Ecotoxicology, **4**, 190–205 (1995)

Extrapolation of the laboratory-based OECD earthworm toxicity test to metal-contaminated field sites

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Received 24 November 1993; revised and accepted 5 March 1994

The effects of cadmium, copper, lead and zinc on survival, growth, cocoon production and cocoon viability of the earthworm *Eisenia fetida* (Savigny) were determined in three experiments. In experiment 1, worms were exposed to single metals in standard artificial soil. For experiment 2, worms were maintained in contaminated soils collected from sites at different distances from a smelting works situated at Avonmouth, south-west England. In experiment 3, worms were exposed to mixtures of metals in artificial soil at the same concentrations as those present in the field soils. A survey of earthworm populations was carried out also. Population densities and species diversities of earthworms declined with proximity to the smelting works. No earthworms were found within 1 km of the factory. Comparison of toxicity values for the metals determined in the experiments indicated that zinc is most likely to be limiting earthworm populations in the vicinity of the works. Zinc was at least ten times more toxic to *E. fetida* in artificial soil than in contaminated soils collected from the field. This difference was probably due to the greater bioavailability of zinc in the artificial soil. The results are discussed in the context of setting 'protection levels' for metals in soils based on laboratory toxicity data.

Keywords: ecotoxicology; *Eisenia fetida*; zinc; toxicity; mapping.

Introduction

Edwards (1983) developed the 'contact filter paper test' and the 'artificial soil test' for use in assessing the acute toxicity of chemicals to earthworms. These tests were principally designed to generate toxicity data for use in risk assessment of new agrochemicals. However, both clearly have potential use in determining the environmental impact of existing pollutants.

The artificial soil and contact filter paper tests were adopted by the OECD (1984) and the EEC (1985) and have been generally accepted for laboratory-based tests with earthworms (Callahan *et al.* 1985). Of the two procedures, the artificial soil test has been used most widely (Goats and Edwards 1988; Grieg-Smith 1992; Reinecke 1992), since it permits the toxicity of chemicals to be determined in a simulated soil medium in the laboratory. Thus, the test was designed to allow direct comparison with effects in the field (Van Gestel and Van Dis 1988; Heimbach 1992).

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In comparative studies, Heimbach (1993) and Van Gestel (1992) concluded that the toxicities of a range of pesticides to earthworms determined in laboratory tests, using the artificial soil test protocol, were comparable with the effects found in field trials with the tested chemicals. The suitability of the OECD protocol for assessing the toxicity of metals to earthworms in the field has not however been examined in detail.

Spurgeon *et al.* (1994) determined the toxicity of cadmium, copper, lead and zinc to *Eisenia fetida* (Savigny) in artificial soils. Toxicity values obtained were related to concentrations of metals in soils in the vicinity of a lead, cadmium and zinc smelting works situated in Avonmouth, south-west England, close to which earthworms are known to be absent or severely reduced in numbers (Hopkin *et al.* 1985; Martin and Bullock, 1994). The areas within which the concentrations of cadmium, copper, lead and zinc in soils exceeded those found to increase mortality and reduce fecundity in laboratory toxicity tests on *E. fetida* were defined. Results indicated that of the four metals, zinc was most likely to be limiting earthworm populations around the smelting works.

In the studies reported in the present paper, three tests with *E. fetida* have been carried out to relate directly the toxicity of metals to worms in artificial and field soils. First, the experiments of Spurgeon *et al.* (1994) have been repeated with the addition of a food source (horse manure) as recommended by Van Gestel *et al.* (1989). Second, *E. fetida* were exposed in the laboratory to soils collected from sites at different distances from the Avonmouth smelter. Third, *E. fetida* were exposed to mixtures of cadmium, copper, lead and zinc in artificial soil at the same concentrations as those in the field soils used in the second experiment.

Materials and methods

The toxicity of cadmium, copper, lead and zinc to *E. fetida* was determined using the modified OECD (1984) toxicity test, described by Van Gestel *et al.* (1989). The soils were held in plastic boxes (dimensions 175 mm × 120 mm × 60 mm).

