

## Soil Invertebrates as Bio-Monitors of Toxic Metals Pollution in Impacted Soils

IMAOBONG I. UDOUSORO\*, I. U. UMOREN, J. M. IZUAGIE,  
C. U. IKPO, S. F. NGERI and E. S. SHAIBU

Chemistry Department, University of Uyo, Uyo, P. M. B 1017 Uyo,  
Postal code: 520001, Akwa Ibom State, Nigeria.

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### ABSTRACT

The bio-indicator potentials of two invertebrate species, *Eudrilus eugeniae* (earthworm) and *Pachybolus ligulatus* (millipede) in toxic metals impacted soils in Akwa Ibom State, in the Niger Delta region of Nigeria were assessed. The study involved collection of *Eudrilus eugeniae* and *Pachybolus ligulatus*, and their site soil samples from different impacted and non-impacted soils in ten locations spread over four Local Government Areas. Toxic metals (Pb, Fe, Zn, Ni and Cd) in soils and in the bio-indicator organisms were analysed using Atomic Absorption Spectrometry, after acid digestion. Geo-accumulation Index (Igeo) was used to measure the extent of soil pollution; Bio-Accumulation Factor (BAF) to compute the metal uptake from soil by bio-indicators; and multivariate statistics to identify interrelationships among metals and the pollution sources. Metals in all soils were higher than the background levels, and were classified as unpolluted to very strongly polluted. Pb, Fe, Zn and Cd were higher in *Eudrilus eugeniae*, and Ni in *Pachybolus ligulatus* at 0.05 level. The organisms absorbed all metals (BAF<1), but *Eudrilus eugeniae* accumulated Cd and Fe and *Pachybolus ligulatus*, Ni and Fe (BAF>1). Metals were clustered into three (*Eudrilus eugeniae*) and two (*Pachybolus ligulatus*) dissimilar groups. Two principal components extracted, each for *Eudrilus eugeniae* and *Pachybolus ligulatus* accounted for 64% and 72% of total variations, respectively. The invertebrates exhibited varying levels of affinity in metals uptake. *Eudrilus eugeniae* appeared to accumulate Pb, Fe and Cd more, and could serve as a better bio-indicator for monitoring these metals. *Pachybolus ligulatus* accumulated 3-folds more Ni, and could provide a monitoring tool especially in agriculture, in the Niger Delta Region of Nigeria, plagued by crude oil pollution of its land and water bodies.

**Key words:** Soil bio-indicators, Pollution, Multivariate analysis, Atomic Spectroscopy.

### INTRODUCTION

From the advent of Agricultural to the Industrial revolution, the scale of natural resource exploitation has progressively exerted pressure on the environment, and the signs of 'weakness' have become very obvious. Consequently, 'sustainable development'<sup>1</sup>, a global concept and panacea, is now in the front burner of all development issues. The concept recognizes the fact that human health and life are inextricably linked to a robust and healthy ecosystem-the soil being an important component. Measurement of the different soil properties or processes provides quality index-indicators that can

be used to estimate soil quality; these include visual, physical, chemical and biological processes (micro and macro-organisms, their activities or functions)<sup>2-4</sup>.

Terrestrial arthropods (e.g. the diplopods like millipedes) and oligochaetes (e.g. earthworms) are ubiquitous and have great diversity of species. These invertebrates which dwell in the various soil layers and are in contact with the soil and/or litters<sup>5</sup>, invariably, are affected by deposition of toxic chemicals like metals, organic compounds and other complex substances. Heavy metals have been found to cause reduction in the abundance

of soil decomposers, and in the diversity of animal communities<sup>6-7</sup>.

Heavy metals deposition in terrestrial invertebrates is reported to depend on the extent of environmental pollution<sup>5,8-12</sup>. Consequently, researchers have used various species of invertebrates including earthworms and millipedes to evaluate contaminated and uncontaminated soils<sup>9,13-14</sup>. Millipedes, which belong to the class of diplopods, are particularly abundant in the tropics; they are herbivores and predominantly nocturnal. Their importance in recycling of nutrients, aeration and fertilization of soil has been well documented<sup>14-19</sup>.

The use of terrestrial invertebrates as bio-indicators of soil pollution has however, focused mainly on the toxic metals aspect<sup>9,13,18-20</sup>. Hopkin<sup>21</sup> and Morgan and Morgan<sup>22</sup> reported a higher uptake of Cu, Zn and Cd by these organisms from contaminated relative to uncontaminated soils; also, Olayinka *et al*<sup>2</sup> recorded higher Pb, Cu, Mn, Zn, Cd and Co around a cement factory compared with the control site. Earthworms from dump site areas in Nigeria have been shown to bio-accumulate high levels of toxic metals<sup>11,20,13</sup>.

Studies have also been conducted on cytotoxic effects of metals<sup>24</sup>; the detoxification mechanism<sup>25</sup>; trait-based approach to depict disturbance impact on soil invertebrate communities<sup>26-28</sup> and ecological remediation<sup>18,29-31</sup>. The histological and histochemical analyses as well as ultrastructural analysis, revealed that chemical substances (both organic and inorganic) are equally toxic to soil invertebrates since the mineralized bodies varied in structural organization within the individual midgut and fat body cells<sup>12,14,17</sup>.

Most authors have used the earthworms to determine soil health, but information on use of millipedes is scarce; and little or no information is available here in Nigeria despite the abundance of different species of millipedes. The aim of the present study was to compare patterns of metals accumulation in these two invertebrates-earthworms (*Eudrilus eugeniae*) and millipedes (*Pachybolus ligulatus*), establish which is a better indicator of soil metal pollution, determine influence of location

on bio-accumulation of the toxic metals by indicator organisms, and subject the database to factor analysis to determine the possible source(s) of pollution; also, to establish the similarities and dissimilarities among locations and metals.

## MATERIALS AND METHODS

### Sample collection and preparation of earthworms (*Eudrilus eugeniae*), millipedes (*Pachybolus ligulatus*) and soil

Soil sample from ten locations in four Local Government Areas (LGAs) in Akwa Ibom State of Nigeria were used for this study. The LGAs were Etinan (around a paint factory, locations 2 and 5), Uyo (the busy state capital, locations 7 [ravine], 6 and 10 [auto-mechanic workshops], 8 [high traffic road] and 9 [agricultural land]), Uruan (an old sea port city, locations 1 and 4) and Ibesikpo (a rural area as control, location 3) (Figure 1).

Earthworms (*Eudrilus eugeniae*) were manually harvested (hand sorted) from soil cores of 100 cm x 100 cm dug to depth of 15 cm with a spade. Each location consisted of four composites in order to obtain enough quantity of *Eudrilus eugeniae* for analysis. Millipedes (*Pachybolus ligulatus*) were picked between the leaf litters and surface soil. *Eudrilus eugeniae* and *Pachybolus ligulatus* (Figure 2A & B) were thoroughly rinsed with de-ionised water and each composite stored in different transparent rubber bowls lined with wet Whatman No. 1 filter paper, and covered with perforated lid.

On reaching the laboratory, *Eudrilus eugeniae* (soft-bodied) and *Pachybolus ligulatus* (hard-bodied) samples were kept in the stored condition for 24 hours, and at laboratory temperature (27°C-30°C) to allow for evacuation of gut content; then oven-dried at 105°C to constant weight. The corresponding soil samples were air-dried at ambient temperature. The dried samples were crushed to fine powder using mortar and pestle, and passed through 0.5 mm sieve.

### Determination of metals in *Eudrilus eugeniae* and *Pachybolus ligulatus*

To 500 mg each of the dried and sieved samples of *Eudrilus eugeniae* and *Pachybolus ligulatus*, 5 ml concentrated HNO<sub>3</sub> (analar grade

Aldrich) was added and kept overnight in Teflon beakers; then heated on a hot plate to near dryness. After cooling, the solution was made up to 25 ml with distilled de-ionised water. Blank solutions were also prepared (without the samples). Metal content was determined using UNICAM 939/959 Atomic Absorption Spectrometer (AAS) for Pb, Zn, Fe, Ni and Cd.

#### Determination of metals in soils

Five hundred milligrammes of the soil samples were digested with mixture of 15 ml concentrated  $\text{HNO}_3$ :5 ml concentrated HCl mixture in a Teflon beaker. After cooling, the solution was made up to 50 ml with distilled-deionized water. Blank solutions were also prepared and the metal content determined as described above. Soil pH and organic matter were determined using methods by IITA<sup>32</sup>.

