Review

Effects of Heavy Metals (Cd, Pb, Cu, Zn, and Ni) on *Ipomoea aquatica* Forsk. Growth in Soil Containing Metal-Biochar Application

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Abstract

The combined heavy metal pollution in vegetable fields poses a threat to food security. In this study, three types of biochar – *Wedelia trilobata* (WB), *Pennisetum sinese* Roxb (PB), and coffee grounds (CB) – were applied to a heavy metal-contaminated soil at rates of 2% and 5% (w/w). A pot experiment was conducted to investigate the effects of these three different types of biochar on the growth two *Ipomoea aquatica* Forsk. cultivars in the soil, and related mechanisms. The results showed that the addition of the three types of biochar significantly increased soil pH and SOM content. WB and PB significantly increased the shoot biomass of *I. aquatica* Forsk. The 5% WB amendment significantly decreased the plant uptake of Cd, Pb, Zn, and Ni. But PB and CB amendments at 2% or 5% showed no consistent effects on plant uptake of Cd, Pb, Cu, Zn, and Ni. The multi-factor variance analysis showed that biochar type significantly influenced Cd, Pb, Cu, Zn, and Ni accumulation in plant shoot and root and Cd, Cu, and Zn concentration in rhizosphere. Interestingly, in some of the treatments with PB and CB amendments, total available heavy metals increased, indicating that heavy metals contained in the biochar might have been released into the soil. The results indicated that biochar feedstock should be ecologically friendly.

Keywords: heavy metal-polluted soil, biochar, *Ipomoea aquatica* Forsk

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Introduction

With the development of industry and the modernization of agricultural production, more and more heavy metals enter into the soil, leading to increasingly serious heavy metal pollution [1, 2]. Through such media as water, air, plants, or the food chain, soil heavy metal pollution reduces crop yield, causes tremendous economic losses, and harms human health [3, 4]. Soil heavy metal pollution has adverse effects on plant growth and biomass quality [5, 6]. Soil heavy metal pollution is long-term, secluded and irreversible [7]. For these reasons, efforts to prevent and control soil heavy metal pollution have always been a focus of international research. In heavy metal-contaminated vegetable fields, compound pollution commonly occurs [8, 9]. Plant growth and quality can be influenced by metal speciation and mobility in soil, which is related to soil type and characteristics [10, 11]. Thus, measures to change the speciation and mobility of heavy metals so as to reduce their accumulation in plants are necessary.

Biochar is the product of thermal degradation of organic materials under an oxygen-limited environment [12]. Biochar could be derived from a variety of biomass, such as agricultural products, crop residue, cattle manure, forestry residues, wood waste, and the organic fraction of municipal solid waste [13]. In many studies, biochar is described as a soil amendment that can improve soil fertility and reduce CO_2 emissions to mitigate climate change [14, 15]. Due to the large surface area and highly microporous structure of biochar, it can adsorb heavy metals and in turn reduce their mobility and bioavailability [13, 16-19]. Thus, biochar could decrease the risk of heavy metals that enter the human body through the food chain [20-22]. Moreover, the bioavailability of heavy metals depends on biochar types and characteristics [23, 24]. Qi et al [25] documented that the acidic biochar did not reduce soil soluble/bioavailable/bioaccessible Cd, but it reduced its mobility under acidic conditions. However, other studies have shown that biochar may be contaminated with contaminants such as heavy metals and polycyclic aromatic hydrocarbons [26, 27]. Thus, the application of these biochars to soil may result in a negative impact on the environment.

Vegetables are an indispensable food of daily life. They are also a very important cash crop of agriculture. However, with the industrial contaminants discharged in the environment, vegetable fields are under the risk of heavy metal pollution [4, 28, 29]. Leaf vegetables are crops susceptible to uptake of heavy metal, and their enrichment factor is higher than rootstock vegetables [4]. In Guangzhou, the capital of Guangdong province, there were 50% vegetables contaminated with heavy metals, and Pb, Cr, Cd are the main heavy metals [30]. Thus, reducing metal accumulation in vegetables is important for food safety. *I. aquatica* Forsk is one of the common leaf vegetables in southern China. And

I. aquatica Forsk had been used in many studies for absorption of heavy metals [31].

In this paper, two varieties of *I. aquatica* Forsk were selected and planted in a soil with compound heavy metal pollution. According to a preliminary experiment, the narrow-leaf (Thailand) *I. aquatica* Forsk (NL (TH)) had the highest endurance capacity in terms of combined heavy metal pollution. The narrow-leaf green-skin (Hong Kong) *I. aquatica* Forsk (NLGS (HK)) had the least endurance capacity. Three types of biochar used in this study were prepared using three different feedstocks: *Wedelia trilobata*, *Pennisetum sinese* Roxb and coffee grounds. *Wedelia trilobata* is a creeping herb native to Central America and has been recognized as a serious invasive weed in southern China [32]. *Pennisetum sinese* Roxb is a fodder crop widely planted in Guangdong Province. Research shows that a series of biochars prepared from *Pennisetum sinese* Roxb had a high adsorption capacity for heavy metals [33]. Coffee grounds is a familiar waste from city life.

This study aimed to investigate the effects of different biochar types prepared with different feedstocks on the growth of two *Ipomoea aquatica* Forsk cultivars and the bioavailability of heavy metals in a heavy metal-polluted soil and related mechanisms about containing-metal-biochar materials used to remediate the heavy metal pollution in soil. The research is expected to be helpful in the treatment of contaminated vegetable soils.

