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# The Toxicity of Zinc to Terrestrial Isopods in a "Standard" Laboratory Test

D. DROBNE* AND S. P. HOPKIN†

*Department of Biology, University of Ljubljana, Vecna pot 111, 61000 Ljubljana, Slovenija; and †Department of Pure & Applied Zoology, University of Reading, P.O. Box 228, Reading RG6 2AJ, United Kingdom

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**A method is described for assessing the effects of metals on the food consumption rate of isopods from measurements of fecal production. The effects of zinc in the diets of two isopod species, *Porcellio scaber* and *Oniscus asellus*, were tested. The metal was fed to the isopods on leaves of field maple (*Acer campestre*) contaminated with concentrations ranging between 1000 and 10,000 $\mu$g Zn g$^{-1}$ leaf dry wt. Significant reductions in feeding rates were observed of the highest concentrations of zinc. The test employed in this study is quick, cheap, and relevant for estimating sublethal effects of metals on isopods.** © 1995 Academic Press, Inc.

## INTRODUCTION

Zinc accumulates in leaf litter and soils in terrestrial ecosystems. When the concentration exceeds "critical" levels it becomes toxic to soil animals (Bengtsson and Tranvik, 1989; Hopkin, 1990; Van Wensem *et al.*, 1992). The total body concentration of this essential metal in isopods depends on rates of consumption, assimilation, and excretion. High levels of zinc in isopods are known to depress reproduction, respiration, and food consumption (Van Capelleveen, 1985; Van Straalen and Van Wensem, 1986; Donker, 1992; Donker *et al.*, 1993; Hopkin and Hames, in press).

In isopods, zinc poisoning may be evaded by an avoidance response, by regulation of the consumption rate (Joosse *et al.*, 1981), by storing metals in the hepatopancreas in an insoluble form (Hopkin, 1989), and/or by fecal and possibly also by urinary excretion (Donker, 1992). Moulting does not change the total zinc concentration of *Porcellio scaber* (Hames and Hopkin, 1991). Hopkin (1990) found that zinc poisoning occurs almost certainly when the storage capacity of the hepatopancreas is exceeded. In this circumstance, zinc is no longer prevented from passing through the organ to other body tissues.

Literature data reveal that there are considerable differences in the concentration of metals in the hepatopancreas between species from the same microhabitat (Hopkin *et al.*, 1985; Morgan *et al.*, 1986; Hopkin *et al.*, 1989). These differences are supposed to be due to ecological and/or physiological factors controlling metal pollutant assimilation, storage, and excretion (Hopkin, 1990; Donker, 1992).

In experiments on survival of *P. scaber*, cadmium and copper were approximately 10 times more toxic than zinc and about 20 times more toxic than lead in the food (Hopkin and Hames, in press). However, in the vicinity of a smelting works at Avonmouth, UK, zinc has a greater relative adverse effect on isopods in the industrially polluted area because it is present in toxic concentrations in leaf litter relative to cadmium, copper, and lead. In leaf litter, the Zn/Cd ratio for example is ca. 100 in all sites. The effects of zinc on terrestrial ecosystems have probably been underestimated (Hopkin, 1993).

The aim of this study was to test sublethal zinc toxicity on two species of terrestrial isopods using the test proposed by Drobne and Hopkin (1994). Fecal production rate as a measure of food consumption, as well as food assimilation efficiency of contaminated food, was followed. The distribution of the assimilated zinc over the tissues of the examined species was investigated also.

## MATERIALS AND METHODS

Specimens of *P. scaber* and *Oniscus asellus* and a large sample of leaves of field maple (*Acer campestre*) were collected from the litter layer of uncontaminated woodlands in the Reading area in February 1993. For the experiment, males and nongravid females were selected at random and were held individually in plastic petri dishes (diameter 9 cm) at 20°C under a 16-hr light, 8-hr dark period.

The leaves were air-dried at room temperature for 48 hr and weighed individually. Solutions of zinc nitrate were applied topically to the leaves as small droplets and allowed to dry overnight at room temperature. The amount of solution applied to each leaf was adjusted to give nominal concentrations of zinc of 1000, 2000, 5000, and 10,000 $\mu$g g$^{-1}$ dry wt. Distilled water was applied to a further batch of leaves which acted as controls.