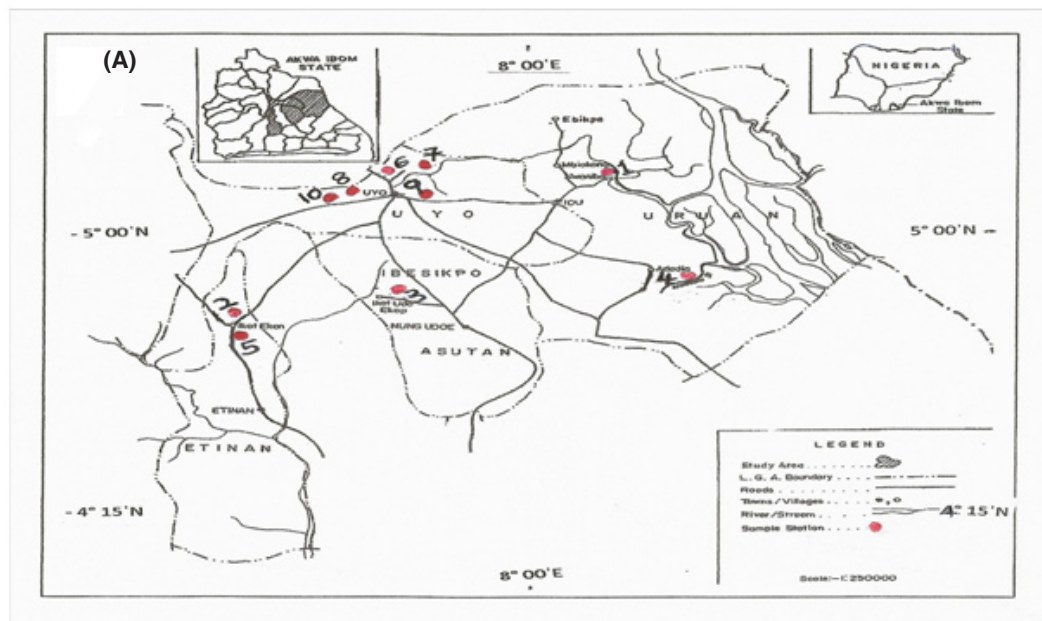


Fig. 1: Sample Locations (A) Study Area (B) Auto-repair workshop

**Geological index of soil pollution (I<sub>geo</sub>)**

This was calculated using geo accumulation index (I<sub>geo</sub>) given by

$$I_{geo} = \frac{\log_2 C_n}{1.5 B_n}$$

Where C<sub>n</sub> = concentration of metal 'n' in soil; B<sub>n</sub> = background concentration of the metal 'n'<sup>33-34</sup>; 1.5 = correction factor to take care of possible variations in background values for a given environment as well as small anthropogenic influence.

I<sub>geo</sub> was divided into seven classes as by Müller<sup>35</sup> as follows: I<sub>geo</sub> ≤ 0 (Class 0) - unpolluted; 0 < I<sub>geo</sub> ≤ 1 (Class 1) - unpolluted to moderately polluted; 1 < I<sub>geo</sub> ≤ 2 (Class 2) - moderately polluted; 2 < I<sub>geo</sub> ≤ 3 (Class 3) - moderately polluted to strongly polluted; 3 < I<sub>geo</sub> ≤ 4 (Class 4) - strongly

polluted; 4 < I<sub>geo</sub> ≤ 5 (Class 5) - strongly polluted to extremely polluted; and I<sub>geo</sub> > 5 (Class 6) - extremely polluted.

**Bio-Accumulation Factor (BAF)**

The Bio-Accumulation Factor (BAF) in earthworms and millipedes was calculated using the formula<sup>36</sup>

$$BAF = \frac{\text{Metal content in Eudrilus eugeniae or Pachybolus ligulatus}}{\text{Total metal content in soil}}$$

**Statistical and data analysis**

Statistical analyses were performed using SPSS Version 10, Statgraphics VII and Microsoft Excel. ANOVA was used to evaluate the effect of sample location and LGA on metal content of *Eudrilus eugeniae* and *Pachybolus ligulatus*. Factor analysis was performed on the data sets. Principal component analysis (PCA) was carried out using Varimax rotation with Kaiser normalization to determine pollution sources; factor loadings on

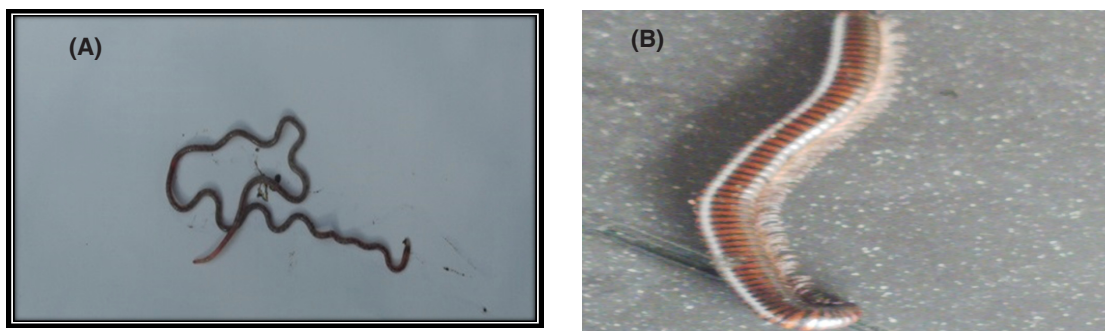


Fig. 2: Test organisms: (A) *Eudrilus eugeniae*, (B) *Pachybolus ligulatus*

Table 1: Summary statistics of metals levels (mg/kg) in soil

	Pb	Fe	Zn	Ni	Cd
Mean	505.58	22260.30	282.43	130.32	12.12
Standard Deviation	168.92	31801.00	83.64	173.25	11.53
Maximum	735.70	109952.00	392.76	512.06	29.00
Minimum	259.10	164.00	160.06	8.19	0.001
Median	480.06	13775.50	288.76	52.20	8.35
Coefficient of Variation	0.33	1.43	0.30	1.33	0.95
Background Concentration*	20.00	46.00	71.00	20.00	0.098
Control location (this study)	385.78	7304	161.75	17.28	0.001

\*Taylor and McLennan (1995), Lo and Fung (1992)

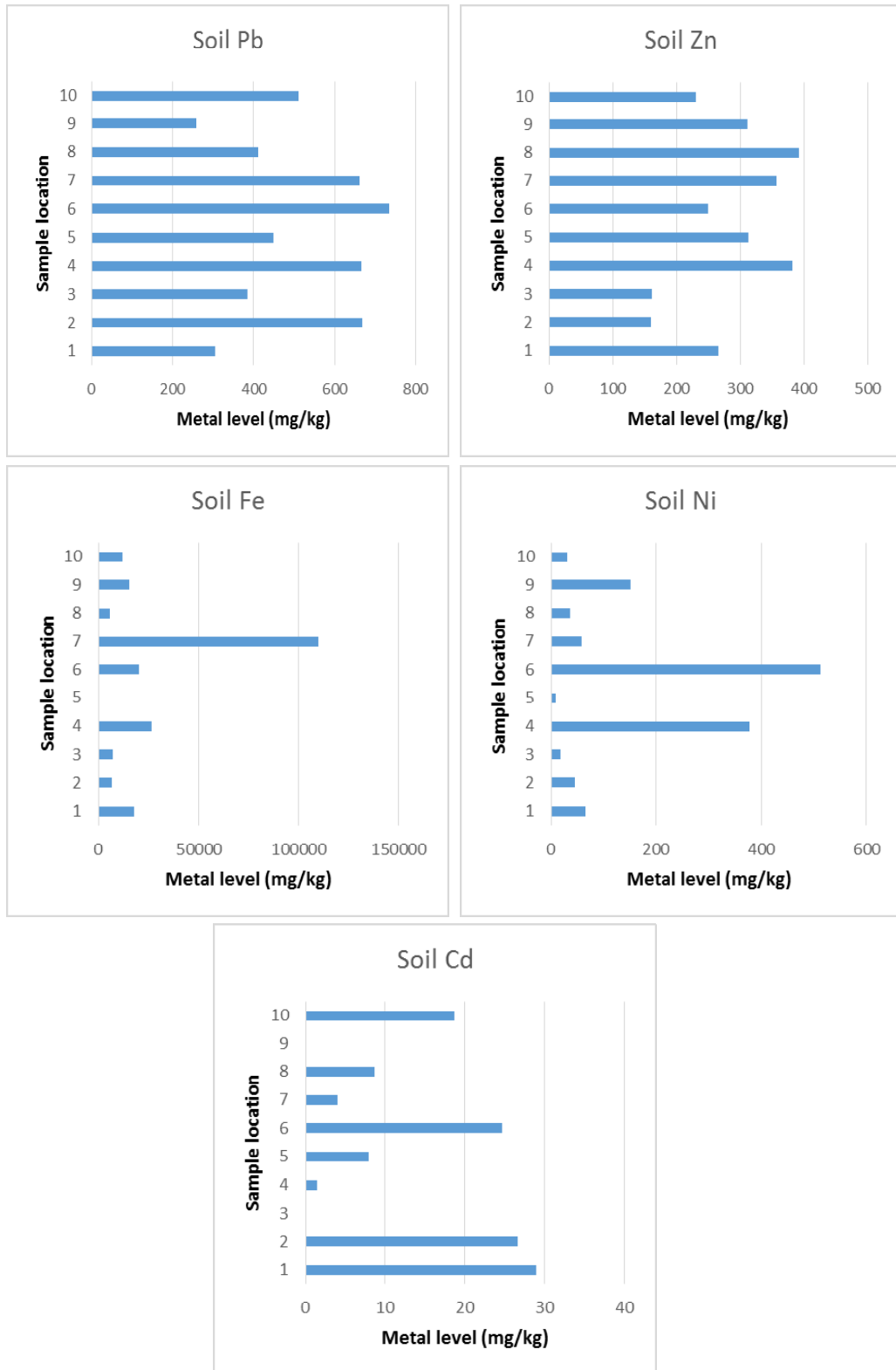


Fig. 3: Toxic metals in soils from different sample locations



principal components were classified as strong (>0.75), moderate (0.75-0.50) and weak (0.50-0.30)37. Cluster analysis (CA) performed on the normalized data sets by means of the Ward's method, using Squared Euclidian distance, was used as a measure of similarity and dissimilarity in patterns of metal accumulation in organisms and soil, and resulted in the dendrogram plots. The analytical quality control measures were the procedural blank

measurements, triplicate analysis of indicator organisms and soil samples, and standardization of analytical instruments.

