Effect of Zn, Cu, Pb, and Cd on Fitness in Snails (*Helix aspersa*)

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Juveniles and adults of the Brown garden snail (*Helix aspersa* Müll.) were fed on an artificial diet contaminated with Zn (ca. 40–12,000 $\mu g \cdot g^{-1}$ dry wt), Cu (ca. 9–1600 $\mu g \cdot g^{-1}$ dry wt), Pb (ca. 0.4–12,700 $\mu g \cdot g^{-1}$ dry wt), Cd (ca. 0.16–145 $\mu g \cdot g^{-1}$ dry wt), and all four metals mixed together for 120 days. Significant negative exponential regressions of food consumption and fecundity on concentrations were found for all treatments. Growth rate was affected significantly only by Zn and mixed treatments. The calculated $EC_{20(consumption)}$ values for juveniles were (in $\mu g \cdot g^{-1}$): Zn, 855; Cu, 248; Pb, 5290; Cd, 60; and for adults: Zn, 1240; Cu, 275; Pb, 3120; Cd, 147. In mixed treatment $EC_{20(consumption)}$ values were substantially lower indicating the additive effect of pollution with these four metals. $EC_{20(consumption)}$ for Zn in mixed treatment was 329 $\mu g \cdot g^{-1}$ for juveniles and 661 $\mu g \cdot g^{-1}$ for adults. The following EC_{20} values were estimated for fecundity (in $\mu g \cdot g^{-1}$): Zn, 1740; Cu, 533; Pb, 6140; Cd, 120; Zn in mixed treatment, 2210. The relative toxicities of the four metals were compared with their ratios in contaminated field sites. Comparing Zn and Cd, for example, even though Zn is ca. 13–24 times less toxic than Cd, it is usually present in plants and forest litter in concentrations ca. 100 times greater than those of Cd. Thus, of these two metals, Zn appeared to be potentially the most important pollutant in ecologically relevant situations. No effect of any treatment on mortality was found during the 4-month experiment. The calculated scenarios of population dynamics under the stress of chronic pollution with mixtures of the four metals revealed that the delayed reproduction due to estivation of snails may be the main cause of population decline at high metal concentrations in food. However, at concentrations at and below ca. 1000 μg Zn $\cdot g^{-1}$ in food, if the reproduction is not delayed the population may persist for a long time (0.25 control number after 50 years). © 1996 Academic Press, Inc.

INTRODUCTION

Snails and slugs are important component of herbivorous and detritivorous fauna in many ecosystems (Russell-Hunter, 1983). According to Jennings and Barkham (1979), more than 8% of the leaf litter input can be consumed yearly by slugs. With population densities of up to 1000 individuals m^{-2} (Mason, 1970), snails can also be important prey for mammals, birds, and large invertebrates (e.g., Symondson and Liddell, 1993; Graveland *et al.,* 1994). On the other hand, as found by Coughtrey and Martin (1976), Hopkin (1989, 1993), and Jones (1991), snails can accumulate substantial amounts of heavy metals in a polluted environment. Thus, even if the concept of a general biomagnification of heavy metals in terrestrial food webs has been criticized (cf. Fagerström, 1991; Laskowski, 1991), snails may represent a ''critical pathway'' for the food-chain transport of heavy metals (cf. Van Straalen and Ernst, 1991).

In a study of invertebrates in the vicinity of the Avonmouth zinc, lead, and cadmium smelting works (UK), *Helix aspersa* was absent in regions of extremely high metal pollution close to the factory (Jones, 1991). The most obvious explanation for this phenomenon is the direct poisoning of snails by metals ingested with food. In laboratory experiments, however, snails and slugs have proved to be fairly tolerant even to very high concentrations of heavy metals in their food. Marigomez *et al.* (1986) did not find any effect of Zn or Cu in diets on the mortality of *Arion ater* at doses of up to 1000 $\mu g \cdot g^{-1}$. Similarly, no response in mortality was found by Russel *et al.* (1981) in *H. aspersa* fed on a diet containing up to 1000 μg Cd $\cdot g^{-1}$. This high tolerance is possibly due to the efficient binding of metal ions by metallothioneins (Ireland, 1981; Taylor *et al.,* 1988; Dallinger *et al.,* 1989) and deposition in insoluble intracellular granules (Howard *et al.,* 1981). Beeby and Richmond (1989) suggested also that the shell could be a short-term 'sink' for Pb. Terrestrial molluscs may also possess physiological mechanisms that allow them to regulate metal assimilation from food (Berger and Dallinger, 1989). Prolonged exposure to heavy metal contamination may also lead to selection of metal-tolerant phenotypes (Beeby and Richmond, 1987; Greville and Morgan, 1991).

These results suggest that mechanisms other than mortality resulting from a direct poisoning may be responsible for the extinction of snails in highly polluted areas. One such possibility is the rejection of food with high concentrations of metals. In this case, the prolonged decrease in food con-

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sumption could cause increased mortality due to starvation rather than poisoning. Such an effect may be delayed in time and, as a result, no increase in mortality would be observed in short-term experiments. Indeed, decreased consumption rates have been observed in snails fed on contaminated diets (Russell *et al.,* 1981; Marigomez *et al.,* 1986). Simkiss and Watkins (1990) suggested that *H. aspersa* is able to detect high concentrations of Zn in the diet and reduce its food intake if contamination is too high. In contrast, Beeby (1985) did not notice any aversion of *H. aspersa* to a Pb-contaminated diet.

The other explanation for the absence of snails in polluted environments could be the effects of metals on their life history. These effects would probably be manifested as decreased growth rate, prolongation of the juvenile period, and decreased fecundity. These kinds of effects were demonstrated in *H. aspersa* fed Cd-contaminated food (Russell *et al.,* 1981). Under field conditions in which more than one pollutant is usually present, the life-history responses probably become more complicated due to antagonism or synergism between metals (Coughtrey and Martin, 1977).

