ELSEVIER   0269-7491(95)00070-4

ACCUMULATION OF Zn, Cu, Pb AND Cd IN THE GARDEN SNAIL (*Helix aspersa*): IMPLICATIONS FOR PREDATORS

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Abstract

Accumulation of Zn, Cu, Pb and Cd was studied in snails fed for 120 days on diets contaminated with each metal separately and with all metals mixed together. The concentrations of Zn in food were in the range 39 to 12 200 mg kg^{-1}, Cu 9–1640 mg kg^{-1}, Pb 0.4–12 700 mg kg^{-1}, and Cd 0.16–146 mg kg^{-1} on a dry weight basis. At the highest concentrations of all metals the consumption rates decreased significantly. For the remaining concentrations, Zn and Cu were accumulated in soft tissue in proportion to their concentrations in food. The lowest treatments of Pb and Cd did not cause any increase in soft tissue concentrations of these metals but at average treatments, a clear increase was observed. Copper was accumulated especially efficiently, exceeding concentrations in food throughout the whole range of treatments. Except for the lower end of experimental treatments, Zn was accumulated approximately in direct proportion to its concentration in the diet. Lead was the most efficiently regulated metal, with soft tissue concentrations always substantially lower than in food. Approximately 60% of Zn, 90% of Cu, 43% of Pb and 68% of Cd on average was assimilated from food. The assimilation efficiency of food alone was ca 74%. The concentrations of metals in shells increased significantly with exposure, but (with one exception) the concentrations in shells did not exceed 5% of those found in soft tissue. We argue that snails are more important as agents of food-chain transport of Cu and Cd, than of Zn or Pb. Our results indicate also that snails are not able to deposit significant quantities of metals in their shells, at least during the time scale of our laboratory experiment.

INTRODUCTION

Snails and slugs, with population densities of up to 1000 individuals m^{-2} (Mason, 1970) can be important prey for mammals, birds and invertebrate predators (Symondson & Lidell, 1993; Graveland *et al.*, 1994). With their high potential for accumulation of pollutants (Coughtrey & Martin, 1976; Hopkin, 1989; Jones,

1991), snails and slugs may provide important links in transfer of chemicals from vegetation or plant litter to carnivores. Such transfer along food chains is one of the most important aspects of ecotoxicology.

With regard to transfer of metals, despite being studied intensively for the last three decades, the question of whether metals 'biomagnify' along trophic chains is still discussed extensively (see Fagerström, 1991; Laskowski, 1991; Van Straalen & Ernst, 1991). Even for PCBs, once believed to biomagnify strongly along food chains, the most recent data suggest that the phenomenon is not general and that biomagnification factors for PCBs vary between 1 and 6 (Sijm *et al.*, 1992). Substantial differences in the mobility of various metals between trophic levels are also well documented (e.g. Grodzińska *et al.*, 1987; Hunter *et al.*, 1987*a*). Van Straalen *et al.* (1987) suggested that the main differences in the ecophysiology of metals are due to their essentiality versus nonessentiality to organisms. Nutritional metals, like Zn and Cu, are regulated, and xenobiotics, like Pb and Cd, are accumulated. However, this rule does not seem to apply to animals with haemocyanin in their oxygen-carrying system, such as isopods and molluscs, which accumulate Cu over a broad range of environmental concentrations (Moser & Wieser, 1979; Hughes *et al.*, 1980; Hopkin, 1990).

This situation is complicated even more by the fact that metals can be deposited in intracellular granules. These granules may be insoluble in the gut of predators and may be voided largely intact in the faeces. The metals are not then accumulated in the tissues of the predator (Hopkin, 1989; Nott & Nicolaidou, 1990). In snails, as suggested by Beeby and Richmond (1989), some metals may be deposited additionally in shells. This would mean that the consequences of metal pollution in snails are significantly different for animals feeding on their soft tissue only (e.g. carabids, shrews) from those eating their shells also (e.g. birds).

In snails, attempts to quantify food chain transfer of metals is even more complicated by the fact that under unfavourable conditions, snails may aestivate and stop feeding (Cain, 1983; Russell-Hunter & Buckley, 1983). Snails fed on diets supplemented with metals may decrease their consumption and hence growth rates (Russell *et al.*, 1981; Marigomez *et al.*, 1986; Simkiss & Watkins, 1990;

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Laskowski & Hopkin, in press). High concentrations of metals in food apparently affect population growth rates (Laskowski & Hopkin, in press), but it is not clear whether this effect is due to direct poisoning or starvation.

At least three important questions arise from the above considerations:

(1) Which metals among common industrial pollutants are of primary concern from the point of view of their transfer through snails to higher trophic levels?

(2) Are the animals that feed on the soft tissues of snails more or less exposed to metal intoxication than those that also eat shells?

(3) Are snails exposed to metal pollution endangered by direct metal poisoning or starvation due to rejection of unpalatable food with high concentrations of metals?

METHODS

Breeding methods

An artificial diet used in the experiment was prepared according to the formula given by Laskowski and Hopkin (in press). The food comprised approximately 5% dry mass and was based on agar powder (Lancaster Synthesis, UK), ground oven-dried carrots, dried skimmed milk (Premier Beverages, UK) and bran, with addition of $CaCO_3$ and fungicide (p-hydroxy benzoic acid methyl ester = methyl paraben, Sigma).

