

## SCREENING OF CARCINOGENIC AND NON-CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBON IN A TYPICAL URBAN CITY CENTER, OWERRI, NIGERIA.

### ABSTRACT

The study screened the carcinogenic and non-carcinogenic PAH in urban city center Owerri, Imo state. Soxhlet extraction-gas chromatography-flame ionization detector (SE-GC-FID) method was accessed to analyze PAH concentrations in the different environmental component to determine its impact. The physicochemical properties of the polluted soil showed that exchangeable base (4.67g/kg), exchangeable acidity (1.25g/kg), organic carbon (6.32g/kg), organic matter (6.45g/kg), percentage nitrogen (0.462%), were higher in the polluted soil however, the effective cation exchange capacity (9.27g/kg), was lower in the polluted soil sample although, the pH value of the polluted soil was acidic. Nevertheless, the water sample properties of turbidity, magnesium, COD and BOD had the mean values of  $9.6 \pm 1.10 \text{mg/l}$ ,  $26.7 \pm 0.08 \text{mg/l}$ ,  $78.8 \pm 14.60 \text{mg/l}$  and  $54 \pm 8.0 \text{mg/l}$  respectively whereas, the bacteria coliform mean value was  $60 \pm 3.50 \text{cfu}$ . The detected PAHs were within the range of  $0.5489 \pm 0.111$  and  $0.8737 \pm 0.108$  for sediment and water samples while, spent engine oil polluted sample and Kanda processed soil sample were found in the range of  $2.2493 \pm 0.110$  and  $0.4983 \pm 0.014$  respectively. However, the level of different carcinogenic PAH obtained from environmental components suggest the toxicity propensity of these pollutant.

**Keywords:** *Polycyclic aromatic hydrocarbons, Carcinogenic, Non-carcinogenic, Environment.*

### INTRODUCTION

The increase in global civilization has led to an up rise in anthropogenic activities and as such resulted to an indiscriminate release of various pollutants in the environment, which include polycyclic aromatic hydrocarbons (PAH) (Mojiri *et al.*, 2019). PAHs can exist as complex mixtures, formed as a result of incomplete combustion of carbon materials such as wood, coal, oil, gas, or biomass, and can emanate from both natural and anthropogenic events (Ivana *et al.*, 2020). The occurrence of polycyclic aromatic hydrocarbon (PAH) in the atmosphere are of two phases; gaseous phase (low molecular weight (LMW) PAHs) and particulate phase (high molecular weight (HMW) PAHs). The rate of precipitation of these compounds was revealed to be high in soil/sediment in view of its hydrophobic nature and low aqueous solubility. Consequently, about 90% availability of PAH in the terrestrial environment can be attributed to soil owing to its proficiency to retain the aforementioned pollutants (Lu *et al.*, 2011; Kuppusamy *et al.*, 2017). Several studies have shown that the main sources of PAHs emission are largely from man-made pollution (Yang *et al.*, 2019; Matar *et al.*, 2018). Considering the places of human clustering, urban areas are exposed more to PAH pollution which are continuously aggravated by high intensity of industrial and human activities, heavy traffic etc. (Xiao *et al.*, 2014). A number of studies have looked into the distribution, sources and risk assessment of PAH pollution in urban soil, suggesting that PAHs in urban soil are influenced by human activities and are often intense in industrial zones and highway.

Therefore, PAHs in urban soil represent a risk to the health of urban population as it can be detrimental to humans through food, inhalation, and dermal interaction. Exposure through any of these routes could result to health challenges of short and long-term effect with some major respiratory and cardiovascular diseases (Ivana *et al.*, 2020; Perez-Padilla *et al.*, 2010). The toxicity imposed by PAHs is considered to be within its metabolites as it can react with cellular protein or DNA to form adduct due to its high affinity (Blaszczyk *et al.*, 2017). This reaction can lead to genetic mutation in animal and plants. Thus, it is essential to screen the carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbon in the environment considering the level of damage it can cause to human health.

## **MATERIALS AND METHOD**

### **Sample collections and reagents.**

Water and sediment samples were collected from Nworie river, spent engine oil polluted soil was collected from Chukwuma Nwoha mechanic workshop and kanda processed soil sample was collected from an abattoir in relieve market. All samples were all obtained in Owerri municipal, Imo state. Solvent including n-hexane, sodium sulphate, magnesium silicate, Soxhlet extraction tubes and other chemicals were obtained from spring board laboratory, Awka, Anambra state.

