Chapter 4

Effects of Biochar on Plant Growth and Cadmium Uptake: Case Studies on Asian Lotus (*Nelumbo nucifera*) and Chinese Sage (*Salvia miltiorrhiza*)

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Additional information is available at the end of the chapter

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Abstract

Application of biochar has many benefits in agriculture, to understand benefits of biochar in crop production and remediation of heavy metal pollution, Asian lotus (*Nelumbo nucifera*) as an aquatic crop and Chinese sage (*Salvia miltiorrhiza*) as a traditional medicinal herb were used to evaluate biochar's effects on plant growth and cadmium (Cd) accumulation in plants in the artificially Cd-polluted condition in containers. In both cases, adding biochar (4% to 32% in soil mix) significantly increased plant biomass. However, its impact on plant physiological traits were unclear. In Asian lotus, the Cd content in rhizomes, petioles, and leaves significantly increased by 69%, 81% and 55%, respectively as 32% biochar added. Meanwhile, a maximum reduction (71%) showed on bioaccumulation coefficient of Cd, and an up to 1.3 folds increase occurred on Cd transfer coefficient of underground to aboveground tissues, which indicated that biochar effectively prevented Cd uptake in major edible parts. In Chinese sage, adding 32% biochar significantly decreased Cd content in leaves and roots by 52.81% and 43.63%, respectively. Therefore, as a valuable soil amendment of improving plant growth and reducing heavy metal uptake, biochar has a huge potential in green agriculture production and remediation of heavy-metal polluted environment.

Keywords: biochar, heavy metal pollution, cadmium, *Nelumbo nucifera*, lotus, *Salvia miltiorrhiza*, Chinese sage, danshen, plant growth, vegetable, medicinal plant

1. Introduction

With the rapid development of urbanization, industry, and agriculture, heavy metal contamination in water, soil, and the atmosphere has been increasing. Heavy metals accumulate in

plants, animals, and human bodies, therefore, human health is exposed to a great potential threat through the food chain. Biochar is an emerging environment functional material and has been proven to be an efficient adsorbent for toxic organic pollutants and heavy metals [1–4]. With excellent physicochemical structure, biochar has great agricultural benefits and the potential for remediation of contaminated soils by improving soil physicochemical properties [5, 6], increasing microbial activity in soil [7, 8], reducing nutrient leaching [9], and enhancing nutrient availability [10], as well as directly or indirectly decreasing the contaminant bioavailability in water and soil. Additionally, biochar is a renewable material that is low cost, sustainable, and environment friendly. It could be an effective way of utilization of agricultural and forestry wastes and has a wide application in sustainable agriculture. Previous studies [1, 11] have shown that biochar improved plant growth and inhibited migration and phytotoxicity of heavy metals. But, it is yet unknown on how to apply biochar in polluted water and how much the accumulation of cadmium (Cd) could be reduced in major aquatic crops such as Asian lotus (*Nelumbo nucifera* Gaertn.). In addition, no data is available about the optimum amount of biochar applied in polluted environment in order to produce safe food and medicine from aquatic plants.

With increasing human health issues caused by heavy metal contamination, a strict standard on the maximum of heavy metal content has been widely established in food and medicinal products. The upper limit set by European Commission in Regulation No. 1881/2006 was at 0.10 and 0.20 mg/kg wet weight in stem vegetables and fresh herbs, respectively. The Chinese Ministry of Health released GB 2762-2012 (Limiting Chemical Contaminants in Food) with a Cd cutoff value of 0.1 and 0.2 mg/kg in root and leafy vegetables, respectively. Cd is one of the top five toxic heavy metals with strong chemical activity, high toxicity, and difficulty to be degraded. Its contamination in major crops such as rice has become a severe problem in China in recent years [12, 13], and excessive Cd content has limited the export of crops and Chinese medicines. Therefore, it is of great scientific value and practical significance to investigate food safety and pollution remediation in heavy metal-contaminated environment.

Asian lotus is widely grown in China, Japan, and other Asian countries as an important vegetable, medicinal, and ornamental plant. Nearly, all parts of lotus plant are edible or used for medicinal purpose [14]. Lotus deserves attention because of its economic potential and because its product quality and economic value are influenced by heavy metal pollution [15, 16]. *Salvia miltiorrhiza* Bunge (danshen in China, red sage or Chinese sage in other areas) has been widely used as one of the most important traditional Chinese herbs in China, Korea, Japan, and other Asian countries [17]. Its dried roots contain major active constituents including various kinds of tanshinones that pharmacologically function as treatment for ischemic cardiovascular diseases, microcirculatory disturbance-related diseases, and coronary disorders [17, 18]. Cd has strong chemical activity and potentially high toxicity that creates challenges in remediation. Contamination of Cd is one of the most serious issues because it is a major biohazard in China.

To investigate effects of biochar on the growth of Asian lotus and Chinese sage, we conducted a trial in artificially Cd-polluted condition in containers to evaluate the toxicity and

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accumulation of Cd in both aquatic and terrestrial plants. The results of this study may provide a useful reference on restoration of heavy metal-polluted environment and production of safe and organic agricultural products.

2. Material and methods

2.1. Site details

The experiment was conducted in a plastic tunnel house and greenhouse, respectively, which are located at Shanghai Chenshan Plant Science Research Center (31°04′37″N; 121°10′35″E), Chinese Academy of Sciences, and Chenshan Botanical Garden, Shanghai of China. The light intensity is 7000 lx (2800–9300 lx) for both tunnel house and greenhouse during daylight hours. The temperature at 28°C (22–42°C) and humidity at 50% (30–70%) are for tunnel house, and the temperature at 25°C (20–36°C) and humidity at 60% (40–80%) are for greenhouse throughout the experiment.

2.2. Plant material

N. *nucifera* 'Taikong Lian 36', one of the most important lotus cultivars in China, and Chinese sage (*S*. *miltiorrhiza* Bunge.), a medicinal plant, were used for experimental plants. The lotus seeds were obtained from Guangchang White Lotus Development Bureau, Jiangxi of China, and those of *S*. *miltiorrhiza* were obtained from Northwest Agriculture and Forestry University, Shanxi, China.

2.3. Trial design and treatments

Pinewood biochar from a local market was sieved to use pieces about 0.5–1.0 cm in size (**Table 1**). Natural soil (loam) was air-dried (**Table 2**) and blended to create a uniform mix after removing rocks, twigs, leaf litters, etc. After recording dry weight, the soil was moistened to 40% maximum field water capacity with deionized water. The biochar was then added and thoroughly mixed with the soil at differing amounts: 0, 4, 8, 16, and 32% biochar-soil (v/v), and labeled as CK (control), B0, B4, B8, B16, and B32, respectively. The lotus seeds germinated in small containers, and the seedlings with three to four floating leaves were transplanted in larger pots without drainage holes for experimental treatment in early June, one plant per pot and six replication. Forty days later, except CK, each pot was added by the solution of 1.09 mg $Cd(NO_3)_2 \cdot 4H_2O$ (3 mg/kg Cd). The pots were placed under a transparent plastic tunnel house to avoid rainfall and regularly watered by tap water to retain the starting water level. No fertilizer was applied throughout trial. For a trial on Chinese sage, the soil-biochar mixture was made same as that in lotus experiment. One month later, the dissolved $Cd(NO_3)_2 \cdot 4H_2O$ (3 mg/kg Cd) was added to soil-biochar mixture other than CK. The lotus seedlings with three to four leaves were transplanted to the experimental pots with saucers in June, one seedling each pot and six pots per treatment. Pots were randomly arranged. Throughout experiment, plants were not fertilized but watered regularly with tap water to maintain soil moisture. To

avoid possible leaching of heavy metal and other nutrients, the leachate from the pots was collected and later reapplied to the pots.

pH	Organic matter (cmol/kg)	Cation exchange capacity (cmol/kg)	Electrical conductivity (mS/cm)	Pore structure (%)	Cd content (mg/kg)
9.2	Immeasurable	23.16	0.69	Large 39%, small 44%	0.17

Table 1. Biochar properties.

Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	pH	Organic matter (cmol/kg)	CEC (cmol/kg)	Electrical conductivity (mS/cm)	Cd content (mg/kg)
5.44	0.65	16.4	6.08	82.46	22.79	0.33	0.38

Table 2. Soil properties.

2.4. Data collection

2.4.1. Plant growth indicators

In lotus experiment, leaf number, leaf area, and plant height (equal to the height of the tallest leaf) were recorded at 35, 75, and 115 d, respectively, after transplanting. Leaf diameter and plant height were measured by a ruler. Six mature emergent leaves were randomly selected from each of the three pots to record leaf diameter ([long + short length diameter]/2), and then leaf area (πR^2) was calculated. Plant height was measured from the top of the pot to the top of the highest leaf in each pot. Seventy days after adding Cd, all the aboveground parts and underground parts (rhizomes only) were separately harvested. After soil removal, the rhizomes were immersed in a 0.2 μm EDTA solution for 30 min, followed by air-drying for 12 h, and then the fresh mass was determined. Thereafter, the weighed rhizomes were immediately placed into envelopes for drying at 65°C for 84 h in an oven (DHG-9240A, Shanghai Yiheng Scientific Instruments Co., Ltd., China), and then the dry mass was recorded.

In Chinese sage experiment, leaf number and area were recorded at 10, 35, and 60 d, respectively, after transplanting. The leaf area of both the largest and smallest mature leaves in each pot was measured by a leaf area meter (YMJ-A, Zhejiang Top Instrument Co., Ltd) to get the average value for each treatment (six plants). Eighty days after transplanting, leaves with stems and roots were harvested separately. After soil removal, root fresh weight was determined before immersing roots in a 0.2 μm EDTA solution for 30 min followed by air-drying for 4 h. Roots were then placed into envelopes and dried at 65°C for 48 h in a drying oven. Finally, the root dry weight was measured.

2.4.2. Plant physiological indicators

All plant samples were collected with a hole puncher (1 cm diameter) at the same position of each fresh leaf. Water extract of leaves was used to measure cell membrane permeability

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by tissue fluid exosmosis conductance method (n = 3/treatment) using a conductivity meter (DDS-11AT, Shanghai, China) [19]. The relative chlorophyll content of fresh leaves was recorded by a handheld chlorophyll meter SPAD-502 Plus (Konica Minolta Sensing, Inc., Japan; n = 6), according to Xiong [19]. Following Xiong [19], the thiobarbituric acid method (n = 3) was used to determine methane dicarboxylic aldehyde (MDA) content in fresh leaves and the NBT photochemical reduction method to analyze superoxide dismutase (SOD) activity. At the same time as the chromogenic reaction, enzyme-extracted leaf liquid was collected and tested at 560 nm OD by an ELISA plate reader (Tecan M200 PRO) (n = 3) [19].

2.4.3. Cd content measurements

Cd contents in the rhizomes, roots, leaves, and petioles were determined by Graphite Furnace Atomic Absorption Spectrometry, according to the "Determination of Cadmium in Foods GB/T 5009.15-2003" [20]. Microwave digestion method was carried out with a PE Analyst AA800 (Perkin Elmer) (n = 3/treatment).

The bioconcentration factor (BCF) reflects the degree or capability of accumulation of heavy metals from soil or water by plants. The biological transfer factor (TF) refers to the transference of heavy metals in plants in vivo Zayed et al. [21]. BCF and TF were calculated by BCF = trace element concentration in plant tissues at harvest/initial concentration of the element in the external nutrient solution and TF = heavy metal content ratio (Cd in aboveground tissues/Cd in rhizomes or roots).

2.5. Data analysis

The averages were determined by Microsoft Excel 2007. The analysis of variance (ANOVA), Duncan's multiple range test, and regression analysis were conducted by statistical package for social scientists (SPSS 16.0). Statistical significance was set at a level of $\alpha = 0.05$.

3. Results

3.1. Biochar promoted the growth of Asian lotus and Chinese sage

3.1.1. Leaf growth

In lotus experiment, no apparent leaf toxicity was observed 5 days after adding Cd. Thirty-five days later, the number, height, diameter, and leaf area of emerging leaves of *N. nucifera* 'Taikong Lian 36' reached the highest in the treatments with 32% biochar (**Table 3**). But, the relationship was unclear between the number of floating leaves and biochar ratio. Forty days later, the same plant indexes in treatment H0 were the lowest, only 32, 39, 59, and 29% of that in treatment H32, respectively. However, differences were not significant between H0 and the control. Therefore, Cd of low concentration could not cause strong toxicity. The relationships between biochar and the number, height, diameter, and leaf area of emerging leaves in lotus could be reflected by (1) $y = -0.03\ x^2 + 1.8\ x + 10.87$ ($R^2 = 0.955$, $P = 0.037$), (2) $y = -0.005\ x^2 + 1.042\ x + 27.047$ ($R^2 = 0.830$, $P = 0.170$), (3) $y = -0.002\ x^2 + 0.231\ x + 13.117$ ($R^2 = 0.836$, $P = 0.164$), and (4) $y = -0.000\ x^2 + 0.06\ x + 0.432$ ($R^2 = 0.962$, $P = 0.037$), respectively. It indicated that the growth of lotus was

Biochar rate (%)	Floating leaf number			Emerging leaf number			Emerging leaf height (cm)			Leaf diameter (cm)			Leaf area (m^2)		
	35 d with biochar	75 d with biochar and Cd	115 d with biochar and Cd	35 d with biochar	75 d with biochar and Cd	115 d with biochar and Cd	35 d with biochar	75 d with biochar and Cd	115 d with biochar and Cd	35 d with biochar	75 d with biochar and Cd	115 d with biochar and Cd	35 d with biochar	75 d with biochar and Cd	115 d with biochar and Cd
CK	8.2 ± 4.4 a	9.5 ± 5.5 a	11.5 ± 4.9 a	6.3 ± 3.7 b	7.5 ± 4.4 b	11.7 ± 6.2 b	8.4 ± 2.5 d	9.0 ± 5.0 c	21.8 ± 10.7 c	6.7 ± 0.7 d	9.2 ± 1.4 c	11.0 ± 1.4 c	0.1 ± 0.0 c	0.1 ± 0.1 c	0.2 ± 0.1 d
H0	7.20 ± 2.1 a	7.8 ± 2.5 a	10.3 ± 2.4 a	5.5 ± 3.4 b	5.8 ± 6.1 b	10.8 ± 11.7 b	8.5 ± 3.6 d	9.0 ± 6.6 c	22.3 ± 10.4 c	7.0 ± 1.1 d	9.4 ± 0.8 c	12.1 ± 2.5 c	0.1 ± 0.0 c	0.1 ± 0.1 c	0.4 ± 0.5 c
H4	7.7 ± 2.3 a	9.0 ± 2.7 a	11.3 ± 2.3 a	8.3 ± 2.6 b	9.8 ± 3.0 b	19.5 ± 6.1 b	16.7 ± 4.3 bc	21.2 ± 7.6 bc	39.5 ± 8.2 b	7.9 ± 0.9 cd	11.3 ± 1.4 bc	15.4 ± 3.1 b	0.1 ± 0.0 c	0.2 ± 0.1 c	0.9 ± 0.4 b
H8	10.5 ± 1.4 a	12.0 ± 3.6 a	15.0 ± 3.3 a	9.2 ± 3.1 b	11.5 ± 5.5 b	19.8 ± 11.9 b	14.5 ± 6.6 cd	17.0 ± 8.7 bc	33.0 ± 18.2 bc	8.4 ± 0.8 bc	11.5 ± 2.7 bc	15.1 ± 3.9 b	0.1 ± 0.1 c	0.3 ± 0.1 c	0.7 ± 0.3 b
H16	10.5 ± 1.6 a	12.5 ± 5.5 a	14.7 ± 5.1 a	15.5 ± 7.3 a	18.8 ± 9.6 a	32.8 ± 8.0 a	22.7 ± 6.4 b	25.3 ± 7.7 b	40.3 ± 12.1 b	9.3 ± 1.3 b	12.9 ± 1.4 b	15.5 ± 1.4 b	0.3 ± 0.1 b	0.5 ± 0.3 b	1.4 ± 0.5 a
H32	7.7 ± 4.3 a	8.0 ± 4.6 a	11.8 ± 3.2 a	20.0 ± 5.8 a	21.3 ± 6.1 a	34.3 ± 9.1 a	34.7 ± 8.4 a	39.0 ± 10.4 a	55.8 ± 5.4 a	11.2 ± 1.5 a	15.2 ± 2.6 a	18.7 ± 1.4 a	0.4 ± 0.2 a	0.7 ± 0.2 a	2.2 ± 0.6 a

CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters in the same column indicate significant difference ($P < 0.05$) among treatments.

Table 3. Effects of biochar on leaf growth of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water.

significantly promoted by biochar added at 16–32% (with Cd added). The higher ratios of biochar promoted more leaf growth but also increased the cost (biochar cost).

In the experiment on Chinese sage, morphological leaf toxicity did not occur 10 d after adding Cd and biochar treatments. Both leaf number and area increased at 35 d after treatment and reached the highest value in the treatments with the highest biochar proportion (B32) at 60 d for the final measurement (**Table 4**). At 60 d, the leaf number of B4, B8, B16, and B32 increased 26.2, 30.9, 37.8, and 42.9%, respectively, while that of CK and B0 increased 19.4 and 26.0%, respectively, compared with those at 10 d. Likewise, leaf area of B4, B8, B16, and B32 increased 36.3, 50.9, 70.7, and 69.9%, respectively, while that of CK and B0 just increased 28.3 and 31.0%, respectively. Therefore, leaf growth of *S. miltiorrhiza* was significantly promoted with the addition of 16–32% biochar (with Cd added). Lower concentrations of Cd did not cause strong toxicity problems but resulted in the smaller leaf size and slower growth of plants.

3.1.2. Plant biomass

Biomass discrepancy is the most direct response to various environmental stimuli for plants. In lotus, our study showed that the total fresh weight, the fresh weight of the aboveground parts, and the fresh and dry weight of rhizomes increased by adding biochar (**Figure 1**). All the highest values of the tested plant growth indexes occurred in treatment H32. Among which, the highest total fresh weight was 4.5 times greater than H0. The fresh weight of underground parts made the greatest contribution to the total fresh weight, which correlated significantly with the proportion of biochar, and the maximum was 6.25 times greater than control values.

In Chinese sage, the total fresh weight, the leaf fresh weight, and the fresh and dry weight of roots increased with the addition of biochar in our study (**Figure 2**). All the highest values of the tested plant growth indices occurred in treatment B32 where the highest total fresh weight was 1.3 times of that in treatment B0. The fresh weight of roots made a greater contribution to

Biochar rate/%	Leaf number			Leaf area (cm^2)		
	10 d	35 d	60 d	10 d	35 d	60 d
CK	19.3 ± 3.2 b	21.8 ± 3.2 c	24.3 ± 4.0 b	37.8 ± 6.7 a	46.7 ± 4.00 ab	48.5 ± 5.6 bc
B0	24.5 ± 3.1 a	26.3 ± 2.9 ab	29.3 ± 7.3 ab	31.5 ± 4.3 a	39.8 ± 3.7 bc	41.3 ± 4.0 c
B4	21.0 ± 1.6 ab	23.5 ± 2.9 bc	26.5 ± 3.4 b	32.2 ± 4.8 a	38.6 ± 5.2 c	43.9 ± 6.4 bc
B8	23.5 ± 1.0 a	27.5 ± 2.1 a	30.8 ± 2.2 ab	33.9 ± 2.7 a	38.8 ± 7.2 c	51.2 ± 4.5 ab
B16	24.5 ± 1.9 a	30.0 ± 2.5 a	33.8 ± 1.5 a	35.1 ± 4.2 a	45.8 ± 4.2 bc	60.0 ± 3.8 a
B32	24.5 ± 1.0 a	29.3 ± 1.0 a	35.0 ± 2.7 a	34.3 ± 4.5 a	52.0 ± 1.2 a	58.1 ± 4.1 a

CK indicates no biochar and no Cd in the soil; B0, B4, B8, B16, and B32 indicate adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters in the same column indicate significant differences ($P < 0.05$) among treatments.

Table 4. Effects of biochar on leaf growth of *S. miltiorrhiza* in Cd-polluted soil ($\bar{x} \pm s$).

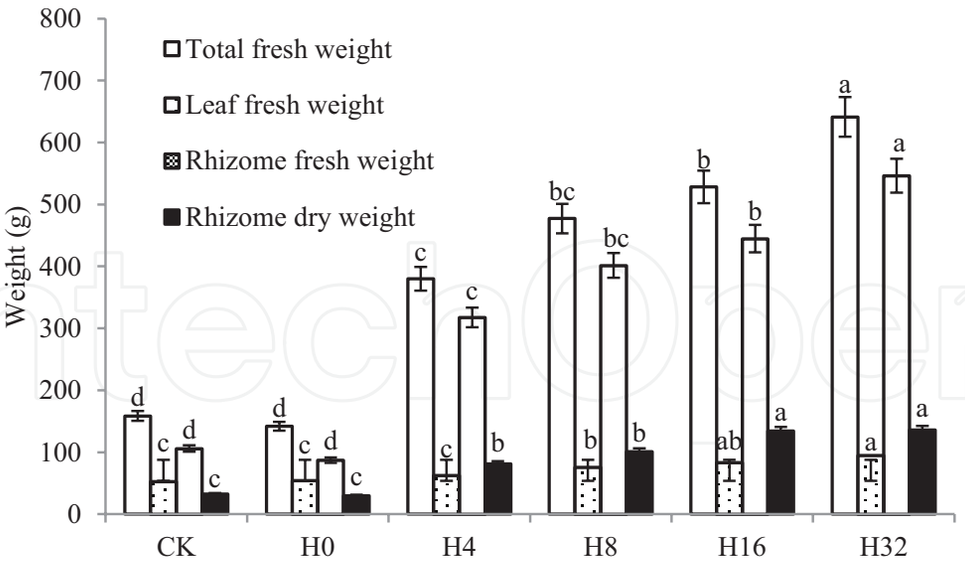

Figure 1. Effects of biochar on plant weight of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water. CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant difference ($P < 0.05$) among treatments.

