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SUSTAINABLE URBAN AGRICULTURE: VECTOR FOR ECOLOGICAL TRANSITION

Heavy metal availability, bioaccessibility, and leachability in contaminated soil: effects of pig manure and earthworms

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Abstract

A pot experiment and a leaching experiment were conducted to investigate the effects of earthworms and pig manure on heavy metals (Cd, Pb, and Zn) immobility, in vitro bioaccessibility and leachability under simulated acid rain (SAR). Results showed manure significantly increased soil organic carbon (SOC), dissolved organic carbon (DOC), available phosphorus (AP), total N, total P and pH, and decreased $CaCl_2$-extractable metals and total heavy metals in water and SAR leachate. The addition of earthworms significantly increased AP (from 0.38 to 1.7 mg kg^{-1}), and a downward trend in $CaCl_2$-extractable and total leaching loss of heavy metals were observed. The combined earthworm and manure treatment decreased $CaCl_2$-extractable Zn, Cd, and Pb. For $Na_4P_2O_7$-extractable metals, Cd and Pb were decreased with increasing manure application rate. Application of earthworm alone did not contribute to the remediation of heavy metal polluted soils. Considering the effects on heavy metal immobilization and cost, the application of 6% manure was an alternative approach for treating contaminated soils. These findings provide valuable information for risk management during immobilization of heavy metals in contaminated soils.

Keywords Metal availability · Leachability · Bioaccessibility · Pig manure compost · Earthworm · Simulated acid rain

Introduction

Soil heavy metal pollution has increased because of mining activities and the application of phosphate fertilizers and sewage sludge (biosolids) to the soil (Ghosh and Singh 2005; Redjala et al. 2009), posing a great threat to public health worldwide. Heavy metals can be transferred through the food chain and accumulate in the human body by intake of heavy metal-contaminated food (Zhuang et al. 2014). In addition, about 40% of the total territory in China is affected by acid rain, such as South China, the Yangtze River Basin, and Sichuan Basin (Huang et al. 2009). Acid rain can mobilize heavy metals and displace exchangeable base cations and accelerate mineral dissolution in the soil. Thus, it is an important and urgent need to seek effective methods to decrease the mobility and bioavailability of heavy metals under acid precipitation and restore the ecosystem in heavy metal-contaminated soils.

In situ immobilization by adding different types of reactive amendments has been considered as a promising soil remediation technique, in which the objective is not to remove heavy metals from soil but to reduce their mobility and bioavailability (Bolan et al. 2003; Paz-Ferreiro et al. 2014). Compost amendments added to contaminated soil can affect the bioavailability and mobility of heavy metals (O'Dell et al. 2007). The application of animal manure compost increased soil N, P, K, and organic matter (Eghball 2002) and the levels of pH (Wu et al. 2016), significantly immobilizing metals by complexation or precipitation (Karlsson et al. 2006). Compost was found to promote plant growth and decrease the uptake of Pb by crops, which was attributed to high metal-binding

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capacities of fulvic and humic acids in compost (Castaldi et al. 2005; O'Dell et al. 2007). Zhang et al. (2015) found that manure addition also increased the mobility (water-extractable fraction) of heavy metals in rhizosphere soil, which was mainly ascribed to the high content of dissolve organic carbon (DOC) in the manure. Furthermore, due to the alteration of soil pH, organic matter, and other inorganic salt content, manure compost amendment was reported to change the soil metal fractionation (Walker et al. 2004). To our knowledge, however, there is limited information about the influence of compost amendment combined with earthworm on heavy metal bioaccessibility in contaminated soils.

Earthworms are considered ecosystem engineers because of the significant role they play in organic matter degradation, nutrient cycling, aggregate stabilization, and soil porosity generation as well as in the transformation of chemical forms of heavy metals (Curry and Schmidt 2007; Jayasinghe and Parkinson 2009). According to Sizmur and Hodson (2009), metal availability was changed after earthworms' activity, due to the stimulation of the soil microbial population, alteration of soil pH, DOC, metal speciation, and sequestration within earthworm tissue. In general, most studies showed that earthworms increase metal mobility and availability (Wang et al. 2006; Wu et al. 2016; Zhu et al. 2014), while a few qualitative studies suggested that earthworm's activity decreased metal availability (Liu et al. 2005; Lukkari et al. 2006).

The objectives of this experiment were (1) to investigate the effect of manure compost and earthworms on soil heavy metal availability, leachability, and in vitro bioaccessibility and (2) to evaluate the impacts of earthworms and manure addition on heavy metal leachability under the SAR condition.

