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Metal accumulation strategies in saprophagous and phytophagous soil invertebrates: a quantitative comparison

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To quantitatively reveal accumulation patterns of environmentally relevant heavy metals in selected saprophagous or phytophagous soil invertebrates, adults of the species *Porcellio scaber* (Isopoda), *Tetrodontophora bielanensis* (Collembola), *Julus scandinavius* (Diplopoda), and *Deroceras reticulatum* (Gastropoda) were exposed to lead-, cadmium-, or zinc-contaminated food and soil for three weeks. The heavy metal concentrations in the food, the substrate, and the bodies of the invertebrates were measured by atomic absorption spectrophotometry (AAS). The investigated species were found to differ in their metal accumulation strategies, which is interpreted as a consequence of different detoxification mechanisms.

Keywords: heavy metals, millipedes, slugs, springtails, woodlice

Introduction

Many studies concerning accumulation of heavy metals in terrestrial invertebrates have been conducted (summarized in Morgan & Morris 1982, Prosi *et al*. 1983, Dallinger & Wieser 1984, Hopkin & Martin 1984, Hopkin 1989, Dallinger 1993), and these have led to the general opinion, that in terrestrial communities, mainly saprophagous (or phytophagous) species are suitable metal accumulators for monitoring studies (e.g. Hopkin 1989). Thus, in field studies, terrestrial woodlice (Wieser *et al*. 1976, Hopkin *et al*. 1989, Dallinger *et al*. 1992), terrestrial gastropods (Berger & Dallinger 1989), and diplopods (Beyer *et al*. 1985, Read & Martin 1990, Köhler & Alberti 1992) have successfully been used as indicators for cadmium, lead, or zinc contamination in soil habitats.

Although these animals have been used as monitor organisms, knowledge of the comparability of metal accumulation in these different taxa is still greatly lacking. The question consequently arising from this uncertainty focuses on the relevance of single-species monitoring studies for the situation in the environment: does a high metal concentration in woodlice indicate a situation comparable to that indicated by a similar concentration of the same element, in, for example, slugs or springtails, and what are the respective conclusions for the habitat? The consideration of the quantitative variations in metal uptake and accumulation in the investigated species should, therefore, be one of the most important aspects of focus for an improved assessment of monitoring studies.

As a contribution to the solution of this problem, in the present study, originally uncontaminated specimens of different soil invertebrate taxa, such as the isopod *Porcellio scaber*, the collembolan *Tetrodontophora bielanensis*, the diplopod *Julus scandinavius*, and the gastropod *Deroceras reticulatum*, were exposed to a graded series of heavy metals in order to reveal the underlying principles of the accumulation patterns of the animals. The study focuses on three rather than one environmentally relevant heavy metal and thus allows the possibility of: (1) relating the quantitative accumulation patterns of the different species mentioned above to one another; and (2) revealing differences in the accumulation behaviour of the three tested metals in the respective species.

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Materials and methods

Conditions and contamination

Adults of *Porcellio scaber* (Isopoda) and *Deroceras reticulatum* (Gastropoda) supplied from a laboratory hatchery, *Tetrodontophora bielanensis* (Collembola) from a minimally contaminated woodland stand in the Neisse valley, eastern Germany (A_h, the upper, organically enriched mineral horizon: 3 mg kg^{-1} Cd, 13 mg kg^{-1} Pb, 87 mg kg^{-1} Zn, author's unpublished data), and *Julus scandinavius* (Diplopoda) originating from an uncontaminated area in Hockenheim near Heidelberg, western Germany (leaf litter < 0.5 mg kg^{-1} Cd, 5–12.5 mg kg^{-1} Pb, < 25 mg kg^{-1} Zn, Müller *et al*. 1987) were exposed to partly decomposed leaf litter material soaked in an aqueous solution of either 10, 50 or 100 mg kg^{-1} Cd^{2+} (as $CdCl_2$), 100, 500 or 1000 mg kg^{-1} Pb^{2+} (as $PbCl_2$), or 500, 1000 or 5000 mg kg^{-1} Zn^{2+} (as $ZnCl_2$) for three weeks. Controls were wetted with tap water. Isopods, diplopods, and collembolans were kept in plastic boxes on a moist gypsum base covered by the above mentioned partly decomposed leaf litter material; for the springtails, the leaf litter was supplemented with yeast flakes, which were also wetted with the respective metal solution. To adjust exposure conditions to the respective preferences of the test species and to meet the field situation as closely as possible, for the woodlice the light/dark photoperiod was 12 h/12 h at 15°C, for the diplopods 12 h/12 h at 7°C and for the collembolans 12 h/12 h at 10°C. The slugs were kept in plastic boxes on a moist soil in a light/dark period of 16 h/8 h at 10°C, the food consisting of lettuce leaves and carrot slices. Both the food and the soil were soaked with the metal solutions mentioned above.

Metal analysis

For atomic absorption spectrophotometric investigations, clearance of the gut was achieved by allowing complete defaecation of the specimens on an uncontaminated, moist gypsum base within two days after the end of the experiment, except for the slugs which were kept on uncontaminated, wetted filter paper. Subsequently, for each species and metal concentration, three individuals were frozen in liquid nitrogen and oven-dried at 60°C for two days. After weighing, the specimens were digested in 200 µl nitric acid (suprapur grade) at 90°C and then analysed for cadmium, lead, and zinc by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer AAS 5000, HGA 500). In order to examine the metal concentration in the food and soil, the heavy metal-soaked material was processed in the same manner as the invertebrate samples. For each experiment, a sample of 500 mg was digested in 10 ml hot nitric acid, filled to 50 ml with H_2O, and analysed for cadmium, lead, and zinc, respectively. To compare the data of the different experiments, a concentration factor (cf) was calculated, which is represented by the quotient of the dry weight heavy metal concentration in the animal ($[hm]_{animal}$) and in the food ($[hm]_{food}$):

$$cf = ([hm]_{animal} \cdot [hm]_{food})^{-1}$$

Statistical handling

The statistical significance of differences in metal concentrations of contaminated food, substrate, and animals and the respective controls (comparison of two independent means) was checked with Student's *t*-test. Significance levels: * for $0.05 > P > 0.01$ (weakly significant), ** for $0.01 > P > 0.001$ (significant), and *** for $P < 0.001$ (highly significant). Regression analysis was conducted with TableCurve (Jandel Scientific).

