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Health Implications of Bioaccumulation of PAHs in Fluted Pumpkin (*Telfairia occidentalis*) and Waterleaf (*Talinum fruticosum*) along the Imo River Watershed, Imo State, Nigeria

Jessica N. Anyahara
Ph.D. Student, Department of Environmental Management,
Federal University of Technology, Owerri, Imo State, Nigeria
C.O. Nwoko
Professor, Department of Environmental Management,
Federal University of Technology, Owerri, Imo State, Nigeria
Dr. R. F. Njoku-Tony
Senior Lecturer, Department of Environmental Management,
Federal University of Technology, Owerri, Imo State, Nigeria
Dr. C.E Ihejirika
Senior Lecturer, Department of Environmental Management,
Federal University of Technology, Owerri, Imo State, Nigeria

Abstract:
The health implication of PAHs has been of great concern due to its contamination of the food chain. This study was carried out to identify the health challenges associated with the bioaccumulation of PAHs in Fluted Pumpkin (Telfairia occidentalis) and Waterleaf (Talinum fruticosum) along Imo River Watershed, Imo State, Nigeria. A total of 8 soil samples (0-30cm) were randomly collected from different activity areas on the watershed and analyzed for the presence of the 16 US-EPA priority PAHs in both soil and vegetable samples. The analysis of PAHs in the soil and vegetable samples were carried out in accordance with EPA 8270: (GC-MSD) and the results revealed that amongst the 16 US-EPA priority PAHs, eight of them were detected in the soil and vegetable samples. The total concentration of PAHs detected in the soil and vegetable samples were Naphthalene(0.146mg/kg), Acenaphthylene(0.010mg/kg), Acenaphthene(0.210mg/kg),Phenanthrene(0.021mg/kg),Anthracene(0.041mg/kg),Flouranthene(0.130mg/kg),Pyrene(0.020mg/kg),Benzo(b)fluoranthene(8.641mg/kg) and Naphthalene(0.090mg/kg),Acenaphthene(0.011mg/kg),Fluorene(0.010mg/kg),Phenanthrene(0.230mg/kg),Anthracene(0.190mg/kg),Flouranthene(0.010mg/kg),Benzo(b)fluoranthene(2.711mg/kg),I(1,2,3-cd)PY(0.540mg/kg) respectively. The decreasing order for individual PAHs concentration in the soil and vegetable samples within the study area were Benzo(b)Flouranthene>Acenaphthene>Naphthalene>Fluoranthene>Anthracene> Phenanthrene> Pyrene> Acenaphthelyne and Benzo(b) Flouranthene>I(1,2,3-cd)PY> Phenanthrene> Anthracene> Naphthalene>Acenaphthene> Fluorene>Fluoranthene respectively. For both soil and vegetables, total PAHs concentrations recorded in Obowo zone were observed to be higher than Okigwe zone. The result also revealed high values of PAHs in the edible vegetable samples collected around abattoirs and abandoned waste dump. It is therefore evident from this study that abattoirs and waste dumps have the potential to emit huge amount of PAHs to soil resource, which is taken up by edible vegetables leading to several health challenges when the vegetables are consumed. Different exposure to PAHs results in immune function suppression, cataracts, kidney and liver damage, skin inflammation, asthma, nausea, vomiting, eye irritation, diarrhea, confusion, lung and other cancers and probably death in chronic cases. There is need for public education on the consumption of vegetables grown in and around abattoirs and areas dominated by waste dumps.

Keywords: Polycyclic aromatic hydrocarbons, soil, physicochemical parameters, bioaccumulation

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a typical group of chemicals that contain two or more aromatic rings and are prevalent in the environment. Because of their persistence, carcinogenicity, toxicity and mutagenicity, they cannot be removed nor destroyed from matrices they contaminate (Muntean et al., 2015). Of the hundreds of PAHs that exist, 16 were identified by the United States Environmental Protection Agency (USEPA) as priority pollutants due to their toxic, mutagenic, and carcinogenic characteristics (Tam et al., 2001). Over the years, bioaccumulation of PAHs has been the focal point of numerous investigations. PAHs can be derived from natural sources, but anthropogenic activity is generally considered to be the major source of PAHs. These PAHs are organic pollutants. They mainly originate from incomplete

combustion processes, such as fuel combustion, biomass combustion and vehicle emissions and are known for their toxic, carcinogenic, mutagenic, bio-accumulative and persistent properties (Feidi et al., 2018; Suman, Sinha & Tarafdar, 2016). Pyrogenic and petrogenic sources are two major origins of anthropogenic PAHs in the environment. Pyrogenic PAHs are formed as trace contaminants by the incomplete combustion of organic matter, such as wood, fossil fuels, asphalt and industrial waste. According to Ying et al. (2009), petrogenic PAHs are generally contained in crude and refined petroleum.

