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EFFECTS OF CADMIUM, COPPER, LEAD AND ZINC ON GROWTH, REPRODUCTION AND SURVIVAL OF THE EARTHWORM *Eisenia fetida* (SAVIGNY): ASSESSING THE ENVIRONMENTAL IMPACT OF POINT-SOURCE METAL CONTAMINATION IN TERRESTRIAL ECOSYSTEMS

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Abstract

The earthworm Eisenia fetida *(Annelida: Oligochaeta) was exposed to a geometric series of concentrations of cadmium, copper, lead and zinc in artificial soil using the OECD recommended protocol. Mortality, growth and cocoon production were measured over 56 days to determine* LC_{50} *and* EC_{50} *values. No observed effect concentrations (NOECs) were also estimated. Furthermore, the percentage of viable cocoons and number of juveniles emerging per cocoon was recorded.*

Cocoon production was more sensitive than mortality for all the metals, particularly cadmium and copper for which NOEC reproduction values were an order of magnitude lower than those for NOEC mortality. However, there was no significant effect of metals on the viability of cocoons.

The weights of earthworms declined in all treatments (including the controls) during the experiment. This was probably due to the lack of suitable food in the OECD standard soil medium used. It was concluded that future experiments should include animal manure in the test medium.

The LC_{50}, EC_{50} *and NOEC values determined in this study were compared with concentrations of metals in soils in the vicinity of a smelting works at Avonmouth, south-west England. The 14-day* LC_{50} *for zinc in* Eisenia fetida *was exceeded in soils covering an area of 75* km^2 *around the works, compared to 4·2* km^2 *for copper and 4·7* km^2 *for lead. Soil values for cadmium did not exceed the* LC_{50} *value anywhere in the region. Similar estimates of relative effects on reproduction confirmed that zinc is most likely to be responsible for the absence of earthworms from sites close to the Avonmouth works. However, the OECD standard test overestimated the potential effects of metals on populations, since earthworms can be found as close as 1 km from the smelting works. The discrepancy between test and field observations was probably due to the greater availability of the metals in the artificial soil.*

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INTRODUCTION

Metal pollution may disturb soil ecosystems by affecting the structure of soil invertebrate populations. Successful protection of communities demands knowledge of the ecophysiology of metals in invertebrates and their susceptibility to metal intoxication (Dallinger and Rainbow, 1993; Hopkin, 1989).

The potential hazards of environmental pollutants to soil invertebrates have been assessed in recent years by the use of the 'earthworm acute toxicity test' (OECD, 1984; EEC, 1985). This 14-day LC_{50} test using the earthworm *Eisenia fetida* has been important for risk assessment and regulation of new and existing chemicals (Becker *et al.*, 1992).

The end point of the 'earthworm acute toxicity test' is mortality. However, mortality is unlikely to be either the most sensitive or ecologically relevant parameter for predicting effects on field populations. Reproductive and/or growth disturbances are far more likely to mediate population effects (Moriarty, 1983). Reproduction is likely to be of particular important in ecotoxicological assessment because of its influence on population dynamics (Joosse & Verhoef, 1983; Kooijman & Metz, 1984; Denneman & Straalen, 1991).

This paper forms part of a wider study into the effects of metal fallout on soil invertebrates in the vicinity of a smelting works situated at Avonmouth, south-west England (for earlier references see Hopkin, 1989, and Martin and Bullock, in press). The standard earthworm acute toxicity test (OECD, 1984) has been used to determine the concentrations of cadmium,copper, lead and zinc that cause specific lethal and sub-lethal effects in the earthworm *Eisenia fetida*. This laboratory-derived toxicity data has been related subsequently to levels of metals in soils around the Avonmouth smelting works. Such an approach has allowed predictions to be made as to which of the four metals is responsible for the absence of certain species of earthworms close to the factory (Hopkin *et al.*, 1985).

The suitability of the OECD test for determining the effects of pollutants beyond the recommended 14-day period is examined also.

