



Potato absorption and phytoavailability of Cd, Ni, Cu, Zn and Pb in sierozem soils amended with municipal sludge compost

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Abstract: Effects of sludge utilization on the mobility and phytoavailability of heavy metals in soil-plant systems have attracted broad attention in recent years. In this study, we analyzed the effects of municipal sludge compost (MSC) on the solubility and plant uptake of Cd, Ni, Cu, Zn and Pb in a soil-potato system to explore the mobility, potato plant uptake and enrichment of these five heavy metals in sierozem soils amended with MSC through a potato cultivation trial in Lanzhou University of China in 2014. Ridge regression analysis was conducted to investigate the phytoavailability of heavy metals in amended soils. Furthermore, CaCl_2 , $\text{CH}_3\text{COONH}_4$, CH_3COOH , diethylene triamine pentacetic acid (DTPA) and ethylene diamine tetraacetic acid (EDTA) were used to extract the labile fraction of heavy metals from the amended soils. The results show that the MSC could not only improve the fertility but also increase the dissolved organic carbon (DOC) content of sierozem soils. The total concentrations and labile fraction proportions of heavy metals increase with increasing MSC percentage in sierozem soils. In amended soils, Cd has the highest solubility and mobility while Ni has the lowest solubility and mobility among the five heavy metals. The MSC increases the concentrations of heavy metals in the root, stem, peel and tuber of the potato plant, with the concentrations being much higher in the stem and root than in the peel and tuber. Among the five heavy metals, the bioconcentration factor value of Cd is the highest, while that of Ni is the lowest. The complexing agent (DTPA and EDTA) extractable fractions of heavy metals are the highest in terms of phytoavailability. Soil properties (including organic matter, pH and DOC) have important impacts on the phytoavailability of heavy metals. Our results suggest that in soil-potato systems, although the MSC may improve soil fertility, it can also increase the risk of soils exposed to heavy metals.

Keywords: municipal sludge compost; amended soils; heavy metals; mobility; ridge regression; phytoavailability

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1 Introduction

Heavy metals, being certain metallic elements or metalloids that have a high density (over 4.5 g/cm^3), are toxic or harmful (Nagajyoti et al., 2010). Many heavy metals (e.g., Cu, Ni, Zn, Mn) needed by plants and humans are essential elements, which are involved in numerous physiological processes (Rengel, 2004; Hänsch and Mendel, 2009). However, some (e.g., Cd, Hg, As, Pb) are non-essential elements and are very toxic to flora and fauna (Sherameti and Varma,

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2011). Essential heavy metals are also toxic when their concentrations exceed certain levels. Thus, sewage sludge-borne high level of heavy metals has been considered to be a risk for its application to soils (Wang, 1997; Singh and Agrawal, 2007). Heavy metals cannot be broken down by microorganisms. They may concentrate in plants through the food chain, thereby threatening human health. If the sewage sludge would be transformed to the sludge compost, it is essential to reduce the concentrations of heavy metals or decrease the availability of heavy metals in sludge. Therefore, many physical, chemical and microbial technologies have been used to reduce the concentrations of heavy metals in sludge (Wang et al., 2004; Gheju et al., 2011). However, all these technologies have high costs and low practicality. Therefore, researchers tried to develop heavy metal-stabilizing technologies in sludge treatment. Among the heavy metal-stabilizing technologies, composting is an effective solution. Mobility and solubility of heavy metals in sludge can be decreased following the composting treatment. Amir et al. (2005) studied the sequential extraction of heavy metals in sludge after applying the composting treatment, and the results indicated that the bioavailable fractions of heavy metals decrease after applying the composting treatment. This conclusion has been reported by many researchers (e.g., Haroun et al., 2007; Zheng et al., 2007; Maňáková et al., 2014; Hazarika et al., 2017). For example, Zheng et al. (2007) found that the availability of Ni and Cr in sludge compost was lower than that in non-composted sludge. Hazarika et al. (2017) revealed that leachable quantities of Cd, Cu, Ni, Pb, Cr and Zn decreased after composting.

Both the activity and plant absorption of heavy metals are affected when composted sludge is applied to soil. Illera et al. (2000) found that sludge could improve the concentrations of exchangeable fractions of Cd, Cu, Pb and Zn in soils. Jalali and Khanlari (2007) pointed out that when sludge is applied to calcareous soils, the primary fractions of Cd, Zn and Pb can be changed from stable to labile fractions. However, Sánchez-Martín et al. (2007) showed that the mobility of Cd, Pb, Ni, Zn, Cu and Cr exhibited no significant difference before and after the application of sludge. It has been previously reported that the sludge compost could reduce the labile fractions of Cd, Cu and Zn in soils (Zubillaga et al., 2012). There have been inconsistent results reported for plant absorption of heavy metals after applying sludge to soils. Many researches showed that sludge or sludge compost increases the plant uptake of heavy metals (e.g., Angin and Yaganoglu, 2012; Gonçalves et al., 2014; Liu et al., 2016), or exhibited no effect on heavy metal concentrations in plant (e.g., Bramryd, 2013; Bouriou et al., 2015). However, McBride et al. (2004) demonstrated that sludge reduced the uptake of Zn, Cd, Cu and Ni by eggplant (*Solanum melongena* L.). The activity and phytoavailability of heavy metals are influenced by element properties, soil properties and plant species characteristics (Kidd et al., 2007). Among which, soil properties (especially pH and organic matter) are primary influencing factors (McBride, 2003; Sánchez-Martín et al., 2007).