## RESULTS

Two types of terrestrial invertebrates namely the soft-bodied earthworms (*Eudrilus eugeniae*) and hard-bodied amber-banded millipedes (*Pachybolus ligulatus*), and their corresponding soils were studied in order to determine the suitability of the organisms as bio-indicators of soil pollution in Akwa Ibom State. The results revealed more accumulation of Pb, Fe, Zn and Cd in *Eudrilus Eugenia*, and Ni in *Pachybolus ligulatus*.

**Table 2: Pollution index ( $i_{geo}$ ) of soils from individual locations and local government areas**

	Pb	Fe	Zn	Ni	Cd
<b>Locations</b>					
1	2.32	5.56	0.92	0.77	5.28
2	3.10	4.60	0.41	0.41	5.20
3	2.55	4.66	0.42	-0.55	-4.99
4	3.10	5.96	1.28	2.53	2.33
5	2.71	3.17	1.08	-1.30	2.06
6	3.20	5.68	0.85	2.84	5.12
7	3.09	7.37	1.21	0.68	3.29
8	2.62	4.42	1.30	0.20	4.08
9	2.16	5.40	1.07	1.62	-4.99
10	2.84	5.18	0.77	0.01	4.85
<b>LGA</b>					
Uruan	2.72	5.05	0.61	0.35	4.84
Etinan	3.10	5.96	1.28	2.53	2.33
Ibesikpo	2.71	3.09	1.08	-1.30	4.00
Uyo	2.85	6.16	1.06	1.66	4.33

### Metals content in soil at individual locations and Local Government Areas (LGAs)

The mean soil metal levels of Pb, Fe, Zn, Ni and Cd in the ten sample locations are presented in Figures 3 and 4, and Table 1; and were in the order Fe>Pb>Zn>Ni>Cd. Analysis of soil samples indicated elevated levels of metals in Uyo (a metropolitan city and State capital with the highest vehicular traffic), the mechanic workshop soil in Uyo and the old sea port city of Uruan. Very high levels (mg/kg) of Pb (735.70), Cd (24.62) and Ni (512.14) in soil were found at location 6 (mechanic village in Uyo). High Pb, Zn and Ni were also obtained around the paint factory (locations 2, 5) and Pb and Cd in location 1 (old sea port) (Figure 3). The highest and lowest mean metal values (mg/kg) in the LGAs were 665.71 (Etinan LGA) and 448.78 (Ibesikpo LGA) for

**Table 3: Locations in each pollution class**

Metals	Pollution Class						
	0	1	2	3	4	5	6
Pb				Locations 1, 3, 5, 8, 9, 10	Locations 2, 4, 6, 7.		
Fe					Location 5.	Locations 2, 3, 8.	Locations 1, 4, 6, 7, 9, 10.
Zn		Locations 1, 2, 3, 6, 10.	Locations 4, 5, 7, 8, 9.				
Ni	Locations 3, 5.	Locations 1, 2, 7, 8, 10.	Locations 9.	Locations 4, 6.			
Cd	Locations 3, 9.			Locations 4, 5.	Locations 7.	Locations 8, 10.	Locations 1, 2, 6.

Pb; 32711 (Uyo LGA) and 164.00 (Ibesikpo LGA) for Fe; 381.82 (Etinan LGA) and 196.04 (Uruan) for Zn; 377.10 (Etinan LGA) and 8.19 (Ibesikpo LGA) for Ni; and 18.55 (Uruan LGA) and 1.51 (Etinan LGA) for Cd

(Figure 4). Iron (Fe), the most abundant metal had levels in the order: Ibesikpo LGA (control)<Uruan LGA<Etinan LGA<Uyo LGA.

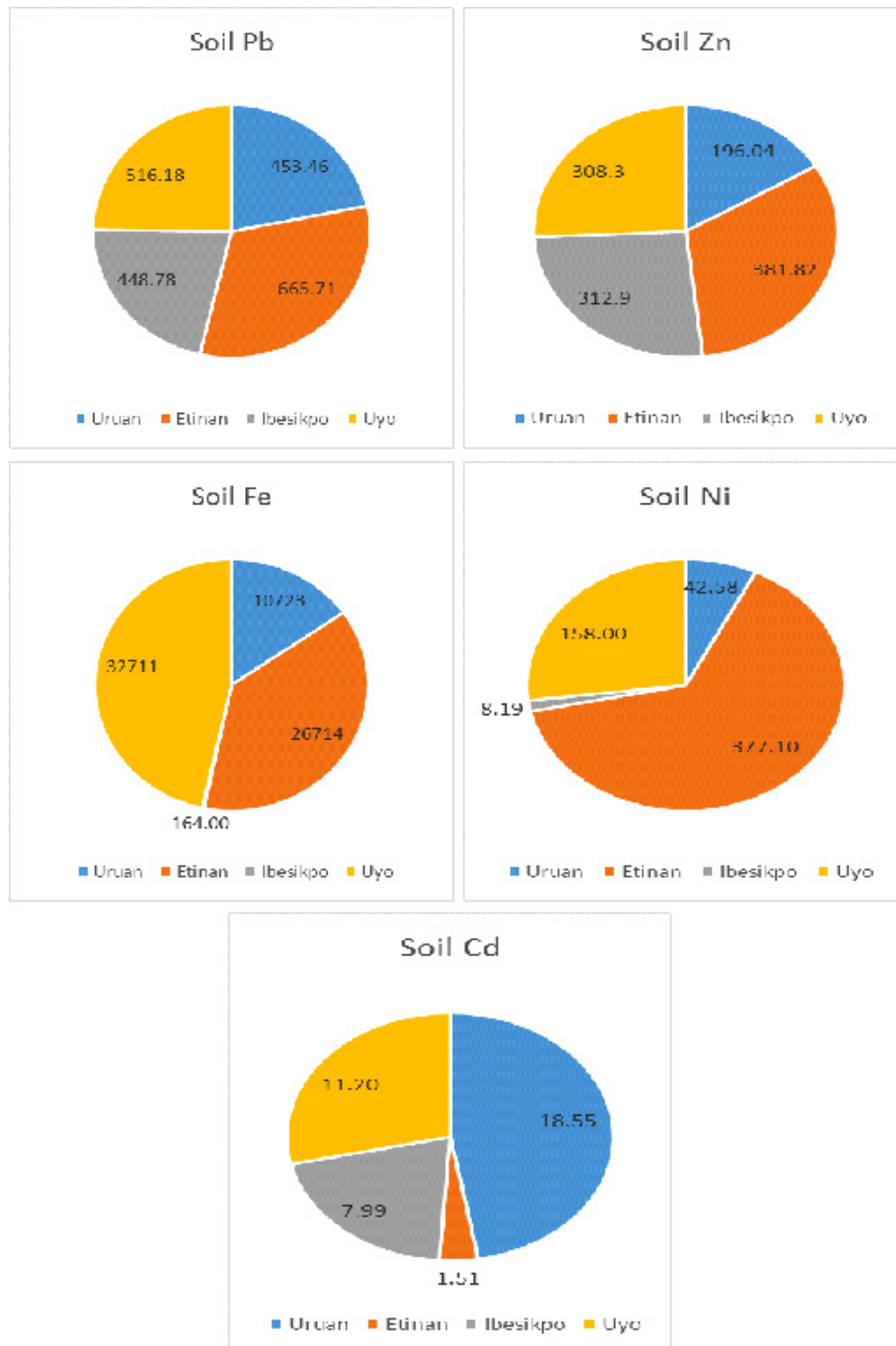


Fig. 4: Toxic metals in soils from different Local Government Areas

**Geological index of soil pollution (Igeo)**

The soil pollution index at each location and LGA are presented in Table 2. The level of soil metal pollution varied from unpolluted to extremely polluted. Table 3 shows the individual locations in the soil pollution classes. Lead (Pb) pollution in soil ranged from class 3 (moderately to strongly polluted) representing 60% of locations-1, 3, 5, 8, 9, 10 and Uruan, Ibesikpo and Uyo LGAs to class 4 (strongly polluted), 40% of the soil locations-2, 4, 6, 7 and Etinan LGA (Table 3). For Fe, 10% of the soil location-5 and Ibesikpo LGA was strongly

