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Effect of Biochar on the Growth, Photosynthesis, Antioxidant System and Cadmium Content of *Mentha piperita* 'Chocolate' and *Mentha spicata* in Cadmium-Contaminated Soil

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Abstract: Cadmium (Cd) is a common heavy metal contaminant which seriously affects plant growth and environmental safety. Biochar, as an organic soil amendment, has been shown to effectively mitigate Cd damage to plants. To study the effectiveness of biochar on mitigating Cd stress, *Mentha piperita* 'chocolate' and *Mentha spicata* were used in a pot experiment of Cd stress with a $CdCl_2$ solution (10 mg Kg^{-1}), while a biochar suspension (0, 40, 80, and 160 g Kg^{-1}) was applied to the soil. The effects of Cd on the growth, physiological and biochemical properties, and Cd content in plant tissues of both mint species were found to be significant. The application of 40 g Kg^{-1}, 80 g Kg^{-1}, 160 g Kg^{-1} biochar significantly alleviated Cd damage to both mint species, increased plant height, leaf length, leaf width, biomass, photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content, and decreased antioxidant enzyme activities (including superoxide dismutase, catalase, peroxidase, and polyphenol oxidase) and non-enzymatic antioxidant content (including flavonoids and total phenols). Biochar effectively reduced the Cd uptake by plants and decreased the migration and transformation capacity of Cd in the soil–plant system. In addition, the available nitrogen (available N), available phosphorus (available P), available potassium (available K), and pH in the soil increased after biochar application compared to non-biochar amended soil. The addition of 160 g Kg^{-1} biochar was shown to have the best performance of the application rates in this experiment and may be considered as an effective way to reduce the damage caused by Cd contamination to *M. piperita* 'chocolate' and *M. spicata*.

Keywords: biochar; cadmium; heavy metal pollution; mint

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1. Introduction

Heavy metals can lead to the deterioration of soil structure and the loss of vegetation cover and microbial diversity and can cause significant public health problems [1]. Industrial waste discharge, sewage irrigation, atmospheric deposition, and long-term application of chemical fertilizers have led to a year-on-year increase in heavy metal content in agricultural soils and other increasingly serious environmental pollution problems [2]. Among the various heavy metal pollutants, Cadmium (Cd) is one of the most toxic pollutants. It is the most potentially dangerous metal pollutant to both plant growth and human health, and has been classified as a class I carcinogen [3]. A survey in China showed that Cd contaminated soil covers an area of 13,000 hm^2, involving 25 areas in 11 provinces, and soil pollution problems are particularly prominent in regions such as the old industrial bases in the northeast [4]. Among currently used methods to improve Cd-contaminated land, phytoremediation has shown to be an excellent method. The use of plants to extract, transform, and immobilize heavy metals not only eliminates heavy metal pollutants but also helps

restore vegetation communities, ecosystems, and local landscapes in areas contaminated with heavy metals [1].

Mint is highly adaptable, tolerates rough treatment, and is an aromatic crop with high economic value. *Mentha piperita* 'chocolate' and *Mentha spicata* grow well in northeast China and can be planted widely. Additionally, studies have shown that mint is tolerant to heavy metals [5,6] and has potential as a plant material for ameliorating Cd-contaminated soil. However, Cd stress has serious toxic effects on plant growth and development as well as physiological and biochemical activities [7]. For example, it causes retardation of plant growth, reduces biomass, inhibits photosynthesis, and induces oxidative damage due to reactive oxygen species (ROS) accumulation in plants [8]. Therefore, it is necessary to investigate ways to mitigate the effects of Cd on plants and promote plant growth under Cd stress. The use of soil additives provides new ideas to promote plant growth under Cd stress.

Biochar is a material produced by the thermal transformation of organic biomass during pyrolysis under low oxygen conditions [9]. Biochar has excellent properties, and some experimental studies have used it instead of traditional substrates to expand its environmental benefits, with good results [10,11]. It has received increasing attention as a soil amendment and has been found to immobilize a variety of heavy metals in the soil, making it a promising material for remediation of heavy metal contaminated soil [12]. The application of biochar has been reported to promote the growth of rice [13], wheat [14], maize [15], sunflowers [16], and other plants on heavy metal contaminated soils. This may be due to the biochar's porous structure, high pH, and high surface charge density, which can immobilize harmful substances in the soil [17], and biochar can passivate heavy metals through various mechanisms such as cation exchange, electrostatic interactions, and physical adsorption, thus reducing their biological effectiveness [18]. In addition, biochar can improve the nutrient content of polluted soil and improve the environment for plant growth. Biochar can reduce plant Cd toxicity by promoting plant growth, improving plant photosynthesis, mitigating plant oxidative damage, and reducing Cd concentration in plants [15]. However, the effect of biochar on *M. piperita* 'chocolate' and *M. spicata* under Cd stress has not been reported, and it is not known whether biochar can promote the growth of *M. piperita* 'chocolate' and *M. spicata* under Cd stress or not.

To date, the role of different types of biochar in reducing the effectiveness of heavy metals in soil and in reducing their uptake by plants has been confirmed by several studies [19–21]. We hypothesized that biochar mitigates Cd stress in both mint species by improving soil physicochemical properties, regulating the morphology of *M. piperita* 'chocolate' and *M. spicata* and their photosynthetic and antioxidant systems, and reducing the accumulation of Cd in the plants. Therefore, in this study, the changes in nutrient content and pH of Cd-contaminated soil after biochar application were determined, in addition to various physiological indicators of *M. piperita* 'chocolate' and *M. spicata* after the addition of Cd and biochar. Plant height, leaf length, leaf width, and biomass were used to assess growth status; chlorophyll content, photosynthetic rate, transpiration rate, and stomatal conductance were used to assess photosynthetic capacity; and superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), flavonoids, and total phenols were used to assess antioxidant capacity. We determined the Cd content in plants and the translocation and bioconcentration factors of Cd. We wanted to explore the effectiveness of biochar in promoting plant growth under Cd stress and to investigate the possible mechanisms used by biochar to mitigate the damage of Cd stress on plants, in order to provide a feasible method and effective concentration for promoting the growth of *M. piperita* 'chocolate' and *M. spicata* under Cd stress.

