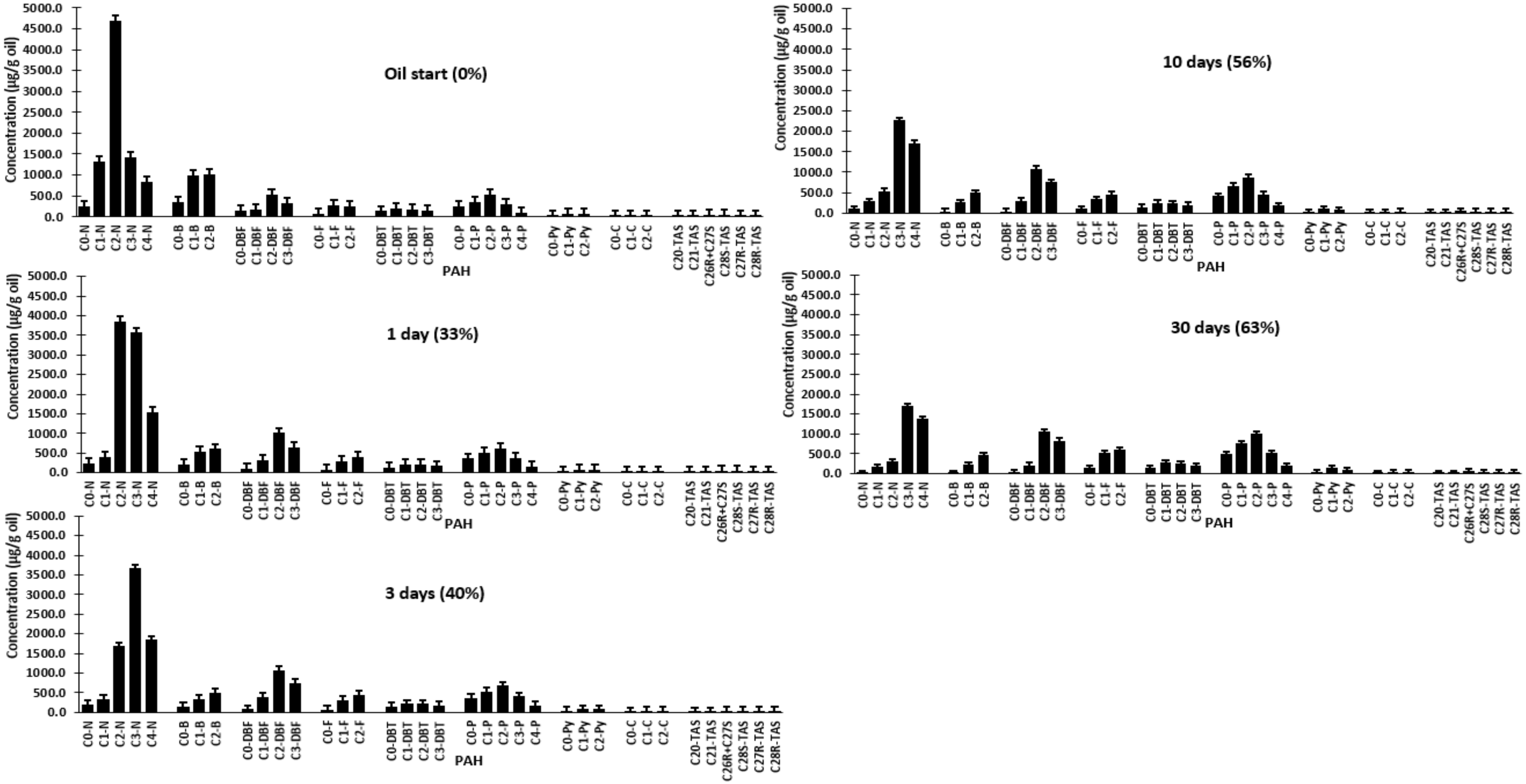


Figure 0.17: Distribution of target alkylated PAHs showing time in days and weathered percentages for Nigerian light oil. N, B, DBF, F, DBT, P, Py, C and TAS represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

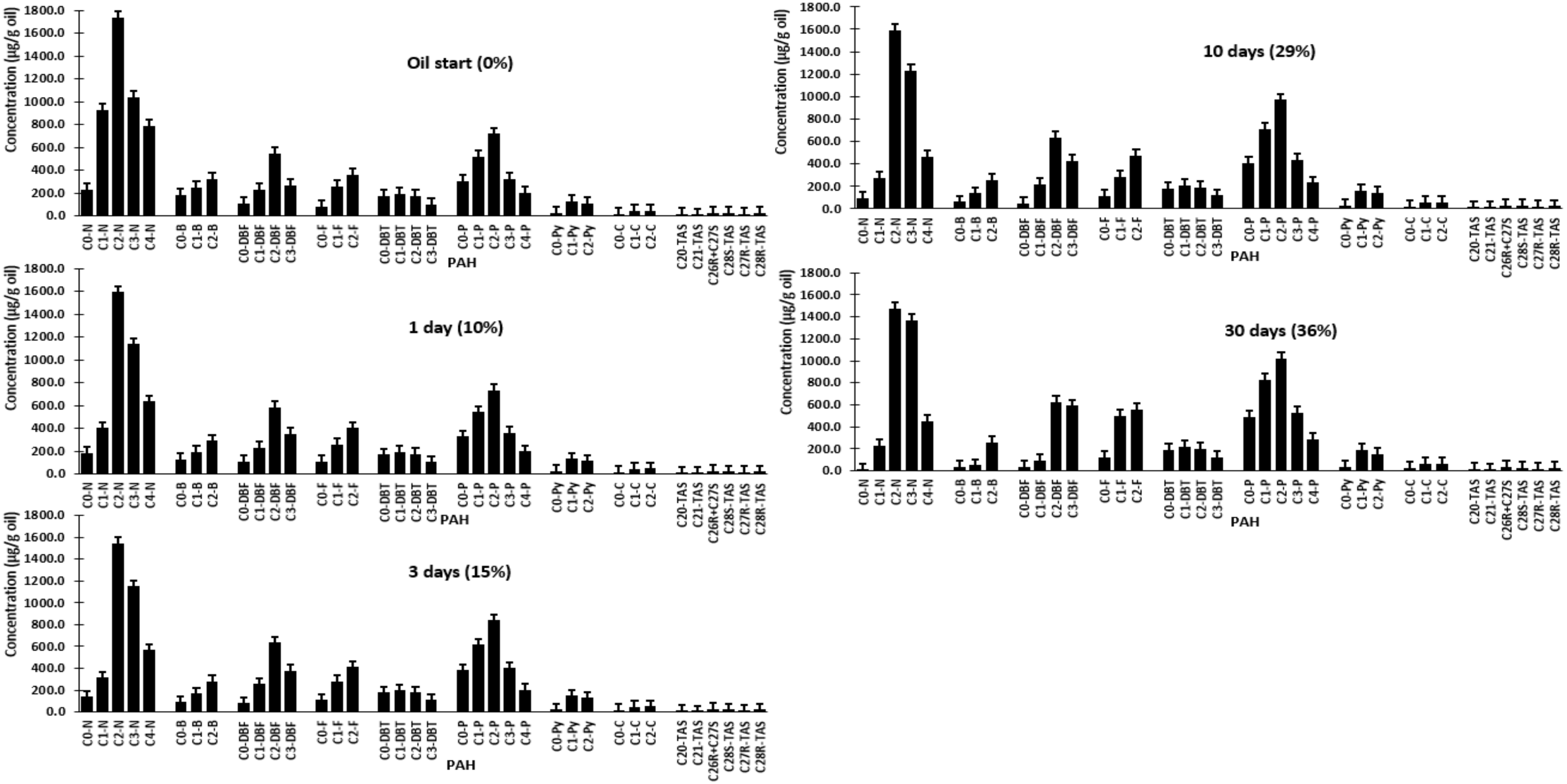


Figure 0.18: Distribution of target alkylated PAHs showing time in days and weathered percentages for Nigerian light oil. N, B, DBF, F, DBT, P, Py, C and TAS represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

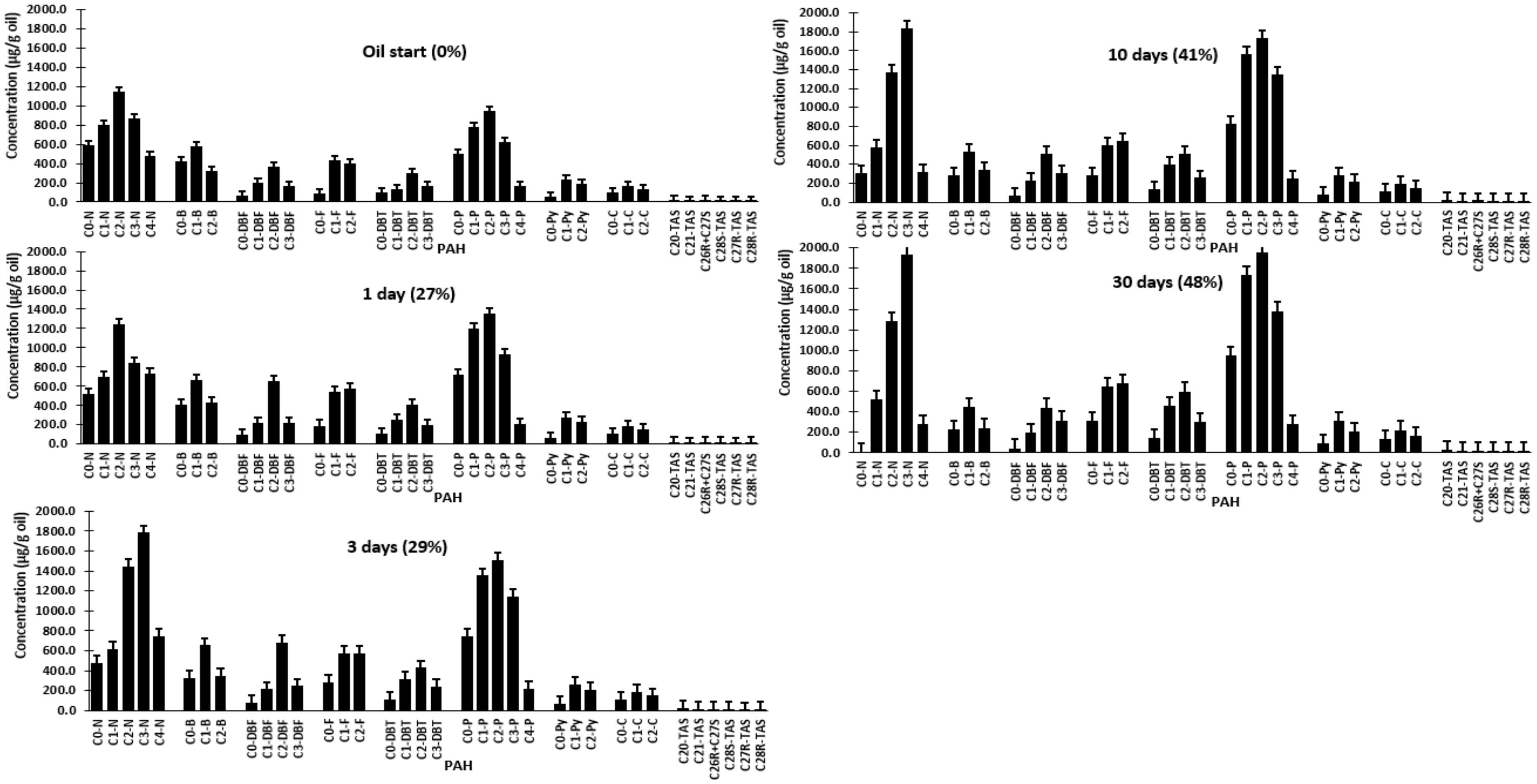


Figure 0.19: Distribution of target alkylated PAHs showing time in days and weathered percentages for Nigerian light oil. N, B, DBF, F, DBT, P, Py, C and TAS represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

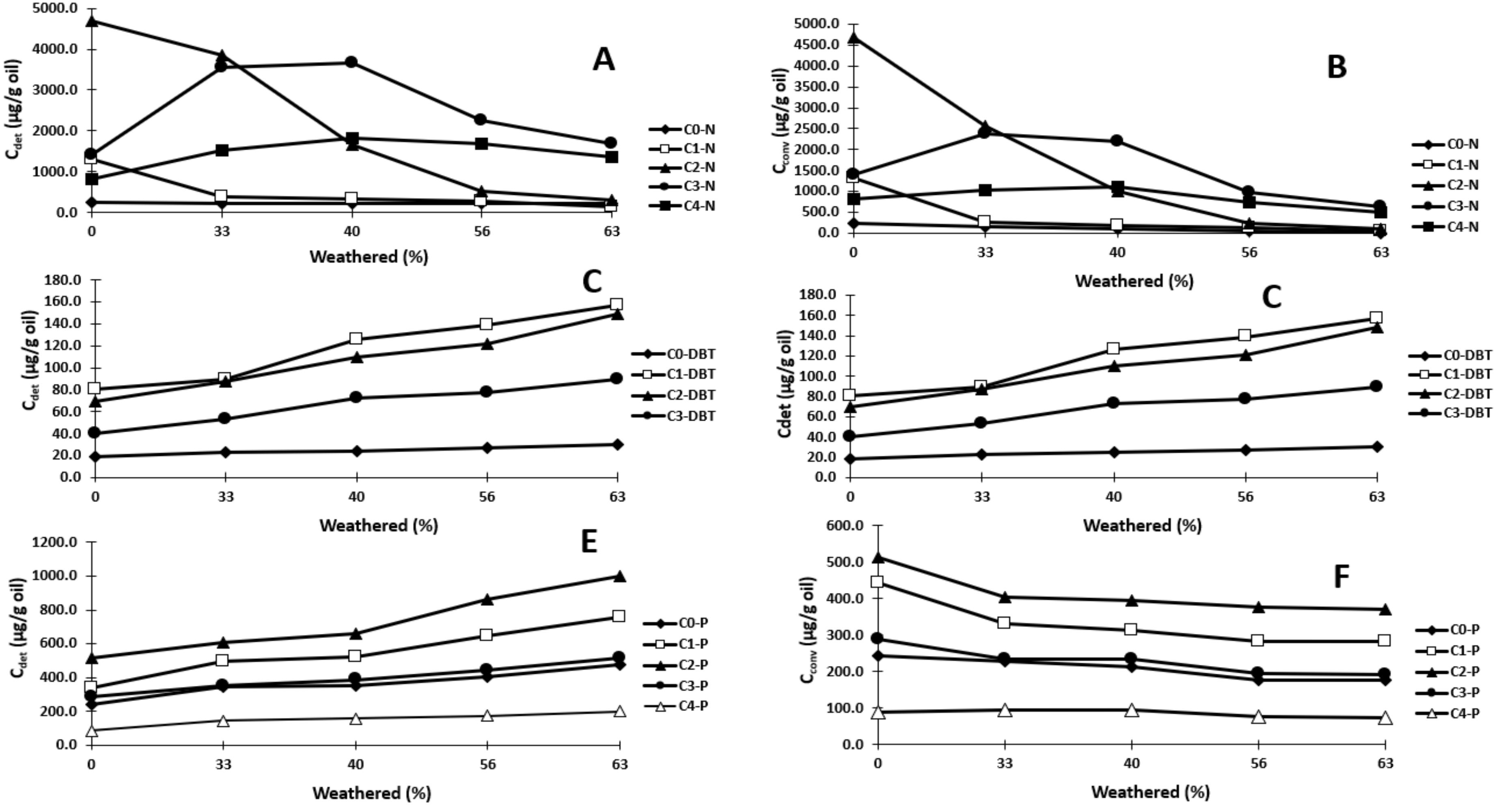


Figure 0.20: Plots of Cdet and Cconv versus weathered percentages for selected alkylated PAHs for Nigerian Light oil. Naphthalenes (A and B), dibenzothiophenes (C and D) and phenanthrenes (E and F)

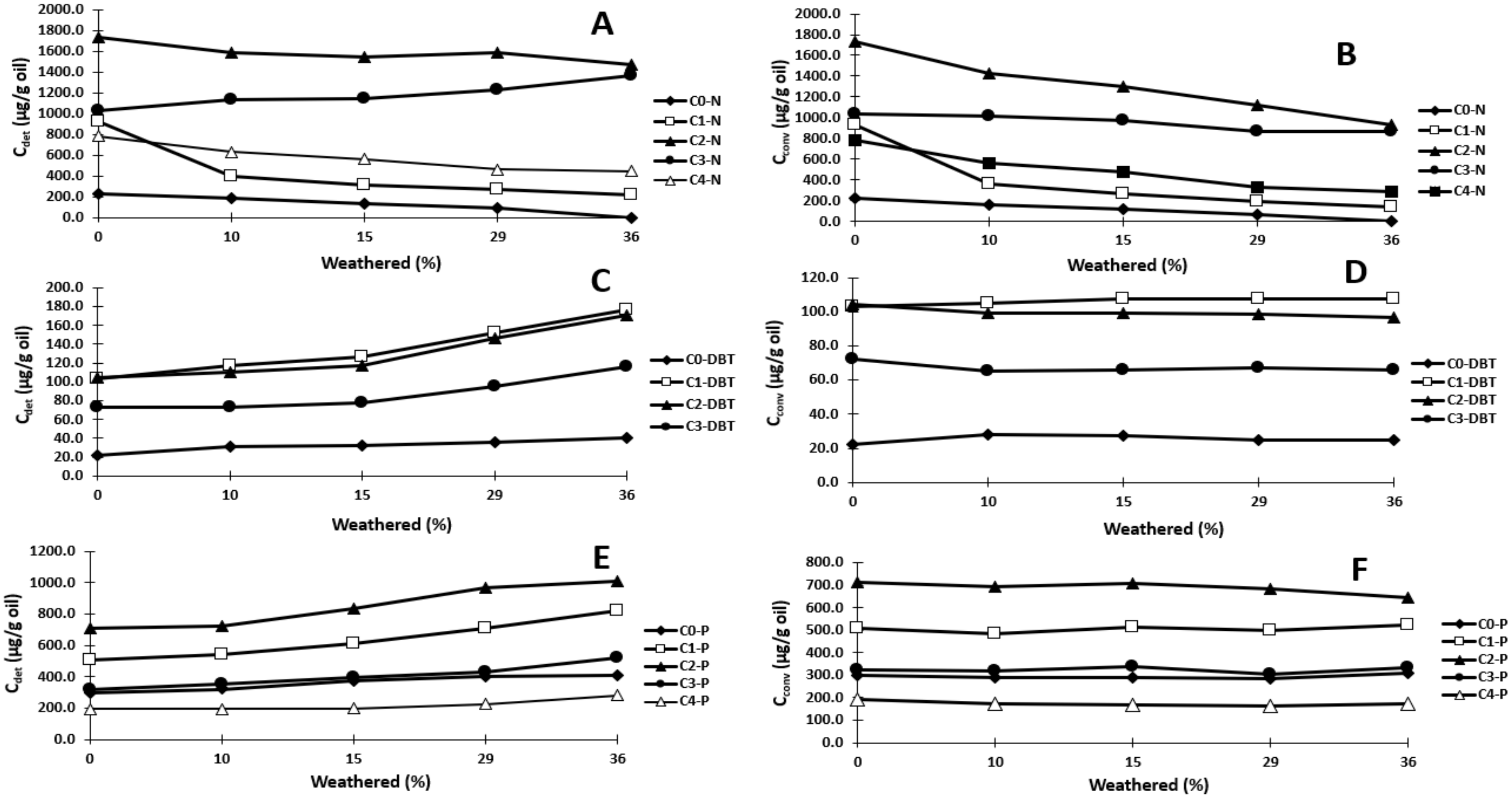


Figure 0.21: Plots of Cdet and Cconv versus weathered percentages for selected alkylated for Nigerian medium oil. Naphthalenes (A and B), dibenzothiophenes (B and C) and Phenanthrenes (E and F).

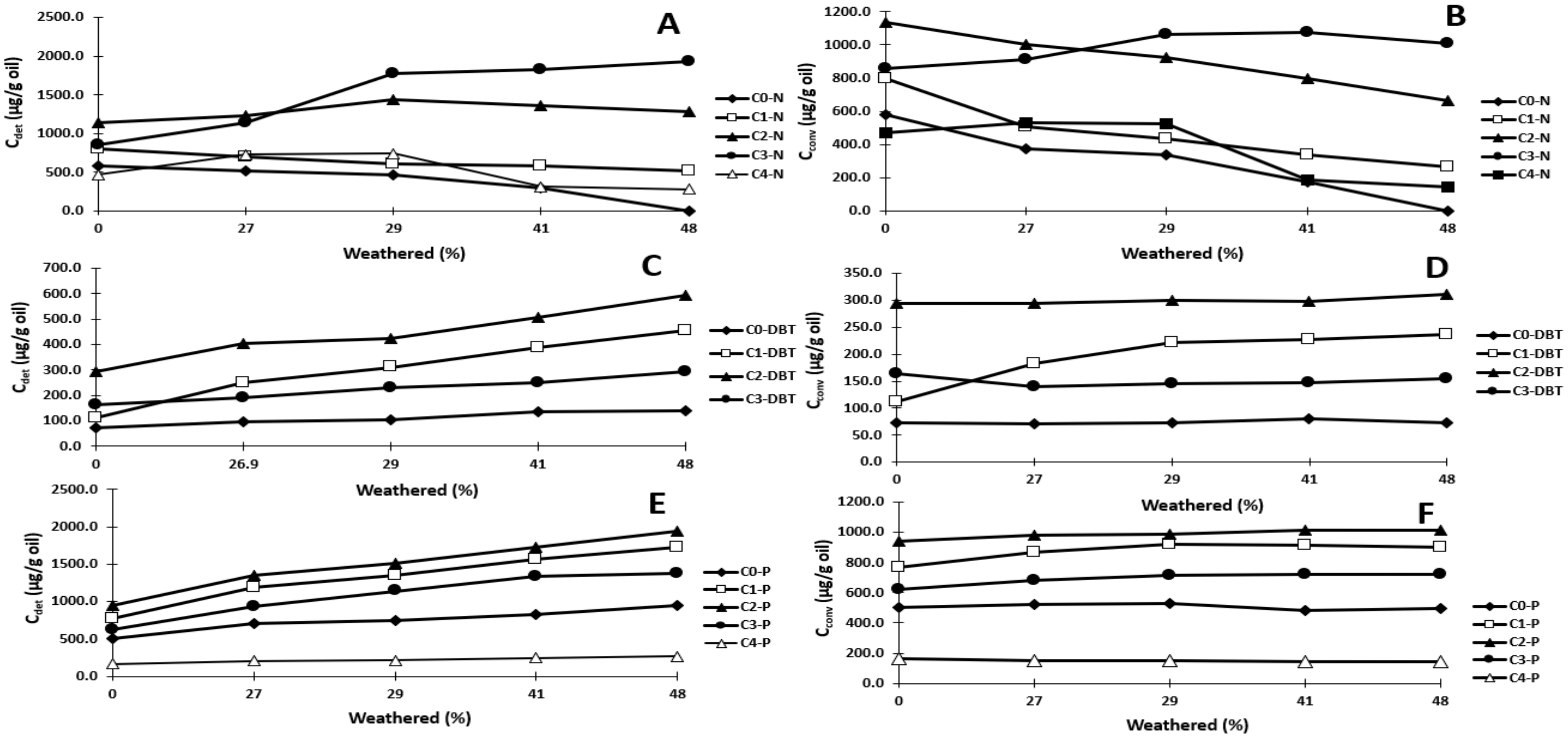


Figure 0.22: Plots of Cdet and Cconv versus weathered percentages for selected alkylated for North Sea oil. Naphthalenes (A and B), dibenzothiophenes (B and C) and Phenanthrenes (E and F).

3.4.6 Biomarker compound composition and concentration changes

Biomarkers are an important group of hydrocarbons in petroleum that have been used for many years for exploratory and reservoir assessment purposes by geochemists such as for the determination of the thermal maturity of oils, type of source material, depositional conditions and history of source material, geological age of source rock, and extent of weathering of oil in the reservoir(Wang *et al.*, 2006). Biomarkers also provide information during forensic investigations for source identification of oil spills, differentiation, correlation and monitoring of degradation and weathering condition of oils under different environmental conditions due to their relatively higher resistance to degradation (Wang *et al.*, 2006). Different sourced oils differ in absolute concentrations and relative distribution patterns, hence, the analysis of biomarkers can provide very on the overall weathering state of spilled oil in the environment (Wang and Fingas, 1995). Steranes and terpenoid biomarkers are the ones most considered during forensic investigation for source identification. Terpanes are saturated terpenoids that occur in petroleum in homologous or pseudo-homologous series classified on the isoprene units they are made up from and they include bicyclic, tricyclic, tetracyclic and pentacyclic compounds (Stout and Wang, 2016a). The distribution of terpanes in petroleum range from C_{19} to C_{35} with $C_{29}\alpha\beta$ and $C_{30}\alpha\beta$-pentacyclic hopanes and C_{23} and C_{24} tricyclic terpanes dominating most times (Wang *et al.*, 2006). Terpanes mainly considered for diagnostic analysis in this project include tricyclic, tetracyclic and pentacyclic terpanes. while others include bicyclic sesquiterpanes and adamantanes.

Terpanes Figure 0.23, Figure 0.24 and Figure 0.25 are the m/z 191 mass chromatograms showing terpanes distribution for the Nigerian Light, Nigerian Medium and North Sea oils respectively. The terpane compound measured concentrations are shown in **Table 0.8**, **Table 0.9** and **Table 0.10**. The peaks numbers 1, 2, 3 and 24 are labelled as S, Q, M and T respectively were observed in the two Nigeria oils, although compound T is also present in North Sea oil.

It is possible that S, Q and M are tricyclic terpanes considering their positions within the tricyclic terpanes region and T within the tetracyclic region. Structural identification of these compounds would form future work. However, the relative abundances of these compounds are very similar to that of other known tricyclic and tetracyclic terpanes and some of which may have been identified, hence, their diagnostic ratios are analysed alongside those of known compounds to ascertain their potentials use as diagnostic compounds. S, M and T are well resolved in the two oils whereas Q in the Nigerian Medium oil is not so resolved. The peak numbers 6, 11, and 19 in the two Nigerian oils (see Figure 0.23 and Figure 0.24) appear to be novel compounds identified in Tertiary deltaic oils reported previously (Samuel *et al.*, 2010). Samuel *et al* (2010), labelled these peaks as X, Y, Z1 respectively, and stated that X and Y were tricyclic terpanes and Z1 was a tetracyclic terpane. Structural details and the possible digenetic history of these compounds were also discussed in that work, peaks 12 and 15 appear to be those labelled as Y1 and Z, respectively, and were also discussed in the work. Unknown peaks were also noted in the North Sea oil (peak numbers 5, 12, 13, and 18) labelled as U, U1, U2 and T respectively. While U is positioned amongst the tricyclic terpanes, U1, U2 and T are amongst the tetracyclic terpanes. The main objective of this work is to analyse the effect of weathering of weathering on all these compounds to assess the extent of their resistance to degradation and potential for use as diagnostic compounds during oil spill investigation (Wang *et al.*, 2006; Douglas *et al.*, 2007; Stout and Wang, 2007b; Wang, 2008). The terpanes were generally unaffected by the extent of the evaporative weathering, this is shown in the plots of the converted concentrations of selected terpanes versus weathered percentages which remained horizontal for the three oil samples (Figure 0.26). The converted concentrations are shown in **Table 0.8Table 0.9Table 0.10** for the Nigeria Light, Nigerian Medium, and North Sea oils respectively.

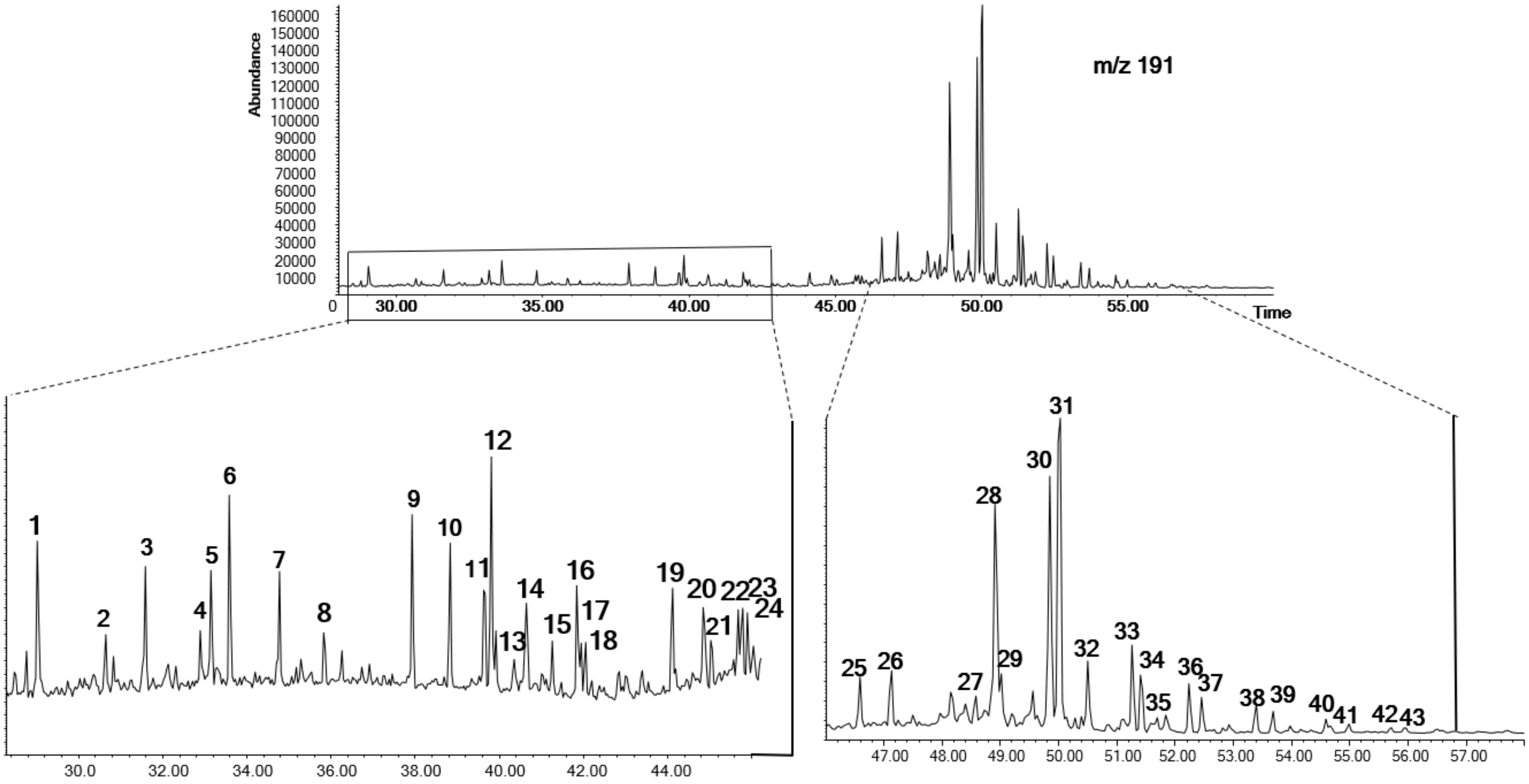


Figure 0.23: m/z 191 mass chromatograms showing the distributions of terpanes in unweathered Nigerian Light oil. The numbers represent peaks with the corresponding labels in Table 0.8**, peak numbers 1, 2, 4, 6, 11, 12 15, 19, and 24 are the suspected novel compounds labelled as S, Q, M, X, Y, Y1, Z, Z1, and T.**

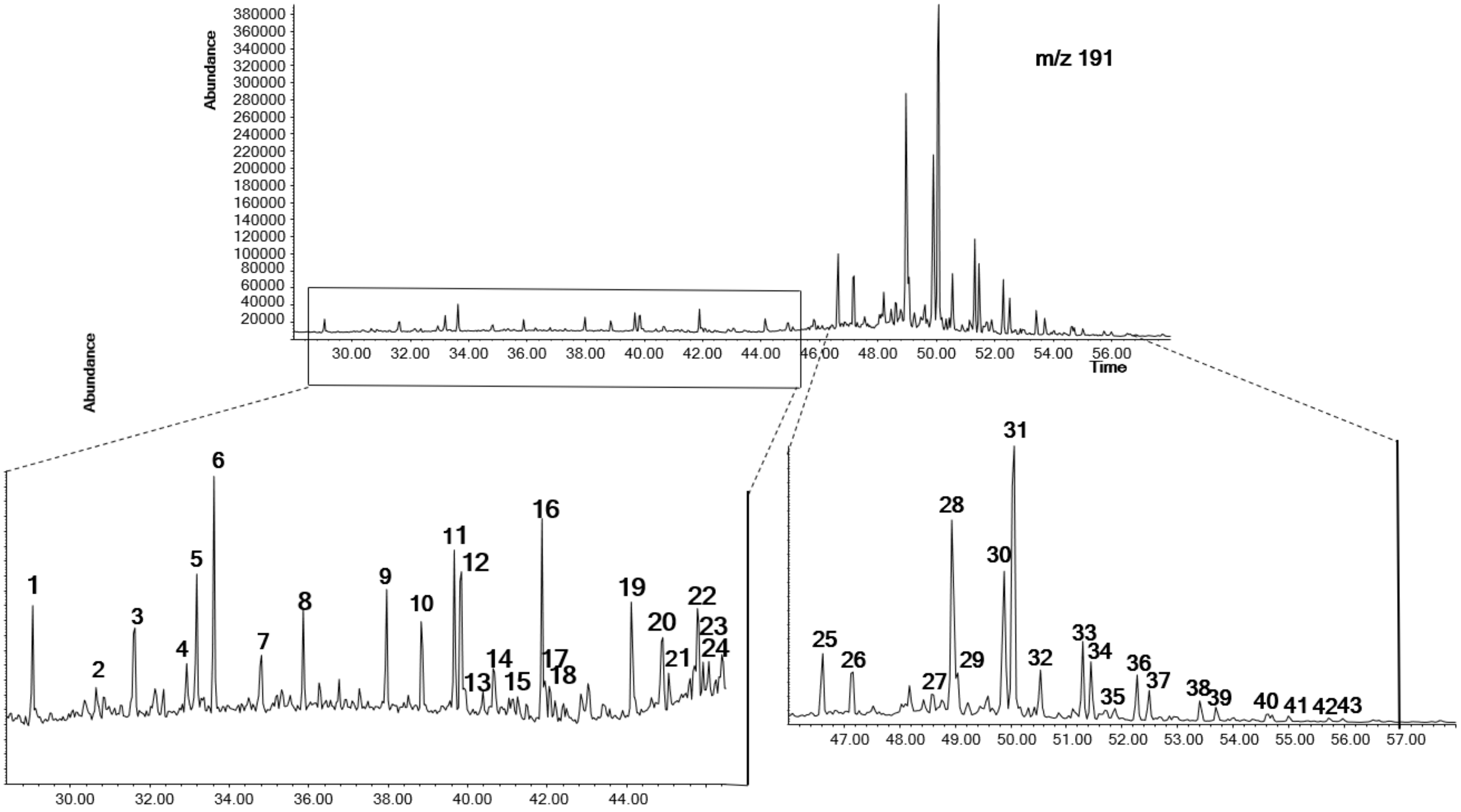


Figure 0.24: m/z 191 mass chromatograms showing the distributions of terpanes in unweathered Nigerian medium oil. The numbers represent peaks with the corresponding labels in Table 0.9**, peak numbers 1, 2, 4, 6, 11, 12 15, 19, and 24 are the suspected novel compounds labelled as S, Q, M, X, Y, Y1, Z, Z1, and T.**

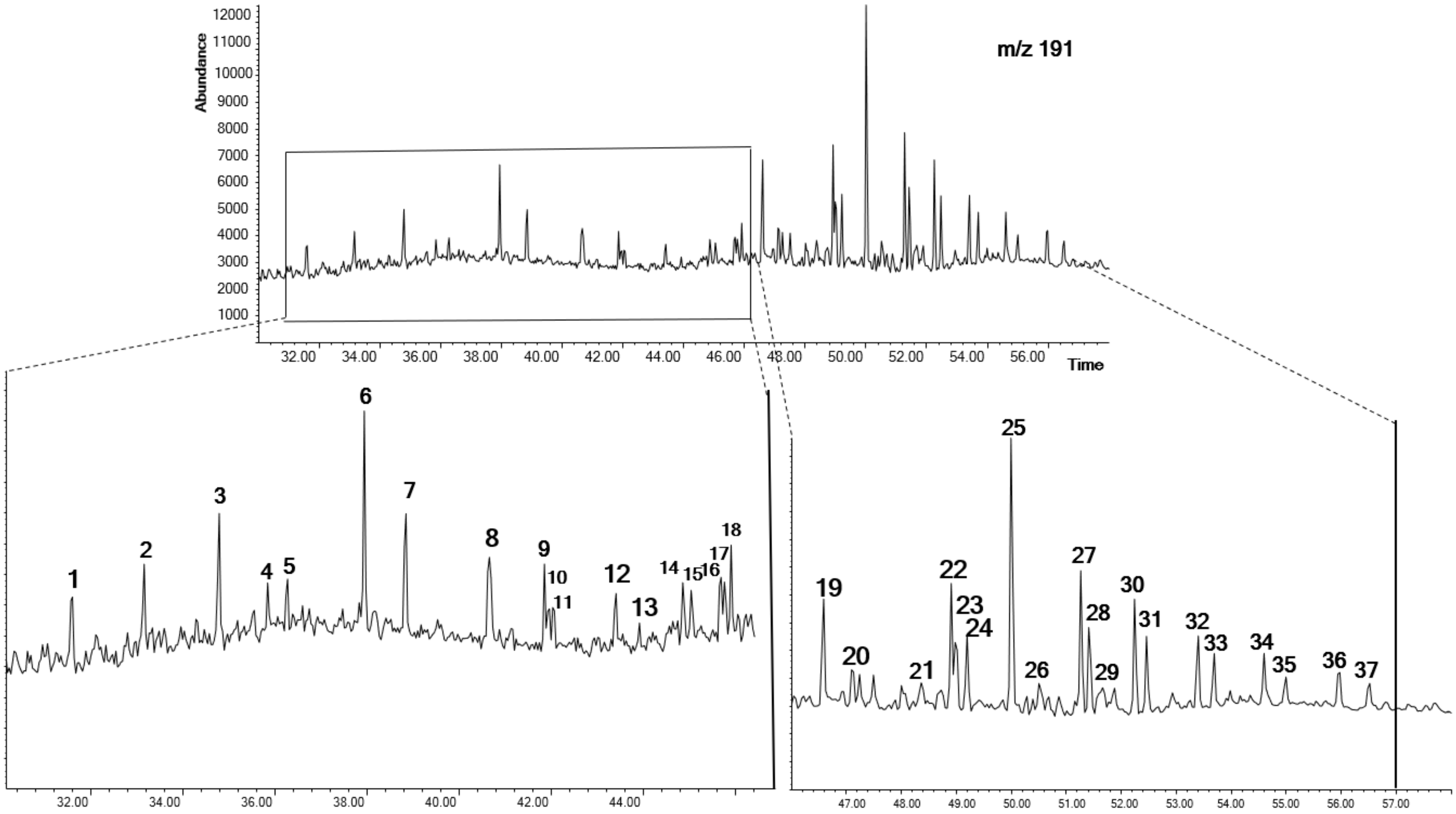


Figure 0.25: m/z 191 mass chromatograms showing the distributions of terpanes in unweathered North Sea oil. The numbers represent peaks with the corresponding labels in Table 0.10, peak numbers 5, 12, 13 and 18 are the suspected novel compounds labelled as U, U1, U2 and T.

Converted concentrations eliminate the effect of molecular build-up of less volatile components due volume decrease and only evaporative weathering effects are reflected.

It can be observed the plots (Figure 0.26) of the suspected novel terpanes compounds S, M, X, Y, Z, U, U1, U2 and T in the three oil samples are very similar in pattern compared to those of known terpanes compounds C_{27}Ts, C_{27}Tm, C_{29}Tm, $C_{30}\alpha\beta$-hopane, and C_{32} (22S)-hopane giving a strong likelihood that these identified compounds can be considered as resistant diagnostic compounds. The plots are parallel to x-axis are an indication of no evaporative loss of these biomarkers up to this weathering extent.

Diagnostic ratio plots of these novel compounds relative to their nearest known terpanes also show a strong indication of their diagnostic potential when compared with that of the known terpanes (see Figure 0.27). The ratio plots of novel compounds S/(S+Q), Q/(Q+C_{19}), X/(X+C_{20}), Y/(Y+C_{24}), Y1/(Y1+C_{24}), Z/(Z+C_{25}B), Z1/(Z1+C_{28}B), U/(U+C_{22}), U1/(U1+U2), and T/(T+C29B) are very similar to ratio plots of known compounds of C23/C24, and the isometric ratios of C_{31} 22S/(22S+22R), C_{32} 22S/(22S+22R), C_{33} 22S/(22S+22R), C_{34} 22S/(22S+22R), and C_{35} 22S/(22S+22R) in the three oils which is a strong indication of their potentials to be used as diagnostic compounds for oil spill source identification where spill samples could be correlated against a candidate source and also for estimating oil depletion of highly weathered oil. The plots are similar in patterns and parallel to the X-axis which means they are virtually unaffected by this extent of weathering conditions which is expected as terpanes are highly resistant to weathering.

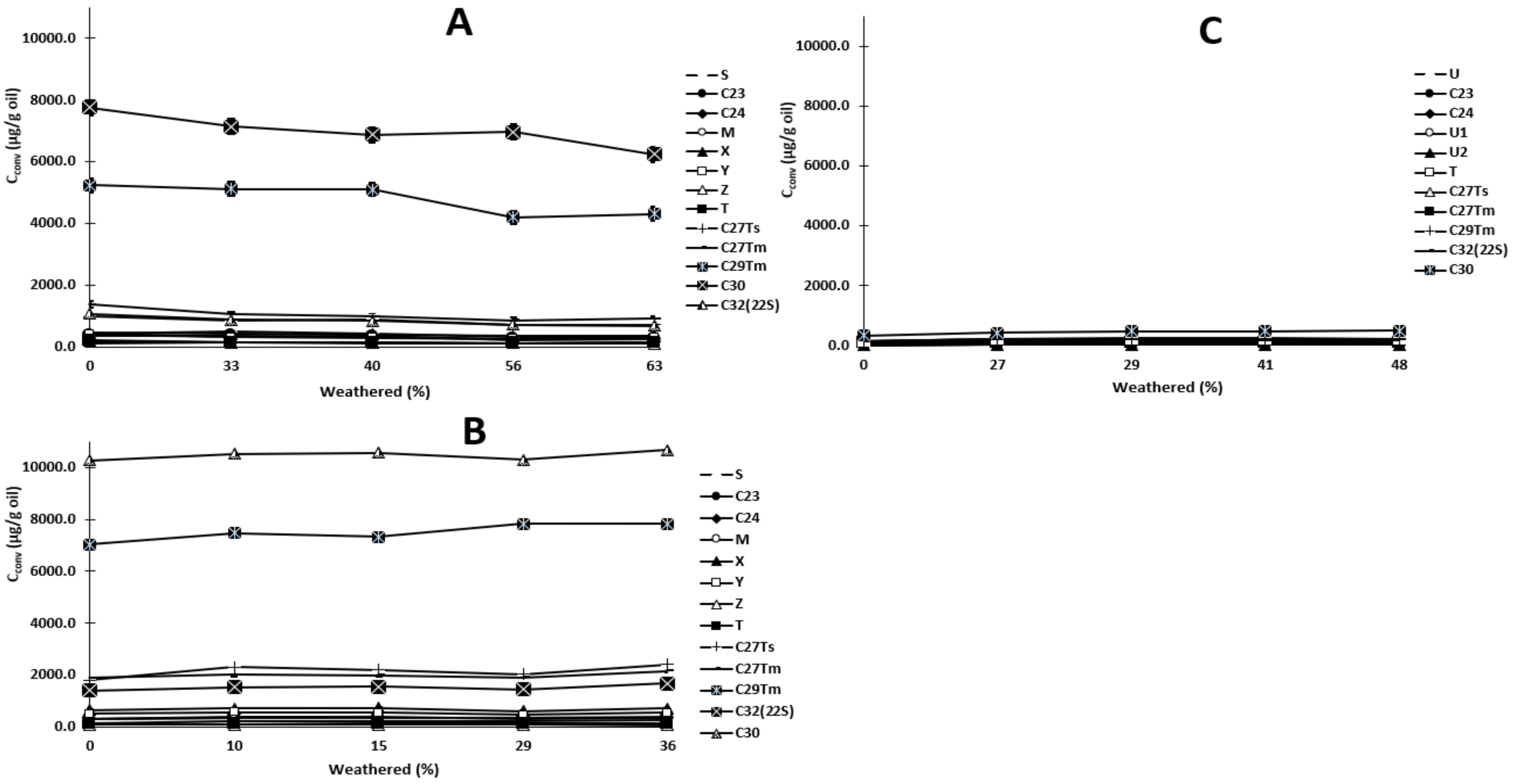


Figure 0.26: Plots of Converted concentrations of selected terpanes versus weathered percentage; (A) Nigerian light, (B) Nigerian medium, (C) North Sea oils respectively.

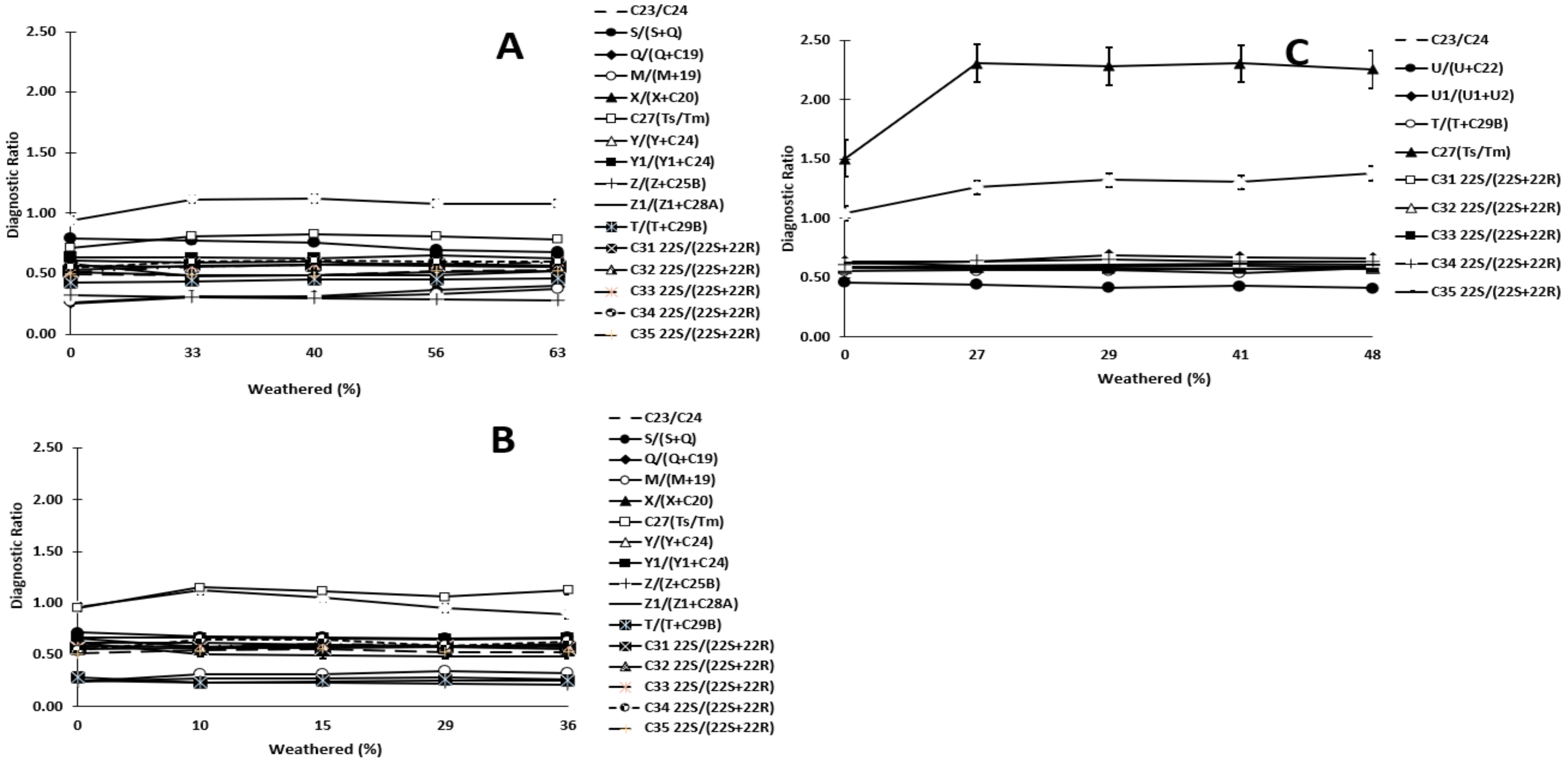


Figure 0.27: Plots of diagnostic ratios of selected known and suspected novel terpanes versus weathered percentage in; (A) Nigerian Light; (B) Nigerian Medium; and (C) North Sea oils.

Steranes are important group of saturated hydrocarbons which are biological marker compounds made up of tetracyclic androstane skeleton with side chain at C_{17} (Fingas, 2015). The C_{27-30} sterols are the known precursors of steranes and sterenes and have their major sources from eukaryotes such as algae and higher plants (Volkman, 1986; Volkman *et al.*, 1998; Fingas, 2015). The steranes have a range of dstribution from C_{20} to C_{30} with C_{27}, C_{28}, and C_{29} 20S/20R homolgous, especially the αββisomers being frequently the most dominant and are considered as reliable source indicators (Wang *et al.*, 1994a; Wang *et al.*, 1994b; Wang *et al.*, 2006; Yang *et al.*, 2006). Steranes were analysed in the three oil samples and their m/z 217 mass chromatograms are shown in Figure 0.28. The mass chromatograms for the two Nigerian Light and Medium oils (Figure 0.28A and Figure 0.28B) are very similar, but different from that of the North Sea oil (Figure 0.28C). The steranes were also relatively unaffected by evaporative weathring like the terpanes discussed earlier. **Table 0.11**,

Peak Number	Steranes	Determined Concentration (Cdet) (M/Z 217)				
		0%	33%	40%	56%	63%
1	C_{20}-5α(H), 14α(H), 17α(H)-sterane	101.8	158.9	174.3	186.2	242.0
2	C_{21}-5α(H), 14β(H), 17β(H)-sterane	189.2	298.9	315.2	478.7	502.1
3	C_{22}-5α(H), 14β(H), 17β(H)-sterane	101.9	174.2	177.0	208.5	261.8
4	C_{27} 20S-13β(H), 17α(H)-diasterane	205.5	325.9	351.4	411.4	521.9
5	C_{27} 20R-13β(H), 17α(H)-diasterane	132.1	201.4	209.4	257.4	337.4
6	C_{27} 20S-13α(H), 17β(H)-diasterane	83.6	104.4	106.0	126.3	160.0
7	C_{27} 20R-13α(H), 17β(H)-diasterane	94.9	124.8	137.9	175.9	239.5
8	C_{28} 20S-13β(H), 17α(H)-diasterane	186.0	203.2	239.3	275.4	346.6

9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	370.4	442.7	482.0	549.6	708.3
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	468.5	535.1	585.5	743.4	892.6
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	289.6	326.9	363.2	429.4	529.3
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	630.7	790.6	831.6	1151.4	1186.1
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	468.0	511.5	602.2	689.5	891.0
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	221.2	191.3	192.8	261.2	325.1
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	455.5	497.8	531.8	642.3	826.1
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	494.1	498.0	567.3	708.8	838.7
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	355.7	410.1	456.6	548.0	679.8
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	282.9	304.6	314.7	364.1	477.4
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	604.1	698.7	775.1	905.1	1135.4
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	688.2	839.0	961.4	1135.0	1383.9
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	581.3	726.6	862.9	973.7	1229.7
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	298.7	394.8	406.1	500.2	602.3
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	894.8	1254.4	1380.3	1538.5	2062.8
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	142.4	120.9	149.3	175.4	196.6
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	65.0	68.9	73.8	108.5	123.7
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	170.8	157.3	171.5	230.1	272.7
	Total	**8576.9**	**10361.1**	**11418.9**	**13774.1**	**16973.0**

Table 0.12, and

Peak Number	Steranes		Determined Concentration (Cdet) -(M/Z 217)			
		0%	10%	15%	29%	36%
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	91.7	132.5	148.9	155.7	176.6
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	151.1	249.6	243.3	251.2	357.3
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	77.2	118.3	121.7	130.0	173.3
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	173.2	244.6	250.4	260.5	340.1
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	95.3	144.4	148.3	163.8	237.3
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	45.3	58.6	62.9	66.8	80.5
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	66.9	93.6	102.2	110.8	141.8
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	120.2	160.3	163.2	176.3	233.3
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	171.9	181.7	190.4	199.8	231.0
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	308.9	357.4	380.1	413.0	550.1
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	154.6	190.1	199.6	213.5	267.5
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	210.9	275.7	327.5	411.6	433.5
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	257.7	336.5	342.1	357.8	481.7
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	199.6	289.5	301.9	314.3	294.8
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	238.3	255.3	269.3	284.4	339.1
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	270.9	283.4	293.1	318.0	410.2
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	192.8	230.9	260.9	271.8	313.3

18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	147.1	128.7	133.2	142.4	195.9
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	205.3	244.6	259.2	301.2	372.5
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	470.7	622.1	675.2	742.7	918.6
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	321.9	424.8	451.8	569.6	621.4
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	172.7	219.6	222.0	231.9	299.6
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	285.1	639.0	666.4	752.9	939.6
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	108.1	124.0	157.6	163.6	171.6
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	36.1	49.8	54.0	61.1	82.8
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	109.1	110.6	113.3	139.0	156.8
	Total	**4682.3**	**6165.4**	**6538.7**	**7203.5**	**8820.2**

Table 0.13 show the measured concnentrations of steranes in Nigerian Light, Nigerian Medium, and North Sea oils respectively, as measured from m/z 217 mass chromatograms. The resistance of steranes to evaporative weathering can be seen in Figure 0.29 as the diagnostic ratios are parallel to x-axis indicating no effect of increased evaporative weathring on steranes, except the for the C_{20}, C_{21}, ans C_{22} steranes (pregnanes) where little effects were observed. The diagnostic ratios plots (Figure 0.29) of C_{20}/C_{21} and C_{21}/C_{22} were not as parallel for all the three oil samples with C_{21}/C_{22} showning the more apparent effect, however, the ratio $C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$ was observed to be slightly affected only in North Sea oil. Aromatic steranes are also an important group of biomarkers compounds that are resistant to degradation and are suitable for use as oil-to-oil correlation and source identification (Peters and Moldowan, 1993b; Wang *et al.*, 2006; Yang *et al.*, 2006).

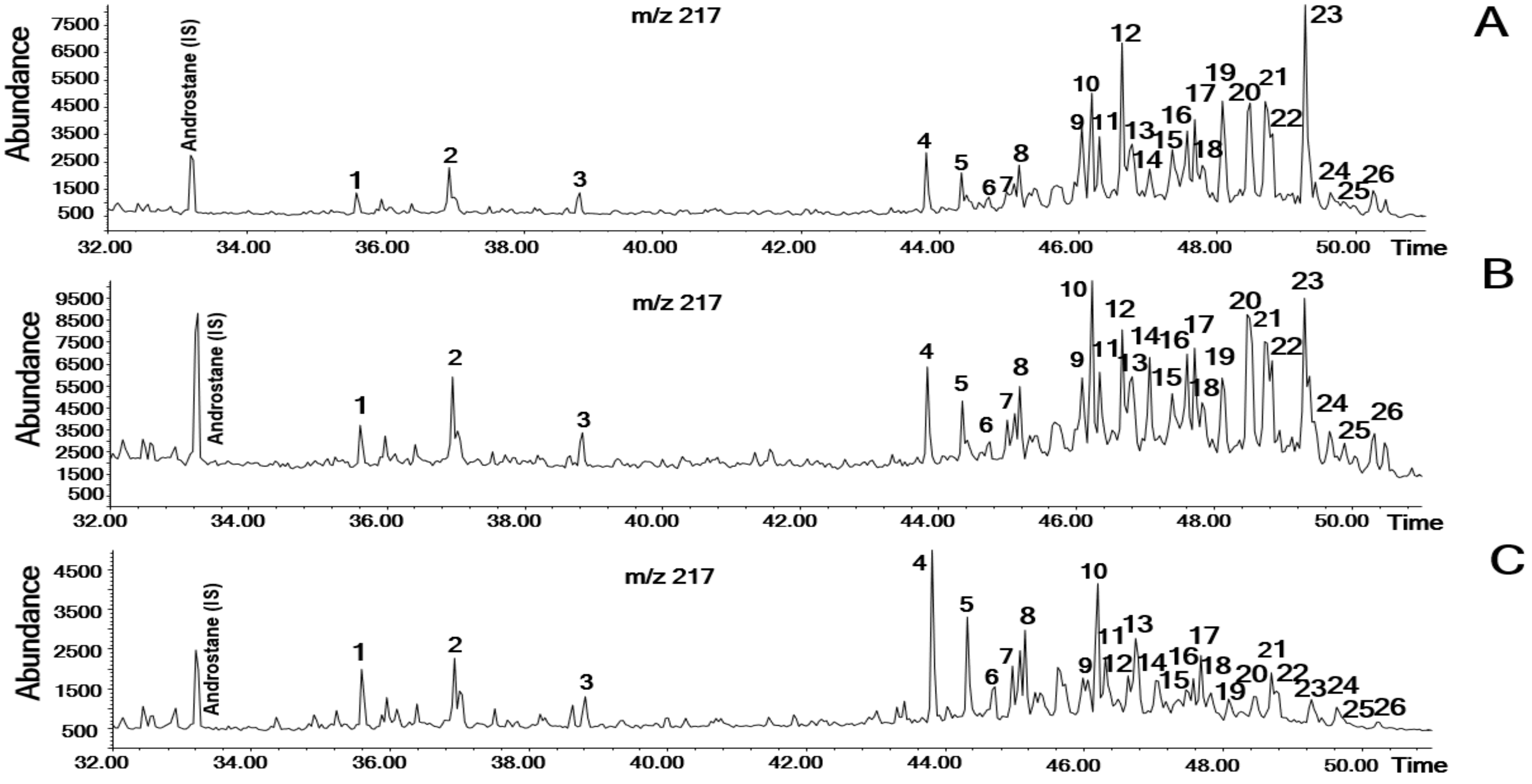


Figure 0.28: m/z 217 mass chromatograms showing the distributions of steranes in unweathered (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils. Numbers in (A) represent the corresponding peak names in Table 0.11, numbers in (B) represent peak names in Table 0.12, and numbers in (C) represent peak names in

Determined Concentration (Cdet) -(M/Z 217)

Peak Number	Steranes	0%	10%	15%	29%	36%
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	91.7	132.5	148.9	155.7	176.6
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	151.1	249.6	243.3	251.2	357.3
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	77.2	118.3	121.7	130.0	173.3
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	173.2	244.6	250.4	260.5	340.1
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	95.3	144.4	148.3	163.8	237.3
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	45.3	58.6	62.9	66.8	80.5
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	66.9	93.6	102.2	110.8	141.8
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	120.2	160.3	163.2	176.3	233.3
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	171.9	181.7	190.4	199.8	231.0
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	308.9	357.4	380.1	413.0	550.1
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	154.6	190.1	199.6	213.5	267.5
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	210.9	275.7	327.5	411.6	433.5
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	257.7	336.5	342.1	357.8	481.7
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	199.6	289.5	301.9	314.3	294.8
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	238.3	255.3	269.3	284.4	339.1
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	270.9	283.4	293.1	318.0	410.2
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	192.8	230.9	260.9	271.8	313.3
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	147.1	128.7	133.2	142.4	195.9
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	205.3	244.6	259.2	301.2	372.5

20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	470.7	622.1	675.2	742.7	918.6
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	321.9	424.8	451.8	569.6	621.4
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	172.7	219.6	222.0	231.9	299.6
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	285.1	639.0	666.4	752.9	939.6
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	108.1	124.0	157.6	163.6	171.6
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	36.1	49.8	54.0	61.1	82.8
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	109.1	110.6	113.3	139.0	156.8
	Total	**4682.3**	**6165.4**	**6538.7**	**7203.5**	**8820.2**

Table 0.13 **respectively.**

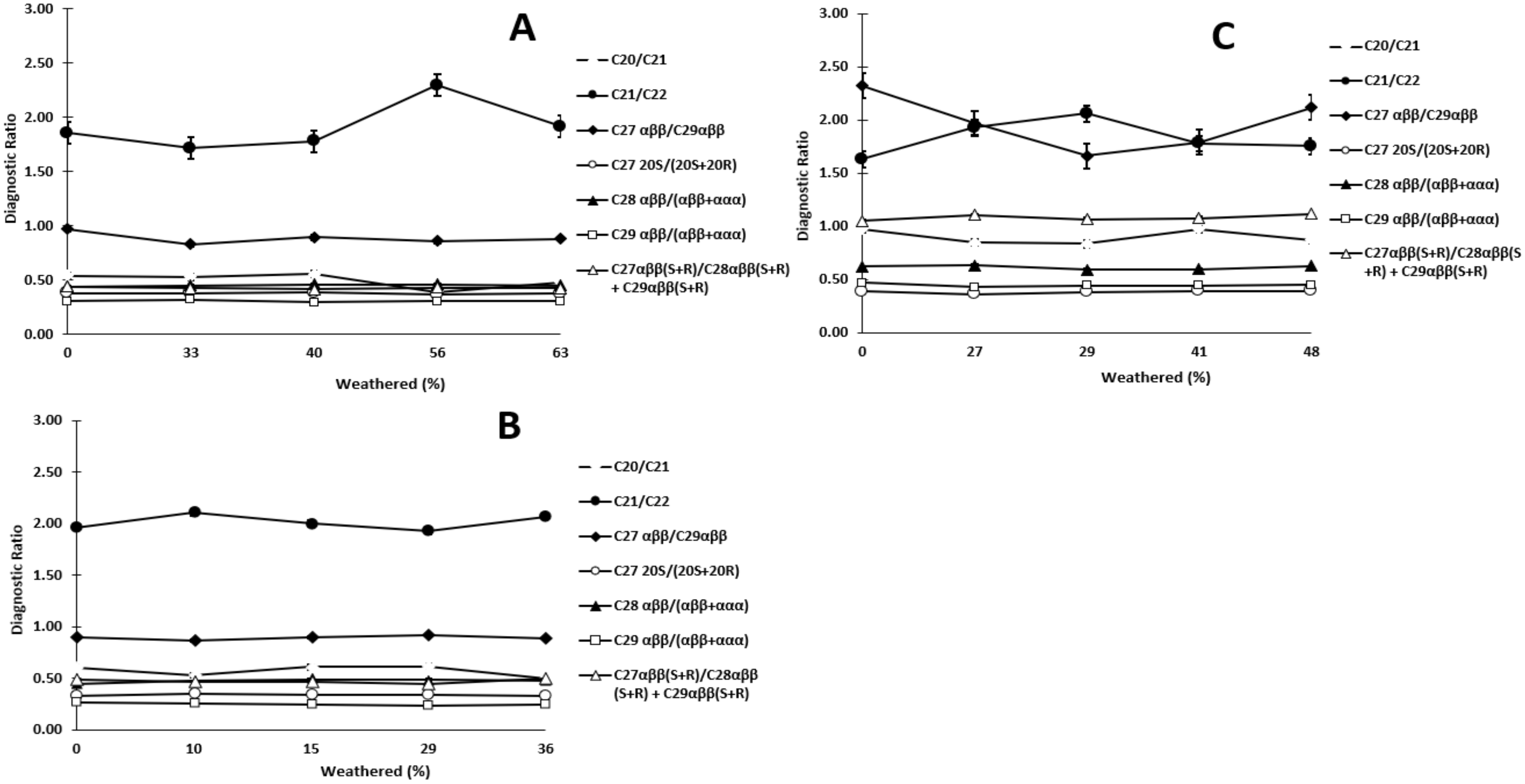


Figure 0.29: Plots of diagnostic ratios of selected steranes versus weathered percentage in; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils respectively.

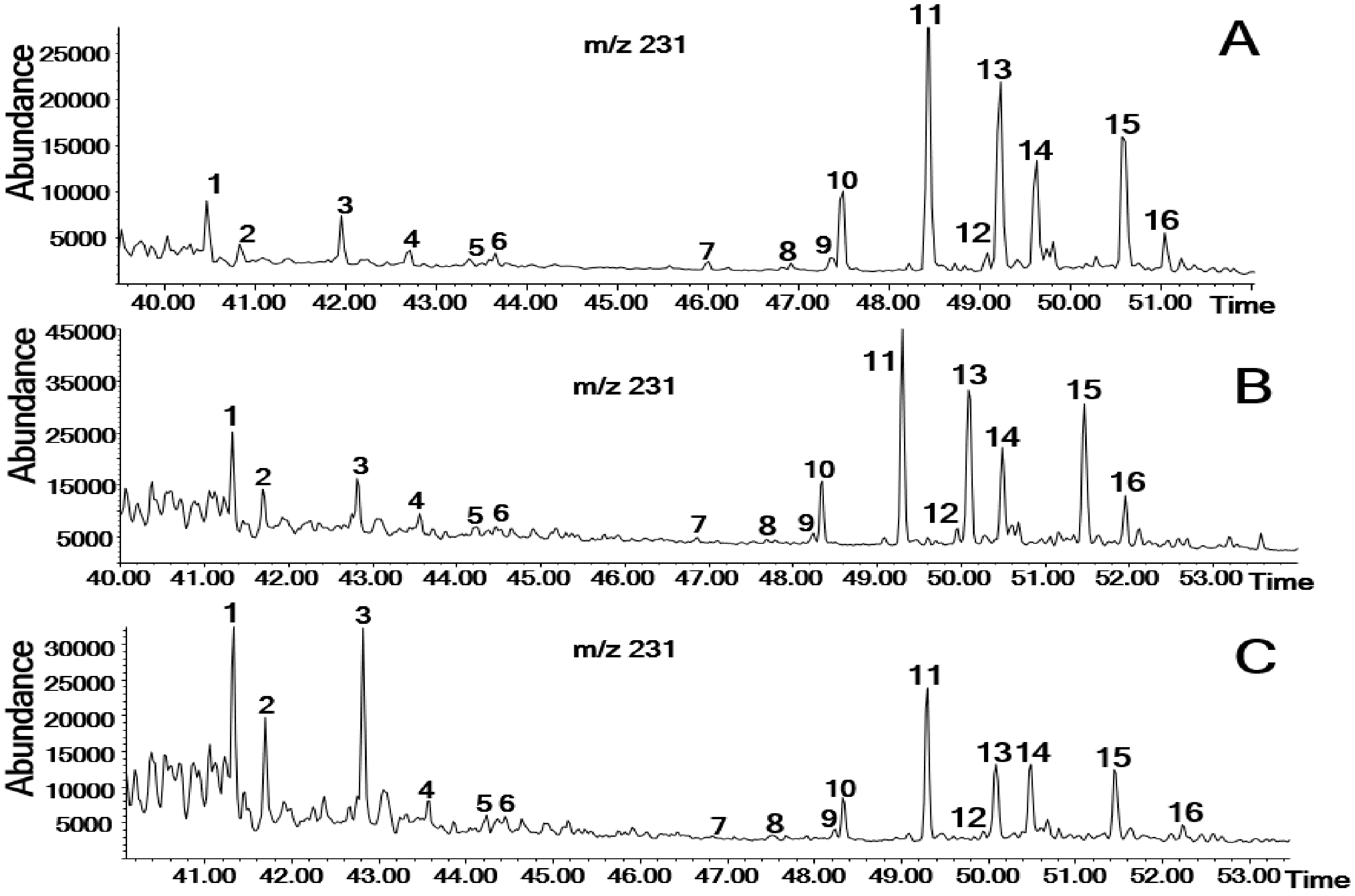


Figure 0.30: m/z 231 mass chromatograms showing the distributions of triaromatic steranes in unweathered Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. Numbers in (A) represent the corresponding peak names in Table 0.14, numbers in (B) represent peak names in Table 0.15, and numbers in (C) represent peak names in Table 0.16 respectively. Numbers 1. 2, 7, 8, 9, and 12 represent unidentified peaks labelled as P, Q, R, S, T, U, and V respectively.

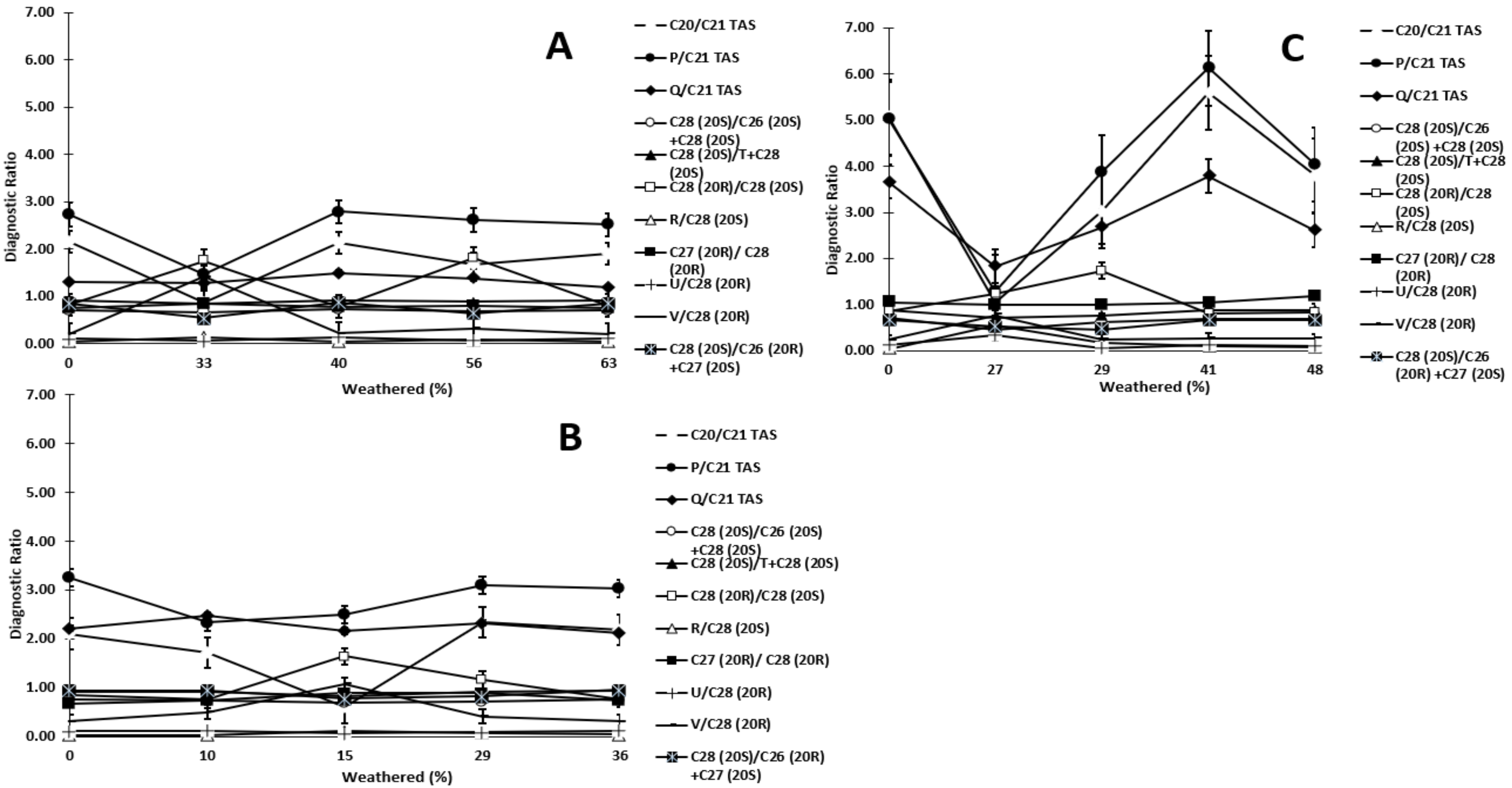


Figure 0.31: Plots of diagnostic ratios of triaromatic steranes versus weathered percentage in; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils respectively.

Triaromatic steranes (TAS) were analysed in the three oil samples and their m/z 231 mass chromatograms are shown in Figure 0.10 and Table 0.14,Table **0.15**,Table **0.16** show the measured concnentrations of the triaromatic steranes (TAS) in the Nigerian Light, Nigerian Medium, and North Sea oils respectively.The diagnostic ratios plots of the known triaromatic steranes (TAS) and that of some unknown peaks are shown in Figure 0.31. The ratios of the known triaromatic steranes were generally unaffected by the evaporative weathering except for the C_{20}/C_{21} TAS ratio, and the ratios of the unknown peaks P/C_{21}, Q/C_{21} TAS, and V/C_{28} TAS were much affected by evaporative weathering. This may be an indication that the peaks P, Q, and V may not triaromatic steranes.

The bicyclic biomarker sesquiterpanes are biomarkers found ubiquitously in crude oils and ancient sediments and contains roughly 15 ('sesqui- ') carbon atoms. They are reported to be of terrigenous origin from higher plants contribution into the sedimentary organic matter (Nytoft *et al.*, 2009; Stout and Wang, 2016a). Bicyclic sesquiterpanes are aliphatic hydrocarbons generally within the C_{14}-C_{16} boiling range with decalin skeleton (Stout and Wang, 2016a). These compounds were analysed in the three oil samples and their m/z 123 mass chromatograms distributions are shown in Figure 0.32 in (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils respectively. Table 0.17Table 0.18Table 0.19, show the measured concentrations of bicyclic sesquiterpanes in Nigerian Light, Nigerian Medium, and North Sea oils respectively, using m/z 123 mass chromatograms. The concentrations of sesquiterpanes in the North Sea oil is lower than in those of the two Nigerian oils. The sesquiterpene most affected by evaporative weathering is 4-decalin (BS1-peak number 1) in the North Sea oil reduced from 7% $\frac{373.0}{5413.6} \times 100)$ in the unweathered oil about the equivalent of 8% in Troll oil, North Sea (e.g. Fingas, 2015), to 1% after 30days (48% oil weight). Compounds A, U_2, B, and D, are novel unidentified sesquiterpanes observed in the two Nigerian samples, and another peak (?) in the North Sea oil. These compounds were first reported in previous work from different oleanane containing oils including Nigerian oil samples (Nytoft *et al.*, 2009). Nytoft et al (2009) reports that A, U_2, B, and D, are C_{15} compounds based on their mass spectral information. The potential of these novel sesquiterpanes to be used as diagnostic markers was assessed in this project by comparing their ratios with those of the known sesquiterpanes.

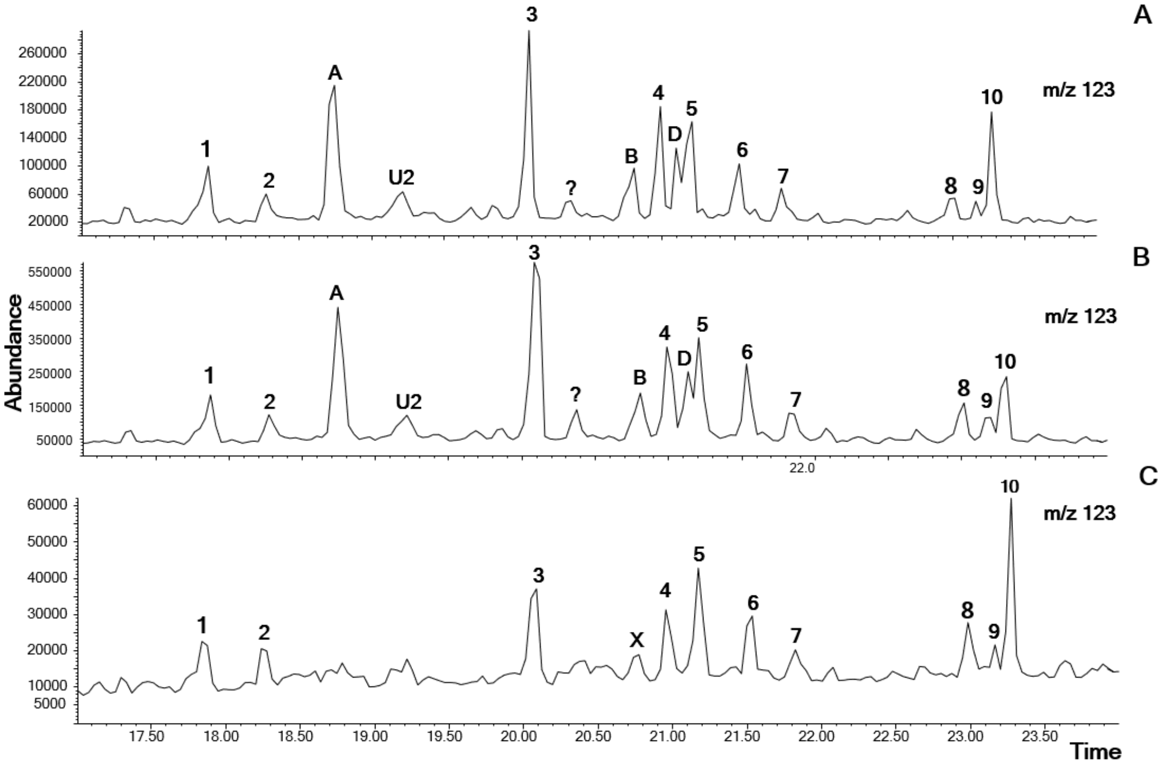


Figure 0.32: m/z 123 mass chromatograms showing the distributions of bicyclic sesquiterpanes in unweathered (A) Nigerian Light; (B) Nigerian Medium; and (C) North Sea oils. NB: A, U_2, B, and D, are novel sesquiterpanes observed in the Nigerian Light and Medium oil.

Figure 0.33 shows the plots of sesquiterpanes diagnostic ratios for: Nigerian light (A); Nigerian Medium (B); and North Sea (C) oils respectively. The ratios patterns for the two Nigerian oils are very similar. The diagnostic ratios pattern of the novel compounds BSA/BS2, BSU2/BS3, BSB/BS4, BSD/BS5, BSA/ $C_{30}\alpha\beta$-hopane, BSU2/$C_{30}\alpha\beta$-hopane, BSB/$C_{30}\alpha\beta$-hopane, and BSD/$C_{30}\alpha\beta$-hopane are very similar to those of the known sesquiterpanes such as BS1/BS2, BS3/$C_{30}\alpha\beta$-hopane, and BS10/$C_{30}\alpha\beta$-hopane with increased weathering for the Nigerian oils.

The diagnostic ratios for the unknown sesquiterpane BSX in the North Sea oil are also like that of the known sesquiterpanes and is unaffected by increased evaporative weathering. BSX/BS10 is the most unaffected ratio and it is similar to BSX/BS3 up to 41% (oil weight)

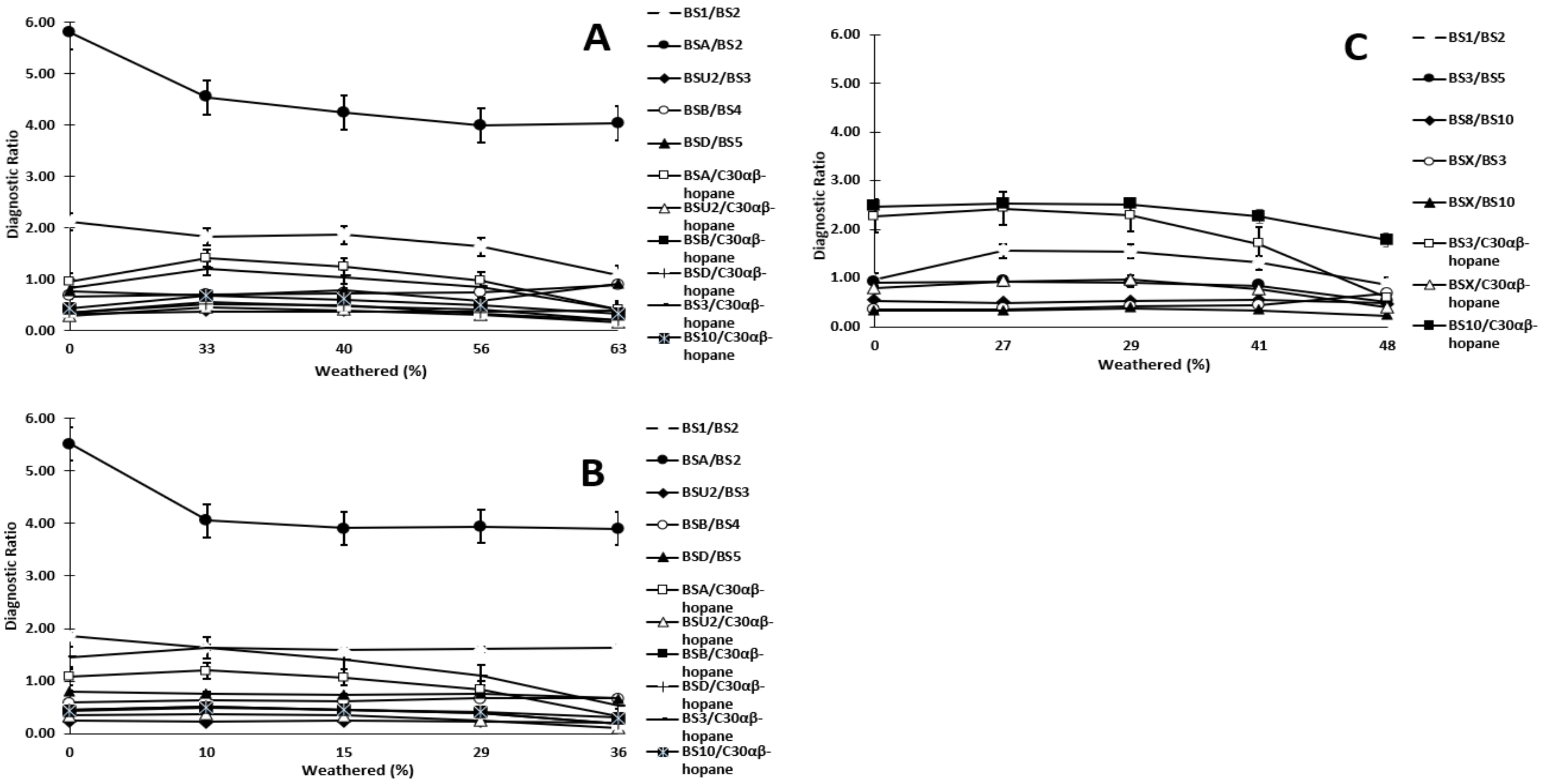


Figure 0.33: Plots of diagnostic ratios of selected and novel bicyclic sesquiterpanes versus weathered percentage in; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils respectively.

weathering, BSX/$C_{30}\alpha\beta$-hopane is similar to BS3/BS5. Except for BS3/ $C_{30}\alpha\beta$-hopane for the North Sea oil where there is significant reduction due to the weathering effect on the ratio from 29 to 48% (oil weight). However, the diagnostic ratios patterns for the three oils are generally similar. Hence, these novel sesquiterpanes could be used as diagnostic compounds for oil-source correlation and differentiation.

Another group of geochemical markers are diamondoids which are saturated hydrocarbons consisting of three or more cyclohexane rings with a ‘diamond-like’ structure, which they derived their name from. Due to their thermodynamic stability, they are useful in oil-source correlation and differentiation where the traditional biomarkers of terpanes and steranes are not present especially in refined products (Mudge *et al.*, 2008; Stout and Wang, 2008; Fingas, 2015) . The simplest diamondoid is adamantane, a tricyclodecane which could be fused to give rise to polymantane homologues such as diamantane, triamantane, and tetramantane (Mudge *et al.*, 2008; Stout and Wang, 2008). Only adamantanes were analysed in this project and their m/z 136 mass chromatograms distributions are shown in Figure 0.34 in Nigerian light (A), Nigerian medium (B), and North Sea (C) oils respectively. The distribution patterns in the two Nigerian oils are similar while that of the North Sea is different. Table 0.20Table 0.21Table 0.22 show the measured concentrations of adamantanes in the Nigerian Light, Nigerian Medium, and North Sea oils respectively. The concentrations of adamantanes in the North Sea oil are much less than those in the Nigerian oils. The analyte most affected by evaporative weathering is adamantane in the Nigerian Light oil which was evaporated from 65.4 µg/g in the unweathered oil to 0 µg/g at 63% (oil weight) weathered percentage, whereas there was an increase in concentration in the Nigerian Medium probably due to molecular build-up from 31.3 µg/g in unweathered oil to 55.1 µg/g at 36% (oil weight). The adamantane in the North Sea oil reduced from 44.9 µg/g to 0.3 µg/g at 48% (oil weight), and similarly, adamantane concentration of 48.6 µg/g in Troll oil, North Sea was reported (Fingas, 2015). Figure 0.35

shows the diagnostic ratios plots for the Nigerian light (A), Nigerian medium (B) and North Sea (C) oils respectively. The ratios of 1-MA/(1,3-DMA), 1-MA/(1-MA+2-MA), and 1-MA/(1-MA+2-MA+2-AE) are the most unaffected by evaporative weathering and are similar in the two Nigerian oils compared to the other ratios considered. The 1-MA/(1-MA+2-MA), 1-DMA/(1,2,5,7-TeMA), and 1-MA/(1-MA+2-MA+2-AE) show similar patterns in the North Sea oil and are unaffected by the weathering.

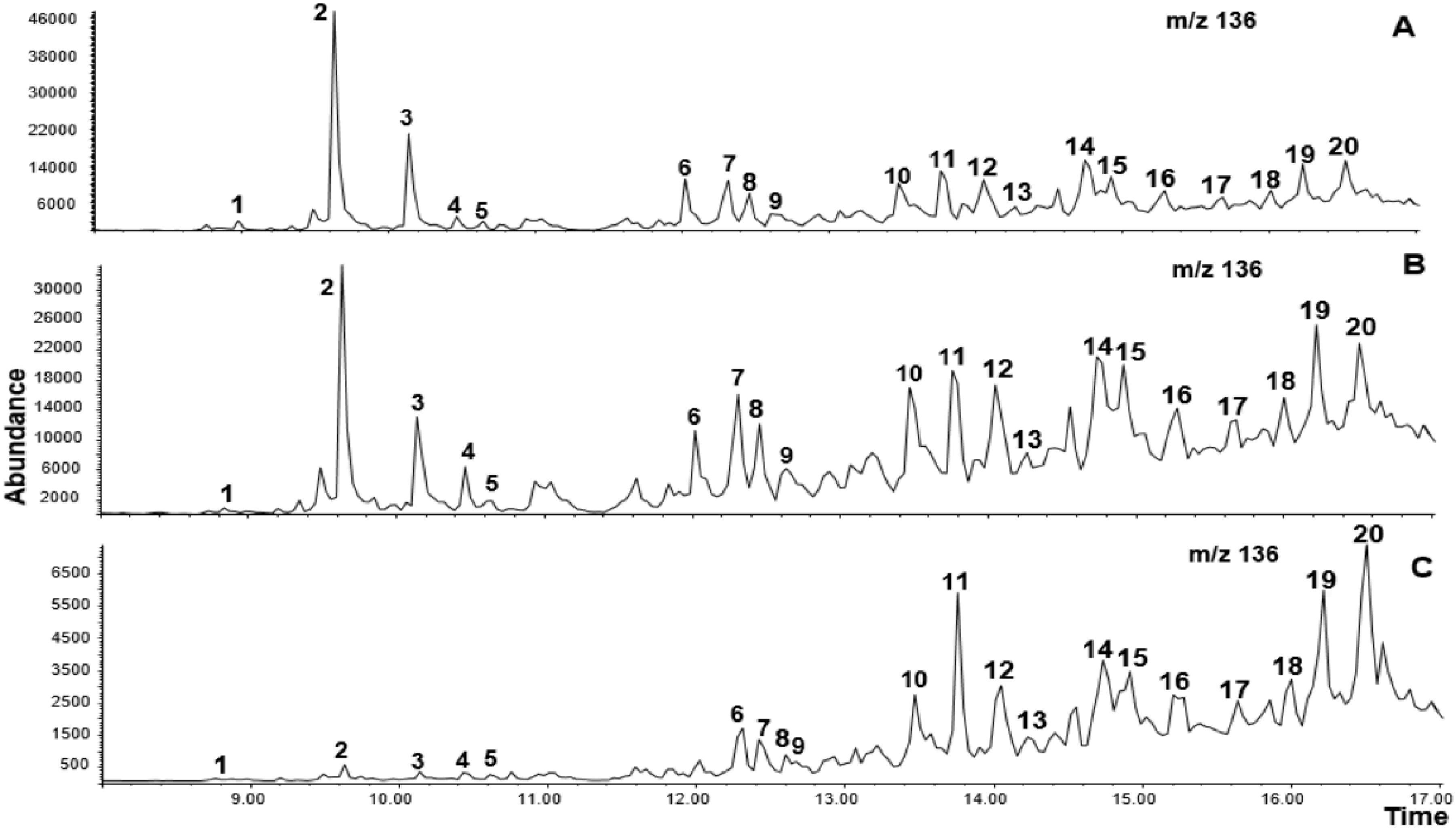


Figure 0.34: m/z 136 mass chromatograms showing the distributions of adamantane in unweathered (A) Nigerian Light; (B) Nigerian Medium; and (C) North Sea oils.

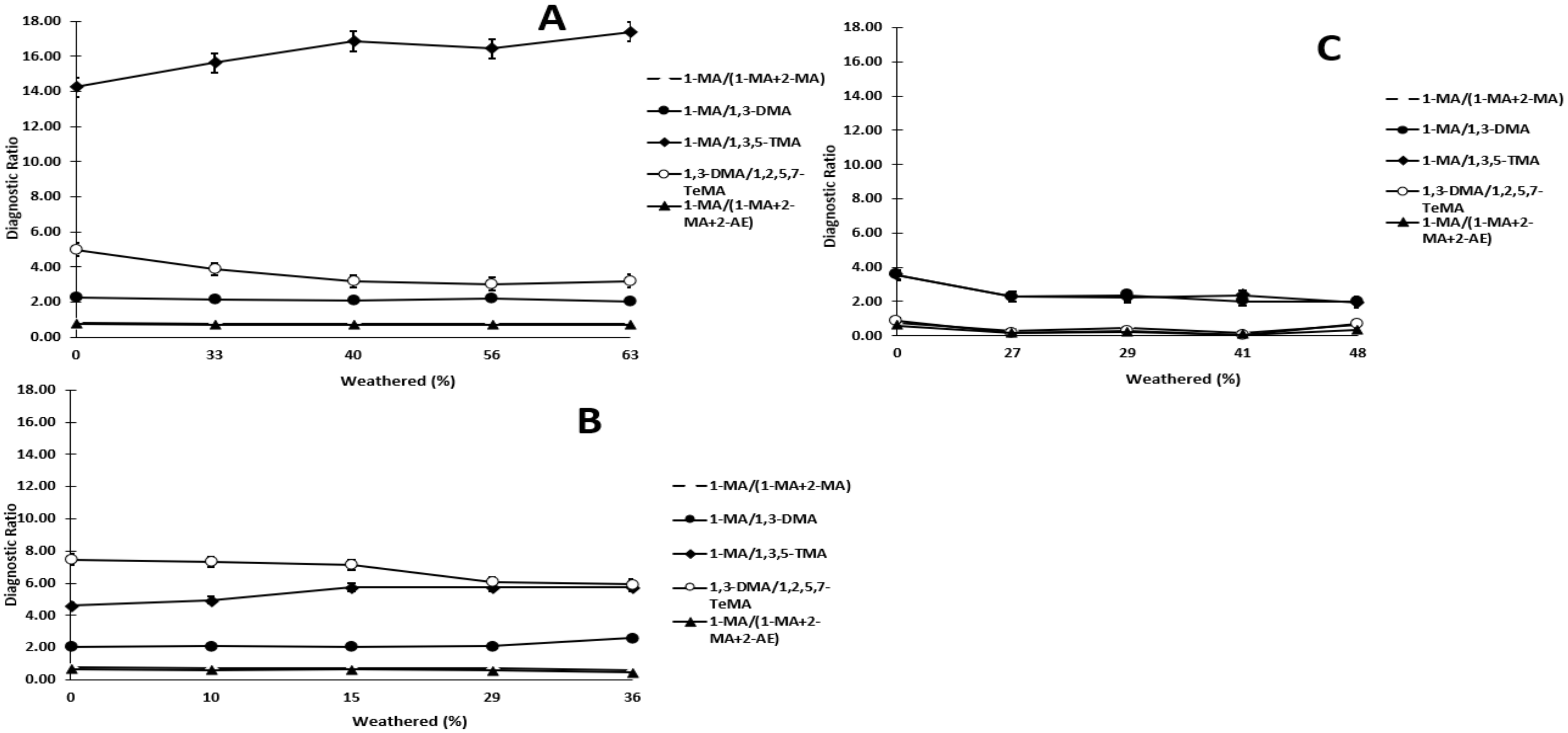


Figure 0.35: Plots of diagnostic ratios of selected adamantanes versus weathered percentage in; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils respectively.

3.4.7 Relative Susceptibility of Selected Molecular Markers

The relative susceptibility of some selected aliphatic and PAH compounds based on their relative elution order was assessed by their ratios shown in **Error! Reference source not found.** in Nigerian Light (A), Nigerian Medium (B), and North Sea (C) oils respectively. The relative susceptibilities of these selected compounds are generally unaffected by evaporative weathering as the plots are observed to be parallel to the x-axis, except for the C_{15} sesquiterpane (BS4) and C_{15} *n*-alkane ratio (BS4/*n*-C_{15}) where the C_{15} sesquiterpane increases relative to *n*-C_{15} and then reduced at 15% evaporation. Table 0.23 shows the description of the selected compounds.

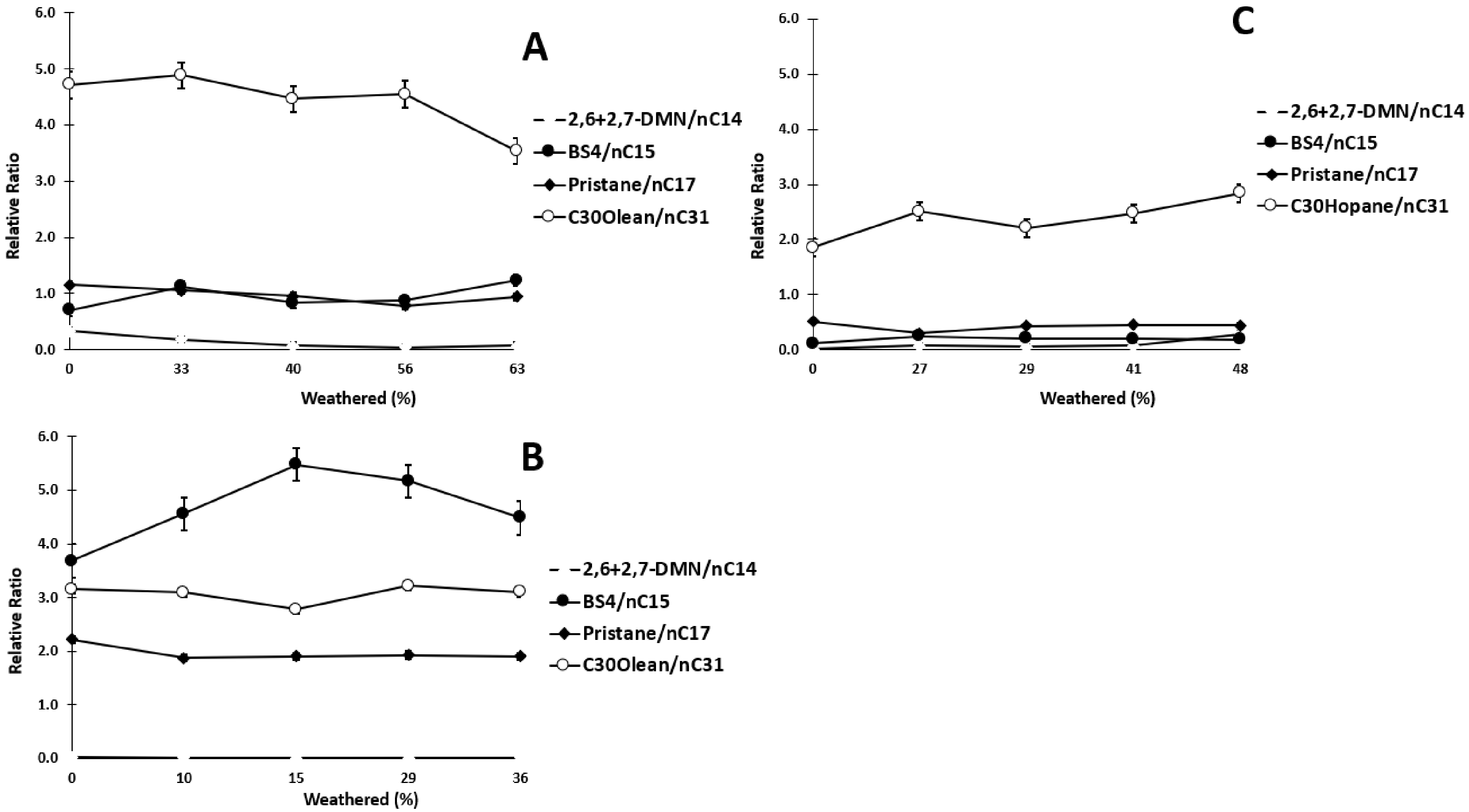


Figure 0.36: Plot of ratios of selected aliphatic/PAH for (A) Nigerian Light, (B) Nigerian Medium, and (C) North Sea oils.

3.4.8 Diagnostic Ratio Plots of Nordtest parameters

Nordtest diagnostic parameters of terpanes, regular steranes and diasteranes, triaromatic steroids and PAHs in the three oils, including the novel tricyclic and tetracyclic terpanes, and novel bicyclic sesquiterpanes identified in the two Nigerian oils were selected and plotted to assess the effect of evaporation for up to 30 days using the “Student’s t” statistical tool. The student’s t distribution is defined by its mean value, $\overline{x}$ as the center of the distribution, and the standard deviation, s signifying the spread of the individual observations around the mean. The variability of the triplicate values of the degraded or weathered oil diagnostic ratios calculated as relative variation at 95% confidence interval or confidence limit (CL) (Daling *et al.*, 2002b). As discussed in section 2.7.4, positive correlation is regarded as match correlation described in the Nordtest methodology whereas the negative correlation is the non-match correlation for the spill and suspected source comparison. Figure 0.37 shows the diagnostic ratio plots of the Nigerian Light oil for evaporation period of 1 day, 3 days, 10 days, and 30 days respectively. The plots of the weathered oil against the fresh oil show a generally positive correlation (i.e., linear regression of the x-y plot) which indicates that these parameters are not affected by this level of evaporative weathering, except for C_{21}TAS and QTAS where variation is observed from 3 days and a slight variation of 4-methyldibenzothiophene/1-methyldibenzothiophene (4-MDBT/1-MDBT) and C_{26} 20R + C_{27} 20S sterane ratios from 10 days. Figure 0.38 shows the diagnostic ratio plots of the Nigerian Medium oil for the same evaporation period. The ratio plots of the molecular markers show a positive match of the fresh oil against the weathered oil, except for the slight variations observed on QTAS and C_{26} 20R + C_{27} 20S sterane ratios at 3 days evaporation only. Figure 0.39 shows the ratio plots for the North Sea weathered oil against the fresh oil for the same evaporation period indicating a positive match or correlation between the weathered and fresh oils except C_{21}TAS and QTAS which are observed to be outliers and thus may not be good for correlation for the Nigerian Light and North Sea oils.

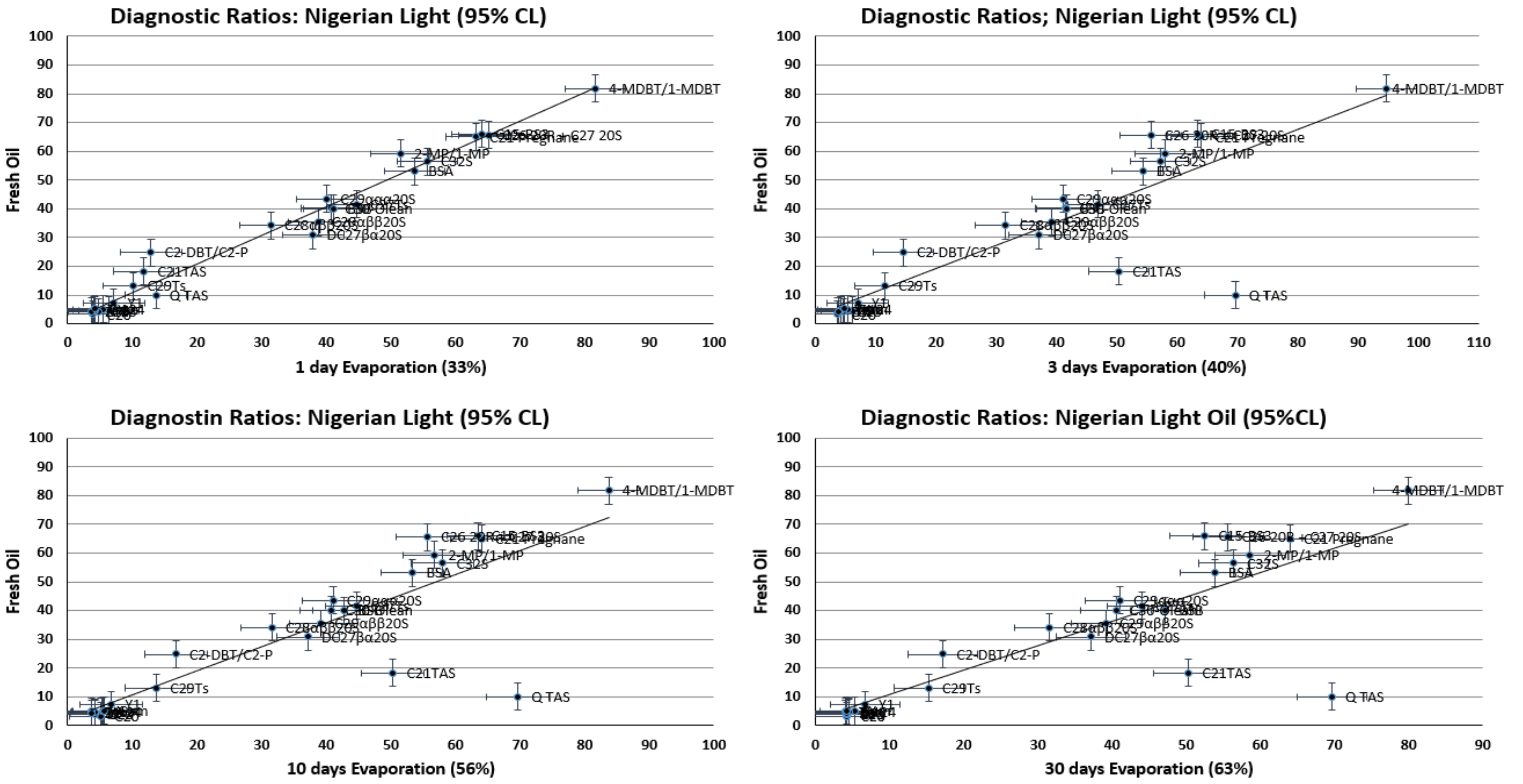


Figure 0.37: Diagnostic Ratio plots of fresh oil vs weathered Nigerian Light oil subjected to evaporation, weathered oil showing positive correlation with the fresh oil up to 30 days using 95% confidence limit.

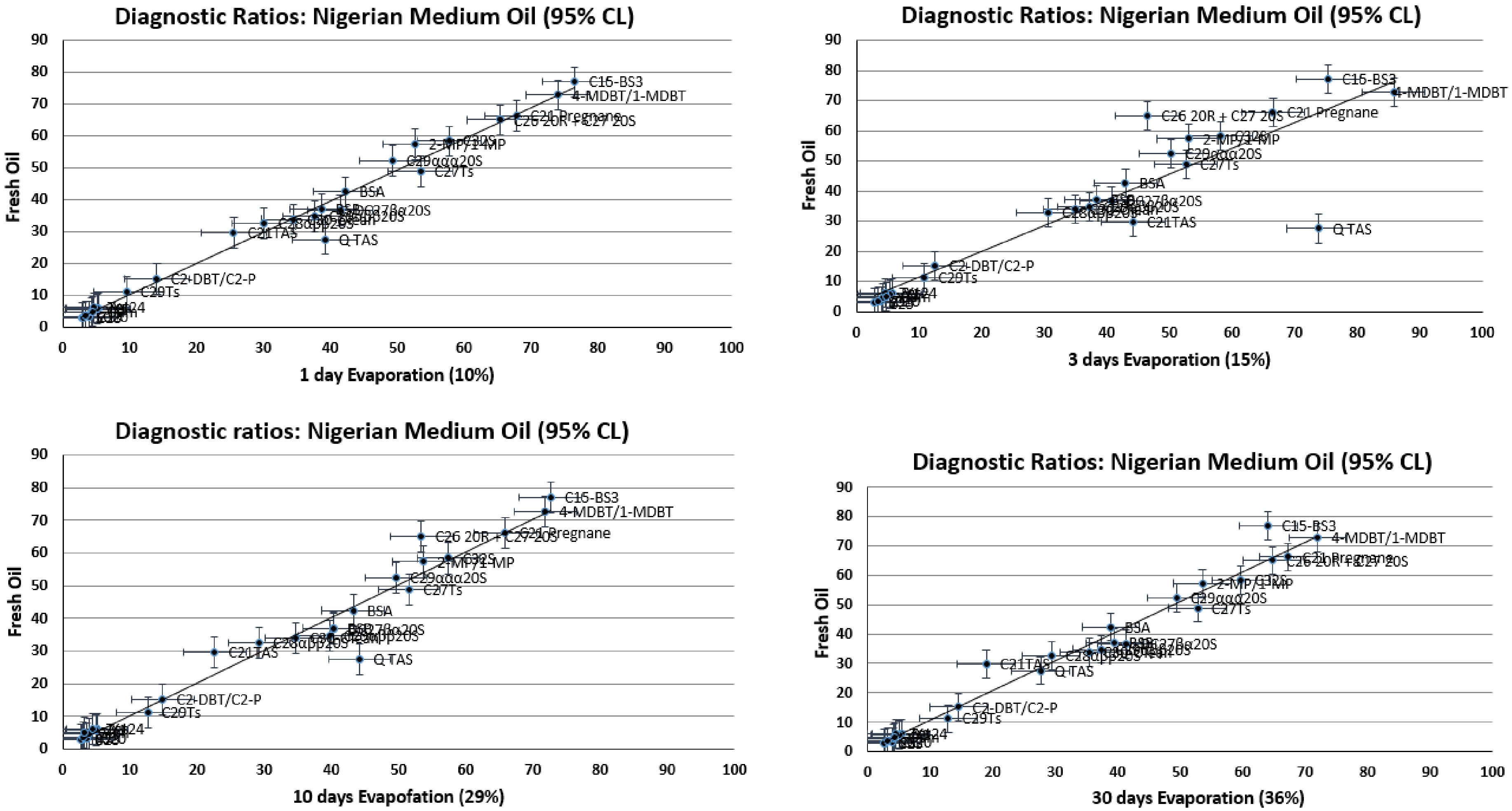


Figure 0.38: Diagnostic Ratio plots of fresh oil vs weathered Nigerian Medium oil subjected to evaporation, weathered oil showing positive correlation with the fresh oil up to 30 days using 95% confidence limit.

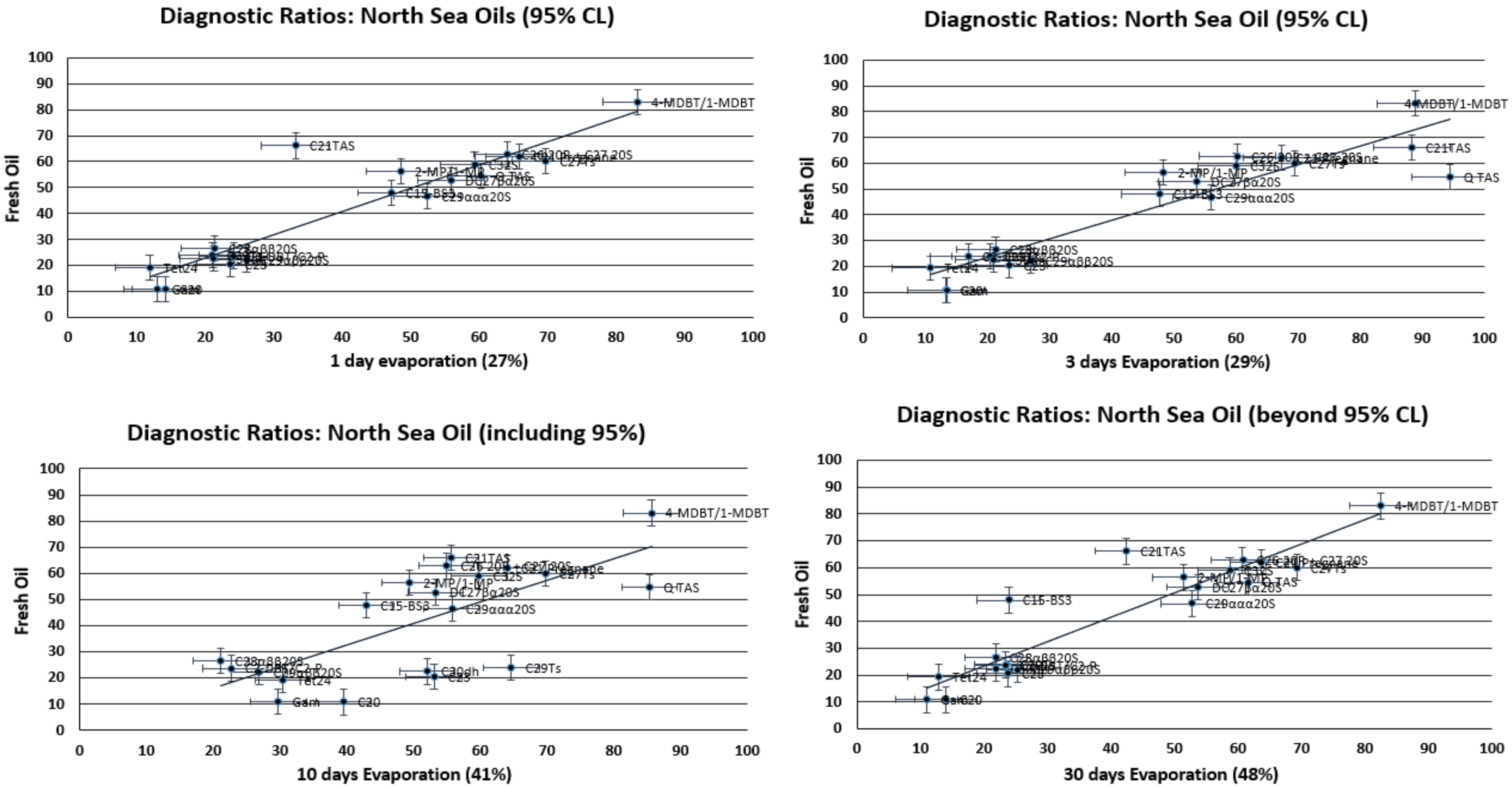


Figure 0.39: Diagnostic Ratio plots of fresh oil vs weathered North Sea oil subjected to evaporation, weathered oil showing positive correlation with the fresh oil up to 30 days using 95% confidence limit.

3.4.9 Conclusions

The low-molecular weight *n*-C_8 to *n*-C_{13} n-alkanes, are completely lost due to evaporative weathering at the 63% oil weight loss stage for the Nigerian Light oil, at 36% for the Nigerian Medium oil, and at 48% for the North Sea oils respectively. There was no evaporative weathering effect on *n*-C_{22} and higher *n*-alkanes for the Nigerian Light, *n*-C_{25} and higher for the Nigerian medium, and *n*-C_{24} and higher for the North Sea oils respectively. The identified suspected novel a,b,c,d,e-pentamethylnaphthalene and v,w,x,y,z-pentamethylnaphthalene have shown a strong potential for being used as molecular markers for correlation and differentiation during oil-source identification. It is generally observed for the three oil samples that the evaporative loss susceptibility order of the aromatic hydrocarbons is naphthalenes > biphenyls > dibenzofurans > dibenzothiophenes > anthracene > phenanthrenes > pyrenes > chrysenes.

The biomarkers are generally unaffected by the evaporative weathering in the order: triterpanes ≤ steranes < bicyclic sesquiterpanes. Apart from the low molecular weight n-alkanes, adamantanes are the most affected of the saturated hydrocarbon geochemical markers measured. The novel tricyclic, tetracyclic terpanes, and bicyclic sesquiterpanes identified in the three oils exhibit strong resistance to weathering, hence, they can be used as diagnostic compounds for oil-source identification depending on the extent of the degradation. The choice of C_{21}TAS, QTAS, and C_{26} 20R + C_{27} 20S sterane as diagnostic ratios based on the observations in section 3.4.8 should be avoided, and the unidentified peak Q may not be a triaromatic sterane.

Table 0.1: Changes in *n*-alkane and acyclic isoprenoid concentrations in weathered Nigerian Light oil (mg/g oil)

***n*-alkane**	**Start (0%)**	**1 day (33%)**	**3 days (40%)**	**10 days (56%)**	**30 days (63%)**
n-C$_{8}$	4.05 (3.2)	1.35 (5.5)	1.03 (2.9)	0.26 (6.2)	0.00 (0!)
n-C$_{9}$	7.31 (1.2)	2.57 (1.5)	1.35 (1.6)	0.37 (2.4)	0.00 (0!)
n-C$_{10}$	8.97 (4.3)	4.62 (2.4)	3.33 (1.4)	0.97 (5.1)	0.00 (0!)
n-C$_{11}$	9.44 (1.8)	5.70 (4.7)	4.44 (0.2)	1.74 (1.9)	0.00 (0!)
n-C$_{12}$	8.77 (0.2)	6.97 (5.0)	5.68 (0.4)	2.47 (2.1)	0.00 (0!)
n-C$_{13}$	7.81 (0.2)	7.59 (0.5)	7.85 (0.4)	4.18 (4.3)	0.00 (0!)
n-C$_{14}$	7.17 (0.5)	7.69 (5.9)	8.35 (2.8)	5.40 (3.4)	0.72 (4.3)
n-C$_{15}$	6.54 (0.2)	7.66 (6.0)	9.17 (4.3)	8.78 (4.7)	3.13 (3.6)
n-C$_{16}$	5.32 (0.4)	6.21 (4.1)	7.33 (6.1)	8.79 (5.5)	9.64 (1.3)
n-C$_{17}$	4.62 (0.1)	6.39 (3.3)	8.33 (6.6)	11.81 (5.4)	9.14 (5.6)
Pristane	5.34 (0.1)	6.76 (3.0)	7.92 (1.0)	9.26 (1.2)	8.61 (1.5)
n-C$_{18}$	4.10 (3.8)	5.11 (4.7)	6.08 (5.5)	7.94 (1.2)	9.55 (0.6)
Phytane	1.75 (2.9)	2.06 (4.2)	2.47 (0.6)	2.95 (4.9)	3.03 (2.0)
n-C$_{19}$	3.55 (2.1)	4.60 (3.8)	5.55 (6.6)	7.37 (2.3)	9.53 (2.4)
n-C$_{20}$	3.26 (2.9)	4.27 (3.2)	5.19 (6.7)	6.76 (2.3)	9.31 (1.8)
n-C$_{21}$	3.27 (2.1)	4.27 (3.2)	5.19 (6.7)	6.39 (1.9)	9.18 (1.7)
n-C$_{22}$	3.46 (1.2)	3.97 (3.2)	4.80 (6.9)	6.35 (0.4)	9.28 (1.2)
n-C$_{23}$	3.12 (3.8)	3.85 (3.1)	4.46 (2.1)	5.90 (3.2)	8.61 (1.2)

***n*-alkane**	**Start (0%)**	**1 day (33%)**	**3 days (40%)**	**10 days (56%)**	**30 days (63%)**
***n*-C_{24}**	2.59 (3.0)	3.52 (2.5)	4.15 (3.8)	5.07 (2.7)	7.65 (0.5)
***n*-C_{25}**	2.81 (0.7)	3.42 (2.0)	4.02 (4.8)	4.80 (1.6)	7.30 (2.8)
***n*-C_{26}**	2.17 (3.1)	2.93 (1.3)	3.45 (3.7)	4.09 (2.4)	6.04 (2.6)
***n*-C_{27}**	2.07 (3.2)	2.79 (1.0)	3.27 (3.4)	3.69 (1.5)	4.97 (5.2)
***n*-C_{28}**	1.71 (1.0)	2.19 (1.9)	2.59 (2.7)	2.94 (4.9)	4.14 (4.8)
***n*-C_{29}**	1.68 (2.9)	1.85 (2.1)	2.33 (2.6)	2.76 (5.3)	4.31 (3.6)
***n*-C_{30}**	1.09 (9.1)	1.29 (0.6)	1.54 (5.1)	1.90 (2.3)	2.93 (4.4)
***n*-C_{31}**	1.24 (1.4)	1.51 (2.7)	1.82 (3.9)	2.07 (3.0)	3.25 (1.4)
***n*-C_{32}**	0.68 (4.7)	0.61 (2.0)	0.76 (5.5)	0.84 (4.9)	1.46 (3.8)
***n*-C_{33}**	0.63 (1.8)	0.33 (3.7)	0.43 (5.1)	0.54 (4.2)	1.23 (6.3)
***n*-C_{34}**	0.24 (4.5)	0.15 (2.6)	0.20 (4.0)	0.21 (2.0)	0.41 (1.3)
***n*-C_{35}**	0.16 (3.2)	0.10 (2.5)	0.14 (2.3)	0.14 (2.3)	0.22 (0.7)
***n*-C_{36}**	0.09 (6.5)	0.05 (4.9)	0.07 (2.5)	0.08 (3.5)	0.14 (4.7)
***n*-C_{37}**	0.06 (3.6)	0.02 (2.9)	0.04 (3.1)	0.04 (6.4)	0.07 (2.1)
***n*-C_{38}**	0.03 (6.6)	0.01 (2.8)	0.02 (3.7)	0.01 (2.7)	0.03 (3.6)
***n*-C_{39}**	0.02 (3.3)	0.01 (5.1)	0.01 (2.9)	0.01 (1.5)	0.02 (6.5)
Sum	115.12	112.44	123.38	126.87	133.93
***n*-C_{17}/pristane**	0.86	0.95	1.05	1.28	1.06
n-C_{18}/phytane	2.35	2.48	2.46	2.69	3.15

n-alkane	Start (0%)	1 day (33%)	3 days (40%)	10 days (56%)	30 days (63%)
pristane/phytane	3.06	3.28	3.21	3.14	2.84
CPI*	1.23	1.16	1.17	1.16	1.18
(C$_8$+C$_{10}$+C$_{12}$+C$_{14}$)/(C$_{22}$+C$_{24}$+C$_{26}$+C$_{28}$)	2.92	1.63	1.23	0.49	0.03

Note: The numbers in brackets after the numbers (concentrations) in the table are relative standard deviations (RSD) (n=3), similarly for Table 0.2 and Table 0.3

Table 0.2: Changes in *n*-alkane and acyclic isoprenoid in the weathered Nigerian Medium oil (mg/g oil)

n-alkane	Start (0%)	1 day (10%)	3 days (15%)	10 days (29%)	30 days (36%)
n-C$_8$	0.44 (4.9)	0.24 (2.6)	0.21 (5.7)	0.00 (0!)	0.00 (0!)
n-C$_9$	0.79 (0.5)	0.27 (4.6)	0.18 (2.9)	0.00 (0!)	0.00 (0!)
n-C$_{10}$	1.00 (4.9)	0.48 (6.3)	0.39 (4.4)	0.00 (0!)	0.00 (0!)
n-C$_{11}$	1.34 (2.7)	1.01 (5.5)	0.67 (0.5)	0.09 (4.5)	0.00 (0!)
n-C$_{12}$	1.70 (1.0)	1.79 (4.5)	1.41 (4.0)	0.58 (3.4)	0.00 (0!)
n-C$_{13}$	1.83 (1.1)	2.30 (3.5)	1.44 (2.8)	0.80 (2.4)	0.00 (0!)
n-C$_{14}$	1.90 (3.2)	2.31 (0.6)	1.65 (2.5)	1.20 (5.5)	0.35 (3.7)
n-C$_{15}$	2.11 (4.3)	2.44 (2.5)	1.91 (0.2)	1.78 (5.2)	1.46 (4.2)
n-C$_{16}$	1.64 (7.5)	2.61 (3.7)	1.98 (1.7)	1.95 (2.9)	2.34 (4.9)
***n*-C17**	2.42 (3.8)	3.20 (0.1)	3.52 (5.5)	3.58 (1.9)	4.52 (3.7)
Pristane	5.36 (5.2)	6.00 (5.3)	6.71 (2.6)	6.92 (2.7)	8.60 (4.5)
n-C$_{18}$	2.63 (0.5)	2.72 (4.0)	3.08 (5.0)	3.40 (5.3)	4.22 (5.4)
Phytane	2.25 (4.6)	2.25 (1.8)	2.53 (4.6)	2.71 (4.0))	3.48 (5.4)

n-alkane	**Start (0%)**	**1 day (10%)**	**3 days (15%)**	**10 days (29%)**	**30 days (36%)**
n-C$_{19}$	2.66 (3.6)	2.71 (4.5)	2.98 (1.9)	3.05 (3.0)	4.11 (1.4)
n-C$_{20}$	2.69 (2.6)	2.88 (5.1)	3.19 (0.6)	3.30 (2.0)	4.41 (1.9)
n-C$_{21}$	2.69 (2.6)	2.88 (5.1)	3.19 (0.6)	3.30 (2.0)	4.41 (2.0)
n-C$_{22}$	2.53 (2.5)	2.70 (4.6)	3.00 (0.8)	3.08 (2.4)	4.22 (0.9)
n-C$_{23}$	2.57 (1.9)	2.79 (6.0)	3.13 (0.5)	3.18 (1.5)	4.35 (1.9)
n-C$_{24}$	2.43 (5.1)	2.78 (5.3)	3.10 (0.4)	3.12 (1.8)	4.39 (1.4)
n-C$_{25}$	2.37 (0.5)	3.08 (2.8)	3.46 (1.7)	3.30 (1.6)	4.49 (0.9)
n-C$_{26}$	2.26 (2.4)	3.01 (0.7)	3.24 (1.8)	3.21 (0.7)	4.31 (1.9)
n-C$_{27}$	2.29 (1.6)	3.06 (3.2)	3.38 (1.6)	3.29 (0.7)	4.41 (2.5)
n-C$_{28}$	2.22 (3.5)	2.69 (3.7)	2.97 (2.3)	2.88 (1.3)	3.92 (5.1)
n-C$_{29}$	2.05 (4.4)	2.76 (4.2)	3.12 (2.9)	2.96 (2.1)	3.93 (3.1)
n-C$_{30}$	1.54 (8.9)	2.13 (4.1)	2.37 (2.6)	2.26 (1.9)	2.98 (2.6)
n-C$_{31}$	1.66 (1.4)	2.38 (3.7)	2.79 (4.6)	2.64 (2.7)	3.53 (3.3)
n-C$_{32}$	0.94 (1.0)	1.32 (4.2)	1.51 (3.4)	1.41 (4.1)	1.84 (1.4)
n-C$_{33}$	0.74 (0.5)	1.03 (3.5)	1.24 (3.8)	1.16 (4.1)	1.65 (1.4)
n-C$_{34}$	0.36 (0.8)	0.52 (4.5)	0.60 (5.8)	0.55 (5.2)	0.73 (2.8)
n-C$_{35}$	0.24 (1.8)	0.36 (5.9)	0.41 (3.2)	0.38 (2.6)	0.54 (3.4)
n-C$_{36}$	0.15 (2.1)	0.24 (1.9)	0.24 (1.0)	0.27 (3.1)	0.35 (1.3)
n-C$_{37}$	0.14 (0.8)	0.18 (4.2)	0.22 (6.3)	0.20 (2.7)	0.25 (2.3)

n-alkane	Start (0%)	1 day (10%)	3 days (15%)	10 days (29%)	30 days (36%)
n-C_{38}	0.13 (0.9)	0.14 (3.1)	0.15 (6.0)	0.14 (0.7)	0.20 (0.2)
n-C_{39}	0.11 (0.4)	0.10 3.5)	0.13 (2.3)	0.11 (0.8)	0.16 (4.5)
Sum	58.16	67.34	70.10	66.79	84.15
n-C_{17}/pristane	0.45	0.53	0.52	0.52	0.53
n-C_{18}/phytane	1.17	1.21	1.22	1.25	1.21
pristane/phytane	2.38	2.66	2.65	2.55	2.47
CPI*	1.11	1.15	1.18	1.17	1.17
(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})	0.53	0.43	0.30	0.14	0.02

Table 0.3: Changes in *n*-alkane and acyclic isoprenoid in the weathered North Sea oil (mg/g oil)

n-alkane	Start (0%)	1 day (27%)	3 days (29%)	10 days (41%)	30 days (48%)
n-C_8	2.96 (5.6)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
n-C_9	3.63 (4.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
n-C_{10}	4.45 (3.1)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
n-C_{11}	4.95 (3.2)	1.35 (1.1)	1.03 (5.8)	0.00 (0!)	0.00 (0!)
n-C_{12}	5.17 (5.3)	3.74 (1.1)	2.67 (4.7)	0.12 (2.3)	0.00 (0!)
n-C_{13}	5.57 (2.9)	4.69 (1.7)	3.73 (5.5)	1.29 (1.6)	0.00 (0!)
n-C_{14}	5.57 (4.5)	6.28 (3.5)	6.29 (1.8)	4.26 (2.1)	0.90 (2.3)
n-C_{15}	5.01 (4.4)	5.90 (5.1)	7.46 (4.7)	6.75 (3.7)	3.36 (2.9)
n-C_{16}	4.13 (1.0)	5.17 (4.6)	6.16 (2.9)	6.45 (4.9)	6.28 (1.7)

***n*-alkane**	**Start (0%)**	**1 day (27%)**	**3 days (29%)**	**10 days (41%)**	**30 days (48%)**
***n*-C17**	3.98 (3.2)	5.08 (5.3)	6.39 (1.1)	6.71 (5.1)	6.44 (3.4)
Pristane	2.02 (3.5)	1.51 (0.6)	2.75 (2.2)	3.03 (1.6)	2.86 (3.5)
***n*-C$_{18}$**	3.37 (1.7)	3.89 (4.6)	4.83 (3.9)	5.03 (4.7)	4.91 (2.7)
Phytane	1.58 (4.9)	2.01 (0.2)	2.19 (5.0)	2.32 (3.7)	2.33 (2.9)
***n*-C$_{19}$**	2.93 (5.1)	3.29 (5.4)	4.06 (2.2)	4.09 (1.4)	4.31 (2.9)
***n*-C$_{20}$**	2.53 (5.5)	2.71 (4.9)	3.25 (1.4)	3.38 (2.4)	3.46 (2.8)
***n*-C$_{21}$**	2.38 (3.4)	2.59 (5.2)	3.25 (1.4)	3.36 (1.7)	3.46 (4.6)
***n*-C$_{22}$**	2.24 (5.1)	2.31 (0.9)	2.95 (1.0)	3.08 (1.0)	3.20 (3.1)
***n*-C$_{23}$**	1.99 (5.2)	2.14 (3.5)	2.70 (0.9)	2.84 (0.9)	2.93 (3.4)
***n*-C$_{24}$**	1.82 (5.5)	2.09 (3.2)	2.58 (2.0)	2.75 (4.5)	2.73 (5.2)
***n*-C$_{25}$**	1.51 (5.6)	1.69 (0.9)	2.20 (1.9)	2.18 (4.0)	2.30 (3.5)
***n*-C$_{26}$**	1.30 (5.4)	1.54 (0.7)	1.98 (2.1)	1.95 (3.6)	2.08 (4.8)
***n*-C$_{27}$**	1.00 (4.8)	1.13 (4.6)	1.46 (1.6)	1.41 (5.6)	1.45 (3.0)
***n*-C$_{28}$**	0.68 (1.9)	0.94 (2.0)	1.14 (2.8)	1.14 (5.2)	1.12 (4.6)
***n*-C$_{29}$**	0.52 (0.9)	0.54 (1.5)	0.60 (3.9)	0.79 (4.8)	0.70 (4.2)
***n*-C$_{30}$**	0.36 (2.3)	0.43 (2.4)	0.55 (4.0)	0.50 (3.0)	0.54 (2.6)
***n*-C$_{31}$**	0.18 (3.8)	0.29 (5.6)	0.36 (4.5)	0.33 (4.4)	0.34 (4.2)
***n*-C$_{32}$**	0.10 (4.1)	0.15 (4.2)	0.19 (3.0)	0.17 (5.0)	0.18 (3.2)
***n*-C$_{33}$**	0.09 (2.0)	0.03 (2.0)	0.05 (5.3)	0.04 (5.0)	0.04 (4.2)

n-alkane	Start (0%)	1 day (27%)	3 days (29%)	10 days (41%)	30 days (48%)
n-C_{34}	0.10 (4.2)	0.25 (1.6)	0.29 (2.9)	0.27 (2.3)	0.293.1)
n-C_{35}	0.05 (5.6)	0.06 (2.5)	0.08 (3.1)	0.07 (2.6)	0.08 (5.0)
n-C_{36}	0.05 (5.4)	0.07 (4.5)	0.09 (3.9)	0.10 (4.4)	0.09 (3.1)
n-C_{37}	0.04 (0.6)	0.04 (4.0)	0.07 (3.8)	0.06 (5.6)	0.06 (4.0)
n-C_{38}	0.03 (0.8)	0.05 (5.5)	0.07 (2.5)	0.05(0.7)	0.07 (4.7)
n-C_{39}	0.03 (1.5)	0.03 (0.0)	0.03 (0.0)	0.03 (2.3)	0.04 (1.2)
Sum	72.32	61.99	71.45	64.56	56.55
n-C_{17}/pristane	1.98	3.36	2.32	2.21	2.25
n-C_{18}/phytane	2.14	1.94	2.20	2.16	2.11
pristane/phytane	1.28	0.75	1.26	1.30	1.23
CPI*	1.04	0.91	0.93	0.95	0.94
$(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})$	3.00	1.46	1.04	0.49	0.10

Table 0.4: Determined (left side) and converted (right side) of target PAH concentrations in weathered Nigerian light oil (µg/g oil)

PAH	Determined Concentration (C_{det})					Converted Concentration (C_{conv})				
	0%	33%	40%	56%	63%	0%	33%	40%	56%	63%
C_0-N	247.3	223.9	180.3	97.0	0.9	247.3	150.0	108.2	42.7	0.3
C_1-N	1314.8	377.8	321.7	275.9	152.0	1314.8	253.1	193.0	121.4	56.2
C_2-N	4680.0	3844.0	1669.5	528.2	296.9	4680.0	2575.5	1001.7	232.4	109.8
C_3-N	1412.8	3552.9	3654.7	2260.8	1688.4	1412.8	2380.4	2192.8	994.7	624.7

C_4-N	828.0	1535.2	1830.9	1693.9	1363.7	828.0	1028.6	1098.5	745.3	504.6
∑Naphthalenes	**8483.0**	**9533.8**	**7657.1**	**4855.8**	**3501.9**	**8483.0**	**6387.7**	**4594.2**	**2136.5**	**1295.7**
C_0-B	344.5	200.6	122.7	32.5	2.5	344.5	134.4	73.6	14.3	0.9
C_1-B	968.4	526.4	331.2	250.6	210.1	968.4	352.7	198.7	110.3	77.8
C_2-B	1005.4	589.3	490.2	480.6	454.0	1005.4	394.9	294.1	211.5	168.0
∑Biphenyls	**2318.3**	**1316.3**	**944.2**	**763.7**	**666.7**	**2318.3**	**881.9**	**566.5**	**336.0**	**246.7**
C_0-DBF	135.3	80.4	60.1	29.1	31.1	135.3	53.8	36.1	12.8	11.5
C_1-DBF	167.9	306.9	376.4	287.6	201.2	167.9	205.6	225.8	126.5	74.4
C_2-DBF	515.9	997.0	1061.9	1068.0	1040.0	515.9	668.0	637.1	469.9	384.8
C_3-DBF	309.8	631.7	719.7	747.1	819.4	309.8	423.3	431.8	328.7	303.2
∑Dibenzofurans	**1128.9**	**2015.9**	**2218.1**	**2131.8**	**2091.6**	**1128.9**	**1350.7**	**1330.8**	**938.0**	**773.9**
C_0-F	52.3	52.3	54.1	96.3	146.8	52.3	35.1	32.4	42.4	54.3
C_1-F	259.0	278.6	284.3	322.8	510.0	259.0	186.6	170.6	142.0	188.7
C_2-F	246.5	377.9	433.3	450.3	596.9	246.5	253.2	260.0	198.1	220.9
∑Fluorenes	**1686.7**	**2724.7**	**2989.8**	**3001.1**	**3345.3**	**1686.7**	**1825.6**	**1793.9**	**1320.5**	**1237.7**
C_0-DBT	18.9	23.2	24.5	27.2	30.3	18.9	15.5	14.7	12.0	11.2
C_1-DBT	80.2	89.3	101.1	139.1	157.3	80.2	59.9	60.7	61.2	58.2
C_2-DBT	69.4	87.7	99.7	121.4	148.8	69.4	58.8	59.8	53.4	55.0
C_3-DBT	40.6	53.0	55.8	77.2	89.2	40.6	35.5	33.5	34.0	33.0
∑Dibenzothiophenes	**209.1**	**253.2**	**281.1**	**364.9**	**425.6**	**209.1**	**169.7**	**168.7**	**160.5**	**157.5**

C_0-P	242.6	343.5	353.5	406.1	474.0	242.6	230.1	212.1	178.7	175.4
C_1-P	441.8	498.6	521.6	649.0	759.8	441.8	334.0	312.9	285.5	281.1
C_2-P	514.0	604.9	660.9	860.5	1000.1	514.0	405.3	396.6	378.6	370.0
C_3-P	287.7	351.6	388.3	445.8	517.5	287.7	235.6	233.0	196.2	191.5
C_4-P	87.3	143.6	158.6	173.3	200.9	87.3	96.2	95.2	76.2	74.3
∑Phenanthrenes	**1573.4**	**1942.2**	**2082.9**	**2534.7**	**2952.3**	**1573.4**	**1301.2**	**1249.7**	**1115.3**	**1092.4**
C_0-Py	9.3	10.5	11.7	13.4	15.4	9.3	7.0	7.0	5.9	5.7
C_1-Py	58.6	69.2	73.9	91.4	102.9	58.6	46.4	44.3	40.2	38.1
C_2-Py	52.2	56.8	61.1	75.5	88.3	52.2	38.0	36.7	33.2	32.7
∑Pyrenes	**120.1**	**136.5**	**146.8**	**180.3**	**206.6**	**120.1**	**91.4**	**88.1**	**79.3**	**76.5**
C_0-C	5.3	6.2	6.4	8.7	8.7	5.3	4.2	3.9	3.8	3.2
C_1-C	15.8	16.4	17.5	18.0	24.2	15.8	11.0	10.5	7.9	8.9
C_2-C	17.9	18.7	19.4	21.2	25.8	17.9	12.5	11.7	9.3	9.5
∑Chrysene	**39.1**	**41.4**	**43.4**	**47.9**	**58.6**	**39.1**	**27.7**	**26.0**	**21.1**	**21.7**
C_0-B(a)A	2.0	2.2	2.4	2.8	2.8	2.0	1.5	1.4	1.2	1.0
C_1-B(a)A	2.4	2.6	2.8	3.1	3.2	2.4	1.7	1.7	1.3	1.2
∑Benzo a anthracene	**4.4**	**4.8**	**5.2**	**5.9**	**6.0**	**4.4**	**3.2**	**3.1**	**2.6**	**2.2**
C_{20}-TAS	4.3	6.1	7.5	8.0	9.3	4.3	4.1	4.5	3.5	3.4
C_{21}-TAS	2.0	2.7	2.6	2.9	4.5	2.0	1.8	1.6	1.3	1.7
$C_{26}R+C_{27}S$	27.7	29.9	31.2	37.6	45.8	27.7	20.0	18.7	16.6	16.9

C_{28}S-TAS	23.3	25.7	28.0	32.4	39.0	23.3	17.2	16.8	14.2	14.4
C_{27}R-TAS	14.6	16.1	18.1	20.7	23.9	14.6	10.8	10.9	9.1	8.8
C_{28}R-TAS	19.7	20.5	22.3	25.3	31.4	19.7	13.7	13.4	11.1	11.6
∑TAS	**91.6**	**100.9**	**109.7**	**126.9**	**153.9**	**91.6**	**67.6**	**65.8**	**55.9**	**56.9**
Anthracene	11.1	15.6	16.4	17.9	19.8	11.1	10.5	9.8	7.9	7.3
Perylene	4.9	5.4	6.0	6.8	7.6	4.9	3.6	3.6	3.0	2.8
2-MN/1-MN	2.9	3.0	2.5	1.4	1.7					
C_0-P/C_0-Anth	21.9	22.0	21.5	22.7	24.0					
	0%	**33%**	**40%**	**56%**	**63%**					
4-MDBT	1.00	1.00	1.00	1.00	1.00					
a,b,c,d,e-PMN	0.43	0.45	0.46	0.49	0.44					
v,w,x,y,z-PMN	1.10	1.14	1.08	1.08	1.08					

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, TAS, a,b,c,d,e-PMN, v,w,x,y,z-PMN represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, triaromatic steranes (TAS), two pentamethylnaphthalenes identified in Nigerian Light oil

Table 0.5: Determined (left side) and converted (right side) of target PAH concentrations in weathered Nigerian medium oil (µg/g oil)

PAH	**Determined Concentration (C_{det})**					**Converted Concentration (C_{conv})**				
	0%	**10%**	**15%**	**29%**	**36%**	**0%**	**10%**	**15%**	**29%**	**36%**
C_0-N	225.3	181.8	135.7	92.1	0.3	225.3	163.6	115.3	65.4	0.2
C_1-N	926.3	398.7	313.5	273.0	221.2	926.3	358.8	266.4	193.8	141.5
C_2-N	1733.9	1589.3	1540.3	1584.6	1467.9	1733.9	1430.4	1309.3	1125.0	939.5
C_3-N	1032.4	1132.5	1145.8	1225.8	1360.9	1032.4	1019.2	973.9	870.3	871.0

C_4-N	781.7	629.7	563.0	460.9	444.2	781.7	566.7	478.6	327.2	284.3
∑Naphthalenes	**4699.5**	**3931.8**	**3698.3**	**3636.3**	**3494.5**	**4699.5**	**3538.6**	**3143.6**	**2581.8**	**2236.5**
C_0-B	174.2	125.8	85.3	58.2	25.2	174.2	113.2	72.5	41.3	16.1
C_1-B	239.0	188.3	166.6	133.2	44.3	239.0	169.5	141.6	94.6	28.3
C_2-B	321.8	285.9	277.6	254.7	249.9	321.8	257.3	235.9	180.9	159.9
∑Biphenyls	**734.9**	**600.0**	**529.5**	**446.1**	**319.4**	**734.9**	**540.0**	**450.1**	**316.8**	**204.4**
C_0-DBF	103.6	105.2	75.5	39.8	32.7	103.6	94.7	64.2	28.3	21.0
C_1-DBF	220.8	227.9	250.6	217.5	90.8	220.8	205.1	213.0	154.4	58.1
C_2-DBF	538.8	580.8	629.2	627.4	622.5	538.8	522.7	534.8	445.4	398.4
C_3-DBF	257.8	348.2	375.9	424.0	585.2	257.8	313.4	319.5	301.0	374.5
∑Dibenzofurans	**1120.9**	**1262.0**	**1331.2**	**1308.7**	**1331.2**	**1120.9**	**1135.8**	**1131.5**	**929.2**	**852.0**
C_0-F	71.8	104.0	106.7	111.7	115.1	71.8	93.6	90.7	79.3	73.6
C_1-F	250.8	252.3	277.7	282.2	492.0	250.8	227.0	236.1	200.3	314.9
C_2-F	354.2	399.4	407.1	467.6	549.2	354.2	359.5	346.1	332.0	351.5
∑Fluorenes	**1797.6**	**2017.8**	**2122.7**	**2170.1**	**2487.4**	**1797.6**	**1816.0**	**1804.3**	**1540.8**	**1592.0**
C_0-DBT	22.1	31.1	32.2	35.3	40.2	22.1	28.0	27.4	25.1	25.7
C_1-DBT	103.2	117.5	126.9	152.5	176.7	103.2	105.7	107.8	108.3	113.1
C_2-DBT	104.4	110.5	117.2	146.6	170.7	104.4	99.5	99.6	104.1	109.3
C_3-DBT	72.5	73.1	77.5	95.2	115.7	72.5	65.8	65.9	67.6	74.0
∑Dibenzothiophenes	**302.2**	**332.2**	**353.8**	**429.6**	**503.3**	**302.2**	**299.0**	**300.7**	**305.0**	**322.1**

C_0-P	297.3	322.5	377.9	401.1	483.1	297.3	290.3	321.2	284.8	309.2
C_1-P	619.2	539.9	609.8	707.3	819.2	619.2	485.9	518.3	502.2	524.3
C_2-P	712.9	726.8	838.6	967.1	1010.6	712.9	654.1	712.8	686.6	646.8
C_3-P	321.9	356.8	398.3	432.1	523.9	321.9	321.1	338.6	306.8	335.3
C_4-P	193.8	194.3	198.7	228.1	283.7	193.8	174.9	168.9	162.0	181.6
∑Phenanthrenes	**2145.2**	**2140.4**	**2423.3**	**2735.7**	**3120.6**	**2145.2**	**1926.3**	**2059.8**	**1942.3**	**1997.2**
C_0-Py	19.9	20.0	23.3	25.1	29.8	19.9	18.0	19.8	17.8	19.1
C_1-Py	120.7	131.0	145.5	156.5	184.0	120.7	117.9	123.6	111.1	117.8
C_2-Py	101.0	111.8	129.3	137.4	146.5	101.0	100.6	109.9	97.5	93.7
∑Pyrenes	**241.7**	**262.7**	**298.0**	**319.0**	**360.3**	**241.7**	**236.5**	**253.3**	**226.5**	**230.6**
C_0-C	13.8	14.1	14.9	15.8	19.0	13.8	12.7	12.6	11.2	12.2
C_1-C	37.1	39.0	44.5	49.6	54.9	37.1	35.1	37.8	35.2	35.1
C_2-C	38.9	42.7	47.5	53.9	59.1	38.9	38.4	40.3	38.2	37.8
∑Chrysene	**89.8**	**95.8**	**106.8**	**119.3**	**133.0**	**89.8**	**86.2**	**90.8**	**84.7**	**85.1**
C_0-B(a)A	4.2	4.3	4.3	5.0	5.1	4.2	3.8	3.7	3.6	3.3
C_1-B(a)A	4.5	4.5	5.3	6.9	7.5	4.5	4.1	4.5	4.9	4.8
∑Benzo a anthracene	**8.7**	**8.8**	**9.6**	**11.9**	**12.6**	**8.7**	**7.9**	**8.2**	**8.5**	**8.1**
C_{20}-TAS	6.1	6.6	6.6	7.8	9.9	6.1	6.0	5.6	5.5	6.4
C_{21}-TAS	2.9	3.0	3.3	3.6	4.4	2.9	2.7	2.8	2.5	2.8
C_{26}R+C_{27}S	22.0	23.0	23.7	24.4	25.7	22.0	20.7	20.1	17.3	16.5

C_{28}S-TAS	20.8	19.1	21.7	22.5	24.3	20.8	17.2	18.5	16.0	15.6
C_{27}R-TAS	11.7	12.0	12.6	13.4	14.3	11.7	10.8	10.7	9.5	9.2
C_{28}R-TAS	17.7	16.3	18.8	18.9	19.7	17.7	14.7	16.0	13.4	12.6
∑TAS	**81.2**	**80.0**	**86.8**	**90.5**	**98.5**	**81.2**	**72.0**	**73.8**	**64.3**	**63.0**
Anthracene	9.6	10.9	11.3	12.1	13.7	9.6	9.8	9.6	8.6	8.8
Perylene	2.6	3.0	3.5	4.5	5.4	2.6	2.7	3.0	3.2	3.5
2-MN/1-MN	1.54	1.35	1.68	1.60	1.61					
C_0-P/C_0-Anth	31.0	29.5	33.5	33.2	35.2					
	0%	**10%**	**15%**	**29%**	**36%**					
4-MDBT	1.00	1.00	1.00	1.00	1.00					
a,b,c,d,e-PMN	0.51	0.53	0.54	0.55	0.54					
v,w,x,y,z-PMN	1.39	1.36	1.37	1.47	1.40					

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, TAS, a,b,c,d,e-PMN, v,w,x,y,z-PMN represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, triaromatic steranes, two pentamethylnaphthalenes identified in Nigerian Medium oil

Table 0.6: Determined (left side) and converted (right side) target PAH concentrations in weathered North Sea oil (µg/g oil)

PAH	**Determined Concentration (C_{det})**					**Converted Concentration (C_{conv})**				
	0%	**27%**	**29%**	**41%**	**48%**	**0%**	**27%**	**29%**	**41%**	**48%**
C_0-N	581.4	511.6	471.6	300.1	0.0	581.4	373.5	334.8	177.1	0.0
C_1-N	799.1	695.7	611.7	576.3	512.1	799.1	507.9	434.3	340.0	266.3
C_2-N	1136.8	1233.5	1442.2	1361.2	1276.7	1136.8	900.4	1024.0	803.1	663.9
C_3-N	857.8	833.5	1779.0	1829.5	1933.1	857.8	608.5	1263.1	1079.4	1005.2

C_4-N	470.6	727.9	738.4	316.4	278.4	470.6	531.4	524.3	186.7	144.8
∑Naphthalenes	**3845.7**	**4002.3**	**5042.9**	**4383.4**	**4000.3**	**3845.7**	**2921.6**	**3580.5**	**2586.2**	**2080.2**
C_0-B	413.6	402.7	321.4	275.3	223.1	413.6	294.0	228.2	162.5	116.0
C_1-B	572.8	658.4	649.8	525.0	441.9	572.8	480.6	461.4	309.7	229.8
C_2-B	318.8	424.2	342.5	339.5	238.3	318.8	309.7	243.2	200.3	123.9
∑Biphenyls	**1305.3**	**1485.3**	**1313.8**	**1139.9**	**903.4**	**1305.3**	**1084.2**	**932.8**	**672.5**	**469.7**
C_0-DBF	63.3	87.1	71.9	63.6	39.1	63.3	63.6	51.0	37.5	20.3
C_1-DBF	194.3	215.3	208.9	221.2	188.2	194.3	157.2	148.3	130.5	97.8
C_2-DBF	360.3	644.8	676.8	506.0	436.4	360.3	470.7	480.6	298.5	226.9
C_3-DBF	171.9	211.0	240.3	297.0	310.2	171.9	154.0	170.6	175.3	161.3
∑Dibenzofurans	**789.8**	**1158.3**	**1197.9**	**1087.8**	**973.8**	**789.8**	**845.5**	**850.5**	**641.8**	**506.4**
C_0-F	78.4	185.4	274.4	274.4	302.4	78.4	135.3	194.8	161.9	157.2
C_1-F	426.0	538.1	568.0	599.3	637.8	426.0	392.8	403.3	353.6	331.6
C_2-F	398.0	564.4	569.9	636.1	672.8	398.0	412.0	404.6	375.3	349.8
∑Fluorenes	**1692.2**	**2446.1**	**2610.1**	**2597.6**	**2586.8**	**1692.2**	**1785.7**	**1853.2**	**1532.6**	**1345.1**
C_0-DBT	73.7	98.1	103.3	136.2	139.2	73.7	71.6	73.3	80.4	72.4
C_1-DBT	112.1	249.8	311.2	387.7	454.8	112.1	182.3	221.0	228.8	236.5
C_2-DBT	293.7	403.5	422.7	506.3	594.0	293.7	294.5	300.1	298.7	308.9
C_3-DBT	163.4	190.1	232.2	250.4	295.2	163.4	138.8	164.8	147.7	153.5
∑Dibenzothiophenes	**642.9**	**941.4**	**1069.4**	**1280.6**	**1483.2**	**642.9**	**687.3**	**759.3**	**755.5**	**771.3**

C_0-P	500.6	713.4	742.3	821.4	947.6	500.6	520.8	527.0	484.6	492.8
C_1-P	769.6	1190.8	1348.6	1559.1	1728.6	769.6	869.3	957.5	919.9	898.9
C_2-P	943.6	1345.2	1505.1	1729.1	1945.0	943.6	982.0	1068.6	1020.2	1011.4
C_3-P	623.4	930.9	1142.4	1342.6	1378.8	623.4	679.6	811.1	792.1	717.0
C_4-P	165.4	206.8	212.9	245.9	270.7	165.4	151.0	151.2	145.1	140.8
∑Phenanthrenes	**3002.6**	**4387.1**	**4951.3**	**5698.1**	**6270.6**	**3002.6**	**3202.6**	**3515.4**	**3361.9**	**3260.7**
C_0-Py	55.2	60.0	63.3	71.5	81.5	55.2	43.8	44.9	42.2	42.4
C_1-Py	224.2	269.9	254.6	281.6	307.1	224.2	197.1	180.8	166.2	159.7
C_2-Py	181.2	225.3	199.6	207.1	202.7	181.2	164.5	141.7	122.2	105.4
∑Pyrenes	**460.6**	**555.2**	**517.4**	**560.2**	**591.3**	**460.6**	**405.3**	**367.4**	**330.5**	**307.5**
C_0-C	96.8	98.4	105.9	110.1	124.8	96.8	71.8	75.2	65.0	64.9
C_1-C	164.1	180.5	185.6	191.3	216.0	164.1	131.8	131.8	112.9	112.3
C_2-C	123.5	142.9	143.8	145.9	162.0	123.5	104.3	102.1	86.1	84.2
∑Chrysene	**384.5**	**421.8**	**435.3**	**447.4**	**502.9**	**384.5**	**307.9**	**309.1**	**264.0**	**261.5**
C_0-B(a)A	13.2	13.2	13.5	15.3	17.9	13.2	9.6	9.6	9.0	9.3
C_1-B(a)A	10.4	10.7	11.7	12.2	13.8	10.4	7.8	8.3	7.2	7.2
∑Benzo a anthracene	**23.6**	**23.9**	**25.1**	**27.4**	**31.7**	**23.6**	**17.4**	**17.8**	**16.2**	**16.5**
C_{20}-TAS	12.9	17.2	17.0	18.0	20.4	12.9	12.5	12.0	10.6	10.6
C_{21}-TAS	2.5	3.5	6.2	6.6	8.2	2.5	2.6	4.4	3.9	4.3
C_{26}R+C_{27}S	11.5	11.5	12.5	12.6	13.2	11.5	8.4	8.9	7.4	6.9

C_{28}S-TAS	7.6	7.6	7.9	8.2	8.8	7.6	5.6	5.6	4.8	4.6
C_{27}R-TAS	6.9	6.4	1.3	4.9	8.6	6.9	4.7	0.9	2.9	4.5
C_{28}R-TAS	6.6	6.8	7.0	8.3	10.4	6.6	5.0	5.0	4.9	5.4
∑TAS	**48.0**	**53.1**	**51.9**	**58.6**	**69.6**	**48.0**	**38.7**	**36.8**	**34.6**	**36.2**
Anthracene	16.5	23.1	24.3	24.9	28.2	16.5	16.9	17.3	14.7	14.7
Perylene	13.4	16.5	16.6	18.4	20.0	13.4	12.0	11.8	10.8	10.4
2-MN/1-MN	1.34	1.57	2.46	1.64	3.02					
C_0-P/C_0-Anth	39.08	40.74	43.94	51.37	52.59					

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, TAS represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, triaromatic steranes.

