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Intake and Assimilation of Zinc, Copper, and Cadmium in the Terrestrial Isopod *Porcellio scaber* Latr. (Crustacea, Isopoda)

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Terrestrial isopods are organisms of choice in metal and pesticide toxicity studies. Laboratory experiments have shown that elevated concentrations of metals in their diet increase mortality, decrease growth, reproduction, feeding rate and moult frequency, and cause changes at tissue and cellular levels in different isopod species (reviewed in Drobne 1997). Among a variety of endpoints used in tests with terrestrial isopods, feeding rate has been the most frequently applied (Drobne and Hopkin 1995). The toxicity of a single metal depends on the duration of exposure, the amount consumed and on mechanisms which prevent toxic effects (reviewed in Hopkin 1989). In isopods, physiological mechanisms that mitigate the toxicity of metals result in assimilation and/or excretion of metals (reviewed in Hopkin 1989). At the behavioural level, isopods regulate metal intake by rejection of metal-contaminated food and by selection of a less contaminated diet (Dallinger 1977). Published data show that concentrations of Cu and Cd in isopods collected in metal-contaminated environments may exceed the concentrations of metals in their food several times, while Zn concentrations in isopods are often lower than in their food (Hopkin and Martin 1982). This suggests that isopods can regulate the intake and assimilation of Zn more efficiently than that of Cu and Cd.

The aim of our work was to study changes in the food consumption rate as a mechanism of metal intake regulation. A change in feeding rate can also be a physiological level response to elevated concentrations of chemicals in the food. In the present paper, we studied Zn, Cu and Cd assimilation efficiency by comparing the intake and accumulation of metals in a pre-adult terrestrial isopod, *Porcellio scaber*. The effects of Zn, Cu, and Cd contaminated food were quantified by measuring feeding rates and described by sigmoidal dose-response curves, EC_{10} and EC_{50}. We reassessed the feeding rate as an effect criterion in toxicity tests with isopods.

MATERIALS AND METHODS

Specimens of *Porcellio scaber*, third generation laboratory raised, were used in the experiment. The original population was collected in an unpolluted

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environment in the vicinity of Ljubljana, Slovenia. Four-month-old isopods, 20-25 mg fresh weight, were placed individually in plastic Petri dishes (diameter 9 cm) together with 100 mg of dry hazel leaf (*Corylus avellana*) (N=15, at each concentration). The leaves were collected from an uncontaminated area in autumn. A filter paper moistened with water was added to each Petri dish to assure constant humidity. Solutions of $ZnCl_2$ (>98 pure Merck, Dermstad, Germany), $CuCl_2*2H_2O$ (≥99 pure Merck, Dermstad, Germany) and $CdCl_2*H_2O$ (>98 pure Merck, Dermstad, Germany) were applied to the leaves by spraying. The amount of solution applied to each leaf was adjusted to give nominal concentrations of 450, 900, 1800, 3600 and 5400 μg Zn g^{-1} dry weight, 300, 600, 1200, 2400 and 3600 μg Cu g^{-1} dry weight and 125, 250, 500 1000 and 1500 μg Cd g^{-1} dry weight. Actual concentrations were within 5% of those desired. The concentrations of Zn, Cu and Cd in the control leaves from the collection site were 40 μg Zn g^{-1} dry weight, 20 μg Cu g^{-1} dry weight and 0.15 μg Cd g^{-1} dry weight. Control animals were fed with untreated food (N=15). Food consumption was measured as the difference in the weight of leaves at the beginning and at the end of the experiment.

After 14 days of exposure to metal treated food, animals were food deprived for 24 hours to empty their guts. Then they were lyophilised, weighed and completely digested in a nitric/ perchloric acid mixture (7:1). After evaporation of the acid the residue was taken up in 0.1% HNO_3. Total Zn, Cu and Cd concentrations in whole animals were determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 100). The leaves were analysed for metals in the same way.

To assure the accuracy of the analytical procedure, samples of plant (hazel leaves) and animal (isopods) material were fortified with Cd, Cu and Zn at three different concentration levels (N=6, at each concentration) and analysed after digestion as described above. The percentage recoveries of these spiking experiments were as follows: for Cd 101.3 ± 0.4 % (plant) and 99.1 ± 0.2 % (animal), for Cu 100.0 ± 0.25 % (plant) and 96.0 ± 1.5 % (animal), and for Zn 103.4 ± 0.5 % (plant) and 98.5 ± 1.6 % (animal).

