Assessment of earthworm activity on Cu, Cd, Pb and Zn bioavailability in contaminated soils using biota to soil accumulation factor and DTPA extraction

Ling Xiao, Ming-Hui Li, Jun Dai, Mikael Motelica-Heino, Xu-Fei Chen, Jia-Long Wu, Lanfeng Zhao, Kexue Liu, Chi Zhang

Ecotoxicology and Environmental Safety xxx (xxxx) xxx-xxx

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: http://ees.elsevier.com

Assessment of earthworm activity on Cu, Cd, Pb and Zn bioavailability in contaminated soils using biota to soil accumulation factor and DTPA extraction

Ling Xiao[a,c,1], Ming-hui Li[a,1], Jun Dai[a,**], Mikael Motelica-Heino[c], Xu-fei Chen[a,b], Jia-Long Wu[a,b], Lanfeng Zhao[a], Kexue Liu[a], Chi Zhang[a,c,*]

[a] Key Laboratory of the Ministry of Agriculture for Arable Land Conservation in South China, Key Laboratory of Guangdong Province for Land Use and Consolidation, College of Natural Resources and Environment, South China Agricultural University, 510642, Guangzhou, China
[b] Land Development and Reserve Bureau of Guangdong Province, 510635, Guangzhou, China
[c] ISTO, UMR 7327, CNRS-Université D'Orléans, 41071, Orléans, France

ARTICLE INFO

Keywords
Earthworms
Heavy metals
Soil
Accumulation factors
Bioavailability
Soil properties

ABSTRACT

The study aims to investigate effect of earthworm activity on metal bioavailability in soils using their BSAF-metals. Based on a microcosmic laboratory experiment, epigeic species *Amynthas corticis (A. corticis)* and endogeic species *Amynthas robustus (A. robustus)* were cultured in two types of soils contaminated by Cd, Zn, Pb and Cu for 120 days. Earthworm characteristics (i.e. numbers, biomass and BSAF), soil properties (i.e. pH, organic C and N contents along with their components such as mineralization and microbial masses) and DTPA extracted metals in soil were determined. After the incubation, the biomass and survival numbers of both earthworm species decreased significantly ($P < 0.05$). The accumulation of Cd, Zn and Pb in earthworm tissues and BSAF-metals were earthworm species dependent. According to two-way ANOVA, BSAF-Pb clearly showed the effect of different species of earthworms while BSAF-Cu indicated an interactive effect of earthworms and soil type. Earthworms changed soil properties significantly, especially for mineralized C (C_{min}), dissolved N (N_{dis}) and pH ($P < 0.05$). Earthworm activity increase DTPA extracted Zn and Cu, and the effect of *A. robustus* were stronger than for *A. corticis*. Redundancy analysis (RDA) showed that BSAF-Cu and BSAF-Pb contributed for respectively 51.9% and 51.7% of soil properties and DTPA metal changes, indicating that the effects of BSAF-Cu and BSAF-Pb on soil properties and on metal bioavailability in soil were similar. BSAF-Cu, indicating the interactive effect of earthworms and soil, accounted for 38.5% and 45.1% of soil properties and soil metal bioavailability changes. BSAF-Pb, representing the effect of earthworm species, accounted for 13.3% and 6.6% of soil property and soil metal bioavailability variations. Stepwise regression indicated that earthworm might change soil properties through their activities and interactions with soil, and hence increase heavy metal bioavailability. It suggested that BSAF is an important indicator for evaluating the effect of earthworm activity on soil metal bioavailability and designing remediation strategies.

1. Introduction

Heavy metal pollution in soil has been a major concern all over the world. The bioavailability of heavy metals plays a vital role in their toxicity and accessibility to organisms (Xiao et al., 2017a, 2017b). Among the various chemical methods to evaluate heavy metal bioavailability in soils, diethylenetriaminepentaacetic acid (DTPA) extraction is an effective and widely used one (Zhang et al., 2016; Han et al., 2019). Hence, using DTPA extracted fraction to assess heavy metal bioavailability facilitates comparison with previous researches. Additionally bio-indicators such as earthworms have become increasingly popular and are now widely used to evaluate the sustainability of soil use (Fusaro et al., 2018) and heavy metal contamination due to the consistent relationships between heavy metal concentrations in earthworm tissues and soils (Suthar et al.,

* Corresponding author. College of Natural Resources and Environment, South China Agricultural University, Guangzhou, 510642, China.
** Corresponding author. College of Natural Resources and Environment, South China Agricultural University, Guangzhou, 510642, China.
E-mail addresses: Jundai@scau.edu.cn (J. Dai); zhangchi2012@scau.edu.cn (C. Zhang)

[1] Joint first authors, Ling Xiao and Minghui Li contribute equally to this work.

https://doi.org/10.1016/j.ecoenv.2020.110513
Received 10 Febraury 2020; Received in revised form 16 March 2020; Accepted 17 March 2020
Available online xxx
0147-6513/© 2020.

2008). The accumulation of heavy metals in earthworm tissues is in fact associated not only with the bioavailable fraction of heavy metals in soil but also with the survival rate, individual biomass and ecological category of earthworms as well as soil quality parameters (Suthar et al., 2008).

The bioavailability of heavy metals in soil is significantly associated to soil properties (i.e. pH, soil organic C and total N along with their components, such as microbial biomass C and N (C_{mic} and N_{mic}), dissolved organic C and N (C_{dis} and N_{dis}), mineralized C and N (C_{min} and N_{min}) and so forth.) (Li et al., 2009; Xiao et al., 2017a, 2017b). Nevertheless, these soil properties can be modified by the activity of earthworms (Gong et al., 2019). For instance it was reported that earthworm activities affected the formation of soil aggregates and hence influenced soil pH, organic C and N along with their components (Fonte et al., 2007; Gong et al., 2019). Under the stress of heavy metal contamination, earthworm activities could also promote N transformation in soils (Xu et al., 2018). Globally earthworms are powerful regulators of soil processes, participating in the maintenance of soil structure and regulation of several ecosystem services (food production, nutrient cycling, contaminants retention and climate regulation) (Liu et al., 2019). They also constitute a major component in soil C and N function through their feeding, burrowing and casting activities (Lavelle et al., 2016). Additionally they can modify heavy metal availability in soil (Zhang et al., 2016) as they are relatively efficient accumulators of both essential and non-essential metals (Wang et al., 2018; Yuvaraj et al., 2020). As the biochemical response of different earthworm species to soil contamination are quite different, they were often used as functional bio-indicators (Jeyanthi et al., 2016; Sinkakarimi et al., 2020). It was reported that biota to soil accumulation factor (BSAF) of earthworms highlights the different "response" strategies of earthworms in contaminated soils (Dai et al., 2004; De Vaufleury et al., 2013). It also reflects the different feeding, burrowing and casting behavior of earthworms in different ecological category (Suthar et al., 2008). Furthermore, it may indicate the distribution of heavy metals in different soil fractions (Dai et al., 2004). Earthworm has been proved to be able to change heavy metal bioavailability in soil, and thus have been frequently used for soil heavy metal remediation (Zhang et al., 2016). Due to different earthworm species, soil types as well as metallic elements, results differed to some extents according to the various studies. For instance, Wen et al. (2004) and Lemtiri et al. (2016) found that addition of earthworms increased soil heavy metal bioavailability, while Li et al. (2019a, 2019b) and reported addition of earthworms reduced it. The relationship between the accumulation of heavy metals in earthworms and heavy metal bioavailability in soil could gain a better insight in the role of internal drives of earthworms in relation to the fate of heavy metals in soil (Suthar et al., 2008). It was found that bioavailable heavy metals were positively correlated with BSAF heavy metals in earthworms (Dai et al., 2004; Wang et al., 2020). However, a negative association between heavy metal bioavailability and accumulation of heavy metals in earthworm tissues was also found by Kavehei et al. (2018). Thus, there is still an uncertainty whether BSAF-heavy metals in earthworm tissues are true indicators of heavy metal bioavailability in contaminated soil. The possibility to use BSAF-heavy metals of earthworms together with biological indicators such as earthworm survival, growth and health has thus to be established. In particular, the association between BSAF-heavy metals of earthworms and soil properties, as well as their effect on heavy metal bioavailability in soil has not been clearly figured out.

