ORIGINAL RESEARCH

Bamboo biochar greater enhanced Cd/Zn accumulation in *Salix psammophila* under non-flooded soil compared with flooded

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Abstract

As a metal immobilizer, biochar can be used to remediate contaminated soil. Biochar's effect on the phytoremediation process in flooded conditions under a scenario of increasing flooding frequency as global climate change is not well understood. This study investigated bamboo biochar (BBC) effects on growth and metal accumulation of *Salix* in multi-metal contaminated soil under non-flooded versus flooded conditions. *Salix* cuttings were cultivated in pots with severely contaminated soil by Cd and Zn, for 120 days, with four treatments including non-flooded treatment, flooded treatment, non-flooded with 3% BBC application (BBC/soil, w/w), and flooded with 3% BBC addition. BBC, flooding, and BBC× flooding significantly decreased the bioavailability of metals in soils ($P < 0.05$). The BBC addition markedly stimulated Cd concentration in leaves under non-flooded (94.20%) and flooded conditions (32.73%) but showed little effect on roots. The BBC significantly boosted Cd and Zn transport from roots to aboveground parts by 68.85% and 102.27% compared with no BBC amendment under non-flooded treatment, while showing insignificant changes under flooded treatment. Although the plant biomass was little affected, BBC significantly increased Cd and Zn accumulation in the whole plant by 52.53% and 28.52% under non-flooded while showing an insignificant impact under flooded conditions. Taken together, BBC enhanced the phytoremediation efficiency of *Salix* to Cd and Zn in severely polluted non-flooded soil, while flooding offset this effect. The results indicated the effects of BBC varied under different soil moisture, which should be considered in the biochar-assisted phytoremediation to variable and complex environments.

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Graphical abstract

Highlights

- Bamboo biochar improved remediation efficiency of Cd/Zn by *Salix* in non-flooded soil.
- Flooding offset the phytoremediation-promoting effect of bamboo biochar on *Salix*.
- Biochar alleviated the inhibition of plant growth caused by the flooded condition.

Keywords Heavy metals · Amendment · *Salix* · Phytoremediation · Soil flooding

1 Introduction

Soil contamination caused by heavy metals (HMs) is a global environmental issue that seriously threatens the ecology, human health, and social sustainability (Ali et al. 2013; Murtaza et al. 2017; Xia et al. 2019). Phytoremediation is an alternative to traditional methods (e.g., excavation and landfilling, soil vapor extraction, soil washing, etc.) as it is cost-effective, environmentally friendly, and in situ. As a green remediation technology, phytoremediation has gained much more attention and can efficiently remove or immobilize toxic contaminants via plants (Antoniadis et al. 2021; Chaney 1983). *Salix* plants have been studied as a potential candidate for phytoremediation/dendroremediation due to

their unique characteristics, including fast growth, deep and extensive roots, short rotation, coppice systems, high antioxidant enzyme activity in the leaves, efficient transpiration, and high nutrient uptake capacity (Marmioli et al. 2011; McBride et al. 2017). Further, *Salix* plants exhibit the ability to cope with several adverse environmental stresses, including HMs, floods, salt, alkali, and drought (Liu et al. 2011b; Tlustoš et al. 2007).

With domestic sewage and industrial wastewater discharge, the lowland zone beside riverside or lakeshore seasonally flooded soil or wetlands were increasingly vulnerable to HMs contamination (Bai et al. 2016). Flooding could alter the soil properties such as soil pH, organic matter, and oxygen content (Chen et al. 2012). Due to the liquid phase of water replacing the gas phase in the soil, the soil oxygen content decreased rapidly, thus limiting the plant root's respiration capacity. Moreover, the photosynthetic rate and photosynthate transport of plants are also obviously inhibited by soil flooding (Pezeshki 2001; Rood et al. 2010). The environmental behaviors of HMs in the soil–plant system were significantly altered by soil flooding (Cao et al. 2017). The *Salix* showed marked tolerance to oxygen shortage in flooded soils with the trait of the presence of adventitious roots (Jackson and Attwood 1996). Yang et al. (2020) reported that *Salix* showed phytostabilization potentials of Cu/Zn under flooded conditions, and the fertile lenticels/adventitious roots of *Salix* conferred the tolerance to

flooding. Previous results suggested that *Salix integra* grown in flooded soil contaminated by Cu could remain relatively high phytoremediation efficiency compared to that in non-flooded soil (Cao et al. 2017). Thus, *Salix* species can be efficient in the dendroremediation of HMs in flooded soil or wetlands.

Biochar, obtained from agricultural, forestry, wastes, and other cheap raw materials, has attracted a wide range of attention in the remediation of HMs-contaminated soil (Wu et al. 2019). Generally, biochar is characterized by high surface area, numerous functional groups, porosity, high cation exchange capacity (CEC), and alkaline pH value (Thomas et al. 2020; Wen et al. 2021; Xiao et al. 2020). Biochar as a good amendment could influence the availability of soil HMs by direct effect (such as electrostatic attraction, ion exchange, complexation, and precipitation) and indirect effect (such as pH, CEC, mineral content, and dissolved organic carbon) (He et al. 2019). Biochar amendment could inhibit the metal uptake and accumulation in edible parts of crops and ensure food safety, widely applied in the remediation of slightly HMs-contaminated farmland (Liu et al. 2021; Rehman et al. 2017; Shu et al. 2016; Xie et al. 2021). And several lines of evidence suggest a potential role of biochar in phytoremediation. Biochar can also improve the environment of the root rhizosphere and enhance plant growth and metal accumulation in heavily polluted soil (Salam et al. 2019). However, these results were mainly obtained from upland soil, which could not reflect the actual circumstance of wetland pollution. Limited information is received on the biochar application in phytoremediation as affected by the combined effects of HMs and soil flooding. Hence, it is crucial to understand the impact of biochar on dendroremediation in flooded soil, further meeting the needs of phytoremediation in HMs-polluted wetland.

