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Sources of Polycyclic Aromatic Hydrocarbons (PAHs) in Water, Sediments, and Body of *Pyxicephalus adspersus edulis* from the Igbekebo River, Southwest Nigeria

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Abstract: Polycyclic Aromatic Hydrocarbons (PAHs) are known contaminants from home and industrial wastes, urban runoff, auto mechanic shops, and agricultural operations. This study measures the concentrations of several PAHs in the water, sediments, and three anatomical sections of male and female *Pyxicephalus adspersus edulis* (frogs) collected from the Igbekebo River. The GC-MS analysis revealed that an average of eighteen (18) distinct PAHs categorised into three groups were found in the water, sediments, and frog samples. Each of the PAHs adds to the complexity of the PAH profile. Dibenzo[a,h]anthracene and Benzo[a]pyrene were identified in the male and female frogs' heads, forelimbs, and hind limbs. The conventional toxicity values for 4 of the PAHs and 8 of the PAHs in female frogs are 3.52±0.25 µg/Kg and 78.31±1.08 µg/Kg, respectively. Both numbers are much higher than the World Standard's recommended threshold values. The small and non-significant differences in PAH values amongst frog body parts indicate that no body part is more important as an indication of PAH accumulation than another. Furthermore, the male and female frogs had comparable PAH values. The cross plots of the source identification ratios of Ant/(Ant+Phe), BaA/(BaA+Chr), and Flu/(Flu+Pyr) are all > 0.5 for the PAHs in the river water, while the values of the three ratios for PAHs in sediments are less than 0.5. These findings indicate that PAHs in river water are primarily from biomass burning, but those in sediments are from petroleum combustion.

Keywords: **Frog, carcinogens, environmental Contaminants, PAHs, and biomarkers**.

Introduction.

In the last twenty years, people have become very interested in polycyclic aromatic hydrocarbons (PAHs), which are grouped under persistent organic Pollutants (POPs), because they are found continuously over time in a lot of waterways (Yin et al, 2008; Sany et al., 2012). PAHs are found in natural habitats and designated contaminated hotspots, and they have a huge environmental impact worldwide. The US Environmental Protection Agency (USEPA) has identified and classified seven out of the sixteen PAHs as carcinogenic to humans, with different degrees of risk (USEPA, 2015). The Agency for Toxic Substances and Diseases Registry (ATSDR, 2003) listed PAHs as ninth on their Substance Priority List (SPL) due to their ability to combine toxicity, and the frequency of exposure to people (ATSDR, 2017). PAHs interact differently and to varied degrees with water, sediments, and biota according to their physicochemical characteristics and lipophilicity. There are different kinds of pollution in a river or dam. Microbes, on the other hand, can break down or destroy some. Many PAHs stay in the environment or build up because of this, which is not healthy for people and many marine species (Zhou et al., 2005; Sany et al., 2012). PAHs get into the bodies of people who eat certain foods. Mirja et al. (2018) and Kamankesh et al. (2015) both found PAHs in smoked meat, grilled meat, smoked fish, and other fish items.

PAHs have also been detected as pollutants in teas, instant noodles, dried vegetables, and coffee (Tran-Lam et al., 2018). The bearing of PAHs in water bodies plus aquatic species poses a serious health risk to people who rely on these water sources, as well as a possible threat to aquatic life. Frogs are also edible, and when a significant number of PAHs accumulate in them, it poses a serious health risk to the animal's consumers via the food chain.

PAH-induced immunological suppression is always linked to a higher risk of getting infectious diseases, different types of cancer, and death (Abdel-Shafy and Mansour, 2016). To be specific, immune system synergism is linked to higher production of cytokines by immune cells, which causes inflammation, which may result into hypersensitivity, allergic reactions, autoimmunity, and the proliferation of tumours (Rodell et al., 2018). PAHs induce cancer, immunotoxicity developmental disorder, oxidative stress, genotoxicity, hormone disruption, and distort cytochrome P450 (CYP) enzyme activity, aided by their hydrophobic nature (Bansal et al, 2014). The International Agency for Research on Cancer (IARC) identifies three PAHs; benzo[a]pyrene (BaP), benzo[a]anthracene (BaA), and dibenz[a,h]anthracene as potential carcinogens.

It has been found that amphibians, like frogs, have very permeable membrane walls and allow free movement of gases, nutrients, and xenobiotics within the water body. Frequently, frogs consume algae, macrophytes, dead plants, and small animals as food, alongside ingesting poisons (Patterson, 2019).

