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RESEARCH ARTICLE

The effect of the earthworm *Lumbricus rubellus* on the bioavailability of cadmium and lead to the springtail *Folsomia candida* in metal-polluted field soils

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Abstract

The bioavailability of metals can be influenced not only by soil properties but also by other species living at polluted sites. However, in laboratory experiments, usually only one test species is used to estimate bioavailability. In this study, a two-species approach was applied to assess the impact of the earthworm *Lumbricus rubellus* on the bioavailability of cadmium and lead to the springtail *Folsomia candida* using natural soils from a gradient of metal pollution. Earthworms were kept in half of the soil replicates for 4 weeks. Subsequently, the uptake and elimination kinetics of cadmium and lead in *F. candida* exposed for 21 days to the soils was determined. Earthworm activity affected soil properties but did not significantly affect metal uptake rate constants in springtails. The slightly higher uptake due to the presence of earthworms, which was consistent in all tested soils and for both metals, suggests that further research is needed on the role of species interactions in affecting metal bioavailability in soil.

Keywords Metal bioavailability · Earthworms · Collembola · Toxicokinetics · Field soil · Soil properties

Introduction

Earthworms are considered ecosystem engineers as their activities, like burrowing, casting and bioturbation, have important effects on soil processes and properties (Jones et al. 1994). They influence among other soil formation, soil structure, nutrient cycling, soil aeration and water dynamics (Blouin et al. 2013). This impact has been a subject of many studies (Zhang and Schrader 1993; Hale et al. 2005; Hedde et al. 2013) including the impact on pollution remediation (Li et al. 2018). Earthworms can survive and reproduce in soils highly polluted with metals (Andre et al. 2010; Giska et al. 2014). Their activity may modify the mobility of metals in contaminated soils and their availability to other organisms (Sizmur and Hodson 2009).

Masoud M. Ardestani and Iwona Giska contributed equally to this work.

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All three recognized ecological types of earthworms, epigeic, anecic and endogeic, have been shown to increase the mobility and bioavailability of metals in soils. The epigeic earthworm *Eisenia fetida* increased the bioavailability of lead measured with Ruby's physiologically based extraction test (Udovic and Lestan 2007). The anecic species *Lumbricus terrestris* was found to increase the uptake of metals (Cu, Pb, Zn) by maize and barley (Ruiz et al. 2011) and ryegrass (Sizmur et al. 2011). The endogeic earthworm *Aporrectodea tuberculata* significantly enhanced the bioaccumulation of metals (Cd, Cu, Zn) by the snail *Helix aspersa* (Coeurdassier et al. 2007). The most often suggested mechanisms by which earthworms influence the bioavailability of metals include alteration of soil pH and increase of soil dissolved organic carbon (DOC) (Wen et al. 2004). Although several studies investigated the impact of earthworms on the bioavailability of metals using plant and animal bioassays, these kinds of experiments are rather sparse, especially regarding animal bioassays.

One of the best methods to study metal bioavailability to soil organisms is the toxicokinetics approach (Peijnenburg et al. 1999). In kinetics studies, chemical uptake and metabolism are integrated over the duration of the exposure time (Lanno et al. 2004). Metal bioaccumulation (uptake phase) and its elimination from the organism (elimination phase) are related to the availability of the metal from the surrounding environment considering the physical-chemical characteristics of the medium. Reaching steady-state tissue concentrations and the rate constants for both phases are important in the process of evaluating metal bioavailability (Van Straalen et al. 2005). For non-essential metals, such as cadmium and lead, increased exposure concentrations generally result in higher metal accumulation in an organism. However, this pattern may be species- and metal-specific (Ardestani et al. 2014).

There are many studies in the literature in which the effects of soil properties have been investigated on metal kinetics in one soil organism, e.g. earthworms and collembolans (Peijnenburg et al. 1999; Spurgeon and Hopkin 1999; Vijver et al. 2001; Ardestani and van Gestel., 2013; Giska et al. 2014). However, so far, no studies investigated the impact of one soil invertebrate species on the uptake and elimination kinetics of metals in another invertebrate species. Therefore, the aim of the present study was to assess the influence of the earthworm *L. rubellus* on the bioavailability of cadmium and lead to the springtail *Folsomia candida* using a toxicokinetics approach. Springtails were exposed to three pine forest soils collected along a gradient of metal pollution in Southern Poland with or without earthworms introduced. We hypothesized that the earlier presence of earthworms in soil would alter metal bioavailability to the collembolans and this impact would be visible from the metal uptake kinetics parameters.

