Title: Evaluation of silkworm excrement and mushroom dreg for the remediation of multiple heavy metal/metalloid contaminated soil using pakchoi

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Number of text pages: 25

Figures: 6

Tables: 1

Abstract

The economical, environmental friendly and efficient materials to remediate the pollution with multiple heavy metals and metalloids are scarce. Silkworm excrement (SE) and mushroom dregs (MD) are two types of agricultural wastes, and they are widely used to improve the soil fertility in many regions of China. A pot experiment with sixteen treatments was set up to assess the possibility of using SE and MD to stabilize heavy metals and metalloids and reduce their uptake in pakchoi cultivated in slightly contaminated soils with arsenic (As), cadmium (Cd), lead (Pb) and zinc (Zn). The results showed that the single addition of SE obviously stimulated the growth of pakchoi, reduced the contents of all tested heavy metals and metalloids in the edible part of pakchoi and availability of Zn and Cd in soil. The single MD treatment showed an inferior ability to enhance the growth and reduce the contents of heavy metals and metalloids in the edible part of pakchoi. The combined utilization of SE and MD appeared not to show better effects than their individual treatment when using them to remediate this contaminated soil. Some potential mechanisms on the stimulation on pakchoi growth and decreasing the accumulation of heavy metals and metalloids in pakchoi subjected to SE were suggested, including: 1) enhancing soil pH to impact the availability of heavy metals and metalloids; 2) improve the fertility of soil; 3) sulfhydryl groups of organic materials in SE play a role in conjugating heavy metals and metalloids to affect their availability in soil; and 4) stimulating the growth of pakchoi so as to show a “dilution effect” of heavy metals and metalloids.

Key Words: silkworm excrement; mushroom dreg; soil contamination; pakchoi; pot experiment

1. Introduction

Anthropogenic activities have lead to the large emissions of heavy metals and metalloids into soil and water systems (Sun et al., **2010**; Tang et al., **2010**; Sun et al., **2011**; Agusa et al., **2006**; Álvarez-Ayuso et al., **2012**). The cleanup of heavy metals and metalloids from contaminated soils is crucial because plants particularly for crops residing in heavy metal and metalloid contaminated soils will convey these accumulated elements to human being through the food chain (Wang et al., **2005**; Yan et al., **2007**; Zheng et al., **2007**). However, it is still a big challenge to control excess influxes of heavy metals and metalloids into the environments around the world, especially in China (Wei and Yang, **2010**).

Many technologies have been developed to remediate the heavy metal and metalloid contaminated soils in order to reduce the their excess accumulation in crops (Guo et al., **2014**), such as phytoremediation (Van Ginneken et al., **2007**; Vamerali et al., **2010**; Tian et al., **2014**), soil replacement (El-Radaideh et al., **2014**), chemical leaching (Bayat and Sari, **2010**; Cheng et al., **2011**) and chemical stabilization (Liang et al., **2013**; Sun et al., **2013a**; Sun et al., **2013b**). Chemical stabilization is a fast in situ technique via mixing various amendments with a contaminated soil to fix the mobile forms of heavy metals and metalloids to lower their uptake in plants (Feng et al., **2013**). Successful chemical

stabilization relies largely on identifying suitable amendments. All kinds of amendments have been widely used in practice to stabilize heavy metals and metalloids to reduce their bio-availability in contaminated soils, including lime, coal ash, zeolite, sepiolite, bentonite, red mud, spodium, calcium magnesium phosphate and silicon calcium fertiliser (Futalan et al., **2011**; Feng et al., **2013**; Liang et al., **2013**). Some potential immobilization mechanisms may be suggested, including precipitating action (Oliver et al., **1998**; Basta et al., **2005**) or being fixed in the oxide lattice interlayer (Hettiarachchi and Pierzynski, **2002**). However, in practice, the application of these amendments is in the face of some disadvantages, such as secondary pollution, short period availability and damages to soil structure. So it is better to screen some amendments characterizing low cost and environment-friendly.

