Soil Biology & Biochemistry 37 (2005) 917–925

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

The effect of two endogeic earthworm species on zinc distribution and availability in artificial soil columns

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Received 5 August 2003; received in revised form 23 August 2004; accepted 1 October 2004

Abstract

The objective of this study was to determine the impact of earthworm bioturbation on the distribution and availability of zinc in the soil profile.

Experiments were carried out with *Allolobophora chlorotica* and *Aporrectodea caliginosa* in 24 perspex columns (∅ 10 cm), filled with 20–23 cm non-polluted soil (OM 2%, clay 2.9%, pH 0.01 M $CaCl_2$ 6.4), that was covered by a 3–5 cm layer of aged zinc spiked soil (500 mg Zn/kg dry soil) and another 2 cm non-polluted soil on top. After 80 and 175 days, columns were sacrificed and each cm from the top down to a depth of 15 cm was sampled. Earthworm casts, placed on top of the soil, were collected. Each sample was analyzed for total and $CaCl_2$-exchangeable zinc concentrations.

Effects of earthworm bioturbation were most pronounced after 175 days. For *A. chlorotica*, total and $CaCl_2$-exchangeable zinc concentrations in the polluted layers were lower with than without earthworms. Total zinc concentrations in the non-polluted layers were higher in columns with earthworms. Casts of *A. chlorotica* collected on the soil surface showed slightly higher total zinc concentrations than non-polluted soil. Casts were found throughout the whole column. For *A. caliginosa* there were no differences in total zinc concentration between columns with and without earthworms. $CaCl_2$-exchangeable zinc concentrations in the polluted layers were lower for columns with earthworms. Casts were mainly placed on top of the soil and contained total zinc concentrations intermediate between those in non-polluted and polluted soil layers.

This study shows that different endogeic earthworm species have different effects on zinc distribution and availability in soils. *A. chlorotica* transfers soil throughout the whole column, effectively mixing it, while *A. caliginosa* decreases metal availability and transfers polluted soil to the soil surface.

Keywords: Earthworms; Heavy metals; Zinc; Bioturbation; Availability; *Allolobophora chlorotica*; *Aporrectodea caliginosa*

1. Introduction

The river Rhine catchment area is one of the most industrialized and most heavily polluted river tributaries in the world. In the past, especially in the 1960s, industries in the Netherlands and Germany (Ruhr-area) caused a diffuse pollution in the floodplains, particularly in the lower parts of the tributary (Middelkoop, 2002). Contaminants, like heavy metals, reached concentrations in soil that can be toxic to organisms.

Nowadays, the industries are cleaner, resulting in lower heavy metal concentrations in the river sediment. Consequently, during flooding conditions the fresh sediment covers the polluted soil and the pollution is embedded below clean soil layers. As a result, the polluted soil layers can now be found at a depth of 5 cm to 1 m (Middelkoop, 2002). This means that soil organisms may still have contact with the polluted soil and take it up or transfer it. Soil transfer by means of soil organisms is called bioturbation. Although total heavy metal concentrations in the polluted soil are high, this does not mean that they directly cause toxic effects. In soil, metals are present in various forms and complexes (Ritchie and Sposito, 1995; Sauvé et al., 2000a),

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0038-0717/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2004.10.012

which are not all available to organisms. Due to this speciation, metal availability to organisms is difficult to assess. There are many factors influencing metal speciation, such as pH, aeration, soil moisture content and salinity (Wolt, 1994; Sauvé et al., 2000b). This means that the soil characteristics have an important impact on heavy metal availability to organisms. In addition, it also means that animal activities, which change soil conditions, may affect metal speciation and consequently metal availability.

Earthworms are known to be able to change soil conditions in various ways. Their burrows improve water infiltration and aeration of the soil (Edwards et al., 1992; Pitkanen and Nuutinen, 1998; Jegou et al., 2001), and due to their effect on litter and microorganisms they are important for decomposition processes and soil fertility (Lee and Pankhurst, 1992; Binet et al., 1998). Some other effects of earthworm bioturbation include stimulation of PCB bioremediation (Singer et al., 2001), soil formation (Johnson, 2002) and the burying of archaeological artifacts (Armour-Chelu and Andrews, 1994). Their burrowing activity can result in a complete turnover of the topsoil in a couple of years. Estimated from casts placed on top of the soil, soil turnover rates vary from about 3.8 kg/m^2 per year in 1-year field experiments, with concurrent influences of drought, rain and frost (Darwin, 1881) to about 41 kg/m^2 per year in a 1-week laboratory experiment at 20 °C (Cook and Linden, 1996).

