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Concentration and Bioaccumulation of Toxic Metals and Polycyclic Aromatic Hydrocarbons in Soil and *Lumbricus Terrestris* in Kolo Creek, Niger Delta, Nigeria

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ABSTRACT

The concentration of toxic metals and polycyclic aromatic hydrocarbons (PAHs) in soil, and their bioaccumulation in *Lumbricus terrestris* in areas around Kolo Creek, Niger Delta, Nigeria were evaluated in this study. 12 soil samples were collected from 4 sites, while *Lumbricus terrestris* were also collected from areas where soil samples were obtained. Toxic metals were extracted using aqua regia and quantified with a flame atomic absorption spectrophotometer. Extraction of PAHs was achieved by liquid-liquid soxhlet extraction, and quantified with a gas chromatograph-mass spectrometer. The bioaccumulation factor (BAF) for both toxic metals and PAHs were calculated. The results obtained in this study showed that the concentration of all studied toxic metals were within their respective regulatory limits in soil. The BAF for all toxic metals were less than 1 in all sites, indicating minimal bioaccumulation in *Lumbricus terrestris*. The Σ16 PAHs ranged from 7.30 to 77.7 mg kg-1 and 1.10 to 4.75 mg kg-1 in soil and *Lumbricus terrestris* across the 4 sites, respectively. The BAF values for individual PAHs were less than 1 in all sites, which indicated that PAH congeners were either not bioaccumulated or accumulated PAH congeners were metabolized. Hence, analysis of PAHs metabolites in *Lumbricus terrestris* is recommended.

INTRODUCTION

Environmental pollution resulting from the negative impact of human activities, is currently a multidimensional global problem (Asonye *et al.*, 2007; Ogamba *et al.*, 2016). Toxic metals and polycyclic aromatic hydrocarbons (PAHs) are among several pollutants that accompany human activities.

Toxic metal is a collective term that applies to a group of metals and some metalloids with density greater than 5 g cm-3, and their toxicities in the environment are a devastating challenge (Koller and Saleh, 2018; Yahaya *et al.*, 2021). They are generally toxic to animal and human health especially if their naturally occurring concentrations are exceeded. The commonest anthropogenic sources of toxic metals are industrial, petroleum contamination and sewage disposal (Santos *et al.*, 2005). Biological processes cannot degrade toxic metals hence; these metals are environmentally persistent, and therefore possess the ability to accumulate in various tissues/organs of aquatic or terrestrial animals.

The PAHs are hydrophobic organic pollutants that are widely distributed in the environment. They are formed from the incomplete burning of fossil fuels or other organic materials such as tobacco and charbroiled meat (Blahova *et al.*, 2010). PAHs may also enter the environment through petrogenic (fuels, oil spill and road construction materials), pyrogenic (incomplete combustion processes), biogenic (produced by organic metabolism) and diagenetic (produced by the transformation process in sediment) (Hylland, 2006; Parra *et al.*, 2009). The presence of PAHs in the ecosystem is of concern specifically because of their toxicity and carcinogenicity (Davi *et al.*, 2016; Honda and Suzuki, 2020; Rengarajan *et al.*, 2015). Effects such as genotoxicity, developmental toxicity, oxidative stress, endocrine disruption and immunotoxicity, have also been reported (Cherr *et al.*, 2017; MacDonald *et al.*, 2013).

Living organisms are reported to be able to accumulate environmental contaminants to a very large extent, thereby making them useful bioindicators (Qari and Hassan, 2014; Al Meelebi, *et al.*, 2014). *Lumbricus terrestris* (earthworms) are permanently in close contact with soil particles, participates in nutrient cycle in terrestrial ecosystem and are significantly affected by pollutants that reach soil systems. Hence, they are well suited for the monitoring of soil contamination (Osioma and Hamilton-Amachree. 2019). Ma *et al.* (1995), reported that earthworm may accumulate fluranthene and to a lesser extent, phenanthrene, in their body tissues; while Malev *et al.* (2016) reported that earthworms can accumulate PAHs arising from their exposure to biochar-treated soils, and transfer them along the food chain.

