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RESEARCH ARTICLE

Effects of soil properties and aging process on the acute toxicity of cadmium to earthworm *Eisenia fetida*

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Abstract
This study was undertaken to investigate the effects of soil properties and aging process on the acute toxicity of cadmium (Cd) to *Eisenia fetida* (*E. fetida*) in 18 Cd-spiked soils. Results showed that the Cd toxicity to *E. fetida* differed in the 18 soils with different characteristics, and median lethal concentration (LC50) values varied from 440.7 to 1520.4 mg/kg in freshly spiked soils. Soil pH and organic matter (OM) content were the two major factors associated with Cd toxicity. The increase in LC50 values and decreases in both exchangeable Cd in soils and tissue Cd concentrations in earthworm whole body indicated that aging (180 and 360 days) could reduce the acute toxicity and bioavailability of Cd to *E. fetida*. Cadmium concentrations in *E. fetida* were positively correlated with exchangeable Cd content in soils, and soil pH and OM were the key factors controlling the distribution and transformation of the exchangeable Cd. The results will provide useful reference information for the risk assessment of Cd in the terrestrial environment.

Keywords Cadmium · Soil properties · Aging process · *E. fetida* · Acute toxicity

Introduction

Agricultural soil cadmium (Cd) contamination has become a widespread environmental problem globally. Cd, as one of the most toxic trace elements in soil, is readily taken up by crop plants and translocated to the shoot and then to the grains. Crop grains are the major source of dietary Cd posing serious health risk to humans (Ok et al. 2011; De Jonge et al. 2012). Two previous investigations conducted separately in 2002 and 2008 in China showed that about 10% of rice samples on the market contained excessive levels of Cd (Zhen et al. 2008). In addition, Cd is also readily accumulated by terrestrial animals and thus, may cause adverse impacts on the terrestrial ecosystems (Panzarino et al. 2016). Therefore, there is a dire need for developing a Cd risk assessment strategy for an effective management of the ecological and health effects associated with soil Cd contamination. The USA and several European countries had carried out the risk assessment and established soil quality standards for Cd contamination (0.36 mg/kg for mammal, 32 mg/kg for plant, and 140 mg/kg for terrestrial invertebrates in ecological soil standard of the USA, and 12 mg/kg as intervention value in the Netherlands) (United States Environmental Protection Agency 2003; European Commission 2003; Hankard et al. 2005). Meanwhile, three classes of benchmark values were specified by the Chinese soil environmental quality standard for heavy metals including Cd (GB15618-1995). Specifically, 0.2 mg/kg as class I value for Cd represents the natural background, 0.3 mg/kg as class II value is set up to ensure agricultural production and human health via the food chain, and 1 mg/kg as class III value is for protection of crops and forests from phytotoxicity where the natural background is elevated. According to a recent survey on the current status of soil Cd contamination in China, up to 7% of the total survey sites exceeded the environmental quality standard for Cd concentration (Ministry of Environmental Protection of the People's Republic of China 2014). In another 132,071 survey samples of Chinese arable soil, the Cd content in 45% of the total survey samples exceeded the class II criterion of the Chinese soil environmental quality

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standard (Zhang et al. 2015). The large number of exceeding rate caused numerous debates on whether the class II value for Cd was overprotective. Furthermore, Cd bioavailability and toxicity depends not directly on the total Cd concentration but on its chemical speciation and varies with soil properties (Boyd and Williams 2003; Ding et al. 2013). Krishnamurti et al. (1995) suggested that Cd bioavailability in soil decreased in the order: exchangeable fraction < carbonate fraction < metal-organic complex fraction < Fe and Mn oxide fraction < mineral lattice fraction. Soil properties could influence the transformation and distribution of Cd chemical speciation (Bur et al. 2010). Since there are numerous soil types in China and they vary greatly in the physicochemical properties, such as soil pH values (4.0 – 9.0) and organic matter contents (3.0 – 132.1 g/kg) (Zhou and Wang 2012), it would be justifiable to take into account the chemical speciation and soil properties for the Cd risk assessment in any future revision of the soil quality standards in China (Boyd and Williams 2003; Panzarino et al. 2016; Rafiq et al. 2014).