In the current study, we investigated plant growth and HMs accumulation of *Salix psammophila* grown in heavily contaminated soil by Cd and Zn, with 3% bamboo biochar (BBC) addition according to previous results (Li et al. 2021), under non-flooded or flooded conditions. The aim of the current study is, (1) to examine the effect of BBC on plants growth and HMs accumulation in *Salix* grown in severely polluted soil under non-flooded and flooded conditions, (2) to explore the possible underlying interactive mechanism of BBC-HMs-*Salix* under non-flooded or flooded conditions. The study results help to broaden our understanding on the interactive mechanism of biochar-flooding-plant, and shed light on the decision-making strategy for application of biochar assisted phytoremediation to severely polluted wetland soils caused by HMs.

2 Materials and methods

2.1 Soil and biochar preparation

The experiment was conducted in an alkaline (pH = 7.65) and multi-metal polluted soil, which was collected from the farmland near a chemical plant of ZnSO₄ reagents nearby Hangzhou City (29° 53' N, 119° 54' E), Zhejiang Province, China. The soil was air-dried, ground, passed through a 2-mm sieve to remove rocks and plant roots, and homogenized for experiments. Total Cd and Zn content in the soil was 60.23 and 2267.39 mg kg⁻¹, respectively, indicating that the sampled soil was heavily contaminated with Cd and Zn.

The BBC used in this experiment was purchased from Yaoshi Charcol Production Company, China, made from bamboo powder with slow pyrolysis at ~ 600 °C then crushed through a 0.25 mm sieve. The biochar was used as supplied without prior washing to remove soluble salts. The properties of the tested soil and BBC are listed in Table 1.

2.2 Pot experiment

The pot experiment was conducted in a greenhouse at the Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, China. This experiment used polyvinyl chloride pots (PVC) with 11 cm in diameter and 25 cm in height. Each pot was filled with 1.5 kg of contaminated sampled soil. Based on our previous research findings (Li et al. 2021), the 3% BBC application rate (BBC/soil, w/w) was selected. The cuttings (approximately 15 cm in length and 0.8 cm in diameter) of *Salix psammophila* were planted and cultivated in the PVC pots. There were four treatments with three replicates were set up in the experiment: NF-0, non-flooded treatment without BBC addition; F-0, flooded

Table 1 The properties of tested soil and bamboo biochar (BBC)

Samples	Soil	BBC
pH	7.65 ± 0.05	9.24 ± 0.001
Organic matter (g kg ⁻¹)/ C _{org} ^a (g kg ⁻¹)	74.93 ± 4.15	554.70 ± 18.45
N (g kg ⁻¹)	3.96 ± 0.01	2.07 ± 0.03
P (g kg ⁻¹)	1.20 ± 0.03	0.07 ± 0.003
K (g kg ⁻¹)	7.33 ± 0.17	0.88 ± 0.05
S (g kg ⁻¹)	—	0.08 ± 0.005
Cd (mg kg ⁻¹)	60.23 ± 1.75	0.11 ± 0.004
Zn (mg kg ⁻¹)	2267.39 ± 65.41	12.22 ± 0.65
Cu (mg kg ⁻¹)	119.59 ± 5.42	19.13 ± 1.77
Pb (mg kg ⁻¹)	217.88 ± 4.88	1.29 ± 0.07

^aC_{org} is the organic carbon in the bamboo biochar; C_{org}/N = 268. Values are means ± standard deviation (n = 3)

treatment without BBC addition; NF-3%, non-flooded treatment with 3% BBC addition; F-3%, flooded treatment with 3% BBC addition. The tested soil maximum water holding capacity was determined prior to experiment as described by Wilcox (1939). During the growth period, 300 mL tap water was watered every other day, to keep the moisture of approximately 70% of the soil water holding capacity. Flooded treatment occurred after initially being cultivated for 2 months, and the water level was set above the topsoil at 10 cm. The total duration of the pot experiment was 4 months.

2.3 Sampling and analysis

After 2 months of soil flooding, the soil and plant samples were collected from each pot. The plants were separated into roots, cuttings, stems, and leaves and washed with deionized water three times. Subsequently, they were dried in an oven at 105 °C for 30 min, and then at 80 °C for 48 h to constant weight, and the dry weight was recorded. All oven-dried samples were ground to a fine powder using a ball mill (Retsch MM400, Germany). Approximately 0.1 g of the ground samples was digested with 6 mL HNO₃ at 120 °C for 45 min and then 2 mL H₂O₂ for 30 min in a hot block system (ED36, Lab Tech, Germany). The concentrations of HMs (Cd, Zn, Cu, and Pb) and mineral elements in solution were determined using an Induced Couple Plasma-mass Spectrometer (ICP-MS, Agilent 7700x, USA).

The soil samples were aired and ground for physico-chemical analysis. Soil pH was determined using a pH meter (PHS-2CW-CN, Bante instrument, Shanghai, China) in 2.5:1 water/soil suspension. The soil organic matter (SOM), total and hydrolytic nitrogen (HN), total and available phosphorus (AP), total and available potassium (AK) were all analyzed according to the protocols as described in Lu (1999). The bioavailable HMs were extracted with buffered DTPA solution (Martens and Lindsay 1990). Briefly, 20 mL diethylenetriaminepentaacetic acid (DTPA)–calcium chloride (CaCl₂)–triethanolamine (TEA) solution (pH 7.3) was added to 10 g soil for extraction, then centrifugated to extract the supernatant. The sampled soil was digested with HNO₃ and HCl mixture (1:3 v/v ratio) using a hot block system (ED36, Lab Tech, Germany) to determine total HMs. The concentrations of Cd, Zn, Cu, and Pb were determined using an Induced Couple Plasma-mass Spectrometer (ICP-MS, Agilent 7700x, USA). Soil anions (including F[−], Cl[−], NO₂[−], NO₃[−], and SO₄^{2−}) were extracted by deionized water and detected by ion chromatography (MIC-5, Metrohm, Switzerland).