Increasing interest has been shown in the immobilization of metals using organic compounds, such as exceptional quality biosolids (Bolan and Duraisamy, **2003**; Bolan et al., **2003a**; Li et al., **2009**), manure compost (Li et al., **2009**) and municipal solid wastes (Do et al., **2006**). Organic fertilizer often contains high levels of organic matters which often play a contrasting role to affect the availability and mobility of heavy metals and metalloids in soils: one role is to reduce their availability and mobility via adsorbing or forming stable complexes with humic substances (Halim et al., **2003**; Liu et al., **2009**) and the other is to increase that through supplying organic chemicals to chelate metals (Zeng et al., **2011**).

In Guangxi province of China, sericulture is the mainstay industry and enriches the people. Many agricultural wastes will be produced in the period of silkworm feeding and

edible fungus cultivation, e.g. silkworm excrement (SE) and mushroom dregs (MD). And the reuse of these “wastes” is worthy of more investigations. SE mainly consists of silkworm faeces, humified mulberry leaf and few of lime. The mulberry stalks are often used to grow edible fungus, and after the cultivation great amounts of MD are produced. The two types of wastes, i.e., SE and MD, have been already used as ameliorants to improve the fertility of farmlands. In the Maonanzu county, Hechi district, Guangxi province, China, some farmlands have been heavily contaminated by heavy metals and metalloids resulted from a sudden wastewater discharge from a tailings dam after flash-flooding. Our previous preliminary survey found that the growth of many crops cultivated in these heavy metal and metalloid contaminated soils were promoted when SE and/or MD are used as basic fertilizers. However, we did not know whether SE and/or MD were the right factors that resulted in the stimulation of crop growth. Therefore, this study was conducted to investigate the ability of SE and MD: 1) to stimulate the growth of pakchoi (Yubaiyihao) residing in multiple metal and metalloid contaminated soil; 2) to stabilize multiple heavy metals and metalloids in soil; 3) to reduce the uptake of heavy metals and metalloids in pakchoi accompanying with its associated mechanisms. To our knowledge, this is the first report using SE and MD as amendments to remediate multiple metal and metalloid contaminated soils.

2. Materials and Methods

2.1 Pretreatment of soil, SE, MD and plant materials

The soil was sampled from a multiple metal and metalloid contaminated paddy field in Daan township, Maonanzu county, Hechi district, Guangxi province, China, and the MD

and SE were obtained from the local farmers. The zinc (Zn), As, Cd and Pb concentrations in the soil were 305 mg kg^{-1} , 33.4 mg kg^{-1} , 1.98 mg kg^{-1} and 228 mg kg^{-1} , being 1.53, 1.11, 6.6 and 2.85 times higher than the individual National Standards of China (GB 15618-2008, pH within a range of 5.5 to 6.5 for a paddy field, 200 mg kg^{-1} for Zn, 30 mg kg^{-1} for As, 0.3 mg kg^{-1} for Cd and 80 mg kg^{-1} for Pb), suggesting that the applied soil was slightly contaminated by As, Zn and Pb, but moderately contaminated by Cd. The methods for the determination of soil properties and organic materials in MD and SE were described in the study of Tang et al. (1999). The soil was air-dried, sieved (2 mm medium sieve), homogenized and stored in the dark before use.

The MD and SE were air-dried and composted for four months before use. The pH values of SE and MD were 8.1 and 7.4, respectively. The contents of Zn, As, Cd and Pb were 152 , 6.2 , 0.8 and 14.2 mg kg^{-1} in the SE, and 48.1 , 3.3 , 0.5 and 4.7 in the MD, respectively, being less than the individual National Secondary Standards of China in imported organic fertilizers [GB 7475 (or 7485)-87, 300 mg kg^{-1} for Zn, 10 mg kg^{-1} for As, 1 mg kg^{-1} for Cd and 100 mg kg^{-1} for Pb]. The organic matter contents in the SE and MD were 130.6 and 198.4 g kg^{-1} , respectively.

