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BIOACCUMULATION OF HEAVY METALS IN SOIL INVERTEBRATES: PART 2: UPTAKE AND ACCUMULATION OF LEAD AND CHROMIUM BY *ACHATINA MARGINATA* (LINNAEUS) AND *LYMNAEA STAGNALIS* (LINNAEUS).

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ABSTRACT

The non-steady state kinetics of lead and chromium in two snails *Lymnaea stagnalis* and *Achatina marginata* were examined. The rates of elimination of Pb and Cr were greater in the gut tissues than in the foot mantles of these snails for different levels of contamination. The rates decreased with increase in metal levels in the guts of *A. marginata*. The studies showed that the snails *L. stagnalis* and *A. marginata* eliminated Cr faster than Pb. Moreover, the gut and the foot mantle of *L. stagnalis* and the foot mantle of *A. marginata* eliminated Cr faster with increase in metal concentration in the tissues. However, *A. marginata* eliminated Cr faster than *L. stagnalis* in both tissues. The elimination of Cr and Pb in both species followed the simple first – order kinetics. The values of rates of elimination calculated were also in agreement with the terminal elimination rate constants. On the basis of elimination factors, the gut tissues of *A. marginata* gave low elimination factors, implying that these tissues in this species accumulated these metals rapidly. *L. stagnalis* and *A. marginata* have low to moderate elimination factors, an indication that these snails might not be suitable for use as bioremediation agents. These snails might however, be used to indicate the presence of lead and chromium in the terrestrial environment.

KEY WORDS: *L. stagnalis; A. marginata;* Lead; Chromium; Biokinetics.

INTRODUCTION

The development of bioremediation processes for wastes and other pollutants is one of the main applications of biological treatment systems (Frank *et al;* 1983). Recently, the application of biologically based technology has branched out to the treatment of metal – containing waste with areas of application covering detoxification of metal bearing from ore processing solutions (Volesky, 1987). Untreated *Saccharomyces cerevisiae* biomass exhibits good metal accumulation properties. It was found (Stoll and Duncan, 1997) that when effluents in excess of the stipulated drinking and river water quality criteria were introduced into tanks containing *S. cerevisiae* biomass, after several hours, the levels of the metals in the tanks were found to be relatively low. Studies on the storage and mechanism of heavy metals in land snails showed high amount of cadmium, lead and zinc in the midgut gland of *Helix pomatia* (Dallinger and Wieser, 1984), *Helix aspersa* (Coughtrey and Matins, 1976). *Cepaea hortensis* (Williamson, 1980) while copper seems to accumulate in the foot-mantle (Dallinger & Wieser). Lead uptake by the shell and digestive gland of *Lymnaea pergra* and the whole soft tissue in *Helix aspersa* followed an asymptotic pattern with time (Everard & Denny, 1984). In a study on the uptake and accumulation of lead and chromium by the land snails *Achatina marginata* and *Lymnaea stagnalis*, we reported high amounts of lead and chromium in the gut tissues and moderate amounts in the foot-mantle of these snails (Omuaru *et al;* 2000).

Studies on the bioaccumulation of mercury in the snail *Eobania vermiculata* showed that at each sampling station, the mercury concentration in the whole soft tissue were comparable to those present in the vegetables on which they fed (Bertani *et al;* 1994) This was different from what accumulated in the shell and in the soft tissue.

The highest mercury levels were however, observed in the gut content. This finding could be due to a mercury extraction via hepatopancreatic granules as observed for lead (Beeby and Richmond, 1987), and could represent a general detoxification mechanism for heavy metals. This paper reports the uptake and elimination of lead and chromium by *Achatina marginata* and *Lymnaea stagnalis* in the gut and foot-mantle (edible portion). The elimination rates, elimination factors and elimination rate constants were determined and the probable use of these snails for bioremediation processes is discussed.