They have a relatively low solubility in water but are highly lipophilic. When dissolved in water or absorbed in particulate matter, PAHs can undergo photodecomposition when exposed to ultraviolet light from solar radiation. Once PAHs are released in soil, they are biodegraded rapidly in an anaerobic environment. However, they may persist longer times under anaerobic conditions or in large concentrations, as they may be toxic to microorganisms (Wang et al., 2016). If they are not biodegraded in soil, some tend to be adsorbed on soil surface based on their octanol-carbon coefficient (K_{oc}) and volatilize based on their vapor pressure from dry soil or from wet soil based on their Henry's law coefficient, while others tend to migrate towards groundwater. (Alexandru, 2021). They may also be degraded by some microorganisms in the soil (ATSDR, 1994). PAHs pollutants have high molecular mass. PAHs of 4 and more condensed aromatic rings are considered more dangerous than 2 and 3 rings PAHs in view of their potential (Kawamura et al., 1994). Heavy PAHs are more stable and more toxic than light PAHs (Kuppusamy et al., 2016). Kim et al. (2013) also noted that PAHs are persistent organic pollutants in which some are low molecular weight compounds consisting of fewer than four rings and others are high molecular weight compounds consisting of four or more rings.

The 16 environmentally significant PAHs are those PAH molecules that contain 2 to 7 benzene rings and are divided into two groups based on their physical, chemical, and biological characteristics (Martinez et al., 2004). The lower-molecular-weight (LMW) PAHs, for example, the 2 to 3 rings of PAHs, such as naphthalenes, fluorenes, phenanthrenes, and anthracenes, have significant acute toxicity to aquatic organisms. The high-molecular-weight (HMW) PAHs, containing 4 to 7 rings from chrysenes to coronenes, do not cause acute toxicity but are known to be carcinogenic. According to Oyo-ita et al. (2016), techniques used to differentiate pyrogenic and petrogenic sources of PAHs include examining the relative amounts of low molecular weight (LMW) and high molecular weight (HMW) compounds. LMW PAHs compounds predominate in petrogenic sources, while HMW compounds predominate in pyrogenic sources (Guo et al., 2021). Due to their low water solubility and high lipophilic nature, PAHs are easily and rapidly absorbed by organisms and can be accumulated in aquatic organisms or adsorbed onto the surface of suspended matter, get deposited on the sea floor and be passed onto the marine food chain (Oyetunji & Francis, 2022).

Once PAHs enter the environment, they can be stable for as short as 48 hours (e.g., naphthalene) or as long as 400 days (e.g., Fluoranthene) in soils (Albert & Juhasz, 2000). Thus, they resist degradation and, thus, remain persistent in sediments and, when in organisms, can accumulate in adipose tissues and further transfer up the tropic chain or web. PAHs, as persistent organic environmental compounds, have been ranked as the ninth most threatening compounds to human health (King et al., 2002). PAHs have been determined to be carcinogenic by several regulatory agencies, like US Environmental Protection Agency (USEPA), US Department of Health and Human Services (DHHS) and the International Agency for Research on Cancer (IARC) (Moorthy et al., 2015). The carcinogenicity and mutagenicity of several PAHs have been proved (Simko, 2005). Furthermore, Kumar et al. (2014) have suggested that PAHs be included in climate regulations at the same level as CO_2 and NO_2, given their longer half-life and chemical properties which give a wide range of toxic derivatives during degradation. PAHs have long transport potential thus, cause adverse environmental effects (Keshavarzifard et al., 2015).

Several health problems are associated with bioaccumulation of PAHs in the food chain (Chen et al., 2018). Long-term exposure to even the simplest PAHs, like Naphthalene (a low molecular weight PAH), can lead to kidney and liver damage and cataracts. It causes redness and inflammation of the skin when in contact and red blood cells get damaged when inhaled, whereas high molecular weight PAHs like Pyrene, Benzo(a)pyrene, etc., are known to be highly carcinogenic.

2. Materials and Methods

2.1. The Study Area

This study was carried out along the Imo River watershed in Imo State, Nigeria. Imo River drains three States, namely: Imo State, Abia State and Rivers State. Imo State is the largest (in terms of size and length) of the rivers. It is the major river in Imo State with a large number of tributaries. Imo River being the most prominent, traverses from north to south of the state with a length of about 225 km. In its upper reach, it receives tributaries. It has its source in the Ideato North Area of Imo State near Osina. Its middle reaches flow north to south for some 80 km in the flat valley through the coastal plain lowland without receiving any significant tributaries. The Imo River drained not only Imo State but also Abia and Akwa Ibom States and emptied into the Atlantic Ocean. It is joined by Aba River flowing from the north and then enters Akwa Ibom State en route to the Ocean. The major tributary of Imo River is the Otamiri River (Okoro et al., 2014).

Imo River is in southeastern Nigeria and flows 240 kilometers (150 mi) into the Atlantic Ocean. Its estuary is about 40 km wide with an annual discharge of 4 Km^3 and 26,000 hectares of wetland. The Imo's tributaries are the Otamiri and Oramirukwa.

There are two prevailing climatic seasons in the study area: the rainy season and the dry season. The rainy season begins in March and ends in October, with a break in August, while the dry season begins in November and ends in February. The rainy season leads to floods and runoffs, which wash various harmful contaminants over the watersheds into the receiving water bodies. The watershed provides the aquifer for groundwater storage because of its high porosity and permeability. The incidence of high porosity and permeability and shallow water table makes the groundwater in the

area very vulnerable to pollution in the presence of poor waste management as practiced in various locations across the study area.

