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Assimilation and Loss of ^{109}Cd and ^{65}Zn by the Terrestrial Isopods *Oniscus asellus* and *Porcellio scaber*

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Terrestrial isopods (woodlice) have been the subject of numerous publications on the dynamics of metals in terrestrial invertebrates (e.g., Alikhan 1989; Morgan et al. 1990; Prosi & Dallinger 1988). They are of particular interest due to the ability of the hepatopancreas of woodlice to accumulate zinc, cadmium, lead and copper to very high concentrations (for a review see Hopkin 1989). In the U.K., Oniscus asellus L. and Porcellio scaber Latreille are the two most common species and have been used to indicate the biological availability of metal pollutants in terrestrial ecosystems (Hopkin et al. 1986). Despite the similarities of diet, and structure and function of the digestive organs of the two species (Hames & Hopkin 1989), there are considerable differences in the net accumulation of metals by O.asellus and P.scaber. For example, in a recent study, Hopkin (1990a) showed that O.asellus from a zinc-contaminated site was able to excrete this metal at a faster rate than P.scaber when both species were fed an uncontaminated diet. In contrast, O.asellus had a greater affinity for cadmium and retained this metal to a much greater extent than P.scaber.

The experiments of Hopkin (1990a) were conducted with uncontaminated leaf litter, and leaves which had been contaminated in the field by aerial fallout of particles containing zinc, cadmium, lead and copper from a smelting works. In this paper, the rates of assimilation and excretion of two of these metals have been quantified by feeding O.asellus and P.scaber on leaves contaminated with radioactive isotopes of zinc and cadmium.

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MATERIALS AND METHODS

Specimens of O.asellus and P.scaber were collected from the Whiteknights campus, University of Reading. They were maintained for 12 weeks prior to the experiment on leaf litter from the collection site in plastic tanks at a constant temperature of 18°C under a 16 hr light/8 hr dark regime.

Ten adult females of each species, at intermolt, were weighed and placed in individual experimental chambers. Each chamber consisted of the base of a plastic petri dish (9 cm in diameter) covered with plastic netting (4 mm mesh). The woodlice and their food were placed on to the netting and were covered with an upturned plastic pot of the same diameter as the petri dish, held in place with elastic bands. This arrangement allowed fecal pellets to drop through to the petri dish below and prevented coprophagy by the isopods. The chambers were kept in a large plastic tank at a constant temperature of 18°C under a 16 hr light/8 hr dark regime. The base of the tank contained a layer of moist tissue paper which maintained a high humidity in the experimental chambers throughout the experiment.

The experimental food consisted of 1 cm by 1 cm squares cut from leaves of Norway Maple (Acer planatoides) collected from the litter layer of Prospect Park, Reading, an uncontaminated site. The solution for contaminating the food was prepared by dissolving cadmium chloride ($2CdCl_2.5H_20$) and zinc chloride ($ZnCl_2$) in distilled water to give a final concentration of 15 µg Cd ml^{-1} and 15 µg Zn ml^{-1}. This solution was spiked with ^{109}Cd and ^{65}Zn (Amersham International) to give a disintegration rate of 3,000 Bq ml^{-1} for each isotope. The food was contaminated by topical application of 50 µl of the solution (containing 0.75 µg of each metal) onto each leaf square. When air-dried to constant weight, each leaf square weighed approximately 7.5 mg. Thus, the concentration of added zinc and cadmium was approximately 100 µg of each metal g^{-1} of leaf and the rate of disintegration of each isotope on the leaf squares was 150 Bq.

