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Comparative Investigation of Some Molecular Biomarker Signatures of Weathered Crude Oils and Automotive Gas Oil

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Abstract

This research was designed to investigate some molecular biomarker signatures of weathered crude oils and automotive gas oil as a means of distinguishing them. Three samples of crude oil were collected from two producing field in the Niger Delta Nigeria and labelled UG7, UG8, AZU. A sample of automotive gas oil was collected from a dispensing station and labelled AGO. The samples were subject to a laboratory oil-weathering technique by rotary evaporation and thereafter to Gas Chromatography Mass Spectroscopy. The biomarker fingerprints of the acyclic isoprenoids and hopanes were used in calculating some diagnostic biomarker ratios such as Pr/Ph, CPI, %$\Sigma(nC_{15} + C_{17} + C_{19})$/Total normal alkanes, $\Sigma(C_{21} - C_{31})/\Sigma(C_{15} - C_{20})$, Pr + nC_{17}, Ph + nC_{18}, Pr + nC_{17}/Ph + nC_{18}, C_{29}Ts/C_{30}-H, Ts/Ts + Tm, C_{35}HH/C_{34}HH, HHI, Moretane Index, Oleanane Index, C_{31}(S)/C_{31}(R), $C_{29}\beta\beta/(\beta\beta + \alpha\alpha)$. These ratios distinguished the samples in terms of the organic matter source, thermal maturity, redox environment degree of weathering and waxiness. Statistical plots such as the ternary plot, (nC_{17} + nC_{18})/Pr + Ph vs Pr + nC_{17}/Ph + nC_{18} obtained from computed ratios gave a clear segregation of the samples. Similarly a dendrogram plot delineated samples UG7, UG8, AZU, and AGO but hit the crux by giving a 0.00% similarity between the crude oils and the AGO.

Keywords: *Biodegradetion; Weathering; Organic matter; Oxic, Biomarkers; Pristane.*

1. Introduction

Environmental molecular fingerprints have been utilized to address issues of source identification, contaminant identification as well as its (contaminant) possible timing of release into petroleum and petroleum derived samples. The combination of chemical fingerprinting to include understanding the types of forensic data, specific site geologic and hydro-geologic conditions as well as their implications or interpretations can give rise to highly effective and tenable argument [1]. Biomarkers are one of the most important hydrocarbon groups in petroleum. They can be detected in low quantities (ppm and sub-ppm levels) in the presence of a wide variety of other types of petroleum hydrocarbons by the use of gas chromatography-mass spectrometry (GC-MS). Relative to other compounds in oil, including the n-alkanes and most aromatics, biomarkers are more degradation-resistant in the environment. The relative content of biomarker compounds in source rocks, and hence crude oils and refined products, depends on the source, maturation, and in-reservoir weathering and biodegradation processes [2].

Thus, biomarkers reveal more information about oil source than do other compounds in oil. Therefore, chemical analysis of biomarkers can be of great importance to environmental forensic investigations for determining the source of spilled oil, differentiating and correlating oils, and monitoring the degradation process and weathering state of oils under a wide variety of conditions [3-4]. Specific biomarkers and their relative abundances are used to identify fossil fuel in reservoirs based on the deposition environment (anoxic or sub-oxic), geological condition of formation (pressure, temperature and residual time) and the mixture of initial organic

matter (terrestrial or marine inputs) with the help of the relative abundances of the specific biomarkers [5]. These biomarkers provide a set of veritable tools for chemical fingerprinting, saturated hydrocarbons and geological biomarkers have been extensively applied as an efficient tool for source correlation studies. The *n*-alkane distribution range can serve as an indicator of crude oil spill or non-crude oil spill (i.e. spill by a refined hydrocarbon product) and due to the resistant nature of the geochemical biomarkers to degradation, different spills can be investigated to have been caused by either a crude oil type , different crude oil types or a crude oil (petroleum) product which form the basis of this work as these geological biomarkers remain traceable during degradation which occur in environmental timescales and natural habitats, thus, are used to differentiate the origin, depositional habitat and organic inputs of petroleum or oil in spills, even when the most labile compounds have degraded [6-7].

Oil spilled into the natural environment tends to experience physical, chemical and biological weathering that changes the oil properties with time. The processes comprise: evaporation, emulsification, natural dispersion, dissolution of water-soluble compounds, photo-oxidation, sedimentation and biodegradation [8-9]. Characteristically, weathering leads to increase in viscosity, density and pour-point of the oil [8]. Consequently, weathering may complicate mechanical clean-up of oil spill, as well as biodegradation, due to reduction on the rate of dispersion of the oils [10]. Biodegradation of oils depends strongly on environmental factors and not only on affecting the indigenous microbial communities, but also the oil properties and behaviour. Reduced temperatures affect oil bioavailability by altering solubility, viscosity and fluidity of the oil, making access to hydrocarbons more difficult for the microorganisms and then leads to slower degradation rates [11-12].

Diesel oil is a complex mixture of normal, branched and cyclic alkanes comprising of between two thousand and four thousands hydrocarbons, usually found in the middle-distillate fraction during petroleum processing [13]. Most of the hydrocarbon compounds are employed as indicators for diesel oil weathering assessment. Alterations in the ratios of the concentration of the hydrocarbons are mostly as a result of processes such as evaporation and dissolution. The polycyclic aromatic hydrocarbons (PAHs) are used as valuable tools to provide for monitoring environmental alterations. Majority of the polycyclic aromatic hydrocarbons (PAHs) compounds in diesel fuel are least affected by weathering. The process of weathering may also be assessed by using total petroleum hydrocarbons (TPH). Achieving this will involve studying the chromatographic profiles of a commercial diesel, which shows resolution profile for all n-alkanes and some other isoprenoid alkanes, such as pristane (2,6,10,14- tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane). However, most important fractions of the diesel oil may appear in the chromatograms as a "hump", also kwon as "unresolved complex mixture (UCM) [14-15].

