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The role of earthworms (*Eisenia fetida*) in influencing bioavailability of heavy metals in soils

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Abstract The effects of earthworm (*Eisenia fetida*) activity on soil pH, dissolved organic carbon (DOC), microbial populations, fraction distribution and bioavailability of heavy metals (Zn, Cu, Cr, Cd, Co, Ni, and Pb) in five Chinese soils were investigated using pot experiments. A three-step extraction procedure recommended by the European Community Bureau of Reference (BCR; now Standards, Measurements and Testing Programme of the European Community) was used to fractionate the metals in soils into water soluble, exchangeable and carbonate bound (B1), Fe-oxides and Mn-oxides bound (B2) and organic matter and sulfide bound (B3). After the soils were treated with earthworms, the soil pH, water-soluble metal fraction and DOC increased. A significant correlation was obtained between the increased DOC and the increased metals in the water-soluble fraction. The heavy metals in fraction B1 increased after earthworm treatments, while those in fraction B3 decreased. No significant differences were observed for heavy metals in fraction B2. The microbial populations in soil were enumerated with the dilution plate method using several media in the presence of earthworms. The microbial populations increased due to earthworm activity. The biomass of wheat shoots and roots, and the heavy metal concentrations in wheat roots and shoots, were also increased due to the earthworm activity. The present results demonstrated that earthworm activity increases the mobility and bioavailability of heavy metals in soils.

Keywords Earthworm · Dissolved organic carbon · Heavy metal fractionation · Microbial populations · Availability

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Introduction

Soil is a complicated heterogeneous system with the predominance of a solid phase constituted of soil organic matter, minerals, plants, microbes, and faunas (Nannipieri and Badalucco 2003). Soil physico-chemical properties are the main factors affecting the mobility, speciation and hence availability and toxicity of some nutrients (such as N and P) and heavy metals (Ge et al. 2000) and these properties depend on the interactions between soils, plants, microbes, and animals. The rhizosphere soil is a unique microenvironment because its properties differ from those of the bulk soil as plant roots exude organic compounds including low-molecular-weight organic acids (Jones 1998; Pinton et al. 2001). Thus, the adsorption-desorption behaviors of heavy metals can be different in the rhizosphere soil with respect to the bulk soil (Naidu and Harter 1998; Shan et al. 2002). In addition, plant uptake of metals is also influenced by rhizosphere bacteria (Whiting et al. 2001).

Earthworms are ubiquitous animals living in soils, affecting soil chemical and physical properties (Lee 1985; Pallant and Hilster 1996; Boyle et al. 1997; Capowiez et al. 2000; Ponder et al. 2000), and the distribution and activity of microbes and soil animals (Binet et al. 1998; Toyota and Kimura 2000; Salmon 2001). In addition, it has been reported that after earthworm activity, the fraction distribution of heavy metals (Zn, Pb, Fe, Mn, Cr, Co, and Cu) is changed significantly, thus affecting the bioavailability of these metals (Devliegher and Verstraete 1996; Cheng and Wong 2002; Ma et al. 2002).

Although the fact that earthworm activity elevates the bioavailability of heavy metals is well documented, the basic mechanisms of how earthworms affect the behaviors of heavy metals in soil are still unclear.

The aims of the present study were to investigate the possible reasons why heavy metals in soils are readily available to plants in the presence of earthworms. We investigated the effects of earthworm inoculation on soil pH, dissolved organic C (DOC), microbial populations in soil, metal fraction distribution, and the bioavailability of metals in soil to wheat roots and shoots.

Materials and methods

Soil sample collection and preparation

Five Chinese cultivated soils were collected from Jiangxi Province, Southern China (Yingtan soil); Beijing, Northern China (Beijing soil); Hubei Province, Central China (Wuhan soil); Shandong Province, Eastern China (Rongcheng soil) and Heilongjiang Province, Northeastern China (Heilongjiang soils). All soil samples were taken from the cultivated surface layer (0–20 cm). The soils were air-dried, ground and screened through a 1-mm nylon fiber sieve to remove stones, plant roots, and other large particles.

