



Influence of biochar derived from Cd polluted silkworm excrement on the phytoavailability of Cd in a paddy soil and its accumulation in mulberry

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ARTICLE INFO

Edited by Dr Muhammad Zia-ur-Rehman

Keywords:

Silkworm excrement
Biochar
Immobilization
Cadmium
Mulberry

ABSTRACT

Developing sericulture industry is a promising model for the utilization of soils heavily contaminated with cadmium (Cd), but the management of polluted silkworm excrement (SE) becomes challenging. This study aimed to evaluate the effects of the SE biochar (SB) with Cd (SB-Cd) and without Cd (SB-Cd free) on the chemical properties of paddy soil, the mulberry leaf quality and the accumulation of Cd in mulberry. The soil incubation experiments showed that the two SBs all raised the acidic soil pH (20.24 %~49.97 %) significantly ($P < 0.05$) with the increasing SB addition rates. The two SBs increased the soil cation exchange capacity (CEC) and played an essential role in reducing the phytoavailability of Cd. The pot experiment elucidated the two SBs all promoted the growth of mulberry, increased the crude protein content and the chlorophyll content, reduced the total sugar content in leaves. The Cd concentrations in root, stem, leaf were decreased with the increase of SB respectively, but no significant differences were found between the same additions of SB-Cd free and SB-Cd. The use of SB-Cd for remediation of the Cd polluted soils could be a reasonable method to address the Cd polluted SE.

1. Introduction

The remediation of the cadmium (Cd) polluted paddy soils has turned into a global environmental problem (Hou et al., 2020; Jiang et al., 2022b). In China, about 2.6×10^7 ha of cultivated land has been contaminated by inorganic contaminants, and the Cd polluted cultivated land is about 1.3×10^4 ha, ranking the first among the inorganic contaminants and also with a mean input concentration of 0.004 mg/kg in the plough layer every year (Lin et al., 2022; Shen et al., 2016; Zou et al., 2021). Cd is one of the extremely toxic environmental pollutants in paddy soils in the south of China, which could result in various toxic effects on plants, animals and the human body for its undegradability and better mobility (Zhao et al., 2015). Soil Cd pollution is mainly caused by human activities, such as the mining, industrial production and agricultural inputs (Ashraf et al., 2019; Lin et al., 2022). There are many treatment solutions for the Cd polluted soils, such as chemical immobilization (Fan et al., 2020; Lin et al., 2022), chemical washing (Feng et al., 2020; Rui et al., 2019), soil replacement (Antonkiewicz and Gworek, 2023; Teng et al., 2020), phytoremediation (Jiang et al., 2022a, 2019b; Zeng et al., 2019) and microorganism remediation (Xu et al.,

2019; Yin et al., 2019). Based on the national conditions of more people and less land in China, in order to better use the cultivated land resources and do not alter its agricultural properties, phytoremediation becomes the innovative and reasonable strategy for using and repairing the Cd heavily polluted farmland (Jiang et al., 2022b; Zhao et al., 2015). Numerous hyperaccumulators have been restricted to large-scale application due to their small plants, slow growth, harsh growing conditions, and accumulation of limited variety of metals and poor subsequent utilization value (Fan et al., 2022; Zeng et al., 2020b). Several studies have utilized woody plants to safely use the metals polluted soils, such as polar (Ancona et al., 2020), willow (Pilipović et al., 2019) and mulberry (Jiang et al., 2022b, 2019b; Lei et al., 2019). China is the earliest country in the world to cultivate mulberry and rear silkworms, and has the richest mulberry resources in the world (Jiang et al., 2017).

Mulberry (*Morus alba* L.), pertaining to the order *Rosales*, family *Moraceae*, genus *Morus*, is a woody, cash and perennial plant with the characteristics of developed root systems, fast growth, high productivity and ease in establishment and management (Jiang et al., 2022b; Lei et al., 2019; Zhou et al., 2015b). Many recent articles have proposed that there are no significant differences in the growth and development of

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Bombyx mori L., and also the production and quality of cocoons under the pressure of heavy metals accumulated by mulberry leaves (Jiang et al., 2020; Lei et al., 2019; Zhou et al., 2015b). Planting mulberry and raising silkworms is a safe, economical, and efficient approach to utilize the Cd polluted areas (Dai et al., 2020; Jiang et al., 2022a; Zeng et al., 2020a). It does make full use of the Cd contaminated farmland and raised farmers' income, and also lessen the hurt to human organs via the food chain (Jiang et al., 2021b, 2022b, 2019b). Meanwhile, to ensure the low Cd content in mulberry leaf, chemical immobilization could be adopted to reduce the phytoavailability and transportation of Cd in soils by adding some organic or inorganic conditioners, such as phosphates-containing materials (Austruy et al., 2014), biochar materials (Du et al., 2024; Li et al., 2022; Shao et al., 2019), clay minerals (Wang et al., 2019), furnace slag (Bashir et al., 2019), Si-rich minerals (Xiong et al., 2018), nano zero-valent iron composites (Mousa et al., 2024) and so on. Biochar material is mainly gained through the pyrolysis process of sewage sludge and agricultural residue in an oxygen-limited atmosphere (von Gunten et al., 2017; Zhang et al., 2019a). Application of biochar could significantly increase soil pH and porosity (Ippolito et al., 2017; Shen et al., 2016), improve plant growth (Wang et al., 2016; Xiao et al., 2018), reduce greenhouse gas emissions (Abhishek et al., 2022; Zhang et al., 2019b) and decrease the extractable heavy metals concentrations in soils (Ippolito et al., 2017; Shen et al., 2016).

