NOVEL APPROACHES TO BIOINDICATION OF HEAVY METALS IN SOILS CONTAMINATED BY OIL SHALE WASTES

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The major sources of heavy metal pollution in Estonia are oil shale industry and oil shale combustion. There is an utmost need to monitor soil pollution with heavy metals in this region. Earthworms are good accumulators of heavy metals and can be used as bioindicators. Endogeic species Aporrectodea caliginosa and Aporrectodea rosea as well as anecic species Lumbricus terrestris are good bioindicators for Zn(II) and Cu(II) ions in soils contaminated by oil waste or fly ash because of the high level of accumulation of these metals in their tissues. Electrochemical methods were used for the determination of heavy metal ions in earthworms as the amount of the sample needed for the analytical work is from 10 to 100 times smaller, when compared to the traditional methods.

Introduction

Soils and sediments are the ultimate sinks for heavy metals. Heavy metals mainly find their way into soils and sediments via airborne particles in the form of dust, smokes and aerosols, resulting from mining, smelting, oil refining, chemical industry and fuel combustion. The build up of toxic metals in the biologically most active part of the soil, the organic topsoil, makes the metals readily accessible to some crops and vegetables [1]. The major sources of heavy metal pollution in Estonia are oil shale industry and oil shale combustion. In North-East Estonia large quantities of Cd, Cu, Zn, Pb and other metals are present in oil shale gangue and fly ash particles [2], from where (in the form of ions) they enter the soil and water. There is an utmost need to monitor the soil pollution with heavy metals in this region.

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Unfortunately, lack of knowledge of the pedofauna is the principal cause and explains why few attempts have been made in this direction [3].

The ideal bioindicator would be simply measured, work equally well in all environments and reliably reveal what problems existed where [4]. According to [5] the best bioaccumulators of heavy metals are the invertebrates that store the substances taken from soil water, particularly earthworms but also springtails, isopods and diplopods. For more than two decades earthworms have played the major role in soil toxicity testing, this group has specially been studied concerning the problem of bioaccumulation of heavy metals [3]. There is also an increased interest in the use of earthworms as tools in ecological assessment, particularly with reference to bioaccumulation/bioavailability of heavy metals [6-8] as well as for monitoring of soil rehabilitation following opencast mining or for ecological restoration of mining areas [9, 10]. Earthworms are essential for the functioning of soil ecosystems, they are distributed everywhere, easy to collect, they survive at low concentrations of contaminant, and the reaction to contaminant is measurable and can be reproduced. They are good accumulators of heavy metals and therefore are good bioindicators [11]. In vivo metal contents in earthworms vary depending on species or metal type. Differences in concentrations have been observed between species [12] and between the individuals of one species [13, 14].

The aim of this study was to determine the most reliable local bioindicators and to ascertain the differences of bioaccumulation of metal ions in the bodies of individuals of different common earthworm species. The test area was selected so that the shale oil combustion period could be clearly defined in this region and no soil pollution with oil wastes had been taken place there. As one of our goals is to develop an early warning system for heavy metal pollution based on biomonitoring, heavily polluted regions were excluded. The distribution of Cd, Cu, Zn and Pb ions was studied due to the availability of electrochemical methodology applicable for rapid and cost-effective determination of these ions (presented below).