Worms were exposed to uncontaminated artificial soil (experiments 1 and 3) or soil from the control site (experiment 2) for 1 week prior to each experiment. After this period, worms were weighed individually and added to the relevant field or artificially contaminated soil. Four replicates were used for each test concentration with ten worms added to each. Containers were covered to prevent water loss and maintained for 21 days at 20°C in constant light. A small pellet (3 g dry weight) of horse manure (collected from an animal that had been grazing uncontaminated pasture and had not undergone any recent medication) was added every week to each container as a source of food as recommended by Van Gestel *et al.* (1989, 1992a).

The number of worms alive in each container was counted after 14 days. Growth was measured by weighing worms at the end of the exposure period, to determine the mean percentage growth of the population relative to the mean initial weight. Cocoons were collected at the end of the experiments by wet-sieving the soil from each container. All cocoons found were maintained on uncontaminated artificial soil for 5 weeks (see method of Van Gestel *et al.* (1988)), to determine the viability, the number of juveniles emerging from each fertile cocoon and, hence, juvenile production rate.

Experiment 1: toxicity of individual metals in artificial soil

Worms were exposed to artificial soil (70% sand, 20% kaolin clay and 10% coarse ground *Sphagnum* peat). The pH of the soil was adjusted to 6.1 with calcium carbonate. Soils were contaminated with solutions of metals (as nitrates) to give dry weight concentrations of 0, 5, 20, 80 and 300 $\mu g\ g^{-1}$ cadmium, 0, 10, 40, 200 and 1000 $\mu g\ g^{-1}$ copper and 0, 100, 400, 2000 and 10 000 $\mu g\ g^{-1}$ lead or zinc. Distilled water was added to give a moisture content of 33% wet weight (for further details, see Spurgeon *et al.* (1994) and Van Gestel *et al.* (1989)).

Experiment 2: toxicity of metal-contaminated field soils

Seven sites in the vicinity of the smelting works were visited on the same day in June 1992 (Fig. 1). All sites were permanent grassland, situated adjacent to minor roads at least 2 m from the kerb. Approximately 4 kg of soil was collected from the top 2 cm layer at each site after removal of surface vegetation and litter. A 'control' sample of soil was collected from an uncontaminated site on the Reading University campus. Metal levels at this site were within the range typical for an 'uncontaminated' soil.

Soil samples were crushed while still damp and placed in an oven at 60°C for 2 days. The dry soils were passed through a 1 mm mesh and 500 g were placed into each experimental container. Distilled water was added to give a moisture content of approximately 50% of the water holding capacity for each soil. The concentrations of cadmium, copper, lead and zinc in the soils were determined by flame atomic absorption spectrometry of nitric acid digests as described by Hopkin (1989). The organic matter content and pH were measured also (Table 1).

Experiment 3: toxicity of mixtures of metals in artificial soil

E. fetida were exposed to mixtures of cadmium, copper, lead and zinc in artificial soil at the same concentrations as those found in the field soils in experiment 2.

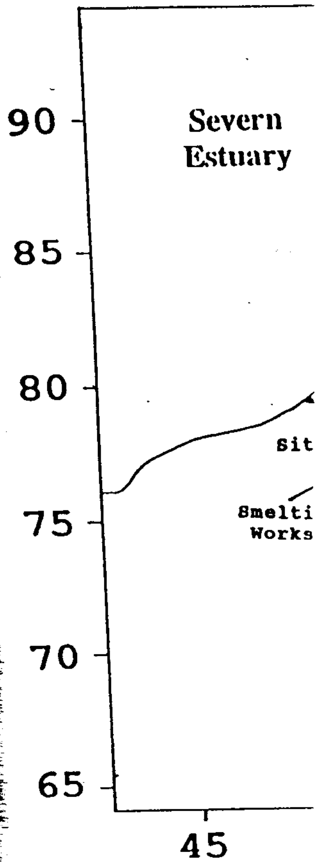
Population density and species diversity of earthworms in the field

Earthworm populations were sampled on the same day in October 1993 at each of the sites from which the soils used in experiment 2 were collected. Four quadrats (each of 25 cm × 25 cm) were marked on the soil surface at each location. The soil was dug out to a depth of 40 cm from within each quadrat. The soil was hand sorted and all earthworms found returned to the laboratory for identification using the key of Sims and Gerard (1985).

