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Effects of Metal-Contaminated Soils on the Growth, Sexual Development, and Early Cocoon Production of the Earthworm *Eisenia fetida,* with Particular Reference to Zinc

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Juvenile *Eisenia fetida* (Savigny) were exposed for 20 weeks to an uncontaminated soil and to soils contaminated with cadmium, copper, lead, and zinc collected from seven sites at different distances from a smelting works at Avonmouth, southwest England. The survival, growth (= weight after 5 weeks exposure), time to sexual maturation (= percentages of adults present after 8 weeks), and reproduction (= number of cocoons produced by the worms) were compared with soil metal concentrations. Of the parameters measured, growth and sexual maturation time had the lowest EC_{50} values. The effects of metal-contaminated soils could be attributed both to the direct toxicity of the metals and to changes in the ''scope for growth'' of the exposed worms. A comparison of the results with those of an earlier toxicity test conducted with adult worms indicated that juveniles are more sensitive to metals than adults. Significant toxic effects on the growth and sexual maturation times of juveniles were detected in soils from sites for which no significant effects on the cocoon production of adults could be detected. The greater sensitivity of juvenile worms indicates the importance of considering effects on a variety of life history stages when conducting a risk assessment of the effects of pollutants in soils. Although *E. fetida* does not occur naturally in soils at Avonmouth, the present study provides evidence to support the suggestion that pollution from the smelter is responsible for the absence of worms within 2 km of the factory. Results presented in this paper, and from previous studies, suggest the observed absence is due to the effects of zinc on the growth and maturation of juveniles and the cocoon production rate of adult worms.

INTRODUCTION

Earthworms are more susceptible to metal pollution than many other groups of soil invertebrates (Bengtsson *et al.*, 1992). High concentrations of cadmium, copper, lead, and zinc in soils can affect the density, viability, cocoon production, growth, and sexual development of worms (Bengtsson and Tranvik, 1989; Bengtsson *et al.*, 1983; Morgan *et al.*, 1993; Spurgeon *et al.*, 1994; Spurgeon and Hopkin, 1995; Tyler *et al.*, 1989; Van Gestel *et al.*, 1989, 1991, 1993). As a result of the relative sensitivity of the worms, particularly for metals such as zinc and copper (Spurgeon *et al.*, 1994; Van Straalen *et al.*, 1994), toxicity data from earthworms will be important in determining ''safe levels'' for metals in soil.

Juvenile earthworms may be more sensitive to pollutants than adults. For example, Van Gestel *et al.* (1991) found a lower sexual development NOEC for copper in juvenile *Eisenia fetida* (56 μg Cu g^{-1}) than for cocoon production in adult worms (60–120 μg Cu g^{-1}). Consequently, the use of toxicity data from adults to estimate ecotoxicological risk, as proposed by Van Straalen and Denneman (1989) and Wagner and Løkke (1991), may underestimate the effects of pollutants on soil invertebrate populations. In this paper, the survival, growth, sexual maturation time, and reproduction of juvenile *E. fetida* were examined in smelter-contaminated field soils for which effects on adults have been recorded in a previous experiment (Spurgeon and Hopkin, 1995). This has allowed the relative sensitivities of adult and juvenile worms to be compared.

Monitoring the growth and development of juvenile *E. fetida* in contaminated soils allows the potential effects of metals on the energy budget of exposed worms to be assessed (Basset, 1993). In a polluted environment, changes to individual energy budgets will occur, as the organism expends energy resisting the contaminant by avoidance, exclusion, removal, or complexing (Donker, 1992; Sibly and Calow, 1989; Widdows and Donkin, 1989). This additional energy requirement will decrease the ''scope for growth'' of the exposed animals (Maltby and Naylor, 1990; Maltby *et al.*, 1990; Widdows and Donkin, 1991), reducing not only growth but also differentiation, cocoon production, and ultimately a number of important life history characteristics such as time to sexual maturity and generation time (Donker *et al.*, 1993a,b).

E. fetida does not occur naturally in soils in the Avonmouth region (Read, 1987); however, it is the opinion of the authors that this earthworm can be considered a suitable model species for predicting the effects of metals on earthworm populations at Avonmouth. Interspecies differences for the toxicity of metals to earthworms have been found in a number of studies. However, these differences almost always result in toxicity values (LC_{50}s, EC_{50}s, NOECs) that are well