Table 1. LC_{50}, EC_{50} cocoon production (with 95% confidence intervals where calculable) and NOEC cocoon production and mortality values for the earthworm *Eisenia fetida* exposed to a geometric series of concentrations of cadmium, copper, lead and zinc (mean of four replicates)

	LC_{50} (14 day)		LC_{50} (56 day)		Estimated NOEC mortality (56 day)		EC_{50} cocoon production (56 day)		Estimated NOEC cocoon production (56 day)	
	µg/g	µmol/g	µg/g	µmol/g	µg/g	µmol/g	µg/g	µmol/g	µg/g	µmol/g
Cadmium	>300	>2·6	>300	>2·6	>300	>2·6	46·3	0·4	39·2	0·35
	—	—	—	—			25·4–91·4	0·23–0·81		
Copper	683	10·7	555	8·7	210	3·3	53·3	0·84	32·0	0·5
	570–812	9–12·8	460–678	7·2–10·7			32·5–186	0·51–2·85		
Lead	4480	21·6	3760	18·1	2190	10·6	1 940	9·4	1 810	8·7
	3 500–6 190	16·9–29·9	2 900–5 180	14–25			—	—		
Zinc	1010	15·4	745	11·4	289	4·4	276	4·2	199	3·4
	780–1 370	11·9–9·21	591–957	9–14·6			202–375	3·1–5·7		

MATERIALS AND METHODS

The artificial soil was prepared following OECD guideline No. 207 (OECD, 1984). The medium consisted (by dry weight) of 70% sand, 20% clay (kaolin clay) and 10% organic matter (as *Sphagnum* peat). Sand was obtained from a local builders' merchant, the kaolin clay from a pottery supplier and the *Sphagnum* peat from a local garden centre. Nitric acid digests of control soils analysed by atomic absorption spectrometry contained <0·1 µgCd/g, 2·4µgCu/g, 12µgPb/g and 65µgZn/g on a dry weight basis (for further details of the analytical method see Hopkin, 1989).

The pH of the medium was adjusted to 6·3 at the start of the experiment with powdered calcium carbonate. By the end of the experiment, the pH had increased to 6·7 in most containers, but this would not have been sufficient to significantly influence growth, mortality or cocoon production (Gestel *et al.*, 1992*a*).

The constituents for the artificial soil were air dried, mixed thoroughly, and weighed into plastic boxes (275 × 155 × 95 mm). Solutions of cadmium nitrate ($Cd(NO_3)_2.4H_2O$), copper nitrate ($Cu(NO_3)_2.3H_2O$), lead nitrate ($Pb(NO_3)_2$) and zinc nitrate ($Zn(NO_3)_2.6H_2O$) (BDH chemicals, Poole, Dorset, UK) were mixed with the dry constituents to give the required percentage water content (35%) and metal concentrations in the soils. The same volume of distilled water was added to the controls. Four replicates were used for each of the test concentrations and the controls (total 80 containers). The concentrations of the four metals in the soils were (in µg/g dry weight of soil): cadmium—5, 20, 80, 300; copper—10, 40, 200, 1000; lead—100, 400, 2000, 10 000; zinc—100, 400, 2000, 10 000. The highest concentrations were chosen to mimic the levels found in the most contaminated soils adjacent to the smelting works where earthworms are absent.

Eisenia fetida were obtained from a local vermiculturist. All worms were adult, at least 8 weeks old and fully clitellate. Worms were incubated in artificial soil for 6 h before use. Ten worms were added to each container. Containers were checked after 6 h to ensure that all the worms had burrowed into the soil.

The experiment was run for 56 days (8 weeks) at 20°C in constant light. Mortality and growth were measured on a weekly basis by counting and weighing the surviving worms in each container. Cocoons were collected by sorting through the soil each week and were incubated on moist filter paper at 20°C until all juveniles had emerged.

Fourteen and 56-day LC_{50} values, 56-day NOEC mortalities, 56-day cocoon production NOECs and EC_{50} values were estimated.

Statistics

LC_{50} values were determined by probit analysis with the SAS software package. EC_{50} values were obtained from a logit model analysis in SAS. Student's *t*–test was used to evaluate the significance of differences between treatments in the viability and number of juveniles that emerged from the cocoons. NOECs were calculated using the Williams test (Williams, 1971, 1972). Strictly speaking a NOEC value should correspond to the highest test concentration causing no significant effect on a measured parameter compared to controls. In this study estimated NOEC values have been calculated by adjusting the mean within the William's test to determine the mean value giving a $P = 0·05$ value. This mean value was then used to estimate the NOEC value by assuming a straight line relationship between the highest concentration causing no significant effect and the lowest concentration giving a significant effect.

RESULTS

Table 1 summarises all LC_{50}, EC_{50} and NOEC values. Figure 1(a)–(d) show the proportion of the initial population of worms still alive in each treatment at weekly intervals.

