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Effects of variations of the organic matter content and pH of soils on the availability and toxicity of zinc to the earthworm *Eisenia fetida*

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Summary. Nine toxicity tests were conducted using a procedure based on the OECD (1984) artificial soil earthworm toxicity test. For each test, *Eisenia fetida* were exposed to zinc in a range of artificial soils with differing pH and/or organic matter (OM) content. Earthworm survival and cocoon production, and the concentrations of zinc in two fractions (nitric acid extractable, water extractable) and the exposed earthworms were measured.
The proportion of total zinc that was soluble was greater in soil with low pH and low OM content. Zinc burdens were greatest in worms maintained in the most contaminated soils. However, the slopes of the relationships between earthworm zinc concentrations and zinc concentrations in soils (total and soluble) were less than one indicating probable regulation of net assimilation of this essential element by *Eisenia fetida.*
Toxic effects of zinc on *Eisenia fetida* as measured by reductions in survival and cocoon production were related more closely to soluble than total metal concentrations in soil. In a previous series of tests, zinc toxicity for *Eisenia fetida* was found to be at least ten times greater in OECD artificial soil when compared to contaminated soils collected from a polluted field site (Spurgeon & Hopkin, 1995). It was concluded that this was due to the greater bioavailability of the metals in the OECD soil. The results of the present paper support this hypothesis.

Key words: Extrapolation, zinc toxicity, bioavailability, cation exchange, binding sites, clay

Introduction

The bioavailability and toxicity of a chemical to soil animals are influenced by physical factors that determine pore water concentrations (Van Gestel 1992, in press). For organic chemicals, toxicity and bio-concentration factors (BCFs) for structurally-related chemicals can be predicted using quantitative structure activity relationships (QSARs) (Belfroid et al. 1993a, 1993b; Larsen et al. 1992; Van Gestel & Ma 1988, 1990, 1993). For metals however, the situation is somewhat more complicated. For a given element, toxicity and BCFs are altered by soil factors that influence availability such as the pH, cation exchange capacity (CEC), clay content and the percentage organic matter (OM) (Hopkin et al. 1993; Ma 1984, 1989; Ma et al. 1983; Van Gestel & Van Dis 1988). Within a given soil, metals are present

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in eight fractions: 1) free metal cations (e.g. Pb^{2+}), 2) inorganic complexes (e.g. $CdCl^{+}$), 3) organo-metal complexes (e.g. $(CH_3)_4$ Pb), 4) organo-complex chelates, 5) in association with high molecular weight organic material, 6) bound as diverse colloids, 7) adsorbed to colloids and 8) within the soil particles (Martin & Bullock 1994). The metals in the first four fractions are present in the soil solution and are more available than those bound to the soil matrix, hence any soil factor that alters relative distribution between fractions will alter bioavailability and hence toxicity and BCFs.

To assess the impact of changes in soil conditions on the bioavailability and as a consequence toxicity of zinc, the effects of this metal on the survival and cocoon production of the earthworm *Eisenia fetida* have been examined in a range of soils with different pH and organic matter content. The measured effects have been compared to the concentrations of zinc in two soil fractions (nitric acid extractable (NAX) and water extractable (WX)), and the tissues of the exposed worms. Water extractable zinc has been used to assess 'bioavailable' metal concentrations, since earlier work has indicated that metal toxicity occurs primarily through uptake across the body wall (Belfroid 1994; Spurgeon et al. 1994; Van Gestel, in press). Nitric acid extracts were used to measure total zinc.

To further investigate the relationship between zinc toxicity and availability, the results of the nine tests conducted have been related to earlier work assessing effects on *Eisenia fetida* measured in contaminated field soils (taken from Spurgeon & Hopkin, in press). These comparisons have allowed differences between the effects of zinc in the laboratory and field soils to be explained in terms of metal solubility. The relevance of using toxicity values calculated from bioavailable metal levels for determining ecologically relevant soil quality criteria is discussed also.

Materials and Methods

Nine toxicity tests were conducted using media based on the artificial soil described by the OECD (1984) ('standard' mix 70% sand, 20% kaolin clay, 10% Sphagnum peat; see Spurgeon et al. (1994) for further details). For the tests, three different percentage organic matter (OM) contents (5, 10 and 15% by weight) and three pHs (4, 5 and 6) were used in factorial combination. To change the percentage OM content, the quantity of *Sphagnum* peat added to the test soils was altered to the desired percentage composition, clay was held constant at 20%, while sand was used to make up the remaining weight. Soil pH was adjusted by varying the quantity of calcium carbonate added to the soil mix. After soil preparation (see OECD 1984 and Van Gestel et al. 1989 for details), 500 g of the soil medium was weighed into each test container. Four replicates for each concentration were used in all tests.

For the soils contaminated with zinc, solutions of the nitrate salt were used to give the required metal concentration and percentage water content in the test soil. Six zinc concentrations were used for all tests; these were adjusted depending on the soil type. Concentrations of 0 µg Zn g^{-1} (control), 190 µg Zn g^{-1}, 350 mg Zn g^{-1}, 620 µg Zn g^{-1} and 1200 µg Zn g^{-1} were used in all tests. In addition, at 15% OM – pH 5.0 and 6.0 and 10% OM – pH 6.0, a soil with 2000 µg Zn g^{-1} was used, while in all other tests the additional test concentration was 100 µg g^{-1}. At 15% OM – pH 6.0, complete mortality did not occur at 2000 µg Zn g^{-1}, therefore an additional concentration of 3600 µg Zn g^{-1} was also tested. The same volume of distilled water was added to the controls.

Eisenia fetida from our laboratory culture were used for all tests. Before the test, the worms were pre-exposed to the relevant test soil for one week to allow acclimatisation (Van Gestel et al. 1989). After this period, ten worms were added to each test replicate. The containers were then covered to prevent water loss and maintained for 21 days as described by the OECD (1984). In all tests, a small pellet (3 g dry weight) of horse manure was added every week to each container as a source of food as recommended by Van Gestel et al. (1989, 1992). The addition of suitable food has been shown to increase cocoon production and growth during tests with *Eisenia fetida* (Spurgeon & Hopkin, in press; Van Gestel et al. 1992). For each test, survival and cocoon production were measured. Cocoon viability and the number of juveniles emerging per fertile cocoon were not recorded since no effects due to zinc were found for these parameters during earlier toxicity tests (Spurgeon & Hopkin, 1995; Spurgeon et al. 1994). LC_{50} and EC_{50} values for each test were determined by probit analysis and logit analysis respectively. Est NOEC values were determined using the derivation of the Williams test (Williams 1971, 1972), used by Spurgeon et al. (1994).

