

Original Research

Effects of Passivators on *Artemisia selengensis* Yield and Cd Stabilization in a Contaminated Soil

Di Zhang*, Aifang Ding, Ting Li, Xiaoxia Wu

Nanjing Xiaozhuang University, Nanjing 211171, Peoples' Republic of China

Received: 22 July 2020

Accepted: 30 August 2020

Abstract

Cadmium (Cd) contamination in agricultural soils has caused serious environmental risks and public health concern; possible eco-friendly immobilization technologies are required to reduce Cd accumulation. A set of pot experiments was carried out with two genotypes of *Artemisia selengensis* grown in a Cd-contaminated soil, which was amended with hydrated lime (L), diatomite (D) and biochar (B), either alone or in combination, to investigate the impacts of these passivators on bioavailability and mobility of Cd in soil and crops. The results demonstrated that hydrated lime and diatomite significantly promoted the immobilization of soil Cd and decreased the accumulation of Cd in *Artemisia selengensis*, and biochar significantly increased *Artemisia selengensis* yield. The residual fraction of Cd was increased 1.35~1.51 times for *Fuqiu Artemisia selengensis* and 0.97~1.05 times for *Dayeqing Artemisia selengensis*, respectively, under the hydrated lime (L) and hydrated lime+diatomite+biochar (LDB) treatments. Correspondingly, *Artemisia selengensis* yields were increased by 44.45%~52.50% and 37.91%~43.36%, respectively, in the treatments of biochar (B) and combination amendment (LDB). The results indicate that combination passivators (LDB) are recommended in practical applications, given their high efficiency for soil remediation and potential benefits. *Fuqiu Artemisia selengensis*, rather than *Dayeqing Artemisia selengensis*, are suggested to be employed for plants in mildly Cd-contaminated soil to ensure vegetable safety. These findings provide stronger evidence and theoretical support for in situ remediation of Cd-contaminated soil.

Keywords: passivator, chemical immobilization, bioavailability, Cadmium, *Artemisia selengensis*

Introduction

Heavy metal contamination has become a global environmental problem in the last few years, which

may be caused by rapid industrial development and agricultural activities [1]. Cd accumulation in Chinese agricultural soils is very serious and widespread. This is a problem of great concern because of the elevated Cd uptake in crops through food chain transfer, which poses risks to human health [2]. Therefore, it is urgently needed to take measures to remediate Cd-contaminated soil. For this purpose, various remediation techniques

*e-mail: zhangdi@njxzc.edu.cn

such as physical approaches, chemical immobilization and phytoextraction, have been explored [3-4]. Chemical immobilization technologies to remediate Cd pollution in agricultural soils have received broad attention because of their cost-effectiveness, short time period required for remediation and minor impact on the ecological environment [5].

The key factor of *in situ* chemical immobilization of heavy metals is the selection of passivators. Many passivators have been developed, such as alkaline materials, organic materials and clay minerals. Hydrated lime, as the most widely adopted amendment, can reduce heavy metal solubility and increase metal sorption to the soil by increasing soil pH, resulting in a reduction in metal bioavailability and a decrease in metal uptake by crops [4, 6]. Organic materials have plenty of biochars, the reactive groups of biochar can provide large areas and high porosities to ameliorate the toxicity of heavy metal fractions [7-8]. Compared to other organic materials, biochar is attractive in quality and inexpensive to remediate heavy metal contamination; however, the immobilizing effect depended on soil conditions, such as CEC and pH [9-10]. The clay mineral amendment such as diatomite belongs to an aluminosilicate mineral and has the characteristics of a large surface area and light porosity [11]. Diatomite could promote the immobilization of heavy metals by ion exchange and adsorption, thereby reducing heavy metal mobility and bioavailability. However, the bioavailability of heavy metal is not only related to the effects of passivators but also strongly linked to the plant source [12]. Moreover, a successful immobilization remediation technique for agricultural soils must maintain low bioavailability of heavy metals and could improve crop productivity effectively [3].

Artemisia selengensis, as medicinal vegetables, are favored because their nutrition and medicinal values are considerably higher than those of ordinary vegetables, and they have been widely planted in southern China in recent years. Previous studies have indicated that *Artemisia selengensis* have a strong ability to accumulate Cd and have low food safety as an edible vegetable [13]. A survey indicated that Cd concentrations in the edible part of *Artemisia selengensis* at all sampling points in the Dongting Lake region significantly exceeded the standard (0.05 mg kg⁻¹) and posed a considerable threat to human health [14]. Li et al. [15] found that Cd concentrations in *Artemisia selengensis* exceeded the food safety standard when the soil Cd content was over 0.5 mg kg⁻¹. Therefore, greater attention should be paid to the safety of *Artemisia selengensis* as edible stem and leaf vegetables in soils with high Cd concentrations.

To date, the majority of studies on this subject have examined the immobilization efficiency of passivators on the bioavailability of Cd in the soil-rice system. Notably few studies have used *Artemisia selengensis* to evaluate the passivating agent's efficiency in decreasing the phytoavailability of Cd. Moreover, the effects of hydrated lime, diatomite and biochar alone on metal

immobilization have been reported; however, the study of co-application of these amendments has been seldom investigated. A detailed study on how these amendments affect the bioavailability of Cd in contaminated soil and *Artemisia selengensis* is required, to decrease the toxicity of Cd to human health through food chains. Meanwhile, economical and environmentally friendly remediating agents have been found to broadly use for Cd contaminated agricultural soils.

Therefore, a set of pot experiments was carried out in a Cd-contaminated soil under the *Artemisia selengensis* system. Hydrated lime, diatomite and biochar were chosen as immobilizing agents to examine their effects on (1) the yield changes in two genotypes of local *Artemisia selengensis*, (2) the accumulation and phytotoxicity of Cd in two genotypes of local *Artemisia selengensis*, and (3) the immobilization of Cd in the soil-vegetable system.

