J Soils Sediments (2018) 18:1–11
DOI 10.1007/s11368-017-1724-2

SOILS, SEC 1 • SOIL ORGANIC MATTER DYNAMICS AND NUTRIENT CYCLING • RESEARCH ARTICLE

Rhizosphere effects of *Loliumperenne* L. and *Beta vulgaris* var. *cicla* L. on the immobilization of Cd by modified nanoscale black carbon in contaminated soil

Yan Lyu[1] · **Yaqin Yu**[1] · **Tong Li**[1] · **Jiemin Cheng**[1]

Received: 13 January 2017 / Accepted: 30 April 2017 / Published online: 15 May 2017

Abstract
Purpose The choice and stability of the immobilization agents in in situ remediation of heavy metal-polluted soil is particularly important. This study aimed at investigating the rhizosphere effects of different plants on the immobilization stability of Cd by modified nanoscale carbon black (MBC) in contaminated soil.
Materials and methods Pot experiments using Cd-tolerant plant ryegrass (*Loliumperenne* L.) and Cd hyperaccumulator leaf red beet (*Beta vulgaris* var. *cicla* L.) were conducted. A rhizobag was applied to investigate the rhizosphere effect on the remediation of Cd by the MBC. After 60 days of cultivation, the pH, DTPA-extractable Cd concentrations, and the fractionation of Cd of rhizosphere and bulk soil were determined. The dry biomass and the concentrations of Cd in the shoots and roots of two plants were also measured.
Results and discussion The results indicated that the biomass of ryegrass significantly increased by 63 and 65% in roots and shoots, respectively, with the addition of MBC. Significant change of the biomass of leaf red beet after adding MBC was not seen. Addition of MBC led to the decreases of soil pH, DTPA-extracted Cd in both rhizosphere and bulk soils, and declined bioavailability of Cd cultivated with either ryegrass or leaf red beet. Compared to the bulk soil, the concentration of DTPA-extracted Cd in rhizosphere reduced by 8.91 and 0.66%, for ryegrass and leaf red beet, respectively, and when MBC was added, decreased by 6.92 and 6.20%, respectively.
Conclusions The rhizosphere process of ryegrass had a negative effect on the immobilization of MBC on Cd. However, there was a promoting function of the immobilization by MBC for Cd in the rhizosphere of leaf red beet. Thus, the Cd-tolerant plant ryegrass should be avoided in the practical application of MBC.

Keywords Cadmium · Immobilization · Nanoscale black carbon · Rhizosphere · Soil

Responsible editor: Xilong Wang

Electronic supplementary material The online version of this article (doi:10.1007/s11368-017-1724-2) contains supplementary material, which is available to authorized users.

✉ Jiemin Cheng
13188941781@163.com

[1] College of Geographic and Environment, Shandong Normal University, Jinan, Shandong, China

1 Introduction

Anthropogenic activities such as smelting, mining, and fertilizer and vehicle exhausts are the main sources of heavy metals in soil (Li et al. 2009; Wang et al. 2015a, b). All these activities have been accelerated during the past several decades leading to extensively occurred soil contamination in China (MLR, MEP 2014). According to the latest national survey, concentrations of heavy metals and many other pollutants in over 16% of soil exceeded the national soil environmental quality standards. Soil contamination and subsequently food safety are major concerned environmental issues in China (Environmental Protection Ministry 2015).

Among various heavy metals and metalloids, cadmium (Cd) is the most concerned contaminant in agricultural soils. Results from the national soil quality survey showed that Cd had the highest exceedance rate among all pollutants (Environmental Protection Ministry 2015). Concentrations of Cd in surface soil ranged from 0.003 to 9.6 mg kg^{-1}in 146 cities of China (Wang et al. 2015a, b). Adverse impacts of Cd on plants, animals, and human beings have also been well demonstrated (Doumett et al. 2008; Shi et al. 2009). It was reported that the Cd concentrations of rice samples ranged from 0.01 to 4.43 mg kg^{-1} in Chenzhou of Southern China, and for an adult, the mean dietary Cd intake from rice was 0.191 mg day^{-1} (Zhai et al. 2008).

To remediate heavy metal-contaminated soils, a number of technologies have been developed and applied (US EPA 1997; Ruttens et al. 2010; Sheoran et al. 2011). Among various technologies, in situ immobilization technology aims to reduce mobility and bioavailability of metals in soil so as to prevent them to cause harmful impacts on soil biota, groundwater, and crop quality. According to the results reported so far, this method is a promising, practical, and economical one (Brown et al. 2005; Xu et al. 2010). One of the remaining issues in in situ immobilization technology is the remobilization potential of the fixed metals as the soil condition changes after the remediation. Therefore, the choice of the immobilization agents is particularly important for in situ remediation of heavy metal-polluted soil.

Nanoscale black carbon, which widely occurs in soil at relatively low content, has huge specific surface area and active adsorption sites. The affinity to metals can be strengthened after modification, which is due to the changes in surface properties of black carbon (Borah et al. 2009; Cheng et al. 2015). For example, the zeta potentials of the modified nanoscale black carbon (MBC) were lower than the unmodified one at the same pH. The specific surface area, pore volume, and mean pore size were obviously increased. The surface functional groups of C=C and O–H were not only significantly increased but also C–O and CNO functional groups were found, compared with the unmodified black carbon. Therefore, the modified nanoscale black carbon (MBC) has good potential to be used as an effective immobilization agent. It was reported that the effectiveness of using nanoscale black carbon for heavy metal fixation in soil can be significantly enhanced by oxidation modification (Zhou et al. 2010; Cheng et al. 2015). For example, it was demonstrated that the sorption capacities of modified black carbon (MBC) for Ni, Cd, and Cu can be elevated by 25.0, 79.4, and 417%, respectively, compared to the unmodified one (Liu and Cheng 2012; Cheng et al. 2015).

Various plants also response differently to the soil metal contamination. This is particularly true for heavy metal-tolerant plants and hyper-accumulating plants, which have developed either tolerance or hyper-accumulation mechanisms (McGrath et al. 2001; Mahar et al. 2016; Thakur et al. 2016). Rhizosphere soil play a key role in plant growth, and behavior and bioavailability of heavy metals in soil are strongly governed by soil properties such as pH, organic acid, microbial activity, etc. (Kim et al. 2010; Wei and Twardowska, 2013; Qasim et al. 2016).

The main objective of this study is to investigate the change of metal bioavailability in rhizosphere of a Cd-contaminated soil with MBC applied and to comparatively analyze the potential rhizosphere effects of different plants on the immobilization process. A cadmium-tolerant plant and a hyper-accumulator were studied in order to achieve the above objectives.

2 Materials and methods

2.1 Soil sample and plant materials

The soil used in the study was brown earth collected from the surface (0–20 cm) in the farm of Shandong Agricultural University (Taian, Shandong Province, China), air dried and ground, and sieved through a 20-mesh sieve prior to use. Brown soil is one of the three main types of soil in Shandong Province, and the Cd pollution of brown soil is serious in vegetable fields, especially in urban suburbs (Jia et al. 2015). The physical and chemical properties of the soil are listed in Table 1, as determined following the method of Lu rukun (2000).

