Determination of Polynuclear Aromatic Hydrocarbons in Water and Sediment of River Benue in Makurdi Metropolis, Nigeria

M. I. Arowojolu[1*], S. M. Tongu[1], A. U. Itodo[1], S. T. Yinusa[2], K. A. Basheeru[3] and S. Mejida[3]

[1]*Department of Chemistry, University of Agriculture, Makurdi, Nigeria.*
[2]*Department of Physics and Engineering Physics, Obafemi Awolowo University, Ile – Ife, Nigeria.*
[3]*Central Research Laboratory, University of Lagos, Akoka, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors MIA and SMT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KAB and SM managed the analyses of the study. Authors AUI and STY managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Polynuclear Aromatic Hydrocarbons (PAHs) were investigated in water and sediment samples from River Benue in Makurdi metropolis. Samples of water and sediment were collected from 6 sites in River Benue during the dry season. The samples were extracted using liquid-liquid extraction and soxhlet extraction methods respectively and analyzed for acenaphthene, fluoranthene, 2-methynaphthalene and naphthalene using gas chromatography coupled to mass spectrometry (GC-MS). The results showed that the samples contained very low concentrations of these PAHs (concentrations varied from 0.06 to 8.02 ppb). So, there was little or no threat to fish, wildlife or

_____

*Corresponding author: E-mail: arowmike@gmail.com;

humans, based on low concentrations determined in the samples but accumulation over time may pose a health risk. Their sources were thought to be natural and anthropogenic, and the varied concentrations at the various points were due to different activities at those points.

1. INTRODUCTION

Polynuclear Aromatic Hydrocarbons (PAHs) are among the major groups of persistent organic pollutants for the environment. They are also known as polycyclic aromatic hydrocarbons. They are widespread and detected in various media, including air, water, food, soil, sediment, industrial effluents, tissues of animals or humans [1-2]. They consist two or more fused benzene rings in a linear or cluster arrangement, with complex chemicals including carbon and hydrogen [3]. Their concentration in water is low due to their lipophilic property. They exhibit high affinity for suspended particulates in water and they eventually settled out of the water column unto the bottom sediments [4].

Generally, PAHs exist as colourless, white or pale yellow-green solids with faint pleasant odour [5]. They occur as complex mixtures under natural and synthesized forms. Based on their formation mechanisms, they are divided into two different categories: pyrolitic and petrogenic forms. Petrogenic PAHs represent their natural forms while pyrolitic PAHs come from incomplete combustion or pyrolysis of organic matter [6-7], crude oil and its products [8].

Over the years, due to development and the increase of human activities, different sources of PAH contamination have been linked to polluted soil, air and water [9]. Some aquatic food products such as fish, can be exposed to PAHs present in water and sediments and the PAH content greatly depends on the ability of the aquatic organisms to metabolize them [10].

There are many types of PAHs, among which are pyrene, fluorine, naphthalene, 2-methylnaphthalene, acenaphthene, fluoranthene. Naphthalene is a crystalline, white solid with formula $C_{10}H_8$ and a structure of two fused benzene rings. It is known as the traditional or primary ingredient of mothballs. Naphthalene is volatile, forms a flammable vapour and readily sublimes at room temperature thus producing a characteristic odour that is detectable at ultra-trace concentrations. Exposure to large amounts of naphthalene causes skin and eyes irritation,

cataracts, headache, vomiting, diarrhea, etc. and may damage red blood cells. 2-Methylnaphthalene is also called beta methyl-naphthalene, is a solid substance like naphthalene. It is an alkylated naphthalene with one methyl group attached and it is a skin, eye and respiratory irritant [11-12].

Acenaphthene is made up of naphthalene and ethylene which is connected at positions 1 and 8. It is a constituent of coal tar and has the formula $C_{10}H_{12}$ and it has three fused rings [13]. Physiologic effects of acenaphthene include irritation of the skin and eyes, it causes mucous membranes and if swallowed in large quantities may cause acute vomiting [14-15]. Fluoranthene is a high molecular weight and an US-EPA Priority Pollutant. Fluoranthene is made up of naphthalene and benzene unit in a four-membered ring with a molecular formula $C_{16}H_{10}$. It is found in many combustion products, along with other PAHs. Its presence is an indicator of less efficient burning temperature and has also been isolated from coal tar pitch [16]. Among the higher molecular weight PAHs, fluoranthene is not classified as cancer causing PAH but can cause mutation in human [17].

