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Correlation of Isoprenoid Hydrocarbon Fingerprints of Ebubu Oil Spill in the Niger Delta

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ABSTRACT

This work was carried out to determine the isoprenoid hydrocarbon indices associated with the Ebubu oil spill site in Rivers State, Nigeria, with a view to correlating fingerprints therefrom with crude oil samples from the oil-rich Niger Delta. The overall objective of the work was to demystify the source of the spill through fingerprinting forensic investigations. Vital diagnostic ratios such as the Pr/Ph, C17/Pr, C18/Ph, C17/C27 and C18/C30 ratios of twelve contaminated soil samples and two background reference samples, were used to correlate those of fresh crude oil samples from the Niger Delta. Other diagnostic parameters such as the carbon preference index (CPI), pollution level index (PLI), terrigenous/aquatic ratio, petroleum load index, average chain length, and weathering ratios were also evaluated. The ratios provided evidence of hydrocarbon source input, biodegradation, extent of weathering and intensity of oil contamination. Oil fingerprints of samples SS5-T and SS5-B showed predominantly bimodal distributions of n-alkanes within the hydrocarbon ranges of nC_{21}–nC_{34} and nC_4–nC_{37}, respectively. Sample SS5-T showed major depletion of resolved n-alkanes and development of a large unresolved complex mixture (UCM), which suggest intense weathering of the contaminated soils. The CPI values for contaminated soil samples varied between 0.69 to 1.71, with average CPI value of 1.00. These results show that the oiled soil samples are biodegraded, indicating a preferential breakdown of odd/even carbons and gradual decrease of CPI values as biodegradation progressed with time. Differences in petroleum load index between the subsoil and topsoil samples highlight variations in hydrocarbon retention, microbial activity and environmental conditions across the oil-spill site. It is hoped that the forensic evidence from this study would support regulatory agencies involved in remediation and oil-spill dispute resolution.

Keywords: Crude oil fingerprints; Isorenoid hydrocarbons; Ebubu oil-spill; Niger Delta

Introduction

Crude oil is a natural resource of immense importance, which in any of its form both as liquid or gas, is used as a source of energy to power transportation machineries and other industrial equipment that sustain human existence and improves economies of various countries[1-4]. In recent years, it has become the major vital source of national economic development, a commodity of strategic importance, a measure of living standard which determines economic health, social and political stability and national security of some countries[5-7].

Petrochemicals, which are by-products of crude oil, are widely used as source of essential raw material feedstocks to produce a wide variety of goods that have improved the overall wellbeing of people across different countries. These raw materials are used in the manufacture goods such as plastics, chemicals, fertilizers, synthetic nylon fibres, polymers and pharmaceuticals, thereby contributing meaningfully to modern living standards and economic development[8].

However, uncontrolled exploration, production, transportation and use of crude oil and its by-products have led to severe environmental contamination across various ecosystems. Crude oil production activities could lead to oil spills, leakage during extraction and transport can contaminate water, soil and surrounding air resulting in long-term ecological disruption, and impact on the health of humans and living organisms[9,10]. Oil could spill into the environment at any stage of the oil life cycle, from drilling operations through disposal of oil and its byproducts after use. Both deliberate or inadvertent oil spills occur on large and small scales depending on factors such as quantity of oil involved and prevailing environmental conditions[11]. Spilled oil contains harmful hydrocarbons that are toxic to humans, organisms, and natural resources in the environment[12,13]. Large-scale oil spills discharge significant volumes of poisonous hydrocarbons into marine and terrestrial ecosystems including seas, oceans, rivers and groundwater leading to widespread damage natural environment, ecological biodiversity, and human death[14].

Nevertheless, frequent spills of small amounts of crude oils could expose the environment to greater cumulative harm than infrequent accidental spills involving larger crude oil volumes[15]. Oil pollution in terrestrial ecosystems results in the inhalation of harmful hydrocarbons, direct disruption of ecological processes, loss of sustenance, and economic stressors that alter intermediary processes, which may lead to community decline[16,17].

Crude oil is formed naturally in subsurface oil reservoirs and consists of complex hydrocarbons mixtures and other compounds exist in liquid, gaseous, or solid states[18,19]. It is generated under different geological conditions, times scales, and depositional environments; as a result, each crude oil has a unique composition and fingerprint[20]. Crude oils originating from same oil field share similar geologic and geochemical histories, including common source rock, maturation stages and processes, and depositional environments[21]. The major hydrocarbons classes in crude oil are alkanes, including isoprenoids; cycloparaffins, aromatics, resins, asphaltenes; and other non-hydrocarbon compounds of sulfur, nitrogen, oxygen, and organometals[22,23].

Isoprenoids are a group of acyclic alkanes in crude oil which are used in forensic oil spill investigations to provide vital information such as origin of organic matter, source rock and depositional environment of crude oils[24]. They are abundant in natural products, microorganisms, animal organisms and in plants[25]. Pristane and Phytane are typical acyclic isoprenoids in crude oil which are derived from phytol sidechain in chlorophyll during diagenetic stages of crude oil formation[26]. Phytol side chain of chlorophyll is derived from phototrophic organisms, bacteriochlorophyll and sulphur bacteria[27]. Pristane-phytane ratio is one of the most widely used diagnostic parameters in the oil spill forensic investigations to correlate spilled oils with source oil and source rocks because it provides information on redox condition, depositional environment and source input of the original oil[14]. It is a geochemical ratio used in forensic environmental pollution and fingerprinting studies for oil-oil, and oil-source rock attribution studies respectively[28]. Their index values are used to indicate redox condition of the oil depositional environment and can provide information on the loss of hydrocarbons and the effects of microbial biodegradation on oil spill impacted areas[19,29].

Oil spills in developing and under-developed nations including Niger Delta region in Nigeria result in culpability disputes and contentions because the spill defaulter may attempt to evade responsibility to avoid litigations, loss of reputation, clean-up cost and victims' compensation payments. Mysterious oil spills are prevalent in both aquatic and terrestrial ecosystems in Niger Delta region and often leading to disputes and contention issue between operators on one hand and with operators and regulatory agencies on the other hand. It is therefore imperative to demystify unknown oil spills, resolve oil-spill disputes and contentions, and provide evidence to defensibly hold oil spillers accountable for legal liabilities, victim compensations, clean-up and oil spill remediations using geochemical fingerprinting techniques[14].

Crude oil analytical fingerprinting technique involving gas chromatographic methods is applied in oil spill forensic investigation for characterizing hydrocarbons in spilled oil, and for accurate and reliable identification of hydrocarbons released into the environment[30,31]. Oil spill fingerprinting analysis is used to assess oil spill contamination[14], and to understand the fate and persistence of spilled oil in the environment[32,33]. In addition, fingerprinting technique is used to trace the origin of spill oil; determine genetic relationship between spilled oil and source oil to establish legal liability against oil spill defaulters[31]. In this study, oil spill forensic investigation approaches using GC-FID fingerprinting technique and isoprenoid geochemical ratios of spilled oil from mysterious spill site in Ebubu town are correlated with those of suspected source oils from various locations in Nigeria to determine the likely source of the oil spill. This study applied oil spill forensic investigation approaches using GC-FID fingerprinting technique and correlation of isoprenoid geochemical ratios to identify the source of oil spill in a mysterious oil spill site in Ebubu town.