Soil pH was measured in de-ionized water using a 1:1 (w:v) soil/solution ratio, organic C by the Walkley-Black procedure (Nelson and Sommers 1982), and cation exchange capacity (CEC) by the method of Rhoades (1982). Amorphous iron (Fe) and aluminum (Al) oxides were determined by the ammonium oxalate extraction method (Blakemore et al. 1987) and crystalline Fe and Al oxides by the oxalate-ascorbic acid extraction method of Shuman (1982). Manganese oxide content was determined by extraction with 0.1 mol l^{-1} hydroxyl-ammonium hydrochloride (Shuman 1982).

Pot experiments

One kilogram of soil was placed in a plastic pot. Before planting, 12 earthworms (*Eisenia fetida*), purchased from China Agricultural University (an average 0.60 g fresh weight each, 3.5–4.5 cm in length) were inoculated in each pot under greenhouse conditions. The soils were initially adjusted to approximately 60% water-holding capacity and de-ionized water was added daily to correct water loss due to evapotranspiration during the incubation. After a 6-week incubation, no mortality was observed and the average weight of each earthworm was still 0.60 g. Wheat seeds (*Triticum aestivum* L.) were thoroughly rinsed with water, and germinated on a filter paper moistened with de-ionized water for 24 h at 20°C in the dark. Uniformly germinated seeds with radical emerged were then sown in all soil types. A total of 20 seeds were sown per pot and subsequently thinned to 15 plants. Winter wheat in pots without earthworms served as controls. All treatments were replicated three times.

Plant shoots and roots were harvested 6 weeks after germination. Still no mortality was observed for earthworms and the average weight of each earthworm was 0.61 g. The plant shoots were cut off with a plastic knife near the soil surface and washed with dilute detergent solution followed by several rinses with de-ionized water. The roots were separated from soil by washing with de-ionized water carefully. The plant materials were then dried at 70°C for 48 h and the dried plant tissue was weighed and finely ground before metal determinations.

The soil collected from each pot after harvest was air-dried, mixed, and then sieved (1-mm) and analyzed for water-soluble heavy metals, organic C and metal speciation by the three-step BCR method.

Water-soluble heavy metals and organic C

Water-soluble metals and organic C were obtained by shaking 1.0 g dried soil with 5.0 ml de-ionized distilled water in 50-ml polypropylene centrifuge tubes for 24 h. After centrifuging at 4,000*g* for 30 min, the supernatant was filtered with 0.45 μm membrane. The heavy metal concentrations and the total organic C in the supernatants were determined by inductively coupled plasma mass spectrometry (ICP-MS) and TOC instruments (Shimadzu 5000), respectively.

Fractionation of heavy metals

Three-stage sequential fractionation as proposed by the European Community Bureau of Reference (BCR; Quevauviller et al. 1993) was carried out. The metal fractions were specified as fraction B1: water soluble, exchangeable and carbonate bound; fraction B2: Fe-Mn oxide bound; and fraction B3: organic matter and sulfide bound. Dried soil (0.50 g) was used for this fractionation. The procedure was replicated three times for each soil sample. Following each extraction, the mixture was centrifuged at 4,000*g* for 30 min to separate the supernatant from the residues. The residues were washed with de-ionized water, and washings were combined with the supernatant fraction. The residues were then subject to the extraction of the next step.

Enumeration of microbial populations

The microbial population enumeration was carried out by using fresh soil because microbial populations are largely influenced by soil moisture. Three subsamples (1.00 g) of fresh soil (Beijing soil) collected from each pot with and without earthworms were suspended in 99 ml sterilized water and ultrasonicated for 20 min at 100 W. A serial tenfold dilution was made and an aliquot of each appropriate dilution was inoculated into plate count agar. Various media were used to incubate bacteria (Bacto beef extract), actinomycetes (Gauze's media no. 1), or fungi (potato dextrose agar; Shen et al. 2001). The inoculated growth media were incubated in the dark at 37°C (for bacteria) and 25°C (for actinomycetes and fungus). Colony forming units (cfu) of microbes were enumerated after 1–3 days (for bacteria) and 4–7 days (for actinomycetes and fungus). At the same time three subsamples were taken from each pot to correct soil moisture.