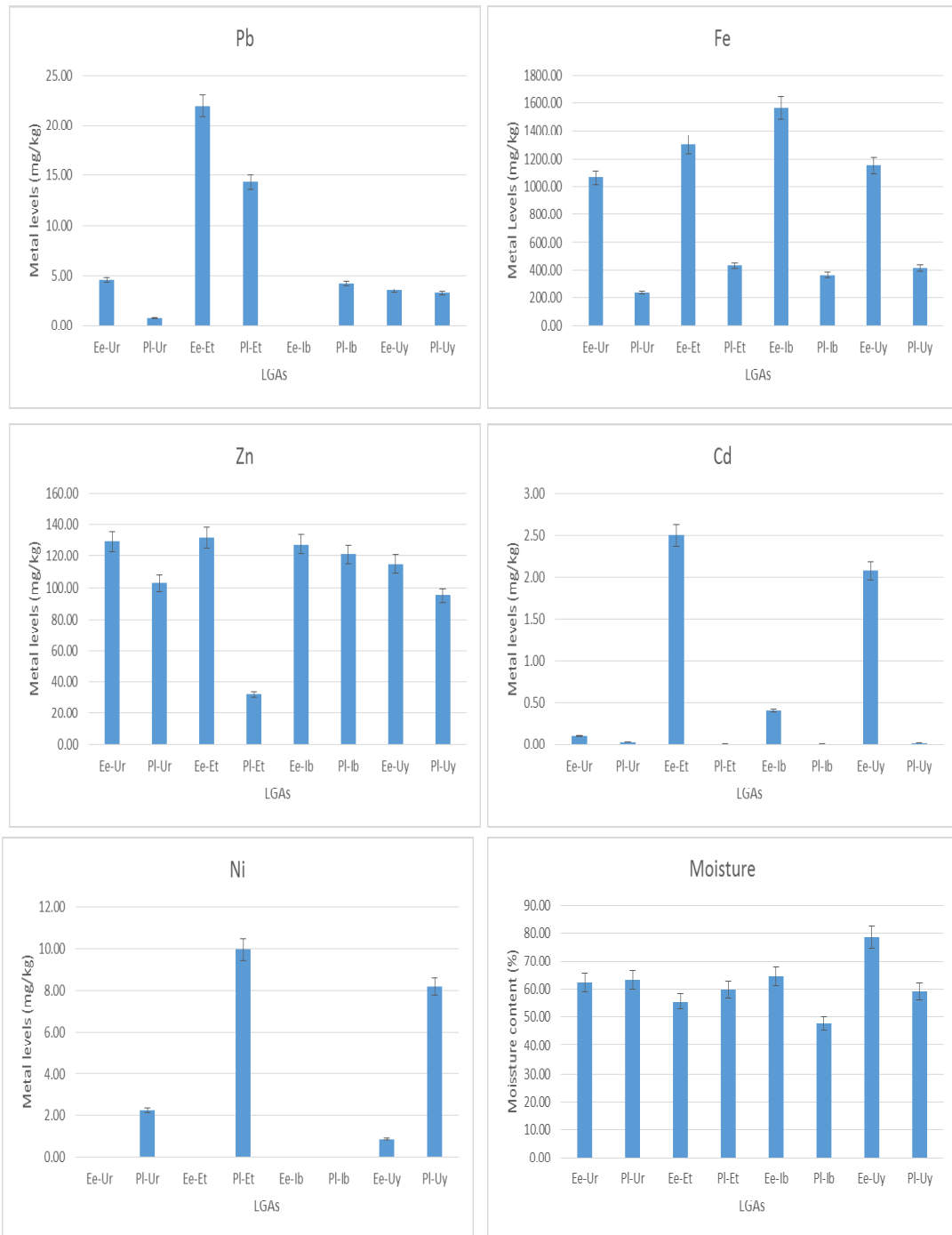
polluted (class 4), 30% locations-2, 3 and 8 strongly to extremely polluted (class 5) and 60% locations-1, 4, 6, 7, 9, 10 and Uruan, Etinan, and Uyo LGAs extremely polluted (class 6). Soil Zn pollution ranged from class 1 (unpolluted to moderately polluted) in 50% of locations-1, 2, 3, 6, 10 and Uruan LGA to class 2 (moderately polluted) in 50% of locations-4, 5, 7, 8, 9 and Etinan, Ibesikpo and Uyo LGAs. Soil Ni pollution ranged from class 0 (zero) to class 3; 20% of soil locations-3 and 5 were unpolluted (class 0), 50% unpolluted to moderately polluted (class 1, locations 1, 2, 7, 8 and 10), 10% moderately polluted

**Table 4: T-test (LSD) results for metal distribution in *Eudrilus eugeniae* and *Pachybolus ligulatus* from different locations**

Location	Bio indicator	Pb	Fe	Zn	Ni	Cd	Moisture
1	Ee	4.590 <sup>a</sup>	1064.2 <sup>a</sup>	129.2 <sup>a</sup>	0.010 <sup>a</sup>	0.100 <sup>a</sup>	62.62 <sup>a</sup>
	PI	0.010 <sup>b</sup>	129.2 <sup>b</sup>	164.95 <sup>b</sup>	0.010 <sup>a</sup>	0.010 <sup>b</sup>	62.40 <sup>a</sup>
	LSD	0.134	2.174	0.9281	0	0.0088	0.2356
2	Ee	29.95 <sup>a</sup>	1303.2 <sup>a</sup>	131.8 <sup>a</sup>	0.01 <sup>a</sup>	2.50 <sup>a</sup>	60.46 <sup>a</sup>
	PI	3.05 <sup>b</sup>	308.6 <sup>b</sup>	113.7 <sup>b</sup>	0.01 <sup>b</sup>	0.01 <sup>b</sup>	55.60 <sup>b</sup>
	LSD	0.0674	2.0823	0.432	0	0.0393	0.3629
3	Ee	0.01 <sup>a</sup>	1565.2 <sup>a</sup>	127.5 <sup>a</sup>	0.01 <sup>a</sup>	0.400 <sup>a</sup>	64.60 <sup>a</sup>
	PI	0.01 <sup>a</sup>	125.4 <sup>b</sup>	98.3 <sup>b</sup>	0.01 <sup>a</sup>	0.100 <sup>b</sup>	63.46 <sup>b</sup>
	LSD	0	14.08	1.02	0	0.0152	0.248
4	Ee	1.10 <sup>a</sup>	1026.1 <sup>a</sup>	110.5 <sup>a</sup>	0.254 <sup>a</sup>	3.15 <sup>a</sup>	76.00 <sup>a</sup>
	PI	0.10 <sup>b</sup>	396.6 <sup>b</sup>	35.5 <sup>b</sup>	9.00 <sup>b</sup>	0.010 <sup>b</sup>	66.81 <sup>b</sup>
	LSD	0.0393	2.504	0.6377	0.4128	0.0278	0.3153
5	Ee	8.90 <sup>a</sup>	1696.6 <sup>a</sup>	155.0 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	189.4 <sup>a</sup>
	PI	14.35 <sup>b</sup>	431.0 <sup>b</sup>	32.15 <sup>b</sup>	9.995 <sup>b</sup>	0.010 <sup>b</sup>	59.8 <sup>a</sup>
	LSD	0.11	1.6998	1.0435	0.184	0.0633	337.75
6	Ee	3.25 <sup>a</sup>	1378.2 <sup>a</sup>	141.5 <sup>a</sup>	0.156 <sup>a</sup>	1.96 <sup>a</sup>	56.43 <sup>a</sup>
	PI	0.01 <sup>b</sup>	562.5 <sup>b</sup>	59.3 <sup>b</sup>	0.010 <sup>b</sup>	0.01 <sup>b</sup>	53.40 <sup>b</sup>
	LSD	0.0708	2.5755	1.7331	0.1088	0.1416	0.3902
7	Ee	1.25 <sup>a</sup>	701.2 <sup>a</sup>	94.4 <sup>a</sup>	0.052 <sup>a</sup>	0.01 <sup>a</sup>	60.3 <sup>a</sup>
	PI	8.55 <sup>b</sup>	365.8 <sup>b</sup>	120.95 <sup>b</sup>	0.010 <sup>b</sup>	0.01 <sup>a</sup>	47.8 <sup>b</sup>
	LSD	0.0576	1.4434	1.0481	0.1166	0	0.3539
8	Ee	0.10 <sup>a</sup>	435.1 <sup>a</sup>	35.5 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	65.4 <sup>a</sup>
	PI	0.10 <sup>a</sup>	83.6 <sup>b</sup>	125.8 <sup>b</sup>	0.01 <sup>a</sup>	0.05 <sup>b</sup>	55.4 <sup>b</sup>
	LSD	0	0.2831	0.8475	0	12x10 <sup>-10</sup>	0.2808
9	Ee	5.60 <sup>a</sup>	1220.6 <sup>a</sup>	149.1 <sup>a</sup>	3.70 <sup>a</sup>	1.50 <sup>a</sup>	60.48 <sup>a</sup>
	PI	9.70 <sup>b</sup>	649.0 <sup>b</sup>	85.7 <sup>b</sup>	12.6 <sup>b</sup>	0.10 <sup>b</sup>	52.4 <sup>b</sup>
	LSD	0.2963	3.9789	0.3867	0.2261	0.0421	0.5545
10	Ee	4.50 <sup>a</sup>	1624.4 <sup>a</sup>	118.3 <sup>a</sup>	0.85 <sup>a</sup>	6.89 <sup>a</sup>	53.0 <sup>a</sup>
	PI	0.01 <sup>b</sup>	507.5 <sup>b</sup>	73.8 <sup>b</sup>	11.90 <sup>b</sup>	0.01 <sup>b</sup>	61.8 <sup>b</sup>
	LSD	0.1388	2.4411	0.1188	0.1188	1.1077	0.4748

