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HYDROCARBONS BIODEGRADATION AND EVIDENCE OF MIXED PETROLEUM SOURCE INPUTS TO SURFACE SEDIMENTS FROM THE CROSS RIVER SYSTEM, S. E. NIGERIA.

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ABSTRACT

Chromatographic analysis of extracts from the Cross River system show evidence of variable composition of biogenic n-alkane profile with dominance of terrigenous over aquatic organic matter present (LHC/SHC-0.36-10.57) at upstream location reflecting the natural background levels and marked levels of petroleum residues (UCM-0.16-11.3) at various sampling stations (downstream). The mild hydrocarbons biodegradation status of these sediment is partly indicated by the pristane/nC_{17} (0.9-2.56) and phytane/nC_{18} (0.10-0.85) ratios. Although the 17α(H)25-norhopane detected in the system represent heavy biodegradation, the n-alkane signatures show some characteristics typical of mild biodegradation, suggesting inputs of mixed/different petroleum origins. In addition, examination of the mass chromatograms of the hopane series shows evidence supportive of inputs from different petroleum source locations, reflected in their thermal history differences, variation in biodegradation patterns and biomarker composition. This distinction is a reflection of utilization of petroleum products derived from different source locations imported into the Nigerian economy.

KEYWORDS: Biodegradation, Mixed Petroleum, Biomarker, Cross River System, Nigeria.

INTRODUCTION

Due to extensive use of petroleum products in Nigeria, almost all compartment of the environment are contaminated by hydrocarbons. Hydrocarbon contamination that has been studied includes microbial degradation processes by bacteria, fungi and yeast (Chaillian *et. al*.,2004; Outdot *et. al*.,2003). Microbial communities play significant roles in the transformation of organic contaminants in marine environments. The role of intrinsic bioremediation in degrading and mitigating the impact of organic contaminants, resulting from industrial and human activities, has been demonstrated (keller and zenger, 2004; Tringe *et al*, 2005; walker et al, 2005 and slater *et al*, 2006). Accurate assessment of the pathway (reactants and products) involved in microbial degradation of organic contaminates is important for effective environmental risk assessment and remediation efforts. These pathways involve changes in contaminant compound distribution such as hopanes (this study) and loses due to complete degradation process (mills *et.al*.,2003).

Due to the relative resistance of biomarker to degradation, comparison of the different amounts of biomarker types has been used to rank the extent of biodegradation as mild, moderate and heavy biodegradation. According to this scale, mildly biodegraded petroleum in contaminated environments exhibit absence of low molecular weight n-alkanes whereas the occurrence of 17α(H) 25-norhopane is indicative of heavy biodegradation. Gas chromatograms of recent contaminated marine sediments often exhibit evidence of admixture of petroleum and biogenic (higher plant, bacteria and algal – derived) hydrocarbons (peters and moldowan 1993).

Previous studies on this system were mostly focused on fisheries ecology (moses, 1987); ecological study (Enyenihi *et. al*., 1987);

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edible clam Egeria radiate (Etim and Akpan 1991). Others include investigation of hydrology of lower Cross River (lowenberg and Kunzel, 1992); distribution of heavy metals and total hydrocarbons (Asuquo _et.al_., 1999); the predominance of n-docosane/docosene in surface sediments (Oyo-Ita *et.al.*, 2006). The main objectives of this present study were to provide evidence of hydrocarbons biodegradation and inputs of mixed petroleum residues from different source locations using biomarker approach.

1.1 The Study Area

The study area (Fig. 1), extends from Mbo river (station CR1) to 1km beyond Itu bridge (station CR10), a distance of approximately 95km covering a remote area (station CR9). Depending on the type of regional impact, the coastal water was divided into three zones (Table 1.).: Zone I (Estuary – near Oron beach- highly populated area, receives a significant amount of untreated sewage, agricultural effluents, possible oil spills and ship/boat traffic pollution.); Zone II (Lower Cross River –near Oku Iboku beach area and thick swamp forest- receives industrial waste effluents, untreated wastewater/discharge. ;Zone III (Upper Cross River –near Itu beach area, receives sewage and agricultural waste as well as engine boat pollution. Niger

The nd latitude 4^{o} 00^{l} N and 8^{o} 00^{l} N, and covers an area of 54,00km^2, of which 14,000km^2 lies in Cameroon and 39,000km^2 is in Nigeria. The river is formed from numerous tributaries arising from the Western slopes of the Cameroon mountains and flows south-westwards into the Atlantic ocean with a discharge of between 879 and 2533m^3/sec (Lowenberg and Kunzel, 1992), the system is exposed to temporal flooding depending on the tides and the season and has large fluctuation in hydrographical conditions (Lowenberg and Kunzel, 1992).

