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

VICTOR O. T. OMUARU, MIABIYE D. SELEMA and ANTHONY E. SOROH
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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 0.025μg/g/week and 0.084μg/g/week in the soft tissues for the lower and higher exposure concentrations respectively. The corresponding rates in the gut were 0.290μg/g/week and 0.697μg/g/week respectively. However, lower rates of accumulation of Pb were calculated for the tissues of the same species at 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 1.847μg/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 relative 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 (Walling, 1983; FEPA; 1991; Sugiyama et al., 1992). The evaluation of this problem involves the investigation of the effects of high metal concentrations in soil and the water on the lower trophic levels of soil and swamp ecosystems and characterizing the biocaccumulation and biokinetics of heavy metals in soil invertebrates (Walling, 1983; Rabitsch, 1995; Walling and Walling, 1983).

Invertebrates have been used as indicators of metal pollution in both aquatic and terrestrial habitats (Daracott and Walling, 1975; Alexander and Young, 1976; Philips, 1976; Coughtry and Martin, 1977). Earthworms were used to test the biological toxicity of soil from hazardous waste sites (Callahan 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 (Boyd, 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 gastropods and isopods accumulated a particular metal at different rates and the sources of variation were attributed to body size, age and season. The two snail species were therefore selected based on body size, as reflected by their masses and also by estimating their age classes (Beeby and Eaves, 1983). 1995 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
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 150
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.

11.16 mg of PbCl₂ were dissolved in 100 cm³ of "Analar"
nitric acid in a 1000 cm³ volumetric flask and the solution
made up to 1000 cm³ with distilled water. Another stock
solution containing 22.18 mg of PbCl₂ was prepared in a
similar manner and was also made up to 1000 cm³ with
distilled water.

The lower concentration of Pb was poured on to
2 kg 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. 4 g 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 polythene
nets to prevent any escape, as well as ensuring proper
aeration. The base (bottom) of the container was
covered with polythene films before diet were put in.
Control containers C-1 and C-1A had 3 kg 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 (2 kg for 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.

12.6 mg of CrCl₃ were also dissolved in a mixture
of 20 cm³ of distilled water and 10 cm³ of "Analar" nitric
acid in a 100 cm³ standard flask and the solution made
up to 100 cm³ with distilled water. This stock solution
was used for the lower exposure treatment. Another stock
solution containing 25.16 mg of CrCl₃ 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-6L, C-9A, were for the higher concentrations.
Containers C-5L and C-8L had L. stagnalis while C-7A
and C-9A had A. marginata. All containers were covered
as previously described. All experiments were under
laboratory conditions of 20°C ± 2°C and 60% ~ 70%
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 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; Neuhauß et al., 1995).
The dried samples were then weighed and digested each
with 25 cm³ of concentrated nitric acid by gently boiling
for 2 hours to dryness. The residue was
redissolved in 10 cm³ of nitric acid, filtered and finally
washed with 5 cm³ of nitric acid. A final volume of
100 cm³ was obtained with double distilled water.
(Watling 1983; Neuhauß et al; 1985). A Pye Unicam
Model Sp-9. Atomic Absorption Spectrometer, using a
deadrnium 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 refrigerators were added to each container
(1 kg) 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.

<p>| 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) |</p>
<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>28</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft</td>
<td>0.119</td>
<td>0.125</td>
<td>0.1333</td>
<td>0.142</td>
<td>0.160</td>
<td>0.171</td>
<td>0.177</td>
<td>0.120</td>
<td>0.303</td>
<td>0.308</td>
<td>0.373</td>
<td>0.378</td>
<td>1.9143</td>
<td>1.154</td>
</tr>
<tr>
<td>Gut</td>
<td>0.009</td>
<td>0.002</td>
<td>0.004</td>
<td>0.002</td>
<td>0.005</td>
<td>0.004</td>
<td>0.005</td>
<td>0.002</td>
<td>0.005</td>
<td>0.004</td>
<td>0.005</td>
<td>0.007</td>
<td>0.009</td>
<td>0.011</td>
</tr>
<tr>
<td>(S.E.)</td>
<td>0.004</td>
<td>0.002</td>
<td>0.004</td>
<td>0.003</td>
<td>0.005</td>
<td>0.008</td>
<td>0.012</td>
<td>0.003</td>
<td>0.011</td>
<td>0.023</td>
<td>0.023</td>
<td>0.031</td>
<td>0.038</td>
<td>0.098</td>
</tr>
</tbody>
</table>

