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Assimilation of Zinc by *Porcellio scaber* (Isopoda, Crustacea) Exposed to Zinc

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The ability of terrestrial isopods to accumulate high amounts of metals, to survive in industrially polluted areas (Hopkin 1989) and respond to environmental contaminants in a dose-dependent manner (Drobne 1996) makes them one of the most favorite experimental organisms for terrestrial ecotoxicology. Understanding metal uptake, assimilation and loss by these animals is important to explain how they cope with polluted environments. Metal uptake depends on the rate of food consumption, on metal availability in the food, on the pH inside the gut (Hopkin 1989; Donker 1992) and some other factors. Isopods respond to high metal concentrations in the food in different ways and try to avoid the negative effects of metal poisoning. Zinc is one of the metals present in high concentrations in industrially polluted areas. Zinc poisoning may be avoided by the regulation of the consumption rate (Joosse et al. 1981; Drobne and Hopkin 1995), by behavioral response (Van Capelleveen 1987; Drobne et al. 1995), by storing metals in the hepatopancreas as insoluble granules (Hopkin 1989), and by fecal, and possibly urinary, excretion (Donker 1992). Zinc in organisms is a constituent of more than 200 metalloenzymes and other metabolic compounds and assures stability of biological molecules and structures (Eisler 1993). High Zn levels in food cause a reduction of feeding rate (Drobne and Hopkin 1995), affect growth and reproduction (Donker 1992), cause changes in the structure of the digestive glands (Drobne 1996) and influence the duration of the molting cycle (Drobne and Strus 1996).

Some authors have investigated uptake and accumulation of Cu, Ni, Mn, Mg by isopods exposed to different concentrations of these metals in food (Alikhan and Storch 1989; Bercovitz and Alikhan 1989). Laboratory data on the uptake and loss of Zn by isopods for areas with low metal concentrations are provided by Hames (1989), and Hames and Hopkin (1991). No comparison so far has been made between isopods exposed to different Zn concentrations in the food.

The aim of present study was to investigate Zn assimilation by *Portellio scaber* exposed to leaves contaminated with radioactively labeled Zn at five different concentrations. The concentrations were chosen on the basis of published data on the effects of Zn on *Portellio scaber* and on the basis of published data on concentrations of Zn in leaf litter (see Hopkin 1989). We aimed to study if the

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amount of assimilated Zn followed the range of Zn concentrations in the food and whether assimilation was influenced by the food consumption rate.

MATERIALS AND METHODS

Specimens of *Porcellio scaber* were collected from a garden in Maribor, Slovenia. Hazel leaves *Corylus avellana* were collected from the litter layer of uncontaminated woodlands near Cerknica. Isopods were maintained at 20° C and under natural autumn diurnal conditions (light-dark cycle 10h:14h) on leaf litter collected from the collection site for 7 weeks prior to the experiment.

The leaves were collected in February, dried for 48 hr at 60° C and kept at room temperature (20° C) and a relative humidity of about 30% for several months. About 60 mg dried leaf weight was placed in a Petri dish (diameter 9 cm). A solution of $ZnCl_2$, neutralized with ammonia to pH 6, was applied topically as small droplets to the leaves and allowed to dry at room temperature (20° C) and relative humidity of about 30% for several days. The amount of solution applied to each leaf was adjusted to give nominal concentrations of 300, 750, 1500, 2100 and 3000 µg Zn g^{-1} dry weight. The concentration of zinc in control leaves from the collection site was 38±4 µg Zn g^{-1} dry weight (mean±SD) as determined by atomic absorption spectroscopy (Perkin-Elmer 2380) *(Zidar p.c.)*. Zn applied to each leaf was labeled with a known concentration of radioactive ^{65}Zn. The specific activity of labeled Zn was measured to be 600 Bq/µg which approximated the range of similar experiments performed on isopods (Hames and Hopkin 1991).