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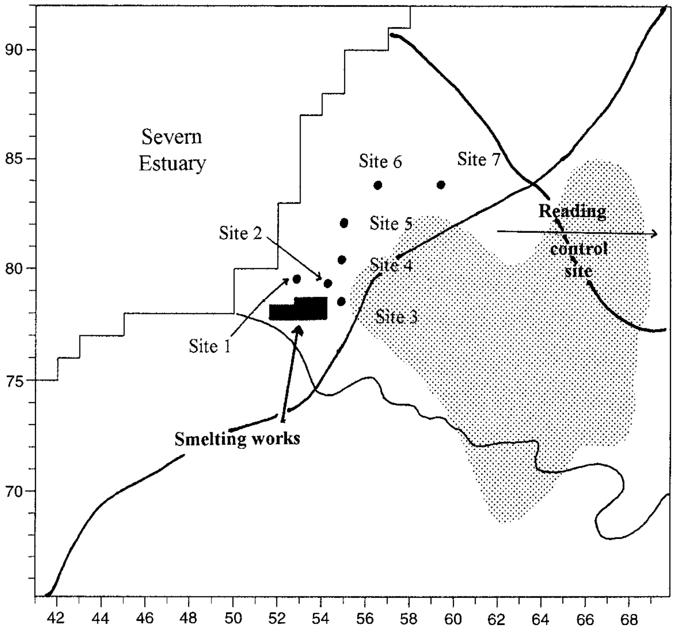

FIG. 1. Location of the sites from which soils were collected and to which juvenile worms were exposed. All sites were situated to the northeast of the Avonmouth smelter. Axes give Ordnance Survey grid reference values in km. Shading indicates the extent of the urban area of Bristol.

within the same order of magnitude (Bengtsson *et al.,* 1986; Ma, 1988). The limited differences in sensitivity to metals between earthworm species and the ease with which *E. fetida* can be reared and handled in the laboratory make this species ideal for predicting the effects of metals on earthworms in the field (Edwards and Coulson, 1992).

MATERIALS AND METHODS

Contaminated soils were collected on the same day from seven sites in the vicinity of the Avonmouth smelting works (Fig. 1). All sites were located on permanent grassland, situated next to minor roads at least 2 m from the curb. About 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 (Hopkin, 1989).

Soil aggregates were crushed while still damp and placed in a fan oven at 60°C for 2 days to dry. A subsample of each soil was collected for the determination of cadmium, copper, lead, and zinc concentrations in the soils by flame atomic absorption spectrometry of nitric acid digests (see Hopkin, 1989) and measurement of percentage loss on ignition (organic matter content) and soil (H_2O) pH. Results of the chemical analysis of soils are given in Table 1. All remaining soil was passed through a 2-mm mesh and 500 g was placed into each of four replicate containers (plastic boxes with dimensions 175 × 120 × 60 mm). Distilled water was added to give a moisture content of approximately 65% of field capacity and the soils left to stabilize for 72 hr prior to the addition of the worms.

To obtain juvenile *E. fetida,* cocoons from laboratory cul-

TABLE 1

Location of the Sites in the Vicinity of the Avonmouth Smelter and the pH, Organic Matter Content, and Concentration of Metals in the Soils Collected (Mean ± SD; n = 6 for Metals, n = 3 for Percentage of Organic Matter)

Site	O. S. Grid Ref.	Distance from smelter (km)	pH	% Organic matter	Cadmium (μg Cd g^{-1})	Copper (μg Cu g^{-1})	Lead (μg Pb g^{-1})	Zinc (μg Zn g^{-1})
1	529794	0.5	6.55	17.2 (±2.8)	312.2 (±81.3)	2610.0 (±230)	16,000 (±2550)	32,900 (±4860)
2	533790	0.5	6.26	22 (±0.4)	129.9 (±22.0)	780.0 (±169)	6,720 (±1360)	7,950 (±1360)
3	535786	0.8	6.35	17.8 (±0.6)	32.4 (±10.2)	159.0 (±46.3)	842 (±246)	1,990 (±246)
4	532803	1.8	6.65	27.1 (±0.8)	33.5 (±12.7)	164.0 (±69.3)	1,250 (±447)	2,790 (±447)
5	537817	3	7.37	18.5 (±9.8)	14.3 (±1.5)	108.0 (±10.5)	930 (±260)	1,850 (±260)
6	552853	5.8	7.36	12.9 (±1.5)	0.9 (±0.5)	36.2 (±14.6)	245 (±93)	657 (±93)
7	578816	7	6.92	19.7 (±2.6)	2.7 (±0.5)	42.3 (±6.5)	290 (±48)	925 (±48)
8 (Control)	737714	110	5.54	9.4 (±0.1)	0.1 (±0.2)	30.9 (±6.4)	30 (±9)	38 (±12)

Note. O. S. Grid Ref., British Ordnance Survey Grid Reference.