The overall goal of this research was to investigate effects of earthworm activity on heavy metal bioavailability in soils using their BSAF-heavy metals. Based upon the above rationale, a microcosmic laboratory experiment was set up with two species of earthworms (i.e. *Amynthas corticis* (*A. corticis*) and *Amynthas robustus (A. robustus)*) and for two types of soils (i.e. based on different contamination levels and soil properties) aiming to 1) evaluate the contribution of BSAF-heavy metals of earthworms to soil properties (pH, soil C and N along with their components); 2) explain the contribution of BSAF-heavy metals of earthworms to heavy metal bioavailability in soil assessed with DTPA extracted heavy metals; 3) assess the association between BSAF-heavy metals of earthworms and soil properties along with their effect on soil heavy metal bioavailability. This study is beneficial to the assessment of soil contamination by using earthworms as bio-indicators and hence can improve future soil heavy metal remediation based on earthworm applications.

2. Materials and methods

2.1. Samplings

The study sites are located close to Dabaoshan mine (24° 30′ N and 113° 45′ E, Guangdong province, South China) which has been operated for 40 years and along Yanghe River that has been seriously polluted by acid mine drainage. Farmlands around Yanghe River have been severely contaminated since it was the main water resource for irrigation. Soil samples were collected from agricultural fields in the upstream (Site A) and downstream (Site B), which were 3 km and 10 km respectively away from the mine cluster. Three soil samples (2 × 2 m) of the surface horizons (0–20 cm) from three paddy rice fields (Spacing is 10 m) were collected at each site. The main physico-chemical properties along with the total and DTPA extraction of metal contents in the soil samples are shown in Supplementary materials (STable1 and STable 2). Generally, soils in both site A and B are quite acid. Soil pH values, organic C and total N contents in site A were significantly higher ($P < 0.05$) than those in site B, while cation exchange capacity (CEC), clay content, and saturated water were much lower than those in site B ($P < 0.05$). Iron and manganese contents for site A were 3.49 and 1.40 times higher than those in site B ($P < 0.05$). Total contents of Zn, Cd, Pb and Cu in site A were 1.10, 2.31, 1.25 and 1.75 folds as those in site B, respectively. Similarly DTPA extraction contents of Zn, Cd, Pb and Cu in site A were 1.07, 2.10, 1.25 and 0.94 times as those in site B ($P < 0.05$), respectively.

A. corticis (C) and *A. robustus* (R) were collected by hand from the uncontaminated fields in Qingyuan, Guangdong Province, Southern China. The soil are well managed and uncontaminated according to our investigation(STable3). According to their physiological characteristics, the earthworm specimens were sent to South China Botanical Garden (Chinese Academy of Sciences) for their accurate identification. Initial heavy metal contents in earthworm tissues is given in STable 3. Generally, there was no significant difference in Cu, Pb and Zn accumulation between these two species of earthworms. However, probably due to higher individual biomass of *A. robustus,* Cd content in *A. robustus* was significantly higher than that in *A. corticis* ($P < 0.01$). Adult earthworms with clitellate were selected and pre-cultured in the soil at 25 °C for one month. In each pot, 8 earthworms of the same species were introduced in 2 kg soil. Average fresh weights of *A. corticis* and *A. robustus* were 0.67 g and 2.02 g, respectively.

The rice straws sampled from the teaching farms of South China Agricultural University were cut into 2 cm segments, soaked into water for one week to remove tannins along with other harmful substances, and applied to pots to feed the earthworms. Organic C and total N contents along with C: N ratio of rice straws was 61.2%, 1.24% and 49.3:1, respectively. Initial heavy metal concentration rice straw are shown in Supplementary (STable 3).

2.2. Experimental design

2 kg of soil with field moisture was placed in 2 L pots (10 × 18 × 15 cm). Orthogonal experiments were designed based on soil type and earthworms species. In total, there were six treat-

ments including two non-incubated controls as following (1) Soil A without earthworms (AS); (2) Soil A with *A. corticis* (AC); (3) Soil A with *A. robustus* (AR); (4) Soil B without earthworms (BS); (5) Soil B with *A. corticis* (BC); and (6) Soil B with *A. robustus* (BR). Four replicates were set for each treatment, and 100 g rice straw (dry weight) was placed on soil surface to feed earthworms.

All pots were placed in the dark and the room temperature was kept at 25 °C for 120 days. Soil moisture was adjusted to field capacity every three days. Living earthworms were collected, counted and weighted at the end of cultural period. Collected earthworms were used to determine their metal accumulation. Soil were mixed and divided into two halves, one of which was air-dried and passed through 2 mm and 0.149 mm sieves for further physical and chemical analysis, while the other one was stored in 4 °C refrigerator at field moisture for the analysis of microbial biomass C and N, dissolved organic C and N along with mineralized C and N.

2.3. Chemical and biological analyses

Soil pH was determined using a 1:2.5 soil-to-water ratio. Clay (< 0.002 mm) content was determined by the pipette method. Organic C was determined by the dichromate digestion method and total N by Kjeldahl digestion (Sparks et al., 1996). The cation exchange capacity was determined by the method of NH_4OAC (1 mol L^{-1}, pH 7.0) exchange and Kjeldahl digestion (Sparks et al., 1996).

Soil organic carbon mineralization rate (C_{min}) was measured by the method of NaOH absorption, and the amount of CO_2 in the microbial carbon source mineralization was determined under the standard condition (Dai et al., 2004). The soil mineralized nitrogen (N_{min}) was extracted by 0.01 mol·$L^{-1}$$CaCl_2$, and the content of ammonia nitrogen and nitrate nitrogen were measured by AAS flowing analyzer, and the value of N_{min} was equal to the sum of ammonia nitrogen and nitrate nitrogen contents. Microbial biomass C and N were determined by the chloroform fumigation extraction method (Vance et al., 1987). Microbial biomass C (C_{mic}) and N (N_{mic}) were calculated according to equations: C_{mic} = Ec/0.38, and N_{mic} = En/0.45 (Jenkinson, 1988), where Ec and En are the difference between extractable C and N from fumigated and non-fumigated samples.

Total Cu, Zn, Pb, Cd contents in soil were determined by flame atomic absorption spectroscopy (FAAS) or graphite furnace atomic absorption spectrometry (GFAAS) after digestion of 1.0 g soil sample with H_2O_2, HF, HNO_3 and $HClO_4$ (Amacher, 1996). Meanwhile, DTPA extraction of soil Cu, Zn, Pb and Cd was conducted based upon Lindsay and Norvell (1978).

After being counted and weighted, earthworms were transported into Petri dishes with one filter paper. A few drops of distilled water were applied to keep earthworms moister. They were kept at 25 °C for 7 days and filter papers were changed daily to allow a complete evacuation of their gut contents. Earthworms were then killed in liquid nitrogen and further oven-dried at 100 °C for 24 h. Heavy metal content in earthworms was determined using 0.2 g of dried and crushed earthworm sample. The sample was mixed with 8 mL concentrated HNO_3 and 2 mL concentrated $HClO_4$ for 12 h and digested at 250 °C for 2 h. After cooling, the solution was made up to 50 mL using ultra-pure distilled water (Dai et al., 2004). Metal contents in earthworms were determined by ICP-OES (Varian 710-ES). The BSAF of a metal in the earthworms was calculated by the following formula: BSAF = the metal content in earthworm tissue/total metal content in soil (Cortet et al., 1999).

