Journal of Scientific Research & Reports
9(4): 1-10, 2016; Article no.JSRR.20594
ISSN: 2320-0227

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Earthworms *Eudrilus eugeniae* as Bio-indicators of Heavy Metal Contamination in Port Harcourt Dumpsites

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Authors' contributions

This work was carried out in collaboration between the authors. Author FI designed the study, wrote the draft of the manuscript, managed the literature searches, authors IMT, AYJ and NJC designed the figures and made statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2016/20594
Editor(s):
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Complete Peer review History: http://sciencedomain.org/review-history/12056

Original Research Article

Received 31st July 2015
Accepted 14th October 2015
Published 2nd November 2015

ABSTRACT

Heavy metal pollution of terrestrial and aquatic ecosystem through improper management of dumpsites and indiscriminate dumping of waste has been recognised as a serious environmental concern in the city of Port Harcourt. It is important to validate the extent of contamination of heavy metals in dumpsites to successfully validate the exposure of humans living around its environs. To investigate heavy metal concentrations in soils of dumpsites, earthworm *Eudrilus eugeniae* was used as bio indicators in dumpsites Wimpy, Mgbuoba and Rumuokwuta in Obio Akpor local government of Port Harcourt. Earthworm *E. eugeniae* and soil were sampled during the wet season (July 2006), digested using concentrated HNO_3, and concentrations of Pb, Cu, Cd, Ni, Zn and Cr were analysed with an atomic absorption spectrophotometer. Correlation between earthworm

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E. eugeniae and soil shows that *E. eugeniae* are good bio indicators of available metal in soil. Concentration of heavy metals in soil samples from dumpsites were within the Canadian environmental guideline for contaminated site remediation and Toronto public health (TPH) guide for soil testing in urban gardens with the exception of Zn which requires remediation action. Bioavailability of metals in soil was shown to be low, suggesting the presence of complex species in soil which reduces mobility of heavy metals.

Keywords: Heavy metals; earthworms; dumpsite; Port Harcourt.

1. INTRODUCTION

In the past, Port Harcourt was known as the garden city of Nigeria because of the beautification and neatness, but today presence of piles of refuse is found around the city. Indiscriminate dumping of commercial, industrial and domestic waste is a common sight around the city. The problem and major environmental concern associated with dispersal of waste is the contamination of soil and ground water through leachate from the dumpsite which endangers both the environment and human life [1,2,3]. Many factors influence leachate composition, including types and composition of waste which include heavy metals, agricultural waste, and organic waste [4,5]. Soil micro-organism can degrade organic contaminants while metal contaminates are non-biodegradable and remain persistent in the environment. Metal accumulation in soil and pollution of ground water causes various diseases and renders the soil unsuitable for plant growth [6,7].

The best Indicators of heavy metal contamination in soil are the organisms directly exposed to them [8]. Among soil species, earthworms are ubiquitous, abundant, and important for soil processes. Earthworms form the largest part of the invertebrate biomass in most soils and play an important role in chemical element transformation [9-12]. They utilize a significant amount of soil organic matter for feeding, produce huge amount of biogenic structures and determine the activities of micro-organisms and other smaller invertebrates [13,14]. Earthworms can be exposed by direct dermal contact with heavy metals in the soil solution or by ingestion of pore water, polluted food and/or soil particles [15,16]. Assimilation of contaminated earthworm tissue by predators may lead to accumulation of toxic chemicals throughout the food chain [17]. Their survival and behaviour in contaminated soils has implications for their use as biological indicators of soil health and as agents of soil restoration [18]. It has been recommended test species to evaluate soil contaminations in acute toxicity [19-23]. *Eudrilus eugeniae* is widely spread in warm regions of tropical Africa and are easy to handle and harvest. They belong to the group of epigeic earthworms that live in the uppermost soil layer and in the litter layer feeding mainly on organic matter and process waste quickly under ideal conditions with high rate of reproduction [24,25]. Because of the shallowness of water table and the soil type in Port Harcourt, it is important to monitor and assess possible impact of contamination from dumpsites in the state. The aim of this study is to;

1. Determine the bioavailability of Pb, Cu, Cd, Ni, Zn and Cr in soil using earth *E. eugeniae* as bio indicators.
2. Identify the extent of contamination of these accumulated heavy metals by comparison of measured field concentrations with international standards.