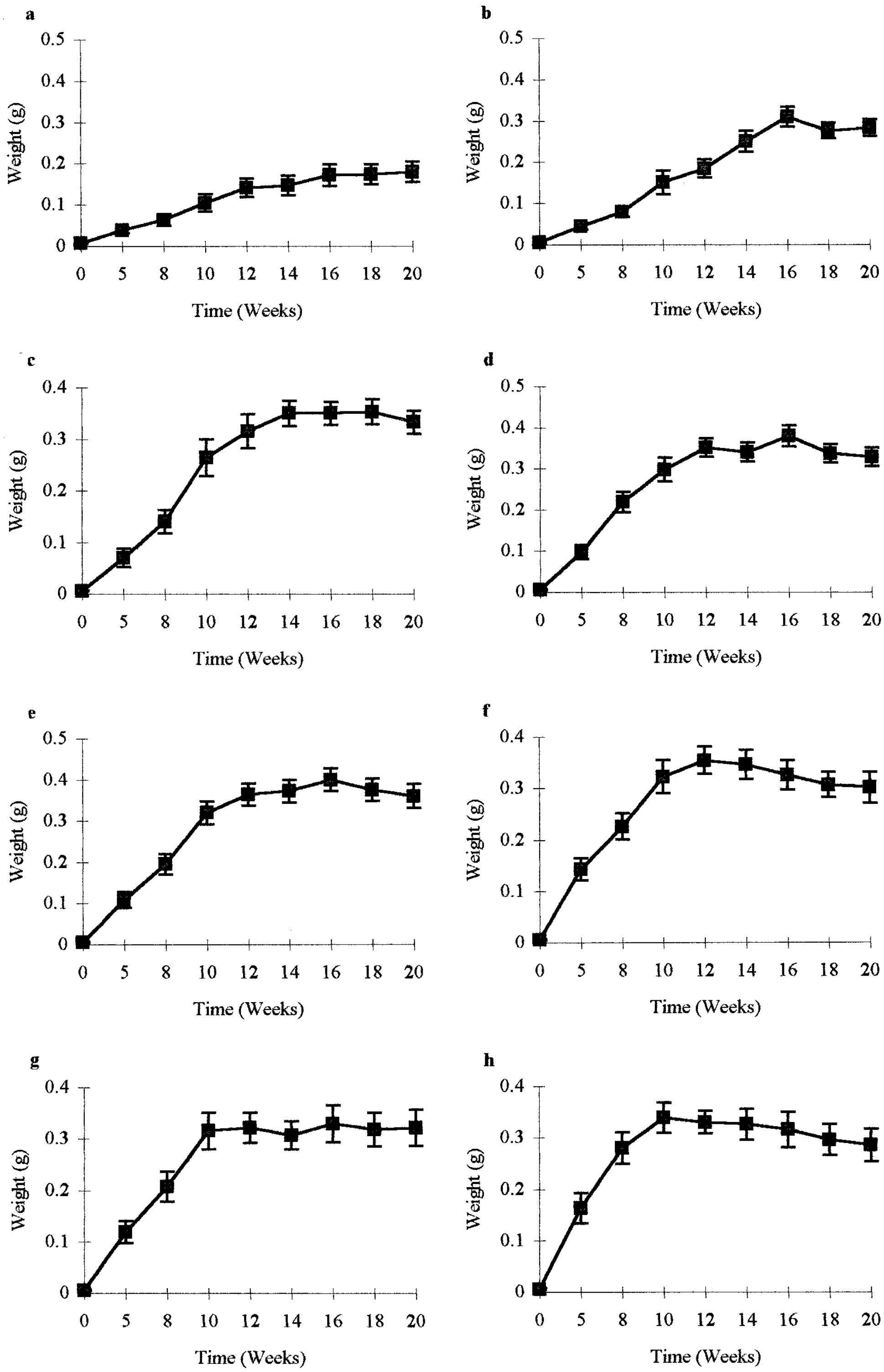

FIG. 2. (a–h) Body growth patterns of juvenile *E. fetida* (mean g fresh wt with SE bars) exposed to soils collected from metal-contaminated sites around Avonmouth Site 1 (a), Site 2 (b), Site 3 (c), Site 4 (d), Site 5 (e), Site 6 (f), Site 7 (g), and a control soil collected from Reading University campus Site 8 (h).

ture were reared in OECD artificial soil for 5 weeks (see method of Van Gestel *et al.,* 1988). Newly emerged worms were sieved from the soil. Ten individuals were weighed and placed into each test replicate. All containers were covered to prevent water loss and maintained at 20°C under constant light for 20 weeks. Throughout the experiment, changes in

TABLE 2
Estimated Growth Parameters for Earthworms Exposed to Contaminated Soils Collected from 8 Sites in the Avonmouth Area

Site	Length of linear growth phase (weeks)	Growth rate (*b*) [g week^{-1} (g day^{-1})]	Growth rate (*b*) [g week^{-1} (g day^{-1})]	Mean final fresh weight (g)
1	20	0.0099	0.0013	0.180
2	16	0.0166	0.0022	0.309
3	14	0.0237	0.0032	0.35
4	12	0.0283	0.0038	0.351
5	12	0.0280	0.0040	0.320
6	12	0.0304	0.0043	0.322
7	10	0.0286	0.0041	0.316
8 (control)	10	0.0341	0.0049	0.339

Soil metal concentration

Note. Mean growth rate is determined as the slope of the regression for weight over the initial linear stage of weight increase. Final fresh weights are given from the mean weight of surviving worms at the end of the linear phase of growth.

soil moisture content were monitored by weighing the containers. Additional water was added if required.