Weathering of oil is the sum total of the physical, chemical and biological processes which oil spills undergo in a marine or terrestrial environment in which there is an interaction between the physical and chemical features of the spilled oil with the physical and biochemical features of the affected environment. Usually, the freshness of the oil is revealed if there exist an identical chemical composition between the unspilled source and the oil residue. Tarr *et al*. [16] stated that some recently accepted research specified that the effect of weathering can give rise to the addition of novel chemical structures (product of photo induced oxidations of the hydrocarbons and non-hydrocarbons in the residues Weathering processes are rapidly facilitated by the presence of water, especially the emulsification and evaporation processes which play a significant role in oil slick density and viscosity. Therefore, oil weathering processes impact changes on the chemical composition and physical characteristics of oil over time [17]. Important weathering processes are evaporation, spreading, emulsification, dissolution, biodegradation and photooxidation processes [18-20]. Meanwhile, evaporation is the single most important and dominant weathering process. The rates of weathering of oil can be very diverse and are controlled by a number of spill conditions and natural processes such as the type of oil spilled, the local environmental conditions, and natural population of indigenous microbial and microbiological activities during and after the spill.

Alkane susceptibility to weathering is clearly correlated with the carbon number or chain length; that is, the shorter the chain length, the more easily it can be weathered. Thus, as the weathered percentages increase, the value of the numerator significantly decreases and the denominator, in contrast, grows larger, resulting in a continuous decrease of the WI values [21].

Weathering index: $$WI = \frac{(n-C_8 + n-C_{10} + n-C_{12} + n-C_{14})}{(n-C_{22} + n-C_{24} + n-C_{26} + n-C_{28})}$$

In this research, the biomarkers in crude oils and petroleum products (AGO) were characterized and their distributions are compared. The effects of evaporative weathering on biomarkers distributions were examined. Some diagnostic ratios of the biomarkers were developed for oil correlation and differentiation. Lastly, the sets samples were used as case studies to illustrate the unique utility of biomarkers for fingerprinting and identification of unknown spills

This study is aimed at carrying a comparative forensic study of the of some molecular biomarker signatures of weathered crude oils and automotive gas oil from the Niger Delta region (Fig. 1).

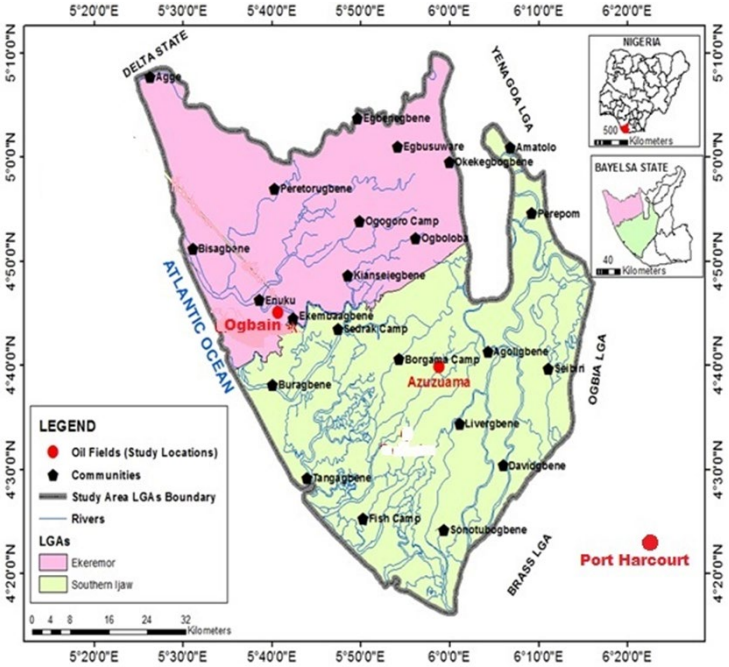

Figure 1. Map of the study area showing Ogbain and Azuzuama in Bayelsa State

2. Materials and methods

2.1. Sample collection, preparation and analysis

Three (3) crude oil samples were collected from two (2) separate onshore oil producing fields in the Niger Delta region of Nigeria into respective glass vials with Teflon caps. A diesel fuel (AGO) sample was collected with a sterilised glass bottles. The samples were respectively labelled as: UG7, UG8, AZU and AGO. A laboratory oil-weathering technique by rotary evaporation was used to artificially weather oils with varying degrees of weight loss. This weathering technique used allows for precise control of the evaporative weight loss for the target oil and can be directly correlated to compositional changes of the oil. The weathered samples were then transferred into different sterile vials and kept in the refrigerator and the temperature adjusted to less than 4°C in preparation for gas chromatography/mass spectrometry analyses. Prior to the analyses, the gas chromatography mass spectrometry was properly calibrated, then about 100µL of each of the samples were measured into four (4) separate solvent rinsed beakers with the aid of a A1000 micropipette and then diluted with dichloromethane (DCM) to make up a volume of 1mL used for analyses.

2.2. Description of the study area

The Niger Delta basin which is the study area for this research is a region characterized with abundant quantity of petroleum resources and presently the only basin that houses commercial quantities of petroleum resources in Nigeria. The geology of the study area is representative of the Niger Delta basin which is made up of three basic geologic formations namely the Agbada, Akata and Benin formations respectively [22]. The Niger Delta geopolitical zone is located within southern coastal Nigerian states. The region extends over about 112,000 Square Km and makes up about 12.0 per cent of Nigeria's landmass [23]. This region is characterized by the magnitude of petroleum activities due to the petroleum-rich nature of the region. Azuzuama is located within the geographical coordinates of 4°45'0" North and 5°57'0" East while Ogbain can be found within 4°45'0" North and 5°40'0" East in Southern Ijaw and Ekeremor Local Government Areas of Bayelsa State.

2.3. Gas chromatography/mass spectrometry (GC/MS) analysis

The aliphatic hydrocarbon fractions were subjected to a gas chromatography - mass spectrometry analyses to observe fragment ions of certain biomarkers using a standard Hewlett Parkard 5890II GC coupled with a split/splitless inlet/injector (kept at 280 °C) connected to HP 5972 MSD (mass spectrometry detector) with an electron voltage of 70 eV, filament current of 220 μA, source temperature of 160 °C, multiplier voltage at 1600 V, and 300 °C for interface temperature (i.e. detector temperature). The collection of data was monitored by an HP Vectra PC chem station computer collectively in full scan mode (FSM) and selected ion modes (SIM). 1μL of the diluted sample was injected into the inlet using the autosampler HP 7673 (290 °C) and the split opened up after 1min. Separation was carried out on a capillary column (30m x 0.25mm ID DB-5) fused with silica, and coated with 0-25μm (film thickness), 5% phenylmethyl silicone (HP-5). The temperature of the GC was set to operate at 40/50-300°C at a count of 4°C per minute and then held for 16.7/20mins as the final temperature. The carrier gas was helium which flowed at 1.0mL/min (nominal) at a pressure of 50KPa, with a slit at 30mL/min. The data was obtained on a digital audio tape (DAT) and was later resolved using a Chem Station G1701 BA software (version B.01.001989-1998) and the peak was integrated using the RTE integrator.