In the United States, the European Union and China, current regulations regarding the utilization of sludge to soils go no further than setting limits on permissible total concentrations of heavy metals in sludge. However, since many soil properties and environmental factors (temperature, precipitation, etc.) greatly affect the phytoavailability of heavy metals, assessment of potential contamination risk from sludge utilization by using total heavy metal concentration alone is inadequate (McLaughlin et al., 2000a). Over several decades, many methods including single chemical extractions (McLaughlin, 2002; Wang et al., 2009; Milićević et al., 2017) and sequential extractions (Tessier et al., 1979; Davidson et al., 1999; Lestari et al., 2018; Xie et al., 2018), have been used to estimate soil heavy metal availability to plants. Single chemical extractions including chelates, neutral salts and dilute acids are widely used because of their operational simplicity (Feng et al., 2005). The commonly used methods for evaluating the phytoavailability of single extractable heavy metal are linear correlation analysis and stepwise regression analysis. Many researchers found that heavy metal fraction extracted by neutral salt extractants, such as MgCl_2 (Chen et al., 2014), CaCl_2 , NaNO_3 , $\text{CH}_3\text{COONH}_4$ (Menzies et al., 2007) and $\text{Ca}(\text{NO}_3)_2$ (Seo et al., 2013), has the highest phytoavailability. Although much research has been carried out on the assessment of heavy metal phytoavailability, no one substance has been recognized as a universal extractant that could be used in any given soil. This is because

plant absorption of heavy metals is influenced by plant species and soil properties (Soriano-Disla et al., 2010).

Sierozem soils are widely distributed in Northwest China, which have low contents of organic matter (OM) and plant nutrients, and high carbonate content and pH value. Thus, the fertility of sierozem soils is low. Municipal sludge, being rich in organic material and nutritive elements, can be supplied to sierozem soils to improve the fertility. Meanwhile, application of municipal sewage sludge to soils is also a method of sludge disposal. However, sewage sludge application will introduce heavy metals into soils. Therefore, it is necessary to understand the mobility and plant absorption of heavy metals in sierozem soils amended with municipal sludge to ensure food safety. Previously, we investigated the wheat uptake of Cd in sierozem soils amended with sludge compost (Liu et al., 2016). Until now, there have been few reports regarding the mobility and plant absorption of heavy metals in sierozem-plant systems with sludge application. In this study, we explored the solubility and potato uptake of Cd, Ni, Cu, Zn and Pb in sierozem soils amended by different amounts of municipal sludge compost (MSC). We also evaluated the appropriateness of five extractants ($\text{CH}_3\text{COONH}_4$, CaCl_2 , CH_3COOH , diethylene triamine pentacetic acid (DTPA) and ethylene diamine tetraacetic acid (EDTA)) for estimating the phytoavailability of Cd, Pb, Cu, Zn and Ni to the potato plant in sierozem soils amended with MSC.

2 Materials and methods

2.1 Experimental materials

The tested plant species is potato (*Solanum tuberosum* L., variety Xindaping), which is widely planted in Northwest China. Dewatered sludge was taken from the Qilihe Sewage Treatment Plant in Lanzhou City, Gansu province, China. The sewage from Lanzhou City was treated using cyclic activated sludge technology. In our experiment, one part of corn straw was added uniformly to eight parts of dewatered municipal sludge. The mixture was composted under aerobic and static conditions. In the composting process, the mixture was turned over every seven days. The MSC was obtained approximately one month later. The chemical properties of MSC are presented in Table 1. Heavy metal concentrations in the MSC were below the limits (3, 300, 500, 1500, 500, 3 and 100 mg/kg for Cd, Pb, Cu, Zn, Cr, Hg and Ni, respectively) for sludge application to farmland in China (Ministry of Housing and Urban-Rural Development of China, 2009). Sierozem soils were obtained from the topsoil layer (0–30 cm) in Yuzhong County of Gansu Province. The soil texture was 16.07% sand, 63.39% silt and 20.54% clay. The collected sierozem soils and MSC were air-dried, ground and passed through a 2-mm sieve for further use.

Table 1 Chemical properties and heavy metal concentrations of municipal sludge compost (MSC)

pH _{1:2.5}	EC _{1:2.5} (μS/cm)	Carbonate (%)	OM (g/kg)	DOC (mg/kg)	P (%)	K (%)
7.66	3215.30	3.08	150.87	2679.43	8.54	3.25
Cd (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Ni (mg/kg)	Cr (mg/kg)	Hg (mg/kg)
1.80	33.61	78.99	353.67	32.86	67.52	1.15

Note: EC, electric conductivity; OM, organic matter; DOC, dissolved organic carbon; P, phosphorus; K, potassium.

2.2 Pot trial design

A pot trial was carried out at Lanzhou University in 2014. Seven soil treatments (0.0%, 0.5%, 1.0%, 2.0%, 4.0%, 6.0% and 8.0% MSC in sierozem soils) with four replicates for each were used in this experiment. Soil treatments were marked as control (0.0% MSC in sierozem soils), S0.5 (0.5% MSC), S1.0 (1.0% MSC), S2.0 (2.0% MSC), S4.0 (4.0% MSC), S6.0 (6.0% MSC) and S8.0 (8.0% MSC). Different weights of sieved MSC were added evenly to the soil in each plastic pot according to proportions mentioned above. The mixed soil dry weight in each pot was 32 kg. The amended sierozem soils were watered and then equilibrated for approximately 6 months. Potato seeds were soaked in 5 mg/L gibberellin solution for 5 min and sprouted in wet silica sand. One piece of sprouted seed tuber was planted in each pot. During the potato growth period,

distilled water was used to irrigate the soil, maintaining soil moisture content at about 30% of field capacity. Potato plants were harvested approximately 15 weeks after sowing.