To ensure growth, maturation, and cocoon production during the experiment, worms were supplied with uncontaminated horse manure as a source of food (see Van Gestel *et al.*, 1992a). Due to the small size of the worms, the supply of food was limited at the beginning of the experiment to prevent a buildup of uneaten material in the test soils. For the first 3 weeks, 1 g (dry wt) of manure was supplied per container, in Weeks 4 to 6, 1.5 g was supplied, and from then until the end of the experiment 2 g were added each week. Cluzeau and Fayolle (1989) concluded that 50 mg/g worm per day of suitable food would be sufficient to allow unrestricted growth, maturation, and cocoon production in *E. andrei.* This corresponds to approximately 1.3 g of food per week supplied to 10 worms of average weight 400 mg. Thus the amount of food used in this test is sufficient to allow unrestricted growth and development.

At intervals after exposure, worms were sorted from the test soil and their survival, weight, and stages of development recorded. Sexual maturation was assessed according to the scheme of Van Gestel *et al.* (1991). Worms with a full clitellum were recorded as adult, those with a full tubercle pubertatis but no clitellum as subadult, and individuals with neither of these reproductive structures as juveniles. Worms were first sampled after 5 weeks. Before this time, they are very difficult to find in the most contaminated soils. Sampling was repeated after 8, 10, 12, 14, 16, 18, and 20 weeks.

Any cocoons produced during the experiment were collected. Weights of individual cocoons were recorded and cocoon production rates calculated for each test replicate. Cocoon viability and the number of juveniles emerging per cocoon were not measured, since no effects due to cadmium, copper, lead, and zinc have been found for these parameters in earlier toxicity tests (Spurgeon and Hopkin, 1995; Spurgeon *et al.*, 1994; Van Gestel *et al.*, 1989, 1992b).

EC_{50} values for metals in soil were determined using the linear interpolation technique developed by the U.S. EPA (Norberg-King, 1993). Results from previous work with adult worms indicate that of the metals present at Avonmouth, zinc is most likely to be limiting the abundance of earthworms close to the smelter (Spurgeon and Hopkin, 1995; Spurgeon *et al.*, 1994). While cadmium is 10 to 30 times more toxic than zinc to *E. fetida* (Malecki *et al.*, 1982; Spurgeon and Hopkin, 1995; Spurgeon *et al.*, 1994; Van Gestel *et al.*, 1993), soil zinc concentrations always exceed those for cadmium by a factor of at least 30 (over 100 times in most cases) (Hopkin and Hames, 1994). Consequently, results from this experiment have been related primarily to the levels of zinc in the field soils.

RESULTS

After 5 weeks, the mortality of worms exposed to soils from Sites 1 and 2 was significantly increased compared to controls. Highest juvenile mortality was recorded in soils collected from Site 2, despite the fact that this soil contained only 7950 μg Zn g^{-1}, while Site 1 soil contained 32,900 μg Zn g^{-1}. However, analysis of the water-soluble zinc fraction in these soils, conducted as part of another experiment (see Spurgeon, 1996; Spurgeon and Hopkin, 1996), demonstrated that Site 2 soil contained a higher concentration of water-extractable zinc than soil from Site 1 (297 μg g^{-1} vs 57.5 μg g^{-1}, respectively). The increased availability of metals at Site 2 almost certainly explained the higher mortality of worms exposed to this soil.

Of the worms that died, most did so during the first 5 weeks of the experiment. This pattern of death could indicate that the juveniles were particularly sensitive during the first weeks of development. However, a similar pattern of mortality, found for adult worms in an earlier toxicity test, was attributed to rapid exposure of the animals due to the uptake of metals across the body wall, rather than from diet (Spurgeon *et al.*, 1994). It is also possible that the metal