A recent study found PAHs in sediments and frogs from the Oluwa River, Igbekebo, in the same geographical zone as the current study, but was unable to determine the variation in PAHs between male and female frog species, even though some of the 16 PAHs were found in them at high concentrations (Ediagbonya et al., 2023). The current study focused on the sources and contributions of various body regions in frogs that are most sensitive to PAH accumulation and their relationships to differences in the molecular weight distribution of such PAHs. This quest has yet to be completely understood or studied.

Methodology

Study Location

The Igbekebo River is found in southwestern Nigeria, known for its socioeconomic impact on the area's logging and fishing activity. The area is the primary supplier of seafood for Ondo State and, to a large part, Nigeria. Chemical and biological inputs have polluted the river, potentially resulting in high concentrations of heavy metals and PAHs. The location, time frame and coordinates of the three sites A, B, C are (i) Location **A** (A1, A2, A3); Time frame (12.51-13.12 H); Coordinate (06^{o} 21.23’N, 04^{o} 51.62’E) (ii)

Location **B** (B_1, B_2, B_3); Time frame (13.18-13.29 H); Coordinate (06° 21.23'N, 04° 48.86'E) and (iii) Location **C** (C_1, C_2, C_3); Time frame 13.36-13.55 H; Coordinate (06° 21.23'N, 04° 51.63'E).

Reagents and Solutions

A purchased analytical grade acetone and hexane (99.9% pure) from Merck Millipore (USA). Acenaphthene (AcP), Benzo[j]fluoranthene (BjFL), Benzo[a]anthracene (BaA), Anthracene (Ant), Benzo[a]pyrene (BaP), Benzo[e]pyrene (BeP), (BkPyrene (InP), Acenaphthylene (AcPy), Benzo[g,h,i]perylene (BghiP), Benzo[b]fluoranthene (BbFL), Benzo[k]fluoranthene Naphthalene (NaP), Pyrene (Pyr), Phenanthrene (Phe), Benzo[e]pyrened12 (BeP-d12) were acquired from Sigma-Aldrich (USA). A set of PAH internal standard mixed solution (1000 mg/mL in toluene) including AcP-d10, Ant-d10, Acpyd8, Bap-d12, BghiP-d12, BkFLd12, Chr-d12, DBA-d14, Flu-d10, FL-d10, BbFL-d12, InP-d12, NaP-d8, Phe-d10, Pyr-d10 and BaA-d12, was acquired from Chiron (Trondheim, Norway). Disposable nylon filters (0.22 mm) were supplied by Merck Millipore (USA)

GC-MS/MS instrumentation and condition

The analysis was conducted using the Shimadzu Gas Chromatography-Mass Spectrometer (GC-MS) QP 2010. Chromatography was performed using a non-polar BPX-5 capillary column with dimensions of 30 m in length, 0.25 mm in internal diameter, and a film thickness of 0.25 μm. The chromatography setup included a splitless injector and a mass spectrometer detector. Helium was used as a carrier gas at a flow rate of 1 mL/min. The samples were introduced into the system using the splitless mode, with the injector temperature set at 280 °C. The oven was first preheated to 50°C for 2 minutes and then maintained at a temperature of 300°C for 10 minutes

Sampling of Water, Sediment and Frog

Nine (9) 1-litre polypropylene Plastic bottles were carefully washed with distilled water to collect samples from three (3) sampling locations A, B, and C, but each location was sampled three (3) times at intervals of 5m point spacing (GPS Logged) at depths ranging from 0 to 15 cm. Nine distinct sediments were sampled surrounding the points where the water was fetched using a soil auger. Six (6) frogs weighing approximately 1.4 kg each were acquired from a fisherman on the Igbekebo River bank on a day in June 2023.

A fisherman identified some males and some females. The only difference between males and females is the skin colour of the throat. Males have loose skin on the throat different from the rest of the belly, while Females possess smooth skin that equates color of their bellies. Frogs were safely transferred to the laboratory for additional processing. The sediments were collected in pre-cleaned glass bottles and rinsed three times with distilled water. To prevent photolytic degradation, the water bottles covered in aluminium foil were kept in a cold refrigerator, the same as the frog specimens then moved to the laboratory in a cooler packed with ice. A scientist from the Biological Sciences Department of Olusegun Agagu University of Science and Technology in Okitipupa identified the species as Anura *Spp*. In the laboratory, it was kept in a refrigerator below 4°C until used (within 24 hours).

