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Zinc, among a 'cocktail' of metal pollutants, is responsible for the absence of the terrestrial isopod *Porcellio scaber* from the vicinity of a primary smelting works

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Porcellio scaber Latreille (Crustacea: Isopoda) of one month in age were reared for a year on leaf litter of field maple (*Acer campestre*) contaminated in the laboratory with a range of concentrations of cadmium, copper, lead or zinc. The metals were applied topically to the leaves as nitrates. Growth and survival, numbers of live offspring produced by females that matured, and concentrations of metals in adult isopods at the end of the experiment were measured.

'Critical concentrations' of metals in food at which all the isopods died before producing offspring were 100 μg Cd g^{-1}, 100 μg Cu g^{-1}, 2000 μg Pb g^{-1} and 1000 μg Zn g^{-1} (on a dry weight basis). The relative toxicities of the four metals in the laboratory were compared with concentrations of cadmium, copper, lead and zinc in surface leaf litter in the vicinity of a primary smelting works at Avonmouth, South West England. The results support the hypothesis that the absence of *Porcellio scaber* from sites in the immediate vicinity of the factory is due to zinc poisoning. Although cadmium is approximately ten times more toxic to isopods than zinc in the laboratory, zinc is most likely to be killing isopods in the field because its concentration is always at least 30 times higher than cadmium in Avonmouth leaf litter, and more than 100 times higher at most sites.

Populations of *Porcellio scaber* survive in field sites where surface leaf litter contains up to 5000 μg Zn g^{-1}. This is at least five times higher than the 'critical concentration' in laboratory experiments. Thus, the methodology for assessing metal toxicity described in this paper, exaggerates the potential effects of metals to isopods in the field. Such differences between laboratory and field toxicities of metals should be taken into account when environmental protection levels for metals are being proposed for soil invertebrates based on ecotoxicological tests conducted in the laboratory.

Keywords: cadmium; copper; isopods; lead; *Porcellio scaber*; zinc.

Introduction

Ecotoxicology is concerned with defining the potential or actual adverse effects of chemical agents on natural ecosystems (Calow 1989). For 'new' chemicals, there are now several laboratory- and field-based tests that must be carried out before a product is licensed. These tests are designed to predict effects on non-target organisms. However, the number of these tests is small and few have reached the stage of being routine.

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For soil ecosystems, the Organisation for Economic Cooperation and Development (OECD) standard test for earthworms is the most widely-used (Van Gestel and Ma 1990; Greig-Smith *et al.* 1992; Spurgeon *et al.* in press). Standard tests have the advantages of repeatability and ease of use. However, the major problem with such tests is extrapolating results to the field situation (Cairns 1992). For example, the species used in the earthworm test, *Eisenia fetida*, is not found in the wild except in manure or compost heaps. An assumption of the test is that 'safe' levels for *E. fetida* will be safe for other earthworm species. Furthermore, the earthworm test may not be appropriate for predicting the effects of a chemical on other soil and leaf litter organisms. While it would clearly be impractical to test every species of soil invertebrate, the current list of test organisms needs to be extended to include representatives of the major trophic groups.

Among leaf litter feeders, isopods would appear to be ideal candidates for standard test organisms (Van Wensem 1989; Donker and Bogert 1991; Eijsackers 1991; Van Wensem and Adema 1991; Van Wensem *et al.* 1991; Donker 1992; Van Wensem *et al* 1992). The most promising species is *Porcellio scaber* which is common in most countries, and is easy to maintain in the laboratory (Hopkin 1991, 1993a, Hopkin *et al.* in press).

In this paper, we report the results of an experiment that was designed to determine the effects of the metals cadmium, copper, lead and zinc on growth, survival and reproduction of *P. scaber*. Toxicity values derived from the experiment have been compared with actual and relative concentrations of the four metals in leaf litter contaminated by aerial deposition of cadmium, copper, lead and zinc from a primary smelting works at Avonmouth, South West England (for further details of this area see Harrison and Williams 1983, Hopkin 1989). The principal aim of the study was to ascertain which of the four metals was most likely to be responsible for the absence of *P. scaber* from the immediate vicinity of the factory where the species would normally be expected to occur.