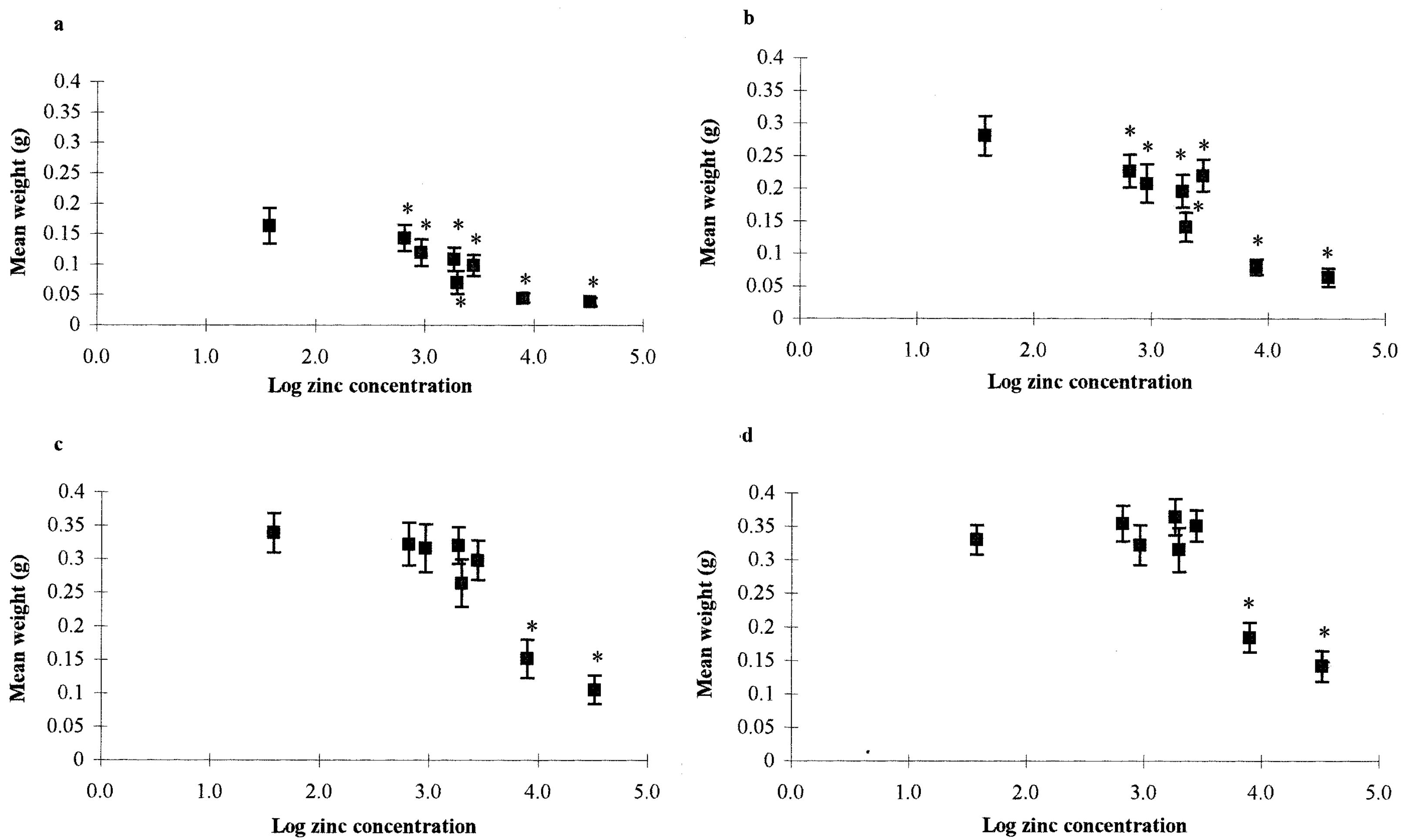

FIG. 3. (a–d) Relationship between mean weight of *E. fetida* and log soil zinc concentration to which the worms were exposed for (a) 5 weeks, (b) 8 weeks, (c) 10 weeks, and (d) 12 weeks. Error bars indicate SE of mean. Asterisks indicate mean weights significantly lower than those for worms exposed to soil from Site 8 (control).

