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Bioaccumulation of heavy metals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils

P.H.F. Hobbelen*, J.E. Koolhaas, C.A.M. van Gestel

Department of Animal Ecology, Faculty of Earth and Life Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

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Earthworms in floodplain soils not only accumulate heavy metals from soluble metal pools.

Abstract

The aim of this study was to determine important metal pools for bioaccumulation by the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in soils with high binding capacity. Cd, Cu and Zn concentrations in soil, pore water and $CaCl_2$ extracts of soil, in leaves of the plant species *Urtica dioica* and in earthworms were determined at 15 field sites constituting a gradient in metal pollution. Variations in the Cu and Cd concentrations in *L. rubellus* and Cu concentrations in *A. caliginosa* were best explained by total soil concentrations, while variation in Cd concentration in *A. caliginosa* was best explained by pore water concentrations. Zn concentrations in *L. rubellus* and *A. caliginosa* were not significantly correlated to any determined variable. It is concluded that despite low availability, earthworms in floodplain soils contain elevated concentrations of Cu and Cd, suggesting that uptake takes place not only from the soluble metal concentrations.

Keywords: Bioaccumulation; Heavy metals; Flood plains; Detritivores; Consumption

1. Introduction

The effects of heavy metals on soil organisms depend on exposure to concentrations that are available for uptake. Therefore it is important to know which metal species can be taken up by organisms and to determine the relative importance of different uptake routes. Earthworms can be exposed by direct dermal contact with heavy metals in the soil solution or by ingestion of pore water, polluted food and/or soil particles (Lanno et al., 2004). Data reported in the literature indicate that soluble metal concentrations are the best descriptors of bioaccumulation in earthworms (Spurgeon and Hopkin, 1996; Peijnenburg et al., 1999). Saxe et al. (2001) and Vijver et al. (2003) also studied the relative contribution of the dermal and the gut exposure route to the uptake of heavy metals in earthworms. Using a modeling approach, Saxe et al. (2001) estimated that the dermal exposure route accounted for more than 96% of the total uptake of Cd and Cu in the earthworm *Eisenia andrei*, and for 82% of the total uptake of Zn, when bioavailable metal concentrations in soil and gut are similar. Cd and Zn concentrations in the earthworm *Lumbricus rubellus*, with their mouth sealed using glue, amounted 83% and 79%, respectively, of internal concentration in earthworms that were able to feed and ingest soil particles, while Cu uptake could be fully ascribed to the dermal route (Vijver et al., 2003). However, other studies suggest an important role of the gut uptake route (Morgan and Morgan, 1992; Morgan et al., 2004).

* Corresponding author. USGS-NWHC, 6006 Schroeder Road, Madison, WI 53711-6223, USA. Tel.: +1 608 270 2486; fax: +1 608 270 2415.
E-mail address: phobbelen@usgs.gov (P.H.F. Hobbelen).

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The aim of this study was to determine which pools in the environment of the earthworms *L. rubellus* and *Aporrectodea caliginosa*, living in a field-polluted soil, could be important for the uptake of Cd, Cu and Zn. *Lumbricus rubellus* belongs to the group of epigeic earthworms, that live in the uppermost 5 cm of the soil layer and in the litter layer feeding mainly on organic matter and decaying material on the soil surface. *Aporrectodea caliginosa* belongs to the group of endogeic earthworms, that live in the top 25 cm of the soil and feed mainly on soil organic matter (Bouché, 1977).

To determine which pools in the environment of the earthworms *L. rubellus* and *A. caliginosa* could be important, metal concentrations in pore water and 0.01 M $CaCl_2$ extracts were measured as indicators of available metal concentrations in the soil solution. Concentrations in stinging nettles were measured as indicator of metal levels in leaf litter and total soil concentrations were measured as indicators of the metal levels in polluted soil particles.

2. Materials and methods

2.1. Field sites, sampling design and methods

This research was conducted in National Park "The Brabantsche Biesbosch" in the Netherlands. The Biesbosch is the delta area of the rivers Rhine and Meuse. During the 1960s and 1970s of the last century, these rivers contained high concentrations of heavy metals. Deposition of polluted particles in the Biesbosch resulted in high total soil concentrations of heavy metals.

Fifteen field sites, constituting a gradient in heavy metal pollution were used. The dominant vegetation at all sites consisted of the stinging nettle, *Urtica dioica*, and common reed, *Phragmites australis*. The field work was conducted in the spring of 2004.

Ten sampling points were laid out per field site using a rectangular grid of 2 × 8 m and a distance of 2 m between adjacent points. At each sampling point, one split corer sample (diameter 10 cm) of the upper 10 cm soil layer (after removal of litter) and one quadrat sample (25 × 25 × 25 cm) were taken, resulting in 10 split corer and 10 quadrat samples per field site. Also, at each sampling point, a number of stinging nettle leaves were picked. Soil from the split corer samples was used to determine organic matter and clay content, pH-$CaCl_2$ and pH of pore water, metal concentrations in soil, pore water and 0.01 M $CaCl_2$ extracts. Soil from the quadrat samples was used to collect earthworms by handsorting. All animals were transported to the laboratory and identified alive using Sims and Gerard (1999). If adult earthworms were present at a sampling point, one individual per species was randomly chosen, kept on moist filter paper for two days to excrete its gut content and frozen.

