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PROFILE AND DISTRIBUTION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN WATER, SEDIMENTS, FISH AND PLANTS OF WARRI RIVER.

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ABSTRACT

This study was to identify the type, concentration and possible risk of exposure to PAHs in the water, sediments, fish and plants in Warri River as PAHs are ubiquitous in the environment and may threaten aquatic life. PAHs were identified and quantified in water, sediments, fish and plant samples from three sites (NPA, Pessu and Mclver) along the Warri River. The samples were analyzed by GC-FID to determine the type and concentrations of PAHs present in the river. The total PAH concentrations ranges from 0.060mg/L to 4.387mg/L for water samples, 0.666mg/kg to 71.498mg/kg for sediment samples, 0.222mg/kg to 0.376mg/kg for fish samples and 0.207mg/kg to 0.827mg/kg for plant samples respectively. The total PAH concentration in the sediment samples was highest when compared to that of water, fish and plant samples: Benzo(a)pyrene which is regarded as a potent animal and probable human carcinogen had a very high concentration of 14.286mg/kg in the sediment samples at NPA (shore). In addition Benzo(a)pyrene concentration in the analyzed fish samples exceeded the recommended EU (European Unit) limit of 0.002mg/kg for Fish considered safe for human consumption. However, these analyses indicates that PAH concentration observed in the analyzed samples could be dangerous to Humans and some aquatic organisms, Particularly during the early stages of development.

INTRODUCTION

PAHs represents a large group of relatively non-polar hydrophobic organic compounds that are composed of two or more fused aromatic rings containing carbon and hydrogen atoms [1 – 2]. PAHs are ubiquitous environmental pollutants that possesses carcinogenic and mutagenic properties [3 - 4]. Due to these properties as well as their persistence in the environment, they have been placed on the list of priority pollutants by the United States Environmental Protection Agency (US EPA) and also the European Environmental Agency [5]. PAH may be synthesized from saturated hydrocarbon under oxygen deficient condition [6], resulting from a stepwise formation of an alkane. The sources of PAHs can be classified as natural or anthropogenic sources. PAH formation from natural sources occur from direct biosynthesis in plants and aquatic micro-organisms and as a result of low to moderate temperature diagenesis of sedimentary organic material to form fossil fuel [7]. The

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anthropogenic sources of PAHs are classified as petrogenic and pyrogenic PAHs. PAHs from pyrogenic sources are generally produced from the incomplete combustion of fossil fuels. whereas, the petrogenic sourced PAHs (from petroleum origin) consist mainly of crude and refined petroleum product [8].

Warri River is one of the most important coastal Rivers of the Niger Delta area of Nigeria. Warri River flows through the southern part of Nigeria, where the drainage and catchments areas are probably very rich in decaying organic matter & humus. The River takes it source from a point, 10km away from Utagba-Uno and between latitudes 5°21' – 6°00'N and Longitude 5°24' – 6°2'E covering a surface area of above 255km^2 with a length of about 150km [9]. Beyond the Warri port, the main channel of the river joins the Forcados Estuary, which empties into the Atlantic Ocean.

The relevant land marks in this River stretch are Enerhen, Aladja (steel town), Warri Ports, Main Warri Market, NNPC Refinery etc.

MATERIALS AND METHODS

Sampling Sites

Three sampling sites located along the Warri River were chosen for this analysis. These sites were Pessu, Mclver and NPA Rivers. The operational area covered latitude 5°30'45.1"N to 5°30'07.46"N and Longitude 5°43'56.1"E to 5°45'14.96"E as shown in the map of the sampling sites in Fig. 1. Relevant human activities in this sites includes commercial sand dredging, fishing, boating for human and goods transportation. In addition, there is also a Market environment close to the River, a boat construction company at the bank of the River that specializes in the building of Tugboats and Barges, a sawmill plant, where the sawdust generated by these sawmills are dumped closed to the river bank and incinerated to reduce the quantity. Solids waste generated from this environment are dumped directly in the River. These activities amongst others generates PAHs.