### **Soil Sample Analysis**

Soil physicochemical properties such as Soil moisture content, Soil textural class, Soil bulk density, Soil porosity, Soil pH, Soil Nitrogen, Available phosphorus, Organic carbon and matter, Available acidity, Base and Effective cation exchange capacity was done according to FAO, (2008).

### **Water Sample Analysis**

The physicochemical properties of the water sample was determined. Physical characteristics e.g. turbidity, pH, color and temperature was ascertained while BOD, COD DO, TSS, TDS, manganese, nitrate and chloride and ammonia was obtained using methods (APHA, 2005). Microbiological analysis was done to obtain the microbial count of bacteria in the water sample. Ten-fold serial dilution was done. An aliquot of dilution factor  $10^{-7}$  was inoculated into the prepared nutrient agar and eosin methylene blue agar using spread plate method. It was incubated at  $37^{\circ}\text{C}$  for 24hrs. it was sub-cultured before taking count of colony forming units. This was done according Cheesbrough, (2000).

### **PAH analysis**

Soxhlet extraction - gas chromatography – flame ionization detection (SE-GC-FID) method developed was utilized for the simultaneous recovery and determination of the 14 PAHs. A Bulk M910 GC-FID system equipped with HP 88 capillary column (100m x 0.25 $\mu\text{m}$  film thickness) were employed for PAHs chromatographic analysis and separation respectively. The extraction of PAHs from water samples was carried out with n-hexane in three extraction sequences (10, 15 and 20ml) and utilized 100ml of milli-q water as matrix for the recovery procedures and clean-up followed immediately (Ajai *et al.*, 2012).

### **Statistics**

Graphical representation of data was done using Microsoft Excel 2010 while standard deviation was obtained with Minitab 17.

## RESULTS

### Physicochemical Properties of Soil Sample

The percentage moisture content, bulk density, and porosity of spent engine oil polluted and unpolluted soil in Figure 1a. Percentage moisture content and bulk density were higher in the polluted soil sample (PSS) than in the unpolluted soil sample (UPSS). The result of porosity showed that the values obtained in the unpolluted soil were higher than that obtained in the polluted soil samples. The percentage soil particle size distribution exhibited variations in the percentage sand, silt and clay distribution for the unpolluted soil sample. Unpolluted soil samples recorded higher values in clay and silt in the range of 3.38g/kg and 6.1g/kg respectively. However, the sands of the polluted and unpolluted were 87.44g/kg and 90.62g/kg respectively. This shows that the soil textural class for both polluted and unpolluted soil samples was sandy. Furthermore, the soil chemical properties of spent engine oil polluted and unpolluted soils are presented in Figure 1b. The results obtained show pH values in polluted and unpolluted soil samples to be 5.1 and 7.4 respectively. The exchangeable base (4.67g/kg), exchangeable acidity (1.25g/kg), Organic Carbon (5.32g/kg) organic matter (6.45g/kg) and percentage nitrogen (0.429%) were higher in the polluted soil than in the unpolluted soil samples. While effective cation exchange capacity and available phosphorus (28.0mg/kg) were higher in the unpolluted oil samples compared to the polluted soil samples.

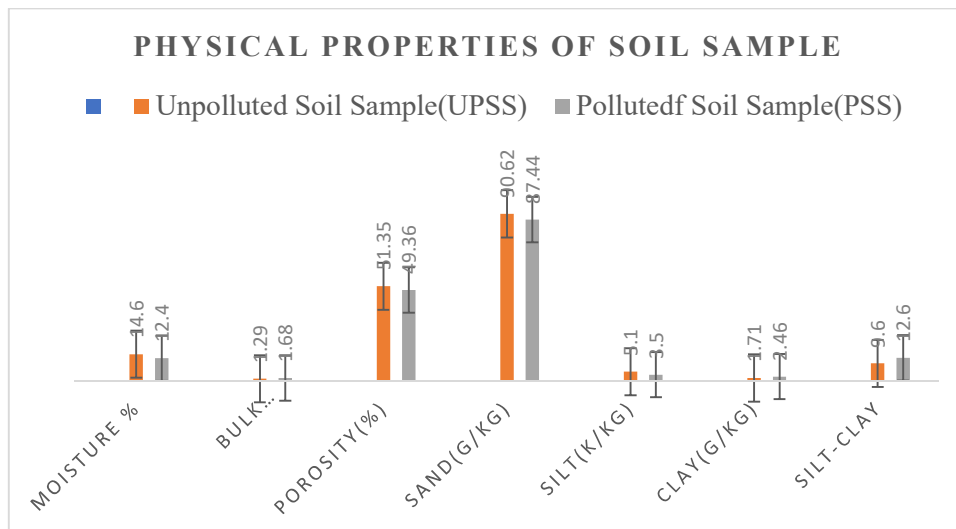


Figure 1a: Physical properties of soil sample.