2.0 Experimental methods

2.1 Sampling

A total of 10 composited surface sediment samples (each a composite of three samples) were collected randomly during the month of January, 1999 from the three zones with the grab samper (0.1m^2). Samples were removed from the middle of the grab, wrapped in aluminium foil and stored frozen at -4^{o}c. Prior to the extraction, the samples were freeze-dried sieved to pass through 230 mesh (<630µm)

To produce the level of contamination, all glasswares were cleansed with soap and water, rinsed with distilled water, heated in an oven at 550^{o}C for 8 hours to combust any traces of surface organic matter and finally rinsed twice with ultra- pure hexane and acetone mixture. Powdered surface sediments samples (50g) were extracted with hexane- acetone (1:1 200ml) in a soxhlet apparatus for 48 hours. Extracts were desulphurised by addition of activated copper (30g) into the round bottom flask during extraction, and evapourated to near dryness using vacuum evaporator. The weight of extract obtained represents the amount of soluble organic matter (SOM). Asphaltenes were precipitated from the extracts with dichloromethane and petroleum ether (b.p 40-60^{o}C) mixture (1:30) and centrifuged at 3000 rpm for about 20 minutes (Schoel *et al*., 1987). The sepeartion of the deasphalted extracts into saturated, aromatic and heterocompounds (NSO) was carried out by column chromatography (column 30 x 1.2cm) using silica gel (20g, 70/230 mesh, activated for 6h at 400^{o} C) and alumina (10g, neutral, activated for 2h at 700^{o}C). The saturated fraction was eluted with hexane (50ml) and finally a mixture of methanol-dichloromethane (1:2, 50ml) was used to remove the hetero fraction (or NSO).

Gas chromatography-mass spectrometry (GC-MS) analysis of the fractions was performed on a Hewlett-Packard Model 6890 GC coupled to a Hewltt-Packard model 5973 quadropole MSD. Separation was achieved on a fused silica capillary column coated with DB5 (30m X 0.2mm i.d.,0.25µm film thickness). The GC operating conditions were as follows: temperature hold at 65^{o}C for 2min increase from 65 to 300^{o}C at a rate of 6^{o}C min^{-1}, with final isothermal hold at 300^{o}C for 20min. Helium was used as carrier gas. The sample was injected in splitless mode with the injector temperature of 300^{o}C. The mass spectrometer was operated in the electron impact mode (EI) at 70Ev ionisation energy and scanned from 50 to 650 dalton.

3.0 Result and discussion

3.1 n-alkanes and Isoprenoid Hydrocarbons

The concentrations (mgkg^{-1}) of n-alkane in the range nC_{12}-nC_{33} and isoprenoid hydrocarbons are presented in table 2. Gas chromatogram of n-alkanes from the relatively prestine upstream station (CR9) indicates hydrocarbon of biogenic origin (fig.2a). This observation is confirmed by

(LHC/SHC=3.45) at state CR9 (Schelske and Silliman,2003;Meyers and Ishiwatari, 1993); an

(terrigenous) over algal detritus in the Cross River system.

Table 1. Samples collected, general characteristics and geochemical composition of aromatic fraction in sediment from Cross River system, Nigeria.

Zones	I				II			III		
Sample code	CR-1	CR-2	CR-3	CR-4	CR-5	CR-6	CR-7	CR-8	CR-9	CR-10
Coordinates	N4^{o} 43.961 E8^{o} 21.327	N4^{o} 46.531 E8^{o} 18.908	N4^{o} 49.927 E8^{o} 15.501	N4^{o} 52.675 E8^{o} 12.742	N4^{o} 56.879 E8^{o}09.334	N5^{o} 00.437 E8^{o} 07.062	N5^{o} 04.318 E8^{o} 06.250	N5^{o} 04.318 E8^{o} 06.250	N5^{o} 12.726 E8^{o} 03.491	N5^{o} 12.258 E8^{o} 00.222
Location name	Oron beach				Oku Iboku beach			Itu beach		
Characteristic features of the environment	Untreated sewage, agricultural waste, oil spills, ship/boat pollution.				Industrial waste effluents, untreated waste water/sewage engine boat pollution.			sewage, agricultural waste.		
Sediment texture	Clayey	clayey	Clayey	silty	clayey	silty	silty	silty	sandy	Silty
TOC (%)	4.03	3.64	4.35	4.56	4.2	4.38	2.77	1.27	2.66	4.26
SOM (mg/kg dry wt.)	3,000	3,680	1,920	2,950	3,710	4,140	2,650	1,510	1,140	1,850

Table 2: Concentrations of n-alkane series and isoprenoid hydrocarbons in surface sediments from Cross River Systems.