The SE and MD were air dried, and individually or jointly mixed with soil according to the designed levels. SE was supplied at the following levels: 0 (SE_0), 0.5% ($\text{SE}_{0.5}$), 1.0% (SE_1) and 2.0 (SE_2). There were four levels for MD as follows: 0 (MD_0), 0.2% ($\text{MD}_{0.2}$), 0.4% ($\text{MD}_{0.4}$) and 0.8% ($\text{MD}_{0.8}$). In total, sixteen treatments were involved in this study as follows: SE_0+MD_0 (control), $\text{SE}_0+\text{MD}_{0.2}$, $\text{SE}_0+\text{MD}_{0.4}$, $\text{SE}_0+\text{MD}_{0.8}$, $\text{SE}_{0.5}+\text{MD}_0$, $\text{SE}_{0.5}+\text{MD}_{0.2}$, $\text{SE}_{0.5}+\text{MD}_{0.4}$, $\text{SE}_{0.5}+\text{MD}_{0.8}$, SE_1+MD_0 , $\text{SE}_1+\text{MD}_{0.2}$, $\text{SE}_1+\text{MD}_{0.4}$,

SE₁+MD_{0.8}, SE₂+MD₀, SE₂+MD_{0.2}, SE₂+MD_{0.4}, SE₂+MD_{0.8}. After mixing, the soil was fully watered with de-ionized water and maintained at equilibrium for 1 week.

One week later, the seeds were sowed into the soil, and before which the seeds were sterilized with 2% NaOCl for 10 min and rinsed with de-ionized water. The seeds of pakchoi were purchased from the Chinese Academy of Agricultural Sciences. When the seedlings developed two true leaves, they were thinned to leave two living seedling in each pot. The seedlings were left to grow for 35 d, and then be harvested. During this process, sterilized deionized water was used to maintain the maximum water holding capacity at 60-70%. The experiments were performed under natural light in a vinylhouse, and the temperatures were in a range of 15 to 30°C. Each treatment was replicated four times.

Upon harvesting, the seedlings of pakchoi were separated into shoots and roots, and the samples were thoroughly rinsed by tap water and then de-ionized water for several times. After that the samples were oven dry at 70 °C for 48h, weight, ground and then digested for the determination of element contents.

2.2 Adsorption isotherm experiment and infrared spectra analysis

To further explore the mechanisms on reducing the uptake of heavy metals and metalloids in packchoi treated with SE and MD, an adsorption isotherm experiment companied with Fourier transform infrared spectra (FT-IR) analysis was set up. The experimental process was as follows: approximately 0.5 g dried soil (sieved through 0.85 mm) was weighted and put in a 50 ml centrifuge bottle. Then the soil in each bottle was treated with 20 ml solution containing different dosages of Cd, Pb, Zn or As, and 0.01

mol L⁻¹NaNO₃ which was considered as supporting electrolyte. The concentrations of Cd [added as Cd(NO₃)₂•4H₂O] in solution were 0.5, 1, 2, 4, 8 and 16 mg L⁻¹; that of Pb [in the form of Pb(NO₃)₂] were 10, 25, 50, 100, 200 and 400 mg L⁻¹; that of Zn [supplied as Zn(NO₃)₂] were 5, 10, 25, 50, 100 and 200 mg L⁻¹; that of As [in the form Na₂HAsO₄•12H₂O] were 0.5, 1, 2, 4, 8 and 16mg L⁻¹. Above series of concentrations of Cd, Pb, Zn and As was denoted as ρ_0 . Next, the centrifuge bottles were sealed, placed in a in a constant temperature water bath oscillator, shaken for 8h at 25 °C. After that, these bottles were centrifuged at 4000 r min⁻¹ for 20 min at 25 °C. The supernatant was filtered (Whatman No.42) and collected for the determination of the concentrations of Cd, Pb, Zn and As. These concentrations were denoted as ρ_e . The equilibrium adsorption capacity (Q , mg g⁻¹) was calculated according to the following equation:

$$Q = \frac{V(\rho_0 - \rho_e)}{m} \quad (1)$$

In which, V , m , ρ_0 , ρ_e , Q denote the solution volume (L), dry mass of SE and MD (g), concentrations of Cd, Pb, Zn and As (mg L⁻¹) prior to and after adsorption, and adsorption amount, respectively. Then the parameters of Q and ρ_e were used to fit the Freundlich equation (equation 2) so as to describe the adsorbing process of heavy metals and metalloids by SE and MD.

$$Q = A\rho_e^B \quad (2)$$

In which, A and B are constants associated with the adsorption capacity of SE and MD.