Materials and Methods

Experimental Materials

Soil samples used in this study were collected from the surface layer (0-20 cm) of a vegetable field in the suburbs of Nanjing City, China. It is classified as yellow-brown soil based on Chinese Soil Taxonomy; while it would be Ferri-Udic Argosols according to the US Soil Taxonomy. Soil physicochemical characteristics were as follows: sand 7.51%, silt 60.24% and clay 32.25%, pH 6.15, cation exchange capacity (CEC) 19.24 cmol kg⁻¹ and soil organic carbon (SOC) 43.17 g kg⁻¹. The total Cd content in the tested soil was 0.82 mg kg⁻¹.

Three materials (hydrated lime, diatomite and biochar) were chosen as soil passivators. The passivators for hydrated lime (L, pH = 11.59) and diatomite (D, pH = 8.45) were directly purchased from a local company of Nanjing. The biochar was derived from rice straw that was prepared according to Zhang et al. [16]. The basic properties of biochar were 154.8 g kg⁻¹ C, 10.25 g kg⁻¹ N, 1.68g kg⁻¹ P, pH (H₂O) of 8.07. The Cd content of biochar was 0.076 mg kg⁻¹.

Pot Experiment

A set of pot experiments was performed in eight treatments: control (without passivators, designed CK), L (hydrated lime), D (diatomite), B (biochar), LD (1:1 hydrated lime: diatomite), LB (1:1hydrated lime: biochar), LB (1:1diatomite: biochar) and LDB (1:1:1 hydrated lime: diatomite: biochar). Each treatment was conducted in triplicate and randomly placed in the greenhouse. The application rate of single passivator was added to the soil at a rate of 5% (w/w, on air-dry weight basis). For the treatment of two passivators, half of the amount of single passivator was applied, for the treatment of three passivators, one-third of the amount of single passivator was applied. Plastic pots measuring

30 cm in height and 30 cm in diameter were used in the pot experiments. 5 kg of dried soil was added to each pot. Urea, potassium dihydrogen phosphate and potassium sulfate were applied as basal fertilizer, and the application rate of N was 150 mg kg⁻¹, P and K was 100 mg kg⁻¹ and 100 mg kg⁻¹, respectively. The passivators (including hydrated lime, diatomite and biochar) and chemical fertilizers were mixed thoroughly and applied to the soils.

Two genotypes of *Artemisia selengensis*, *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*, were employed in this study. The seedlings were raised by cutting. When the true leaves growing on the stems of *Artemisia selengensis* measured approximately 1 cm, the seedlings with the same growth were transplanted, with three plants per pot. *Artemisia selengensis* were watered according to the normal growth requirements during the experimental period. *Artemisia selengensis* were harvested 60 days after transplanting. Then, the aboveground parts (leaves and stems) and the roots were separated. The leaves, stems and roots were washed with running water, rinsed 3~4 times with distilled water and followed by air-dried for 24 h. The fresh weights of roots, stems and leaves were measured per pot and then they were homogenized by pulp refiner, respectively, and stored in plastic bottles prior to chemical analysis.

Soil samples of 0~20 cm were collected from the pots after removing *Artemisia selengensis* plants. The soil was air-dried and then ground to pass through a 10-mesh sieve for soil pH, CEC and available Cd analysis. A subsample was ground further to sieve through a 100-mesh sieve to analyse SOC and Cd speciation.

Chemical Analysis

Soil pH was determined using soil to water ratio at 1:2.5 (w/v). Soil CEC was determined by BaCl₂/NH₄Cl compulsive exchange method as described by Gillman and Sumpter [17]. SOC was measured using a TOC (total organic carbon) analyzer (Multi N/C 3100, Analytik Jena, Germany). Soil available Cd was determined by 0.01 mol L⁻¹ CaCl₂ extraction as described by Houben et al. [18], and the redistribution of Cd in soil was determined using the European Community Bureau of Reference (BCR) sequential extraction method which was described in detail by Xu et al. [19]. Four different fractions designated by this method included: acid soluble, reducible, oxidizable, and residual fraction, of which the first three were extracted by 0.11 mol L⁻¹ HAc, 0.5 mol L⁻¹ NH₂OH·HCl and 1.0 mol L⁻¹ NH₄OAc, respectively, while the last was obtained by digesting the residual with HNO₃-HF-HClO₄ mixture. Cd concentrations in soil samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500, USA). A soil certified reference material (GBW07403, National Research Center for Certified Reference Materials,

China) was used to ensure the precision of the analytical procedure.

The Cd concentrations in *Artemisia selengensis* were measured using ICP-MS after digestion by HNO₃/H₂O₂ according to the Determination of Cadmium in Foods, National Food Safety Standard of China (GB/T 5009.15-2014). The digestion process is as follows: samples of 0.5000 g were weighed into a polytetrafluoroethylene inner tank, and 4 mL of guaranteed pure HNO₃ was added to incubate overnight, then 3 mL of H₂O₂ (30%) was added. The inner cover was closed and the stainless steel coat was tightened, then, the samples were placed into a constant temperature dryer and kept for 4~6 h at 140°C. After that, the samples were placed onto an electricity plate and digested to the near dry. The samples were then diluted to 25 mL with HNO₃ (1%). A plant certified reference material (GBW10015, National Research Center for Certified Reference Materials, China) was used to ensure the precision of the analytical procedure. The bioaccumulation factor (BAF) and the translocation factor (TF) were calculated as follows:

$$\text{BAF} = \frac{C_{\text{plant}}}{C_{\text{soil}}}$$

$$\text{TF} = \frac{C_{\text{aboveground}}}{C_{\text{belowground}}}$$

...where, C_{plant} is the Cd concentration in *Artemisia selengensis* (mg kg⁻¹), C_{soil} is the Cd concentration in soil (mg kg⁻¹), C_{aboveground} is the Cd concentration in *Artemisia selengensis* leaf and stem (mg kg⁻¹), and C_{belowground} is the Cd concentration in *Artemisia selengensis* root (mg kg⁻¹).

Statistical Analysis

All data were determined in duplicate. The means (n = 3) and standard deviations (S.D.) of the soil physicochemical properties, Cd concentrations and *Artemisia selengensis* yield were presented. One-way analysis of variance (ANOVA) was conducted using SPSS 20.0 statistical software. The least significant difference (LSD) test was carried out at the significance level of 0.05.

