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BIOACCUMULATION OF HEAVY METALS IN SOIL INVERTEBRATES: PART 1: UPTAKE AND ACCUMULATION OF LEAD AND CHROMIUM BY *ACHATINA MARGINATA* (LINNAEUS) AND *LYMNAEA STAGNALIS* (LINNAEUS).

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ABSTRACT

Achatina marginata and *Lymnaea stagnalis* were each exposed to 4.15µg/g and 8.26µg/g diet of lead and chromium respectively over a period of 28 days. Comparative accumulation studies were carried out on the edible (soft) and gut tissues of both snails. The rates of accumulation of Cr by *L. stagnalis were 0.025*µg/g/week and 0.084µg/g/wee in the soft tissues for the lower and higher exposure concentrations respectively. The corresponding rates in the gut were 0.290µg/g/week and 0.537µg/g/week respectively. However, lower rates of accumulation of Pb were calculated for the tissues of the same species at the two exposure levels. Higher concentrations of Cr and Pb were measured in the gut tissues than the soft tissues. The rates of accumulation of Cr by *A. marginata* were 0.341µg/g/week and 0.518µg/g/week for the soft tissues at the lower and higher exposure concentrations respectively while the corresponding rates for the gut were 1.347µg/g/week (lower) and 1.477µg/g/week (higher). These values are 13.6 times and 6.2 times greater than those of *L. stagnalis* at the lower and higher concentrations of exposure respectively. A similar trend in the bioaccumulation of Pb by *a. marginata* rative to *L. stagnalis* was also observed. Bioaccumulation rates of Pb and Cr were greater in the gut than the soft tissues for both species. The moderate accumulation factors calculated for *L. stagnalis* and high values for *A. marginata* suggest that these species can be used as indicators of metal pollution in field experiments.

KEY WORDS: *L. stagnalis; A. marginata;* Lead; Chromium; Bio-accumulation.

INTRODUCTION

Snails, earthworms, slugs and other soil invertebrates are important components in the food chain since they form a significant proportion of the diet of other animals. The increased indiscriminate dumping of domestic, municipal and inappropriately treated industrial wastes, together with spillages and leakages of petroleum related products on lands and rivers have raised concern over the increase in metal concentrations in soils and water bodies. The infiltration of potentially toxic metals into terrestrial and aquatic ecosystems in therefore a common phenomenon (Flegal *et al;* 1990; Khwaja *et. Al.*, 1997; IPCS, 1992). This process is likely to lead to bioaccumulation of metals via the food chain (Watling, 1983; FEPA; 1991; Sugiyama *et al.*, 1992). Evaluation of this problem involves the investigation of the effects of high metal concentrations in soil and /or water on the lower tropic levels of soil and swamp ecosystems and characterizing the bioconcentration and biokinetics of heavy metals in solid invertebrates (Watling, 1983; Rabitsch, 1995; Watling and Watling, 1983).

Invertebrates have been used as indicators of metal pollution in both aquatic and terrestrial habitats (Darracott and Watling, 1975; Alexander and Young, 1976; Philips, 1976; Coughtrey and Martin, 1977). Earthworms were used to test the biological toxicity of soil from hazardous waste sites (Callaham *et al;* 1985). The period for these tests ranged from 14 days to 28 days (Karnak and Hamelink, 1982). Molluscs have been preferred in these studies because they accumulate higher concentrations of metals than other groups (Williamson and Evans, 1972; Beeby and Eaves, 1983). This ability is probably a function of their physiology and feeding habits (Boyden, 1974). Tests using earthworms as indicators of toxicity to soil biota have been developed.

In this study, the accumulation of Lead and Chromium by *Achatina marginata*(Linnaeus) and *Lymnaea stagnalis* (Linnaeus) was investigated. A comparison of their rates of accumulation under the same controlled experimental conditions is presented, and the effect of concentration variation on uptake is discussed. The possible use of these snails as indicators of metal pollution in terrestrial environments is also discussed.

