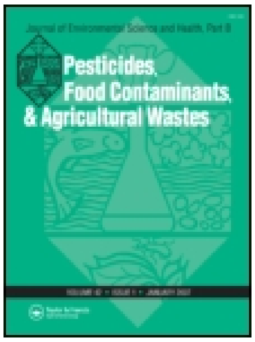

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METAL ACCUMULATION FROM CONTAMINATED FOOD AND ITS EFFECT ON GROWTH OF JUVENILE LANDSNAILS *HELIX ENGADDENSIS*

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ABSTRACT

Metal accumulation by juvenile landsnails, *Helix engaddensis*, and its effect on growth rate was studied over a 5-week period of exposure and 2 weeks of recovery. An artificial food contaminated with Cu (4–2500 $\mu g \cdot g^{-1}$), Cd (50–800 $\mu g \cdot g^{-1}$), Pb, and Zn (20–12 500 $\mu g \cdot g^{-1}$) was used. During the 7 weeks of the experiment, mortality rates were 20, 27, 30, and 38% among snails fed Cu-, Pb-, Zn-, and Cd-contaminated food, respectively. According to the ability to inhibit growth, metals were found to have the following order: Cd > Zn > Cu = Pb. Inhibitory effects of dietary metals started to be significant from the third week of exposure on. Inhibition of growth by Pb and Cu was found to be reversible, and within the first week of recovery, snails erupted their aestivation and resumed feeding and growth to gain weights similar to those of the control groups. Snails fed Cd- or Zn-contaminated food failed to resume growth during the 2 weeks of recovery. This indicates that in the case of Cu and Pb, growth inhibition was mainly due to starvation due to food rejection and aestivation. On the other hand, growth inhibition caused by Cd and Zn may have been resulting from irreversible toxicity. Therefore, snails were assumed to be sensitive to Cd and Zn but tolerant to Cu and Pb. Accumulation of Cu and Pb was significant only at the highest concentrations. At low and medium concentrations, no signs of accumulation were observed, indicating regulation at these concentrations. Cd and Zn accumu-

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lation starts at low concentrations but became significant at medium and high levels indicating accumulation of these metals.

Key Words: Metals; Accumulation; Landsnails; *Helix engaddensis*; Growth; Contamination

INTRODUCTION

Snails are among invertebrates that can concentrate heavy metals in their tissues to remarkably high levels and, therefore, are considered as good test animals to study the kinetics of metal accumulation and detoxification (1,2). The kinetics of metal accumulation and detoxification are still subjected to discussion, and there is a lack in consensus regarding metal toxicity in snails (3). Berger and Dallinger (1) found that terrestrial snails might regulate some metals assimilated from food. Van Straalen et al. (4) suggested that nutritional metals might be regulated, while xenobiotic metals are accumulated. Using snails in toxicity bioassays is an attractive method because snails are easy to culture in the laboratory, can be fed artificial diets with the desired amounts of metals, and respond quickly to metal contamination in the range of sublethal doses. However, this might be complicated by the fact that snails fed on diets supplemented with metals may decrease food consumption or even aestivate and stop feeding and, hence, decrease growth rates (3,5–7). According to Gomot (2), the mechanism involved in growth inhibition of snails fed metal-supplemented food is still unknown. In a study about the effect of cadmium on the growth of the snail *H. aspersa*, she suggested that growth inhibition could be due to inhibition of the production of a growth hormone essential for the growth of *Helix*. Szücs et al. (8) suggested that the chronic exposure of neurons of the nerve collar of the snail *Lymnaea stagnalis* to Cd can irreversibly modify the structure of the Ca channel.

In previous studies, Swaileh and Ezzughayyar (6,7) studied the effect of dietary metals (Cu, Cd, Pb, and Zn) on adult *Helix engaddensis* landsnails. According to Gomot (2), juvenile snails could be more resistant to short-term environmental pollution because they are more homogeneous than adults. Therefore, the present study was conducted in order to (a) evaluate the dose-dependent impact of metal-supplemented food (Cu, Cd, Pb, and Zn) on growth rate of juvenile landsnails, *H. engaddensis*, and examine the suitability of this snail to be used in laboratory short-term toxicity bioassays, (b) examine the accumulation patterns of the four metals by the juvenile snails, (c) inspect whether growth inhibition caused by metals is reversible or not when the metals are no longer added to the food of the snail, and finally (d) link results of the present study to those obtained earlier for adult snails of the same species.