Kolo Creek is a non-tidal fresh water which transverses through several communities including Otuasaga, Oruma, Imiringi, Kolo, Emeyal 2, etc. (Ineginte *et al.*, 2010). Although, Toxic metals and total petroleum hydrocarbons have been reported in and around Kolo Creek (Aghoghoviwia and Ayatari, 2012; Aghoghovwia and Chijoke, 2012; Osioma and Hamilton-Amachree, 2019), the distribution and bioaccumulation of toxic metals and PAHs in *Lumbricus terrestris* has not been investigated. Thus, this present research was aimed at (i) determining the toxic metals and PAHs content in soils and soft tissue of *Lumbricus terrestris* (ii) profiling the concentration of PAHs in soils and (iii) determining the bioaccumulation factors of toxic metals and PAHs in *Lumbricus terrestris* in areas around Kolo Creek, Niger

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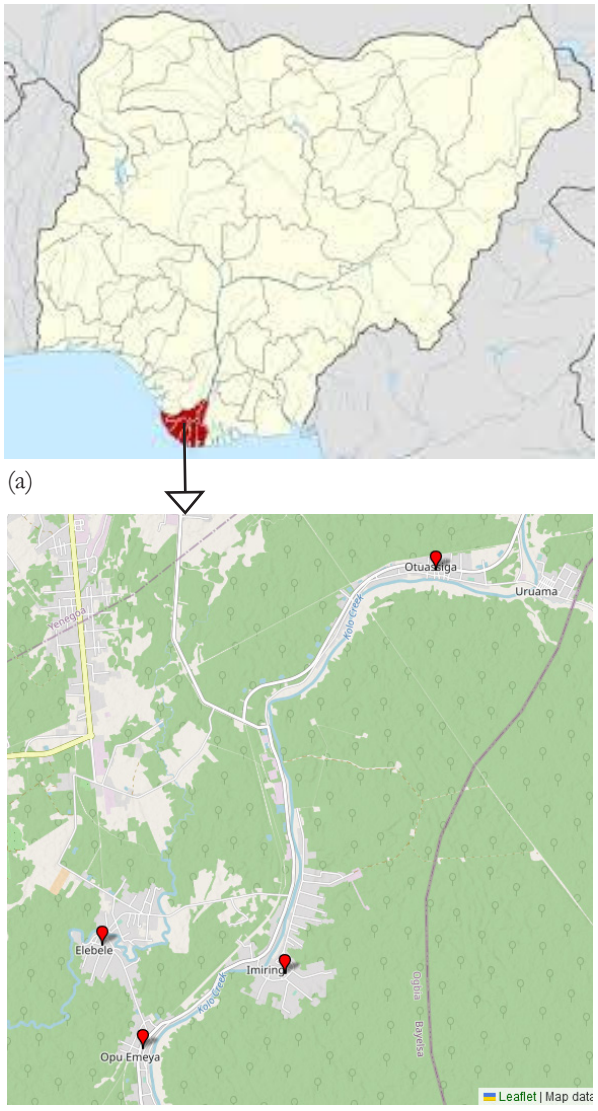
METHODOLOGY

Sampling Sites

Samples used for this study were collected from four different communities around Kolo Creek in Ogbia Local Government Area, Bayelsa State, and were labeled accordingly as A – Emeyal, B – Imiringi, C - Elebele, and D - Otuasega. A map of Nigeria showing Bayelsa State and that of the sampled sites in Ogbia Local Government Area, are shown in Figure 1.



(a)

(b)

Figure 1: Map of (a) Nigeria showing Bayelsa State (b) Study area indicating the sampled sites

Collection of *Lumbricus terrestris*

Samples of *Lumbricus terrestris* were collected from the 4 identified locations using spade and forceps to dig beneath the earth, then handpicked into a sterile plastic universal container and labeled accordingly. From each site, samples from 3 locations were obtained by pooling together 10 worms.

Collection of Soil Samples

Top-soill samples were obtained from the sites where the *Lumbricus terrestris* were collected into aluminium foils with a stainless steel soil auger, and labeled accordingly. In the laboratory, soil samples were-dried soil at ambient temperature, sieved using a 2 mm mesh, and ground to smaller particles using agate mortar and pestle, and refrigerated until extraction.

Extraction of Toxic Metals

Digestion of *Lumbricus terrestris* Samples

Exactly 2.00 g of *Lumbricus terrestris* was weighed into a conical flask and 20 mL of aqua regia and 10 mL of HClO4 were added, and a dish was used to cover the flask. The mixture was heated in a thermostat block at 150 ºC until the sample dissolved completely. Afer hating, the mixture was allowed to attain ambient temperature. A Whatman No. 4 filter paper was used for filtering the mixture in a 100 mL volumetric flask, and a 1M HNO3 was used to make up the mixture to mark (Csuros and Csuros, 2020). The filtrates were quantified for metals (Pb, Cu, Zn, Mn and Fe) using a Flame Atomic Absorption spectrophotometer (Thermo Jarrell Ash A.A. 12E).