MATERIALS AND METHODS

a. *marginata* (Linnaeus) were purchased at Mile 1 Market Diobu, in Port Harcourt. They were separated into taxonomic categories and to species level. Williamson (1979) showed that terrestrial gastropds and isopods accumulated a particular metal at different rates and the sources of variation were attributed to body size, age and season. The two snaii species were therefore selected based on body size, as reflected by their masses and also by estimating their age classes (Beeby and Eaes, 1983). 1985 snails each of the two species were allowed to evacuate their gut contents in separate plastic trays and also to get acclimatized to the laboratory conditions. These snails were then

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randomly separated into ten (10) groups. Two groups had 5 snails of *L. stagnalis* and *A. marginata* as control while 8 groups had 45 snails each of the two species respectively. There were therefore 180 snails of *L. stagnalis* and 180 snails of *A. marginata* for the uptake and elimination study. They were all washed with distilled water prior to food preparation.

11.16mg of $PbCl_2$ were dissolved in $10cm^3$ of "Analar" nitric acid in a $100cm^3$ volumetric flask and the solution made up to $100cm^3$ with distilled water. Another stock solution containing 22.18mg of $PbCl_2$ was prepared in a similar manner and was also made up to $100cm^3$ with distilled water.

The lower concentration of Pb was poured on to 2kg of a mixture of mashed pawpaw fruits and chopped cabbage leaves in a dry and clean plastic bucket. This contaminated diet was then stirred with a wooden spoon, 1m long. The 2kg-treated diet was transferred into one of the wooden containers (1m x 1m x 0.5m) specifically constructed for the study. The diet was further stirred with the wooden spoon to effect even distribution of the pollutant in this container, C–21. 4g of this diet was taken for analysis while 45 snails (*L. stagnalis)* previously washed with distilled water, were then placed in the container, C-21. the top of the wooden container was then covered with polyethene nets to prevent any escape, as well as ensuring proper aeration. The base (bottom) of the container was covered with polyethene films before diet were put in. Control containers C-1L and C-1A had 3kg each of untreated diet for 5 snails of *L. stagnalis* and 5 snails of *A. marginata*. Similarly, 45 snails each of both species were kept in treated diets (2kg diet each) in C-3A, C-4L and C-5A; where C-2L and C-3A were containers for diets of lower exposure concentrations for *L stagnalis* and *A. marginata* respectively; C-4L and C-5A were for higher exposure concentrations of Pb for *L.stagnalis* and *A. marginata* respectively.

12.6mg of $CrCl_3$ were also dissolved in a mixture of $20cm^3$ of distilled water and $10cm^3$ of "Analar" nitric acid in a $100cm^3$ standard flask and the solution made up to $100cm^3$ with distilled water. This stock solution was used for the lower exposure treatment. Another stock solution containing 25.16mg of $CrCl_3$ was also prepared in a similar manner for the higher exposure treatment for the snails.

Treatments of diets with stock solutions were as described earlier. the same weights of diets were collected for analysis. 45 snails each were put in containers C-61; C-7A and C-8L, where C-6L and C-7A represented containers for the lower concentrations and C-8L, C-9A were for the higher concentrations. Containers C-6L and C-8L had *L. stagnalis* while C-7A and C-9A had *A. marginata*. All containers are covered as previously described. All experiments were under laboratory conditions of 29°C ± 2°C and 80 – 90% relative humidity. Each of the containers had a wet filter paper as a source of moisture.

