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Seasonal Changes of N-Alkanes in Water and Suspended Particulate Matter from Cross River Estuary, South-east Nigeria

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Abstract:
Several studies worldwide have attributed the seasonal variation in aromatic compound levels to changes in riverine discharges and rainfall, yet similar correspondence with n-alkanes distributions is scarcely undertaken. Assessment of the behavior of n-alkanes in water and suspended particulate matter from Cross River estuary, South-South Nigeria, with depth in reaction to anthropogenic pressure/hydrological cycles and their source contributions were carried out by gas chromatography–flame ionization detector. Higher mean levels of n-alkanes in water and suspended particulate matter were recorded in the wet season than in the dry season, linked to the effects of higher wet precipitation and surface run-off as well as proximity to point sources where anthropogenic activities were at the peak of the rainy season. Evaluation of diagnostic indices values such as long-chain hydrocarbon/short-chain hydrocarbon, terrestrial aquatic ratio, carbon preference index, carbon maximum, proxy aquatic ratio, $\Sigma n\text{-}alkanes/C_{16}$ and unresolved complex mixture as well as multi-variate statistics [eg. Multiple linear regression and principal component analysis] revealed that vascular plant/wood combustion predominated with moderate-to-minor contributions from macrophytes, degraded oil, petrochemicals and algae/photosynthetic bacteria. Changes in hydrological parameters, including suspended sediment concentration, temperature, salinity, and anthropogenic pressure, influenced n-alkanes distribution in the estuary.

Keywords: Human-induced activity, n-alkanes, hydrological parameter, salinity, source

1. Introduction

Organic carbon inputs from different sources undergo intense biogeochemical alteration in coastal regions, which are known active sites, forming a remarkable component of the world carbon record (Middleburg et al., 2004; Duarte et al., 2004; Borges et al., 2005). This region is loaded with considerable continental inputs and increased marine production. In addition, organic pollutants being one of the major constituents of land-borne material and oil spills from offshore operations, are introduced into coastal regions with attendant impact on public health and ecosystems (Gogou et al., 1999).

N-alkanes are broadly utilized as molecular markers for source correlation purposes in differentiating between autochthonous and allochthonous organic matter (OM) as well as discriminating among autochthonous sources due to their resistance towards diagenesis and preservation during sedimentation (Oyo-Ita et al., 2016). Carbon maximum (C_{max}), proxy aquatic ratio (P_{aq}), carbon preference index (CPI), long-chain/short-chain hydrocarbons (LHC/SHC), $\Sigma n\text{-}alkanes/C_{16}$ and unresolved complex mixture (UCM) are some of the common indices used by several researchers worldwide for this purpose (e.g., Kennicult et al. 1987; Zhu et al. 2005; Maioli et al. 2010; Oyo-Ita et al. 2016).

The influx of *n*-alkanes into the water column over time leads to variation in the quantity/quality of organic carbon at different water levels, implying their distributions should vary in response to hydrology and anthropogenic pressure. Thus, it is imperative to examine the distribution of *n*-alkanes in the water column to understand their relationship with estuarine hydrological parameters such as temperature, salinity and suspended sediment concentration (SSC). In-depth investigations of transient trends in aromatic hydrocarbon distribution in water and SPM have been

worldly documented (e.g., Doong and Lin, 2004; Silva et al., 2007; Liu et al., 2017) and most reveal negative or positive and weak or strong relationships amongst these variables. Nevertheless, corresponding information regarding *n*-alkanes is scanty or rather poorly discussed, especially for tropical West Africa. However, in the past decades, little attention has been given to assessing the impact of activities of humans on *n*-alkanes distribution in estuaries.

The present study is part of an overall regional project entitled "Organic Tracers of Pollution in the Environment of Niger Delta (OTPEND)," designed to characterize the sources, fate and distributions of organic pollutants in the region. The main objectives of the present study were to assess the seasonal changes in the concentrations, compositions and source contributions of *n*-alkanes and examine their responses to hydrological cycles and anthropogenic pressure.

Past investigations in the estuary concentrated mainly on the origins and distributions of triterpene ethers and acetates (Oyo-Ita et al., 2010 a), aliphatic hydrocarbons and ketones (Oyo-Ita et al., 2010 b), PAHs (Ekpo et al., 2011) and other biomarkers of natural and anthropogenic origins (Pisani et al., 2013) as well as phthalates and plastics additives (Oyo-Ita et al., 2014), all in surface sediments. Other studies include the distribution of heavy metals in water (Asuquo et al., 1998) and mollusk response to anthropogenic impacts (Asuquo & Anyanwu, 2018).

The increasing population density, the occasional oil spill from offshore operations, rapid urbanization and industrialization, as well as run-off from the tourist city (Calabar, the capital of Cross River State), including other adjoining towns (e.g., Oron and Mbo), make the estuary sensitive to *n*-alkanes loading. The central estuary near Parrot Island (Figure 1), where sampling was carried out, was chosen because the hydrographic conditions represent a midpoint between the ocean water and freshwater (Lowenberg & Kunzel, 1992). The inhabitants of Parrot Island and other adjoining coastal towns (e.g., Oron and Mbo) mainly engage in commercial fishery using canoes and boats as well as roasting and drying fish for both local consumption and export purposes, especially during the peak of the wet season (i.e., June) when optimal silver catfish, *Chrysichthys nigrodigitatus,* catch occurs.

In the study, alternating bimonthly depth-profile sampling was carried out in Edik Ekpu area ($04^0 49'$E and $08^0 17'$N) along Parrot Island within a period of about 1 year, including the beginning and peak of wet and dry seasons, from June 2016 to April 2017.

2. Materials and Methods

2.1. Materials

Dichloromethane (DCM; CH_2Cl_2), iso-octane (C_8H_{18}), ethyl acetate ($CH_3COOCH_2CH_3$), methanol (MeOH), *n*-hexane (C_6H_{14}) and acetone (CH_3COCH_3) were the analytical-grade solvents used, made available by Dr. Ehrenstofer (GmbH-Augsburg, Germany). Anhydrous sodium sulfate (Na_2SO_4), alumina (Al_2O_3) active neutral (0.063-0.200 mm) and silica gel (SiO_2) were used as stationary phase (were from Merck - Hohenbrunn, Germany) while per-deuterotetracosane was used as external standard (obtained from Cambridge Isotope Laboratory (USA). All glassware was pre-washed with acetone and $CH_3COOCH_2CH_3$ and then heated at 450^0C overnight.

2.1.1. Sampling

Comprehensive information about the study area is described in Oyo-Ita et al. (2010 b). Briefly, the Cross River estuary (Figure 1) lies between longitude $20^0 03'$ E and $10^0 00'$ E and latitudes $4^0 00'$ N and $8^0 00'$ N and is one of the largest estuaries in the south-east Niger-Delta, Nigeria. The estuary receives freshwater from three main rivers, namely the Great Kwa, Calabar, and Cross Rivers, as well as numerous tributaries and saline water from the Atlantic Ocean with a discharge of between 880 m^3sec^{-1} and 2533 m^3sec^{-1} (Lowenberg & Kunzel, 1992). The Cross River system experiences temporal flooding, which depends on the tidal and seasonal cycles (dry/wet). The region experiences a rainy season between April and the beginning of October, having 80 % of the annual rainfall, which peaks between June and August. The average temperature ranges from 24 ^{0} C in August to 30 ^{0} C in February and relative humidity is high between 80-100% (Eze & Efiong, 2010). Changes in the season and tides significantly influence the Cross River estuary hydrology (Asuquo & Anyanwu, 2018).

2.2. Water

Samples were taken at 5 different depths in the flood tide (surface, 2, 4, 6 and 8 m) in the water column labeled as W1, W2, W3, W4 and W5, respectively, using a stainless-steel Nansen sampler fitted with a thermometer. About 3 liters of water (a composite of 5-6 samples) was collected at each depth, poured into a brown glass bottle, cooled in ice, moved to the laboratory and kept in a freezer at -20 ^{0} C until further protocols.