Digestion of Soil Samples

Exactly 3.00 g of pulverized soil sample was weighed into a conical flask and 20 mL of aqua regia and 10 mL of HClO4 were added, and a dish was used to cover the flask. The mixture was heated in a thermostat block at 150 ºC until the volume of the solution was approximately 10 mL. This was left to attain ambient temperature. A Whatman No. 4 filter paper was used for filtering the mixture in a 100 mL volumetric flask, and a 1M HNO3 was used to make up the mixture to mark (Csuros and Csuros, 2020) The filtrates were quantified for metals (Pb, Cu, Zn, Mn and Fe) using a Flame Atomic Absorption spectrophotometer (Thermo Jarrell Ash A.A. 12E).

Soil Pollution Assessment Methods

Geoaccumulation Index (Igeo)

This is a common approach for estimating metals enrichment in soil above background concentrations, as proposed by Ntekim *et al.*, (1993). It assesses contamination by comparing current and pre-industrial metal levels. It was evaluated by calculating the base 2 logarithm of the metal concentration against its background level:
where:

$$Igeo = log_2 \frac{C_n}{1.5B_n} \qquad (1)$$

- Cn is the concentration of element (n) in the soil sample;
- Bn is the crustal abundance for element (n); and
- The factor, '1.5' is a correction factor that is normally introduced to reduce the effect of possible variations in the background value due to lithological variations. The Igeo consists of 7 classes described as follows: Igeo > 5 (class 6) – extremely contaminated; 4 - 5 (class 5) – strongly-to-moderately contaminated; 3 - 4 (class 4)

– strongly contaminated; 2 - 3 (class 3) – moderately-to-strongly contaminated; 1 - 2 (class 2) – moderately contaminated; 0 - 1 (class 1) – uncontamination-to-moderately contaminated; while, < 0 indicates uncontamination (Buccolieri *et al.*, 2006).

Contamination Factor

A contamination factor (Cf) quantifies the degree of contamination relative to measured values from geologically similar but uncontaminated areas (Tijani *et al.*, 2004). It was computed using the expression (Håkanson 1980):

Where Cs is the concentration of metal from the study

$$Cf = \frac{C_s}{C_n} \qquad (2)$$

site and Cn is the background level of metals (DPR, 2002). Values less < 1 indicate contamination range, while values > 1 indicate pollution range. The contamination factor is described by the following terminologies: Cf < 0.1 – very slightly contaminated; 0.10 – 0.25, slightly contaminated; 0.26 – 0.5, moderately contaminated; 0.51 – 0.75, severely contaminated; 0.76 – 1.00, very severely contaminated; 1.1 – 2.0, slightly polluted; 2.1 – 4.0, moderately polluted; 4.1 – 8.0, severely polluted, 8.1 – 16.0, very severely polluted; and > 16 excessively polluted (Lacatusu, 2000).

Pollution Load Index

The pollution load index (PLI) was used to evaluate the extent of metals contamination in the soils. It was evaluated for each site using the equations described by Tomlinson *et al.* (1980):

$$PLI = (CF1xCF2xCF3x.....CFn)^{1/n} \qquad (3)$$

Where:

n = number of metals and CF = contamination factor.

PLI value was classified into four groups (Wang *et al.*, 2010). PLI < 1 - no pollution, 1.0 < 2.0 - moderately polluted, 2.0 < 3.0 – heavily polluted, and ≥ 3.0 extremely polluted.

Extraction and quantification of polycyclic aromatic hydrocarbons (PAHs) in soil and *Lumbricus terrestris*

Extraction of PAHs was done according to the USEPA Method 3550C (US EPA, 2007). Exactly 10.00 g of well–mixed sample was measured. 30 mL of extraction solvent (n–hexane and methylene chloride, 3:1v/v) was then added to the sample and spiked with ortho –terphenyl,. This mixture was shaken in a vortex mixer for 1 - 5 minutes, and sonicated for about 5 - 10 minutes at 70 ºC. The extract was filtered through a glass wool, dried with anhydrous Na2SO4 and transferred to a Teflon–lined screw cap vial for analysis.