Determination of heavy metals

Heavy metal (Cu, Pb, Cr, Mn, Co, Ni, Cd, and Zn) concentrations of wheat shoots and roots, soil and each soil fraction were determined by ICP-MS (VG Elemental, Winsford, United Kingdom). ^{115}In was

Table 1 Selected properties of studied soils in China

Soil	Order	Sand (%)	Silt (%)	Clay (%)	Fe (%)		Al (%)		Mn (mg kg^{-1})	CEC (cmol kg^{-1})	pH	Organic C (%)
					Crystal	Amorph.	Crystal	Amorph.				
Yingtan	Ultisol	19.5	38.7	42.4	3.52	0.72	1.39	0.28	27.6	14.24	4.56	0.89
Wuhan	Alfisol	52.9	21.4	25.7	23.5	0.73	1.05	0.012	409	22.8	6.73	1.72
Beijing	Mollisols	37.6	51.6	10.8	0.88	0.32	0.36	0.11	112	15.71	6.90	0.78
Rongcheng	Mollisols	89.7	3.9	6.4	11.68	0.33	0.97	0.011	475	9.82	6.08	0.39
Helongjiang	Mollisols	9.8	62.8	27.4	1.50	0.40	1.6	0.34	326	26.00	7.35	3.71

added as an internal standard to monitor matrix effects and signal drifting. General instrumental operating conditions and sample digestion method have been already described (Wen and Shan 2002; Wen et al. 2001). The analyses were carried out in triplicate.

Reagents and statistics

All reagents used were of analytical reagent grade. Statistical analyses were conducted with the software SPSS 11.5 for Windows (SPSS, Chicago, Ill.). Soil pH values, concentrations of water-soluble heavy metals and organic C, heavy metal fractionations of soils, metal concentrations of wheat roots and shoots, and biomass of dry wheat are reported as mean ± standard errors. One-way ANOVA analyses (Student's *t*-test and *F*-test) were undertaken to establish significant differences between treatments. Linear regression analyses were conducted using the least-squares method.

Results and discussion

Soil analysis

The soils represented a wide range of chemical and physical properties (Table 1). Yingtan soil was the most acidic and Rongcheng soil slightly acidic, whereas the other three soils had near neutral pH values. Heilongjiang soil had the highest organic C content and CEC, whereas Rongcheng soil had the lowest organic C and clay contents, thus the lowest CEC. The total metal concentrations of soils are shown in Table 2.

Effect of earthworms on soil pH

Earthworm activity increased pH of all soils from 0.2 to 1.1 units (P <0.1; Fig. 1a). The pH of Beijing soil was affected most significantly since it increased from 6.9 to 8.0, while the pH of Heilongjiang soil was only slightly elevated from 7.3 to 7.5. For Yingtan, Wuhan, and Rongcheng soils, the pH values increased by 0.8, 0.6, and 0.5 units, respectively.

It is well established that soil pH is a key factor affecting the adsorption-desorption behaviors and hence bioavailability of heavy metals in soil. Therefore, it is important to determine the pH change due to earthworm activity. Hu et al. (1998) observed that earthworm activity increased soil pH due to excretion of calcium compounds into the environment by calciferous glands. However, it must be pointed out that all earthworms with or without calciferous glands increase soil pH (Cheng and Wong 2002) due to their alkaline urine (Salmon 2001). Although the results obtained by Cheng and Wong (2002) showed that earthworm (*Pheretima* sp.) activity decreased the pH of red acidic soil, such as the Yingtan soil used in this study, no decrease but an increase in soil pH was observed in the Yingtan soil. This discrepancy may be due to the different type of earthworm inoculated.