Mortality

For cadmium, there was no significant mortality at the highest test concentration of 300 µg/g (Fig. 1(a)). Of

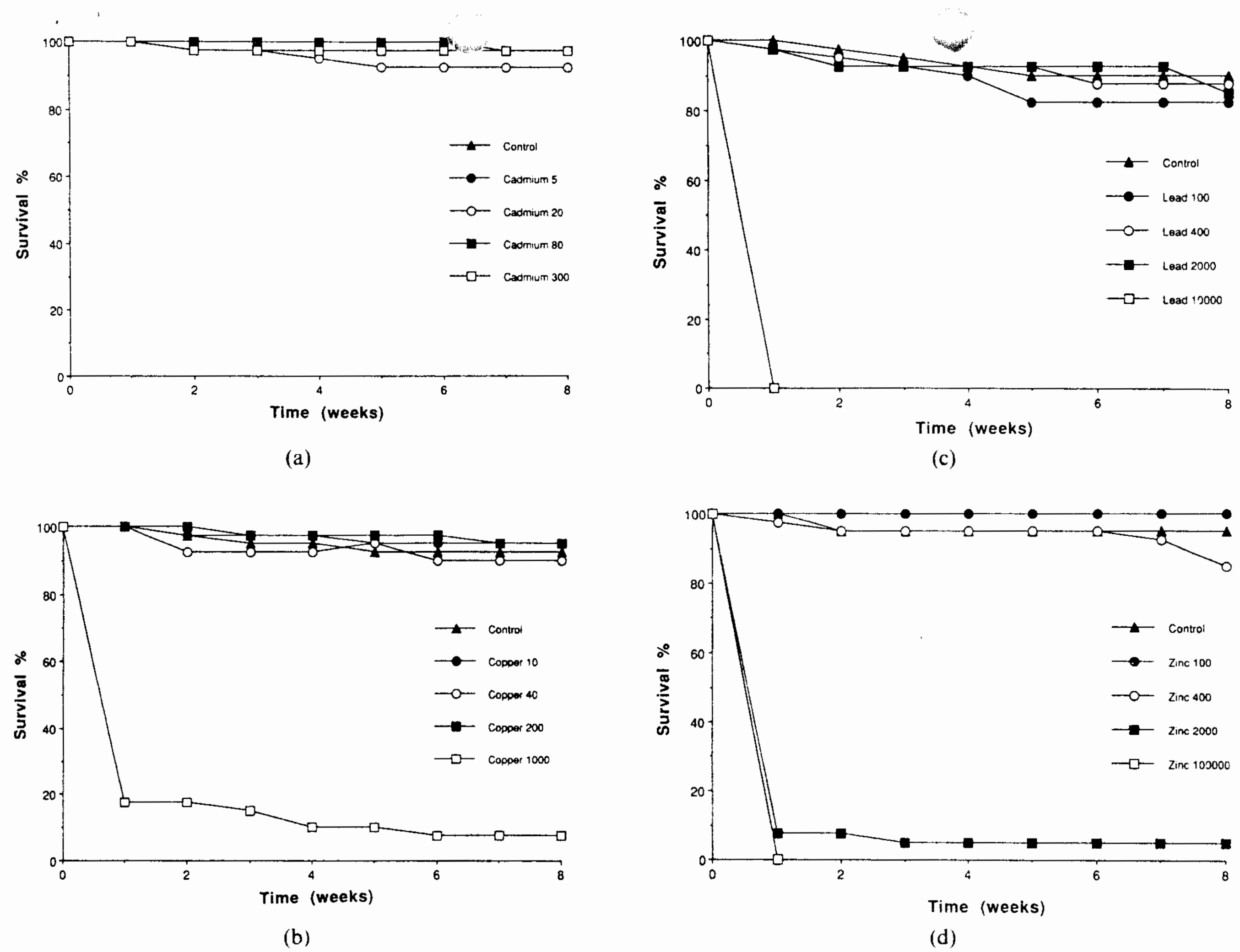

Fig. 1. Survival of *Eisenia fetida* in artificial soil contaminated with a geometric series of concentrations of (a) cadmium, (b) copper, (c) lead, and (d) zinc (μg/g dry wt). Each point represents the mean of four replicates, each comprising 10 worms at the start of the experiment (i.e. 40 worms per treatment).

the three other metals, copper was the most toxic with significant mortality at 1000 μg/g ($P < 0{\cdot}001$) (Fig. 1(b)), and 14- and 56-day LC_{50} values of 683 μg/g and 555 μg/g (Table 1). Zinc produced a slight but significant ($P < 0{\cdot}05$) increase in mortality at 400 μg/g in week 8 compared to controls (Fig. 1(d)), and a highly significant increase in mortality at 2000 μg/g ($P < 0{\cdot}001$) after only 1 week. The 14- and 56-day LC_{50} values for zinc were higher than those for copper being 1010 μg/g and 745 μg/g, respectively (Table 1). Lead was the least toxic metal. Significant mortality was recorded only at 10 000 ($P < {\cdot}001$) (Fig. 1(c)) with 14- and 56-day LC_{50} values of 4480 μg/g and 3760 μg/g.