Sample Collection

The sampling were carried out on the 5th to 6th of November 2014 and the sampling plan was designed to include the collection of Water, Sediments, Plants and Fish samples from near shore and the middle location of the River. Water samples were collected from the surface using a pre-cleaned (washed with warm water and liquid soap using a brush, after which, it was properly rinsed with tap water and dried) glass bottle by hand.

Sediment samples were collected from the selected sampling locations with the aid of a locally made stainless steel grab sampler which was deployed manually from a speed boat to obtain sediment samples with a maximum penetration of about 5cm. The collected sediment

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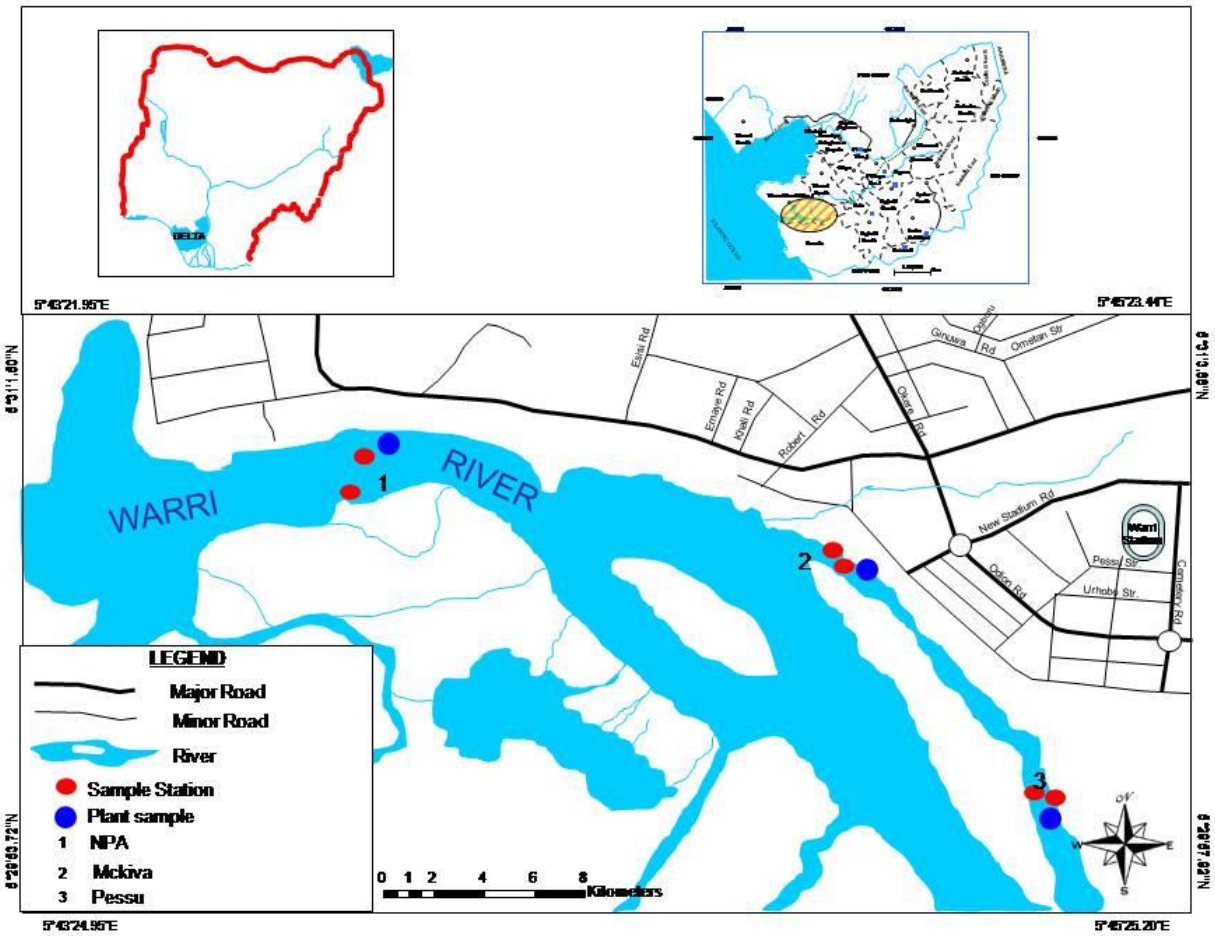
samples were transferred into a cleaned labelled polythene bags and transported to the laboratory, where the individual grab samples of the sediments from each sampling sites were carefully homogenized into composite samples with the removal of twigs and stones.