Materials and methods

Test soils

Natural polluted soils were collected from three sites (OL1, OL2, OL5; collectively called Olkusz soils) in a pine forest along a metal pollution gradient near Olkusz in Southern Poland (approximately 50° 16′–50° 19′N, 19° 29′–19° 32′E). Sampling of soils has been described by Giska et al. (2014). Soil samples were air-dried, sieved (2 mm mesh) and homogenized prior to the experiment, and soil moisture content was adjusted to 50% of the maximum water holding capacity (WHC). The soils were acidic and rich in organic matter (Table 1).

Test animals

L. rubellus were obtained from a commercial earthworm supplier who collected them from an unpolluted site in The Netherlands (Nijkerkerveen). The collembolan species *F. candida* (Isotomiidae) were cultured on a moist substrate of plaster of Paris with charcoal. The animals were from the parthenogenetic 'Berlin strain' which had been maintained at the Vrije Universiteit, Amsterdam, for more than 10 years. Animals were fed twice a week with dry baker's yeast granules and kept in climate chamber for synchronization at 20 °C, 75% relative humidity (RH) and 12:12 h light:dark regime. For the main experiment, 20-day (± 2) age-synchronized animals were used.

Table 1 Physicochemical properties of the test soils used in the experiment. The values are mean ± standard deviation (SD; $n = 3$ for pH and organic matter content (OM), and $n = 2$ for cation exchange capacity (CEC)). Part of the data was taken from Giska et al. (2014)

	Soil characteristics			Total metal concentration (mg kg^{-1})	
Test soil	pH_{H2O}	OM (%)	CEC ($cmol_c$ kg^{-1})	Cd	Pb
OL1	5.49	45.1 ± 1.3	37.7 ± 0.0	63.2 ± 3.0	3041 ± 158
OL2	4.78	53.5 ± 0.4	34.4 ± 0.3	49.1 ± 1.1	2060 ± 37
OL5	4.83	36.3 ± 0.7	26.3 ± 0.6	12.1 ± 0.7	708 ± 12

Test design

Two clitellate *L. rubellus* (total biomass ~ 2 g) were introduced to half of the soil replicates, consisting of 200 g dry weight placed in a 600 mL glass jar. Since the test soils had high organic matter contents (on average 50%), the bulk density was low. Therefore, the density of two earthworms in each test container was higher than their natural abundance in the field (see e.g. Klok 2007). In total, eighteen (3 soils × 2 treatments × 3 replicates each) replicates were used. All replicates, with and without earthworms (treatments), were kept at the same conditions in the climate chamber at 16 °C, 75% RH and 16:8 h light:dark regime. After 4 weeks, the earthworms were removed and mortality was checked. Each soil replicate in which both earthworms survived was divided into seven replicates for the toxicokinetics experiment with collembolans, each replicate being used for a single sampling time. This resulted in three replicates for each time point (Fig. S1, Supporting Information). Five age-synchronized *F. candida* were introduced into these replicates. Each replicate consisted of 7 g dry weight of soil equivalent in a 100-mL glass jar. A few small grains of dried baker's yeast were added at the beginning of the test as food for the collembolans. Uptake kinetics of cadmium and lead in *F. candida* was assessed during 21 days of exposure to the test soils. The tests were kept in a climate chamber at 20 °C, 12:12 h light:dark and 75% RH. Metal uptake was determined by sampling one or two springtails per replicate for metal analysis at days 0, 1, 2, 4, 7, 10, 15 and 21. Initial metal concentration measured in *F. candida* at the beginning of the test was used as C_0 in the kinetics modelling (Eq. 1).