MATERIALS AND METHODS

Collection and Culture of Snails

Helix engaddensis is one of the common landsnails in Palestine. It is smaller in size than European *Helix* species. Juvenile snails were collected from a house garden in Qalqilia city and were cultured in the laboratory in large glass aquaria. Snails were fed

Table 1. Nominal Treatments and Actual (Measured) Concentrations of Metals (Mean ± SE, n = 3) in the Experimental Food and Average Weight (g ± SE) of Snails in Each Group (n = 10) at the Start of the Experiment

	Metal Concentration in the Diet ($\mu g \cdot g^{-1}$)		
Group and Metal	Nominal	Actual	Average Weight
G1-Pb	0 (control)	1.25 ± 0.250	0.38 ± 0.031
G2-Pb	20	19.90 ± 0.33	0.40 ± 0.030
G3-Pb	100	96.20 ± 2.60	0.40 ± 0.032
G4-Pb	500	454.5 ± 11.1	0.41 ± 0.027
G5-Pb	2500	2274 ± 141.2	0.40 ± 0.028
G6-Pb	12500	12104 ± 519	0.40 ± 0.028
G1-Zn	0 (control)	14.60 ± 1.50	0.41 ± 0.030
G2-Zn	20	30.90 ± 2.30	0.39 ± 0.024
G3-Zn	100	104.1 ± 6.00	0.43 ± 0.022
G4-Zn	500	521.7 ± 35.0	0.39 ± 0.022
G5-Zn	2500	2517 ± 151.1	0.39 ± 0.032
G6-Zn	12500	12615 ± 415	0.39 ± 0.020
G1-Cu	0 (control)	5.59 ± 1.23	0.43 ± 0.022
G2-Cu	4	6.93 ± 0.22	0.43 ± 0.015
G3-Cu	20	28.9 ± 1.22	0.44 ± 0.017
G4-Cu	100	121.5 ± 17.02	0.44 ± 0.021
G5-Cu	500	468.3 ± 16.46	0.44 ± 0.022
G6-Cu	2500	2158 ± 69.76	0.44 ± 0.016
G1-Cd	0 (control)	0.32 ± 0.012	0.43 ± 0.022
G2-Cd	50	58.9 ± 7.35	0.43 ± 0.021
G3-Cd	100	87.7 ± 1.19	0.44 ± 0.020
G4-Cd	200	172.4 ± 3.50	0.44 ± 0.019
G5-Cd	400	391.0 ± 7.63	0.45 ± 0.022
G6-Cd	800	705.1 ± 39.17	0.43 ± 0.014

carrots and lettuce and were kept at room temperature. Before starting the experiment, snails of similar weight (Table 1) were selected and cleaned, and each 10 snails were kept in a transparent plastic box (size 17×13×7 cm). Boxes were perforated at their sides to allow proper aeration. The bottom of each box was covered by a thin sponge soaked with deionised water to keep 100% humidity. The experiment was run in a growth chamber at 15 °C and 16/8 hours light/dark period. Before starting the experiments, snails were offered an artificial control food for 3 days in order to acclimatize.

Food Preparation and Feeding of Snails

Metal stock solutions (1 g/L) were prepared using cupric chloride dihydrate ($CuCl_2.2H_2O$), cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$], lead nitrate [$Pb(NO3)_2$], and zinc chloride ($ZnCl_2$). An artificial food containing ca. 5% dry mass was prepared by mixing 4 g Cerelac-with vegetables, baby food (Nestlé, Belgium), and 1 g Agar (Sigma, USA) with solutions containing the required concentrations of metals (Table 1) to give

100 ml of agar medium. A fungicide (*p*-hydroxy benzoic acid methyl ester = methyl paraben, Sigma) was added to the solutions as 0.3 ml/100 ml food. Each 100 ml medium was divided equally between four Petri dishes (25 ml/dish). After cooling, Petri dishes were kept in the refrigerator. Control food was prepared the same way except using distilled water, instead of the metal solutions above. From each treatment, three Petri dishes were taken randomly, and food was dried in an oven at 60 °C until constant weights were observed. Thereafter, dried food was ground to powder, and subsamples of 0.2 g were taken from each treatment and digested using a mixture of 1:1 nitric:perchloric acids (Suprapur, Merck) until the mixture became clear. Samples were diluted with deionized distilled water, and volumes were adjusted to 25 ml in volumetric flasks. Finally, concentrations of the four metals were measured using ICP-spectrophotometry (Table 1).