2.4 Data analyses

The translocation factor (*TF*) was calculated to evaluate the HMs transport capacity of *Salix psammophila* as affected by BBC and soil flooding.

$$TF = C_{aboveground\ parts} / C_{roots}$$

where $C_{aboveground\ parts}$ and C_{roots} are the contents of a specific metal in the aboveground parts and roots, respectively (Martina et al. 2003).

All statistical analyses were performed using Data Processing System software (DPS13.01, Zhejiang University, Hangzhou, China) by two-way analysis of variance (ANOVA) with a least significant difference (LSD) at a significance level of 0.05. The figures were plotted with Origin 2021 (Originlab Corporation, Northampton, MA, USA) and Microsoft Excel 2016 (Microsoft Corporation, USA).

3 Results

3.1 Soil properties and HMs bioavailability

The addition of BBC showed little effect on soil pH under non-flooded conditions while significantly increasing the soil pH (0.20 units) in F-3% treatment compared with F-0 treatment under flooded conditions. The BBC amendment markedly increased SOM by 33.46%, and AK by 156.02% in NF-3% than in NF-0 treatment, increased SOM by 22.19%, and AK by 57.10% in F-3% treatment compared to those treated with F-0 treatment, respectively. BBC addition (F-3%) in flooded soil significantly decreased AP content (15.38%) compared to the F-0 treatment (Table 2). The two-way analysis results revealed significant interactions among the BBC and flooding treatment on soil pH, AP, and AK content ($P < 0.05$).

The total HM contents in soil were not significantly altered with BBC or flooding treatment compared with NF-0 treatment (Table 3). The BBC addition (NF-3%) significantly decreased the bioavailable contents of Cd and Zn under non-flooded conditions compared with NF-0 treatment, while it (F-3%) showed an insignificant effect under flooded conditions (F-0). Relative to F-0 treatment, BBC addition (F-3%) reduced the proportion of bioavailable Cd in the soil more significantly compared to NF-0 treatment. A significant interaction between BBC and flooding treatment was observed in the bioavailable Cd, Zn, and Cu contents and the ratio of bioavailable Pb to total Pb in the soil. The BBC addition (NF-3%) increased F[−], NO₂[−], NO₃[−] and SO₄^{2−} contents by 12.22%, 95.57%, 8.17% and 57.48% in non-flooded soil compared with NF-0 treatment, respectively. F-3% increased F[−], NO₂[−] and NO₃[−] contents by 6.56%, 292.40%, and 7.64% compared with F-0, respectively, while decreased SO₄^{2−} content by 36.53%. Compared with NF-0 treatment, F-0 significantly increased the soil F[−] content by 17.99% while decreasing the contents of Cl[−],

Table 2 Effect of BBC and flooding on physical and chemical properties of soil

Physicochemical properties	Treatment				Significance B/F/BF
	NF-0	F-0	NF-3%	F-3%	
pH	7.65 ± 0.05b	7.70 ± 0.11b	7.61 ± 0.03b	7.90 ± 0.07a	ns/**/*
Organic matter/(g kg ⁻¹)	74.93 ± 4.15b	80.23 ± 4.30b	100.00 ± 8.87a	98.03 ± 2.95a	***/*ns/ns
Total nitrogen/(g kg ⁻¹)	3.96 ± 0.01a	3.98 ± 0.10a	3.83 ± 0.03b	3.92 ± 0.02ab	*/ns/ns
Total phosphors/(g kg ⁻¹)	1.20 ± 0.03a	1.23 ± 0.06a	1.23 ± 0.06a	1.21 ± 0.06a	ns/ns/ns
Total potassium/(g kg ⁻¹)	7.33 ± 0.17a	7.59 ± 0.33a	7.47 ± 0.55a	7.50 ± 0.40a	ns/ns/ns
Hydrolytic N/(mg kg ⁻¹)	402.67 ± 8.74a	368.33 ± 19.35ab	384.67 ± 17.79ab	359.00 ± 27.87b	ns/*/*ns
Available P/(mg kg ⁻¹)	34.62 ± 1.66b	42.58 ± 1.36a	36.28 ± 0.96b	36.03 ± 0.18b	***/*/*/*
Available K/(mg kg ⁻¹)	65.23 ± 6.10c	115.00 ± 7.00b	167.00 ± 9.17a	180.67 ± 13.20a	***/*/*/*

Different letters (a, b, c, d) indicate statistically significant differences of the same index between the groups (ANOVA, LSD test, $P < 0.05$), values are means ± standard deviation (n=3). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant)