Compound Type	Compound Name	MW	Molecular formula	Concentration in mg/kg (ppm)									
				CR 1	CR 2	CR 3	CR 4	CR 5	CR 6	CR 7	CR 8	CR 9	CR10
Isoprenoid (Pristane)	2,6,10,14-Tetramethyl Pentadecan	268	$C_{19}H_{40}$	7.40	6.45	8.90	1.60	1.85	0.78	0.70	1.77	0.03	0.04
Isoprenoid (Phytane)	2,6,10,14-Tetramethyl Hexadecane	282	$C_{20}H_{42}$	4.05	2.95	6.28	1.43	1.80	0.65	0.53	0.78	0.02	0.02
Isoprenoid (Norpristane)	2,6,10,14Tetramethyl Tetradecane	254	$C_{18}H_{38}$	nd	nd	2.96	nd	nd	nd	nd	nd	nd	nd
n-Alkanes	n-tetradecane	198	$C_{14}H_{30}$	nd	nd	0.33	nd	nd	nd	nd	nd	nd	nd
	n-pentadecane	212	$C_{15}C_{32}$	nd	nd	0.83	0.74	nd	0.51	0.65	0.97	0.85	0.93
	n-hexadecane	226	$C_{16}H_{34}$	1.92	2.24	3.15	3.96	1.15	1.39	1.95	1.70	0.18	0.61
	n-Heptadecane	240	$C_{17}H_{36}$	3.14	3.35	3.73	0.99	1.85	0.64	0.83	1.20	0.09	0.30
	n-Octadecane	254	$C_{18}H_{38}$	4.41	5.35	7.79	0.41	4.33	2.70	1.87	2.42	0.39	1.50
	n-nonadecane	268	$C_{19}H_{40}$	1.12	2.49	3.82	0.66	0.67	0.65	1.49	1.00	0.06	0.30
	n-Eicosane	282	$C_{20}H_{42}$	2.61	2.12	1.15	5.66	4.84	2.11	1.26	0.65	0.28	0.13
	n-Heneicosane	296	$C_{21}H_{44}$	3.02	3.23	3.03	1.16	1.59	0.58	0.30	0.83	0.08	0.30
	n-Docosane	310	$C_{22}H_{46}$	3.36	2.73	3.72	4.36	6.81	1.56	5.82	5.21	0.21	0.62
	n-tricosane	324	$C_{23}H_{48}$	5.36	1.35	3.20	3.12	2.84	0.93	0.24	0.59	0.09	0.60
	n-tetracosane	338	$C_{24}H_{50}$	5.31	0.34	3.41	3.91	2.95	1.14	0.54	0.76	0.15	0.30
	n-pentacosane	352	$C_{25}H_{52}$	6.84	0.31	2.72	3.27	1.92	0.68	0.18	0.38	0.07	0.30
	n-hexacosane	366	$C_{26}H_{54}$	4.65	0.22	1.72	2.07	1.25	0.64	0.33	0.48	0.07	0.20
	n-heptacosane	380	$C_{27}H_{56}$	6.42	nd	2.45	3.13	1.40	0.61	0.17	0.20	0.06	0.20
	n-octacosane	394	$C_{28}H_{58}$	3.83	nd	4.80	1.53	0.83	0.41	0.15	0.24	0.07	0.18
	n-Nonacosane	408	$C_{29}H_{60}$	16.42	nd	10.58	13.02	3.38	2.26	0.84	0.31	0.31	0.60
	n-triacontane	422	$C_{30}H_{62}$	3.46	nd	0.79	1.80	1.08	0.51	0.40	0.13	0.06	0.10
	Hentriacontane	436	$C_{31}H_{64}$	8.47	nd	6.91	9.17	1.85	1.43	0.86	0.19	0.28	0.32
	Dotriacontane	450	$C_{32}H_{66}$	1.34	nd	1.11	0.81	0.50	0.31	1.06	0.23	0.02	0.15
	n-tritriacontane	464	$C_{33}H_{66}$	nd	nd	nd	nd	0.57	0.97	nd	nd	nd	nd
∑ Alkanes				4.90	3.55	3.84	3.02	2.17	1.02	1.05	0.99	0.14	0.35

N/B: nd- not detected

Table 3: Concentrations of hopane series in the surface sediments of Cross River System