2.4. Statistical analysis

All data were expressed as mean ± standard deviation. Multivariate analysis was carried out using R and CANOCO 5.0 (R Development Core Team, 2009; Sadyś et al., 2015). Univariate analysis was conducted using SAS 8.0 software. One-way or two-way ANOVA was applied if the data were in a normal distribution. Kruskal Wallis and Scheiner-Ray-Hare tests were used if the data did not conform to the normal distribution. Multiple comparisons were also conducted by means of Duncan's test, when significant difference was found by means of one-way ANOVA. Residual normality and homoscedasticity were verified using Kolmogrov-Smirnov and Bartlett tests. To gain further insight into the growth of earthworms and metal accumulation in earthworm tissues as well as the influence of soil C and N parameters on heavy metal availability in soil, redundancy analysis (RDA) was used with the CANOCO 5.0 software. In order to analyze the influence of earthworms on soil properties, survival number, biomass, and BSAF–heavy metal values of earthworms were set as explanatory variables, while soil organic C and N, along with their components were set as response variables. For analyzing the influence of earthworms on metal bioavailability, survival number, biomass, along with BSAF values of earthworms were used as explanatory variables while DTPA extracted metals were used as responsible variables. To maintain the consistency of the results, original data were transformed to lg (x + 1) for correlation coefficients and stepwise regression analysis.

3. Results

3.1. Growth, metal accumulation and heavy metal bio-concentration factor of earthworm

3.1.1. Characteristics of earthworm growth

Biomass of *A. corticis* significantly decreased by 70.6% (AC) and 64.2% (BC) in both soils ($p < 0.05$), respectively. In contrast biomass of *A. robustus* in soil A was fairly stable ($p > 0.05$), while it decreased by 32.1% in B soil (Table 1). Generally, the quantity of the two earthworm species decreased in all treatments. Compared to *A. robustus,* the quantity of which decreased by 25% ($P < 0.05$) and 12.5% ($P > 0.05$) in soil A and B, respectively, the numbers of *A. corticis* decreased more drastically by 50% ($P < 0.05$) and 62.5% ($P < 0.05$), respectively in soil A and soil B.

3.1.2. Accumulation and BSAF of metals in earthworm tissue

After 120 days of cultivation, the accumulation of Zn, Cd, Pb and Cu in *A. corticis* tissue increased by 1.99–2.98, 1.34–2.82, 27.0–87.0 and 13.4–15.8 times, respectively, compared to their initial concentrations in that earthworm. The accumulation of Zn, Cd, Pb and Cu in *A. robustus* increased by 0.79–0.81, 1.75–1.88, 124–233 and 4.23–6.53 times, respectively, compared to their initial concentrations. Heavy metal accumulation in earthworm tissue was associated with

Table 1
Variation of earthworm biomass and quantity (mean ± S.D., n = 4).

		Biomass		Quantity	
		g·pot $^{-1}$		worms·pot $^{-1}$	
		0 d	120 d	0 d	120 d
A. corticis	soil A	5.34 ± 0.07 Aa	1.57 ± 0.88 Ab	8 ± 0 Aa	4 ± 3 Ab
	soil B	5.31 ± 0.08 Aa	1.90 ± 1.59 Ab	8 ± 0 Aa	3 ± 2 Bb
A. robustus	soil A	16.2 ± 0.09 Aa	16.6 ± 1.51 Aa	8 ± 0 Aa	7 ± 1 Aa
	soil B	16.2 ± 0.11 Ab	11.0 ± 3.00 Bb	8 ± 0 Aa	6 ± 1 Ab

Different capital letters indicate the significant difference ($P < 0.05$) between the treatments with different soil and same earthworm, Different lower letters indicate the significant difference ($P < 0.05$) in the same treatment before and after incubation.

earthworm species and soil type (Table 2). Zn accumulation in earthworm tissue was species dependent. Accumulation of Zn in *A. corticis* was significantly higher than that of *A. robustus* ($p < 0.05$). Cd accumulation in earthworm tissue however, was related to soil types. Cd contents in both earthworm species cultivated in soil A were significantly higher than those in soil B ($P < 0.05$). Although the initial content of Cd in *A. robustus* was significantly higher than that in *A. corticis* (STable3), after 120 day of incubation in heavy metal contaminated soil, the contents of Cd in both earthworms were similar (Table 2). It was suggested that *A. corticis* may readily accumulate Cd from contaminated soil. According to two-way ANOVA, accumulations of Pb was significantly related to earthworm species ($P < 0.05$), while accumulation of Cu was significantly affected by the interactive effect of soil type and earthworm species ($P < 0.05$).

Cd-BSAF ranged from 4.38 to 11.5, and the highest value occurred in treatment with *A. robustus* in soil B. Zn-BSAF were 1.39–4.57, and Zn-BSAF of *A. corticis* was significantly higher than that of *A. robustus* in both soils ($P < 0.05$). Pb-BSAF ranged from 0.222 to 11.4. Pb-BSAF of *A. robustus* was significantly higher than that of *A. corticis* in both soils. Cu-BSAF were 0.15–0.48. Two-way ANOVA indicated a significant interaction occurred in Cu-BSAF between earthworm species and soil types ($P < 0.05$).

3.2. Soil pH, organic C and N along with their components

After 120 days of incubation, variations of soil properties in the different treatment are given in Table 3. Soil pH ranged from 3.83 to 5.23 in the soils treated with earthworms. Generally, the addition of earthworms could reduce soil pH to some extent. In particular, the addition of *A. robustus* significantly decreased soil pH by 0.21–0.45 units ($P < 0.05$). Two-way ANOVA showed that soil pH was significantly associated with earthworm species and soil type.

Though the addition of earthworms did not change soil C_{org} significantly ($P > 0.05$) in soil A, it changed C_{min} and C_{mic} significantly ($P < 0.05$). In soil A, adding *A. corticis* and *A. robustus* increased C_{min} by 27.0% and 28.3% in average, respectively, while incubation with *A. corticis* and *A. robustus* respectively reduced C_{mic} by 19.9% and 44.4% in average. In soil B, the addition of *A. corticis* and *A. robustus* significantly increased soil C_{org} by 26.2% and 23.8%, respective ($P < 0.05$). Incubation with two species of earthworms also resulted in a significant increase in C_{mic} (44.5% vs.61.3% respectively for *A. corticis* and *A robustus*). Independent t tests showed that soil type also affected organic C and its components. C_{org} was significantly higher in treatments with earthworms in soil B than that in soil A ($P < 0.05$). C_{min} in all treatments with soil A was significantly higher than that with soil B ($P < 0.05$), while C_{mic} in treatment with soil A was significantly lower than that with for soil B ($P < 0.05$). Two-way ANOVA also indicated that interactive effect between soil type and earthworm species occurred in C_{org} and C_{mic} ($P < 0.05$).

Addition of earthworms significantly changed soil N_{tot} and its components in both soils ($P < 0.05$). The addition of *A. corticis* and *A. robustus* significantly increased N_{tot} in soil A by 11.6% and 17.0%, respectively. The addition of *A. corticis* and *A. robustus* significantly increased N_{tot} in soil B by 9.6% and 13.9%, respectively ($P < 0.05$). The addition of *A. robustus* in both soils resulted in a significant increase of N_{dis} ($P < 0.05$). Incubation with two species of earthworms significantly increased N_{min} for both soils ($P < 0.05$), while incubation with these earthworms significantly reduced N_{mic} ($P < 0.05$). Based upon independent *t*-test, N_{dis} was found to be significantly higher in treatment with *A. robustus* in soil B than that in soil A. N_{min} was significantly higher (more than 2 times) in all treatment with soil B than that in soil A ($P < 0.05$).

3.3. Heavy metal bioavailability

DTPA extracted Zn, Cd, Pb and Cu were 12.3–21.8, 0.115–0.4, 37.2–52.1 and 61.9–104.5 mg kg^{-1}, respectively (Table 4). DTPA extracted Zn was significantly higher in treatment AR than AS, indicating that *A. robustus* increased the soil bioavailability of Zn. DTPA extracted Cu increased by 4.0 and 5.0 mg kg^{-1} in treatments with A. corticis in soil A. In soil B, Cu-DTPA content was significantly higher in treatment BR compared to BS ($P < 0.05$), indicating that *A. robustus* significantly increased bioavailability of Cu. Compared to BS, Cu-DTPA in treatment BR increased by 9.6%.