2.3 Plant and soil analyses

Plant samples were washed carefully with deionized water to remove soil particles and other impurities. The potato plant was cut into four parts: root, stem (including stalk and leaves), peel and tuber. Then, the fresh weight of the tuber was measured. The plant samples were oven-dried at 105°C for 40 min, and then kept at 75°C until constant weights were obtained. Samples were ground and homogenized in a mill and then dispensed into sample bottles. Dry samples were digested with HNO₃ using microwaves (Gardea-Torresdey et al., 2004). The Cd, Pb, Cu, Zn and Ni concentrations of the potato plant were determined with an atomic absorption spectrometer equipped with a graphite furnace atomizer (AAS, Type M6MKII, Thermo Electron Corporation, USA). Approximately 500 g of soil was taken from each pot. Soil samples were air-dried and ground. The soil texture was measured by the hydrometer method (Liu, 1996). The pH and electric conductivity (EC) in water (1.0:2.5) were measured using a combined glass calomel electrode (Ministry of Agriculture of China, 2006). The organic matter (OM) content was determined by oxidation with potassium dichromate and colorimetric determination (Nelson and Sommers, 1996). The dissolved organic carbon (DOC) content was determined following Kaiser et al. (1996). The titration method was used to determine the carbonate content (Ministry of Agriculture of China, 1988). Soil samples and MSC samples were digested with HNO₃ and HCl (2.7:1.0) in a laboratory microwave system (Kidd et al., 2007) to determine the P (phosphorus) content. In order to determine the K (potassium) content, and concentrations of Cd, Pb, Cu, Zn, Cr and Ni, we digested soil samples and MSC samples with HNO₃ and HClO₄ (2:1) in a laboratory microwave system (Udom et al., 2004). The Hg concentration in the MSC was determined using the method conducted by Horvat et al. (1991). Available phosphorus (AP) was determined by bicarbonate extraction (Olsen et al., 1954). Available potassium (AK) was determined by extraction with CH₃COONH₄ (Page, 1982). The single extraction analysis procedure was selected to investigate the phytoavailability of heavy metals in amended sierozem soils. The CaCl₂, CH₃COONH₄, CH₃COOH, DTPA and EDTA were used to extract the labile fraction of heavy metals from the soils (Quevauviller, 1998; Sanka and Dolezal, 2006). The heavy metal concentrations and K content in amended sierozem soils were determined with an atomic absorption spectrometer equipped with a graphite furnace atomizer (AAS, Type M6MKII, Thermo Electron Corporation, USA). The Hg concentration in amended sierozem soils was determined with an atomic fluorescence spectrometer (AFS, Type RGF8780, Bohui Innovation Technology Corporation, Beijing). The P content in amended sierozem soils was determined with an ultraviolet spectrophotometer (Evolution 300 Security UV-Vis Spectrophotometer, Thermo Electron Corporation, USA). Furthermore, certified reference samples, bush leaf material (GBW-07603) and yellow soil material (GBW-07408), were employed in quality control. The difference between measured and certified concentrations of Cu, Zn, Ni, Pb and Cd was no more than 10%.

2.4 Statistical analysis

Mean value of each treatment was obtained by averaging four replicates, and the relative error was less than 10%. We used one-way analysis of variance (ANOVA) followed by Duncan's test ($P < 0.05$) to evaluate the significant differences between experimental data. In addition, the following data analysis methods were also used in this study.

2.4.1 Bioconcentration factor (BCF)

The BCF is one of the key components representing a plant's capacity for enrichment with heavy metals (Jamali et al., 2009). The BCF was determined using the Equation 1:

$$BCF = C_P / C_S, \quad (1)$$

where C_P and C_S are the heavy metal concentrations (mg/kg) in the plant and soil, respectively.

2.4.2 Ridge regression analysis

The effects of multicollinearity on the least squares estimate of regression coefficients are well

known. Multicollinearity can result in regression coefficient estimates with high variance that may consequently be far removed from the true population values. In addition, the least squares estimates may be too large in absolute values, and it is possible that some of them will even be of the wrong sign, resulting in unreliable regression equation (Timmermans, 1981). Stepwise regression analysis is usually used to overcome the multicollinearity problem. However, there is a consensus that stepwise regression analysis does not always succeed in selecting the best subset of predictor variables in terms of maximizing explained variance. To select the best predictor variables, Hoerl and Kennard (1970) introduced the ridge regression analysis. This approach has been successfully applied in many research fields. For the potato plant, root is the first plant organ exposed to soil metals. Heavy metal concentration in the root is considered to be a good indicator of phytoavailability, whereas the concentration in the other parts of the plant may not necessarily reflect heavy metal supply (Soriano-Disla et al., 2010). Thus, the heavy metal concentration in the root was selected as the plant absorption parameter in this study. Furthermore, it is commonly recognized that the extractable concentration of heavy metal is a useful variable to assess the potential risk of heavy metals in amended soils (McBride, 2003). Soil properties, such as OM content and pH value, also have an important impact on the phytoavailability of heavy metals (Li et al., 2003). Moreover, DOC could affect the plant uptake of heavy metals. Thus, we selected the extractable concentration of heavy metals, soil pH value, and soil OM and DOC contents as the independent variables in this study. Multicollinearity is present among these independent variables. In response, ridge regression was used to analyze the phytoavailability of heavy metals in the mixed soil.

3 Results

3.1 Properties of amended sierozem soils

The difference analysis results of properties of amended sierozem soils indicate significant variations in EC, OM, DOC, P, K, AP and AK among different treatments (Table 2). However, no significant difference was found between pH value and carbonate content. Compared with control treatment, the EC, OM, DOC, P, K, AP and AK increased in MSC treatments. The EC, OM, DOC, P, K, AP and AK were 104.4%, 162.2%, 521.6%, 711.1%, 42.1%, 695.7%, and 272.2% higher in S8.0 than in control treatment, respectively. As the MSC percentage in sierozem soils increased, the concentrations of Cu, Zn, Cd and Pb in amended soils increased correspondingly, while the concentration of Ni did not change significantly (Table 2). When the MSC percentage increased to 8.0% in sierozem soils, the concentrations of Cd, Pb, Cu, Zn and Ni increased by 92.3%, 13.0%, 22.2%, 32.2% and 8.1%, respectively, compared with the control treatment. Nevertheless, the heavy metal concentrations were within the limit (0.6, 350, 100, 300 and 60 mg/kg for Cd, Pb, Cu, Zn and Ni, respectively) for farmland soils ($\text{pH} > 7.5$) in China (Ministry of Environmental Protection of China, 1995).

3.2 Labile fraction proportions of heavy metals

Single extractions of Cd, Pb, Cu, Zn and Ni concentrations in amended sierozem soils are shown in Figure 1. Duncan's test indicates that each extractable concentration of heavy metal increased significantly with increasing MSC percentage in sierozem soils. For Zn, Pb, Cu and Cd, the complexing agent (DTPA and EDTA) extractable concentrations were highest, but for Ni, the CH_3COOH extractable concentration was the highest. For all five heavy metals, the CaCl_2 extractable concentration was the lowest. Different heavy metals had different solubility in amended sierozem soils. Furthermore, the mean labile fraction proportion of each heavy metal was also investigated (Table 3). The result shows that the extractable labile fraction proportion of the five heavy metals was in the following order: $\text{Ni} < \text{Zn}$, Cu and $\text{Pb} < \text{Cd}$.

