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Comparisons of metal accumulation and excretion kinetics in earthworms (*Eisenia fetida*) exposed to contaminated field and laboratory soils

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Abstract

The uptake and excretion kinetics of cadmium, copper, lead and zinc were studied for *Eisenia fetida* exposed to mixtures of these metals in field and OECD artificial soil. Body burdens in worms exposed to all contaminated soils increased over the duration of the experiment. Highest accumulation rates were for worms exposed to the most polluted soils. Pronounced differences were found in the uptake and excretion patterns for essential and non-essential elements (particularly in field soils). For cadmium and lead (non-essential), an equilibrium plateau was not reached during the uptake study and slow excretion was found on transfer of worms to clean soil. For copper and zinc (essential), fast initial uptake was followed by equilibrium after only a few days exposure. Rapid excretion was found after transfer to clean soil, with half-lives of less than 1 day for both metals. A previous study of the effects of metals on worms exposed in OECD and field soils had indicated a higher toxicity in the artificial medium. Thus, in the present study, it was anticipated that greater toxicity would be reflected by increased body burdens for worms in OECD soil. This was, however, not the case. Explanations are given that might account for the fact that the greater toxicity in OECD soil is not invariably accompanied by higher metal burdens. These include the presence of high concentrations of very toxic and highly available ions in laboratory tests and potential differences in the importance of soluble and total metal concentration for determining toxicity and body burdens. © 1999 Published by Elsevier Science B.V.

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1. Introduction

A range of factors has been found to determine the levels of pollutants accumulated by soil invertebrates. These include: the characteristics of the chemical (Belfroid et al., 1993; Janssen and Hogervorst, 1993), the concentration present in the soil (Hopkin, 1993; van Brummelen et al., 1996), substrate properties (Ma, 1987; Perämäki et al., 1992), temperature (van Hattum et al., 1993; Spurgeon et al., 1997), size of the individual (van Hattum et al., 1991) and the physiology of the species (Janssen et al., 1991; Morgan and Morgan, 1991; Hopkin et al., 1993). For some chemicals such as persistent lipophilic organic compounds, accumulation can also be related to the trophic status of the exposed species (Walker and Livingstone, 1992). However, for metals, body bur-

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dens are probably linked more strongly to the physiology of the species than to trophic level (van Straalen and van Wensem, 1986; Laskowski and Maryanski, 1993).

Studies of steady-state metal burdens have indicated that some elements are present at increased concentrations in earthworms inhabiting contaminated soils (for references see Ireland, 1983; Morgan et al., 1993; Romijn et al., 1994; Spurgeon and Hopkin, 1996a). The capacity of earthworms to assimilate metals has led to this group being recommended for monitoring the spatial distribution and effects of pollutants in the field (Samiullah, 1990). However, despite the volume of data that details metal concentrations in earthworms collected from contaminated soils, relatively little is known of the accumulation and excretion kinetics of individual metals.

Studies of accumulation kinetics and the factors that influence uptake and loss have a key role in ecotoxicology, as they can be used to predict the physiological fate of a pollutant (Walker et al., 1996). An approach to studying uptake and excretion has been described by Moriarty and Walker (1987). This system, which is based on the use of compartment models can be applied to calculate pollutant fluxes within the tissues of the exposed organism. A number of studies has used kinetic models to describe the accumulation of xenobiotics by soil invertebrates. Belfroid et al. (1995) modelled the accumulation of halogenated aromatic hydrocarbons in *Eisenia fetida*, while Janssen et al. (1991) studied the uptake and loss of cadmium by four soil arthropod species. Both studies demonstrated that compartmental models can be used to describe uptake and excretion. Similar approaches have been used in this study to assess the accumulation and excretion of two essential (copper and zinc) and two non-essential (cadmium and lead) metals.

2. Materials and methods

The accumulation and excretion kinetics of the four metals were assessed in time series studies with the earthworm *Eisenia fetida* in two soils. For the first experiment, uptake and excretion were determined in worms exposed to contaminated field soils collected from sites located in the area around a primary zinc/lead/cadmium smelting works situated at Avonmouth in south-west England and an uncontaminated (control) soil collected from the campus of the University of Reading. Previous studies have indicated that soils adjacent to the Avonmouth factory contain elevated levels of cadmium, copper, lead and zinc (for full details of metal contamination in the region and the accompanying effects on wildlife see Hopkin, 1989; Martin and Bullock, 1994 and Spurgeon and Hopkin, 1996b). In the second experiment, worms were exposed to mixtures of cadmium, copper, lead and zinc added to OECD (1984) artificial soil in similar ratios to those found in soils in the Avonmouth region.

2.1. Experiment 1: Accumulation and excretion in contaminated field soils

Field soils were collected from three of the eight sites used in a previous experiment conducted by Spurgeon and Hopkin (1995) to determine toxic effects on the survival and cocoon production of *Eisenia fetida* (for locations see Fig. 1 in Spurgeon and Hopkin, 1995 and Table 1 of this paper). The sites selected were Site 1, a heavily polluted location at which earthworms are absent; Site 4, a moderately polluted site at which there is a reduced earthworm fauna and Site 8, a clean soil collected from the campus of the University of Reading in which a normal earthworm fauna exists (Spurgeon and Hopkin, 1996b). All sites were permanent grassland situated adjacent to minor roads at least 2 m from the kerb.

At each site, a large volume of soil sufficient to obtain 20 kg after drying was collected from the top 2 cm layer after removal of surface vegetation and litter. After transfer to our laboratory, soil aggregates were broken up while still damp and placed in an oven at 60°C until dry. Soils were then sieved through a 4 mm mesh and 10 kg placed into a single experimental container for each site ($450 \times 340 \times 240\ mm^3$). After sieving, a sample of soil was taken for analysis of pH, percentage loss on ignition (% LOI), metal content, maximum water holding capacity and field capacity. Sufficient distilled water was then added to each container to give a moisture content of 60% field capacity. Finally, soils were left to stabilise for 1 week prior to the introduction of sufficient earthworms. The worms used for the experiment were *Eisenia fetida* collected from cultures maintained in our laboratory. All individuals

Table 1
Ordnance survey grid reference (OSGR) or control and polluted sites and the ph, % loss on ignition and concentrations of metals (on a dry weight basis) in the collected field soils and the soil mix used for the elimination study

Site no.	Site location		Soil parameters					
	OSGR	Distance from smelter (km)	pH (range)	% loss on ignition	Cadmium (μg Cd g^{-1})	Copper (μg Cu g^{-1})	Lead zinc (μg Pb g^{-1})	Zinc (μg Zn g^{-1})
8	737 714 (Reading campus)	110	5.93–6.08	9.5	0.084	17.4	37.9	56.6
4	532 803	1.8	6.04–6.56	14.1	44.5	401	3100	7310
1	529 794	0.5	5.95–6.67	15	325	2800	19400	43300
Elimination soil	–	–	5.78–6.1	26.5	0.1	46	189	125
OECD 3	–	–	–	–	<0.05	1.8 ±0.2	7.95 ±1.08	20.4 ±0.3
OECD 2	–	–	–	–	4.6 ±0.3	39.4 ±0.7	322 ±53	312 ±4
OECD 1	–	–	–	–	13.7 ±0.4	115 ±4	656 ±32	1420 ±60

Mean ± SE values, all values based on six replicates).
Actual measured metal concentration are also given for the OECD soils.
* = Metals for which mean concentrations differ by a factor of at least 2 between the accumulation and elimination phase soil batches.

were at least 8 weeks old and weighed between 150–800 mg fresh weight.