Of the worms that died, most did so during the first week of the experiment. Thus, all worms died at 10 000 μg/g lead and zinc, 82·5% at 1000 μg/g copper and 92·5% at 2000 μg/g zinc during the first week (Figs 1(a)–(d)). After the first week however, the rates of mortality reduced greatly. Where high but not complete mortality occurred (e.g. 1000 μg/g copper and 2000 μg/g zinc), many of the remaining animals survived for the full duration of the experiment. This pattern of mortality suggests that the main toxic effect of the metals tested was exerted via uptake across the body wall, rather than through dietary metal assimilation. For example, worms placed on soil containing the highest concentration of zinc (10 000 μg/g) were initially very active, but all died within 10 min.

Growth

Control worms and those on the lowest concentration of metals had slightly increased in weight after 1 week. However, in subsequent weeks their weight declined, and after 56 days there were no statistically significant differences ($P > 0{\cdot}05$), between the mean weights of worms in the control soil and any of the metal treatments (Figs 2(a)–(d)). Growth data for 1000 μg/g copper and 2000 μg/g zinc is not shown as too few worms survived to permit reliable statistical analysis.

The initial increases in weight were due probably to ingestion of soil. Although worms were pre-exposed to the artificial soil for 6 h before being placed in the treatment containers, this was evidently of insufficient length to allow them to fill their guts.

Cocoon production

Cocoon production was significantly lower than controls at the highest metal concentrations (Figs 3(a)–(d)).