Means with the same letters are not significantly different; Ee= *Eudrilus eugeniae*; PI= *Pachybolus ligulatus* ; LSD= Least Significant Level





Ee = *Eudrilus eugeniae*, Pl = *Pachybolus ligulatus*, Ur = Uruan, Et = Etinan, Ib = Ibesikpo Asutan, Uy = Uyo

**Fig. 5: Metal and moisture content in *Eudrilus eugeniae* and *Pachybolus ligulatus* from different Local Government Areas**

(class 2, location 9) and 20% moderately to strongly polluted (class 3, locations 4, 6). Soils from Ibesikpo LGA were unpolluted with Ni; Uruan LGA revealed unpolluted to moderately polluted; Uyo, moderate pollution and Etinan LGA, strong pollution. For Cd, 20% were unpolluted (class zero, locations 3 and 9), 20% moderately to strongly polluted (class 3, locations 4 and 5), 10% strongly polluted (class 4, location 7), 20% strongly polluted to extremely polluted (class 5, locations 8 and 10) and 30% extremely polluted (class 6, locations 1, 2, and 6). In the LGAs lgeo increased in the following order: Pb, Etinan > Uyo > Uruan > Ibesikpo; Fe, Uyo > Etinan > Uruan > Ibesikpo; Zn, Etinan > Ibesikpo > Uyo > Uruan; Ni, Etinan > Uyo > Uruan > Ibesikpo and Cd, Uruan > Uyo > Ibesikpo > Etinan.

#### Metals accumulation in potential bio-indicators of soil pollution at different locations

Mean metal levels in *Eudrilus eugeniae* and *Pachybolus ligulatus* at the individual locations were significantly different from each other at  $\alpha=0.05$  (Table 4) except for locations 3 and 8 for Pb, 1, 3 and 8 for Ni and 7 for Cd. The highest level (mg/kg) of Pb was found in the paint factory (locations 2 and 5). Pb was generally higher in *Eudrilus eugeniae* than in *Pachybolus ligulatus* at all the locations except for locations 5 (paint factory), 7 and 9 (Uyo, a busy city) where Pb was more in *Pachybolus ligulatus* samples. *Eudrilus eugeniae* accumulated more Fe (2-24-folds) at all locations than *Pachybolus ligulatus* (Tables 4 and 5). The paint factory (location 5 in Etinan) followed by mechanic village (location 10 in Uyo) had

**Table 5: Locations with maximum and minimum metals distribution in bio- indicators**

Metal	Bio indicator	Maximum (mg/kg)	Location	Minimum (mg/kg)	Location
Pb	<i>Eudrilus eugeniae</i>	21.95	2	0.01	3, 8
	<i>Pachybolus ligulatus</i>	14.35	5	0.01	3, 4, 6, 8
Fe	<i>Eudrilus eugeniae</i>	1696.6	5	435.13	8
	<i>Pachybolus ligulatus</i>	649	9	83.6	8
Zn	<i>Eudrilus eugeniae</i>	155.05	5	35.5	8
	<i>Pachybolus ligulatus</i>	164.95	2	32.5	5
Ni	<i>Eudrilus eugeniae</i>	3.7	9	0.01	1, 2, 3, 8
	<i>Pachybolus ligulatus</i>	12.6	9	0.01	1-3, 6, 7, 8
Cd	<i>Eudrilus eugeniae</i>	6.89	10	0.01	1, 5, 7, 8
	<i>Pachybolus ligulatus</i>	0.1	3	0.1	1, 2, 4, 5, 6, 7, 9, 10

**Table 6: Anova-LSD Results Of Metals In Bio-indicators At All Locations**

Bio indicators	Pb	Fe	Zn	Ni	Cd	Moisture content
<i>Eudrilus eugeniae</i> (n=50)	5.12 <sup>a</sup>	1201.5 <sup>a</sup>	119.3 <sup>a</sup>	0.61 <sup>a</sup>	1.75 <sup>a</sup>	73.25 <sup>a</sup>
<i>Pachybolus ligulatus</i> (n=50)	3.57 <sup>b</sup>	345.97 <sup>b</sup>	91.03 <sup>b</sup>	4.35 <sup>b</sup>	0.023 <sup>b</sup>	59.52 <sup>b</sup>
LSD	0.027	1.100	0.201	0.039	0.080	24.23

Means with same letters are not significantly different at  $\alpha=0.05$ .

the highest levels of Fe in *Eudrilus eugeniae* (Tables 4 and 5). Seventy percent (70%) of the locations recorded higher values of Zn in *Eudrilus eugeniae* while *Pachybolus ligulatus* recorded higher levels of Zn at locations 1, 7 and 8 (30%). Ni accumulated much more (greater than 3-folds) in *Pachybolus ligulatus*, ranging 0.01-12.6 mg/kg, while *Eudrilus eugeniae* ranged 0.01-3.70 mg/kg. Cadmium (Cd) was much higher in *Eudrilus eugeniae* (6.89 mg/kg) than in *Pachybolus ligulatus* (0.10 mg/kg). Lead (Pb), Fe, Zn and Cd levels were higher in *Eudrilus eugeniae* (feeds on soil), and Ni higher in *Pachybolus ligulatus* (herbivore, feeds on litters) (Table 5). Moisture content of *Eudrilus eugeniae* was higher

for most locations except location 10 with higher values for *Pachybolus ligulatus*. However, moisture content in the body of *Eudrilus eugeniae* (73.25%) though higher than in *Pachybolus ligulatus* (59.52%), was not significant at  $\alpha=0.05$  (Table 6). The areas of the paint factory, mechanic workshop and Uyo city high vehicular traffic roads had the greatest impact on soil as evidenced in the higher levels of metals in the bio-indicators. ANOVA results indicated a significant influence of location ( $p<0.0001$ ) on accumulation of metals by the indicator organisms ( $p<0.0001$ ). Interaction between these two factors were significant at  $\alpha = 0.05$ , implying that there are differences in Pb, Fe, Zn, Ni and Cd accumulation

