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HEAVY METALS CONCENTRATIONS IN EARTHWORMS (*Eudrilus eugeniae*) EXPOSED TO WATER AND SEDIMENTS FROM OGUN RIVER

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ABSTRACT

The bioavailability of heavy metals plays a vital role in their toxicity and accessibility to organisms. This study aimed at evaluating heavy metal levels in the earthworms (Eudrilus eugeniae) exposed to water and sediment from the Ogun River. Earthworms were collected using the handpicking and sorting method from the Botanical Garden of the Federal University of Agriculture, Abeokuta. Fifteen (15) earthworms each were inoculated into three plastic culture bins a few centimeters below the surface of the beddings and treated with sediments and water from Ogun River and distilled water, respectively. The treatment was applied at 72 hours (3 days) intervals for the duration of thirty (30) days. Sample digestion for heavy metals was by wet oxidation, thereafter analyzed using atomic absorption spectrophotometer. Highest Lead (Pb) concentration was observed in the tissue of earthworms exposed to river sediments (20.89 mg/kg) while the highest Copper (Cu) concentration was obtained in the control (7.81 mg/kg). Cadmium (Cd) concentration (0.15 mg/kg) was similar in earthworms exposed to river sediments and the control. The highest Chromium (Cr) and Iron (Fe) concentration was observed from those exposed to river sediments (7.66 and 12.30 mg/kg) respectively while Zn had the highest concentration in those exposed to river water (0.72 mg/kg). The highest CN concentration was determined in those of the control (0.65 mg/kg). The level of heavy metals was evident in the earthworm samples affirming that the safety threshold for metals in the environment can be determined via earthworm toxicity tests.

Keywords: metal load, metal exposure, earthworm tissue, earthworm toxicity

INTRODUCTION

Rapid urbanization, industrialization, economic growth, population expansion, and wastewater output have all contributed to environmental pollution, a major global health concern that has inspired several scientific research globally (Famuyiwa *et al.*, 2022; 2023). Surface water such as rivers, lakes, ponds and oceans accounts for 80 percent of the water utilized by humans (Young, 2018) and is presently undergoing a serious pollution threat. Freshwater pollution especially that of the river is an intense environmental problem in developing countries (Famuyiwa *et al.*, 2023). Similarly, sediments are increasingly recognized as sources of contaminants in aquatic ecosystems and as well as carriers of toxins, serving as receptacles for dangerous chemical species that have been discharged (Osakwe and Peretiemo-Clarke, 2013). A mix of factors (river hydrodynamics, biogeochemical processes, and environmental conditions) leads to sediment's capacity to take up contaminants (Enuneku *et al.*, 2017).

Heavy metals (HMs) are environmental pollutants with high density/nucleon number. They can dissolve in soil solutions, surface and interstitial waters, where they are adsorbed in sediment through cation exchange processes and become readily available to plants and aquatic organisms (Ladigbolu and Balogun, 2011). The pollution caused by heavy metals is a serious problem due to their toxicity and bio-accumulating ability negatively impacting environment and human health (Salniko *et al.*, 2013).

Earthworms are large megadrile that belong to Phylum Annelida. In Nigeria, Ephyriodrilus afroccidentalis, Eudrilus eugeniae, and Hyperiodrilus africanus are the most common earthworms (Owa *et al.*, 2013). Eudrilus eugeniae is an earthworm species that originated from West Africa and are popularly called "African night crawler" (Dedeke *et al.*, 2018). They are widely found on the soil's surface layer (Epigeic) characterized with rich organic matters (Ali and Naaz, 2013; Dedeke *et al.*, 2018). Earthworm has been widely adopted in various studies as a bio-indicator of environmental pollution (Owagboriaye *et al.*, 2015; Amadi *et al.*, 2019; Latifi *et al.*, 2020). Ogun River is a prominent river with records of an array of heavy metal pollution and monitoring this river is pivotal. Therefore, the study was conducted, aiming at determining the levels of some heavy metals in earthworm exposed to river water and sediments.

MATERIALS AND METHODS

Study Area

Ogun River is a permanent river with a total area of 22.4 km and a fairly large flow of about 393m3/s during the wet season (Oketola *et al.*, 2013). The river originates from Oyo State (3' 28" E and 8'41" N) near Shaki flows through Ogun State and discharges into the Lagos Lagoon, the final sink. Ogun River is used for agriculture, transportation, human consumption, various industrial activities and domestic purposes (Ayoade *et al.*, 2004). The river serves as raw water for Ogun and Lagos States water works, which treats it before dispensing it to the public. Along the river basin, effluents from breweries, abattoir, dyeing industry and domestic wastewaters is discharged (Oketola *et al.*, 2013). This study was carried out between the months of September and November, 2023 at the Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Ogun State.