Each of the 20 isopods was provided with one square of contaminated leaf and had access to no other food material for 115 hr. At the end of this period, each isopod had consumed most of the leaf square (Table 1). The remains of the trial leaf squares were removed and replaced with pieces of uncontaminated Norway Maple. The gamma emissions from assimilated ^{109}Cd and ^{65}Zn, and the weights of living individual isopods, were measured at regular intervals (Fig. 1a, b). Live weights were converted to dry weights from a plot of wet weight

against dry weight of ten isopods of each species taken from the stock tanks. The weights and radioactivity of fecal pellets and the trial leaf remnants were determined also. Gamma emissions from the decay of ^{109}Cd and ^{65}Zn in each sample were collected for 300sec with an LKB Ultrogamma 1280. Corrections were made for background and natural decay of isotopes during the experiment.

Uncontaminated leaves of Norway Maple analyzed by flame atomic absorption spectrometry contained about 100 µg Zn g^{-1} dry wt and 1.0 µg Cd g^{-1} dry wt. Ten specimens of each species removed from the stock tank at the start of the experiment were analyzed also. The mean concentrations of zinc and cadmium in whole P.scaber was 250 µg g^{-1} dry wt and 2 µg g^{-1} dry wt, respectively. In whole O.asellus, the concentrations of zinc and cadmium were 140 µg g^{-1} dry wt and 12 µg g^{-1} dry wt, respectively. These values indicate that the site from which the animals were collected was 'uncontaminated' with zinc and cadmium according to the indices of Hopkin et al. (1986, 1989a).

RESULTS AND DISCUSSION

There are clear differences between O.asellus and P.scaber in the rates of uptake and loss of cadmium and zinc (Figs. 1,2). The period 0 hr to 16 hr, when similar amounts of cadmium were detected in each species, reflects the initial filling of the gut with the trial food. At 115 hr the concentration of each labelled metal was at it's maximum, and P.scaber contained higher concentrations of cadmium and zinc than O.asellus. In every individual, more cadmium was accumulated than zinc.

At 133 hr, 18 hr after the labelled food was replaced with unlabelled leaf, the counts were reduced in all isopods, compared with the counts at 115 hr. This resulted primarily from the voiding in the feces of the last residues of labelled food from the hindguts of the woodlice. There was a large (approximately 50%) reduction in the concentration of labelled zinc in O.asellus between 115 hr and 133 hr, compared with only a 10% reduction of labelled cadmium over the same period (Fig. 2). In P.scaber approximately 10% of the labelled cadmium and 20% of the labelled zinc were lost between 115 hr and 133 hr. The concentrations of labelled cadmium in O.asellus were maintained from 133 hr onwards while labelled zinc continued to be excreted. Less than 5% of the zinc remained in O.asellus at the end of the experiment, compared to the amount present at 115 hr. Labelled cadmium was excreted from P.scaber at a similar rate to zinc, which was slower than the rate of