**Table 7: Bio-accumulation factor (BAF) of metals at different locations and LGAs**

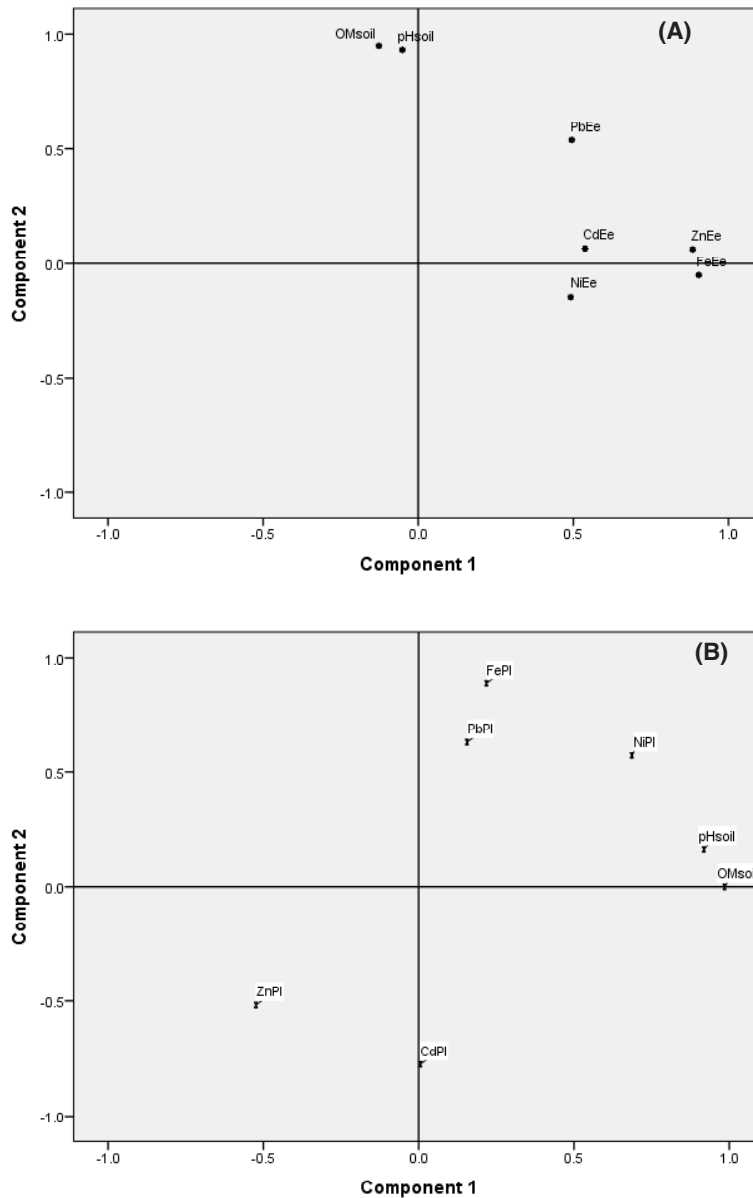
	Pb		Fe		Zn		Ni		Cd	
	BAF- Ee	BAF-PI	BAF-Ee	BAF-PI	BAF- Ee	BAF-PI	BAF- Ee	BAF-PI	BAF- Ee	BAF-PI
<b>Locations</b>										
1	0.016	3.3E-05	0.059	0.007	0.485	0.619	1.5E-04	1.5E-04	0.003	3.4E-04
2	0.328	0.005	0.190	0.045	0.824	0.710	2.2E-04	2.2E-04	0.094	3.8E-04
3	2.6E-05	2.6E-05	0.214	0.017	0.789	0.608	5.8E-04	5.8E-04	0.040	0.100
4	0.002	1.5E-05	0.038	0.015	0.289	0.093	6.7E-04	0.025	2.086	0.007
5	0.020	0.032	1.031	0.262	0.496	0.103	0.112	1.215	0.125	0.001
6	0.004	1.4E-05	0.068	0.028	0.568	0.238	3.1E-04	2.0E-05	0.08	4.1E-04
7	0.002	0.013	0.006	0.003	0.264	0.339	8.8E-04	1.7E-04	0.003	0.003
8	2.4E-05	2.4E-05	0.076	0.015	0.090	0.320	2.7E-04	2.7E-04	0.001	0.006
9	0.002	0.037	0.08	0.042	0.479	0.276	0.024	0.083	1.50	0.010
10	0.009	2.0E-05	0.132	0.041	0.512	0.319	0.028	0.394	0.368	5.3E-04
Mean	0.038	0.009	0.189	0.048	0.480	0.362	0.017	0.172	0.430	0.013
SD	0.102	0.014	0.302	0.076	0.225	0.214	0.035	0.386	0.740	0.031
Min	2.4E-05	1.4 E-05	0.006	0.003	0.09	0.093	1.5 E-04	2.0 E-05	0.001	3.4E-04
Max	0.328	0.037	1.031	0.262	0.824	0.710	0.112	1.215	2.086	0.100
Median	0.003	3.0E-05	0.078	0.022	0.490	0.319	6.2E-04	4.2E-04	0.087	0.002
CV	2.66	1.63	1.59	1.61	0.47	0.59	2.10	2.24	1.72	2.39
<b>Local Government Areas (LGAs)</b>										
Uruan	0.01	0.002	0.099	0.022	0.659	0.526	2.3E-04	0.053	0.005	0.002
Etinan	0.323	0.001	0.049	0.009	0.343	0.27	2.6E-05	0.006	1.656	0.021
Ibesikpo	2.2E-05	0.01	9.543	2.232	0.409	0.387	0.001	0.001	0.950	0.001
Uyo	0.007	0.006	0.035	0.013	0.373	0.308	0.005	0.052	0.185	0.002
Mean	0.085	0.005	2.431	0.569	0.446	0.373	0.002	0.028	0.699	0.006
SD	0.158	0.004	4.741	1.108	0.144	0.113	0.002	0.028	0.758	0.009
Min	2.2E-05	0.001	0.035	0.009	0.343	0.27	2.6 E-05	0.001	0.005	0.001
Max	0.323	0.010	9.543	2.232	0.659	0.526	0.005	0.053	1.656	0.021
Median	0.008	0.004	0.074	0.017	0.391	0.347	0.0006	0.029	0.567	0.002
CV	1.86	0.86	1.94	1.94	0.32	0.30	1.48	1.01	1.08	1.48

Ee= *Eudrilus eugeniae*; PI=*Pachybolus ligulatus*

pattern of the two indicator organisms, *Eudrilus eugeniae* and *Pachybolus ligulatus*. Variations among the LGAs are presented in Figure 5. Cadmium (Cd) in *Eudrilus eugeniae* and Ni in *Pachybolus ligulatus* in Etinan and Uyo LGAs were significantly different from those in Ibesikpo Asutan and Uruan LGAs.

**Bio-Accumulation Factor (BAF) of metals in *Eudrilus eugeniae* and *Pachybolus ligulatus***

Bio-Accumulation Factors (BAFs) are presented in Table 7. Generally, BAFs were <1 for the selected metals in both *Eudrilus eugeniae* and *Pachybolus ligulatus* at the different locations,



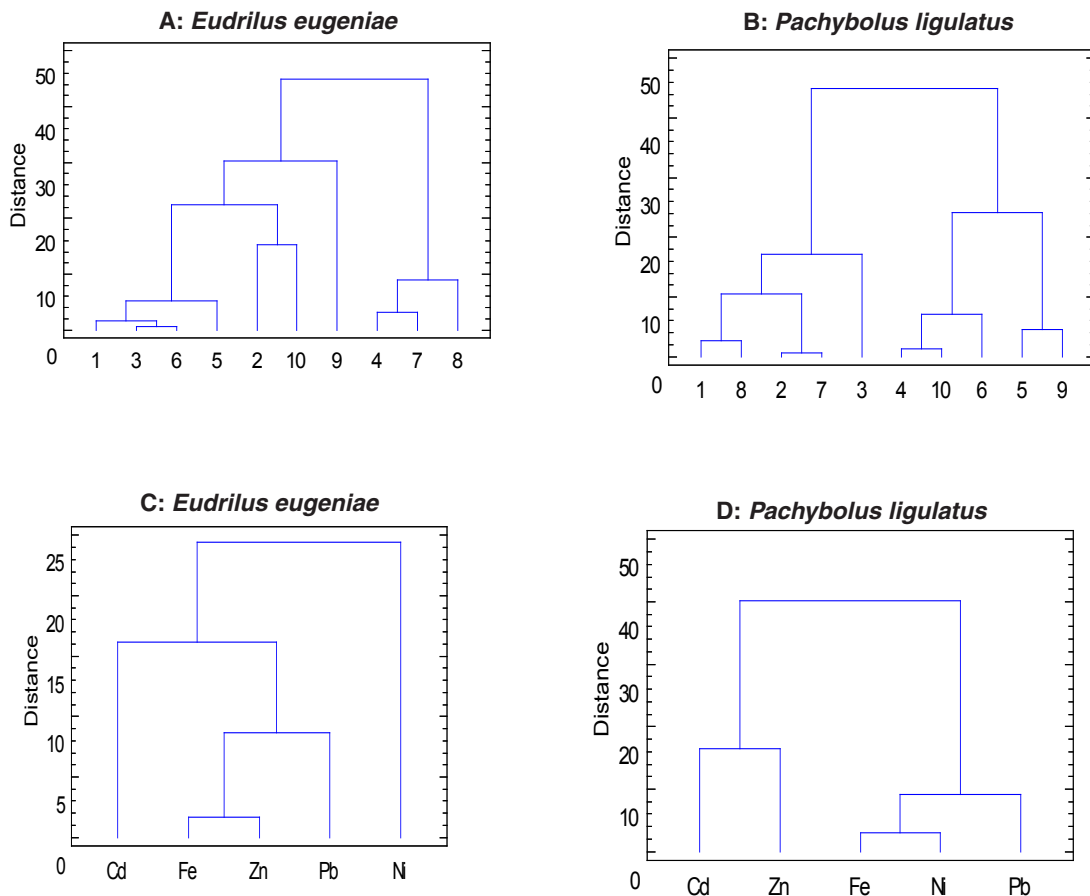
**Fig. 6: Component plot of factors 1 and 2 for metals in (A) *Eudrilus eugeniae* and (B) *Pachybolus ligulatus***