Results

Effects of Passivators on *Artemisia selengensis* Yield

Fig.1 demonstrated that there were different effects of three passivators on edible part yields of *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*. *Artemisia selengensis* yields were the highest in the treatment of biochar (B), which were 44.45%~52.50% higher than those in the control. However, there was no dramatically different in the treatments of B, LB and LDB in two genotypes of *Artemisia selengensis*. Compared to the control, the

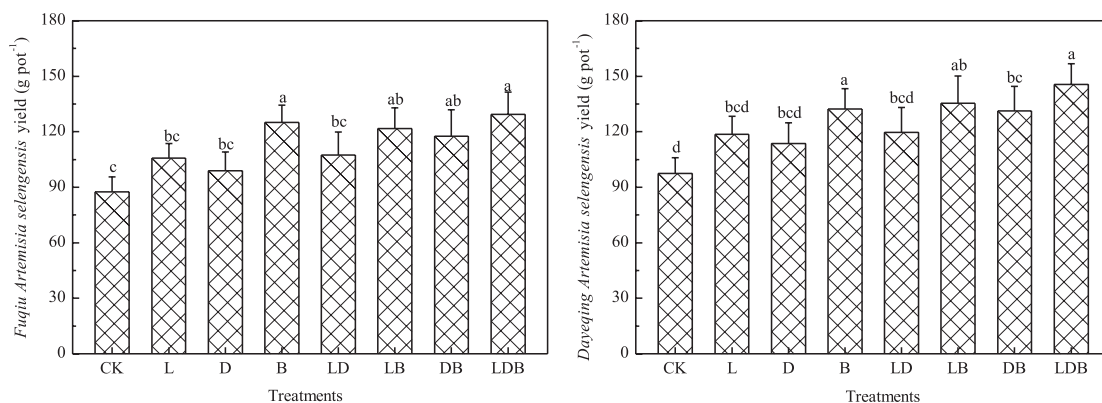


Fig. 1. Edible part yields of *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis* grown in tested soil. CK, control; L, hydrated lime; D, diatomite; B, biochar; LD, hydrated lime + diatomite; LB, hydrated lime + biochar; DB, diatomite + biochar; LDB, hydrated lime + diatomite + biochar. Values are mean±SD, and different lowercase letters indicate significant difference among passivator treatments at $p < 0.05$ level ($n = 3$, LSD test).

effects of hydrated lime and diatomite on *Artemisia selengensis* yields were not significant.

Effects of Passivators on Cd Bioaccumulation and Translocation in *Artemisia selengensis*

The Cd concentrations in the roots, stems and leaves (fresh weight) were significantly reduced by the application of passivators in the two genotypes of *Artemisia selengensis* (Fig. 2). Compared to the control, the Cd concentrations in roots, stems and leaves decreased by 36.17%~56.72%, 41.18%~76.80% and 47.89%~84.51%, respectively, in the soil with *Fuqiu Artemisia selengensis* after the application of passivators. Similarly, for *Dayeqing Artemisia selengensis*, the Cd concentrations in roots, stems and leaves decreased by 25.69%~57.93%, 36.26%~71.32% and 43.16%~73.54%, respectively. Under the control treatment (CK), Cd concentrations in stems (edible part of *Artemisia selengensis*) were 0.125 mg kg⁻¹

and 0.182 mg kg⁻¹ with *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*, respectively, exceeding the maximum limit (0.05 mg kg⁻¹) of the Food Quality Standard of China (GB2762-2017). The Cd concentrations in the stems of *Fuqiu Artemisia selengensis* were below 0.05 mg kg⁻¹ in the amendment treatments, except for the treatment of biochar addition (B). The minimum Cd concentrations were observed in the L and LDB treatments, which caused 76.80%~78.4% reductions compared to the control. Similarly, Cd contents in the stems of *Dayeqing Artemisia selengensis* were below 0.05 mg kg⁻¹ in the treatments of L, LD and LDB, and the Cd concentrations were decreased by 77.47%, 75.27% and 80.22%, respectively.

The BAF of roots, stems and leaves was significantly decreased by the application of passivators in the two genotypes of *Artemisia selengensis* (Table 1). The lowest root, stem and leaf BAF was observed with the L and LDB passivators were 53.13%~56.69%, 77.17%~73.91% and 69.23%~84.62% lower than the control value with

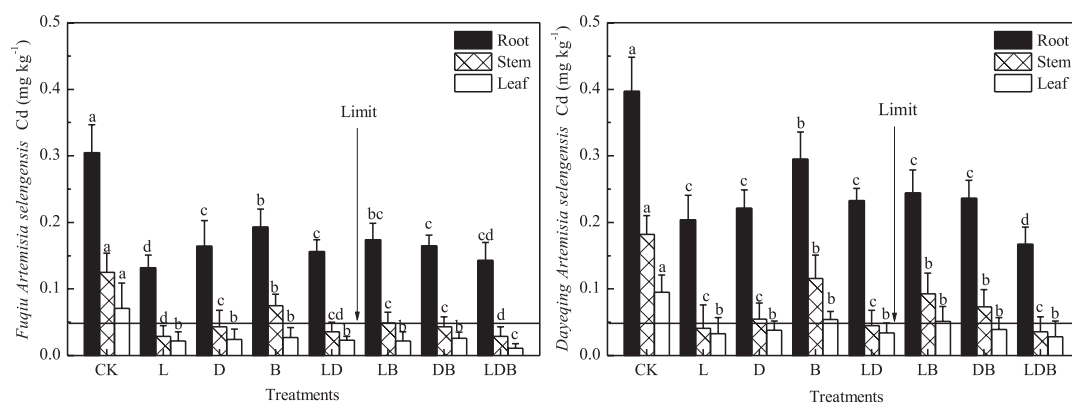


Fig. 2. Cd concentrations of *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis* grown in tested soil. CK, control; L, hydrated lime; D, diatomite; B, biochar; LD, hydrated lime + diatomite; LB, hydrated lime + biochar; DB, diatomite + biochar; LDB, hydrated lime + diatomite + biochar. Values are mean ± SD, and different lowercase letters indicate significant difference among passivator treatments at $p < 0.05$ level ($n = 3$, LSD test). The horizontal line means that the maximum Cd limit of the National Food Quality Standard of the People's Republic of China is 0.05 mg kg⁻¹ in vegetables.