Statistics

LC_{50}s and EC_{50}s were determined by probit analysis and logit analysis, respectively, using the SAS software package. NOEC values were determined using the derivation of the Williams (1971, 1972) test, used by Spurgeon *et al.* (1994). Recently there has been some discussion about the legitimacy of NOECs (Hoekstra and Van Ewijk 1993a, 1993b; Van Straalen *et al.* 1994), however the use of such values has been retained in this paper to maintain consistency with previous work.

Calculations of LC_{50}, EC_{50} and NOEC values were based on the assumption that the metals acted independently with no additive toxic effects. This approach is supported by a number of studies in the literature. For example, Berger *et al.* (1993) concluded that the uptake of cadmium and zinc in the gastropod *Helix pomatia* were similar after



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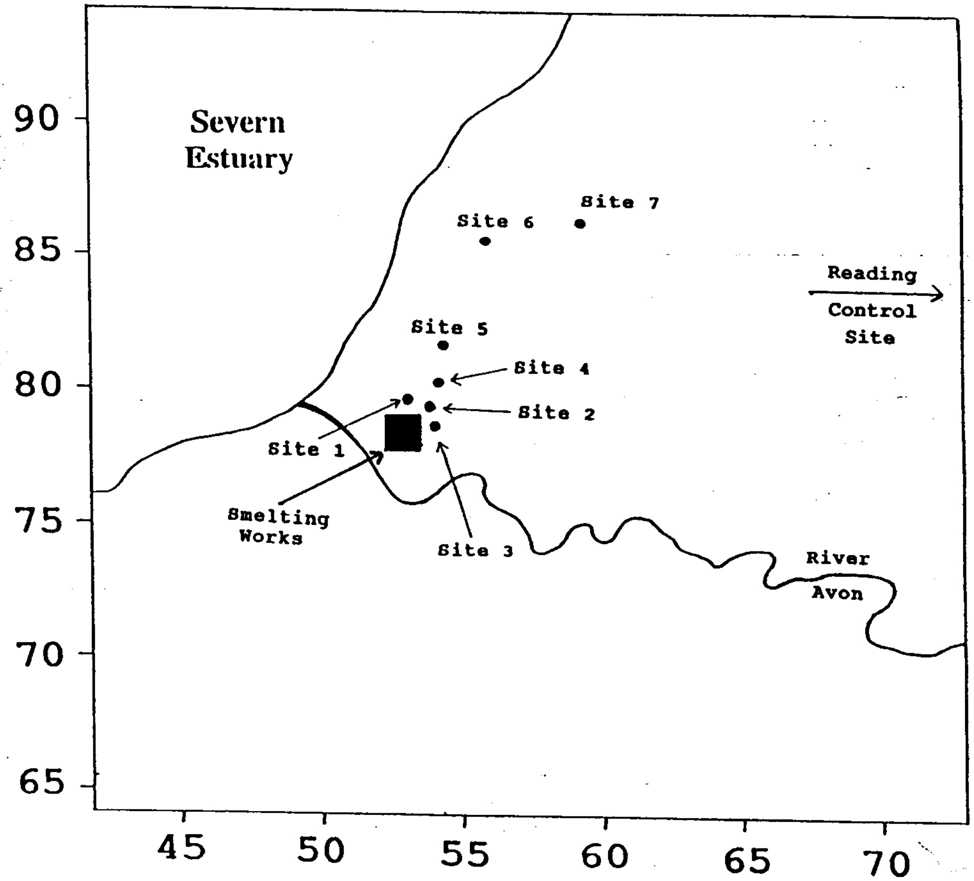

Fig. 1. Map to show location of sampling sites to the north-east of a zinc, cadmium and lead smelting works situated at Avonmouth, south-west England (Ordnance Survey grid references are given in km).

exposure to these metals individually and in combination. Furthermore, Kraak *et al.* (1993) concluded that the chronic effects of mixtures could not be predicted from their short-term effects, nor from the chronic effects of the metals tested individually. Thus, it seems that metal species may utilize novel uptake pathways and have different modes of action, which preclude additive toxic effects.