At present, the ecotoxicological data on Cd contamination based on different soil types is relatively scarce in China, especially for terrestrial invertebrates such as earthworm and collembolan (Adams et al. 2004; Kirkham 2006; Ye et al. 2014). Previous study showed that Cd median lethal concentration (28-day LC50) values of *Paronychiurus kimi* (Lee) (Collembola) were 53.3, 63.8, and 115.9 mg/kg in Cd-spiked artificial soils at pH 4.5, 5.8, and 7.0, respectively (Son et al. 2007). Lock and Janssen (2001a) reported a 3.2-fold variation in the 14-day LC50 of *E. fetida* and the total added Cd was from 477 to 1520 mg/kg in the three soils. Bur et al. (2010) showed that Cd median inhibition effect (EC50) values of Collembola *Folsomia candida* were 182, 111, and 107 mg/kg in the three Cd-spiked cultivated and forested soils with different pH values (4.5 – 8.2) and organic matter contents (16 – 165 g/kg). However, the published data were insufficient to be used for adequate quantification of the effects of soil properties on the toxicity and bioavailability of Cd to terrestrial invertebrates. Achieving this goal requires a systematic toxicity study and soils with a wide range of soil properties should be concerned. In addition, the changes of heavy metal speciation distribution with time, known as aging, cannot be ignored in evaluating the risks of heavy metal contamination in soil (Alexander 2000). With the passage of time, the mobility and bioavailability of heavy metal gradually decrease, and water-soluble fraction of heavy metal is readily converted to less labile fraction (iron-manganese oxide fractions and mineral lattice fractions) via various mechanisms including microporous diffusion, cavity entrapment, and surface precipitation or coprecipitation in solid phase (Ma et al. 2006a, b; Lock et al. 2001b). Many previous studies of Cd risk assessment to terrestrial invertebrates were based on the freshly spiked soils, which may lead to overestimate Cd toxicity in field soils with long-term contamination history (Ma et al. 2006a, b; Zhou et al. 2014).

As the important terrestrial model organisms for toxicity testing to assess environmental pollution, earthworms could serve as useful biological indicators of contaminated soils because of the fairly consistent correlation between the concentration of contaminants in their tissues and corresponding soils (Spurgeon et al. 2005; Kilic 2011; Voua Otomo et al. 2011). By direct soil ingestion or transdermal uptake, soil Cd could cause lethal and sublethal toxicities such as avoidance behavior and physiological disruption to earthworms. The present study was conducted to investigate the acute toxicity of Cd to earthworm (*E. fetida)* in a large set of 18 Chinese soils with different properties. The main objectives were to test and explore the effects of soil properties and aging process and underlying mechanisms on Cd toxicity to *E. fetida* and correlation between the toxicity thresholds and soil properties. The present study could provide baseline data support for the development of robust soil ecological screening values of Cd in China.

Materials and methods

Earthworm

Adult earthworm *E. fetida* was purchased from the earthworm-rearing farm in Nanjing. The earthworms were fed cow manure in the fertile soil and acclimated in an environment-controlled chamber at 20 ± 1 °C in dark with 70% relative humidity. The earthworms were depurated on a moist filter paper for 24 h and rinsed with deionized (DI) water, prior to the toxicity tests.

Soil sampling and treatment

Clean soils were sampled from 18 locations situated in different regions of China (Table 1). The 18 soils are the representative of the major soil types in these regions and cover a wide range of soil properties. Surface soils (0 – 20 cm) were air-dried, grinded, and sieved to < 5 mm. On the basis of preliminary experiment, each soil was spiked with a range of seven Cd concentrations (control + six Cd doses). The maximum target concentrations varied from 700 to 1700 mg/kg. The concentrations of Cd-spiked soils were specified in Table S1. To ensure even distribution of the Cd solution in soil, Cd was applied by spraying appropriate amount of $CdCl_2$ solution (50 g Cd/L) diluted with DI water to the oven dry soil at a ratio of 100 mL/kg according to the methodology proposed by Rooney et al. (2006). After adding Cd, each soil was incubated at 70% water holding capacity for 2, 180, and 360 days before the toxicity tests. Such three incubation time periods were used to evaluate the effects of aging process on the acute toxicity of Cd to *E. fetida.*

Table 1 Selected properties of Chinese soils used in the toxicity tests

Soil location	Soil type	pH	WHC (%0	OM (g/kg)	CEC (cmol/kg)	Clay (%)	Silt (%)	Sand (%)	Cd (mg/kg)
Yingtan	Red earth	4.69	56.5	37.9	14.5	13.8	51.3	34.9	0.41
Leshan	Purple soil	5.52	44.4	20.5	13.0	34.3	57.5	8.2	0.46
Xuyi	Yellow brown soil	5.02	47.8	22.0	25.6	12.7	80.1	7.2	0.2
Changsha	Paddy soil	4.62	65.7	43.6	6.9	20.0	67.0	13.0	0.48
Shaoguan	Latosolic red earth	5.51	43.2	18.5	16.2	22.9	41.4	35.7	0.27
Quanzhou	Paddy soil	6.14	45.3	14.3	9.2	12.6	47.1	40.3	0.30
Zhaoqing	Latosol	5.33	58.6	40.0	13.4	27.1	53.7	19.2	0.34
Hechi	Red earth	5.40	64.9	58.2	9.9	32.5	43.2	24.3	2.27
Yichang	Paddy soil	5.94	44.8	26.5	7.0	11.9	64.5	23.6	0.39
Weifang	Cinnamon soil	7.03	44.6	7.9	18.0	10.4	57.7	31.9	0.26
Shenyang	Brown earth	7.64	42.9	5.7	15.7	47.0	49.8	3.2	0.33
Ningbo	Paddy soil	8.09	46.2	24.6	18.4	22.2	59.6	18.2	0.23
Chongzuo	Latosol	7.43	44.9	33.1	16.5	41.3	29.5	29.2	0.97
Hefei	Paddy soil	8.20	44.1	5.6	19.1	21.4	72.7	5.9	0.27
Dujiangyan	Paddy soil	7.03	54.4	31.7	26.1	13.6	61.8	24.6	0.42
Baoding	Fluvo-aquic soil	8.69	42.0	5.7	10.9	13.1	61.5	25.4	0.27
Xiangxiang	Paddy soil	7.99	53.1	38.6	14.8	29.5	48.9	21.6	0.93
Guiyang	Yellow earth	7.60	45.5	81.1	9.9	48.1	41.4	10.5	1.14

