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Effects of earthworms on decomposition and metal availability in contaminated soil: Microcosm studies of populations with different exposure histories

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Abstract

Population-specific differences in the responses of earthworms to simultaneous exposure to Cu and Zn were studied in microcosm experiments. Two populations of *Aporrectodea caliginosa tuberculata* (Eisen) with different metal exposure histories were chosen for the studies. Microcosms were prepared containing either uncontaminated soil or soils with low or high combined Cu/Zn -concentrations (79/139 or 178/311 mg kg^{-1} dry mass of soil, respectively). Earthworms from each population were introduced to the microcosm treatments with some microcosms serving as controls without earthworms. One series of microcosms was destructively sampled after 16 weeks incubation in a climate chamber. Survival, growth, reproduction and decomposition by earthworms in each treatment were measured. An additional microcosm series was sampled for soil and earthworm measurements at four weeks intervals to determine temporal changes in the availability of metals in the soils and their accumulation into earthworms. Cu and Zn were sequentially extracted from the soil samples of both microcosm series to estimate mobility and availability of the metals in the soil. Earthworms with long-term exposure history to metal-contaminated soil seemed to tolerate higher soil metal concentrations than earthworms without earlier exposure. Both earthworms and metals affected soil respiration (CO_2 production) and nitrogen mineralization. In addition, earthworms seemed to decrease the mobility and bioavailability of metals in the soil through their burrowing activity.

Keywords: Microcosms; Earthworms; Aporrectodea caliginosa tuberculata; Metal exposure history; Metal availability; Decomposition

1. Introduction

Earthworms are valuable and reliable animals for environmental monitoring of soil quality and contamination assessment (Van Gestel et al., 1997; Kula and Larink, 1998). Responses of earthworms to soil contamination vary depending on the species and even exposure history of individuals (Morgan and Morgan, 1999; Reinecke et al., 1999; Lukkari et al., 2005; Lukkari and Haimi, 2005). Possible acclimatization of earthworms and selection for increased resistance during long-term field exposure are the two important factors affecting results obtained from laboratory and field ecotoxicological studies (Eckwert and Köhler, 1997; Lukkari et al., 2004a). Therefore the species and even the population used in studies utilized in risk assessment procedures should be considered in addition to environmental conditions and the properties of harmful substances.

Microcosms are a useful tool for revealing the responses of organisms to harmful compound(s) in their natural environment, and for tracing the effects of these responses at higher levels of biological organisation, such as regulation of community structure, decomposition processes and primary production (Moore et al., 1996; Verhoef, 1996). Microcosm studies measure many variables and endpoints at the same time in the same environment, and so, offer more information for higher tiers of risk assessment than standardised single species toxicity tests or field monitoring (Cairns, 1984; Sheppard, 1997; Løkke and Van Gestel, 1998; Olesen and Weeks, 1998). Because results derived from standardised toxicity tests and field monitoring do not

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clearly support each other in all cases (Spurgeon, 1997; Van Gestel, 1997), microcosms are an especially useful tool for ecotoxicological research.

The degree of soil metal contamination is generally evaluated by total metal concentrations (Allen, 2002). However, this usually provides little information on the bioavailability and mobility of metals in the soil and, as a result, ignores actual exposure of soil organisms to metals. Soil characteristics and other environmental conditions strongly affect toxicity, speciation and behaviour of metals, e.g. those of Cu and Zn (Peijnenburg et al., 1997; Allen, 2002). These metals, while being essential trace elements, are toxic at high concentrations (Hopkin, 1989). They may be present in the soil in several chemical forms, each having a specific mobility and availability to soil organisms (Brusseau, 1997). Consequently, risk assessment for soil organisms should not be based on total metal concentrations only. Sequential extraction with increasing strength of extraction solution fractionates metals into forms of different solubility and mobility, which can be used when predicting metal availability to soil biota and their movements in the soil profile (Cooksey and Barnett, 1979; Tessier et al., 1979). Because sequential extraction procedures provide valuable information on differently behaving metal forms (Ure, 1996), they should be preferred for ecotoxicological studies (Ure et al., 1993a; Van Gestel and Weeks, 2004).