Within artificial soil, OM is the main factor that determines water holding capacity (WHC). Consequently, in the tests with 5% or 15% OM, the quantity of water added to the test soil was modified. All soils were moistened to approximately 50% of their WHC to maintain consistency with the conditions described by the OECD. For the tests with 5% OM, 125 ml of water was added, 175 ml was used for the 10% OM soil, while 250 ml was added to the 15% OM medium. Altering the percentage water content of the test soil would not affect zinc availability (Van Gestel, pers. comm.).

Raising OM content also increases total soil volume. This resulted in a decrease in the number of worms per unit volume in the 10% and 15% containers compared to those at 5% OM. In the tests with 15% OM, soil volume was 830 cm^3, with 10% OM it was 650 cm^3 and at 5% OM 450 cm^3. For *Eisenia fetida*, both growth and cocoon production are density-dependent. Reinecke & Viljoen (1990) found that *Eisenia fetida* required at least 20 g of manure for unrestricted growth over 48 days; this corresponds to a volume per worm of approximately 80 cm^3. In the tests with 5% and 10% OM, the volume of soil available for each worm is 45 cm^3 and 65 cm^3, below the optimum. However, this was not found to seriously impair cocoon production, since no consistent differences in cocoon production were recorded between the 5%, 10% and 15% OM control soils for each pH.

To measure metal availability in test soils, a variety of selective extractants have been suggested, however from the review of Ross (1994) it is clear that no technique for assessing the 'bioavailable' fraction has gained widespread acceptance. Consequently for this study zinc concentrations were only measured in the total and water-extractable fractions. Total zinc was assessed by digesting soils in boiling nitric acid according to the technique described by Hopkin (1989). Solutions were analysed by flame and flameless atomic absorption spectrometry (Varian Spectra 30, GTA 95). Water-extractable metal levels were measured by transferring 3–4 grams of soil and 50 ml of double distilled water into a graduated 100 ml Pyrex conical flask. The solutions were allowed to stand overnight to allow partial extraction to begin and then shaken for a one hour on a Luckham R100 Rotatest shaker set at maximum shaking speed. Flasks were stood for 24 hours to allow the soils to settle from the water and 10 ml filtered into a test tube, carefully avoiding disturbance of the sediment in each flask.

Zinc concentrations were also assessed in the exposed worms. For the analysis, eight worms (two from each replicate) were starved to allow the animals to void their gut contents (Morgan & Morgan 1988). Each worm was placed in a numbered acid washed test tube with 2 ml of concentrated Analar grade nitric acid. The solution was left to stand overnight before boiling on a hot plate until all tissue had been digested. Once cool, each digest was diluted to 10 ml with double distilled water and analysed on a Varian Spectra 30 atomic absorption spectrophotometer using the technique described by Hopkin (1989).

The results from the nine tests conducted in this study have been related to those for *Eisenia fetida* exposed to soil collected from eight sites in the Avonmouth area (see Spurgeon & Hopkin, 1995). To allow these comparisons to be made, water extractable zinc concentrations were analysed in the field soils and tissues of *Eisenia fetida* exposed to these soils for three weeks. This has allowed the fraction that best describes toxic effects on *Eisenia fetida* in a range of soils to be assessed.

Results

Zinc concentrations in soil nitric acid and water extractable fractions

The concentrations of zinc in the WX fraction were greater at high NAX levels (Table 1). A comparison of log NAX and log WX zinc levels using a linear model indicated a strong positive correlation between the two fractions ($P < 0.001$) (Fig. 1). WX metal levels were also influenced by soil conditions, since at the same levels of added zinc, concentrations were lower at higher percentage OM and soil pH values (Table 1). For example at 620 µg Zn g^{-1}, 7.6 µg Zn g^{-1} was water soluble at 15% OM – pH 6.0, while 123 µg Zn g^{-1} was extracted from the 5% OM – pH 4.0 soil (Table 1). A similar relationship between cadmium solubility and pH and OM content was found by Crommentuijn (1994).

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Table 1. The concentration of water-extractable zinc present in the test soils used in nine toxicity tests conducted with the earthworm *Eisenia fetida* ±SD. All values are based on means for six replicate samples taken from soil collected and pooled from each of the four test replicates. Values in brackets give the percentage of total zinc (boiling nitric acid soluble) in the water fraction

Nominal zinc concentrations	15% OM pH 6.0	10% OM pH 6.0	5% OM pH 6.0	15% OM pH 5.0	10% OM pH 5.0	5% OM pH 5.0	15% OM pH 4.0	10% OM pH 4.0	5% OM pH 4.0
Control (0 µg Zn g^{-1}) (% zinc extracted)	1.32 ± 0.3 (10.2)	1.83 ± 0.34 (9.1)	1.54 ± 0.75 (15)	1.27 ± 0.1 (13.2)	0.83 ± 0.1 (10.9)	0.21 ± 0.15 (1.3)	0.5 ± 0.2 (3.8)	0.59 ± 0.42 (3.6)	1.48 ± 0.2 (8.4)
100 µg Zn g^{-1}	–	–	3.33 ± 0.49 (4.5)	–	3.63 ± 0.44 (3.5)	4.84 ± 0.74 (4.6)	3.98 ± 0.99 (3.7)	4.68 ± 0.92 (4.1)	6.38 ± 0.35 (4.9)
190 µg Zn g^{-1}	1.83 ± 0.1 (1.1)	2.89 ± 0.45 (2.4)	7.39 ± 1.06 (7.1)	5.56 ± 0.43 (5.3)	8.89 ± 0.84 (6.4)	12.4 ± 0.77 (10.6)	7.2 ± 0.52 (7.5)	11.2 ± 0.67 (13.9)	13.1 ± 0.8 (9.4)
350 µg Zn g^{-1}	3.49 ± 0.43 (1.2)	8.4 ± 0.52 (2.4)	18.8 ± 2.82 (7.1)	14.1 ± 1.22 (5.3)	22.3 ± 2.26 (6.4)	34.2 ± 5.31 (10.6)	21.45 ± 2.78 (7.5)	52.5 ± 21.46 (13.9)	36.9 ± 8.82 (9.4)
620 µg Zn g^{-1}	7.6 ± 1.47 (1.6)	17 ± 4.89 (3.7)	54.7 ± 10.7 (14)	54.5 ± 12.9 (9.1)	74.7 ± 9.11 (12.7)	82.3 ± 9.72 (19.3)	59 ± 3.35 (13.5)	96.9 ± 18.3 (14.6)	123 ± 8.9 (21.2)
1200 µg Zn g^{-1}	22.7 ± 2.74 (2.2)	82.5 ± 19.5 (9.1)	206 ± 39.8 (27.1)	129 ± 20.8 (15.5)	266 ± 80.5 (26.5)	241 ± 33.7 (24)	184 ± 31.7 (21.3)	–	–
2000 µg Zn g^{-1}	59.4 ± 15.86 (3.9)	304 ± 38 (22.3)	–	440 ± 57.4 (29.8)	–	–	–	–	–
3600 µg Zn g^{-1}	–	–	–	–	–	–	–	–	–

