Sublethal effects, accumulation capacities and elimination rates of As, Hg and Se in the manure worm, *Eisenia fetida* (Oligochaeta, Lumbricidae)

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With 3 figures

Synopsis: Original scientific paper

Lethal concentrations, sublethal effects, incorporation and elimination of As, Hg and Se, introduced into the substratum as potassium arsenate, mercuric chloride and sodium selenite, were studied in *Eisenia fetida*, using a laboratory vermicomposting system.

*E. fetida* tolerated 25 mg · kg⁻¹ potassium arsenate, 50 mg · kg⁻¹ sodium selenite and 100 mg · kg⁻¹ mercuric chloride in the wet substratum without lethal effect. Both juvenile mass gain and adult cocoon production were decreased significantly by sublethal concentrations of arsenate and selenite; however, mercury resulted in a significant enhancement of cocoon production.

The concentrations of the toxic elements increased in the worms with enhanced concentrations in their surroundings, while the concentration factors decreased. The maximal accumulation capacities measured at sublethal concentrations in long-term studies were 902, 337 and 156 mg · kg⁻¹ dry mass for As, Se and Hg, respectively. No decrease of As content and only 5–6% loss of the accumulated Se content could be observed at the end of the 8-week recovery period.

Key words: earthworms, arsenic, mercury, selenium, sublethal effects, accumulation capacity, elimination.

1. Introduction

In many parts of the world earthworms are the principal organisms involved in translocating and mixing soil constituents. GISH & CHRISTENSEN (1973), VAN HOOK (1974) and IRELAND (1975) were first to indicate that earthworms can accumulate some heavy metals from the soil which will result in greater than normal concentration in their bodies. Since then numerous reports have proved that most elements that are abundant in soil are also abundant in worms; while some elements including a few relatively toxic ones tend to be concentrated by earthworms (BEYER, 1981; BEYER et al., 1978, 1990; FLECKENSTEIN & GRAFF, 1982; IRELAND, 1983). These characteristics of earthworms make them potentially attractive organisms for monitoring the biological availability of natural and anthropogenic trace elements in soil (CARTER et al., 1980; HELMKE et al., 1979; MORGAN & MORGAN, 1988).

In addition, earthworms are important link in the natural food chain. They can accumulate and transfer potentially hazardous materials from soil to predators (IRELAND, 1977). Oligochaete worms are among the species with the highest capacity for accumulation of some toxic elements, but this capacity is highly variable depending on the sort of element (BEYER, 1981) and on soil properties (MA, 1982).

The present study deals with the manure worm, *Eisenia fetida* (SAVIGNY), a worm with a potential use in management of biotical wastes and sludges. With respect to this species, the study concerns the determination of

(1) the lethal concentrations,

(2) the sublethal effects,
(3) the maximal accumulation capacities, and
(4) the rate of elimination of the toxic elements As, Hg and Se using a laboratory vermicomposting system.

2. Materials and methods

2.1. Animals, substratum and treatment

Specimens of *Eisenia fetida* (SAVIGNY) were obtained from stock culture in our laboratory. The substratum consisted of peaty marshland soil and horse manure in the proportions 1:1 (m/m), and was wetted to attain a moisture content of approximately 65–70%. A more detailed description of the substratum is given in Fischer, 1989. Plastic bags containing 500 g wet mass substratum were used and 10 worms were introduced into each bag at the start of experiments. The average initial age of the worms was five weeks and the average biomass was 176 mg. Experiments were carried out at room temperatures (19–22 °C). 200 specimens were used as controls and 20–60 for each toxication experiment. Potassium arsenate, mercuric chloride and sodium selenite were dissolved in water and mixed into the substratum in different concentrations. Concentrations of the compounds are given on a wet mass basis.

2.2. Growth and reproduction studies

The numbers of survivors and their live mass were recorded weekly. The cocoons were also counted weekly and removed from the culture bags. Cultures were preserved for 8–10 weeks. Samples of toxicated worms were kept in unpolluted substrata in order to examine their ability to recover after toxication.

2.3. Incorporation and elimination studies

Worms toxicated for 8 weeks were used for examination of the incorporation of elements. For elimination studies toxicated worms were transferred to unpolluted substratum for 8 weeks before elemental analysis. Controls and toxicated worms were kept on moist filter paper for one or two days in order to void their gut content. Then worm and substratum samples were dried at 105 °C. The As, Hg and Se content of the samples were determined with an energy-dispersive X-ray emission spectrometer. Emission was excited with 109Cd. Addition method was used for quantitative analysis. Concentrations of elements are given on a dry mass basis.