2. Materials and Methods

2.1. Test Materials and Growth Conditions

The test soil (sandy loamy) used was for garden cultivation, and the basic physicochemical properties, obtained from this experiment, are shown in Table 1. The test biochar (Hongyuan

Jialianhe Biomass Energy Co., Ltd., Jilin City, China) was made from rice husks with a carbonization temperature of 450 °C. The basic physicochemical properties, obtained from this experiment, are shown in Table 1. The mint was from cuttings, and the parent material is kept in the garden nursery of Northeast Forestry University. The experiment was conducted in a randomized complete block design. The following treatments were used for pot experiments: (1) control: biochar 0, Cd 0; (2) C0Cd10: biochar 0, Cd 10 mg Kg^{-1}; (3) C4Cd10: biochar 40 g Kg^{-1}, Cd 10 mg Kg^{-1}; (4) C8Cd10: biochar 80 g Kg^{-1}, Cd 10 mg Kg^{-1}; (5) C16Cd10: biochar 160 g Kg^{-1}, Cd 10 mg Kg^{-1}. Amounts of 0 g Kg^{-1}, 40 g Kg^{-1}, 80 g Kg^{-1}, 160 g Kg^{-1} biochar were added to the air-dried soil, and $CdCl_2$ (Maclean Biochemical Technology Co., Ltd., Shanghai, China) aqueous solution was added and mixed thoroughly to bring the Cd concentration in the substrate to 10 mg Kg^{-1}, and the substrate was then allowed to stabilize for two months [22]. Three replicates were set up for each treatment, and the well-mixed substrate was packed into plastic pots (13 cm in diameter and 11 cm in height) with 400 g of soil per pot. One-month old cuttings of 6–8 cm uniformly growing *M. piperita* 'chocolate' and *M. spicata* seedlings were transplanted into the above substrate, and four plants were planted in each pot. Watering was done every five days to keep the soil moisture content at 75% of the maximum water holding capacity in the field, with an average temperature of 20 °C and 12 h of light. Plant samples were collected after 30 days to determine various physiological and biochemical characteristics.

Table 1. Selected properties of soil and biochar.

Properties	Soil	Biochar
Bulk density (g cm^{-3})	0.59	0.19
Water holding capacity (%)	59.68	252.72
Total porosity (%)	66.38	72.02
Cd (mg Kg^{-1})	ND	ND
Available N (μg g^{-1})	98.87	51.3
Available P (μg g^{-1})	12.51	205.42
Available K (μg g^{-1})	263.07	3022.39
pH	5.57	9.92
Total organic carbon (%)	15.69	53.11

ND = Not detected.

2.2. Determination of Assays and Contents

After plant harvesting, soil samples from the pots were air-dried through a 2 mm sieve and subjected to physical and chemical analysis. Soil pH was determined in deionized water (w_{soil}:v_{water} = 1:2.5); soil available nitrogen (available N) was determined by the alkaline diffusion method; soil available phosphorus (available P) was determined by the sodium bicarbonate leaching method; and soil available potassium (available K) was determined by the ammonium acetate extraction flame method. Physical properties of the substrate were determined by cutting ring method [16]. The plant height was measured with a straightedge and the leaf length and width with a Vernier calipers. After the plants were harvested, the fresh biomass was weighed on an analytical balance (Mettler Toledo, Shanghai, China), and the dry biomass was weighed after drying to a constant weight.

Fresh leaves (0.3 g) and 10 mL of 0.05 mol L^{-1} phosphate buffer (pH 7.8) were homogenized, and the supernatant was centrifuged to obtain the enzyme solution. Superoxide dismutase (SOD) activity was measured by the nitrogen blue tetrazolium (NBT) method by taking 0.05 mL of enzyme solution, 1.5 mL of 0.05 mol L^{-1} phosphate buffer, 0.3 mL of 130 mmol L^{-1} Met (Sigma-Aldrich, Burlington, MA, USA), 0.3 mL of 750 μmol L^{-1} NBT (Sigma-Aldrich), 0.3 mL of 100 μmol L^{-1} EDTA-Na_2 (Yongda Chemical Reagent Co., Ltd., Changzhou, China), 0.3 mL of 2.0 μmol L^{-1} riboflavin (Yongda Chemical Reagent Co., Ltd.), and 0.25 mL of distilled water to obtain the SOD reaction mixture. The reaction mixture was exposed for color development reaction, and the absorbance at 560 nm was recorded [23]. The peroxidase (POD) activity was determined by the guaiacol method by taking 0.1 mL of enzyme solution, 1.5 mL of 0.05 mol L^{-1} phosphate buffer, 1 mL of 0.05 mol L^{-1} guaiacol solu-