Cd^{2+} was added to the soil described above by spiking with $CdCl_2 \cdot 2.5H_2O$ solution to contain 15 mg Cd kg^{-1}, which is the same as most of the literature (Li et al. 2007; Wei and Twardowska 2013; Wang et al. 2015a, b). Adding deionized water to soils achieved a moisture content of 70% field capacity. To ensure even dispersion of various Cd fractions, the soils were thoroughly mixed and equilibrated at room temperature (at about 20 °C) for 2 months.

Ryegrass (*Loliumperenne L.*) and leaf red beet (*Beta vulgaris* var. *cicla L.*) seeds used in the experiments were purchased from Jinan Dajiang Seed Company, Shandong Province, China. Ryegrass can grow in the environment with high-level accumulation of heavy metals, belonging to the "metal-tolerant plants." Leaf red beet can accumulate large quantities of Cd, belonging to the "Cd hyper-accumulator plants".

2.2 Preparation of the modified nanoscale black carbon

A commercial MBC with particle size of 20–70 nm and the specific surface areas of 1259 m^2 g^{-1} was purchased from Jinan Black carbon factory, Shandong Province, China. The black carbon was washed with deionized water to remove the

Table 1 The physical and chemical properties of the soil for pot experiment

Properties	Values
Organic matter content ($g \cdot kg^{-1}$)	0.92
pH	6.64
<0.01 mm clay content (%)	23.3
Total Cd ($mg \cdot kg^{-1}$)	0.20
Available Cd ($mg \cdot kg^{-1}$)	0.042

surface impurity, dried to constant weight at 105–110 °C in a vacuum oven (DHG-9245, Shanghai, China). This black carbon was further oxidized (following the method of Cheng et al. 2015) with acidic $KMnO_4$ and 65% HNO_3 for modification by refluxing 10 g of black carbon with 74 ml $KMnO_4$ (0.3 mol L^{-1}) and 36 ml HNO_3 (65%) added in order in a conical flask, then sealed with plastic film and put into water bath (SHA—C, Jintan, Jiangsu Province, China) at 90 °C for 180 min. The MBC was filtered, washed with deionized water until the pH of the filtrate became stable (pH is about 5.5), and finally dried to constant weight in a vacuum oven at 80 °C. Characterizations of MBC were similar to those reported by Cheng et al. (2015).

2.3 Pot experiments

In the pot experiments of this study, a rhizobag method was conducted to investigate the rhizosphere effect on the remediation of Cd of the MBC. The rhizobag was a cylindrical water-permeable nylon 500-mesh bag ($C = 16$ cm, $H = 12$ cm) applied to keep the rhizosphere soil separate from the bulk soil. There were a total of four treatments, including control of two plants without MBC addition (CK), and treatments adding 2% MBC compared with control (MBC). The experiments were made in three replicates.

In each pot, there was 1.5 kg of soil in total; therein, 0.2 kg was placed in the rhizosphere zone. For each pot, 1.317 g K_2HPO_4 and 0.644 g urea were added as fertilizer and mixed thoroughly. The plants of all pots were watered by deionized water to keep the soil at 70% of field water holding capacity throughout the growth period of 60 days.

For the treatment groups, based on our previous studies on the passivation effect of different concentrations of MBC from 0.5 to 5% (Wang et al. 2009; Cheng et al. 2015), 2% MBC was added into soil, mixed thoroughly. After 1 week, two kinds of plant seeds were sowed into the rhizosphere zone of each pot, respectively. Fifteen seedlings of ryegrass and five of leaf red beet were persisted for each pot, respectively, after seed germination.

At the end of the experiment, all plants in each pot were harvested, removed adhering soil thoroughly, washed carefully, and separated into roots and shoots, then oven-dried at 105 °C for 30 min and weighed regularly until constant weight. Dried plant shoots and roots were weighed respectively. The soil subsamples distinguished as the rhizosphere and the bulk were air-dried and ground to pass through a 10-mesh nylon sieve prior to use.

2.4 Sample analysis

Soil pH was measured in deionized water extracts (1:2.5 *w/v*) by a DZS-706 multi-parameter analyzer (Shanghai, China). Soil organic matter was determined by H_2SO_4–$K_2Cr_2O_7$ with external heating using total organic carbon analyzer (TOC-VCPH, Shimadzu, Japan). The soil samples to analyze available Cd content were mixed into diethylenetriaminepentaacetic acid (DTPA) (1:2 *w/v*) and shaken at 180 rpm at 25 °C for 2 h, then filtered with filter paper, the filtrate was analyzed by atomic absorption spectrophotometer (AAS, TAS-990, Beijing, China) (Shi 1996). Total Cd concentrations were determined by HNO_3–$HClO_4$–HF extraction, followed by AAS. The fractions of Cd in soil were performed following the Tessier method (1979). Oven-dried plant samples were digested with concentrated HNO_3 and 60% of $HClO_4$ (5:1, by volume), analyzed for Cd concentration of shoots and roots using AAS. All reagents in this study were of analytical grade purchased from Sinopharm Chemical Reagent Co., Ltd.

2.5 Data analysis

The results were calculated using Microsoft EXCEL, presented as means ± standard deviations. One-way analysis of variance and bivariate correlation analysis were performed using SPSS v.13.0 (SPSS Inc., USA) at a significance level of 0.05.

3 Results and discussion

3.1 Changes of soil pH in the bulk and rhizosphere soils

Soil pH is often under influences of plant growth through various processes such as respiration, absorption, and secretion, as well as activity of root system-associated microorganisms (Luo et al. 2000; Hinsinger et al. 2005). On the other hand, any change in soil pH can affect fractionation and consequently bioavailability of trace metals in soil (Fitz et al. 2003; Gonzaga et al. 2009).

Figure 1 shows pH values in both bulk and rhizosphere soils after 60 days of cultivation of either ryegrass or leaf red beet with or without MBC spiked. The measured pH values of the bulk soils cultivated with either ryegrass or leaf red beet for 60 days increased significantly from 6.64 ± 0.04 of the raw soil to 7.49 ± 0.04 and 7.42 ± 0.02 ($p < 0.05$). Compared with the bulk soil, the pH changes in rhizosphere soils were even larger and the measured pH values in the rhizosphere soils were 7.75 ± 0.05 and 7.51 ± 0.01 for the ryegrass or leaf red beet grown soils, which were not only significantly higher than that of the raw soil but also higher than corresponding bulk soils after the cultivation ($p < 0.05$ for ryegrass and $p < 0.10$ for leaf red beet). The stronger effect on rhizosphere soils, which were under direct influence of root system, was well expected. For instance, Kim et al. (2010) found that soil solution pH increased by 1.4 U in acidic soil growing Indian mustard. It was often reported the influence of roots are limited to several millimeters of rhizosphere soil (Weng et al. 2014).

