Earthworm as Bio-indicator of Heavy Metal Pollution around Lafarge, Wapco Cement Factory, Ewekoro, Nigeria

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Abstract

Heavy metal pollution of terrestrial and aquatic ecosystems has long been recognized as a serious environmental concern. The use of earthworm as bio-index of soil heavy metal pollution was hereby examined. Using the Lagos/Abeokuta express road as transect, four replicates each of soil and earthworm samples were collected from five points: 100m north, 500m south, 1000m north, 1000m south, 2000m north of the Lafarge WAPCO cement factory, Ewekoro and a sixth point at the Arboretum, University of Agriculture, Abeokuta (control site). Using spectro-photometry method, heavy metal concentrations (Pb, Cu, Mn, Zn, Cd, Co) were measured in all samples of soil and earthworm. Histological studies were conducted on earthworm sections. All data obtained were subjected to analysis of variance and post hoc tests to establish significance at ($p<0.05$). Heavy metal concentrations in earthworm and soil decreased significantly ($p<0.05$) with increasing distance from the factory. The heavy metal concentrations in soil and earthworm around the factory were significantly higher than that obtained at the control site ($P<0.05$). Histological studies on the earthworm body wall revealed dark spots and patches on the tissues of the earthworm samples collected from the cement factory area compared with clear earthworm tissues at the control site. Bioaccumulation of heavy metal in soil and earthworm was noticeable around West Africa Portland Cement factory, Ewekoro, Nigeria and could serve as a possible bio-index of heavy metal pollution.

Key words: Bio-indicator, Bio-index, Earthworm, Heavy metal, Spectro-photometry

Introduction

Soil is the region on the earth's crust where Geology and Biology meet; it is the land surface that provides a home to plant animal and microbial life (Pelczar *et al*., 1993). Soil samples differ in their content of heavy metal and pH depending on climate, soil origin, composition and human activities (Hashem and Al-Obaid, 1996). Heavy metals in the soil have received attention as environmental contaminants because of their extended persistence, and toxicity to many organisms (Hashem and Al-Obaid, 1996).

Heavy metal contaminated soils constitute a huge problem as it affects human health and environmental quality (Otitoloju., 2009). The anthropogenic sources of the heavy metals, in soils are either primary sources, i.e. the heavy metals are added to the soil as an outcome of working the soil, such as fertilization or secondary sources where heavy metals are added to the soil as a consequence of a nearby activity, such as smelting or aerial deposition (Ferguson, 1990).

Cement dusts are particulate air pollutant generated from cement production processes. They are produced during blasting of raw materials, grinding of cement clinker and packaging or loading of finished cement. It is one of the major means by which heavy metal could be added to the soil. Notably among the heavy metals added to the soil through cement production includes: cadmium, copper, lead and zinc (Shemback, 2009).

Earthworms contribute to the aeration of the soil, hence the need for its study. In earthworm toxicity testing, the organisms are in close contact with soil and can be used to evaluate bioavailability (Connor, 1988). In addition, the earthworm is a representative of soil fauna and recognized to be a relatively sensitive indicator (Connor, 1988). In soils, earthworms constitute 60–80% of the animal biomass and play a critical ecological role (Double and Brown, 1988). For example, it is estimated that under favourable conditions, earthworms can move up to 18 tons of soil per acre per year. Being continuously exposed to the soil through direct dermal contact and ingestion of soil materials, earthworms are therefore in close contact with exogenous dusts (Double and Brown, 1988). This study is therefore aimed at establishing the level of bioaccumulation of heavy metal in earthworm species found around the cement manufacturing industry.

Study Location

The experimental area is LAFARGE Cement factory, WAPCO, Ewekoro, within Latitude 6º 54'N and Longitude 3º 13' E. The cement factory is situated within Ewekoro Local Government area of Ogun state, SW Nigeria between June and September, 2010. Six soil sites were selected viz: 100meters north of the dust stack, 500meters south of the dust stack, 1000meters north of the dust stack, 1000meters south of the dust stack and 2km south of the factory. University of Agriculture Abeokuta arboretum was used as control.