Table 3 Effect of BBC and flooding on bioavailable heavy metals in soil

Metals	Treatment	Cd	Zn	Cu	Pb
Total metals in soil (mg kg ⁻¹)	NF-0	60.23 ± 1.75a	2267.39 ± 65.41a	119.59 ± 5.42a	217.88 ± 4.88a
	F-0	55.27 ± 7.15a	2109.54 ± 281.96a	112.90 ± 11.74a	198.13 ± 23.72a
	NF-3%	57.41 ± 1.46a	2174.70 ± 84.25a	117.33 ± 5.10a	206.66 ± 3.77a
	F-3%	58.33 ± 1.84a	2192.85 ± 29.83a	117.37 ± 3.55a	211.59 ± 5.07a
Significance	B/F/BF	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns
Total metals in water (mg L ⁻¹)	F-0	0.012 ± 0.008a	0.271 ± 0.138a	0.034 ± 0.018a	0.014 ± 0.008a
	F-3%	0.002 ± 0.0006a	0.095 ± 0.008a	0.015 ± 0.003a	0.004 ± 0.002a
Bioavailable metals content (mg kg ⁻¹)	NF-0	26.95 ± 0.2a	149.01 ± 0.84a	30.12 ± 1.69a	17.28 ± 1.19b
	F-0	21.82 ± 0.27c	143.57 ± 0.99c	24.37 ± 1.26c	15.13 ± 0.46b
	NF-3%	24.57 ± 0.04b	145.92 ± 1.07b	27.77 ± 0.6b	24.46 ± 4.29a
	F-3%	21.87 ± 0.29c	144.16 ± 0.23c	29.97 ± 0.65a	17.89 ± 1.43b
Significance	B/F/BF	***/*/*/*	*/****/*	*/****	*//*/*ns
Ratio of Bioavailable/total metals in soil %	NF-0	44.77 ± 1.68a	6.58 ± 0.23a	25.22 ± 1.88a	7.94 ± 0.61b
	F-0	39.97 ± 5.69ab	6.90 ± 1.00a	21.76 ± 2.70b	7.70 ± 0.87b
	NF-3%	42.81 ± 1.14ab	6.72 ± 0.24a	23.70 ± 1.21ab	11.82 ± 1.88a
	F-3%	37.51 ± 1.15b	6.57 ± 0.08a	25.54 ± 0.48a	8.45 ± 0.52b
Significance	B/F/BF	ns/*/*ns	ns/ns/ns	ns/ns/*	*//*/*

Different letters (a, b, c, d) indicate statistically significant differences of the same index between the groups (ANOVA, LSD test, $P < 0.05$), values are means ± standard deviation (n=3). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: not significant)

NO₂⁻ and SO₄²⁻ by 68.03%, 54.97%, and 40.70%, respectively. The F-3% treatment increased the soil F⁻ content by 12.05% while decreasing the Cl⁻, NO₂⁻ and SO₄²⁻ contents by 68.58%, 9.30%, and 76.10% compared with NF-3% treatment, respectively (Fig. 1).

3.2 Salix growth in different treatments

Salix plants maintained normal growth under all treatments and showed no visual symptoms of HMs toxicity and flooding stress in this study (Fig. 2). The BBC addition (NF-3%,

F-3%) showed little effect on plant growth (plant height, stem diameter) and biomass (aboveground biomass, root biomass, and total biomass) compared with non-flooded and flooded treatments (NF-0, F-0), respectively. Flooding (F-0) markedly decreased the plant height by 9.34%, biomass of the aboveground part by 22.85%, the total biomass of roots by 52.10%, and total biomass of the whole plant by 27.85% compared with NF-0. As a comparison, flooding with BBC addition (F-3%) only decreased the plant height by 9.07%, biomass of the aboveground part by 13.47%, the total biomass of roots by 40.12%, and total biomass of

Fig. 1 Effect of BBC and flooding on common anions content in the soil. The data indicate the means (red dot) $\pm$ standard deviation ($n=3$). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown (* $P<0.05$;

** $P<0.01$; *** $P<0.001$; ns: not significant). Different letters (a, b, c, d) indicate statistically significant differences between the groups (ANOVA, LSD test, $P<0.05$), $n=3$

Fig. 2 Effect of BBC and flooding on plant growth. The data indicate the means $\pm$ standard deviation ($n=3$). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown (* $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns: not signifi-

cant, A: aboveground part; R: underground and aerial root; T: total biomass). Different letters (a, b, c, d) indicate statistically significant differences of index in the same organs between the groups (ANOVA, LSD test, $P<0.05$), $n=3$

the whole plant by 18.02% compared with NF-0. And the ratio of the underground roots to whole roots biomass was increased in F-3% treatment (46.50%) than that in F-0 treatment (31.88%), while the ratio of the underground roots to the total biomass of the whole plant was 5.81% and 3.62% in F-3% and F-0 treatment, respectively. Flooding significantly decreased the plant biomass, while the significant interactive effect of BBC and flooding was not observed.

3.3 Contents of mineral elements in *Salix*

The BBC addition reduced the N contents in aboveground and underground parts of the plant under non-flooded and flooded conditions (Fig. 3). NF-3% treatment reduced N content by 21.49% and by 29.69% in aboveground parts and underground roots compared with NF-0 treatment, respectively. F-3% treatment also reduced the N content by 9.48% and 16.77% in aboveground parts and underground roots of the plant compared with F-0 treatment. Moreover, the F-3% treatment decreased N content by 18.20% in aerial roots compared with the F-0 treatment. When the plants were

Fig. 3 Nutrient contents in *Salix* plants. The data indicate the means (red dot) $\pm$ standard deviation ($n=3$). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown (* $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns: not significant). Different letters (a, b, c, d) indicate statistically significant differences of index in the same organs between the groups (ANOVA, LSD test, $P<0.05$), $n=3$

cultivated in the F-0 and F-3% treatments, the N contents were 24.03% and 12.41% less in the aboveground tissues of the plant compared with the plants grown in the respective non-flooding treatments (NF-0 and NF-3%). In contrast, the N contents were 36.62% and 61.71% greater in the plant's underground roots than those grown in the respective non-flooded treatment (NF-0 and NF-3%).

The plants in the NF-3% treatment markedly decreased the P accumulation by 27.19% and 31.51% in the aboveground parts and roots compared with those in the NF-0 treatment. The plants grown in the F-3% treatment also decreased P contents by 25.78% and 28.41% in aerial and underground roots compared with those exposed to the F-0 treatment. When the plants were subjected to flooded conditions, P contents were increased by 142.93% and 153.93% in underground roots of the plants under F-0 and F-3% treatments compared with the plants cultivated in the respective non-flooded conditions (NF-0 and NF-3%).