Soil and animal analyses

Prior to the experiment, pH_{H2O}, organic matter content (OM), cation exchange capacity (CEC), and total metal concentrations in the test soils were measured (Table 1). Before the toxicokinetics experiment, after removal of the earthworms, we measured the following characteristics of the soils incubated with and without earthworms: 0.01 M $CaCl_2$ extractable and porewater metal concentrations, pH_{H2O} and DOC. The analytical methods have been described earlier (Dohrmann 2006; Diez-Ortiz et al. 2010; Ardestani and van Gestel 2013; Giska et al. 2014). Total metal concentrations in the test soils were measured using a flame atomic absorption spectrophotometer (F-AAS, Perkin Elmer, AAnalyst 100), those in extracts and porewater samples by graphite furnace AAS (GF-AAS, Perkin Elmer, 5100 PC).

After freeze-drying and weighing (dry weight) the springtails individually, the animals were digested with a 7:1 mixture of ultra-pure nitric acid (HNO_3 Ultrex II, J.T. Baker, 69%) and perchloric acid ($HClO_4$ Ultrex II, J.T. Baker, 70%). Internal metal concentrations were subsequently measured using GF-AAS.

Data analysis and modelling

Metal uptake and elimination rate constants were calculated by fitting a one-compartment kinetics model to the data (Atkins 1969; Skip et al. 2014). We performed nonlinear regression fitting of the following equation:

$$C_{\text{body}} = C_0 * e^{(-k_2 * t)} + \frac{k_1}{k_2} * C_{exp} * \left(1 - e^{(-k_2 * t)}\right) \quad (1)$$

where:

C_{body}, internal metal concentration in the springtails at sampling time t (μg g^{-1});

C_0, initial metal concentration in the springtails at time t_0 (μg g^{-1});

k_1, uptake rate constant (g_{soil} g_{animal}^{-1} day^{-1});

k_2, elimination rate constant (day^{-1});

C_{exp}, exposure concentration (μg g^{-1});

t, time (day).

Kinetics parameters were calculated based on total and $CaCl_2$ extractable concentrations in soil and porewater concentrations of the two metals analysed. The significance of differences in kinetics parameters (k_1, k_2) between treatments with and without earthworms was assessed with the likelihood ratio test (Sokal and Rohlf 1995). A *t* test was used to compare the effects of earthworms on soil properties (pH and DOC). All analyses were run in IBM SPSS 24.0.

Bioaccumulation factors (BAF) were calculated as the ratio of k_1 and k_2 values obtained from the fitting of the kinetics model to the metal concentrations in the springtails related to total or 0.01 M $CaCl_2$ extractable concentrations in soil. Bioconcentration factors (BCF) were calculated from k_1 and k_2 values based on porewater concentrations.

Results

Impact of earthworms on soil characteristics

Earthworm presence did increase soil pH_{CaCl2} and dissolved organic carbon content, which was significant (*t* test, $p < 0.05$) except for DOC in soil OL2 (Table 2). The 0.01 M $CaCl_2$ extractable and porewater metal concentrations did not show a consistent pattern of changes due to the introduction of *L. rubellus* to the soils (Table 2).

Impact of earthworms on metal bioavailability

For cadmium, average uptake rate constants based on total soil concentrations ranged between 0.015 and 0.061 g_{soil} g_{animal}^{-1} day^{-1} the for different test soils and were always slightly but not significantly higher in the treatments that contained earthworms

Table 2 The effect of the earthworm *Lumbricus rubellus* on physicochemical properties of the test soils used in the toxicokinetics experiment with *Folsomia candida*: pH_{CaCl2} (pH measured in 0.01 M $CaCl_2$); DOC, dissolved organic carbon. Treatments are the test soils incubated with or without earthworms for 4 weeks before the start of the toxicokinetics experiment. The values are means ± standard deviations (SD; $n = 3$)