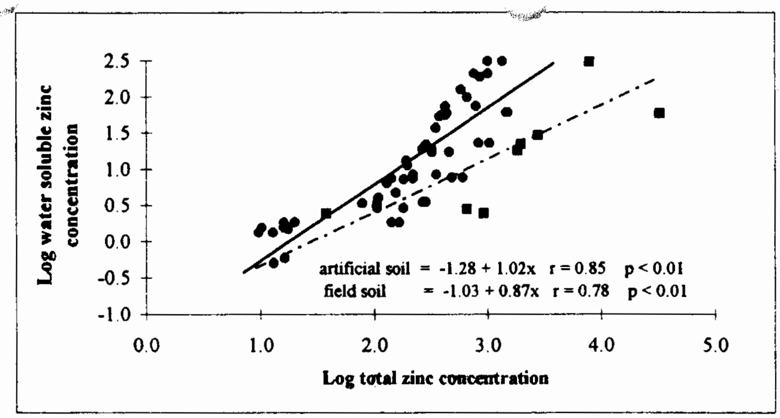

Fig. 1. Zinc concentrations in two soil fractions (water extractable and nitric acid extractable) in nine toxicity tests in which organic matter content and pH were varied (circles), or in contaminated field soils from Avonmouth (squares). Regression lines for artificial (solid) and field soils (dashed) and regression equations are given

The concentrations of metals in the tissues of exposed earthworms

Analysis of exposed worms indicated that tissue zinc concentrations were greatest at high NAX and WX concentrations (Figs. 2a and 2b). Zinc burdens were significantly increased at 190 µg Zn g^{-1} in one test, at 350 µg Zn g^{-1} in three tests, at 620 µg Zn g^{-1} in three tests and at 1200 and 2000 µg Zn g^{-1} in one test each, respectively. Relating log earthworm zinc to log NAX and WX values using a linear model, indicated a significant positive correlation for both fractions ($p < 0.05$ and $p < 0.01$). However, in all cases the slope parameters for these relationships were considerably below one indicating that zinc is regulated by *Eisenia fetida*. The correlation coefficient for water extractable zinc ($r = 0.39$) was marginally higher than that for nitric acid extractable metal ($r = 0.35$) indicating that worm burdens were related more closely to water soluble than NAX concentrations.

Effects on survival and cocoon production in relation to zinc availability

Mortality. Survival in control soils was not affected by pH or OM content and mortality was less than five percent in every test. Survival was however reduced at high NAX and WX zinc concentrations (Figs. 3a and 3b). There was a less clear relationship between earthworm zinc concentration and mortality (Fig. 3c). The relationship between log zinc concentration and survival was analysed using logistic regression, since for both fractions, a threshold concentration existed below which effects on mortality did not occur. For total zinc, this threshold concentration was approximately 350 µg Zn g^{-1}, while for soluble zinc, the value was 40 µg Zn g^{-1} (Figs. 3a and 3b). If the log-likelihood ratios for the logistic relationships are compared for each regression, results indicate that inclusion of the water-extractable zinc in the NAX regression significantly improves the fit compared to that for the NAX zinc alone ($P < 0.05$). However, for the WX regression the inclusion of the nitric acid values does not significantly improve the regression. The inclusion of worm zinc levels in the regression failed to improve the fit for both NAX and WX metal. Thus, it is clear that soluble zinc levels gave a better indication of effects on mortality than NAX values (compare Fig. 3b with 3a). Earthworm zinc does not describe effects on mortality as closely as either the WX or NAX fractions (compare Fig. 3c with Fig. 3a and 3b).
Toxicity values calculated from total zinc concentrations were highest at high pH and OM contents. Highest LC_{50} and estNOECs were found in the 15% OM – pH 6.0 test, while

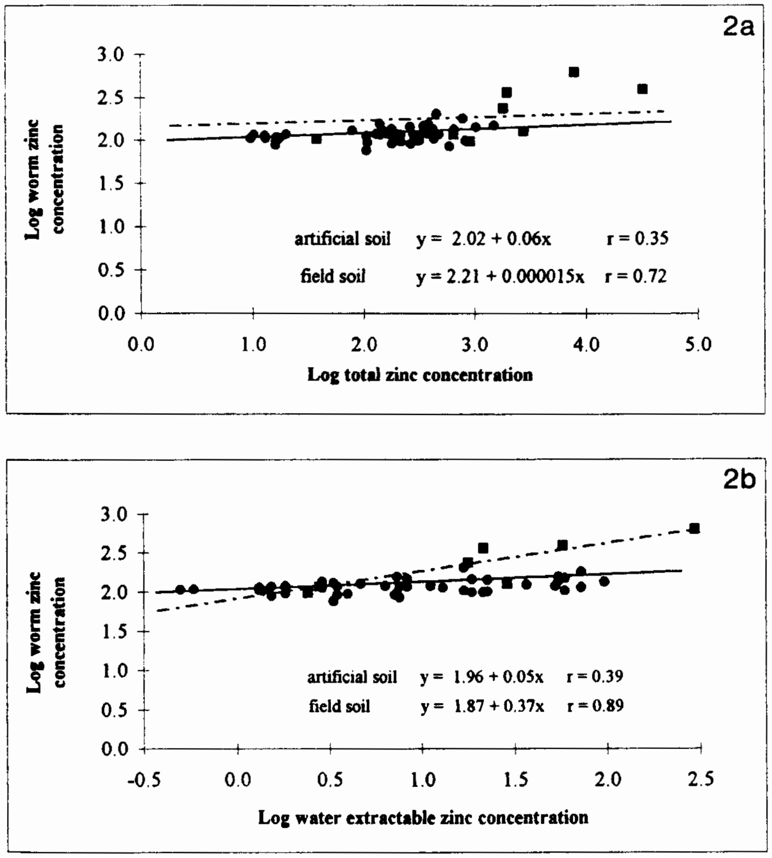

Fig. 2a – b. Zinc concentrations in *Eisenia fetida* and the nitric acid extractable (Fig. 2a) and water extractable soil fractions (Fig. 2b) from nine toxicity tests in which organic matter and pH were varied. Linear regression lines and equations for the artificial (solid line, circles) and field soils (dashed line, squares) are given