MATERIALS AND METHODS

The snails *a. marginata* (Linnaeus) and *L. stagnalis* (Linnaeus) were purchased at Mile 1 Market, Diobu, in Port Harcourt. The approach adopted in these experiments was to allow 185 snails each of the two species to evacuate their gut contents in separate plastic plates for 24 hours. The snails were then separated into ten groups: 8 groups had 45 snails each of the two species while 2 groups had 5 snails each of *L.*

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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

stagnalis and *A. marginata* as control snails. There were therefore 180 snails of *L. stagnalis* and 180 snails of *A. marginata* for the uptake and elimination study. Diet for the snails was a mixture of mashed pawpaw fruits and chopped cabbage leaves. Stock solution of $PbCl_2$ and $CrCl_3$ were prepared at two different concentrations. The lower concentration of Pb was poured unto 2kg of the diet in a dry, clean plastic bucket, stirred with a wooden spoon and then transferred into one of the 8 wooden containers of dimension (1m x 1m x 0.5m) specially constructed for this study. The diet was further stirred in this container to ensure even distribution of the pollutant (Omuaru *et al;* 2000). The treated diet had concentration of 4.15µg/g diet of lead when 4g of the diet was digested and analysed (Neuhauser *et al;* 1995). Using stock solution of 22.18mg of lead chloride, 12.64mg of chromium chloride and 25.16mg of chromium chloride respectively, diets that contained 8.26µg/g, 4.15µg/g and 8.26µg/g of the elements were prepared (Omuaru *et al;* 2000).

For the uptake study, 45 snails of each of the two species were transferred into each of the containers with 2kg of treated diets of the elements respectively. Individuals of both snail species were exposed to the two concentrations of each element, 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, and 28. On day – O, three of each "control" snail species were separately digested and analysed, while four of each of the species were used on subsequent days. At the end of each experiement, the wet tissues of individuals were removed from their shells, separated into soft and gut tissues and frozen prior to analysis.

The metal contents of the snails tissues and treated diet were determined by dry ashing the samples at 300°C (Frank *et al;* 1983; Neuhauser *et al;* 1995). Dried samples were then reweighed, digested and the solution made up to $100cm^3$ with doubly distilled water. A Pye Unicam Model Sp-9 Atomic Absorption Spectrometer was used for metal analysis and the results expressed in µg/g metal in dry tissue.

For the elimination study, the two snail species

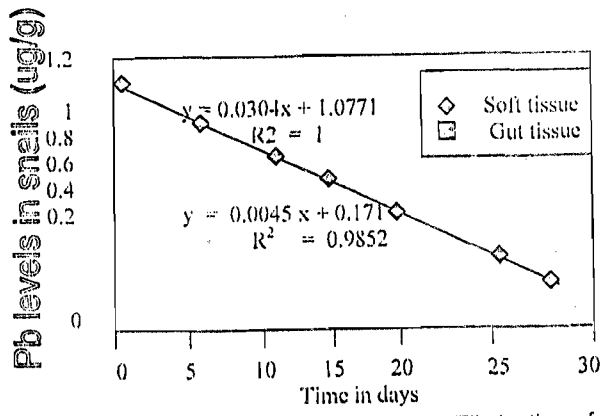

Fig. 1a: Terminal Eliminatio Rate Constnt-Elimination of Pb by L.stagnalis (ug/g/day) for concentration (A).

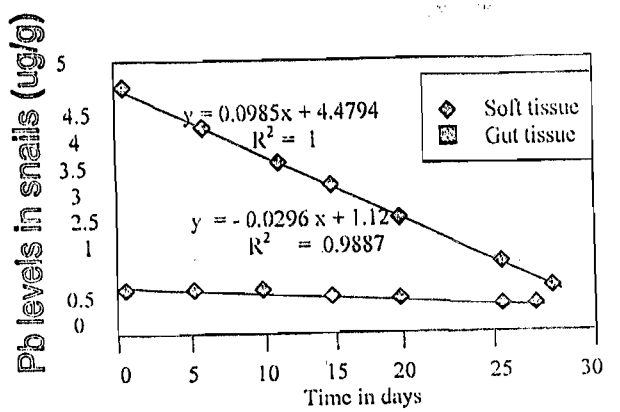

Fig. 1b: Terminal Elimination Rate Constant-Elimination of Pb by A.marginata (ug/g/day) for concentration (A).