Figure 1: Map showing the location of sampling site in Warri River



The plant (*Eichhornia Crassipes)* samples were collected by hand and where shaken to remove sand, leaves and particulate matter that will interfere with the analysis. The plant (*Eichhornia crassipes)* samples were also transferred into a clean labelled polythene bag and were transported to the laboratory for analysis.

Fresh Fish samples were purchased from local fishermen from each location. A fairly big size of Nile Tilapia was obtained from Pessu Market and Bony Fishes, whiting of similar sizes were obtained from both Mclver Market and NPA. The Fish samples were wrapped with a clean labelled Aluminum foil and were transported to the laboratory. All samples collected from all locations were stored in the Refrigerator at 4^{o}C

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prior to further analysis. For the plant and fish samples, one sample was collected for each from each Sampling location.

Chemicals

Dichloromethane (Riedel-de Haen) purchased from sigma Aldrich laboratory Chemi Kalien GMBH, Germany. Hexane (Kermel) (China), Acetone (JHD) and Anhydrous sodium sulfate (JHD) were purchased from Guandong Gruanghua Chemical factory China. All solvents used for sample processing and analysis were of HPLC grade. All glasswares were prewashed and rinsed with distilled water and finally with organic solvents (Acetone and dichloromethane) before use.

Sample Preparation, Extraction and Clean Up

In the laboratory, using liquid-liquid extraction, the total amount of each Water samples was filtered with a whatman filter paper to remove debris and suspended materials. 50ml of the water sample was measured with a graduated cylinder and poured into a separatory funnel. 50ml of equal mixture of Acetone and Dichloromethane (1:1v/v) was added to the sample and was shaken vigorously for 1 to 2 minutes with periodic venting before a two phase separation was achieved. The water phase was drained into a 500ml beaker. The organic phase (extracts) was collected in a round bottom flask and was concentrated using a rotary evaporator to about 2ml. the extract was transferred to a Teflon-lined screw cap vial, labeled and refrigerated prior to analysis.

For the sediments samples, 5g wet weight of the homogenized composite samples from each sampling sites was weighed into a 100ml solvent rinsed glass beaker in which 25ml of equal mixture of dichloromethane and Acetone (1:1v/v) was added to the sediment samples prior to extraction.

Samples of fresh Fish and Plants (*Eichhornia crassipes*) were ground into a homogenous mixture by mortar and pestle. 5g wet weight of each of the homogenized fish and plant mixtures from each sampling site were also weighed into a 100ml solvent rinsed glass beaker in which 25ml of an equal mixture of Dichloromethane and Acetone (1:1v/v) was added to the each homogenized Fish and Plant samples.

Extraction of the sample (Sediments, Fish and Plant) mixtures were carried out by an ultrasonic sonication. The weighed samples of Sediments, Fish and Plant were extracted twice in 50ml mixture of Acetone and Dichloromethane. Firstly, 5g of each of the samples containing the mixture were extracted in an ultrasonic sonicator (LDR Ultrasonic T – 14 sonicator), with a frequency of 20KHz in a water bath (10 – 40^{o}C) for 15miunutes and 10g of anhydrous sodium sulfates was added to the sample mixture. The extract was decanted into a round bottom flask. The extraction was repeated with an additional 25ml of the solvent mix, sonicated, allowed to

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settle in the beaker and decanted into the same round bottom flask. The extracts were concentrated using a rotary evaporator (RE – 52A) prior to extract clean up.