the lowest values were from the 5% OM – pH 4.0 soil (Tables 2a and 2b). Comparisons of LC_{50} and estNOECs for a given pH or percentage OM, indicated that both factors affect the toxicity of zinc in artificial soil. For example, when the total zinc LC_{50} and estNOEC for each OM content are plotted against pH using a linear model (Fig. 4a, Fig. 4c), the slope parameter is highest for the 15% OM tests, intermediate for the 10% OM tests and lowest for the 5% tests. Thus increases in soil pH have a stronger effect on zinc toxicity as OM content increases.

LC_{50} and estNOEC calculated from WX concentrations gave values below those determined from NAX zinc concentrations (Tables 3a and 3b). Furthermore, no clear trends were apparent in the calculated values as a result of changes in soil OM content and pH. For example, the LC_{50} value of 71.8 µg Zn g^{-1} in the test with 15% OM – pH 6.0 is almost identical to that calculated in the test with 5% OM – pH 4.0 (67.7 µg Zn g^{-1}). If regression parameters for the relationship between the LC_{50} and estNOECs and soil pH are determined for each OM content (Figs. 4b and 4d), these indicate no significant relationships. The slope parameters for these relationships are low for all OM contents indicating no effects on toxicity values calculated from soluble zinc concentrations due to changes in pH or OM content.

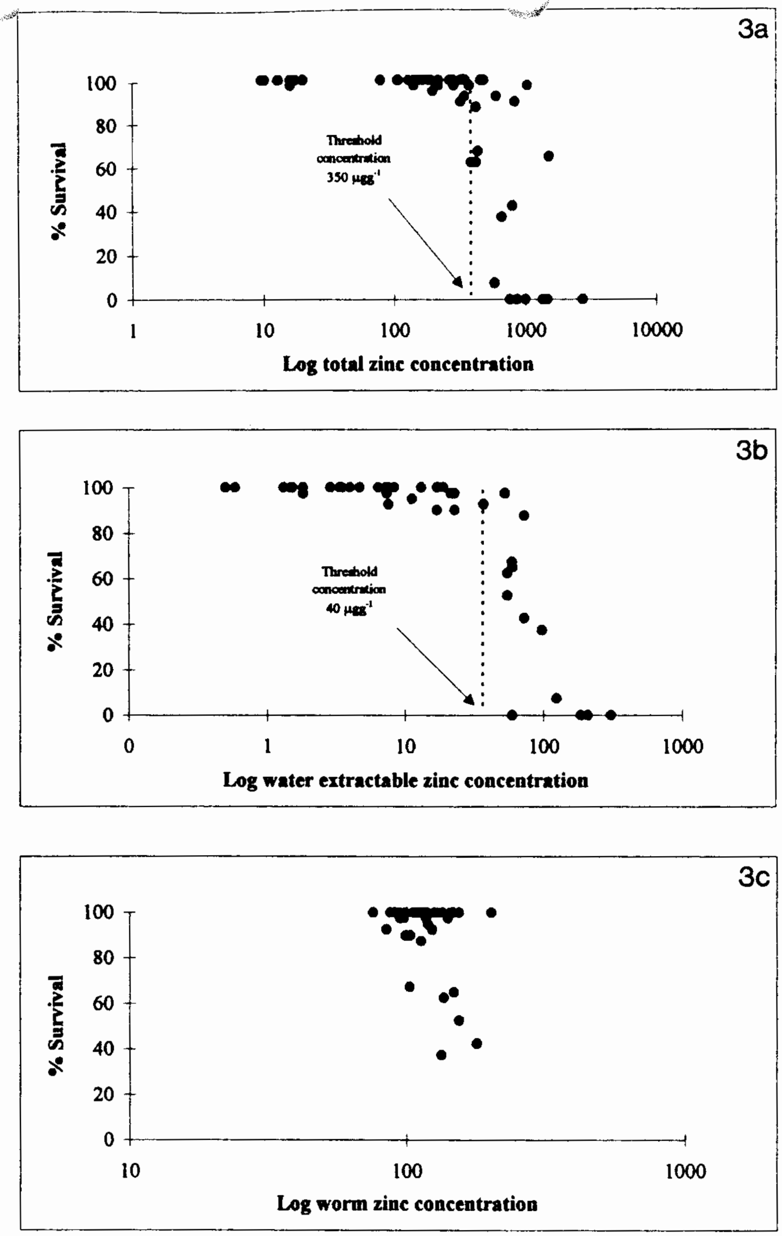

Fig. 3a – c. Percentage survival versus (a) log nitric acid extractable zinc concentration, (b) log water extractable zinc concentration, (c) log zinc concentration in the tissues of exposed worms at each concentration in nine zinc toxicity tests with varied pH and organic matter contents

Cocoon production

Cocoon production in control soils did not differ significantly at pH 5.0 and 6.0 ($p > 0.05$). However, at pH 4.0 rates were significantly lower than for the higher pH soils. Crommentuijn (1994) has suggested that the results of tests in which soil acidity is altered to change metal

Table 2a–d. LC_{50}, EC_{50} and estNOEC values for mortality and cocoon production calculated from total zinc concentrations (with 95% CIs where calculable) for *Eisenia fetida* exposed to a geometric series of zinc concentrations in artificial soils with three OM content and three pH values. Table 2a gives LC_{50} values, Table 2b mortality estNOECs, Table 2c cocoon production EC_{50}s and Table 2d cocoon production estNOECs

	5% Organic Matter	10% Organic Matter	15% Organic Matter		5% Organic Matter	10% Organic Matter	15% Organic Matter
2a				**2b**			
pH 6.0	620	791	1613	pH 6.0	274	702	1048
95% CI	(510–679)	(–)	(1515–1794)				
pH 5.0	591	601	992	pH 5.0	366	256	368
95% CI	(–)	(528–704)	(913–1106)				
pH 4.0	451	617	474	pH 4.0	197	168	184
95% CI	(411–493)	(545–730)	(440–539)				
2c				**2d**			
pH 6.0	136	462	592	pH 6.0	97	553	484
pH 5.0	199	343	548	pH 5.0	85	183	414
pH 4.0	142	189	230	pH 4.0	115	161	223