**Material and methods**

**Earthworm collection**

The sampling area was located in the town of Saue (North Estonia, location of sampling area 59°19’12”, 24°33’94”) including territory of smaller industrial enterprises surrounding deciduous forest. From 1992 to 2002 shale oil containing traces of heavy metals was utilized for supplying the town of Saue with thermal energy. In 2002 oil was substituted by natural gas. The soils of the studied area are limestone and pebble rendzinas (Rendzic and Cambi-Rendzic Leptosols) with sandy loam texture. The earthworms were collected on 5 plots 50×50 cm using 15% solution of mustard as verminifuge [15]. The collected earthworms were washed, kept 48 hours in refrigerator and weighted; the species were identified according to [16, 17]. Five
composite samples of common species individuals were composed (Table 1): epigeic species *Dendrobaena octaedra* (Savigny, 1826) and *Lumbricus rubellus* (Hoffmeister 1843), endogeic species *Aporrectodea rosea* (Savigny, 1826) and *Aporrectodea caliginosa* (Savigny, 1826), anecic species *Lumbricus terrestris* (Linnaeus, 1758). Non-identified juvenile individuals were included in one separate sample. Soil samples were taken with a soil corer Ø 85 mm (depth of soil 0–15 cm) from the studied plots and the soil was mixed for composite sample. Concentration factors CF (Concentration_{tissue}/Concentration_{soil}) were calculated by van Hook method [18]. Data analysis was performed using nonparametric statistical methods (dispersion analysis of Kruskall-Wallis, Mann-Whitney U-test) and programs STATISTICA 7 and Microsoft Excel.

**Table 1. Characteristics of earthworm samples**

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>No in sample (total of 5 plots)</th>
<th>Biomass, g (total of 5 plots)</th>
<th>Mean biomass of individual, g ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aporrectodea caliginosa</td>
<td>Mature</td>
<td>5</td>
<td>0.248</td>
<td>0.050±0.018</td>
</tr>
<tr>
<td>Aporrectodea rosea</td>
<td>Mature</td>
<td>11</td>
<td>0.218</td>
<td>0.020±0.017</td>
</tr>
<tr>
<td>Dendrobaena octaedra</td>
<td>Mature</td>
<td>11</td>
<td>0.175</td>
<td>0.016±0.009</td>
</tr>
<tr>
<td>Lumbricus rubellus</td>
<td>Mature</td>
<td>9</td>
<td>0.217</td>
<td>0.024±0.012</td>
</tr>
<tr>
<td>Lumbricus terrestris</td>
<td>Mature</td>
<td>7</td>
<td>2.739</td>
<td>0.391±0.126</td>
</tr>
<tr>
<td>Lumbricus sp</td>
<td>Juvenile</td>
<td>6</td>
<td>0.218</td>
<td>0.036±0.028</td>
</tr>
</tbody>
</table>

**Apparatus and reagents**

An Autolab potensiotstat PGSTAT 10 with a Metrohm VA663 Stand system connected to the computer was used to perform an electrochemical analysis of the environmental samples. The reagents used were of analytical grade produced by Merck and Aldrich. The Milli-Q Plus grade deionised water was used throughout the analysis.

**Analytical method**

The content of Pb(II), Cd(II), Zn(II) and Cu(II) ions in soil and earthworm samples was measured electrochemically applying a hanging mercury drop electrode (HMDE) as a very reliable tool for this kind of multielemental analysis. The samples were dried in a drying oven for 48 hr at 71 °C and then crushed in a mortar with a pestle both carefully cleaned before with a blend of H₂O₂ (30% w/w) and 1 M HNO₃ (pH ca. 0.8). 0.1 g of the milled sample was weighed for the wet digestion. The sample was first quantitatively transferred with a 2×0.5 ml of Milli-Q water from the weighing glass to a carefully cleaned Teflon-made crucible. The water from the obtained suspension was carefully evaporated by heating this blend on the hotplate. Before the sample became completely dry, 1.5 ml concentrated nitric acid (65% w/w) was added. Additionally 1.0 ml conc. HNO₃, 0.55 ml
Novel Approaches to Bioindication of Heavy Metals in Soils Contaminated by Oil Shale Wastes