3.4. Multivariate statistical analysis of the effect of earthworms on soil properties

After 999 times Monte Carlo test, Pb-BSAF and Cu-BSAF were selected as significant factors affecting the variations of soil properties (Fig. 1). Cu-BASF and Pb-BSAF reflected 51.9% of total eigenvalue. Explaining 38.5% of changes in soil pH, organic C and N along with their components, Cu-BSAF was the major factor that affected soil property changes. Pb-BSAF explained 13.3% of soil property variations. The axis 1 of RDA, positively associated with Cu-BSAF, indicated 40% of the dependent variable changes. Axis 1 represented the effect of Cu accumulation capacity of earthworms on soil properties. Axis 2 was positively correlated with Pb-BSFA, reflecting 11.9% of dependent variable variations. Axis 2 represented the impact of Pb accumulation capacity of earthworms on soil properties. Moreover, distribution of the treatments along axis 1 suggested that Cu-BSAF played a major role in variations of soil properties, Cu-BSAF of earthworms being associated with the interactive effect of soil types and earthworm species. Distribution of the treatments along axis 2 suggested that Pb-BSAF also had a great impact on soil property changes and was related to earthworm species.

3.5. Multivariate statistical analysis of influence of earthworms on heavy metal bioavailability

Through forward selection by 999 times of Monte Carlo test, Pb-BSAF and Cu-BSAF were selected as the significant factors influencing the bioavailability of heavy metals in soils (Fig. 2). Pb-BSAF and Cu-BSAF explained 51.7% of the total eigenvalues. This result is coupled with the effect of earthworms on soil properties. Cu-BSAF explained 45.1% of DTPA extracted metals and hence has a dominant effect on metal bioavailability. Pb-BSAF reflected 6.6% of DTPA extracted metals and also affected soil metal bioavailability significantly. Axis 1 was positively correlated with Cu-BSAF, explaining 47.6% of dependent variable changes. Hence, axis 1 represented the influence of Cu accumulation capacity of earthworms on heavy metal availability. Axis 2 was positively associated with Pb-BSAF, explaining 4.1% of dependent variable changes. Axis 2 reflected the impact of Pb accumulation capacity on heavy metal bioavailability. Distribution of treatments along Axis 1 indicated that Cu-BSAF had a major impact on metal bioavailability and was associated with soil types. Distribution of treatments along Axis 2 demonstrated that Pb-BSAF also affected metal bioavailability and was related to earthworm species.

Based upon the relationship between soil properties and BSAF-heavy metals of earthworms along with the relationship between DTPA extracted heavy metals and BSAF-heavy metals, it is obvious that earthworms could affect soil properties (i.e. C components and pH) and hence influenced DTPA extracted heavy metals. Stepwise regression was employed to analyze the comprehensive effect of soil properties and BSAF-heavy metals of earthworms (Table 5). In general, DTPA extracted heavy metals in soil could be regressed by soil

Table 2
Variation of earthworm metal accumulation and BSAF in treatments (mean ± S.D., n = 4).

			Metals accumulation				BSAF			
			mg·kg $^{-1}$							
			Zn	Cd	Pb	Cu	Zn	Cd	Pb	Cu
	AC		627 ± 206a	24.6 ± 6.77a	93.2 ± 42.3c	72.8 ± 12.9a	3.48 ± 1.44a	6.70 ± 1.84 b	0.56 ± 0.25c	0.233 ± 0.04c
	AR		270 ± 8.90 b	24.7 ± 3.75a	576 ± 206 b	51.1 ± 2.90 b	1.50 ± 0.05 b	6.72 ± 1.02 b	3.45 ± 1.23 b	0.163 ± 0.01 d
	BC		470 ± 148 ab	15.1 ± 2.16 b	300 ± 165bc	61.6 ± 7.53 ab	2.89 ± 0.91a	9.48 ± 1.36a	2.24 ± 1.23bc	0.344 ± 0.04 b
	BR		272 ± 68.4 b	10.1 ± 1.82 b	1080 ± 325a	73.5 ± 10.5a	1.67 ± 0.42 b	6.34 ± 1.14 b	8.07 ± 2.43a	0.411 ± 0.06a
One way ANOVA		*p*	0.014	0.0005	0.0001	0.015	0.021	0.025	0.004	< 0.0001
Two way ANOVA	Earthworm	*p*	0.002	0.078	0.002	0.310	0.001	0.043	< 0.0001	0.949
	Soil	*p*	0.325	< 0.001	< 0.001	0.250	0.534	0.108	0.0003	< 0.0001
	Earthworm*Soil	*p*	0.244	0.050	0.732	0.003	0.342	0.040	0.475	0.007

A and B referred to the treatments using soil A and soil B. C and R referred to treatment with *A. corticis,* and *A robsutus*.
The different letters indicate significant differences in different treatment ($P < 0.05$).

Table 3
Variation of soil organic C and N along with their components in treatments (mean ± S.D., n = 4).

Treatment			pH	C_{org}	C_{dis}	C_{min}	C_{mic}	N_{tot}	N_{dis}	N_{min}	N_{mic}
				g·kg $^{-1}$	mg·kg $^{-1}$	mg C–CO_2·g $^{-1}$soil per day	mg·kg $^{-1}$	g·kg $^{-1}$	mg·kg $^{-1}$	mg·kg $^{-1}$	mg·kg $^{-1}$
	AS		5.12 ± 0.121A ^{a}	20.3 ± 4.86A ^{a}	26.8 ± 15.9A ^{a}	23.3 ± 3.42B ^{a}	67.5 ± 4.03A ^{a}	1.12 ± 0.042B ^{a}	43.1 ± 10.4 B ^{a}	9.65 ± 4.08B ^{b}	30.6 ± 1.[illegible]
	AC		5.05 ± 0.123AB ^{a}	19.2 ± 1.10A ^{b}	24.0 ± 2.35A ^{a}	29.6 ± 4.22A ^{a}	54.1 ± 5.06B ^{a}	1.25 ± 0.070A ^{a}	50.9 ± 5.55 AB ^{a}	19.5 ± 2.09A ^{b}	19.0 ± 4.[illegible]
	AR		4.91 ± 0.114B ^{a}	20.5 ± 0.266A ^{b}	18.0 ± 2.60A ^{b}	29.9 ± 3.40A ^{a}	37.5 ± 6.51C ^{b}	1.31 ± 0.052A ^{a}	58.9 ± 6.72 A ^{b}	23.2 ± 5.85A ^{b}	5.00 ± 4.[illegible]
	BS		4.49 ± 0.090a ^{b}	17.2 ± 1.26b ^{a}	21.3 ± 9.72a ^{a}	15.7 ± 2.90a ^{b}	35.7 ± 5.20b ^{b}	1.15 ± 0.016b ^{a}	44.9 ± 21.1b ^{a}	25.9 ± 4.81c ^{a}	32.9 ± 14[illegible]
	BC		4.31 ± 0.208ab ^{b}	21.7 ± 1.37a ^{a}	28.7 ± 11.3a ^{a}	19.6 ± 3.74a ^{b}	51.6 ± 4.23a ^{a}	1.26 ± 0.114a ^{a}	62.4 ± 8.89b ^{a}	48.5 ± 16.4b ^{a}	9.15 ± 7.[illegible]
	BR		4.04 ± 0.206b ^{b}	21.3 ± 0.563a ^{a}	31.7 ± 9.69a ^{a}	17.2 ± 1.76a ^{b}	57.6 ± 10.0a ^{a}	1.31 ± 0.023a ^{a}	90.5 ± 15.0a ^{a}	88.3 ± 17.4a ^{a}	16.0 ± 15[illegible]
Two way ANOVA	Earthworm	*p*	0.006	0.06	0.353	0.004	0.696	< 0.0001	0.004	< 0.0001	< 0.00[illegible]
	Soil	*p*	< 0.0001	0.362	0.381	< 0.0001	0.167	0.655	0.033	< 0.0001	0.745
	Earthworm*Soil	*p*	0.247	0.012	0.103	0.204	< 0.0001	0.638	0.205	0.798	0.902

A and B referred to the treatments using soil A and soil B. S, C and R referred to treatment with no earthworm (control), *A. corticis*, and *A robsutus*. The different capital letters indicate significant differences in treatment of soil A ($P < 0.05$). The different lower letters indicated significant differences in treatment of soil B ($P < 0.05$). The different superscript letters indicated significant difference in different soil type with same earthworm species.