3. Results and discussion

The gas chromatography analyses (GC-MS) of samples UG7, UG8, AZU and AGO in full and selected ion modes m/z 71, m/z 191, m/z 217 showed the distribution of *n*-alkanes, acyclic isoprenoids (pristane and phytane) and terpanes (hopanes) respectively. The results obtained in this work are reported as shown in Tables 1 to 4.

Table 1. Result of the normal alkanes and acyclic isoprenoids

Biomarker	UG7	UG8	AZU	AGO
C10	-	-	4125367	2546168
C11	-	-	7733822	6601003
C12	624202	2066575	1536350	4277743
C13	880684	12793663	7360263	10782612
C14	808174	7596112	6874100	5584682
C15	1221232	8909372	6375853	14652659
C16	1546932	11846001	5752602	18707535
C17	925443	8601475	6194955	17933763
Pristane (Pr)	3925108	12420974	10310631	2854830
C18	1282727	10553209	4423063	2215338
Phytane (Ph)	1819985	4690150	3721647	2269847
C19	1248672	9711502	4173854	13708608
C20	1237543	10061596	3718894	13454358
C21	1559816	10394350	4033926	11521973
C22	1143558	12262829	3869384	10795842

Biomarker	UG7	UG8	AZU	AGO
C23	1077391	13902760	3572436	9864236
C24	1062551	14342500	3559368	7880791
C25	819934	13366773	3358984	4816381
C26	372100	11091122	2573445	1337340
C27	216866	5362944	2109502	-
C28	-	6297309	1351027	-
C29	-	3026658	1248111	-
C30	-	3230742	716817	-
C31	-	2052578	568948	-
C32	-	1423291	-	-
C33	-	802527	-	-

Table 2. Some diagnostic ratios of acyclic isoprenoids and *n*-alkane

Ratios	UG7	UG8	AZU	AGO
Pr/Ph	2.16	2.65	2.77	1.26
Pr/nC_{17}	4.24	1.44	1.66	0.16
Ph/nC_{18}	1.42	0.44	0.84	1.02
(nC_{17} + nC_{18})/Pr + Ph	0.38	1.12	0.76	3.93
Pr + nC_{17}/Ph + nC_{18}	1.56	1.38	2.03	4.63
nC_{25}/nC_{18}	0.64	1.27	0.76	2.17
Weathering Index (WI)	0.56	0.22	1.10	0.62
Weathering Ratio (WR)	0.19	0.61	0.25	0.18
$\Sigma(C_{21}- C_{31})/\Sigma(C_{15}- C_{20})$	0.12	0.66	0.46	0.41
$\Sigma(C_{25}- C_{35}/\Sigma C_{13}- C_{20}$	0.15	0.58	0.27	0.06
CPI	0.98	0.98	1.21	1.35
%(C_{15} + C_{17} + C_{19})/TNA	16%	14%	17%	29%
%(C_{27} + C_{29} + C_{31} + C_{33})/TNA	1%	6%	4%	ND
(C_{21} - C_{33}) ODD/(C_{21} - C_{33})EVEN	1.43	1.01	1.23	1.31

CPI: Carbon Preference Index = sum of odd carbon numbered n-alkanes/ sum of odd carbon numbered n-alkanes, Pr/Ph: Pristane/Phytane, TNA: Total N-alkanes, Isoprenoids/n-alkanes: Pr/nC_{17} and Ph/nC_{18}, n-alkanes/isoprenoids: (nC_{17} + nC_{18})/Pr + Ph, Degree of wax: $\Sigma(C_{21}- C_{31})/\Sigma(C_{15}- C_{20})$,
UG7- UGBAIN7TBG, UG8- UGBAIN8LS, AZU- AZUZONMA, AGO- AUTOMOTIVE GAS OIL

Table 3. Results of the hopane biomarkers

Biomarkers	UG7	UG8	AZU	AGO
Ts	239532	163621	221044	-
Tm	278621	41146	325890	-
C_{28} Bisnorhopane	24877	17120	21212	-
C_{29} Nor-25-hopane	554562	211546	290033	-
C_{29} Norhopane	85620	87033	124313	1036234
C_{29} Ts-hopane	40858	43875	46510	806563
C_{30} Diahopane	-	-	-	54794
C_{29} Normoretane	-	16914	24524	326263
C_{30} Oleanane(αβ)	26624	495441	1240774	74497
C_{30} Hopane	1471510	399688	1059350	642574
C_{30} Moretane	102890	156958	192325	-
C_{31} Homohopane(22S)	133321	141892	237498	54907
C_{31} Homohopane(22R)	92228	65991	161312	60871
C_{30} Gammacerane	-	27669	-	15085
C_{32} Homohopane(22S)	25927	64406	111263	18922
C_{32} Homohopane(22R)	15402	42858	84652	17133
C_{33} Homohopane(22S)	15249	39490	15279	67508
C_{33} Homohopane(22R)	10479	6164	9315	21511
C_{34} Homohopane(22S)	10224	6380	8931	-
C_{34} Homohopane(22R)	5548	3248	5369	-
C_{35} Homohopane(22S)	5975	3592	5214	-
C_{35} Homohopane(22R)	2513	1964	3610	-