Table 1. Bioaccumulation factor (BAF) and translocation factor (TF) of Cd in plants grown in tested soil with *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*.

Treatments	BAF _{Fuqiu}			TF _{Fuqiu}			BAF _{Dayeqing}			TF _{Dayeqing}		
	Root	Stem	Leaf	Stem	Leaf	Root	Stem	Leaf	Stem	Leaf	Stem	Leaf
CK	0.224±0.032a	0.092±0.008a	0.052±0.011a	0.425±0.036a	0.233±0.018a	0.292±0.055a	0.135±0.022a	0.070±0.006a	0.458±0.021a	0.239±0.019a	0.458±0.021a	0.239±0.019a
L	0.097±0.015c	0.024±0.012d	0.016±0.008b	0.220±0.012d	0.127±0.013b	0.151±0.022cd	0.032±0.011de	0.024±0.008c	0.226±0.015e	0.132±0.013b	0.226±0.015e	0.132±0.013b
D	0.121±0.013bc	0.032±0.005cd	0.018±0.007b	0.263±0.018c	0.136±0.011b	0.163±0.018cd	0.038±0.007de	0.033±0.004bc	0.265±0.014d	0.146±0.006b	0.265±0.014d	0.146±0.006b
B	0.142±0.020b	0.055±0.009b	0.020±0.006b	0.330±0.015b	0.140±0.002b	0.217±0.014b	0.079±0.021b	0.041±0.015b	0.342±0.023b	0.148±0.008b	0.342±0.023b	0.148±0.008b
LD	0.115±0.017bc	0.026±0.006cd	0.018±0.007b	0.231±0.023cd	0.129±0.006b	0.171±0.016bc	0.034±0.007de	0.026±0.006c	0.243±0.015de	0.133±0.023b	0.243±0.015de	0.133±0.023b
LB	0.128±0.009bc	0.043±0.012bc	0.019±0.005b	0.333±0.019b	0.130±0.009b	0.179±0.008bc	0.065±0.006bc	0.035±0.007bc	0.331±0.005bc	0.137±0.005b	0.331±0.005bc	0.137±0.005b
DB	0.121±0.011bc	0.032±0.011cd	0.019±0.013b	0.261±0.014c	0.133±0.007b	0.174±0.015bc	0.054±0.008cd	0.028±0.009bc	0.310±0.009c	0.145±0.016b	0.310±0.009c	0.145±0.016b
LDB	0.105±0.007c	0.021±0.009cd	0.008±0.005b	0.205±0.017d	0.078±0.008c	0.123±0.022d	0.025±0.002e	0.021±0.003c	0.216±0.012e	0.077±0.007c	0.216±0.012e	0.077±0.007c

CK, control; L, hydrated lime; D, diatomite; B, biochar; LD, hydrated lime + diatomite; LB, hydrated lime + biochar; DB, diatomite + biochar; LDB, hydrated lime + diatomite + biochar. Values are mean ± SD, and different lowercase letters indicate significant difference among passivator treatments at $p < 0.05$ level (n = 3, LSD test).

Fuqiu Artemisia selengensis ($p < 0.05$), respectively. For *Dayeqing Artemisia selengensis*, the lowest root, stem and leaf BAF was also observed with hydrated lime (L) addition being 48.29%, 76.12% and 65.71% ($p < 0.05$) of the highest value observed in the control, respectively. The translocation factor of Cd in the amended soil with two genotypes of *Artemisia selengensis* had a significant decreasing effect ($p < 0.05$) over the control (Table 1).

Effects of Passivators on Soil Properties

Table 2 indicated that significant changes were observed in soil chemical properties after the application of different passivators. Compared to the control, soil pH in the tested soil with two genotypes of *Artemisia selengensis* was significantly increased with the addition of passivators ($p < 0.05$). The highest value of soil pH was found in the L treatment among all the passivator treatments. Soil pH was increased over the control by 0.37 and 0.33 units in the tested soil with *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*, respectively. The application of biochar (B) led to significantly higher soil SOC concentration than that in the control ($p < 0.05$) with *Fuqiu Artemisia selengensis*. However, the soil SOC concentrations of all treatments with biochar addition (B, LB, DB and LDB) were significantly higher than that of other treatments with *Dayeqing Artemisia selengensis*. No significant difference was found in CEC contents among all treatments. The maximum soil CEC was found in the LDB treatment.

Effects of Passivators on CaCl_2 -Cd Concentration

The application of passivators effectively reduced soil Cd bioavailability (Fig. 3). Soil CaCl_2 -Cd concentrations in all passivator treatments were significantly reduced in the two genotypes of *Artemisia selengensis*, particularly in the L treatment. Compared with the control, the L and LDB treatments with *Fuqiu Artemisia selengensis* decreased the CaCl_2 -Cd concentration by 37.50% and 29.17%, respectively. Similarly, the L and LDB treatments with *Dayeqing Artemisia selengensis* decreased the CaCl_2 -Cd concentration by 50.0% and 45.45%, respectively. There was no significant difference in the treatments of D, B, LB and DB. In general, soil CaCl_2 -Cd concentrations with *Fuqiu Artemisia selengensis* were lower than those with *Dayeqing Artemisia selengensis*.

Effects of Passivators on Soil Cd Distribution

The application of passivators altered the fraction distribution of Cd in the soil with *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis* (Fig. 4). The acid soluble fractions were reduced by 9.34%~30.11% for *Fuqiu Artemisia selengensis* and 13.33%~30.77% for *Dayeqing Artemisia selengensis*.

