EFFECT OF BIOCHAR ON SOIL CADMIUM CONTENT AND CADMIUM UPTAKE OF COTTON (*GOSSYPIUM HIRSUTUM* L.) GROWN IN NORTHWESTERN CHINA

ZHU, Y. Q. – WANG, H. J.* – LV, X. – SONG, J. H. – WANG, J. G. – TIAN, T.

Agricultural College, Shihezi University, Shihezi, Xinjiang 832003, China
(e-mail: shzuyongqizhu@hotmail.com – Y. Q Zhu; phone: +86-131-5040-7812)

**Corresponding author*
e-mail: wanghaijiang@shzu.edu.cn; phone: +86-189-6382-7056

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Abstract. In order to investigate the effects of biochar on soil cadmium (Cd) content in northwestern China, we used cotton (*Gossypium hirsutum* L.) straw charcoal in a 2-year experiment involving different doses of both biochars (1.5% (C1) and 3% (C2)) and Cd (1 mg·kg^{-1} (H1), 2 mg·kg^{-1} (H2), and 4 mg·kg^{-1} (H3)) in addition to the treatments, control plots were set up with no biochar application. We grew cotton (*Gossypium hirsutum* L.) in pots for each treatment and examined soil pH, Cd forms, and Cd accumulations in aboveground plant organs. Compared to no added biochar, the addition of biochar significantly increased soil pH, but there was no notable difference in soil pH between the C1 and C2 treatments. Biochar significantly decreased soil-available Cd with this effect increasing with higher biochar doses. The lowest amounts of soil-available Cd in all Cd treatments (H0–H3), including the control, occurred later in the study, typically at 90 to 150 days post treatment. In the cotton (*Gossypium hirsutum* L.), Cd preferentially accumulated the most in leaves, then in stems, and then in bolls and the amount of Cd in cotton and soil-available Cd significantly correlated. The addition of biochar promoted the transformation of exchangeable and carbonate-bound Cd to organic matter-bound Cd at 30, 60, and 90 days, and the exchangeable and carbonate-bound Cd to Fe-Mn oxide-bound Cd at 120 and 150 days. In conclusion, biochar addition decreases both Cd bioavailability and the accumulation of Cd in cotton (*Gossypium hirsutum* L.) aboveground organs.
Keywords: *cotton straw biochar, soil pH, Cd forms, Cd bioavailability*

Introduction

Widely found in farmland soils, cadmium (Cd) is a highly toxic heavy metal that biomagnifies through the food chain, thus ultimately endangering human health (Li et al., 2010). Studies proved that metals such as copper, lead, zinc, cobalt, nickel, chromium, and mercury which have been considered as hazardous heavy metals are very toxic elements (Ghassabzadeh et al., 2010). Wastewater, fertilization, and irrigation using wastewater containing Cd are the main sources of Cd pollution in China’s soils, contributing to both soil and water pollution in the Xinjiang area of northwest China, where chemical fertilizers containing Cd are used. Such repeated fertilization over many years often leads to an accumulation of heavy metals in the soil and unacceptable amounts of Cd have been found in Xinjiang soil (Wang et al., 2016). When subjected to Cd exposure, plants display shortened root length, decreased chlorophyll amount, and decreased fruit number (Renyuan et al., 2018).

Biochar, a charcoal with a pore structure, high aromatization, and high carbon amount (Aslam et al., 2017), is beneficial in agricultural and environmental contexts, especially since the use of biochar as a soil amendment may potentially help to mitigate global warming (Gaunt, 2008), improve soil quality (Fellet et al., 2011), reduce the bioavailability of organic contaminants (Li et al., 2010), and increase nutrient and water

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retention capacity of soil (Abel et al., 2013; Zheng et al., 2013), thereby increasing crop yield (Zhang et al., 2013). Song et al. (2017) showed that modified walnut shell biochar is a catalyst for the catalytic removal of organic sulfur and arsenic. Other research has shown that biochar decreases the mobility of heavy metals by altering soil pH to control heavy metal mobilization (Tong et al., 2020; Ma et al., 2020; Kim et al., 2015; Zhu et al., 2015). Also, Zhu et al. (2015) demonstrated that 0.5% wine lees-derived biochar decreased exchangeable Cd in soil by 48.14% and Wang et al. (2016) showed that biochar derived from tea branches promoted the growth of *Lolium multiflorum* and reduced antimony and Cd bioavailability.

Cotton *(Gossypium hirsutum* L.*)*, a dominant commercial crop in Xinjiang, was planted in 53.46% of the farmed area and provided 67.3% of the total Chinese cotton harvest (Gaunt, 2008). Long-term, continuous cotton cropping, coupled with the use of agricultural chemicals, have enriched Xinjiang farm soils with heavy metals. Luckily, abundant cotton straw accumulation, common in this large, cultivated area, presents an important opportunity to reduce soil pollution by converting it into biochar and using it as a soil amendment. Therefore, using heavy metal contaminated cotton stalks converted to biochar to both absorb Cd and provide nutrition in cotton field soil would not only reduce the risk of Cd contaminating farmland, but would also usefully reuse Cd-contaminated cotton stalks.

We applied cotton straw biochar to Cd-contaminated, Xinjiang, cotton field soil to examine the effects of biochar on various soil Cd forms and how Cd levels in soil varied over time, as well as cotton’s absorption of Cd and how it varied over time. Our results provide basic data and technical reference for controlling Cd pollution in northwestern China farmland soil.

Materials and methods

Soil and biochar collection and their physicochemical properties

We conducted our experiments at the Agricultural College of Shihezi University in Shihezi City, Xinjiang Province, China (86°03′E, 45°19′N) for a two-year continuous remediation. The temperate was of continental climate, with an average annual temperature of 7.5 ~ 8.2 ℃, sunshine duration of 2318 ~ 2732 h, frost-free period of 147 ~ 191 d, annual rainfall of 180 ~ 270 mm, and annual evaporation of 1000 ~ 1500 mm. The heavy metals in the soil of this area come mostly from chemical fertilizers, agricultural chemicals such as pesticides, and long-term continuous cropping. To manage and control potential errors, we used pots filled with loamy soil from test station cotton fields, where cotton had been continuously planted for more than 25 years. Here, because of long-term continuous cropping, the soil had various levels of heavy metals. Before collecting the samples, the depth of the soil sampling was 0-20 cm, we have prepared 800 kg of soil sample we removed soil debris by hand and then air dried the soil before passing it through a 2 mm-mesh sieve, keeping a sub-sample to determine the soil’s physicochemical characteristics. The hydrometer method was used to investigate soil particle size distribution, subsequently finding that the soil texture was clay loam (Bouyoucos, 1962), and then used the Walkley–Black method described by Nelson and Sommers (1982) to determine soil organic carbon. We used a soil–water suspension (w/v, 1:2.5), shaken for 1 h, to measure soil pH using a calibrated pH meter (WTW 7110, Weilheim, Germany) (Muhammad, 2019). Total nitrogen (N), phosphorous (P) and potassium (K) concentrations were determined using the Kjeldahl

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protocol (Bremner and Mulvaney, 1982), the Watanabe and Olsen method (1965), and the $HF-HClO_4-H_2SO_4$ digestion method (Page et al., 1982), respectively (*Table 1*).