Table 4. Calculated hopane biomarker diagnostic ratios

Biomarker ratios	UG7	UG8	AZU	AGO
Ts/Ts+Tm	0.46	0.80	0.40	ND
$C_{28}BNH/C_{30}H$	0.02	0.04	0.02	ND
$C_{29}N\text{-}25\text{-}H/C_{30}H$	0.38	0.53	0.27	ND
$C_{30}\text{-}Ole/C_{30}H$	0.02	1.24	1.17	0.12
$C_{30}Mor/C_{30}H$	0.07	0.39	0.18	ND
$C_{30}Gam/C_{30}H$	0.00	0.07	0.00	0.02
$C_{31}HH(22S)/C_{31}HH(22R)$	1.45	2.15	1.47	0.90
$C_{32}HH(22S)/C_{32}HH(22R)$	1.68	1.50	1.31	1.10
$C_{33}HH(22S)/C_{33}HH(22R)$	1.46	6.41	1.64	3.14
$(C_{31}\text{-}C_{35}H)/C_{30}H$	0.22	0.94	0.61	0.37
$C_{35}H/C_{34}H$	0.54	0.58	0.62	ND
$C_{29}Ts/C_{30}H$	0.03	0.11	0.04	1.26
$C_{31}HH(22S)/22R+22S$	0.59	0.68	0.60	0.47
$C_{32}HH(22S)/22R+22S$	0.63	0.60	0.57	0.52
$C_{33}HH(22S)/22R+22S$	0.59	0.86	0.62	0.76
Ts/Tm	0.86	3.98	0.68	ND
$C_{31}R/C_{30}\text{-}H$	0.06	0.17	0.15	0.09

Ts: 18α(H)-22,29,30-trisnorneohopane, Tm: 17α(H)-22,29,30-trisnorhopane, C_{30}-Ole/C_{30}H: C_{30} Oleanane/C_{30} Hopane, (C_{31}-C_{35}H)/C_{30}H: Homohopane Index, C_{30}Mor/C_{30}H: C_{30}Moretane/C_{30}Hopane, C_{29}Ts/C_{30}H: C_{29}Ts- hopane/C_{30} Hopane, C_{31}HH(22S)/22R+22S: C_{31}Homohopane(22S)/C_{31}Homohopane(22R)+C_{31}Homohopane(22S), C_{30}Gam/C_{30}H: C_{30}Gammacerane/C_{30}Hopane

Carbon number distribution typical of crude oil usually ranges between C_3 – C_{44} (from lower to very high molecular weight components), lubricating oils (C_{20} – C_{44}), heavy fuel oils (C_{10} – C_{40}), diesel fuel (C_9 – C_{25}), kerosene/jet fuels (C_6 – C_{16}), Naphtha (C_6 – C_{10}) and gasoline (C_3 –C_{10}). However, the *n*-alkane distribution from the m/z 71 fragmentogram showed the absence of C_1- C_9 in all the oil samples, this might be due to biodegradation or evaporation as the lighter components easily evaporated in the cause of weathering. Three (3) of the samples UG7, UG8 and AZU have a carbon range between C_{10} – C_{33} typical of their crude oil nature while AGO showed a carbon range of C_{10} – C_{26} which is much narrower than the carbon range for crude oil, but typical of a refined hydrocarbon product, most likely diesel fuel as its carbon range assumes that of a diesel fuel (C_9 – C_{26}) (Figure 2a-d).