Results

Dry weight metal content of the substrate

As expected from previous studies (Weigmann *et al.* 1985, Köhler *et al*. 1992), soaking dry leaf litter material with heavy metal solutions resulted in an approximate four to five fold concentration of the respective metal in the food dry weight. The metal concentration in the slugs' food material increased by an average of five and half fold. For details, see Tables 1 and 2.

Metal concentrations in the invertebrates

Exposure to different concentrations of cadmium, lead, and zinc resulted in uptake and accumulation of these heavy metals by the animals. Concentrations in the investigated invertebrates did not surpass the respective metal concentrations in the food within three weeks of exposure, with the exception of the lowest applied cadmium concentration. In this case only the cadmium concentrations in *P. scaber* and in *D. reticulatum* exceeded the concentrations in the food. Usually, the metal concentrations in the animals were found to be dose-dependent and increased with the metal concentration in the food. In woodlice and diplopods, this relationship was found to follow a saturation curve: above a distinct metal concentration in the food/substrate, the animals did not show a further increase in metal accumulation in their body even under conditions of extreme contamination. To be more precise, *P. scaber* from an uncontaminated site did not seem able to reach body concentrations higher than about 215 mg kg^{-1} cadmium, which is far from the lethal body concentration of 2117 mg kg^{-1} cadmium (van Straalen 1995), 260 mg kg^{-1} lead, and 300 mg kg^{-1} zinc within three weeks of exposure. This observation was mirrored by *J. scandinavius* with approximate limits of 28 mg kg^{-1} cadmium, 240 mg kg^{-1} lead, and 650 mg kg^{-1} zinc. In contrast, springtails and slugs showed dose-dependent accumulation patterns even in the experiments with the

Table 1. Heavy metal concentrations in the dry weight of the soaked food (leaf litter material) and in the bodies of *P. scaber*, *T. bielanensis* and *J. scandinavius*. Means ± standard deviations

Soaking solution	Resulting metal concentration in the substrate (dry weight)	Metal concentration in *P. scaber* (dry weight)	Metal concentration in *T. bielanensis* (dry weight)	Metal concentration in *J. scandinavius* (dry weight)
Control	0.4 mg Cd kg^{-1}	5.1 ± 3.7 mg Cd kg^{-1}	0.8 ± 0.4 mg Cd kg^{-1}	0.9 ± 0.3 mg Cd kg^{-1}
10 mg Cd l^{-1}	57.3 mg Cd kg^{-1}	107.9 ± 89.2 mg Cd kg^{-1}	2.9 ± 2.0 mg Cd kg^{-1}	8.7 ± 2.0 mg Cd kg^{-1}**
50 mg Cd l^{-1}	222.2 mg Cd kg^{-1}	214.9 ± 42.3 mg Cd kg^{-1}**	5.5 ± 1.5 mg Cd kg^{-1}***	27.8 ± 5.6 mg Cd kg^{-1}**
100 mg Cd l^{-1}	418.6 mg Cd kg^{-1}	195.4 ± 76.6 mg Cd kg^{-1}**	17.8 ± 5.2 mg Cd kg^{-1}	18.6 ± 13.6 mg Cd kg^{-1}
Control	7.1 mg Pb kg^{-1}	2.9 ± 2.3 mg Pb kg^{-1}	34.1 ± 12.8 mg Pb kg^{-1}	2.6 ± 0.3 mg Pb kg^{-1}
100 mg Pb l^{-1}	517.1 mg Pb kg^{-1}	73.5 ± 26.4 mg Pb kg^{-1}**	29.6 ± 22.2 mg Pb kg^{-1}	87.9 ± 65.7 mg Pb kg^{-1}
500 mg Pb l^{-1}	2777.5 mg Pb kg^{-1}	211.0 ± 67.2 mg Pb kg^{-1}***	143.3 ± 57.2 mg Pb kg^{-1}*	240.2 ± 15.0 mg Pb kg^{-1}**
1000 mg Pb l^{-1}	7676.0 mg Pb kg^{-1}	263.7 ± 68.2 mg Pb kg^{-1}***	215.9 ± 209.5 mg Pb kg^{-1}	91.7 ± 25.7 mg Pb kg^{-1}**
Control	4.0 mg Zn kg^{-1}	170.7 ± 59.4 mg Zn kg^{-1}	365.0 ± 174.4 mg Zn kg^{-1}	151.9 ± 40.9 mg Zn kg^{-1}
500 mg Zn l^{-1}	1975.6 mg Zn kg^{-1}	252.9 ± 89.4 mg Zn kg^{-1}	387.3 ± 84.1 mg Zn kg^{-1}	228.5 ± 75.1 mg Zn kg^{-1}
1000 mg Zn l^{-1}	5729.7 mg Zn kg^{-1}	221.3 ± 104.6 mg Zn kg^{-1}	459.4 ± 175.7 mg Zn kg^{-1}	156.6 ± 57.0 mg Zn kg^{-1}
5000 mg Zn l^{-1}	22 052.3 mg Zn kg^{-1}	302.4 ± 18.3 mg Zn kg^{-1}**	620.3 ± 377.3 mg Zn kg^{-1}	649.6 ± 61.8 mg Zn kg^{-1}***

Statistical significance compared to the respective controls was checked with Student's *t*-test. *** highly significant ($P < 0.001$); ** significant ($0.001 < P < 0.01$); * weakly significant ($0.01 < P < 0.05$).