The BBC addition showed an insignificant effect on K accumulation in the parts under non-flooded and flooded conditions. The BBC addition (F-3%) increased the K content in the aerial roots by 39.95% compared with F-0. Soil flooding (F-0) decreased the K accumulation

in aboveground parts by 26.90%, while increased the K accumulation in underground roots by 35.17% than that in NF-0 treatment. In comparison, F-3% treatment decreased K content in aboveground parts by 8.87% and increased the K accumulation in underground roots by 42.89% than that in NF-0 treatment. And the F-3% treatment decreased the K accumulation by 9.51% in aboveground parts while it increased by 75.06% in underground roots compared with the NF-3% treatment (Fig. 3).

The BBC amendment decreased Ca and Na contents in the aboveground tissues of the plants under non-flooded and flooded conditions (Fig. 4). The Ca and Na contents were significantly reduced by 8.81% and 15.74% in NF-3% treatment compared with NF-0 treatment and decreased by 8.70% and 14.71% in F-3% treatment compared with F-0 treatment, respectively. Soil flooding reduced Ca, Mg, and S contents in aboveground parts of plants (decreased by 14.08%, 9.82%, and 20.80% in F-0 compared with NF-0, decreased by 13.98%, 19.01%, and 13.85% in F-3% compared with NF-3%), while increased Fe and Na contents (increased by 255.54% and 33.72% in F-0 compared with NF-0, increased by 218.48% and 35.35% in F-3% compared with NF-3%) significantly. F-3% decreased the contents of Mg and Na in the aerial roots by 23.11% and 42.61% compared with F-0 treatment, respectively. BBC decreased the Al, B, Ca, Fe, Mg, and Na contents in underground roots (by 19.97–46.67% in NF-3% compared with NF-0, by 21.89–44.42% in F-3% compared with F-0, respectively). The contents of Al, B, Ca, Fe, Mg, Mn, Na, and S were increased significantly in underground roots after soil flooding compared with non-flooded treatment (by 39.17–699.03% in F-0 compared with NF-0, by 39.70–3887.56% in F-3% compared with NF-3%, respectively). Significant interactions between BBC and flooding treatments were observed on Mn content in the aboveground part and underground roots.

3.4 Heavy metals contents in *Salix*

Under the non-flooded treatment, the BBC addition (NF-3%) significantly increased the Cd contents in cuttings, stems, and leaves by 45.33%, 107.40%, and 94.20%, respectively, compared with the NF-0 treatment (Fig. 5). NF-3% significantly increased the Zn contents in stems, and leaves by 27.51%, and 47.43%, respectively, compared with the NF-0 treatment. NF-3% treatment insignificantly affected the Cd and Zn contents in the root, while the BBC significantly decreased Cu and Pb contents in roots by 53.77% and 79.01% compared with NF-0 treatment. Under the flooded condition, the BBC (F-3%) stimulated the accumulation of Cd in the aboveground part of *Salix* plants compared with F-0, while the significant effect was only observed in leaves (+32.73%). F-3% treatment significantly increased the Zn content in underground roots by 14.61% while decreasing

Fig. 4 Mineral elements accumulation in *Salix* plants in different treatments. The data indicate the means (red dot) $\pm$ standard deviation ($n=3$). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown ($*P<0.05$;

$**P<0.01$; $***P<0.001$; ns: not significant). Different letters (a, b, c, d) indicate statistically significant differences of index in the same organs between the groups (ANOVA, LSD test, $P<0.05$), $n=3$

by 20.21% in aerial roots compared with F-0 treatment. Pb content in underground roots decreased by 18.21%, while significantly increased by 137.65% in aerial roots in F-3% treatment compared with F-0 treatment.

Soil flooding (F-0) significantly increased the Cd and Zn contents in underground roots of *Salix* by 72.48% and 336.31% than NF-0 treatment, respectively. F-3% treatment increased the Cd and Zn contents in underground roots by 57.42%, and 704.22% than NF-3% treatment, respectively.

Soil flooding (F-0) significantly decreased Cd and Zn contents in cuttings by 71.61% and 36.89%, in stems by 66.83% and 58.08%, in leaves by 48.26% and 44.30% than those in NF-0 treatment, respectively. F-3% treatment also significantly decreased Cd and Zn contents by 78.12% and 50.15% in cuttings, 78.67% and 66.80% in stems, and 64.64% and 59.40% in leaves than those in NF-3%, respectively. Lead content in underground roots and cuttings significantly increased by soil flooding by 213.78% and 145.05% in F-0

Fig. 5 Effect of BBC and flooding on HMs content (mg kg^{-1}) in plants. The data indicate the means (red dot) $\pm$ standard deviation ($n=3$). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown ($*P<0.05$;

$**P<0.01$; $***P<0.001$; ns: not significant). Different letters (a, b, c, d) indicate statistically significant differences of index in the same organs between the groups (ANOVA, LSD test, $P<0.05$), $n=3$

treatment compared with NF-0 treatment, and by 1122.66% and 90.74% in F-3% treatment compared with NF-3% treatment, while there were no apparent changes in stems and leaves (Fig. 5).

3.5 HMs bioaccumulation and translocation

BBC (NF-3%) increased TF values of Cd, Zn, and Pb by 68.85%, 102.27%, and 366.67% relative to NF-0 (Fig. 6). In F-0 compared with NF-0, the TF values for Cd and Zn decreased by 60.66% and 73.86%, respectively, whereas in F-3% compared with NF-3%, they decreased by 73.79% and 91.57%, respectively. The BBC addition (F-3%) did not improve the TF values of Cd and Zn for the *Salix* plant under flooded conditions (F-0) (Fig. 6). In comparison with NF-0, NF-3% treatment significantly increased Cd and Zn accumulation by 59.10% and 36.71% in aboveground parts of the *Salix* plant, 52.53% and 28.52% in the whole plant, respectively, while decreasing Cu and Pb by 30.06% and 71.31% in the roots, 19.75% and 1.23% in aboveground parts, and 25.22% and 62.90% in the whole plant, respectively (Fig. 7). The accumulation of Cd and Zn in F-3% was not significantly different from F-0 except for aerial roots. BBC additions (F-3%) with flooded conditions significantly increased

Fig. 6 Effect of BBC and flooding on the translocation of HMs for the *Salix* plant. The data indicate the means (red dot) $\pm$ standard deviation ($n=3$). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown ($*P<0.05$; $**P<0.01$; $***P<0.001$; ns: not significant). Different letters (a, b, c, d) indicate statistically significant differences of index between the groups (ANOVA, LSD test, $P<0.05$), $n=3$

the Pb content of aerial roots, underground roots, and the whole plant by 142.86%, 52.15%, and 68.49%, respectively, when compared with F-0 treatment. The BBC amendment and flooding treatments significantly influenced Cd accumulation in the aboveground part and the whole plant.