About 2 g of MD or SE was weighted for the IR analysis, which was placed in 100 ml centrifuge bottle. 80 ml solution containing 16 mg L⁻¹ Cd [Cd(NO₃)₂•4H₂O], or 400 mg L⁻¹ Pb [Pb(NO₃)₂], or 200 mg L⁻¹ Zn [Zn(NO₃)₂], or 16 mg L⁻¹ As [Na₂HAsO₄•12H₂O] was added into the centrifuge bottles. After that, the centrifuge bottles were sealed, placed in a constant temperature water bath oscillator, and shaken for 8h at 25 °C. Following that, these bottles were centrifuged at 4000 r min⁻¹ for 20 min at 25 °C. The supernatant was abandoned and the residues were collected and air-dried. The residues were analyzed using infrared spectrometer (Magana ESP 560, Nicolet Corporation) according to the methods described in the study of Liang et al. (Liang et al., 2011).

2.3 Determination of heavy metals and metalloids in plant tissues and soil

Sieved soil (< 0.147 mm) was digested with concentrated HNO₃ and HF (V:V was 7:1), but ground plant tissues, MD and SE (0.3000 g) were digested using concentrated HNO₃ and 30 % H₂O₂ (V:V was also 7:1) (Lo and Sakamoto, 2005; Li et al., 2013; Moreno-Jiménez et al., 2014).. The digestion processes of soil and plant samples were both performed in a microwave digestion instrument (MARS, CEM). The available Cd, Zn and Pb in soil were assessed by 100 mmol L⁻¹ CaCl₂ (Menzies et al., 2007), but the available As by 50 mmol L⁻¹ NaH₂PO₄ (Huang et al., 2006). The extraction procedure of available different heavy metals and metalloids in soil was: approximate 5 g dried soil (< 2 mm) was transferred into a 50 ml plastic bottle, after which 25 ml 10 mmol L⁻¹ CaCl₂ (or 50 mmol L⁻¹ NaH₂PO₄) was added into the bottle. Then sealed bottle was shaken in a

reciprocating shaker for 2 h at 160 rpm at 25 ± 0.5 °C, and then the supernatant was filtered through Whatman No.42.

Inductively coupled plasma mass spectrometry (ICP-MS, Agilent7500a, USA) was employed to assay the contents of heavy metals and metalloids at the Central Laboratory of the Tianjin Academy of Agricultural Sciences of China. Standard reference material (GBW07603, GSV-2) was used to evaluate the analysis accuracy, which was purchased from the Centre for Standard Reference of China.

2.4 Data analysis

One-way with multi-comparisons by Tukey's test at $P \leq 0.05$ was used to compare the means among different treatments and two-way ANOVA with Tukey's test ($P \leq 0.05$) to analyze the interactive effects between SE and MD. The two-way ANOVA analysis was performed by SAS software, and SPSS software was used to conduct the one-way ANOVA analysis. The plots were generated using SigmaPlot (SigmaPlot 10.0). The values in this study were means of three replications.

3. Results

3.1 The biomass responses to multiple heavy metal and metalloid contamination and different amendments

As compared to the control, the treatments containing SE all significantly increased the shoot biomass of pakchoi besides the treatment of $SE_{0.5}+MD_{0.8\%}$; however the addition of SE did not significantly enhance the root biomass except for the $SE_1+MD_{0.4\%}$ and $SE_1+MD_{0.8\%}$ treatments (Fig. **1a, b**). When SE was not added into the soil, the shoot and root biomass of pakchoi was almost non-significantly enhanced by increasing MD levels