2.3. Suspended Particulate Matter

The water samples collected bi-monthly were sieved through a glass fiber filter (Whatmann Grade GF/C: 47mm diameter and 1.2 μm porosity), which were previously pre-heated in an oven at 450 ^{0}C for 6 hrs., pre-cleaned in acetone, their weight predetermined, stored in a desiccator containing silica gel, post determined to obtain the total suspended particulate matter (SPM) and stored in a desiccator until laboratory protocols.

2.4. Determination of Physicochemical Properties

According to Iwouha et al. (2012), temperature readings were taken *in-situ*, while salinity was measured using the combined pH/conductivity meter (Jenway 3540 Meters).

2.4.1. Extraction and Clean-up
Water and SPM samples were exposed to extraction and clean-up protocols as follows:

2.4.1.1. Water
One liter of filtered water sample was turned over to a separating funnel and about 60 mL of redistilled dichloromethane was put into it. The mixture was shaken vigorously with periodic venting for 2 mins. to release pressure and then allowed to stand for 10 minutes. The organic layer was allowed to flow out slowly, and the obtained aqueous layer was re-extracted 2 x. The extracts were concentrated *in-vacuo* and subsequently fractionated by open column adsorption chromatography using silica gel activated at 450 ^{0}C with alumina activated at 550 ^{0}C as adsorbent and washed out with 10 mL of *n*-hexane: dichloromethane (4:1 v/v) to recover the aliphatic fraction (Silva et al., 2007). The fractions were then evaporated to near dryness *in-vacuo* and subjected to GC-FID analysis.

2.5. Suspended Particulate Matter
Before the extraction protocol, a solution of known concentrations made up of the target compounds was 'spiked' onto the dried, filtered samples and extracted thrice by ultrasonification for 30 minutes with 15 mL (*n*-hexane: DCM; 4:1 v/v). All extracts were combined and evaporated *in-vacuo* to near-dryness and made into a solution with *n*-hexane and separated into polar and non-polar fractions with DCM: MeOH (1:1 v/v) and *n*-hexane, respectively, using a mini-column loaded with Al_2O_3 and SiO_2 prior to GC-FID.

2.5.1. Instrumental Analysis
The non-polar fractions were analyzed according to Oyo-Ita et al. (2010 b). A 30 m, 0.25 mm i.d. fused silica capillary column (Ultra 2: 5% diphenyl-dimethyl-polysiloxane) connected with FID of Hewlett Packard model 5890 II series in a splitless injection mode was used for quantitative and qualitative determination of individual *n*-alkanes. The flow rate of the carrier gas (N_2) was 1 mL min^{-1}. The injector and column program temperatures were as presented in Oyo-Ita et al. (2010 b).

X-calibur Thermo Finnigan software (San Jose, California, USA) was used to process data picked up in the selected ion monitoring (SIM) modes and full scan with 6 mins. solvent delay. Compounds identification was based on a comparison of their retention times with authentic samples. Quantitation was made possible with areas of peaks converted to mass of compound applying external standard: perdeuterotetracosane peak area. Surrogate values were used to calculate percentage recovery. To determine their loss during the analytical procedure, the recovery study (n = 3) was carried out by spiking a standard solution containing the target compounds onto a clean sand matrix. The percentage mean recovery for the range of *n*-alkane compounds determined was 85±0.98 % (60–130 %).

2.6. Data Analysis
The target analytes (*n*-alkane compounds determined) were statistically analyzed by t-test, Pearson correlation analysis, cluster analysis, principal component analysis (PCA) and multiple linear regression (MLR) using the software SPSS 20. Comprehensive information about the extraction procedures of PCA was presented by Oyo-Ita et al. (2016). Determination of the relationship between paired variables was done using Pearson correlation analysis, while the t-test was to delineate significant differences between wet and dry season data sets. The application of cluster analysis was used to sort the profile samples into specific areas with known properties, while PCA and MLR models in the present study were used to categorize the *n*-alkane sources and estimate their source contributions, respectively (Larser & Baker, 2003).

3. Results

3.1. Seasonal Variation in N-Alkanes Concentrations in Water
ΣC_8-C_{40} levels of *n*-alkanes in water for the wet season ranged between 0.04 and 7.20 ng/L minimizing at 4 m (AprW3) in the month of April and a maximum at the surface (AW1) in the month of August (mean = 3.44±1.92 ng/L). ΣC_8-C_{40} concentration of *n*-alkanes in water for the dry season ranged from 0.74 ng/L at 4 m (FW3) in the month of February to 3.80 ng/L at the surface (OW1) in the month of October with a mean of 2.54±0.50 ng/L (Fig 2 a).

The highest mean *n*-alkanes concentration in water (4.81 ng/L) was found in the month of August, while the lowest mean concentration of 0.72 ng/L was recorded in April, both in the wet season (Table 1). Correlation analysis revealed a poor positive relationship between the wet and the dry seasons' *n*-alkane concentrations (r= 0.0777; Table 2).

3.2. Seasonal Variation in N-Alkanes Concentrations in Suspended Particulate Matter
ΣC_8-C_{40} levels of *n*-alkanes in SPM for the wet season were in the range 41.32 - 2502 ng/g with a minimum at 4 m (AprSP3) in the month of April and a maximum at 2 m (ASP2) in the month of August (mean=1271±618.48 ng/g). In the case of the dry season, a range of 81.39 - 1274 ng/g was found with a minimum at 8 m (FSP5) in the month of February and a maximum at 6 m (DSP4) in the month of December (mean =649.18±240.68 ng/g; Figure 3). Correlation analysis revealed a weak positive relationship between the wet and the dry seasons' *n*-alkane concentrations (r= 0.3168; Table 2).

3.3. Source Analysis of N-Alkanes with Season
Source analysis of *n*-alkanes for water and SPM was carried out using various distribution indices presented below.

3.3.1. Water

Table 1 shows the bi-monthly mean diagnostic indices used in identifying *n*-alkanes sources in water. LHC/SHC was utilized to assess the proportions of higher plants/macrophyte-originated and phytoplankton/algae- or bacteria-originated OM influx to the estuary. LHC/SHC values > 1 indicate a higher fraction of vascular plant/macrophyte, while values < 1 imply a higher amount from phytoplankton/algae or bacteria (Commendatore et al., 2012; Oyo-Ita et al., 2016). The results revealed the lowest (3.57) and the highest (12.69) values in April and August (wet season), respectively.

In the study, *n*-alkanes distributions in water exhibited an odd over even carbon numbered predominance in some samples (Figures 4), characteristic of OM influx from higher plant/wood combustion, while in others, an even over odd carbon numbered predominance prevailed, characteristic of input from micro-organisms (Ekpo et al., 2005).

UCM, also known as 'hump,' is a component often found in gas chromatographic data of extracts from organisms exposed to oils and is composed of unresolved cyclic and branched hydrocarbons (Frysinger et al., 2003). The extent of anthropogenic pressure on the estuary is reflected in the variation in UCMs with the season in the representative chromatograms (Figures 4).

For water, besides C_8, C_{10} or C_{11} occurring in high abundances in a few samples in the wet season, a minor C_{max} (C8-C20) occurred in most samples (Figure 4). In the case of the dry season, no C_{max} (C8-C20) was detected in most samples except in DW4. However, C_{max}(C21-C40) was predominated by C_{27}, C_{29} and C_{31} in most samples (Figure 4).

TAR (calculated with the expression - TAR =$[(C_{27}+C_{29}+C_{31})/(C_{15}+C_{17}+C_{19})]$ was applied to investigate further the terrestrial/aquatic blend of hydrocarbons in water samples. In the study, the bimonthly mean TAR values ranged between 15.66 in December to 34.64 in August (Table 1). P_{aq} expressed as $C_{23}+C_{25}/(C_{23}+C_{25}+C_{29}+C_{31}$ was applied to further evaluate the proportion of vascular plants waxes and macrophytes. Here, the bimonthly mean P_{aq} results revealed intermediate values (0.38±0.09 in the wet season, and0.36±0.02 in the dry season; Table 1).

The $\sum n$-alkanes/C_{16} ratio was also applied to estimate the comparative proportion of natural or biogenic and anthropogenic origins of OM in the water samples. Here, the results of bi-monthly values for water ranged from 176.66 in April to 637.18 in August (wet season; mean= 439.97±193.39), while those of the dry season ranged from 90.40 in October to 165.69 in December (dry season; mean = 135.80±32.64; Table 1).