				0.01 M $CaCl_2$ extractable concentration (mg kg^{-1})		Porewater concentration (μg L^{-1})	
Soil	Treatment	pH_{CaCl2}	DOC (mg L^{-1})	Cd	Pb	Cd	Pb
OL1	Without worms	5.38 ± 0.03 ^{A}	73.2 ± 9.0 ^{A}	0.89 ± 0.02	0.55 ± 0.01	24.8 ± 1.07	125 ± 11.5
	With worms	5.45 ± 0.01 ^{B}	115 ± 16 ^{B}	0.83 ± 0.01	0.55 ± 0.02	21.9 ± 0.81	196 ± 23.2
OL2	Without worms	4.12 ± 0.02 ^{A}	119 ± 15 ^{A}	3.69 ± 0.05	1.98 ± 0.05	81.0 ± 3.57	230 ± 19.4
	With worms	4.19 ± 0.01 ^{B}	155 ± 37 ^{A}	3.38 ± 0.04	1.22 ± 1.06	69.7 ± 4.86	276 ± 25.6
OL5	Without worms	4.40 ± 0.02 ^{A}	178 ± 4.8 ^{A}	0.69 ± 0.01	0.53 ± 0.02	13.0 ± 0.18	231 ± 15.7
	With worms	4.53 ± 0.01 ^{B}	303 ± 5.6 ^{B}	0.57 ± 0.02	0.33 ± 0.29	15.4 ± 1.02	299 ± 72.5

Values marked with the different capital letters (A or B) show significant differences between treatments (with/without earthworms) for each soil (*t* test, $p < 0.05$)

(Table 3). The highest uptake rate constants were observed for OL5 soil. Based on 0.01 M $CaCl_2$ extractable concentrations, average cadmium uptake rate constants varied between 0.327 and 1.52 $g_{soil}\ g_{animal}^{-1}\ day^{-1}$, with the lowest values observed for OL2 (Table S1, Supporting Information). The same trend was observed for the k_1 values based on porewater concentrations, which ranged from 5.34 to 25.7 L $g_{animal}^{-1}\ day^{-1}$. For 0.01 M $CaCl_2$ extractable and porewater concentrations, uptake rate constants were higher for the treatments with earthworms (Table S1). Internal cadmium concentrations in the springtails reached up to approximately 6 μg g^{-1} dry weight at the end of uptake phase (Fig. 1a, c, e). In the least polluted OL5 soil, concentrations in the springtails were lower than in the other test soils (Fig. 1e).

For lead, internal concentrations in the springtails reached approximately 100 μg g^{-1} dry weight after 21 days of exposure (Fig. 1b, d, f), with the values being much lower for the least polluted OL5 soil (Fig. 1f). The average uptake rate constants based on total soil concentrations were between 0.003 and 0.019 $g_{soil}\ g_{animal}^{-1}\ day^{-1}$ and higher for OL2 soil compared with the other test soils (Table 3). Again, higher k_1 values were calculated for the treatments which did contain earthworms. When related to 0.01 M $CaCl_2$ extractable concentrations, average lead uptake rate constants ranged between 5.64 and 15.2 $g_{soil}\ g_{animal}^{-1}\ day^{-1}$ for treatments without earthworms and between 16.5 and 41.6 $g_{soil}\ g_{animal}^{-1}\ day^{-1}$ with earthworms (Table S2, Supporting Information). Based on porewater

Table 3 The effect of the earthworm *Lumbricus rubellus* on the kinetics parameters describing the uptake of cadmium and lead in *Folsomia candida* related to the total soil concentrations: uptake rate constant (k_1) and elimination rate constant (k_2) in *F. candida* exposed to polluted field soils for 21 days in soils previously incubated with or without earthworms. Kinetics parameters were estimated by fitting of the one-compartment model (Eq. 1). The values in parentheses are 95% confidence intervals. Bioaccumulation factors (BAF) estimated from the ratio of k_1 and k_2