exposed to two different concentrations of each metal (Pb and Cr) for 28 days were then transferred to eight 8) separate containers with uncontaminated diets (3kg of a mixture of mashed pawpaw fruits and chopped cabbage leaves each). Each of these containers had 21 snails of each species. From these 3 snails each of *L. stagnalis* and *A. marginata* were then killed by freezing for Day – O experiments. Metal determinations were then made on the snails on Days – O, 5, 10, 15, 20, 25 and 28, to

TABLE 5: Rates of metal loss (µg/g/week) for a 4-week exposure of contaminated snails to "Control" (uncontaminated) food.

Species	Tissue Type	(A) 4.15 µg/g Exposure	(B) 8.26 µg/g Exposure	Metal
Lymnaea stagnalis	Foot mantle	0.033 (0.033)	0.067 (0.066)	Pb
	Gut	0.213 (0.213))	0.408 (0.408)	
Achatina marginata	Foot mantle	0.218 (0.217)	0.326 (0.325)	
	Gut	0.490 (0.689)	0.473 (0.472)	
L. stagnalis	Foot mantle	0.042 (0.044)	0.085 (0.087)	Cr
	Gut	0.271 (0.239)	0.492 (0.553)	
A. marginata	Foot mantle	0.287 (0.288)	0.423 (0.423)	
	Gut	0.897 (0.896)	0.762 (0.763)	

measure snail metal loss after the snails were placed in uncontaminated diets. The metals, Pb and Cr were evaluated in the foot mantle and gut tissues of the snails. Three (3) snails of each species were used for the study on other days also (Days 5, 10, 15, 20, 25 and 28).

At the end of each experiment, the wet tissues of individuals were removed from their shells, separated into soft (foot mantle) and gut tissues and frozen. Metal concentrations in separate tissues of each snail species were determined as described above.

The approach adopted in these experiments was therefore to obtain snails with low metal concentrations and measure snail metal uptake after the snails were placed in diets with high metal concentrations, and to obtain snails with high metal concentrations and measure snail metal loss after the snails were placed in uncontaminated diets. The uptake experiment has been reported in a previous paper (Omuaru *et al;* 2000). Snails remaining in the respective cages (containers) after 28 days of exposure to diets of high metal concentration were therefore used for the elimination experiments. Thus, the highest concentrations in the tissues of these snails were on Day – O, which was the first day of the metal loss experiments, while the lowest concentrations were on Day – 28.

RESULTS

The mean tissues metals loss of the two snail species placed in uncontaminated diets are summarized in Tables 1 to 4. In order to compare the tissue elimination rates of different metals by a given species and the elimination rates of a single element by different species, snails of different metal contamination levels were used for the experiments. The rate of elimination (µg/g/week) of each element by each species was calculated as follows:

$$Kw = \frac{Co - C_{28}}{4} \quad (1)$$

TABLE 6. Elimination factors for a 4-week exposure of contaminated snails to uncontaminated food

Species	Tissue Type	(A) 4.15 µg/g Test	(B) 8.26 µg/g Test	Metal
Lymnaea stagnalis	Foot mantle	3.9	3.5	Pb
	Gut	4.8	6.2	
Achatina marginata	Foot mantle	4.1	4.3	
	Gut	2.6	1.6	
L. stagnalis	Foot mantle	3.7	3.6	Cr
	Gut	5.1	6.4	
A. marginata	Foot mantle	4.3	4.3	
	Gut	2.6	1.9	