Table 2. Heavy metal concentrations in the dry weight of the soaked food (lettuce leaves and carrot slices) and substrate, and in the body of *D. reticulatum*. Means ± standard deviations

Soaking solution	Resulting metal concentration in the substrate (dry weight)	Resulting metal concentration in the food (dry weight)	Metal concentration in *D. reticulatum* (dry weight)
Control	0.1 ± 0.0 mg Cd kg^{-1}	0.7 ± 0.1 mg Cd kg^{-1}	7.8 ± 8.9 mg Cd kg^{-1}
10 mg Cd l^{-1}	1.4 ± 0.0 mg Cd kg^{-1}***	59.0 ± 1.9 mg Cd kg^{-1}***	71.4 ± 49.7 mg Cd kg^{-1}
50 mg Cd l^{-1}	5.8 ± 0.1 mg Cd kg^{-1}***	358.0 ± 7.9 mg Cd kg^{-1}***	121.8 ± 7.5 mg Cd kg^{-1}*
100 mg Cd l^{-1}	14.1 ± 0.2 mg Cd kg^{-1}***	750.7 ± 13.0 mg Cd kg^{-1}***	245.9 ± 135.0 mg Cd kg^{-1}***
Control	86.4 ± 22.3 mg Pb kg^{-1}	28.6 ± 7.7 mg Pb kg^{-1}	7.6 ± 9.3 mg Pb kg^{-1}
100 mg Pb l^{-1}	103.1 ± 9.3 mg Pb kg^{-1}	405.4 ± 6.4 mg Pb kg^{-1}***	30.2 ± 8.5 mg Pb kg^{-1}
500 mg Pb l^{-1}	174.6 ± 9.4 mg Pb kg^{-1}**	2456.4 ± 71.8 mg Pb kg^{-1}***	178.7 ± 115.2 mg Pb kg^{-1}
1000 mg Pb l^{-1}	292.3 ± 1.4 mg Pb kg^{-1}***	3750.6 ± 10.6 mg Pb kg^{-1}***	1168.6 ± 1532.3 mg Pb kg^{-1}*
Control	81.1 ± 1.5 mg Zn kg^{-1}	5.2 ± 9.0 mg Zn kg^{-1}	92.8 ± 69.1 mg Zn kg^{-1}
500 mg Zn l^{-1}	136.4 ± 13.4 mg Zn kg^{-1}**	1873.7 ± 31.3 mg Zn kg^{-1}***	436.7 ± 427.3 mg Zn kg^{-1}
1000 mg Zn l^{-1}	86.9 ± 2.7 mg Zn kg^{-1}*	9305.1 ± 117.7 mg Zn kg^{-1}***	393.1 ± 192.0 mg Zn kg^{-1}
5000 mg Zn l^{-1}	490.9 ± 7.3 mg Zn kg^{-1}***	14 915.0 ± 779.2 mg Zn kg^{-1}***	4252.4 ± 2785.5 mg Zn kg^{-1}***

Statistical significance compared to the respective controls was checked with Student's *t*-test. *** highly significant ($P < 0.001$); ** significant ($0.001 < P < 0.01$); * weakly significant ($0.01 < P < 0.05$).

highest metal concentrations. For detailed data see Table 1 and Figures 1 and 2.

In all cases, the concentration factors decreased with increasing heavy metal content of the substrate except in lead-exposed individuals of *D. reticulatum*. In this case, the concentration factor increased with increasing lead concentration in the food/substrate up to 0.31. For details see Figure 3.

All the applied heavy metal concentrations were sublethal (< LC_{30}) for the test species within 21 days with the exception of Zn^{2+} at the highest applied concentration, which exceeded the LC_{30} in two species. In *D. reticulatum*, 4250 mg zinc kg^{-1} dry weight in the food caused copious secretion of mucus in the first days of exposure and the mortality was 36% after just 9 days. In *J. scandinavius*, mortality was 76% after 21 days exposure to 4250 mg zinc kg^{-1} dry weight.