Metal	Test soil	Treatment	k_1 ($g_{soil}\ g_{animal}^{-1}\ day^{-1}$)	k_2 (day^{-1})	BAF	R^2
Cd	OL1	Without worm	0.015 (0.003–0.027)^{aA}	0.150 (− 0.025–0.326)	0.10	0.54
		With worm	0.021 (0.003–0.040)^{aA}	0.208 (− 0.028–0.444)	0.10	0.54
	OL2	Without worm	0.026 (0.004–0.049)^{aA}	0.223 (− 0.029–0.474)	0.12	0.37
		With worm	0.035 (0.007–0.063)^{aA}	0.273 (0.001–0.545)	0.13	0.43
	OL5	Without worm	0.040 (0.010–0.069)^{aA}	0.201 (0.013–0.388)	0.20	0.65
		With worm	0.061 (− 0.014–0.136)^{aA}	0.249 (− 0.129–0.627)	0.25	0.31
Pb	OL1	Without worm	0.003 (− 0.002–0.007)^{aA}	0.085 (− 0.203–0.372)	0.04	0.22
		With worm	0.008 (− 0.008–0.023)^{aA}	0.257 (− 0.300–0.912)	0.03	0.17
	OL2	Without worm	0.005 (− 0.007–0.017)^{aA}	0.220 (− 0.452–0.892)	0.02	0.08
		With worm	0.019 (− 0.014–0.051)^{aA}	0.424 (− 0.433–1.281)	0.05	0.14
	OL5	Without worm	0.004 (− 0.007–0.015)^{aA}	0.124 (− 0.391–0.640)	0.03	0.12
		With worm	0.011 (− 0.008–0.030)^{aA}	0.272 (− 0.298–0.843)	0.04	0.12

[a] Values marked with the same lower case letters show no significant differences between soils for each metal (likelihood ratio test, $p < 0.05$)

[A] Values marked with the same capital letters show no significant differences between treatments (with/without worms) for each metal (likelihood ratio test, $p < 0.05$)

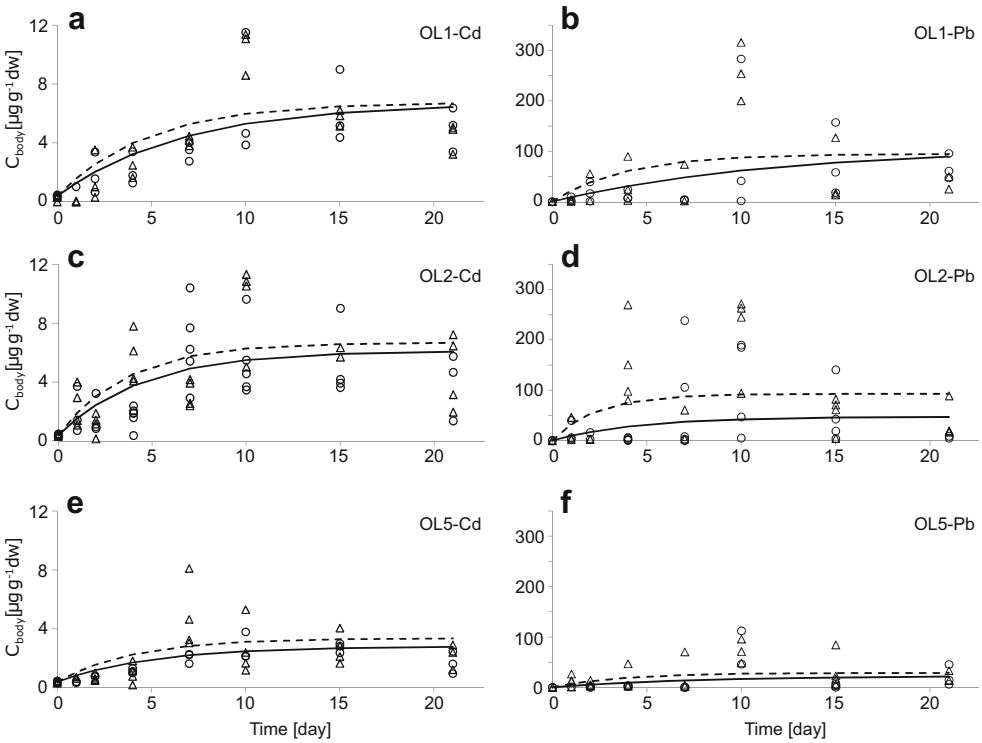

Fig. 1 Uptake kinetics of cadmium (**a**, **c**, **e**) and lead (**b**, **d**, **f**) in *Folsomia candida* exposed to three natural soils (OL1, OL2, OL5) from a gradient of metal pollution. Circles represent measured internal metal concentration in the treatments without earthworms and triangles treatments with earthworms. Curves were estimated according to the one-compartment kinetics model based on total metal concentrations in the soils (Eq. 1). Solid curves represent treatments without earthworms, dashed curves treatments with earthworms. Estimated kinetics parameters are shown in Table 3

concentrations, average lead uptake rate constants ranged from 6.80 to 30.1 L g_{animal}^{-1} day^{-1} without earthworms and from 14.4 to 52.1 L g_{animal}^{-1} day^{-1} with earthworms.