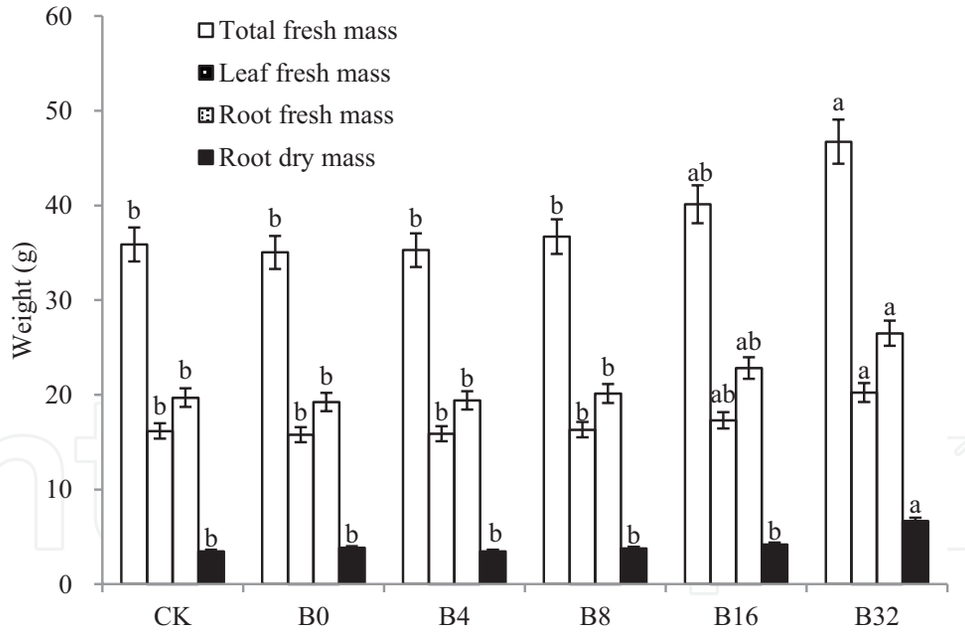

Figure 2. Effects of biochar on mass of *S. miltiorrhiza* in Cd-polluted soil. CK indicates no biochar and no Cd in soil; B0, B4, B8, B16, and B32 indicate adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments.

the total fresh weight than that of the leaves and stems, and it correlated significantly with the biochar proportion. The maximum root fresh and dry weight observed in B32 was 1.4 times of that in the control, which further verified that biochar promoted root growth.

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3.2. Effects of biochar on physiological status

3.2.1. Chlorophyll content

The relative content of chlorophyll can be used to evaluate plant growth, physiological changes, and nitrogen nutrition. Three consecutive measurements on leaf chlorophyll were taken by SPAD at 20, 50, and 80 d after adding biochar (**Table 5**). The relative chlorophyll contents in leaves of treated plants were significantly higher than those in the control, but no significant difference was found between biochar treatments. The highest values in each treatment occurred at 10 d after adding Cd when the leaf growth reached its peak. The highest chlorophyll content (82%) in H0, followed by 62% in H16, could be explained by that Cd did not reach a toxic stress concentration affecting the enzyme activity relevant to chlorophyll formation. On the contrary, low concentration of Cd promoted chlorophyll synthesis. Biochar increased the relative chlorophyll content of lotus leaf. The chlorophyll formation was significantly promoted at 20 and 50 d after biochar being added. Fifty days later, it decreased slowly as plants started senescence.

In Chinese sage, the relative chlorophyll content was tested at 10, 35, and 60 d, respectively, after transplanting (**Table 6**). Relative chlorophyll contents in the leaves of treated plants were higher than those in the control, but no difference was found between the treatments of biochar ratios. The highest chlorophyll content in all treatments occurred at 35 d when the leaves were at the peak stage of growth. The highest increase rate of chlorophyll content from 10 to 35 d was in treatment B32 (42%), indicating that Cd might affect the enzyme activity relevant to chlorophyll synthesis, and it stimulated the chlorophyll formation at a certain concentration. Sixty days after biochar treatment, the relative chlorophyll content began to decrease slowly as the plants gradually senesced.

3.2.2. Cell membrane permeability

In both lotus and Chinese sage, the maximum of cell membrane conductivity occurred in treatment H0 and was significantly different from the control and biochar treatments (**Figure 3A** and **B**). This

Biochar rate (%)	Relative chlorophyll content (20 d with biochar)	Relative chlorophyll content (50 d with biochar)	Relative chlorophyll content (80 d with biochar)
CK	12.7 ± 0.8 ab	20.0 ± 2.1ab	15.8 ± 0.5c
H0	11.8 ± 2.0a	21.4 ± 0.9b	16.0 ± 1.4abc
H4	14.2 ± 0.9ab	22.0 ± 1.6a	14.9 ± 0.7 ab
H8	13.7 ± 0.8 ab	21.6 ± 1.3a	15.9 ± 0.8 abc
H16	14.1 ± 0.8 ab	22.7 ± 0.5 a	15.3 ± 0.9 a
H32	13.1 ± 1.8b	20.8 ± 1.6ab	14.8 ± 0.27 bc

CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16 and 32% of biochar in soil, respectively. Different lower-case letters in the same column indicate significant difference ($P < 0.05$) among treatments.

Table 5. Effects of biochar on relative chlorophyll content of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water.

Biochar rate (%)	Relative chlorophyll content		
	10 d	35 d	60 d
CK	16.9 ± 0.2 a	22.1 ± 0.4 a	14.5 ± 0.4 ab
B0	16.6 ± 0.8 ab	22.5 ± 0.8 a	13.0 ± 0.5 ab
B4	16.0 ± 1.55 b	22.1 ± 0.7 a	12.4 ± 0.9 bc
B8	16.5 ± 0.8 ab	22.9 ± 1.2 a	12.0 ± 0.9 c
B16	16.2 ± 0.5 ab	22.3 ± 1.3 a	12.3 ± 0.9 bc
B32	16.2 ± 0.5 ab	23.0 ± 0.6 a	13.6 ± 0.4 a

CK indicates no biochar and no Cd in soil; B0, B4, B8, B16, and B32 indicate adding 0, 4, 8, 16, and 32% of biochar in soil, respectively; and different lower-case letters in the same column indicate significant differences ($P < 0.05$) among treatments.

Table 6. Effects of biochar on relative chlorophyll content of *S. miltiorrhiza* in Cd-polluted soil.

demonstrated that 3 mg/kg of Cd stressed the plants resulting in an increase of membrane permeability. Our data showed that adding biochar could reduce cell membrane conductivity, but no change trend is related to the biochar ratio.

3.2.3. MDA content

Figure 4 showed the effects of different proportions of biochar on MDA content of *N. nucifera* 'Taikong Lian 36' grown for 20 d in Cd-polluted water and *S. miltiorrhiza* grown for 30 d in Cd-polluted soil. The highest MDA content was seen in the treatment H8 and the lowest in H32 for lotus (**Figure 4A**). However, no significant differences in MDA content were found between treatments where both Cd and biochar were added, which indicated that the amount of Cd did not produce a great impact on the cell membrane lipid peroxidation. The other possible reason was that the physiological metabolic activity of *N. nucifera* 'Taikong Lian 36' was disturbed by Cd, leading to the accumulation of oxidation product MDA, while the MDA accumulation was inhibited by biochar, which alleviated the damage of the membrane system caused by lipid membrane peroxidation.

In Chinese sage, the highest MDA content was seen in CK (**Figure 4B**), which indicated that the amount of Cd did produce some impact on the lipid peroxidation of cell membranes. Less MDA content occurred in biochar treatments, and the lowest was seen in B16, although no differences were found among the treatments with both Cd and biochar. The possible reason for no difference in treatments might be that the physiological metabolic activity was disturbed by the MDA accumulation due to Cd addition, while the MDA accumulation was inhibited by biochar, which alleviated the damage of the membrane system caused by lipid membrane peroxidation.