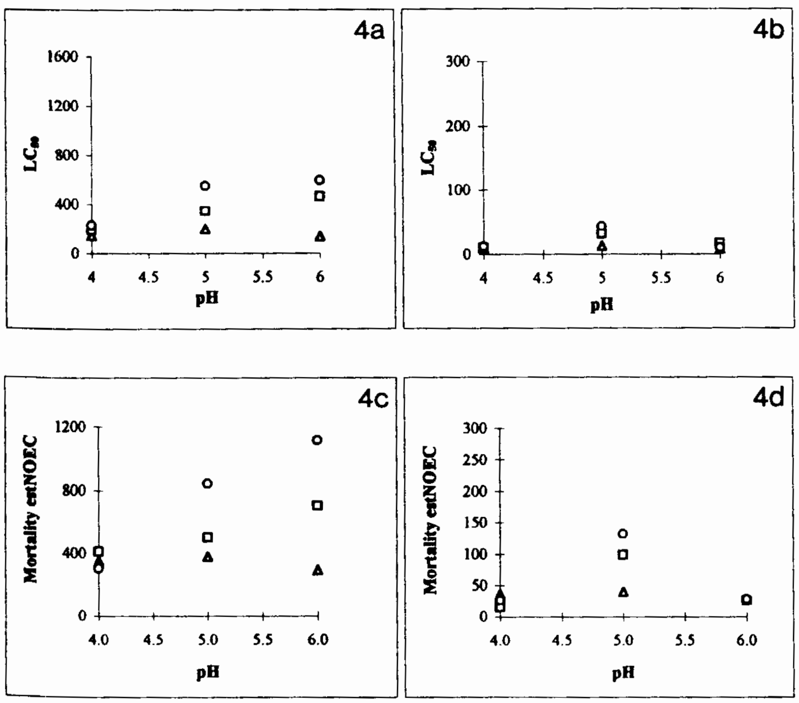

Fig. 4a–d. Toxicity values for mortality in the earthworm *Eisenia fetida* exposed to a geometric series of zinc concentrations in artificial soils. Values for each organic matter content have been plotted against soil pH; 5% organic matter values are shown as triangles, 10% organic matter as squares and 15% organic matter as circles. Figs. 4a and b give total and water soluble LC_{50} values. Figs. 4c and d give total and soluble zinc mortality estNOECs

Table 3a–d. LC_{50}, EC_{50} and estNOEC values for mortality and cocoon production calculated from soluble zinc concentrations (with 95% CIs where calculable) for *Eisenia fetida* exposed to a geometric series of zinc concentrations in artificial soils with three OM contents and three pH values. Table 3a gives LC_{50} values, Table 3b mortality estNOECs, Table 3c cocoon production EC_{50}s and Table 3d cocoon production estNOECs

	5% Organic Matter	10% Organic Matter	15% Organic Matter		5% Organic Matter	10% Organic Matter	15% Organic Matter
3a				**3b**			
pH 6.0	91.5	71.4	71.8	pH 6.0	21.1	26.2	23.3
95% CI	(78.5 – 109.9)	(–)	(58.9 – 129.5)				
pH 5.0	82.6	117.1	187.6)	pH 5.0	37.8	13.7	26.2
95% CI	(–)	(93 – 150.6)	(156 – 236.3)				
pH 4.0	67.7	95	67.9	pH 4.0	20.5	8.9	7.2
95% CI	(54.9 – 83.7)	(78.3 – 137.3)	(58.5 – 83.1)				
3c				**3d**			
pH 6.0	7.7	17.4	10.5	pH 6.0	4.5	14.8	7.5
pH 5.0	12.9	31.6	43.9	pH 5.0	3.9	7.2	31.7
pH 4.0	9.6	10.2	12.4	pH 4.0	5.8	8.3	12.7

availability can frequently be attributed the direct effects of pH, rather than changes in solubility. To account for such pH effects, the impact of total and soluble zinc have been assessed by comparing cocoon production as a percentage of the control rate for each test soil.

Relative cocoon production was lowest at high NAX and WX zinc concentrations in the test soil. Analysis of the relationships between the log concentrations of zinc in the three measured fractions and log percentage cocoon production using the logistic model common to dose-response relationships, indicated that the best fit for the model is obtained by relating cocoon production to WX zinc (Fig. 5b). For this fraction, a greater proportion of the variance in cocoon production is obtained than for either NAX or worm zinc (Fig. 5a and 5c). A particularly poor correlation was obtained for the relationship with worm tissue zinc concentrations.

EC_{50} and estNOECs for cocoon production calculated from total zinc were greatest at the highest pH and OM contents of the test soil (Table 2c and 2d). If the toxicity values for each percentage OM content calculated from total zinc are plotted against pH (Fig. 6a and 6c), the steepest slope is found for the 15% OM tests. This indicates that low soil pH has a stronger effect on cocoon production at high OM contents, a pattern similar to that found for LC_{50} and mortality estNOEC values (Figs. 4a and 4c). If EC_{50} and estNOECs for cocoon production are calculated from the concentration of water extractable zinc, however, values no longer increase with increasing pH or OM content (Fig. 6b and 6d).