For the experiment 50 intermolt adult males of approximately 1 cm length and 63,2±13,6 mg fresh weight (mean±SD) were placed individually into plastic Petri dishes. A water container (diameter 1 cm and height 1 cm) filled with distilled water was placed in each Petri dish. A piece of filter paper, placed in the container, allowed isopods access to wet filter paper ad libitum. The Petri dishes were stacked in large covered glass tanks which were maintained at a temperature of 20° C and a relative humidity of 100%. Isopods were exposed to treated leaves for ten days. After this period they were transferred to uncontaminated leaves.

Radioactivity on assimilated ^{65}Zn in isopods along with their body weight were measured every second day of the experiment. Fecal pellets of the previous 48 hr were removed from each Petri dish and dried. As fecal production is, generally, proportional to food consumption (Drobne and Hopkin 1995), the weight and radioactivity of the fecal pellets were also measured. Gamma emissions from the decay of ^{65}Zn in each sample were collected for up to 600 sec (to ensure a statistical error under 2%) with an Ortec Well Type HP Ge detector and Canberra 90 analyzer. Corrections were made for background gamma emissions and natural decay of the radionuclide during the experiment. The weight of assimilated Zn was calculated using the method of isotope dilution (Tölgyessy and Kyrs 1989). To convert live body weight to dry body weight 10 intermolt adult males were dried, according to the method of Hames (1989). Dry body weight represented 26±0.9 % (mean±SE) of live body weight of animals.

Concentration factors (CF) were calculated as a ratio of Zn concentration in the isopods to Zn concentrations in the leaves (Ma 1994). We also calculated a modified concentration factor (CF), where Zn concentrations in isopods were taken two days after the transfer to uncontaminated leaves. In both cases Zn concentrations in the leaves were those applied at the beginning of the experiment.

All data were checked for normality of distribution (Kolmogorov-Smirnoff test). Within assimilated Zn levels, fecal production rates and Zn concentrations in feces comparisons were made using the Kruskal-Wallis test.

RESULTS AND DISCUSSION

Concentrations of assimilated Zn were significantly lower (Kruskal-Wallis test, $p \leq 0.05$) following 10 days after the exposure in isopods allowed access to 300 µg Zn g^{-1} dry weight than in other groups of isopods (Fig. 1). In isopods exposed to 750 and 1500 µg Zn g^{-1} dry weight, the concentrations of assimilated Zn did not differ significantly during the experiment. From the sixth day onwards the isopods exposed to 2100 µg Zn g^{-1} dry weight showed statistically significant differences in amounts of assimilated Zn in comparison with isopods exposed to 300, 750 and 1500 µg Zn g^{-1} dry weight concentrations. However, isopods exposed to 3000 µg Zn g^{-1} dry weight showed statistically significant differences from the fourth day onwards in amounts of assimilated Zn in comparison with isopods exposed to 300, 750 and 1500 µg Zn g^{-1} dry weight concentrations.

It was evident that the concentrations of assimilated Zn in the different groups of isopods did not follow the trend of Zn concentrations in the food. At the end of the exposure to contaminated leaves, isopods on 1500 µg Zn g^{-1} dry weight assimilated approximately twice as much Zn as the animals fed on five times lower Zn concentrations (300 µg Zn g^{-1} dry weight, Fig. 1). Isopods exposed to 3000 µg Zn g^{-1} dry weight assimilated approximately five times more Zn than those exposed to a ten times lower Zn concentration (300 µg Zn g^{-1} dry weight).

Concentrations of assimilated Zn in isopods depend on many factors; the most important being the rate of uptake, assimilation and loss of the metal. The differences in concentrations of Zn in different groups of isopods may arise from a reduced rate of Zn uptake, because of a reduced feeding rate and because animals could find less Zn contaminated edges of leaves. Isopods exposed to 1500 µg Zn g^{-1} dry weight assimilated only 1.4 times more Zn after feeding on uncontaminated leaves for 50 days than animals exposed to five times lower concentrations (300 µg Zn g^{-1} dry weight). Isopods exposed to 3000 µg Zn g^{-1} dry weight assimilated 2.2 times more Zn than the animals exposed to ten times lower concentration (300 µg Zn g^{-1} dry weight). Thus, it is evident that after exposure to uncontaminated leaves the differences in the concentrations of assimilated Zn between the isopods exposed to 3000 µg Zn g^{-1} dry weight and those exposed to 300 µg Zn g^{-1} dry weight were lower than that observed at the start of the exposure to uncontaminated leaves.