Materials and methods

Soils, manure compost, and earthworms

The soils contaminated by heavy metals (Cd, Pb, and Zn) were sampled from the plow layers (0–20 cm) of a cropland area, near a zinc/lead mine in the suburb of Lechang (25° 10′ N and 113° 20′ E), Guangdong Province, China. After being air-dried and homogenized, part of the soil samples were sieved to < 2 mm before physicochemical analysis, and some were passed through a 1-mm mesh sieve for the pot experiment to cultivate earthworms. The pig manures were obtained from an organic farm in Maoming (Guangdong Province). After being air-dried and ground, the manures were passed through a 0.149-mm mesh sieve. A part of the manures was used to conduct general analyses, and the rest was mixed with the subsample soils in sufficient amounts for the experiment. The selected chemical characteristics of the soil and pig manure compost are listed in Table 1. The epigeic earthworm *Eisenia fetida*, widely used in ecotoxicological studies, was chosen in this experiment due to its effect on soil physicochemical properties by consuming soil organic matter and forming aggregates. Earthworms were obtained from a farm breeding earthworms in Guangzhou. Before the experiment was started, earthworms were washed in distilled water and dried gently on filter paper, and then adult worms after species identification were placed in a pot for a week to adapt to the experimental conditions.

Pot experiment

The experiment, conducted in a temperature-controlled chamber, was in a randomized block design with four replicates for each treatment. The treatments were as follows: (1) control soil without pig manure and earthworms (CK); (2) soil amended with 3% (*w/w*) pig manure (M1), (3) soil amended with 6% pig manure (M2); (4) soil amended with 12% (M3) pig manure; (5) soil with earthworms only (E); (6) soil amended with both earthworms and 3% (*w/w*) pig manure (EM1); (7) soil amended with both earthworms and 6% pig manure (EM2); and (8) soil amended with both earthworms and 12% pig manure (EM3).

Before transfer into plastic pots (15 cm in diameter and 12 cm in height), the sample soil, weighing 300 g, was completely mixed with the pig manure. Then, distilled water was added to each pot to keep the soil at 60% of water holding capacity, and the wetted soil was equilibrated for 3 days. Finally, six adult earthworms weighing about 200 mg after gut voiding were placed in the pots. In order to provide the earthworms a favorable environment, the pots were moved into a temperature-controlled chamber with no light and a temperature of 20 °C. To maintain 60% of water holding capacity, all the pots were weighed every 2 days, and distilled water was added to the soils in order to replenish moisture losses. This pot culturing experiment lasted for a month.

Leaching experiment

The leaching period was designed to represent 4 years of local annual precipitation (1680 mm) with a 30% surface runoff loss. Water and SAR were chosen as the leaching solutions for the leaching experiment, which lasted for 30 days. In South China, acid rain is primarily made up of H_2SO_4 and HNO_3 with a volumetric ratio of 4:1 (Fang et al. 2013). In Guangzhou, acid rain has been measured to have a pH value of 3 (Fang et al. 2013).

After culturing the pots for 1 month as described above, all 32 pots were taken from the chamber, and the earthworms were removed. The soils in each pot were sieved to < 2 mm after air-drying. A fraction of each soil was saved for chemical analyses, and the remainder was divided into two parts to conduct the leaching experiment. Long neck funnels fitted with two pieces of filter paper were filled with 50 g soil.

Table 1 Chemical characteristics of soil and pig manure compost ($n = 3$)

Material	pH	OM (%)	Total N (%)	Total P (%)	Available P (mg kg^{-1})	Total Cd (mg kg^{-1})	Total Pb (mg kg^{-1})	Total Zn s(mg kg^{-1})
Soil	3.64 ± 0.16	3.64 ± 0.19	0.53 ± 0.03	0.08 ± 0.00	0.39 ± 0.04	1.53 ± 0.12	2088 ± 60.9	1438 ± 24.8
PMC	9.81 ± 0.67	33.8 ± 1.48	3.30 ± 0.12	2.70 ± 0.07	2917 ± 40.5	0.11 ± 0.01	31.7 ± 1.10	52.5 ± 8.47

PMC denotes pig manure compost

There were 64 funnels needed, 32 for water leaching and 32 for SAR leaching. Before the first leaching step, the air-dried soil in the funnel was brought to 60%water holding capacity with distilled water. According to local annual precipitation (1680 mm), and assuming 30% surface runoff loss, the equivalent total leaching volume of leaching solution was estimated to be 900 ml. So, 30-ml leaching solution was used for each day in the leaching experiment.

To understand the dynamic change of heavy metal leaching, heavy metal concentrations were measured in the leachate sampled on days 1, 8, 15, 22, and 29 after the first SAR (or distilled water) addition. In order to measure the total metals leached for the entire leaching procedure, leachate was collected each day for each funnel, and 1/10 of the leachate was reserved in a glass beaker. The same beakers were used to accumulate this daily leachate until the experiment was finished.