The recent interest in environmental risk as regards to heavy metal contamination has been attributed to public concern towards health issues as well as keeping the environment safe. This is especially important when considering the toxic potential, from not properly managed dumpsites located in residential areas in Port Harcourt. This provides useful information to estimate the health risk to humans and the environment in order to prevent/ avoid a health epidemic as reported in the past [24,26-28].The study intends to use earthworm *E. euginiae* because it is widely spread in the soil of Port Harcourt and practicable with ease of interpretation. This helps to estimate with greater confidence the extent of ecological risk of these heavy metals.

2. MATERIALS AND METHODS

Two dumpsites were sampled in Obio Akpor local government in Port Harcourt; Wimpy and Mgbuoba along with an open dump in Rumuokwuta which consists of hospital, market and house waste (Fig. 1). Obio Akpor local government of Port Harcourt was sampled because it is densely populated and dumpsites are located in residential areas in the heart of the city surrounded by buildings and business activities. Mgbuoba was the only active dumpsite in the city as at the time of this study. It was commissioned in June 2003, closed temporarily in January 2005 and re-opened June 2006. Wimpy was commissioned in 2004 and closed early 2006. Both dumps are partially fenced with refuse spilling out of the constricted area into roads and residential quarters.

2.1 Sampling, Pre-treatment and Analysis

Soil and earthworms were sampled randomly by using number tables marked on the map of the study area on each dumpsite at regular interval of three days, morning and night in the month of July (wet season) 2006 [29]. Soils from the upper soil layer were sampled using a spade and earthworms in the soil were collected by hand sorting. A number of earthworms were collected from each dumpsite and washed free of adhering soil particles using distilled water. The earthworms were divided into two decontaminated glass jars; one, containing 4% formalin to preserve the earthworm for taxonomy and the other was empty for metal analysis. The jars were decontaminated by soaking in 1% nitric acid for 24 hours. Earthworms for metal analysis were refrigerated overnight to allow time to purge the soil in the guts [30]. They were then rinsed and placed in decontaminated jars and frozen. Soil samples collected were allowed to dry at room temperature for 14 days and passed through a 2 mm sieve after manual removal of stones, woods and other objects.