These differences cannot be explained only on the basis of either a reduced feeding rate or because animals could select less Zn-contaminated food but also

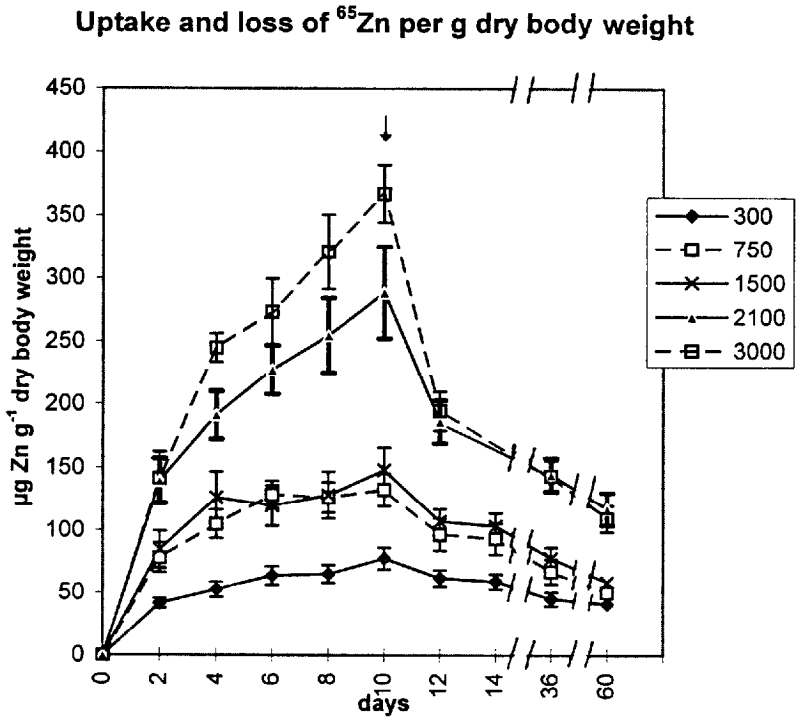

Figure 1. Concentrations of labeled Zn (means±SE) in Porcellio scaber exposed for ten days to Hazel leaves contaminated with 300, 750, 1500, 2100 and 3000 µg Zn g^{-1} dry mass, followed by 50 days (arrow) feeding on uncontaminated leaves. N=10, after the tenth day N=8.

by higher rates of Zn loss by isopods exposed to 2100 and 3000 µg Zn g^{-1} dry weight.

The relation between the amount of Zn in the animal and the amount of Zn in the food expressed as a concentration factor (CF) decreases with increasing Zn concentrations in leaves (Table 1). The concentrations of Zn in animals at the end of exposure to contaminated food were used to calculate the CF. In the literature concentration factors mostly refer to concentrations of metals in tissues (Van Hook 1974; Ma 1994). However, immediately after exposure to contaminated food isopods contained some contaminated food in the gut, in the gland lumen and in the hemolymph. Concentration factor was calculated in order to express more adequately the concentrations of Zn in tissues.

Two days after the isopods were transferred to uncontaminated leaves, concentrations of assimilated Zn were reduced in all animals. According to Hames and Hopkin (1991a) the amount of Zn lost by *Portellio scaber* in the same period, when transferred to uncontaminated leaves, was about 20 % of the total Zn concentration in the animal. Our results for animals exposed to up to