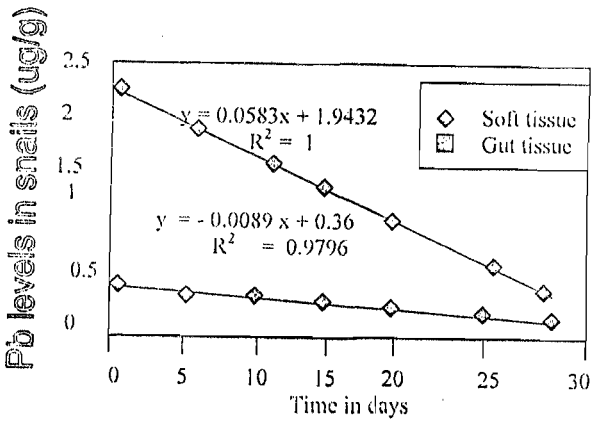

Fig. 2a: Terminal Elimination Rate Constant-Elimination of Pb by A.marginata (ug/g/day) fo concentration (B).

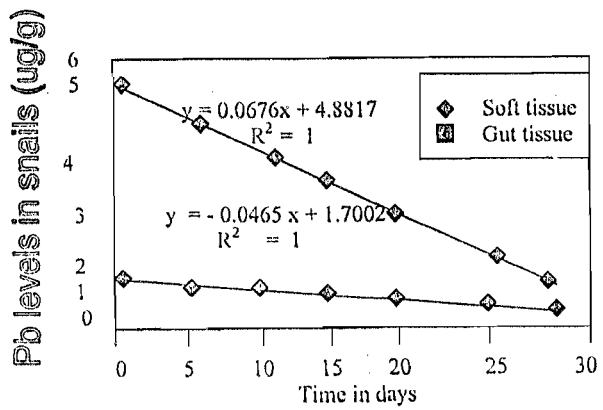

Fig. 2b: Terminal Elimination Rate Constant-Elimination of by A.marginata (ug/g/day) for concentration (B).

where, Kw is the rate of elimination; Co is the mean concentration of the study element in a tissue on Day – O and C28 is the mean concentration of the study element in a tissue on the 28th day of the metal loss experiments. These elimination rates are summarised in Table 5. The elimination patterns were also treated graphically and presented as Figures 1 – 4, and fitted to a two-dimensional model:

$$Y = mx + C \quad (2)$$

in order to examine the kinetic profiles of these two metals. From equation (2), m is the gradient and represents the rate of elimination. The respective correlation coefficients, R^2, are given in the Figures 1 – 4. the values of m, give the terminal elimination rates in ug/g/day (k_o).

TABLE 7. Rates of metal loss ((µg/g/week) for a 8-week exposure of control snails to uncontaminated control diet.

Species	Tissue Type	Pb Lead Loss		Cr Chromium		Pb Levels Day 0	Day 56	Cr Levels Day 0	Day 56
Lymnaea stagnalis	Foot mantle	0.013	0.014	0.119	0.012	0.131	0.019		
	Gut	0.017	0.019	0.164	0.032	0.186	0.032		
A. marginata	Foot mantle	0.006	0.007	0.120	0.075	0.138	0.83		
	Gut	0.025	0.025	0.042	0.301	0.436	0.101		

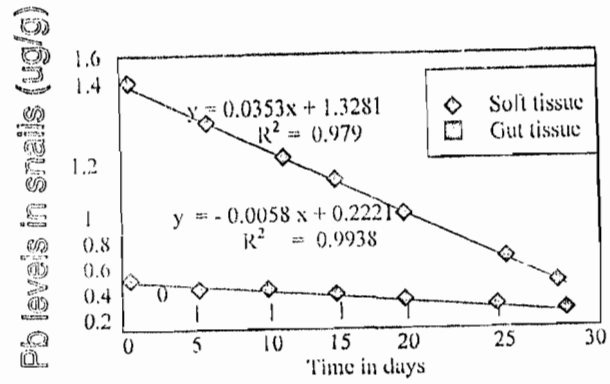

Fig. 3a: Terminal Elimination Rate Constant-Elimination of Cr by L. stagnalis (ug/g/day) for concentration (A).