To assess the uptake and loss of the four metals, time series studies of accumulation and excretion were undertaken. For the accumulation experiment, earthworms were sampled from the culture prior to the start of the experiment (Day 0) and from the three field soils at 1, 3, 7, 10, 14, 21, 28, 35 and 42 days after introduction. At each sample interval, eight earthworms (reduced to six after 10 days in Site 1 soil due to high mortality) were sorted from each test soil and starved on moist filter for 24 h to allow them to void their gut. The paper was changed twice during the starvation period (at 6 and 18 h). After starvation, the worms were stored at −20°C to await analysis. For the excretion study it was first necessary to 'pre-load' worms with metals. Thus, worms were exposed to the three field soils for 42 days to allow uptake. At the end of this accumulation period, surviving worms were transferred into a clean soil for the excretion phase of the experiment. The soil used for the excretion phase was a mixture of commercially available topsoil (Rockall, Wokingham, UK) and 4 mm sieved Sphagnum peat (Bullrush, Bellaghy, UK) in a 3 : 1 by weight ratio. This soil mixture has been found to be a suitable medium for the long-term incubation of *Eisenia fetida* in previous experiments conducted in our laboratory. Eight worms were sampled from each soil at 1, 3, 7, 14, 21, 30, 40, 50, 60, 80 and 100 days after the start of the excretion phase. The worms were starved and frozen to await analysis.

Eisenia fetida usually live in organic-rich localities such as manure or compost heaps and are unable to obtain sufficient nutrition from either natural field soils or the OECD (1984) artificial medium (van Gestel et al., 1992). Thus, to avoid excessive weight loss over the duration of the experiment, which can affect pollutant concentrations (Spacie and Hamelink, 1985), 0.25 g dry weight food per worm per week (fresh horse manure dried and re-wetted to 80% water holding capacity) was added to soils throughout the experiment. This quantity of food was considered to be sufficient to maintain earthworm weights (Cluzeau and Fayolle, 1989), while limiting the build up of uneaten organic material that may reduce metal availability in the soils. The use of uncontaminated food would not be expected to affect metal accumulation rates since available evidence suggests that the accumulation of metal in earthworms occurs primarily by direct diffusion from soil solution rather than from food (Spurgeon and Hopkin, 1996c; van Gestel, 1997).

2.2. Experiment 2: Accumulation in OECD artificial soil

In addition to studying the uptake of metals from contaminated field soils, worms were also exposed to mixtures of cadmium, copper, lead and zinc in artificial soil. The artificial soil used in this study was developed by the OECD (1984). This medium, which consists of a mixture of 70% fine (silver) sand, 20% kaolin clay and 10% coarse ground *Sphagnum* peat with powdered calcium carbonate added to adjust soil pH to 6.1 ± 0.2, has been widely used for toxicity testing with earthworms (for further details of preparation of the test soils, see van Gestel et al., 1989 and Spurgeon et al., 1994). Two contaminated and one uncontaminated soil were used for the experiment, with 10 kg of soil in one test container used for each concentration. To allow accumulation rates in the artificial soil to be compared with those in contaminated field soil, metals were added to the soil in similar ratios to those found at Avonmouth. Analysis of the data of Jones (1991), who measured metal levels in soils from 88 sites in the region, indicated that the ratios for cadmium, copper, lead and zinc were approximately 1: 10: 60: 100 on a weight basis. Thus, metals were added to the artificial soil in similar ratios. Ratios were calculated as mean values of the 88 sites sampled by Jones (1991), since this would ensure that values were relevant to those present across the contaminated region around the smelter, rather than the limited number of sites selected for this study.

Previous experiments comparing relative toxicity of cadmium, copper, lead and zinc with metal levels in soils at Avonmouth have indicated that zinc is the metal most likely to cause deleterious effects on earthworms (Spurgeon and Hopkin, 1995; Spurgeon et al., 1994) and other soil species (Hopkin and Hames, 1994; Laskowski and Hopkin, 1996a; Sandifer and Hopkin, 1996). Thus, if mixtures of metals are prepared in artificial soils based on the metal concentrations at Avonmouth toxic effects on exposed worms would be attributed primarily to added zinc. Studies of zinc toxicity in OECD soil have demonstrated that significant mortality of *Eisenia fetida* occurs at concentrations in excess of 1200 $\mu g\ g^{-1}$ dry weight (van Gestel et al., 1993; Spurgeon et al., 1997; Spurgeon and Hopkin, 1996c). Thus the highest nominal soil zinc concentration used in the experiment was 1160 $\mu g\ g^{-1}$ (Table 2). Metals were added to the artificial soils as solutions of the nitrate salts. Sufficient water was also added to produce the required water content. After addition of the water and metals, the soils were left to stabilise for 1 week prior to the addition of *Eisenia fetida* (100 in the two least contaminated soils, 150 in the most contaminated). Earthworm maintenance and sampling followed the procedures described for the field soil accumulation experiment, with six earthworms sorted from the test soil after 1, 3, 7, 10, 14, 21, 28, 35 and 42 days.

2.3. Analytical procedures for soil and earthworms

Field soil samples for analysis of pH, weight loss on ignition (% LOI) and metal content were collected immediately after drying and sieving, while artificial soils for metal analysis were collected at the end of the 1 week stabilisation period. Soil % LOI was measured to estimate the organic matter content (%OM). To measure % LOI, dried soils were heated for 12 h at 500°C. Soil pH was measured in water following the procedure of Haynes (1982). For the analysis of soil metal content, approximately 1 g of test soil was placed into a conical flask with 10 ml of concentrated nitric acid. The flask was heated until all organic matter had been digested and the digests were then diluted to 100 ml with double distilled water. The solution was analysed by flame atomic absorption spectrometry (Varian Spectra 30 AAS). The measured concentration was verified by comparison with standard reference materials as described in Spurgeon and Hopkin (1996a) and was within 10% of certified values.

Analysis of the earthworm metal burdens was conducted using a method adapted from the procedure of Hopkin (1989). Whole worms were placed into acid-washed test tubes and dried to constant weight. 2 ml of Analar grade nitric acid was added to each tube and the solution heated until all tissue had been digested. Once cool, the digest was diluted to 10 ml with double distilled water and analysed for cadmium, copper, lead and zinc by flame AAS.

2.4. Statistical analysis of bioaccumulation and excretion

To model metal accumulation and allow accumulation and excretion rates to be calculated, a linear

Table 2
Kinetic parameter estimates for cadmium, copper, lead and zinc uptake and excretion by the earthworm *Eisenia fetida* exposed to three polluted field soils and three OECD soils contaminated by the addition of metals as nitrate salts.