Comparison of current toxicity results with zinc toxicity in polluted field soils

The results from the nine toxicity tests conducted with the artificial soil have been used to clarify the results of an earlier toxicity test conducted with field soils collected from the area around a smelting works situated at Avonmouth in south-west England. Spurgeon and Hopkin (1995), exposed *Eisenia fetida* to soils collected at eight sites along a transect

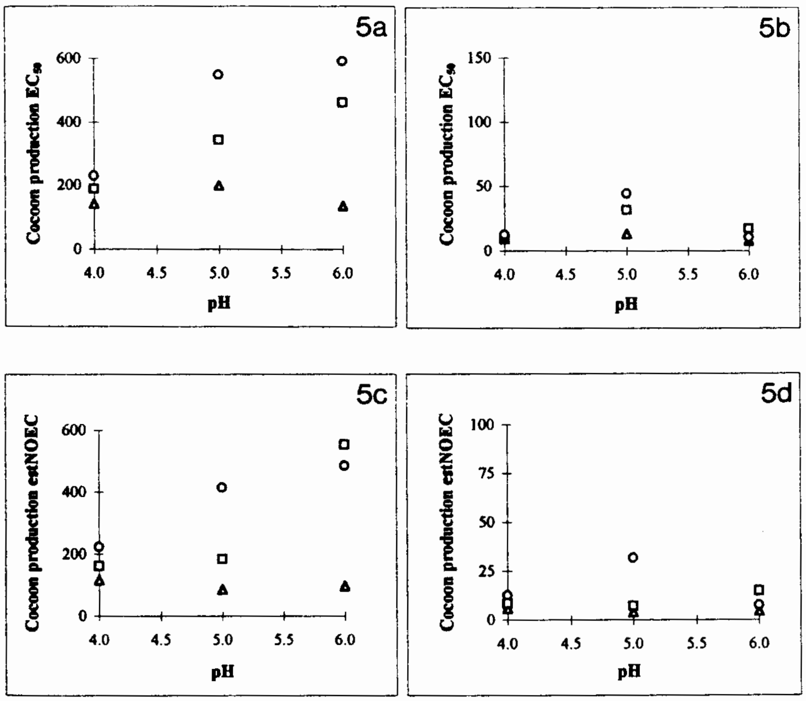

Fig. 5a – d. Toxicity values for cocoon production measured in the earthworm *Eisenia fetida* exposed to a geometric series of zinc concentrations in artificial soils. Values for each organic matter content have been plotted against soil pH, 5% organic matter values are shown as triangles, 10% organic matter as squares and 15% organic matter as circles. Figs. 5a and b give total and water soluble LC_{50} values. Fig. 5c and d give total and soluble zinc mortality estNOECs

from the smelter. Survival and cocoon production were assessed. The soils used for this experiment were analysed for total and soluble zinc using the same procedures as in the artificial soil tests described in the present paper.

Water-extractable zinc in the field soils was significantly increased at high NAX concentrations (Table 4). Comparisons of log NAX and WX zinc concentrations indicated a significant positive linear relationship (Fig. 1). Local variations between sites were also found to influence solubility. For example, the WX zinc level in Site 2 soil was higher than for Site 1, despite the fact that the latter soil contained more than four times the concentration of NAX metal (Table 4). The increase in zinc availability in soil from Site 2 is reflected in the results of the toxicity test, since lower cocoon production was recorded for this site than in the more contaminated soil collected from Site 1. Zinc burdens in earthworms exposed to field soils were highest for the most contaminated sites. However, individual variability was high, and hence there was no site for which a significant increase was found (Table 4). Log worm tissue zinc concentrations were related significantly to log NAX and WX concentrations in field soils ($P < 0.05$ and $p < 0.01$) (Figs. 2a and 2b). The correlation coefficient was higher for WX zinc ($r = 0.89$) than for NAX ($r = 0.72$) indicating that body burdens were related more closely to soluble metal concentrations.

No significant mortality in the field soils was recorded for any of the sites used (Table 4). This was despite the fact that zinc concentrations in field soils from the most contaminated site were over 10 times higher than the LC_{50} value calculated from the 15% OM – pH 6.0 test. Since survival was not reduced in any soil tested, LC_{50}s, and associated relationships between NAX and WX zinc in field soils could not be assessed. Cocoon production in the

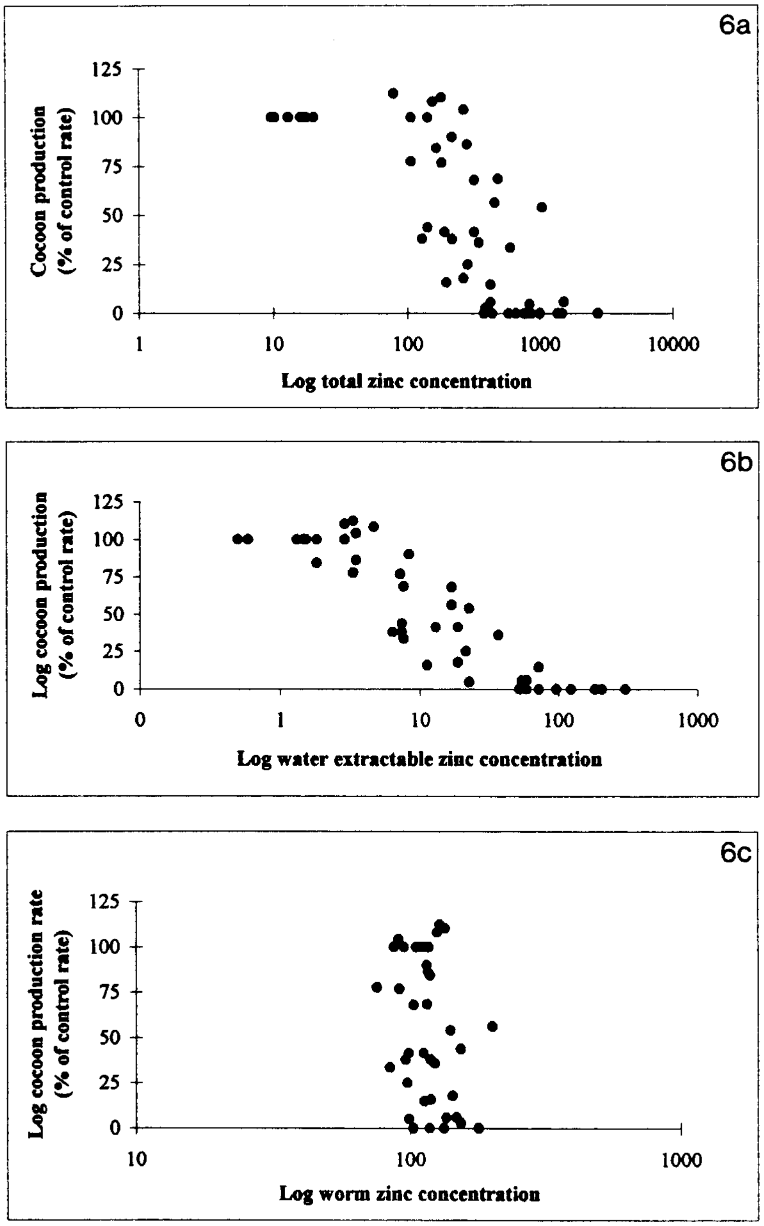

Fig. 6a–c. Cocoon production rate (calculated as a percentage of control rate) versus (a) log nitric acid zinc, (b) log water extractable zinc and (c) log worm tissue zinc concentrations at each concentration in nine zinc toxicity tests with varied pH and organic matter contents

field soils was substantially lower in the four most contaminated soils (Table 4). Analysis of the interaction between cocoon production and NAX and WX zinc using a logistic model, indicated a better fit for soluble than for total metal levels. Thus, WX levels of zinc appear to give a better indication of effects on cocoon production in the field soils as well as for the range of artificial soils used.