Like other PAHs, Naphthalene, 2-methynaphthalene, acenaphthene and fluoranthene are readily metabolized in humans and most animals, therefore less bioaccumulative [18], however, the little-accumulated concentration could be health threatening. Their occurrence raises major concerns for human health, even at trace concentration. They are known to be carcinogenic, mutagenic, teratogenic and can cause other health problems in humans, wildlife and aquatic beings [19-22].

Environmental monitoring studies which include pesticide residues and trace metals within the study area have been reported respectively by Ogah et al. [23] and Akaahan et al. [24]. Some other environmental studies have been carried out to determine the level of contamination of the river, but there is no information concerning PAHs in the river in the Makurdi metropolis. Thus, this study was carried out to determine the

levels of PAHs in water and sediment of River Benue.

2. MATERIALS AND METHODS

2.1 Study Area

River Benue, the second largest river in Nigeria, serves as the major source of municipal water supplies for towns and villages along its course. The sources of pollution in the river among others include industrial and municipal wastes. Situated along the river are Benue Brewery Limited (BBL) and Nigeria Bottling Company (NBC), Benue State University zoological garden, Wurukum abattoir and Wadata market, with various activities that can pollute the river in one way or the other. These activities and possible natural sources of pollution in the river body, therefore, call for various analyses of the water and sediment of the said river since the water from the river is used by some inhabitants of the location for domestic and industrial purposes. The fishes and other aquatic organisms from the river that are regularly made available to the public by the fishermen equally make it necessary to characterize and know the pollution level of the river [25-26]. A map illustrating the study area is presented in Fig. 1.

2.2 Samples Collection, Treatment and Conservation

The samples were collected from River Benue starting from Benue brewery bank to Wadata market bank in Makurdi metropolis. The river was divided into 6 sampling points based on the major activities going on along its banks. These points were: Benue brewery (R_1), BSU zoo (R_2), Wurukum abattoir (R_3), St. Joseph Technical College (R_4), NASME Barracks (R_5) and Wadata market abattoir (R_6). Composite water samples were collected downstream in the river between the depth of 1-5 cm below the surface at both banks and middle of the river at each point into thoroughly washed amber bottles. pH and temperature of the water samples were measured immediately as collected, 5 mL nitric acid was then added to each sample to prevent degradation of analytes in the samples and stored below 4°C in a cooler [27]. Sediment samples were collected at benthic (0 – 2 cm) at the same points the water samples were collected in thoroughly washed amber bottles and stored below 4°C in a cooler [28]. The water samples were immediately stored below 4°C in a freezer while the sediment samples were oven dried at 150°C to 200°C for about 30 min, homogenized, sieved through a mesh of 1 mm to remove stones and other debris and stored at -20°C in a freezer in the laboratory [27-28]. Mm

2.3 Determination of Percentage Organic Carbon

The organic matter analysis of sediment samples was carried out using Loss-on-Ignition (LOI) method. A representative sample of the sediment (4 g) was positioned in a clean, dry crucible of known mass with its lid securely in placed. The mass of the crucible and sample was determined
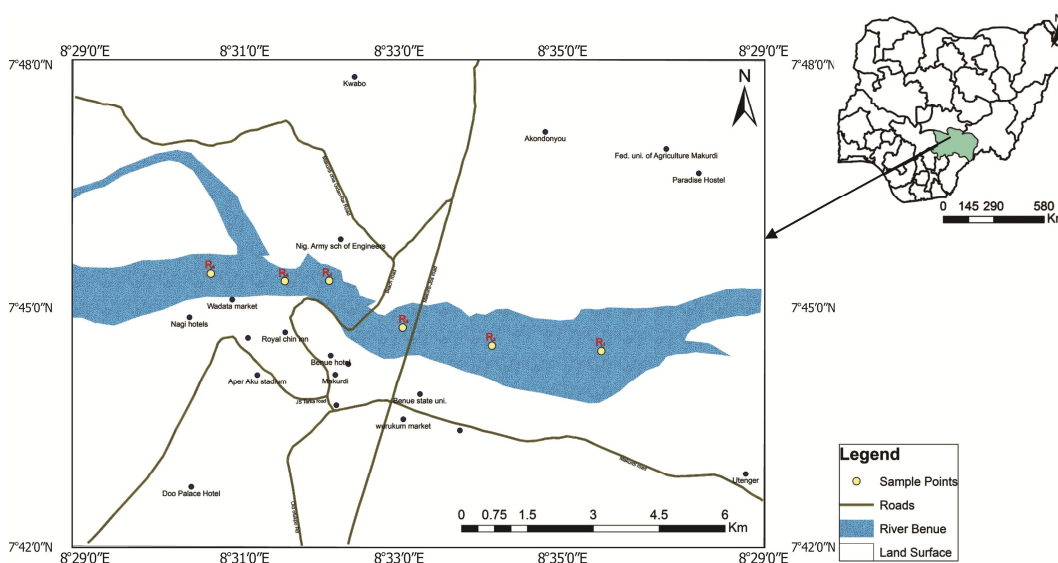