with the exceptions of Fe (1.031) and Cd (2.086) in *Eudrilus eugeniae* from locations 5 (paint factory) and 4 (a defunct old sea port), respectively; and Ni (1.215) in *Pachybolus ligulatus* from location 5 (paint factory), showing the impact of locations on metal bio-accumulation in the organisms. At the LGAs, BAF values of Pb, Fe, Zn and Cd in *Eudrilus eugeniae* were higher than those in *Pachybolus ligulatus* except for Pb in Ibesikpo LGA; Ni on the other hand was higher in *Pachybolus ligulatus* in all LGAs except for Ibesikpo (the control LGA/location). BAF>1 was obtained for Fe in both *Eudrilus eugeniae* (9.543) and *Pachybolus ligulatus* (2.232) in Ibesikpo LGA, and Cd in *Eudrilus eugeniae* (1.656) in Etinan LGA. BAF values (mean metal  $\pm$ SD, range) mg/kg at all locations followed the pattern: Zn (0.480 $\pm$ 0.225, 0.09-0.824)> Fe (0.189 $\pm$ 0.302, 0.006-1.031)> Cd (0.430 $\pm$ 0.740, 0.001-2.086)> Pb (0.038 $\pm$ 0.102, 2.4E-05-0.0328)> Ni (0.017 $\pm$ 0.035, 1.5E-04-0.112)

for *Eudrilus eugeniae*, and Zn (0.362 $\pm$ 0.214, 0.093-0.710)> Ni (0.172 $\pm$ 0.386, 2.0E-05-1.215)> Fe (0.048 $\pm$ 0.076, 0.003-0.262)> Pb (0.009 $\pm$ 0.014, 1.4E-05-0.037)> Cd (0.013 $\pm$ 0.031, 3.4E-4-0.100) for *Pachybolus ligulatus*.

**Factor Analysis**

Principal Component Analysis (PCA) of metals in *Eudrilus eugeniae* and *Pachybolus ligulatus* after Varimax rotation with Kaiser normalization resulted in the extraction of two Principal Components (PCs), each of which was used in interpreting the sources of pollution of the indicator organisms and the corresponding soil. PCs with Eigen values >1 are considered significant<sup>38</sup>. Table 8 shows the variance explained by the PCs for metals in *Eudrilus eugeniae* and *Pachybolus ligulatus*. Two PCs obtained with Eigen values >1 explained more than 64.116% of the total variance of metals in *Eudrilus eugeniae*. PC1



**Fig. 7: Dendrograms showing clustering of sample locations (A and B) and metals in indicator organisms (C and D)**



and PC2 accounted for 34.212% and 29.904% of variation, respectively. PC1 showed strong positive factor loading on Fe and Zn, and moderate positive factor loading on Cd; while PC2 showed strong positive factor loading on soil organic matter and soil pH, and moderate positive factor loading on Pb (Figure 6A). On the other hand, two PCs with Eigen values >1 obtained for metals in *Pachybolus ligulatus*, explained 71.863% of the total variance with PC1 accounting for 37.558% of the variance, and PC2 34.304%. PC1 showed strong positive factor loading on Ni, soil pH and soil organic matter (OM); and PC2 strong positive factor loading on Pb and Fe, and strong negative factor loading on Cd (Figure 6B).

Cluster analysis (CA) performed on *Eudrilus eugeniae* and *Pachybolus ligulatus* data sets grouped similar sampling sites (spatial variability) in the ten locations across the four Local Government Areas (LGAs) (Figures 7A and B), and the variables (metals) within the bio-indicators (Figures 7C and D). The spatial variability, that is, similarity and dissimilarity between locations where bio-indicators were collected, showed four clusters for *Eudrilus eugeniae* and three for *Pachybolus ligulatus*. For *Eudrilus eugeniae* data set, cluster one was made of locations 1, 3, 6 and 5; cluster two, locations 2 and 10; cluster three, location 9 only; and cluster four contained locations 4, 7 and 8 (Figure 7A). *Pachybolus ligulatus* data set contained

**Table 8: Total variance explained by PCA for metals in *Eudrilus eugeniae* and *Pachybolus ligulatus***

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of variation	Cummulative %	Total	% of variation	Cummulative %	Total	% of variation	Cummulative %
<b><i>Eudrilus eugeniae</i></b>									
1	2.411	34.441	34.441	2.411	34.441	34.441	2.395	34.212	34.212
2	2.077	29.675	64.116	2.077	29.675	64.116	2.093	29.904	64.116
<b><i>Pachybolus ligulatus</i></b>									
1	3.620	51.720	51.720	3.620	51.720	51.720	2.629	37.558	37.558
2	1.410	20.143	71.863	1.410	20.143	71.863	2.401	34.304	71.863

Extraction Method: Principal Component Analysis

**Table 9: Comparison of metals levels (mg/kg) in impacted soils with the present study**

Pb	Fe	Zn	Ni	Cd	Sites
27.8 - 82.3	-	60.4 - 110.6	-	-	Beijing parks, China <sup>35</sup>
7.85 - 50.32	-	14.3 - 107.2	-	2.82 - 10.35	Cement factory, Ewekoro, Nigeria <sup>12</sup>
38.0 - 120.0	0.28 - 4.10	35.0 - 112.0	-	0.01 - 3.00	Mechanic village, Makurdi, Nigeria <sup>48</sup>
21.83 ± 1.82	-	31.48 ± 2.28	-	0.05 ± 0.02	Clean (campus)-Control, UK <sup>13</sup>
291 ± 54	-	408 ± 22	-	5.93 ± 0.24	Smelter (Hallen Hull) UK <sup>13</sup>
10,200 ± 690	-	6,050 ± 416	-	60.8 ± 3.1	Mine (Shipham) UK <sup>13</sup>
259 - 736	164 - 109952	160 - 393	17.28 - 512	0.001 - 29.00	This study

Table 10: Comparison of metals accumulation (mg/kg) in bio-indicators with the present study

Pb	Fe	Zn	Ni	Cd	Sites (bio-indicator)
10.07 - 25.13	-	42.45 - 216.9	-	7.67 - 21.89	Cement factory, Ewekoro Nigeria ( <i>Eudrilus eugeniae</i> ) <sup>2</sup>
0.0081 - 0.0327	0.0052 - 0.9899	0.0336 - 0.0746	-	0.01; 0.045	Dumpsite, Zaria, Nigeria ( <i>Lumbricus terrestris</i> ) <sup>23</sup>
0.0025 - 0.0689	0.015 - 0.8985	0.0070 - 0.0489	-	0.0001 - 0.0410	Dumpsite, Kaduna, Nigeria ( <i>Lumbricus terrestris</i> ) <sup>23</sup>
0.0017	-	1.07	-	0.0009	Groundnut field, India (Earthworms) <sup>19</sup>
0.0011	-	1.76	-	0.0011	Sugar cane field, India (Earthworms) <sup>19</sup>
0.0002	-	-	-	0.0012	Paddy field, India (Earthworms) <sup>19</sup>
5.01 - 265.4	-	105.8 - 380.9	-	0.55 - 8.13	Dump sites Zaria, Nigeria ( <i>Lumbricus terrestris</i> ) <sup>11</sup>
0.01 - 21.95	435.1 - 1697	35.5 - 155.0	0.01 - 3.70	0.01 - 6.89	This Study ( <i>Eudrilus eugeniae</i> )
0.01 - 14.35	83.6 - 649	32.15 - 164.95	0.01 - 12.60	0.01 - 0.10	This Study ( <i>Pachybolus ligulatus</i> )

in cluster one, locations 1, 8, 2, 7 and 3; Cluster two, locations 4, 10 and 6; and cluster three, locations 5 and 9 (Figure 7B). Cluster one had more locations in both dendrograms. Metals in bio-indicator organisms showed three clusters for *Eudrilus eugeniae*: Cluster one with Cd only, cluster two had Fe, Zn and Pb and cluster three Ni only (Figure 7C). For *Pachybolus ligulatus*, two clusters emerged: Cluster one contained Cd and Zn and cluster two, Fe, Ni and Pb (Figure 7D).

Pearson's product moment correlations between significant pairs of variables at  $\alpha = 0.05$  were: Fe/Zn in *Eudrilus eugeniae* ( $r = 0.8160$ ,  $P = 0.0040$ ); Cd in *Eudrilus eugeniae*/soil organic matter ( $r = 0.6646$ ,  $P = 0.0360$ ); and Fe/Ni ( $r = 0.6639$ ,  $P = 0.0363$ ) in *Pachybolus ligulatus*. Positive correlations were found between Fe in *Pachybolus ligulatus*/Ni in *Eudrilus eugeniae* ( $r = 0.6586$ ,  $P = 0.0384$ ); and Ni in *Eudrilus eugeniae*/Ni in *Pachybolus ligulatus* ( $r = 0.7258$ ,  $P = 0.0175$ ). Soil organic matter correlated positively with soil pH ( $r = 0.8800$ ,  $P = 0.0008$ ); Zn in soil correlated negatively with Fe in *Eudrilus eugeniae* ( $r = -0.672$ ,  $P = 0.0330$ ); while Ni in soil correlated positively with Fe in soil ( $r = 0.7580$ ,  $P = 0.0110$ ).