Silkworm excrement (SE) is one of the main by-products of sericulture industry. In China, there would be over 4.5 million tons SE every year (Jiang et al., 2019a). SE has been mainly used as composting material for its richness in organic matter (OM) and macro-elements, such as nitrogen (N), phosphorus (P) and potassium (K) (Lei et al., 2018; Shi et al., 2023b). And the SE compost can be used for remediation of the heavy metals polluted soils (Chen et al., 2021; Shi et al., 2023b). Also, several studies reported that the SE could be utilized to prepare carbon materials, such as nanosheets for high-performance supercapacitors (Lei et al., 2018), biochar for adsorption of bisphenol A (Li et al., 2017) and restoration of vegetation and soil ecology in metals heavily polluted mining soils (Shi et al., 2023a), porous carbon for the continuous emission of the pesticide (Wei et al., 2017). In fact, SE is randomly discarded and has a low degree of resource utilization in most rural areas in China, which increases the probability of sericulture disease occurrence. What is more, the heavy metals in abandoned SE could also result in secondary pollution (Jiang et al., 2019a). Pyrolysis is an important disposal, utilization methods for the biomass containing heavy metals (Cui et al., 2024; Fan et al., 2024; Hu et al., 2024; Liu and Tran, 2021) and biochar could be used for the remediation of heavy metal polluted soils for its high cation exchange capacity (CEC), large specific surface area and functional groups (Blenis et al., 2023; Long et al., 2024; Nguyen et al., 2023). However, few studies have investigated the reasonable treatment of SE with Cd. And the effects of SE biochar (SB) (SB-Cd, SB-Cd free) on the chemical properties of paddy soils, the speciation of Cd in contaminated soils and Cd distribution in mulberry are unclear. In this study, the soil incubation experiments were carried out to study the effects of the two SBs on the chemical properties of paddy soil that had been naturally contaminated by Cd and chemical forms of Cd. The influences of the two kinds of SBs on the growth of mulberry, the quality of mulberry leaf, the accumulation and distribution of Cd in mulberry were also examined by pot experiments.

2. Materials and methods

2.1. Soil and the preparation and characterization of SB

The soil heavy metals pollution in Zhuzhou City, the second biggest city in Hunan Province, mainly originates from the discharge, sedimentation of industrial wastes to the surrounding environment and irrigation with wastewater from the rivers close to the industrial sites (Tian et al., 2025; Wang and Stuanes, 2003). A large number of industrial wastewater containing a lot of heavy metals from the Qingshuitang,

a very important industrial base in Zhuzhou, was discharged into the Xiang River in the past (Chen et al., 2016). The polluted soils were sampled from the farmland in a village (113°03'19"E, 27°53'48"N) near the Qingshuitang, Shifeng District, Zhuzhou City, Hunan Province, China. The soils in arable layer (0–20 cm) were collected in plastic bags, air-dried at room temperature, removed the debris and passed across a 2-mm nylon sieve for further use. The collected soil with a sandy clay texture (USDA classification) belongs to Anthrosols according to World Reference Base for Soil Resources (WRB) (Mantel et al., 2023). The SE without Cd was collected at the fifth instar of silkworm from the Changsha branch of National Silkworm Improvement Center, in the Sericultural Research Institute of Hunan province. The Cd contaminated SE was sampled from Yangjiazhai (109°40'E, 28°58'N), Huayuan County, Xiangxi Tujia and Miao Autonomous Prefecture, Hunan province, China (Jiang et al., 2022a). After removing the impurities such as the broken leaves and lime particles from the SE, they were dried in an oven at 70 °C to stable weight, respectively. A tube furnace (OTF-1200X-80, Hefei Kejing Material Technology Co., LTD, China) was utilized to produce SB. For each pyrolysis experiment, about 20 g of the dried SE were placed into a quartz boat (6 cm diameter, 15 cm long). The N₂ (400 mL/min) was used to keep the tube inert anaerobic atmosphere. Then the temperature was set to 450°C at an ascent rate of 7°C/min and held at 450°C for 2 h (Tan et al., 2015). The slow (10 °C/min) and low temperature (300–550 °C) pyrolysis not only obtained more biochar production, but also this biochar showed higher CEC and more functional groups, which were suitable for the remediation of heavy metal contaminated soil (Blenis et al., 2023). The obtained SB was slightly grounded and passed across a 2-mm sieve, then stored in airtight containers before use. The contents of element C, H, N, S, O in SB were determined through an elemental analyzer (3H-2000PM2, Best instrument technology (Beijing) Co., Ltd). Specific surface areas (SSA) and total pore volume were analyzed by a physical adsorption analyzer (MicroActive for ASAP 2460, Shanghai, China) in the N₂ atmosphere by adsorption and desorption technique. The selected basic physicochemical properties of the paddy soil and SBs were shown in Table 1.

2.2. Soil incubation experiment

Forty grams prepared soils were added to the 50-mL plastic centrifuge tube. The SB was added at the rate of 0.5 %, 1 %, 3 % and 5 % (w/w, dry basis) and the tubes without SB were used as the control. The soil and SB were mixed absolutely and added the deionized water to reach 70 % of field soil moisture capacity. Each treatment was repeated three times. All tubes were covered with corresponding plastic lids which were drilled a few small holes to enable gas exchange and reduce moisture loss. Then the all tubes were incubated at 20 ± 1 °C for 1 year in a dark environment. After incubation, the soil samples were removed from the tubes for the following experiments. The pH, OM, CEC of the paddy soil were determined by the routine test methods (Lu, 1999). For the speciation of Cd, 1 g (dry base) soil from each tube was sequentially extracted by the methods of sequential batch extraction (Tessier et al., 1979). The concentration of Cd in the extraction solutions was detected by Atomic Absorption Spectrometry (AAS) (Thermo Fisher ICE-3400, America) (Jiang et al., 2020). For quality control, GBW07401(GSS-1) and GBW07603 (GSV-2) from the China National Standard Materials Center were used as certified reference materials for soils and plants, respectively. Cd standard solutions (GBW(E) 082822, 1000 mg/L, 50 mL) were supplied by Tanmo Quality Inspection Standard Material Center.

2.3. Pot experiment

The pot experiment was conducted in a greenhouse at the Sericultural Research Institute of Hunan Province. The Cd polluted soils from the Section 2.1 were fully mixed with SB-Cd free and SB-Cd at five doses (0 %, 0.5 %, 1 %, 3 % and 5 %, m/m, dry weight), respectively. Nine

Table 1
Some physical and chemical properties of soils and SB.