In vitro evaluation of bioaccessibility

In vitro bioaccessibilities of Cd, Pb, and Zn were measured using the physiologically-based extraction test (PBET) method, which was modified from the previously described method (Ruby et al. 1993; Zhuang et al. 2016). The PBET contains two phases which simulate the digestive processes in the stomach and small intestines of human. The simulated gastric solution contained 1.25 g L^{-1} pepsin, 0.50 g L^{-1} citric acid, 0.50 g L^{-1} maleic acid, 420 μl L^{-1} DL-lactic acid, and 500 μl L^{-1} acetic acid dissolved in water and was adjusted to pH 1.5 with HCl. The soil-gastric solution mixture was put in an incubator-rotary shaker at 150 rpm for 1 h at 37 °C. Finally, before analysis, the solution was filtered with Whatman no. 42 paper after being centrifuged at 3000 rpm for 10 min. In the second stage, the gastric solution was adjusted to pH 7 with saturated $NaHCO_3$ and 52.5 mg bile salts, and 15 mg pancreatin was added to simulate gastrointestinal digestion. All the samples were put in a thermostatic bath maintained at 37 °C for 4 h, and then the obtained supernatant was filtered and analyzed for Cd, Pb, and Zn by ICP-MS (Agilent 7700×, Agilent Scientific Technology Ltd., USA). The in vitro bioaccessibility of Cd, Pb, and Zn in each soil sample was defined as the ratio of the bioaccessible fraction to the total concentration:

$$\text{Bioaccessibility}\ (\%) = \frac{\text{Bioaccessible metal concentration}}{\text{Total metal concentration in soil}} \times 100$$

Soil chemical analyses

The air-dried soil samples were measured for pH (using a pH meter with 1:2.5 (*w*/*v*) soil to water ratio), organic carbon (potassium dichromate oxidation and titration with ferrous ammonium sulfate), available P (molybdate blue colorimetric method, using 0.03 M NH_4F–0.025 M HCl extraction), total N and total P (micro-Kjeldahl digestion followed by colorimetric determination, FIA, Lachat Instruments, USA), and dissolved organic carbon (DOC, using 0.5 M potassium sulfate with 4:1 (*v*:*w*) to soil, measured by Shimadzu TOC VSCH). The mobile forms of Cd, Pb, and Zn in the soils were extracted by 0.01 M $CaCl_2$ (Ure et al. 1995), and chemical extraction with 0.1 M $Na_4P_2O_7$ was selected to measure the organically bound metals (Silva et al. 1993). Total soil Cd, Pb, and Zn were determined (digested with HNO_3-$HClO_4$-HF) according to the methods described by Zhuang et al. (2009). All the metal concentrations were analyzed using an ICP-OES spectrometer (Optima 2000). A standard reference material GBW08303 was used for quality assurance with the recovery rates for Cd (91 to 103%), Pb (90 to 104%), and Zn (94 to 104%).

Statistical analysis

Statistical analyses (calculation of means and standard deviations, differences between treatments) were performed using SPSS 20.0 package. Some variables were transformed to achieve normality and homoscedasticity before data analysis when necessary. Differences of means were tested using a two-way ANOVA with the presence/absence of earthworms and the type of amendment (different levels of pig manures) as factors. Fisher's unprotected Least Significant Difference (LSD) test was used to test for significant differences between treatments.

Result and discussion

Soil chemical properties

Soil chemical properties of the different treatments in the pot experiment are shown in Table 2. The contents of organic C, total N, total P, available P, and pH level in the presence of manure or combined with earthworms significantly increased ($p < 0.01$) with the increasing application rates of manures. The contents of organic C, total N, total P, and DOC in M3 treatment increased by 1.64, 2.02, 6.18, and 3.25 times compared to the control. This result was consistent with previous studies (Blanchet et al. 2016; Eghball 2002; Zhou et al. 2015). Compared to manure application, earthworm activity did not affect the contents of SOC, total N, total P, pH, and DOC. In the presence of earthworms and manure (EM treatment), the contents of SOC, total P, DOC, and pH level were lower than those in the M3 treatment. However, earthworms significantly increased available P (from 0.38 mg kg^{-1} in the control to 1.70 mg kg^{-1} in E treatment), suggesting that the activity of earthworms had a role in changing forms of phosphorus. This could be explained by the increased mineralization of organic P due to earthworm activity, resulting in high levels of soluble P in the earthworm casts and causing higher available P in the bulk soils (Vos et al. 2014). In general, earthworms tend to change soil properties depending on soil conditions and earthworm ecological types (Brown et al. 2000).