Materials and methods

Leaves of field maple (*Acer campestre*) collected from the litter layer of an uncontaminated woodland in Prospect Park, Reading (Ordnance Survey Grid Reference SU 689 728), were air-dried at room temperature for three days and weighed individually. Solutions of nitrates of cadmium, copper, lead and zinc (BDH Chemicals, Poole, Dorset) were applied topically to the leaves as small droplets and allowed to dry overnight at room temperature. The amounts of metals applied to the leaves were adjusted to give different concentrations of each metal in the dry leaves after allowing for the natural metal content of the leaves (Table 1). Preliminary experiments had shown that most isopods would survive on the lowest concentration of each metal for a year, and all would die at the highest concentration of each metal before the end of the experiment.

Approximately 10 g of leaves (air-dried weight) were placed into each of 13 plastic containers (17 × 11 × 6 cm – one for each metal concentration plus a control containing uncontaminated leaves). A piece of bark approximately 15 × 10 cm from a fallen beech tree was placed into each container to provide the isopods with a substrate to cling to when they were not feeding. A fine spray of distilled water was applied to the lid of each container and they were left for 48 hours to allow the leaves to rehydrate.

Table 1. Measured concentrations of metals (μg g^{-1} dry weight) in leaves of field maple (*Acer campestre*) fed to *Porcellio scaber*, and number of adult and juvenile isopods alive at the end of the experiment

Treatment	Measured concentrations in leaves (μg g^{-1} dry weight)				Survivors at day 360				Juveniles at day 360
	Cd	Cu	Pb	Zn	Males	Gravid females	Non-gravid females	Total	
Control	0.1	11.3	43	102	15	6	17	38	182
Cd10	10.8	12.2	77	154	20	8	14	42	97
Cd50	46.1	9.5	67	138	6	1	15	22	105
Cd100	107	10.0	81	111	0	0	0	0	0
Cu20	0.1	23.4	53	158	13	9	15	37	210
Cu50	0.1	57.0	47	144	11	3	16	30	86
Cu100	0.1	96.2	55	121	0	0	0	0	0
Pb500	0.1	11.7	540	168	8	5	16	29	100
Pb1000	0.1	13.2	1100	163	14	2	15	31	157
Pb2000	0.1	11.1	1920	109	0	0	0	0	0
Zn500	0.1	12.5	67	611	11	6	19	36	146
Zn1000	0.1	9.8	52	1090	0	0	0	0	0
Zn2000	0.1	11.0	51	2350	0	0	0	0	0

At the end of this period, 50 juvenile *P. scaber* of approximately one month in age were introduced into each container. These young isopods were taken from a laboratory stock culture of female isopods collected from Prospect Park. The experiment was conducted in a constant temperature room at 20 °C under a 16 h light/8 h dark regime. The containers were examined three times a week and a spray of distilled water added to the lids of the containers to maintain humidity. Every 30 days, the numbers of surviving isopods were counted by searching through the leaf litter (Fig. 1). New leaves with the same metal concentrations were supplied to each container when the isopods had eaten about 50% of those in the containers.

Towards the end of the experiment, some of the (now sexually mature) female isopods developed brood pouches and released offspring. After one year, the numbers of live juveniles, adult males and gravid and non-gravid females were counted (Table 1) and the experiment was stopped.

Six adult isopods from each treatment (three males, three non-gravid females) were dissected into three tissue fractions (hepatopancreas, gut and rest), dried overnight at 60 °C and analysed for cadmium, copper, lead and zinc by flame or flameless atomic absorption spectrometry (Table 2) using the methodology described by Hopkin (1989). The results, however, are expressed as concentrations on a whole animal basis since the proportions of metals in each tissue fraction were similar to those found in previous studies (Hopkin 1990, 1993b). Samples of uncontaminated and laboratory-contaminated leaves were analysed in a similar manner.