Numerical data in this study are given as means ± SD. The results were subjected to statistical evaluation with Students’s t-test.

3. Results

3.1. Mortality and sublethal effects

Arsenate resulted in marked mortality in much less concentration than selenite (fig. 1) but sublethal effects, resulting in significant decrease both of the mass gain (fig. 2) and of cocoon production (fig. 3), could be observed at about equal concentrations of arsenate and selenite. Thus, similar concentrations of arsenate result in sublethal and lethal effects on *E. fetida*, but the concentrations of selenite which had corresponding effects were widely different.
Fig. 1. Mortality effects of different concentrations of toxicants on *Eisenia fetida* during the 8-week exposure period. (1) 500 mg·kg⁻¹ mercuric chloride lost its mortality effect during 2 weeks preincubation in the substratum.

Fig. 2. The effects of sublethal concentrations of toxicants on mass gain by juvenile *Eisenia fetida*.
Mercuric chloride shows some peculiar effects in these toxicological studies. We found about 25% mortality already in the first week of the toxication using 250 mg : kg\(^{-1}\) mercuric chloride without further mortality until the end of the experiment. However, all worms survived the 8-week experiment in a substratum contaminated with 500 mg : kg\(^{-1}\) mercuric chloride when the contaminated substratum had been preincubated for two weeks before introducing the worms (fig. 1). Another unusual effect observed was that sublethal concentrations of mercuric chloride resulted in a marked stimulation of cocoon production in comparison with the control (fig. 3).

3.2. Incorporation and elimination of elements

Selenium content in the body of the worms increased with increased environmental selenium load, while the concentration factor decreased (table 1) and sank below 1.0 when the environmental concentration reached the 300 – 350 mg : kg\(^{-1}\) level. Maximal Se content measured in the toxicated worms was 337.6 mg : kg\(^{-1}\). Our results demonstrate a significant ability of the worms to concentrate arsenic from the substratum. Near the lethal concentration the concentration factor was over 10.0, resulting in more than 900 mg : kg\(^{-1}\) As content in the dry mass of the worm. Mercury could not be concentrated in the worms in relation to the substratum as the concentration factor was less than 1.0 at sublethal environmental mercury content (table 1). The observed accumulation in the worms was 156 mg : kg\(^{-1}\).

No elimination of As and a rather slow one of Se could be observed during the 8-week experiment (table 2). Elimination of Hg was not measured in this work.
Table 1. Incorporation of As, Hg, and Se in the manure worm, *Eisenia fetida* in a laboratory vermicomposting system.

<table>
<thead>
<tr>
<th>Introduced compounds: Concentration:</th>
<th>Concentration:</th>
<th>mg · kg(^{-1}) dry mass ± S.D.</th>
<th>Concentration factor(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concen.</td>
<td>in the substratum</td>
<td>in the worms</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>--------------------</td>
<td>---------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Potassium arsenate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>23.0 ± 9.0</td>
<td>418.0 ± 34</td>
<td>18.10</td>
</tr>
<tr>
<td>25</td>
<td>50.0 ± 14.0</td>
<td>643.0 ± 51</td>
<td>12.80</td>
</tr>
<tr>
<td>50</td>
<td>87.0 ± 18.5</td>
<td>902.0 ± 73</td>
<td>10.30</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>243.0 ± 37.0</td>
<td>1560 ± 25</td>
<td>0.64</td>
</tr>
<tr>
<td>Sodium selenite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>14.5 ± 1.5</td>
<td>76.5 ± 12</td>
<td>5.27</td>
</tr>
<tr>
<td>50</td>
<td>73.3 ± 9.1</td>
<td>145.1 ± 31</td>
<td>1.98</td>
</tr>
<tr>
<td>100</td>
<td>136.1 ± 14.0</td>
<td>165.2 ± 25</td>
<td>1.12</td>
</tr>
<tr>
<td>250</td>
<td>347.2 ± 24.0</td>
<td>337.6 ± 43</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\(^1\) Concentration factor (storage ratio) = concentration in earthworms / concentration in substratum

Table 2. Elimination of As, and Se from the toxiicated manure worms, *Eisenia fetida* during 8 weeks in uncontaminated substratum.