Table 1. *Basic physical-chemical properties of biochar and soil used in the experiments*

Property	Biochar	Soil
pH	9.50	7.76
Total nitrogen ($g \cdot kg^{-1}$)	0.89	0.46
Total P ($g \cdot kg^{-1}$)	2.54	28.42
Organic matter ($g \cdot kg^{-1}$)	625	14.73
Total K ($g \cdot kg^{-1}$)	8.62	246.83
Total Cd ($mg \cdot kg^{-1}$)	0.021	0.25
Total salinity ($g \cdot kg^{-1}$)	-	3.36
Carboxyl ($mmol \cdot g^{-1}$)	0.20	-
Lactone ($mmol \cdot g^{-1}$)	0.25	-
Phenolic hydroxyl ($mmol \cdot g^{-1}$)	0.21	-

Biochar was prepared using anaerobic pyrolysis of cotton straw at 450 °C for 6 h, with a resultant biochar conversion rate of 37.5% (Parinda et al., 2016). That biochar was then dried, crushed, and screened through a 2-mm sieve and its pH was measured in a 10 $mmol \cdot L^{-1}$ $CaCl_2$ solution (solid: solution = 1:2.5 (w/v)) using a glass electrode and a Corning pH 10 portable pH meter (Acton, MA, USA). We used the same methods to determine total N, total P, total K, and organic carbon concentration as we used for the soil (*Table 1*). Biochar pore structure is illustrated in *Figure 1*.

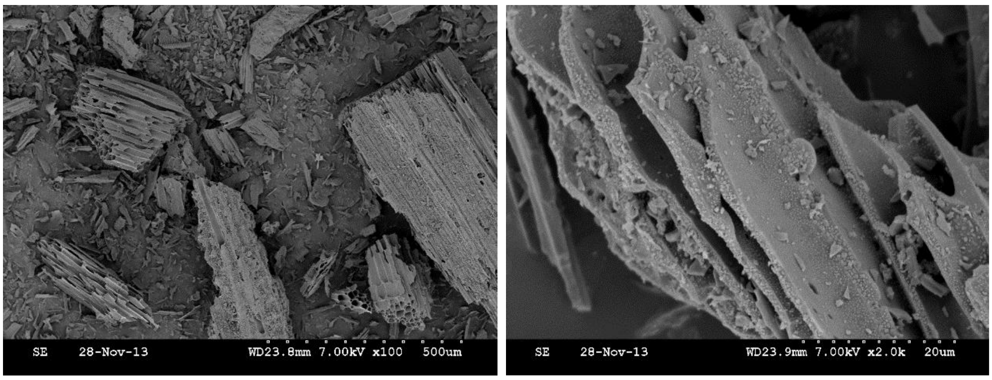

Figure 1. *Representative microporous structure of biochar magnified 100× (500 µm (left)) and 2,000× (20 µm (right))*

Experiment pot design

Before beginning the experiment, the soil was air dried and screened through a 2-mm sieve. We prepared contaminated soil by first dissolving our Cd source, $CdCl_2 \cdot 2.5H_2O$ (2.44 g, analytical reagent, Merck, Germany), in distilled water. We shook the preparation to ensure complete dissolution and then diluted it to 1000 mL, resulting in a 1.2 $g \cdot L^{-1}$ solution of Cd^{2+}. 10, 20, or 40 mL of that stock solution were each mixed with 12 kg soil samples, thus producing experimental samples with 1, 2, and 4 $mg \cdot kg^{-1}$ exogenous Cd^{2+} amount levels. Those levels correspond to 1-, 2-, and 4-times China's

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Level III Soil Environmental Quality standard for Cd. Control soil with no added Cd and the 1, 2, and 4 $mg \cdot kg^{-1}$ Cd samples were named H0, H1, H2, and H3, respectively. Next, we used a plastic container and a spatula to mix biochar into the soil at 1.5% and 3% w/w (equivalent to 23.4 $t \cdot ha^{-1}$ and 46.8 $t \cdot ha^{-1}$), respectively. Biochar levels of 0%, 1.5%, and 3% (mass ratio) were named C0, C1, and C2, respectively. Finally, we made 12 experimental soil treatments by completely mixing either H0, H1, H2, or H3 $CdCl_2$ soils with either C0, C1, or C2 biochar soils on a plastic cloth. For each experimental soil treatment, we put 12 kg of soil into a pot (25 cm × 30 cm), repeating each treatment 5 times for a total of 60 pots. The pots were placed in a randomized block design and maintained in identical conditions for 10 weeks (*Fig. 2*). Each pot received recommended doses of $N–P_2O_2–K_2O$ (180-150-210 $kg \cdot hm^2$ urea, diammonium phosphate, and potassium sulfate, respectively). All the P and K and half of the N was applied before crop sowing and the remaining N was applied after crop establishment.

Figure 2. *Pot culture experiment*

Planting and sample collection

This study was conducted under open air. We first bought seeds of a local cotton variety (LuYan 24) and then disinfected and sterilized (with 2.5% sodium hypochlorite) same-sized seeds. Twenty seeds were planted in each plastic pot and, once they had 3-5 true leaves, seedlings were thinned to 5 per pot and grown for 150 days. To prevent recontamination, we use deionized water for irrigation. We collected soil and leaves and stems plant samples at 30, 60, 90, 120, and 150 days of culture, and collected cotton bells at 60, 90, 120, and 150 days of culture. Soil samples were collected by sampling 3 points in each pot to a maximum soil depth of 20 cm, then the collected samples from each pot were combined and air-dried, the amount of the collected soil sample was

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180 g. Cotton stems, leaves, and bolls were collected and rinsed with deionized water and then weighed on a digital scale to determine each sample's wet weight, before they were oven dried (85 °C) to a constant dry weight.