3.2.4. SOD activity

When both *N. nucifera* 'Taikong Lian 36' and *S. miltiorrhiza* grew for 30 d in Cd-contaminated environment, the effects of biochar on SOD activity in fresh leaves reached a significant level

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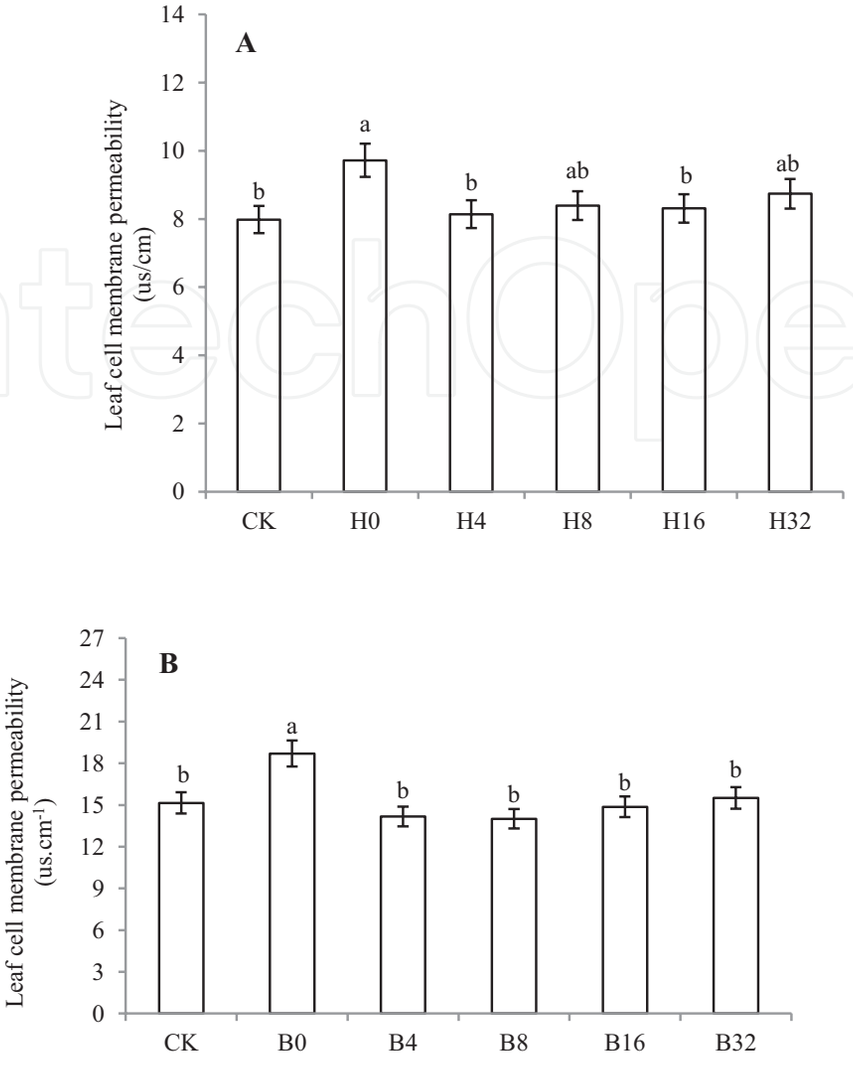

Figure 3. Effects of biochar on leaf cell membrane permeability of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water (A) and *S. miltiorrhiza* in Cd-polluted soil (B). CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant difference ($P < 0.05$) among treatments.

(**Figure 5**). The highest SOD activity appeared in treatment H0, which indicated that Cd significantly enhanced the SOD activity of plants. However, after adding biochar, SOD activity declined significantly, with the highest decrease by 53% in treatment H8 for lotus (**Figure 5A**) and by 29% in treatment B16 for Chinese sage (**Figure 5B**), comparing to the control H0, though a decrease trend was inconsistent with the proportion of biochar.

3.3. Cd content

The Cd accumulation in plants clearly increased after adding Cd but significantly decreased when biochar is added (**Figures 6** and **7**). For *N. nucifera* 'Taikong Lian 36', the Cd content in rhizomes, petioles, and leaves increased by 12.81, 17.35, and 7.56 folds, respectively, in treatment H0, compared with the control (**Figure 6**). With increasing biochar, Cd in plant tissue decreased correspondingly. In H32 treatment, the Cd content in rhizomes was 0.125 mg/kg,

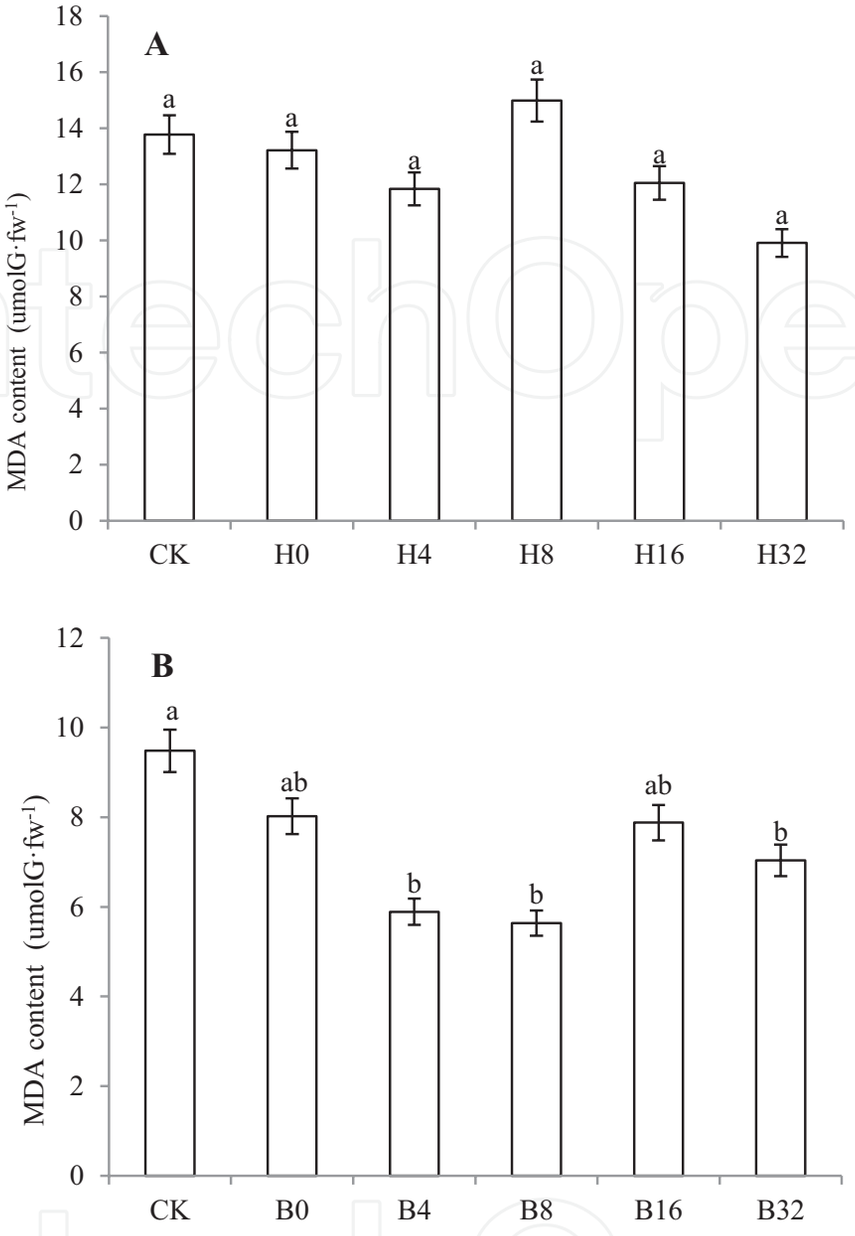

Figure 4. Effects of biochar on MDA content in leaf of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water (A) and *S. miltiorrhiza* in Cd-polluted soil (B). CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant difference ($P < 0.05$) among treatments.

only 68.51% of that in H0. It was more reduced in petioles from 0.694 to 0.129 mg/kg, a decrease of 81.41%. The lowest Cd content in leaves was 0.209 mg/kg in H32 and decreased by 54.66% of the highest in H0. However, the Cd content in all evaluated plant parts remained above the limit set by the European Commission regulations.