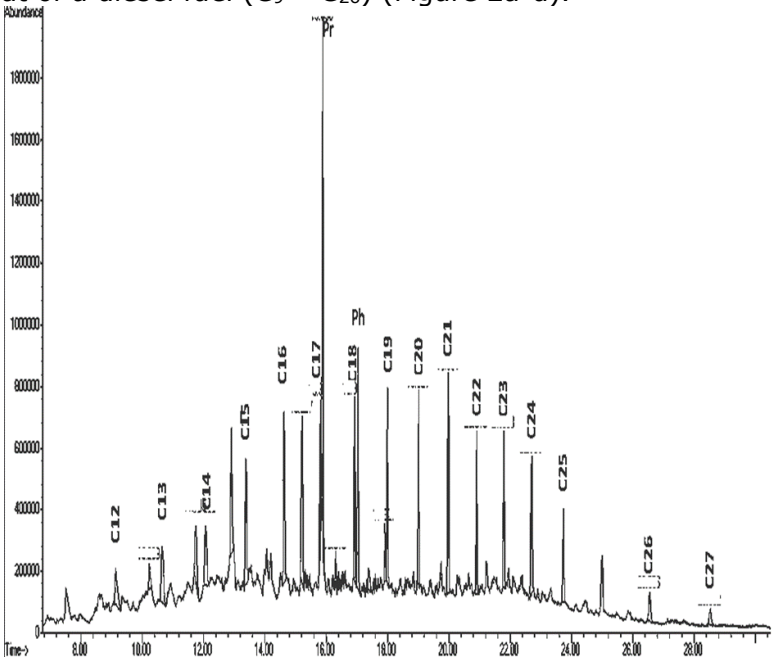

Figure 2(a). Mass chromatogram showing *n*-alkane and isoprenoids of OG7

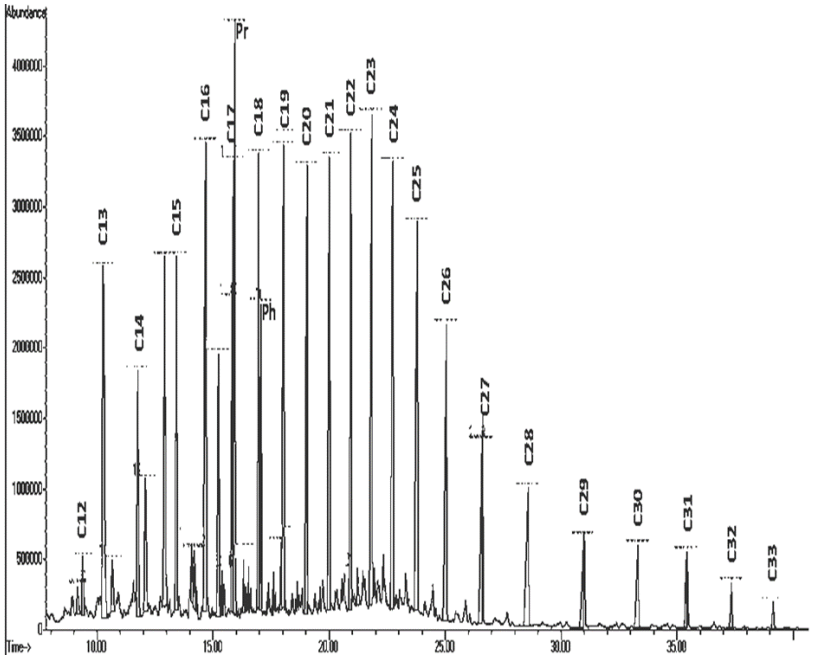

Figure 2(b). Mass Chromatogram showing *n*-alkane and isoprenoids of OG8

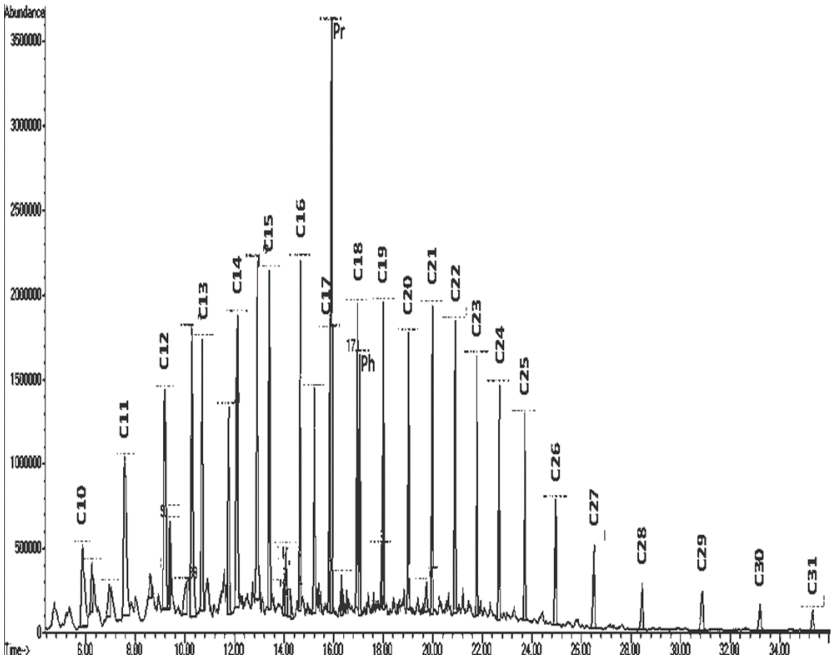

Figure 2(c). Mass Chromatogram showing *n*-alkane and isoprenoids of AZU

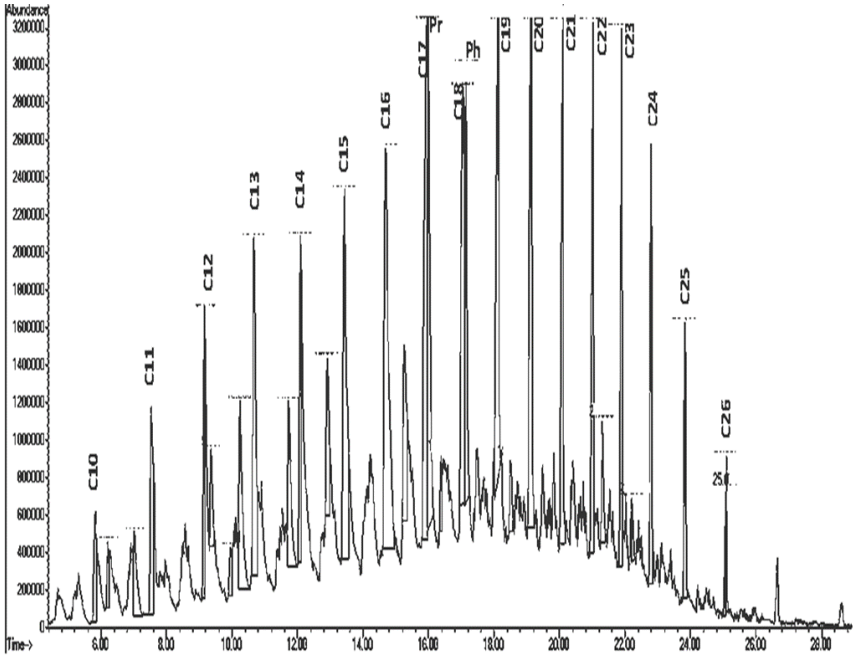

Figure 2(d). Mass Chromatogram showing *n*-alkane and isoprenoids of AGO

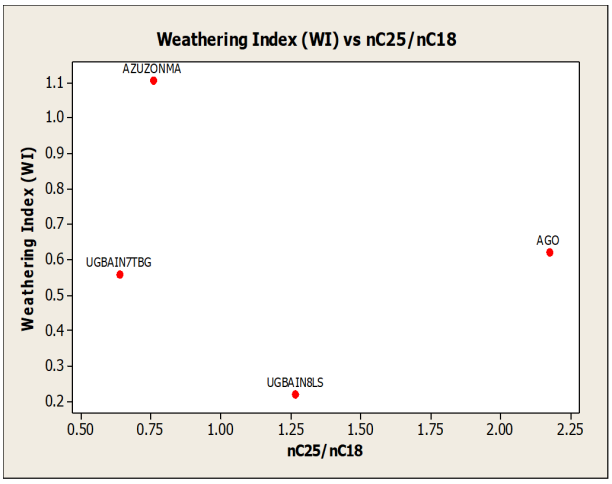

Figure 3. Plot of weathering index versus nC_{25}/nC_{18} indicating degree of weathering

The loss of the lighter fraction (C_1-C_9) in AGO can be attributed to the process of refining. With close uniformity and carbon number ranges of (C_{12} – C_{27}) and (C_{12} - C_{33}) for UG7 and UG8 respectively, the two oils may be related or suggested to have similar generation histories, but have undergone different rate of biodegradation [24-25]. A plot of weathering index versus nC_{25}/nC_{18} as seen in Figure 3 affirms the degree of weathering of most of the samples but did not actually distinguish the oil samples.

The C_{17}/pristane and C_{18}/phytane and the pristane/phytane ratios have been considered as oil weathering and source indicators, respectively [26].

3.1. Carbon Preference Index

A plot of CPI versus Pr/Ph as seen in Figure 4 can be used to discriminate oils from its product as the oils tend to have an almost similar characteristics and a seemingly triangular cluster. Carbon preference index values of the oil samples shown in Table 2, range between 0.98 – 1.35. UG7 and UG8 have the same odd to even carbon numbered preference as they showed the same CPI value of 0.98 typifying high thermal maturity [2, 27]. AZU with CPI values of 1.20 is typical of crude oil of low maturity while AGO with CPI of 1.35 can be suggested to be obtained from immature crude oil source as also reported by some researchers [2, 27-28].