The annual temperature ranges from 22°C to 33°C across the study area. The study area vegetation is a mixture of mangrove and rainforest with a mean annual rainfall of 2400mm and mean humidity of 85%. Some parts are rich in savannah vegetation, with bamboo (Dendrocalamus strictus) dominating the typical grass specie.
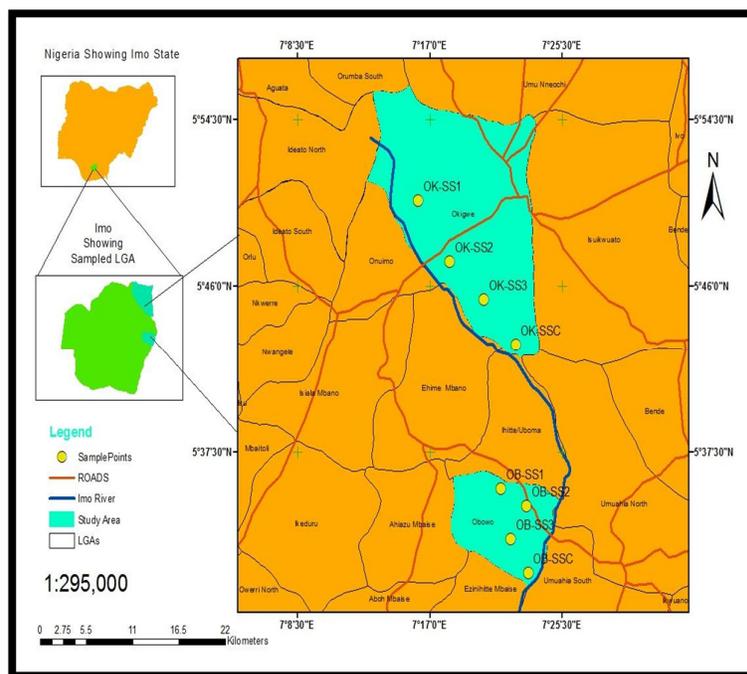

Figure 1: Map of Nigeria Showing Imo State, Okigwe and Obowo
Local Government Areas, the Study Area and Sampling Points

2.2. Sampling Plan

The study area was divided into two zones: Okigwe and Obowo. Each zone was further divided into four sampling sites, namely:

- OKSS1 = Okigwe Soil Sample 1 (Imo State Water Scheme premises)
- OKSS2 = Okigwe Soil Sample 2 (Abandoned waste dump)
- OKSS3 = Okigwe Soil Sample 3 (River bank)
- OKSSC = Okigwe Soil Sample Control (1KM from the river bank)
- OBSS1 = Obowo Soil Sample 1 (Abattoir)
- OBSS2 = Obowo Soil Sample 2 (Spare Parts Market)
- OBSS3 = Obowo Soil Sample 3 (River bank)
- OBSSC = Obowo Soil Sample Control (1KM from the river bank)
  The vegetable samples were labeled as:
- OK/VEG/ W = Okigwe waterleaf,
- OK/VEG/ P = Okigwe pumpkin,
- OB/VEG/ W = Obowo waterleaf,
- OB/VEG/ P = Obowo pumpkin,

2.3. Sample Collection

Soil samples were collected in triplicate into sterilized containers using a 30-cm marked metal soil auger from each of the sampling points within the zones and then a control site. A total of forty-eight (48) batch soil samples made into thirty-two (16) different composite samples were collected for both seasons at a depth of 0-30 cm (subsurface soils), after the removal of the exposed surface with the use of a machete, according to MPCA (2008). This range of soil depth was chosen because it comprises surface and subsurface soils which are the first locus of input of contaminants, where they tend to accumulate on a relatively long-term basis, according to Abenchi et al. (2010). Krishna and Grovil (2007) also added that pollutants normally contaminate the upper layer of the soil at a depth of 0-40cm. The soil auger was appropriately cleaned between samples to avoid potential errors in results due to cross-contamination. Control samples were similarly collected from a distance of 1km away from the river bank where no activities existed. Two edible vegetable samples: Fluted Pumpkin (Telfairia occidentalis) and waterleaf (Talinum fruticosum), were also collected from each zone using sterilized hand gloves and were changed for each zone to avoid potential errors due to cross-contamination and stored in a sterilized Polycyclicthene bag before taken to the laboratory for further analysis.

2.4. Analysis of Physicochemical Properties in Soil Samples

The physicochemical analysis was carried out on the soil samples to assess their impact on some physicochemical variables such as soil pH, electrical conductivity, total organic carbon, organic matter and nitrate. Soil pH was determined using the HANNA (HI83099) multi-parameter. Soil Electrical Conductivity was determined using the Hanna Conductivity meter. Cation Exchange Capacity was obtained by summating the concentrations of exchangeable base cations and exchangeable acidity. The soil nitrate was determined using the HANNA (HI83099) Multi-Parameter Bench Photometer and the test method was ALPHA 4500. Soil Organic Carbon and organic matter were determined by the Walkley-Black Method.