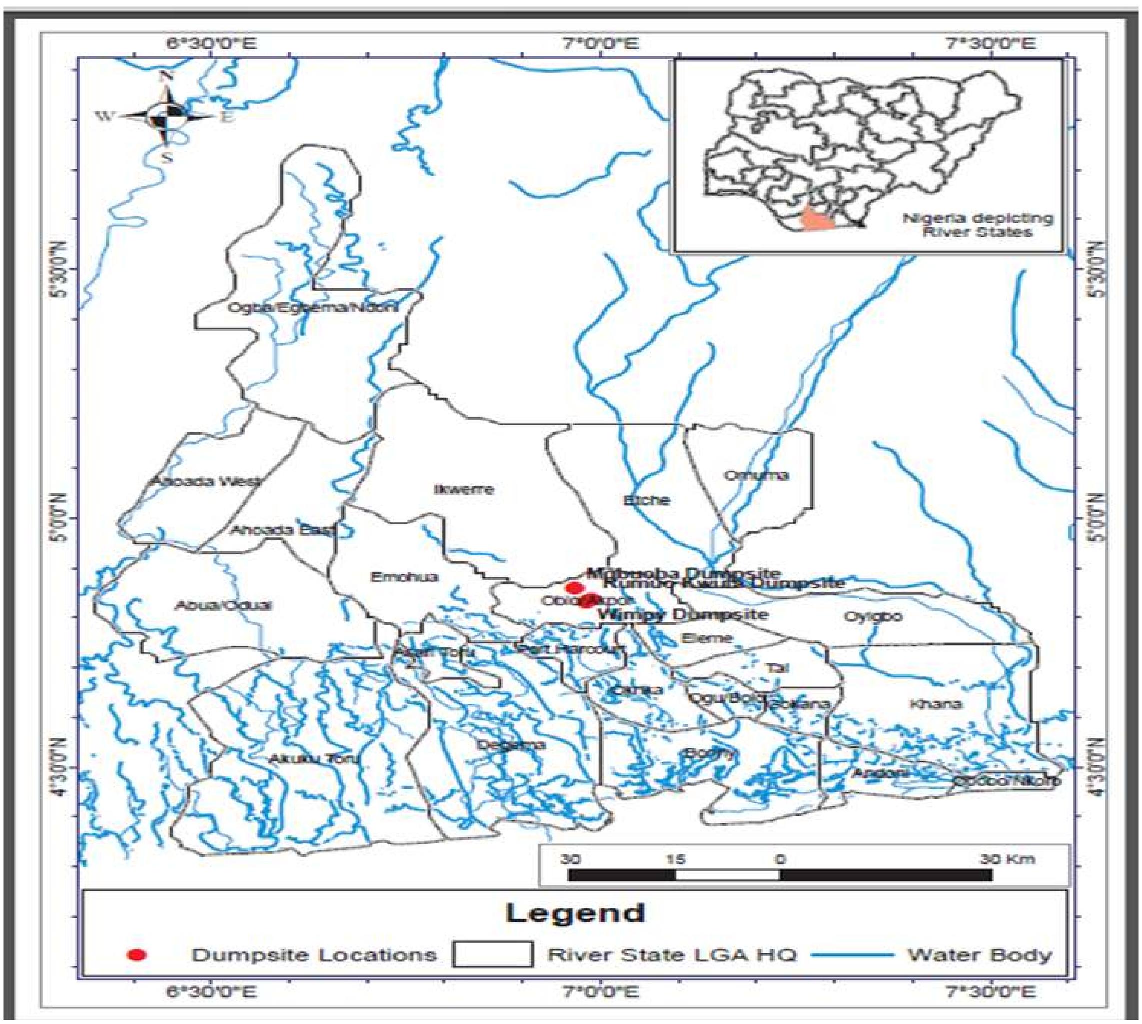

Fig. 1. Map of the study area

Thawed earthworms (*Eudrilus eugeniae*) from each dumpsite were macerated using previously cleaned and decontaminated laboratory mortar to get a compost sample and 3 g was measured for analysis. Residues of lead, zinc cadmium, chromium, nickel and copper in the tissue were extracted using 2 ml of concentrated nitric acid. It was heated to dryness on a hot plate and the digest was re-dissolved in 1 ml concentrated nitric acid and made up to 50 ml with distilled water in a volumetric flask. Agilent 240 AAAtomic Absorption Spectrometer was used for the determination of all the elements, simultaneous background corrections were made using a deuterium lamp.

5 g of sieved soil sample was weighed, 10ml concentrated nitric acid was added and digested over a hot plate for 2 hours. The suspension was filtered and the solution was made up 25ml with distilled water in a volumetric flask. Heavy metals were estimated using Agilent 240 AAAtomic Absorption Spectrophotometer. A blank determination and quality control samples were carried out using the above procedure to monitor the instrument performance and correct for contamination and impurities. Quality control samples were prepared in identical fashion with spikes of known concentrations of heavy metal solution for each metal; and percentage recoveries between 94-101% were obtained. The spikes and calibration standards were prepared using 100 mg/L stock solution (Merck). The blanks and spikes were introduced after every 10 samples analysed, and in case of deviation of more than 10%, the equipment was recalibrated.

2.2 Soil pH

Soil pH was determined in air dried soil in a 1:1 w/v ratio with distilled water. 10 g of air dried soil was weighed into 50ml beaker and 10ml of distilled water added. The mixture was stirred thoroughly for 1 minute and allowed to stand for 30 minutes to allow suspended particles to settle before pH reading. The pH was determined using a calibrated pH meter.

2.3 Organic Matter Determination

IITA [31] procedure was adopted for the determination of organic matter. 0.5 g of air dried soil was weighed into a 100 ml conical flask, 5ml 1 M potassium dichromate solution was added. 10 ml concentrated sulphuric acid was immediately added. This was mixed by swirling the flask for about a minute and left to stand for 30 minutes. 20 ml of deionized water was added and cooled with tap water. 3 drops of ferroin indicator were then added. The solution was titrated with 0.5 N ferrous sulphate solution until the colour changed from deep green through bright green to reddish brown (maroon). A blank was carried out through the procedure to correct for impurities from reagent and contamination during the process.