Extracts were quantified for PAHs using a gas chromatograph (6890 N Agilent technologies) coupled with a mass selective detector (Agilent 5975B). The GC (6890 N Agilent technologies) Coupled with a mass selective detector (Agilent 5975B) (GC–MS) fitted with an Agilent HP-5 − 60 to 325 °C GC column (30 m × 320 µm × 0.25 µm film thickness) was used to quantify PAHs in the sample extracts. The temperature programming of the GC was 280 °C and 300 °C, respectively for the injection and detection temperatures. The initial column temperature was set at 50 °C for 2 min, then increased to 150 °C at 20 °C/min, raised to 160 °C at 10 °C/min and finally to 300 °C at 5 °C/min for 15 min. The total runtime of the chromatographic separation was 32.25 min. Helium gas flowing steadily at a rate of 0.8 mL/min served as the carrier gas. A 1-µL sample was introduced into the GC–MS in splitless mode. The selected ion monitoring mode was used for data acquisition by Agilent Chemstation software.

Bioaccumulation Factor (BAF)

The BAF was evaluated as the concentration of contaminant in the soft tissues of *Lumbricus terrestris* using the method of Cortet *et al.* (1999) as indicated in the equations below:

$$BAF_{(Toxic\ metals)} = C_{HME} / C_{HMS} \qquad (4)$$

$$BAF_{(PAHs)} = C_{PAHE} / C_{PAHS} \qquad (5)$$

Where;

$BAF_{(Toxic\ metals)}$ = Bioaccumulation factor for toxic metals analysed; C_{HME} = Heavy metal concentration in *Lumbricus terrestris* (mg kg-1); C_{HMS} = Heavy metal concentration in soil (mg kg-1);

$BAF_{(PAH)}$ = Bioaccumulation factor for PAHs analysed; C_{PAHE} = PAH concentration in *Lumbricus terrestris* (mg kg-1); CPAHS = PAHs concentration in soil (mg kg-1).

Source Identification of PAHs

The diagnostic ratios of some PAHs isomers have been employed in identifying their potential sources. The diagnostic ratios of PAHs isomers used in this study and their interpretations in relation to the result of source evaluation of PAHs include the following ratios: phenanthrene/anthracene (values <10 indicates pyrolytic source while values >10 indicates petrogenic source), fluoranthene/pyrene (values <1 indicates petrogenic origin while values >1 indicates pyrolytic origin),and low molecular weight PAHs/high molecular weight PAHs (values <1 denotes combustion of fossil fuels or woods while values >1 denotes petrogenic source of PAHs) (Lawal, 2017).

RESULTS AND DISCUSSION

Concentration of Toxic Metals in Soil

The concentration of toxic metals studied in soils from all experimental sites are depicted in Table 1. Lead (Pb) content (2.37±0.01 mg kg-1) in site D was significantly higher ($p<0.05$) than that of the other studied locations. However, sites B and C had comparable ($p>0.05$) Pb concentrations. The average concentration of copper (Cu) increased ($p<0.05$) in the following order with respect to locations C < A < D < B. Site D showed the highest (22.98 ±0.03 mg/kg) concentration of Zn. Table 2 also indicated that the mean manganese (Mn) value of site A (78.71± 0.08 mg/kg) were statistically higher than

that of sites B, C and D. Significant ($p<0.05$) difference was observed in iron (Fe) concentration from all studied sites with location D having the highest Fe concentration while site A had the lowest.

Soil Pollution Assessment Methods

Geoaccumulation Index

Table 1: Toxic metals concentration in soil

Sites	Concentration (mg kg-1)				
	Pb	**Cu**	**Zn**	**Mn**	**Fe**
A	1.43±0.01a	5.28±0.02a	20.94±0.03a	78.71±0.08a	1650.44±0.12a
B	0.56±0.03b	23.40±0.01b	2.89±0.01b	53.36±0.29b	1875.96±0.76b
C	0.41±0.01b	0.87±0.01c	0.03±0.0001c	59.06±0.01c	2075.00±1.57c
D	2.37±0.01c	15.49±0.01d	22.98±0.03d	27.76±0.11d	2476.60±0.27d

Results are expressed as Mean±SD; n = 4.

Means that differ significantly at p < 0.05 do not share the same superscript alphabet in a given column

A = Emeyal ; B = Imiringi; C = Elebele; D = Otuasega

The geoaccumulation index of toxic metals in soils from all the studied sites is shown in Table 2. The results of the Igeo index for all studied metals across the experimental sites are less than zero (< 0).

