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ABSTRACT

The bioaccumulative properties of heavy metals increase their chances of being incorporated into the food chain. In this study, the bioaccumulation and biotransfer of lead (Pb) was studied in an experimental food chain involving the earthworm, *Eudrilus eugeniae* and the catfish, *Clarias gariepinus*. Earthworms were exposed to soils contaminated with Pb at different concentrations for 14 and 28 days: Control (Group 1), 3000 (Group 2), 4500 (Group 3) and 5000 ppm (Group 4). Earthworms from the 14 days exposure were subsequently fed to four corresponding groups of *C. gariepinus* for 28 days and the fish organs were analysed for Pb content. The highest earthworm Pb load was observed in group 4 at 1774 ppm on day 14 and 1898 ppm on day 28. In fish, Pb concentration was in the order of gut > bone > muscle. Highest Pb load was observed in the group 4 fish gut (1.40 ppm) while group 4 fish bone had 1.17 ppm. All results showed significant differences ($p < 0.05$) from the control group. Bioaccumulation and biotransfer of Pb from earthworm to fish was observed to be concentration and time dependent. The Pb load in muscles of exposed fish exceeds the FAO (1983) permissible limit for Pb in fish. Heavy metals, however, need not bioaccumulate in large amounts to exert their toxic effects on living organisms, hence, bioaccumulation at low concentrations should not be ignored.

Keywords: Bioaccumulation, Biotransfer, Lead, *Eudrilus eugeniae*, *Clarias gariepinus*.

INTRODUCTION

The quality of life on earth is linked inextricably to the overall quality of the environment which has, unfortunately, deteriorated over the years due to anthropogenic activities that threaten the wellbeing and survival of other components of the environment. These activities which range from industrial, domestic and agricultural processes are intimately associated with the

extensive use of a wide array of chemicals (Gerald, 2004) which include heavy metals.

Lead is identified as one of the prominent heavy metals in industrial effluents discharged into the Nigerian environment and it also featured prominently in water and sediment samples of effluents receiving water from storm drains (Oyewo, 1998). It is released into the Nigerian environment mainly through exhaust pipes of automobiles

as a result of its presence in gasoline (Nsofor *et al* 2007). Other sources of lead in the environment include mining and burning of fossil fuels, lead-based paint, batteries, refuse dump sites and so on (Doyle, 1998; EFSA, 2010). The contamination of soil with lead presents a health hazard to living organisms and is a difficult problem to address due to the persistent and ubiquitous nature of lead in the environment (Doyle, 1998). Organisms that inhabit these polluted environments are thereby, exposed to the contamination through several routes, one of which includes diet (Hayashi, 2009).

Animals can be exposed directly through diet when a contaminated food crop is ingested or by consumption of an organism in the lower trophic level of the food chain, which has accumulated to a certain level of the pollutant in its body system (Nusseý, 2000; Mupo *et al* 2008; Akan *et al* 2012). Setyorini *et al* (2001) noted that soil contaminated by lead may pose a threat to human health if incorporated into the food chain. Due to their vast use in industrial, agricultural and even domestic activities, it is imperative to not only determine their amounts in the environment but also their bioavailable concentrations. Several studies have revealed that earthworms are capable of accumulating lead from contaminated soil substrate (Spurgeon *et al* 1996; Rosciszewska *et al* 2003). Oyeleke *et al* (2006) reported that lead may have no apparent adverse health effects on the earthworms themselves but the risk is to the ultimate level of consumers (humans in most cases) considering the important position that they occupy in the food chain.

Bioaccumulation of lead by earthworms is a matter of concern because of its role as a prey to a large number of animals. They are commonly used as baits for fishes. Besides their importance as a vital source of protein for man, fishes play a key role in the aquatic food chain and are sensitive to heavy metals contamination (Kotze *et al*, 1995). Several research studies have revealed that fishes are also capable of accumulating heavy metals like lead in their tissues and organs. More worrisome is the fact that the muscle, which is the part of the fish consumed by man,

is also capable of accumulating these metals. The results of a study by (Kusemiju *et al*, 2012), amidst other research works are proofs that food fishes like *C. gariepinus* are also capable of bio accumulating metals in tissues and organs when exposed to polluted water.

A lot of the studies carried out on bioaccumulation of metals by *C. gariepinus* are focused on the water-borne route of exposure and rarely on the diet-borne route. Also, there are several evidences on the prey-predator relationship between earthworm and fish; with paucity of information on the transfer of lead and other pollutants in this trophic relationship. While the effects of diet borne metal are not as well studied as waterborne metals, a number of studies illustrate that dietary exposure to metals can result in toxicity (Drexel *et al*, 2003). This study determines the rate of bioaccumulation and biotransfer of lead in an experimental food chain involving earthworm, *Eudrilus Eugeniae* and catfish, *C. Gariepinus*.

