Environmental Pollution 159 (2011) 722–728

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Lumbricus terrestris L. activity increases the availability of metals and their accumulation in maize and barley

E. Ruiz [a], J. Alonso-Azcárate [b], L. Rodríguez [a,*]

[a] *Department of Chemical Engineering, School of Civil Engineering, University of Castilla-La Mancha, Avenida Camilo José Cela, s/n, 13071 Ciudad Real, Spain*

[b] *Department of Physical Chemistry, Faculty of Environmental Sciences, University of Castilla-La Mancha, Avenida Carlos III, s/n, 45071 Toledo, Spain*

Lumbricus terrestris L. activity increases the bioavailability of metals in soils and total, shoot and root metal concentrations for maize and barley.

ARTICLE INFO

Article history:
Received 17 July 2010
Received in revised form
13 November 2010
Accepted 24 November 2010

Keywords:
Soil pollution
Lumbricus terrestris
Earthworms
Sequential extraction
Metal availability

ABSTRACT

The effect of the earthworm *Lumbricus terrestris* L. on metal availability in two mining soils was assessed by means of chemical extraction methods and a pot experiment using crop plants. Results from single and sequential extractions showed that *L. terrestris* had a slight effect on metal fractionation in the studied soils: only metals bound to the soil organic matter were significantly increased in some cases. However, we found that *L. terrestris* significantly increased root, shoot and total Pb and Zn concentrations in maize and barley for the soil with the highest concentrations of total and available metals. Specifically, shoot Pb concentration was increased by a factor of 7.5 and 3.9 for maize and barley, respectively, while shoot Zn concentration was increased by a factor of 3.7 and 1.7 for maize and barley, respectively. Our results demonstrated that earthworm activity increases the bioavailability of metals in soils.

1. Introduction

The impact of earthworms on soil properties and plant growth has been widely studied during the last two decades (Edwards and Bater, 1992; Zhang and Schrader, 1993; Scheu, 2003; Amador and Gorres, 2005). Earthworm soil activities such as burrowing, casting and mixing of litter and soil (bioturbation) have important consequences for soil processes and properties. Earthworms improve soil structure due to the increase of macroporosity with effects on aeration, water dynamics, soil organic matter dynamics and provision of paths for root exploration (Amador and Gorres, 2005; Wen et al., 2006; Suthar et al., 2008). They can alter the ecology of the soil by suppressing plant pathogens and/or promoting growth of microflora and fauna that may be beneficial to crops (Clapperton et al., 2001). Moreover, earthworms are capable of increasing the bioavailability of several soil nutrients such as C, N and P (Devliegher and Verstraete, 1996; Cheng and Wong, 2002; Chaoui et al., 2003), as well as metal availability (Devliegher and Verstraete, 1996; Cheng and Wong, 2002; Ma et al., 2002; Wen et al., 2004; Udovic and Lestan, 2007; Sizmur and Hodson, 2009).

Furthermore, earthworms are relatively efficient accumulators of certain essential and non-essential metals, such as Cd, Cu, Zn and Pb (Morgan et al., 1989; Morgan and Morgan, 1999; Lanno et al., 2004; Yu et al., 2005). Chemical analysis of earthworms exposed to or collected in soils containing toxic substances such as metals can also give an indication of the bioavailable amount of a selected toxicant (Maleri et al., 2008).

Availability of metals in soils is considered an important parameter for the effectiveness of the uptake and accumulation of metals by plants (Zhang and Shan, 2000). Chelating agents, such as EDTA and its structural analogues, e.g. DTPA, EGTA and NTA, have been used to enhance metal solubility in soils and hence increase metal accumulation in plants (Meers et al., 2005a). However, side-effects related to the addition of chelates, e.g. metal leaching, may cause additional health, safety and environmental concerns (Kos and Lestan, 2003). Some researchers have assessed the feasibility of using earthworms to modify the metal availability in polluted soils and enhance plant uptake (Cheng and Wong, 2002; Wen et al., 2006; Ruiz et al., 2009). However, further research is necessary to study the differences due to different earthworm species and to assess the mechanisms for metal mobilization (Sizmur and Hodson, 2009).

The main objective of the present study is to assess the ability of the earthworm species *Lumbricus terrestris* L. to change metal availability in polluted soils from an old Pb–Zn mining area by combining chemical extraction methods, i.e. single extraction with 0.01 M $CaCl_2$ and water and BCR sequential extraction, and a plant bioassay using crop plants.

---

* Corresponding author.
E-mail address: Luis.Rromero@uclm.es (L. Rodríguez).