Acute toxicity tests

Acute toxicity tests were conducted following the standard methods of ISO 11268-1 and OECD-207 (ISO 1993; OECD 1984). The earthworms were individually placed into 500 g dry weight spiked soil in a 1-L high-density polyethylene container in the environment-controlled chamber. The relative humidity, temperature, and light intensity were adjusted to 70%, 20 ± 1 °C, and 400 – 800 lx, respectively. In order to prevent the escape of the earthworms from the soil, plastic containers were covered with a plastic film having several tiny holes to keep ventilation and minimize the moisture evaporation. All treatments were conducted in quadruplicate, with ten earthworms per quadruplicate. The soil moisture content was adjusted twice a week by replenishing weight loss with the appropriate amount of DI water. During 14-day exposure, the dead earthworms, which did not respond to gentle needle probing in the tail, were immediately removed and their number was recorded. The earthworms that survived at the end of exposure, were collected, depurated on a moist filter paper for 24 h, rinsed with DI water, and stored at − 70 °C for further analysis.

Analytical methods

Soil pH was measured in 0.01 M $CaCl_2$ (1:2.5 soil:solution ratio). The maximum water holding capacity (%, *w*/*w*) was measured by sealing the dry soil with a filter paper immersed in DI water for 24 h. Soil organic matter (OM) was measured by the method (potassium dichromate oxidation-ferrous sulfate titrimetry) proposed by Walkley and Black (1934). Soil texture (sand, silt, and clay percentages) was analyzed by a laser particle size analyzer (Beckman LS 13320, USA). Cation exchange capacity (CEC) was measured by the ammonium acetate centrifugal exchange method. The values of background Cd and spiked Cd in soils were determined by the inductively coupled plasma mass spectrometry (ICP-MS; Thermo ICAP Q, USA) with the detection limit of 0.01 μg/L and the inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin Elmer Optima 2000DV, USA) with the detection limit of 0.002 mg/L at a wavelength of 214.44 nm after digestion by mixed acid (HNO_3-$HClO_4$-HF), respectively. The certified sediment reference material (GBW07456, the National Research Center for Standard Materials of China) with a Cd concentration of 0.56 ± 0.04 mg/kg was used for controlling the quality of our analysis and the recovery rates were 94–103%. The heavy metal contents (Table S2) in the 18 soils prior to Cd spiking were analyzed by ICP-OES following mixed-acid digestion (HNO_3-$HClO_4$-HF), and the results suggested that the sampled soils were clean and uncontaminated by heavy metals; thus, the interference effects from other heavy metals on the Cd toxicity tests were avoided in the current study.

Exchangeable Cd in spiked soils was extracted with 0.01 M $CaCl_2$ (1:10 soil:solution ratio) and determined by ICP-OES. The Cd concentrations in *E. fetida* (mg Cd/kg worm, dry weight) were analyzed by ICP-OES after digestion in a 9:2 (*v*/*v*) mixture of HNO_3 and H_2O_2 in a microwave oven (Ethos One, Milestone, Italy). The certified reference material (TORT-2, lobster hepatopancreas, National Research Council Canada) with a Cd concentration of 26.7 ± 0.6 mg/kg was concurrently digested and the recovery rates were 96 – 105%.

Statistical analyses

The Cd lowest observed effect concentrations (LOECs) to *E. fetida* were calculated by using Dunnett's test based on a significant differences from the control group ($p < 0.05$) (OECD 2006). The Cd 10% lethal concentrations (LC10s), 20% lethal concentrations (LC20s), and 50% lethal concentrations (LC50s) based on spiked Cd were calculated by using the probability regression method. All statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). The data and linear or non-linear regression curves were plotted using SigmaPlot 10.0, and especially, the dose-response (mortality) data were fitted by a sigmoidal curve according to the methodology proposed by Haanstra et al. (1985) as follows:

$y = y_0 / (1 + \exp(-(x - x_0) / b))$, (1)where y is the mortality of *E. fetida* (%); x is the spiked Cd concentration (mg/kg); y_0, b, and x_0 are the parameters to be fitted; and x_0 is the median lethal concentration (LC50) (mg/kg).