Materials and Methods

Study site

The oil spill site is in Ebubu town in Eleme Local Government Area of Rivers State **(Figure 1)** situated about 20 kilometers from Port Harcourt a town in Niger Delta region of Nigeria[34]. The spill site in Ebubu town is good for oil spill environmental forensic investigation because several oil production installations and equipment are located within the Ebubu town. Niger Delta

region is located at the southern part of the Nigeria bordering the Atlantic Ocean, directly situated on the West African continental margin at the apex of the Gulf of Guinea. It is the main oil producing region of Nigeria with estimated reserve of 23 billion barrels of oil and gas reserve of 183 cubic feet[35].

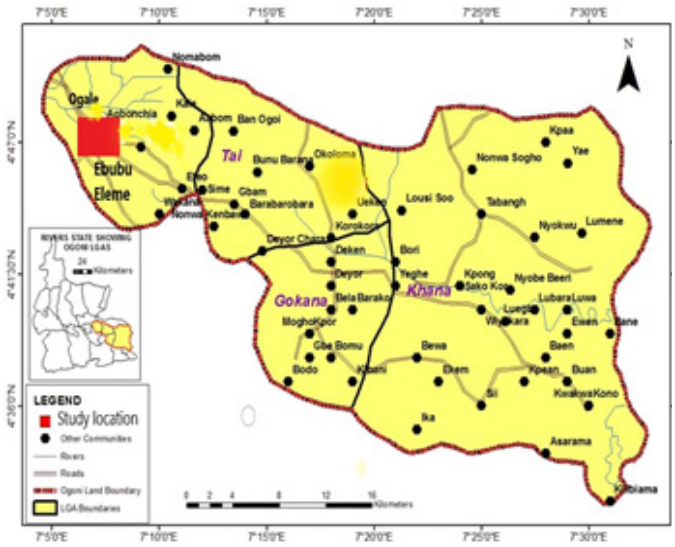

Figure 1: Map of the study area of Rivers State in the Niger Delta showing the study location at Ebubu

Sample collection and preparation

Fourteen contaminated soil samples were collected from seven sampling points at two different depths of 0-15cm and 15-35cm, indicating that one sample each was collected from two different depths at each of the seven sampling points in the oil spill site. Five crude oil samples were obtained from five different oil field locations in three states in Niger Delta region of Nigeria namely Akwa-Ibom, Bayelsa, Delta and Rivers. Five Crude oil samples were collected in collected in glass vails capped with Teflon seals and labelled as BAS, FLD, FRD, NEM, and QIB, while the fourteen contaminated soil samples were collected in ember glass bottles, capped with Teflon seals and labelled as SS1-T, SS1-B, SS2-T, SS2-B, SS5-T, SS5-B, SS6-T, SS6-B, SS9-T, SS9-B, SS10-T and SS10-B. Sample information (sample ID, location and coordinates) is presented in **(Table 1)**.

Column chromatography for crude oil and soil samples

One gram (1.0g) of each crude oil sample was fractionated using column chromatography with 1:1 volume mixture of dichloromethane (DCM) and hexane to separate the aromatics and aliphatic components respectively[14]. The aliphatic fractionate was passed through glass funnel stuff with glass wool covered with sodium anhydrous sulphate to trap moisture while being filtered through Whatman 41 filter paper to remove impurities and waxes. The aliphatic fractionate was allowed to concentrate and evaporated in a solvent sand bath and excess solvent was removed with the aid of nitrogen gas. Sample extract was reconstituted to 2ml and transferred into a vial for gas chromatographic analysis.

Ten grams (10g) of homogenized soil was weighed and added to 100ml equal mixture of dichloromethane and hexane in a 250ml extraction bottle covered, shook for proper homogenization in vortex mixer for 20 mins. The supernatant solvent was allowed to settle and passed through glass funnel stuff with glass wool covered with sodium anhydrous sulphate to trap moisture while being filtered through Whatman 41 filter paper to remove soil matrixes, impurities and waxes. The aliphatic fractionate was allowed to concentrate and evaporated in a solvent sand bath and excess solvent was removed with the aid of nitrogen gas. Sample extract was reconstituted to 2ml and transferred into a vial for gas chromatographic analysis.

Table 1: Showing the GPS coordinates of sample locations.

	Sampling Coordinates		
Samples	**Latitude**	**Longitude**	**Depth (cm)**
BRS	4° 4’ 6.56”N	6° 17’ 4.56”E	Surface
FLD	4° 48’ 43.64”N	7° 9’ 8.68”E	Surface
FRD	5° 9’ 59.76”N	5° 10’ 59.88”E	Surface
NEM	4° 32’ 12.85”N	6° 24’ 22.36”E	Surface
QIB	4° 32’ 54.24”N	8° 0’ 45.72”E	Surface
SS1T	4° 48’ 44.18”N	7° 9’ 7.57”E	0-15
SS1B	4° 48’ 44.18”N	7° 9’ 7.57”E	15-30
SS2T	4° 48’ 45.3”N	7° 9’ 6.7”E	0-15
SS2B	4° 48’ 45.3”N	7° 9’ 6.7”E	15-30
SS5T	4° 48’ 44.41”N	7° 9’ 7.44”E	0-15
SS5B	4° 48’ 44.41”N	7° 9’ 7.44”E	15-30
SS6T	4° 48’ 43.13”N	7° 9’ 8.18”E	0-15
SS6B	4° 48’ 43.13”N	7° 9’ 8.18”E	15-30
SS9T	4° 48’ 43.34”N	7° 9’ 8.15”E	0-15
SS9B	4° 48’ 43.34”N	7° 9’ 8.15”E	15-30
SS10T	4° 48’ 44.91”N	7° 9’ 7.33”E	0-15
SS10B	4° 48’ 44.91”N	7° 9’ 7.33”E	15-30
SSCT	4° 48’ 44.29”N	7° 9’ 7.65”E	0-15
SSCB	4° 48’ 44.29”N	7° 9’ 7.65”E	15-30

Gas Chromatography (GC-FID) analysis

Aliphatic fraction obtained by column chromatography of crude oil and soil samples were subjected to gas chromatography fingerprinting analysis. The gas chromatographic analysis of the aliphatic hydrocarbons (carbon ranges from nC3-nC40) identification and quantification were performed using Agilent 7890B gas chromatography (GC) system incorporated with flame ionization detector (FID)[36]. The GC system was fitted with a 30 meter long, 0.32 mm internal diameter capillary column coated with a 0.25um of 100% dimethyl siloxane stationary phase (J & W scientific Co. Ltd., USA). A temperature programmable oven housing the capillary column with the gas heated split /splitless column inlet and flame ionization detector. The oven housing the capillary column with gas heated split/splitless column inlet and flame ionization detector was programmed with an initial temperature of 65˚C (held 2.0 minutes) and is ramped at 15˚C / min to 320˚C, with initial hold time of 1 min and final hold time of 10 minutes. The injector temperature was set at 300 degrees Celsius to vaporize the hydrocarbon components of the oil. Helium is used as the carrier gas while oxygen and hydrogen were used as combustion gases. Separated mobile hydrocarbon vapours were carried by the helium carrier gas through the capillary column inside the oven to the detector.