Category	pH	OM (g/kg)	Element content (%)					Specific surface area (m ² /g)	Average pore diameter (nm)	Total pore volume (mL/g)	Cd content (mg/kg)
			C	H	O	N	S				
SB-Cd free	9.45	799	41.74	3.02	26.85	2.13	0.24	6.16	17.53	0.027	0.13
SB-Cd	9.21	711	46.31	3.38	26.53	1.33	0.16	8.30	16.30	0.034	3.92
Soil	6.22	19.6	CEC	Nutritional elements (mg/kg)				Size distribution (%)			9.33
				available nitrogen	available phosphorus	available potassium		sand	silt	clay	
			3.04	226	4.6	52	57.86	5.17	36.97		

treatments were as follows: (1) no SB application (CK), (2) SB-Cd free (0.5 %), (3) SB-Cd free (1 %), (4) SB-Cd free (3 %), (5) SB-Cd free (5 %), (6) SB-Cd (0.5 %), (7) SB-Cd (1 %), (8) SB-Cd (3 %) and (9) SB-Cd (5 %). The mixed soils were loaded to plastic pots (10 cm bottom diameter×15 cm upper diameter×15 cm height) filled with 1.0 kg soils. The pot experiment was set up in randomized complete block design with three replicates each treatment. The soils were incubated in the pots for 60 days with 70 % water holding capacity with deionized water. Yuesang 11, *Morus atropurpurea* Roxb., was provided by the Sericultural & Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China. Subsequently, one one-year-old mulberry tree was planted into each pot. Three months later, the mulberry was harvested to determine fresh biomass and Cd content. The treatment and Cd concentrations analysis of the soil and plant samples were followed by a previous study (Jiang et al., 2020). The plant height was measured by meter rule. The crude protein was detected by Automatic Kjeldahl Apparatus (HaiNeng K1100, China). The total sugar and chlorophyll content were measured by the reagent kits from Beijing Solarbio Science & Technology co., Ltd.

2.4. Statistical analyses

Each treatment was carried out in three replicates and all the data was shown as means±SD (n = 3). The SPSS software (version 17.0) was utilized for the data analysis and Duncan's multiple range test ($P < 0.05$) was applied to compare the significant differences among the mean values of different treatments. The relative standard deviation (RSD), limit of detection (LOD), limit of quantification (LOQ) and extraction recovery (ER) were used to determine the validation of the method (Haq et al., 2024). The recovery rates of Cd in the standard reference materials were 88–96 % and 99–113 % in soil and plant samples, respectively. The recovery of Cd obtained by sequential batch extraction ranged from 95 % to 125 %. The values of RSD in all replicates were less than 10 % and the LOD, LOQ were 0.08 and 0.25 ug/L, respectively.

3. Results and discussion

3.1. Soil chemical properties

The soil pH is one of the particularly important items in changing the soil surface electric charge and also the chemical forms of heavy metal in soil (Jiang et al., 2018, 2024; Shi et al., 2023b; Wu et al., 2020). In this study, the SB-Cd and SB-Cd free all increased the acidic soil pH significantly ($P < 0.05$) and the pH values were increased with the increasing application rate of SB (Fig. 1A). The pHs of the soil with SB-Cd free (5 %) and SB-Cd (5 %) were 7.90 and 7.77, 49.97 % and 47.44 % higher than the control group, respectively (Fig. 1A). The alkalinity of biochar is a more valuable parameter than its pH in increasing the acidic soil pH (Dai et al., 2017). During the pyrolysis process of biochar, the reduction of the acidic functional groups (Xiang et al., 2021) and the production of -COO- and -O- functional groups increased the biochar alkalinity (Dai et al., 2017; Wei et al., 2020). Also, the volatile substances in SE escaped, the organic matter reduced, the pH and CEC of biochar increased (Wei

et al., 2020), which made it an outstanding soil amendment for the acidic soils. Once the soil pH elevated, the acidic groups in the soil would be deprotonated, such as the phenolic, hydroxyl groups, which made the soil particles be more negatively-charged (He et al., 2019). The more negatively-charged sites in soil, the more attraction for metal Cd²⁺ (Wu et al., 2020), the more content of iron-manganese oxides in polluted paddy soil and then increased the adsorption capacity for Cd (Chen et al., 2022; Fidel et al., 2017).

The soil CEC is an important indicator to evaluate the soil fertility and buffering performance (Jiang et al., 2018; Yu et al., 2024). In this paper, the soil CEC was increased no matter the application of SB-Cd or SB-Cd free (Fig. 1B), indicating that the nutrient retention in the incubated soil was increased (Gul et al., 2015). The SB-Cd free treatments all significantly increased the soil CEC compared with the control group, and when the SB-Cd free content was 5 %, the soil CEC was 3.96 cmol (+)/kg, 2.65 times of the control (Fig. 1B). Though the SB-Cd treatments all increased the soil CEC compared with the control, the CEC values among the treatments were not significantly different (Fig. 1B). The increase of the soil CEC in SB treatments could be attributed to the high surface area, highly porous structure (Hansen et al., 2016), surface oxygen-containing functional groups (Yu et al., 2024) of SB and the increase of base cation concentration after pyrolysis (Fidel et al., 2017). The increased soil CEC by SB may result in strong adsorption for Cd and decrease the Cd phytoavailability (Wu et al., 2020). The increased soil pH, CEC indicated the liming potential of SB.

The content of soil OM is another key item to influence the phytoavailability of heavy metals through some physical chemistry reactions to change the activity of heavy metals, such as adsorption, complexation, ion exchange, oxidation-reduction (Jiang et al., 2018). In this study, compared with the control, the soil OM decreased first and then increased slowly with the increase of SB (Fig. 1C). However, the OM values between the control, SB-Cd and SB-Cd free showed no significant differences (Fig. 1C). The soil OM increases or reduces the phytoavailability of Cd is determined by the types of OM. Dissolved OM could generate explicable compounds with Cd, reduce the adsorption of Cd on soils and increase the availability of Cd, while particulate OM could increase the adsorption of Cd on soil and then decrease the mobility and bioavailability of Cd (Jiang et al., 2018; Wu et al., 2020).