Results

The concentrations of cadmium, copper, lead and zinc in artificial soil that increased mortality and reduced growth and cocoon production in *E. fetida* (Table 2), are consistent with those obtained in previous studies on earthworms (Ma 1983, 1984, 1988;

Table 1. Location of study sites in the vicinity of Avonmouth and the pH, organic matter content and concentrations of metals in soils used in experiment 2

Site number	Distance from smelter (km)	OS Grid reference	Site soil (pH)	Site soil organic matter (%)	Soil Cd concentration ($\mu g\ g^{-1}$)	Soil Cu concentration ($\mu g\ g^{-1}$)	Soil Pb concentration ($\mu g\ g^{-1}$)	Soil Zn concentration ($\mu g\ g^{-1}$)
1	0.5	529794	6.6	17.2	312.2	2609.4	15 996	32 871
2	0.5	533790	6.3	22.0	129.9	779.9	6723	7945
3	0.9	535786	6.4	17.8	32.4	159.3	842	1987
4	1.8	532803	6.6	27.1	33.5	163.5	1245	2793
5	3	537817	7.3	18.5	14.3	107.5	930	1848
6	5.8	552853	7.3	12.9	0.9	36.2	245	657
7	7	578816	6.9	19.7	2.7	42.3	290	925
Control	110	737714	5.5	9.4	0.1	30.9	30	38

Table 2. EC_{50}[a] and estimated NOEC[b] determined in three toxicity experiments with the earthworm *E. fetida*

Cadmium	Copper	Lead	Zinc

Table 2. EC_{50}[a] and estimated NOEC[b] determined in three toxicity experiments with the earthworm *E. fetida*

	Cadmium		Copper		Lead		Zinc	
	EC_{50}	Estimated NOEC	EC_{50}	Estimated NOEC	EC_{50}	Estimated NOEC	EC_{50}	Estimated NOEC
Experiment 1								
Mortality	>300	>300	836 (721–939)	293	>10 000	4793	1078 (789–1449)	442
Cocoons	–295	152	–716	29	1629	608	–357	237
% Growth	215 (167–292)	207	601 (383–5823)	725	2249	1966	>400	>400
Experiment 2								
Mortality	>312	>312	>2609	>2609	>15 996	>15 996	>32 871	>32 871
Cocoons	–40	18	–296	119	–2131	492	3605	1879
% growth	211 (172–264)	83	1763 (1392–2303)	481	10 830 (8839–13521)	4064	22 371	5444
Experiment 3								
Mortality	>16.0 (13.0–18.5)	4.3	110 (101–118)	51	759 (688–825)	375	1730 (1599–1830)	1047
Cocoons	–5.6	2.1	–59	40	–391	275	–1001	833
% Growth	1.7 (1.4–2.2)	1.7	38 (37–40)	39	258 (234–273)	265	740 (641–827)	777

[a] Concentration of each metal predicted to cause a 50% increase in mortality in 14 days or a 50% reduction in cocoon production or growth rate in 21 days.
[b] Concentrations predicted to cause an exactly significant increase in mortality or decrease in cocoon production or growth rate.
See materials and methods for details of experimental design. Values for each metal in experiments 2 and 3 are determined using a non-additive model.

Table 3. Effects of contaminated field soils on the survival over 14 days, growth over 21 days as a percentage of initial weight and cocoon production over 21 days and cocoon viability over 35 days from the end of the experiment of the earthworm *E. fetida* in a laboratory toxicity test (experiment 2)

	% survival (14 days)	% growth	Cocoons or worms per week	% cocoons hatching	Number of juveniles per cocoon	Juveniles or worms per week
Site 1	100	21*	0.033***	100[1]	2.25[1]	0.08***
Site 2	100	20*	0.017***	100[1]	1.0[1]	0.02***
Site 3	100	65	0.042***	80[1]	2.5[1]	0.08***
Site 4	100	74	0.133**	81.3	2.54	0.28**
Site 5	92.5	61	0.288	87.5	2.1	0.54
Site 6	100	60	0.25	76.6	2.14	0.41
Site 7	100	46	0.345	82.9	2.32	0.66
Control	100	52	0.375	88.8	2.75	0.92

* Significantly different from controls at $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[1]Less than six replicate cocoons available.