Table 0.7: Change in concentrations (µg/g oil) of pentamethylnaphthalenes with corresponding percentage compositions for Nigerian Light and Medium oils.

	Nigerian Light oil						Nigerian Medium oil				
PAH	**0%**	**33%**	**40%**	**56%**	**63%**	**PAH**	**0%**	**10%**	**15%**	**29%**	**36%**
4-MDBT	22.66	34.99	38.86	39.19	39.19	**4-MDBT**	28.00	30.61	31.31	34.78	41.49
a,b,c,d,e-PMN	9.67	15.60	18.32	19.07	17.07	**a,b,c,d,e-PMN**	14.41	16.28	17.02	19.03	22.49
3+2-MDBT	17.81	27.87	14.47	31.03	31.03	**3+2-MDBT**	25.64	27.52	18.44	33.97	38.48
v,w,x,y,z-PMN	25.02	39.75	41.78	42.23	42.23	**v,w,x,y,z-PMN**	38.94	41.75	42.94	51.17	58.13
1-MDBT	5.05	7.88	2.19	7.59	7.59	**1-MDBT**	10.48	10.71	5.16	13.54	16.15
∑MDBT	**80.20**	**126.10**	**115.63**	**139.11**	**137.11**	**∑MDBT**	**117.47**	**126.88**	**114.88**	**152.48**	**176.74**

				Percentage composition (%)							
4-MDBT	28.3	**27.75**	33.61	28.17	28.58	**4-MDBT**	23.84	24.13	27.26	22.81	33.61
a,b,c,d,e-PMN	12.1	12.37	15.85	13.71	12.45	**a,b,c,d,e-PMN**	12.27	12.83	14.82	12.48	15.85
3+2-MDBT	22.2	22.11	12.51	22.31	22.63	**3+2-MDBT**	21.82	21.69	16.05	22.28	12.51
v,w,x,y,z-PMN	31.2	31.52	36.14	30.36	30.80	**v,w,x,y,z-PMN**	33.15	32.91	37.38	33.56	36.14
1-MDBT	6.3	6.25	1.90	5.46	5.54	**1-MDBT**	8.92	8.44	4.49	8.88	1.90

Table 0.8 Measured concentrations of terpanes in weathered Nigerian Light oil (µg/g oil)

	Terpanes	**Determined Concentration (C_{det})**					**Converted Concentration (Cconv)**				
Peak Number	**Tricyclic Terpanes**	**0%**	**33%**	**40%**	**56%**	**63%**	**0%**	**33%**	**40%**	**56%**	**63%**
1	**S**	429.5	727.9	741.4	751.6	790.9	429.5	487.7	444.8	330.7	292.6
2	**Q**	114.0	216.6	234.6	330.9	532.2	114.0	145.1	140.7	145.6	196.9
3	**C_{19}**	330.0	483.1	521.9	573.5	683.1	330.0	323.7	313.1	252.3	252.7
4	**M**	118.5	220.2	226.8	284.2	413.5	118.5	147.6	136.1	125.0	153.0
5	**C_{20}**	295.9	435.2	444.1	738.0	749.6	295.9	291.6	266.5	324.7	277.3
6	**X**	462.1	630.4	661.9	788.8	944.2	462.1	422.4	397.1	347.1	349.4
7	**C_{21}**	284.0	438.5	449.9	455.0	704.8	284.0	293.8	270.0	200.2	260.8
8	**C_{22}**	172.7	201.0	250.8	285.4	333.7	172.7	134.7	150.5	125.6	123.5
9	**C_{23}**	368.4	536.9	563.6	604.9	727.9	368.4	359.7	338.1	266.2	269.3

10	**C_{24}**	391.0	481.8	583.7	488.1	709.9	391.0	322.8	350.2	214.7	262.6
11	**Y**	415.3	465.4	505.2	596.8	788.7	415.3	311.8	303.1	262.6	291.8
12	**Y1**	684.0	823.8	872.1	988.7	1206.5	684.0	552.0	523.3	435.0	446.4
13	**C_{25}A**	105.0	195.8	205.0	213.5	274.6	105.0	131.2	123.0	93.9	101.6
14	**C_{25}B**	392.2	454.8	511.5	639.6	739.9	392.2	304.7	306.9	281.4	273.8
15	**Z**	184.3	202.9	213.5	259.4	285.2	184.3	135.9	128.1	114.1	105.5
16	**Tet24**	446.4	452.5	510.1	514.2	707.8	446.4	303.2	306.1	226.3	261.9
17	**C_{26}(S)**	56.2	131.8	206.4	254.5	299.1	56.2	88.3	123.8	112.0	110.7
18	**C_{26}(R)**	181.6	226.4	228.0	258.3	311.0	181.6	151.7	136.8	113.6	115.1

		Determined Concentration (C_{det})					**Converted Concentration (C_{conv})**				
	Tetracyclic Terpanes	**0%**	**33%**	**40%**	**56%**	**63%**	**0%**	**33%**	**40%**	**56%**	**63%**
19	**Z1**	387.1	411.6	447.2	514.6	728.8	387.1	275.7	268.3	226.4	269.6
20	**C_{28}A**	274.9	448.6	477.7	543.9	657.4	274.9	300.5	286.6	239.3	243.2
21	**C_{28}B**	135.1	223.4	251.3	284.1	392.9	135.1	149.7	150.8	125.0	145.4
22	**C_{29}A**	198.0	271.5	304.1	354.5	410.8	198.0	181.9	182.5	156.0	152.0
23	**C_{29}B**	276.4	279.5	290.7	334.6	420.3	276.4	187.3	174.4	147.2	155.5
24	**T**	203.7	219.6	238.5	278.3	366.2	203.7	147.1	143.1	122.4	135.5

Determined Concentration (C_{det}) **Converted Concentration (Cconv)**

	Pentacyclic Terpanes	**0%**	**33%**	**40%**	**56%**	**63%**	**0%**	**33%**	**40%**	**56%**	**63%**
25	**C_{27}Ts**	977.8	1280.8	1449.9	1585.7	1915.8	977.8	858.1	869.9	697.7	708.9
26	**C_{27}Tm**	1374.9	1584.9	1637.3	1955.9	2436.1	1374.9	1061.9	982.4	860.6	901.3
27	**C_{29}nor25H**	1022.8	950.4	1067.5	1308.8	1849.2	1022.8	636.7	640.5	575.9	684.2
28	**C_{29}Tm**	6236.0	7679.2	8521.0	9606.9	11621.4	6236.0	5145.1	5112.6	4227.0	4299.9
29	**C_{29}Ts**	1317.8	1215.2	1494.3	2173.3	3032.3	1317.8	814.2	896.6	956.2	1121.9
30	**C_{30}: 18α-Oleanane**	5867.9	7387.5	8142.9	9424.8	11484.8	5867.9	4949.6	4885.8	4146.9	4249.4
31	**$C_{30}\alpha\beta$**	8748.1	10725.4	11471.2	13657.2	16823.2	8748.1	7186.0	6882.7	6009.2	6224.6
32	**C_{30}-Mor**	1561.4	1934.9	2055.4	2468.9	2979.6	1561.4	1296.4	1233.2	1086.3	1102.4
33	**C_{31}S**	1760.3	2273.8	2471.0	2795.9	3212.6	1760.3	1523.4	1482.6	1230.2	1188.6
34	**C_{31}R**	1569.7	1728.0	1852.0	2114.1	2547.1	1569.7	1157.8	1111.2	930.2	942.4
35	**Gam**	472.6	465.5	568.1	745.4	817.1	472.6	311.9	340.9	328.0	302.3
36	**C_{32}S**	1067.8	1308.1	1399.0	1651.7	1856.0	1067.8	876.4	839.4	726.7	686.7
37	**C_{32}R**	825.0	1045.4	1041.9	1195.9	1437.1	825.0	700.4	625.2	526.2	531.7
38	**C_{33}S**	670.4	911.6	960.1	1148.4	1313.0	670.4	610.8	576.1	505.3	485.8
39	**C_{33}R**	645.4	734.7	715.6	831.5	892.0	645.4	492.2	429.4	365.8	330.0
40	**C_{34}S**	382.2	523.2	479.9	628.4	748.9	382.2	350.6	288.0	276.5	277.1
41	**C_{34}R**	309.9	343.5	309.9	425.1	497.9	309.9	230.1	186.0	187.1	184.2
42	**C_{35}S**	229.3	238.9	248.2	337.6	264.1	229.3	160.1	148.9	148.6	97.7

43	**C_{35}R**	232.1	246.6	264.7	308.5	350.3	232.1	165.2	158.8	135.8	129.6
	Total (µg/g oil)	**42177.6**	**51756.7**	**56089.8**	**65699.3**	**80257.5**	**42177.6**	**34677.0**	**33653.9**	**28907.7**	**29695.3**

NB: S, Q, M, X, Y, Y1, Z, Z1 and T are peaks noted

Table 0.9. Measured concentrations of terpanes in weathered Nigerian Medium oil (µg/g oil)

	Terpanes	**Determined Concentration (C_{det})**					**Converted Concentration (C_{conv})**				
Peak Number	**Tricyclic Terpanes**	**0%**	**10%**	**15%**	**29%**	**36%**	**0%**	**10%**	**15%**	**29%**	**36%**
1	**S**	303.3	409.6	430.1	465.1	585.0	303.3	367.0	363.9	328.8	372.6
2	**Q**	120.6	196.2	214.5	243.7	286.2	120.6	175.8	181.5	172.3	182.3
3	**C_{19}**	379.6	528.9	582.0	608.4	794.3	379.6	473.9	492.3	430.1	505.9
4	**M**	124.2	240.7	271.0	316.5	379.7	124.2	215.7	229.2	223.8	241.9
5	**C_{20}**	344.3	580.8	588.5	605.5	833.2	344.3	520.4	497.9	428.1	530.8
6	**X**	651.5	798.5	862.6	869.7	1133.5	651.5	715.4	729.8	614.9	722.0
7	**C_{21}**	226.8	364.1	372.6	273.5	404.7	226.8	326.2	315.2	193.4	257.8
8	**C_{22}**	280.1	336.5	390.5	395.2	487.3	280.1	301.5	330.4	279.4	310.4
9	**C_{23}**	294.5	436.7	441.6	446.6	543.8	294.5	391.3	373.6	315.7	346.4
10	**C_{24}**	304.8	387.1	419.7	467.9	552.7	304.8	346.9	355.1	330.8	352.1
11	**Y**	498.2	635.9	635.7	645.1	848.3	498.2	569.8	537.8	456.1	540.4
12	**Y1**	617.7	765.2	809.7	846.4	1079.9	617.7	685.6	685.0	598.4	687.9

13	**$C_{25}A$**	93.3	121.5	139.1	146.1	172.2	93.3	108.8	117.7	103.3	109.7
14	**$C_{25}B$**	252.7	335.6	351.3	392.9	433.6	252.7	300.7	297.2	277.8	276.2
15	**Z**	85.1	104.1	107.8	115.0	119.4	85.1	93.3	91.2	81.3	76.1
16	**Tet24**	669.0	680.7	697.1	741.3	937.7	669.0	609.9	589.7	524.1	597.3
17	**C_{26}(S)**	57.6	100.0	118.5	138.9	230.3	57.6	89.6	100.3	98.2	146.7
18	**C_{26}(R)**	128.8	160.4	187.0	200.2	266.9	128.8	143.8	158.2	141.5	170.0

		Determined Concentration (C_{det})					**Converted Concentration (C_{conv})**				
	Tetracyclic Terpanes	**0%**	**10%**	**15%**	**29%**	**36%**	**0%**	**10%**	**15%**	**29%**	**36%**
19	**Z1**	366.3	495.8	505.3	518.6	660.1	366.3	444.2	427.5	366.7	420.5
20	**$C_{28}A$**	229.6	484.9	522.2	547.0	692.0	229.6	434.5	441.8	386.8	440.8
21	**$C_{28}B$**	163.5	164.8	168.4	190.8	265.9	163.5	147.7	142.5	134.9	169.4
22	**$C_{29}A$**	132.6	241.6	258.7	275.5	393.1	132.6	216.5	218.9	194.8	250.4
23	**$C_{29}B$**	315.8	450.1	454.1	510.7	635.8	315.8	403.3	384.2	361.1	405.0
24	**T**	121.6	132.1	147.2	175.6	209.5	121.6	118.4	124.5	124.2	133.4

		Determined Concentration (C_{det})					**Converted Concentration (C_{conv})**				
	Pentacyclic Terpanes	**0%**	**10%**	**15%**	**29%**	**36%**	**0%**	**10%**	**15%**	**29%**	**36%**
25	**C_{27}Ts**	1791.7	2573.5	2603.1	2861.3	3781.7	1791.7	2305.8	2202.2	2023.0	2409.0

26	**C_{27}Tm**	1875.8	2236.9	2345.0	2686.7	3375.6	1875.8	2004.2	1983.9	1899.5	2150.2
27	**C_{29}-nor25h**	945.9	1218.6	1358.0	1762.2	1996.5	945.9	1091.8	1148.8	1245.9	1271.8
28	**C_{29}Tm**	7037.1	9461.2	9833.4	11064.3	13868.3	7037.1	8477.3	8319.1	7822.4	8834.1
29	**C_{29}Ts**	1288.1	1477.8	1747.5	2320.8	2944.9	1288.1	1324.1	1478.4	1640.8	1875.9
30	**C_{30}-Olean**	5254.3	7350.7	7746.9	8505.6	10932.5	5254.3	6586.2	6553.9	6013.5	6964.0
31	**C_{30}**	10284.7	13975.5	14381.6	15978.4	19892.8	10284.7	12522.0	12166.8	11296.7	12671.7
32	**C_{30}-Mor**	1553.5	2052.9	2179.1	2425.8	3123.1	1553.5	1839.4	1843.5	1715.0	1989.4
33	**C_{31}S**	2135.8	2720.1	3080.7	3540.7	4206.2	2135.8	2437.2	2606.3	2503.3	2679.3
34	**C_{31}R**	1678.7	2116.6	2383.6	2650.3	3312.8	1678.7	1896.5	2016.6	1873.8	2110.2
35	**Gam**	526.6	662.7	710.7	535.3	895.7	526.6	593.8	601.3	378.5	570.6
36	**C_{32}S**	1392.2	1717.8	1829.3	2030.5	2615.3	1392.2	1539.2	1547.6	1435.5	1666.0
37	**C_{32}R**	992.7	1256.0	1313.9	1503.4	1768.3	992.7	1125.4	1111.6	1062.9	1126.4
38	**C_{33}S**	688.0	889.8	983.5	1128.4	1436.1	688.0	797.2	832.0	797.8	914.8
39	**C_{33}R**	505.3	636.5	648.4	796.3	1067.6	505.3	570.3	548.5	563.0	680.1
40	**C_{34}S**	360.2	518.8	640.4	580.7	829.8	360.2	464.9	541.7	410.6	528.6
41	**C_{34}R**	300.1	282.1	352.7	415.9	486.2	300.1	252.8	298.4	294.1	309.7
42	**C_{35}S**	224.8	260.1	335.7	406.8	497.3	224.8	233.1	284.0	287.6	316.8
43	**C_{35}R**	213.1	218.3	266.5	364.7	443.5	213.1	195.6	225.5	257.8	282.5
	Total (µg/g oil)	**45810.1**	**60778.1**	**64415.5**	**71694.2**	**90419.4**	**45810.1**	**54457.1**	**54495.5**	**50687.8**	**57597.2**

NB: S, Q, M, X, Y, Y1, Z, Z1 and T are peaks noted

Table 0.10: Measured concentrations of terpanes in weathered North Sea oil (µg/g oil)

	Terpanes	**Determined Concentration (C_{det})**					**Converted Concentration (C_{conv})**				
Peak Number	**Tricyclic Terpanes**	**0%**	**27%**	**29%**	**41%**	**48%**	**0%**	**27%**	**29%**	**41%**	**48%**
1	**C_{19}**	55.8	119.9	130.2	135.2	147.4	55.8	87.7	92.6	79.4	76.9
2	**C_{20}**	40.8	120.4	124.5	148.4	158.8	40.8	88.0	88.5	87.1	82.9
3	**C_{21}**	81.3	199.1	202.2	218.1	243.7	81.3	145.5	143.8	128.1	127.2
4	**C_{22}**	34.3	76.6	87.2	90.1	111.8	34.3	56.0	62.0	52.9	58.4
5	**U**	29.3	60.5	62.3	68.0	78.0	29.3	44.2	44.3	39.9	40.7
6	**C_{23}**	86.6	225.7	248.6	256.4	304.7	86.6	165.0	176.8	150.5	159.1
7	**C_{24}**	83.3	179.1	187.9	196.7	221.2	83.3	130.9	133.6	115.5	115.5
8	**C_{25}B**	89.1	182.2	202.0	220.3	237.2	89.1	133.2	143.6	129.3	123.8
9	**Tet24**	80.5	97.9	98.1	99.2	144.3	80.5	71.6	69.7	58.2	75.3
10	**C_{26}(S)**	37.3	50.4	55.4	57.0	64.7	37.3	36.9	39.4	33.5	33.8
11	**C_{26}(R)**	54.5	66.3	79.6	83.3	89.5	54.5	48.4	56.6	48.9	46.7
		Determined Concentration (C_{det})					**Converted Concentration (C_{conv})**				
	Tetracyclic Terpanes	**0%**	**27%**	**29%**	**41%**	**48%**	**0%**	**27%**	**29%**	**41%**	**48%**
12	**U1**	41.0	66.4	88.8	91.3	123.7	41.0	48.6	63.2	53.6	64.6
13	**U2**	23.7	38.1	40.7	44.6	62.9	23.7	27.8	28.9	26.2	32.8

14	**C_{28}A**	34.4	89.3	114.5	123.7	135.1	34.4	65.3	81.4	72.6	70.5
15	**C_{28}B**	32.5	74.7	86.6	95.7	99.4	32.5	54.6	61.6	56.2	51.9
16	**C_{29}A**	46.4	108.5	111.5	119.9	140.3	46.4	79.3	79.2	70.4	73.3
17	**C_{29}B**	38.7	85.7	89.7	102.6	104.3	38.7	62.7	63.8	60.2	54.5
18	**T**	48.8	109.9	114.1	119.6	146.1	48.8	80.3	81.1	70.2	76.2

		Determined Concentration (C_{det})					**Converted Concentration (C_{conv})**				
	Pentacyclic Terpanes	**0%**	**27%**	**29%**	**41%**	**48%**	**0%**	**27%**	**29%**	**41%**	**48%**
19	**C_{27}Ts**	149.8	305.4	313.3	331.4	414.2	149.8	223.2	222.8	194.5	216.2
20	**C27Tm**	99.7	132.5	137.5	143.8	183.8	99.7	96.9	97.7	84.4	95.9
21	**C_{29}nor25H**	30.5	125.5	133.5	139.2	162.6	30.5	91.7	94.9	81.7	84.9
22	**C_{29}Tm**	145.1	332.8	409.3	414.9	424.0	145.1	243.3	291.0	243.5	221.3
23	**C_{29}Ts**	105.6	194.2	206.8	246.4	299.9	105.6	141.9	147.0	144.6	156.5
24	**C_{30}dh**	97.8	196.1	214.2	226.6	274.9	97.8	143.3	152.3	133.0	143.5
25	**C_{30}-Hopane**	337.0	724.9	806.8	826.8	977.3	337.0	529.9	573.6	485.3	510.2
26	**C_{30}-Mor**	58.9	121.1	127.2	137.0	175.2	58.9	88.5	90.4	80.4	91.5
27	**C_{31}S**	177.0	343.7	365.2	385.3	446.0	177.0	251.3	259.7	226.2	232.8
28	**C_{31}R**	128.8	255.0	269.5	283.7	333.7	128.8	186.4	191.6	166.5	174.2
29	**Gam**	40.9	96.3	109.8	125.1	120.5	40.9	70.4	78.1	73.4	62.9

30	**C_{32}S**	155.9	283.7	294.0	315.0	364.0	155.9	207.4	209.0	184.9	190.0
31	**C_{32}R**	108.8	194.4	195.5	211.4	256.1	108.8	142.1	139.0	124.1	133.7
32	**C_{33}S**	112.2	198.3	218.8	232.3	269.3	112.2	145.0	155.6	136.4	140.6
33	**C_{33}R**	78.3	142.3	158.8	170.6	188.7	78.3	104.0	112.9	100.1	98.5
34	**C_{34}S**	68.4	171.3	195.8	206.9	218.9	68.4	125.2	139.2	121.5	114.3
35	**C_{34}R**	42.8	97.0	104.0	118.3	128.3	42.8	70.9	73.9	69.5	67.0
36	**C_{35}S**	80.1	157.0	184.9	213.9	226.8	80.1	114.7	131.4	125.5	118.4
37	**C_{35}R**	48.6	106.4	120.8	134.0	148.8	48.6	77.8	85.9	78.7	77.7
	Total (µg/g oil)	**3004.9**	**6128.5**	**6689.6**	**7132.8**	**8226.1**	**3004.9**	**4479.9**	**4756.3**	**4187.0**	**4294.0**

NB: U, U1, U2 and T are peaks noted.

Table 0.11: Measured concentrations of steranes (m/z 217) in weathered Nigerian Light oil (µg/g oil)

			Determined Concentration (Cdet) (M/Z 217)			
Peak Number	**Steranes**	**0%**	**33%**	**40%**	**56%**	**63%**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	101.8	158.9	174.3	186.2	242.0
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	189.2	298.9	315.2	478.7	502.1
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	101.9	174.2	177.0	208.5	261.8
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	205.5	325.9	351.4	411.4	521.9
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	132.1	201.4	209.4	257.4	337.4
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	83.6	104.4	106.0	126.3	160.0

7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	94.9	124.8	137.9	175.9	239.5
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	186.0	203.2	239.3	275.4	346.6
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	370.4	442.7	482.0	549.6	708.3
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	468.5	535.1	585.5	743.4	892.6
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	289.6	326.9	363.2	429.4	529.3
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	630.7	790.6	831.6	1151.4	1186.1
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	468.0	511.5	602.2	689.5	891.0
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	221.2	191.3	192.8	261.2	325.1
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	455.5	497.8	531.8	642.3	826.1
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	494.1	498.0	567.3	708.8	838.7
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	355.7	410.1	456.6	548.0	679.8
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	282.9	304.6	314.7	364.1	477.4
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	604.1	698.7	775.1	905.1	1135.4
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	688.2	839.0	961.4	1135.0	1383.9
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	581.3	726.6	862.9	973.7	1229.7
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	298.7	394.8	406.1	500.2	602.3
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	894.8	1254.4	1380.3	1538.5	2062.8
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	142.4	120.9	149.3	175.4	196.6
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	65.0	68.9	73.8	108.5	123.7
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	170.8	157.3	171.5	230.1	272.7
	Total	**8576.9**	**10361.1**	**11418.9**	**13774.1**	**16973.0**

Table 0.12: Measured concentrations of steranes (m/z 217) in weathered Nigerian Medium oil (μg/g oil)

			Determined Concentration (Cdet) -(M/Z 217)			
Peak Number	**Steranes**	**0%**	**10%**	**15%**	**29%**	**36%**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	91.7	132.5	148.9	155.7	176.6
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	151.1	249.6	243.3	251.2	357.3
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	77.2	118.3	121.7	130.0	173.3
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	173.2	244.6	250.4	260.5	340.1
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	95.3	144.4	148.3	163.8	237.3
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	45.3	58.6	62.9	66.8	80.5
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	66.9	93.6	102.2	110.8	141.8
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	120.2	160.3	163.2	176.3	233.3
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	171.9	181.7	190.4	199.8	231.0
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	308.9	357.4	380.1	413.0	550.1
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	154.6	190.1	199.6	213.5	267.5
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	210.9	275.7	327.5	411.6	433.5
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	257.7	336.5	342.1	357.8	481.7
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	199.6	289.5	301.9	314.3	294.8
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	238.3	255.3	269.3	284.4	339.1
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	270.9	283.4	293.1	318.0	410.2
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	192.8	230.9	260.9	271.8	313.3

18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	147.1	128.7	133.2	142.4	195.9
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	205.3	244.6	259.2	301.2	372.5
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	470.7	622.1	675.2	742.7	918.6
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	321.9	424.8	451.8	569.6	621.4
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	172.7	219.6	222.0	231.9	299.6
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	285.1	639.0	666.4	752.9	939.6
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	108.1	124.0	157.6	163.6	171.6
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	36.1	49.8	54.0	61.1	82.8
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	109.1	110.6	113.3	139.0	156.8
	Total	**4682.3**	**6165.4**	**6538.7**	**7203.5**	**8820.2**

Table 0.13: Measured concentrations of steranes (m/z 217) in weathered North Sea oil (µg/g oil)

		Determined Concentration (Cdet) (M/Z 217)				
Peak Number	**Steranes**	**0%**	**27%**	**29%**	**41%**	**48%**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	126.0	284.9	331.0	367.4	443.0
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	128.9	336.0	395.9	376.6	505.4
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	78.9	174.0	191.9	211.4	287.6
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	309.2	653.1	692.1	726.4	882.7
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	219.9	404.4	433.9	452.4	573.0
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	101.8	205.3	210.5	231.8	268.4
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	103.9	203.6	202.7	217.2	266.4

8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	166.2	292.3	293.2	299.6	354.5
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	109.5	135.5	146.7	157.9	226.3
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	289.1	530.1	598.5	625.6	759.7
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	184.8	303.0	371.2	405.8	494.5
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	113.1	198.6	204.5	220.7	266.0
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	228.4	447.8	499.6	519.9	609.1
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	145.9	254.2	277.5	297.4	347.7
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	86.5	127.9	190.3	196.8	206.4
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	102.6	114.8	121.2	128.3	172.1
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	143.1	223.9	278.2	292.4	347.3
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	105.4	107.1	172.0	183.1	200.2
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	78.3	132.0	158.5	175.3	190.4
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	90.1	205.3	274.9	282.0	286.0
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	124.7	258.6	283.2	308.8	369.3
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	79.5	153.6	223.0	226.3	233.2
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	103.1	186.0	216.1	223.7	256.5
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	73.7	176.1	223.8	231.2	256.2
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	10.4	24.9	26.2	30.5	36.5
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	23.6	35.4	38.5	50.3	55.4
	Total	**3326.6**	**6168.3**	**7055.1**	**7438.8**	**8893.8**

Table 0.14: Measured concentrations of triaromatic steranes (m/z 231) in weathered Nigerian Light oil (µg/g oil)

Peak Number	Triaromatic Steranes (TAS)	Determined Concentration (C_{det}) -(M/Z 231)					Converted Concentration (C_{conv})				
		0%	**33%**	**40%**	**56%**	**63%**	**0%**	**33%**	**40%**	**56%**	**63%**
1	**C_{20}**	116.7	693.4	1204.4	2231.2	2966.8	116.7	464.6	722.6	981.7	1097.7
2	**Q**	55.9	613.9	646.7	1180.9	1405.4	55.9	411.3	388.0	519.6	520.0
3	**C_{21}-TAS**	92.4	417.3	919.9	1433.6	2238.3	92.4	279.6	552.0	630.8	828.2
4	**P**	42.8	475.1	431.3	852.0	1179.5	42.8	318.3	258.8	374.9	436.4
5	**C_{22}A-TAS**	25.8	132.5	192.3	310.4	658.4	25.8	88.8	115.4	136.6	243.6
6	**C_{22}B-TAS**	30.5	117.3	127.9	470.7	667.9	30.5	78.6	76.7	207.1	247.1
7	**R**	24.5	38.6	192.8	164.4	508.8	24.5	25.8	115.7	72.3	188.2
8	**S**	15.3	43.3	133.3	163.9	331.5	15.3	29.0	80.0	72.1	122.6
9	**T**	40.4	44.6	382.2	262.3	866.8	40.4	29.9	229.3	115.4	320.7
10	**C_{26}(20S) TA**	206.7	135.4	1552.6	966.8	4194.0	206.7	90.7	931.5	425.4	1551.8
11	**C_{26} (20R) + C_{27}(20S) TA**	596.3	495.6	4702.8	3315.2	11644.7	596.3	332.0	2821.7	1458.7	4308.5
12	**U**	44.0	27.6	466.7	264.4	966.5	44.0	18.5	280.0	116.4	357.6
13	**C_{28} (20S) TA**	503.0	267.5	4093.7	2094.6	9937.4	503.0	179.2	2456.2	921.6	3676.8
14	**C_{27}(20R) TA**	313.0	395.5	2520.7	3052.4	6079.4	313.0	265.0	1512.4	1343.1	2249.4
15	**C_{28} (20R) TA**	413.4	468.2	3227.9	3791.6	8002.1	413.4	313.7	1936.8	1668.3	2960.8
16	**V**	85.4	660.7	711.4	1203.6	1664.0	85.4	442.7	426.8	529.6	615.7

	Total	**2606.1**	**5026.3**	**21506.5**	**21758.2**	**53311.4**	**2606.1**	**3367.6**	**12903.9**	**9573.6**	**19725.2**

Table 0.15: Measured concentrations of triaromatic steranes (m/z 231) in weathered Nigerian Medium oil (µg/g oil)

		Determined Concentration (C_{det}) (M/Z 231)					**Converted Concentration (C_{conv})**				
Peak Number	**Triaromatic Steranes (TAS)**	**0%**	**10%**	**15%**	**29%**	**36%**	**0%**	**10%**	**15%**	**29%**	**36%**
1	**C_{20}**	193.4	450.8	532.0	1334.7	2997.9	193.4	405.7	452.2	947.7	1918.6
2	**Q**	131.6	475.2	463.2	1001.5	2095.3	131.6	427.7	393.7	711.1	1341.0
3	**C_{21}-TAS**	125.2	332.2	127.9	1004.0	2150.9	125.2	299.0	108.7	712.9	1376.6
4	**P**	59.4	192.2	213.5	429.3	985.7	59.4	172.9	181.4	304.8	630.9
5	**C_{22}A-TAS**	45.5	102.4	116.3	233.6	439.0	45.5	92.1	98.9	165.8	280.9
6	**C_{22}B-TAS**	21.0	81.3	76.3	150.4	391.9	21.0	73.2	64.8	106.8	250.8
7	**R**	13.9	28.1	18.1	93.1	258.9	13.9	25.3	15.4	66.1	165.7
8	**S**	11.7	33.9	16.7	65.8	185.3	11.7	30.5	14.2	46.7	118.6
9	**T**	32.5	59.0	33.9	125.4	377.4	32.5	53.1	28.8	89.1	241.6
10	**C_{26}(20S) TA**	132.7	251.0	73.1	468.5	1754.7	132.7	225.9	62.2	332.6	1123.0
11	**C_{26} (20R) + C_{27}(20S) TA**	449.8	774.6	210.9	1525.8	5725.2	449.8	697.1	179.3	1083.3	3664.1
12	**U**	40.9	69.8	18.3	131.5	507.7	40.9	62.8	15.5	93.3	325.0
13	**C_{28} (20S) TA**	428.7	737.0	163.9	1264.1	5484.3	428.7	663.3	139.3	897.5	3509.9

14	**C_{27}(20R) TA**	242.3	410.5	242.7	1330.6	3118.7	242.3	369.5	206.3	944.7	1995.9
15	**C_{28} (20R) TA**	362.6	558.1	270.6	1476.6	4221.0	362.6	502.3	230.0	1048.4	2701.4
16	**V**	112.1	281.0	289.5	614.7	1344.9	112.1	252.9	246.1	436.5	860.7
	Total	**2403.2**	**4837.1**	**2866.9**	**11249.9**	**32038.7**	**2403.2**	**4353.4**	**2436.9**	**7987.5**	**20504.8**

Table 0.16: Measured concentrations of triaromatic steranes (m/z 231) in weathered North Sea oil (µg/g oil)

		Determined Concentration (C_{det}) (M/Z 231)					**Converted Concentration (C_{conv})**				
Peak Number	**Triaromatic Steranes (TAS)**	**0%**	**27%**	**29%**	**41%**	**48%**	**0%**	**27%**	**29%**	**41%**	**48%**
1	**C_{20}**	306.5	1209.0	2608.0	2988.6	2872.0	306.5	882.5	1851.7	1763.3	1493.5
2	**Q**	223.5	1728.8	1806.7	1846.1	1856.0	223.5	1262.1	1282.7	1089.2	965.1
3	**C_{21}-TAS**	308.8	981.4	2034.9	2725.2	2698.1	308.8	716.4	1444.8	1607.8	1403.0
4	**P**	60.9	947.7	674.0	487.6	710.3	60.9	691.8	478.5	287.7	369.4
5	**C_{22}A-TAS**	39.9	453.2	431.9	370.3	368.1	39.9	330.8	306.6	218.5	191.4
6	**C_{22}B-TAS**	34.3	315.4	258.8	257.9	291.8	34.3	230.2	183.8	152.2	151.7
7	**R**	7.5	57.1	53.4	113.0	88.9	7.5	41.7	37.9	66.6	46.2
8	**S**	12.1	63.2	63.4	106.1	111.9	12.1	46.2	45.0	62.6	58.2
9	**T**	23.4	38.5	95.1	156.7	149.7	23.4	28.1	67.5	92.5	77.8
10	**C_{26}(20S) TA**	77.6	121.4	197.5	541.5	501.6	77.6	88.6	140.2	319.5	260.8

11	C_{26} (20R) + C_{27}(20S) TA	280.1	190.3	662.2	1823.7	1757.1	280.1	138.9	470.1	1076.0	913.7
12	U	18.7	41.1	28.6	116.9	105.9	18.7	30.0	20.3	69.0	55.1
13	C_{28} (20S) TA	185.6	102.1	309.2	1212.8	1156.4	185.6	74.5	219.6	715.6	601.3
14	C_{27}(20R) TA	165.9	125.6	541.8	1018.5	1135.7	165.9	91.7	384.7	600.9	590.6
15	C_{28} (20R) TA	158.2	125.6	538.1	981.2	963.1	158.2	91.7	382.1	578.9	500.8
16	V	36.9	96.7	133.0	267.9	259.3	36.9	70.6	94.4	158.1	134.8
	Total	**1939.9**	**6597.1**	**10436.5**	**15014.0**	**15025.8**	**1939.9**	**4815.9**	**7409.9**	**8858.2**	**7813.4**

Table 0.17: Measured concentrations of bicyclic sesquiterpanes (m/z 123) in weathered Nigerian light oil (µg/g oil)

				Determined Concentration (C_{det})				
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Formula**	**0%**	**33%**	**40%**	**56%**	**63%**
1	**C_4-decalin (C14)**	**BS1**	**C14H26**	3019.4	6035.0	5560.7	5394.7	1870.2
2	**C_{14} Sesquterpane**	**BS2**	**C14H26**	1425.5	3306.5	2990.2	3302.5	1731.5
Unknown	**A Sesquiterpane**	**BSA**	**C15H28**	8270.3	15028.2	14234.1	13200.0	6981.1
Unknown	**U2 Sesquiterpane**	**BSU2**	**C15H28**	2459.5	4869.1	4535.6	4160.2	2603.0
3	**C_{15} Sesquiterpane**	**BS3**	**C15H28**	7314.7	12954.2	11976.9	11523.1	6793.4
Unknown	**B Sesquiterpane**	**BSB**	**C15H28**	3052.0	6004.9	5437.4	5703.4	3456.0
4	**C_{15} Sesquiterpane**	**BS4**	**C15H28**	4586.7	8588.3	7624.8	7624.2	3861.5
Unknown	**D Sesquiterpane**	**BSD**	**C15H28**	3127.9	5459.6	5563.1	4545.3	3524.6
5	**8β(H)-drimane**	**BS5**	**C15H28**	4096.5	7997.1	7140.3	7822.8	3892.9
6	**C_{15} Sesquiterpane**	**BS6**	**C15H28**	2853.2	5361.6	4905.7	4829.9	3118.5

7	**C_{16} Sesquiterpane**	**BS7**	**C16H30**	1548.4	3530.0	3210.3	3628.9	2259.0
8	**C_{16} Sesquiterpane**	**BS8**	**C16H30**	1711.8	3419.7	3163.3	3393.4	2508.2
9	**C_{16} Sesquiterpane**	**BS9**	**C16H30**	1171.6	1931.7	1858.3	2011.4	1448.1
10	**8β(H)-Homodrimane**	**BS10**	**C16H30**	3775.6	7257.9	6922.8	6628.8	5431.6
	Total			**48413.2**	**91743.7**	**85123.5**	**83768.5**	**49479.4**

Table 0.18: Measured concentrations of bicyclic sesquiterpanes (m/z 123) in weathered Nigerian medium oil (μg/g oil)

				Determined Concentration (C_{det})				
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Formula**	**0%**	**10%**	**15%**	**29%**	**36%**
1	**C_4-decalin**	**BS1**	**C14H26**	3734.6	6773.2	6224.6	5273.1	2093.2
2	**C_{14} Sesquiterpane**	**BS2**	**C14H26**	2007.0	4132.0	3929.3	3275.2	1275.4
Unknown	**A Sesquiterpane**	**BSA**	**C15H28**	11070.7	16745.1	15361.5	13499.4	6440.9
Unknown	**U2 Sesquiterpane**	**BSU2**	**C15H28**	3638.2	5147.9	5020.3	4125.1	2184.6
3	**C_{15} Sesquiterpane**	**BS3**	**C15H28**	15024.1	22847.8	20359.6	17717.6	10493.1
Unknown	**B Sesquiterpane**	**BSB**	**C15H28**	4577.9	7031.0	6510.4	6213.6	4424.9
4	**C_{15} Sesquiterpane**	**BS4**	**C15H28**	7794.8	11122.2	10446.6	9205.1	6541.5
Unknown	**D Sesquiterpane**	**BSD**	**C15H28**	4707.9	7261.8	6465.7	6366.4	3681.1
5	**8β(H)-drimane**	**BS5**	**C15H28**	5858.8	9467.7	8786.4	8355.5	5516.6
6	**C_{15} Sesquiterpane**	**BS6**	**C15H28**	5563.3	7580.0	7257.6	6758.1	5209.3
7	**C_{16} Sesquiterpane**	**BS7**	**C16H30**	2976.4	4700.1	4215.9	4213.4	3290.1

				Determined Concentration (C_{det})				
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Formula**	**0%**	**10%**	**15%**	**29%**	**36%**
8	**C_{16} Sesquiterpane**	**BS8**	**C16H30**	3570.1	5464.8	5019.4	4980.7	4289.8
9	**C_{16} Sesquiterpane**	**BS9**	**C16H30**	3030.1	3861.8	3493.6	3407.1	3018.7
10	**8β(H)-Homodrimane**	**BS10**	**C16H30**	4507.2	6987.8	6641.7	6661.7	6081.3
	Total			**78061.1**	**119123.1**	**109732.7**	**100052.2**	**64540.4**

Table 0.19: Measured concentrations of bicyclic sesquiterpanes (m/z 123) in weathered North Sea oil (μg/g oil)

				Determined Concentration (C_{det})				
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Formula**	**0%**	**27%**	**29%**	**41%**	**48%**
1	**C_4-decalin**	**BS1**	**C14H26**	373.0	1265.7	1246.0	525.2	79.8
2	**C_{14} Sesquiterpane**	**BS2**	**C14H26**	389.5	814.6	807.9	400.8	92.4
3	**C_{15} Sesquiterpane**	**BS3**	**C15H28**	765.8	1863.1	1850.7	1407.5	572.6
Unknown	**C_{15} Sesquiterpane**	**BSX**	**C15H28**	267.2	674.2	777.1	628.8	392.6
4	**C_{15} Sesquiterpane**	**BS4**	**C15H28**	541.9	1431.9	1486.4	1292.3	634.8
5	**8β(H)-drimane**	**BS5**	**C15H28**	840.9	2017.9	2073.8	1699.3	1140.1
6	**C_{15} Sesquiterpane**	**BS6**	**C15H28**	482.9	1066.4	1156.9	1071.9	746.3
7	**C_{16} Sesquiterpane**	**BS7**	**C16H30**	201.9	874.9	1013.4	976.4	697.7
8	**C_{16} Sesquiterpane**	**BS8**	**C16H30**	445.4	994.9	1052.3	1012.3	828.1
9	**C_{16} Sesquiterpane**	**BS9**	**C16H30**	271.8	633.8	680.9	633.5	621.8
10	**8β(H)-Homodrimane**	**BS10**	**C16H30**	833.3	2083.2	2030.0	1870.5	1740.1

Total	**5413.6**	**13720.6**	**14175.5**	**11518.4**	**7546.2**

Table 0.20: Measured concentrations of adamantanes (m/z 136) in weathered Nigerian Light oil (µg/g oil)

			Determined Concentration (C_{det})				
Peak Number	**Adamantanes**	**Code**	**0%**	**33%**	**40%**	**56%**	**63%**
1	**Adamantane**	**A**	65.4	61.4	36.0	26.2	0.0
2	**1-Methyladamantane**	**1-MA**	1466.1	1951.9	1883.3	1468.3	657.8
3	**1,3-Dimethyladamantane**	**1,3-DMA**	653.9	908.5	902.8	666.6	329.2
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	102.9	124.9	111.7	68.5	19.2
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	71.7	67.7	71.2	47.6	14.2
6	**2-Methyladamantane**	**2-MA**	342.4	558.8	584.7	480.8	224.6
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA(cis)**	422.5	710.8	676.1	503.2	186.0
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA(trans)**	277.0	450.7	425.3	350.7	111.4
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	99.7	308.8	299.6	286.1	118.4
10	**1,2-Dimethyladamantane**	**1,2-DMA**	505.6	561.3	519.1	447.8	225.0
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA(cis)**	516.0	697.2	687.5	460.8	126.3
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA(trans)**	416.1	744.3	680.9	555.7	256.6
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	131.3	235.3	284.0	220.9	103.0
14	**1-Ethyladamantane**	**1-AE**	717.0	1131.3	1095.5	1018.7	472.4
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	223.8	428.4	357.0	472.8	247.0
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	204.0	546.1	512.5	476.0	207.5

17	**2-Ethyladamantane**	**2-AE**	74.4	189.0	176.5	111.7	53.5
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	95.4	224.6	196.1	194.9	106.6
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	344.9	461.1	395.0	309.6	139.8
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	201.8	459.6	435.7	262.3	66.8
	Total		**6932.0**	**10821.6**	**10330.4**	**8429.1**	**3665.4**

Table 0.21: Measured concentrations of adamantanes (m/z 136) in weathered Nigerian Medium oil (µg/g oil)

			Determined Concentration (C_{det})				
Peak Number	**Adamantanes**	**Code**	**0%**	**10%**	**15%**	**29%**	**36%**
1	**Adamantane**	**A**	31.3	14.4	12.6	9.3	55.1
2	**1-Methyladamantane**	**1-MA**	516.6	782.6	739.5	500.9	82.6
3	**1,3-Dimethyladamantane**	**1,3-DMA**	254.6	377.3	361.8	240.2	32.0
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	112.6	157.8	129.0	58.1	8.9
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	30.0	71.0	50.7	39.7	31.9
6	**2-Methyladamantane**	**2-MA**	169.8	308.5	288.3	223.7	58.2
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA(cis)**	329.7	552.3	475.0	310.6	40.9
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA(trans)**	209.0	353.1	296.0	187.3	43.4
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	97.5	241.0	213.4	118.5	62.3
10	**1,2-Dimethyladamantane**	**1,2-DMA**	477.2	498.6	459.2	376.8	60.4

			Determined Concentration (C_{det})				
Peak Number	**Adamantanes**	**Code**	**0%**	**10%**	**15%**	**29%**	**36%**
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA(cis)**	461.5	523.3	427.9	201.1	44.7
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA(trans)**	357.1	576.3	526.6	358.8	59.8
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	79.2	135.8	109.1	116.3	83.7
14	**1-Ethyladamantane**	**1-AE**	590.9	885.2	813.1	526.9	137.4
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	278.9	575.0	540.4	440.9	95.7
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	207.8	326.4	283.9	219.7	41.8
17	**2-Ethyladamantane**	**2-AE**	68.5	165.8	143.3	94.1	35.0
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	89.7	207.0	192.1	152.7	68.1
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	328.0	516.2	475.7	330.9	57.4
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	186.7	276.4	243.1	195.6	160.7
	Total		**4865.1**	**7544.2**	**6780.7**	**4702.0**	**1259.9**

Table 0.22: Measured concentrations of adamantanes (m/z 136) in weathered North Sea oil (µg/g oil)

			Determined Concentration (C_{det})				
Peak Number	**Adamantanes**	**Code**	**0%**	**27%**	**29%**	**41%**	**48%**
1	**Adamantane**	**A**	44.9	4.5	3.0	2.9	0.3
2	**1-Methyladamantane**	**1-MA**	102.3	14.0	26.7	2.0	1.6
3	**1,3-Dimethyladamantane**	**1,3-DMA**	28.8	11.3	11.3	1.1	0.8
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	29.0	12.8	12.1	0.8	0.8

5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	20.5	8.9	7.1	4.5	0.5
6	**2-Methyladamantane**	**2-MA**	35.2	31.9	28.2	8.6	0.8
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA(cis)**	105.0	100.2	83.5	12.8	1.2
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA(trans)**	71.3	69.8	64.3	9.3	1.1
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	28.5	31.2	23.5	14.4	1.1
10	**1,2-Dimethyladamantane**	**1,2-DMA**	137.8	151.4	126.7	27.8	1.6
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA(cis)**	176.1	195.6	159.0	33.5	1.2
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA(trans)**	119.2	179.9	126.3	21.5	2.1
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	33.2	64.5	38.8	31.0	1.2
14	**1-Ethyladamantane**	**1-AE**	166.6	217.1	195.0	46.5	2.3
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	88.4	184.6	174.0	47.8	3.1
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	58.2	111.8	95.7	469.1	2.4
17	**2-Ethyladamantane**	**2-AE**	32.1	57.4	59.2	18.9	2.3
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	37.3	76.4	71.4	34.3	3.5
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	139.8	203.0	184.9	80.5	9.7
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	104.7	232.7	218.0	77.5	11.8
	Total		**1543.9**	**1959.0**	**1708.9**	**944.8**	**49.3**

Table 0.23: Identification

Compound	Assignment
2,6, + 2,7-DMN	2,6+2,7 Dimethylnaphthalene

BS4	C15-Sesquiterpane
C_{30}Olean	C30-18α(H) Oleanane

Chapter 4: Effects of water washing on hydrocarbon composition of crude oils

4.1 Introduction

Determination of the quantity, composition, and distribution of hydrocarbons in a given sedimentary area are important aspects of the geochemical investigation involved in petroleum explorations (Kuo, 1994). Controlling factors that influence the composition of petroleum include; type of organic matter, source rock maturity, hydrocarbon migration, and post-generation alteration of crude oils (Kuo, 1994; Jones, 2013). Alteration of crude oil composition due to water washing involves the removal of water-soluble compounds from the oil as they partition differently between the aqueous and oil phases, whereas components with relatively high aqueous solubilities partitioned into water phase while the insoluble components are retained in the oil phase with dissolution playing a very key role in this process (Bobra, 1992). Water washing and other post-generation alteration processes such as microbial degradation, thermal alteration, gas diffusion, de-asphalting, migration, thermochemical sulphate reduction, evaporative fractionation etc., are said to occur mainly in a pooled reservoired oil in the sub-surface (Tissot and Welte, 1984; Killops and Killops, 1993). While thermal maturation and microbial degradation thermo-chemical or biochemical processes, water washing, preferential loss of light compounds by diffusion, de-asphalting and new compounds addition during migration are physical alteration processes. (Tissot and Welte, 1984). Water washing and biodegradation are commonly observed in pooled oils usually invaded by surface sourced meteoric waters and while the two processes not necessarily connected, they are usually observed in combination because the effects are not easily distinguished for C_{15+} components of the crude oil (Hall *et al.*, 2014). The sub-surface water usually removes the water-soluble compounds at the oil-water contact thereby altering the composition of the oil in the reservoir and thereby affecting its economic value (Tissot and

Welte, 1984; Lafargue and Barker, 1988). Understanding these alteration processes, their effects on oil composition and proper documentation is vital for oil-oil and oil-source correlation to categorise oils with high degree of accuracy into various genetically related (e.g., cogenetic) families within a given area (Lafargue and Barker, 1988). In addition to its effect on pooled reservoired oil, water washing may affect the composition of petroleum at various stages of formation from the source rock during diagenesis (Lafargue and Barker, 1988). It has been previously observed and reported that laboratory water washing experiments is affected by factors such as temperature, salinity and nature of the oil considered (Lafargue and Barker, 1988). The aqueous solubilities of hydrocarbon compounds were reported increased with temperature, whereas reduced solubilities were recorded with increase in salinity of the water (Price, 1976; Lafargue and Barker, 1988; Wang *et al.*, 2016b).

4.2 Aim and Objectives

The aim of the experiments detailed in this chapter is to investigate the effect of water washing on the hydrocarbon compositions of the three oil samples with different chemical and physical properties. Water washing is an important weathering process which may affect the distributions of geochemical markers used in oil correlations.

The specific objectives were;

1. To observe water washing effects on the oil samples with different chemical compounds and physical properties.
2. To obtain detailed compositional information of the oils samples such as *n*-alkanes, acyclic isoprenoids, biomarker compounds, polynuclear aromatic hydrocarbons (PAHs) and their alkyl homologues and any relevant less well-known compounds identified.

3. To quantitatively determine the concentrations of the analytes in (2) above and compare the compositional and concentrations changes with respect to different levels of water washing.
4. To quantitatively examine ratios of compounds used in oil-oil correlations, and relate the water washing levels with the concentration changes of target analytes for weathering evaluations.

4.3 Material and Methods

4.3.1 Reagents and Materials

Chromatographic-grade distilled solvents used included petroleum ether (BP 40-60°C), dichloromethane (DCM) and methanol. Silica gel 60 (230-400 mesh ASTM) and aluminium oxide 90 active neutral (70-230 mesh ASTM) were purchased from Merck, Darmstadt, Germany and 500 gr white non-absorbent cotton wool purchased from Fisher Scientific, UK The silica gel, the aluminium oxide and the cotton wool were pre-extracted in a Soxtec solvent extractor using an azeotropic DCM-methanol solvent mixture. The silica gel and the aluminium oxide were stored in glass bottles, covered with aluminium foil and activated in an oven over night at 130°C before use, e.g. (Later *et al.*, 1985; Bundt *et al.*, 1987). Aliphatic hydrocarbon standards were: squalane (99% purity) and 5α-cholestane (98% purity) obtained from Aldrich, Steinem, Germany, *n*-heptadecylcyclohexane (98% purity) obtained from Lancaster Synthesis, Morecambe, UK, 5α-androstane (98% purity) obtained from Sigma-Aldrich, Steinem, Germany. PAH standard were 1,1’-binaphthyl (98% purity) obtained from Lancaster Synthesis, White Lund, UK and *p*-terphenyl (99% purity) obtained from Fluka Chemika, Buchs, Switzerland. The sodium chloride (NaCl) analytical reagent grade obtained from Fisher Chemical. Two crude (light and medium) oil samples from the Niger Delta region of Nigeria and one from the North Sea were obtained.

4.3.2 Water washing experiments

Three oil samples were used for this experiment which include; Nigerian Light, Nigerian Medium, and North Sea oils with API gravities of 40.3º, 26.3º, and 40º respectively. The Nigerian Light oil was obtained at Nigerian Agip Oil Company's (NAOC) Oshie Flow Station, Akala-Olu, Ahoada L.G.A, Rivers State, Nigeria, while the Nigerian Medium oil obtained at the NAOC's Ebocha Oil Center, Rivers State, Nigeria. The samples were collected in duran bottles with Teflon lined lids and shipped. The North Sea oil from the Andrew Oilfield was a standard oil sample available in the laboratory at Newcastle. 100 ml glass separating funnels were washed and rinsed in deionised water and set up on a clamp. 50 ml of 1.7% saline water (17 g NaCl made up to 1 L with deionised water) was introduced into each separating funnel and 10 ml of each oil samples were added to the separating funnels in triplicates. The funnels were shaken vigorously shaken and then allowed for oil-water layer to clearly separate for about 1hour at room temperature, before the bottom aqueous layer water removed. The above was repeated with fresh aliquots (50 ml) of saline water each time and oil samples were taken for analysis after washing five times, ten times, fifteen times, and twenty times respectively. The water salinity of 1.7% was adopted as the average salinity of brackish water (Ukpaka and Akuboye, 2016) found within the 28,000 km^2 coastal area or estuaries of the Niger Delta region (see section 1.5, chapter 1) where most of the oil spills occurred (see Figure 1, chapter 1).

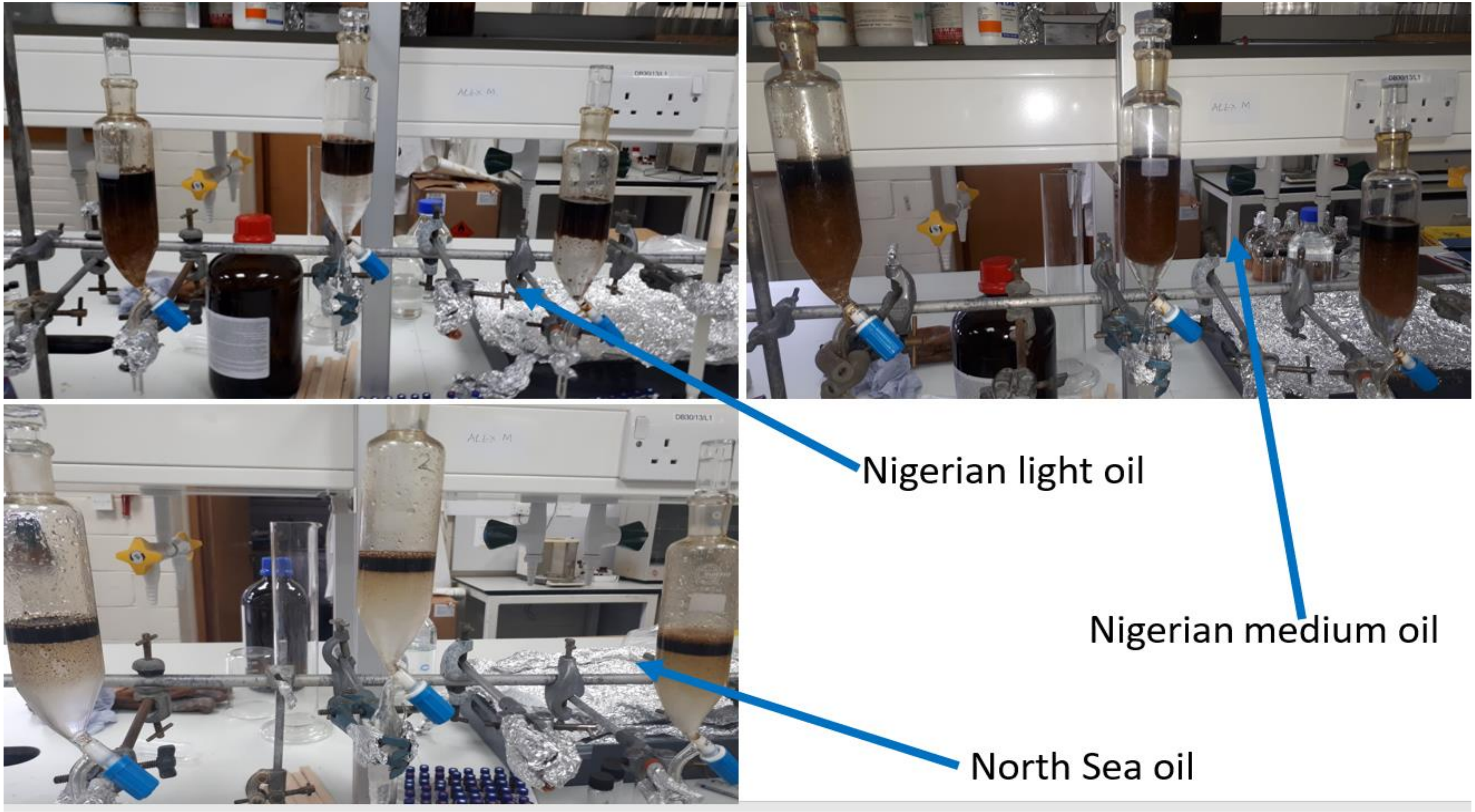


Figure 0.1: Water washing experimental set-up

4.3.3 Column chromatographic fractionation of hydrocarbons

Glass chromatography columns (28 cm l x 10 mm i.d.) were set-up on clamps and pre-extracted cotton wool was used to plug the bottom of the columns. A slurry of silica gel in petroleum ether was poured into the columns followed by slurry of alumina at the top to form alumina/silica (1:4) columns. Weighed aliquots (~50 mg) of the water washed crude oil samples from the separating funnels were adsorbed onto small quantities (~1g) of alumina and then charged onto the alumina/silica column. The columns were eluted with petroleum ether (70 ml) to provide an aliphatic hydrocarbon fraction (F1) for *n*-alkanes and biomarker analysis, followed by elution of a PAHs-containing fraction (F2) (70 ml 50% DCM in petroleum ether) for analysis of PAHs similar to that previously reported (e.g. Wang *et al.*, 1994a; Wang *et al.*, 1994b; Wang *et al.*, 2004; Wang *et al.*, 2005).

4.3.5 Gas chromatography and gas chromatography - mass spectrometry

Gas chromatography with flame ionisation detection (GC-FID) analysis of the aliphatic hydrocarbon fractions was performed on an Agilent 5890 GC in split-less mode, with the injector at 280°C, FID at 300°C. The sample (1ul) in hexane was injected by an HP7673 auto sampler and the split opened after 1 minute. After the injection, the GC temperature programme and data acquisition commenced. Separation was performed on a fused silica capillary column (30m x 0.25mm i.d.) coated with 0.25um dimethyl poly-siloxane (HP-5 phase). The GC was temperature programmed from 50°C-310°C at 5°C min and held at final temperature for 20 minutes with hydrogen as the carrier gas (flow 1ml/min, pressure of 50kPa, split at 30 mls/min). The GC data was acquired on a Thermo-Atlas laboratory chromatography data system.

Gas chromatography-mass spectrometry (GC-MS) analysis of the aliphatic hydrocarbon and PAH fractions was performed on an Agilent 7890B GC with a split/split less injector (280°C) linked to a Agilent 5977B MSD (electron voltage 70eV, source temperature 230°C, quadrupole temperature 150°C multiplier voltage 1200V, interface temperature 310°C). The acquisition was controlled using Chemstation software, initially in full scan mode (50-600 amu/sec) or in selected ion mode (20 ions, 3.0 cps, 20 ms dwell) for greater sensitivity. The sample (1 ul) in hexane was injected by an Agilent7683B auto sampler and the split opened after 1 minute. After the solvent peak had passed the GC temperature programme and data acquisition commenced. Separation was performed on an Agilent fused silica capillary column (30 m x 0.25 mm i.d) coated with 0.25 um dimethyl polysiloxane (HP-5) phase. The GC was temperature programmed from 50-310°C at 5°C min and held at final temperature for 10 minutes with helium as the carrier gas (flow rate of 1ml/min, initial pressure of 50kPa, split at 20 mls/min). The acquired data was stored for later data processing, integration and printing. Peaks were identified and labelled after comparison of their elution order with known standard mixtures or from relevant published data, as indicated in the appropriate results sections and also for the PAH by comparison of their mass spectra with those of the NIST05 library. The NIST Mass Spectral Library is a software and peer-reviewed databases that are developed and supported by the National Institute for Standards and Technology, USA.

4.4 Results and Discussion

The water washing effects on the three oil samples were analysed into the following component compositions.

4.4.1 *n*-Alkane and acyclic isoprenoid composition and concentration changes

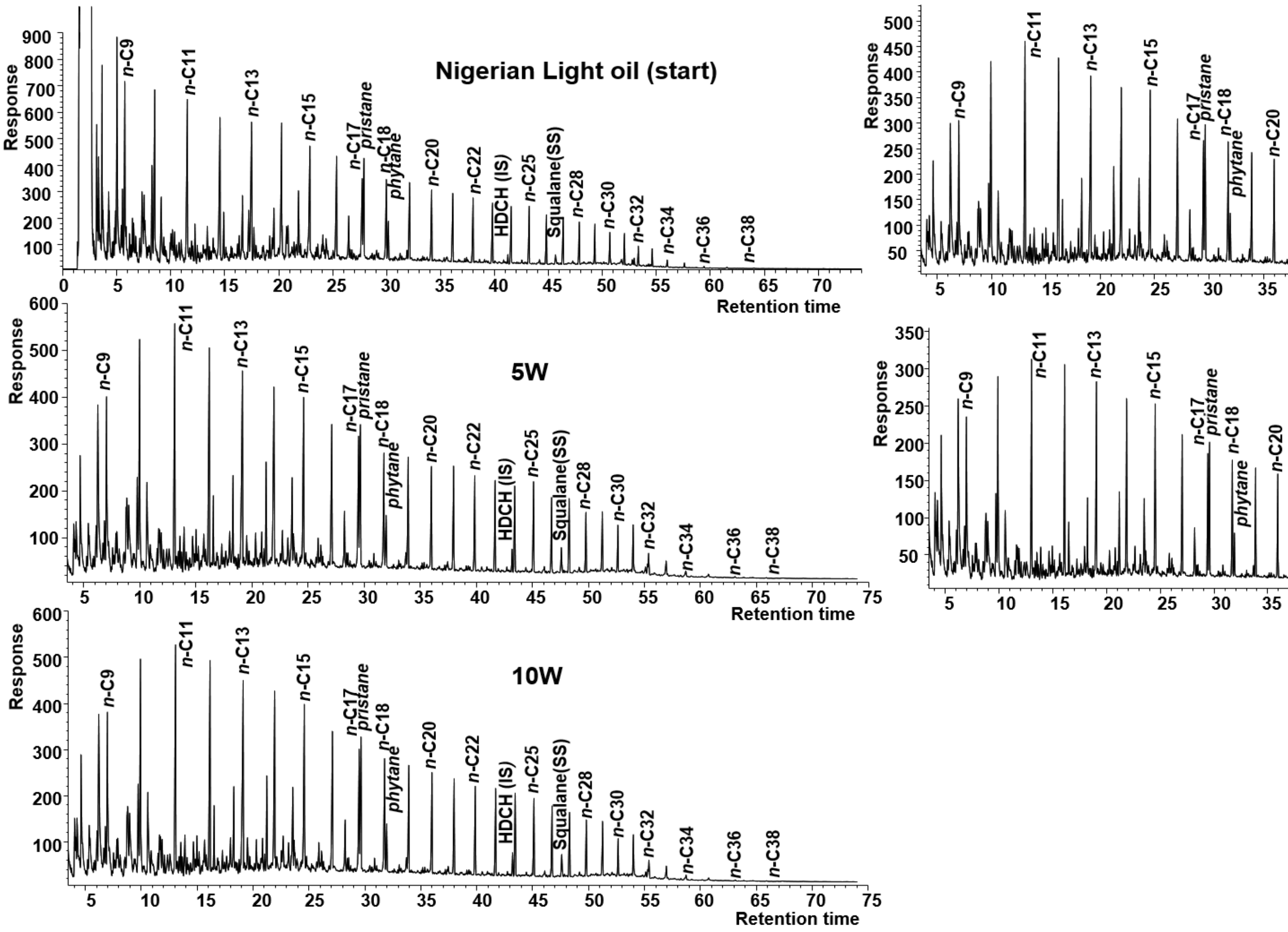


Figure 0.2**,** Figure 0.3**,** and Figure 0.4 are GC-FID chromatograms of the aliphatic (F1) fractions of the Nigerian Light and Medium oils, and the North Sea oil respectively, showing the effects of water washing on the *n*-alkanes in the oils. The *n*-alkanes and isoprenoids are generally less affected by water washing compared to the evaporative weathering observed after 30 days (see section 3.4.2) after washing twenty times. The chromatograms show clearly resolved peaks and no prominent “hump” is an indication that these components of the oils are not much weathered by water washing for the Nigerian light and North Sea oils as compared with effect of evaporation, except the Nigerian medium that was observed to have been slightly degraded from source as stated in chapter 3 (see section 3.4.2) The low molecular weight *n*-alkanes of C_{15} and below (*n*-C_{15-}) in particular, are indicators used to identify secondary

processes of oil alterations in the reservoir such as biodegradation and water washing (Thompson, 1979; Thompson, 1983; Whelan *et al.*, 1984; Thompson, 1987; Lafargue and Le Thiez, 1996; López and Lo Mónaco-Trupiano, 2016), but are relatively less affected by water washing (x20) compared to evaporative weathering for 30 days (see Figures 3.4, 3.5 and 3.6, Tables 3.1, 3.2, and 3.3, Section 3.4.2) for all the three oil samples.

Table 0.1, Table 0.2, Table 0.3 show the summary of the identified *n*-alkanes (*n*-C_8 to *n*-C_{39}), pristanes and phytanes and their respective concentrations for the weathered Nigerian Light, Nigerian Medium, and North Sea oils respectively, obtained by comparison of integrated peak areas against the peak areas of known amounts of internal standard (*n*-heptadecylcyclohexane) using GC-FID. A recovery or surrogate standard (squalane) was also used to compute the percentage recoveries for the analytical procedures, which were over 90% for all the samples. The mean values (n=3) from triplicates with relative standard deviation (RSD) in brackets after the numbers, are shown in Table 0.1, Table 0.2, and Table 0.3 respectively. The quantitations were carried out as described in chapter 3 (see equations 3.1, 3.2, 3.3 and 3.4 in section 3.4.2).

The n-alkanes are generally less susceptible to water washing (x20) as compared to evaporative weathering (for 30 days) as shown in Tables 4.1, 4.2 and 4.3 respectively. For the Nigerian Light oil, a reduction in concentrations is observed from *n*-C_8 to *n*-C_{11} as the number water washing increased to 20 times, whereas *n*-alkanes greater than *n*-C_{11}, pristane and phytane are not so affected as aqueous solubility of n-alkanes decrease with increase in carbon number (cf. Ferguson *et al.*, 2009). Similarly, *n*-C_8 and *n*-C_9 are the most affected for the Nigerian Medium oil, and *n*-C_8 to *n*-C_{13} for the North Sea oil respectively. Studies have shown that water washing largely affects the light ends of oils from C_{15} fraction and below (C_{15-}), and the most affected are usually components lighter than *n*-C_8 (C_4-C_7 *n*-alkanes) which are not assessed in this work usually analysed using whole oil gas chromatography (WOGC) (Whelan *et al.*, 1984; Thompson, 1987; Lafargue and Barker, 1988; Elliott, 2015). Although the *n*-alkanes are less affected by water washing weathering compared to that of evaporative weathering, the *n*-alkanes concentration sum with after washing (x20) is observed to approximately equal for the Nigerian Light (see Table 0.1) compared with that in the 30 day evaporation experiments (see Table 3.1) as *n*-C_8 to *n*-C_{39} were not completely removed after washing (x20), but *n*-C_8 to *n*-C_{13} completely evapotated after 30 days probaly due to higher-molcular weight build-up. The *n*-alkanes sum (see Table 0.2) after washing (x20) was slightly less than that of evaporation experiments after 30 days (See Table 3.3) for the Nigerian Medium oil.

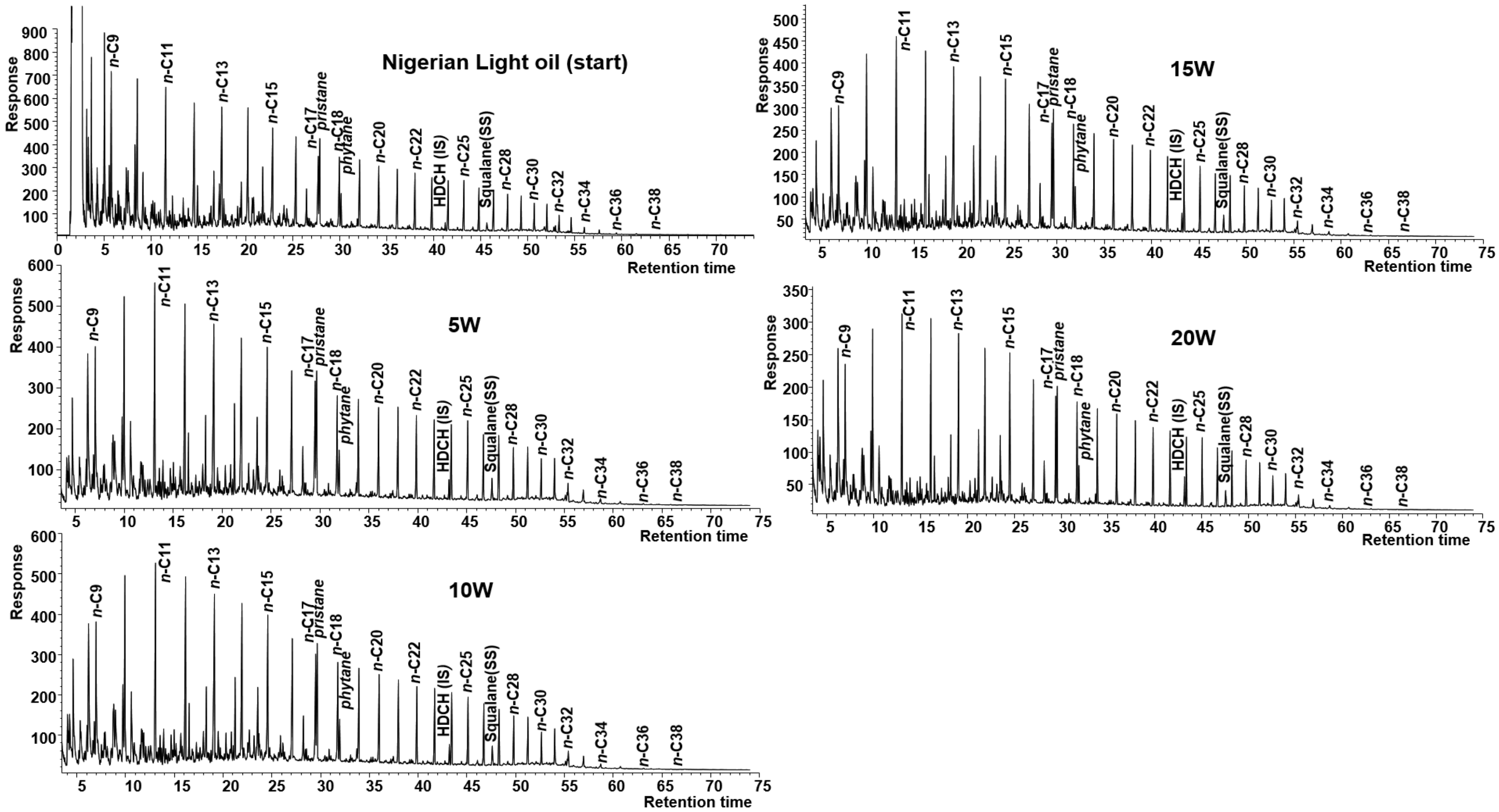


Figure 0.2: GC-FID chromatograms of aliphatic fractions of the Nigerian Light oil showing the number of water washing steps and corresponding components losses. Note: 5W, 10W, 15W and 20W are the number of sample water washing steps 5, 10, 15 and 20 times respectively.

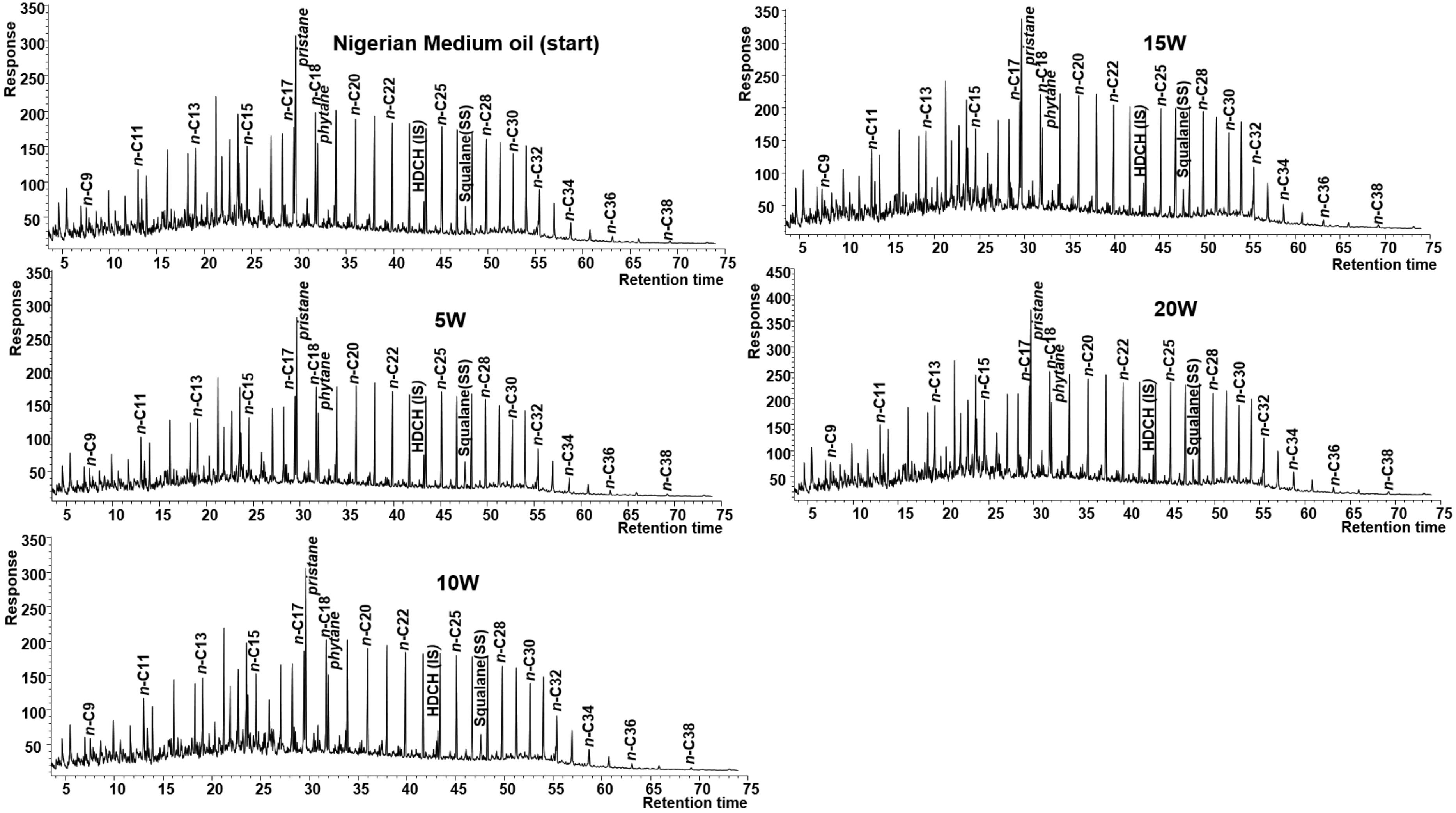


Figure 0.3: GC-FID chromatograms of aliphatic fractions of the Nigerian Medium oil showing the number of water washing steps and corresponding components losses. Note: 5W, 10W, 15W and 20W are the number of sample water washing steps 5, 10, 15 and 20 times respectively.

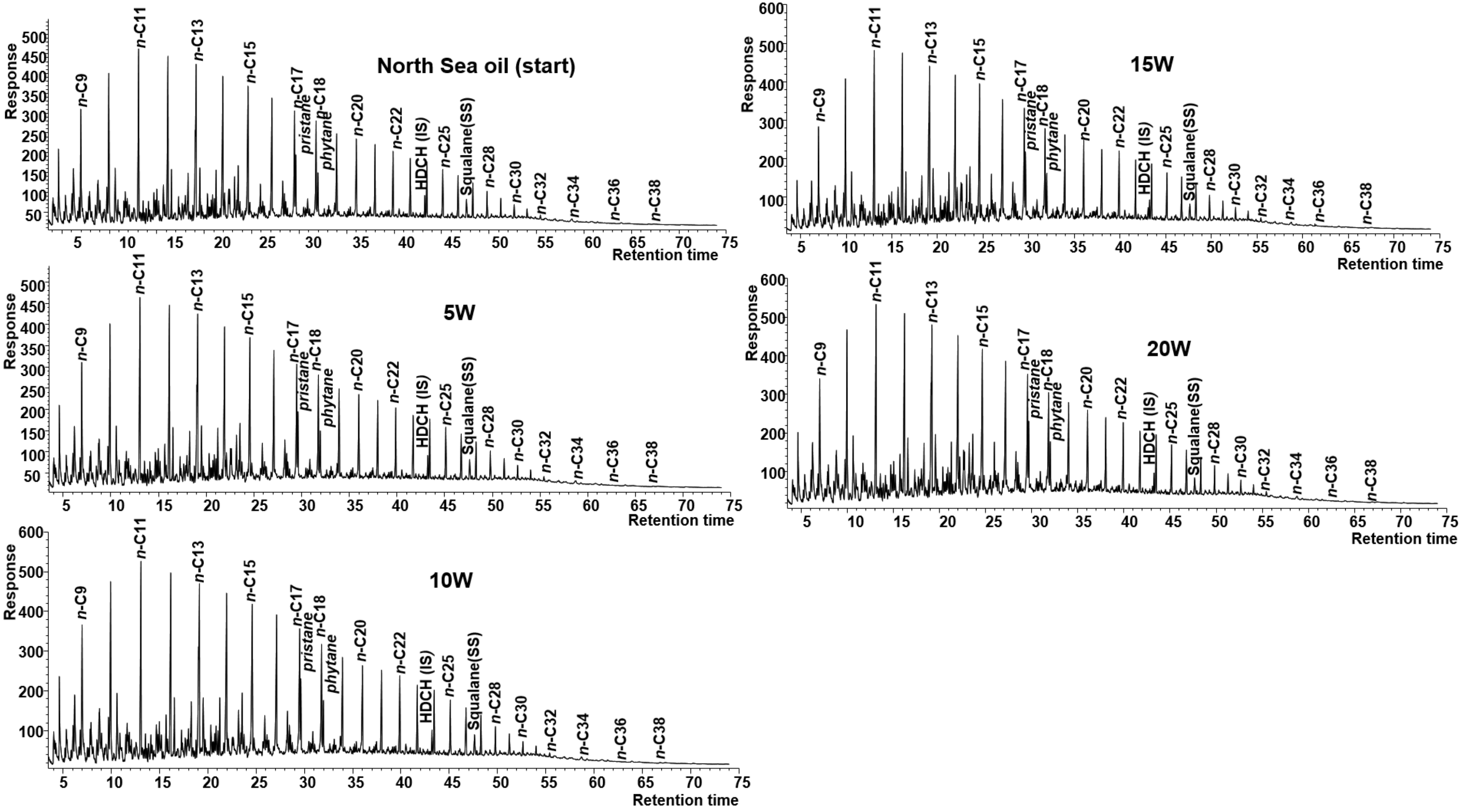


Figure 0.4: GC-FID chromatograms of aliphatic fractions of the Nigerian Light oil showing the number of water washing steps and corresponding components losses. Note: 5W, 10W, 15W and 20W are the number of sample water washing steps 5, 10, 15 and 20 times respectively.

The sum difference was however was much for North Sea as the *n*-alkanes sum (see Table 0.3) after washing (x20) was about twice as much as that of the evaporation experiments (see Table 3.3). The values of the ratios *n*-C_{17}/pristane, *n*-C_{18}/phytane, pristane/phytane, and CPI* are very similar for both the water washing (see Table 0.1, Table 0.2, and Table 0.3) and evaporation experiements (see Tables 3.1, 3.2 and 3.3) with the water washing ratios slightly more preserved, except for the weathering index ratio (WI) $(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})$ which is more affected by evaporative weathering than water washing due to much greater loss of the low-molecular weight (numerator) *n*-alkanes to evaporation. Figure 0.5, Figure 0.6, and Figure 0.7 are graphical representations of the *n*-alkanes and isoprenoids concentrations for the Nigerian Light and Medium, and North Sea oils respectively. The light ends *n*-alkanes are observed as not so affected compared to that of evaporative weathering (see Figures 3.7, 3.8, and 3.9).

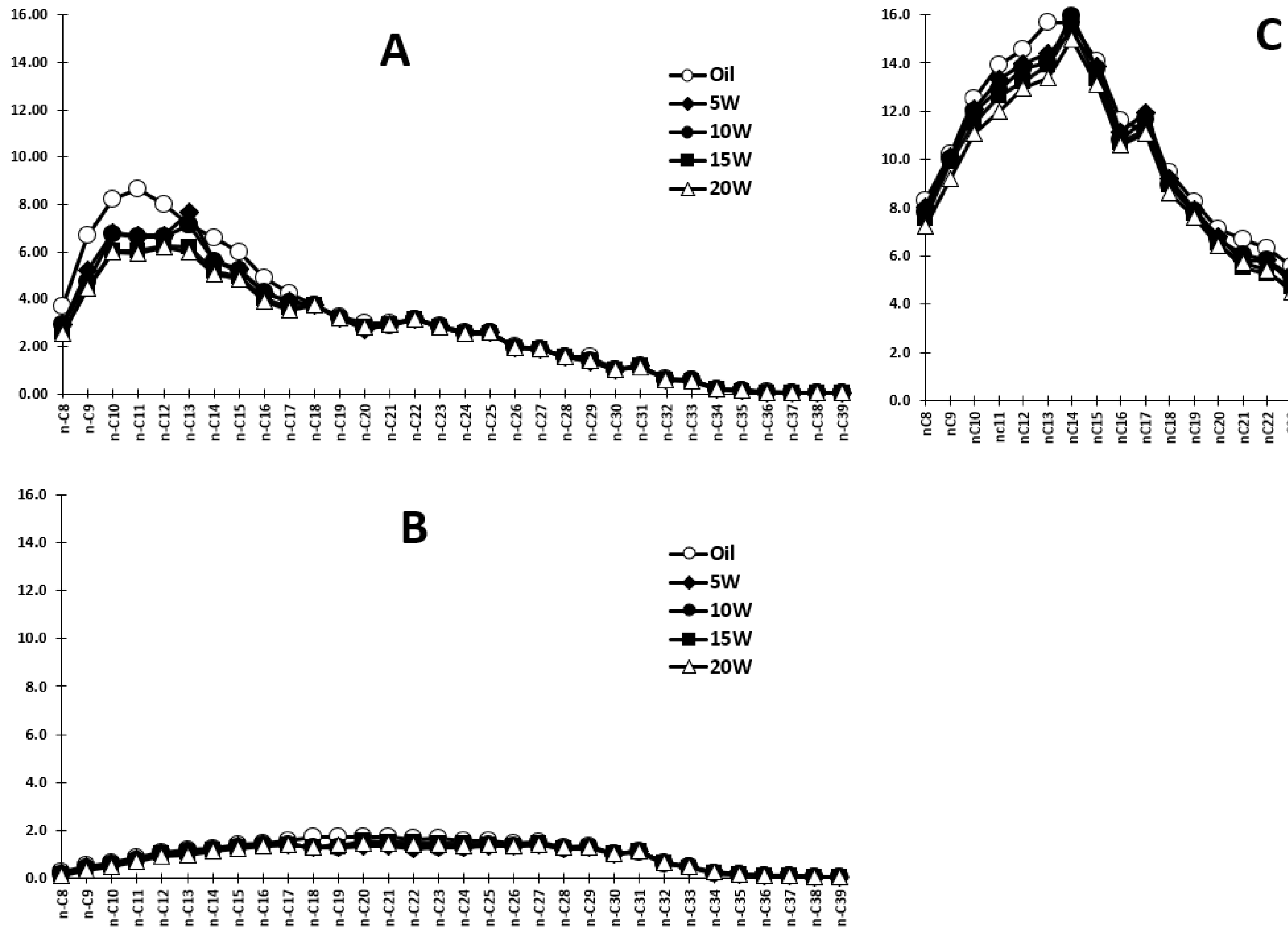


Figure 0.8 shows the plots of abundances or concentrations (see Table 0.1, Table 0.2, and Table 0.3) of *n*-alkane in the weathered oils relative to *n*-C_{30} for the three oils. The effect of water washing is observed to be more noticeable within the *n*-C_{15-} range (or less) for the Nigerian light (A), Nigerian Medium (B), and North Sea (C) oils respectively. Slight variation of the plot of the unweathered oil plot from that of the weathered oil at the peak of the Nigerian medium oil plots. Cycloalkanes were not considered in this work but it has been established that they tend to be slightly more soluble than the normal alkanes discuss in this work (e.g., Bobra, 1992).

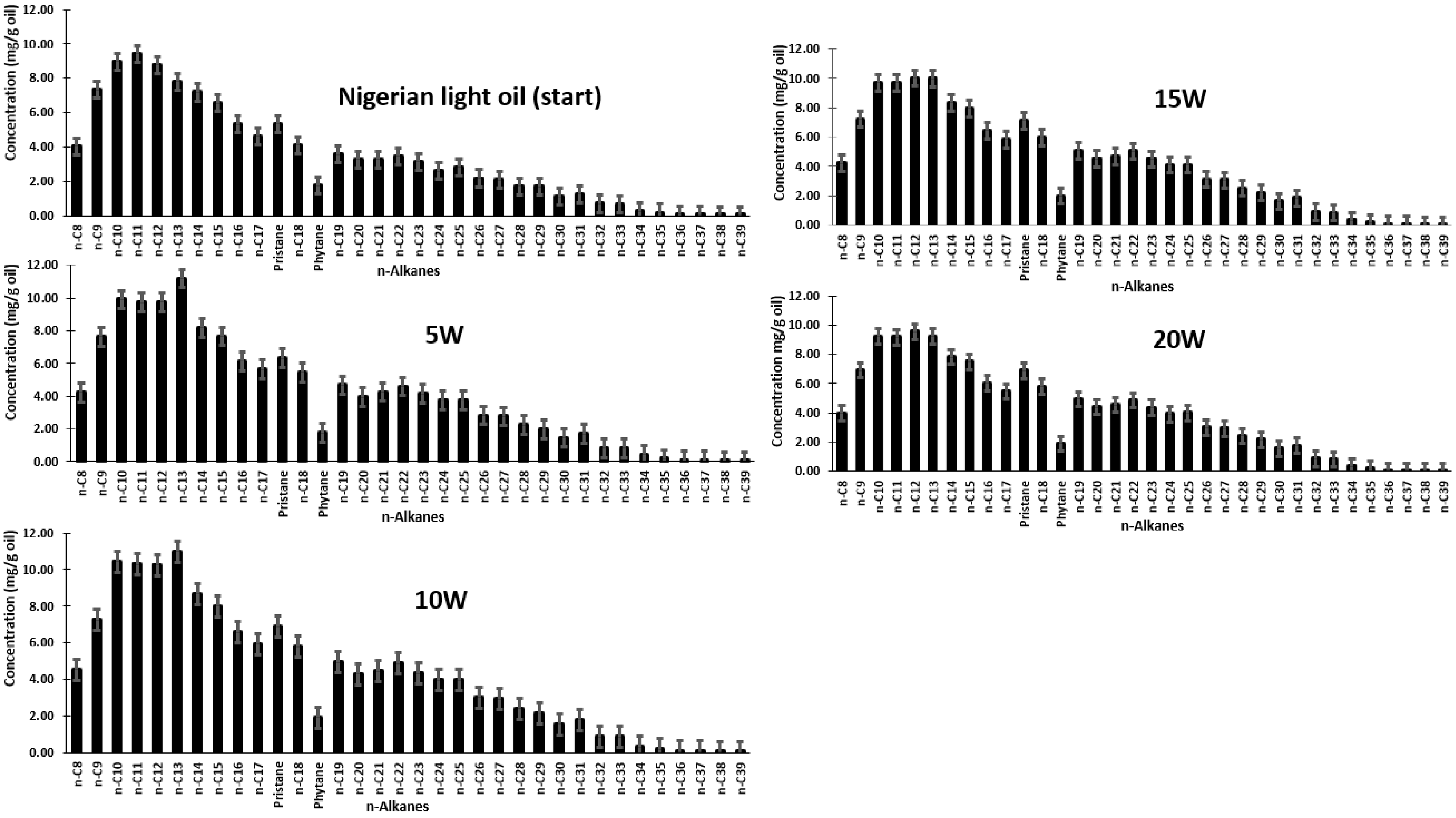


Figure 0.5: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane for Nigerian Light oil after repeated water washings (5W, 10W, 15W and 20W).

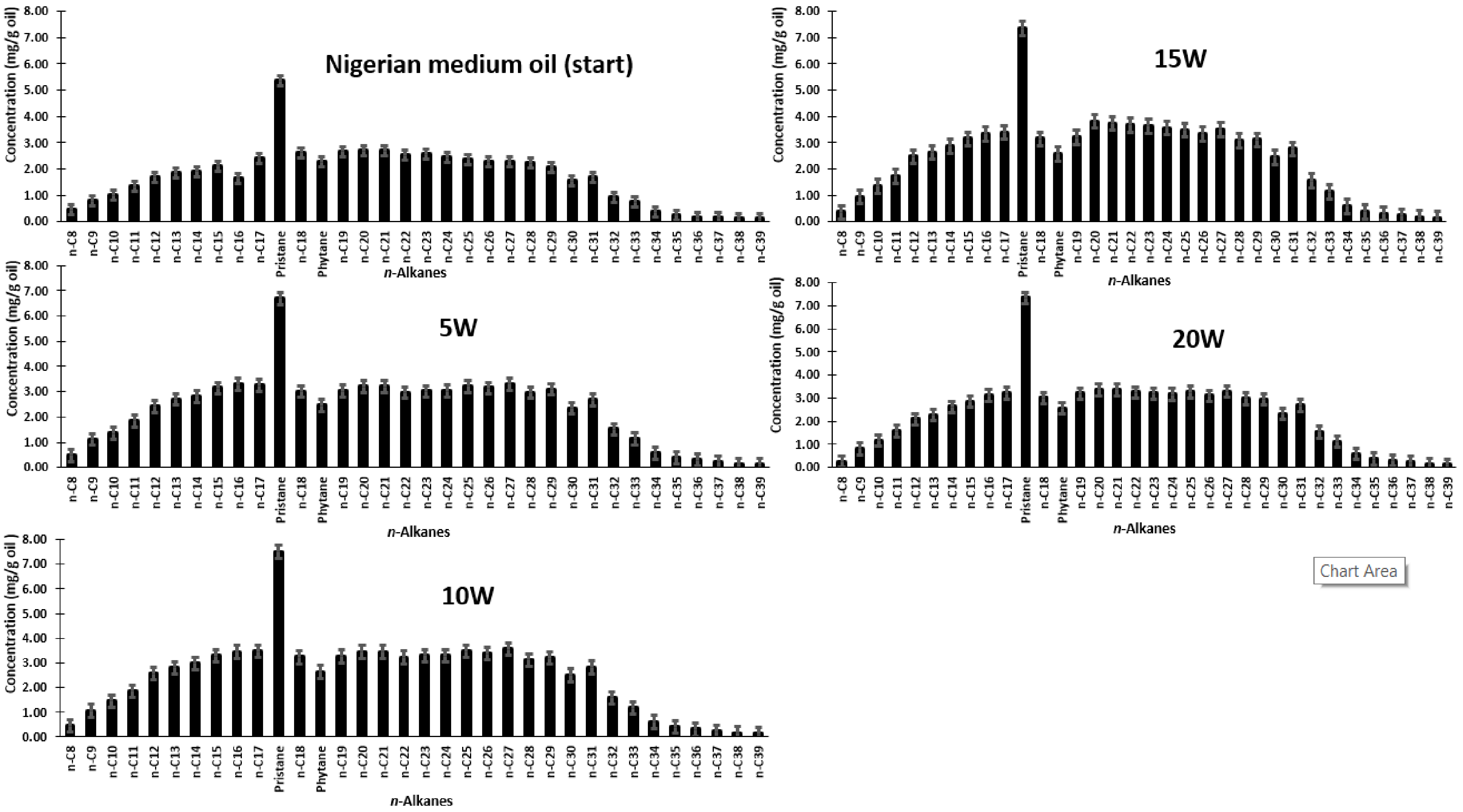


Figure 0.6: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane for Nigerian Medium oil after repeated water washings (5W, 10W, 15W and 20W).

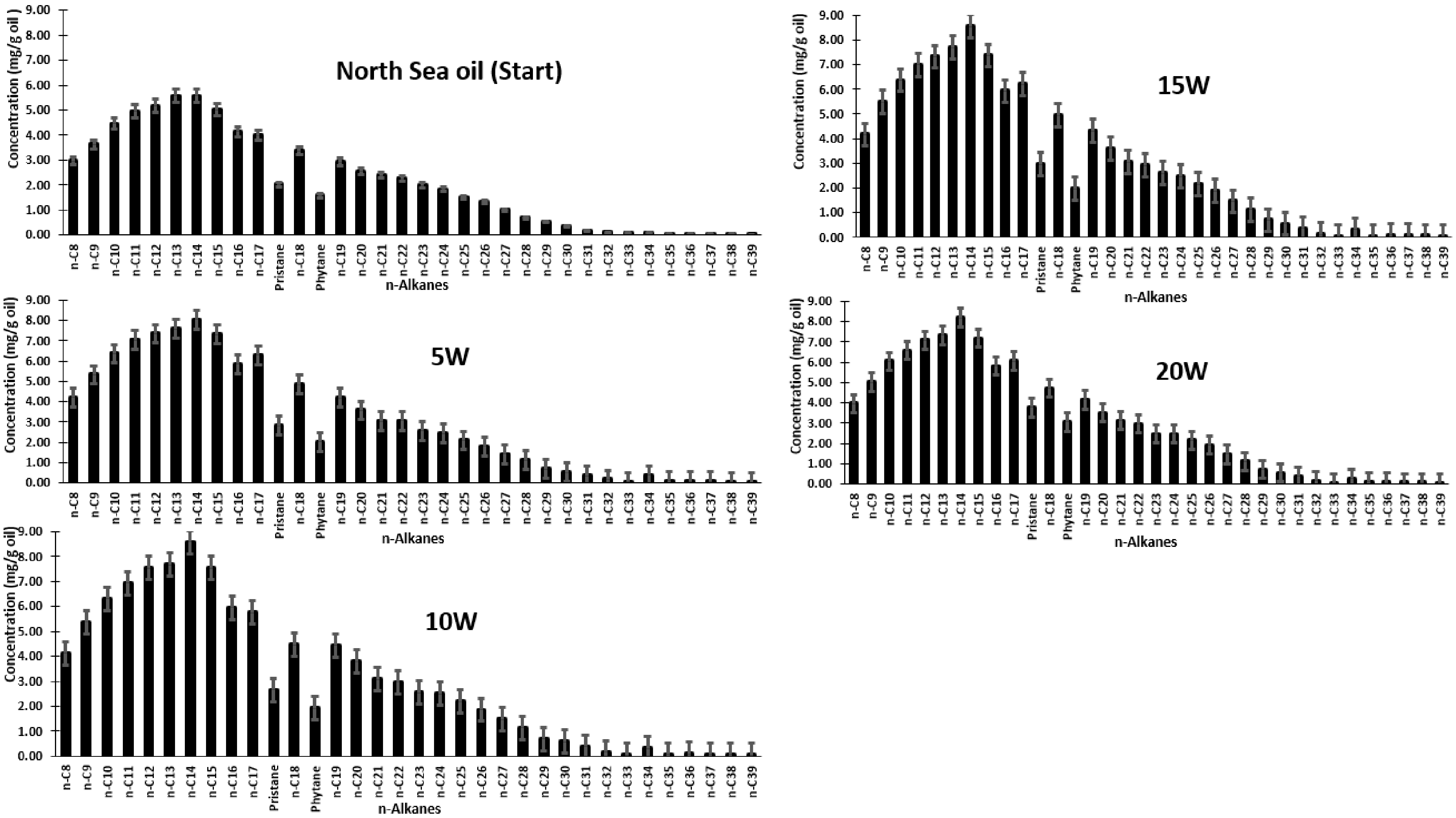


Figure 0.7: Graphical representation of concentration changes of *n*-alkanes, pristane and phytane for North Sea oil after repeated water washings (5W, 10W, 15W and 20W).

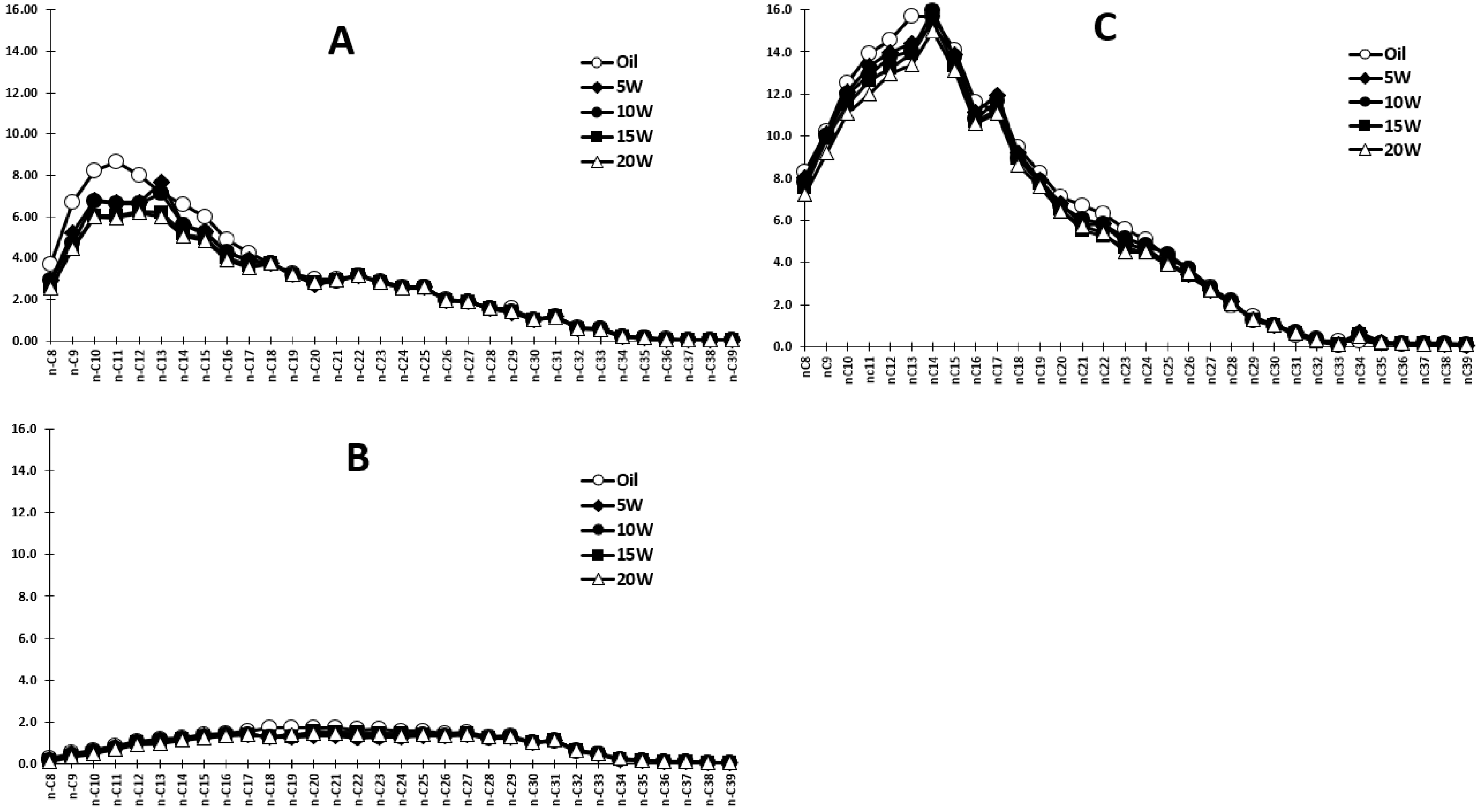


Figure 0.8: Plots of abundances of *n*-alkane in weathered oils relative to *n*-C_{30} for Nigerian light (A), Nigerian medium (B), and North Sea (C) oils after repeated water washings (5W, 10W, 15W and 20W).

4.4.2 Aromatic hydrocarbons composition and concentration changes

The aromatic fractions (F2) were also analysed and the GC/MS TIC chromatograms (in SIM mode) weathered by water washing; the results from 5 (5W), 10 (10W), 15 (15W), and 20 (20W) times washes, are shown in Figure 0.9, Figure 0.10, and Figure 0.11 for the Nigerian Light and Medium, and North Sea oils respectively. The summary of the quantitations of the measured concentrations of the target PAHs are shown in Table 0.4, Table 0.5, and Table 0.6 for the Nigerian light and medium, and North Sea oils respectively, which are mean values of triplicate samples with average relative standard deviations (RSD) of 5%, and up to 9% for some samples at 20 times (20W) number of washings. The measured concentrations of the PAHs were calculated as described in chapter 3 (see equations 3.7 and 3.8 in section 3.4.5). Homologous series of the alkylated PAHs were integrated with straight base lines carefully maintained for each peak for consistency (Yang *et al.*, 2014) and calculated based on mean values of triplicate samples for the Nigerian light, Nigerian medium and North Sea oils respectively . The relative response factors (RRFs) used were as described in chapter 4 (see section 3.4.3) as samples were analysed under the same GC/MS operating conditions.

Polycyclic aromatic hydrocarbons (PAH) and their alkylated homologous were analysed, including the well-known EPA priority pollutants Naphthalene, phenanthrene, dibenzothiophene, fluorene and chrysene amongst others are important pollutants to consider at all levels of risk assessment and remedial decision-making, as well as in the field of environmental forensics, for oil source identification and spill assessment and to distinguish petrogenic sources from biological and combustion derived sources of PAHs in the environment (Venkatesan, 1988; Wang *et al.*, 1999; Wang *et al.*, 2001; Bertilsson and Widenfalk, 2002; Laane *et al.*, 2006; Boehm *et al.*, 2007; Jiao *et al.*, 2009; Antle *et al.*, 2014; Stogiannidis and Laane, 2015). The detailed effects of water washing weathering on these alkylated PAHs are analysed and discussed in this chapter. The parent PAHs (Table 0.4,Table

0.5,Table 0.6) such as naphthalene (C_0-N), biphenyl (C_0-B), dibenzofuran (C_0-DBF) etc. are more affected by water washing than their alkylated homologues such as methylnaphthalenes (C_1-N), dimethylnaphthalenes (C_2-N), and trimethylnaphthalenes (C_3-N), tetramethylnaphthalanes (C_4-N), pentamethylnaphthalenes (C_5-N), methylbiphenyls (C_1-B), dimethylbiphenyls (C_2-B) etc because the parent PAHs are more soluble, hence are more susceptible to weathering than their daughter homologues (Lafargue and Barker, 1988; Wang and Fingas, 1995; Overton *et al.*, 2016; Tarr *et al.*, 2016). Susceptibility to weathering or solubility by the parent PAHs however generally decreased with increase in carbon number (Lafargue and Barker, 1988; Wang and Fingas, 1995). Aromatic compounds are generally known to be far more soluble than alkanes with same carbon number or molecular weight (Bobra, 1992). Table 0.4 shows a decrease in absolute concentrations of naphthalene and it alkylated homologues with increased washing for the Nigerian Light oil. However, the relative compositions (%) shows an increase in compositions for the alkylated homologues as parent naphthalene (C_0-N) has a reduced relative composition from 3% ($\frac{247.3}{8483.0} \times 100$) in the starting oil to 2% after washing 20 times (20W) (see Table 0.4). Similarly, an increase from 16% to 18% was observed for C_1-N, a decrease of 56% to 52% for C_2-N, an increase from 17% to 18% for C_3-N, and an increase from 10% to 11% for C_4-N respectively. Considering the percentage loss of the homologous naphthalenes of the starting oils after washing (x20), 45% $(\frac{247.3-136.8}{247.3}) \times 100$ of naphthalene (C_0-N) was loss, and similarly, 5% methylnaphthalenes (C_1-N), 22% dimethylnaphthalenes (C2-N), 10% trimethylnaphthalenes (C_3-N), and 5% tetramethylnaphthalenes (C_4-N) respectively. The aqueous solubility of the methylnaphthalenes is very low comparing the solubilities of 55 mg/L for naphthalene, 50 mg/L for methylnaphthalenes and 4 mg/L for dimethylnaphthalenes perhaps as a result of sampling of non-homogenous portion or inadequate turbulence during shaking (e.g., Lafargue and Barker, 1988). For the Nigerian medium oil, the relative composition (%) of naphthalene

(C_0-N) decreased from 5 to 2%, C_1-N from 20 to 7%, C_2-N from 37 to 36% respectively, whereas C_3-N increased from 22 to 33%, and C_4-N from 17 to 22% respectively (see Table 0.5), and 68% losses for naphthalene (C_0-N), 68% for methylnaphthalenes (C_1-N), 15% for dimethylnaphthalenes (C_2-N), whereas trimethylnaphthalenes (C_3-N) increased by 28%, and tetramethylnaphthalenes (C_4-N) by 14% respectively. The aqueous solubilities of naphthalene (55 mg/L), methylnaphthalene (50 mg/L) and dimethylnaphthalene (4 mg/L) relative to the percentage losses for the Nigerian Medium oil were as expected (e.g., Lafargue and Barker, 1988). For the North Sea oil (see Table 0.6), the relative composition (%) of naphthalene (C_0-N) also decreased with number of washing from 15 to 6%, C_1-N from 21 to 20%, whereas C_2-N increased from 23 to 33%, 22 to 30%, and C_4-N from 12.2 to 12.4% respectively, and these are similar to results reported by Chang *et al*, and Overton *et al*, (2016) (Chang *et al.*, 2016; Overton *et al.*, 2016). All the naphthalenes in the North Sea oil were almost loss to about 99% of the starting oil, and the aqueous solubility reduced with increased in homologous series. The naphthalenes are observed to be more affected by evaporative weathering than water washing except for the tetramethylnaphthalanes. This is similar for other target PAHs except that the relative variation reduced with increase in aromatic rings as solubility or degradation reduces with increase in rings, with the chrysenes showing the least changes (Wang and Fingas, 1995; Stogiannidis and Laane, 2015). The heteroatom-containing aromatics dibenzofurans (DBFs) and dibenzothiophenes (DBTs) for all the three oils are observed to be affected by water washing due to their relative susceptibility to water washing, but the effect reduced with increase alkyl carbon number (e.g., Palmer, 1993; Wang *et al.*, 2016a).

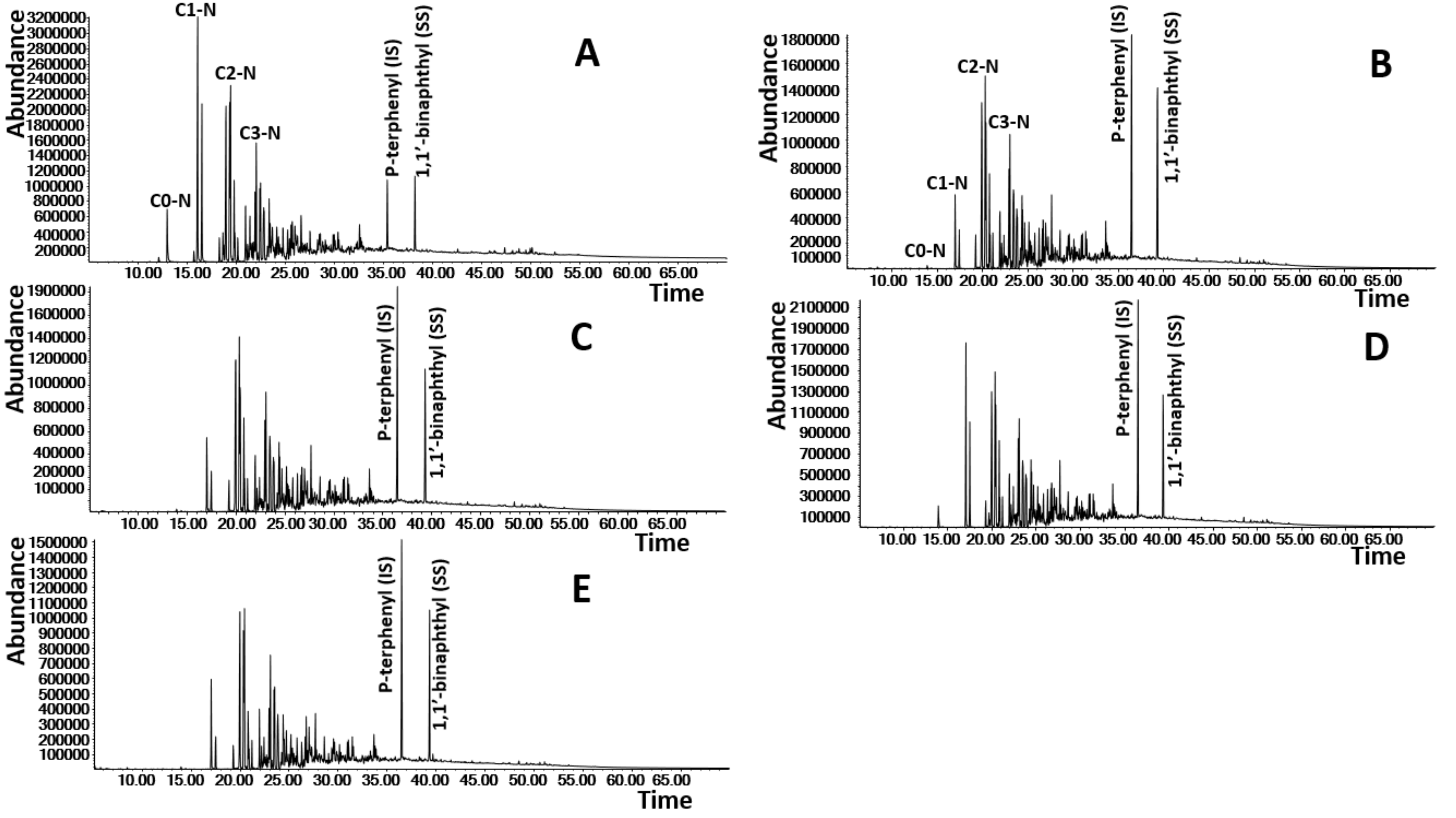


Figure 0.9: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian Light oil weathered by water washing, (A) start, (B) 5W, (C) 10W, (D) 15W, (E) 20W, respectively.

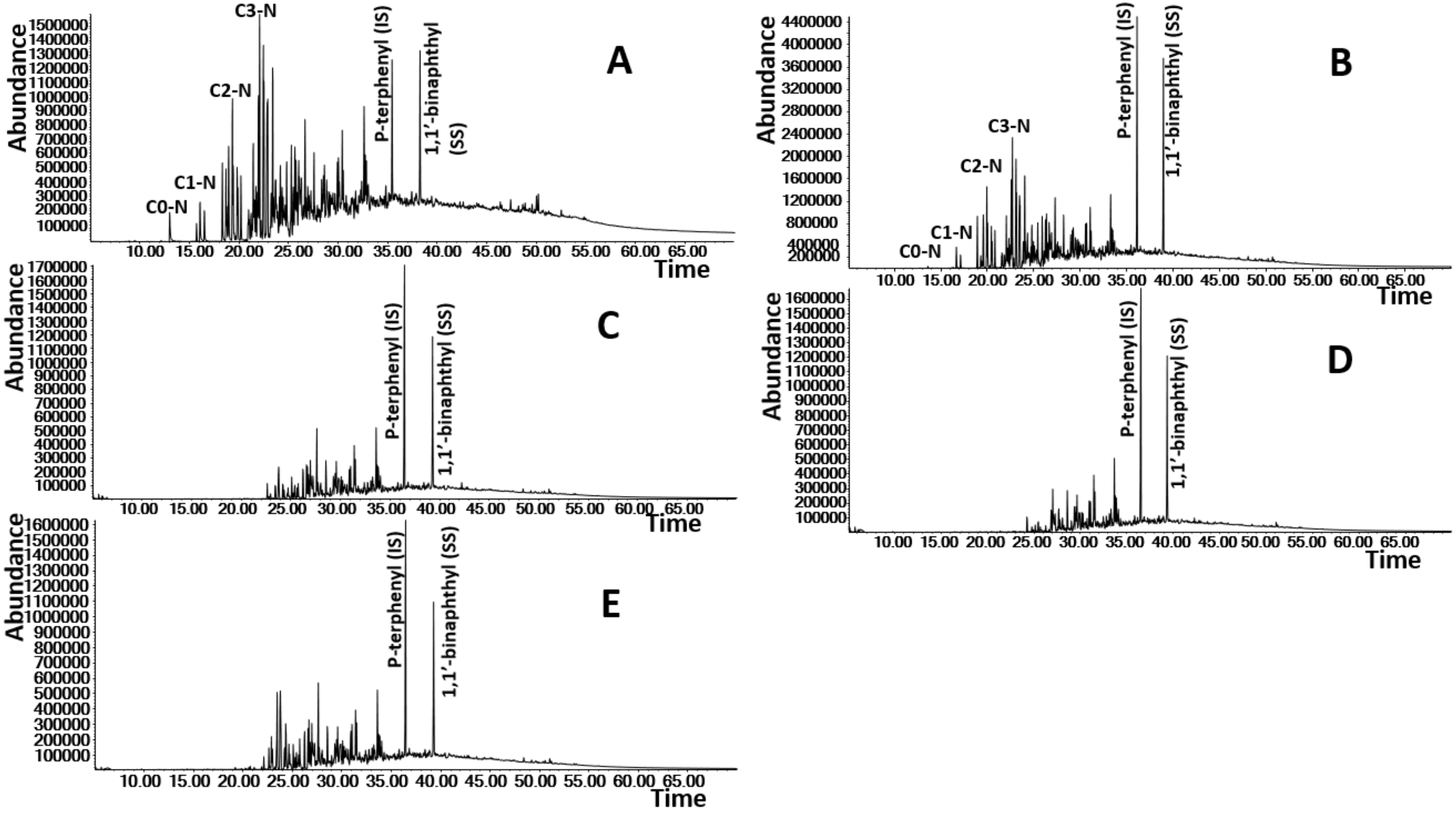


Figure 0.10: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian Medium oil weathered by water washing, (A) start, (B) 5W, (C) 10W, (D) 15W, (E) 20W, respectively.

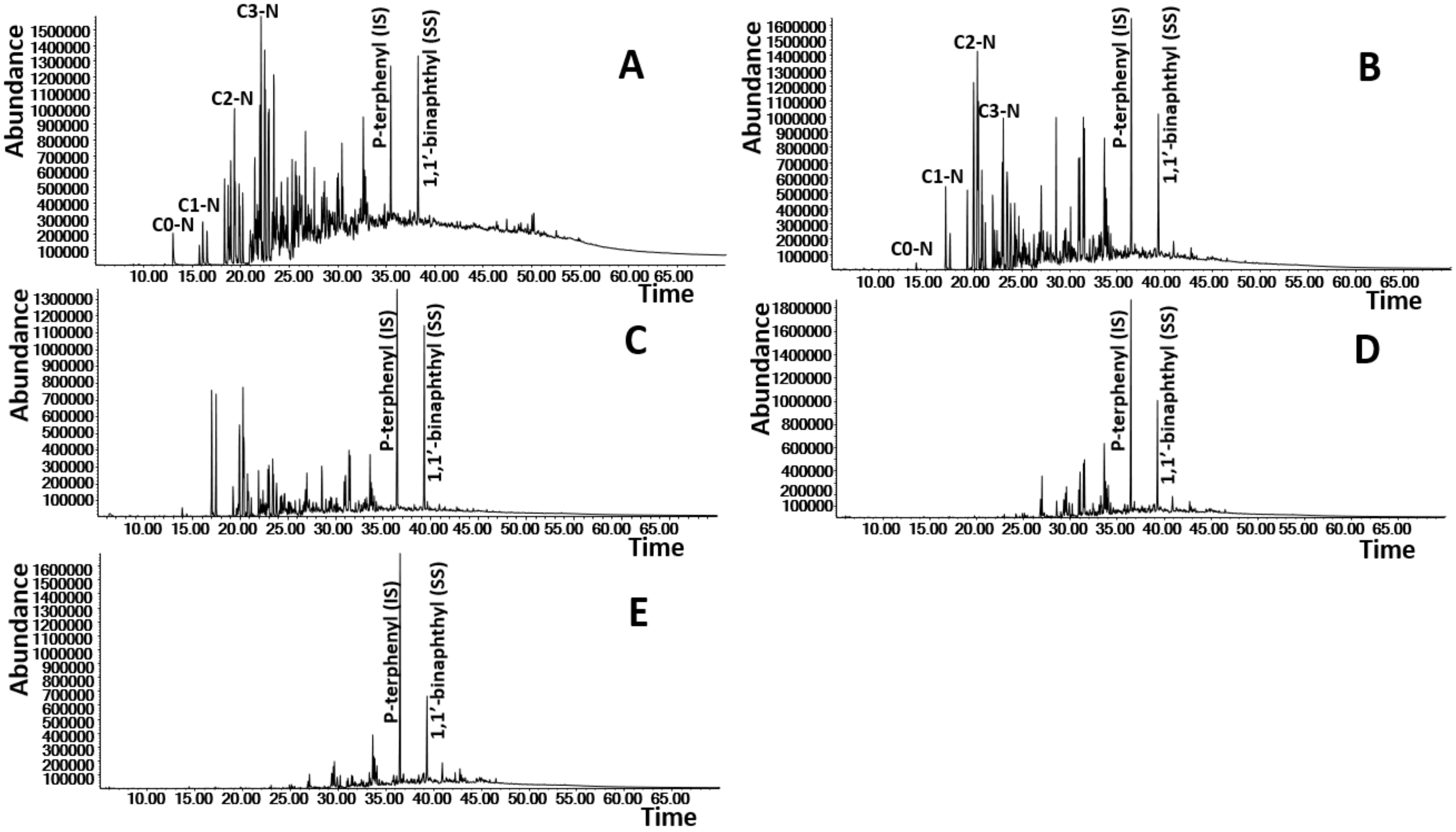


Figure 0.11: GC/MS SIM/TIC Chromatograms of aromatic compounds in the North Sea oil weathered by water washing, (A) start, (B) 5W, (C) 10W, (D) 15W, (E) 20W, respectively.

The methylnaphthalene (2-MN/1-MN) ratio, which is unaffected by light weathering and also used to distinguish products with different methylnaphthalene compositions (Wang and Stout, 2007; Stogiannidis and Laane, 2015) and phenanthrene/anthracene (C_0-N/C_0-Anth) ratio, used to distinguish petrogenic and pyrogenic sources of PAHs (Grimmer *et al.*, 1981; Guo *et al.*, 2007; Stogiannidis and Laane, 2015) for all the three oil samples are relatively unaffected by the weathering (see Table 0.4Table 0.5Table 0.6). Figure 0.12Figure 0.13Figure 0.14 show the distribution of the target alkylated PAHs for the Nigerian Light and Medium, and North Sea oils respectively, which have "bell shaped" alkylated homologues distributions, a characteristic signature for PAHs hydrocarbons of petrogenic origin (Wang *et al.*, 1999; Stogiannidis and Laane, 2015) .

The effect of water washing on target isomers of methylbiphenyls (C_1-B) and methylphenanthranes (C_1-P) were assessed (see Figure 0.15Figure 0.16) and the concentrations are shown in Table 0.7,Table 0.8, Table 0.9 for Nigerian Light and Medium, and North Sea oils respectively. The 3-methylbiphenyl (3-MBp) isomer dominates in the Nigerian Light and North Sea oils, while the 4-methylbiphenyl (4-MBp) isomer dominates in the Nigerian medium oil. The distribution patterns of Nigerian Light and Medium oils are similar and different from that of North Sea oil. It is visibly obvious that the most affected isomers with increased water washing are that of the North Sea oil, while that of the Nigerian light and medium oils are less affected. The distribution pattern of the methylphenanthrene isomers for Nigerian light and medium oils are very similar with clearly higher concentrations of the methylphenanthrene isomers in the undegraded oil than the degraded isomers, while that of the North Sea oil is different (see Figure 0.16). It is however observed that the five methylphenanthrene isomers are relatively little affected by water washing.

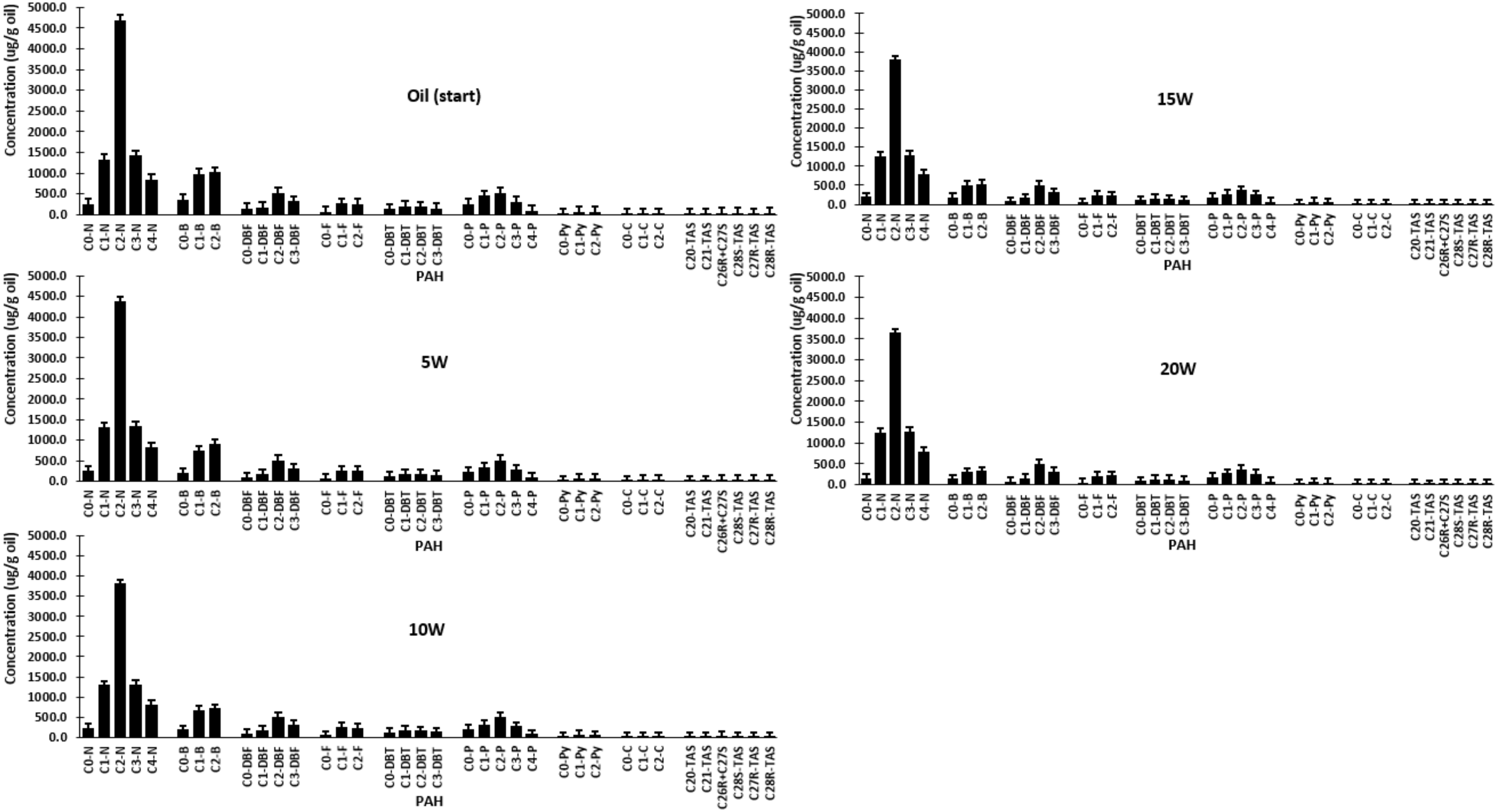


Figure 0.12: Distribution of target alkylated PAHs showing the number of water washing for Nigerian light oil. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

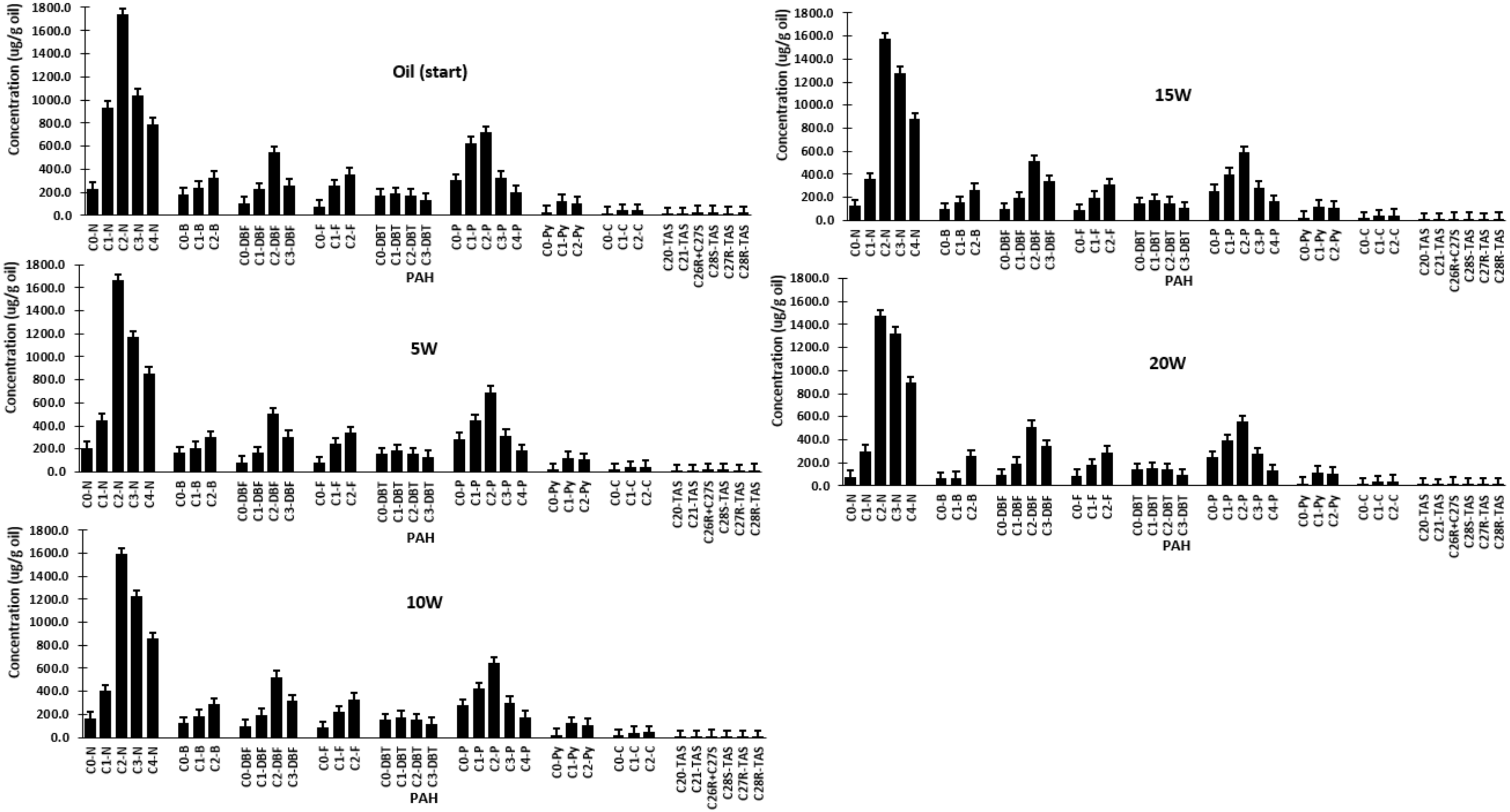


Figure 0.13: Distribution of target alkylated PAHs showing the number of water washing for Nigerian medium oil. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

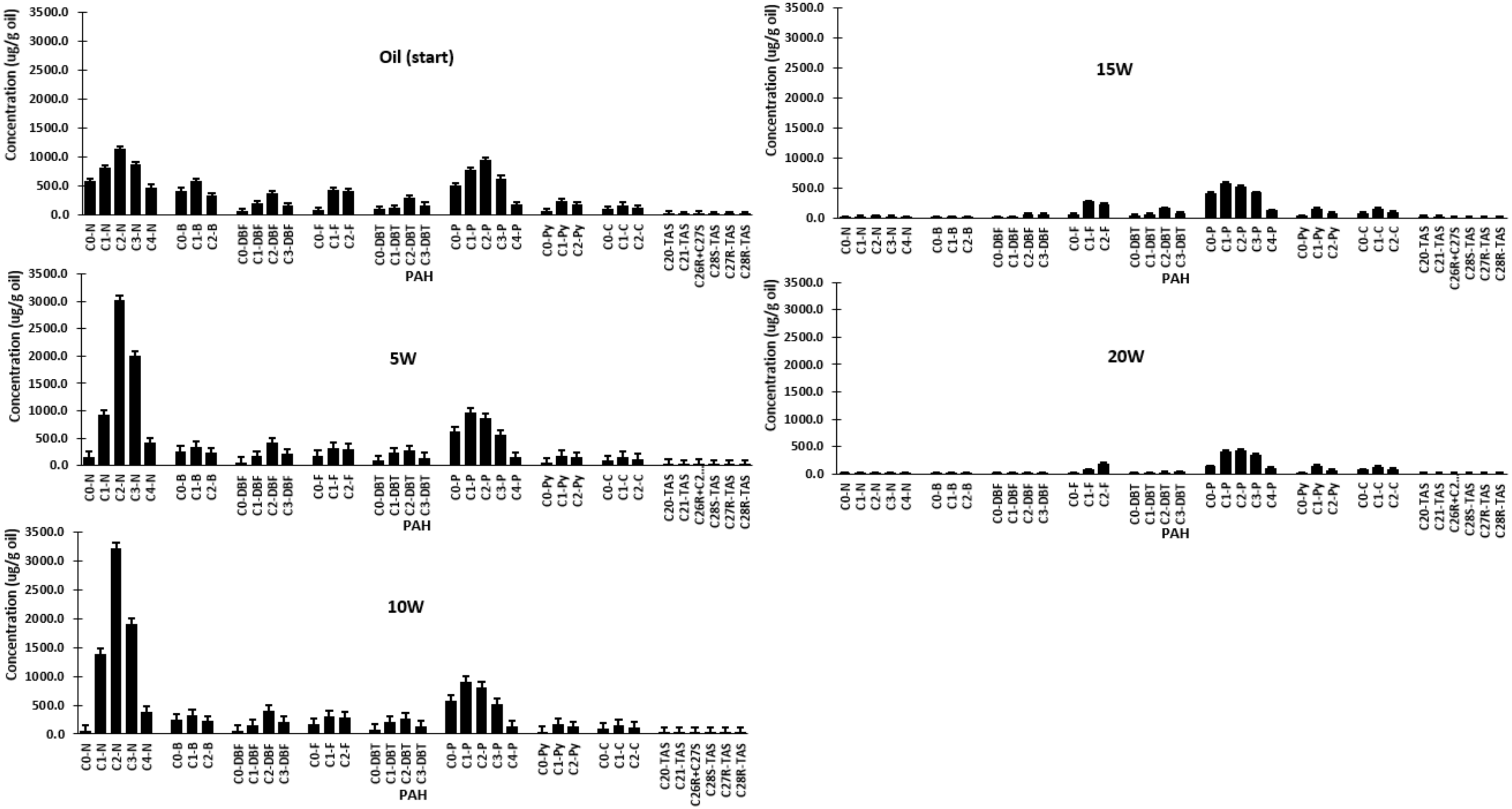


Figure 0.14: Distribution of target alkylated PAHs showing the number of water washing for Nigerian medium oil. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

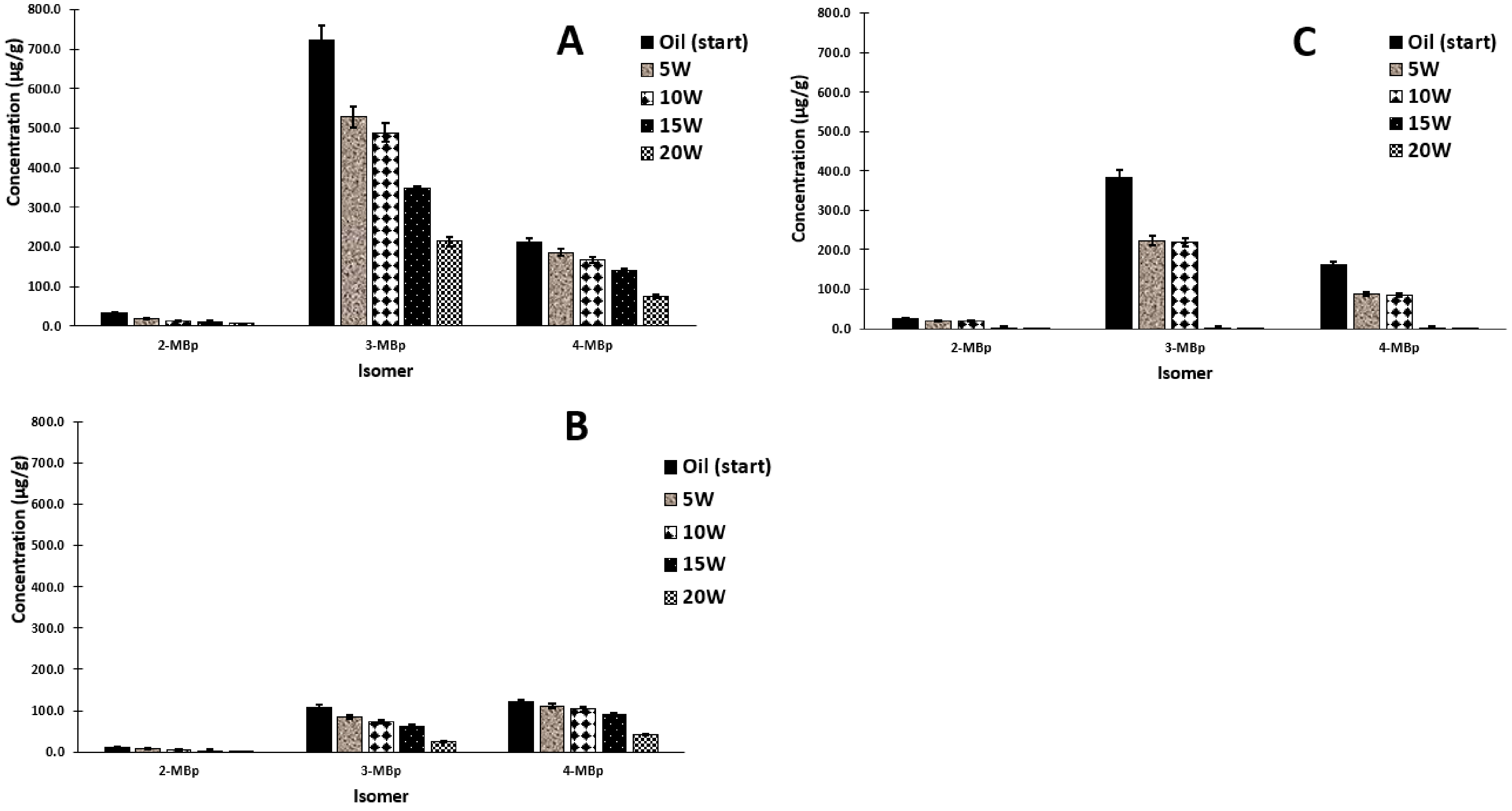


Figure 0.15: Effect of water washing on methylbiphenyl isomers for (A) Nigerian light, (B) Nigerian medium, (C) North Sea oil respectively.

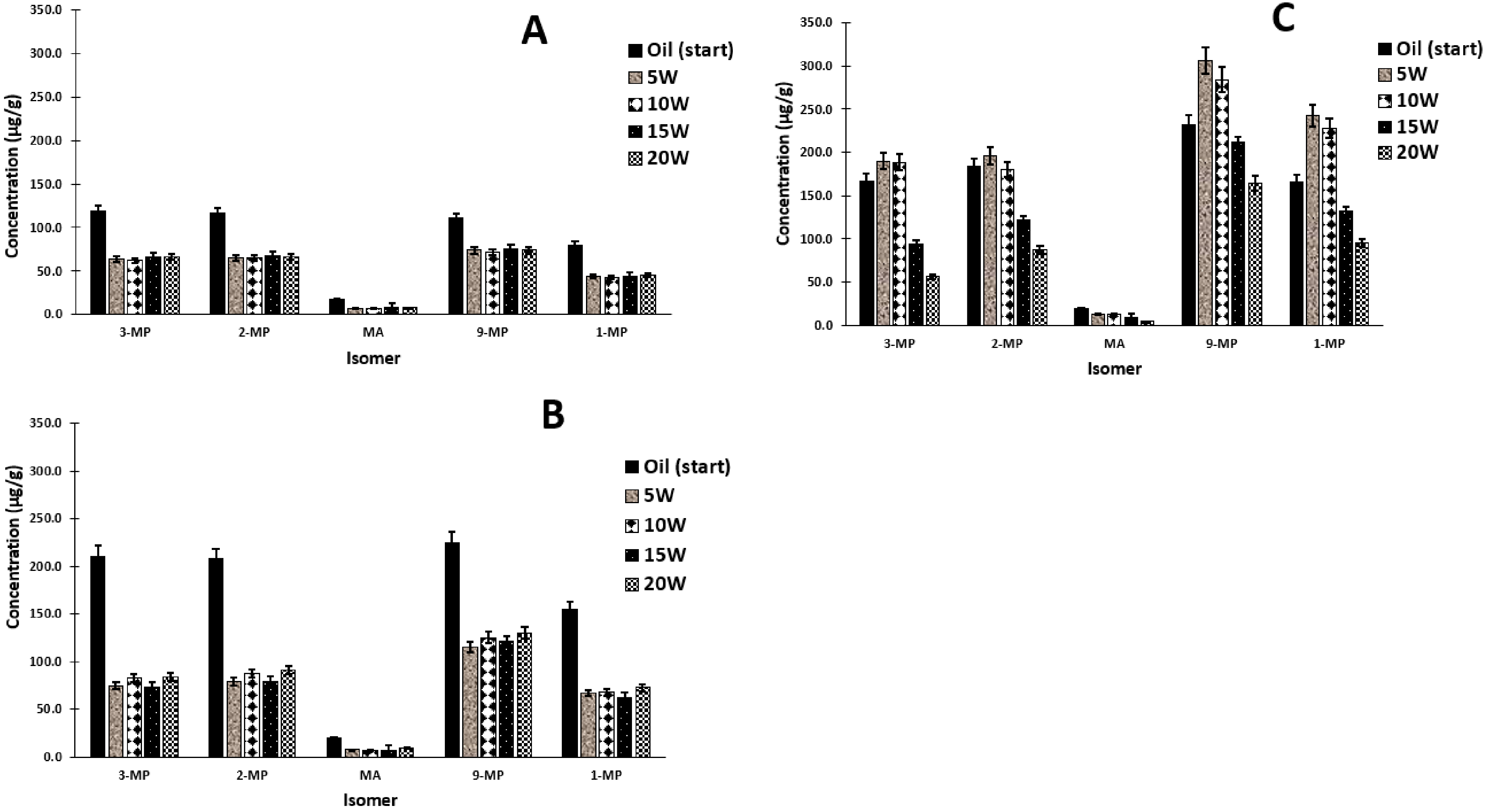


Figure 0.16: Effect of water washing on phenanthrene isomers for (A) Nigerian light, (B) Nigerian medium, (C) North Sea oil respectively

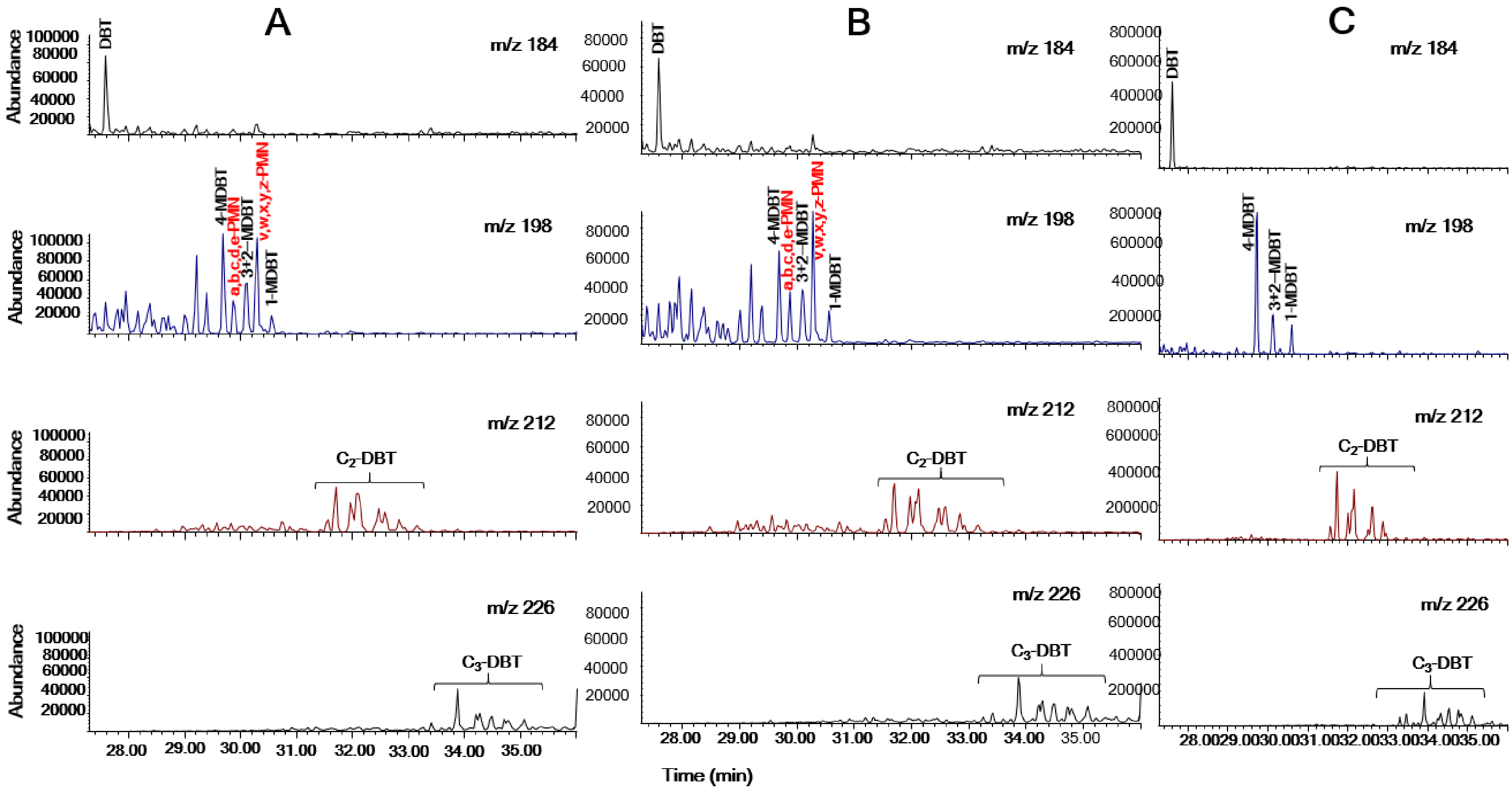


Figure 0.17: Chromatograms showing two identified alkylated PAHs (a,b,c,d,e-PMN and v,w,x,y,z-PMN) observed in Nigerian Light oil (A) and Nigerian Medium oil (B) which are not obvious in North Sea oil (C)

Along with the methylddibenzothiophenes, two additional peaks were observed in the m/z 198 ion chromatograms of the two Nigerian oils, which are not so resolved in the North Sea oil, these are most likely to be pentamethylnaphthalenes isomers and are, labelled as a,b,c,d,e-PMN and v,w,x,y,z-PMN in Figure 0.17. Their potential as diagnostic ratios for oil-oil correlation and differentiation were tested by normalising each with respect to the most well resolved 4-methyldibenzothiophene for a double ratio (S/U) assessment as a more thermodynamically stable isomer used for oil spill identification and differentiation (Wang and Fingas, 1995; Mitra *et al.*, 1999; Wang *et al.*, 1999; Stout, 2007; Stogiannidis and Laane, 2015). These ratios for each of the three oil samples (see Table 0.4,Table 0.5, and Table 0.6) remained relatively similar to that of evaporative weathering in chapter 3 (see Tables 3.4, 3.5, and 3.6 Section 3.4.5), and the 4-MDBT: a,b,c,d,e-PMN: v,w,x,y,z-PMN ratios as 1.00:0.43:1.10, 1.00:0.41:1.10, 1.00:0.41:0.80, 1.00:0.42:0.79, and 1.00:0.42:0.79 for start oil, 5W, 10W, 15W, and 20W respectively for the Nigerian Light oil and 1.00:0.51:1.39, 1.00:0.53:1.33, 1.00:0.51:1.41, 1.00:0.52:1.40, and 1.00:0.50:1.34 for start oil, 5W, 10W, 15W, and 20W respectively for Nigerian Medium oil. Table 0.10 shows the concentrations and relative percentage compositions (%) of the methyldibenzothiophenes and pentamethylnaphthalenes isomers used to calculate the ratios, and the reasonably consistent relative percentage compositions (%) for the two Nigerian oils is a strong positive indicator of their potential as diagnostic ratios for oil spill identification and differentiation.

