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# APPLICATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN CHEMICAL FINGERPRINTING: THE NIGER DELTA CASE STUDY

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## ABSTRACT

Chemical fingerprinting is an aspect of environmental forensic investigation which involves chemical analysis of contaminants and associated chemicals to provide source specific information. Polynuclear Aromatic Hydrocarbons (PAHs) in the environment have 3 categories of sources namely petrogenic, pyrogenic and biogenic sources. Petrogenic PAHs are generated from geochemical alterations of organic mater. Pyrogenic PAHs originate when organic matter is incompletely combusted. Biogenic PAHs originate as a result of oxidation of microbial or plant derived compounds in older and deeper sediments. PAHs fingerprinting involves the determination of a number of quantitative diagnostic ratios of source specific marker PAH compounds. These quantitative diagnostic ratios may be used to distinguish petrogenic PAHs including phenanthrene/anthracene; benz(a)anthracene/chrysene; flouranthene/pyrene; phenanthrene/(phenanthrene+anthracene) and indeno(1,2,3-cd) pyrene/indeno (1,2,3-cd) pyrene + benzo (ghi) perylene from other sources. In this research over 40 environmental samples from the Niger Delta region were subjected to chemical fingerprinting employing some of the quantitative diagnostic ratios above with the aim of ascertaining the precise nature and source the contaminants. It was found that the PAHs contamination in the Niger Delta is not only emanating from petrogenic sources but other sources contribute significantly.

**Key words:** Crude oil; Fingerprinting; PAHs; Niger Delta

## INTRODUCTION

Polynuclear Aromatic Hydrocarbons (PAHs) are a class of diverse organic compounds containing two or more fused aromatic rings of carbon and hydrogen atoms. They are ubiquitous environmental contaminants found in air, water and soil (Yang *et al.,* 1991; Kennicutt *et al.,* 1994; DuoAbul *et al.,* 1997; Dejmek *et al.,* 2001; Anyakora *et al.,* 2005) and are always found as a mixture of individual compounds. PAHs comprise the largest class of chemical compounds known to be cancer-causing agents. Some, while not carcinogenic, may act as synergists (Anyakora *et al*., 2008). Owing to their low solubility and high affinity for particulate matter, PAHs are not usually found in water in notable concentrations; hence their presence in surface water or groundwater is an indication of a source of pollution.

PAHs are very difficult to degrade; the difficulty is due to the complexity and stability of their molecular structures (Yun and Xinhong, 2003; Arbabi *et al.,* 2004; Nasseri *et al.,* 2010). They are classified among the Semi-Volatile Organic Compounds (SVOC) having boiling points greater than 200°C. There are several hundred different PAHs, which always occur as complex

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mixtures of different PAHs. Some PAHs are considered as priority because they are supposed to be more harmful than the other PAHs, more information is available on them and there is a greater possibility of people being exposed to them. These PAHs include: acenaphthene, acenaphthylene, anthracene, benz (a) anthracene, benzo (a) pyrene, benzo (b) flouranthene, benzo (g, h, i) perylene, benzo (k) flouranthene, chrysene, dibenz (a,h) anthracene, flouranthene, flourene, indeno(1,2,3-c,d)pyrene, naphthalene, phenanthrene and pyrene. Their chemical structures are shown in Fig. 1.

PAHs are highly stable and have multiplicity of which could be broadly classified as diagenetic in origin, pyrogenic in origin or petrogenic in origin (Neff, 1979; Mazeas and Budzinski, 2001; Wang *et al.,* 2004; Anyakora and Coker, 2006). They are classified as diagenetic in origin when they occur as a transformation product of natural sources such as volcanic eruption and microbial degradation of organic matter. When PAHs occur as a result of incomplete combustion processes of organic matter such as combustion of wood, oil, vehicular emissions, industrial emission and forest fires and so on, they are classified as pyrogenic. But when the contamination occurs from petroleum sources as a result of natural or anthropogenic causes such as oil spill and petroleum production, they are referred to as petrogenic in origin.

In the recent years, toxicity and carcinogenicity of PAHs have been considered as major issues (Gladen *et al.,* 2000; Dejmek *et al.,* 2001; Vassilev and Klotz, 2001; Anyakora *et al.,* 2004; Arbabi *et al*., 2009). Also, the environmental awareness has grown tremendously, hence appropriate regulatory bodies both at national and international levels have continued to promulgate and implement stricter environmental regulations in this regard. Several researchers have published on the adverse health impacts on human as a result of PAHs contamination. Even with the effort of the regulatory bodies, PAHs contamination cannot be completely eliminated because their occurrence is attached to our normal daily living, but its enforcement can be further implemented if there is a way to determine an objective defensible source specific information on a given PAH contamination.