Results

Acute toxicity of Cd to *E. fetida*

Acute toxicity of Cd to *E. fetida* in the 18 freshly spiked soils (2-day incubation) was investigated. Figure 1 showed that *E. fetida* survived during the entire experiment period, when spiked Cd was relatively low. LOECs in spiked soils varied from 300 mg/kg in red earth (Yingtan) to 1300 mg/kg in yellow earth (Guiyang) (Table S3). Sigmoidal dose-effect curves were observed between the *E. fetida* mortality and spiked Cd concentrations as depicted in Fig. 1 ($R^2 \geq 0.97$, $p < 0.05$). LC10s and LC20s varied from 331.4 to 1278.7 mg/kg and 364.7 to 1355.0 mg/kg, respectively, representing 3.9-fold and 3.7-fold variations among the soils (Table S4 and S5). LC50s were also different in the different spiked soils with the minimum value of 440.7 mg/kg in red earth (Yingtan) and the maximum value of 1520.4 mg/kg in yellow earth (Guiyang), representing 3.5-fold variations among the soils (Table S6). By using LC50s as the biological toxicity indicators of Cd to *E. fetida*, the Cd toxicity in the 18 spiked soils showed that red earth and acid paddy soils in some parts of southern China were more susceptible to the toxicity of Cd.

Effects of aging process on acute toxicity of Cd to *E. fetida*

The Cd toxicity to *E. fetida* was significantly reduced by aging process in the 18 soils, as LOECs varied from 400 to 1400 mg/kg (180-day aging) and 400 to 1500 mg/kg (360-day aging) (Table S3). Additionally, the LC10s, LC20s, and LC50s were also significantly increased with aging process (Table S4, S5, and S6). For instance, LC50s were increased to 506.4 and 1234.0 mg/kg (180-day aging) and 553.7 and 1309.0 mg/kg (360-day aging) in red earth (Yingtan) and paddy soil (Dujiangyan), respectively, representing 1.15-fold and 1.13-fold (180-day aging) and 1.26-fold and 1.20-fold (360-day aging) increases compared to the freshly spiked soil treatments (2-day aging). The mortalities were below 50% with aging (180 and 360 days) as compared to 90% mortality in the freshly spiked soils with the maximum target concentration in yellow earth (Guiyang).

Effects of soil properties on acute toxicity of Cd to *E. fetida*

Stepwise multiple regressions were carried out to determine the relative contributions of soil properties (pH, OM, CEC, and clay) to Cd toxicity thresholds of LC50s. Significantly positive correlations were observed between soil pH, OM and Cd LC50s in the freshly spiked soils. These two factors can account for 89.2% variance for Cd LC50s (Table 2). Furthermore, soil pH was the most appropriate predictor for the LC50s with the increase in aging time.

Exchangeable Cd in soils and Cd accumulation in *E. fetida*

Exchangeable Cd in freshly spiked soils and Cd accumulation in *E. fetida* were concurrently determined. The concentrations of exchangeable Cd were substantially different in different soils at the same Cd spiking concentration of 400 mg/kg (Fig. 2). For example, the maximum value of exchangeable Cd was 283.6 mg/kg in red earth (Yingtan, Fig. 2(a)) and the minimum value was 5.4 mg/kg in paddy soil (Xiangxiang, Fig. 2(q)), representing 52.5-fold variations among the soils. The exchangeable Cd concentrations were significantly influenced by soil pH which accounted for 90% variance for the exchangeable Cd in the soils (Table S8). A significantly positive correlation with a correlation coefficient R^2 value of 0.956 was observed between the exchangeable Cd concentrations in spiked soils and the whole body Cd concentrations in *E. fetida* (Fig. 3(a)). Cadmium bioavailability and its toxicity to *E. fetida* can be predominantly determined by exchangeable Cd, and the variations of exchangeable Cd values in the different soils were attributed to the differences of soil properties, especially soil pH.