Tests and assays

We used the Tessier 5 step continuous extraction method (Tessier et al., 1979) to sequentially extract trace metals, including Cd, for subsequent measurement at the end of each step. Each 0.2000 g air-dried soil sample was subjected to the following operations, each designed to extract a fraction of the metals in the soil:

1. Exchangeable: Soil samples were oscillated continuously for 1 h with 1 mol L^{-1} $MgCl_2$ solution (pH = 7).
2. Bound to carbonates: The residue in step (1) was oscillated continuously for 5 h with 1 mol·L^{-1} CH_3COONa solution (pH = 5).
3. Bound to iron (Fe) and manganese (Mn) oxides (Fe-MnO): The residue in step (2) was oscillated continuously for 6 h at 96 ± 3 °C with 0.04 mol·L^{-1} $NH_2OH·HCl$ diluted in 25% acetum solution.
4. Bound to organic matter: The residue in step (3) was oscillated for 2 h at 85 ± 2 °C with 3% H_2O_2 (adjusted to pH = 2 using HNO_3) and 0.02 mol·L^{-1} HNO_3 (volume ratio). Then 3% H_2O_2 (pH = 2) was added, and the mixture was oscillated continuously for 3 h at 85 ± 2 °C. After cooling, the mixture was oscillated continuously for 0.5 h with 3.2 mol·L^{-1} CH_3COONH_4 diluted in 20% HNO_3 (volume ratio).
5. The residue form: Using the subtraction method, after the fractions from the previous 4 extractions were subtracted from the sample, the remaining amount was the residue.

Between each step, the mixture was centrifuged at room temperature for 15 min at 2000 rpm and the supernatant was transferred into a 25 mL centrifugal tube to maintain constant volume. Then we used a Hitachi Z2000 graphite atomic absorption spectrophotometer (Hitachi, SiChuan., Tokyo, Japan) to detect metals in each supernatant sample.

We determined available Cd in the soil by performing diethyline-triamine-penta acetic acid extraction and then testing the extract with a graphite atomic absorption spectrophotometer (Mahanta et al., 2011). We then used microwave digestion-graphite atomic absorption spectrometry to determine Cd levels in the cotton stems, leaves, and bolls (Parinda et al., 2016). Soil pH was determined with the soil pH-potential method, in which soil: water = 2.5:1 (Yan et al., 2000).

In addition, soil water content was determined gravimetrically by comparing the wet and dry weights of soil samples (collected at the end of every cotton growing stage) to determine the amount of soil irrigation (the field moisture capacity of 60%-70%) (Wang et al., 2020).

Data analysis and visualization

The data were compiled in Excel 2016 and two-way analysis of variance (ANOVA) was performed using SPSS 23.0. Multiple comparisons between different treatments were conducted using Duncan's new multiple range test (α = 0.05). Charts were drawn using Origin 8.0 (OriginLab, MA, USA).

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Results and analyses

Changes in soil pH

The addition of biochar significantly increased soil pH ($P < 0.05$, *Table 2*), which rose in line with increasing biochar amounts. However, interactions between exogenous Cd and biochar had no significant effects on soil pH ($P < 0.05$) and, as time elapsed, soil pH decreased. The maximum pH at 30 days was in the C2H3 treatment (pH = 8.56), thus showing that added biochar increased pH than those in the C0 treatments (controls, no biochar) ($P < 0.05$). Overall, the experimental groups pH were higher than those of the control groups, but none of them were significantly different ($P < 0.05$). For instance, pH was not significantly different between the C0H0 treatment and the C1H0, C1H1, and C1H3 treatments after 150 days. After 30 days, the C0H0, C0H1, C0H2, and C0H3 treatments pH were 7.48, 7.44, 7.55, and 7.49, respectively, and this trend remained at 60, 90, 120, and 150 days.

Table 2. *Effects of biochar addition on soil pH*

Cd content (mg·kg^{-1})	Biochar (%)	pH				
		30d	60d	90d	120d	150d
H0	C0	7.48b	7.66b	7.54b	7.35b	7.12b
	C1	8.46a	8.24a	8.09a	7.99a	7.46b
	C2	8.49a	8.25a	8.28a	8.16a	7.98a
H1	C0	7.44b	7.56b	7.35b	7.33b	7.33b
	C1	8.48a	8.29a	7.94a	7.46b	7.46b
	C2	8.5a	8.31a	8.26a	8.09a	7.93a
H2	C0	7.55b	7.58b	7.52b	7.35b	7.33b
	C1	8.51a	8.32a	8.24a	7.92a	7.63ab
	C2	8.53a	8.36a	8.37a	8.00a	7.96a
H3	C0	7.49b	7.43b	7.69b	7.34b	7.36b
	C1	8.44a	8.44a	8.30a	8.07a	7.37b
	C2	8.56a	8.49a	8.27a	8.19a	8.03a
Regression analysis (significance)						
Cd content (H)		ns	ns	ns	ns	ns
Biochar ©		**	**	**	**	**
Interaction (H×C)		ns	ns	ns	ns	ns

C0, no added biochar; C1, 1.5% added biochar; C2, 3 % added biochar; H0, no added Cd; H1, 1 mg kg^{-1} Cd added; H2, 2 mg·kg^{-1} Cd added; H3, 4 mg·kg^{-1} Cd added. Different lowercase letters in the same column indicate significant differences ($P < 0.05$) in pH among individual treatments. **, $P < 0.01$; ns, $P \geq 0.05$

Effects of biochar on Cd forms in the soil

Throughout our sampling periods, Cd existed mostly as residue, accounting for 35.24%–63.02% of the total Cd (*Fig. 3*). Compared to C0 (no biochar), the biochar treatments exhibited reduced proportions of exchangeable and carbonate-bound Cd. After 30 days, compared to the C0H0 treatment, the proportion of exchangeable Cd decreased by 1.26% and 2.51% in the C1HO and C2HO treatments, respectively, and the proportion of carbonate-bound Cd decreased by 5.02% and 7.39% in the C1HO and