Results and Discussion

Results

(Tables 2 and 3) contain the results of the acyclic isoprenoid hydrocarbons in the crude oil samples and in the contaminated

soil samples respectively. The analyses are presented contain Pr/Ph, C17/Pr, C18/Ph, C17/C27 and C18/C30 ratios for both the crude oil and soil samples respectively. **(Figure 2)** contains the chromatograms of five source crude soil samples, twelve oil-contaminated soil samples and two background reference samples.

Table 2: Acyclic isoprenoid hydrocarbon ratios of crude oil samples.

Sample	Pr/Ph	CPI	TAR	C17/Pr	C18/Ph	C17/C27	C18/C30	WR	ACL	Pet/Bio
BAS	1.58	1.07	0.59	0.83	1.61	1.26	1.79	0.65	28.86	1.62
FLD	1.35	1.07	0.71	0.53	1.1	1.09	1.45	0.84	28.74	1.06
FRD	0.84	0.69	0.64	0.95	0.54	2.12	1.02	0.58	28.33	2.21
NEM	2.2	1.26	0.6	0.46	1.24	1.5	1.67	0.79	29.26	1.42
QIB	1.77	1.01	0.53	0.7	1.41	1.28	1.9	0.61	29.13	1.87

Table 3: Showing the isoprenoid and isoprenoid-alkane ratios of contaminated soil samples.

Sample	Pr/Ph	CPI	**TAR**	**C17/Pr**	**C18/Ph**	**C17/C27**	**C18/C30**	**PLI**	**WR**	**ACL**	**Pet/Bio**
SS1B	0.58	1.3	5.39	0.92	0.55	0.26	0.23	0.9	3.43	29.61	0.15
SS1T	1.13	1.71	3.13	0.33	0.53	0.51	0.4	1.02	2.27	28.9	0.25
SS2B	2.03	0.74	0.94	1	0.88	1.67	0.37	1.18	0.93	28.59	0.41
SS2T	1.97	0.69	1.1	1.03	0.74	1.69	0.57	1.36	0.74	28.45	0.45
SS5B	2.87	0.97	1.02	0.29	0.8	0.83	0.8	1.13	1	28.56	0.37
SS5T	0.58	1.15	5.87	1.94	2.04	0.26	0.26	0.87	4.12	29.26	0.12
SS6B	0.76	0.8	1.28	0.52	0.37	0.8	0.51	1.07	0.91	28.76	0.35
SS6T	0.88	0.95	1.49	0.4	0.4	0.64	0.64	1.09	1.01	28.87	0.35
SS9B	1.26	1.03	1.22	0.86	0.92	1.12	0.77	1.02	0.79	28.52	0.43
SS9T	0.87	0.9	1.06	0.71	0.55	0.96	0.7	1.05	0.75	28.59	0.42
SS10B	1.13	0.83	0.94	0.63	0.79	1.06	0.83	1.06	0.7	28.53	0.46
SS10T	0.89	0.94	0.76	1.35	0.72	1.8	0.82	1.26	0.68	28.51	0.46
SSCB	1.55	0.74	3.64	0.62	0.88	0.4	0.45	-	-	28.65	0.18
SSCT	1.37	0.67	4.2	0.48	0.41	0.35	0.38	-	-	27.95	0.18

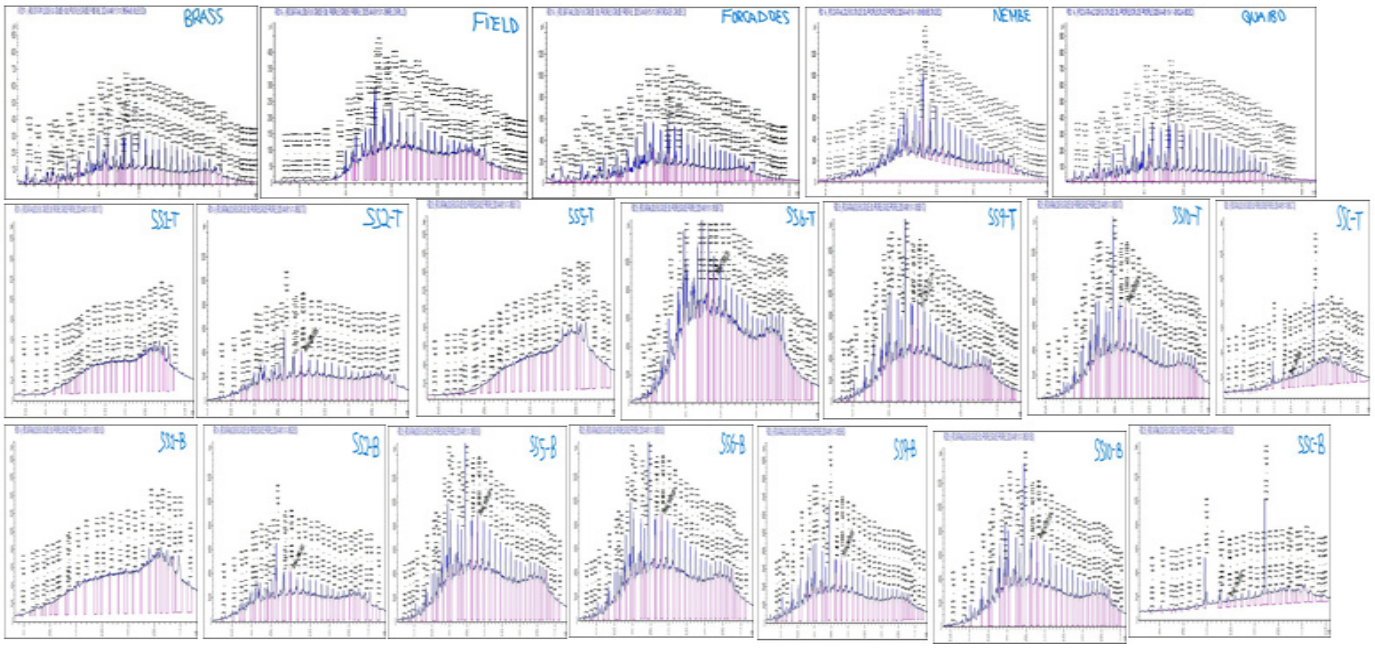

Figure 2: GC-FID fingerprints of five source crude oils, twelve oil-contaminated soil samples and two background control samples

Discussion

Fingerprinting of crude oil is an important aspect of oil spill forensic investigations for correlating and differentiating environmental spilled oil sources with known source crude oils[30]. Gas Chromatography with Flame Ionisation Detection is an important tool in oil spill forensic investigation used to separate, identify and quantify constituent chemical hydrocarbons, which are fingerprints in crude oil.