The lack of a consensus regarding metal toxicity in snails encouraged undertaking studies to assess effects of metal pollution on the population dynamics of *H. aspersa.* Because population dynamics may be affected through changes in the rates of reproduction, somatic growth, and mortality, all these parameters were measured. The separate effects of two essential (Zn and Cu) and two nonessential metals (Pb and Cd) and mixtures of all four metals were studied. Results have been compared with the concentrations of Zn, Cu, Pb, and Cd in food of snails in a contaminated field site (Avonmouth) to assess which of the four metals is most likely to be responsible for the absence of *H. aspersa* in the vicinity of the smelting works.

MATERIALS AND METHODS

Diets and Metal Treatment

Juvenile and mature specimens of the Brown garden snail (*H. aspersa* Müll.) were obtained from the Sciento (Manchester, UK). In the experiments, an artificial diet was used for two reasons: (a) it assured an even distribution of metal salts in the medium, and (b) it was expected to decrease the within-group variance in consumption and growth rates (Whelan, 1982). The food containing ca. 5% dry mass was prepared according to the following formula: 1 g agar powder (Lancaster Synthesis, UK) + 3 g ground oven-dried carrots + 0.5 g dried skimmed milk (Premier Beverages, UK) + 0.5 g bran + 0.01 g $CaCO_3$ (BDH Ltd., Poole, UK) + 0.3 ml fungicide (*p*-hydroxy benzoic acid methyl ester = methyl paraben, Sigma) were mixed with a solution of the metal(s) being tested in double-distilled water to give 100

g of medium containing the required concentration of metals. The following were added as nitrate salts: $Zn(NO_3)_2 \times 6H_2O$, $Cu(NO_3)_2 \times 3H_2O$, $Pb(NO_3)_2$, and $Cd(NO_3)_2 \times 4H_2O$ (BDH Ltd., Analar grade).

Five concentrations of each metal were used. For Zn and Pb, the nominal concentrations were 20, 100, 500, 2500, and 12,500 $\mu g \cdot g^{-1}$ (on a dry mass basis); for Cu: 2, 10, 50, 250, and 1250 $\mu g \cdot g^{-1}$; and for Cd: 0.2, 1, 5, 25, and 125 $\mu g \cdot g^{-1}$. The same concentrations of metals were used in ascending order of the five combinations of Zn, Cu, Pb, and Cd in the intermetallic interactions experiments. In this way, 25 different treatments were used plus a control. In the remainder of this paper, the treatments are described as Zn-20, Zn-100, Zn-500, and so on for the other metals. For combinations of metals, the following abbreviations were used: MIX-1, 20 + 20 + 2 + 0.2 $\mu g \cdot g^{-1}$ Zn + Pb + Cu + Cd, respectively, and so on up to MIX-5.

Solutions containing predefined concentrations of metals 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 experiments, discs of agar were cut off with a stainless-steel cork borer and placed in the boxes containing snails.

Three samples of each type of food were analyzed by flame atomic absorption spectrometry (AAS) to determine actual concentrations of metals (Varian-Techtron, SpectrAA-30). Samples were digested for 24 hr in Pyrex glass tubes in 2 ml of Analar grade HNO_3 (BDH Ltd.) at room temperature and then boiled until clear. After cooling, the digests were diluted to 10 ml with double-distilled water. Samples were analyzed against standards in 20% HNO_3 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 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.

Two separate experiments were carried out to follow mortality, consumption, and growth in juvenile snails, and mortality, consumption, and reproduction in adults. All experiments were conducted at a constant temperature of 15°C under a constant light regime of 16-hr light, 8-hr darkness.

Juveniles

Juvenile, approx 8-month-old snails were kept individually in small transparent plastic boxes that were 8.5 cm in diameter and were perforated for ventilation. The average

TABLE 1
The Nominal Treatments and Actual (Measured) Concentrations of Zn, Cu, Pb, and Cd (Mean $\pm$ SD) in the Experimental Food

Treatment	$\mu g \cdot g^{-1}$			
	Zn	Cu	Pb	Cd
0 (Control)	39 ± 15	15 ± 2.6	0.4 ± 0.08	0.16 ± 0.03
Zn-20	80 ± 15			
Zn-100	128 ± 25			
Zn-500	483 ± 47			
Zn-2,500	2,650 ± 60			
Zn-12,500	12,200 ± 1,380			
Cu-2		15 ± 1.0		
Cu-10		26 ± 4.0		
Cu-50		76 ± 2.6		
Cu-250		337 ± 28		
Cu-1,250		1640 ± 149		
Pb-20			23 ± 5.1	
Pb-100			100 ± 3.8	
Pb-500			591 ± 99	
Pb-2,500			2,680 ± 293	
Pb-12,500			11,400 ± 800	
Cd-0.2				0.28 ± 0.08
Cd-1				1.11 ± 0.07
Cd-5				5.32 ± 0.37
Cd-25				25.90 ± 2.12
Cd-125				145.00 ± 20.5
MIX-1	43 ± 2	9 ± 0.2	16 ± 3.8	0.38 ± 0.14
MIX-2	136 ± 44	24 ± 0.4	99 ± 3.5	1.21 ± 0.10
MIX-3	446 ± 19	67 ± 5.4	474 ± 34	5.38 ± 0.28
MIX-4	2,070 ± 67	294 ± 8.6	2,275 ± 231	25.80 ± 2.03
MIX-5	10,900 ± 542	1320 ± 90	12,700 ± 854	132.00 ± 4.56

Note. Because no contamination with metals other than those used for the treatments was detected, only the values for metals used in particular treatments are given.

fresh weight of snails was 1.06 g (range 0.319–2.03 g). The boxes were covered with transparent lids. Breeding boxes were kept in partly closed large transparent plastic tanks. A sponge soaked with water was placed at the bottom to maintain the humidity at 100%. For all metal treatments, five replicates were used. Ten animals were fed the control diet. Altogether, 135 juvenile snails were used in the experiment.