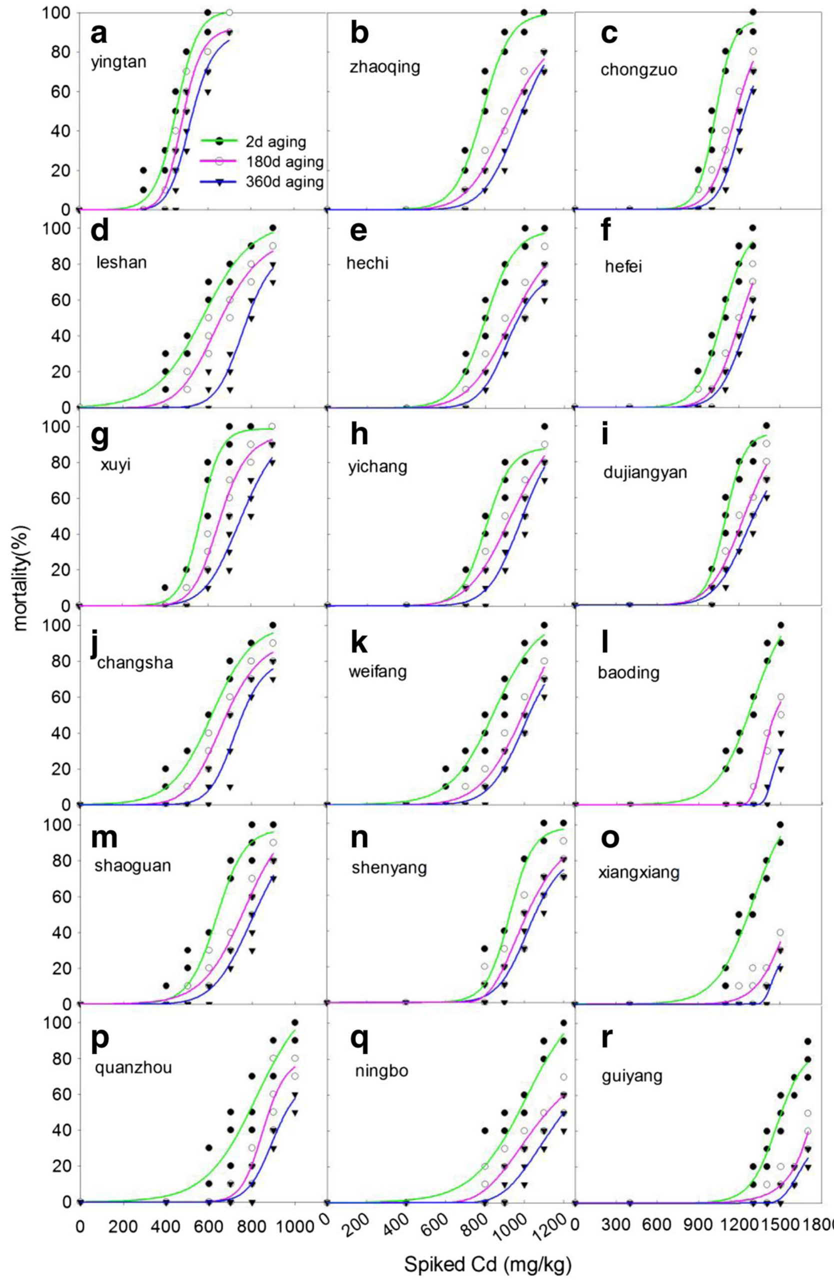

Fig. 1 Dose-response curves between the mortality of *E. fetida* and spiked Cd after aging (2, 180, and 360 days). Symbols represent all replicated data points and lines are the fitted sigmoidal curves

There were obvious decreases in exchangeable Cd in the spiked soils and whole body Cd concentrations in *E. fetida* with aging process (Fig. 2 and Fig. S1). The exchangeable Cd values (Fig. 2(a)) significantly decreased from 283.6 ± 11.4 to 249.5 ± 7.5 (180 days) and 244.4 ± 8.6 (360 days) mg/kg with the aging process, when the earthworms were exposed to red

Table 2 Simple and multiple stepwise regressions between LC50 of Cd to *E. fetida* (mg/kg) and soil properties

Regression type	Aging time (days)	Regression equation	R^2	p
Simple	2	1 logLC50 = 2.311 + 0.093pH	0.737	< 0.001
Stepwise	2	2 logLC50 = 2.155 + 0.104pH + 0.003OM	0.892	< 0.001 0.001
Simple	180	3 logLC50 = 2.452 + 0.080pH	0.757	< 0.001
Stepwise	180	4 logLC50 = 2.266 + 0.098pH + 0.003OM	0.826	< 0.001 0.042
Simple	360	5 logLC50 = 2.544 + 0.071pH	0.702	< 0.001
Stepwise	360	6 logLC50 = 2.388 + 0.086pH + 0.002OM	0.777	< 0.001 0.066