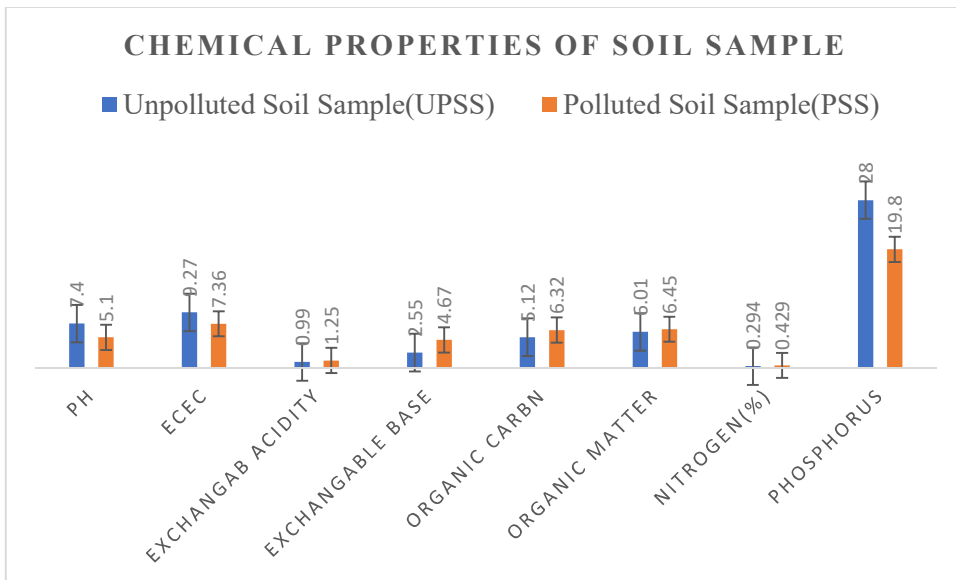


Figure 1b: Chemical properties of soil sample.

### Physicochemical and Microbiological Properties of Water

The results of the physical, chemical and microbiological analysis carried out on the water sample are shown on tables 1a and 1b. All the tested physicochemical properties of Nworie river water samples were within the world health organization (WHO) permissible limit except for turbidity, magnesium, COD and BOD with the mean values of  $9.6 \pm 1.10$ ,  $26.7 \pm 0.08$ ,  $78.8 \pm 14.60$  and  $54 \pm 8.0$  respectively. Also the bacteriological analysis revealed that Nworie River has a high number of coliform bacteria with a mean value of  $60 \pm 3.50$ .

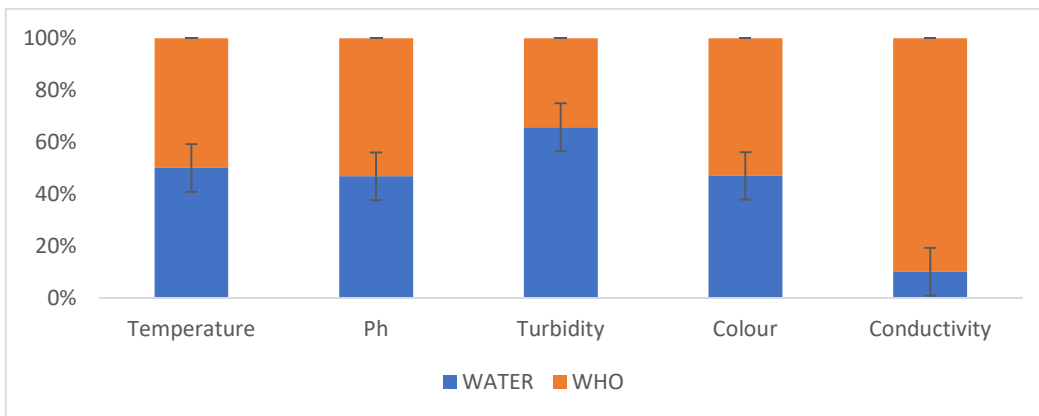


Figure 2a: Physical Quality of Water Sample.