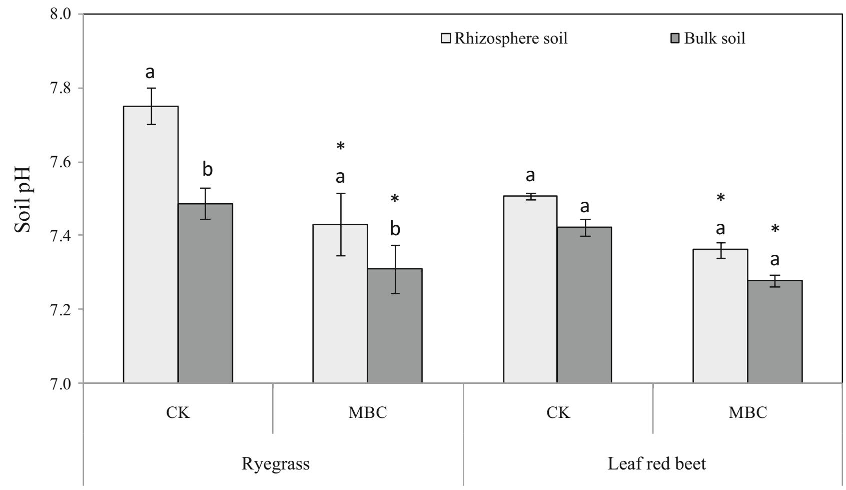

Fig. 1 Soil pH values in both rhizosphere and bulk soils after 60 days of cultivation of ryegrass and leaf red beet with or without MBC spiked (the results are shown as means and standard deviations. *Significantly different from the CK ($p < 0.05$). Different letters over column bar indicate significant differences between rhizosphere and bulk soil ($p < 0.05$) (same below))

For the two plants, the pH changes in both rhizosphere and bulk soils cultivated with ryegrass were larger than those cultivated with leaf red beet ($p < 0.05$). Such a strong influence on soil pH could be facilitated by the stress of the Cd added. The effect of Cd stress on the secretion of protons by plant roots has been reported previously. Since metal solubility can be generally reduced in alkaline condition, the slowing down of the proton release from plant roots under Cd stress is believed to be a function to alleviate the rhizosphere acidification process (Tu et al. 1989). It appears that under Cd stress, Cd-tolerant ryegrass can respond better than leaf red beet could to reduce Cd solubility in soil. On the other hand, the strategy of leaf red beet, as a Cd hyperaccumulator, is not to prevent the absorption by activating Cd in soils. It was revealed that the hyperaccumulator can often secrete proton or organic acid to acidify the rhizosphere soil (Kinnersley 1993; Yang et al. 2002). Unfortunately, controlled experiment without Cd spiking was not conducted in this study and it is impossible to distinguish the influence of Cd stress from the physiological effect of plant root.

Soil pH was also affected strongly by the MBC. In all cases of both bulk and rhizosphere soils with ryegrass or leaf red beet cultivated, pH increase during the cultivation was weakened by the addition of MBC ($p < 0.05$). This is particularly true for rhizosphere soil of ryegrass grown soil, and the increase in pH was reduced from 7.75 ± 0.05 to 7.43 ± 0.08. Liu and Cheng (2012) tested the effect of MBC on pH of a brown soil and found similar trend of reducing pH change. It was believed that MBC has lower negative zeta potential, which augments the electrostatic adherence with cation (Zhou et al. 2010). Such an effect can be explained by the abundance of oxygen-containing functional groups on the surface of the MBC (Borah et al. 2008).

3.2 The DTPA extractable Cd in the soils

The measured concentrations of DTPA extractable Cd, as indicators of bioavailable fraction of Cd in the soil, are shown in Fig. 2 for two plant-cultivated soils with or without MBC spiked, including rhizosphere and bulk soils. It appears that

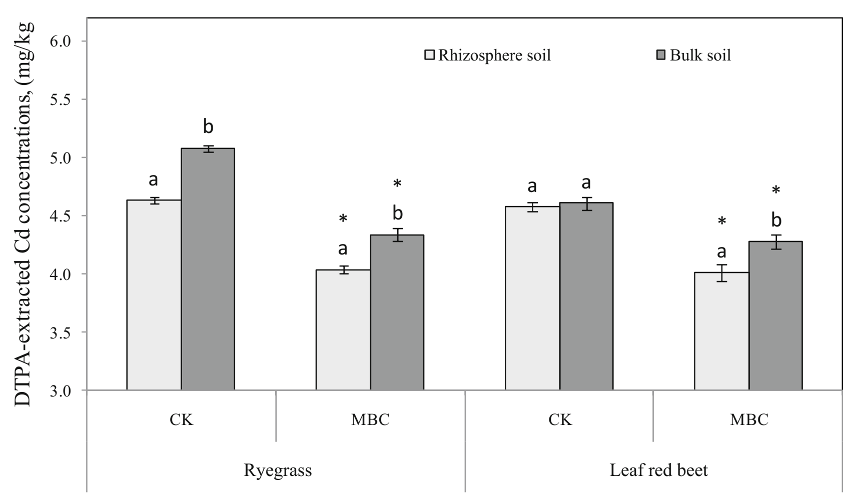

Fig. 2 DTPA-extractable Cd concentrations in rhizosphere and bulk soils for two plant-cultivated soils with or without MBC spiked

compared with the soils grown leaf red beet, soil cultivation with ryegrass resulted in higher concentration of the DTPA extractable Cd in the bulk soil, and the difference is significant.

The concentrations of DTPA extractable Cd in rhizosphere soil cultivated with ryegrass significantly reduced by 8.91% compared with those in bulk soils. This finding was similar to those reported previously in the literature. For instance, Weng et al. (2014) also observed that the content of the DTPA-extractable Cd decreased significantly in the rhizosphere of *Kandelia obovata* (S., L.) *Yong*, compared to those in the bulk soil. On the other hand, only slightly non-significant difference of the DTPA-extractable Cd between rhizosphere and bulk soil was observed for the leaf red beet cultivated soil. Taking into consideration that the planned leaf red beet absorbed more Cd from the soil (will be discussed in the back section), largely from the rhizosphere, than the ryegrass did, the difference between the two plants was more obvious.

Such changes appears to be pH dependent. The 60 days of cultivation led to pH increase in the soil and decrease in bioavailable fraction of Cd. The effect was stronger in the rhizosphere, which was under direct effect of root system, than that in the bulk soil. The similar trend was found for other soils, either acidic or alkaline. The increase of pH after plant growth reduced the Cd solubility, in particular, stable Cd-DOC complexes formed DOC excreted by plant roots with Cd resulted in the reduction of free Cd ions in soil (Kim et al. 2010; Qasim et al. 2016). A number of the processes involved in bioavailability reduction of heavy metal are adsorption on soil particles, precipitation as phosphates or hydroxides, or formation of the complex, which are positively promoted by alkalization (Luster et al. 2008).