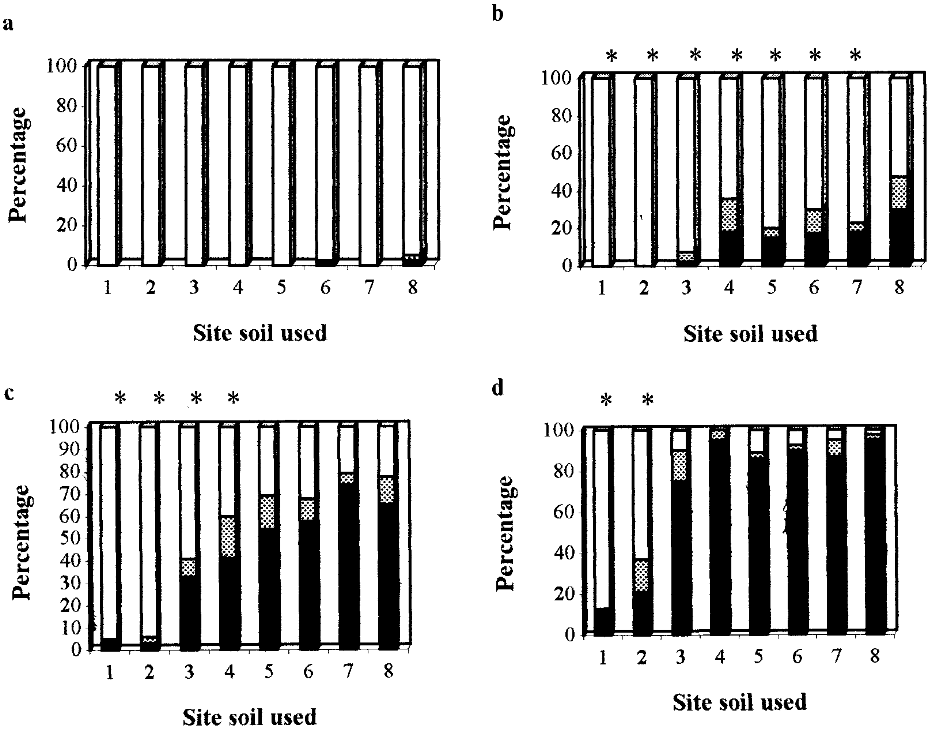

FIG. 4. (a–d) Influence of seven soils collected from the Avonmouth area and an uncontaminated control soil on the sexual development of *E. fetida* after 5 weeks (a), 8 weeks (b), 10 weeks (c), and 12 weeks (d). Light shading indicates juveniles, stippled shading subadults, and dark shading adults. For bars marked with an asterisk, the number of sexual mature worms is significantly lower ($P < 0.05$) than in the Site 8 (control) soil.

availability decreases with time throughout the experiment, as the metals become more firmly bound to soil particles. The patterns of mortality for both juvenile and adult earthworms exposed to soils from Avonmouth indicate that mortality of earthworms in metal contaminated soils is not strongly influenced by the duration of exposure.

The worms exhibited a sigmoidal pattern of growth, although the base of the curve was absent due to the lack of sampling intervals in the early stage of the experiment (Figs. 2a–2h). Thus, during the initial phase of the experiment, there was a strong linear phase of growth that continued until maximum weight was reached. At this point, which corresponded to the onset of sexual maturity and the beginning of cocoon production, growth rate reduced and the worms maintained, or even lost, weight as reproductive effort increased (Figs. 2a–2h, also Table 3). The growth curves for worms exposed to the most contaminated soils was much flatter than for control soil-exposed worms, indicating that they spent longer in the linear phase of growth. For example, worms exposed to soils collected from Sites 7 and 8 revealed linear growth for 10 weeks, Sites 4–6 for 12 weeks, Site 3 for 14 weeks, and Site 2 for 16 weeks, while worms in Site 1 soil did not reach maximum weight by end of the experiment (Figs. 2a–2h).

Because earthworm growth had a strong linear phase, the simplest way to determine growth rate is to use a linear regression with no intercept constant for data over the period when weight was increasing. Modeling growth in this way would allow the use of the slope parameter (b) as a prediction of growth rate (g week^{-1} or g day^{-1}). Calculation of growth in this way indicated a reduction in rates for worms in the most polluted soils, with lowest growth in the most heavily contaminated soils (Table 2).

Changes in growth rates ultimately result in higher fresh weights for worms in the least contaminated soils (Figs. 3a–3d). At 5 and 8 weeks, mean weight was reduced in all Avonmouth soils compared to control values (Figs. 3a and 3b). However, as the experiment progressed, worms in soils from Sites 3, 4, 5, 6, and 7 caught up with the controls, so that after 10 and 12 weeks, only worms in soils from Site 1 and 2 weighed less than controls (Figs. 3c and 3d) and after 16 weeks only animals in Site 1 soil were smaller. Calculations of zinc EC_{50}s for the effects of zinc on average worm weights indicated that the lowest value (with 95% confidence intervals) of 3120 (1920–4830) μg Zn g^{-1} was for the 5-week sample interval.

Control worms reached sexual maturity earlier than those exposed to the most contaminated soils (Figs. 4a–4d). At 8 weeks, the number of adults was significantly reduced in soils from all Avonmouth sites (Fig. 4b), at 10 weeks, adult number was reduced in soils from Sites 1, 2, 3, and 4 (Fig. 4c), while subsequent to this only the two most contaminated

TABLE 3

Cocoon Production Rates (Cocoon/Worm/Week) and Total Number of Cocoons Produced for *Eisenia fetida* Exposed to Soils Collected from Seven Metal-Contaminated Sites Close to the Avonmouth Smelter and an Uncontaminated Control (See Fig. 1 for Location of Sites)

Site		8 weeks	10 weeks	12 weeks	14 weeks	16 weeks	18 weeks	20 weeks	Total No. Cocoons
1	Soil metal concentration	0	0	0	0.188**	0	0.290**	0.220**	19
2		0	0	0	0.222**	0.400**	0.090**	0.147**	24
3		0	0.077*	0.207**	0.722	1.382	1.316	1.303*	370
4		0.048	0.313*	0.264**	0.595	1.220	1.620	1.840	430
5		0.278	0.500	0.672**	0.824	1.840	2.050	1.960	563
6		0.095	0.365	0.389**	0.579	1.410	1.680	1.920	484
7		0.048	0.196	0.667**	0.838	1.510	1.750	1.940	488
8 (control)		0.083	0.75	1.092	1.038	1.688	1.900	2.050	659

Note. Rates significantly different from controls at $*P < 0.05$ and $**P < 0.01$.

soils had significantly fewer adult worms than the controls (Fig. 4d). All worms, except those exposed to soils from Site 1 and 2, were adult after 16 weeks. For Site 1 and 2 soils, the rate of sexual development was slowed to the extent that by the end of the test, only 62 and 94% of worms had reached maturity respectively (Table 5). The zinc EC_{50} values for the percentage of adults present were lowest after 8 weeks and were 1860 (480–4190) μg Zn g^{-1}.