Identified numbered	**Compound name**	**MW**	**Molecular formula**	**Concentration (mg/kg)**									
				CR1	**CR2**	**CR3**	**CR4**	**CR5**	**CR6**	**CR7**	**CR8**	**CR9**	**CR10**
4	17α(H)21β(H)trisnorhopane (Tm)	370	$C_{27}H_{42}$	0.95	1.10	1.02	0.43	0.30	0.25	0.36	0.42	nd	0.21
5	17α(H)21β(H)trisnorneohopane (Ts)	370	$C_{27}H_{42}$	0.45	0.51	0.50	0.21	0.15	0.12	0.18	0.21	nd	0.10
6	17α(H)21β(H)bisnorhopane	384	$C_{28}H_{44}$	0.23	0.25	0.20	0.10	0.08	0.06	nd	nd	nd	nd
7	17α(H)21β(H)25-norhopane	398	$C_{29}H_{48}$	1.20	2.00	1.00	0.65	0.44	0.38	2.53	2.15	nd	1.90
8	Oleanane	412	$C_{30}H_{52}$	1.25	2.30	1.05	0.79	0.56	0.47	nd	nd	nd	nd
9	17α(H)21β(H)hopane	412	$C_{30}H_{52}$	1.36	2.58	1.21	0.85	0.61	0.51	0.75	0.83	nd	0.42
10	17β(H)21β(H)hopane	412	$C_{30}H_{52}$	0.65	0.83	0.45	0.29	0.29	0.20	nd	nd	nd	nd
11	17α(H)21β(H)homohopane (S)	426	$C_{31}H_{54}$	0.61	0.89	0.49	0.31	0.25	0.25	0.84	0.75	nd	0.34
12	17α(H)21β(H)homohopane (R)	426	$C_{31}H_{54}$	0.64	0.91	0.51	0.35	0.29	0.29	0.19	0.64	nd	0.79
13	17β(H)21α(H)moretane	426	$C_{31}H_{54}$	0.62	0.80	0.41	0.26	0.18	0.18	nd	nd	nd	nd
14	17β(H)21β(H)bishomohopane	440	$C_{32}H_{56}$	1.40	2.60	1.26	0.93	0.65	nd	nd	nd	nd	nd
15	17α(H)21β(H)bishomohopane (S)	440	$C_{32}H_{56}$	0.60	0.76	0.38	0.20	0.10	0.12	0.61	0.18	nd	0.35
16	17α(H)21β(H)bishomohopane (R)	440	$C_{32}H_{56}$	0.54	0.70	0.30	0.15	0.08	0.10	0.54	0.69	nd	0.28
17	17β(H)21β(H)trishomohopane	454	$C_{33}H_{58}$	0.45	0.83	0.41	0.30	0.25	0.20	nd	nd	nd	nd
18	17α(H)21β(H)trishomohopane (S)	454	$C_{33}H_{58}$	nd	nd	nd	nd	nd	nd	0.48	0.53	nd	0.20
19	17α(H)21β(H)trishomohopane (R)	454	$C_{33}H_{58}$	nd	nd	nd	nd	nd	nd	0.40	0.48	nd	0.15
20	17α(H)21β(H)tetrahomohopane (S)	468	$C_{34}H_{60}$	nd	nd	nd	nd	nd	nd	0.38	0.40	nd	0.13
21	17α(H)21β(H)tetrahomohopane (R)	468	$C_{34}H_{60}$	nd	nd	nd	nd	nd	nd	0.34	0.35	nd	0.10