As shown in Fig. 2, the bioavailability of Cd in the soil was significantly reduced by addition of the MBC, for both rhizosphere and bulk soils, cultivated with either ryegrass or leaf red beet. This shows that MBC has a better immobilization effect on Cd in soil. In the meantime, the difference between the bulk soil and rhizosphere of ryegrass was lower than that without MBC added, i.e., the concentrations of the bioavailable Cd in the rhizosphere soils decreased by 6.92% compared with those in the bulk soils ($p < 0.05$). For leaf red beet, the bioavailable Cd concentrations of the rhizosphere significantly lower than those in bulk soils with MBC added ($p < 0.05$). And, this difference increased from 0.66% without MBC to 6.20% with MBC added.

As discussed before, addition of the MBC brought down soil pH due to the existence of oxygen-containing functional groups on the surface of the MBC (Borah et al. 2008), which can reduce bioavailability of Cd by facilitating the binding of the free metal ions (Liu and Cheng 2012). Navari-Izzo and Quartacci (2001) also suggested that the complexation of metals with organic ligands secreted from the plant roots is one of the important mechanisms of the plant to avoid overstressing. More specifically, oxalic acid was found to be the dominant organic acid secreted from ryegrass roots and the quantity of the secretion was correlated with its Cd stress tolerance (Xie et al. 2009; Lou et al. 2015). In fact, chelation of metals by oxalic acid to form stable Cd^{2+}-chelate complex can prevent Cd ions into the root cell (Nazar et al. 2012) and avert the combination with MBC, which had a negative effect on the immobilization process of MBC on Cd ions.

However, for hyperaccumulator plants such as the leaf red beet, small molecular weight organic acids secreted from roots can also chelate metals to increase metal solubilities and facilitate the accumulation process of plants on metal ions (Kinnersley 1993; Yang et al. 2002). This may also facilitate the combination of cadmium with MBC. Therefore, the rhizosphere process of leaf red beet had a positive effect on the immobilization by MBC on Cd in this study.

3.3 Fractionation of Cd in the soils

In addition to the DTPA-extractable fraction, Cd fractionation in the studied soil samples were characterized by sequential extraction according to Tessier et al. (1979), and five fractions were extracted for both bulk and rhizosphere soil samples. The relative contributions of the five fractions to the total Cd concentrations in the soil are shown in Fig. S1 (Electronic Supplementary Material) for both the rhizosphere and the bulk soils cultivated with either ryegrass or leaf red beet and with or without the MBC added. In general, the fractionation of Cd in the samples was similar to one another, no matter which plants were cultivated or which samples were collected from the rhizosphere or bulk soil, or whether the MBC was added or not, and the concentrations of Cd in each fraction are given in Table 2.

It is generally believed that among the five fractions extracted, the first two, e.g., exchangeable and carbonate, are indicators to the bioavailable fractions (Lee et al. 2011; Wang et al. 2014; Rosado et al. 2016). As shown in Fig. 3, the total concentrations of the exchangeable and the carbonate fractions in the rhizosphere significantly decreased compared to that in bulk soils for all cases except the leaf red beet cultivated samples with the MBC added ($p < 0.05$). On the other hand, there were no significant differences in the total concentrations of these two fractions between the soils cultivated with different plants. Zhang et al. (2014) found that the bioavailable fraction in the rhizosphere soil of two ecotypes of *A. wardii* was lower than in the bulk soil or soil before planting under different Cd levels. In contrast, in the soil spiked with different doses of Cd, the bioavailable fraction of Cd hyperaccumulator *Rorippaglobosa* rhizosphere was higher than in non-accumulator *Rorippapalustris* and the bulk soil (Wei and Twardowska 2013).

Table 2 The concentrations of Cd in each fractionation in the bulk and rhizosphere soils of ryegrass and leaf red beet and with or without the MBC added (mg·kg^{-1})

Fractionation Treatment			EXC	CARB	Fe/Mnox	OM	R
Ryegrass	CK	Bulk	3.99 ± 0.03	4.03 ± 0.03	2.71 ± 0.04	0.66 ± 0.04	3.61 ± 0.05
		Rhizosphere	3.40 ± 0.01	4.01 ± 0.03	2.65 ± 0.05	0.64 ± 0.03	4.30 ± 0.13
	MBC	Bulk	3.46 ± 0.15	3.79 ± 0.10	2.95 ± 0.19	0.70 ± 0.04	4.10 ± 0.27
		Rhizosphere	3.36 ± 0.03	3.18 ± 0.30	3.00 ± 0.15	0.74 ± 0.03	4.72 ± 0.44
Leaf red beet	CK	Bulk	3.94 ± 0.07	4.18 ± 0.06	2.81 ± 0.08	0.75 ± 0.06	3.33 ± 0.28
		Rhizosphere	3.84 ± 0.05	3.95 ± 0.06	2.85 ± 0.07	0.81 ± 0.05	3.54 ± 0.23
	MBC	Bulk	3.49 ± 0.13	3.71 ± 0.07	3.07 ± 0.26	0.78 ± 0.00	3.95 ± 0.32
		Rhizosphere	3.02 ± 0.18	3.75 ± 0.03	3.19 ± 0.16	0.79 ± 0.01	4.24 ± 0.30

EXC exchangeable, *CARB* carbonate, *Fe/Mnox* Fe/Mn oxides, *OM* organic matter, *R* Residual

Addition of the MBC to the soil can also alter the total concentrations of the exchangeable and the carbonate fractions. Statistically significant decrease of the bioavailable fractions in soils, both bulk and rhizosphere, was caused by the addition of the MBC. This is again similar the DTPA extraction.

3.4 Absorption and accumulation of Cd by the plants

The measured concentrations of Cd in the shoots and roots of both ryegrass and leaf red beet cultivated for 60 days in Cd-contaminated soils are shown in Fig. 4. The results are presented for both experiments with or without the MBC spiked. The result of an analysis of variance indicates significant differences between the shoots and the roots, between the two plants, and between the two experiments with or without the spiking ($p < 0.05$). In the case that no MBC was added, ryegrass can absorb a relatively large amount of Cd in its roots (158.76 ± 3.86 mg kg^{-1}), compared with that of leaf red beet (56.60 ± 3.25 mg kg^{-1}). One main reason is different root systems of these two plants. The surface area of the taproot of leaf red beet is much smaller than that of the fibrous root of ryegrass. The large surface areas of the root system not only allow faster absorption but also provide large specific area for adsorption, which cannot be ruled out in this study. Another reason for the significant difference is the fact that the translocation of Cd from the roots to the shoots of leaf red beet was much faster than that of ryegrass. In fact, the total concentrations of Cd in leaf red beet are significantly higher than that in ryegrass. As a Cd hyperaccumulator, leaf red beet can accumulate an enormous amount of the metal. For example, in soil spiked by 20 mg kg^{-1} Cd, the Cd concentration in shoot of *B. vulgaris* var. *cicla* L (leaf red beet) was up to 159.79 mg kg^{-1}(Li et al. 2007). In this study, Cd concentration in the shoots of leaf red beet reached 223 mg kg^{-1}, which was 3.93-fold higher than that in the roots and 10.13 times of that in the ryegrass shoots, indicating strong accumulation potential. In fact, the concentration was more than two times higher than 100 mg kg^{-1}, the critical threshold defined for Cd hyperaccumulator plants (Baker and Brooks 1989). Different mechanism may be involved in the accumulation. For example, the results of several studies