	Soil	Co (μg worm^{-1})	*a* (μg worm^{-1} day^{-1})	*k* (day^{-1})	Half-life (days)	Equilibrium content (μg g^{-1})
Cadmium	Site 8	0.181	0.000003	0	∞	∞
	Site 4	0.427	0.0255	0	∞	∞
	Site 1	0.467	0.034	0	∞	∞
	OECD 3	0.0127	0	0	ND	0.0127
	OECD 2	0.0718	0.0249	0.0808	8.58	0.38
	OECD 1	0.0143	0.0295	0.0263	26.4	1.27
Copper	Site 8	0.419	0	0	ND	0.419
	Site 4	1.002	1.33	1.63	0.43	1.82
	Site 1	0.901	1.08	1.19	0.59	1.81
	OECD 3	0.305	0	0	ND	0.305
	OECD 2	0.3	0	0	ND	0.3
	OECD 1	0.307	0.0116	0.0274	25.2	0.73
Lead	Site 8	−0.066	0.419	0.387	1.79	1.02
	Site 4	0.092	5.56	1.15	0.603	4.97
	Site 1	0.236	1.187	0.106	6.54	11.41
	OECD 3	−0.0596	0.0537	0.134	5.16	0.34
	OECD 2	−0.0789	0.066	0.0241	28.8	2.66
	OECD 1	−0.0522	0.0887	0.465	14.9	1.86
Zinc	Site 8	4.89	0	0	nd	4.89
	Site 4	4.9	2.44	1.34	0.52	6.72
	Site 1	4.88	6.3	1.84	0.378	8.31
	OECD 3	2.67	0.0282	1.1	0.631	4.41
	OECD 2	2.62	0	0	ND	2.62
	OECD 1	2.6	0	0	ND	2.6

For details of soil charactersitics see Table 1. For definitions of the parameter esimates and details of procedure for their calculation see Eqs. (1) and (2).
Negative values for *a* and *k* are given as zero values. Half-life is calculated by ln 2/*k* and equilibrium content by Co + (*a*/*k*) according to Atkins (1969).
ND indicates that a value could not be determined.

compartment model was fitted to the data (Atkins, 1969). Previous studies of the uptake of metals by soil arthropods (Janssen et al., 1991) and earthworms (Posthuma and Notenboom, 1996; Marinissen, 1997) have indicated that uptake can be described by a one-compartment model. To model uptake changes in earthworm metal burdens over both the uptake and excretion phases of the experiment, least-squared fitting of the data was conducted using the following two equations:

$$\text{for } t \leq tc: \; Q_t = C_0 + \frac{a}{k}\left[1 - \mathrm{e}^{kt}\right] \tag{1}$$

$$\text{for } t > tc: \; Q_t = C_0 + \frac{a}{k}\left[1 - \mathrm{e}^{kt}\right] - \frac{a}{k}\left[1 - \mathrm{e}^{-k(t-tc)}\right] \tag{2}$$

where:

Q_t	Total amount of metal in the animal (μg)
t	time (days)
C_0	Amount of metal at $t = 0$
a	Accumulation rate (μg day^{-1})
k	Excretion rate (day^{-1})
tc	Time at which animals were transferred to clean soil

For the statistical analysis of pollutant kinetics, it is recommended that body burdens rather than actual concentrations be used (Moriarty, 1983), since the latter can be affected by rapid changes in the weight of the organism (Spacie and Hamelink, 1985). Kinetic trends and parameters have therefore been determined on the basis of body burdens throughout this paper. The calculations of accumulation and excretion parameters were conducted using the SAS statistical software package.

3. Results

3.1. Experiment 1: Accumulation and excretion in contaminated field soils

3.1.1. Characteristics of field soils

Measurements of the three field-collected soils indicated similar pHs, with values ranging from 5.93–6.67. The % LOI was also found to be broadly similar for the three soils. The lowest value of 9.5% was found for soil collected from Site 1, while the highest values of 15% was for the Site 8 soil (Table 1). The similarities found in soil pH and % LOI indicate that these factors are unlikely to contribute to any difference found in metal kinetics in the three selected soils. Highest metal concentrations were found in soils collected from Site 1, where total metal content was approximately 6.5% of the dry weight (Table 1). Site 4 showed intermediate (although still elevated) levels of metals, while concentrations in the control (Site 8) soil were within the range typical for an 'uncontaminated' soil (Merian, 1991). The pH for the soil mix used in the elimination phase of the study was similar to the values found in the three field soils (Table 1). However, % LOI was higher due to the use of peat in the mix. Analysis of metal levels in the elimination soil indicated that cadmium levels were similar to the control soil, while copper, lead and zinc levels were somewhat higher, although still within the range of uncontaminated soil.

3.2. Uptake patterns and accumulation and excretion kinetics

For worms exposed to the clean soils, no consistent trends of uptake were found for any of the metals examined in this study (Fig. 1(a), Fig. 2(a), Fig. 3(a), Fig. 4(a)). However, in the worms exposed to the two polluted soils, the four metals were generally accumulated. Thus, cadmium, copper, lead and zinc burdens were higher at the end of the accumulation period than at Day 0 (Fig. 1(b,c), Fig. 2(b,c), Fig. 3(b,c) and Fig. 4(b,c)). Different time-dependent patterns of uptake were found for non-essential and essential elements. For the xenobiotic metals cadmium and lead, body burdens failed to reach equilibrium in all cases except for lead in Site 4 soil (Fig. 1(b,c), Fig. 3(b,c)). However, for the essential elements, copper and zinc, equilibrium was reached within the first 7 days of exposure in all soils (Fig. 2(b,c) and Fig. 4(b,c)). From the available data it was possible to calculate a number of the kinetic parameters – initial concentration, accumulation and excretion rate, half-life and theoretical equilibrium concentration. Calculation of accumulation rates for the selected metals gave highest values in the polluted soils (Table 2). Differences were also found in the accumulation rates of the selected metals. Generally values were low for cadmium, intermediate for copper and lead and high for zinc (Table 2). Neuhauser et al. (1995) found similar patterns of uptake and excretion by *Eisenia fetida* exposed to these metals added singly to natural soils.

For each sample interval, highest body burdens for all metals were generally found in worms exposed to the heavily polluted soil from Site 1 indicating that uptake was related to soil metal concentration. The dependence of uptake on soil metal concentrations is clear if theoretical equilibrium concentrations are determined for worms exposed to the three field soils using the equation $C_0 + a/k$, where C_0 is the amount of metal at $t = 0$, a is the accumulation rate and k is the excretion rate (Atkins, 1969). Calculated values for lead and zinc indicate higher equilibrium concentrations in worms exposed to the most contaminated soils; for copper, values in the two polluted soils are similar, but greatly exceed control soil values (Table 2). However, for cadmium, equilibrium concentration could not be calculated due to the fact that the calculated elimination rate was effectively zero in the three field soils. The absence of elimination in this study indicates that cadmium body burdens over 42 days at a given soil concentration will be dependent on the duration of exposure. It currently remains unclear if such time-dependent accumulation will extend over longer exposure periods.