0269-7491/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2010.11.032

2. Materials and methods

2.1. Soil sample collection and preparation

Two different soils, taken from arable land (S1) and mine tailings (S2) in the surrounding area of an abandoned Pb–Zn mine in Spain (San Quintín mine), were used in this study. Soils were sampled from the top 0–25 cm soil layer, air-dried at ambient temperature for 72 h, disaggregated and, finally, sieved to <2 mm and <63 μm. The <2 mm fraction was used for plant pot experiments and for the measurement of the soil's physico-chemical properties. The <63 μm fraction was used to analyze the total metal content, as well as BCR sequential extraction, water and 0.01 M $CaCl_2$ extractions.

Soil pH and electrical conductivity (EC) were measured in a 1:5 soil/water mixture; organic carbon was determined using a total organic carbon analyzer (Shimadzu TOC-VCSH, Columbia, USA); particle-size distribution (clay, silt and sand content) was determined using laser diffractometry (Beckman Coulter LS, Fullerton, USA); cation exchange capacity (CEC) was measured by the ammonium acetate saturation method (MAPA, 1994). To determine the total concentration of Cu, Pb and Zn in the soils (<63 μm fraction), 0.5 g of sample was digested with a mixture of acids (9 mL of concentrated HNO_3 + 3 mL of concentrated HCl) in a microwave unit (CEM MARS 5, Matthews, USA), according to the EPA 3051A method (USEPA, 1996a).

2.2. Pot experiments

Plastic pots were filled with 1 kg of the air-dried soils sieved to <2 mm. Six different experimental series were carried out simultaneously for each soil. The experimental series were: control soil, without plants and earthworms (C); soil with earthworms and without plants (E); soil with maize, without earthworms (M); soil with barley, without earhworms (B); soil with maize plus earthworms (ME); and soil with barley plus earthworms (BE). Each series consisted of three replicates (using three separate pots); earthworms from each pot were treated separately.

Barley (*Hordeum vulgare*) and maize (*Zea mays*) seeds were pregerminated in a germination chamber (ASL IBERCEX, Madrid, Spain) for 3 days at 20–24 °C, 60% relative humidity and 46.8 $\mu mol/m^2/s$ light intensity. Next, a total of 20 barley and 10 maize seeds were sown per pot and later reduced to 10 and 5 seeds per pot, respectively. The soil was adjusted to approximately 60% of its water-holding capacity, and water loss was compensated daily by the addition of deionized water. After three days the E, ME and BE series were inoculated with 12 *L. terrestris* worms. The earthworms were obtained from a local commercial supplier (Armeria20, Toledo, Spain), who imported them from a commercial vermiculture supplier (Vivastic, Elsenheim, France). All individuals were adult worms with clitellum and a mean fresh weight of 436 ± 51 mg.

The pot experiment was carried out with continuous light (24 h day^{-1}) to keep the earthworms in the soil. Since the optimal temperature for *L. terrestris* development is between 15–20 °C (Lowe and Butt, 2005), the pot experiments were conducted at a constant temperature of 17 °C.

Plants were harvested 4 weeks after planting. Plant shoots and roots were thoroughly washed with de-ionized water, weighed, stored at −80 °C, freeze-dried, finely ground to fine powder by a ball mill (Retsch MM200, Haan, Germany) and sealed in plastic bags for subsequent heavy metal analysis. The dry plants were digested in the above mentioned microwave unit using a mixture of $HNO_3/HCl/H_2O_2$, according to the 3052 EPA method (USEPA, 1996b). The total metal accumulation rate (TMAR) in maize and barley was calculated by the following equation:

$$\mathrm{TMAR} = \frac{([M]_{\mathrm{root}}\mathrm{DW}_{\mathrm{root}}) + ([M]_{\mathrm{shoot}}\mathrm{DW}_{\mathrm{shoot}})}{(\mathrm{DW}_{\mathrm{root}} + \mathrm{DW}_{\mathrm{shoot}})}$$

where $[M]_{\mathrm{shoot}}$ and $[M]_{\mathrm{root}}$ represent the total metal concentration in plant shoots and roots (mg kg^{-1}), respectively, while $\mathrm{DW}_{\mathrm{root}}$ and $\mathrm{DW}_{\mathrm{shoot}}$ represent the weight of plant roots and shoots (kg), respectively.

At the end of experiment, the earthworms were washed and placed in Petri dishes for 48 h to remove ingesta residues in the guts (Cheng and Wong, 2002; Udovic and Lestan, 2007); earthworm casts were removed each day. Later, the earthworms were weighed, stored at −80 °C and freeze-dried. The dried earthworms were digested in the microwave unit using 10 mL of HNO_3 (EPA 3051A method). As was pointed out above, all the earthworms from each pot were treated as separate pools. Simultaneously, three specimens of *L. terrestris*, not used in an experiment, were washed, depurated, dried and digested as described above. These specimens were used as a control (control earthworms, CE) to establish the accumulation of metals by earthworms in the pot experiment.