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Evidence of additive

Neuhauser *et al.* 1985; Bengtsson *et al.* 1986; Van Gestel *et al.* 1989, 1991, 1992b; Spurgeon *et al.* 1994). Cocoon production was the most sensitive parameter, while mortality was the least sensitive.

No significant mortality of *E. fetida* occurred in any of the soils collected from the field (Table 3). The lack of mortality in the most polluted soils had not been expected. For example, the concentration of zinc in the soil from site 1 was 30 times greater than the 14 day LC_{50} value determined for this metal in artificial soil (Table 2). Furthermore, the level of zinc at site 1 was three times higher than the concentration that caused all *E. fetida* to die within 5 min in an earlier artificial soil test (Spurgeon *et al.* 1994).

Cocoon production rates were significantly reduced in soils from the four most contaminated sites (sites 1–4) (Table 3). A negative correlation was found between cocoon production rate and the log-concentration of each metal in the soils ($p < 0.02$ in all cases). No significant effects on the viability of cocoons or numbers of juveniles emerging per fertile cocoon were observed (Table 3). Worms exposed to soils from sites 1 and 2 grew significantly less than those maintained in soils collected further from the smelting works (Table 3).

To determine which metal in soils in the field is reducing the performance of worms in the vicinity of the factory a simple approach using a comparison of toxicity values from the field (experiment 2) and the single-metal artificial soil experiment (experiment 1) (expressed as ratios) was used. Multiple regression analysis to identify the element principally responsible for the observed effects could not be applied, as the concentrations of the four metals in the soils at each site were very highly correlated ($p < 0.001$).

Since no increase in mortality was observed in field soils in the laboratory, ratios for LC_{50} values cannot be determined. However, toxicity values for the most sensitive sublethal parameter (cocoon production rate) can be used for such comparisons. The ratios for the effects of cadmium, copper, lead and zinc on cocoon production (field soil : artificial soil) were 0.14:1, 0.4:1, 1.3:1 and 10.1:1 for EC_{50}s and 0.12:1, 4.1:1, 0.8:1 and 7.9:1 for estimated NOECs, respectively. If the reduction in toxicity for each metal is similar in the field soil, the metal with the largest ratio is likely to be the most limiting element. Since ratios were highest for zinc, this indicates that this metal is most likely to be affecting earthworm reproduction and, consequently, population viability at sites close to the smelting works.

Toxic effects on earthworms were less severe in field soils than in artificial soils containing mixtures of metals at the same concentrations (Tables 3 and 4). In the mixture test, mortality was increased significantly in soils containing metal levels similar to or above those found at site 5. All worms exposed to artificial soils containing metal levels similar to those at sites 1–4 died. Effects on sublethal parameters were also more severe than in the comparable field soils (Table 4). Cocoon production and growth rates were reduced significantly at all sites, except the least contaminated (site 6) and control sites.

Ratios of toxicity values between the mixture and single-metal experiment for cadmium, copper, lead and zinc were (mixture test : single metal test), <0.05:1, 0.13:1, <0.08:1 and 1.61:1 for LC_{50}s, 0.02:1, 0.08:1, 0.24:1 and 2.8:1 for cocoon production EC_{50}s and 0.01:1, 1.38:1, 0.4:1 and 3.5:1 for cocoon production estimated NOECs, respectively. The highest ratios were for zinc. Thus, it was almost certainly zinc that was responsible for the effects on earthworms in the artificial soils containing mixtures of metals.

Evidence of additive toxic effects of the metals on *E. fetida* was not found in this

Table 4. Effects of soils contaminated with mixtures of metals at the same concentrations as those present in field soils on survival, growth and cocoon production and viability of the earthworm *E. fetida* in laboratory toxicity tests (experiment 3)

	% survival (14 days)	% growth	Cocoons per worm per week	% cocoons hatching	Number of juveniles per cocoon	Juveniles per worm per week
Site 1	0***	–	0**	–	–	–
Site 2	0***	–	0**	–	–	–
Site 3	0***	–	0**	–	–	–
Site 4	0***	–	0**	–	–	–
Site 5	60***	−4.4**	0.06**	60	2.0	0.072**
Site 6	100	17.7	0.383	76	2.06	0.6
Site 7	92.5	6.1*	0.34*	76	2.39	0.618
Control	100	28.1	0.567	79	2.09	0.936

See legend of Table 3 for details of parameters measured.