Table 2 Total heavy metal concentrations of soils (μg g^{-1})[a]

Soils	Cr	Co	Ni	Zn	Cu	Cd	Pb
Yingtan	45.30	3.93	20.77	269.95	48.47	2.17	36.69
Wuhan	53.76	13.55	33.81	254.54	62.29	4.19	22.40
Beijing	34.05	10.10	30.36	136.9	38.49	3.65	23.07
Rongcheng	16.38	8.66	29.75	181.5	68.63	3.28	43.46
Helongjiang	46.91	25.35	27.66	191.5	83.30	3.63	39.77

[a] RSD less than 10% for six measurements

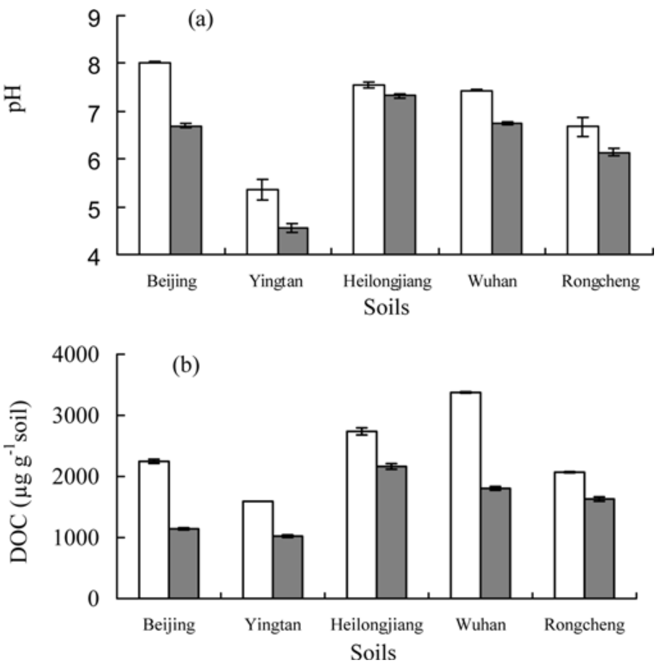

Fig. 1 Effects of earthworm activity on soil pH (**a**) and dissolved organic carbon (DOC; **b**). *White column* Earthworms, *black column* no earthworms; *bars* represent standard errors

Effect of earthworms on DOC

The increase in DOC can lead to high desorption of rare earth elements and Cr (Jones 1997). Therefore, the effect of earthworms on DOC was measured in this study. Earthworm activity significantly (P <0.1) enhanced DOC to different extents in the five soils (Fig. 1b). DOC increased by 1.97, 1.56, 1.26, 1.79, and 1.27 times in Beijing, Yingtan, Heilongjiang, Wuhan, and Rongcheng soils, respectively, in the presence of earthworms.

Effect of earthworm activity on water-soluble heavy metals

The bioavailability, and hence potential toxicity, of trace metal ions in soils can depend on metal concentrations in the soil solution (McLaren et al. 1998). Therefore, a comparison of metal concentrations in the water-soluble fraction was made between soils with and without earthworms (Fig. 2). Treatment of all five soils by earthworms significantly (P <0.05) increased the water-soluble Cr, Co, Ni, Zn, Cu, Cd, and Pb by 1.3–2.7, 1.7–3.8, 1.3–2.5, 1.4–3.5, 2.1–4.3, 1.34–2.12, and 1.7–4.5 times, respectively. Among the soils studied, the increase in the concentrations of water-soluble heavy metals due to earthworm activity was the greatest in Beijing soil, and the lowest in the Heilongjiang soil.

Generally, the increased soil pH due to earthworm activity would enhance the affinity of soils for heavy metals due to pH-dependent surface-charge density on colloids, leading to lower concentrations of heavy metals in soil solution (Cao et al. 2001; Shan et al. 2002). However, this phenomenon was not observed in our experiments. In view of the pH increase of the soils and the elevation of water-soluble heavy metals, one may deduce that there must be some heavy-metal-chelating metallophores produced by earthworms.