Fig. 7 Effect of BBC and flooding on HMs accumulation in the *Salix* plant. The data indicate the means $\pm$ standard deviation ($n=3$). P values of ANOVAs of BBC amendment (B), flooding treatments (F), and their interactions (BF) were shown (* $P<0.05$; ** $P<0.01$;

*** $P<0.001$; ns: not significant, A: aboveground part; R: underground and aerial root; T: total accumulation). Different letters (a, b, c, d) indicate statistically significant differences of index in the same organs between the groups (ANOVA, LSD test, $P<0.05$), $n=3$

Soil flooding (F-0) significantly decreased Cd accumulation in roots (sum of underground and aerial roots), aboveground parts, and the whole plant by 57.99%, 74.00%, and 70.06%, respectively. F-3% treatment decreased Cd accumulation in roots, aboveground parts, and the whole plant by 42.54%, 64.67%, and 59.22% compared with NF-0 treatment, respectively. F-0 treatment decreased Zn accumulation in roots, aboveground parts, and the whole plant by 0.32%, 60.07%, and 49.02% compared with NF-0 treatment, respectively. F-3% treatment also decreased Zn accumulation in aboveground parts and the whole plant by 57.42% and 32.82%, while increased that by 75.58% in roots compared with NF-0 treatment, respectively. Differing from Cd and Zn, the Cu accumulation was decreased more significantly when with the BBC addition under flooded conditions. F-0 treatment reduced Cu accumulation in roots, aboveground parts, and the whole plant by 65.28%, 40.33%, and 53.58%, while F-3% decreased that by 69.17%, 43.29%, and 57.03% compared with NF-0 treatment, respectively. F-0 treatment decreased Pb accumulation by 33.69% and 30.01% in roots and the whole plant compared with NF-0 treatment, while F-3% treatment increased that by 16.85% and 17.93%, respectively (Fig. 7).

A principal component analysis (PCA) was performed using the indices of the total accumulation of HMs and the TF values (Fig. 8). PC1 and PC2 accounted for 66.70% and 16.33% of the investigated variation, respectively. The total accumulation of Cd and Zn and the TF values of Cd and Zn were more influenced by PC1, while the total accumulation of Cu and Pb, and the TF values of Cu were

Fig. 8 Principal component analysis (PCA) plots of HMs phytoremediation (accumulation and translocation) of plant with BBC amendment (0 and 3%) under non-flooded or flooded treatment. TA-HM: total accumulation of HM in *Salix* plants; TF-HM: the translocation factor of willow to HMs

more influenced by PC2. The differentiation of BBC treatment was more evident in the absence of non-flooded than flooded treatment.

4 Discussion

4.1 Effect of BBC and flooding on soil properties

The current study reflected both BBC addition and soil flooding that directly influenced soil properties (Table 2). As a kind of soil amendment, BBC usually enhanced soil pH value because its original pH is higher than acid soil (Marris 2006; Yuan et al. 2011). In this study, the soil pH changed slightly after adding BBC, attributed to the alkaline character (7.65) of the tested soil and the low dosage of BBC. It is worth noting that the BBC addition (F-3%) significantly increased the soil pH (0.20 units) in the F-3% treatment compared with the F-0 treatment under flooded conditions. As a result of the decreased soil oxidation–reduction status (Eh) with flooding, Fe^{3+} eventually becomes Fe^{2+} in the soil, consuming a significant amount of H^+ (Hue and Amien 1989).

The rich organic carbon of BBC contributed to the significantly increased soil organic matter content. The BBC addition reduced the total N content in the soil as well because the BBC content was lower than that in the soil tested. According to this study, NO_3^- and NO_2^- contents in the soil increased with the BBC amendment, which implied that the BBC amendment weakened HN leaching. The HN content was affected more significantly by soil flooding. Soil flooding and oxygen deficiency would induce the nitrate reduction, convert NO_3^- and NO_2^- into NH_4^+ and decrease HN content in the soil. Moreover, the slight decrease of HN with the BBC amendment can be explained by the fact that the BBC enhanced the soil C/N ratio value caused by the high organic carbon of the BBC, which enhanced the biological fixation of NH_4^+ in the soil (Lehmann et al. 2003). The enhanced soil C/N ratio value caused by the BBC amendment might result in biological fixation of AP and reduced P availability in soil (Lehmann et al. 2003). Wang et al. (2015) reported that flooding could stimulate soil P's release and increase the AP content. It appears that BBC weakened the impact of flooding, which was also observed in the interaction between BBC amendment and flooding. The iron oxide colloid formed by the ferric irons in the soil could absorb the phosphorus in the colloid solution and then reduce the AP content (Yan et al. 2017). Reducing Fe^{3+} to Fe^{2+} released more free phosphate radicals, thus increasing the soil AP under flooded conditions. There is ample calcium in soil, making AP challenging to fix to soil (Khadem et al. 2021). We observed that the BBC application increased the AK content in the soil, partly attributed to the direct addition of

the K-rich ash fraction in BBC and the reductions in runoff and leaching (Chen et al. 2017; Khadem et al. 2021; Laird et al. 2010).