Elimination rate constants (calculated based on the uptake phase) for cadmium ranged between 0.150 and 0.273 day^{-1} and for lead between 0.085 and 0.424 day^{-1} (Table 3, Tables S1 and S2). In all cases, elimination rate constants showed large variation, making it impossible to draw conclusions on differences between soils and between treatments with and without earthworms.

BAF ranged from 0.10 to 0.25 for cadmium and from 0.02 to 0.05 for lead based on total soil concentrations in different soils (Table 3). The BCF varied between 23.9 and 105 for cadmium and between 52.9 and 358 for lead (Tables S1 and S2).

Discussion

Effect of earthworms on soil pH and DOC

Earthworm activity increased soil pH and DOC content (Table 2). Soil pH increase was slight (0.07–0.13 units) but significant and consistent across all three soils. Numerous studies have already shown that earthworm presence increased soil pH, which is attributed to excretion of calcium compounds by calciferous glands, mucus secretion, and alkaline urine (Hu et al., 1998; Schrader, 1994; Salmon 2001; Cheng and Wong 2002; Ma et al. 2002). However, there are also studies reporting a decrease of soil pH by earthworms (e.g. McColl, 1982; Yu et al., 2005). As stated by Sizmur and Hodson (2009) this may mean that the alteration of soil pH is not the main mechanism by which earthworms affect metal bioavailability in soil. DOC content was a factor of 1.3–1.7 higher in soils incubated with *L. rubellus* with the largest change, similar to pH, in soil OL5 (Table 2). Comparable increases of DOC content have been reported due to the presence of *E. fetida* in Chinese cultivated soils (Wen et al. 2004). The higher DOC levels in the treatments with earthworms could be due to their role in the transport and breakdown of organic matter. This effect could be particularly relevant for the test soils used in our study, which were rich in organic matter.

Earthworm presence and bioavailability of metals

The results of this study showed that lead and cadmium uptake in the springtails slightly increased due to the presence of earthworms (Table 3, Fig. 1). Uptake rate constants were approximately 1.4–1.5 times higher for cadmium and 2.7–3.8 times higher for lead in the soils incubated with earthworms compared with earthworm-free soils (Table 3). Similarly, in the soils with earthworms introduced, elimination rate constants were 1.2–1.4 times higher for cadmium and 1.9–3.0 times higher for lead. However, differences between treatments were not significant, neither for the uptake rate constants nor for the elimination rate constants as both parameters generally had very wide confidence intervals. Similar conclusions can be drawn for the BAF and BCF values. Because of their activity, such as burrowing and bioturbation, earthworms may affect the availability of metals

in soil and subsequently increase metal bioavailability to other co-occurring soil organisms (Sizmur and Hodson 2009; Ruiz et al. 2011; Sizmur et al. 2011).

It should be noted that earthworm density in the present study was fairly high, with 2 g biomass per 200 g dry soil; this corresponds to a density of approximately 400 g m^{-2}. In optimum field conditions, a maximum biomass of 200–400 g m^{-2} may be found, which includes all earthworm species and all life stages present (Curry 1998; Lavelle and Spain 2001; Zorn et al. 2005). We however, used only adult earthworms of one (epigeic) species. As a consequence, earthworm activity may have been overestimated compared with that in natural field soils. This should be taken into account when interpreting the results of our study.