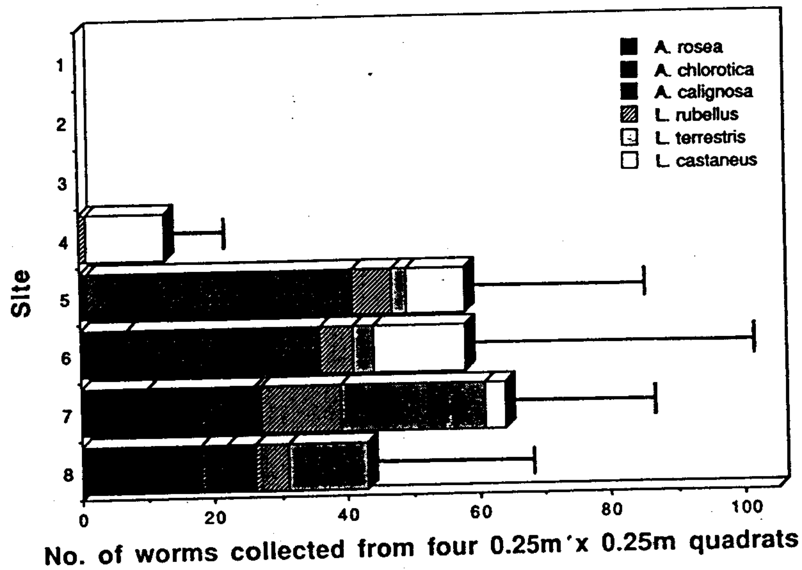

Fig. 2. Number of species of earthworms collected from the sites shown in Fig. 1. Error bars indicate SD of population mean determined from four replicates.

Table 5. Areas over which zinc concentrations in surface soils in the Avonmouth area exceed toxicity values calculated from experiments on *E. fetida* shown in Tables 2–4 (compare with Fig. 3)

	Area exceeded (km^2)			
	Mortality LC_{50}	Mortality estimated NOEC	Cocoon production EC_{50}	Cocoon production estimated NOEC
Artificial soil toxicity test (experiment 1)	77	299	411	681
Field soil toxicity test (experiment 2)	0	0	1	18
Mixtures toxicity test (experiment 3)	29	80	96	121