The snails were offered food *ad libitum.* The culture was examined daily and food was supplemented as required. Once every 7 days the animals were weighed, and breeding boxes were cleaned of mucus. At the same time, feces and uneaten food were collected and oven dried at 70°C. Fresh agar discs were weighed and given to the snails. Each time a new portion of food was offered, at least 10 similar agar discs were oven dried to estimate the actual dry matter content. This enabled the amount of dry matter consumed by the snails to be calculated. The experiment was stopped after 90 days, and the animals were left for another 3 days without food to void their digestive tracts. Eventually, all snails were weighed and killed by deep freezing.

Adults

Adult snails of an average weight of 8.3 g (range 6.2–11.4 g) were held in plastic boxes (ca. 18 × 12 × 7 cm) with perforated transparent lids. Four snails were kept in each box. During the first 90 days of the experiment, each box contained a piece of wet sponge (ca. 10 × 8 cm) to maintain the humidity at 100%. The snails were fed *ad libitum* on the same diet as that for juveniles. For all treatments, three replicates were used. Altogether, 312 adult snails were used in these experiments.

The experiments were examined daily and food was supplied as required. Every 7 days, all boxes and sponges were cleaned in hot tap water. For the first 90 days of the experiment, the food offered, feces, and uneaten food were collected and oven dried at 70°C. Snails were weighed at the beginning and end of the experiment. Eggs, if found, were collected, counted, and transferred to petri dishes, and subsequent hatchlings were counted also.
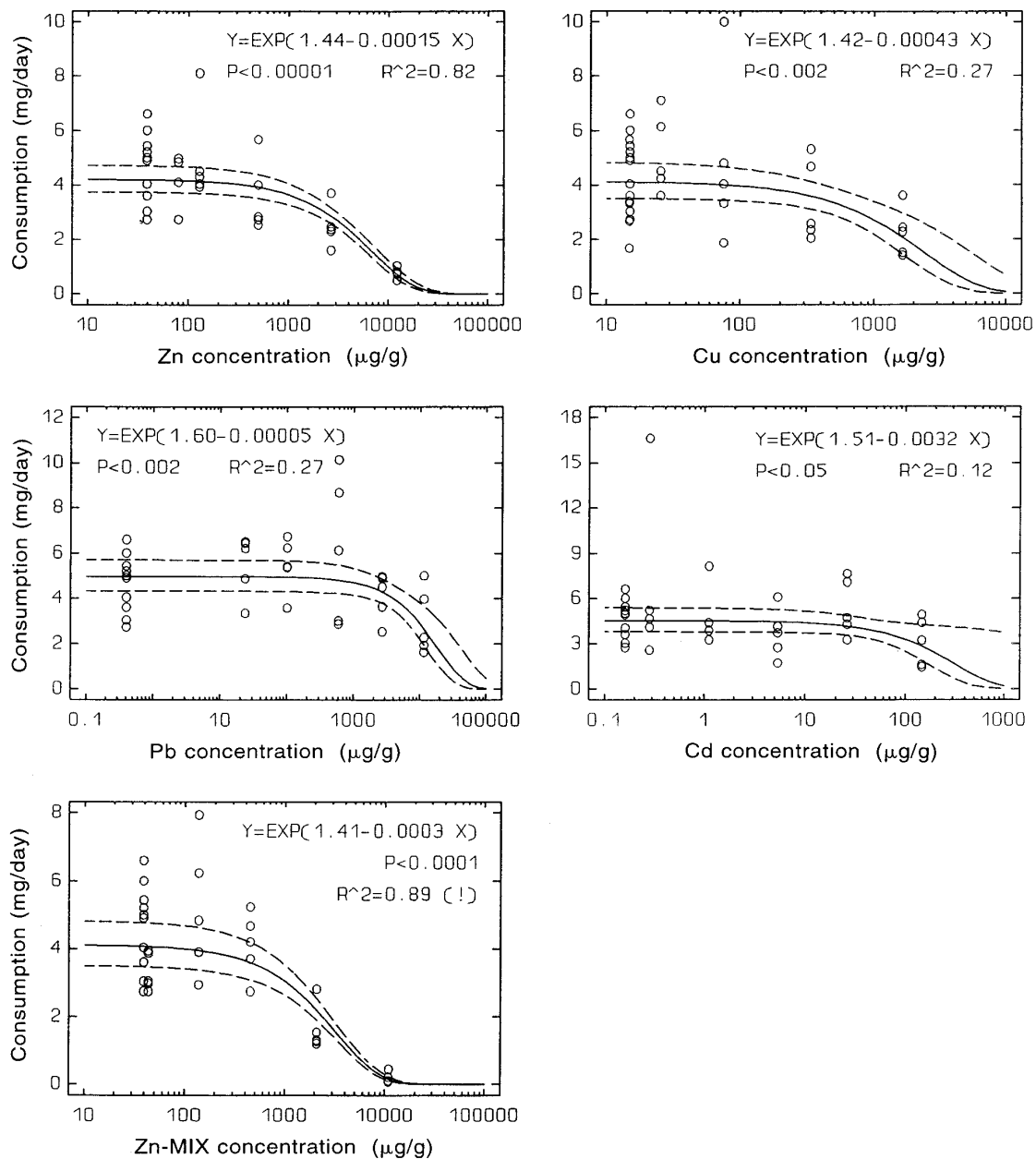
After 3 months, all boxes with adults were supplied with

FIG. 1. Regressions of consumption rates in juvenile snails on concentration of metals in food. Zn–MIX, concentration of zinc in MIX treatments; broken lines, 95% confidence intervals for mean; !, significant ($P < 0.05$) lack of fit.

a 3 cm-thick layer of soil to provide them with conditions suitable for laying eggs. Soil was collected at the Reading University campus, frozen at $-20°C$, and thawed. Snails were offered food as described earlier, but the food, feces and uneaten food were no longer weighed. After 14 days, the soil was searched for eggs. The procedure was repeated after another 14 days. Each egg clutch was placed in a separate petri dish on wet tissue paper and the eggs were counted. Petri dishes with eggs were placed in a light-tight box and

examined daily, and the number of hatchlings was counted. The experiment was stopped after 120 days.