The relationship for the proportion of biochar (x) relative to Cd content (y) in rhizomes could be reflected by (5) $y = -0.0002\ x^3 + 0.008\ x^2 - 0.075\ x + 0.3999$ ($R^2 = 0.999$, $P = 0.351$). Based on this relationship, a higher level of biochar possibly further reduces Cd uptake and makes the

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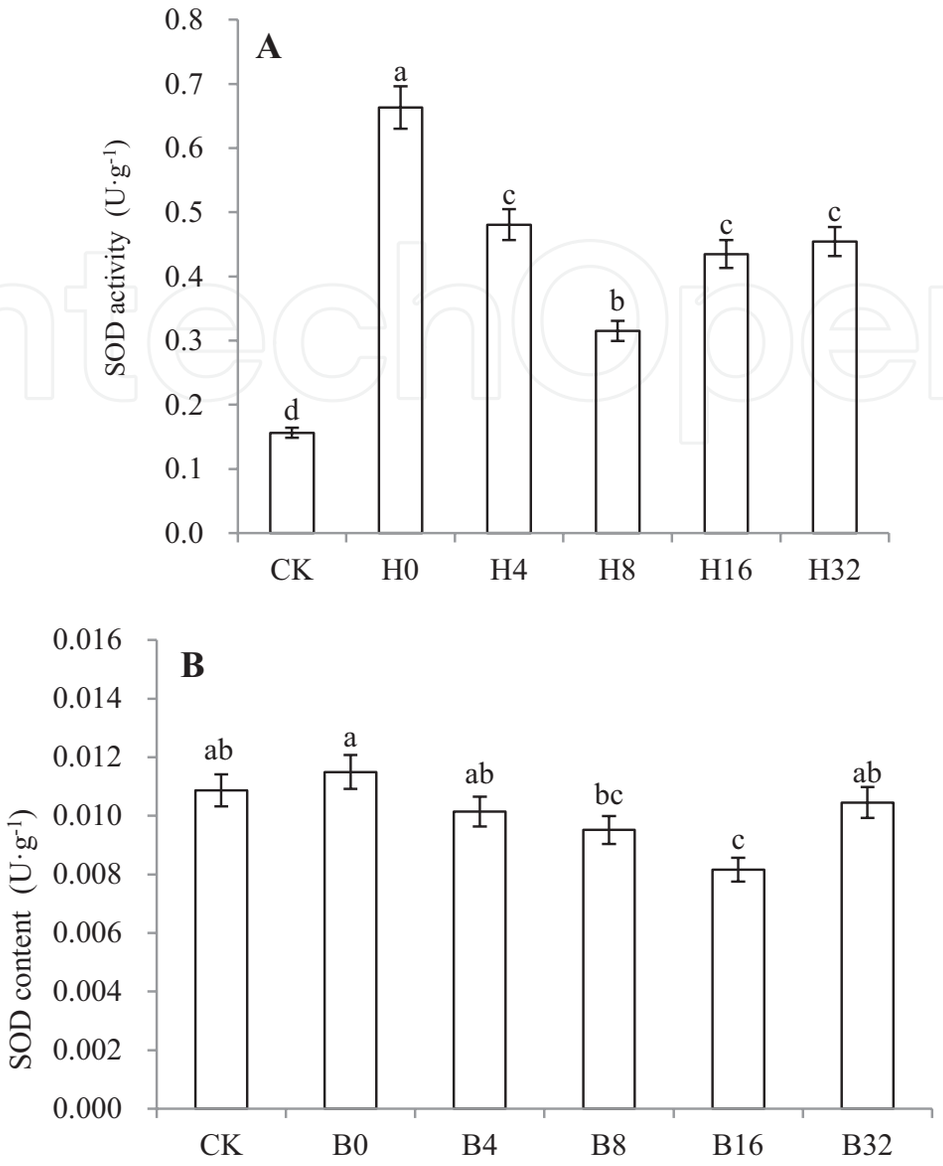

Figure 5. Effects of biochar on SOD activity in the leaf of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water (A) and *S. miltiorrhiza* in Cd-polluted soil (B). CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant difference ($P < 0.05$) among treatments.

rhizomes of lotus grown in Cd contaminated condition meet food safe standards. The relationship between the proportion of biochar (x) and Cd content (y) in petioles and leaves could be presented by (6) $y = -0.0003\ x^3 + 0.012\ x^2 - 0.142\ x + 0.674$ ($R^2 = 0.965$, $P = 0.238$) and (7) $y = -0.00004\ x^3 + 0.002\ x^2 - 0.047\ x + 0.489$ ($R^2 = 0.793$, $P = 0.559$), respectively.

In Chinese sage, Cd content in roots and leaves was 0.57 and 0.18 mg/kg, respectively, in treatment B0 and increased to 2.2 and 2.0 folds compared with the control (**Figure 7**). However, with increasing biochar, Cd content in plant tissues decreased correspondingly. In roots, Cd content was the lowest 0.25 mg/kg in B32, only 43.6% of that in B0. It was much lower in leaves

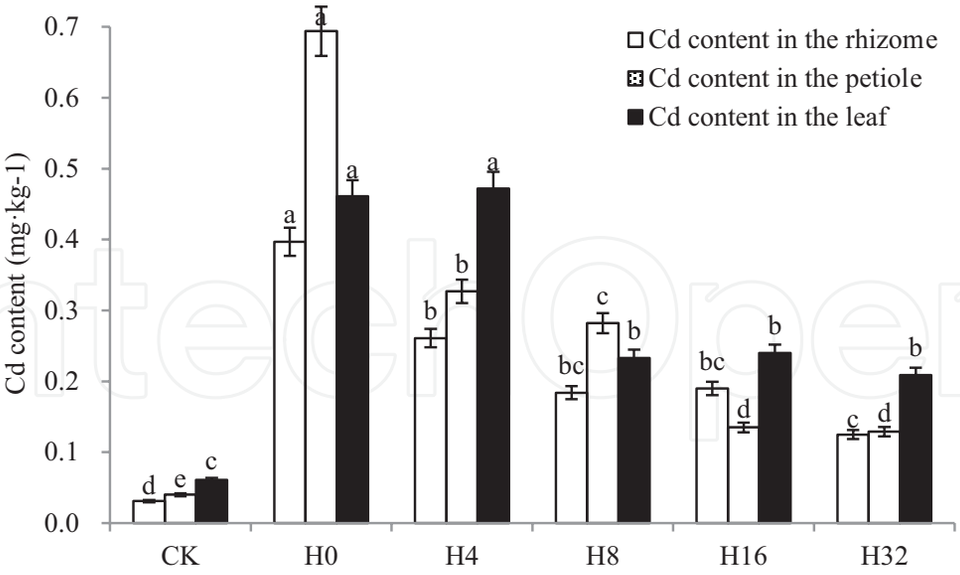

Figure 6. Effects of biochar on Cd content in the root, petiole, and leaf of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water. CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant difference ($P < 0.05$) among treatments.