Feeding rates of the isopods could not be recorded accurately since the faecal pellets of the juvenile animals were too small to separate from leaf fragments. Weighing the leaves before and after feeding was also an unreliable method due to fluctuations in moisture content and the fact that in the early stages of the experiment, consumption was small in relation to the total amount of food present. However, judging by the appearance of the leaves and amounts of faeces present, it was clear that the isopods on the highest concentrations of all four metals consumed much less food than those on the medium and lowest concentrations. Thus mortality on the highest concentrations was probably caused by a combination of metal poisoning and starvation through reluctance of the isopods to eat the heavily contaminated diet.

Levels of metals corresponding to some of those used in the experiments were

Table 2. Dry weight (mg) and concentrations of metals ($\mu g\ g^{-1}$ dry weight) in whole *Porcellio scaber* from treatments on which adults survived for 360 days (N=6, mean ± standard errors). There were no significant differences ($p < 0.05$) in weights of isopods on the different treatments at the end of the experiment

Treatment	mg	Cd	Cu	Pb	Zn
Control	15.10 ± 1.82	2.24 ± 0.40	237 ± 38	4.35 ± 0.73	198 ± 28
Cd10	17.30 ± 1.97	78.6 ± 10.5	173 ± 27	4.01 ± 0.90	269 ± 34
Cd50	14.32 ± 1.94	349 ± 47	91.1 ± 11.5	2.70 ± 0.61	241 ± 30
Cu20	17.53 ± 2.10	4.76 ± 0.91	289 ± 39	5.43 ± 0.88	300 ± 27
Cu50	16.00 ± 1.86	3.22 ± 0.72	724 ± 230	3.71 ± 0.52	256 ± 36
Pb500	14.89 ± 1.83	1.80 ± 0.38	185 ± 36	97.4 ± 17.9	187 ± 21
Pb1000	15.97 ± 2.10	1.95 ± 0.50	211 ± 30	186 ± 41	235 ± 21
Zn500	16.94 ± 2.01	3.61 ± 0.74	306 ± 41	2.78 ± 0.33	772 ± 116