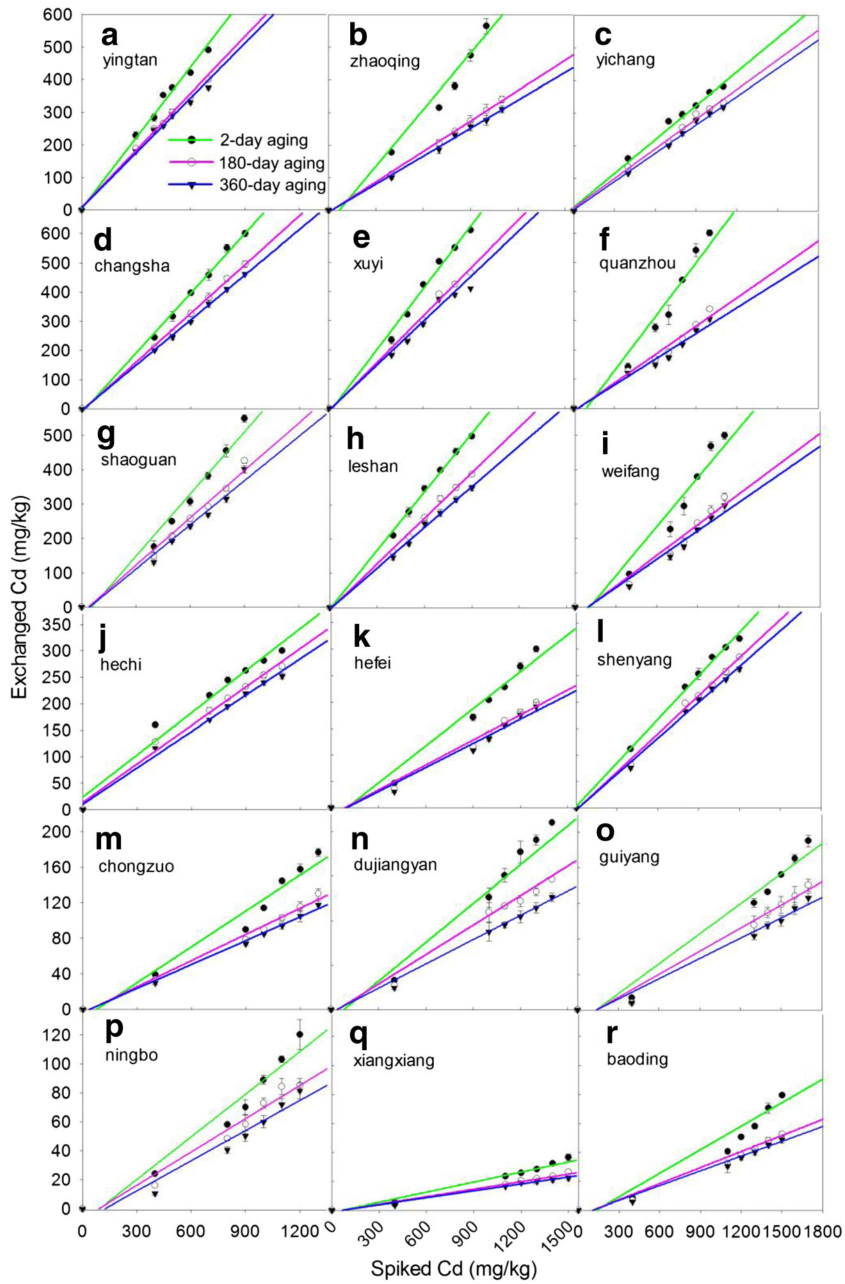

Fig. 2 Correlation between the exchangeable Cd and spiked Cd after aging (2, 180, and 360 days) in 18 soils. Data are shown as mean ± SD ($n = 4$)

earth (Yingtan) spiked with 400 mg/kg Cd, representing a decrease of 12 and 14%, respectively. Similarly, Cd concentrations in the whole body tissue of *E. fetida* decreased from 680.1 ± 14.5 to 550.4 ± 10.3 and 508.6 ± 12.5 mg/kg for the corresponding soil aging treatment groups, respectively, representing 19 and 25% decreases. When the earthworm was exposed to paddy soil (Xiangxiang, Fig. 2(q)) spiked with 400 mg/kg Cd, the exchangeable Cd values decreased from 5.4 ± 0.2 to 4.1 ± 0.1 and 3.5 ± 0.1 mg/kg with aging process, respectively, representing 24 and 35% decreases. Correspondingly, the whole body tissue Cd concentrations in *E. fetida* decreased from 278.3 ± 26.1 to 216.9 ± 15.3 and 196.5 ± 17.0 mg/kg, respectively, representing 22 and 29% decreases. The decrease extents were larger within the first 180 days than the following 180 days, which indicated that the initial Cd aging process in the freshly spiked soils was faster and the rate was gradually decreased. The significantly positive correlations with the correlation coefficients R^2 values of 0.957 and 0.959 were observed between the exchangeable Cd in the spiked soils and the whole body tissue Cd concentrations in *E. fetida* exposed to spiked Cd 400 mg/kg with aging processes (180 and 360 days) (Fig. 3(b, c)). As reflected by the changes of the whole body tissue Cd concentrations in *E. fetida* exposed to each soil spiked with a series of Cd (Fig. S1), cadmium accumulation in *E. fetida* was conformed to obey the first-order dynamics. Significantly