3.2. Cd speciation

The speciation of heavy metal in soils is important to the environmental impacts (Jiang et al., 2024). The total Cd in soil could be partitioned into five fractions: exchangeable (F1), carbonated-bound (F2), Fe-Mn oxides phase (F3), complexed with organic matter (F4), and residual fraction (F5) by Tessier sequential extraction method (Tessier et al., 1979). In this study, the effects of SB-Cd and SB-Cd free on the ratio of F1, F2, F3, F4, and F5 of Cd in the polluted paddy soil were showed in Fig. 2. For SB-Cd free, compared with the control, the increasing SB reduced the ratio of F1, increased the ratio of F2, F3 and the ratio of F4 and F5 showed a few changes (Fig. 2). However, compared to the control, the application of SB-Cd made the ratio of F1 decrease, the ratio of F2, F3, F4 increase, the F5 keep the relatively

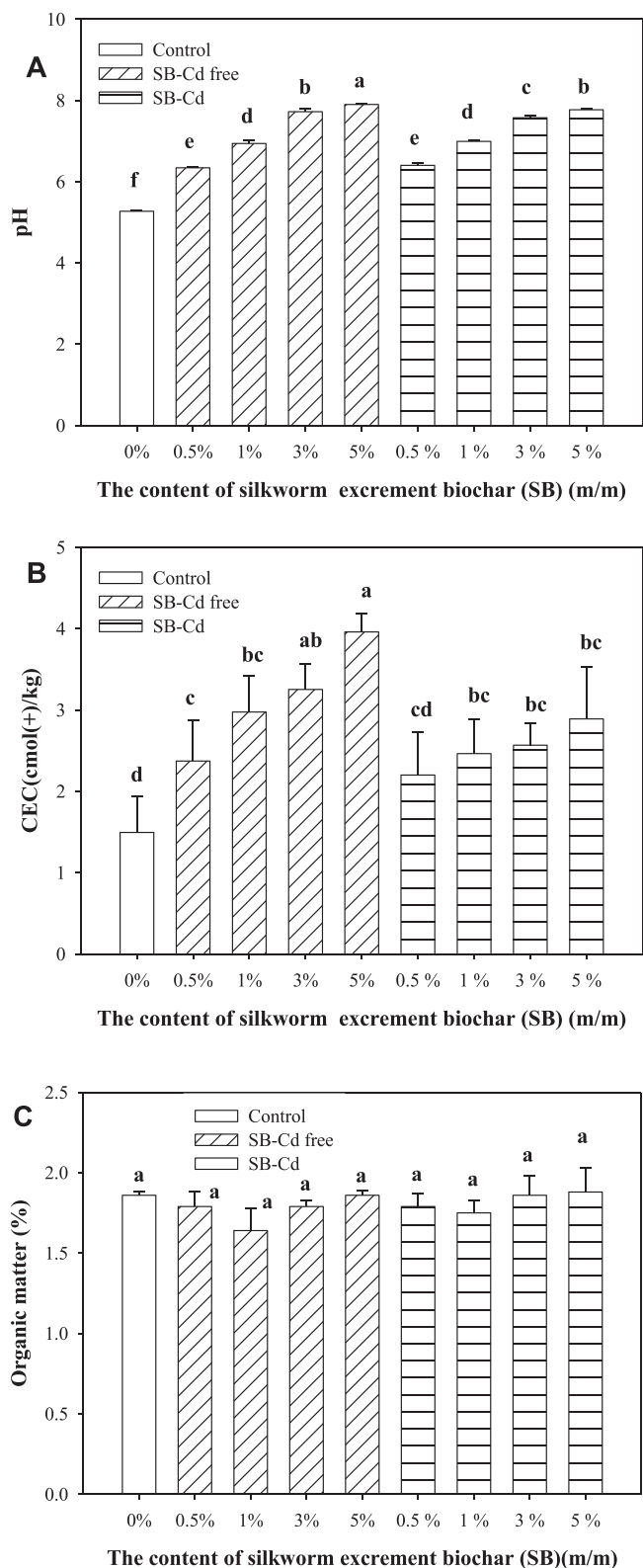


Fig. 1. Effect of SB on the chemical properties of the Cd-contaminated paddy soil.

stable (Fig. 2). Among the five fractions of Cd in soil, F1 and F2 are more phytoavailable and toxic (Fan et al., 2024; Hou et al., 2020; Mehdizadeh et al., 2021). In this study, the content of F1 and F2 fractions in the control, SB-Cd free (5 %) and SB-Cd (3 %) occupied the total soil Cd

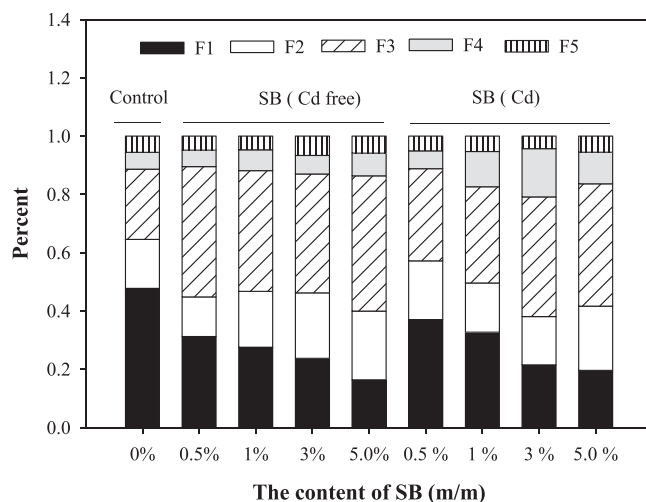


Fig. 2. Effect of SB on the ratio of (F1) exchangeable, (F2) carbonate-bound, (F3) Fe-Mn oxide, (F4) organically bound, and (F5) residual fractions of Cd in the polluted paddy soil.

