Full Length Research Paper

Source predictions of polycyclic aromatic hydrocarbon (PAHs) concentration in water, sediment, and biota (FISHES) from Ethiope River, Delta State, Southern Nigeria

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Received 30 April, 2020; Accepted 14 July, 2020

This study was carried out to predict the source of the sixteen priority polycyclic aromatic hydrocarbon (PAH) compounds in water, sediment, and biota samples from River Ethiope, Delta State, Southern Nigeria. The samples were extracted using soxhlet extraction and analysed with GC/FID. Results obtained for total concentration of the sixteen priority PAHs ranges from 0.185 to 3.679 mg/kg (sediment), 0.000 to 27.353 µg/l (water), and 0.053 to 6.060 µg/kg (biota samples). The result indicated that the concentration level in the water, sediment, and biota are considerably low. Although the PAH were below the USEPA standard, the observed levels can cause adverse effects for lower dwelling aquatic organisms, which are exposed to the sediments daily. Therefore, persistent monitoring and strict adherence to responsible waste discharge should be upheld by all manufacturing and agro-industries in the catchment of the river to avoid deleterious effects on biodiversity and to ensure the safety of the consumers. From the source prediction, the results obtained show the sources are quite similar across the sample sites. This is an indication that the PAHs in the water samples are mostly of pyrogenic origin, except the water samples from Abraka site 2, which are petrogenic. All river sediment samples show pyrogenic origins.

Keywords: Source prediction, polycyclic aromatic hydrocarbons (PAHs), combustion, River Ethiope.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous organic compounds consisting of two or more fused aromatic rings. They are mostly hydrophobic and are capable of bio-accumulating in animal and human tissues (Zheng et al., 2007). PAH is one of the several pollutants released into the environment during crude oil exploration and production. However, studies have revealed that there are also natural sources of PAHs...
Other sources of PAHs are from pyrolytic activities involving industrial and commercial burning of fuel or hydrocarbons in oil, certain cooking practices such as broiling of food over charcoal, frying and smoking (Teaf, 2008; Bayowa and Agbozu, 2016). PAHs from pyrolytic sources are produced due to incomplete combustion and have been shown to be a major source of PAHs intake by humans (European Food Safety Authority, 2008).

PAHs, which are classified as persistent organic pollutants commonly occurring in the environment are considered bone of the most challenging organic contaminants to remediate (Edwards, 1983; Cerniglia, 1992; Weissenfels et al., 1992). This may be due to their toxic, mutagenic and carcinogenic properties; they pose a significant environmental risk to public health (Chen and Liao, 2006; Mekuley et al., 2018). The partitioning behaviour of PAHs between water, sediments, particulate, and dissolved organic material has been documented and severely predicted (Cornelissen et al., 2006). The toxicity of polycyclic aromatic hydrocarbons (PAHs) and their widespread distribution has led to more interest in the presence of these compounds in the aquatic and terrestrial environment (Aderemi et al., 2003).

In recent times, the water quality in water bodies in areas of industrial activities is to a great extent adversely affected by build-up of traffic-generated organic compounds on road surfaces, leading to their presence in water runoff and sediments. The accumulation of PAH metabolites is more toxic than the parent compound as stated by Christensen et al. (1997) and Nwineewii and Ibok (2014). Although largely insoluble in water, some of them are soluble and dissolve in water and sorb into groundwater from ash, tar or creosote improperly disposed of landfills. Waste products containing significant amounts of PAHs are indiscriminately dumped into water, on land or buried at subsurface sites. Airborne particulates resulting from PAHs activities are transported in the atmosphere and are usually deposited in soils and sediments of the aquatic system (Christensen et al., 1997; Christensen and Bzdusek, 2005; Nwineewii and Ibok, 2014). In general, PAHs dissolved in pure water are accumulated in sediments, and these sediments which surround the biota may play an important role in the uptake of PAHs by some species. The fraction of freely dissolved PAHs is usually assumed to be readily available for uptake by organisms.