Chemical fingerprinting is one way of determining source specific information through thorough chemical analysis of contaminants. PAHs show different molecular distribution depending on their origin. This attribute is a very important tool in determining source specific information for PAHs. Even though, molecular distribution of PAHs cannot be said to be 100% accurate, since

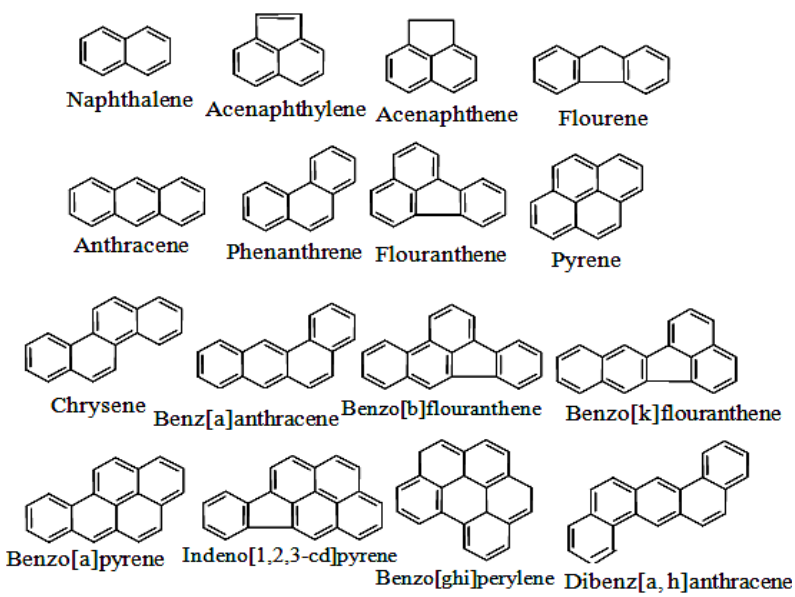


Fig. 1: The chemical structures of 16 priority PAHs

other factors can affect the molecular distribution over time, such as; evaporation, dissolution, photo oxidation and biodegradation which can lead to compound specific degradation (Neff, 1979; Baek *et al.,* 1991). This notwithstanding fairly good source specific information can be provided based on molecular distribution of PAHs.

Several quantitative diagnostic ratios have been defined to differentiate between different PAHs sources. Since focus is made on priority PAHs, those quantitative ratios that deal with 16 priority PAHs would be considered which include: phenanthrene/anthracene (Ph/An), fluoranthene/pyrene(Fl/Py), benz[a] anthracene/ chrysene(BaA/Ch), phenanthrene/(phenanthrene +anthracene) Ph/(Ph+An) and indeno[1,2,3-cd] pyrene/(indeno[1,2,3-cd]pyrene+benzo[ghi] perylene).

In this research work, some of these diagnostic ratios to environmental samples from the Niger Delta region of Nigeria were applied with the aim of ascertaining the source of PAHs contamination. Even though petroleum production and crude exportation are the primary activities done in the area, our findings show that there may be multiple sources of PAH contamination in the area.

## MATERIALS AND METHODS

*Study site*

The Niger Delta is a wide expanse of swamp forest ecoregion extending from the Atlantic Ocean to River Niger. It is located between longitude 5° and 9° E and between latitude 4° and 6° N. Along its southern side, Niger Delta is separated from the Atlantic Ocean by a band of Mangrove which can reach up to 10 kilometer inland. In front of the mangrove belt and close to the sea are ephemeral coastal barrier islands often clothed in transitional vegetation. The ecoregion's total area of approximately 15,000 $km^2$ is contained in three states of Nigeria namely: Rivers, Bayelsa and Delta. It contains both fresh inland waters and a highly urbanized brackish ecosystem impacted by municipal and industrial activities that have significantly increased in the past decades, But very much impacted by crude oil due to great activities of import and export of petroleum related products. The choice of the location was influenced by the fact that it is one of the most significant environments in Nigeria. The map of the Niger Delta is as shown in Fig. 2.

