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PREDOMINANCE OF N-DOCOSANE/DOCOSENE AS MOLECULAR INDICATORS OF MICROBIAL AND RECENT BIOGENIC ORGANIC MATTER INCORPORATION INTO SURFACE SEDIMENTS OF CROSS RIVER ESTUARY S.E NIGER DELTA OF NIGERIA

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ABSTRACT

Surface sediments of Cross River estuary were analysed and found to have predominant n-docosane/docosene biomarkers only at stations CR5 and CR7. The concentrations of n-docosane ranged between 0.21 and 6.82 mg/kg dry sediment weight with all overall average of 3.14 ± 4.15 mg/kg dry sediment weight. The high standard deviation most likely reflects variation in the sediment grain size and differences in quantity and quality of organic matter source. N-docosene was detected at station CR5, CR6, CR7, CR8, CR9 and CR10 with concentrations of 0.65, 0.07, 0.71, 0.05, 0.07 and 0.02mg/kg dry sediment weight respectively. The CPI values at stations where docosane/docosene predominance was observed were 0.9 and 0.8 respectively suggesting a contribution of petroleum/microbial input at these stations. The observed Unresolved Complex Mixture (UCM) at these two stations gives supportive evidence of petroleum contamination. The predominance of n-docosane at these two stations could either be attributed to its synthesis by petroleum degrading bacteria or as a consequence of bacterial alteration of algal detritus. The origin of n-docosene was thought to be a consequence of diagenetic defunctionalization of unsaturated fatty acids contained in certain species of bacteria. Its occurrence could be utilized as molecular indicator of recent incorporation of biogenic organic matter in sediments.

KEYWORDS: Docosane, Predominance, docosene, n-heptadecane and biomarkers

INTRODUCTION

Bulk chemical and molecular marker approaches offer detailed information on organic matter (OM) sources in aquatic sediments but sometimes lack the specificity needed for source assessments for complex aquatic systems where organic matter input assessments beyond the terrestrial and microbial end members are needed (Gordon and Goni, 2003; Jaffe *et al*, 2005). Traditionally, the dynamics of organic matter inputs, transport and transformation in aquatic ecosystem can often by explained through conceptual models, which are primarily based on the distribution of allochthonous and autochthonous organic matter (Yunker *et al*, 1993; Jaffe *et al*, 2005).

An odd-over-even carbon number predominance in the n-alkanes $>C_{23}H_{48}$ has often been used as a molecular marker for a direct input of terrestrial plant wax into geological and environmental samples (Simoneit and Radzi bin Abas 1997). On the other hand, an even-over-odd predominance, although much less common, has been reported in recent years and seems to be associated with organism already containing this predominance such as bacteria and diatoms (Elias *et al* 1997). The distribution of n-alkanes is believed to be from a mixed source of microorganisms, higher plant and petroleum. The lower molecular weight n-alkanes are believed to be derived from microorganism (Shucheng, *et al* 2003).

The Cross River estuary lies between latitude 4°30` and 5°15`N and longitude 6°00` and 8°.00`E. Most of the area is covered by mangrove swamp forest. The estuary is influenced by semi-diurnal tides that go upstream to about 80km. Influx of organic species into the coastal waters are enhanced by two principal ocean currents: northward rising Benquela current and eastward flowing Guinea current (Asuquo *et al*, 1999). Cross River estuary receives organic pollutants from diverse sources including petrochemical pollution from boat/ship traffic, domestic untreated sewage/waste water, atmospheric fallout and agricultural waste waters.

The main objectives of this preliminary investigation were to report on the predominance of docosane/docosene as molecular indicators of recent incorporation of biogenic organic matter into this estuary. This is the first time specific organic compounds are reported in this estuary. Previous work were mainly focused on: Fishery ecology (Moses 1987); ecology (Enyinihi *et al*, 1987); metals in edible clam *Egerlia radiata* (Etim and Akpan 1991); water quality (Akpan and Offem, 1993) and distribution of heavy metals and total hydrocarbon in sediments (Asuquo *et al*, 1999).