When PAHs enter into an aquatic environment, they may remain in water or accumulate in organisms and migrate as water flows. Meanwhile, sediment acts as a local scale collector for environmental contaminants (Froehner et al., 2018; Cardoso et al., 2019). PAHs adsorbed on the sediment would be retained in sediment for a long time or released into water columns causing secondary pollution. Therefore, it is quite necessary to routinely monitor concentration levels of PAHs in an aquatic environment and evaluate their potential risks (Olaikan et al., 2014). It is therefore necessary to assess the source of PAHs in the biota as their bioaccumulation in aquatic biota could serve as a good indication of pollution problems in the lagoon (Mitra et al., 1999).

The main aim of this study was to predict the source and assess the level of PAHs in water, sediment and biota (fishes) from River Ethiope in the Niger Delta region of Southern Nigeria.

**MATERIALS AND METHODS**

**Study area**

The study area was River Ethiope in the Niger Delta area of Delta State, Nigeria, which is the second largest Delta in the world and the largest mangrove swamps in Africa. It spans over 20,000 km². The Niger Delta region is located at latitudes 5°31’N and 5°33’N and longitude 5°30’E and 5°32’E. The Niger Delta covers an area of 70,000 km² of marshland, creeks and tributaries that drains the River Niger into the gulf of Guinea in the Atlantic Ocean. The coastal region cuts across nine (9) states in Southern Nigeria. This region has an estimated population of over 30 million people, with fishing and farming as the primary source of livelihood and sustenance. Economic activities include oil and gas exploration and exploitation, fishing industries, agriculture and tourism.

**Sampling**

Samples were collected across Ethiope River along its bank at six different locations as shown in Figure 1. A total of six samples each of water and sediment were collected, while four fish samples of two different species were collected at each location making a total of twenty-four from the different sampling locations.

**Water samples**

The grab sampling technique was employed for the collection of all the water samples at about 1 m below the surface using a 1.0 L amber bottle. The samples were collected and then stored in an ice chest and later moved to the laboratory, and then kept at <4°C until further analysis.

**Sediment samples**

Sediment samples were collected from the same locations as water samples. Wet sediment samples were collected in the river bed with a Petite Ponar Grab sampler. Samples were put in clean glass bottles and kept in an ice chest during transportation, and then kept at <4°C in the laboratory while awaiting sample preparation and analysis.

**Fish samples**

Fish samples of African Sharptooth catfish (Clarias gariepinus) and Redbelly Tilapia (Tilapia zillii) were purchased at each sampling location from fishermen. The samples were immediately kept in pre-cleaned polythene bags, which were sealed and stored in an ice box until further analysis. The samples were identified in the Department of Environmental Management and Toxicology, FUPRE, Delta State, Nigeria.
Extraction of samples

The process of sample extraction in this study is similar to the extraction process carried out by Mekuleyi et al. (2018). As stated subsequently.

**Extraction and fractionation of PAHs in water samples**

A total of 250 ml each of the water sample was transferred into a separating funnel. The pH was adjusted to <pH 2. The solution was then extracted twice with 15 ml methylene chloride. The extract was dried with 5 g anhydrous sodium sulphate and concentrated to 1 ml in a rotary evaporator. The concentrate was fractionated over silica gel column, first eluted with 10 ml hexane and collected as an aliphatic fraction, and then with 15 ml methylene chloride, and collected as aromatic fraction.

**Extraction and fractionation of sediment samples**

Sediment samples were air-dried for 3 days and sieved with 0.5 mm mesh sieves (Ogunfowokan et al., 2003; Olalekan et al., 2014). A total of 10 g of the sediment sample was blended with 10 g of anhydrous sodium sulphate. The mixture was placed in an extraction thimble and refluxed for 4 h with 50 ml methylene chloride. Thereafter the solution was cooled, dried with 5 g anhydrous sodium sulphate, and concentrated to 1 ml in a rotary evaporator. The concentrate was fractionated over silica gel column, first eluted with 10 ml hexane and collected as an aliphatic fraction, and then with 15 ml methylene chloride, and collected as aromatic fraction.

