Accumulation and elimination of cadmium, chromium and zinc and effects on growth and reproduction in *Eisenia andrei* (Oligochaeta, Annelida)

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**ABSTRACT**

Accumulation and elimination of cadmium, chromium and zinc and effects on the growth and reproduction of the earthworm *Eisenia andrei* were determined in an artificial soil. Cadmium at the lowest soil concentration tested (10 mg/kg) significantly reduced cocoon production, whereas cadmium concentrations in the earthworms at the end of the 3-week exposure period were significantly increased in a dose-related manner at all concentrations tested. Reproduction was completely recovered at the end of a 3-week recovery period in untreated soil, although cadmium concentrations in the earthworms were still significantly elevated. Apparently, the remaining cadmium residues in the worms were strongly bound, and therefore no longer exerted a toxic effect on the reproduction of earthworms. Chromium was significantly accumulated and earthworm reproduction significantly reduced at soil concentrations of 100 mg/kg and higher. Chromium was completely eliminated and reproduction no longer affected at the end of a 3-week recovery period. Zinc significantly reduced reproduction at soil concentrations of 560 and 1000 mg/kg, and induced the production of malformed cocoons. Earthworms were able to regulate their body content of zinc; only at 1000 mg/kg dry soil were zinc concentrations significantly increased. At the end of a 3-week recovery period zinc concentrations in the earthworms had returned to the control level, and reproduction was completely recovered.

**Key words:** Earthworm reproduction; Metal toxicity; Internal effect concentration; Bioaccumulation; Artificial Soil test

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INTRODUCTION

Recently, a new earthworm reproduction toxicity test has been developed (Van Gestel et al., 1989). This test method uses the same earthworm species and artificial soil substrate as prescribed by existing guidelines for acute toxicity testing with earthworms (OECD, 1984; EEC, 1985). In the test method, earthworms are exposed to chemical substances for 3 weeks and effects on growth and reproduction are assessed. The method also allows for inclusion of a (3-week) recovery period. To gain experience with this test method, a number of chemical substances, including cadmium and chromium (III), were tested; results have been described before (Van Gestel et al., 1992a).

From the literature, it appeared that hardly any data are available on the earthworm toxicity of zinc. One acute (Neuhauser et al., 1985) and three sublethal toxicity studies (Hartenstein et al., 1981; Malecki et al., 1982; Neuhauser et al., 1984) have been described. In the latter three tests, earthworms were exposed in a system of untreated soil covered by a layer of treated substrate. Earthworms may be able to avoid toxic substances (Edwards and Lofty, 1977); so, the results of these studies are difficult to interpret. The newly developed test method was therefore also applied to zinc and results are presented in this paper.

During the last decade many papers have described differences in the bioaccumulation of different metals and the ability of organisms to regulate their body concentrations of essential metals such as zinc. These studies mainly concentrated on aquatic organisms (e.g., Rainbow, 1988; Rainbow and White, 1989; Kraak, 1992). Many data are available on metal concentrations in earthworms in relation to soil pollution. No systematic studies have been made, however, of the uptake and elimination characteristics of metals in earthworms, and the relationship between body metal concentrations and the effects on growth and reproduction. In the reproduction toxicity studies on cadmium and chromium described by Van Gestel et al. (1992a) and the present study on zinc, therefore, also the uptake and elimination of these metals were studied. Results are presented in this paper.

MATERIALS AND METHODS

Earthworms

The earthworms used for the experiments were adult, with a well developed clitellum and were of the species *Eisenia andrei* (Bouché, 1972). All worms were obtained from our own culture, and were grown on horse
dung at an ambient temperature of 20 ± 5°C. Worm ages ranged between 9.5 and 15.5 weeks.

**Chemicals**

Cadmium chloride (CdCl$_2$·2H$_2$O), chromium(III)nitrate (Cr(NO$_3$)$_3$·9H$_2$O) and zinc chloride (ZnCl$_2$) were obtained from Baker and Merck and were ≥ 98% pure.