4.4.3 Biomarker compound composition and concentration changes.

Biomarkers are an important group of hydrocarbons in petroleum that have been used for many years for exploration and reservoir assessment purposes by geochemists. These purposes include the determination of thermal maturity of oils, type of source material, depositional conditions and history of source material, geological age of source rock, determination of oil mixing from different sources, and extent of alteration of oil in the reservoir (Seifert and Moldowan, 1978; Mackenzie *et al.*, 1980; Seifert and Moldowan, 1980; Mackenzie *et al.*, 1981; Mackenzie, 1984; Rullkötter *et al.*, 1984; Peters *et al.*, 2005; Wang *et al.*, 2006). Biomarkers also provide information during forensic investigations for source identification of oil spills, differentiation, correlation and monitoring of degradation and weathering of oils under different environmental conditions due to their relatively high resistance to degradation (Peters *et al.*, 2005; Wang *et al.*, 2006). Oils from different sources differ in biomarker absolute concentrations and relative distribution patterns, hence, their analysis can provide information on the overall weathering state of spilled oil in the environment (Rullkötter *et al.*, 1984; Wang and Fingas, 1995). Steranes and terpenoid biomarkers are the ones most commonly considered during forensic investigation for source identification. Terpanes are saturated hydrocarbon terpenoids that occur in petroleum in homologous or pseudo homologous series classified on the isoprene units that they are made up from and they include bicyclic, tricyclic, tetracyclic and pentacyclic compounds (Stout and Wang, 2016a). Whereas the steroids include the regular and rearranged steranes, mono- and triaromatic steranes, and also, many PAHs (Seifert and Moldowan, 1978; Malmborg, 2017). Other biomarkers such as bicyclic sesquiterpanes, and also non-biomarker geochemical markers (because they have no obvious biological precursors) such as diamondoids and some monoaromatic compounds which are mostly found in condensates and petroleum products or distillates because of their low ranges of boiling points are used for spill source identification for light petroleum products (Stout *et al.*, 2005; Wang *et al.*, 2005; Wang *et al.*, 2006; Yang *et al.*, 2006; Wang and Stout, 2007; Wang, 2008; Yang *et al.*, 2009; Yang *et al.*, 2012; Malmborg, 2017). The terpanes mainly considered for diagnostic analysis in this project include tricyclic, tetracyclic and pentacyclic terpanes, while others include bicyclic sesquiterpanes. Adamantane geochemical markers were also considered.

Figure 0.18, Figure 0.19Figure 0.20 are the m/z 191 mass chromatograms showing terpane distributions for the Nigerian light, Nigerian medium, and North Sea oils, respectively. The terpane concentrations measured are shown in biomarkers is main objective of this chapter, to ascertain their extent of resistance and potentials as diagnostic compounds during spill investigations as well as extent of oil alteration in the reservoir (Thompson, 1979; Thompson, 1987; Lafargue and Barker, 1988; Kuo, 1994; ZHANG and ZHANG, 2000; López and Lo Mónaco-Trupiano, 2016; Wang *et al.*, 2016a). The terpanes are generally unaffected by water washing as the concentrations generally increased slightly relative in the weathered oils with increased water washing for all three oil probably as a result of the decrease in the concentrations of the more soluble compounds (see Table 0.11Table 0.12Table 0.13)

,Table 0.12Table 0.13. The numbers in Figure 0.18, Figure 0.19, Figure 0.20 represent the terpanes listed in biomarkers is main objective of this chapter, to ascertain their extent of resistance and potentials as diagnostic compounds during spill investigations as well as extent of oil alteration in the reservoir (Thompson, 1979; Thompson, 1987; Lafargue and Barker, 1988; Kuo, 1994; ZHANG and ZHANG, 2000; López and Lo Mónaco-Trupiano, 2016; Wang *et al.*, 2016a). The terpanes are generally unaffected by water washing as the concentrations generally increased slightly relative in the weathered oils with increased water washing for all three oil probably as a result of the decrease in the concentrations of the more soluble compounds (see Table 0.11Table 0.12Table 0.13)

, Table 0.12, and Table 0.13 and the labelled peaks represent unidentified compounds. The peak numbers 1, 2, 3 and 24, labelled as S, Q, M, and T are the unidentified peaks discussed in chapter 3 (see section 3.4.6), with peaks S (1), Q (2), and M (3) in the two Nigeran oils and T (24) in all the three oils. It is possible that peaks S (1), Q (2), and M (3) are tricyclic terpanes, and peak T (24) is tetracyclic terpanes due to their elution times within the regions of other identified terpanes. Further verification and structural identification of these compounds would form future work. Due to the similarities in relative abundances of these unidentified peaks to that of other known terpanes, their diagnostic ratios were measured to assess their diagnostic potentials as discussed in chapter 3. The peak numbers 6, 11, and 19 in the two Nigerian oils (see Figure 0.18Figure 0.19) appear to be the novel terpanes identified in tertiary deltaic oils previously reported (Samuel *et al.*, 2010). Samuel *et al* (2010), labelled the peaks as X (6), Y (11), and Z1 (19) respectively, and that X (6) and Y (11) are tricyclic terpanes while Z1 (19) a tetracyclic terpane, and also peaks Y1 (12) and Z (15) as tricyclic terpanes identified in the work as explained in chapter 3 (see section 3.4.6). Unidentified peaks were also found in the North Sea oil with peaks numbers 5, 12, 13 and 18 labelled as U, U1, and U2 respectively, subject to further verification. Assessing the effect of weathering by water washing on these

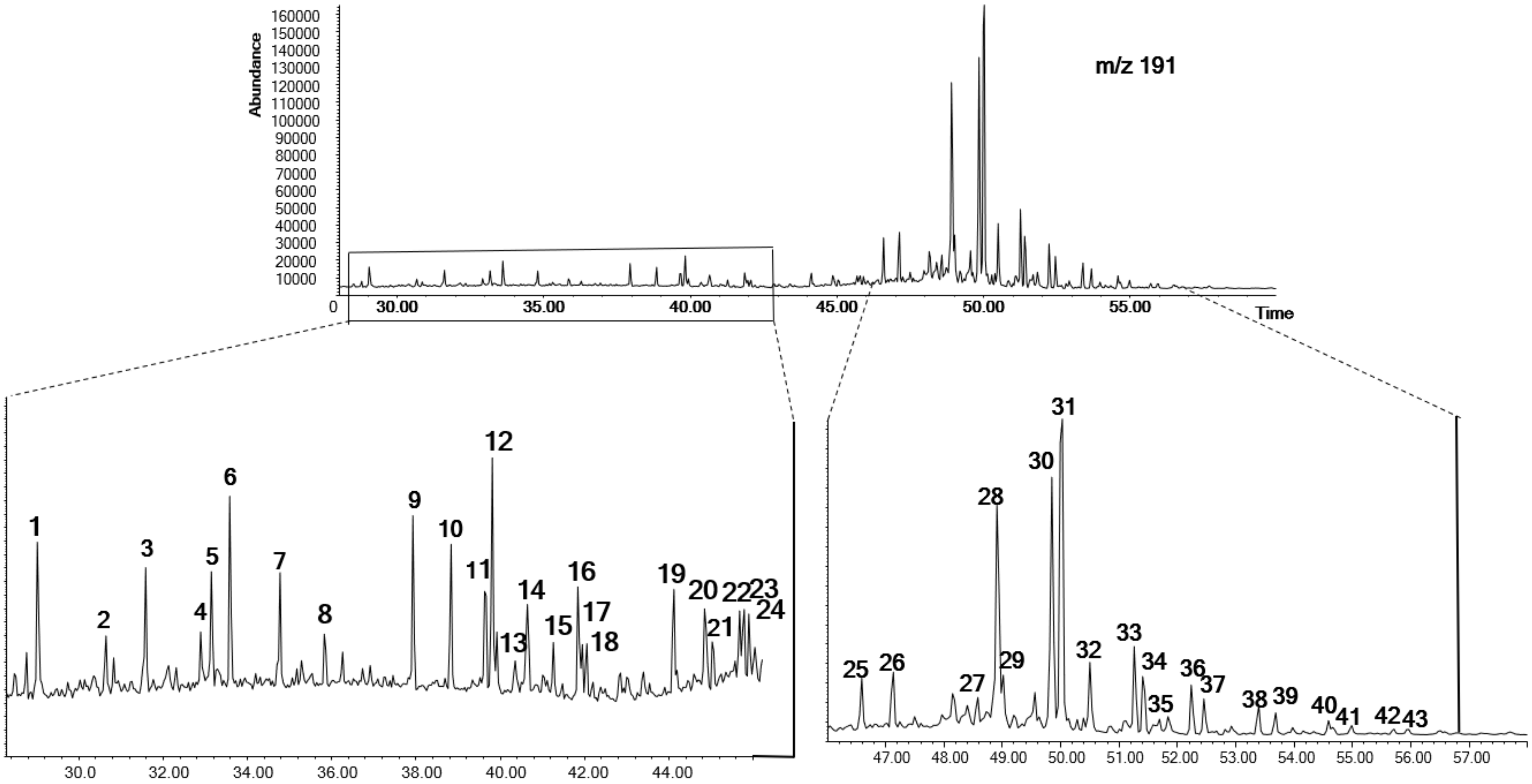


Figure 0.18: m/z 191 mass chromatograms showing terpane distributions from unweathered Nigerian Light oil. The numbers represent the corresponding peak names listed in biomarkers is main objective of this chapter, to ascertain their extent of resistance and potentials as diagnostic compounds during spill investigations as well as extent of oil alteration in the reservoir (Thompson, 1979; Thompson, 1987; Lafargue and Barker,

1988; Kuo, 1994; ZHANG and ZHANG, 2000; López and Lo Mónaco-Trupiano, 2016; Wang *et al.*, 2016a). The terpanes are generally unaffected by water washing as the concentrations generally increased slightly relative in the weathered oils with increased water washing for all three oil probably as a result of the decrease in the concentrations of the more soluble compounds (see Table 0.11Table 0.12Table 0.13)

. Numbers in red represent unidentified peaks.

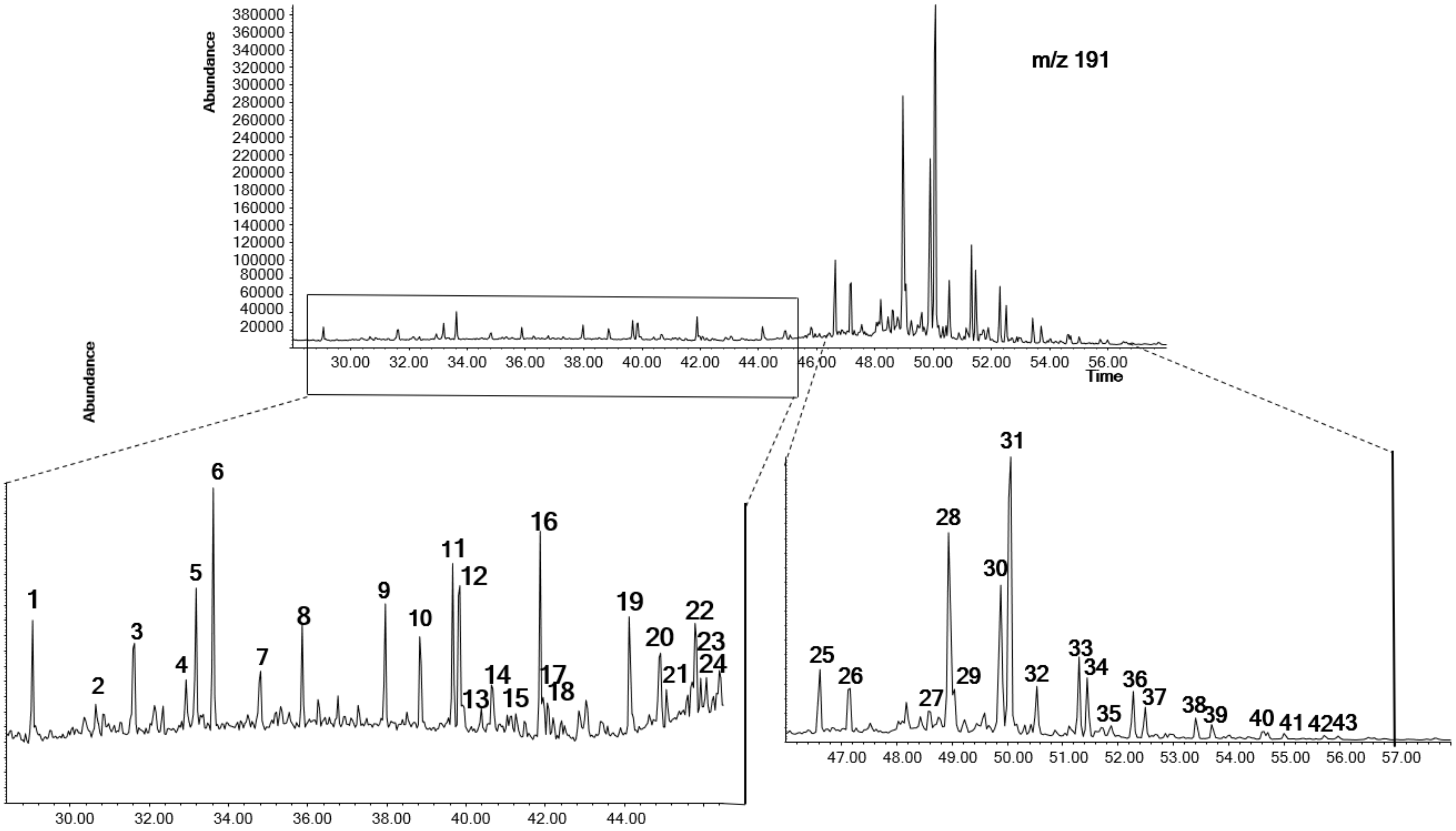


Figure 0.19: m/z 191 mass chromatograms showing terpane distributions from unweathered Nigerian Medium oil. The numbers represent the corresponding peak names listed in Table 0.12. Numbers in red represent unidentified peaks.

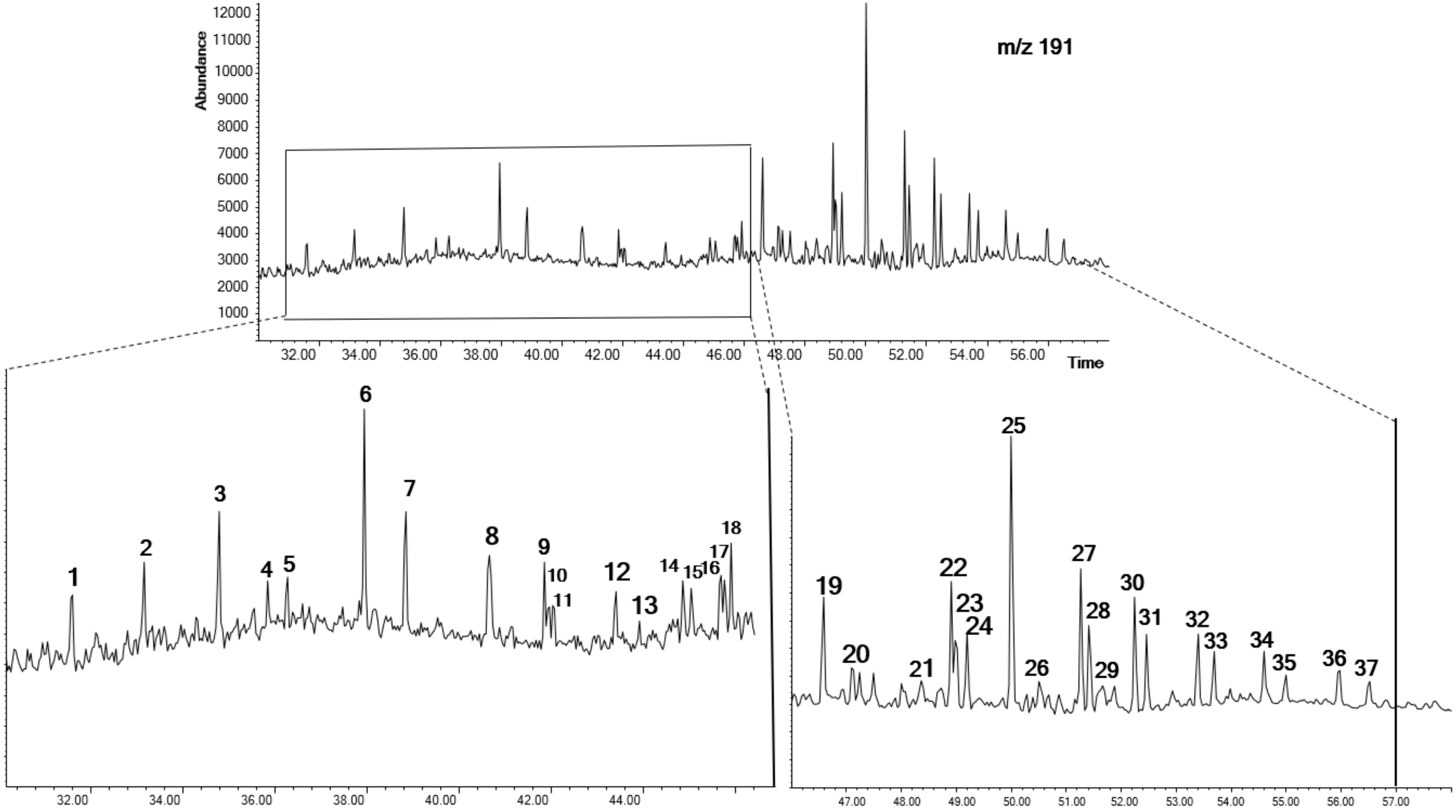


Figure 0.20: m/z 191 mass chromatograms showing terpane distributions from unweathered North Sea oil. The numbers represent the corresponding peak names listed in Table 0.13. Numbers in red represent unidentified peaks.

biomarkers is main objective of this chapter, to ascertain their extent of resistance and potentials as diagnostic compounds during spill investigations as well as extent of oil alteration in the reservoir (Thompson, 1979; Thompson, 1987; Lafargue and Barker, 1988; Kuo, 1994; ZHANG and ZHANG, 2000; López and Lo Mónaco-Trupiano, 2016; Wang *et al.*, 2016a). The terpanes are generally unaffected by water washing as the concentrations generally increased slightly relative in the weathered oils with increased water washing for all three oil probably as a result of the decrease in the concentrations of the more soluble compounds (see Table 0.11Table 0.12Table 0.13)

Plots of diagnostic ratios of the both the identified terpanes and the unidentified terpanes to assess their potential as diagnostic ratios are shown in Figure 0.21. The diagnostic ratio plots of the unidentified terpanes peaks S (1), Q (2), M (4), X (6), Y (11), Y1 (12), Z (15), Z1 (19), and T (24) for the two Nigerian oils (see Figure 0.21 A and B) are equally as unaffected by the weathering as the plots of the identified terpanes such as the C_{23}, C_{24}, C_{27} Tm and Ts, C_{31} 22S and 22R etc. in the ratio forms of C_{23}/C_{24},S/(S+Q), Q/(Q+C_{19}), M/(M+C_{19}), X/(X+C_{19}), C_{27}(Ts/Tm), Y/(Y+C_{24}), Y1/(Y1+C_{24}), Z/(Z+C_{25}B), Z1/(Z1+C_{28}A), T/(T+C_{29}B) and C_{31} 22S/(22S+22R). The horizontal plots indicate the terpanes resistance to weathering by water washing and it is a good indication of potentials of the unidentified peaks as diagnostic ratios. Similarly, the unidentified peaks in the North Sea oil U (5), U1 (12), U2 (13) and T (18) are unaffected by the weathering as the diagnostic ratio plots are also horizontal compared to that of the identified terpanes in the two Nigerian oils stated above (see Figure 0.21C). The diagnostic ratio plots for the water washing weathering for all the three oil samples (Figure 0.21) are relatively as unaffected (Kuo, 1994; Wang *et al.*, 2016a) compared to that of the evaporative weathering (see Figure 3.28, section 3.4.6). Diagnostic ratio plots of C_{27}(Ts/Tm) and C_{23}/C_{24} for the North Sea oil increased by 50% with increased water washing which is not observed in the two Nigeria oils perhaps Tm becomes slightly more soluble than Ts with increased washing and similarly for C_{24} and C_{23} tricyclic terpanes which indicate inconsistency due to increased water washing (e.g., Wang *et al.*, 1994b).

Steranes were analysed in the three oil samples and the m/z 217 mass chromatograms showing their distributions are presented in Figure 0.22 with their peak assignments and measured concentrations shown in Table 0.14, Table 0.15, andTable 0.16 respectively for the three oils. The steranes like the terpanes are notably unaffected by increased level of water washing and the concentrations remained relatively unchanged. Figure 0.23 shows the diagnostic ratio plots of the steranes which include C_{20}/C_{21}, C_{21}/C_{22}, $C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$, $C_{27}20S/(20S+20R)$, $C_{28}\alpha\beta\beta/(\alpha\beta\beta+\alpha\alpha\alpha)$, $C_{29}\alpha\beta\beta/(\alpha\beta\beta+\alpha\alpha\alpha)$, and $C_{27}\alpha\beta\beta(S+R)/C_{28}\alpha\beta\beta(S+R) + C_{29}\alpha\beta\beta(S+R)$ for the three oils. The plots are almost horizontal too, which shows the resistance of the steranes to degradation by the level of water washing conducted in this experiment (Kuo, 1994; Wang *et al.*, 2016a). The water washing effect on steranes is relatively like that of evaporative

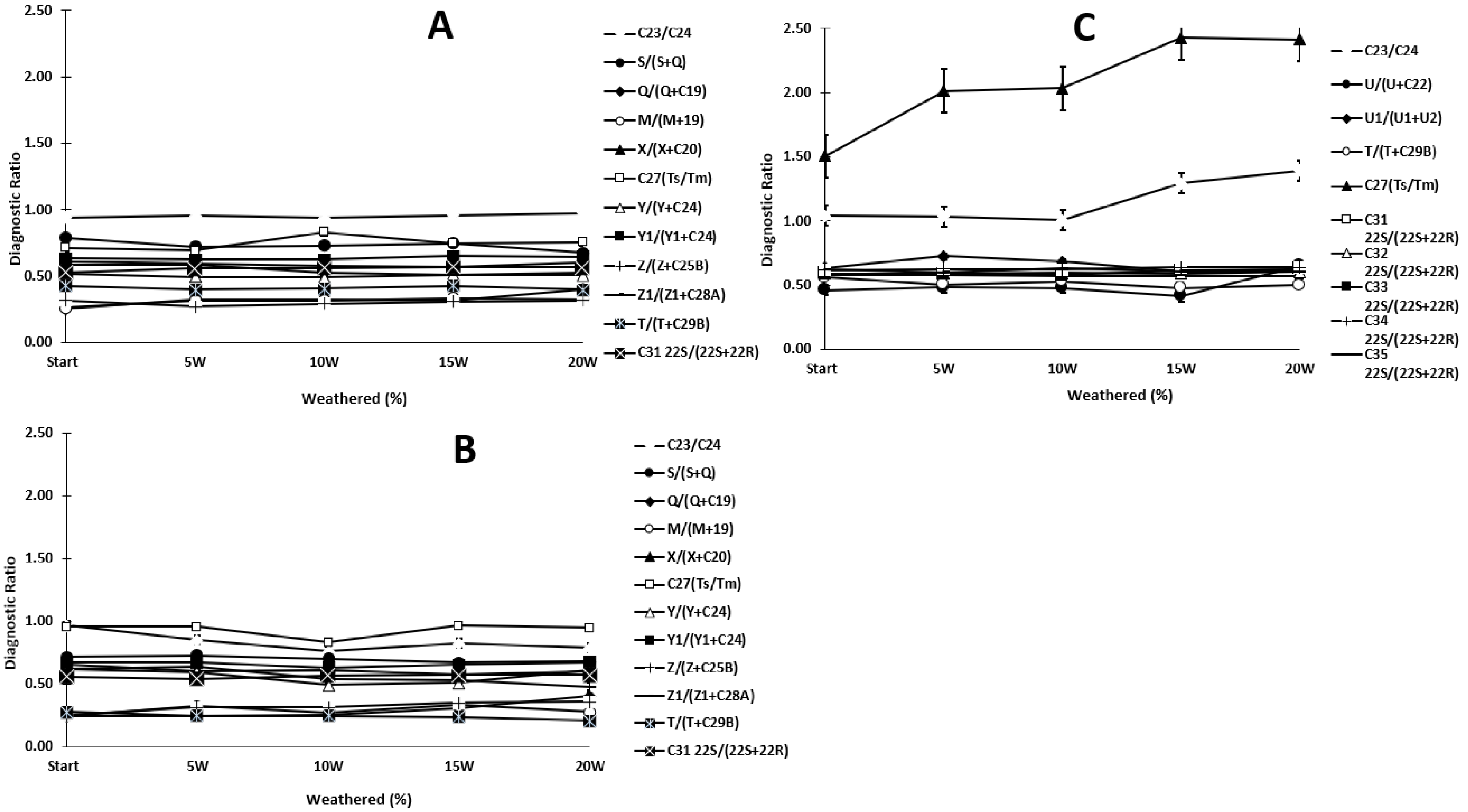


Figure 0.21: Plots of diagnostic ratios of selected identified and unidentified terpanes versus weathered oil (Number of washing) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils

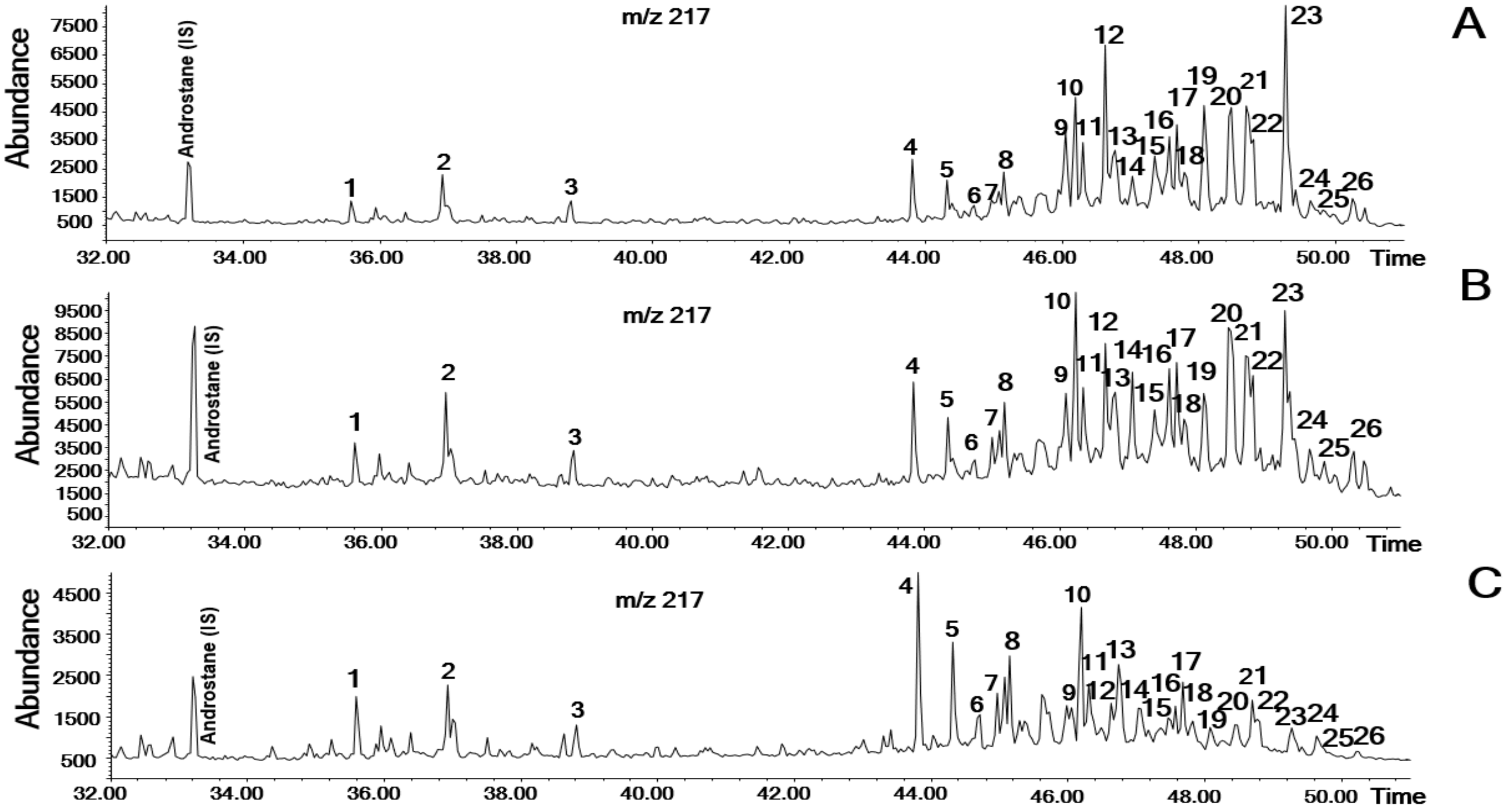


Figure 0.22: m/z 217 mass chromatograms showing the distributions of steranes in unweathered Nigerian light (A), Nigerian medium (B), and North Sea (C) oi

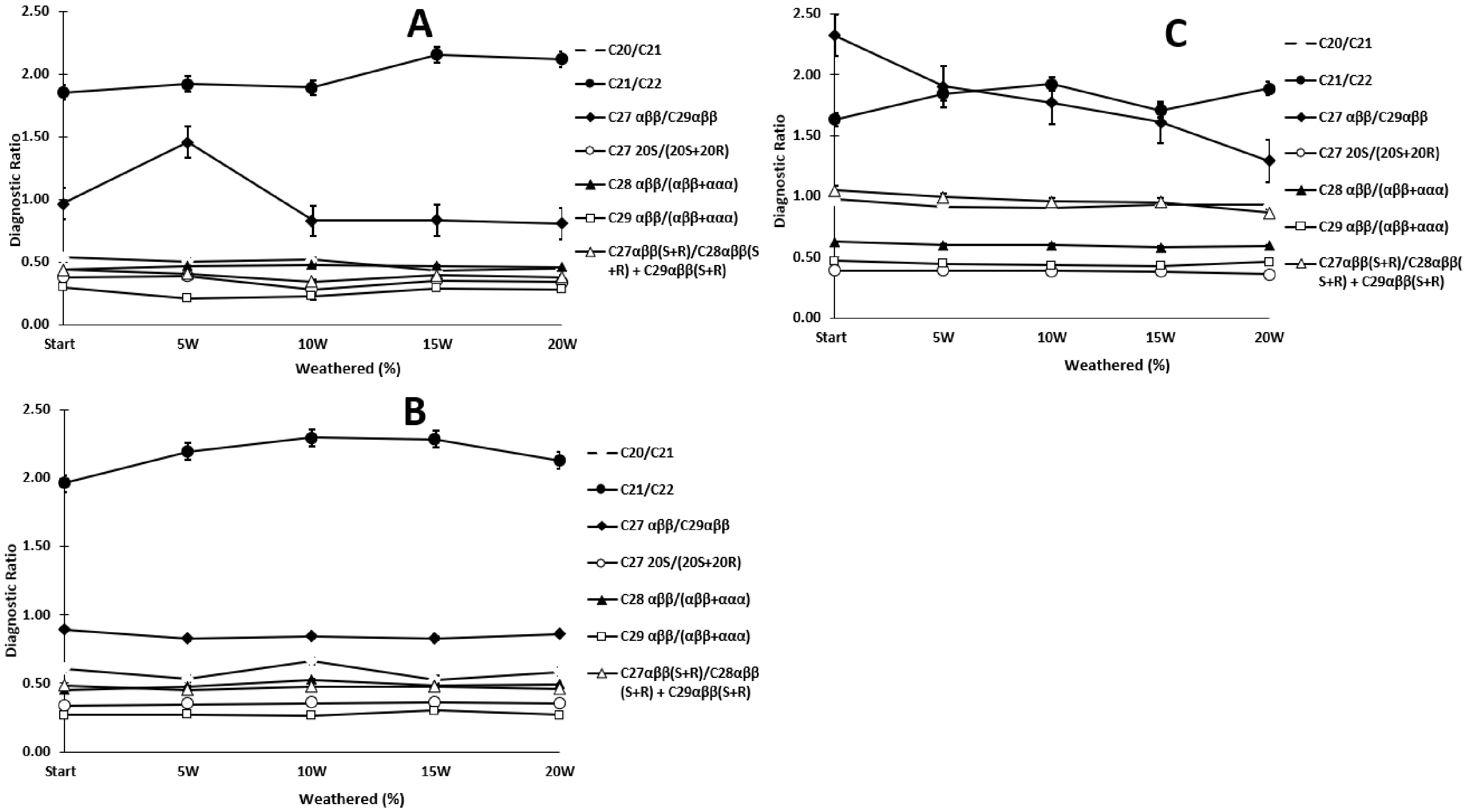


Figure 0.23:Pots of diagnostic ratios of selected identified and unidentified steranes versus weathered oil (Number of washing) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oil

weathering except for the C_{21}/C_{22} (pregnanes) which is affected by increased washing for all the three oils due to their susceptibilities to weathering, and $C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$ ratio for the Nigerian Light and North Sea oils are slightly affected by water washing as the plot drops with increased washing more notable compared to that of evaporative weathering (see Figure 0.23C) this could be as a result of decrease in aqueous solubility $C_{27}\alpha\beta\beta$ relative to $C_{29}\alpha\beta\beta$ steranes.

Triaromatic steranes (TAS) were also analysed in the three oil samples and the m/z 231 mass chromatograms showing their distributions are presented in Figure 0.24 with their peak assignments and measured concentrations shown in Table 0.17, Table 0.18, and Table 0.19 respectively for the three oils. Figure 0.25 shows the diagnostic ratio plots of the triaromatic steranes (TAS) and the diagnostic ratio plots C_{20}/C_{21} TAS, P/C_{21} TAS, and Q/C_{21} TAS are also observed to be affected by water washing for all the three oils as was observed in evaporative weathering (see Fig 3.31, section 3.4.6 in chapter 3) which is also a confirmation that the unidentified peaks P, Q, and V may not be triaromatic steroids (TAS) as discussed in chapter 3, but ratio V/C_{28}(20R) is not affected water washing in the Nigerian oils and those of North Sea oil particularly diminished with increased washing. The alteration of C_{20}/C_{21} plot is possibly due to depletion of C_{20} and C_{21} TAS relative to their C27 and C28 TAS counterparts due to both water washing and biodegradation (Bata *et al.*, 2018). All the other ratios are not affected by water washing in the Nigerian oils, except for the Nigerian Medium oil where a little effect is observed at 5W, and it may be due to sampling error or inadequate homogeneity after shaking due to its waxy and viscous nature. The other ratios in the North Sea oil were observed to be unaffected by water washing up to 10W and then became affected and diminished at 15W which was unexpected as triaromatic steranes (TAS) have been reported to be unaffected by water washing (cf. Chang Xiangchun, 2017; Chang *et al.*, 2018).

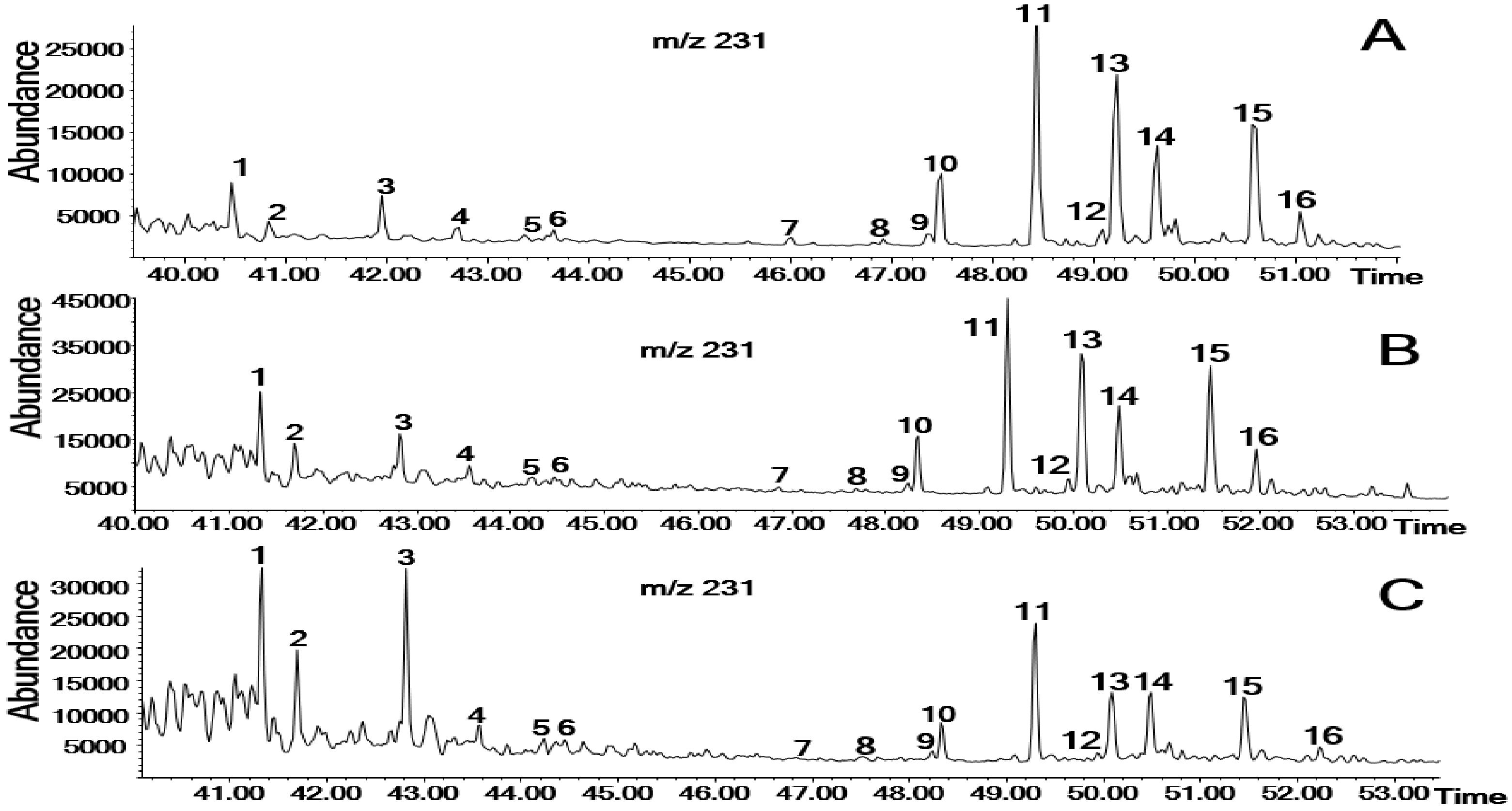


Figure 0.24: m/z 231 mass chromatograms showing the distributions of triaromatic steranes in unweathered Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. Numbers in (A) represent the corresponding peak names in Table 0.17, numbers in (B) represent peak names in Table 0.18, and numbers in (C) represent peak names in Table 0.19 respectively. Numbers 1. 2, 7, 8, 9, and 12 represent unidentified peaks labelled as P, Q, R, S, T, U, and V respectively.

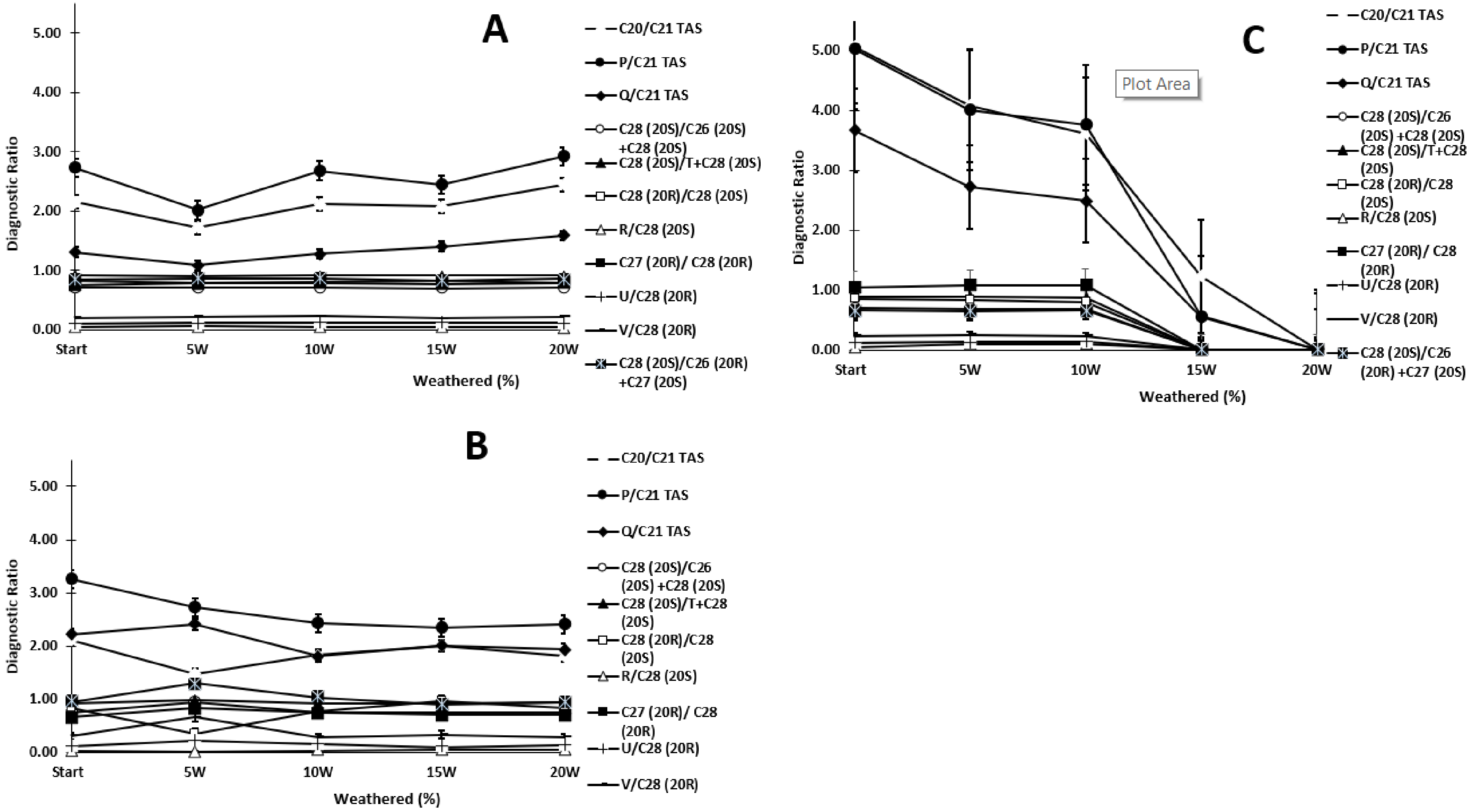


Figure 0.25: Pots of diagnostic ratios of selected identified and unidentified triaromatic steranes versus weathered oil (Number of washing) for; (A) Nigerian light; (B) Nigerian Medium; and (C) North Sea oil

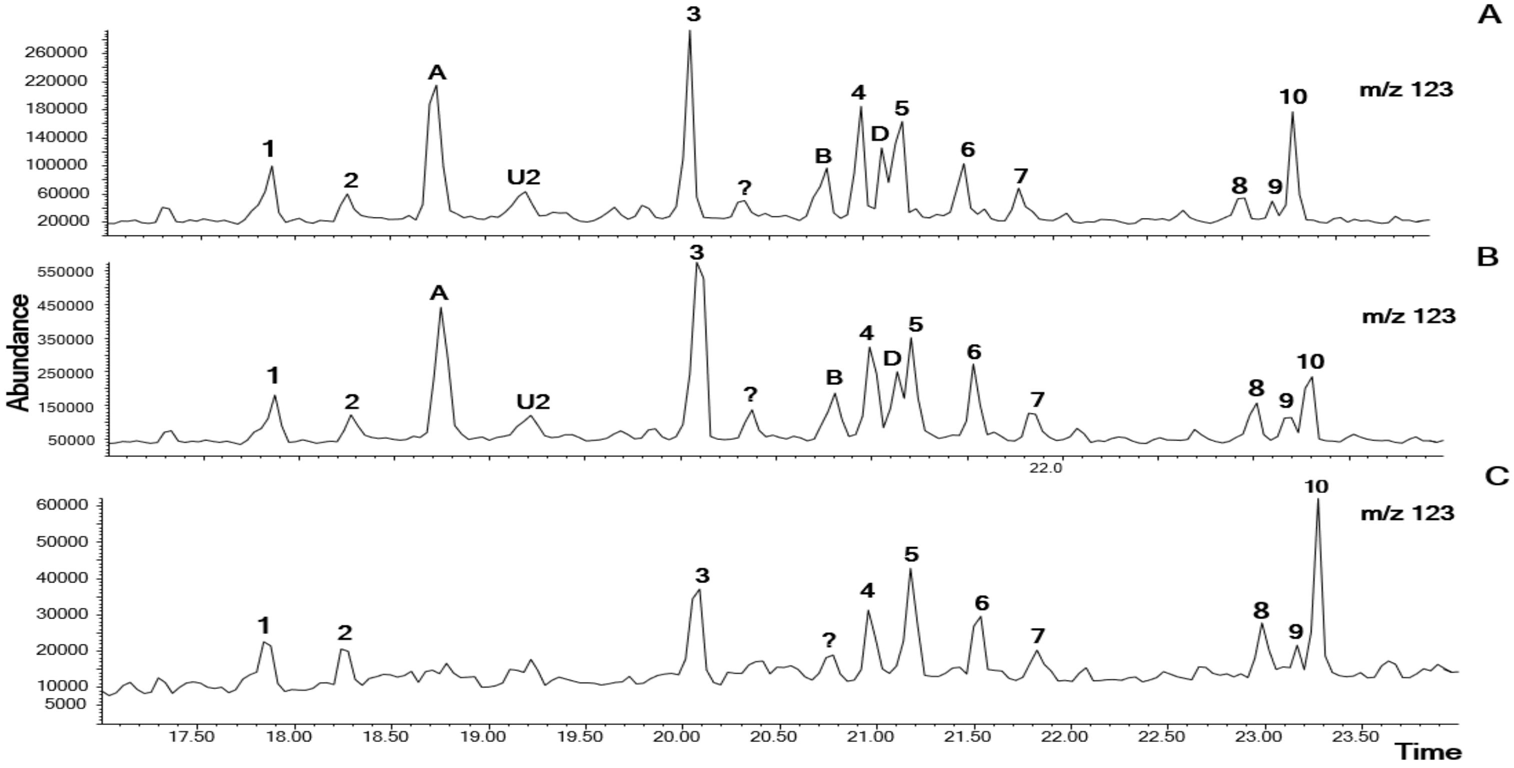


Figure 0.26:m/z 123 mass chromatograms showing the distributions of bicyclic sesquiterpanes in unweathered (start); (A) Nigerian light oil; (B) Nigerian medium; and (C) North Sea oils. Note: Peaks; A, U2, B, and D are novel sesquiterpanes observed in Nigerian light and medium oils.

Bicyclic sesquiterpanes were also analysed in the three oil samples and the m/z 123 mass chromatograms showing their distributions are shown in Figure 0.26 with the peak assignments, and measured concentrations shown in Table 0.20Table 0.21, and Table 0.22 for the Nigerian light and medium oils, and North Sea oils respectively. Novel bicyclic sesquiterpanes identified and discussed in chapter 3 (see section 3.4.6) were also analysed, and the sesquiterpanes abbreviations correspondingly represented by the peak numbers as follows; BS1 (1) for C4-decalin, BS2 (2) for C14 sesquiterpane, BSA (A) for novel sesquiterpane labelled "A"; BSU2 (U2) for novel sesquiterpane "U2", BS3 (3) for C15 sesquiterpane, BSY(Y) for novel "Y" sesquiterpane, BSB (B) for novel "B" sesquiterpane, BS4 (4) for C15 sesquiterpane, BSD (D) for novel "D" sesquiterpane, BS5 (5) for 8β(H)-drimane, BS6 (6) for C15 sesquiterpane, BS7 (7) for C16 sesquiterpane, BS8 (8) for C16 sesquiterpane, BS9 (9) for C16 sesquiterpane, and BS10 (10) for 8β(H)-homodrimane respectively for the two Nigerian oils. The listed known bicyclic sesquiterpanes in the Nigeria oils also present in North Sea oil with one unidentified peak found in the North Sea oil as BSX (X) for "X" sesquiterpane. An abrupt decrease in concentrations of the weathered oils relative to the original oils after washing five times (5W) is observed for the three oils samples, possibly due to effect of water washing but the relative concentrations remained unaffected with further washing. This effect is reflected in the diagnostic ratio plots of BS3/C30αβ-hopane, BSX/C30αβ-hopane, and BS10/C30αβ-hopane for all the three oil samples and the most notable decrease on North Sea oil plot (see Figure 0.27C) suggesting that the sesquiterpanes are affected by water washing relative to the C30αβ-hopane, while the remaining ratio plots of the sesquiterpanes are relatively unaffected (Kuo, 1994) and similar to but slightly more affected than the plots of evaporation experiment in chapter 3 (see Figure 3.32). The adamantanes discussed in chapter 3 were analysed in the three oil samples and the m/z 136 mass chromatograms showing their distributions are shown in Figure 0.28.

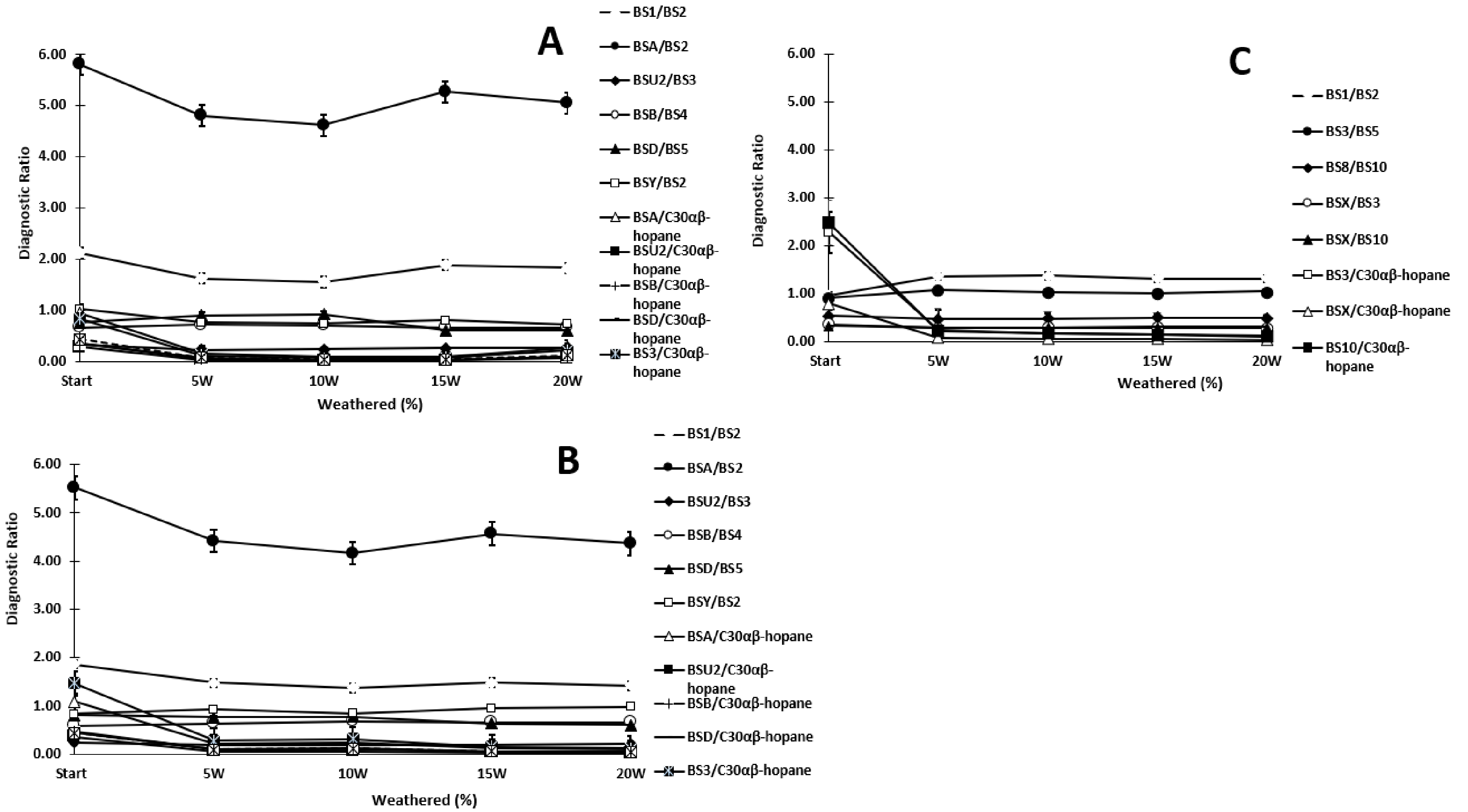


Figure 0.27: Pots of diagnostic ratios of selected bicyclic sesquiterpanes versus number of washings for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils

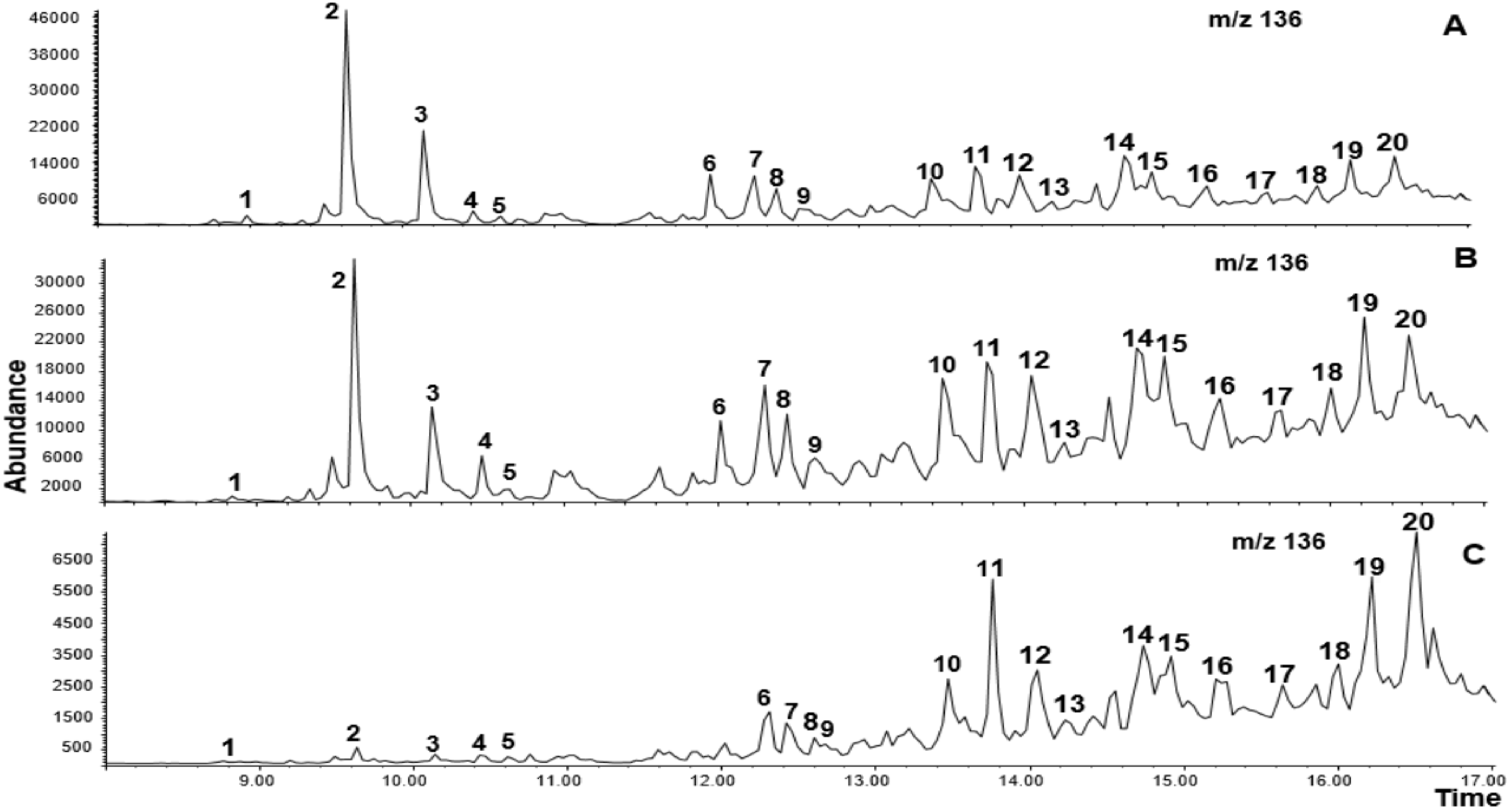


Figure 0.28: m/z 136 mass chromatograms showing the distributions of adamantanes in unweathered (start); (A) Nigerian light oil; (B) Nigerian medium; and (C) North Sea oils.

The peak numbers and the corresponding adamantanes assignments, together with their concentrations are shown in Table 0.24, Table 0.25Table 0.26. The weathering effects due to water washing on the adamantanes were assessed by plotting the diagnostic ratios of the relative concentrations as shown in Figure 0.29. Adamantanes are diamondoids (collective term for adamantane, diamantane and their alkyl homologous series) classified as saturated geochemical markers and are used for oil-source correlation and differentiation because they are thermodynamically stable, most importantly in petroleum products where terpanes and steranes are absent but they retained the relative distribution of their unrefined crude oils (Fingas, 2015; Wang *et al.*, 2016b). The following adamantanes abbreviations used for the ratio plots with their corresponding peak numbers are as follows; 1-MA (2), 1,3-DMA (3), 1,3,5-TMA (4), 2-MA (6), 1,2,5,7-TeMA (13), and 2-AE (17) respectively (see Table 0.24Table 0.25, and Table 0.26). The diagnostic ratio plots include; 1-MA/(1-MA+2-MA), 1-MA/1,3-DMA, 1-MA/1,3,5-TMA, 1,3-DMA/1,2,5,7-TeMA, and 1-MA/(1-MA+2-MA+2-AE), and these plots remain parallel as diamondoids are poorly soluble in water and unaffected by water washing (cf. Jiang *et al.*, 2020). The ratio 1-MA/1,3,5-TMA is observed to increase with washing for all the three oil samples, suggesting that 1,3,5-TMA is relatively more soluble than 1-MA, as signified by horizontal plots with increased weathering in Figure 0.29 except the ratio 1,3-DMA/1,2,5,7-TeMA which decrease with washing for Nigerian Medium oil. The effect of water washing on the adamantanes as observed from the ratio plots for the Nigerian light (see Figure 0.29A) are very similar to that evaporative weathering described in chapter 3 (see section 3.4.6), whereas slight variation is observed on ratios 1,3-DMA/1,2,5,7-TeMA and 1-MA/1,3,5-TMA for Nigerian medium (see Figure 0.29 B), while 1-MA/1,3-DMA and 1-MA/1,3,5-TMA ratios are slightly less affected by water washing compared to that of evaporation for North Sea (see Figure 0.29C).

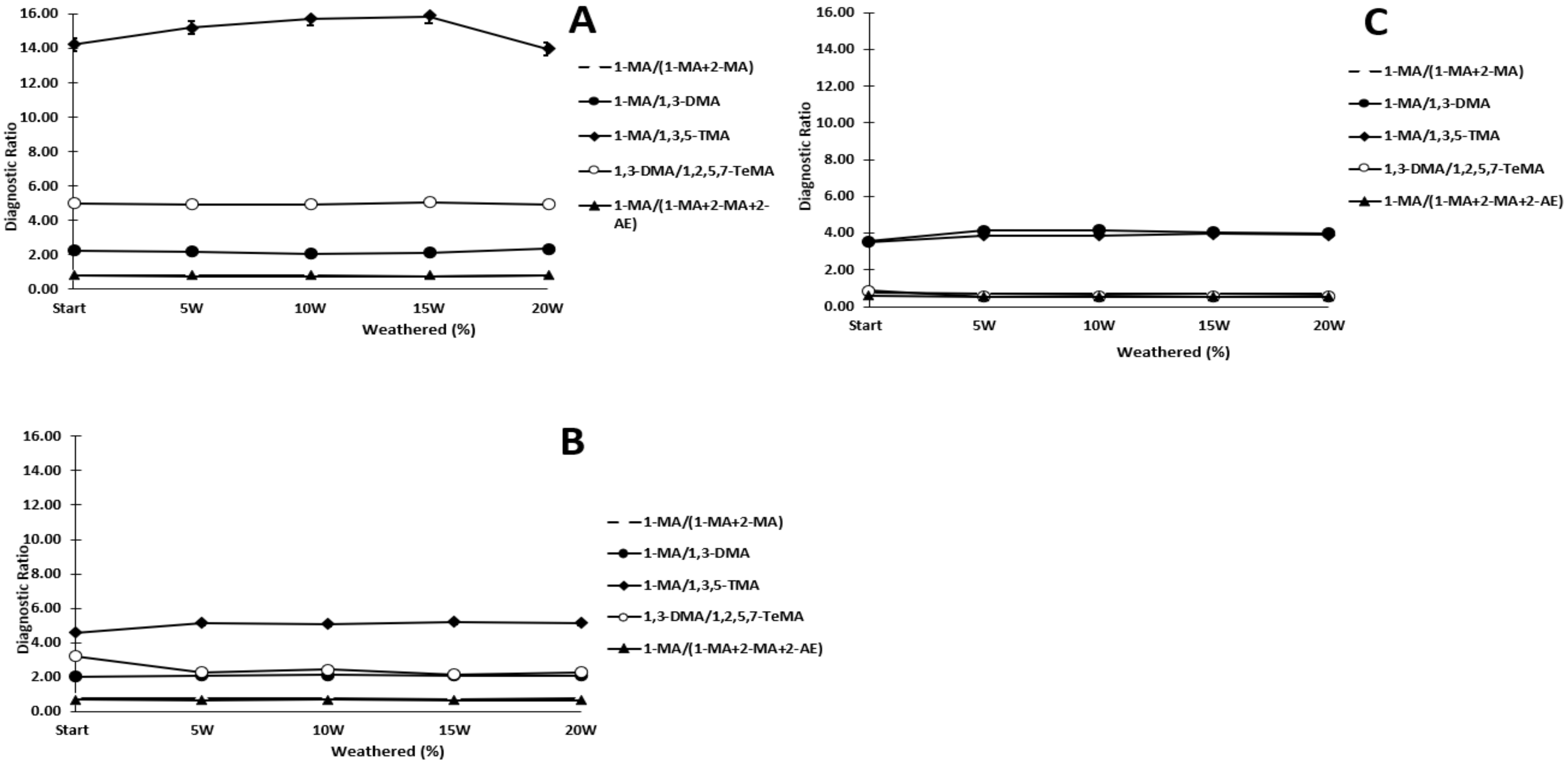


Figure 0.29: Pots of diagnostic ratios of selected identified and unidentified bicyclic adamantanes versus weathered oil (Number of washing) for; (A) Nigerian Light; (B) Nigerian Medium; and (C) North Sea oils

4.4.4 Relative Susceptibility of Selected Molecular Markers

The relative water washing susceptibilities of compounds from different classes but with similar volatilities were assessed using the ratios of measured concentrations of the following compounds: C_{14}, C_{15}, C_{17} and C_{31} n-alkanes, C_{15} bicyclic sesquiterpene (BS4), pristane, and 2,6+2,7-dimethylnaphthalane, for the three oils, while C_{30}-oleanane was used for the two Nigerian oils C_{30}-hopane was used for the North Sea oil. Figure 4.28 shows the ratio plots of these compound classes against the number of washings. The plots are fairly horizontal which indicate that the ratios are not so affected by this level of water washing, except for BS4/*n*-C_{15} ratio which is observed that C_{15} bicyclic sesquiterpene (BS4) is more soluble than *n*-C_{15} at the start of water washing but stabilised after washing five times for the Nigerian Medium oil. A slight initial decrease and then increase with washing in C_{30}-oleanane/*n*-C_{31} ratios for the Nigerian oils and C_{30}-hopane/*n*-C_{31} for the North Sea oil could be as a result of build-up of less soluble long-chain n-alkanes at the initial stage of washing, but becomes more soluble with increase in washing relative to C_{30}-oleanane and C_{30}-hopane as the ratios began to rise, a pattern that is not similar to those of evaporative weathering. The pristane/*n*-C_{17} ratios showed a similar pattern especially for the Nigerian Medium and North Sea oils, suggesting that *n*-C_{31} and *n*-C_{17} becomes more soluble relative to C_{30}-oleanane, C_{30}-hopane and pristane with further washing. A general look at the different isomers of the naphthalenes homologous considered, showed that solubility decreased from the parent naphthalene to alkyl homologous tetramethylnaphthalenes with increase in washing and aqueous solubility varies within isomers, most of the tetramethylnaphthalene were unaffected for the Nigerian Light oil. Comparatively, only the parent, methylnaphthalenes and the dimethylnaphthalenes were affected more by evaporative weathering, but trimethyl and tetramethylnaphthalenes were affected more by water washing as observed in the Nigerian Light oil. For the Nigerian Medium oil, the naphthalene isomers were generally affected more by water washing than evaporative weathering possibly due to its viscous and waxy nature where “skin” formation might have slowed down evaporative weathering (Wang and Fingas, 1995), and aqueous solubility also varies within isomer groups most of which agreed with available data on aqueous solubility (Mackay et al., 1980). Similarly, the naphthalene isomers in the North Sea oil were the most affected of the three oils by water washing and the effect of evaporative weathering was much less on dimethyl, trimethyl and tetramethylnaphthalenes.

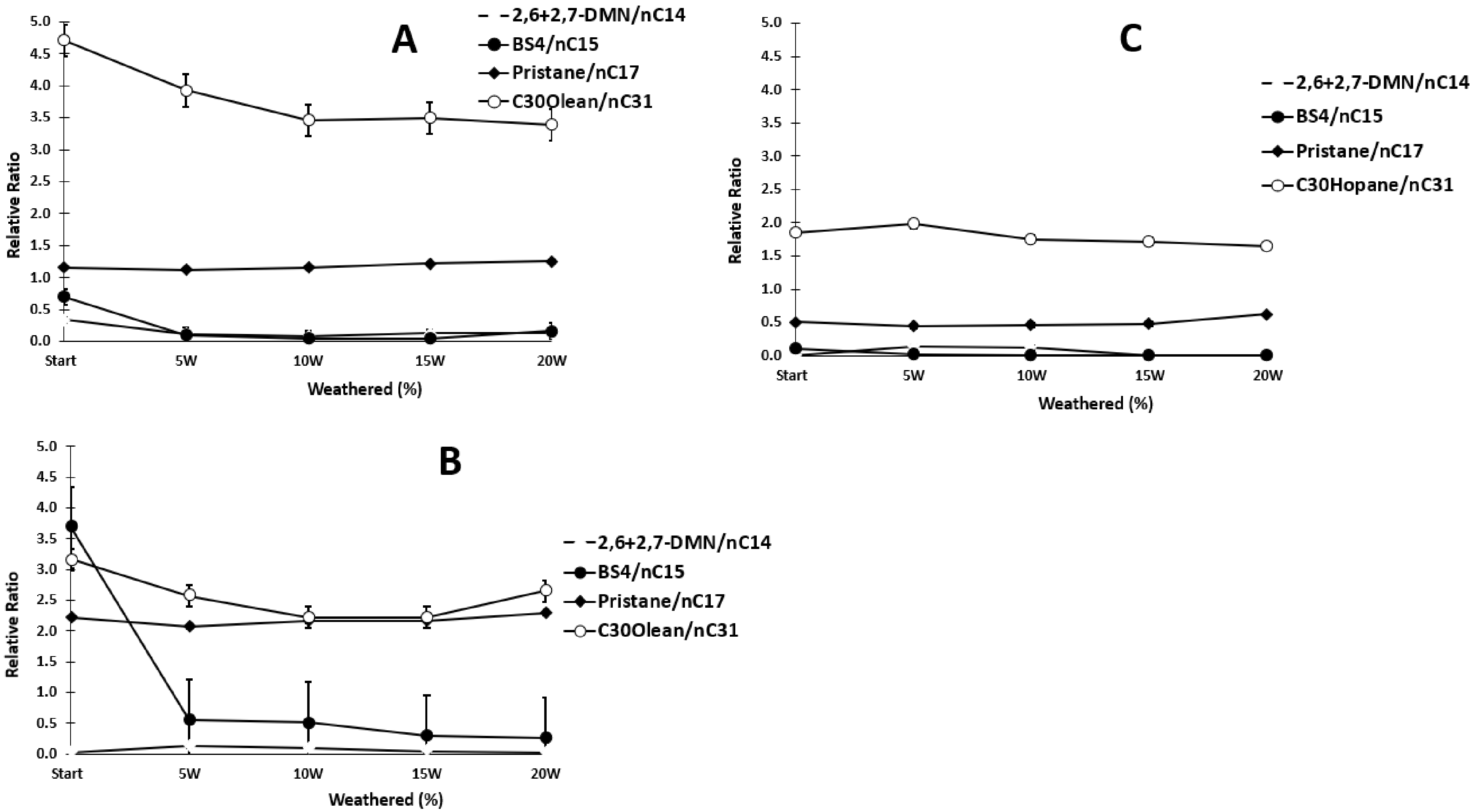


Figure 0.30: Plots of ratios of selected aliphatic/PAH for (A) Nigerian Light, (B) Nigerian Medium, and (C) North Sea oils

4.4.5 Diagnostic Ratio Plots of Nordtest Parameters

The diagnostic ratios of the parameters plotted for evaporative weathering (see section 3.4.8) were also plotted in this chapter to assess the effect of water washing on the selected parameters. Figure 0.31 shows the diagnostic ratio plots of the fresh oil versus Nigerian Light weathered by washing 5, 10, 15, and 20 times respectively. The positive correlation indicates that the parameters are not affected by this level of water washing. Similarly, Figure 0.32 shows the diagnostic plots for Nigerian Medium oil with a positive correlation which is also not affected by this level of water washing, except for ratios Q TAS and C_{26} 20R + C_{27} 20S sterane where variations were observed after washing 10 times only. Figure 0.33 shows the plots for the North Sea oil with generally a positive correlation, but ratios Q TAS, C_{26} 20R + C_{27} 20S, C_{21} TAS, and 4-MDBT/1-MDBT show variations from washing 15-20 times, which is consistent with being affected by water washing. These parameters were observed to be slightly affected by evaporation as discussed in section 3.4.8.

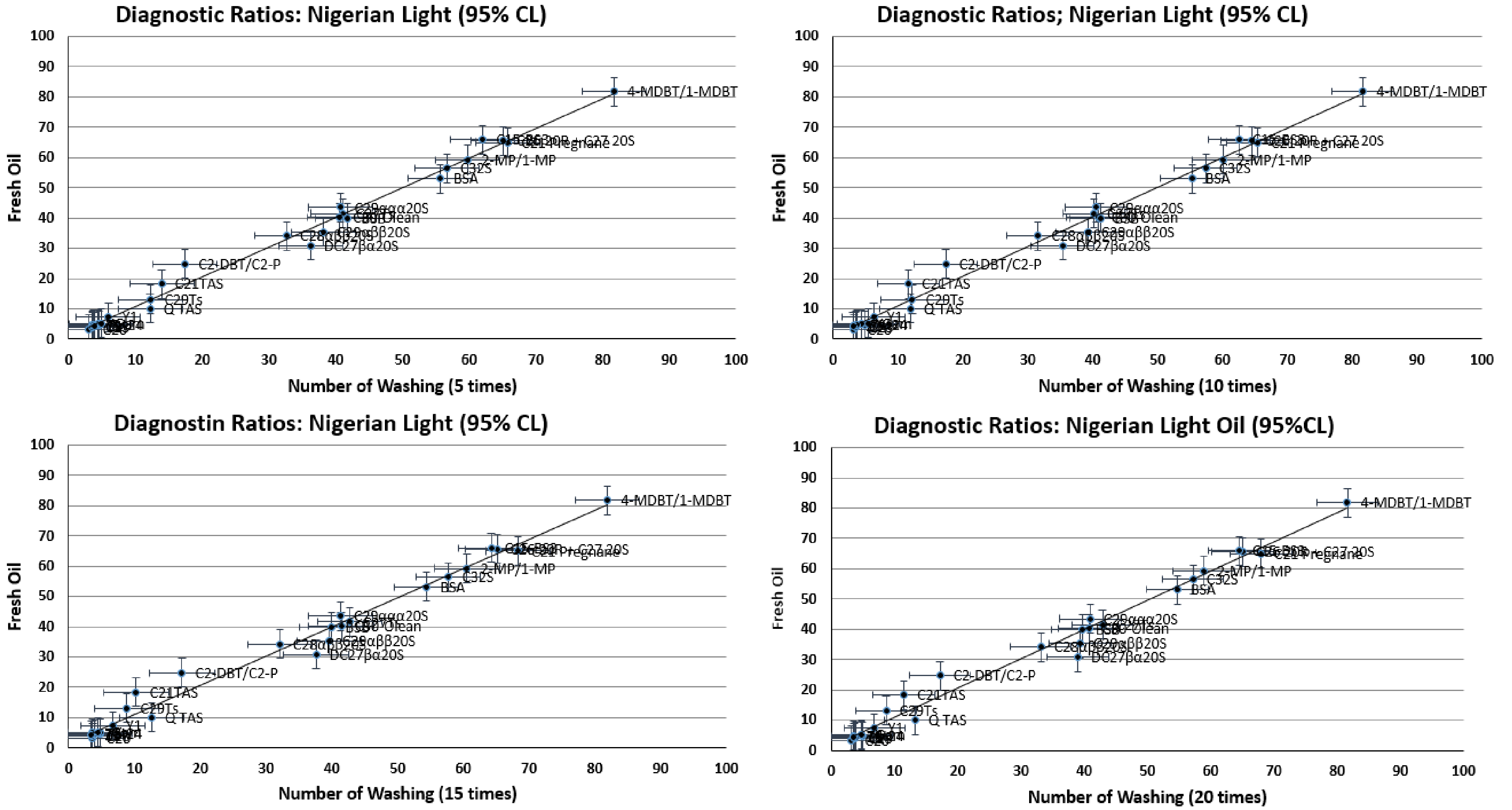


Figure 0.31: Diagnostic Ratio plots of fresh oil vs weathered Nigerian Light oil subjected to water washing, weathered oil showing positive correlation with the fresh oil for maximum washing of 20 times using 95% confidence limit.

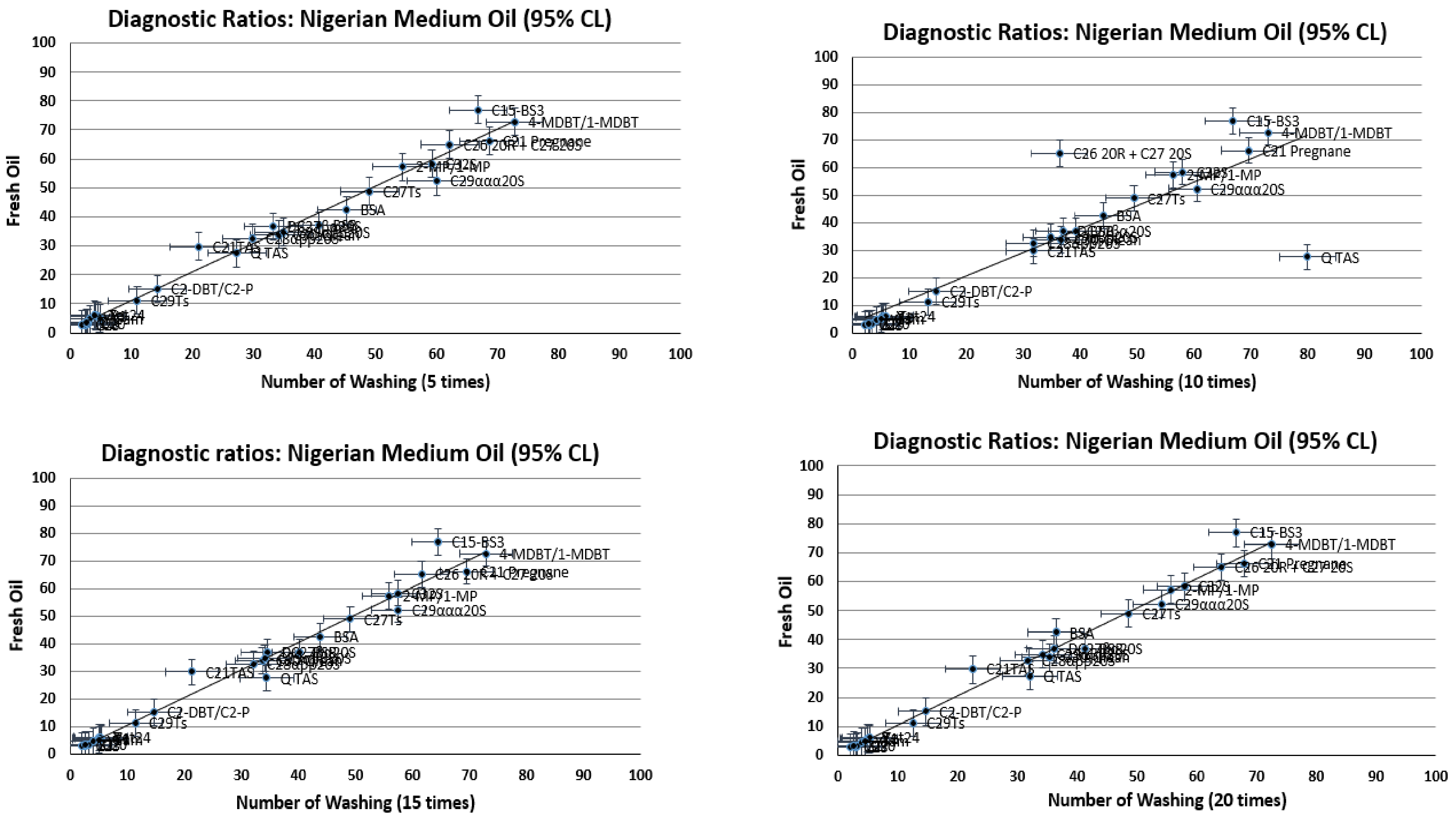


Figure 0.32: Diagnostic Ratio plots of fresh oil vs weathered Nigerian Medium oil subjected to water washing, weathered oil showing positive correlation with the fresh for maximum washing of 20 times using 95% confidence limit.

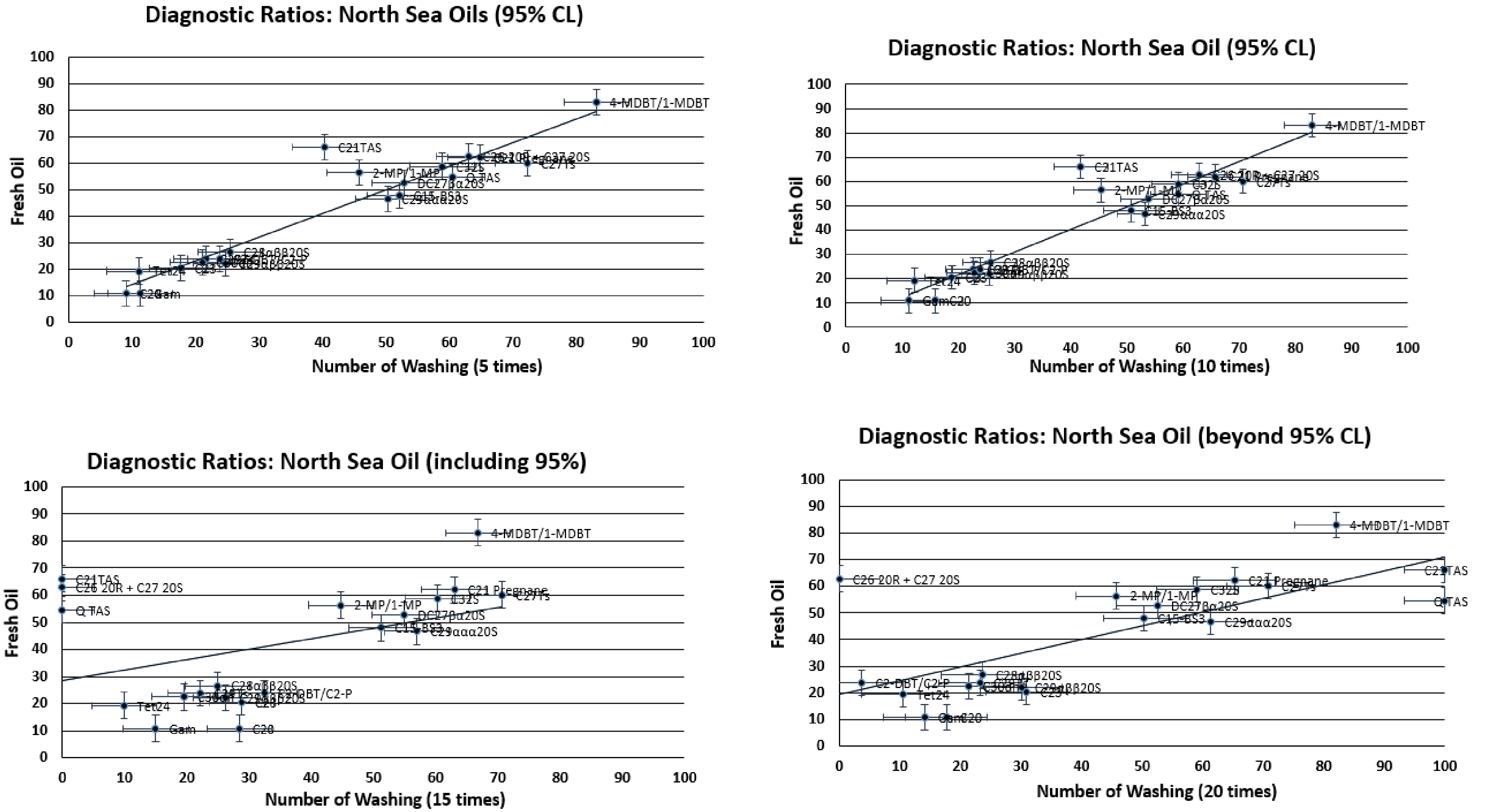


Figure 0.33: Diagnostic Ratio plots of fresh oil vs weathered North Sea oil subjected to water washing, weathered oil showing positive correlation with the fresh oil for maximum washing of 20 times using 95% confidence limit.

4.5 Water washing as a weathering process

Water washing weathering processes are mainly physical involving the loss by dissolution of the most water-soluble components of the oil, but results in both physical and chemical changes as the physico-chemical characteristics of the oil are altered over time. When crude oil is spilled in water environment weathering starts with evaporation of volatile components and dissolution of soluble (mostly low molecular weight) components of the crude oil which are physical weathering processes. The resulting residue then becomes more viscous, and with further weathering including mixing with water to form emulsions, can form very viscous residues (NRC, 2003). Further weathering including by both microbial and photooxidation can result in oxidized components which form semi solid residues such as tarballs, surface residue balls, and mats, which are very resistant to further weathering processes including water washing (NRC, 2003; Tarr *et al.*, 2016).

4.6 Effects of unknown compounds and their use for correlation

While it was noted in section 4.4.3 and 4.4.4 that some unknown compounds (e.g., BS4) appeared to have been affected by water washing, these compounds (and the others suggested as being useful for correlation purposes) should be structurally identified and information about their origin ascertained in the future work (see section 3.4.6) to ensure that they are suitable compounds molecular markers that can be used for oil-oil and oil-source correlation. Critical evaluation for selection of a diagnostic compound is dependent on high specificity and diversity among oils, resistance to weathering, and high analytical precision (Daling *et al.*, 2002a; Daling *et al.*, 2004).

4.7 Conclusion

The *n*-alkanes measured are observed to be less susceptible to weathering by water washing compared to evaporative weathering. The measured *n*-alkanes (*n*-C_8 to *n*-C_{39}) peaks were only slightly affected and non was completely lost after water washing twenty times for the three oils whereas the low-molecular weight *n*-alkanes (*n*-C_8 to *n*-C_{13}) were completely lost due to evaporative losses of 63%, 36%, and 48% evaporation for the Nigerian Light and Medium, and North Sea oils respectively. The *n*-alkanes are observed to be relatively less susceptible to water washing which is confirmed by the relative increase in the summed concentrations of *n*-alkanes compared to those by evaporative weathering. The effects of water washing on the *n*-alkanes are more on the low-molecular weight components and is observed to decrease with increase in carbon number (cf. Ferguson *et al.*, 2009).

Certain PAHs are noted to be more affected by evaporative weathering than water washing, with as example, the methylnaphthalene ratios (2-MN/1-MN) are more preserved in the water washing experiments than evaporative weathering ones, especially in the Nigerian oils, as the aqueous solubilities of the methylnaphthalene isomers are almost equal (cf. Mackay *et al.*, 1980). Parent PAHs are more susceptible to water washing than their alkylated homologous. Water washing effects amongst PAH isomers was observed and methyl biphenyl isomers were affected for all the three oils, though the North Sea oil was the most affected. The methylphenanthrene isomers were virtually unaffected for the Nigerian oils and the North Sea oil only slightly affected possibly due to higher number of aromatic rings compared to methylphenyls and thus overall lower solubility. The double ratio (S/U) solubility assessment of the two pentamethylnaphthalenes identified in the two Nigerian oils relative to 4-methyldibenzothiophene as potential diagnostic geochemical makers compares relatively well with that of evaporative weathering.

The terpanes, steranes and bicyclic sesquiterpanes were observed to be generally unaffected by water washing. As discussed in section 3.4.9, ratios Q TAS, C_{26} 20R + C_{27} 20S, C_{21} TAS, and 4-MDBT/1-MDBT should be avoided when considering diagnostic parameters for correlation.

Table 0.1: Changes in *n*-alkane and acyclic isoprenoid concentrations in weathered Nigerian light oil (mg/g oil)

***n*-Alkane**	**Start**	**5W**	**10W**	**15W**	**20W**
***n*-C_8**	4.05 (3.2)	4.23 (6.1)	4.55 (6.1)	4.20 (3.8)	3.96 (3.3)
***n*-C_9**	7.31 (1.2)	7.63 (4.0)	7.25 (7.2)	7.21 (5.1)	6.89 (6.4)
***n*-C_{10}**	8.97 (4.3)	9.92 (6.9)	10.45 (5.3)	9.68 (7.4)	9.22 (2.8)
***n*-C_{11}**	9.44 (1.8)	9.73 (5.4)	10.28 (3.3)	9.69 (6.2)	9.16 (0.9)
***n*-C_{12}**	8.77 (0.2)	9.75 (4.4)	10.26 (1.7)	10.01 (1.5)	9.54 (0.6)
***n*-C_{13}**	7.81 (0.2)	11.20 (4.6)	10.99 (3.1)	9.98 (6.3)	9.20 (7.0)
***n*-C_{14}**	7.17 (0.5)	8.17 (3.2)	8.67 (0.6)	8.32 (4.9)	7.82 (1.0)
***n*-C_{15}**	6.54 (0.2)	7.65 (2.9)	8.01 (1.8)	7.94 (5.1)	7.48 (1.8)
***n*-C_{16}**	5.32 (0.4)	6.13 (3.4)	6.58 (1.8)	6.44 (7.4)	6.03 (2.6)
***n*-C_{17}**	4.62 (0.1)	5.65 (3.8)	5.95 (1.0)	5.83 (5.0)	5.46 (2.3)
Pristane	5.34 (0.1)	6.33 (2.9)	6.89 (4.0)	7.10 (1.4)	6.87 (2.4)
n-C_{18}	4.10 (3.8)	5.45 (2.6)	5.80 (5.2)	5.97 (6.7)	5.79 (3.0)
Phytane	1.75 (2.9)	1.76 (3.2)	1.94 (6.1)	1.97 (3.7)	1.89 (2.9)
***n*-C_{19}**	3.55 (2.1)	4.67 (3.6)	4.93 (5.5)	5.07 (4.0)	4.93 (2.3)
***n*-C_{20}**	3.26 (2.9)	3.94 (3.9)	4.29 (3.9)	4.51 (3.1)	4.38 (1.9)
***n*-C_{21}**	3.27 (2.1)	4.23 (3.9)	4.45 (3.9)	4.67 (3.1)	4.53 (1.9)
***n*-C_{22}**	3.46 (1.2)	4.59 (3.8)	4.88 (3.1)	5.04 (6.1)	4.83 (1.7)

n-C$_{23}$	3.12 (3.8)	4.14 (3.6)	4.32 (2.8)	4.50 (3.6)	4.35 (1.6)
n-C$_{24}$	2.59 (3.0)	3.74 (3.5)	3.96 (2.5)	4.10 (6.7)	3.91 (1.5)
n-C$_{25}$	2.81 (0.7)	3.74 (3.7)	3.97 (2.2)	4.10 (5.6)	3.99 (1.4)
n-C$_{26}$	2.17 (3.1)	2.81 (4.4)	3.02 (1.5)	3.11 (2.9)	2.98 (1.3)
n-C$_{27}$	2.07 (3.2)	2.76 (4.4)	2.94 (1.3)	3.06 (1.9)	2.93 (1.0)
n-C$_{28}$	1.71 (1.0)	2.24 (4.6)	2.40 (0.9)	2.52 (3.7)	2.42 (1.3)
n-C$_{29}$	1.68 (2.9)	1.98 (5.2)	2.13 (0.4)	2.21 (2.5)	2.15 (1.4)
n-C$_{30}$	1.09 (9.1)	1.46 (5.6)	1.55 (0.5)	1.61 (5.5)	1.55 (1.6)
n-C$_{31}$	1.24 (1.4)	1.70 (5.4)	1.80 (0.9)	1.85 (4.8)	1.76 (1.2)
n-C$_{32}$	0.68 (4.7)	0.83 (5.2)	0.86 (6.8)	0.89 (6.4)	0.86 (5.8)
n-C$_{33}$	0.63 (1.8)	0.82 (6.7)	0.86 (9.9)	0.85 (2.2)	0.83 (3.7)
n-C$_{34}$	0.24 (4.5)	0.41 (6.1)	0.32 (3.2)	0.33 (3.0)	0.32 (0.9)
n-C$_{35}$	0.16 (3.2)	0.18 (9.1)	0.19 (2.6)	0.18 (2.6)	0.18 (4.7)
n-C$_{36}$	0.09 (6.5)	0.06 (4.3)	0.06 (4.9)	0.06 (6.0)	0.06 (4.9)
n-C$_{37}$	0.06 (3.6)	0.06 (3.1)	0.07 (5.0)	0.07 (8.8)	0.07 (6.8)
n-C$_{38}$	0.03 (6.6)	0.03 (5.6)	0.03 (4.5)	0.03 (5.6)	0.03 (3.5)
n-C$_{39}$	0.02 (3.3)	0.02 (6.4)	0.02 (6.4)	0.02 (3.6)	0.02 (5.6
Sum	115.12	138.00	144.65	143.12	136.39
n-C$_{17}$/pristane	0.86	0.89	0.86	0.82	0.79

n-C_{18}/phytane	2.35	3.10	2.99	3.03	3.07
pristane/phytane	3.06	3.59	3.56	3.60	3.64
CPI*	1.23	1.21	1.21	1.21	1.21
(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})	2.92	2.40	2.38	2.18	2.16

Note: 5W, 10W, 15W and 20W are as explained in

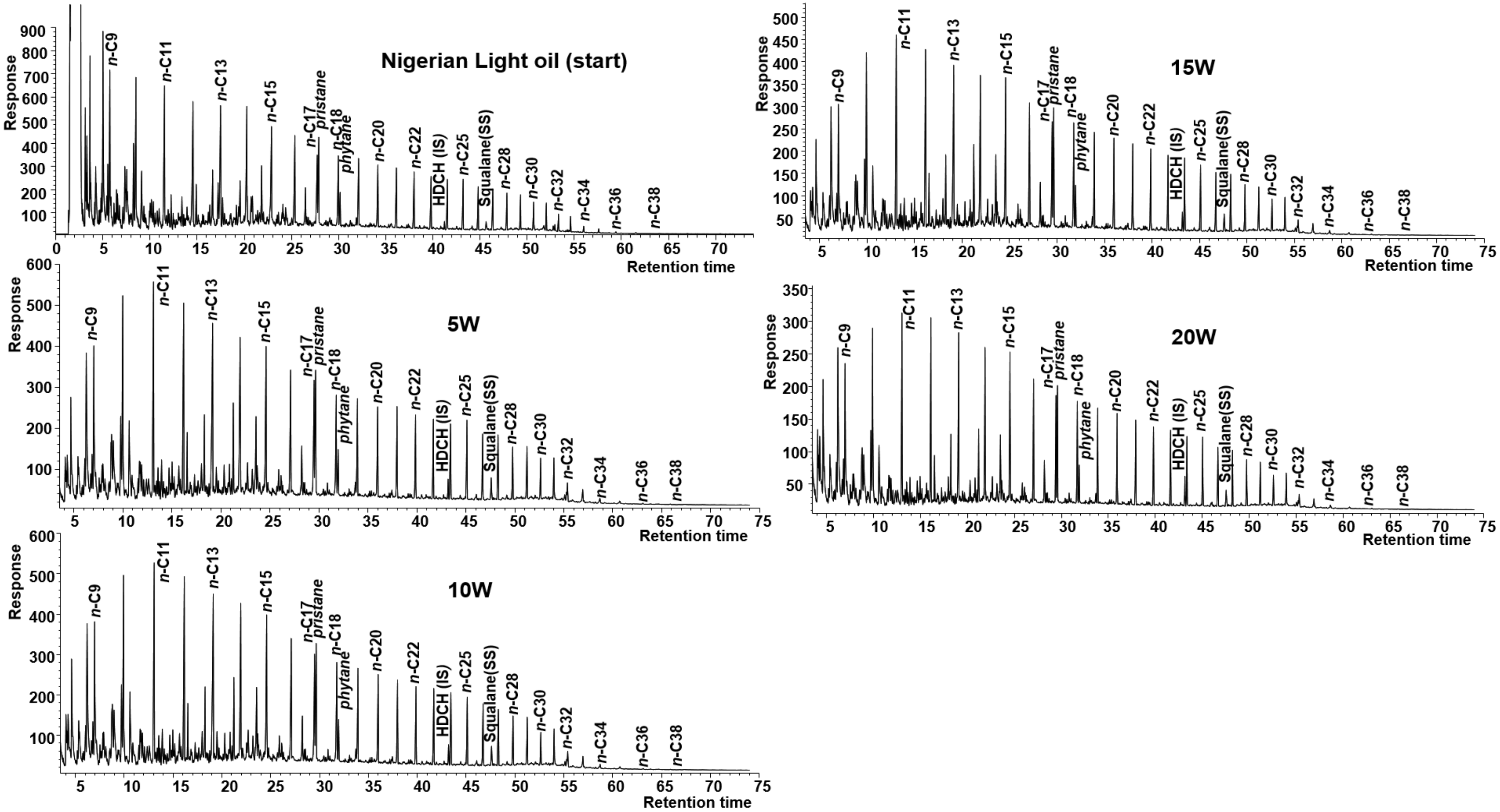


Figure 0.2. The numbers in brackets after the numbers (concentrations) in the table are relative standard deviations (RSD) (n=3), similarly for Table 0.2 and Table 0.3.

Table 0.2: Changes in n-alkane and acyclic isoprenoid concentrations in weathered Nigerian medium oil (mg/g oil)

***n*-Alkane**	**Oil (start)**	**5W**	**10W**	**15W**	**20W**
***n*-C_{8}**	0.44 (4.9)	0.48 (6.2)	0.45 (4.2)	0.35 (5.5)	0.24 (1.1)
***n*-C_{9}**	0.79 (0.5)	1.11 (8.6)	1.05 (1.6)	0.94 (5.9)	0.81 (0.3)
***n*-C_{10}**	1.00 (4.9)	1.35 (5.4)	1.44 (9.1)	1.34 (4.4)	1.16 (1.7)
***n*-C_{11}**	1.34 (2.7)	1.85 (1.9)	1.86 (9.1)	1.73 (3.8)	1.57 (3.4)
***n*-C_{12}**	1.70 (1.0)	2.41 (7.2)	2.56 (9.0)	2.47 (3.2)	2.09 (3.5)
***n*-C_{13}**	1.83 (1.1)	2.70 (8.8)	2.80 (9.9)	2.63 (2.9)	2.27 (3.8)
***n*-C_{14}**	1.90 (3.2)	2.82 (3.0)	2.98 (7.7)	2.87 (6.7)	2.61 (3.8)
***n*-C_{15}**	2.11 (4.3)	3.14 (3.6)	3.28 (3.2)	3.15 (4.7)	2.85 (3.7)
***n*-C_{16}**	1.64 (7.5)	3.29 (4.6)	3.45 (7.9)	3.33 (5.4)	3.11 (2.6)
***n*-C_{17}**	2.42 (3.8)	3.25 (2.1)	3.46 (5.6)	3.39 (3.2)	3.21 (7.6)
Pristane	5.36 (5.2)	6.70 (7.5)	7.50 (7.0)	7.35 (2.7)	7.34 (5.2)
n-C_{18}	2.63 (0.5)	3.00 (6.1)	3.23 (9.7)	3.14 (4.7)	3.00 (7.8)
Phytane	2.25 (4.6)	2.45 (3.7)	2.63 (6.6)	2.57 (2.8)	2.55 (4.1)
***n*-C_{19}**	2.66 (3.6)	3.02 (9.1)	3.26 (9.4)	3.20 (2.0)	3.20 (3.2)
***n*-C_{20}**	2.69 (2.6)	3.19 (8.3)	3.44 (8.9)	3.81 (1.3)	3.37 (1.1)
***n*-C_{21}**	2.69 (2.6)	3.19 (8.3)	3.44(8.9)	3.72 (1.3)	3.37 (1.1)
***n*-C_{22}**	2.53 (2.5)	2.96 (8.2)	3.21 (9.4)	3.68 (1.5)	3.25 (1.4)

n-C$_{23}$	2.57 (1.9)	3.01 (8.1)	3.30 (9.0)	3.63 (1.1)	3.20 (1.6)
n-C$_{24}$	2.43 (5.1)	3.02 (7.5)	3.28 (8.8)	3.56 (1.4)	3.16 (5.8)
n-C$_{25}$	2.37 (0.5)	3.21 (7.3)	3.46 (9.0)	3.47 (2.4)	3.26 (1.6)
n-C$_{26}$	2.26 (2.4)	3.14 (8.5)	3.37 (9.8)	3.33 (1.3)	3.10 (0.8)
n-C$_{27}$	2.29 (1.6)	3.30 (6.8)	3.55 (7.6)	3.51 (0.7)	3.28 (0.2)
n-C$_{28}$	2.22 (3.5)	2.96 (6.2)	3.11(7.5)	3.08 (1.2)	2.98 (0.4)
n-C$_{29}$	2.05 (4.4)	3.09 (5.6)	3.20 (9.1)	3.10 (3.3)	2.94 (1.8)
n-C$_{30}$	1.54 (8.9)	2.34 (6.5)	2.50 (7.3)	2.45 (1.6)	2.31 (0.7)
n-C$_{31}$	1.66 (1.4)	2.67 (7.2)	2.81(7.8)	2.76 (0.8)	2.67 (1.1)
n-C$_{32}$	0.94 (1.0)	1.51 (2.8)	1.59 (8.6)	1.55 (2.3)	1.53 (0.9)
n-C$_{33}$	0.74 (0.5)	1.12 (6.4)	1.16 (4.7)	1.13 (1.5)	1.11 (2.4)
n-C$_{34}$	0.36 (0.8)	0.56 (7.7)	0.60 (4.1)	0.59 (8.7)	0.58 (1.2)
n-C$_{35}$	0.24 (1.8)	0.38 (5.7)	0.40 (8.1)	0.37 (9.0)	0.36 (3.1)
n-C$_{36}$	0.15 (2.1)	0.29 (8.9)	0.30 (5.4)	0.30 (2.4)	0.29 (2.5)
n-C$_{37}$	0.14 (0.8)	0.21 (7.9)	0.22 (7.9)	0.22 (0.9)	0.21 (4.7)
n-C$_{38}$	0.13 (0.9)	0.14 (3.6)	0.16 (6.2)	0.15 (3.4)	0.15 (1.4)
n-C$_{39}$	0.11 (0.4)	0.11 (5.6)	0.12 (5.5)	0.12 (4.5)	0.12 (4.7)
Sum	58.16	77.98	83.16	82.97	77.26
n-C$_{17}$/pristane	0.45	0.48	0.46	0.46	0.44

***n*-C$_{18}$/phytane**	1.17	1.23	1.22	1.22	1.18
pristane/phytane	2.38	2.74	2.85	2.86	2.88
CPI*	1.11	1.15	1.15	1.14	1.14
(C$_{8}$+C$_{10}$+C$_{12}$+C$_{14}$)/(C$_{22}$+C$_{24}$+C$_{26}$+C$_{28}$)	0.53	0.58	0.57	0.52	0.49

Table 0.3: Changes in n-alkane and acyclic isoprenoid concentrations in North Sea oil (mg/g oil)

n-Alkane	Start	5W	10W	15W	20W
***n*-C$_{8}$**	2.96 (5.6)	4.22 (8.5)	4.13 (6.7)	4.17 (7.3)	3.95 (8.2)
***n*-C$_{9}$**	3.63 (4.9)	5.33 (7.6)	5.37 (7.1)	5.49 (8.0)	5.02 (4.4)
***n*-C$_{10}$**	4.45 (3.1)	6.37 (8.2)	6.30 (6.4)	6.37 (6.7)	6.05 (3.8)
***n*-C$_{11}$**	4.95 (3.2)	7.04 (8.7)	6.93 (6.1)	6.99 (7.8)	6.57 (7.1)
***n*-C$_{12}$**	5.17 (5.3)	7.36 (9.5)	7.54 (9.2)	7.32 (2.1)	7.08 (4.3)
***n*-C$_{13}$**	5.57 (2.9)	7.60 (3.0)	7.67 (9.3)	7.69 (6.8)	7.32 (3.5)
***n*-C$_{14}$**	5.57 (4.5)	8.04 (6.6)	8.59 (6.8)	8.54 (2.0)	8.19 (4.8)
***n*-C$_{15}$**	5.01 (4.4)	7.31 (6.5)	7.54 (5.6)	7.37 (8.8)	7.18 (2.8)
***n*-C$_{16}$**	4.13 (1.0)	5.87 (4.9)	5.94 (5.0)	5.92 (3.0)	5.80 (5.5)
***n*-C$_{17}$**	3.98 (3.2)	6.30 (7.6)	5.75 (7.6)	6.21 (6.0)	6.06 (5.6)
Pristane	2.02 (3.5)	2.81 (6.5)	2.65 (5.5)	2.97 (2.8)	3.76 (4.5)
n-C$_{18}$	3.37 (1.7)	4.84 (2.9)	4.46 (4.7)	4.94 (8.2)	4.70 (2.6)
Phytane	1.58 (4.9)	2.03 (3.7)	1.93 (6.6)	1.96 (5.0)	3.05 (3.1)
***n*-C$_{19}$**	2.93 (5.1)	4.19 (4.1)	4.44 (3.8)	4.31 (0.6)	4.14 (2.6)

***n*-C$_{20}$**	2.53 (5.5)	3.58 (6.0)	3.79 (5.9)	3.61 (4.8)	3.51 (3.2)
***n*-C$_{21}$**	2.38 (3.4)	3.05 (6.0)	3.07 (7.8)	3.05 (4.8)	3.12 (3.2)
***n*-C$_{22}$**	2.24 (5.1)	3.06 (5.9)	2.97 (4.6)	2.91 (4.4)	2.94 (2.1)
***n*-C$_{23}$**	1.99 (5.2)	2.58 (5.6)	2.54 (7.1)	2.59 (3,9)	2.46 (2.4)
***n*-C$_{24}$**	1.82 (5.5)	2.45 (5.3)	2.50 (8.4)	2.47 (3.7)	2.46 (3.0)
***n*-C$_{25}$**	1.51 (5.6)	2.10 (3.5)	2.19 (3.5)	2.16 (3.9)	2.14 (0.8)
***n*-C$_{26}$**	1.30 (5.4)	1.81 (6.3)	1.86 (4.8)	1.87 (4.0)	1.92 (2.0)
***n*-C$_{27}$**	1.00 (4.8)	1.42 (5.2)	1.47 (7.0)	1.47 (3.0)	1.46 (3.2)
***n*-C$_{28}$**	0.68 (1.9)	1.12 (4.5)	1.13 (3.4)	1.12 (4.2)	1.12 (2.4)
***n*-C$_{29}$**	0.52 (0.9)	0.68 (4.6)	0.69 (5.2)	0.70 (2.8)	0.70 (3.4)
***n*-C$_{30}$**	0.36 (2.3)	0.53 (5.9)	0.58 (6.8)	0.55 (4.3)	0.55 (1.3)
***n*-C$_{31}$**	0.18 (3.8)	0.36 (5.5)	0.38 (6.4)	0.36 (6.0)	0.36 (2.0)
***n*-C$_{32}$**	0.10 (4.1)	0.18 (3.9)	0.17 (3.7)	0.13 (3.9)	0.16 (6.6)
***n*-C$_{33}$**	0.09 (2.0)	0.05 (3.1)	0.05 (4.4)	0.05 (5.3)	0.05 (8.6)
***n*-C$_{34}$**	0.10 (4.2)	0.38 (3.2)	0.32 (3.8)	0.32 (7.3)	0.27 (7.8)
***n*-C$_{35}$**	0.05 (5.6)	0.11 (3.9)	0.08 (7.8)	0.06 (3.7)	0.11 (7.1)
***n*-C$_{36}$**	0.05 (5.4)	0.09 (7.2)	0.09 (7.3)	0.09 (6.6)	0.08 (2.0)
***n*-C$_{37}$**	0.04 (0.6)	0.07 (5.3)	0.08 (6.7)	0.08 (4.3)	0.07 (5.3)
***n*-C$_{38}$**	0.03 (0.8)	0.06 (7.2)	0.07 4.2)	0.07 (5.4)	0.06 (4.3)

***n*-C_{39}**	0.03 (1.5)	0.03 (4.3)	0.06 (5.8)	0.05 (6.3)	0.05 (5.4)
Sum	72.32	103.00	103.33	103.97	102.47
***n*-C_{17}/pristane**	1.98	2.24	2.17	2.09	1.61
***n*-C_{18}/phytane**	2.14	2.39	2.31	2.52	1.54
pristane/phytane	1.28	1.39	1.37	1.51	1.23
CPI*	1.04	0.95	0.97	0.98	0.96
(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})	3.00	3.08	3.14	3.15	2.99

Table 0.4: Measured concentrations of target PAH in weathered Nigerian Light oil (µg/g oil)

PAH	Oil sample	5W	10W	15W	20W
C_0-N	247.3	230.2	217.0	189.5	136.8
C_1-N	1314.8	1294.9	1282.3	1251.1	1243.6
C_2-N	4680.0	4371.1	3802.3	3781.6	3642.1
C_3-N	1412.8	1319.1	1301.2	1285.6	1265.5
C_4-N	828.0	800.0	793.2	786.0	782.6
∑Naphthalenes	**8483.0**	**8015.4**	**7395.9**	**7293.8**	**7070.6**
C_0-B	344.5	192.5	175.9	170.0	135.0
C_1-B	968.4	733.3	670.2	496.5	295.2
C_2-B	1005.4	892.3	700.9	519.2	315.5
∑Biphenyls	**2318.3**	**1818.1**	**1547.0**	**1185.6**	**745.7**
C_0-DBF	135.3	80.4	78.8	71.1	60.1

C_1-DBF	167.9	160.9	158.7	153.8	149.4
C_2-DBF	515.9	497.0	491.2	489.8	485.6
C_3-DBF	309.8	301.7	305.8	302.8	301.8
∑Dibenzofurans	**1128.9**	**1039.9**	**1034.4**	**1017.5**	**996.8**
C_0-F	52.3	48.3	45.6	39.2	36.3
C_1-F	259.0	248.6	242.9	229.9	196.4
C_2-F	246.5	237.9	224.9	218.2	214.0
∑Fluorenes	**557.8**	**534.8**	**513.3**	**487.2**	**446.7**
C_0-DBT	122.8	108.2	101.5	97.5	76.9
C_1-DBT	180.2	169.3	158.2	145.0	122.4
C_2-DBT	169.4	157.7	146.7	134.3	113.9
C_3-DBT	140.6	129.0	118.5	107.4	86.6
∑Dibenzothiophenes	**612.9**	**564.2**	**524.8**	**484.2**	**399.8**
C_0-P	242.6	221.0	195.3	184.1	172.2
C_1-P	441.8	322.5	297.1	260.4	258.2
C_2-P	514.0	498.8	493.8	388.8	358.8
C_3-P	287.7	271.5	262.6	256.4	251.6
C_4-P	87.3	74.4	72.7	66.5	57.9
∑Phenanthrenes	**1573.4**	**1388.3**	**1321.5**	**1156.1**	**1098.7**
C_0-Py	9.3	6.4	6.4	6.5	6.6

C_1-Py	58.6	56.2	55.5	55.2	54.5
C_2-Py	52.2	47.9	44.7	41.9	39.9
∑Pyrenes	**120.1**	**110.5**	**106.6**	**103.6**	**101.0**
C_0-C	5.3	5.1	4.9	5.0	5.1
C_1-C	15.8	9.2	9.2	9.6	9.9
C_2-C	17.9	11.3	10.6	10.7	10.8
∑Chrysene	**39.1**	**25.6**	**24.7**	**25.3**	**25.7**
C_0-B(a)A	2.0	1.6	1.5	1.5	1.6
C_1-B(a)A	2.4	0.9	1.0	1.1	1.1
∑Benzo a anthracene	**4.4**	**2.6**	**2.5**	**2.6**	**2.7**
C_{20}-TAS	4.3	3.6	3.5	3.6	4.6
C_{21}-TAS	2.0	2.1	1.7	1.6	2.1
C_{26}R+C_{27}S	27.7	18.9	18.1	19.0	24.4
C_{28}S-TAS	23.3	16.3	15.7	16.3	20.3
C_{27}R-TAS	14.6	10.1	9.9	10.2	13.1
C_{28}R-TAS	19.7	12.9	12.6	13.0	16.7
∑TAS	**91.6**	**63.9**	**61.6**	**63.7**	**81.3**
Anthracene	11.06	9.7	9.9	8.9	8.9
Perylene	4.9	2.7	2.5	6.8	2.5
2-MN/1-MN	2.87	2.82	2.80	2.79	2.74

C_0-P/C_0-Anth	21.9	22.7	19.7	20.8	19.4

	Start	5W	10W	15W	20W
4-MDBT	1.00	1.00	1.00	1.00	1.00
a,b,c,d,e-PMN	0.43	0.41	0.41	0.42	0.42
v,w,x,y,z-PMN	1.10	1.10	0.80	0.79	0.79

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes (TAS). Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes 2 alkyl carbons (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbons (C_5-N). a,b,c,d,e-PMN and v,w,x,y,z-PMN are unidentified peaks found in the two Nigerian oils

Table 0.5: Measured concentrations of target PAH in weathered Nigerian medium oil (μg/g)

PAH	Oil sample	5W	10W	15W	20W
C_0-N	225.3	202.6	161.2	120.6	72.8
C_1-N	926.3	448.2	401.6	351.0	294.9
C_2-N	1733.9	1659.5	1589.2	1567.5	1469.3
C_3-N	1032.4	1168.4	1221.8	1274.8	1321.2
C_4-N	781.7	850.6	855.3	874.6	887.9
∑Naphthalenes	**4699.5**	**4329.4**	**4229.1**	**4188.4**	**4046.2**
C_0-B	174.2	160.6	121.0	91.0	57.0
C_1-B	239.0	205.1	180.6	151.4	65.4
C_2-B	321.8	294.2	281.2	260.0	252.5

∑Biphenyls	**734.9**	**659.9**	**582.8**	**502.5**	**375.0**
C_0-DBF	103.6	101.0	95.1	93.3	89.2
C_1-DBF	220.8	218.3	192.8	190.0	188.9
C_2-DBF	538.8	565.9	520.5	508.4	507.9
C_3-DBF	257.8	303.4	314.2	332.4	338.3
∑Dibenzofurans	**1120.9**	**1188.5**	**1122.6**	**1124.0**	**1124.2**
C_0-F	71.8	74.1	80.0	82.4	84.2
C_1-F	250.8	238.1	214.1	190.9	177.7
C_2-F	354.2	332.6	326.6	304.3	284.2
∑Fluorenes	**676.8**	**644.8**	**620.7**	**577.6**	**546.1**
C_0-DBT	164.2	152.2	149.6	138.9	134.5
C_1-DBT	183.2	178.7	170.2	166.7	146.0
C_2-DBT	168.4	151.2	148.3	145.0	134.8
C_3-DBT	129.5	127.0	114.0	103.4	89.5
∑Dibenzothiophenes	**645.3**	**609.2**	**582.1**	**554.0**	**504.7**
C_0-P	297.3	281.9	271.2	250.6	243.1
C_1-P	619.2	442.9	419.6	394.2	386.7
C_2-P	712.9	688.6	639.4	583.3	550.6
C_3-P	321.9	311.6	295.6	281.6	271.6
C_4-P	193.8	181.4	170.2	156.6	124.5

∑Phenanthrenes	**2145.2**	**1906.5**	**1796.0**	**1666.3**	**1576.5**
C_0-Py	19.9	18.0	17.0	16.9	16.2
C_1-Py	120.7	118.4	117.3	114.1	110.9
C_2-Py	101.0	102.4	103.9	103.9	104.1
∑Pyrenes	**241.7**	**238.8**	**238.2**	**234.9**	**231.2**
C_0-C	13.8	13.8	14.0	13.6	13.3
C_1-C	37.1	36.0	36.8	33.1	32.9
C_2-C	38.9	39.0	41.0	36.7	35.0
∑Chrysene	**89.8**	**88.7**	**91.8**	**83.3**	**81.1**
C_0-B(a)A	4.2	3.9	3.6	3.4	3.2
C_1-B(a)A	4.5	4.2	4.1	4.0	3.9
∑Benzo a anthracene	**8.7**	**8.1**	**7.6**	**7.4**	**7.1**
C_{20}-TAS	6.1	5.7	5.4	5.1	5.4
C_{21}-TAS	2.9	3.1	3.1	2.8	3.2
C_{26}R+C_{27}S	22.0	13.9	8.0	9.8	13.9
C_{28}S-TAS	20.8	14.5	1.4	9.6	13.2
C_{27}R-TAS	11.7	8.4	4.5	5.9	7.7
C_{28}R-TAS	17.7	10.6	5.2	8.8	10.7
∑TAS	**81.2**	**56.3**	**27.6**	**42.0**	**54.2**
Anthracene	9.58	4.7	3.0	2.5	2.3

Perylene	2.6	2.5	2.5	2.5	2.7
2-MN/1-MN	1.54	1.86	1.80	1.79	1.79
C_0-P/C_0-Anth	31.0	29.5	33.5	33.2	35.2

	Oil sample	**5W**	**10W**	**15W**	**20W**
4-MDBT	1.00	1.00	1.00	1.00	1.00
a,b,c,d,e-PMN	0.51	0.53	0.51	0.52	0.50
v,w,x,y,z-PMN	1.39	1.33	1.41	1.40	1.34

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS). Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes 2 alkyl carbons (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbons (C_5-N). a,b,c,d,e-PMN and v,w,x,y,z-PMN are unidentified peaks found in the two Nigerian oils.

Table 0.6: Measured concentrations of target PAH in weathered North Sea oil (µg/g)

PAH	**Oil sample**	**5W**	**10W**	**15W**	**20W**
C_0-N	581.4	152.3	55.5	2.8	1.6
C_1-N	799.1	913.5	1374.0	13.2	5.4
C_2-N	1136.8	3003.2	3212.2	22.9	9.0
C_3-N	857.8	1987.8	1904.4	13.7	8.2
C_4-N	470.6	403.3	379.7	3.9	3.4
∑Naphthalenes	**3845.7**	**6460.1**	**6926.0**	**56.6**	**27.7**
C_0-B	413.6	254.2	238.6	1.2	0.7

C_1-B	572.8	331.9	325.7	2.2	1.7
C_2-B	318.8	220.4	211.6	1.9	2.5
∑Biphenyls	**1305.3**	**806.4**	**775.8**	**5.3**	**4.9**
C_0-DBF	63.3	48.7	45.2	0.3	0.2
C_1-DBF	194.3	155.5	147.7	1.3	1.3
C_2-DBF	360.3	401.6	387.8	44.6	10.9
C_3-DBF	161.9	196.7	199.1	52.9	9.0
∑Dibenzofurans	**779.8**	**802.4**	**779.8**	**99.1**	**21.4**
C_0-F	78.4	168.9	157.5	51.1	8.0
C_1-F	426.0	315.7	301.2	263.1	78.2
C_2-F	398.0	291.4	277.5	213.6	186.3
∑Fluorenes	**902.4**	**776.0**	**736.2**	**527.8**	**272.5**
C_0-DBT	98.7	79.0	75.0	30.7	1.0
C_1-DBT	112.1	217.8	203.6	52.2	4.5
C_2-DBT	293.7	266.3	251.1	153.0	23.0
C_3-DBT	163.4	130.4	120.9	71.6	28.5
∑Dibenzothiophenes	**667.9**	**693.5**	**650.6**	**307.5**	**57.1**
C_0-P	500.6	603.4	559.7	404.0	135.1
C_1-P	769.6	947.9	893.7	568.2	407.6
C_2-P	943.6	852.8	797.7	511.6	427.9

C_3-P	623.4	551.6	507.4	411.8	351.6
C_4-P	165.4	142.7	119.4	113.7	106.6
∑Phenanthrenes	**3002.6**	**3098.3**	**2878.0**	**2009.4**	**1428.8**
C_0-Py	55.2	44.5	35.3	20.5	12.7
C_1-Py	224.2	168.3	157.0	145.8	140.8
C_2-Py	181.2	145.3	114.6	73.1	62.7
∑Pyrenes	**460.6**	**358.1**	**306.9**	**239.3**	**216.2**
C_0-C	96.8	85.1	82.9	74.3	71.1
C_1-C	164.1	153.9	146.0	138.5	127.0
C_2-C	123.5	107.9	105.0	94.4	87.8
∑Chrysene	**384.5**	**346.9**	**333.9**	**307.2**	**285.8**
C_0-B(a)A	13.2	9.5	9.3	9.1	8.8
C_1-B(a)A	10.4	8.6	7.5	7.1	6.4
∑Benzo a anthracene	**23.6**	**18.0**	**16.9**	**16.1**	**15.3**
C_{20}-TAS	12.9	10.8	11.2	4.8	0.0
C_{21}-TAS	2.5	2.1	3.2	4.9	0.0
C_{26}R+C_{27}S	11.5	7.3	7.8	0.0	0.0
C_{28}S-TAS	7.6	4.9	5.2	0.0	0.0
C_{27}R-TAS	6.9	4.2	4.6	0.0	0.0
C_{28}R-TAS	6.6	4.0	4.2	0.0	0.0

∑TAS	**48.0**	**33.5**	**36.2**	**9.7**	**0.0**
Anthracene	16.5	4.9	3.6	2.1	1.3
Perylene	13.4	7.4	8.7	11.5	12.5
2-MN/1-MN	1.34	1.65	1.73	1.69	1.56
C_0-P/C_0-Anth	30.43	30.87	30.50	32.95	33.60

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS). Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes 2 alkyl carbons (C_2-N) and so on.

Table 0.7: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing water washing, for Nigerian Light oil.

	Oil (start)	5W	10W	15W	20W
2-MBp	32.5	18.23	13.95	8.50	6.55
3-MBp	723.0	528.91	489.09	347.99	213.62
4-MBp	212.9	186.13	167.21	139.99	75.03
	968.4	**733.27**	**670.25**	**496.49**	**295.19**
3-MP	118.4	63.7	61.7	65.7	65.9
2-MP	116.2	64.8	64.5	67.6	65.6
MA	17.2	7.0	7.0	7.8	7.5
9-MP	110.5	73.6	71.3	75.2	73.8
1-MP	79.5	43.5	42.6	44.0	45.4

	441.8	**252.5**	**247.1**	**260.4**	**258.2**

Table 0.8: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing water washing, for Nigerian Medium oil.

	Oil (start)	**5W**	**10W**	**15W**	**20W**
2-MBp	10.6	8.62	5.06	1.01	0.17
3-MBp	107.9	85.23	72.26	60.40	23.55
4-MBp	120.4	111.24	103.29	90.01	41.70
	238.95	**205.10**	**180.61**	**151.42**	**65.42**
3-MP	210.8	74.4	82.7	73.3	83.5
2-MP	208.1	79.2	87.1	79.5	91.3
MA	20.1	7.5	6.7	7.3	9.1
9-MP	225.1	115.0	125.4	121.5	130.2
1-MP	155.1	66.8	67.7	62.6	72.7
	819.24	**342.91**	**369.64**	**344.16**	**386.69**

Table 0.9: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing water washing, for North Sea oil.

	Oil (start)	**5W**	**10W**	**15W**	**20W**

2-MBp	26.5	20.57	19.78	0.27	0.17
3-MBp	383.3	223.10	219.44	1.32	1.02
4-MBp	163.0	88.18	86.47	0.56	0.53
	572.83	**331.85**	**325.69**	**2.15**	**1.71**
3-MP	167.6	189.9	188.6	93.5	56.5
2-MP	184.1	196.5	180.4	121.6	87.7
MA	19.8	12.8	12.4	8.3	4.3
9-MP	232.0	305.9	284.4	212.6	164.1
1-MP	166.1	242.8	227.8	132.1	94.9
	769.58	**947.88**	**893.75**	**568.18**	**407.60**

Table 0.10: Change in concentrations (μg/g) with increased water washing, of the a,b,c,d,e-PMN, v,w,x,y,z-PMN, and methyldibenzothiophenes isomers with corresponding percentage compositions for Nigerian Light and Medium oils.

Nigerian light oil						Nigerian medium oil					
PAH	**Oil (Start)**	**5W**	**10W**	**15W**	**20W**	**PAH**	**Oil (start)**	**5W**	**10W**	**15W**	**20W**
4-MDBT	22.66	20.25	19.52	20.74	20.56	**4-MDBT**	28.00	20.83	22.51	19.63	23.29
a, b, c, d, e-PMN	9.67	8.29	7.95	8.66	8.57	**a, b, c, d, e-PMN**	14.41	11.00	11.51	10.12	11.72
3+2-MDBT	17.81	15.79	15.56	16.36	16.29	**3+2-MDBT**	25.64	18.36	21.06	18.18	20.92
v, w, x, y, z-PMN	25.02	22.33	20.77	21.66	22.34	**v, w, x, y, z-PMN**	38.94	27.79	31.78	27.40	31.24
1-MDBT	5.05	4.54	4.38	4.57	4.63	**1-MDBT**	10.48	7.76	8.30	7.32	8.81
∑MDBT	**80.2**	**71.20**	**68.18**	**139.11**	**72.40**	**∑MDBT**	**117.47**	**85.74**	**95.16**	**82.66**	**95.99**

					Relative Percentage composition (%)						
4-MDBT	28.3	28.4	28.6	14.9	28.4	**4-MDBT**	23.8	24.3	23.7	23.8	24.3
a, b, c, d, e-PMN	12.1	11.6	11.7	6.2	11.8	**a, b, c, d, e-PMN**	12.3	12.8	12.1	12.2	12.2
3+2-MDBT	22.2	22.2	22.8	11.8	22.5	**3+2-MDBT**	21.8	21.4	22.1	22.0	21.8
v, w, x, y, z-PMN	31.2	31.4	30.5	15.6	30.9	**v, w, x, y, z-PMN**	33.1	32.4	33.4	33.1	32.5
1-MDBT	6.3	6.4	6.4	3.3	6.4	**1-MDBT**	8.9	9.0	8.7	8.9	9.2

Note: 1-MDBT, 3+2-MDBT, and 4-MDBT are 1-methyldibenzothiophene, 2/3-methyldibenzothiophene, and 4-methyldibenzothiophene respectively

Table 0.11: Measured concentrations of terpanes in weathered Nigerian light oil (µg/g)

	Terpanes	**Measured Concentration**				
Peak Number	**Tricyclic Terpanes**	**Start**	**5W**	**10W**	**15W**	**20W**
1	**S**	429.5	465.5	456.7	463.3	446.4
2	**Q**	114.0	179.7	168.7	160.4	145.1
3	C_{19}	330.0	376.8	353.2	352.7	330.3
4	**M**	118.5	171.5	162.5	176.0	160.1
5	C_{20}	295.9	310.8	298.8	346.4	287.4
6	**X**	462.1	452.2	414.9	459.7	434.1
7	C_{21}	284.0	281.2	257.2	332.8	318.0
8	C_{22}	172.7	158.6	148.8	152.2	147.4
9	C_{23}	368.4	351.8	325.0	342.5	336.6

10	**C_{24}**	391.0	368.5	346.1	357.5	345.3
11	**Y**	415.3	362.4	333.2	372.1	354.5
12	**Y1**	684.0	622.3	598.3	652.7	636.2
13	**C_{25}A**	105.0	154.2	146.3	161.0	145.6
14	**C_{25}B**	392.2	416.6	370.4	381.4	340.7
15	**Z**	184.3	157.6	149.6	168.3	156.2
16	**Tet24**	446.4	369.4	349.9	334.2	319.8
17	**C_{26}(S)**	56.2	133.6	114.9	168.2	162.2
18	**C_{26}(R)**	181.6	191.8	173.8	164.3	156.4

		Measured Concentration				
	Tetracyclic Terpanes	**Start**	**5W**	**10W**	**15W**	**20W**
19	**Z1**	387.1	389.6	282.8	328.2	316.2
20	**C_{28}A**	274.9	277.6	252.0	320.3	288.4
21	**C_{28}B**	135.1	203.6	170.2	203.8	190.4
22	**C_{29}A**	198.0	237.9	207.3	220.4	170.4
23	**C_{29}B**	276.4	292.0	265.6	250.8	253.8
24	**T**	203.7	192.7	181.9	186.2	167.4

		Measured Concentration				
	Pentacyclic Terpanes	**Start**	**5W**	**10W**	**15W**	**20W**

25	**C_{27}Ts**	977.8	964.5	882.7	983.2	926.8
26	**C_{27}Tm**	1374.9	1383.5	1314.7	1319.7	1233.4
27	**C_{29}nor25H**	1022.8	847.1	833.2	783.6	746.2
28	**C_{29}Tm**	6236.0	6614.5	6110.3	6804.7	6460.3
29	**C_{29}Ts**	1317.8	1373.1	1254.2	886.7	824.5
30	**C_{30}: 18α-Oleanane**	5867.9	6669.0	6220.9	6456.3	5966.5
31	**C_{30}αβ**	8748.1	9761.8	9018.5	9122.3	8681.7
32	**C_{30}-Mor**	1561.4	1621.4	1441.5	1461.4	1406.1
33	**C_{31}S**	1760.3	2092.9	1882.9	1946.5	1883.7
34	**C_{31}R**	1569.7	1639.3	1464.1	1462.0	1432.3
35	**Gam**	472.6	498.1	516.5	421.0	440.0
36	**C_{32}S**	1067.8	1169.5	1053.5	1055.2	1012.6
37	**C_{32}R**	825.0	894.4	781.1	771.1	757.4
38	**C_{33}S**	670.4	793.7	724.4	701.1	678.2
39	**C_{33}R**	645.4	568.5	534.4	487.9	457.9
40	**C_{34}S**	382.2	438.9	396.4	364.3	355.3
41	**C_{34}R**	309.9	261.4	230.7	222.3	196.7
42	**C_{35}S**	229.3	182.4	154.4	141.8	131.6
43	**C_{35}R**	232.1	199.0	181.9	168.4	167.0

Total (ppm)	42177.6	45091.0	41525.0	42614.8	40367.0

Table 0.12: Measured concentrations of terpanes in weathered Nigerian medium oil (µg/g)

	Terpanes	Measured Concentration				
Peak Number	**Tricyclic Terpanes**	**Start**	**5W**	**10W**	**15W**	**20W**
1	**S**	303.3	325.7	341.2	326.2	408.8
2	**Q**	120.6	121.2	144.8	159.7	197.4
3	**C_{19}**	379.6	374.7	425.3	369.7	471.8
4	**M**	124.2	178.1	155.3	183.8	181.2
5	**C_{20}**	344.3	382.8	380.4	415.5	440.2
6	**X**	651.5	567.5	600.0	648.3	673.9
7	**C_{21}**	226.8	209.6	206.1	222.3	302.4
8	**C_{22}**	280.1	240.5	262.6	283.9	303.9
9	**C_{23}**	294.5	257.8	249.1	260.4	271.1
10	**C_{24}**	304.8	303.6	326.6	316.7	345.6
11	**Y**	498.2	437.6	479.8	518.7	531.6
12	**Y1**	617.7	612.7	624.1	691.1	713.1
13	**C_{25}A**	93.3	98.8	115.8	123.5	114.5
14	**C_{25}B**	252.7	238.9	256.0	238.0	257.7

15	**Z**	85.1	109.4	116.7	128.1	144.3
16	**Tet24**	669.0	552.7	658.6	681.0	721.2
17	**C_{26}(S)**	57.6	106.0	136.0	134.6	146.6
18	**C_{26}(R)**	128.8	139.7	90.7	82.9	92.0

		Measured Concentration				
	Tetracyclic Terpanes	**Start**	**5W**	**10W**	**15W**	**20W**
19	**Z1**	366.3	371.1	303.7	329.2	344.1
20	**C_{28}A**	229.6	213.7	260.7	295.3	374.9
21	**C_{28}B**	163.5	145.0	143.6	150.1	171.4
22	**C_{29}A**	132.6	154.5	200.2	142.4	152.4
23	**C_{29}B**	315.8	410.4	389.9	448.4	477.5
24	**T**	121.6	134.1	127.5	138.4	124.1

		Measured Concentration				
	Pentacyclic Terpanes	**Start**	**5W**	**10W**	**15W**	**20W**
25	**C_{27}Ts**	1791.7	1764.0	1843.6	2036.5	2078.9
26	**C_{27}Tm**	1875.8	1833.5	1883.2	2111.4	2194.1
27	**C_{29}nor25H**	945.9	1122.4	1059.9	993.4	1127.3
28	**C_{29}Tm**	7037.1	7646.0	7421.1	8242.8	8804.7
29	**C_{29}Ts**	1288.1	1625.6	1647.5	1556.3	1856.9

30	**C_{30}: 18α-Oleanane**	5254.3	6868.7	6232.6	6125.2	7080.8
31	**$C_{30}αβ$**	10284.7	13171.3	10783.1	12077.3	12917.8
32	**C_{30}-Mor**	1553.5	1461.6	1376.2	1573.3	1644.0
33	**$C_{31}S$**	2135.8	2291.6	2302.4	2336.3	2661.0
34	**$C_{31}R$**	1678.7	1989.5	1752.1	1755.4	2004.0
35	**Gam**	526.6	668.2	564.7	646.7	624.3
36	**$C_{32}S$**	1392.2	1694.6	1395.6	1403.2	1581.3
37	**$C_{32}R$**	992.7	1166.0	1007.0	1040.5	1144.2
38	**$C_{33}S$**	688.0	837.5	740.1	732.6	837.7
39	**$C_{33}R$**	505.3	603.9	514.7	510.4	614.6
40	**$C_{34}S$**	360.2	485.7	400.2	436.8	470.8
41	**$C_{34}R$**	300.1	223.7	232.3	208.7	263.2
42	**$C_{35}S$**	224.8	236.1	211.5	189.2	245.5
43	**$C_{35}R$**	213.1	164.2	158.4	180.4	141.3
	Total (ppm)	**45810.1**	**52540.1**	**48521.1**	**51444.6**	**56254.3**

Table 0.13: Measured concentrations of terpanes in weathered North Sea oil (µg/g)

	Terpanes	**Measured Concentration**				
Peak Number	**Tricyclic Terpanes**	**Start**	**5W**	**10W**	**15W**	**20W**

1	**C_{19}**	55.8	108.1	107.7	92.6	113.7
2	**C_{20}**	40.8	70.4	126.0	235.7	134.1
3	**C_{21}**	81.3	146.7	151.3	76.6	44.0
4	**C_{22}**	34.3	43.1	44.3	64.1	71.4
5	**U**	29.3	40.1	40.3	45.4	134.0
6	**C_{23}**	86.6	152.9	154.4	230.4	250.3
7	**C_{24}**	83.3	147.9	153.4	178.5	179.6
8	**C_{25}B**	89.1	150.2	145.3	179.2	219.8
9	**Tet24**	80.5	88.8	91.8	64.8	73.4
10	**C_{26}(S)**	37.3	48.9	50.6	39.5	48.9
11	**C_{26}(R)**	54.5	63.2	64.6	62.5	81.3

		Measured Concentration				
	Tetracyclic Terpanes	**Start**	**5W**	**10W**	**15W**	**20W**
12	**U1**	41.0	86.5	90.8	51.4	83.8
13	**U2**	23.7	32.6	42.7	33.8	50.0
14	**C_{28}A**	34.4	61.8	56.5	108.4	123.0
15	**C_{28}B**	32.5	71.5	62.6	68.5	78.2
16	**C_{29}A**	46.4	87.4	90.4	65.3	77.2
17	**C_{29}B**	38.7	89.3	79.6	72.3	75.8

18	**T**	48.8	91.1	89.6	66.3	75.8

	Pentacyclic Terpanes	**Measured Concentration**				
		Start	**5W**	**10W**	**15W**	**20W**
19	**C_{27}Ts**	149.8	198.1	219.4	230.9	208.1
20	**C27Tm**	99.7	98.4	107.9	95.3	86.3
21	**C_{29}nor25H**	30.5	64.2	50.5	57.7	51.0
22	**C_{29}Tm**	145.1	294.4	284.7	282.8	268.8
23	**C_{29}Ts**	105.6	196.2	195.0	189.4	169.5
24	**C_{30}dh**	97.8	189.8	196.4	170.8	144.4
25	**C_{30}-Hopane**	337.0	709.4	663.8	625.0	594.8
26	**C_{30}-Mor**	58.9	109.0	97.0	86.4	75.8
27	**C_{31}S**	177.0	325.6	309.4	286.1	255.6
28	**C_{31}R**	128.8	238.8	236.1	212.6	190.9
29	**Gam**	40.9	90.5	84.0	102.0	105.1
30	**C_{32}S**	155.9	259.0	258.6	231.7	201.7
31	**C_{32}R**	108.8	180.7	177.9	160.3	133.2
32	**C_{33}S**	112.2	194.7	183.4	177.8	164.1
33	**C_{33}R**	78.3	136.9	134.0	112.5	98.1
34	**C_{34}S**	68.4	134.0	127.4	122.8	106.0

35	**C_{34}R**	42.8	80.7	77.4	70.1	60.6
36	**C_{35}S**	80.1	139.2	131.7	122.9	101.1
37	**C_{35}R**	48.6	93.1	77.4	79.8	66.3
	Total (ppm)	**3004.9**	**5313.2**	**5253.9**	**5152.3**	**4995.4**

Table 0.14: Measured steranes concentrations in unweathered (start) and weathered Nigerian Light oil (µg/g oil)

Peak Number	**Steranes**	**Determined Concentration (Cdet) -(M/Z 217)**				
		Start	**5W**	**10W**	**15W**	**20W**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	101.8	130.1	118.9	123.2	123.8
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	189.2	255.8	228.0	285.7	276.3
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	101.9	132.8	120.5	132.5	130.4
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	205.5	273.1	244.3	263.9	246.1
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	132.1	161.9	144.6	182.6	174.5
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	83.6	86.7	81.9	91.7	87.3
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	94.9	104.6	97.5	131.7	117.5
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	186.0	175.8	155.0	193.7	178.7
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	370.4	393.2	362.9	398.8	357.9
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	468.5	468.2	432.7	479.7	434.7
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	289.6	297.6	170.7	261.0	223.9
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	630.7	717.3	657.3	670.7	618.4
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	468.0	433.4	398.6	454.5	414.9

14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	221.2	170.5	163.7	172.8	161.4
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	455.5	441.2	405.1	411.3	404.0
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	494.1	467.3	395.2	470.3	446.8
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	355.7	391.2	373.7	367.7	345.8
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	282.9	224.6	196.1	237.5	224.8
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	604.1	623.9	579.8	636.5	581.4
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	688.2	777.8	709.4	767.5	706.9
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	581.3	799.1	783.9	720.3	663.0
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	298.7	203.8	205.1	312.8	277.5
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	894.8	1128.9	1041.9	1089.5	1020.2
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	142.4	146.9	143.6	132.3	134.2
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	65.0	67.6	63.5	69.9	73.7
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	170.8	139.6	137.5	142.8	143.2
	Total	**8576.9**	**9212.7**	**8411.4**	**9200.7**	**8567.3**

Table 0.15: Measured steranes concentrations in unweathered (start) and weathered Nigerian Medium oil (µg/g oil)

Peak Number	**Steranes**	**Determined Concentration (Cdet) -(M/Z 217)**				
		Start	**5W**	**10W**	**15W**	**20W**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	91.7	104.7	132.6	116.0	129.6
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	151.1	197.1	199.7	220.1	221.9
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	77.2	90.0	87.0	96.4	104.4

4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	173.2	208.4	197.4	204.4	220.7
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	95.3	116.3	119.6	129.1	132.7
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	45.3	48.4	53.4	54.4	59.5
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	66.9	73.4	82.3	87.2	85.8
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	120.2	116.6	103.0	126.7	143.0
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	171.9	156.0	167.9	152.3	157.6
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	308.9	321.8	308.7	343.2	344.3
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	154.6	169.4	169.9	193.6	183.9
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	210.9	263.7	288.0	262.8	270.7
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	257.7	270.5	249.2	299.0	314.7
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	199.6	203.9	202.6	207.1	230.6
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	238.3	225.1	209.9	241.6	243.0
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	270.9	298.4	263.2	301.8	316.3
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	192.8	203.2	227.3	226.4	234.0
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	147.1	151.0	130.6	149.9	162.5
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	205.3	240.8	222.8	223.3	256.6
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	470.7	552.1	559.2	549.5	587.2
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	321.9	389.5	324.3	366.1	381.5
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	172.7	205.3	202.0	234.1	213.7
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	285.1	391.2	390.5	452.1	461.9
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	108.1	117.1	112.3	102.2	116.4

25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	36.1	18.6	16.3	16.4	20.2
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	109.1	130.1	131.4	127.2	142.6
	Total	**4682.3**	**5262.8**	**5151.0**	**5483.1**	**5735.2**

Table 0.16: Measured steranes concentrations in unweathered (start) and weathered North Sea oil (μg/g oil)

		Determined Concentration (Cdet) -(M/Z 217)				
Peak Number	**Steranes**	**Start**	**5W**	**10W**	**15W**	**20W**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	126.0	276.9	289.1	253.6	273.3
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	128.9	302.5	319.4	272.6	293.6
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	78.9	164.0	166.3	160.0	155.6
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	309.2	625.8	634.0	589.0	479.6
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	219.9	384.2	396.2	374.8	295.9
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	101.8	173.2	190.7	169.3	138.6
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	103.9	184.9	186.0	178.9	150.6
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	166.2	274.3	280.4	250.7	233.5
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	109.5	143.1	144.2	129.0	110.7
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	289.1	552.1	541.8	490.6	449.9
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	184.8	351.1	340.0	299.6	250.2
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	113.1	186.8	180.5	170.9	137.5
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	228.4	460.4	448.0	446.3	363.3
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	145.9	258.3	249.3	250.1	196.9

15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	86.5	208.2	210.9	197.4	166.9
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	102.6	146.2	146.4	129.6	112.5
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	143.1	314.1	316.8	275.2	244.7
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	105.4	225.3	225.6	208.1	176.1
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	78.3	172.6	184.1	159.3	136.9
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	90.1	230.3	251.1	250.6	230.4
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	124.7	262.4	267.0	239.1	260.7
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	79.5	184.5	192.3	186.5	194.1
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	103.1	227.4	221.2	189.3	144.8
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	73.7	144.6	149.8	212.2	152.2
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	10.4	34.7	52.9	81.9	55.6
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	23.6	51.0	73.0	56.1	64.3
	Total	**3326.6**	**6539.0**	**6656.9**	**6220.6**	**5468.5**

Table 0.17: Measured triaromatic steranes concentrations in unweathered (start) and weathered Nigerian Light oil (μg/g oil)

Peak Number	**Triaromatic Steranes (TAS)**	**Measured Concentration (Cdet) -(M/Z 231)**				
		Start	**5W**	**10W**	**15W**	**20W**
1	**P**	116.7	911.4	990.2	578.4	538.5
2	**Q**	55.9	490.4	474.6	331.5	292.0
3	**C20-TAS**	92.4	777.6	780.7	490.1	449.5
4	**C21-TAS**	42.8	451.5	369.4	236.1	184.1

5	**C22A-TAS**	25.8	237.9	215.8	146.0	126.5
6	**C22B-TAS**	30.5	212.9	195.5	130.6	105.7
7	**R**	24.5	213.8	169.2	108.8	88.6
8	**S**	15.3	157.8	134.8	94.1	78.5
9	**T**	40.4	338.0	309.5	201.9	185.3
10	**C26(20S) TA-cholestane**	206.7	1445.2	1422.5	900.9	818.2
11	**C26 (20R) cholastena + C27(20S) TA-ergostane**	596.3	4078.3	4046.9	2610.8	2363.0
12	**U**	44.0	344.5	361.1	236.0	189.8
13	**C28 (20S) TA-stigmastane**	503.0	3530.5	3510.3	2176.8	2027.7
14	**C27(20R) TA-ergostane**	313.0	2186.7	2219.8	1402.2	1264.3
15	**C28 (20R) TA-stigmastane**	413.4	2792.8	2813.4	1821.3	1606.9
16	**V**	85.4	621.0	670.0	365.8	359.9
	Total	**2606.1**	**18790.3**	**18683.8**	**11831.3**	**10678.3**

Table 0.18: Measured triaromatic steranes concentrations in unweathered (start) and weathered Nigerian Medium oil (µg/g oil)

Peak Number	Triaromatic Steranes (TAS)	Measured Concentration (Cdet) -(M/Z 231)				
		Start	**5W**	**10W**	**15W**	**20W**
1	**P**	193.4	301.9	349.2	362.2	467.5
2	**Q**	131.6	265.9	260.2	310.0	377.7

3	**C20-TAS**	125.2	163.3	263.3	310.7	351.5
4	**C21-TAS**	59.4	110.6	143.7	154.7	194.3
5	**C22A-TAS**	45.5	48.0	47.4	57.1	70.6
6	**C22B-TAS**	21.0	51.1	46.3	61.7	63.2
7	**R**	13.9	11.9	30.6	29.5	45.6
8	**S**	11.7	11.8	21.0	21.1	24.1
9	**T**	32.5	12.9	54.2	46.5	53.9
10	**C26(20S) TA-cholestane**	132.7	36.6	211.8	202.1	258.8
11	**C26 (20R) cholastena + C27(20S) TA-ergostane**	449.8	513.7	664.5	653.8	841.9
12	**U**	40.9	51.6	87.7	61.6	88.1
13	**C28 (20S) TA-stigmastane**	428.7	666.4	692.5	590.8	798.0
14	**C27(20R) TA-ergostane**	242.3	198.4	403.9	406.6	471.5
15	**C28 (20R) TA-stigmastane**	362.6	236.5	537.5	569.9	667.6
16	**V**	112.1	156.5	152.5	193.4	190.6
	Total	**2403.2**	**2837.0**	**3966.4**	**4031.5**	**4964.9**

Table 0.19: Measured triaromatic steranes concentrations in unweathered (start) and weathered North Sea oil (µg/g oil)

Peak Number	**Triaromatic Steranes (TAS)**	**Measured Concentration (Cdet) -(M/Z 231)**				
		Start	**5W**	**10W**	**15W**	**20W**

1	**P**	306.5	2491.8	2553.9	42.3	0.0
2	**Q**	223.5	1692.3	1695.8	40.8	0.0
3	**C20-TAS**	308.8	2537.3	2454.5	91.5	0.0
4	**C21-TAS**	60.9	620.6	679.4	74.0	0.0
5	**C22A-TAS**	39.9	349.3	342.4	14.2	0.0
6	**C22B-TAS**	34.3	283.7	244.8	27.4	0.0
7	**R**	7.5	110.8	124.7	0.0	0.0
8	**S**	12.1	86.5	104.6	0.0	0.0
9	**T**	23.4	137.6	159.3	0.0	0.0
10	**C26(20S) TA-cholestane**	77.6	484.3	529.7	0.0	0.0
11	**C26 (20R) cholastena + C27(20S) TA-ergostane**	280.1	1692.1	1744.8	0.0	0.0
12	**U**	18.7	118.4	136.3	0.0	0.0
13	**C28 (20S) TA-stigmastane**	185.6	1106.1	1170.6	0.0	0.0
14	**C27(20R) TA-ergostane**	165.9	988.5	1031.9	0.0	0.0
15	**C28 (20R) TA-stigmastane**	158.2	917.6	945.4	0.0	0.0
16	**V**	36.9	231.2	221.4	0.0	0.0
	Total	**1939.9**	**13848.2**	**14139.6**	**290.2**	**0.0**

Table 0.20: Measured sesquiterpanes concentrations in unweathered (start) and weathered Nigerian Light oil (µg/g oil)

				Measured Concentration				
Peak Number	**Bicyclic Sesquiterpanes**	**Abbreviations**	**Formula**	**Start**	**5W**	**10W**	**15W**	**20W**

1	**C_4-decalin (C14)**	**BS1**	**C14H26**	3019.4	484.3	241.8	269.3	804.8
2	**C_{14} Sesquterpane**	**BS2**	**C14H26**	1425.5	299.1	156.0	142.9	441.5
Unknown	**A Sesquiterpane**	**BSA**	**C15H28**	8270.3	1437.7	720.1	752.6	2229.8
Unknown	**U2 Sesquiterpane**	**BSU2**	**C15H28**	2459.5	259.6	134.8	165.9	504.5
3	**C_{15} Sesquiterpane**	**BS3**	**C15H28**	7314.7	1147.2	582.0	629.3	1844.7
Unknown	**B Sesquiterpane**	**BSY**	**C15H28**	1452.2	227.1	114.5	114.4	320.8
Unknown	**C_{15} Sesquiterpane**	**BSB**	**C15H28**	3052.0	534.5	261.6	274.7	793.1
4	**D Sesquiterpane**	**BS4**	**C15H28**	4586.7	743.3	371.9	411.8	1205.9
Unknown	**8β(H)-drimane**	**BSD**	**C15H28**	3127.9	621.4	314.5	253.0	737.5
5	**C_{15} Sesquiterpane**	**BS5**	**C15H28**	4096.5	692.6	340.1	413.2	1214.0
6	**C_{16} Sesquiterpane**	**BS6**	**C15H28**	2853.2	473.1	233.2	258.4	746.4
7	**C_{16} Sesquiterpane**	**BS7**	**C16H30**	1548.4	317.6	167.8	162.4	471.2
8	**C_{16} Sesquiterpane**	**BS8**	**C16H30**	1711.8	304.6	151.5	144.2	412.2
9	**8β(H)-Homodrimane**	**BS9**	**C16H30**	1171.6	168.5	81.9	59.0	168.9
10	**C_4-decalin (C14)**	**BS10**	**C16H30**	3775.6	701.6	348.1	350.1	1015.3
	Total			**49865.4**	**8412.3**	**4219.9**	**4401.3**	**12910.5**

Table 0.21: Measured sesquiterpanes concentrations in unweathered (start) and weathered Nigerian Medium oil (µg/g oil)

				Measured Concentration				
Peak Number	**Bicyclic Sesquiterpanes**	**Abbreviations**	**Formula**	**Start**	**5W**	**10W**	**15W**	**20W**
1	**C_4-decalin (C14)**	**BS1**	**C14H26**	3734.6	901.0	867.7	461.8	359.5
2	**C_{14} Sesquterpane**	**BS2**	**C14H26**	2007.0	612.7	632.6	311.9	253.7
Unknown	**A Sesquiterpane**	**BSA**	**C15H28**	11070.7	2704.2	2630.3	1424.4	1106.3
Unknown	**U2 Sesquiterpane**	**BSU2**	**C15H28**	3638.2	659.1	617.8	347.3	290.4
3	**C_{15} Sesquiterpane**	**BS3**	**C15H28**	15024.1	3669.0	3353.0	1786.1	1450.5
Unknown	**B Sesquiterpane**	**BSY**	**C15H28**	1652.2	560.8	535.1	295.2	249.4
Unknown	**C_{15} Sesquiterpane**	**BSB**	**C15H28**	4577.9	1106.6	1131.2	600.0	493.0
4	**D Sesquiterpane**	**BS4**	**C15H28**	7794.8	1748.1	1668.0	918.8	755.1
Unknown	**8β(H)-drimane**	**BSD**	**C15H28**	4707.9	1199.6	1295.1	548.2	427.9
5	**C_{15} Sesquiterpane**	**BS5**	**C15H28**	5858.8	1590.0	1693.4	875.5	721.8
6	**C_{16} Sesquiterpane**	**BS6**	**C15H28**	5563.3	1281.1	1291.3	699.2	584.1
7	**C_{16} Sesquiterpane**	**BS7**	**C16H30**	2976.4	725.6	692.3	363.0	301.8
8	**C_{16} Sesquiterpane**	**BS8**	**C16H30**	3570.1	852.1	816.2	432.9	342.0
9	**8β(H)-Homodrimane**	**BS9**	**C16H30**	3030.1	574.3	579.1	286.5	250.0
10	**C_4-decalin (C14)**	**BS10**	**C16H30**	4507.2	1226.6	1177.1	624.0	467.3
	Total			**79713.3**	**19410.9**	**18980.1**	**9974.7**	**8052.6**

Table 0.22: Table 0.23: Measured sesquiterpanes concentrations in unweathered (start) and weathered North Sea oil (µg/g oil)

				Measured Concentration				
Peak Number	**Bicyclic Sesquiterpanes**	**Abbreviations**	**Formula**	**Start**	**5W**	**10W**	**15W**	**20W**
1	**C_4-decalin**	**BS1**	**C14H26**	373.0	84.8	57.8	44.9	32.4
2	**C_{14} Sesquiterpane**	**BS2**	**C14H26**	389.5	62.8	42.3	34.4	24.9
3	**C_{15} Sesquiterpane**	**BS3**	**C15H28**	765.8	165.5	108.0	86.2	62.7
Unknown	**C_{15} Sesquiterpane**	**BSX**	**C15H28**	267.2	45.0	30.8	24.6	16.9
4	**C_{15} Sesquiterpane**	**BS4**	**C15H28**	541.9	99.3	65.5	54.4	38.4
5	**8β(H)-drimane**	**BS5**	**C15H28**	840.9	155.8	105.2	86.5	60.4
6	**C_{15} Sesquiterpane**	**BS6**	**C15H28**	482.9	86.5	58.3	47.8	32.3
7	**C_{16} Sesquiterpane**	**BS7**	**C16H30**	201.9	58.0	40.5	31.0	21.4
8	**C_{16} Sesquiterpane**	**BS8**	**C16H30**	445.4	71.9	50.0	41.3	27.7
9	**C_{16} Sesquiterpane**	**BS9**	**C16H30**	271.8	38.2	26.5	20.2	14.1
10	**8β(H)-Homodrimane**	**BS10**	**C16H30**	833.3	157.2	106.6	83.8	57.4
	Total			**5413.6**	**1025.0**	**691.6**	**555.1**	**388.7**

Table 0.24: Measured adamantanes concentrations in unweathered (start) and weathered Nigerian light oil (µg/g oil)

			Measured Concentration				
Peak Number	**Adamantanes**	**Abbreviations**	**Start**	**5W**	**10W**	**15W**	**20W**
1	**Adamantane**	**A**	65.4	16.3	7.6	4.3	3.6
2	**1-Methyladamantane**	**1-MA**	1466.1	387.2	187.9	130.0	98.7

3	**1,3-Dimethyladamantane**	**1,3-DMA**	653.9	179.3	92.4	55.7	47.0
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	102.9	25.5	11.9	8.2	7.1
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	71.7	13.7	8.1	4.0	3.6
6	**2-Methyladamantane**	**2-MA**	342.4	95.7	46.1	30.5	24.6
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	422.5	122.1	61.6	39.4	31.9
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	277.0	72.0	36.0	22.6	20.0
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	99.7	33.5	21.8	11.0	12.1
10	**1,2-Dimethyladamantane**	**1,2-DMA**	505.6	118.1	66.8	38.6	34.4
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	516.0	115.2	59.0	37.2	31.0
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	416.1	105.2	58.8	33.9	31.4
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	131.3	36.6	18.8	11.4	9.3
14	**1-Ethyladamantane**	**1-AE**	717.0	168.5	92.6	55.3	48.2
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	223.8	117.3	52.6	38.4	26.7
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	204.0	62.4	34.1	21.0	17.6
17	**2-Ethyladamantane**	**2-AE**	74.4	25.5	13.6	8.5	7.5
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	95.4	36.2	18.0	11.3	9.5
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	344.9	93.3	43.6	30.5	20.6
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	201.8	103.1	56.6	34.0	29.0
	Total		**6932.0**	**1926.6**	**987.9**	**625.8**	**513.8**

Table 0.25: Measured adamantanes concentrations in unweathered (start) and weathered Nigerian medium oil (µg/g oil)

Peak Number	Adamantanes	Abbreviations	Measured Concentration (Cdet)				
			Start	5W	10W	15W	20W
1	**Adamantane**	**A**	20.0	5.6	5.3	2.9	2.5
2	**1-Methyladamantane**	**1-MA**	516.6	136.5	127.6	82.0	68.7
3	**1,3-Dimethyladamantane**	**1,3-DMA**	254.6	65.9	60.6	39.7	33.1
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	112.6	26.7	25.3	15.8	13.4
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	30.0	7.6	8.2	4.7	4.3
6	**2-Methyladamantane**	**2-MA**	169.8	45.3	41.7	28.9	24.6
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	329.7	85.3	77.7	51.9	43.2
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	209.0	49.5	49.9	33.2	29.0
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	97.5	25.5	31.0	21.5	18.0
10	**1,2-Dimethyladamantane**	**1,2-DMA**	477.2	113.9	108.7	68.5	56.5
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	461.5	89.0	87.2	55.6	46.4
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	357.1	90.4	88.6	54.0	45.8
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	79.2	29.0	25.0	17.5	15.4
14	**1-Ethyladamantane**	**1-AE**	590.9	128.4	151.9	73.7	63.0
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	278.9	111.7	78.5	66.7	55.8
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	207.8	56.4	52.3	32.8	27.4
17	**2-Ethyladamantane**	**2-AE**	68.5	25.4	22.8	15.5	12.8

18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	89.7	32.0	29.1	20.1	16.3
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	328.0	96.8	88.2	59.2	48.3
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	186.7	75.6	79.5	51.2	41.1
	Total		**4865.1**	**1296.7**	**1239.2**	**795.4**	**665.5**

Table 0.26: Measured adamantanes concentrations in unweathered (start) and weathered North Sea oil (µg/g oil)

Peak Number	Adamantanes	Abbreviations	Measured Concentration				
			Start	5W	10W	15W	20W
1	**Adamantane**	**A**	29.9	1.3	1.3	1.0	1.0
2	**1-Methyladamantane**	**1-MA**	102.3	7.8	7.4	6.2	6.0
3	**1,3-Dimethyladamantane**	**1,3-DMA**	28.8	1.9	1.8	1.5	1.5
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	29.0	2.0	1.9	1.6	1.5
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	20.5	1.2	1.3	0.8	0.8
6	**2-Methyladamantane**	**2-MA**	35.2	3.3	2.9	2.6	2.5
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	105.0	9.4	8.7	7.3	7.4
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	71.3	6.4	5.8	5.2	5.1
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	28.5	3.9	3.5	3.1	2.3
10	**1,2-Dimethyladamantane**	**1,2-DMA**	137.8	10.1	8.9	8.0	7.6
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	176.1	13.4	11.9	10.6	10.6
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	119.2	10.9	9.8	8.5	8.4
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	33.2	3.6	3.2	2.9	2.6
14	**1-Ethyladamantane**	**1-AE**	166.6	16.8	14.3	13.3	13.4
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	88.4	7.9	9.2	7.0	6.2
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	58.2	6.2	5.5	5.0	5.0
17	**2-Ethyladamantane**	**2-AE**	32.1	3.5	3.0	2.9	2.7

18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	37.3	4.2	3.8	3.5	3.4
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	139.8	12.7	11.2	10.3	10.4
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	104.7	16.1	15.2	12.8	13.7
	Total		**1543.9**	**142.6**	**130.6**	**114.2**	**112.3**

Table 0.27: Identification

Compound	Assignment
2,6, + 2,7-DMN	2,6+2,7 Dimethylnaphthalene
BS4	C_{15}-Sesquiterpane
C_{30}Olean	C_{30}-18α(H) Oleanane

Chapter 5: Effects of aerobic biodegradation on composition of crude oils

5.1 Introduction

Biodegradation is an important petroleum hydrocarbon weathering processes which involved the microbial (predominantly bacteria, then some fungi and microalgae) oxidation of petroleum as source of carbon and energy to hydrocarbons degrading organisms (Connan, 1984a; Lee and Levy, 1989; Head *et al.*, 2003; Prince and Walters, 2016). There have been numerous biodegradation studies involve two interconnected research areas. One involves biodegradation studies in the laboratory and field, whereas the other involves the study of petroleum degradation in the deep sub-surface or reservoir systems, which by far has longer potential time scales compared to the laboratory and environmental studies(Bailey *et al.*, 1973; Connan, 1984a; Volkman *et al.*, 1984; Wardroper *et al.*, 1984; Rowland *et al.*, 1986; Prince and Walters, 2016). The degree of occurrence of biodegradation depends of limiting factors such as the oil type, microbial population, temperature, availability of nutrients and oxygen (Prince and Walters, 2016). Biodegradation takes longer time to occur and more complex to predict, but it is considered a more effective hydrocarbons pollutant removal process compared to evaporation and water washing (Prince and Walters, 2016). Decomposition or mineralisation of organic matter by microbes could be aerobic where oxygen supply is needed and the rate of decomposition is faster, or anaerobic where oxygen is absent or limited (Floodgate, 1972; Atlas, 1981; Killops and Killops, 1993). This chapter focuses effect of aerobic biodegradation on crude oils compositions from microcosms simulated in the laboratory. In oxic environments, where oxygen is the oxidant or the terminal electron acceptor (TEA), aerobic condition is created and it is the most rapid and complete degradation of different hydrocarbon compounds in oils into final or intermediate products (Aitken *et al.*, 2004b). Aliphatic hydrocarbons are degraded through aerobic biodegradation pathway which starts by enzymes called oxygenases and peroxidases would add oxygen molecules to the hydrocarbon molecules, which leads to formation of primary alcohols which are further oxidized intermediate carboxylic acids which are then biosynthesised to acetyl-CoA, and then finally mineralised to the most thermodynamically stable products CO_2 and H_2O and the energy utilised for biomass cell growth(Watkinson and Morgan, 1991; Fritsche and Hofrichter, 2000; Widdel and Rabus, 2001; Prince and Walters, 2016). Similarly, degradation of aromatic compounds starts with the addition of oxygen molecules by mono or di-oxygenases to produce intermediate products such as benzyl alcohol, phenol e.tc., which in turn undergo ring cleavage by different oxygenases to yield carboxylic acids, which subsequently yield acetyl-CoA and succinyl-CoA as degradation continues(Fritsche and Hofrichter, 2000; Fuchs *et al.*, 2011). While low-molecular weight compounds are the most affected components during evaporation and water washing or dissolution weathering processes, wide range of selective n-alkanes molecular compounds are subjected to microbial degradation as noted in spill impacted recent sediments (Youngblood and Blumer, 1973; Atlas, 1981), crude oils degraded in the laboratory(Bailey *et al.*, 1973; Jones *et al.*, 1983; Watson *et al.*, 2002), and biodegraded in the reservoir (Connan, 1984a; Volkman *et al.*, 1984). The microbial degradation results in continues depletion of gas chromatography (GC) resolvable components of the crude oils such as the n-alkanes, acyclic isoprenoids, aromatics result in production of unresolved complex mixture (UCM) or the 'hump' (Jones *et al.*, 1983; Gouch *et al.*, 1992), which is an indication of microbial degradation of the crude oil and it is also applied to differentiate an oil polluted sediment from an unpolluted sediment (Jones *et al.*, 1983). Biodegradation alters the normal light crude to heavy and heavy oils, and bitumen by elimination of the light hydrocarbons in the reservoir (Connan, 1984a; Peters and Moldowan, 1993a; Larter *et al.*, 2006a; Larter *et al.*, 2012). Biodegradation effect can be ascertained by the presence or absence of certain hydrocarbon compounds in crude oils, isomer distributions, or the relative composition of some compound class (Huang *et al.*, 2019). Crude oils have been ranked in terms of relative scale of biodegradation with the most common one developed by Peters and Moldowan (1993) abbreviated as “PM level” whereby biodegradation levels of crude oils are ranked on a scale of 1 as the least affected to 10 being the most altered, successfully used for light oils and condensates (Moldowan and Peters, 1993; Larter *et al.*, 2012; Huang *et al.*, 2019). Another scale was also developed by Wenger et al (2001) based on the consideration of a more comprehensive list of compounds, their classes and carbon ranges (Wenger *et al.*, 2001; Larter *et al.*, 2012; Huang *et al.*, 2019), and its modification (Head *et al.*, 2003). To address the inadequacy of the aforementioned schemes such as various aromatic compound classes not being included in the “PM level”, variability in oil quality with higher levels of biodegradation (Larter *et al.*, 2012), local variability in biodegradation systems such as aerobic vs anaerobic (Wardlaw *et al.*, 2011), or the different anaerobic degradation pathway (Head *et al.*, 2003), Larter et al (2012) proposed the “Manco” (Modular Analysis and Numerical Classification of Oils) biodegradation scale. It involves qualitative examination of compound classes and provides a much higher resolution scale to distinguish biodegradation effects than the other scales and provides details on the extent of biodegradation of heavy oils and bituminous sands(Larter *et al.*, 2012; Huang *et al.*, 2019).