The values in a parenthesis are the rates of elimination (ug/g/week) for the first 15 days. The rate of loww (ug/g/day), k_d, was calculated as follows:

$$K_d = \frac{C_o - C_{15}}{15}$$

where C_o is as defined above; C_{15} is the mean concentration of the study element in the tissue on the 15th day. Multiplying this value by 7 gives the rate of elimination per week (ug/g/week).

The elimination factor is the ratio of the mean concentration of the study metal in the tissues of treated individuals on Day – O, to the mean concentration in the tissues of treated individuals that eliminated the elements up to Day – 28 (Watling, 1983). These elimination factors are summarized in Table 6, and were obtained from C_o/C_{28}.

Two snails each of both species remained in the uncontaminated diet of pawpaw fruits and chopped cabbage leaves for the 56 days of uptake and elimination studies. The rates of metal loss (ug/g/week) for this 8-week exposure of these control snails to uncontaminated diets are summarized in Table 7.

DISCUSSION

From our experimental design, the elimination studies could be expected to give data that might be described by a simple shift from one steady state to another. Lead and chromium in our studies consistently followed simple first-order shifts in steady states as illustrated in Tables 1-4 and Figs. 1-4. There was a steady decrease in metal concentrations in the foot-mantle and gut tissues of each species. In order to establish this first-order shift, the rates of metal loss (ug/g/week) for a 4-week elimination experiments were calculated (Table 5), for the two species. The foot mantle of *L. stagnalis* eliminated Pb at the rate of 0.033ug/g/week while the value for the gut tissue was 0.213ug/g/week, for the exposure concentration for 4.15ug/g(A). At the higher exposure concentration of 8.26ug/g (B), the rates of lead loss were 0.067ug/g/week and 0.408ug/g/week for the foot mantle and gut respectively.

The rates of lead loss for the first 15 days were also calculated for this species (in parenthesis). The values are in good agreement with those for the 4-week experiments. The elimination rates were faster in the gut than the foot mantle for both exposure concentrations in *L. stagnalis*. Moreover, the elimination rates increased with increase in concentration. Similar trends were observed for the foot mantle and gut of *A. marginata*.

As illustrated in Figures 1-4, the terminal elimination rate constants are remarkably consistent. The value for the foot mantle of *L. stagnalis*, K_e is 0.0045ug/g/day for exposure (A) and 0.0089 $ug.g^{1}$, day^{1}, for exposure concentration (B). these values are in very good agreement with the rates of metal loss recorded in Table 5. Thus, K_e values increase with increasing metal levels, as previous reported for earthworms, (Neuhaus, *et. al;* 1995). The rates of elimination by A. marginata, of Pb for both exposure concentrations were greater than values for *L. stagnalis*. For example, the foot mantle of *A. marginata* eliminates Pb about 6.6 times more than that of *L. stagnalis* at concentration (A) while at concentration (B), the relative ratio was 4.9. However, the gut of *A. marginata* eliminates Pb about 3.2 times for concentration (A) and 1.2 times at concentration (B) more than the values for *L. stagnalis*. By comparison, it may be inferred that at both concentrations, the foot mantle of *L. stagnalis* showed higher elimination capacity than the gut tissues. This implies that bioconcentration is greater in the gut than in the foot mantle in this species. This agrees with our previous findings (Omuaru, *et al;* 2000). This is further confirmed by the fact that rates of eliminated of lead in the gut tissues of *A. marginata* decreased with increase in pollutant concentration (Table 5.).