**Reproduction toxicity tests**

Methods used to determine the effect of cadmium and chromium on the reproduction and growth of earthworms have been described in detail by Van Gestel et al. (1992a). Methods for the reproduction toxicity test on earthworms with zinc followed those used for cadmium and chromium. After a 1-week pre-incubation period (phase A) in untreated artificial soil, adult earthworms were exposed for 3 weeks to concentrations of 0, 100, 180, 320, 560 and 1000 mg Zn/kg dry soil in artificial soil (phase B). At the end of this period, cocoon production was assessed and the worms were transferred to untreated artificial soil for a 3-week recovery period (phase C). Cocoons were incubated in untreated artificial soil for 5 weeks as described by Van Gestel et al. (1988) to assess hatchability. At the start and end of each test phase, body weight (fresh weight) of the earthworms was determined, and growth during each phase was calculated as the % increase of body weight. During all three test phases, worms were fed untreated cow dung, which was supplied in a hole in the middle of the soil. Four replicate jars were used for each test concentration.

**Bioaccumulation and elimination studies**

To determine the bioaccumulation of the metals tested, earthworms were sampled at the end of the 3-week exposure period (phase B). Soil samples were taken and analysed at the start and end of phase B. Elimination rates were determined by analysing worms at the end of phase C. Before analysis, all earthworms were incubated on wet filter paper in the dark at 15°C for 48 h to allow the gut to empty; after 24 h the filter paper was renewed. Elimination rates were expressed as half-life times, which were calculated assuming first-order elimination kinetics.

For the analysis of chromium concentrations, soil and earthworm samples were digested in HNO$_3$ at 180°C for 16 h and analysed by ICP-AES and AAS, respectively. Detection limits were 1.4 and 0.07 mg Cr/kg dry weight, respectively. To determine cadmium and zinc levels, soil and earthworm samples were digested in HNO$_3$//HCl (1:3) for 2.5 h at 200°C and analysed.
by AAS. Detection limits were 0.01 and 0.5 mg Cd/kg dry weight and 0.5 and 2.0 mg Zn/kg dry weight of soil and earthworms, respectively.

Statistical analysis

EC$_{50}$ values for the effect of zinc on earthworm reproduction were calculated according to a logit model. No-Observed-Effect-Concentrations (NOEC) were calculated using William’s test or Dunnett’s test in the TOXSTAT software package and Student’s $t$ test.

RESULTS

Cadmium

At the lowest test concentration (10 mg Cd/kg dry soil) cocoon production was significantly ($P < 0.05$) reduced (Van Gestel et al., 1992a), while the number of juveniles/worm per week was reduced from 18 mg Cd/kg dry soil onwards (Fig. 1). Growth was not significantly affected, but it tended to show a dose-related increase.

In phase B of this test, the worms were fed either untreated cow dung or cow dung treated with cadmium at the same concentrations as mixed through the test soils. Cadmium levels in the worms fed treated cow dung were between 6 and 24% higher compared to those in worms fed untreated cow dung. Only at a concentration of 10 mg Cd/kg dry soil was this difference significant ($P < 0.05$), suggesting that only a small part of the cadmium uptake by earthworms is via the food. Also at the end of the 3-week recovery period worms fed treated food tended to contain the highest cadmium levels. As the differences were small, in Fig. 1 the results of the studies with treated and untreated food are combined.

From the results in Fig. 1, it can be concluded that at all dose levels cadmium concentrations in the earthworms were significantly higher than control. Worms taken from our laboratory culture contained on average 2.9 mg Cd/kg dry weight; the control worms, kept for 3 weeks in untreated artificial soil, contained 3.1 mg Cd/kg dry weight. At 10 mg Cd/kg dry soil, earthworms contained 115 mg Cd/kg dry weight at the end of phase B. Cadmium levels in the worms were dose-relatedly increased, reaching a level of 325 mg Cd/kg dry weight at the highest soil concentration tested. Bioconcentration factors decreased with increasing soil concentration, resulting in BCF values of 10 and 3.5 at the lowest and highest soil concentrations, respectively. Untreated soil contained 0.1 mg Cd/kg dry weight; BCF value for earthworms kept in this soil therefore was 31.