3.3 Plant absorption of heavy metals

The Cd, Pb, Cu, Zn and Ni concentrations of the root, stem, peel and tuber of the potato plant are presented in Figure 2. As the MSC percentage in sierozem soils increased, the heavy metal

concentrations in each part of the potato plant increased correspondingly. Concentrations of Cd, Pb, Cu, Zn and Ni in the potato plant were much higher in the stem and root than in the peel and tuber (Fig. 2). The calculations of BCF were conducted to compare the plant enrichment ability of each heavy metal. Since the heavy metal concentration in different parts of the potato plant showed differences, we used the metal concentration of the whole plant to calculate the BCF. The BCF value of each heavy metal was in the following order: Ni<Zn, Cu and Pb<<Cd (Table 4).

Table 2 Chemical properties and heavy metal concentrations of amended sierozem soils

Item	Control	S0.5	S1.0	S2.0	S4.0	S6.0	S8.0
pH _{1:2.5}	8.33±0.52 ^a	8.23±0.03 ^a	8.22±0.06 ^a	8.12±0.26 ^a	8.03±0.43 ^a	7.98±0.52 ^a	7.77±0.32 ^a
EC _{1:2.5} (μS/cm)	713.7±30.0 ^c	962.7±62.9 ^d	1030.1±60.2 ^{cd}	1121.7±39.7 ^c	1173.7±43.5 ^{bc}	1304.7±86.4 ^b	1457.2±67.7 ^a
Carbonate (%)	13.12±0.12 ^a	13.26±0.35 ^a	13.28±0.20 ^a	13.36±0.38 ^a	13.35±0.59 ^a	13.43±0.71 ^a	12.78±0.58 ^a
OM (g/kg)	7.60±0.39 ^f	8.63±0.81 ^{ef}	9.51±0.61 ^e	11.37±1.09 ^d	13.52±0.33 ^c	16.11±1.09 ^b	19.93±0.58 ^a
DOC (mg/kg)	24.23±1.65 ^g	35.65±1.83 ^f	42.79±1.90 ^c	51.68±4.44 ^d	86.49±8.08 ^c	120.47±8.39 ^b	150.61±12.67 ^a
P (%)	0.09±0.01 ^g	0.14±0.01 ^f	0.19±0.01 ^e	0.27±0.01 ^d	0.45±0.03 ^c	0.59±0.05 ^b	0.73±0.03 ^a
K (%)	1.59±0.13 ^b	1.89±0.01 ^b	2.00±0.12 ^a	2.02±0.08 ^a	2.05±0.19 ^a	2.18±0.01 ^a	2.26±0.10 ^a
AP (mg/kg)	19.78±0.74 ^g	34.84±0.67 ^f	39.41±1.69 ^c	47.89±2.31 ^d	52.46±0.63 ^c	87.5±5.16 ^b	157.39±3.56 ^a
AK (mg/kg)	59.12±4.82 ^c	97.44±8.83 ^d	139.08±1.77 ^c	152.71±13.95 ^c	153.06±9.68 ^c	188.09±1.83 ^b	220.02±6.13 ^a
Cd (mg/kg)	0.13±0.01 ^d	0.14±0.01 ^d	0.16±0.01 ^c	0.18±0.01 ^c	0.21±0.01 ^b	0.23±0.02 ^{ab}	0.25±0.02 ^a
Pb (mg/kg)	16.86±0.74 ^b	17.56±1.13 ^{ab}	17.66±1.14 ^{ab}	17.86±1.21 ^{ab}	18.16±1.15 ^{ab}	18.79±1.78 ^{ab}	19.05±0.40 ^a
Cu (mg/kg)	23.95±1.13 ^c	24.96±1.92 ^c	25.25±0.81 ^c	25.66±2.01 ^{bc}	26.46±1.25 ^{bc}	27.26±0.10 ^b	29.26±0.51 ^a
Zn (mg/kg)	68.75±3.27 ^c	71.58±1.09 ^c	72.68±2.48 ^c	75.48±4.58 ^{bc}	81.67±1.57 ^b	85.45±6.31 ^{ab}	90.89±2.21 ^a
Ni (mg/kg)	25.57±0.70 ^a	26.19±2.01 ^a	26.55±0.50 ^a	27.02±0.78 ^a	27.25±0.25 ^a	27.42±1.58 ^a	27.65±1.89 ^a

Note: EC, electric conductivity; OM, organic matter; DOC, dissolved organic carbon; AP, available phosphorus; AK, available potassium. Control, 0.0% MSC in sierozem soils; S0.5, 0.5% MSC; S1.0, 1.0% MSC; S2.0, 2.0% MSC; S4.0, 4.0% MSC; S6.0, 6.0% MSC; S8.0, 8.0% MSC. Different lowercase letters in the same row indicate significant differences among soil treatments at $P<0.05$ level according to the Duncan's test. Mean±SD, $n=4$.

Table 3 Mean labile fraction proportions of the five heavy metals using different extractants

Heavy metal	Labile fraction proportion of heavy metal (%)				
	CaCl ₂	CH ₃ COONH ₄	CH ₃ COOH	DTPA	EDTA
Cd	1.53±0.25 ^a	19.25±2.79 ^a	7.50±1.37 ^a	48.44±5.36 ^a	32.51±6.08 ^a
Pb	0.67±0.09 ^b	1.61±0.17 ^b	3.79±0.58 ^b	4.86±1.18 ^b	7.31±1.01 ^b
Cu	0.64±0.11 ^b	2.42±0.30 ^b	2.86±0.57 ^b	3.60±0.36 ^b	5.74±0.80 ^b
Zn	0.77±0.13 ^b	1.96±0.18 ^b	2.92±0.32 ^b	4.10±0.73 ^b	6.82±0.86 ^b
Ni	0.45±0.08 ^c	0.69±0.08 ^c	1.43±0.35 ^c	0.92±0.17 ^c	1.63±0.29 ^c

Note: DTPA, diethylene triamine pentacetic acid; EDTA, ethylene diamine tetraacetic acid. Different lowercase letters in each column indicate significant differences among different heavy metals at $P<0.05$ level according to the Duncan's test. Mean±SD, $n=28$.