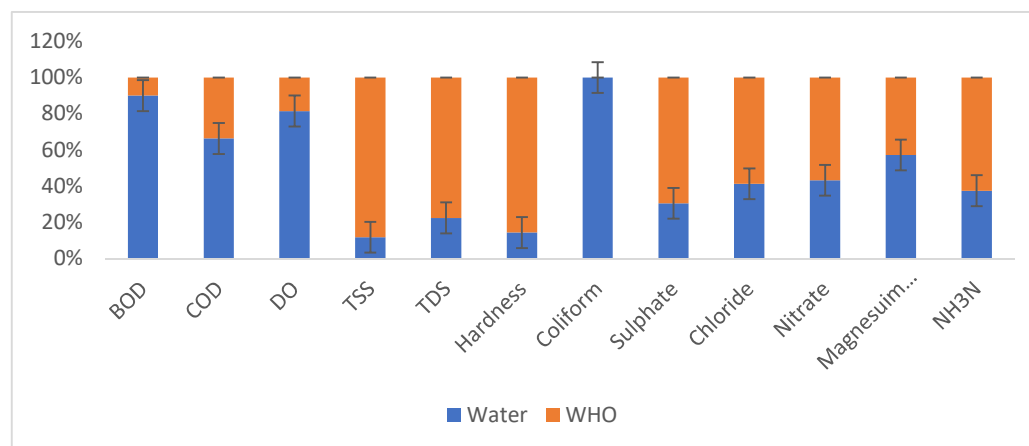


Figure2b: Chemical and Bacteriological Quality Water Sample.

### PAH Compositions in a Typical Urban Environment

The results of PAH chemical compounds obtained from different urban Environmental components are presented on Table 1a. The result shows that eleven (11) chemical compounds of PAH were detected in water sample from urban environment, compared to other environmental components. Acenaphthylene, phenanthrene, 1-2 benzanthrene, acenaphthene, benzo (k) fluoranthene, benzo (a) pyrene, fluorene, naphthalene, dibenzyl (a-h) anthracene, anthracene and benzo (g-h-i) perylene were detected in water sample with acenaphthylene and phenanthrene having the highest value (0.8737mg/ml and 0.6850mg/ml), compared to other individual environments, however, anthracene had the least value (0.0030mg/ml). Furthermore, Sediment sample had seven (7) PAHs; Acenaphthylene, phenanthrene, 1-2 benzanthrene, acenaphthene, benzo (k) fluoranthene, benzo (a) pyrene and benzo (b) fluoranthene. Of the PAHs detected, phenanthrene had the highest value (0.55mg/ml), this is followed by acenaphthylene (0.53mg/ml) and the lowest value of PAH compound was benzo (k) fluoranthene (0.18mg/ml). Moreover, in spent engine oil polluted soil sample nine (9) PAHs were detected. Acenaphthylene, phenanthrene, 1-2 benzanthrene, benzo (k) fluoranthene, benzo (a) pyrene, benzo (b) fluoranthene, naphthalene, benzo (g-h-i) perylene and fluoranthene, with fluoranthene having the highest value of 2.25mg/ml and phenanthrene having the lowest value (0.16mg/ml). While PAH detected in Kanda processed soil includes acenaphthylene, 1-2 benzanthrene, benzo (k) fluoranthene, benzo (g-h-i) perylene and pyrene. PAHs such as acenaphthalene, 1-2 benzathene, and benzo (k) fluoranthene were detected across all the samples. The different PAH concentrations obtained from the different land use and surface water of urban environment was compared with the United State environmental protection agency (USEPA) threshold limit of PAH in water and sediments, and the classification of PAH as strong, weak and non-carcinogenic as shown on Table 1b. Carcinogenic PAH identified in surrounding urban environment include; benzo(k)fluoranthene, benzo(b)fluoranthene and dibenzyl(a, h)anthracene. Of all the three PAH benzo(k)fluoranthene was found in all the different land use, and water body in higher quality. The kanda processed soil did not record any benzo(a)pyrene and benzo(b)fluoranthene. Benzo(a)pyrene which was classified as strong carcinogenic was also obtained in water, sediment and spent engine oil polluted soil in quality greater than the standard limit of USEPA (2006). Fluoranthene which is a weak carcinogenic substance was also obtained in spent engine oil polluted soil.

Table 1a: PAHs concentration from different urban environmental component.