MATERIALS AND METHODS

Biological material

Adult species of *Eudrilus eugeniae* with an average weight of 0.9g were collected randomly from moist soil around Eleyele River, Ibadan, Oyo State. Digging and hand sorting method according to (Owa, 1992) was employed in the collection of earthworms and transported to the laboratory for one week acclimation under laboratory conditions in perforated plastic pots containing soil and dried poultry manure at a temperature of $25 \pm 2^\circ\text{C}$. Worms were fed 10% of their body weight twice a week with fermented poultry dung (Dynes, 2003). The feed was spread on top of the substrate and water was sprinkled on soil to maintain its moisture.

Clarias gariepinus of $17 \pm 2\text{g}$ average weight were obtained from Oyo State Fisheries, Agodi Gardens, Ibadan. The fish specimens were transported to the laboratory in two large aerated plastic containers and acclimatized for fourteen days during which they were fed with crude protein pellet feed (Durante fish feed) 5% of their body weight twice daily.

The soil samples used for this study were collected from the surroundings of the Animal house, University of Ibadan where they were air dried, sieved and weighed into experimental plastic pots. The sites of earthworm and experimental soil collections were chosen because there is no evidence of industrial, agricultural or domestic waste discharge at this site.

Experimental set up

Anhydrous lead nitrate $Pb(NO_3)_2$ of analytical reagent grade obtained from Donald Scientific Suppliers, Agbowo, Ibadan, Nigeria, was used as the test substance. Soils weighed into the plastic pots were spiked with $Pb(NO_3)_2$ according to the methods of (Davies *et al.*, 2003) and (OECD, 2009). The experimental set-up consisted of four groups with a replicate each as follows; Group 1 (Control group): soil not treated with lead nitrate.

Group 2: soil treated with 3000 ppm of $Pb(NO_3)_2$

Group 3: soil treated with 4500 ppm of $Pb(NO_3)_2$

Group 4: soil treated with 5000 ppm of $Pb(NO_3)_2$

Ten (10) earthworms per kg of soil were randomly collected and introduced into the four groups of spiked soils for 14 and 28 days consecutive days and lead analysis was carried out. Four groups of eight (8) numbers of *C. garipienus* in replicate were also set up for fish and were feed with exposed earthworm based on the groups. Eight live earthworms per day from the 14 days exposure group were used and the period of fish exposure lasted for 28 days

LEAD ANALYSIS

*Earthworm analysis

Metal content analysis of the earthworms was done according to the method prescribed by (Adamu, 1997). Earthworms were collected at the end of the exposure, rinsed and killed by freezing at below 0°C and then oven dried (48 hours at 70°C) to constant weight. The dried

earthworms were crushed (0.2 mm) and digested performed by mixing 100 mg of the ground sample with HNO_3 and HCl in the ratio of 2:1 (4 ml HNO_3). The mixture was covered and left to stand for 12 h at ambient temperature. The mixture was then heated progressively to near dryness in a furnace mantle and the solution was made up to 50 ml with distilled water after cooling, then measure with Buck Scientific Atomic Absorption Spectrophotometer Model 2010/2011 VGP.

Soil analysis

The soil samples were air-dried at ambient temperature, and sieved (<2 mm). The soil samples were crushed to 0.2 mm and digested using the same acid mixture and procedure as described for earthworm analysis. Digests were then measured by Buck Scientific Atomic Absorption Spectrophotometer Model 2010/2011 (Adamu, *et al.* 1997).

Fish tissues analysis

Fish were dissected ventrally and body tissues collected. Digestion was done by a modified method of [10]. The bone, gut and muscle of each fish samples were digested with tri-acid mixture ($HNO_3:HClO_4:H_2SO_4 = 10:4:1$) at a rate of 5 mL/per 0.5 g of sample and were placed on a hot plate at 100°C temperature. Digestion was continued until the liquor became clear. All the digested liquors were filtered through Whatmann 541 filter paper and diluted to 25 mL with distilled water. Determination of Pb was made directly on each final solution using Buck Scientific Atomic Absorption Spectrophotometer Model 2010/2011 VGP.

Data Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0. Results were expressed as the mean of the replicates $\pm$ standard error. Analysis of variance (ANOVA) was used to test for statistical differences between the species of different group which was followed by Duncan multiple tests to

compare the experimental groups and the control. Values were considered significant at $p < 0.05$.

RESULTS

The Physico-chemical parameters of the unexposed soil were presented in Table 1. The percentage Organic carbon content and water holding capacity were 1.0% and 62% respectively with pH of 6.8. The background lead concentration is 315 ppm.