The excretion phase of the study further demonstrated differences in the kinetics of non-essential and essential metals. For cadmium, body levels were not reduced for worms previously exposed to soils from Site 1 (Fig. 1(c)) and showed only a slow decrease in the Site 4 worms (Fig. 1(b), Table 2). For lead, no

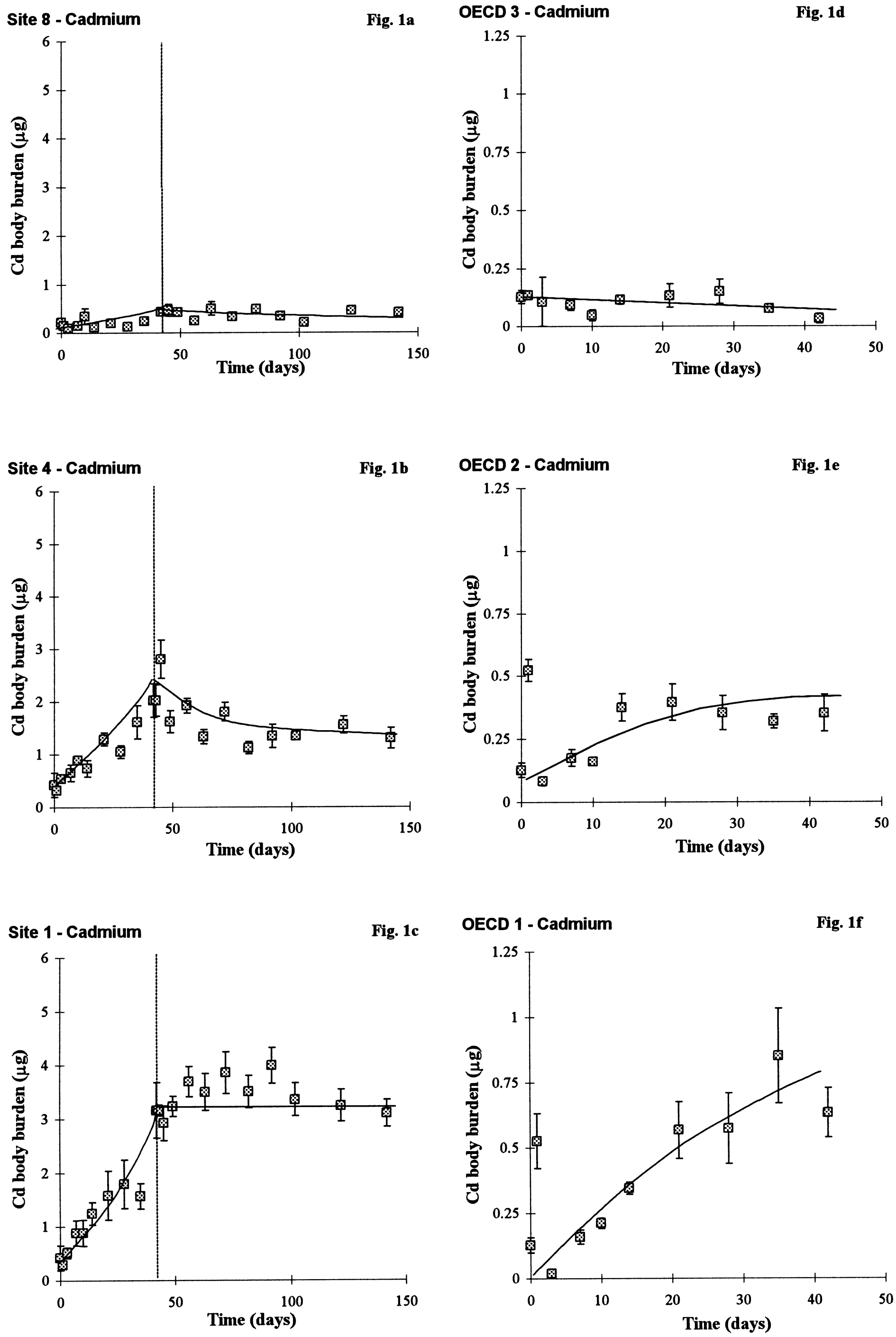

Fig. 1. (a)–(c). Accumulation and excretion of cadmium in *Eisenia fetida* exposed to field soils collected from the three sites located at different distances from a smelting works situated at Avonmouth, UK, and (d)–(f) uncontaminated and spiked artificial soil. For a full description of the location of the sites and chemical parameters, including metal concentrations, in the collected soils see Table 1. Original data are given as means of 8 (6 from Day 10–Day 42 in Site 1 soil) replicate worms with their standard errors. ——— (solid line) indicates the fitted one compartment model, - - - (dashed line) indicates the start of the elimination period.