Table 4. Total, soluble (concentration and percentage of total) and earthworm tissue zinc levels, in soils used in nine toxicity tests conducted with *Eisenia fetida*. The effects of the contamined field soils on the survival and cocoon production of the exposed worms over 21 days are also detailed

	Nitric acid extractable zinc ($\mu g\ g^{-1}$)	Water extractable zinc ($\mu g\ g^{-1}$)	% of nitric acid extractable zinc in the water fraction	Worm zinc concentration 21 days ($\mu g\ g^{-1}$)	Survival (14 days)	Cocoon production rate (21 days)
Site 8	38 ($\pm$12)	2.43 ($\pm$0.5)	6.39	102.9 ($\pm$20.3)	100	0.375
Site 7	925 ($\pm$48)	2.42 ($\pm$0.5)	0.26	96.2 ($\pm$30.4)	100	0.345
Site 6	657 ($\pm$93)	2.78 ($\pm$1.2)	0.42	115.2 ($\pm$17.8)	100	0.25
Site 5	1848 ($\pm$260)	17.9 ($\pm$2.3)	0.96	235 ($\pm$363)	100	0.288
Site 4	2793 ($\pm$447)	29 ($\pm$5.9)	1.04	126.6 ($\pm$40.9)	92.5	0.133
Site 3	1987 ($\pm$246)	21.6 ($\pm$3.4)	1.09	361.7 ($\pm$334)	100	0.042
Site 2	7945 ($\pm$1360)	296.5 ($\pm$9.9)	3.73	619 ($\pm$504)	100	0.017
Site 1	32871 ($\pm$4890)	57.5 ($\pm$7.2)	0.18	389.3 ($\pm$299)	100	0.033

The calculation of cocoon production EC_{50} and estNOECs for the field soil, based on total and WX zinc concentrations, gave values of 3605 μg Zn g^{-1} and 1879 μg Zn g^{-1} and 14.2 μg Zn g^{-1} and 20.9 μg Zn g^{-1} respectively. Thus, the values calculated from total zinc levels are higher than in any of the artificial soils (see Tables 2c and 2d), while the water soluble values are comparable to WX values determined in laboratory tests (see Tables 3c and 3d). Thus it is apparent that effects on cocoon production can be related to water soluble zinc levels in a range of soil types.

Discussion

The effects of pH and OM on zinc solubility

The concentration and percentage of soluble zinc was greatest at low soil pH and OM content (Table 1). Such changes in metal solubility can be attributed to changes in the cation exchange capacity (CEC) of the artificial soil. Two of the most important factors determining metal availability in soils are OM and clay content, since these components bear surface negative charges that bind metals from solution and reduce availability (Wild 1993). These surface charges can be permanent or temporary. If permanent, the charges are not altered by pH, however if temporary, they are broken down at low pH and CEC is reduced (Wild 1993). The clays illite, smectite and vermiculite have permanent charges, while for kaolinite and soil OM matter the charges are temporary. Thus, the two components of artificial soil that bind metals both have reduced CECs at low pHs. In the pH range used for this study, it is likely that the reduction in the adsorptive capacity of the artificial soils at low pH results from the disruption of surface carboxyl groups of the soil OM and kaolinite (Brady 1990).

In addition to the disruption of surface binding sites, low soil pH also affects metal binding by competing directly for the available negative charges. Kiewet & Ma (1991) found a positive correlation between pH and binding of lead. This was attributed to antagonism between the H^+ and Pb^{2+} ions. At low pHs, the H^+ ion concentration is raised. This increases competition for negative sites resulting in a reduction in the binding of lead. Thus at low pH, a combination of decreasing numbers of negative sites and an increase in competition from H^+ ions at low pHs reduce binding and as a result increase water soluble zinc. The increase in zinc solubility at low OM contents is due to a reduction in the number of available sites for the binding of the metal ions.

Avonmouth soils have a higher CEC capacity than any of the artificial soils tested. For example, there were only four sites from which more than one percent of total zinc was present in the WX fraction, while at least one percent of total zinc was always extracted from the artificial soils (see Table 4 and Table 1). The increase in the binding capacity of the field soils can be attributed directly to their composition. All field soils have a high OM content (12.9 – 27.1%, see Spurgeon and Hopkin, 1995). Furthermore, this OM is likely to be more degraded and thus have a greater surface area/mass ratio than the Sphagnum peat used in the artificial medium. This will increase the binding capacity of the soil, since negative sites occur only at the particle surface.

Field soils also have a higher clay content than the artificial soils used (Alloway pers. comm.). In addition, the clays common in natural soils have a greater binding capacity than kaolin. For example, the CEC of kaolin is 0.02 – 0.06 $mol \cdot kg^{-1}$ compared to values of 0.3 $mol \cdot kg^{-1}$ and 1.4 $mol \cdot kg^{-1}$ for the clay minerals illite and vermiculite (Wild 1993). Thus, the binding capacity of vermiculite is over 20 times that of kaolin. The presence of such clays in field soils will further decrease metal availability.

Zinc accumulation in the tissues of exposed worms

Log worm zinc levels showed a significant positive relationship ($P < 0.05$) with both NAX and WX zinc (Figs. 2a and 2b), although the strongest correlation between tissue and soil zinc was for soluble metal levels in both the artificial and field soils. Zinc was not strongly accumulated in the exposed worms. Concentration factors (earthworm zinc concentrations/total zinc concentrations in soil) decreased as the concentration of zinc in the soil was increased. The relatively low extent of net zinc accumulation found in this study can be attributed to the regulation of this metal by the physiological mechanisms involved in the control of this essential element (Morgan & Morgan 1988, 1989).

For earthworm metal burdens to be useful for estimating the available metal fraction, tissue concentrations must both increase at contaminated sites and differ as availability changes. Thus for zinc, worm body burdens are not a good indicator of zinc exposure, since the low level of accumulation in contaminated soils precludes the prediction of soluble metal concentrations from earthworm burdens. This is indicated by the poor correlations that exist between tissue zinc and toxicity (Fig. 5c and 6c).