Individuals of both snail species were exposed to the two concentrations of each element. Uncontaminated snails were therefore fed on diets with low and higher metal concentrations, in order to measure snail metal uptake, during 28 days of exposure. Metal determinations were made on the snails on days 0,5,10,15,20,25 and 28. Pb and Cr levels in all treated diets were determined also, as described above. At the end of each experiment, the wet tissues of individuals were removed from their shells, separated into soft and gut tissues and frozen preparatory to chemical analysis. The frozen specimens were thawed, weighed into clean, dry flasks and oven-dried at 90°C for 24 hours, after which the temperature was raised by 20°C increments every 30 minutes until 300°C was reached (Frank *et al;* 1983; Neuhauser *et al;* 1995). The dried samples were then reweighed and digested each with $25cm^3$ of concentrated nitric acid by gently boiling for 2 hours to near dryness. The residue was redissolved in $10cm^3$ of nitric acid, filtered and finally washed with $5cm^3$ of nitric acid. A final volume of $100cm^3$ was obtained with double distilled water. (Watling 1983; Neuhauser *et al;* 1985). A Pye Unicam Model Sp-9. Atomic Absorption Spectrometer, using a deadtrium lamp to correct for background interference, was used. Treated diets were digested and analyzed in a similar manner, as described above. Food which were preserved in retrigerators were added to each container (1kg) from previously treated diets on Day 15.

On Day-0, three (3) of each snail species were separately digested and analyzed while on Days 5, 10, 15, 20, 25 and 28, four (4) of each snail species were digested and analyzed. The results are expressed in µg/g metal in dry tissue.

TABLE 1: Mean Lead Concentrations in *L. stagnalis* and *A. marginata* Tissues Following 28 Day Exposure to 4.15 µg/g of Contaminant in Food Complex (µg/g Dry Tissue)

	Lymnaea stagnalis							*Achatina marginata*						
Days / Tissue	0	5	10	15	20	25	28	0	5	10	15	20	25	28
Soft Tissue	0.119	0.125	0.1333	0.142	0.160	0.171	0.177	0.120	0.303	0.488	0.673	0.858	1.0143	1.154
(S.E)	0.003	0.002	0.004	0.002	0.005	0.004	0.006	0.002	0.002	0.004	0.005	0.007	0.009	0.011
Gut Tissue	0.164	0.201	0.238	0.277	0.816	0.979	1.077	0.301	1.490	1/794	2.540	3.286	4.032	4.479
(S.E)	0.004	0.002	0.004	0.003	0.006	0.008	0.012	0.003	0.011	0.023	0.051	0.063	0.098	0.111

S. E. = Standard Error; n = 4.

TABLE 2. Mean Lead Concentrations in *L. stagnalis* and *A. maginata* Tissues Following 28 Day Exposure To 8.26µg/g of Contaminant in Food Complex. (µg/g Dry Tissue)

	Lymnaea stagnalis							*Achatina marginata*						
Days / Tissue	0	5	10	15	20	25	28	0	5	10	15	20	25	28
Soft Tissue	0.119	0.153	0.187	0.222	0.301	0.347	0.37	0.120	0.401	0.683	0.966	1.248	1.531	1.700
(S.E)	0.003	0.002	0.004	0.002	0.005	0.004	0.004	0.002	0.003	0.012	0.015	0.011	0.018	0.017
Gut Tissue	0.164	0.201	0.238	0.277	0.816	0.979	1.943	0.301	1.117	1.935	2.754	3.572	4.390	4.881
(S.E)	0.004	0.002	0.004	0.003	0.006	0.008	0.017	0.003	0.015	0.018	0.019	0.012	0.101	0.112

TABLE 3. Mean Lead Concentrations in *L. stagnalis* and *A. marginata* Tissues Following 28 – Day Exposure To 4.15µg/g of Contaminant in Food Complex. (µg/g Dry Tissue)

	Lymnaea stagnalis							*Achatina marginata*						
Days / Tissue	0	5	10	15	20	25	28	0	5	10	15	20	25	28
Soft Tissue	0.131	0.142	0.167	0.192	0.197	0.217	0.230	0.138	0.366	0.613	0.861	1.108	1.356	1.700
(S.E)	0.006	0.008	0.009	0.010	0.011	0.011	0.012	0.004	0.003	0.012	0.01	0.011	0.018	0.017
Gut Tissue	0.186	0.217	0.272	0.802	1.014	1.225	1.346	0.436	1.288	2.273	3.258	4.243	4.390	4.881
(S.E)	0.004	0.009	0.011	0.011	0.013	0.012	0.014	0.005	0.002	0.004	0.011	0.013	0.017	0.112