Table 2. Effects of passivators on soil pH, soil organic carbon (SOC) and CEC in the tested soil with *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*

Treatments	<i>Fuqiu Artemisia selengensis</i>			<i>Dayeqing Artemisia selengensis</i>		
	pH	SOC	CEC	pH	SOC	CEC
CK	6.16±0.03e	43.55±3.06b	19.33±2.01a	6.15±0.06c	43.45±3.11b	19.79±2.19a
L	6.53±0.04a	42.84±2.83b	22.21±2.04a	6.48±0.11a	43.31±2.07b	23.71±1.50a
D	6.38±0.05bcd	42.97±3.32b	20.49±1.64a	6.32±0.06b	43.51±3.98b	22.73±1.51a
B	6.29±0.03d	54.23±4.69a	20.68±1.32a	6.29±0.06bc	56.87±5.75a	20.41±1.95a
LD	6.42±0.05b	43.42±3.22b	22.57±3.67a	6.38±0.05ab	44.25±2.33b	22.48±3.18a
LB	6.39±0.09bc	47.09±6.01ab	20.60±2.93a	6.31±0.08b	50.29±2.73a	22.64±2.56a
DB	6.31±0.03cd	49.41±3.82ab	21.47±2.06a	6.33±0.06b	50.91±4.81a	21.61±0.14a
LDB	6.36±0.06bc	50.27±5.29ab	22.94±2.31a	6.41±0.13ab	54.85±2.36a	23.76±1.73a

CK, control; L, hydrated lime; D, diatomite; B, biochar; LD, hydrated lime + diatomite; LB, hydrated lime + biochar; DB, diatomite + biochar; LDB, hydrated lime + diatomite + biochar. Values are mean±SD, and different lowercase letters indicate significant difference among passivator treatments at $p < 0.05$ level ($n = 3$, LSD test).

The percentage of the reducible fractions increased from 11.03% to 44.78% in the soil with *Fuqiu Artemisia selengensis* and from 11.21% to 29.80% with *Dayeqing Artemisia selengensis*. In the L and LDB treatments, the residual fractions of Cd were increased 1.35~1.51 times for *Fuqiu Artemisia selengensis* and 0.97~1.05 times for *Dayeqing Artemisia selengensis*. These results demonstrate that some of the acid soluble and reducible Cd fractions were transformed into oxidizable and residual fractions, indicating a decrease in their bioavailability.

Discussion

The purpose of this research was to examine the impact of different passivators on *Artemisia selengensis* yield and Cd accumulation in contaminated soil and

plants. This study found that the effects of biochar in increasing *Artemisia selengensis* yield were better than those of hydrated lime and diatomite. Biochar is a net source of organic matter, N, P and K, which can provide multiple nutrients to plant and improve soil structure to promote plant growth [20-21]. Previous study found that hydrated lime could effectively increase crop yield in severely acidic soils [3]; but the tested soil is slightly acidic in this research and the yield-increasing effect of hydrated lime is thus limited. Except for crop biomass, heavy metal bioavailability in the edible part of crops is an important indicator for assessing efficiency in the in situ immobilization [6]. In this study, the application of the three passivators decreased Cd concentrations in the roots, stems and leaves of *Artemisia selengensis* at different degrees. The effects of hydrated lime and diatomite in reducing Cd accumulation of *Artemisia selengensis* were stronger than those with biochar

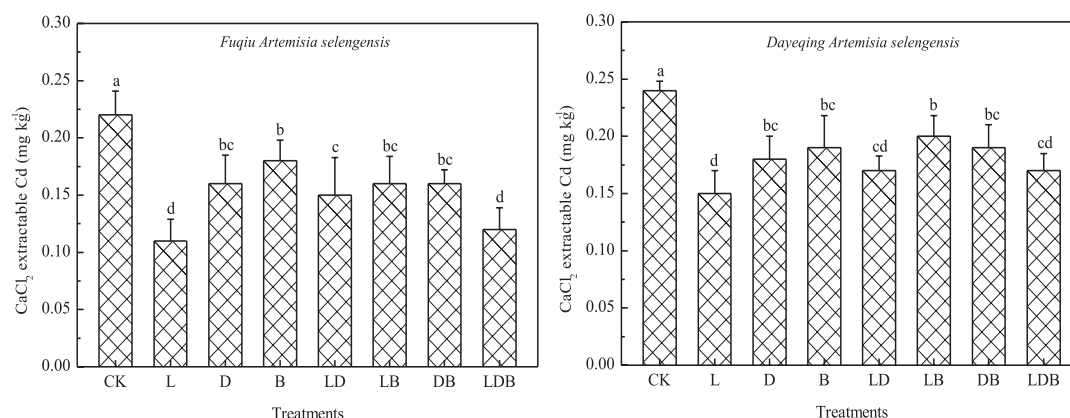


Fig. 3. Concentrations of available Cd in soils with *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*. CK, control; L, hydrated lime; D, diatomite; B, biochar; LD, hydrated lime + diatomite; LB, hydrated lime + biochar; DB, diatomite + biochar; LDB, hydrated lime + diatomite + biochar. Values are mean±SD, and different lowercase letters indicate significant difference among passivator treatments at $p < 0.05$ level ($n = 3$, LSD test).

(Fig. 2). The reduced uptake of Cd by the *Artemisia selengensis* grown in the soils applied with passivators was associated with the enhancement of soil pH and depended on the available Cd concentration in soils (Fig. 3). Hydrated lime significantly increased soil pH compared to other passivators (Table 1), decreased accumulation of Cd in soil and restrained Cd uptake by plants. Diatomite plays an important part in surface complexation because of its higher specific surface, which can promote metal sorption via surface complexation processes [22-23]. Biochar decreased the Cd concentration, most likely due to adsorption by soil organic matter and strong inner-sphere complexation with Cd [24-25]. However, compared with employing a single amendment, the application of mixed passivators appears to be more efficacious in reducing Cd accumulation in the edible part of *Artemisia selengensis* (Fig. 2) and improving the growth of two genotypes of *Artemisia selengensis* (Fig. 1). This finding may be ascribed to the increment of soil nutrients and the alleviation of Cd stress.