Mean time to sexual maturity (MTSM) of the exposed juvenile worms was calculated from the number of adults in each test soil at every sample interval. For Sites 1 and 2, the calculation was based on the assumption that the worms that were not mature after 20 weeks would have matured at 22 weeks. The time taken to reach sexual maturity in *E. fetida* was increased at the most contaminated sites (Table 5). Analysis of data for MTSM using a one way ANOVA indicated that the soil to which the worms were exposed had a significant effect on MTSM ($P < 0.001$). Maturation was particularly slowed in worms exposed to soils from Sites 1 and 2. These animals took on average 5 and 8 weeks longer, respectively, to reach maturity than controls. However, calculation of median maturation time for all sites indicated a lengthening of maturation time of worms exposed to soils from Sites 3 and 4 (Table 5).

The quicker sexual maturation of worms in the control and less contaminated soils resulted in an earlier production of cocoons than for animals exposed to soils collected from the most heavily contaminated sites located close to the smelter. Worms in soils from Sites 4, 5, 6, 7, and 8 produced cocoons after 8 weeks of exposure, worms in Site 3 after 10 weeks, while for Sites 1 and 2, cocoons were not collected until 14 weeks after the start of the test (Table 3). Rates of cocoon production were also reduced in worms exposed to soils collected from sites close to the smelting works. The reduction in cocoon production was particularly pronounced in soils from Sites 1 and 2. For these sites, a total of only 19 and 24 cocoons, respectively, were produced in the four test replicates throughout the 20-week duration of the test. In contrast, worms exposed to soil from Site 8 produced a total of 659 cocoons (Table 3).

Cocoon production at 12 weeks was significantly reduced in all soils collected from Avonmouth (Table 3) and the zinc EC_{50} value (with 95% confidence intervals) was 637 (497–1933) μg Zn g^{-1} for this sample interval. In contrast after 20 weeks, only worms in the three most contaminated soils produced fewer cocoons, and a zinc EC_{50} value of 4950 μg Zn g^{-1} could be calculated. A reduction in cocoon production at the most contaminated Avonmouth sites is in agreement with the results of an earlier toxicity test conducted with adult worms (Spurgeon and Hopkin, 1995).

Mean cocoon weight showed a slight increase as the metal concentration of the soil increased (Table 4). The largest cocoons were collected from the most contaminated sites and

TABLE 4

Mean Weight of Cocoons Collected in Weeks 12–20 from Juvenile *Eisenia fetida* Exposed to Soils Collected from Seven Sites in the Avonmouth Area and a Control Site Situated at Reading University Campus

Site		Total No. of cocoons weighed	Mean cocoon weight (g) ± SE
1	Soil metal concentration	575	0.01049 ± 0.00014
2		430	0.01009 ± 0.00016
3		484	0.01047 ± 0.00016
4		563	0.01157 ± 0.00014
5		430	0.01112 ± 0.00015
6		370	0.01086 ± 0.00012
7		24	0.01197 ± 0.00057
8 (control)		19	0.01129 ± 0.00077

Note. Only cocoons from which it was certain that no juveniles had emerged were weighed.

TABLE 5

Percentage of adults Reaching Sexual Maturity after 20 Weeks and Mean (±SE) and Median Sexual Development Times for *Eisenia fetida* Exposed to Soils Collected from Seven Sites in the Avonmouth Area and a Control Site Situated on the University of Reading Campus

Site		Percentage of worms reaching maturity	Mean time to sexual maturity (weeks)	Median sexual development time (weeks)
1	Soil metal concentration	62	18.207 ± 0.285	20
2		94	15.111 ± 0.324	15
3		100	11.842 ± 0.297	12
4		100	10.947 ± 0.305	12
5		100	10.811 ± 0.299	10
6		100	10.589 ± 0.296	10
7		100	10.333 ± 0.690	10
8 (control)		100	10.125 ± 0.743	10

Note. Mean times to sexual maturity for Sites 1 and 2 have been calculated assuming that worms that were not mature after 20 weeks would have reached maturity at 22 weeks.

the smallest from Site 2. Analysis of variance indicated that a significant relationship ($P < 0.01$) existed between mean cocoon weight and the soil in which the cocoons were laid.

DISCUSSION

In an earlier test in which adults were exposed to soils from the same sites, no significant mortality was found (Spurgeon and Hopkin, 1995). The results of the present work indicate that the survival of juvenile *E. fetida* is more sensitive to metal-contaminated soils than that of mature worms, as significantly increased mortality was found in the most heavily contaminated soils. Despite the increased sensitivity of juveniles, it is unlikely that direct mortality has a major effect on earthworm distribution in the Avonmouth region. However, the increased mortality of worms in Site 2 soil indicates that soil conditions that alter metal bioavailability can increase toxicity causing direct mortality at some locations.