tion (Yongda Chemical Reagent Co., Ltd.), and 1 mL of 2% H_2O_2 (Yongda Chemical Reagent Co., Ltd.) to obtain the POD. The change in absorbance of the reaction mixture at 470 nm was then recorded [24]. The catalase (CAT) activity was determined according to the method of Seckin et al. [25] by taking 0.1 mL of enzyme solution, 1 mL of 0.05 mol L^{-1} phosphate buffer, and 1.7 mL of distilled water to obtain the CAT reaction mixture. The reaction mixture was left at 25 °C for 3 min. Then, 0.2 mL of 200 mmol L^{-1} H_2O_2 was added, and the change in absorbance of the reaction mixture at 240 nm was recorded. Polyphenol oxidase (PPO) was determined by the catechol colorimetric method by taking 0.1 mL of enzyme solution, 3.9 mL of 0.05 mol L^{-1} phosphate buffer, and 1 mL of 0.1 mol L^{-1} catechol solution to obtain the PPO reaction mixture. The change in the absorbance of the reaction mixture at 525 nm was recorded [26]. Total phenols were determined by the method of Kim et al. [27] by taking 0.5 g of powdered dried leaves, adding 10 mL of 80% methanol solution (Yongda Chemical Reagent Co., Ltd.), extracting with an ultrasound for 30 min and centrifuging to obtain the supernatant. Supernatant (1 mL), 10 mL of 7% Na_2CO_3 (Yongda Chemical Reagent Co., Ltd.), 1 mL of Folin–Ciocalteu phenol reagent (Sigma-Aldrich), and distilled water were added to increase the total volume to 25 mL, and the absorbance of the reaction mixture at 750 nm after 90 min was recorded. The flavonoid content was determined according to the method of Jia et al. [28] by extracting 1 g of powdered dried leaves with 100 mL of distilled water in a Soxhlet extractor for 1 h. The extract was filtered. A volume of 5 mL of the extract, 0.3 mL of 5% $NaNO_2$ (Yongda Chemical Reagent Co., Ltd.), 3 mL of 10% $AlCl_3$ (Yongda Chemical Reagent Co., Ltd.), and 2 mL of 1 mol L^{-1} NaOH (Yongda Chemical Reagent Co., Ltd.) were combined, and then distilled water was added until the total volume was 10 mL, and the absorbance of the reaction mixture at 510 nm was recorded.

Plant samples were dried at 105 °C for 30 min and then dried at 65 °C until a constant weight was obtained. Oven-dried powder from plants above and below ground was passed through 0.15 mm sieve. Plant power (0.05 g) was digested with 5 mL of nitric acid-perchloric acid (v/v = 4:1) solution. This solution was then subjected to nitrification at 160 °C until the liquid became transparent, with a final volume of 20 mL. The concentration of Cd was determined by an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkins Elmer, Norwalk, CT, USA). The detection limit ranges were <0.1 μg L^{-1}, and the spiked sample recovery was 99.6–103%. The equation of the standard curve for Cd is: y = 6942.7x + 158.91 R^2 = 0.9993 [29].

Translocation factors (TF) = shoot Cd content/root Cd content; bioconcentration factor (BCF) = Cd content in plants/soil Cd content [18,30].

2.3. Statistical Analysis

Statistical charts were produced by Origin 2019 (OriginLab, Northampton, MA, USA). Data were analyzed using SPSS 26.0 (Statistical Product and Service Solutions, IBM, Amonk, NY, USA) for one-way analysis of variance (ANOVA), and Duncan's method was used to analyze the significance of differences between the means [29].

3. Results

3.1. Soil Property

According to the analysis of the soils with different treatments, it can be seen from Table 2 that the nutrient element content of the soil did not change significantly after Cd contamination, and the nutrient elements in the soil increased to some extent after the application of biochar, among which the available P and available K increased significantly. Cd contamination decreased soil pH, and the application of biochar can significantly increase soil pH. Compared with the treatment with Cd stress alone, its application of different ratios of biochar increased soil pH by 0.59–1.18 units, which facilitated the fixation of heavy metal Cd, with the most pronounced effect in the group treated with 160 g Kg^{-1} biochar.

Table 2. Effect of biochar on soil characteristics under Cd stress.

Plant	Treatments	Available N (μg g^{-1})	Available P (μg g^{-1})	Available K (μg g^{-1})	pH
Mentha piperita 'chocolate'	Control	84.88 ± 2.45 c	6.83 ± 0.8 d	107.19 ± 0.42 d	5.7 ± 0.06 d
	C0Cd10	88.14 ± 3.51 c	6.59 ± 0.71 d	106.21 ± 0.46 d	5.31 ± 0.08 e
	C4Cd10	101.91 ± 3.45 b	20.81 ± 0.65 c	155.1 ± 0.79 c	5.9 ± 0.11 c
	C8Cd10	107.63 ± 4.94 ab	28.73 ± 2.48 b	164.12 ± 0.58 b	6.06 ± 0.06 b
	C16Cd10	113.11 ± 3.16 a	41.37 ± 1.5 a	182.57 ± 0.89 a	6.22 ± 0.05 a
Mentha spicata	Control	97.71 ± 1.46 ab	10.02 ± 1.08 d	93.1 ± 3.23 d	5.49 ± 0.27 c
	C0Cd10	95.38 ± 4.13 b	8.31 ± 0.81 d	91.01 ± 4.05 d	5.13 ± 0.06 d
	C4Cd10	98.53 ± 5.05 ab	20.95 ± 0.58 c	130.13 ± 9.05 c	5.93 ± 0.12 b
	C8Cd10	103.89 ± 3.52 a	30.54 ± 2.09 b	148.24 ± 1.27 b	6.08 ± 0.13 ab
	C16Cd10	102.26 ± 4.06 ab	43.85 ± 2.56 a	177.16 ± 0.83 a	6.31 ± 0.05 a

Data in the table are mean ± standard deviation (SD) (n = 3), different lowercase letters in the same column indicate significant differences between treatments, and differences are considered significant when $p < 0.05$.

3.2. Growth Characteristics

Compared with the control group, Cd stress caused a significant decrease in plant height, leaf length, and leaf width of both mint species. Cd stress severely affected the growth of mint, and the addition of biochar promoted the growth of the mint under Cd contamination, significantly increasing the plant height, leaf length, and leaf width of the mint. The most significant promotion was achieved by the addition of 160 g Kg^{-1} biochar treatment under Cd stress, which increased the height, leaf length and leaf width of *M. piperita* 'chocolate' by 60.63%, 17.37%, and 24.66%, respectively, and the height, leaf length and leaf width of *M. spicata* by 47.99%, 21.67%, and 21.95%, respectively. (Table 3, Figure 1)

Table 3. Effect of biochar on the growth of two mint species under Cd stress.