Water and Sediments' Collection

For the purpose of the experiment, water and sediment sample were collected from the Lafenwa section of Ogun River, South west, Nigeria (Figure 1). Water samples were collected using sterile plastic labelled and transported to the laboratory for storage until use. Sediments were collected using a shovel allowed to drain, and then placed in a sterile polythene sampling bag. At the laboratory, the sediments were allowed to sun-dry for one week, followed by passes through 2 mm mesh, then storing until use (Latifi *et al.*, 2020).

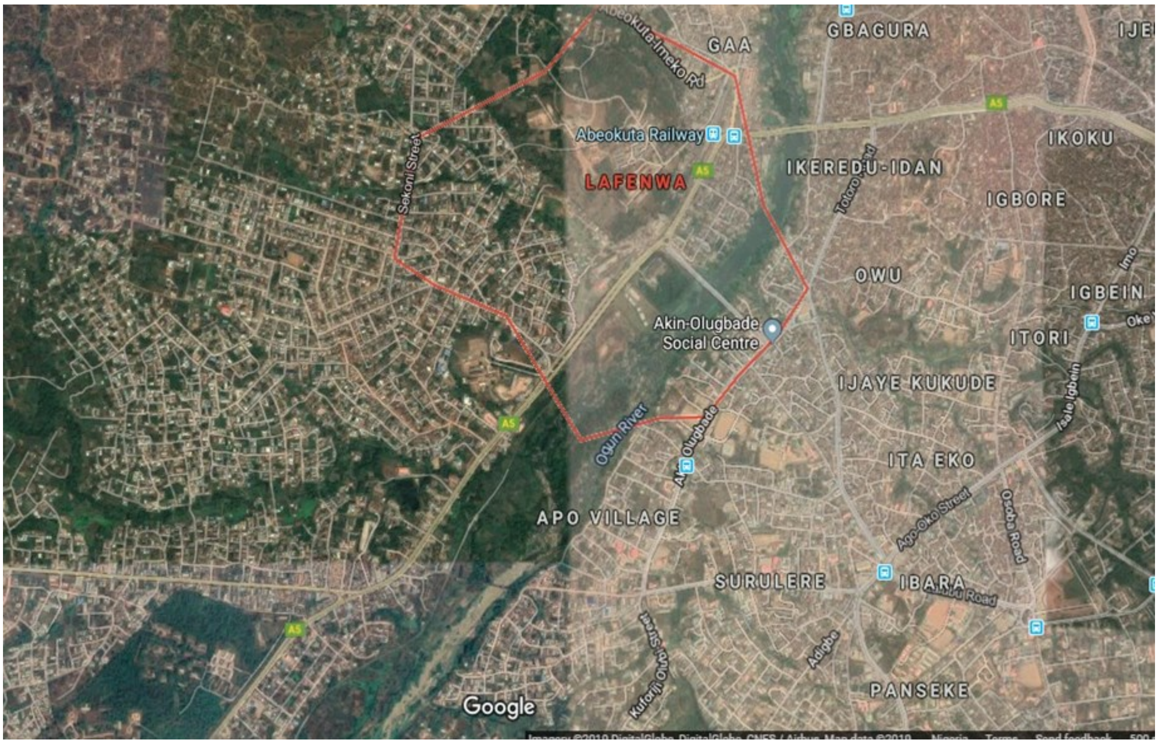

Fig. 1: Water and sediments collection site at Lafenwa, Abeokuta, Nigeria

Experimental Animals model

Collection of earthworms

The collection of earthworms was done as described by Owa *et al*. (2013) and Owagboriaye *et al*. (2015). Earthworms were collected from the Botanical Garden of the Federal University of Agriculture, Abeokuta. The earthworms were collected from the same site in other to reduce variability in biotype. The soil was carefully turned using a trowel while the healthy earthworms were handpicked and transported to the laboratory in plastic buckets along with the soil in which they were collected and supplemented with half-boiled, ground waterleaf (Talinium triangulare) - Fafioye and Owa, (2000). They were thereafter moistened with distilled water. The earthworms were identified using earthworm identification keys.

Earthworm culture

Collected earthworms were housed in a rectangular plastic culture bin (depth- 5cm, width- 8cm and length- 11cm) which was left for 10 days to acclimatize prior to selection (Owa *et al*., 2008). A total of nine plastic culture bins, each filled with 1.2 kg of top soil (humus) previously oven dried to kill the soil microbes, were placed in each compartment of a wooden stand (triplicate). The earthworms were fed with 50 g of cow dung (dried) every 3 days' intervals.

Experimental design

Fifteen earthworms were inoculated into each plastic culture bin a few centimeters below the surface of the beddings, so as to prevent inviting predatory insects such as the doryline ants, e.g., Dorylus sp. The first and the second earthworm culture was treated with sediments (S) and water (W) collected from Ogun River, respectively. The treatment was added at 72 hours (3 days) interval. Additionally, the third culture was treated with distilled water to serve as control (C).