(Fig. 1a, b). Also, the interactive effects between SE and MD did not significantly affect the shoot and root biomass (Table 1). However, under certain conditions, the combined addition of SE and MD showed a stronger ability to stimulate the growth of pakchoi than their individual single treatment. Because the highest shoot biomass appeared at the treatment of SE₂+MD_{0.2}, significantly increasing up to 2130% compared to the control, which was followed by SE₁+MD_{0.8} (2098%), SE₂+MD₀ (1975%), SE₁+MD_{0.2} (1841%), SE₁+MD_{0.4} (1809%), SE₂+MD_{0.4} (1738%), SE₁+MD₀ (1640%), SE₂+MD_{0.8} (1629%), SE_{0.5}+MD₀ (1386%), SE_{0.5}+MD_{0.2} (1347%) and SE_{0.5}+MD_{0.4} (1343%). Most treatments non-significantly increased the root biomass as compared to the control, except for the SE₁+MD_{0.4}, SE₁+MD_{0.8} and SE₂+MD_{0.2} treatments where the root biomass increased up to 4398%, 3972% and 3003%, respectively.

3.2 The pH values and concentrations of available heavy metals and metalloids in soil

The addition of SE and MD and their interaction all showed significant effects on the soil pH value (Table 1). Regardless of the MD levels in soil, the addition of SE with high pH value significantly increased the soil pH (Fig 1c). Unlike the effects of SE on soil pH, only the highest level of MD as 0.8% to the soil treated without SE or with 0.5% SE obviously enhanced the soil pH when compared to the control and the SE_{0.5}+MD₀ treatment, respectively. Other levels of MD appeared to show non-significant effects on the pH values of soil treated with or without SE soil (Fig. 1c).

When no SE was added in the soil, only 0.8% MD significantly reduced the available Zn and Cd concentration in the soil solution (Fig. 2a, c); however, with certain

level of SE present in the soil (for example: 1%), the addition of MD appeared to have no significant effects on the available Zn and Cd concentrations. When the soil was treated with a certain level of MD (for example: 0.2%), increasing SE levels significantly reduced the soil Zn and Cd available concentrations (Fig. **2a, c**).

With or without SE presence, increasing MD levels did not significantly affect the available As concentration (Fig. **2b**). Regardless of the presence of MD in the soil or not, increasing SE levels all significantly increased the available As concentration (Fig. **2b**).

In the absence/presence of SE in the soil, increasing MD levels did not significantly affect the available Pb concentration (Fig. **2d**). When the soil treated without MD or with 0.8% MD, increasing SE levels non-significantly enhanced the available Pb concentration; however, when the soil was treated with 0.2% and 0.4% MD, further addition of 1% SE significantly enhanced but 2% SE did not significantly affect the available Pb concentration in the soil as compared to the treatment of MD_{0.2%}+SE₀ and MD_{0.4%}+SE₀, respectively (Fig. **2d**).

3.3 Results of FT-IR analysis and adsorption isotherm experiment

The results of FT-IR analysis showed that the spectrum for SE samples in the control treatment exhibited an obvious peak at 2516.83 cm⁻¹ (Fig. **3a**), which contributed to the stretching vibration of structural S–H groups; however, when the SE samples were treated with Pb and Cd, the peak was apparently weakened, suggesting that sulfhydryl groups in SE might bond with Pb and Cd. In MD samples, similar stretching vibration of structural S–H groups was not observed (Fig. **3b**).

The results of adsorption isotherm experiment were shown in Fig. 4. The constant A in the Freundlich equation for SE samples treated with Cd, Pb and Zn were all much higher than that for MD samples (Fig. 4c, d, e, f, g, h), suggesting a stronger ability of SE to adsorb Cd, Pb and Zn than that of MD.

3.4 The contents of Zn, Cd, As and Pb in different tissues of pakchoi

The contents of Zn, Cd, As and Pb in the shoots and roots were all significantly affected by the SE treatment, MD treatment and the interaction between SE and MD, except for the Zn in the roots and Cd in the shoots where the MD treatment did not show significant effects on their contents (Table 1).

When no SE was added in the soil, increasing MD levels significantly reduced the Zn content in the shoots but non-significantly reduced it in the roots (Fig. 5a, b). However, when SE was present at a certain level (for example: 0.5%), increasing MD levels did not significantly affect the shoot and root Zn content. When MD was added at a certain level (for example: 0.2%), increasing SE level significantly reduced the shoot and root Zn content (Fig. 5a, b).