Plant	Treatments	Height (cm)	Leaf Length (mm)	Leaf Width (mm)
Mentha piperita 'chocolate'	Control	14.9 ± 0.2 d	31.66 ± 0.29 c	23.06 ± 0.27 c
	C0Cd10	13.03 ± 0.31 e	30.62 ± 0.33 d	21.45 ± 0.36 d
	C4Cd10	15.77 ± 0.35 c	31.94 ± 0.44 c	23.68 ± 0.15 bc
	C8Cd10	18.67 ± 0.25 b	33.27 ± 0.14 b	24.56 ± 0.22 b
	C16Cd10	20.93 ± 0.65 a	35.94 ± 0.65 a	26.74 ± 1.18 a
Mentha spicata	Control	14.97 ± 0.21 d	30.6 ± 0.55 c	20.89 ± 0.43 c
	C0Cd10	13.4 ± 0.46 e	28.52 ± 1.09 d	18.86 ± 0.35 d
	C4Cd10	16.37 ± 0.45 c	31.7 ± 0.27 bc	21.4 ± 0.38 c
	C8Cd10	17.67 ± 0.4 b	32.69 ± 0.57 b	22.04 ± 0.17 b
	C16Cd10	19.83 ± 0.42 a	34.7 ± 0.92 a	23 ± 0.09 a

Data in the table are mean ± SD (n = 3); different lowercase letters in the same column indicate significant differences between treatments, and differences are considered significant when $p < 0.05$.

Figure 2 shows that Cd stress treatment without biochar addition significantly reduced both the dry and fresh shoot and root biomass in both mint species compared to the control. The fresh shoot and root biomass of *M. piperita* 'chocolate' decreased by 16.89% and 26.71% and dry biomass by 13.16% and 20.00%, respectively, while the fresh shoot and root biomass of *M. spicata* decreased by 22.13% and 20.11%, and dry biomass by 28.57% and 24.00%, respectively. In contrast, all treatments with biochar application increased the biomass of mint under Cd stress and showed a gradual increase with the increase of biochar addition ratio (Figure 2), which indicated that the application of biochar could alleviate the inhibitory effect of Cd stress on the growth of mint.

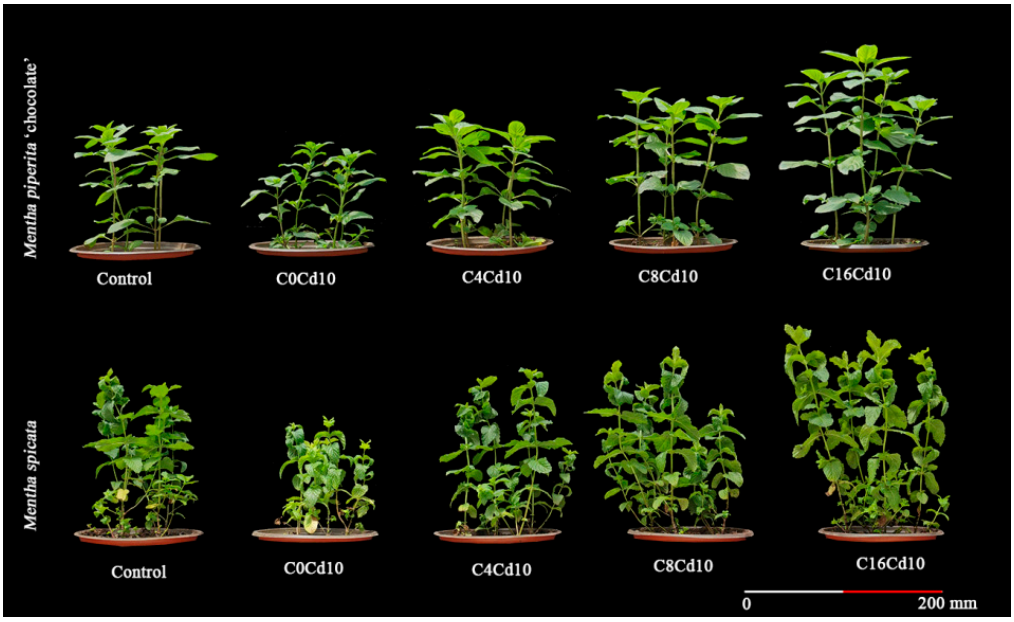
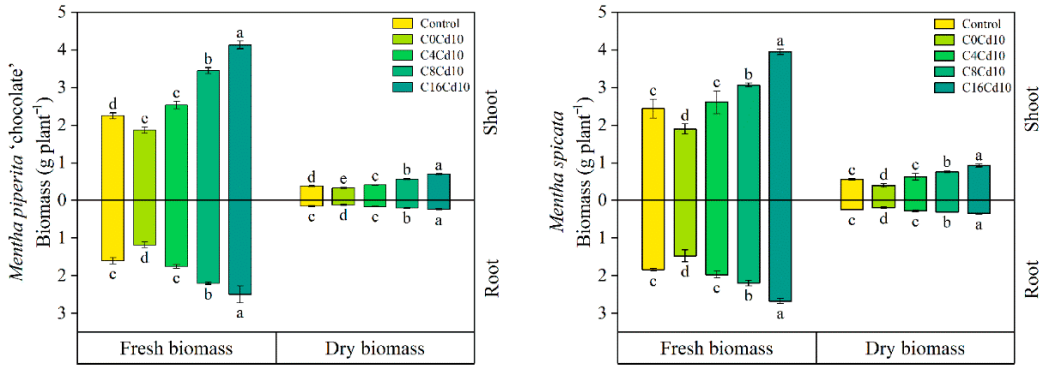


Figure 1. Effect of biochar on two mint species under Cd stress.



(**a**) (**b**)

Figure 2. Effect of biochar on the biomass of *M. piperita* 'chocolate' (**a**) and *M. spicata* (**b**) under Cd stress. Error lines are standard deviations from the mean (n = 3) and different letters indicate significantly different values between treatments, $p < 0.05$.