Water Samples

Water sampling was conducted on a sunny day in the first week of June 2023. The first step was to collect a water sample from the target surface water source to detect PAHs in surface water with GC-MS (Agilent 7820A). The sample was then ready to extract PAHs from water using the appropriate solvent. After introducing the extracted sample into a gas chromatogram, the individual compounds were separated based on their physicochemical attributes. The peaks of the separated chemical species are then transmitted to a mass spectrometer (5975C) where they are ionized and the mass-to-charge ratio is calculated. Specific PAHs in surface water samples were identified and quantified by comparing the mass spectra of the isolated compounds with established standards.

Sediment Samples Preparation

Ten grammes (10g) of wet sediment was measured in a cellulose thimble and spiked with 50 μL of anthracene d10 internal standards using a micro syringe. A 250 ml round-bottom flask of Dichloromethane (DCM) held 0.1 g of anti-bumping material was fitted into the soxhlet extractor flask. Soxhlet extracted samples after 8 hours on a heated mantle (Masood et al., 2016). Dichloromethane boils at 42°C at 50 rpm, therefore a 40°C rotary evaporator used lowered the solvent Boiling point. Hydrocarbon extract fractionation was achieved using a 1.1 cm × 25 cm glass column chromatography with silica gel (0.040-0.063 mm). The extract was eluted from silica gel using the 1:1 solvent ratios.

The Frog Samples and Preparation

Each frog sample was dissected into three parts: the head, hands, and limbs. The items were dehydrated separately in an electric oven adjusted to 70-80°C for 12 hours. The materials were then homogenised using a grinder, as described by Anyakora et al. (2005). 10 grams of each homogenised sample were placed into a 50-milliliter solvent consisting of a 50:50 combination of acetone and methylene chloride in a beaker that had been washed with solvent. 1.0 mL of surrogate mix was added to the mix. The specimen was inserted into a DFT-56 Sonicator and subjected to sonication at a frequency of 20 kHz for about 10 to 15 minutes at a temperature of 70 degrees Celsius. A quantity of 10 grams of anhydrous Na_2SO_4 was introduced into the frog samples until a clean extract was obtained. The solvent recovered was transferred into a round bottom flask. A further 50 mL of the solvent mixture was used for sonication, followed by another set of clear solvents in the same round bottom flask. The extract was condensed, substituted with hexane, and then re-condensed to about 3 mL. The materials were then divided into aliphatic and aromatic fractions by passing them through columns loaded with 10 g of 100-200 mesh silica gel. The silica gel had been preheated (baked) at 105°C overnight. A mixture was created by combining silica gel with hexane, resulting in a slurry. The samples were then analysed for PAHs using a Shimadzu Gas Chromatography-Mass Spectrometer (GC-MS) QP 2010, with each sample analysed twice.

Results

PAHs Categorization

All the PAHs were found in all the samples, with a high prevalence in frogs from the research area (Table 1). Naphthalene had the greatest concentration in the group, nearly double that of the others, while the remaining three were less than 13 µg/kg. There are no much changes in the mean values of PAHs tested in different sections of the female frog (Table 1). There is no much differences in the mean values recorded between males and females (Fig. 1).

Low molecular weight PAHs

Some PAHs of Low molecular weights are those between Naphthalene and Pyrene (Table 1). They are known to exhibit relatively higher water solubility than other PAHs. They show elevated concentrations in the frogs compared to those observed in river water and sediments. The concentrations of Anthracene, Pyrene, Phenanthrene, and Fluoranthene in sediments, water, and male and female frogs ranged from 0.02-0.40 µg/kg with the highest of all the four in the river water (Fig. 1).