conc. \( \text{HClO}_4 \), 1.0 + 1.0 ml conc. \( \text{HNO}_3 \) and finally 0.5 ml conc. \( \text{HClO}_4 \) (70% w/w) were added to the almost dry matter, which was dissolved in 25.0 ml 1.0 mM aqueous solution of \( \text{HNO}_3 \). The above described sample preparation is based upon Dabeka method [19] but has been improved to have a more complete decomposition of the worm-sample. Finally, 10 ml of the obtained sample solution (to determine zinc ions only 1.0 ml of sample was diluted with water up to 10 ml) was taken for the electrochemical analysis. Then acetate buffer solution was added to the sample solution so that the final concentration of the supporting electrolyte solution was 0.1 M. The pH was ca 4.2. The HMDE as a working electrode (WE), glassy carbon rod as a counter electrode (CE), and Ag/AgCl (3 M KCl, aq.) as a reference electrode (RE) were used. Electrochemical conditions: 300 s of electrolysis at the WE potential –1.45 V vs. RE, then differential-pulse anodic stripping sweep until the WE potential 0.2 V vs. RE (modulation amplitude: 50 mV, modulation time: 0.7 s and interval time: 0.3 s). The method was similar to the one used by Moreno et al [20]. Argon gas (99.993%) was used to de-aerate the solutions. The presented analytical results were background corrected. The measurement uncertainty was within ±10%.

Results

Applying electrochemical methods the concentrations of Zn(II), Cd(II), Pb(II) and Cu(II) ions in earthworm body tissues and soils were measured. The contents of metal ions in earthworm bodies are presented in Table 2 and concentration factors CF in figure. The concentration of heavy metal ions differed in the tissues of earthworm species. They accumulated more Zn(II) and Cd(II) ions (CF 28.6–45.1 and 12.2–26.8, respectively) in the body tissues in comparison with Pb(II) and Cu(II) ions (CF 0.21–1.0 and 0.66–2.8, respectively). The content of Zn(II) ions in the tissues was higher in the individuals of endogeic (Aporrectodea caliginosa and A. rosea) and anecic species (Lumbricus terrestris) compared to epigeic species. The content of Cd(II) ions in the tissues was the highest in the individuals of epigeic species Lumbricus rubellus and endogeic species Aporrectodea caliginosa. The

Table 2. Concentrations of metals in earthworms’ bodies and soil

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zn(II) ppm±SE</th>
<th>Cd(II) ppm±SE</th>
<th>Pb(II) ppm±SE</th>
<th>Cu(II) ppm±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aporrectodea caliginosa (N = 5)</td>
<td>509±49</td>
<td>2.54±0.19</td>
<td>2.66±0.40</td>
<td>7.55±0.77</td>
</tr>
<tr>
<td>Aporrectodea rosea (N = 11)</td>
<td>492±18</td>
<td>1.99±0.03</td>
<td>8.13±0.62</td>
<td>7.43±0.51</td>
</tr>
<tr>
<td>Dendrobaena octaedra (N = 11)</td>
<td>343±11</td>
<td>1.33±0.03</td>
<td>4.69±0.25</td>
<td>4.26±0.19</td>
</tr>
<tr>
<td>Lumbricus rubellus (N = 9)</td>
<td>319±4.8</td>
<td>2.92±0.09</td>
<td>2.75±0.18</td>
<td>2.75±0.18</td>
</tr>
<tr>
<td>Lumbricus terrestris (N = 7)</td>
<td>510±13</td>
<td>1.62±0.06</td>
<td>1.72±0.14</td>
<td>7.55±0.77</td>
</tr>
<tr>
<td>Lumbricidae juveniles (N = 6)</td>
<td>508±24</td>
<td>1.92±0.08</td>
<td>2.37±0.15</td>
<td>1.76±0.10</td>
</tr>
<tr>
<td>Soil (composite sample)</td>
<td>11.29±0.36</td>
<td>0.109±0.002</td>
<td>8.16±0.16</td>
<td>2.68±0.21</td>
</tr>
</tbody>
</table>
Fig. Concentration Factors (CF) of Zn, Cd, Pb and Cu in bodies of individuals of different earthworm species. ACAL – *Aporrectodea caliginosa*; AROS – *Aporrectodea rosea*; DOCT – *Dendrobaena octaedra*; LRUB – *Lumbricus rubellus*; LTER – *Lumbricus terrestris*.

content of Pb(II) ions was the highest in the bodies of endogeic *Aporrectodea rosea*. The content of Cu(II) ions was the highest in the individuals of endogeic species *Aporrectodea caliginosa* and *A. rosea* and anecic species *Lumbricus terrestris*.