3.4. Suspended Particulate Matter

The bi-monthly values of LHC/SHC ratios for SPM ranged between 3.04 in April to 18.19 in August (wet season; mean = 11.50±6.31), while that of the dry season ranged from 3.11 in February to 5.71 in October (mean=4.69±1.13; Table 1 b).

The bi-monthly mean CPI values ranged between 0.92 in April to 1.28 in June (wet season; mean= 1.11±0.15; Table 1). In contrast, those for the dry season were in the range of 1.02-1.16 (mean= 1.10±0.06), with minimum and maximum recorded for October and February, respectively. Similar UCM profiles were observed in SPM, particularly in the detection of bimodal distributions in JSP2, DSP4 and ASP1.

Generally, Cmax(C8-C20) was predominated by C10 and C20 in most SPM samples in the wet season except in APrSP1, JSP2 and ASP2 with Cmax(C8-C20) found at C11, C17 and C18, whereas Cmax(C8-C20) at C17, C18 and C10 or C20 predominated in most SPM in the dry season except in OSP4, DSP1, DSP4 and DSP5 with Cmax(C8-C20) at C11 and/or C13. C16 and C18 occurring in the range Cmax(C8-C20) were also observed in addition to C10 or C20 in most February samples. The results indicated that seasonal changes did not only affect the quantity of OM flux but also the OM quality. On the other hand, Cmax(C21-C40) was predominated by C27, and C29 in most SPM in the wet season except in JSP4, JSP5 and most August samples with Cmax at C26, C28, C30, C34 and C36. APrSP1, JSP4 and ASP2 exhibited Cmax at C23 and C25 in addition, indicating that higher input of macrophytes occurred in the wet season. Besides the occurrence of Cmax at C29 or C31 in a few SPM in the dry season, Cmax at C24, C26, C28, C30 and C32 exhibiting even over-odd carbon predominance were found in most SPM samples.

The bi-monthly mean TAR values recorded for SPM ranged between 6.05 in April and 50.15 in August. The bi-monthly mean Paq values recorded for SPM ranged between 0.27 in February and 0.39 in August (Table 2). While 0.36±0.03 and 0.31±0.03 were the mean Paq values recorded for the SPM in the wet and dry seasons, respectively (Table 1 b).

Ratios of Σn-alkanes/C16 were also calculated for SPM, and the bi-monthly mean results indicated values ranging from 111.31 in April to 714.29 in August (Table 2). In the case of SPM, the values ranged from 111.31 in April to 714.29 in August (wet season; mean= 412±246.17), while those of the dry season ranged from 139.66 in October to 184.34 in December (dry season; mean= 155.33±20.54; Table 2).

3.5. Principal Component Analysis

Principal component analysis (PCA) of n-alkanes data for water and SPM performed using SPSS 20 was expected to identify those compounds that co-vary. The application of varimax rotation on the first three principal components was carried out to streamline the interpretation of PCA predictions and optimize the loading of each variable on each principal component while the patterns are still retained (Yunker et al., 2011). Source identification of n-alkanes was carried out as follows:

3.5.1. Water

The first 3 principal components (PC1, PC2 and PC3; Table 3) were identified through the use of Eigenvalues >1, which made up 84.23% of total variability. PC1 had 65.22% of the overall variability and weighted heavily with LHC, including C_{27}/C_{29}/C_{31} and C_{36}/C_{38}/C_{39}/C_{40}, as well as MHC such as C_{23}/C_{25} (abundant in high levels in macrophytes; Ficken

et al., 2000) and SHC, including C_{15} and C_{19} (typically present in high abundance in photosynthetic bacteria; Meyers 1997). PC2 was made up of 10.94% of the total variability, was highly weighted by certain SHC such as $C_{16}/C_{17}/C_{18}$ and PC3 and was moderately weighted with other SHC, which included $C_8/C_{11}/C_{12}$, accounting for 8.07% of the total variability.

3.6. Suspended Particulate Matter

The first 3 principal components (Table 4) were identified through the use of Eigenvalues >1, which accounted for 84.41% of the total variability. PC1 made up of 52.32% of the total variability and was highly loaded with $C_{27}/C_{29}/C_{31}/C_{33}/C_{35}$ and $C_{34}/C_{36}/C_{38}/C_{39}$. PC2 accounted for 20.40% of the total variability and had high loading of other SHC, including $C_{14}/C_{15}/C_{16}/C_{17}/C_{18}/C_{19}/C_{20}$. PC3 had 11.69% of the overall variability and was moderately loaded by $C_9/C_{10}/C_{11}/C_{12}$.

3.6.1. Seasonal Variation in Source Contribution by Multiple Linear Regression

Multiple linear regression (MLR) was applied for quantification of the contributions of different *n*-alkanes sources. Due to the complexity inherent in *n*-alkanes contributions from natural biogenic and anthropogenic origins to the study site, components of known characteristics sources were extracted and then subjected to MLR. Although this approach does not provide the exact source contributions to the estuary, it, however, gave a fair estimate of these contributions. For instance, $\sum C_{27}+C_{29}+C_{31}$, $\sum C_{23}+C_{25}$, $\sum C_{15}+C_{17}+C_{19}$, $\sum C_{36}-C_{40}$ and $\sum C_8-C_{14}$ corresponded to inputs from vascular plant/wood combustion, macrophyte, algae/photosynthetic bacteria, degraded oil and petrochemical, respectively. In water, *n*-alkanes of vascular plant/wood combustion, degraded oil, macrophytes, petrochemicals and algae/bacteria origins contributed 47.24%, 18.92%, 18.65%, 14.13% and 1.59%, respectively, in the wet season, while 51.98%, 19.88%, 14.95 %, 9.45% and 3.73% were the contributions from vascular plant/wood combustion, macrophytes, degraded oil, petrochemicals and algae/bacteria, respectively in the dry season (Figures 4 a and b).

In SPM, *n*-alkanes of vascular plant/wood combustion, macrophyte, degraded oil, petrochemicals and algae/bacteria origins contributed 49.89 %, 19.64 %, 15.80 %, 9.03 % and 5.64 %, respectively, in the wet season, while 46.72 %, 20.93 %, 14.89 %, 12.48 % and 4.98 % were the contributions from vascular plant/wood combustion, petrochemicals, macrophytes, degraded oil and algae/bacteria, respectively in the dry season (Figures 4 c and d).

3.6.2. Relationship amongst Samples by Cluster Analysis

The measurement regarding similarities and/or differences within molecular compositions of *n*-alkanes in different samples by cluster analysis was carried out to identify point sources of *n*-alkanes.

3.7. Water

The result of cluster analysis for water is shown on the dendrogram (Figure 5 a) with three main distinguishable clusters A, B and C observed. A which comprised AprW1, AprW2, Aprw3, AprW5 and, FW3 combined first with the smallest distance and increased slightly to OW2, OW4, OW5, DW2, DW3, DW5, FW2, JW5 and then slightly to FW4 and AprW4. The second cluster B comprised AW3 and AW4 having the shortest distance between them and extended slightly to OW1, OW3, FW5 and DW1, and then the distance further extended slightly to JW1, JW3, AW2 and AW5. Finally, the third cluster C consisted of AW1, DW4 and JW2, with great similarity among them and the distance extended slightly to JW4 and FW1.

3.8. Suspended Particulate Matter

The result of cluster analysis of *n*-alkanes data applied in the categorization of the SPM samples into definite zones with distinct properties is as shown on the dendogram (Figure 6). Like in the case of water samples, the complete linkage method indicated three main clusters: A, B and C. Cluster A, which comprised FSP1, FSP3, FSP4, FSP5, AprSP1, AprSP3 and AprSP4, shared first with the shortest space or great resemblance between them. The linking space increased slightly to OSP1, OSP2, OSP3, OSP4, DSP1, DSP2, DSP3, DSP5, AprSP2, AprSP5 and FSP2. The second cluster B consisted of OSP5 and DSP4 combined at a great distance from the former and showed great dissimilarity in source contribution compared to the latter, while the third main cluster C comprised JSP1, JSP2, JSP3, JSP4, JSP5, ASP1, ASP2, ASP3, ASP4 and ASP5.