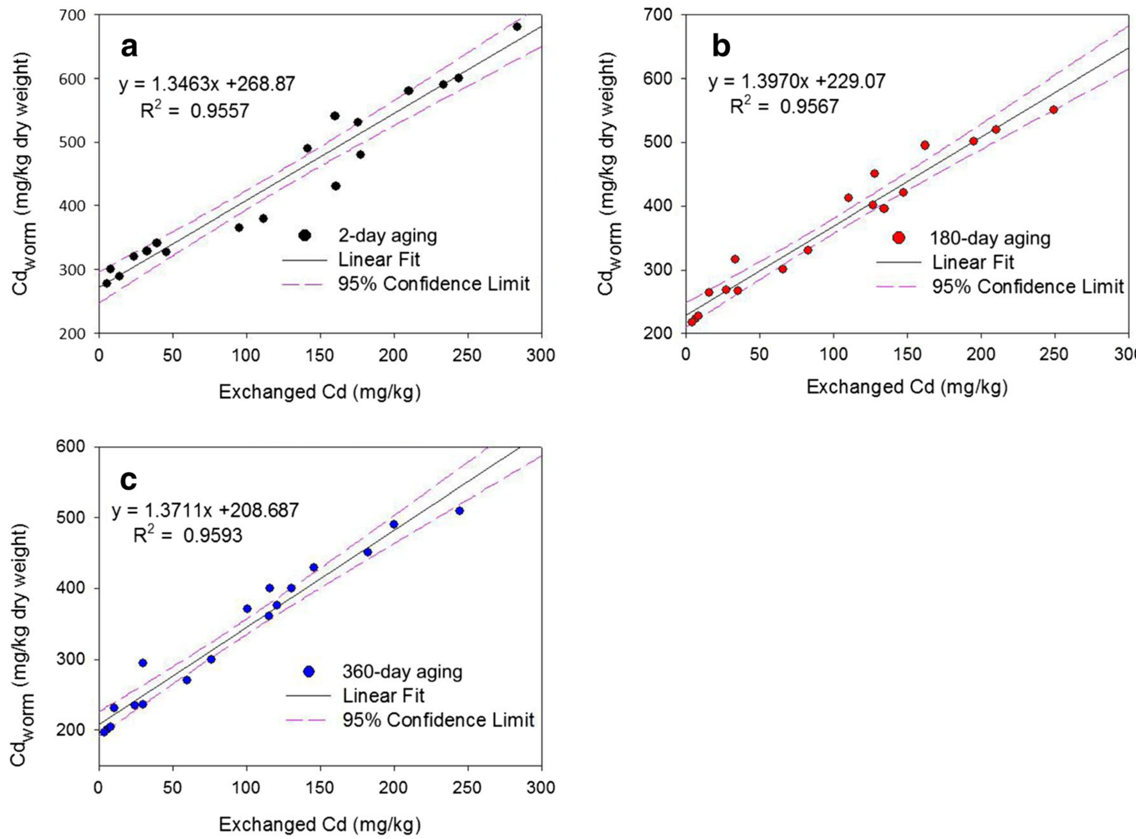

Fig. 3 Correlation between the exchangeable Cd after aging (2, 180, and 360 days) and whole body tissue concentrations of Cd in *E. fetida* after 14-day exposure to 18 soils spiked with 400 mg/kg Cd

positive correlations with all values (R^2) higher than 0.90 were observed between the whole body tissue concentrations of Cd in *E. fetida* and exchangeable Cd ($p < 0.05$) in the 18 soils with aging processes (2, 180, and 360 days), except yellow earth (Guiyang). Therefore, it was supported that soil properties affected the Cd bioavailability and toxicity to *E. fetida* mainly by controlling the distribution of the exchangeable Cd fraction.

Discussion

The present study has provided a systematic investigation regarding the soil factors controlling the expression of Cd toxicity to *E. fetida*, using a wide range of soils collected from 18 locations in China. The results of LC50s showed the sharp differences of Cd toxicity to *E. fetida* in different types of soils. LC50 values varied from 440.7 to 1520.4 mg/kg in the 18 freshly spiked soils. Previous studies showed the nickel median inhibition effect values (EC50s) of cocoon production of *E. fetida*, which varied from 53.7 to 2050.0 mg/kg in 13 European spiked soils (Van Eeckhout et al. 2005). Van Gestel and Koolhaas (2004) showed that Cd LC50 values to *Folsomia candida* were ranged from 665 and 1307 mg/kg in seven soil pH combinations.

Soil properties induced dramatic effects on the Cd bioavailability and toxicity to *E. fetida* and thus should be taken into account for the purpose of risk assessment. Significantly positive correlations between toxicity thresholds and soil pH and OM were obtained in the freshly spiked soils. In our study, soil pH and OM accounted for 89.2% variance for Cd LC50s. For validation of the empirical ecotoxicity model, we have collected datasets from published literature that reported Cd LC50s to *E. fetida* in the spiked field or artificial soils. The datasets have been provided in the supplementary information (Table S7). The model validation was performed with literature data on LC50, pH, and OM. The linear regression was drawn between model predicted and measured values of LC50 in literature (Fig. 4). The correlation coefficient R^2 value of 0.77 indicated that this model could be utilized to well predict acute toxicity of Cd to *E. fetida* in freshly spiked soils for a large variety of artificial soils and realistic soils sampled around world. The relationships between toxicity thresholds and soil pH suggested that the toxicity of Cd to *E. fetida* is relatively low in the alkaline soils due to the availability of more negatively charged sites in oxide minerals (Al-, Fe-, and Si-oxides) and humic constituents of these soils for strongly adsorbing cationic metals (Bradl 2004). It was also reported that the fraction of strongly adsorbed Cd was increased with an increase in pH in two Oxisols (Mena and Malanda) which