Sample clean up was carried out for all the extracts. This was done using a silica gel column. The column was packed with a 10g of 100 – 200mesh silica gel preconditioned (baked) at 105°C overnight in a desiccator. Then 40ml of hexane was added to the silica gel to form a slurry. The concentrated extracts were eluted with a 10ml of dichloromethane to obtain PAH fractions.

The extracts were concentrated to 1 to 3ml in a rotary evaporator, solvent exchange with hexane and stored in a refrigerator prior to analysis.

Sample Analysis

PAH analysis in the extract were analyzed by an HP6890 gas chromatograph fitted with an Agilent PH – 5 (crosslinked PHME Siloxane) 19091J-413 capillary column with a length of 30m, an internal diameter of 0.32mm, a film thickness of 0.25µm coupled with a flame ionization detector (GC/FID). Helium was used as the carrier gas with a splitless injector mode. The detector temperature set point was maintained at 300°C where hydrogen gas was introduced at a rate of 35ml/min with an inflow of air (neutral air) at a rate of 350ml/min and make up gas (Helium) was allowed to flow through at 25ml/min. All injection volumes were 1µL. The quantitative analysis was done by internal calibration method and the PAHs identification was performed by comparing their retention time with those of the corresponding retention times of PAHs standards. No independent method of conformation was applied.

Table 1 provides the concentrations of PAHs in water samples from three different sites (NPA, Pessu, and McIver) at the shore and middle locations of the Warri River. The results reveal variations in PAH concentrations both between the sites and within different locations at each site. All 16 PAHs listed by EPA were present, with Pessu (shore) recording the highest total PAH concentration of 4.387 mg/L, notably from Benzo(a)pyrene (1.142 mg/L) and other high molecular weight PAHs (HPAHs). NPA and McIver also showed higher PAH concentrations at the shore compared to the middle, though less extreme than Pessu, suggesting that shoreline activities and runoff such as agricultural and urban run-off, atmospheric deposition etc., are major sources of contamination [10 – 11]. HPAHs like Benzo(a)pyrene and Benzo(a)anthracene had higher concentrations than low molecular weight PAHs (LPAHs) such as Naphthalene and Anthracene, indicating pyrogenic sources, such as combustion processes, as major contributors to PAH pollution [12]. The total PAH values ranged from 0.060 mg/L at Pessu (middle) to 4.387 mg/L at Pessu (shore), exceeding values reported from Ekpan Creek of Warri River (0.02439–0.2836 mg/L) [13], Rivers Niger and Benue in Lokoja of Kogi State

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(0.000264 – 0.000958 mg/L) [14] and the Citarum irrigation system in Indonesia (0.0018–0.0023 mg/L) [15]. Also permissible limit for total PAHs in drinking water (0.0002 mg/L) was exceeded [16]. Additionally, permissible limit of 0.2 µg/L set by Agency for Toxic Substances and Disease Registry [17] for benzo(a)pyrene also was exceeded, indicating high contamination levels in the Warri River.

PAHs in sediment samples

RESULTS AND DISCUSSION

PAHs in water samples

Table 1. Concentrations of PAHs (mg/L) in Water samples collected from 3 sites in Warri River.