3.8.1. Effects of Hydrological Cycles and Anthropogenic Activities on n-alkanes Distribution

The bi-monthly mean SSCs in the rainy season were in the range of 0.12 ng/L- 0.33 ng/L with an overall mean of 0.21 ± 0.07 ng/L and 0.01 ng/L to 0.14 ng/L in the dry season (overall mean = 0.14 ± 0.21 ng/L). The bi-monthly mean salinity ranged between 5.50 ‰ and 23.50 ‰ with an overall mean of 13.43 ± 6.23 ‰, minimizing in the month of June (rainy season) and a maximum in the month of February (dry season). The bi-monthly mean temperature varied from 20 $^{\circ}C$ to 36 $^{\circ}C$ (overall mean = 27.25 ± 6.11 $^{\circ}C$), with higher values recorded in the wet season. In the study, the bi-monthly mean $\sum C_8-C_{40}$ levels in water and SPM were higher in the rainy season (mean=1.56 ± 1.40 ng/L and 577.76 ± 588.16 ng/L, respectively), compared to those in the dry season (mean=1.15 ± 1.11 ng/L and 295.08 ± 250.88 ng/L, respectively; Figure 6). Also, the bi-monthly mean LHC concentrations were lower in the dry season (2.23 ng/L) than in the rainy season (3.98 ng/L) and those for SHC concentrations were also lower in the dry season (0.32 ng/L) than in the rainy season (0.38 ng/L) in water.

Here, Pearson correlation analysis was employed to understand the relationship between hydrological parameters and *n*-alkanes distributions in water for the two seasons. The results for the wet season showed that $\sum C_8-C_{40}$ concentrations exhibited a strong positive correlation with temperature (r=0.8473) and good negative and positive correlations with salinity (r=-0.3829) and SSC (r=0.4235), respectively. In the dry season, temperature exhibited a weak

positive correlation with $\sum C_8$-C_{40} (r=0.0412) and good negative and positive correlations existed between $\sum C_8$-C_{40} and salinity (r=-0.4386) and SSC (r=0.4256), respectively, (Table 2).

4. Discussion

4.1. Seasonal Variation in N-Alkanes Concentrations

Results indicate that the mean *n*-alkanes concentrations in the wet and dry seasons exhibited a remarkable seasonal variation (p< 0.05; Figure 2). Correlation analysis revealed a poor positive relationship between the wet and the dry seasons' *n*-alkane concentrations (r= 0.0777; supplementary information-SI 1), suggesting changes in the quantity and quality of OM inputs in the two seasons.

The total *n*-alkane levels (0.04 to 7.20 ng/L) in water in the present study were comparatively lower than Daliao river system, China which ranged from 13.39-283.62 µg/L (Guo et al., 2010), Gulf of Tunis, Tunisia which ranged from 701.00 to 741.70 ng/L (Mzoughi & Chouba, 2011) and Shatt Al-Arab River, Iraq (8.81 to 35.58 µg/L; Al-Hejuje et al. 2015). This lower level may be due to the location of the sampling points, relatively further from anthropogenic activity and low urbanization.

Comparing data with that of water samples, the observed large standard deviation values recorded for SPM *n*-alkanes concentrations may be a consequence of the wider changes in the quantity and quality of OM source. As shown in table 1 b, although the bi-monthly mean concentrations of *n*-alkanes in the wet and dry seasons somewhat indicated a seasonal variation, t-test analysis revealed there was no statistical remarkable difference between the concentrations in the 2 seasons (p > 0.05). Furthermore, correlation analysis revealed that a weak positive relationship existed between the wet and dry seasons' *n*-alkane concentrations (r= 0.3168), suggesting variation in the *n*-alkanes source contributions to SPM in the two seasons (Table 2).

4.2. Source Analysis of N-Alkanes with Season

N-alkanes derive their sources from both biogenic production and anthropogenic activities (Aboul-Kasim & Simoneit, 1995; Oyo-ita et al., 2020). Short-chain *n*-alkanes mainly occur in micro-organisms (e.g., algae, bacteria or plankton), whereas mid- and long-chain counterparts mainly originate from macrophyte and vascular plants, respectively (Meyers, 1997; Ficken et al., 2000).

Results of LHC/SHC for water and SPM indicate a higher contribution of vascular plant waxes/macrophytes- over phytoplankton/algae- or bacterial-derived OM in the wet season than in the dry season. The lowest value recorded in April can be attributed to the fact that April being the beginning of the wet season, usually exhibits the lowest wet precipitation and run-off.

For several years, carbon preference index (CPI) has been widely utilized worldwide to discriminate among biogenic inputs and distinguish between anthropogenic and biogenic sources (Meyers et al., 1997). CPI values around unity are indicative of petroleum contamination, while values less than 1 imply input from micro-organisms and above 1 imply contribution from vascular plants (Pies et al., 2008; Kanzari et al., 2014; Oyo-Ita et al., 2017). The highest CPI value recorded for water (1.26) in the month of April suggests a greater wash-in of higher plant waxes to the estuary in the month. In the case of SPM, CPI values indicate a relatively higher contamination by petroleum hydrocarbons in the month of April than in other months and the highest influx of higher plant in the month of June.

The variation in the UCM profiles shown in figures 2 and 3 supports the earlier assertion that petroleum/petrochemical contamination was heavier in the wet season than in the dry season. The observed bimodal UCM profiles for water (JW2, DW4 and AW1) and SPM (JSP2, DSP4 and ASP1; Figures 2 and 3) indicate an additional minor source input most likely of coal tar origin (Zhang et al., 2004; Oyo-Ita et al. 2016) utilized in the nearby towns such as Calabar and Oron for road construction.

The investigative parameter that gives supportive proof of the occurrence of natural inputs to the aquatic environment is the carbon maximum (C_{max}). For instance, C_{max} of 31 in sediments is an indication that the watershed is populated with grasses, whereas C_{27} and C_{29} are more abundant in aquatic sediments where trees dominate the vicinity of the lake. Although the range ($C_{max(C21-C40)}$) recorded for water was predominated by C_{27} and C_{29} in some samples in the wet season, most samples showed an even/odd carbon numbered preference (C_{26}, C_{28}, C_{30} and C_{32}; Figure 5) with C_{max} at C_{23} and C_{25} occurring in JW2 and AW5 (wet season), indicating input from micro-organisms and macrophyte, respectively. In the case of the dry season, C_{max} at C_{27}, C_{29} and C_{31} indicate a predominance of higher plant waxes over micro-organisms in these samples (Figure 6), whereas $C_{max(C8-C20)}$ was predominated by C_{10}, C_{11}, C_{17}, C_{18}, and C_{20} in most SPM samples in the wet season, most likely derived from petrochemicals and micro-organisms.

However, inter and intra-plant variations show that most of the C_{max} approaches have been on a premise of a slim set of data. A larger set of analyzed data on grasses and trees of different species from different parts of the globe shows that C_{27}, C_{29} and C_{31} are largely inconstant within plant families, in a way that chemotaxonomic dissimilarities between woody plants and grasses are difficult to make on the bases of abundances of *n*-alkane (Bush & Mcinerney, 2013).

Values of TAR greater than 1 are suggestive of dominant inputs from terrestrial OM, while those less than 1 imply a major contribution from aquatic OM (Simoneit, 2002). In the study, TAR values for water (Table 1 a) support lower aquatic than terrestrial OM inputs in the dry than in the wet season, while those for SPM not only imply higher input of terrestrial over aquatic OM but also the fact that this higher contribution peaked in the wet season.

P_{aq} values ranging between 0.01 and 0.23 are ascribed to higher plant leaf waxes, while ratios in the range of 0.48-0.94 are linked to floating/submerged macrophytes (Ficken et al., 2000). The observed intermediate P_{aq} values recorded

for the water and SPM samples imply an almost equal amount of terrestrial plant and macrophytes with higher input of macrophyte found in the month of June (wet season).