Effect of earthworms on the metal fraction distribution

The soils with and without earthworms were subjected to BCR sequential extraction and the distributions of heavy metals among B1, B2, and B3 fractions were compared in order to investigate the changes in the binding site of heavy metals. This could help investigate the basic mechanisms of how earthworms affect heavy metal behaviors in soils. For the five soils studied, heavy metals in the water soluble, exchangeable and carbonate bound fraction (B1) increased ($P<0.1$) by factors of 4.2–5.7, 2.2–4.5, 1.3–2.8, 2.3–3.9, 3.3–7.2, 1.1–7.1, and 4.5–6.6 for Cr, Co, Ni, Zn, Cu, Cd, and Pb, respectively, after earthworm treatment. Since it has been shown that B1 represents the most bioavailable form of heavy metals to plants (Zhang et al. 1998; Wang et al. 2002), we can hypothesize that earthworms increased bioavailability in our soils.

Cheng and Wong (2002) showed that the presence of earthworms increased organic bound Zn. They regarded the organic-associated metals to be readily available to plants because organic components are easily decomposed by soil microorganisms with the consequent release of the metal into solution. However, no significant correlation was observed between heavy metal contents of plants and the metal content of the B3 fraction (Wang et al. 2002). In our study, organic matter and the sulfide-bound fraction (B3) of Cr, Co, Ni, Zn, Cu, Cd, and Pb were decreased significantly ($P<0.1$) by 10–15%, 38–54%, 32–52%, 32–43%, 31–41%, 15–25% and 19–21%, respectively, when the treatments with and without earthworms were compared. Metal concentrations of the B2 fraction slightly decreased in the presence of earthworms. However, this decrease was not significant ($P>0.1$). Similar trends were observed in all five soils and all metals studied, and thus we only reported the effects of earthworm activity on the distribution of heavy metals among B1, B2, and B3 fractions of the Beijing soil in Fig. 3. The increase in heavy metal concentrations in B1 and the decrease in B3 suggested a change in heavy metal speciation.