The effect of biochar on the bioavailable HMs is multifaceted. Due to the electrostatic adsorption or complexation with HMs, biochar amendment reduced HM mobility and bioavailability (Bashir et al. 2018; Tong et al. 2011), which caused a significant decrease in NF-3% than NF-0 treatment. And the BBC addition (F-3%) made HMs leach less from soil to accumulated water than F-0 treatment because of the BBC adsorption under flooded conditions. On the other hand, the immobilization effect of the BBC amendment on HMs was influenced by some other factors. For example, the iron plaque formed on the surface of plant roots and the organic acids secreted from roots affected the balance between passivation and activation effect of HMs when the soil was treated with BBC amendment and flooding (Lefevre et al. 2013; Lei et al. 2011).

Moreover, soil anions also play a crucial role in the adsorption–desorption of HMs. The existence of Cl^- in soil stimulated the release of HMs from the solid phase into the solution phase (Collins et al. 1999; Li et al. 2019a). In this study, flooding treatment reduced the content of Cl^- in soil, and the content of bioavailable HMs decreased accordingly. SO_4^{2-} made a more decisive role in Cu adsorption in soil, explaining the variation of bioavailable Cu content in different treatments. To some degree, the increased accumulation of Cd and Zn in *Salix* may be caused by reduced bioavailable Cd and Zn. The soil's reduced Eh and increased pH with the BBC addition and flooding substantially decrease metal solubility (Kashem and Singh 2001). Flooding leads to increased hydrous Fe and Mn through a reductive dissolution process, which is thought to provide sites for HMs adsorption and immobilization in soil (Iu et al. 1981). The reduction of bioavailable HMs after flooding indicated that the creation of anaerobic conditions in the soil resulted in converting the bioavailable forms of HMs into the stable fraction (Kashem and Singh 2004).

4.2 Effect of BBC and flooding on *Salix* growth

BBC showed little effect on plant growth and plant biomass in the current study (Fig. 2), which may be ascribed to the severe pollution of HMs and a low dose of BBC amendment. We noted that soil flooding significantly reduced the *Salix* plants biomass, which could be ascribed to the reduced gas exchange rate for the plant root system and the decreased chlorophyll content of leaves, which further limited the photosynthetic rate, stomatal aperture, and photochemical quantum efficiency of the plant (Carvalho and Amancio 2002; Edwards et al. 2003). The BBC addition under flooded condition (F-3% treatment) alleviated the flooding inhibition in

plant growth, significantly increased by 86.27% in the underground root biomass and 13.63% in total biomass compared with the without-BBC addition treatments under flooding conditions (F-0 treatment). This may be due to the improvement in soil quality, like the increased organic matter, AP, and AK contents in the soil. It is suggested that biochar may reduce soil bulk density and increase soil porosity, facilitating a favorable environment for the root system (Laird et al. 2010), and promote soil enzymes activity and improve the root environment of plants (Knicker 2007; Kolton et al. 2011), which can help to reduce plant's oxidative stress and increase the antioxidant enzyme activities of plants to reduce HMs toxicity (Abbas et al. 2018).

4.3 Effect of BBC and flooding on mineral elements in *Salix*

The accumulation and distribution of mineral elements in plants were significantly affected by BBC and flooding treatments (Figs. 3, 4). BBC increased nutrient retention in the soil, but a fraction of those nutrients were not readily perceived by the plant, reducing mineral elements accumulation in the *Salix* plant. In contrast, the contents of mineral elements in plants were usually increased after biochar application to soil or nutrient solution in other results (Jia et al. 2019). Soil flooding could deteriorate soil physicochemical properties (Kozłowski 1997) and inhibit substantially plant growth, contributing to the lower element content in plants aboveground in soil flooding than non-flooded treatment in this study. With the progress of soil flooding, the transpiration of *Salix* plants was decreased, which may negatively affect the translocation of nutrients to the aboveground parts of plants. Consistent with the previous study, the alterations of nutrient contents (except for K) in the aboveground part of the *Salix* plants were decreased by BBC addition and soil flooding. One explanation for the abnormal performance in plant nutrients is the inhibition impact of the iron plaque on nutrient absorption and transport under flooded conditions. This also partially explained the elevated concentration of mineral elements in the roots after flooding, i.e., a large of mineral elements were retained in the roots due to disrupted translocation. Another reason for the high content of mineral elements in the roots could be the response of *Salix* to flooding stress. For example, high content of elements such as Ca, K, and Na can better sustain plant activity under flooding stress (Rubio et al. 1997; Xiong et al. 2002).

With the supplement of BBC, the absorption of Na^+ in *Salix* plants was decreased, which reflected the increased ability of the plant antioxidant response under complex conditions (Thomas et al. 2013). F-3% treatment decreased the Fe content in the plant roots compared with F-0 treatment, indicating that the addition of BBC could reduce the

formation of iron plaques on the *Salix* plant's roots (Li et al. 2020). The interaction between BBC and flooding on Mn uptake by underground roots also verified the possibility of the BBC reducing iron plaques' formation. When the plants were exposed to flooding conditions, the contents of Fe and S in the plant root were increased, which could be due to the increased precipitation of Fe/Mn oxide and sulfide on the plant root surface (Du Laing et al. 2009; Zimmer et al. 2011). A previous study demonstrated that the uptake of P in the roots was positively correlated with Fe content in roots (McKevlin et al. 1987), which was similar to our results.