Fig. 1. River Benue in Makurdi metropolis showing the sampling sites

3

using a weighing balance. Thereafter, the lid was removed and the crucible was placed in an oven maintained at 550°C for 6 hrs. The crucible was transferred to evaporating dish containing silica gel as the drying agent to obtain a constant weight. The mass of the crucible and dried sample was measured. The % organic matter of the sediment was calculated using the following expression:

% Organic Matter

$$= \frac{moist\ soil - dry\ soil}{moist\ soil} \times 100 \qquad (1)$$

2.4 Extraction of Samples

Exactly 150 mL water sample was transferred into a 250 mL separating funnel, and 9 mL of dichloromethane was added. The separating funnel was shaken for a period of 2 min, and the organic layer was separated and collected in a 50 mL flat-bottomed flask containing 2 g of anhydrous sodium sulfate. The extraction step was repeated twice, and the extracts were combined and concentrated to a volume of approximately 1 mL using a rotary evaporator then transferred into 5 mL vail, wrapped with foil and stored below 4°C [29].

Exactly 10 g of each sediment sample was extracted for 2 hours with soxhlet apparatus, using 250 mL acetone and methanol mixture (1:1). The extracts were concentrated to about 5 mL using rotary evaporator and purified by silica gel column chromatography. Each extract was loaded onto the column and eluted with 10 mL dichloromethane. The first 1.0 mL of eluate was discarded, about 5 mL were collected into vials, rapped with aluminum foil and kept in the refrigerator until instrumental analysis [30-32].

2.5 GC-MS Conditioning

An Agilent Technologies GC-MS comprises of 7890A gas chromatography and MS 5975C mass spectrometer detector was used in this study. The instrument comprises a column thickness of 0.25 µm and Helium as carrier gas at the rate of 1 mL/min. temperature programme of initial temperature at 60ºC hold for 0.5 min then ramp to 240ºC at the rate of 10ºC/min to the final temperature at 300ºC hold for 6 min.

2.6 Characterization of Samples

PAHs in samples were identified and quantified using gas chromatograph coupled with mass spectrometer [33]. A standard containing 100 ppm each of naphthalene, 2-methynaphthalene, acenaphthene and fluoranthene was used to calibrate the instrument and the chromatogram of this is shown in Fig. 1. The limit of detection of the instrument was 0.06 ppb for naphthalene, 0.45 ppb for 2-methylnaphthalene, 0.12 ppb for acenaphthene and 0.06 ppb for fluoranthene. The chromatogram of standard used to calibration of the instrument is presented in Fig. 2.
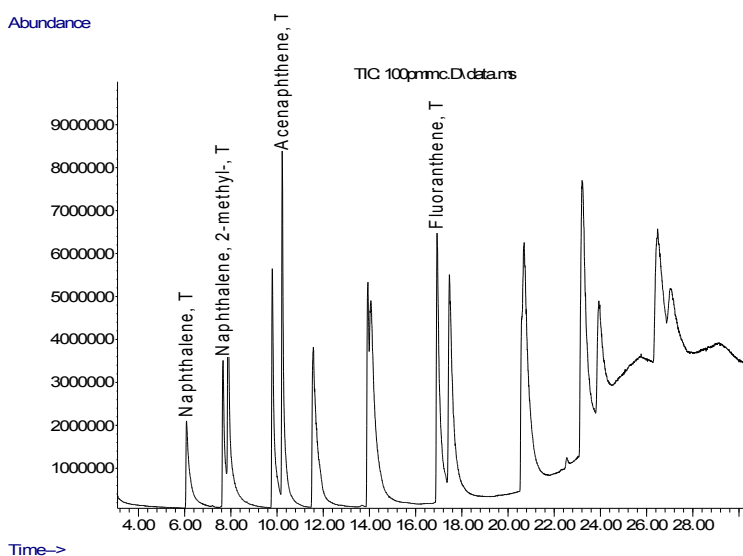