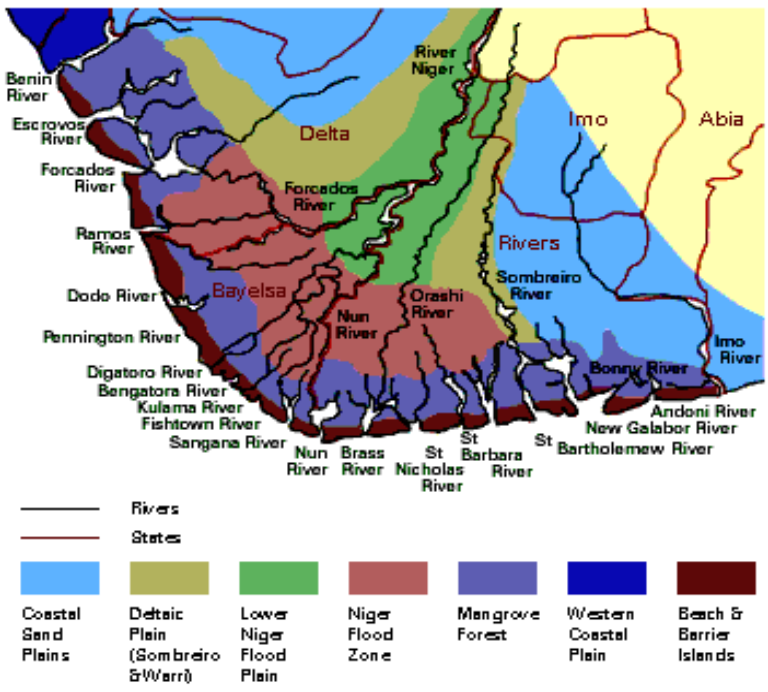


Fig. 2: The map of the Niger Delta

*Reagents*

All chemicals and reagents were of analytical grade and of highest purity possible. LC-grade dichloromethane used for extractions was obtained from Fischer Scientific. A PAH standard mixture (NIST, Baltimore, MD) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a] pyrene, benzo[ghi]perylene dibenz[a, h] anthracene and indeno[1,2,3-cd]pyrene was used in this study. A mixture containing four isotopically labeled PAHs (ChemService, Westchester, PA) namely acenaphthalene-$_{d10}$, chrysene-$_{d12}$, phenanthrene-$_{d10}$ and perylene-$_{d12}$ was used as an internal standard.

*Collection of samples*

Three categories of environmental samples were used in this study. These include, water samples, sediment samples and fish samples. Thirty nine (39) samples were analyzed altogether, including: fish 13, sediment 13, and water 13. Sediment samples were collected from the river bed, the fish samples were collected from their natural habitat while water samples were collected between 0 and 1 m depth.

*Preparation of standard solution*

Five standard solutions each containing 16 target compounds were prepared by diluting the standard mixture (1647 mix from NIST) to desired concentrations with HPLC grade dichloromethane. To all these solutions 0.5 µg of each of the four internal standards namely acenaphthene-$d_{10}$, chrysene-$d_{12}$, phenanthrene-$d_{10}$ and perylene-$d_{12}$. were added These solutions were transferred to a capped and sealed vial as ready for analysis.

*Extraction*

Different extraction methods were used for the sample-liquid-liquid extraction and soxhlet extraction. The extraction of the samples was carried out by liquid-liquid extraction method (US EPA, 1994). The apparatus consisted of a 100 mL separating funnel mounted on a retort stand. The separating funnel was thoroughly washed and dried over night in a muffle furnace at an elevated temperature. Prior to use, the funnel was rinsed vigorously with dichloromethane for several minutes. This was removed and allowed to drain and dry completely in fume cupboard. 20 mL of water sample to be extracted was transferred to the separating funnel and 20 mL of dichloromethane was added to it; this was shaken vigorously for 2 minutes and allowed to separate and settle. After 10 minutes, the organic layer was removed and the process was repeated with the aqueous layer twice. The three portions of the organic phase were combined and evaporated to 1 mL volume using a rotary evaporator.

In the case of sediment and fish samples, soxhlet extraction method was carried out using a modified form of the EPA 3540 method (US EPA, 1994) with 150 mL dichloromethane for 16 h. The extract was concentrated in a rotavap. Specifically for fish, the fillet was homogenized in an equal volume of $Na_2SO_4$ (Mottier *et al*., 2000) until a completely dry homogenate was obtained prior to extraction.

*Calibration*

Several dilutions of the standard PAH mixture were analyzed to determine the Limit of Detection (LOD), Limit of Quantization (LOQ), Limit of Linearity (LOL), Relative Standard Deviation (RSD) and regression coefficient ($r^2$). LOD was determined by the signal to noise ratio of 3:1. LOQ was determined by the signal to noise ratio of 10:1 and LOL was determined from the plot of the concentration versus response. The RSD for the sixteen compounds were determined by triplicate of each analysis. $r^2$ was determined for each compound using excel formula software.

*Analysis by GC/MS*

GC/MS analysis was carried out on a Finnigan Magnum instrument equipped with a CTC A200S autosampler and a 30 m, 0.25 ID DB-5 MS fused silica capillary column (J and W Scientific, Folson CA). Helium was used as the carrier gas and the column head pressure was maintained at 10 psi to give an approximate flow rate of 1 mL/min. The injector and transfer line were maintained at 290°C and 250°C, respectively. All injection volumes were 1 µL in the splitless mode. The

column temperature was initially held at 70°C for 4 minutes, ramped to 300°C at a rate of 10°C/min and then the temperature was held at 300°C for 10 minutes. The mass spectrometer detector was used in electron ionization mode and all spectra were acquired using a mass range of 50-400 m/z and Automatic Gain Control (AGC).