SB-Cd (3 %) played a better role in reducing the phytoavailable Cd content (Fig. 2). These results theoretically indicated that SB could reduce the phytoavailability of Cd in soils effectively whatever the SB contained Cd or not. Also, other plants residues like the rice husk biochar and sunflower seedshells-derived biochar significantly reduced the bioavailability of Cd in paddy soils by 12 % and 24 %, respectively (Khalique et al., 2024). Biochar could be regarded as the most efficacious tool for immobilization Cd in soil than the rest of amendments as proclaimed by Irfan et al. (Irfan et al., 2021) with different assumed mechanisms, such as surface adsorption, ion exchange, complexation, precipitation and electrostatic interaction (Khalique et al., 2024; Zhang et al., 2023).

3.3. Mulberry growth

The effects of SB amendments on the mulberry growth were showed in Fig. 3. From the physical growth image of the mulberry trees, the application of SB-Cd free and SB-Cd all promoted plant growth, increased the mulberry height (Fig. 3A). But as shown in Fig. 3B, different contents of SB-Cd free or SB-Cd had different effects on the mulberry height. As for SB-Cd free, the addition content of 0.5 %, 1 % and 3 % SB significantly increased the height of mulberry compared with the control, but the treatment of SB-Cd free (5 %) did not significantly improved the mulberry height in comparison with the control (Fig. 3B). On the contrary, compared with the control, the SB-Cd (0.5 %), SB-Cd (1 %), SB-Cd (3 %) did not significantly promote the plant height, but the SB-Cd (5 %) increased the plant height significantly (Fig. 3B).

It could not evaluate the plant growth properly just only from the plant height because that a lower plant may have more branches. The plant biomass is another important parameter to reflect the plant growth. As shown in Table 2, compared to the control, accept the treatment of SB-Cd (5 %) that significantly increased the root biomass, the other treatments with SB had no significant effects on the root or stem or leaf of mulberry. Though the total biomass of mulberry treated with SB showed without significant differences compared with that of the control, the treatments of SB-Cd increased the total biomass and it was increased with the increasing SB-Cd content (Table 2). The SB with high CEC was a stellar soil amendment and it could improve the soil physicochemical properties, provide the essential nutrients to plants and be helpful for plant growth (Fan et al., 2024; Khosravi et al., 2024; Yu et al., 2024). This study reported the positive influences of SB on the plant growth, including the SB-Cd, but some studies reported the

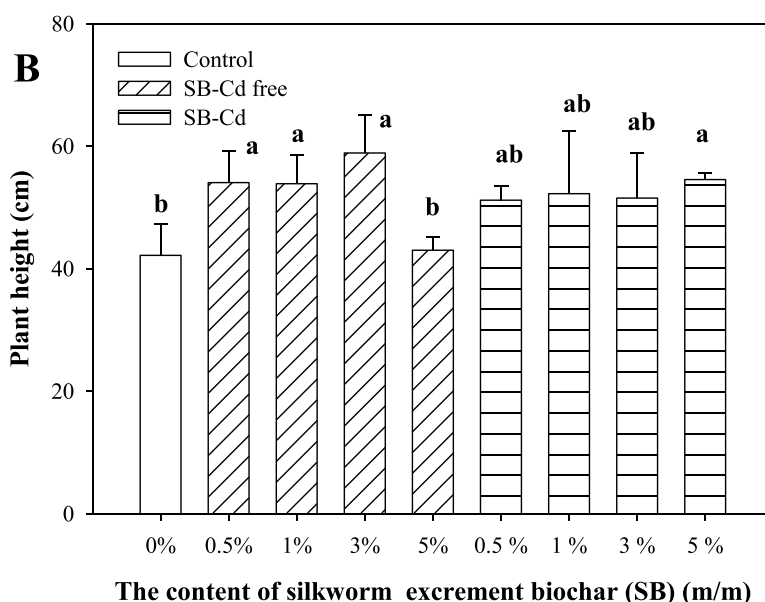
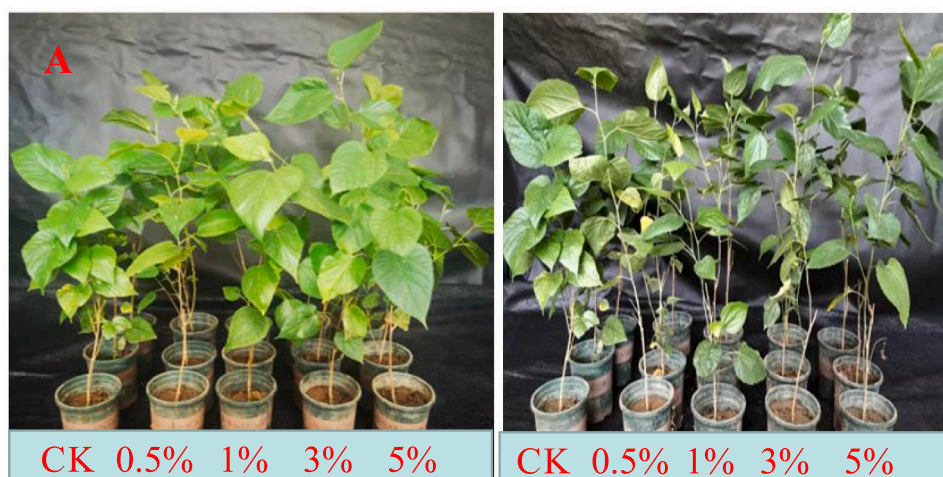


Fig. 3. Effect of SB on the plant height of mulberry planted in the Cd-contaminated paddy soil.

Table 2
Effect of SB on the biomass and moisture content of mulberry planted in the Cd-contaminated paddy soil.