3.3. Gas Exchange Parameters and Chlorophyll Content

Cd stress resulted in a significant decrease in gas exchange parameters and chlorophyll content in both mint species compared to the control. Application of biochar significantly increased the photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content of mint under Cd contamination. Among them, the addition of 160 g Kg^{-1} biochar treatment (C16Cd10) under Cd stress improved the effect most significantly. *M. piperita* 'chocolate' photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content increased by 113.00%, 96.37%, 80.00%, and 39.37% compared to the Cd stress treatment without the addition of biochar (C0Cd10), *M. spicata* photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content increased by 113.21%, 101.46%, 96.97%, and 49.48% compared to the Cd stress treatment without the addition of biochar (C0Cd10) (Table 4).

Table 4. Effect of biochar on gas exchange parameters and chlorophyll content of two mint species under Cd stress.

Plant	Treatments	Photosynthetic Rate ($\mu mol\ m^{-2}\ s^{-1}$)	Transpiration Rate ($mmol\ m^{-2}\ s^{-1}$)	Stomatal Conductance ($mol\ m^{-2}\ s^{-1}$)	Chlorophyll Content ($mg\ g^{-1}$ FW)
Mentha piperita 'chocolate'	Control	10.852 ± 0.08 d	1.719 ± 0.005 c	0.045 ± 0.001 c	2.265 ± 0.046 d
	C0Cd10	8.275 ± 0.366 e	1.268 ± 0.032 e	0.035 ± 0.005 d	2.037 ± 0.006 e
	C4Cd10	11.562 ± 0.562 c	1.549 ± 0.018 d	0.042 ± 0.001 c	2.564 ± 0.016 c
	C8Cd10	13.832 ± 0.334 b	2.054 ± 0.003 b	0.053 ± 0.002 b	2.639 ± 0.029 b
	C16Cd10	17.589 ± 0.297 a	2.49 ± 0.004 a	0.063 ± 0.002 a	2.839 ± 0.045 a
Mentha spicata	Control	6.404 ± 0.205 d	1.468 ± 0.032 c	0.046 ± 0.001 b	2.338 ± 0.01 d
	C0Cd10	5.706 ± 0.273 e	1.23 ± 0.116 d	0.033 ± 0.006 c	2.009 ± 0.025 e
	C4Cd10	7.299 ± 0.211 c	1.827 ± 0.003 b	0.043 ± 0.001 b	2.644 ± 0.041 c
	C8Cd10	9.006 ± 0.297 b	2.406 ± 0.001 a	0.061 ± 0.001 a	2.781 ± 0.045 b
	C16Cd10	12.166 ± 0.269 a	2.478 ± 0.006 a	0.065 ± 0.002 a	3.003 ± 0.026 a

Data in the table are mean ± SD (n = 3); different lowercase letters in the same column indicate significant differences between treatments, and differences are considered significant when $p < 0.05$. FW, fresh weight.

3.4. Antioxidant Defense Responses

As can be seen from Figure 3, Cd stress resulted in a significant increase in superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO) in *M. piperita* 'chocolate' and *M. spicata* compared to the control, indicating that the plants themselves responded to Cd stress by increasing their own antioxidant enzyme activities. In this study, the antioxidant enzyme activity decreased gradually with increasing biochar concentration in the soil under Cd stress, with the treatment adding 160 g Kg^{-1} biochar (C16Cd10) causing the most significant decrease in enzyme activity. SOD activity decreased by 26.30% (Figure 3a), CAT activity decreased by 61.29% (Figure 3b), POD activity decreased by 67.17% (Figure 3c), and PPO activity decreased by 46.45% (Figure 3d) in *M. piperita* 'chocolate'; SOD decreased by 43.46% (Figure 3a); CAT decreased by 54.87% (Figure 3b) in spearmint; POD decreased by 68.39% (Figure 3c), and PPO activity decreased by 29.28% (Figure 3d) in *M. spicata*.

As can be seen from Figure 4, the addition of Cd significantly increased the content of non-enzymatic antioxidants (flavonoids and total phenols) in both mint species compared to the Control. The total phenols and flavonoids play a very important role as non-enzymatic antioxidants in plants to resist Cd stress. The application of biochar significantly reduced the contents of flavonoids and total phenols of both mint species under Cd stress, and the differences in the contents of total flavonoids and phenols between different concentrations of biochar treatments did not reach significance. The flavonoid content of *M. piperita* 'chocolate' was reduced by 9.25~14.73% (Figure 4a) and total phenolic content by 9.51~11.73% (Figure 4b); the flavonoid content of *M. spicata* was reduced by 7.45~8.51% (Figure 4a), and total phenolic content decreased by 12.43~14.69% (Figure 4b) under Cd contamination with the addition of biochar compared to without the addition of biochar.