Fig. 2. Chromatogram of standard used to calibration of the instrument

2.7 Statistical Analyses

Mean and standard deviation were used as data treatment. One-way analysis of variance (ANOVA) of the resulting PAHs concentrations and their maximum allowable concentrations in sediment and water was carried out using SPSS at 0.05 level of significance [34]. LOD was calculated as follow using concentration of the blank sample run.

$$LOD = \frac{3s_b}{a} \qquad (2)$$

Where s_b stands for the standard deviation of the blanks and a is the slope of the calibration curve

3. RESULTS AND DISCUSSION

3.1 pH and Temperature

Table 1 presents the pH and the temperature of River Benue water. The study revealed that the water was slightly above neutral. This suggested that PAHs in the river could be optimally biodegradable [35]. The water temperature is ordinarily ambient and good for consumers who choose cool to warm water. This temperature is good for specific reasons since the high temperature of water adversely impact water quality by improving the growth of micro-organisms which may advance taste, odour, colour and corrosion problems [36].

Generally, this range of Soil pH values is well suited for the availability of most essential macro-nutrients. Some nutrients become 'tied up' in the soil at particular pH levels. Phosphorus, nitrogen, potassium and sulphur are expected to be present in the region. Alkaline soils, on the other hand, may lead to deficiencies in iron, manganese, boron, copper and zinc [37].

3.2 Characterization of Samples Based on PAHs

The results of PAHs in the water (Table 2) showed that the total concentration of PAHs (tPAHs) ranged from 0.0.27 ppb in RW_4 to 0.47 ppb in RW_3 while those in the sediment (Table 3) ranged from 0.22 ppb in RS_1 to 17.99 ppb in RS_5.

The concentrations of PAHs studied at various points in River Benue were very low indicating small amounts of the pollutants in the river due to their lipophilic nature. This is in agreement with the results of Teaf [38] and Fagbote and Olanipekun [28]. Emissions from a host of mechanisms including stormwater runoff, direct deposition, and surface runoff from roadways and discharges from boats contribute heavily to aquatic pollution.

The prevalence of 4-6 rings PAHs in an environment showed that they were likely to be formed by pyrolysis (i.e. anthropogenic combustion). Among the PAHs investigated for, fluoranthene (4-membered ring) was dominant in distribution in the sediment having been detected across all the six points along the river. It was, however, below detectable limit in water at all the points except at point RW_4. This implies that the analyte is more strongly held in sediment than in water and would settle at the bottom of the river when it enters the river. Fluoranthene that was detected in all sediment samples also confirmed that the major source of PAHs in the river was anthropogenic. Fluoranthene is one of the high molecular weight PAHs that resist biodegradation and tend to accumulate in sediment [19]. The 2 or 3- membered ring PAHs that were not detected in most of the sediment samples suggested that the PAHs loading of the river might not be connected to the petrogenic source of PAHs but anthropogenic such as various activities involving the use of refined petroleum products. The sediment sample from point 5 was densely populated with PAHs compared to others indicating the higher introduction of PAH-containing substances into the river at that point.

2-methylnaphthalene being the only alkylated analyte in the present study is only detected in RS_1 out of the 12 water and sediment samples (Fig. 3). This corroborates Wilkes [39] that alkylated PAHs are more common than the parent compounds in petrogenic samples and less common than the parent compounds in pyrolytic samples. 2 or 3 rings PAHs are usually from petrogenic (i.e. production, transport and use of petroleum and its refined products) sources while 4-6 rings PAHs are from pyrolytic sources.