5.2 Aim and Objectives

The aim of the experiments detailed in this chapter is to investigate the effects of aerobic biodegradation on the chemical compositions of the three different oil samples used to spike the sediment microcosms. The importance of this work is to ascertain at what point biodegradation as a weathering process affects the correlation efficiency of the diagnostic parameters.

The specific objectives were.

1. To observe aerobic biodegradation effects on the different oil samples in the sediment microcosms.
2. To determine the hydrocarbons background concentrations and their sources from the sediment before spiking.
3. To obtain detailed compositional information of the oils samples such as *n*-alkanes, acyclic isoprenoids, biomarker compounds, polynuclear aromatic hydrocarbons (PAHs) and their alkyl homologues and any relevant less well-known compounds identified.
4. To quantitatively determine the concentrations of the analytes in (3) above and compare the compositional and concentrations changes with respect to different levels or extent of biodegradation.
5. To quantitatively examine ratios of compounds used and their changes relative to the biodegradation levels for weathering evaluations.

5.3 Material and Methods

5.3.1 Laboratory biodegradation of crude oil

Three oil samples were used for this experiment which include; Nigerian Light, Nigerian Medium, and North Sea oils with API gravities of 40.3º, 26.3º, and 40º respectively. The Nigerian Light oil was obtained at Nigerian Agip Oil Company's (NAOC) Oshie Flow Station, Akala-Olu, Ahoada L.G.A, Rivers State, Nigeria, while the Nigerian Medium oil obtained at the NAOC's Ebocha Oil Center, Rivers State, Nigeria. The samples were collected in duran bottles with Teflon lined lids and shipped. The North Sea oil from the Andrew Oilfield as a standard oil was available in the laboratory at Newcastle. Seawater and marine sediment ('clean' sand) were collected from St Mary's Island (Whitley Bay, Tyne and Wear, UK) and, both stored at 4°C in the dark before use. And Sediment from Bodo, Niger Delta region of Nigeria, stored in the dark. A total of 126 100 ml fisher brand conical flasks were used for the experiments. 63 flasks for Whitley sediment and the remaining 63 flasks for Nigerian sediment respectively. The contents of the flasks were as follows: 150 µl crude oil + 10 g sediment (Whitley or Nigerian) + 25 ml seawater + 25 ml nutrients. The final nutrient concentrations in the flasks were; $NaNO_3$ 200 mg/l, KH_2PO_4 20 mg/l, $MgCl_2.6H_2O$ 13.7 mg/l, $CaSO_4.2H_2O$ 17 mg/l, $CoCl_2.6H_2O$ 0.0183 mg/l, $Na_2MoO_4.2H_2O$ 0.0106 mg/l, $MnSO_4.2H_2O$ 0.08 mg/l, $CuSO_4.5H_2O$ 0.022 mg/l, $FeSO_4.7H_2O$ 0.08 mg/l, $ZnSO_4.7H_2O$ 0.035 mg/l, KI 0.07 mg/l (e.g. Rosenberg *et al.*, 1992; Watson *et al.*, 2002). $MgCl_2.6H_2O$ 13.7 mg/l was used in this work in place of $MgCl_2.7H_2O$ 15 mg/l used in the previous works due to unavailability of the later. Similarly, $CoCl_2.6H_2O$ 0.0183 mg/l was used in place of $CoCl_2$ 0.010 mg/l, and $Na_2MoO_4.2H_2O$ 0.0106 mg/l was used in place of the $NaMoO_4$ 0.008 mg/l in the previous works respectively. The flasks were stoppered with non-absorbent cotton wool and incubated under aerobic conditions in the dark at room temperature on an orbital shaker operated at 110 rev/min. For each of the three oil samples, there were 21 triplicate samples incubated, extracted and analysed at days 5, 10, 20, 50, 100, and 300 respectively, and autoclaved sterilised controls to account for non-biodegradation also extracted and analysed at day 300. A total of 63 samples for Whitely sediment + 63 samples for Nigerian sediment.

5.3.2 Extraction method

Aliphatic hydrocarbon standard Squalane (99% purity) and 5α-cholestane (98% purity) obtained from Aldrich, Steinem, Germany, and PAH standard 1,1'-binaphthyl (98% purity) obtained from Lancaster Synthesis, White Lund, UK and *p*-terphenyl (99% purity) obtained from Fluka Chemika, Buchs, Switzerland was added as recovery standards just before extraction. 1ml slurry of each sample was taken after shaking and stored in -20C freezer for DNA analysis. The aqueous layer decanted into separating funnels and the sediment in the flasks rinsed with 15 ml methanol into cleaned centrifuged tubes, sonicated for 5 minutes and then centrifuged for 8minutes at 3500 rev/min and the supernatant decanted into the separating funnels. This extraction cycle was repeated using 15 ml (2:1) and then 15 ml (4:1) DCM/methanol, and finally three times with 15 ml DCM. The contents were shaken and allowed to be separated gravimetrically and the bottom organic layer collected into 100 ml RBFs leaving the top polar layer (e.g. Birk *et al.*, 2012). 30ml DCM and 50 ml deionised water again added to the bottom organic layer into separating funnel and re-separated (see Figure 0.1). The extracts rotary evaporated to about 5 ml and anhydrous sodium sulphate (Na2SO4) from BDH added to remove trace of moisture, and made up to 6ml in measuring cylinder where 2 ml aliquot each were taken for fractionation, total petroleum hydrocarbon (TPH) and extractible organic matter (EOM) determination respectively.

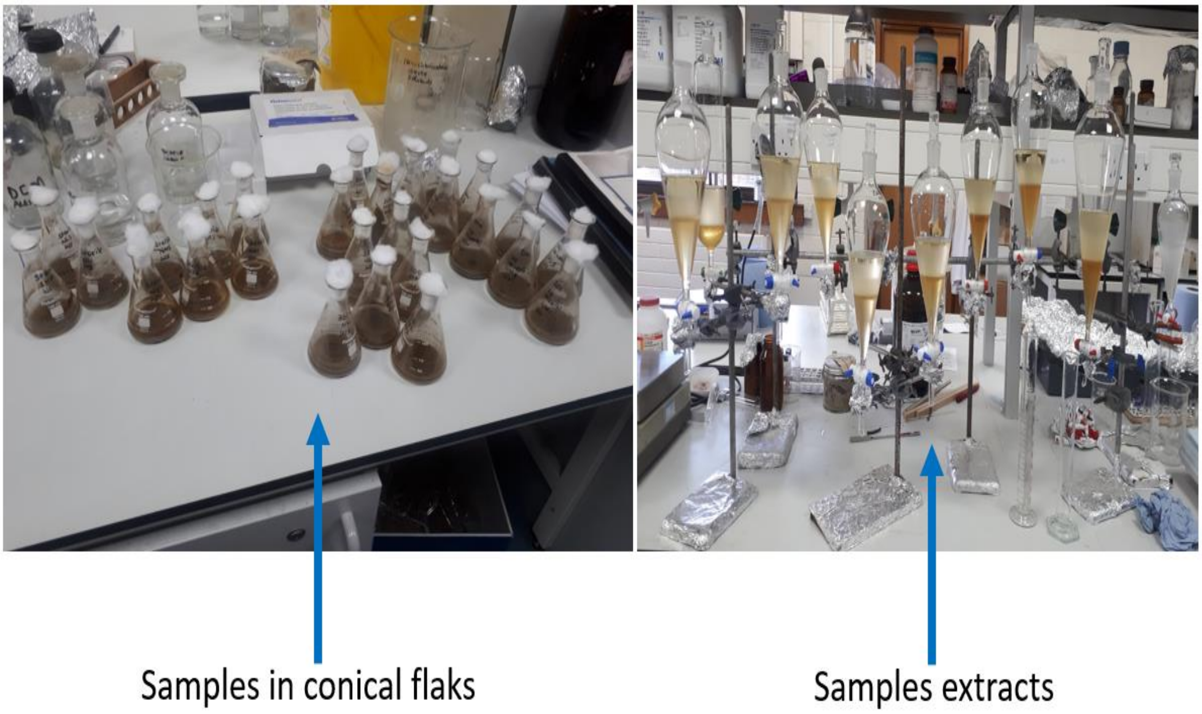


Figure 0.1.: Aerobic biodegradation experimental Set up.

5.3.3 Reagent and Materials

Chromatographic-grade distilled solvents used include petroleum ether (BP 40-60°C), dichloromethane (DCM) and methanol. Silica gel 60(230-400 mesh ASTM) and aluminium oxide 90 active neutral (70-230 mesh ASTM) were purchased from Merck, Darmstadt, Germany and 500gr white non-absorbent cotton wool purchased from Fisher Scientific, UK The silica gel, the aluminium oxide and the cotton wool were pre-extracted in a Soxtec solvent extractor using an azeotropic (DCM-methanol) solvent mixture. The silica gel and the aluminium oxide were stored in glass bottles, covered with aluminium foil and activated in an oven over night at 130°C before use (e.g., Later et al., 1985; Bundt et al., 1987).

5.3.4 Column chromatographic fractionation of hydrocarbons

Chromatography columns (28 cm l x 10 mm i.d.) were set-up on clamps and pre-extracted cotton wool were used to plug the bottom of the columns. A slurry of silica gel in petroleum ether was poured into the columns followed by slurry of alumina at the top to form alumina/silica (1:4) column. The 2ml of the extract adsorbed onto small quantities (~1g) of alumina and then charged onto the alumina/silica column. The columns were eluted with petroleum ether (70ml) as aliphatic fraction (F1) for n-alkanes and biomarker analysis, followed by elution of a PAHs-containing fraction (F2) (70ml 50% DCM in petroleum ether) for analysis of PAHs similar to that previously reported (e.g. Wang *et al.*, 1994a; Wang *et al.*, 1994b; Wang *et al.*, 2004; Wang *et al.*, 2005). Aliphatic standard *n*-heptadecylcyclohexane (98% purity) obtained from Lancaster Synthesis, Morecambe, UK, 5α-androstane (98% purity) obtained from Sigma-Aldrich, Steinem, Germany, and PAH standard *p*-terphenyl (99% purity) obtained from Fluka Chemika, Buchs, Switzerland were added to respective fractions as internal standards.

5.3.5 Gas chromatography and gas chromatography - mass spectrometry

Gas chromatography with flame ionisation detection (GC-FID) analysis of aliphatic samples was performed on an Agilent 5890 GC in split-less mode, with the injector at 280°C, FID at 300°C. The sample (1ul) in hexane was injected by an HP7673 auto sampler and the split opened after 1 minute. After the injection, the GC temperature programme and data acquisition commenced. Separation was performed on a fused silica capillary column (30 m x 0.25 mm i.d.) coated with 0.25 um dimethyl poly-siloxane (HP-5 phase). The GC was temperature programmed from 50°C-310°C at 5°C min and held at final temperature for 20 minutes with hydrogen as the carrier gas (flow 1ml/min, pressure of 50kPa, split at 30 mls/min). The GC data was acquired on a Thermo-Atlas laboratory data system on a dedicated channel.

Gas chromatography-mass spectrometry (GC-MS) analysis of the aliphatic and PAH fractions was performed on an Agilent 7890B GC with a split/split less injector (280°C) linked to a Agilent 5977B MSD (electron voltage 70eV, source temperature 230°C, quad temperature 150°C multiplier voltage 1200V, interface temperature 310°C). The acquisition was controlled by a HP Z240 computer using Chemstation software, initially in full scan mode (50-600 amu/sec) or in selected ion mode (20 ions, 3.0 cps, 20ms dwell) for greater sensitivity. The sample (1ul) in hexane was injected by an Agilent7683B auto sampler and the split opened after 1 minute. After the solvent peak had passed the GC temperature programme and data acquisition commenced. Separation was performed on an Agilent fused silica capillary column (30m x 0.25mm i.d) coated with 0.25um dimethyl polysiloxane (HP-5) phase. The GC was temperature programmed from 50-310°C at 5°C min and held at final temperature for 10 minutes with Helium as the carrier gas (flow rate of 1ml/min, initial pressure of 50kPa, split at 20 mls/min). The acquired data was stored for later data processing, integration and printing. Peaks were identified and labelled after comparison of their elution order with known standard mixtures or from geochemistry literature and also for the PAH by comparison of their mass spectra with those of the NIST05 library. The NIST Mass Spectral Library is a software and peer-reviewed databases that are developed and supported by the National Institute for Standards and Technology, USA.

5.4 Results and Discussion

The effect of biodegradation on different compound classes in the three oil samples are also considered in this chapter as previously discussed in sections 3.4.2 and 4.4.1 on effects of evaporation and water washings respectively. Biodegradation effect could be evaluated by using the absence or presence of certain hydrocarbon compound classes, isomer distributions and relative abundances of compound classes (Huang *et al.*, 2019).

5.4.1 *n*-Alkane and acyclic isoprenoid composition and concentration changes

Figure 0.2, Figure 0.3, and Figure 0.4 are GC-FID chromatograms of the aliphatic hydrocarbon (F1) fractions of Whitley Sediment spiked with Nigerian light and medium, and the North Sea oils, and Figure 0.5, Figure 0.6Figure 0.7 are GC-FID chromatograms of the aliphatic hydrocarbon (F1) fractions of Nigerian Sediment spiked the same oils respectively, showing the effects of biodegradation on these compound classes. The background concentrations of *n*-alkanes and isoprenoids in the sediments were observed to be quite negligible in concentrations compared to that of spiked sediments measured with respect to extractable organic matter (EOM), with *n*-C_{21} (82 µg/g EOM) as the peak concentration in Nigerian sediment and *n*-C_{17} (106.9 µg/g EOM) as the peak concentration for the Whitley sediment (see Figure 0.8). The *n*-alkanes in the two sediments range from *n*-C_{14} to *n*-C_{37} and it is within the range of *n*-alkanes (*n*-C_{15} to *n*-C_{35}) commonly found in most sediments (Volkman *et al.*, 1992). The effects of biodegradation are clearly indicated by the compositional changes as the chromatograms are altered as biodegradation increases with time much more in the Whitley sediment samples than it is observed in that of the Nigerian Sediment. The resolved peaks continue to diminish until the unresolved complex mixture (UCM) or "hump" dominates and peaked at 300 days for the three oil samples in Whitley sediment which signifies that the oils have undergone biodegradation which could also be used to distinguish polluted sediments from unpolluted ones (Jones *et al.*, 1983; Gouch *et al.*, 1992; Volkman *et al.*, 1992; Yang *et al.*, 2012; Dembicki, 2017), but not so much biodegradation is observed in the Nigerian sediment where only the light end (*n*-C_8 to *n*-C_{13}) were degraded this is obviously as a result of the grain sizes of the sediments as Whitley Bay sediment is sandy while the Nigerian sediment is clay-loam, and studies shows that biodegradation in clay samples are less than in sandy samples as microbial activity is influenced by sediment type (Aelion, 1996), because soils with low permeability as clay hinders water transportation and distribution, nutrient and oxygen needed by the microbes (Sihag *et al.*, 2014). The *n*-alkanes and isoprenoids are most effected by biodegradation as compared to the evaporative weathering after 30 days (see section 3.4.1) and water washing after twenty times which is the least affected and n-alkanes were completely preserved for the three oils (see section 4.4.1). The low molecular weight *n*-alkanes *n*-C_8 to *n*-C_{10} are observed to have been biodegraded completely after 5 days, and *n*-C_8 to *n*-C_{17} after 50 days for the Nigerian light oil respectively (see Table 0.1), whereas *n*-C_8 was still present after 10 days of evaporative weathering (see Table 3.1). It took up to 30 days for *n*-C_8 to *n*-C_{13} to weather away completely under evaporative weathering. The *n*-alkanes and isoprenoids are observed to have been biodegraded completely after 300 days while *n*-C_{13} to *n*-C_{39} n-alkanes and isoprenoids are observed to have been preserved after 300 days in the sterilised control sample. Similarly, the Nigeria medium oil had *n*-C_8 and *n*-C_9 observed to be biodegraded after 5 days, *n*-C_8 to *n*-C_{16} after 50 days, complete mineralisation of *n*-alkanes after 300 days, and *n*-C_{12} to *n*-C_{39} preserved for the sterilised control respectively (see Table 0.2), whilst only *n*-C_8 to *n*-C_{10} was completely weathered after 10 days and *n*-C_8 to *n*-C_{13} after 30 days due to evaporative weathering (see Table 3.2). And for the North Sea oil, *n*-C_8 to *n*-C_{10} completely biodegraded after 5days, *n*-C_8 to *n*-C_{13} after 50 days, completely degraded after 300 days, and *n*-C_{12} to *n*-

C_{39} preserved for the sterilised control after 300 days respectively (see Table 0.3), whilst the North Sea oil was the most susceptible of the three oils to evaporative weathering where *n*-C_8 to *n*-C_{10} was completely weathered after 1day and *n*-C_8 to *n*-C_{13} after 30 days respectively (see Table 3.3). While for the Nigerian sediment, only *n*-C_8 to *n*-C_9 are degraded after 5days of incubation and *n*-C_8 to *n*-C_{13} after 300 days for the three oil samples (see Table 0.4Table 0.5, andTable 0.6).

Table 0.1, Table 0.2Table 0.3, Table 0.4Table 0.5Table 0.6 show the summary of the identified *n*-alkanes (*n*-C_8 to *n*-C_{39}), pristane and phytane and their concentrations for the biodegraded Nigerian light and medium, and North Sea oils in both Whitley and Nigerian sediment respectively, by comparison of integrated peak areas against the peak areas of known internal standard (*n*-heptadecylcyclohexane) using GC-FID. A recovery or surrogate standard (squalene) was also used to compute the percentage recoveries for the analytical procedures, which were 80% and above for all the samples. The mean values (n=3) from triplicates, with relative standard deviation (RSD) in brackets after the numbers are shown in Table 0.1Table 0.2Table 0.3, Table 0.4Table 0.5Table 0.6 respectively. The quantitations were carried out using the equations as described in chapter 3 (see equations 3.1, 3.2. 3.3 and 3.4 in section 3.4.2). A sudden decrease in the sum of *n*-alkanes with increase in biodegradation indicate the *n*-alkanes mineralisation (Jones *et al.*, 1983; Lee and Levy, 1989; Jackson *et al.*, 1996; Liu *et al.*, 2020) and are mostly affected by biodegradation than by evaporation and water washing as the *n*-alkanes sums increased with increase in evaporation (see Tables 3.1, 3.2, and 3,3) and water washing (see Tables 4.1, 4.2, and 4.3) respectively. Consequently, the ratios *n*-C_{17}/pristane, *n*-C_{18}/phytane, pristane/phytane, CPI and the weathering index (WI) are much more altered by increased biodegradation than evaporative weathering and water washing for the three oil samples with the alterations occurring between 50- and 100-days incubation period for the Whitley sediment, however, not much alterations were observed in the case of Nigerian sediment.

Figure 0.9Figure 0.10Figure 0.11Figure 0.12Figure 0.13Figure 0.14 are graphical representations of the *n*-alkanes and isoprenoids concentrations for the Nigerian light and medium, and North Sea oils in both the Whitley and Nigerian sediments, respectively. These compound classes are greatly affected much more compared to that of evaporative weathering (see Figures 3.7, 3.8, and 3.9) and water washing (see Figures 4.5, 4.6, and 4.7) as discussed earlier. The well-known biodegradation assessment classification system developed by Peters and Moldowan (1993) (PM scale) and its attendant modifications were applied to rank the biodegradation levels of the components of the crude oils assessed (Moldowan and Peters, 1993; Head *et al.*, 2003; Larter *et al.*, 2012). The effect of biodegradation on the three oil samples in Whitley sediment could be ranked using the *n*-alkanes depletion as PM level 0 (intact) for the undegraded oil, PM level 1 (very slight) for 5-, 10-, and 20-days incubated samples, PM level 2-3(slight to moderate) for 50, and 100 days incubation period, and PM level 4 (moderate to heavy) for 300 days incubation period where the *n*-alkanes are completely degraded as observed from the

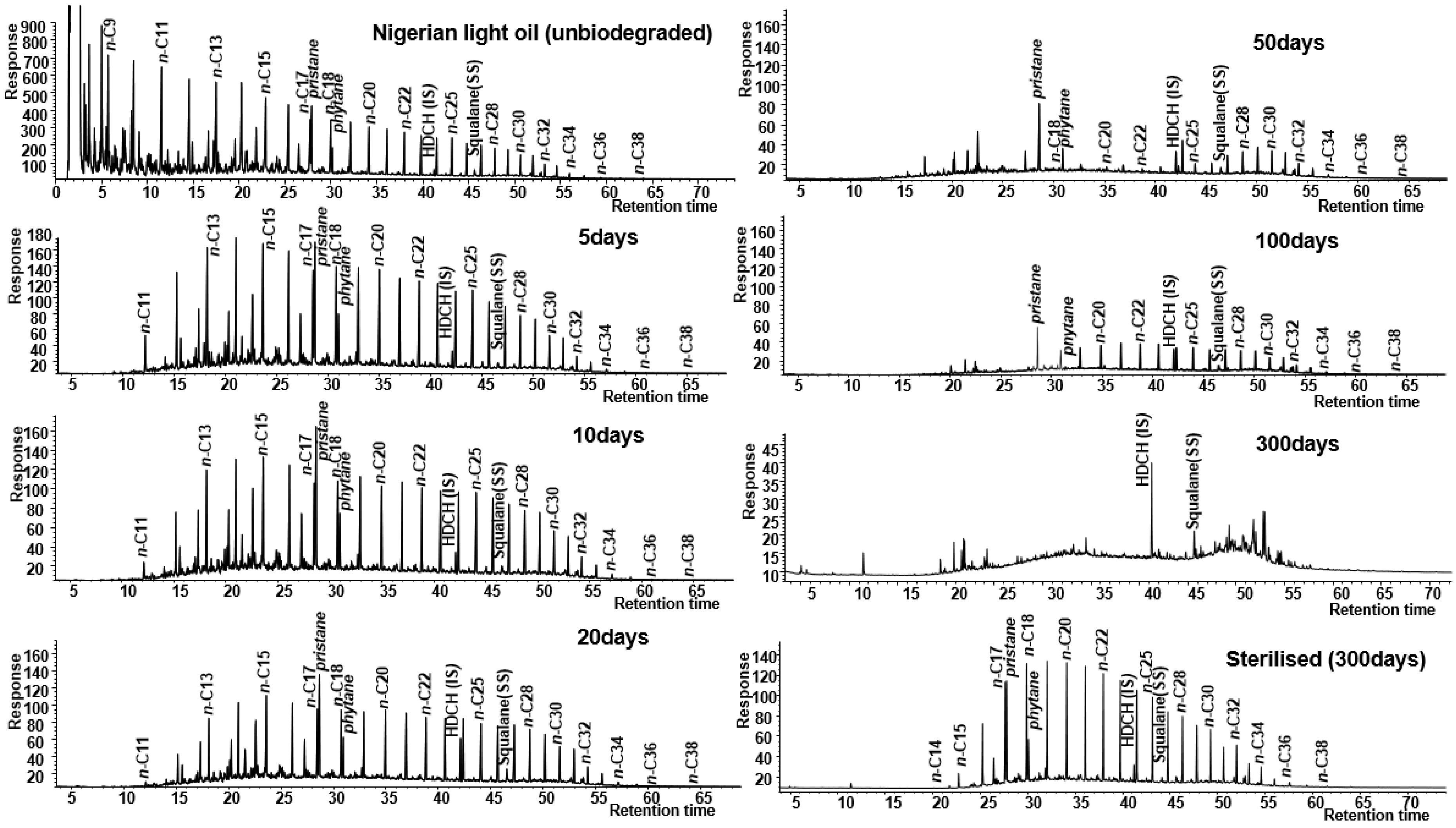


Figure 0.2: GC-FID chromatograms of aliphatic fractions of the Nigerian light in Whitley Sediment oil showing days of biodegradation.

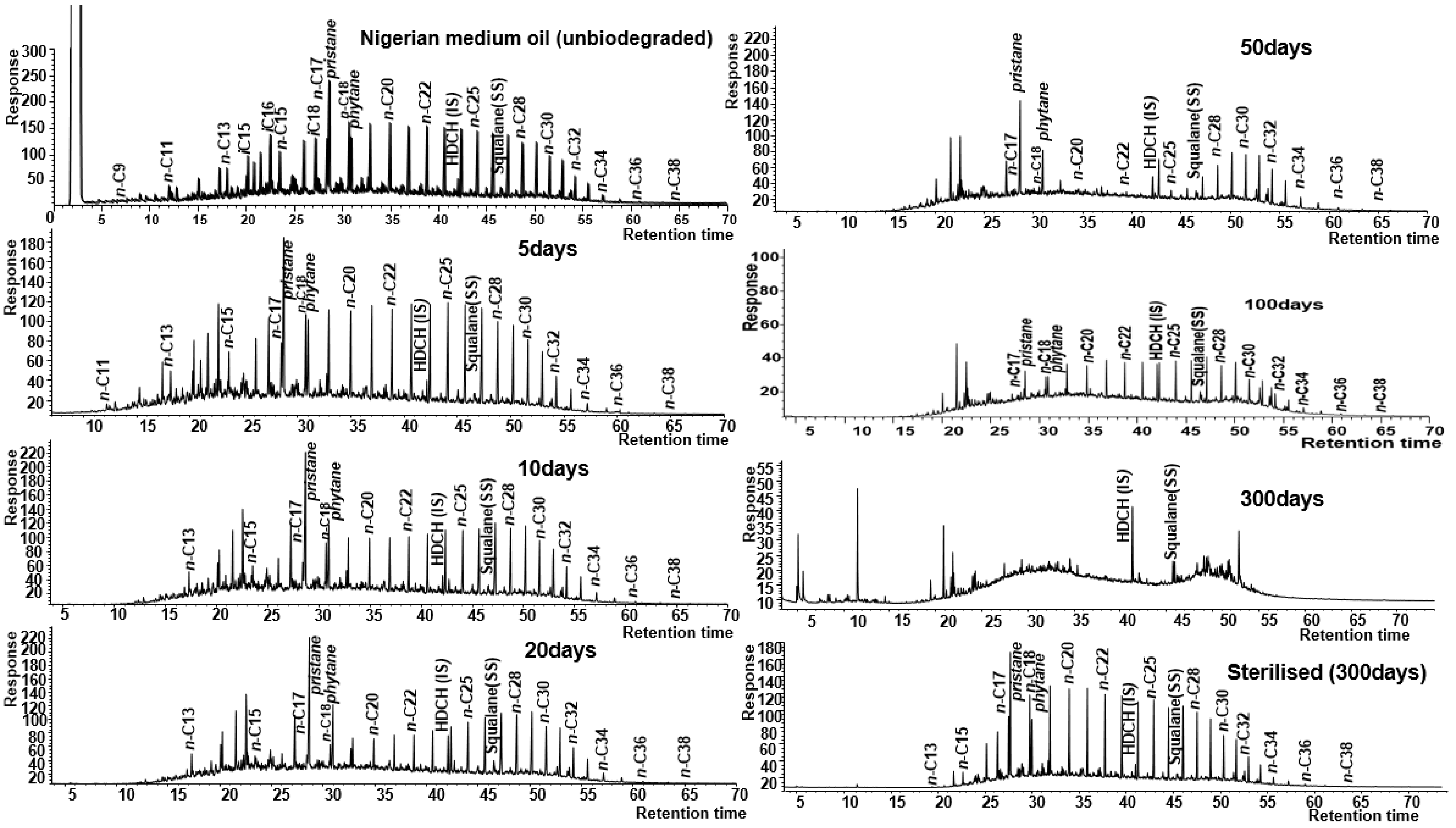


Figure 0.3: GC-FID chromatograms of aliphatic fractions of the Nigerian medium oil in Whitley sediment showing days of biodegradation.

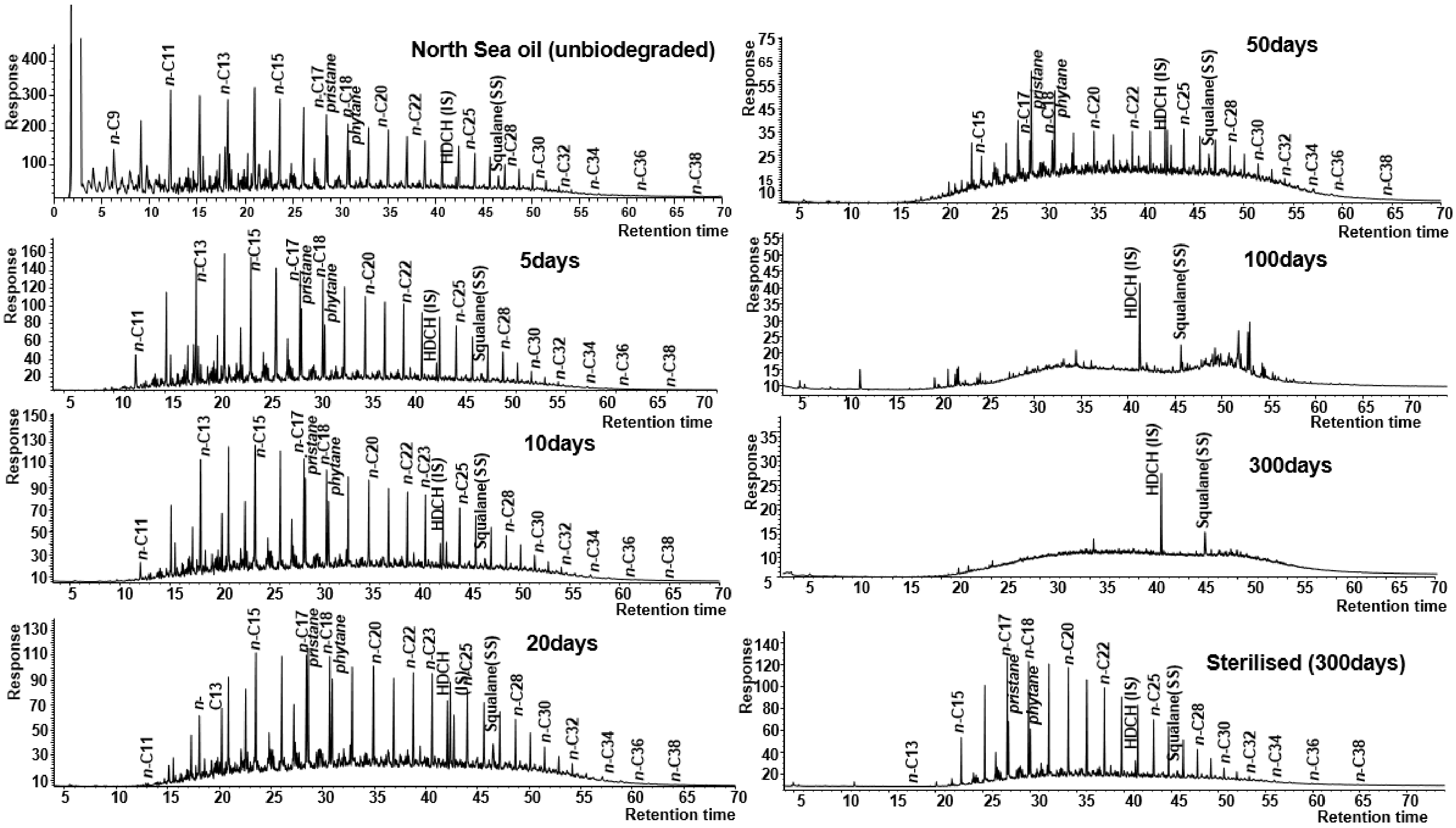


Figure 0.4: GC-FID chromatograms of aliphatic fractions of the North Sea oil in Whitley sediment showing days of biodegradation.

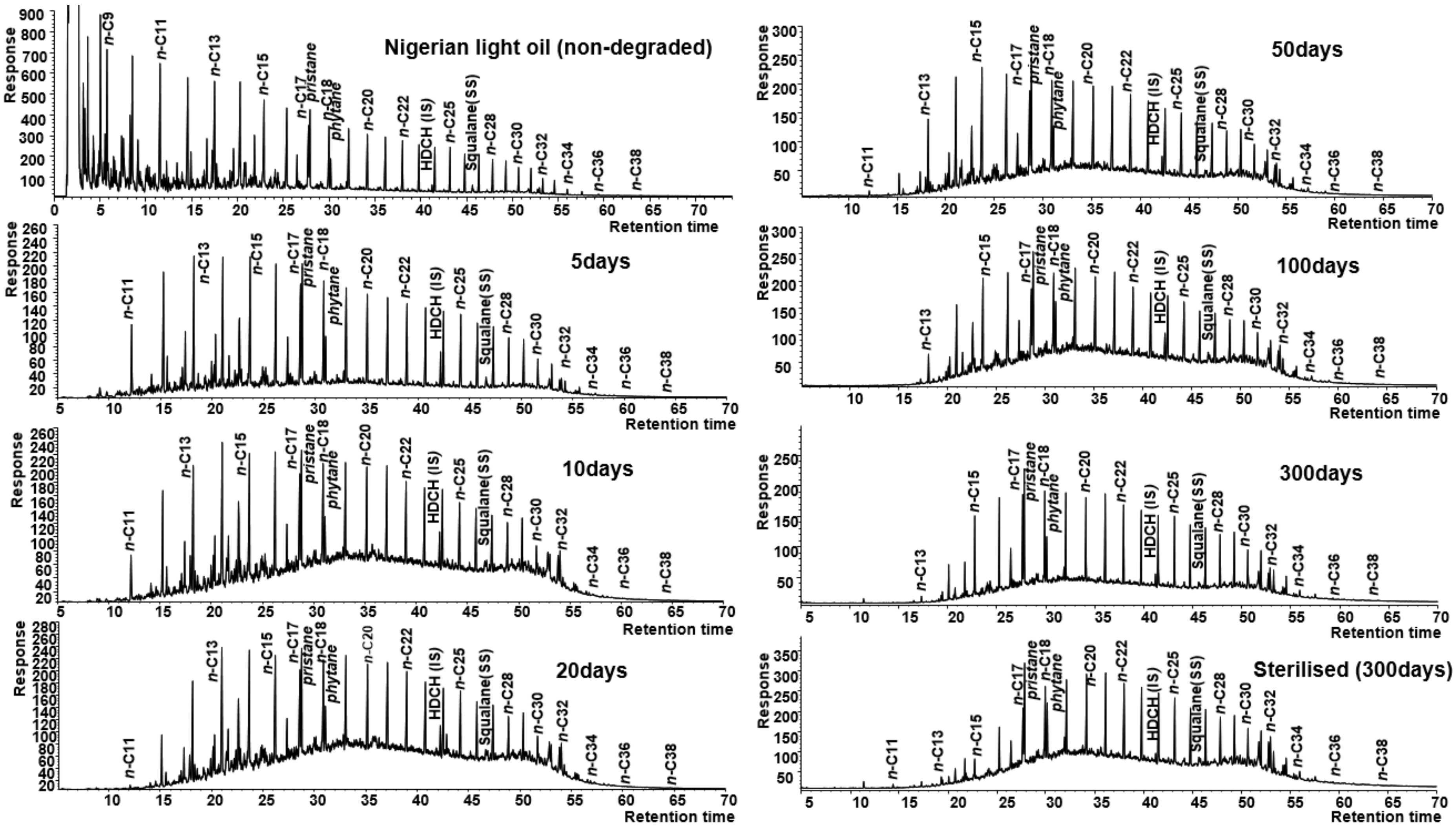


Figure 0.5: GC-FID chromatograms of aliphatic fractions of the Nigerian light oil in Nigerian sediment showing days of biodegradation.

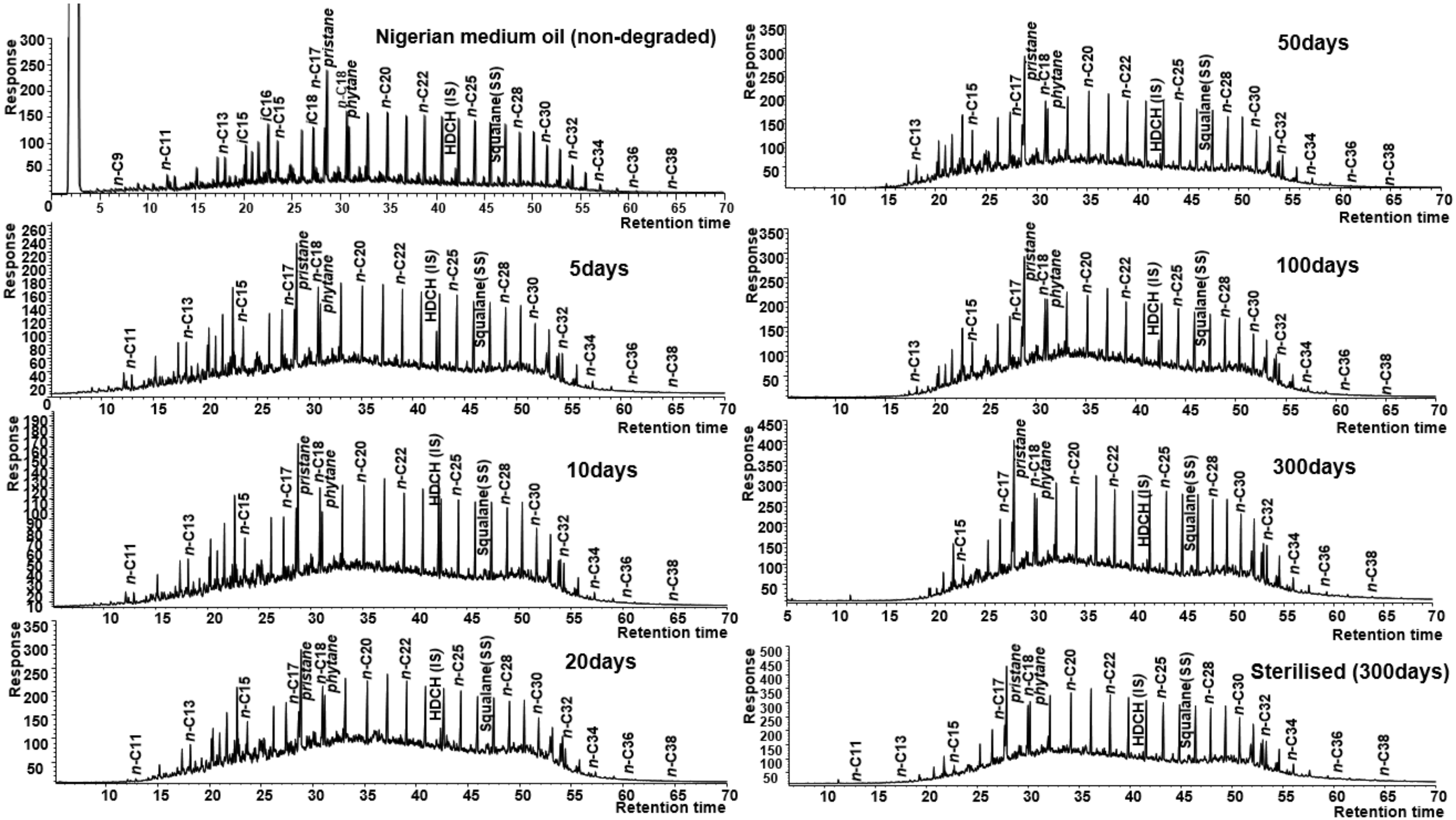


Figure 0.6: GC-FID chromatograms of aliphatic fractions of the Nigerian medium oil in Whitley sediment showing days of biodegradation.

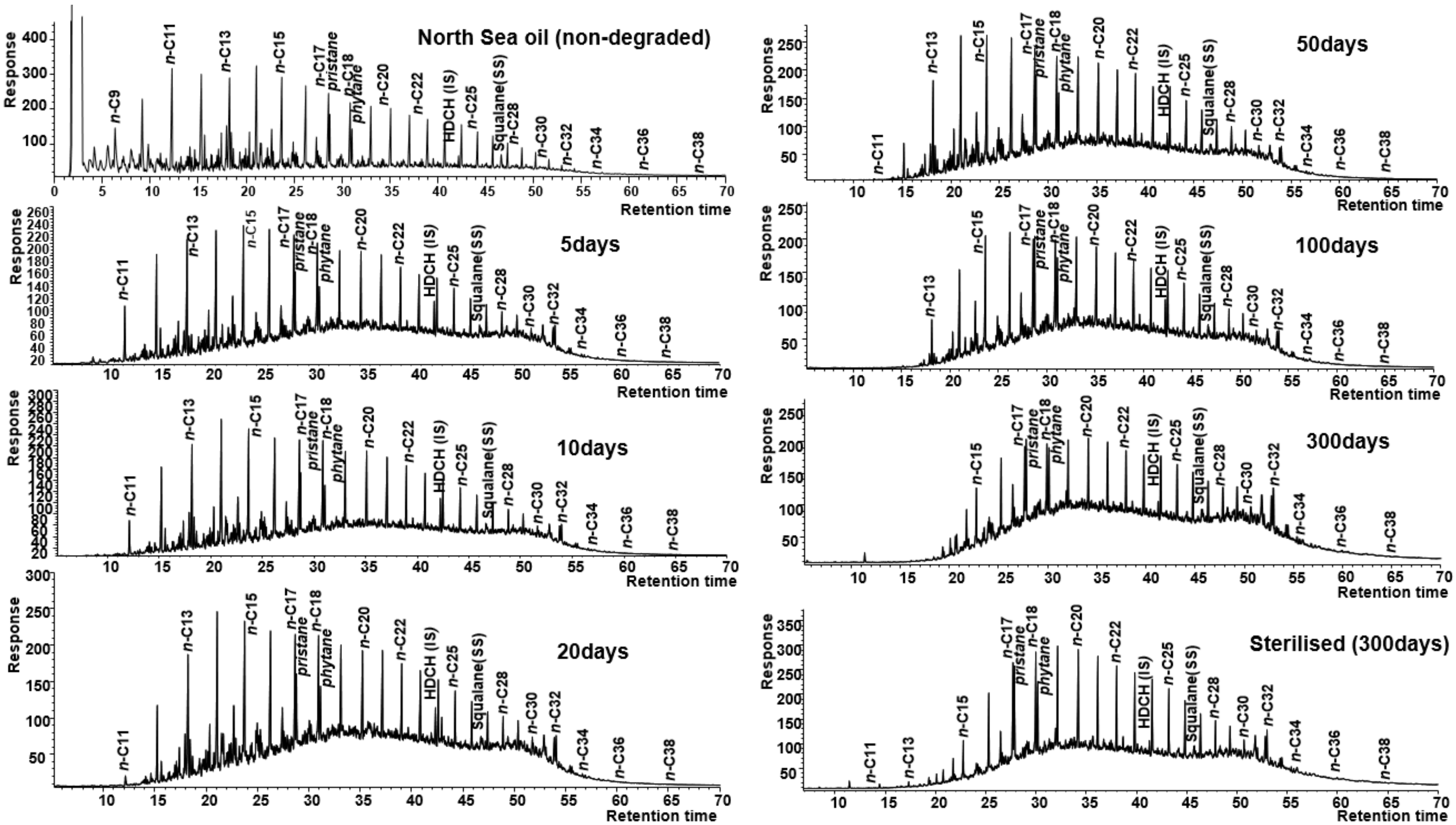


Figure 0.7: GC-FID chromatograms of aliphatic fractions of the North Sea oil in Whitley sediment showing days of biodegradation.

Nigerian light and medium oils (see Figure 0.2 and Figure 0.3). Similar trend is observed from the North Sea oil (see Figure 0.4) with PM level 4 starting from 100 days incubation period as the only difference which is an indication higher degradation rate. The biodegradation level is however low in the case of the Nigerian sediment where PM level 1 (very slight) could be assigned to 5, 10, 20, 50 and 100 days incubation period, and PM level 2-3 (slight) for 300 days incubation period for all the three oil samples. Figure 0.15 is a plot of abundances or concentrations (see Table 0.1, Table 0.2Table 0.3) of n-alkanes in the biodegraded oils relative n-C_{30} for the three oil samples in Whitley sediment, whereas Figure 0.16 is a plot of abundances or concentrations (see Table 0.4, Table 0.5,Table 0.6) of *n*-alkanes in the biodegraded oils relative n-C_{30} for the three oil samples in Nigerian sediment. The choice of *n*-C_{30} for the normalisation is as discussed in chapter 3 (see section 3.4.3).

The low molecular weight n-alkanes are observed to be biodegraded after 5 days incubation period for all the three oil samples in Whitley sediment. The abundances continue to decrease as biodegradation progressed with incubation period of up to 300 days with highest loss of n-alkanes for the three oil samples. Slight built up of high-molecular weight n-alkanes with increase in incubation period are observed with the sterilised controls for all the three oil samples possibly due to low microbial activities due to the kill. It is further observed that the biodegradation becomes less effective at *n*-C_{29} for the Nigerian light oil, and at *n*-C_{30} for the Nigerian medium and North Sea oils respectively. However, due to the low biodegradation rate, only the low molecular weight *n*-alkanes for the three oil samples in Nigerian sediment are observed to degrade from 5 to 300 days incubation period and the biodegradation becomes less effective at *n*-C_{30} for all the three oil samples. Less built-up of high-molecular weight *n*-alkanes in the sterilised controls were observed in the Nigerian oils compared to that of the North Sea oil possibly due to low rate of biodegradation.

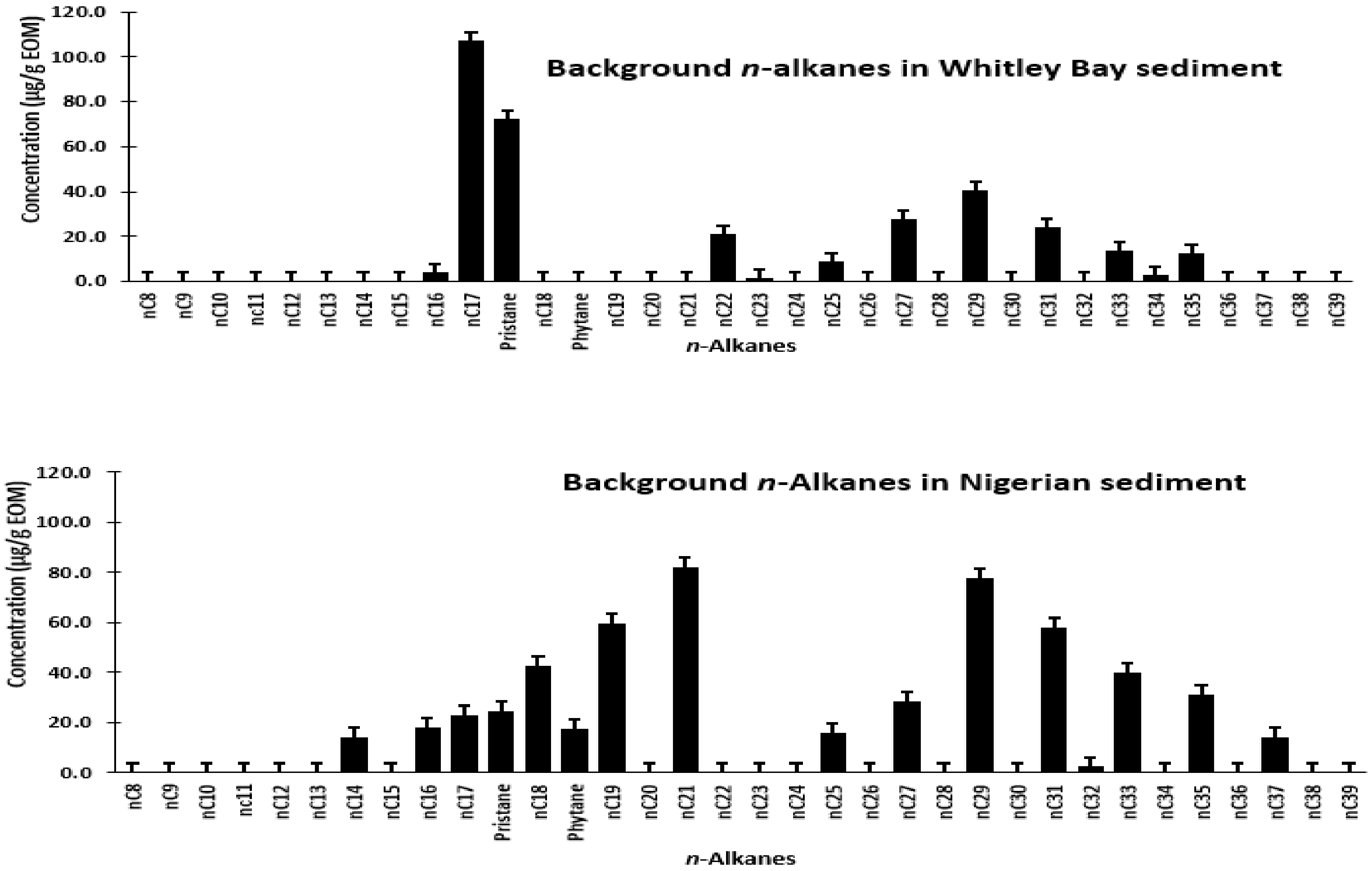


Figure 0.8: Graphical representation of background concentrations of *n*-alkanes, pristane, and phytane in Whitley and Nigerian sediment, measured with respect to extractable organic matter (EOM).

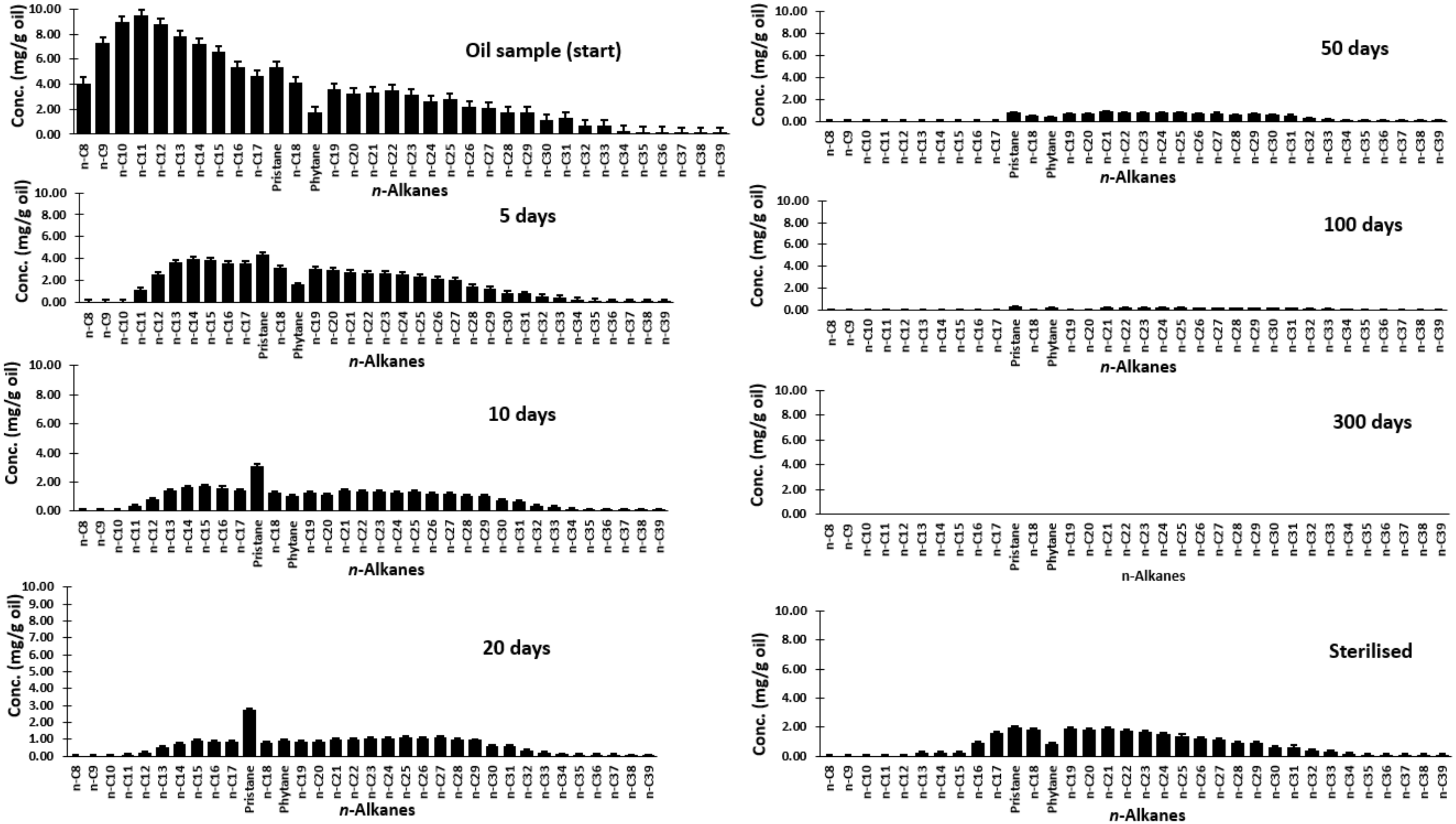


Figure 0.9: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian light oil in Whitley sediment.

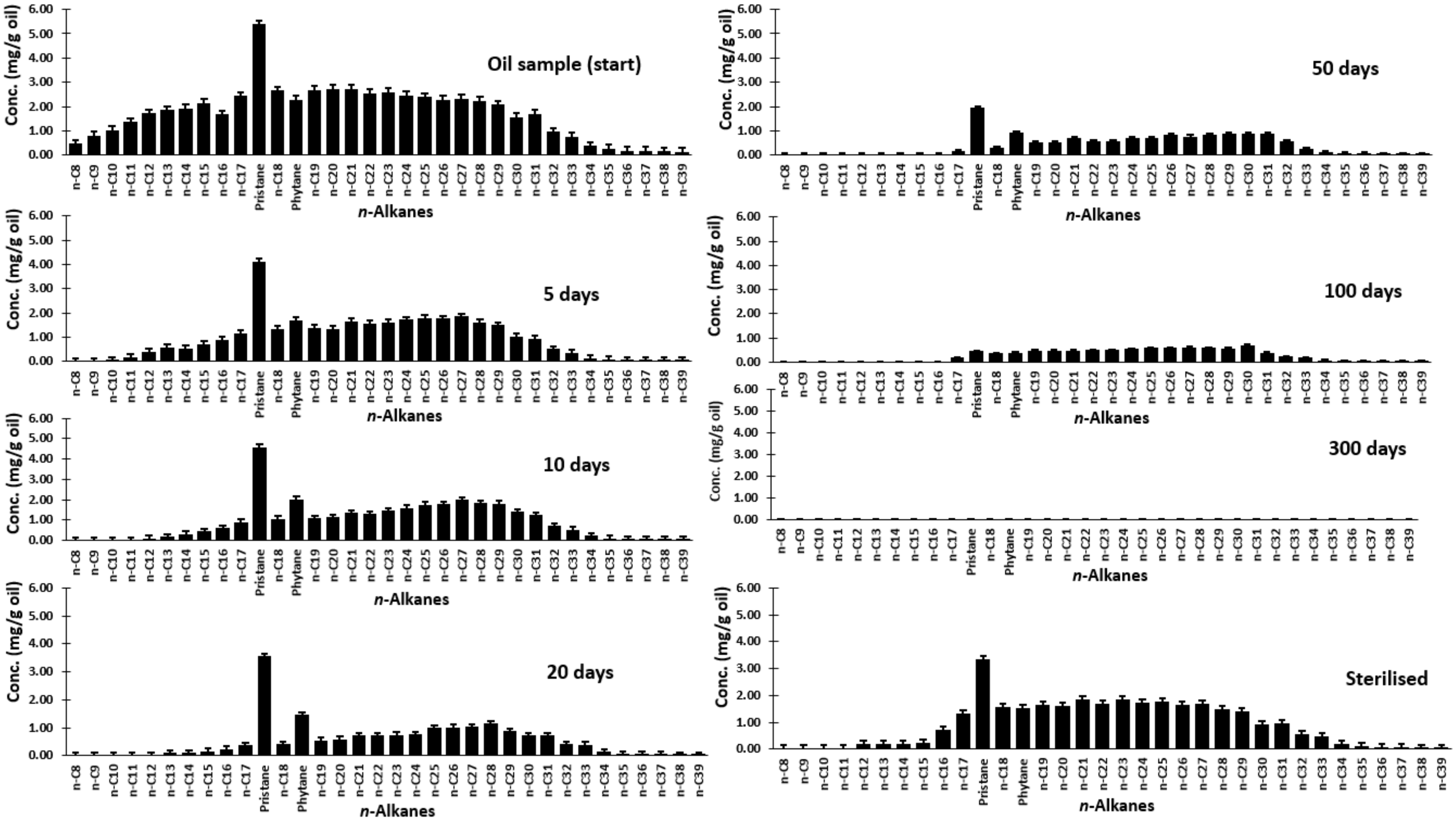


Figure 0.10: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian medium oil in Whitley.

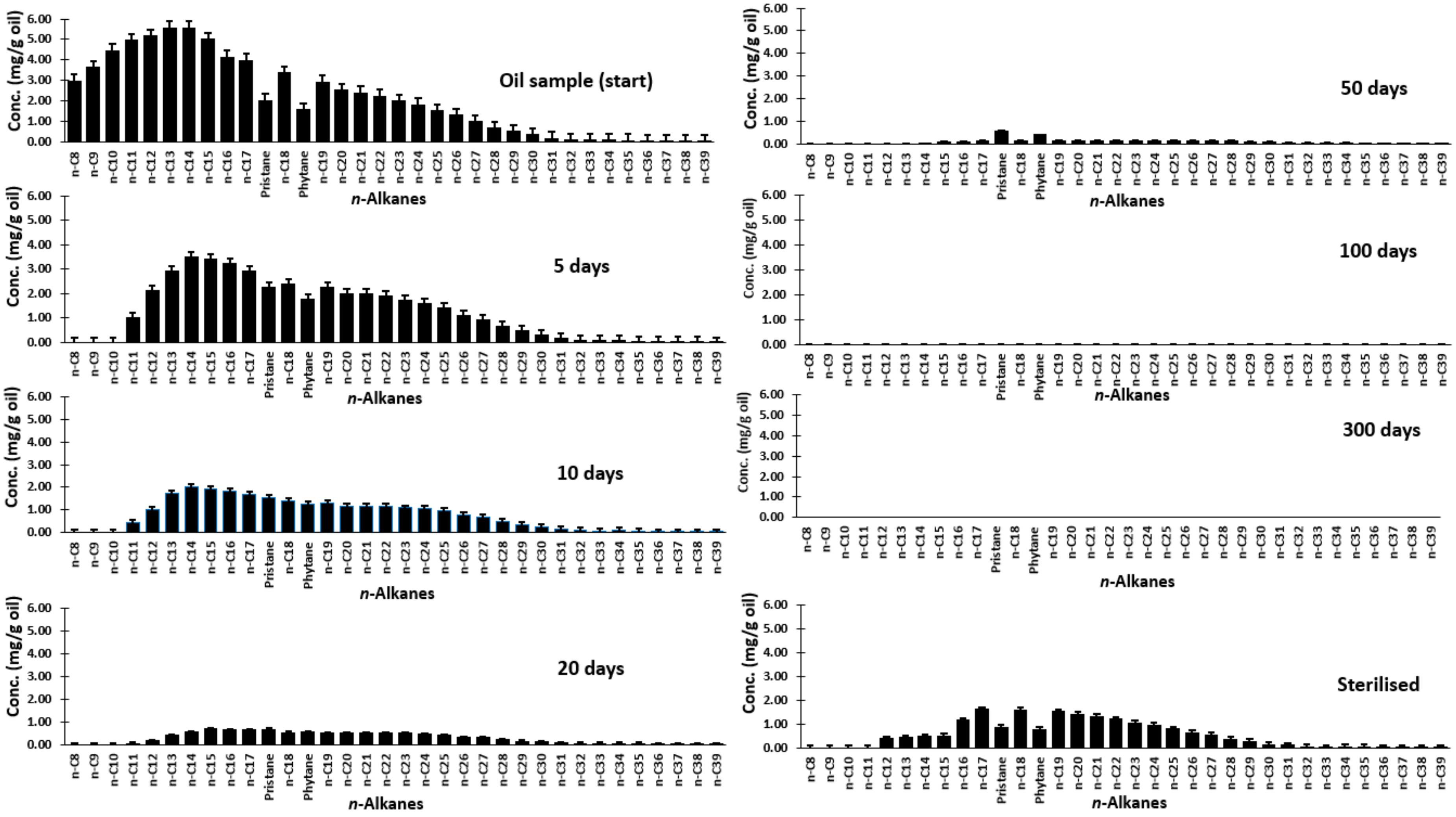


Figure 0.11: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian medium oil in Whitley sediment.

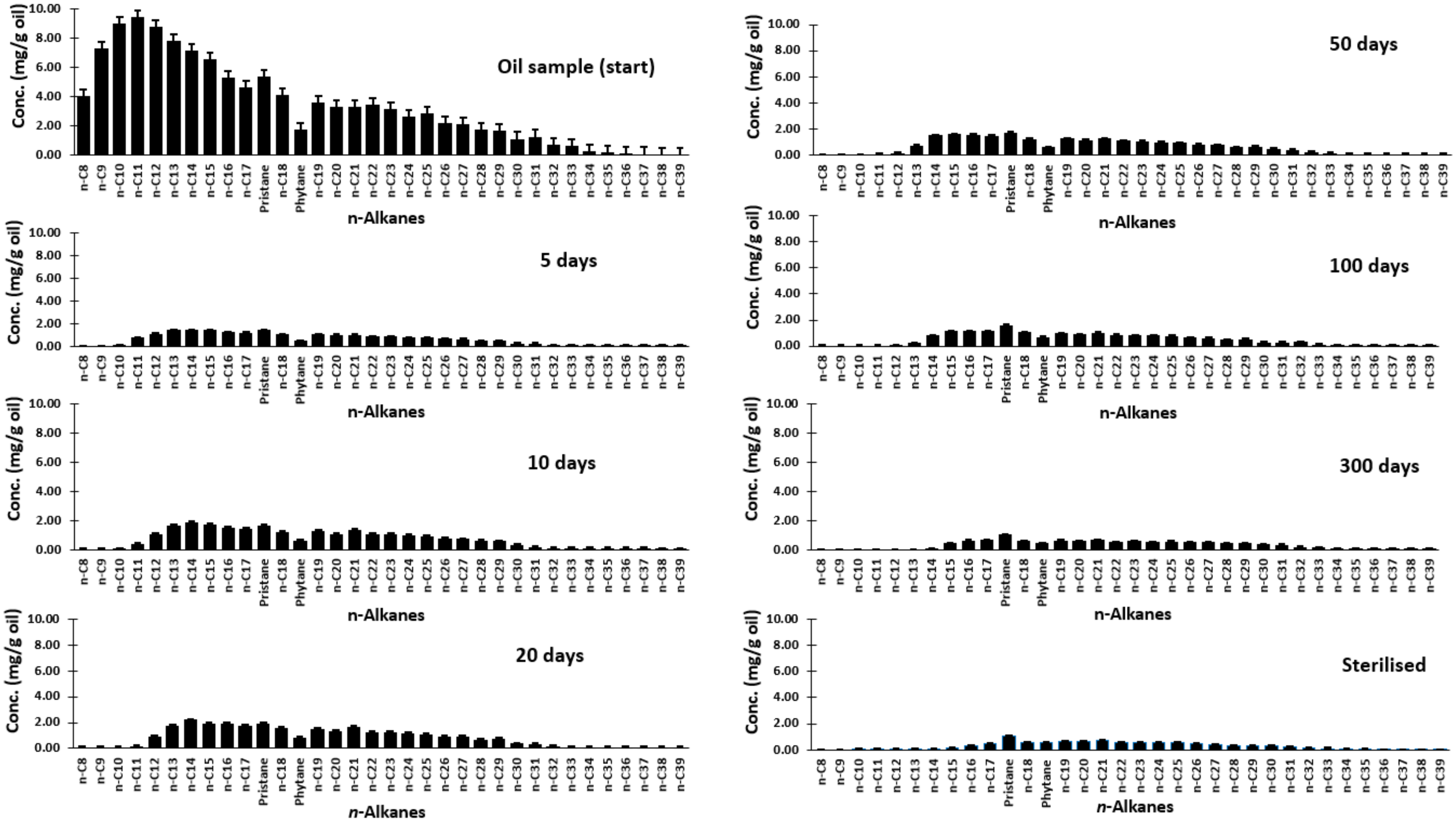


Figure 0.12: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian light oil in Nigerian sediment.

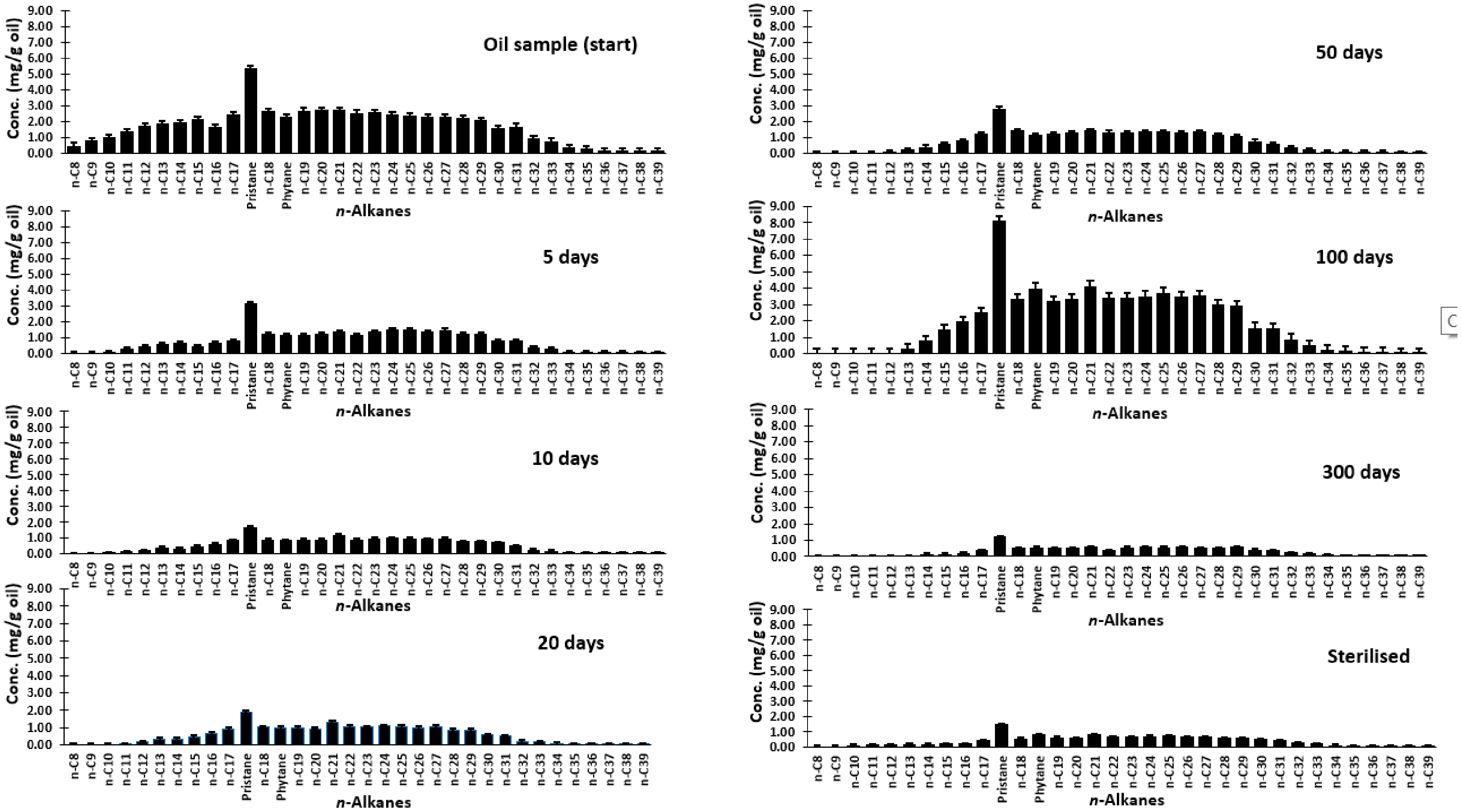
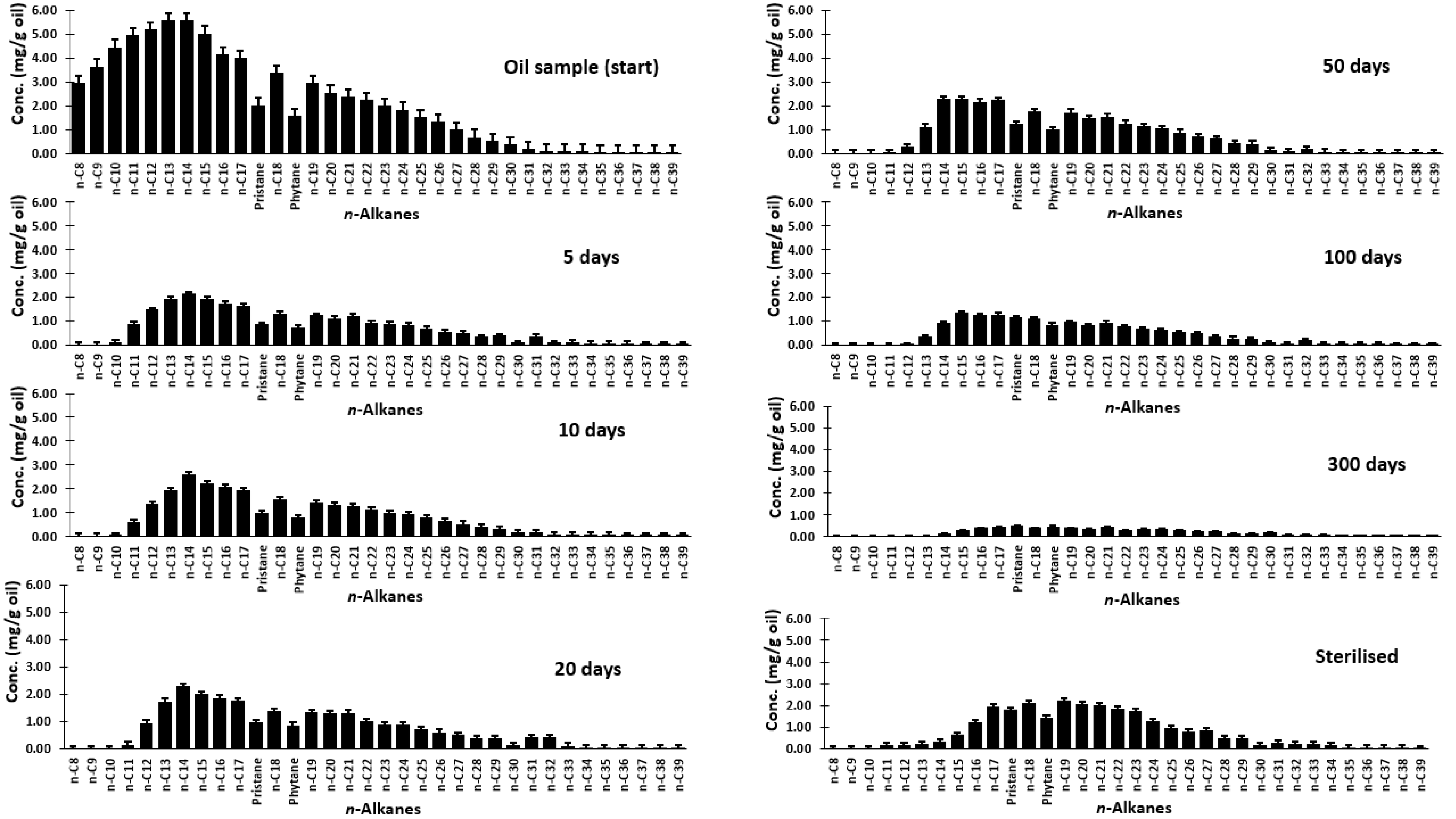


Figure 0.13: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian medium oil in Nigerian sediment.



Oil sample (start)

5 days

10 days

20 days

50 days

100 days

300 days

Sterilised

Figure 0.14: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for North Sea oil in Nigerian sediment.

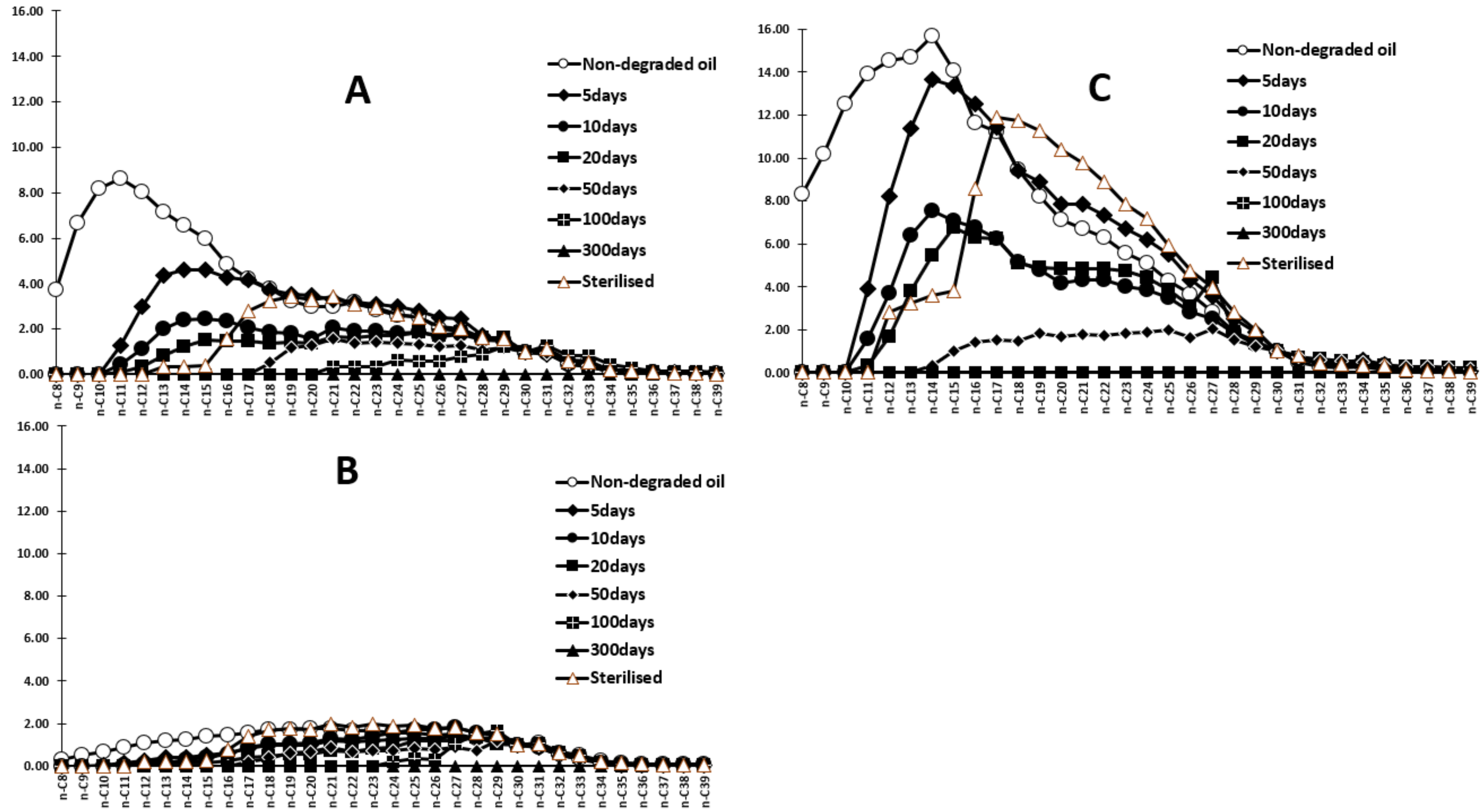


Figure 0.15: Plots of abundances of n-alkane in weathered oils in Whitley sediment relative to C30 for Nigerian light (A), Nigerian medium (B), and North Sea (C) oils.

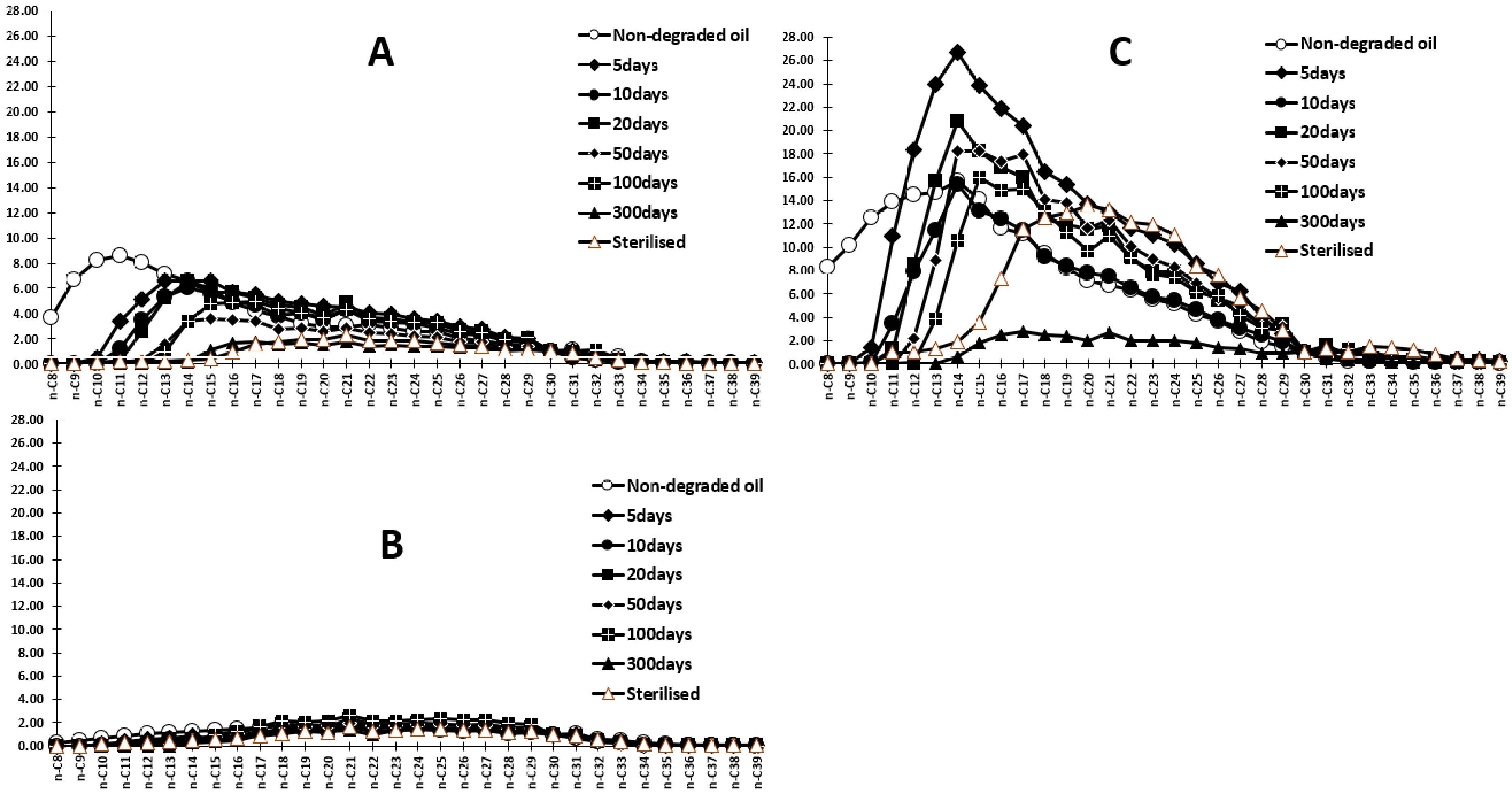


Figure 0.16: Plots of abundances of n-alkane in weathered oils in Nigerian sediment relative to C30 for Nigerian light (A), Nigerian medium (B), and North Sea (C) oils.

5.4.2 Aromatic hydrocarbons composition and concentration changes

The aromatic fractions (F2) were also analysed and the GC/MS TIC chromatograms (in SIM mode) weathered by biodegradation ; the results for the incubated samples for the period of 5, 10, 20, 50, 100, and 300 days and sterilised controls incubated for 300 days respectively, are shown in Figure 0.17Figure 0.18, and,Figure 0.19 for the Nigerian light and medium, and North Sea oils, in Whitley sediment and Figure 0.20,Figure 0.21Figure 0.22 for the three oil samples in Nigerian sediment respectively. The measured concentrations of the target PAHs are shown in Table 0.7Table 0.8Table 0.9 for the Nigerian light and medium, and North Sea oils in Whitley sediment and Table 0.10Table 0.11Table 0.12 for the three oil in Nigerian sediment respectively, which are mean values of triplicate samples with average relative standard deviations (RSD) of 6%, but increases to about 10% as biodegradation increased. Table 0.7Table 0.10 also show the background concentrations of target PAHs in the Whitley and Nigerian sediments respectively, which are quite negligible compared to the concentrations of PAHs in the undegraded oils. The Whitley sediment is dominated by the naphthalenes, dibenzofurans, and phenanthrenes, whereas the Nigerian sediment is dominated by the chrysenes, pyrenes, phenanthrenes, and then relatively small dibenzofurans and fluorenes. Figure 0.23 shows the distribution of the background target PAHs in both Whitley and Nigerian sediments. The low background concentration could be as a result of aerated mineralisation of the hydrocarbons due to high oxygen supply through the sandy particle size of the sediment (Killops and Killops, 1993). The measured concentrations of the PAHs were calculated as described in chapter 3 (see equations 3.7 and 3.8 in section 3.4.5). Homologous series of the alkylated PAHs were integrated with straight base lines carefully maintained for each peak for consistency (e.g., Yang *et al.*, 2014) and calculated based on mean values of triplicate samples for the Nigerian light, Nigerian medium and North Sea oils respectively. The relative response factors (RRFs) used were as

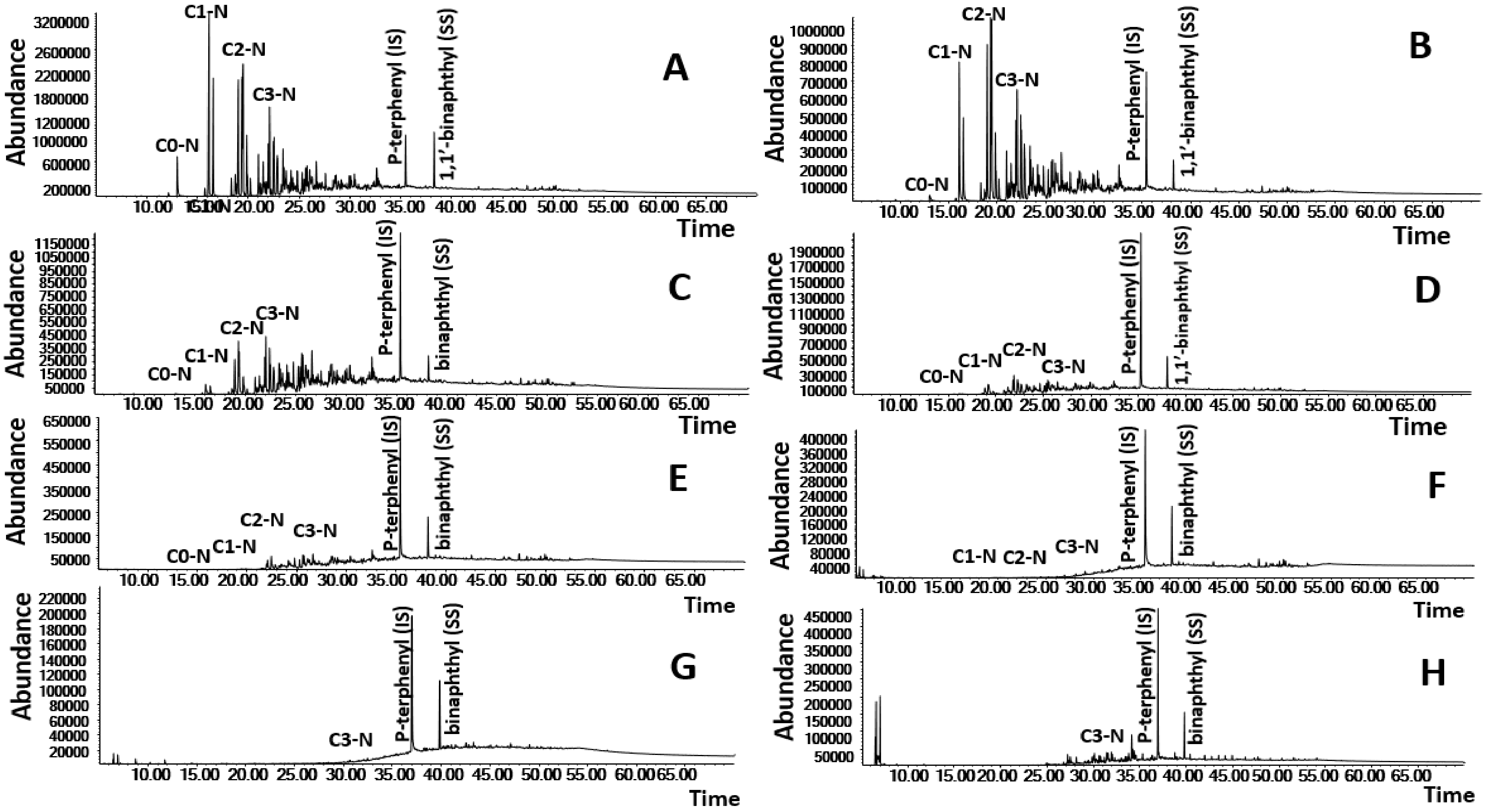


Figure 0.17: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian light oil weathered by biodegradation in Whitley sediment, (A) start, (B) 5 days, (C) 10 days, (D) 20 days, (E) 50 days, (F) 100 days, (G) 300days, and (H) sterilised control at 300 days, respectively.

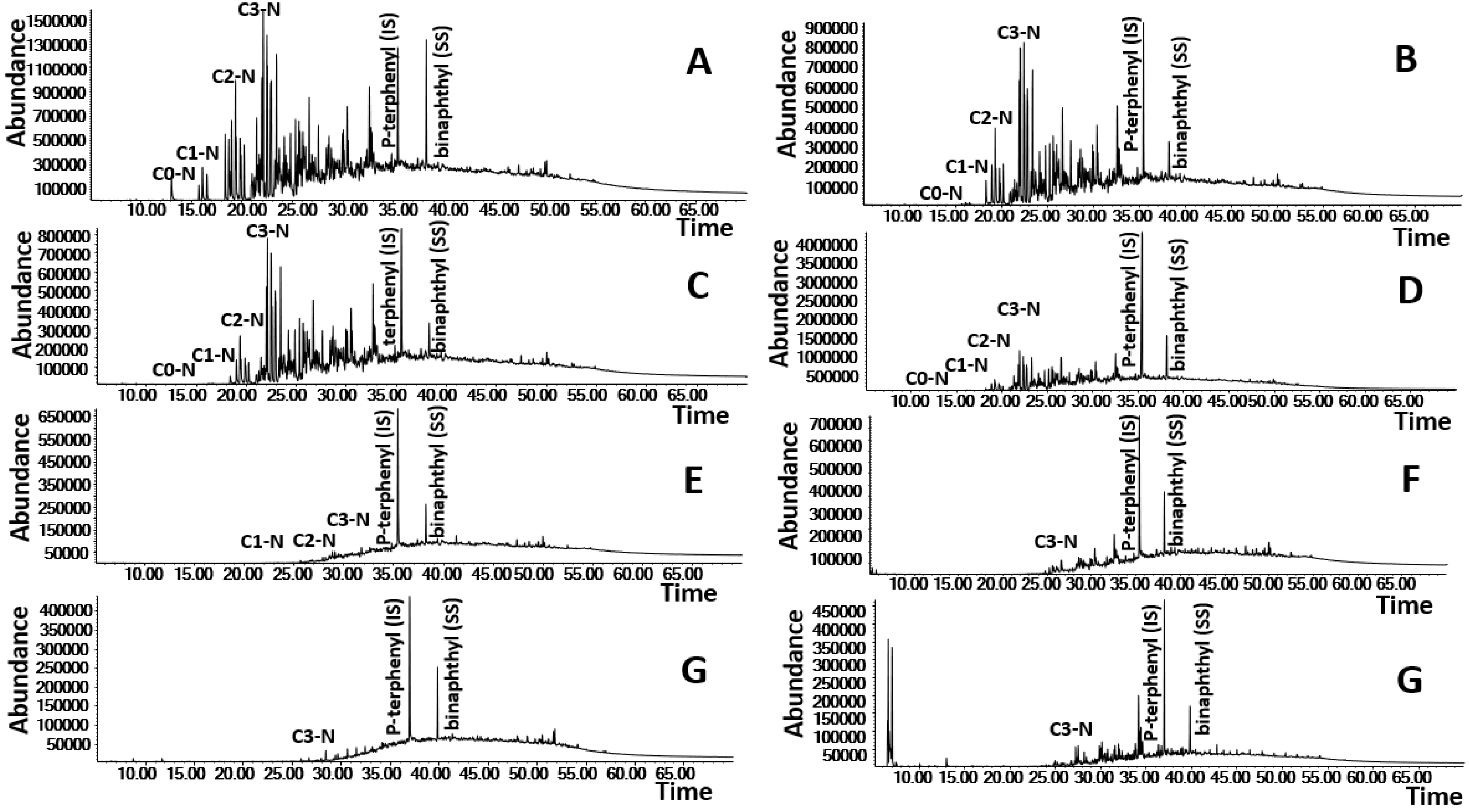


Figure 0.18: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian medium oil weathered by biodegradation in Whitley sediment, (A) start, (B) 5 days, (C) 10 days, (D) 20 days, (E) 50 days, (F) 100 days, (G) 300 days, and (H) sterilised control at 300 days, respectively.

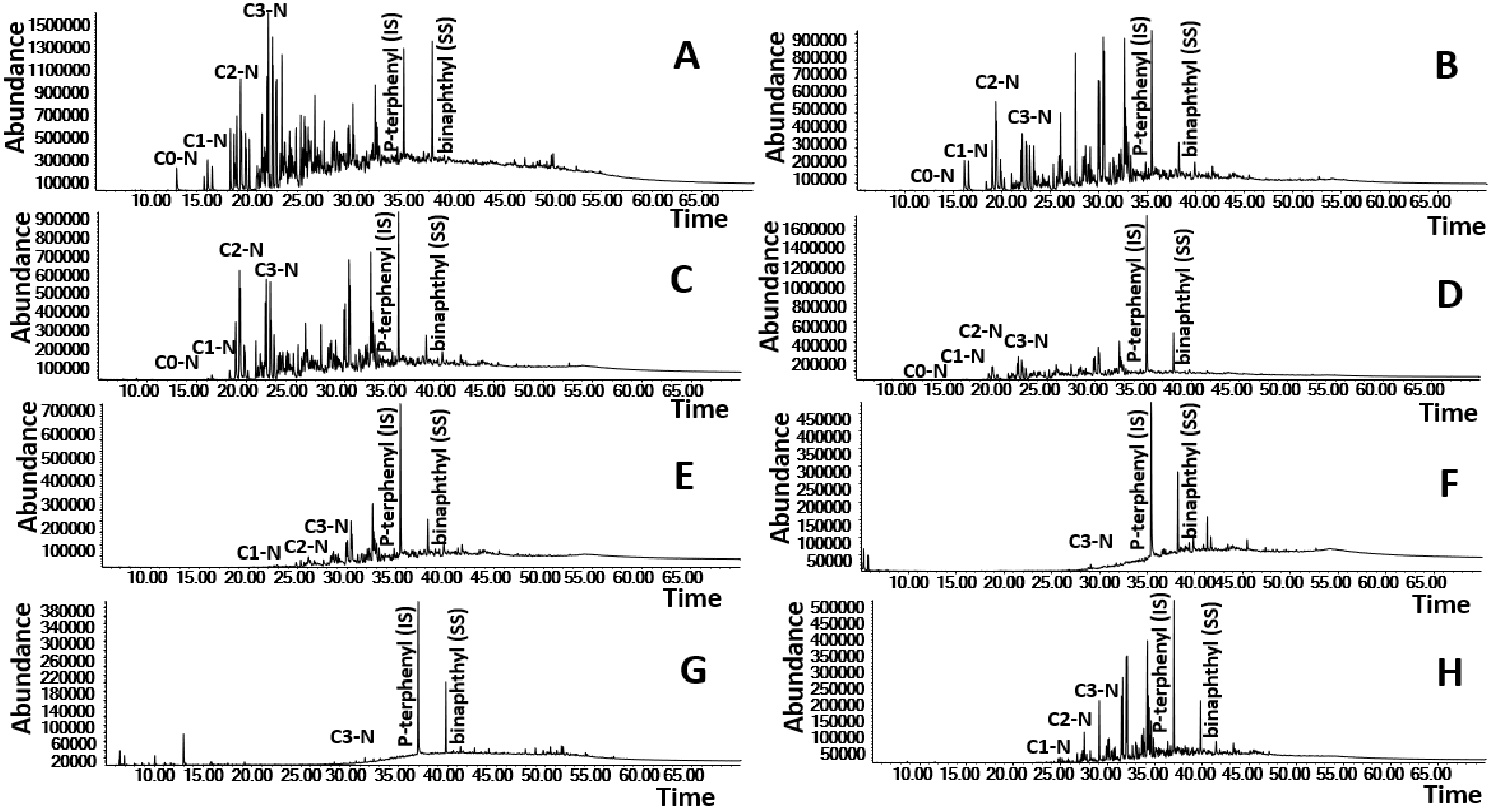


Figure 0.19: GC/MS SIM/TIC Chromatograms of aromatic compounds in North Sea oil weathered by biodegradation in Whitley sediment, (A) start, (B) 5 days, (C) 10 days, (D) 20 days, (E) 50 days, (F) 100 days, (G) 300 days, and (H) sterilised control at 300 days, respectively.

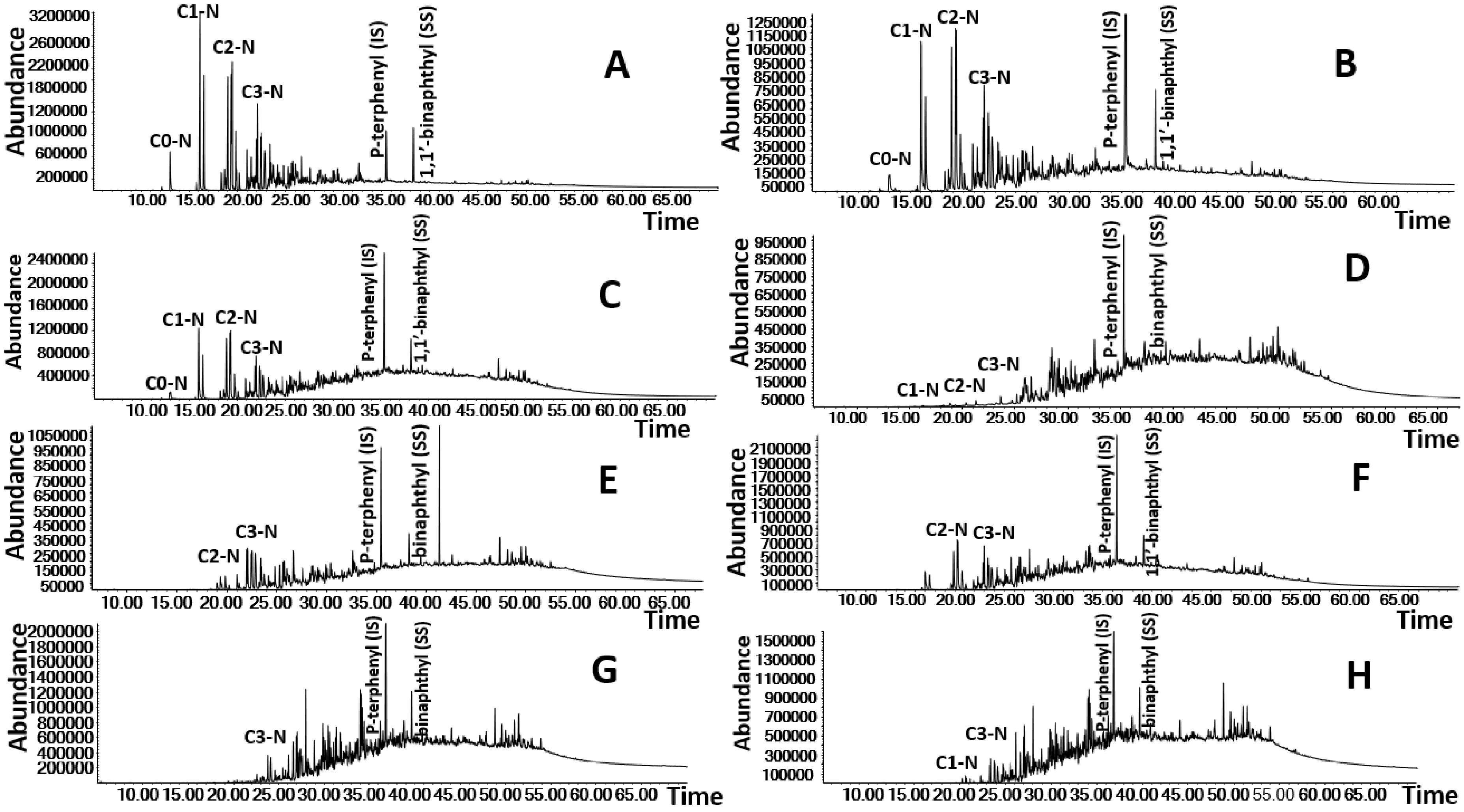


Figure 0.20: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian light oil weathered by biodegradation in Nigerian sediment, (A) start, (B) 5 days, (C) 10 days, (D) 20 days, (E) 50 days, (F) 100 days, (G) 300 days, and (H) sterilised control at 300 days, respectively.

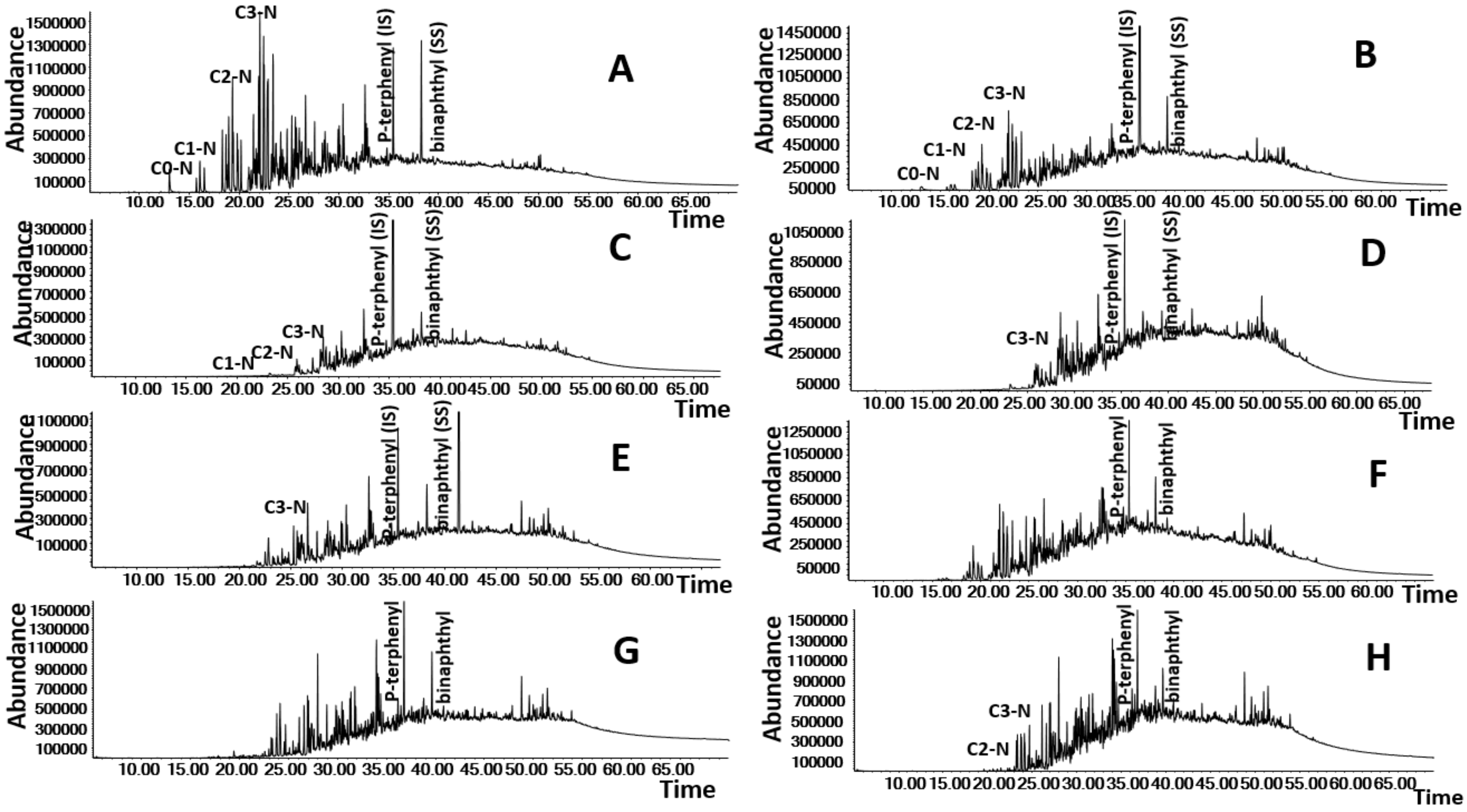


Figure 0.21: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian medium oil weathered by biodegradation in Nigerian sediment, (A) start, (B) 5 days, (C) 10 days, (D) 20 days, (E) 50 days, (F) 100 days, (G) 300 days, and (H) sterilised control at 300 days, respectively.

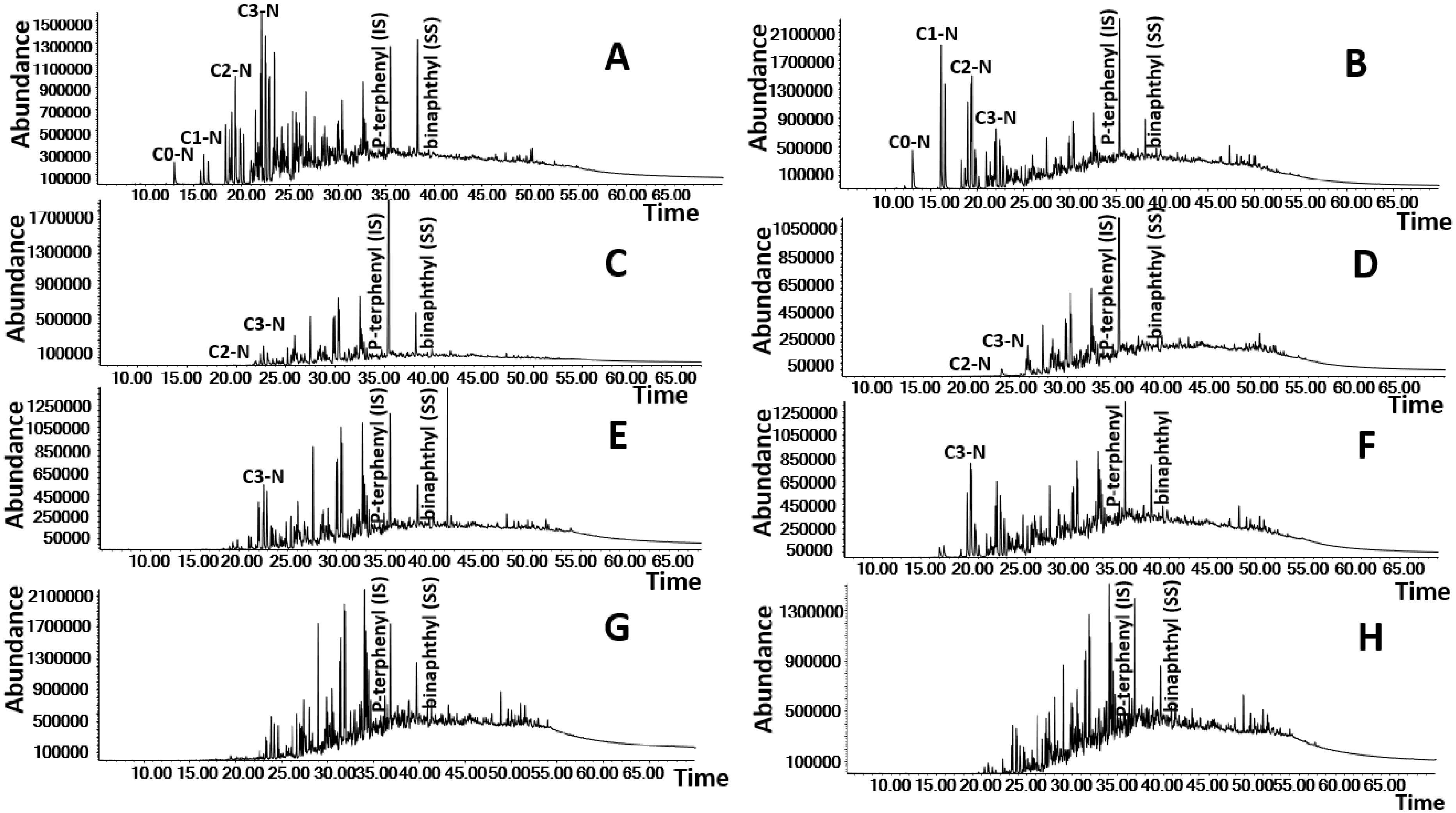


Figure 0.22: GC/MS SIM/TIC Chromatograms of aromatic compounds in North Sea oil weathered by biodegradation in sediment, (A) start, (B) 5 days, (C) 10 days, (D) 20 days, (E) 50 days, (F) 100 days, (G) 300 days, and (H) sterilised control at 300 days, respectively.

described in chapter 3 (see section 3.4.3) as samples were analysed under the same GC/MS operating conditions.

The importance and application of analysing PAHs in environmental samples is as discussed in chapter 4 (see section 4.4.2) and the detailed effects of weathering by aerobic biodegradation on these alkylated PAHs are analysed and discussed in this chapter. The PAHs are observed to be affected most by biodegradation than by evaporative and water washing weathering discussed in chapters 3 and 4 respectively.

Some of the parent and alkylated naphthalenes, biphenyls, dibenzofurans and fluorenes were completely mineralised after 100 days incubation period for the Nigerian light oil in Whitley sediment (see Table 0.7), whereas that of Nigerian sediment, naphthalene is observed to be completely degraded after 20days (see Table 0.10). The parent PAHs are more affected by biodegradation than the alkylated PAHs as observed in evaporative weathering discussed in chapter 3 (see section 3.4.5) and water washing in chapter 4 (see section 4.4.2) as it is generally accepted, that the biodegradation of PAHs simultaneously decreases with increase in the PAHs ring numbers and alkylation (Burns and Mankiewicz; Stout *et al.*, 2001; Stogiannidis and Laane, 2015). The relative composition (%) of naphthalene (C_0-N) was observed to reduce from 3% $(\frac{247.3}{8483.0}) \times 100$ (see Table 0.7Table 0.10) for the undegraded Nigerian light oil to 0% after 300 days incubation period and the sterilised microcosm for both sediments. Similarly, the percentage composition of naphthalene for Nigerian medium and North Sea oils in both Whitley and Nigerian sediments reduced from 5 to 0% and 15 to 0% respectively (see Table 0.8Table 0.9,Table 0.12). The alkylated homologous naphthalenes were observed to be more resistant to biodegradation than the parent naphthalenes as the alkyl carbon numbers increased as widely reported sequence of biodegradation rates which decrease with increase in ring numbers and degree of alkylation (Mazeas *et al.*, 2002; Bennett and Larter, 2008; Larter *et al.*, 2012; Huang *et al.*, 2019). The methylnaphthalene (2-MN/1-MN) and phenanthrene/anthracene (C_0-N/C_0-Anth) ratios are quite affected by biodegradation unlike that of evaporation (Chapter 3) and water washing (Chapter). The biodegradation effect on the methylnaphthalene (2-MN/1-MN) ratios is more on the oils in the Nigerian sediment (see Table 0.10Table 0.11Table 0.12) than that of the Whitley sediments (see Table 0.7Table 0.8Table 0.9). This generally confirms the susceptibility of aromatic compounds in crude oils to microbial transformation as reported in a number of literature in the surface and sub-surface environments (Claret *et al.*, 1977; Rubinstein, 1977; Higgins and Gilbert, 1978; Cain, 1980; Kimes *et al.*, 2014). The ratios of the 4-methyldibenzothiophene to that of the two identified pentamethylnaphthalnes (a, b, c, d, e-PMN and v, w, x, y, z-PMN) is also observed to be slightly affected by biodegradation than by dehydration and water washing. This indicates a strong possibility of using these ratios for oil correlation and differentiation. Figure 0.24Figure 0.25,Figure 0.26Figure 0.27Figure 0.28Figure 0.29 show the distribution of the target alkylated PAHs for the Nigerian light and medium, and North Sea oils in Whitley and Nigerian sediments, respectively. The effect of biodegradation is observed by the alteration of the characteristic “bell shape” distribution pattern with increase in degradation time. The most noticeable alteration of the distribution pattern could be observed in the napthalenes and their alkyl homologous where the pattern changed with increasing biodegradation time with the Parent naphthalenes degraded first and then followed by the less alkylated homologues for all oils in both sediments. Example of such is the degradation of naphthalene (C_0-N) from 3% composition in the undegraded oil 0% at 300 days degradation (PM Level 5), whereas C_1-N naphthalenes increased from 15% in the starting oil to 26% in 100 days and then to 0% in 300 days degradation, while C_2-N naphthalenes decreased much slower than C_0-N and C_1-N from 55% in starting oil to 31% after 100 days and finally to 0% in 300 days. While C_3-N naphthalenes increased significantly from 17% in the starting oil to 65% at 300 days degradation, and finally the C_4-N naphthalenes also increased from 10% in undegraded 35% at 300 days (see Table 0.7). This apparent variability in alteration could be attributed to a number of factors such as variable microbial communities, oil type, water chemistry, nutrient accessibility and temperature (Larter *et al.*, 2012). The next susceptible alkyl homologous are the biphenyls which were degraded similarly but much slower than the naphthalenes. The PAHs in Whitley sediments are observed to be more affected by biodegradation than those in Nigerian sediment, and it is characterised by the well-known degradation sequence starting by the rapid depletion of the parent PAHs, followed by less alkylated homologues, whereas the more alkylated homologues tend to increase which began to deplete with increase biodegradation (e.g., Wang *et al.,* 1998). Higher ring PAHs such as chrysenes and triaromatic steroids are more resistant to biodegradation and tend to increase with increase biodegradation relative to lower ring PAHs, this effect is more pronounced with PAHs in the Whitley sediment (see Figure 0.24Figure 0.25, andFigure 0.26) which indicate a higher degradation than observed with PAHs in the Nigerian sediment (see Figure 0.27,Figure 0.28Figure 0.29). Pyrenes, chrysenes, and triaromatic steroids of Nigerian light and North Sea oils in Whitley sediment increased remarkably relative to their corresponding lower ring PAHs from 50 days to 300 days degradation period (see Figure 0.24Figure 0.26) this indicate the highest degree of degradation (e.g., Wang *et al.*, 1998; Stogiannidis and Laane, 2015).

Methylbiphenyls and methylphenanthrene isomers were analysed to assess the effects of biodegradation as previously discussed in chapter 4 (see section 4.2.2) and the concentrations are shown in Table 0.13Table 0.14Table 0.15Table 0.16Table 0.17Table 0.18 and the distribution for the methylbiphenyls are shown in Figure 0.31 and the methylphenanthrenes in Figure 0.32 respectively. The methylbiphenyls in the Whitley sediment (Figure 0.31 A, B, and C) were relatively more affected by biodegradation than the ones in Nigerian sediment (Figure 0.31 D, E, and F). The methylbiphenyls in the Nigerian medium oil amongst the oils is the

Whitley sediment (Figure 0.31B) are the least affected by biodegradation. Similarly, the methylphenanthrenes in the Whitley sediment (Figure 0.32 A, B, and C) are also more degraded than those in Nigerian sediment (Figure 0.32 D, E, and F). Just like the methylbiphenyls, the methylphenanthrenes in the Nigerian medium oil (Figure 0.32 B) in is the least affected for the oils in Whitley sediment whereas methylphenanthrenes in the North Sea oil are the least biodegraded for the oils in Nigerian sediment (Figure 0.32 F).

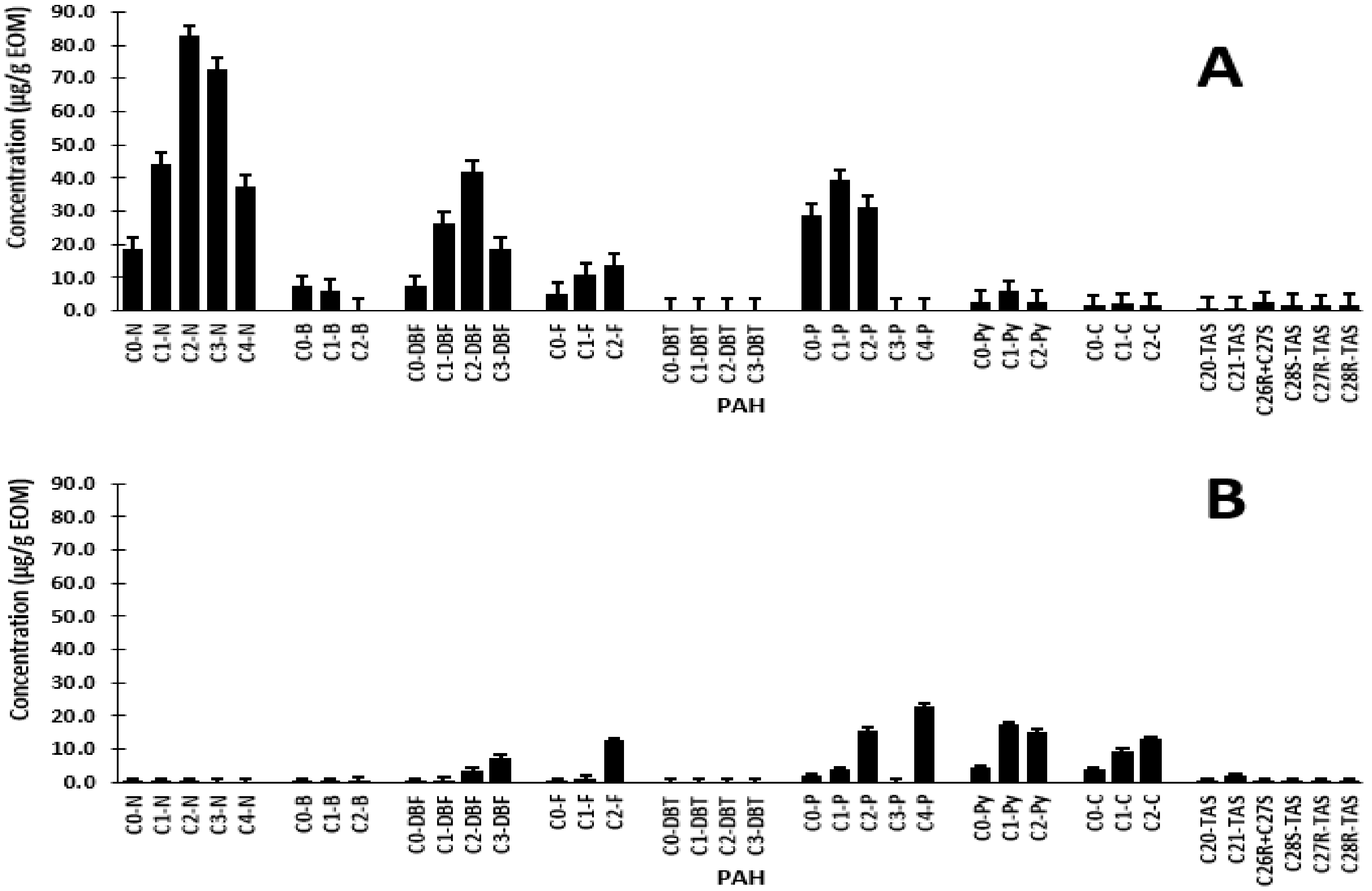
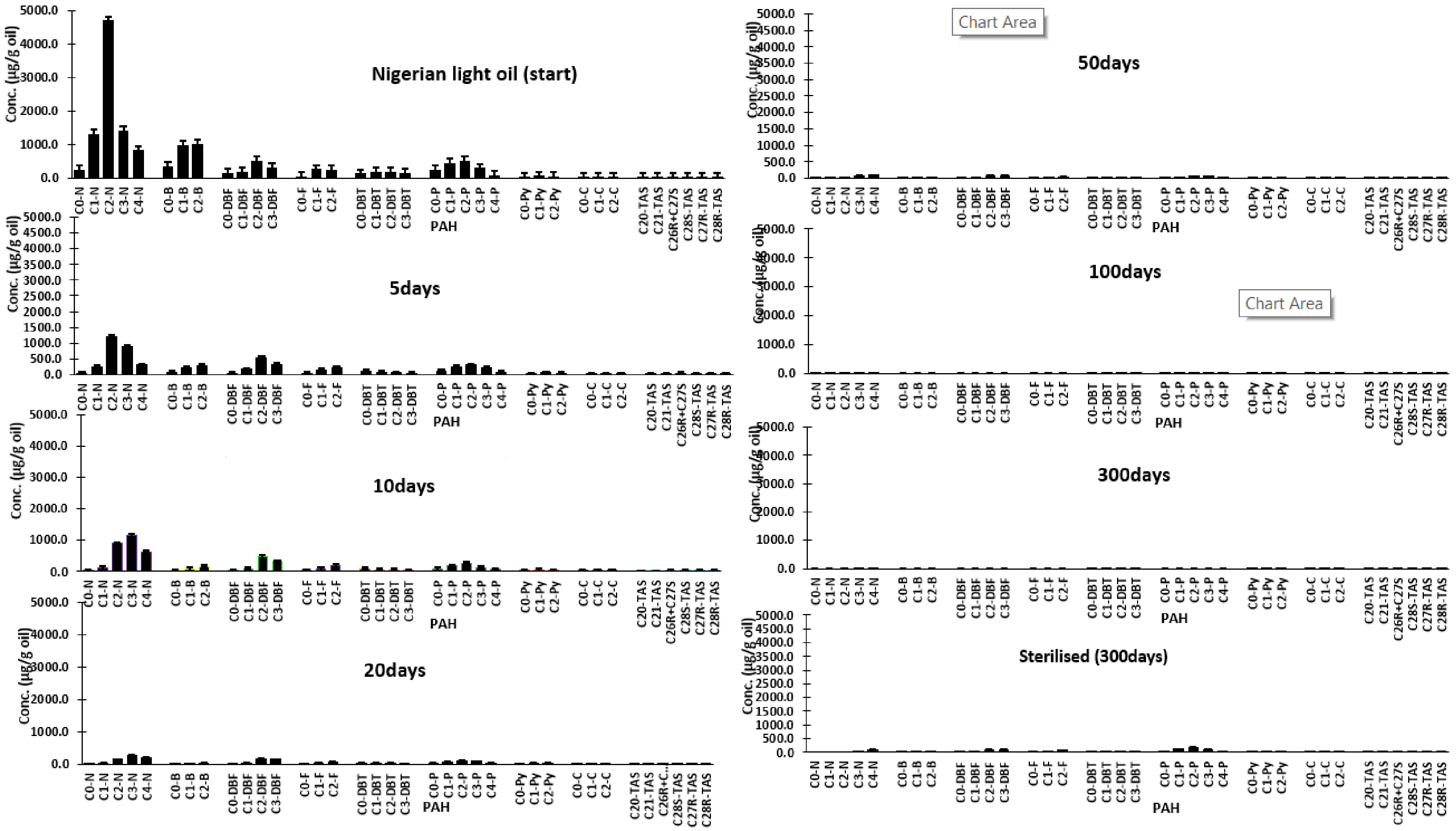


Figure 0.23: Distribution of background target alkylated PAHs in (A) Whitely sediment, (B) Nigerian sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene and chrysene respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.



Nigerian light oil (start)

5days

10days

20days

50days

100days

300days

Sterilised (300days)

Figure 0.24: Distribution of target alkylated PAHs showing the biodegradation incubation period for Nigerian light oil in Whitley sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

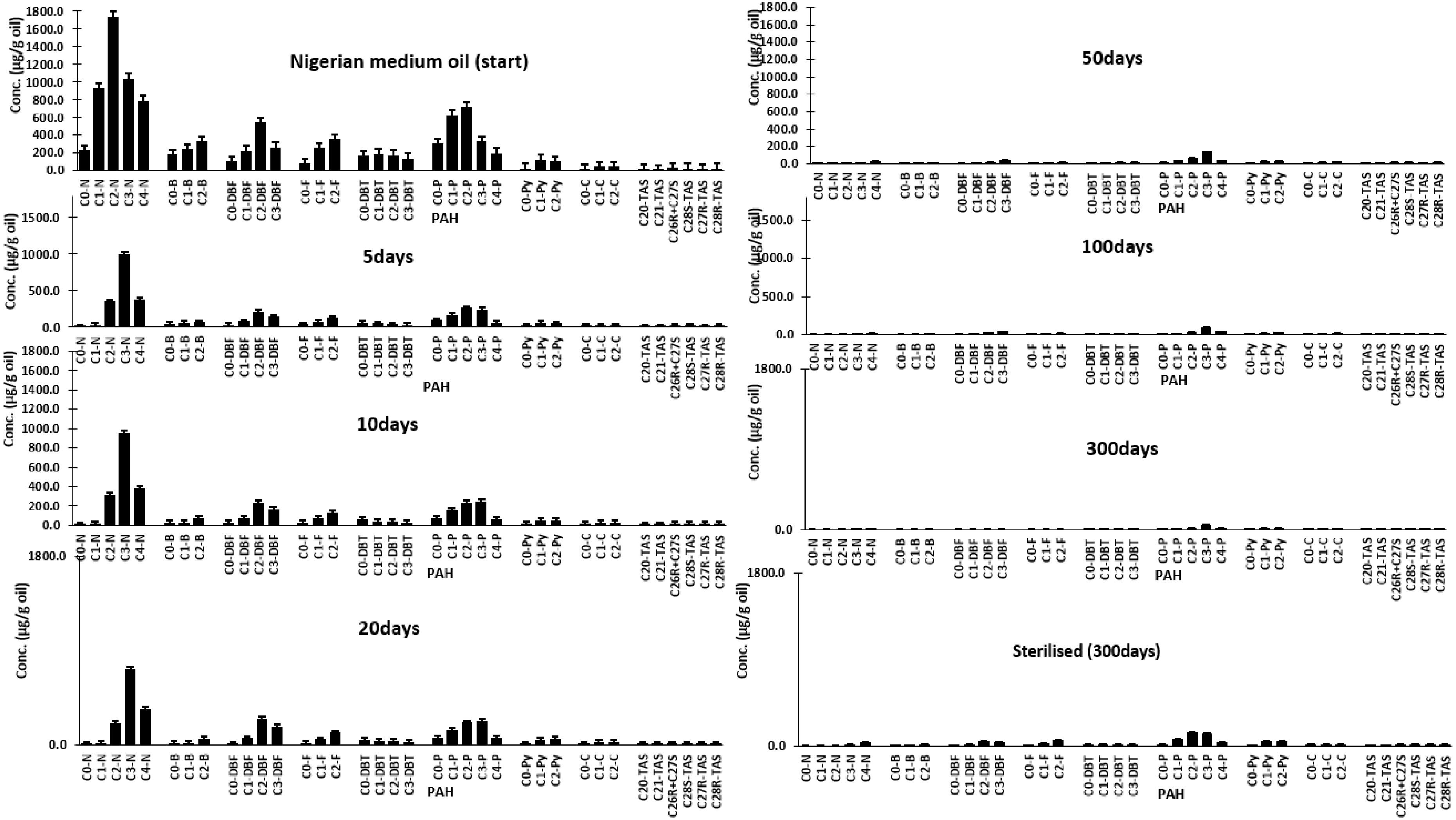


Figure 0.25: Distribution of target alkylated PAHs showing the biodegradation incubation period for Nigerian medium oil in Whitley sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

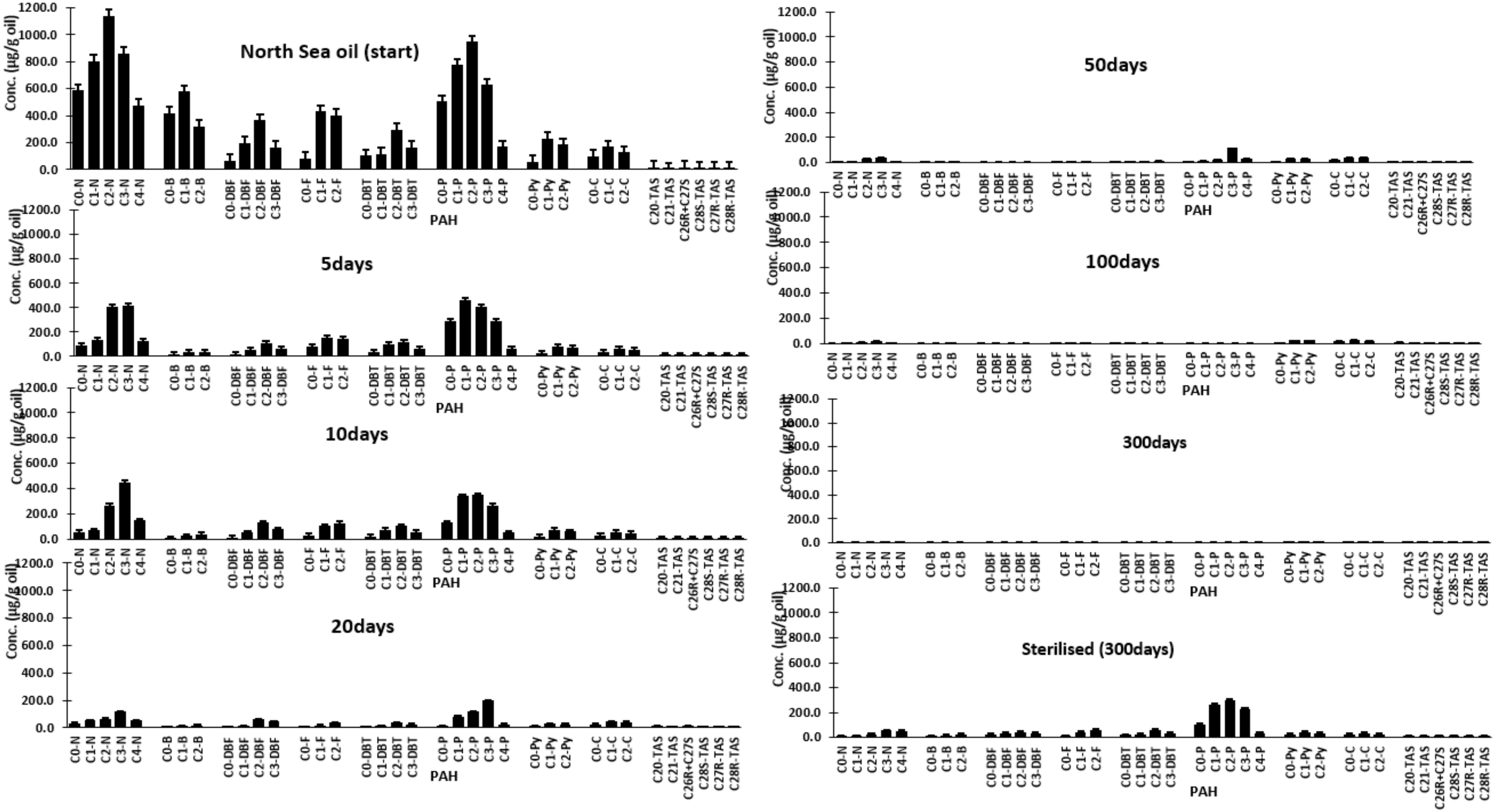


Figure 0.26: Distribution of target alkylated PAHs showing the biodegradation incubation period for North Sea oil in Whitley sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

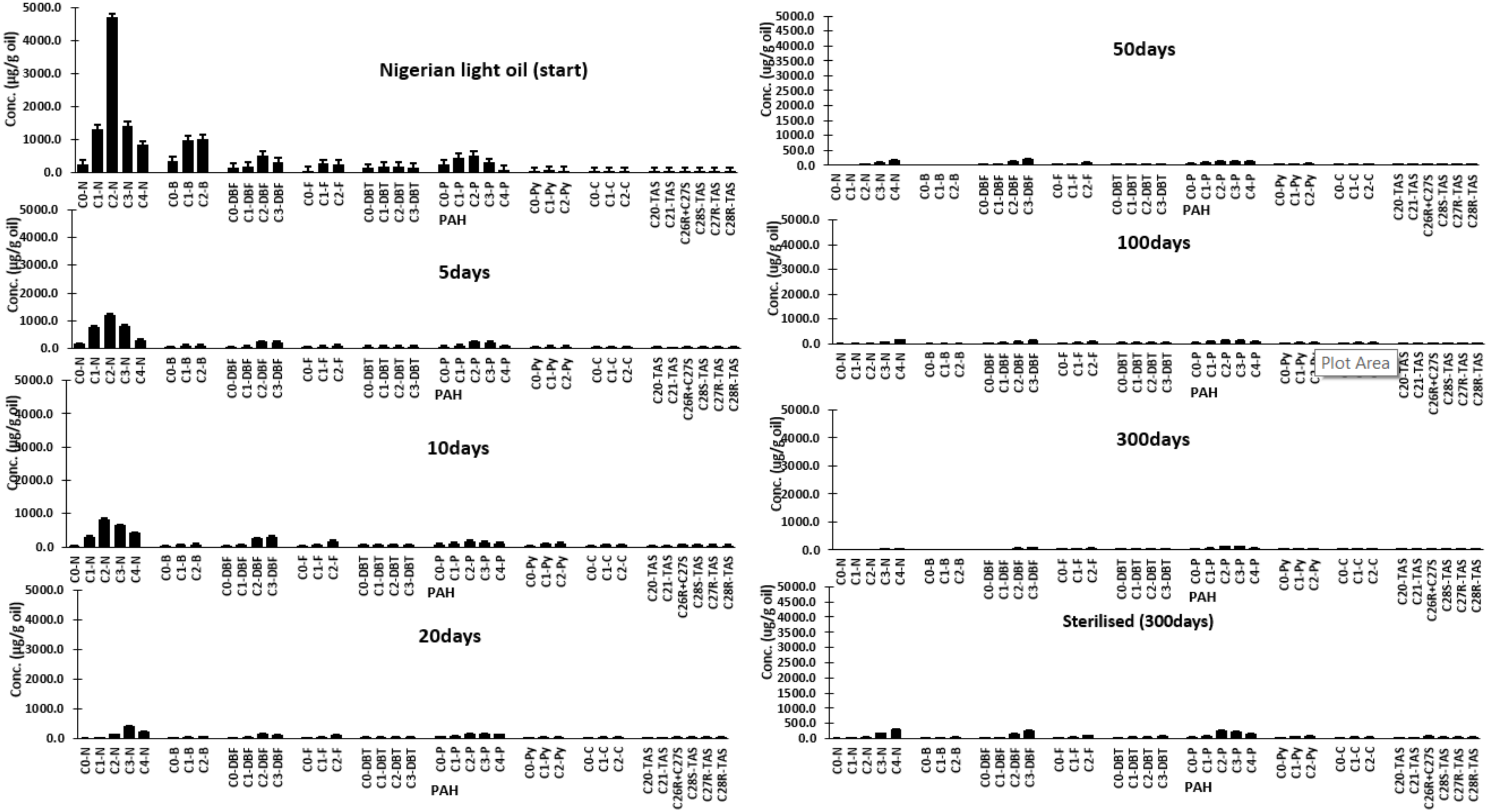
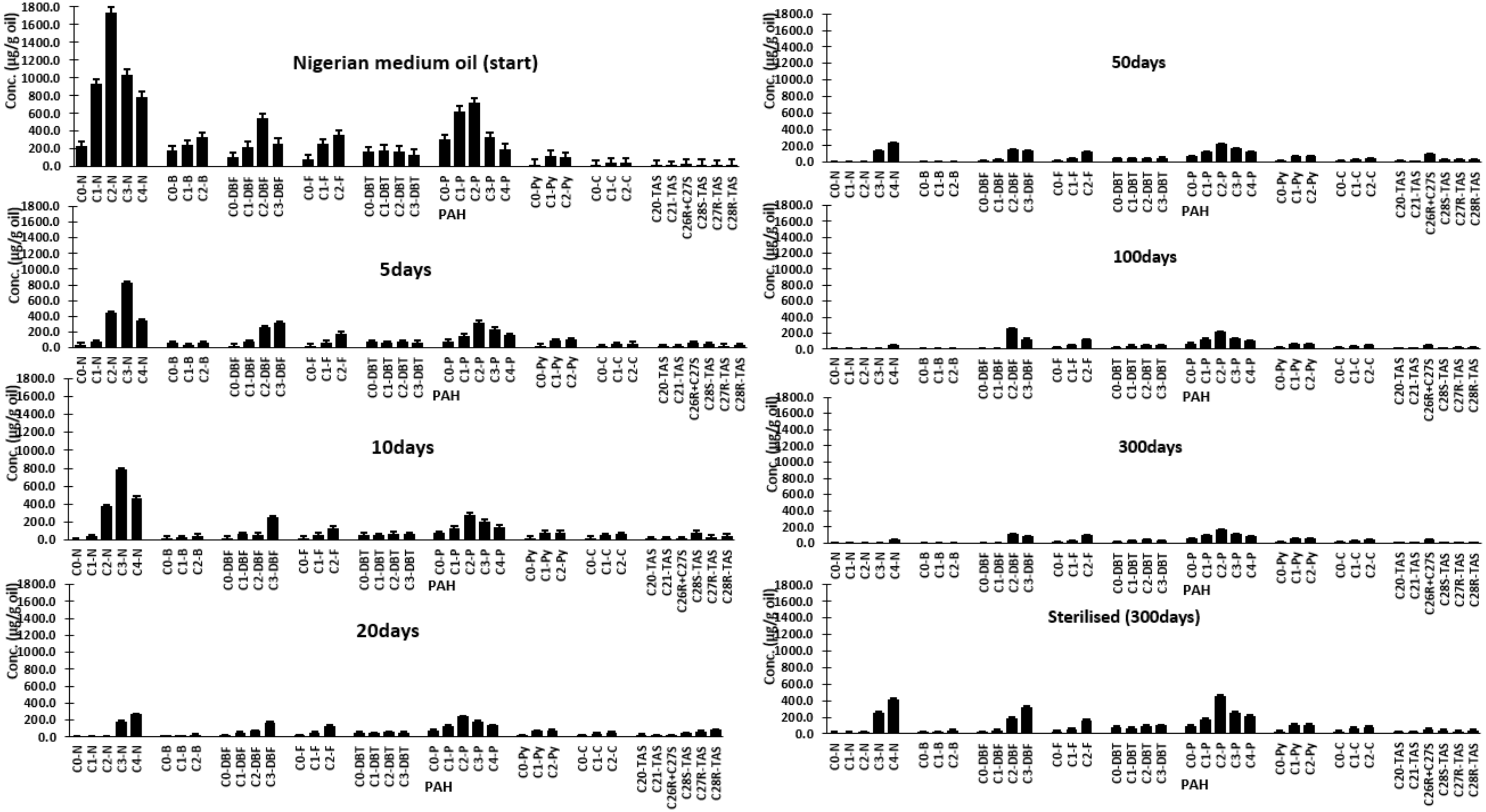


Figure 0.27: Distribution of target alkylated PAHs showing the biodegradation incubation period for Nigerian light oil in Nigerian sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.