The leaves were rehydrated at 100% relative humidity



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for 48 hr and then placed individually into petri dishes. A single specimen of either *P. scaber* or *O. asellus* of ca. 40 mg fresh wt was placed with each leaf. There were 12 replicates of each zinc concentration and 12 controls for each species giving a total of 120 petri dishes. Humidity in the petri dishes was maintained by regularly spraying the internal side of the lids with distilled water. The petri dishes were stacked in large covered plastic tanks which maintained the relative humidity at 100%.

The isopods ate about 90% of the leaves in 5 weeks. At the end of this period, 6 of the 12 isopods from each treatment were dissected into three tissue fractions (hepatopancreas, gut and "rest") using the technique of Hopkin (1990). The remaining 6 isopods from each treatment were transferred to untreated leaves for 2 weeks and then dissected in the same manner.

Every day of the experiment, the fecal pellets voided during the previous 24 hr were counted and removed from each petri dish. In the group of animals transferred to clean leaves the fecal pellets were collected in separate dishes and analyzed subsequently for zinc. At the end of the experiment, the fecal pellets and leaf remains from

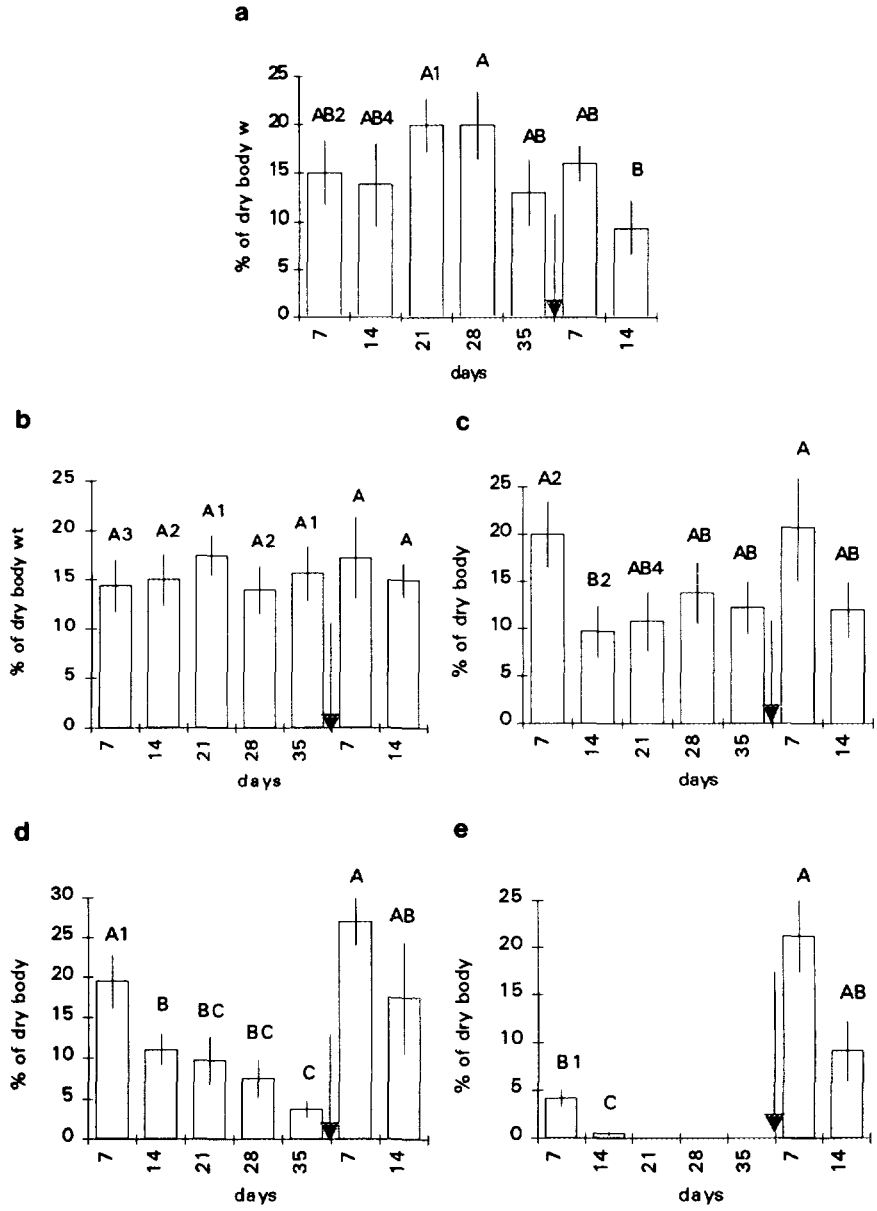


FIG. 1. *Porcellio scaber*. Weekly fecal production rates of isopods (expressed as percentage dry body wt) feeding on (a) uncontaminated leaves and leaves of field maple contaminated with (b) 1000, (c) 2000, (d) 5000, and (e) 10,000 $\mu$g Zn $g^{-1}$ dry wt for 5 weeks, followed by 2 weeks (arrow) on uncontaminated leaves. Means and standard error bars ($N$ = 12 until Day 28, $N$ = 6 thereafter) are not significantly different at the 5% level ($t$ test) if they have the same letter (A, B). Numbers after these letters indicate the number of animals that were moulting during the 7-day period.

each petri dish were weighed. There is very little variation in weights of fecal pellets in individual isopods (<5%). Thus, daily fecal production of each isopod can be determined (without having to weigh every sample) from the formula $W/T \times D$ (where $W$ is total weight of fecal pellets produced by an individual during the experiment, $T$ is total number of fecal pellets, and $D$ is number of fecal pellets produced during the previous 24 hr). Assimilation efficiency (AE) was calculated for each individual as $AE = (C - F)/C$, where $C$ is consumption and $F$ is defecation in milligram dry weight. Consumption rate was not measured directly (earlier experiments have demonstrated that food consumption is directly proportional to fecal production rates). Thus, the latter are the values given in Figs. 1 and 2. Fecal production rate is presented as a percentage of dry body weight to allow for individual weight differences and is given on a weekly basis.

Each sample of woodlice tissue was digested in 2 ml of boiling concentrated Analar-grade nitric acid (BDH