Heavy metal availability extracted by $CaCl_2$ and $Na_4P_2O_7$

Soil $CaCl_2$-extractable and $Na_4P_2O_7$-extractable metals in the pot experiment are shown in Table 3. The concentrations of $CaCl_2$-extractable Cd, Pb, and Zn were 34.4 μg kg^{-1} and 5.65 and 6.09 mg kg^{-1}, respectively, in the control. Substantial reductions by more than 10, 100, and 50 times, respectively, of $CaCl_2$-extractable Cd, Pb, and Zn were observed in the presence of manure compost compared to the untreated soils, meaning that heavy metals of exchangeable fraction were obviously decreased with manure addition. These results indicated that manure addition played a vital role in decreasing soil heavy metal availability. The addition of manure compost may have increased heavy metal immobilization through the following reactions, all of which are enhanced by the raised pH of the manure-amended soil (Table 2). Firstly, the manure compost amendment-induced metal immobilization is ascribed to the increases in surface negative charge (Bolan et al. 2003a) resulting from the added organic matter. Then, the enhancement of metal retention could also be attributed to the presence of carbonates, phosphates, Al, and Fe oxides as well as other inorganic minerals in manure compost (Katoh et al. 2014). Lastly, metal adsorption is increased by the formation of organo-metal complexes when organic amendments are added (Pare et al. 1999). It is notable that the reductions of $CaCl_2$-extractable Cd, Pb, and Zn in M2 treatment (6% manure) were 92.4, 99.3, and 98.5%, respectively, compared with the control treatment. However, there were no significant differences in $CaCl_2$-extractable metals between M2 and M3 treatments, suggesting 12% manure addition had a similar effect on metal immobilization in comparison with 6% manure addition. Fleming et al. (2013) also reported that soil Pb extraction by ammonium acetate was reduced by compost amendment. In the present study, earthworms did not change the concentrations of $CaCl_2$-extractable metals compared to the untreated soil (Table 3), revealing that earthworms had no potential to immobilize soil heavy metals. The manure compost–earthworm-combined treatment showed no significant effect on $CaCl_2$-extractable metals compared to the manure compost alone.

In the untreated soils, the concentrations of $Na_4P_2O_7$-extractable Cd, Pb, and Zn were 27.8 μg kg^{-1} and 49.1 and 20.2 mg kg^{-1}, respectively. Just like the $CaCl_2$-extractable heavy metals, the presence of manure compost also significantly decreased $Na_4P_2O_7$-extractable Cd ($p < 0.01$), Pb ($p < 0.01$), and Zn (non-significant changes in M3 and EM3) when compared to the untreated soils, suggesting that the organic-bond fraction of heavy metals were also affected by manure addition (manure addition alone or combining with earthworms). In the present study, the trend for $Na_4P_2O_7$-extractable Zn was different (decreased in M1 and then increased in the higher compost treatments) from Cd and Pb (decreased more in the higher compost treatments). We speculated that more Zn tended to bind with organic matter with increasing manure addition, while Cd and Pb were inclined to bind with other components, like Fe and Mn oxide. A similar result by Quenea et al. (2009) noticed that Zn was more likely to associate with organic matter, and a strong interaction was found between Cd and Pb and other soil components, like oxides and minerals.

With the bioturbation by earthworms, $Na_4P_2O_7$-extractable Cd, Pb, and Zn were significantly decreased by 10, 32.4, and 44.3%, respectively, compared to CK ($p < 0.01$). It could be partly explained by the significant increase of available P (Table 2), which might compete with organic matter to bind heavy metals by forming phosphate complexes. In the present study, there was a similar trend in $Na_4P_2O_7$ extraction when comparing the treatment combining manure with earthworms and manure only, suggesting earthworm activity had no remarkable effect on mobility of metals (Table 3). Our previous study (Wu et al. 2016) also suggested that the presence of earthworms had little effect on exchangeable and organic-bound fractions of Cd in soil aggregates.

Generally, for the same treatment, the concentrations of Cd, Pb, and Zn extracted by $Na_4P_2O_7$ were higher than those extracted by $CaCl_2$. Pare et al. (1999) observed that stabilized organic matter formed strong complexes with metals, a

Table 2 Soil chemical properties after 30 days of incubation

Treatments	Organic C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Available P (mg kg^{-1})	Soil pH	DOC (mg kg^{-1})
CK	35.5 ± 1.16e	2.60 ± 0.08e	0.79 ± 0.02e	0.38 ± 0.04g	3.62 ± 0.03f	243 ± 17.5d
M1	37.1 ± 0.86e	3.07 ± 0.15d	1.64 ± 0.12d	14.1 ± 0.54e	4.58 ± 0.04e	216 ± 18.8ef
M2	44.2 ± 0.65cd	3.87 ± 0.09b	2.45 ± 0.11b	137 ± 4.63c	5.93 ± 0.02c	372 ± 22.8c
M3	58.2 ± 1.21a	5.24 ± 0.11a	4.88 ± 0.20a	638 ± 34.2a	6.56 ± 0.07a	791 ± 70.9a
E	36.4 ± 1.92e	2.49 ± 0.12e	0.79 ± 0.01e	1.70 ± 0.12f	3.70 ± 0.04f	215 ± 2.67de
EM1	40.8 ± 1.13d	3.40 ± 0.09cd	1.58 ± 0.04d	30.9 ± 1.53d	4.82 ± 0.04d	181 ± 7.25f
EM2	45.3 ± 1.06c	3.70 ± 0.14bc	1.89 ± 0.08c	163 ± 10.9b	5.90 ± 0.08c	347 ± 12.6c
EM3	52.9 ± 1.40b	5.03 ± 0.15a	2.64 ± 0.09b	600 ± 13.6a	6.27 ± 0.08b	610 ± 14.4b
Analysis of variance (*p* values)						
Manures (M)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Earthworms (E)	0.704	0.88	< 0.001	0.003	0.004	0.003
M × E	0.011	0.123	< 0.001	0.001	< 0.001	0.033

Data are means ± SE ($n = 4$). Means followed by different small letters within the different treatment column are significantly different ($p < 0.05$, LSD test). *p* values are listed for treatment effects based on two-way ANOVA analysis

DOC, soil dissolved organic carbon; *CK*, control; *M1*, 3% manure; *M2*, 6% manure; *M3*, 12% manure; *E*, earthworms; *EM1*, earthworms + 3% manure; *EM2*, earthworms + 6% manure; *EM3*, earthworms + 12% manure

process that was responsible for decreases in metal mobility and availability. Therefore, the reductions of $CaCl_2$-extractable metals associated with manure amendments in our results could be explained by increases of organic C, total N, available P, and the soil pH, all of which are strongly correlated with metal availability (Wu et al. 2016; Zhou et al. 2015).