For other metals, earthworm tissue metal concentrations may be useful for predicting available metal levels. For cadmium, accumulation in earthworm tissues occurs even at low ambient levels (Morgan & Morgan 1988). Furthermore, uptake can be suppressed in soils with a high OM content and pH (Beyer et al. 1987; Ma 1982, 1987; Ma et al. 1983; Perämäki et al. 1992). Thus, tissue cadmium may be a useful indicator of available soil concentrations. Tissues lead levels also increase in contaminated soils (Morgan & Morgan 1988). Accumulation of this metal is known to be influenced by soil pH, CEC and calcium content (Ireland 1983; Ma 1982; Morgan & Morgan 1988). Consequently, for lead, as for cadmium, worm metal burden may be used to indicate available metal. For copper, only low level accumulation occurs even at high soil concentrations (Morgan and Morgan 1988). Additionally, it is generally accepted that soil conditions have only a minor effect on copper accumulation (Ma 1983; Ma et al. 1983; Morgan & Morgan 1988). Consequently, earthworm copper levels are unlikely to be useful for assessing water soluble metal concentrations.

The relationships between NAX, WX and earthworm zinc and toxicity for ***Eisenia fetida***

Survival was not affected by soil pH, however, cocoon production was reduced in the pH 4.0 soils. Percentage OM had no effect on survival or cocoon production. This is in agreement with the work of Bengtsson et al. (1986) who found cocoon production, but not survival of *Dendrobaena rubida*, to be reduced at pH 4.5. Clearly, for cocoon production, low pH acts as an additional stressor in *Eisenia fetida*, although this may not be the case for ubiquitous species such as *Lumbricus rubellus*. Thus in toxicity tests with pH sensitive species, direct effects must be separated from effects on metal solubility if valid conclusions on the effects of changes in availability are to be made (Crommentuijn 1994).

High total and water soluble zinc concentrations decreased both the survival and relative cocoon production of *Eisenia fetida*. For survival, a threshold concentration existed below which effects did not occur. This value is approximately 350 µg Zn g^{-1} for total zinc, while for soluble zinc a threshold concentration of 40 µg Zn g^{-1} can be estimated. For cocoon production, the effects of metals followed a more normal dose response pattern.

Survival and cocoon production in both the artificial and field soil were correlated more strongly with water soluble zinc than NAX or worm zinc (Figs. 3a, 3b, 6a and 6b). This suggests that effects on *Eisenia fetida* are exerted primarily by the uptake of pore water zinc (Van Gestel 1992, in press). If it is assumed that the toxicity of zinc is exerted through pore water concentrations, then toxicity values calculated from soluble zinc should be comparable between the test soils (Crommentuijn 1994). This appears to be the case, since LC_{50}s, cocoon production EC_{50}s and cocoon production and mortality estNOECs determined from the soluble zinc were not raised in the high pH or OM soils (Figs. 4b, 4d, 5b, 5d), while values calculated from total metal increased (Figs. 4a, 4c, 5a, 5c).

The calculation of cocoon production EC_{50}s and estNOECs for total zinc in Avonmouth soils gave higher values than for the artificial medium. This was due to a greater binding capacity of the field soils. However, values calculated from soluble zinc concentrations are similar to those calculated for these parameters in artificial soil. This indicates that water soluble zinc concentrations can be used to predict effects on earthworms over a range of soil types in which soil conditions and CECs differ. Thus, water soluble zinc could be useful for predicting impact on earthworm populations at contaminated field sites, especially if sensitivity differences between *Eisenia fetida* and natural soil species can be reconciled.

Using available metal toxicity values to calculate HC5s

The influence of environmental conditions on metal toxicity can be problematic when attempts are made to derive ecologically-relevant soil quality criteria. Van Straalen & Denneman (1989) have developed an approach using laboratory toxicity data to calculate 'hazardous concentrations for 5% of species' (HC5s) for metals in soils (see also Van Straalen 1993; Aldenberg & Slob 1993). However, the values of 0.2 µg Cd g^{-1}, 2.7 µg Cu g^{-1} and 77 µg Pb g^{-1} derived by Van Straalen (1993) using this method, seem low when compared to the concentrations that affect soil invertebrates at polluted sites close to a smelter at Avonmouth, Southwest England (Drobne & Hopkin 1994; Hopkin 1989; Hopkin & Hames 1994; Spurgeon & Hopkin, 1995). The differences that exist between the proposed HC5s and metal toxicity at this field site can be attributed, at least in part, to the effects of soils conditions on availability (Spurgeon and Hopkin, 1995).

Two possible explanations, related to the influence of metal availability, exist for the low values of the proposed HC5s. Firstly, the data used to derive HC5s are taken from laboratory studies, particularly with lumbricid worms (see Table 1, Van Straalen 1993). Comparison of the results of OECD (1984) artificial soil tests with the effects of metals in soil from Avonmouth on *Eisenia fetida*, suggests that the laboratory procedure may overestimate toxicity due to the increased availability in the laboratory medium (Spurgeon & Hopkins, 1995, Crommentuijn 1994). Secondly, the HC5 represents a general value for all soils.

Consequently, in less sensitive areas, populations may exist in soils containing metal concentrations well in excess of the HC5 value with no deleterious effects, while in habitats that are for example acidic or have a low OM content, detrimental effects may occur at the HC5 level.

HC5s calculated using toxicity values determined from total metal concentrations may not predict effects at a range of sites, due to differences in availability between the test and field soils and variations in soil conditions at contaminated sites (Van Gestel, in press). However, if an HC5 could be based on the concentration of available metal, this should allow a value to be calculated that can be compared between laboratory tests and field soils and across a range of different sites. Van Wensem et al. (1994) suggested that the internal threshold concentration (ITC) of exposed species could be used to calculate ecologically relevant quality criteria. This technique was found to be useful for estimating an HC5 for cadmium. However, the results of the work conducted in the present paper (and those of Morgan and Morgan 1988) indicate that at least for zinc, earthworm burdens are unlikely to be useful in calculating an ecologically relevant HC5 due to poor accumulation and weak correlations between internal zinc and toxic effects. The work outlined here indicates that for zinc, soluble concentrations best describe toxic effects for earthworms, hence use of concentrations in this fraction would be most useful for calculating ecologically relevant HC5 values for zinc in soils.

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