*Identification and quantitation*

Identification of the compounds was based on the retention time match and mass spectra match against the calibration standards. Quantitation was performed by the method of internal standardization using acenaphthene-d10, chrysene-d12, phenanthrene-d10 and perylene-d12. Acenaphthene-d10 was used as the internal standard for naphthalene, acenaphthylene, acenaphthene and fluorene. Phenanthrene-d10 was used as the internal standard for phenanthrene, anthracene, fluoranthene and pyrene. Chrysene-d12 was used for benz[a] anthracene and chrysene. Perylene-d12 was used for benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h] anthracene and indeno[1,2,3-cd]pyrene.

The quantitation was based on the ratio of the peak height of the quant ion to that of the corresponding internal standard. The possibility of selected ion chromatogram enabled us to detect the target ion without ambiguity, despite the complexity of the samples.

## RESULTS

Chemical fingerprinting is very important to effectively determine and identify the source of PAH contamination, making use of different diagnostic ratios. But for the reliability of the fingerprinting results, it is extremely important that the analytical data to be used in qualitative and quantitative determination of PAHs, be subjected to thorough Quality Assurance and Quality Control (QA/QC) procedure. These QA/QC procedures include instrument calibration, matrix spike recoveries, replicate analyses, LOD and LOQ. In order to determine the linearity of response for the target compounds a 5 point calibration curve was plotted for each of the sixteen US EPA priority PAHs which are the target compounds. Fig. 3 shows the calibration curve for naphthalene which serves as the representative calibration curve for the sixteen target compounds. The sixteen compounds were all found to be linear with the regression coefficient ($r^2$) ranging from 0.994-1.000.

The GC-MS analysis conditions employed in this study achieved a good separation of the sixteen target compounds in less than 33 min. Fig. 4 shows the total ion chromatogram of the National Institute of Standard and Technology standard solution, containing sixteen PAHs. This chromatogram confirms the appropriateness of the analytical method used. All the analyses were done in triplicate and the standard deviation for

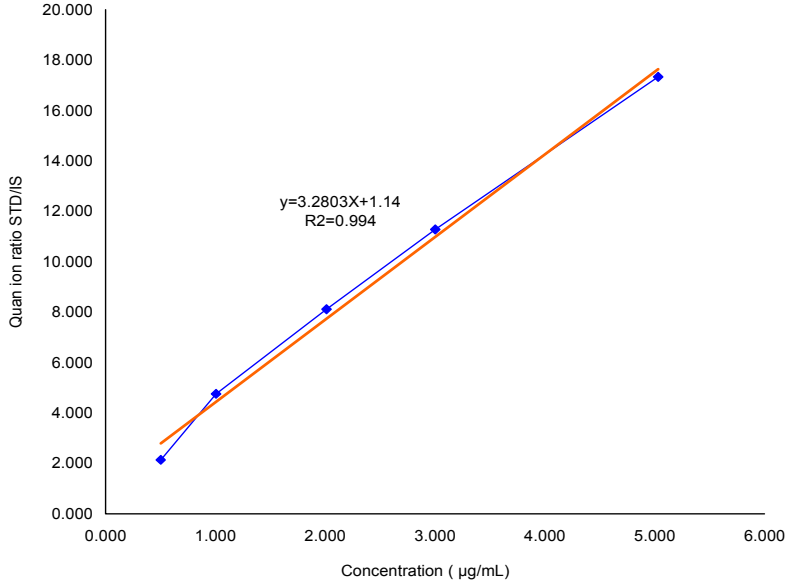


Fig. 3: Calibration curve for naphthalene via GC-Mass

the target compounds were mostly below 10%. Internal standardization was employed to enhance the accuracy and precision of the results. LOD and the LOQ were in the range of 0.06-1.7 and 0.2-5 µg/mL, respectively. Fig. 5 shows the total ion chromatogram of one of the environmental samples as a representative of all the analyzed samples.

The conditions gave good enough separation to enable identification, quantitation and determination. The selected ion chromatogram capability of GC-MS helped in improving precision in the determination of the target compounds in the environmental samples. Table 1 shows the summary of the analytical characteristics.

Sometimes it is possible to have multiple sources of PAHs in a given environment, thereby making the ratio relative. Fig. 6 shows the phenanthrene/ anthracene ratio for various environmental samples from the Niger Delta region.