**Extraction and fractionation of fish samples**

The fish sample was homogenized using a blender. A 2 g portion of each sample of the homogenate was saponified with 200 ml methanol/KOH (12% KOH in 95% methanol) solution in an ultrasonic bath at 60°C, for 30 min. The sample was cooled and filtered through glass wool into a separatory funnel. The filtrate was extracted twice with 100 ml hexane. The extract was washed with methanol/water (4:1) mixture, and then concentrated to 1 ml with a rotary evaporator. The concentrate was fractionated through a silica gel column, first eluted with 10 ml hexane to collect the aliphatic hydrocarbon fraction, and then with 15 ml methylene chloride to collect the aromatic hydrocarbon fraction. Both fractions were concentrated to 1 ml, capped in GC vials and stored.

**Analysis of samples**

Analysis was done using Gas Chromatography coupled with FID (Thermo Scientific-Trace GC Ultra). A 2 μl of the concentrated sample was injected by means of Hamilton micro syringe through
rubber septum into the column. Separation occurs as the vapour constituent partition between the gas and stationary phases. The sample is automatically detected as it emerges from the column by a Flame Ionisation Detector FID. PAH quantification was carried out by CLARITY-GC interfaced software.

**RESULTS AND DISCUSSION**

The results obtained from the analysis of the various PAHs concentration in water and sediment from River Ethiope are as shown in Figure 2.

On the individual basis of the compound of PAHs analysed, it was observed that a good number of compounds were not detected (ND), which indicates either absence or that they were below the detection levels for each sample according to the instrument of detection used (Figure 2).

From the results obtained, six out of the sixteen PAHs compounds were found to be undetected (ND) across all the sampling points. These compounds are Naphthalene, Acenaphthylene, Acenaphthene, benzo (g, h, i) perylene, Indeno (1, 2, 3-cd) pyrene, and Dibenz (a, h) anthracene. It is observed that 50% of the undetected PAHs are two ringed and three ringed PAHs compounds, which are Naphthalene (two ringed PAHs), Acenaphthylene, Acenaphthene, Anthracene, Phenanthrene and Flourene (three ringed PAHs compound). They were below the detection limit which is contrary to the study carried out by Mekuleyi et al. (2018), although flourene and anthracene were present in sample location EE with a concentration above detection limit in four sampling locations (KK, HH, EE, and G) with values of 0.089, 0.088, 0.111 and 0.087 mg/kg. At the control point, values for three-ringed PAH could be said to be evenly distributed among the sample locations due to their values. Thus, it could be said that higher ringed PAHs were detected more than lower ringed PAHs in the sampled locations. Due to the presence of other ringed PAHs, it could be deduced that Naphthalene, Acenaphthylene, and Acenaphthene may be present in very low concentration below detection limit or may have been evaporated since they are volatile or degraded by microbial actions of microbes in the sediment, thus cannot not be detected when analysed due to its lower molecular weight when compared with other compound present (Bayowa and Agbozu, 2016). In addition, it could be said to be as a result of volatilisation or biodegradation as these are the major removal process for lower molecular weight PAHs in aquatic environments (Neff et al., 2005; Bayowa and Agbozu, 2016).

The four-ringed PAHs analysed in this study were fluoranthene, chrysene, benzo (a) anthracene and pyrene. Their value ranged from <0.000 to ≤ 1.358 mg/kg. Four-ringed PAHs have been classified as semi-volatile compounds and could be said to possess characteristics in between the lower molecular weight and higher molecular weight PAHs (Neff et al., 2005; Bayowa and Agbozu, 2016). Their character however is mostly determined by those of the substrates to which they are attached and the medium (Neff et al., 2005; Bayowa and Agbozu, 2016). Fluoranthene and pyrene are the major PAHs compounds present in all sample locations, with values ranging from 0.104 to 0.179 mg/kg and 0.035 to 0.083 mg/kg, this may be due to their
unique characteristics. This finding is similar to the study carried out at Limpopo province by Olalekan et al. (2014). Benz (a) anthracene was also present in all sample locations ranging from 0.164 to 0.284 mg/kg, with an exception of sample location DD and II where it was found to be undetected (ND). Chrysene on the other hand was only present at two sample locations (HH and EE) with a value of 1.358 and 0.488 mg/kg, respectively but was below the detection limit in other locations.