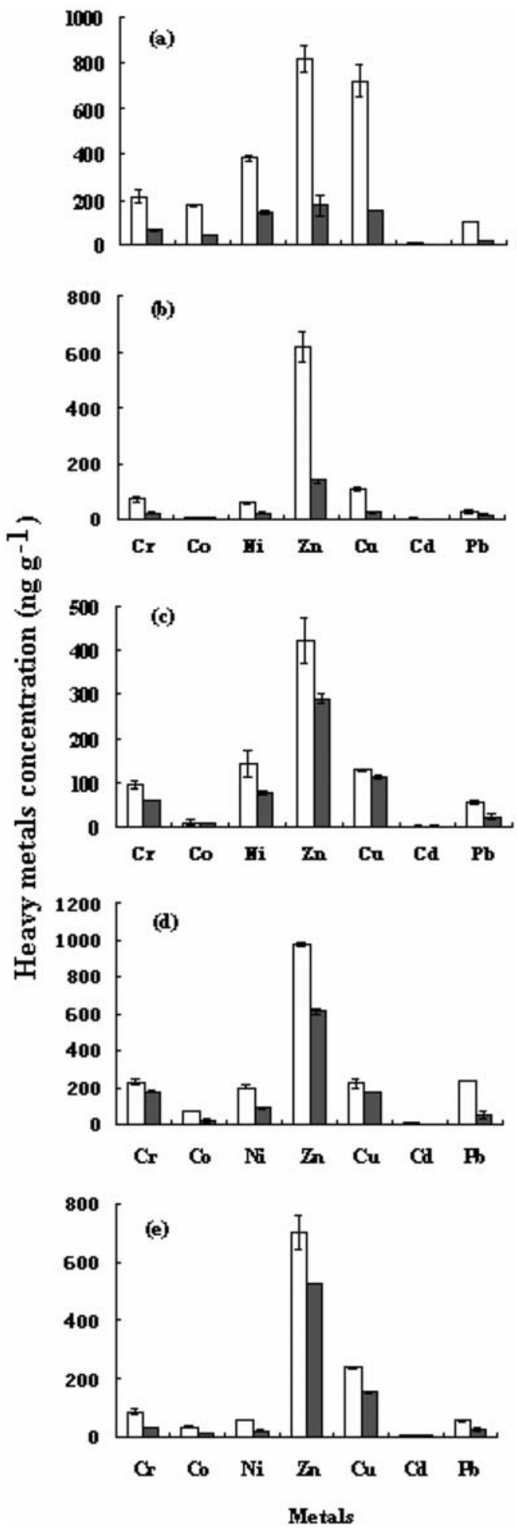

Fig. 2a–e Effect of earthworm activity on the concentration of water-soluble heavy metals. *White column* Earthworms, *black column* no earthworms; *bars* represent standard errors. **a** Beijing soil; **b** Yingtan soil; **c** Heilongjiang soil; **d** Rongcheng soil; **e** Wuhan soil

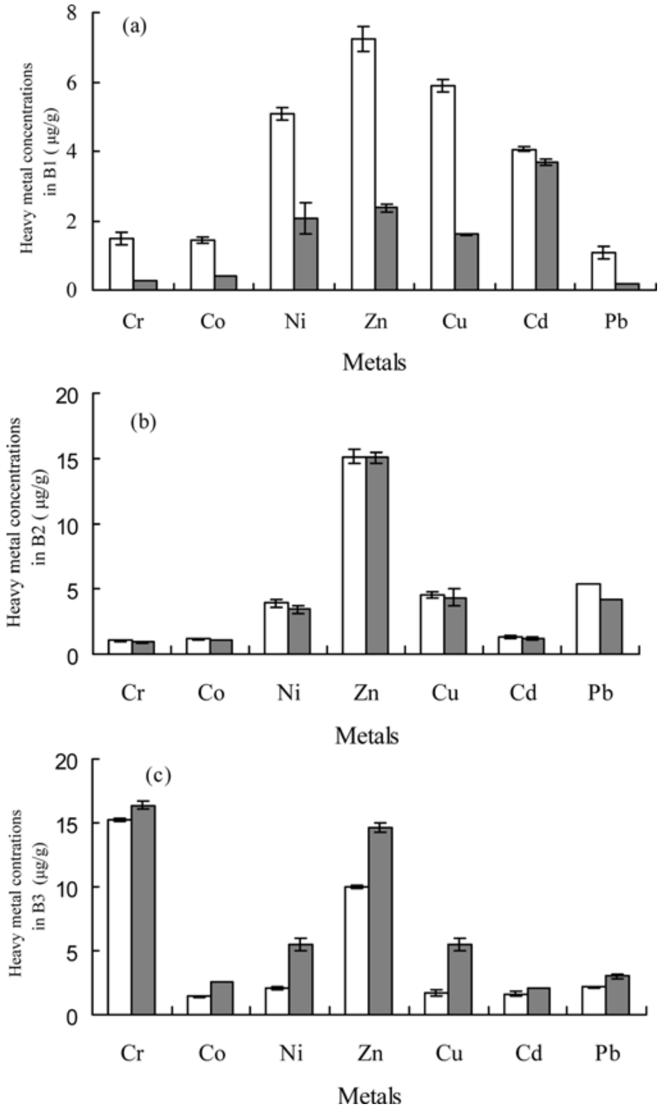

Fig. 3 Effect of earthworms on the distribution of heavy metals in the B1 (**a**), B2 (**b**), and B3 fractions (**c**) of Beijing soil (*white column* earthworms, *black column* no earthworms; *bars* represent standard errors)

Effect of earthworms on soil microbial populations

A comparison of microbial populations of the Beijing soil between the treatments with and without earthworms is shown in Table 3. Significant increases ($P<0.05$) in bacteria, actinomycetes, and fungus populations were found in the presence of the earthworm.