Five experimental doses were used for each metal. For Zn and Pb the nominal concentrations were: 20, 100, 500, 2500 and 12 500 mg kg^{-1} (on a dry weight basis); for Cu: 2, 10, 50, 250 and 1250 mg kg^{-1}; and for Cd: 0.2, 1, 5, 25 and 125 mg kg^{-1}. The same doses of metals were used in combinations of all four metals for the intermetallic interactions experiments. In this way 25 different treatments were obtained plus control. In the following text the treatments are described as follows: Zn–20; Zn–100 and so on for the other metals. For mixed metals: MIX-1 = 20 + 20 + 2 + 0.2 mg kg^{-1} Zn + Pb + Cu + Cd, respectively, and so on up to MIX-5.

Solutions of the pre-defined concentrations of metals (as nitrates) were prepared and 40 ml of each solution was mixed while boiling with 1 g of agar. For the control diet 40 ml of double distilled water was used. This was mixed with 59 g of a pulp consisting of distilled water, carrots, dried milk, bran, $CaCO_3$ and methyl paraben, and poured into Petri dishes to form a thin layer. When set, the agar plates were stored at 4°C. During the experiment discs of agar were cut with a stainless steel cork-borer and offered to the animals.

Adult snails with an initial fresh weight of 6.2 to 11.4 g were held in plastic boxes (ca 18×12×7 cm) with perforated transparent lids. Four animals selected at random were kept in each box. During the first three months of the experiment, every box contained a piece of a wet sponge (ca 10×8 cm) to maintain the humidity at 100%. After three months all boxes were supplied with a 3 cm thick layer of uncontaminated soil collected from Reading University campus to provide snails with conditions suitable for laying eggs. The snails were examined daily. Food was supplied ad libitum. Once a week, all boxes and sponges were cleaned in hot water. For the first three months of the experiment, the food offered, faeces and uneaten food were collected and oven dried at 70°C. The experiment was run at 15°C under a 16:8 L:D regime. For all treatments, three replicates were used. A total of 312 adult individuals were used in the experiment. After four months, snails were washed twice in distilled water and left in clean empty boxes for 4 days to void their digestive tracts. Finally, the snails were killed by deep freezing.

Chemical analyses

Three samples of each type of food and one sample of faeces from each replicate were digested in 2 ml of boiling Analar grade HNO_3 (BDH Ltd, Poole, UK). After cooling, the samples were diluted to 10 ml with double distilled water. One snail from each replicate was separated from its shell, and the samples digested individually in 100 ml Pyrex conical flasks in 20 ml of boiling HNO_3. After cooling, these digests were diluted to 100 ml with double distilled water. Samples of food, faeces and soft tissues were analysed by flame atomic absorption spectrometry (AAS; Varian–Techtron, SpectrAA-30) against 20% HNO_3 standards with deuterium lamp background correction. Blank samples were run every 15 samples, and after 40 samples one standard was measured to check for the consistency in measurements. The same method was applied for Zn in shells, while Cu, Pb and Cd in shells were analysed by graphite furnace AAS (GTA-95) using the standard additions method. The spectrometer was recalibrated every 10 samples and blank samples were run every 20 samples. The AAS was calibrated using standard reference materials of lobster hepatopancreas, bovine liver and tomato leaves as described by Hopkin (1990). Values for Zn, Cu, Pb and Cd were within 5% of the certified values.

Calculations and statistical analysis

Relationships between concentrations of metals in food and soft tissue, shells and faeces were studied by regression analysis of log-transformed data. In most cases significant lack-of-fit was detected due to significantly decreased consumption rates in snails fed the highest concentrations of metals (cf Laskowski & Hopkin, in press). Accordingly, averages and 95% confidence intervals for means were calculated for consumption rates. Concentrations at which consumption rates were significantly lower than at all other treatments were excluded from the regression analyses. In this way all the highest concentrations of separately applied metals and two highest concentrations of MIX treatment were excluded. Exclusion of these values was considered appropriate as earlier studies (Laskowski & Hopkin, in press) have shown that at the highest concentrations used in this experiment, snails cease to breed in the laboratory and the population would not persist in the field.
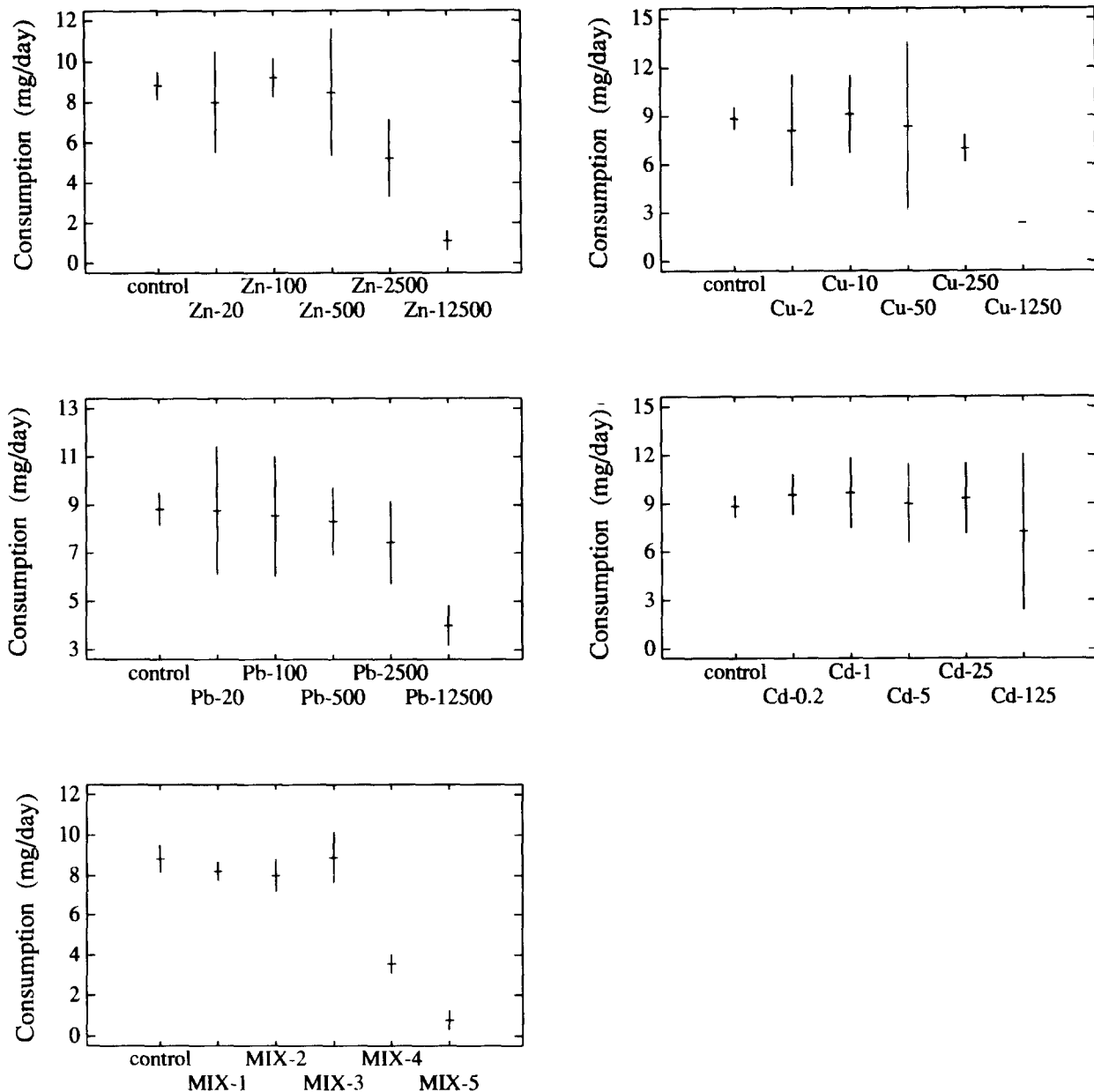
Fig. 1. Effects of metal treatment on consumption rate in snails: mg dry wt per day per 1 g fresh weight of snail (mean and 95% confidence intervals); see text and Tables 1–4 for further details of dietary concentrations of metals.