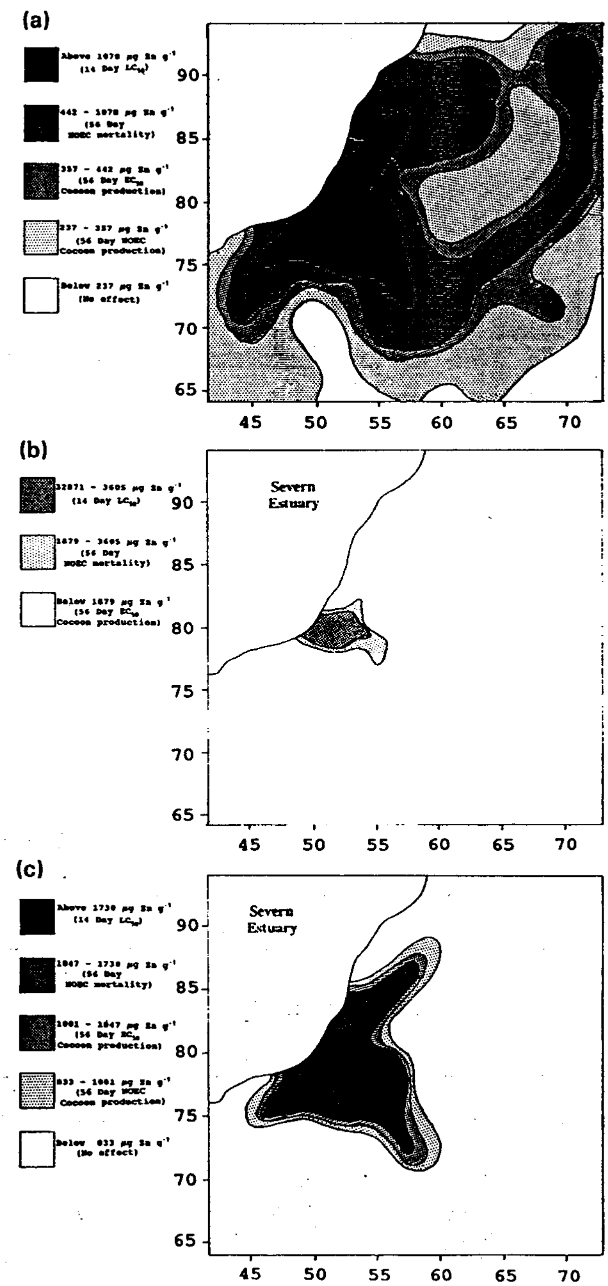

Fig. 3. Toxicity values of zinc for *E. fetida* determined in (a) experiment 1, (b) experiment 2 and (c) experiment 3 superimposed on concentrations of zinc in field soils from Avonmouth, south-west England determined by Jones (1991).

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study. Comparisons of LC_{50}s for zinc in the single-metal and mixture experiments, indicate that the toxicity of zinc may be slightly depressed in the presence of other metals. The LC_{50} value determined in the mixture experiment was above the upper 95% confidence interval of the LC_{50} value determined in the single-metal test (Table 2). However, further work will be required if the impact of mixtures of pollutants on earthworms at complexly polluted sites are to be fully understood.

No earthworms were present at sites 1–3 and only two species were present at site 4 (Fig. 2). At site 5 and sites further away from the smelting works, species diversities and numbers were similar to those at the control site. At sites 1–4, a thick layer of undecomposed leaf litter was present on the soil surface. At site 4, all the worms were confined to this material and there were none present in the underlying soil.

EC_{50} and estimated NOEC values determined for zinc in each experiment have been superimposed onto maps of concentrations of the metal (nitric acid digests) determined in soil samples collected from the vicinity of the smelting works as part of an earlier survey (Jones 1991) (Fig. 3). Maps of similar parameters for cadmium, copper and lead have not been drawn since the potentially affected areas would be overestimated due to the presence of zinc.

Areas over which concentrations of zinc in soils exceed calculated toxicity values for each experiment have been determined (Table 5). For some parameters (zinc cocoon production EC_{50} and estimated NOEC in the single-metal test), the potentially affected areas merged into unmapped regions over 25 km from the smelting works.

Discussion

Comparisons of toxicity values for each metal determined from experiments 1 and 2 indicate that of the four metals released by the smelting works, zinc is most likely to be preventing earthworms from colonizing soils in the vicinity of the factory. Toxicity values for zinc obtained in artificial soils were at least an order of magnitude lower than in field soils (Table 2), indicating that the toxicity of zinc is substantially lower in the field soil. The increased sensitivity of worms to zinc in artificial soil was probably due to differences in the bioavailability of this metal between the two soil types.

Differences in the availability of zinc between the soils can be explained by the characteristics of the respective soils and the experimental protocol used. Although the pHs of the soils were similar (6.1 in the artificial soil, 5.5–7.3 in the field soils), the artificial soil had a lower organic matter content than any of the field soils used (10% in the artificial soil, 12.9–27.15% in the field soils). Furthermore, the kaolin clay used in the artificial soil (which is uncommon in soils in Europe), has a low adsorption capacity for cations compared to more common clay minerals such as montmorillonite, illite and vermiculite. Thus, the artificial soil has a lower adsorption capacity for metals than the field soils.

The form in which the test chemical is added may also be critical in determining the decreased toxicity of metals in the artificial soil. Zinc was added to the test soil as a soluble nitrate salt. Although a short period (3 days) was allowed for the metal to stabilize, it is unlikely that the sorption kinetics had reached equilibrium after this time. Thus, in the laboratory test with artificial soil, the worms were exposed to high concentrations of unnatural metal species which they would not normally encounter in the field.