4.4 Effect of BBC and flooding on phytoremediation capacity of *Salix*

A schematic mechanism diagram of BBC effects on the *Salix* phytoremediation was supplied (Fig. 9). Biochar usually acted as an amendment to reduce the bioavailability of HMs in soil and the accumulation in crops (Beesley et al. 2013; Cui et al. 2011). Under the non-flooded condition in this study, the decrease of bioavailability ratio of HMs with the BBC amendment reduced the uptake of HMs by roots. The amendment of BBC decreased the HMs toxicity in rhizospheric soil and soil pore water that close contacted with *Salix* roots in severely polluted soil (Lebrun et al. 2017), which improved the rhizosphere condition and stimulated plant physiological activities, including the increase of plant transpiration and gas exchanges (Li et al. 2021), and finally enhanced the xylem transport of Cd and Zn from roots to aboveground tissues (Habiba et al. 2015). Previous studies revealed that the biochar amendment could reduce the proportion of cell wall-bound Cd in roots, which caused easier Cd translocation from the cortex to the stele in the root and up to the aboveground parts (Li et al. 2019b). Biochar increased soil porosity and provided more habitat for microorganisms (Pathy et al. 2020). This measure may also stimulate the associated soil microorganisms, thus facilitating the transport of Cd and Zn from the plant to the aboveground. Soil microorganisms, such as *Bacillus* sp. and *Aspergillus niger* were confirmed to enhance antioxidant defenses of *Salix* and promote HMs bioavailability and improve transport coefficients finally (Niu et al. 2021). As a HMs remediation tree species, *Salix* has high tolerance and accumulation capacity for HMs. The addition of 3% BBC to the soil had little effect on the balance between the uptake and transport of HMs by roots. These results ensured HMs were continuously transported through the root system to *Salix* aboveground part, the leaves especially.

The effect of the BBC amendment on Pb levels in *Salix* plants was slight, similar to Lebrun et al. (2017), who also reported that the biochar derived from pine wood reduced Pb uptake in *Salix viminalis*. It suggests that biochar has different effects on the phytoremediation of various HMs.

Fig. 9 The underlying mechanism of BBC enhancing *Salix* phytoremediation to Cd/Zn in the heavily polluted soil

Meanwhile, the absorbed Pb in roots of *Salix* plants was primarily restricted in the cortex (Wang et al. 2021), which determines a small amount of Pb translocation within the *Salix* plants to the aboveground.

As we previously reported, soil flooding significantly increased Cu accumulation in the roots of *Salix jiangsuensis* cv. J-172 and *Salix babylonica* L. (Chen et al. 2012). Kissoon et al. (2010) also found the flooding significantly increased metal (Cu, Fe, and Mn) uptake by *Rumex Crispus* L. It was suggested that iron plaque formed on root surface in flooding conditions reduced the uptake of metals by plants because of its iron hydroxide functional groups (Batty et al. 2000; Liu et al. 2011a). The increase in HMs in the roots was partly due to the retention effect of HMs by iron plaques on the root surface. The transport of HMs in plants was confirmed to be driven by transpiration (Cao et al. 2020; Welch

1995). Flooding weakened the ability of plants to transport HMs to aboveground parts, leading to parts of HMs retention in the root system.

The content of AK related to HMs absorption and transport in plants under the metal-induced stresses was increased in soil and plant aboveground parts under F-3% treatment compared with F-0, which further improved the activity of HMs tolerance-related enzymes (Ahmad et al. 2016). The interaction between the BBC and flooding treatment on the TF values of Cd and Zn was apparent in this study. BBC stimulated more Cd and Zn transporting to aboveground tissues of *Salix* plants, while soil flooding weakened this impact because of the negative effect on plant growth and the reduction of HMs bioavailability. This result indicated that HMs uptake through the plants grown in flooded soil was more susceptible than cultivated

in the only BBC amended soil treatments. The negative effect on plant growth and the worsened soil properties caused by soil flooding restricted the accumulation and transport of HMs by *Salix* plants. Meanwhile, flooding weakened the positive effect of BBC on soil properties in non-flooded soils. F-3% treatment slightly increased the contents of Cd and Zn in the shoots of *Salix* plants compared with F-0, which proved BBC still could improve the soil environment and enhance the vigor of *Salix* plants as discussed above to a certain extent when the soil was flooded. This result would indicate the role of BBC might be different when soil moisture conditions varied.

The total accumulated HMs in the *Salix* plant was determined by the plant biomass and the contents of HMs in organs. Ernst (1995) illustrated that HMs contents in plants are not the most appropriate method of evaluating HMs uptake due to the differences in biomass-affected contents because of dilution effects. Kissoon et al. (2010) suggested the uptake of HMs in plants can only be accurately measured as the total amount of element per plant, mainly when significant differences in biomass occur between the groups being compared. In this study, the BBC significantly increased Cd and Zn accumulation in *Salix*, while flooding restricted the proliferation of the metals except for Pb. The previous study proved that biochar could substantially inhibit the formation of iron plaques on plant roots and thus reduce Pb immobilized by the plaques (Li et al. 2020). The effect of the BBC amendment and flooding on the phytoremediation potential of *S. psammophila* was significantly observed in PCA results. The BBC significantly influenced the phytoremediation of *Salix* under non-flooded condition, while flooding weakened this impact. The different responses of HMs in *Salix* to BBC and flooding treatments may be due to the various storage features in the plant (Cloutier-Hurteau et al. 2014; Cao et al. 2020; Wang et al. 2021).

5 Conclusion

The use of BBC significantly increased the organic matter and AK in soil and offset the inhibition of flooding on the *Salix* plant growth. BBC stimulated the accumulation and transport of Cd and Zn by *Salix psammophila* in heavily contaminated soil under non-flooded conditions while inhibiting Cu and Pb accumulation. Under the flooded treatments, the BBC increased Pb accumulation by *Salix* but had slight effect on Cd, Zn, and Cu accumulation. The combination of BBC and soil flooding had an interactive effect on the accumulation and distribution of HMs in *Salix psammophila*. Flooding can offset the promoting effect of BBC on the phytoremediation capacity of *Salix psammophila*. This study demonstrated that BBC in non-flooded soils was superior

to flooded soils in promoting phytoremediation of severely HMs-contaminated soils, which provided some insight into biochar application for the remediation of contaminated soils under different moisture conditions.

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Data availability All data generated or used during the study appear in the submitted article.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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