Five to six-ring PAHs and above are classified as the higher molecular weight fractions when compared with other PAHs compounds analysed for this study. Those analysed in this study includes: benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a h)anthracene, indeno(1,2,3)cd pyrene and benzo (g, h, i) perylene. Their values ranged from 0.234 to 1.118 mg/kg. Three of these compounds were below the detection limit for all sample locations. Benzo (b) fluoranthene which was detected in three sample locations with values of 0.443 mg/kg (EE), 0.540 mg/kg (GG) and 0.795 mg/kg (KK) which was the maximum concentration in the sediments analysed. A similar result was obtained in EE, GG, and KK for benzo (a) pyrene, with a value of 1.118 mg/kg which is the highest concentration of the five-six ringed PAHs compound, 0.390 and 0.619 mg/kg, respectively.

As shown in Figure 3, the highest percent of PAHs in the sediment was found to be sampling location EE with 36%. HH and KK had a percent of 22 and 21%, respectively, while GG with a 17% was a fourth addition to the total concentration of PAHs compounds present in the sediment of River Ethiope analysed. DD and II which were sparsely distributed to a percent value of 4 and 2%, respectively was of minute contribution to the total concentration observed.

These findings suggest the differences in the natural and anthropogenic activities at the various sampling locations. It could be said that locations with higher concentrations of PAHs may be more contaminated due to industrial and domestic activities in such area (Banan et al., 2018; Olayinka et al., 2018), although they are within threshold limits and may not pose health threat but proper monitoring measures can be put in place in sample locations such as KK, GG, EE and HH, which were observed to be the major contributors of high concentration of poly aromatic hydrocarbons (PAHs). The concentration may increase over time in the sediments which may then pose detrimental effects on biota and humans when they interact with the polluted aquatic environment.

At sample location AW1, total concentration was 0.667 µg/L with only the four member-ringed PAHs compounds found within and above the detection limits (Figure 4). The result obtained at this location were fluoranthene (0.185 µg/L), pyrene (0.065 µg/L), and chrysene (0.417 µg/L), which happens to be the maximum concentration at the location. While other PAHs compounds analysed were below the detection limits. The total concentration of PAHs compounds at sample location UW4 was 6.761 µg/L, having values ranged from 0.071 to 3.453 µg/L. It was observed to have the highest number of PAHs compound analysed from a sample location, with a total number of 11 compounds which cut across two-six ringed PAHs compounds.

From the results obtained, it was observed that the 16 PAHs compounds analysed at sample locations UM3 and AMW were below the detection limits (ND), this may be as a result of the less contaminating human activities around these regions, which includes peasant farm practices, thus making these locations less contaminated.
At sample location EW, concentrations of PAH compounds range from 0.096 to 9.375 µg/L and gave a total concentration of 12.487 µg/L. The highest concentration of 9.375 µg/L was chrysene, which happens to be the second maximum concentration of PAHs compounds in all sample sites after benzo(b)fluoranthene (13.438 µg/L), found at sample AW2 location.

Figure 5 shows the total concentrations of PAHs distribution from the various sample locations, with the percentage distribution of the PAHs (ΣPAHs = 100%). The highest percent of PAHs in the water analysed from River Ethiope is found to be 58% in location AW2. Locations EW and UW4 had a percent of 27 and 14%, respectively, while AW1 with a percent was the fourth addition to the total concentration of PAHs compounds present in the water sample of River Ethiope. The percentage distribution at sample location AW1 was sparsely distributed in minute quantity to the total concentration observed.

While AMW and UM3 were of no contribution to the total PAHs concentration present in all the sample locations. Thus, the general distribution of PAHs compound in descending order of the water samples across all sample locations can be expressed as AW2 > EW > UW4 > AW1 > AMW=UM3.