LMW/HMW indexes (low molecular weight PAHs (2-3rings)/high molecular weight PAHs (4-6 rings)) were calculated for all samples. The values of LMW/HMW < 1 indicate PAHs pollution of pyrolytic origin and vice-versa for petrogenic origin [40]. The results of this study showed that the LMW/HMW values for all the water samples were zero as most analytes were below detection limit as at the time of study. As shown in Table 3,

three out of the 6 sediment samples have LMW/HMW > 1, suggesting that there were some petrogenic PAHs in the river.

All PAHs concentrations obtained from water and sediment of River Benue were lower than the recommended limit. Concentrations of the studied PAHs (Tables 2 and 3) and their respective maximum allowable concentrations as presented in Table 4 are not statistically significant. This suggested that the river is safe from health problems associated with studied PAHs. The linear regression analysis between mean concentrations and percentage organic carbon was carried out on sediment samples in this study, the results are presented in Fig. 4 and it showed no significant correlation ($r2 = 0.3522$). This indicated that the sites were specifically contaminated to different levels. It also suggested that pollution of sediment with PAHs in the zone was from various anthropogenic sources.
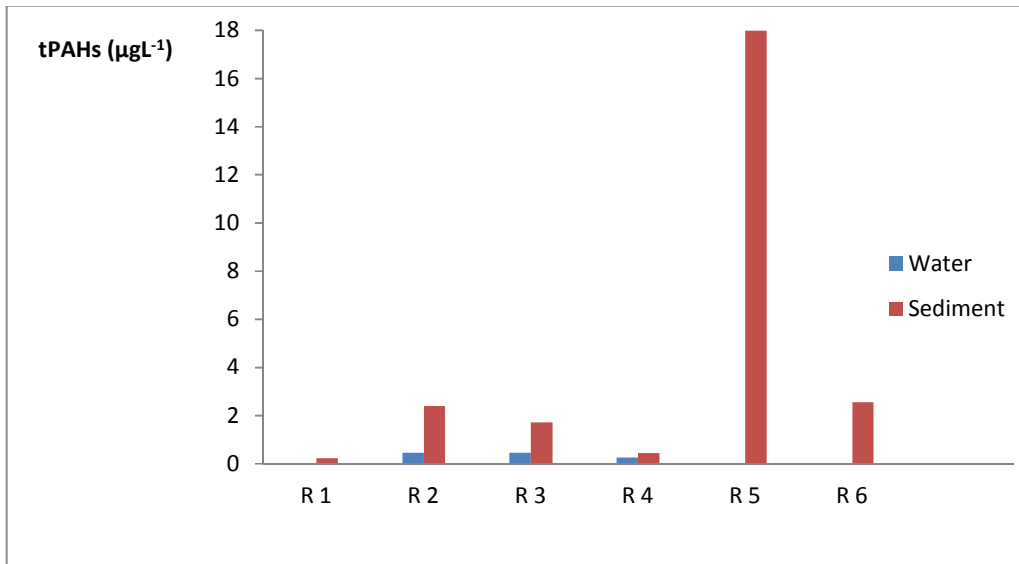

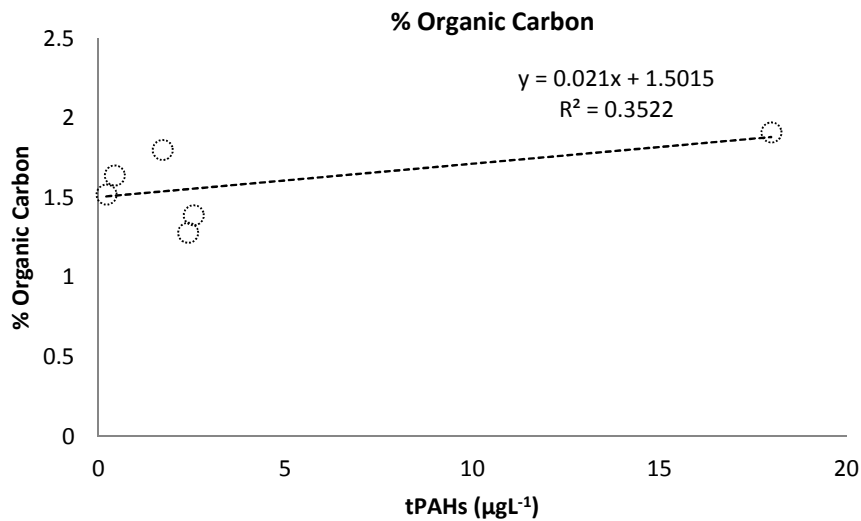**Fig. 3. Evolution of tPAHs in the water and sediment in sampling sites**