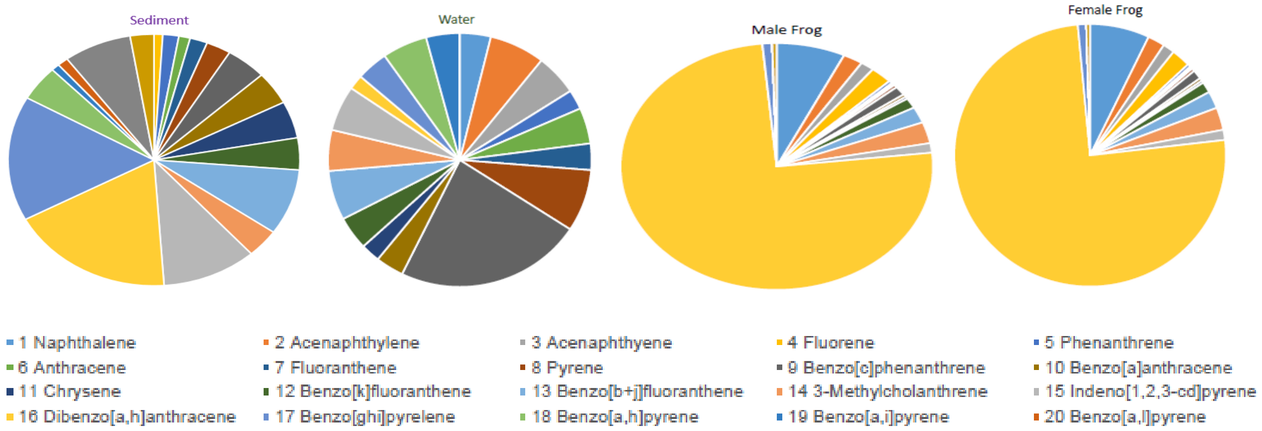
Medium molecular weight PAHs

PAHs from Benzo{c]phenanthrene to Indeno[1,2,3-cd]pyrene are the medium molecular weight PAHs. Some members in the group exhibit comparable concentration patterns to the low molecular weight PAHs concentrated in frog samples. The PAHs' concentrations found in the frogs range between 15 to 48 orders of magnitude higher than those reported in sediment and water samples. Benzo[c]phenanthrene was of the highest concentrations in water (Figure 1) but also found in all body parts of frogs at variable mean concentrations, ranging from 0.32 µg/kg in the forelimbs to 1.12 µg/kg in the heads. Benz(a)anthracene was discovered throughout the body, with mean values ranging from 0.25 mg/kg in the forelimbs to 0.62 mg/kg in the head. Benzo[k]fluoranthene was found in all body regions, with average values ranging from 12.80 µg/kg in the head to 15.20 µg/kg in the forelimbs. Benzo[b+j] fluoranthene was found in all body parts, with mean values ranging from 0.43 µg/kg in the front limbs to 1.99 µg/kg in the heads.

Table 1: The mean ± SD or SE9 (Decide?) Concentrations of PAHs in the three segmented body parts of the Female Frog from Igbekebo River.

S/N	**PAHs**	**HEAD** Mean (µg/kg)	**FORELIMBS** Mean (µg/kg)	**HINDLIMBS** Mean (µg/kg)
1	Naphthalene	6.72±0.24	6.63±0.24	6.62±1.39
2	Acenaphthylene	1.92±0.06	1.92±0.03	1.74±0.09
3	Acenaphthene	1.34±0.05	1.28±0.23	1.29± 0.03
4	Fluorene	2.16±0.07	2.15±0.04	1.93±0.05
5	Phenanthrene	3.60±0.08	3.30±0.70	5.06±0.23
6	Anthracene	0.16±0.05	0.15±0.06	0.16±0.01
7	Fluoranthene	0.15±0.04	0.14±0.09	0.17±0.04
8	Pyrene	0.35±0.03	0.25±0.01	1.08±0.02
9	Benzo{c]phenanthrene	1.12±0.02	0.57±0.05	0.32±0.02
10	Benz(a)anthracene	0.62±0.01	1.25±0.01	0.25±0.04
11	Chrysene	0.27±0.05	1.25±0.35	1.25±0.05
12	Benzo[k]fluoranthene	1.20±0.04	1.52±0.04	1.44±0.09
13	Benzo[b+j)fluoranthene	0.02±0.01	0.46±0.07	0.46±0.08
14	3-Methylcholanthrene	0.08±0.01	0.07±0.01	0.07±0.01
15	Indeno[1,2,3-cd]pyrene	1.18±0.08	0.93±0.05	0.28±0.05
16	Dibenzo{a,h]anthracene	70.3±0.09	72.2±1.90	72.9±0.08
17	Benzo[ghi]perylene	0.55±0.01	0.93±0.02	0.93±0.02
18	Benzo{a,h]pyrene	N.D	N.D	N.D
19	Benzo[a,i]pyrene	N.D	N.D	N.D
20	Benzo{a,1]pyrene	N.D	N.D	N.D
21	Benzo[e]pyrene	N.D	N.D	N.D
22	Benzo[a]pyrene	0.46±0.02	0.28±0.05	0.28±0.02
	Total PAHs (Σ PAHs)	**92.2±0.96**	**94.71±3.97**	**96.07±2.3**