Table 4
DTPA extracted heavy metals in soil of treatments (mean ± S.D., n = 4).
Expand

Treatment				Zn-DTPA	Cd-DTPA	Pb-DTPA	Cu-DTPA
				$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$
	AS			19.5 ± 0.892B [a]	0.333 ± 0.024A [a]	50.1 ± 2.19A [a]	96.0 ± 2.97B [a]
	AC			20.4 ± 0.251AB [a]	0.335 ± 0.015A [a]	50.2 ± 1.11A [a]	100 ± 2.86A [a]
	AR			21.1 ± 0.665A [a]	0.345 ± 0.022A [a]	48.1 ± 0.850A [a]	101 ± 1.69A [a]
	BS			13.5 ± 0.536a [b]	0.140 ± 0.006a [b]	40.2 ± 1.39a [b]	64.8 ± 2.41b [b]
	BS			14.3 ± 1.570a [b]	0.135 ± 0.014a [b]	40.1 ± 2.20a [b]	68.6 ± 2.38ab [b]
	BR			14.6 ± 0.781a [b]	0.133 ± 0.010a [b]	38.3 ± 0.871a [b]	71.0 ± 3.01a [b]
Two way ANOVA		Earthworm	*p*	0.008	0.784	0.215	0.0002
		Soil	*p*	<0.0001	<0.0001	<0.0001	<0.0001
		Earthworm*Soil	*p*	0.745	0.39	0.922	0.922

A and B referred to the treatments using soil A and soil B. S, C and R referred to treatment with no earthworm (control), *A. corticis*, and *A robsutus*. The different capital letters indicate significant differences in treatment of soil A ($P < 0.05$). The different lower letters indicated significant differences in treatment of soil B ($P < 0.05$). The different superscript letters indicated significant difference in different soil type with same earthworm species.

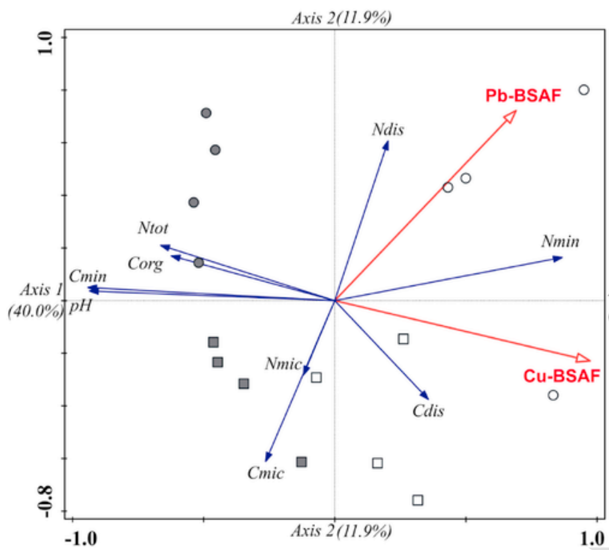

Fig. 1. Redundancy analysis (RDA) of soil properties and BSAF-heavy metals of earthworms. Squares and circles referred to soil treated with A. corticis and *A. robustus*, respectively. Color white and grey referred to soil A and soil B. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

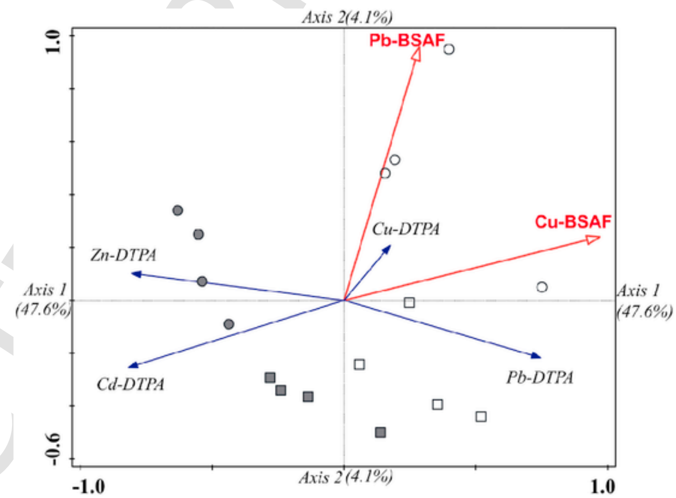

Fig. 2. Redundancy analysis (RDA) of DTPA extracted heavy metals in soil and BSAF-heavy metals of earthworms. Squares and circles referred to soil treated with *A. corticis* and *A. robustus*, respectively. Color white and grey referred to soil A and soil B. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

properties (i.e. C_{min}, pH, and N_{dis}) and BSAF of earthworms (i.e. Cu-BSAF and Pb-BSAF). Soil pH and Cu-BSAF along with N_{dis} were significant factors that affected Zn-DTPA content in soil. Soil pH and Pb-BSAF had an important impact on Cu-DTPA contents in soil. Cd-DTPA and Pb-DTPA in soil was controlled by soil C_{min}. These results indicated that earthworms may affect soil properties and hence influence heavy metal bioavailability in soil.

4. Discussion

4.1. Characteristics of earthworms in different species

A. corticis and *A. robustus* are quite different in terms of behavior. *A corticis* is a typical epigeic species, which lives on the surface of soil (0–5 cm) and tends not to make burrows (Xiao et al., 2019). Biomass of mature individual of *A. corticis* was about 1/4 of *A. robustus* (Table 1). As an endogeic species (Lin et al., 2016), *A. robustus* feeds on soil mineral particles and makes burrows through soil to

Table 5
Stepwise regression between DTPA extracted heavy metals, soil organic C and N along with the components as well as BSAF of earthworms.
Expand

Element	Equations	R^2	R^2adj	P
Zn	DTPA-Zn = −0.063 + 0.159 pH + 0.391N_{dis}-0.998Cu-BSAF	0.911	0.888	<0.001
Cd	DTPA-Cd = −0.309 + 0.289C_{min}	0.805	0.791	<0.001
Pb	DTPA-Pb = 1.086 + 0.409C_{min}	0.846	0.835	<0.001
Cu	DTPA-Cu = 1.198 + 0.152 pH + 0.140 Pb-BSAF	0.716	0.696	<0.001

move around (Lin et al., 2012; Xu et al., 2019). Not only the biomass of *A. robustus* individual was higher than that of *A. corticis*, but also the survival rate of *A. robustus* was higher than that of *A. corticis* (Table 1). These results indicated that *A. robustus* is more tolerant to heavy metal contamination compared to *A. corticis*, probably due to its higher individual biomass. In addition, the burrowing capacity of endogeic species, e.g. *A. robustus* enhances their toleration to heavy metal contamination (Lukkari et al., 2004). Therefore, under the stress of heavy metal contamination, the variation of biomass and survival rates of *A. robustus* were more stable than that of *A. corticis*.