MATERIALS AND METHODS

Surface sediments of Cross River estuary were obtained using Van-Veen grab sampler. Sampling locations (Fig 1) were divided into three zones on the basis of the anthropogenic input and sediment grain size. Each zone comprised of three or four sampling stations (Table 1). Extraction of ground, sieved and dried sediment samples (50g) was carried out in soxhlet extractor using mixture of dichloromethane and methanol (2:1). These extracts were desulphurised using activated copper before fractionated into saturated, aromatic and NSO fractions using column chromatography packed with activated silica-alumina (2:1)as stationary phase (silica gel below and alumina on top). The saturated fraction was eluted with hexane (50ml), aromatic fraction eluted with dichloromethane and then finally a mixture of methanol-dichloromethane (2:1) was used to isolate NSO or resins.

The gas chromatographic-mass spectrometric (GS-MS) analysis of saturated fraction was performed on a Hewlett-Packard model 5890 GC coupled Hewlett-Packard model 5973 quadrupole MSD. Separation was achieved on a fused silica capillary column coated with DB5 (0.25µm thin film thickness). The GC operating conditions were as follows: temperature hold at 65°C to 300°C for 20minutes. Helium was used as carrier gas. The samples were injected in splitless mode with injection temperature of 300°C. The mass

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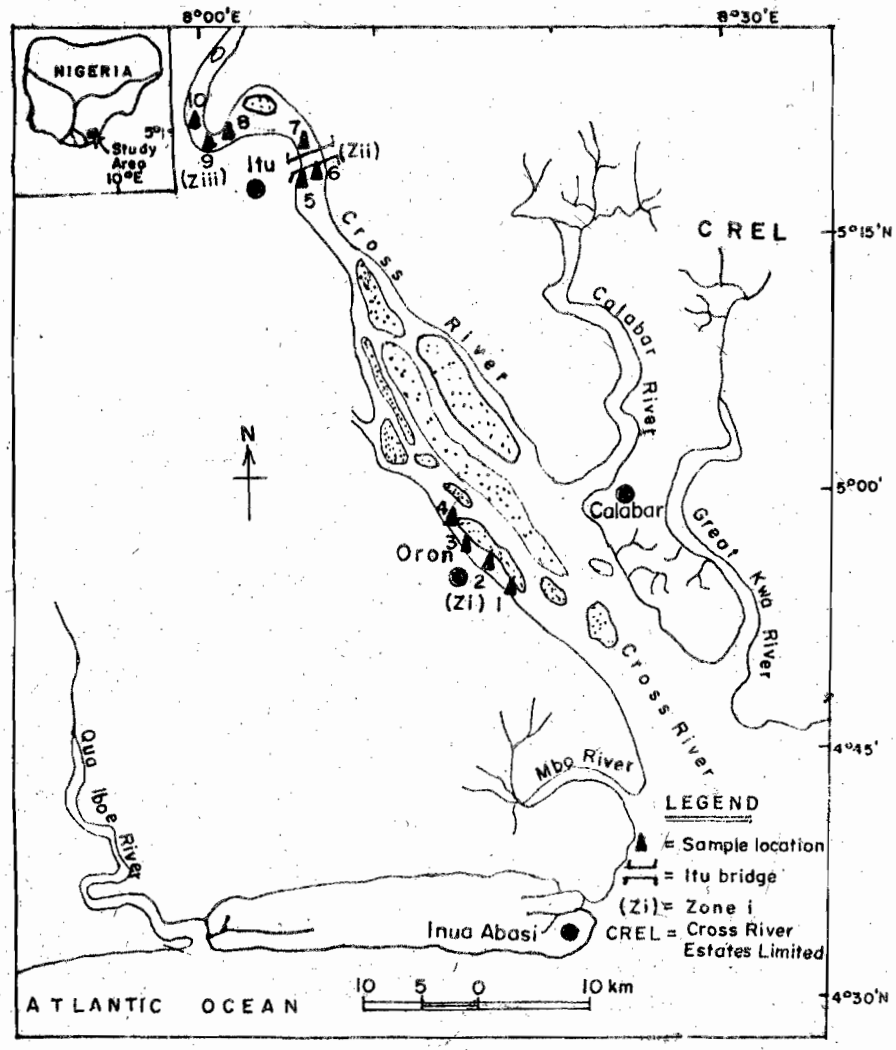

FIG. 1 Map of Cross River Estuary Showing Sample Locations and River Channels (Adapted from Akpan and Offem, 1993).