Significantly lower average weights after 5 weeks were found for worms exposed to all Avonmouth soils in comparison to mean control weights (Fig. 3a). A number of studies on soil invertebrates have recorded a reduction in growth in animals exposure to metal-contaminated diets. However, such effects can frequently be attributed to a reduction in consumption due to avoidance of the contaminated food (Drobne and Hopkin, 1995; Laskowski and Hopkin, 1996). For the current study, uncontaminated food was added to all test containers to avoid complication of results due to differences in metal availability between the test soils and food. The use of uncontaminated food means that the effects on growth observed in this experiment are almost certainly due to the direct effects of metal on the physiology of the exposed earthworms.

High levels of a given pollutant can alter growth by both direct and indirect effects. Growth can be impaired by direct toxic effects on the physiology of exposed worms, or by changes in the energy budget as an individual attempts to prevent accumulation in sensitive tissues. In earthworms, cadmium, lead, and some zinc are detoxified by binding in granules (chloragocytes) or metallothionein-like proteins in the chloragogenous tissue (Morgan and Morgan, 1988; Morgan *et al.*, 1989). In contrast, copper and the remaining zinc are eliminated by an excretion mechanism (Morgan and Morgan, 1990, 1991). The mechanisms of metal sequestration and elimination have metabolic costs, both in the development of the system and for maintenance and repair. This increased requirement for maintenance energy will ultimately result in a reduction in the energy available for growth and development (Donker *et al.*, 1993a).

Disruption to the energy budget in the worms exposed to contaminated soils could also underlie differences in sexual maturation and cocoon production. Energy is required for maintenance, growth, and two reproductive processes, differentiation to reach maturity and egg production (Kooijman, 1986). If there is an increased energy demand for metal sequestration and elimination, this will decrease the energy available for sexual maturation and ultimately slow maturation in animals exposed to the most contaminated soils. However, it should also be noted that the direct toxicological impact of metals could also cause the observed delay.

Cocoon production rates were particularly sensitive during the early period of reproduction, with a significant decrease found in all Avonmouth soils. However, at 20 weeks, effects on cocoon production rates were similar to those found in an earlier toxicity experiment conducted with adult worms by Spurgeon and Hopkin (1995). In the toxicity test with adults, cocoon production was significantly reduced in worms exposed to the four most contaminated soils, while in the current experiment, cocoon production was significantly lower than in the controls for worms exposed to soils from Sites 1, 2, and 3 (Table 3). The apparent sensitivity of cocoon production in early adulthood is closely linked to the delayed maturation of worms in the most contaminated soils, since cocoon production increases markedly in the first weeks of adulthood (Table 3).

Average cocoon size increased slightly with exposure to metal-contaminated soil. It is not clear if this relationship is a response of earthworms to the presence of high metal levels, or if cocoons are larger due to the decrease in cocoon production rates in the heavily contaminated soils. An increased reproductive output in individual broods has been found in the aquatic isopod *Asellus aquaticus* (Maltby, 1991) and in Collembola collected from sites around a smelting works (Bengtsson and Rundgren, 1988). This indicates that organisms exposed to toxic stress may undertake fewer reproductive events, but with a greater amount of energy allocated to each. Since it is not known if increased cocoon size is a result of phenotypic plasticity in cocoon production rate, or due to greater energy availability for each cocoon due to a reduction in reproductive rate caused by disruption of the cocoon formation process, it is unclear whether *E. fetida* adopts such a strategy when exposed to metals.

Metal-contaminated soils have a profound effect on many parameters governing the development of juvenile earthworms. Thus, it must be supposed that there is considerable disruption to the life history of worms present at metal-contaminated sites in the field. Effects are primarily governed by the direct effects of metal intoxication and/or a reduction in energy available for growth. Changes in the life history characteristics of individual earthworms could affect field populations in a variety of ways. For example, the increase in the MTSM at polluted sites could result in a reduction in the available time for reproduction. This may be particularly important as it may result in a loss in the seasonal synchrony of a species (Posthuma *et al.,* 1993). Such an effect has already been observed for carabid beetle populations at Avonmouth (Read *et al.,* 1987).

CONCLUSION

The results of this study indicate that juvenile *E. fetida* are more sensitive to zinc-contaminated soils than adults. For adults, the EC_{50} for the most sensitive parameter (cocoon production) was 3600 μg Zn g^{-1}, while for juveniles, the EC_{50} for the most sensitive parameter, (sexual development) was 1860 μg Zn g^{-1}. The increased sensitivity of development in juvenile earthworms compared to cocoon production in adults supports the findings of earlier toxicity tests conducted by Van Gestel *et al.* (1991).

The lower toxicity values determined in juveniles suggest that it may be important to consider effects on juveniles when proposing safe levels for metals in soils. Juvenile growth rate and sexual development are important life history parameters for earthworms, since they may modify the dynamics of earthworm populations in the field. Data from juvenile development experiments should therefore also be considered when proposing a safe environmental concentration for a pollutant.

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