Materials and Methods

All the six sites used were randomly sampled using a quadrant to collect the soil samples. The top 10cm of soil was sampled and hand sorted to remove root and litters. This was done in four replicates with each replicate obtained from an area of $50cm^2$. The samples were stored in labeled polythene bags prior to analysis. 1.00g of thoroughly mixed soil samples were digested using 15ml of concentrated $HN0_3$, 5ml of concentrated Hydrochloric Acid (HCl) at 100^oC for 20 min. This was done after drying the soil samples at 80^oC in a digestion microwave oven. The samples were then filtered using cotton wool and diluted to 50 ml with distilled water and kept in sample bottles.

The digested samples were analyzed for heavy metals using Atomic Absorption Spectrophotometer, (VGR System model 210). Co, Cu, Pb, Zn, Cd and Mn were recorded in parts per million. The result in parts per million were calculated as follows:

$$\text{PPM (mineral)} = \frac{\text{Reading from AAS} \times \text{Volume of digest}}{\text{Weight of Sample}}$$

Earthworm Collection and Analysis

Earthworms were collected using the quadrant $50cm^2$. Sampling was done on four different points on each of the six sites from a depth of 10cm. Five to ten earthworms from each of the six locations were collected and identified using the description of Owa (1992). The earthworms collected were kept in aerated plastic containers with wet soil samples from the point of collection.

The dry earthworms were crushed, and then digested as follows: 1.00g of sample was mixed with 15ml conc. HNO3 and 5 ml conc. HCl, and heated progressively to 150^oC at 800 kPa in 250ml conical flask using a hot plate for 2hrs. After cooling at ambient temperature, the solution was filtered using cotton wool and made up to 50 ml with distilled water. The metal contents were determined by Atomic Absorption Spectrophotometer, (VGR System model 210) for Co, Cu, Pb, Zn,Cd and Mn.The result in parts per million were calculated as follows:

$$\text{PPM (mineral)} = \frac{\text{Reading from AAS} \times \text{Volume of digest}}{\text{Weight of Sample}}$$

Histological Analysis of Earthworm Samples

Earthworm sample from each of the five locations which were stored in FAA were later taken and washed with distilled water after which the abdominal portion of each earthworm were dehydrated by passing them through increasing concentration of ethanol 50%, 70%, 90%, and 100% one hour each. This was done to remove water from the tissues. The dehydrate portions were then cleared in three changes of xylene to remove excess alcohol from the tissues. The portions were thereafter impregnated in three changes of molten wax to remove xylene from the tissue and to replace it with paraffin wax. The portions were embedded in molten wax and allowed to solidify. The block were mounted on the microtome and sectioned.

Statistical Analysis

For all the parameters that were tested, comparisons of means were analyzed statistically, using one-way Analysis of Variance (ANOVA) and Pearson chi–square statistics at probability of $P<0.05$. Relationships were then tested using the Pearson correlation index at the same probability. All statistical analysis was performed using SPSS 16.0 software.

Table 1: Heavy Metal Concentration in Soil across the Six Locations (X±SD)

	Cd	Co	Cu	Mn	Pb	Zn
100m NF	10.35±1.02^{c}	11.2833±11.28^{c}	2.3333±0.29^{b}	208.52±27.01^{b}	50.3167±0.55^{e}	98.3833±2.24^{c}
1000m NF	7.6±0.96^{b}	1.2167±0.03^{a}	2.35±0.1^{b}	104.48±33.9^{a}	34.3167±1.99^{c}	82.7167±8.0^{b}
2000m NF	4.7333±1.38^{a}	3.7±0.43^{a}	1.3167±0.08^{a}	85.6167±0.55^{a}	19.0333±2.64^{b}	16.4167±0.49^{a}
500m SF	9±3.0^{c}	5.6667±0.29^{b}	8.2167±1.4^{c}	183.951.2^{b}	44.9000±0.13^{d}	107.23±0.55^{c}
1000m SF	4.3±2.04^{a}	1.15±0.26^{a}	1.5± 0.1^{a}	88.1833±21.59^{a}	16.5333±2.07^{b}	19.2833±8.18^{a}
UNAAB	2.825±0.81^{a}	1.6933±0.02^{a}	0.9167±0.8^{a}	68.575±13.9^{a}	7.85±3.55^{a}	14.3±0.07^{a}

a, b, c indicates significant difference in metal concentration across the column ($P<0.05$); Data are in mg/kg dry weights; NF: North of factory; SF: South of factory