Table 1: Sample locations, description of anthropogenic activities and grain size values of Cross River estuary

Sample Locations	Description of anthropogenic activities	Grain size values
Zone (1) (Stations CR1, 2,3 and 4) - near Oron beach	Domestic sewage/agricultural waste and ship/boat traffic	Predominantly silty: [approximately 52% silty, (2-50 μ m), 38% clayey (<2 μ m) and 10% sandy (>50 μ m)]
Zone (ii) (Stations CR5, CR6 and CR7) - near Oku Iboku paper industry and thick forest	Industrial waste water/sludge and ship/boat traffic	Predominantly clayey [Aproximately 73% clayey (<2 μ m), 27% silty (2-50 μ m) and 7% sandy (>50 μ m)
Zone (iii) (Stations CR8, CR9 and CR10) - near Itu bridge	Domestic sewage and agricultural waste	Predominantly sandy: [Aproximately 75%. sandy (>50 μ m), 22% silty (2-50 μ m) and 3% clayey (<2 μ m)

spectrometry was operated in the electon impact mode (EI) at 70eV and scanned for 50 to 650 dalton. Data was acquired and processed with the chemstation software. Individual compounds were identified by comparison of their retention times with that of authentic standard compounds. The INCOS computer software program was used to quantify the area of each identified peak and the internal standard which is always "spiked" with a known amount of a standard solution.

RESULTS AND DISCUSSION

The organic matter found in surface sediments along Cross River estuary reflects both biogenic and anthropogenic inputs as well as diagenetic processes taking place within the water column and during transport and sedimentation. The concentrations of n-docosane (nC_{22}) ranged between 0.21 and 6.82mg/kg dry sediment weight maximizing at station CR5 and a minimum at station CR9, with an overall average of 3.14 ± 4.15mg/kg dry sediment weight. The high standard deviation most likely reflects variation in the sediment grain size and the differences in quantity and quality of organic matter source. Table 1 shows the approximate grain size values for the three zones of the estuary while Fig 1 is the map of the study area indicating the sampling locations. The spatial variation trend observed points to the fact that there appear to be a direct correlation between n-alkane accumulation in sediments and sediment grain size. Thus, supporting the believe that clay sediments has the greater capacity of adsorption of organic matter than silty or sandy sediments. This observation was supported by the mean n-alkane composition in these three zones being 3.54, 4.74, and 1.92mg/kg dry sediment weight respectively. N-docosene was detected at stations CR5, CR6, CR7, CR8, CR9 and CR10 with concentrations of 0.65, 0.07, 0.71, 0.05, 0.07 and 0.02mg/kg dry sediment weight respectively (Table 2). Fig 2a shows a GC total ion current (TIC) of n-alkanes distribution indicating unresolved complex mixture (UCM-indicator of petroleum contamination at station CR5 and CR7) while Fig 2b and 2c show the GC-MS of docosane and docosene respectively.

Table 2: Molecular weight (MW), Molecular formular and concentrations of n-docosane, n-docosane and n-heptadecane at each station along Cross River estuary

Compound name	MW	Molecular formular	Concentration in mg/kg sediment weight									
			Station CR1	Station CR2	Station CR3	Station CR4	Station CR5	Station CR6	Station CR7	Sta-Tion CR8	Sta-tion CR9	Sta-tion CR10
n-docosane	310	$C_{22}H_{41}$	3.36	2.73	3.72	4.36	6.82	1.56	5.82	4.93	0.21	0.62
n-docosene	298	$C_{22}H_{44}$	nd	nd	nd	nd	0.65	0.07	0.71	0.05	0.07	0.02
n-heptadecane	240	$C_{17}H_{36}$	3.14	3.35	3.73	0.99	5.85	0.64	6.83	1.20	0.09	0.30