<table>
<thead>
<tr>
<th>Average element content of the toxicated worms:</th>
<th>Body mass alteration in relation of the original mass: %</th>
<th>Average element content after 8 weeks: mg · kg(^{-1}) dry mass</th>
<th>Calculated concentration on the original body mass basis mg · kg(^{-1}) dry mass</th>
<th>Percentage of elimination during 8 weeks: %</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg · kg(^{-1}) dry mass</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>643.0</td>
<td>+22.9</td>
<td>560.2</td>
<td>688.0</td>
</tr>
<tr>
<td>Se</td>
<td>145.1</td>
<td>-10.3</td>
<td>151.0</td>
<td>135.4</td>
</tr>
<tr>
<td></td>
<td>165.2</td>
<td>+80.1</td>
<td>86.5</td>
<td>155.8</td>
</tr>
</tbody>
</table>

4. Discussion

Toxicity of elements and organic compounds to earthworms may be tested at sublethal levels by the retarding effects on the mass gain of juveniles and on the reproduction of adults (FISCHER, 1989; LOFS-HOLMIN, 1980; MOLNAR et al., 1989; NEUHAUSER et al., 1984; VENTER & REINECKE, 1984). Our results prove, however, that the differences between sublethal and lethal concentrations of toxicants may vary considerably. Consequently, retarding effects on growth and reproduction are very sensitive signals for these toxicants which are characterized by a wide difference between sublethal and lethal levels (e.g. selenium), but they are less sensitive signals if the concentrations which result in significant sublethal effects differ only to a slight extent from the lethal concentrations (e.g. arsenic).

We found higher tolerance of *E. fetida* for mercuric chloride than that was found by BEYER et al. (1985) using methylmercuric chloride. ABBASI & SONTI (1983) toxicated the earthworm *Octochaetus pattoni* with mercuric chloride and established that the LC\(_{50}\) was 0.79 mg · kg\(^{-1}\) mercury over a 66-day exposure period. FLECKENSTEIN & GRAFF (1982) found 28 mg · kg\(^{-1}\) Hg in juvenile *E. fetida* sampled from municipal waste compost containing 7 mg · kg\(^{-1}\) Hg. In our experiments *E. fetida* tolerated 243 mg · kg\(^{-1}\) dry mass mercury without lethal effect during the 56-day exposure, and much higher concentrations were tolerated when the introduced mercuric chloride had been preincubated in the substratum for 2 weeks before worms were placed in it. These differences may be explained either by differences in the composition of the substratum and in the species used, or by the differences in the mercuric compounds used.
ABBASI & SONI (1983) established that as the mortality of adult earthworms progressed throughout the experimental period of mercury toxication, the earthworms still alive reproduced more than the controls. In our experiments the reproduction of *E. fetida* was significantly enhanced by mercury at sublethal level. Neither the reason nor the mechanism of this effect is known yet.

Results of BEYER *et al.* (1985), FLECKENSTEIN & GRAFF (1982), GISSEL-NIELSEN & GISSEL-NIELSEN (1975) and HELMKE *et al.* (1979) prove that all three elements used in our experiments may be concentrated in the body of earthworms if compared with the concentrations of their surrounding soil, resulting in a concentration factor greater than 1.0. Most of these data concern earthworms living in polluted habitats with relatively low concentrations of toxic elements. MOLNÁR *et al.* (1989) established that the chromium content of *E. fetida* is positively related to the chromium concentration of the substratum, if all other experimental conditions are identical. However, the concentration factors and environmental concentrations were inversely related. Our results for potassium arsenate and sodium selenite confirm these relations.

Results of laboratory experiments on the maximal accumulation capacities of earthworms for toxic elements are scarce. Figures for maximal concentrations in earthworms in the literature were 25 mg · kg⁻¹ As, 28 mg · kg⁻¹ Hg (FLECKENSTEIN & GRAFF, 1982) and 78 mg · kg⁻¹ Se (BEYER *et al.*, 1987). However, in *Arenicol a marina* a polychaete worm, 665 mg · kg⁻¹ As was found (JENNER & BOWMER, 1990). In our experiments we found much higher concentrations of As, Hg and Se in surviving *E. fetida* than those described previously. It may be concluded that earthworms are able to concentrate these elements to levels that are seriously hazardous to their predators. Furthermore, earthworms may retain toxic elements such as As and Se in their bodies for a long period because of their restricted ability for elimination. The great accumulation capacity and slow elimination rate are probably related to the well developed cellular immobilization mechanisms which occur in the earthworm’s body (HOPKIN, 1989), or in their chloragogenous tissue (IRELAND, 1983; JANSSEN, 1989; MOLNÁR *et al.*, 1989; MORGAN & MORGAN, 1988).

5. Acknowledgements

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6. References


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