Snails were offered food *ad libitum*. Boxes were examined daily, and food was offered as required. Once every 7 days, the snails were weighed, and the boxes were cleaned. During the first 5 weeks of the experiment, snails were fed metal-contaminated food. Thereafter, all groups of snails were fed food with no metals added (control food) for another 2 weeks.

Statistical Analysis

All statistical tests were performed using SYSTAT for Windows 5.02 (SYSTAT, Evanston, IL, USA, 1993). Each week, average weights of groups were tested for differences in their mean weights using ANOVA test. Thereafter, Tukey test was performed for pairwise comparisons between groups.

RESULTS AND DISCUSSION

Mortality

During the 7 weeks of the experiment, a total of 69 snails out of 240 died. These death cases were from all groups. The death rates were 20, 27, 30, and 38% among snails fed Cu-, Pb-, Zn-, and Cd-contaminated diets, respectively. Death cases started to appear during the first week of exposure and increased with time of the experiment (Figure 1). In similar studies on adult snails of the same species (6,7), death rates were 2, 18, 50, and 17% for Cu, Pb, Zn, and Cd, respectively. Obviously, death rates among adults were much less than juveniles, except for Zn. Gomot (2) observed much lower death rates among *H. aspersa aspersa* and *H. aspersa maxima* juveniles fed Cd-contaminated diet (range 0–800 μg Cd/g food). During the 4 weeks of exposure, 5% of *H. aspersa aspersa* and 6.7% of *H. aspersa maxima* died. Deaths were distributed among all groups including the control. Laskowski and Hopkin (3) studied the effect of Zn, Cu, Pb, and Cd on fitness of *H. aspersa*. Mortality rate among all groups was 6.7% among juveniles and 1.9% among adults during the 4 months of the experiment. As shown in Figure 1, snails were less sensitive to Cu than other metals. Snails are able to accumulate large quantities of Cu (9), hence the lowest mortality rate observed among snails fed Cu-contaminated food compared to other metals. Gastropod snails are known to have the Cu-containing respi-

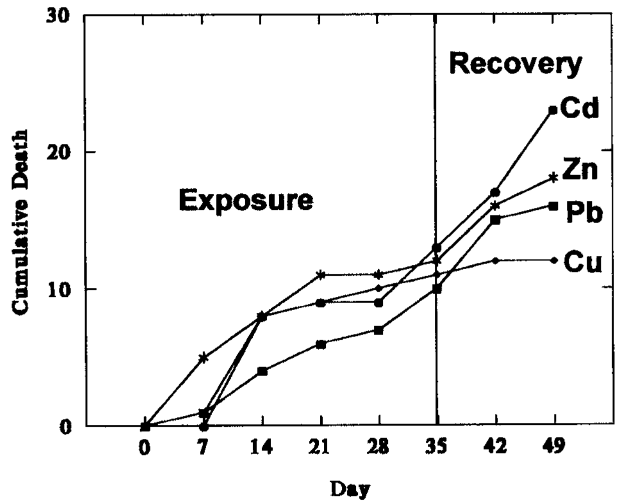

Figure 1. Mortality of juvenile *Helix engaddensis* snails from all groups during the 7-week experiment. Total number of snails subjected to each metal (including the control group) is 60.

ratory pigment (haemocyanin), which could explain the ability to accumulate Cu to high levels (9,10).