PAHs	NPA		PESSU		MCIVER	
	Shore	**Middle**	**Shore**	**Middle**	**Shore**	**Middle**
Naphthalene	0.090	0.027	0.169	0.000	0.009	0.008
Anthracene	0.000	0.002	0.411	0.000	0.003	0.007
Acenaphthylene	0.001	0.002	0.187	0.000	0.001	0.006
Fluorene	0.004	0.014	0.121	0.000	0.000	0.001
Phenanthrene	0.048	0.000	0.100	0.000	0.009	0.009
Acenaphthene	0.001	0.000	0.009	0.003	0.007	0.003
Total LPAH	**0.144**	**0.045**	**0.997**	**0.003**	**0.029**	**0.034**
Pyrene	0.058	0.000	0.066	0.000	0.066	0.007
Fluoranthene	0.003	0.024	0.069	0.000	0.000	0.001
Chrysene	0.001	0.002	0.294	0.000	0.000	0.001
Benzo(a)anthracene	0.024	0.000	0.434	0.000	0.002	0.002
Benzo(b)fluoranthrene	0.034	0.007	0.297	0.000	0.000	0.014
Benzo(k)fluoranthrene	0.024	0.047	0.391	0.055	0.019	0.005
Benzo(a)pyrene	0.003	0.002	1.142	0.000	0.000	0.004
Dibenzo(a,h)anthracene	0.003	0.024	0.342	0.002	0.000	0.037
Indeno(1,2,3-cd) perylene	0.006	0.000	0.195	0.000	0.002	0.000
Benzo(g,h,i) perylene	0.045	0.002	0.160	0.000	0.116	0.011
Total HPAH	**0.2**	**0.108**	**3.39**	**0.057**	**0.205**	**0.082**
Total PAHs	**0.345**	**0.153**	**4.387**	**0.060**	**0.234**	**0.116**

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Table 2: Concentration of PAHs (mg/kg) in sediment samples collected from 3 sites in Warri River.

PAHs	NPA		PESSU		MCIVER	
	Shore	Middle	Shore	Middle	Shore	Middle
Napthalene	0.894	0.064	0.017	0.044	0.045	0.002
Acenapthylene	0.000	6.777	0.020	0.022	0.005	0.357
Acenapthene	0.000	0.000	0.002	0.007	0.007	0.006
Fluorine	1.583	4.196	0.277	0.009	0.137	0.263
Phenanthrene	1.197	0.024	0.025	0.038	0.035	0.002
Anthracene	0.325	0.235	0.025	0.020	0.048	0.021
Total LPAH	**3.999**	**11.296**	**0.366**	**0.140**	**0.277**	**0.651**
Fluoranthene	3.802	0.042	0.051	0.025	0.028	0.004
Pyrene	6.812	0.109	0.045	0.057	0.083	0.012
Benzo[a]anthracene	1.167	0.003	0.017	0.018	0.037	0.008
Chrysene	4.479	0.257	0.016	0.098	0.032	0.005
Benzo[b]fluoranthene	3.953	0.000	0.000	0.000	0.035	0.016
Benzo[k]fluoranthene	9.943	0.002	0.037	0.032	0.097	0.001
Benzo[a]pyrene	14.286	0.000	0.082	0.105	0.182	0.002
Indeno[1,2,3-cd] pyrene	2.671	0.157	0.000	0.015	0.022	0.001
Dibenzo[a,h] anthrancene	5.264	0.381	0.056	0.029	0.107	0.008
Benzo[g,h,i] perylene	15.122	0.014	0.150	0.147	0.251	0.009
Total HPAH	**67.499**	**0.965**	**0.454**	**0.526**	**0.874**	**0.066**
Total PAH	**71.498**	**12.261**	**0.820**	**0.666**	**1.151**	**0.717**

The concentrations of the 16 PAHs of concern in the surface sediment of three sites (NPA, Pessu, and McIver) at the shore and middle locations of the Warri River, expressed on a dry weight (dw) basis, are given in Table 2. The results reveal that sediment samples from the Warri River exhibit significant PAH contamination, particularly at the shore of the NPA site, where total PAH concentrations reach 71.498 mg/kg. This high contamination is predominantly for high molecular weight PAHs (HPAHs) like Benzo[g,h,i]perylene and Benzo[a]pyrene. Both the Pessu and McIver sites show lower contamination levels, with shore concentrations of 0.820 mg/kg and 1.151 mg/kg, respectively. The results also indicate that PAH concentrations in the sediments of Warri River are higher at the shore than in the middle of the river at all sites. This suggests that PAH concentrations in the shore sediments are influenced by lateral transport, such as urban runoff and water transportation due to daily rainfall, burning of garbage on land, and indiscriminate dumping of domestic waste on the shore. These shore areas receive both burnt materials (pyrogenic) and oil products (petrogenic) which also contributes to increase in PAH level [18]. Moreover, the presence of carcinogenic PAHs, particularly Benzo(a)pyrene with a concentration of 14.286 mg/kg at NPA (shore), poses substantial environmental and health risks. This necessitates regular monitoring, stricter pollution control