N/B: nd – Not detectable

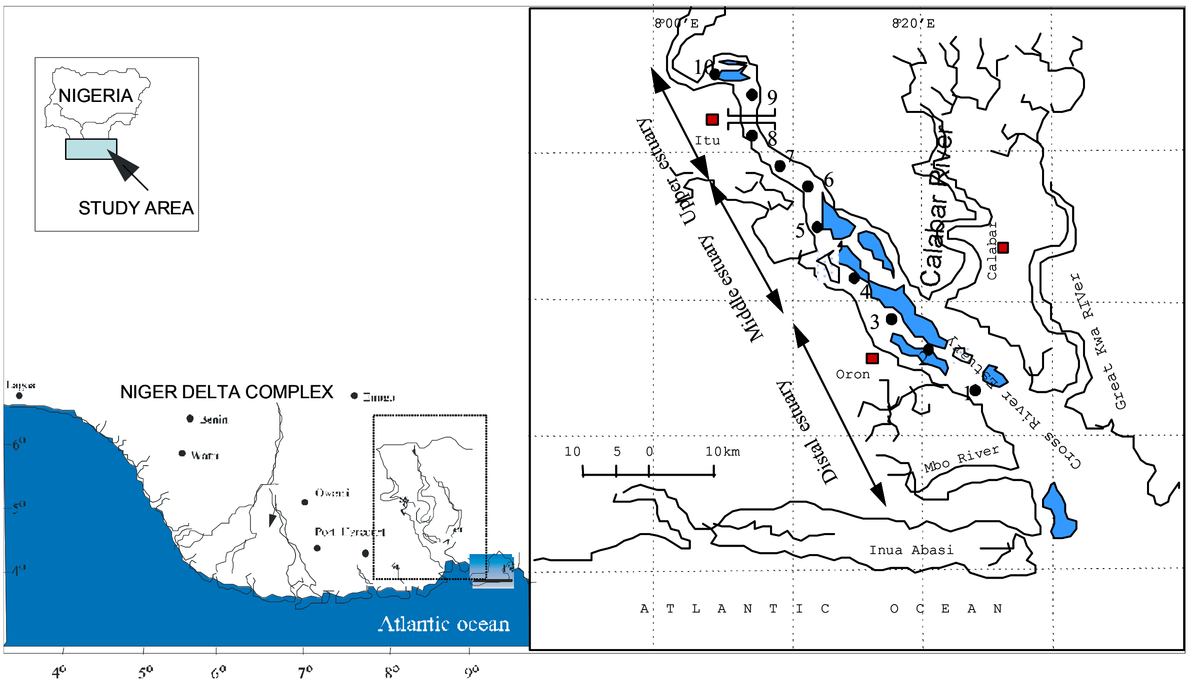
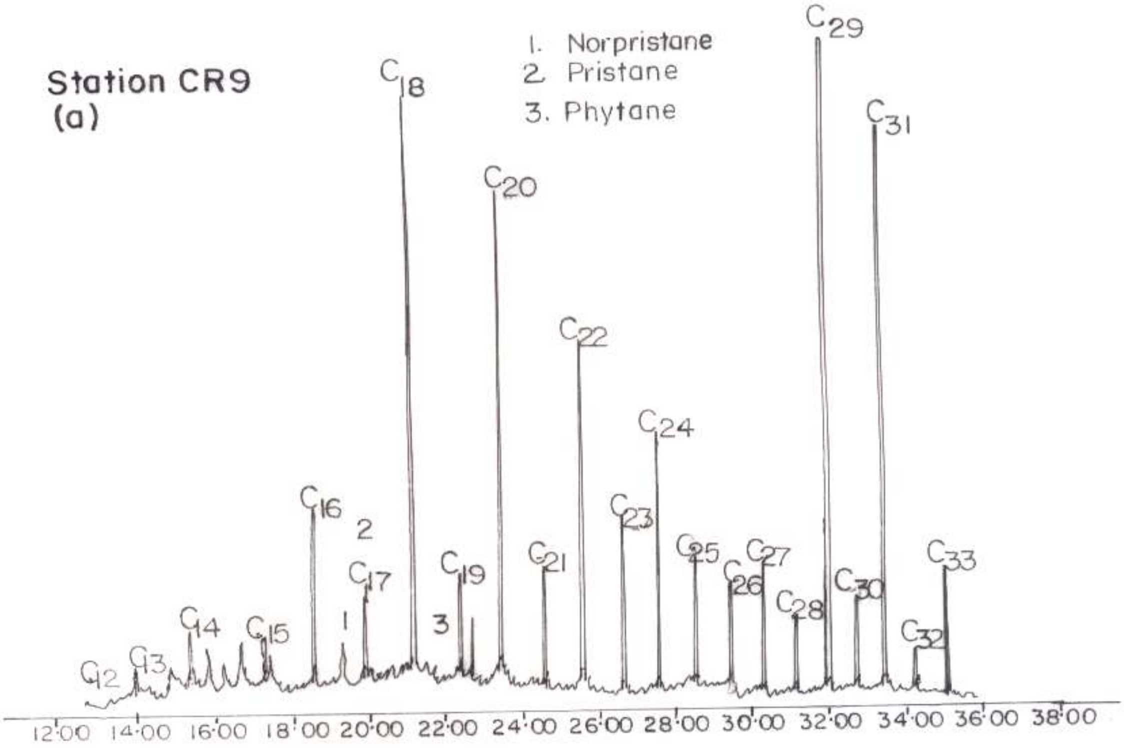


Fig. 1: Map of the Niger Delta of Nigeria showing the sampling locations in the Cross River system.