Table 2: Heavy Metal Concentration in Earthworm (X±SD)

	Cd	Co	Cu	Mn	Pb	Zn
	ENP	ENP	ENP	ENP	ENP	ENP
100m NF	21.866±2.01^{c}	2.63±0.06^{b}	6.8±1.04^{c}	131.33±18.8^{d}	25.13±0.81^{c}	216.87±0.84^{d}
1000m NF	9.933±0.06^{a}	0.667±0.06^{a}	1.93±0.05^{a}	106.13±2.31^{c}	15.47±3.8^{b}	84.83±0.40^{b}
2000m NF	9.966±0.06^{a}	2.7±0.12^{b}	4±0^{b}	79.06±0.58^{b}	24±3.46^{c}	133.27±1.0^{c}
500m SF	14.7±3.16^{b}	0.867±0.81^{a}	4.367±1.76^{b}	67.96±9.4^{a}	21.3±0.61^{c}	142.57±0.55^{d}
1000m SF	7.667±1.1^{a}	0.5±0.1^{a}	1.2±0^{a}	52.6±11.1^{a}	10.07±1.52^{a}	42.45±0.21^{a}
UNAAB						

a, b, c indicates significant difference in metal concentration across the column ($P<0.05$); Data are in mg/kg dry weights; NF: North of factory; SF: South of factory; ENP: Earthworm not present

Results and Discussion

Concentration of heavy metals tested in soil samples collected from the six locations decreased with increasing distance from the factory area (Table 1). Cd, Co, Cu, Mn, Pb and Zn showed a decreasing metal concentration from 100m NF, 1000m NF and 2000m NF. Metal concentrations at 500m SF were higher than 1000m SF. Soil samples collected from UNAAB arboretum had the least metal concentration for all the metals tested. The heavy metal concentrations in soil samples collected from the six locations decreased in this order; Mn> Zn> Pb> Cd> Co> Cu. A steady decrease in the concentration of Cd, Co, Cu, Pb and Zn was recorded from the earthworm, *Hyperiodrilus africanus* as we moved away North of the Factory, 100m NF, 1000m NF and 2000m NF with the least concentrations of the heavy metals recorded from the earthworm samples collected from UNAAB Arboretum as shown in Table 2.

It was further observed that nearly in all sampling locations the concentrations of the heavy metals in the earthworm samples were higher than soil samples from the same location whereas earthworms and soil samples from the UNAAB Arboretum (Control location) showed only slight variation in the concentrations of the heavy metals. On another hand, Mn and Co concentrations were exceptionally higher in the soil samples of the Arboretum than the earthworm samples. This might probably be due to some form of mechanism in the earthworms which increases the concentration of these two heavy metals in the earthworms collected from the non contaminated soils of the Arboretum.

On a comparative basis the concentration of Mn was the highest (68.58 mg/kg) for all the metals analyzed while the lowest was Cu with a concentration of 0.92 mg/kg in the soil samples. This trend of a highest Mn concentration and a lowest Cu concentration obtained in this study is similar to the reported work of Anderson and Laursen (1982) who worked *on Lumbricus terrestris* in uncontaminated soils.

In contrast to the earlier observation of earthworm metals concentration being higher than soil metal concentration in the control site, it was observed that in nearly all cases, the concentration of metals in the soil samples were higher than those of the earthworm samples, with the exception of Cd and Zn concentrations which had higher concentrations in earthworms than in soil samples.

This observation may be due to the chemical changes which occur in the alimentary tracts of earthworms and hence render various metals more available to plants. It may also be as a result of in built earthworm body mechanism which reduces the rate at which the earthworm absorbs heavy metal from contaminated soils. Also mineralization of dead earthworms releases accumulated heavy metals back to the environment (Morgan and Morgan 1993).