Calculations and Statistical Analysis

Regression analysis was used to determine relationships between doses of particular metals and consumption, growth, or reproduction rates. The linear and exponential models were tested and those selected were ones that did not reveal
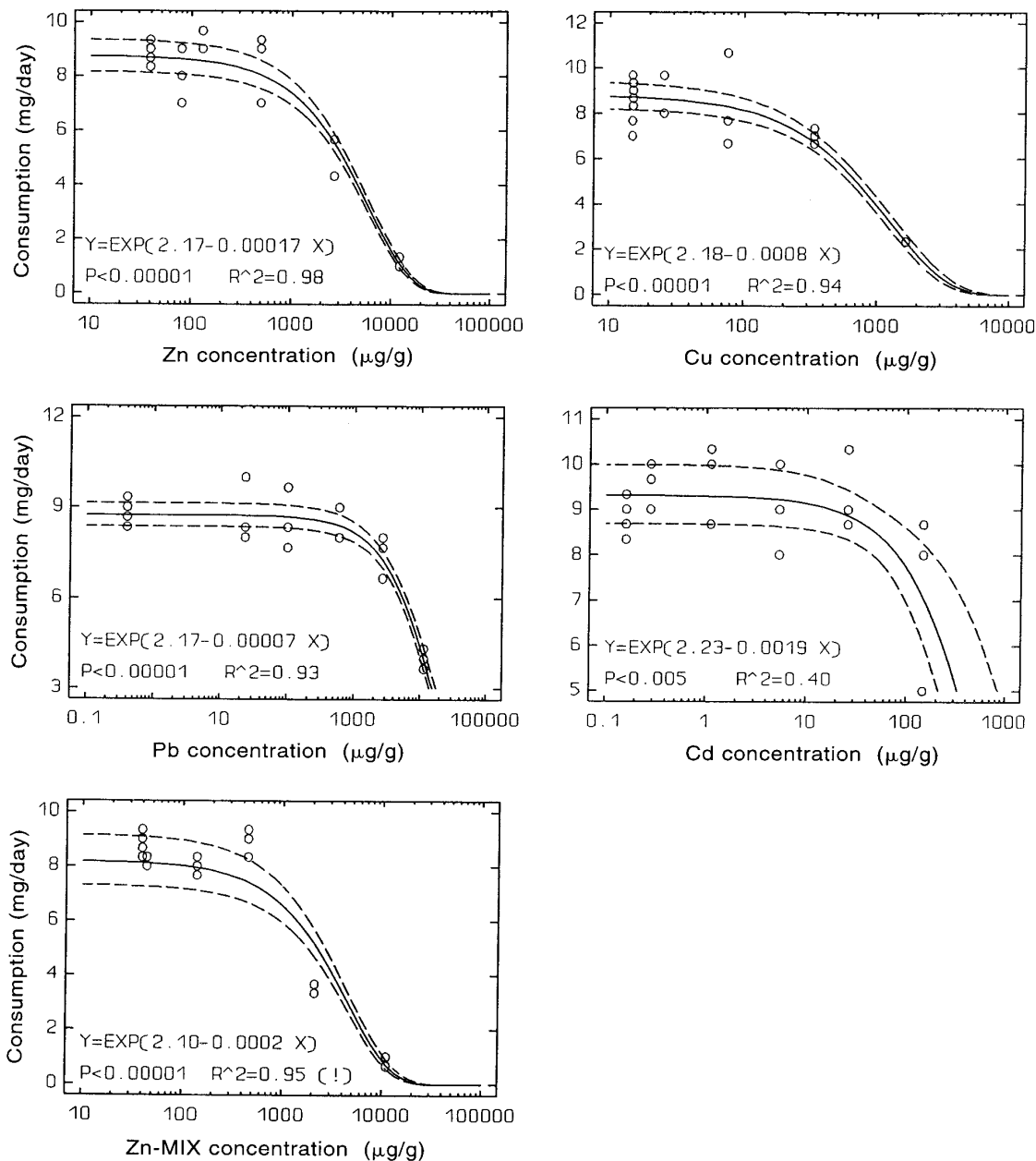
FIG. 2. Regressions of consumption rates in adult snails on concentration of metals in food. Zn–MIX, concentration of zinc in MIX treatments; broken lines, 95% confidence intervals for mean; !, significant ($P < 0.05$) lack of fit.

significant ($P < 0.05$) lack of fit (if possible) and had higher determination coefficients (R^2). The regression equations obtained were used to calculate concentrations that caused 20% (EC_{20}) and 50% (EC_{50}) reduction in consumption, growth, and reproduction rates. These estimates were used to compare the toxicities between metals. The intrinsic rates of increase in affected populations were also calculated. For the latter calculation, literature data were used to construct the initial projection matrix (Peake, 1978; Daguzan, 1981;

Godan, 1983; Tompa, 1984). According to the literature, both fertility and survival rates are highly variable among different populations of snails (Cain, 1983; Russell-Hunter and Buckley, 1983). Thus, even using literature data, somewhat arbitrary values were assigned to the parameters in the matrix. These inaccuracies, however, do not affect the conclusions because the procedure used (see below) is based on ratios between control and experimental populations rather than on the raw data.