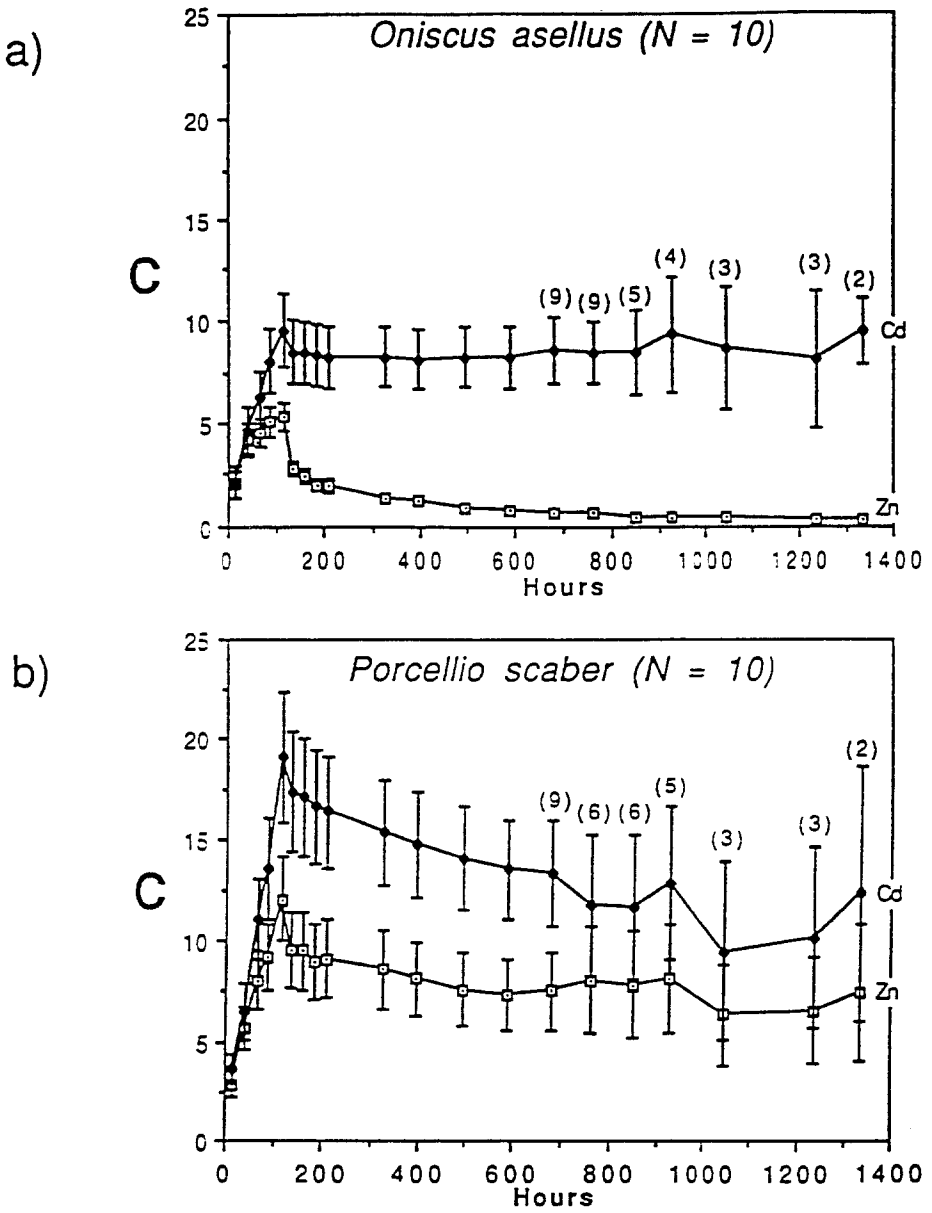

Figure 1. Concentrations (C, µg g^{-1} dry wt) of isotope-labelled cadmium (♦) and zinc (▫) in a) Oniscus asellus and b) Porcellio scaber (mean ± standard error). The standard error is not shown where the value is less than 0.35 µg g^{-1} dry weight. Figures in parentheses represent the number of individuals alive when less than ten.