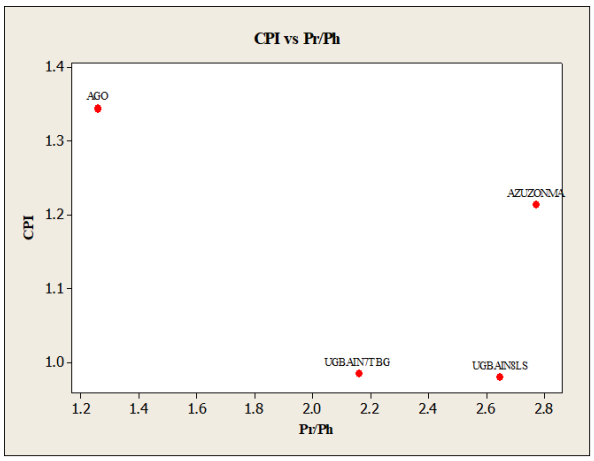

Figure 4. Plot of CPI vs Pr/Ph showing a distinct separation between the oils and its product

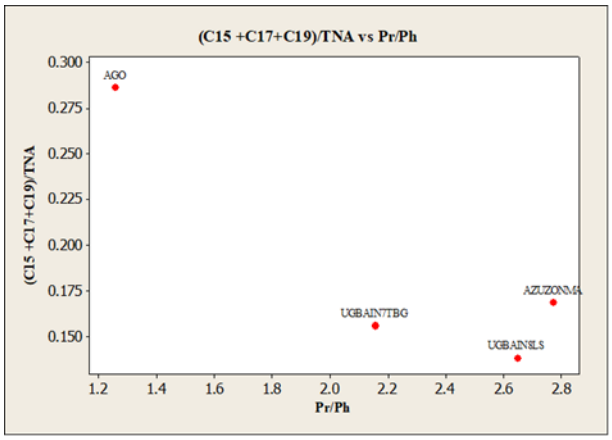

Figure 5. A plot of (C_{15} +C_{17}+C_{19})/TNA vs Pr/Ph showing the distribution of the oil samples.

3.2. Assessment of the depositional environment using n-alkanes and their ratios

The distributions of *n*-alkanes, their ratios and isoprenoids/*n*-alkane ratios have been applied to assess the organic matter contribution to source rock for hydrocarbon generation [25, 29]. The organic contribution to hydrocarbon generation can be determined by *n*-alkane concentration of major marine and terrestrial biomarkers. The %∑(nC_{15} + C_{17} + C_{19})/Total normal alkanes less than percent (< 5%) depicts low marine contribution while values greater than five percent (> 5%) typify major contribution from marine sources [30]. Also, %∑(nC_{27} + C_{29} + C_{31} + C_{33})/Total normal alkanes less than five percent (< 5%) indicate low terrestrial input while values greater than five percent (> 5%) indicate major terrestrial input to hydrocarbon generation [31-32].

It can be inferred from a plot of (C_{15} + C_{17} + C_{19})/TNA vs Pr/Ph (Figure 5) that the oils have similar characteristics hence a close proximity clustering but this not the case with the product (AGO) as it can be observed to be positioned afar off the cluster. This plot can serve as a useful tool in petroleum and petroleum product discrimination. However, from Table 2 above, the percentage relative abundances of the major marine markers were calculated to be greater than five percent (5%) for samples UG7, UG8 and AZU (16%, 14% and 17% respectively), but low percentage relative abundance of the terrestrial markers typify low terrestrial contribution to source.

The gas chromatography fingerprints showed a characteristic dominance of the short chain-alkanes (nC_{13} – nC_{20}) over the long chain *n*-alkanes (nC_{25} – nC_{33}). Long chain *n*-alkanes are typical of wax from terrestrial higher plant origin while short chain alkanes are more prevalent in marine-derived crude oils [33]. The Table 2 above shows that ∑(*n*-C_{25} – *n*-C_{33})/∑(*n*-C_{13} – *n*-C_{20}) for the samples UG7, UG8 and AZU oils are 0.15, 0.58, 0.27 respectively. This shows a higher relative abundance of the short chain alkanes than the long chain alkanes and this depicts the crude oils were generated from major marine organic origin [33]. In AGO sample, the extremely low value of ∑(nC_{25} – nC_{33})/∑ (nC_{13} – nC_{20}) ratio as seen in Table 2 above was as a result of the disappearance of long chain *n*-alkane hydrocarbon which were lost to cracking process to produce more short *n*-alkanes [33]. Evidently, a plot of ∑(C_{25}- C_{35}/∑C_{13}-C_{20} vs Pr/Ph can also be applied to an extent in segregating crude oils from its products as observed in Figure 6. The discrimination can also be attributed to the carbon range of diesel and low abundance of C_{25} – C_{30} in the diesel chromatogram (Figure 2d).

Biogenic and petrogenic hydrocarbons can be differentiated based on their precursor or source. Blumer *et al*.[34] identified Pr/Ph > 1.00, CPI value approximately 1.00 and *n*-alkane distribution of more abundance of odd numbered paraffin than even numbered paraffin in the range C_{21} – C_{33} are associated with oil of biogenic input (algae and bacterial material) in marine debris. Figure 7, which is a plot of nC_{25}/nC_{18} vs Pr/Ph is prime in distinguishing oils as it gives

a higher fraction of AGO relative to the oils hence, a cluster of close proximity between the crude oils denoting similar characteristics while the AGO is far off. The automotive gas oil has high C_{25} because it is from a higher paleoenvironment. Table 2 shows UG8 with a weak odd preference over even carbon number for C_{21} – C_{33} carbon range depicting almost equal contributions from bacteria and rock sediments. Both UG7 and AZU can be suggested to be of algae and bacterial material deposited in a marine environment [34].