In Fig. 7, the three environmental samples used in this

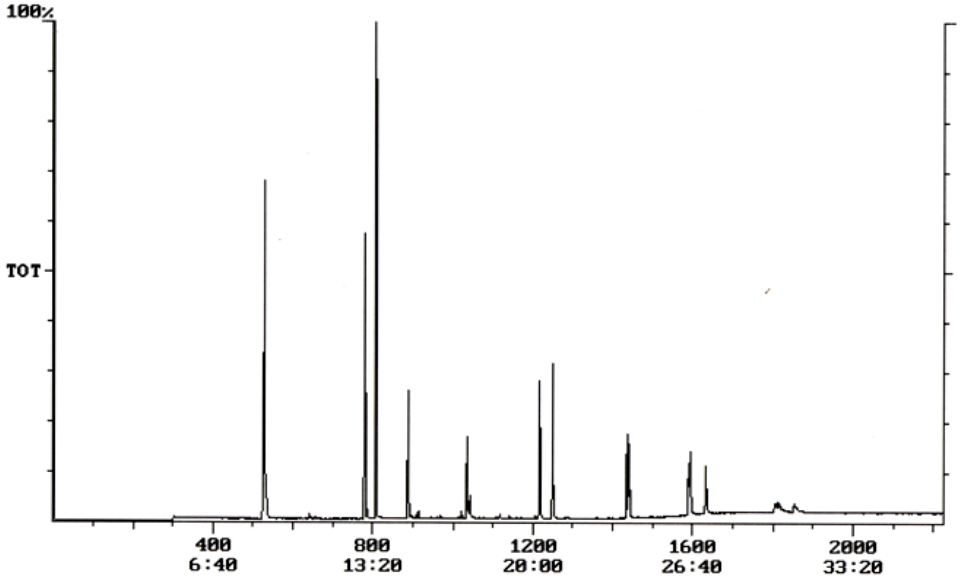


Fig. 4: Total ion chromatogram of the 16 PAHs namely: naphthylene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene., dibenzo[a, h]anthracene and indeno[1,2,3-cd]pyrene

Table 1: Chromatographic characteristic of the target compounds

| Compound | Retention time (min) | Linear range | Slope | Intercept | Regression coefficient | RSD (%) | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| Naphthalene | 8.46 | 0.503-5.033 | 3.280 | 1.143 | 0.994 | 4.76 | 0.06 | 0.20 |
| Acenaphthylene | 13.00 | 0.387-3.888 | 6.930 | 0.074 | 0.997 | 1.70 | 0.02 | 0.06 |
| Acenaphthene | 13.26 | 0.519-5.193 | 4.653 | 0.342 | 0.996 | 0.89 | 0.02 | 0.06 |
| Flourene | 14.49 | 0.119-1.188 | 4.024 | 0.084 | 0.997 | 6.20 | 0.02 | 0.06 |
| Phenanthrene | 17.14 | 0.086-0.855 | 5.063 | 0.105 | 0.997 | 7.17 | 0.03 | 0.09 |
| Anthracene | 17.22 | 0.020-0.198 | 5.272 | 0.01 | 0.997 | 4.92 | 0.02 | 0.06 |
| Flouranthene | 20.16 | 0.191-1.910 | 6.108 | 0.243 | 0.998 | 2.48 | 0.04 | 0.12 |
| Pyrene | 20.49 | 0.212-2.118 | 6.269 | 0.664 | 0.998 | 4.40 | 0.04 | 0.12 |
| Benz[a]anthracene | 23.55 | 0.102-1.023 | 5.354 | -0.134 | 0.999 | 5.36 | 0.06 | 0.20 |
| Chrysene | 24.00 | 0.092-0.918 | 5.388 | 0.089 | 0.996 | 4.26 | 0.06 | 0.20 |
| Benzo[b]flouranthene | 26.30 | 0.104-1.043 | 10.249 | -0.159 | 0.997 | 1.69 | 0.10 | 0.30 |
| Benzo[k]flouranthene | 26.35 | 0.118-1.180 | 13.24 | -0.417 | 0.999 | 2.71 | 0.15 | 0.50 |
| Benzo[a]pyrene | 27.18 | 0.123-1.228 | 6.884 | -0.411 | 0.995 | 2.11 | 0.15 | 0.50 |
| Benzo[ghi]perylene | 30.06 | 0.354-0.885 | 1.377 | -0.167 | 0.995 | 10.16 | 0.75 | 2.50 |
| Dibenz[a,h]anthracene | 30.17 | 0.368-0.920 | 0.922 | -0.108 | 0.997 | 15.79 | 0.90 | 2.70 |
| Indeno[1,2,3-cd]pyrene | 30.55 | 0.428-1.070 | 1.307 | -0.122 | 1.000 | 4.77 | 1.70 | 5.00 |