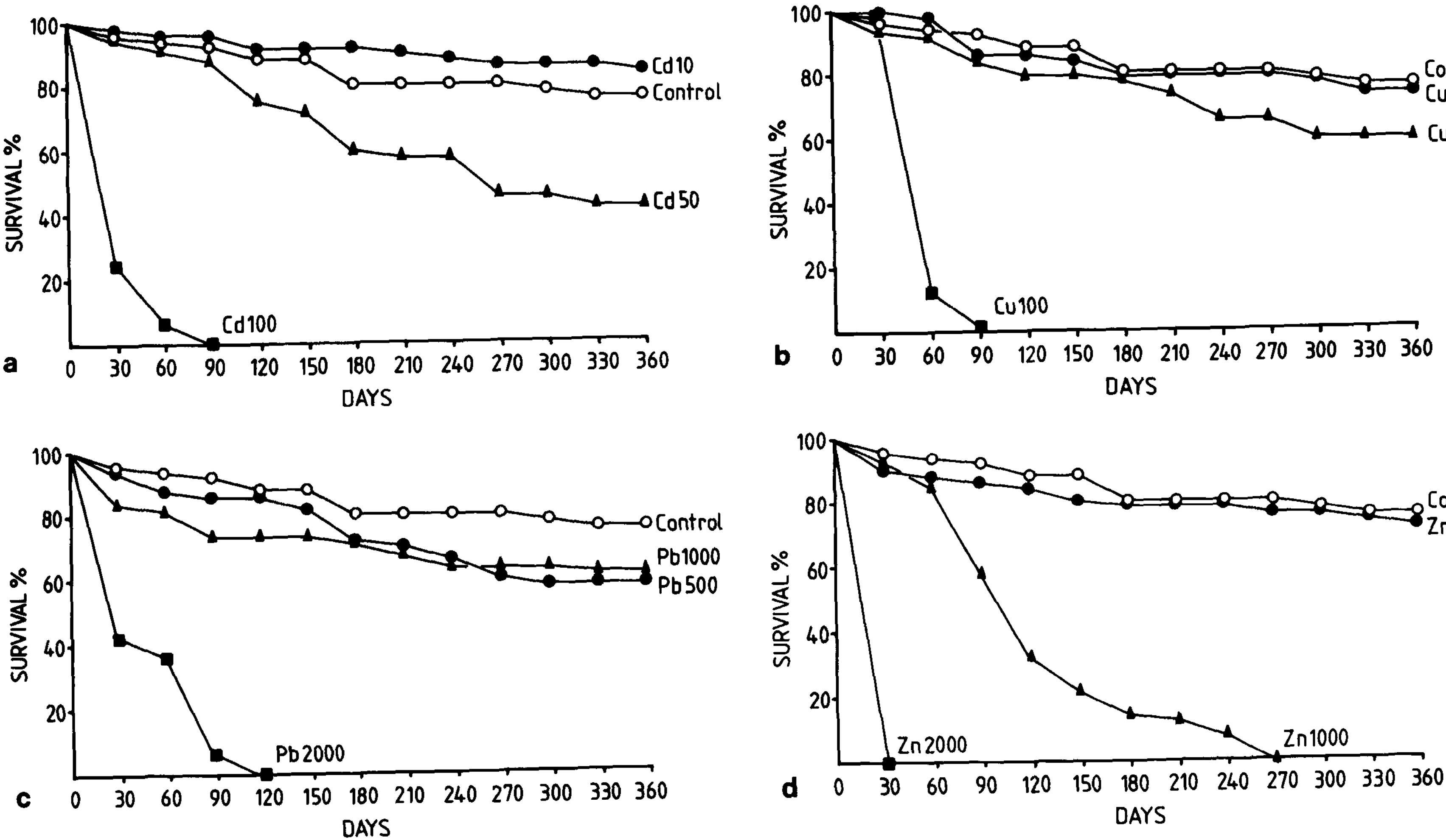

Fig. 1 Survival of *Porcellio scaber* on diets of leaves of field maple (*Acer campestre*) contaminated with different concentrations (µg g^{-1} dry weight) of a) cadmium, b) copper, c) lead and d) zinc (for actual concentrations, see Table 1). Each point represents the percentage of the initial population of 50 juveniles alive at intervals of 30 days.