Table 4 BCF (bioconcentration factor) values of the five heavy metals under different soil treatments

Treatment	BCF value				
	Cd	Pb	Cu	Zn	Ni
Control	3.98±0.57 ^a	0.31±0.05 ^b	0.30±0.04 ^b	0.39±0.04 ^b	0.10±0.01 ^c
S0.5	5.23±1.00 ^a	0.43±0.07 ^b	0.40±0.04 ^b	0.46±0.09 ^b	0.14±0.02 ^c
S1.0	5.49±0.65 ^a	0.50±0.06 ^b	0.49±0.06 ^b	0.52±0.10 ^b	0.17±0.03 ^c
S2.0	5.64±0.71 ^a	0.50±0.09 ^b	0.52±0.06 ^b	0.51±0.08 ^b	0.18±0.02 ^c
S4.0	6.29±0.72 ^a	0.56±0.09 ^b	0.60±0.07 ^b	0.54±0.08 ^b	0.23±0.03 ^c
S6.0	5.89±0.67 ^a	0.54±0.07 ^b	0.61±0.08 ^b	0.55±0.07 ^b	0.24±0.03 ^c
S8.0	2.73±0.51 ^a	0.54±0.08 ^b	0.65±0.09 ^b	0.57±0.11 ^b	0.25±0.03 ^c

Note: Different lowercase letters in each row indicate significant differences among different heavy metals at $P<0.05$ level according to the Duncan's test. Mean±SD, $n=4$.

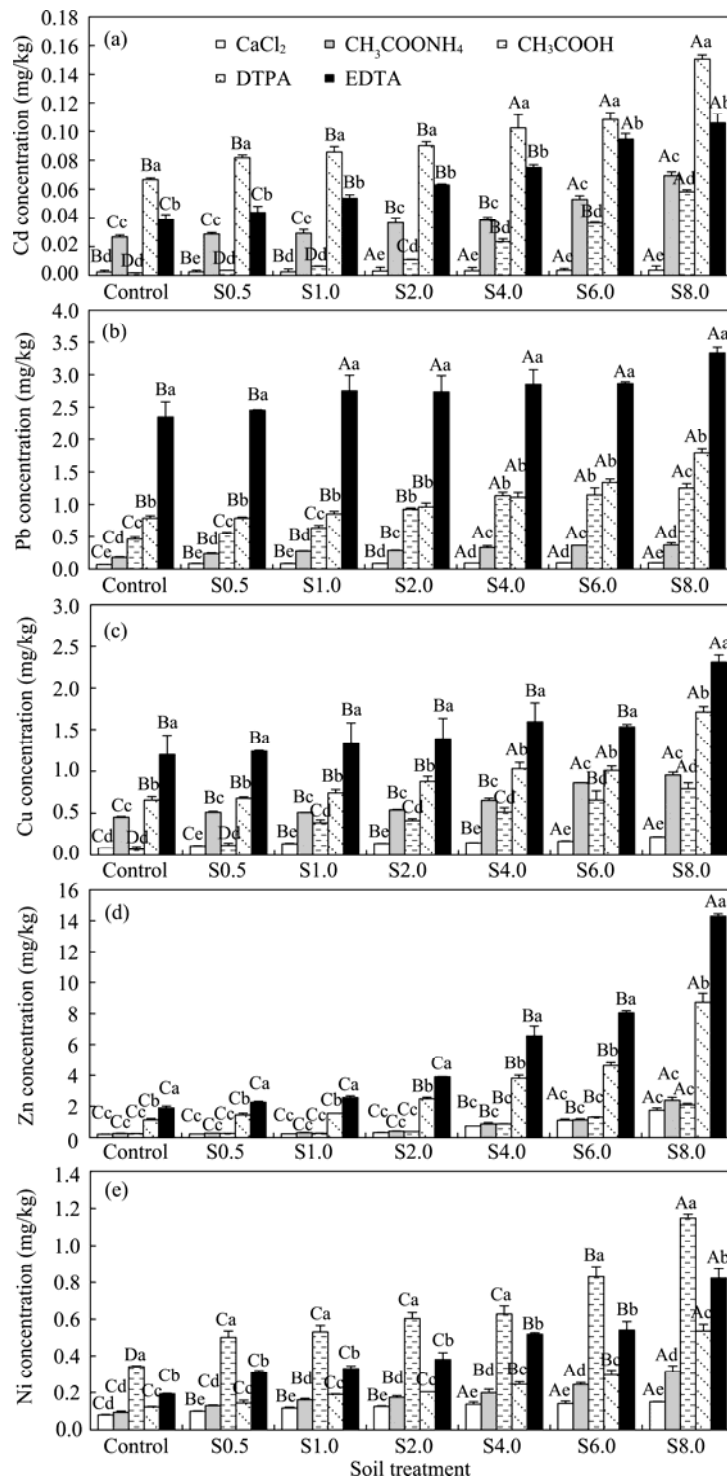


Fig. 1 Concentrations of Cd (a), Pb (b), Cu (c), Zn (d) and Ni (e) in amended sierozem soils extracted using CaCl₂, CH₃COONH₄, CH₃COOH, diethylene triamine pentacetic acid (DTPA) and ethylene diamine tetraacetic acid (EDTA) under different soil treatments. Control, 0.0% MSC (municipal sludge compost) in sierozem soils; S0.5, 0.5% MSC; S1.0, 1.0% MSC; S2.0, 2.0% MSC; S4.0, 4.0% MSC; S6.0, 6.0% MSC; S8.0, 8.0% MSC. Different capital letters indicate significant differences among different soil treatments at *P* < 0.05 level for the same extractant, and different lowercase letters indicate significant differences among different extractants at *P* < 0.05 level for the same soil treatment. Error bar means standard deviation. *n* = 4.