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C2HO treatments, respectively, while organic-bound Cd increased by 11.57% and 15.21%, respectively. This trend was the same in the H1, H2, and H3 groups (*Fig. 3a*). At 60 days, the proportion of exchangeable and carbonate-bound Cd in the C1H0 and C2H0 treatments decreased by 2.61% and 1.15% (C1H0), and 3.38% and 6.74% (C2H0) respectively, as organic-bound Cd increased by 2.65% and 5.86%, respectively in each treatment. Again H1, H2, and H3 trends for those tests mirrored these findings (*Fig. 3b*). The change of Cd forms after 90 days trended similarly to the 30 and 60-day results, indicating that biochar promoted the transformation of exchangeable and carbonate-bound Cd to organic-bound Cd (*Fig. 3c*). *Figure 3d* and *e* show changes of Cd forms in soils after 120 and 150 days, respectively. At the 120-day mark, the C1H0 and C2H0 treatments had decreased proportions of exchangeable and carbonate-bound Cd (5.01%, 7.69% (C1H0) and 2.37%, 5.52% (C2H0), respectively) and increased proportions of Fe-Mn oxide-bound Cd (9.98% and 18.07%, respectively, *Fig. 3d*).

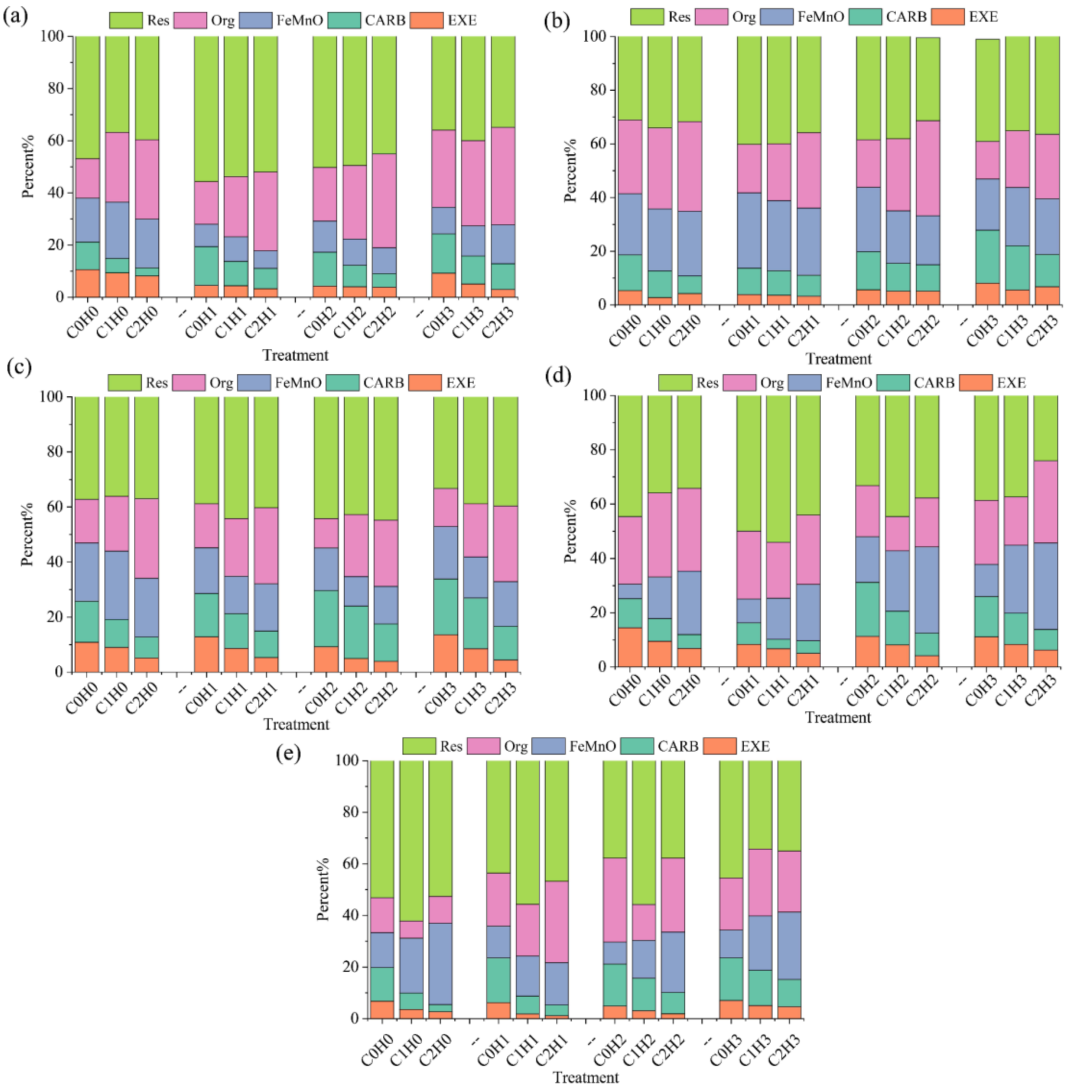

Figure 3. *Effects of biochar on Cd distribution in the soil after 30 days (a), 60 days (b), 90 days (c), 120 days (d), and 150 days (e). Res, Cd residue; Org, fraction bound to organic matter; FeMnO, fraction bound to Fe-Mn oxides; CARB, fraction bound to carbonates; EXE, exchangeable fraction; C0, no added biochar; C1, 1.5% added biochar; C2, 3% added biochar; H0, no added Cd; H1, 1 mg·kg^{-1} Cd added; H2, 2 mg·kg^{-1} Cd added; H3, 4 mg·kg^{-1} Cd added*

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This trend was similar in the H1, H2, and H3 groups, and for all treatments at 150 days (*Fig. 3e*) as well. So, after about 120 days of growth, exchangeable and carbonate-bound Cd in biochar treated soils were being converted to Fe-Mn oxide-bound Cd, instead of to organic-bound Cd, as was seen in earlier periods.