Although a larger evidence base suggests an increase in metal availability due to earthworm activity in soils (Leveque et al. 2014; Wang et al. 2006), there are a few examples of earthworm activity decreasing metal availability in contaminated soils (Liu et al. 2005). These results were in agreement with the report by Lukkari et al. (2006) who found that earthworms decreased metal mobility and availability through burrowing activity. Ma et al. (2006) also reported that the activity of earthworm *Pheretima guillelmi* decreased the concentrations of Pb and Zn in ammonium acetate (NH_4OAc) extractions. It is well-known that earthworm activity directly influences soil properties by burrowing and ingesting soils (Brown et al. 2000). Therefore, reduction in heavy metal availability with earthworm activity might relate to the

Table 3 $CaCl_2$-extractable and $Na_4P_2O_7$-extractable Cd, Pb, and Zn of soil after 30 days of incubation

Treatments	$CaCl_2$-Cd (μg kg^{-1})	$Na_4P_2O_7$-Cd (μg kg^{-1})	$CaCl_2$-Pb (mg kg^{-1})	$Na_4P_2O_7$-Pb (mg kg^{-1})	$CaCl_2$-Zn (mg kg^{-1})	$Na_4P_2O_7$-Zn (mg kg^{-1})
CK	34.4 ± 0.39a	27.8 ± 0.57a	5.65 ± 0.23a	49.1 ± 1.95a	6.09 ± 0.18a	20.2 ± 1.13a
M1	13.4 ± 1.47b	20.9 ± 0.50c	0.51 ± 0.06c	27.8 ± 0.63c	2.39 ± 0.21b	14.7 ± 0.97b
M2	2.60 ± 0.11c	17.4 ± 0.46d	0.04 ± 0.00d	22.7 ± 1.31d	0.09 ± 0.01de	15.4 ± 0.39b
M3	2.15 ± 0.10c	15.2 ± 0.35e	0.03 ± 0.00d	15.6 ± 0.68f	0.03 ± 0.00g	18.9 ± 1.06a
E	34.0 ± 0.13a	25.0 ± 0.48b	5.12 ± 0.04b	33.2 ± 0.94b	5.93 ± 0.17a	11.3 ± 0.05c
EM1	11.7 ± 0.60b	20.3 ± 0.19c	0.37 ± 0.03c	23.0 ± 1.20d	1.90 ± 0.09c	11.5 ± 0.04c
EM2	2.50 ± 0.10c	17.7 ± 0.93d	0.04 ± 0.00d	21.7 ± 1.84de	0.08 ± 0.01ef	15.7 ± 1.61b
EM3	2.25 ± 0.10c	15.5 ± 0.73e	0.04 ± 0.00d	18.6 ± 1.66ef	0.06 ± 0.00f	20.8 ± 0.91a
Two-way ANOVA *p* values						
Manures (M)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Earthworm (E)	0.226	0.086	0.011	<0.001	0.093	< 0.001
M × E	0.437	0.044	0.014	<0.001	0.151	< 0.001

Data are means ± SE ($n = 4$). Means followed by different small letters within the different treatment column are significantly different ($p < 0.05$, LSD test). *p* values are listed for treatment effects based on two-way ANOVA analysis

CK, control; *M1*, 3% manure; *M2*, 6% manure; *M3*, 12% manure; *E*, earthworms; *EM1*, earthworms + 3% manure; *EM2*, earthworms + 6% manure; *EM3*, earthworms + 12% manure increase in organic C, total N, and available P and the decrease in DOC after earthworm activity. Cheng and Wong (2002) found that earthworm activity enhanced organic-Zn and $MgCl_2$-extractable Zn in a hydragric paddy soil, while the same earthworm type was found to decrease organic-Zn but increase iron oxide binding of Zn in an alluvial soil.