Contamination Factor

Evaluating soil contamination was based on contamination factors, and presented in Table 3. Site C had the highest contaminated factor for Pb (0.16), Zn (0.30) and Fe (0.06) as compared with all sites. Results from Table 3 also indicated that site B had the highest contaminated factor for Cu and Site A for Mn.

Concentration of Toxic Metals in *Lumbricus terrestris*

The concentrations of toxic metals in *Lumbricus terrestris* from studied areas are shown in Table 4. Pb, Cu and Zn contents in site B were significantly elevated ($p < 0.05$) as compared with other experimental sites (A, C & D). The results also revealed that site A had the highest concentration of Mn while Fe content in *Lumbricus terrestris* from site D was statistically higher ($p < 0.05$) than the other three sites (A, B & C) under investigation.

Table 2: Geoaccumulation index of toxic metals in soil

Location	Geoaccumulation index				
	Pb	**Zn**	**Cu**	**Mn**	**Fe**
A	-3.86	-2.41	-3.82	-4.17	-5.22
B	-5.15	-5.28	-1.68	-4.74	-5.03
C	-5.71	-11.87	-6.41	-4.59	-4.88
D	-3.51	-2.29	-2.27	-5.67	-4.63

A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Table 3: Contamination factor of toxic metals in soil

Location	Geoaccumulation index				
	Pb	**Zn**	**Cu**	**Mn**	**Fe**
A	0.10	0.27	0.11	0.082	0.040
B	0.04	0.03	0.47	0.056	0.045
C	0.03	0.0004	0.02	0.062	0.050
D	0.16	0.30	0.31	0.029	0.060

A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Table 4: Concentration of toxic metals in Lumbricus terristris

Sites	Concentration (mg kg-1)				
	Pb	**Cu**	**Zn**	**Mn**	**Fe**
A	0.0026±0.0001a	0.43±0.008a	1.92±0.028a	9.82±0.009a	16.09±0.02a
B	0.08±0.001b	0.63±0.005b	2.38±0.013b	8.14±0.045ab	0.84±0.009b
C	0.0026±0.001a	0.15±0.008c	0.042±0.028c	0.042±0.0008c	0.67±0.008c
D	0.02±0.005c	0.38±0.008d	0.67±0.009d	7.35±0.041b	25.93±0.013d

Results are expressed as Mean±SD, n = 4. Means that differ significantly at p < 0.05 do not share the same superscript alphabet in a given column

A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Bioaccumulation Factor (BAF) And Pollution Index (PLI) Of Metals

The BAF and PLI of toxic metals from studied sites are indicated in Table 5. *Lumbricus terrestris* accumulated Pb and Mn more in sites B; Cu and Zn in site C and Fe in both site A and D. The pollution load index recorded for all sites were less than one (i.e., PLI < 1).

Table 5: Bioaccumulation factors and pollution load index of toxic metals

Toxic metals	A	B	C	D
Lead (Pb)	0.002	0.14	0.006	0.01
Copper (Cu)	0.08	0.03	0.20	0.03
Zinc (Zn)	0.10	0.82	1.40	0.03
Manganese(Mn)	0.13	0.15	0.001	0.3
Iron (Fe)	0.01	0.001	0.0003	0.01
Pollution Load Index (PLI)	0.10	0.07	0.014	0.12

A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Concentration and Distribution of PAHs in Soil from Experimental Sites

Table 6 shows the concentrations of 16 detected PAHs in soil samples from studied areas. The concentration of 2 – rings PAHs (Nap) was highest in site B; while site A had the highest concentration of 3 – rings PAHs. 4 and 5 –ring PAHs were found more in site A. The results also indicated that 6- rings PAHs had the highest concentration in site B.

The profile and ratios of PAHs in soil samples from experimental sites are depicted in Table 7. Higher percentages of LMW PAHs, i.e. Naphthalene,

Acenapthylene, Acenaphthene, Fluorene, Phenanthrene and Anthracene were present at site D. However, site A had higher proportion of medium molecular weight PAHs (MMW PAHs), i.e. Flouranthene, Pyrene, Benzo[a]anthracene and Chrysene. The results in Table 8 also indicated that the percentage of HMW PAHs, i.e. Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a] pyrene, Indeno[1,2,3-c,d]pyrene, Dibenzo[a,h]anthracene and Benzo[g,h,i]perylene was highest in site B. The results also revealed the decreasing order of the sum of 7 carcinogenic PAHs as follows: site B (53.28%) > site A (44.95%) > site C (26.71%) > site D (18.67%).