Effects of biochar on available Cd

In our study, the interaction of exogenous Cd with biochar significantly affected the amount of soil-available Cd (*Table 3*, $P < 0.05$). Available Cd increased along with increasing exogenous Cd concentrations and, as time passed, the proportions of available Cd initially decreased and then stabilized. In the C0H0, C0H1, C0H2, C0H3 treatments, available Cd changed little over time, varying from 0.0002–0.0143 mg·kg^{-1}, while available Cd in the C1 and C2 groups differed significantly through time ($P < 0.05$). The C0H0 treatment had the least available Cd (0.0808 mg·kg^{-1}) at 90 days, but it increased after that. The least available Cd in the C1H0 treatment (0.0702 mg kg^{-1}) was measured at 60 days and it stabilized after that, increasing to 0.0703 mg·kg^{-1} at 150 days. At 90 days, the C2H0 treatment had its lowest available Cd level (0.1107 mg·kg^{-1}), but that level later stabilized. So, available Cd in biochar treated soil without added Cd (C1H0 and C2H0) reduced significantly ($P < 0.05$), but available Cd in the biochar treatments with the smallest amount of added Cd (C1H1 and C2H1) were lower than that in the C0H1, no biochar/lowest added Cd treatment ($P < 0.05$). The lowest Cd amount for C1H1 (0.1107 mg·kg^{-1}) was measured at 90 days, while the lowest for C2H1 (0.0819 mg·kg^{-1}) was at 120 days. After reaching those minimum values, available Cd in both C1H1 and C2H1 stabilized and then decreased. In the H2 groups, available Cd in the C1H2 and C2H2 treatments was lowest at 120 days (0.1732 mg·kg^{-1} and 0.1108 mg·kg^{-1}, respectively), Changes in available Cd in the H3 groups trended similarly to those in the H2 groups. Available Cd in the C1H3 and C2H3 treatments was significantly less than that in the C0H3 treatment at 30, 60, 90, 120, 150 days. After 120 days, compared with C0H3, available Cd in the C2H3 treatment had decreased by 82.52% ($P < 0.05$).

Effects of biochar on Cd amounts in cotton plants

Biochar can reduce available Cd in soil by absorption, complexation, and precipitation, thus preventing cotton plants from absorbing Cd. *Figures 4, 5,* and *6* show that cotton leaves preferentially enrich in Cd; however, after adding biochar, cotton's aboveground Cd uptake decreased. In the C0H0 treatment, Cd uptake into cotton leaves, stems, and bolls increased from 0.0737, 0.0144, and 0.0103 mg·kg^{-1} at 30 days to 0.0834, 0.0151, and 0.0114 mg·kg^{-1} at 120 days, respectively, and then stabilized. We observed the same increasing trend in the other H groups. But Cd uptake in the C1 and C2 groups decreased as cultivation time increased. For example, Cd uptake into cotton leaves, stems, and bolls in the C1H3 and C2H3 treatments decreased between 30 days to 150 days ($P < 0.05$) (C1H3: 0.7414, 0.0791, and 0.0538 mg·kg^{-1} to 0.5822, 0.0687, and 0.0519 mg·kg^{-1}, respectively, and C2H3: 0.7049, 0.0744, and 0.0496 mg·kg^{-1} to 0.5449, 0.0621, and 0.0471 mg·kg^{-1}, respectively). We observed a similar trend in the C1H1, C1H2, C2H1, and C2H2 treatments. In the CIH0 and C2H0 treatments, the lowest uptake of Cd into cotton leaves (0.0563 mg·kg^{-1} and 0.0427 mg·kg^{-1}), stems (0.0129 mg·kg^{-1} and 0.0113 mg·kg^{-1}), and bolls (0.009 mg·kg^{-1} and 0.0078 mg·kg^{-1}) occurred 90 days after biochar addition, corresponding to 30.58%, 49.28%, 15.69%, 26.14%, and 18.92%, 29.73% lower, respectively, than those of the C0 groups

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(P < 0.05). At the H1 level, Cd uptake into cotton leaves, stems, and bolls after 90 days was the lowest in the C1 treatment (0.1811, 0.0339, and 0.0156 mg·kg^{-1}, respectively), which was 23.01%, 13.96%, and 25.12% lower than in the C0H1 treatment. However, Cd uptake into cotton leaves, stems, and bolls reached its lowest point in the C2H1 treatment after 120 days (0.1551, 0.0300, and 0.0141 mg·kg^{-1}), which were 33.72%, 26.11%, and 32.86% lower than in the control groups. In the CIH0 and C2H0 treatments, the lowest uptake of Cd into cotton leaves (0.4197 mg·kg^{-1} and 0.4034 mg·kg^{-1}), stems (0.0632 mg·kg^{-1} and 0.0601 mg·kg^{-1}), and bolls (0.0385 mg·kg^{-1} and 0.0351 mg·kg^{-1}) decreased by 21.39%, 24.43%, 11.11%, 15.47% and 12.51%, 20.23%, respectively, then uptakes in the C0H2 treatment occurred 90 days after biochar addition. The H3 uptakes trended similarly to H2, showing that biochar reduces the uptake of Cd by cotton, most likely through Cd adsorption.

Table 3. *Effects of biochar on available Cd in the soil at different time points*

Cd content (mg·kg^{-1})	Biochar (%)	Available Cd (mg·kg^{-1})				
		30d	**60d**	**90d**	**120d**	**150d**
H0	C0	0.0934a	0.0862a	0.0808a	0.0871a	0.1016a
	C1	0.0759b	0.0702b	0.0702b	0.0702b	0.0703b
	C2	0.0682b	0.0598c	0.0439c	0.0439c	0.0439c
H1	C0	0.1641a	0.1573a	0.1518a	0.1498a	0.1493a
	C1	0.1340b	0.1242b	0.1107b	0.1107b	0.1108b
	C2	0.1087c	0.0939c	0.0896c	0.0819c	0.0819c
H2	C0	0.2285a	0.2261a	0.2303a	0.2248a	0.2239a
	C1	0.2051b	0.1935b	0.1761b	0.1732b	0.1733b
	C2	0.1513c	0.1474c	0.1260c	0.1108c	0.1107c
H3	C0	1.1145a	1.1208a	1.1194a	1.1106a	1.1138a
	C1	0.8966b	0.8297b	0.8150b	0.793b	0.7931b
	C2	0.3952c	0.3386c	0.2195c	0.1959c	0.1958c
Regression analysis (significance)						
Cd content (H)		**	**	**	**	**
Biochar (C)		**	**	**	**	**
Interaction (H×C)		**	**	**	**	**

H0, no added Cd; H1, 1 mg·kg^{-1} Cd added; H2, 2 mg·kg^{-1} Cd added; H3, 4 mg·kg^{-1} Cd added; C0, no added biochar; C1, 1.5% added biochar; C2, 3% added biochar; Different lowercase letters in the same column indicate significant differences ($P < 0.05$) in pH among individual treatments. **, $P < 0.01$