Syakti et al. (2013) stated that values of $\sum n$-alkanes/C_{16} ratio greater than 50 suggest biogenic origin, whereas those less than 15 indicate petrogenic origin. The mean values for the water and SPM samples indicate a predominance of biogenic over anthropogenic OM inputs, with the highest biogenic flux recorded in the wet season (Tables 1 a and b).

4.3. Principal Component Analysis

Results for water indicated that PC1 was heavily weighted by LHCs and characterized by input from vascular plant/wood combustion and degraded oil Linuma et al., 2007; Oyo-Ita et al., 2016 as well as MHCs and SHCs characterized by input from macrophytes (Ficken et al., 2000) and photosynthetic bacteria, respectively (Meyers, 1997). Therefore, PC1 can be said to originate from a mixed source of vascular plant/wood combustion, degraded oil, macrophytes and bacteria. PC2, heavily weighted by certain SHCs, was associated with inputs from micro-organisms (e.g., algae; Choudhary et al., 2010), while PC3 was moderately weighted by different SHCs associated with inputs from petrochemicals (e.g., gasoline/kerosene; Kang et al., 2016).

Similarly, for SPM, PC1 was heavily loaded by LHCs characteristics of vascular plant/wood combustion and degraded oil inputs Linuma et al. 2007; Oyo-Ita et al. 2015 as well as MHCs typically abundant in high levels in macrophytes (Ficken et al., 2000) and certain SHCs characteristics of input arising from bacterial degradation of algal OM (Elias et al., 1997). PC2 had high loading of other SHCs typically present in high abundance in micro-organisms (e.g., photosynthetic bacteria and algae; Choudhary et al., 2010), while PC3 was moderately loaded with SHCs typical of petrochemicals input such as gasoline/kerosene (Kang et al., 2016).

4.4. Seasonal Variation in Source Contribution by Multiple Linear Regression

Generally, natural biogenic OM input overwhelmed the contribution from anthropogenic activities to the estuary. However, the anthropogenic input in water was lower in the dry season than in the wet season, whereas the reverse was the case for SPM, in which higher anthropogenic pressure was found in the dry season. In other words, a lower natural biogenic contribution was found for SPM in the dry season than in the wet season, whereas in water, the reverse was the case.

4.5. Relationship amongst Samples by Cluster Analysis

The three main distinguishable clusters A, B and C observed for water in which sampling points in Cluster A impacted mainly by LHCs and MHCs were related to a common mixed source of vascular plant/wood combustion, degraded oil and macrophyte. The second cluster B comprised sampling points mainly influenced by algae and photosynthetic bacteria related to inputs from a certain analogue of SHCs, while the third cluster C consisted of sampling points impacted mainly by petrochemicals (e.g., gasoline and kerosene) related to input from different SHCs analogue (Figures 5 a and b).

Similarly, for SPM cluster A the sampling points were mainly influenced by a mixed common source of vascular plant/wood combustion, degraded oil and macrophyte (Figure 5 b) related to inputs from LHCs and MHCs. The second cluster B consisted of sampling points mainly impacted by micro-organisms such as algae and photosynthetic bacteria linked to inputs from certain SHCs analogue, while the third cluster C comprised of sampling points impacted majorly by petrochemicals such as gasoline and kerosene linked to inputs from different SHCs analogue.

4.6. Effects of Hydrological Cycles and Anthropogenic Activities on N-Alkanes Distribution

In the Cross River estuary, the riverine discharges are regulated by the rainfall in the river basin, as about 80% of the yearly rainfall accentuates in the rainy season. In 1992, the water discharge for each month ranged between 880 m^3sec^{-1} in February and 2533 m^3sec^{-1} in June (mean=1860 m^3/s) (Lowenberg & Kunzel, 1992). Moreover, with the recent dredging of Calabar River by the Nigerian Port Authority for larger ship navigation and enhanced flood episodes caused by the recent intense rainfall occasioned by climate change, river discharges are expected to rise in the estuary. Hydrological cycles have been reported to reflect variations in the estuarine characteristics, including suspended sediment concentration (SSC), temperature and salinity (Liu et al., 2017). In the study, the estuarine parameters exhibited remarkable seasonal variation (Figure 6). Several studies have attributed the seasonal variation in aromatic compound levels to changes in riverine discharges and rainfall (e.g. Doong and Lin, 2004; Sun et al., 2009; Zakaria et al., 2002; Liu et al., 2017). Similar correspondence with n-alkanes distributions is scarce.

In the study, the bi-monthly mean $\sum C_8$-C_{40}, LHCs and SHCs levels in water and SPM were higher in the rainy season than in the dry season (Figure 6). This was attributable to higher wet precipitation and surface run-off carrying terrigenous materials (e.g., street dust, plant debris and domestic/commercial wastes) adsorbed onto solid matrix into the estuary as well as the fact that fishery activity involving boating and fish roasting/drying by utilization of charcoal (i.e., wood combustion) mainly take place in the peak of the wet season (June). This became possible due to the hydrophobic nature of n-alkanes with high adsorption capacity towards solid matrix. It is obvious that part of the enhanced n-alkanes loads in the estuary originated due to anthropogenic activities. Occasional oil spills from offshore operations, and utilization of charcoal/petrochemicals for fishery/boating, particularly in the vicinity of the sampling point, resulted in more anthropogenic pressure on the estuary in the wet season.

Anthropogenic n-alkanes distribution, mobility and partition are expected to differ because of variations in the physico-chemical characteristics of estuaries. The higher temperature recorded in the wet season (Figure 6) caused the desorption of n-alkanes from SSC into the aqueous phase. It appeared that increasing salinity in the estuary caused more n-

alkanes desorption from SSC in the dry season than in the rainy season. The relatively good correlations that existed between SSC and $\sum C_8$-C_{40} in water in the two seasons not only supported the high affinity of *n*-alkanes for the solid matrix but also the fact that the SHC series might not have adsorbed onto SSC to the same extent as the LHC counterpart because of differential water solubility. In other words, a considerable SSC coincided with a reasonable amount of $\sum_{C8-C40}$ in both wet and dry seasons. Such a behavior is most likely linked to the influence of temperature and salinity in the estuary, associated with relatively high-water solubility of the SHC congeners (Liu et al., 2017). This implied that higher SSC contributed to higher LHC levels in the wet season.

5. Conclusions

In this work, an assessment of the behaviors of n-alkanes in water and SPM from the Cross River estuary with depth in reaction to anthropogenic pressure and hydrological cycles was undertaken. The levels of n-alkanes in water and SPM were higher in the rainy season than in the dry season, attributed to higher wet precipitation and surface run-off as well as proximity to fish settlements where charcoal utilization for fish roasting/drying takes place in the peak of the wet season.

Evaluation of LHC/SHC, TAR, CPI, Cmax, Paq, Σn-alkanes/C16, and UCM, as well as PCA and MLR, indicated changes in the quantity and quality of OM inputs with the season. The results revealed similar source contributions for water (dry season) and SPM (wet season) as: vascular plant/wood combustion> macrophyte > degraded oil > petrochemicals > algae/photosynthetic bacteria, while dissimilar source contributions were found for water in the wet season (vascular plant/wood combustion > degraded oil > macrophyte > petrochemical > algae/photosynthetic bacteria) and SPM in the dry season (vascular plant/wood combustion > petrochemical > macrophyte > degraded oil > algae/photosynthetic bacteria). In the study, it was demonstrated that organic contaminants in estuarine biogeochemical systems were susceptible to hydrological variations and anthropogenic activities, thereby stimulating more studies in the face of worldwide environmental changes.

6. Acknowledgements

7. Author Contributions

- The project design was done by Orok E. Oyo-Ita.
- Sampling, material preparation, analysis and data collection were mainly performed by Patience Y. Nkom.
- Statistical analysis was carried out by Samuel U. Ugim.
- The investigation, review and editing of the manuscript were carried out by Patience Y. Nkom, Inyang O. Oyo-Ita, Samuel U. Ugim and Orok E. Oyo-Ita.

8. Declarations

Conflict of interest: The authors declare that they have no conflicting interests.