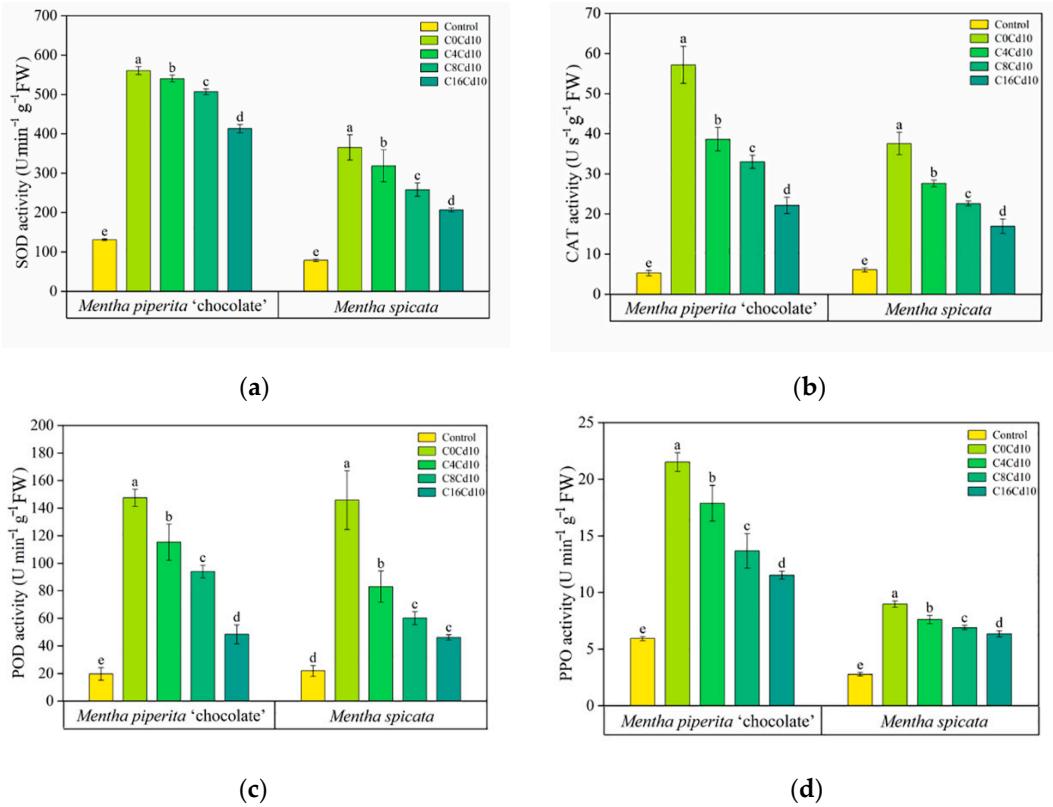


Figure 3. Effect of biochar on SOD activity (**a**), CAT activity (**b**), POD activity (**c**), and PPO activity (**d**) of two mint species under Cd stress. The error line is the standard deviation of the mean (n = 3) and different letters indicate significantly different values between treatments for the same plant, $p < 0.05$. FW, fresh weight.

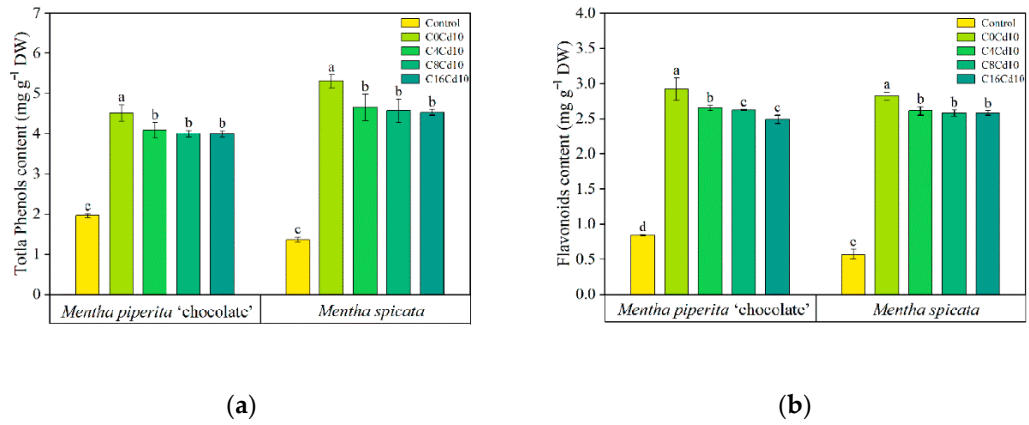


Figure 4. Effect of biochar on total phenols (**a**) and flavonoids (**b**) contents of two mint species under Cd stress. Error lines are standard deviations from the mean (n = 3), different letters indicate significantly different values between treatments for the same plant, $p < 0.05$. DW, dry weight.

3.5. Cd Absorption and Distribution

As seen in Figure 5, the Cd content in the roots of both mint species treated with Cd stress was significantly higher than that in the shoot, indicating that Cd was more enriched in the roots of the mint. The application of biochar in Cd-contaminated soil significantly reduces Cd content in the roots and shoots of mint. The Cd content of *M. piperita* 'chocolate' shoots significantly decreased by 21.14%, 37.40%, and 48.78%, respectively (Figure 5a), and the roots Cd content significantly decreased by 14.19%, 26.97%, and 38.22%, respectively (Figure 5b); the Cd content in the shoots of *M. spicata* was significantly reduced by 26.06%,

33.8%, and 47.89% (Figure 5a), and the root Cd content significantly decreased by 12.75%, 22.62%, and 29.02% (Figure 5b), when 40 g Kg^{-1}, 80 g Kg^{-1}, and 160 g Kg^{-1} biochar was added compared to no biochar treatment (C0Cd10). This indicates that biochar can reduce the uptake of Cd from the soil by the plant body and lower the Cd content in the plant.

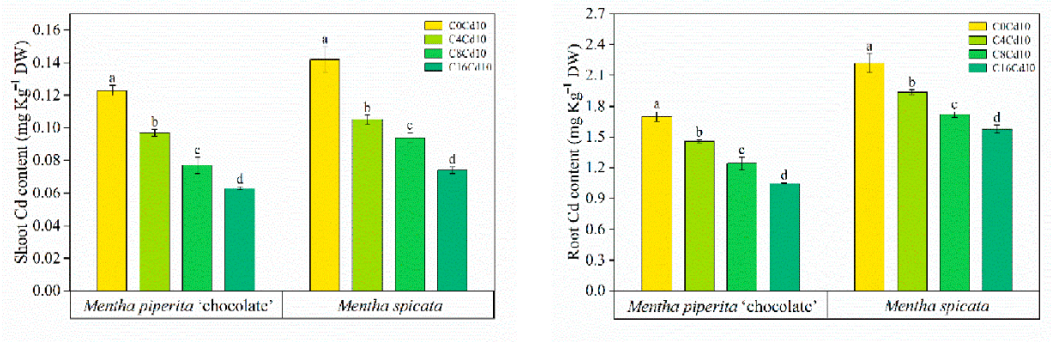


Figure 5. Effect of biochar addition on Cd content in shoot (**a**) and Cd content in root (**b**) of two mint species under Cd contamination. Error lines are standard deviations from the mean (n = 3), and different letters indicate significantly different values between treatments for the same plant, $p < 0.05$. DW, dry weight.