Treatment Group: Culture I: A 50 g of sediments + 100 ml of distilled water, Culture II: 100 ml of river water only

Control Group: 100 ml of distilled water only

Earthworm scarification was done after 30 days' exposure; each earthworm was rinsed and then killed by freezing for 4 hours.

Heavy Metal Analysis

Sample digestion and determination of the heavy metals in the digested samples followed the method previously adopted by Owagboriaye *et al.* (2015) and the Association of Analytical Chemists (AOAC, 2019). The heavy metals were: Cadmium; Chromium; Copper, Iron; Nickel, Lead and Zinc. Sample digestion was by wet oxidation. To 0.2 g of earthworm, 2 mL sulphuric acid, 4 mL perchloric acid and 20 mL nitric acid were added and the mixture was heated to boil on heating mantle until the mixture turned colourless. The mixture was allowed to cool and then diluted with distilled water to 100 mL. Following sample digestion, an atomic absorption spectrophotometer (Perkin Elmer Model 403, North Chicago USA.) was used to determine the concentrations of heavy metals (Cd, Cr, Cu, Fe, Ni, Pb, Zn) in the digested earthworm tissues.

Quality control

All chemical used were of analytical grade, laboratory glass and plastic wares were sterilized and pretreated with 5% nitric acid before use. All instruments were calibrated according to the manufacturer's guidelines.

Data Analysis

Data collected were subjected to statistical analysis, which includes descriptive (Mean±SD) and inferential (Analysis of Variance (ANOVA) statistics using the Statistical Package for Social Sciences (SPSS) Version 21.0. And Microsoft Excel 2013 version. Post Hoc Test was done using Duncan Multiple Range Test (DMRT), to determine significant differences in mean values. $P \leq 0.05$

RESULTS

Heavy Metal Levels

The concentration of Pb in the study ranged from 13.93 to 17.92 mg/kg (Table 1). The Pb concentration was significantly higher with the tissue of earthworm exposed to river sediments (20.89 mg/kg) having the highest significant value. The concentration of Cd in the study ranged between 0.12 and 0.15 mg/kg; the level in the earthworms exposed to river sediments (0.15±0.04 mg/kg) was similar to that in the control earthworms (0.15±0.03 mg/kg) These values showed no statistical difference from the mean of Cd (0.12±0.02 mg/kg) in the earthworms exposed to the river water (Table 1).

Concentration of Cu in the study varied from 5.87 to 7.81 mg/kg and was significantly lower in the control earthworms (7.81±0.54 mg/kg) than in the sediment and river water 5.87±0.48 mg/kg) exposed earthworms (Table 1). Concentration of Cr in the study ranged from 5.81 to 7.66 mg/kg. There was no significant difference in the Cr concentrations in the earthworms from the river sediments (7.66 mg/kg) and the 5.81 mg/kg from river water (Table 1).

Concentration of Ni in the study ranged from 11.86 to 13.83 mg/kg and was not significantly different between any of the samples. Highest Ni concentration was observed in the control (14.19 mg/kg), with the lowest values observed from the river sediments (11.86 mg/kg). The concentrations of Fe in the study ranged from 11.61 to 12.30 mg/kg. No significant difference was observed between the Ni concentration in the earthworm tissues from the sediments, control and river water. The highest Fe concentration was observed from earthworms exposed to river sediments (12.30 mg/kg) while the lowest amount (11.61 mg/kg) was recorded from the river water (Table 1). The concentration of CN in the study ranged from 0.48 to 0.65 mg/kg. The highest CN concentration was recorded from the control (0.65 mg/kg) while the lowest was recorded from the river sediment (0.48 mg/kg). The concentration of Zn in the study ranged from 0.54 to 0.72 mg/kg. The highest Zn concentration was recorded from the tissue of earthworm exposed to water from the river (0.72 mg/kg) while the lowest (0.54 mg/kg) was from the control (Table 1).

Table 1: Heavy Metal Concentrations in the Earthworm Tissues (n=3)