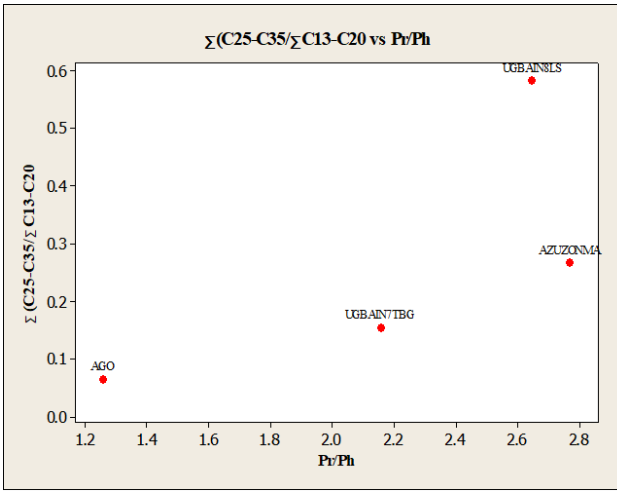

Figure 6. A discriminative plot of $\Sigma(C_{25}\text{-}C_{35}/\Sigma C_{13}\text{-}C_{20}$ vs Pr/Ph of oil samples

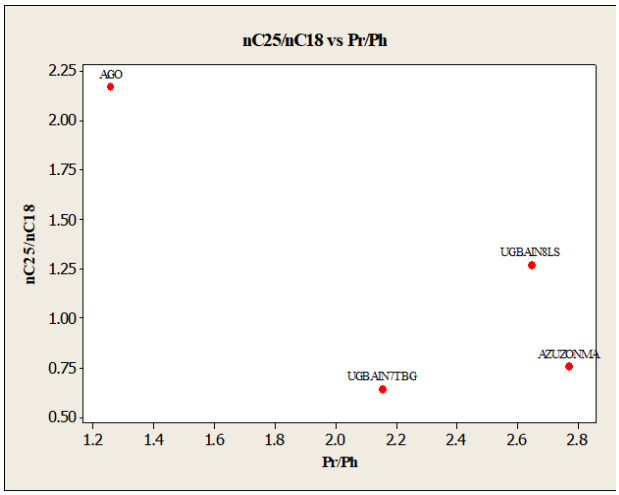

Figure 7. A plot of nC_{25}/nC_{18} vs Pr/Ph discriminating crude oil from its product.

3.3. The isoprenoid and terpane biomarker depicting depositional environment

The ratio of Pr/Ph remains the commonly used isoprenoid diagnostic ratio for evaluating the redox conditions of depositional environment. Pristane and Phytane are products of phytol side chain reaction of chlorophyll under an oxic and anoxic condition respectively [35]. Pristane to Phytane ratio has proven to be an effective and widely used diagnostic parameter for correlating oil depositional environment [36]. Pr/Ph < 1.0 (unity) indicates oil origin of highly reducing depositional, hypersaline environments, Pr/Ph ranging between 1.0 – 3.0 shows a suboxic depositional environment, but a Pr/Ph > 3.0 is an indication of terrestrial sediments input under an oxic condition [19]. Furthermore, according to Lijmbach [37], low Pr/Ph less than 2.0 is typical of oil of aquatic depositional environments to include fresh, brackish and marie water under reducing conditions, oils from coastal swamp generally have Pr/Ph ratios between 2.0 – 4.0 while very high Pr/Ph values as high as 10.0 is typical of peat swamp i.e. oxidizing condition [38]. Pr/Ph ratios ≥ 3.00 indicate an oxidizing and terrestrial or higher plant input, ratios in the range 1.00 – 3.00 have been reported to be consistent with oxidizing, siliclastic marine environment.

The GC fingerprints (Figures 2 a-d) of all the samples showed a high predominance of pristane over phytane, providing a first-hand information of an oxic paleoenvironment as the formation of pristane is favoured by the oxidation of the phytol side chain reaction of chlorophyll molecule Eneogwe and Ekundayo [39], this is not the case with the AGO sample where the relative abundances of pristane to phytane are almost equal with Pr/Ph ratio of 1.26. The Pr/Ph ratios of UG7, UG8 and AZU were calculated as 2.16, 2.65 and 2.77 (Table 2) respectively suggesting the oils were deposited under an oxic environment and high relative abundance of the marine *n*-alkane (C_{15} – C_{20}) markers in the three crude oil samples [19]. Hopane homologous ratios $C_{31}(S)/C_{31}(R)$, $C_{32}(S)/C_{32}(R)$, $C_{33}(S)/C_{33}(R)$ and homohopane indices (HHI) were further applied to confirm their redox conditions (Table 2) showed consistent trend with oxic paleoenvironment. The ratios of $C_{31}(S)/C_{31}(R)$, $C_{32}(S)/C_{32}(R)$, $C_{33}(S)/C_{33}(R)$ and HHI less than unity (< 1.0) typify oxic condition while values greater than unity (> 1.0) indicate anoxic environment [2, 5,40].

Terpane biomarker ratios for the evaluation of redox condition of depositional paleoenvironment include homohopane index (HHI), $C_{35}HH/C_{34}HH$ as well as the variation in the relative concentration of the extended hopanes (C_{31} – C_{35} HH). Sonibare *et al*.[41] reported that HHI and $C_{35}HH/C_{34}HH$ less than unity with a gradual decrease in the relative concentration of the extended hopanes from C_{31} – C_{35} are effective indicator of oils embedded in an oxic depositional environment. The relative abundance of the extended hopanes gradually decreased from C_{31} to C_{35} homohopanes for all the samples. It can be observed from Table 4 that the values of the HHI and $C_{35}HH/C_{34}HH$ for UG7, UG8, AZU and AGO range from 0.22 – 0.94 and 0.54 – 0.62. They all have values less than unity (< 1.0) and this trend is consistent with an oxidizing depositional environment confirming the relatively high pristane concentrations for all the analyzed oil samples [41].

3.4. Degree of waxiness

Peters and Moldowan [2] quantitatively determined wax content to assess depositional environment using the expression $\sum(C_{21} - C_{31})/\sum(C_{15} - C_{20})$. Ratios less than unity (1.00) indicate low wax content typical of marine-derived oils while ratios above unity typify oils of high wax content consistent with terrigenous organic matter. UG7 (0.12), UG8 (0.66), AZU (0.46) have ratios less than unity indicating they are marine-related oils while AGO (0.41) can be suggested to be obtained from oil of marine origin.