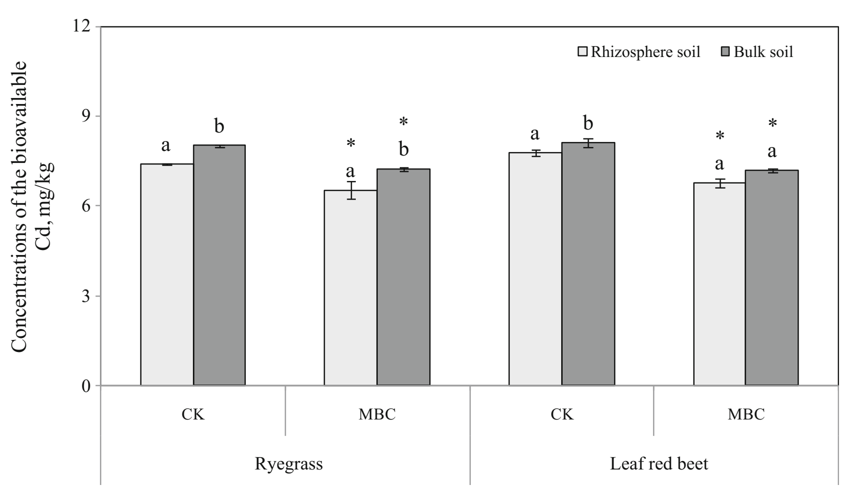

Fig. 3 The bioavailable Cd concentrations (i.e., the sum of the exchangeable and the carbonate fractions) in the rhizosphere and bulk soils cultivated with either ryegrass or leaf red beet and with or without the MBC added

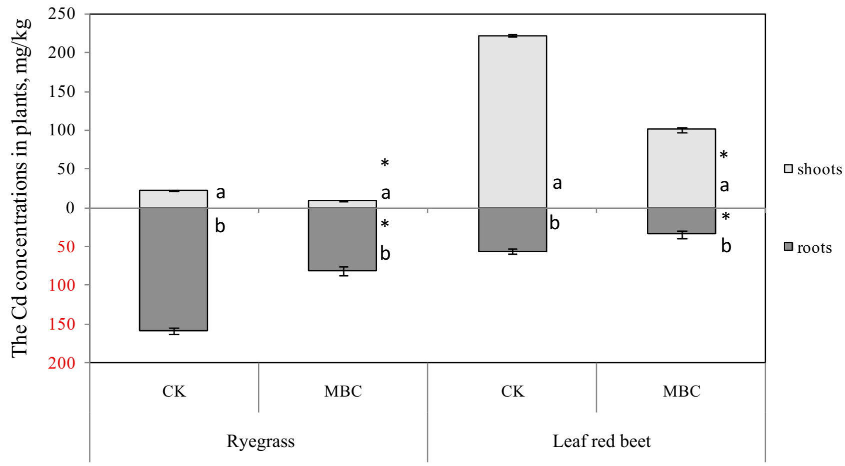

Fig. 4 The Cd concentrations in the shoots and roots of two plants, with or without the MBC added

revealed that metal in forms of ions or metal chelates can be transported into shoots via root symplastic pathway and specific or universal ion carrier channel proteins through the xylem (Tester and Leigh 2001; Ghosh and Singh 2005; Saxena and Misra 2010). In addition, plants might reduce metal uptake by means of the chelation with PCs and compartmentation into cytosol and the remaining metal in the root cell wall or/and transporting metal from the cell wall to the soil (Nishizono et al. 1987; Cobbett and Goldsbrough 2002). In case of leaf red beet, the translocation towards shoots appears to be the dominant process (Li et al. 2007).

The metal accumulation in plants can be characterized by calculating enrichment factors, which is defined as the ratio of metal concentration in the shoots to that in soil (Wei et al. 2005). Translocation factor, the ratio of element concentration in the shoots to that in the roots, is also used to indicate the transport capacity of plants to metal element (Ladislas et al. 2012). As shown in Fig. 5, for the two plants studied, the Cd enrichment factor was 1.46 for ryegrass, slightly greater than 1 and significantly lower than that (14.83) for leaf red beet ($p < 0.05$). On the other hand, the Cd translocation factors were 0.14 and 3.93 for ryegrass and leaf red beet, respectively, showing again a significant difference between the two plants. Although the Cd enrichment factor of the ryegrass was slightly greater than 1, the Cd translocation factor was below 1, suggesting that this plant is not a Cd hyperaccumulator plant. On the other hand, for leaf red beet, both factors of the Cd enrichment and translocation well exceeded 1 and the accumulated Cd concentration in plant shoot was as high as 223 mg kg^{-1}, exhibiting typical hyper-accumulation behavior of the plant for Cd. This result agree with what was found by Li et al. (2007), who reported Cd accumulation by *B. vulgaris* var. *cicla* L (leaf red beet) using a pot experiment.

With the MBC added in the Cd-contaminated soil at the rate of 15 mg kg^{-1}, Cd concentrations in the shoots and roots of both ryegrass and leaf red beet were reduced compared with those without the addition ($p < 0.05$). Although the addition of the MBC brought pH down in both the bulk and rhizosphere soils, which is favorable for metal absorption by the plants

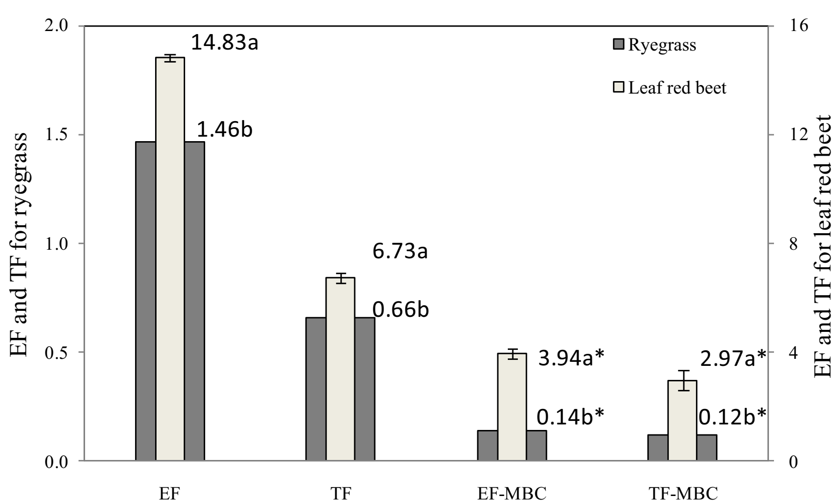

Fig. 5 Enrichment factor (EF) and translocation factor (TF) for ryegrass and leaf red beet after the 60 days of cultivation with or without the MBC added