Nigerian medium oil (start)

5days

10days

20days

50days

100days

300days

Sterilised (300days)

Figure 0.28: Distribution of target alkylated PAHs showing the biodegradation incubation period for Nigerian medium oil in Nigerian sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

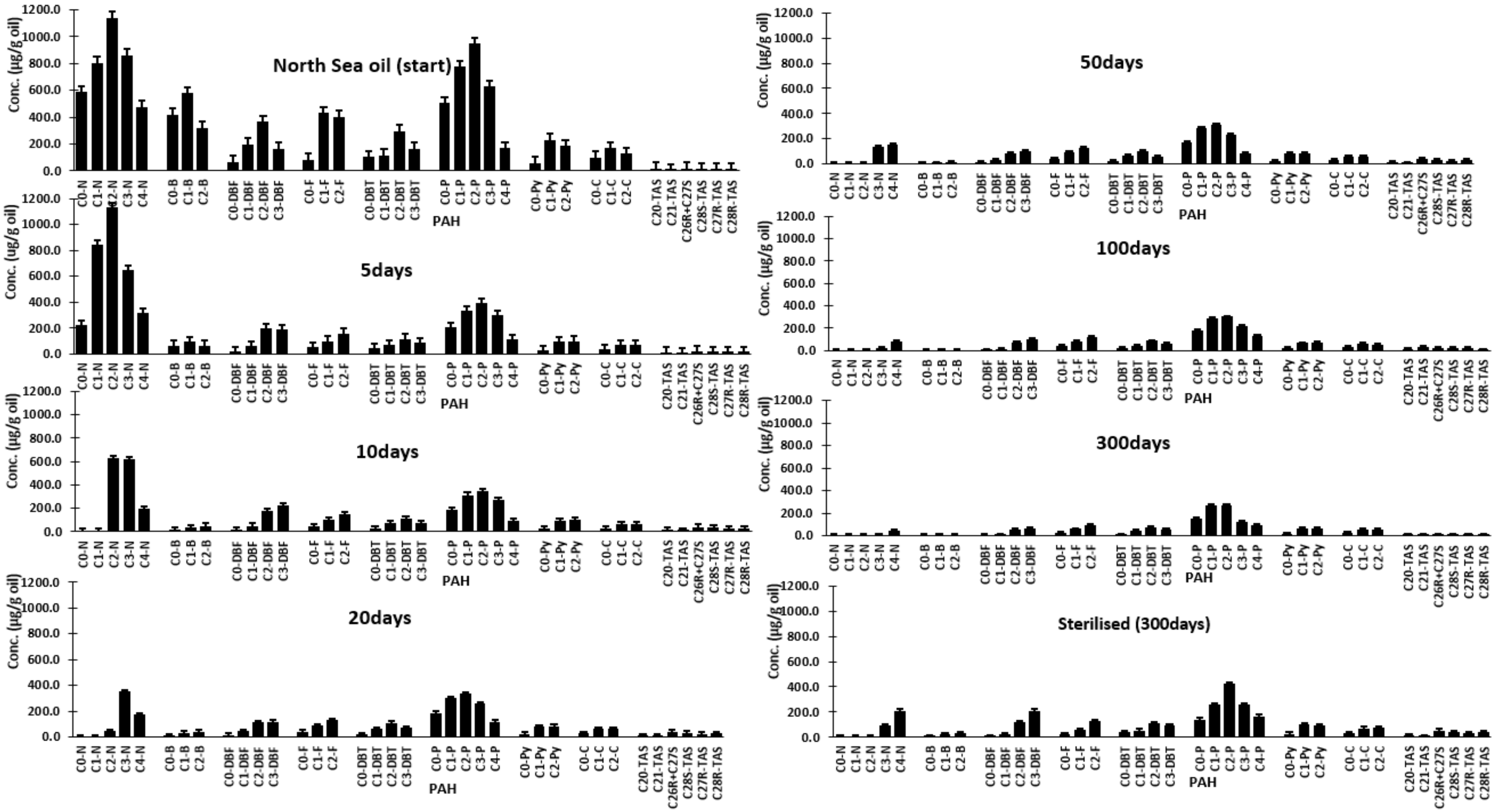


Figure 0.29: Figure 0.30: Distribution of target alkylated PAHs showing the biodegradation incubation period for North Sea oil in Nigerian sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs.

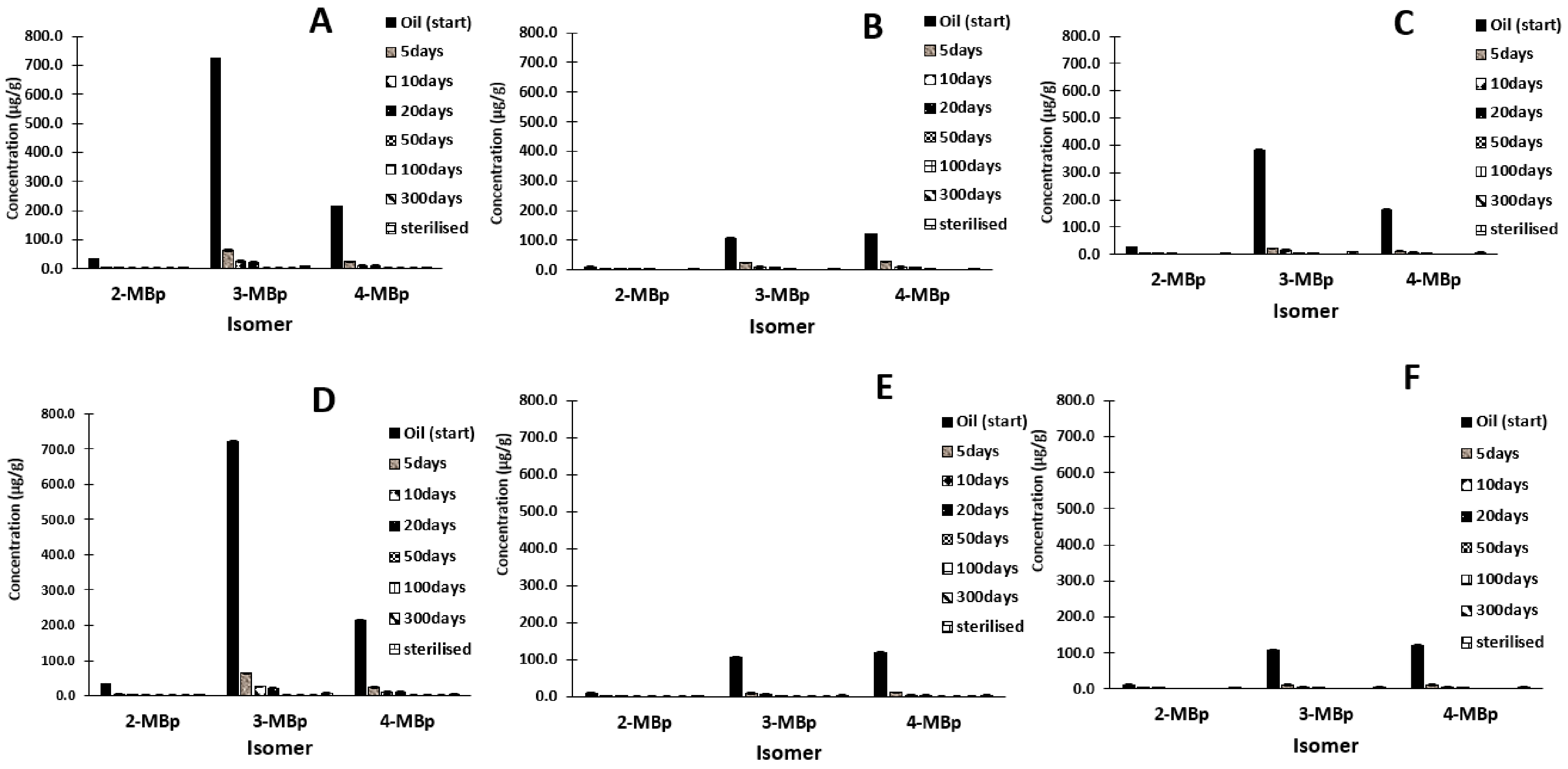


Figure 0.31: Effect of biodegradation on methylbiphenyls isomers for (A) Nigerian light, (B) Nigerian medium, (C) North Sea oils in Whitley sediment, and (D) Nigerian light, (E) Nigerian medium, (F) North Sea oils in Nigerian sediment respectively.

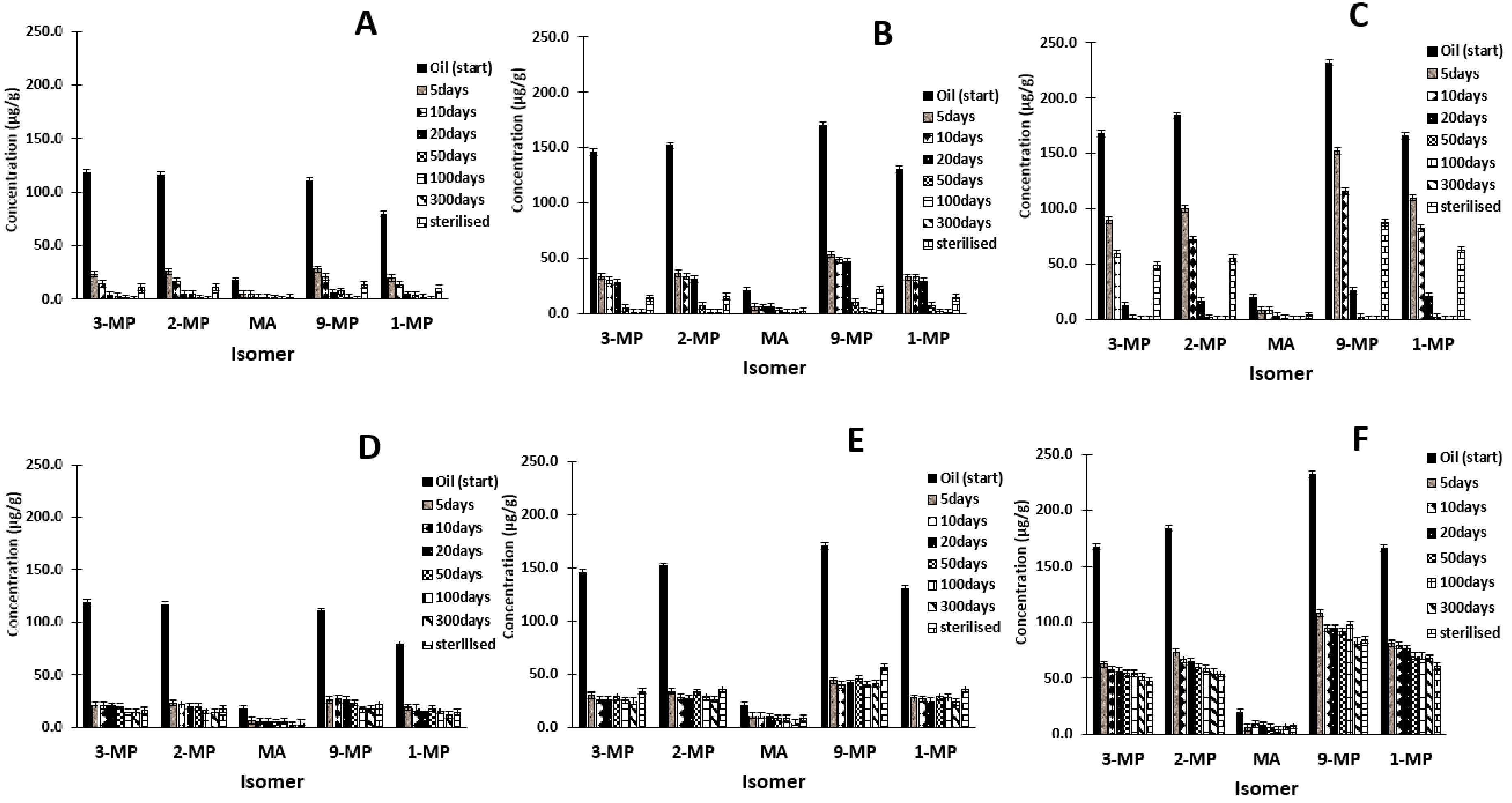


Figure 0.32: Effect of biodegradation on methylphenanthrene isomers for (A) Nigerian light, (B) Nigerian medium, (C) North Sea oils in Whitley sediment, and (D) Nigerian light, (E) Nigerian medium, (F) North Sea oils in Nigerian sediment respectively.

These isomers are were observed to be more affected by biodegradation than evaporation as discussed in chapter 3 and water washing as discussed in chapter 4 respectively. The choice of methylphenanthrene isomers is due their abundances in source rock extracts and oils, and their variable resistance to biodegradation make them useful for assessment of biodegradation extent (Connan, 1984a; Volkman *et al.*, 1984; Rowland *et al.*, 1986; Chen *et al.*, 2013). Susceptibility to biodegradation between methyl biphenyls and methylphenanthrenes isomers in terms of their relative percentage composition were assessed for the Nigerian light oil in Whitley sediment (Table 0.130). The 2-methylbiphenyl isomer reduces from 3% $(\frac{32.5}{968.4}) \times 100$ in the undegraded oil 2% in 50days and the 0% in 100days, whereas the 3-methylbiphenyl isomer decreased from 75% to 60% in 50days, however, the 4-methylbiphenyl isomer was observed to be the least susceptible of the three isomers. Similarly, the 9-mthylphenethrene and 1-methylphenenthrene isomers were observed to be more resistant to biodegradation as they increased from 25 to 31%, and 18 to 32% respectively in 100days compare to 2-methylphenenthrene and 3-methylphenanthrene which decreased 27 to 13%, and 26 to 13% correspondingly. This is not the case for all the case due to isomer variable susceptibility. The phenanthrene isomer variability is due to the impact of alkyl substituent positioning on the rate of biodegradation, consequently, 9-methylphenanthrene have been identified as the generally more resistant to biodegradation compared to the other three isomers (Rowland *et al.*, 1986; Cassani and Eglinton, 1991; Chen *et al.*, 2013), although a contrary sequence of removal have been reported (Bennett and Larter, 2008). Bennett and Later (2008) reported 9-methylphenanthrene exhibiting a selective resistance to degradation compared to 1-methylphenenthrene, but instances where 9-methylphenanthrene have been degraded before 1-methylphenenthrene and a situation where both are removed before 3- and 2-methylphenenthrene respectively.

5.4.3 Biomarker compound composition and concentration changes.

It is well-known that biomarkers are relatively more resistant to weathering and degradation compared to other components of the crude (Peters *et al.*, 2005; Wang *et al.*, 2006), biomarkers have been reported to survive certain levels of biodegradation (Rubinstein, 1977; Seifert and Moldowan, 1979) on the surface (Stout *et al.*, 2005) and sub-face environments (Reed, 1977; Jones *et al.*, 2008). Consequently, biomarkers are important sources of fingerprints for correlation during forensic studies or investigation of biodegraded oils as biomarkers such as tricyclic terpanes have been reported to survive heavy biodegradation while regular steranes are destroyed (Seifert and Moldowan, 1979). This research looks at the effect of biodegradation by diagnostic analysis of biomarkers which include tricyclic, tetracyclic and pentacyclic terpanes, sesquiterpanes, steranes, a non-biomarker geochemical maker adamantane and triaromatic steranes.

Figure 0.33Figure 0.34Figure 0.35 are the m/z 191 mass chromatograms showing terpanes distributions for the non-biodegraded Nigerian light, Nigerian medium, and North Sea oils respectively. Figure 0.36 is are the m/z 191 mass chromatograms showing terpanes distributions for the North Sea oil added to Nigerian sediment incubated for 5 days showing the sediment background effect, and the measured concentrations are shown in Table 0.19Table 0.20Table 0.21 for the Nigerian light, Nigerian medium, and North Sea oils in Whitley sediment, and Table 0.22,Table 0.23Table 0.24 for the Nigerian light, Nigerian medium, and North Sea oils in Nigerian sediment respectively. Background concentrations in the two sediments were also measured to assess the influence of indigenous hydrocarbons and were found to be of much lower amounts compared to the corresponding concentrations in the non-degraded or starting oils. Concentrations of biodegraded samples incubated up 300 days with sterile controls are also shown in the tables. The coloured labelled peaks are the unidentified peaks discussed in

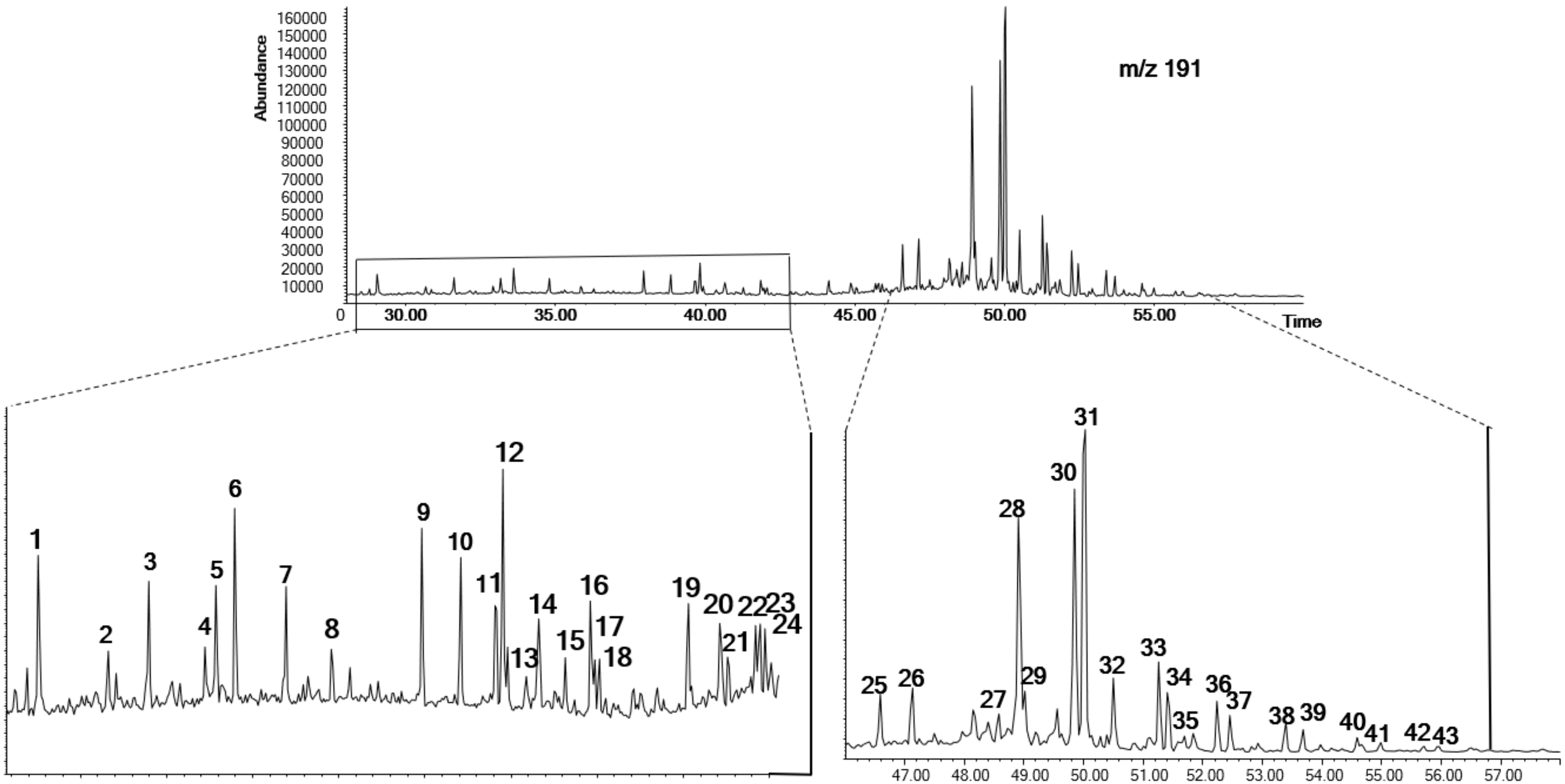


Figure 0.33: m/z 191 mass chromatograms showing terpanes distribution from non-biodegraded Nigerian light oil. The numbers represent the corresponding peak names listed in Table 0.19 and Table 0.22. Letters represent unidentified peaks.

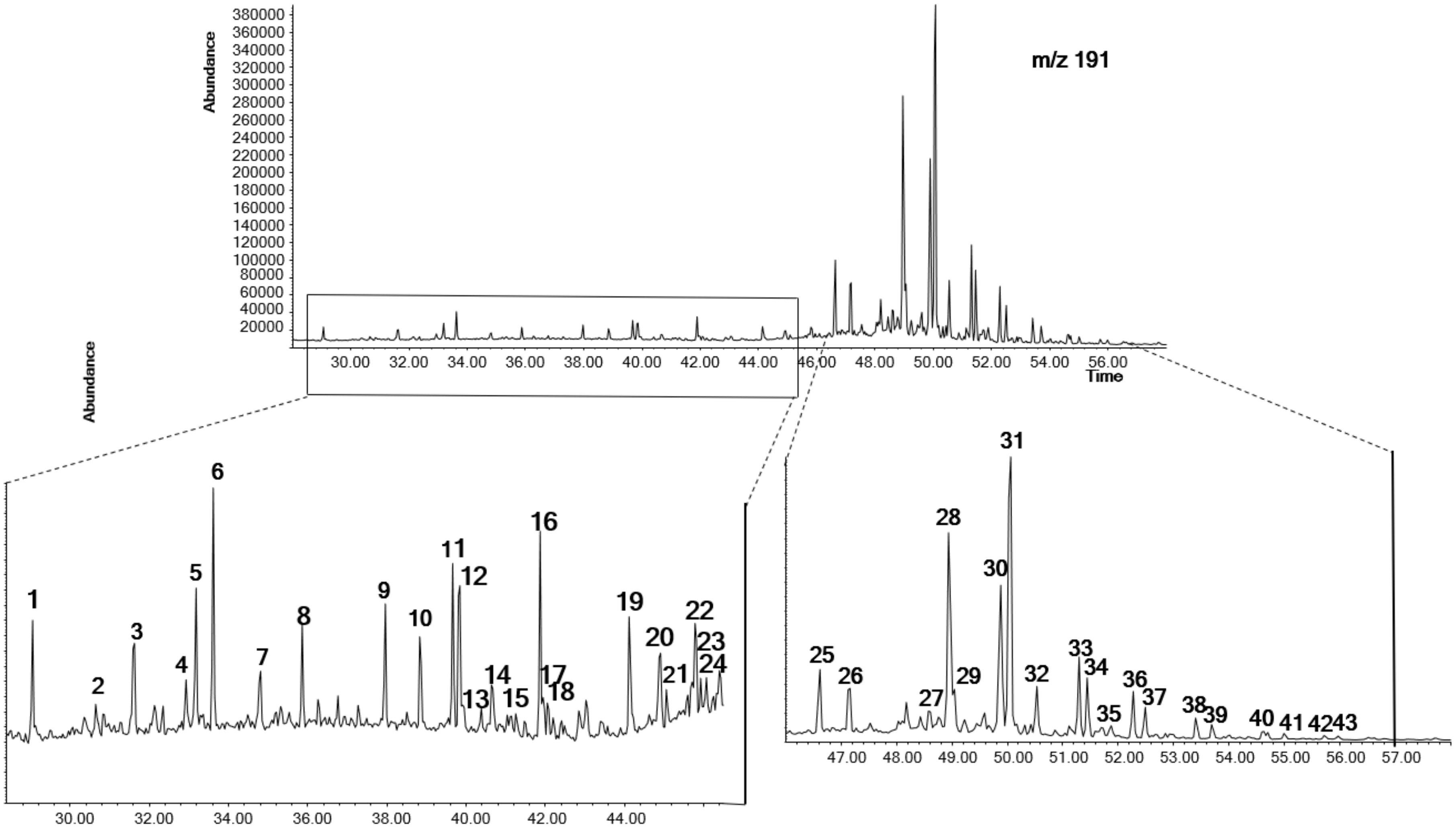


Figure 0.34: m/z 191 mass chromatograms showing terpanes distribution from non-biodegraded Nigerian medium oil. The numbers represent the corresponding peak names listed in Table 0.20Table 0.23. The letters represent unidentified peaks.

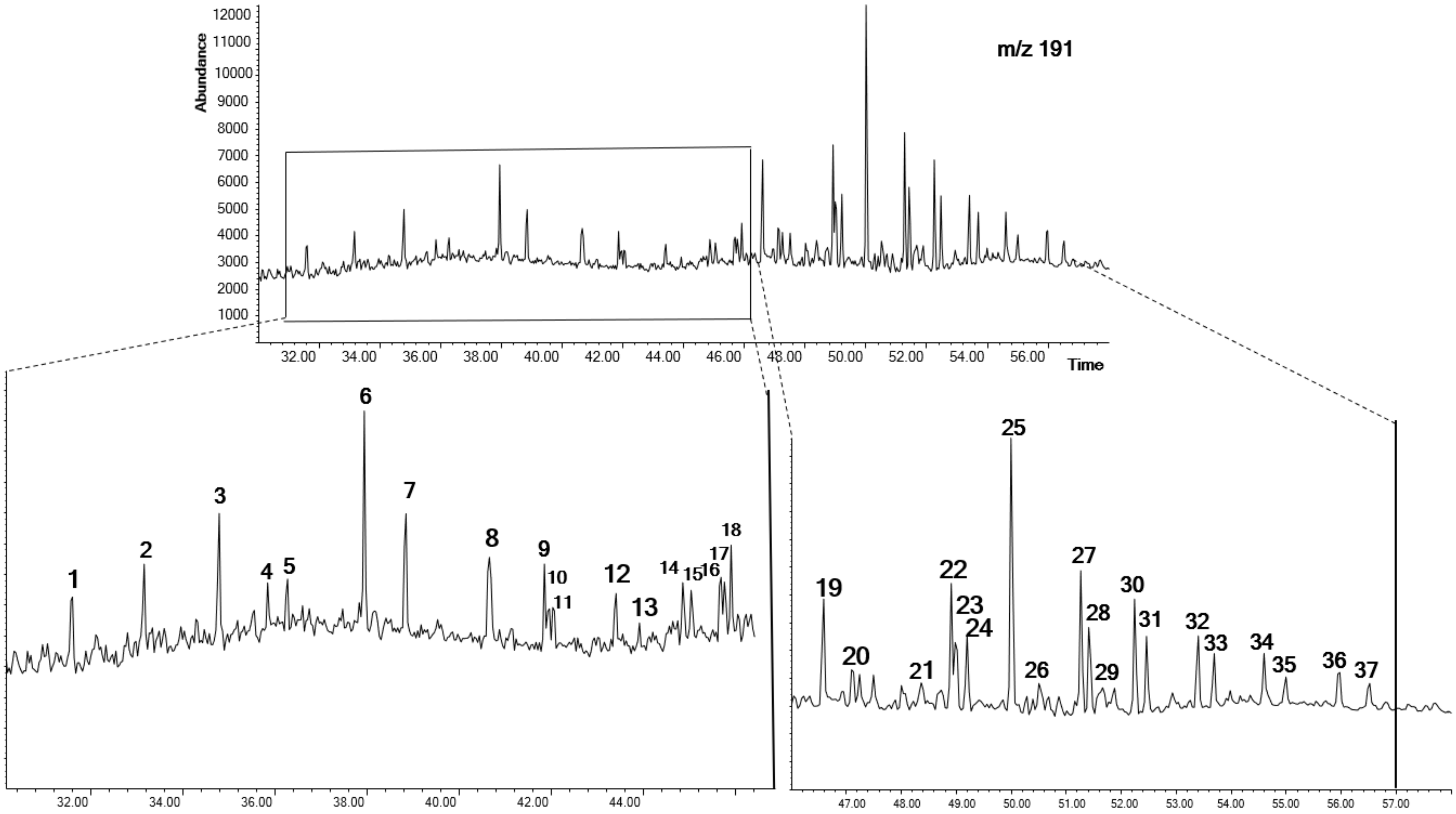


Figure 0.35: m/z 191 mass chromatograms showing terpanes distribution from non-biodegraded North Sea oil. The numbers represent the corresponding peak names listed in Table 0.21. The letters represent unidentified peaks.

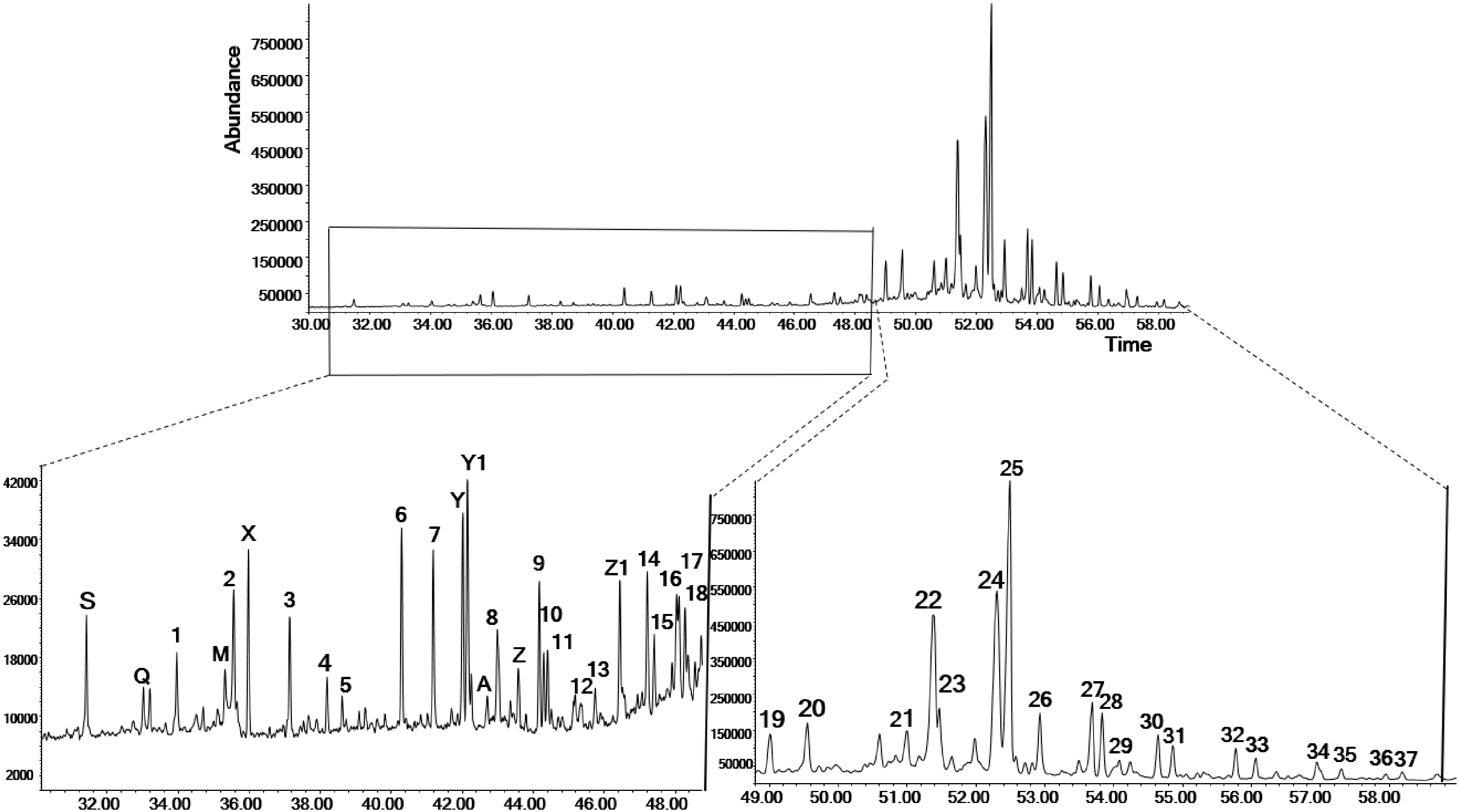


Figure 0.36: m/z 191 mass chromatograms showing terpanes distribution from biodegraded North Sea oil (5 days) added to Nigerian sediment. The numbers represent the corresponding peak names listed in Table 0.24. The letters represent unidentified peak.

chapters 3 (section 3.4.6) and 4 (section 4.4.3), the peak numbers 1, 2, and 4, labelled as S, Q, and M are the unidentified peaks observed in the two Nigerian oils within the tricyclic terpanes elution times, while peak number 24 labelled T was observed in the three oil samples within the tetracyclic terpanes elution times (see Figure 0.33 and Table 0.19). Further verification of these unidentified peaks remains the future focus of this work and their diagnostic ratios are analysed together with that of the known terpanes to also assess the effect of biodegradation on them as well as their diagnostic potentials. Whereas peak numbers 6, 11, 12 and 15 labelled as X, Y, Y1, and Z are tricyclic terpanes and 19 labelled Z1 is tetracyclic terpane are novel terpanes reported by Samuel et al, (2010) as discussed in chapters 3 (section 3.4.6) and 4 (section 4.4.2) and this chapter investigates the effect of biodegradation on these terpanes to assess their potentials as 'source-specific marker' compounds (Seifert and Moldowan, 1979; Wang *et al.*, 1999; Wang *et al.*, 2006) . Unidentified peaks were also observed in the North Sea oil with peaks numbers 5, 12, 13 and 18 labelled as U, U1, and U2 (see Table 0.21) respectively, subject to further verification. The effect of biodegradation on these compound classes was assessed using their diagnostic ratios for the three oil samples in Whitley and Nigerian sediments. However, higher degree of biodegradation was observed in the Whitley sediment than the Nigerian sediment in sections 5.4.1 and 5.4.2 above. The terpanes showed a similar trend where most of the terpanes concentrations decreased with degradation times for the Nigerian light oil in Whitley sediment (see Table 0.19) whereas the terpanes in Nigerian sediment (see Table 0.22) increased in concentrations with increased degradation time which is quite different from the terpanes concentrations obtained from evaporative and water washing weathering in chapters 3 and 4 respectively (e.g., Wang *et al.*, 1998). This is possible as it has been reported that hydrocarbons are preferentially attacked by microbial community during biodegradation (Connan, 1984a; Wenger *et al.*, 2001; Head *et al.*, 2003). This applies for the Nigerian medium and North Sea oils respectively.

Diagnostic ratio plots of C_{23}/C_{24},S/(S+Q), Q/(Q+C_{19}), M/(M+C_{19}), X/(X+C_{19}), C_{27}(Ts/Tm), Y/(Y+C_{24}), Y1/(Y1+C_{24}), Z/(Z+C_{25}B), Z_1/(Z_1+C_{28}A), T/(T+C_{29}B) and C_{31} 22S/(22S+22R)carried out (see Figure 0.37Figure 0.38) also showed a greater effect of biodegradation on some of the terpanes more than what was observed in evaporative and water washing weathering as the biodegradation plots are not as parallel .The effect is more on the samples in the Whitley sediment with C_{27}(Ts/Tm) in North Sea (Figure 0.37C) being the most affected followed by C_{23}/C_{24} ratio for all the oils after 300 days (Figure 0.37), this is an indication biodegradation, although generally moderate (PM Level 4) and the alteration is not much. Irregular trends observed from the beginning the plots are due to variable background concentrations from the sediment and the greatest effect of biodegradation is quite visible at 300 days. The samples in the Nigerian sediment were not so affected. The plots are observed to be nearly parallel (see Figure 0.38), which is an indication the terpanes were not affected or altered by biodegradation, and this could have been due to the long period of time the sediment was stored at room temperature and that could have affected the microbes. One unique observation is that the background of terpanes concentrations in the Nigerian sediment are quite similar to the concentrations in the two Nigerian oils, with the Nigerian light oil (Figure 0.38A) fitting perfectly well. The Nigerian sediment was taken from an oil impacted side and this could have been oils that share the same or similar origin with the Nigerian light oil, however, a marked contrast of background concentrations is observed in the Nigerian sediment spiked with North Sea oil (Figure 0.38C) where peaks S, Q, M, X, Y, Y1, C_{25}A, Z, and Z1 are absent in the starting oil but present in the in the sediment about the same amount. The terpanes, can be said to be slightly affected by the degree of biodegradation undergone in this experiment as terpanes have been reported to be resistant to degradation (e.g., Reed, 1977; Seifert and Moldowan, 1979).

Figure 0.39 shows the m/z 217 mass chromatograms of steranes with the peaks numbered and the measured concentrations and the corresponding peak names shown in Table 0.25Table 0.26,Table 0.27 for the three oils in Whitley sediment, and Table 0.28Table 0.29Table 0.30 for the oils in Nigerian sediment respectively. It is observed that while the steranes in the Whitley sediment were slightly affected preferentially by biodegradation as concentrations decreased slightly with degradation time with Nigerian medium oil being the least affected (see Table 0.26), the steranes in the Nigerian sediment are unaffected by biodegradation as concentrations increased from the start and began to decreased after 50 days degradation period (see Table 0.28Table 0.29Table 0.30). Figure 0.40Figure 0.41 show the diagnostic ratio plots of the steranes in the three oils in Whitley and Nigerian sediments which include C_{20}/C_{21}, C_{21}/C_{22}, C_{27} αββ/C_{29}αββ, C_{27} 20S/(20S+20R), C_{28} αββ/(αββ+ααα), C_{29} αββ/(αββ+ααα), and C_{27}αββ(S+R)/C28αββ(S+R) + C_{29}αββ(S+R) which were also used to analyse the effect of evaporation and water washing in chapters 3 and 4 respectively. These ratios are observed to be much more affected by biodegradation than by evaporation and water washing as discussed in chapters 3 and 4 respectively. The ratios variation for the oils in the Whitley sediment is quite greater than the oils in the Nigeria n sediment owing the to the lesser degree of biodegradation observed in the Nigerian sediment. The C_{21}/C_{22} ratio is noted to be slightly affected by evaporation, water washing and slightly severe by biodegradation, whereas ratio C_{27}αββ/C_{29}αββ is observed as the most affected by biodegradation in both Whitley and Nigerian sediments, and C_{27}αββ/C_{29}αββ for the Nigerian light oil is also observed to be slightly affected by water washing, as C_{21}- C_{22} steranes and C_{27}-C_{29} diasteranes are the most susceptible of this class to biodegradation (e.g., Connan, 1984a). Generally, biodegradation effect is observed to be more pronounced on steranes than terpanes (Seifert and Moldowan, 1979; Connan, 1984a) albeit this depends on the specific biodegradation conditions (Wenger *et al.*, 2001; Jones *et al.*, 2008).

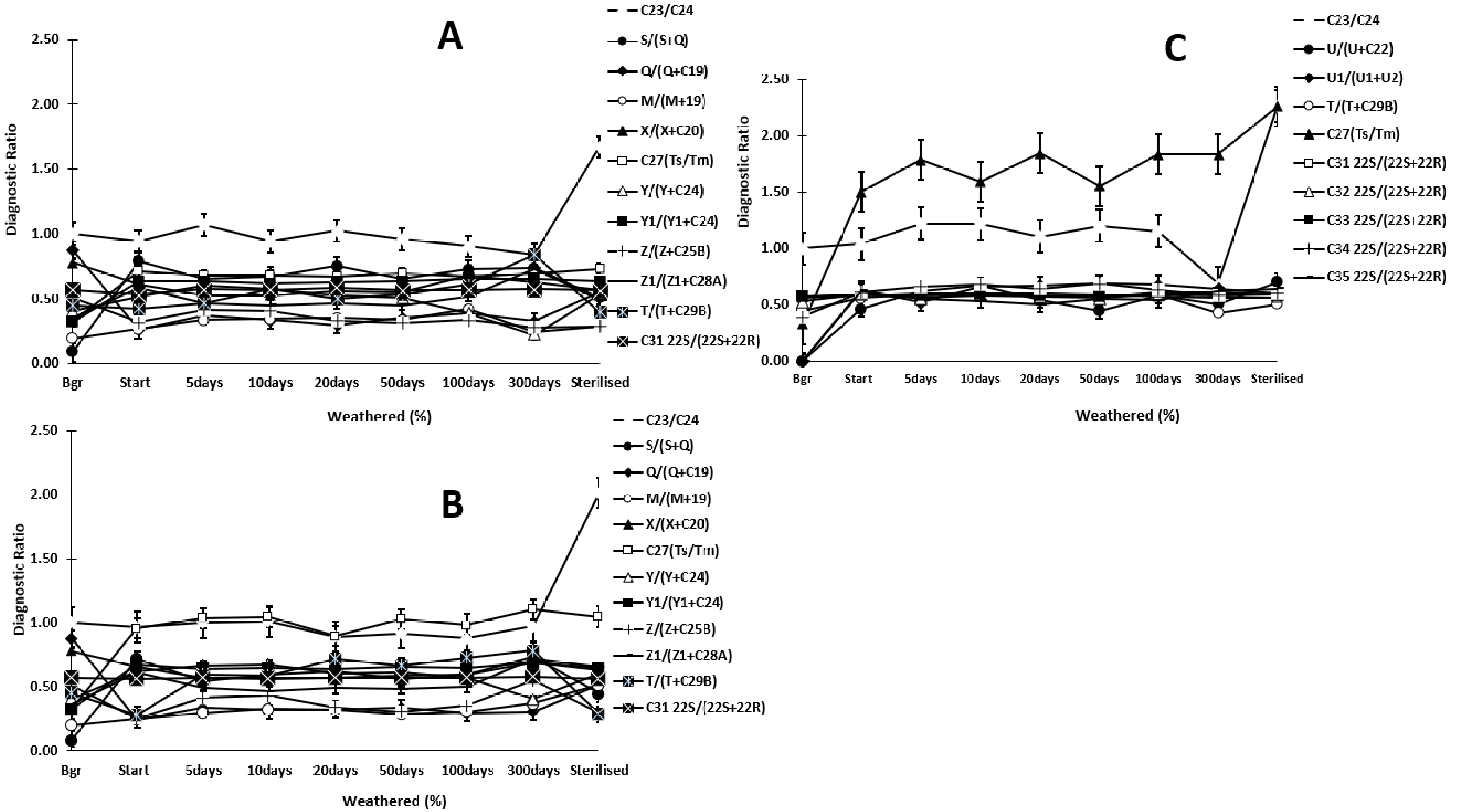


Figure 0.37: Plots of diagnostic ratios of selected terpanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Whitley sediment.

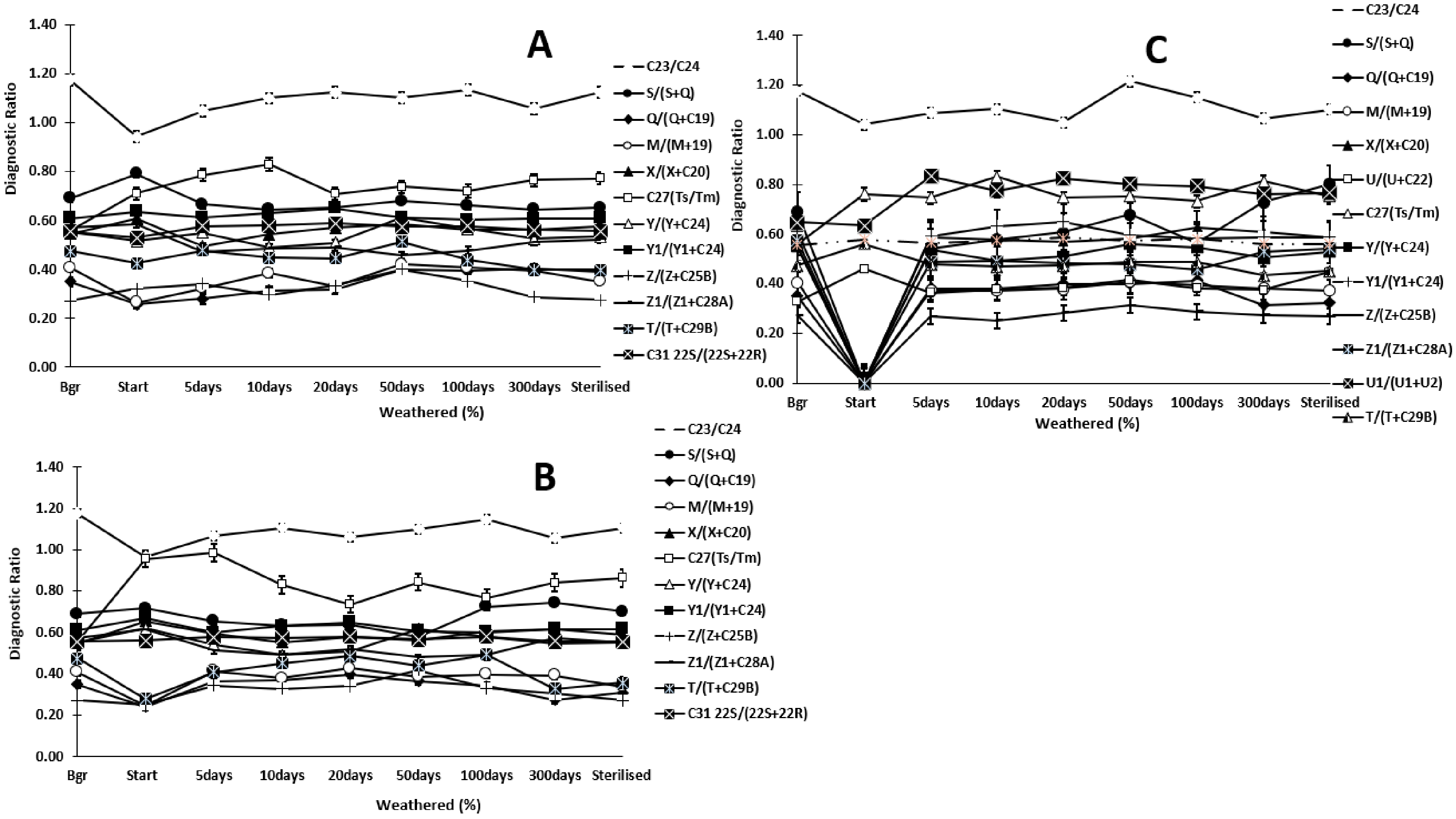


Figure 0.38: Plots of diagnostic ratios of selected terpanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Nigerian sediment.

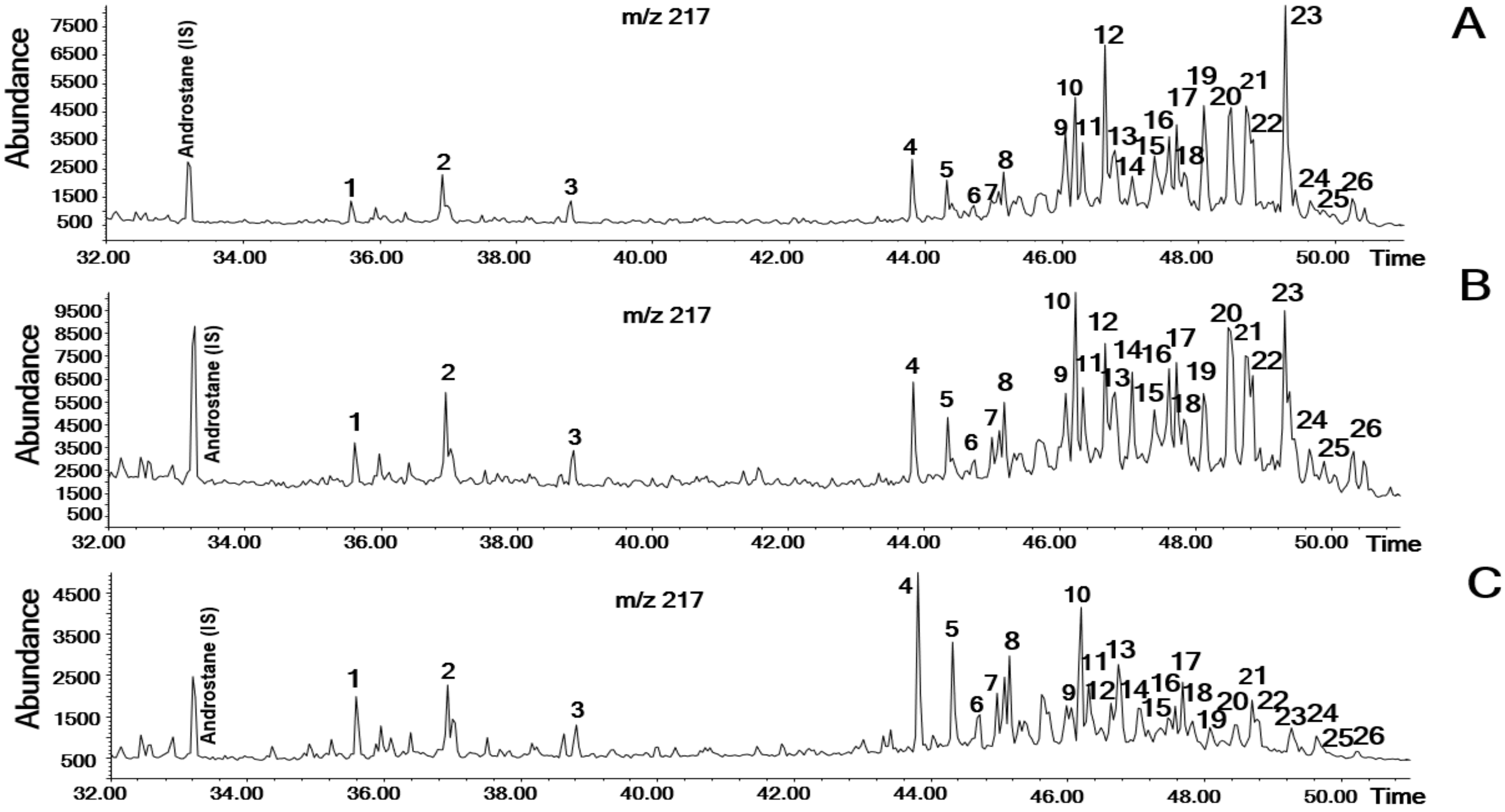


Figure 0.39: m/z 217 mass chromatograms showing the distributions of steranes in non-biodegraded Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. Numbers in (A) represent the corresponding peak names in Table 0.25Table 0.28, numbers in (B) represent peak names in Table 0.26Table 0.29, and numbers in (C) represent peak names in Table 0.27Table 0.30 respectively.

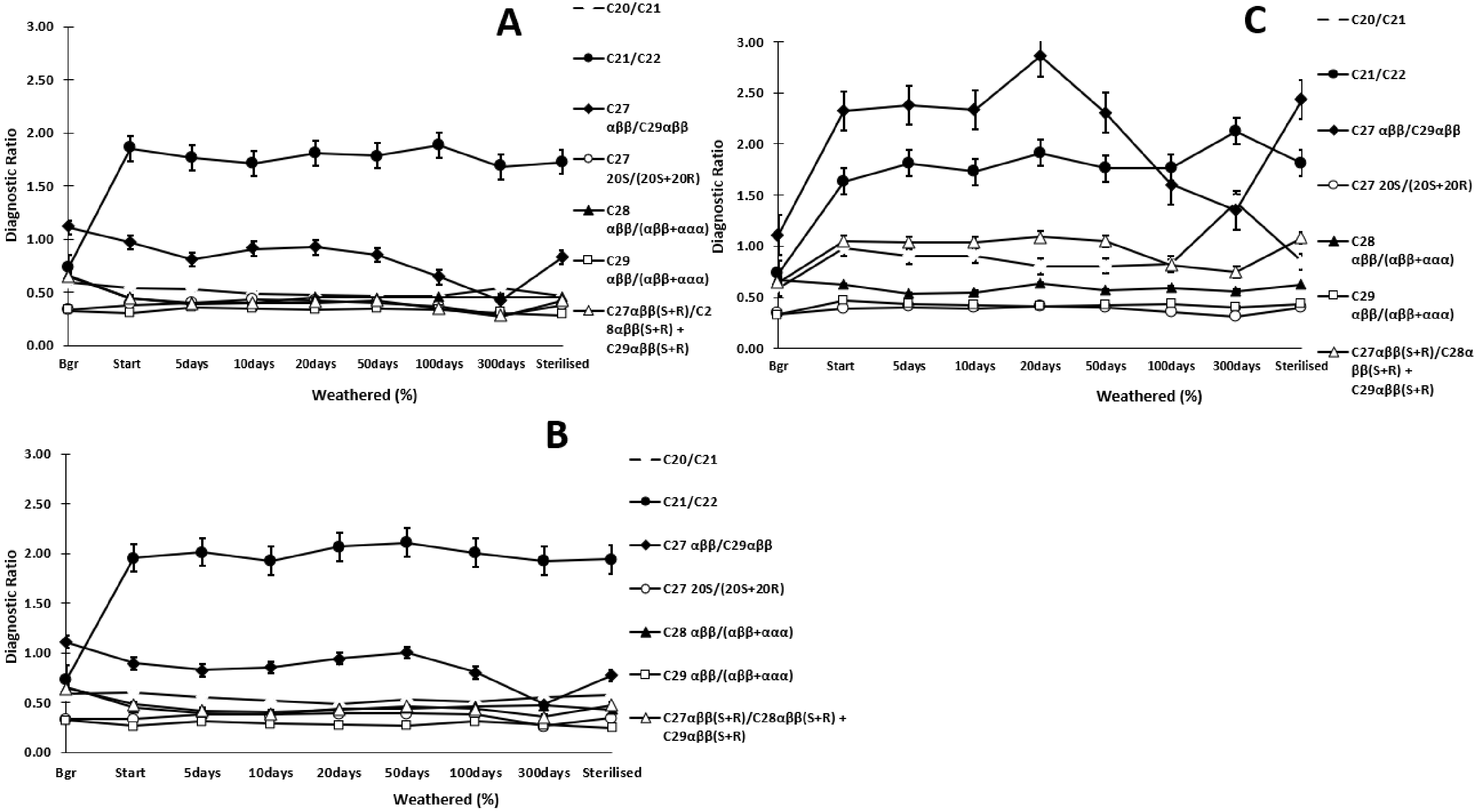


Figure 0.40: Plots of diagnostic ratios of selected steranes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Whitley sediment.

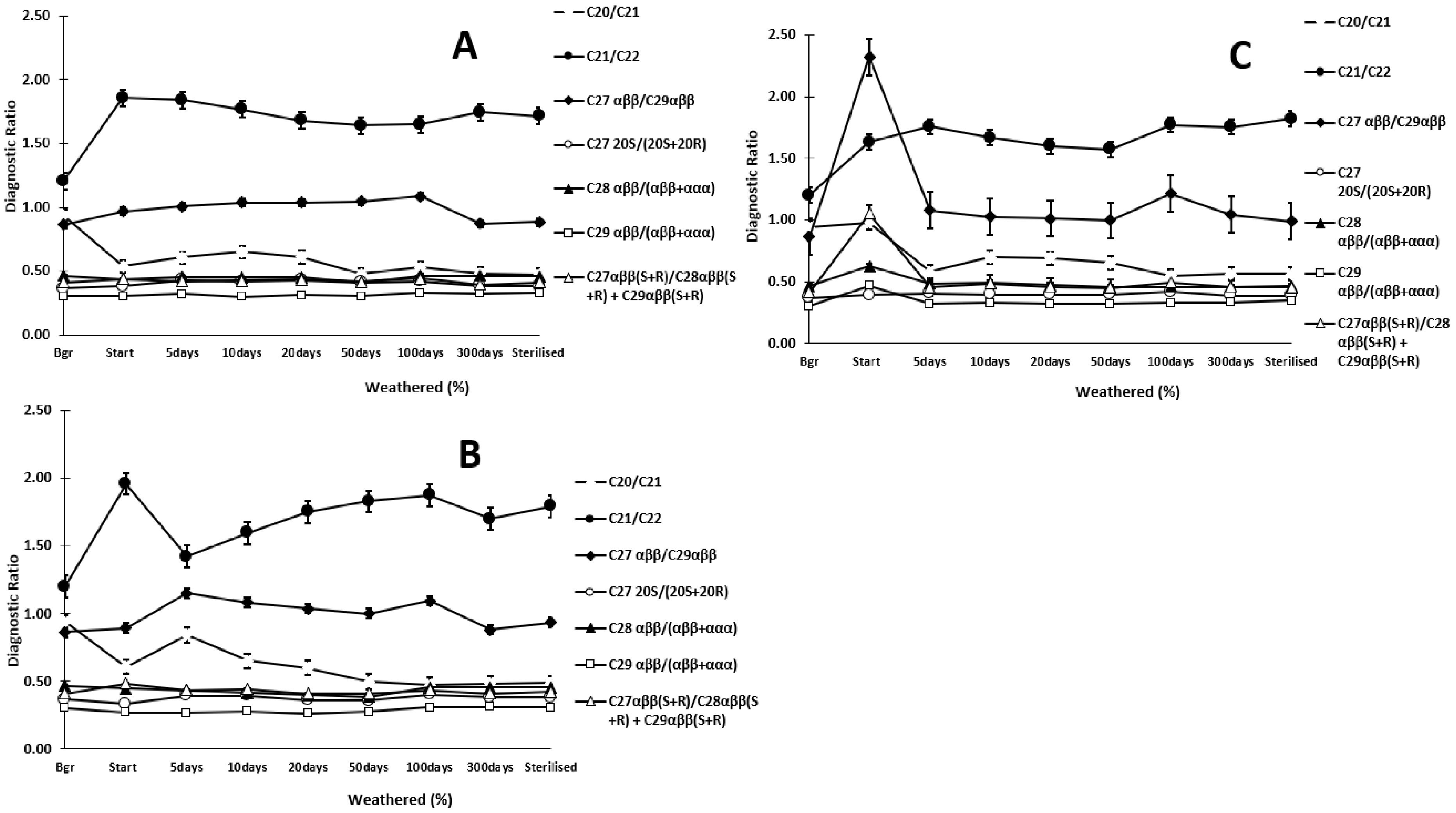


Figure 0.41: Pots of diagnostic ratios of selected steranes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Nigerian sediment.

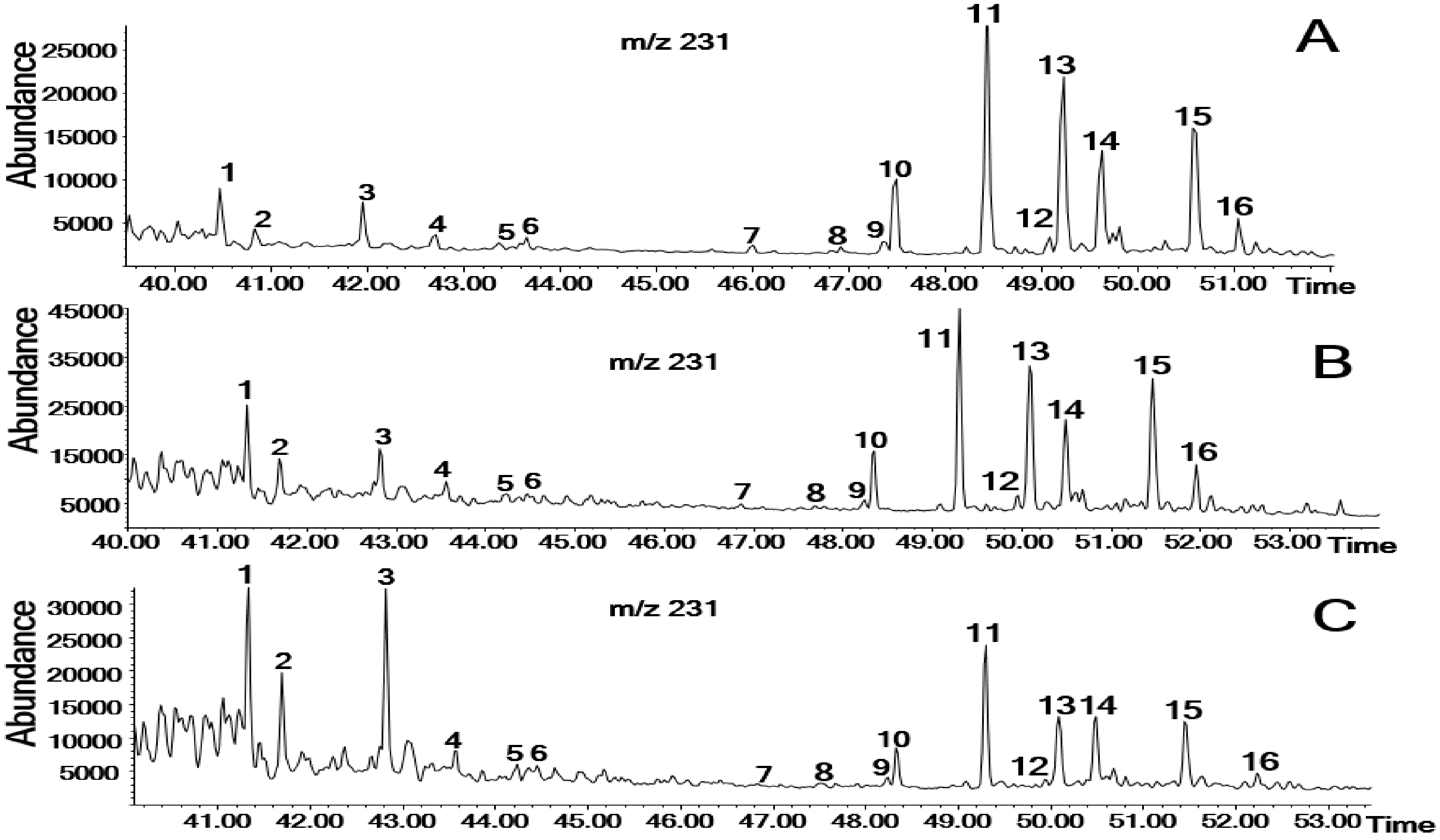


Figure 0.42: m/z 231 mass chromatograms showing the distributions of triaromatic steranes in non-biodegraded Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. Numbers in (A) represent the corresponding peak names in Table 0.31Table 0.34, numbers in (B) represent peak names in Table 0.32Table 0.35, and numbers in (C) represent peak names in Table 0.33Table 0.36 respectively. Numbers in red represent unidentified peaks.

Triaromatic Steranes (TAS) were also analysed and Figure 0.42 shows the m/z 231 mass chromatograms distribution of triaromatic steranes with the numbered peaks and the measured concentrations and the corresponding peak names are shown in Table 0.31Table 0.32Table 0.33 for the three oils in Whitley sediment and Table 0.34Table 0.35, andTable 0.36 for the three oils in Nigerian sediment respectively. P, Q, R, S, T, U, and V are unidentified peaks considered with the known triaromatic steranes to assess their potential for use as markers for source correlations. The effect of biodegradation on triaromatic steranes is similar to that of steranes earlier discussed in this chapter, as the concentrations decreased with increased degradation time for all the three oils in Whitley sediment (see Table 0.31Table 0.32Table 0.33) while the concentrations of the triaromatic steranes (see Table 0.34Table 0.35Table 0.36) for the three oils in Nigerian sediment increased with degradation time respectively. Figure 0.43Figure 0.44 show the diagnostic ratio plots of the triaromatic steranes versus degradation time for the three oils in Whitley and Nigerian sediments. Triaromatic steranes are aromatised steroids which are known to be unaffected by the most severe oil biodegradation conditions of PM level 10 and this makes them suitable biomarkers for correlation and maturity assessment (Peters *et al.*, 2005; Yang *et al.*, 2013; Xu *et al.*, 2018). The three ratios C_{20}/C_{21}, P/C_{21}, and Q/C_{21} are the quite affected by biodegradation for all the three oils in both the Whitley and Nigerian sediments while the other ratios not so affected, possibly owing to the susceptibility of C_{20} and C_{21} TAS, and P and Q would be subject to molecular identification to ascertain their properties to help draw conclusion regarding their susceptibilities. Similarly, ratios C_{20}/C_{21}, P/C_{21}, and $Q/C_{21,}$ and were also affected by evaporation, and ratio $C_{28}(R)/C_{28}(S)$ also affected by evaporation for the two Nigerian oils. However, these ratios were unaffected by water washing for the two Nigerian oils, but ratios for the North Sea oil were affected as discussed in chapter 4. This perhaps could be an indication that unidentified peaks P and Q may be unsuitable for use as diagnostic compounds.

Bicyclic sesquiterpanes were also analysed and their m/z 123 mass chromatograms distributions are shown in Figure 0.45 Figure 0.46 with the peaks numbered, and the measured concentrations with the corresponding peak names shown in Table 0.37Table 0.38Table 0.39 for the oils in Whitley sediment, and Table 0.40Table 0.41Table 0.42 for the oils in the Nigerian sediment respectively. More details about the peaks are as discussed in sections 3.4.6 and 4.4.2 in chapters 3 and 4 respectively. The sesquiterpanes are generally affect by biodegradation as the concentrations steadily reduces with degradation time for both the Whitley and Nigerian sediments. Figure 0.47Figure 0.48 show the diagnostic ratio plots of the sesquiterpanes versus degradation time for the three oils in Whitley and Nigerian sediments. The ratios indicate that the sesquiterpanes are affected by biodegradation than by evaporation and water washing discussed in chapters 3 and 4 respectively, with those in Whitley sediment being the more affected than those in Nigeria sediment. The North Sea oil in Whitley sediment (Figure 0.47C) is the most affected as ratios BS10/$C_{30}\alpha\beta$-hopane, BS3/$C_{30}\alpha\beta$-hopane, BS1/BS2 were noted to be quite variable and decreased with degradation unlike those of the two Nigerian oils (Figure 0.47A & B) which are low and parallel. However, ratio BSA/BS2 for the two Nigerian oils was affected at 100 days and this was also the case with BSY/BS2 in the Nigerian medium oil. The sesquiterpanes in the Nigerian sediment were relatively unaffected except for ratio BSA/BS2 in the North Sea oil (Figure 0.48C).

Adamantanes were also analysed and their m/z 136 mass chromatograms distributions are shown in Figure 0.49 with the peaks numbered, and the measured concentrations with the corresponding peak names shown in Table 0.43Table 0.44Table 0.45 for the oils in Whitley sediment, and Table 0.46Table 0.47Table 0.48 for the oils in the Nigerian sediment respectively. Figure 0.50Figure 0.51 show the diagnostic ratio plots of the adamantanes versus degradation time for the three oils in Whitley and Nigerian sediments. Adamantanes are the most affected by biodegradation in both the Whitley and Nigerian sediments as most adamantanes were completely degraded after 20 days in the Whitley sediment which confirms the degree of biodegradation was higher in the Whitley sediment than the Nigerian sediment as stated earlier in this chapter. The ratios in the two sediments were quite altered except for 1-MA/(1-MA+2-MA) and 1-MA/(1-MA+2-MA+2-AE) which were not so affected.

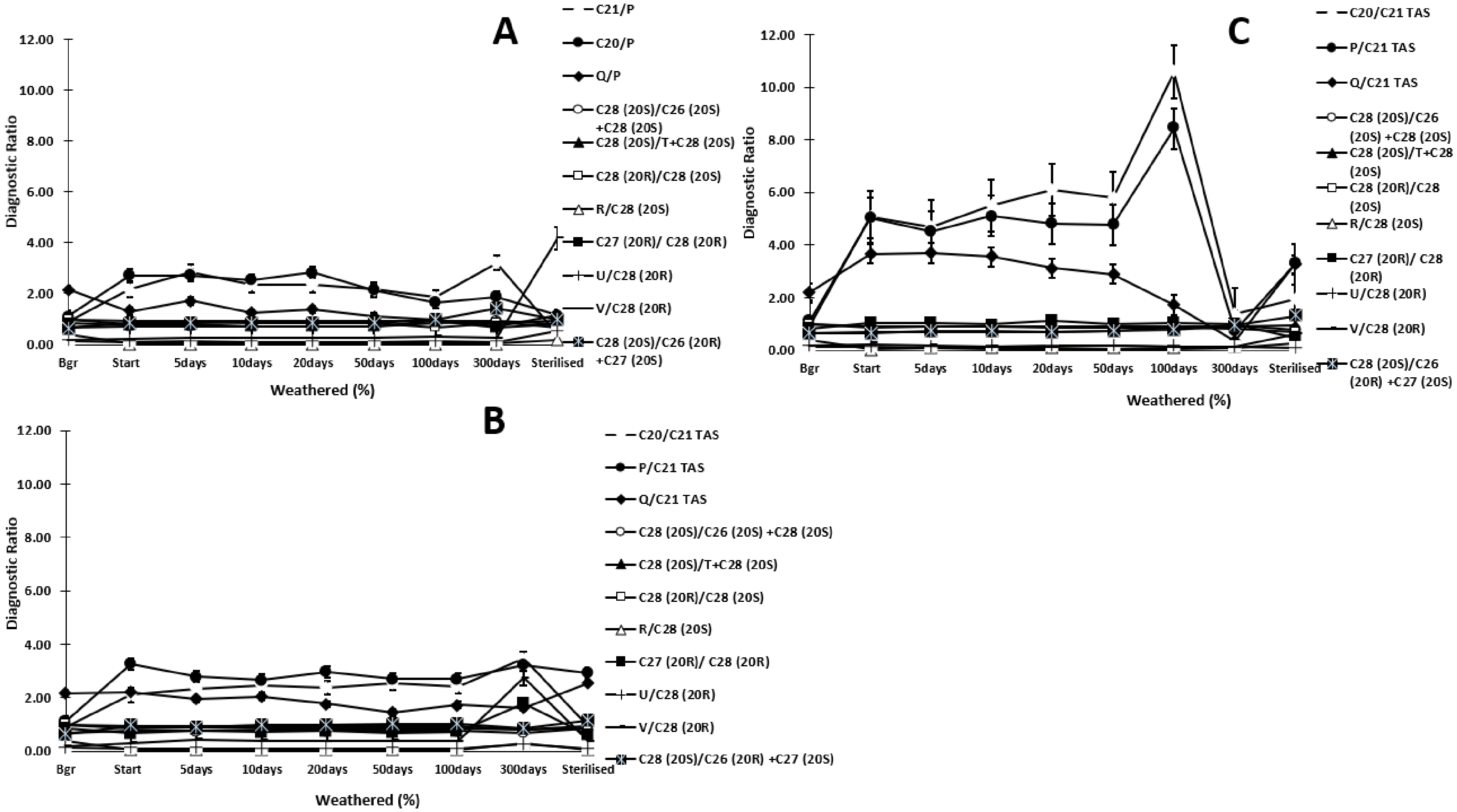


Figure 0.43: Pots of diagnostic ratios of selected triaromatic steranes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Whitley sediment.

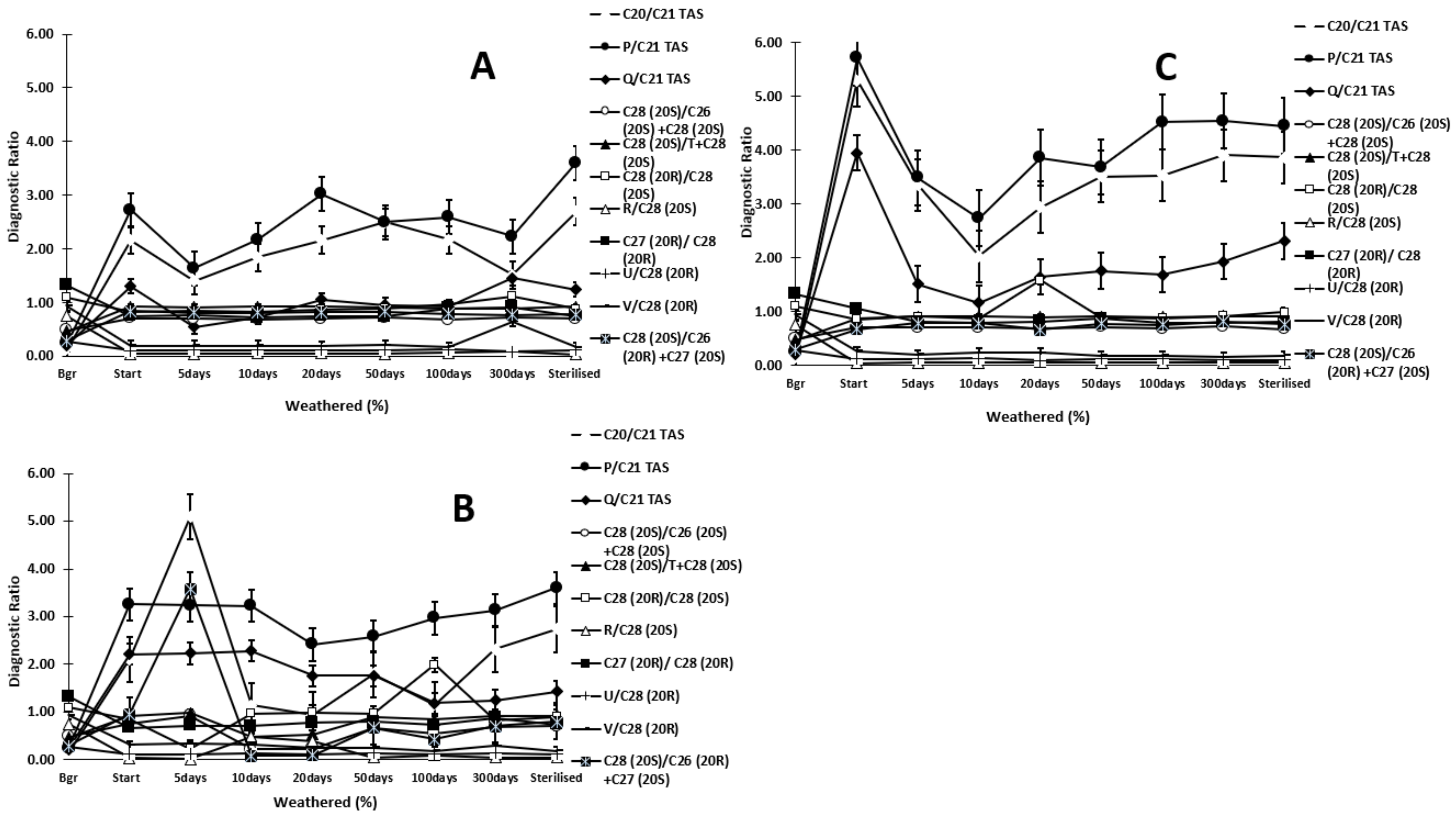


Figure 0.44: Pots of diagnostic ratios of selected triaromatic steranes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Nigerian sediment.

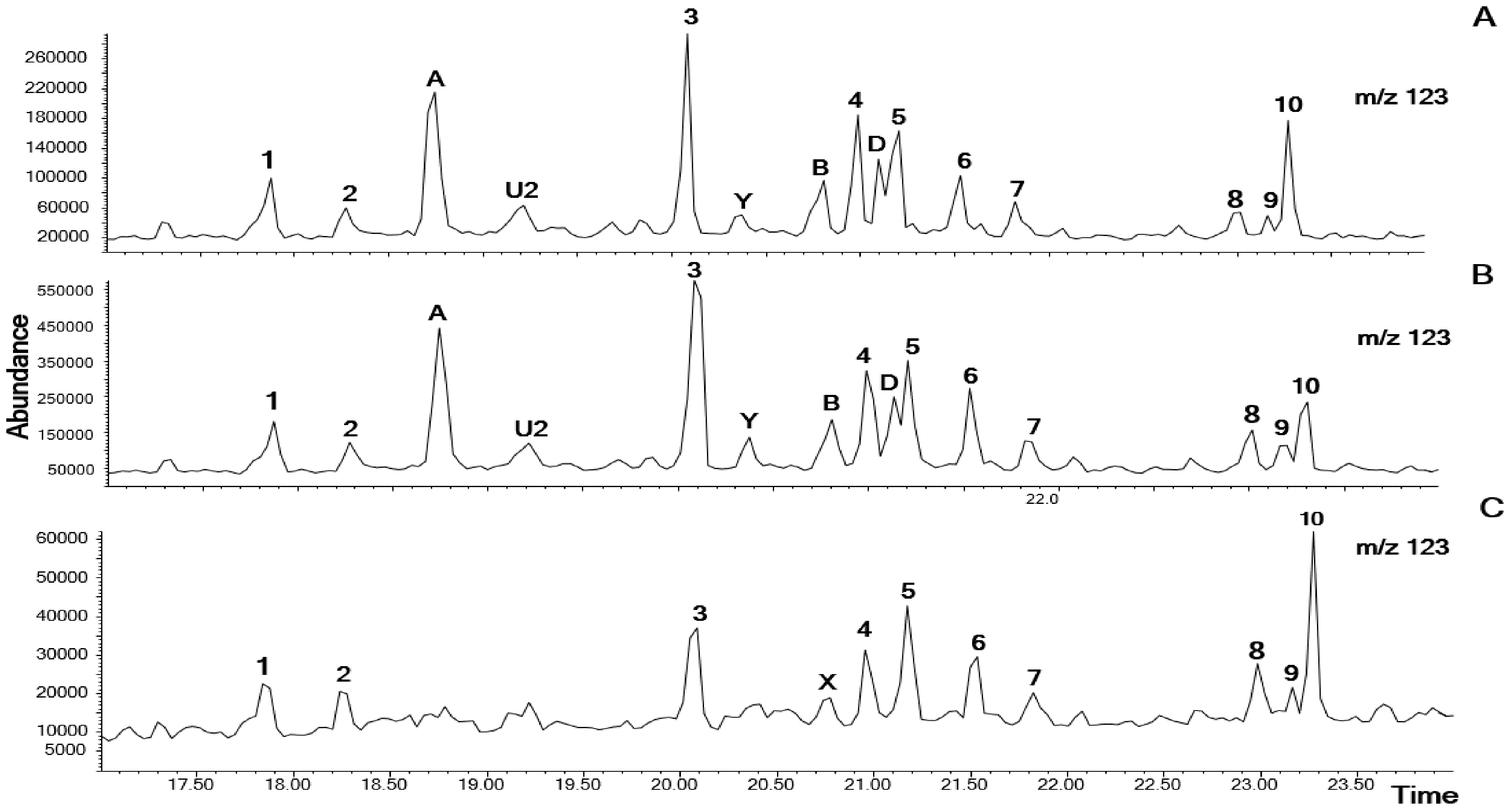


Figure 0.45: m/z 123 mass chromatograms showing the distributions of bicyclic sesquiterpanes in non-biodegraded Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. A, U2, B, and D, are novel sesquiterpanes observed in Nigerian Light and Medium oils. Numbers in (A) represent the peak names in Table 0.37, numbers in (B) represent peak names in Table 0.38, and numbers in (C) represent peak names in Table 0.39 respectively.

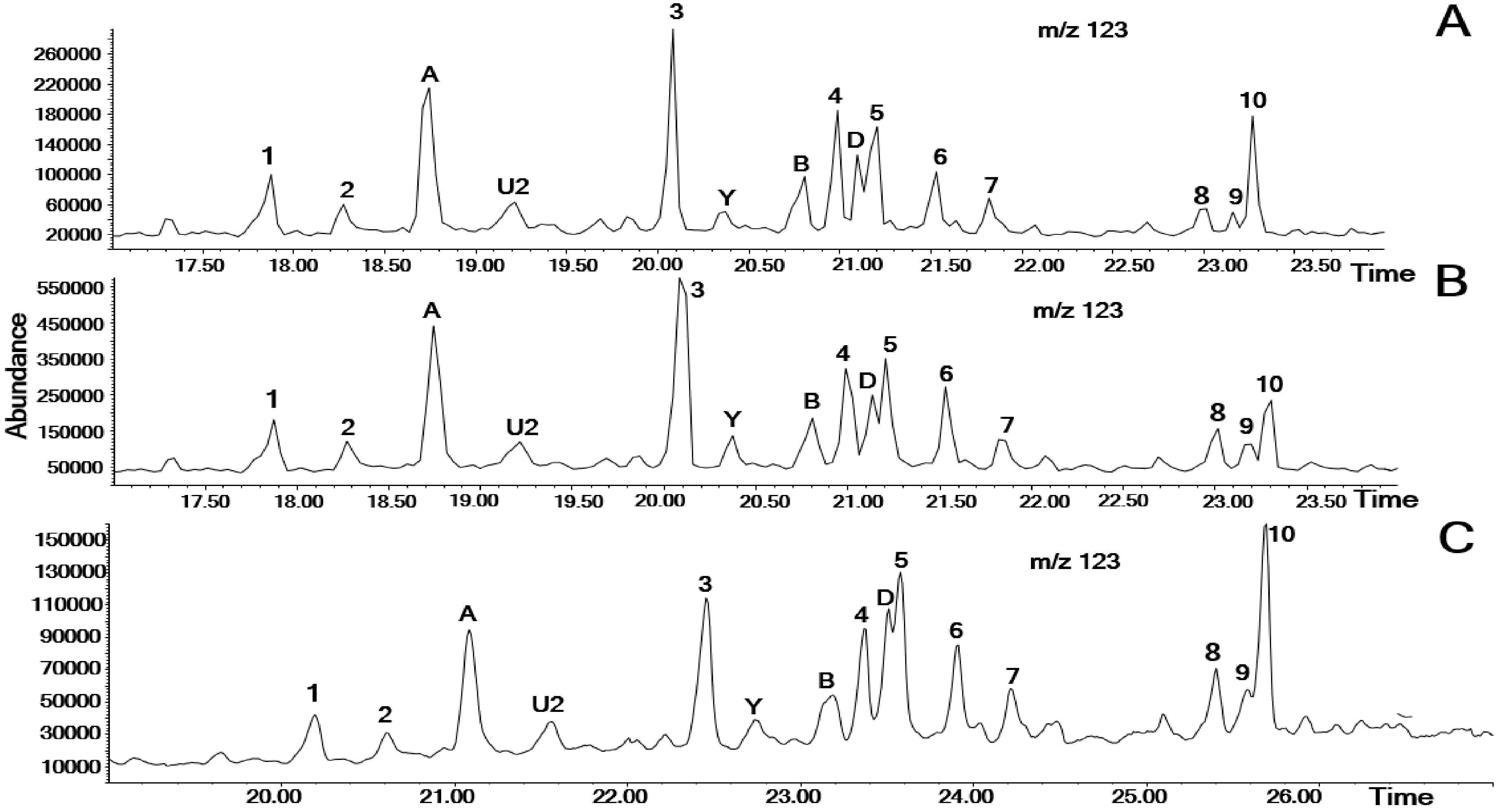


Figure 0.46: m/z 123 mass chromatograms showing the distributions of bicyclic sesquiterpanes in biodegraded Nigerian light (A), Nigerian medium (B), and North Sea (C) oils in Nigerian sediment. Numbers in (A) represent the peak names in Table 0.40, numbers in (B) represent peak names in Table 0.41, and numbers in (C) represent peak names in Table 0.42 respectively.

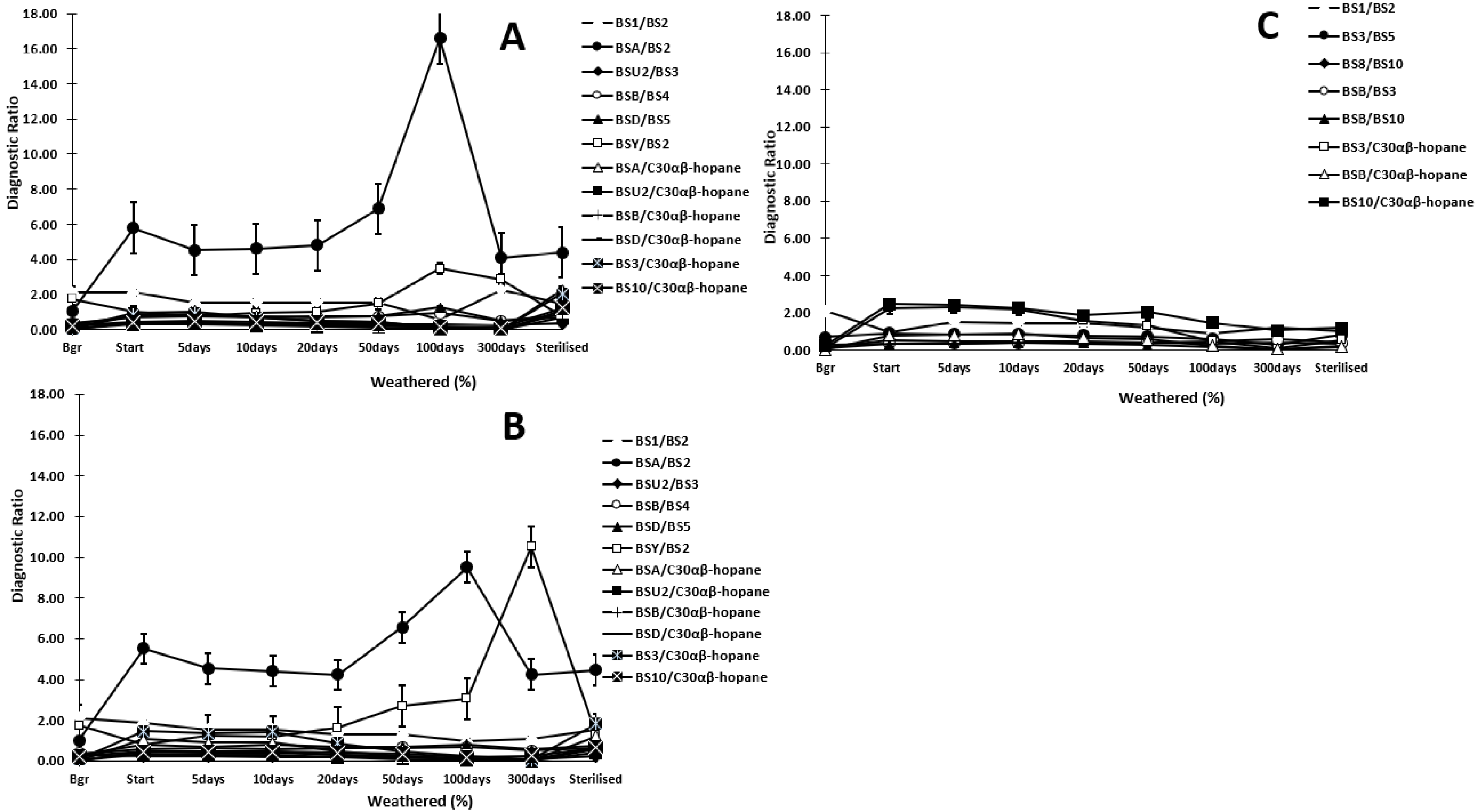


Figure 0.47: Pots of diagnostic ratios of selected bicyclic sesquiterpanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Whitley sediment.

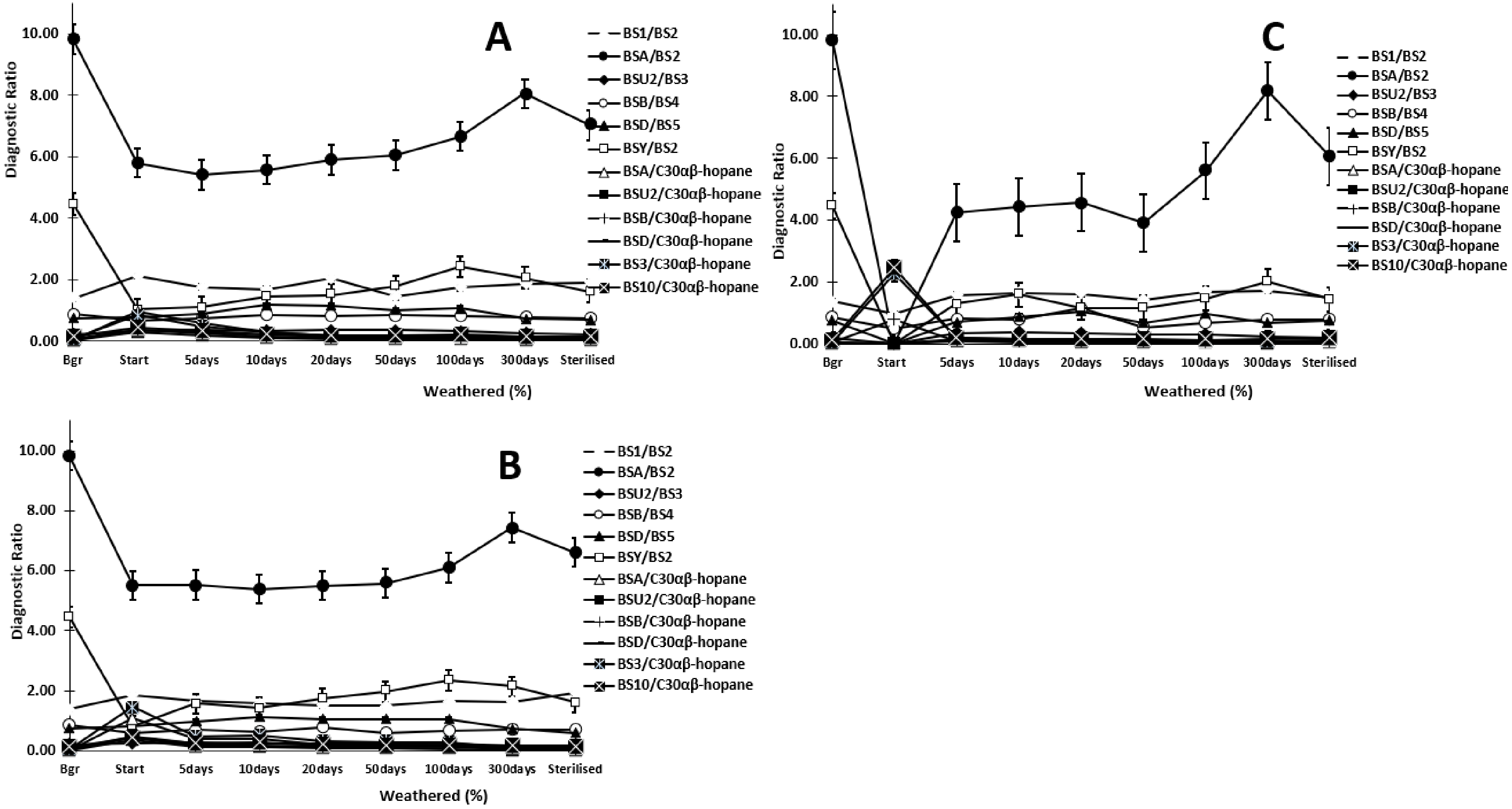


Figure 0.48: Pots of diagnostic ratios of selected bicyclic sesquiterpanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Nigerian sediment.

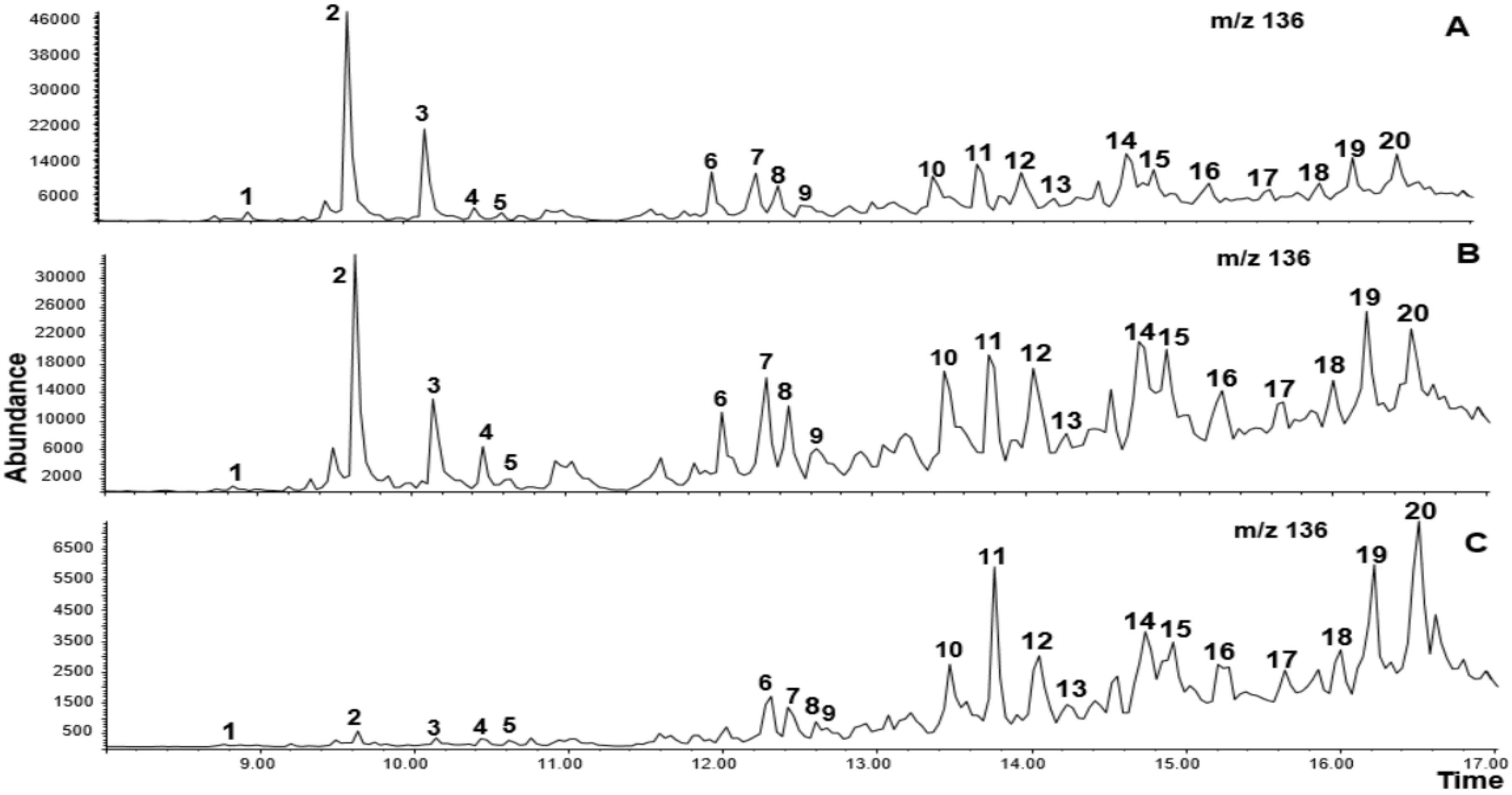


Figure 0.49: m/z 136 mass chromatograms showing the distributions of adamantanes in non-biodegraded; (A) Nigerian light oil; (B) Nigerian medium; and (C) North Sea oils. Numbers in (A) represent the corresponding peak names in Table 0.43Table 0.46, numbers in (B) represent peak names in Table 0.44Table 0.47, and numbers in (C) represent peak names in Table 0.45Table 0.48 respectively

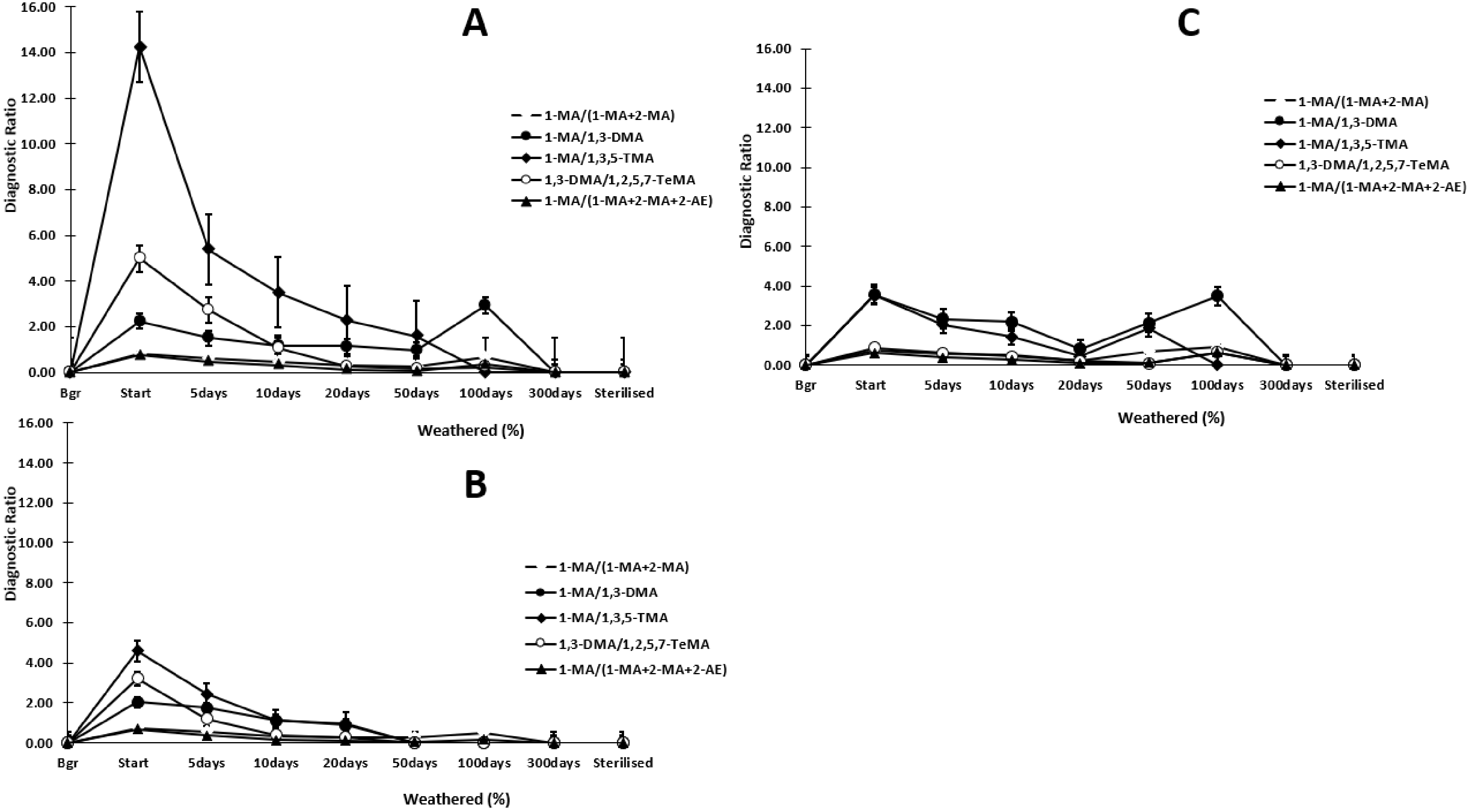


Figure 0.50: Pots of diagnostic ratios of selected Adamantanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Whitley sediment.

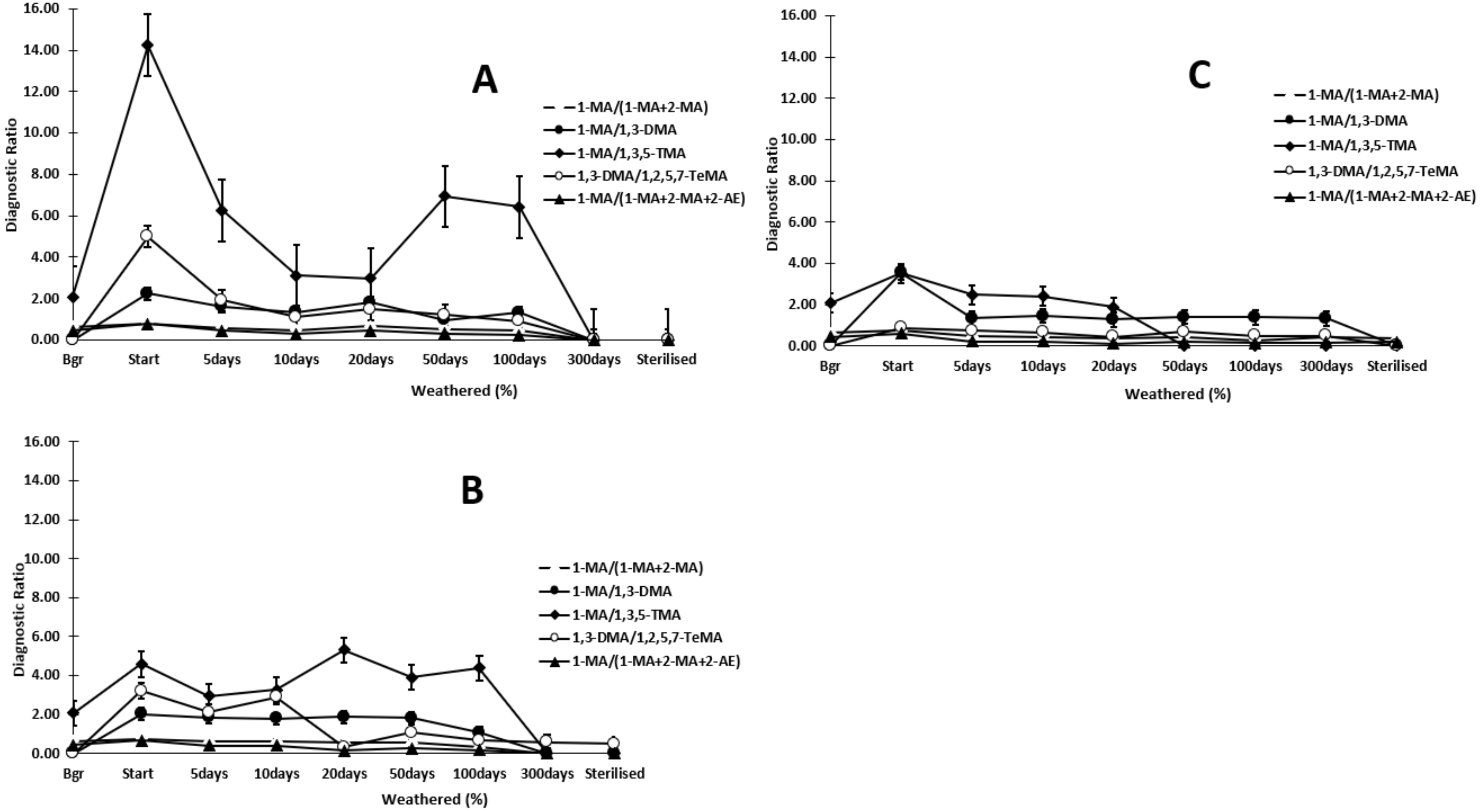


Figure 0.51: Pots of diagnostic ratios of selected Adamantanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Nigeria sediment

5.4.4 Relative susceptibility

Relative susceptibility to biodegradation of some selected aliphatic and a PAHs based on their relatively elution order was assessed using their ratios (see Figure 0.52, and Figure 0.53) for the three oils in the two sediments. The ratios indicate a greater relative susceptibility due to the effect of biodegradation on selected compounds much more than the effect of evaporation (section 3.4.7) and water washing (section 4.4.4) which are quite unaffected as discussed in chapters 3 and 4 respectively. The plots of these selected compounds of Whitley Bay sediment were observed to be more affected by biodegradation than those of the Nigerian sediment after 100 days of incubation. But the Nigerian light oil for the Whitley Bay sediment (see Figure 0.52A) has three of the ratio plots are affected at 50 days except the C_{30} Olean/*n*-C_{31} ratio plot. The North Sea oil ratio plots (see Figure 0.52C) are the least affected by biodegradation.

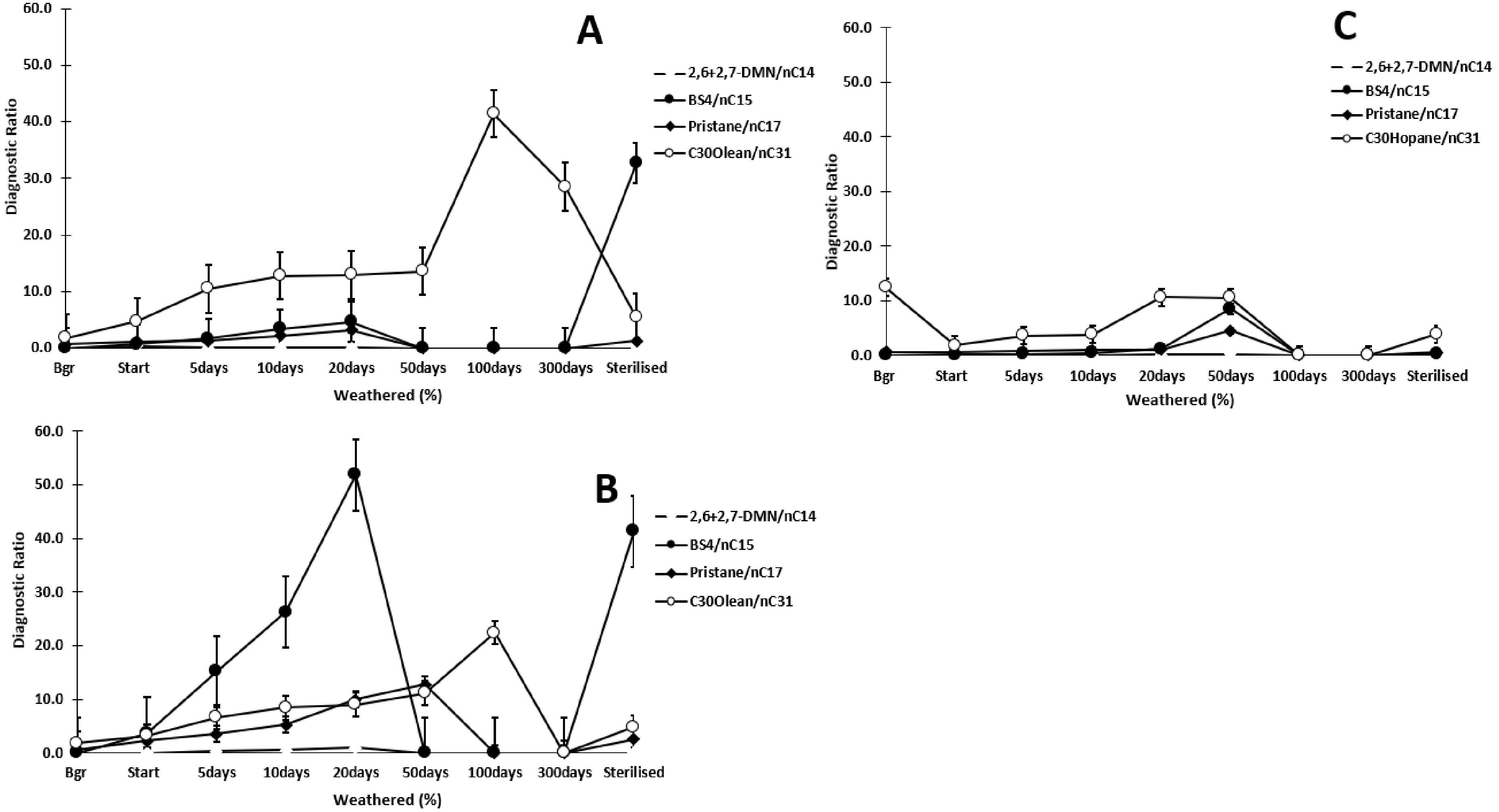


Figure 0.52: Ratios of selected aliphatics/PAH for (A) Nigerian light, (B) Nigerian medium, and (C) North Sea oils in Whitley sediment

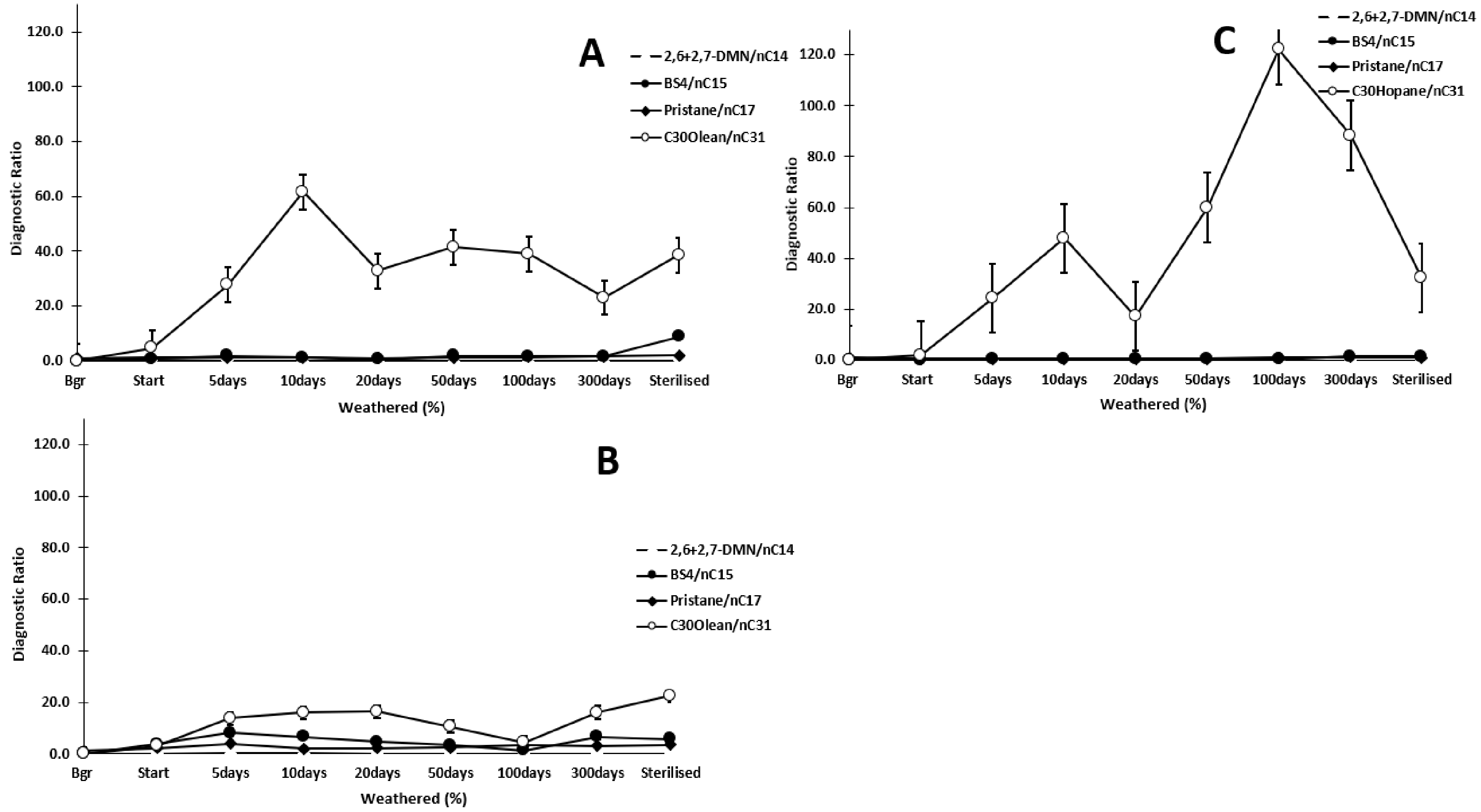


Figure 0.53: Ratios of selected aliphatics/PAH for (A) Nigerian light, (B) Nigerian medium, and (C) North Sea oils in Nigerian sediment.

5.4.5 Diagnostic Ratio plots of selected Nordtest parameters

The oils in the aerobic biodegradation microcosms using the Whitley Bay sediment were observed to be the most affected by biodegradation of all the weathering experiments conducted in this work where degradation was up to PM level 4 (moderate to heavy) after 300 days incubation (see section 5.4.1) based on the *n*-alkanes and PM level 6-10 based on dibenzothiophenes and phenanthrenes. The Whitley Bay sediment has the least background hydrocarbon concentration and an average extractable organic matter (EOM) of 6.5mg, followed by the Tyne sediment with an average of 40.7mg EOM and Nigerian sediment with average of 98.4mg EOM from triplicate 10g sediment (dry weight) samples, respectively. Hence, the effect of high background concentration on the efficiency of the correlation (Daling *et al.*, 2002b; Stout *et al.*, 2005) is minimised in Whitley Bay microcosms. Some selected Nordtest diagnostic parameters of terpanes, regular steranes and diasteranes, triaromatic steroids and PAHs in the three oils, including the novel tricyclic and tetracyclic terpanes, and novel bicyclic sesquiterpanes identified in the two Nigerian oils were plotted using the "Student's t test" statistical tool. The student's t test is defined by its mean value, $\overline{x}$ as the center of the distribution, and the standard deviation, s signifying the spread of the individual observations around the mean. The variability of the triplicate values of the degraded oil diagnostic ratios is calculated as relative variation at 95% confidence interval or confidence limit (CL) (Daling *et al.*, 2002b). Figure 0.54 shows the diagnostic ratio plots of Nigerian Light oil in the Whitley Bay sediment microcosms from 5 days to 50 days incubation with positive correlation at 95% confidence limit which shows that biodegradation has no effect on the biomarkerr used, while Figure 0.55 is the same microcosms incubated for 100 days, 300 days and the control with a negative correlation at 98% confidence limit which shows that the parameters were divergent from the linear regression x-y plots as PAHs and bicyclic sesquiterpanes are affected by biodegradation. The positive correlation is the same as the match correlation described in the Nordtest methodology whereas the negative correlation is the non-match correlation for the spill and suspected source comparison (Daling *et al.*, 2002a). The positive correlation within 95% confidence level is however observed to occur within 20 days of incubation for the Nigerian Medium (See Figure 0.56) and North Sea (See Figure 0.58) oils, while the negative correlation is observed from 50 days incubation for the Nigerian Medium (see Figure 0.57) and North Sea oils (see Figure 0.59) with a variation beyond the 98% confidence limit due to biodegradation effect on mostly the PAHs and the bicyclic sesquiterpanes, followed by the C_{21} pregnane. The novel tricyclic and tetracyclic terpanes identified in the two Nigerian oils were observed to be unaffected by the degradation like the selected Nordtest diagnostic parameters. The variability observed in the oil samples is as result of the effect of biodegradation on the selected diagnostic parameters similar to a previous work where diagnostic ratios of adamantanes were analysed in heavily weathered oil for 90, 180, 270, and 360 days respectively (Han *et al.*, 2019), but only the 90 days weathered samples showed positive correlation with the original oil. However, moderately weathered oils have been observed to unaffected with positive correlation in other studies (cf Daling *et al.*, 2002a; Faksness *et al.*, 2002a; Faksness *et al.*, 2002b; Daling *et al.*, 2004; Handbook, 2016), and even weathered oil in a riser was also found to give a positive correlation (Misselwitz *et al.*, 2013). Nordtest forensic studies were conducted on the Exxon Valdez spill in Prince William Sound and the Gulf of Alaska (USA) for over 25 years after the spill occurrence, where biodegradation is expected to dominate based on the time scale. It was found that the Alaska North Slope crude oil (ANSCO) that was spilled in 1989 matched the field data better than other correlated sources, with the selected biomarkers considered generally conserved (Carls *et al.*, 2016).

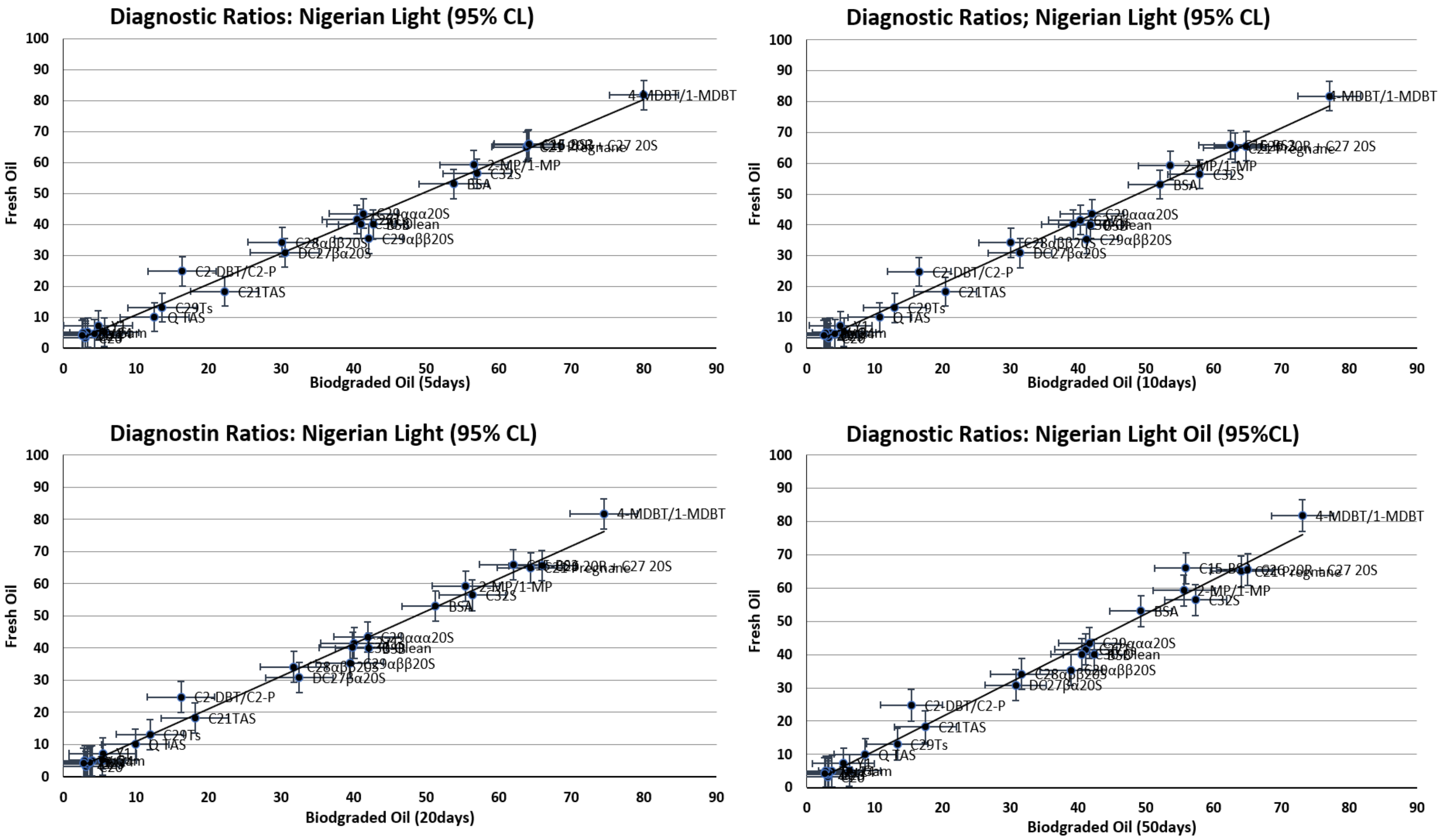


Figure 0.54: Diagnostic Ratio plots of fresh oil vs degraded oil Nigerian Light oil in Whitley Bay sediment, degraded oil showing positive correlation with the fresh oil up to 50 days incubation using 95% confidence limit.

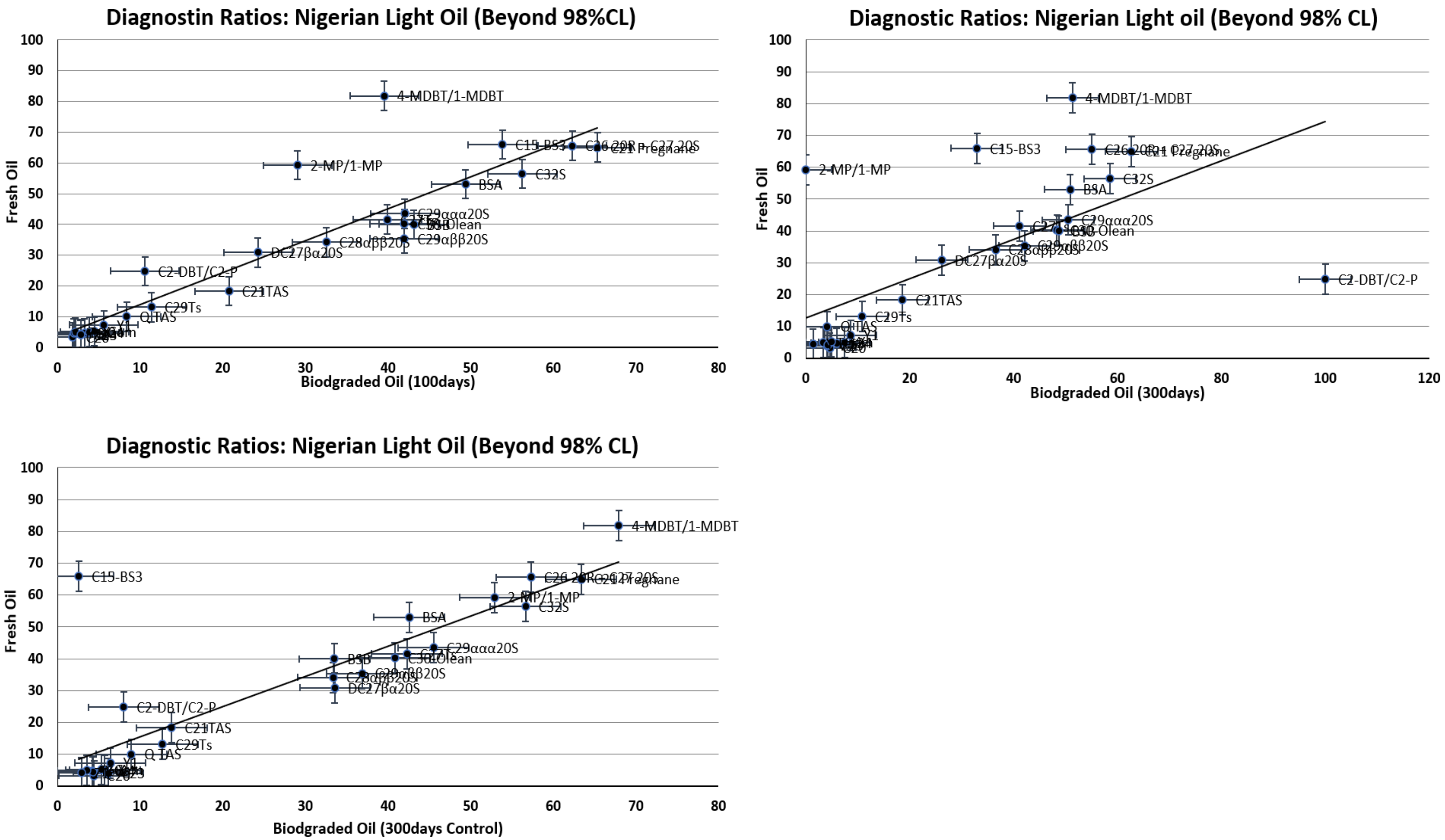


Figure 0.55: Diagnostic Ratio plots of fresh oil vs degraded oil Nigerian Light oil in Whitley Bay sediment, degraded oil showing negative correlation with the fresh oil from 100 – 300 days incubation using 98% confidence limit.

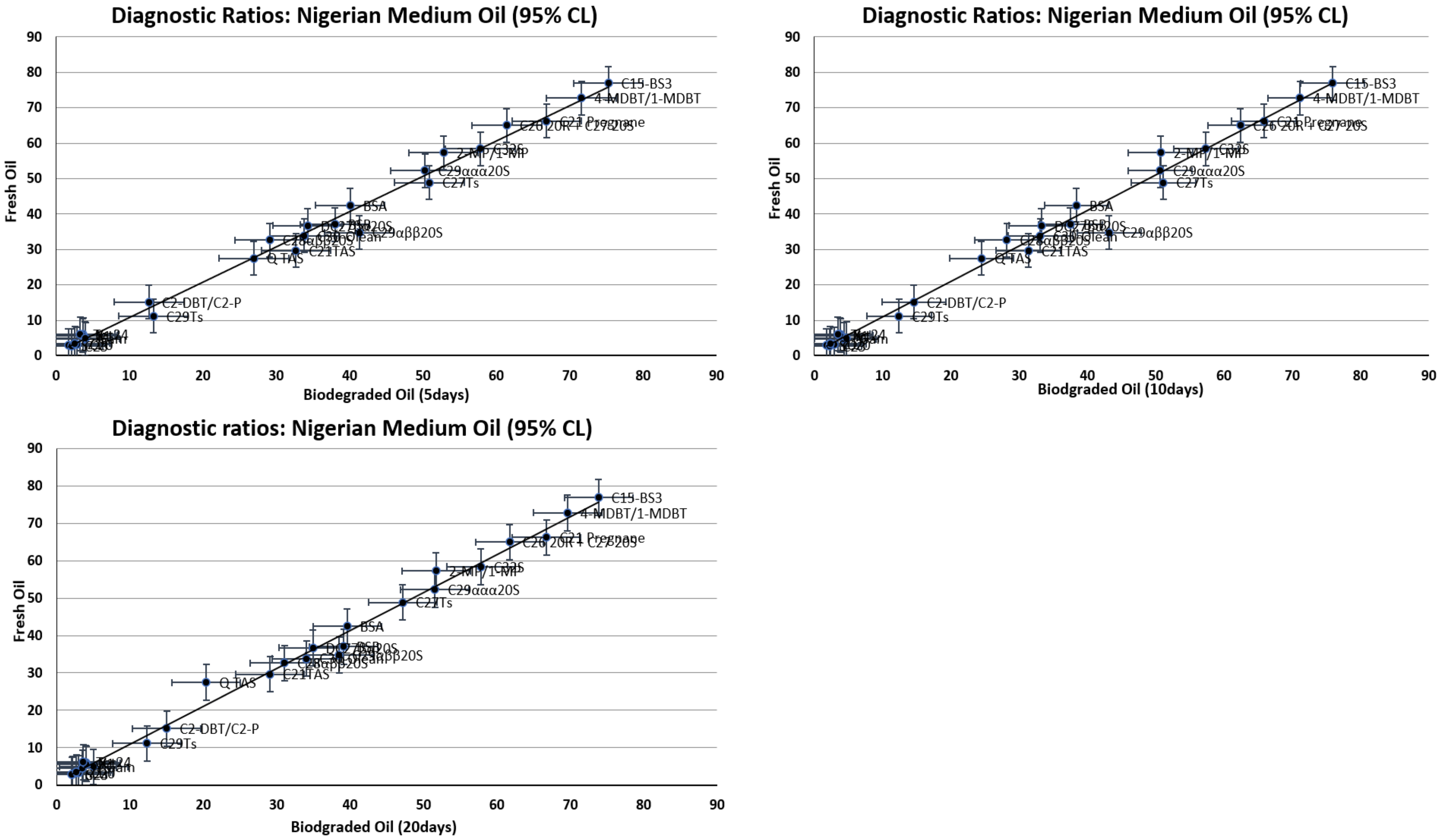


Figure 0.56: Diagnostic Ratio plots of fresh oil vs degraded oil Nigerian Medium oil in Whitley Bay sediment, degraded oil showing positive correlation with the fresh oil up to 20 days incubation using 95% confidence limit.

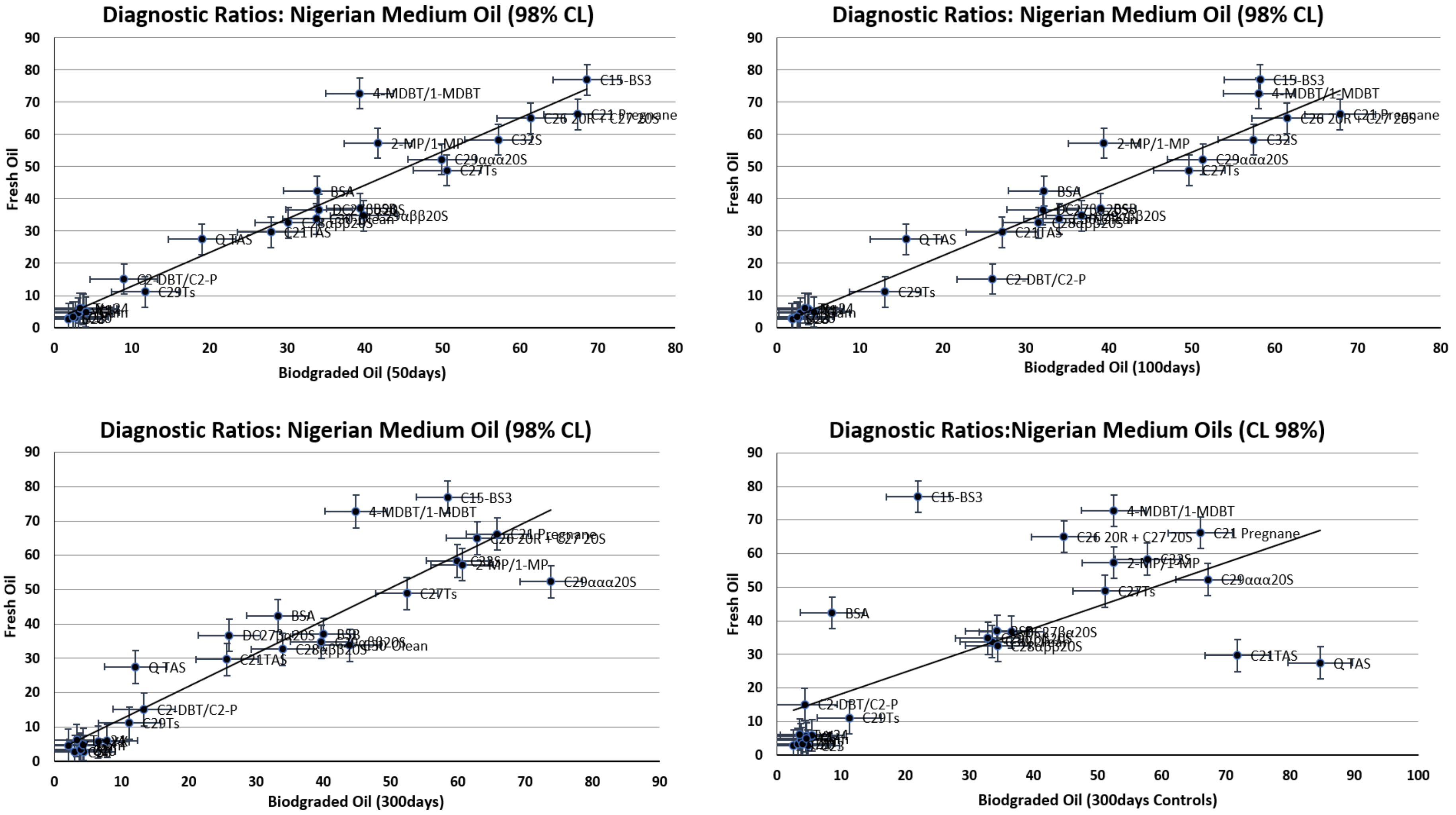


Figure 0.57: Diagnostic Ratio plots of fresh oil vs degraded oil Nigerian Medium oil in Whitley Bay sediment, degraded oil showing negative correlation with the fresh oil from 50 - 300 days incubation using 98% confidence limit.

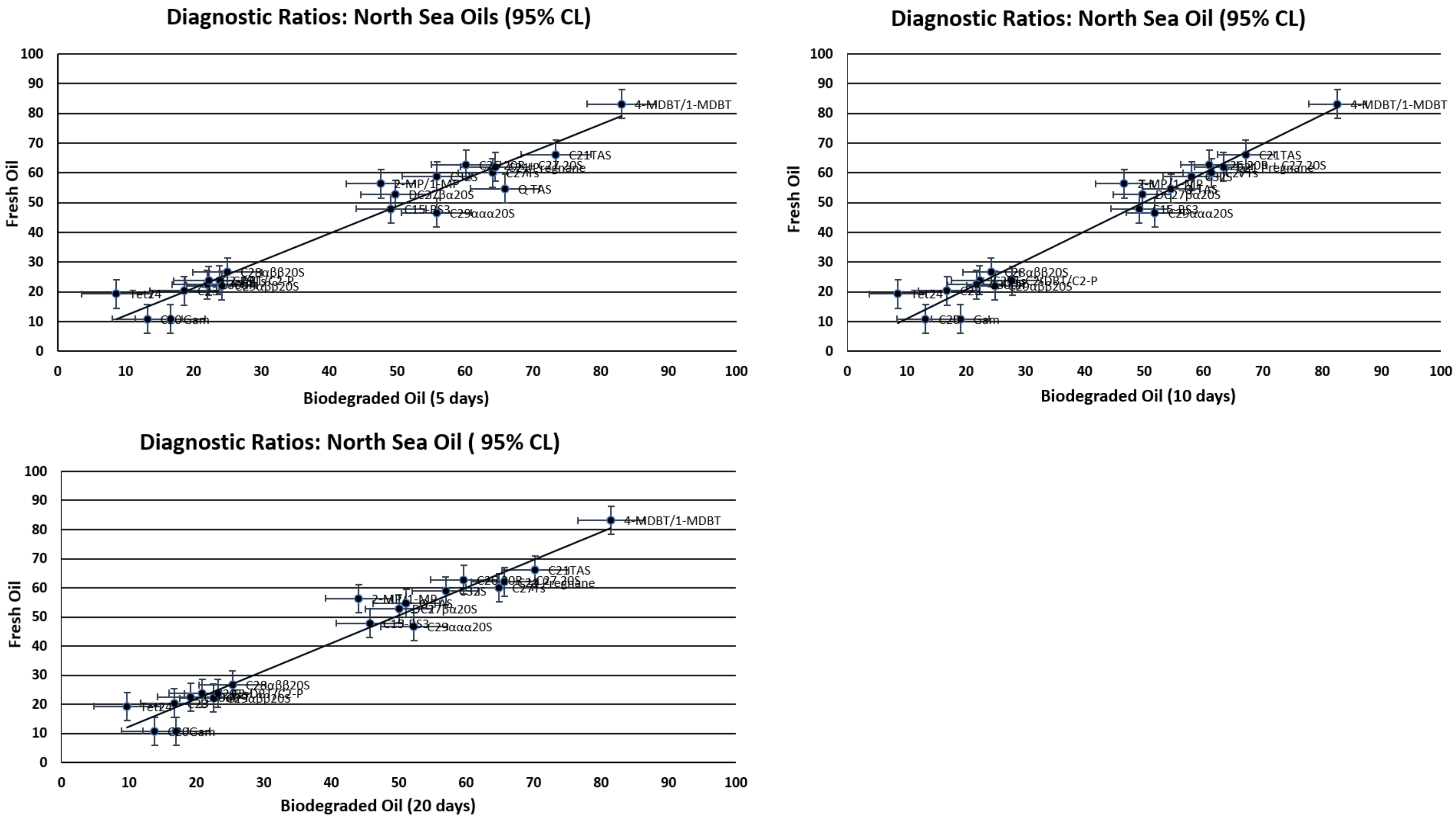


Figure 0.58: Diagnostic Ratio plots of fresh oil vs degraded oil North Sea oil in Whitley Bay sediment degraded oil showing positive correlation with the fresh oil from 5 - 20 days incubation using 95% confidence limit.

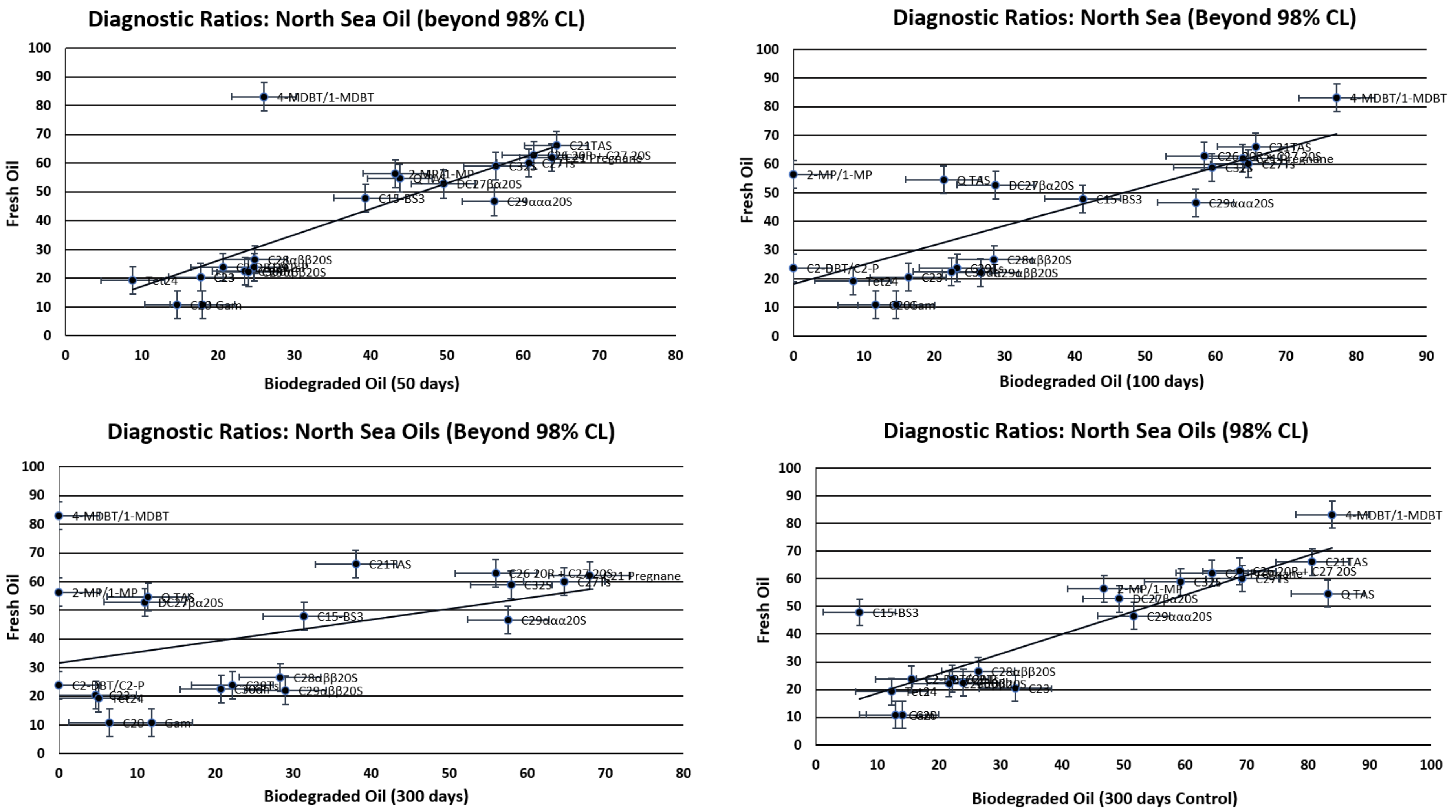


Figure 0.59: Diagnostic Ratio plots of fresh oil vs degraded oil North Sea oil in Whitley Bay sediment degraded oil showing negative correlation with the fresh oil from 50 - 300 days incubation using 98% confidence limit.

5.4.6 Sensitivity Check on the Nordtest Methodology

Sensitivity analysis was carried on some selected biomarkers to assess the influence of background hydrocarbon concentrations that could affect or interfere with the efficiency of the correlation (e.g.,Daling *et al.*, 2002b; Douglas *et al.*, 2007). Table 0.56Table 0.57Table 0.58 show the background concentrations of some selected biomarkers which include; C_{19} and C_{26} (S) tricyclic terpanes, $C_{30}\alpha\beta$ hopane, C_{20}-ααα-sterane, C_{30} 20R-sterane, C_4-decalin and 8β(H)-homodrimane (which are bicyclic sesquiterpanes), in the Whitley Bay, Nigerian and Tyne sediments and their concentrations in the starting three oils respectively. The relative ratios of the concentrations in the starting oil (µg/g oil) from 50 mg oils added to the sediments (10 g dry weight) to the sediment background concentrations in terms of extractable organic matter (µg/g EOM) of these biomarkers in the sediment, were assessed. Therefore, 50 mg oil added to 10g sediment constitutes 0.5% oil in the sediments (i.e., 5000 ppm). The ratios of oil (50 mg) to background sediment (10 g) are as shown in the tables and some are observed to be as low as 0.5 for $C_{30}\alpha\beta$ hopane of North Sea oil in Nigerian sediment (see Table 0.57) and these low ratios will not be suitable for correlation. For effective oil-oil correlation the oil derived markers should be much more abundant in the oil than the in the background in the sediment, e.g., 50 times more, as lower detection limit, to determine the minimum amount of oil (ppm) in the sediment to be able to be detected using the presence of these biomarkers. The last columns of the Table 0.56Table 0.57, and Table 0.58 show the minimum amounts of oils (ppm) required to be added to each of the sediments to be able to detect the presence of these biomarkers for effective correlation normalised to 50, as reliable correlation needs several marker parameters. The concentration ratios of several marker parameters would need to be taken into account and the detection/correlation limit would be determined by the worst of them, and this would be a limitation of the method, though it may guide a user to avoid using some marker parameters in order to improve correlation efficiency.

5.4.7 Conclusion

Biodegradation is an important affecting the composition of spilled crude oils in surface environments and understanding its detailed effects is required in order to assess the fate of the oils for source identification and remedial purposes (e.g., Jones *et al.*, 1986; Rosenberg *et al.*, 1992; Wang *et al.*, 2006).

Gas chromatograms (see Figure 0.2,Figure 0.3Figure 0.4) and concentrations (see Table 0.2,Table 0.3, andTable 0.3) of aliphatic fractions of the two Nigerian and North Sea oils in Whitley sediment incubated for 300days showed a visible effect of biodegradation on the samples. Many of the *n*-alkanes are observed to have been biodegraded completely from *n*-C_8 to *n*-C_{20} in the Nigerian light oil and *n*-C_8 to *n*-C_{23} in the Nigerian medium oil after 100 days, whereas *n*-alkanes the North Sea oil were completely degraded while *n*-alkanes in the sterile control were almost conserved. A preferential bacterial oxidation of the normal alkanes over the isoprenoids starting with the low-molecular weight *n*-alkanes is observed (e.g., Jones *et al.*, 1986). The level of biodegradation is considered moderate on the PM scale as level 4. However, not as much bacterial activity was observed in the samples containing the same oils in the Nigeria sediment as only *n*-C_8 to *n*-C_{13} *n*-alkanes were oxidised after 300 days (see Figure 0.5, Figure 0.6,Figure 0.7), which could be attributed to the long period of sediment storage under room temperature before setting up the experiments. The *n*-alkanes were not as affected by evaporation and water washing as discussed in chapters 3 and 4 respectively, with water washing having the least effect.

The concentrations of the aromatic fractions components were also observed to be more affected in the Whitley Bay sediment (see Table 0.7,Table 0.8Table 0.9) than in the Nigeria sediment (see Table 0.10Table 0.11Table 0.12) as parent and alkyl homologous of naphthalene, biphenyl, and phenanthrene were generally observed to decreased with degradation time, while those of pyrenes, chrysenes and triaromatic steranes (TAS) relatively increased with degradation time and the sterile controls remained unaltered. Some PAH isomers also showed variable susceptibility to microbial oxidation. The PAHs like the n-alkanes were generally affected by biodegradation more than by evaporation and water washing.

The alkane molecular markers and biomarkers were generally unaffected by biodegradation except for the adamantanes, bicyclic sesquiterpanes, C_{21}-C_{22} steranes and C_{27}-C_{29} diasteranes.

Relative susceptibility to biodegradation of some selected aliphatics and PAH based on their relatively elution order was assessed using their ratios (see Figure 0.52, and Figure 0.53) for the three oils in the two sediments with the concentrations shown in Table 0.49,Table 0.50,Table 0.51,Table 0.52,Table 0.53,Table 0.54 .The ratios indicate a greater relative susceptibility due to the effect of biodegradation on selected compounds than the effect of evaporation and water washing which were generally unaffected as discussed in chapters 3 and 4 respectively. Table 0.55 shows the identification of some the compounds for further information.

The diagnostic ratio plots of selected Nordtest parameters (see section 5.4.5) shows the effect of biodegradation on these compounds within the required statistical confidence limit as well as the sensitivity check on the Nordtest methodology (see section 5.4.6) to ascertain the sensitivity limit of the selected parameters for correlation efficiency.

The Nordtest analysis was carried out only on Whitley Bay sediment samples because it was observed to be the most affected by biodegradation.