RESULTS

Almost all regressions calculated using the whole range of experimental concentrations revealed significant lack-of-fit at the 0.05 level, apparently due to decreased food consumption at the highest concentrations. Although consumption rates remained relatively constant over a broad range of experimental concentrations, at the highest concentrations of Zn and MIX treatments, snails almost completely rejected the food offered. Significant decreases in consumption rates when compared to the control (95% confidence intervals for mean) also occurred in the Zn–2500, Cu–250 and Cu–1250, Pb–12500, and MIX-4 treatments (Fig. 1). In many cases this resulted in lower concentrations of metals in snails fed diets with the highest treatments compared to those exposed to lower

concentrations (Tables 1–4). Because of this, and the predicted extinction of field populations at the highest concentrations (Laskowski & Hopkin, in press), the highest treatments were excluded from the regression analysis.

Such calculated regressions revealed good fit in most cases and were highly significant (Tables 1–4). For soft tissue concentrations the regressions explained from 44% (Cd in MIX) to 92% (Zn in Zn-treatment) of total variability. Concentrations of metals in faeces were also related to concentrations in food, with R^2 between 0.87 (Cu and Cd in MIX) and 0.97 (Zn in Zn-treatment). Although concentrations of metals in shells were significantly related to those in food, on average this relationship explained only 50% of the variability (range from 35% for Cd in Cd-treatment to 64% for Pb in Pb-treatment) (Tables 1–4).

Table 1. The nominal treatments and actual (measured) concentrations of Zn in the experimental food, soft tissue, shells and faeces of snails (average ±SD); in brackets are the slopes and significance levels for regressions of log metal concentration in soft tissue/shells/faeces on log concentration in food

Treatment	Food (mg kg⁻¹)	Soft tissue (mg kg⁻¹)	Shells (mg kg⁻¹)	Faeces (mg kg⁻¹)
0 (control)	39 ± 15	131 ± 20	3.5 ± 1.0	75 ± 10
		(0.63***)	(0.21*)	(0.85***)
Zn-20	80 ± 15	187 ± 49	3.8 ± 0.2	112 ± 21
Zn-100	128 ± 25	208 ± 105	5.5 ± 2.6	157 ± 63
Zn-500	483 ± 47	548 ± 158	10.0 ± 8.4	718 ± 43
Zn-2500	2650 ± 6	1830 ± 376	7.9 ± 2.9	2320 ± 271
Zn-12500	12200 ± 1380	1400 ± 1580	15.4 ± 2.0	3310 ± 1660
		(0.63***)	(0.34**)	(0.83***)
MIX-1	43 ± 2	137 ± 34	2.8 ± 0.5	107 ± 31
MIX-2	136 ± 44	246 ± 98	5.4 ± 1.2	202 ± 45
MIX-3	446 ± 19	645 ± 183	7.2 ± 2.9	648 ± 30
MIX-4	2070 ± 67	699 ± 221	10.5 ± 4.3	1120 ± 330
MIX-5	10900 ± 542	452 ± 183	28.0 ± 6.2	2260 ± 2230

Regressions calculated with the highest concentrations excluded—see Methods. $* = p < 0.05$, $** = p < 0.005$, $*** = p < 0.0001$; $N = 4$ for control, $N = 3$ for other treatments.