sediment and water samples. Benzo[c]phenanthrene was of the highest concentrations in water (Fig. 1), but also found in all body parts of frogs at variable mean concentrations, ranging from 0.32 µg/kg in the forelimbs to 1.12 µg/kg in the heads. Benz(a)anthracene was discovered throughout the body, with mean values ranging from 0.25 mg/kg in the forelimbs to 0.62 mg/kg in the head. Benzo[k]fluoranthene was found in all body regions, with average values ranging from 12.80 µg/kg in the head to 15.20 µg/kg in the forelimbs. Benzo[b+j] fluoranthene was found in all body parts, with mean values ranging from 0.43 µg/kg in the front limbs to 1.99 µg/kg in the heads.



the high molecular weight PAHs have very low Log Kow values, reason for them to be bio-accumulated in frogs and hence non-detected.

There are two PAHs whose concentrations are unique in the frogs. dibenzo [a,h]anthracene has the highest concentration and is approximately ten (10) times that of all other PAHs in the Frogs (Fig. 1). Also, benzo[a]pyrene was detected in all frog body sections, with varied mean amounts. Its concentrations ranged between 2.08 µg/kg in the hind limbs and 4.60 µg/kg in the heads (Table 1).

Importantly, Benzo[a]pyrene's very high molecular weight did not prevent it from accumulating in a quantifiable amount in the frogs, even when its concentration in the sediment and water was low (near to non-detectability).

The near-zero solubility of benzo[a]pyrene (an environmental contaminant and known carcinogen) in water could explain the fairly high concentrations in frogs (Fig. 1). Benzo[a]pyrene has been discussed and used in numerous cancer research models (Revel et al. 2003). Its carcinogenesis is based on its oxidation by CYP1A1 to benzo[a]pyrenediolepoxide, which forms depurating adducts with DNA and dissociates to generate abasic lesions. Benzo[a]pyrene is an aryl hydrocarbon receptor (AhR) ligand, and some of its biological effects, such as CYP1A1 upregulation, appear to be mediated by AhR activation (Xiong et al. 2021).

Discussion

Amphibians have a good probability of bio-accumulating the chemical pollutants because they spend more time in the water, and the frogs' skin may efficiently absorb and store these PAHs. The female frog in this study had a total PAHs of (92.2 - 96.07) µg/Kg which is nearly double the quantity reported in a previous study (Ediagbonya et al., 2023), the reason for this large deviation is not yet understood. The accumulation of PAHs does not differ in their mean values throughout the frog's body regions (Table 1). The abundance of Dibenzo (a,h) anthracene accounts for a higher percentage of the total PAHs. PAHs in the research locations (Igbekoda Rivers) can be traced back to various possible sources. Local industrial operations, particularly those involving the oil and gas sector. These locations are closer to Nigeria's Niger Delta, where there has been substantial oil and gas drilling (Ukpebor, 2021).

The partition coefficient (log Kow) or the n-octanol/water partition is a constant for the molecule in its neutral state. The ratio is an important measure in chemical risk assessment and it is frequently used to predict a compound's environmental fate, bioavailability, exposure and toxicity (Okere & Semple, 2012). All four PAHs in the high molecular weight category above have almost the same log Kow (approximately 4.5), which corresponds to their molecular weights.

When PAHs adsorb more easily to organic debris in sediments, it must be due to their low affinity for water (<1.9). However, the PAHs with fairly high log Kow values (≥4.5) are of more concern since they can bio-concentrate in living creatures such as frogs, with the idea that their absorption is influenced by their hydrophobicity.

PAHs can infiltrate aquatic ecosystems via oil spills, leaks, and discharge from drilling and production sites. PAHs get into the brackish environment through different sources, such as during oil refining (legal/illegal), atmospheric deposition and fallouts, effluents from industrial and treatment plants, and sewage settle in estuary sediments and bio-accumulate over time in these organisms that feed on sediments by filtering large quantity of water (Diacono and Montemurro, 2010).