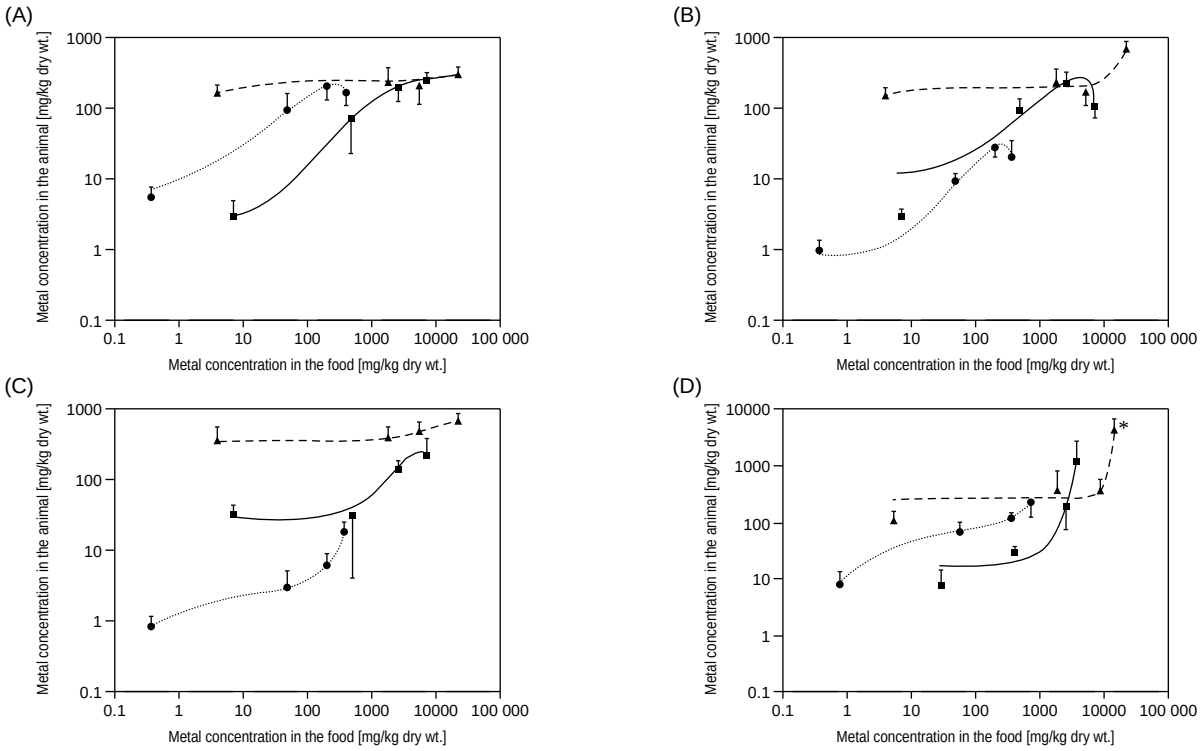

Figure 1. Comparison of the concentrations of Cd (circles), Pb (squares), and Zn (triangles) in the bodies of *P. scaber* (A), *J. scandinavius* (B), *T. bielanensis* (C), and *D. reticulatum* (D) after three weeks of exposure. * data obtained after 9 days of exposure.

Discussion

Most terrestrial invertebrates take up mineral substances from the food by absorption via their intestinal epithelia, showing a concentration gradient between food pulp and resorptive tissue (Wieser 1967). In this context, the impact of toxicants depends on the bioavailability of the respective substances for the organism, which is determined by characteristics of the environment, the toxicant, the organism itself (Crommentuijn *et al*. 1994), and the specific composition of the intestinal microflora (Hopkin & Martin 1984). Furthermore, the degree of resorption of these substances depends, of course, predominantly on the concentration in the diet (Dallinger 1993). In most soil invertebrates, heavy metals are selectively concentrated in only one or a few organs, or in specific parts of a tissue and, typically, these organs are part of the digestive apparatus (Dallinger 1993). For instance, in slugs (Coughtrey & Martin 1976, Williamson 1980, Ireland 1981, 1982, 1984, Dallinger & Wieser 1984, Berger & Dallinger 1989), millipedes (Köhler & Alberti 1992), isopods (Hopkin & Martin 1982a,b, Dallinger & Prosi 1988), and collembola (Pawert *et al*. 1996) the midgut epithelium and the midgut gland (providing one is present) are the main targets of heavy metals.

It is well known that the uptake of trace metals from the food pulp by terrestrial invertebrates is not under the control of the animal (Hopkin 1993). However, some terrestrial invertebrates are able to discriminate their diet and regulate the rate of consumption to avoid the uptake of heavy metal contaminated food, as shown for springtails (Joosse & Verhoef 1983), millipedes (Hopkin *et al*. 1985, Read & Martin 1990), woodlice (Ullrich *et al*. 1993, van Straalen 1995), and also gastropods (Russell *et al*. 1981). Much more important for poisoning of the animals by heavy metals is the possibility of detoxification of unwanted elements and, therefore, the capacity to inactivate, to store, and/or to excrete them. In invertebrates, there are two main mechanisms of detoxification: (1) the activation of metal binding proteins such as metallothioneins (Hunziker and Kägi 1985, Kägi 1987, Dallinger *et al*. 1989a, Janssen and Dallinger 1991); and (2) the compartmentalization of metals as intracellular granules of different types (Simkiss 1976, Taylor & Simkiss 1984, Hopkin 1989, Dallinger 1993). The latter mechanism has been described for woodlice (Hopkin & Martin