Heavy metals in effluent after leaching with water and SAR

The concentrations of Cd, Pb, and Zn in the leachate after leaching with water and SAR are illustrated in Fig. 1. In general, Cd and Zn concentrations in both leachates of all treatments decreased over the 29-day leaching time. The Pb concentration in both leachates showed a different pattern, increasing from day 1 to 15 (for both control and earthworm treatments) or 8 (for all the other treatments), then decreasing. After repeated leaching with water and SAR, metal concentrations in both leachates reached a steady state near the end of leaching experiment. The present results indicate that manure application contributed to strong metal binding in soil with less leaching loss. The maximum concentrations of Cd, Pb, and Zn in leachates from the control, and earthworm treatments were higher than grade V (0.01, 0.1, and 2.0 mg L^{-1} for Cd, Pb, and Zn, respectively) of Chinese national environmental quality standards for surface water (SEPAC, GB 3838-2002) (Fig. 1). The application of manure significantly reduced the leaching loss from the both water and SAR effluent. In fact, the Cd and Zn concentrations in leachates were below the grade III (0.005, 0.05, and 1.0 mg L^{-1} for Cd, Pb, and Zn, respectively) after amending soil with 6 and 12% manure (Fig. 1). These results suggest that Cd, Pb, and Zn leached from mine spoil-contaminated soils could pose a risk of water pollution. Manure addition was found to reduce the risk of heavy metals leaching into the environment, with the application of 6% manure (M2) appearing to be an appropriate amendment to mitigate metal leaching.

The cumulative leaching losses of soil Cd, Pb, and Zn in the leachates are shown in Table 4. Compared to the untreated soil, a pronounced decrease of total Cd, Pb, and Zn in water and SAR leachate was observed in the presence of manure compost ($p < 0.01$); for example, there were reductions of 59.3 and 96.4% of Cd in water leachate in the M1 and M3 treatment, respectively. The leaching losses of metals with SAR as the leaching solution were higher than those with pure water (Table 4). Ling et al. (2007) reported that about 34, 46, 20, and 77% of the original exchangeable soil Ca^{2+}, Mg^{2+}, K^+, and Na^+, respectively, are leached out of soil by SAR at pH 2.5 after 21 days. The H^+ ion in acid rain promotes not only base cation but also heavy metal desorption by displacing metal cations from their binding sites and consequently promotes the leaching process (Wilson and Bell 1996).

Schwab et al. (2007) found that aged cattle manure amendments increased Cd, Pb, and Zn in leachate due to the high content of water soluble organic carbon. However, our results indicated that manure amendment had beneficial effect on retaining heavy metals in the soil, which was in agreement with Zhang et al. (2015), who found that organic amendments decreased heavy metal solubility in sediments. Manure application increased soil organic matter and pH, which led to heavy metal immobilization, attributed mainly to adsorption, complexation, and surface precipitation (Liu et al. 2009; Zhou and Haynes 2010). Additionally, manure amendment may lead to an increment of carbonate, phosphate, and other salts, resulting in precipitation of insoluble metal compounds and decreased metal solubility (Walker et al. 2003). In addition, an increase of soil pH due to manure addition may have an important role in metal retention by increasing metal sorption onto negatively charged surface sites (Bradl 2004). Nevertheless, the increase of DOC resulting from manure addition can at the same time enhance the complexation of metals by soluble organic ligands, resulting in an increase of metal concentrations in the leachate (Houben et al. 2012). Therefore, the overall effect of manure addition on heavy metal mobility depends on the result of the counteracting effects of metal retention and metal mobilization. The present results indicate that application of manure compost decreases the risk of water pollution with heavy metals due to metal retention. Balancing the beneficial effects with cost, 6% manure compost addition appears to be an appropriate soil amendment to protect groundwater from toxic metals.

In vitro bioaccessibility of heavy metals

The soil concentrations of in vitro bioaccessible Cd, Pb, and Zn in the gastric and gastrointestinal fractions (determined by in vitro PBET methods) are presented in Table 5. In the untreated soil, the in vitro bioaccessibility of Cd, Pb, and Zn was 28.2, 59.8, and 6.25% in gastric phase and 12.2, 3.91, and 2.30% in gastrointestinal phase, respectively. Cadmium, Pb, and Zn bioaccessibility in the gastric fractions was significantly higher ($p < 0.01$) than those in the gastrointestinal fractions for all the treatments. These results can be explained by the fact that metals solubilized by the quite low pH of the gastric phase were partially precipitated and/or resorbed in the higher pH gastrointestinal phase (Mounicou et al. 2002). Application of manure or earthworms did not change Cd bioaccessibility except for M3 treatment. The Cd bioaccessibility in both the gastric and gastrointestinal phase increased after amendment by 12% manure. Similarly, Cui et al. (2011) found Cd bioaccessibility was increased by mustard leaf addition in the gastric and small intestinal phase due to the higher content of DOC. In this study, based on the extraction rates and leaching loss (Table 4), we found that the solubility of Cd (in both G and GI fractions), Pb (in GI fraction), and Zn (in G fraction) by