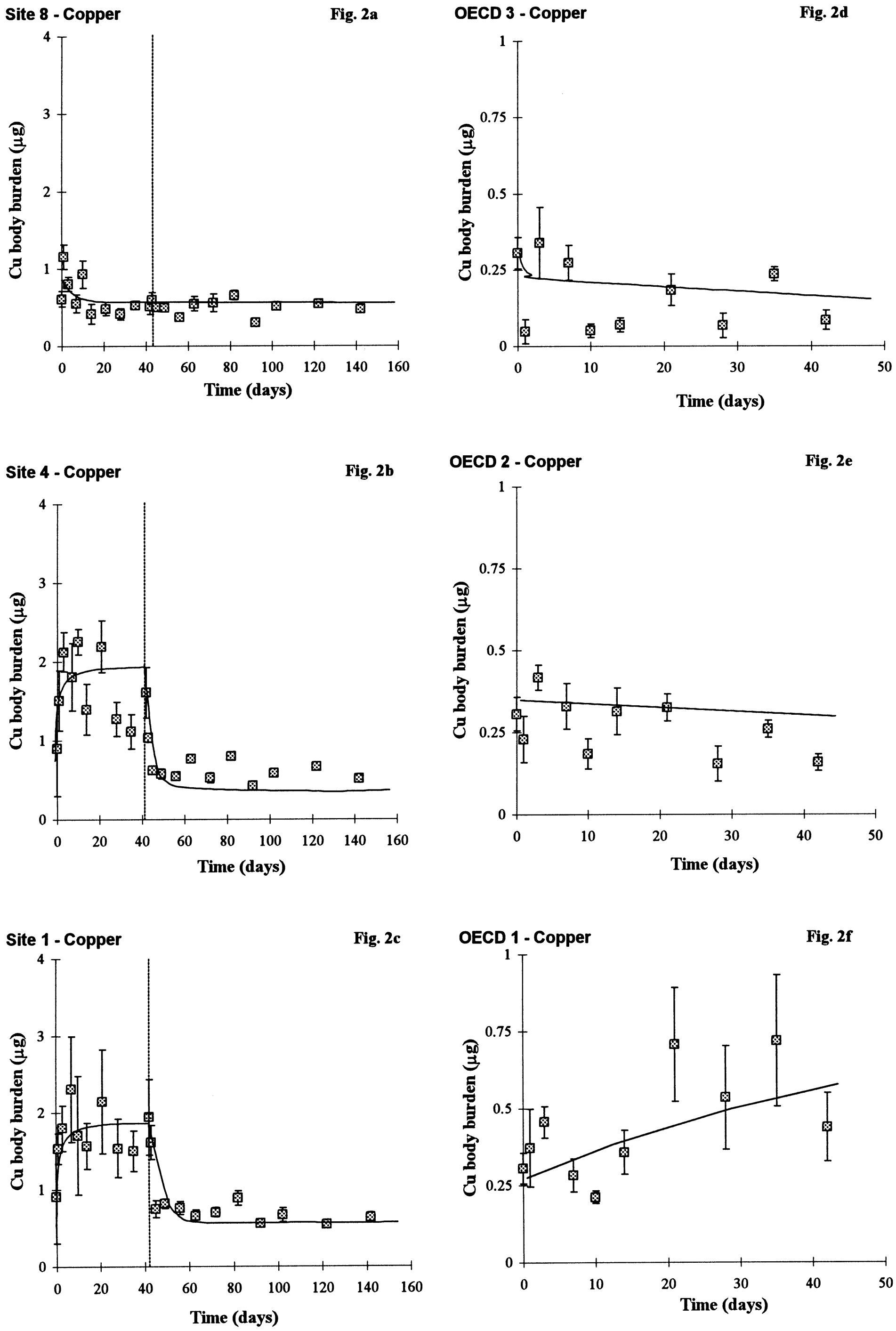

Fig. 2. (a–c) Accumulation and excretion of copper in *Eisenia fetida* exposed to field soils collected from the three sites located at different distances from a smelting works situated at Avonmouth, UK and (d–f) uncontaminated and spiked artificial soil. For further details see legend for Fig. 1.

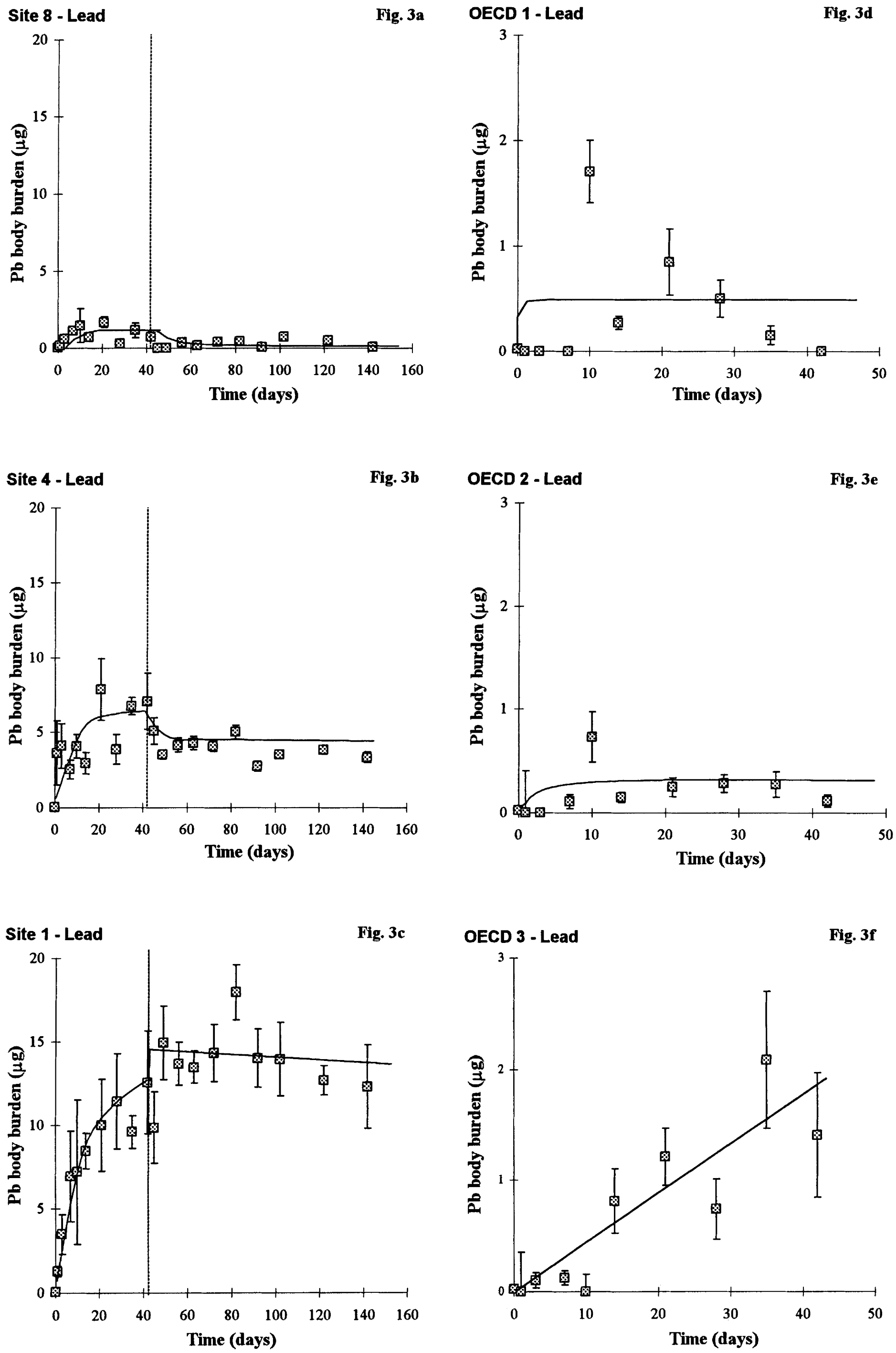

Fig. 3. (a–c) Accumulation and excretion of lead in *Eisenia fetida* exposed to field soils collected from the three sites located at different distances from a smelting works situated at Avonmouth, UK and (d–f) uncontaminated and spiked artificial soil . For further details see legend for Fig. 1.