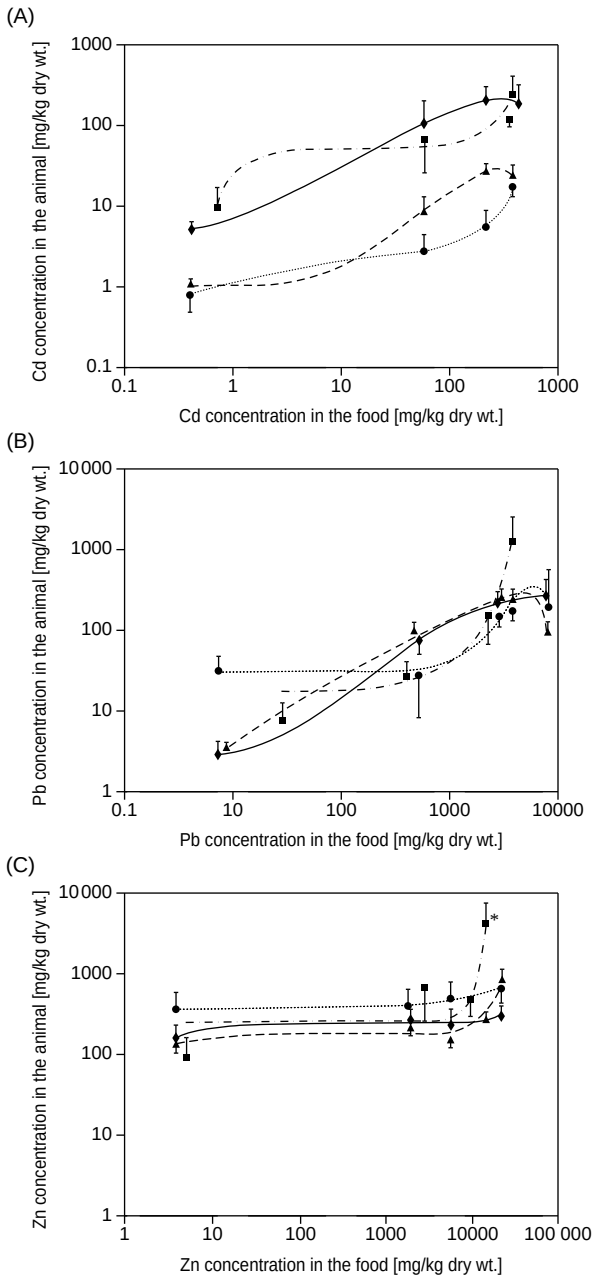

Figure 2. Accumulation of Cd (A), Pb (B), and Zn (C). Comparison of *P. scaber* (diamonds), *T. bielanensis* (circles), *J. scandinavius* (triangles) and *D. reticulatum* (squares). * data obtained after 9 days of exposure.

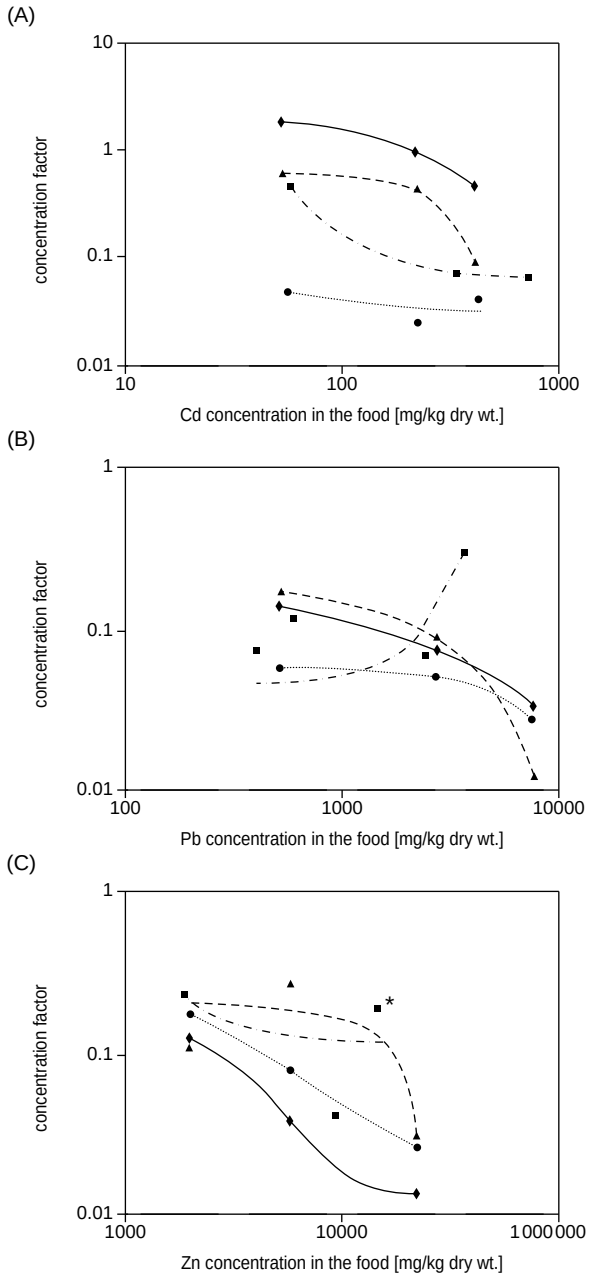

Figure 3. Concentration factors of Cd (A), Pb (B), and Zn (C). Comparison of *P. scaber* (diamonds), *T. bielanensis* (circles), *J. scandinavius* (triangles) and *D. reticulatum* (squares). * data obtained after 9 days of exposure.

1982b, Dallinger & Prosi 1988, Prosi & Dallinger 1988), diplopods (Hubert 1979, Köhler *et al*. 1995), slugs (Howard & Simkiss 1981) and springtails (Pawert *et al*. 1996). After storage in digestive cells, the metals can be excreted from the body either by direct release of the vesicle contents into the lumen of the gut and subsequent discharge with the faeces (Dallinger 1993), and/or by replacement of the whole midgut epithelium (Hubert 1979) during moulting. In the present study, it seems that these mechanisms were important for determining the accumulation behaviour of the investigated invertebrates.