The translocation of Cd in plants organ plays a key role in the accumulation of Cd in edible part of *Artemisia selengensis*. In our study, Cd concentrations in different tissues of two genotypes of *Artemisia selengensis* decreased in the order of root>stem>leaf, and the translocation factor (TF) of Cd was lower than 0.4. These results indicated that the Cd absorbed by the *Artemisia selengensis* plants from the soil was mainly concentrated in the root system, and the amount of Cd transported to the aerial part was less than that in the underground part, which is similar to the conclusion of other studies [15, 26]. The reason for this finding may be that Cd could accumulate in the cell walls, vacuoles and nuclei of roots in the insoluble fraction. Moreover, the migration and accumulation of Cd in roots, stems and leaves were in similar forms, and the ability of the former organ to immobilize or intercept Cd has a great influence on the accumulation of Cd in the next organ [21]. Cd concentrations in the edible part of *Artemisia selengensis* are closely related to food safety and human health. According to the results, Cd concentrations in the edible part of *Fuqiu Artemisia selengensis* met the

food safety standard (0.05 mg kg^{-1}) in the amendment treatments except for biochar addition, while Cd concentrations in *Dayeqing Artemisia selengensis* were below 0.05 mg kg^{-1} only in L, LD and LDB treatments (Fig. 2). These findings may be correlated to the lower Cd accumulation ability of *Fuqiu Artemisia selengensis* than *Dayeqing Artemisia selengensis* after passivating agents' application, and previous research has identified large genotypic differences in Cd accumulation and translocation among *Artemisia selengensis* species [15]. Meanwhile, there are complex and intensive interactions among soil organisms, roots and heavy metals, resulting in redistribution of Cd fractions in the rhizosphere soil [27-28]. Therefore, *Fuqiu Artemisia selengensis* should be employed as a plant in mildly Cd-contaminated soil to decrease the risk of Cd contamination in vegetables.

Researches on chemical immobilization need to pay more attention to the changes of soil properties and Cd accumulation in contaminated soil after the application of passivators, which may affect the accumulation of Cd in crops. In present study, hydrated lime, diatomite and biochar significantly affected soil physicochemical properties and Cd bioavailability. Previous studies have reported that soil pH, SOC and CEC are important factors affecting heavy metal bioavailability, especially soil pH, which plays the most important role in controlling Cd availability compared with other soil properties [29-30]. Soil pH had a prominent effect on heavy metal speciation due to its dissolution and precipitation [8, 31]. The increase in soil pH led to the generation of more negatively charged sorption sites and the precipitation of Cd as CdCO_3 or $\text{Cd}(\text{OH})_2$ [32]. Previous studies demonstrated that there was significantly negatively correlated between soil pH and CaCl_2 extractable Cd [33-34]. In this research, the soil CaCl_2 -Cd was notably reduced in the amended soil, particularly in the treatment of hydrated lime addition, possibly because the added hydrated lime has high pH and promotes an increase in soil pH. In addition, increasing CEC and SOC played an important part in enhancing soil Cd immobilization and reducing the bioavailability of Cd. This is agreed with Hoben et al. [19], who found there were strong and significant

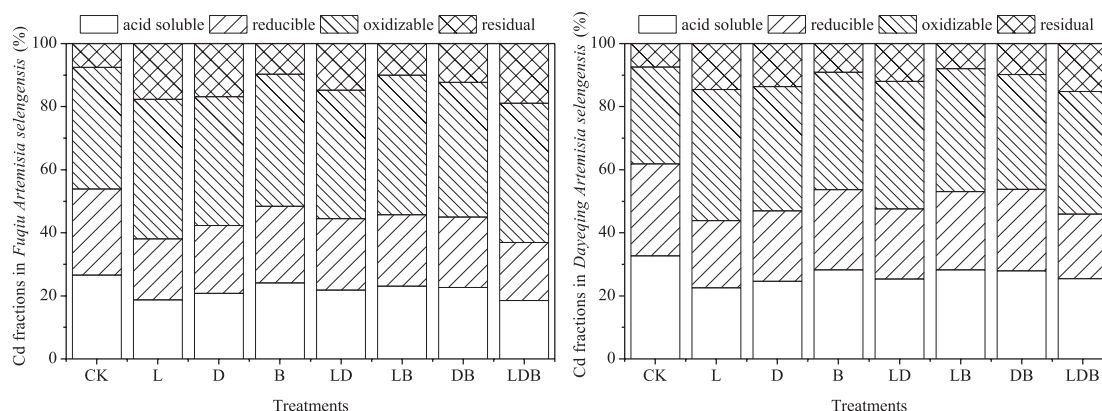


Fig. 4. Cd chemical fractions in tested soil with *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis*.

negative correlations among available Cd concentration and SOC and CEC. Metal sequential extraction technique is commonly regarded as the assessment method to evaluate the remediation efficacy, because the bioavailability of soil heavy metals varies with their chemical speciation [35-36]. Our results indicated that the speciation of Cd was clearly changed by the addition of passivators. Different passivators exhibited different Cd immobilization strengths, and the application of hydrated lime changed the soil Cd from the acid-soluble fraction to more stabilized residual fractions than other passivators (Fig. 4). This finding is in agreement with the decreased Cd uptake by *Artemisia selengensis* (Fig. 2).

The chemical remediation of Cd-contaminated soil requires not only technological advances but also economic efficiency evaluations and environmental friendliness. Although price of hydrated lime is relatively low and the remediation effect for Cd is quite well, long-term application may pose a potential risk to the environment, such as soil compaction and hazardous substance accumulation [37], its application in large scale is hindered. The prices of diatomite and biochar are 2~2.5 times higher than that of hydrated lime, and the remediation efficiency of diatomite or biochar alone on Cd is lower than hydrated lime. Therefore, it is not recommended that diatomite and biochar are used alone for remediation of Cd-contaminated soil. In most cases, the environmental risks and economic cost may be controlled by the application of suitable combined passivators; therefore, developing combined passivators was regarded as an effective way to immobilize soil heavy metal. Furthermore, the remediation efficiency and vegetable growth need to be taken into account simultaneously [38-39]. In the present study, hydrated lime and diatomite could effectively decrease Cd accumulation in *Artemisia selengensis* and soil. Biochar may provide nutrients for *Artemisia selengensis* growth and have the ability to improve nutrient status. Meanwhile, biochar can increase soil Cd adsorbing capability due to a large specific surface area [40]. According to the present results, Cd concentrations (0.029 and 0.038 mg kg⁻¹, respectively) in the edible part of *Fuqiu Artemisia selengensis* and *Dayeqing Artemisia selengensis* met the food safety standard (0.05 mg kg⁻¹) in the amended treatment of hydrated lime + diatomite + biochar (LDB). Therefore, in the view of the effects of passivators on Cd accumulation and *Artemisia selengensis* yield, the cost as well as the potential benefits expected, combination passivators such as LDB are recommended for practical applications.