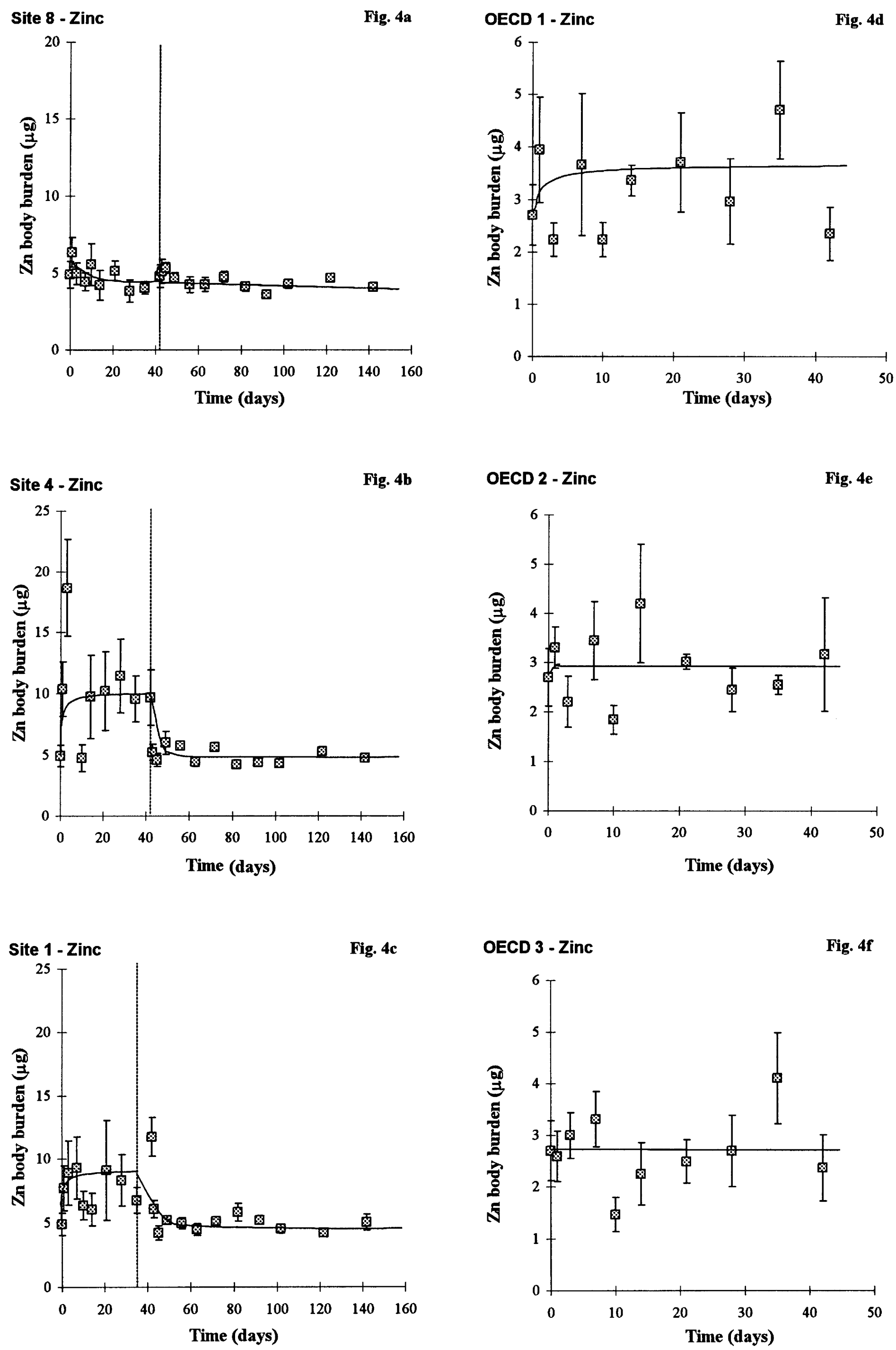

Fig. 4. (a–c) Accumulation and excretion of zinc in *Eisenia fetida* exposed to field soils collected from the three sites located at different distances from a smelting works situated at Avonmouth, UK and (d–f) uncontaminated and spiked artificial soil. For further details see legend for Fig. 1.

excretion was observed in the Site 1 worms (Fig. 3(c)), while for the Site 4 worms an initial faster period of excretion was followed by stabilisation at an elevated level (Fig. 2(b)). In contrast to the two xenobiotic metals, rapid rates of excretion were found in all cases for the essential metals, copper and zinc (Figs. 2 and 4, Table 2). Thus, the levels of these decreased to values close to those found in unexposed worms within 3 days (Figs. 2 and 4). Subsequent to this, the levels of copper and zinc stabilised at concentrations comparable to worms in the control soil and showed no further fluctuations over the excretion period.

Observed differences in the excretion patterns for each metal are confirmed by an estimation of biological half-lives calculated from ln 2/k (Atkins, 1969). For worms exposed to the heavily polluted soil collected from Site 1, half-lives of 0.59 days for copper, 6.54 for lead and 0.378 for zinc can be calculated (Table 2). A value could not be calculated for cadmium, since the excretion rate were effectively zero for this metal. Comparisons of calculated half-lives with the results of the excretion study indicate good agreement for copper and zinc, where rapid excretion was recorded and for cadmium for which no excretion was found. For lead, poor agreement was found between the calculated half-lives and the results of the excretion study. Thus, it was predicted that body burdens would decrease by 50% in 6.54 days, whilst in fact no excretion was found over 100 days (Fig. 3(c)). Differences between predicted and actual excretion can be accounted for by the presence of lead in a mobile pool, which can be excreted immediately after entering the body, but which is subsequently immobilised and stored.

3.3. Experiment 2: Accumulation in OECD artificial soil

3.3.1. Measured metal concentrations

Measured metal concentrations were generally in good agreement with nominal values for all metals (Table 2). Measurement of the control soils indicated the presence of very low levels of lead, copper and zinc, but no cadmium was detected. Measured metal concentrations have been used in all statistical calculations.

3.3.2. Patterns of metal accumulation from contaminated OECD soils

No consistent patterns of increase were found for any of the four elements in the control worms (Fig. 1(d), Fig. 2(d), Fig. 3(d), Fig. 4(d)), while in the contaminated OECD soils, body burdens were generally increased over the exposure period for all metals except zinc (Fig. 1(e,f), Fig. 2(e,f), Fig. 3(e,f), Fig. 4(e,f)). Highest accumulation rates were usually found for worms exposed to the heavily contaminated OECD 1 soil (Table 2). However, these trends were not as clear as for the field soils. Calculation of theoretical equilibrium concentrations ($C_0 + a/k$) for the four metals indicated clear concentration dependence only for cadmium. For lead, the values for the contaminated OECD 1 and 2 soils cannot be distinguished, while for copper similar values were found in the two least polluted (OECD 2 and 3) soils (Table 2). No trend was found for zinc, for which the highest values were calculated for the clean soil (Table 2).

For the non-essential metals, cadmium and lead, metal accumulation patterns were similar to those observed in the contaminated field soils (Figs. 1 and 3), although some differences in the kinetic parameters were recorded. For cadmium, accumulation rates in the contaminated OECD soils were comparable to those in the polluted field soils (Table 2), despite the fact that the latter soils contained up to 90 times as much cadmium as in the artificial soils. However, unlike in the polluted field soils where excretion was found effectively to be zero, excretion rates could be calculated for this metal. From these values half-lives of 8.58 and 26.4 days were determined for cadmium in the OECD 2 and OECD 1 soils, respectively (Table 2). For lead, the kinetic parameters were estimated excluding obvious outlier values at Days 3 and 14. Calculation indicated that both accumulation and excretion rates were lower in the contaminated OECD soil than in the polluted field soils (Table 2). This is to be expected, since the latter soils contained higher levels of this metal. Half-lives for lead in the contaminated artificial soils were 14.9 and 28.8 days and were thus longer than those for the polluted field soils.

Accumulation patterns for the essential metals showed marked differences between the OECD and field soils. In the field soils, a rapid initial period of accumulation occurred after which body burdens reached equilibrium (Figs. 2 and 4). However, in the two contaminated OECD soils, no clear trend for accumulation could be observed for zinc (Fig. 4(e,f)), while for copper an almost linear trend of uptake was found in OECD 1 (Fig. 2(f)) and no accumulation was found in the OECD 2 soil (Fig. 2(d)). Due to the absence of consistent patterns of uptake for essential metals in OECD soil, it was not possible to calculate the kinetic parameters for these metals, with the exception of copper in the OECD 1 test (Table 2).