As shown in Table 5, the addition of biochar to Cd-contaminated soil significantly reduced the Cd translocation factors (TF) in *M. piperita* 'chocolate' and *M. spicata* plants. This shows that the application of biochar can reduce the uptake of Cd in plants, limit the transfer of Cd absorbed in plants from roots to shoots, and reduce the TF of Cd in plants. The bioconcentration factors (BCF) of both mint species were significantly reduced after biochar application and gradually decreased with the increase of biochar additive. Among them, the BCF of *M. piperita* 'chocolate' treated with 40 g Kg^{-1}, 80 g Kg^{-1}, and 160 g Kg^{-1} biochar decreased by 14.84%, 27.47%, and 39.01%, respectively, compared to those under Cd stress treatment (C0Cd10) without the addition of biochar. The BCF of *M. spicata* with the addition of 40 g Kg^{-1}, 80 g Kg^{-1}, and 160 g Kg^{-1} biochar treatments were reduced by 13.56%, 23.31%, and 30.08% compared to the Cd stress treatment without biochar (C0Cd10). It indicates that the application of biochar can significantly reduce the uptake of Cd by plants as a way to alleviate Cd stress, and the addition of 160 g Kg^{-1} biochar (C16Cd10) was the most effective in reducing the uptake of Cd by plants in this study.

Table 5. Effect of biochar application on the TF and BCF of two mint species under Cd contamination.

Plant	Treatments	Translocation Factors (TF)	Bioconcentration Factors (BCF)
Mentha piperita 'chocolate'	C0Cd10	0.073 ± 0.003 a	0.182 ± 0.005 a
	C4Cd10	0.066 ± 0.002 b	0.155 ± 0.002 b
	C8Cd10	0.062 ± 0.002 c	0.132 ± 0.007 c
	C16Cd10	0.06 ± 0.001 c	0.111 ± 0.001 d
Mentha spicata	C0Cd10	0.064 ± 0.001 a	0.236 ± 0.01 a
	C4Cd10	0.054 ± 0.002 b	0.204 ± 0.002 b
	C8Cd10	0.055 ± 0.001 b	0.181 ± 0.003 c
	C16Cd10	0.047 ± 0.001 c	0.165 ± 0.004 d

Data in the table are mean ± SD (n = 3), different lowercase letters in the same column indicate significant differences between treatments, and differences are considered significant when $p < 0.05$.

4. Discussion

Heavy metal pollution is one of the most serious environmental problems facing the world today, and many studies have proven that Cd pollution has a variety of negative effects on plant growth and development [31]. Our study found that the growth of *M. piperita*

'chocolate' and *M. spicata* plants under Cd contamination was significantly inhibited, with significant reductions in plant height, leaf length, leaf width, and dry and fresh biomass compared to the control (Table 3, Figure 2). The application of biochar significantly alleviated the inhibitory effect of Cd stress on the growth of mint plants and played a decisive role in mitigating the deleterious effects of Cd stress on mint plants. The positive effects of biochar on plant growth performance under Cd contamination was similarly obtained in studies on rice [13] and wheat [14]. The enhancement of plant growth indicators in Cd-polluted environments was also associated with an increase in nutrient uptake. The results of this study showed that the application of biochar increased the content of available soil nutrients, especially available P and available K, compared with the treatment without biochar (Table 2). This is due to the high concentration of available P and available K in the biochar itself. Biochar can reduce nitrogen leaching and provide better adsorption sites for NH_4-N and NO_3-N adsorption, and some studies have shown that there is an aging process of biochar particles in soil, and more nitrogen is mineralized in aged biochar compared with fresh biochar [32]. This may be the reason why biochar itself has low available N content but can enhance the available N content of soil. A large number of studies have also found that mixing biochar and soil increased soil enzyme activity and had a positive effect on soil microorganisms, and that mineralization in biochar soil mixtures was enhanced, which is the reason for the increase in available N, available P, and available K in the soil [33,34]. The release of nutrients from biochar can continuously supply soil fertility and provide a good nutritional environment for plants [35]. The beneficial effects of biochar on plant growth and soil nutrient environment were also found in a study by Turan et al. [36]. Our study found that applying biochar enhanced the transpiration rate of both mint species. Plants absorb nutrients by absorbing ions from water, and transpiration is the driving force for water uptake by plants, so biochar can also promote nutrient uptake by plants.

In addition to transpiration rate, biochar also influenced some other key indicators of response of photosynthetic intensity. Photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content can reflect the response of plant photosynthetic physiology to adversity and are basic indicators that can evaluate the intensity of photosynthesis. In this study, we found that Cd stress severely affected the gas exchange parameters and chlorophyll content of mint (Table 4), resulting in a significant decrease in the photosynthetic intensity of the plants. The effect of Cd stress on photosynthesis has yielded similar results in studies on rice [37]. This is due to the fact that Cd can reduce plant photosynthetic intensity by enhancing enzymatic degradation to inhibit chlorophyll synthesis, or by limiting stomatal opening and closing of plant leaves. The addition of a biochar treatment significantly increased photosynthetic rate, transpiration rate, stomatal conductance, and chlorophyll content of the mint under Cd stress (Table 4), alleviating the inhibitory effect of Cd on photosynthesis in the mint. Similar results have been found in studies on maize [38]. This may be because biochar retains the Cd in the soil, protects plant chlorophyll and photosynthetic apparatus, mitigates Cd damage to leaf stomata, and enhances photosynthesis in plants under Cd contamination.