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measures, and increased public awareness to mitigate these risks. The total PAH concentrations in the sediments of Warri River (0.666–71.498 mg/kg) are higher than some of the reported literature, such as Brisbane River, Australia (148–3079 ng/g dw) [19], River in Chongqing, China (221–3205 ng/g dw) [20] and Sele River, Southern Italy (331.75–871.96 ng/g) [21]. However, the sedimentary PAHs concentrations were lower than those found in Huai River, China (810–28,228 ng/g dw) [22] and Yinma River Basin, China (895.6 to 2,518.2 ng/g) [23].

PAHs in fish samples

Table 3: Concentration of PAHs (mg/kg) in Fish samples collected from 3 sites in Warri River.

Component	NPA River	Pessu River	McIver River
Naphthalene	0.015	0.025	0.010
Anthracene	0.028	0.014	0.019
Acenaphthylene	0.013	0.015	0.009
Fluorene	0.008	0.027	0.005
Phenanthrene	0.017	0.045	0.011
Acenaphthene	0.008	0.004	0.005
Total LPAH	**0.089**	**0.13**	**0.059**
Pyrene	0.011	0.030	0.007
Fluoranthene	0.006	0.019	0.004
Chrysene	0.015	0.013	0.010
Benzo(a)anthracene	0.020	0.013	0.013
Benzo(b)fluoranthrene	0.019	0.023	0.012
Benzo(k)fluoranthrene	0.032	0.017	0.021
Benzo(a)pyrene	0.056	0.041	0.037
Dibenzo(a,h)anthracene	0.026	0.023	0.017
Indeno(1,2,3-cd) perylene	0.009	0.016	0.006
Benzo(g,h,i) perylene	0.052	0.051	0.034
Total HPAH	**0.248**	**0.246**	**0.161**
Total PAH (mg/kg)	**0.335**	**0.376**	**0.220**

All 16 PAHs were detected in all locations in the analyzed fish samples. The total concentrations of these PAHs in this locations are 0.335 mg/kg at NPA, 0.376 mg/kg at Pessu and 0.220 mg/kg at McIver respectively. Benzo(a)pyrene, a known carcinogenic PAH has the highest concentration of 0.056 mg/kg at NPA, 0.041 mg/kg at Pessu and 0.037 mg/kg at McIver respectively. The occurrence of PAHs in fish is an indication of PAH contamination in river waters. Both low and high molecular weight PAHs were observed. The aquatic organisms get exposed to these

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contaminants which bioconcentrate in their bodies (across their gills and skin) [24], and ingestion of PAH-contaminated particulate matter along with food [25], as PAHs readily adsorb onto particulate organic matter [26 – 27]. PAHs are lipophilic and so they accumulate in the fatty tissues of fish following their uptake [28]. The concentration of benzo(a)pyrene in all the fish samples analyzed in all the sites exceeded the EU limit of 0.002mg/kg. This could be an indication of possible anthropogenic pollution in the environment including oil from ships and fishing boats, combustion of petroleum, burning of garbage and automobile tyre on land, and wood and vehicle emissions [29]. In addition, the ratio of the low molecular weight PAHs to high molecular weight PAHs in all the fish samples analyzed was less than one, further indicating that the sources of these PAHs in the fishes analyzed are mainly pyrogenic [30]. The total PAHs levels obtained in fish from this study were lower than 45.9–171.9 µg/kg reported in seafood from Niger Delta coastal waters [31]. Also, our values were lower than the total PAHs (48.75–166.79 µg/kg) reported in fish from Ghana coastal waters [32].

PAHs in Plant Samples

Table 4: Concentration of PAH (mg/kg) in plant samples collected from 3 sites in Warri River.