Fig. 4. Regression relationship between % organic carbon and pahs concentration in sediment samples

Table 1. pH and temperature properties of River Benue water

Parameter	RW_1	RW_2	RW_3	RW_4	RW_5	RW_6
pH	7.40 ± 0.17	7.63 ± 0.29	7.50 ± 0.20	7.43 ± 0.21	7.55 ± 0.49	7.53 ± 0.29
Temperature ℃	24.33 ± 0.58	24.67 ± 0.58	24.67 ± 0.58	24.67 ± 0.58	24.00 ± 0.00	24.67 ± 0.58

$RW_1 – WR_6$ means river water at points 1 – 6 respectively

Table 2. Concentration of PAHs studied in water samples

Analyte	RW_1 (μgL^{-1})	RW_2 (μgL^{-1})	RW_3 (μgL^{-1})	RW_4 (μgL^{-1})	RW_5 (μgL^{-1})	RW_6 (μgL^{-1})	tPAH (μgL^{-1})
Naphthalene	BDL	0.61	0.47	BDL	BDL	BDL	1.08
2-methylnaphthalene	BDL	BDL	BDL	BDL	BDL	BDL	0.00
Acenaphthene	BDL	0.45	BDL	BDL	BDL	BDL	0.45
Fluoranthene	BDL	BDL	BDL	0.27	BDL	BDL	0.27
tPAHs	0	1.06	0.47	0.27	0	0	1.80
LMW/HMW	0	0	0	0	0	0	

$RW_1 – WR_6$ means river water at points 1 – 6 respectively, LMW is low molecular weight PAHs, HMW is High molecular weight PAHs, BDL is below the detection limit.

Table 3. Concentration of PAHs studied in sediment samples

Analyte	RS_1 (μgkg^{-1})	RS_2 (μgkg^{-1})	RS_3 (μgkg^{-1})	RS_4 (μgkg^{-1})	RS_5 (μgkg^{-1})	RS_6 (μgkg^{-1})	tPAH (μgkg^{-1})
Naphthalene	BDL	BDL	1.05	BDL	8.04	0.48	9.57
2-methylnaphthalene	BDL	BDL	BDL	BDL	7.49	BDL	7.49
Acenaphthene	BDL	BDL	0.42	BDL	1.38	1.13	2.93
Fluoranthene	0.22	2.40	0.25	0.44	1.08	1.00	6.86
tPAHs	0.22	2.40	1.72	0.44	17.99	2.55	26.85
% Organic Carbon	1.52±0.56	1.28±0.05	1.80±0.62	1.64±0.24	1.91±0.56	1.39±0.21	
LMW/HMW	0	0	6.00	0	15.66	1.55	

$RS_1 – WS_6$ means river sediment at points 1 – 6 respectively, LMW is low molecular weight PAHs, HMW is High molecular weight PAHs, BDL is below the detection limit.

Table 4. Maximum allowable concentrations (macs) of pahs in soil and water

Analyte	Water (PPB)	Sediment (PPB)
Naphthalene	3000	1000
2-methylnaphthalene	8	8
Acenaphthene	3000	3000
Fluoranthene	3000	3000

Source: [41]