GC-FID Chromatograms also known as fingerprints from the GC-FID provide a descriptive information or depictions of the various dominant hydrocarbons in each sample **(Figure 2)**. These chromatograms provide details on individual saturated hydrocarbons including n-alkanes (C2-C40) and selected acyclic isoprenoids such as pristane and phytane. GC-FID chromatograms provide

information on oil type characterization and reveal the overall carbon number range of the sample, which is informative when choosing which biomarker would be relevant for further oil spill forensic identification. For each sample, several isoprenoid and n-alkane ratios have been calculated including Pr/Ph, nC17/Pr, nC18/Ph, C17/C27, C18/C30, (C17+C18)/(pri+phy) CPI, TAR, WR, ACL, and Pet/Bio. The results for these ratios for source crude oils and oil-contaminated soil samples are presented in **(Tables 2 and 3)** respectively.

GC-FID of source crude oils: Hydrocarbon distribution profiles in chromatograms could be distinguished from one another by careful visual analysis of peak patterns with emphasis on the following parameters, low and high retention times, overall profile of the shape of the peaks, carbon number ranges for each sample profile and the existence of unresolve complex mixtures in fingerprint profiles[14,30,37]. Chemical fingerprints of all five source crude oils including Brass, Field, Forcadoes, Nembe and Qua-Ibo, were evaluated using GC-FID analysis of saturated hydrocarbon fractionates from these crude oils[37]. Figure 2 shows that hydrocarbon fingerprints of four of the source crude oil samples including Brass, Nembe, Forcadoes and Qua-Ibo samples predominantly bimodal n-alkane distributions dominated by saturates in the range of nC3 - nC39. For each of these crude oils, the figure also shows two conspicuous chromatographic baseline humps known as unresolved complex mixtures, whose presence indicate loss of smaller n-alkane molecules due to evaporation weathering process[38]. GC-FID Chromatogram for Field source crude oil revealed a predominantly bimodal n-alkane distributions dominated by saturates in the range of nC11- nC40, showing two unequal unresolved complex mixtures. The preferential depletion of lower-molecular weight alkanes and the consequential presence of unresolve complex mixture can be attributed to compositional alteration due to environmental weathering processes[31,39].

Based on visual inspection of the fingerprint characteristics such as profile shapes, low retention times, high retention times of the components and carbon number distributions for each source crude oil. Figure 2 also revealed that fingerprints of all 5 source crude oils are dissimilar from one another. The figure further showed that although GC-FID fingerprints for Field, Nembe and Qua-Ibo source crude oils samples share certain similarities, their fingerprint profiles are these three samples are different from each other in profile shapes, retention times and profile carbon number distributions. In addition, their fingerprints are dissimilar from those of Forcadoes and Brass source crude oils **(Figure 2)**. Furthermore, Figure 2 showed that although Forcadoes and Brass source oil fingerprints are similar in profile shapes at high retention times and carbon number distributions, however, there are dissimilarities in their profile shapes at low retention times. Indicating that all five crude oil sources may not be genetically related. However, this demonstrates visual inspection of GC-FID fingerprint profile alone is not adequate tool for source correlation among petroleum sources, additional assessment approach is needed for a reliable and defensible source attribution[40].

GC-FID of spill-impacted soil samples: GC-FID fingerprints of 12 oil-contaminated soil samples from spill impacted site and 2 control samples collected from 100m away from spill site were evaluated using GC-FID analysis of saturated hydrocarbon fractionates from these soil samples are presented in Figure 2. Figure 2 contains fingerprints of all 12 spill-impacted soil samples consisting of SS1-T, SS1-B, SS2-T, SS2-B, SS5-T, SS5-B, SS6-T, SS6-B, SS9-T, SS9-B, SS10-T and SS10-B samples. The figure revealed a predominately bimodal n-alkane distributions which is dominated by saturates in the range of nC5 - nC40, showing varying sizes of two unequal unresolved complex mixtures (UCM), indicating variations in the extent and impact of environmental weathering processes on oil-contaminated soil samples[31,38].

GC-FID fingerprints of samples SS1-T and SS1-B showed predominantly bimodal distributions of n-alkane hydrocarbons within the carbon range nC_{26}–nC_{34}, with substantial loss of resolved n-alkanes and development of a noticeable unresolved complex mixture (UCM), which indicates extensive petroleum weathering and biodegradation[41]. The chromatographic profiles of both samples demonstrated strong similarities in peak patterns at low and high retention times, suggesting a common hydrocarbon source and similar environmental alteration processes. The disappearance of lower molecular weight n-alkanes approximately below nC_{25}, along with elevated UCM intensity, indicates preferential degradation and loss of labile hydrocarbons during extended environmental exposure, which is characteristic of advanced stages of oil spill weathering and biodegradation[31,38].

GC-FID fingerprints of samples SS2-T and SS2-B exhibited predominantly bimodal distributions of n-alkane hydrocarbons within carbon range of nC_6–nC_{33}, with minor loss of lower molecular weight n-alkanes and the advance of two unequal unresolved complex mixtures (UCMs), which may indicate moderate evaporation weathering processes[14]. The fingerprint profiles of both samples showed similar peak distribution patterns at low and high retention times, signifying a common petroleum source and similar oil weathering history. Moderate degradation of isoprenoids and resolved n-alkanes up to around nC_{10} was evident, whereas the persistence of higher molecular weight hydrocarbons and comparable bimodal UCM intensities reflect intermediate stages of oil weathering and biodegradation. These chromatographic characteristics are consistent with partial loss of volatile hydrocarbons through evaporation together with progressive environmental alteration of petroleum hydrocarbons[38,41].

GC-FID fingerprints of samples SS5-T and SS5-B showed predominantly bimodal distributions of n-alkanes within the carbon-number ranges of nC_{21}–nC_{34} and nC_4–nC□□, respectively. Sample SS5-T showed major depletion of resolved n-alkanes along with the development of a large unresolved complex mixture (UCM), signifying advanced petroleum weathering processes[41]. The chromatographic profile exhibited extensive loss of isoprenoids and n-alkanes up to around nC□□, revealing elevated UCM intensity, and marked reductions in peak abundances of heavier hydrocarbon components, thereby confirming severe environmental alteration of the spilled oil. In contrast, sample SS5-B showed comparatively minor depletion of lower molecular weight saturates up to nearly nC_8 together with two unequal UCMs characteristic evidence of slight evaporation weathering and minor microbial degradation[38,40]. The dissimilarities observed in fingerprint profile shapes at both low and high retention times between SS5-T and SS5-B indicate varying extent of environmental exposure and hydrocarbon compositional alteration.

GC-FID chromatgrams of samples SS6-T and SS6-B exhibited predominantly bimodal distributions of alkane hydrocarbons within carbon range of nC_{11}–nC_{32}, with depletion of lower molecular weight n-alkanes, together with development of two unequal unresolved complex mixtures (UCMs), indicating early stages of petroleum weathering. The fingerprint profiles of both samples displayed similar peak distribution patterns at low and high retention times, indicating a common hydrocarbon source and similar environmental weathering processes. Minor biodegradation of isoprenoids and resolved n-alkanes up to around nC_{11} were observed in both topsoil and bottom samples. Although significantly unequal UCM intensities were noticed between the two chromatograms, however, the similarity in carbon-number distributions and hydrocarbon peak abundances signifies comparable petroleum composition and weathering history despite differences in sampling depth. These chromatographic characteristics are consistent with early-stage evaporation, together with mild microbial degradation processes affecting the spilled oil[14,31].