Results from Table 7 also indicated that the ratio between the LMW PAHs/HMW PAHs was highest in site D and lowest in site B. Site A had the highest Fluoranthene/ Pyrene ratio while site C had the highest ratio for Phenanthrene/Anthracene.

Concentration and bioaccumulation of PAHs in Lumbricus terresyris

Table 6: Concentration of PAHs in soil samples from experimental sites

PAHs	Number of rings	Concentration (mg kg-1)			
		A	B	C	D
Naphthalene	2	1.00	2.05	0.35	0.25
Acenaphthylene	3	1.95	3.05	0.70	0.95
Acenaphthene	3	17.1	0.10	2.00	2.25
Fluorene	3	2.20	0.35	0.35	0.65
Phenanthrene	3	1.10	0.20	0.50	0.40
Anthracene	3	1.20	0.15	0.35	0.45
Fluoranthene	4	16.2	1.10	0.40	0.50
Pyrene	4	1.50	2.05	0.45	0.35
Chrysene	4	5.40	1.65	0.10	0.05
Benzo[a]anthracene	4	0.35	0.45	0.05	0.05
Benzo[b]fluoranthene	5	1.10	0.10	0.35	0.20
Benzo[k]fluoranthene	5	25.7	1.70	0.40	0.20
Benzo[a]pyrene	5	1.40	3.70	0.55	0.65
Indeno[1,2,3-c,d]pyrene	6	0.70	4.55	0.15	0.15
Dibenzo[a,h]anthracene	5	0.30	1.25	0.35	0.10
Benzo[g,h,i]perylene	6	0.55	2.70	0.25	0.30
Σ16 PAHs		77.7	25.2	7.30	7.50
Σ 2- rings		1.00	2.05	0.35	0.25
Σ 3- rings		23.5	3.85	3.90	4.70
Σ 4- rings		23.5	5.25	1.00	0.95
Σ 5- rings		28.5	6.75	1.65	1.15
Σ 6 – rings		1.25	7.25	0.40	0.45

A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Table 7: Profile and ratios of concentrations of PAHs in soil samples from experimental sites

	A	B	C	D
LMW PAHs	24.55 (31.58%)	5.90 (23.46%)	4.25 (58.22 %)	4.95 (66%)
MMW PAHs	23.45 (30.16%)	5.25 (20.87%)	1.00 (13.70%)	0.95 (12.67%)
HMW PAHs	29.75 (38.26%)	14.00 (55.67%)	2.05 (28.08%)	1.60 (21.33%)
Σ 7cPAHs	34.95 (44.95%)	13.4(53.28%)	1.95 (26.71%)	1.4 (18.67%)
Σ PAHs	77.75	25.15	7.30	7.5
RATIOS				
LMW PAHs / HMW PAHs	0.83	0.42	2.07	3.09
Fluoranthene/ Pyrene	10.8	0.54	0.89	1.43
Phenanthrene /Anthracene	0.92	1.33	1.43	0.89

A = Emeyal; B = Imiringi; A = Elebele; C = Otuasega; cPAHs = Carcinogenic PAHs

Concentrations of polycyclic aromatic hydrocarbon in *Lumbricus terrestris* from experimental sites is presented in Table 8. Earthworm from site D had the highest concentration of 2-rings and 6 – rings PAHs. *Lumbricus terrestris* from sites A, B and C did not accumulate 2 –ring PAHs. Results also showed that 3 – rings and 5 –rings PAHs were present in high concentrations in earthworm from site A. The highest concentration of 4 – rings PAHs was seen in *Lumbricus terrestris* from site B.

Bioaccumulation of PAHs by *Lumbricus terrestris* from experimental sites is shown in Table 9. From the results, accumulation of individual PAHs by *Lumbricus terrestris* were less than or equal to unity (i.e., ≤ 1).