Generally, the concentrations of the applied heavy

metals in the bodies of the investigated invertebrates were found to increase with the concentration of the respective elements in the diet, probably caused by a higher rate of diffusion. The accumulation pattern, however, differs among the test species and, therefore, justifies the division into two different groups. The first group, to which belong the isopod *Porcellio scaber* and the diplopod *Julus scandinavius*, seems not to exceed a constant saturation level independent of even the highest metal concentration in the food. In the second group (the collembolan *Tetrodontophora bielanensis* and the slug *Deroceras reticulatum*), the highest heavy metal accumulation corresponded to the highest metal concentration of the respective metal in the food. Vice versa, the concentration factors usually decreased with increasing metal concentrations (with the exception of lead for *D. reticulatum*) and led to the suggestion that this effect might be a consequence of an accelerated saturation of the uptake mechanisms in animals subjected to extremely high metal burdens. The difference between the two groups might be explained by a reduction in food uptake by isopods and diplopods. Although the production of faeces pellets proved feeding activity in all experiments, food consumption rates may be diminished in these animals; by contrast, the collembolans showed extreme tolerance to the metal-containing food (no mortality in all experiments), which might not cause any need to reduce food uptake. Furthermore, gastropods are known to absorb metals via the skin (Cavallero & Ravera 1966, Ryder & Bowen 1977, Ireland 1982) and, therefore, are not able to prevent further metal impact by terminating feeding, which may explain the continuous increase in the body's metal concentration even under extreme conditions where mortality was high.

In the present study, woodlice showed the highest cadmium concentration of all investigated species. Cadmium may be taken up by the same pathway as copper (Hopkin 1989) and the assimilation mechanism of copper as an essential metal for isopods (Bonaventura and Bonaventura 1980) must be very efficient, since only low concentrations of this metal litter can normally be found in natural leaf litter (Hopkin & Martin 1982a). According to Hopkin (1989), the ability of isopods to accumulate cadmium at a rather high rate might be explained by two different cadmium pools in the hepatopancreas, one of which is immobilized by storage in intracellular granules, which cannot be excreted. A second, more mobile protein-bound cadmium pool can be excreted at low rates (Dallinger 1993). These cadmium-binding components of low molecular weight have not been identified as metallothioneins, but one of them was found to probably be a glycoprotein (Dallinger 1993). In terrestrial gastropods, however, the involvement of metallothioneins in metal detoxification has been shown by Dallinger *et al*. (1989b). The capacity of these animals to accumulate cadmium is due mainly to these metal binding proteins (Dallinger *et al*. 1989a) and ranks, as shown by the present study, second to woodlice. In comparison to these two taxa, the cadmium accumulation of diplopods and colembolans was found to be lower. Contrary to isopods, whose hepatopancreas cannot be moulted, in millipedes (Hubert 1979) and springtails (Joosse & Buker 1979, van Straalen *et al*. 1987, Kronshage 1991) the exfoliation of the midgut epithelium is an important mechanism for excretion of the large amounts of metals deposited here (Seifert 1979, Joosse 1981). This mechanism is predominantly relevant for long-term exposure of these animals, since the specimens investigated in the present study did not moult during exposure time. Thus, the comparatively low accumulation of cadmium in millipedes and collembola leads to the suggestion that additional mechanisms for exclusion of cadmium from deposition in tissues must be present in these animals. This mechanism must not necessarily be of a biochemical nature but could also be based on behavioural aspects. As mentioned above, as a general response to metal enriched food, the decreasing cadmium content in the woodlice and millipedes after exposure to the highest metal concentration might be a consequence of decreasing food consumption rates to avoid further uptake of the toxin.

Compared to cadmium and lead, high amounts of zinc have also been found in uncontaminated control animals of all investigated species, which is most probably caused by the fact that zinc is essential for the function of a variety of enzymes. In parallel, concentration factors for zinc are usually rather low, and in the present study the lowest concentration factors of all investigated metals have been calculated for zinc in *P. scaber* and *J. scandinavius*. These results correspond to the observations of Hopkin (1989), who found much lower concentration factors for zinc than for cadmium in isopods of the same site. This may be due to the fact that zinc is usually present in higher concentrations in uncontaminated leaf litter (Hopkin & Martin 1982a) than, for example, cadmium, thus allowing a sufficient zinc supply even in the presence of less efficient uptake mechanisms (Hopkin 1989). In this study, the highest concentration factors for zinc were found in the species *T. bielanensis* and *D. reticulatum*, although

in gastropods excretion rates for zinc of up to 56% via faecal material have been described (Williamson 1980). Obviously, especially the slugs' capability of detoxifying high zinc levels was overcharged in the group exposed to the highest zinc concentrations. In this context, uptake not only via the food but also via the whole surface of the animal must be considered, leading also to the increased mortality rate. A similar overcharging can also be postulated for the mortality rate of the diplopods at the highest zinc exposure concentrations. Diplopods mainly store zinc in a subcuticular cell layer (Hopkin 1989) and manage to excrete about one third of the zinc taken up by the food (Köhler *et al*. 1995). Studies have shown that extreme zinc burdens led to pathological cell damage in the intestinal tract in the investigated species (e.g. Berkus *et al*. 1994, Köhler *et al*. 1996, Triebskorn & Köhler 1996), which, however, was most moderate in the comparatively tolerant collembolan species *T. bielanesis* (Pawert *et al*. 1996).

Regarding lead, the factors enhancing or limiting accumulation may in principle be similar to those already mentioned for the other tested metals. Thus, the lower body content of diplopods after exposure to the highest lead concentrations was probably caused by a lower rate of food consumption. In the gastropods, the highest concentration factor also corresponds to the highest lead concentration in the food as a consequence of metal absorption through the skin, which cannot be shut off by the animal. Although a specific lead carrier has not yet been found in these animals and only a few percent of the ingested lead is usually assimilated (Hopkin 1989), considerable amounts of this metal were accumulated in all investigated animals during three weeks of exposure, the degree of which, however, depended on the species. Thus, passive uptake routes, probably after microbial metabolization in the intestinal tract, must be considered for lead as well as, probably, for all the metals investigated in the present study.

As shown by the present comparisons, accumulation of metals in soil animals depends: (1) on the type of the metal; and (2) on the respective species influenced by the metal. Soil quality assessment, therefore, requires at least consideration of the different accumulation patterns shown above. However, in order to bridge the gap between presence and toxic impact of metals in the environment, it should additionally be based on monitoring studies of ultrastructural effects.