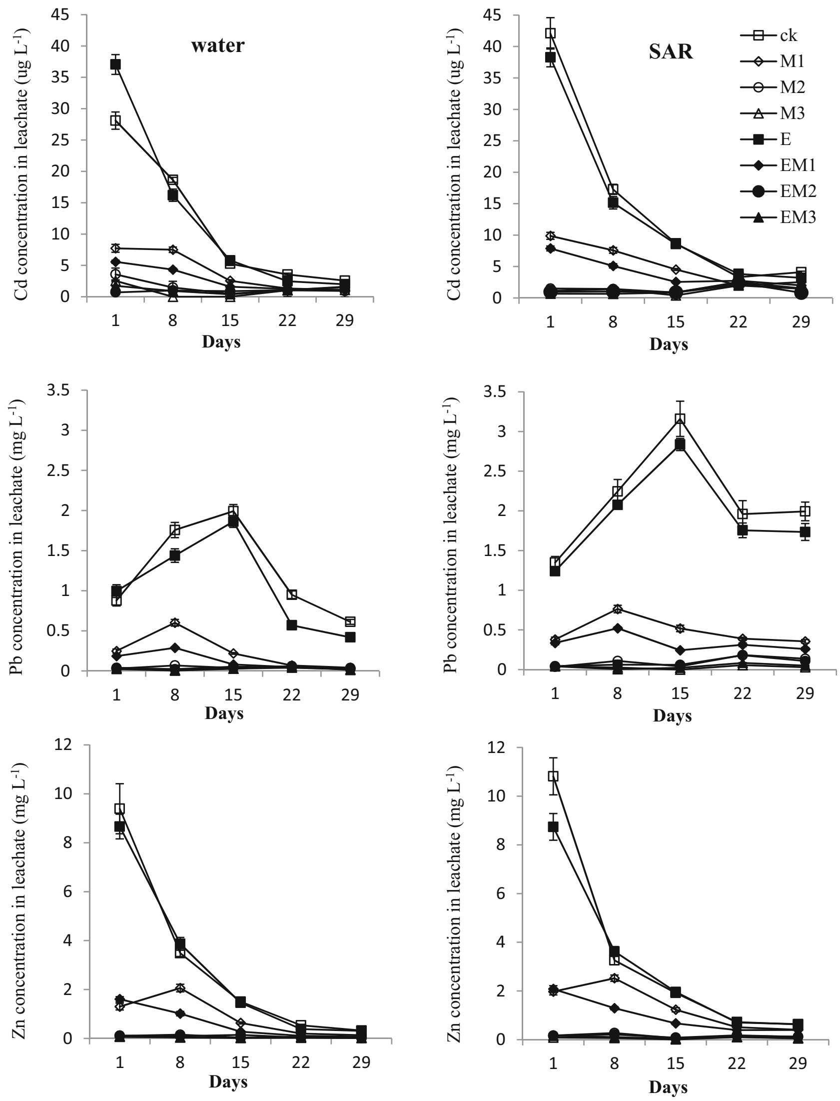

Fig. 1 Concentration of Cd, Pb, and Zn in water and SAR (simulated acid rain) leachate for the different treatment. CK, control; M1, 3% manure; M2, 6% manure; M3, 12% manure; E, earthworms; EM1, earthworms + 3% manure; EM2, earthworms + 6% manure; EM3, earthworms + 12% manure

PBET method can reflect the results of leaching loss in the untreated soils. We suggested that PBET method can be used to predict the in vitro bioaccessibility of heavy metals in contaminated soils. These results were consistent with Li and Zhang (2013), who reported besides EDTA extraction, the PBET method could be applied to establish the in vitro Cu, Pb, and Zn bioaccessibility in mildly acidic soils.

In the presence of manure or earthworms, the bioaccessibility of Pb in both gastric and gastrointestinal fractions and Zn bioaccessibility in the gastrointestinal fraction were lower than those in the control. Conversely, Zn bioaccessibility in the gastric phase showed an upward trend as the application rate of manure increased. A similar trend was observed in the $Na_4P_2O_7$-extractable Zn fraction in treated soils with the application of manure or combination with earthworms. In vitro bioaccessibilities estimated from the gastrointestinal phase of the PBET assay tend to reflect more differences depending on element and soil type in comparison with those of the gastric phase (Li and Zhang 2013). Soil factors, such as pH, SOC, cation exchange capacity, and soil particle size distribution, strongly affect the in vitro bioaccessibility of metals (Luo et al. 2012). It has been suggested that earthworms affect metal speciation and in vitro bioaccessibility (Tica et al. 2013), a result expected from their influence on soil properties (Tang et al. 2008). The results in this study indicated earthworm activities did not obviously affect the in vitro bioaccessibility of heavy metals.

Conclusion

The pot experiment revealed that soil organic C, DOC, Total N, total P, available P, and pH in the presence of manure were significantly higher than those in the control soil. Earthworm activities did not change the above mentioned soil chemical properties; whereas, they remarkably increased available P.