Table 2. The nominal treatments and actual (measured) concentrations of Cu in the experimental food, soft tissue, shells and faeces of snails (average ±SD); in brackets are the slopes and significance levels for regressions of log metal concentration in soft tissue/shells/faeces on log concentration in food

Treatment	Food (mg kg⁻¹)	Soft tissue (mg kg⁻¹)	Shells (mg kg⁻¹)	Faeces (mg kg⁻¹)
0 (control)	15 ± 2.6	101 ± 35	0.7 ± 0.3	6.3 ± 0.6
		(0.54***)	(0.40**)	(0.72***)
Cu-2	15 ± 1.0	134 ± 58	1.1 ± 0.2	10.1 ± 0.5
Cu-10	26 ± 4.0	285 ± 46	2.1 ± 0.8	9.8 ± 2.2
Cu-50	76 ± 2.6	223 ± 57	1.8 ± 0.6	20.9 ± 2.6
Cu-250	337 ± 28	703 ± 54	3.3 ± 0.4	73.5 ± 8.2
Cu-1250	1640 ± 149	740 ± 166	3.7 ± 0.9	239.0 ± 76.3
		(0.61***)	(0.58*)	(0.65***)
MIX-1	9 ± 0.2	98 ± 17	1.2 ± 0.5	6.8 ± 1.4
MIX-2	24 ± 0.4	136 ± 35	2.0 ± 0.5	10.5 ± 0.3
MIX-3	67 ± 5.4	300 ± 53	2.9 ± 1.6	21.7 ± 3.0
MIX-4	294 ± 8.6	308 ± 58	2.3 ± 0.5	91.1 ± 22.1
MIX-5	1320 ± 90	203 ± 65	3.3 ± 0.1	271.0 ± 306

Regressions calculated with the highest concentrations excluded—see Methods. $* = p < 0.05$, $** = p < 0.005$, $*** = p < 0.0001$; $N = 4$ for control, $N = 3$ for other treatments.

Table 3. The nominal treatments and actual (measured) concentrations of Pb in the experimental food, soft tissue, shells and faeces of snails (average ±SD); in brackets are the slopes and significance levels for regressions of log metal concentration in soft tissue/shells/faeces on log concentration in food

Treatment	Food (mg kg⁻¹)	Soft tissue (mg kg⁻¹)	Shells (mg kg⁻¹)	Faeces (mg kg⁻¹)
0 (control)	0.4 ± 0.08	22 ± 2.8	5.3 ± 1.1	12.1 ± 6.0
		(0.38**)	(0.16**)	(0.66***)
Pb-20	23 ± 5.1	8 ± 6.4	5.7 ± 1.4	43.2 ± 6.5
Pb-100	100 ± 3.8	46 ± 1.9	9.5 ± 1.9	201.0 ± 53
Pb-500	591 ± 99	224 ± 47	11.7 ± 2.8	832.0 ± 157
Pb-2500	2680 ± 293	640 ± 100	27.7 ± 14.3	4380.0 ± 172
Pb-12500	11400 ± 800	1240 ± 441	27.0 ± 10.2	23800.0 ± 1320
		(0.32**)	(0.11**)	(0.58***)
MIX-1	16 ± 3.8	21 ± 5.0	9.5 ± 4.0	44.2 ± 4.2
MIX-2	99 ± 3.5	167 ± 213	8.4 ± 6.0	166.0 ± 8.5
MIX-3	474 ± 34	251 ± 25	13.2 ± 2.3	876.0 ± 75
MIX-4	2280 ± 231	361 ± 135	13.9 ± 3.9	2300.0 ± 676
MIX-5	12700 ± 854	156 ± 105	23.4 ± 5.9	3390.0 ± 2240

Regressions calculated with the highest concentrations excluded—see Methods. $* = p < 0.05$, $** = p < 0.005$, $*** = p < 0.0001$; $N = 4$ for control, $N = 3$ for other treatments.

Table 4. The nominal treatments and actual (measured) concentrations of Cd in the experimental food, soft tissue, shells and faeces of snails (average ± SD); in brackets are the slopes and significance levels for regressions of log metal concentration in soft tissue/shells/faeces on log concentration in food

Treatment	Food (mg kg^{-1})	Soft tissue (mg kg^{-1})	Shells (mg kg^{-1})	Faeces (mg kg^{-1})
0 (control)	0.16 ± 0.03	7.3 ± 2.9 (0.46***)	0.010 ± 0.002 (0.19*)	0.4 ± 0.08 (0.72***)
Cd-0.2	0.28 ± 0.08	2.9 ± 0.5	0.008 ± 0.001	0.5 ± 0.29
Cd-1	1.11 ± 0.07	7.5 ± 1.3	0.008 ± 0.002	0.9 ± 0.30
Cd-5	5.32 ± 0.37	18.0 ± 5.7	0.009 ± 0.001	2.3 ± 0.12
Cd-25	25.90 ± 2.12	55.3 ± 17.0	0.040 ± 0.009	19.6 ± 6.55
Cd-125	146.00 ± 20.5	154.5 ± 85.7 (0.28*)	0.127 ± 0.093 (−0.35*)	106.0 ± 24.4 (0.56***)
MIX-1	0.38 ± 0.14	6.1 ± 0.7	0.018 ± 0.003	0.8 ± 0.24
MIX-2	1.21 ± 0.10	6.1 ± 1.7	0.011 ± 0.012	0.8 ± 0.10
MIX-3	5.38 ± 0.28	21.3 ± 7.9	0.002 ± 0.002	3.3 ± 0.18
MIX-4	25.80 ± 2.03	18.6 ± 8.3	0.018 ± 0.003	7.7 ± 0.89
MIX-5	132.00 ± 4.56	9.8 ± 6.5	0.052 ± 0.006	37.3 ± 46.7