Table 0.1: Changes in *n*-alkane and acyclic isoprenoid concentrations in biodegraded Nigerian light oil in Whitley sediment (mg/g oil).

n-Alkane	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
***n*-C$_8$**	4.05 (3.2)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_9$**	7.31 (1.2)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{10}$**	8.97 (4.3)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{11}$**	9.44 (1.8)	1.09 (6.9)	0.30 (3.6)	0.05 (9.4)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{12}$**	8.77 (0.2)	2.51 (3.4)	0.75 (6.7)	0.20 (8.0)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{13}$**	7.81 (0.2)	3.66 (3.0)	1.37 (4.7)	0.48 (4.4)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.18 (2.2)
***n*-C$_{14}$**	7.17 (0.5)	3.88 (5.1)	1.61 (7.8)	0.70 (6.3)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.20 (3.1)
***n*-C$_{15}$**	6.54 (0.2)	3.85 (5.4)	1.67 (7.4)	0.87 (8.4)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.20 (2.1)
***n*-C$_{16}$**	5.32 (0.4)	3.56 (8.3)	1.57 (8.6)	0.86 (0.1)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.87 (1.4)
***n*-C$_{17}$**	4.62 (0.1)	3.48 (8.5)	1.39 (7.6)	0.86 (3.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	1.54 (4.9)
Pristane	5.34 (0.1)	4.32 (5.1)	3.09 (4.0)	2.73 (3.2)	0.80 (5.6)	0.29 (3.8)	0.00 (0!)	1.94 (4.0)
n-C$_{18}$	4.10 (3.8)	3.12 (6.9)	1.25 (4.4)	0.78 (9.0)	0.46 (2.4)	0.00 (0!)	0.00 (0!)	1.80 (4.1)
Phytane	1.75 (2.9)	1.55 (2.7)	1.02 (4.4)	0.91 (5.0)	0.33 (3.8)	0.21 (3.0)	0.00 (0!)	0.78 (4.4)
***n*-C$_{19}$**	3.55 (2.1)	2.98 (5.2)	1.23 (7.0)	0.83 (7.5)	0.63 (2.1)	0.00 (0!)	0.00 (0!)	1.88 (3.3)
***n*-C$_{20}$**	3.26 (2.9)	2.93 (6.7)	1.06 (5.9)	0.80 (2.1)	0.69 (3.2)	0.00 (0!)	0.00 (0!)	1.81 (4.9)
***n*-C$_{21}$**	3.27 (2.1)	2.76 (6.9)	1.39 (8.9)	0.96 (3.7)	0.87 (7.6)	0.21 (3.1)	0.00 (0!)	1.88 (4.7)
***n*-C$_{22}$**	3.46 (1.2)	2.65 (5.8)	1.28 (7.7)	0.93 (2.2)	0.75 (8.4)	0.20 (0.6)	0.00 (0!)	1.69 (4.9)

n-C$_{23}$	3.12 (3.8)	2.60 (6.8)	1.30 (7.4)	1.00 (1.1)	0.77 (9.9)	0.19 (5.9)	0.00 (0!)	1.62 (4.5)
n-C$_{24}$	2.59 (2.9)	2.52 (9.0)	1.24 (5.0)	1.00 (4.4)	0.75 (3.4)	0.19 (1.7)	0.00 (0!)	1.46 (4.4)
n-C$_{25}$	2.81 (0.7)	2.35 (6.4)	1.27 (5.6)	1.07 (7.1)	0.72 (3.8)	0.19 (9.7)	0.00 (0!)	1.38 (4.2)
n-C$_{26}$	2.17 (3.1)	2.11 (6.6)	1.13 (6.9)	1.00 (4.3)	0.66 (7.7)	0.18 (9.1)	0.00 (0!)	1.16 (4.9)
n-C$_{27}$	2.07 (3.2)	2.04 (5.6)	1.18 (4.8)	1.07 (4.4)	0.71 (5.1)	0.18 (5.7)	0.00 (0!)	1.11 (4.4)
n-C$_{28}$	1.71 (1.0)	1.45 (7.9)	1.01 (5.1)	0.94 (1.0)	0.58 (8.5)	0.17 (1.5)	0.00 (0!)	0.90 (3.7)
n-C$_{29}$	1.68 (2.9)	1.24 (0.6)	0.99 (5.2)	0.93 (2.5)	0.65 (4.5)	0.17 (6.6)	0.00 (0!)	0.86 (3.8)
n-C$_{30}$	1.09 (3.1)	0.84 (4.0)	0.68 (5.0)	0.58 (3.3)	0.55 (7.4)	0.16 (2.0)	0.00 (0!)	0.55 (3.3)
n-C$_{31}$	1.24 (1.4)	0.76 (6.6)	0.63 (5.4)	0.59 (3.3)	0.51 (4.2)	0.16 (5.0)	0.00 (0!)	0.62 (3.0)
n-C$_{32}$	0.68 (4.7)	0.49 (3.2)	0.31 (5.0)	0.29 (2.6)	0.28 (7.1)	0.12 (4.9)	0.00 (0!)	0.33 (2.6)
n-C$_{33}$	0.63 (1.8)	0.38 (6.9)	0.24 (6.6)	0.21 (5.6)	0.20 (9.5)	0.12 (7.0)	0.00 (0!)	0.30 (2.5)
n-C$_{34}$	0.24 (4.5)	0.17 (4.8)	0.08 (7.5)	0.08 (6.6)	0.06 (3.7)	0.06 (8.1)	0.00 (0!)	0.11 (2.0)
n-C$_{35}$	0.16 (3.2)	0.14 (4.1)	0.04 (6.6)	0.04 (2.1)	0.04 (4.3)	0.04 (3.2)	0.00 (0!)	0.08 (4.6)
n-C$_{36}$	0.09 (6.5)	0.04 (0.9)	0.03 (7.4)	0.03 (6.6)	0.02 (5.5)	0.02 (1.2)	0.00 (0!)	0.04 (3.3)
n-C$_{37}$	0.06 (3.6)	0.04 (2.5)	0.03 (3.8)	0.02 (2.3)	0.02 (2.2)	0.02 (2.4)	0.00 (0!)	0.03 (3.6)
n-C$_{38}$	0.03 (6.6)	0.02 (5.1)	0.02 (4.0)	0.02 (3.2)	0.02 (3.2)	0.01 (3.2)	0.00 (0!)	0.01 (2.2)
n-C$_{39}$	0.02 (3.3)	0.02 (2.7)	0.01 (4.4)	0.01 (5.0)	0.01 (4.2)	0.01 (3.2)	0.00 (0!)	0.01 (4.9)
Sum	115.12	59.57	29.18	20.85	11.05	2.90	0.00	25.37
n-C$_{17}$/pristane	0.86	0.81	0.45	0.31	0.00	0.00	#DIV/0!	0.79

***n*-C$_{18}$/phytane**	2.35	2.01	1.23	0.86	1.42	0.00	#DIV/0!	2.31
pristane/phytane	3.06	2.78	3.02	2.99	2.43	1.40	#DIV/0!	2.49
CPI*	1.23	1.13	1.16	1.18	1.15	1.06	#DIV/0!	1.19
(C$_8$+C$_{10}$+C$_{12}$+C$_{14}$)/(C$_{22}$+C$_{24}$+C$_{26}$+C$_{28}$)	2.92	0.73	0.50	0.23	0.00	0.00	#DIV/0!	0.04

Note: The numbers in brackets after the numbers (concentrations) in the table are relative standard deviations (RSD) (n=3), similarly for Tables 5.2 and 5.3.

Table 0.2: Changes in *n*-alkane and acyclic isoprenoid concentrations in biodegraded Nigerian medium oil in Whitley sediment (mg/g oil).

***n*-Alkane**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
***n*-C$_8$**	0.44 (4.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_9$**	0.79 (0.5)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{10}$**	1.00 (4.9)	0.02 (4.4)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{11}$**	1.34 (2.7)	0.17 (1.6)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{12}$**	1.70 (1.0)	0.36 (6.9)	0.08 (4.8)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.18 (2.4)
***n*-C$_{13}$**	1.83 (1.1)	0.55 (3.0)	0.17 (6.3)	0.09 (2.2)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.18 (3.5)
***n*-C$_{14}$**	1.90 (3.2)	0.52 (4.0)	0.29 (2.3)	0.09 (5.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.20 (4.1)
***n*-C$_{15}$**	2.11 (4.3)	0.69 (1.2)	0.42 (3.1)	0.14 (0.5)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.21 (7.8)
***n*-C$_{16}$**	1.64 (7.5)	0.86 (7.5)	0.57 (4.3)	0.22 (0.8)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.70 (4.5)
***n*-C$_{17}$**	2.42 (3.8)	1.15 (4.4)	0.87 (7.9)	0.36 (3.7)	0.15 (3.6)	0.16 (2.6)	0.00 (0!)	1.29 (3.1)
Pristane	5.36 (5.2)	4.11 (2.4)	4.55 (6.4)	3.54 (3.8)	1.93 (1.6)	0.45 (4.3)	0.00 (0!)	3.32 (2.6)
***n*-C$_{18}$**	2.63 (0.5)	1.33 (5.9)	1.04 (7.4)	0.39 (6.0)	0.26 (2.5)	0.35 (7.4)	0.00 (0!)	1.56 (8.1)

Phytane	2.25 (4.6)	1.68 (2.4)	2.00 (4.9)	1.45 (5.2)	0.91 (1.8)	0.36 (3.8)	0.00 (0!)	1.51 (1.4)
n-C$_{19}$	2.66 (3.6)	1.38 (3.3)	1.06 (4.7)	0.54 (6.3)	0.48 (2.1)	0.46 (4.6)	0.00 (0!)	1.65 (8.4)
n-C$_{20}$	2.69 (2.6)	1.34 (2.8)	1.10 (6.9)	0.57 (2.9)	0.51 (1.4)	0.46 (6.1)	0.00 (0!)	1.60 (8.9)
n-C$_{21}$	2.69 (2.6)	1.65 (4.3)	1.34 (1.5)	0.70 (3.5)	0.68 (1.4)	0.46 (5.8)	0.00 (0!)	1.85 (5.6)
n-C$_{22}$	2.53 (2.5)	1.54 (3.9)	1.28 (1.1)	0.71 (2.4)	0.53 (2.1)	0.48 (5.6)	0.00 (0!)	1.66 (6.7)
n-C$_{23}$	2.57 (1.9)	1.60 (4.4)	1.43 (1.2)	0.72 (2.7)	0.53 (2.3)	0.49 (3.1)	0.00 (0!)	1.84 (5.9)
n-C$_{24}$	2.43 (5.1)	1.70 (2.6)	1.57 (3.5)	0.75 (5.4)	0.67 (2.5)	0.52 (3.7)	0.00 (0!)	1.71 (1.8)
n-C$_{25}$	2.37 (0.5)	1.78 (3.7)	1.73 (2.7)	0.98 (2.2)	0.69 (4.1)	0.58 (4.1)	0.00 (0!)	1.76 (0.3)
n-C$_{26}$	2.26 (2.4)	1.75 (3.1)	1.77 (7.6)	1.00 (6.3)	0.79 (2.7)	0.58 (5.7)	0.00 (0!)	1.64 (0.3)
n-C$_{27}$	2.29 (1.6)	1.83 (2.2)	1.96 (5.1)	1.01 (2.2)	0.74 (4.1)	0.59 (6.6)	0.00 (0!)	1.69 (0.5)
n-C$_{28}$	2.22 (3.5)	1.58 (1.5)	1.81 (4.6)	1.13 (5.1)	0.82 (3.0)	0.57 (4.9)	0.00 (0!)	1.45 (0.8)
n-C$_{29}$	2.05 (4.4)	1.48 0.5)	1.79 (4.4)	0.85 (2.4)	0.85 (3.7)	0.56 (5.0)	0.00 (0!)	1.41 (1.6)
n-C$_{30}$	1.54 (8.9)	1.02 (6.8)	1.38 (7.0)	0.71 (2.2)	0.85 (2.6)	0.69 (5.2)	0.00 (0!)	0.92 (2.2)
n-C$_{31}$	1.66 (1.4)	0.91 (1.2)	1.21 (3.5)	0.70 (3.8)	0.85 (3.1)	0.37 (2.8)	0.00 (0!)	0.95 (3.2)
n-C$_{32}$	0.94 (1.0)	0.50 (1.9)	0.68 (6.5)	0.40 (4.2)	0.54 (4.1)	0.22 (6.4)	0.00 (0!)	0.56 (3.7)
n-C$_{33}$	0.74 (0.5)	0.35 (1.3)	0.50 (6.2)	0.38 (3.9)	0.21 (4.1)	0.17 (4.9)	0.00 (0!)	0.44 (4.9)
n-C$_{34}$	0.36 (0.8)	0.13 (6.3)	0.21 (5.2)	0.13 (1.5)	0.10 (1.9)	0.05 (6.9)	0.00 (0!)	0.17 (7.5)
n-C$_{35}$	0.24 (1.8)	0.08 (3.5)	0.08 (3.1)	0.05 (2.0)	0.04 (7.8)	0.04 (4.9)	0.00 (0!)	0.11 (6.2)
n-C$_{36}$	0.15 (2.1)	0.05 (3.1)	0.04 (3.8)	0.03 (2.3)	0.03 (2.0)	0.02 (7.7)	0.00 (0!)	0.06 (4.5)

***n*-C$_{37}$**	0.14 (0.8)	0.03 (3.2)	0.02 (7.7)	0.02 (2.8)	0.02 (8.6)	0.01 (1.7)	0.00 (0!)	0.04 (4.4)
***n*-C$_{38}$**	0.13 (0.9)	0.02 (3.1)	0.02 (5.4)	0.01 (6.6)	0.01 (7.3)	0.01 (6.6)	0.00 (0!)	0.03 (4.8)
***n*-C$_{39}$**	0.11 (0.4)	0.02 (2.1)	0.01 (3.2)	0.01 (2.4)	0.01 (3.0)	0.01 (4.1)	0.00 (0!)	0.02 (1.6)
Sum	58.16	32.18	31.00	17.69	13.21	8.66	0.00	30.71
***n*-C$_{17}$/pristane**	0.45	0.28	0.19	0.10	0.08	0.36	#DIV/0!	0.39
***n*-C$_{18}$/phytane**	1.17	0.79	0.52	0.27	0.29	0.97	#DIV/0!	1.03
pristane/phytane	2.38	2.45	2.27	2.44	2.12	1.24	#DIV/0!	2.20
CPI*	1.11	1.12	1.12	1.08	0.99	0.97	#DIV/0!	1.16
(C$_{8}$+C$_{10}$+C$_{12}$+C$_{14}$)/(C$_{22}$+C$_{24}$+C$_{26}$+C$_{28}$)	0.53	0.14	0.06	0.03	0.00	0.00	#DIV/0!	0.06

Table 0.3: Changes in *n*-alkane and acyclic isoprenoid concentrations in biodegraded North Sea oil in Whitley sediment (mg/g oil)

***n*-Alkane**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
***n*-C$_{8}$**	2.96 (5.6)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{9}$**	3.63 (4.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{10}$**	4.45 (3.1)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{11}$**	4.95 (3.2)	1.01 (4.2)	0.43 (5.6)	0.03 (6.7)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{12}$**	5.17 (5.3)	2.11 (5.6)	1.01 (2.5)	0.17 (5.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.38 (3.5)
***n*-C$_{13}$**	5.57 (2.9)	2.93 (3.3)	1.74 (4.7)	0.39 (4.6)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.44 (5.7)
***n*-C$_{14}$**	5.57 (4.5)	3.51 (6.2)	2.04 (5.6)	0.56 (2.7)	0.03 (3.3)	0.00 (0!)	0.00 (0!)	0.49 (6.7)

n-C$_{15}$	5.01 (4.4)	3.43 (5.1)	1.92 (5.7)	0.69 (5.5)	0.08 (8.0)	0.00 (0!)	0.00 (0!)	0.51 (7.7)
n-C$_{16}$	4.13 (1.0)	3.22 (6.1)	1.83 (6.7)	0.64 (4.7)	0.12 (7.3)	0.00 (0!)	0.00 (0!)	1.16 (9.6)
n-C$_{17}$	3.98 (3.2)	2.94 (4.2)	1.69 (4.5)	0.63 (6.0)	0.13 (4.0)	0.00 (0!)	0.00 (0!)	1.61 (3.6)
Pristane	2.02 (3.5)	2.27 (3.9)	1.55 (5.3)	0.65 (5.6)	0.56 (4.6)	0.00 (0!)	0.00 (0!)	0.86 (4.0)
n-C$_{18}$	3.37 (1.7)	2.41 (4.9)	1.39 (6.0)	0.52 (3.3)	0.13 (3.9)	0.00 (0!)	0.00 (0!)	1.59 (3.2)
Phytane	1.58 (4.9)	1.77 (4.6)	1.26 (3.0)	0.53 (0.8)	0.41 (5.4)	0.00 (0!)	0.00 (0!)	0.79 (4.2)
n-C$_{19}$	2.93 (5.1)	2.28 (5.3)	1.30 (4.2)	0.50 (2.4)	0.15 (4.0)	0.00 (0!)	0.00 (0!)	1.52 (3.3)
n-C$_{20}$	2.53 (5.5)	2.01 (4.3)	1.18 (4.1)	0.49 (5.3)	0.15 (5.6)	0.00 (0!)	0.00 (0!)	1.40 (3.9)
n-C$_{21}$	2.38 (3.4)	2.01 (4.3)	1.17 (5.3)	0.49 (1.4)	0.15 (2.8)	0.00 (0!)	0.00 (0!)	1.32 (3.3)
n-C$_{22}$	2.24 (5.1)	1.89 (6.9)	1.17 (3.6)	0.49 (3.3)	0.15 (3.2)	0.00 (0!)	0.00 (0!)	1.20 (3.6)
n-C$_{23}$	1.99 (5.2)	1.73 (5.8)	1.09 (3.0)	0.48 (1.9)	0.15 (3.1)	0.00 (0!)	0.00 (0!)	1.06 (3.2)
n-C$_{24}$	1.82 (5.5)	1.59 (5.8)	1.05 (2.1)	0.45 (2.0)	0.15 (7.6)	0.00 (0!)	0.00 (0!)	0.97 (3.4)
n-C$_{25}$	1.51 (5.6)	1.42 (4.8)	0.95 (5.7)	0.39 (3.9)	0.16 (7.9)	0.00 (0!)	0.00 (0!)	0.80 (2.9)
n-C$_{26}$	1.30 (5.4)	1.11 (5.8)	0.76 (6.2)	0.31 (4.7)	0.16 (6.8)	0.00 (0!)	0.00 (0!)	0.64 (3.4)
n-C$_{27}$	1.00 (4.8)	0.91 (3.2)	0.69 (4.0)	0.30 (0.5)	0.17 (4.7)	0.00 (0!)	0.00 (0!)	0.54 (2.2)
n-C$_{28}$	0.68 (1.9)	0.65 (5.1)	0.50 (3.7)	0.19 (4.5)	0.12 (7.2)	0.00 (0!)	0.00 (0!)	0.38 (3.4)
n-C$_{29}$	0.52 (0.9)	0.49 (5.5)	0.36 (5.8)	0.14 (2.9)	0.10 (5.1)	0.00 (0!)	0.00 (0!)	0.27 (3.4)
n-C$_{30}$	0.36 (2.3)	0.29 (6.3)	0.27 (4.1)	0.11 (3.3)	0.09 (1.7)	0.00 (0!)	0.00 (0!)	0.14 (6.1)
n-C$_{31}$	0.18 (3.8)	0.18 (4.8)	0.17 (4.7)	0.07 (3.4)	0.06 (8.8)	0.00 (0!)	0.00 (0!)	0.11 (4.1)

n-C_{32}	0.10 (4.1)	0.09 (5.7)	0.09 (6.2)	0.04 (5.2)	0.04 (7.0)	0.00 (0!)	0.00 (0!)	0.06 (9.7)
n-C_{33}	0.09 (2.0)	0.09 (5.1)	0.08 (4.1)	0.04 (4.2)	0.04 (4.4)	0.00 (0!)	0.00 (0!)	0.02 (5.1)
n-C_{34}	0.10 (4.2)	0.10 (5.7)	0.09 (2.5)	0.04 (5.4)	0.03 (5.4)	0.00 (0!)	0.00 (0!)	0.05 (8.9)
n-C_{35}	0.05 (5.6)	0.05 (4.6)	0.04 (3.7)	0.03 (7.9)	0.02 (4.3)	0.00 (0!)	0.00 (0!)	0.04 (3.6)
n-C_{36}	0.05 (5.4)	0.03 (5.7)	0.02 (4.0)	0.01 (8.7)	0.01 (3.5)	0.00 (0!)	0.00 (0!)	0.01 (6.8)
n-C_{37}	0.04 (0.6)	0.02 (6.0)	0.02 (3.4)	0.01 (4.1)	0.01 (5.9)	0.00 (0!)	0.00 (0!)	0.01 (5.5(
n-C_{38}	0.03 (0.8)	0.02 (5.5)	0.02 (5.3)	0.01 (3.1)	0.01 (5.3)	0.00 (0!)	0.00 (0!)	0.01 (4.1)
n-C_{39}	0.03 (1.5)	0.01 (3.2)	0.01 (3.1)	0.01 (2.0)	0.01 (3.5)	0.00 (0!)	0.00 (0!)	0.01 (8.9)
Sum	72.32	42.57	25.89	9.41	3.38	0.00	0.00	18.36
n-C_{17}/pristane	1.98	1.30	1.09	0.96	0.22	#DIV/0!	#DIV/0!	1.86
n-C_{18}/phytane	2.14	1.36	1.11	0.97	0.32	#DIV/0!	#DIV/0!	2.02
pristane/phytane	1.28	1.28	1.24	1.22	1.38	#DIV/0!	#DIV/0!	1.10
CPI*	1.04	1.10	1.08	1.09	1.07	#DIV/0!	#DIV/0!	1.08
(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})	3.00	1.07	0.88	0.51	0.04	#DIV/0!	#DIV/0!	0.27

Table 0.4: Changes in *n*-alkane and acyclic isoprenoid concentrations in biodegraded Nigerian light oil in Nigerian sediment (mg/g oil).

n-Alkane	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
n-C_8	4.05 (3.2)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
n-C_9	7.31 (1.2)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
n-C_{10}	8.97 (4.3)	0.10 (0.7)	0.02 (2.8)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.04 (3.2)

***n*-C$_{11}$**	9.44 (1.8)	0.72 (4.1)	0.38 (4.1)	0.10 (3.2)	0.07 (1.9)	0.00 (0!)	0.00 (0!)	0.06 (4.1)
***n*-C$_{12}$**	8.77 (0.2)	1.09 (2.6)	1.05 (5.1)	0.86 (4.0)	0.15 (2.1)	0.01 (1.0)	0.00 (0!)	0.07 (3.2)
***n*-C$_{13}$**	7.81 (0.2)	1.41 (2.5)	1.61 (3.5)	1.75 (3.2)	0.70 (0.8)	0.21 (6.2)	0.00 (0!)	0.08 (4.5)
***n*-C$_{14}$**	7.17 (0.5)	1.40 (2.4)	1.85 (1.0)	2.17 (1.4)	1.49 (6.7)	0.77 (4.7)	0.05 (3.3)	0.09 (1.9)
***n*-C$_{15}$**	6.54 (0.2)	1.39 (5.1)	1.70 (1.1)	1.91 (2.6)	1.57 (5.4)	1.10 (3.7)	0.42 (3.2)	0.11 (7.1)
***n*-C$_{16}$**	5.32 (0.4)	1.21 (2.2)	1.50 (2.0)	1.89 (3.8)	1.51 (5.1)	1.11 (2.3)	0.61 (2.9)	0.29 (5.2)
***n*-C$_{17}$**	4.62 (0.1)	1.16 (3.6)	1.42 (1.3)	1.74 (6.1)	1.47 (4.9)	1.13 (0.6)	0.65 (3.2)	0.49 (5.4)
Pristane	5.34 (0.1)	1.39 (6.7)	1.63 (1.3)	1.90 (1.7)	1.71 (5.3)	1.58 (4.8)	0.99 (4.8)	1.02 (4.6)
***n*-C$_{18}$**	4.10 (3.8)	1.05 (5.6)	1.18 (7.2)	1.57 (6.6)	1.21 (1.7)	1.01 (3.1)	0.60 (1.2)	0.54 (4.0)
Phytane	1.75 (2.9)	0.44 (2.4)	0.59 (2.2)	0.77 (5.6)	0.59 (3.8)	0.67 (4.5)	0.39 (6.2)	0.55 (3.1)
***n*-C$_{19}$**	3.55 (2.1)	1.00 (2.8)	1.22 (2.2)	1.49 (3.8)	1.24 (5.2)	0.93 (0.7)	0.60 (4.0)	0.61 (4.6)
***n*-C$_{20}$**	3.26 (2.9)	0.96 (4.1)	1.05 (4.9)	1.29 (5.6)	1.12 (4.6)	0.85 (6.1)	0.56 (7.7)	0.61 (5.5)
***n*-C$_{21}$**	3.27 (2.1)	0.96 (2.9)	1.31 (1.8)	1.61 (4.2)	1.24 (3.7)	0.99 (3.9)	0.67 (6.2)	0.72 (7.8)
***n*-C$_{22}$**	3.46 (1.2)	0.85 (0.6)	1.03 (0.7)	1.23 (5.2)	1.08 (5.1)	0.82 (1.4)	0.52 (6.3)	0.57 (4.4)
***n*-C$_{23}$**	3.12 (3.8)	0.83 (2.7)	1.01 (1.8)	1.20 (4.4)	1.03 (4.5)	0.79 (2.6)	0.54 (5.9)	0.58 (5.6)
***n*-C$_{24}$**	2.59 (2.9)	0.76 (3.1)	0.98 (2.9)	1.12 (5.4)	0.97 (3.7)	0.77 (1.5)	0.50 (7.2)	0.58 (5.1)
***n*-C$_{25}$**	2.81 (0.7)	0.72 (7.0)	0.86 (4.0)	1.07 (5.3)	0.92 (4.7)	0.76 (1.0)	0.53 (5.3)	0.53 (7.5)
***n*-C$_{26}$**	2.17 (3.1)	0.63 (5.4)	0.72 (6.4)	0.90 (3.5)	0.77 (4.8)	0.59 (1.2)	0.48 (6.1)	0.45 (0.6)
***n*-C$_{27}$**	2.07 (3.2)	0.58 (3.9)	0.69 (1.7)	0.86 (6.1)	0.75 (3.7)	0.57 (1.7)	0.48 (4.7)	0.44 (3.9)

_n_-C_{28}	1.71 (1.0)	0.44 (8.6)	0.56 (3.2)	0.65 (6.2)	0.59 (3.7)	0.45 (2.5)	0.40 (7.8)	0.34 (4.9)
_n_-C_{29}	1.68 (2.9)	0.43 (7.7)	0.54 (2.8)	0.67 (4.9)	0.62 (6.4)	0.48 (1.5)	0.41 (4.3)	0.35 (7.3)
_n_-C_{30}	1.09 (3.1)	0.21 (7.5)	0.30 (4.0)	0.33 (3.1)	0.43 (3.8)	0.23 (3.8)	0.37 (4.0)	0.31 (3.6)
_n_-C_{31}	1.24 (1.4)	0.22 (0.4)	0.15 (3.6)	0.27 (6.6)	0.35 (2.2)	0.24 (6.5)	0.31 (7.8)	0.25 (7.5)
_n_-C_{32}	0.68 (4.7)	0.09 (2.3)	0.08 (4.3)	0.10 (3.1)	0.18 (2.2)	0.26 (2.3)	0.16 (5.7)	0.14 (5.3)
_n_-C_{33}	0.63 (1.8)	0.06 (6.6)	0.04 (6.6)	0.07 (1.4)	0.13 (4.0)	0.09 (4.0)	0.13 (5.3)	0.11 (7.9)
_n_-C_{34}	0.24 (4.5)	0.05 (2.4)	0.04 (5.6)	0.04 (2.4)	0.04 (4.9)	0.04 (5.1)	0.05 (6.1)	0.04 (6.7)
_n_-C_{35}	0.16 (3.2)	0.05 (8.8)	0.04 (6.5)	0.03 (4.0)	0.03 (2.1)	0.03 (6.1)	0.03 (4.8)	0.03 (4.2)
_n_-C_{36}	0.09 (6.5)	0.04 (5.0)	0.03 (5.4)	0.02 (6.5)	0.02 (2.1)	0.02 (5.6)	0.02 (5.4)	0.02 (3.6)
_n_-C_{37}	0.06 (3.6)	0.03 (1.8)	0.03 (4.6)	0.02 (5.3)	0.02 (3.8)	0.02 (4.7)	0.02 (2.2)	0.02 (5.3)
_n_-C_{38}	0.03 (6.6)	0.03 (2.3)	0.02 (3.4)	0.02 (3.2)	0.02 (3.2)	0.02 (3.2)	0.02 (4.3)	0.01 (4.4)
_n_-C_{39}	0.02 (3.3)	0.02 (3.2)	0.02 (2.4)	0.02 (2.1)	0.02 (4.2)	0.01 (2.1)	0.01 (3.9)	0.01 (6.8)
Sum	115.12	20.31	23.63	27.70	22.05	16.54	10.51	10.06
_n_-C_{17}/pristane	0.86	0.83	0.88	0.92	0.86	0.72	0.66	0.48
_n_-C_{18}/phytane	2.35	2.40	1.99	2.04	2.05	1.51	1.53	0.97
pristane/phytane	3.06	3.17	2.74	2.46	2.90	2.35	2.54	1.84
CPI*	1.23	1.18	1.10	1.20	1.16	1.14	1.13	1.12
$(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})$	2.92	0.96	0.89	0.78	0.48	0.30	0.02	0.10

Table 0.5: Changes in *n*-alkane and acyclic isoprenoid concentrations in biodegraded Nigerian medium oil in Nigerian sediment (mg/g oil).

n-Alkane	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
***n*-C$_8$**	0.44 (4.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_9$**	0.79 (0.5)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{10}$**	1.00 (4.9)	0.10 (4.2)	0.03 (5.5)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.09 (3.2)
***n*-C$_{11}$**	1.34 (2.7)	0.29 (5.3)	0.13 (5.2)	0.03 (4.5)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.12 (2.5)
***n*-C$_{12}$**	1.70 (1.0)	0.45 (6.2)	0.21 (0.8)	0.15 (1.6)	0.04 (1.5)	0.00 (0!)	0.00 (0!)	0.14 (4.6)
***n*-C$_{13}$**	1.83 (1.1)	0.56 (2.9)	0.37 (4.3)	0.31 (6.8)	0.23 (4.9)	0.26 (2.9)	0.00 (0!)	0.15 (3.6)
***n*-C$_{14}$**	1.90 (3.2)	0.65 (5.7)	0.29 (4.5)	0.32 (3.9)	0.38 (4.5)	0.78 (3.2)	0.12 (4.0)	0.16 (6.5)
***n*-C$_{15}$**	2.11 (4.3)	0.44 (2.5)	0.44 (6.8)	0.44 (2.9)	0.55 (3.8)	1.43 (5.7)	0.13 (4.7)	0.17 (7.3)
***n*-C$_{16}$**	1.64 (7.5)	0.62 (1.6)	0.59 (3.9)	0.65 (2.9)	0.79 (4.7)	1.91 (3.4)	0.20 (1.0)	0.19 (1.9)
***n*-C$_{17}$**	2.42 (3.8)	0.79 (5.8)	0.86 (4.6)	0.89 (6.6)	1.19 (5.5)	2.51 (1.0)	0.38 (4.4)	0.42 (7.3)
Pristane	5.36 (5.2)	3.14 (4.2)	1.66 (5.7)	1.90 (0.6)	2.81 (1.9)	8.05 (1.8)	1.20 (4.9)	1.45 (3.7)
***n*-C$_{18}$**	2.63 (0.5)	1.23 (2.0)	0.88 (5.5)	1.00 (5.1)	1.40 (3.6)	3.28 (2.4)	0.50 (1.4)	0.52 (5.8)
Phytane	2.25 (4.6)	1.17 (5.9)	0.83 (6.3)	0.95 (5.9)	1.16 (4.6)	3.96 (2.0)	0.54 (5.0)	0.79 (4.2)
***n*-C$_{19}$**	2.66 (3.6)	1.16 (7.5)	0.89 (4.2)	0.98 (4.3)	1.23 (2.9)	3.18 (2.8)	0.50 (4.1)	0.60 (5.9)
***n*-C$_{20}$**	2.69 (2.6)	1.22 (7.4)	0.87 (5.5)	0.93 (5.3)	1.28 (4.4)	3.29 (3.2)	0.51 (4.2)	0.57 (6.0)
***n*-C$_{21}$**	2.69 (2.6)	1.36 (4.9)	1.17 (6.1)	1.28 (6.8)	1.44 (2.9)	4.11 (2.7)	0.56 (6.8)	0.77 (6.9)
***n*-C$_{22}$**	2.53 (2.5)	1.16 (6.3)	0.90 (7.1)	1.03 (5.6)	1.31 (3.5)	3.36 (1.2)	0.39 (1.8)	0.61 (6.8)

n-C$_{23}$	2.57 (1.9)	1.35 (7.0)	0.97 (6.3)	1.01 (5.2)	1.28 (2.6)	3.37 (3.6)	0.54 (3.1)	0.63 (5.3)
n-C$_{24}$	2.43 (5.1)	1.51 (4.1)	0.99 (4.2)	1.07 (7.3)	1.35 (3.2)	3.48 (4.3)	0.58 (5.6)	0.67 (6.5)
n-C$_{25}$	2.37 (0.5)	1.48 (2.9)	0.97 (1.6)	1.02 (5.8)	1.37 (1.4)	3.69 (2.1)	0.54 (4.5)	0.68 (4.3)
n-C$_{26}$	2.26 (2.4)	1.37 (5.3)	0.91 (4.6)	0.98 (4.9)	1.26 (4.2)	3.46 (6.5)	0.59 (6.6)	0.63 (6.4)
n-C$_{27}$	2.29 (1.6)	1.46 (1.6)	0.94 (2.3)	1.03 (5.5)	1.32 (3.2)	3.54 (1.7)	0.53 (7.9)	0.66 (7.5)
n-C$_{28}$	2.22 (3.5)	1.21 (7.2)	0.77 (6.2)	0.85 (5.4)	1.10 (1.2)	2.95 (2.4)	0.53 (4.8)	0.55 (4.0)
n-C$_{29}$	2.05 (4.4)	1.24 (6.4)	0.78 (5.1)	0.84 (2.2)	1.08 (3.1)	2.89 (1.0))	0.56 (4.0)	0.59 (6.8)
n-C$_{30}$	1.54 (8.9)	0.78 (3.0)	0.70 (3.3)	0.55 (3.6)	0.74 (4.3)	1.55 (5.4)	0.41 (2.2)	0.48 (2.1)
n-C$_{31}$	1.66 (1.4)	0.75 (6.2)	0.49 (4.5)	0.49 (5.5)	0.58 (0.6)	1.51 (1.0)	0.39 (5.0)	0.40 (6.5)
n-C$_{32}$	0.94 (1.0)	0.33 (2.5)	0.25 (2.6)	0.19 (7.6)	0.33 (6.3)	0.85 (2.3)	0.24 (6.6)	0.24 (6.9)
n-C$_{33}$	0.74 (0.5)	0.25 (3.7)	0.17 (4.3)	0.16 (4.7)	0.22 (6.3)	0.46 (4.9)	0.19 (7.4)	0.19 (4.3)
n-C$_{34}$	0.36 (0.8)	0.07 (5.0)	0.06 (1.9)	0.06 (4.7)	0.05 (4.1)	0.17 (3.3)	0.08 (4.4)	0.08 (6.2)
n-C$_{35}$	0.24 (1.8)	0.06 (3.1)	0.04 (4.7)	0.02 (4.8)	0.04 (4.2)	0.12 (3.2)	0.05 (6.5)	0.05 (5.6)
n-C$_{36}$	0.15 (2.1)	0.03 (6.0)	0.03 (5.5)	0.02 (6.2)	0.02 (4.8)	0.06 (6.5)	0.03 (4.1)	0.04 (4.8)
n-C$_{37}$	0.14 (0.8)	0.03 (6.8)	0.02 (6.4)	0.02 (6.6)	0.02 (4.9)	0.03 (5.1)	0.02 (5.7)	0.03 (4.2)
n-C$_{38}$	0.13 (0.9)	0.02 (5.2)	0.02 (4.2)	0.02 (1.3)	0.02 (4.5)	0.02 (6.5)	0.01 (4.4)	0.03 (7.5)
n-C$_{39}$	0.11 (0.4)	0.02 (3.2)	0.02 (3.2)	0.01 (3.5)	0.01 (3.6)	0.01 (4.3)	0.01 (3.6)	0.03 (6.1)
Sum	58.16	26.29	18.27	19.23	24.58	64.30	10.30	12.73
n-C$_{17}$/pristane	0.45	0.25	0.51	0.47	0.42	0.31	0.31	0.29

***n*-C$_{18}$/phytane**	1.17	1.05	1.06	1.06	1.21	0.83	0.91	0.65
pristane/phytane	2.38	2.69	1.99	1.99	2.42	2.03	2.21	1.83
CPI*	1.11	1.19	1.09	1.15	1.14	1.17	1.07	1.12
(C$_8$+C$_{10}$+C$_{12}$+C$_{14}$)/(C$_{22}$+C$_{24}$+C$_{26}$+C$_{28}$)	0.53	0.23	0.15	0.12	0.08	0.06	0.06	0.16

Table 0.6: Changes in n-alkane and acyclic isoprenoid concentrations in biodegraded North Sea oil in Nigerian sediment (mg/g oil).

***n*-Alkane**	**Start**	**5days**	**10days**	**20days**	**50days**	**100days**	**300days**	**Sterilised**
***n*-C$_8$**	2.96 (5.6)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_9$**	3.63 (4.9)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{10}$**	4.45 (3.1)	0.11 (2.6)	0.02 (4.0)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)	0.00 (0!)
***n*-C$_{11}$**	4.95 (3.2)	0.87 (3.5)	0.59 (6.1)	0.15 (7.1)	0.01 (6.2)	0.00 (0!)	0.00 (0!)	0.17 (2.1)
***n*-C$_{12}$**	5.17 (5.3)	1.46 (2.9)	1.33 (2.6)	0.93 (5.7)	0.27 (2.7)	0.01 (4.0)	0.00 (0!)	0.16 (3.6)
***n*-C$_{13}$**	5.57 (2.9)	1.91 (2.0)	1.92 (1.2)	1.72 (5.0)	1.11 (5.2)	0.33 (3.4)	0.00 (0!)	0.22 5.2)
***n*-C$_{14}$**	5.57 (4.5)	2.13 (6.3)	2.57 (6,2)	2.28 (4.0)	2.27 (2.9)	0.89 (3.3)	0.10 (6.2)	0.31 (2.9)
***n*-C$_{15}$**	5.01 (4.4)	1.90 (5.0)	2.20 (4.6)	2.00 (2.0)	2.27 (5.2)	1.35 (4.3)	0.28 (5.2)	0.60 (4.0)
***n*-C$_{16}$**	4.13 (1.0)	1.74 (4.2)	2.07 (2.0)	1.85 (5.7)	2.16 (5.2)	1.26 (2.8)	0.39 (3.4)	1.22 (2.9)
***n*-C$_{17}$**	3.98 (3.2)	1.63 (6.9)	1.91 (1.5)	1.76 (7.6)	2.23 (3.5)	1.26 (1.1)	0.42 (4.7)	1.92 (1.9)
Pristane	2.02 (3.5)	0.85 (1.7)	0.97 (5.7)	0.96 (3.7)	1.23 (3.8)	1.14 (5.4)	0.51 (5.4)	1.76 (6.5)

***n*-C$_{18}$**	3.37 (1.7)	1.31 (2.9)	1.54 (4.7)	1.36 (3.7)	1.76 (6.2)	1.11 (2.4)	0.38 (7.4)	2.09 (4.3)
Phytane	1.58 (4.9)	0.74 (2.3)	0.76 (2.9)	0.84 (5.2)	0.99 (0.8)	0.84 (6.0)	0.46 (3.7)	1.43 (2.3)
***n*-C$_{19}$**	2.93 (5.1)	1.22 (6.6)	1.40 (1.2)	1.32 (5.0)	1.72 (2.7)	0.95 (2.0)	0.36 (4.0)	2.19 (0.4)
***n*-C$_{20}$**	2.53 (5.5)	1.09 (3.7)	1.30 (4.3)	1.27 (4.7)	1.45 (6.6)	0.81 (1.6)	0.31 (5.9)	2.02 (3.3)
***n*-C$_{21}$**	2.38 (3.4)	1.19 (4.5)	1.26 (7.7)	1.31 (5.9)	1.54 (4.1)	0.93 (1.5)	0.41 (3.6)	1.99 (4.4)
***n*-C$_{22}$**	2.24 (5.1)	0.92 (7.0)	1.09 (1.7)	1.00 (2.1)	1.25 (4.6)	0.77 (3.2)	0.30 (3.4)	1.83 (6.7)
***n*-C$_{23}$**	1.99 (5.2)	0.88 (5.9)	0.97 (3.0)	0.87 (5.6)	1.12 (5.6)	0.65 (2.7)	0.31 (2.6)	1.74 (4.1)
***n*-C$_{24}$**	1.82 (5.5)	0.82 (7.7)	0.91 (2.8)	0.87 (7.3)	1.04 (7.9)	0.63 (3.7)	0.31 (2.1)	1.26 (6.6
***n*-C$_{25}$**	1.51 (5.6)	0.69 (1.6)	0.79 (1.5)	0.71 (3.2)	0.86 (5.3)	0.52 (5.8)	0.27 (1.8)	0.94 (3.9)
***n*-C$_{26}$**	1.30 (5.4)	0.55 (5.4)	0.63 (2.6)	0.60 (7.3)	0.70 (2.2)	0.49 (5.5)	0.21 (4.6)	0.77 (3.5)
***n*-C$_{27}$**	1.00 (4.8)	0.49 (4.8)	0.51 (4.0)	0.50 (5.9)	0.62 (0.8)	0.35 (2.9)	0.20 (5.4)	0.82 (3.2)
***n*-C$_{28}$**	0.68 (1.9)	0.32 (5.1)	0.41 (3.5)	0.37 (3.7)	0.40 (6.1)	0.26 (3.8)	0.15 (4.2)	0.47 (1.3)
***n*-C$_{29}$**	0.52 (0.9)	0.36 (7.0)	0.31 (7.3)	0.37 (6.1)	0.39 (2.0)	0.24 (5.2)	0.14 (5.5)	0.47 (4.9)
***n*-C$_{30}$**	0.36 (2.3)	0.08 (1.0)	0.17 (4.6)	0.11 (1.8)	0.12 (4.4)	0.08 (6.8)	0.15 (4.6)	0.17 (5.5)
***n*-C$_{31}$**	0.18 (3.8)	0.34 (1.8)	0.14 (1.3)	0.40 (3.5)	0.08 (2.8)	0.06 (1.5)	0.07 (3.5)	0.26 (3.7)
***n*-C$_{32}$**	0.10 (4.1)	0.08 (5.5)	0.08 (4.1)	0.40 (7.6)	0.18 (3.1)	0.19 (5.6)	0.04 (4.0)	0.23 (4.2)
***n*-C$_{33}$**	0.09 (2.0)	0.08 (6.6)	0.04 (2.5)	0.10 (4.8)	0.05 (5.8)	0.05 (4.1)	0.06 (4.0)	0.20 (4.6)
***n*-C$_{34}$**	0.10 (4.2)	0.05 (5.5)	0.04 (1.8)	0.03 (5.1)	0.03 (4.5)	0.03 (1.0)	0.03 (4.3)	0.14 (0.3)
***n*-C$_{35}$**	0.05 (5.6)	0.05 (4.1)	0.04 (3.3)	0.03 (2.4)	0.03 (3.1)	0.02 (3.8)	0.02 (2.6)	0.07 (7.4)

n-C$_{36}$	0.05 (5.4)	0.04 (4.1)	0.03 (1.2)	0.03 (3.8)	0.02 (3.9)	0.02 (3.1)	0.02 (5.9)	0.05 (6.0)
n-C$_{37}$	0.04 (0.6)	0.03 (0.9)	0.03 (1.2)	0.02 (5.5)	0.02 (6.3)	0.02 (4.6)	0.02 (2.9)	0.04 (7.8)
n-C$_{38}$	0.03 (0.8)	0.03 (7.0)	0.03 (1.2)	0.02 (5.5)	0.02 (4.8)	0.02 (4.6)	0.01 (5.7)	0.03 (2.8)
n-C$_{39}$	0.03 (1.5)	0.02 (3.2)	0.02 (3.2)	0.02 (4.1)	0.02 (2.1)	0.01 (3.4)	0.01 (5.3)	0.03 (6.2)
Sum	72.32	24.00	26.06	24.21	25.97	15.30	5.97	25.59
n-C$_{17}$/pristane	1.98	1.93	1.98	1.83	1.82	1.10	0.84	1.09
n-C$_{18}$/phytane	2.14	1.78	2.02	1.62	1.77	1.32	0.82	1.46
pristane/phytane	1.28	1.15	1.27	1.14	1.24	1.37	1.09	1.23
CPI*	1.04	1.45	1.08	1.12	1.11	0.95	1.07	1.22
(C$_{8}$+C$_{10}$+C$_{12}$+C$_{14}$)/(C$_{22}$+C$_{24}$+C$_{26}$+C$_{28}$)	3.00	1.42	1.28	1.13	0.75	0.42	0.11	0.11

Table 0.7: Measured concentrations of target PAH in biodegraded Nigerian light oil (µg/g oil) and background concentration (µg/g EOM) in Whitley sediment.

PAH	Nigerian light oil (start)	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised 300 days	Background
C$_0$-N	247.3	36.9	8.8	3.2	1.6	0.0	0.0	0.0	18.6
C$_1$-N	1314.8	238.5	89.9	7.5	3.9	1.1	0.0	0.0	44.2
C$_2$-N	4680.0	1200.6	369.9	176.6	5.2	9.0	0.0	0.0	82.5
C$_3$-N	1412.8	898.1	465.5	211.5	3.8	89.2	2.8	4.8	72.6
C$_4$-N	828.0	301.2	254.6	82.6	1.9	90.5	1.5	35.5	37.4
∑Naphthalenes	**8483.0**	**2675.3**	**1188.7**	**481.4**	**189.8**	**4.3**	**16.5**	**40.3**	**255.3**
C$_0$-B	344.5	30.4	4.3	0.4	0.2	0.0	0.0	0.1	7.1

C_1-B	968.4	90.3	30.0	5.5	0.7	0.0	0.0	0.7	5.9
C_2-B	1005.4	110.7	65.6	17.1	13.3	0.0	0.0	10.4	0.0
∑Biphenyls	**2318.3**	**231.4**	**99.8**	**23.1**	**14.2**	**0.0**	**0.0**	**11.2**	**13.0**
C_0-DBF	135.3	13.9	4.1	0.7	0.4	0.0	0.0	0.1	7.1
C_1-DBF	167.9	63.2	38.4	10.4	9.9	0.0	0.0	3.3	26.1
C_2-DBF	515.9	218.7	187.7	64.5	69.8	0.0	0.0	42.7	41.5
C_3-DBF	309.8	127.7	127.9	60.9	55.6	0.0	0.0	39.1	18.4
∑Dibenzofurans	**1128.9**	**423.5**	**358.2**	**136.4**	**135.7**	**0.0**	**0.0**	**85.2**	**93.1**
C_0-F	52.3	12.7	4.3	0.7	0.2	0.0	0.0	0.4	4.9
C_1-F	259.0	57.7	32.0	7.7	6.4	0.0	0.0	9.5	10.8
C_2-F	246.5	87.5	74.1	22.8	26.9	0.0	0.0	30.0	13.5
∑Fluorenes	**557.8**	**157.9**	**110.4**	**31.2**	**33.6**	**0.0**	**0.0**	**39.9**	**29.3**
C_0-DBT	122.8	42.6	35.5	9.6	11.6	0.4	0.4	6.7	0.0
C_1-DBT	180.2	27.5	22.6	7.6	7.4	0.7	0.5	6.9	0.0
C_2-DBT	169.4	23.6	21.7	7.0	9.0	1.1	0.5	6.3	0.0
C_3-DBT	140.6	13.9	14.0	5.8	6.5	1.4	0.7	5.5	0.0
∑Dibenzothiophenes	**612.9**	**107.5**	**93.8**	**29.9**	**34.5**	**3.6**	**2.1**	**25.3**	**0.0**
C_0-P	242.6	44.7	31.7	10.0	4.2	1.8	0.0	14.2	28.7
C_1-P	441.8	101.7	70.2	20.6	20.8	4.3	0.0	47.1	39.1
C_2-P	514.0	121.4	109.8	36.4	49.8	9.6	0.0	72.3	30.9

C_3-P	287.7	201.5	131.6	81.6	51.6	10.6	0.0	91.6	0.0
C_4-P	87.3	26.8	27.3	11.4	14.9	10.4	0.0	15.7	0.0
∑Phenanthrenes	**1573.4**	**496.1**	**370.5**	**160.0**	**141.3**	**36.8**	**0.0**	**240.9**	**98.7**
C_0-Py	9.3	4.1	6.1	3.3	1.4	0.6	1.1	1.7	2.6
C_1-Py	58.6	22.9	26.4	9.1	12.3	4.2	3.8	11.4	5.6
C_2-Py	52.2	17.7	21.8	6.9	9.6	3.9	4.9	9.0	2.5
∑Pyrenes	**120.1**	**44.7**	**54.3**	**19.3**	**23.4**	**8.2**	**9.7**	**22.1**	**10.7**
C_0-C	5.3	6.9	3.3	1.9	1.5	0.5	1.7	1.2	1.3
C_1-C	15.8	14.2	7.3	2.7	3.5	1.7	2.6	2.6	1.9
C_2-C	17.9	17.5	7.9	3.2	4.3	1.8	3.8	3.3	1.7
∑Chrysene	**39.1**	**38.6**	**18.6**	**7.8**	**9.3**	**4.0**	**8.1**	**7.0**	**4.9**
C_0-B(a)A	2.0	0.9	1.8	1.1	0.4	0.2	0.3	0.5	0.9
C_1-B(a)A	2.4	0.8	1.2	0.4	0.5	0.3	0.2	0.4	0.4
∑Benzo a anthracene	**4.4**	**1.7**	**3.0**	**1.6**	**0.9**	**0.5**	**0.5**	**0.9**	**1.3**
C_{20}-TAS	4.3	4.1	1.8	1.8	1.5	1.4	0.5	3.3	0.5
C_{21}-TAS	2.0	1.8	0.7	0.7	0.6	0.5	0.9	3.1	0.6
C_{26}R+C_{27}S	27.7	24.2	10.2	9.8	8.4	7.4	1.0	10.9	2.4
C_{28}S-TAS	23.3	20.6	8.7	8.5	7.2	7.7	0.9	11.5	1.6
C_{27}R-TAS	14.6	13.2	5.4	5.3	4.5	4.6	0.7	6.9	1.3
C_{28}R-TAS	19.7	17.4	7.3	7.2	6.1	6.6	1.0	9.5	1.5

∑TAS	**91.6**	**81.4**	**34.1**	**33.3**	**28.4**	**28.1**	**5.0**	**45.2**	**7.9**
Anthracene	11.06	2.0	2.3	1.7	1.3	0.4	0.0	0.6	1.7
Perylene	4.9	1.8	1.8	1.3	1.9	1.5	1.1	1.2	0.0
2-MN/1-MN	2.87	1.80	1.79	1.75	1.80	1.74	#REF!	#REF!	1.29
C_0-P/C_0-Anth	21.9	22.1	13.6	5.9	3.2	4.1	#DIV/0!	23.9	17.3

	Oil sample	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised 300 days
4-MDBT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
a,b,c,d,e-PMN	0.43	0.50	0.77	0.94	1.22	2.30	0.91	2.91
v,w,x,y,z-PMN	1.10	1.27	1.97	2.87	3.26	4.59	2.67	7.47

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene. Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the amount of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). a, b, c, d, e-PMN and v, w, x, y, z-PMN are unidentified found in the two Nigerian oils. 0! represent result of values divided by 0 (undefined)

Table 0.8: Measured concentrations of target PAH in biodegraded Nigerian medium oil (µg/g) in Whitley sediment.

PAH	Nigerian medium oil (start)	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised 300 days
C_0-N	225.3	2.6	1.6	1.2	0.7	0.0	0.0	0.0
C_1-N	926.3	27.5	11.0	11.4	2.2	0.0	0.0	0.0
C_2-N	1733.9	355.0	303.1	207.6	3.1	0.0	0.0	0.0
C_3-N	1032.4	994.2	950.7	722.0	5.6	4.6	2.6	9.3
C_4-N	781.7	375.5	375.3	339.0	26.6	15.0	1.3	31.6

∑Naphthalenes	**4699.5**	**1754.7**	**1641.6**	**1281.1**	**38.3**	**19.6**	**0.0**	**40.9**
C_0-B	174.2	37.7	15.9	13.3	0.0	0.0	0.0	0.1
C_1-B	239.0	53.6	18.0	16.3	0.1	0.0	0.0	0.7
C_2-B	321.8	60.8	65.0	59.4	0.3	0.6	0.0	14.6
∑Biphenyls	**734.9**	**152.0**	**98.9**	**89.1**	**0.4**	**0.6**	**0.0**	**15.4**
C_0-DBF	103.6	27.9	15.9	9.7	0.0	0.0	0.0	0.4
C_1-DBF	220.8	79.1	71.8	61.1	0.0	1.0	0.0	5.4
C_2-DBF	538.8	209.5	232.7	250.7	14.7	25.3	0.0	48.6
C_3-DBF	257.8	143.4	158.6	173.4	38.2	37.0	0.0	33.2
∑Dibenzofurans	**1120.9**	**460.0**	**479.0**	**495.0**	**52.9**	**63.3**	**0.0**	**87.6**
C_0-F	71.8	30.8	19.4	13.6	0.1	0.1	0.0	2.5
C_1-F	250.8	72.3	61.8	52.6	1.9	3.4	0.0	21.3
C_2-F	354.2	123.6	119.7	115.1	10.3	20.5	0.0	52.1
∑Fluorenes	**676.8**	**226.6**	**200.9**	**181.3**	**12.3**	**24.0**	**0.0**	**75.9**
C_0-DBT	164.2	57.4	54.9	49.6	2.0	5.4	0.3	5.2
C_1-DBT	183.2	45.0	38.3	36.0	4.5	7.2	0.9	9.2
C_2-DBT	168.4	37.4	38.3	37.2	6.6	9.8	2.1	6.0
C_3-DBT	129.5	24.5	25.5	27.1	9.2	8.6	3.8	7.4
∑Dibenzothiophenes	**645.3**	**164.4**	**157.0**	**150.0**	**22.4**	**30.9**	**7.2**	**27.7**
C_0-P	297.3	96.3	70.2	66.0	6.6	3.6	2.8	15.1

C_1-P	619.2	161.6	149.9	142.0	31.3	5.2	3.3	67.0
C_2-P	712.9	257.9	225.0	210.6	67.3	27.9	13.8	132.4
C_3-P	321.9	241.4	234.1	225.3	133.8	84.3	50.8	122.3
C_4-P	193.8	58.0	60.9	64.5	30.2	38.7	11.7	32.3
∑Phenanthrenes	**2145.2**	**815.2**	**740.2**	**708.5**	**159.7**	**269.1**	**82.4**	**369.1**
C_0-Py	19.9	10.4	9.8	10.5	3.7	3.3	1.5	2.6
C_1-Py	120.7	58.3	48.1	47.8	20.5	17.2	7.3	42.0
C_2-Py	101.0	49.7	46.8	58.4	27.8	25.1	7.4	40.8
∑Pyrenes	**241.7**	**118.4**	**104.7**	**116.7**	**52.0**	**45.7**	**16.1**	**85.4**
C_0-C	13.8	10.7	9.5	8.6	3.5	3.9	1.2	8.2
C_1-C	37.1	11.9	18.9	22.5	11.7	11.2	3.8	8.6
C_2-C	38.9	14.5	22.9	24.4	17.3	13.1	5.1	11.4
∑Chrysene	**89.8**	**37.2**	**51.3**	**55.5**	**32.5**	**28.2**	**10.1**	**28.2**
C_0-B(a)A	4.2	1.9	1.8	2.1	2.7	0.9	0.3	1.8
C_1-B(a)A	4.5	2.7	2.3	2.8	3.4	1.5	0.6	2.1
∑Benzo a anthracene	**8.7**	**4.6**	**4.1**	**4.9**	**6.1**	**2.4**	**0.9**	**3.9**
C_{20}-TAS	6.1	3.3	3.2	3.2	2.7	3.1	1.4	3.4
C_{21}-TAS	2.9	1.3	1.3	1.3	1.1	1.0	0.4	1.4
C_{26}R+C_{27}S	22.0	9.2	8.4	8.6	8.2	9.1	3.6	9.2
C_{28}S-TAS	20.8	9.0	8.1	8.5	8.1	9.6	5.0	8.2

C_{27}R-TAS	11.7	5.6	5.1	5.2	5.1	5.9	2.6	5.2
C_{28}R-TAS	17.7	7.8	7.0	7.5	7.2	8.4	4.2	7.1
∑TAS	**81.2**	**36.2**	**33.1**	**34.4**	**32.4**	**37.2**	**17.1**	**34.6**
Anthracene	9.58	2.0	2.7	4.5	0.8	0.9	0.1	0.8
Perylene	2.6	2.2	2.5	3.8	2.3	2.7	1.05	1.2
2-MN/1-MN	2.87	1.16	1.22	1.17	1.1	#REF!	#REF!	#REF!
C_0-P/C_0-Anth	31.0	49.3	25.7	14.6	4.6	7.4	31.1	19.7

	Oil sample	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised 300 days
4-MDBT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
a, b,c,d,e-PMN	0.51	0.60	0.65	0.73	1.87	1.21	1.59	6.75
v,w,x,y,z-PMN	1.39	1.57	1.72	1.99	5.54	3.43	3.36	16.22

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene. Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). a, b, c, d, e-PMN and v, w, x, y, z-PMN are unidentified found in the two Nigerian oils. 0! represent result of values divided by 0 (undefined).

Table 0.9: Measured concentrations of target PAH in biodegraded North Sea oil (μg/g) in Whitley sediment.

PAH	N0rth Sea oil (start)	5days	10days	20days	50days	100days	300days	Sterilised 300days
C_0-N	581.4	83.3	51.3	30.3	2.1	0.0	0.0	0.0
C_1-N	799.1	126.4	63.7	46.7	4.3	4.3	0.0	0.1
C_2-N	1136.8	401.2	259.6	60.5	24.5	7.0	0.0	21.3

C_3-N	857.8	415.0	443.7	114.3	36.6	14.1	3.4	51.9
C_4-N	470.6	117.7	143.5	51.8	4.2	2.0	1.5	49.6
∑Naphthalenes	**3845.7**	**1143.6**	**961.8**	**303.6**	**69.6**	**27.4**	**0.0**	**122.7**
C_0-B	413.6	13.5	3.9	1.0	0.3	0.0	0.0	5.2
C_1-B	572.8	33.6	20.5	7.3	0.4	0.0	0.0	13.9
C_2-B	318.8	32.0	33.9	14.0	2.0	0.2	0.0	17.4
∑Biphenyls	**1305.3**	**79.2**	**58.2**	**22.3**	**2.6**	**0.2**	**0.0**	**17.4**
C_0-DBF	63.3	11.8	5.4	1.1	0.0	0.0	0.0	20.4
C_1-DBF	194.3	46.7	45.7	11.2	0.0	0.0	0.0	26.5
C_2-DBF	360.3	100.7	125.8	56.5	0.0	0.0	0.0	41.2
C_3-DBF	161.9	58.1	72.4	38.9	0.0	0.0	0.0	26.8
∑Dibenzofurans	**779.8**	**217.3**	**249.4**	**107.6**	**0.0**	**0.0**	**0.0**	**114.9**
C_0-F	78.4	72.9	25.4	0.6	0.3	0.2	0.0	6.7
C_1-F	426.0	144.6	100.2	12.2	1.2	0.6	0.0	36.6
C_2-F	398.0	141.2	120.0	34.2	3.0	1.4	0.0	59.1
∑Fluorenes	**902.4**	**358.7**	**245.7**	**47.0**	**4.4**	**2.2**	**0.0**	**102.4**
C_0-DBT	98.7	29.4	14.7	3.4	1.2	0.6	0.0	15.9
C_1-DBT	112.1	91.0	68.8	10.7	3.8	0.4	0.0	23.2
C_2-DBT	293.7	114.3	101.3	34.3	4.1	0.0	0.0	54.9
C_3-DBT	163.4	57.4	52.2	21.5	13.9	0.0	0.0	31.5

∑Dibenzothiophenes	**667.9**	**292.1**	**237.0**	**69.9**	**23.0**	**1.0**	**0.0**	**125.5**
C_0-P	500.6	283.7	124.4	8.3	3.3	0.0	0.0	101.8
C_1-P	769.6	459.0	337.8	77.3	6.8	0.0	0.0	258.1
C_2-P	943.6	400.0	345.3	114.4	15.7	0.0	0.0	295.9
C_3-P	623.4	286.8	263.1	196.4	111.4	45.5	0.0	227.3
C_4-P	165.4	53.6	47.9	20.1	29.3	5.9	0.0	28.7
∑Phenanthrenes	**3002.6**	**1483.1**	**1118.6**	**416.5**	**51.4**	**166.6**	**0.0**	**911.8**
C_0-Py	55.2	19.0	17.7	4.9	2.9	1.5	1.3	16.7
C_1-Py	224.2	72.0	67.8	26.0	28.6	20.3	3.1	37.0
C_2-Py	181.2	62.6	57.5	22.2	29.8	21.6	2.6	31.1
∑Pyrenes	**460.6**	**153.7**	**143.0**	**53.1**	**61.3**	**43.5**	**7.0**	**84.9**
C_0-C	96.8	30.0	27.2	22.0	19.3	15.3	0.9	16.9
C_1-C	164.1	58.9	53.5	42.8	37.9	23.1	1.6	32.3
C_2-C	123.5	46.2	40.8	36.6	33.7	17.2	1.8	23.4
∑Chrysene	**384.5**	**135.0**	**121.5**	**101.4**	**90.9**	**55.5**	**4.2**	**72.7**
C_0-B(a)A	13.2	3.6	3.6	12.0	1.3	0.6	0.3	2.0
C_1-B(a)A	10.4	3.7	3.5	1.4	2.7	1.5	0.2	1.8
∑Benzo a anthracene	**23.6**	**7.3**	**7.1**	**13.5**	**4.1**	**2.1**	**0.5**	**3.9**
C_{20}-TAS	12.9	4.8	4.8	4.4	4.8	5.8	2.1	5.9
C_{21}-TAS	2.5	1.1	0.9	0.9	0.8	1.0	1.3	1.5

C_{26}R+C_{27}S	11.5	4.1	3.4	3.7	4.3	4.8	4.9	5.3
C_{28}S-TAS	7.6	2.9	2.9	3.2	3.2	3.6	4.9	6.0
C_{27}R-TAS	6.9	2.7	2.9	3.1	2.7	3.2	3.2	4.1
C_{28}R-TAS	6.6	2.6	2.2	3.0	2.6	3.0	4.4	4.0
∑TAS	**48.0**	**18.1**	**17.1**	**18.3**	**18.5**	**21.4**	**20.8**	**26.9**
Anthracene	16.45	1.0	1.5	1.3	0.3	0.0	0.0	0.6
Perylene	13.4	7.0	6.8	2.6	7.6	8.0	0.85	3.8
2-MN/1-MN	2.87	1.28	1.16	1.36	1.87	#REF!	#REF!	1.5
C_0-P/C_0-Anth	30.4	287.2	85.5	6.3	9.9	#DIV/0!	#DIV/0!	164.4

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene. Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C0-N), whereas a daughter PAH e.g. methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). 0! represent result of values divided by 0 (undefined).

Table 0.10: Measured concentrations of target PAH in biodegraded Nigerian light oil (µg/g oil) and background concentration (µg/g EOM) in Nigerian sediment.

PAH	Nigerian light oil (start)	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised 300 days	Sediment only
C_0-N	247.3	154.6	9.4	0.0	0.0	0.0	0.0	0.0	0.1
C_1-N	1314.8	757.0	289.7	1.7	0.0	0.0	0.0	0.3	0.1
C_2-N	4680.0	1203.5	815.8	128.7	3.6	0.0	0.0	34.8	0.1
C_3-N	1412.8	794.3	645.6	409.2	82.6	55.3	4.3	166.1	0.0
C_4-N	828.0	301.6	408.3	219.5	160.7	133.6	38.4	300.9	0.0
∑Naphthalenes	**8483.0**	**3211.1**	**2168.8**	**759.2**	**246.8**	**188.9**	**42.7**	**502.0**	**0.2**

C_0-B	344.5	34.6	9.1	4.6	0.0	0.0	0.0	1.4	0.0
C_1-B	968.4	88.8	37.5	31.3	0.0	0.0	0.0	11.2	0.0
C_2-B	1005.4	99.5	63.7	52.9	0.0	0.0	0.0	27.7	0.5
∑Biphenyls	**2318.3**	**223.0**	**110.3**	**88.9**	**0.0**	**0.0**	**0.0**	**40.2**	**0.5**
C_0-DBF	135.3	10.9	8.2	8.0	2.0	0.2	0.0	2.2	0.1
C_1-DBF	167.9	58.0	46.8	39.6	17.8	15.7	0.0	19.3	0.4
C_2-DBF	515.9	238.2	233.1	139.5	116.4	66.4	51.3	140.0	3.3
C_3-DBF	309.8	202.6	285.9	117.7	203.5	114.2	77.0	248.5	7.1
∑Dibenzofurans	**1128.9**	**509.8**	**573.9**	**304.8**	**339.7**	**196.5**	**128.3**	**410.0**	**10.9**
C_0-F	52.3	10.4	9.0	7.6	5.4	5.4	3.7	3.5	0.2
C_1-F	259.0	49.7	49.8	36.2	35.7	34.0	22.3	24.9	1.0
C_2-F	246.5	101.3	157.3	103.7	103.5	76.6	61.9	93.8	12.4
∑Fluorenes	**557.8**	**161.4**	**216.0**	**147.5**	**144.6**	**116.0**	**87.8**	**122.2**	**13.6**
C_0-DBT	122.8	59.2	52.2	38.8	33.8	28.8	25.4	9.0	0.0
C_1-DBT	180.2	43.9	43.8	33.3	27.0	22.7	20.5	36.1	0.0
C_2-DBT	169.4	62.4	52.2	36.5	34.8	27.2	19.7	52.0	0.0
C_3-DBT	140.6	57.4	52.9	35.1	30.3	23.5	21.9	60.2	0.0
∑Dibenzothiophenes	**612.9**	**223.0**	**201.1**	**143.6**	**125.8**	**102.3**	**87.5**	**157.3**	**0.0**
C_0-P	242.6	61.4	58.2	54.5	43.1	33.9	31.9	37.6	1.8
C_1-P	441.8	95.2	93.5	86.4	84.7	67.8	58.5	73.1	3.7

C_2-P	514.0	230.9	157.4	154.4	134.6	126.0	111.6	252.3	15.5
C_3-P	287.7	202.3	124.7	138.6	131.3	127.2	119.5	211.9	0.0
C_4-P	87.3	75.1	89.9	127.4	119.9	80.6	61.3	138.4	22.6
∑Phenanthrenes	**1573.4**	**664.8**	**523.6**	**561.3**	**513.5**	**435.4**	**382.8**	**713.4**	**43.7**
C_0-Py	9.3	10.8	11.3	17.5	15.5	8.3	6.3	14.2	4.2
C_1-Py	58.6	56.2	73.0	51.3	38.3	32.4	29.9	57.6	17.2
C_2-Py	52.2	62.5	87.0	48.6	44.9	37.9	35.7	62.6	14.9
∑Pyrenes	**120.1**	**129.5**	**171.3**	**117.4**	**98.7**	**78.6**	**71.9**	**134.4**	**36.2**
C_0-C	5.3	6.8	10.4	14.8	8.2	5.3	5.1	9.6	3.6
C_1-C	15.8	25.2	37.3	27.1	18.0	17.2	14.6	31.8	9.2
C_2-C	17.9	20.7	36.4	49.6	33.0	24.9	20.2	45.3	12.7
∑Chrysene	**39.1**	**52.8**	**84.2**	**91.5**	**59.2**	**47.5**	**39.8**	**86.8**	**25.5**
C_0-B(a)A	2.0	2.2	3.2	2.2	1.6	1.5	1.2	2.7	1.0
C_1-B(a)A	2.4	3.5	4.6	6.8	4.3	2.9	2.7	4.9	1.2
∑Benzo a anthracene	**4.4**	**5.7**	**7.8**	**9.0**	**5.9**	**4.5**	**4.0**	**7.6**	**2.2**
C_{20}-TAS	4.3	5.3	8.1	7.4	3.3	1.7	1.7	8.7	0.2
C_{21}-TAS	2.0	2.4	2.7	5.0	2.4	1.1	0.7	3.3	1.8
$C_{26}R+C_{27}S$	27.7	32.3	44.3	33.9	20.3	9.7	3.9	62.5	0.2
$C_{28}S$-TAS	23.3	25.9	33.9	24.0	16.5	8.3	2.8	49.0	0.2
$C_{27}R$-TAS	14.6	18.6	25.0	23.0	10.8	5.3	3.2	33.6	0.3

C_{28}R-TAS	19.7	24.2	31.3	29.1	14.0	7.0	3.8	43.2	0.3
∑TAS	**91.6**	**108.7**	**145.2**	**122.4**	**67.3**	**33.1**	**16.1**	**200.2**	**3.0**
Anthracene	11.1	13.7	9.2	2.5	1.7	1.5	1.5	3.8	0.8
Perylene	4.9	8.0	10.8	15.9	7.2	8.7	5.5	10.8	2.9
2-MN/1-MN	2.87	1.71	1.30	1.10	#DIV/0!	#DIV/0!	#DIV/0!	1.48	1.14
C_0-P/C_0-Anth	21.9	6.6	19.5	29.0	21.5	4.3	20.9	10.0	2.2

	Oil sample	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised 300 days**
4-MDBT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
a,b,c,d,e-PMN	0.43	0.94	0.75	0.62	0.97	1.28	1.87	1.28
v,w,x,y,z-PMN	1.10	2.15	1.63	1.53	2.24	2.77	4.39	3.28

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene. Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g. methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). 0! represent result of values divided by 0 (undefined).

Table 0.11: Measured concentrations of target PAH in biodegraded Nigerian medium oil (µg/g) in Nigerian sediment.

PAH	**Nigerian medium oil (start)**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised 300 days**
C_0-N	225.3	34.5	0.0	0.0	0.0	0.0	0.0	0.0
C_1-N	926.3	68.2	36.1	0.0	0.0	0.0	0.0	0.0
C_2-N	1733.9	435.6	370.1	0.0	0.0	0.0	0.0	10.1
C_3-N	1032.4	815.5	778.9	181.3	131.8	0.0	0.0	249.8

C_4-N	781.7	333.3	463.4	268.0	229.7	47.0	36.1	407.4
∑Naphthalenes	**4699.5**	**1687.1**	**1648.5**	**449.3**	**361.5**	**47.0**	**36.1**	**667.3**
C_0-B	174.2	50.5	17.0	1.2	0.0	0.0	0.0	3.5
C_1-B	239.0	24.1	21.2	3.1	0.0	0.0	0.0	7.8
C_2-B	321.8	54.8	41.9	19.8	0.0	0.0	0.0	32.3
∑Biphenyls	**734.9**	**129.5**	**80.2**	**24.1**	**0.0**	**0.0**	**0.0**	**43.6**
C_0-DBF	103.6	20.3	18.2	15.3	9.2	0.0	0.0	7.3
C_1-DBF	220.8	64.3	58.2	50.7	31.4	0.0	0.0	33.6
C_2-DBF	538.8	249.7	54.0	64.3	145.7	252.0	108.8	184.7
C_3-DBF	257.8	311.3	248.1	163.8	138.0	121.3	86.2	318.9
∑Dibenzofurans	**1120.9**	**645.6**	**378.5**	**294.0**	**324.3**	**373.3**	**195.0**	**544.5**
C_0-F	71.8	21.4	19.4	17.3	15.3	13.4	11.8	14.5
C_1-F	250.8	59.6	54.5	47.4	45.0	44.5	33.9	48.2
C_2-F	354.2	176.4	130.9	125.5	123.9	113.1	91.3	148.0
∑Fluorenes	**676.8**	**257.4**	**204.9**	**190.2**	**184.3**	**171.0**	**137.0**	**210.7**
C_0-DBT	164.2	70.2	52.1	46.2	45.0	13.9	8.6	74.0
C_1-DBT	183.2	52.8	49.5	44.6	39.0	36.6	32.0	62.7
C_2-DBT	168.4	68.8	64.0	52.7	46.1	43.4	42.4	88.8
C_3-DBT	129.5	63.2	60.8	49.3	46.8	42.7	33.7	93.4
∑Dibenzothiophenes	**645.3**	**255.0**	**226.4**	**192.7**	**176.8**	**136.6**	**116.7**	**318.8**

C_0-P	297.3	75.4	74.9	72.5	67.5	65.2	54.6	84.4
C_1-P	619.2	145.8	131.1	128.5	124.8	119.8	101.4	170.1
C_2-P	712.9	315.2	274.1	241.5	221.4	209.8	167.3	444.4
C_3-P	321.9	232.7	205.7	178.2	163.6	123.4	102.7	251.6
C_4-P	193.8	149.9	143.5	133.7	123.2	100.0	88.7	205.2
∑Phenanthrenes	**2145.2**	**918.9**	**829.3**	**754.5**	**700.5**	**618.2**	**514.6**	**1155.8**
C_0-Py	19.9	18.3	18.0	17.3	13.7	13.0	12.0	21.1
C_1-Py	120.7	86.1	78.8	70.9	64.5	55.0	54.8	96.1
C_2-Py	101.0	100.4	82.3	73.6	68.7	61.6	59.1	97.1
∑Pyrenes	**241.7**	**204.9**	**179.1**	**161.8**	**146.9**	**129.5**	**125.9**	**214.3**
C_0-C	13.8	13.9	16.8	11.5	11.4	9.0	9.0	18.2
C_1-C	37.1	39.4	46.5	36.6	31.5	31.0	27.2	57.0
C_2-C	38.9	49.0	61.8	48.7	43.9	42.3	37.1	74.3
∑Chrysene	**89.8**	**102.3**	**125.2**	**96.9**	**86.8**	**82.3**	**73.3**	**149.6**
C_0-B(a)A	4.2	3.7	2.9	2.7	2.5	2.3	2.0	4.3
C_1-B(a)A	4.5	6.9	7.7	5.4	5.1	4.9	4.5	8.5
∑Benzo a anthracene	**8.7**	**10.7**	**10.6**	**8.2**	**7.6**	**7.2**	**6.5**	**12.7**
C_{20}-TAS	6.1	7.3	9.8	21.0	8.8	4.9	2.3	8.4
C_{21}-TAS	2.9	3.9	5.0	8.5	3.1	3.0	3.0	3.1
C_{26}R+C_{27}S	22.0	55.2	2.0	9.2	98.6	48.9	35.3	47.6

C_{28}S-TAS	20.8	44.4	81.4	37.7	27.3	5.2	1.3	37.8
C_{27}R-TAS	11.7	21.1	31.0	59.5	25.7	11.9	2.6	25.3
C_{28}R-TAS	17.7	27.6	39.5	78.4	32.5	14.2	3.1	34.0
∑TAS	**81.2**	**159.5**	**168.6**	**214.3**	**196.0**	**88.1**	**47.7**	**156.1**
Anthracene	9.58	12.3	2.1	1.4	3.1	16.1	3.1	5.1
Perylene	2.6	12.1	10.3	15.9	8.0	12.4	6.9	12.2
2-MN/1-MN	1.54	1.21	1.31	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
C_0-P/C_0-Anth	31.0	6.1	35.2	50.4	21.0	4.2	17.9	16.6

	Oil sample	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised 300 days
4-MDBT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
a,b,c,d,e-PMN	0.51	0.68	0.84	0.95	1.07	1.24	1.30	0.82
v,w,x,y,z-PMN	1.39	1.80	1.97	2.33	2.48	3.25	2.93	2.10

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene. Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g. methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). 0! represent result of values divided by 0 (undefined).

Table 0.12: Measured concentrations of target PAH in biodegraded North Sea oil (µg/g) in Nigeria sediment.

PAH	North Sea oil (start)	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised 300 days
C_0-N	581.4	217.8	0.0	0.0	0.0	0.0	0.0	0.0
C_1-N	799.1	840.9	0.0	0.0	0.0	0.0	0.0	0.0

C_2-N	1136.8	1127.0	625.1	41.8	0.0	0.0	0.0	0.0
C_3-N	857.8	645.3	613.5	348.7	131.4	15.8	0.0	85.6
C_4-N	470.6	312.7	193.8	167.9	148.6	77.9	41.5	206.2
∑Naphthalenes	**3845.7**	**3143.8**	**1432.3**	**558.4**	**280.0**	**93.7**	**41.5**	**291.9**
C_0-B	413.6	62.4	8.5	4.0	0.0	0.0	0.0	2.0
C_1-B	572.8	93.4	32.8	30.5	0.9	0.0	0.0	15.7
C_2-B	318.8	61.2	45.1	37.3	3.9	0.0	0.0	26.0
∑Biphenyls	**1305.3**	**217.0**	**86.4**	**71.8**	**4.8**	**0.0**	**0.0**	**26.0**
C_0-DBF	63.3	14.2	12.2	11.9	5.3	1.5	0.0	3.3
C_1-DBF	194.3	54.4	44.7	44.3	24.5	12.5	5.2	20.7
C_2-DBF	360.3	192.5	168.9	113.2	75.0	68.9	49.9	115.8
C_3-DBF	161.9	182.8	221.6	114.3	99.2	98.8	60.5	205.4
∑Dibenzofurans	**779.8**	**444.0**	**447.5**	**283.7**	**203.9**	**181.7**	**115.6**	**345.2**
C_0-F	78.4	48.4	39.8	38.3	35.5	34.5	23.7	19.6
C_1-F	426.0	95.4	95.2	83.3	84.5	76.9	55.0	56.6
C_2-F	398.0	154.2	145.4	129.3	120.1	114.5	89.8	123.8
∑Fluorenes	**902.4**	**297.9**	**280.3**	**250.8**	**129.3**	**111.4**	**144.7**	**199.9**
C_0-DBT	98.7	40.1	20.3	18.4	16.8	15.1	4.7	37.8
C_1-DBT	112.1	65.5	65.6	59.7	62.0	40.9	39.1	49.4
C_2-DBT	293.7	111.9	104.1	105.7	94.9	81.8	73.7	106.2

C$_3$-DBT	163.4	80.9	71.0	67.6	55.2	57.3	51.3	89.4
∑Dibenzothiophenes	**667.9**	**298.4**	**261.1**	**251.4**	**228.9**	**195.0**	**168.8**	**282.8**
C$_0$-P	500.6	202.0	185.1	182.1	169.6	173.8	150.3	135.6
C$_1$-P	769.6	330.9	309.0	300.9	282.1	284.3	265.6	254.2
C$_2$-P	943.6	390.8	343.1	330.4	308.7	297.7	263.4	421.8
C$_3$-P	623.4	294.3	267.6	253.4	228.5	211.9	119.2	251.6
C$_4$-P	165.4	111.1	87.9	116.1	78.3	129.8	91.0	162.1
∑Phenanthrenes	**3002.6**	**1329.0**	**1192.7**	**1182.9**	**1067.2**	**1097.4**	**889.5**	**1225.4**
C$_0$-Py	55.2	23.1	20.6	21.5	18.0	17.1	15.2	23.5
C$_1$-Py	224.2	89.4	86.0	76.4	78.5	63.2	59.0	94.0
C$_2$-Py	181.2	94.6	93.5	79.9	78.8	67.3	59.2	91.0
∑Pyrenes	**460.6**	**207.2**	**200.1**	**177.8**	**175.3**	**147.5**	**133.4**	**208.5**
C$_0$-C	96.8	29.0	25.0	28.4	26.0	26.0	22.7	26.8
C$_1$-C	164.1	65.0	61.8	61.4	53.8	55.0	51.2	66.4
C$_2$-C	123.5	66.2	59.7	61.0	54.0	50.5	49.8	71.0
∑Chrysene	**384.5**	**160.2**	**146.5**	**150.8**	**133.9**	**131.6**	**123.7**	**164.3**
C$_0$-B(a)A	13.2	3.9	3.9	4.2	3.2	3.6	3.3	4.7
C$_1$-B(a)A	10.4	7.6	6.1	5.1	4.9	5.0	4.3	7.2
∑Benzo a anthracene	**23.6**	**11.5**	**10.0**	**9.3**	**8.1**	**8.7**	**7.6**	**11.9**
C$_{20}$-TAS	12.9	9.5	10.2	9.3	7.9	7.8	4.2	11.6

C_{21}-TAS	2.5	2.9	3.0	5.0	2.6	2.4	2.2	3.3
C_{26}R+C_{27}S	11.5	19.0	36.3	38.8	36.0	25.0	3.4	50.0
C_{28}S-TAS	7.6	11.8	28.5	29.2	27.3	19.5	3.5	35.9
C_{27}R-TAS	6.9	14.1	20.6	21.9	20.7	17.5	4.6	29.1
C_{28}R-TAS	6.6	17.6	25.4	27.1	25.7	21.0	5.3	36.4
∑TAS	**48.0**	**74.8**	**124.1**	**131.3**	**120.3**	**93.2**	**23.2**	**166.3**
Anthracene	16.45	9.2	2.0	1.3	3.1	5.8	2.5	6.9
Perylene	13.4	10.2	10.2	10.7	5.3	10.6	5.24	8.5
2-MN/1-MN	1.34	1.27	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
C_0-P/C_0-Anth	30.4	20.1	76.1	130.3	65.0	34.6	68.4	19.5

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene. Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). 0! represent result of values divided by 0 (undefined).

Table 0.13: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing biodegradation for Nigerian light oil in Whitley sediment.

Isomer	Oil (start)	5days	10days	20days	50days	100days	300days	Sterilised
2-MBp	32.5	1.0	0.3	0.2	0.01	0.0	0.0	0.0
3-MBp	723.0	64.4	20.8	3.6	0.40	0.0	0.0	0.4
4-MBp	212.9	24.9	8.9	1.7	0.26	0.0	0.0	0.3
∑Isomer	**968.4**	**90.3**	**30.0**	**5.5**	**0.7**	**0.0**	**0.0**	**0.7**
3-MP	118.4	23.5	14.3	3.8	2.9	0.6	0.0	10.9

2-MP	116.2	25.8	16.3	4.9	4.7	0.6	0.0	10.9
MA	17.2	4.7	4.8	1.8	1.9	0.4	0.0	2.0
9-MP	110.5	27.9	21.1	5.8	7.5	1.4	0.0	13.5
1-MP	79.5	19.8	13.7	4.3	3.8	1.4	0.0	9.8
∑Isomer	441.8	101.7	70.2	20.6	20.8	4.3	0.0	47.1

Table 0.14: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing biodegradation for Nigerian medium oil in Whitley sediment.

Isomer	Oil (start)	5days	10days	20days	50days	100days	300days	Sterilised
2-MBp	10.6	1.62	0.32	0.70	0.02	0.0	0.0	0.0
3-MBp	107.9	23.71	8.69	7.86	0.04	0.0	0.0	0.3
4-MBp	120.4	28.27	9.00	7.77	0.03	0.0	0.0	0.4
∑Isomer	**239.0**	**53.6**	**18.0**	**16.3**	**0.1**	**0.0**	**0.0**	**0.7**
3-MP	145.9	33.5	30.2	28.3	4.7	0.8	0.8	13.8
2-MP	151.7	36.4	33.5	31.3	6.8	1.1	0.8	15.3
MA	20.7	5.7	5.5	6.0	2.4	0.4	0.4	2.2
9-MP	170.4	53.3	48.2	47.1	10.0	1.7	0.8	21.7
1-MP	130.5	32.6	32.6	29.2	7.4	1.2	0.5	13.9
∑Isomer	619.2	**161.6**	**149.9**	**142.0**	**31.3**	**5.2**	**3.3**	**67.0**

Table 0.15: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing biodegradation for North Sea oil in Whitley sediment.

Isomer	Oil (start)	5days	10days	20days	50days	100days	300days	Sterilised
2-MBp	26.5	0.5	0.5	0.4	0.0	0.0	0.0	0.2
3-MBp	383.3	22.0	14.3	4.6	0.4	0.0	0.0	8.3
4-MBp	163.0	11.1	5.6	2.2	0.0	0.0	0.0	5.4
***∑Isomer**	**572.8**	**33.6**	**20.5**	**7.3**	**0.4**	**0.0**	**0.0**	**13.9**
3-MP	167.6	89.6	59.3	12.3	0.8	0.0	0.0	48.9
2-MP	184.1	99.8	72.2	16.3	1.3	0.0	0.0	55.2
MA	19.8	7.7	7.9	2.6	1.0	0.0	0.0	3.4
9-MP	232.0	152.1	115.9	25.5	1.9	0.0	0.0	87.8
1-MP	166.1	109.8	82.4	20.7	1.7	0.0	0.0	62.7
∑Isomer	769.6	459.0	337.8	77.3	6.8	0.0	0.0	258.1

Table 0.16: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing biodegradation for Nigerian light oil in Nigerian sediment.

Isomer	Oil (start)	5days	10days	20days	50days	100days	300days	Sterilised
2-MBp	32.5	2.4	1.6	0.0	0.00	0.0	0.0	0.1
3-MBp	723.0	63.0	25.6	22.1	0.00	0.0	0.0	8.0
4-MBp	212.9	23.4	10.4	9.2	0.00	0.0	0.0	3.1
∑Isomer	**968.4**	**88.8**	**37.5**	**31.3**	**0.0**	**0.0**	**0.0**	**11.2**
3-MP	118.4	20.9	20.4	20.1	19.8	14.3	13.8	16.0

2-MP	116.2	23.3	21.7	20.0	19.3	15.8	13.7	17.1
MA	17.2	6.1	5.5	5.0	4.6	5.4	2.3	4.5
9-MP	110.5	25.9	27.7	26.2	23.3	17.0	17.2	21.5
1-MP	79.5	19.0	18.1	15.2	17.7	15.3	11.5	14.0
∑Isomer	441.8	95.2	93.5	86.4	84.7	67.8	58.5	73.1

Table 0.17: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing biodegradation for Nigerian medium oil in Nigerian sediment.

Isomer	Oil (start)	5days	10days	20days	50days	100days	300days	sterilised
2-MBp	10.6	1.74	1.39	0.00	0.00	0.0	0.0	0.2
3-MBp	107.9	10.58	5.63	0.78	0.00	0.0	0.0	3.8
4-MBp	120.4	11.82	4.23	2.36	0.00	0.0	0.0	3.8
∑Isomer	**239.0**	**24.1**	**11.2**	**3.1**	**0.0**	**0.0**	**0.0**	**7.8**
3-MP	145.9	30.0	25.8	25.6	29.3	25.4	24.8	33.7
2-MP	151.7	33.8	28.2	27.2	32.9	29.1	26.1	36.0
MA	20.7	10.9	11.1	9.2	8.7	8.5	4.3	8.0
9-MP	170.4	43.7	39.6	41.6	45.9	40.4	41.0	56.5
1-MP	130.5	27.4	26.4	24.9	29.0	27.8	23.7	35.8
∑Isomer	619.2	145.8	131.1	128.5	145.8	131.1	119.8	170.1

Table 0.18: Target isomer (methylbiphenyls and methylphenanthrenes) concentration changes with increasing biodegradation for North Sea oil in Nigerian sediment.

Isomer	Oil (start)	5days	10days	20days	50days	100days	300days	sterilised
2-MBp	26.5	8.4	0.1	0.00	0.00	0.0	0.0	0.4
3-MBp	383.3	59.1	15.4	21.64	0.39	0.0	0.0	10.3
4-MBp	163.0	25.9	17.3	8.88	0.48	0.0	0.0	5.0
∑Isomer	**572.8**	**93.4**	**32.8**	**30.5**	**0.9**	**0.0**	**0.0**	**15.7**
3-MP	167.6	62.4	58.2	56.5	54.2	54.5	51.1	47.6
2-MP	184.1	72.9	67.3	65.2	59.7	58.3	56.2	54.0
MA	19.8	6.0	9.5	8.3	6.4	4.0	6.9	7.6
9-MP	232.0	108.2	94.6	94.5	91.7	97.5	83.0	84.5
1-MP	166.1	81.5	79.5	76.4	70.0	70.0	68.3	60.5
∑Isomer	769.6	330.9	309.0	300.9	282.1	284.3	265.6	254.2

Table 0.19: Measured concentrations (µg/g oil) of terpanes in biodegraded Nigerian light oil in Whitley sediment

Peak Number	**Terpanes**	**Measured Concentration (C_{det}) (m/z 191)**								
	Tricyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**S**	8.2	429.5	141.4	155.1	179.4	133.0	138.3	105.8	88.2

2	**Q**	88.2	114.0	75.7	75.5	59.9	70.9	52.4	37.9	86.3
3	**C_{19}**	12.5	330.0	130.7	151.6	142.5	129.7	84.2	78.7	71.6
4	**M**	3.0	118.5	65.4	79.7	77.5	65.4	60.5	25.6	28.8
5	**C_{20}**	4.7	295.9	143.5	164.3	147.1	129.7	78.3	80.5	91.3
6	**X**	16.5	462.1	160.4	178.0	188.6	158.0	165.4	135.2	120.6
7	**C_{21}**	6.1	284.0	105.3	114.4	124.8	103.4	81.6	68.1	67.1
8	**C_{22}**	7.9	172.7	61.2	65.6	64.2	55.3	38.6	34.7	42.2
9	**C_{23}**	53.9	368.4	143.6	153.4	160.4	129.0	119.0	71.2	129.8
10	**C_{24}**	53.8	391.0	134.2	163.1	156.6	134.5	131.8	84.8	77.7
11	**Y**	37.1	415.3	204.6	217.9	175.0	136.2	82.7	24.1	88.3
12	**Y1**	25.7	684.0	230.4	261.9	265.9	232.3	244.6	157.7	135.5
13	**C_{25}A**	12.4	105.0	64.1	73.4	44.3	66.6	67.0	27.2	53.6
14	**C_{25}B**	11.1	392.2	135.9	158.1	152.0	133.4	131.7	107.7	99.2
15	**Z**	11.4	184.3	97.1	108.7	75.3	60.3	66.7	41.1	39.9
16	**Tet24**	57.0	446.4	127.5	141.2	138.9	117.6	92.2	59.0	73.7
17	**C_{26}(S)**	45.7	56.2	65.1	70.9	71.8	59.2	55.3	19.0	22.1
18	**C_{26}(R)**	60.4	181.6	68.1	75.7	75.3	62.5	46.8	34.1	34.9

Measured Concentration (C_{det})
(m/z 191)

	Tetracyclic Terpanes	Background (µg/g EOM)	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
19	**Z1**	24.8	387.1	121.0	129.9	132.0	109.2	119.0	74.6	58.8
20	**C_{28}A**	44.8	274.9	141.3	158.4	155.0	134.3	111.3	27.7	60.7
21	**C_{28}B**	36.1	135.1	85.5	86.8	89.2	72.5	56.3	29.0	42.0
22	**C_{29}A**	46.8	198.0	56.3	88.2	40.7	30.9	37.6	18.5	45.3
23	**C_{29}B**	53.4	276.4	121.3	93.8	110.5	90.7	67.6	12.4	64.9
24	**T**	43.6	203.7	106.7	127.6	110.7	101.9	105.3	65.5	43.3

Measured Concentration (C_{det})
(m/z 191)

	Pentacyclic Terpanes	Background (µg/g EOM)	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
25	**C_{27}Ts**	69.9	977.8	422.9	481.9	472.9	414.8	409.1	187.2	211.5
26	**C_{27}Tm**	211.8	1374.9	623.6	712.4	707.0	594.3	614.9	268.2	288.9
27	**C_{29}nor25H**	21.2	1022.8	450.4	472.6	404.4	380.1	341.7	180.4	195.6
28	**C_{29}Tm**	368.8	6236.0	3251.7	3305.2	3157.2	2776.1	2714.4	1227.8	1344.3
29	**C_{29}Ts**	40.9	1317.8	722.3	745.3	627.5	627.3	530.6	202.1	288.5
30	**C_{30}: 18α-Oleanane**	43.9	5867.9	3189.7	3238.9	3068.4	2778.4	2981.2	1566.4	1370.7
31	**C_{30}αβ**	296.6	8748.1	4595.5	5006.3	4625.6	4080.5	4123.9	1672.6	1986.9
32	**C_{30}-Mor**	100.6	1561.4	755.7	929.2	860.0	744.7	543.1	236.2	328.0

33	**C_{31}S**	119.2	1760.3	1054.2	1120.1	1035.9	970.5	928.1	361.7	430.1
34	**C_{31}R**	91.2	1569.7	782.6	856.3	740.1	748.1	709.7	268.5	333.7
35	**Gam**	19.4	472.6	273.9	290.3	263.1	273.0	192.9	86.0	111.0
36	**C_{32}S**	71.2	1067.8	569.6	640.3	562.9	553.1	557.7	203.4	241.3
37	**C_{32}R**	61.8	825.0	429.9	464.7	436.2	412.1	433.2	143.8	184.7
38	**C_{33}S**	25.3	670.4	421.0	437.0	440.2	389.7	388.1	143.8	180.2
39	**C_{33}R**	19.1	645.4	267.9	335.4	319.8	307.9	269.2	86.0	131.7
40	**C_{34}S**	14.8	382.2	257.8	297.8	267.9	242.9	204.8	42.4	101.1
41	**C_{34}R**	23.0	309.9	215.5	185.0	196.1	174.1	129.7	36.6	65.2
42	**C_{35}S**	0.0	229.3	149.5	138.0	114.8	134.9	128.6	34.8	49.9
43	**C_{35}R**	0.0	232.1	154.0	136.5	120.9	156.0	91.3	24.0	61.6
	Total (µg/g oil)	**2363.6**	**42177.6**	**21374.1**	**22886.5**	**20937.5**	**18002.1**	**18137.4**	**8286.2**	**10842.6**

Table 0.20: Measured concentrations (µg/g oil) of terpanes in biodegraded Nigerian medium oil in Whitley sediment.

Terpanes		**Measured Concentration (C_{det}) (m/z 191)**								
Peak Number	**Tricyclic Terpanes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**S**	8.2	303.3	102.1	107.1	127.3	110.7	111.4	120.5	92.2

2	**Q**	88.2	120.6	87.6	73.9	77.5	87.0	76.9	45.0	115.6
3	**C_{19}**	12.5	379.6	176.8	158.9	165.6	173.6	184.1	103.5	106.6
4	**M**	3.0	124.2	73.9	76.4	78.6	68.8	80.6	60.0	115.4
5	**C_{20}**	4.7	344.3	175.4	177.8	176.1	162.6	166.3	101.9	118.0
6	**X**	16.5	651.5	233.2	234.2	234.9	226.5	240.8	222.1	201.8
7	**C_{21}**	6.1	226.8	89.5	87.4	82.6	83.4	84.7	52.2	77.3
8	**C_{22}**	7.9	280.1	116.3	107.0	101.1	108.1	106.9	51.8	82.3
9	**C_{23}**	53.9	294.5	130.5	133.2	120.3	112.2	118.8	81.7	175.4
10	**C_{24}**	53.8	304.8	130.9	132.3	134.9	122.2	135.6	84.0	87.1
11	**Y**	37.1	498.2	255.4	269.6	207.5	188.9	180.5	56.2	133.9
12	**Y1**	25.7	617.7	229.9	238.4	237.2	233.3	243.8	183.4	161.7
13	**C_{25}A**	12.4	93.3	45.6	42.3	40.9	43.9	47.2	80.0	43.8
14	**C_{25}B**	11.1	252.7	103.2	99.1	93.9	97.1	96.5	21.5	82.5
15	**Z**	11.4	85.1	72.3	75.0	48.0	42.3	52.1	26.4	33.5
16	**Tet24**	57.0	669.0	207.2	212.0	212.0	202.2	214.7	91.1	128.2
17	**C_{26}(S)**	45.7	57.6	56.1	54.4	57.3	52.8	53.7	22.6	45.0
18	**C_{26}(R)**	60.4	128.8	62.9	58.4	60.0	54.2	54.9	29.2	46.0

Measured Concentration (C_{det})
(m/z 191)

	Tetracyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
19	**Z1**	24.8	366.3	158.5	141.1	155.2	146.0	152.8	107.2	145.9
20	**C_{28}A**	44.8	229.6	163.4	162.7	163.3	157.6	155.9	43.0	78.4
21	**C_{28}B**	36.1	163.5	72.0	63.4	62.8	62.4	60.4	47.3	58.4
22	**C_{29}A**	46.8	132.6	75.6	62.8	43.3	43.0	65.4	21.4	46.3
23	**C_{29}B**	53.4	315.8	118.3	125.2	75.8	89.3	72.7	28.6	97.4
24	**T**	43.6	121.6	171.4	178.1	191.6	175.8	191.1	102.1	38.7

Measured Concentration (C_{det})
(m/z 191)

	Pentacyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
25	**C_{27}Ts**	69.9	1791.7	949.2	905.2	857.9	901.3	886.2	481.4	544.0
26	**C_{27}Tm**	211.8	1875.8	917.2	866.7	960.7	881.4	897.8	436.6	519.6
27	**C_{29}nor25H**	21.2	945.9	497.4	533.1	475.3	470.1	503.2	319.7	272.9
28	**C_{29}Tm**	368.8	7037.1	3906.5	3978.4	3797.3	3953.2	3891.9	1832.2	2251.3
29	**C_{29}Ts**	40.9	1288.1	929.4	847.2	795.5	785.1	924.1	328.3	443.8
30	**C_{30}: 18α-Oleanane**	43.9	5254.3	3106.9	2959.7	2928.8	3019.7	3186.6	2055.0	1766.1
31	**C_{30}αβ**	296.6	10284.7	6080.4	5987.4	5669.8	5932.7	6174.5	2621.4	3502.1
32	**C_{30}-Mor**	100.6	1553.5	916.5	838.7	871.1	855.3	905.6	303.0	496.2

33	**$C_{31}S$**	119.2	2135.8	1280.9	1262.1	1266.5	1260.2	1306.7	490.5	727.9
34	**$C_{31}R$**	91.2	1678.7	961.2	981.5	949.5	967.5	997.4	356.9	564.8
35	**Gam**	19.4	526.6	248.9	295.2	298.3	251.1	287.4	116.9	168.8
36	**$C_{32}S$**	71.2	1392.2	775.3	795.1	760.9	758.5	793.4	317.1	436.2
37	**$C_{32}R$**	61.8	992.7	565.1	590.7	556.5	568.3	588.3	212.4	319.2
38	**$C_{33}S$**	25.3	688.0	424.7	457.2	416.9	462.2	447.7	181.5	237.2
39	**$C_{33}R$**	19.1	505.3	314.0	328.2	310.2	321.9	317.1	112.0	176.5
40	**$C_{34}S$**	14.8	360.2	276.5	239.5	286.4	298.0	245.1	81.5	146.1
41	**$C_{34}R$**	23.0	300.1	171.3	181.4	174.7	165.7	164.0	53.7	80.2
42	**$C_{35}S$**	0.0	224.8	144.2	142.4	161.3	167.5	174.1	49.0	85.2
43	**$C_{35}R$**	0.0	213.1	131.4	124.8	100.2	112.4	131.1	42.1	69.8
	Total (µg/g oil)	**2363.6**	**45810.1**	**25705.1**	**25385.4**	**24481.7**	**23843.4**	**25207.6**	**12054.0**	**15755.8**

Table 0.21: Measured concentrations (µg/g oil) of terpanes in biodegraded North Sea oil in Whitley sediment.

	Terpanes	**Measured Concentration (C_{det}) (m/z 191)**								
Peak Number	**Tricyclic Terpanes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C_{19}**	12.5	55.8	34.8	28.8	34.7	36.1	31.6	7.9	24.9

2	**C_{20}**	4.7	40.8	37.5	38.0	46.3	44.6	38.7	10.4	27.1
3	**C_{21}**	6.1	81.3	37.4	39.1	50.5	40.9	45.4	10.3	30.3
4	**C_{22}**	7.9	34.3	24.3	14.7	18.3	22.5	14.2	5.1	16.4
5	**U**	0.0	29.3	40.1	29.6	22.0	18.1	19.6	5.2	38.9
6	**C_{23}**	53.9	86.6	56.5	50.5	57.9	55.9	57.0	7.4	79.5
7	**C_{24}**	53.8	83.3	46.2	41.5	52.6	46.5	49.5	10.7	35.1
8	**C_{25}B**	11.1	89.1	47.9	44.4	52.6	49.7	51.1	14.6	42.7
9	**Tet24**	57.0	80.5	23.3	23.3	31.1	25.1	27.0	8.0	23.2
10	**C_{26}(S)**	45.7	37.3	17.7	17.3	22.1	17.7	18.2	8.3	15.1
11	**C_{26}(R)**	60.4	54.5	19.6	19.7	24.7	19.3	16.7	10.5	19.9

Measured Concentration
(C_{det}) (m/z 191)

	Tetracyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
12	**U1**	0.0	41.0	24.7	25.9	27.9	29.1	12.6	15.2	20.2
13	**U2**	0.0	23.7	22.8	13.3	13.7	13.2	5.9	8.5	13.3
14	**C_{28}A**	44.8	34.4	23.9	23.0	28.2	26.9	11.9	13.0	20.9
15	**C_{28}B**	36.1	32.5	22.3	20.8	24.1	24.2	13.8	14.3	21.0
16	**C_{29}A**	46.8	46.4	30.2	30.3	34.8	38.6	21.0	13.9	21.7
17	**C_{29}B**	53.4	38.7	20.1	19.8	21.9	22.7	14.6	18.7	28.9

18	**T**	43.6	48.8	29.3	30.0	33.0	31.1	20.9	13.8	29.8

		Measured Concentration (C_{det}) (m/z 191)								
	Pentacyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
19	**C_{27}Ts**	69.9	149.8	86.0	84.8	88.8	85.7	98.3	26.2	65.4
20	**C27Tm**	211.8	99.7	48.2	53.4	48.1	55.3	53.6	14.3	29.0
21	**C_{29}nor25H**	21.2	30.5	40.6	41.2	38.3	46.3	34.7	12.6	22.7
22	**C_{29}Tm**	368.8	145.1	107.1	133.1	112.6	123.3	122.3	65.7	71.6
23	**C_{29}Ts**	40.9	105.6	77.1	71.7	76.1	85.4	88.6	43.0	47.1
24	**C_{30}dh**	43.9	97.8	69.6	69.5	68.5	79.9	84.7	39.3	52.3
25	**C_{30}-Hopane**	296.6	337.0	246.6	250.2	289.3	260.9	292.4	150.8	165.8
26	**C_{30}-Mor**	100.6	58.9	32.3	35.8	36.0	33.5	37.1	30.9	28.2
27	**C_{31}S**	119.2	177.0	145.2	148.9	157.5	152.5	148.7	74.0	83.2
28	**C_{31}R**	91.2	128.8	94.8	99.6	106.3	105.7	109.2	57.6	64.7
29	**Gam**	19.4	40.9	49.0	58.8	59.1	57.1	49.9	20.2	24.6
30	**C_{32}S**	71.2	155.9	101.0	103.3	111.8	103.5	119.1	58.0	69.4
31	**C_{32}R**	61.8	108.8	79.8	74.7	84.3	79.8	81.1	42.2	47.6
32	**C_{33}S**	25.3	112.2	77.8	81.2	78.9	85.8	91.4	44.5	52.9
33	**C_{33}R**	19.1	78.3	56.2	55.5	57.4	65.2	57.0	28.6	37.2

34	**C_{34}S**	14.8	68.4	76.1	77.0	86.4	86.9	76.0	25.3	33.5
35	**C_{34}R**	23.0	42.8	38.8	36.0	47.5	40.0	44.4	18.4	22.1
36	**C_{35}S**	0.0	80.1	62.2	59.6	62.2	64.8	54.8	27.8	41.5
37	**C_{35}R**	0.0	48.6	50.0	51.4	61.7	51.7	46.7	17.0	23.3
	Total (µg/g oil)	**2136.3**	**3004.9**	**2097.0**	**2095.6**	**2266.9**	**2225.4**	**2159.8**	**992.2**	**1490.9**

Table 0.22: Measured concentrations (µg/g oil) of terpanes in biodegraded Nigerian light oil in Nigerian sediment.

Terpanes		**Measured Concentration (C_{det}) (m/z 191)**								
Peak Number	**Tricyclic Terpanes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**S**	89.6	429.5	246.6	272.9	297.9	489.5	424.2	291.0	355.5
2	**Q**	40.2	114.0	123.9	150.7	157.9	254.1	217.9	159.4	189.9
3	**C_{19}**	74.8	330.0	320.0	329.6	339.1	461.3	336.0	234.6	294.7
4	**M**	51.2	118.5	153.3	205.8	167.7	334.5	230.4	153.5	158.9
5	**C_{20}**	154.8	295.9	330.2	360.5	351.0	570.6	416.2	366.3	416.1
6	**X**	186.5	462.1	325.5	427.3	425.3	797.5	535.2	474.4	569.8
7	**C_{21}**	129.0	284.0	215.0	297.6	271.4	475.4	392.3	303.2	385.7
8	**C_{22}**	58.3	172.7	112.3	168.9	166.2	281.8	181.1	152.8	195.6

9	**C_{23}**	200.4	368.4	329.2	477.8	456.7	749.7	602.9	398.7	595.3
10	**C_{24}**	170.4	391.0	313.8	434.3	406.6	680.9	530.8	376.6	529.7
11	**Y**	209.6	415.3	382.6	545.9	552.9	1065.0	692.1	417.4	607.3
12	**Y1**	266.5	684.0	491.8	680.0	617.9	1073.1	805.4	580.4	827.3
13	**C_{25}A**	45.7	105.0	83.9	144.5	157.2	266.6	62.8	131.7	148.9
14	**C_{25}B**	165.1	392.2	275.8	403.2	364.1	626.0	420.0	367.1	531.5
15	**Z**	61.6	184.3	142.7	169.3	183.4	416.4	229.1	146.8	202.1
16	**Tet24**	156.8	446.4	256.9	329.1	324.5	580.1	443.4	310.5	464.6
17	**C_{26}(S)**	69.0	56.2	146.3	180.1	188.5	332.6	247.6	155.3	240.3
18	**C_{26}(R)**	90.2	181.6	156.7	194.5	193.2	350.3	265.1	190.6	282.5

					Measured Concentration (C_{det}) (m/z 191)					
	Tetracyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
19	**Z1**	155.7	387.1	273.3	399.1	383.0	605.9	489.8	306.8	453.7
20	**C_{28}A**	116.8	274.9	305.8	421.3	397.4	714.7	534.9	293.2	414.3
21	**C_{28}B**	78.0	135.1	172.5	234.1	236.9	391.9	299.1	203.3	298.8
22	**C_{29}A**	92.9	198.0	86.0	122.9	114.5	438.8	132.5	243.2	356.7
23	**C_{29}B**	92.9	276.4	231.6	344.9	335.6	498.5	423.8	292.5	443.0
24	**T**	83.9	203.7	210.8	279.5	268.5	525.7	330.9	193.8	292.4

		Measured Concentration (C_{det}) (m/z 191)								
	Pentacyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
25	**C_{27}Ts**	411.8	977.8	902.7	1277.2	1163.6	2007.5	1314.3	1068.7	1479.4
26	**C_{27}Tm**	739.4	1374.9	1148.7	1834.1	1639.9	2721.5	1823.7	1397.8	1914.4
27	**C_{29}-nor25h**	684.7	1022.8	1077.9	1784.6	1631.3	2635.3	1789.9	1082.3	1515.8
28	**C_{29}Tm**	2781.4	6236.0	5428.5	8550.2	7972.1	13556.5	8659.8	6308.0	8486.2
29	**C_{29}Ts**	617.7	1317.8	1382.0	1922.7	1554.5	3063.8	1884.7	1587.1	2038.3
30	**C_{30}-Olean**	3102.9	5867.9	6151.8	9473.2	8799.3	14482.0	9326.9	7091.4	9745.0
31	**C_{30}**	3228.6	8748.1	7906.7	11753.3	10290.4	19553.5	12408.4	9008.1	12304.5
32	**C_{30}-Mor**	619.1	1561.4	1462.7	2033.9	1658.8	3115.1	2039.8	1593.8	2218.2
33	**C_{31}S**	714.8	1760.3	1826.9	2545.5	2215.7	4084.5	2590.3	1967.6	2658.2
34	**C_{31}R**	574.1	1569.7	1341.3	1854.2	1544.2	3027.9	1897.8	1536.6	2111.4
35	**Gam**	272.0	472.6	424.9	652.9	458.7	952.8	634.5	352.1	456.6
36	**C_{32}S**	443.9	1067.8	973.2	1309.0	1138.6	2209.9	1361.2	1097.1	1508.1
37	**C_{32}R**	348.6	825.0	766.8	989.9	848.8	1681.3	1045.5	834.1	1161.0
38	**C_{33}S**	317.5	670.4	598.0	920.7	756.6	1400.3	912.6	741.5	1039.5
39	**C_{33}R**	213.7	645.4	500.1	612.8	520.9	1069.4	631.0	521.5	695.1
40	**C_{34}S**	175.6	382.2	582.7	810.3	723.4	1293.7	753.0	406.5	598.9

41	**C_{34}R**	118.4	309.9	273.3	404.0	331.9	604.1	433.0	284.3	382.1
42	**C_{35}S**	78.3	229.3	167.6	257.9	231.6	429.5	271.1	193.7	231.7
43	**C_{35}R**	91.0	232.1	246.8	262.9	235.7	448.9	55.5	235.6	342.7
	Total (µg/g oil)	**18373.3**	**42177.6**	**38849.1**	**56823.0**	**51073.8**	**91318.7**	**59076.5**	**44050.7**	**60141.6**

Table 0.23: Measured concentrations (µg/g oil) of terpanes in biodegraded Nigerian medium oil in Nigerian sediment.

Terpanes		**Measured Concentration (C_{det}) (m/z 191)**								
Peak Number	**Tricyclic Terpanes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**S**	89.6	303.3	481.9	311.8	259.8	160.4	255.7	232.3	300.7
2	**Q**	40.2	120.6	253.4	180.3	149.0	118.5	97.9	80.1	128.8
3	**C_{19}**	74.8	379.6	446.0	306.1	225.7	206.6	190.1	211.6	288.3
4	**M**	51.2	124.2	314.0	185.4	167.5	130.4	125.3	136.5	146.4
5	**C_{20}**	154.8	344.3	551.7	412.1	283.1	246.4	239.9	274.6	400.9
6	**X**	186.5	651.5	801.6	507.1	386.6	317.5	365.7	435.1	569.7
7	**C_{21}**	129.0	226.8	554.3	291.9	220.5	154.4	241.7	254.2	348.3
8	**C_{22}**	58.3	280.1	331.8	205.0	157.7	139.8	126.7	155.3	204.0
9	**C_{23}**	200.4	294.5	958.0	499.2	399.7	284.6	371.8	363.9	516.9

10	**C_{24}**	170.4	304.8	898.4	451.4	376.6	259.0	324.5	344.4	468.0
11	**Y**	209.6	498.2	1057.0	645.1	489.2	418.2	442.1	416.5	575.2
12	**Y1**	266.5	617.7	1334.2	719.4	557.7	396.5	486.4	556.4	754.8
13	**C_{25}A**	45.7	93.3	261.0	161.5	121.2	97.0	62.4	106.3	147.8
14	**C_{25}B**	165.1	252.7	767.6	407.2	321.2	214.2	260.2	305.1	464.2
15	**Z**	61.6	85.1	401.1	196.7	165.3	154.5	127.6	132.3	173.0
16	**Tet24**	156.8	669.0	832.3	399.1	323.3	271.2	296.6	339.3	489.5
17	**C_{26}(S)**	69.0	57.6	398.6	196.2	165.7	120.1	156.6	151.4	208.1
18	**C_{26}(R)**	90.2	128.8	446.0	215.0	176.8	137.2	166.7	191.6	264.4

Measured Concentration (C_{det})
(m/z 191)

	Tetracyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
19	**Z1**	155.7	366.3	951.4	441.0	375.5	263.3	311.6	323.2	444.6
20	**C_{28}A**	116.8	229.6	902.8	455.3	348.9	286.3	322.8	241.6	362.7
21	**C_{28}B**	78.0	163.5	474.1	266.5	194.1	151.4	180.1	175.1	292.1
22	**C_{29}A**	92.9	132.6	266.5	121.3	109.6	100.4	93.7	211.9	309.7
23	**C_{29}B**	92.9	315.8	830.7	433.6	286.2	295.3	262.7	346.3	441.1
24	**T**	83.9	121.6	573.2	355.2	270.0	231.0	255.3	169.1	245.3

Measured Concentration (C_{det})
(m/z 191)

	Pentacyclic Terpanes	Background (µg/g EOM)	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
25	**C_{27}Ts**	411.8	1791.7	2009.4	1146.2	1202.3	1177.6	1053.6	1164.4	1688.3
26	**C_{27}Tm**	739.4	1875.8	2040.0	1537.1	1633.7	1396.8	1375.7	1385.8	1956.1
27	**C_{29}-nor25h**	684.7	945.9	1966.6	1473.1	1443.4	1077.1	1284.0	982.4	1369.8
28	**C_{29}Tm**	2781.4	7037.1	10177.0	7656.9	7277.1	6709.6	6624.9	5987.4	8670.2
29	**C_{29}Ts**	617.7	1288.1	2259.5	1381.6	1582.1	1474.6	1393.1	1363.8	1890.4
30	**C_{30}-Olean**	3102.9	5254.3	10331.1	7880.1	7931.1	6126.7	6448.9	6288.1	9090.2
31	**C_{30}**	3228.6	10284.7	14413.1	10004.9	10633.6	9872.0	9272.6	8769.8	12643.2
32	**C_{30}-Mor**	619.1	1553.5	2246.1	1634.6	1555.3	1457.8	1417.7	1482.2	2135.2
33	**C_{31}S**	714.8	2135.8	2984.5	2105.1	2032.3	1990.9	1928.4	1901.2	2666.2
34	**C_{31}R**	574.1	1678.7	2197.6	1572.6	1474.5	1523.0	1409.2	1500.9	2138.2
35	**Gam**	272.0	526.6	842.2	493.4	454.6	448.9	473.5	361.8	448.1
36	**C_{32}S**	443.9	1392.2	1601.3	1143.9	1059.5	1084.2	1023.6	1092.8	1589.2
37	**C_{32}R**	348.6	992.7	1180.9	898.3	803.1	822.4	781.0	833.3	1174.2
38	**C_{33}S**	317.5	688.0	1074.8	779.5	684.2	704.7	677.9	698.2	997.0
39	**C_{33}R**	213.7	505.3	712.7	501.4	438.2	480.2	465.0	477.3	671.4
40	**C_{34}S**	175.6	360.2	1002.2	634.6	629.5	667.6	586.9	395.5	556.2
41	**C_{34}R**	118.4	300.1	414.4	289.4	264.7	271.1	268.3	263.1	352.8
42	**C_{35}S**	78.3	224.8	276.2	201.3	214.4	211.8	92.1	197.8	261.4

43	**C_{35}R**	91.0	213.1	315.6	215.3	177.5	189.7	185.0	210.8	313.8
	Total (µg/g oil)	**18373.3**	**45810.1**	**73132.8**	**49912.7**	**48022.1**	**42841.0**	**42525.4**	**41510.9**	**59156.2**

Table 0.24: Measured concentrations (µg/g oil) of terpanes in biodegraded North Sea oil in Nigerian sediment.

Terpanes		**Measured Concentration (C_{det}) (m/z 191)**								
Peak Number	**Tricyclic Terpanes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
	S	89.6	0.0	127.8	168.8	178.9	174.9	181.5	140.0	239.1
	Q	40.2	0.0	67.5	107.6	103.0	109.6	70.1	60.1	139.0
1	**C_{19}**	74.8	55.8	136.9	165.7	167.8	197.5	147.1	126.8	175.9
	M	51.2	0.0	93.9	100.1	100.9	128.7	90.1	74.8	104.1
2	**C_{20}**	154.8	40.8	160.2	196.0	233.1	224.5	201.0	186.2	227.6
	X	186.5	0.0	220.9	308.9	328.3	375.7	293.8	261.8	309.5
3	**C_{21}**	129.0	81.3	156.4	194.1	227.9	267.1	207.2	183.6	216.7
4	**C_{22}**	58.3	34.3	90.2	104.3	112.6	134.7	102.8	85.3	115.1
5	**U**	28.3	29.3	51.5	63.0	70.1	95.4	63.0	50.9	92.5
6	**C_{23}**	200.4	86.6	311.1	255.1	316.8	417.0	372.4	277.2	363.9
7	**C_{24}**	170.4	83.3	210.0	302.2	330.0	362.8	292.0	251.9	342.8

	Y	209.6	0.0	267.6	382.6	354.5	432.0	297.5	281.9	399.7
	Y1	266.5	0.0	307.6	420.2	495.3	501.6	415.5	358.5	477.2
	C_{25}A	45.7	0.0	68.9	80.4	84.1	102.5	73.3	63.7	97.6
8	**C_{25}B**	165.1	89.1	255.2	257.7	292.6	301.8	237.7	196.3	280.7
	Z	61.6	0.0	94.5	101.0	108.0	122.4	90.2	86.5	97.8
9	**Tet24**	156.8	80.5	222.7	229.8	267.5	290.7	201.2	162.4	276.4
10	**C_{26}(S)**	69.0	37.3	124.3	128.8	168.2	169.9	115.9	96.2	152.3
11	**C_{26}(R)**	90.2	54.5	141.8	144.8	178.2	186.5	132.5	107.6	164.6

		Measured Concentration (C_{det}) (m/z 191)								
	Tetracyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
	Z1	155.7	0.0	197.7	265.5	288.9	299.5	232.4	178.6	292.8
12	**U1**	43.6	41.0	75.5	77.2	118.3	128.7	95.3	62.2	102.3
13	**U2**	23.8	23.7	15.3	22.8	25.4	32.1	25.0	19.7	31.4
14	**C_{28}A**	116.8	34.4	315.0	286.4	195.6	347.1	207.2	167.6	298.5
15	**C_{28}B**	78.0	32.5	111.8	164.1	199.6	212.9	144.3	132.8	186.8
16	**C_{29}A**	92.9	46.4	88.4	93.2	94.3	161.0	86.4	68.4	147.9
17	**C_{29}B**	92.9	38.7	147.2	207.3	246.3	262.8	195.7	168.7	230.1
18	**T**	83.9	48.8	149.7	140.7	224.8	249.0	186.4	138.5	203.1

		Measured Concentration (C_{det}) (m/z 191)								
	Pentacyclic Terpanes	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
19	**C_{27}Ts**	411.8	149.8	1025.2	795.0	825.8	840.6	878.1	770.3	1040.7
20	**C_{27}Tm**	739.4	99.7	1375.8	1035.2	1108.0	698.7	1200.8	948.8	1386.6
21	**C_{29}nor25H**	684.7	30.5	1316.7	1056.8	1111.7	719.8	1207.2	800.0	1331.8
22	**C_{29}Tm**	2781.4	145.1	5850.9	4393.7	4869.9	3269.6	5186.0	4131.2	6739.2
23	**C_{29}Ts**	617.7	105.6	1467.9	1210.9	1204.5	835.7	1282.9	1012.2	1396.2
24	**C_{30}: 18α-Oleanane**	3102.9	97.8	7007.8	5309.0	5706.9	3580.7	5610.9	4790.8	7088.5
25	**$C_{30}\alpha\beta$**	3228.6	337.0	8356.8	6583.9	6804.2	4952.5	7360.9	6009.5	8325.0
26	**C_{30}-Mor**	619.1	58.9	1446.0	1093.5	1158.7	776.8	1272.3	1080.7	1489.4
27	**C_{31}S**	714.8	177.0	1824.9	1467.1	1512.5	1071.8	1628.2	1310.2	1824.6
28	**C_{31}R**	574.1	128.8	1418.2	1091.4	1077.0	795.9	1182.9	1025.3	1437.2
29	**Gam**	272.0	40.9	445.2	369.3	336.7	285.5	432.8	260.7	317.3
30	**C_{32}S**	443.9	155.9	1006.9	776.7	773.2	553.6	851.5	761.9	1024.8
31	**C_{32}R**	348.6	108.8	718.1	527.4	571.2	460.4	636.5	580.9	785.7
32	**C_{33}S**	317.5	112.2	672.8	534.3	521.7	434.7	586.9	516.0	725.3
33	**C_{33}R**	213.7	78.3	505.8	355.4	345.8	264.5	390.4	361.4	494.9

34	**C_{34}S**	175.6	68.4	629.4	467.8	477.6	370.0	545.5	296.7	398.3
35	**C_{34}R**	118.4	42.8	263.9	202.3	208.5	167.3	242.4	191.3	270.5
36	**C_{35}S**	78.3	80.1	179.0	134.5	147.1	103.4	154.0	118.5	180.3
37	**C_{35}R**	91.0	48.6	223.4	156.2	155.0	122.3	166.6	164.0	226.5
	Total (µg/g oil)	**18468.9**	**3004.9**	**39944.4**	**32528.1**	**34426.9**	**26592.3**	**35574.3**	**29118.7**	**42248.0**

Table 0.25: Measured steranes concentrations (µg/g oil) in biodegraded Nigerian light oil in Whitley sediment.

		Determined Concentration (C_{de}t) -(M/Z 217)								
Peak Number	Steranes	Background (µg/g EOM)	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	2.6	101.8	30.5	31.8	41.7	31.1	33.2	28.4	23.9
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	4.3	189.2	58.1	64.9	88.5	67.0	71.9	52.6	51.3
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	5.9	101.9	32.9	37.9	49.0	37.5	38.1	31.2	29.7
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	29.6	205.5	67.6	76.4	99.0	75.4	56.9	26.0	59.3
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	14.9	132.1	47.0	55.4	72.0	50.1	22.9	15.9	37.1
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	11.1	83.6	30.1	29.1	39.9	29.9	17.9	8.9	14.6

7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	15.3	94.9	39.6	46.0	51.9	44.2	24.7	9.1	17.7
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	19.9	186.0	61.7	63.2	86.7	66.3	61.9	27.89	41.5
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	25.4	370.4	179.9	197.1	197.7	153.3	98.5	37.7	101.3
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	35.3	468.5	156.2	163.4	204.9	168.3	158.3	84.6	119.3
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	17.9	289.6	104.8	124.1	150.7	114.0	90.8	33.6	71.7
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	42.8	630.7	216.9	220.0	280.7	222.5	109.4	56.5	158.9
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	34.4	468.0	164.0	187.3	237.2	179.2	190.6	113.8	120.8
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	13.9	221.2	49.3	46.4	52.1	40.4	48.8	51.0	60.2
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	9.6	455.5	179.4	200.2	217.0	161.5	168.0	105.5	106.1
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	17.2	494.1	162.8	171.0	212.7	173.2	179.9	115.1	124.8

17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	19.0	355.7	120.7	131.3	178.7	131.3	138.3	86.8	88.8
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	19.0	282.9	94.4	101.8	133.5	101.8	109.8	71.4	69.5
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	21.0	604.1	199.3	209.2	277.7	209.3	191.8	96.6	151.6
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	32.9	688.2	238.0	256.5	327.5	256.7	278.6	184.2	215.9
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	30.4	581.3	265.3	278.2	323.7	239.8	269.3	152.6	149.5
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	16.1	298.7	130.1	136.3	163.6	133.9	141.4	80.4	86.5
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	24.2	894.8	338.3	353.3	453.9	359.3	385.3	180.5	258.6
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	5.7	142.4	31.9	29.9	41.8	29.6	30.1	28.3	34.4
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	103.6	65.0	37.7	37.2	37.6	27.1	26.7	14.1	25.0

26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	298.8	170.8	61.9	62.9	87.6	63.3	67.6	47.5	44.5
	Total (µg/g oil)	**870.6**	**8576.9**	**3098.3**	**3310.7**	**4107.0**	**3165.8**	**3010.7**	**1740.3**	**2262.2**

Table 0.26: Measured steranes concentrations (µg/g oil) in biodegraded Nigerian medium oil in Whitley sediment.

		Determined Concentration (C_{det}) -(M/Z 217)								
Peak Number	**Steranes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	2.6	91.7	44.3	40.2	45.6	41.0	38.8	30.3	29.9
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	4.3	151.1	79.0	77.4	94.5	77.3	76.2	54.7	52.1
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	5.9	77.2	39.2	40.1	45.7	36.7	37.9	28.4	26.8
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	29.6	173.2	80.8	82.3	99.0	74.2	78.5	28.5	58.3
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	14.9	95.3	56.0	55.0	62.3	51.3	53.3	17.6	38.7
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	11.1	45.3	35.7	21.1	31.0	23.2	30.4	7.7	15.9

7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	15.3	66.9	43.9	47.7	47.5	32.2	41.2	12.6	29.6
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	19.9	120.2	70.0	74.8	76.5	67.9	64.4	28.28	41.5
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	25.4	171.9	143.4	162.5	133.9	103.4	108.6	32.4	60.6
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	35.3	308.9	160.8	171.4	190.0	162.0	152.2	96.8	108.2
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	17.9	154.6	101.5	104.4	121.8	104.4	93.5	34.6	58.4
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	42.8	210.9	114.8	113.2	138.4	106.6	110.7	42.0	80.4
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	34.4	257.7	145.3	152.6	167.5	145.1	133.3	101.6	93.2
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	13.9	199.6	105.3	105.2	116.7	105.9	88.5	77.1	78.1
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	9.6	238.3	167.9	192.5	172.2	142.8	122.3	80.3	93.2
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	17.2	270.9	147.7	153.5	177.7	152.2	143.0	97.8	107.1

17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	19.0	192.8	110.4	117.8	135.5	112.4	107.8	71.4	70.4
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	19.0	147.1	84.5	89.4	94.1	82.8	78.1	59.5	49.0
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	21.0	205.3	117.8	119.6	137.3	114.1	110.5	53.3	73.7
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	32.9	470.7	273.6	298.4	326.1	275.0	259.6	187.0	224.8
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	30.4	321.9	243.3	293.6	285.5	204.3	194.9	126.9	94.4
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	16.1	172.7	122.8	121.7	128.5	103.7	116.5	71.1	75.0
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	24.2	285.1	270.4	291.1	327.5	260.8	243.9	66.3	109.9
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	5.7	108.1	50.7	55.7	59.8	46.7	44.1	39.4	47.2
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	103.6	36.1	51.1	68.1	35.3	42.5	38.3	20.1	18.8

26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	298.8	109.1	72.0	77.5	85.4	71.4	64.4	54.4	54.6
	Total (µg/g oil)	**870.6**	**4682.3**	**2931.9**	**3126.7**	**3335.6**	**2740.0**	**2631.1**	**1519.9**	**1790.0**

Table 0.27: Measured steranes concentrations (µg/g oil) in biodegraded North Sea oil in Whitley sediment.

			Determined Concentration (C_{det}) -(M/Z 217)							
Peak Number	**Steranes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	2.6	126.0	79.0	76.6	80.0	75.7	85.1	59.3	61.1
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	4.3	128.9	88.0	84.4	100.0	94.0	102.8	41.4	72.3
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	5.9	78.9	48.6	48.8	52.3	53.3	58.2	19.5	39.9
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	29.6	309.2	183.6	174.9	205.9	199.7	62.1	8.9	153.6
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	14.9	219.9	127.7	121.0	142.9	134.8	54.5	8.7	86.4
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	11.1	101.8	58.8	61.2	71.8	66.8	38.3	9.4	44.0

7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	15.3	103.9	67.7	64.5	74.5	64.1	24.5	2.6	50.3
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	19.9	166.2	106.6	105.9	118.4	112.1	103.2	37.16	75.6
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	25.4	109.5	147.1	134.1	143.9	151.2	91.5	39.0	57.6
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	35.3	289.1	187.4	182.1	205.6	204.9	187.4	97.7	149.2
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	17.9	184.8	126.8	117.8	143.0	136.2	102.5	44.4	97.9
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	42.8	113.1	67.9	59.3	66.6	61.8	28.5	24.9	57.4
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	34.4	228.4	166.7	163.5	200.4	189.0	201.8	97.7	129.5
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	13.9	145.9	93.3	90.2	98.6	101.2	106.5	66.1	79.2
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	9.6	86.5	81.5	73.5	60.7	77.6	76.9	42.8	45.2
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	17.2	102.6	61.5	53.5	65.6	63.3	72.8	40.1	50.5
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	19.0	143.1	93.0	89.3	104.2	102.2	111.6	54.4	74.9
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	19.0	105.4	61.4	62.4	60.3	69.7	77.7	48.2	54.5

19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	21.0	78.3	46.0	41.1	41.0	48.4	47.6	29.0	41.2
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	32.9	90.1	71.3	70.3	71.9	81.8	84.8	49.8	52.7
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	30.4	124.7	96.0	96.3	100.6	100.8	107.7	63.9	62.7
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	16.1	79.5	53.3	50.5	50.0	59.1	63.9	32.8	40.2
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	24.2	103.1	56.5	65.3	65.7	63.9	63.5	36.8	49.1
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	5.7	73.7	48.3	53.0	46.2	54.3	55.5	30.5	37.2
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	103.6	10.4	7.8	5.1	20.0	15.3	18.4	1.9	1.8
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	298.8	23.6	15.5	17.7	18.4	17.2	20.8	10.5	12.3
	Total (µg/g oil)	**870.6**	**3326.6**	**2241.3**	**2162.2**	**2408.6**	**2398.5**	**2048.1**	**997.5**	**1676.1**

Table 0.28: Measured steranes concentrations (µg/g oil) in biodegraded Nigerian light oil in Nigerian sediment.

		Determined Concentration (C_{det}) -(M/Z 217)								
Peak Number	**Steranes**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**

1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	52.6	101.8	90.9	118.4	120.0	153.4	143.3	119.3	149.7
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	56.2	189.2	149.5	181.8	196.8	320.9	271.2	247.7	315.2
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	46.8	101.9	81.2	103.0	117.3	195.8	164.4	141.9	184.0
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	117.0	205.5	189.9	256.7	271.2	407.7	319.8	238.1	337.5
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	67.8	132.1	132.1	193.4	222.3	268.6	223.4	148.8	200.0
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	41.9	83.6	84.0	96.2	112.5	139.0	137.1	75.8	97.2
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	47.4	94.9	98.4	138.6	154.3	216.0	184.8	89.0	147.8
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	100.2	186.0	193.9	266.6	284.7	407.1	325.5	205.01	287.6
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	115.8	370.4	294.0	451.2	479.7	899.7	688.7	425.4	587.1
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	173.8	468.5	355.5	520.7	560.9	868.1	695.2	516.2	692.5
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	100.4	289.6	265.9	374.3	426.2	607.8	507.6	325.8	430.8
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	154.6	630.7	377.8	502.9	538.9	963.2	881.3	652.5	879.0

13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	198.8	468.0	417.4	607.9	643.7	921.4	699.1	490.5	674.1
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	92.7	221.2	128.2	197.2	230.0	296.3	207.6	227.5	324.6
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	166.8	455.5	351.8	517.0	544.7	958.7	652.5	501.6	661.8
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	195.0	494.1	366.1	570.4	604.9	943.7	705.5	579.6	764.8
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	144.9	355.7	296.2	423.0	449.6	672.6	530.5	433.6	561.2
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	117.8	282.9	250.4	351.1	389.6	584.5	408.9	335.5	430.0
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	151.1	604.1	375.1	495.0	531.8	934.7	777.4	643.6	848.1
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	268.4	688.2	553.6	850.1	910.9	1342.7	951.0	775.3	984.6
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	210.3	581.3	554.6	745.8	814.4	1334.8	1010.2	736.8	920.8
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	116.3	298.7	264.1	360.6	411.7	581.5	467.8	374.4	487.5
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	245.7	894.8	715.4	1031.5	1110.0	1778.3	1344.7	955.6	1274.2
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	74.3	142.4	61.4	113.1	127.6	158.3	105.8	128.6	164.7

25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	31.1	65.0	87.4	114.3	130.9	207.1	119.2	65.0	97.0
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	86.7	170.8	188.5	301.9	352.3	496.9	313.3	213.6	288.8
	Total (µg/g oil)	**3174.3**	**8576.9**	**6923.2**	**9882.7**	**10736.9**	**16658.9**	**12835.8**	**9646.8**	**12790.6**

Table 0.29: Measured steranes concentrations (µg/g oil) in biodegraded Nigerian medium oil in Nigerian sediment.

		Determined Concentration (C_{det}) -(M/Z 217)								
Peak Number	**Steranes**	**Background (µg/g EOM)**	**Start**	**5days**	**10days**	**20days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	52.6	91.7	139.7	94.8	91.8	78.0	104.7	96.0	139.8
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	56.2	151.1	165.8	145.8	153.2	156.9	222.1	197.9	287.8
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	46.8	77.2	116.5	91.3	87.4	85.8	118.4	116.3	160.5
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	117.0	173.2	267.3	209.0	203.5	212.7	215.4	195.6	307.3
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	67.8	95.3	180.6	137.7	145.2	150.2	157.0	120.3	180.1
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	41.9	45.3	107.5	87.6	92.3	93.8	95.1	54.6	80.7

7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	47.4	66.9	174.3	96.3	118.7	115.6	109.8	77.6	130.8
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	100.2	120.2	358.0	208.3	231.7	238.3	196.9	165.85	251.6
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	115.8	171.9	552.6	346.6	362.5	381.7	435.8	327.7	500.5
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	173.8	308.9	695.2	407.3	435.7	468.1	467.7	418.3	631.6
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	100.4	154.6	448.6	266.2	247.5	258.7	313.1	258.1	390.1
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	154.6	210.9	525.2	342.9	338.3	405.3	555.4	475.9	711.8
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	198.8	257.7	828.1	451.9	494.7	500.5	421.4	400.0	622.6
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	92.7	199.6	434.2	183.8	222.1	233.1	153.5	215.1	317.0
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	166.8	238.3	686.8	427.2	491.4	509.7	451.7	403.5	611.5
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	195.0	270.9	696.0	417.0	465.1	473.8	467.9	468.1	672.2
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	144.9	192.8	522.9	302.2	321.4	319.3	344.4	335.6	510.5
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	117.8	147.1	474.3	257.0	267.3	275.6	266.3	262.9	393.1

19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	151.1	205.3	584.5	349.3	349.8	691.8	487.2	474.8	713.2
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	268.4	470.7	1067.1	635.6	675.4	690.2	644.6	644.0	944.0
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	210.3	321.9	1026.2	561.1	651.5	731.9	691.7	550.7	813.5
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	116.3	172.7	390.5	245.9	238.6	258.6	285.9	293.0	417.3
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	245.7	285.1	1236.6	757.1	793.6	850.0	889.1	741.7	1099.5
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	74.3	108.1	131.2	89.6	90.3	104.6	83.0	116.5	159.2
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	31.1	36.1	158.6	110.3	115.9	122.7	97.8	58.7	85.3
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	86.7	109.1	431.9	256.4	283.8	314.4	210.4	173.4	261.0
	Total (µg/g oil)	**3174.3**	**4682.3**	**12400.3**	**7478.4**	**7968.7**	**8721.3**	**8486.3**	**7642.3**	**11392.6**

Table 0.30: Measured steranes concentrations (µg/g oil) in biodegraded North Sea oil in Nigerian sediment.

Peak Number	**Steranes**	**Determined Concentration (C_{det}) -(M/Z 217)**								
		Background (µg/g EOM)	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100days**	**300days**	**Sterilised**

1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	52.6	126.0	117.0	114.5	105.1	103.3	103.3	97.3	124.5
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	56.2	128.9	213.2	197.0	152.1	148.6	184.7	149.8	222.5
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	46.8	78.9	120.3	112.2	95.1	89.2	101.5	95.3	126.9
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	117.0	309.2	263.4	291.3	222.0	238.8	234.5	224.5	279.1
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	67.8	219.9	191.5	201.7	156.0	192.7	129.2	160.2	156.3
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	41.9	101.8	103.6	99.0	84.2	90.6	72.6	89.9	86.7
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	47.4	103.9	118.3	129.3	104.2	105.0	107.6	109.5	108.5
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	100.2	166.2	235.3	252.1	192.0	205.4	172.3	196.8	195.14
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	115.8	109.5	419.6	345.6	267.8	259.6	291.3	278.0	329.0
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	173.8	289.1	514.9	478.0	382.8	377.6	377.6	384.1	455.3
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	100.4	184.8	366.6	327.0	248.1	246.3	237.4	250.5	287.7
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	154.6	113.1	570.4	409.0	322.6	308.5	407.3	348.5	448.6

13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	198.8	228.4	478.8	524.0	424.1	415.2	371.5	436.7	455.6
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	92.7	145.9	143.7	165.8	129.7	151.7	185.7	142.9	242.4
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	166.8	86.5	433.0	401.6	328.9	289.2	327.6	350.6	402.6
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	195.0	102.6	501.8	487.0	392.5	382.3	385.7	398.8	469.2
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	144.9	143.1	375.6	378.2	291.6	276.3	282.8	298.3	342.6
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	117.8	105.4	289.1	304.5	228.6	221.9	222.7	236.6	283.0
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	151.1	78.3	508.8	404.8	317.6	287.0	393.4	332.9	457.3
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	268.4	90.1	623.4	642.1	528.0	495.4	454.0	533.1	553.2
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	210.3	124.7	608.7	600.8	441.6	389.0	446.2	467.2	539.4
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	116.3	79.5	302.1	303.4	245.2	240.3	240.2	251.7	275.6
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	245.7	103.1	847.5	767.9	622.8	587.2	565.7	637.8	671.6
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	74.3	73.7	82.1	84.0	82.8	76.5	87.8	88.2	112.3

25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	31.1	10.4	73.6	76.4	72.7	63.4	44.9	82.7	51.7
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	86.7	23.6	214.9	259.7	208.1	193.0	147.3	218.0	171.2
	Total (µg/g oil)	**3174.3**	**3326.6**	**8717.0**	**8356.9**	**6645.9**	**6434.0**	**6574.7**	**6859.9**	**7848.0**

Table 0.31: Measured triaromatic steranes concentrations (µg/g oil) in biodegraded Nigerian light oil in Whitley sediment.

		Determined Concentration (C_{det}) -(M/Z 231)								
Peak Number	**Triaromatic Steranes (TAS)**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**P**	39.4	116.7	22.8	51.0	75.5	28.0	24.2	26.8	52.6
2	**Q**	78.7	55.9	14.3	25.1	36.8	14.5	14.3	10.1	52.9
3	**C20-TAS**	31.9	92.4	23.8	47.4	62.7	28.2	27.4	45.8	19.2
4	**C21-TAS**	36.2	42.8	8.4	20.2	26.7	13.1	14.8	14.3	46.2
5	**C22A-TAS**	9.9	25.8	6.5	14.6	19.0	9.2	6.8	14.7	14.1
6	**C22B-TAS**	11.2	30.5	8.2	16.1	25.8	9.3	7.3	13.9	54.2
7	**R**	35.2	24.5	4.4	10.3	14.9	8.0	6.1	11.8	3.6
8	**S**	29.3	15.3	3.4	7.8	11.1	7.1	5.7	5.5	7.5
9	**T**	2.2	40.4	8.4	18.6	31.7	13.5	12.2	14.7	5.7
10	**C26(20S) TA-cholestane**	46.2	206.7	40.4	83.8	138.5	63.2	28.3	28.5	10.7
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	143.8	596.3	115.8	245.0	387.2	180.6	157.9	163.6	21.3

12	**U**	13.7	44.0	10.5	18.7	31.4	13.4	12.3	21.8	10.8
13	**C28 (20S) TA-stigmastane**	92.8	503.0	100.5	209.2	334.7	154.4	156.1	234.7	21.2
14	**C27(20R) TA-ergostane**	77.4	313.0	65.1	133.2	200.1	97.1	95.7	133.4	15.9
15	**C28 (20R) TA-stigmastane**	92.6	413.4	83.7	184.7	282.6	133.1	104.5	200.1	19.6
16	**V**	15.0	85.4	21.3	48.5	70.7	33.1	32.3	49.9	81.8
	Total (µg/g oil)	755.6	2606.1	537.5	1134.2	1749.6	805.8	706.0	989.4	437.2

Table 0.32: Measured triaromatic steranes concentrations (µg/g oil) in biodegraded Nigerian medium oil in Whitley sediment.

Peak Number	**Triaromatic Steranes (TAS)**	**Determined Concentration (C_{det}) -(M/Z 231)**								
		Background (µg/g EOM)	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**P**	39.4	193.4	66.1	77.9	95.8	76.4	65.6	60.8	156.5
2	**Q**	78.7	131.6	45.9	59.3	56.9	40.8	42.2	30.7	137.2
3	**C20-TAS**	31.9	125.2	54.9	72.2	76.8	72.1	59.2	65.3	51.2
4	**C21-TAS**	36.2	59.4	23.7	29.4	32.2	28.3	24.3	18.9	53.7
5	**C22A-TAS**	9.9	45.5	15.1	17.5	17.8	21.8	16.2	22.0	24.6

6	**C22B-TAS**	11.2	21.0	16.7	20.4	20.4	21.5	17.9	25.5	47.2
7	**R**	35.2	13.9	4.9	6.5	7.7	8.3	7.0	7.8	10.1
8	**S**	29.3	11.7	3.4	5.5	7.3	6.9	5.5	7.1	7.9
9	**T**	2.2	32.5	10.2	15.4	17.0	15.0	12.7	5.3	17.0
10	**C26(20S) TA-cholestane**	46.2	132.7	41.8	60.1	72.2	68.9	56.6	11.3	34.8
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	143.8	449.8	139.4	188.8	227.7	218.3	176.9	29.2	196.4
12	**U**	13.7	40.9	12.5	16.9	19.5	21.5	15.7	5.3	19.3
13	**C28 (20S) TA-stigmastane**	92.8	428.7	124.4	183.1	222.0	222.1	180.2	24.7	224.1
14	**C27(20R) TA-ergostane**	77.4	242.3	87.7	113.9	141.3	136.5	111.5	36.1	115.9
15	**C28 (20R) TA-stigmastane**	92.6	362.6	113.0	158.2	187.4	193.9	153.0	20.1	189.2
16	**V**	15.0	112.1	47.5	59.2	69.8	72.4	56.6	54.9	69.7
	Total (µg/g oil)	755.6	2403.2	807.0	1084.4	1271.9	1224.6	1001.1	223.3	1128.4

Table 0.33: Measured triaromatic steranes concentrations (µg/g oil) in biodegraded North Sea oil in Whitley sediment.

Determined Concentration (C_{det}) -(M/Z 231)

Peak Number	Triaromatic Steranes (TAS)	Background (µg/g EOM)	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
1	**P**	39.4	306.5	85.1	114.4	130.9	104.3	131.2	24.8	239.7
2	**Q**	78.7	223.5	69.5	79.9	85.1	63.2	26.8	11.7	237.1
3	**C20-TAS**	31.9	308.8	88.4	123.5	166.3	126.6	164.8	46.4	141.2
4	**C21-TAS**	36.2	60.9	18.8	22.4	27.2	21.8	15.6	34.2	73.0
5	**C22A-TAS**	9.9	39.9	16.5	16.9	12.6	11.6	21.0	13.8	74.0
6	**C22B-TAS**	11.2	34.3	10.7	10.9	8.2	9.1	12.0	13.0	43.7
7	**R**	35.2	7.5	2.9	4.4	4.2	3.8	4.3	8.2	13.2
8	**S**	29.3	12.1	3.5	4.7	4.5	6.5	3.8	4.2	10.7
9	**T**	2.2	23.4	3.8	8.0	10.5	9.2	12.8	10.6	2.7
10	**C26(20S) TA-cholestane**	46.2	77.6	15.2	28.4	33.5	31.8	18.5	6.0	12.7
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	143.8	280.1	50.1	92.8	116.2	111.8	125.1	95.9	36.5
12	**U**	13.7	18.7	2.9	8.4	11.1	10.9	12.3	9.2	2.8
13	**C28 (20S) TA-stigmastane**	92.8	185.6	35.9	66.6	81.4	80.9	98.7	90.9	47.8
14	**C27(20R) TA-ergostane**	77.4	165.9	33.2	59.3	78.7	70.3	89.2	75.4	16.5
15	**C28 (20R) TA-stigmastane**	92.6	158.2	32.0	60.4	70.5	70.1	86.0	75.6	33.8
16	**V**	15.0	36.9	6.2	8.8	8.8	11.4	10.9	9.2	20.8
	Total (µg/g oil)	755.6	1939.9	474.6	710.1	849.7	743.2	833.0	529.1	1006.4

Table 0.34: Measured triaromatic steranes concentrations (μg/g oil) in biodegraded Nigerian light oil in Nigerian sediment.

Peak Number	Triaromatic Steranes (TAS)	Determined Concentration (C_{det}) -(M/Z 231)								
		Background (μg/g EOM)	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
1	**P**	9.4	116.7	196.1	199.5	274.4	241.7	263.7	574.0	3831.1
2	**Q**	8.5	55.9	65.3	67.9	95.9	92.4	93.9	373.4	1320.9
3	**C20-TAS**	5.4	92.4	168.4	170.1	196.6	241.4	220.9	390.0	2863.7
4	**C21-TAS**	39.3	42.8	119.6	91.9	90.7	96.5	101.6	256.6	1063.5
5	**C22A-TAS**	4.7	25.8	46.4	48.5	49.7	65.3	68.5	119.8	711.7
6	**C22B-TAS**	5.8	30.5	34.0	48.3	57.6	52.0	41.2	197.9	574.5
7	**R**	1.9	24.5	42.2	55.5	62.7	65.2	68.4	66.5	642.2
8	**S**	2.2	15.3	29.5	35.2	44.1	53.0	59.0	51.2	452.4
9	**T**	2.8	40.4	78.5	79.5	80.9	117.2	111.1	87.8	1339.7
10	**C26(20S) TA-cholestane**	2.6	206.7	358.6	424.9	454.9	498.1	460.8	275.6	6908.2
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	8.5	596.3	1029.9	1166.8	1286.0	1572.2	1241.0	998.8	20361.0
12	**U**	0.8	44.0	88.4	93.9	111.2	144.9	123.7	74.6	1640.0

13	**C28 (20S) TA-stigmastane**	2.5	503.0	838.2	946.8	1055.4	1311.1	991.2	769.5	15949.5
14	**C27(20R) TA-ergostane**	3.6	313.0	549.7	559.8	695.7	867.9	861.5	789.0	10599.1
15	**C28 (20R) TA-stigmastane**	2.7	413.4	710.4	777.6	927.9	1170.8	959.6	862.8	14077.3
16	**V**	2.6	85.4	146.8	155.6	180.6	249.9	169.0	564.4	2447.6
	Total (µg/g oil)	103.2	2606.1	4502.1	4921.8	5664.3	6839.8	5835.2	6451.9	84782.4

Table 0.35: Measured triaromatic steranes concentrations (µg/g oil) in biodegraded Nigerian medium oil in Nigerian sediment.

Peak Number	**Triaromatic Steranes (TAS)**	**Determined Concentration (C_{det}) -(M/Z 231)**								
		Background (µg/g EOM)	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**P**	9.4	193.4	196.6	196.1	198.4	232.7	436.5	2684.5	3070.2
2	**Q**	8.5	131.6	135.4	138.5	144.8	158.8	174.2	1067.1	1214.9
3	**C20-TAS**	5.4	125.2	309.0	69.2	78.1	161.9	169.9	1985.8	2331.0
4	**C21-TAS**	39.3	59.4	60.7	60.7	82.1	90.2	147.2	856.6	851.6
5	**C22A-TAS**	4.7	45.5	44.6	56.8	59.6	76.7	98.8	513.5	603.8
6	**C22B-TAS**	5.8	21.0	28.1	28.7	35.4	44.5	55.3	331.1	333.6
7	**R**	1.9	13.9	16.6	21.3	28.0	33.0	75.7	306.7	474.8

8	**S**	2.2	11.7	58.2	10.0	11.1	18.2	23.8	266.2	329.0
9	**T**	2.8	32.5	40.6	46.1	61.2	72.6	141.5	550.6	914.7
10	**C26(20S) TA-cholestane**	2.6	132.7	137.5	152.3	241.6	337.2	615.8	3062.7	4446.6
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	8.5	449.8	460.8	522.5	764.0	963.0	1745.1	9874.1	13161.8
12	**U**	0.8	40.9	46.6	54.4	70.4	87.3	176.3	789.2	1164.1
13	**C28 (20S) TA-stigmastane**	2.5	428.7	1649.1	44.2	71.1	641.8	752.2	6946.6	10458.4
14	**C27(20R) TA-ergostane**	3.6	242.3	278.4	304.8	455.2	499.1	1093.5	5053.8	6984.3
15	**C28 (20R) TA-stigmastane**	2.7	362.6	386.8	433.0	588.6	624.5	1502.1	5761.4	9396.7
16	**V**	2.6	112.1	128.2	141.7	153.5	159.0	287.9	1673.8	1799.9
	Total (µg/g oil)	103.2	2403.2	3977.2	2280.4	3043.3	4200.3	7495.8	41723.5	57535.3

Table 0.36: Measured triaromatic steranes concentrations (µg/g oil) in biodegraded North Sea oil in Nigerian sediment.