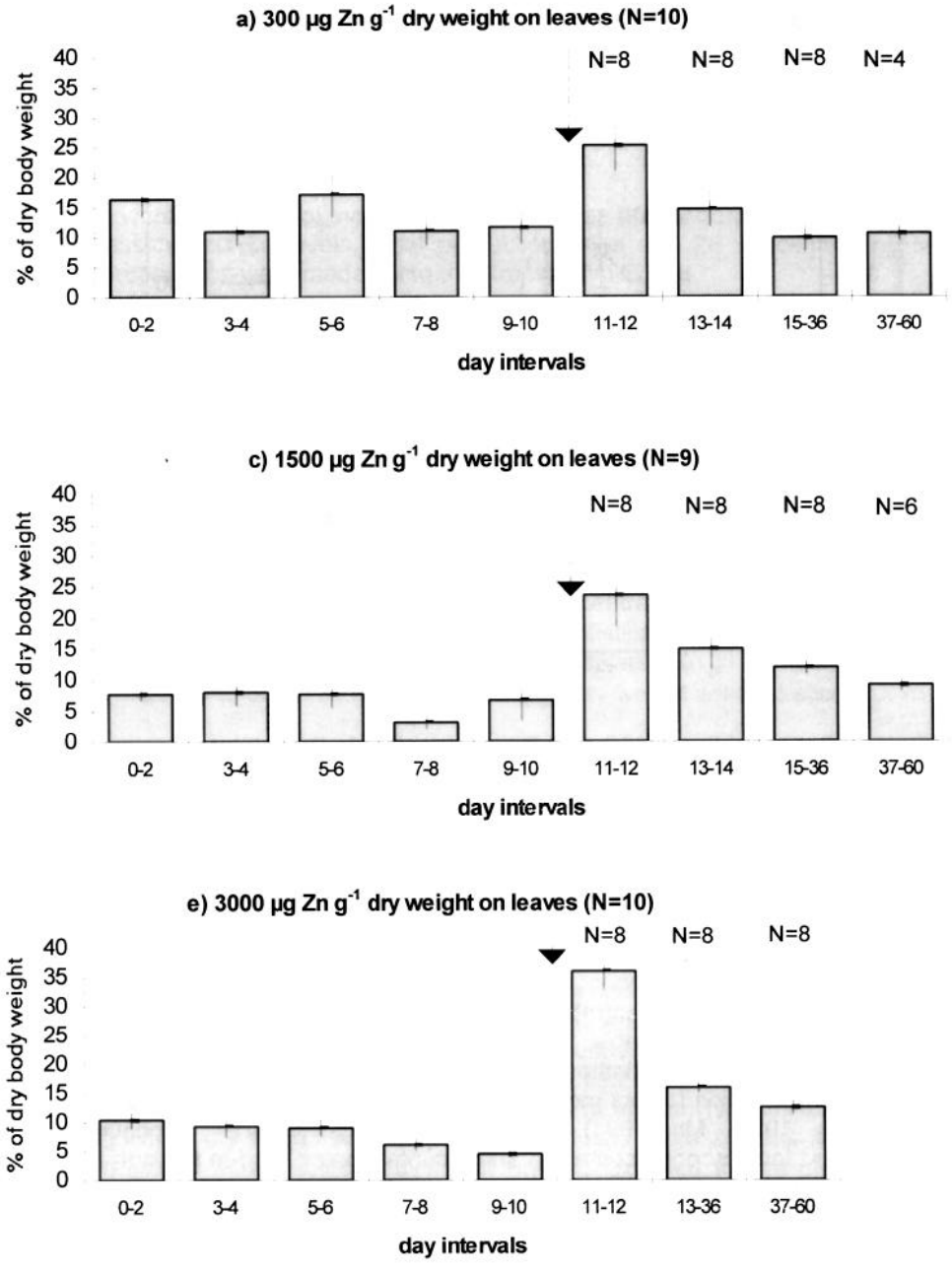

Figure 2. Daily fecal production rates (means±SE), expressed as percentage of dry body weight in Porcellio scaber, feeding on Hazel leaves contaminated with (a) 300 (c) 1500 and (e) 3000 µg Zn g^{-1} dry weight for ten days, followed by feeding for 50 days (arrow) on uncontaminated leaves. Numbers indicate the number of animals that were molting during that period.