Regressions calculated with the highest concentrations excluded—see Methods. * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.0001$; $N = 4$ for control, $N = 3$ for other treatments.
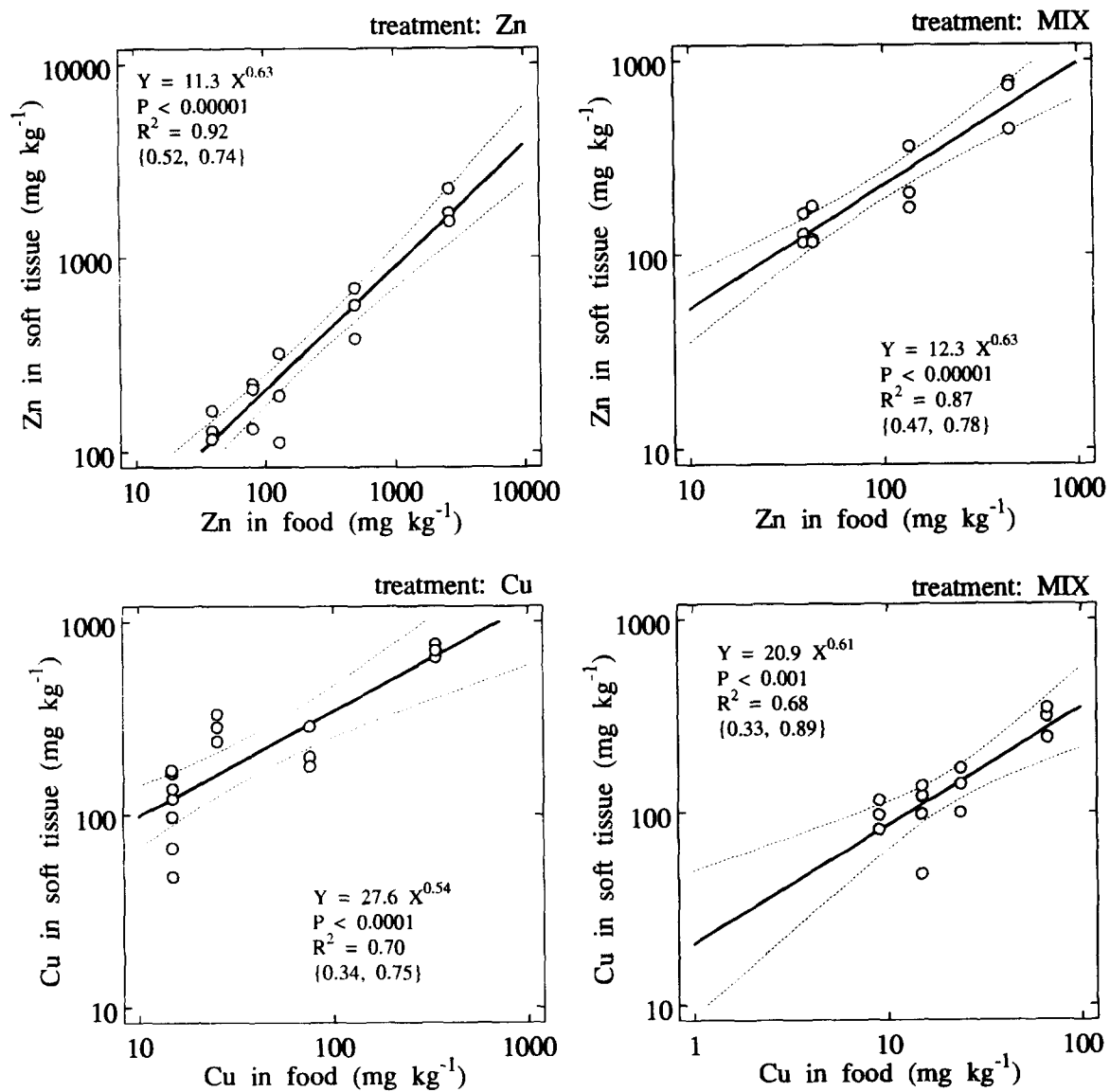