PAH in biota samples

The total concentration of the PAHs in the various sample locations of the two different species of fishes (C. gariepinus and T. zilli) varies from one location to another. As shown in Figure 6, there is similarity between the two species.

The occurrence of pollutants in the fish samples depends largely on environmental concentrations of PAH compounds and on the physiology and ecological characteristics of the species (Meador et al., 2006). This could explain the reason for the slight variation of concentrations in the two different species (C. gariepinus and T. zilli). The total PAH Concentrations reported in this study shows that Abraka 1 had the highest value of 6.06 µg/kg in T. zilli while the lowest value of total PAH concentration was at Umutu 1 with a value of 0.053 µg/kg. Total concentration of PAH at Umutu 2, Abraka 2, Amukpe and Sapele in T. zilli were 1.492, 3.882, 3.724 and 5.636 µg/kg, while total PAH concentrations in C. gariepinus for these locations were 1.995, 2.016, 2.801 and 4.755 µg/kg. The PAH concentration of C. gariepinus at Umutu 1 and Abraka 1 had values of 0.756 and 4.218 µg/kg.

Source identification

PAHs can be used as anthropogenic geochemical tracers and are used to identify the origins of pollutants (Olalekan et al., 2014). The sources of PAHs are widely considered to be very important for studying the transportation and
fate of pollutants in the environment. The isomer ratios are effective indicators for identifying PAH sources because the isomers from the same source undergo the same mitigation process, since the distributions of the homologues are strongly associated with the formation mechanisms of carbonaceous aerosols with similar characteristics to organic species. The ratios of the specific individual PAHs can provide information about anthropogenic sources of PAHs (Zhang et al., 2018). Considering the aforementioned established facts, the implication of the results of this work is hereby presented and interpreted accordingly.

**PAHs diagnostic ratios**

PAH sources have been predicted using diagnostic ratios conventionally and reported in several studies (Yunker et al., 2002; Agbozu et al., 2017). PAHs of molecular mass 178 and 202 are commonly used to distinguish between combustion and petroleum sources (Agbozu et al., 2017). These ratios include but not limited to Ant/Ant + Phe; Fluo/Fluo+Pyr, BaA/BaA+Chry and Ind/Ind+BghiP. The Fluo/Fluo+Pyr ratio presumes that ratios in the range ≥ 0.4 and ≤ 0.5 indicate petroleum combustion, ratios < 0.4 indicate petroleum sources while ratios > 0.5 indicate
Table 1. PAHs diagnostic ratio for sediment and water samples.

| Sampling location | PAHs diagnostic ratio for sediment and water samples |
|-------------------|-----------------------------------------------------|
|                   | BaA/BaA+Chry sediment | Flu/Fluo+Pyr sediment | BaA/BaA+Chry water | Flu/Fluo+Pyr water |
| Umutu 2           | 1                      | 0.57                  | 0                   | 0.74               |
| Umutu 3           | 0.1                    | 0.75                  | 0.57                | 0.64               |
| Abraka 1          | 1                      | 0.82                  | 0                   | 0                  |
| Abraka 2          | 0                      | 0.78                  | 1                   | 0.36               |
| Amukpe            | 0.37                   | 0.77                  | 0                   | 0                  |
| Sapele 1          | 1                      | 0.56                  | 0.03                | 0.58               |

In this study fluoranthene/(fluoranthene + pyrene) ratios (Yunker et al., 2002; Olalekan et al., 2014) and BaA/BaA+Chry were calculated for all the samples (Table 1). From Table 1, diagnosis of the PAHs ratios showed that Flu/Fluo+Pyr for sediment samples within Ethiope River ranging from 0.56 to 0.82. This shows PAHs from here to be of combustion origin from grasses, coal and wood. While the Flu/Fluo+Pyr for the water samples ranged from 0 to 0.74 within the Ethiope River. This shows PAHs from here to be of petroleum and combustion origin from grasses, coal and wood. The BaA/BaA+Chry ratio for the water samples ranged from 0 to 0.1 within the Ethiope River; this indicates that the PAHs are of mixed sources and combustion sources. While the BaA/BaA+Chry ratio for the sediment samples ranged from 0 to 1.0 within the Ethiope River. This also shows PAHs from Ethiope River to be of mixed sources which are petroleum sources, petroleum combustion sources and grass wood and coal combustion sources. From this analysis, it could be said that PAHs in sediment and water samples within Ethiope River were majorly of combustion sources from mixed origin which could be petroleum or grass wood and coal origin; also at the control point the PAHs were of combustion origin (Teaf, 2008).