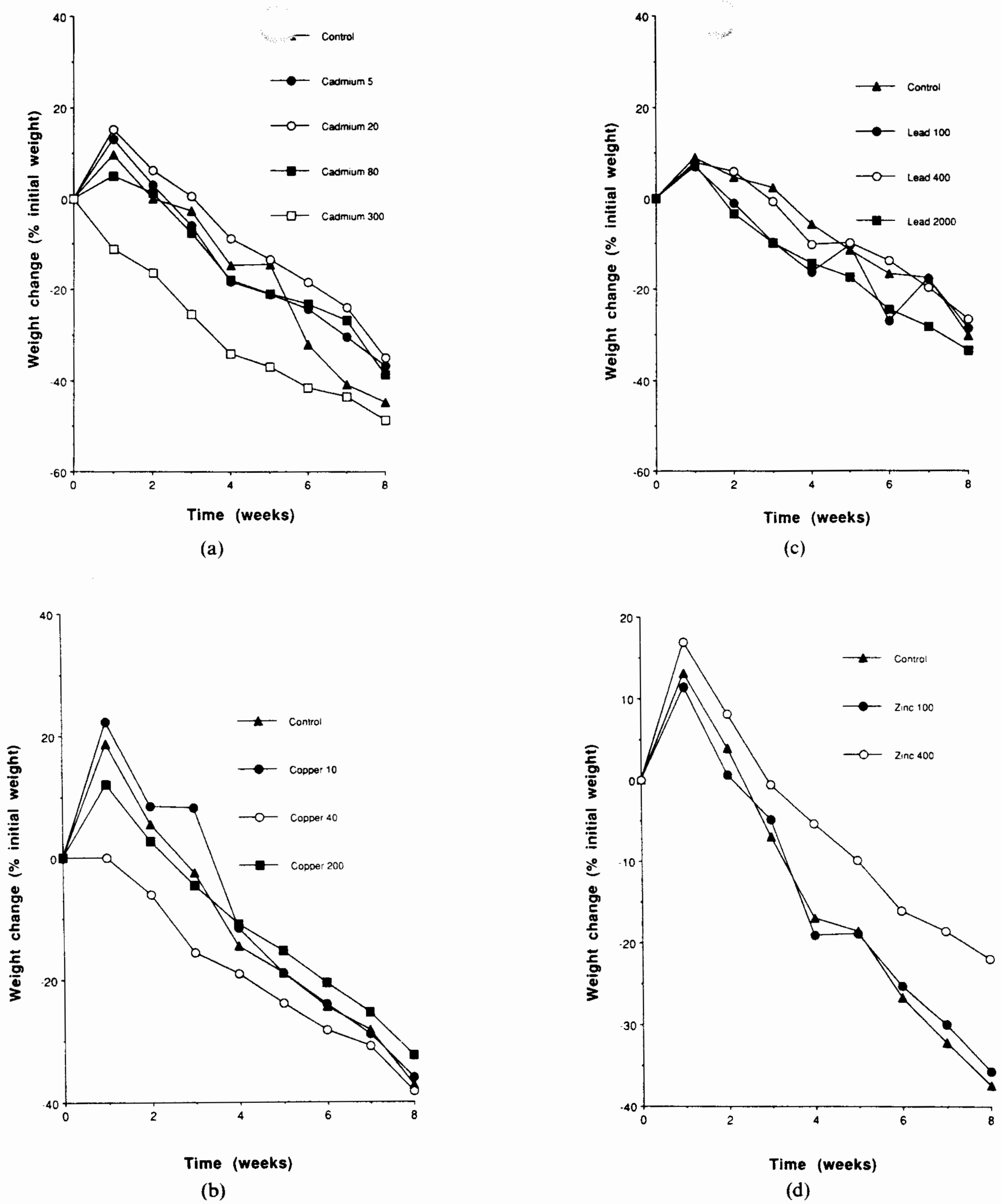

Fig. 2. Changes in fresh weight of *Eisenia fetida* in artificial soil contaminated with (a) cadmium, (b) copper, (c) lead, and (d) zinc (μg/g dry wt). Each point represents the mean of four replicates, each comprising 10 worms at the start of the experiment (i.e. 40 worms per treatment).

Interestingly, there was a significant ($P < 0{\cdot}05$) increase in cocoon production at 10 μg/g copper (Fig. 3(b)). No enhancement of reproduction was recorded at low concentrations of cadmium, lead or zinc (Figs 3(a)–(d)).

For cadmium and copper, cocoon production was an order of magnitude more sensitive to metals than mortality, whereas for lead and zinc, reproductive sensitivity was only marginally more sensitive than mortality (Table 1).

Overall, cocoon production rates were low (only 151 were collected during the experiment) due probably to the lack of suitable food in the artificial soil. Gestel *et al.* (1989), recorded a production rate of 0·2 cocoons per worm per week in worms not supplied with animal manure, compared with the 1·2 to 2·0 cocoons per worm per week in those supplied with food.

Viability of cocoons

The viability of cocoons (i.e. the proportion from which juveniles emerged) was high in all samples and