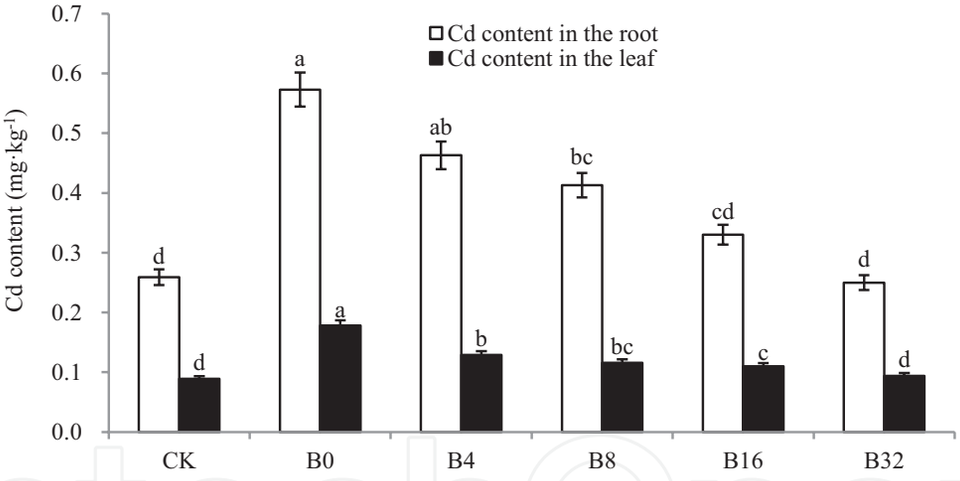

Figure 7. Effects of biochar on Cd content in the root and leaf of *S. miltiorrhiza* in Cd-polluted soil. CK indicates no biochar and no Cd in soil; B0, B4, B8, B16, and B32 indicate adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant differences ($P < 0.05$) among treatments.

in the same treatment, down to 0.09 mg/kg, a decrease of 52.8% of the highest in B0, and was below the upper limit value set by the *Chinese Pharmacopoeia* [22].

The regression equations for the proportion of biochar (x) related to Cd content (y) in roots and leaves, respectively, were

$$y = -0.00007x^3 + 0.003x^2 - 0.046x + 0.067 \ (R^2 = 0.997,\ P = 0.067) \quad (8)$$

$$y = -0.00007x^3 + 0.002x^2 - 0.023x + 0.177 \ (R^2 = 0.991,\ P = 0.119) \quad (9)$$

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The increasing proportion of biochar in 3 mg/kg Cd-contaminated planting mix likely further prevents Cd uptake to make the roots (a major medicinal part) meet safe standards of Chinese medicine.

3.4. Cd BCF and S/R

The BCF shows the capacity of plants to adsorb heavy metals. In lotus case, the BCF was the lowest in the rhizomes of the control, only 7.6% of the highest value of H0 (**Figure 8**). However, after adding biochar, the BCF decreased significantly, with the lowest (0.042) in H32, about one-third of that in the control. The highest BCF (0.385) of the above-ground parts occurred in H0 and was 11 times greater than that in the control. Likewise, the above-ground BCF was reduced significantly, with the biggest decrease seen in H32, down to 70.65% of that in the control. The petiole's BCF was significantly less than that of the leaves.

The transfer coefficient from roots to shoots (S/R) showed that Cd was transported to different organs of plants. The S/R rose with the increasing biochar proportions and the highest value (3.06) was observed in H32. The increase of S/R was not significant, but it indicated that biochar could affect Cd transference and reduce its accumulation in rhizomes.

In Chinese sage, the highest BCF (0.19) of the roots occurred in B0, 2.2 times that of the control (**Figure 9**), indicating that roots were able to accumulate Cd. However, after adding biochar, the BCF of both root and leaf decreased with the rate of biochar. The lowest root BCF (0.08) in treatment B32 was only 43% of the highest observed in B0. Likewise, the lowest BCF of leaf

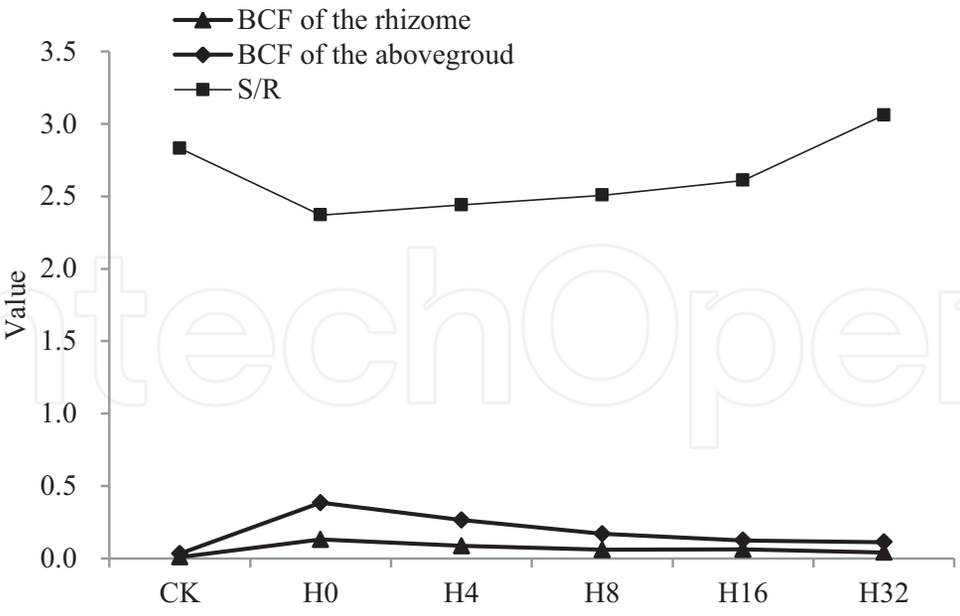

Figure 8. Effects of biochar on Cd bioconcentration factor (BCF) and biological transfer coefficient (S/R) of *N. nucifera* 'Taikong Lian 36' in Cd-polluted water. CK refers to none of biochar and Cd added to the soil; H0, H4, H8, H16, and H32 refer to adding 0, 4, 8, 16, and 32% of biochar in soil, respectively. Different lower-case letters indicate significant difference ($P < 0.05$) among treatments.

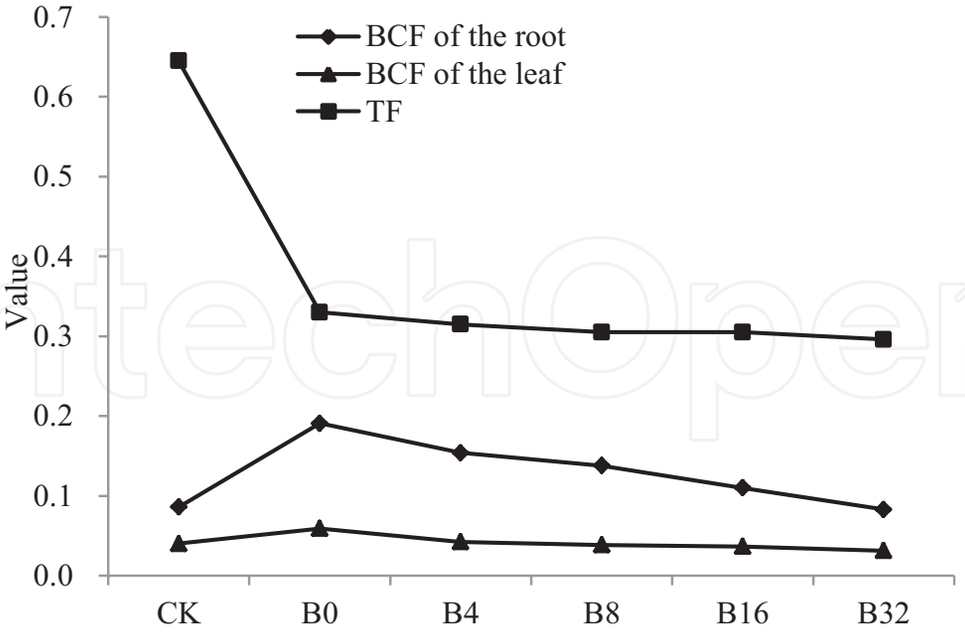

Figure 9. Effects of biochar on Cd bioconcentration factor (BCF) and biological transfer factor (TF) of *S. miltiorrhiza* in Cd-polluted soil. CK refers to no biochar and no Cd in soil; B0, B4, B8, B16, and B32 indicate adding 0, 4, 8, 16, and 32% of biochar in soil, respectively.

also occurred in B32, 77.5% of the highest in B0. It is possibly due to the capacity of biochar to inhibit absorption of Cd in plants.