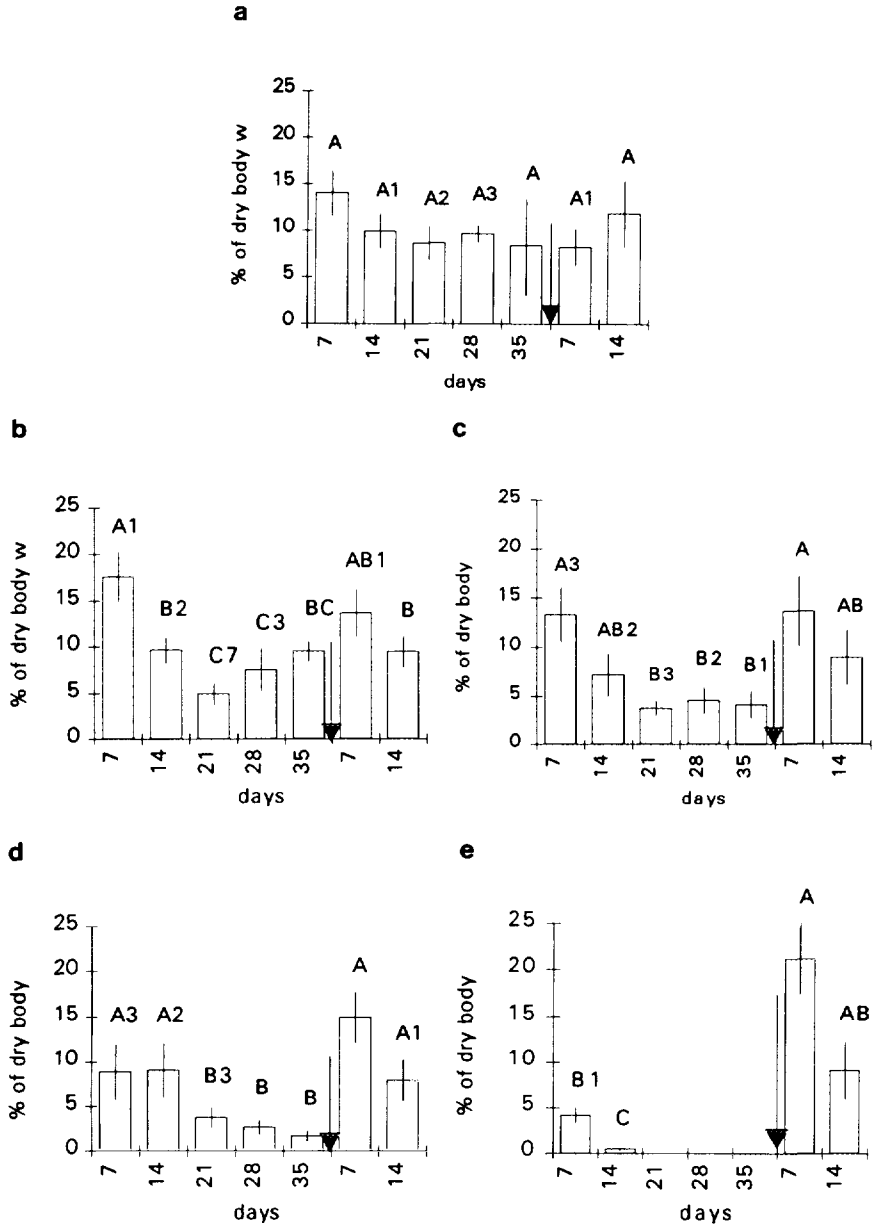


FIG. 2. *Oniscus asellus*. Weekly fecal production rates of isopods (expressed as percentage dry body wt) feeding on (a) uncontaminated leaves and leaves of field maple contaminated with (b) 1000, (c) 2000, (d) 5000, and (e) 10,000 $\mu$g Zn $g^{-1}$ dry wt for 5 weeks, followed by 2 weeks (arrow) on uncontaminated leaves. Means and standard error bars ($N$ = 12 until Day 28, $N$ = 6 thereafter) are not significantly different at the 5% level ($t$ test) if they have the same letter (A, B). Numbers after these letters indicate the number of animals that were moulting during the 7-day period.

TABLE 1

**Mean Concentrations of Zinc in the Hepatopancreas (Hep), Gut, and Rest Body Fractions of *Porcellio scaber* and *Oniscus asellus* Exposed to Four Different Zinc Concentrations for 5 Weeks**

| Treatment μg Zn g-1 dry wt | Hep | Gut | Rest | Total |
|---|---|---|---|---|
| | | *P. scaber* | | |
| C ± SE | 4,190 ± 295 | 215 ± 25 | 55 ± 5 | 260 ± 15 |