Treatments	Content (m/m)	Root		Stem		Leaf		Total biomass (dry weight/g)
		dry weight/g	moisture content	dry weight/g	moisture content	dry weight/g	moisture content	
Control	0 %	1.10 ± 0.04b	0.57 ± 0.02b	1.89 ± 0.05ab	0.58 ± 0.03c	1.27 ± 0.21a	0.78 ± 0.00a	4.26 ± 0.17a
SB-Cd free	0.50 %	1.19 ± 0.24b	0.60 ± 0.03ab	1.75 ± 0.34ab	0.62 ± 0.00abc	1.19 ± 0.36a	0.79 ± 0.01a	4.13 ± 0.92a
	1 %	1.12 ± 0.16b	0.63 ± 0.01a	2.09 ± 0.34ab	0.62 ± 0.04abc	1.24 ± 0.46a	0.80 ± 0.01a	4.45 ± 0.90a
	3 %	1.43 ± 0.04ab	0.62 ± 0.04ab	2.49 ± 0.13a	0.61 ± 0.01bc	1.45 ± 0.12a	0.78 ± 0.01a	5.36 ± 0.27a
	5 %	1.16 ± 0.11b	0.63 ± 0.02a	1.59 ± 0.10b	0.64 ± 0.01ab	1.34 ± 0.12a	0.79 ± 0.02a	4.09 ± 0.12a
SB-Cd	0.50 %	1.25 ± 0.40ab	0.63 ± 0.03a	1.93 ± 0.36ab	0.61 ± 0.04abc	1.37 ± 0.35a	0.76 ± 0.04a	4.54 ± 0.40a
	1 %	1.20 ± 0.28b	0.62 ± 0.02ab	2.10 ± 0.98ab	0.62 ± 0.02abc	1.33 ± 0.18a	0.78 ± 0.02a	4.63 ± 1.42a
	3 %	1.35 ± 0.36ab	0.60 ± 0.01ab	2.11 ± 0.47ab	0.62 ± 0.00abc	1.20 ± 0.36a	0.78 ± 0.02a	4.66 ± 1.19a
	5 %	1.65 ± 0.18a	0.62 ± 0.02ab	2.25 ± 0.12ab	0.66 ± 0.01a	1.60 ± 0.21a	0.77 ± 0.02a	5.50 ± 0.44a

Note: Data are mean values ± SD (n = 3). Means with the same letter in the same column did not differ significantly (Duncan's test, p < 0.05). The table below was the same.

potential harms of biochar to plants, such as the inhibitory effect on the plant growth by heavy metals in biochar (Bai et al., 2022; Xiang et al., 2021), the toxic effects on plant or microorganism for the presence of phenolic compounds (Xiang et al., 2021; Zhang et al., 2019b), the hindrance to nutrient absorption because of the adsorption of N, P by mineralizable compounds in biochar and the increase of soil pH by

biochar (Mohamed et al., 2023; Xiang et al., 2021). So the kind of feedstock, pyrolysis rate, pyrolysis temperature, the structure and particle size of biochar should be carefully chosen to decrease the bioavailability of heavy metals in biochar (Mehdizadeh et al., 2021; Xiang et al., 2021). Especially the SB-Cd in this study, though the pyrolysis of polluted SE stabilized Cd in biochar and reduced SB-Cd

toxicity, it is essential to systematically contemplate the relationship between the pyrolysis parameters, physicochemical properties of SB and the environmental conditions to minimize the ecological risks of SB due to the presence of Cd. Therefore, although the metals in most biochar are indeed safe, it is still recommended to conduct toxicity assessments and long-term monitoring on the biochar before large scale field use (Fan et al., 2024).

3.4. Mulberry leaf quality

Mulberry leaves are the main feed for silkworms (*Bombyx mori* L.), and the quality of mulberry leaves plays a key role in the growth and development of silkworms (Chandang et al., 2017). Moisture content of mulberry leaves is one of the important items in determining the nutritional value of mulberry leaves (Rahmathulla et al., 2006). Usually, the moisture content in silkworms is 80–87 % and it maintains balance relying on the moisture in mulberry leaves. The moisture content in fresh mulberry leaves is 75–82 %, and it could be changed under various conditions, such as different developmental stage, various mulberries, the soil quality and mulberry garden management level. The water contents in the mulberry leaves were found significantly declined with the increasing soil Cd, and when the Cd content in soil was 75.8 mg/kg, the mulberry leaf showed wilting (Wang et al., 2004). In this paper, the moisture content of leaves in both all treatments and the control were 76–80 %, and the moisture content in all the SB treatments showed without significant differences compared with the control (Table 2). These results indicated that SB could alleviate the water stress of mulberry trees induced by Cd.

It has been declared that Cd could hinder adversely the synthesis of chlorophyll, resulting in the decrease of proteins and carbohydrates (Prince et al., 2002). The protein content in mulberry leaves is closely related to the nutrition of silkworms and cocoon quality. Compared to the control, all the treatments (SB-Cd and SB-Cd free) increased the crude protein contents in mulberry leaves and the crude protein content in SB-Cd free (5 %) was 25.07 %, the highest among all the treatments, 21.26 % higher than the control (Fig. 4). The treatments of SB-Cd free (1 %), SB-Cd free (3 %), SB-Cd free (5 %), SB-Cd (0.5 %), SB-Cd (1 %) and SB-Cd (3 %) all increased the crude protein content in leaf significantly ($P < 0.05$) (Fig. 4). As for the total sugar content and the chlorophyll content in mulberry leaves, the treatments of SB-Cd free and SB-Cd all showed without significant differences with the control (see supplementary material, Fig.S1 and Fig.S2). An overall reduction in chlorophyll content associated with dwindling carbohydrate and protein content in mulberry leaf was found with the increasing concentrations of

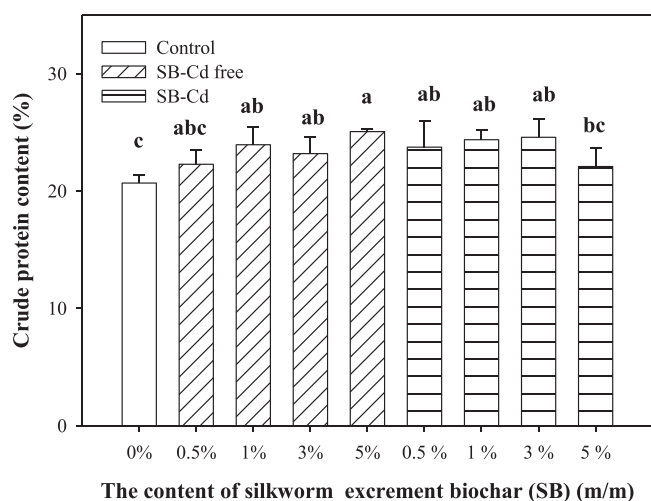


Fig. 4. Effect of SB on the crude protein content of mulberry leaf obtained from the Cd-contaminated paddy soil.