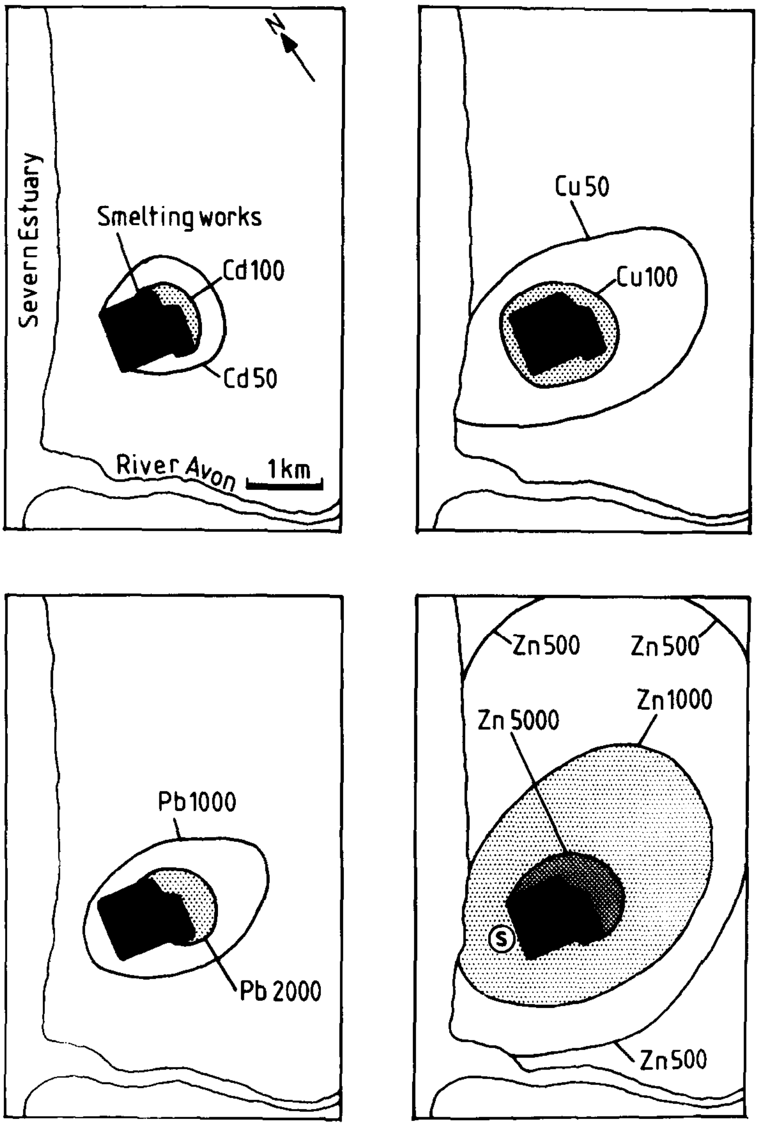

Fig. 2 Regions within which concentrations of metals in surface leaf litter are exceeded around the Avonmouth smelting works (the area covered by the factory is shown in solid black). The area from which *Porcellio scaber* are absent (1.5 km^2 including the land covered by the smelting works) is indicated by dense stipple on the map for zinc. This corresponds with sites where concentrations of zinc in surface leaf litter exceed 5000 $\mu g\ g^{-1}$. *Porcellio scaber* is present in all other sites, including areas within which laboratory experiments predict that populations should not be able to persist (light stipple on the map for zinc). The St. Andrew's Road site (S), where concentrations of zinc in leaf litter are 4150 $\mu g\ g^{-1}$, is the closest point to the smelting works where isopods can be found.