Station CR9
(a)
1. Norpristane
2. Pristane
3. Phytane
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
C24
C25
C26
C27
C28
C29
C30
C31
C32
C33
1
2
3
12:00
14:00
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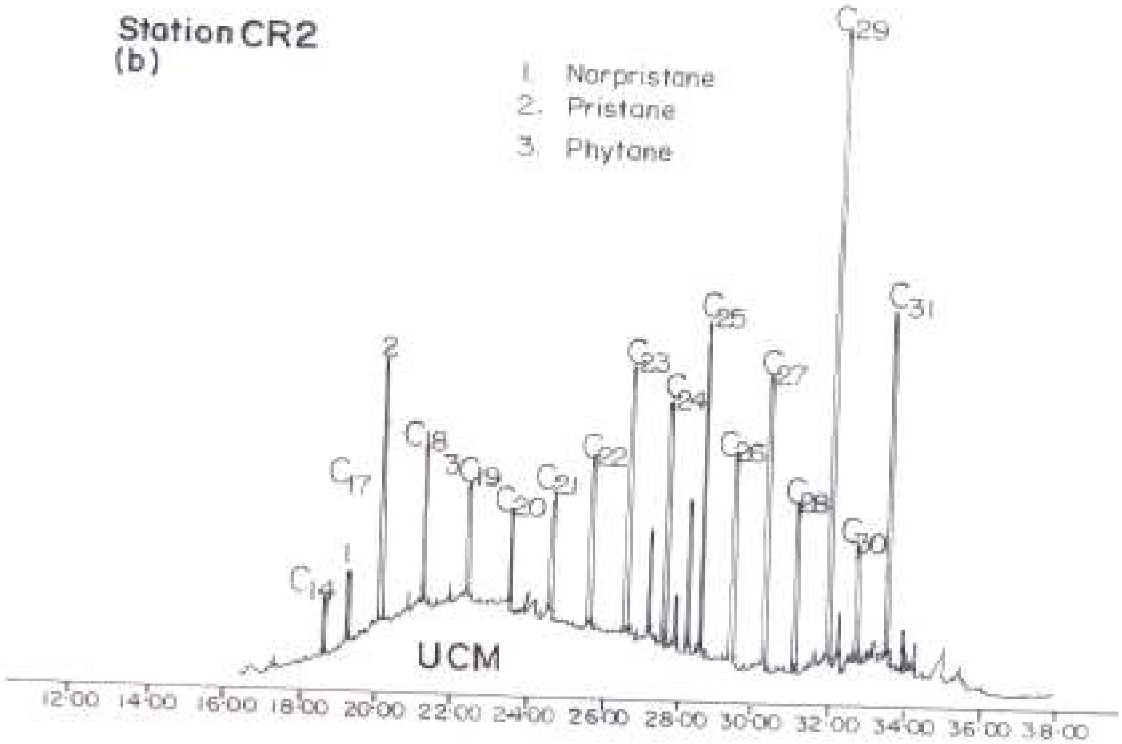
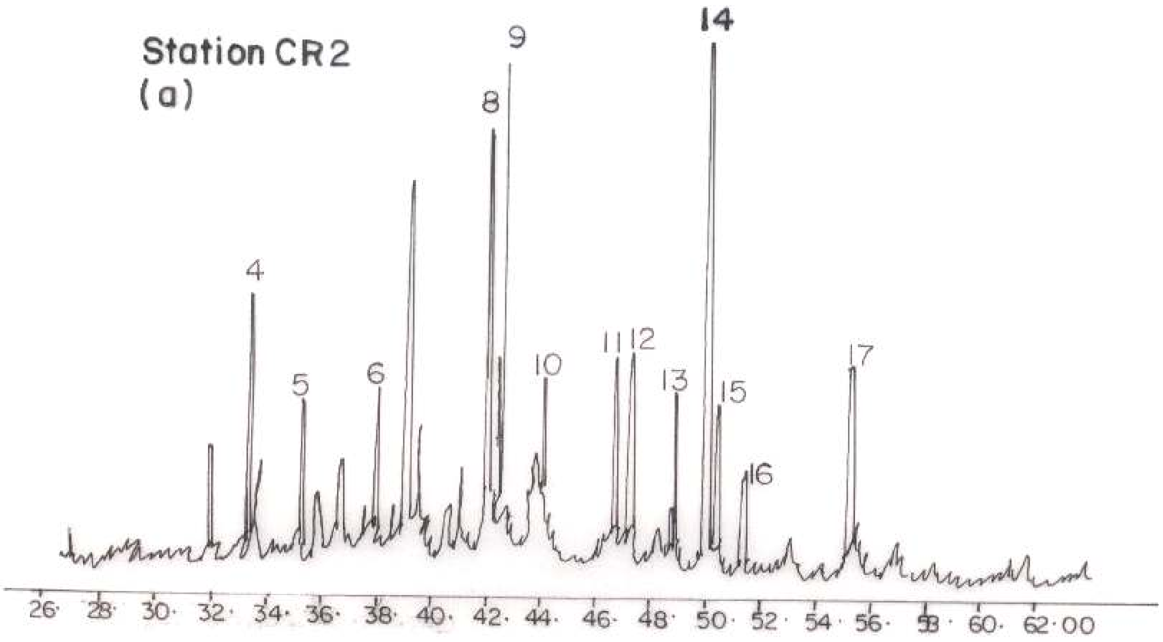