The percentage organic carbon is given by the equation:

$$\frac{(\text{MeK}_2\ \text{Cr}_2\ 0_7 - \text{MeFeSO}_4) \times 0.0031 \times 100 \times F}{\text{Mass (g) of air dried soil}}$$

F = Correction factor (1.33)
Me = Normality of solution x ml of solution used
% organic matter in soil = % organic carbon x 1.729.

2.4 Statistical Analysis

Mean concentrations of heavy metals in earthworms and soil samples for each day sampled were calculated and used for statistical analysis. Pearson correlation analysis was used to identify all possible combinations to determine the bioaccumulation of heavy metals by earthworms in soil. Correlation analysis of soil and earthworms for all the metals in each dumpsite was carried out. The equation for the correlation plots is: Yi=p1*xi+p2, for i=1,2,3,...,6, where p1 and p2 are the coefficient of the metal concentration, x represents individual metals, and y is the basic fitting. The fittings are done within the accuracy of 95% confidence Interval. The 'y' in this equation is thus used to draw the correlation line on the data scattered plots. The fiitings are derived by first computing the coefficient, p1 and the constant, p2 from the pearson correlation equation. In this study, rather than show those values of p1 and p2, we presented the fittings as the effects of p1 and p2, because either way the result is the same.

3. RESULTS

Table 1 shows the percentage organic matter and soil pH in the soil. Mean concentrations of heavy metals in both earthworms and soil is shown in fig 2-4. Soil pH was acidic in Wimpy dumpsite (4.63) while soil pH at Rumuokwuta and Mgbuoba were neutral with values 6.52 and

7.43 respectively. All soils sampled had low organic matter content with range between 1.16-2.68%. Mean metal concentrations in soil ranged between 13-47 mg Pb/kg, 57.3-72.5 mg Cu/kg, 0-0.5 mg Cd/kg, 4.9-42 mg Ni/kg, 6.88-10.1 mg Cr/kg and >100 mg Zn/kg. Pb and Cd concentrations in earthworms were relatively higher than mean concentrations in soil with the exception of Cd at Rumuokwuta where earthworm concentration was 0.46 mg Cd/kg. Mean concentrations of Cu, Ni, Zn and Cr in earthworms in all the dumpsites were lower than concentrations in soil.

Table 1. Soil pH and percentage Organic matter in soil samples from contaminated dumpsites in Port Harcourt

Dumpsite	pH	% Organic matter
Wimpy	4.63±0.01	1.16±0.23
Mgbuoba	7.43±0.44	2.68±0.04
Rumuokwuta	6.52±0.17	2.43±0.13

Mean metal concentrations in soil were positively correlated with the exception of Cu which was negatively correlated (Fig. 5). There was no significant difference with concentration in earthworms *Eudrilus eugeniae* with soil p=.08 (Pb), p=.48 (Cd), p=.54 (Ni), p=.19 (Zn), p=.18 (Cr) and p=.28(Cu).

4. DISCUSSION

The correlation between metals Pb, Cd, Ni, Zn, Cu and Cr of both earthworm *E. eugeniae* and soil are in agreement with data reported in literature [32-34] which suggests that earthworm *E. eugeniae* are good bioindicator of available metal concentration. Estimated population and type of waste dumped were factors that influenced the concentration of heavy metals in soil and earthworms *E. eugeniae* at the dumpsites. Wimpy, although closed as at the time of the study was found to have the highest concentration of Pb (Fig. 2). This can be attributed to the fact that Wimpy was the highest populated area sampled and had the highest volume of waste generated according to Ayotamuno [35] which includes waste from automobile workshops, industries and domestic waste as a major contributor to increased level of Pb in both soil and earthworm *E. eugeniae*. Mgbuoba which was the only active dumpsite as at the time of the study had the highest concentration of Cu, Zn and Cr (Figs. 2 and 4). Rumuokwuta was characterised with hospital waste and this was evident in the highest concentration of Ni and Cd (Fig. 3) found in soil at the dumpsites. The major source of Cd-Ni contamination in soil is from dumping of Ni/Cd batteries used as rechargeable or secondary power source [36].