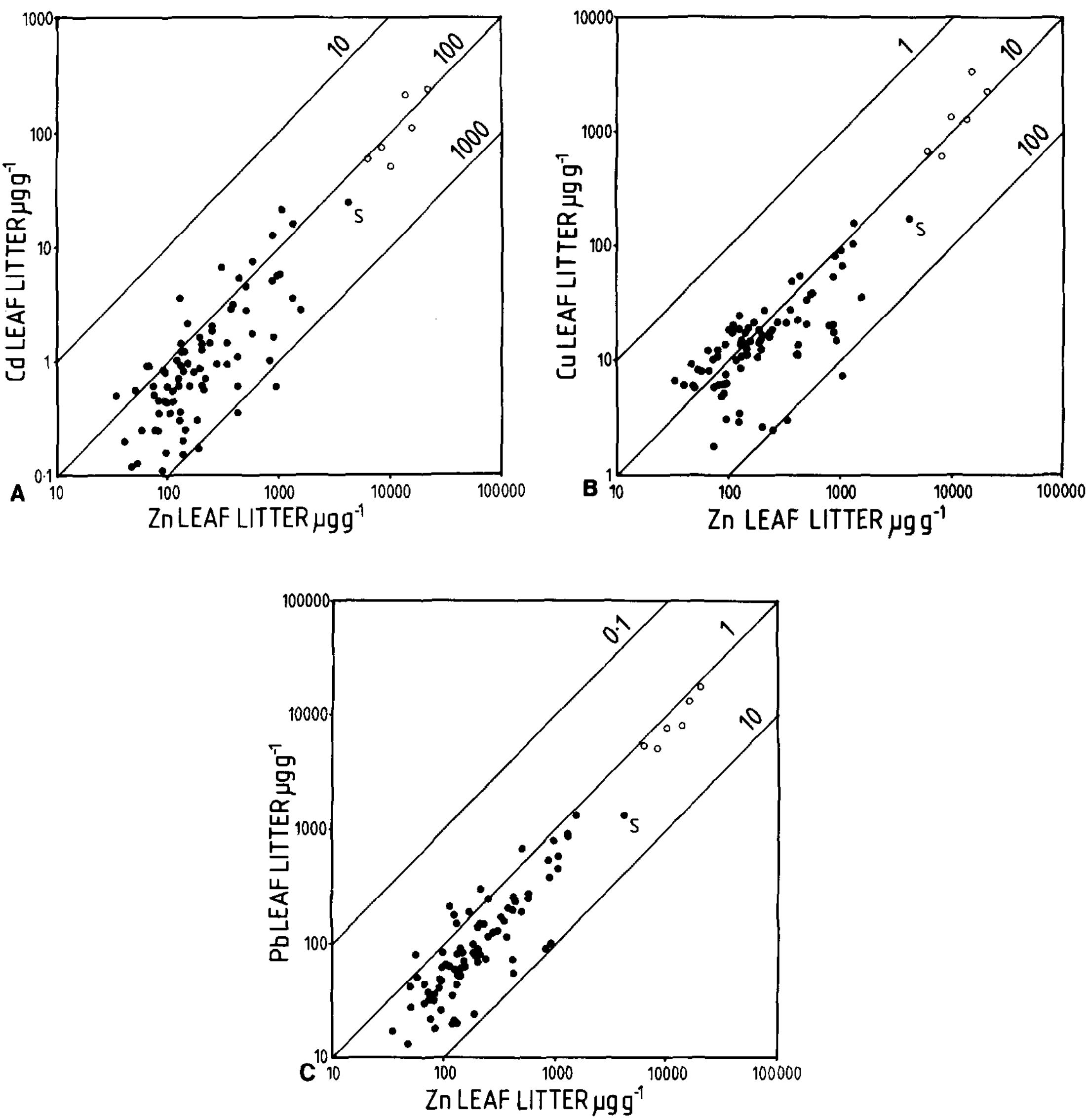

Fig. 3 Scatter diagrams relating concentrations of zinc to a) cadmium, b) copper and c) lead (µg g^{-1} dry weight) in surface leaf litter in the region contaminated by aerial fallout from the Avonmouth smelting works. (●) *Porcellio scaber* present, (○) *Porcellio scaber* absent. S, St. Andrew's Road site (see Fig. 2). Data from Hopkin *et al.* (1986) plus seven additional samples collected in 1992. The diagonal lines on each graph represent the ratios between the concentration of zinc and the other metal.

superimposed onto maps of the concentrations of cadmium, copper, lead and zinc in surface leaf litter in the vicinity of a primary smelting works at Avonmouth, South West England (measured during an earlier study by Hopkin *et al.* 1986) (Fig. 2). The concentrations of cadmium, copper and lead, relative to zinc, in samples of leaf litter from 87 sites in the region, were plotted also (Fig. 3).

All concentrations are expressed on a dry weight basis.

Results

No woodlice survived to reproductive age on leaves with 100 $\mu g\ g^{-1}$ of cadmium or copper (Figs. 1a, b), 2000 $\mu g\ g^{-1}$ of lead (Fig. 1c) or 1000 $\mu g\ g^{-1}$ of zinc (Fig. 1d). Thus, cadmium and copper were approximately ten times more toxic than zinc, and about 20 times more toxic than lead, to *P. scaber*.