| 1,000 ± SE | 5,910 ± 735 | 410 ± 110 | 65 ± 5 | 385 ± 45 |
| 2,000 ± SE | 8,960 ± 1,690 | 540 ± 180 | 75 ± 5 | 495 ± 55 |
| 5,000 ± SE | 8,160 ± 1,795 | 620 ± 250 | 95 ± 10 | 540 ± 110 |
| 10,000 ± SE | 4,945 ± 1,245 | 250 ± 80 | 150 ± 20 | 435 ± 55 |
| | | *O. asellus* | | |
| C ± SE | 620 ± 125 | 115 ± 10 | 60 ± 2 | 95 ± 10 |
| 1,000 ± SE | 2,085 ± 595 | 440 ± 125 | 55 ± 2 | 160 ± 25 |
| 2,000 ± SE | 2,985 ± 710 | 440 ± 155 | 60 ± 5 | 180 ± 20 |
| 5,000 ± SE | 3,255 ± 550 | 850 ± 290 | 60 ± 5 | 230 ± 35 |
| 10,000 ± SE | 2,420 ± 410 | 950 ± 380 | 115 ± 10 | 270 ± 35 |

*Note.* $N = 12$; SE, standard error.

Chemicals) and diluted to 10 ml with double-distilled water. The leaves were digested in 20 ml of boiling Analar nitric acid and diluted to 100 ml. The digests were analyzed for zinc by flame atomic absorption spectrometry (Varian Spectra 30). Concentrations of zinc in control leaves were approximately 70 $\mu g\ g^{-1}$. The levels of zinc in contaminated leaves were within 5% of the expected values.