The transfer factor (TF) from roots to leaves showed that Cd can be absorbed from soil by *S. miltiorrhiza* and transported among different plant organs. The highest TF was observed in the control (0.65). TF decreased with the addition of Cd. This could be explained by Cd potentially inhibiting growth and metabolism of *S. miltiorrhiza*, therefore slowing the absorbed Cd from the roots. With the increasing biochar proportion, TF decreased correspondingly. The lowest TF was 0.3 in B32, less than half of that in the control, indicating that biochar could affect the transfer of Cd from roots to leaves.

4. Discussion

4.1. Effects of biochar on plant growth

In this study, 3-mg/kg Cd added to the soil or soil-water mix did not elicit overt symptoms of heavy metal poisoning such as leaf chlorosis or stunting in lotus but resulted in treated plants being stunted and undersized in Chinese sage when compared to CK and biochar treatments. The lack of observed toxicity in *N. nucifera* may be due to its strong resistance of the polluted environments, such as in a Cd-polluted case study [23]. For both cases of our study, the leaf growth, total fresh weight, aboveground fresh weight, and fresh and dry weight of underground parts of plants increased with biochar ratio, and nearly all the highest values appeared in the treatment with the highest amount of biochar. Therefore, biochar can

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promote plant growth, which has also been reported in the previous studies [7, 24]. Because no fertilizer was applied after adding biochar, the biomass increase suggests that biochar might work as a fertilizer [25] or fertilizer enhancer [24], by ameliorating soil pH, CEC, base saturation, and other physicochemical properties, by affecting soil nutrient availability, such as the effectiveness of K, Ca, Na, Mg, N, P, and other elements [26], or by increasing the rhizosphere and soil nitrogen retention indirectly [27]. Yet some studies also showed that biochar had no beneficial effect on plant biomass, which is related to the type of soil and biochar, fertility levels, and other relevant experimental conditions [5, 28, 29]. Although Cd inhibited the underground growth of lotus, adding biochar offsets the stress of Cd and significantly promoted the rhizome enlargement and biomass increase. The 16–32% of biochar added in soil was more beneficial for growth of both lotus and Chinese sage. However, more biochar addition will increase production cost.

4.2. Effects of biochar on physiological status

Cell membrane permeability, relative chlorophyll content, MDA, and SOD served as markers to represent plant reactions under stress conditions. Our study showed that Cd added to water or soil produced a stress on cell membrane structure of plant, but no significant difference occurred among treatments with different proportions of biochar. Therefore, no optimal biochar level could be determined based on this point. Possibly, the beneficial effects of biochar on membrane permeability were also affected by other environmental factors, such as pH, EC, etc., making the membrane permeability change irregularly. The chlorophyll content in plant leaves increased with biochar amount in all treatments, which coincided with the previous report, in which low Cd concentration facilitated chlorophyll formation and high Cd concentration reduced chlorophyll content [23, 30]. This phenomenon also occurred in other heavy metal study cases [31]. In the early period of plant growth, promotion effect of biochar on chlorophyll was not evident. However, leaf chlorophyll content increased in the middle period, followed by a relatively big decrease in the latter. Therefore, biochar promoted plant growth and advanced plant senescence simultaneously. In lotus case, after adding Cd and biochar, the MDA contents showed no difference among treatments because low Cd concentrations did not increase cell membrane peroxidation. Therefore, a mitigating effect of biochar could not be determined. But, in the case of Chinese sage, the significant differences showed in MDA content between treatments with and without biochar, which suggested that biochar alleviated cell membrane peroxidation caused by Cd. In both cases, SOD activity significantly increased by adding Cd, indicating that Cd stimulated the physiological defense mechanisms and physiological resistance activities and improved activity of antioxidant and its enzyme [30, 32]. However, the addition of biochar significantly decreased SOD activity, which suggested that biochar can ameliorate Cd hazards.

4.3. Effects of biochar on Cd content in plants

Cd accumulation was observed in the organs and tissues of *N. nucifera* 'Taikong Lian 36' and *S. miltiorrhiza* in artificially polluted environment, and the accumulative concentrations increased more than 10 folds in some treatments when compared with the control. The uptake of Cd was

significantly reduced by adding biochar in all treatments, but the maximum effect was observed in H32. The Cd content in roots of *S. miltiorrhiza* could be reduced to meet the maximum allowed value set by the *Chinese Pharmacopoeia* [22]. In both cases, Cd content in plants could possibly be further decreased with more biochar added, extrapolating from the regression equation of biochar and Cd content. The role of biochar on reducing the heavy metal accumulation in plants has been demonstrated in the previous reports [33, 34]. It might be related to huge specific surface area and strong adsorption functions of biochar to form insoluble chelates or cause Cd precipitation. Biochar reduced the bioavailability of Cd in soil or water, by affecting pH, CEC (cation exchange capacity), water-holding capacity, and other physicochemical properties of the soil. Although the Cd-contaminated lotus rhizomes in our study did not meet the edible standard, the regression equation of biochar and Cd content indicated that it was possible to further reduce the Cd content to the safe range if more biochar would be applied or the environment is less polluted. Combined with Cd contents in different organs of *N. nucifera* 'Taikong Lian 36', all the lowest BCFs in various organs occurred in the controls, while the highest in treatments H0. In Chinese sage, BCFs of the root and leaf decreased with the addition of biochar indicate that besides its strong ability of adsorbing Cd, biochar also reduced the bioavailability of heavy metals or immobilized heavy metals by changing the physicochemical property of soil [35]. The highest S/R values were observed in the treatment with 32% biochar (H32), and the lowest showed in the treatment H0. Based on the values of Cd content, Cd BCF, and S/R, 32% of biochar was the optimum amount for applying to demonstrate biochar's potential in plant growth promotion, ventilation and water retention, fertilizer efficiency, and pollution reduction.

5. Conclusion

In this study, the effects of biochar were evaluated on edible and medicinal plants. The major goals were to understand growth-promoting effects of biochar and investigate how biochar alleviates the toxicity and accumulation of Cd in artificially Cd-amended pot culture. The results showed that the contents of Cd in rhizomes, petioles, and leaves of *N. nucifera* 'Taikong Lian 36' and in roots, leaves, and stems of *S. miltiorrhiza* were significantly reduced and the transference of Cd in plants was inhibited by adding biochar. Although the impacts of biochar on plant physiological indicators were not very clear, biochar not only promoted plant growth but also alleviated toxic effects of Cd in Cd-contaminated environment. Therefore, as a valuable soil amendment, biochar has a huge potential in green agriculture production and remediation of heavy metal-polluted water or soil due to its positive effects of improving plant growth and reducing heavy metal uptake. Further studies are needed to evaluate effects of biochar on safe production of lotus in the field and other important vegetables, fruit trees, and Chinese medicinal plants and to establish the optimum of physiologically and economically feasible amount of biochar to add for environmental remediation.

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