The numbers of juveniles found was similar in all treatments in which woodlice survived until day 360 (Table 1). The females had produced enough viable offspring to at least maintain the populations in the containers although it is not known whether these would have been sufficient to allow the species to persist over several generations in the wild.

Isopods that survived had accumulated considerable amounts of metals in comparison to the controls (Table 2). The mean whole body concentration of those fed on 500 μg Zn g^{-1} had reached almost 800 μg Zn g^{-1}, the level at which the most contaminated individuals in a population begin to die of zinc poisoning (Hopkin 1990). The hepatopancreas of one of the six isopods contained 19 500 μg Zn g^{-1}, close to the 'critical' lethal level of 25 000 μg Zn g^{-1} for *P. scaber* (Hopkin 1990; Donker 1992).

Concentration factors after 360 days (mean concentration of metal in whole isopods (Table 2)/concentration of metal in food (Table 1)) were 7.3 and 7.6 for cadmium, 12.4 and 12.7 for copper, 0.18 and 0.17 for lead and 1.26 for zinc. These values are within the ranges of concentration factors found for the four metals in the Avonmouth area in an earlier study (Fig. 7 in Hopkin *et al.* 1986).

There was clear evidence of antagonism between cadmium and copper. Isopods fed on a cadmium-enriched diet contained lower levels of copper than controls fed on uncontaminated leaves (Table 2). This supports the suggestion of Hopkin (1993b), who concluded that the lower than expected concentrations of copper in isopods from an old zinc and cadmium mining area were caused by antagonism from high levels of cadmium in the diet. No other intermetallic effects were apparent.

In this study, the 'critical concentration' can be defined as the level of a metal in the diet at which all isopods died before reaching reproductive age. At Avonmouth, the greatest area within which critical concentrations were exceeded in leaf litter was for zinc (Fig. 2). Although cadmium was at least ten times more toxic than zinc in the laboratory experiments (Figs. 1a, d), zinc is most likely to be killing isopods in the field because its concentration is always 30 times higher than cadmium in Avonmouth leaf litter, and more than 100 times higher in most sites (Fig. 3a). At some sites close to the smelting works, levels of copper may be high enough to affect *P. scaber* (Fig. 3b). However, the area covered by the critical concentration in leaf litter of 100 μg Cu g^{-1} is smaller than that for zinc (Fig. 2), and it is therefore zinc which is most likely to be having the greatest effect. There are no sites close to the smelting works where concentrations of lead are greater than those of zinc in leaf litter (Fig. 3c).

Detailed surveys by Hopkin *et al.* (1986) and Jones (1991) have shown that *P. scaber* is absent from sites where concentrations of zinc in surface leaf litter exceed 5000 μg g^{-1} dry weight (Fig. 2). This corresponds to an area of approximately 1.5 km^2 (including the land occupied by the smelting works). The closest that *P. scaber* has been found to the smelting works is on grassland adjacent to St. Andrew's Road to the west of the smelting works (Fig. 2) where levels of zinc in surface leaf litter are 4150 μg g^{-1}. Thus, populations of *P. scaber* are absent from a much smaller area (1.5 km^2) than that predicted by the laboratory experiments (9 km^2) (Fig. 2).

Discussion

The results reported in this paper support the suggestion of Hopkin (1990) that contamination of leaf litter by zinc is most likely to be responsible for the absence of *P. scaber* from the vicinity of the Avonmouth smelting works. Spurgeon *et al.* (in press) came to a similar conclusion regarding earthworms.