Earthworms can be divided into three ecological groups, following different morphological and behavioral characteristics (Bouché, 1977; Lavelle, 1983; Sims and Gerard, 1999): epigeic, endogeic and anecic species. Because of their different burrowing behaviors, the different groups of earthworms can have a different contribution to bioturbation. Endogeic species burrow through the soil searching for food, which consists of dead roots, root exudates and decaying organic matter. This group is quite heterogeneous, with species, like *Allolobophora chlorotica*, living in the top 10 cm of the soil, in close association with plant roots, species, like *Octolasion* spec., searching also deeper soil layers, preferring mineral and humus rich soils, and species, like *Aporrectodea caliginosa*, with a very broad range of habitats (Sims and Gerard, 1999; Martinucci et al., 1983).

The objective of this study was to determine the impact of earthworm bioturbation on the distribution and availability of zinc in a soil profile. Endogeic species were expected to show the highest impact, because these species burrow through the soil in search for food. The impact of two endogeic earthworm species on metal distribution was studied in columns packed with a layer of zinc polluted soil between layers of non-polluted soil. *A. chlorotica* and *A. caliginosa* were selected, because of their different habitat preference, while both species can co-exist in high densities in floodplain areas. Effects on metal availability were estimated by measuring total and $CaCl_2$-exchangeable zinc concentrations in different soil layers and in earthworm casts.

2. Materials and methods

2.1. Soil columns

Perspex columns ($n=24$, ∅ 10 cm, length 40 cm) with some small holes on top and bottom to allow air and water flow, were constructed in such a way that they could be split in two lengthwise. This made it possible to sample soil at specific depths.

Non-polluted soil and 10-year aged zinc spiked soil (approximately 500 mg/kg dry soil) were collected from an earlier semi-field experiment, carried out by Smit et al. (1997). Soil, as described by Smit and coworkers, contained 2% organic matter, 2.9% clay and had a pH (0.01 M $CaCl_2$) of approximately 6.4. The soil was sieved, air dried and remoistened to 50% of the maximum water holding capacity, corresponding with a moisture content of 15% of dry weight (w/w).

2.2. Earthworms

Two earthworm species, *Allolobophora chlorotica* (Savigny) and *Aporrectodea caliginosa* (Savigny), were obtained from non-polluted field sites.

A. chlorotica (green morph) was collected from an agricultural grassland soil near Drachten, Friesland, the Netherlands. *A. caliginosa* was collected from a natural grassland soil in a floodplain near Druten, Gelderland, the Netherlands. Both species were maintained in potting compost in a climate room (14 °C) until used. Earthworms were placed on wet filter paper, 24 h before use, to empty their guts.

2.3. *A. chlorotica*

The 24 perspex columns were filled with non-polluted soil to a depth of 20 cm, followed by a polluted layer of 3 cm and finally a 2 cm layer of non-polluted soil on top. Soil density was about 1.40 g/cm^3.

In 12 columns, five earthworms, with an average weight of 140 mg (SD ± 20 mg; $n=60$) each, of the species *A. chlorotica* were introduced, the other 12 columns did not receive any earthworms. The columns were placed in a dark climate room at a temperature of approximately 14 °C. After 40, 80 and 175 days, respectively 10, 8 and 6 soil columns were opened (equal number of columns with and without earthworms were used each time), inspected for casts, destructively sampled and soil was taken in one cm layers from the top down to a depth of 15 cm. The earthworms were collected, kept overnight for 20 h on wet filter paper, and weighed. At 175 days, casts produced on top of the soil columns with earthworms were sampled. Soil samples were analyzed for moisture content, $CaCl_2$-exchangeable and total zinc concentrations. The percentage of the total zinc concentration that was $CaCl_2$-exchangeable was termed available fraction. Casts were analyzed only for total zinc