TABLE 4. Mean Lead Concentrations in *L. stagnalis* and *A. maginata* Tissues Following 28 Day Exposure To 8.26µg/g of Contaminant in Food Complex. (µg/g Dry Tissue)

	Lymnaea stagnalis							*Achatina marginata*						
Days / Tissue	0	5	10	15	20	25	28	0	5	10	15	20	25	28
Soft Tissue	0.131	0.187	0.243	0.301	0.368	0.431	0.468	0.138	0.491	0.863	1.236	1.608	1.981	2.210
(S.E)	0.006	0.007	0.006	0.008	0.005	0.011	0.011	0.004	0.009	0.014	0.016	0.017	0.016	0.021
Gut Tissue	0.186	0.469	0.937	1.325	1.712	2.100	2.332	0.436	1.379	2.459	3.539	4.619	5.699	6.340
(S.E)	0.004	0.011	0.016	0.018	0.019	0.022	0.021	0.005	0.018	0.021	0.024	0.027	0.033	0.039

RESULTS

Mean tissue metal concentrations of *A. marginata* and *L. stagnalis* exposed to lead and chromium are summarized in Tables 1 to 4. In a similar investigation on the accimulation of seven metals by oysters and mussels, a solution concentration of 100µg/dm^3 of each of the elements was used (Watling, 1983). Solution concentrations of 0.1µg/dm^3 and 0.05µg/dm^3 have also been used by others (Schuster and Pringle, 1969), for the accumulation of Zinc, copper, cadmium and chromium by *Crassostrea virginica*. Exposure concentrations of 4.15 µg/g and 8.26µg/g of lead and chromium from diets were used in our study. These levels of the metals in the diets are comparable to the mean range (1.15-3.61µg/g) obtained in surface soils around flowstations in the Niger Delta areas of Nigeria (IPS, 1996; Geolink, 1998).

Uncontaminated snails of each species were placed in contaminated diets. The contaminants were lead and chromium at two different exposure concentrations of 4.15µg/g and 8.26µg/g of each of the elements, in order to compare the uptake rates of the different metals by a given species and the uptake rates of a single element by different species. The rate of accumulation (µg/g/week) of each element by each species was calculated as follows:

$$K = \frac{C\_Cc}{\frac{28}{4}}$$

Where K = rate of accumulation

C_{28} = mean concentration of study element in tissue on Day – 28

C_o = mean concentration of element in tissue of snail on Day 0.

There accumulation rates are summerised in Table 5. An accumulation factor is defined as the ratio of the mean concentration of the study element in the tissues of treated individuals to the mean concentration in the tissues of "Control" individuals which have not been exposed to that element (Watling, 1983), and is an index for indicating metal pollution in a polluted environment The accumulation factors are summerised in Table 6

DISCUSSION

The results indicate that the two elements are accumulated to a greater or lesser extent by each of the two species (Tables 1-4). The uptake rates of Cr by *L.*

TABLE 5: Rates of Metal Accumulation (µg/g week) for a 4-week Exposure to Different contaminations of one of two elements.

Species	Tissue Type	4.15 µg/g Exposure	8.26 µg/g Exposure	Metal
L. stagnalis	Edible (soft)	0.015	0.064	Pb
	Gut	0.228	0.445	
A. marginata	Edible (soft)	0.259	0.395	
	Gut	0.228	1.145	
L. stagnalis	Edible (soft)	0.025	0.084	Cr
	Gut	0.290	0.537	
A. marginata	Edible (soft)	0.341	0.518	
	Gut	1.347	1.477	