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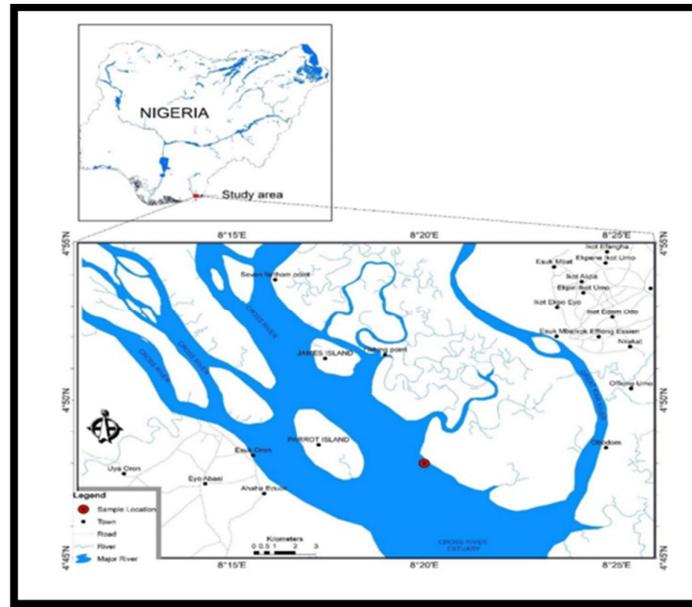
Appendices

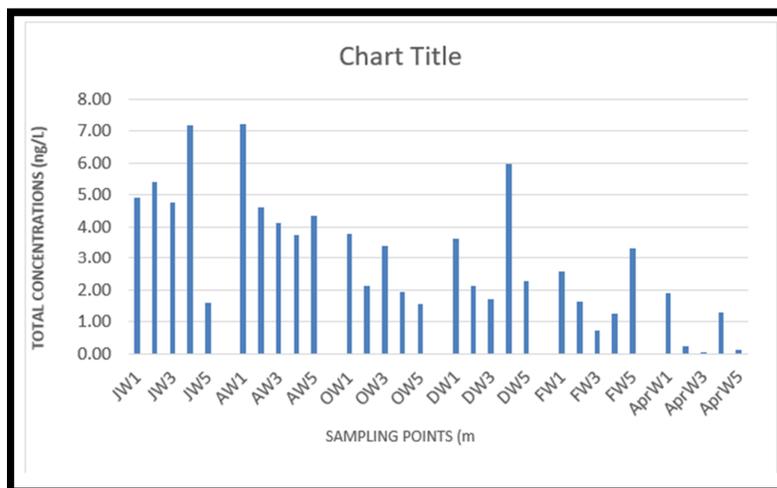*Figure 1: Map of the Cross River Estuary Showing Sampling Location*

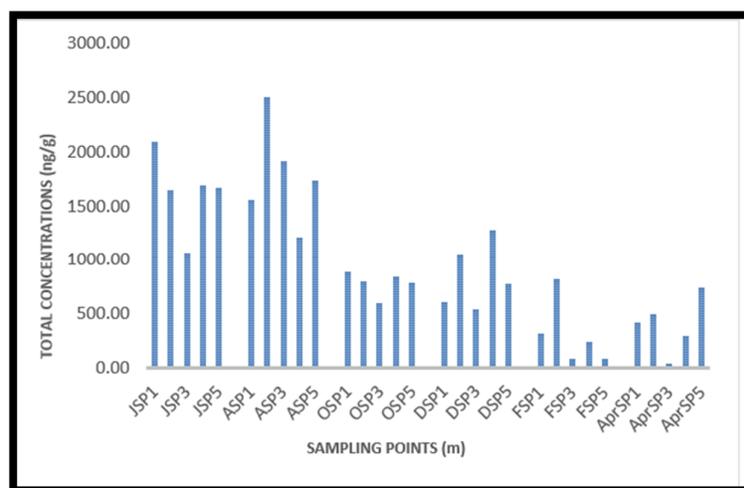*Figure 2: Total N-Alkanes Concentrations in Water from the Cross River Estuary*