From Figure 7, the Flu/(Fluo+Pyr) ratios indicate that the sources of PAHs are of petroleum and combustion origin from grasses, coal and wood (Yunker et al., 2002; Olalekan et al., 2014). For the water samples which ranged from 0 to 0.74 within the Ethiope River which show PAHs to be of petroleum and combustion origin from grasses, coal and wood. Sampling station Abraka 1 and Amukpe indicated petroleum sources with a source ratio of 0 while the other sampling station shows combustion origin from grasses, coal and wood. The source ratio for the sampling stations is as follows: Umutu 2 (0.74), Umutu 3(0.64), Abraka 2 (0.36), Sapele 1 (0.58). While for the sediment samples which ranged from 0.56 to 0.82 within the Ethiope River show PAHs to be mainly of combustion origin from grasses, coal and wood. The source ratio for the sampling stations is as follows: Umutu 2 (0.57), Umutu 1 (0.75), Abraka 1 (0.82), Abraka 2 (0.78), Amukpe (0.77), Sapele 1 (0.58). The maximum source ratio for both water and sediment samples are Umutu 2 (0.74) and Abraka 1 (0.82), respectively.

The ratios of BaA/BaA+Chry are also as shown in Figure 8. For the water samples which ranged from 0 to 1 within the Ethiope River which show PAHs to be of petroleum and combustion origin from grasses, coal and wood. All sampling stations except Umutu 3 and Abraka 2 indicated combustion origin from grasses, coal and wood while the other sampling station indicated petroleum sources with a source ratio within the range of 0 and 0.03. The source ratio for the sampling stations is as follows: Umutu 2 (0), Umutu 3 (0.57), Abraka 2 (1.0), Sapele 1 (0.032), Abraka 1 (0), Amukpe (0). While for the sediment samples which ranged from 0 to 1 within the Ethiope River also indicate the sources of PAHs shown to be of petroleum and combustion origin from grasses, coal and wood. Two sampling stations indicated petroleum sources, which are Umutu 3 and Abraka 2 with source ratio 0.1 and 0, respectively. The other four sampling stations indicated from combustion origin (Zhenhua et al., 2017). The source ratio for the sampling stations is as follows: Umutu 2 (1.0), Abraka 1 (1.0), Amukpe (0.37), Sapele 1 (1.0).

Conclusion

The sixteen priority individual compounds of polycyclic aromatic hydrocarbon concentrations in the water, sediment and biota from Ethiope River are considerably low and below risky levels. Although, the polycyclic aromatic hydrocarbons were below the threat level which indicated no danger status from the consumption or dermal contact for humans but the concentration level can cause adverse effects for lower aquatic organisms.
which are exposed to the sediments on a daily basis. Therefore, persistent monitoring and strict adherence to responsible waste discharge should be upheld by all manufacturing and agro industries in the catchment of the river in order to avoid deleterious effects of the biodiversity in these water bodies as well as ensuring safety of the consumers. From the source prediction, the results obtained show the sources are quite similar with some sample locations indicating the PAHs in the water and sediment samples are of petroleum and combustion origin from grasses, coal and wood. This is in line with car washing and other commercial activities within the vicinity and upstream of the site.

CONFLICT OF INTERESTS
The authors have no conflict of interests.

ACKNOWLEDGEMENT
The authors wish to acknowledge the support of Nigerian Institute for Oceanography and Marine Research, 3 Wilmot Point Road, Victoria Island, Lagos where the GC analysis was carried out.

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