In our experiment, earthworms were kept in the soil for 4 weeks, and the clearly visible burrows evidenced they were active in all soils. However, the time of earthworms' presence could have been too short to have a significant effect on the bioavailability of metals. We used 4 weeks as this is the most common duration of soil ecotoxicological tests. Nevertheless, the effect we observed was consistent for both cadmium and lead in all three soils (see also Fig. S2, Supporting Information). This effect could be caused by the change of soil properties, especially increased DOC content as it reduces metal adsorption onto the soil solid phase. DOC competes for the free metal ions and forms soluble organo-metallic complexes, which however, may not be available for uptake by springtails. In addition, metals can be preferentially adsorbed onto the soil surfaces leading to increased metal concentrations in the soil solution. Antoniadis and Alloway (2002) showed that increased DOC concentrations increased extractability of metals from soil and their availability to plants.

It is well known that handling a soil may lead to major disturbances of chemical equilibriums, and often leads to an increased availability of chemicals. The handling of the soil (dividing over replicate smaller portions) before introduction of the springtails might have partly eliminated the effects of the earthworms.

Toxicokinetics of metals in *F. candida*

In earthworm-free soils, average uptake rate constants of 0.003–0.005 g_{soil} g_{animal}^{-1} day^{-1} for lead and 0.015–0.040 g_{soil} g_{animal}^{-1} day^{-1} for cadmium, based on total soil concentrations, were observed for *F. candida*. For lead uptake in *L. rubellus*, similar values were reported based on total soil concentrations (0.004 to 0.009 g_{soil} g_{animal}^{-1} day^{-1}) using the same field-contaminated soils (Giska et al. 2014) but higher values of 0.032–0.060 g_{soil} g_{animal}^{-1} day^{-1} were found for cadmium. The values reported by Giska et al. (2014) for cadmium are comparable with the cadmium uptake rate constants we observed in springtails kept in earthworm-treated soils: 0.021–0.061 g_{soil} g_{animal}^{-1} day^{-1}, especially for soil OL5. It could mean that earthworms in the experiment of Giska et al. (2014) made metals more available to themselves. However, this effect was observed only for cadmium but not for lead. Nahmani et al. (2009) reported uptake rate constants of 0.001–0.023 g_{soil} g_{animal}^{-1} day^{-1} for lead and 0.022–4.92 g_{soil} g_{animal}^{-1} day^{-1} for cadmium, based on total soil concentrations, in the earthworm *E. fetida* exposed to different field-contaminated soils. Ardestani and van Gestel (2013) reported uptake rate constants ranging from 0.33 to 0.99 g_{soil} g_{animal}^{-1} day^{-1} based on total cadmium concentrations, for *F. candida* exposed for 21 days in natural soils at different pH levels. Uptake rate constants for cadmium calculated based on 0.01 M $CaCl_2$ extractable concentrations (Table S1) are within the range of the values reported by Ardestani and van Gestel (2013) for *F. candida*; however, the values based on porewater concentrations are much higher in the present study. One of the factors affecting the bioavailability of metals is route along which soil organisms are taking up metals. Soil pore water is assumed to be the main route of exposure for soil organisms and the ventral tube and cuticle probably are the main pathways that collembolans use to take up metals from soil (Fountain and Hopkin 2001, 2005). Physiological processes inside the body may affect metal bioaccumulation as they may determine the extent to which organisms are able to store and excrete metals (Depledge and Rainbow 1990). These mechanisms usually involve the use of special proteins to bind metals, like metallothioneins (e.g., Stürzenbaum et al. 2004), which help control the rate of incoming elements. It should be noted that our uptake and elimination rate constants were obtained by fitting the one-compartment model to data only including an uptake phase, making especially the estimated elimination rate constants less reliable. A study on the internal sequestration of the metals in the animals and perhaps including some physiological endpoints to unravel such mechanisms could provide more information on the physiological aspects of lead and cadmium excretion/detoxification mechanisms in the test animals.

Conclusions

The earthworm *L. rubellus* influenced the physicochemical characteristics of metal-polluted field soils. Cadmium and lead uptake was slightly but not significantly higher in *F. candida* exposed to the soils in which earthworms were previously kept compared with soils without earthworms. Although the differences in kinetics parameters between treatments were not significant, the observed pattern was consistent but small for both metals in all three experimental soils.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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