GC-FID fingerprints of samples SS9-T and SS9-B showed predominantly bimodal distributions of saturated hydrocarbons within carbon range of nC_8–nC_{31}, with minor loss of lower molecular weight n-alkanes and existence of two unequal unresolved complex mixtures (UCMs), which could indicate early stages of spilled oil weathering. The chromatographic profiles of both samples showed slight similarities at low and high retention times, suggesting comparable but unidentical hydrocarbon alteration histories. Minor degradation of isoprenoids and resolved n-alkanes up to around nC_8 were observed in both soil samples, which reflects early-stage evaporation and microbial weathering processes[14]. However, dissimilarities in carbon-number distributions and hydrocarbon peak abundances were evident, with sample SS9-B exhibiting greater reductions in resolved hydrocarbon peaks between nC_6 and nC_{32} compared with SS9-T. These differences likely show varying extents of environmental exposure, weathering intensity, or biodegradation rates between the topsoil and bottom samples despite originating from the same sampling point.

GC-FID chromatograms of samples SS10-T and SS10-B exhibited predominantly bimodal distributions of saturated hydrocarbons within carbon number range of nC_7–nC_{32}, with minor depletion of lower molecular weight n-alkanes and the existence of unresolved complex mixtures (UCMs), which indicate early stages of oil weathering. The fingerprint profiles of both samples displayed strong similarities at low and high retention times, together with relatively comparable UCM intensities, reflecting a common hydrocarbon source with similar weathering degradation history. Minor degradation of isoprenoids and resolved n-alkanes up to around nC_8 were observed in both topsoil and bottom samples, demonstrating early-stageo of evaporation and biodegradation weathering[14,31]. The similar carbon-number distributions and hydrocarbon peak abundances between the fingerprints further indicate slight differences in hydrocarbon alteration despite sampling at different depths from the same sampling point.

Fingerprints of the background control samples (SSC-T and SSC-B) revealed predominantly unimodal n-alkane distributions within the range of nC_7–nC_{31}, with dominance of saturated hydrocarbons and enrichment of long-chain hydrocarbons, which characterises biogenic organic matter derived from terrestrial higher plant inputs[42,43].

Degree of waxiness: Degree of waxiness of crude oils is a diagnostic ratio in GC-FID fingerprinting used for forensic correlation between crude oil and source oils by characterizing the amount of terrestrial organic matter inputs in oil from source rocks based on the abundance and distribution of high molecular weight alkanes[14,44]. Higher molecular weight n-alkanes which are terrestrial derived organic materials in crude oils are useful organic source indicators for correlation of oils and source rocks[45,46]. Oil waxiness is a parameter used in oil spill forensic investigation to indicate the source, type and weathering stage of spilled oil. Typical of crude oils from Niger Delta, the five crude oil samples including Brass, Field, Forcadoes, Nembe and Qua-Ibo exhibited waxiness index values ranging from 1.16 and 1.34, which indicates moderately waxy oils probably from mixed marine and terrestrial source inputs[31,44]. The degree of waxiness of oil spill in polluted samples including SS1-T, SS1-B, SS2-T, SS2-B, SS5-T, SS5-B, SS6-T, SS6-B, SS9-T, SS9-B, SS10-T and SS10-B exhibited a wide range of values ranging from 1.09 and 5.83 with a mean value of 1.93.

These indicate increases in the degree of waxiness for the spilled oils majorly because of the weathering effects which results in preferential removal of low-chain n-alkanes in the oil leaving behind the higher molecular weight paraffins and increasing waxiness[31,44]. Control samples SSC-T and SSC-B showed a degree of waxiness values of 3.28 and 3.52 respectively, indicating highly waxy hydrocarbons source predominantly from terrestrial higher plants[31].

Pollution level Index (PLI): Petroleum load index is an environmental assessment parameter commonly used in forensic oil spill investigations to evaluate intensity and overall level of crude oil hydrocarbon contamination in oil-spill impacted area. It is an environmental index employed to trace changes in oil pollution in sediments, soils and aquatic environments over a period and to compare the extent of contamination between sites[37,47]. In environmental forensic investigations, it is typically applied to evaluate spatial distribution and intensity of petroleum contamination, monitor effectiveness of remediation and support environmental risk assessment around industrial and petrochemical areas, as well as within oil spill sites[19]. PLI is used to compare the relative concentrations of petroleum hydrocarbons between impacted sites and background or reference levels, thereby providing a unified measure of oil contamination assessment across affected areas[19,48].

Petroleum load index: Petroleum load indices for contaminated soil samples were calculated using n-alkane fingerprint data for oil-impacted samples along with corresponding fingerprint data from background soil samples. Table 1 shows PLI values of oil-impacted soil samples with highest and lowest values of 1.36 and 0.87 respectively, and with average value of 1.08, progressive deterioration or oil pollution of the affected area. The Table also shows that most of the soil samples have PLI values more than 1 confirming oil contamination of the affected area. Samples SS1B and SS5T have PLI values lower than 1 which likely indicate significant biodegradation of the spilled in the polluted soil samples[37].

Table 1 also shows variations in the PLI values for samples collected from different depths corresponding to topsoil and subsoil for each oil spill sampling points. These variations in PLI values with respect to sampling depth are caused by strong variations in environmental factors such as availability of oxygen

and nutrients, effects of microbial activities, moisture and type of soil texture. Comparison of trends in PLI values between topsoil and subsoil for each sampling points indicate that higher PLI values were obtained for topsoil samples SS1-T, SS2-T, SS6-T SS9-T and SS10-T, when compared to their corresponding subsoil samples including SS1-B, SS2-B, SS6-B, SS9-B and SS10-B respectively. Such trend is attributable to scenarios where spilled oil is trapped at the topsoil due to underlining factors such as high organic matter, low permeability, recent contamination, or limited oil downward infiltration. These factors result in accumulation and retention of hydrocarbons within the upper soil surface across oil-impacted areas[49-51]. Higher PLI value of 1.13 was obtained for subsoil SS5B, compared to PLI value of 0.87 for SS5T. This tendency may be attributed to intense biodegradation together with other environmental weathering processes occurring in the topsoil for sample SS5T, where hydrocarbons are exposed to enhanced microbial activity, oxygenation, evaporation, and photooxidation[19,37].

Alkane chain ratio (ACL): Average chain length (ACL) of organic hydrocarbons is another geochemical parameter used in environmental forensics to identify sources of hydrocarbons by distinguishing between petroleum hydrocarbons from hydrocarbons from biogenic sources[38,52-54]. It is also used to identify the source of saturated hydrocarbons in the environment based on the abundance of the odd-numbered-carbon alkanes in higher plants[55]. ACL is used to reflect the dominant molecular size of hydrocarbons present in the organic source of the pollutants[29]. Table 2 shows ACL values for suspected soil samples while table 3 contains different ACL values for contaminated soil samples. The Average carbon chain length (ACL) values for crude oils ranged from 28.33 to 29.26, with average value of 28.86 **(Table 2)**. ACL values for the contaminated soil samples ranged from 28.45 to 29.61, with average value of 28.76 **(Table 3)**. The two background soil samples (SSCB and SSCT) have ACL values of 28.65 and 27.95 respectively **(Table 3)**. These results show that lower average ACL value was obtained for contaminated soil samples compared to the average ACL values for the source crude oil samples. Comparison between these average ACL values indicate that varying amounts of oil contamination of natural background soil resulted in lower ACL values, with wider ACL range for contaminated soil samples[54]. Table 3 further shows variations in the ACL values between topsoil and subsoil samples. The trend for most sample pairs from each sampling point revealed that ACL increases with increased degree of weathering as reflected in increased ACL values for samples with higher WR values. This shoes the impact of environmental weathering processes, which preferentially remove lower molecular weight hydrocarbons, leading to increase in WR values[29]. Consequentially, higher WR values result in dominance of higher molecular weight hydrocarbons with corresponding increase in ACL values[54].