The concentrations of Cu, Pb and Zn in plant, soil and earthworm extracts were analyzed by ICP-OES using a Thermo ICAP 6500 spectrometer (Thermo Electron, Cambridge, UK).

2.3. Single and sequential extraction procedures

Single extraction with water and 0.01 M $CaCl_2$ and the BCR sequential extraction method were applied to the air-dried soil samples (<63 μm fraction) collected after harvesting the plants.

The BCR sequential extraction scheme was applied in this work to assess the changes in the chemical partitioning of Cu, Pb and Zn in the soil after the treatments (Sahuquillo et al., 1999). It basically consisted of three extraction steps, namely: (i) Step 1, extraction with acetic acid 0.11 M (to obtain the F1 fraction); (ii) Step 2, extraction with hydroxylamine hydrochloride 0.5 M at pH 1.5 (F2 fraction); and (iii) Step 3, reaction with 8.8 M H_2O_2 followed by an extraction with 1.0 M ammonium acetate at pH 2 (F3 fraction). Residue from the third step was digested by the above-mentioned 3051A EPA method (to obtain the F4 fraction). The F1 fraction is usually called 'exchangeable' and accounts for water-extractable, exchangeable and carbonate-bound metals; the F2 fraction is called 'reducible' and it mainly consists of metals bound to Fe–Mn oxides; the F3 fraction is called 'oxidizable' and it accounts for metals bound to organic matter and sulphides; finally, the F4 fraction is called 'residual' and consists of metals bound to mineral lattices (Zemberyová et al., 2006).

The quality of the analytical data for the BCR sequential extraction procedure was assessed by carrying out analyses of the BCR-701 certified reference material (LGC Promochem, Barcelona, Spain). At least one reference material and a blank sample (taken through the complete procedure but in the absence of a sample) were included in each batch of samples. Measured values of the reference material were typically ±15% of certified values; an accuracy that can be considered as acceptable (Marguí et al., 2004; Cappuyns et al., 2007).

Two additional single extraction methods were used. Water-extractable metals were determined by shaking 2 g of soil with 20 mL of milli-Q water for 2 h and then centrifuging at 3000*g* for 10 min. The 0.01 M $CaCl_2$-extractable metals were determined according to the method reported by Novozamsky et al. (1993): 2 g of soil in 20 mL of 0.01 M CaCl2, shaking for 2 h.

All the extractions were carried out in triplicate and reagent blanks were included in every batch. Finally, water- and $CaCl_2$-extractable Cu, Pb and Zn concentrations were measured by ICP-MS in a Thermo XSeries II spectrometer. The concentration of metals extracted in the BCR sequential extraction was analyzed by ICP-OES using the above-mentioned Thermo spectrometer.

2.4. Statistical analysis

All statistical analyses were carried out with the program Statistica 6.0 (Statsoft Inc., Tulsa, USA). A Shapiro-Wilk W-test for normality was performed to determine if the data came from a population with normal distribution. Then, one-way ANOVA was used to assess the effect of earthworm presence on metal fractionation in studied soil and to compare earthworm weights before and after the experiments. ANOVA followed by Tukey's HSD tests were performed to determine significant differences between the Cu, Pb and Zn accumulation in the earthworms belonging to different experimental series. The same statistical method was used to compare metal uptake of barley and maize in the S1 and S2 soils with and without earthworms.

3. Results and discussion

3.1. Soil characterization

Table 1 shows the main physico-chemical properties, total metal concentrations and mineralogical analysis for the S1 and S2 soil samples.

The S1 soil was almost neutral while the S2 soil had a slightly alkaline pH value. Total organic carbon (TOC) content of the S1

Table 1
Land use, selected physicochemical properties (<2 mm fraction) and total metal concentration (<63 μm fraction) of the two soils used in this study.

Parameter	Soil	
	S1	S2
Land use	Arable land	Mine tailing
pH-H_2O	6.30	7.51
EC (μs cm^{-1})	150	83.9
Clay (%)	16.2	12.1
Silt (%)	51.1	21.4
Sand (%)	32.7	66.5
CEC[a] (meq 100 g^{-1})	16.7	8.56
TOC[b] (%)	0.94	1.46
Water-Holding Capacity (%)	38.5	26.1
Total Cu (mg kg^{-1})	34.9	38.7
Total Pb (mg kg^{-1})	133	821
Total Zn (mg kg^{-1})	299	674

[a] Cation exchange capacity.

[b] Total organic carbon content.