TABLE 2

Estimated EC_{20} and EC_{50} Values for Consumption Rates in Juveniles and Adults and Fecundity of Adults

Estimate	$\mu g \cdot g^{-1}$				
	Zn	Cu	Pb	Cd	Zn–MIX
$EC_{20 \text{ (consumption)}}$					
Juveniles	855	248	5,290	60	329
Adults	1240	275	3,120	147	661
$EC_{50 \text{ (consumption)}}$					
Juveniles	4080	1350	13,900	206	1880
Adults	3980	859	10,000	396	2760
$EC_{20 \text{ (fecundity)}}$	1740	533	6,140	120	2210
$EC_{50 \text{ (fecundity)}}$	5970	1050	8,300	183	5240

Note. Zn–MIX: values estimated for Zn taking intermetallic effect into account.

One-year-wide age classes were taken for constructing the projection matrix. Fertilities were assumed to be constant throughout the mature life of the snails and were set at 75 per year. Survival rates for age classes above class 0 are also reported to be fairly constant in *H. aspersa* and were initially set at 0.05 for class 0 and 0.2, 0.25, 0.25, and 0.2 for older classes. The survival rates in the matrix were then adjusted by iteration to represent a stable population ($\lambda = 1$):

$$
\begin{matrix}
0 & 0 & 75 & 75 & 75 & 75 \\
0.0505 & 0 & 0 & 0 & 0 & 0 \\
0 & 0.2 & 0 & 0 & 0 & 0 \\
0 & 0 & 0.25 & 0 & 0 & 0 \\
0 & 0 & 0 & 0.25 & 0 & 0 \\
0 & 0 & 0 & 0 & 0.15 & 0
\end{matrix}
$$

The numbers in the first row of the projection matrix are the estimated age-specific fertilities for unaffected population of *H. aspersa* (F_i), and the numbers in the subdiagonal indicate the age-specific probabilities of survival from one age class to the next (P_i).

The effect of treatment (δ) can be estimated as

$$
\delta = \frac{F_i^E}{F_i^C},
$$

where F_i is the age-specific fertility at age i (matrix entry at row 1, column i), and the superscripts denote mean fertility values for experimentally treated (E) and control (C) animals. Thus, new entries for matrices for treated populations ($F^{(T)}$) were calculated according to the formula

$$
F_i^{(T)} = F_i \delta.
$$

Such parametrized matrices were used to prepare scenarios for populations under environmentally relevant pollution stresses related to concentrations of metals known to occur in the food of snails in contaminated field sites.

RESULTS

Actual (measured) concentrations of Zn, Cu, Pb, and Cd in experimental food are given in Table 1. The mean relative ratios of mean concentrations of metals in food were 94 Zn:15 Cu:78 Pb:1 Cd.

There was no relationship between mortality of juveniles or adults and concentration of any metal or combinations of metals in food of snails. Only 6.7% of juveniles and 1.9% of adults died during the experiment.

The effect of treatment on consumption rate was negligible over a broad range of lower concentrations; however, at higher concentrations a rapid drop in consumption was observed (Figs. 1 and 2). This resulted in significant regressions of consumption rates on metal concentration in food for both juveniles and adults. In all cases, the exponential model gave better fit. The effect was especially well pronounced in adults; for Zn, Cu, Pb, and MIX treatments the regressions explained over 93% of total variability in the consumption rates (Fig. 2). The average consumption rate in control juveniles was 4.65 mg · day^{-1} per 1 gram of snail (95% confidence interval for mean = 3.75–5.56). Consumption rates at both the EC_{20} and the EC_{50} levels differed significantly from this mean (one-sample *t* test). $EC_{20\text{(consumption)}}$ and $EC_{50\text{(consumption)}}$ respectively were as follows: Zn, 855 and 4080 $\mu g \cdot g^{-1}$ dry mass food; Cu, 248 and 1350 $\mu g \cdot g^{-1}$; Pb, 5290 and 13900 $\mu g \cdot g^{-1}$; Cd, 60 and 206 $\mu g \cdot g^{-1}$. The toxicity of metals in the MIX
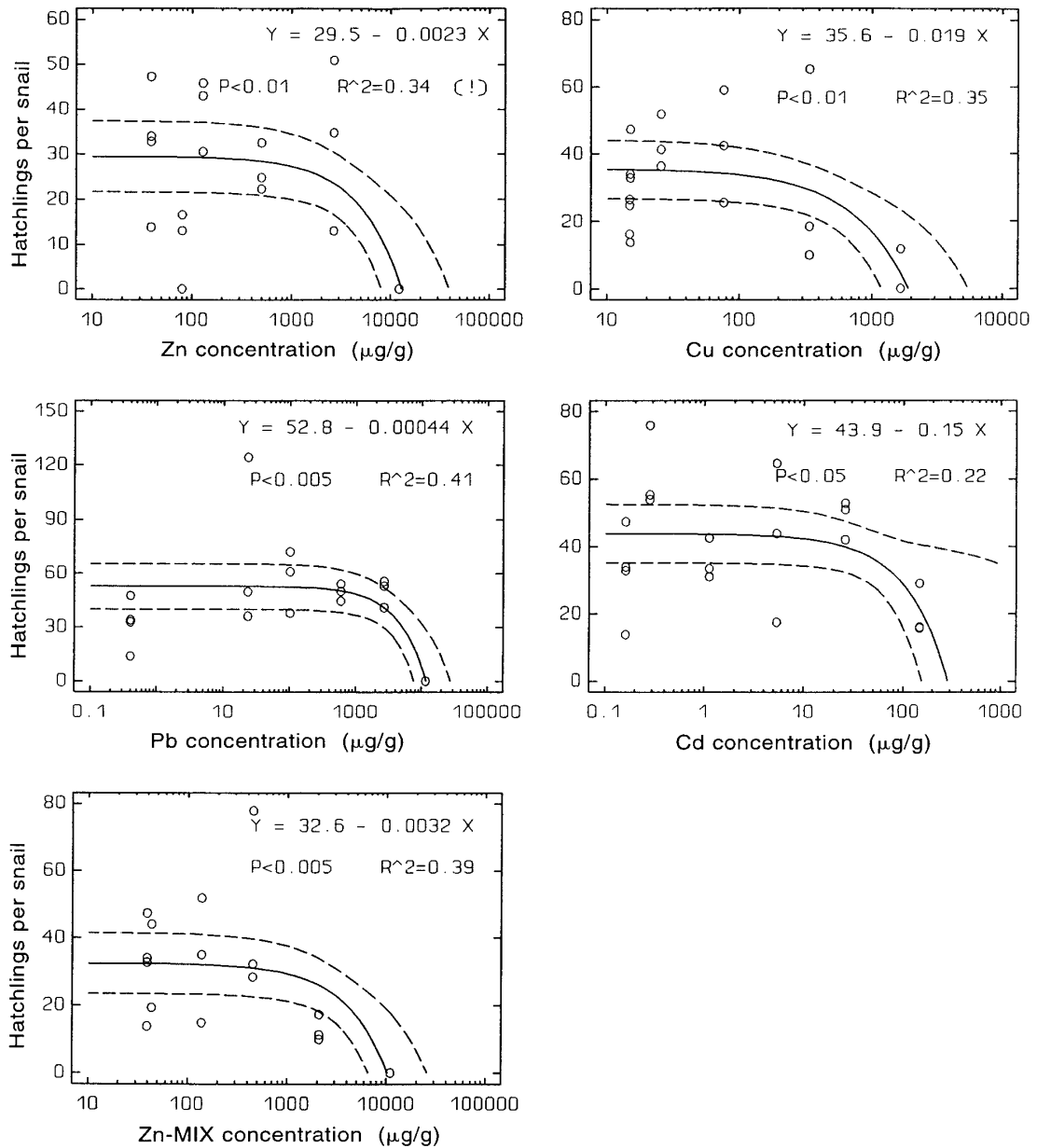
FIG. 3. Regressions of fecundity (number of hatchlings per snail) on concentration of metals in food; Zn−MIX, concentration of zinc in MIX treatments; broken lines, 95% confidence intervals for mean; !, Significant ($P < 0.05$) lack of fit.