The elimination of chromium for both exposure concentrations in both species also followed the simple first order kinetics. For concentration (A), the rate constants were 0.006ug/g/day and 0.035ug/g/day for the

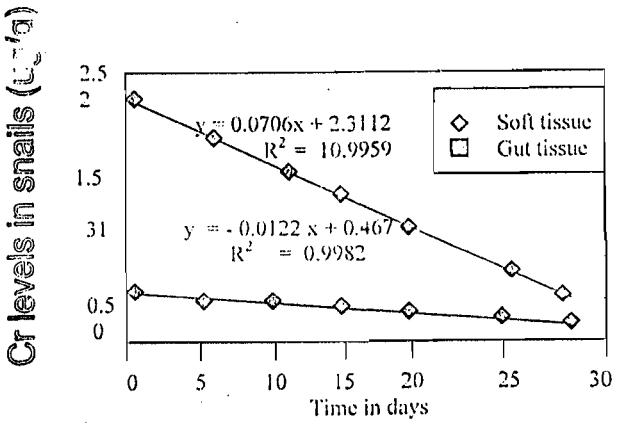

Fig. 4a: Terminal Elimination Rate Constant-Elimination of Cr by L.stagnalis (ug/g/day) for concentration (B).

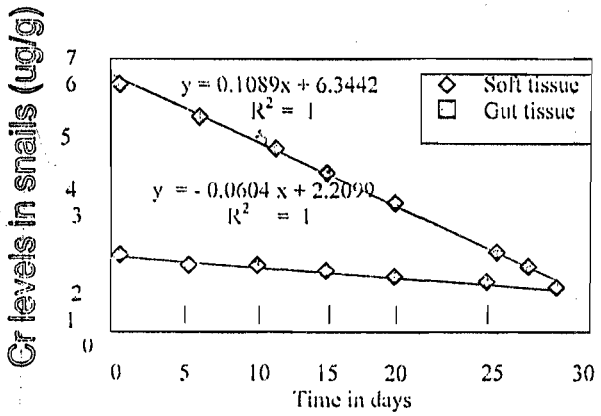

Fig. 4b: Terminal Elimination Rate Constant-Elimination of Cr by A. marginata (ug/g/day tor concentration (B).

foot mantle and gut tissues respectively of *L. stagnalis*, while the K_e values of *A. marginata* were 0.041 ug/g/day and 0.13 ug/g/day for foot mantle and gut tissues respectively (Figs. 3a and 3b). The K_e values for L. stagnalis increased with increase in concentration of Cr in the foot mantle. However, the K_e values in the gut tissues of *A. marginata* decreased with increase in concentrastion of the pollutant. (Figs 4a and 4b). this trend was equally observed in the elimination of Pb by *A. marginata.*

The pattern of uptake and elimination were consistent with the result that might be expected for these two metals which were bioconcentrated in the tissues of these snails. In this study, Cr was eliminated faster than Pb by both snails for the exposure concentrations considered (Table 5). However, *A. marginata* eliminated Cr faster than *L. stagnalis* in both tissues. In their studies on the uptake and elimination of heavy metals in the earthworm, Neuhauser *et al;* (1995) reported that lead and nickel were eliminated in the worms rapidly between days 0 and 7 and then tended to a plateau or increased slightly. This complex kinetic profile was not observed for Pb in the elimination of this metal in the snails, *L. stagnalis* and *a. marginata.* Beeby and Eaves (1983) and Williamson (1980) reported slower rates of loss of Pb and Zn in Helix aspersa than those for Cepaea hortensis and attributed the slower loss to the turnover rates experienced by the snails in their environment. The rate of loss of Pb in *Mytilus edulis* has also been shown to depend on tissue concentrations rather than the rate of uptake (Schulz-Baldes, 1974). These factors among others, may affect the kinetics of metal elimination in molluscs.

Considering the elimination factors (Table 6), it was found that the gut tissues of A. marginata had low elimination factors under the experimental conditions. This implies that these elements are accumulated moderately by the gut of this snail. The foot mantle of *L. stagnalis* also had moderate elimination factors and therefore eliminated chromium and lead moderately.