All the acquired data were checked for normality of distribution (Kolmogorov-Smirnov test. Data on food consumption and metal concentration were compared applying one-way analyses of variance (ANOVA) with Tukey's test. For the determination of the median effective concentration (EC_{50}) and 10% effective concentration (EC_{10}) for Zn, Cu and Cd, a log-logistic model (Haanstra et al. 1985) was used. All computations were executed using the SPSS for Windows and S-PLUS 4.5 programs. Zn, Cu and Cd assimilation efficiencies (AE) were calculated as the increase of a single metal concentration in isopods per amount of metal consumed (Dallinger and Wieser 1977) by the formula $AE = ((C_a - BG)/(C_f*FC))*100$, where: C_a = concentration of metal in animals after the experiment, BG = mean background metal concentration in control animals, C_f = concentration of metal in food, FC = amount of food consumed.

RESULTS AND DISCUSSION

The isopods exposed to 450 and 900 μg Zn g^{-1} dry food weight consumed the same amount of food as the animals from the control group (Fig. 1A). The isopods exposed to 1800 or more μg Zn g^{-1} dry food weight consumed significantly (ANOVA, Tukey test $p<0.05$) less food than the control isopods and those exposed to 450 and 900 μg Zn g^{-1} dry weight.

The results are comparable with toxicity data obtained in similarly designed experiments. The reduction in feeding rate was noticeable in adult isopods exposed for two weeks to hazel leaves at a concentration of 1500 μg Zn g^{-1} dry weight (Bibič et al. 1997) or for five weeks at 2000 μg Zn g^{-1} dry weight (Drobne and Hopkin 1995). In contrast, Donker et al. (1996) found a reduction in food consumption only in adult isopods exposed to poplar leaves at 3500 μg Zn g^{-1} dry weight for six weeks.

Concentrations of Zn accumulated in isopods were not proportional to Zn concentrations in the food (Fig. 2A). In the isopods fed with 900 μg Zn g^{-1} dry food weight no reduction of the feeding rate was observed and they accumulated as much Zn as the animals fed with 450 μg Zn g^{-1}. This could be explained by lower assimilation and/or higher loss of Zn from the body due to the higher Zn content in the food. A similar phenomenon was observed by Bibič et al. (1997).

The assimilation efficiency (AE) of Zn in animals fed differently Zn-dosed food appears to be non-linear (Table 1). It could be independent of feeding rate.

The isopods exposed to 300 and 600 μg Cu g^{-1} dry food weight consumed the same amount of food as the control animals (Fig. 1B). The isopods exposed to 1200 μg Cu g^{-1} dry weight consumed significantly (ANOVA, $p<0.05$) less food than the control animals. Published data show that the effect of Cu on feeding rate is a consequence of the exposure time and the concentration of Cu in the food. For example, isopods that were exposed to Cu-dosed maple leaves for four weeks showed reduced feeding at 500 μg Cu g^{-1} dry food weight (Farkas et al. 1996), while over six weeks of exposure to Cu-dosed birch leaves the feeding rate decreased at 282 μg Cu g^{-1} dry weight (Hassall and Rushton 1982).

The concentration of Cu in the isopods exposed to 600 μg Cu g^{-1} dry food weight and above did not increase with increasing concentrations of Cu in their food (Fig. 2B). The internal Cu concentration was maintained at around 400 μg Cu g^{-1} dry body weight, while the feeding rate was reduced (Fig. 1B).

AE of Cu in *P. scaber* decreased with increasing Cu concentration in the food. Contrary to the findings of Dallinger and Wieser (1977), who reported a dose-related increase of AE of Cu up to 87%, the AE of Cu in our experiment did not exceed 20% (Table 1).