Table 4 Total metals in the leachates of different treatments after 30 days of leaching

Treatments	Total Cd in the leachate (μg)		Total Pb in the leachate (mg)		Total Zn in the leachate (mg)	
	Water	SAR	Water	SAR	Water	SAR
CK	16.3 ± 1.62a	18.4 ± 0.66a	1.80 ± 0.07a	3.12 ± 0.17a	3.90 ± 0.26a	4.44 ± 0.27a
M1	6.64 ± 0.68b	7.97 ± 0.48b	0.33 ± 0.03c	0.76 ± 0.06b	1.64 ± 0.15b	2.23 ± 0.13b
M2	1.60 ± 0.11d	1.74 ± 0.09d	0.06 ± 0.00fg	0.08 ± 0.00de	0.09 ± 0.01d	0.22 ± 0.02d
M3	0.59 ± 0.04f	0.62 ± 0.04f	0.05 ± 0.00g	0.05 ± 0.00f	0.04 ± 0.00f	0.09 ± 0.01g
E	14.5 ± 0.87a	16.3 ± 0.51a	1.45 ± 0.06b	2.83 ± 0.06a	3.54 ± 0.12a	4.16 ± 0.12a
EM1	2.90 ± 0.14c	5.38 ± 0.26c	0.18 ± 0.01d	0.47 ± 0.04c	0.82 ± 0.02c	1.28 ± 0.04c
EM2	1.32 ± 0.07e	1.50 ± 0.11d	0.06 ± 0.00fg	0.09 ± 0.01d	0.08 ± 0.01de	0.17 ± 0.01e
EM3	0.69 ± 0.04f	0.83 ± 0.09e	0.07 ± 0.01ef	0.07 ± 0.00e	0.07 ± 0.00e	0.14 ± 0.01f
Analysis of variance (*p* values)						
Manures (M)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Earthworms (E)	< 0.001	< 0.001	< 0.001	0.011	0.002	0.001
M × E	0.001	0.001	<0.001	0.037	0.003	0.001

Data are means ± SE ($n = 4$). Means followed by different small letters within the different treatment column are significantly different ($p < 0.05$, LSD test). *p* values are listed for treatment effects based on two-way ANOVA analysis

CK, control; *M1*, 3% manure; *M2*, 6% manure; *M3*, 12% manure; *E*, earthworms; *EM1*, earthworms + 3% manure; *EM2*, earthworms + 6% manure; *EM3*, earthworms + 12% manure; *SAR*, simulated acid rain

Application of manure effectively reduced $CaCl_2$-extractable and $Na_2P_4O_7$-extractable metals, bioaccessible metals, and leaching losses, as a result of metal immobilization. It was noteworthy that SAR leached more heavy metals than pure water, indicating that more attention should be paid to groundwater quality of heavy metal-contaminated sites impacted by acid rain. Considering the cost and the efficacy of heavy metal immobilization and leachability, 6% manure application is an appropriate soil treatment to immobilize heavy metals and prevent leaching. The results of the present study provide insight and guidance for soil remediation using manure compost.

Table 5 Bioaccessibility of Cd, Pb, and Zn (%, in gastric and gastrointestinal phase) of the soil after a 30-day pot cultivation measured by PBET method

Treatments	Cd		Pb		Zn	
	Gastric	Gastrointestinal	Gastric	Gastrointestinal	Gastric	Gastrointestinal
CK	28.2 ± 0.70b	12.2 ± 0.37b	59.8 ± 1.79a	3.91 ± 0.34a	6.25 ± 0.42b	2.30 ± 0.06a
M1	29.8 ± 0.69b	13.2 ± 0.32b	49.8 ± 1.31c	3.35 ± 0.10ab	5.46 ± 0.29cd	1.58 ± 0.06bc
M2	28.0 ± 1.40b	13.1 ± 0.85b	49.5 ± 0.09c	2.66 ± 0.11cd	6.44 ± 0.10b	0.41 ± 0.06e
M3	33.0 ± 1.53a	16.8 ± 1.41a	46.9 ± 0.52cd	3.12 ± 0.20bc	10.5 ± 0.44a	0.12 ± 0.01f
E	27.1 ± 1.12b	12.4 ± 1.21b	56.1 ± 2.42b	3.54 ± 0.43ab	5.45 ± 0.03cd	2.03 ± 0.15ab
EM1	27.6 ± 0.70b	11.1 ± 0.98b	45.6 ± 0.81d	3.04 ± 0.13bc	4.88 ± 0.08d	1.44 ± 0.06c
EM2	26.8 ± 0.79b	13.1 ± 0.70b	47.5 ± 0.46cd	2.90 ± 0.16bcd	6.32 ± 0.07b	0.72 ± 0.07d
EM3	28.8 ± 0.81b	12.5 ± 0.74b	46.2 ± 0.51cd	2.49 ± 0.12d	10.3 ± 0.40a	0.37 ± 0.03e
Two-way ANOVA *p* values						
Manures (M)	0.008	0.041	$p < 0.001$	0.001	$p < 0.001$	$p < 0.001$
Earthworm (E)	0.006	0.023	0.006	0.109	0.04	0.474
M × E	0.418	0.067	0.494	0.315	0.621	0.001

Data are means ± SE ($n = 4$). Means followed by different small letters within the different treatment column are significantly different ($p < 0.05$, LSD test). *p* values are listed for treatment effects based on two-way ANOVA analysis

CK, control; *M1*, 3% manure; *M2*, 6% manure; *M3*, 12% manure; *E*, earthworms; *EM1*, earthworms + 3% manure; *EM2*, earthworms + 6% manure; *EM3*, earthworms + 12% manure

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