PAH Components	SUSW(mg/ml)	USW(mg/ml)	SEOPS(mg/ml)	KPSS(mg/ml)
Acenaphthylene	0.5302 ±0.010	0.8737 ±0.108	0.1720 ±0.051	0.4983 ±0.014
Phenanthrene	0.5489 ±0.111	0.6850 ±0.101	0.1619 ±0.052	ND
1-2 Benzanthrene	0.2141 ±0.016	0.0222 ±0.141	0.3219 ±0.077	0.0007 ±0.000
Acenaphthene	0.3788 ±0.119	0.0047 ±0.001	ND	ND
Benzo (k) fluoranthene	0.1810 ±0.031	0.1801 ±0.007	0.1643 ±0.057	0.1294 ±0.026
Benzo (a) pyrene	0.1950 ±0.097	0.1246 ±0.019	0.2806±0.028	ND
Benzo (b) fluoranthene	0.4371 ±0.047	ND	0.2391 ±0.040	ND
Fluorene	ND	0.0048 ±0.001	ND	ND
Naphthalene	ND	0.0047 ±0.000	0.2607 ±0.122	ND
Dibenzyl (a-h) anthracene	ND	0.3067 ±0.153	ND	ND
Anthracene	ND	0.0030 ±0.001	ND	ND
Benzo (g-h-i) perylene	ND	0.2016 ±0.064	0.1871 ±0.023	0.2627 ±0.078
Fluoranthene	ND	ND	2.2493 ±0.110	ND
Pyrene	ND	ND	ND	0.1789 ±0.007
Cumulative Total Concentrations	2.4851	2.6032	6.0369	1.0701

Legend: ±Standard deviation; n = 14. ND = not detected; SUSW: Sediment from urban surface water; USW: Urban surface water; SEOPS: Spent engine oil polluted soil; KPSS: Kanda processed soil samples.

Table 1b: Quality threshold limits of PAH in water and sediment for the protection of aquatic life

PAH Component	Carcinogenicity	Water mg/ml	Sediment (µg/g)		
		WOG (x10 <sup>-5</sup> )	ISQG	PEL	FSSB
<b>Acenaphthylene</b>	NC	-	0.00587	0.12800	0.0059
<b>Phenanthrene</b>	NC	0.4	0.04190	0.51500	0.2040
<b>1-2 Benzanthrene</b>	NC	-	-	-	-
<b>Acenaphthene</b>	NC	5.8	0.00671	0.08890	0.0067
<b>Benzo (k) fluoranthene</b>	C	-	-	-	0.2400
<b>Benzo (a) pyrene</b>	SC	0.015	0.03190	0.78200	0.1500
<b>Benzo (b) fluoranthene</b>	C	-	-	-	0.0272
<b>Fluorene</b>	NC	3.0	0.02120	0.14400	0.0774
<b>Naphthalene</b>	NC	1.1	0.03460	0.39100	0.1760
<b>Dibenzyl (a-h) anthracene</b>	C	-	0.00622	0.13500	0.0330
<b>Anthracene</b>	NC	0.12	0.04690	0.24500	0.0572
<b>Benzo (g-h-i) perylene</b>	NC	-	-	-	0.0170
<b>Fluoranthene</b>	WC	0.04	0.11100	2.35500	0.4230
<b>Pyrene</b>	NC	0.025	0.05300	0.87500	0.1950

NC: Non-Carcinogenic, C: Carcinogenic, WC: Weakly Carcinogenic, SC: Strong Carcinogenic. ISQG: Interim Sediment Quality Guideline. PEL: Probable Effect level. FSSB: Freshwater Sediment Screening Benchmark. WOG: Water Quality Guideline. Adapted from USEPA 2006.

## DISCUSSION

### **The Impact of Spent Engine Oil Pollutant on Soil Samples**

Physico-chemical characteristics of soil polluted with spent engine oil indicates the trends of soil textural class, moisture content, bulk density, pH, organic carbon, nitrogen, etc. This reveals the low moisture content of polluted soil sample when compared to the unpolluted soil sample. This might be as a result of the pollutant present in the soil as its hydrophobic nature facilitates the loss of water through evaporation. According to Ahamefule *et al.* (2014) hydrophobic property of spent engine oil impedes the adherence of water molecule to soil particles which leads to water loss through evaporation. Bulk density was shown to be high when compared to the unpolluted soil samples. The consequence of increased bulk density leads to soil compaction thereby affecting soil porosity (Grossman & Reinsch, 2002). However, the textural class which is sandy appears not to be affected by the spent engine oil pollutants. This supports Onweremadu, (2012) who reported that the textural class of contaminated soil is not affected by pollutant. The polluted soil pH is acidic which indicates its necessity as metal cations are made soluble and available for plant uptake. However, the input of microbial degradation in formation of organic acid can also make the soil pH acidic (Osuji & Nwoye, 2007). The increase in organic carbon and organic matter in the polluted soil was probably due to hydrocarbon content in spent engine oil while increase in nitrogen may be due to the high organic matter of the contaminated soil. This agrees with Tanimu *et al.* (2019) who reported an increase in organic carbon and nitrogen content of oil polluted soil. The effective cation exchange capacity (ECEC) which shows the capability of Ca, Mg, Na and K to displace other cations was low probably because of the sandy textural class of the polluted soil and its low pH. This indicates low fertility of the contaminated soil (Uchendu & Ogwo, 2014). The available phosphorus of polluted soil was low demonstrating that the soil physicochemical property was altered by the spent engine oil as it inhibits microbial transformation of organic matter (Nwite & Alu, 2015).