Conclusions

This study demonstrated that the application of hydrated lime and diatomite effectively immobilized Cd, and biochar could significantly increase *Artemisia selengensis* yield. Combination passivators, such as

hydrated lime + diatomite + biochar, are recommended in practical applications in Cd-contaminated soil, considering their low cost and high efficiency for remediation. *Fuqiu Artemisia selengensis* is suggested for plants in mildly Cd-contaminated soil to ensure vegetable safety compared to *Dayeqing Artemisia selengensis*. However, the immobilization effects of these applied passivators need to be evaluated under field conditions.

Acknowledgements

This research was supported by the Natural Science Research Project for Colleges and Universities of Jiangsu Province, China (19KJB610005) and the Scientific Research Project of Nanjing Xiaozhuang University (2018NXY52).

Conflict of Interest

The authors declare no conflict of interest.

References

- ZHOU R., LIU X.C., LUO L., ZHOU Y.Y., WEI J.H., CHEN A.W., TANG L., WU H.P., DENG Y.C., ZHANG F.F., WANG Y.Y. Remediation of Cu, Pb, Zn and Cd-contaminated agricultural soil using a combined red mud and compost amendment. *Int. Biodeter. Biodegr.* **118**, 73, **2017**.
- KHAN A., KHAN S., KHAN M.A., QAMAR Z., WAQAS M. The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk, a review. *Environ. Sci. Pollut. Res.* **22** (18), 13772, **2015**.
- GUO F.Y., DING C.F., ZHOU Z.G., HUANG G.X., WANG X.X. Effects of combined amendments on crop yield and cadmium uptake in two cadmium contaminated soils under rice-wheat rotation. *Ecotoxicol. Environ. Saf.* **148**, 303, **2018**.
- BOLAN N., KUNHIKRISHNAN A., THANGARAJAN R., KUMPIENE J., PARK J., MAKINO T., KIRKHAM M.B., SCHECKEL K. Remediation of heavy metal(loid)s contaminated soils-to mobilize or to immobilize? *J. Hazard. Mater.* **266**, 141, **2014**.
- XU C.B., QI J., YANG W.J., CHEN Y., YANG C., HE Y.L., WANG J., LIN A.J. Immobilization of heavy metals in vegetable-growing soils using nano zero-valent iron modified attapulgite clay. *Sci. Total Environ.* **686**, 476, **2019**.
- HUANG L.M., YU G.W., CAI X., LONG X.X. Immobilization of Pb, Cd, Cu and Zn in a multi-metal contaminated acidic soil using inorganic amendment mixtures. *Inter. J. Environ. Res.* **11** (4), 425, **2017**.
- ZHANG D., DING A.F. Effects of passivating agents on the availability of Cd and Pb and microbial community function in a contaminated acidic soil. *Bull. Environ. Contam. Toxicol.* **103** (1), 98, **2019**.
- BASHIR S., SHAABAN M., HUSSAIN Q., MEHMOOD S., ZHU J., FU Q.L., AZIZ O., HU H.Q. Influence