2.5. Analysis of Polycyclic Aromatic Hydrocarbons in Soil and Vegetable Samples

The Polycyclic aromatic hydrocarbon analysis of the soil and vegetable samples collected from the study area was carried out in accordance with EPA 8270: Standard Test Method for Determination of Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water Using Solid-Phase Micro-extraction and Gas Chromatography/Mass Spectrometry in Mass Selective Detection Mode, which describes the procedure for analysis of extractable Polycyclic Aromatic Hydrocarbon (PAH) in surface water, groundwater and soil/sediment. In the case of this study, polycyclic aromatic hydrocarbons (PAHs) were detected in the soil samples by Gas Chromatography-Mass selective detector (GC-MSD).

2.6. Analysis of Microbial Population in Soil Samples

The associated bacteria were isolated and identified using microscopic/ physical observation and Biochemical tests, as detailed in the table above.

CFU/g = No. of Colony counted x dilution factor x volume plated / weight of sample used.

2.7. Statistical Analysis

All data collected were analyzed using Minitab statistical package (16th Edition) and boxplots, which were used to represent variations in soil chemical characteristics and the concentration of PAHs in the study area (Musa et al., 2020). Two-Way Analysis of Variance (ANOVA) was used to partition means and significant means separated using Tukey range test and 95% Confidence. Pearson correlation coefficient® was used to determine the possible relationship between PAH concentrations and the physicochemical attribute.

3. Results and Discussion

3.1. Physicochemical Properties of Soil Samples Analyzed in the Study Area

The result of the analysis is presented in table 1. The physicochemical parameters showed significant differences across the sampling locations ($P < 0.05$). The pH ranged from 5.50±0.0 to 6.33±0.05, showing that the soil samples all had low pH and their mean values varied slightly across the sampling locations. EC ranged from 128.0±0.06 to 325.3±0.57μs/cm, TOC ranged from 0.02±0.006 to 0.06±0.006%, TOM ranged from 0.030±0.0006 to 0.17±0.063%. NO3 ranged from 0.32±0.006 to 1.15±0.006mg/kg.

Sampling Location	Parameters				
	NO$_3$ (mg/kg)	TOC (%)	pH	EC (μs/cm)	OM (%)
OK/SS/1	1.04±0.006b	0.02±0.006c	6.10±0.006b	325.3± 0.57a	0.033± 0.006c
OK/SS/2	1.04±0.006b	0.06±0.006a	5.60±0.06ge	131.3± 0.57g	0.13±0.06b
OK/SS/3	0.67±0.006e	0.06±0.006a	6.33±0.05a	128.0± 0.06h	0.17±0.063a
OK/SS/C	1.02±0.0c	0.03±0.0006b	6.03±0.06c	219.3± 0.56c	0.050± 0.0006c
OB/SS/1	0.32±0.006g	0.02±0.006c	5.60± 0.0e	263.7± 0.6b	0.030± 0.0006c
OB/SS/2	0.69±0.006d	0.03±0.0b	5.50±0.0f	140.3± 0.6e	0.050± 0.0006c
OB/SS/3	0.65±0.006f	0.031±0.0012b	5.60±0.10e	136.3± 0.6f	0.050± 0.0006c
OB/SS/C	1.15±0.006a	0.03±0.0b	5.80±0.006d	217.3± 0.56d	0.050± 0.001c

Table 1: Concentrations of Soil Physicochemical Parameters Detected in the Soil Samples with Their Mean Separations across the Sampling Locations

3.2. Polycyclic Aromatic Hydrocarbon Concentration Level in the Soil Samples

The Polycyclic Aromatic Hydrocarbons (PAHs) detected in the soil samples from the study area and their concentrations are recorded in table 1. The result revealed that only Naphthalene (0.146mg/kg), Acenaphthylene (0.010mg/kg), Acenaphthene (0.210mg/kg), Phenanthrene (0.021mg/kg), Anthracene (0.041mg/kg), Flouranthene (0.130mg/kg), Pyrene (0.020mg/kg) and Benzo(b)fluoranthene (8.641mg/kg) amongst the 16 EPA priority PAHs studied were detected in the eight soil samples analyzed. The individual PAH concentrations ranged from negligible to 3.1mg/kg, with Benzo(b)fluoranthene concentration level being the highest among the individual PAHs across the sampling locations. The highest individual PAH concentrations were recorded in OB/SS/1.

The result revealed that there were significant variations in the level of Polycyclic Aromatic Hydrocarbons across the sampling locations. Obowo zone had the highest number of individual PAHs. The individual PAHs present in the study area were Naphthalene, Acenaphthylene, Acenaphthene, Phenanthrene, Anthracene, Flouranthene, Pyrene and Benzo(b)fluoranthene.