Our objective was to use microcosms mimicking field conditions to determine how *Aporrectodea caliginosa tuberculata* (Eisen) (Lumbricidae) populations with and without earlier metal exposure respond to simultaneous Cu and Zn contamination. In addition, decomposition processes and metal availability were studied. The sequential extraction method developed by Tessier et al. (1979), but improved with ultrasound-assisted extraction to decrease time and labour needed, was applied to describe the availability of Cu and Zn in the soil.

2. Materials and methods

2.1. Earthworms

Individuals of *A.c. tuberculata* were collected by hand at two sites. The population with earlier exposure to metal-contaminated soil was collected 1 km from the Imatra Steel Oy Ab -steel smelter (Imatra, SE Finland, 61°12′N, 28°48′E). The smelter was founded in 1937 and until 1944 there was also a copper factory next to the smelter. Currently the smelter produces steel by smelting recycled metal. The soil close to the smelter contains moderately increased concentrations of several metals, and earthworm populations at the site have been exposed to metals for generations (Lukkari et al., 2004b). The earthworms without earlier exposure to elevated concentrations of metals were collected from deciduous forest in Jyväskylä, central Finland (62°14′N, 25°44′E) (for soil metal concentrations e.g. Lukkari et al., 2004a). Collected earthworms were transferred without delay to the laboratory and were stored in a climate chamber (15 °C, darkness) not more than seven days before the experiment began. Individuals used in the experiment were adults with well-developed clitella or subadults with clear signs of developing tubercula pubertasis (0.5–1 g in fresh weight).

2.2. Test soils

Organic rich soil for the experiment was collected from deciduous forest in Jyväskylä approximating (in properties other than metal concentrations) both soils in which the experimental earthworms had lived. The soil was homogenised by passing it through a 0.5 cm sieve. Physicochemical properties, soil pH (H_2O), organic matter content (loss on ignition at 550 °C for 4 h) and moisture content (48 h in 80 °C) were measured both before and after the experiment from randomly taken samples with three replicates. Total soil metal concentrations were analysed and published previously (Lukkari et al., 2004a). In the present study, soil metal concentrations were analysed with a sequential extraction procedure (see below) during and after the experiment.

2.3. Microcosms

Microcosms were plastic jars (volume 2 l) with lids, and 1.2 kg homogenised soil (fresh mass) which was either uncontaminated (control) or simultaneously spiked with Cu and Zn was added to each microcosm. Two contamination treatments, low and high, (79/139 and 178/311 Cu/Zn mg kg^{-1} dry mass of soil, respectively) were created by adding to the soil appropriate amounts of copper chloride ($CuCl_2 \cdot 2H_2O$, pro analysis quality by J.T.Baker, Deventer, the Netherlands) and zinc chloride ($ZnCl_2$, GR for analysis by Merck KGaA, Darmstadt, Germany) in water so that soil moisture content was ca. 25% of fresh mass. Two experimental series of microcosms were established. The main series contained 54 microcosms (18 microcosms without and 36 with earthworms). Six replicates of each microcosm soil treatment (control, low, or high contamination) were designated to earthworm treatment: no earthworms, 4 earthworms from the population without earlier metal exposure or 4 earthworms from the population with exposure history. The additional microcosm series contained 30 microcosms (15 microcosms without and 15 with earthworms). Five replicates of each microcosm soil treatment (control, low or high contamination) were designated to earthworm treatment: no earthworms or 4 earthworms from the population without metal exposure history. Earthworms from the population with metal exposure history were not used in this part of the experiment. At the beginning of the experiment, all microcosms received 2 g (fresh mass) of grass to serve as