FIG. 2a & b: Gas chromatograms indicating (a) non-depletion and (b) depletion of low molecular weight n-alkanes ($<nC_{15}$) respectively with predominance of pristane over phytane from the Cross River System



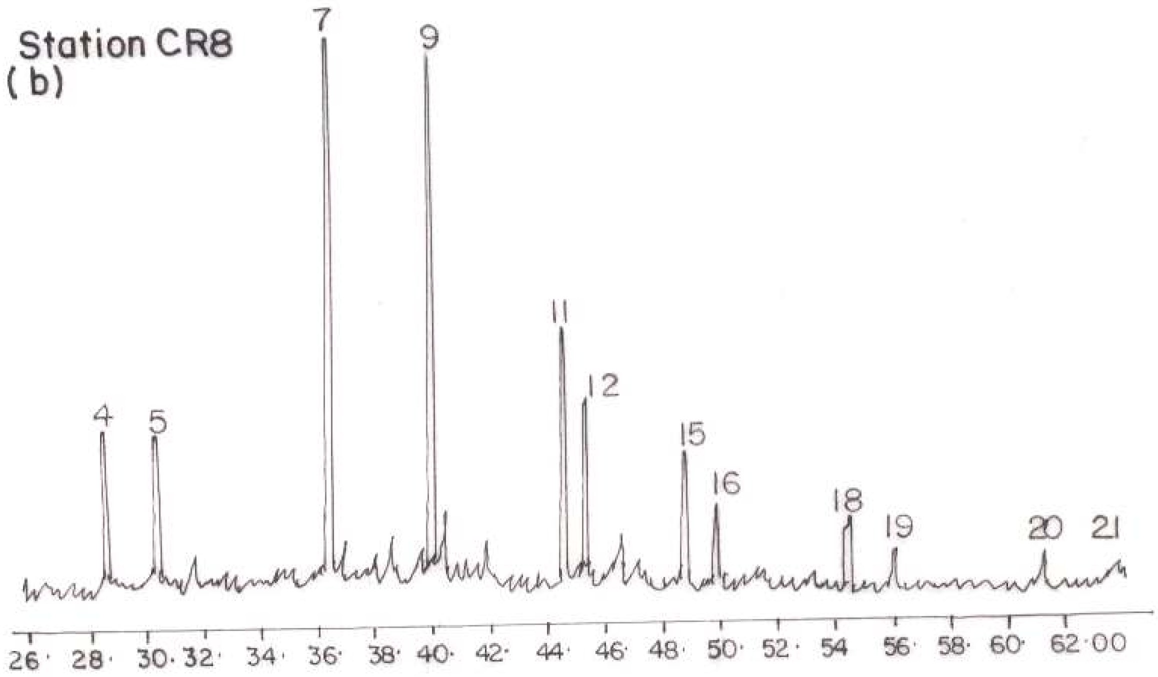


FIG. 3a & b: Mass chromatograms of (a) Hopane series for Zone I (b) Hopane series for Zone III of surface sediments from the

Sediment samples at the estuary are more vulnerable to petroleum contamination (eg station CR2) due to their proximity to ship/boat traffic channels. Diagnostic evidence for petroleum contamination of these sediments is provided by the measurement of unresolved complex mixture (UCM- o.16-11.3; fig.2b; Table3). This UMC is therefore being used as diagnostic indicator of petroleum contamination of the system (Peters and Moldowan 1993).

During the study, mild biodegradation is observed typified in the depletion of low molecular weight n-alkanes ($<C_{15}$) with no significant alteration of acyclic isoprenoid hydrocarbons (eg. Pristance and phytane). In our results, input of this mildly biodegraded petroleum is supported by higher pristane/nC_{17} and phytane/nC_{18} ratio at CR2 (2.56 and 0.78 respectively, Table 3). The non-detection of other petroleum biomarkers such as hopanes in the sediment sample from the upstream station (CR9 Table 3 suggests that the relatively low level of the isoprenoid hydrocarbons at station CR9 (fig 2a) may have originated from diagenesis of chlorophyll phytyl chain rather than direct petroleum input. A much higher level of pristane and phytane measured at station CR2 (fig. 2b) may reflect a combination of diagenetic process and direct petroleum input to the Cross River system.