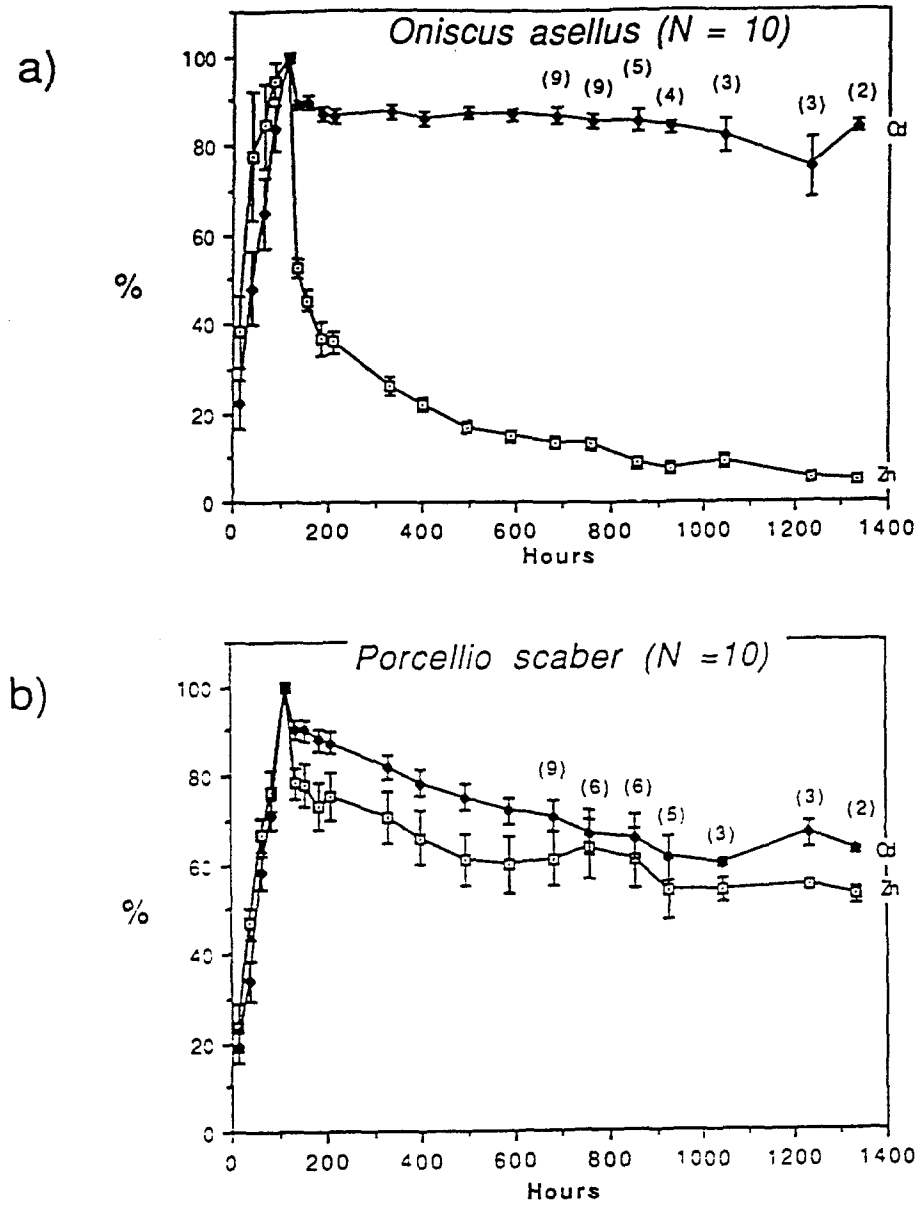

Figure 2. Amounts (%) of isotope-labelled cadmium (♦) and zinc (▫) retained in a) Oniscus asellus and b) Porcellio scaber (mean ± standard error). Results are expressed as percentages of the concentrations at 115 hr, when labelled food was replaced with unlabelled leaf. Figures in parentheses represent the number of isopods alive when less than ten.

excretion from O.asellus.

The mean assimilation rate for labelled cadmium ingested by O.asellus up to 115 hr was 49.1%, and in P.scaber 58.5%. For zinc the assimilation rate was 29.4% in O.asellus and 36.7% in P.scaber (Table 1). Within each species, assimilation rates for cadmium and zinc were shown by Student's t-tests to be significantly different ($p < 0.001$). When assimilation rates were compared between O.asellus and P.scaber, the rates for zinc were significantly different ($p < 0.001$), but not for cadmium ($p > 0.05$). Differences in rates of ingestion between the two species were not significant ($p > 0.05$). The similar rate of assimilation of cadmium by P.scaber when compared to O.asellus was surprising in view of previous findings where O.asellus was shown to accumulate substantially more cadmium than P.scaber (Hopkin 1990a). However, this result (Table 1) could have arisen from the presentation of cadmium in a highly available form in the food in the woodlice, compared to what they would experience in a field site. In addition, the concentration of cadmium in the diet relative to zinc was much higher than that which pertains in metal-polluted sites, which may also have distorted the findings. The experiments are currently being repeated, using a range of concentrations of cadmium and zinc, to determine whether antagonism or synergism between the metals takes place.