Peak Number	**Triaromatic Steranes (TAS)**	**Determined Concentration (C_{det}) -(M/Z 231)**								
		Background (µg/g EOM)	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**P**	9.4	328.8	262.7	403.2	262.1	250.6	221.8	1431.4	2338.9
2	**Q**	8.5	227.1	113.7	170.9	111.6	119.4	82.6	608.3	1214.9
3	**C20-TAS**	5.4	304.6	252.7	299.0	199.8	238.7	173.3	1231.5	2031.7

4	**C21-TAS**	39.3	57.5	75.3	147.2	67.9	68.0	49.0	315.0	525.3
5	**C22A-TAS**	4.7	39.5	58.2	88.8	51.3	48.1	48.9	277.9	363.6
6	**C22B-TAS**	5.8	35.0	37.0	55.3	35.4	39.0	26.0	243.5	292.4
7	**R**	1.9	7.0	39.0	71.4	19.8	30.6	25.3	203.6	282.7
8	**S**	2.2	12.3	29.7	59.2	20.9	22.0	25.0	158.9	264.1
9	**T**	2.8	24.7	75.7	134.8	39.2	62.3	60.9	351.9	523.9
10	**C26(20S) TA-cholestane**	2.6	74.9	315.8	582.5	152.5	239.2	249.8	1572.4	2810.3
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	8.5	269.8	958.6	1711.8	526.3	755.2	722.9	4959.6	7657.3
12	**U**	0.8	18.1	83.5	152.9	50.2	66.4	59.1	384.7	604.0
13	**C28 (20S) TA-stigmastane**	2.5	180.0	754.2	1349.1	346.0	588.0	542.7	4058.7	5823.7
14	**C27(20R) TA-ergostane**	3.6	162.7	551.2	926.9	446.7	460.6	375.0	2951.6	4636.8
15	**C28 (20R) TA-stigmastane**	2.7	154.5	683.1	1168.7	545.6	528.9	477.6	3706.2	5760.5
16	**V**	2.6	40.2	139.6	274.6	126.5	94.6	86.5	545.5	1036.9
	Total (µg/g oil)	103.2	1936.6	4430.2	7596.2	3001.8	3611.6	3226.5	23000.7	36166.9

Table 0.37: Measured concentrations (µg/g oil) of bicyclic sesquiterpanes in biodegraded Nigerian light oil in Whitley sediment.

					Determined Concentration (C_{det}) (m/z 123)						
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C4-decalin (C14)**	**BS1**	21.3	3019.4	1595.6	1242.9	717.8	376.9	12.6	1.6	1527.3

2	**C14 Sesquiterpane**	**BS2**	10.1	1425.5	1055.9	790.0	463.2	244.0	21.5	0.7	1012.8
Unknown	**A Sesquiterpane**	**BSA**	10.3	8270.3	4779.6	3636.3	2229.3	1683.4	356.6	2.9	4440.3
Unknown	**U2 Sesquiterpane**	**BSU2**	6.9	2459.5	1453.1	1125.1	757.4	521.9	103.4	0.8	1423.0
3	**C15 Sesquiterpane**	**BS3**	22.3	7314.7	4112.9	3454.1	2294.8	1728.8	344.4	3.9	4087.5
Unknown	**Y Sesquiterpane**	**BSY**	17.6	1452.2	832.2	751.9	462.6	362.1	74.8	2.0	778.6
Unknown	**B Sesquiterpane**	**BSB**	5.5	3052.0	1901.9	1651.5	1209.2	937.6	334.3	5.4	1934.8
4	**C15 Sesquiterpane**	**BS4**	15.3	4586.7	2562.8	2274.2	1646.8	1237.7	352.5	10.8	2693.7
Unknown	**D Sesquiterpane**	**BSD**	9.6	3127.9	2023.5	1623.5	1147.3	933.2	425.0	9.6	2205.3
5	**8β(H)-drimane**	**BS5**	32.7	4096.5	2605.4	2210.9	1629.1	1187.4	333.7	19.6	2582.8
6	**C15 Sesquiterpane**	**BS6**	13.2	2853.2	1574.1	1447.1	1069.5	831.1	315.1	11.0	1748.2
7	**C16 Sesquiterpane**	**BS7**	7.5	1548.4	1165.8	1086.6	832.2	615.3	184.2	12.3	1279.7
8	**C16 Sesquiterpane**	**BS8**	10.3	1711.8	1024.5	957.2	855.1	657.7	284.5	32.1	1105.0
9	**C16 Sesquiterpane**	**BS9**	5.8	1171.6	649.7	597.2	519.5	367.2	153.6	21.1	689.8
10	**8β(H)-Homodrimane**	**BS10**	65.5	3775.6	2303.7	2121.0	1806.8	1479.6	701.7	147.0	2448.9
	Total (μg/g oil)		**253.9**	**49865.4**	**29640.7**	**24969.4**	**17640.6**	**13163.9**	**3997.9**	**280.8**	**29957.7**

Table 0.38: Measured concentrations (μg/g oil) of bicyclic sesquiterpanes in biodegraded Nigerian medium oil in Whitley sediment.

			Determined Concentration (C_{det}) (m/z 123)								
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (μg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C4-decalin (C14)**	**BS1**	21.3	3734.6	1857.7	1887.8	782.0	256.2	68.3	4.4	1461.3
2	**C14 Sesquiterpane**	**BS2**	10.1	2007.0	1209.0	1220.6	593.4	196.2	72.3	4.1	940.5
Unknown	**A Sesquiterpane**	**BSA**	10.3	11070.7	5490.4	5392.7	2507.4	1286.8	687.2	17.4	4204.7
Unknown	**U2 Sesquiterpane**	**BSU2**	6.9	3638.2	1800.2	1760.5	965.3	474.5	137.1	9.7	1387.3
3	**C15 Sesquiterpane**	**BS3**	22.3	15024.1	8211.2	8656.7	4900.0	2712.3	1382.0	185.3	6420.6
Unknown	**Y Sesquiterpane**	**BSY**	17.6	1652.2	1483.2	1468.8	966.9	531.4	222.0	43.2	1204.4
Unknown	**B Sesquiterpane**	**BSB**	5.5	4577.9	2476.1	2562.2	1784.6	1168.3	533.0	113.1	2118.3
4	**C15 Sesquiterpane**	**BS4**	15.3	7794.8	4039.3	4271.4	2744.7	1831.7	798.4	216.6	3298.7
Unknown	**D Sesquiterpane**	**BSD**	9.6	4707.9	2628.2	2840.0	1627.2	1287.7	540.8	144.8	2127.1
5	**8β(H)-drimane**	**BS5**	32.7	5858.8	3743.1	3513.2	2321.9	1860.0	664.7	257.8	2804.6
6	**C15 Sesquiterpane**	**BS6**	13.2	5563.3	2705.9	2788.1	1982.6	1554.2	587.8	217.0	2237.1
7	**C16 Sesquiterpane**	**BS7**	7.5	2976.4	1673.5	1780.7	1295.6	992.2	352.8	179.7	1427.3

8	**C16 Sesquiterpane**	**BS8**	10.3	3570.1	1843.8	1968.8	1634.0	1359.7	582.4	391.1	1674.0
9	**C16 Sesquiterpane**	**BS9**	5.8	3030.1	1321.4	1449.1	1173.5	982.9	460.1	314.6	1192.9
10	**8β(H)-Homodrimane**	**BS10**	65.5	4507.2	2701.0	2748.2	2242.7	1948.1	980.9	658.1	2274.2
	Total (μg/g oil)		**253.9**	**79713.3**	**43184.2**	**44308.6**	**27521.9**	**18442.2**	**8069.7**	**2756.8**	**34772.9**

Table 0.39: Measured concentrations (μg/g oil) of bicyclic sesquiterpanes in biodegraded North Sea oil in Whitley sediment.

			Determined Concentration (C_{det}) (m/z 123)								
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (ug/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C4-decalin (C14)**	**BS1**	21.3	373.0	361.5	337.6	282.9	161.2	27.8	1.9	25.3
2	**C14 Sesquiterpane**	**BS2**	10.1	389.5	236.3	231.0	196.2	132.3	30.7	1.6	25.1
3	**C15 Sesquiterpane**	**BS3**	22.3	765.8	571.6	549.0	456.4	343.5	142.7	12.1	90.4
Unknown	**B Sesquiterpane**	**BSB**	5.5	267.2	201.1	216.4	189.6	145.9	68.2	7.0	35.2
4	**C15 Sesquiterpane**	**BS4**	15.3	541.9	354.1	352.4	325.1	278.2	153.5	15.2	76.3
5	**8β(H)-drimane**	**BS5**	32.7	840.9	674.6	654.8	572.2	473.9	234.1	41.6	109.3

6	**C15 Sesquiterpane**	**BS6**	13.2	482.9	339.7	343.9	307.5	216.9	140.4	26.7	54.1
7	**C16 Sesquiterpane**	**BS7**	7.5	201.9	241.8	231.9	238.3	175.4	152.8	23.1	35.9
8	**C16 Sesquiterpane**	**BS8**	10.3	445.4	255.8	250.3	234.3	201.7	151.1	59.6	66.8
9	**C16 Sesquiterpane**	**BS9**	5.8	271.8	215.3	200.1	170.3	153.5	105.5	36.2	34.8
10	**8β(H)-Homodrimane**	**BS10**	65.5	833.3	591.9	566.9	542.1	530.0	427.7	158.6	197.4
	Total (µg/g oil)		**209.4**	**5413.6**	**4043.7**	**3934.4**	**3514.8**	**2812.4**	**1634.4**	**383.6**	**750.6**

Table 0.40: Measured concentrations (µg/g oil) of bicyclic sesquiterpanes in biodegraded Nigerian light oil in Nigerian sediment.

				Determined Concentration (C_{det}) (m/z 123)							
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C4-decalin (C14)**	**BS1**	14.7	3019.4	1480.0	1009.1	685.2	780.5	495.3	174.3	318.8
2	**C14 Sesquiterpane**	**BS2**	10.6	1425.5	841.4	599.3	334.7	544.5	286.6	94.7	167.0
Unknown	**A Sesquiterpane**	**BSA**	103.7	8270.3	4549.8	3341.9	1971.2	3294.6	1909.5	762.6	1174.5
Unknown	**U2 Sesquiterpane**	**BSU2**	29.3	2459.5	1411.0	1082.4	662.6	1240.9	693.6	188.0	237.5
3	**C15 Sesquiterpane**	**BS3**	141.8	7314.7	3866.7	3179.9	1928.3	3580.0	2249.7	758.6	1186.9

Unknown	**Y Sesquiterpane**	**BSY**	47.1	1452.2	923.3	860.3	502.4	973.9	694.3	194.7	265.6
Unknown	**B Sesquiterpane**	**BSB**	137.6	3052.0	1940.7	1652.2	1040.6	2309.4	1442.2	533.0	717.1
4	**C15 Sesquiterpane**	**BS4**	157.1	4586.7	2575.1	1920.2	1280.4	2761.4	1746.0	679.3	979.3
Unknown	**D Sesquiterpane**	**BSD**	153.1	3127.9	2245.4	2458.4	1485.9	2990.4	2000.0	612.1	805.6
5	**8β(H)-drimane**	**BS5**	203.4	4096.5	2542.5	2089.2	1287.2	2990.0	1877.8	847.6	1180.4
6	**C15 Sesquiterpane**	**BS6**	152.3	2853.2	1678.6	1651.6	1025.8	2060.6	1556.1	658.9	874.8
7	**C16 Sesquiterpane**	**BS7**	134.9	1548.4	1383.6	1361.1	845.6	1895.9	1356.6	520.2	667.1
8	**C16 Sesquiterpane**	**BS8**	131.6	1711.8	1238.3	1049.9	805.0	1746.4	1254.2	458.3	607.5
9	**C16 Sesquiterpane**	**BS9**	115.7	1171.6	771.4	903.8	625.4	1095.8	946.6	339.8	480.0
10	**8β(H)-Homodrimane**	**BS10**	392.8	3775.6	2611.9	2450.0	1671.9	3539.4	2642.2	1326.4	1608.8
	Total (µg/g oil)		**1925.5**	**49865.4**	**30059.9**	**25609.5**	**16152.2**	**31803.8**	**21150.6**	**8148.6**	**11270.8**

Table 0.41: Measured concentrations (µg/g oil) of bicyclic sesquiterpanes in biodegraded Nigerian medium oil in Nigerian sediment.

			Determined Concentration (C_{det}) (m/z 123)								
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5days**	**10days**	**20days**	**50days**	**100days**	**300days**	**Sterilised**
1	**C4-decalin (C14)**	**BS1**	14.7	3734.6	1670.7	1222.7	636.4	543.1	438.8	165.9	289.6
2	**C14 Sesquiterpane**	**BS2**	10.6	2007.0	1005.6	771.8	421.5	362.5	265.2	101.4	150.3

Unknown	**A Sesquiterpane**	**BSA**	103.7	11070.7	5554.7	4154.2	2320.9	2020.8	1618.9	755.0	994.3
Unknown	**U2 Sesquiterpane**	**BSU2**	29.3	3638.2	1834.8	1350.2	837.7	764.3	664.9	170.4	246.0
3	**C15 Sesquiterpane**	**BS3**	141.8	15024.1	6707.7	5280.8	3528.4	2975.9	2571.6	984.4	1348.0
Unknown	**Y Sesquiterpane**	**BSY**	47.1	1652.2	1576.2	1104.3	740.6	717.7	624.3	217.3	241.3
Unknown	**B Sesquiterpane**	**BSB**	137.6	4577.9	2468.8	1895.1	1406.5	1209.1	1200.5	597.9	669.0
4	**C15 Sesquiterpane**	**BS4**	157.1	7794.8	3582.4	2924.6	1991.2	1806.6	1750.2	848.9	968.0
Unknown	**D Sesquiterpane**	**BSD**	153.1	4707.9	3665.1	2910.6	1975.5	1773.8	1666.1	714.6	732.0
5	**8β(H)-drimane**	**BS5**	203.4	5858.8	3691.8	2556.3	1871.8	1674.2	1559.2	981.4	1260.0
6	**C15 Sesquiterpane**	**BS6**	152.3	5563.3	2789.8	2288.2	1567.7	1384.2	1321.0	816.6	854.8
7	**C16 Sesquiterpane**	**BS7**	134.9	2976.4	1886.0	1551.3	1138.7	1024.5	1006.9	642.9	698.7
8	**C16 Sesquiterpane**	**BS8**	131.6	3570.1	2002.1	1604.0	1238.0	1144.9	1007.8	643.0	734.5
9	**C16 Sesquiterpane**	**BS9**	115.7	3030.1	1485.7	1380.7	960.1	946.5	871.9	519.5	552.7
10	**8β(H)-Homodrimane**	**BS10**	392.8	4507.2	3328.1	2609.6	2065.1	1766.9	1637.7	1495.1	1761.6
	Total (µg/g oil)		**1925.5**	**79713.3**	**43249.5**	**33604.2**	**22700.3**	**20114.8**	**18204.9**	**9654.2**	**11500.8**

Table 0.42: Measured concentrations (µg/g oil) of bicyclic sesquiterpanes in biodegraded North Sea oil in Nigerian sediment.

			Determined Concentration (C_{det}) (m/z 123)								
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**C4-decalin (C14)**	**BS1**	14.7	373.0	533.7	387.7	337.0	209.3	167.1	123.7	258.8
2	**C14 Sesquiterpane**	**BS2**	10.6	389.5	341.7	236.2	208.0	124.3	116.4	71.5	173.3
Unknown	**A Sesquiterpane**	**BSA**	103.7	0.0	1450.3	1049.2	949.1	695.9	585.2	454.6	1050.2
Unknown	**U2 Sesquiterpane**	**BSU2**	29.3	0.0	545.8	413.5	328.2	237.5	178.9	131.0	243.2
3	**C15 Sesquiterpane**	**BS3**	141.8	765.8	1583.7	1043.1	922.4	737.2	558.0	542.7	1143.2
Unknown	**Y Sesquiterpane**	**BSY**	47.1	0.0	441.7	377.2	245.0	183.0	144.7	135.8	250.2
Unknown	**B Sesquiterpane**	**BSB**	137.6	267.2	843.8	627.1	449.8	369.8	397.3	311.5	711.7
4	**C15 Sesquiterpane**	**BS4**	157.1	541.9	1040.6	776.7	579.1	533.9	494.3	392.9	908.1
Unknown	**D Sesquiterpane**	**BSD**	153.1	0.0	1015.8	795.9	802.1	667.5	447.9	436.4	819.4
5	**8β(H)-drimane**	**BS5**	203.4	840.9	1443.5	919.0	762.6	679.7	665.4	643.3	1091.5
6	**C15 Sesquiterpane**	**BS6**	152.3	482.9	996.8	668.5	589.3	552.7	518.5	442.4	838.6

7	**C16 Sesquiterpane**	**BS7**	134.9	201.9	755.9	542.6	387.7	381.4	431.0	304.1	693.5
8	**C16 Sesquiterpane**	**BS8**	131.6	445.4	766.7	515.2	423.6	404.4	368.2	284.8	578.2
9	**C16 Sesquiterpane**	**BS9**	115.7	271.8	528.0	412.7	358.8	348.8	291.3	270.3	439.6
10	**8β(H)-Homodrimane**	**BS10**	392.8	833.3	1643.4	1099.0	1014.2	1017.5	1000.2	734.9	1568.7
	Total (µg/g oil)		**1925.5**	**5413.6**	**13931.2**	**9863.4**	**8356.8**	**7142.9**	**6364.4**	**5279.9**	**10768.2**

Table 0.43: Measured concentrations (µg/g oil) of Adamantanes in biodegraded Nigerian light oil in Whitley sediment.

			Determined Concentration (C_{det}) (m/z 136)								
Peak Number	**Adamantanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**Adamantane**	**A**	0.0	65.4	6.5	1.0	0.0	0.0	0.0	0.0	0.0
2	**1-Methyladamantane**	**1-MA**	0.0	1466.1	118.5	38.4	6.2	0.6	1.0	0.0	0.0
3	**1,3-Dimethyladamantane**	**1,3-DMA**	0.0	653.9	79.2	33.1	5.4	0.7	0.3	0.0	0.0
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	0.0	102.9	22.0	11.0	2.7	0.4	0.0	0.0	0.0

5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	0.0	71.7	11.0	4.2	0.0	0.0	0.0	0.0	0.0
6	**2-Methyladamantane**	**2-MA**	1.4	342.4	81.0	48.0	13.4	2.0	0.6	0.0	0.0
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	2.6	422.5	114.4	74.5	23.9	2.1	0.6	0.0	0.0
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	2.0	277.0	80.1	53.5	17.4	2.3	0.4	0.0	0.0
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	1.8	99.7	30.0	18.3	7.2	1.7	0.5	0.0	0.0
10	**1,2-Dimethyladamantane**	**1,2-DMA**	5.3	505.6	151.5	108.2	40.2	11.4	3.4	0.0	0.0
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	2.8	516.0	145.6	114.3	59.7	13.3	1.4	0.0	0.0
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	7.9	416.1	133.1	107.4	62.0	14.7	5.3	0.0	0.0
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	3.0	131.3	29.1	31.4	23.5	4.8	1.6	0.0	0.0

14	**1-Ethyladamantane**	**1-AE**	11.8	717.0	221.7	188.1	102.9	16.4	5.2	0.0	0.0
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	6.8	223.8	166.8	146.1	80.4	6.5	3.3	0.0	0.0
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	6.3	204.0	70.9	63.6	37.3	9.6	2.4	0.0	0.0
17	**2-Ethyladamantane**	**2-AE**	6.4	74.4	50.4	33.1	30.0	6.6	1.4	0.0	0.0
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	5.5	95.4	65.1	49.3	31.8	12.7	5.8	0.0	0.0
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	9.4	344.9	84.2	85.2	69.7	24.9	10.0	0.0	0.0
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	4.5	201.8	106.9	108.2	84.9	13.8	4.0	0.0	0.0
	Total (µg/g oil)		**77.5**	**6932.0**	**1768.0**	**1317.0**	**698.7**	**144.4**	**47.3**	**0.0**	**0.0**

Table 0.44: Measured concentrations (μg/g oil) of Adamantanes in biodegraded Nigerian medium oil in Whitley sediment.

			Determined Concentration (C_{det}) (m/z 136)								
Peak Number	**Adamantanes**	**Code**	**Background (μg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**Adamantane**	**A**	0.0	20.0	1.2	0.6	0.0	0.0	0.0	0.0	0.0
2	**1-Methyladamantane**	**1-MA**	0.0	516.6	55.0	9.9	5.3	0.4	0.6	0.0	0.0
3	**1,3-Dimethyladamantane**	**1,3-DMA**	0.0	254.6	31.6	8.8	6.0	0.0	0.0	0.0	0.0
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	0.0	112.6	22.5	8.7	5.4	0.0	0.0	0.0	0.0
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	0.0	30.0	8.9	2.9	1.7	0.0	0.0	0.0	0.0
6	**2-Methyladamantane**	**2-MA**	1.4	169.8	42.8	22.7	13.5	0.9	0.6	0.0	0.0
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	2.6	329.7	89.8	51.3	32.0	1.2	0.4	0.0	0.0

8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	2.0	209.0	65.4	40.7	25.0	2.2	0.6	0.0	0.0
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	1.8	97.5	21.1	14.4	9.6	1.2	0.7	0.0	0.0
10	**1,2-Dimethyladamantane**	**1,2-DMA**	5.3	477.2	122.9	77.0	64.2	9.5	4.7	0.0	0.0
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	2.8	461.5	126.1	89.0	73.6	5.1	0.4	0.0	0.0
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	7.9	357.1	125.4	99.5	81.2	11.5	4.4	0.0	0.0
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	3.0	79.2	26.5	22.9	22.4	4.3	1.3	0.0	0.0
14	**1-Ethyladamantane**	**1-AE**	11.8	590.9	186.6	144.5	125.8	13.6	4.6	0.0	0.0
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	6.8	278.9	164.2	132.7	107.3	7.3	4.6	0.0	0.0
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	6.3	207.8	80.6	67.6	52.4	10.0	5.9	0.0	0.0

17	**2-Ethyladamantane**	**2-AE**	6.4	68.5	47.3	38.3	38.2	6.9	2.9	0.0	0.0
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	5.5	89.7	38.6	41.7	33.1	15.0	7.7	0.0	0.0
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	9.4	328.0	92.2	93.5	84.1	20.5	10.9	0.0	0.0
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	4.5	186.7	87.9	75.5	55.7	13.4	7.4	0.0	0.0
	Total (µg/g oil)		**77.5**	**4865.1**	**1436.5**	**1042.2**	**836.7**	**123.0**	**57.9**	**0.0**	**0.0**

Table 0.45: Measured concentrations (µg/g oil) of Adamantanes in biodegraded North Sea oil in Whitley sediment.

						Determined Concentration (C_{det}) (m/z 136)					
Peak Number	**Adamantanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**Adamantane**	**A**	0.0	29.9	3.0	0.7	0.0	0.0	0.0	0.0	0.0
2	**1-Methyladamantane**	**1-MA**	0.0	102.3	16.0	9.8	1.6	0.5	0.9	0.0	0.0

3	**1,3-Dimethyladamantane**	**1,3-DMA**	0.0	28.8	6.9	4.5	2.0	0.2	0.2	0.0	0.0
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	0.0	29.0	7.8	6.9	3.7	0.3	0.0	0.0	0.0
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	0.0	20.5	5.1	2.6	1.7	0.0	0.0	0.0	0.0
6	**2-Methyladamantane**	**2-MA**	1.4	35.2	12.4	9.7	6.4	0.2	0.1	0.0	0.0
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	2.6	105.0	40.9	35.4	23.7	1.8	0.2	0.0	0.0
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	2.0	71.3	28.5	23.1	15.0	1.7	0.1	0.0	0.0
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	1.8	28.5	9.7	9.5	6.2	0.9	0.1	0.0	0.0
10	**1,2-Dimethyladamantane**	**1,2-DMA**	5.3	137.8	49.3	40.5	29.9	6.7	1.2	0.0	0.0
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	2.8	176.1	64.3	55.7	39.1	3.2	0.2	0.0	0.0

12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	7.9	119.2	47.4	42.1	35.1	8.4	1.2	0.0	0.0
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	3.0	33.2	11.3	10.7	10.3	2.6	0.4	0.0	0.0
14	**1-Ethyladamantane**	**1-AE**	11.8	166.6	65.2	61.5	46.1	10.3	0.7	0.0	0.0
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	6.8	88.4	61.1	57.1	43.5	9.5	0.6	0.0	0.0
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	6.3	58.2	29.0	26.3	20.3	4.8	0.4	0.0	0.0
17	**2-Ethyladamantane**	**2-AE**	6.4	32.1	15.5	17.3	14.9	3.9	0.4	0.0	0.0
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	5.5	37.3	19.6	20.7	16.3	6.0	2.4	0.0	0.0
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	9.4	139.8	43.9	36.9	31.0	16.2	1.0	0.0	0.0
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	4.5	104.7	80.8	60.9	45.9	11.2	1.2	0.0	0.0

Total (µg/g oil)	**77.5**	**1543.9**	**617.7**	**531.8**	**392.6**	**88.5**	**11.4**	**0.0**	**0.0**

Table 0.46: Measured concentrations (µg/g oil) of Adamantanes in biodegraded Nigerian light oil in Nigerian sediment.

			Determined Concentration (C_{det}) (m/z 136)								
Peak Number	**Adamantanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**Adamantane**	**A**	0.3	65.4	3.1	1.9	0.8	0.7	0.0	0.0	0.0
2	**1-Methyladamantane**	**1-MA**	0.3	1466.1	74.0	15.7	10.0	16.6	3.4	0.0	0.0
3	**1,3-Dimethyladamantane**	**1,3-DMA**	0.0	653.9	45.5	11.7	10.3	9.2	2.5	0.0	0.0
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	0.1	102.9	11.8	5.0	3.4	2.4	0.5	0.0	0.0
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	0.1	71.7	5.7	3.3	1.0	0.7	0.0	0.0	0.0
6	**2-Methyladamantane**	**2-MA**	0.2	342.4	49.6	17.5	8.5	8.4	4.0	1.3	2.8
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	0.1	422.5	71.8	29.3	12.2	10.8	4.9	2.6	3.1

8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	0.1	277.0	51.9	22.8	9.8	7.8	3.6	2.0	3.7
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	0.1	99.7	17.3	7.1	3.8	2.1	1.1	1.75	2.7
10	**1,2-Dimethyladamantane**	**1,2-DMA**	0.2	505.6	76.1	43.3	20.3	18.7	10.0	5.5	12.8
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	0.3	516.0	117.9	51.1	24.6	18.5	4.7	2.8	5.9
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	0.2	416.1	104.8	48.2	24.8	19.9	11.1	7.9	13.10
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	0.3	131.3	23.7	10.7	8.5	6.2	2.8	3.0	6.1
14	**1-Ethyladamantane**	**1-AE**	0.2	717.0	164.1	81.8	47.3	43.2	21.8	11.8	21.3
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	0.7	223.8	137.9	63.4	37.3	35.6	17.8	6.8	11.2
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	0.2	204.0	55.9	30.7	18.9	15.7	7.1	6.6	11.2
17	**2-Ethyladamantane**	**2-AE**	0.2	74.4	39.1	22.6	14.0	11.9	6.8	6.4	7.5

18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	0.1	95.4	53.6	20.2	19.8	17.3	7.4	5.5	10.1
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	0.1	344.9	75.1	40.4	37.4	35.5	16.2	9.4	16.2
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	0.1	201.8	101.5	41.0	36.4	31.1	11.9	4.5	14.8
	Total (µg/g oil)		**3.9**	**6932.0**	**1280.5**	**567.6**	**349.3**	**312.2**	**137.6**	**78.0**	**142.3**

Table 0.47: Measured concentrations (µg/g oil) of Adamantanes in biodegraded Nigerian medium oil in Nigerian sediment.

			Determined Concentration (C$_{det}$) (m/z 136)								
Peak Number	**Adamantanes**	**Code**	**Background (µg/g EOM)**	**Start**	**5 days**	**10 days**	**20 days**	**50 days**	**100 days**	**300 days**	**Sterilised**
1	**Adamantane**	**A**	0.3	20.0	1.8	0.5	0.0	0.0	0.0	0.0	0.0
2	**1-Methyladamantane**	**1-MA**	0.3	516.6	53.9	31.6	7.3	2.0	1.4	0.0	0.0
3	**1,3-Dimethyladamantane**	**1,3-DMA**	0.0	254.6	28.9	17.6	4.0	1.1	1.7	1.3	2.3

4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	0.1	112.6	18.2	9.7	1.4	0.5	0.3	0.0	0.0
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	0.1	30.0	6.1	3.7	0.6	0.0	0.0	0.0	0.0
6	**2-Methyladamantane**	**2-MA**	0.2	169.8	33.9	19.0	6.1	1.6	2.8	2.5	4.1
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	0.1	329.7	58.4	36.1	11.0	2.8	4.0	2.7	2.9
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	0.1	209.0	46.8	28.2	10.2	2.4	3.0	1.4	2.1
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	0.1	97.5	13.8	7.9	3.5	0.0	0.0	0.00	0.0
10	**1,2-Dimethyladamantane**	**1,2-DMA**	0.2	477.2	71.9	56.1	19.9	5.9	6.9	5.3	8.2
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	0.3	461.5	87.0	59.6	26.1	10.5	3.9	2.8	7.0
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	0.2	357.1	83.0	56.4	26.6	11.7	8.1	6.5	11.41

13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	0.3	79.2	13.6	6.1	3.7	3.3	3.0	1.9	4.7
14	**1-Ethyladamantane**	**1-AE**	0.2	590.9	118.7	87.5	45.8	23.7	19.8	13.5	16.5
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	0.7	278.9	109.1	72.6	36.8	20.5	16.2	5.6	10.1
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	0.2	207.8	52.5	38.2	21.0	9.9	6.5	6.4	10.2
17	**2-Ethyladamantane**	**2-AE**	0.2	68.5	39.1	27.3	15.2	9.6	3.9	3.2	5.0
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	0.1	89.7	31.6	20.0	10.9	8.1	7.0	4.8	8.1
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	0.1	328.0	72.4	56.0	34.7	25.3	14.3	10.2	18.4
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	0.1	186.7	68.6	47.4	30.0	21.3	6.2	3.9	13.3
	Total (µg/g oil)		**3.9**	**4865.1**	**1009.4**	**681.3**	**314.9**	**160.2**	**108.9**	**71.9**	**124.3**

Table 0.48: Measured concentrations (µg/g oil) of Adamantanes in biodegraded North Sea oil in Nigerian sediment.

Peak Number	Adamantanes	Code	Determined Concentration (C_{det}) (m/z 136)								
			Background (µg/g EOM)	Start	5 days	10 days	20 days	50 days	100 days	300 days	Sterilised
1	**Adamantane**	**A**	0.3	29.9	1.4	0.0	0.0	0.0	0.0	0.0	0.0
2	**1-Methyladamantane**	**1-MA**	0.3	102.3	7.2	4.3	3.6	0.7	0.8	1.1	1.5
3	**1,3-Dimethyladamantane**	**1,3-DMA**	0.0	28.8	5.4	3.0	2.5	0.6	0.8	0.0	0.0
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	0.1	29.0	3.1	1.8	1.2	0.0	0.0	0.0	0.0
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	0.1	20.5	1.0	0.8	0.0	0.0	0.0	0.0	0.0
6	**2-Methyladamantane**	**2-MA**	0.2	35.2	8.1	5.2	4.4	1.1	2.1	1.5	2.9
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	0.1	105.0	19.3	13.4	8.1	1.7	3.1	1.3	1.5

8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	0.1	71.3	13.1	12.8	6.7	1.8	2.4	0.7	0.6
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	0.1	28.5	0.0	0.0	0.0	0.0	0.0	0.00	0.0
10	**1,2-Dimethyladamantane**	**1,2-DMA**	0.2	137.8	23.7	25.9	12.4	2.8	5.4	4.1	5.5
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	0.3	176.1	40.4	25.7	18.6	3.9	3.3	2.0	3.2
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	0.2	119.2	32.1	20.4	16.1	4.6	6.5	5.3	6.53
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	0.3	33.2	7.2	4.6	3.7	1.3	1.6	2.1	2.5
14	**1-Ethyladamantane**	**1-AE**	0.2	166.6	49.9	36.2	28.7	10.6	14.3	8.1	12.5
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	0.7	88.4	38.0	26.7	26.6	9.3	10.8	4.8	7.1
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	0.2	58.2	20.4	14.4	13.4	5.5	6.0	4.4	5.8

17	**2-Ethyladamantane**	**2-AE**	0.2	32.1	17.7	10.9	11.0	5.2	4.2	2.5	3.5
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	0.1	37.3	18.8	12.3	7.9	6.7	4.0	4.8	5.3
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	0.1	139.8	31.9	20.0	23.1	11.1	11.2	8.6	11.3
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	0.1	104.7	50.1	34.3	31.4	20.0	9.8	2.6	7.4
	Total (μg/g oil)		**3.9**	**1543.9**	**388.8**	**272.5**	**219.5**	**87.1**	**86.2**	**54.0**	**77.0**

Table 0.49: Concentrations of selected aliphatics/PAH for Nigerian light oil in Whitley sediment.

Compound	Background (ug/g EOM)	Start	5days	10days	20days	50days	100days	300days	Sterilised
2,6+2,7-DMN	16.2	2406.0	345.5	92.7	35.1	5.1	2.3	0.0	0.0
nC14	0.0	7171.3	3881.8	1606.2	705.0	0.0	0.0	0.0	195.0
BS4	15.3	4586.7	2562.8	2274.2	1646.8	1237.7	352.5	10.8	2693.7
nC15	0.0	6535.7	1547.1	671.0	351.8	0.0	0.0	0.0	82.5
Pristane	72.0	5337.3	1734.8	1244.3	1099.6	321.7	86.5	0.0	783.8
nC17	106.9	4615.3	1398.0	559.0	346.3	0.0	0.0	0.0	619.3
C30Olean	43.9	5867.9	3189.7	3238.9	3068.4	2778.4	2981.2	1566.4	1370.7
nC31	23.9	1244.8	304.1	253.4	237.2	204.3	72.0	54.9	250.8

Table 0.50: Concentrations of selected aliphatics/PAH for Nigerian medium oil in Whitley sediment.

Compound	Background (ug/g EOM)	Start	5days	10days	20days	50days	100days	300days	Sterilised
2,6+2,7-DMN	16.2	51.1	71.8	57.5	32.9	2.0	0.0	0.0	0.0
nC14	0.0	1899.5	201.7	111.3	36.5	0.0	0.0	0.0	75.2
BS4	15.3	7794.8	4039.3	4271.4	2744.7	1831.7	798.4	216.6	3298.7
nC15	0.0	2114.7	265.8	162.9	52.9	0.0	0.0	0.0	79.8
Pristane	72.0	5357.8	1583.0	1749.9	1370.3	743.5	174.0	0.0	1283.8
nC17	106.9	2415.0	443.6	334.8	137.4	58.2	0.0	0.0	500.5
C30Olean	43.9	5254.3	3106.9	2959.7	2928.8	3019.7	3186.6	2055.0	1766.1
nC31	23.9	1663.6	466.7	350.4	326.1	271.0	142.1	0.0	368.0

Table 0.51: Concentrations of selected aliphatics/PAH for North Sea oil in Whitley sediment.

Compound	Background (ug/g EOM)	Start	5days	10days	20days	50days	100days	300days	Sterilised
2,6+2,7-DMN	16.2	46.3	87.9	48.7	31.6	2.2	0.0	0.0	1.0
nC14	0.0	5572.1	1377.8	802.2	218.9	10.0	0.0	0.0	189.8
BS4	15.3	541.9	354.1	352.4	325.1	278.2	153.5	15.2	76.3
nC15	0.0	5013.2	1344.4	754.5	270.7	32.6	0.0	0.0	200.9
Pristane	72.0	2016.3	890.1	610.4	257.7	221.3	0.0	0.0	339.0

nC17	106.9	3984.8	1153.8	665.5	248.4	49.1	0.0	0.0	630.2
C30-Hopane	296.6	337.0	246.6	250.2	289.3	260.9	292.4	150.8	165.8
nC31	23.9	181.9	69.4	66.0	27.1	24.7	0.0	0.0	42.7

Table 0.52: Concentrations of selected aliphatics/PAH for Nigerian light oil in Nigerian sediment.

Compound	Background (ug/g EOM)	Start	5days	10days	20days	50days	100days	300days	Sterilised
2,6+2,7-DMN	0.0	2406.0	136.9	120.0	40.0	1.3	0.0	0.0	9.2
_n_C14	14.2	7171.3	1396.9	1847.8	2174.5	1488.7	774.4	46.4	88.8
BS4	157.1	4586.7	2575.1	1920.2	1280.4	2761.4	1746.0	679.3	979.3
_n_C15	0.0	6535.7	1390.0	1698.3	1914.8	1566.0	1101.9	415.5	111.6
Pristane	24.3	5337.3	1389.7	1627.1	1899.1	1712.4	1575.0	993.6	1022.8
_n_C17	22.8	4615.3	1158.0	1424.3	1742.5	1472.8	1129.9	652.5	492.5
C30Olean	3102.9	5867.9	6151.8	9473.2	8799.3	14482.0	9326.9	7091.4	9745.0
_n_C31	0.0	1244.8	221.1	153.8	268.5	349.8	238.6	307.6	252.0

Table 0.53: Concentrations of selected aliphatics/PAH for Nigerian medium oil in Nigerian sediment.

Compound	Background (µg/g EOM)	Start	5days	10days	20days	50days	100days	300days	Sterilised
2,6+2,7-DMN	0.0	51.1	97.2	90.0	0.0	0.0	0.0	0.0	10.1
_n_C14	14.2	1899.5	647.2	292.7	318.3	377.8	783.0	123.1	157.5

BS4	157.1	7794.8	3582.4	2924.6	1991.2	1806.6	1750.2	848.9	968.0
***n*C15**	0.0	2114.7	443.5	438.9	440.2	545.9	1434.3	131.0	168.6
Pristane	24.3	5357.8	3140.6	1664.0	1895.9	2812.6	8050.1	1199.1	1450.2
***n*C17**	22.8	2415.0	793.1	855.9	892.4	1188.8	2507.4	376.9	422.3
C30Olean	3102.9	5254.3	10331.1	7880.1	7931.1	6126.7	6448.9	6288.1	9090.2
***n*C31**	0.0	1663.6	754.4	493.7	485.2	579.6	1507.3	392.1	404.8

Table 0.54: Concentrations of selected aliphatics/PAH for North Sea oil in Nigerian sediment.

Compound	Background (µg/g EOM)	Start	5days	10days	20days	50days	100days	300days	Sterilised
2,6+2,7-DMN	0.0	46.3	276.8	234.0	13.0	0.0	0.0	0.0	0.0
nC14	14.2	5572.1	2128.6	2570.7	2283.6	2274.7	893.6	102.8	311.5
BS4	157.1	541.9	1040.6	776.7	579.1	533.9	494.3	392.9	908.1
nC15	0.0	5013.2	1900.6	2195.3	2004.0	2268.6	1348.7	282.8	602.4
Pristane	24.3	2016.3	845.2	967.2	959.9	1230.7	1142.4	505.7	1756.3
nC17	22.8	3984.8	1628.5	1910.9	1756.4	2235.0	1261.4	424.8	1921.0
C30-Hopane	3228.6	337.0	8356.8	6583.9	6804.2	4952.5	7360.9	6009.5	8325.0
nC31	0.0	181.9	343.7	137.3	397.1	82.6	60.3	68.0	257.8

Table 0.55: Identification

Compound	Assignment

2,6,+2,7-DMN	2,6+2,7 Dimethylnaphthalene
BS4	C15-Sesquiterpane
C30Olean	C30-18α(H) Oleanane

Table 0.56: Background concentrations of selected biomarkers in Whitley Bay sediment and in the starting oils.

Biomarker	EOM (mg)	Backgr. Conc. (µg/g EOM)	Backgr. Conc. (µg/10g)	Starting Oil (µg/g oil)	Backgr amt. (in 10g sed) + 50mg Starting Oil (ug)	Ratio of oil (50mg) to backgr (10g)	Oil Added to Sed. (ppm)	Assumed Ratio=50	Min. Amnt. of oil (ppm)
Nigerian Light Oil									
C19 Terpane	6.5	12.5	0.0	330.0	16.5	2020	5000	50	124
C26(S) Terpane	6.5	45.7	0.0	56.2	2.8	94	5000	50	2654
C30αβ Hop	6.5	296.6	0.2	8748.1	437.6	2257	5000	50	111
C20-ααα-ster	6.5	2.6	0.0	101.8	5.1	3052	5000	50	82
C30 20R-ster	6.5	298.8	0.2	170.8	8.7	44	5000	50	5714
C4-decalin	6.5	21.3	0.0	3019.4	151.0	10863	5000	50	23
8β(H)-Homo	6.5	65.472	0.043	3775.599	188.8	4413	5000	50	57
Nigerian Medium Oil									
C19 Terpane	6.5	12.5	0.0	379.6	19.0	2324	5000	50	108
C26(S) Terpane	6.5	45.7	0.0	57.6	2.9	97	5000	50	2590
C30αβ Hop	6.5	296.6	0.2	10284.7	514.4	2654	5000	50	94
C20-ααα-ster	6.5	2.6	0.0	91.7	4.6	2747	5000	50	91
C30 20R-ster	6.5	298.8	0.2	109.1	5.6	28	5000	50	8950

C4-decalin	6.5	21.3	0.0	3734.6	186.7	13436	5000	50	19
8β(H)-Homo	6.5	65.5	0.0	4507.2	225.4	5269	5000	50	47
				North Sea Oil					
C19 Terpane	6.5	12.5	0.0	55.8	2.8	341	5000	50	732
C26(S) Terpane	6.5	45.7	0.0	37.3	1.9	62	5000	50	4001
C30αβ Hop	6.5	296.6	0.2	337.0	17.0	87	5000	50	2875
C20-ααα-ster	6.5	2.6	0.0	126.0	6.3	3775	5000	50	66
C30 20R-ster	6.5	298.8	0.2	23.6	1.4	6	5000	50	41320
C4-decalin	6.5	21.3	0.0	373.0	18.7	1342	5000	50	186
8β(H)-Homo	6.5	13.2	0.0	833.3	41.7	4844	5000	50	52

Table 0.57: Background concentrations of selected biomarkers in Nigerian sediment and in the starting oils

				Nigerian Light Oil					
Biomarker	**EOM (mg)**	**Backgr. Conc. (μg/g EOM)**	**Backgr. Conc. (μg/10g)**	**Starting Oil (μg/g oil)**	**Backgr amt. (in 10g sed) + 50mg Starting Oil (ug)**	**Ratio of oil (50mg) to backgr (10g)**	**Oil Added to Sed. (ppm)**	**Assumed Ratio=50**	**Min. Amnt. of oil (ppm)**
C19 Terpane	98.4	74.8	0.7	330.0	17.2	22.4	5000	50	11155
C26(S) Terpane	98.4	69.0	0.7	56.2	3.5	4.1	5000	50	60354
C30αβ Hop	98.4	3228.6	31.8	8748.1	469.2	13.8	5000	50	18158
C20-ααα-ster	98.4	52.6	0.5	101.8	5.6	9.8	5000	50	25428
C30 20R-ster	98.4	86.7	0.9	170.8	9.4	10.0	5000	50	24969

C4-decalin	98.4	14.7	0.1	3019.4	151.1	1047.1	5000	50	239
8β(H)-Homo	98.4	392.8	3.9	3775.6	192.6	48.8	5000	50	5118
Nigerian Medium Oil									
C19 Terpane	98.4	74.8	0.7	379.6	19.7	25.8	5000	50	9697
C26(S) Terpane	98.4	69.0	0.7	57.6	3.6	4.2	5000	50	58899
C30αβ Hop	98.4	296.6	2.9	10284.7	517.2	176.2	5000	50	1419
C20-ααα-ster	98.4	52.6	0.5	91.7	5.1	8.8	5000	50	28249
C30 20R-ster	98.4	86.7	0.9	109.1	6.3	6.4	5000	50	39107
C4-decalin	98.4	14.7	0.1	3734.6	186.9	1295.1	5000	50	193
8β(H)-Homo	98.4	392.8	3.9	4507.2	229.2	58.3	5000	50	4287
North Sea Oil									
C19 Terpane	98.4	74.8	0.7	55.8	3.5	3.8	5000	50	66016
C26(S) Terpane	98.4	69.0	0.7	37.3	2.5	2.7	5000	50	91005
C30αβ Hop	98.4	3228.6	31.8	337.0	48.6	0.5	5000	50	471335
C20-ααα-ster	98.4	52.6	0.5	126.0	6.8	12.2	5000	50	20557
C30 20R-ster	98.4	86.7	0.9	23.6	2.0	1.4	5000	50	180553
C4-decalin	98.4	14.7	0.1	373.0	18.8	129.3	5000	50	1933
8β(H)-Homo	98.4	392.8	3.9	833.3	45.5	10.8	5000	50	23190

Table 0.58: Background concentrations of selected biomarkers in Tyne sediment and in the starting oils.

Nigerian Light Oil

Biomarker	EOM (mg)	Backgr. Conc. (µg/g EOM)	Backgr. Conc. (µg/10g)	Starting Oil (µg/g oil)	Backgr amt. (in 10g sed) + 50mg Starting Oil (ug)	Ratio of oil (50mg) to backgr (10g)	Oil Added to Sed. (ppm)	Assumed Ratio=50	Min. Amnt. of oil (ppm)
C19 Terpane	40.7	51.3	0.2	330.2	16.7	79.0	5000	50	3164
C26(S) Terpane	40.7	63.9	0.3	56.5	3.1	10.8	5000	50	23114
C30αβ Hop	40.7	1383.0	5.6	8753.7	443.0	77.7	5000	50	3217
C20-ααα-ster	40.7	32.0	0.1	102.0	5.2	39.1	5000	50	6397
C30 20R-ster	40.7	20.8	0.1	170.9	8.6	101.0	5000	50	2476
C4-decalin	40.7	43.3	0.2	3019.5	151.1	856.8	5000	50	292
8β(H)-Homo	40.7	220.1	0.9	3776.5	189.7	210.7	5000	50	1187
Nigerian Medium Oil									
C19 Terpane	40.7	51.3	0.2	379.8	19.2	90.9	5000	50	2751
C26(S) Terpane	40.7	63.9	0.3	57.9	3.1	11.1	5000	50	22556
C30αβ Hop	40.7	1383.0	5.6	10290.4	519.9	91.4	5000	50	2737
C20-ααα-ster	40.7	32.0	0.1	91.8	4.7	35.2	5000	50	7107
C30 20R-ster	40.7	20.8	0.1	109.1	5.5	64.5	5000	50	3878
C4-decalin	40.7	43.3	0.2	3734.7	186.9	1059.7	5000	50	236
8β(H)-Homo	40.7	220.1	0.9	4508.1	226.3	251.5	5000	50	994
North Sea Oil									
C19 Terpane	40.7	51.3	0.2	56.0	3.0	13.4	5000	50	18721
C26(S) Terpane	40.7	63.9	0.3	37.6	2.1	7.2	5000	50	34852
C30αβ Hop	40.7	1383.0	5.6	342.6	22.5	3.0	5000	50	83511

C20-ααα-ster	40.7	32.0	0.1	126.1	6.4	48.3	5000	50	5171
C30 20R-ster	40.7	20.8	0.1	23.7	1.3	14.0	5000	50	17904
C4-decalin	40.7	43.3	0.2	373.2	18.8	105.8	5000	50	2362
8β(H)-Homo	40.7	220.1	0.9	834.2	42.6	46.5	5000	50	5376

Chapter 6: Effects of anaerobic biodegradation on the composition of crude oils

6.1 Introduction

Hydrocarbons are activated by microorganisms in anaerobic or anoxic conditions through biochemical mechanisms quite different from what is obtained through aerobic hydrocarbon degradation. Degradation rates of anaerobic microorganisms utilising hydrocarbons are broadly slower in oxygen-free or anoxic conditions than in aerobic conditions (Grishchenkov *et al.*, 2000; Sherry *et al.*, 2014). Hydrocarbon anaerobic degradation usually occur dominantly in the subsurface environments (Aitken *et al.*, 2004a; Jones *et al.*, 2008; Gray *et al.*, 2010; Widdel *et al.*, 2010; Sherry *et al.*, 2014), where they are degraded to methane by methanogenic microbial group (Townsend *et al.*, 2003; Jones *et al.*, 2008; Gray *et al.*, 2011; Aitken *et al.*, 2013; Sherry *et al.*, 2014) and by sulfate-reducing conditions (Aeckersberg *et al.*, 1991; Cravo-Laureau *et al.*, 2004; Davidova *et al.*, 2006; Gieg *et al.*, 2010; Sherry *et al.*, 2013). It is now widely known that many simple hydrocarbon compounds can be degraded under different oxygen deficient conditions such as sulphate-reducing, nitrate-reducing, perchlorate-reducing, ferric ion-reducing, humic acid-reducing, and methanogenic conditions (Widdel and Rabus, 2001; Townsend *et al.*, 2003; Jones *et al.*, 2008; Sherry *et al.*, 2014; Hazen *et al.*, 2016) not only through enzymes activated aerobic biodegradation as previously thought. Methanogenic degradation involves the stoichiometric transformation of constituent hydrocarbons in crude oil to methane and CO_2 where intermediate products are also produced in the process (Gray *et al.*, 2011; Aitken *et al.*, 2013). Substantial amounts of biodegraded oils have been reported in reservoirs around the world with depreciated economic value as a result of biodegradation such as decrease in API gravity, increase in acidity, sulphur content, metal content, cost of refining etc., (Cassani and Eglinton, 1991; Head *et al.*, 2003; Jones *et al.*, 2008), it is however an important process where the generated gases could reintroduce the needed pressure lift for oil recovery from the reservoir, or an economical conversion of unrecoverable residual oil to gas, as well as provide attenuation process of petroleum contaminants in anoxic subsurface or reservoirs (Dolfing *et al.*, 2008; Gray *et al.*, 2011). In this chapter, the focus is on the effect of anaerobic biodegradation on crude oils compositions on laboratory simulated microcosms. In anoxic environments, hydrocarbons are activated in syntropic mode by anaerobic degraders mostly bacteria (facultative and obligate) reducing nitrate, sulphate, or ferric ion-reducing as terminal electron acceptors other than the oxygen, and the archaea will then rummage on the remaining intermediate substrates (H_2 or acetate) to produce methane and CO_2 under the conditions of methanogenesis (Widdel *et al.*, 2010). Crude oil hydrocarbons methanogenic degradation is an important process the deep petroleum reservoir and anoxic petroleum contaminated environments whereby hydrocarbons are converted via different pathways such as complete oxidation of alkanes to H_2 and CO_2, linked to methanogenesis from CO_2 reduction; oxidation of alkanes to acetate and H_2, linked to acetoclastic methanogenesis and CO_2 reduction; oxidation of alkanes to acetate alone, linked to syntrophic acetate oxidation and methanogenesis from CO_2 reduction etc., which provide an opportunity of choice to evaluate the most thermodynamically feasible condition-specific pathway (Dolfing *et al.*, 2008). These thermodynamically feasible chemical reactions are mediated by microbes through their catalytic abilities to sustain the energy supply needed to maintain the ecosystem in a given suitable environmental conditions, whereby the fraction of the energy released is used by the microbes (Hanselmann, 1991).

6.2 Aim and Objectives

The aim of the experiments detailed in this chapter is to investigate the effects of anaerobic biodegradation on the chemical compositions of the three different oil samples used to spike the sediment microcosms.

The specific objectives are follows.

6. Observe the occurrence of anaerobic biodegradation by production of methane and CO2
7. To observe anaerobic biodegradation effects on the compounds in the oil samples in the sediment microcosms.
8. To determine the hydrocarbons background concentrations in the sediment.
9. To obtain detailed compositional information of the oils samples such as *n*-alkanes, acyclic isoprenoids, biomarker compounds, polycyclic aromatic hydrocarbons (PAHs) and their alkyl homologues and other unidentified hydrocarbon compounds.
10. To quantitatively determine the concentrations of the analytes in (4) above and compare the compositional and concentrations changes with respect to different levels or extent of biodegradation.
11. To quantitatively examine ratios of compounds used and their changes relative to the biodegradation levels for weathering evaluations.

6.3 Material and Methods

6.3.1 Laboratory biodegradation of crude oil

The crude oil samples were obtained as described in section 5.3.1. Anoxic sediment cores (~70 cm long, 5cm in diameter) were collected in December, 2018 from River Tyne at Dunston (54° 57’ 51.1” N, 001° 40’ 58.5” W), Newcastle Upon Tyne, Tyne and Wear, UK and stored at 4°C in the dark before use. The microcosms were prepared in 120 ml sterile glass serum bottles for the experiments from the laboratory cabinet (Coy Laboratory Products Inc., Milwaukie, USA), and a total of 36 serum bottles were set up in triplicates for three oil samples and each of the 27 serum bottles contains 10 g of sediment, 150ul of crude oil samples, and 50ml of media and 60 ml headspace. The media was made up from a stock solution (basal media) prepared consisting NaCl (14g), KH_2PO_4 (0.4g), $CaCl_2.2H_2O$ (0.3g),

$MgCl_2.6H_2O$ (0.8g), KCL (1g), NH_4Cl (0.5g) in 2L double distilled water (bidest water) and autoclaved + 2 ml supplement of selenite-tungstate solution consisting NaOH (0.4g), $Na_2SeO_3.5H_2O$ (6mg), $Na_2WO_4.2H_2O$ (8 mg) in 1000 ml bidest water, 2 ml non-chelated trace element consisting 8.5 ml HCl (37%=12.1M), $FeSO_4.7H_2O$ (2.1g) in 800 ml bidest water, 2 ml vitamin solution consisting 250 ml Na_2HPO_4/NaH_2PO4 (25mM, PH 7.1) buffer, 4-Aminibenzoic acid (10mg), D(+)-Biotin (2.5 mg), Nicotin acid (250 mg), Calcium-D(+)-pantothenate (12.5 mg), pyridoxal hydrochloride (37.5 mg), Di-α-lipoic acid (3.8 mg), Folic acid (10 mg), 2-Mercaptoethanesulfonic acid (62.5 mg) sterile filtered through 0.2 µm nitrocellulose filters and stored at 4°C , 2 ml vitamin B12 consisting cyanocobalamin (12.5 mg) in 250 ml bidest water, sterile filtered and stored at 4°C, 60 ml 1M $NaHCO_2$ + Sulfide + 8 ml of 0.5M Na_2S stock solution as oxygen scavenger. The remaining 9 serum bottles were sterile controls, and each contained the same contains as the 27 serum bottles and 500 ul 1M Bromoethane Sulphonic Acid (BES) to inhibit methanogenesis and 500 ul 1M Sodium Molybdate as sulphate reducing inhibitor sterile filtered and autoclaved (e.g. Widdel and Bak, 1992; Zengler *et al.*, 1999; Aitken *et al.*, 2013; Heider and Schühle, 2013; Sherry *et al.*, 2020). The samples were kept in the dark to ensure anaerobic conditions were maintained and incubated for 100, 300 and 600 days respectively. The 600 days samples were not analysed due to time constraints and the 300 days and the sterile controls samples were extracted after 301 days. Methane and CO_2 were analysed using gas GC-MS (MD800), and the aliphatic (F1) and aromatic (F2) fractions were analysed using GC-FID and GC-MS respectively.

6.3.2 Extraction method

Aliphatic hydrocarbon standard Squalane (99% purity) and 5α-cholestane (98% purity) obtained from Aldrich, Steinem, Germany, and PAH standard 1,1’-binaphthyl (98% purity) obtained from Lancaster Synthesis, White Lund, UK and *p*-terphenyl (99% purity) obtained from Fluka Chemika, Buchs, Switzerland were added as recovery standards just before extraction. 1ml slurry of each sample was taken after shaking and stored in -20C freezer for DNA analysis. The aqueous layer decanted into separating funnels and the sediment in the flasks rinsed with 15 ml methanol into cleaned centrifuged tubes, sonicated for 5minutes and then centrifuged for 8minutes at 3500 rev/min and the supernatant decanted into the separating funnels. This extraction cycle was repeated using 15 ml (2:1) and then 15 ml (4:1) DCM/methanol, and finally three times with 15 ml DCM. The contents were shaken and allowed to separate gravimetrically, and the bottom organic layer collected into 100 ml RBFs leaving the top polar layer. 30 ml DCM and 50 ml deionised water again added to the bottom organic layer into separating funnel and re-separated. The extracts rotary evaporated to about 5ml and anhydrous sodium sulphate (Na2SO4) added to remove trace of water, and made up to 6 ml in measuring cylinder where 2ml aliquot each were taken for fractionation, total petroleum hydrocarbon (TPH) and extractible organic matter (EOM) determination respectively.

Figure 0.1: Anaerobic biodegradation experimental set up.

6.3.3 Reagent and Materials

Chromatographic-grade distilled solvents used include petroleum ether (BP 40-60°C), dichloromethane (DCM) and methanol. Silica gel 60(230-400 mesh ASTM) and aluminium oxide 90 active neutral (70-230 mesh ASTM) were purchased from Merck, Darmstadt, Germany and 500 gr white non-absorbent cotton wool purchased from Fisher Scientific, UK The silica gel, the aluminium oxide and the cotton wool were pre-extracted in a Soxtec solvent extractor using an azeotropic (DCM-methanol) solvent mixture. The silica gel and the aluminium oxide were stored in glass bottles, covered with aluminium foil and activated in an oven over night at 130°C before use (e.g., Later et al., 1985; Bundt et al., 1987).

6.3.4 Column chromatographic fractionation of hydrocarbons

Chromatography columns (28 cm l x 10 mm i.d.) were set-up on clamps and pre-extracted cotton wool were used to plug the bottom of the columns. A slurry of silica gel in petroleum ether was poured into the columns followed by slurry of alumina at the top to form alumina/silica (1:4) column. The 2 ml of the extract adsorbed onto small quantities (~1 g) of alumina and then charged onto the alumina/silica column. The columns were eluted with petroleum ether (70 ml) as aliphatic fraction (F1) for n-alkanes and biomarker analysis, followed by elution of a PAHs-containing fraction (F2) (70ml 50% DCM in petroleum ether) for analysis of PAHs similar to that previously reported (e.g., Wang *et al.*, 1994a; Wang *et al.*, 1994b; Wang *et al.*, 2004; Wang *et al.*, 2005). Aliphatic standard *n*-heptadecylcyclohexane (98% purity) obtained from Lancaster Synthesis, Morecambe, UK, 5α-androstane (98% purity) obtained from Sigma-Aldrich, Steinem, Germany, and PAH standard *p*-terphenyl (99% purity) obtained from Fluka Chemika, Buchs, Switzerland was added to respective fractions as internal standards.

6.3.5 Headspace gas analysis, gas chromatography, and gas chromatography - mass spectrometry

The gas chromatography-mass spectrometry (gas-GCMS-MD800) analysis of the biogenic gases (CH_4 and CO_2) produced was performed on a Fisons 8060 GC using split injection (150°C) linked to a Fisons MD800 MS (electron voltage 70eV, filament current 4A, source current 800uA, source temperature 200°C, multiplier voltage 300V, interface temperature 150°C). The acquisition was controlled by a Compaq Deskpro computer using Xcalibur software; in full scan mode (1-150 amu/sec dwell 10ms/amu) or in SIM mode 20 ions (dwell 100 ms/ion) for greater sensitivity. The headspace sample (100 ul) was injected in split mode and the GC programme and MS data acquisition commenced. Separation was performed on a HP-PLOT-Q capillary column (30 m x 0.32 mm i.d) packed with 20 um Q phase. The GC was held isothermally at 35°C with Helium as the carrier gas. (flow 1ml/min, pressure of 65kPa, split at 100 mls/min). The chromatograms of the separated gases (CH_4, CO_2, N_2O, SF_6 etc) were integrated and quantified.

Gas chromatography with flame ionisation detection (GC-FID) analysis of aliphatic samples was performed on an Agilent 5890 GC in split-less mode, with the injector at 280°C, FID at 300°C. The sample (1 ul) in hexane was injected by an HP7673 auto sampler and the split opened after 1 minute. After the injection, the GC temperature programme and data acquisition commenced. Separation was performed on a fused silica capillary column (30 m x 0.25 mm i.d.) coated with 0.25 um dimethyl poly-siloxane (HP-5 phase). The GC was temperature programmed from 50°C-310°C at 5°C min and held at final temperature for 20 minutes with hydrogen as the carrier gas (flow 1ml/min, pressure of 50 kPa, split at 30 mls/min). The GC data was acquired on a Thermo-Atlas laboratory data system on a dedicated channel.

Gas chromatography-mass spectrometry (GC-MS) analysis of the aliphatic and PAH fractions was performed on an Agilent 7890B GC with a split/split less injector (280°C) linked to a

Agilent 5977B MSD (electron voltage 70 eV, source temperature 230°C, quad temperature 150°C multiplier voltage 1200 V, interface temperature 310°C). The acquisition was controlled by a HP Z240 computer using Chemstation software, initially in full scan mode (50-600 amu/sec) or in selected ion mode (20 ions, 3.0 cps, 20 ms dwell) for greater sensitivity. The sample (1 ul) in hexane was injected by an Agilent7683B auto sampler and the split opened after 1 minute. After the solvent peak had passed the GC temperature programme and data acquisition commenced. Separation was performed on an Agilent fused silica capillary column (30 m x 0.25 mm i.d) coated with 0.25 um dimethyl polysiloxane (HP-5) phase. The GC was temperature programmed from 50-310°C at 5°C min and held at final temperature for 10 minutes with Helium as the carrier gas (flow rate of 1ml/min, initial pressure of 50 kPa, split at 20 mls/min). The acquired data was stored for later data processing, integration and printing. Peaks were identified and labelled after comparison of their elution order with known standard mixtures or from geochemistry literature and for the PAH by comparison of their mass spectra with those of the NIST05 library. The NIST Mass Spectral Library is a software and peer-reviewed databases that are developed and supported by the National Institute for Standards and Technology, USA.

6.4 Results and Discussion

6.4.1 Headspace Methane and CO_2

Methane (measured ion m/z 15) and CO_2 (ion m/z 44) generation from the microcosms confirmed the occurrence of methanogenesis as shown in Figure 0.2 as it is observed that methane increase slightly from start to 100 days but drastically increased from 100 days to 300 days with the Nigerian light oil (NL) making the most methane generated (273.2 µmoles) of 60 ml headspace) whereas the North Sea oil (NS) with least methane generated (109.5 µmoles). No methane was however generated in the amended microcosms for the three oil samples up to 300 days as methane production was completely inhibited whereas CO_2 was generated in both the amended and non-amended microcosms, and perhaps CO_2 could have been observed if the amended samples were analysed at 100 days. Although methanogenic degradation of crude oil affects other components, aliphatic fraction is quantitatively the most important (Dolfing *et al.,* 2008) and as compounds are preferentially degraded (Head *et al.*, 2003; Jones *et al.,* 2008), alkanes have been reported to stoichiometrically transformed to methane and CO_2 (Jones *et al.,* 2008; Gray *et al.,* 2011) through established number of pathways with attendant thermodynamic potentials using chemical reaction equations (Hanselmann, 1991; Dolfing *et al.,* 2008; Dolfing *et al.*, 2009). It could be observed that while methane increased slowly from start to 100 days, CO_2 dramatically increased with corresponding decrease in *n*-alkanes and vice versa from 100 days to 300 days possibly due to hydrogentrophic methanogenesis where acetate have to be oxidized syntrophically to CO_2 and hydrogen before conversion to methane and surge in CO_2 could have been maintained by the concomitantly produced hydrogen in the closed system (e.g.Jones *et al.*, 2008), and this resulted in dramatic removal of n-alkanes (Figure 0.2C). Whereas the gradual decrease in CO_2 after 100 days (Figure 0.2B) is as a result of the attendant CO_2 conversion to methane which results in corresponding increase

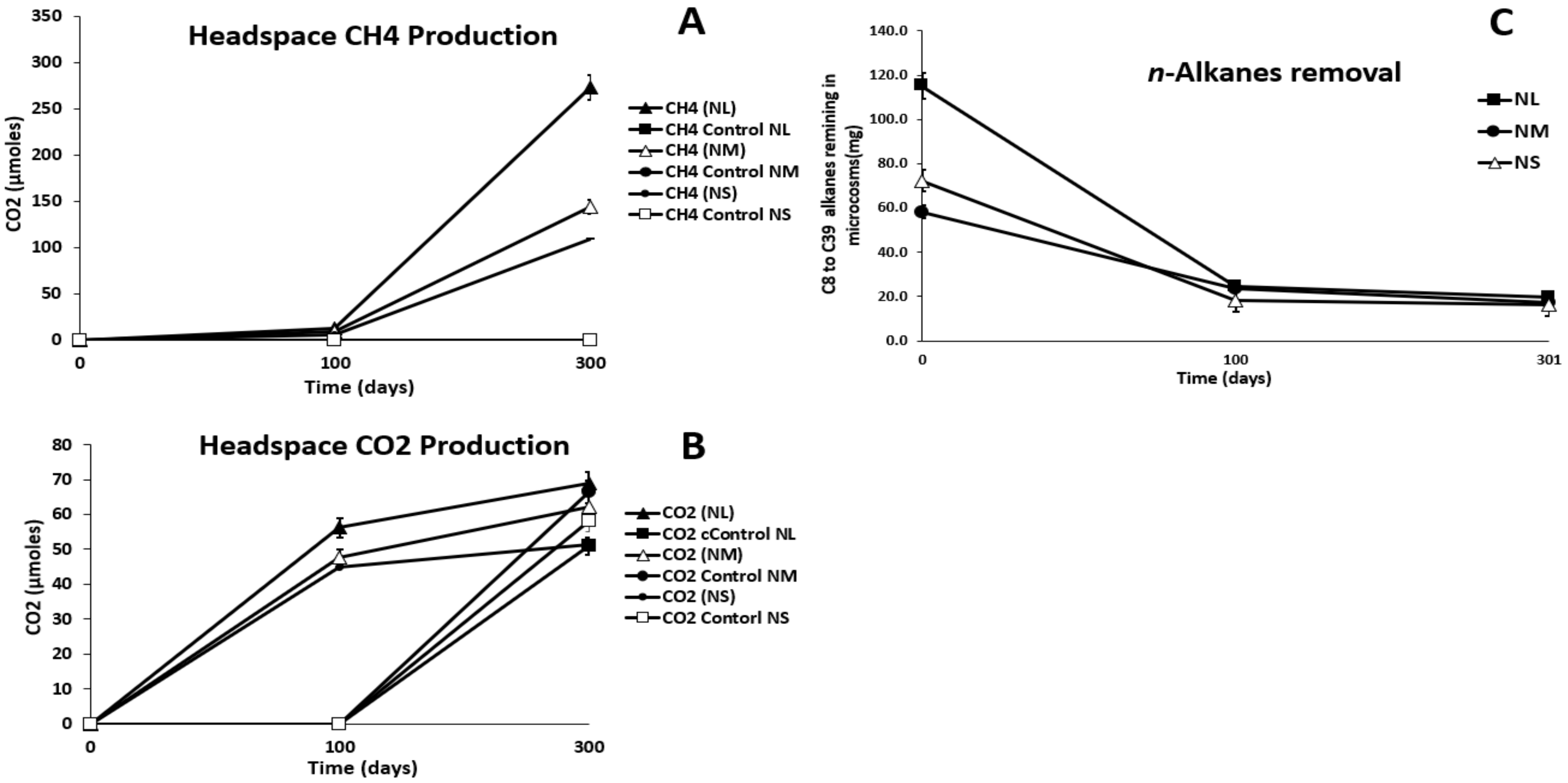


Figure 0.2: Alkanes removal versus methane and CO_2 generation during methanogenic oil degradation. NL and NM represent Nigerian light and medium oils, and NS North Sea oil incubated under methanogenic conditions with Tyne sediment as the inoculum in laboratory microcosms for 300 days with (A) methane, (B) CO_2 generated, and (C) *n*-Alkanes removal respectively. The error bars show ±1 standard error of replicate microcosms (n = 3). The control microcosms were treated with BES and sodium molybdate to inhibit methanogenesis and sulfate reduction, respectively, and then autoclaved at 121°C for 15mins. The controls were only analysed at 300 days.

in methane (Figure 0.2A) from 100 days to 300 days to account for the thermodynamic balance. For inexplicable reasons, CO_2 were produced in the controls at 300 days (see Figure 0.2B) but the sulfate-reducing bacteria (SRB) remained inhibited and methanogens absent. Some spore forming fermentative bacteria may have survived and re-established themselves slowly, producing fatty acids, CO_2 and hydrogen from organic matter degradation, otherwise it could have been an unidentified process that resulted in the CO_2 generation which will be subject to further findings. The methane produced is stoichiometrically more than the conversion from *n*-alkanes, as other compounds undergo methanogenic degradation simultaneously, especially the branched *n*-alkanes though at a slower rate compared to *n*-alkanes (e.g. Larter *et al.*, 2003; Larter *et al.*, 2006b; Jones *et al.*, 2008).

6.4.2 *n*-Alkane and acyclic isoprenoid composition and concentration changes

Figure 0.3,Figure 0.4, andFigure 0.5 are GC-FID chromatograms of the aliphatic hydrocarbon (F1) fractions of Tyne Sediment spiked with Nigerian light and medium, and the North Sea oils respectively, showing the effects of anaerobic biodegradation on *n*-alkanes and isoprenoids. Figure 0.6 shows the background concentrations of *n*-alkanes and isoprenoids in the sediments were observed to be quite negligible in concentrations compared to that of spiked sediments measured with respect to extractable organic matter (EOM), with C_{27} to C_{31} dominance possibly as a result of contribution from plants (Bush and McInerney, 2013) with C_{29} (299.4 µg/g EOM) as the peak concentration. The *n*-alkanes range from C_{12} to C_{35} in the Tyne sediment which is typical of most sediments (Volkman *et al.*, 1984). Slight compositional change is observed as a result of the anaerobic biodegradation on the chromatograms as biodegradation increased with time as expected. Table 0.1Table 0.2Table 0.3 show the summary of the identified *n*-alkanes (*n*-C_8 to *n*-C_{39}), pristanes and phytanes, and their concentrations for the Nigerian light and medium, and North Sea oils respectively, obtained by comparison of the integrated peak areas against the peak areas of known amounts of internal standard (n-heptadecylcyclohexane) using GC-FID. Recovery standard (squalane) was used to compute the percentage recoveries for the analytical procedures, which were over 90% for all the samples. These concentrations are mean values (n=3) from triplicates with relative standard deviation (RSD) shown in brackets after the numbers as shown in Table 0.1Table 0.2Table 0.3 respectively. The quantitations were also carried out as described in chapter 3 (see equations 3.1, 3.2, 3.3 and 3.4 in section 3.4.2). The degradation effect is observed to be quite low as C_8 to C_{39} *n*-alkanes are still visible after 300 days for all the three oils. The *n*-alkanes are observed to be slightly affected but the entire range are preserved for the three oil samples as shown on the chromatograms (see Figure 0.3Figure 0.4Figure 0.5). Consequently, the ratios n-C_{17}/pristane, n-C_{18}/phytane, pristane/phytane, CPI and the weathering index (WI) (see Table 0.1,Table 0.2,Table 0.3) are not so altered compared to that of evaporation weathering (see section 3.4.2) and aerobic biodegradation (see section 5.4.1) but however more altered than that of water washing (see section 4.4.1). However, the sum of the *n*-alkanes (C_8 to n-C_{39}) drastically decreased from the start to 100days by 79%, 60%, and 75% for the Nigerian light and medium, and north Sea oils respectively, which is an indication of the effect methanogenic degradation (see Figure 0.2) (Jones *et al.*, 2008; Gray *et al.*, 2011; Wang *et al.*, 2011).

Figure 0.7Figure 0.8Figure 0.9 are graphical representations of the *n*-alkanes and isoprenoids concentrations for the Nigerian light and medium, and North Sea oils in Tyne sediment which indicate the n-alkanes and isoprenoids compositional changed with degradation time, whereas Figure 0.10 shows the plots of abundances of the *n*-alkanes which also present a graphical view of the effect of methanogenic degradation as discussed in section 6.4.1 above. A decrease in *n*-alkanes removal with time is observed which decreased with increase in chain length until the effects diminished around *n*-C_{29}.

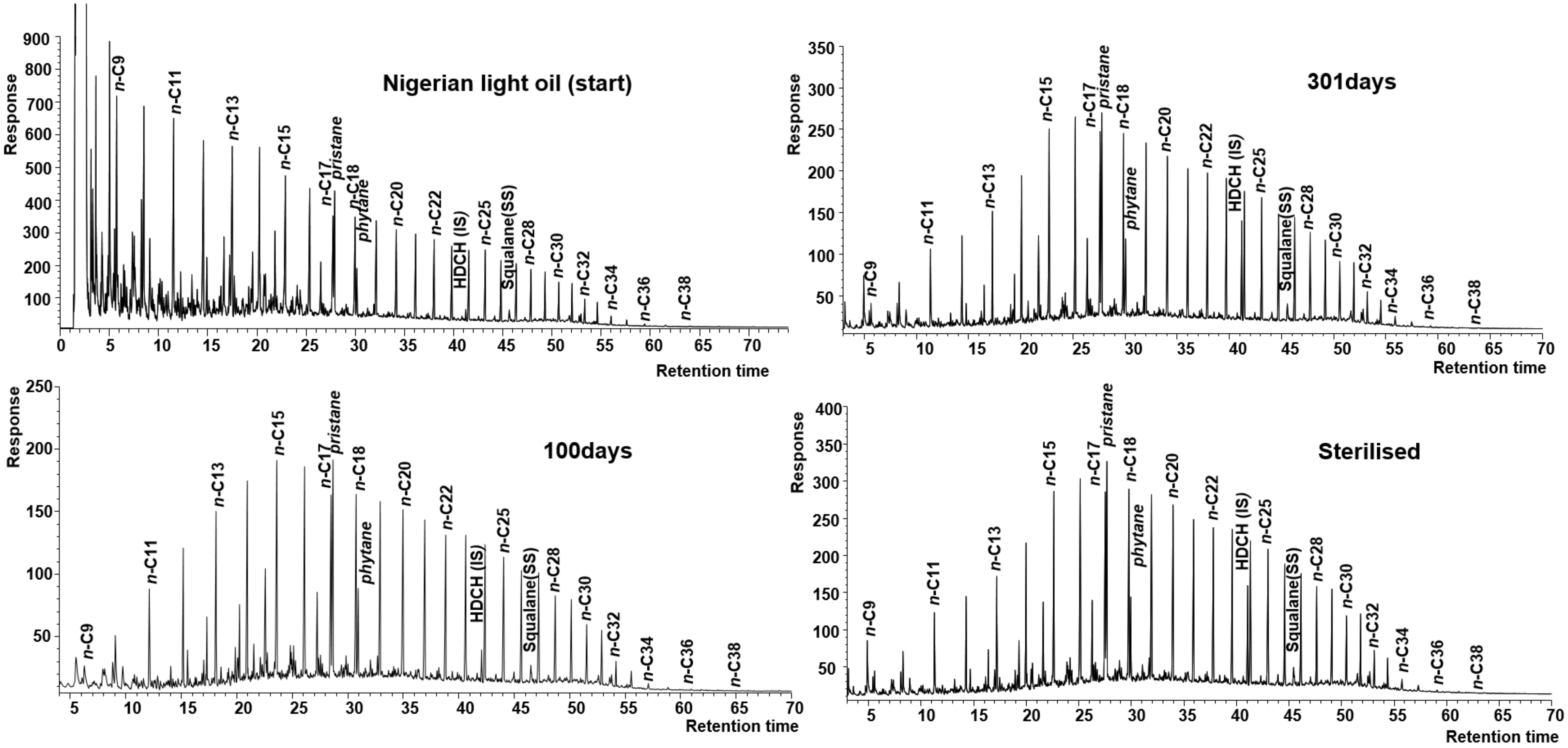


Figure 0.3: GC-FID chromatograms of aliphatic fractions of the Nigerian light oil in Tyne sediment showing days of biodegradation

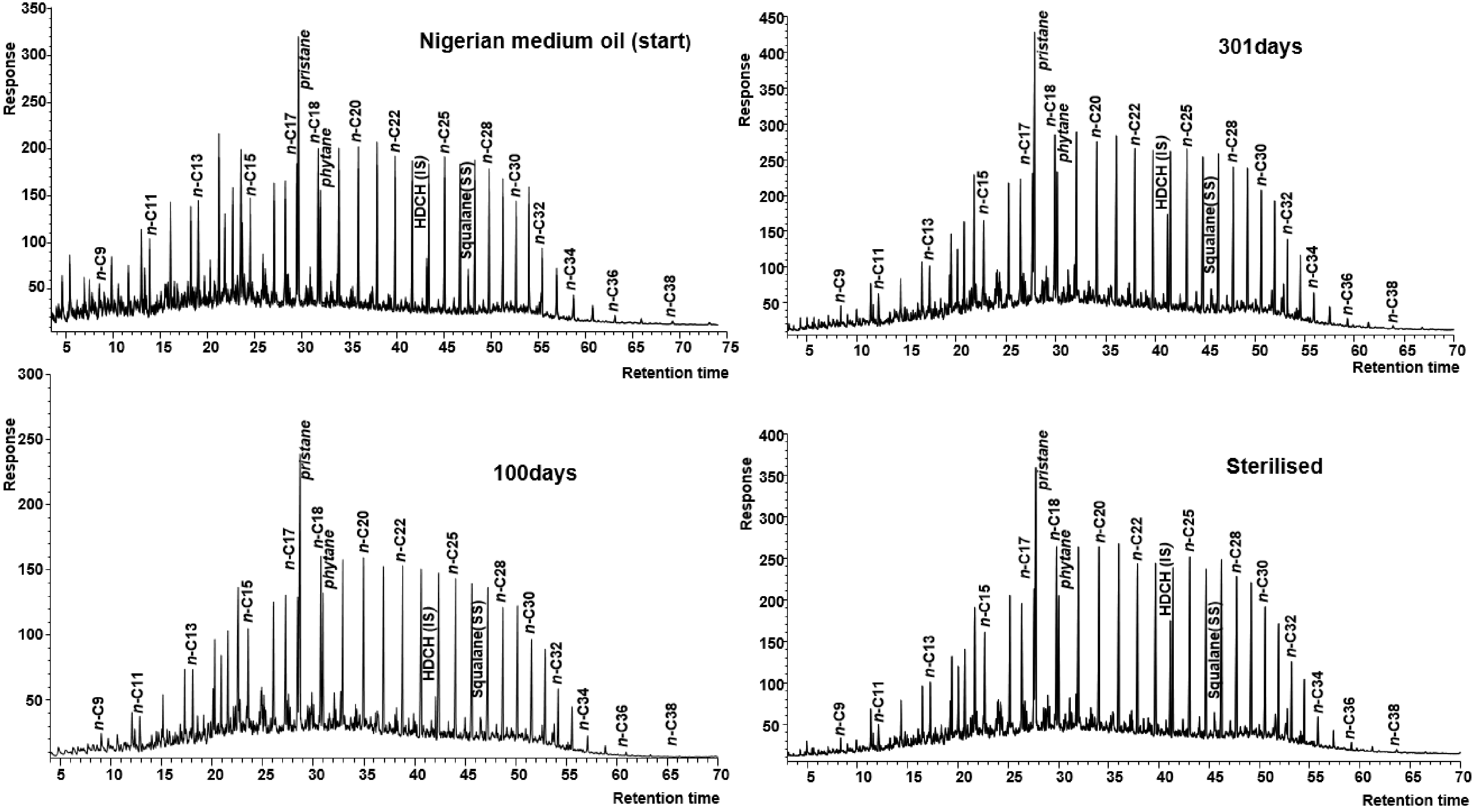


Figure 0.4: GC-FID chromatograms of aliphatic fractions of the Nigerian medium oil in Tyne sediment showing days of biodegradation

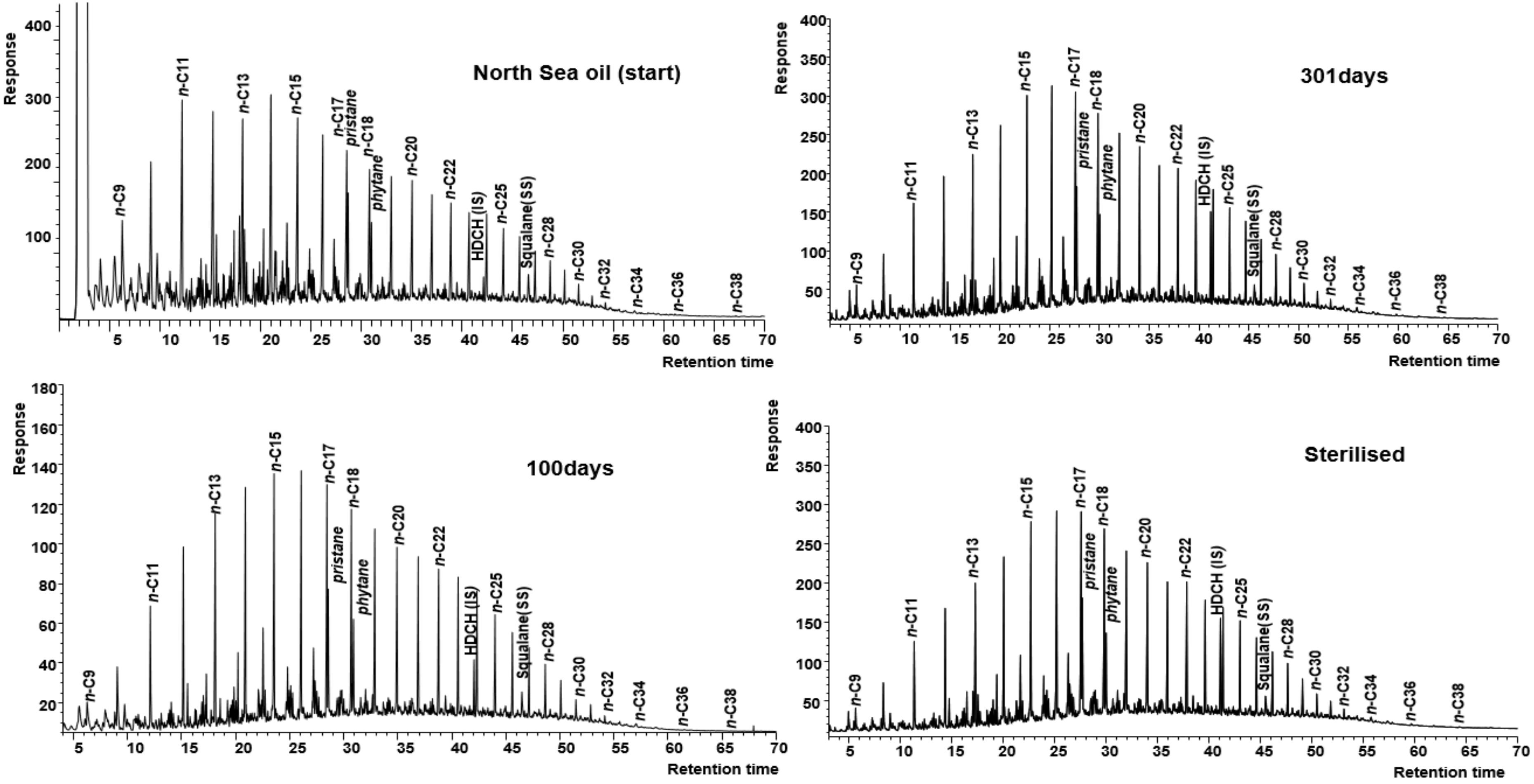


Figure 0.5: GC-FID chromatograms of aliphatic fractions of the North Sea oil in Tyne sediment showing days of biodegradation

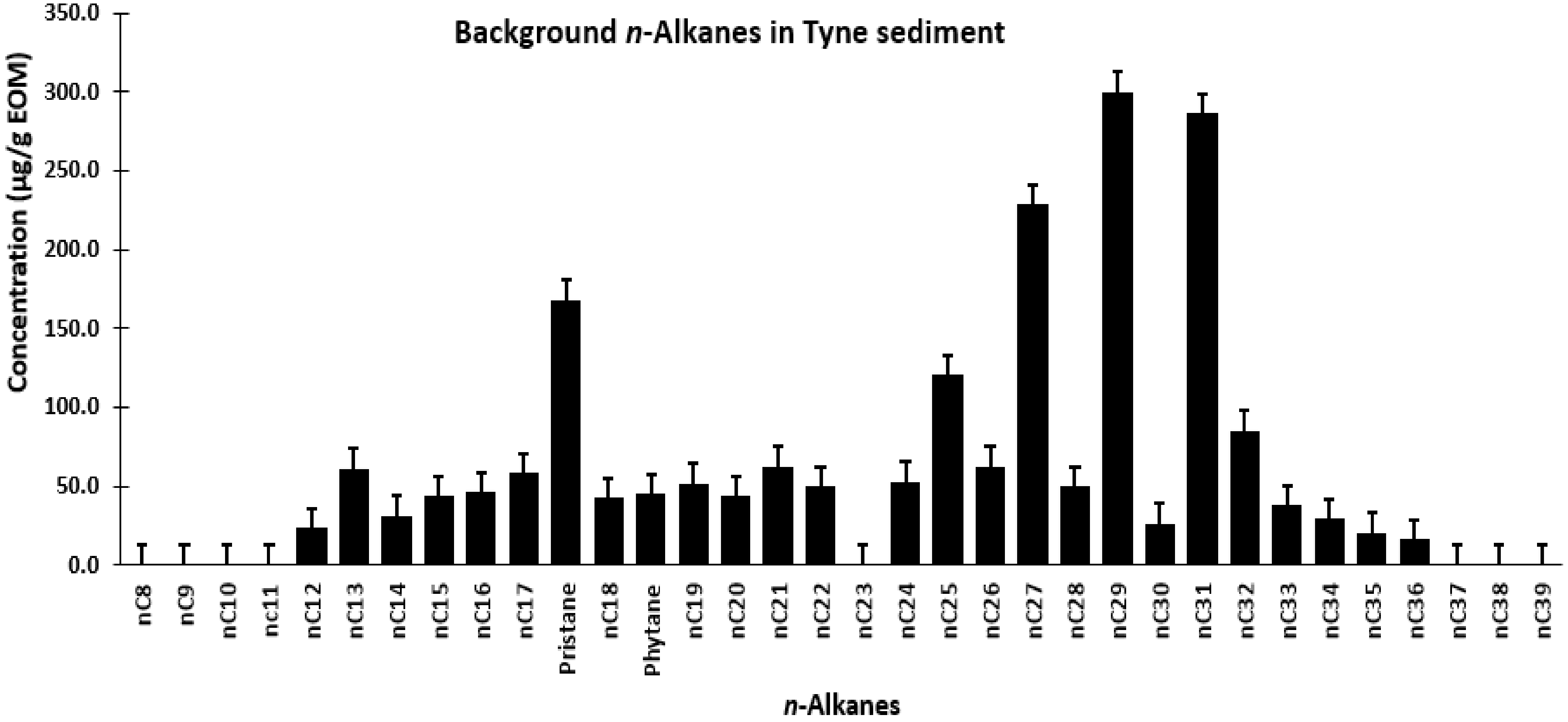


Figure 0.6: Graphical representation of background concentrations of *n*-alkanes, pristane, and phytane in Tyne sediment, measured with respect to extractable organic matter (EOM). The error bars show ± 1 standard error of replicate samples (n = 3)

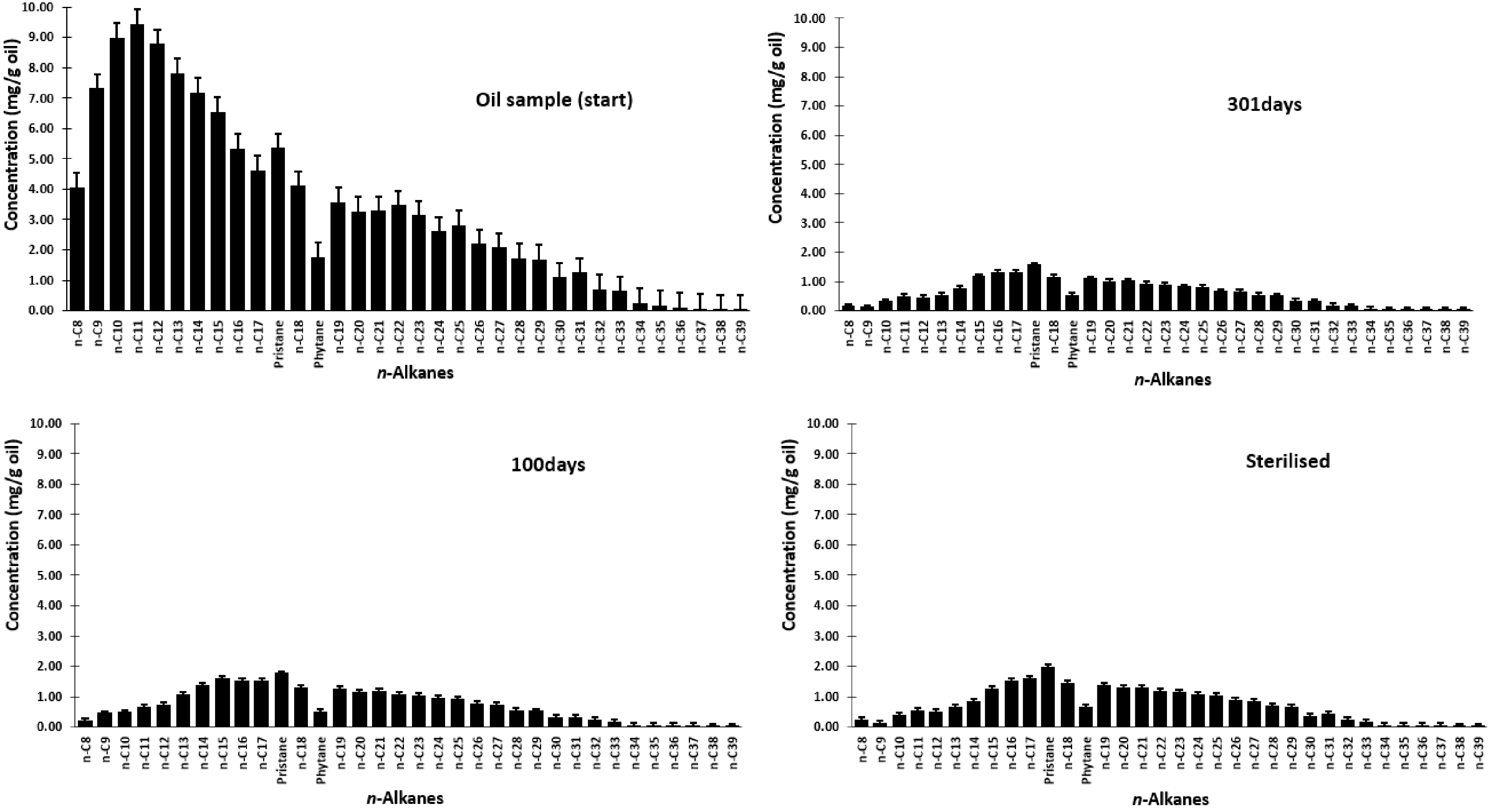


Figure 0.7: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian light oil in Tyne sediment. The error bars show ± 1 standard error of replicate microcosms (n = 3).

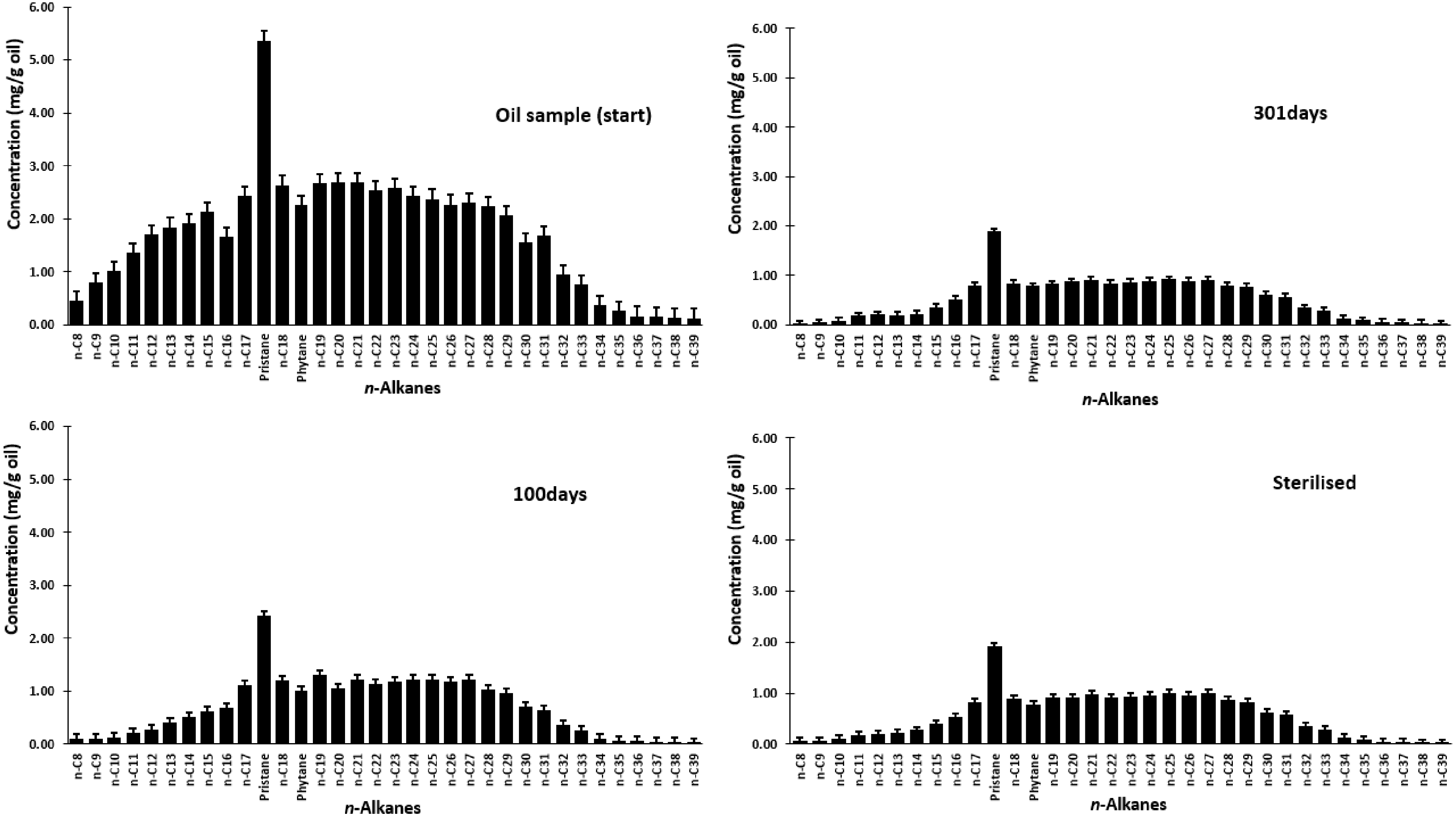


Figure 0.8: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian medium oil in Tyne sediment. The error bars show ± 1 standard error of replicate microcosms (n = 3).

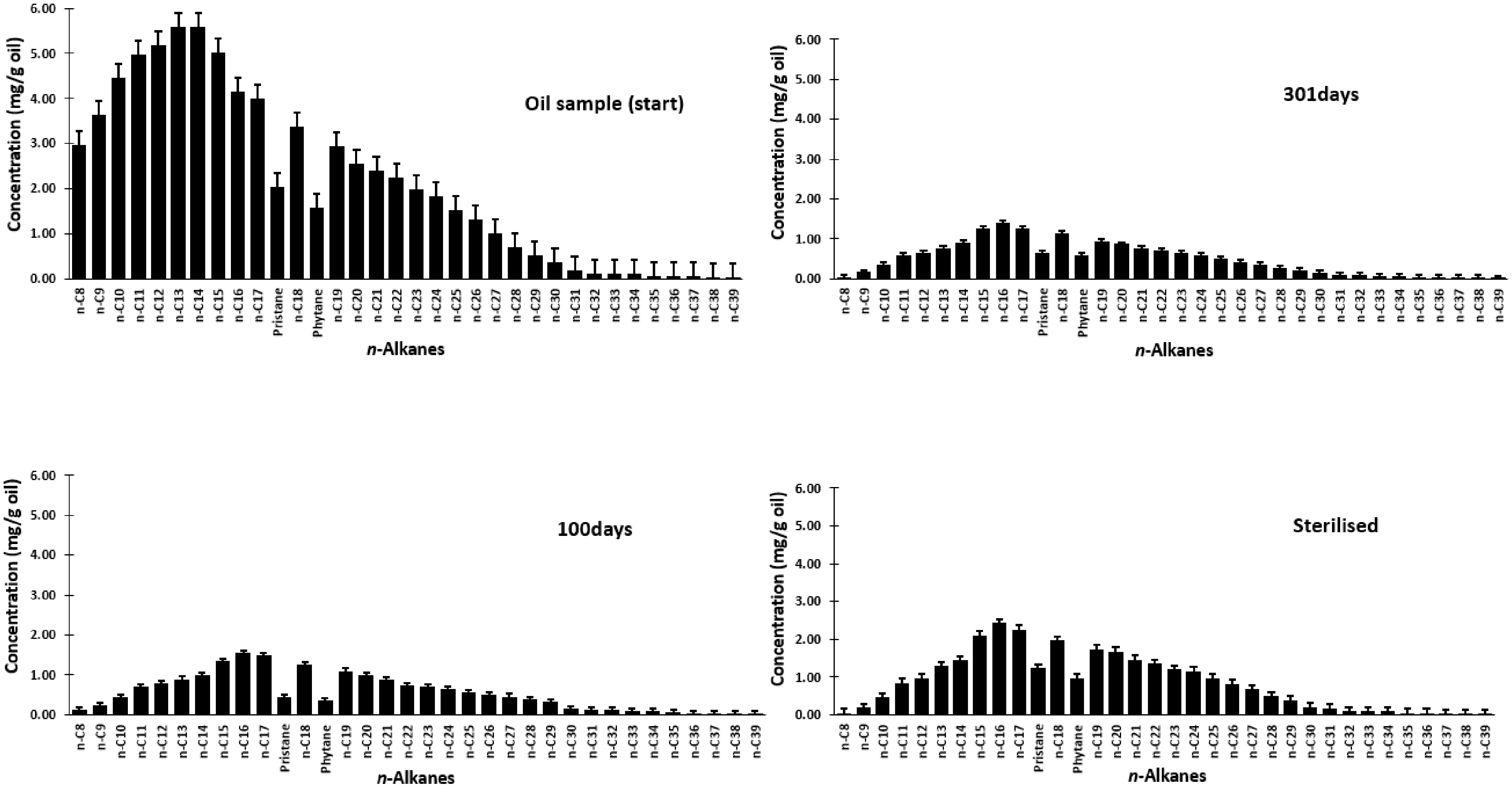


Figure 0.9: Graphical representation of concentration changes of *n*-alkanes, pristane, and phytane with increasing biodegradation for Nigerian medium oil in Tyne sediment. The error bars show ± 1 standard error of replicate microcosms (n = 3).

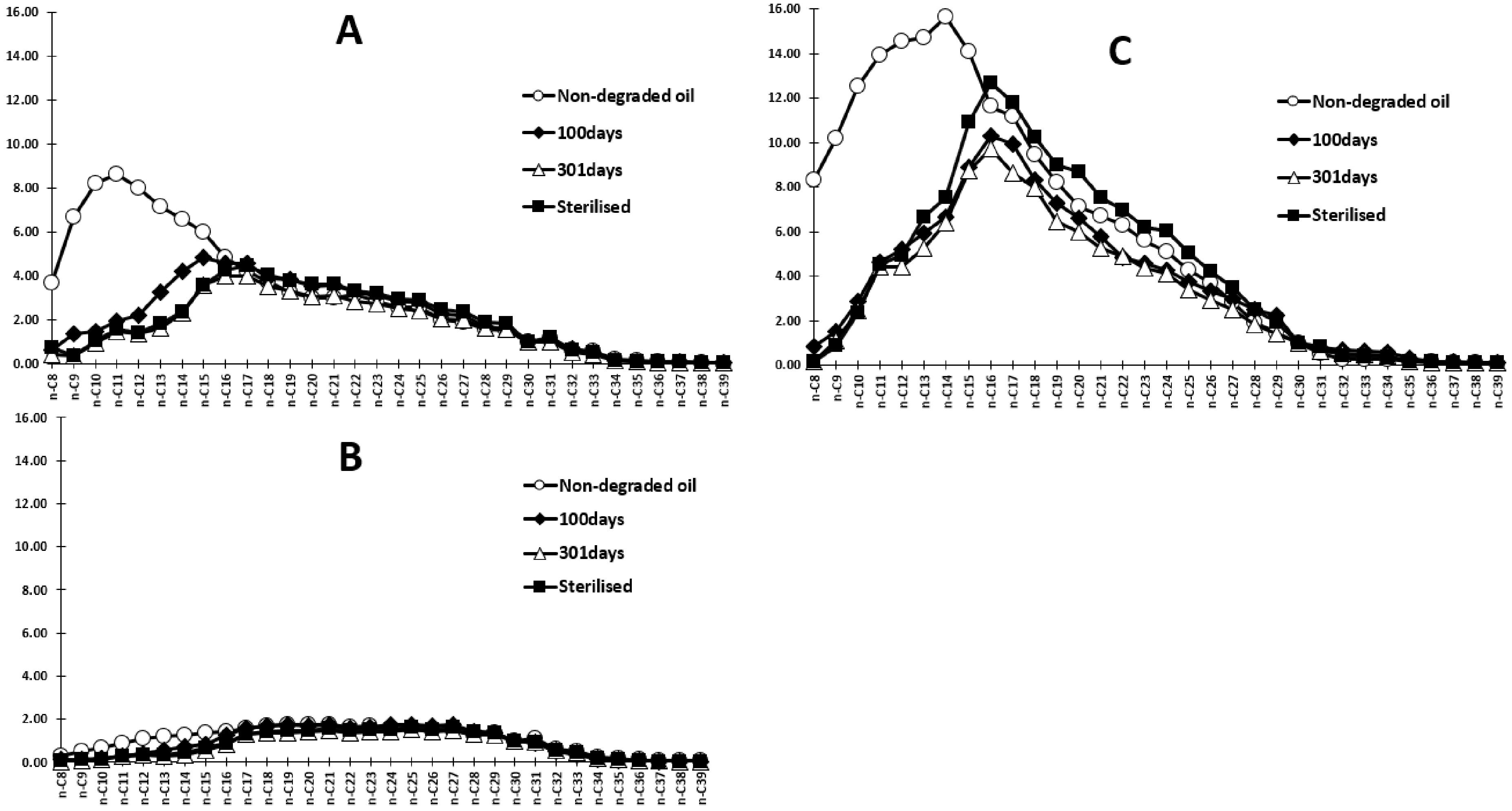


Figure 0.10: Plots of abundances of n-alkane in weathered oils in Tyne sediment relative to C_{30} for Nigerian light (A), Nigerian medium (B), and North Sea (C) oils.

6.4.3 Aromatic hydrocarbons composition and concentration changes

The aromatic fractions (F2) were also analysed and the GC/MS TIC chromatograms (in SIM mode) are shown in Figure 0.11Figure 0.12, andFigure 0.13 for the Nigerian light and medium, and North Sea oil respectively. The samples weathered by methanogenic degradation and the microcosms were incubated for the period of 100, 301 days and sterile controls for 301 days. The summary of the quantitations of the measured concentrations of the target PAHs are shown in Table 0.4,Table 0.5Table 0.6 for the Nigerian light and medium, and North Sea oils respectively, which are mean values of triplicate samples with average relative standard deviations (RSD) of 6%, for the samples. The measured concentrations of the PAHs were calculated as described in chapter 3 (see equations 3.7 and 3.8 in section 3.4.5). Homologous series of the alkylated PAHs were integrated with straight base lines carefully maintained for each peak for consistency (e.g. Yang *et al.*, 2014) and calculated based on mean values of triplicate samples for the Nigerian light, Nigerian medium and North Sea oils respectively . The relative response factors (RRFs) were as described in chapter 3 (see section 3.4.3) as samples were analysed under the same GC/MS operating conditions. Table 0.4 shows the background concentrations of target PAHs in the in the Tyne sediment, which is dominated by phenanthrenes, pyrenes and chrysenes smaller amounts of naphthalenes, biphenyls, dibenzofurans, fluorenes, dibenzothiophenes, and TAS with their alkylated homologous which is an indication of petrogenic sources though slightly degraded based on their distribution pattern (see Figure 0.14) in addition to other sources of target PAH (Stogiannidis and Laane, 2015). Detailed discussion on the importance and application of analysing PAHs in environmental samples was carried in chapter 4 (see section 4.4.2), however this chapter focussed on effects of methanogenic degradation on the target PAHs and their alkylated homologous. The PAHs are observed to be affected most by biodegradation than by evaporative and water washing weathering discussed in chapters 3 and 4 respectively.

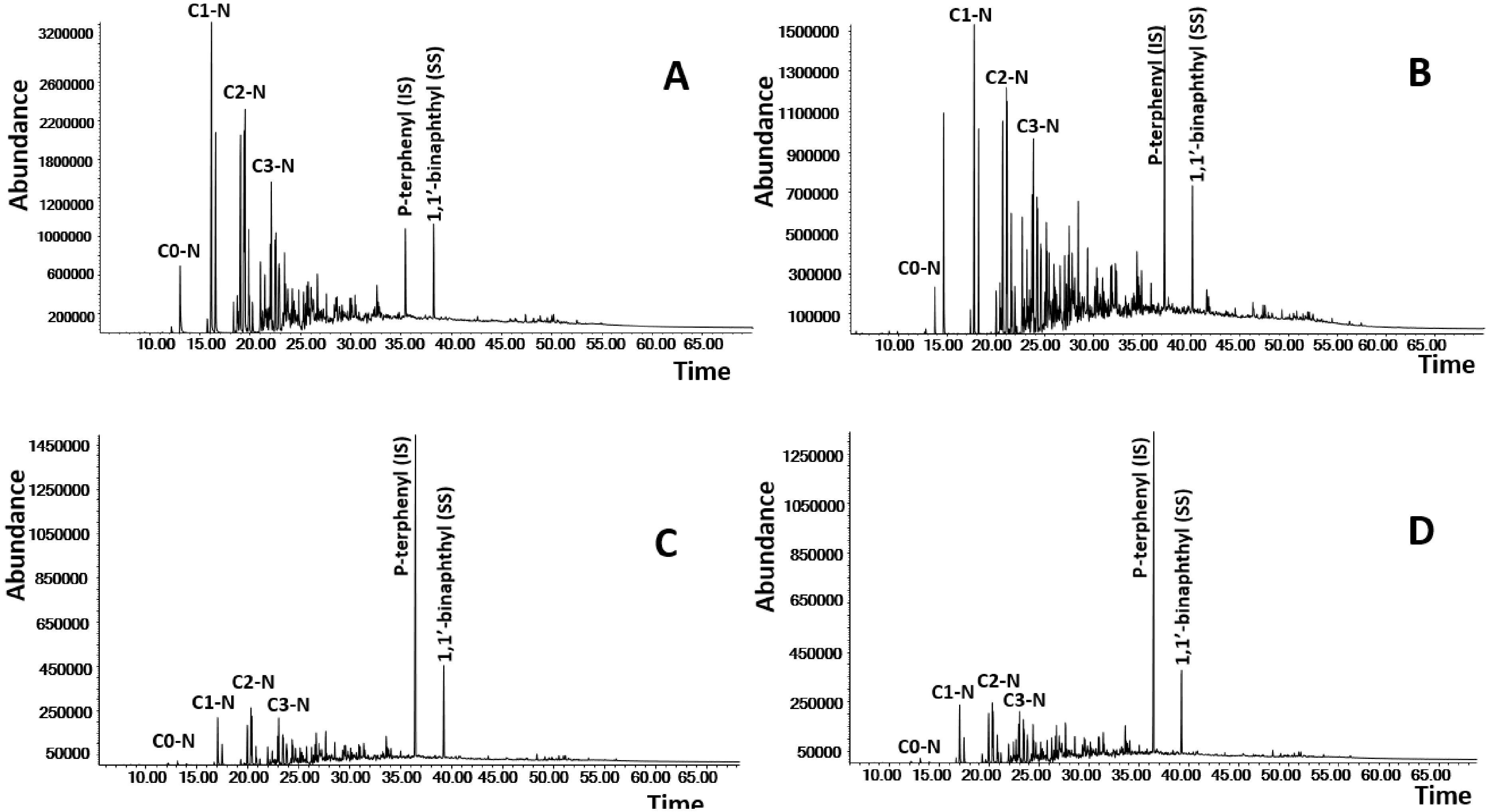


Figure 0.11: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian light oil weathered by biodegradation in Tyne sediment, (A) start, (B) 100days, (C) 301days, and (D) sterilised control at 301days, respectively.

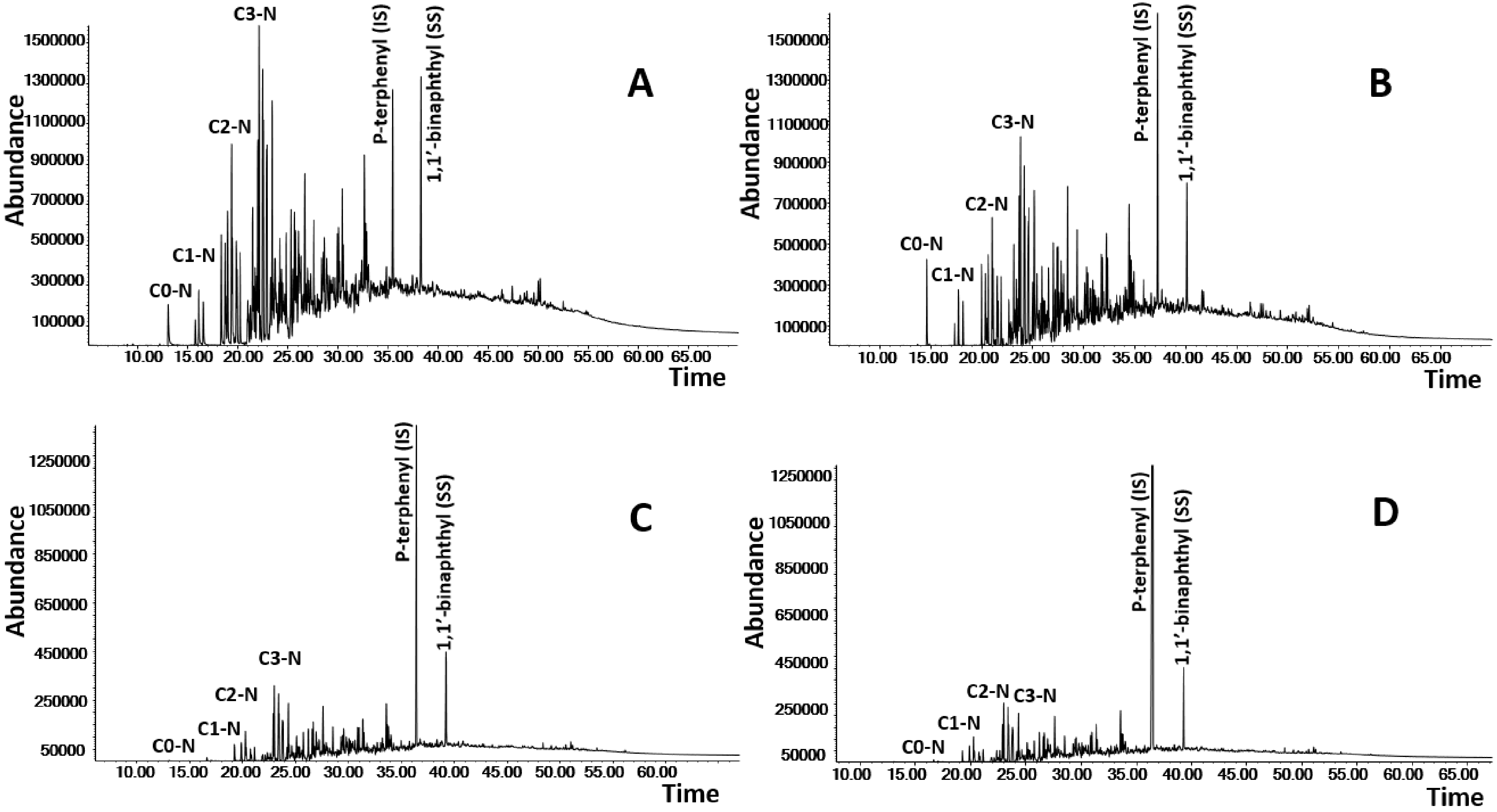


Figure 0.12: GC/MS SIM/TIC Chromatograms of aromatic compounds in Nigerian medium oil weathered by biodegradation in Tyne sediment, (A) start, (B) 100days, (C) 301days, and (D) sterilised control at 301days, respectively.

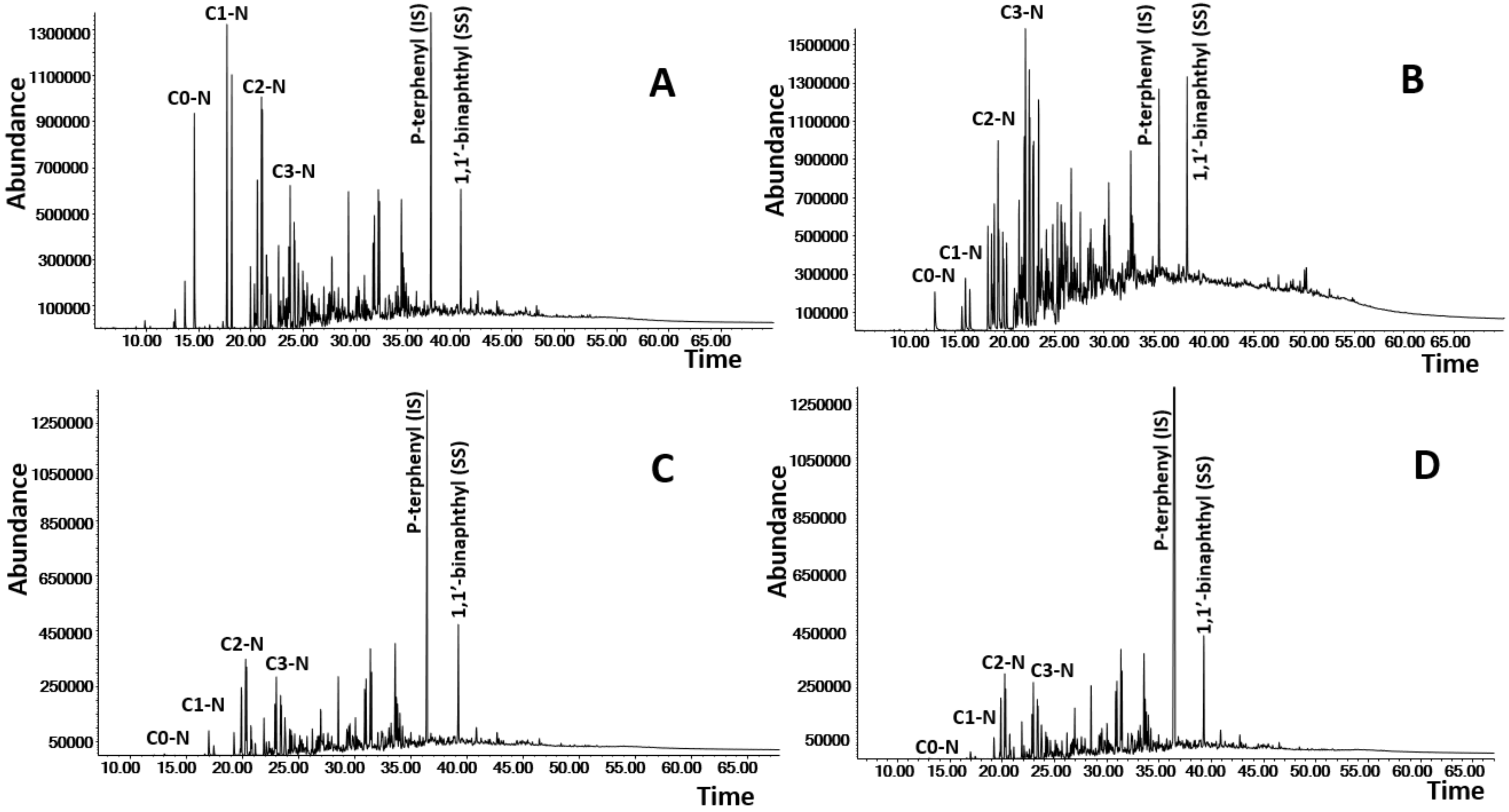


Figure 0.13: GC/MS SIM/TIC Chromatograms of aromatic compounds in North Sea oil weathered by biodegradation in Tyne sediment, (A) start, (B) 100days, (C) 301days, and (D) sterilised control at 301days, respectively.

The target PAHs and their homologous are observed to be affected by methanogenic PAH degradation for all the three oil samples. Naphthalene is observed to be more susceptible to degradation than other target PAHs considered with that of the Nigerian light oil having the least amount remaining (5.4 µg/g) after 301 days (see Table 0.4Table 0.5Table 0.6) but this is however a higher when compared with that of evaporation weathering and aerobic biodegradation in chapters 3 and 5 respectively but the naphthalene amount remaining for water washing in chapter 4 is much higher. Naphthalene was observed to decrease in re from 3% to 1%, 5% to 1%, and 15% to 3% after 301days for the Nigerian light and medium, and North Sea oils respectively. Methyl naphthalenes are also affected as the 2-MN/1-MN ratios are observed to decreased with degradation time for the Nigerian oils (see Table 0.4Table 0.5) whereas the North Sea oil shows a different trend as the ratios increased with degradation time as these isomers are degraded differently. Relative compositions of dimethylnaphthalenes also decreased from 55% to 37% and 37% to 19% for the Nigerian light and medium oils, whilst that of the North Sea oil increased from 30% to 41% this is likely due to the preferential microbial degradation of hydrocarbon compounds (e.g. Head *et al.*, 2003; Jones *et al.*, 2008). However, relative compositions of trimethylnaphthalenes and tetramethylnaphthalenes increased with degradation time for all the oils as variation in degradation decrease with increasing in aromatic rings and alkyl substituents (e.g. Volkman *et al.*, 1984; Rowland *et al.*, 1986; Cassani and Eglinton, 1991; Wang and Fingas, 1995), and this extend of degradation could be ranked as slight on PM levels 2-5 (e.g. Dolfing *et al.*, 2009). The degradation variation decreased with higher ring PAHs with increasing resistance to degradation such as dibenzofurans, fluorenes., dibenzothiophenes, phenanthrenes, pyrenes, chrysenes, and TAS which are more resistant to degradation and could be more affected by higher level of degradation. This variation could be as a result of many thermodynamic potential routes of PAHs methanogenic degradation are linked to (Dolfing *et al.*, 2009). Consequently, the effect of methanogenic degradation on the naphthalenes are less affected compared to evaporative weathering and aerobic biodegradation. Figure 0.15Figure 0.16Figure 0.17 show the distribution of the target alkylated PAHs for the Nigerian light and medium, and North Sea oils in Tyne sediments. Methanogenic degradation is observed to be quite low because it has little effect of alteration on the characteristic “bell shape” distribution pattern with increase in degradation time compared with aerobic biodegradation for Whitley sediment (see section 5.4.2) where the distribution patterns were greatly altered after 300 days. The characteristic distribution patterns are however almost preserved for all the target PAHs and their alkylated homologous except for the naphthalenes that show a slight alteration after 301 days as expected. The ratios of the 4-methyldibenzothiophene to that of the two identified pentamethylnaphthalnes (a, b, c, d, e-PMN and v, w, x, y, z-PMN) are unaffected with degradation time, which is a further validation of their possibility of being used as ratios for oil correlation and differentiation as discussed in chapters 3, 4, and 5 respectively. Figure 0.18 and Figure 0.19 show methylbiphenyls and methylphenanthrene isomers analysed to assess the effect of methanogenic degradation as previously discussed in chapters 3, 4, and 5, respectively. The methylbiphenyls isomers were slightly affected compared to the effect of biodegradation on oils in Whitley sediment in chapter 5 for all the three oil samples, and like the water washing effect discussed in chapter 4 and Nigerian sediment discussed in chapter 5. Similarly, methylphenanthrene isomers were also slightly affected as previously discussed in chapters 3, 4, and 5 respectively because of their high resistance to degradation with the North Sea oil being the least affected.

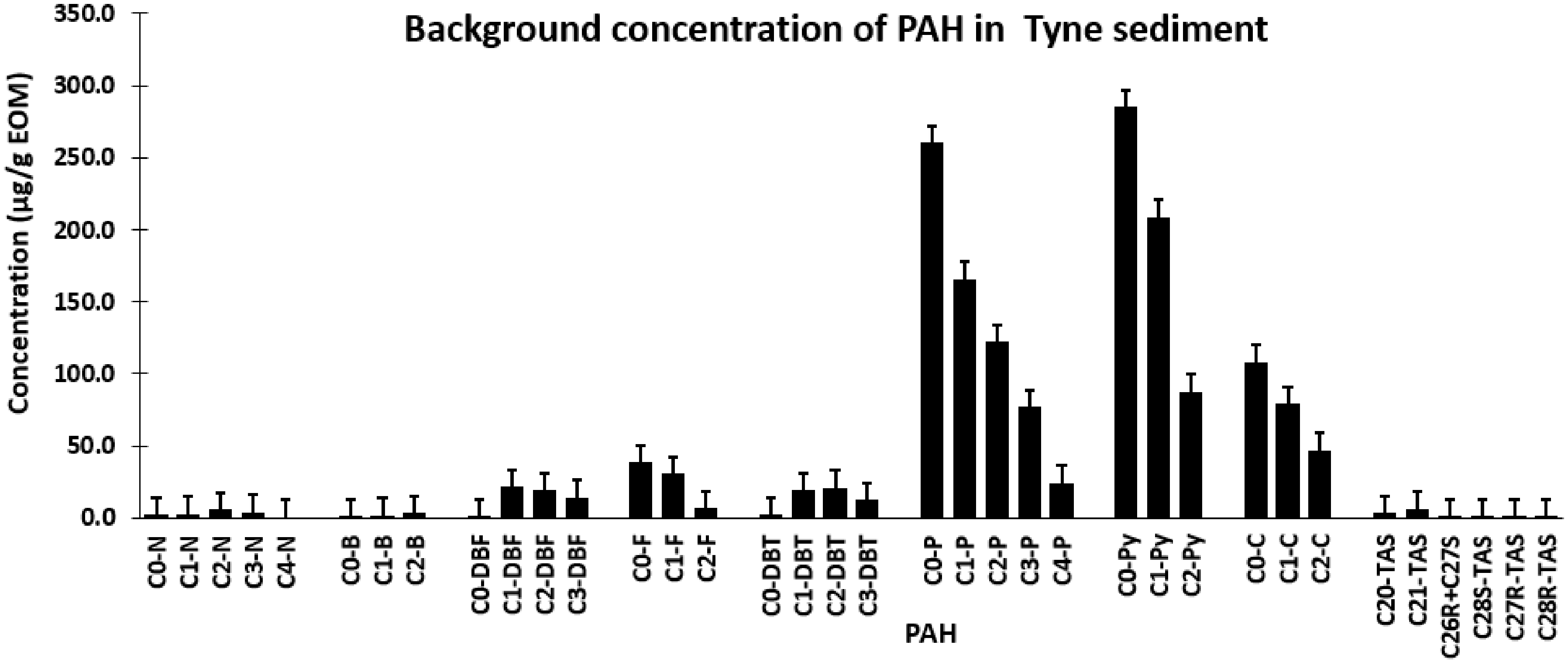


Figure 0.14: Distribution of background concentration of target alkylated PAHs in (A) Tyne sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs. **The error bars show ± 1 standard error of replicate microcosms (n = 3).**

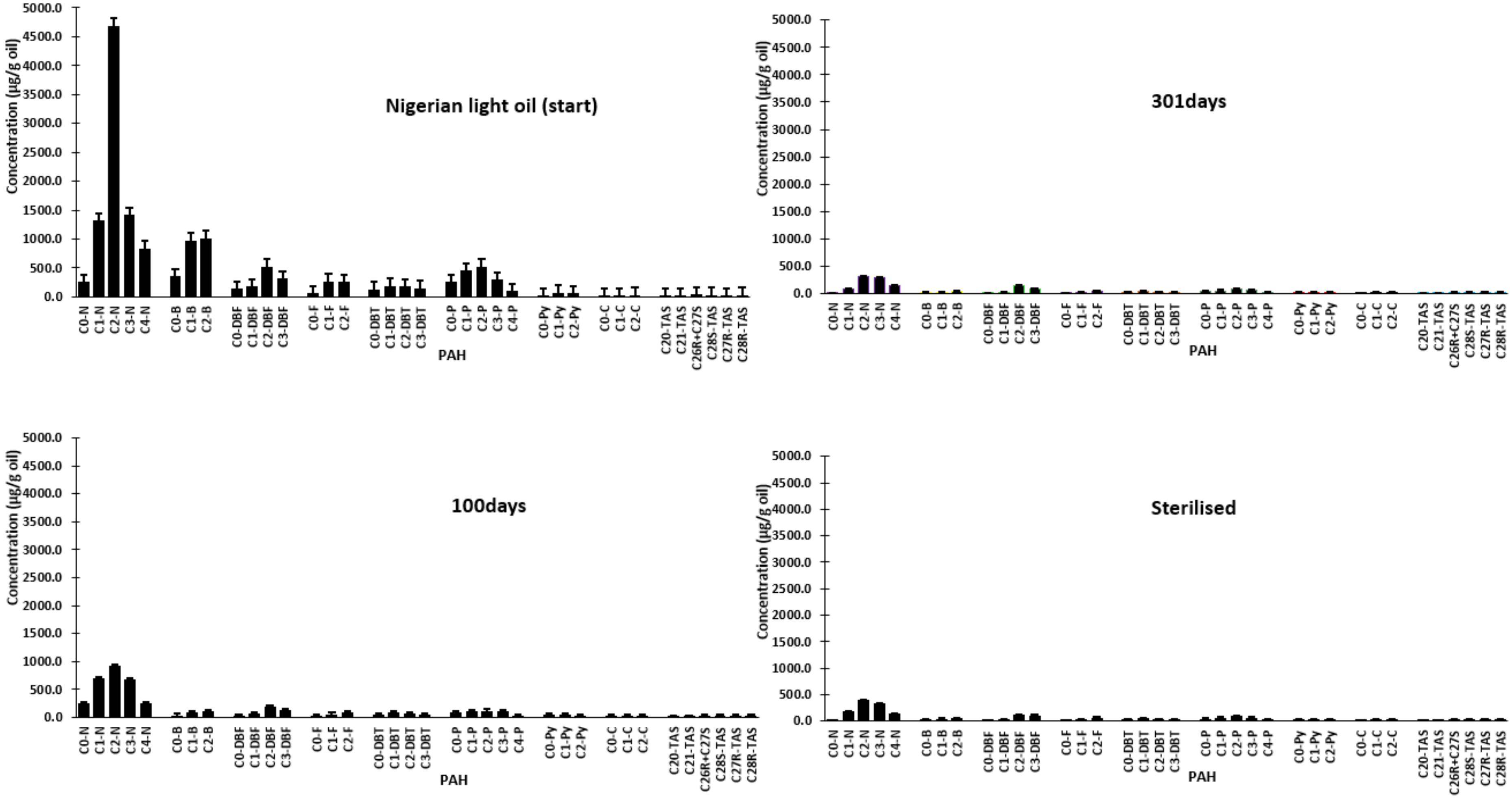


Figure 0.15: Distribution of target alkylated PAHs showing the biodegradation incubation period for Nigerian light oil in Tyne sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs. The error bars show ± 1 standard error of replicate microcosms (n = 3).

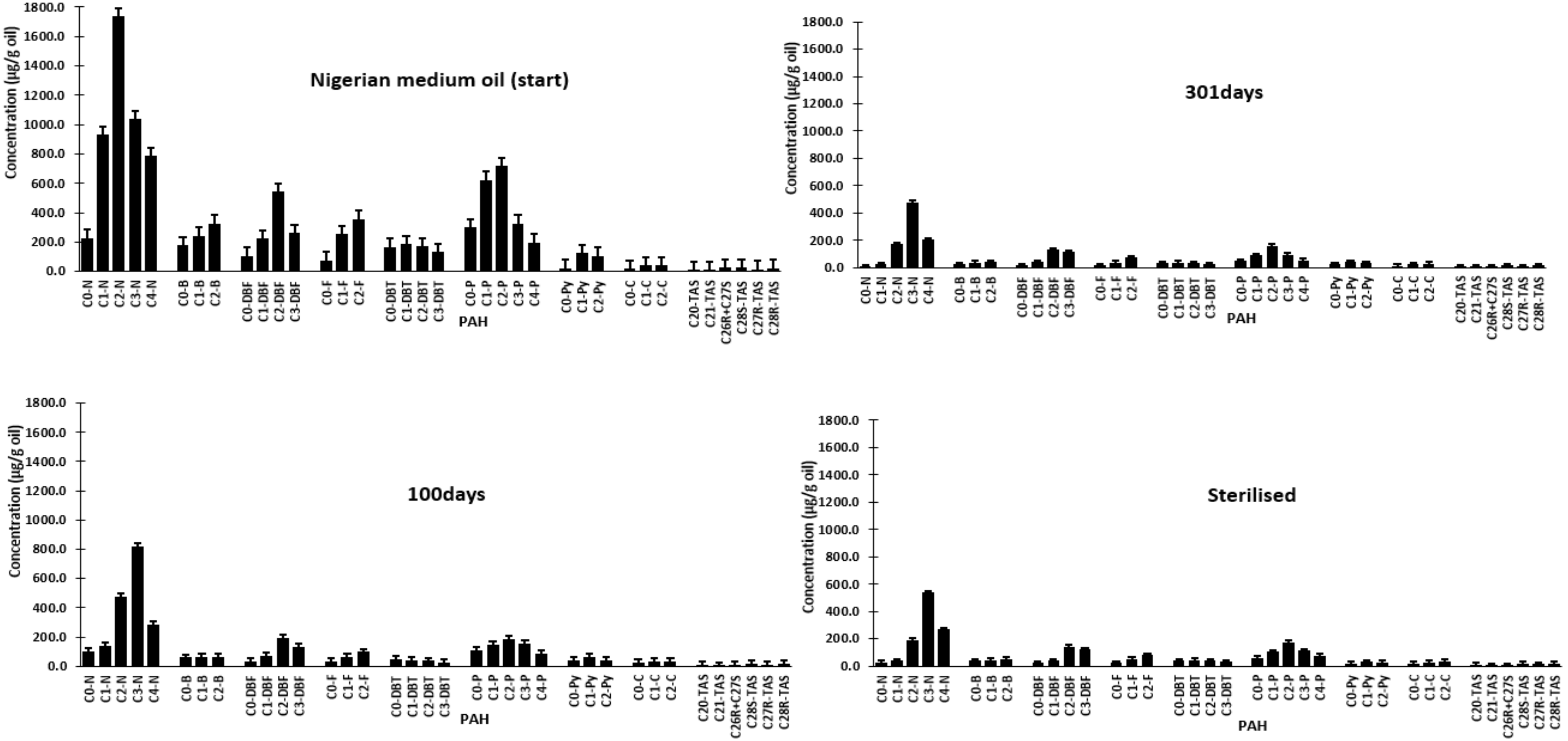


Figure 0.16: Distribution of target alkylated PAHs showing the biodegradation incubation period for Nigerian medium oil in Tyne sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs. The error bars show ± 1 standard error of replicate microcosms (n = 3).

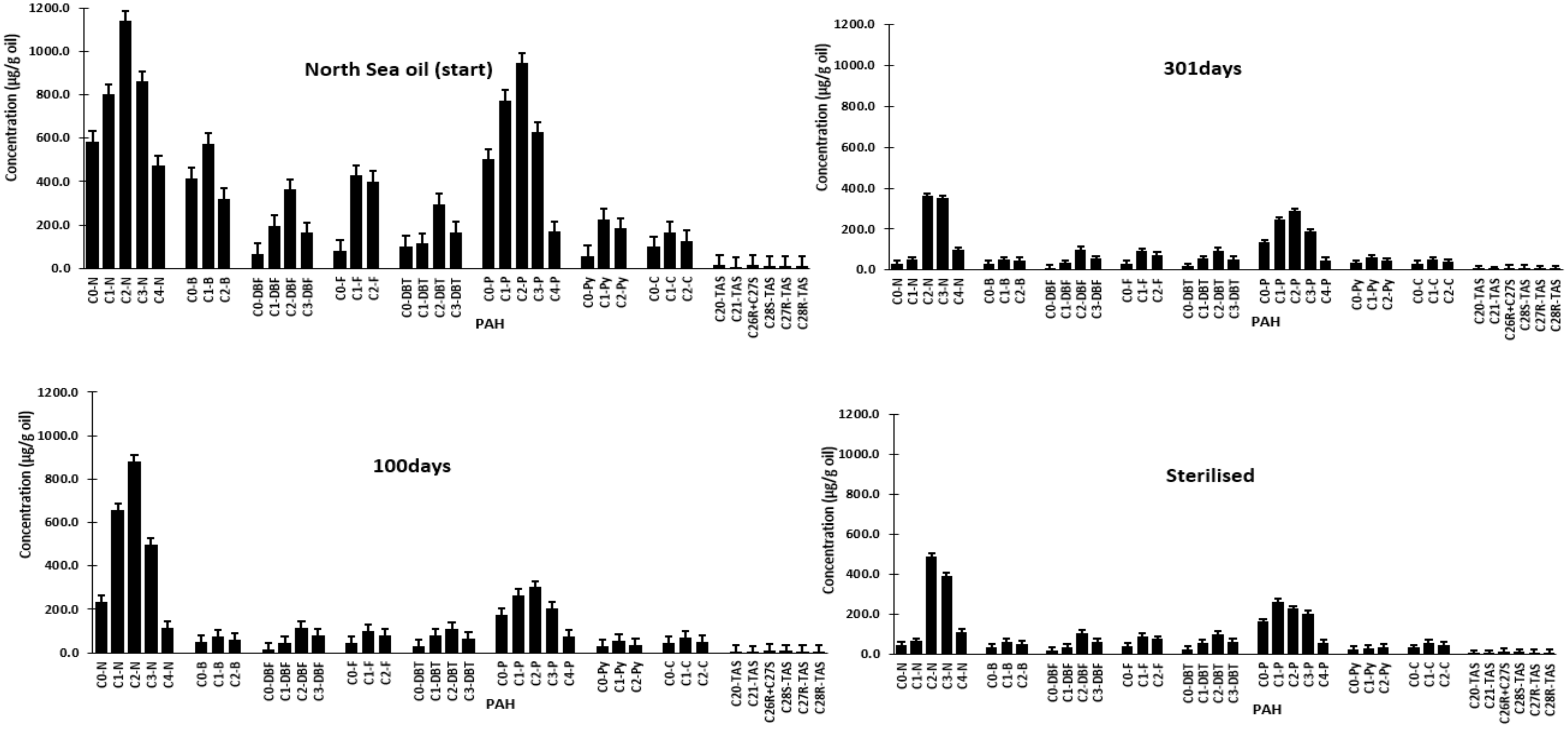


Figure 0.17: Distribution of target alkylated PAHs showing the biodegradation incubation period for North Sea oil in Tyne sediment. N, B, DBF, F, DBT, P, Py and C represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, and triaromatic steranes (TAS) respectively. 0, 1, 2, 3, and 4 represent alkyl group carbon numbers in alkylated PAHs. The error bars show ± 1 standard error of replicate microcosms (n = 3).

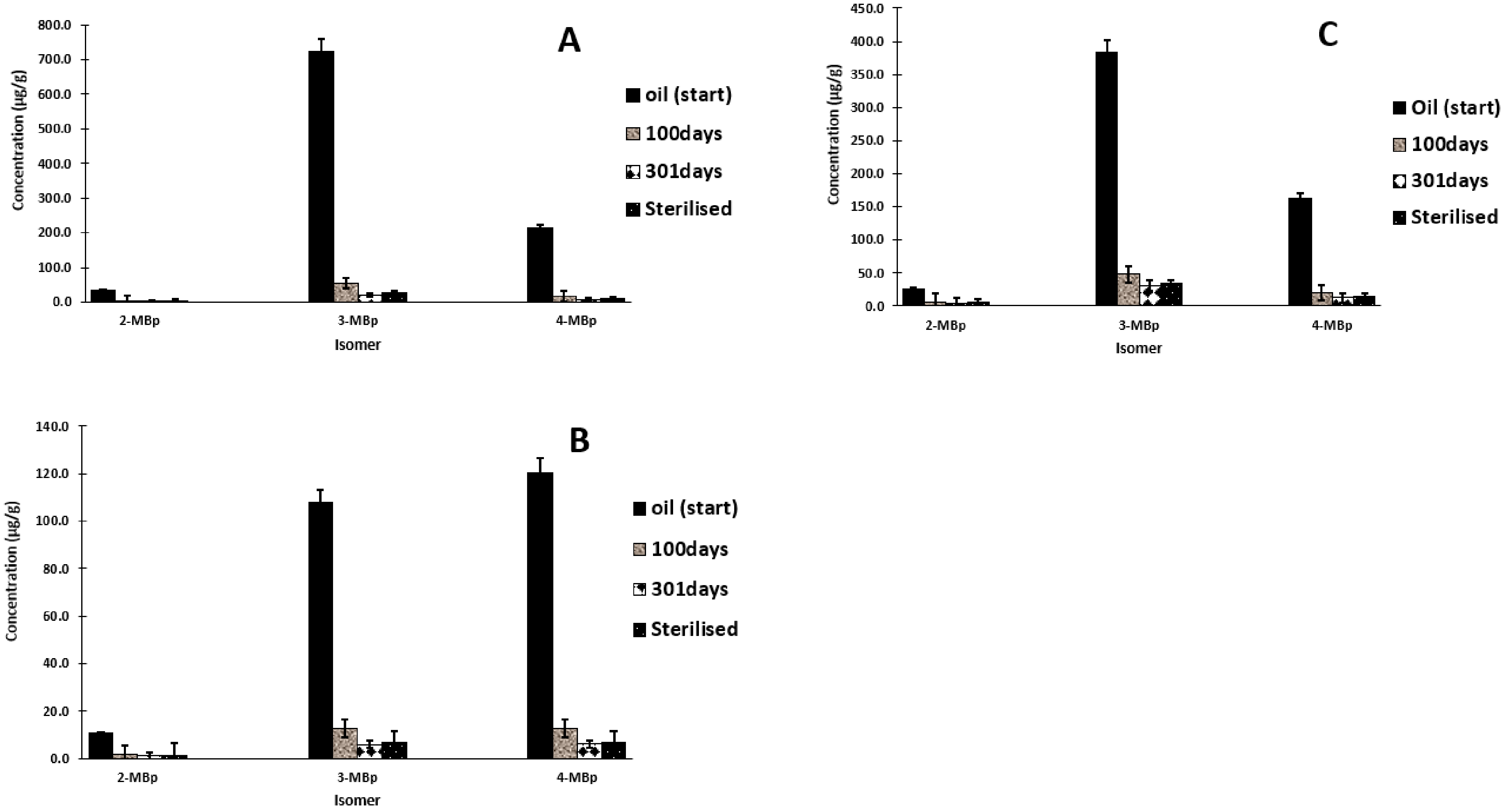


Figure 0.18: Effect of methanogenic degradation on methylbiphenyls isomers for (A) Nigerian light, (B) Nigerian medium, (C) North Sea oils in Tyne sediment. **The error bars show ± 1 standard error of replicate microcosms (n = 3).**

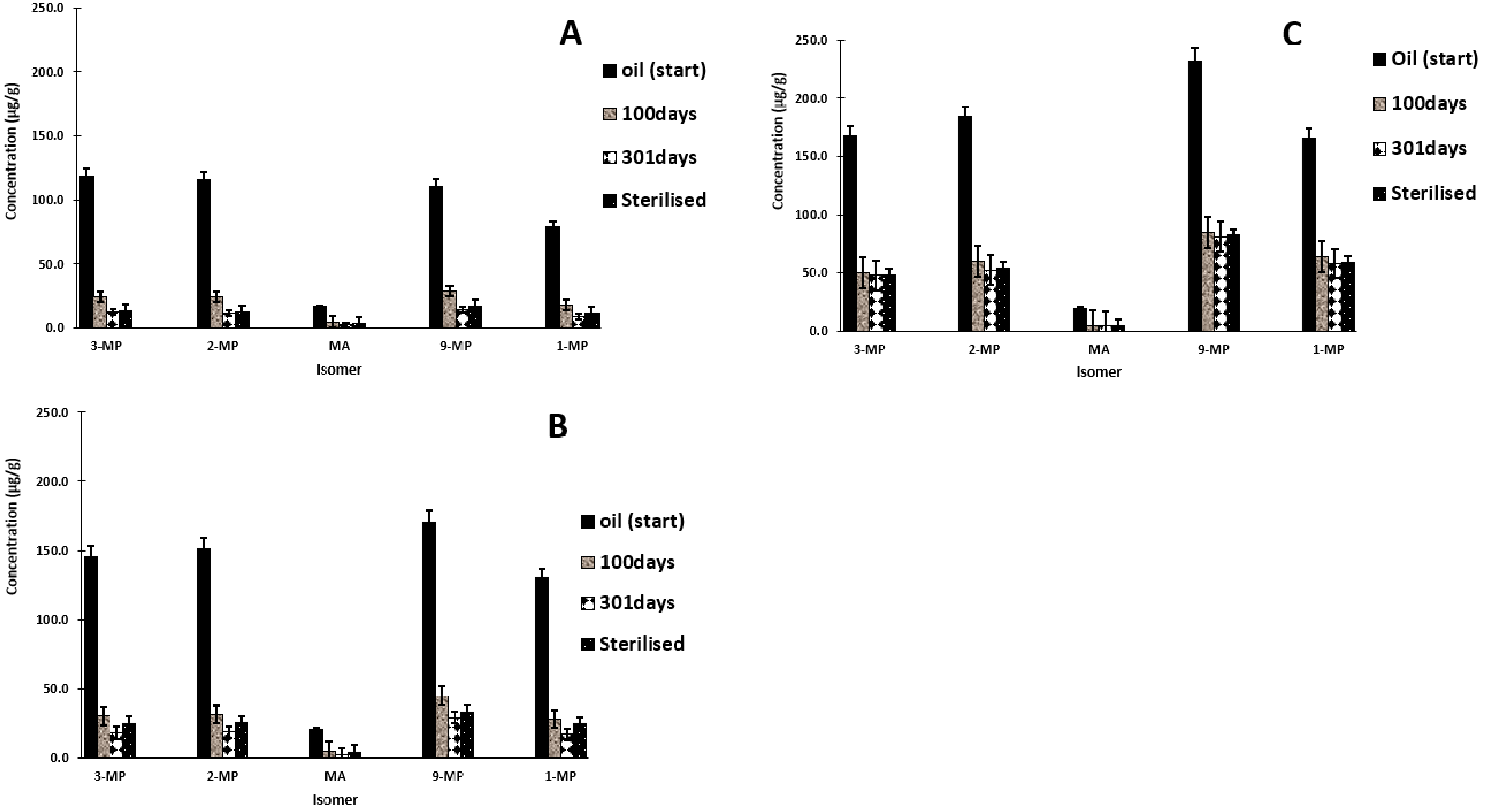


Figure 0.19: Effect of methanogenic degradation on methylphenanthrene isomers for (A) Nigerian light, (B) Nigerian medium, (C) North Sea oils in sediment. **The error bars show ± 1 standard error of replicate microcosms (n = 3).**

6.4.4 Biomarker compound composition and concentration changes.

The effects of microbial degradation on biomarkers are well-known and have reported severally as discussed in chapter 5 (see section 5.4.3). Regular steranes, hopanes and tricyclic terpanes have reported to degrade under microbial attack both on the surface and in the subsurface under anoxic conditions (Reed, 1977; Seifert *et al.*, 1984; Cassani and Eglinton, 1991). This work looks at these biomarkers such as tricyclic, tetracyclic and pentacyclic terpanes, sesquiterpanes, steranes, a non-biomarker geochemical maker adamantane and triaromatic steranes and how anaerobic biodegradation alter their suitability for being used for correlation and differentiation using diagnostic analysis. Figure 0.20, Figure 0.21Figure 0.22 are the m/z 191 mass chromatograms showing terpanes distributions for the non-degraded Nigerian light, Nigerian medium, and North Sea oils respectively, whereas Table 0.7, Table 0.8Table 0.9 show the measured concentrations for the Nigerian light, Nigerian medium, and North Sea oils in Tyne sediment respectively. Background concentrations of terpanes in the sediment were also measured to assess the influence of indigenous hydrocarbons and were found to be of much lower amounts compared to the corresponding concentrations in the non-degraded or starting oils similar to that of Whitley and Nigerian sediments discussed in chapter 5 (see section 5.4.3). The tables show the concentrations of degraded anaerobic microcosms incubated for 100 and 300 days with sterile controls. The coloured numbers (see Figure 0.20Figure 0.21Figure 0.22) represent the unidentified peaks with their corresponding letters (see Table 0.7Table 0.8, and Table 0.9) as discussed comprehensively in chapters 3, 4, and 5 respectively. The effect of methanogenic degradation on these compound classes was assessed using their diagnostic ratios for the three oil samples as discussed in previous chapters. Figure 0.23 show the diagnostic ratio plots for the three oil samples which include C_{23}/C_{24}, S/(S+Q), Q/(Q+C_{19}), M/(M+C_{19}), X/(X+C_{19}), C_{27}(Ts/Tm), Y/(Y+C_{24}), Y1/(Y1+C_{24}), Z/(Z+C_{25}B), Z_1/(Z_1+C_{28}A), T/(T+C_{29}B) and C_{31} 22S/(22S+22R).

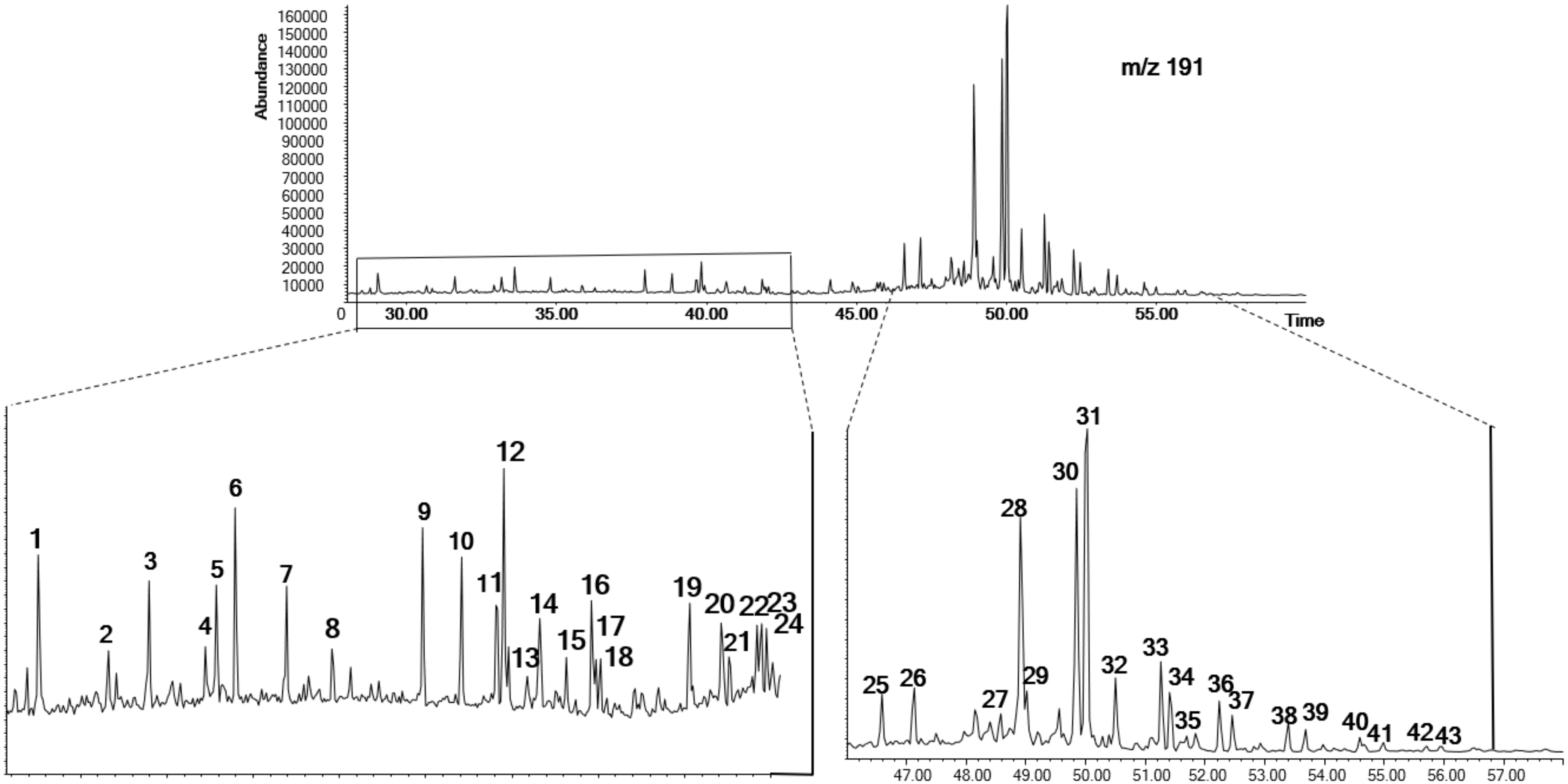


Figure 0.20: m/z 191 mass chromatograms showing terpanes distribution from non-biodegraded Nigerian light oil. The numbers represent the corresponding peak names listed in Table 0.7. Numbers 1, 2, 4, 6, 11, 12, 15, 19, and 24 are the unidentified peaks.