Hopane series

Evidence that the surface sediment from the Cross River system are contaminated by petroleum from different location is provided by comparison of m/z-191 mass chromatograms of extract from stations CR2 (fig.3b) . A close examination of fig 3b indicate a regular pattern of decreasing levels of 17α(H)21 (H)homohopanes ($C_{31}>C_{32}>C_{33}>C_{34}$) with the 22R epimers being more susceptible to biodegradation than their counterparts in the S-configuration. This regular biodegradation pattern is absent in sediment sample from station CR 2 (fig. 3a) and shows an unexpected reversed pattern for c_{31}-homohopane where the S-counterpart is more biodegraded. This implies that the system is contaminated by the petroleum of different origin that must have experienced variable extent of biodegradation in their respective reservoirs. Comparison of the chromatograms of two representative sediment sample (fig.3a and 3b) shows differences in the extent of biodegradation of the petroleum hydrocarbons,. While sample from station CR2 exhibits a distribution indicating a higher abundance of 17α(H)21 β(H)hopane (C_{30}) than 17α(H)25-norphopane(C_{29}), a reversed pattern prevails in sample from state CR8, reflecting variable extent of biodegradation by different microorganisms in reservoir. In addition, the detection of oleanane at station CR2 ,a useful specific indicator compound for Nigeria crude oil products analogous to the petroleum source characteristic, 17α(H)18 β (H)21β (H)-28,30-bisnorhopane in crude oil of western United State, Los Angeles (simoneit *et al*., 1988 and its absent at station CR8 suggests input of petroleum with different source organic facies (terrestrials versus marine). This difference is reflective of the variation in organic matter source for this petroleum product. Furthermore, the present of 17β(H)21a(H)-moretane (C_{31}) and other thermally immature hopane compound in sample from station CR2 (fig 3a) and their absence in sample from station CR8 (fig.3b) support the idea that the Cross River system is contaminated by petroleum from different source locations. These differences in the petroleum source input at various sampling point may be attributed to utilization of other petroleum products including Nigeria refined product imported into the economy.

To further support the contamination of the cross river system by petroleum of different origins, the two representative sample (fig.3a and 3b) show difference in extent of biodegradation of hopanes. For instance, it has been shown that 17α(H)28,30-bisnorhopane is demethylated during biodegradation to 17α(H)25,28,30-trisnorhopane (moldowan *et al*., 1984) similar trend is observed in the contaminated sediment from the cross River system where the absence of 17α(H) 28, 30-bisnorhopane may be associated with its degradation to 17α(H)25,28,30-trisnorhopane in the in the reservoir (fig.3b).17α(H)25-norhopane (C_{29}) detected in some sediment from the Cross River system is a typical biomarker compound found in most but not all heavily biodegraded petroleum (Trendel *et at*., 1990). The compound appears to result from bacterial removal of methyl group ta c-10 from the regular hopane (Peter and Moldowan, 1993). Although the 17α(H)25-norhopane, accepted by many petroleum geochemists as an indicator of heavy biodegradation, was detected in sediments from the Cross River system, the gas chromatogram of n-alkanes that indicate evidence of petroleum contamination (high UCM – fig.2b) show some characteristics of mild biodegradation due to depletion of only the low molecular weight n-alkanes while the isopreniod hydrocarbons remain. This scenario can be explained by considering that the surface sediments from the Cross River system, exemplified in sample from station CR2, are contaminated with mixed petroleum residues, comprising of both mildly (a consequence of low molecular weight n-alkanes depletion) and heavily biodegraded (due to detection of 17a(H) 25-norhopane) petroleum. In other words if this sample were contaminated only by heavily biodegraded petroleum, them the GC n-alkanes signatures would have reflected not only depletion of low molecular weight n-alkanes but also those of high molecular weight including the isoprenoid hydrocarbons. This mixed petroleum concept sometimes occurs in some petroleum reservoirs (pan et al.,2003) All these sum up to the idea that the surface sediments from the Cross River system are not only contaminated with mixed petroleum (eg.CR2) but also with petroleum residues at other stations (eg.CR 8) that had experienced different thermal history and variable biodegradation behaviour in reservoirs.

CONCLUSION

Gas chromatograms of n-alkanes distribution show evidence of not only biogenic (dominance of terrigenous over aquatic) compositions in surface sediments from the upstream remote station, reflecting the natural background of the Cross River system but also input from anthropogenic activity (e.g. petroleum) at other sampling locations. Although the presence of 17α(H)25-norhpones in some sediment samples indicates heavy biodegradation of petroleum hydrocarbons, the n-alkane profiles show some characteristic typical of mild biodegradation at these sampling stations, suggesting contamination by mixed petroleum origin. The mass chromatograms of hopane series show evidence of inputs of petroleum from different origins or locations that had experienced variable extent of biodegradation and different thermal history in reservoirs, a consequence of utilization of different petroleum products imported into the Nigeria economy.

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