TABLE 6: Accumulation Factors

Species	Tissue Type	4.15 µg/g Exposure	8.26 µg/g Exposure	Metal
L. stagnalis	Edible (soft)	1.5	3.1	Pb
	Gut	6.6	11.9	
A. marginata	Edible (soft)	9.6	14.2	
	Gut	14.9	16.2	
L. stagnalis	Edible (soft)	1.8	3.6	Cr
	Gut	7.2	12.5	
A. marginata	Edible (soft)	10.9	16.0	
	Gut	13.4	14.6	

stagnalis were greater than the uptake of Pb in the edible (soft) part of this snail, for the two concentrations studied. Similarly, the uptake rates of Cr by this snail in the gut tissues were greater when compared to Pb, for both exposures. However, the rates of accumulation in the soft and gut tissues increased with increase in concentration of pollutants. A similar trend has been observed by Walting & Walting (1983). The accumulation of higher concentrations of Pb and Cr in the gut tissue is worthy of mention, especially when we consider that humans eat the soft tissue and not the gut. Complications arise however, along the food chain since the whole tissue is eaten by other animals consumed by humans (Walting, 1983; Sugiyama *et al.*, 1992). During the 4-2eek exposure, *A. marginata* accumulated chromium at a greater rate than lead in the soft and gut tissues. Table 5 shows that *Achatina marginata* accumulated chromium in the soft tissue about 13.6 times greater than *L. stagnalis* at the lower concentration and 6.2 times at the higher concentration to which both snails were exposed. Similarly, lead was accumulated by *A. marginata* in the soft tissue (about 17.3 and 6.2 times at the lower and higher concentrations of exposure respectively) as compared to *L. stagnalis*.

The uptake rates of lead (Pb) and chromium (Cr) by the gut tissue of *A. marginata* were faster with increase in concentration, than the soft tissue. A similar trend was observed for *L. stagnalis*. The figures in Table 5 can therefore be used to compare the uptake rates of different metals by a given species or the uptake rates of a single metal by different species. Generally, *A. marginata* and *L. stagnalis* accumulated lead and chromium at faster rates in the gut than the edible part. It is likely that higher concentrations of these metals may be accumulated by these experimental animals during long exposure periods (Watling, 1983) or the chemical or physical form of the metals in solution may also play an important role in the uptake mechanism. Further experiments on the accumulation of these metals associated with sediment particles or complexed by

naturally occurring organic substances, complemented by biochemical studies could clarify the mechanism by which these metals are normally accumulated.

The health hazards associated with eating these snails particularly *A. marginata,* will be discussed in a subsequent paper that would deal with the biokinetics of chromium and lead in these species.

Watling (1983), reported that zinc, copper, cadmium and chromium were accumulated by *Crsssostrea virginic.* The rates of accumulation for the first three weeks were 122, 46, 10.3 and 1.3µg/L respectively. The rates of accumulation for the first three weeks of lead and chromium in *Crassostrea gigas* were 0.44 and 0.15 µg/g week; *Crassostrea marginata* 0.57 and 0.13, *Perna perna* 0.90 and 0.19, *Choromytilus meridionalis* 1.06 and 0.09 µg/g/ week, the solution concentration being 100µg/1. In our study, the rates of accumulation of lead in the gut of both species were higher but similar rates were obtained in the edible parts, for a 3-week exposure. Our results also show that the relative order of accumulation is Cr > Pb. This order is the reverse of that obtained previously by the four species above (Watling, 1983; Shuster & Pringle, 1969). The reversal of this order may be due to species differences, experimental conditions, e.g. the use of food particles, probable complexation with food particles or the chemical or physical form of the metals used (Watling, 1983).

The accumulation factors for each species and metal have been calculated from Tables 1-4 and presented as Table 6. Based on these accumulation factors, lead and chromium appear slowly accumulated at low concentrations of exposure by factors of 1.5 and 1.8 respectively in the soft tissue of *L. stagnalis,* but with moderate accumulation factors of 3.1 and 3.6 respectively at the higher concentration. The gut tissues for the snail, *A. marginata* have high accumulation factors for both metals while the gut tissues for *L. stagnalis* have moderate values. These results suggest that lead and chromium are accumulated rapidly in the gut tissue of *a. marginata,* and at an intermediate rate in the soft tissue of the species under the experimental conditions. The gut tissue of *L. stagnalis* also accumulated these metals at an intermediate rate. Consequently, *A. marginata* and *L. stagnalis* could be used to indicate the presence of these metals in a terrestrial environment, or for field and laboratory experiments.

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