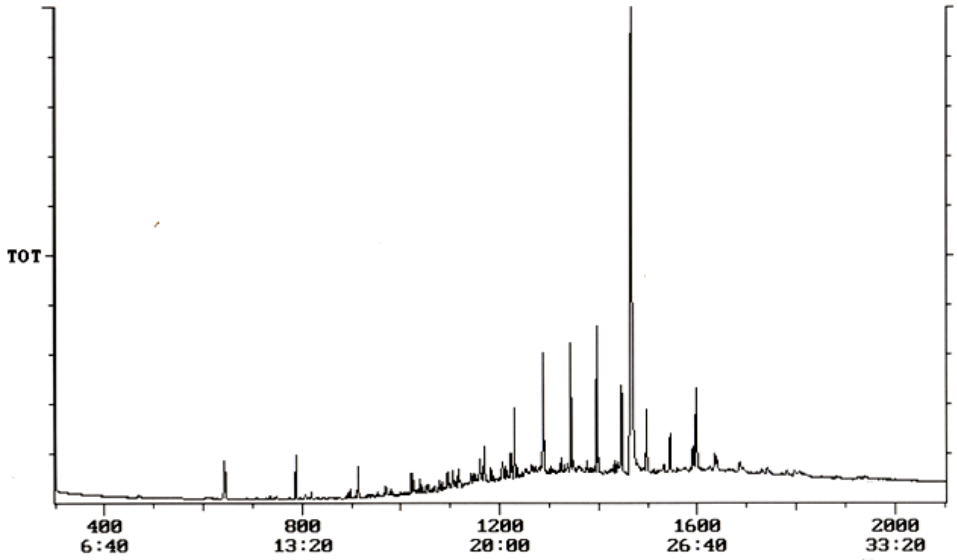


Fig. 5: Total ion chromatogram of a fish extract

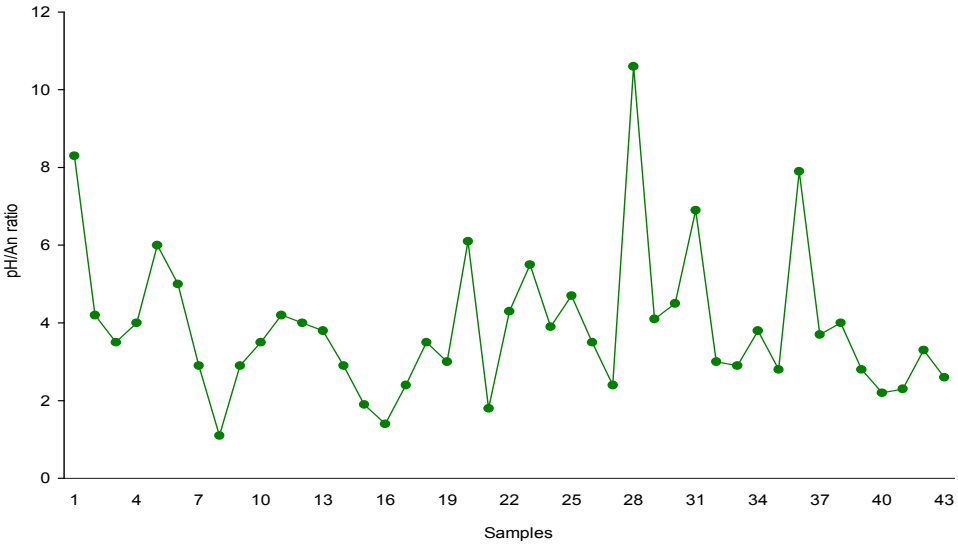


Fig. 6: Phenathrene/anthracene ratio for environmental samples from the Niger Delta region

study namely fish; sediment and water samples are separately studied to compare their distribution.

Figs. 8-10 show the different diagnostic ratios for phenanthrene/anthracene (Ph/An), fluoranthene/pyrene (Fl/Py) and phenanthrene/(phenanthrene + anthracene) Ph/(Ph+An)) for fish samples, sediment samples and water samples respectively, indicating the kind of relationship that exist between the diagnostic ratios.

## DISCUSSION

In this study determination and quantitation of PAHs were focused on sixteen priority PAHs and in the same way the quantitative diagnostic ratios used in this study for the determination of source specific information of PAHs were restricted to those sixteen. The quantitative diagnostic ratios for PAHs that fall within this category include phenanthrene/anthracene (Ph/An), fluoranthene/pyrene (Fl/Py), (phenanthrene/ {phenanthrene + anthracene}) (Ph/{Ph+An}), benz[a]anthracene/chrysene (BaA/Ch) and indeno[1,2,3-cd]pyrene/{indeno[1,2,3-cd]pyrene