Sampling Locations	PAH Components (mg/kg)							
	NAPH	ACENATHYLN	ACE	PHTHN	ANTH	FLTHN	PY	B(b)FLTHN
OK/SS/1	0.07±0.0006a	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0b	1.20±0.006c
OK/SS/2	0.0±0.0c	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.1±0.006f
OK/SS/3	0.0067±0.006b	0.0±0.0 b	0.21±0.006a	0.0±0.0b	0.01±0.0b	0.11±0.0a	0.0±0.0b	1.01±0.006d
OK/SS/C	0.0±0.0c	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.12±0.0f
OB/SS/1	0.07±0.00a	0.01±0.0a	0.0±0.0b	0.021±0.0012a	0.031±0.002a	0.02±0.006b	0.02±0.06a	1.5±0.0b
OB/SS/2	0.0±0.0c	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0b	3.1±0.0006a
OB/SS/3	0.0±0.0c	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0b	1.20±0.006c
OB/SS/C	0.0±0.0c	0.0±0.0b	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0c	0.0±0.0b	0.41±0.006e

Table 2: Concentration of PAHs Detected in the Soil Samples with
Their Mean Separations across the Sampling Locations

NAPH = Naphthalene. ACENATHYLN = Acenaphthylene. ACE = Acenaphthene, PHTHN = Phenanthrene. ANTH = Anthracene, FLTHN = Flouranthene, PY = Pyrene and B(b)FLTHN = Benzo(b)fluoranthene. OKSSI = Okigwe Soil Sample 1, OKSS2 = Okigwe Soil Sample 2, OKSS3 = Okigwe Soil Sample 3, OKSSC = Okigwe Soil Sample Control, OB/SS/I = Obowo Soil Sample 1, OBSS2 = Obowo Soil Sample 2, OBSS3 = Obowo Soil Sample 3, OBSSC = Obowo Soil Sample Control.

Means with different alphabets as superscripts for each soil sample are significantly different, while those with the same alphabets are the same.

3.3. Polycyclic Aromatic Hydrocarbon Concentration Level in the Vegetable Samples

The result revealed that Acenephthylene, Pyrene, Benzo (a)anthracene, Chrysene, Benzo(b)anthracene, Benzo(a)pyrene, Benzo(g,h,i)perylene and Dibenzo(a,h)anthracene among the sixteen environmental significant PAHs were not detected in the vegetable samples. Moreover, only Naphthalene, Fluoranthene, Acenaphthene, Fluorene, Anthracene, Benzo(b)fluoranthene, Indino{1,2,3-cd}Pyrene and Phenanthrene were detected in the vegetables. The individual PAH concentrations in the vegetable samples ranged from negligible to 0.97mg/kg, with Benzo(b)fluoranthene concentrations level being the highest among the individual PAHs across the sampling locations. In summary, the highest PAH mean value was recorded in OB/VEG/P, while the lowest PAH mean value was recorded in OK/VEG/W.
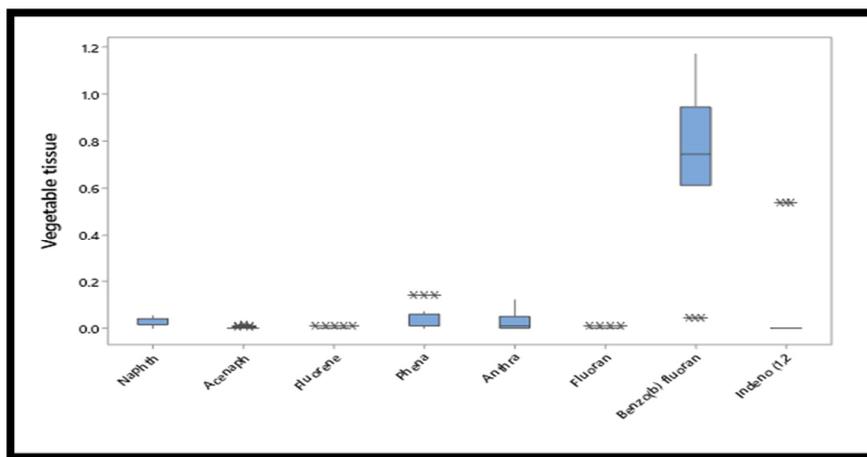

Figure 2: Box Plot on PAH Concentrations of Vegetable Samples

Sampling Locations	PAH Components (mg/kg)							
	NAPH	ACE	FL	PHTHN	ANTH	FLTHN	B(b)FLTHN	I (1,2,3-cd)PY
OK/VEG /W	0.0003±0.06c	0.0003±0.05b	0.003±0.05b	0.010± 0.006c	0.0± 0.0d	0±0.0b	0.041±0.001d	0.0±0.0b
OK/VEG/ P	0.04±0.006b	0.0103±0.05a	0.0±0.0b	0.07±0.006b	0.06±0.06b	0.0±0.0b	0.87±0.001b	0.0±0.0b
OB/VEG/ W	0.01±0.0d	0.0 ±00b	0.0 ±0.0b	0.01±0.0c	0.01±0.0c	0.01±0.0a	0.83±0.001c	0.0±0.0b
OB/VEG /P	0.04±0.0a	0.0003±0.06b	0.01±0.0a	0.14±0.0a	0.12±0.0a	0.0± 0.0b	0.97±0.001a	0.54±0.0a

Table 3: Concentration of PAHs Detected in Fruited Pumpkin and
Waterleaf Samples with
Their Mean Separations across the Study Area

Means with different alphabets as superscripts for each soil sample are significantly different, while those with the same alphabets are the same. OK/VEG/W = Okigwe waterleaf, OK/VEG/P = Okigwe pumpkin, OB/VEG/W = Obowo waterleaf, OB/VEG/P = Obowo pumpkin, NAPH = Naphthalene. ACE = Acenaphthene, FL = Fluorene, ANTH = Anthracene, B(b)FLTHN = Benzo(b)fluoranthene, I{1,2,3-cd}PY = Indino{1,2,3-cd}Pyrene, FLTHN = Fluoranthene and PHTHN = Phenanthrene.