After transfer to clean soil, cadmium was eliminated with half-life times of 26–32 days. Elimination rates appeared not to be concentration-
Fig. 1. Influence of cadmium on the growth, reproduction and cadmium accumulation by *Eisenia andrei* after 3 weeks exposure (phase B) followed by a 3-week elimination period (phase C) in an artificial soil substrate.
dependant. At the end of the 3-week recovery period, concentrations in the earthworm were still significantly enhanced compared to the control, with concentrations ranging between 73 and 190 mg Cd/kg dry weight at the lowest and highest dose level, respectively. Reproduction was completely recovered at the end of phase C, at all concentrations tested.

**Chromium**

Figure 2 summarizes the results of the toxicity and bioaccumulation study with chromium. At concentrations of 100 mg Cr/kg dry soil and higher, reproduction was significantly reduced, while growth was only significantly reduced at the highest concentration tested (1000 mg Cr/kg dry soil). Comparison of the growth data from Fig. 2 with those of Fig. 1 shows that growth rate was considerably higher in the test on chromium; reproduction was, however, much lower. The negative relationship between growth and cocoon production described by Van Gestel et al. (1992b) may explain this difference.

From Fig. 2 it can be concluded that chromium levels in the earthworms at the end of the 3-week exposure period showed a dose-related increase: chromium levels in the worms at the three highest dose levels were significantly increased compared to controls. In the control worms, chromium concentration was 0.3 mg/kg dry weight; in the exposed worms concentrations ranged between 0.8 and 18 mg Cr/kg dry weight at the lowest and highest exposure level, respectively. BCF values for the accumulation of chromium in the worms were 0.031–0.047 at the three lowest and 0.016–0.019 at the two highest dose levels. Control soil contained 6.3 mg Cr/kg dry weight; the BCF value for worms kept in this soil therefore is 0.048.

At the end of the 3-week recovery period, chromium levels had returned to normal in all dose groups, and ranged between 0.3 and 1.1 mg Cr/kg dry weight. From these data, it can be concluded that chromium was eliminated from the earthworms with half-life times of 51–109 days for the two lowest dose groups and of 5–7 days for the three highest dose groups. During the 3-week recovery period, reproduction was almost completely recovered. Growth showed, however, a dose-related increase; this increase was significantly different from the control in the highest dose group only.

**Zinc**

Table 1 gives the results of the reproduction toxicity study with zinc. It can be concluded that growth tended to show a dose-related increase and was significantly stimulated at the highest zinc concentration in soil. Cocoon production was significantly reduced at 560 and 1000 mg Zn/kg dry soil.
Fig. 2. Influence of chromium on the growth, reproduction and chromium accumulation by Eisenia andrei after 3 weeks exposure (phase B) followed by a 3-week elimination period (phase C) in an artificial soil substrate.
TABLE 1

Effect of zinc on the growth and reproduction of *Eisenia andreii* in artificial soil (average of four replicates)

<table>
<thead>
<tr>
<th>Conc. (mg Zn/kg)</th>
<th>% growth</th>
<th>Number cocoons/ worm/week</th>
<th>% fertile cocoons</th>
<th>Number juveniles/ fert. cocoon</th>
<th>Number juveniles/ worm/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.4 ± 1.7</td>
<td>0.98 ± 0.14</td>
<td>87 ± 5</td>
<td>2.06 ± 0.26</td>
<td>1.78 ± 0.44</td>
</tr>
<tr>
<td>100</td>
<td>5.4 ± 0.8</td>
<td>0.95 ± 0.17</td>
<td>87 ± 6</td>
<td>2.38 ± 0.13</td>
<td>1.98 ± 0.43</td>
</tr>
<tr>
<td>180</td>
<td>4.6 ± 1.4</td>
<td>1.00 ± 0.15</td>
<td>97 ± 4</td>
<td>2.32 ± 0.39</td>
<td>2.20 ± 0.31</td>
</tr>
<tr>
<td>320</td>
<td>5.7 ± 2.4</td>
<td>0.99 ± 0.25</td>
<td>87 ± 3</td>
<td>2.35 ± 0.26</td>
<td>1.99 ± 0.41</td>
</tr>
<tr>
<td>560</td>
<td>5.8 ± 2.2</td>
<td>0.68 ± 0.10*</td>
<td>92 ± 9</td>
<td>1.69 ± 0.36</td>
<td>1.03 ± 0.15*</td>
</tr>
<tr>
<td>1000</td>
<td>7.9 ± 1.1</td>
<td>0.11 ± 0.07**</td>
<td>50 ± 35*</td>
<td>1.38 ± 0.44*</td>
<td>0.09 ± 0.10**</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01.