Table 1. Isopod weight (Isopods, mg), food and metal intake.

	Oniscus asellus N=10	Porcellio scaber N=10
Isopods, mg	22.8 ±1.0	13.2 ±0.9
Leaf, mg	4.4 ±0.6	4.1 ±0.5
Food intake	4.0% ±0.6	6.7% ±0.8
^{109}Cd & ^{65}Zn ingested	0.4 ±0.05	0.4 ±0.05
Cd, 0 hr to 115 hr	49.1% ±6.2	58.5% ±8.1
Zn, 0 hr to 115 hr	29.4% ±2.9	36.7% ±3.8

[labelled leaf ingested (Leaf, mg), food consumed per 24 hr as % of dry wt of isopod (Food intake), weight of labelled cadmium and zinc ingested by the isopods (^{109}Cd & ^{65}Zn ingested, µg) and assimilation rates for cadmium (Cd, 0 hr to 115 hr) and zinc (Zn, 0 hr to 115 hr). All values are mean ± standard errors.]

None of the isopods molted while they were feeding on the labelled food. However, about one third of the woodlice molted while feeding on the unlabelled diet. Nevertheless, cadmium and zinc retention and loss was similar to that of woodlice that did not molt. No radioactive metals were detected in molted exoskeletons.

Loss of ^{65}Zn from O.asellus after replacement of contaminated with uncontaminated food was initially rapid; almost 50% of the labelled zinc was voided in the feces within 18 hr (Fig. 1a). Comparison with P.scaber (Fig. 1b) indicates that probably only 10% of this loss can be attributed to unassimilated ^{65}Zn in the lumen of the hindgut. Previous work by Lauhachinda & Mason (1979) on Armadillidium vulgare has shown that this isopod was able to excrete 50% of ^{65}Zn within 2 days when the label was injected directly into the haemolymph. Thus, the greater ability of O.asellus to excrete zinc compared to P.scaber may account for the differences observed in the concentrations of the metal between the two species in the field (Hopkin et al. 1989a).

In both O.asellus and P.scaber cadmium was taken up more rapidly than zinc, but the subsequent loss of cadmium from the body was slow. Whereas a continued low rate of loss was observed in P.scaber throughout the experiment, no loss of cadmium was detected from O.asellus after residual trial food had been cleared from the hindgut. For O.asellus, this supports the suggestion of Hopkin (1989) that once cadmium has been deposited in association with sulphur in type B copper-containing granules in the hepatopancreas, it is retained until the animal dies. In the study described by Hopkin (1990a) when woodlice from a contaminated site were provided with litter from an uncontaminated site, the concentration of cadmium in O.asellus did not decrease over a five-month period.

Measurements of uptake and loss of zinc and cadmium labelled with isotopes ^{109}Cd and ^{65}Zn have revealed profound differences between O.asellus and P.scaber. These findings are not accounted for by differences in the structure of the digestive system in O.asellus and P.scaber, since it is similar in both species (Hames & Hopkin 1989). The two cell types present in the hepatopancreas display different functional characteristics which may influence assimilation and loss of metals in this tissue (Hopkin et al. 1989b). Cadmium is usually stored in the S cells which retain metal-containing granules throughout the life of an isopod, whereas zinc is sequestered in S cells and B cells (Hopkin 1990b). In a recent paper, Hames & Hopkin (in press) have shown that the B cells of O.asellus and P.scaber undergo considerable apocrine secretion every 24 hr, whereas the S cells were never observed to discharge any material into the lumen of the hepatopancreas. Thus, if O.asellus accumulated a greater proportion of assimilated ^{65}Zn in B cells than in S cells in comparison to P.scaber, this could account for the greater rate of loss of the isotope from O.asellus when it was transferred to an uncontaminated

diet. This possibility is currently being examined by autoradiography in the light and electron microscopes.

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