treatments was clearly dominated by the effect of Zn, but the additive effect of metals was also noticeable. The EC_{20}'s and EC_{50}'s for Zn in the MIX treatment were 2.1–2.6 times lower than those in Zn-only treatments (329 and 1880 $\mu g \cdot g^{-1}$ food, respectively; Table 2).

The average consumption rate in control adults was 8.83 $mg \cdot day^{-1}$ per 1 gram of snail (95% confidence interval for mean = 8.15–9.52). Consumption rates at EC_{20} (7.07 $mg \cdot day^{-1}$) and EC_{50} (4.42 $mg \cdot day^{-1}$) were significantly different at $P = 0.05$ from the average for controls (one-

sample t test). $EC_{20(consumption)}$ and $EC_{50(consumption)}$ respectively were as follows: Zn, 1240 and 3980 $\mu g \cdot g^{-1}$; Cu, 275 and 859 $\mu g \cdot g^{-1}$; Pb, 3120 and 10,000 $\mu g \cdot g^{-1}$; Cd, 147 and 396 $\mu g \cdot g^{-1}$. As in the juveniles, the effect in the MIX treatment was dominated by Zn toxicity. A 20% reduction in consumption rate was found at 661 $\mu g \cdot g^{-1}$ Zn, and the EC_{50} was 2760 $\mu g \cdot g^{-1}$ (Table 2).

Despite these sharp decreases in consumption rates in all food contamination experiments, significant effects on growth rate were found only in juveniles fed with food

TABLE 3

Estimated Effects on Consumption, Growth, and Fecundity Rates and Intrinsic Rate of Increase (λ) in Populations of *H. aspersa* at Three Different Concentrations of Zn When Mixed with Cu, Pb, and Cd

Trait	Zn concentration in $\mu g \cdot g^{-1a}$		
	1000	2000	3000
Juveniles			
Consumption	−35	−52	−64
Growth	−10	−12	−13
Adults			
Consumption	−26	−41	−52
Fecundity	−8	−18	−28
λ_F	−2.5 (0.975)	−5.8 (0.942)	−9.4 (0.906)
$\lambda_{F,R}$	−29.4 (0.706)	−31.2 (0.688)	−33.2 (0.668)

Note. λ_F, Only fecundity decrease taken into account; $\lambda_{F,R}$, fecundity and 1-year delay in reproduction taken into account (cf. text).

[a] Effect as percentage change relative to control (λ value).

treated with Zn ($P < 0.04$) or mixtures of all four metals ($P < 0.01$). Adults did not reveal any significant changes in their mass that could be related to pollution (in all cases $P > 0.4$).

In adults, the decrease in consumption rate was reflected in a sharp drop in fecundity at high concentrations of all metals (Fig. 3). In fact, in all replicates of Zn-12,500, in two Pb-12,500, and in one MIX-5 the animals did not produce darts that may indicate decreased or ceased mating activity. In the highest concentrations of other treatments, even if animals did mate and the darts were found, in most cases they did not produce any eggs. Among the highest treatments, eggs were found only in one replicate of Cu-1250 and in all Cd-125 experiments. It is worth noticing, however, that contamination of food with metals had little effect on fecundity over a broad range of concentrations (Fig. 3). The estimated $EC_{20(fecundity)}$ and $EC_{50(fecundity)}$ were respectively as follows: Zn, 1740 and 5970 $\mu g \cdot g^{-1}$; Cu, 533 and 1050 $\mu g \cdot g^{-1}$; Pb, 6140 and 8310 $\mu g \cdot g^{-1}$; Cd, 120 and 183 $\mu g \cdot g^{-1}$. In contrast to the consumption rate, there was no clear additive effect on fecundity in the MIX treatment. Although the calculated EC_{50} for Zn in MIX was lower than that in Zn-alone treatment (5240 $\mu g \cdot g^{-1}$), the EC_{20} was higher (2210 $\mu g \cdot g^{-1}$) (Table 2).