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Differences in the bioavailability of zinc in the field may cause problems when attempts are made to set 'critical concentrations' for this metal in soils. For example, *Lumbricus rubellus* (Hoffmeister) has been found living in mine spoil containing more than 180 000 $\mu g\ g^{-1}$ Zn (Corp and Morgan 1991; Morgan *et al.* 1993) and 40 000 $\mu g\ g^{-1}$ Zn (Morgan and Morgan 1991). At Avonmouth, earthworms survive in soils containing zinc concentrations of up to 3000 $\mu g\ g^{-1}$ Zn, but are absent from more heavily contaminated sites (Table 1, Fig. 2). Beyer *et al.* (1987) found that all earthworms were absent from plots 2 years after they had been treated with zinc chloride to give a concentration of only 470 $\mu g\ g^{-1}$ Zn. In the artificial soil test the LC_{50} for adult *E. fetida* was 1078 $\mu g\ g^{-1}$ Zn and the estimated NOEC for cocoon production was only 237 $\mu g\ g^{-1}$ Zn (Table 2). Thus, variation in bioavailability between soil types has a profound effect on the relative toxicity of a particular concentration of zinc to earthworms.

The results of the population survey indicate that some species of earthworms are more sensitive to metal pollution than others (Fig. 2). *Aporrectodea rosea* (Savigny) and *Allolobophora chlorotica* (Savigny) appear to be more sensitive to metal contamination than *L. rubellus* or *Lumbricus castaneus* (Savigny), since these species are absent from soils containing ~2800 $\mu g\ g^{-1}$ Zn, whereas *L. rubellus* or *L. castaneus* survive in such soils. Species-specific responses to metal pollutants have been found in many groups of soil invertebrates including earthworms (Morgan and Morgan 1988, 1991), isopods (Hopkin, 1990; Jones and Hopkin 1991; Hopkin *et al.* 1993) and Collembola (Bengtsson and Rundgren 1988; Tranvik and Eijsackers 1989; Tranvik *et al.* 1993). These results indicate that it is important to adopt a species-specific approach in ecotoxicological studies (Van Straalen and Ernst 1991; Hopkin 1993).

Several papers have been published recently in which models have been described for establishing 'acceptable levels' for pollutants in ecosystems. Van Straalen and Denneman (1989) and Wagner and Løkke (1991) have proposed that the 'acceptable level' should be set at a concentration above which only 5% of species are adversely affected (that is, hazardous concentration for 5% of species; 'HC5' value). Van Straalen (1993) used a model based on an assumption of a log-logistic distribution of reproductive NOEC toxicity data to predict HC5 values of 0.2 $\mu g\ g^{-1}$ Cd, 2.7 $\mu g\ g^{-1}$ Cu and 77 $\mu g\ g^{-1}$ Pb. An HC5 value could not be determined for zinc since insufficient toxicity data were available from the literature.

The toxicity values used in all models are reproductive NOECs obtained during laboratory toxicity tests. In the work of Van Straalen (1993), much of the toxicity data used was from reproductive tests on earthworms (three out of eight values for cadmium, four out of seven for copper and four out of eight for lead). The experiments described in this paper (Tables 2–5) show that laboratory tests may be over-sensitive in predicting effects on earthworms at polluted sites. This is due probably to differences in bioavailability between the test and field soils. Thus, the HC5 approach may yield 'safe levels' which seem overcautious in comparison to the concentrations of metals that actually cause effects on individuals and populations at polluted sites. This is indicated by the fact that there is currently no evidence of deleterious effects on any species caused by the concentrations currently proposed as HC5 values for metals in soils (Hopkin 1993). There is clearly an urgent need to develop our knowledge of the effects on metal toxicity of differences in bioavailability under both test and field conditions.

Acknowledgements

The authors wish to th
was supported by rese
for D.J.S.

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Acknowledgements

The authors wish to thank Emma Stabler for help with earthworm sampling. This work was supported by research grants from NERC and a research studentship from SERC for D.J.S.

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