3.4. Microbial Population of Soil Samples across the Study Area

The result of total hydrocarbon-degrading bacteria and total heterotrophic bacteria count indicated that THDB mean values ranged from negligible to 5.50CFU/g, while the THBC mean value ranged from 3.45CFU/ml to 7.25CFU/ml. The result further revealed that the highest THDB mean value was recorded at OB/SS/2, while the lowest mean value was recorded at OK/SS/C. Also, the highest THBC mean value was recorded at OK/SS/1, while the lowest mean value was recorded at OB/SS/C. The mean values of both the THDB and the THBC were significantly different (p<0.05) from each other. The presence of THDB and THBC indicates the presence of PAH because they use PAHs as sources of carbon and energy.

Location	THDB (CFU/g)	THBC (CFU/ml)	% HDB
OK/SS/1	$2.88 \times 10^3 \pm 0.58f$	$7.25 \times 10^6 \pm 1a$	0.04
OK/SS/2	$3.45 \times 10^3 \pm 0.58e$	$6.45 \times 10^6 \pm 1c$	0.05
OK/SS/3	$4.21 \times 10^3 \pm 0.58d$	$6.55 \times 10^6 \pm 1b$	0.06
OK/SS/C	$0.00 \pm 0.00h$	$4.25 \times 10^6 \pm 1g$	Nil
OB/SS/1	$4.70 \times 10^4 \pm 0.6c$	$6.42 \times 10^6 \pm 1d$	0.73
OB/SS/2	$5.50 \times 10^4 \pm 0a$	$5.95 \times 10^6 \pm 0e$	0.92
OB/SS/3	$4.8 \times 10^4 \pm 0.6b$	$5.44 \times 10^6 \pm 1f$	0.89
OB/SS/C	$2.08 \times 10^2 \pm 0.0g$	$3,45 \times 10^6 \pm 1h$	0.01

*Table 4: Mean Concentration of HDB and HBC in the
Soil Samples across the Study Area*

Means with different alphabets as superscripts for each soil sample are significantly different, while those with the same alphabets are the same. OKSSI = Okigwe Soil Sample 1(Imo State Water Scheme), OKSS2 = Okigwe Soil Sample 2 (Abandoned waste dump), OKSS3 = Okigwe Soil Sample 3 (River Bank), OKSSC = Okigwe Soil Sample Control (1KM away from the river bank, OBSSI = Obowo Soil Sample 1(Abattoir), OBSS2 = Obowo Soil Sample 2 (Spare Parts Market), OBSS3 = Obowo Soil Sample 3 (River Bank), OBSSC = Obowo Soil Sample Control (1KM away from the river bank), THDB = Total Hydrocarbon Degrading Bacteria, THBC = Total heterotrophic bacteria count, %HDB=% Hydrocarbon degrading bacteria.

3.5. Correlation between Physicochemical Parameters and Individual PAHs

Karl Pearson's correlation (r) was used to evaluate the possible relationships between the chemical parameters and individual PAHs analyzed in the study area. The correlation results presented in table 5 revealed that a relationship existed between the PAHs and soil chemical properties. Thus, the availability and distribution of PAHs in the study area were influenced by some physicochemical properties. The result further revealed that at $P \leq 0.001$ with a confidence level of significance of 99.9%, NAPH, ACENTHLYN, PHTHN, ANTH and B(b)FLTHN correlated positively with soil Nitrate (NO3)with a correlation coefficient r of 0.000, 0.033, 0.008, 0.003, 0.027 respectively. Also, NAPH correlated positively and averagely with Electrical Conductivity (EC) with a correlation coefficient r of 0.006 and no relationship existed between the Soil pH, total organic carbon and soil organic matter with PAHs obtained in the soil of the study area.