PAH environmental issues are often associated with PAHs that have molecular weights between 128.16 g/mol (such as naphthalene, which has a 2-ring structure) and 300.36 g/mol (such as coronene, which has a 7-ring structure). Unsubstituted PAH compounds with fewer rings (2 or 3) cause notable acute toxicity and other negative effects in certain organisms, but they do not have the ability to cause cancer. On the other hand, PAH compounds with more rings (4 to 7) are considerably less toxic, but many of these compounds are known to be carcinogenic, mutagenic, or teratogenic to various organisms such as fish, amphibians, birds, and mammals. (USEPA, 2000). Despite their great lipid solubility, PAHs have limited potential to bio-magnify in food chains, most likely due to fast metabolization. Individual PAH reactions vary greatly between and within species and are considerably influenced by several inorganic and organic chemicals, PAHs as well. The interaction effects are yet to be distinguished.

The United States Environmental Protection Agency (USEPA) identified 16 of the PAHs whose amounts were determined in the current investigation as priority pollutants in the late nineteenth century (USEPA, 2015). Among these key PAHs, B[a]P is a known human carcinogen and has been extensively studied (Di Bella et al., 2020).

Several PAH diagnostic ratios have been widely used to identify the most likely sources of PAHs in ecosystems (Kapsimalis et al., 2014; Jiao et al., 2015). PAHs with molecular masses ranging from 178 to 202 are widely employed to distinguish between combustion and petroleum sources of PAHs (Tehrani et al., 2013). Traven (2013) discovered four types of isomer ratios for PAHs: geogenic, petrogenic, pyrolytic, and biogenic. The LMW/HMW ratio and the fraction of physicochemically identical isomers may provide qualitative information about the contamination sources. An LMW/HMW ratio of more than 1.0 indicates petroleum sources, while a ratio less than 1.0 indicates combustion sources (Soclo et al., 2000).

HMW PAH ratios, such as BaA/(BaA + Chr) and Flu/(Flu + Pyr), are commonly employed due to their stability than LMW ratios. To evaluate the likely PAHs source, the ratios of Ant/(Ant + Phe) and BaA/(BaA + Chr) against Flu/(Flu + Pyr) are displayed in **Fig. 2a** and **Fig 2b**. An Ant/(Ant+Phe) ratio of less than 0.1 implies petroleum pollution, while a ratio of more than 0.1 indicates pyrogenic sources (Lee et al., 2021). A BaA/(BaA+ Chr) ratio <0.2 suggests that oil emissions are the main cause of PAH contamination. A BaA/(BaA+ Chr) ratio of 0.35 implies combustion-dominant sources. A ratio of these two statistics reflects a combination of oil and combustion pollution (Yunker et al., 2002).

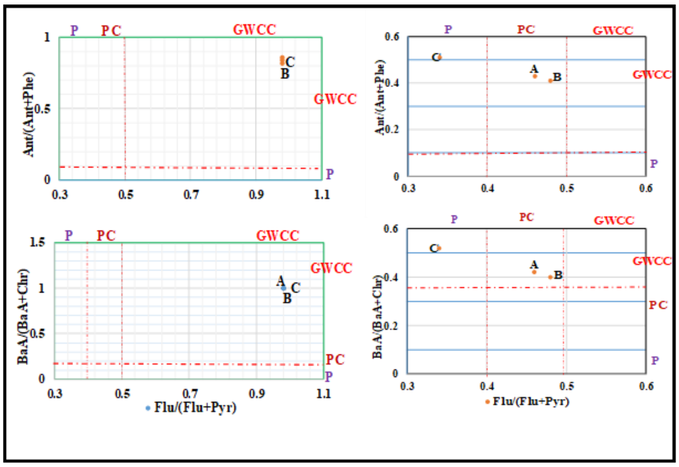


Figure 2: Cross plots of PAHs source identification (**P-Petroleum, PC-Petroleum combustion, GWCC-Grass, Wood and Coal combustion)**

However, a Flu/(Flu+Pyr) ratio of <0.4 indicates petroleum origin or oil spills. A Flu/(Flu+Pyr) ratio of 0.4 or less indicates the presence of petroleum combustion sources (Li, 2012). A Flu/(Flu+Pyr) > 0.5 suggests biomass combustion sources (Ontiveros-Cuadras et al., 2019). **Fig. 2a** reveals that the sources of PAHs in the river water examined from Sites A, B, and C all came from biomass combustion rather than petroleum or its combustion sources—the values of the ratios on the Cross plot were near '1' for both parameters. Similarly, **Fig. 2b** on the sediments demonstrates that the PAHs from Site 'A' are of petroleum origin, whilst those from Sites 'B' and 'C' are of petroleum combustion origin.