The accumulation of heavy metals differed significantly in earthworm tissues differed for *A. corticis* and *A. robustus* ($P < 0.05$, Table 2). These results indicated that the species of earthworms affected heavy metal accumulation in their tissue. Suthar et al. (2008) also observed species specific metal accumulation pattern. They found that endogeic earthworms accumulated more heavy metals in their tissue than anecic earthworms. Species-specific ingestion behavior, burrowing habit as well as earthworm niche structure have a great impact on heavy metal accumulation in earthworm tissues (Suthar et al., 2008; Wang et al., 2018). However, it should be noted that the accumulation patterns is element specific. For instance, Zn-BSAF and Cd-BSAF of *A. corticis* were significantly higher than those of *A. robustus* though Pb-BSAF of *A. corticis* was significantly lower than that of *A. robustus*, indicating that heavy metal accumulation capacity of earthworms was element dependent. As an essential element, the accumulation of Zn in plants was relatively high (Wang et al., 2016). Cd tends to be the most mobile element among the studied heavy metals in soils, hence it could be more accessible to plants (Dai et al., 2004). As the major origin of soil organic matter is the plants growing on it (Wang et al., 2014), Cd and Zn in soil organic matter are relatively high. In addition, exogenous Cd and Zn are easily absorbed into soil organic matter (Fan et al., 2016; Zhou et al., 2018), which *A. corticis* feed on, may lead to the accumulation of Cd and Zn. Higher accumulation of Pb in *A. robustus* than that in *A. corticis* was also found because of their different feeding behavior. *A. robustus* mainly feed on soil particles which were the main sink of Pb (Li et al., 2009). An interactive effect between soil type and earthworm species could be also observed in the variation of Cu-BSAF, indicating that different earthworm species played an important role in Cu accumulation in earthworms for the two types of soil. Based upon the above discussion, BSAF was found to be a good indicator to evaluate the ecological characteristics of earthworms. It also partially reflected interactive effect of earthworm species and soil types. Therefore, it is reasonable to use BSAF to assess the influence of earthworms on soil properties and heavy metal bioavailability.

4.2. Soil property variations and their influencing factors

Soil pH in treatment AR and BR was significantly lower than that in the control, indicating that *A. robustus* could significantly reduce soil pH. García-Montero et al. (2013) found that earthworms (*Prosellodrilus* sp.) increase soil pH by their cast production and activity, which is quite different from our results. However the effect earthworms on soil pH should depend on site-specific factors including pH and category of the original soil (Cheng and Wong, 2002; Yu et al., 2005; Oo et al., 2015; F Li et al., 2019a, 2019b). RDA showed that soil pH and Cu-BSFA were significantly correlated with Axis 1, suggesting Cu-BSFA which reflected the influence of both earthworm species and soil types had a major impact on soil pH change.

Earthworms changed C and N transportation in soil (Hoang et al., 2016; Sheehy et al., 2019). Two-way ANOVA showed a significant influence of earthworm on C_{min} ($P < 0.01$, Table 3), suggesting that earthworms affected soil C and N components by influencing soil microbial activities. Earthworm intestinal microorganisms were associated with redistribution of C and N in soil (Sheehy et al., 2019). *A. corticis* could increase soil C mineralization through their promotion of soil respiration (Snyder et al., 2009). Enzymes associated with C mineralization, for instance, β-glucosidase, cellobiohydrolase, xylanase were activated in burrows of endogeic earthworm like *A. robustus* (Hoang et al., 2016). The present study showed that the effects of *A. corticis* and *A. robustus* on C_{min} in soil were similar (Fig. 1), indicating that although the feeding behavior and burrowing behavior of these two earthworms were different, due to their different ecological category, their influence on C_{min} were similar. C_{min} was negatively correlated with Cu-BSAF, indicating that soil C_{min} might be also associated with the interactive effect between soil types and earthworm species. C_{min} in soil A was quite higher than that in soil B (Table 3 and Fig. 1), which is in line with higher heavy metal content in soil A than that in soil B, suggesting that soil heavy metal contamination might stimulate C mineralization to some extent. Similar phenomenon has been reported in previous studies (Flieβbach et al., 1994; Dinesh et al., 2012). Two-way ANOVA showed that soil N and its components were associated with earthworm species. In particular, soil N_{dis} was positively correlated with Pb-BSAF (Fig. 1), demonstrating that earthworms with higher Pb-BSAF (i.e. *A. robustus*) tends to impact N_{dis} greater than those with lower Pb-BSAF (i.e. *A corticis*). So far, several possible mechanisms explaining endogeic earthworm-stimulated N_{dis} have been put forward. Burrowing activity of earthworms could mediate soil aeration, modify soil moisture, decompose organic matter, and hence change the microbial communities and N cycles (Kim et al., 2017). Earthworm epidermal mucus contains NO_3^--N and NH_4^+-N, which could directly increase soil dissolved N content by burrowing activities (Zhang et al., 2009; Xu et al., 2018). Through burrowing activities, endogeic earthworms could have enhanced microbial activity and accelerate N mineralization (Ernst et al., 2009).

RDA showed that 51.9% of soil property changes could be explained by Cu-BSAF and Pb-BSAF of earthworms, suggesting that heavy metal accumulation capacity of earthworms played an important role in soil property variations. Compared to biomass and survival number of earthworms, the species along with soil types were the most significant factors affecting soil properties. Thus, further studies regarding effect of earthworms on soil properties should consider intrinsic features of earthworm ecological category (i.e. BSAF of heavy metals).

4.3. Influence of earthworms on DTPA extracted heavy metals

Zn-DTPA in treatment AR along with Cu-DTPA in treatment AR and BR were significantly higher than those of control ($P < 0.05$), indicating that *A. robustus* increased Zn and Cu availability in soil. Nevertheless, *A. corticis* increased Cu availability in soil A. These results demonstrated that *A. robustus* might have stronger capability in activating heavy metal availability than *A. corticis*. This evidence has been supported by previous study that earthworms of endogeic species activated heavy metal availability (Dai et al., 2004). Heavy metals combined with soil minerals might be released during intestinal digestion by endogeic earthworms (Sizmur and Hodson, 2009). Compared to epigeic earthworms, endogeic earthworms tend to make burrows and move frequently in soil (Lin et al., 2012; Xu et al., 2019)., and hence have greater impact on heavy metal bioavailability in soil.

RDA result showed that Cu-BSAF and Pb-BSAF of earthworms could explain 51.7% of DTPA extracted heavy metal changes, indicating that Cu and Pb accumulation capacity of earthworms were the most significant factors impacting heavy metal bioavailability in soil. As BSAF –Pb and Cu-BSAF respectively reflected the effect of earthworm species and the interactive effect between soil type and earthworm species, these results demonstrated that heavy metal bioavailability was affected not only by earthworm species but also by the interaction of earthworms with the studied soils. Significant differences occurred between soil A and soil B in soil properties (i.e. pH, SOC, total N, clay, CEC, total Fe and total Mn) and DTPA heavy metals (STable 1 and 2), which might result in an interactive effect with earthworm species and subsequently affected heavy metal bioavailability in soil. For instance, pH was reported to be the most important factors affecting earthworm survival and hence leading to the change of heavy metal accumulation in earthworm and heavy metal bioavailability in soil (Bradham et al., 2006). CEC was also recognized as a significant factor influencing heavy metal toxicity to earthworms and further impacting bioavailable heavy metal in soil (Lanno et al., 2019).

It should be noted that Cu-BSAF and Pb-BSAF could explain 51.9% and 51.7% of soil property and DTPA extracted heavy metal changes respectively by means of RDA, and these two analyses were similar (Figs. 1 and 2). These results suggested that the accumulation of Cu and Pb in earthworms have similar impact on soil properties and heavy metal bioavailability. On one hand, the accumulation of Cu and Pb in earthworms affected soil properties and hence influenced DTPA extracted heavy metals. Stepwise regression indicated that increase of pH, C_{min} and N_{dis} could enhance heavy metal availability in soil (Table 5), and these soil properties were all associated with earthworm activities based upon our above analysis. The increase of C_{min} indicated an increase of organic C decomposition, which is conducive to heavy metal availability (Zhang et al., 2016). The change of soil pH altered heavy metal bioavailability in soil (Xiao et al., 2017a, 2017b). The decomposition of soil urea was proved to be associated with soil heavy metal bioavailability (Li et al., 2013). These changes of soil properties reflected the synergetic effect of earthworms and microorganisms, which finally led to an augment of heavy metal bioavailability (Liu et al., 2017). On the other hand, it should be noticed that the Pb-BSAF explained 13.3% of soil property change while it only explained 6.6% of DTPA extracted heavy metals in soil, while Cu-BSAF explained 38.5% of soil property change and 45.1% of DTPA extracted heavy metals in soil. These results implied that earthworm activity have a great impact on the variation of soil properties and DTPA extracted heavy metals, while the interaction between different earthworm species and soil types played a dominant role in soil properties and heavy metal changes.