1500 µg Zn g^{-1} dry weight confirmed these data. But the animals exposed to 2100 and 3000 µg Zn g^{-1} dry weight lost higher amounts of Zn in these two days.

Table 1. Concentration factors for Zn (mean±SE) in isopods exposed to five different Zn concentrations.

Cone. of Zn in leaves (µg Zn g^{-1} dry weight)	CF	CF
300	0.258±0.029	0.206±0.022
750	0.177±0.017	0.129±0.017
1500	0.099±0.012	0.071±0.007
2100	0.137±0.017	0.089±0.008
3000	0.122±0.008	0.065±0.005

CF: Concentration factor
CF.: Modified concentration factor (see Materials and Methods)

The fecal production rate in isopods exposed to 300 and to 750 µg Zn g^{-1} dry weight was very similar and did not change significantly during the experiment (Fig. 2a). The isopods exposed to 1500 and 2100 µg Zn g^{-1} dry weight produced half as much feces during the first ten days as did isopods exposed to 300 and 750 µg Zn g^{-1} dry weight (Fig. 2c). This shows that their food consumption was low, and as a result lower amounts of Zn were assimilated by isopods exposed to 1500 µg Zn g^{-1} dry weight than animals exposed to 750 µg Zn g^{-1} dry weight, Isopods exposed to 3000 µg Zn g^{-1} dry weight had reduced in fecal production immediately after exposure to contaminated leaves, and this continued to the tenth day (Fig. 2e). The results are supported by literature data where reduction of feeding rate was noticeable in isopods exposed to leaves with concentrations of 3000 µg Zn g^{-1} dry leaf weight (Donker 1992; Joosse et al. 1983). Drobne and Hopkin (1995) reported a reduction of feeding rate at concentrations of 2000 µg Zn g^{-1} dry leaf weight.

Table 2. Concentrations of labeled Zn (mean±SE) in µg Zn g^{-1} dry weight in feces of Porcellio scaber feeding for ten days on hazel leaves contaminated with 300, 750, 1500, 2100 and 3000 µg Zn g^{-1} dry weight. In the interval from the tenth to twelfth day, isopods were transferred to uncontaminated leaves (N=10, after the tenth day N=8)

	Days					
Group	0-2	3-4	5-6	7-8	9-10	11-12
300	404±57	378±26	393±30	380±32	340±29	28±5
750	776±66	927±47	933±48	1009±70	1004±47	58±9
1500	1183±127	1373±148	1694±140	1434±198	1718±169	111±27
2100	1988±131	2349±134	3066±159	3107±251	3001±222	273±69
3000	1750±176	2631±136	3092±180	3477±197	3464±205	227±44

The concentrations of labeled Zn in feces in isopods exposed to 300 and to 750 µg Zn g^{-1} dry weight remained identical over the ten-day period and were close to the concentrations of labeled Zn in the leaves (Table 2). The concentrations of

labeled Zn in feces produced after the first two days by isopods exposed to 3000 µg Zn g^{-1} dry weight were significantly lower (Kruskal-Wallis test, $p<0.01$) than the concentrations of labeled Zn in the leaves. Up to the sixth day there was an increase in the concentration of Zn in feces and it exceeded the concentration of Zn in the leaves at the end of exposure. In feces produced between the tenth and the twelfth day, when isopods were already feeding on uncontaminated leaves, the concentrations of Zn were an order of magnitude lower than when feeding on contaminated leaves (Table 2).

Isopods exposed to 3000 µg Zn g^{-1} dry weight assimilated lower amounts of Zn in the first two days of the experiment than later on (Fig. 1). They also consumed lower amounts of Zn than expected during this period. This was supported by low concentrations of Zn in feces during the first two days in the isopods exposed to 3000 µg Zn g^{-1} dry weight (Table 2). Ther could be explained this by the fact that Zn was not applied completely homogeneously on the leaves. We noticed that isopods first consumed the edges of the leaves, which probably contained lower amounts of Zn than the rest. Results of other experiments showed that the animals could detect high Zn concentrations in the food (Drobne et al. 1995).

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