Using the regressions described previously, the effects on consumption, growth, and fecundity rates were calculated for a mixture (MIX) of heavy metals in food at three concentrations of Zn: 1000, 2000, and 3000 $\mu g \cdot g^{-1}$ (Table 3). The results indicate a rapid drop in fecundity over this range of concentrations and a relatively slower drop in the growth

rate. Nevertheless, the drop in the growth rate was statistically significant ($P < 0.01$) and the estimated values were 10–13% lower than the average for control animals. Thus, a possibility exists that the animals can lose a year in reproduction due to the delay in maturation. Because it cannot be clearly stated whether this decrease in the growth rate is sufficient to delay the maturity, two scenarios are provided for each concentration: one taking into account only the estimated effect on fecundity and the other including a 1-year delay in maturity (Fig. 4).

DISCUSSION

The results of a breeding experiment by Daguzan (1981) with 1000 individuals of *H. aspersa* revealed higher numbers of fertile eggs per clutch (mean = 111.8; SD = 32.4) than in control animals in this experiment (mean = 70.8; SD = 16.8). The reason for this discrepancy may be connected with the relatively poor nutritional quality of the artificial food used in the experiment in comparison to a ''natural'' diet (Whelan, 1982). In addition, the temperature during experiments was lower (15°C) than that in Daguzan's study (20°C). The mean hatchability was, however, higher in the culture: 90.8% (SD = 7.2) compared to the 81.3% (SD = 8.4) obtained by Daguzan (1981). The adult mortality during the 120-day experiment (including treated animals) was much lower (1.9%) than that reported by Daguzan during 14 weeks (39.6%). These data indicate that the culture conditions were suitable for *H. aspersa*.

Increased mortality of snails fed a diet containing elevated concentrations of metals has not been reported previously (Russell *et al.,* 1981; Marigomez *et al.,* 1986; Berger *et al.,* 1993). However, this high ''tolerance'' to metals is probably not principally due to physiological mechanisms enabling snails to exclude metals from passing into body tissues. Indeed, terrestrial gastropods are considered to be ''macroconcentrators'' of metals (Coughtrey and Martin, 1977; Russell *et al.,* 1981; Greville and Morgan, 1991; Jones, 1991; Dallinger *et al.,* 1993). There are two other possible explanations for the low mortality in snails exposed to a metal-polluted food: (a) efficient binding of metals to metallothioneins and other low-molecular-weight proteins (Cooke *et al.,* 1979; Berger *et al.,* 1986; Dallinger *et al.,* 1993); and (b) resistance of snails to unfavorable environmental conditions because of their ability to estivate (Godan 1983).

During estivation, the metabolic activity of snails declines and they stop feeding. In natural, uncontaminated conditions, estivation allows snails to survive during unfavorable periods. This may lead to differences in time required to reach maturity by as much as 4 years (Cain, 1983). However, when exposed to a permanently contaminated food, snails are endangered by a prolonged decrease in consumption rate that
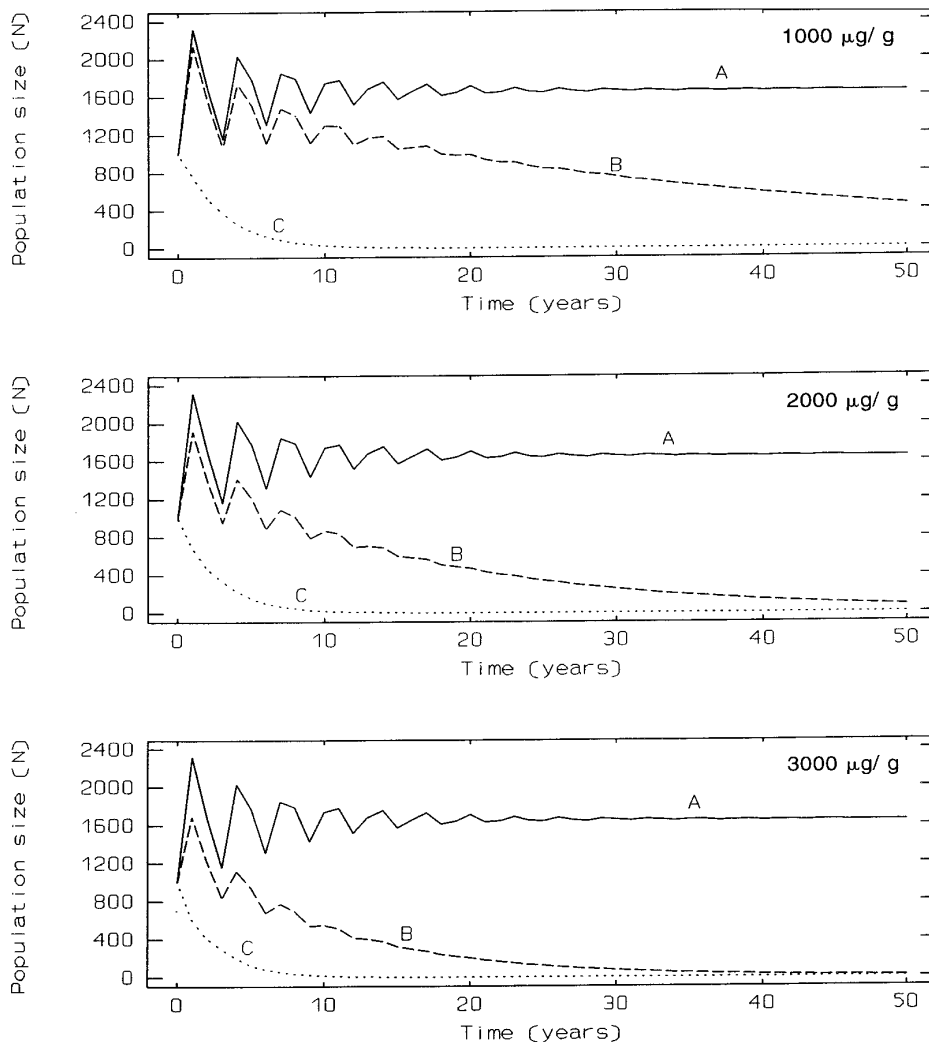
FIG. 4. Scenarios for the dynamics of hypothetical populations of *H. aspersa* at three pollution levels—1000, 2000, and 3000 μg Zn$\cdot$g^{-1} dry wt food—when additive intermetallic effect is taken into account; A, undisturbed population; B, only decrease in fecundity taken into account; C, decrease in fecundity and 1-year delay (lost) in reproduction.