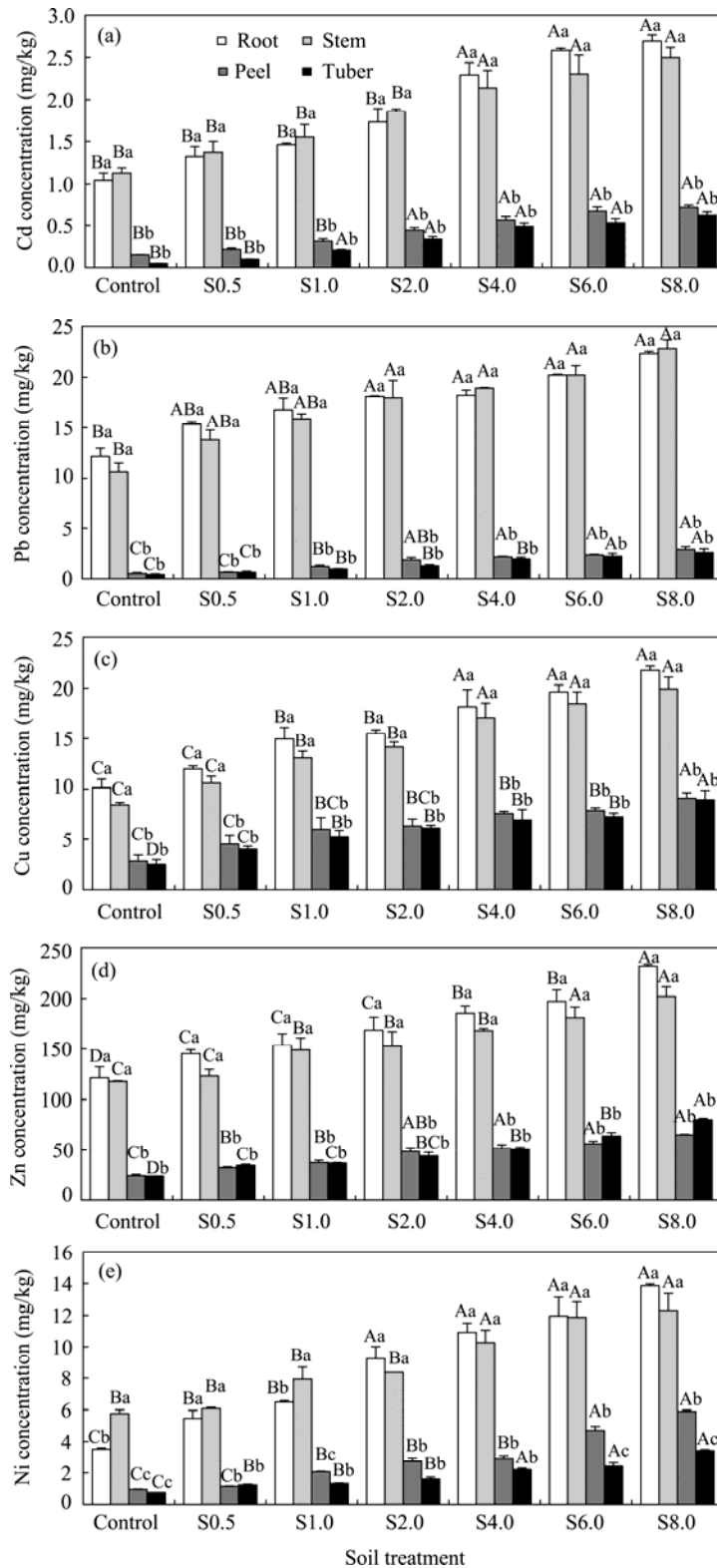


Fig. 2 Concentrations of Cd (a), Pb (b), Cu (c), Zn (d) and Ni (e) in different parts of the potato plant under different soil treatments. Different capital letters indicate significant differences among different soil treatments at $P < 0.05$ level for the same plant part, and different lowercase letters indicate significant differences among different plant parts at $P < 0.05$ level for the same soil treatment. Error bar means standard deviation. $n=4$.

3.4 Ridge regression analysis

The best-fit predictive equations for the five heavy metals using ridge regression analysis are shown in Table 5. A suitable indicator was found by DTPA extraction for Cd and Pb, and by EDTA extraction for Cu, Zn and Ni in the root of the potato plant. The complexing extractions can be used to predict the phytoavailability of heavy metals in amended sierozem soils. The regression coefficients of OM and DOC contents were positive for all heavy metals, indicating that the increases of OM and DOC contents could lead to the increases of heavy metal concentrations in the root. However, the regression coefficients of soil pH were negative for all heavy metals, showing that an increase of pH value could lead to the decreases of heavy metal concentrations in the root.

Table 5 Ridge regression analysis of heavy metal concentration in the root, soil pH value, soil OM content, soil DOC content and extractable concentration of heavy metal

Heavy metal	Ridge regression equation	R^2
Cd	$C_{\text{root}} = 2.370C_{\text{DTPA}} + 0.043C_{\text{OM}} - 0.657\text{pH} + 0.005C_{\text{DOC}} + 6.080$	0.953*
Pb	$C_{\text{root}} = 0.916C_{\text{DTPA}} + 0.309C_{\text{OM}} - 1.728\text{pH} + 0.019C_{\text{DOC}} + 25.318$	0.952*
Cu	$C_{\text{root}} = 0.838C_{\text{EDTA}} + 0.307C_{\text{OM}} - 4.829\text{pH} + 0.027C_{\text{DOC}} + 47.829$	0.959*
Zn	$C_{\text{root}} = 1.540C_{\text{EDTA}} + 2.389C_{\text{OM}} - 43.565\text{pH} + 0.188C_{\text{DOC}} + 472.084$	0.972*
Ni	$C_{\text{root}} = 1.390C_{\text{EDTA}} + 0.292C_{\text{OM}} - 4.861\text{pH} + 0.022C_{\text{DOC}} + 42.460$	0.958*

Note: * means that the correlation coefficient is significant difference at $P < 0.05$ level. C_{root} , heavy metal concentration in the root (mg/kg); C_{DTPA} , DTPA-extractable concentration of corresponding heavy metal in amended sierozem soils (mg/kg); C_{EDTA} , EDTA-extractable concentration of corresponding heavy metal in amended sierozem soils (mg/kg); C_{OM} , soil organic matter content (g/kg); C_{DOC} , soil dissolved organic carbon content (g/kg).