(Kinnersley 1993), strong affinity of the added MBC to Cd competed effectively with plant root absorption. It appears that the former (pH reduction effect) was totally suppressed by the latter (MBC adsorption), leading to a remarkable reduction in plant accumulation. As a result, the Cd enrichment factors were reduced from 1.46 to 0.66 and from 14.83 to 6.73 for ryegrass and leaf red beet, respectively (Fig. 5). Kim et al. (2010) have demonstrated negative correlation between the Cd uptake by plants and the rhizosphere pH in the alkaline soils. In this study, however, no dependence of absorption on

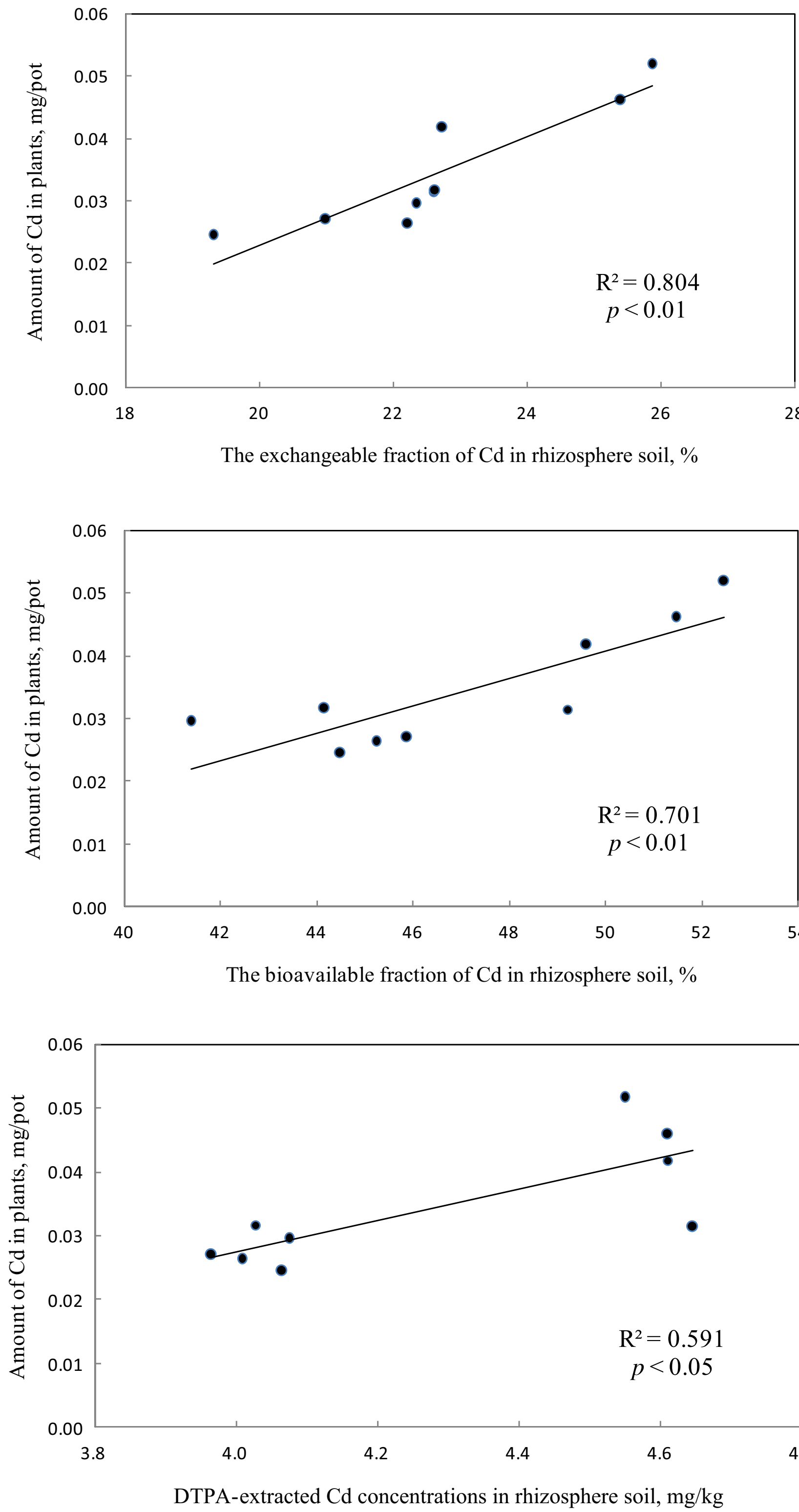

Fig. 6 Dependences of the absorption of Cd by the plants on the exchangeable fraction, the bioavailable fractions of Cd, and the DTPA-extractable concentration in the rhizosphere soils

pH was found. The bioavailability is primarily associated with Cd fractionation.

Due to high cost and low repeatability of biological experiments, chemical approaches are often used as a fast screening method to mimic plant absorption (Bacon et al. 2005; Antoniadis et al. 2008). It was well established that the levels of bioavailable rather than the total concentrations of the metals in soil are key to the plant absorption (Kim et al. 2010). This is well demonstrated in this study as well. Although the total concentrations were identical across the experiments, adsorptions of Cd by the plant vary extensively between the plants and are significantly affected by the addition of the MBC. To address the influence of metal fractionation on the bioavailability, both DTPA extraction and the five-step sequential extraction methods were adopted to investigate bioavailability of Cd in the studied soil and adsorption potential of Cd by the two plants. As discussed above, there were significant differences in the quantities of Cd extracted by either simple DTPA extraction or the first two steps of the sequential extraction between the two plants tested and between the rhizosphere and bulk soils. Differences were strongly associated with the absorption of Cd by the plants. As shown in Fig. 6, the absorption of Cd by the plants depend positively on the bioavailable fractions of Cd in the rhizosphere, the sum of the exchangeable and carbonate fractions, characterized by either the exchangeable fraction or the DTPA-extractable fraction. Among the three, the first two steps of the sequential extraction appear to be a better indicator to the bioavailability, while the DTPA-extractable fraction was not normally distributed.

3.5 Impact of Cd contamination on plant growth

Among various adverse impacts of metals on plant, growth reduction is a critical one, which can be characterized by plant biomass (Lou et al. 2013). For ryegrass and leaf red beet cultivated in this study, the dry biomass of the harvested plants, both aboveground and belowground tissues, were weighted after the 60 days of cultivation. The results for those with or without addition of the MBC are shown in Fig. 7.

With the addition of the MBC, the biomass of both roots and aboveground tissues of the ryegrass increased 63 ± 9 and 65 ± 11%, respectively, and the differences were statistically significant ($p < 0.05$). On the other hand, the difference of the biomass of leaf red beet with or without MBC spiking was not significant at the level of 0.05, even though a slight increase in root (13%) and aboveground tissue (15%) biomass was observed. It appears that the growth inhibition of ryegrass caused by Cd was alleviated significantly by the MBC, which competes with root for Cd in soil. The phenomenon was not observed for the leaf red beet as a Cd hyperaccumulator. Similar results have been previously reported for ryegrass and leaf red beet. For instance, it was reported that heavy metals including Cd can cause plant dwarfism and biomass decline of Italian ryegrass (Juknys et al. 2012; Zhu et al. 2015). On the other hand, the results of a cultivation experiment conducted by Li et al. (2007) also suggested that leaf red beet as a typical hyperaccumulator plant can accumulate a large quantity of Cd in its shoot while causing very weak physicological influences including biomass change.