The background lead concentration is 315 ppm. The initial and final lead concentrations of the soil are presented in Table 2. Reduction in the soil lead concentrations was recorded on Day 14 after earthworm exposure. The highest final concentration of 4430 ppm was recorded in group 4 soil compared the initial concentration of 5315 ppm. A reduction in the group 1 soil (control) was also recorded, from 315 ppm to 255 ppm.

Highest earthworm lead load of 1774 and 1898 ppm were recorded in group 4 on Days 14 and 28 (fig. 1). The least

earthworm lead concentrations were observed in group 1 soil (control); 104.5 and 150.5 ppm on Days 14 and 28. Groups 2 and 3 earthworms also indicate increase in the bioaccumulation of lead based on increase in concentrations and exposure period (fig. 1).

Progressive concentration dependent increase was also recorded in the level of bioaccumulation of lead in fish organs. The least concentrations were seen in the control group with values approximately equal to 0.02 to 0.08 ppm. Fish lead concentration was in the increasing pattern of gut > bone > muscle. Group 4 fish fed with the highest lead contaminated worms had the highest lead load and the concentrations of lead detected in this group were 0.98, 1.17 and 1.40 ppm in the muscle, bone and gut respectively. This was followed by the fish in group 3 with lead load of 0.95, 1.05 and 1.07 ppm in the muscle, bone and gut respectively. Lead concentrations of 0.65, 0.76 and 0.83 ppm were detected in the muscle, bone and gut of fish in group 2.

Table 1: Background Physico-chemical parameters of the soil sample

Parameters	Values
pH	6.8
Organic Carbon Content	1.0%
Water Holding Capacity	62%
Lead	315 ppm

Table 2: Soil Lead Concentrations at Days 0 and 14 (ppm)

Group	Day 0	Day 14
1	315 $\pm$ 0.0 ^a	255 $\pm$ 1.0 ^b
2	3315 $\pm$ 0.0 ^a	2490 $\pm$ 1.0 ^b
3	4815 $\pm$ 0.0 ^a	3220 $\pm$ 0.0 ^b
4	5315 $\pm$ 0.0 ^a	4430 $\pm$ 1.0 ^b

Data on same row with different superscripts are significantly different ($P < 0.05$)

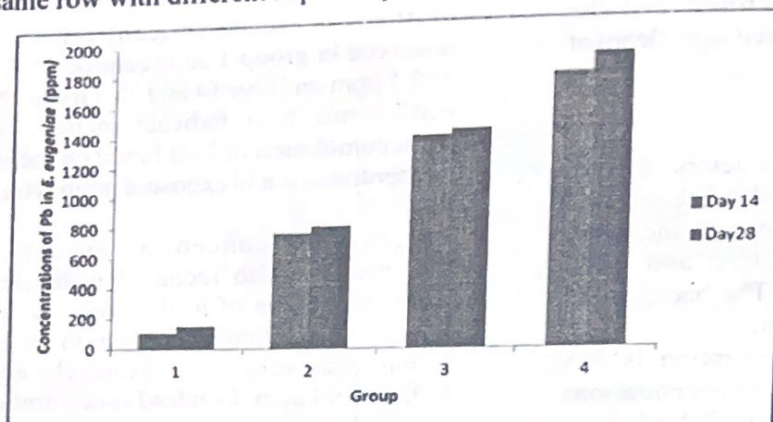


Figure 1: Concentrations of lead in *E. eugeniae* at Days 14 and 28

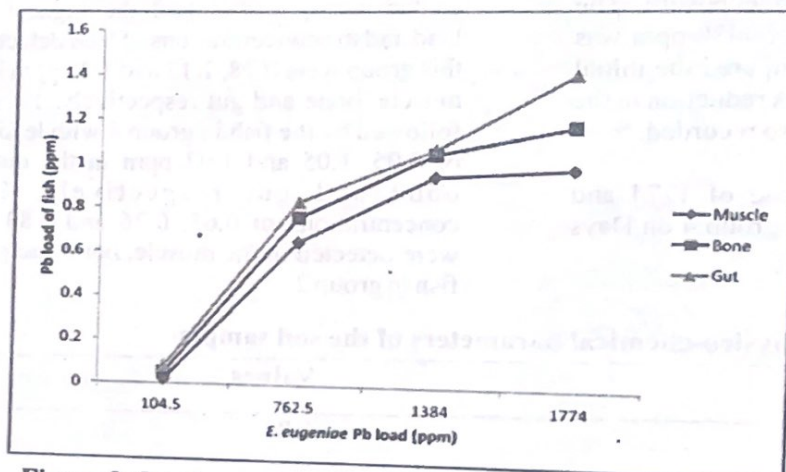


Figure 2: Lead concentrations in fish organs exposed to contaminated worms

DISCUSSION

The soil organic carbon and water holding capacity indicate that the soil is rich in organic matter according to the soil rating method of (Edwards *et al.*, 1996) and the pH value obtained falls within the tolerable range for earthworms as reported by (EPA, 2006). It can therefore be suggested that the soil is habitable for earthworms. The background level of lead (315 ppm) in the soil used falls within the range of natural soil lead level (50-400 ppm) as reported by (Peijnenburg *et al.*, 1999).