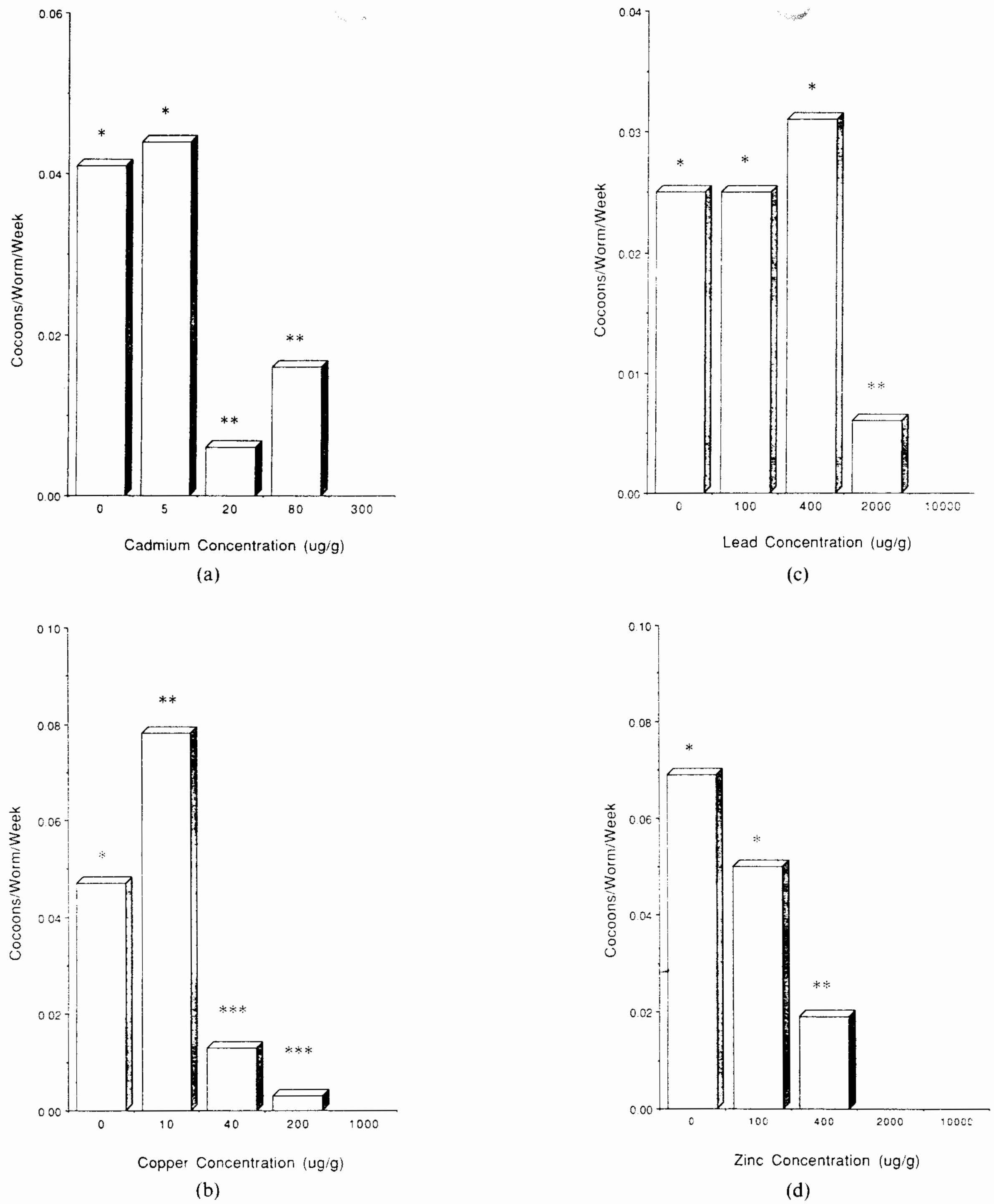

Fig. 3. Rate of cocoon production by *Eisenia fetida* exposed to (a) cadmium, (b) copper, (c) lead, and (d) zinc (μg/g dry wt). Bars with the same number of asterisks (*) were not significantly different at $P < 0{\cdot}05$.

was always >88% (*cf.* 96% found by Gestel *et al.*, 1988) for cocoons incubated on artificial soil). No significant differences were found between treatments.

Number of juveniles per cocoon

No significant differences were found between treatments in the mean numbers of juveniles that emerged from cocoons. The average number of juveniles per cocoon was 2·01 (range 1–5). Gestel *et al.* (1988) obtained a higher mean value of 2·8 juveniles per cocoon, but these were derived from adults supplied with animal manure.

Mapping (Figs 4(a)–(d))

All of the values shown in Table 1 (with the exception of 56-day LC_{50} values) were superimposed onto the concentrations of cadmium, copper, lead and zinc in soils in the Avonmouth area measured in 1988 and 1989 (total nitric acid digests) (Jones, 1991). Areas within which these values were exceeded were calculated (Table 2). In some cases (56-day NOEC mortality for zinc, and 56-day EC_{50} cocoon production and 56-day NOEC cocoon production for zinc and copper), the area could not be determined as it extended into