As illustrated in Table 7, the elimination rate of chromium for a 56 day elimination experiment was 0.014 ug/g/week in the foot mantle of *L. stagnalis.* The K_e value of 0.002 ug/g/day is consistent with the rate of chromium loss calculated. This rate of loss of Cr in the uncontaminated snails is lower by a factor of 3 for the lower concentration (A) and by a factor of 6 for the higher concentration (B) of contaminated snails. The rate of lead loss for the gut was 0.01 ug/g/week for uncontaminated snails, *L. stagnalis.* Similar trends were recorded for *A. marginata.* These results suggest that the rates of elimination of these metals are faster in contaminated snails than those of uncontaminated snails, probably induced by the necessity to detoxify.

From the results of rats of metal loss in our study, L. stagnalis and A. marginata have low to moderate elimination factors. Thee values indicate that these snails may not be suitable as agents for bioremediation processes but could be used to indicate the presence of chromium and lead in the terrestrial environment. The moderate rates of metal loss of A. marginata suggest also that snails bought for consumption could be left for some days to induce detoxification of the foot mantle before their use for food. Longer periods of exposure of these snails to higher exposure concentrations might reveal kinetic profiles different from that obtained in this study.

REFERENCES

Beeby, A. and Eaves, s.L., 1983. Short-term changes in Ca, Pb, Zn and Cd concentrations of the garden snail *Helix aspersa* (Muller) and its relevance to the monitoring of heavy metal contamination of the environment. *Oecologia,* 27: 67 – 74.

Beeby, A. and Richmond, L., 1987. Adaptation by an urban population of the snail. *Helix aspersa* to a diet contaminated with lead, *Environ. Pollut.,* 46: 73 – 82.

Beeby, A. and Richmond, L., 1989. The shell as a lead deposition *helix aspersa.* Arch. Environ. Comtam. Toxixicol; 18: 623 – 628.

Bertani, R., Cosiml, C; Delisp, a., Ferrara, R., Maserti, B.E., Trifoglio, M and zuccoreli, D., 1994. Mercury in a primary consumer, *Eobania vermiculata* collected near a Chlor-Alkali Complex. Environ. Tech. 15: 1095 – 1000.

Coughtrey, P.J. and Martin, M.H., 1976. The distribution of lead, zinc, cadmium and copper within the pulmonate mollusck *Helix aspersa* (Muller) *Oecologia 23:* 315 – 322.

Dallinger, R. and Wieser, W., 1984. Pattern of accumulation, distribution and libertion of Zinc, Copper, Cadmium and Lead in different organs of the land snail, *Helix pomatia*. J. Comp. Biochem. Physiol; 79C: 17 – 24.

Everard, M. and Denny, D., 1984. the transfer of lead by freshwater snail in Ullswater, Cumbia. Environmental Pollut. *35:* 2299 – 2314.

Frank, R., Kaluck, C. and Stonefield, K.I., 1983. metal transfer in vermicomposting of sewage sludge and plant wastes. Bull. Environ. Contam. & Toxicol., 31: 673 – 679.

Neuhauser, E.F., Cukic, Z. V., Malecki, M.R., Leohr, R.C. and Durkin, P.R., 1995. Bioconcentration and biokinetics of heavy metals in the earthworm. Environ. Pollution, 89: 293 – 301.

Omuaru, V.O.T., Selema, M.D. and Soroh, A.E., 2000. Bioaccumulation of heavy metals in soil invertebrates: Part 1 – uptake and accumulation of lead and chromium by *Lymnaea stagnalis* and *Achatina marginata.* Global J. Pure & Applied Sci. (in press).

Schulz-Baldes, M., 1974. Lead uptake from sea water and food, and lead loss in the common mussel *Mytilus edilus.* Marine Biol; 25: 177 – 193.7

Stoll, A. Duncan, J. R., 1997. Implementation of a continuous flow stirred bioreactor system in the bioremediation of heavy metals from industrial waste waters. Environ. Pollut.; 97: 247 – 251.

Volesky, B., 1987. Biosorbents for metal recovery. Trend in Biotechnology; 5: 96 – 100.

Williamson, P., 1980. Variables affecting body burdens of lead, zinc and cadmium in a road population of the snail *Cepaea hortensis* (Muller). *Oecologia*, (Berl.) 44: 213 – 220.