**Discussion**

Soft-bodied soil-dwelling organisms are exposed to metals either through direct dermal contacts with metals in soil solution or by ingestion of bulk soil or specific soil fractions [21]. Almost every type of soil contains individuals of at least one earthworm *Lumbricidae* species. They are numerous large-bodied individuals, resistant enough and sensitive enough to contaminants, which make them good bioindicators [22–24]. Because of limited mobility they have adapted to life in a certain soil depth under certain soil condition. In temperate climate several ecological groups – epigeic, endogeic and anecic earthworms [25] are found. Based on feeding habitats, earthworms can be divided into detrivores (epigeic and anecic species) and geophages (endogeic species) [26]. Epigeic earthworms (*Dendrobaena octaedra, Lumbricus rubellus*) feed on decay on the soil surface. Anecic earthworms (*Lumbricus terrestris*) feed on plant material on the surface but they live in deep burrows in the soil. Endogeic earthworms (*Aporrectodea caliginosa, Aporrectodea rosea*) digest the organic matter with soil microorganisms in the upper 30 cm mineral soil layer. Food sources for different ecological groups of earthworms are differently exposed to heavy metal contamination and thereof the species belonging to various ecological groups assimilate metal ions differently. Our results are in excellent agreement with [27, 28] and other authors. According to Lee [29], earthworms are able to
accumulate higher concentrations (CF>10) of Zn(II) and Cd(II) ions and lower concentrations of Pb(II) and Cu(II) ions in their bodies. The measured concentration factor CF is 9 to 188 for Cd and 2.8 to 8.3 for Zn [3]. Earlier in the 1990ties we measured the concentrations of heavy metal ions in earthworms (species were not identified) from the same sampling site by atom adsorption spectrophotometry and obtained the following results: Zn(II) ions – 723 ppm, Cd(II) ions – 1.34 ppm, Pb(II) ions – 2.9 ppm and CF for Zn(II) and Cd(II) ions – 43.3 and 27.0, respectively. In areas not polluted with oil and flying ash (Kambja, South-Estonia) the relevant concentrations were significantly lower: Zn(II) ions – 530 ppm, Pb(II) ions – 2.4 ppm and CF for Zn(II) and Cd(II) ions – 14.7 and 20.0, respectively. These are in good agreement with the results obtained in the current study despite of differences in analytical methods.

According to [1] accumulation of hazardous substances by several organisms has become an important component of bioindication as this allows the presence of low levels of chemicals in the environment to be identified and quantified. The earthworms are one of the best bioindicators of trace metals amongst soil invertebrates because they are able to accumulate metal ions in the body tissues [3, 14, 30]. It is important to study the individuals of different species separately and to know ecological characteristics of species and soil characteristics as different species have several different mechanisms of accumulation and excretion of metal ions [27, 29].

According to our preliminary results, we can make some conclusions about the ability of earthworms to indicate the heavy metal contamination in soil. Our results showed that endogeic species *Aporrectodea caliginosa* and *Aporrectodea rosea* as well as anecic species *Lumbricus terrestris* can be used for bioindication of Zn(II) and Cu(II) ions in contaminated soil. The concentration factor of Cd is high in the case of all earthworm species but the earthworms cannot be used as Pb(II) indicators because of the low level of accumulation of this metal ions in the tissues. Additionally, applying electrochemical methods is one of the most cost-effective and reliable ways to perform analysis of a large variety of trace-metal ions in environmental samples.

This study showed that the content of heavy metals in earthworms collected in the town of Saue tends to decrease by 20-30%, if compared to our earlier measurements, carried out in 1993. The decrease in metal concentrations might be explained by the fact that from 2002 Saue receives its thermal energy by the means of natural gas burning and soil pollution originating from shale oil combustion is excluded.

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REFERENCES


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