Growth

The effect of dietary metals on growth of juvenile *H. engaddensis* landsnails is shown in Figure 2. The four metals were found to negatively affect growth of snails. Growth inhibition of snails fed Cu- or Pb-contaminated food became statistically significant only during the fifth week of the experiment and at the highest concentrations of the two metals (12,500 ppm for Pb and 2500 ppm for Cu). All other groups continued to have average weights that did not show any statistical difference with those of the control groups. In previous works (6,7), adult snails of the same species were found to be more sensitive to Cu and Pb because growth inhibition was significant during the second and third weeks of exposure for Pb and Cu, respectively. When juvenile snails in group 6 were offered control food, they started to grow again, and 1 week of recovery was enough for snails to have body weights similar to other groups including the control (Figure 2). Similar results were observed for adults of *H. engaddensis* (6,7). However, growth of adult snails fed Pb-contaminated diets during the recovery period was much slower than that of the juveniles. This indicates that growth inhibition was due to starvation caused by rejection of metal-contaminated food followed by aestivation and was not due to irreversible toxicity. The ability of snails to distinguish metal-contaminated food is documented in many studies (5–7,11) and is shared by other test organisms. For example, the woodlouse, *Porcellio laevis*, was found to be able to distinguish and avoid cadmium-contaminated plant leaves (12).

Juvenile *H. engaddensis* snails seem to be much more sensitive to Zn and Cd than Pb and Cu (Figure 2). Besides, snails were more sensitive to Cd than Zn. The inhibitory

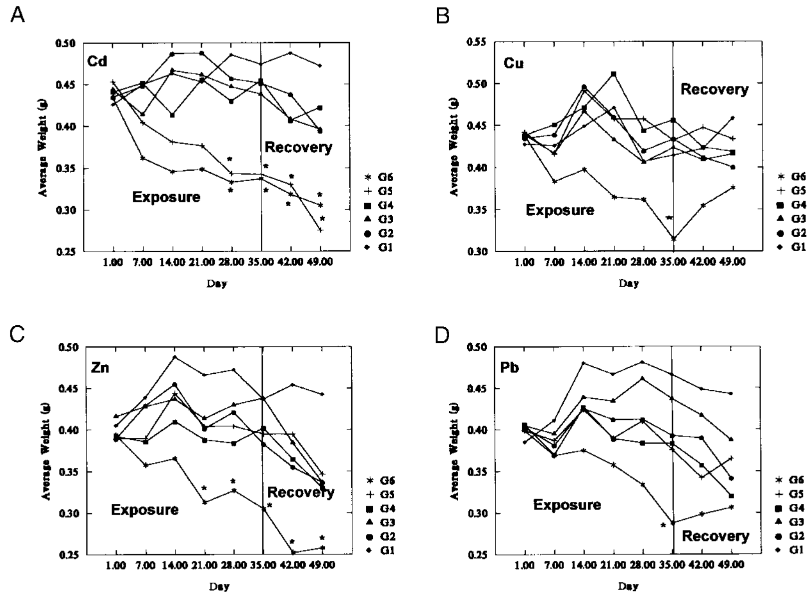

Figure 2. Growth curves of juvenile *Helix engaddensis* snails exposed to Cu-, Cd-, Pb-, and Zn-contaminated food for 35 days (exposure) and offered control food for another 14 days (recovery). For metal concentrations in the diet of each group, see Table 1. Values represent means ± standard errors. Initial number of snails in each group was 10. *Indicates values that are significantly different ($p < 0.01$) from that of the control group.

effect of the Cd and Zn on the growth of juvenile snails started to be significant during the third (Zn) and fourth (Cd) weeks of exposure. This effect was obvious in groups 6 (Zn) and 5 and 6 (Cd). Moreover, the inhibitory effect continued during the 2 weeks of recovery when snails were offered control food. This could be an indication of irreversible toxicity caused by th 2 metals. Adult snails of the same species failed also to resume growth after being exposed to the two metals for 4 weeks and offered control food for 2 weeks (6,7). The mechanism involved in growth inhibition of organisms exposed to elevated dietary metals is still unknown (2). In snails of the genus *Helix*, a growth hormone, necessary for growth, is secreted by the neurosecretory cells of the mesocerebrum-supraesophageal part of the nerve collar (13). Cadmium may disturb the function of the neurosecretory cells of the mesocerebrum causing ''growth stoppage'' (2). Szücs et al. (8) found that acute exposure to Cd of neurons of the nerve collars of *Lymnaea stagnalis* can reversibly block the Ca channels, whereas chronic exposure can irreversibly modify the structure of the channel. Other studies suggest that Cd may block calcium uptake through the gut, causing calcium deficiency with disturbance of Ca^{++} homeostasis (14), or may alter food intake by an inhibiting action on the nerve centers (2). Metals were found to reduce growth of other test organisms like the earthworm *Eisenia fetida* (15). They attributed the effect of metals on growth and maturation time to the direct toxicity of metals and to changes in the ''scope of growth'' of the exposed worms. Copper-contaminated microalgae were found to affect growth of rotifers causing a delay of 1 or 2 days in populational development (16).