Pristane-Phytane Ratio (Pr/Ph): Pristane-phytane ratio is one of the most widely used isoprenoid diagnostic parameters in the oil spill forensic investigations to correlate spilled oils with source oil because it provides information on redox condition, depositional environment and source oil input[14]. Pristane and phytane isoprenoids are derived from phytol sidechain in chlorophyll during diagenetic stages of crude oil formation[26]. Phytol side chain of chlorophyll is primarily generated from phototrophic organisms, bacteriochlorophyll and sulphur bacteria[27]. Pristane is formed by decarboxylation of phytol under oxidizing condition, while phytane is generated by phytol dehydration under reducing condition. They are petroleum diagnostic indicators used in assessing extent of oil degradation based on their resistance to biodegradation during early stages of environmental weathering[14,37]. Pristane-phytane ratios are commonly applied in petroleum geochemistry and oil spill forensics to indicate type of organic materials that formed petroleum sources such as crude oils, sediments and rock extracts[29]. Pristane-phytane index value less than 1 (Pr/Ph<1) is used to indicate anoxic source-rock deposition environment[56]. Ratio values more than 1 are indicative of Oxic depositional environment, while higher values more than or equal to 3 indicate oxic conditions associated with terrestrial organic material input[29]. Table 3 shows pristane-phytane ratios obtained for 14 contaminated soil samples and Table 3 contains Pr/Ph values for 5 source crude oil samples including Brass, Field, Forcadoes, Nembe and Qua-Ibo. Pristane-phytane index values for source crude oils range from 0.84 to 2.24, with average value of 1.55. Table 3 further shows a highest Pr/Ph value of 2.20 for Nembe crude oil and the lowest value of 0.84 obtained for Forcadoes crude oil. This shows that four of the five crude oil samples exhibited Pristane-Phytane ratios greater than 1.0, indicating organic material generated under Oxic conditions in possible normal marine shelf and deltaic environments. While as, Forcadoes crude oil with ratio value of 0.84, may suggest that the oil was generated in Sub-oxic/anoxic, slightly reducing condition during deposition[29,44,56,57]. The highest pristane-phytane ratio value of 2.2 was obtained for Nembe source crude oil indicating possible oxic conditions under normal marine shelf or deltaic environment[29,54,58]. Field source crude oil sample has a Pr/Ph ratio of 1.35 which indicates oil generated in Transitional suboxic to oxic depositional condition. Brass and Qua-Ibo source crude oils have Pr/Ph ratios of 1.58 and 1.77 respectively, reflecting possible oxic to sub-oxic conditions under normal marine shelf or deltaic environment[29].

Pristane-phytane ratio values obtained for 14 oil-contaminated soil samples range between 0.57 to 2.87, with average value of 1.25, as shown in Table 3. The table shows reducing changes in pristane-phytane ratios obtained for contaminated soil samples largely due to different microbial and weathering effects on the spilled oil as weathering progresses with time[14]. Compared to the pristane-phytane ratio value of 0.84 for Forcadoes source crude oil sample, the Pr/Ph ratio increased in some cases for some soil samples because of preferential biodegradation of phytane instead of the less stable pristane isoprenoids[41,59].

Carbon preferential index (CPI): The carbon preferential index, (CPI) (C25–C33) is a petroleum geochemical parameter used to assess source input and maturity of n-alkanes in petroleum sources[26] and interpret source depositional environmental conditions[29]. It is also used to determine odd-to-even carbon predominance in long-chain alkanes and to differentiate between alkanes from vascular land plants and alkanes from bacteria or petroleum sources[60]. Studies demonstrates that a predominance of long chain odd-numbered-carbon alkane in petroleum sources is indicative of terrigenous inputs, whereas the predominance of long chain even-numbered carbon alkane is typically considered as indicator of organic matter derived from marine, algae, bacteria or petrogenic sources[29,42,61]. CPI values presented in Table 1 were calculated from n-alkane distributions in the

studied source crude oils and contaminated soil samples. CPI values obtained for all five crude oil samples range between 0.69 to 1.26, with average value of 1.02. All crude oil samples except Forcadoes oil have CPI values above 1.0, which might reflect crude oils with petroleum-like distribution in thermally mature organic source with predominance of marine inputs[29].

CPI value of 0.69 obtained for Forcadoes crude oil could indicate oil of petrogenic origin with an even-carbon enrichment from thermally matured hydrocarbon source. This indicates possible characteristics of its source rock, or alteration of the odd carbon alkanes due to weathering and biodegradation processes[29,31]. The CPI values for contaminated soil sample varies between 0.69 to 1.71, with average CPI value of 1.00. These values reflect biodegradation process on the spilled oil hydrocarbons on the soils, suggesting preferential breakdown of odd/even carbons and gradual decrease of CPI values to 1 as biodegradation progresses with time[60]. Also, CPI value of 1 is a diagnostic petrogenic origin of crude oil[43,62]. The CPI value of the background soil samples varies between 0.67 and 0.74, with an average value of 0.71.

Terrigenous aquatic ratio (TAR): TAR is diagnostic geochemical ratio applied in oil spill forensics to trace the source of spilled oil assessment of the predominance of terrigenous against aquatic organic matter inputs in petroleum systems[63,64]. It can be used to identify changes in the abundances of terrigenous versus aquatic hydrocarbons in petroleum sources such as rock extracts and crude oils[29,63]. TAR is a ratio of long-chain n-alkanes with carbon atoms consisting of nC_{27}, nC_{29}, nC_{31} typically from higher plant and other terrestrial inputs, against short-chain n-alkanes consisting of nC_{15}, nC_{17}, nC_{19} derived from algae, bacteria, planktons, aquatic input and microbial lipids[26].