In the absence of SE in the soil, increasing MD levels significantly reduced the shoot and root As content; however, with the presence of SE, increasing MD levels did not significantly affect the As contents both in the shoots and roots (Fig. 5c, d). When MD was not added in the soil, increasing SE levels significantly reduced the As contents both in the shoots and roots (Fig. 5c, d). However, when MD was present in the soil, increasing SE levels did not significantly influence the shoot As content (Fig. 5c), but significantly reduced the root As content (Fig. 5d).

With or without MD in the soil, increasing SE levels significantly reduced the shoot and root Cd contents (Fig. **6a, b**). When no SE was added in the soil, increasing MD levels significantly reduced the shoot Cd content but enhanced the root Cd content (Fig. **6a, b**); however, when SE was added in the soil, increasing MD levels appeared to have no significant effects on the shoot and root Cd content (Fig. **6a, b**).

When the soil was treated with $< 0.4\%$ MD, increasing SE levels significantly reduced the shoot Pb content; however, when $\geq 0.4\%$ MD was added, the addition of SE non-significantly reduced the shoot Pb content (Fig. **6c**). When no SE was added in the soil, increasing MD levels significantly reduced the shoot Pb content; however, when SE was present in the soil, increasing MD levels did not significantly affect the shoot Pb content (Fig. **6c**). With or without MD in the soil, the increasing SE levels significantly reduced the root Pb content except for the treatments containing 0.2% MD, where the decrease in the root Pb content was not significant (Fig. **6d**). When no SE was added in the soil, increasing MD levels significantly reduced the root Pb content; however, when SE was added at a certain level in the soil (for example: 0.5%), increasing MD levels did not significantly affect the root Pb content (Fig. **6d**).

4. Discussion

This study was carried out to assess the possibility of using agricultural wastes (SE and MD) to reduce the uptake and accumulation of heavy metals and metalloids in pakchoi subjected to slightly contaminated soil with multiple heavy metals and metalloids. The significant increases in the shoot biomass after SE addition might suggest the beneficial effects of SE on the growth of pakchoi; however, MD showed limited ability to stimulate

pakchoi growth judging from the unremarkable changes in the biomass of pakchoi treated with MD. Documents have shown that SE is often used in China as an excellent organic fertilizer, which contains high levels of organic matters (occupying 77.68-81.15% of total dry weight), nitrogen (N, 2.97%), phosphorus (P_2O_5 , 1.03%) and potassium (K_2O , 3.52%) (Zhou et al., **2008**). Therefore, the stimulation on the shoot dry biomass after SE addition might partially ascribe to the enhanced soil fertility in this study. Similar stimulating effects of SE on the growth of some vegetables were also reported by (Qu et al., **2010**), in whose study the yields, chlorophyll content, reducing sugar content and vitamin C content were all significantly enhanced by SE in three leafy vegetables (Chinese cabbage, lettuce and spinach) when compared with conventional inorganic fertilizers and organic-inorganic fertilizers.

Thing deserved to be mentioned was that the content of organic matter in MD was 1.52 times higher than that in SE, but this high level of organic matter did not result in the significant increases in the shoot and root biomass of pakchoi. In this study the supply of SE, which was a complex containing lime, has resulted in the significant increases in soil pH; however, the MD treatment appeared to show a relatively weaker ability than SE to increase the soil pH value. Furthermore, the tested soil was more heavily contaminated by Cd than other elements, and the growth of pakchoi might be mainly affected by the behaviors of Cd rather than As, Zn and Pb in the soil. Reports have shown that lime is an efficient way to immobilize Cd by way of enhancing soil pH and Cd adsorption onto soil particles so as to alleviate Cd phytotoxicity (Bolan et al., **2003b**). Therefore, the weak

abilities of MD relative to SE to enhance soil pH, reduce soil Cd availability and decrease Cd contents in pakchoi resulted in MD failing to counteract the toxicity of Cd to pakchoi.