The effects of earthworms on the composition and activity of soil microbes have been well documented (Binet et al. 1998; Toyota and Kimura 2000). Whiting et al. (2001) investigated the effectiveness of microbes on the mobilization of Zn and deduced that an increase in the solubility of Zn in soil may be due to compounds such as Zn-chelating metallophores produced by the microbes. Neilands and Leong (1986) also reported that metallophores are commonly produced by strains of *Pseudomonas* and *Enterobacter*, which often exist in soils. Therefore, we speculated that earthworm activity could increase heavy metal bioavailability through its effect on microbial activity.

Table 3 Effect of earthworm activity on the microbial population of Beijing soil (cfu g^{-1})[a]

	Earthworms	No earthworms
Bacteria	$(4.3\pm0.9)\times10^{8}$	$(4.0\pm1.3)\times10^{7}$
Actinomycetes	$(3.3\pm1.1)\times10^{6}$	$(1.3\pm0.2)\times10^{5}$
Fungus	$(1.0\pm0.3)\times10^{6}$	$(8.3\pm1.6)\times10^{4}$

[a] Mean ± standard error ($n=9$)

Table 4 Correlation coefficients between the water-soluble $\text{metals}_{\text{earthworms}}$/water-soluble $\text{metals}_{\text{no earthworms}}$ ratio (B) and the $\text{DOC}_{\text{earthworms}}/\text{DOC}_{\text{without earthworms}}$ ratio (C) for the five soils

Element	Regression equation	Correlation coefficient
Cr	$B=1.76\,C-0.80$	0.99**
Co	$B=2.65\,C-1.87$	0.91*
Ni	$B=1.61\,C-0.57$	0.98**
Zn	$B=2.23\,C-1.15$	0.91*
Cu	$B=2.68\,C-1.40$	0.89*
Cd	$B=0.16\,C-0.93$	0.92*
Pb	$B=3.38\,C-2.10$	0.90*

* Significant at 0.05 probability level
** Significant at 0.01 probability level

DOC and metals in the water-soluble fraction as affected by the earthworm

The comparison between the DOC and metal contents of the water-soluble fraction can give an idea of the effect of dissolved organic C on the solubility of heavy metals. To prove the role of earthworms we calculated the respective ratio values with and without earthworms. In the case of Cr, the ratio values of $\text{DOC}_{\text{earthworms}}/\text{DOC}_{\text{no earthworms}}$ were 1.97, 1.56, 1.26, 1.79, and 1.27 for Beijing, Yingtan, Heilongjiang, Wuhan, and Rongcheng soils, respectively (Fig. 1b), while the ratios of water-soluble $\text{Cr}_{\text{earthworms}}/\text{Cr}_{\text{no earthworms}}$ were 2.72, 1.90, 1.57, 2.32, and 1.33, respectively (Fig. 2). Significant correlation ($r=0.99$, $P<0.01$) was found between the $\text{DOC}_{\text{earthworms}}/\text{DOC}_{\text{no earthworms}}$ ratio values and the water-soluble $\text{Cr}_{\text{earthworms}}$/water-soluble $\text{Cr}_{\text{no earthworms}}$ ratio values. Similar results were obtained when the other heavy metals were considered (Table 4; $P<0.05$).