5. Conclusion

The addition of earthworms decreased soil pH, increased soil C_{min}, and increased soil N along with its components (i.e. N_{dis} and N_{min}). The addition of *A. robustus* enhanced Zn-DTPA in soil A along with Cu-DTPA in both soil A and soil B, while addition of *A. corticis* increased Cu-DTPA in soil A. Cu-BSAF and Pb-BSAF could reflect 51.9% of soil property change, and they could explain 51.7% of DTPA extracted heavy metal variation. Pb-BSAF, indicating the influence of earthworm activities of different species, had a great impact on soil property and heavy metal bioavailability change. Nevertheless, Cu-BSAF, reflecting the interaction between earthworm species and soil types, had a dominant effect on the variation of soil property and heavy metal bioavailability. RDA and stepwise regression analysis indicated that earthworm activities changed soil properties (i.e. pH, C_{min} and N_{dis}) and thus enhanced heavy metal bioavailability. Collectively these results imply that BSAF is an important indicator for evaluating the effect of earthworm activity on soil heavy metal bioavailability and assessing the capacity of earthworms for soil heavy metal remediation. Further study may consider a field investigation to evaluate the contribution of BSAF-heavy metals of earthworms on heavy metal bioavailability for in situ condition.

CRediT authorship contribution statement

Ling Xiao: Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Ming-hui Li:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Jun Dai:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing - review & editing. **Mikael Motelica-Heino:** Formal analysis, Writing - review & editing. **Xu-fei Chen:** Data curation, Methodology. **Jia-Long Wu:** Data curation, Writing - original draft.. **Lanfeng Zhao:** Data curation. **Kexue Liu:** Data curation. **Chi Zhang:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Funding acquisition.

Declaration of competing interest

We declare that we have no conflicts of interest to this work.

Acknowledgements

This work was financially supported by the Natural Science Foundation of China (Grant No. 41201305 and 41601575), National Science and Technology Fundamental Resources Investigation Program of China (2018FY100300), National Key Research and Development Program of China (2016YFD0201301 and 2016YFD0201200) and China Scholarship Council in China. The author Dr. Ling Xiao thanks Chengqian Gong for his consistent support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.110513.

References

Amacher, M C, 1996. Nickel, cadmium, and lead. Method. Soil Anal. Part 3—Chem. Method. 739–768 methodsofsoilan3.
Bradham, K D, Dayton, E A, Basta, N T, Schroder, J, Payton, M, Lanno, R P, 2006. Effect of soil properties on lead bioavailability and toxicity to earthworms. Environ. Toxicol. Chem.: Int. J. 25 (3), 769–775.
Cheng, J, Wong, M H, 2002. Effects of earthworms on Zn fractionation in soils. Biol. Fertil. Soils 36 (1), 72–78.
Cortet, J, Gomot-De Vauflery, A, Poinsot-Balaguer, N, Gomot, L, Texier, C, Cluzeau, D, 1999. The use of invertebrate soil fauna in monitoring pollutant effects. Eur. J. Soil Biol. 35 (3), 115–134.
Dai, J, Becquer, T, Henri Rouiller, J, Reversat, G, Bernhard-Reversat, F, Nahmani, J, Lavelle, P, 2004. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. Soil Biol. Biochem. 36 (1), 91–98.
De Vaufleury, A, Gimbert, F, Gomot, L (Eds.), 2013. Bioaccumulation, bioamplification des pollutants dans la faune terrestre-Un outil pour la biosurveillancedes écosystèmes: Un outil pour la biosurveillance des écosystèmes. EDP sciences,2013.
Dinesh, R, Anandaraj, M, Srinivasan, V, Hamza, S, 2012. Engineered nanoparticles in the soil and their potential implications to microbial activity. Geoderma 173, 19–27.
Ernst, G, Henseler, I, Felten, D, Emmerling, C, 2009. Decomposition and mineralization of energy crop residues governed by earthworms. Soil Biol. Biochem. 41 (7), 1548–1554.
Fan, T, Wang, Y, Li, C, He, J, Gao, J, Zhou, D, Friedman, S P, Sparks, D L, 2016. Effect of organic matter on sorption of Zn on soil: elucidation by Wien effect measurements and EXAFS spectroscopy. Environ. Sci. Technol. 50 (6), 2931–2937.
Fließbach, A, Martens, R, Reber, H H, 1994. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol. Biochem. 26 (9), 1201–1205.
Fonte, S J, Kong, A Y Y, van Kessel, C, Hendrix, P F, Six, J, 2007. Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agroecosystem management. Soil Biol. Biochem. 39 (5), 1014–1022.
Fusaro, S, Gavinelli, F, Lazzarini, F, Paoletti, M G, 2018. Soil Biological Quality Index based on earthworms (QBS-e). A new way to use earthworms as bioindicators in agroecosystems. Ecol. Indicat. 93, 1276–1292.
García-Montero, L G, Valverde-Asenjo, I, Grande-Ortíz, M A, Menta, C, Hernando, I, 2013. Impact of earthworm casts on soil pH and calcium carbonate in black truffle burns. Agrofor. Syst. 87 (4), 815–826.
Gong, X, Wang, S, Wang, Z, Jiang, Y, Hu, Z, Zheng, Y, Chen, X, Li, H, Hu, F, Liu, M, Scheu, S, 2019. Earthworms modify soil bacterial and fungal communities through enhancing aggregation and buffering pH. Geoderma 347, 59–69.