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References

Berger B, Dallinger R. 1989 Accumulation of copper and cadmium by terrestrial snail *Arianta arbustorum* L.: kinetics and budgets. *Oecologia* **79,** 60–65.

Berkus M, Gräff S, Alberti G, Köhler H-R. 1994 The impact of heavy metals, lead, zinc, and cadmium on the ultrastructure of midgut cells of *Julus scandinavius* (Diplopoda). *Verh dtsch Zool Ges* **87,** 321.

Beyer WN, Pattee OH, Sileo L, Hoffman DJ, Hulhern BM. 1985 Metal contamination in wildlife living near two zinc smelters. *Environ Pollut* **38A,** 63–86.

Bonaventura J, Bonaventura C. 1980 Hemocyanins: relationship in their structure, function and assembly. *Am Zool* **20,** 7–17.

Cavallero R, Ravera O. 1966 Biological indicators of manganese-54 contamination in terrestrial environments. *Nature* **209,** 1259.

Coughtrey PJ, Martin MH. 1976 The distribution of Pb, Zn, Cd and Cu within the pulmonate mollusc *Helix aspersa* Müller. *Oecologia* **23,** 315–322.

Crommentuijn T, Doodeman CJAM, Doornekamp A, *et al*. 1994 Lethal body concentrations and accumulation patterns determine time-dependent toxicity of cadmium in soil arthropods. *Environ Toxicol Chem* **13,** 1781–1789.

Dallinger R. 1993 Strategies of metal detoxification in terrestrial invertebrates. In: Dallinger R, Rainbow PS, eds. *Ecotoxicology of Metals in Invertebrates*. Boca Raton: Lewis Publishers; 246–289.

Dallinger R, Prosi F. 1988 Heavy metals in the terrestrial isopod *Porcellio scaber* Latreille. II. Subcellular fractionation of metal-accumulating lysosomes from hepatopancreas. *Cell Biol Toxicol* **4,** 97.

Dallinger R, Wieser W. 1984 Molecular fractionation of zinc, copper, cadmium and lead in the midgut gland of *Helix pomotioa* L. *Comp Biochem Physiol* **79**, 125–129.

Dallinger R, Berger B, Bauer-Hilty A. 1989a Purification of cadmium-binding proteins from related species of terrestrial Helicidae (Gastropoda, Mollusca): A comparative study. *Mol Cell Biol* **85,** 135–145.

Dallinger R, Janssen HH, Bauer-Hilty A, Berger B. 1989b Characterization of an inducible cadmium-binding protein from hepatopancreas of metal-exposed slugs *(Arionidae, Mollusca). Comp Biochem Physiol* **92C,** 355–360.

Dallinger R, Berger B, Birkel S. 1992 Terrestrial isopods. Useful biological indicators of urban metal pollution. *Oecologia* **89,** 32–41.

Hopkin SP. 1989 *Ecophysiology of Metals in Terrestrial Invertebrates*. London: Elsevier Applied Sciences; 1–366.

Hopkin SP. 1993 Deficiency and excess of copper in terrestrial isopods. In: Dallinger R, Rainbow PS, eds. *Ecotoxicology of Metals in Invertebrates*. Boca Raton: Lewis Publishers.

Hopkin SP, Martin MH. 1982a The distribution of zinc, cadmium, lead and copper within the woodlouse *Oniscus asellus* (Crustacea, Isopoda). *Oecologia* **54,** 227–232.

Hopkin SP, Martin MH. 1982b The distribution of zinc, cadmium, lead and copper within the hepatopancreas of a woodlouse. *Tiss Cell* **14,** 703–715.

Hopkin SP, Martin MH. 1984 Heavy metals in woodlice. *Symp Zool Soc Lond* **53,** 143–166.

Hopkin SP, Watson K, Martin MH, Mould ML. 1985 The assimilation of heavy metals by *Lithobius variegatus* and *Glomeris marginata* (Chilopoda, Diplopoda). *Bijdr Dierk* **55,** 88–94.

Hopkin SP, Hames CAC, Bragg S. 1989 Terrestrial isopods as biological indicators of zinc pollution in the Reading area, South East England. *Monit Zool Ital* **4,** 477–488.

Howard B, Simkiss K. 1981 Metal binding by *Helix aspersa* blood. *Comp Biochem Physiol* **70A,** 559–561.

Hubert M. 1979 Localization and identification of mineral elements and nitrogenous waste in diplopoda. In: Camatini M, ed. *Myriapod Biology*. London: Academic Press; 127–133.

Hunziker PE, Kägi JHR. 1985 Metallothionein. In: Harrison PM, ed. *Metalloproteins Part 2, Metal Proteins with Non-redox Roles*. Basingstoke: Macmillan Press; 149–181.

Ireland MP. 1981 Uptake and distribution of cadmium in the terrestrial slug *Arion ater* (L.). *Comp Biochem Physiol* **68A,** 37–41.

Ireland MP. 1982 Sites of water, zinc and calcium uptake and distribution of these metals after cadmium administration in *Arion ater* (Gastropoda: Pulmonata). *Comp Biochem Physiol* **73A,** 217–221.

Ireland MP. 1984 Effect of chronic and acute lead treatment in the slug *Arion ater* on calcium and delta-aminolaevulinic acid dehydratase activity. *Comp Biochem Physiol* **79C**, 287–290.

Janssen HH, Dallinger R. 1991 Diversification of cadmium-binding proteins due to different levels of contamination in *Arion lusitanicus*. *Arch Environ Contamin Toxicol* **20,** 132–137.