4. Discussion

4.1. Importance of accumulation and excretion in elucidating metal metabolism

4.1.1. Physiological fates of metals

Kinetic studies can be used to predict the physiological fates of pollutants in exposed organisms. In particular, analysis of excretion patterns gives useful information on probable detoxification mechanisms. Three potential pathways exist for the removal of chemicals from sensitive tissues; elements can be regulated by excretion from the body, bound within the matrix of inorganic granules or attached to proteins or other ligands (Tessier et al., 1994). If metals are detoxified primarily by excretion, body concentrations should decrease when previously exposed individuals are transferred to a clean environment. However, if an element is bound in an inorganic matrix or to organic ligands, metal levels may remain constant, even after exposure has ceased.

The differences found in the excretion kinetics of essential and xenobiotic elements in *Eisenia fetida* suggest contrasting physiological fates for these metals. The high excretion rates found for copper and zinc indicate that these metals are detoxified primarily by excretion. Rapid rates of loss of copper and zinc have been found in studies with a number of invertebrate species (Hopkin, 1989; Dallinger, 1993), although for haemocyanin-dependent groups such as molluscs and isopods, copper excretion rates may be lower (Hopkin, 1990; Laskowski and Hopkin, 1996b). The ability of earthworms to eliminate excess copper and zinc is probably dependent on the essential nature of the element (Hopkin, 1995). Thus, physiological pathways, possibly based on carrier systems, already exist for the physiological control of these elements (Morgan and Morgan, 1991).

For the xenobiotic metals, cadmium and lead, excretion was slow or absent. Thus for these metals, it is probable that the main detoxification pathways are sequestration within inorganic matrices or binding to organic ligands. The presence of storage detoxification systems for cadmium and lead have been demonstrated in a number of earthworm species (Morgan et al., 1989, 1995; Cancio et al., 1995). The main site of storage is the chlorogogenous tissue, which is a sheath of modified peritoneal cells that line the outer wall of the gut (Fischer and Molnar, 1993). X-ray microanalytical studies of this tissue have revealed that the cells contain two types of granules (chloragosomes). The first granule form is represented by phosphate-rich complexes containing calcium, and to a lesser extent zinc (Prentø, 1979; Morgan and Morgan, 1988). These granules are involved in the binding of type A or borderline metals such as lead by a process involving exchange with the matrix-associated calcium (Morgan and Morgan, 1988, 1993). The second granule-type contains sulphur-donating ligands probably in the form of residues of metallothionein-like proteins (Suzuki et al., 1980; Morgan et al., 1989). Evidence that these proteins, which bind type B metals, such as cadmium, occur at increased levels in worms collected from contaminated soils has been found by Stürzenbaum et al. (1996).

4.1.2. Effects on equilibrium concentrations

Janssen et al. (1991) studied the uptake and excretion of cadmium from four soil invertebrate species (*Platynothrus peltifer*, *Orchesella cincta*, *Neobisium muscorum*, and *Notiophilus biguttatus*). From this work, it was concluded that the equilibrium concentration was much more strongly affected by the excretion rate than the rate of metal assimilation. The importance of the excretion rate for determining the equilibrium concentration is evident from the results of this work. Of the metals studied, calculation of the equilibrium concentrations in the heavily polluted (Site 1) soils indicated higher values for lead than for copper or zinc (Table 2). Since the accumulation rate for lead (1.187 μg worm^{-1} day^{-1}) calculated in this soil is comparable to the value for copper (1.08 μg worm^{-1} day^{-1}) and is well below that for zinc (6.3 μg worm^{-1} day^{-1}), the higher equilibrium concentration cannot be related to uptake rates. Instead, it is the difference in excretion, 0.106 day^{-1} for lead compared to 1.19 and 1.84 day^{-1} for copper and zinc, respectively, that underlies the higher equilibrium concentrations calculated for this metal.

4.2. Importance of accumulation and excretion patterns in ecotoxicological studies

4.2.1. Relationship to toxic effects

The differences found in accumulation and excretion patterns for the selected metals may have profound effects on the relationship between toxicity and exposure time. If the uptake pattern of a chemical is linear, such as found for cadmium and lead in the field soils study, body concentration would reach equilibrium (if at all) only over an extended exposure period. Thus, for these elements, the likelihood that body concentrations will exceed a toxicity threshold (resulting in an accompanying effect) will be higher in longer duration tests. The increased probability of an effect when the exposure duration is long will mean that toxic effect concentrations (e.g. LC50, EC50, NOEC) will tend to decrease as the length of a test increases. As a result, toxicity values will be dependent, both on the concentration and duration of exposure (Crommentuijn et al., 1994).

An example of an increase in toxic effects with longer exposure duration was found by Hopkin and Martin (1984). In this study, the probability of mortality due to cadmium and zinc toxicity in the isopod *Oniscus asellus* was found to be higher as the period of exposure increased. This time-dependent pattern for toxicity can be explained by the nature of the metal detoxification mechanisms found in isopods. Woodlice are known to store both cadmium and zinc in granules in the hepatopancreas (Hopkin, 1989). However, the capacity for metal storage within this organ is limited. Thus, for animals exposed to high concentrations of metals over a long period, the storage capacity of the hepatopancreas may become saturated, allowing metals to pass into the haemolymph and interfere with sensitive biochemical processes.

For pollutants that show non-linear patterns of uptake, such as copper and zinc in the field soil experiment (Figs. 2 and 4), a different relationship can be anticipated between toxicity and exposure time. For these chemicals, internal body concentrations rapidly reach equilibria that are dependent primarily on external concentrations. Thus, the probability that body concentration will exceed the toxicity threshold will rise only during the net accumulation period. As a result of non-linear accumulation, toxic effect concentrations will only be time-dependent when the test duration does not exceed the time taken to reach equilibrium. Subsequent to this, toxic effects will be determined only by the available concentration of the pollutant in the external medium.

Differences in metal accumulation patterns and the resultant effects on the relationship between exposure time and toxic effect concentrations have important consequences for the design of toxicity studies. Most laboratory tests are conducted using short-term exposure over only a few hours, days or weeks. However, for long-lived organisms such as earthworms, exposure to persistent chemicals in the field may occur over the life-span of the organisms which can be up to 4 years (Edwards and Bohlen, 1996). For cadmium, which shows continuous linear accumulation in polluted field soils, toxic body concentrations may only be reached when exposure duration is long. Thus, the potential effects of these metals may be underestimated when determined by short-term studies. For lead, toxic effects may occur only at equilibrium. However, due to the long equilibrium time for this metal, this concentration may not be reached within the time-span of current standard toxicity tests and effects may again be incorrectly predicted. For copper and zinc, which rapidly reach an internal equilibrium, the suitability of short-term tests for predicting long-term effects will depend on the relationship between exposure and equilibrium time. If exposure duration exceeds equilibrium time, the test will be suitable for predicting toxicity in the field. However, if the test length is shorter than equilibrium time, effects in the field may be underestimated.