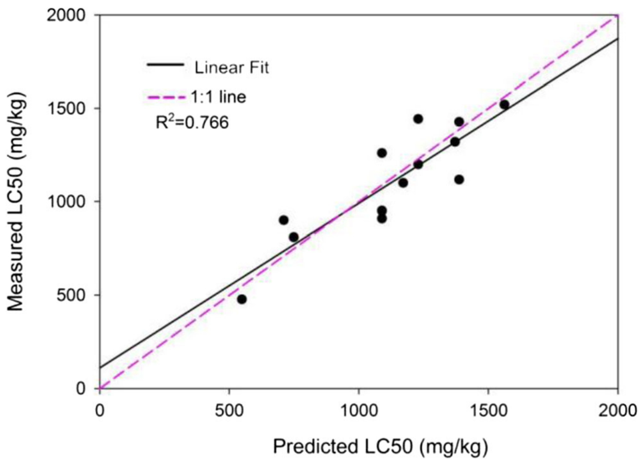

Fig. 4 Correlation between measured (data from literature) and predicted LC50 of Cd to *E. fetida*. The dash line indicates the 1:1 line

was mainly attributed to the increased negative surface charge (Naidu et al. 2010). The relationships between toxicity thresholds and soil OM suggested that the Cd toxicity to *E. fetida* can be alleviated by OM, and this alleviation is possibly attributed the complexation between Cd and OM (Zhou et al. 2014). Our previous results showed that various low molecular weight organic acid (LMWOA) Cd complex species were not accumulated in earthworm, and thus, they were considered to be unavailable and nontoxic to *E. fetida* in simulated soil solution (Liu et al. 2016). However, organic matter-bound Cd was also demonstrated to be toxic to a soil alga, *Chlorococcum* sp. (Vig et al. 2004). The inconsistence may be related to the tested species and conditions. In short, these findings in this present study are basically consistent with the previous studies that the OM and clay were the main factors affecting Cd toxicity to soil nematode *Caenorhabditis elegan* while pH and OM were the major factors for Cd bioavailability to *Daucua carota* L. (Boyd and Williams 2003; Ding et al. 2013). Our results are also in consensus with the previous studies of agricultural heavy metal (Cd and Cu) pollution remediation by applying alkaline materials (lime, apatite, and charcoal) or organic fertilizer to immobilize heavy metals in soils through regulating soil pH and organic matter (Cui et al. 2014; Farrell and Jones 2010).

Many previous studies have found that the total metal concentrations are not good predictors of Cd bioavailability and toxicity, which is mainly due to their dependency on its chemical speciation, especially the dissolved Cd in soil solution (Lock and Janssen 2001a; Peijnenburg and Jager 2003). Our results showed that significantly positive correlations were observed between the whole body tissue Cd concentrations in *E. fetida* and exchangeable Cd ($p < 0.05$) in all the 18 spiked soils. The large variations of exchangeable Cd concentrations in different soils with the same dosage were mainly caused by the differences in soil properties, especially soil pH and OM. The extracted Cd by a 0.01 mol/L $CaCl_2$ solution as the extracting agent in the present study could be implemented to directly reflect the soil dissolved Cd in natural soils, and the $CaCl_2$ method was recommended to be the best method as compared to other extracting agents, such as diethylene triamine pentacetic acid (DTPA) and HCl (Pueyo et al. 2004; Menzies et al. 2007).

The significant influence of aging processes (180 and 360 days) on acute Cd toxicity and bioavailability to *E. fetida*, as indicated by the increased LC50s and decreased exchangeable Cd in soils in present study, was in agreement with previous reports that aging significantly alleviated heavy metal toxicity (Ma et al. 2013; Sayen and Guillon 2014). Moreover, the decrease of EDTA exchangeable fractions of Cd in aged soils has also been reported (Udovic and Lestan 2009). In addition, Tang et al. (2006) showed that a sharp decreased bio-accessibility of Cd after 2-month aging in five typical Chinese soils might be mainly attributed to the gradual decrease in the exchangeable Cd fraction and the conversion to less labile fraction (carbonate-bound, Fe/Mn oxide-bound, organic-bound, and residual Cd fractions). It was also reported that about 16.7% of initial $MgCl_2$-exchangeable Cd transformed to EDTA-extractable (regarded as unlabile fraction) and residual forms in a Mollisol within 1 month, and the slow process of transformation was attributed to inner-sphere surface complexation via partial or complete dehydration of surface species (Ma and Uren 1998). Clearly, our study indicated that acute Cd toxicity and bioavailability to *E. fetida* were significantly lower with the prolonged aging time.

Conclusion

Soil properties substantially influenced the expression of Cd toxicity to *E. fetida*, which further resulted in a wide range of toxicity median lethal thresholds reported among different soils. The values of LC50s varied by 3.5-fold in *E. fetida* test with the freshly spiked Cd in the 18 soils sampled from different regions in China. Multiple regression analysis showed that soil pH and OM were the dominant factors and could be used to predict Cd LC50s. Cadmium bioaccumulation in *E. fetida* was positively correlated with exchangeable Cd in soils. Exchangeable Cd was more suited to assess bioavailability of Cd to *E. fetida*. The increase in LC50s and decrease in both the exchangeable Cd in soils and tissue Cd concentrations in earthworm whole body indicated that aging processes (180 and 360 days) could reduce the acute toxicity and bioavailability of Cd to *E. fetida*. Moreover, soil pH could be an ideal indicator to predict Cd LC50s in the soils after aging. The results of this study confirmed that the toxicity thresholds should be normalized on the key soil properties (pH and OM) prior to be utilized for Cd risk assessment. Meanwhile, the application of these models may lead to a more robust quantitative risk assessment of Cd-contaminated soils.

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