N/B: nd- not detectable

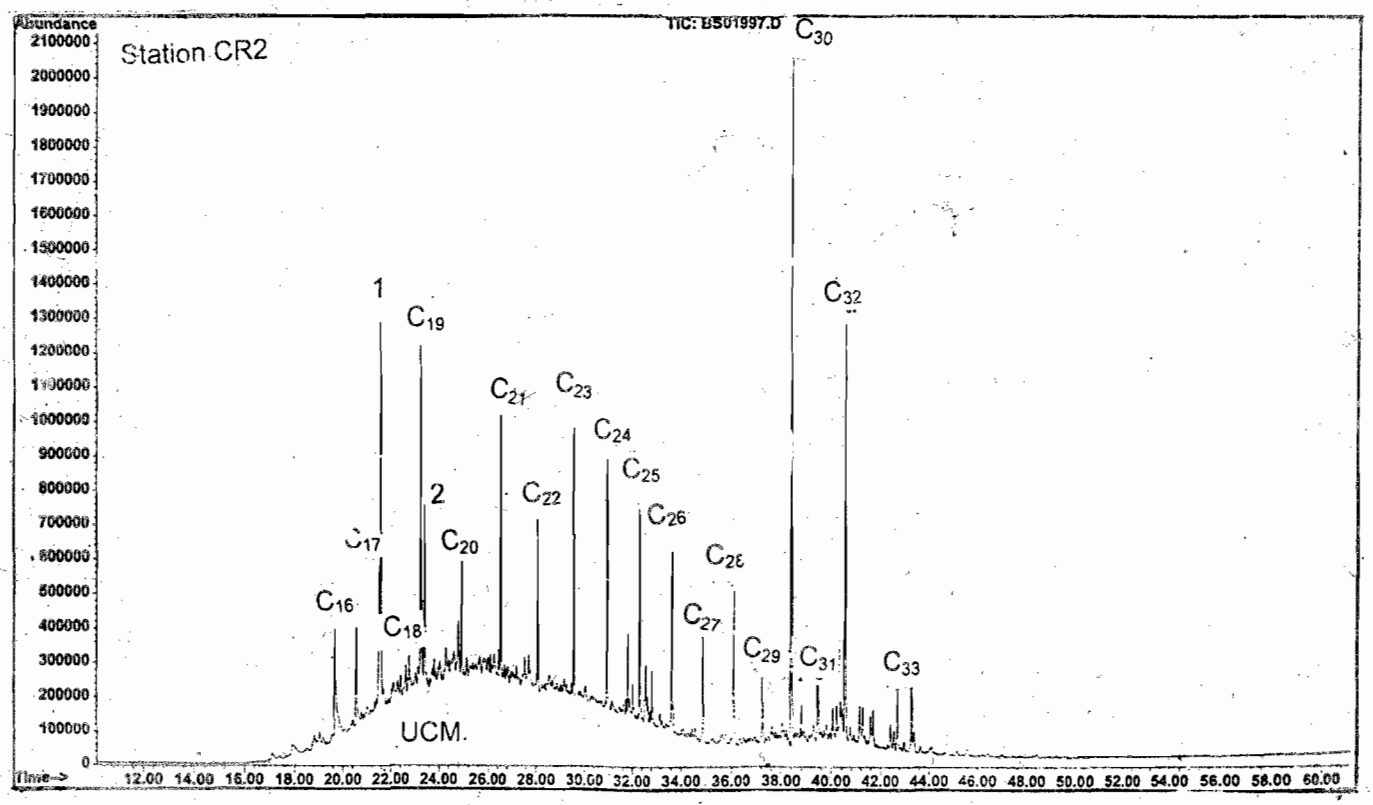

Fig 2A: Gas chromatogram of n- alkanes indicating unresolved complex mixture (UCM)