Zinc is a common component of industrial waste. However, zinc has not been included among the United States Environmental Protection Agency's list of metals for which emissions from incinerators must be controlled (Carnes *et al.* 1992). The effects of zinc on terrestrial ecosystems have probably been underestimated since it is usually found associated with the more toxic metal cadmium. Indeed, some areas of the world suffer from zinc deficiency (Boardman and McGuire 1990a, 1990b), and it is this aspect of the metal which has hitherto been considered to be of more concern in terrestrial ecosystems. In his paper discussing critical concentrations for metals in soils, Van Straalen (1993) was unable to propose a value for zinc due to the lack of experimental data.

In this paper, we have demonstrated the importance of comparing the relative toxicities of metals with their concentrations in the field. In our laboratory experiments, cadmium was about ten times more toxic than zinc to *P. scaber* (Fig. 1a, d). If the relative toxicities of the two metals were similar in the laboratory and the field, cadmium would limit the distribution of *P. scaber* only when the ratio of zinc : cadmium in leaf litter is less than 10 : 1. At Avonmouth, the zinc : cadmium ratio in leaf litter is always more than 30 : 1, and in most sites is more than 100 : 1 (Fig. 3a). Thus, zinc is most likely to be responsible for the absence of *P. scaber* from around the factory (Fig. 2).

After feeding for one year on the laboratory-contaminated diets, concentration factors in surviving adults were much higher for cadmium (7.3 and 7.6) and copper (12.4 and 12.7) than for zinc (1.25) or lead (0.17 and 0.18). These values are in agreement with the ranges reported in Hopkin *et al.* (1986) for field populations of *P. scaber* in the Avonmouth area. Thus the experimental design used in the present paper was able to predict the 'bioavailability' of the metals to *P. scaber*, within certain limits, and hence, the potential exposure of their predators to cadmium, copper, lead and zinc. Nevertheless, it is important to recognize that there is considerable between-site variation in concentration factors in the field (Hopkin *et al.* 1986). For accurate determination of the concentrations of metals in isopods at individual sites, it is essential that the concentrations in the animals should be analysed directly, rather than be predicted from concentrations in soil or leaf litter using a concentration factor.

The isopods died before reaching reproductive age on leaf litter with a much lower concentration of zinc (1000 μg g^{-1}) than that on which populations are able to survive in the field (ca 5000 μg g^{-1}). The zinc was present as a nitrate salt in the laboratory.

This form of the metal may have been a greater feeding deterrent than the form in which zinc is associated with leaf litter in the field. Additionally, the zinc as a nitrate may have been released more rapidly into the digestive fluids of the isopods than the more gradual release that might occur from field-contaminated litter. Detoxification systems in the cells of the hepatopancreas may be able to cope with 'chronic' release of zinc from food over several hours, but cannot respond rapidly enough to an 'acute' influx of the same amount of zinc in the laboratory (Dallinger 1993).

Some degree of tolerance to metals may have been developed in *P. scaber* at Avonmouth but this would not have been sufficient to account for the five-fold field : laboratory difference in toxicity values for zinc observed in our experiment (Donker and Bogert 1991; Donker *et al.* 1993).

The design of this study fulfils some of the criteria required for a standard ecotoxicological test. *P. scaber* is easy to maintain in the laboratory, is widespread in Europe, and has been introduced to many other countries where it is common (Hopkin 1991; Dallinger *et al.* 1992; Hopkin *et al.* in press). The experiment is easy to perform and is relevant for assessing effects at the population level since reproductive success is measured. Field maple is a common tree species that is native to Britain. However, leaves from other suitable trees could be used as a food source, providing the isopods found them 'palatable'.

Nevertheless, there are drawbacks with the experiment as described. First, because *P. scaber* has such a long life cycle in comparison to other test species currently in use, it takes at least ten months before the results of the effects of a chemical on reproduction can be assessed. Second, food consumption rates are difficult to measure in the very young animals; it is difficult to ascertain whether juvenile isopods die through poisoning, or from starvation following reluctance to ingest contaminated food. The test could be shortened by measuring adult mortality. However, the saving in time would not be large since individual *P. scaber* can survive for up to 180 days without food (Donker 1992).

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