The general trend of the metals concentrated either in the soil or in the earthworms show that Mn (131.33 - 208 mg/kg) had the highest concentration while Cu had the lowest (5.217 -6.8 mg/kg) and follow a general trend of Mn > Zn > Pb > Cd > Co > Cu. Though this trend may not be exclusive, it follows to an extent the reported trend of Sposito (1992) in his work on trace metal in arid zone soils amended with sewage sludge of which the trend Mn > Zn > Pb > Cu > Cr > Cd was reported.The main variation observed lies with the cadmium concentration. In this work, rather than Cadmium being the least in terms of heavy metal concentration, copper had the least concentration. This is however in conformity with the work of Al-Yemeni and Hashem (2006) who had reported that cadmium concentration was more than cobalt which was also more than nickel in soil samples collected around Aramco Gulf Operation Company Saudi Arabia.

PLATE 1: Transverse section of control earthworm showing normal tissue arrangement. The outer ring (pink) consists of the epidermis, circular muscles, and longitudinal muscle (radiating lines). (X400)
NOTE:
A: EPIDERMIS
B: LONGITUDINAL MUSCLES
C: COELOM
D: TYPHLOSOL

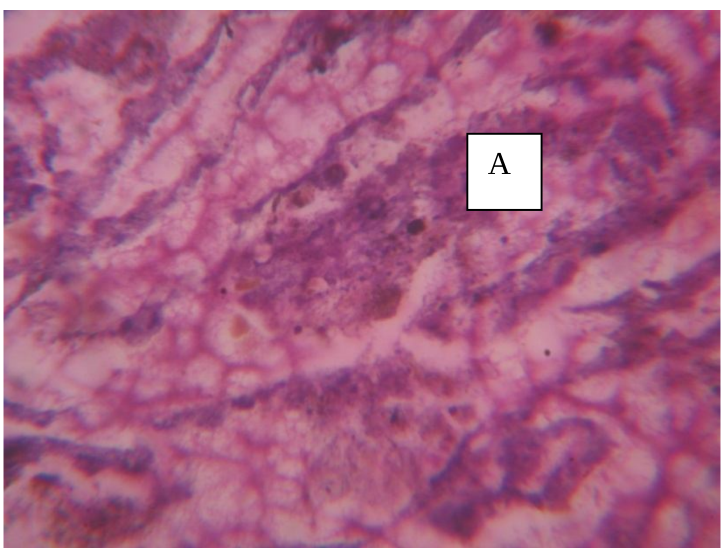

PLATE 2: Transverse section of earthworm collected from 500meter south of the dust stack. (X400)
NOTE: A: DARK SPOTS WHICH LOOKS LIKE ANTHRACOSIS AND SILICOSIS OF THE LUNGS

Furthermore, this present study revealed that heavy metal concentration in soil and earthworm decreased with increasing distance away from the factory area. The histological studies showed that earthworm samples collected at 500meter south of the dust stack, 1000meters south of the dust stack, 1000meters north of the factory and 2000meters north of the factory showed dark patches on their tissues (plate 2). Whereas earthworm samples collected at UNAAB arboretum have clear surface (Plate 1). The histological studies revealed on the body wall of the earthworms what seemed like anthracosis and silicosis of the human lungs. Anthracosis was described by McGraw-Hill (2002) as a generic term for the blackening of tissues, often understood to mean carbon dust deposition in the lung and lymph nodes, which does not itself cause disease, and is usually present in urban dwellers, and in those working in certain occupations eg, coal mining. The condition was also described as heavy black deposits of carbon; a common necropsy found in the lung tissues of dogs which have passed a busy working life in a heavily industrialized city (Saunders, 2007).The dark spots were visibly present in all the body wall tissues of the earthworm collected from the factory area as seen from plates 2. However, plate 1 showed that earthworms collected from control site (UNAAB Arboretum) had clearer tissues when compared with the ones from the factory area.

In conclusion, this study confirms that the earthworm (*Hyperiodrilus africanus*) can serve as a bio-indicator in polluted environments.

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