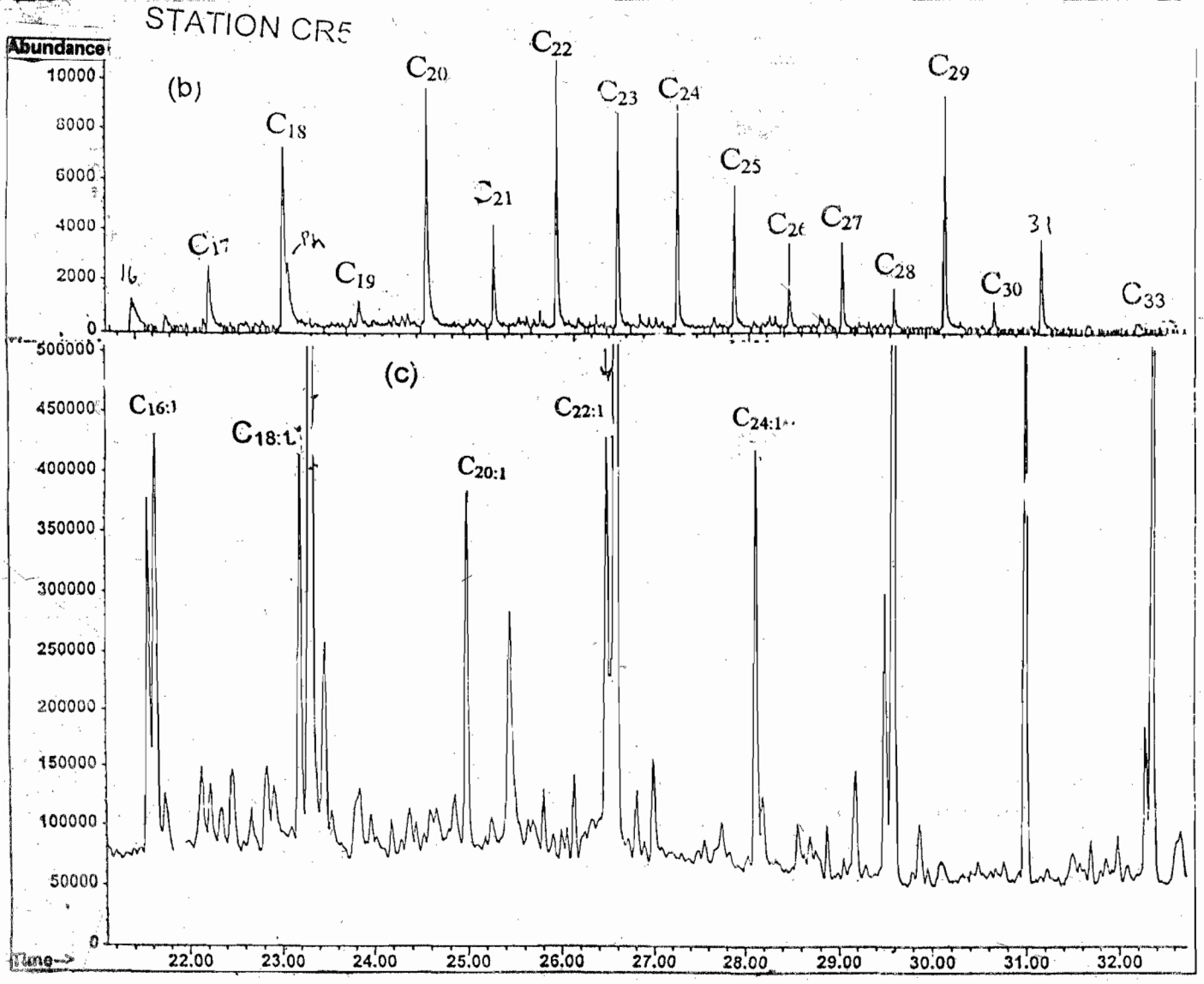

Fig.2b, c Mass Chromatogram indicating predominance of docosane and docosene occurrence respectively.

Carbon preference index (CPI), a measure of biologically synthesized n-alkanes (Simoneit and Radzi bin Abas, 1997) indicates the relative contribution of n-alkanes from biogenic/terrestrial (CP1 >1) compared to anthropogenic (e.g. petroleum pollution – CPI < 1). CPI was calculated according to Bray and Evans (1961) and are shown in Table 3 for all sampling stations of Cross River estuary. The determination of carbon maximum (C_{max}) for every sample also gives an indication of the relative source input, where C_{max} >C_{25} for n-alkanes reflects the incorporation of higher plant wax and C_{max} at lower carbon number (C_{max} < C_{25}) indicates a major contribution from microbial sources. The CPI values at stations CR5 and CR7 where docosane/docosene predominates were 0.9 and 0.8 respectively. These stations are major navigational channels (Table 1) for boat/ship traffic where petrochemicals (lubricating oil, diesel and gasoline from vehicular exhaust emission) enhanced microbial activity.

Table 3: Characteristic n-alkane parameters from surficial sediments of Cross River estuary, Nigeria

Sample number	CR1	CR2	CR3	CR4	CR5	CR6	CR7	CR8	CR9	CR10
Carbon range	C_{16}-C_{32}	C_{15}-C_{32}	C_{17}-C_{32}	C_{16}-C_{33}	C_{16}-C_{32}	C_{16}-C_{32}	C_{14}-C_{32}	C_{14}-C_{32}	C_{16}-C_{32}	C_{16}-C_{32}
C_{max}	C_{27}, C_{29}, C_{31}	C_{18}, C_{20}	C_{18}, C_{29}, C_{31}	C_{29}, C_{31}	C_{18}, C_{20}, C_{22}	C_{18}, C_{20}, C_{29}	C_{18}, C_{20}, C_{22}	C_{29}, C_{18},	C_{29}, C_{31}	C_{18}, C_{20}, C_{29}, C_{31}
CPI	2.0	1.8	4.0	3.9	0.9	2.0	0.8	1.4	2.7	3.8

Certain microorganisms are known to be hydrocarbon or petroleum utilizers while others inhabiting environments where petroleum contamination occurs are hydrocarbons synthesizers (Peters and Moldowan, 1993). The predominance of n-docosane at stations CR5 and CR7 could likely be a consequence of its synthesis by bacterial inhabiting this petroleum contaminated estuary.