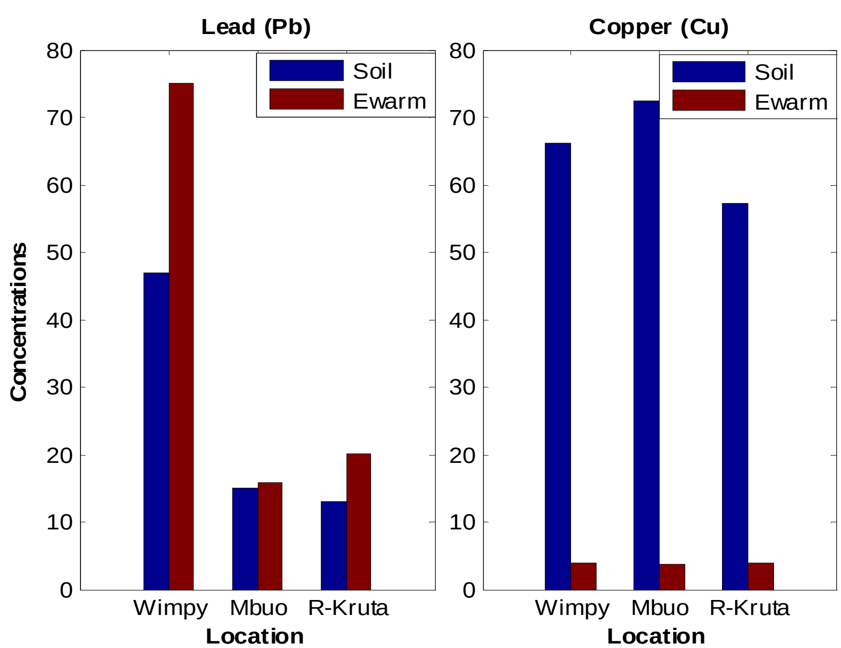

Fig. 2. Concentration of lead (Pb) and copper (Cu) in the three dumpsites

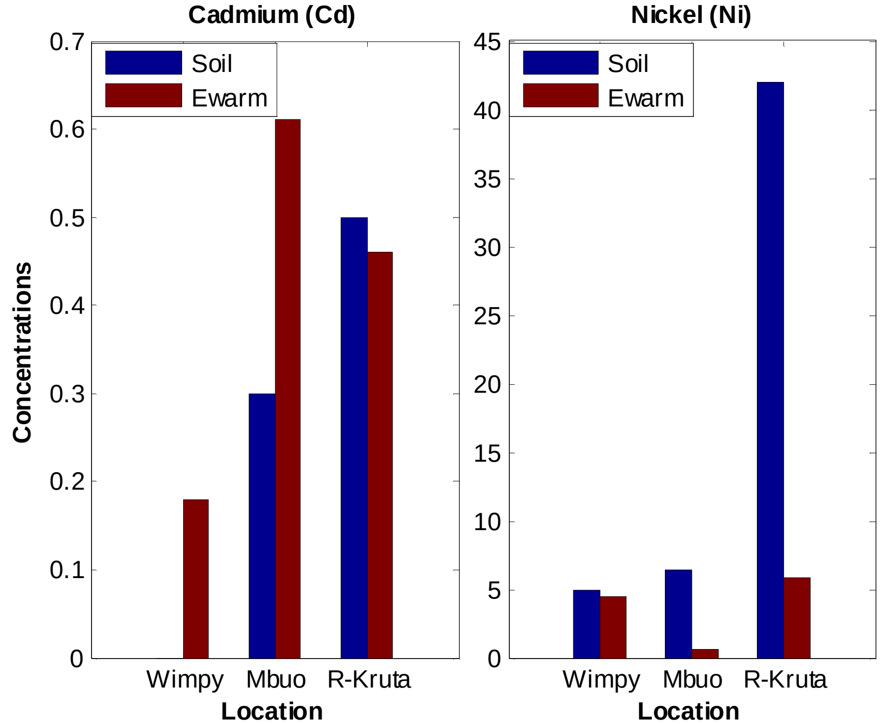

Fig. 3. Concentration of cadmium (Cd) and nickel (Ni) in the three dumpsites

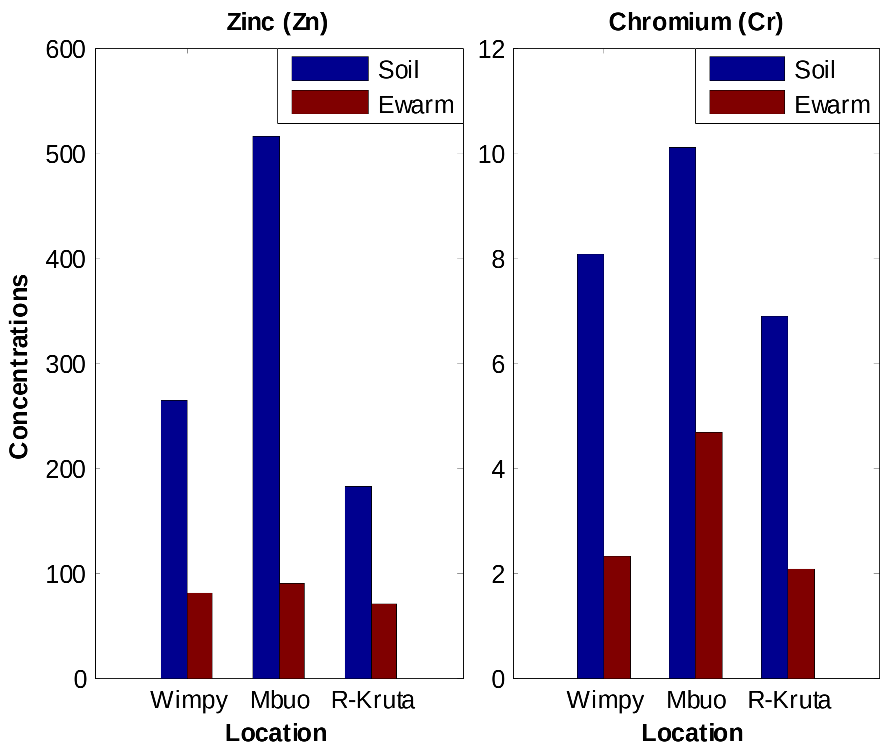

Fig. 4. Concentration of zinc (Zn) and chromium (Cr) in the three dumpsites

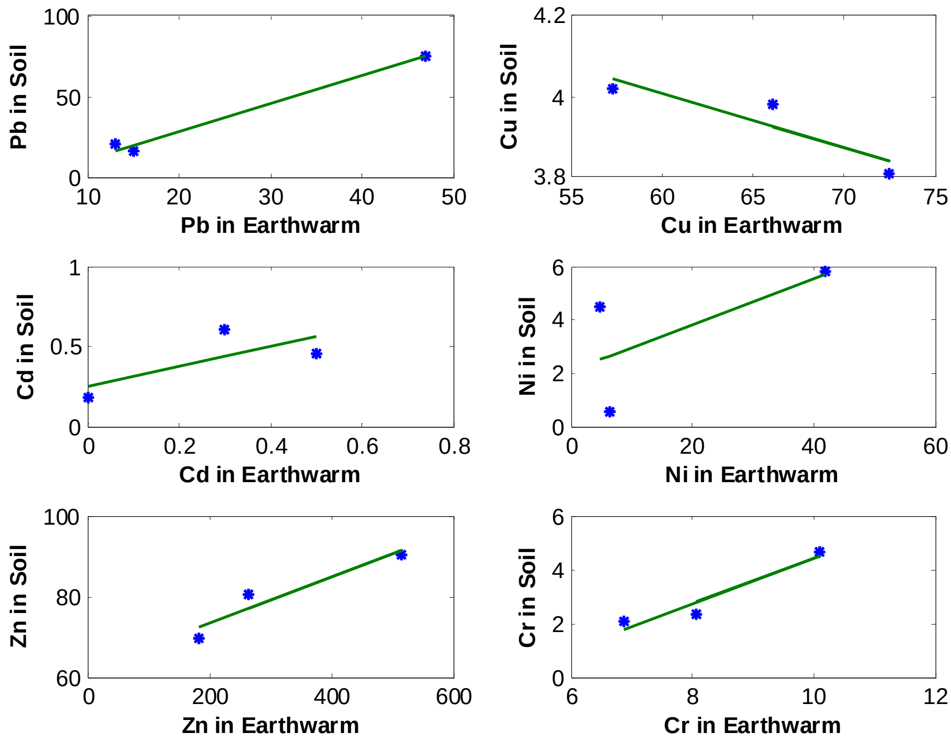

Fig. 5. Correlation of metals concentrations in soil and earthwarms in dumpsites

Though organic matter found in soil of sampled dumpsites were found to be low and pH ranged from acidic to neutral, accumulation of heavy metals by earthworm *E. eugeniae* were low compared to concentrations found in soil [37] with the exception of Pb and Cd. This suggests the presence of complex species in the soil solution which can significantly affect the transport of metals through the soil matrix relative to the free metal ion [38]. Pb was relatively more mobile than other metals studied. Mobility of Pb could be as a result of sulphate reduction in contaminated soil, which may mobilize Pb through formation of sulphide colloids [39]. Another possible explanation is the decrease in sorption of Pb in the presence of complexing ligands and competing cations. Pb also has a strong affinity for organic ligands and the formation of such complexes may greatly increase the mobility of Pb in soil [38]. Cd was not detected in soil at Wimpy dumpsite but concentrations of 0.18 mg/l was found in earthworms *E. eugeniae,* this is as a result of the solubility of Cd under acidic condition (4.63 at Wimpy dumpsite) with very little adsorption of Cd by soil thereby enhancing Cd mobility.