4 Discussion

4.1 Effects of MSC on properties of amended sierozem soils

As the MSC percentage in amended sierozem soils increased, the soil EC, OM, DOC, P, K, AP, and AK increased correspondingly. Previous studies have reported similar results (Antonious et al., 2011; Yilmaz and Temizgöl, 2012). Soil EC represents total salt content in soils. Generally, the suitable soil EC for plant growth is less than 1500 $\mu\text{S}/\text{cm}$ (Casado-Vela et al., 2006). In this study, the highest EC of amended sierozem soils was less than this threshold, indicating that the total salt content of amended sierozem soils is suitable for plant growth. DOC, the primary component of dissolved organic matter, plays an important role in the transport of metals in soils (Kalbitz et al., 2000). DOC can form a stable chemical complex with heavy metals; therefore it improves the solubility of soil heavy metals (Sherence, 2009). Thus, the increase of DOC content in amended sierozem soils may enhance the solubility of heavy metals. Soil OM, P and K contents are important indicators of soil fertility. AP and AK, respectively representing the P and K, can be directly taken up by plants. Although the soil OM content in this study (7.60–19.93 g/kg) was lower than the average value in Gansu Province (22.62 g/kg), MSC did effectively promote the fertility and phytoavailability of P and K in amended sierozem soils.

4.2 Influence of MSC on mobility of heavy metals

Single extraction can be used to determine the labile fraction of heavy metals in soils. It is a common method for investigating the mobility of heavy metals and exploring the potential risk of heavy metals. Generally speaking, the labile fractions of heavy metals in soils are composed of water-soluble, exchangeable and acid-soluble (bound to carbonate) components (Luo and Christie, 1998; Davidson et al., 1999). In this study, we used CaCl_2 , $\text{CH}_3\text{COONH}_4$, CH_3COOH , DTPA and EDTA as extractants to extract the labile fractions of heavy metals. Both CaCl_2 and $\text{CH}_3\text{COONH}_4$ extractants are neutral salt solutions. Neutral salts could extract metals in soil solutions and from soil particles (McLaughlin et al., 2000b). Phytoavailable metals are mainly absorbed on the surface of soil particles. They can be exchanged by other cations and therefore be released to soil solutions. Neutral salt extractions are considered to be directly absorbed by plants (McBride,

2003). The CH_3COOH extractant is a dilute acid solution. It can extract water and acid-soluble heavy metals (Luo and Christie, 1998). Both DTPA and EDTA are complexing agents that can form strong complexes with metals (Menzies et al., 2007). Chelating agents and dilute acids could extract more metals compared to neutral salts (McBride, 2003). Chelating agents and dilute acids are usually used to assess the potential toxicity of heavy metals, and the heavy metals extracted by chelating agents and dilute acids could represent the long-term available metals in soils (McLaughlin et al., 2000a).

The labile fraction proportion of heavy metals increased with increasing MSC percentage in amended sierozem soils (Bhat et al., 2011; Delgado et al., 2012; Arvas et al., 2013). Zubillaga et al. (2012) reported that the labile fraction proportion of heavy metals in soil-sludge mixtures decreased with increasing soil OM content. In this study, soil OM content increased significantly with an increase of MSC percentage in amended sierozem soils, and the labile fraction proportion of each heavy metal increased with increasing soil OM content. This may be attributed to the DOC in the MSC. As MSC percentage in amended sierozem soils increased, the DOC content increased correspondingly. DOC can form stable complexes in soil solution with metal cations, metallic oxides and metal hydroxides, and then the sorption of metal ions on the soil solid phase is suppressed (Sherence, 2009). Thus, DOC can increase the solubility of metals. In amended sierozem soils, the increased DOC content could form a complex in soil solution with heavy metals, and increase the concentration of water-soluble metals. In addition, carbonate also influences the labile fraction proportion of heavy metals. Heavy metals are very effectively retained by carbonate surfaces at low soil metal concentrations via the chemisorption mechanism (Thakur et al., 2006), while they can precipitate with carbonates at high soil metal concentrations (Ouhadi et al., 2010). Yuan and Lavkulich (1997) found that carbonates in soils with low OM content have a stronger sorption capacity of heavy metals than those in soils with high OM content. The amended sierozem soils in this study had a high carbonate content and a low OM content. More heavy metals tended to combine with carbonate than combine with OM. In summary, MSC could increase the DOC content in amended sierozem soils, and therefore increase the water-soluble fraction of heavy metals. Moreover, the high carbonate content in amended sierozem soils increased the acid-soluble fraction of heavy metals. Thus, the labile fraction proportion of heavy metals increased with increasing amounts of MSC addition in sierozem soils.

Different extractants had various extractable capacities. The complexing agent (DTPA and EDTA) extractable concentrations were highest for Cu, Pb, Cd and Zn. The CH_3COOH -extractable concentration was highest for Ni, and the CaCl_2 -extractable concentration was lowest for all heavy metals. Similar results have been reported by Menzies et al. (2007) and Soriano-Disla et al. (2010). These findings might be attributable to the nature of each extractant. DTPA, EDTA and CH_3COOH have high extractable capacities, and their extractions represent the long-term available pools of metals. However, CaCl_2 mainly extracts water-soluble and exchangeable metals in soils, and its extraction tends to represent the short-term available pools of metals. Thus, there are few metals in amended sierozem soils that could be directly absorbed by the potato plant. However, this does not mean that plant absorption of heavy metals is low and that CaCl_2 extraction can best be used to evaluate the phytoavailability of heavy metals. There are other factors that influence the plant absorption of heavy metals, such as soil properties, mineralization of OM, and plant species. These factors require further evaluation of the relationship between metal concentrations of the potato plant and extractable concentrations of heavy metals, in order to decide which extractable fraction is the highest in phytoavailability and to elucidate the influencing factors.