Table 1 shows TAR ratios for five crude oil sample, 14 soil samples including 2 background soil samples used as control to monitor oil pollution on the site under investigation. TAR values for the five crude oil samples is between 0.53 and 0.71, with an average value of 0.61. This reflects dominance of aquatic input possibly from algal/ bacterial organic matter, because crude oil and aquatic inputs have predominance of short- and mid-chain n-alkanes[43,65,66]. TAR values for 12 contaminated soil samples range between 0.76 and 5.39, with average value of 2.02, these increases in the values of TAR values of contaminated soil samples compared to the suspected Field crude oil with a TAR of 0.7, is as a result of weathering and biodegradation processes which result in loss of aquatic labile short-chain alkane components from the oil-contaminated soil samples. Oil weathering and biodegradation process degrades short-chain alkanes, leave behind long-chain alkanes and creates the wrong impression of enhanced terrestrial inputs with higher-than-normal values of TAR[31,43,53,66]. TAR values obtained for the two background soil samples ranged between 3.64 and 4.20, with average value of 3.94, these values reflect the predominance of territorial inputs in the organic matter of the background soil samples. Average TAR value of 3.94 in the background soil is an indication of predominance of odd-number long chain alkanes from higher terrestrial wax hydrocarbons, typical of natural soils not contaminated with crude oil pollution[31,42,43,66]. This presents a clear difference with spilled oil, which has TAR values around 1 or less prior to weathering and biodegradation processes.

Weathering ratio (WR): Weathering ratios are geochemical indicators used in oil spill investigations to evaluate the extent of compositional alteration of petroleum sources and support oil spill source attribution based on the relative concentration indices between labile and conservative hydrocarbons. These ratios are applied to compare the relative abundances of weathering-susceptible hydrocarbons with more degradation-recalcitrant hydrocarbons to evaluate environmental weathering processes such as evaporation, biodegradation, dissolution, and photooxidation[14,67,68].

Conclusion

This study demonstrated the effectiveness of GC-FID fingerprinting technique and application of isoprenoid indices in environmental oil spill investigations for source attribution of mysterious spills in Niger Delta region of Nigeria. Comparative assessment of fingerprints and diagnostic ratios between oil-contaminated soil samples and suspected source crude oils revealed significant variations associated with petroleum composition, depositional environment and weathering processes. Petroleum geochemical parameters including pristane/phytane ratio, carbon preference index, terrigenous/aquatic ratio, petroleum load index, average chain length, and weathering ratios provided vital evidential information regarding hydrocarbon source input, biodegradation, weathering extent and intensity of oil contamination. The contaminated soil samples exhibited critical evidence of extensive environmental weathering characterized by preferential depletion of low molecular weight hydrocarbons and consequential enrichment of long chain hydrocarbons due to biodegradation, evaporation, dissolution, and photooxidation processes. Differences in petroleum load index between subsoil and topsoil samples reflected variations in hydrocarbon retention, microbial activity and environmental conditions across the oil-spill site. This study has established that integration of GC-FID technique with isoprenoid diagnostic ratios is a comprehensive, reliable and defensible tool for reliable oil-source attribution, environmental forensics, oil-pollution monitoring and spill liability assessment in oil-producing environments. This approach provides forensic evidence that support regulatory agencies, oil spill dispute resolution, spill remediation strategies and enhances environmental protection in oil-producing regions.

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References

1. Cherubini F, Bird ND, Cowie A, Jungmeier G, Schlamadinger B, Woess-Gallasch S. Energy- and greenhouse gas-based LCA of biofuel and bioenergy systems: Key issues, ranges and recommendations. Resources, Conservation and Recycling 2009;53(8):434-447.

2. Etkin D. Weather-related disasters and oil spills: Causes, consequences, and management. Springer 2011.

3. Kujawinski EB, Kido Soule MC, Valentine DL, et al. Fate of Dispersants Associated with the Deepwater Horizon Oil Spill. Environmental Sci Tech 2011;45(4):1298-1306.

4. Ozokede EV, Awogbemi TO. The Impact of Crude Oil Price Volatility on Economic Growth in Nigeria. Int J Res and Innovation in Social Science 2025;9(9):7385-7396.
5. Hamilton JD. The historical role of oil shocks in the U.S. economy. Energy J 2014;35:1-28.
6. Kilian L. Oil Price Shocks: Causes and Consequences. Annual Review of Resource Economics 2014;6(1):133-154.
7. Xue Y, Liu X, Wang J. The impact of crude oil price fluctuations on economic growth and energy consumption. Energy Policy 2011;39(9):5357-5364.
8. American Chemistry Council. Petrochemicals and their role in everyday life. American Chemistry Council 2022.
9. Encyclopaedia Britannica. Oil spill. In Oil spill 2024.
10. US Energy Information Administration. Oil and the environment (Oil and the Environment). U.S. Energy Information Administration 2023.
11. Fingas M. The basics of oil spill cleanup (2nd ed.). CRC Press 2001.
12. Jafarinejad S. Oil spill waste management: A review of technologies and environmental impacts. J Environmental Manag 2017;201:33-46.
13. Simecek-Beatty D, Leah W. Oil spill modelling for environmental response. Marine Pollution Bulletin 2017;115(1-2):5-15.
14. Wang Z, Fingas MF. Development of oil hydrocarbon fingerprinting and identification techniques. Marine Pollution Bulletin 1999;38(9):753-760.
15. Yang Z, Wang Z, Fingas M, Brown C. Review of major oil spill incidents and their environmental impacts. Marine Pollution Bulletin 2015;96(1-2):5-15.
16. Aguilera F, Méndez J, Pásaro E, Laffon B. Review on the effects of exposure to spilled oils on human health. J Applied Toxicology 2010;30(4):291-301.
17. Webler T, Lord F. Planning for the Human Dimensions of Oil Spills and Spill Response. Environmental Management 2010;45(4):723-738.
18. Fingas M. Oil spill science and technology (2nd ed.) (2nd ed.). Gulf Professional Publishing 2016.
19. Wang Z, Stout SA, Fingas M. Forensic Fingerprinting of Biomarkers for Oil Spill Characterization and Source Identification. Environmental Forensics 2006;7(2):105-146.
20. Curtis JB, Michael B. Petroleum geochemistry and geology. Blackwell Science 2000.
21. Ekpo BO, Essien N, Neji PA, Etsenake RO. Geochemical fingerprinting of western offshore Niger Delta oils. J Petro Sci Eng 2018;160:452-464.
22. Rossini FD, Mair BJ. Hydrocarbons from petroleum. In A. Weissberger (Ed.), Technique of organic chemistry. John Wiley & Sons 1959;5.
23. Hunt JM. Petroleum geochemistry and geology (2nd ed). W. H. Freeman 1996.
24. Volkman JK. Biological marker compounds as indicators of the depositional environments of petroleum source rocks. Geological Society, London, Special Publications 1988;40(1):103-122.
25. Connolly JD, Hill RA. Triterpenoids. In Second Supplements to the 2nd Edition of Rodd's Chemistry of Carbon Compounds. Elsevier 1991:555-596.
26. Waples DW. Reappraisal of Anoxia and Organic Richness, with Emphasis on Cretaceous of North Atlantic. AAPG Bulletin 1983;67(6):963-978.
27. Powell TG, McKirdy DM. Relationship between ratio of pristane to phytane, crude oil composition and geological environment in Australia. Nature Physical Science 1973;243:37-39.
28. Wang Z, Zhang L, Liu Q. Hydrocarbon composition and distribution in crude oils. J Petro Sci Eng 2016;145:200-215.
29. Peters K, Walters C, Moldowan M. The Biomarker Guide: Biomarkers and Isotopes in Petroleum Exploration and Earth History (2nd ed.) (2nd Edition). Cambridge University Press 2005;1.
30. Bayona JM, Domínguez C, Albaigés J. Analytical developments for oil spill fingerprinting. Trends in Environmental Analytical Chemistry 2015;5:26-34.
31. Wang Z, Fingas MF. Development of oil hydrocarbon fingerprinting and identification techniques. Marine Pollution Bulletin 2003;47(9-12):423-452.
32. Kingston PF. Long-term Environmental Impact of Oil Spills. Spill Science & Technology Bulletin 2002;7(1-2):53-61.
33. Samsuria NNC, Ismail WZW, Nazli MNWM, et al. Problems, Effects, and Methods of Monitoring and Sensing Oil Pollution in Water: A Review. Water 2025;17(9):1252.
34. Ollorwi O. The People of Ebubu in History. Paper presented by Osaro Ollorwi on the occasion of Cultural Fiesta at Leaders Foundation School, Ebubu 2016.
35. Sonibare O, Alimi H, Jarvie D, Ehinola OA. Origin and occurrence of crude oil in the Niger delta, Nigeria. J Petro Sci Eng 2008;61(2-4):99-107.
36. Environmental Protection Agency EPA. Nonhalogenated organics by gas chromatography. EPA Methods 8015C. U.S. Environmental Protection Agency EPA 2007.
37. Fingas MF. Oil Spill Science and Technology: Prevention, Response, and Cleanup. Elsevier 2011.
38. Peters K, Walters C, Moldowan MJ. The Biomarker Guide: Vol 1 (First edition). Cambridge University Press 2005.
39. El-Sheshtawy HS, Khalil N, Farouk S. Biodegradation Effect on the Campanian Oil Shale of Egypt. Geomicrobiology J 2020;37(8):746-752.
40. Stout SA, Wang Z. Oil Spill Environmental Forensics Case Studies ((1st ed.)). Academic Press, Cambridge, UK 2018.
41. Zhendi W, Merv F, Gary S. Study of 22-Year-Old Arrow Oil Samples Using Biomarker Compounds by GC/MS. Environmental Science & Technology 1994;28(9):1733-1746.
42. Eglinton G, Hamilton RJ. Leaf Epicuticular Waxes: The waxy outer surfaces of most plants display a wide diversity of fine structure and chemical constituents. Sci 1967;156(3780):1322-1335.
43. Meyers PA. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Organic Geochemistry 1997;27(5-6):213-250.
44. Barakat AO, Mostafa AR, Qian Y, Kennicutt Ii MC. Application of Petroleum Hydrocarbon Chemical Fingerprinting in Oil Spill Investigations-Gulf of Suez, Egypt. Spill Science & Technology Bulletin 2002;7(5-6):229-239.
45. El Diasty WSh, Moldowan JM. Application of biological markers in the recognition of the geochemical characteristics of some crude oils from Abu Gharadig Basin, northwestern Desert – Egypt. Marine and Petroleum Geology 2012;35(1):28-40.
46. Hedberg HD. Significance of High-Wax Oils with Respect to Genesis of Petroleum. AAPG Bulletin 1968;52(5):736-750.
47. Arzaghi E, Abbassi R, Garaniya V, Binns J, Khan F. An ecological risk assessment model for Arctic oil spills from a subsea pipeline. Marine Pollution Bulletin 2018;135:1117-1127.
48. Tomlinson DL, Wilson JG, Harris CR, Jeffrey DW. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. Helgoländer Meeresuntersuchungen 1980;33(1-4):566-575.
49. Atlas RM, Bartha R. Hydrocarbon Biodegradation and Oil Spill Bioremediation. In K. C. Marshall (Ed.), Advances in Microbial Ecology. Springer US 1992;12:287-338.