## RESULTS

The highest zinc concentrations in food decreased consumption rates as indicated by decreased fecal production (Fig. 1 and Fig. 2). High zinc concentrations in food had a greater effect in *O. asellus* than in *P. scaber*. Fecal production was reduced in both species exposed to 2000 mg Zn $g^{-1}$ in the food (Fig. 1d and Fig. 2d). This is in agreement with results obtained by Donker (1992) where 54 mmol Zn $g^{-1}$ (3500 mg Zn $g^{-1}$) affected the consumption rate of *P. scaber*. Most individuals of *P. scaber* exposed to 10,000 mg Zn $g^{-1}$ stopped eating after 4 days of exposure. However, most of the *O. asellus* stopped eating between the 7th and 14th day of exposure. After transfer to clean leaves, the feeding activity was restored in all treatments. High zinc concentrations in the food affected the moulting cycle also. Moulting of *P. scaber* occurs at intervals of about 28 days (Steel, 1980). In groups of *P. scaber* exposed to 200, 500, (unpublished results), 1000, and 2000 mg Zn $g^{-1}$ dry wt of food, almost all the individuals moulted within 7 weeks. In groups exposed to 5000 and 10,000 mg Zn $g^{-1}$, only one animal moulted. In *O. asellus* moulting was not affected by high zinc concentration in the food except in the group exposed to 10,000 mg Zn $g^{-1}$.

Interspecific differences in the reduction of food consumption, complete absence of feeding, and the alteration of the moulting cycle appeared mostly at higher zinc concentrations in the food.

Although the two species were brought from the same micro environment and reared on identical diets in the laboratory, they exhibited some differences in zinc concentrations in their tissues (Table 1). The ratio between mean zinc concentration in the hepatopancreas of *P. scaber* and *O. asellus* and zinc in the control (the least contaminated food) was about 7, but in the food contaminated with zinc this ratio was lower on treatments with the highest zinc concentrations. The amount of zinc accumulated in the hepatopancreas during the exposure was higher in *O. asellus* than in *P. scaber*. The same trend for the whole body fraction of animals exposed to different zinc concentrations was observed by Hopkin

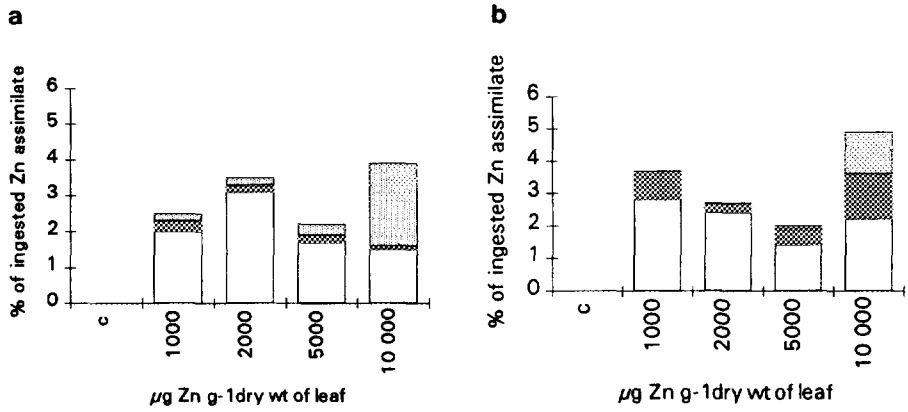


FIG. 3. Mean percentage of total amounts of zinc ingested with the food (100%) that were present in the three tissue fractions of (a) *Porcellio scaber* and (b) *Oniscus asellus* after the first 5 weeks of the experiment (dot shading = "rest," line shading = gut, and unshaded = hepatopancreas). $N = 12$ for each treatment. Note that most of the zinc is stored in the hepatopancreas and that the overall assimilation rate is low (always <6%).