Correlations were described by the following regression equation:

$$B=KC+a$$

where B is the water-soluble $\text{metals}_{\text{earthworms}}$/water-soluble

Table 5 Mean and range of heavy metals in wheat grown in the five soils with and without earthworm treatments

Element	Concentration of heavy metals in roots and shoots ($\mu g\ g^{-1}$)							
	With earthworms (in roots)		Without earthworms (in roots)		With earthworms (in shoots)		Without earthworms (in shoots)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Cd	8.68	6.44–11.0	3.93	2.77–4.95	0.32	0.22–0.47	0.21	0.16–0.29
Co	6.98	3.21–10.6	3.98	1.07–7.26	0.59	0.32–0.81	0.25	0.20–0.29
Cr	19.33	16.01–23. 6	11.0	8.98–14.1	1.91	0.99–3.67	0.54	0.28–1.09
Cu	44.4	25.46–66.1	33.9	15.93–45.0	20.1	9.07–41.5	8.00	5.00–14.8
Ni	9.89	7.68–11.9	5.46	3.01–7.68	6.97	1.97–11.9	4.54	1.23–7.68
Pb	8.83	5.18–12.2	2.94	2.33–7.68	0.97	0.50–1.29	0.53	0.23–0.70
Zn	138.6	80.1–190.6	76.9	47.8–120.8	26.9	6.39–44.5	19.9	7.30–31.5

$metals_{no\ earthworms}$ ratio, C is the $DOC_{earthworms}/DOC_{no\ earthworms}$ ratio, and K and a are constants. All the correlation coefficients were higher than 0.89 (Table 4).

Jones (1997) suggested that DOC may bind Cr, thus promoting the desorption of Cr. Indeed, various components of the DOC phase can bind heavy metals and affect the heavy metal sorption-desorption equilibrium. We have shown that the DOC increase by earthworms may contribute to an increase in the metal concentrations of the water-soluble fractions.

Effects of earthworms on wheat biomass and metal accumulation in wheat roots and shoots

The effect of earthworms on the total dry weight of wheat shoots and roots is shown in Fig. 4. The results indicated that earthworm treatments significantly ($P<0.1$) increased the yield of wheat shoots and roots by 1.5–1.9 and 1.2–1.5 times, respectively, in the five soils.

The mean and range of the concentrations of heavy metals in wheat roots and shoots with and without earthworms are given in Table 5. The heavy metal content of roots increased in the presence of earthworms by 40%, 60%, 50%, 30%, 50%, 100%, and 120% for Cr, Co, Ni, Zn, Cu, Cd, and Pb, respectively, in respect to the values without earthworms. Significant increases were also found for the Zn, Cu, and Cr concentrations ($P<0.05$), and for the Ni and Pb concentrations ($P<0.1$) of wheat shoots. No significant differences were observed for the Co and Cd contents of wheat shoots as compared to the controls ($P>0.1$). Earthworm treatments increase heavy metal contents of wheat to a different extent for different soil types (data not shown).

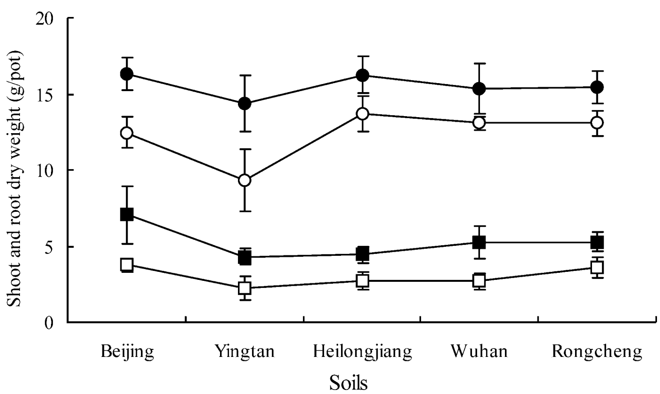

Fig. 4 Effect of earthworms on wheat dry weights (*filled circle* shoots with earthworms, *open circle* shoot without earthworms, *filled square* root with earthworms, *open square* roots without earthworms; *bars* represent standard errors)

Conclusion

DOC in soils is likely to be increased by earthworms through: (1) decomposition of organic matter into smaller and water-soluble components; (2) increase of microbial activity with release of microbial metabolites by increasing mobility of metals in soils. Thus earthworms could potentially be used in phytoremediation and phytomining of metals from soils. Of course the earthworm species used should tolerate high concentrations of heavy metals in soil and should be capable of living in soil

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