DISCUSSIONS

Table 8: Concentration of PAHs in *Lumbricus terrestris* from experimental sites

PAHs	Number of rings	Concentration (mg/kg)			
		A	B	C	D
Naphthalene	2	<0.001	<0.001	<0.001	0.25
Acenaphthylene	3	<0.001	<0.001	<0.001	0.45
Acenaphthene	3	1.00	0.10	0.30	0.25
Fluorene	3	0.85	0.05	0.05	0.60
Phenanthrene	3	0.10	<0.001	0.10	0.40
Anthracene	3	0.50	<0.001	0.10	0.45
Fluoranthene	4	0.20	0.05	0.20	0.05
Pyrene	4	<0.001	0.05	0.15	0.35
Chrysene	4	0.15	0.25	<0.001	0.05
Benzo[a]anthracene	4	0.15	0.30	<0.001	0.05
Benzo[b]fluoranthene	5	0.70	<0.001	0.05	0.20
Benzo[k]fluoranthene	5	0.65	0.15	<0.001	0.20
Benzo[a]pyrene	5	0.10	0.05	<0.001	0.65
Indeno[1,2,3-c,d]pyrene	6	0.05	0.05	<0.001	0.15
Dibenzo[a,h]anthracene	5	0.05	0.20	0.15	0.10
Benzo[g,h,i]perylene	6	0.25	<0.001	<0.001	0.30
Σ16 PAHs		4.75	1.25	1.10	4.50
Σ 2- rings		0.00	0.00	0.00	0.25
Σ 3- rings		2.45	0.15	0.55	2.15
Σ 4- rings		0.50	0.65	0.35	0.50
Σ 5- rings		1.50	0.40	0.20	1.15
Σ 6 – rings		0.30	0.05	0.00	0.45

A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Table 9: Bioaccumulation factors of PAHs

PAHs	Number of rings	Concentration (mg/kg)			
		A	B	C	D
Naphthalene	2	-	-	-	1.00
Acenaphthylene	3	-	-	-	0.47
Acenaphthene	3	0.06	1.00	0.15	1.00
Fluorene	3	0.39	0.14	0.14	0.92
Phenanthrene	3	0.09	-	0.20	1.00
Anthracene	3	0.42	-	0.29	1.00
Fluoranthene	4	0.012	0.05	0.50	0.1
Pyrene	4	-	0.02	0.33	1.00
Chrysene	4	0.027	0.15	-	1.00
Benzo[a]anthracene	4	0.43	0.67	-	1.00
Benzo[b]fluoranthene	5	0.64	-	0.143	1.00
Benzo[k]fluoranthene	5	0.025	0.09	-	1.00
Benzo[a]pyrene	5	0.071	0.014	-	1.00
Indeno[1,2,3-c,d]pyrene	6	0.071	0.01	-	1.00
Dibenzo[a,h]anthracene	5	0.17	0.16	0.143	1.00
Benzo[g,h,i]perylene	6	0.46	-	-	1.00
Σ PAHs		2.866	2.304	1.896	14.50
Σ 2- rings		-	-	-	1.00
Σ 3- rings		0.96	1.14	0.78	5.39
Σ 4- rings		0.469	0.89	0.83	3.10
Σ 5- rings		0.906	0.264	0.286	4.00
Σ 6 – rings		0.31	0.01	-	2.00

A = Emeyal; B = Imiringi; C = Elebele; D = Otuasega

Polluted soil is a worldwide concern due to its likely impact on the rapid growth of crops, the environment and human health (Zhoa *et al.*, 2014; Emurotu and Onianwa, 2017). The mean concentrations of toxic metals obtained and as depicted in Table 1 indicated that Pb, Zn and Fe were more abundant in Otuasega (site D); while Cu was predominantly higher in Imiringi and Mn at Emeyal. These heavy metal concentrations in the studied areas were below the NESREA (2009) regulatory limits and hence, may not be regarded as polluted by

toxic metals. However, Fe, appears to be relatively more abundant in soil of Kolo Creek. Inengite *et al.* (2010) reported exceptionally high concentrations of Fe in Kolo Creek and classified the Creek as very polluted in terms of Fe. The high Fe content in soils from the experimental sites is responsible for the brownish coloration observed in groundwater from the study area.

The estimated metals enrichment in soil above background concentrations as indicated by the geoaccumulation index values obtained for all experimental sites weare less than zero (< 0) which means uncontamination. The contamination factor for sites A and D indicated very slight contamination for Pb, while slight contamination for Zn and Cu. Site B (Imiringi) had moderate contamination for Cu. The values obtained for the pollution load index (PLI) for all experimental sites (A, B,C & D) are less than one (< 1) which indicates no pollution.