S. E. = Standard Error; n = 4.
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 accumulation of seven metals by oysters and mussels, a solution concentration of 100 µg/cm² of each of the elements was used (Waring, 1983). Solution concentrations of 0.1 µg/cm² and 0.05 µg/cm² 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 8.26 µg/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 (1.15-3.61 µg/g) obtained in surface soils around flowstations in the Niger Delta areas of Nigeria (IPS, 1998; Geonip, 1998).

Uncontaminated snails of each species were placed in contaminated diets. The contaminants were lead and chromium at two different exposure concentrations of 4.15 µg/g and 8.26 µg/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:

\[
\text{C}_{\text{Cc}} = \frac{C_{\text{0}}}{K^{28}}
\]

Where \( K = \) rate of accumulation

\( C_{\text{28}} = \) mean concentration of study element in tissue on Day - 28

\( C_{\text{0}} = \) mean concentration of element in tissue of snail on Day 0.

There accumulation rates are summarised 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 (Waring, 1983), and is an index for indicating metal pollution in a polluted environment. The accumulation factors are summarised 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. stagnalis* and *A. marginata* are more rapid than those for Pb. This indicates that chromium is more mobile in the soil than lead. The accumulation of lead in the snails is faster than that of chromium. This is consistent with the results of other studies (Waring, 1983; Schuster and Pringle, 1969).
**TABLE 5:** Rates of Metal Accumulation (μg/g week) for a 4-week Exposure to Different contaminations of one of two elements.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue Type</th>
<th>4.16 μg/g Exposure</th>
<th>8.26 μg/g Exposure</th>
<th>Metal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. stagnalis</em></td>
<td>Edible (soft)</td>
<td>0.015</td>
<td>0.064</td>
<td>Pb</td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>0.228</td>
<td>0.445</td>
<td></td>
</tr>
<tr>
<td><em>A. marginata</em></td>
<td>Edible (soft)</td>
<td>0.259</td>
<td>0.395</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>0.228</td>
<td>1.145</td>
<td></td>
</tr>
<tr>
<td><em>L. stagnalis</em></td>
<td>Edible (soft)</td>
<td>0.025</td>
<td>0.084</td>
<td>Cr</td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>0.290</td>
<td>0.537</td>
<td></td>
</tr>
<tr>
<td><em>A. marginata</em></td>
<td>Edible (soft)</td>
<td>0.341</td>
<td>0.518</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>1.347</td>
<td>1.477</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 6:** Accumulation Factors

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue Type</th>
<th>4.16 μg/g Exposure</th>
<th>8.26 μg/g Exposure</th>
<th>Metal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. stagnalis</em></td>
<td>Edible (soft)</td>
<td>1.5</td>
<td>3.1</td>
<td>Pb</td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>6.6</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td><em>A. marginata</em></td>
<td>Edible (soft)</td>
<td>9.6</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>14.9</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td><em>L. stagnalis</em></td>
<td>Edible (soft)</td>
<td>1.8</td>
<td>3.6</td>
<td>Cr</td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>7.2</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td><em>A. marginata</em></td>
<td>Edible (soft)</td>
<td>10.3</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gut</td>
<td>13.4</td>
<td>14.6</td>
<td></td>
</tr>
</tbody>
</table>

*L. 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 Watling & Waitling (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 (Watling, 1983; Sugiyama et al., 1992). During the 4-week 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 Crassostrea virginica. The rates of accumulation for the first three weeks were 122, 46, 10.3 and 1.3 μg/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/L week; Crassostrea marginata 0.57 and 0.13; Penaeus monodon and 1.98; Chlamys miliarius medionigra 1.06 and 0.09 μg/g week; and the solution concentration being 100 μg/l. 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, environmental 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.

ACKNOWLEDGEMENT

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