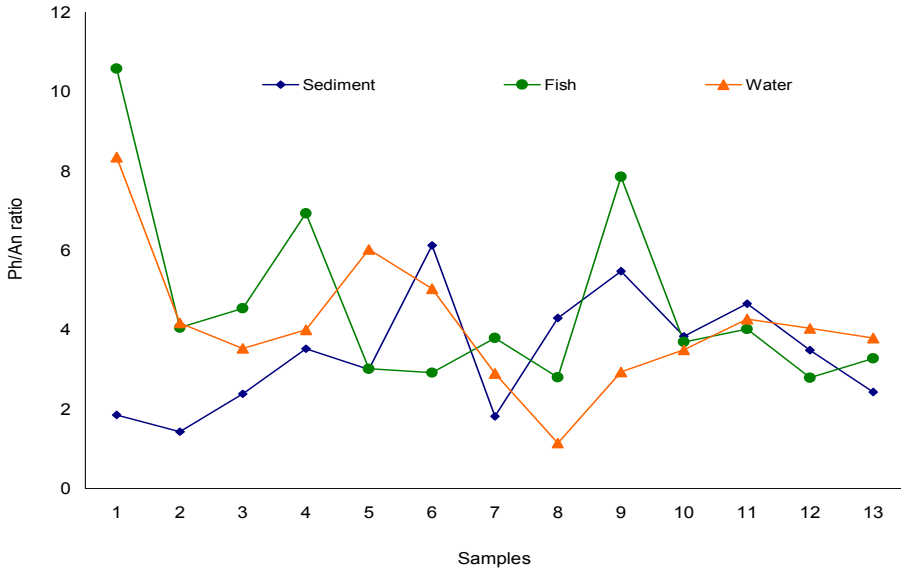


Fig. 7: Comparison of the phenanthrene/anthracene ratios for fish, sediment and water

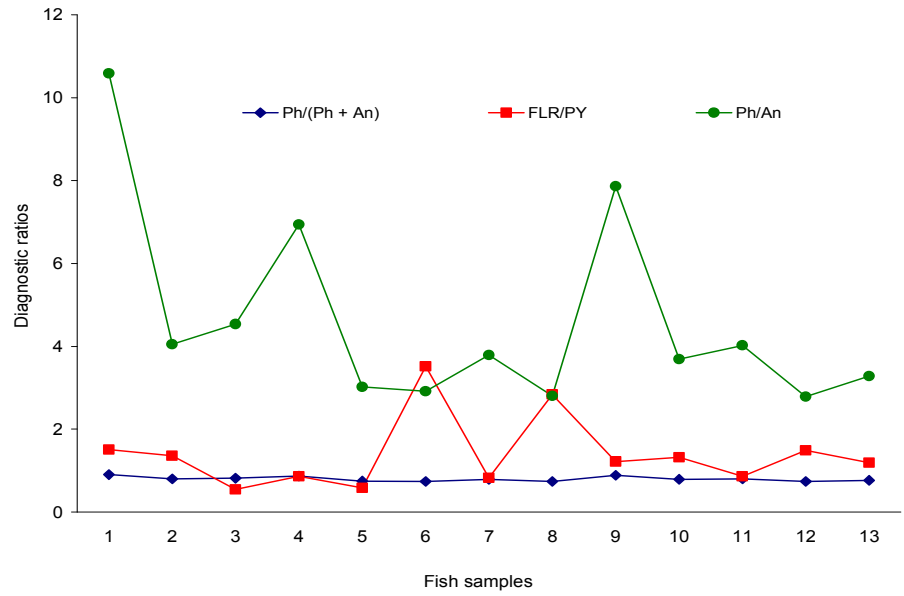


Fig. 8: Phenanthrene/anthracene; flouranthene/pyrene and phenanthrene/(phenanthrane + anthracene) ratios of fish samples from the Niger Delta region

+ benzo[ghi]perylene}) (IP/{IP + BP}) (Prahl, and Carpenter, 1983; Sicre et al., 1987; Behlahcen *et al.*, 1997). But in this study only the first three were focused. Pyrogenic sources are suspected when the phenanthrene/anthracene has a value less than 10. For values above 10, it points to pyrogenic sources (Behlahcen *et al*., 1997).

With just one exception, all the samples had the phenanthrene/anthracene ratio of less than 10, pointing to pyrogenic sources despite heavy petroleum activities in the area. Our explanation for this is that combination of various sources has different contributions to the overall ratio. The more the input of petrogenic sources, the close to 10 the ratio becomes. Sample 30 on Fig. 6 shows a very high petrogenic input while sample 9 shows much less input from petrogenic sources.