2.2. Soil texture and soil pH

To determine organic matter content, dried soil was placed in a combustion furnace (Heraeus) and heated for 1 h at 200 °C, for 1 h at 400 °C and for 6 h at 500 °C. Organic matter content was calculated as the loss on ignition. Clay (0–8 μm), silt (8–63 μm) and sand (63–2000 μm) content of the mineral soil fraction were determined using a Fritsch 'Laser Particle Sizer' A22[2] as described by Konert and Vandenberghe (1997). To measure pH-$CaCl_2$, soil was dried at 40 °C for 24 h and shaken with a 0.01 M $CaCl_2$ solution for 2 h at 200 rpm, using a solution:soil (w/w) ratio of 5:1. After sedimentation of soil particles and filtering over a Schleicher and Schuell 0.45 μm membrane, pH was measured using a Consort p907 meter. The same pH-meter was used to measure the pH of pore water. Pore water was collected using a Kontron TGA-6 centrifuge. Soil was centrifuged for 45 min with a relative centrifugal force of approximately 2000 × *g* over a Schleicher and Schuell 0.45 μm membrane filter, placed inside the tubes.

2.3. Total soil, available and internal concentrations of heavy metals

For the determination of the total soil concentrations of heavy metals, dried soil was digested in a 1:3:1 mixture of demineralized water, HCl 37% (Baker) and HNO_3 65% (Riedel-de Haën), using a microwave oven (CEM MARS 5). The 0.01 M $CaCl_2$-extractable and pore water concentrations of heavy metals were measured in the same extracts and pore water as used for the determination of the pH. Frozen earthworms were freeze-dried for 2 days and weighed to the nearest microgram on a Mettler Toledo UMT2. Earthworms were digested in a 7:3 mixture of HNO_3 65% (Riedel-de Haën) and demineralized water using a microwave oven (CEM MARS 5). Stinging nettle leaves were dried and ground using a Retsch MM 200 ball mill. The ground leaves were digested in a 4:1 mixture of HNO_3 65% (Riedel-de Haën) and HCl 37% (Baker) in closed Teflon bombs using an Ehret TK 3064 oven. Concentrations of heavy metals in soil extracts, animal and plant digests were measured using a Perkin–Elmer atomic absorption spectrometer (AAS), type 1100 B. Flame AAS was used if concentrations were high enough, otherwise graphite furnace AAS was used. Reference material Dolt II (animal tissue), Olive leaves (plant tissue) and Setoc soil were used to determine the accuracy of the analytical procedures. Average metal concentrations in soil varied between minus 1.2 and minus 23% of certified values. Average metal concentrations in animal tissue varied between plus 3.5 and plus 8.3% of certified values. Average Cd and Cu concentrations in plant tissue were within 12.7% of certified values. Zn concentrations in plant tissues were 45% lower than certified values and therefore not taken into account.

2.4. Statistical analysis

Pearson's correlation test and partial correlations were used to identify all possible (combinations of) predictor variables, significantly correlated to metal concentrations in pore water and 0.01 M $CaCl_2$ extracts of the soil, earthworms and plant leaves. Next, linear regression was used to determine the amount of variance in these metal concentrations that could be explained by models, containing the significantly correlated variables as predictors. Silt and sand content were not taken into account in the statistical analysis.

3. Results

3.1. Soil characteristics and metal concentrations

Tables 1–3 show the soil characteristics and metal concentrations in the soils, plant leaves and earthworms. Soil pH-$CaCl_2$ did not show large differences between the test sites and ranged between 7.34 and 7.74, while pore water pH levels were somewhat higher (7.60–8.20) (Table 1). No correlation was found between pH-$CaCl_2$ and pH levels in pore water. All soils had high organic matter contents (15.1–30.0%). Clay contents were higher than 25% at all sites except for site 1 (14.7%). Silt content showed little variation between field sites (44.1–54.6%), but was lower at site 1 (24.6%) and site 2 (37.1%). Sand content ranged from 1.82–37.9%, but was much higher at site 1 (60.7%).

Total metal concentrations in soil (Table 2) were high, and ranged between 9.9 and 24.4 mg Cd/kg, between 61 and 307 mg Cu/kg and were >1000 mg Zn/kg dry soil, except for site 1 where the Zn level was 654 mg/kg dry soil. Cd, Cu and Zn concentrations in the pore water of site 15 were relatively high, probably as a result of the low moisture content of the soil from this site. The amount of pore water collected from soil sampled at site 15 was sometimes too low to allow for a reliable measurement of concentrations of all metals.