4 Conclusions

The present study demonstrated the significant effects of MBC on soil pH, the fractionation and bioavailability of Cd, plant biomass, and the uptake of Cd by the two plants in contaminated soil. The results evidenced the effect of MBC as immobilization agent and the effect of rhizosphere on Cd immobilization by MBC. As a hyperaccumulator, the rhizosphere process of leaf red beet had a positive effect on the immobilization of MBC on Cd. However, for Cd-tolerant plants, a negative effect on immobilization for Cd by MBC was revealed in the rhizosphere of ryegrass. Therefore, it is

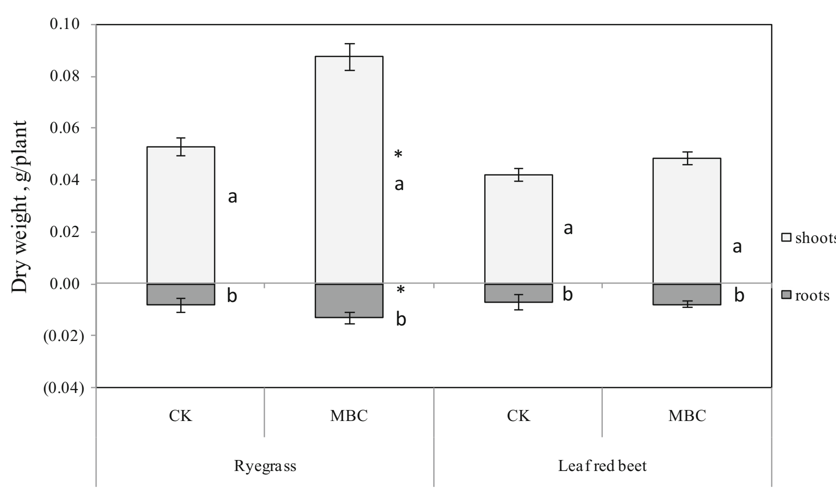

Fig. 7 Dry biomass of ryegrass and leaf red beet after the 60 days of cultivation with or without addition of the MBC

suggested that the Cd-tolerant plants should be avoided in the practical application of MBC. These findings are of importance for a better understanding of the immobilization effect of MBC on Cd and for future application of MBC in contaminated soil remediation.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Grant No. 41471255 and 41171251) and the Shandong Province Natural Science Foundation of China (Grant No. ZR2016YL002).

References

Antoniadis V, Robinson JS, Alloway BJ (2008) Effects of short-term pH fluctuations on cadmium, nickel, lead, and zinc availability to ryegrass in a sewage sludge-amended field. Chemosphere 71(4):759–764

Bacon JR, Hewitt IJ, Cooper P (2005) Reproducibility of the BCR sequential extraction procedure in a long-term study of the association of heavy metals with soil components in an upland catchment in Scotland. Sci Total Environ 337:191–205

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metal elements: a review of their distribution, ecology, and phytochemistry. Biorecovery 1:81–126

Borah D, Satokawa S, Kato S, Kojima T (2008) Characterization of chemically modified carbon black for sorption application. Appl Surf Sci 254:3049–3056

Borah D, Satokawa S, Kato S, Kojima T (2009) Sorption of As(V) from aqueous solution using acid modified carbon black. J Hazard Mater 162:1269–1277

Brown S, Christensen B, Lombi E, McLaughlin M, McGrath S, Colpaert J, Vangronsveld J (2005) An inter-laboratory study to test the ability of amendments to reduce the availability of Cd, Pb, and Zn in situ. Environ Pollut 138:34–45

Cheng JM, Yu L, Li T, Liu Y Zh, Lu Ch X, Li TT, Wang HW (2015) Effects of nanoscale carbon black modified by HNO3 on immobilization and phytoavailability of Ni in contaminated soil. J Chem 1–7

Cobbett C, Goldsbrough P (2002) Phytochelation and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Doumett S, Lamperi L, Checchini L, Azzarello E, Mugnai S, Mancuso S, Petruzzelli G, Del Bubba M (2008) Heavy metal distribution between contaminated soil and *Paulownia tomentosa*, in a pilot-scale assisted phytoremediation study: influence of different complexing agents. Chemosphere 72:1481–1490

Environmental Protection Ministry, PR China (2015) (http://www.bbc.com/news/world-asia-china-27076645)

Fitz WJ, Wenzel WW, Zhang H, Nurmi J, Stipek K, Fischerova Z, Schweiger P, Kollensperger G, Ma LQ, Stingeder G (2003) Rhizosphere characteristics of the arsenic hyperaccumulator Pterisvittata L. and monitoring of phytoremoval efficiency. Environ Sci Technol 37:5008–5014

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of it's by products. Asian J Energy Environ 6(4):18

Gonzaga MIS, Ma LQ, Santos JAG, Matias MIS (2009) Rhizosphere characteristics of two arsenic hyperaccumulating Pteris ferns. Sci Total Environ 407:4711–4716

Hinsinger P, Plassard C, Jaillard B (2005) Rhizosphere: a new frontier for soil biogeochemistry. J Geochem Explor 88:210–213

Jia L, Guo DF, Wang LL (2015) Characteristic of heavy metal accumulation in vegetables in Jinan vegetable fields. J Anhui Agri Sci 43(2): 117–119

Juknys R, Vitkauskaitė G, Račaitė M, Venclovienė J (2012) The impacts of heavy metals on oxidative stress and growth of spring barley. Cent Eur J Biol 7:299–306

Kim KR, Owens G, Naidu R (2010) Effect of root-induced chemical changes on dynamics and plant uptake of heavy metals in rhizosphere soils. Pedosphere 20:494–504

Kinnersley AM (1993) The role of phytochelates in plant growth and productivity. Plant Growth Regul 12:207–218

Ladislas S, El-Mufleh A, Gérente C, Chazarenc F, Andrès Y, Béchet B (2012) Potential of aquatic macrophytes as bioindicators of heavy metal pollution in urban stormwater runoff. Water Air Soil Pollut 223:877–888

Lee SH, Park H, Koo N, Hyun S, Hwang A (2011) Evaluation of the effectiveness of various amendments on trace metals stabilization by chemical and biological methods. J Hazard Mater 188:44–51

Li YS, Sun LN, Sun TH, Wang H (2007) Cadmium hyperaccumulator *Beta vulgaris var. cicla* L. and its accumulating characteristics. J Agro-Environ Sci 26:1386–1389

Li P, Wang X, Allinson G, Li X, Xiong X (2009) Risk assessment of heavy metals in soil previously irrigated with industrial wastewater in Shenyang, China. J Hazard Mater 161:516–521

Liu YZ, Cheng JM (2012) Adsorption kinetics and isotherms of Cu (II) and Cd (II) onto oxidized nano carbon black. Adv Mater Res 529: 579–584