The soil lead analysis revealed significant reduction in soil lead concentrations at Day 14 when compared to the initial concentrations at Day 0. Similarly, soil lead concentration in Groups 2, 3 and 4 decreased by 25%, 33% and 17% respectively. This irregular trend in percentage reduction of soil lead can be attributed to the non-uniform distribution of soil bacteria and organic components. The reduction in concentrations of soil lead can be linked to the bioaccumulative action of earthworms and the biochemical action of certain soil bacteria. This report agrees with the observation of (Panday *et al.*, 2012) where

the concentrations of metal in soil decreased with time. Bamgbose *et al* (2005) also reported a decrease in soil lead concentration due to the vermicomposting and bioaccumulative activities of three earthworm species, *Eisenia fetida*, *Perionyx excavatus*, and *Lampito mauritii*.

This study also reveals that lead bioaccumulation by earthworms is directly proportional to the soil lead concentration and period of exposure. This is in concordance with the report of Andre *et al* (2009a) where earthworm was studied as a bio-indicator of metal pollution. Andre *et al* (2009b) also reported an increase in accumulation of lead in *Eisenia fetida* with an increase in soil dose and exposure period. The dose and time dependent bioaccumulation of lead by earthworms can be linked to a lead storage mechanism made possible by lead nodules and proteins such as metallothioneins (Bamgbose *et al*, 2005). A contrast report by FAO (1983) show a decline in the accumulation of lead by *E. Fetida* at the highest exposed concentration. The reason adduced for this may be as a result of decrease in bioavailability of lead after 14 days exposure period and interference of Cobalt (used in the study) with lead availability to the earthworms due to its contamination. The level of lead concentration in *C. gariepinus* fed with Pb-contaminated *E. Eugeniae* revealed a biotransfer in the increasing order of muscle < bone < gut. The level of lead biotransfer to fish was found to be dependent on the lead load in the earthworm diet. There was significant difference ($p < 0.05$) amongst each group and the concentrations in muscles of the exposed fish were above the permissible limit (0.05 mg kg⁻¹) of lead in fish (FAO, 1983). Similar report in the biotransfer of Cadmium to a hybrid of catfish, *C. macrocephalus* and *C. gariepinus* were observed as the concentration of cadmium in the zooplankton diet, *Moina macrocopa* increased (Ruangsomboon *et al*, 2006). Muscle tissues were also reported to have least accumulated lead in an experiment where *C. gariepinus* fingerlings were exposed to sub lethal concentrations of lead nitrate and in a study of the

biotransfer of heavy metals in a soil-plant-chicken food chain (Zhuang *et al* 2009; Kusemiju *et al*, 2012).

The high gut concentration of lead can be attributed to the route of uptake of the metal which, in this case, is dietary. Metal concentrations in the digestive tract of fish are related to the dietary uptake route as reported by Al-Mohanna (1994), Kock *et al* (1998) and Giguere *et al* (2004). The pattern of lead accumulation among the three fish parts in this study is in concordance with the report of Jezierska *et al* (2006) which states that high levels of metal are sometimes found in bones while the muscles, compared to the other tissues, usually show low concentrations of metals. According to Oronsaye *et al* (1995), once the pollutants are absorbed, they are transported by the blood to either a storage point such as bone to the liver for transformation and storage. Bone has been reported to serve as a reservoir of lead and this is possibly due to its ability to act in a similar manner to calcium ions (Dikranian, 2001).

CONCLUSION AND RECOMMENDATION

The rate of bioaccumulation of lead in *E. eugeniae* and bio-transfer to *C. gariepinus* which is dose and time dependent indicates that higher level of pollution in the environment brings higher chances of contamination of the food chain. The public is more concerned with the acute exposure of contaminants in the environment, oblivious of the fact that chronic exposure which can come through the food chain is also an unknown danger. This is therefore, a wakeup call for action in developing Nations like Nigeria, to evaluate the possibility of food chain transfer of pollutants before giving any clean bill of health. There should be enforcement of laws and monitoring policies against indiscriminate environmental pollution with proper sanction of violators. The role of non-governmental organizations (NGOs) in reducing environmental pollution can also not be overlooked, considering the impacts that they have made in

ameliorating environmental pollution in developed countries.

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CONFLICT OF INTEREST

Authors declare no conflict of interest amongst themselves

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