The fertility of cocoons was significantly reduced at the highest soil concentration; at this concentration cocoons appeared to be malformed, with cocoon shells not being completely finished. Malformed cocoons appeared to be infertile. As an overall result, the number of juveniles/worm per week was significantly (and dose-relatedly) reduced at 560 and 1000 mg Zn/kg dry soil. *EC*₅₀ values for the effect of zinc on cocoon production and the number of juveniles per worm per week are 659 (552–786) and 512 mg Zn/kg dry soil, respectively.

Figure 3 shows the results of the sublethal toxicity and bioaccumulation study with zinc. It can be concluded that at the end of the 3-week exposure period zinc concentrations in the earthworms were somewhat increased at 320 and 560 mg Zn/kg dry soil, resulting in concentrations of 130–150 mg/kg dry weight, and about doubled at the highest dose level (231 mg/kg dry weight). BCF values showed a dose-related decrease, being 1.4 and 0.21–0.23 at 100 and 560–1000 mg Zn/kg dry soil, respectively. Control soil contained 1.4 mg Zn/kg dry weight; the BCF value for worms kept in this soil therefore is 72.

During the 3-week recovery period in untreated artificial soil, the accumulated zinc was completely eliminated, resulting in earthworm concentrations of 73–89 mg Zn/kg dry weight. At the highest dose level, zinc was eliminated with a half-life time of 13 days. Growth was significantly reduced in all dose groups during the recovery period; as growth was rather high in the control and was not dose-relatedly affected, this was considered not to be related to zinc exposure. Reproduction was completely recovered.
Fig. 3. Influence of zinc on the growth, reproduction and zinc accumulation by Eisenia andrei after 3 weeks exposure (phase B) followed by a 3-week elimination period (phase C) in an artificial soil substrate.
at the end of phase C, and was significantly increased in the groups previously exposed to 560 and 1000 mg Zn/kg dry soil.

DISCUSSION

Toxicity of zinc for earthworms

Neuhauser et al. (1985) reported a 14-day LC$_{50}$ of 662 mg/kg dry soil for the toxicity of zinc for *E. fetida* in an artificial soil test according to OECD guideline 207 (OECD, 1984). We used the same artificial soil. From our study, an NOEC of 320 mg Zn/kg dry soil can be derived, indicating that for zinc the difference between acute and sublethal effect levels is small. The studies of Malecki et al. (1982) and Neuhauser et al. (1984) resulted in an NOEC value for the effect of zinc on the reproduction of *E. fetida* of 1000 mg/kg dry substrate. They, however, performed their tests by placing a layer of treated substrate (horse dung) on top of a layer of untreated soil, thus enabling the earthworms to escape from exposure. Beyer et al. (1987) found no earthworms on a loam soil containing 470 mg Zn/kg, and attributed this to the earthworm toxicity of zinc.

Surprisingly, zinc affected the formation of cocoons; such an effect of zinc in earthworms has not been reported before. Ravera (1991), in a review, mentioned that zinc significantly affected the reproduction of *Biomphalaria glabrata* by reducing the number and hatchability of egg capsules and by increasing embryonic development time.