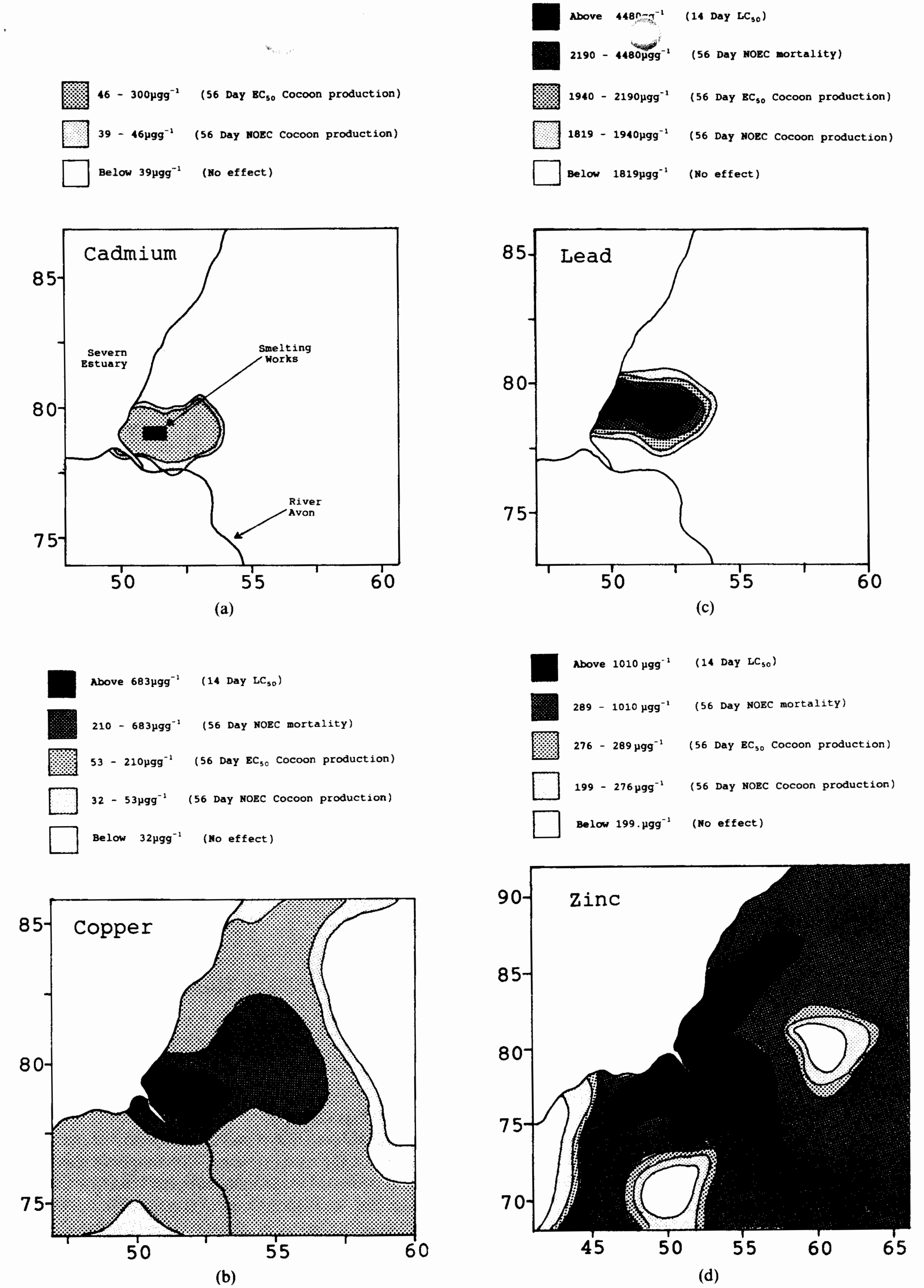

Fig. 4. Maps showing the areas within which estimated toxicity values for (a) cadmium, (b) copper, (c) lead, and (d) zinc in *Eisenia fetida* are exceeded in soils at Avonmouth, south-west England. X and Y values are Ordnance Survey Grid Reference numbers expressed in km (see also Table 2).

Table 2. Area over which experimentally determined LC_{50}, EC_{50} and NOEC values for the earthworm *Eisenia fetida* are exceeded for the metals cadmium, copper, lead and zinc in soils at Avonmouth (see Figs 4(a)–(d))

	Cadmium (km^2)	Copper (km^2)	Lead (km^2)	Zinc (km^2)
14-Day LC_{50}	0	4·2	4·7	75
56-Day NOEC mortality	0	21·9	6·9	>320
Estimated 56-Day EC_{50} cocoon production	6·2	>108	8·1	>368
Estimated 56-day NOEC cocoon production	7·4	>117	9·9	>394

regions of 'background' concentrations some distances from the smelting works.

DISCUSSION

Copper caused higher mortality than lead or zinc in *Eisenia fetida* when present at the same concentration (Table 1). LC_{50} and NOEC values for cadmium could not be determined since no significant mortality was observed at the highest test concentration used (300 μg/g). Gestel and Dis (1988) recorded a 14-day LC_{50} for cadmium of >1000 μg/g in artificial soil, while Neuhauser *et al.* (1985) calculated a 14-day LC_{50} of 1843 μg/g. Thus, the relative toxicity of cadmium in terms of mortality is somewhere between those for zinc and lead. The high tolerance of earthworms to cadmium poisoning is due probably to detoxification by metallothionein proteins in the posterior alimentary canal (Morgan *et al.*, 1989).

Cadmium had the greatest detrimental effect on cocoon production, followed by copper, zinc and lead (Table 1, Figs 3(a)–(d)). This order of toxicity has been found in other studies on the effects of metals on reproduction in earthworms (Malecki *et al.*, 1982; Ma, 1983; Bengtsson *et al.*, 1986; Gestel *et al.*, 1991). The results also indicate that where cocoons were produced, the viability and numbers of juveniles that emerged were not affected by any of the four metals at the concentrations used.