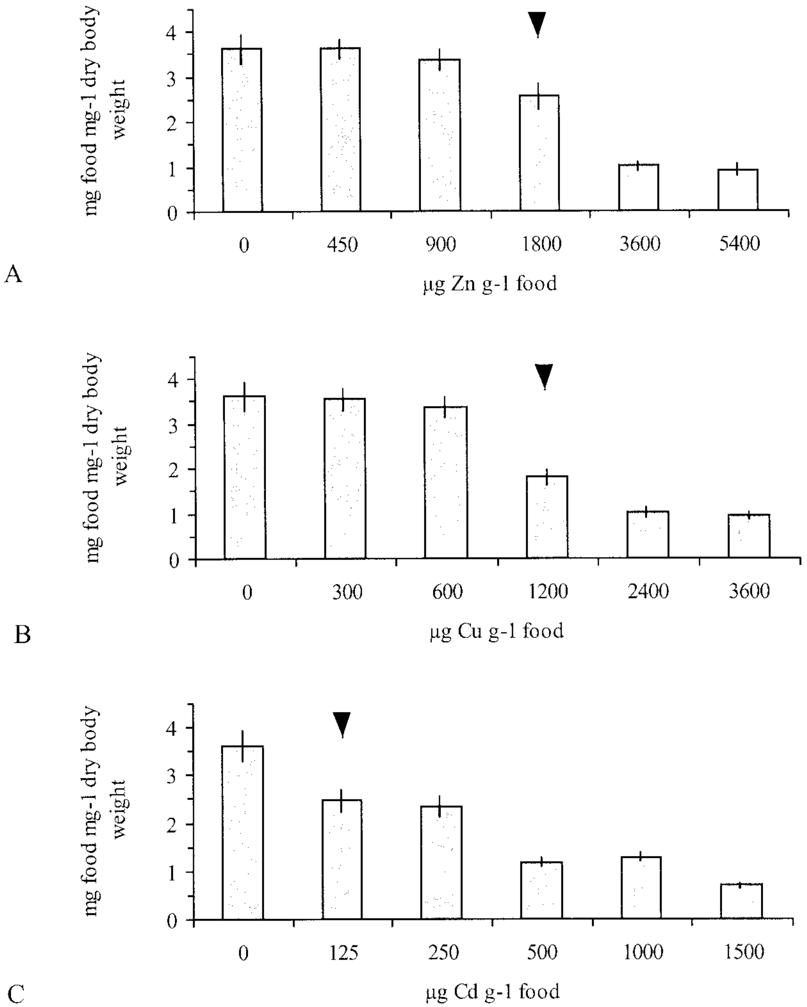

Figure 1. Mean food consumed in 14 days (±SE) in control isopods and isopods exposed to Zn (A), Cu (B) and Cd (C) dosed food (N=15, at each concentration; arrowhead - statistically significant lower food consumption: ANOVA, $p<0.05$).

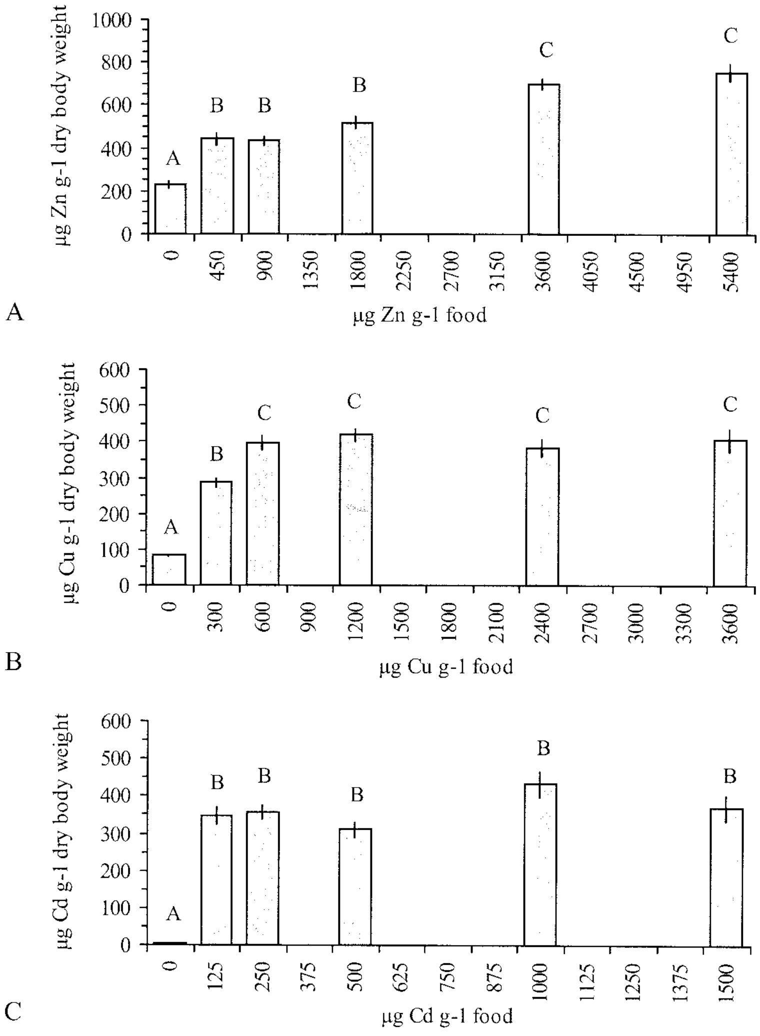

Figure 2. Body concentrations of Zn (A), Cu (B) and Cd (C) in isopods after 14 days of exposure to metal dosed food (mean±SE; N=15, at each concentration; different letters mean statistically significant differences between groups, ANOVA, Tukey test, $p<0.05$).