PAHs	NPA	PESSU	MCIVER
Napthalene	0.008	0.002	0.006
Acenapthylene	0.000	0.000	0.000
Acenapthene	0.002	0.000	0.001
Fluorine	0.073	0.018	0.058
Phenanthrene	0.008	0.002	0.006
Anthracene	0.000	0.000	0.000
Total LPAH	**0.091**	**0.022**	**0.071**
Fluoranthene	0.037	0.009	0.029
Pyrene	0.005	0.001	0.004
Benzo[a]anthracene	0.022	0.006	0.018
Chrysene	0.101	0.025	0.080
Benzo[b]fluoranthene	0.075	0.019	0.059
Benzo[k]fluoranthene	0.215	0.054	0.170
Benzo[a]pyrene	0.027	0.007	0.021
Indeno[1,2,3-cd] pyrene	0.004	0.001	0.003
Dibenzo[a,h] anthrancene	0.049	0.012	0.039
Benzo[g,h,i] perylene	0.204	0.051	0.161
Total HPAH	**0.736**	**0.185**	**0.584**
Total PAH	**0.827**	**0.207**	**0.655**

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The plant *(Eichhornia crassipes*) samples analyzed from Warri River also contains a mixture of high molecular weight PAHs and low molecular weight PAHs. The total PAH values recorded for three locations are 0.827 mg/kg at NPA, 0.207mg/kg at Pessu and 0.6550 mg/kg at McIver respectively. Benzo(k)fluoranthenes has the highest concentration of 0.215mg/kg at NPA. Acenapthylene and anthracene were also not detected in the plant samples that was analyzed at NPA, Pessu and McIver location of the Warri River. The PAH levels in the *Eichhornia crassipes* samples were lower than those reported in the leaves of *Artemesia santolina schrenk* from the Aojiang River Waterways in Wenzhou, China (1,584.8 ng/g – 3,538.6 ng/g) [33], as well as those found in the shoots and leaves of mint plants (902,347 ng/g) at the Konin garden in Poland [34]. However, the values of PAHs from this study were higher than those reported in the shoots and leaves of lavender (24,501.4 ng/g) and parsley (92,644.5 ng/g) plants from Konin and Poznan gardens in Poland, respectively [35]. The predominance of HMW PAHs over LMW PAHs in the *Eichhornia crassipes* samples suggests a significant contribution from combustion processes, likely originating from pyrogenic sources [36].

CONCLUSION

All 16 PAHs were detected in the water sample in all the sites except in some cases where the concentration levels of PAHs within the shore or middle locations of the River were below detection limits. The total concentration of PAHs in the analyzed water samples were higher than the recommended fresh water guidelines by USEPA and ANZECC.

The sediments samples analyzed in Warri River contains at least 15 out of the 16 toxic PAHs. Acenaphthene and benzo[b]fluoranthene were not detected in the NPA and Pessu location of the River. The total concentration of PAHs in Warri River was higher in the sediment sample when compared to that of the other samples.

The fish samples analyzed contains all 16 PAHs. The total concentration of PAHs in fish samples analyzed in McIver and NPA location of the Warri River were a little higher than the water samples analyzed in the same location. Because fishes metabolized these substance, this observation could be attributed to bioaccumulation by the fish samples.

14 PAHs out of 16 PAHs analyzed for, were present in the analyzed plant samples of Warri River. Acenaphthylene and Anthracene were not detected in all locations of the River. But the concentration of high molecular weight PAHs were predominant over low molecular weight PAHs in all location of the Analysis. The predominance of high molecular weight PAHs in all is an indication that PAH contamination in Warri River are from direct influence from anthropogenic processes which are mainly from pyrogenic sources.

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The result obtained in the present studies shows that the level of PAHs detected in the water, sediment, Fish and Plant samples of Warri River are high and this may be detrimental to humans and aquatic lives. Therefore, future monitoring and coastal management programme must be put in place around this region, to guarantee the safe usage of the River for all purposes, because levels of PAHs can exceed the present concentration level in the nearest future, if not properly monitored.

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