Uptake and regulation of metals by earthworms

Cadmium and chromium were dose-relatedly accumulated by *E. andrei*, whereas the earthworms seemed to be capable of regulating their body concentration of zinc. These results for cadmium and zinc are supported by the findings of other authors (e.g., Rainbow, 1988; Rainbow and White, 1989; Kraak, 1992; Bryan and Hummerstone, 1973; Bryan et al., 1986), reporting similar results for a number of aquatic organisms. On the other hand, Rainbow and White (1989) demonstrated that aquatic amphipods and barnacles were not able to regulate their body concentrations of zinc. The decapod *Palaemon elegans* regulated its body concentration to a level of 80–90 mg Zn/kg dry weight; only at high exposure concentrations does it lose its capacity to regulate zinc concentrations (Rainbow and White, 1989). Background levels of 110–170 mg Zn/kg dry weight were reported for lobsters, polychaetes and mussels (Kraak, 1992; Bryan and Hummerstone, 1973; Bryan et al., 1986). In *E. andrei*, zinc concentration is regulated to a constant level of about 80–100 mg/kg dry weight. Apparently, many organisms have a similar zinc level. This is supported by White and
Rainbow (1985), who estimated that, on the basis of their enzymatic requirements, the minimal zinc body content of aquatic crustaceans and molluscs should be about 35 mg/kg dry weight. When zinc is also needed for e.g. haemocyanine or other blood proteins, another 36–58 mg/kg dry weight is needed.

In other earthworm species, often much higher zinc concentrations were found. Beyer et al. (1987) found 320, 400 and 310 mg Zn/kg dry weight in Aporrectodea tuberculata, A. turgida and Lumbricus rubellus on a non-polluted soil. Ma (1983) found an average concentration of zinc of 336 mg/kg dry weight in newly-hatched and 639 mg/kg in full-grown adults of L. rubellus from a non-polluted sandy loam soil.

Relation between internal concentrations and effects

Reproduction is significantly reduced when cadmium concentrations in the earthworms reach a level of 115–150 mg/kg dry weight (Fig. 1). At the end of the recovery period, cadmium concentrations in the earthworms exposed to the two highest dose levels in soil still exceeded this value. Nevertheless, reproduction was completely recovered. Apparently, the earthworms have succeeded in detoxifying cadmium. It is well known that earthworms are capable of binding cadmium in their chloragogen tissue (Morgan and Morris, 1982), and that metallothioneins are involved (Suzuki et al., 1980). The binding of cadmium to metallothioneins may also be responsible for the slow elimination rate of cadmium after transfer of the earthworms to untreated soil; similar findings for aquatic organisms have been described by Rainbow (1988). This study demonstrates that detoxification of cadmium reduces the sensitivity of E. andrei for this metal. It is, however, not certain what the energy costs of this detoxifying capacity are, and what would be the long-term effects of sustaining such a cadmium-binding capacity.

Chromium exhibited effects on the earthworm’s reproduction at soil concentrations of 100 mg/kg and higher; at these dose levels chromium concentrations in the earthworms were significantly increased. It can be concluded that earthworm concentrations exceeding 3 mg Cr/kg dry weight are toxic for E. andrei. Chromium is rapidly eliminated from the worms, suggesting that no strong binding within the earthworms occurred. No literature data are available to confirm this. It may be concluded that the rapid elimination of chromium from the worms allowed a complete recovery of the earthworm’s growth and reproduction.

Zinc only had an effect on E. andrei at soil concentrations of 560 and 1000 mg/kg. Only at the latter concentration were zinc concentrations significantly elevated. After a 3-week recovery period, zinc levels had
returned to normal, suggesting a rapid elimination. Zinc may also be associated with metallothioneins, but the binding of zinc to metallothioneins is reversible, allowing for a rapid release of zinc (Williams, 1984). Also for zinc, the rapid elimination allowed for a complete recovery of the earthworm's reproduction.

CONCLUSIONS

Earthworms are able to regulate their body concentration of zinc at soil concentrations up to 560 mg/kg. Cadmium and chromium were not regulated, although chromium concentrations in the earthworms were only significantly and dose-relatedly increased at concentrations of 100 mg Cr/kg dry soil and higher.

Internal cadmium concentrations in the earthworms could not predict toxic effects, as reproduction was completely recovered after a 3-week recovery period in clean soil, while earthworm concentrations were still significantly increased.

NOEC values for effects of cadmium, chromium, and zinc on the growth and reproduction of the earthworm *E. andrei* in artificial soil are <10, 32, and 320 mg/kg dry soil, respectively.

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REFERENCES


Ravera, O., 1991. Influence of heavy metals on the reproduction and embryonic development of freshwater pulmonates (Gastropoda; Mollusca) and cladocerans (Crustacea; Arthropoda). Comp. Biochem. Physiol., 100C: 2150–2190.