Correlations between soil pH, Cd forms, and Cd amounts in cotton

Cd amounts in cotton leaves and stems were significantly correlated with Cd amounts in the soil (r = 0.977 and 0.915, respectively; P < 0.01) and Cd amounts in cotton leaves, stems, was positively correlated with the amounts of exchangeable and available Cd in the soil (*Table 4*). However, Cd amounts in cotton leaves, stems, and bolls was significantly negatively correlated with the organic-bound Cd amount in the soil (r = -0.633, -0.608, and -0.968, respectively; (P < 0.01)), as were pH with the amounts of available, exchangeable, and carbonate-bound Cd in the soil (r = -0.66, -

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0.543, and -0.555, respectively; $P < 0.01$). Total Cd correlated highly with all Cd forms, while available Cd also correlated highly with both exchangeable and carbonate-bound Cd forms ($r = 0.943$ and 0.716, respectively; $P < 0.01$). Fe-Mn oxide-bound Cd amounts were significantly correlated with organic-bound, but not with available, exchangeable, and carbonate-bound, Cd amounts.

Table 4. *Relationships between soil pH, and Cd contents in the soil and in cotton samples*

Indicators	Soil pH	Soil Cd	Leaf Cd	Stem Cd	Available Cd	EXE	CARB	FeMnO	Org
Soil pH	1								
Soil Cd	0.138	1							
Leaf Cd	-0.126	0.977**	1						
Stem Cd	-0.139	0.915**	0.973**	1					
Available Cd	-0.66**	0.892**	0.797**	0.688**	1				
EXE	-0.543**	0.951**	0.668**	0.871**	0.943**	1			
CARB	-0.555**	0.821**	0.211	0.174	0.716**	0.815**	1		
FeMnO	0.244	-0.642**	-0.265	-0.254	-0.225	-0.191	-0.208	1	
Org	0.254	-0.778**	-0.633**	-0.608**	-0.240	-0.238	-0.209	0.304*	1

Org, Cd fraction bound to organic matter; FeMnO, Cd fraction bound to Fe-Mn oxides; CARB, Cd fraction bound to carbonates; EXE, exchangeable fraction of Cd. **, $P < 0.01$; *, $P < 0.05$

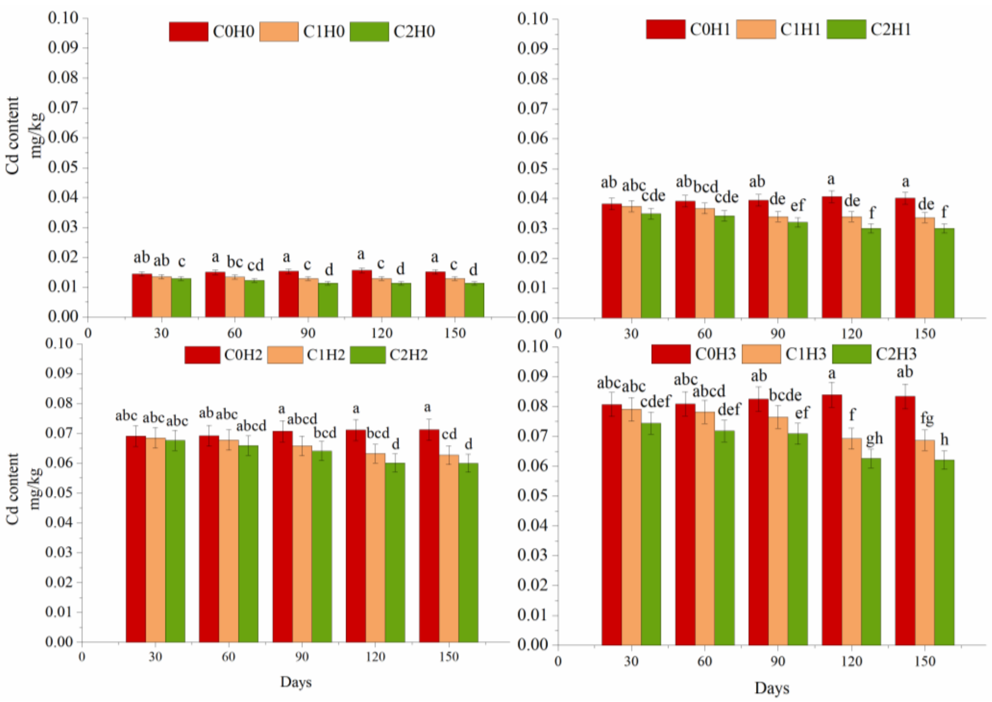

Figure 4. *Effects of biochar on Cd contents in cotton leaves over time. C0, no added biochar; C1, 1.5% added biochar; C2, 3% added biochar; H0, no added Cd; H1, 1 mg·kg^{-1} Cd added; H2, 2 mg·kg^{-1} Cd added; H3, 4 mg·kg^{-1} Cd added. Error bars represent SD, Different letters within the same variety and column indicate significant difference at 0.05 level*

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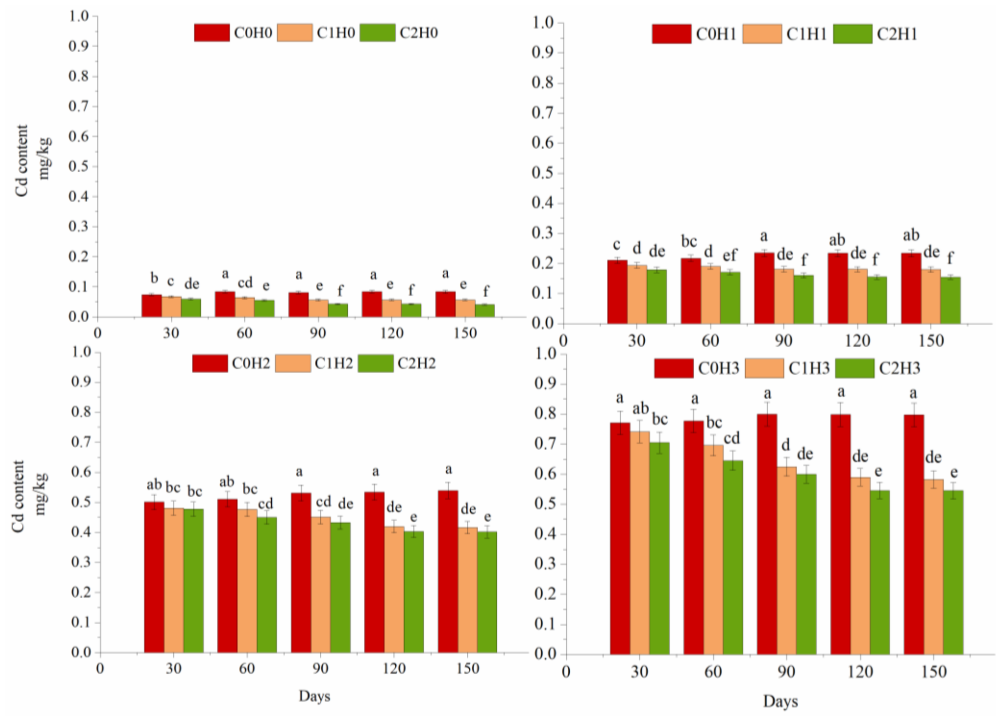