50. Kostecki PT, Calabrese EJ. Hydrocarbon Contaminated Soils and Groundwater: Analysis, Fate, Environmental & Public Health Effects, & Remediation. Routledge 2022;1.

51. Yuniati MD. Bioremediation of petroleum-contaminated soil: A Review. IOP Conference Series: Earth and Environmental Science 2018;118:012063.

52. Bence A, Burns W. Fingerprinting Hydrocarbons in the Biological Resources of the Exxon Valdez Spill Area. In P. Wells, J. Butler, & J. Hughes, Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters. ASTM International100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 1995:19428-2959.

53. Killops SD, Killops VJ. Introduction to organic geochemistry. Wiley-Blackwell 2013.

54. Wang Z, Fingas MF, Stout SA. Oil spill identification and characterization using petroleum biomarkers. Environmental Forensics 2007;8(1-2):105-146.

55. Cochran JR, Stow DAV, Poynter J, Eglinton G. Proceedings of the Ocean Drilling Program, 116 Scientific Results. Ocean Drilling Program 1990;116.

56. Didyk BM, Simoneit BRT, Brassell SC, Eglinton G. Organic geochemical indicators of palaeoenvironmental conditions of sedimentation. Nature 1978;272(5650):216-222.

57. Hughes WB, Holba AG, Dzou LIP. The ratios of dibenzothiophene to phenanthrene and pristane to phytane as indicators of depositional environment and lithology of petroleum source rocks. Geochimica et Cosmochimica Acta 1995;59(17):3581-3598.

58. Tissot BP, Welte DH. Petroleum formation and occurrence (2nd Edition). Springer-Verlag 1984.

59. Butler EL, Prince RC, Douglas GS. Hopane, a new chemical tool for measuring oil biodegradation. In R. E. Hinchee & R. F. Olfenbuttel (Eds.), On-Site Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment. Butterworth-Heinemann 1991.

60. Bray EE, Evans ED. Distribution of n-paraffins as a clue to recognition of source beds. Geochimica et Cosmochimica Acta 1961;22(1):2-15.

61. Volkman JK, Johns RB, Gillan FT, Perry GJ, Bavor HJ. Microbial lipids of an intertidal sediment—I. Fatty acids and hydrocarbons. Geochimica et Cosmochimica Acta 1980;44(8):1133-1143.

62. Huang W-Y, Meinschein WG. Sterols as ecological indicators. Geochimica et Cosmochimica Acta 1979;43(5):739-745.

63. Rielley G, Collier RJ, Jones DM, Eglinton G. The biogeochemistry of Ellesmere Lake, U.K.-I: Source correlation of leaf wax inputs to the sedimentary lipid record. Organic Geochemistry 1991;17(6):901-912.

64. Jaffé R, Mead R, Hernandez ME, Peralba MC, DiGuida OA. Origin and transport of sedimentary organic matter in two subtropical estuaries: A comparative, biomarker-based study. Organic Geochemistry 2001;32(4):507-526.

65. Bourbonniere RA, Meyers PA. Sedimentary geolipid records of historical changes in the watersheds and productivities of Lakes Ontario and Erie. Limnology and Oceanography 1996;41(2):352-359.

66. Volkman JK, Holdsworth DG, Neill GP, Bavor HJ. Identification of natural, anthropogenic, and petroleum hydrocarbons in aquatic sediments. Science of the Total Environment 1992;112:203-219.

67. Killops SD, Killops VJ. Introduction to organic geochemistry (2nd Edition). Blackwell Publishing 2005.

68. Waples DW. Geochemistry in Petroleum Exploration. Springer Netherlands 1985.