The amount of metals accumulated within earthworm tissues is partly dependent on the absolute concentration of metal within a given soil and physiochemical interactions [40]. Cu concentration in earthworm *E. eugeniae* and soil were negatively correlated, indicating a decrease in earthworm *E. eugeniae* as soil concentration increases in the dumpsites. This could be explained by the ability of Cu to form strong complexes in soil [36]. Also, Joan and Bert [38] reported that Cu is adsorbed to a great extent by soil and soil constituent compared to other metals. Though concentrations of Zn in earthworm *E. eugeniae* were positively correlated with concentrations in soil with no significant difference, levels in earthworm *E. eugeniae* were found to be relatively lower compared to soil. This indicates the ability of earthworms to regulate Cu and Zn which are essential elements in soil [41]. Ni and Cr showed similar trends with Zn in all the dumpsites as concentrations in soil and earthworm *E. eugeniae* were positively correlated with no significant difference.

Reduced mobility and bioavailability of Cr in soil could be attributed to the transitional nature of Cr in soil and the anaerobic reduction of Cr(VI) to Cr(III) [42]. Low bioaccumulation of Ni in earthworms *E. eugeniae* at the dumpsites (Fig. 3) was also reported by Agbaire and Emoyan

[43] who reported that Ni was least accumulated by earthworms *Lumbricus Terrestris* in dumpsites in Abraka. Heavy metal contamination of soil samples in Wimpy and Mgbuoba followed the same trend Zn>Cu>Pb>Cr>Ni>Cd while Rumuokwuta was in the order Zn>Cu>Ni>Pb>Cr>Cd. Earthworm *E. eugeniae* bioaccumulation of heavy metals in Wimpy and Rumuokwuta followed the trend Zn>Pb>Ni>Cu>Cr>Cd while Mgbuoba had