Figure 5. *Effects of biochar on Cd contents in cotton stems over time. C0, no added biochar; C1, 1.5% added biochar; C2, 3% added biochar; H0, no added Cd; H1, 1 mg·kg^{-1} Cd added; H2, 2 mg·kg^{-1} Cd added; H3, 4 mg·kg^{-1} Cd added. Error bars represent SD, Different letters within the same variety and column indicate significant difference at 0.05 level*

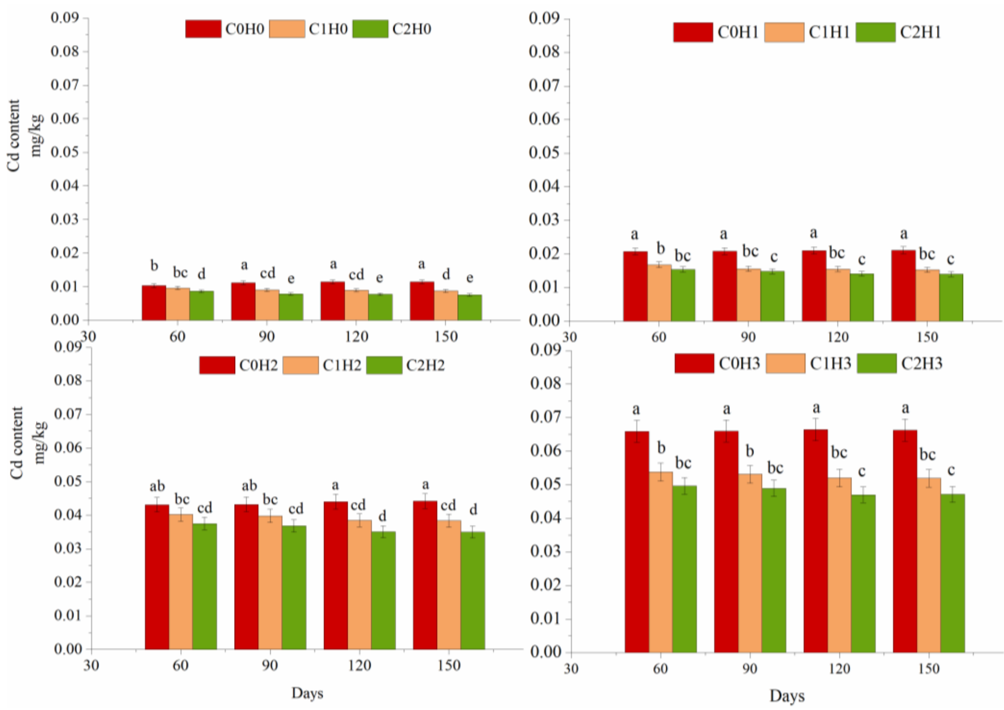

Figure 6. *Effects of biochar on Cd content in cotton bolls over time. C0, no added biochar; C1, 1.5% added biochar; C2, 3% added biochar; H0, no added Cd; H1, 1 mg·kg^{-1} Cd added; H2, 2 mg·kg^{-1} Cd added; H3, 4 mg·kg^{-1} Cd added. Error bars represent SD, Different letters within the same variety and column indicate significant difference at 0.05 level*

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Discussion

Biochar has a rich microporous structure that forms a large specific surface area (Rehman et al., 2021; Ma et al., 2020; Eissa et al., 2019), and its ash contains much soluble calcium, magnesium, potassium, sodium, and other salt-based ions. Biochar added to soil improves the soil salt-based saturation to some extent as salt-based ions interact with both hydrogen and exchangeable aluminum ions. Within this ion-exchange interaction, soil pH increases as the amount of both hydrogen and aluminum ions decrease (Kookana et al., 2011). In our study, the addition of biochar significantly increased soil pH, and pH increases paralleled increasing amounts of added biochar, peaking in the 3% added biochar treatment (0.97–1.47 units relative to the blank). Guo et al. (2017) found that biochar promoted soil sediment formation ($CdCO_3$, $Cu(OH)_2$, and $Pb_5(PO_4)_3OH$) as soil pH increased, thereby reducing the soil Cd amount. In our study, biochar addition also reduced the amount of available Cd in the soil. The more biochar was added, the better the adsorption of soil Cd was. Without biochar and exogenous Cd, soil pH initially increased but then decreased with time. Soil pH also initially increased and then decreased, finally stabilizing with the addition of exogenous Cd without biochar. Irrigation affects the process of soil reduction, ultimately resulting in the consumption of H^+ ions with an accompanying rapid soil pH increase. However, the combined effects of the resultant intermediate product (organic acid) and the final carbonate end product (CO_2) produced during watering causes a gradual decrease in soil pH over time (Yang et al., 2016; Cao et al., 2011).