Figure 3: Total N-Alkanes Concentrations in SPM from the Cross River Estuary
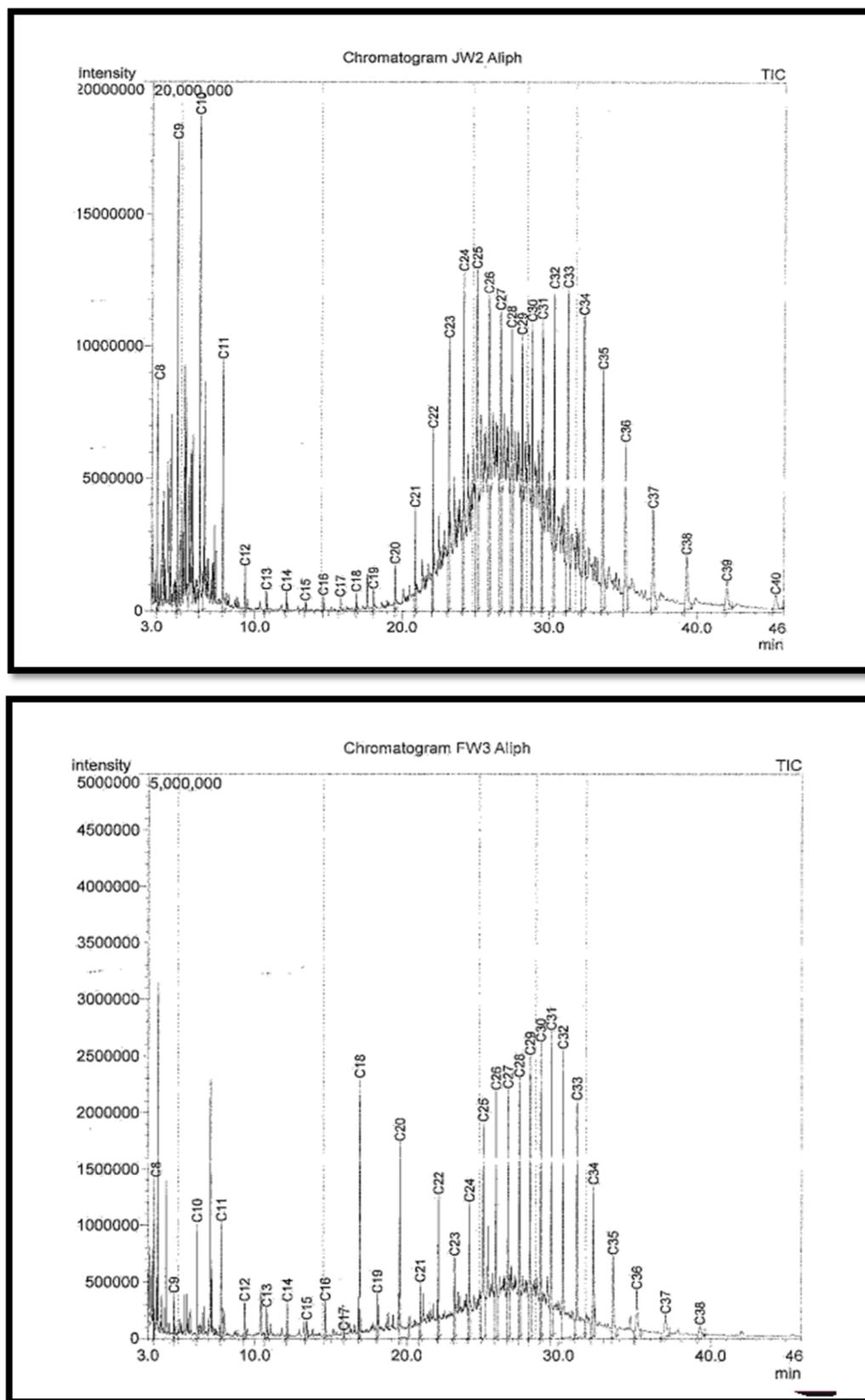
Figure 4: Representative Gas Chromatograms of N-Alkanes Showing
(A) Relatively High UCM with a Bimodal Distribution in the Wet Season and
(B) Relatively Low Abundant UCM with an Even Over Odd Carbon Numbered Predominance in the Dry Season
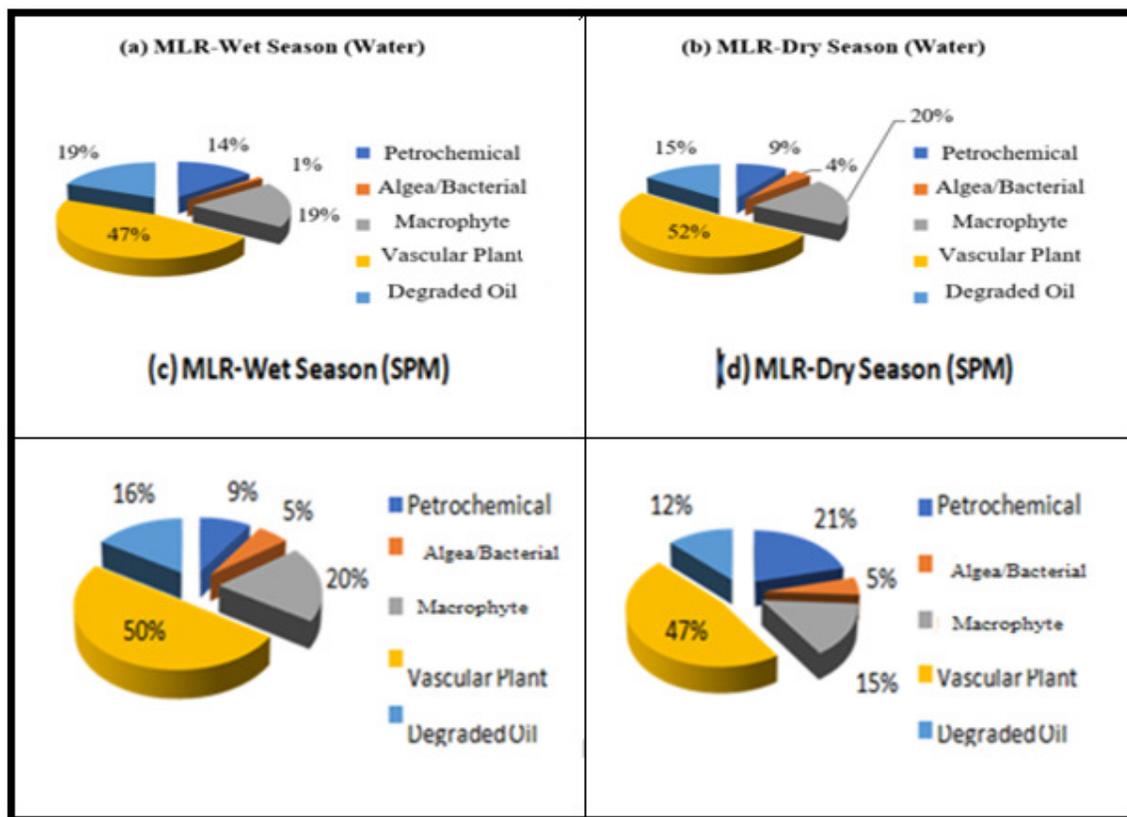
Figure 5: Source Contributions of N-Alkanes in Water [(A) Wet and (B) Dry Seasons] and SPM [(C) Wet and (D) Dry Seasons]
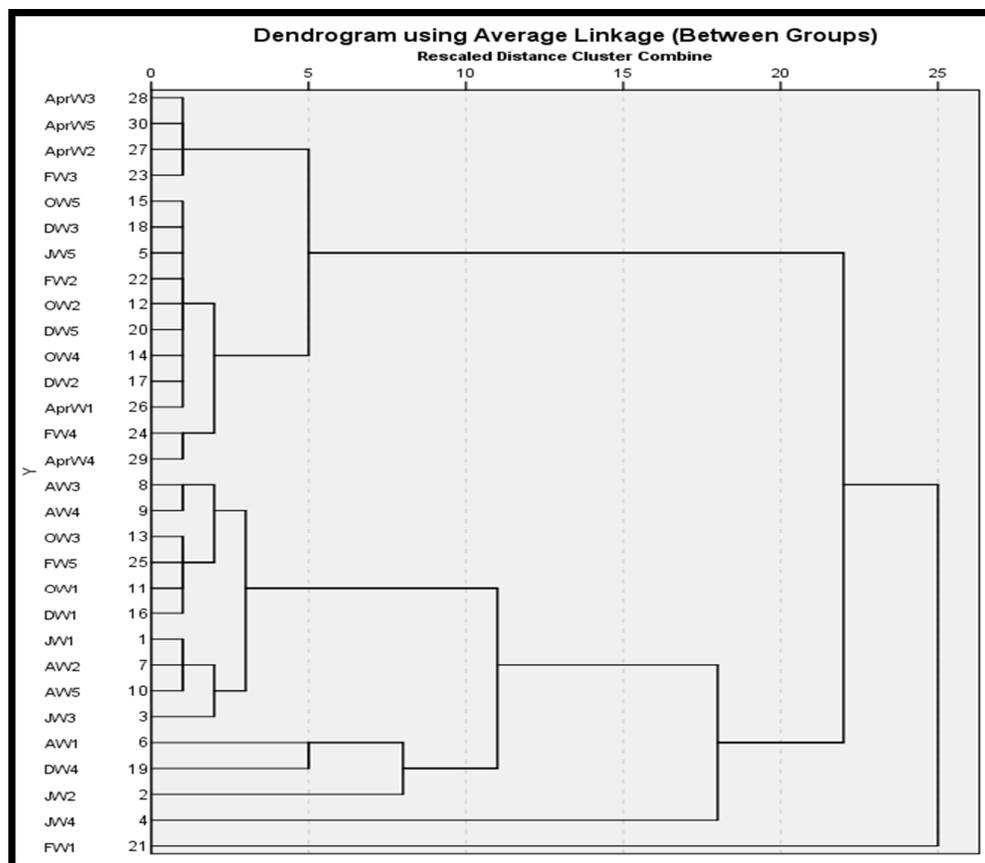

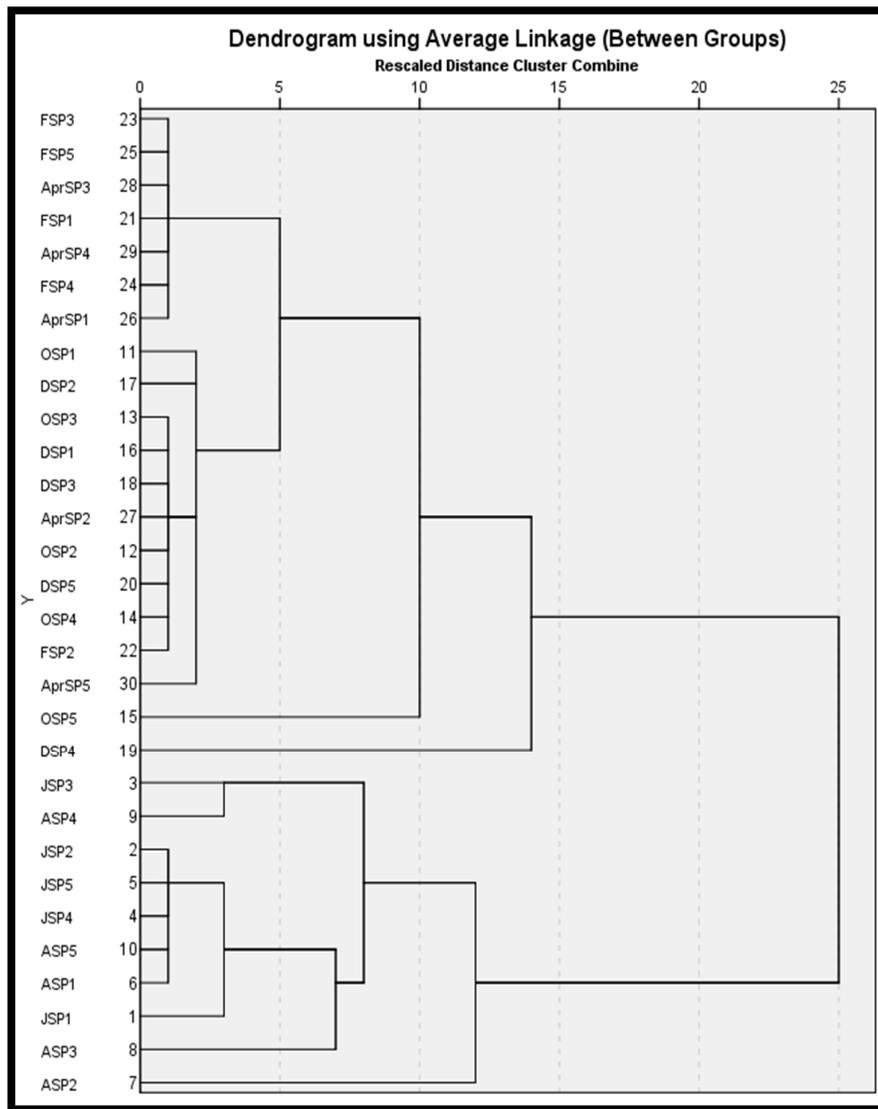*Figure 6: Cluster Dendrogram for N-Alkanes in Water*