eventually may lead to death by starvation rather than contamination. This effect is, however, difficult to assess in a typical, i.e., short, ecotoxicological experiment because snails are able to estivate for many weeks. To measure actual effects of metal pollution on mortality, the experiment should last for at least 2 years.

Significant decreases in food consumption of snails in response to metal contamination have been found by many authors. Russell *et al.* (1981) exhibited a linear negative regression of food consumption compared to dose when subadult *H. aspersa* were fed diets contaminated with CdCl$_2$. According to these authors, snails fed on a diet containing more than 25 μg Cd$\cdot$g^{-1} dry weight had reduced shell growth and reproductive activity and increased estivation in comparison to controls. Simkiss and Watkins (1990) found

substantial (approx 68%) decreases in consumption of food by *H. aspersa* supplemented with only 98 μg Zn$\cdot$g^{-1} fresh weight. In the experiment on *Arion ater* by Marigomez *et al.* (1986), food consumption decreased at doses of 1000 μg$\cdot$g^{-1} Zn, 100 μg$\cdot$g^{-1} Cu, and 300 μg$\cdot$g^{-1} Hg. They did not find any effect on consumption of the highest concentration of Pb tested (1000 μg$\cdot$g^{-1}). In the present studies, the estimated EC$_{20(consumption)}$ values for juveniles were 855 μg$\cdot$g^{-1} Zn, 248 μg$\cdot$g^{-1} Cu, 5290 μg$\cdot$g^{-1} Pb, and 60 μg$\cdot$g^{-1} Cd. For adults, the respective values were as follows: Zn, 1250 μg$\cdot$g^{-1}; Cu, 275 μg$\cdot$g^{-1}; Pb, 3120 μg$\cdot$g^{-1}; Cd, 147 μg$\cdot$g^{-1}. These results indicate that snails are very insensitive to Pb in their diet. In his studies in the neighborhood of the zinc and lead smelter in Avonmouth, England, Jones (1991) found the highest concentrations of these four heavy metals

in nettle leaves (*Urtica dioica*) to be approx 3000 $\mu g\ Zn \cdot g^{-1}$, 250 $\mu g\ Cu \cdot g^{-1}$, 2000 $\mu g\ Pb \cdot g^{-1}$, and 30 $\mu g\ Cd \cdot g^{-1}$. Thus, the $EC_{20(consumption)}$ estimated in the present experiment was only exceeded for Zn. In terms of importance as an industrial pollutant, Cu can also be of major importance, especially when the intermetallic effects are taken into account (Hopkin and Hames, 1994) (Table 3).

In polluted environments (Jones, 1991), the relative ratios of metals in nettle leaves are approx 100 Zn:8 Cu:67 Pb:1 Cd. When calculated according to the magnitude of the effect on consumption, taking the average from the estimated $EC_{20(consumption)}$ and $EC_{50(consumption)}$ values for juveniles and adults, the following ratios were obtained: 13 Zn:4 Cu:51 Pb:1 Cd. This indicates the primary significance of Zn as an industrial pollutant to primary consumers in the Avonmouth area. Although Zn is approximately 13 times less toxic than Cd to *H. aspersa* in the laboratory, it occurs in food in the field at concentrations of as much as 100 times greater than that of Cd. The fecundity of snails seems to be generally less sensitive to metals in the diet than the consumption rate (Table 2), but the mean ratios of toxicity are similar: 24 Zn:5 Cu:48 Pb:1 Cd. Similar conclusions regarding the primary importance of Zn in restricting the distribution of terrestrial isopods and earthworms were reached by Hopkin and Hames (1994), Spurgeon and Hopkin (1996), and Spurgeon *et al.* (1994).

The values of $EC_{20(consumption)}$ and $EC_{50(consumption)}$ estimated for Zn in the MIX treatments (i.e., taking into account the intermetallic effects) were substantially lower than those estimated using data from Zn-only treatments. This indicates the importance of intermetallic effects in ecotoxicological studies that, unfortunately, often consider only one-element effects (e.g., Russell *et al.,* 1981; Dallinger and Wieser, 1984; Marigomez *et al.,* 1986; Berger and Dallinger, 1989).

CONCLUSIONS

The scenarios presented in Fig. 4 indicate the crucial importance of losing 1 year of reproduction in *H. aspersa.* However, even if the animals did not delay their reproduction, at concentrations of approx 3000 $\mu g \cdot g^{-1}$ Zn and above (when in mixture with Cu, Pb, and Cd) the rapid decline in population numbers leads to almost complete extinction after ca. 30 years of pollution. In contrast, at lower concentrations (below ca. 1000 $\mu g \cdot g^{-1}$ Zn in mixtures of metals), the decrease in population numbers is slow and it may be possible for snails to develop some genetic resistance to pollutants (Beeby and Richmond, 1987, 1989; Greville and Morgan, 1991). According to the present model, if there is no delay in reproduction at this concentration even after 50 years of exposure, the population number would still be approximately one-fourth of control. It should be stressed, however,

that the models may appear rather ''optimistic'' because any estimates of pollution-caused mortality could not be included. Although this was neither observed during the experiment nor has it been reported by other authors, it seems possible that it would be found if the experiments lasted longer because the consumption decrease was substantially larger than the decrease in growth rate and fecundity.

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