## DISCUSSION

The soil ecosystem is in equilibrium between its abiotic and biotic components; any change in one adversely affects the other<sup>14, 26,39,40</sup>. Toxic metals on release into the soil (the ultimate sink of pollutants) disrupt this equilibrium. Their accumulation in soils is as a result of anthropogenic activities-from rapid industrialization, agriculture and urbanization.

The mean soil metal (Zn, Pb, Cd, Ni, and Fe) levels in the study areas were higher than the background levels<sup>33-34</sup>. High levels were especially recorded in the urban city of Uyo (with higher vehicular emissions), the mechanic workshops and the paint factory. This confirms reports by previous researchers<sup>5,41-43</sup> that most areas near busy cities, industries and road systems have high concentration of toxic metals. Heavy metals may persist in the soil for up to a hundred decades; and can directly pollute soil and endanger the ecosystem by availability to flora and fauna, transfer to food chain and

contamination of surface and ground water<sup>26,39,40,44,45</sup>. Soil characteristics such as pH and organic matter content affect the heavy metals adsorbed in soil<sup>46</sup>. According to Souza *et al.*<sup>47</sup>, decreasing the pH made available metals that were strongly adsorbed in organic matter of the soil. Trace elements are generally more solubilized in acid soil conditions. The present study revealed high positive correlations between soil organic matter and/soil pH ( $r = 0.8800$ ); also, between soil Zn/Fe ( $r = 0.7580$ ), Cd in *Eudrilus eugeniae*/soil organic matter ( $r = 0.6646$ ,  $P = 0.0360$ ). The total soil Pb and Cd were higher than values reported by Olayinka *et al.*<sup>2</sup> and Iorungwa *et al.*<sup>8</sup> from a cement factory area and waste dump site in a mechanic village, respectively both in Nigeria, and Spurgeon and Hopkin<sup>13</sup> in a smelter plant area in the UK; but lower than from a mine in the UK<sup>13</sup>. Fe had values higher than reported by Iorungwa *et al.*<sup>8</sup>; Cd was higher than values reported in soil from a smelter plant but lower than from a mine, both in the UK<sup>13</sup> (Table 9).

Igeo values for Pb, Fe, Zn, Ni and Cd metals in soil were generally higher in soils around the paint factory and Uyo (a metropolitan city). The study areas soil quality varied from strongly polluted (soils from the paint factory, auto-mechanic village, and high traffic areas) to unpolluted (in the control). Qingjie *et al.*<sup>5</sup> reported soil quality varying from polluted to unpolluted in Beijing Parks, from the center to the outskirts of Beijing City (Table 9).

Regular exposure of soil invertebrates to metals in the superficial layers of soil causes them to accumulate metals in their tissues<sup>49</sup>, hence the organisms can be used for monitoring soil pollution. Heikens *et al.*<sup>9</sup>, further reported that the concentration of metals in terrestrial invertebrates were in the order  $Pb > Cd > Cu$ ; which is closely similar to the trend observed in this study with *Eudrilus eugeniae* ( $Pb > Cd$ ). Our results also, are in line with findings that earthworms in impacted areas-near road sides, factories, mechanic villages and recreational areas agro-ecosystem and dump sites areas accumulate much more metals<sup>11-13,19,23,35,48</sup> (Table 10). The variation in metals levels in the two organisms may be due to factors including pathway of metal uptake-dermal and/or oral, the body composition/metabolism and feeding habit<sup>50</sup>. Diplopods have been reported to metabolize and/or

accumulate various types of metal ions in the midgut, and the fat body<sup>8</sup> which is distributed throughout the body cavities. Accumulation of more Pb, Fe, Zn and Cd in *Eudrilus eugeniae* tissues than *Pachybolus ligulatus* may be explained in their feeding habits. *Pachybolus ligulatus* species are herbivores and live between the soil surface and litters, feeding mainly on decayed plants materials very rich in organic matter, while *Eudrilus eugeniae* species are burrow animals that feed on soil particles with lesser organic matter down the soil profile. Soil being a sink for pollutants, could be responsible for the higher concentrations of metals in earthworms (*Eudrilus eugeniae*) than in millipedes (*Pachybolus ligulatus*).

Bio-accumulation in terrestrial invertebrates often has been the sum of the metals adsorbed to the body wall and absorbed into the body. However, Vijver *et al.*<sup>1</sup>, in their work on earthworm and isopod reported that adsorption of Cd and Zn to surface of the organisms were negligible. They further showed that the metal uptake levels in the organisms were mainly the internalised metals with little or no external component; contrary to previous report<sup>52</sup>. Issues of variation and/or affinity of metals therefore could reside in the internal mechanisms/processes of the organism. The highest concentration of Cd (6.89mg/kg) in *Eudrilus eugeniae* compared with 0.10 mg/kg in *Pachybolus ligulatus* may result from its localization in the chloragogenous tissue<sup>9,50</sup> which is unique to oligochaetes, and has a function in metal storage and trafficking<sup>53</sup>; and essential metals e.g. Zn when involved, also function as buffer for the coelomic fluid and blood. On the other hand, for arthropods, the hepatopancreas appears to be the most important storage organ for metals. The hepatopancreas is packed with different types of granules like the chloragogenous tissue in earthworms. Metals are precipitated in different chemical forms in these granules<sup>21,54</sup>. The binding chemicals of the hepatopancreas in millipedes may have more affinity for Ni (12.60mg/l) than the chloragogenous tissue of earthworms (3.70 mg/l), hence the higher concentration of Ni in *Pachybolus ligulatus* than *Eudrilus eugeniae*. Other factors that could influence uptake of metals in the bio-indicators include the solubility of the soil metal bearing mineral<sup>41,55</sup>. The higher the solubility, the higher the accumulation of metals in invertebrates. This study revealed high positive correlations

between soil organic matter/soil pH ( $r = 0.8800$ ); also, between Cd in *Eudrilus eugeniae*/soil organic matter ( $r = 0.6646$ ). Another important factor in bio-accumulation of metals in terrestrial invertebrates is their ability to eliminate/excrete excess of metals. For the xenobiotic metals e.g. Cd and Pb, it is reported that the excretion is slow or absent<sup>9</sup>, which could also be responsible for higher accumulation of Pb, Cd in *Eudrilus eugeniae* and Ni in *Pachybolus ligulatus*.

Soils, and consequently the indicator organisms are impacted by activities of the paint factory, auto-mechanic workshop and the municipal city. PCA revealed that for *Eudrilus eugeniae* Eigen values  $>1$  obtained explained more than 64.116% of the total variation of metals. PC1 showed strong positive factor loading on Fe and Zn, and moderate positive factor loading on Cd; and PC2, strong positive factor loading on soil organic matter and soil pH, and moderate positive factor loading on Pb. For metals in *Pachybolus ligulatus*, PC1 accounted for 37.558% of the total variation and showed strong positive factor loading on Ni, soil organic matter (OM) and soil pH; which could also explain the preferential accumulation of organically bound metal (Ni) by *Pachybolus ligulatus* while *Eudrilus eugeniae* appears to be indicator for the inorganic species of metals. On the other hand, PC2 explained 34.304% of the total variation, with strong positive factor loading on Pb and Fe; and strong negative factor loading on Cd. These suggest anthropogenic input of metals from waste from the paint factory, mechanic workshop, agricultural activities, decomposed organic wastes and acid precipitation.

### CONCLUSION

The invertebrates exhibited (preferential) levels of affinity in metals uptake. *Eudrilus eugeniae*

(soil feeder) appears to accumulate the inorganic metals species - Pb, Fe and Cd more, and could serve as bio-indicator of these metals; while millipede (decayed litter feeder) accumulated 3-folds more Ni (organically bound metal), thus presenting the organism as a suitable indicator of Ni-polluted soils. The endowment of *Pachybolus ligulatus* for accumulation of nickel makes the invertebrate both suitable and choice bio-indicator of crude oil pollution. This could provide a monitoring tool for agriculture in the Niger Delta, a region of Nigeria that is plagued by decades-long oil pollution of its water bodies and land.

We are of the opinion that despite other possible factors, preference in metals uptake/storage in the organisms may reside largely at the level of the chloragogenous and hepatopancreas tissues of the earthworm (*Eudrilus eugeniae*) and millipede (*Pachybolus ligulatus*), respectively. A more detailed research on the affinity of the tissues for metals would be required. In the overall, the activities in the areas studied clearly contributed to the pollution of locations.

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