a trend of Zn>Pb>Cr>Cu>Cd>Ni. Using the Canadian environmental guideline for contaminated site remediation [44] and Toronto public health (TPH) guide for soil testing in urban gardens [45]. It was shown that metals did not exceed the permissible level for all the dumpsites with the exception of Zn in Mgbuoba dumpsite.

5. CONCLUSION

The result shows correlation between heavy metal concentrations in earthworm *E. eugeniae* and soil indicating that earthworms *E. eugeniae* are good bio indicator of heavy metal contamination in dumpsites. Concentrations of heavy metals in dumpsites were influenced by type of waste, population around the dumpsite and duration of usage. Concentration of heavy metals in soil samples from dumpsites were within the Canadian environmental guideline for contaminated site remediation and Toronto public health (TPH) guide for soil testing in urban gardens with the exception of Zn which requires remediation action. Bioavailability and mobility of heavy metals were reduced, resulting to low bioaccumulation of heavy metals by earthworm *E. eugeniae*. This suggests the presence of complex species which limits the mobility of heavy metals. As a result, the dumpsite does not pose an immediate threat to humans, however, due to the absence of a proper engineered leachate collection system in the dumpsites, possibilities of contamination of groundwater and erosion into nearby water bodies are worthy of concern.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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(Accessed 27 August 2014).

Peer-review history:
The peer review history for this paper can be accessed here:
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