Effects of cadmium and copper on the reproductive rate of *Eisenia fetida* were particularly acute. NOEC cocoon production values were an order of magnitude lower than those for NOEC mortality (Table 1), thus, mortality is not a reliable indicator of the potential effects of cadmium and copper on reproduction. However, NOEC cocoon production values for lead and zinc were comparable to those for NOEC mortality.

The results of this experiment are in close agreement with those reported in the literature by other researchers (Ma, 1983; Neuhauser *et al.*, 1985; Bengtsson *et al.*, 1986; Gestel & Dis, 1988; Gestel *et al.*, 1989, 1991, 1992*b*). Copper has been the most widely-studied metal. Neuhauser *et al.* (1985) determined a 14-day LC_{50} for copper of 643 μg/g, while NOEC reproduction values of between 30–120 μg/g have been calculated in natural and artificial soils (Ma, 1983, 1984, 198[illegible] Bengtsson *et al.*, 1986; Gestel *et al.*, 1989). Cadmium toxicity has been widely surveyed also. Gestel *et al.* (1991) determined a 12-week LC_{50} for cadmium of 253 μg/g, whilst Neuhauser *et al.* (1985) recorded a 14-day LC_{50} of 1843 μg/g, NOEC reproduction values for cadmium range from <10 to 100 μg/g (Ma, 1983; Bengtsson *et al.*, 1986; Gestel *et al.* (1992*b*). Less information on the lethal and sub-lethal effects of lead is available. Neuhauser *et al.* (1985) found a 14-day LC_{50} of 5941 μg/g for lead while Ma (1983) and Bengtsson *et al.* (1986) determined NOEC reproduction values of 500 μg/g and 200 μg/g, respectively. The toxicity of zinc to earthworms has received relatively little attention. Neuhauser *et al.* (1985) determined a 14-day LC_{50} of 662 μg/g in artificial soil. However, the authors are unaware of any comparable data concerning the sub-lethal effects of zinc.

Toxicity values determined in experiments in which earthworms were exposed to a metal contaminated food source (sewage sludge or animal dung) overlaying a pollutant-free soil medium cannot be compared directly with this study (e.g. Hartenstein *et al.*, 1981; Malecki *et al.*, 1982; Neuhauser *et al.*, 1984). Earthworms are able to avoid polluted soil (Ma, 1988; Wentsel & Guelta, 1988). Thus, it seems likely that in these studies the earthworms were able to escape from the metal contaminated food into the underlying clean soil, and hence were not exposed continuously to elevated concentrations of metals (Gestel *et al.*, 1991).

There are obvious problems with the OECD method if experiments are continued beyond the recommended 14 days. Principally, worms at all concentrations lost weight after the second week (Figs 2(a)–(d)). Although the medium contained 10% organic matter (*Sphagnum* peat) this was clearly an unsuitable source of food. In addition, it was not necessary to continue the experiment for 8 weeks since EC_{50} and NOEC levels calculated after 28 days were comparable with those found after 56 days.

The test procedure clearly requires modification if it is to be used for longer term toxicity studies. The addition of a food source (e.g. animal manure) is essential. Furthermore, a longer soil acclimation period is required to overcome the initial increase in weight following filling of the gut with soil. The authors would, therefore, support the use of the standard reproduction toxicity test proposed by Gestel *et al.* (1989). In this protocol a small pellet of animal manure is supplied to which the worms have free access during the three phases of the experiment. Initially worms are preconditioned for 1 week (phase A). This allows them to acclimatise to the artificial soil. The acclimation period is followed by a 3-week exposure to the chemical under test (phase B). During the recovery period (phase C) worms are returned to uncontaminated soil. Growth and cocoon production are monitored during phases B and C.

The effects of pollution at particular sites can be assessed by comparing toxicity values for metals determined in the laboratory with concentrations in soils in the contaminated area (Figs 4(a)–(d)). The area over which these values are exceeded can then be calculated (Table 2) and used in environmental impact assessment.

Preliminary observations on the soil fauna of the Avonmouth area have shown that at least one species of earthworm is present within the area where 14-day LC_{50} values for zinc in *Eisenia fetida* is exceeded in soil (Hopkin *et al.*, 1985), this is to be expected since under the experimental conditions used in this study, the metals were much more available than they would be in the field. If we assume that the extent of this discrepancy between field and laboratory observations is similar for all four metals, it is clear that zinc is likely to be exerting the greatest deleterious effects on earthworms at Avonmouth relative to cadmium, copper and lead (Figs 4(a)–(d)).

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