4. CONCLUSION

This study revealed that PAH concentrations obtained from water and sediment of River Benue were lower than the recommended limit. Therefore the study concluded that River Benue is free from immediate health problems associated with the analytes studied. PAHs were probably from anthropogenic sources, including pyrolysis based on the predominance of fluoranthene (containing 4 – 6 rings) in the investigated samples and the regression analysis (Fig. 4) carried out. Despite the fact that PAHs concentration is low, accumulation overtime may pose a health risk to both animals and humans. Therefore, it is recommended that these toxic substances are investigated in the environment from time to time.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gao YZ, Zhu LZ. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere. 2004;55: 1169–1178.
2. Layshock JA, Wilson G, Anderson KA. Ketone and quinone-substitued polycyclic aromatic hydrocarbons in mussel tissue, sediment, urban dust, and diesel particulate matrices. Environ. Toxicol. Chem. 2010;29(11):2450-2460.
3. Sexton K, Sahmas JJ, MacDonald TJ, Gowen RMZ, Miller RP, McCormick JB, Fisher-Hosh SP. PAHs in maternal unbilical cord blood from pregnant hispanic women living in Brownsville, Texas. International Journal Environmental Research and Public Health. 2011;8(8): 3365–3379.
4. Farshid K, Amir HS, Rokhsareh M. Determination of polycyclic aromatic hydrocarbons (PAHs) in water and sediments of the Kor River, Iran. Middle-East Journal of Scientific Research. 2011;10(1):1-7.
5. Agency for Toxic Sub stances and Disease Registry, "Polynuclear Aromatic Hydrocarbon (PAH) Toxicity," Case Studies in Environmental Medicine., Atlanta, GA; 1993.
6. Masih A, Saini R, Taneja A. Contamination and exposure profiles of priority polycyclic aromatic hydrocarbons (Pahs) in groundwater in a Semi-Arid Region in India. International Journal of Water. 2008; 4(1-2):136-147.
7. Duke O. Source determination of polynuclear aromatic hydrocarbons in water and sediment of a creek in the Niger Delta Region. African Journal of Biotechnology. 2008;7(3):282-285.
8. Mahmoodi M, Safahieh A, Nikpour Y, Ghanemi K. Distribution and sources of polycyclic aromatic hydrocarbons in the sediment of bushehr coastal Zone-Iran. Iranica Journal of Energy & Environment. 2012;3(2):173-179.
9. World Health Organization (WHO). Standard Methods for the Examination of Water and waistewater. American Public Health Association, Washington, DC; 2005.
10. Plaza- Bolanos P, Frenich AG, Vidal JLM. Polycyclic aromatic hydrocarbons in food and beverages. Analytical Methods and Trends, J. of Chromatogr. 2010;1217: 6303 – 6326.
11. Gosselin RE, Smith RP, Hodge HC. Clinical toxicology of commercial products. 5th ed. Baltimore: Williams and Wilkins. 1984;III-309.
12. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. Division of Toxicology/ Toxicology Information Branch, Atlanta, Georgia; 2005.
13. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile of polycyclic aromatic hydrocarbons. Department Of Health And Human Services, Public Health Service. Atlanta, Georgia; 1995.
14. Clayton GD, Clayton FE. (eds.). Patty's industrial hygiene and toxicology. Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons. 1982;3353.

15. Sax NI. Dangerous properties of industrial materials. 5th ed. New York: Van Nostrand Rheinhold. 1979;331.

16. Florida Spectrum Environmental Services Incorporation (FSESI), Chemical fact sheet.
Available:http://www.speclab.com/compound/c206440.htm
(Accessed 16th October, 2016)

17. Barfknecht TR, Hites RA, Cavaliers EL, Thilly WG. Human cell mutagenicity of polycyclic aromatic hydrocarbon components of diesel emissions. Dev Toxicol Environ Sci. 1982;10:277-294.

18. Ramesh A, Walker SA, Hood DB, Guillen MD, Schneider K, Weyand EH. Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. International Journal of Toxicology. 2004;23(5):301–333.

19. Ryan B. Source apportionment of polycyclic aromatic hydrocarbons in sediment cores from the humber estuary using molecular distribution. M. Sc. Thesis presented to school of School of Environmental Sciences, University of East Anglia, Norwich. 2013;73.

20. Prycek J, Ciganek M, Srinek Z. Clean up of extracts for nitrated derivatives of polycyclic aromatic hydrocarbons analysis prior their gas chromatography determination. Journal Brazilian Chemical Society. 2007;18:1125 – 1131.

21. Gaga E. Deposition of polycyclic aromatic hydrocarbon in Ankara. PhD Thesis, Department of Chemistry, The Middle East Technical University. 2004;254.