Joosse ENG. 1981 Ecological strategies and population regulation of Collembola in heterogenous environments. *Pedobiologia* **21,** 346–356.

Joosse ENG, Buker JB. 1979 Uptake and excretion of lead by litter-dwelling Collembola. *Environ Pollut* **18,** 235–240.

Joosse ENG, Verhoef SC. 1983 Lead tolerance in collembola. *Pedobiologia* **25**(1), 11–18.

Kägi JHR. 1987 *Metallothioneins II*. Basel: Birkhäuser.

Köhler H-R, Alberti G. 1992 The effect of heavy metal stress on the intestine of diplopods. In: Meyer E, Thaler K, Schedl W, eds. *Advances in Myriapodology*. Innsbruck: Ber nat-med Ver. Suppl. 10; 257–267.

Köhler H-R, Storch V, Alberti G. 1992 The impact of lead on the assimilation efficiency of laboratory-held Diplopoda (Arthropoda) preconditioned in different environmental situations. *Oecologia* **90,** 113–119.

Köhler H-R, Körtje K-H, Alberti G. 1995 Content, absorption quantities and intracellular storage sites of heavy metals in Diplopoda (Arthropoda). *BioMetals* **8,** 37–46.

Köhler H-R, Hüttenrauch K, Berkus M, Gräff S, Alberti G. 1996 Cellular hepatopancreatic reactions in *Porcellio scaber* (Isopoda) as biomarkers for the evaluation of heavy metal toxicity in soils. *Appl Soil Ecol* **3,** 1–15.

Kronshage J. 1991 Experiments on the influence of lead compounds and acids on collembola. *Zool Beiträge* **34,** 289–311.

Morgan AJ, Morris B. 1982 The accumulation and intracellular compartmentation of cadmium, lead, zinc and calcium in two earthworm species (*Dendrobaena rubida* and *Lumbricus rubellus*) living in highly contaminated soil. *Histochemistry* **75,** 589–598.

Müller G, Haamann L, Kubat R, Noe K. 1987 Schwermetalle und Nährstoffe in den Böden des Rhein-Neckar-Raums: Ergebnisse flächendeckender Untersuchungen. *Heidelberger Geowissenschaftliche Abhandlungen* **13,** 1–346.

Pawert M, Triebskorn R, Gräff S, *et al*. 1996 Cellular alterations in collembolan midgut cells as a marker of metal exposure: ultrastructure and intracellular metal distribution. *Sci Tot Environ* **181**, 187–200.

Prosi F, Dallinger R. 1988 Heavy metals in the terrestrial isopod *Porcellio scaber* Latreille. I. Histochemical and ultrastructural characterization of metal containing lysosomes. *Cell Biol Toxicol* **4,** 81.

Prosi F, Storch, V, Janssen HH. 1983 Small cells in the midgut glands of terrestrial isopoda: Sites of heavy metal accumulation. *Zoomorphology* **102,** 53–64.

Read HJ, Martin MH. 1990 A study of millipede communities in woodlands contaminated with heavy metals. In: Minelli A, Brill EJ, eds. *Proceedings of the 7th International Congress of Myriapodology*. Leiden; 289–298.

Russell LK, De Haven JI, Botts RP. 1981 Toxic effects on the garden snail (*Helix aspersa*). *Bull Environ Contam Toxicol* **26,** 634–640.

Ryder TA, Bowen ID. 1977 The slug foot as site of uptake of copper mulluscicide. *J Invert Pathol* **30,** 381–386.

Seifert G. 1979 Considerations about the evolution of excretory organs in terrestrial arthropods. In: Camatini M, ed. *Myriapod Biology*. London: Academic Press; 353–372.

Simkiss K. 1976 Intracellular and extracellular routes in bio-mineralization. *Symp Soc Exo Biol* **30,** 423–444.

Taylor MG, Simkiss K. 1984 Inorganic deposits in invertebrate tissues. *Environ Chem* **3,** 102–138.

Triebskorn R. Köhler H-R. 1996 The impact of heavy metals on the grey garden slug, *Deroceras reticulatum* (Müller); metal storage, cellular effects and semi-quantitative evaluation of metal toxicity. *Environ Pollut*, in press.

Ullrich B, Storch V, Reiß S, *et al*. 1993 Die Auswirkungen von Umweltbelastungen auf die Energetik von Landasseln. *Verh Ges Ökol* 22, 443–450.

van Straalen NM. 1995 The use of critical body concentrations to evaluate and compare the risk of persistent pollutants in ecosystems. *SETAC–Europe 6th Annual Meeting*, Plymouth, September 5–6, 1995.

van Straalen NM, Burghouts TBA, Doornhof MJ, *et al*. 1987 Efficiency of lead and cadmium excretion in populations of *Orchesella cincta* collembola from various contaminated forest soils. *J Appl Ecol* **24**(3), 953–968.

Weigmann G, Gruttke H, Kratz W, Papenhausen U, Richarski G. 1985 Zur Wirkung von Umweltchemikalien auf den Abbau von *Solidago gigantea* Streu. *Verh Ges Ökol* **13,** 631–636.

Wieser W. 1967 Conquering terra firma: the copper problem from the isopods' point of view. *Helgol Wiss Meeresunters* **15,** 282.

Wieser W, Busch G, Büchel L. 1976 Isopods as indicators of the copper content of soil and litter. *Oecologia* **23**, 107–114.

Williamson P. 1980 Variables affecting body burdens of lead, zinc and cadmium in a roadside population of the snail *Cepaea hortensis* Müller. *Oecologia* **44,** 213–220.