- of organic and inorganic passivators on Cd and Pb stabilization and microbial biomass in a contaminated paddy soil. *J. Soil Sediment.* **18** (9), 2948, **2018**.
9. JUANG K.W., HO P.C., YU C.H. Short-term effects of compost amendment on the fractionation of cadmium in soil and cadmium accumulation in rice plants. *Environ. Sci. Pollut. Res.* **19** (5), 1696, **2012**.
 10. GUO G.H., LEI M., CHEN T.B., YANG J.X. Evaluation of different amendments and foliar fertilizer for immobilization of heavy metals in contaminated soils. *J. Soil Sediment.* **18** (1), 239, **2017**.
 11. HAMID Y., TANG L., SOHAIL M.I., CAO X., HUSSAIN B., AZIZ M.Z., USMAN M., HE Z.L., YANG X. An explanation of soil amendments to reduce cadmium phytoavailability and transfer to food chain. *Sci. Total Environ.*, **660**, 80, **2019**.
 12. GU J.F., ZHOU H., TANG H.L., YANG W.T., ZENG M., LIU Z.M., PENG P.Q., LIAO B.H. Cadmium and arsenic accumulation during the rice growth period under in situ remediation. *Ecotoxicol. Environ. Saf.* **171**, 451, **2019**.
 13. DONG M., ZHAO Y.L., LEI C.X., DAI M.B., YI H.C., KU W.Z. Growth responses and phytoremediation potential of *Artemisia selengensis* to Cd stress. *Acta Sci. Circumst.* **32**, 1473, **2012** [In Chinese].
 14. DONG M., ZHAO Y.L., LEI C.X., ZHOU X.M., KU W.Z. Physiological mechanism of Cd-tolerance of *Artemisia selengensis* grown in Dongting Lake Wetland. *Asian J. Ecotox.* **8**, 111, **2013** [In Chinese].
 15. LI X., ZHANG Y.L., WEI Y.G., XU M.X., DI G.J., BU D.R. Genotypic differences in Cd accumulation and translocation in *Artemisia selengensis*. *J. Agro-Environ. Sci.* **32**, 2132, **2013** [In Chinese].
 16. ZHANG D., DING A.F. Effects of combined passivators on remediation of Cd and Pb compound-contaminated soil. *J. Agro-Environ. Sci.* **37**, 2718, **2018** [In Chinese].
 17. GILLMAN G.P., SUMPTER E.A. Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Aust. J. Soil Res.* **24**, 61, **1986**.
 18. HOUBEN D., EVRARD L., SONNET P. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere.* **92** (11), 1450, **2013**.
 19. XU C., CHEN H.X., XIANG Q., ZHU H.H., WANG S., ZHU Q.H., HUANG D.Y., ZHANG Y.Z. Effect of peanut shell and wheat straw biochar on the availability of Cd and Pb in a soil-rice (*Oryza sativa* L.) system. *Environ. Sci. Pollut. Res.* **25** (2), 1147, **2018**.
 20. NIGAM N., KHARE P., YADAV V., MISHAR D., JAIN S., KARAK T., PANJA S., TANDON S. Biochar-mediated sequestration of Pb and Cd leads to enhanced productivity in *Mentha arvensis*. *Ecotoxicol. Environ. Saf.* **172**, 411, **2019**.
 21. CHENG J.Z., LI Y.L., GAO W.C., CHEN Y., PAN W.J., LEE X.Q., TANG Y. Effects of biochar on Cd and Pb mobility and microbial community composition in a calcareous soil planted with tobacco. *Biol. Fert. Soils.* **54** (3), 373, **2018**.
 22. SUN Y.B., SUN G.H., XU Y.M., LIU W.T., LIANG X.F., WANG L. Evaluation of the effectiveness of sepiolite, bentonite, and phosphate amendments on the stabilization remediation of cadmium-contaminated soils. *J. Environ. Manage.* **166**, 204, **2016**.
 23. NAMOR A.F.D., GAMOUZ A.E., FRANGIE S., MARTINEZ V., VALIENTE L., WEBB O.A. Turning the volume down on heavy metals using tuned diatomite. A review of diatomite and modified diatomite for the extraction of heavy metals from water. *J. Hazard. Mater.* **241**, 14, **2012**.
 24. BIAN R.J., JOSEPH S., CUI L.Q., PAN G.X., LI L.Q., LIU X.Y., ZHANG A.F., RUTLIDGE H., WONG S.W., CHIA C. MARJO C., GONG B., MUNROE P., DONNE S. A three-year experiment confirms continuous immobilization of cadmium and lead in contaminated paddy field with biochar amendment. *J. Hazard. Mater.* **272**, 121, **2014**.
 25. LIU Y.X., WANG Y.Y., LU H.H., LONAPPAN L., BRAR S.K., HE L.L., CHEN J.Y., YANG S.M. Biochar application as a soil amendment for decreasing cadmium availability in soil and accumulation in *Brassica chinensis*. *J. Soil Sediment.* **18** (7), 2511, **2018**.
 26. JIANG P. Research of the growth and quality security of Seleng Wormwood in Cd polluted soil and the corresponding adjustment measures. Nanjing Agricultural University. **2009** [In Chinese].
 27. HAMIDPOUR M., AFYUNI M., KALBASI M., KHOSHGOFTARMANES A.H., INGLEZAKIS V.J. Mobility and plant-availability of Cd(II) and Pb(II) adsorbed on zeolite and bentonite. *Applied Clay Sci.* **48** (3), 342, **2010**.
 28. SESHADRI B., BOLAN N.S., NAIDU R. Rhizosphere-induced heavy metal (loid) transformation in relation to bioavailability and remediation. *J. Soil Sci. Plant Nutr.* **15**, 524, **2015**.
 29. ZHANG F., WANG X., YIN D.X., PENG B., TAN C.Y., LIU Y.G., TAN X.F., WU S.X. Efficiency and mechanisms of Cd removal from aqueous solution by biochar derived from water hyacinth (*Eichornia crassipes*). *J. Environ. Manage.* **153**, 68, **2015**.
 30. GAO L.Y., DENG J.H., HUANG G.F., LI K., CAI K.Z., LIU Y., HUANG F. Relative distribution of Cd²⁺ adsorption mechanisms on biochars derived from rice straw and sewage sludge. *Bioresour. Technol.* **272**, 114, **2019**.
 31. GUO F.Y., DING C.F., ZHOU Z.G., HUANG G.X., WANG X.X. Stability of immobilization remediation of several amendments on cadmium contaminated soils as affected by simulated soil acidification. *Ecotoxicol. Environ. Saf.* **161**, 164, **2018**.
 32. XUE Y., GAO B., YAO Y., INYANG M., ZHANG M., ANDREW R.Z., KYOUNG S.R. Hydrogen peroxide modification enhances the ability of biochar (hydrochar) produced from hydrothermal carbonization of peanut hull to remove aqueous heavy metals, batch and column tests. *Biochem. Eng. J.* **200-202**, 673, **2012**.
 33. ZHENG R.L., CAI C., LIANG J.H., HUANG Q., CHEN Z., HUANG Y.Z., ARP H.P.H., SUN G.X. The effects of biochars from rice residue on the formation of iron plaque and the accumulation of Cd, Zn, Pb, As in rice (*Oryza sativa* L.) seedlings. *Chemosphere.* **89** (7), 856, **2012**.
 34. YANG X., LIU J.J., MCGROUTHER K., HUANG H.G., LU K.P., GUO X., HE L.Z., LIN X.M., CHE L., YE Z.Q., WANG H.L. Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. *Environ. Sci. Pollut. Res.* **23** (2), 974, **2016**.
 35. SHAHID M., XIONG T.T., MASOOD N., LEVEQUE T., QUENEA K., AUSTRUY A., FOUCAULT Y., DUMAT C. Influence of plant species and phosphorus amendments on metal speciation and bioavailability in a smelter impacted soil, a case study of food-chain contamination. *J. Soil Sediment.* **14** (4), 655, **2013**.
 36. KIM R.Y., YOON J.K., KIM T.S., YANG J.E., OWENS G., KIM K.R. Bioavailability of heavy metals in soils,

- definitions and practical implementation--a critical review. *Environ. Geochem. Health.* **37** (6), 1041, **2015**.
37. HE H., NORA F.Y.T., YAO A.J., QIU R.L., LI W.C., YE Z.R. Effects of alkaline and bioorganic passivators on cadmium, lead, zinc, and nutrient accumulation in brown rice and grain yield in acidic paddy fields contaminated with a mixture of heavy metals. *Environ. Sci. Pollut. Res.* **23**, 23551, **2016**.
38. ZHAO F.J., MA Y.B., ZHU Y.G., TANG Z., MCGRATH S.P. Soil contamination in China, current status and mitigation strategies. *Environ. Sci. Technol.* **49**, 750, **2015**.
39. YU Y., WAN Y.N., WANG Q., LI H.F. Effect of humic acid-based amendments with foliar application of Zn and Se on Cd accumulation in tobacco. *Ecotoxicol. Environ. Saf.* **138**, 286, **2017**.
40. LU K.P., YANG X., SHEN J.J., ROBINSON B., HUANG H.G., LIU D., BOLAN N., PEI J.C., WANG H.L. Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to *Sedum plumbizincicola*. *Agric. Ecosyst. Environ.* **191**, 124, **2014**.