Fig. 2. Relationships between Zn and Cu concentrations in soft tissue and concentrations of metals in food in treatments with single elements and in a mixture of Zn, Cu, Pb and Cd (MIX); broken lines = 95% confidence intervals for mean; 95% confidence intervals for the slope estimate are given in brackets.
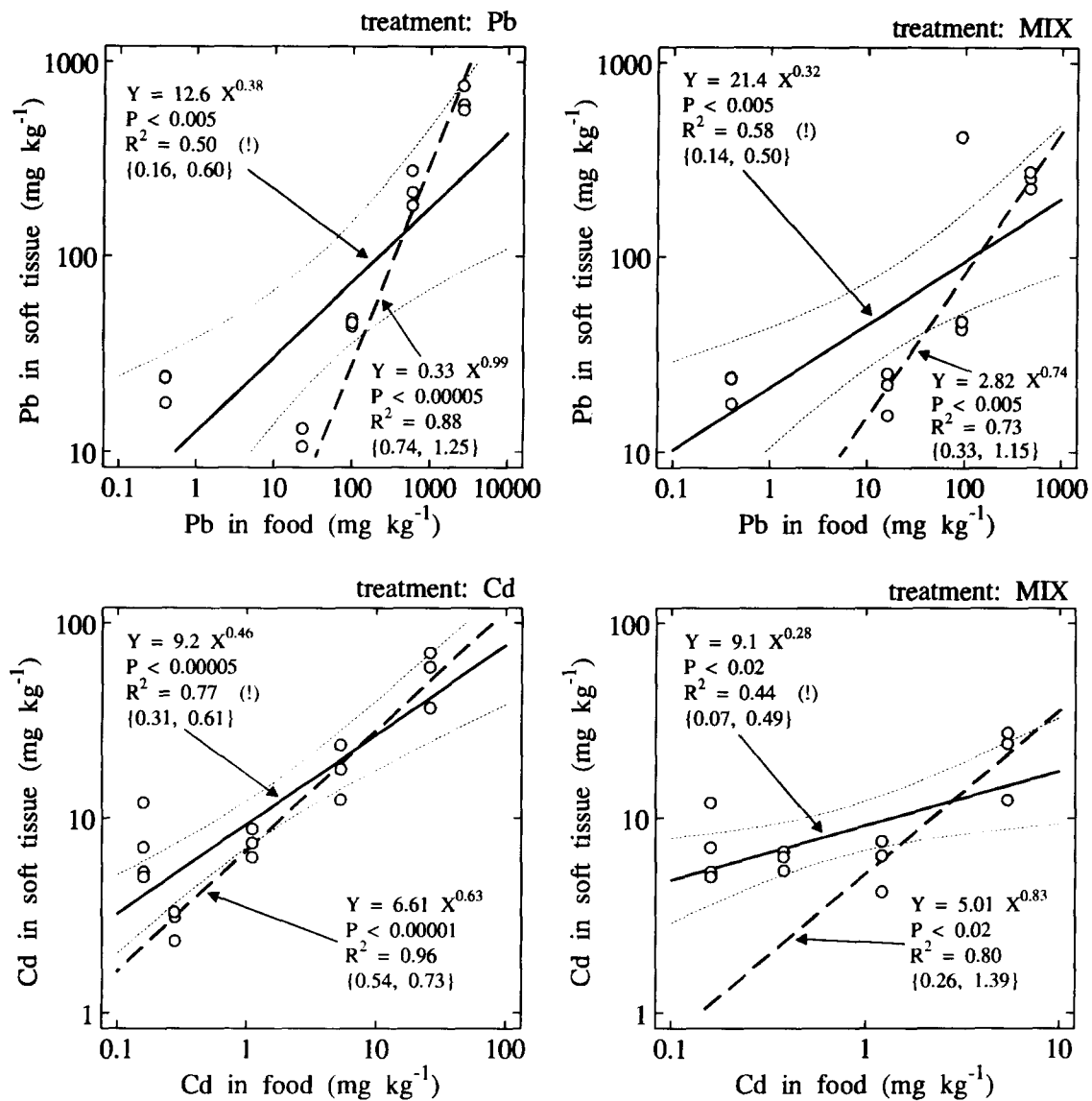
treatment: Pb

Y = 12.6 X$^{0.38}$
P < 0.005
R^2 = 0.50 (!)
{0.16, 0.60}

Y = 0.33 X$^{0.99}$
P < 0.00005
R^2 = 0.88
{0.74, 1.25}

Pb in soft tissue (mg kg^{-1})

Pb in food (mg kg^{-1})

treatment: MIX

Y = 21.4 X$^{0.32}$
P < 0.005
R^2 = 0.58 (!)
{0.14, 0.50}

Y = 2.82 X$^{0.74}$
P < 0.005
R^2 = 0.73
{0.33, 1.15}

Pb in soft tissue (mg kg^{-1})

Pb in food (mg kg^{-1})

treatment: Cd

Y = 9.2 X$^{0.46}$
P < 0.00005
R^2 = 0.77 (!)
{0.31, 0.61}

Y = 6.61 X$^{0.63}$
P < 0.00001
R^2 = 0.96
{0.54, 0.73}

Cd in soft tissue (mg kg^{-1})

Cd in food (mg kg^{-1})

treatment: MIX

Y = 9.1 X$^{0.28}$
P < 0.02
R^2 = 0.44 (!)
{0.07, 0.49}

Y = 5.01 X$^{0.83}$
P < 0.02
R^2 = 0.80
{0.26, 1.39}

Cd in soft tissue (mg kg^{-1})

Cd in food (mg kg^{-1})

Fig. 3. Relationships between Pb and Cd concentrations in soft tissue and concentrations of metals in food in treatments with single elements and in a mixture of Zn, Cu, Pb and Cd (MIX); solid line—regression with all data included with 95% confidence intervals for mean (thin broken lines); thick broken line—regression with one (Pb, Pb-MIX and Cd-treatments) or two (Cd-MIX) initial values excluded due to significant lack-of-fit of the primary model; 95% confidence intervals for the slope estimate are given in brackets; (!)—significant lack-of-fit at $p = 0.05$.

The concentrations of all metals in soft tissues, shells and faeces increased with exposure but the rate of increase differed markedly among metals. In all treatments, the soft-tissue concentrations of nutritional metals had steeper slopes than Pb and Cd. However, due to the broad 95% confidence intervals for slope estimates, the difference was statistically significant in one case only (Figs 2 and 3). Additionally, both xenobiotic metals revealed clearly two-stage relation of soft-tissue concentrations to concentrations in food. The lowest treatments did not affect body concentrations, indicating some regulation of Pb and Cd levels. Although all regressions were significant, this resulted in significant lack-of-fit for all Cd and Pb regressions. Exclusion of the lowest concentrations from the analysis resulted in steeper regressions but they still remained substantially below regressions for Cu and Zn (Fig. 3).

metal in soft tissue (mg kg^{-1})

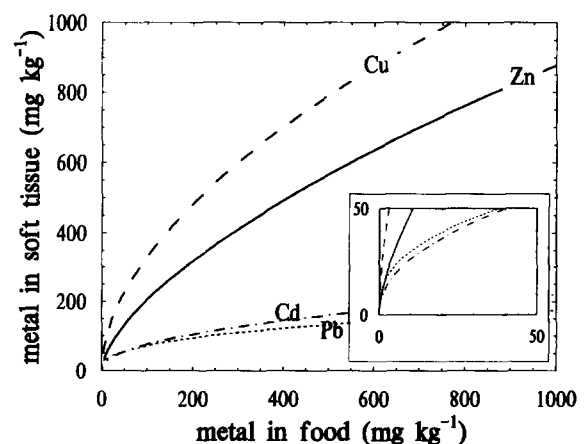metal in food (mg kg^{-1})

Fig. 4. Idealized plot of the dynamics of Zn, Cu, Pb and Cd in soft tissue of snails exposed to metal-contaminated food; the insert shows metal dynamics at range of concentrations environmentally relevant for Cd.