3.5. Assessment of the degree of biodegradation

Biodegradation of crude oil involves series of microbial activities which alter the alkanes, isoprenoids and biomarker profile in crude oils and source rocks [42]. It results in the gradual wearing away of the long-chain hydrocarbons and occurs in the presence of dissolved oxygen in water, absence of hydrogen sulphide and at a temperature range of 65°C – 80°C. According to Onojake *et al*. [43] Pr/nC_{17} and Ph/nC_{18} < unity strongly suggest no biodegradation has occurred. A plot of Pr/nC_{17} vs Ph/nC_{18} shown in Figure 8 did not clearly distinguishing the oils and its product but it gives the extent of biodegradation.

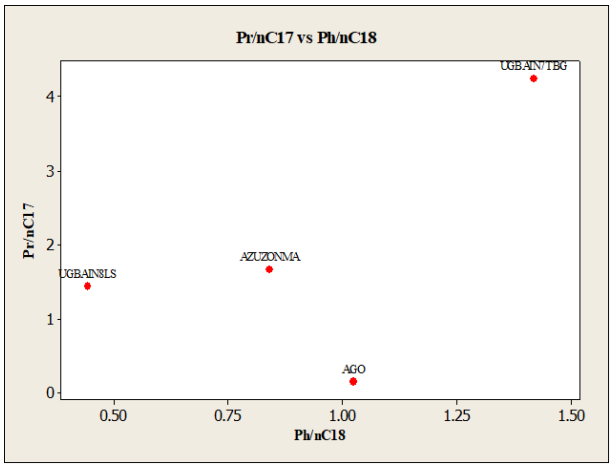

Figure 8. Plot of Pr/nC_{17} vs Ph/nC_{18} showing the extent of biodegradation of the oil samples.

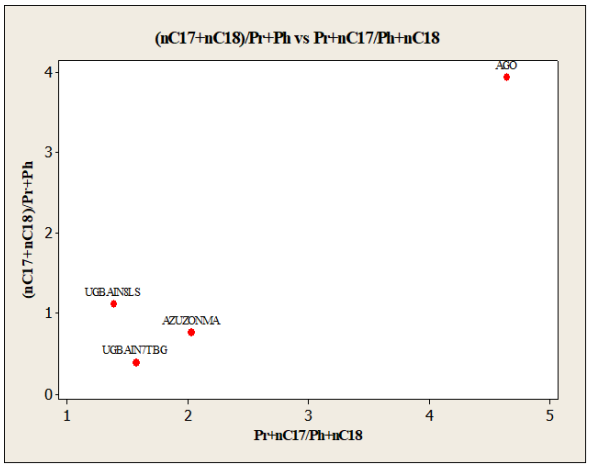

Figure 9. Plot of (nC_{17} + nC_{18})/Pr + Ph vs Pr + nC_{17}/Ph + nC_{18} showing a distinct discrimination of the oil samples.

Table 2 shows Pr/nC_{17} ratios for all the samples were computed to be above unity while Ph/nC_{18} for UG8 and AZU showed inconsistency with ratios unexpectedly less than unity (0.44 and 0.84). Sample UG7 can be said to be the most biodegraded with Pr/nC_{17} and Ph/nC_{18} ratios of 4.24 and 1.42 respectively as shown by its short *n*-alkane nC_{12} – nC_{27} carbon range. Samples UG8 and AZU were fairly biodegraded while AGO showed biodegradation ratios typical of an unweathered oil sample. However, a plot of (nC_{17} + nC_{18})/Pr + Ph vs Pr + nC_{17}/Ph +

nC_{18} (Figure 9) is vital as it shows a cluster of the crude oil samples depicting similar characteristics between the oil samples and it clearly separates the petroleum product making it a useful plot for discriminating refined and unrefined products as it applies to this study.

3.6. Dendrogram and star plot for similarity and differentiation of analyzed samples

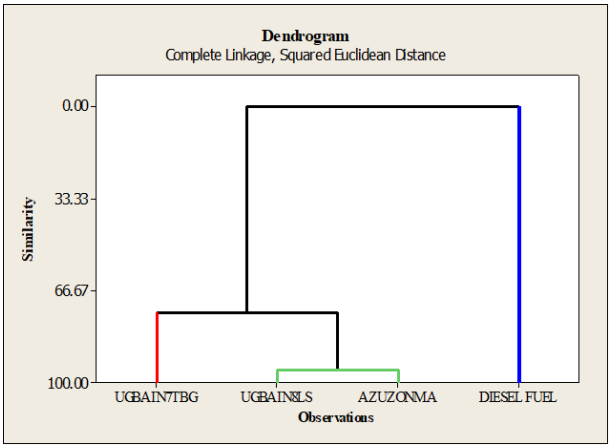

Figure10. A dendrogram showing the level of similarity among the samples

A dendrogram is a higher statistical application for segregation of clusters based on hierarchy, it is interpreted in the form of percentage similarity. In Figure 10 it can be observed that at 74.91% the three (3) crude oil samples UG7, UG8 and AZU are related but as it goes higher the degree/level of similarity between UG8 and AZU is very close at 95.29%. However, the diesel (AGO) and the crude oil samples show no level of similarity as it is at 0.00% showing no degree of similarity. This plot is vital in discriminating petroleum and petroleum products.

4. Conclusion

The comparative investigation of some molecular biomarker signatures of crude oil samples UG7, UG8, AZU and AGO using gas chromatography mass spectrometry provided a means of discriminating the samples. The examination of the *n*-alkanes, isoprenoids (pristane and phytane) and hopanes (pentacyclic triterpanes), and elaborated the respective characteristics of the samples. The samples were within their respective carbon range marginally or totally, in terms of CPI values the samples samples were marked by marine organic contributions from shale source rock and were obtained from terrigenous higher plants deposited under oxic environmental conditions. Biodegradation was severe in UG7 but fair in UG8 and AZU and not pronounced in AGO. The ability to identify the different characteristic fingerprints of the studied samples based on values calculated from computed data and their respective chromatograms which gave an insight in terms of oil-oil correlation, oil- source correlation, which serves as a very useful tool in event of an oil spill in order to trace the source either to a crude oil or its refined product(s) using these traits. Also, geochemical analysis of weathered samples can serve as an alternative to the complex forensic analysis of spills if properly handled as it is economically friendly.

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