Han, L, Zhao, X, Jin, J, Gao, B, Yang, Y, Sun, K, Li, F, 2019. Using sequential extraction and DGT techniques to assess the efficacy of plant- and manure-derived hydrochar and pyrochar for alleviating the bioavailability of Cd in soils. Sci. Total Environ. 678, 543–550.
Hoang, D T T, Razavi, B S, Kuzyakov, Y, Blagodatskaya, E, 2016. Earthworm burrows: kinetics and spatial distribution of enzymes of C-, N- and P- cycles. Soil Biol. Biochem. 99, 94–103.
Jeyanthi, V, Paul, J A J, Selvi, B K, Karmegam, N, 2016. Comparative study of biochemical responses in three species of earthworms exposed to pesticide and metal contaminated soil. Environ. Process. 3 (1), 167–178.
Kavehei, A, Hose, G C, Gore, D B, 2018. Effects of red earthworms (Eisenia fetida) on leachability of lead minerals in soil. Environ. Pollut. 237, 851–857.
Kim, Y, Robinson, B, Lee, K, Boyer, S, Dickinson, N, 2017. Interactions between earthworm burrowing, growth of a leguminous shrub and nitrogen cycling in a former agricultural soil. Appl. Soil Ecol. 110, 79–87.
Lanno, R, Oorts, K, Smolders, E, Albanese, K, Chowdhury, M, 2019. Effects of soil properties on the toxicity and bioaccumulation of lead in soil invertebrates. Environ. Toxicol. Chem. 38 (7), 1486–1491.
Lavelle, P, Spain, A, Blouin, M, Brown, G, Decaëns, T, Grimaldi, M, Jiménez, J J, McKey, D, Mathieu, J, Velasquez, E, 2016. Ecosystem engineers in a self-organized soil: a review of concepts and future research questions. Soil Sci. 181 (3/4), 91–109.
Lemtiri, A, Liénard, A, Alabi, T, Brostaux, Y, Cluzeau, D, Francis, F, Colinet, G, 2016. Earthworms Eisenia fetida affect the uptake of heavy metals by plants Vicia faba and Zea mays in metal-contaminated soils. Appl. Soil Ecol. 104, 67–78.
Li, F, Li, Z, Mao, P, Li, Y, Li, Y, McBride, M B, Wu, J, Zhuang, P, 2019. Heavy metal availability, bioaccessibility, and leachability in contaminated soil: effects of pig manure and earthworms. Environ. Sci. Pollut. Control Ser. 26 (20), 20030–20039.
Li, M, Cheng, X, Guo, H, 2013. Heavy metal removal by biomineralization of urease producing bacteria isolated from soil. Int. Biodeterior. Biodegrad. 76, 81–85.
Li, Y, Becquer, T, Dai, J, Quantin, C, Benedetti, M F, 2009. Ion activity and distribution of heavy metals in acid mine drainage polluted subtropical soils. Environ. Pollut. 157 (4), 1249–1257.
Li, Y, Wang, S, Lu, M, Zhang, Z, Chen, M, Li, S, Cao, R, 2019. Rhizosphere interactions between earthworms and arbuscular mycorrhizal fungi increase nutrient availability and plant growth in the desertification soils. Soil Tillage Res. 186, 146–151.
Lin, Z, Li, X, Li, Y, Huang, D, Dong, J, Li, F, 2012. Enhancement effect of two ecological earthworm species (Eisenia foetida and Amynthas robustus E. Perrier) on removal and degradation processes of soil DDT. J. Environ. Monit. 14 (6), 1551–1558.
Lin, Z, Zhen, Z, Wu, Z, Yang, J, Zhong, L, Hu, H, Luo, C, Bai, J, Li, Y, Zhang, D, 2016. The impact on the soil microbial community and enzyme activity of two earthworm species during the bioremediation of pentachlorophenol-contaminated soils. J. Hazard Mater. 301, 35–45.
Lindsay, W L, Norvell, W A, 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper 1. Soil Sci. Soc. Am. J. 42 (3), 421–428.
Liu, G, Ling, S, Zhan, X, Lin, Z, Zhang, W, Lin, K, 2017. Interaction effects and mechanism of Pb pollution and soil microorganism in the presence of earthworm. Chemosphere 173, 227–234.
Liu, T, Chen, X, Gong, X, Lubbers, I M, Jiang, Y, Feng, W, Li, X, Whalen, J K, Bonkowski, M, Griffiths, B S, 2019. Earthworms coordinate soil biota to improve multiple ecosystem functions. Curr. Biol. 29 (20), 3420–3429.
Lukkari, T, Taavitsainen, M, Väisänen, A, Haimi, J, 2004. Effects of heavy metals on earthworms along contamination gradients in organic rich soils. Ecotoxicol. Environ. Saf. 59 (3), 340–348.
Oo, A N, Iwai, C B, Saenjan, P, 2015. Soil properties and maize growth in saline and nonsaline soils using cassava-industrial waste compost and vermicompost with or without earthworms. Land Degrad. Dev. 26 (3), 300–310.
Sadyś, M, Strzelczak, A, Grinn-Gofroń, A, Kennedy, R, 2015. Application of redundancy analysis for aerobiological data. Int. J. Biometeorol. 59 (1), 25–36.
Sheehy, J, Nuutinen, V, Six, J, Palojärvi, A, Knuutila, O, Kaseva, J, Regina, K, 2019. Earthworm Lumbricus terrestris mediated redistribution of C and N into large macroaggregate-occluded soil fractions in fine-textured no-till soils. Appl. Soil Ecol. 140, 26–34.
Sinkakarimi, M H, Solgi, E, Colagar, A H, 2020. Interspecific differences in toxicological response and subcellular partitioning of cadmium and lead in three earthworm species. Chemosphere 238, 124595.
Sizmur, T, Hodson, M E, 2009. Do earthworms impact metal mobility and availability in soil? – a review. Environ. Pollut. 157 (7), 1981–1989.
Snyder, B A, Boots, B, Hendrix, P F, 2009. Competition between invasive earthworms (Amynthas corticis, Megascolecidae) and native North American millipedes (Pseudopolydesmus erasus, Polydesmidae): effects on carbon cycling and soil structure. Soil Biol. Biochem. 41 (7), 1442–1449.
Sparks, D L, Helmke, P A, Page, A L, 1996. Methods of Soil Analysis: Chemical Methods. SSSA.
Suthar, S, Singh, S, Dhawan, S, 2008. Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: is metal bioaccumulation affected by their ecological category? Ecol. Eng. 32 (2), 99–107.
Team, D C R, 2009. A Language and Environment for Statistical Computing. R Foundation for statistical Computing, Vienna, Austria http://www. R-project. org. (Accessed 16 October 2009).
Vance, E D, Brookes, P C, Jenkinson, D S, 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6), 703–707.
Wang, F, Liu, X, Shi, Z, Tong, R, Adams, C A, Shi, X, 2016. Arbuscular mycorrhizae alleviate negative effects of zinc oxide nanoparticle and zinc accumulation in maize plants–a soil microcosm experiment. Chemosphere 147, 88–97.
Wang, G, Xia, X, Yang, J, Tariq, M, Zhao, J, Zhang, M, Huang, K, Lin, K, Zhang, W, 2020. Exploring the bioavailability of nickel in a soil system: physiological and histopathological toxicity study to the earthworms (Eisenia fetida). J. Hazard Mater. 383, 121169.
Wang, H, Guan, D, Zhang, R, Chen, Y, Hu, Y, Xiao, L, 2014. Soil aggregates and organic carbon affected by the land use change from rice paddy to vegetable field. Ecol. Eng. 70, 206–211.
Wang, K, Qiao, Y, Zhang, H, Yue, S, Li, H, Ji, X, Liu, L, 2018. Bioaccumulation of heavy metals in earthworms from field contaminated soil in a subtropical area of China. Ecotoxicol. Environ. Saf. 148, 876–883.
Wen, B, Hu, X, Liu, Y, Wang, W, Feng, M, Shan, X, 2004. The role of earthworms (*Eisenia fetida*) in influencing bioavailability of heavy metals in soils. Biol. Fertil. Soils 40 (3), 181–187.
Xiao, L, Guan, D, Peart, M R, Chen, Y, Li, Q, 2017. The respective effects of soil heavy metal fractions by sequential extraction procedure and soil properties on the accumulation of heavy metals in rice grains and brassicas. Environ. Sci. Pollut. Control Ser. 24 (3), 2558–2571.
Xiao, L, Guan, D, Peart, M R, Chen, Y, Li, Q, Dai, J, 2017. The influence of bioavailable heavy metals and microbial parameters of soil on the metal accumulation in rice grain. Chemosphere 185, 868–878.
Xiao, Z, Jiang, L, Chen, X, Zhang, Y, Defossez, E, Hu, F, Liu, M, Rasmann, S, 2019. Earthworms suppress thrips attack on tomato plants by concomitantly modulating soil properties and plant chemistry. Soil Biol. Biochem. 130, 23–32.
Xu, H, Bai, J, Li, W, Zhao, L, Li, Y, 2019. Removal of persistent DDT residues from soils by earthworms: a mechanistic study. J. Hazard Mater. 365, 622–631.
Xu, H, Chen, H, Wang, X, Zhang, Y, Wang, J, Li, N, Li, Y, 2018. Earthworms stimulate nitrogen transformation in an acidic soil under different Cd contamination. Ecotoxicol. Environ. Saf. 165, 564–572.
Yu, X, Cheng, J, Wong, M H, 2005. Earthworm–mycorrhiza interaction on Cd uptake and growth of ryegrass. Soil Biol. Biochem. 37 (2), 195–201.
Yuvaraj, A, Karmegam, N, Tripathi, S, Kannan, S, Thangaraj, R, 2020. Environment-friendly management of textile mill wastewater sludge using epigeic earthworms: bioaccumulation of heavy metals and metallothionein production. J. Environ. Manag. 254, 109813.
Zhang, C, Mora, P, Dai, J, Chen, X, Giusti-Miller, S, Ruiz-Camacho, N, Velasquez, E, Lavelle, P, 2016. Earthworm and organic amendment effects on microbial activities and metal availability in a contaminated soil from China. Appl. Soil Ecol. 104, 54–66.
Zhang, S, Hu, F, Li, H, Li, X, 2009. Influence of earthworm mucus and amino acids on tomato seedling growth and cadmium accumulation. Environ. Pollut. 157 (10), 2737–2742.
Zhou, T, Wu, L, Luo, Y, Christie, P, 2018. Effects of organic matter fraction and compositional changes on distribution of cadmium and zinc in long-term polluted paddy soils. Environ. Pollut. 232, 514–522.