The idealized graph of calculated regressions (Fig. 4) shows that Cu is accumulated at a high rate throughout the whole range of concentrations, resulting in soft-tissue concentrations substantially higher than concentrations in food. Zinc, with a high accumulation rate at lower concentrations, is assimilated approximately in direct proportion to the exposure at higher contamination levels. Regressions calculated for Pb and Cd suggest that body-concentrations of these metals are efficiently regulated over a broad range of exposure levels. However, it has to be stressed that at environmentally relevant concentrations, Cd is accumulated at a relatively high rate which leads to soft-tissue concentrations exceeding those in food (see insert in Fig. 4). Thus, when considering levels of contamination typical of those found in the snails' environment for particular metals, the regressions indicate that snails are accumulators of Cu and Cd, but not of Zn and Pb.

Accumulation rates of metals in shells were low and for all metals in all treatments the calculated slopes were in the range of 0.11 for Pb in MIX to 0.58 for Cu in MIX-treatment. For Cd in MIX a negative slope was found (Tables 1–4). Slopes of regressions of metal concentrations in faeces on concentrations in food were in all cases higher than accumulation rates for soft tissues or shells (Tables 1–4).

On average, approximately 91% of Cu was assimilated from the food ingested (or 89% excluding doses not used in regression analysis). Next in terms of assimilation efficiency was Cd, with average assimilation 68%, and Zn with assimilation 60% (excluding the highest doses in both cases). The metal regulated most efficiently by snails was Pb; only 43% of Pb was assimilated from food during the experiment.

When mixed together, the four metals were obviously additive in their effects, which was reflected in faster decrease in consumption rates in MIX-treated snails (Fig. 1; *cf* also Laskowski & Hopkin, in press).

DISCUSSION

Some data suggest that animals are able to regulate their body levels of nutritional metals over a broad range of environmental concentrations, while xenobiotics are accumulated (e.g. Van Straalen *et al.*, 1987). However, this is certainly not a general characteristic of terrestrial animals. The most important exceptions to this 'rule' are probably invertebrates which contain haemocyanin in their blood. High Cu accumulation has been found both in terrestrial isopods (e.g. Hopkin, 1990) and gastropods (e.g. Moser & Wieser, 1979). One of the probable reasons for the efficient assimilation of Cu by snails and isopods irrespective of its concentration in diets may be the fact that in natural environments Cu always occurs in concentrations near to the minimum nutritional requirements of these animals (Hopkin, 1993*a,b*). Thus, they are probably preadapted to extract Cu from food very efficiently. However, in Cu-polluted environments, this may lead to Cu being particularly

toxic for haemocyanin-dependent invertebrates. Indeed, Berger and Dallinger (1989) found assimilation efficiencies for Cu exceeding 95% at concentrations of Cu in food up to 723 mg kg^{-1} dry wt. In our experiment, mean Cu-assimilation was in the range of 89–91%, leading to soft-tissue concentrations as high as 740 mg kg^{-1} dry wt (Table 2).

The assimilation efficiency of Cd was in the range 68–72%, and was similar to the average assimilation efficiency for food (74%). This was substantially higher than the range 7–59% found by Russell *et al.* (1981), but these authors used Cd concentrations from 25 to 1000 mg kg^{-1}, and the lowest assimilation (7%) was found at the highest Cd concentration. In our studies we have limited the experimental Cd concentrations to environmentally relevant levels, i.e. up to *ca* 125 mg kg^{-1}. When the assimilation efficiencies for Cd obtained in our studies were compared with the 59% showed by Russell *et al.* (1981) at 25 mg Cd kg^{-1}, the agreement between the two figures was better. Thus, we may conclude that at levels of Cd found in polluted field sites, assimilation is in the range 59–72%. Even though assimilation rate of Cd did not differ substantially from the assimilation rate of food, its concentrations in snail tissues after a four-month exposure were higher than those in food.

Dallinger *et al.* (1993) considered snails as 'macroconcentrators' of Zn and Cd, but in our experiments, Zn was not accumulated in snail tissues to concentrations exceeding its levels in food. Slopes for the relation between Zn concentrations in soft tissue and food indicate that snails assimilate this metal in direct proportion to the amount of food assimilated. Nevertheless, concentrations in the hepatopancreas were probably greater than in food, due to storage of metals in this organ (Hopkin, 1989). In our experiment only Cu and Cd were clearly concentrated in soft tissues in comparison to their concentrations in food.

The most efficiently regulated metal was Pb. Concentrations in faeces consistently exceeded those in the diet. Similar results were obtained by Beeby and Richmond (1987) who suggested that Pb assimilation and excretion is under control of a physiological mechanism that is able to adapt to high concentrations of this metal in food. Williamson and Evans (1973) indicated that the widespread tolerance of soil invertebrates to Pb contamination may be due to the fact that animals are exposed to relatively high concentrations under natural, uncontaminated conditions. According to these authors, Pb concentrations in some types of natural unpolluted soils may reach up to 200 mg kg^{-1}. Thus soil and epigeic invertebrates could be evolutionary, 'preadapted' to tolerate high Pb concentrations. Alternatively, the solubility of Pb in the gut of snails may be much lower than the other three metals, hence less will be assimilated by the hepatopancreas.