The negative impact of heavy metals on plants also comes from the induction of excessive accumulation of reactive oxygen species in plants, which leads to plasma membrane damage, affects normal cellular physiological and biochemical functions, and inhibits plant growth [39]. The plant body itself has powerful antioxidant defense mechanisms, including antioxidant enzymes and non-enzymatic antioxidants. Under stress conditions, plants accumulate more antioxidant substances to scavenge ROS and mitigate oxidative damage under environmental stress [40]. The results of the study revealed that the activities of SOD, CAT, POD, and PPO were significantly increased in mint grown in Cd contaminated environments compared to the two mint types in the control treatment (Figure 3). A significant increase in flavonoid and total phenolic content also occurred under Cd stress (Figure 4). This suggests that both mint species adapt to Cd stress by enhancing antioxidant enzyme activity and elevating the content of non-enzymatic antioxidants in the body. The addition of biochar significantly reduced

the antioxidant enzyme activities and the contents of flavonoids and total phenols in both mint species under Cd stress compared to the treatment with Cd stress alone. Because the addition of biochar enhanced the adsorption capacity of the soil for heavy metals, the Cd in the soil was blunted; the uptake of Cd by the plant body was reduced, thus weakening the damage of Cd to the plant plasma membrane; the plant body self-regulated, the degree of antioxidant defense was reduced, and the corresponding antioxidant enzyme activity and non-enzymatic antioxidant content decreased. Similar patterns have been found in studies on sunflowers [16] and *Withania somnifera* [41]. The protective effect of biochar against oxidative stress induced by heavy metal stress has been well documented in many plant species [42,43].

In this study, the Cd content of both *M. piperita* 'chocolate' and *M. spicata* roots and shoots under Cd stress was significantly reduced by biochar application (Figure 4), with the treatment with 160 g Kg^{-1} biochar application being the most effective in inhibiting Cd uptake by both mint species. The addition of biochar also significantly reduced the BCF and TF of Cd in both mint species compared to the Cd treatment alone (Table 5). The BCF indicates the ability of the plant to take up heavy metals from the soil, and the TF indicates the ability of heavy metals to be transferred in the root-shoot part of the plant. The results of this study showed that biochar inhibited the uptake of Cd from soil in both mint species and could inhibit the translocation of Cd from roots to shoots in mint. The application of biochar effectively reduced the risk of excessive Cd accumulation in plants grown on Cd-contaminated soil and effectively decreased the toxicity of Cd in *M. piperita* 'chocolate' and *M. spicata*. Sajid et al. [44] in sesame, Khan et al. [45] in cabbage, and Nigam et al. [7] in mint (*Mentha arvensis*) reported similar patterns of reduction in heavy metal content in plants after the addition of biochar. Surface precipitation, surface complexation, ion exchange, electrostatic attraction, and physical adsorption are the main mechanisms for reducing the metal mobility [7]. Biochar exhibits effective passivation of heavy metals, and it has been shown that biochar has a large surface area, high ion exchange capacity and abundant functional groups, which can convert the highly biotoxic exchangeable Cd to the weakly biotoxic inert Cd [46]. In the studies on the effects of biochar on various heavy metals (Ni, Cd, Co, Cr, Pb, etc.), it was found that biochar significantly reduced the content of heavy metals in plants, which is similar to our findings [47].

Moreover, this study found that the application of biochar significantly increased the pH of the soil (Table 2), with the most significant effect at 160 g Kg^{-1} of biochar. Ma et al. [48] also found that the addition of biochar can increase soil pH because biochar ash is rich in alkali ions that can absorb H^+ and exchangeable Al^{3+} from the soil [49], and there is also an alkaline component in biochar that leads to an increase in soil pH [50]. It has been shown that soil pH plays a dominant role in plant biomass production and Cd fixation in heavy metal contaminated soils [51]. This is due to the alkaline components carbonate, oxide, and hydroxide in biochar, which increase soil pH, promote the precipitation of $Cd(OH)_2$ and $CdCO_3$, facilitate soil fixation of the heavy metal Cd, and reduce the biological effectiveness of Cd [52].

5. Conclusions

This study confirmed that the application of biochar could promote the growth of *M. piperita* 'chocolate' and *M. spicata* in Cd-contaminated soil and could significantly reduce the toxic effects of Cd on both mint species. The results showed that (1) the addition of biochar increased the nutrient content of the soil and raised the pH value of the soil. (2) Different ratios of biochar significantly promoted the growth of *M. piperita* 'chocolate' and *M. spicata* under Cd stress. (3) Biochar alleviated the physiological stress of heavy metal Cd on two mint species and improved photosynthesis of *M. piperita* 'chocolate' and *M. spicata* under Cd stress. (4) Biochar reduced the activity of antioxidant enzymes (SOD, CAT, POD, and PPO) and the content of non-enzymatic antioxidants (flavonoids and total phenols) under Cd stress. (5) Biochar significantly reduced the uptake of Cd from soil and

the translocation of Cd from roots to shoots in both mint species and reduced the migration and accumulation of Cd in mint. In addition, as the amount of added biochar increases, the mitigation effect on Cd toxicity becomes more and more obvious, with the reduction rate reaching 48.78%. In conclusion, biochar can effectively reduce the toxic effects of Cd on *M. piperita* 'chocolate' and *M. spicata* and promote the growth of both mint species on Cd-contaminated soil. The application of biochar is an effective technique to promote the growth of mint in Cd-contaminated environments and can be used for ecological and plant landscape restoration in cadmium-contaminated areas. However, we recommend optimizing the amount of biochar additives; focusing on how to effectively reduce the cost of biochar production in the next studies; and based on the results of this preliminary study, conducting further studies to validate the results obtained with the actual effects of adding biochar under field conditions.

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