Relative solubilities and other factors may explain the abundance of some PAHs in sediments while others remain undetectable in both water and sediments (Okere and Semple, 2012).

4.2 Use of PAHs indicator for PAH Signaling in Frogs

Based on a study of some data from Member States, the European Food Safety Authority's (EFSA) CONTAM Panel found in 2008 that benzo[a]pyrene was not an appropriate indication for the presence of PAHs in food.

TABLE 2: Risk of (a) PAH4 (ΣPAHs) and (b) PAH8 (ΣPAHs) in the female frog from Igbekebo River, Ondo State.

S/N	**PAHs**	(a) **PAHs in Frogs (µg/Kg)**	(b) **PAHs in Frogs (µg/Kg)**
1	Benz[a]anthracene	1.95±0.02	1.95±0.02
2	Chrysene	0.92±0.15	0.92±0.15
3	Benzo[b]fluoranthene	0.31± 0.05	0.31± 0.05
4	Benzo[k]fluoranthene	Nil	1.39± 0.06
5	Benzo[ghi] perylene	Nil	0.80± 0.02
6	Dibenz[a,h]anthracene	Nil	71.8± 0.69
7	Indeno[1,2,3-cd]pyrene	Nil	0.80± 0.06
8	Benzo[a] Pyrene	0.34±0.03	0.34±0.03
Total	**Σ 4PAH**	**3.52±0.25**	
PAHs	**Σ 8PAHs**		**78.31±1.08**

The Panel found that PAH4 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) and PAH8 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi] perylene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) were the most suitable indicators for PAHs in food, with PAH8 not providing much-added value compared to PAH4 (EFSA, 2008). Regulation 2065/2003 on smoke flavourings used or intended for use in or on foods establishes a maximum allowable amount of 10 ppb for benzo[a]pyrene and 20 ppb for benz[a]anthracene in smoke flavoring in the EU Muscle meat from smoked fish and smoked fisheries products, excluding those indicated in entry points 6.1.6 and 6.1.7. (ECR, 2006). The upper limit for smoked crustaceans pertains to the muscular tissue in their appendages and the belly. As of September 1st, 2014, the concentrations of benzo(a)pyrene (2 µg/Kg) and PAH4 (45) (12 µg/Kg) were recorded (FSAI, 2015).

The benzo[a]pyrene and benz[a]anthracene concentrations (Table 2) are significantly higher than the EU's permitted criteria of 10 ppb and 20 ppb, respectively (FSA, 2012). Similarly, the value of the total PAHs for the 4PAHs in the frog samples (Table 2) is significantly beyond the 30 µg/Kg (old) or even 12 µg/Kg recently proposed by the EU (ECR, 2006). This means that the four PAHs may have carcinogenic health consequences on people through the intake of frog meat. The results of this investigation, while unique, and the concentrations of the 4PAHs are significantly greater than those obtained by Dokubo and Igwe (2019) in their examinations of certain commonly consumed shellfish in River State, Nigeria.

The high level of maritime activity in the studied area could be a significant source of PAH pollution (Nwaichi and Ntorgbo, 2016; Nnaji and Ekwe, 2018). Apart from the possibility of incomplete combustion of fossil fuels, burning of biomass, municipal trash, unintentional spills, and organic matter decomposition (Guo et al. 2006, 2007) and from local vessel traffic in general.

Conclusion

Collecting data on sediment quality and ecological risk conditions is crucial due to the potential hazards posed by polluted sediment and its chemical and biological interactions with the surrounding water body. The three PAH categories reflect the complicated profiles obtained in the water, sediment, and frogs. The sources of the PAHs in the water and sediment have been evaluated using some notable PAH cross plots. Most PAHs have biomass-burning sources in the water and petrol combustion sources in the sediment. Periodically, we should enhance our comprehension of the spatial distribution and potential hazards associated with pollutants in river ecosystems to maintain and rehabilitate their ecological integrity and safeguard human well-being. The complexity of pollutant fluxes at the sediment-water interface is often disregarded when addressing biological absorption. The current study demonstrates that frogs can significantly absorb contaminants from riverbank sediments and water. Therefore, frogs could quickly absorb PAHs through the food chain, posing major health risks. As a result, there is a need to properly monitor PAH levels in commercial food supply sourced from these bodies of water.

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