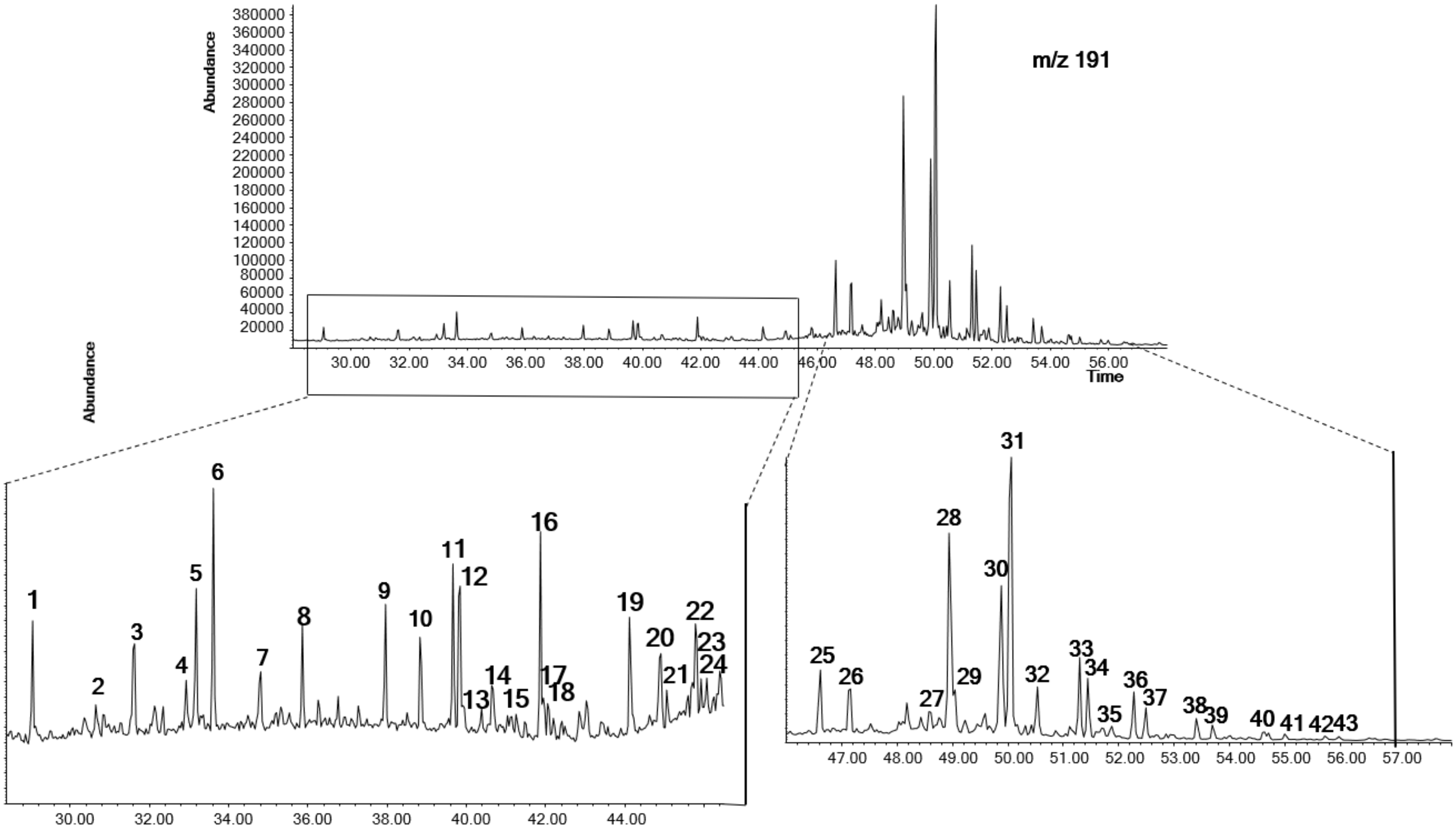


Figure 0.21: m/z 191 mass chromatograms showing terpanes distribution from non-biodegraded Nigerian medium oil. The numbers represent the corresponding peak names listed in Table 0.8. Numbers 1, 2, 4, 6, 11, 12, 15, 19, and 24 are the unidentified peaks.

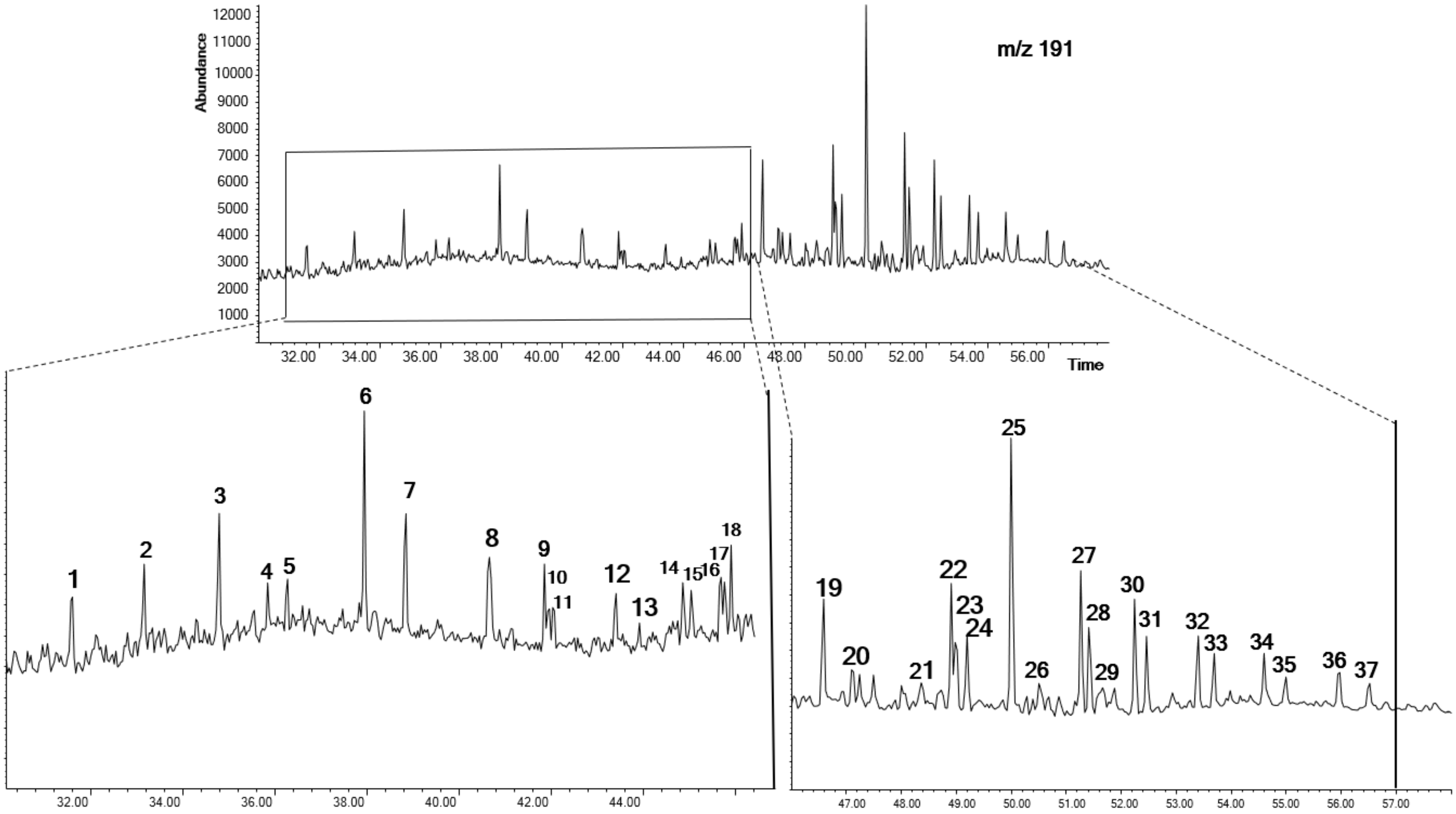


Figure 0.22: m/z 191 mass chromatograms showing terpanes distribution from non-biodegraded North Sea oil. The numbers represent the corresponding peak names listed in Table 0.9. Numbers 5, 12, 13, and 18 are the unidentified peaks.

The ratios are quite unaffected as expected, it is observed that the plots remained parallel or unaffected from the start (undegraded oils) through to 301days and the sterilised controls, a degradation as earlier regarded as slight (PM Level 2-3). The only variation is from the sediment background concentrations which was quite irregular due to different sources of contribution into the River Tyne. Terpanes have been reported to be one of the most resistant to microbial attack under deep reservoir systems mostly under anoxic conditions (e.g. Cassani and Eglinton, 1991).

Figure 0.24 shows the m/z 217 mass chromatograms of steranes with the peaks numbered and the measured concentrations and the corresponding peak names shown in Table 0.10,Table 0.11, and Table 0.12 for the three oil samples. Figure 0.24 shows the diagnostic ratio plots of the steranes in the three oils C_{20}/C_{21}, C_{21}/C_{22}, C_{27} αββ/C_{29}αββ, C_{27} 20S/(20S+20R), C_{28} αββ/(αββ+ααα), C_{29} αββ/(αββ+ααα), and C_{27}αββ(S+R)/C28αββ(S+R) + C_{29}αββ(S+R) were analysed to assess the effect of anaerobic biodegradation as discussed in chapters 3, 4, and 5 respectively. The steranes were also unaffected by the methanogenic biodegradation as compared with aerobic biodegradations in chapter 5. The only variation was from the background concentration of the sediment, and this was quite expected. Whilst the concentrations of the steranes in Nigerian light oil remained, that of the Nigerian medium and North Sea oils slightly increased with degradation time.

Figure 0.26 shows the m/z 231 mass chromatograms distribution of triaromatic steranes with the numbered peaks and the measured concentrations and the corresponding peak names are shown in Table 0.13,Table 0.14Table 0.15 respectively. TAS being one of the most resistant steroids to biodegradation (Peters *et al.*, 2005), are observed to be unaffected as the concentrations increased with degradation time in for all the three oil samples. Figure 0.27 shows the diagnostic ratio plots of the triaromatic steranes versus degradation time for the three oils and the ratios are unaffected by anaerobic biodegradation except for the two ratios P/C_{21}, and Q/C_{21} which are affected which were affected by both evaporation and aerobic biodegradation in chapters 3 and 5 respectively, as well as C_{20}/C_{21} which is noted to be unaffected by methanogenic degradation in this chapter. Consequently, peaks P and Q may not be TAS as they are observed to be more susceptible and may not be suitable for use as diagnostic compounds for correlation. The only variation is in the sediment background concentrations. Bicyclic sesquiterpanes were also assessed similarly, and Figure 0.28 shows the m/z 123 mass chromatograms distributions for the three oil samples, and the measured concentrations with the corresponding peak names shown in Table 0.16,Table 0.17Table 0.18 respectively. The concentrations are observed to decrease with degradation, the novel sesquiterpanes from the two Nigerian oils are as discussed in chapters 3, 4, and 5 respectively. The diagnostic ratio plots for the three oil samples are shown in Figure 0.29 and are quite unaffected by anaerobic degradation unlike that of aerobic biodegradation in chapter 5 for all the three oil samples, except for BS3/$C_{30}\alpha\beta$-hopane for North Sea oil which decreased slightly after 100 days as the sesquiterpane (BS3) was slightly degraded relative to $C_{30}\alpha\beta$-hopane. The only variation is in the background concentration of the sediment. There seem to be not much studies carried out on the sesquiterpanes under reservoir conditions despite literature search.

Adamantanes were also analysed and Figure 0.30 shows the m/z 136 mass chromatograms distributions whereas Table 0.19, Table 0.20Table 0.21 show the measured concentrations with the corresponding peak names for the three oil samples respectively. The concentrations are observed to decrease with degradation time as a result of methanogenic degradation effect. Figure 0.31 shows the diagnostic ratio plots of the adamantanes versus degradation time for the three oils in Tyne sediment. Some adamantanes ratios are observed to be quite affected by anaerobic biodegradation which include 1,3-DMA/1,2,5,7-TeMA in the two Nigerian oils, and 1-MA/ (1-MA + 2-MA) in Nigerian medium oil, 1-MA-/1,3,5-TMA slightly affected in all the three oils.

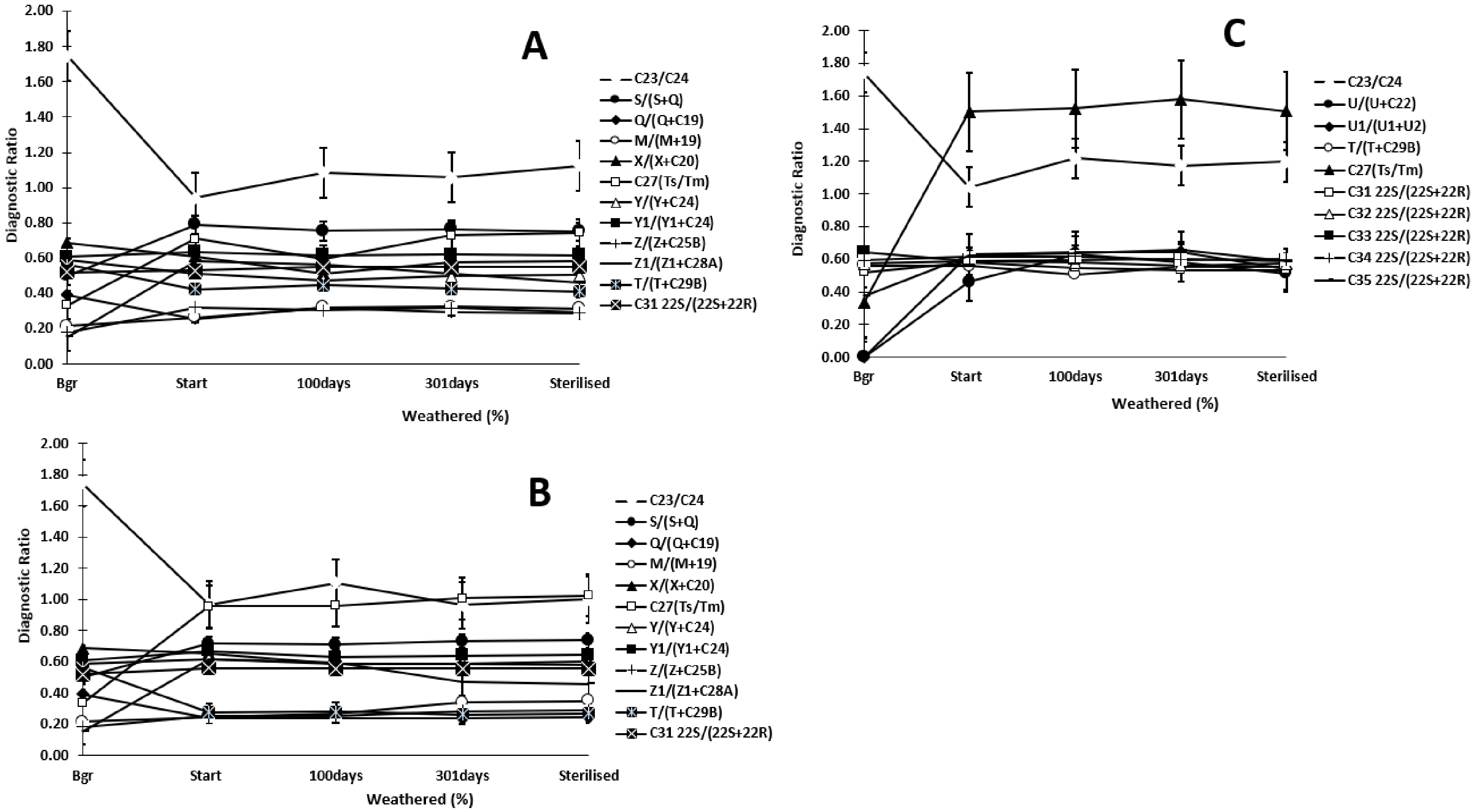


Figure 0.23: Plots of diagnostic ratios of selected terpanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Tyne sediment.

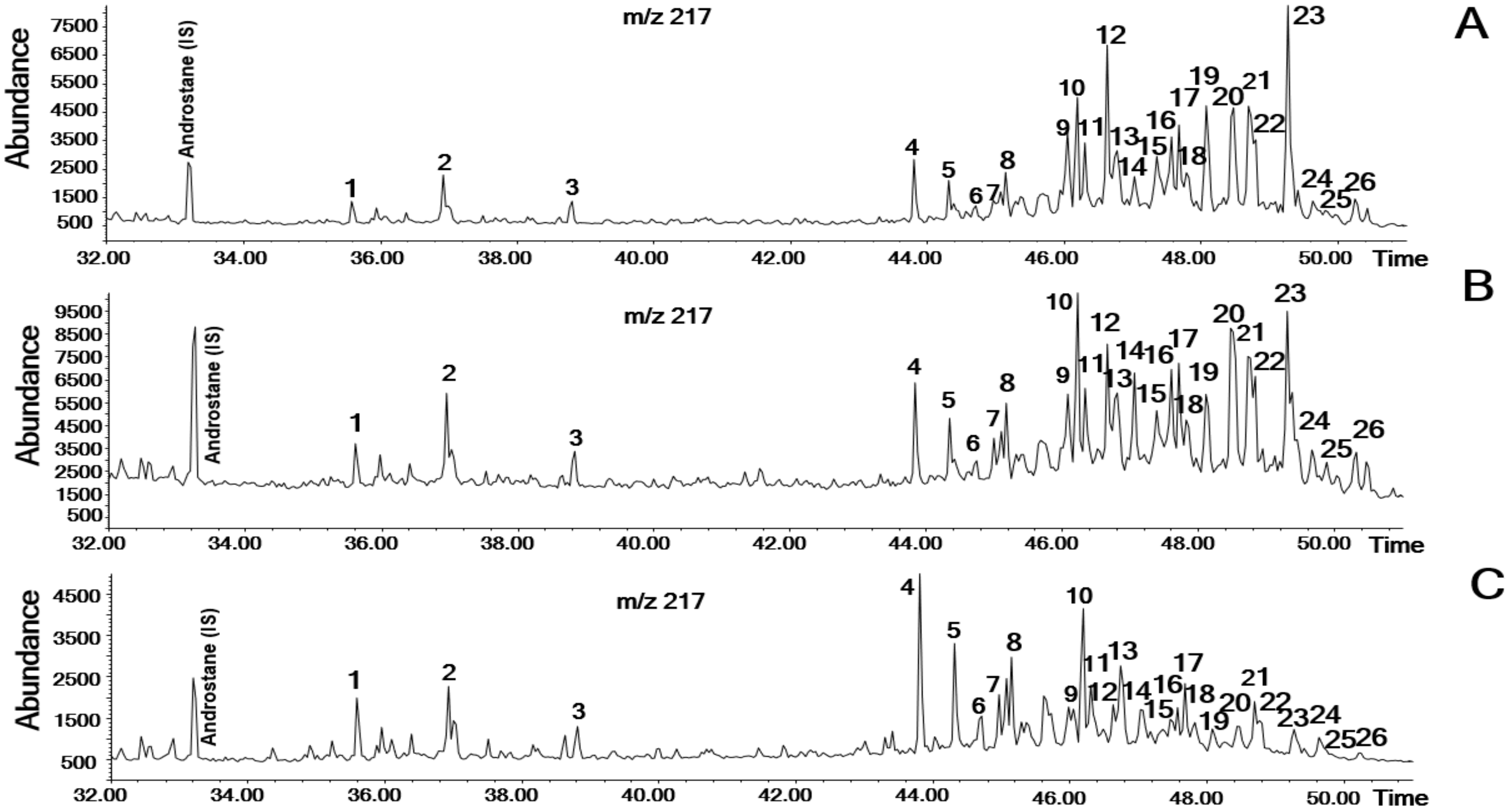


Figure 0.24: m/z 217 mass chromatograms showing the distributions of steranes in non-biodegraded Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. Numbers in (A) represent the corresponding peak names in Table 0.10, numbers in (B) represent peak names in Table 0.11, and numbers in (C) represent peak names in Table 0.12 respectively.

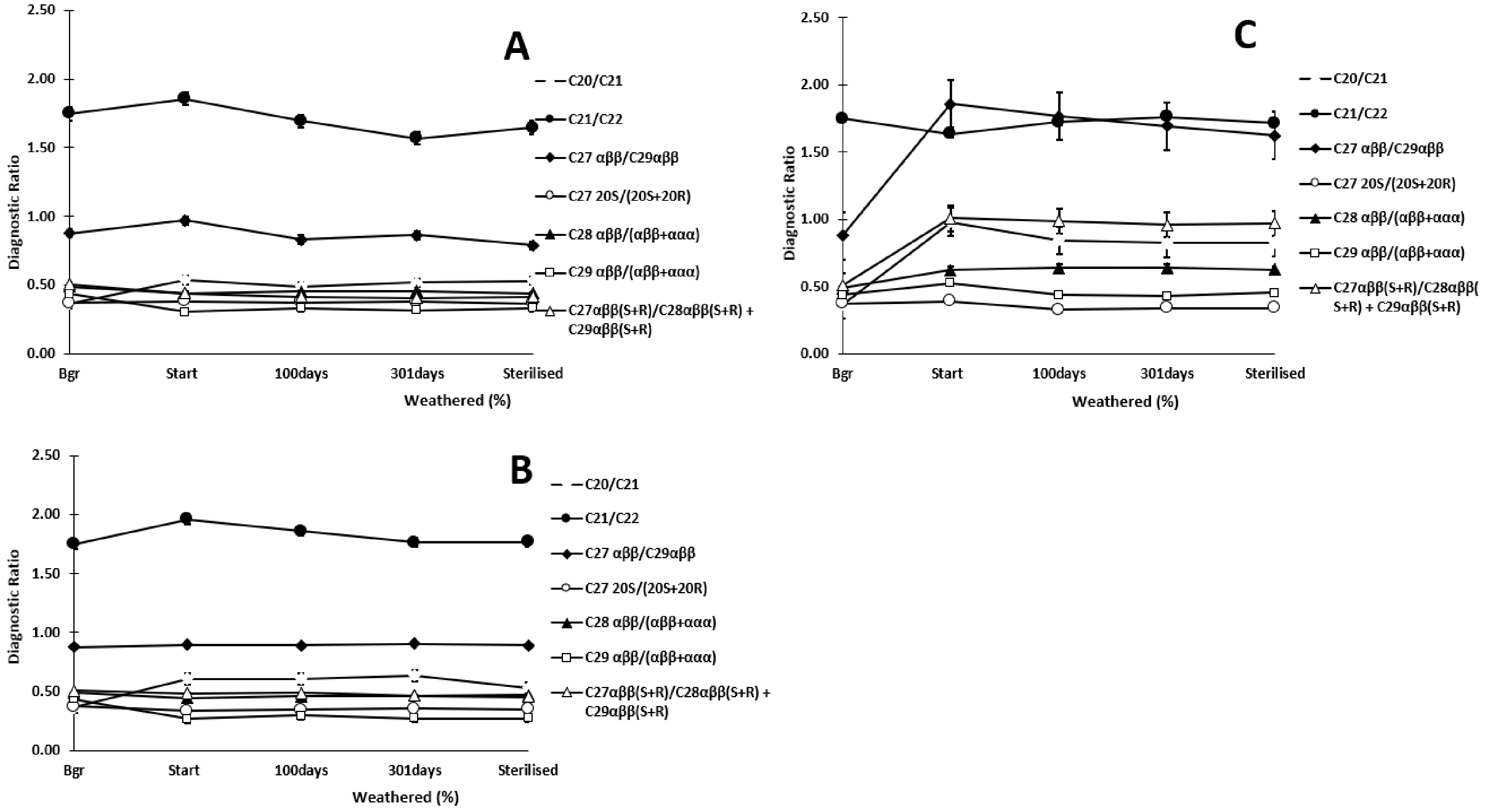


Figure 0.25: Pots of diagnostic ratios of selected steranes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Tyne sediment.

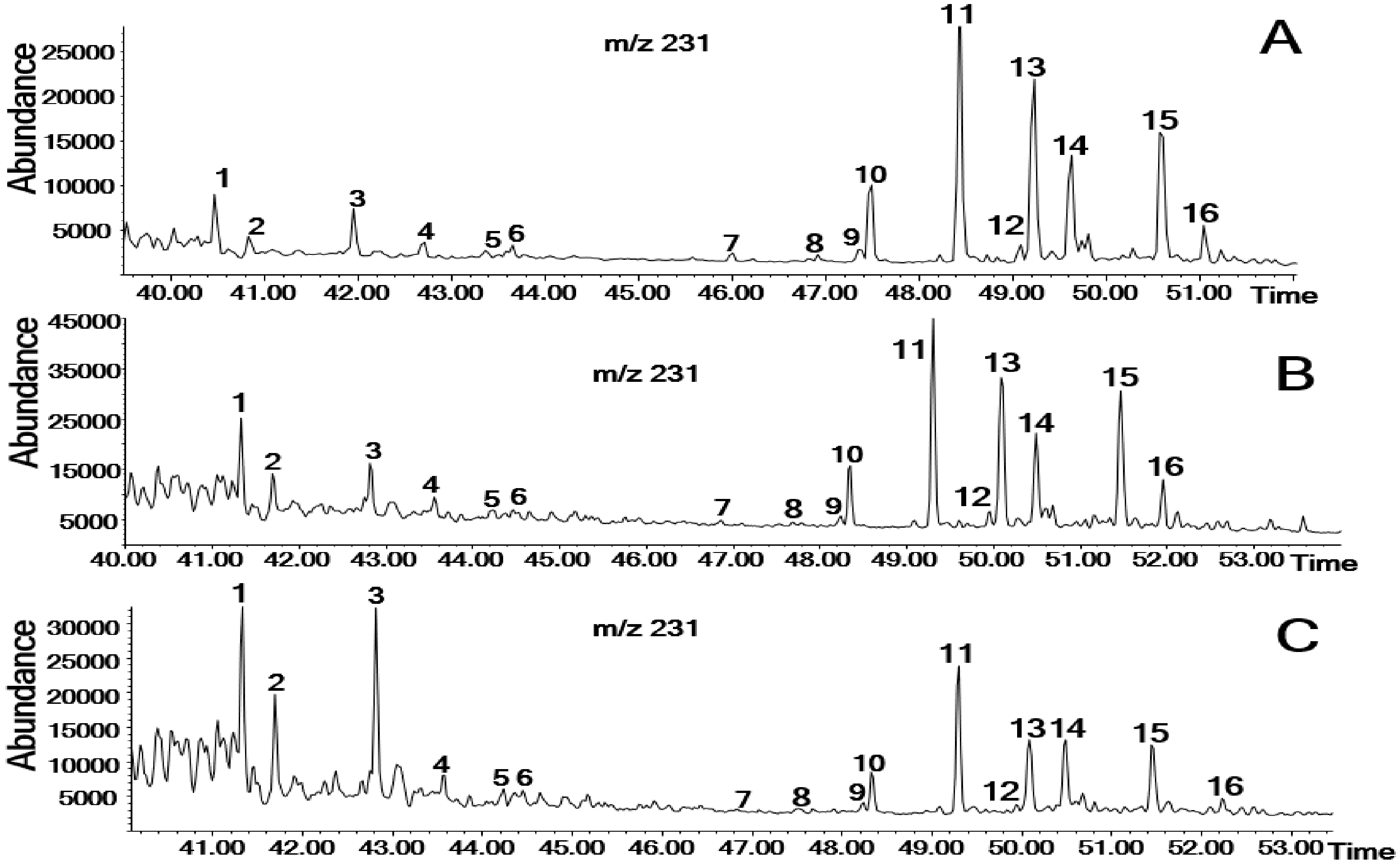


Figure 0.26: m/z 231 mass chromatograms showing the distributions of triaromatic steranes in non-biodegraded Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. Numbers in (A) represent the corresponding peak names in Table 0.13, numbers in (B) represent peak names in Table 0.14, and numbers in (C) represent peak names in respectively. Numbers 1, 2, 7, 8, 9, and 12 are the unidentified peaks.

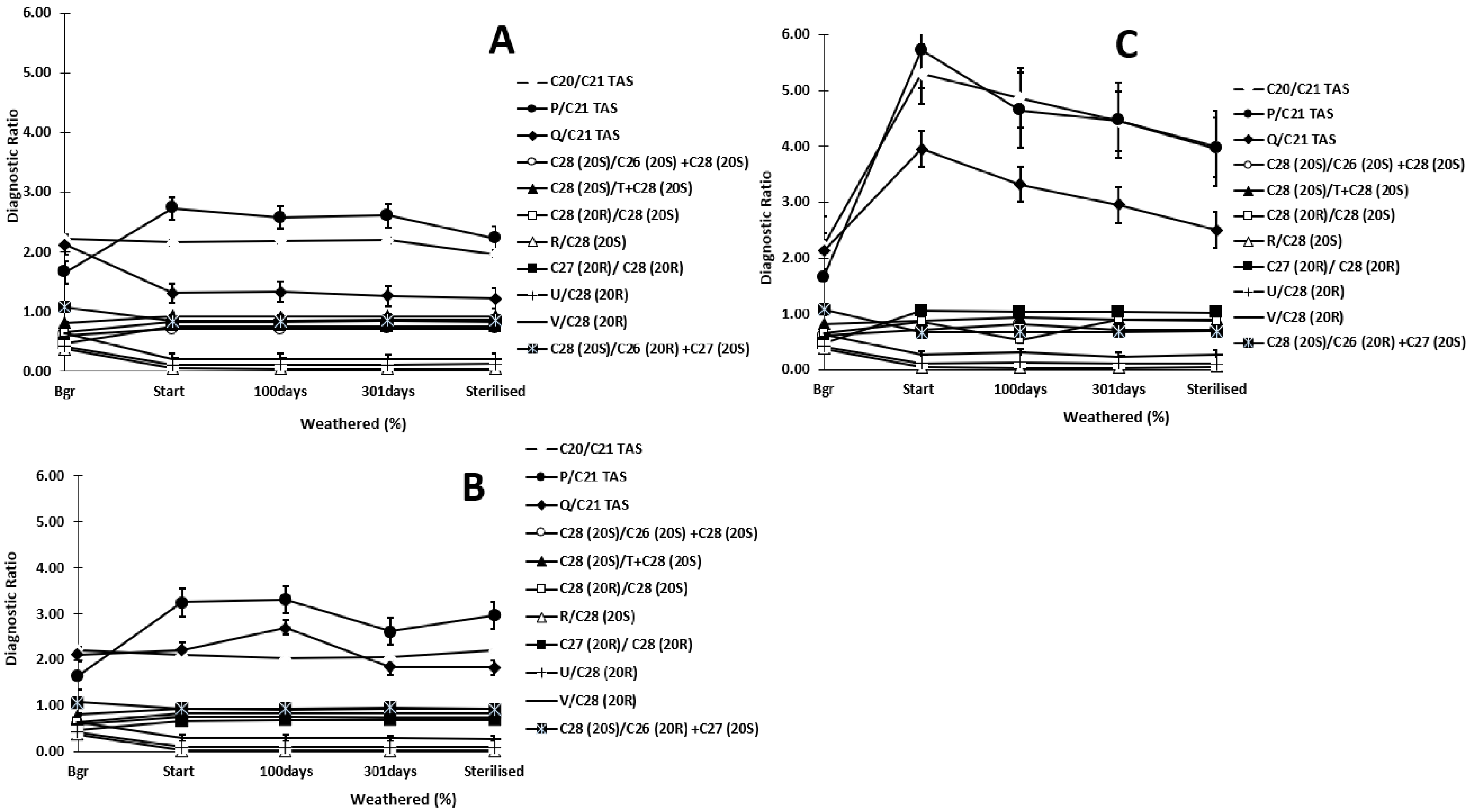


Figure 0.27: Pots of diagnostic ratios of selected triaromatic steranes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Tyne sediment.

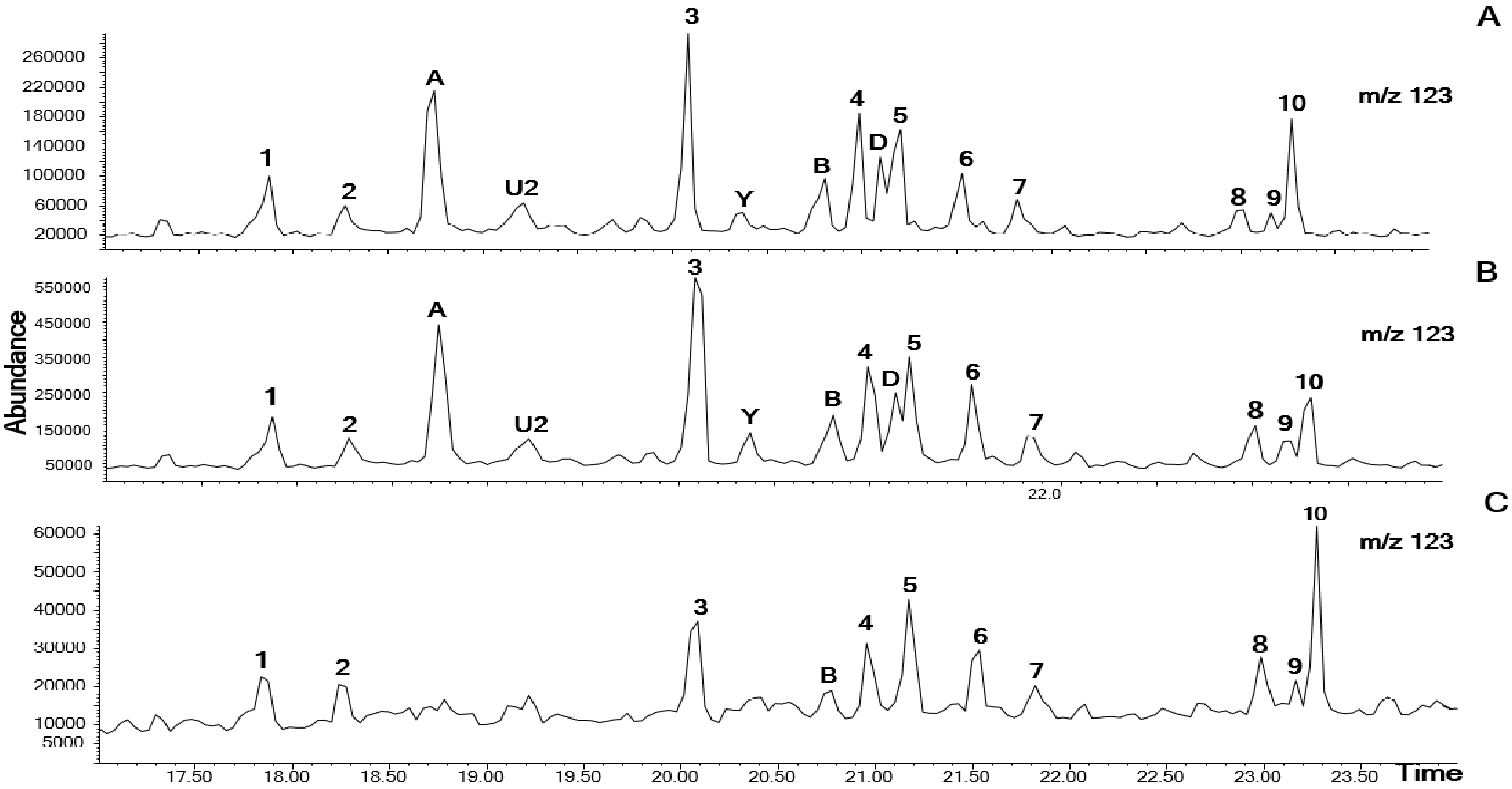


Figure 0.28: m/z 123 mass chromatograms showing the distributions of bicyclic sesquiterpanes in non-biodegraded Nigerian light (A), Nigerian medium (B), and North Sea (C) oils. A, U2, B, and D, are novel sesquiterpanes observed in Nigerian light and medium oils and B in North Sea oil. Numbers in (A) represent the peak names in Table 0.16, numbers in (B) represent peak names in Table 0.17, and numbers in (C) represent peak names in Table 0.18 respectively.

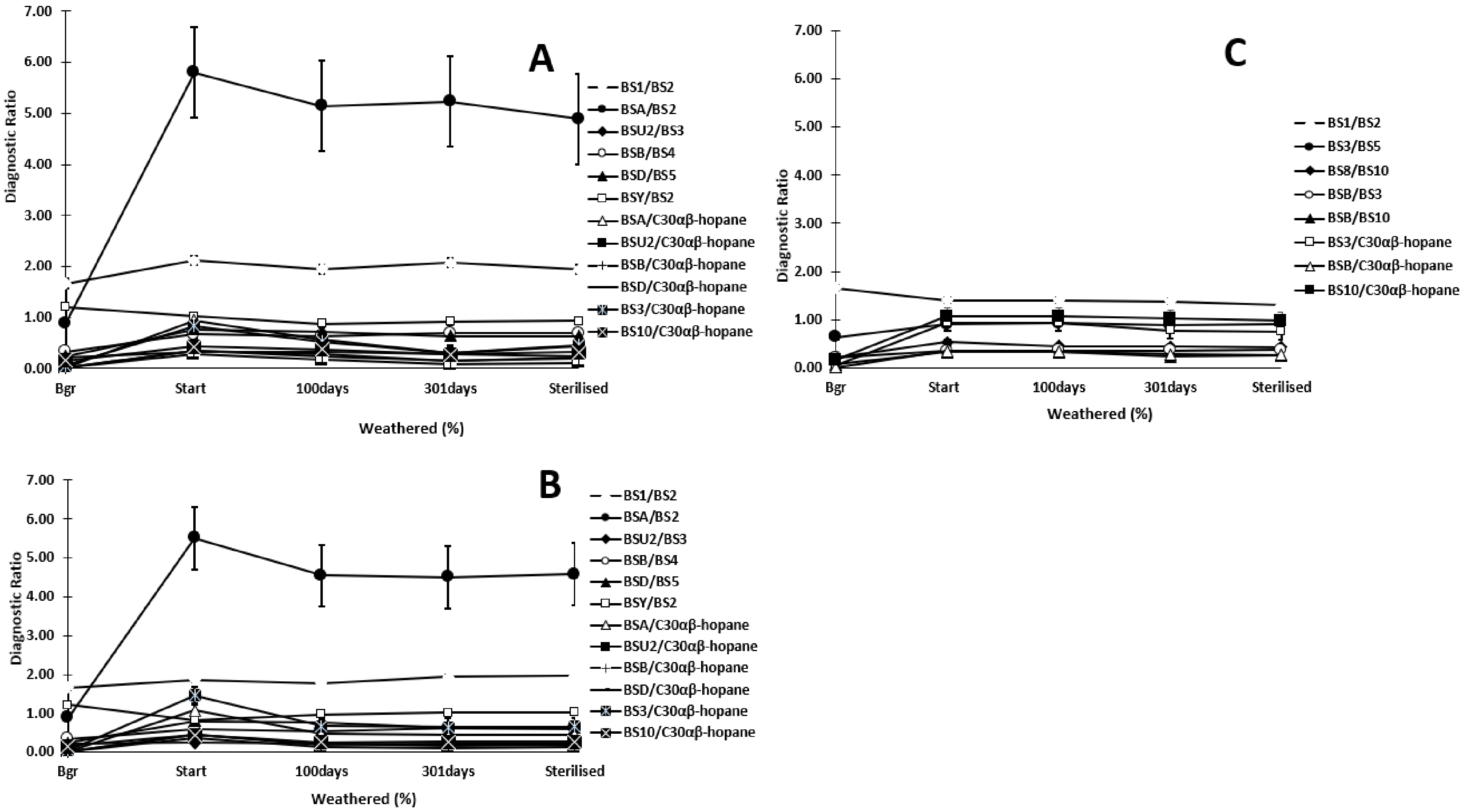


Figure 0.29: Pots of diagnostic ratios of selected bicyclic sesquiterpanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Tyne sediment.

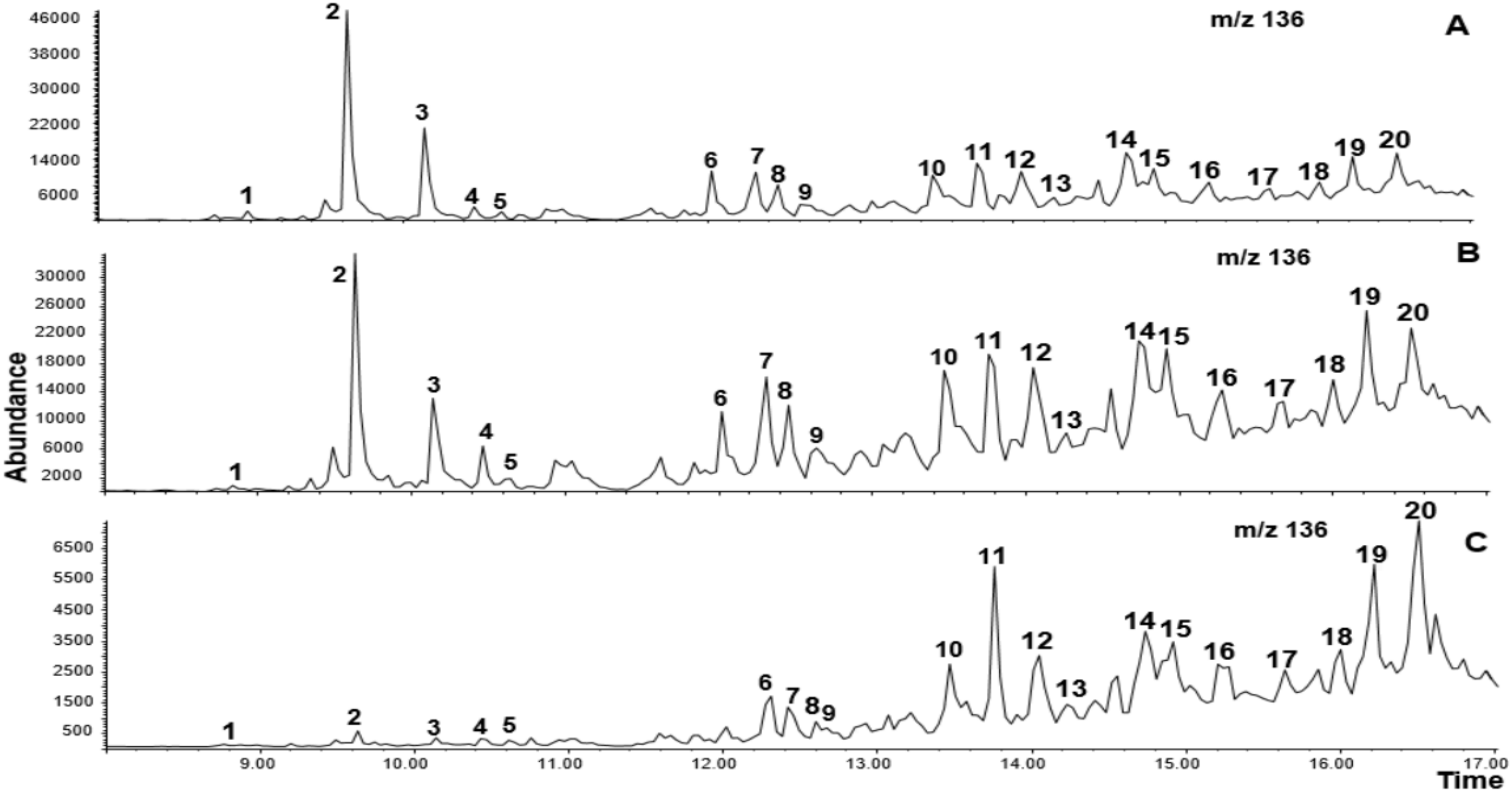


Figure 0.30: m/z 136 mass chromatograms showing the distributions of adamantanes in non-biodegraded; (A) Nigerian light oil; (B) Nigerian medium; and (C) North Sea oils. Numbers in (A) represent the corresponding peak names in Table 0.19, numbers in (B) represent peak names in Table 0.20, and numbers in (C) represent peak names in Table 0.21 respectively

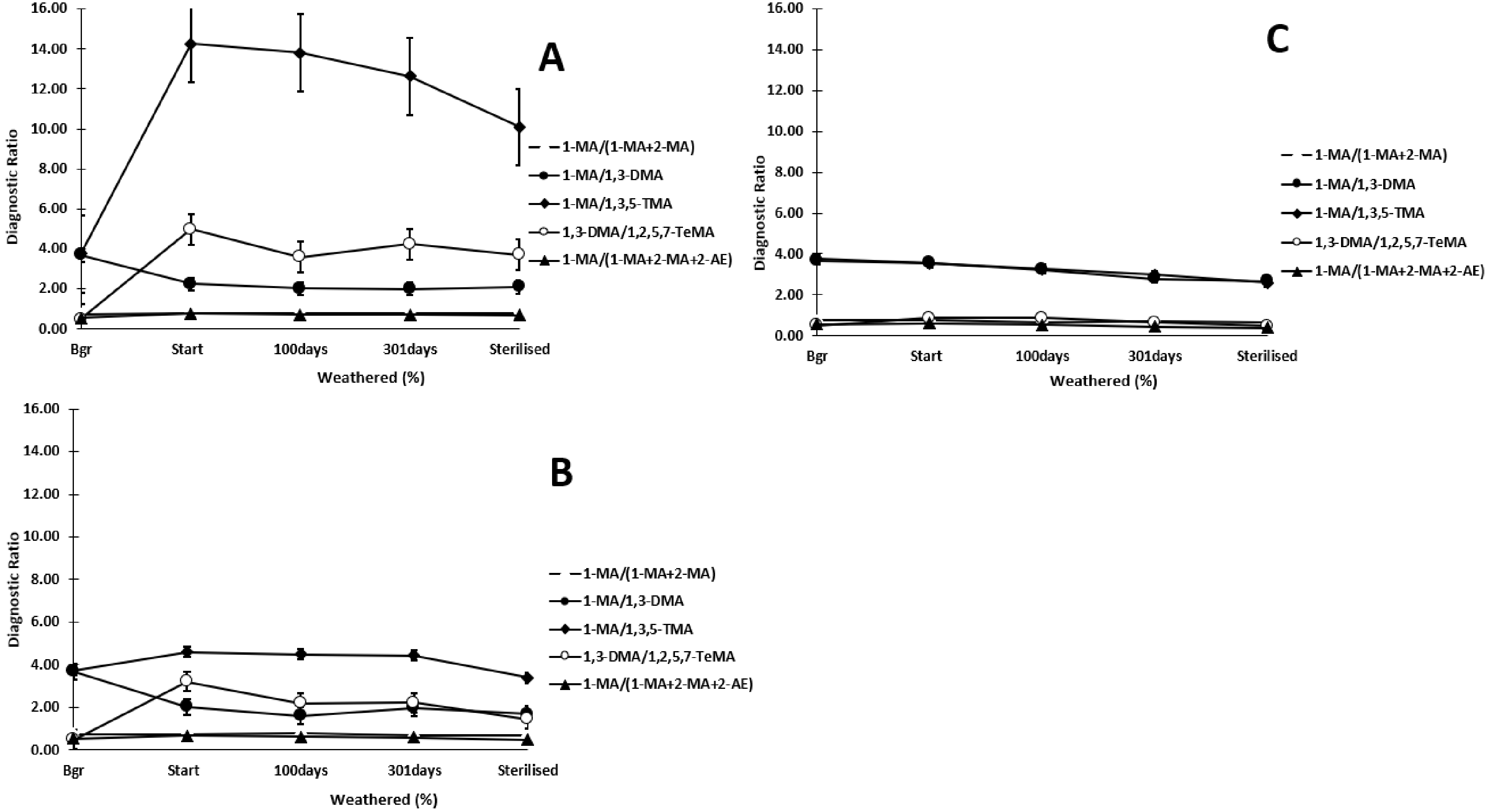


Figure 0.31: Pots of diagnostic ratios of selected Adamantanes versus degradation time (days) for; (A) Nigerian light; (B) Nigerian medium; and (C) North Sea oils in Tyne sediment.

6.4.5 Relative Susceptibility of Selected Molecular Markers

The relative susceptibilities to biodegradation of some selected aliphatic and PAH molecular markers based on their relatively close elution order was assessed using their ratios shown in **Error! Reference source not found.** as discussed in chapter 5. The ratio plots are much less affected by anaerobic degradation compared to aerobic biodegradation, during which were very altered by microbial attack in all the three oil samples. The biochemical reactions and mechanisms by which the anaerobic biodegradation takes place is much slower than in aerobic condition, this accounts for why the ratios of the selected molecular markers are fully conserved and unaffected by anaerobic biodegradation after 301 days, unlike in the aerobic condition discussed in section 5.4.4.

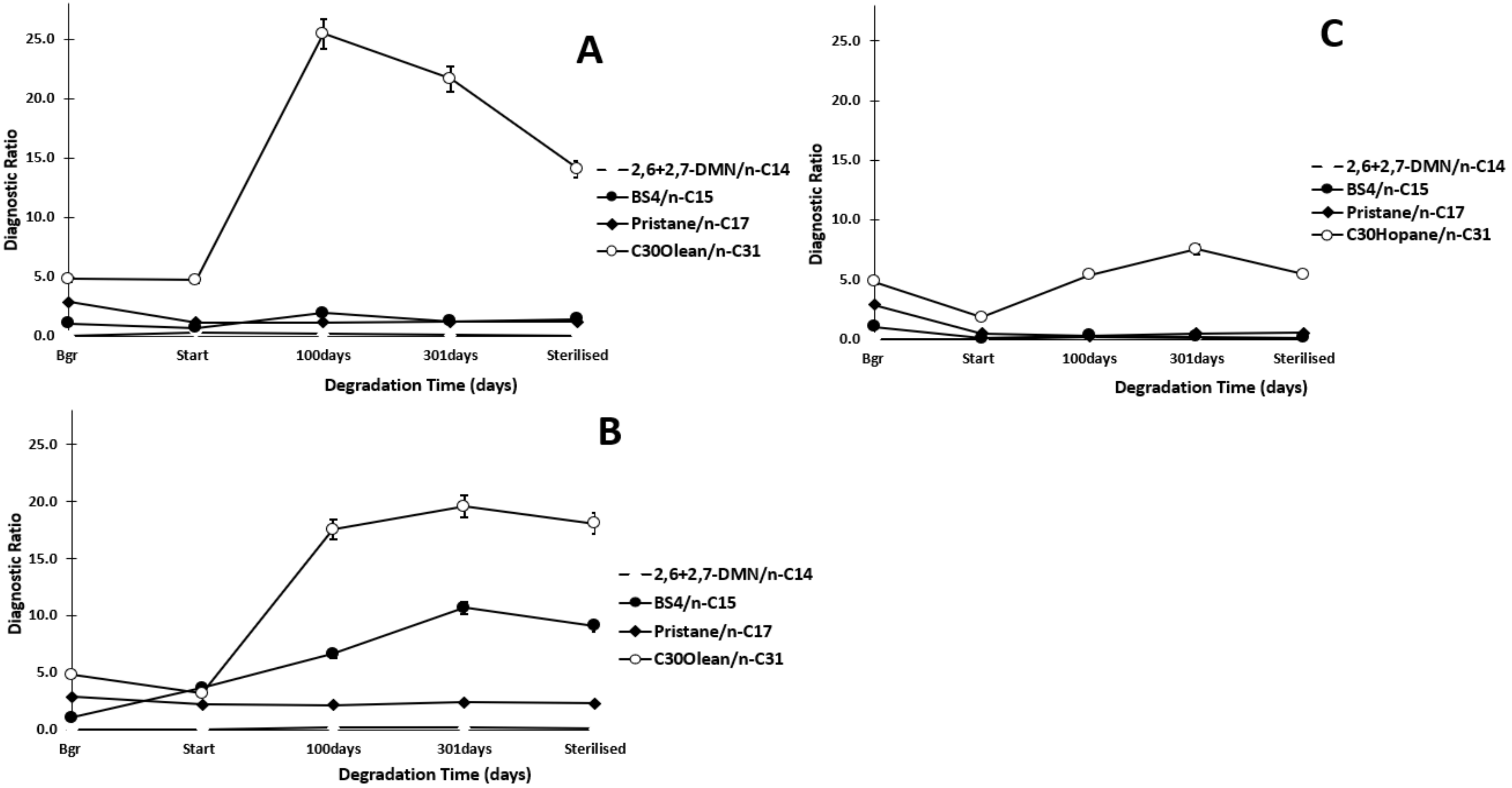


Figure 0.32: Ratios of selected aliphatics/PAH for (A) Nigerian light, (B) Nigerian medium, and (C) North Sea oils in Tyne sediment.

Note: 2,6+2,7-DMN: 2,6+2,7-Dimethylnaphthalene, BS4: C_{15}-Sesquiterpane, C_{30}Olean: C_{30}-18α(H) Oleanane. The error bars show ±1 standard error of replicate microcosms (n = 3)

6.4.6 Diagnostic Ratio plots of selected Nordtest parameters

The same selected Nordtest diagnostic parameters analysed for the aerobic biodegradation (section 5.4.5) are also plotted for the anaerobic biodegradation in this chapter. Figure 0.33 shows the diagnostic ratio plots of Nigerian Light oil in the Tyne sediment microcosms for 100 days and 301 days incubation with positive correlation (fitted error bars on x-y plot) at 95% confidence limit, which show that the anaerobic biodegradation has had no effect on the selected parameters. Figure 0.34 shows the diagnostic ratio plots of Nigerian Medium oil in the Tyne sediment microcosms for 100 days and 301 days incubation with positive correlation at 95% confidence limit, which show that the anaerobic biodegradation had no effect on the selected parameters, (apart from the C_{21} TAS parameter, which shows a little variability) after 301 days and the control. Figure 0.35 shows the diagnostic ratio plots of the North Sea oil in the Tyne sediment microcosms for 100 days and 301 days incubation with positive correlation at 95% confidence limit and with C_{21} TAS as the only misfitted parameter. This result is as expected because anaerobic biodegradation is a much slower biochemical reaction compared to aerobic biodegradation discussed in section 5.4.5.

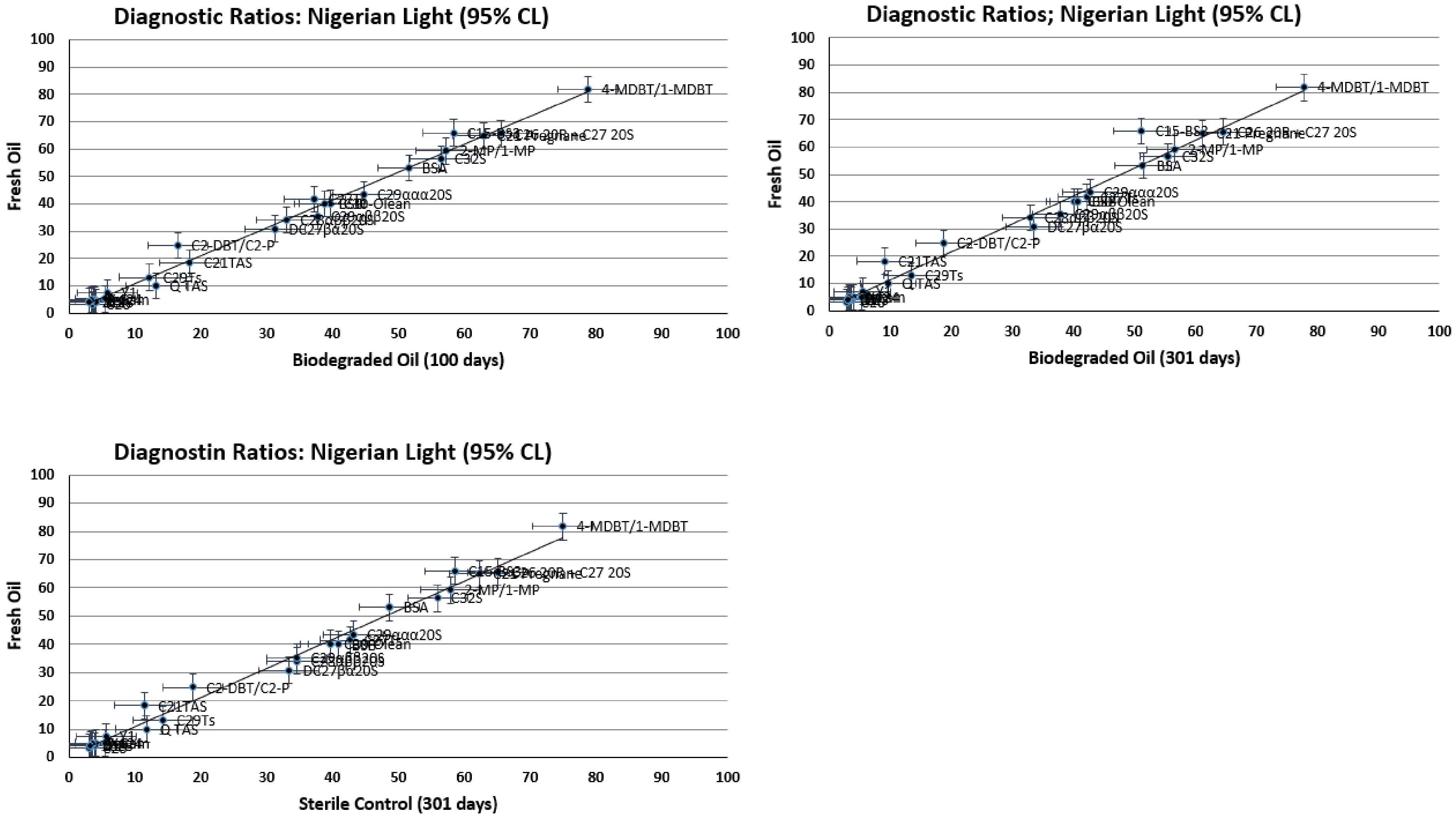


Figure 0.33: Diagnostic Ratio plots of fresh oil vs degraded oil Nigerian Light oil in Tyne sediment, degraded oil showing positive correlation with the fresh oil for 100 days, 301 days incubation and control using 95% confidence limit.

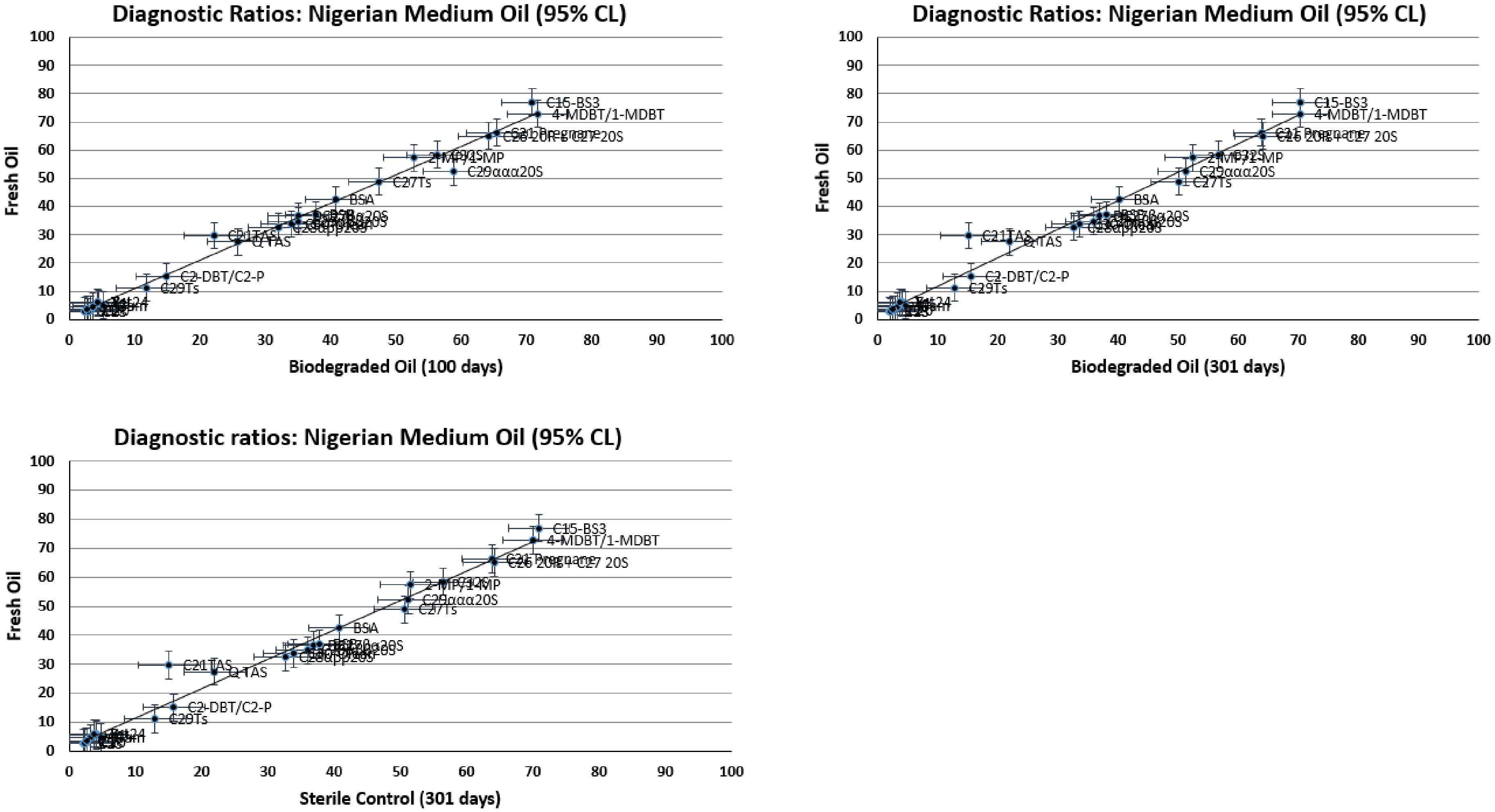


Figure 0.34: Diagnostic Ratio plots of fresh oil vs degraded oil Nigerian Medium oil in Tyne sediment, degraded oil showing positive correlation with the fresh oil for 100 days, 301 days incubation and control using 95% confidence limit.

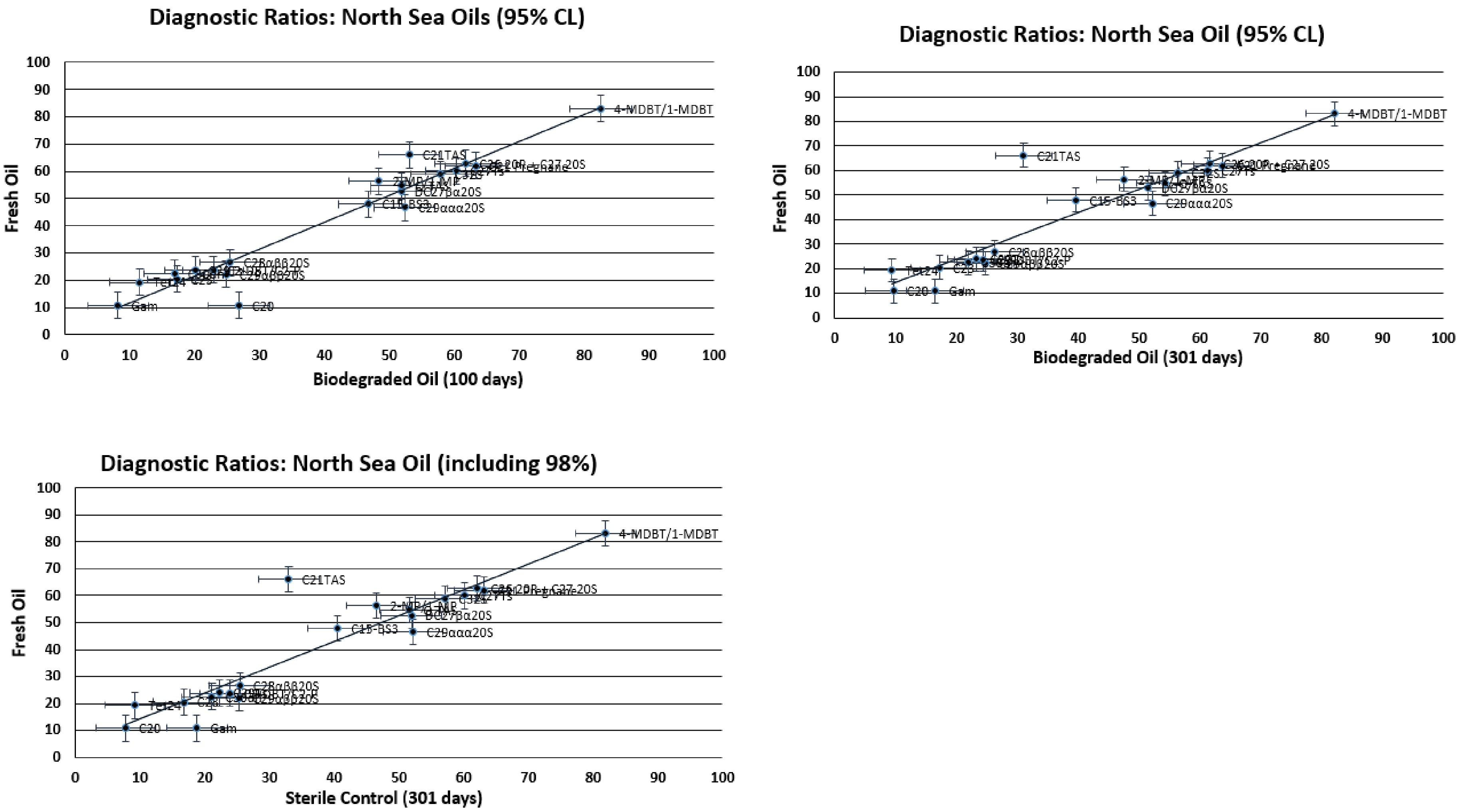


Figure 0.35: Diagnostic Ratio plots of fresh oil vs degraded oil North Sea oil in Tyne sediment degraded oil showing positive correlation with the fresh oil for 100 days, 301 days incubation and control using 95% confidence limit.

6.4.7 Conclusion

Methanogenic degradation showed no remarkable alterations on the compound compositions in the oils incubated under anaerobic conditions compared to aerobic biodegradation as expected. The occurrence of methanogenesis in the anaerobic microcosms resulted in the production of methane and CO_2 with the corresponding *n*-alkane removal. The degradation was assessed as a slight and ranked at PM level 2-3 where the *n*-alkane removal was slight in the *n*-C_8 to *n*-C_{39} range, PAHs, biomarkers and non-biomarker molecular markers were preserved compared to that of the aerobic biodegradation, the naphthalenes and adamantanes were the most affected in these compound classes.

The relative susceptibilities to biodegradation of some aliphatic hydrocarbons and PAH based on their relatively close elution order was assessed using their ratios shown in Figure 0.32 as discussed in chapter 5. The ratio plots are quite unaffected by methanogenic degradation compared to aerobic biodegradation which were very altered by microbial attack for all the three oil samples.

The diagnostic ratio plots of the selected Nordtest parameters generally show a positive correlation for the three oil samples.

Table 0.1: Changes in n-alkane and acyclic isoprenoid concentrations in biodegraded Nigerian light oil in Tyne sediment (mg/g oil).

n-Alkane	Start	100 days	301 days	Sterilised
***n*-C8**	4.05 (3.2)	0.21 (2.3)	0.14 (3.0)	0.25 (2.9)
***n*-C9**	7.31 (1.2)	0.44 (3.5)	0.12 (1.4)	0.13 (7.6)
***n*-C10**	8.97 (4.3)	0.48 (3.8)	0.30 (3.7)	0.37 (6.9)
***n*-C11**	9.44 (1.8)	0.64 (2.5)	0.48 (4.6)	0.55 (7.0)
***n*-C12**	8.77 (0.2)	0.72 (1.2)	0.44 (6.1)	0.51 (5.4)
***n*-C13**	7.81 (0.2)	1.08 (3.7)	0.52 (6.1)	0.65 (4.9)
***n*-C14**	7.17 (0.5)	1.37 (4.8)	0.76 (5.3)	0.83 (5.0)
***n*-C15**	6.54 (0.2)	1.58 (5.5)	1.16 (2.9)	1.26 (3.7)
***n*-C16**	5.32 (0.4)	1.50 (4.8)	1.30 (1.4)	1.52 (5.6)
***n*-C17**	4.62 (0.1)	1.50 (4.8)	1.30 (1.8)	1.59 (3.8)
Pristane	5.34 (0.1)	1.76 (3.5)	1.56 (2.9)	1.95 (3.4)
***n*-C18**	4.10 (3.8)	1.28 (5.7)	1.15 (3.7)	1.44 (2.7)
Phytane	1.75 (2.9)	0.51 (5.5)	0.51 (7.5)	0.64 (4.3)
***n*-C19**	3.55 (2.1)	1.25 (4.3)	1.08 (1.5)	1.35 (2.8)
***n*-C20**	3.26 (2.9)	1.15 (5.7)	0.99 (0.9)	1.29 (5.0)
***n*-C21**	3.27 (2.1)	1.17 (4.6)	1.01 (1.7)	1.29 (1.9)
***n*-C22**	3.46 (1.2)	1.07 (3.9)	0.92 (1.7)	1.18 (1.6)
***n*-C23**	3.12 (3.8)	1.02 (3.4)	0.88 (2.1)	1.13 (1.5)

n-C$_{24}$	2.59 (2.9)	0.94 (3.8)	0.81 (2.9)	1.05 (1.0)
n-C$_{25}$	2.81 (0.7)	0.91 (4.5)	0.79 (2.5)	1.02 (1.3)
n-C$_{26}$	2.17 (3.1)	0.75 (3.0)	0.67 (3.2)	0.87 (0.9)
n-C$_{27}$	2.07 (3.2)	0.72 (3.7)	0.65 (3.4)	0.84 (0.8)
n-C$_{28}$	1.71 (1.0)	0.55 (2.5)	0.53 (3.0)	0.68 (1.4)
n-C$_{29}$	1.68 (2.9)	0.52 (1.5)	0.51 (3.1)	0.65 (2.0)
n-C$_{30}$	1.09 (3.1)	0.33 (0.8)	0.33 (0.6)	0.35 (3.8)
n-C$_{31}$	1.24 (1.4)	0.32 (0.8)	0.32 (3.1)	0.44 (2.4)
n-C$_{32}$	0.68 (4.7)	0.23 (0.3)	0.17 (2.7)	0.22 (2.3)
n-C$_{33}$	0.63 (1.8)	0.17 (3.1)	0.14 (3.1)	0.18 (3.5)
n-C$_{34}$	0.24 (4.5)	0.05 (3.6)	0.05 (4.5)	0.06 (4.6)
n-C$_{35}$	0.16 (3.2)	0.04 (5.0)	0.03 (7.8)	0.04 (3.3)
n-C$_{36}$	0.09 (6.5)	0.04 (6.5)	0.02 (3.7)	0.04 (3.9)
n-C$_{37}$	0.06 (3.6)	0.03 (7.0)	0.02 (5.4)	0.03 (7.1)
n-C$_{38}$	0.03 (6.6)	0.02 (5.0)	0.02 (5.0)	0.03 (5.1)
n-C$_{39}$	0.02 (3.3)	0.02 (3.1)	0.01 (5.9)	0.02 (5.1)
Sum	115.12	24.38	19.71	24.45
n-C$_{17}$/pristane	0.86	0.85	0.84	0.81
n-C$_{18}$/phytane	2.35	2.52	2.23	2.24
pristane/phytane	3.06	3.46	3.03	3.05

CPI*	1.23	1.16	1.17	1.21
(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})	2.92	0.84	0.56	0.52

Note: The numbers in brackets after the numbers (concentrations) in the table are relative standard deviations (RSD) (n=3), similarly for Table 0.2Table 0.3

Table 0.2: Changes in n-alkane and acyclic isoprenoid concentrations in biodegraded Nigerian medium oil in Tyne sediment (mg/g oil).

***n*-Alkane**	**Start**	**100days**	**301days**	**Sterilised**
n-C_8	0.44 (4.9)	0.10 (2.3)	0.01 (3.6)	0.06 (4.3)
n-C_9	0.79 (0.5)	0.10 (1.2)	0.03 (5.1)	0.06 (3.5)
n-C_{10}	1.00 (4.9)	0.12 (3.9)	0.07 (3.3)	0.09 (3.8)
n-C_{11}	1.34 (2.7)	0.21 (2.0)	0.18 (4.2)	0.17 (5.3)
n-C_{12}	1.70 (1.0)	0.27 (5.7)	0.19 (6.8)	0.20 (2.7)
n-C_{13}	1.83 (1.1)	0.39 (7.4)	0.19 (3.8)	0.21 (7.4)
n-C_{14}	1.90 (3.2)	0.50 (5.9)	0.21 (0.7)	0.27 (5.2)
n-C_{15}	2.11 (4.3)	0.60 (6.6)	0.34 (2.3)	0.39 (5.7)
n-C_{16}	1.64 (7.5)	0.67 (5.4)	0.51 (7.2)	0.53 (4.3)
n-C_{17}	2.42 (3.8)	1.10 (7.3)	0.78 (5.5)	0.82 (5.4)
Pristane	5.36 (5.2)	2.42 (4.2)	1.88 (6.4)	1.91 (6.7)
n-C_{18}	2.63 (0.5)	1.19 (7.7)	0.83 (9.6)	0.87 (3.6)
Phytane	2.25 (4.6)	0.99 (1.4)	0.78 (4.4)	0.76 (6.5)
n-C_{19}	2.66 (3.6)	1.29 (2.9)	0.82 (5.5)	0.89 (8.2)

n-C$_{20}$	2.69 (2.6)	1.04 (0.8)	0.86 (5.0)	0.91 (3.7)
n-C$_{21}$	2.69 (2.6)	1.21 (2.8)	0.90 (4.2)	0.97 (5.4)
n-C$_{22}$	2.53 (2.5)	1.12 (1.0)	0.83 (3.5)	0.90 (5.6)
n-C$_{23}$	2.57 (1.9)	1.16 (5.2)	0.86 (1.8)	0.92 (5.0)
n-C$_{24}$	2.43 (5.1)	1.21 (6.6)	0.87 (1.7)	0.95 (5.3)
n-C$_{25}$	2.37 (0.5)	1.22 (2.7)	0.91 (3.4)	0.99 (4.6)
n-C$_{26}$	2.26 (2.4)	1.18 (6.1)	0.87 (3.6)	0.95 (4.5)
n-C$_{27}$	2.29 (1.6)	1.21 (4.7)	0.89 (2.8)	0.99 (4.7)
n-C$_{28}$	2.22 (3.5)	1.01 (5.5)	0.78 (6.9)	0.85 (3.9)
n-C$_{29}$	2.05 (4.4)	0.96 (4.9)	0.76 (7.4)	0.82 (4.1)
n-C$_{30}$	1.54 (8.9)	0.71 (6.7)	0.61 (3.9)	0.61 (3.5)
n-C$_{31}$	1.66 (1.4)	0.62 (4.1)	0.56 (6.9)	0.58 (4.7)
n-C$_{32}$	0.94 (1.0)	0.35 (5.0)	0.33 (7.3)	0.34 (6.5)
n-C$_{33}$	0.74 (0.5)	0.25 (2.7)	0.28 (6.8)	0.28 (3.8)
n-C$_{34}$	0.36 (0.8)	0.10 (3.7)	0.12 (5.5)	0.12 (4.9)
n-C$_{35}$	0.24 (1.8)	0.05 (0.9)	0.08 (6.3)	0.08 (6.5)
n-C$_{36}$	0.15 (2.1)	0.05 (6.8)	0.04 (3.6)	0.04 (7.4)
n-C$_{37}$	0.14 (0.8)	0.03 (4.2)	0.03 (4.2)	0.03 (4.3)
n-C$_{38}$	0.13 (0.9)	0.02 (2.3)	0.02 (6.2)	0.02 (3.0)
n-C$_{39}$	0.11 (0.4)	0.01 (2.4)	0.01 (3.1)	0.02 (8.0)

Sum	58.16	23.46	17.44	18.58
n-C_{17}/pristane	0.45	0.46	0.42	0.43
n-C_{18}/phytane	1.17	1.21	1.08	1.15
pristane/phytane	2.38	2.44	2.42	2.50
CPI*	1.11	1.11	1.12	1.13
(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})	0.53	0.22	0.14	0.17

Table 0.3: Changes in n-alkane and acyclic isoprenoid concentrations in biodegraded North Sea oil in Tyne sediment (mg/g oil).

***n*-Alkane**	**Start**	**100days**	**301days**	**Sterilised**
n-C_8	2.96 (5.6)	0.12 (3.2)	0.02 (2.8)	0.03 (4.8)
n-C_9	3.63 (4.9)	0.22 (1.7)	0.16 (3.1)	0.16 (3.7)
n-C_{10}	4.45 (3.1)	0.43 (2.4)	0.35 (6.7)	0.44 (3.4)
n-C_{11}	4.95 (3.2)	0.69 (0.8)	0.57 (2.3)	0.82 (2.2)
n-C_{12}	5.17 (5.3)	0.77 (5.0)	0.63 (5.5)	0.94 (4.7)
n-C_{13}	5.57 (2.9)	0.88 (2.8)	0.75 (6.7)	1.27 (6.4)
n-C_{14}	5.57 (4.5)	0.99 (4.0)	0.91 (5.6)	1.43 (6.6)
n-C_{15}	5.01 (4.4)	1.32 (6.0)	1.24 (6.6)	2.08 (5.7)
n-C_{16}	4.13 (1.0)	1.53 (8.8)	1.39 (6.5)	2.42 (3.1)
n-C_{17}	3.98 (3.2)	1.47 (3.5)	1.23 (6.3)	2.25 (2.5)
Pristane	2.02 (3.5)	0.43 (5.0)	0.64 (6.2)	1.22 (6.6)

***n*-C$_{18}$**	3.37 (1.7)	1.23 (3.4)	1.13 (3.4)	1.95 (5.7)
Phytane	1.58 (4.9)	0.33 (6.8)	0.58 (1.5)	0.96 (4.4)
***n*-C$_{19}$**	2.93 (5.1)	1.08 (2.3)	0.92 (6.4)	1.71 (1.9)
***n*-C$_{20}$**	2.53 (5.5)	0.98 (6.1)	0.85 (5.9)	1.65 (5.5)
***n*-C$_{21}$**	2.38 (3.4)	0.86 (1.9)	0.75 (6.7)	1.44 (3.2)
***n*-C$_{22}$**	2.24 (5.1)	0.72 (2.4)	0.70 (6.8)	1.33 (3.4)
***n*-C$_{23}$**	1.99 (5.2)	0.68 (1.6)	0.63 (5.8)	1.18 (4.9)
***n*-C$_{24}$**	1.82 (5.5)	0.64 (5.4)	0.58 (5.6)	1.15 (4.6)
***n*-C$_{25}$**	1.51 (5.6)	0.56 (2.4)	0.48 (6.7)	0.96 (6.5)
***n*-C$_{26}$**	1.30 (5.4)	0.50 (7.1)	0.41 (6.7)	0.81 (6.9)
***n*-C$_{27}$**	1.00 (4.8)	0.44 (2.3)	0.36 (5.8)	0.66 (6.1)
***n*-C$_{28}$**	0.68 (1.9)	0.37 (4.6)	0.26 (4.4)	0.48 (5.8)
***n*-C$_{29}$**	0.52 (0.9)	0.30 (6.4)	0.20 (3.5)	0.36 (4.1)
***n*-C$_{30}$**	0.36 (2.3)	0.15 (4.2)	0.14 (4.3)	0.19 (7.1)
***n*-C$_{31}$**	0.18 (3.8)	0.11 (1.1)	0.09 (5.1)	0.16 (7.6)
***n*-C$_{32}$**	0.10 (4.1)	0.10 (2.1)	0.07 (3.9)	0.08 (7.9)
***n*-C$_{33}$**	0.09 (2.0)	0.09 (5.3)	0.07 (1.9)	0.07 (3.8)
***n*-C$_{34}$**	0.10 (4.2)	0.08 (5.2)	0.06 (4.2)	0.07 (3.6)
***n*-C$_{35}$**	0.05 (5.6)	0.04 (1.6)	0.02 (7.4)	0.03 (2.5)
***n*-C$_{36}$**	0.05 (5.4)	0.02 (5.0)	0.02 (5.5)	0.03 (4.1)

n-C_{37}	0.04 (0.6)	0.02 (5.7)	0.01 (4.5)	0.02 (5.1)
n-C_{38}	0.03 (0.8)	0.02 (6.2)	0.01 (4.0)	0.02 (7.1)
n-C_{39}	0.03 (1.5)	0.01 (3.2)	0.01 (5.8)	0.01 (3.2)
Sum	72.32	18.19	16.26	28.40
n-C_{17}/pristane	1.98	3.41	1.93	1.85
n-C_{18}/phytane	2.14	3.71	1.94	2.04
pristane/phytane	1.28	1.30	1.10	1.27
CPI*	1.04	1.06	1.04	1.09
$(C_8+C_{10}+C_{12}+C_{14})/(C_{22}+C_{24}+C_{26}+C_{28})$	3.00	1.04	0.98	0.76

Table 0.4: Measured concentrations of target PAH in biodegraded Nigerian light oil (µg/g oil) and background concentration (µg/g EOM) in Tyne sediment.

PAH	Nigerian light oil (start)	100 days	30 1days	Sterilised 300 days	Sediment
C_0-N	247.3	241.9	5.4	6.7	1.4
C_1-N	1314.8	683.6	88.7	175.7	2.3
C_2-N	4680.0	905.8	312.3	392.8	4.7
C_3-N	1412.8	675.9	296.0	332.7	3.3
C_4-N	828.0	242.6	138.9	133.9	0.0
∑Naphthalenes	**8483.0**	**2749.8**	**841.3**	**1041.8**	**11.7**
C_0-B	344.5	29.0	9.3	14.8	0.7
C_1-B	968.4	74.4	27.5	33.8	1.0

C_2-B	1005.4	93.2	44.9	52.0	2.7
∑Biphenyls	**2318.3**	**196.6**	**81.8**	**100.5**	**4.3**
C_0-DBF	135.3	15.4	4.8	6.4	0.3
C_1-DBF	167.9	57.3	24.4	29.8	20.8
C_2-DBF	515.9	181.7	135.8	110.3	18.7
C_3-DBF	309.8	116.0	88.0	99.8	13.7
∑Dibenzofurans	**1128.9**	**370.3**	**252.9**	**246.3**	**53.5**
C_0-F	52.3	14.3	5.4	7.6	37.7
C_1-F	259.0	50.2	24.9	32.0	30.0
C_2-F	246.5	73.9	44.8	56.3	6.0
∑Fluorenes	**557.8**	**138.4**	**75.0**	**95.9**	**73.7**
C_0-DBT	122.8	35.6	20.2	24.7	1.8
C_1-DBT	180.2	76.6	35.4	46.3	18.8
C_2-DBT	169.4	53.4	18.0	19.7	20.0
C_3-DBT	140.6	33.0	11.6	13.0	11.5
∑Dibenzothiophenes	**612.9**	**198.6**	**85.2**	**103.8**	**52.2**
C_0-P	242.6	83.7	33.1	35.6	259.6
C_1-P	441.8	100.8	50.8	55.1	164.9
C_2-P	514.0	111.1	78.2	85.4	121.8
C_3-P	287.7	95.3	61.3	57.8	76.4

C_4-P	87.3	24.9	21.7	23.1	23.5
∑Phenanthrenes	**1573.4**	**415.7**	**245.2**	**257.0**	**646.2**
C_0-Py	9.3	37.7	16.6	18.2	284.5
C_1-Py	58.6	40.9	18.4	19.8	208.0
C_2-Py	52.2	20.9	11.7	13.3	87.3
∑Pyrenes	**120.1**	**99.4**	**46.6**	**51.3**	**579.8**
C_0-C	5.3	14.9	5.5	5.7	107.4
C_1-C	15.8	14.7	11.9	13.5	78.4
C_2-C	17.9	12.0	10.3	14.9	46.3
∑Chrysene	**39.1**	**41.6**	**27.7**	**34.1**	**232.1**
C_0-B(a)A	2.0	2.9	3.7	4.2	100.6
C_1-B(a)A	2.4	3.0	2.9	3.3	14.3
∑Benzo a anthracene	**4.4**	**5.9**	**6.6**	**7.5**	**114.8**
C_{20}-TAS	4.3	3.1	4.4	4.5	3.0
C_{21}-TAS	2.0	1.1	1.2	1.2	5.4
C_{26}R+C_{27}S	27.7	17.1	15.7	14.5	0.4
C_{28}S-TAS	23.3	16.0	15.3	16.2	0.4
C_{27}R-TAS	14.6	11.7	11.5	14.4	0.6
C_{28}R-TAS	19.7	14.9	17.0	18.8	0.3
∑TAS	**91.6**	**64.0**	**65.1**	**69.6**	**10.1**

Anthracene	11.06	2.5	1.2	1.7	0.0
Perylene	4.9	0.7	2.0	1.9	2.1
2-MN/1-MN	2.87	2.22	2.06	2.40	1.68
C_0-P/C_0-Anth	21.9	33.4	26.5	21.4	#DIV/0!

	Oil sample (start)	100 days	301 days	Sterilised	Sediment
4-MDBT	1.00	1.00	1.00	1.00	1.00
a, b, c, d, e-PMN	0.43	0.43	0.51	0.51	0.15
v, w, x, y, z-PMN	1.10	1.04	1.25	1.31	0.14

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, triaromatic steranes (TAS). Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). a, b, c, d, e-PMN and v, w, x, y, z-PMN are unidentified found in the two Nigerian oils. 0! represent result of values divided by 0 (undefined)

Table 0.5: Measured concentrations of target PAH in biodegraded Nigerian medium oil (µg/g) in Tyne sediment.

PAH	Nigerian medium oil (start)	100days	301days	Sterilised 300days
C_0-N	225.3	98.2	7.2	24.0
C_1-N	926.3	135.1	23.5	35.9
C_2-N	1733.9	471.3	165.5	186.6
C_3-N	1032.4	814.1	474.8	534.2
C_4-N	781.7	282.9	200.1	262.8

∑Naphthalenes	**4699.5**	**1801.5**	**871.0**	**1043.4**
C_0-B	174.2	55.8	23.1	36.5
C_1-B	239.0	57.1	33.0	40.1
C_2-B	321.8	61.2	36.3	49.0
∑Biphenyls	**734.9**	**174.1**	**92.4**	**125.6**
C_0-DBF	103.6	27.5	10.9	17.7
C_1-DBF	220.8	68.3	34.9	37.4
C_2-DBF	538.8	190.1	126.2	136.1
C_3-DBF	257.8	127.8	109.5	116.3
∑Dibenzofurans	**1120.9**	**413.7**	**281.6**	**307.5**
C_0-F	71.8	27.6	12.8	19.6
C_1-F	250.8	57.8	34.6	47.9
C_2-F	354.2	94.1	68.2	77.2
∑Fluorenes	**676.8**	**179.5**	**115.6**	**144.7**
C_0-DBT	164.2	43.0	30.1	34.8
C_1-DBT	183.2	34.5	33.7	39.5
C_2-DBT	168.4	32.3	29.0	34.4
C_3-DBT	129.5	20.5	21.0	25.7
∑Dibenzothiophenes	**645.3**	**130.4**	**113.9**	**134.4**
C_0-P	297.3	103.4	46.5	57.5

C_1-P	619.2	140.6	86.2	100.6
C_2-P	712.9	184.3	156.8	169.8
C_3-P	321.9	151.6	91.3	109.6
C_4-P	193.8	84.2	50.3	72.6
∑Phenanthrenes	**2145.2**	**664.1**	**431.0**	**510.1**
C_0-Py	19.9	37.5	20.7	15.3
C_1-Py	120.7	61.3	37.0	28.7
C_2-Py	101.0	37.5	30.1	23.7
∑Pyrenes	**241.7**	**136.3**	**87.8**	**67.6**
C_0-C	13.8	18.8	10.0	16.6
C_1-C	37.1	29.2	23.1	24.1
C_2-C	38.9	28.9	25.3	33.2
∑Chrysene	**89.8**	**76.9**	**58.5**	**73.9**
C_0-B(a)A	4.2	4.2	5.4	3.9
C_1-B(a)A	4.5	4.5	4.1	4.0
∑Benzo a anthracene	**8.7**	**8.7**	**9.6**	**7.8**
C_{20}-TAS	6.1	5.4	4.8	5.6
C_{21}-TAS	2.9	1.3	2.4	3.3
$C_{26}R+C_{27}S$	22.0	5.8	2.5	2.0
$C_{28}S$-TAS	20.8	15.5	12.4	15.9

C_{27}R-TAS	11.7	8.2	7.4	9.1
C_{28}R-TAS	17.7	14.6	12.0	15.6
∑TAS	**81.2**	**50.8**	**41.4**	**51.6**
Anthracene	9.58	3.3	2.0	2.0
Perylene	2.6	0.9	3.2	2.3
2-MN/1-MN	2.87	2.13	1.88	2.03
C_0-P/C_0-Anth	31.0	31.7	23.0	28.5

PAH	Oil sample (start)	100days	301days	Sterilised
4-MDBT	1.00	1.00	1.00	1.00
a, b, c, d, e-PMN	0.51	0.52	0.58	0.59
v, w, x, y, z-PMN	1.39	1.25	1.51	1.50

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, triaromatic steranes (TAS). Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) up to pentamethylnaphthalenes with 5 alkyl carbon (C_5-N). a, b, c, d, e-PMN and v, w, x, y, z-PMN are unidentified found in the two Nigerian oils. 0! represent result of values divided by 0 (undefined)

Table 0.6: Measured concentrations of target PAH in biodegraded North Sea oil (µg/g) in Tyne sediment.

PAH	North Sea oil (start)	100days	301days	Sterilised 300days
C_0-N	581.4	234.2	28.3	45.6

C_1-N	799.1	653.4	48.3	62.5
C_2-N	1136.8	877.5	358.7	487.1
C_3-N	857.8	494.2	349.4	388.1
C_4-N	470.6	111.5	95.2	107.0
∑Naphthalenes	**3845.7**	**2370.8**	**879.9**	**1090.3**
C_0-B	413.6	49.8	27.8	31.5
C_1-B	572.8	74.8	48.7	58.7
C_2-B	318.8	60.0	43.9	48.6
∑Biphenyls	**1305.3**	**184.6**	**120.4**	**138.7**
C_0-DBF	63.3	13.2	8.0	16.0
C_1-DBF	194.3	44.2	30.7	33.6
C_2-DBF	360.3	112.9	96.8	103.9
C_3-DBF	161.9	79.9	53.2	61.2
∑Dibenzofurans	**779.8**	**250.2**	**188.7**	**214.7**
C_0-F	78.4	41.6	28.2	36.4
C_1-F	426.0	99.6	88.7	86.5
C_2-F	398.0	79.4	70.7	72.8
∑Fluorenes	**902.4**	**220.7**	**187.6**	**195.7**
C_0-DBT	98.7	28.3	15.5	21.7
C_1-DBT	112.1	77.9	52.8	55.4

C_2-DBT	293.7	109.3	92.0	98.8
C_3-DBT	163.4	62.9	49.8	58.4
∑Dibenzothiophenes	**667.9**	**278.4**	**210.1**	**234.2**
C_0-P	500.6	171.1	131.0	159.4
C_1-P	769.6	264.1	243.6	259.3
C_2-P	943.6	299.6	285.2	224.4
C_3-P	623.4	201.4	183.4	199.6
C_4-P	165.4	72.1	44.1	56.0
∑Phenanthrenes	**3002.6**	**1008.3**	**887.2**	**898.7**
C_0-Py	55.2	27.3	31.8	22.8
C_1-Py	224.2	52.7	59.0	25.8
C_2-Py	181.2	31.6	40.6	31.2
∑Pyrenes	**460.6**	**111.5**	**131.5**	**79.7**
C_0-C	96.8	44.0	27.7	30.0
C_1-C	164.1	68.7	46.6	54.5
C_2-C	123.5	48.6	38.2	43.1
∑Chrysene	**384.5**	**161.3**	**112.4**	**127.6**
C_0-B(a)A	13.2	6.6	7.8	5.0
C_1-B(a)A	10.4	2.9	3.5	3.6
∑Benzo a anthracene	**23.6**	**9.5**	**11.3**	**8.7**

C_{20}-TAS	12.9	3.9	2.7	2.5
C_{21}-TAS	2.5	0.8	0.5	0.5
C_{26}R+C_{27}S	11.5	7.0	8.3	9.3
C_{28}S-TAS	7.6	6.4	7.9	7.9
C_{27}R-TAS	6.9	5.4	4.9	5.9
C_{28}R-TAS	6.6	5.3	5.9	5.8
∑TAS	**48.0**	**28.9**	**30.4**	**31.8**
Anthracene	16.45	1.9	1.6	1.8
Perylene	13.4	0.7	2.4	1.7
2-MN/1-MN	1.34	1.57	1.60	1.98
C_0-P/C_0-Anth	30.4	91.5	81.6	87.4

Note: N, B, DBF, F, DBT, P, Py, C, B(a)A, f-MDBT, g-MDBT represent naphthalene, biphenyl, dibenzofuran, fluorene, dibenzothiophene, phenanthrene, pyrene, chrysene, triaromatic steranes (TAS). Numbers (0,1,2,3, and 4) preceding the PAH abbreviations represent the number of alkylation. A parent PAH e.g., naphthalene (N) has 0 alkyl carbon (C_0-N), whereas a daughter PAH e.g., methylnaphthalene has 1 alkyl carbon (C_1-N), dimethylnaphthalenes has 2 alkyl carbon (C_2-N) etc.

Table 0.7: Measured concentrations (µg/g oil) of terpanes in degraded Nigerian light oil in Tyne sediment

	Terpanes				**Measured Concentration (C_{det}) (m/z 191)**		
Peak Number	**Tricyclic Terpanes**	**Code**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**S**	TRR	33.6	429.5	329.4	243.9	212.4
2	**Q**	TRQ	33.3	114.0	107.6	75.6	70.6

3	**C_{19}**	TR19	51.3	330.0	229.7	180.6	177.5
4	**M**	TRM	14.2	118.5	108.2	86.4	81.6
5	**C_{20}**	TR20	34.1	295.9	301.5	203.1	190.8
6	**X**	TRX	74.7	462.1	320.1	279.0	265.4
7	**C_{21}**	TR21	40.6	284.0	228.9	191.8	165.1
8	**C_{22}**	TR22	31.0	172.7	115.3	101.2	101.9
9	**C_{23}**	TR23	233.9	368.4	346.5	252.4	252.5
10	**C_{24}**	TR24	134.1	391.0	318.9	238.8	224.3
11	**Y**	TRY	190.0	415.3	290.5	237.4	227.2
12	**Y1**	TRY1	208.0	684.0	512.4	391.8	363.3
13	**C_{25}A**	TR25A	48.3	105.0	36.9	106.0	89.8
14	**C_{25}B**	TR25B	49.0	392.2	317.6	248.4	244.1
15	**Z**	TRZ	10.8	184.3	138.8	115.2	101.5
16	**Tet24**	TET24	123.0	446.4	291.8	224.5	221.7
17	**C_{26}(S)**	TR26A	63.9	56.2	144.8	112.7	107.5
18	**C_{26}(R)**	TR26B	63.9	181.6	145.6	134.7	117.2

			Measured Concentration (C_{det}) (m/z 191)				
	Tetracyclic Terpanes	**Code**	**Background (µg/g EOM)**	**Start**	**100days**	**301days**	**Sterilised(301 days)**
19	**Z1**	**TRZ**	13.6	387.1	254.3	214.1	205.6

20	**C_{28}A**	**TR28A**	74.8	274.9	195.6	201.1	243.3
21	**C_{28}B**	**TR28B**	63.8	135.1	139.2	133.3	137.5
22	**C_{29}A**	**TR29A**	74.3	198.0	177.7	152.9	145.7
23	**C_{29}B**	**TR29B**	60.4	276.4	211.1	182.5	199.3
24	**T**	**TRT**	77.8	203.7	170.3	136.0	139.9

			Measured Concentration (C_{det}) (m/z 191)				
	Pentacyclic Terpanes	**Code**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
25	**C_{27}Ts**	**Ts**	211.3	977.8	889.7	737.6	680.5
26	**C_{27}Tm**	**Tm**	631.6	1374.9	1495.4	1013.3	913.8
27	**C_{29}nor25H**	**Nor25H**	85.6	1022.8	686.3	568.4	449.2
28	**C_{29}Tm**	**C29Tm**	1420.4	6236.0	5773.9	4649.9	4172.1
29	**C_{29}Ts**	**C29Ts**	231.9	1317.8	1143.8	1064.1	1024.8
30	**C_{30}: 18α-Oleanane**	**OL**	0.0	5867.9	5396.5	4600.4	4049.8
31	**$C_{30}\alpha\beta$**	**H30**	1383.0	8748.1	8218.3	6859.2	6154.5
32	**C_{30}-Mor**	**M30**	342.0	1561.4	1466.4	1266.3	1165.4
33	**C_{31}S**	**H31S**	680.4	1760.3	1919.1	1592.6	1461.8
34	**C_{31}R**	**H31R**	625.3	1569.7	1564.6	1305.0	1189.5
35	**Gam**	**Gam**	238.0	472.6	480.2	382.8	359.7
36	**C_{32}S**	**H32S**	425.0	1067.8	1071.0	964.3	873.6

37	**$C_{32}R$**	**H32R**	314.8	825.0	825.1	771.5	688.4
38	**$C_{33}S$**	**H33S**	331.1	670.4	740.5	623.9	590.7
39	**$C_{33}R$**	**H33R**	183.7	645.4	515.0	455.2	452.0
40	**$C_{34}S$**	**H34S**	157.5	382.2	366.8	352.6	367.9
41	**$C_{34}R$**	**H34R**	107.0	309.9	252.8	255.7	267.3
42	**$C_{35}S$**	**H35S**	88.0	229.3	237.8	214.0	151.7
43	**$C_{35}R$**	**H35R**	146.5	232.1	143.3	142.9	187.5
	Total (μg/g oil)		**9404.9**	**42177.6**	**38619.4**	**32263.4**	**25200.2**

Table 0.8: Measured concentrations (μg/g oil) of terpanes in biodegraded Nigerian medium oil in Tyne sediment.

	Terpanes		**Measured Concentration (C_{det}) (m/z 191)**				
Peak Number	**Tricyclic Terpanes**	**Code**	**Background (μg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised(301 days)**
1	**S**	TRR	33.6	303.3	275.4	227.3	229.7
2	**Q**	TRQ	33.3	120.6	112.0	83.3	81.7
3	**C_{19}**	TR19	51.3	379.6	350.8	268.4	252.1
4	**M**	TRM	14.2	124.2	126.9	138.6	135.4

5	**C_{20}**	TR20	34.1	344.3	340.1	316.0	292.3
6	**X**	TRX	74.7	651.5	489.1	456.7	443.2
7	**C_{21}**	TR21	40.6	226.8	184.7	169.7	161.5
8	**C_{22}**	TR22	31.0	280.1	196.0	207.2	200.9
9	**C_{23}**	TR23	233.9	294.5	301.8	245.3	247.6
10	**C_{24}**	TR24	134.1	304.8	273.1	254.8	247.3
11	**Y**	TRY	190.0	498.2	386.8	367.9	345.8
12	**Y1**	TRY1	208.0	617.7	461.9	455.8	451.2
13	**C_{25}A**	TR25A	48.3	93.3	47.6	79.8	83.7
14	**C_{25}B**	TR25B	49.0	252.7	228.7	208.0	212.1
15	**Z**	TRZ	10.8	85.1	78.9	83.4	85.4
16	**Tet24**	TET24	123.0	669.0	444.8	427.6	415.3
17	**C_{26}(S)**	TR26A	63.9	57.6	127.9	115.8	116.2
18	**C_{26}(R)**	TR26B	63.9	128.8	119.9	121.2	124.2

			Measured Concentration (C_{det}) (m/z 191)				
	Tetracyclic Terpanes	**Code**	**Background (µg/g EOM)**	**Start**	**100days**	**301 days**	**Sterilised (301 days)**
19	**Z1**	**TRZ**	13.6	366.3	307.2	288.6	280.3
20	**C_{28}A**	**TR28A**	74.8	229.6	206.8	325.3	332.8
21	**C_{28}B**	**TR28B**	63.8	163.5	119.5	123.4	130.0

22	**C_{29}A**	**TR29A**	74.3	132.6	152.2	161.9	149.7
23	**C_{29}B**	**TR29B**	60.4	315.8	313.4	354.8	342.6
24	**T**	**TRT**	77.8	121.6	123.3	127.5	126.3

			Measured Concentration (C_{det}) (m/z 191)				
	Pentacyclic Terpanes	**Code**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
25	**C_{27}Ts**	**Ts**	211.3	1791.7	1811.3	1770.8	1712.4
26	**C_{27}Tm**	**Tm**	631.6	1875.8	1886.0	1760.1	1667.1
27	**C_{29}nor25H**	**Nor25H**	85.6	945.9	914.9	856.4	861.1
28	**C_{29}Tm**	**C29Tm**	1420.4	7037.1	7304.2	6958.1	6808.7
29	**C_{29}Ts**	**C29Ts**	231.9	1288.1	1471.4	1601.9	1555.1
30	**C_{30}: 18α-Oleanane**	**OL**	0.0	5254.3	5631.7	5516.7	5356.8
31	**$C_{30}\alpha\beta$**	**H30**	1383.0	10284.7	10929.7	10876.3	10421.6
32	**C_{30}-Mor**	**M30**	342.0	1553.5	1692.0	1746.6	1714.1
33	**C_{31}S**	**H31S**	680.4	2135.8	2451.9	2507.6	2412.8
34	**C_{31}R**	**H31R**	625.3	1678.7	1937.9	1989.1	1927.5
35	**Gam**	**Gam**	238.0	526.6	597.8	530.3	517.6
36	**C_{32}S**	**H32S**	425.0	1392.2	1427.1	1581.6	1452.7
37	**C_{32}R**	**H32R**	314.8	992.7	1105.0	1204.7	1119.4
38	**C_{33}S**	**H33S**	331.1	688.0	810.3	870.9	859.5

39	**C_{33}R**	**H33R**	183.7	505.3	560.9	618.0	580.8
40	**C_{34}S**	**H34S**	157.5	360.2	400.8	477.7	476.2
41	**C_{34}R**	**H34R**	107.0	300.1	265.5	278.4	295.0
42	**C_{35}S**	**H35S**	88.0	224.8	134.8	176.4	162.6
43	**C_{35}R**	**H35R**	146.5	213.1	224.0	212.8	195.3
	Total (µg/g oil)		**9404.9**	**45810.1**	**47325.9**	**47142.8**	**40096.4**

Table 0.9: Measured concentrations (µg/g oil) of terpanes in biodegraded North Sea oil in Tyne sediment.

Terpanes			**Measured Concentration (C_{det}) (m/z 191)**				
Peak Number	**Tricyclic Terpanes**	**Code**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**C_{19}**	TR19	51.3	55.8	61.8	70.8	85.4
2	**C_{20}**	TR20	34.1	40.8	224.8	69.4	72.7
3	**C_{21}**	TR21	40.6	81.3	96.5	94.3	127.7
4	**C_{22}**	TR22	31.0	34.3	26.8	33.6	46.4
5	**U**	TRU	0.0	29.3	45.5	46.2	49.1
6	**C_{23}**	TR23	233.9	86.6	129.1	135.1	173.6
7	**C_{24}**	TR24	134.1	83.3	105.9	115.1	144.9

8	C_{25}B	TR25B	49.0	89.1	116.8	140.8	161.1
9	Tet24	TET24	123.0	80.5	80.4	67.7	87.9
10	C_{26}(S)	TR26A	63.9	37.3	31.3	48.0	59.7
11	C_{26}(R)	TR26B	63.9	54.5	48.0	52.5	69.3

			Measured Concentration (C_{det}) (m/z 191)				
	Tetracyclic Terpanes	Code	Background (µg/g EOM)	Start	100 days	301 days	Sterilised (301 days)
12	U1	TRU1	0.0	41.0	51.0	55.1	66.7
13	U2	TRU2	0.0	23.7	28.0	30.2	56.3
14	C_{28}A	TR28A	74.8	34.4	47.6	73.7	86.7
15	C_{28}B	TR28B	63.8	32.5	54.1	55.0	75.9
16	C_{29}A	TR29A	74.3	46.4	60.4	81.8	95.8
17	C_{29}B	TR29B	60.4	38.7	63.9	70.3	89.5
18	T	TRT	77.8	48.8	65.4	87.1	110.1

			Measured Concentration (C_{det}) (m/z 191)				
	Pentacyclic Terpanes	Code	Background (µg/g EOM)	Start	100 days	301 days	Sterilised (301 days)
19	C_{27}Ts	Ts	211.3	149.8	202.0	204.6	263.5
20	C27Tm	Tm	631.6	99.7	132.6	129.5	174.8
21	C_{29}nor25H	Nor25H	85.6	30.5	72.7	94.1	136.9
22	C_{29}Tm	C29Tm	1420.4	145.1	342.8	351.2	448.4

23	**C_{29}Ts**	**C29Ts**	231.9	105.6	154.1	197.8	248.0
24	**C_{30}dh**	**H30dh**	0.0	97.8	124.6	181.8	231.2
25	**C_{30}-Hopane**	**H30**	1383.0	337.0	613.2	651.0	862.7
26	**C_{30}-Mor**	**M30**	342.0	58.9	96.0	125.0	177.8
27	**C_{31}S**	**H31S**	680.4	177.0	285.7	335.4	442.6
28	**C_{31}R**	**H31R**	625.3	128.8	233.4	293.7	382.8
29	**Gam**	**Gam**	238.0	40.9	54.9	129.6	200.3
30	**C_{32}S**	**H32S**	425.0	155.9	213.0	278.7	346.5
31	**C_{32}R**	**H32R**	314.8	108.8	154.6	216.2	259.6
32	**C_{33}S**	**H33S**	331.1	112.2	161.1	217.6	293.8
33	**C_{33}R**	**H33R**	183.7	78.3	108.8	142.7	202.4
34	**C_{34}S**	**H34S**	157.5	68.4	100.4	160.7	197.8
35	**C_{34}R**	**H34R**	107.0	42.8	62.1	108.1	132.4
36	**C_{35}S**	**H35S**	88.0	80.1	103.7	167.5	200.0
37	**C_{35}R**	**H35R**	146.5	48.6	59.0	87.9	141.3
	Total (µg/g oil)		**8778.5**	**3004.9**	**4612.1**	**5399.6**	**7001.7**

Table 0.10: Measured steranes concentrations (µg/g oil) in degraded Nigerian light oil in Tyne sediment.

		Determined Concentration (C_{det}) -(M/Z 217)				
Peak Number	**Steranes**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**

1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	32.0	101.8	92.7	86.2	84.9
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	87.6	189.2	188.8	166.9	159.6
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	50.2	101.9	111.5	106.4	97.0
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	123.7	205.5	222.9	203.8	195.5
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	73.9	132.1	138.9	128.3	120.1
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	41.3	83.6	79.6	71.1	64.3
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	46.6	94.9	93.6	95.0	92.4
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	85.8	186.0	185.9	164.6	160.7
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	132.9	370.4	424.9	350.3	337.7
10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	224.9	468.5	496.6	409.9	398.7
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	133.2	289.6	298.2	250.0	230.0
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	125.2	630.7	608.8	493.3	479.0
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	198.2	468.0	485.2	426.5	400.8
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	101.3	221.2	233.2	179.0	186.2
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	149.7	455.5	462.6	387.9	380.7
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	197.8	494.1	516.6	426.0	411.2
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	142.7	355.7	380.2	324.2	299.6
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	128.5	282.9	313.0	254.2	262.6
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	115.4	604.1	602.4	506.8	482.3
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	196.2	688.2	721.9	620.1	584.6
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	215.0	581.3	665.4	572.8	510.6

22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	152.0	298.7	358.3	289.7	291.7
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	197.1	894.8	891.8	830.0	770.0
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	54.4	142.4	148.1	96.3	83.1
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	11.4	65.0	55.5	76.7	72.0
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	20.8	170.8	80.0	148.5	126.2
	Total (µg/g oil)	**3037.8**	**8576.9**	**8856.3**	**7664.6**	**7281.5**

Table 0.11: Measured steranes concentrations (µg/g oil) in degraded Nigerian medium oil in Tyne sediment.

		Determined Concentration (C_{det}) -(M/Z 217)				
Peak Number	**Steranes**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**C_{20}-5α(H), 14α(H), 17α(H)-sterane**	32.0	91.7	96.5	101.3	88.4
2	**C_{21}-5α(H), 14β(H), 17β(H)-sterane**	87.6	151.1	159.1	158.9	166.7
3	**C_{22}-5α(H), 14β(H), 17β(H)-sterane**	50.2	77.2	85.5	90.0	94.3
4	**C_{27} 20S-13β(H), 17α(H)-diasterane**	123.7	173.2	208.4	190.4	191.6
5	**C_{27} 20R-13β(H), 17α(H)-diasterane**	73.9	95.3	123.9	116.9	123.1
6	**C_{27} 20S-13α(H), 17β(H)-diasterane**	41.3	45.3	60.2	58.0	53.5
7	**C_{27} 20R-13α(H), 17β(H)-diasterane**	46.6	66.9	84.4	84.9	91.0
8	**C_{28} 20S-13β(H), 17α(H)-diasterane**	85.8	120.2	153.8	146.5	149.6
9	**C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane**	132.9	171.9	228.0	198.7	208.0

10	**C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane**	224.9	308.9	400.5	356.4	350.4
11	**C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane**	133.2	154.6	212.5	194.3	187.2
12	**C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane**	125.2	210.9	274.9	242.6	253.9
13	**C_{29} 20S-13β(H), 17α(H)-diasterane**	198.2	257.7	328.1	315.3	311.5
14	**C_{30} 20S-13β(H), 17α(H)-diasterane**	101.3	199.6	241.1	234.9	223.0
15	**C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane**	149.7	238.3	285.1	272.4	266.1
16	**C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane**	197.8	270.9	356.6	329.0	323.2
17	**C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane**	142.7	192.8	243.7	239.4	235.9
18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	128.5	147.1	207.1	192.9	184.1
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	115.4	205.3	269.9	258.3	261.4
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	196.2	470.7	564.4	570.8	551.1
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	215.0	321.9	418.2	395.9	406.6
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	152.0	172.7	238.9	214.3	210.0
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	197.1	285.1	395.3	533.5	524.5
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	54.4	108.1	140.7	97.3	105.1
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	11.4	36.1	50.2	68.0	63.8
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	20.8	109.1	132.1	113.0	120.7
	Total (µg/g oil)	**3037.8**	**4682.3**	**5958.8**	**5773.9**	**5744.6**

Table 0.12: Measured steranes concentrations (µg/g oil) in degraded North Sea oil in Tyne sediment.

Peak Number	Steranes	Determined Concentration (C_{det}) -(M/Z 217)				
		Background (µg/g EOM)	Start	100 days	301 days	Sterilised (301 days)
1	C_{20}-5α(H), 14α(H), 17α(H)-sterane	32.0	126.0	145.2	185.6	168.3
2	C_{21}-5α(H), 14β(H), 17β(H)-sterane	87.6	128.9	172.3	225.1	203.5
3	C_{22}-5α(H), 14β(H), 17β(H)-sterane	50.2	78.9	99.9	128.0	118.7
4	C_{27} 20S-13β(H), 17α(H)-diasterane	123.7	309.2	361.0	460.7	407.1
5	C_{27} 20R-13β(H), 17α(H)-diasterane	73.9	219.9	216.5	272.8	248.7
6	C_{27} 20S-13α(H), 17β(H)-diasterane	41.3	101.8	115.8	157.2	129.5
7	C_{27} 20R-13α(H), 17β(H)-diasterane	46.6	103.9	115.0	160.6	134.8
8	C_{28} 20S-13β(H), 17α(H)-diasterane	85.8	166.2	192.8	254.0	224.4
9	C_{27} 20S-5α(H), 14α(H), 17α(H)-cholestane	132.9	109.5	123.2	159.2	139.4
10	C_{27} 20R-5α(H), 14β(H), 17β(H)-cholestane	224.9	289.1	361.2	455.3	399.3
11	C_{27} 20S-5α(H), 14β(H), 17β(H)-cholestane	133.2	184.8	176.0	236.8	207.5
12	C_{27} 20R-5α(H), 14α(H), 17α(H)-cholestane	125.2	113.1	117.7	161.0	130.1
13	C_{29} 20S-13β(H), 17α(H)-diasterane	198.2	228.4	312.3	406.6	362.9
14	C_{30} 20S-13β(H), 17α(H)-diasterane	101.3	145.9	173.6	234.1	196.8
15	C_{28} 20S-5α(H), 14α(H), 17α(H)-ergostane	149.7	86.5	93.2	128.1	116.2
16	C_{28} 20R-5α(H), 14β(H), 17β(H)-ergostane	197.8	102.6	110.2	147.0	120.6
17	C_{28} 20S-5α(H), 14β(H), 17β(H)-ergostane	142.7	143.1	166.1	225.0	193.0

18	**C_{29} 20R-13α(H), 17β(H)-diasterane**	128.5	105.4	115.5	157.7	143.2
19	**C_{28} 20R-5α(H), 14α(H), 17α(H)-ergostane**	115.4	78.3	83.5	120.8	104.5
20	**C_{29} 20S-5α(H), 14α(H), 17α(H)-stigmastane**	196.2	90.1	127.7	187.0	154.2
21	**C_{29} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	215.0	124.7	169.1	209.2	184.7
22	**C_{29} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	152.0	99.5	99.7	139.9	128.0
23	**C_{29} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	197.1	103.1	116.3	170.9	141.2
24	**C_{30} 20R-5α(H), 14β(H), 17β(H)-stigmastane**	54.4	73.7	91.7	137.8	109.0
25	**C_{30} 20S-5α(H), 14β(H), 17β(H)-stigmastane**	11.4	10.4	9.4	27.7	23.9
26	**C_{30} 20R-5α(H), 14α(H), 17α(H)-stigmastane**	20.8	23.6	26.0	43.2	37.4
	Total (µg/g oil)	**3037.8**	**3346.6**	**3890.8**	**5191.4**	**4526.8**

Table 0.13: Measured triaromatic steranes concentrations (µg/g oil) in degraded Nigerian light oil in Tyne sediment.

		Determined Concentration (C_{det}) -(M/Z 231)				
Peak Number	**Triaromatic Steranes (TAS)**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**P**	34.1	116.7	237.6	1387.2	1406.5
2	**Q**	43.6	55.9	122.7	674.5	772.5
3	**C20-TAS**	45.6	92.4	201.3	1166.3	1241.9
4	**C21-TAS**	20.6	42.8	92.2	531.9	631.9
5	**C22A-TAS**	15.6	25.8	94.4	322.3	361.8

6	**C22B-TAS**	18.2	30.5	81.2	497.5	415.5
7	**R**	7.9	24.5	47.3	267.5	266.3
8	**S**	8.8	15.3	40.9	190.5	187.4
9	**T**	4.9	40.4	94.9	484.0	465.8
10	**C26(20S) TA-cholestane**	13.6	206.7	423.9	2494.0	2340.2
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	19.7	596.3	1249.5	7302.9	6850.0
12	**U**	5.9	44.0	99.8	585.2	600.5
13	**C28 (20S) TA-stigmastane**	21.3	503.0	1051.2	6308.3	5868.4
14	**C27(20R) TA-ergostane**	6.6	313.0	656.2	4001.0	3670.4
15	**C28 (20R) TA-stigmastane**	14.0	413.4	870.9	5338.4	4903.9
16	**V**	8.9	85.4	181.1	1088.5	1007.0
	Total (µg/g oil)	289.4	2606.1	5545.1	32640.1	30990.1

Table 0.14: Measured triaromatic steranes concentrations (µg/g oil) in degraded Nigerian medium oil in Tyne sediment.

		Determined Concentration (C_{det}) -(M/Z 231)				
Peak Number	**Triaromatic Steranes (TAS)**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**P**	34.1	193.4	416.1	2041.7	2209.7
2	**Q**	43.6	131.6	339.4	1433.8	1366.6
3	**C20-TAS**	45.6	125.2	255.8	1600.4	1638.2

4	**C21-TAS**	20.6	59.4	125.7	778.0	744.0
5	**C22A-TAS**	15.6	45.5	140.3	349.2	333.5
6	**C22B-TAS**	18.2	21.0	70.6	265.2	269.8
7	**R**	7.9	13.9	30.0	153.1	182.9
8	**S**	8.8	11.7	26.1	135.9	129.3
9	**T**	4.9	32.5	77.1	370.5	352.9
10	**C26(20S) TA-cholestane**	13.6	132.7	311.2	1619.8	1585.7
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	19.7	449.8	1029.7	5153.3	5186.2
12	**U**	5.9	40.9	93.4	467.2	456.9
13	**C28 (20S) TA-stigmastane**	21.3	428.7	977.0	4999.7	4868.7
14	**C27(20R) TA-ergostane**	6.6	242.3	573.6	2990.7	2898.5
15	**C28 (20R) TA-stigmastane**	14.0	362.6	813.4	4243.4	4149.8
16	**V**	8.9	112.1	245.4	1262.6	1199.2
	Total (µg/g oil)	289.4	2403.2	5524.8	27864.4	27571.9

Table 0.15: Measured triaromatic steranes concentrations (µg/g oil) in degraded North Sea oil in Tyne sediment.

		Determined Concentration (C_{det}) -(M/Z 231)				
Peak Number	**Triaromatic Steranes (TAS)**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**P**	34.1	306.5	1606.7	3710.2	2975.5

2	**Q**	43.6	223.5	1147.7	2448.7	1880.5
3	**C20-TAS**	45.6	308.8	1683.3	3701.6	2995.5
4	**C21-TAS**	20.6	60.9	345.8	830.6	751.4
5	**C22A-TAS**	15.6	39.9	380.1	590.3	461.4
6	**C22B-TAS**	18.2	34.3	450.4	497.9	337.0
7	**R**	7.9	7.5	60.3	62.1	93.9
8	**S**	8.8	12.1	71.9	128.3	105.1
9	**T**	4.9	23.4	129.4	240.9	203.6
10	**C26(20S) TA-cholestane**	13.6	77.6	407.5	858.2	712.4
11	**C26 (20R) cholestane + C27(20S) TA-ergostane**	19.7	280.1	2512.2	3062.6	2549.9
12	**U**	5.9	18.7	115.8	205.0	161.0
13	**C28 (20S) TA-stigmastane**	21.3	185.6	1689.0	2063.7	1760.8
14	**C27(20R) TA-ergostane**	6.6	165.9	933.4	1905.6	1555.4
15	**C28 (20R) TA-stigmastane**	14.0	158.2	902.5	1845.9	1523.9
16	**V**	8.9	36.9	273.6	424.3	406.7
	Total (µg/g oil)	289.4	1939.9	12709.7	22575.9	18473.8

Table 0.16: Measured concentrations (µg/g oil) of bicyclic sesquiterpanes in degraded Nigerian light oil in Tyne sediment.

			Determined Concentration (C_{det}) (m/z 123)				
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (µg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**C_4-decalin (C14)**	**BS1**	43.3	3019.4	1747.1	848.9	1066.2

2	**C_{14} Sesquterpane**	**BS2**	26.1	1425.5	898.3	409.3	548.6
A	**A Sesquiterpane**	**BSA**	23.1	8270.3	4622.3	2141.0	2684.2
U2	**U2 Sesquiterpane**	**BSU2**	16.8	2459.5	1419.1	585.1	665.5
3	**C_{15} Sesquiterpane**	**BS3**	73.7	7314.7	4344.2	2025.0	2828.9
Y	**Y Sesquiterpane**	**BSY**	31.6	1452.2	786.4	375.8	510.9
B	**B Sesquiterpane**	**BSB**	15.5	3052.0	1965.9	1011.9	1276.0
4	**C_{15} Sesquiterpane**	**BS4**	46.1	4586.7	3092.0	1470.4	1846.8
D	**D Sesquiterpane**	**BSD**	28.6	3127.9	2245.3	1032.6	1214.0
5	**8β(H)-drimane**	**BS5**	116.1	4096.5	3080.1	1612.5	1917.1
6	**C_{15} Sesquiterpane**	**BS6**	48.0	2853.2	2004.4	1007.0	1272.9
7	**C_{16} Sesquiterpane**	**BS7**	32.3	1548.4	1323.7	742.9	854.2
8	**C_{16} Sesquiterpane**	**BS8**	47.0	1711.8	1397.3	662.1	778.3
9	**C16 Sesquiterpane**	**BS9**	25.2	1171.6	824.6	360.1	468.7
10	**8β(H)-Homodrimane**	**BS10**	220.1	3775.6	3094.7	1933.4	2000.2
	Total (μg/g oil)		**793.6**	**49865.4**	**32845.3**	**16218.0**	**19932.6**

Table 0.17: Measured concentrations (μg/g oil) of bicyclic sesquiterpanes in degraded Nigerian medium oil in Tyne sediment.

			Determined Concentration (Cdet) (m/z 123)				
Peak Number	**Bicyclic Sesquiterpanes**	**Code**	**Background (μg/g EOM)**	**Start**	**100days**	**301days**	**Sterilised(301days)**

1	**C_4-decalin (C14)**	**BS1**	43.3	3734.6	2053.2	2045.5	2020.9
2	**C_{14} Sesquterpane**	**BS2**	26.1	2007.0	1150.6	1055.2	1029.8
A	**A Sesquiterpane**	**BSA**	23.1	11070.7	5229.6	4752.4	4717.1
U2	**U2 Sesquiterpane**	**BSU2**	16.8	3638.2	1574.9	1188.9	1264.2
3	**C_{15} Sesquiterpane**	**BS3**	73.7	15024.1	7370.0	7036.5	6845.4
Y	**Y Sesquiterpane**	**BSY**	31.6	1652.2	1115.0	1075.4	1055.2
B	**B Sesquiterpane**	**BSB**	15.5	4577.9	2178.9	2239.9	2156.5
4	**C_{15} Sesquiterpane**	**BS4**	46.1	7794.8	3986.4	3632.4	3558.6
D	**D Sesquiterpane**	**BSD**	28.6	4707.9	2432.1	2106.8	2028.4
5	**8β(H)-drimane**	**BS5**	116.1	5858.8	3194.7	3398.5	3237.2
6	**C_{15} Sesquiterpane**	**BS6**	48.0	5563.3	2726.8	2610.6	2483.7
7	**C_{16} Sesquiterpane**	**BS7**	32.3	2976.4	1550.3	1521.7	1420.8
8	**C_{16} Sesquiterpane**	**BS8**	47.0	3570.1	1969.1	1736.0	1632.4
9	**C16 Sesquiterpane**	**BS9**	25.2	3030.1	1256.6	1218.6	1148.5
10	**8β(H)-Homodrimane**	**BS10**	220.1	4507.2	2754.6	2963.9	2801.0
	Total (µg/g oil)		**793.6**	**79713.3**	**40542.7**	**38582.3**	**37399.6**

Table 0.18: Measured concentrations (µg/g oil) of bicyclic sesquiterpanes in degraded North Sea oil in Tyne sediment.

				Determined Concentration (Cdet) (m/z 123)			

Peak Number	Bicyclic Sesquiterpanes	Code	Background (µg/g EOM)	Start	100days	301days	Sterilised(301days)
1	**C_4-decalin (C14)**	**BS1**	43.3	373.0	309.7	269.1	217.8
2	**C_{14} Sesquterpane**	**BS2**	26.1	389.5	220.9	194.7	168.4
3	**C_{15} Sesquiterpane**	**BS3**	73.7	765.8	574.6	506.2	434.8
B	**B Sesquiterpane**	**BSB**	15.5	267.2	209.5	181.2	160.3
4	**C_{15} Sesquiterpane**	**BS4**	46.1	541.9	411.6	305.0	256.2
5	**8β(H)-drimane**	**BS5**	116.1	840.9	622.7	574.6	472.7
6	**C_{15} Sesquiterpane**	**BS6**	48.0	482.9	375.6	322.7	272.1
7	**C_{16} Sesquiterpane**	**BS7**	32.3	201.9	203.6	225.6	188.9
8	**C_{16} Sesquiterpane**	**BS8**	47.0	445.4	294.4	336.0	271.7
9	**C_{16} Sesquiterpane**	**BS9**	25.2	271.8	137.0	149.2	135.7
10	**8β(H)-Homodrimane**	**BS10**	220.1	833.3	652.8	769.7	637.2
	Total (µg/g oil)		**693.5**	**5413.6**	**4012.4**	**3834.2**	**3215.8**

Table 0.19: Measured concentrations (µg/g oil) of Adamantanes in degraded Nigerian light oil in Tyne sediment.

Peak Number	Adamantanes	Code	Determined Concentration (C_{det}) (m/z 136)				
			Background (µg/g EOM)	Start	100 days	301 days	Sterilised (301 days)
1	**Adamantane**	**A**	1.6	65.4	10.2	6.7	7.0
2	**1-Methyladamantane**	**1-MA**	4.3	1466.1	191.5	213.1	134.3
3	**1,3-Dimethyladamantane**	**1,3-DMA**	1.2	653.9	94.7	106.6	64.1
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	1.1	102.9	17.7	16.9	13.3

5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	5.4	71.7	11.6	9.8	7.8
6	**2-Methyladamantane**	**2-MA**	1.4	342.4	57.9	59.6	34.8
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	2.9	422.5	68.8	72.6	64.4
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	2.2	277.0	49.0	48.2	41.5
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	5.8	99.7	19.0	28.6	17.2
10	**1,2-Dimethyladamantane**	**1,2-DMA**	3.9	505.6	69.8	69.7	55.1
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	3.7	516.0	80.3	78.0	64.1
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	3.1	416.1	76.5	75.0	60.3
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	2.3	131.3	26.4	25.1	17.4
14	**1-Ethyladamantane**	**1-AE**	3.5	717.0	116.0	118.5	89.1
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	7.0	223.8	53.3	67.0	55.7
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	2.4	204.0	50.2	53.1	41.0
17	**2-Ethyladamantane**	**2-AE**	2.2	74.4	24.2	23.7	23.3
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	4.3	95.4	29.5	31.6	28.7
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	4.6	344.9	60.1	48.4	45.7
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	3.3	201.8	60.0	60.1	51.3
	Total (μg/g oil)		**66.3**	**6932.0**	**1166.7**	**1212.1**	**915.9**

Table 0.20: Measured concentrations (μg/g oil) of Adamantanes in degraded Nigerian medium oil in Tyne sediment.

Peak Number	Adamantanes	Code	Determined Concentration (C_{det}) (m/z 136) Background μg/g EOM)	Start	100 days	301 days	Sterilised (301 days)
1	**Adamantane**	**A**	1.6	20.0	4.0	4.6	3.2
2	**1-Methyladamantane**	**1-MA**	4.3	516.6	168.6	90.4	91.0
3	**1,3-Dimethyladamantane**	**1,3-DMA**	1.2	254.6	104.0	45.6	53.6
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	1.1	112.6	37.6	20.4	26.8
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	5.4	30.0	16.2	6.7	10.1
6	**2-Methyladamantane**	**2-MA**	1.4	169.8	49.9	42.2	43.8
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	2.9	329.7	114.9	65.0	105.3
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	2.2	209.0	82.8	48.6	73.6
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	5.8	97.5	48.7	21.5	44.9
10	**1,2-Dimethyladamantane**	**1,2-DMA**	3.9	477.2	150.1	65.8	134.2
11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	3.7	461.5	146.1	77.4	141.1
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	3.1	357.1	151.7	72.6	128.6
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	2.3	79.2	47.2	20.6	36.9
14	**1-Ethyladamantane**	**1-AE**	3.5	590.9	223.2	103.3	203.1
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	7.0	278.9	131.6	56.7	134.6
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	2.4	207.8	110.8	50.7	103.1
17	**2-Ethyladamantane**	**2-AE**	2.2	68.5	52.0	21.2	49.6

18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	4.3	89.7	73.2	28.5	66.2
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	4.6	328.0	112.8	64.5	108.5
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	3.3	186.7	112.0	30.9	106.8
	Total (μg/g oil)		**66.3**	**4865.1**	**1937.4**	**937.2**	**1665.1**

Table 0.21: Measured concentrations (μg/g oil) of Adamantanes in degraded North Sea oil in Tyne sediment.

			Determined Concentration (C_{det}) (m/z 136)				
Peak Number	**Adamantanes**	**Code**	**Background (μg/g EOM)**	**Start**	**100 days**	**301 days**	**Sterilised (301 days)**
1	**Adamantane**	**A**	1.6	29.9	10.2	4.5	2.5
2	**1-Methyladamantane**	**1-MA**	4.3	102.3	30.3	28.1	17.3
3	**1,3-Dimethyladamantane**	**1,3-DMA**	1.2	28.8	10.9	10.1	6.5
4	**1,3,5-Trimethyladamantane**	**1,3,5-TMA**	1.1	29.0	9.3	9.4	6.7
5	**1,3,5,7-Tetramethyladamantane**	**1,3,5,7-TeMA**	5.4	20.5	9.4	5.8	4.5
6	**2-Methyladamantane**	**2-MA**	1.4	35.2	13.9	12.2	8.8
7	**1,4-Dimethyladamantane, cis-**	**1.4-DMA (cis)**	2.9	105.0	37.7	44.0	30.2
8	**1,4-Dimethyladamantane, trans-**	**1,4-DMA (trans)**	2.2	71.3	28.4	29.2	20.3
9	**1,3,6-Trimethyladamantane**	**1,3,6-TMA**	5.8	28.5	11.1	9.6	8.2
10	**1,2-Dimethyladamantane**	**1,2-DMA**	3.9	137.8	37.2	44.7	37.1

11	**1,3,4-Trimethyladamantane, cis-**	**1,3,4-TMA (cis)**	3.7	176.1	53.0	64.3	48.5
12	**1,3,4-Trimethyladamantane, trans-**	**1,3,4-TMA (trans)**	3.1	119.2	40.6	49.1	39.8
13	**1,2,5,7-Tetramethyladamantane**	**1,2,5,7-TeMA**	2.3	33.2	12.6	15.8	13.5
14	**1-Ethyladamantane**	**1-AE**	3.5	166.6	42.6	58.8	42.8
15	**1-Ethyl-3-methyladamantane**	**1-E-3-MA**	7.0	88.4	29.4	41.3	31.9
16	**1-Ethyl-3,5-dimethyladamantane**	**1-E-3,5-DMA**	2.4	58.2	27.3	36.6	27.4
17	**2-Ethyladamantane**	**2-AE**	2.2	32.1	14.0	21.8	19.0
18	**1,3,5,6-Tetramethyladamantane**	**1,3,5,6-TeMA**	4.3	37.3	18.3	30.8	19.3
19	**1,2,3,5-Tetramethyladamantane**	**1,2,3,5-TeMA**	4.6	139.8	38.8	43.7	35.7
20	**1-Ethyl-3,5,7-Trimethyladamantane**	**1-E-3,5,7-TMA**	3.3	104.7	39.5	66.5	49.3
	Total (μg/g oil)		**66.3**	**1543.9**	**514.5**	**626.4**	**469.3**

Chapter 7: General Discussion and Conclusion

7.1 Discussion

The effects of weathering processes on biomarker fingerprinting initially investigated used by the geochemists for oil-source rock characterisation, genetic family, identification of deposits, migration, and maturation purposes, which later evolved into a forensic environmental geochemical application for source identification in oil spill studies and the settling of legal disputes and liability relating to environmental pollution (Kaplan *et al.*, 1997; Wang *et al.*, 1999; Wang *et al.*, 2004; Reyes *et al.*, 2014). The global wide usage of petroleum and its products resulted in large quantities of the petroleum making its way into the environment such as the seas, ground waters, sediments, etc. causing considerable damage to human health, ecosystem, and natural resources (Wang *et al.*, 1999; NRC, 2003). Biomarker fingerprinting provides evidence to unambiguously identify the spilled oils and relate them to a known or suspected source due to their degradation resistance, which is key for environmental damage assessment, prediction of probable long-term effects, and taking the most effective remedial action (Wang *et al.*, 1999; Wang *et al.*, 2004). Spilled oils in the environment undergo physico-chemical and biological processes including dissolution, evaporation, microbial degradation, photooxidation etc. (collectively termed ‘weathering’) that alters the chemical composition of the oil, and the level and rate of the weathering greatly depends on the type of oil, environmental conditions, and microbial conditions (Wang *et al.*, 1999; NRC, 2003; Wang *et al.*, 2004; Tarr *et al.*, 2016). This work investigates the effects on molecular markers of the main weathering processes including evaporation, water washing, aerobic and anaerobic biodegradation and their effects on correlation efficiency of the biomarkers in the two Nigerian crude oils (light and medium) and a North Sea. Evaporation is quantitatively often the most important oil spill removal process on the marine surface or the terrestrial environment that starts in the period of hours to days (Fingas, 1995; NRC, 2003; Overton *et al.*, 2016), and it is dependent on the oil type or composition, air and sea temperature, wind velocity, intensity of solar radiation, physical state of the oil (thick slicks or “chocolate mousse” (Wang *et al.*, 1999; Reyes *et al.*, 2014). This was evident as the three oil samples were observed to evaporate at different rates of 33%, 40%, 56%, and 63% for the Nigerian Light oil, 10%, 15%, 29%, and 36% for the Nigerian Medium oil, and 27%, 29%, 41%, and 49% for the North Sea oil, in the laboratory evaporation periods of 1, 3, 10, and 30 days respectively (section 4.4.1). The low molecular weight *n*-alkanes (*n*-C_8 to *n*-C_{13}) for the three oils was completely lost due to evaporation after 30 days (cf. Overton *et al.*, 2022), and the Nigerian medium oil was least affected due its waxy nature. The most affected PAHs observed in the three oil samples were the parent naphthalene (C_0-N) and biphenyl (C_0-B) and the C_1-alkyl naphthalenes (cf. Overton *et al.*, 2022), whereas the other PAHs were less affected. The biomarkers are generally unaffected by these level of evaporation except for C_{21} TAS and Q TAS. However, these results could change if the environmental evaporation conditions are altered.

In aquatic environments, oil spilled on the water surface will immediately start to undergo evaporation, while oil released in subsurface waters will undergo dissolution in addition to evaporation by soluble and low-molecular weight oil compounds (mostly C_1 to C_{10}) and the oil becomes more viscous and forms droplets and patches of floating residues (Overton *et al.*, 2016; Tarr *et al.*, 2016). A reduction in *n*-alkane concentrations was observed in the water washing experiments from *n*-C_8 to *n*-C_{11} for the Nigerian light oil and *n*-C_8 to *n*-C_{13} for the North Sea oil and *n*-C_8 to *n*-C_9 for the Nigerian Medium oil which was the least affected due to its waxy nature, indicate that both evaporation and dissolution losses took place (cf. Overton *et al.*, 2022). Although effect of dissolution is substantially less than that of evaporation, it also depends on same variables as evaporation, while varying solubility, and rapid emulsion formation on water surface can be enabled by oxy hydrocarbons produced as a result of photooxidation (Overton *et al.*, 2022). Although BTEX was not analysed in this work, a very small effect of water washing was observed on the naphthalene and some of its alkylated homolog for the three oil samples studied this may be influenced by temperature or the salinity as aqueous solubility is known to increase with temperature and reduce with salinity increase (Lafargue and Barker, 1988), and altering these conditions would therefore yield different results (e.g., Overton *et al.*, 2016; Tarr *et al.*, 2016; Overton *et al.*, 2022). Biomarkers were generally not affected by water washing (mainly evaporation and dissolution) in this work, however studies have shown that biomarkers are sometimes affected in the environment based on particular environmental conditions (Wang *et al.*, 2001; Bata *et al.*, 2018). The absence of unresolved complex mixtures in the chromatograms is an indication that biodegradation did not occur (Wang *et al.*, 1999).

Biodegradation was evident in both the Whitley Bay and the Nigerian sediments under aerobic conditions, but the Whitley Bay sediment is the most affected by microbial activity due to it sandy nature as microbial activity is influenced by the particle size and distribution of the sediment type (e.g., Aelion, 1996; Atlas and Hazen, 2011). Whereas the Nigerian sediment texture is that of clay-loam and its low permeability may limit water transportation and distribution, of nutrients and oxygen required by the microbes within the sediment particles (Sihag *et al.*, 2014). Although biodegradation could be slowed down with depth in sandy sediments due to oxygen deficiency in some environmental conditions (Schiewer *et al.*, 2015), aerobic biodegradation which often occurs at the surface, can be observed within a very short time scale unlike the anaerobic degradation (Aitken *et al.*, 2004a). The presence of unresolved complex mixture UCM) or hump in the gas chromatograms is evidence of aerobic biodegradation in both the Whitley and Nigeria sediment (Jones *et al.*, 1983; Wang *et al.*, 1999; Wang *et al.*, 2001), and the *n*-alkanes and other aliphatics are usually more affected than the PAHs in order of susceptibility to biodegradation (Si-Zhong *et al.*, 2009; Das and Chandran, 2011). Biodegradation is an important process for the restoration of impacted environments, but it is an unwanted process for crude oil production as it affects the quantity and quality of the oil in the deep subsurface reservoirs (Head *et al.*, 2003; Rabus *et al.*, 2016).

Anaerobic biodegradation occurs in anoxic (oxygen deficient) terrestrial soils and sediments, marine sediments, deep subsurface sediments and oil reservoirs with viable anaerobic degradation processes (Widdel and Rabus, 2001; Rabus *et al.*, 2016). Anaerobic biodegradation studies on natural environments, microcosms, and enrichment cultures have been carried out and reported to occur much slower than in aerobic conditions and anaerobes degrade much more limited range of hydrocarbon compounds compared to aerobic microorganisms (e.g., Widdel and Rabus, 2001; Aitken *et al.*, 2004a; Si-Zhong *et al.*, 2009; Sherry *et al.*, 2014; Rabus *et al.*, 2016). This explains why the compounds in the oil samples analysed in this work less affected by the biodegradation experiments carried out under anaerobic conditions compared to those under aerobic conditions.

7.2 Flow chart/timeline weathering processes

The timelines of the weathering effects on the biomarker and non-biomarker compounds analysed in this work is shown Figure 0.1. The compounds in brackets are either completely weathered or are found to be outside the 95% confidence limits of Nordtest parameter plots (i.e., affected correlation efficiencies). The timelines shown are for the most affected compounds of the three oil samples, because the biomarkers susceptibilities to different weathering processes vary (Das and Chandran, 2011; Carls *et al.*, 2016). For instance, the PAHs (naphthalenes and biphenyls) in the North crude are more affected by water washing than the PAHs in the two Nigerian oils. It is worthy of note that evaporation and water washing/dissolution results in preferential loss of low molecular weight components while under aerobic microbial activity crude oil a wide range of compounds are readily biodegradable

(Jones *et al.*, 1983; Prince and Walters, 2016), and the sequence of susceptibility of the components to microbial attack (with initial *n*-alkanes loss) is as reported in many publications with terpanes, steranes being the least affected by weathering processes (Connan, 1984b; Wang and Fingas, 2003; Das and Chandran, 2011; Overton *et al.*, 2016). Low molecular weight PAHs are observed to be more soluble in water than their equivalent aliphatic components (Overton *et al.*, 2016). Weathering of low molecular weight *n*-alkanes and PAHs starts from hours for evaporation and day one for biodegradation where some components of *n*-alkanes biodegrade completely from 5 days whereas some PAHs started to degrade completely from 10 days similar to those reported in previous work (cf. Jones *et al.*, 1983). None of the steranes and the TAS listed are completely degraded by any of the weathering processes, though the effect of the weathering shifted the correlation measurements outside the 95% confidence limits. Photooxidation may not have played an important role in this experiment as the samples were not exposed to high sunlight irradiation and because degradation was observed to decrease with larger more photosensitive PAHs compared to the aliphatics, unlike in photooxidation where degradation increased with increase in aromatic rings and alkylation (Garrett *et al.*, 1998; Overton *et al.*, 2016). It is also observed that only low molecular weight PAHs were affected by evaporation (Wang and Fingas, 1995), which is also consistent with the possibility of photooxidation effects.

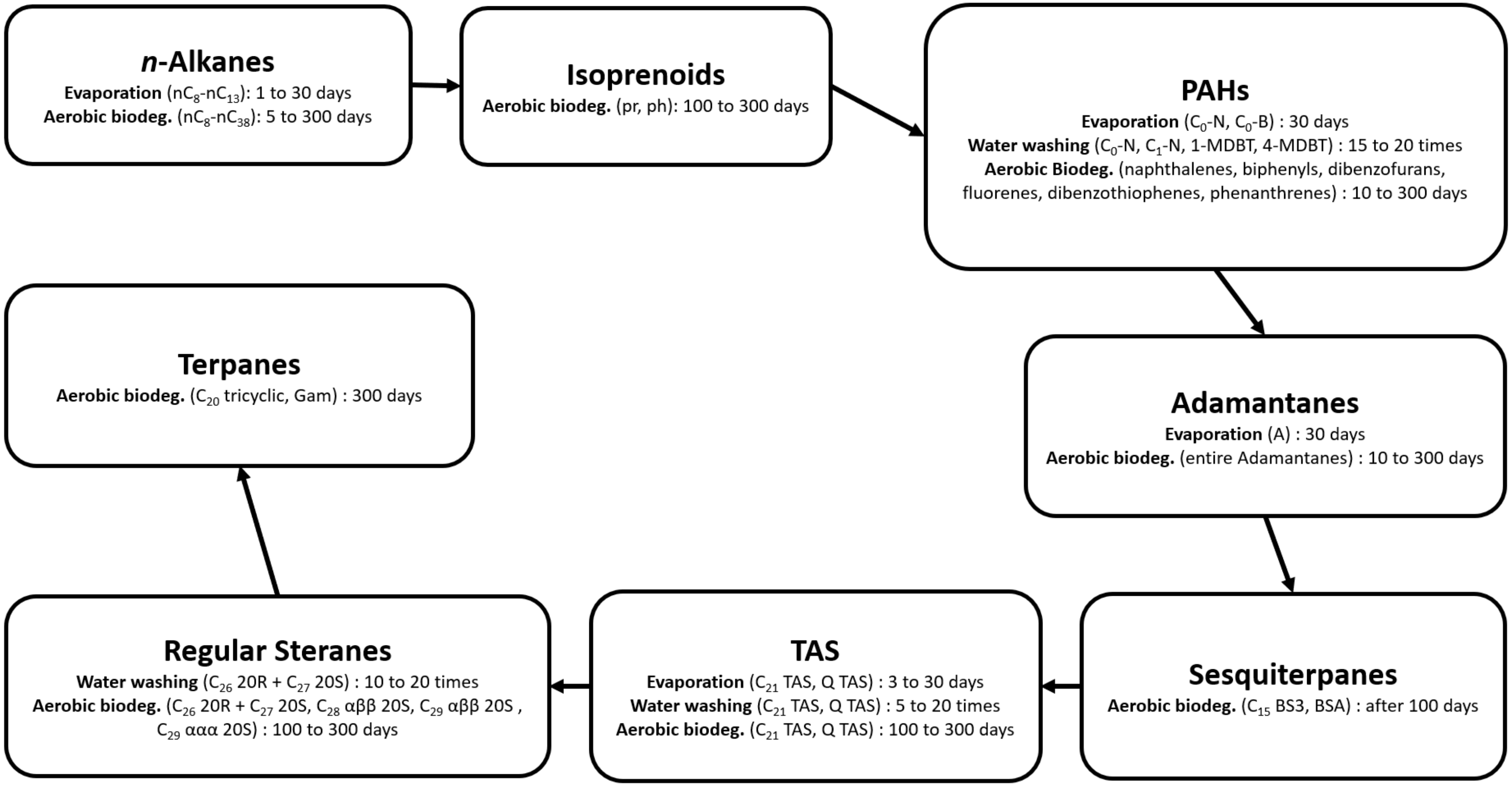


Figure 0.1: Timelines showing the effects of weathering on biomarker and non-biomarker compounds in the crude oil samples analysed. The listed compounds and the period are for the most weathered oil samples (worst case). NB: Details of the compounds are found in the tables at the end of chapters 3, 4, 5, and 6 respectively.

7.3 Importance of sampling and sample preservation

In addition to the normal precautions (clean equipment etc.) to avoid sample contamination during sample collection, seasonal variation should be considered during the preparation for sample collection for oil spill identification purposes. In brackish environment such as the estuaries of the Niger Delta, salinity and hydrocarbon concentrations variable due to seasonal change (Benson and Essien, 2009; Ukpaka and Akuboye, 2016) as high and low tide influences the mixing and dilution of sea water and the receiving fresh water from the river courses. Studies have shown higher TPH in the dry season (February) than in the wet season (June) probably as result of dilution and weathering processes (Benson and Essien, 2009). Well aerated sediment samples can be collected using a hand trowel or shovel or a core sampler into the sample containers, whereas submerged sediments can be collected using appropriate probe or longer core samplers to maintain the distinction between the bottom sediment and the water interface.

While the time between an oil spill and the collection of sediment samples that are thought to be affected by it, for forensic oil correlation purposes should ideally be as short as possible, the work presented in this thesis showed that different oil components may be usable for different periods of time. *n*-alkanes and low molecular aromatic components can be affected within 5 days, components like acyclic isoprenoids, some sequiterpenoid alkanes and PAH are potentially usable for up to 100 days post-spill, while some sterane and triterpene biomarkers can be used after more than 300 days in aerobic sediments. In anaerobic sediments these times would be considerably longer, without affecting many of the key parameters used for oil-oil correlation used in the Nordtest methodology.

7.4 Review of Research Questions

This work investigated the effects of the main weathering processes including evaporation, water washing, aerobic and anaerobic biodegradation, on molecular markers used in oil-source correlation and, focusing on biomarkers and non-biomarkers in two crude oils (light and medium) from the Niger Delta region of Nigeria and one from the North Sea.

Aerobic biodegradation was identified as the main weathering process that affected many of the key geochemical parameters used for oil-oil correlations, including those used in the standard Nordtest methodology.

This work carried out included a sensitivity analysis on some selected biomarkers to assess the influence of background hydrocarbon (biogenic or combustion-derived) concentrations that could affect or interfere with the efficiency of the correlation, and detection/correlation limits was established.

Novel compounds, possibly tricyclic terpanes, labelled S, Q, M, and T were identified in the two Nigerian oils and had correlation efficiencies like other known tricyclic terpanes. Another set of compounds X, Y, and Z1 which were potentially useful for correlation purposes were also noted which had been previously reported (Samuel *et al.*, 2010). Samuel *et al* (2010) stated that X and Y were tricyclic terpanes and Z1 was a tetracyclic terpane and their structures were also identified. Two other tentatively identified peaks were also observed in the two Nigerian oils and labelled as a,b,c,d,e-pentamethylnaphthalene and v,w,x,y,z-pentamethylnaphthalene and were observed to give a positive correlation with other markers.

The sensitivity checks indicated the limitations in the Nordtest methodology by the determination of the detection/correlation limits which serves as a guide to a user to avoid using particular marker parameters in order to improve correlation efficiency.

7.5 Future Work

Statistical multivariate analysis of these data could be carried out where many more correlation parameters could potentially be used from the same chromatographic data in order to further assesses ways of improving oil-oil correlations. However, the effects of analytical variability would need to be thoroughly investigated to avoid minor unidentified peaks from this causing correlation artefacts.

Structural and geochemical identification of the novel unidentified compounds that could be used for correlation purposes would be beneficial to enable them to be more widely applied.

Further weathering studies on a wider range of oils of different compositions and using different sediment inocula would be useful to confirm the generality of the findings in this work.

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