Cd (0–160 mg/kg) (Prince et al., 2002). But when the Cd concentration in soil was 8.49 mg/kg, the crude protein contents in mulberry leaf were higher than that of the control. When soil Cd concentrations were above 22.3 mg/kg, the crude protein gradually decreased with increasing Cd in soil (Wang et al., 2004). The soluble sugar contents were higher than that of the control when soil Cd was at 8.49 and 22.3 mg/kg. And a gradual decrease of was found at Cd concentrations in soil over 40.6 mg/kg (Wang et al., 2004). In this study, the application of SB improved the quality of mulberry leaf under the stress of Cd, as indicated by increased crude protein (Fig. 4) and chlorophyll content (Fig.S1), as well as a decrease in total sugar content in mulberry leaves (Fig.S2).

3.5. Cd concentrations in Mulberry

The effects of SB-Cd free and SB-Cd amendments on the Cd concentrations in root, stem and leaf of mulberry planted in the Cd-contaminated paddy soil were shown in Table 3. The SB decreased the Cd content in root, stem and leaf, and it was decreased with the increase of SB (Table 3). The Cd concentrations in mulberry root were all above that of the paddy soil, indicating that the Cd in soil was easy to be absorbed by mulberry root and 86–92 % of the Cd in mulberry was accumulated in the root (Table S1). The SB-Cd free (3 %), SB-Cd free (5 %), SB-Cd (1 %), SB-Cd (3 %) and SB-Cd (5 %) significantly decreased the Cd concentrations in root, and the Cd content in root in the treatments of SB-Cd free (5 %) and SB-Cd (5 %) were 9.38 and 13.15 mg/kg, 71.66 % and 60.27 % less than that of the control, respectively (Table 3). The SB significantly decreased the Cd concentrations in leaf, but for the same addition of SB-Cd free and SB-Cd, they did not have the significant differences (Table 3). The effects of the SB on the percentage of Cd in root and stem showed without significant differences compared with the control, but it significantly decreased in leaf (Table S1). These results were consistent with those in Section 3.2, lower phytoavailability of Cd in the soil, less Cd content in mulberry plants.

The application of biochar in polluted soils could boost the immobilization and reduce the absorption of metals by crops for the specific properties, such as high recalcitrance, stability, microporous structure (Chen et al., 2024; Cui et al., 2021; Shi et al., 2023b). Although the SB-Cd could also effectively reduce the content of Cd in mulberry, the application of SB-Cd in farmland soil still requires a full life cycle assessment because that the interactions between the soil environment and biochar can alter the availability of Cd in biochar. For example, the aging of biochar could significantly extend the SSA and enrich oxygen-containing functional groups, decrease the pH, increase the mobility and phytoavailability of heavy metals in biochar, thus resulting in potential environmental risks (Cui et al., 2021). Meanwhile, even if the heavy metals were bonded with the biomass component strongly and this stable combination made leaching more difficult, this stable biochar

Table 3
Effect of SB on the Cd content in root, stem and leaf of mulberry planted in the Cd-contaminated paddy soil.

Treatments	SB content	Cd concentration (mg/kg)		
		root	stem	leaf
control	0.0 %	33.10 ± 2.35a	2.13 ± 0.35a	1.22 ± 0.23a
SB-Cd free	0.5 %	26.72 ± 6.34ab	2.00 ± 0.85ab	0.55 ± 0.32b
	1.0 %	21.96 ± 6.96abc	1.33 ± 0.51ab	0.26 ± 0.05bcd
	3.0 %	19.65 ± 5.82bc	1.25 ± 0.42ab	0.13 ± 0.08d
	5.0 %	9.38 ± 1.60c	0.54 ± 0.29b	0.07 ± 0.02d
SB-Cd	0.5 %	28.06 ± 4.21ab	1.44 ± 0.58ab	0.47 ± 0.30bc
	1.0 %	17.73 ± 5.37bc	1.08 ± 0.64ab	0.22 ± 0.08 cd
	3.0 %	15.45 ± 2.60bc	1.00 ± 0.51b	0.11 ± 0.01d
	5.0 %	13.15 ± 4.72c	0.59 ± 0.28b	0.11 ± 0.03d