The relationship between test length and validity of the acquired toxicity data for predicting the long-term consequences of pollution increases the complexity of attempts to extrapolate laboratory results to the field. Spurgeon et al. (1994) compared the toxicity of cadmium, copper, lead and zinc in short-term artificial soil tests to metal concentrations found in soils at Avonmouth. From this work it was concluded that the greatest effects of metals in the region was due to soil zinc, with the potential effects of the remaining metals decreasing in the order copper > lead > cadmium. The data used for these comparisons were obtained in tests conducted in OECD (1984) artificial soil using the protocol of van Gestel et al. (1989). LC_{50}s were recorded after 14 days and cocoon production EC_{50}s after 21 days' exposure. Results from the present study indicate that after 21 days, internal copper and zinc levels would have been at equilibrium, while for cadmium and lead concentrations would still be increasing. Thus, when earthworms are exposed for a long period of time in the field, toxic effects due to cadmium and lead may be of more importance than would be anticipated from the results of short-term tests.

4.2.2. *Comparisons of uptake in different soil-types*

A previous study of the effects of cadmium, copper, lead and zinc on *Eisenia fetida* indicated that these metals are more toxic in OECD artificial soil than in soils collected from Avonmouth (Spurgeon and Hopkin, 1995). For example, calculation of the ratios of cocoon production EC_{50}s for zinc indicated that the toxicity of this metal in the OECD medium was higher than that found in the field soil by a factor of 10. The higher relative toxicity found for zinc in the OECD test can be attributed to a number of factors related to the experimental design, of which the most important was the increased bioavailability of metals in the artificial soil due to its low binding capacity and the relatively short contact time between the soils and added metal ions (Spurgeon and Hopkin, 1996c).

For the present study it was anticipated that the greater availability and toxicity of metals in artificial soil would be reflected by higher rates of uptake resulting in higher internal concentration. Indeed, such differences in the accumulation of cadmium and zinc by the encytraid *Enchytraeus cryptices* were found in a field and artificial soil by Posthuma and Notenboom (1996). To determine if the greater availability and toxicity of metals in OECD soil is reflected by increased accumulation and ultimately higher body burdens the preferred technique would be to compare metal levels in worms exposed to similar concentrations of metals in the two soil-types. In the present study, however, such comparisons are not possible, since metal concentrations present even in the moderately polluted field soil exceed those in the most contaminated OECD soil by up to a factor of 5.27 (Table 3). Accumulation and theoretical equilibrium contents must therefore be compared in relation to the relative concentrations of metals in the different soils.

To compare uptake and body burdens in the two soil-types, ratios for accumulation rate and theoretical body burdens in the moderately polluted and OECD 1 soil were related to ratios of soil metal concentrations. If, as hypothesised, the uptake in the artificial soil is higher than in field soils, this would be expected to result in a reduction in the ratio of accumulation rate and equilibrium content when compared to metal concentrations ratios. However, calculations for this study do not indicate such a trend (Table 3). For accumulation rates, ratios for copper and lead were greater than those for metal concentration in the selected soils, while the value for cadmium was comparable (a value could not be calculated for zinc). For theoretical equilibrium concentration, ratios were lower than those for soil metal concentration for copper, lead and zinc (a value could not be calculated

Table 3
Ratios for the accumulation rates and theoretical equilibrium concentrations (calculated from the one compartment model of Atkins, 1969

	Ratio for accumulation rate	Ratio for theoretical equilibrium concentration	Ratios for soil metal concentration
	Site 4 : OECD 1	Site 4 : OECD 1	Site 4 : OECD1
Cadmium	0.86 : 1	ND	1.41 : 1
Copper	115 : 1	2.67 : 1	3.62 : 1
Lead	62.7 : 1	2.49 : 1	5.2 : 1
Zinc	ND	2.58 : 1	5.27 : 1

See Eqs. (1) and (2) for cadmium, copper, lead and zinc in *Eisenia fetida* exposed for 42 days to the moderately contaminated field soil collected from Site 4 of Spurgeon and Hopkin (1995) and the most contaminated OECD 1 soil (see Table 1 for a description of soils). Ratios of total (nitric acid-extractable) metal concentration in the two soils are also given.

for cadmium) (Table 3). However, these differences were relatively small, suggesting that the metal burdens of worms exposed in the two soils were primarily dependent on total metal levels and did not increase as a result of higher availability in the OECD soil. Results therefore indicate that although toxicity is greater in OECD soil by up to a factor of 10 (Spurgeon and Hopkin, 1995, 1996c), these increased effects are not reflected by higher accumulation rates or theoretical equilibrium concentrations.

A number of explanations could account for the fact that the greater toxicity observed for metals in OECD soil is not invariably accompanied by higher metal burdens. Firstly, it is possible that the increased toxicity of metals found in artificial soil results from exposure to higher concentration of very toxic metal ions. In artificial soils tests, metals are added to the soil as a solution of a soluble salt. Although a short period (up to 1 week) is allowed for stabilisation, it is unlikely that sorption kinetics reach equilibrium within this time (van Wensem et al., 1994; Smit and van Gestel, 1996). Thus, worms may be exposed to high concentrations of the selected metal species (such as perhaps the Zn^{2+} ion) than they would encounter in the field. Increased exposure to these species could result in greater toxic effects in the laboratory soil than would be anticipated from an accumulation study.

A second explanation for the absence of higher accumulation in artificial soil is that metal uptake may be more closely related to total soil metal levels, while toxicity is determined by soluble concentrations. Thus in OECD and field soils with similar metal concentrations, accumulation may be comparable, while toxic effects may be more severe in OECD soil. Evidence that earthworm metal burdens are determined primarily from total soil metal levels is given by Marinissen (1997), who found a higher correlation for worm metal burdens with total concentrations than soluble values. Dependence of toxic effects on the concentrations of metals in the soluble fraction has been demonstrated by Spurgeon and Hopkin (1996c), who found that effects on survival and cocoon production of *Eisenia fetida* were determined by water-extractable concentration. Although it is not possible at present to suggest which of these factors may be most important in mediating the relationship between toxicity and body burdens, it is clear a number of factors may be involved and that key factors may vary between metals.

4.2.3. Relevance of data for measurement of earthworm metal levels

The results presented in this paper have implications for studies that aim to measure metal burdens in earthworms collected from polluted sites in the field. To accurately measure tissue metal concentrations, it is first necessary to starve worms to remove soil present in the gut. Previous studies conducted to measure the metal burdens have used a wide range of starvation protocols, although the techniques can be divided into two principle types. Soil can be removed directly from the gut by dissection and washing, or animals can be maintained on moist filter paper or in distilled water until the gut contents are voided (Denneman, 1993). For the latter technique, starvation periods of up to 13 days have been used. As a result, large differences exist in the time between the sampling of worms and their preparation for analysis. Differences in the time taken between sampling and preparation for analysis do not present a problem when measuring cadmium and lead levels in worms, since the excretion rates of these metals are low. However, preparation time may have important implications for measured copper and zinc concentrations, since these metals are rapidly excreted. Indeed, in the current study levels of these metals in *Eisenia fetida* returned to control values after only 3 days' excretion even for worms previously exposed to the most heavily contaminated soil (Fig. 2(c)Fig. 4(c)).

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