The study revealed that fish samples in the Niger Delta region have higher phenanthrene/anthracene ratios indicating higher contribution of PAHs contamination from petrogenic sources. Sediment samples have the least contribution from

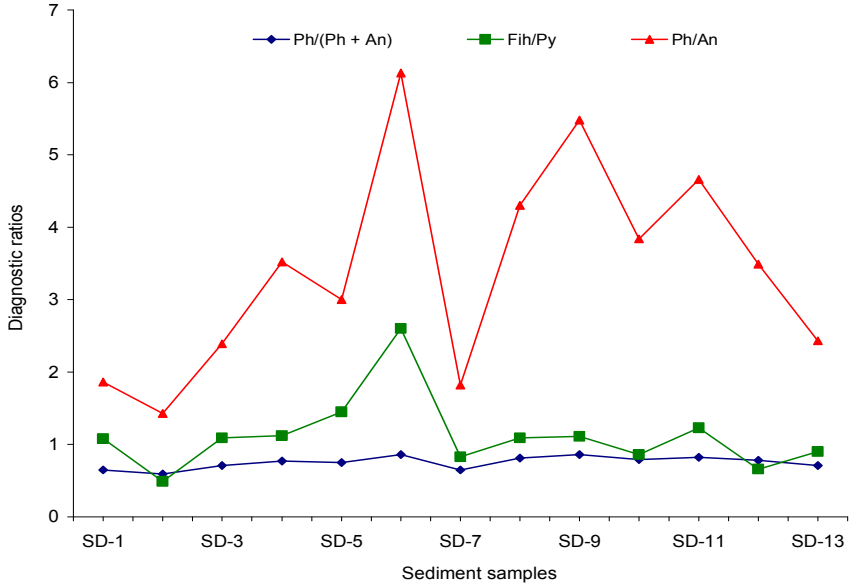


Fig. 9: Phenanthrene/anthracene; flouranthene/pyrene and phenanthrene/(phenanthrane + anthracene ratios of sediment samples from the Niger Delta region

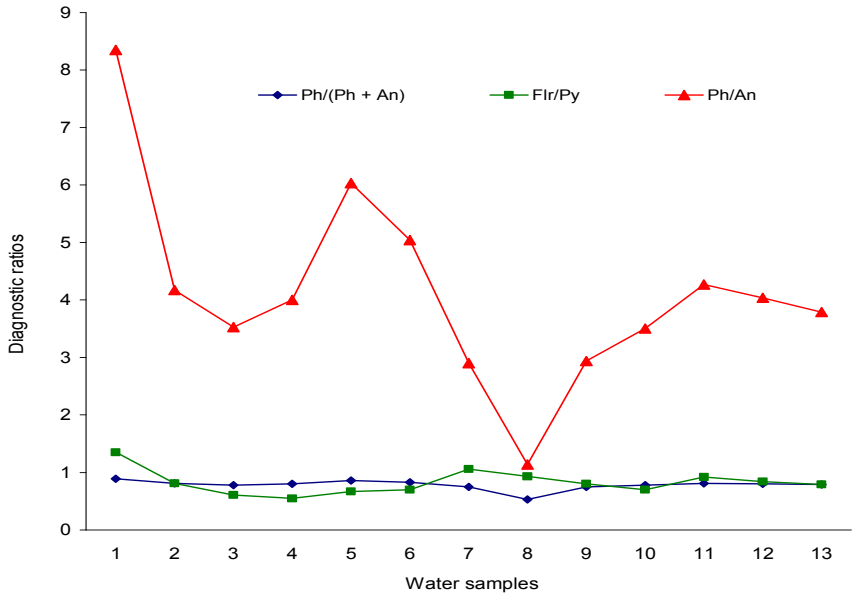


Fig. 10: Phenanthrene/anthracene; flouranthene/pyrene and phenanthrene/(phenanthrane + anthracene ratios of water samples from the Niger Delta region

petrogenic sources. This can be explained by the fact the fish have definite life time spanning just months while sediment samples are more static in nature and the sediment is like a sink for PAHs, which means any contamination from any source is accumulated, while in fish it depends on the contamination of the water samples which are much less static.

It is also known that the fluoranthene/pyrene less than one indicates petrogenic source of contamination while a ratio of more than one indicates pyrogenic sources (Behlahcen *et al*., 1997) . But the findings in this study showed most of the ratios to be above one. In the same way this may be due to multiple sources of contamination.

This study attempted to apply some already established diagnostic ratios for PAHs for the determination of source specific information for PAHs. Even though the Niger Delta is known to

be highly polluted by PAHs and given the level of petroleum production in the area, the expectation was to have petrogenic sources as the sole reason for the PAHs. But this study revealed that other sources of PAH contamination could contribute to different degrees, hence opening up a new argument on how to determine sources of PAH contamination when there are multiple sources and how to apportion contribution of each source. This work is still on going in its effort to assign a specific percentage contribution to a given source. Also this work revealed that more static samples (like sediment samples) are more appropriate in giving a long term history of contamination in the area, since they act like sinks for PAHs and are able to capture contamination of a longer time, unlike fish and water that have much shorter lifespan.

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