The solubility and mobility of Cd were highest and those of Ni were lowest among the five heavy metals. Sánchez-Martín et al. (2007) reported that Cd is more easily transferred than other heavy metals in soils following sludge application. This trend may be attributed to the nature of heavy metals. Several studies have confirmed that Cd is the most mobile heavy metal than other heavy metals in soils, and it is largely bound to readily leached exchangeable and acid-soluble fractions (e.g., Harrison et al., 1981; Chlopecka et al., 1996). Studies on arid soils (properties are

similar to the loessal soil) in Gansu Province showed that the labile fraction proportion of Cd is greater than those of Zn, Ni, Pb and Cu (Li et al., 2006; Wang et al., 2010). Likewise, in this study, Cd also had higher solubility and mobility than other heavy metals. This might affect the absorption of heavy metals by the potato plant.

4.3 Effects of MSC on plant absorption of heavy metals

In this research, MSC promoted the potato uptake of heavy metals, which is similar to the results of Bozkurt et al. (2010) and Bhat et al. (2011). This might be attributed to the increased labile fraction proportion of heavy metals. Water-soluble and exchangeable heavy metals can be directly absorbed by plants. Moreover, in addition to DOC, there was some easily decomposable OM in the MSC. During the growth period of the potato plant, the labile OM in amended sierozem soils was degraded by microorganisms, and therefore heavy metals that were combined with the labile OM can be released into the soil solution. Under the influence of two substances (DOC and labile OM), heavy metals can be directly absorbed by the potato plant following an increase in MSC addition in sierozem soils. Also, acid-soluble heavy metals can be absorbed by the potato plant. Plassard et al. (2000) found that the retention capacity of highly carbonated soil for heavy metals was large but the retention force was weak, and therefore a decrease in soil pH would increase the risk of metal release. Root-induced acidification can reduce the rhizosphere pH by two to three units compared to bulk soil (Marschner, 1995). Therefore, heavy metals that were bound to carbonates can be released into the soil solution. MSC improved the labile fraction proportion of heavy metals, and thus increased the amount of heavy metals absorbed by the root. Meanwhile, the change in root metal concentrations was the primary factor affecting the metal concentrations of the stem, peel and tuber.

Previous studies have demonstrated that the heavy metal concentration varies among different plant parts (Singh et al., 2006; Nedjimi and Daoud, 2009). Specifically, the heavy metal concentration is higher in nutritive organs (root and stem) and lower in edible parts (peel and tuber). Similar results were obtained in this study. Root is the first plant organ to be exposed to heavy metals. In the root zone, heavy metals are bound to root tissue, where heavy metals are mainly combined with sulfur (Isaure et al., 2006). Leaf and shoot (stem) are the primary tissues of plant transpiration, where water evaporates from the stem but solid mass is retained. Thus, high metal concentration is observed in the stem (Li et al., 2016). Although potato tuber grows in soils like the root, it cannot absorb substances from soils; instead, it receives substances transferred from the stem. Since most heavy metals are retained in the root and stem, few heavy metals were transferred to the tuber and peel. Thus, heavy metal concentrations in the tuber and peel were lower than those in the root and stem.

The order of BCF value was similar to that of the labile fraction proportion for the five heavy metals. The BCF value of Cd was far higher than those of other heavy metals (Turek et al., 2005). In amended sierozem soils, Cd had higher solubility and mobility than other heavy metals. Thus, Cd was more easily absorbed by the potato plant. In addition, the BCF value of Cd was greater than 1.0 in this study, indicating that the potato plant has a strong Cd enrichment ability. Cd is not an essential element for the growth of potato plants, but it can accumulate easily in plants due to high solubility in amended sierozem soils. Although the total concentration and labile fraction proportion of Cd in amended sierozem soils were far lower than those of other heavy metals, the enrichment ability of potato plants was highest for Cd. Thus, attention should be focused on Cd when considering sludge application to sierozem soils in this region, i.e., Lanzhou City. The BCF value of Ni was the lowest among all heavy metals, which indicated that the potato plant has a weak Ni enrichment ability (Tack, 2014; Musilova et al., 2015). Ni uptake by plants varies between species. Dixon et al. (1975) reported that leguminous and cucurbitaceous plants have higher Ni enrichment abilities than other plant species, since their urease synthesis process requires Ni element. In contrast, the potato plant has a low Ni enrichment ability.

4.4 Phytoavailability of heavy metals

The neutral salt extraction can be used to predict the phytoavailability of heavy metals (Menzies

et al., 2007). This may be because the neutral salt extraction can be directly absorbed by plants. In this study, the DOC and labile OM were presented in the MSC, leading to increases in labile fraction proportion and potato plant absorption of heavy metals. Thus, the neutral salt extraction did not reflect the plant uptake of heavy metals. In contrast, the complexing agent extraction did reflect the phytoavailability of heavy metals, since it represented the potential available pools of heavy metals. According to the ridge regression equations, the heavy metal concentration of the root increased with increasing soil OM content. Chaudri et al. (2007) and Zhou et al. (2010) have reported that increases in soil OM content can suppress the plant uptake of heavy metals. In this study, soil OM content and potato plant uptake of heavy metals increased as the MSC percentage increased in amended sierozem soils. The regression equations can better reflect the actual results. It should be noted that the complexing agent (EDTA and DTPA) extraction can be used to predict the phytoavailability of heavy metals, with soil OM, pH and DOC being important impacting factors.

5 Conclusions

The MSC improves the fertility of sierozem soils, increases DOC content, and enhances the solubility of Cd, Ni, Cu, Zn and Pb in sierozem soils. For the five heavy metals in amended sierozem soils, Cd has the highest solubility and mobility while Ni has the lowest solubility and mobility. The MSC significantly promotes the potato plant uptake of heavy metals. Most heavy metals accumulate in the root and stem, while few metals accumulate in the tuber and peel. The potato plant has a strong enrichment ability for Cd and a weak enrichment ability for Ni. The extractable concentration for complexing agents (DTPA and EDTA) is a suitable indicator for the phytoavailability of Cd, Ni, Cu, Zn and Pb in amended sierozem soils, and soil OM, pH and DOC are important impacting factors. Our results suggest that soils amended with MSC may improve soil fertility but increase the exposure risk of soils to heavy metals. Thus, further research should be conducted to investigate the mechanism of heavy metal release from OM decomposition, and to find methods to delay or inhibit the release of sludge-borne metals or retain the released metals.

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