with heavy metals should also be treated as a potentially dangerous contaminant (Xiang et al., 2021). However, in one of our previous studies, the circulation model of mulberry planting and silkworm raising in heavy metals polluted mine soils has been proposed (Jiang et al., 2022a). It suggests that the application of SB-Cd in remediation of the Cd heavily polluted soils is feasible. Therefore, we thought that the circulation model of mulberry planting and silkworm rearing was also suitable for the Cd heavily contaminated paddy soils (Fig. 5), especially the mine soils. The main reasons were as follows: 1) The utilization model of planting mulberry and raising silkworms in heavy metals polluted farmland has not changed the agricultural properties of the soil, which is in line with national policy in China (Jiang et al., 2021a); 2) Planting mulberry and raising silkworm is easy to operate, easy to manage, and utilizes the polluted land while continuously increases the farmers' income (Jiang et al., 2020; Lei et al., 2019); 3) The Cd is not degraded by soil microorganisms and only migrates from one place to another, from one chemical form to another (Borges et al., 2018), and both the mulberry and silkworm have higher tolerance to Cd (Jiang et al., 2020; Zhou et al., 2015a); 4) Adding the SB-Cd to the repaired paddy soil again did indeed increase the total Cd content of the repaired soil, but the total Cd content was decreased compared to that of the pre-repaired soil. Moreover, the increasing pyrolysis temperature decreases the phytoavailability of Cd in biomass and then reduces the environmental risk of biochar (Fan et al., 2024). Also, the Cd concentration in the SB was lower than that in the heavily contaminated soil (NY/T 3499–2019, China). But the strict verification is required for the annual application amount, continuous application years and ecological risk assessment of SB-Cd (GB 4284–2018, China). With the development of science and technology, gene editing can be also used to knock out the Cd absorbing genes in mulberry, solving the problem of Cd pollution in sericulture products (Fan et al., 2022; Tang et al., 2022). 5) The Cd absorbed by mulberry trees is mainly accumulated in the roots, and is rarely found in leaves (Dai et al., 2020; Huang et al., 2018), making it easier for further safe utilization (Jiang et al., 2021a). So the ordinary supervision of the silkworm rearing mode is necessary, that is, safety evaluation of silk with the Technical specifications of ecological textiles (the extractable content, limited value, 0.1 mg/kg) (GB/T 18885–2020, China) and

pupae with the Food safety national standard food contaminant limit (limited value, 0.1 mg/kg) (GB 2762–2022, China) or the Hygienical standard for feeds (limited value, 2 mg/kg) (GB13078–2017, China) (Fig. 5).

4. Conclusions

This study aims to investigate the effects of silkworm excrement (SE) biochar (SB) (SB-Cd, SB-Cd free) on the chemical properties of paddy soils, the speciation of Cd in contaminated soils and Cd distribution in mulberry. Application of SB significantly increased the soil pH, enhanced the soil CEC but without significant effects on the soil OM. The SB-Cd all promoted plant growth, increased the mulberry plant height, the crude protein content and the chlorophyll content, reduced the total sugar content in mulberry leaves. The SB-Cd decreased the phytoavailability of Cd in soils and SB-Cd (3 %) played the best, thus reducing the absorption and accumulation of the Cd in mulberry. These findings suggest that SB-Cd may serve as an effective immobilization agent to stabilize the Cd in heavily polluted soil and the SB-Cd may be a reasonable disposal method for the SE contaminated with Cd. Additionally, pyrolysis technology is an important method for processing and utilizing agricultural residues from phytoremediation of heavy metals contaminated soils. Pyrolysis could reduce the volume of agricultural residues and stabilize the heavy metals in it, and then accelerate the application process of phytoremediation. The circular planting and breeding model of sericulture in Cd heavily polluted farmland was proposed, but further field experiments are needed to verify the attained results. Also, it is necessary to determine the maximum continuous usage of SB-Cd in paddy soils and further evaluate the environmental risk of Cd in SB.

Funding

This work was supported by National Natural Science Foundation of China (31901190) and the Hunan Provincial Natural Science Foundation of China (2020JJ5275).

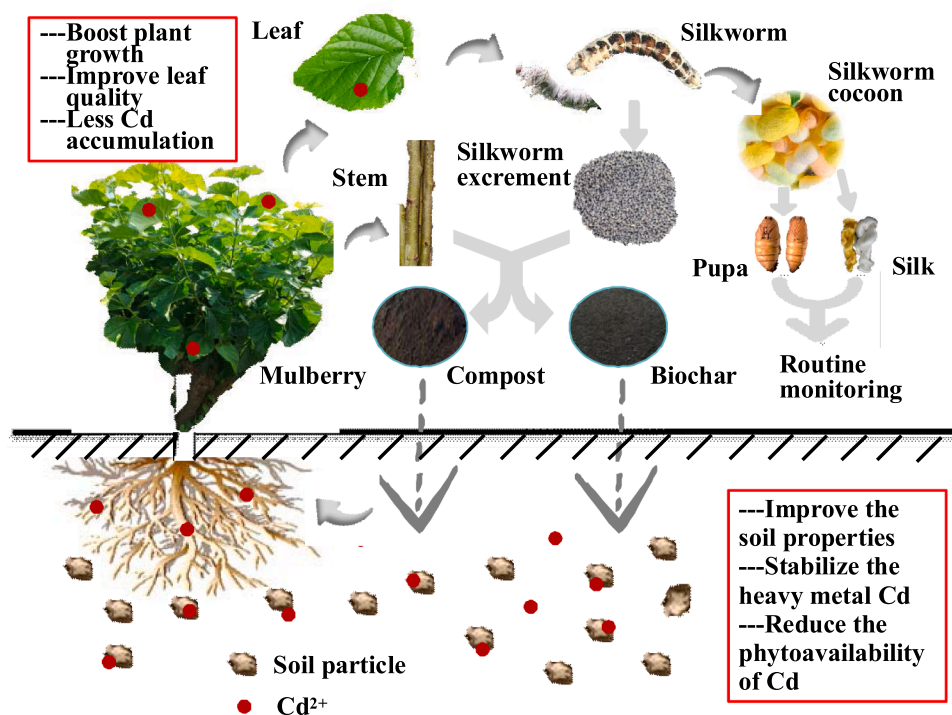


Fig. 5. The circulation model of mulberry planting and silkworm rearing in the Cd heavily contaminated paddy soils.

CRedit authorship contribution statement

Zhangbao Li: Methodology, Data curation. **Shimeng Jiang:** Methodology, Investigation, Data curation. **Xiyun Yang:** Writing – review & editing, Supervision. **Ming Wang:** Resources, Formal analysis. **Hui Cao:** Methodology, Investigation. **yongbing jiang:** Writing – original draft, Methodology, Funding acquisition.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors do not use the AI tools to analyse and draw insights from data as part of the research process.

Declaration of Competing Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Acknowledgements

We thank Professor Zhibin Hu (Hunan Agricultural University) and his student Haibo Sun for their help.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2024.117455](https://doi.org/10.1016/j.ecoenv.2024.117455).

Data Availability

Data will be made available on request.

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