The fact that toxic metals are biologically non-degradable and possess bioaccumulation tendencies in the environment makes them deleterious to animals inhabiting such environments, and they may remain a potential threat for many years (Isirimah, 2000). The concentration of toxic metals in *Lumbricus terrestris* from our results indicated that Pb, Cu, and Zn were elevated ($p < 0.05$) in soft tissues of earthworm from site B (Imiringi) as compared with other experimental sites. This may be connected with long years of petroleum exploration activities in the Kolo Creek environment since the introduction of toxic metals into the environment has been reported to be related to oil and gas pollution (Essoka *et al.*, 2006; Chinedu and Chukwumemeka, 2018; Nnamdi *et al* 2021). The bioaccumulation factor of toxic metals in all experimental sites is less than a unity (< 1). Earthworms inhabiting these areas may have not accumulated toxic metals because the environment is very slightly contaminated according to results obtained for contamination factor. Similar low bioaccumulation of metals by *Lumbricus terrestris* has been previously reported for dumpsite soils (Uba *et al.*, 2009; Agbaire, 2012; Latifi *et al.*, 2020).

Like toxic metals, PAHs are widely distributed contaminants, which have drawn great concerns due to their well-established toxicities and bioaccumulative tendencies in animals (Meador *et al.*, 1995). From the results of this study, 2- rings and 6- rings PAHs are predominant in Imiringi (site B) while 3- to 5 – rings PAHs are dominant at site A (Emeyal). The profiling of PAHs indicated that LMW PAHs were found more at site D (Otuasega, 66%), followed by site C (58.22%), site A (31.58%) and site B (23.46%). The range of HMW PAHs in the experimental sites are as follows: site B > site A > site C > site D. Increased concentration of LMW PAHs indicated that the environment could be contaminated with naturally occurring petrogenic PAHs while the presence of HMW PAHs suggests pyrolytic sources (Helfrich and Armstrong, 1986).

The ratios of LMW PAHs/HMW PAHs are greater than one (> 1) at sites C and D. This suggests petrogenic and petroleum-based compounds as the major sources of PAHs (Yap *et al.*, 2012). Phenanthrene to anthracene ratios (Phe/Ant) in all sites (A, B, C & D) are less than 10 (< 10) indicating that the presence of PAHs could be as a result of combustion process. However, Fluoranthene to pyrene ratio (Flt / Pyr) were less than I (< 1) in sites (B, C & D) which suggests PAHs from petrogenic sources but site A had Flt / Pyr value of 10.8 (> 1) implying or indicating PAHs from pyrolytic origins. From the above results, it could be concluded that PAHs from the experimental areas may originate from both pyrolytic and petrogenic sources.

The percentage range of carcinogenic PAHs (Table 7) are as follows: site B (53.28%) > site A (44.95%) > site C (26.71%) > site D (18.67%). Several environmental risk associated with PAHs are related to the carcinogenic character expressed by them. The carcinogenic PAHs are transported into cells because of their hydrophobicity and induce gene expression of cytochrome P450 (CYP450) enzyme group (28 -31 PAH E4). The numerous intermediates formed in this metabolic pathway can bind to DNA and become mutagenic / carcinogenic.

The exposure of *Lumbricus terrestris* to PAHs can be evaluated by determining the PAHs concentrations in their tissue. Earthworm has been reported to accumulate PAHs (Malev *et al* 2015, Ma *et al* 1995). The concentration of 2 – and 6 – rings were highest in earthworm from site D; while 3 – and 5 –rings in earthworm from site A and 4 – ring PAHs in *Lumbricus terrestris* from site B. Values for bioaccumulation factor were less than or equal to 1 (≤ 1) in earthworm from all the experimental sites. This indicates that either earthworm did not accumulate PAHs or accumulated PAHs are metabolized

CONCLUSION

The concentrations of toxic metals (Pb, Cu, Zn and Mn) in soil samples around Kolo Creek were below specified limits. However, Fe concentration appears to be very high in the experimental areas. Contamination factor indicated very slight contamination for Pb (Emeyal and Otuasega), while the PLI indicates no pollution for all sites under investigation. Although, there is the presence of toxic metals in soft tissue of Lumbricus terretris but such were not accumulated to any significant extent..

The study also showed that LMW PAHs were predominant at Otuasega (site D) while higher percentage of HMW PAHs are found in Imiringi (site B). Based on the following isomeric ratios: LMW PAHs/ HMW PAHs, Phe/Ant and Flt/ Pyr; the origin of PAHs could be both pyrolytic and petrogenic sources. Carcinogenic PAHs were highest at site B (Imiringi), which could pose environmental risk. Again, on bioaccumulation of PAHs in soft tissues of earthworm, it was observed that either PAHs were not accumulated or accumulated PAHs were metabolized. Hence, it is recommended that for future study of this nature, PAH metabolites should be analysed.

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