Parameters	PAH Components (mg/kg)							
	NAPH	ACENATHLYN	ACE	PHTHN	ANTH	FLTHN	PY	B(b) FLTHN
Ph	-0.110	-0.073	0.043	-0.037	-0.054	-0.028	0.006	-0.050
	0.465	0.632	0.778	0.806	0.720	0.852	0.966	0.744
NO3	0.719*	0.315*	0.043	0.388*	0.434*	0.209	0.169	0.327*
	0.000	0.033	0.776	0.008	0.003	0.164	0.263	0.027
TOC	0.016	-0.096	-0.089	-0.086	-0.049	0.024	-0.032	-0.162
	0.918	0.528	0.555	0.568	0.749	0.872	0.835	0.283
EC	0.398*	0.006	-0.196	0.053	0.071	-0.096	-0.151	-0.002
	0.006	0.967	0.192	0.724	0.641	0.524	0.317	0.987
OM	-0.105	-0.106	-0.139	-0.041	-0.036	0.011	-0.051	-0.151
	0.486	0.482	0.357	0.786	0.812	0.943	0.734	0.317

Table 5: Correlation (R) Between PAHs and Physicochemical Parameters Measured in the Soil Samples

3.6. Correlation between Polycyclic Aromatic Hydrocarbons (PAHs) and Microbial Population Measured in the Soil Samples across the Study Area

Karl Pearson's correlation (r) was used to evaluate the possible relationships between the Hydrocarbon degrading bacterial (HDB), Heterotrophic bacterial count (HBC) and individual PAHs analyzed in the study area. The correlation results are presented in table 6.

The result revealed that at $P \leq 0.05$, NAPH correlated negatively with HBC with a correlation coefficient r of -0.440 and I(1,2,3-cd)PY correlated positively and averagely with HDB with a correlation coefficient r of 0.535.

This indicates that the higher concentration of Naphthalene, the lower the populations of heterotrophic bacteria count and the higher the concentration of Indeno(1,2,3-cd)pyrene, the higher the population of hydrogen-degrading bacteria.

Generally, the result showed moderate negative and positive correlations between the PAH components and the microbial population of the soil samples.

	NAPH	ACENATHLYN	ACE	FL	PHTHN	ANTH	FLTHN	PY	B (b) FLTHN	I(1,2,3-cd) PY
HBC	-0.44	0.182	0.145	0.121	0.184	0.096	0.116	0.142	0.182	-0.221
	0.031*	0.396	0.499	0.573	0.39	0.656	0.59	0.507	0.396	0.299
HDB	0.046	0.287	0.229	0.191	0.316	0.341	0.382	0.344	0.287	0.535
	0.829	0.174	0.282	0.371	0.133	0.103	0.065	0.099	0.174	0.007*

*Table 6: Correlation (R) between PAHs and Microbial Population
Measured in the Soil Samples across the Study Area*

3.7. Discussion

Sixteen PAHs recommended by the United State Environmental Protection Agencies (USEPA) were investigated in this study and only Naphthalene, Acenaphthelyn, Acenaphthene Phenanthrene, Acenaphthene, Fluoranthene, Pyrene and Benzo(b)fluoranthene were detected in the soil samples. The highest concentrations of PAHs were recorded in the soil and vegetable samples of Obowo zone and the lowest concentrations were recorded in the soil of Okigwe zone. The highest total concentration for individual PAHs in the soil and vegetable samples was Benzo(b)Flouranthene at 8.641mg/kg and 2.711mg/kg, respectively, while the least individual PAH was Acenaphthelyn at 0.010mg/kg and Fluorene at 0.010mg/kg respectively. The decreasing order for individual PAHs concentration in the soil samples within the study area was Benzo(b)Flouranthene > Acenaphthene > Naphthalene > Fluoranthene > Anthracene > Phenanthrene Pyrene> Acenaphthelyn. Obowo Zone had the highest concentration levels of PAHs in the study area indicating pollution input from the abattoir and spare parts market. This may lead to PAH of the watershed, therefore polluting the water body itself and accumulating in the sediments.

3.8. Characteristics of Soil Physicochemical Properties

The quality of any environmental medium is generally influenced by its physicochemical factors. Therefore, monitoring these factors is imperative for both long-term and short-term environmental management of pollutant inputs. Also, the distribution and productivity levels of organisms are largely determined by physicochemical factors in these ecosystems. Accordingly, several authors have investigated the direct effect of interactions of many frequently measured physicochemical variables on biotic residents of these ecosystems (Jonnalagadda & Mhere, 2001). Also, Ogbuagu et al. (2011) revealed that the physicochemical attributes of the Imo River varied based on the gradient. According to Biswas and Mojid (2018), soil parameters such as pH and redox conditions largely affect the adsorption and desorption of chemical contaminants in the soil. However, these sorption processes are dependent on the aforementioned soil parameters and other soil parameters. The occurrence, fate, and transport of chemical pollutants in soils are largely dependent on the properties of the soil and pollutants as well.

3.9. Properties of Polycyclic Aromatic Hydrocarbon (PAH) Compounds

The physical and chemical properties of PAHs vary with their molecular structure (number of rings) and molecular weight. This means that as the number of rings increases, the molecular weight also increases. They vary with high melting and boiling points, low vapour pressure, very low aqueous solubility and tend to decrease with increasing molecular weight. PAHs are generally classified as Low Molecular Weight and High Molecular Weight PAHs. According to Wick et al. (2011), PAHs with low molecular weight are those with two or three fused benzene rings, while high molecular weight PAHs are those with four or more fused benzene rings. Pandey et al. (1997) stated that PAHs with low molecular weight easily degrade and volatilize faster than high molecular weight PAHs. The higher the molecular weight of PAHs, the higher its hydrophobicity, toxicity, and lipophilic property, the lower its solubility in water, vapor pressure, and degradability (Wick et al., 2011). Although, low molecular weight PAHs of 3-rings and high molecular weight of 5-rings dominated the study area.