No Observed Effect Concentrations (NOEC) of Zn in food reported for mortality of mammals range from 730 to 20 000 mg kg^{-1} dry wt, and for growth 5000 mg kg^{-1} (Clayton & Clayton, 1981). For Cu, Clayton and

Clayton (1981) give 66 to 417 mg kg^{-1} as mortality NOECs and 50 mg kg^{-1} as a growth NOEC for mammals. The same authors report 1490 mg kg^{-1} as an NOEC on mortality for Pb in mammals (Clayton & Clayton, 1981), and Jacquet and Gerber (1979) give 3340 mg kg^{-1} as a growth NOEC. The NOECs of Cd reported for growth of birds are from 2 to 75 mg kg^{-1} (Supplee, 1961; Richardson et al., 1974), and for mammals from 3 to 50 mg kg^{-1} (Cousins et al., 1973; Nomiyama et al., 1987). Mortality NOEC for Cd in mammals given by Fitzhugh and Meiller (1941) is 45 mg kg^{-1}. These ranges of concentrations were reached for Zn, Cu and Cd in our experimental snails as well as in H. aspersa from the Avonmouth area, with Cu and Cd exceeding even the highest NOECs reported. This implies that animals feeding for only a few months on diets highly contaminated with these three metals may constitute a serious threat to birds and mammals feeding on snails.

Summarizing these results, it appears that snails are more important pathways for transfer along food chains of Cu and Cd than of Zn and Pb. Carnivores feeding on snails are not exposed to concentrations of Zn and Pb higher than those in the snails' diet.

The importance of the apparent role of snails in Cu and Cd transfer along food chains requires additional studies on the form in which the metals are stored in the snails. A number of studies have shown that Cu can be deposited in insoluble intracellular granules in the hepatopancreas of most terrestrial invertebrates, although 'copper granules' have not been found in H. aspersa (Hopkin, 1989). If such granules were insoluble in the guts of predators, snails could be regarded as 'detoxifiers' rather than potential sources of contamination for higher trophic levels. Such an effect has been already found in marine molluscs (Nott & Nicolaidou, 1990). In contrast, the main mechanism for Cd detoxification in snails is based on binding metal ions to metallothioneins and other low molecular-weight proteins (Cooke et al., 1979; Ireland, 1981; Berger et al., 1986). All these proteins are easily soluble, the metal being thus readily available for higher trophic levels. Indeed, Hunter et al. (1987b) have found that although both Cu and Cd were highly mobile in their transfer from soil and plants to invertebrates, only Cd retained this mobility in transfer to the next trophic level—small mammals.

Distribution of metals between soft tissue and shell indicates that contamination in soft tissues is a much more important threat to higher trophic levels. Although in most cases metal concentrations in shells increased with increased exposure, even at the highest treatments they remained well below the concentrations in the diet and soft tissues. Beeby and Richmond (1989) suggested that the shell may be used by snails in Pb detoxification as a short-term 'sink' for the metal. However, with concentrations as low as those found in our study, only a negligible amount of Pb was deposited in the shell over the period of exposure in the experiments. At dietary concentrations of Pb above 100 mg

kg^{-1}, the concentrations in shells did not exceed 5% of those found in soft tissue.

In the highest treatments, concentrations of metals in soft tissues were lower (Zn–12 500 and all metals at MIX-5) or similar (Cu–1250 and most metals at MIX-4) to those at smaller doses due to decreased food consumption. Significant decreases in food consumption as a result of metal pollution have been found also by Russell et al. (1981), Marigomez et al. (1986) and Simkiss and Watkins (1990). These results, together with those presented by Laskowski and Hopkin (in press) suggest that starvation may be the direct cause of population extinction in areas highly contaminated with metals because snails aestivate under unfavourable environmental conditions. Due to the long life-span of many species of snails (e.g. 5–6 years in H. aspersa), some questions addressed in our studies cannot be finally answered without longer term experiments. For instance, although we have found no significant accumulation of metals in shells, the situation might be different if the snails were fed on contaminated diets for a year or more. During experiments lasting only a few months, shell growth and the opportunity to transport metals to the shell is negligible. Animals exposed to metal contaminated food for their whole lives, may incorporate metals into their shells as they grow. This problem can be examined to some extent by measuring metal concentrations in soft tissue and shells in field populations that have been exposed to different levels of pollution. Even more difficult is to answer the question concerning the direct causes of extinction of populations of snails in metal polluted environments. Under laboratory conditions both increase in metal concentrations in tissues and decreased food consumption are observed. At the same time no effect on mortality was recorded as snails may aestivate for many weeks. This should not be considered, however, as proof that there is no effect of metal pollution on mortality in snails. Since snails refuse to eat highly contaminated food, if the experiments were carried out long enough, some mortality would certainly occur due to starvation. Alternatively, snails could die of direct poisoning if forced eventually to consume the contaminated food. Highest metal concentrations found in apparently viable populations of H. aspersa at Avonmouth 1 km from the zinc-and-lead smelter were (in mg kg^{-1} dry wt): Zn 898, Cu 530, Pb 327 and Cd 80 (Jones, 1991). These values are comparable to the highest values measured in experimental snails which showed no evidence of decreased food consumption. This suggests that the absence of H. aspersa from the immediate vicinity of the factory is due to a combination of metal toxicity and prolonged aestivation due to rejection of aerially-contaminated food by snails.

ACKNOWLEDGEMENTS

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