Lou YH, Luo HJ, Hu T, Li HY, Fu JM (2013) Toxic effects, uptake, and translocation of Cd and Pb in perennial ryegrass. Ecotoxicology 22: 207–214

Lou YH, Yang Y, Hu LX, Liu HM, Xu QG (2015) Exogenous glycinebetaine alleviates the detrimental effect of Cd stress on perennial ryegrass. Ecotoxicology 24:1330–1340

Lu RK (2000) The analytical methods of soil agricultural chemistry. Chinese Agricultural Press, China

Luo YM, Christie P, Baker AJM (2000) Soil solution Zn and pH dynamics in non-rhizosphere soil and in the rhizosphere of Thlaspicaerulescens grown in a Zn/Cd-contaminated soil. Chemosphere 41:161–164

Luster J, Menon M, Hermle S, Schulin R, Georg-Gunthardt MS, Nowack B (2008) Initial changes in refilled lysimeters built with metal polluted topsoil and acidic or calcareous subsoils as indicated by changes in drainage water composition. Water Air Soil Pollut 8:163–176

Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Li RH, Zhang ZQ (2016) Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicol Environ Saf 126:111–121

McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207–214

MLR, MEP (2014) The communique of national investigation of soil contamination. Ministry of Land and Resources of the People's Republic of China. Ministry of environmental protection of the People's Republic of China

Navari-Izzo F, Quartacci MF (2001) Phytoremediation of metals: tolerance mechanisms against oxidative stress. Minerva Biotecnologica 13:73

Nazar R, Iqbal N, Masood A, Khan MIR, Syeed S, Khan NA (2012) Cadmium toxicity in plants and role of mineral nutrients in its alleviation. Am J Plant Sci 3:1476–1489

Nishizono H, Ichilawa H, Suzuki S, Ishii F (1987) The role of the root cell wall in the heavy metal tolerance of Athyrium yokoscense. Plant Soil 101:15–20

Qasim B, Motelica-Heino M, Bourgerie S, Gauthier A, Morabito D (2016) Rhizosphere effects of *Populus euramericana Dorskamp* on the mobility of Zn, Pb and Cd in technosols. J Soils Sediments 16:811–820

Rosado D, Usero J, Morillo J (2016) Ability of 3 extraction methods (BCR, Tessier and protease K) to estimate bioavailable metals in sediments from Huelva estuary (southwestern Spain). Mar Pollut Bull 102:65–71

Ruttens A, Adriaensen K, Meers E, De Vocht A, Geebelen W, Carleer R, Mench M, Vangronsveld J (2010) Long-term sustainability of metal immobilization by soil amendments: cyclonic ashes versus lime addition. Environ Pollut 158:1428–1434

Saxena P, Misra N (2010) Remediation of heavy metal contaminated tropical land. In: Sherameti I, Varma A (eds) Soil Heavy Metals Soil Biology. pp 431–477

Sheoran V, Sheoran A, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Crit Rev Environ Sci Technol 41:168–214

Shi RH (1996) The analytical methods of soil agricultural chemistry. Chinese Agricultural Press, Beijing

Shi WY, Shao HB, Shao MA, Li H, Du S (2009) Progress in the remediation of hazardous heavy metal-polluted soils by natural zeolite. J Hazard Mater 170:1–6

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51: 844–851

Tester M, Leigh RA (2001) Partitioning of nutrient transport processes in roots. J Exp Bot 52(suppl 1):445–457

Thakur S, Singh L, Wahid ZA, Siddiqui MF, Atnaw SM, Md-Din MF (2016) Plant-driven removal of heavy metals from soil: uptake, translocation, tolerance mechanism, challenges, and future perspectives. Environ Monit Assess 188:206

Tu SI, Nungesser E, Brauer D (1989) Characterization of the effects of divalent cations on the coupled activities of the H^+-ATPase in tonoplast vesicles. Plant Physiol 90(4):1636–1643

U.S. Environmental Protection Agency (1997) Recent developments for in situ treatment of metal contaminated soils. Solid waste and emergency response (20460), Washington DC

Wang HW (2009) Application of modified nano-particle black carbon for the remediation of soil heavy metal pollution. Master's thesis of Shandong Normal University

Wang FL, Wei OY, Hao FH, Lin CY, Song NN (2014) In situ remediation of cadmium-polluted soil reusing four by-products individually and in combination. J Soils Sediments 14:451–461

Wang L, Cui XF, Cheng HG, Chen F, Wang JT, Zhao XY, Lin CY, Pu X (2015a) A review of soil cadmium contamination in China including a health risk assessment. Environ Sci Pollut Res 22:16441–16452

Wang X, Liang CH, Yin Y (2015b) Distribution and transformation of cadmium formations amended with serpentine and lime in contaminated meadow soil. J Soils Sediments 15(7):1–7

Wei SH, Twardowska I (2013) Main rhizosphere characteristics of the Cd hyperaccumulator *Rorippa globosa* (Turcz.) Thell. Plant Soil 372: 669–681

Wei SH, Zhou QX, Wang X, Zhang KS, Guo GL, Ma LQ (2005) A newly-discovered Cd-hyperaccumulator *Solanum nigrum* L. Chin Sci Bullet 50:33–38

Weng BS, Huang Y, Liu JC, Lu HL, Yan CL (2014) Alleviated toxicity of cadmium by the rhizosphere of Kandeliaobovata (S., L.) Yong. Bull Environ Contam Toxicol 93:603–610

Xie MJ, Yan CL, Ye J, Wei L (2009) Impact of phenanthrene on organic acids secretion and accumulation by perennial ryegrass, *Loliumperenne* L., root. Bull Environ Contam Toxicol 83:75–80

Xu YM, Liang XF, Sun GH, Sun Y, Qin X, Wang L, Dai XH (2010) Effects of acid and heating treatments on the structure of sepiolite and its adsorption of lead and cadmium. Environ Sci 31:560–1567

Yang XE, Long XX, Ni WZ (2002) Physiological and molecular mechanisms of heavy metal uptake by hyperaccumulting plants. Plant Nutr Fert Sci 8:8–15

Zhai LM, Liao XY, Chen TB, Yan XL, Xie H, Wu B, Wang LX (2008) Regional assessment of cadmium pollution in agricultural lands and the potential health risk related to intensive mining activities: a case study in Chenzhou City, China. J Environ Sci-China 20:696–703

Zhang SJ, Li TX, Zhang XZh YHY, Zheng ZC, Wang YD, Hao XQ, Pu Y (2014) Changes in pH, dissolved organic matter and Cd species in the rhizosphere soils of Cd phytostabilizer Athyriumwardii (hook.) Makino involved in Cd tolerance and accumulation. Environ Sci Pollut Res 21:4605–4613

Zhou DM, Wang YJ, Wang HW, Wang SQ, Cheng JM (2010) Surface-modified nanoscale carbon black used as sorbents for Cu(II) and Cd(II). J Hazard Mater 174:34–39

Zhu TY, Fu DF, Yang F (2015) Effect of Saponin on the Phytoextraction of Pb, Cd and Zn from soil using Italian ryegrass. Bull Environ Contam Toxicol 94:129–133