It is well established that soil pH is believed to be an important factor to affect the mobility and availability of heavy metals and metalloids in soil (Appel and Ma, **2002**; Zeng et al., **2011**). However, the effects of pH on the availability of heavy metals and metalloids will differ with different elements. In soil solution, Cd, Zn and Pb often present as cations while As mainly appears as anion, therefore, the changes in soil pH values might result in different effects on the availability of these four elements. Reports have shown that alkaline pH released substantial proportions of As into soil solution (Masscheleyn et al., **1991**); however, soil pH value within a certain range was found to be significantly negatively correlate to the availability of Zn, Pb (Zeng et al., **2011**) and Cd (Appel and Ma, **2002**). Similar results were also observed in this study. For example, the available concentrations of Zn and Cd in the soil both significantly decreased with the addition of increasing SE levels (Fig. **2a, c**) which resulted in the increases in soil pH (Fig. **1c**), but the available concentration of As was apparently enhanced by increasing SE levels (Fig. **2b**) regardless of the addition of MD. Interestingly, upon certain level of MD in the soil, the available Pb concentration was enhanced by $\leq 1\%$ SE, but it began to decrease at a higher SE level as 2% (Fig. **2d**). This increase in the availability of Pb in soil might be connected to the increasing pH after SE addition which was a complex containing lime. Similar increases in Pb availability in soil after lime addition were also observed in the study of Khan and Jones (**2009**).

Apart from soil pH, soil organic matter is another factor affecting the availability of heavy metals and metalloids in soil (Zeng et al., **2011**). Alkaline materials with low total and bio-available metal content were thought to be an effective sink for reducing the bioavailability of metals in contaminated soil (Bolan and Duraisamy, **2003**). However organic matter quantity was not the key factor to affect the availability of soil heavy metals and metalloids in this study. Because in the presence of SE in the soil, the addition of different levels of MD showed non-significant effects on the available concentrations of Zn, As, Cd and Pb, which suggesting that the extra addition of MD (meaning high levels of organic matters) did not help SE reduce the availability of heavy metals and metalloids in soil. In this study, some groups of organic matters in SE samples might influence the availability of some soil heavy metals and metalloids. Because the results of FT-IR analysis might suggest the participation of S–H groups derived from SE in binding with Pb and Cd (Fig. **3a**). However, similar stretching vibration of structural S–H groups was not observed in MD samples (Fig. **3b**), suggesting S–H groups in MD were not involved in binding with heavy metals and metalloids in this study. Above results were well in line with the weaker ability of MD than that of SE to affect the availability of heavy metals and metalloids in soil in this study. In fact, mercapto functionalized materials have been reported to be able to provide a potential remedy for Pb and Cd contamination in soil and water (Liang et al., **2011**). The results of adsorption isotherm experiment demonstrated a stronger ability of SE to adsorb Cd, Pb and Zn than that of MD, thus, affect the availability of these heavy metals and metalloids in soil.

Inconsistent with the changes in the availability of heavy metals and metalloids in soil after the addition of MD, single treatment of MD significantly reduced the Zn and Cd contents in the shoots, and As and Pb contents both in the shoots and roots. Here, we did not know why the single addition of MD could significantly reduce the contents of As and Pb in the shoots and roots of pakchoi, which was not supported by the associated results of non-significantly enhanced availability of As and Pb. However, the significantly suppressed Zn and Cd contents in the shoots were well line with the apparently decreased availability of Zn and Cd in the soil treated with single MD treatments. When certain level of SE was supplied to the soil, increasing MD levels did not help SE to reduce the contents of all tested heavy metals and metalloids both in the shoots and roots of pakchoi.

Similar to MD, the SE showed negative effects on the contents of heavy metals and metalloids in pakchoi, showing as the significantly decreased contents of all heavy metals and metalloids both in the shoots and roots after the single treatment of SE (Fig. 5, 6). However, the decreased contents of As and Pb both in the shoots and roots of pakchoi subjected to single SE treatment did not match well with the increased availability of As and Pb, respectively. We speculated that it might be partially related with the increases in the biomass of shoots and roots, and result in a “dilution effect” of heavy metals and metalloids. Additionally, the extra addition of SE appeared to be able to help MD to further reduce the accumulation of some heavy metals and metalloids in some tissues of pakchoi.