However, the origin of docosane according to Elias et al, (1997) is somewhat uncertain but Schenck, (1969) mentioned that it is predominant in a few genera of algae such as *Crytophyceace* and *Syracosphaera*. Sediment samples from deltaic environment in Spain also have this unusual nC_{22} predominance and it was attributed to microbial degradation of algal detritus. Castro (1994) analysed lipids from different organisms of geochemical interest and also found the C_{max} at nC_{22} in a few genera, but in this case, it was attributed to contamination from unknown origin (Elias *et al*, 1997).

Moreover, laboratory experiments involving plant and sediment samples both contain initially n-alkanes typical of plant waxes (i.e. odd over even) carbon predominance and C_{max} at C_{27}, C_{29}, and C_{31}, under aerobic conditions produced unimodal n-alkanes distribution with a C_{max} at C_{22} (John and Calder, 1973), which is further evidence for bacterial origin of docosane. In this respect, stations CR5 and CR7 resemble those for the Ebro delta (Spain) and Amazon Shell (Brazil) where C_{max} at C_{22} was found to predominate. It therefore seems reasonable that the same natural processes could account for input of docosane where this predominance is inferred to be of bacterial alteration of algal detritus. This deduction is a consequence of the abundance of algal biomarker, n-heptadecane, in this estuary especially at these two stations. This inference can be contrasted with example of sediments from hypothermal system in middle valley, northeast Pacific, which contains this predominance as a result of reductive process occurring during the hydrothermal alteration of immature organic matter (Elias *et al*, 1997).

N-docosene ($nC_{22:1}$), detected in the surface sediments of Cross River estuary was also observed in Amozonia River sediments, Egypt (Aboul-Kassim and Simoneit, 1996). N-docosenes are not dominant components of plant wax and were inferred to be derived from biomass fuel (Mazurek and Simoneit 1997). The entry of n-docosenes into this estuary probably via atmospheric fallout was initially considered to be a result of dehydration of docosanol (sometimes occurring in ester-bound form in higher plant wax). Docosanols are easily dehydrated by high temperature to docosene during incomplete combustion processes. However, more recently, Ekpo *et al* (2005) called for an explanation of the predominance of even-number carbon compound in surface sediments of Calabar River, one of the tributaries of Cross River. According to these authors, the possibility of synthesis of this even numbered alkene from waxes of higher plants by defunctionalization of alkanol seems unlikely as no samples exhibited even carbon predominance in the range $nC_{25} - C_{35}$ assigned to waxes of higher plant (Aboul-Kassim and Simoneit 1996). Though, the origin of docosene in this estuary remains inconclusive, it was considered to be derived from diagenetic defunctionalization of unsaturated fatty acid contained in certain species of micro organisms such as bacteria. However, the absence of docosene in some of the sampling stations and the low levels in others reflect its instability in the estuary. This compound is rapidly oxidized and degraded in the environment and could be utilized as molecular indicator of recent incorporation of organic matter into sediments.

CONCLUSION

The determination of C_{max} provides the strongest indication of contemporary biogenic components in sediments, mainly in the n-alkanes series. The CPI is a diagnostic parameter, where a low CPI < 1 indicates a major incorporation of petroleum/microbial input into sediments. Thus, coupling C_{max}, CPI and biomarker identification allows the classification of natural biogenic and anthropogenic components of environmental samples. The CPI values of 0.9 and 0.8 indicate petroleum contamination at these two stations The predominance of docosane at these two stations was considered either to originate as a consequence of bacterial alteration of algal detritus or a consequence of its synthesis by petroleum degrading bacterial inhabiting these sediments. On the other hand, the predominance of docosene was thought to be a consequence of diagenetic defunctionalization of unsaturated fatty acid contained in certain species of micro organism such as bacteria. The occurrence of n-docosene could be utilized as molecular indicator of recent incorporation of biogenic organic matter in sediments.

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