Metal Accumulation

Metal accumulation patterns in juvenile *H. engaddensis* snails are shown in Figure 3. Strong correlation coefficients for the relationships between dietary metal concentration and snails' metal concentration were observed. A remarkably strong correlation coefficient ($r = 0.96$) between metal concentration in food and that in snail tissue was observed for Pb. Less strong, but statistically significant, correlation coefficients existed for the other three metals. In addition, snails of groups 4, 5, and 6 (for Cd and Zn) and 5 and 6 (for Pb and Cu) were found to contain significantly higher metals in their tissues than the control groups. Figure 3 indicates that snails might be able to regulate Pb and Cu at concentrations below 2500 ppm (for Pb) and 500 ppm (for Cu). This is because statistically significant accumulation of the two metals started at these concentrations. On the other hand, accumulation of Zn and Cd starts earlier and becomes statistically significant at 200 ppm (for Cd) and 500 ppm (for Zn). In other words, this indicates that juvenile *H. engaddensis* snails might have the ability to regulate Cu and Pb to a certain limit. Beyond this limit, the ability breaks and significant accumulation starts. Such ability seems to be lacking when Cd and Zn are considered. This again, along with the results in Figures 1 and 2, explains the higher sensitivity of juvenile *H. engaddensis* snails to Cd and Zn than Cu and Pb. Accumulation of Cu, Cd, Pb, and Zn

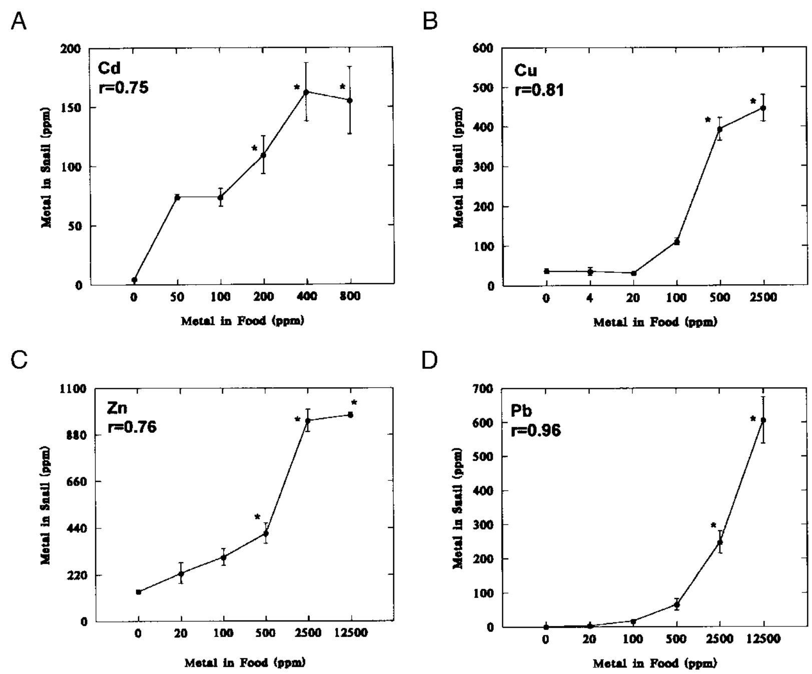

Figure 3. Accumulation of Cu, Cd, Pb, and Zn by juvenile *Helix engaddensis* snails in relation to dietary metal concentration after 5 weeks of exposure and 2 weeks of recovery. Values represent means ± standard errors of three specimens. *Indicates values that are significantly different ($p < 0.01$) from that of the control group. r = Correlation coefficient.

in *H. aspersa* was studied (17). They found that Pb was the most efficiently regulated metal of the four, while Zn was accumulated approximately in direct proportion to its concentration in the diet. Other studies considered snails as macroconcentrators of Zn and Cd (18).

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