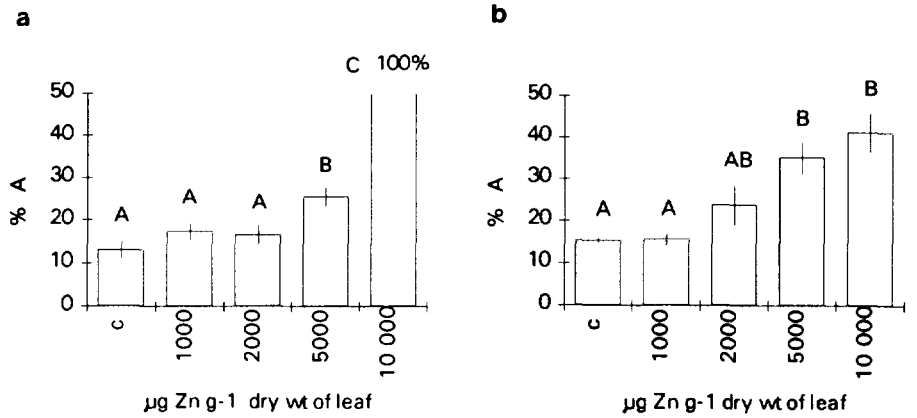


FIG. 4. Mean food assimilation efficiencies (AE) in (a) *Porcellio scaber* and (b) *Oniscus asellus* feeding for 5 weeks on leaves of field maple contaminated with zinc. Means and standard error bars are not significantly different at the 5% level (*t* test) if they have the same letter (A, B). $N = 12$ for each treatment.

(1989) and exactly the same ratio in control animals was reported by Hopkin (1990). Most of the zinc assimilated during 5 weeks of the exposure was stored in the hepatopancreas (Fig. 3). The percentage of assimilated zinc retained in this organ ranged from 2.8 to 4.7% in *O. asellus* and from 2.7 to 5.3% in *P. scaber*.

The lowest food assimilation rate was found in both species at the lowest zinc concentration in the food (control). Food assimilation efficiency in *P. scaber* increased significantly at 5000 mg Zn $g^{-1}$. At 10,000 mg Zn $g^{-1}$ the animals stopped eating (Fig. 1d) and food assimilation rate was at the maximum (Fig. 4a). However, this is probably an effect of retention of the food for a long period in the gut. In *O. assellus*, assimilation rate was affected at lower concentrations of zinc in the food (Fig. 4b) but was not as different as in *P. scaber*. In all groups of animals expect in those exposed to 10,000 mg Zn $g^{-1}$ the food assimilation rate was between 15 and 35%. This is in agreement with other similar studies (Van Capelleveen, 1987; Van Straalen and Verweij, 1991).

## DISCUSSION

The relationship between the dose of the toxicant and the response of the organism is perhaps the central relationship in ecotoxicology.

A decreased food consumption at higher concentrations of metal in the food indicates an avoidance response (Joosse, 1981). In this test, isopods responded by lowering food consumption when exposed to 2000 mg Zn $g^{-1}$ and very evidently at 5000 mg Zn $G^{-1}$ in the food. The same response was found also in a toxicity test with cobalt (Drobne and Hopkin, 1994). *O. asellus* proved to be more affected by higher cobalt concentrations as measured by reduction in food consumption and metal accumulation. In the zinc toxicity test described in this paper, *P. scaber* proved to be more sensitive to higher zinc concentration in the food. *O. asellus* accumulated less coablt in the hepatopancreas than *P. scaber*, while it was just the opposite in the zinc toxicity test. The animals were collected in the same microhabitat and offered the same diet during the experiment. Hopkin *et al.* (1985, 1989) reported that *P. scaber* and *O. asellus* from the same collection sites have been found to contain different concentrations of zinc. The two species may have different mechanisms to cope with high levels of metals in the food (Hopkin, 1990; Hames and Hopkin, 1991).

The "critical concentration" of zinc in food in laboratory tests at which all juvenile isopods reared for 1 year die before producing offspring is 1000 mg Zn $g^{-1}$ (Hopkin and Hames, in press). This is at least five times lower than the concentration in leaf litter in the field at which populations of *P. scaber* persist (Hopkin and Hames, in press). The test employed in this study demonstrated the significant critical concentration to be between 2000 and 5000 mg Zn $g^{-1}$, much closer to the level in the field at which isopods die.

## CONCLUSIONS

Feeding rate reduction was a common response of isopods to higher metal concentration of the offered food. The laboratory test employed in this study is relevant for estimating sublethal effects of metals on isopods. Other test systems must be developed to gain more understanding of effects in the natural environment.

## ACKNOWLEDGMENT

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