5. Conclusions

The results of this study indicated that the SE is an excellent amendment to ameliorate the toxicity of multiple heavy metals and metalloids to pakchoi cultivated in such slightly contaminated soil. Its application can significantly reduce the contents of As, Zn, Cd and Pb in the edible part of pakchoi. The SE was more efficient than MD to remediate the multiple metal and metalloid polluted soils, due to the significantly enhanced biomass of pakchoi and reduced heavy metal and metalloid content in the shoots of pakchoi despite that the content of organic matter in MD was much higher than that in SE. The extra addition of MD to SE treatments appeared not to remarkably help SE stimulate the growth of pakchoi and reduce the accumulation of heavy metals and metalloids in the shoots. Therefore, based on the results of shoot biomass of pakchoi and contents of all heavy metals and metalloids, and in view of enhancing soil fertility and ameliorating the contaminated soil, 2% SE treatment was recommended in this study upon using SE and MD to remediate As, Cd, Pb and Zn contaminated soil.

Acknowledgements

This research was supported by National High Technology Research and Development Program of China (2012AA101404-5), the National Science Foundation of China (41373128), the Tianjin Natural Science Foundation (12JCYBJC14900) and the Central Public Research Institute Basic Fund for Research and Development (2014-szjj-frw-08).

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Table 1 Results of the two-way ANOVA and Tukey multiple range tests for the effects of silkworm excrement (SE) and mushroom dreg (MD) on the dry weight, soil pH, total contents of heavy metals in different tissues of pakchoi, and available concentrations of heavy metals in soil.

		Source of variation		
		ANOVA F-values		
	Tissues	SE treatment	MD treatment	SE*MD interaction
Biomass	shoots	74.68**	0.30ns	0.92ns
	roots	21.70**	1.07ns	1.81ns
Zn	shoots	271.42**	5.34**	3.80**
	roots	211.33**	1.44ns	2.97**
As	shoots	19.18**	4.01*	3.52**
	roots	204.97**	20.50**	17.97**
Cd	shoots	80.66**	2.65ns	3.51*
	roots	298.38**	13.23**	8.07**
Pb	shoots	40.42**	7.08**	5.64**
	roots	301.25**	23.53**	8.96**
Available Zn in soil		313.17**	5.57**	4.81**
Available As in soil		85.63**	1.27ns	1.11ns
Available Cd in soil		200.78**	2.72ns	1.32ns
Available Pb in soil		25.66**	0.53ns	2.12*
Soil pH		1485.72**	10.02**	4.66**

⁽¹⁾ F-values for the SE*MD interaction, SE treatment and MD treatment. ns: not significant F ratio

($p < 0.05$); * and ** indicate significant at $P < 0.05$ and 0.01 , respectively

Fig. 1 Effects of silkworm excrement and mushroom dreg on the dry weight of pakchoi and pH values of soil. Each treatment was replicated three times. The bars are standard deviations for the mean of three replications. Different letters above bars indicate significant difference at $P < 0.05$.

Fig. 2 Effects of silkworm excrement and mushroom dreg on the available concentrations of heavy metals in soil. Each treatment was replicated three times. The bars are standard deviations for the mean of three replications. Different letters above bars indicate significant difference at $P < 0.05$.

Fig. 3 FT-IR spectra of silkworm excrement (a) and mushroom dreg samples (b).

Fig. 4 The fitted curves and associated equations for the adsorption of As, Cd, Pb, Zn by SE and MD.

Fig. 5 Effects of silkworm excrement and mushroom dreg on the contents of Zn and As in the shoots and roots of pakchoi. Each treatment was replicated three times. The bars are standard deviations for the mean of three replications. Different letters above bars indicate significant difference at $P < 0.05$.

Fig. 6 Effects of silkworm excrement and mushroom dreg on the contents of Cd and Pb in the shoots and roots of pakchoi. Each treatment was replicated three times. The bars are standard deviations for the mean of three replications. Different letters above bars indicate significant difference at $P < 0.05$.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

The fitted curve for As, Cd, Pb, Zn adsorbing by SE and MD

Fig. 5

Fig. 6