Availability of heavy metals is an important indicator of heavy metal environmental behavior, and the addition of biochar can effectively reduce that availability. After 2 years, Bian et al. (2014) found that wheat straw biochar continuously reduced the available Cd in paddy soil. By increasing soil pH, biochar promotes ion exchange on its surface (e.g., Ca^{2+}, K^+, and Mg^{2+} in the form of oxides or carbonates) and therefore reduces the mobility of exchangeable and carbonate-bound Cd in the soil (Aslam et al., 2017), an effect that increases along with increasing soil pH (Zhang et al., 2013; Kim et al., 2018). The adsorption of Cd to biochar also plays a non-negligible role in sequestering Cd. In our study, available soil Cd initially decreased and then stabilized, as time passed. The extent of that reduction also differed between the different amounts of exogenous Cd. With no exogenous Cd, the available Cd in the soil decreased to its lowest level at 150 days, but Cd amounts in soil with 1 $mg \cdot kg^{-1}$ added Cd^{2+} were the lowest after 120 and 150 days for treatments with 1.5% and 3% biochar, respectively. As exogenous Cd amounts increased (2 $mg \cdot kg^{-1}$ Cd^{2+} and 4 $mg \cdot kg^{-1}$ Cd^{2+}), the treatments with 1.5% and 3% biochar experienced their lowest Cd amounts at 120 days. We found that the treatments with less added biochar and greater exogenous Cd reached equilibrium quicker than in other treatments, thus indicating that the ability of biochar to adsorb metals decreased over time (Bian et al., 2014). This stabilization at a later period, Kookana et al. (2010) called it “aging”, in which natural organic molecules lead to the “aging” of biochar adsorption capacity, resulted in a balance of available Cd amount in soils (Xu et al., 2017; Mousavi et al., 2010).

Many studies have shown that exchangeable, carbonate-bound, and Fe-Mn oxides of heavy metals are available states and exhibit bioavailability under certain conditions. The effect of biochar on Cd form differs in different regions (Bashir et al., 2017). For example, Zhu et al. (2015) found that biochar decreased exchangeable Cd and increased Fe-Mn oxide-bound Cd in Shenyang paddy soil, while Gu et al. (2018) studied the transformation of Cd form in Hunan Province farmland soils and found that biochar

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decreased exchangeable Cd but increased both organic-bound and residual Cd. Our results showed that, after biochar addition, the proportion of exchangeable and carbonate-bound Cd decreased; organic-bound Cd increased after 30, 60, and 90 days; and the proportion of exchangeable and carbonate-bound Cd decreased at 120 and 150 days, respectively, while the proportion of Fe-Mn oxide-bound Cd increased. The differing results of each study may be because of soil differences and the raw materials used to make biochar (e.g., different proportions of cellulose, hemicellulose, and lignin) between regions result in both inconsistent Cd speciation and effects of biochar on heavy metal Cd forms (Cantrell et al., 2012). However, when soil pH exceeds the zero charge of an Fe-MnO colloid, the charge on the colloidal surface will change from positive to negative, thus increasing the Cd-adsorptive capacity of the colloid and possibly explaining the increase of Fe-Mn oxide-bound Cd (Neumann et al., 2001).

Cd is transported to aboveground plant organs via root absorption. Generally, after biochar addition the Cd amount in those organs decreased, but the effects of each biochar treatment on the accumulation of Cd differed (Zong et al., 2021). Abid et al. (2017) found that Cd amounts in tomato roots were 33% lower than in the control when 1% biochar was added and Jin et al. (2011) showed that Cd amounts in Indian shepherd's purse buds were 76.1%, 82.2%, and 96.3% lower than in the control when 1%, 5%, and 15% chicken manure biochar, respectively, was added. In our study, biochar addition significantly reduced Cd accumulation in cotton, with the largest decrease occurring in the C2H3 treatment (49.28%) and resembling results were published by Rehman et al. (2021). The accumulation and distribution of heavy metals in plants depend on plant species, element species, chemical and biological availability, redox, pH, cation exchange capacity, dissolved oxygen, temperature, and root secretions (Jin et al., 2011). Because of its large biomass, cotton has great tolerance to Cd and the accumulation of Cd, but cotton seedling growth was inhibited when the exogenous soil Cd amount was 20 $mg \cdot L^{-1}$. As their Cd amount increased from 20 $mg \cdot L^{-1}$ to 80 $mg \cdot L^{-1}$, the seedlings' stem and root lengths decreased significantly, but they still grew normally. Given this resilience, cotton is considered a good crop to grow for heavy metal remediation (Jin et al., 2011). Overall, our cotton exhibited good Cd absorption capacity, even without biochar, a result consistent with Ma et al. (2017). In our study, Cd primarily accumulated in cotton leaves, with a maximum uptake in the C0H3 trial (0.7992 $mg \cdot kg^{-1}$). Coincidentally, Cd uptake differed between different parts of various other crops, too. In pakchoi, Cd accumulated more in leaves than in roots and more in leaves than in petioles (Chen et al., 2012), but in maize and wheat, more Cd accumulated in leaves than in grain, and wheat grain enriched in Cd more than corn grain (Wang et al., 2017). In our study, Cd preferentially accumulated in leaves, then in stems and then in bolls. While soil pH did not correlate greatly with total Cd, it did correlate significantly with available Cd, primarily because biochar affected the distribution of heavy metal forms by affecting soil pH (Guo et al., 2017). That correlation showed that available Cd in soil could be directly absorbed by plants, resulting in a significant effect of available Cd on the Cd amount in cotton leaves and stems. Since biochar reduces the availability of soil Cd, it most likely reduces the accumulation of heavy metals in the ground.

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Conclusions

Soil pH increased and available Cd decreased significantly with the addition of biochar, when the Cd dosage was 1 mg·kg^{-1}, available Cd in the 1.5% biochar treatment was the lowest at 120 days and at 150 days for the 3% biochar treatment. When Cd dosages were 2 and 4 mg·kg^{-1}, soil-available Cd reached minima at 120 days for both the 1.5% and 3% biochar treatments. Exchangeable Cd in the soil was the main source of accumulated Cd in aboveground plant organs. Biochar increased the proportion of organic-bound Cd (30, 60, and 90 days) and Fe-Mn oxide-bound Cd (120 and 150 days), then, biochar promoted the transformation of exchangeable and carbonate-bound Cd to organic-bound and Fe-Mn oxide-bound Cd. Finally, biochar (1.5% and 3%) reduced soil available Cd content and slowed Cd uptake by cotton (*Gossypium hirsutum L.*). The physiological mechanism of biochar on *Gossypium hirsutum* L. growth promotion and Cd stress tolerance is not clear. The next step is to determine the effect of biochar on metabolism and metagenome of *Gossypium hirsutum* L. to determine the role of biochar under heavy metal stress.

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Conflict of interests. The authors declare that they have no conflict of interests.

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