22. U.S. Environmental Protection Agency (US-EPA). Ecological soil screening levels for polycyclic aromatic hydrocarbons (PAHs). Interim Final. OSWER Directive 9285.7-78; 2007.
Available:https://www2.epa.gov/sites/production/files/2015-09/documents/eco-ssl_pah.pdf

23. Ogah E, Eneji IS, Odo R, Ushie OA, Longbap BD. Analysis of diazinon, chlorpyrifos, mevinfos in river benue using gas chromatography equipped with flame photometric detector (GC-FPD). International Journal of Chemistry and Aquatic Sciences (IJCA). 2016;2(2):1-6.

24. Akaahan TJA, Olabanji FM, Azua ET. Studies on contamination of surface waters of river benue with trace elements at Makurdi, Benue State, Nigeria. Journal of Environmental Chemistry and Ecotoxicology. 2015;7(5):49-55.

25. Akpen GD, Eze RAM. Water pollution modeling of the river benue in the reach of Makurdi Town. Journal of Nigeria Society of Engineers, NSE Technical Transaction. 2006;41(2).

26. Apeh S, Ekenta OE. Assessment of surface water quality of River Benue at Makurdi. Journal of Emerging Trends in Engineering and Applied Sciences, (JETEAS). 2012;3(6):904-913.

27. Anyakora C, Coker H. Determination of polynuclear aromatic hydrocarbons (PAHs) in selected water bodies in the Niger Delta. African Journal of Biotechnology. 2006; 5(21):2024-2031.

28. Fagbote EO, Olanipekun EO. Levels of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in sediment of bitumen deposit impacted area. International Journal of Environmental Science and Technology. 2010;7(3):561-570.

29. Díaz-Moroles NE, Garza-Ulloa HJ, Castro-Ríos R, Ramírez-Villarreal EG, Barbarín-Castillo JM, Salazar-Cavazos ML, Torres N. A comparison of the performance of two chromatographic and three extraction techniques for the analysis of PAHs in sources of drinking water. Journal of Chromatographic Science. 2007;45:57-62.

30. U.S. Environmental Protection Agency (US-EPA), "Ecological Soil Screening Levels for Polycyclic Aromatic Hydrocarbons (PAHs), Interim Final. OSWER Directive 9285.7-78; 2007.
Available:https://www2.epa.gov/sites/production/files/2015-09/documents/eco-ssl_pah.pdf

31. Ana REEG, Sridhar MKC, Emerole GO. A comparative assessment of soil pollution by polycyclic aromatic hydrocarbons in two Niger Delta communities, Nigeria. African Journal of Pure and Applied Chemistry. 2009;3(3):031-041.

32. Fagbote EO, Olanipekun EO. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in soils of Agbabu, Nigeria. 1st Annual International Interdisciplinary Conference, AIIC. 24-26 April, Azores, Portugal; 2013.

33. Elaine AL, Herman H. Multicomponent analyte determination. Gas Chromatography - USEPA Method 8270; 2000.

34. Braun WJ. Naive analysis of variance. Journal of Statistics Education. 2012;20(2): 1-17.

35. Pawar RM. The effect of soil pH on bioremediation of polycyclic aromatic hydrocarbons (PAHS). Journal of Bioremediation & Biodegradation. 2015;6(3):291-304.

36. UNICEF. UNICEF Handbook on Water Quality. United Nations Children's Fund (UNICEF). New York, USA. 2008;179.

37. United States Department of Agriculture (USDA). Soil quality indicators, pH, soil quality information sheet, U.S. Department of Agriculture. 1998;2.

38. Teaf CM. Polycyclic aromatic hydrocarbons (PAHs) in Urban Soil; A flourida risk assessment perspective. Int. J. Soil. Sed. Water. 2008;1(2):1-15.

39. Wilkes E. Isotopic and molecular methods sourcing environmental PAHs: A review. Dartmouth Undergraduate Journal of Science. 2012;13:33-36.

40. Magi E, Bianco R, Ianni C, Carro MD. Distribution of polycyclic aromatic hydrocarbons in the sediments of the Adriatic Sea. Environmental Pollution. 2002;119:91–98.

41. Sibiya PN. Modification, development and application of extraction methods for pahs in sediments and water. A PhD thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg. 2012;227.

_____