Heavy Metals	Exposure samples	Mean±SD (mg/kg)	Min	Max	Standard Value WHO (mg/l)	Standard Value UK (mg/kg)
Pb	Control	16.02±2.00^{a}	13.93	17.92		
	River sediments	20.89±1.39^{b}	19.72	22.43		450
	River water	17.85±1.36ab	16.93	19.41	0.01	
Cd	Control	0.15±0.03^{a}	0.13	0.18		
	River sediments	0.15±0.04^{a}	0.11	0.19		150
	River water	0.12±0.02^{a}	0.10	0.13	0.03	
Cu	Control	7.81±0.54^{c}	7.20	8.22		
	River sediments	6.87±0.31^{b}	6.67	7.22		_
	River water	5.87±0.48^{a}	5.33	6.22	2.00	
Cr	Control	7.50±1.47^{a}	5.81	8.47		
	River sediments	7.66±2.25^{a}	5.32	9.80		200
	River water	5.81±0.44^{a}	5.32	6.17	0.05	
Ni	Control	14.19±2.06^{a}	12.81	16.56		
	River sediments	11.86±1.94^{a}	10.09	13.94		_
	River water	13.83±1.98^{a}	11.54	15.00		
Fe	Control	12.23±0.56^{a}	11.62	12.71		
	River sediments	12.30±2.20^{a}	10.39	14.70		_
	River water	11.61±0.88^{a}	10.64	12.34	1.00	
CN	Control	0.65±0.16^{a}	0.49	0.81		
	River sediments	0.48±0.00^{a}	0.44	0.51		_
	River water	0.61±0.14^{a}	0.44	0.70	0.1	
Zn	Control	0.54±0.12^{a}	0.42	0.66		
	River sediments	0.67±0.08^{a}	0.59	0.74		_
	River water	0.72±0.14^{a}	0.60	0.87	3.00	

Mean value with different superscript in column are significant at $p \leq 0.05$

Correlation matrix of heavy metals

Pearson correlation analysis showed significant correlations only between Cr and Fe, and Zn and Cu (Table 2). The matrix shows strong, positive and significant correlation between chromium and iron (r=0.867; P <0.01; Table 2) while zinc has a negative, but significant correlation with copper (r=-0.753; P <0.05; Table 2). The relationship between Pb and Cd, Pb and Cr, Pb and Zn, Pb and Cn are weak and not significant (Table 2).

Table 2: Heavy Metal correlation matrix

	Pb	Cd	Cu	Cr	Ni	Fe	CN	Zn
Pb	1							
Cd	0.068	1						
Cu	-0.217	0.496	1					
Cr	0.412	0.069	0.316	1				
Ni	-0.428	-0.103	0.186	0.030	1			
Fe	0.404	0.024	0.180	0.**867****	0.412	1		
CN	-0.293	-0.065	0.437	-0.067	0.578	0.135	1	
Zn	0.350	-0.082	-0.**753***	-0.205	-0.628	-0.261	-0.571	1

* and **Correlation is significant at the 0.05 level and 0.01 level (2-tailed) respectively

DISCUSSION

In ecotoxicology, earthworms are good indicators of metal pollution (Sivakumar, 2015). The uptake of metals by earthworms depends on various factors such as species type (epigeic, anecic and endogeic), the earthworms' living habitat and their food choice, the speciation, bioavailability and mobility of the metals involved in the process (Singh *et al.*, 2011). Earthworms from the study had higher concentrations of Pb, Cd, Cr and Fe on exposure to the river sediments. This was lower than the guideline value stated by the UK for Pb, Cd and Cr (Umoren *et al.*, 2024).

Sediments are components of our environment that serve as repositories for deleterious chemical species because of anthropogenic wastes discharged into water bodies (Osakwe and Peretiemo-Clarke, 2013). The highest Zn concentration was recorded from earthworms exposed to river water, which was lower than the WHO standard (WHO, 2011; Famuyiwa *et al.*, 2023). The ability to detect a single heavy metal can be attributed to the time and period of water collection. Correlation between heavy metals is a good model for suggesting the source of the heavy metals in the environment (Famuyiwa *et al.*, 2023. The correlation between Fe and Cr; Zn and Cu suggests the possible sources of these metals i.e., from both natural and man-made origins.

CONCLUSION

Earthworms may not be significantly impacted by the bioaccumulation of metals in the food chain, but at higher trophic levels, this process could cause major harm. Therefore, safety thresholds for substances in the environment can be determined via earthworm toxicity tests. The initial basic mortality test in environmental risk assessments is expanded to include growth and reproduction, chronic, and field test trials.

The level of heavy metals in observed in earthworms from the study is an affirmation that earthworm can be employed as a bio-indicator and safety threshold for metals in the environment (aquatic and terrestrial) can be determined through earthworm toxicity testing. The observable difference between the control and exposure samples is indicative of the level of pollution from the Lafenwa section of Ogun River and the exposed earthworms are indicative of what to expect from aquatic species being harvested from such water for consumption.

The study has a limitation as a result of not determining the level of heavy metals in the exposure media (water and sediments) in order to determine the bioaccumulation factors (BAF) of each heavy metal in the earthworm tissue. Therefore, it is recommended that further studies should evaluate the heavy metal load in the exposure media as well as assess the BAF. Comparison of the heavy metal load in earthworms from the sampling site at Lafenwa and the Botanical Garden as well as exposure studies are also desirable.

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