Table 1. Assimilation efficiency (AE, %) for Zn, Cu and Cd in *Porcellio scaber* (mean ±SE).

Conc. of Zn in food ($\mu g\ g^{-1}$)	450	900	1800	3600	5400
AE (%) Zn	14.5 (±2.4)	7.2 (±0.9)	7.2 (±0.9)	16.0 (±2.3)	13.2 (±1.9)
Conc. of Cu in food ($\mu g\ g^{-1}$)	300	600	1200	2400	3600
AE (%) Cu	19.9 (±1.2)	16.2 (±0.9)	16.7 (±1.1)	12.8 (±0.9)	9.7 (±0.7)
Conc. of Cd in food ($\mu g\ g^{-1}$)	125	250	500	1000	1500
AE (%) Cd	112.4 (±6.6)	63.8 (±3.6)	54.3 (±4.7)	32.7 (±2.7)	35.4 (±2.9)

The consumption of Cd-dosed food was affected even at the lowest concentration and appears to be dose dependent (Fig. 1C), whereas the Cd concentrations in isopods were not (Fig. 2C). The AE of Cd was several times higher than that for Zn and Cu even at the highest concentration used (Table 1). Hames and Hopkin (1991) reported an AE for Cd (60% at 100 μg Cd g^{-1} dry food weight) roughly double that for Zn. In the isopods exposed to 125 μg Cd g^{-1} dry food weight AE exceeded 100%. Unreasonably high AE values can be explained only by additional Cd intake from feces and fungal hyphae (Hassall and Rushton 1982; Hopkin 1993) or possibly from other sources (Drobne and Fajgelj 1993). Therefore, the reliability of AE values obtained in this way (see Materials and Methods) is questionable.

Various toxicity studies conducted with terrestrial isopods differed in endpoint selection and subsequently in the duration of exposure. In order to compare our data with those of other studies, we used EC_{10} values (Table 2) as a substitute for no-observed-effect-concentrations (NOEC) (Hoekstra and Van Ewijk 1993). The EC_{10} value determined in our experiments for Zn (900 μg Zn g^{-1} dry weight), corresponds to the Zn concentration in food that did not affect the reproduction or survival of *P. scaber* (Beyer and Anderson 1985; Hopkin and Hames 1994) even on longer exposure. We calculated an EC_{10} for Cd (35 μg Cd g^{-1} dry weight), which is similar to the NOEC for growth and mortality following chronic exposure (Hopkin and Hames 1994; Crommentuijn et al. 1994). In our study, the EC_{10} for Cu was more than three times higher than the Cu concentration in food that caused reduction in growth (Farkas et al. 1996) or survival (Hopkin and Hames 1994) of isopods on longer exposure. It appears that toxicity data for Zn and Cd obtained in different tests are similar. In contrast, the toxicity data for Cu may depend more on the endpoint selected.

Laboratory toxicity data show that EC_{10} and EC_{50} for Zn are evidently higher than those for Cu and Cd (Table 2), but the potential toxicity of Zn in a polluted environment should not be overlooked. Namely, in the environment, concentrations of Zn can be more than 100-times higher than those of Cd and Cu.

Table 2. Ten percent effective concentration (EC_{10}) and median effective concentration (EC_{50}) with 95% confidence intervals for Zn, Cu and Cd based on the feeding rate of pre-adult *P. scaber* in a short-term laboratory test (*graphically determined).

	Zn	Cu	Cd
EC_{10} ($\mu g\ g^{-1}$)	900	326	35*
95% conf. int.	440-1360	118-534	/
EC_{50} ($\mu g\ g^{-1}$)	2600	1335	350
95% conf. int.	2060-3140	982-1688	218-496

On the basis of our results, we can summarize that a reduced feeding rate is one mechanism of metal intake regulation. However, the reduction of feeding seems to be independent of metal storage capacities. Feeding rate is reduced in a dose-dependent manner after the consumption of Zn, Cd and Cu contaminated food. In our experiments, again, a change in feeding rate is confirmed as a suitable effect criterion in laboratory toxicity studies with isopods, firstly because data are obtained within a short time and secondly, EC_{10} values for the feeding rate can be used to anticipate the population level NOEC.

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