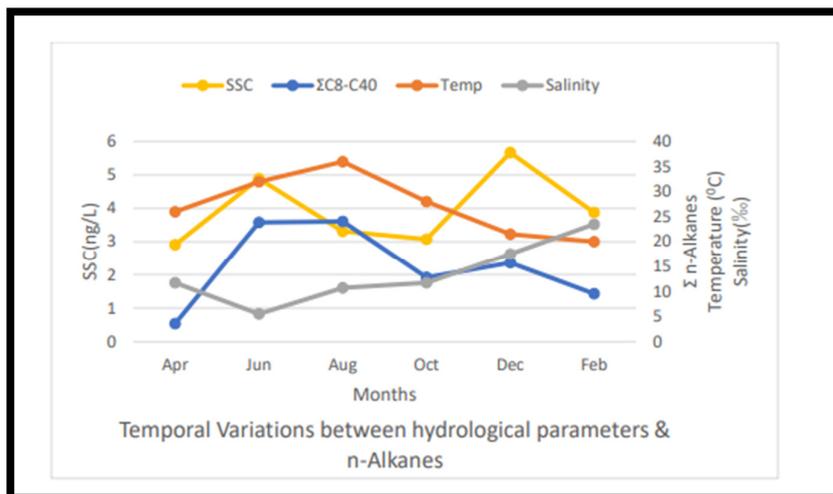*Figure 7: Cluster Dendrogram for N-Alkanes in Suspended Particulate Matter*

Figure 8: Temporal Variations in Hydrological Parameters with N-Alkane Concentrations in Water (Adapted From Oyo-Ita Et Al. 2021)

(a) water									
Season	Month	$\sum C_8\text{-}C_{40}$ (Mean)	SHC	LHC	LHC/SHC	CPI	TAR	P_{aq}	$\Sigma n\text{-alkanes}/C_{16}$
Wet	APR	0.72	0.11	3.59	3.57	1.26	17.44	0.27	176.66
Wet	JUNE	4.78	0.59	4.04	7.42	1.05	21.6	0.48	503.08
Wet	AUG	4.81	0.45	4.23	12.69	1.15	34.64	0.39	637.18
Wet	Mean	3.44±1.92	0.38±0.20	3.95±0.27	7.89±3.74	1.15±0.09	24.56±7.32	0.38±0.09	439.973±193.39
Dry	OCT	2.56	0.27	2.23	8.54	1.09	16.86	0.33	90.4
Dry	DEC	3.14	0.46	2.6	6.75	1.12	15.66	0.38	151.32
Dry	FEB	1.91	0.24	1.64	7.52	1.21	17.28	0.38	165.69
Dry	Mean	2.54±0.50	0.32±0.10	2.16±0.40	7.60±0.73	1.14±0.05	16.6±0.69	0.36±0.02	135.80±32.64
(b) Suspended Particulate Matter									
Season	Month	MEAN $C_8\text{-}C_{40}$	SHC	LHC	LHC/SHC	CPI	TAR	P_{aq}	$\Sigma n\text{-alkanes}/C_{16}$
Wet	APR	400.85	108.76	284.28	3.04	0.92	6.05	0.31	111.31
Wet	JUNE	1629.71	111.15	1475.37	13.28	1.28	30.99	0.37	413.29
Wet	AUG	1782.64	97.4	1641.27	18.19	1.13	50.15	0.39	714.29
Wet	Mean	1271.06±618.48	105.77±5.10	1133.64±604.39	11.50±6.31	1.11±0.15	29.06±18.06	0.36±0.03	412±246.17
Dry	OCT	784.24	121.38	647.39	5.71	1.02	11.84	0.32	139.66
Dry	DEC	852.23	180.26	655.18	5.25	1.12	12.58	0.33	184.34
Dry	FEB	311.08	76.79	228.49	3.11	1.16	13.12	0.27	141.98
Dry	Mean	649.18±240.68	126.14±42.38	510.35±199.33	4.69±1.13	1.10±0.06	12.51±0.52	0.31±0.03	155.33±20.54

Table 1: Bi-Monthly Mean N-Alkanes Concentrations (Ng/L) and
Their Diagnostic Indices in (A) Water and (B) Suspended Particulate Matter

Components	PC 1	PC 2	PC 3
Eigenvalues	21.52	3.61	2.66
Variance %	65.22	10.94	8.07
Source	Mixed (Vascular plants, macrophytes, algae and degraded oil)	Algae/ Bacteria	Petrochemicals (gasoline/kerosene)
C24	0.984	-0.02	-0.07
C23	0.98	-0.083	-0.108
C22	0.978	-0.024	-0.112
C34	0.977	-0.021	-0.135
C35	0.971	-0.095	-0.172
C26	0.969	0.047	-0.164
C36	0.968	-0.116	-0.178
C37	0.961	-0.118	-0.19
C32	0.96	0.09	-0.134
C28	0.959	0.151	-0.077
C38	0.953	-0.157	-0.176
C21	0.946	-0.138	-0.123
C27	0.942	0.108	-0.029
C39	0.942	-0.124	-0.164
C30	0.924	0.187	-0.12
C25	0.923	-0.035	0.039
C29	0.921	0.215	-0.058
C40	0.914	-0.267	-0.083
C31	0.854	0.247	0.096
C19	0.784	0.087	-0.073
C15	0.732	0.284	-0.035
C12	0.703	-0.185	**0.627**
C10	0.694	-0.355	0.511
Components	PC 1	PC 2	PC 3

C13	0.681	0.028	0.587
C11	0.655	-0.263	**0.639**
C9	0.591	-0.317	0.428
C33	0.538	-0.064	0.134
C18	0.036	**0.934**	0.145
C16	0.103	**0.905**	0.149
C17	0.229	**0.827**	-0.098
C20	0.436	0.605	0.15
C8	0.503	-0.114	**-0.601**
C14	0.487	0.264	0.553

Table 2: Eigenvalues, Total Variability and Factorial Weight Matrix Explained by PCA for N-Alkanes in Water

Parameter	T_W (°C)	S_W (%)	SSC_W (g/L)	T_D (°C)	S_D (%)	SSC_D (g/L)	MCW_D (ng/L)	MCS_D (ng/g)
$\sum C_8$-C_{40}	0.8473	-0.3829	0.4235	0.4235	-0.4386	0.4256	-	-
MCW_W (ng/L)	-	-	-	-	-	-	0.0777	-
MCS_W (ng/g)	-	-	-	-	-	-	-	0.3168

Table 3: Correlation Coefficients (R) Among Determined Parameters in
Water and Suspended Particulate Matter for the Two Seasons

N/B: MCW_D – Mean concentrations of n- alkanes in water for the dry season
MCS_D – Mean concentrations of n-alkanes in SPM for the dry season
MCW_W – Mean concentrations of n-alkanes in water for the wet season
MCS_W – Mean concentrations of n-alkanes in SPM for the wet season
T_W – Temperature in wet season
T_D – Temperature in dry season
SSC_W – Suspended sediment concentrations in wet season
SSC_D – Suspended sediment concentration in dry season
S_W – Salinity in wet season
S_D – Salinity in dry season