### **The Effect Physicochemical and Microbiological Properties of Nworie River**

The pH of Nworie river water sample was slightly alkaline while the turbidity was high as against the permissible limit by world health organization and this may be due to anthropogenic activities within the river basin. However, the pH was within the standard limit of world health organization (Virendra *et al.*, 2013). According to Nure *et al.*, (2019) the acceptable pH range for aquatic growth is within 6.8-9.0 respectively. This indicates that the Nworie River's pH can support life forms. Moreover, influx of particles water bodies can influence its turbidity. The high concentration of Magnesium in Nworie River designates the hardness of the water. According to Onojake & Abrakasa, (2012), the hardness of the water may be as a result of presence of dissolved salts (calcium and magnesium) from pollution. The biochemical and chemical oxygen demand (BOD and COD) of the water sample exceeded the permissible limit of WHO and surface water regulation ( $\leq 6\text{mg/l}$  and  $40\text{mg/l}$ ). This may be due to the presence of chemical which may be organic and inorganic caused by domestic, livestock and industrial waste that contains elevated levels of organic pollutant. The high number of coliform in Nworie Rivers probably because of the indiscriminate release of domestic sewage into the river. This result is in line with Akpan-Idot *et al.*, (2012) who observed that Okpauku River in Yala, Cross River state contains high number of coliform bacteria.

### **Assessment of PAH Contents in Different Environmental Component**

The total PAH samples in the environment shows that they were higher than the permissible limit as indicated by United State Environmental Protection Agency (USEPA, 2006) regulatory guide. The PAH levels in water samples of Nworie river were observed to be higher than those measured

in corresponding sediment samples. This is in contradiction to the high levels of PAH that have been reported in sediments (Ma *et al.*, 2013; Hong *et al.*, 2016). The difference can be attributed to presence of the low molecular weight (LMW) PAH (2-3 rings PAH) which are soluble and volatile in water and have been more detected in water samples of Nworie River. This observed pattern of PAH in water samples shows that three rings PAHs appears to be more while sediment is mostly dominated by four ring PAHs. This phenomenon between water and sediment sample might be attributed to activities such as road construction, oil spill etc. In sediment samples, the high level of phenanthrene (3-ringed PAH) detected shows a major constituent of crude oil and coal tar. According to Santos *et al.*, (2018), an increase level of phenanthrene in aqueous sediment was linked to atmospheric deposition and petroleum contamination. Phenanthrene is known to cause endocrine and reproductive disruption, neurotoxicity, genotoxicity, oxidative damage, cytotoxicity and growth impairment in fish has been reported (Machado *et al.*, 2014). In spent engine oil polluted soil (SEOPS), the HMW PAH (4-5) had the highest proportion. The availability of the HMW PAHs relative to LMW PAH in SEOPS has been shown to emanate from pyrogenic activities and activities of mechanic workshop such as photochemical smog from automobile exhaust (Nekhavambe *et al.*, 2014). The carcinogenic PAHs detected in water and sediment samples of Nworie River is unsafe for both aquatic organisms and humans as its reactive metabolites can bind to proteins and DNA. Thus, altering the nucleotide sequence which can cause cell damage. Igwe & Ukaogo (2015) reported that carcinogenic PAH can cause biochemical disruption and cell damage.

### Conclusion

In the different urban environment of Owerri municipal, PAH compounds were observed to be above the permissible limit of USEPA especially in water samples. Therefore, it is necessary to monitor the environment in terms of pollution control to avoid contamination of the environment with these toxic compounds which can be detrimental to life forms because of its bioaccumulation and recalcitrant nature.

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#### COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.