3.10. Polycyclic Aromatic Hydrocarbons (PAHs) in Vegetables

Fluted pumpkin (TelfairiaOccidentalis) and waterleaf (Talinum fruticosum) were the two vegetables used for this study. The Variations observed in concentrations of PAHs in the vegetable samples indicated that the vegetable samples harvested from the study area harbored a significant number of PAHs. Eight individual PAHs were detected in the vegetable samples. The highest individual PAH concentrations in the study area detected in vegetable samples were recorded for Benzo(b)Flouranthene at 0.97mg/kg. The decreasing order for individual PAHs concentration in the vegetable samples within the study area is Benzo(b)Flouranthene > Naphthalene > Indino(1,2,3cd)Pyrene > Phenanthrene > Anthracene > Acenaphthene > Flourenee > Fluoranthene. High values of total PAHs were generally recorded in the vegetable samples of Obowo zone; this may be as a result of input from the abattoir and spare parts market. The accumulation of PAHs by vegetables may be an indirect exposure pathway to humans. Most plants take up PAHs from the soil through their roots and the PAHs bioaccumulate in the plant roots and are trans-located to their storage organs which are usually consumed by man and other organisms (Adetunde et al., 2018).

3.11. Characteristics of Microbial Population

Hydrocarbon-degrading bacteria are ubiquitous in nature and can utilize hydrocarbons as sources of carbon and energy, thus are exploited for the remediation of hydrocarbon-contaminated environments. In this study, the total hydrocarbon-degrading bacteria mean values obtained from all the locations in both rainy and dry seasons varied. This is because levels of activities vary from one location to another with different levels of deposition, which confirms that different activities emit different concentrations of PAHs. So in assessing the usefulness of the Imo River watershed area for agricultural purposes, the availability of PAHs in the vegetables planted along the watershed is an indicator that Fluted pumpkin (TelfairiaOccidentalis) and waterleaf (Talinum fruticosum) accumulates PAHs.

3.12. Health Risk Assessment of PAHs Contamination

PAHs are pollutant of extreme health concern in the environment as it poses a direct threat to living organisms, including humans. The major exposure routes of PAHs to humans and other living organisms are through ingestion, dermal contact and inhalation. Research by Choi et al. (2010) shows that Carcinogenic PAHs are found in all surface soils. These PAHs are majorly known to be carcinogenic, teratogenic, genotoxic, cytotoxic and immunosuppressant (Ogoko, 2014) and cause both acute and chronic health effects in humans. Watershed contamination by PAHs may be due to the runoff of PAHs from various urban activities across the watershed into the water body, during which a significant amount of pollutant infiltrates into the soil, thereby contaminating plants and crops. This water body is often used by the dwellers in the study area for different purposes, including domestic purposes and this may pose health risks to them and the aquatic ecosystem.

Also, the PAH concentrations in the study area were compared with standards of 0.5mg/kg for individual PAHs and 10mg/kg for total PAHs stipulated by National Environmental Standards and Regulations Enforcement Agency (NESREA) and soil quality guidelines for the protection of the environment and human health to determine the level of toxicity of PAHs in the study area.

In this study, the mean concentrations of individual PAHs observed in soil samples were all lower than 0.5 mg/kg, except B(b)FLTHN, whose concentration was 8.64mg/kg, while those observed in vegetable samples were all lower than 0.5 mg/kg, except B(b)FLTHN and I(1,2,3-cd)PY whose concentrations were 2.711mg/kg and 0.540mg/kg respectively.

4. Conclusion

This study assessed the health implications of PAH bioaccumulation in two edible vegetables along Imo River watershed in Imo State and it was evident that the study area accommodates high levels of contaminants that exceeded the maximum permissible limits of 0.5mg/kg for individual PAHs recommended by National Environmental Standards and Regulations Enforcement Agency (NESREA). PAHs released from abattoirs and automobile repairs activities affect soil quality and the surrounding water body (Ekanem et al., 2019). The continuous use of such soil for agricultural purpose will lead to contaminated agricultural produces, which in turn can constitute a serious health risk, as well as contaminate the food chain. Of the 16 individual PAHs on the US EPA priority list that were analyzed, the individual concentrations of PAHs ranged from negligible to 3.1mg/kg. Higher values of total PAHs were generally recorded in the soil samples of Obowo zone, indicating pollution input from the abattoir and spare parts market. The high concentrations of the PAH components recorded in the soil samples can readily be linked to high levels of petrogenic (petroleum sources) and pyrogenic (combustion sources) activities going on in the study area, such as the indiscriminate abattoirs located in various locations within the watershed, as well as other automobile and industrial activities. This indicates that PAH contamination in the study area may have greatly risen from these activities.

Therefore, this study shows that abattoirs and automobile repair activities have the potential to emit huge amounts of Benzo(b)Fluoranthene to soil resources, which is a probable carcinogen to humans. It has been shown to cause lung, liver and skin cancer in animals.

5. References

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