Material and Methods

Biochar and Soil Characteristics

Biochar was produced from three different feedstocks: *Wedelia trilobata*, *Pennisetum sinese* Roxb, and coffee grounds. *Wedelia trilobata* and *Pennisetum sinese* Roxb were planted in a heavy metal-polluted soil at the Experiment Station of South China Agricultural University. The spent coffee grounds were supplied by Starbucks, Guangzhou. Each feedstock was air-dried and then pyrolyzed at 500ºC for 2 h using an in-house batch pyrolysis unit in the presence of flowing nitrogen gas. The obtained *Wedelia trilobata* biochar (WB), *Pennisetum sinese* Roxb biochar (PB), and coffee grounds biochar (CB) were ground to pass through a 2-mm sieve and homogenized before use. In addition, the biochars were characterized with a scanning electron microscope (SEM) and the biochar powders were mounted onto aluminum stubs with conductive carbon tape. The specimens were then sputter coated with Au-Pd and examined with a JEOL 6400 V (JEOL USA, Inc., Peabody, MA) scanning electron microscope.

The soil used was collected from the plow-layer (0-20 cm depth) in a vegetable field at the Experiment Station of South China Agricultural University. Soil samples were air-dried, homogenized, sieved (<2 mm), and stored before use. The soil is a loam, and it had

Table 1. Heavy metals total content in soil, the availability of heavy metals in the soil, *Wedelia trilobata* biochar (WB), *Pennisetum sinese* Roxb biochar (PB) and coffee grounds biochar (CB).

	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Ni (mg kg^{-1})	Cu (mg kg^{-1})
Total	2.83±0.01.	344.88±9.57	379.41±12.89	59.40±1.46	127.76±9.36
Availability	1.10±0.32	12.33±1.08	61.10±6.43	7.60±0.37	15.67±1.21
WB	0.63±0.03	10.82±1.18	83.23±6.91	2.50±0.24	76.65±19.25
PB	1.29±0.03	21.04±2.04	141.36±8.54	7.75±0.66	22.86±2.89
CB	0.62±0.05	9.28±2.82	91.45±9.29	3.26±0.37	67.39±8.75

the following main properties: pH of 5.65 and total N, P, and K contents of 35.90, 16.45, and 63.20 g kg^{-1}, respectively. The contents of heavy metals in the soil and biochars are shown in Table 1.

Pot Experiment

Plastic pots (210 mm diameter, 185 mm high) were filled with 2 kg of soil, and amended with the WB, PB, and CB biochars at rates of 0%, 2% or 5% (w/w). Each treatment was replicated three times. After application of the biochars to the soil, deionized water was added to the pots to bring soil moisture to 50% of the field water-holding capacity (WHC). Then the pots were preincubation for 7 days.

The seeds of *I. aquatica* Forsk were purchased from a seed market in Guangzhou in southern China. Two varieties, namely Thailand narrow-leaf *I. aquatica* Forsk (TH) and Hongkong narrow-leaf green-stem *I. aquatica* Forsk (HK), were selected for this experiment due to their different tolerances to heavy metal stress in our preliminary experiment. Uniform-sized seedlings were transplanted directly from the seedbeds into the pots and 3 seedlings were planted in each pot.

After two months when the plants grew to be commercially harvestable, the aboveground parts (leaves and stem) were harvested by cutting the stems at the soil surface. The roots were removed carefully from the soil. After that, the roots were washed with deionized water and oven-dried. Both rhizosphere and non-rhizosphere soil samples were collected, air-dried, homogenized, and sieved (16 mesh and 100 mesh) [34]. The soils were sieved through a 16 mesh sieve for determining pH, and through a 100 mesh sieve for determining SOM content and heavy metals (Cd, Pb, Cu, Zn, and Ni).

Measurement of Plant and Soil Characteristics

Shoot height and root length were measured for every plant in each pot. For dry weight determination, aboveground parts and roots were dried first at 105ºC (15-20 min) and then at 70-80ºC until constant weight [34].

The determination of soil pH was carried out according to Li et al [11]: 4.00 g soil (<1 mm) was mixed with 10 ml deionized water and pH was measured with a pH meter (PB-10, Sartorius, Germany). SOM was measured using the potassium dichromate heating method according to Li et al [11].

For heavy metal determination, the plant samples were digested with HNO_3 -$HClO_4$ (4:1) [34], while the soil samples were digested with HNO_3-HF-$HClO_4$ (5:5:3) after primary treatment with HCl [35]. Heavy metals in the digest were analyzed by atomic absorption spectrometry (AAS) (ZEEnit700P, Analytik Jena AG, Germany). For quality assurance, certified reference materials of soli (GBW07410) and plant (BGW(E)100349) were included.

Statistical Analysis

The experimental data are presented as mean ± stand error and analyzed using multi-way analysis of variance (ANOVA) of SPSS 17.0 software package SPSS Inc., Chicago, IL, USA). Means were separated using Duncan's multiple range test at the significant level of $P<0.05$.

Results

The Microstructure of Biochar and the Effect of Biochar on Soil pH and SOM

SEM is a good technique for studying the morphology of solid materials. The SEM images of the three types of the biochar show good structure of the WB and PB with plenty of internal pores, which provided a large specific surface area for adsorption of heavy metals (Fig. 1).

The addition of biochar significantly increased soil pH (Fig. 2a). In particular, the pH in NL(TH) soils with 2% WB and 5% WB amendment was raised to 7.08 and 7.30, 1.49 and 1.71 units higher than that in control (5.59), respectively. For NLGS (HK) soils, the pH in 2% WB and 5% WB amendment was raised to 6.95 and 7.82, 1.34 and 2.21 units higher than in control (5.61), respectively.
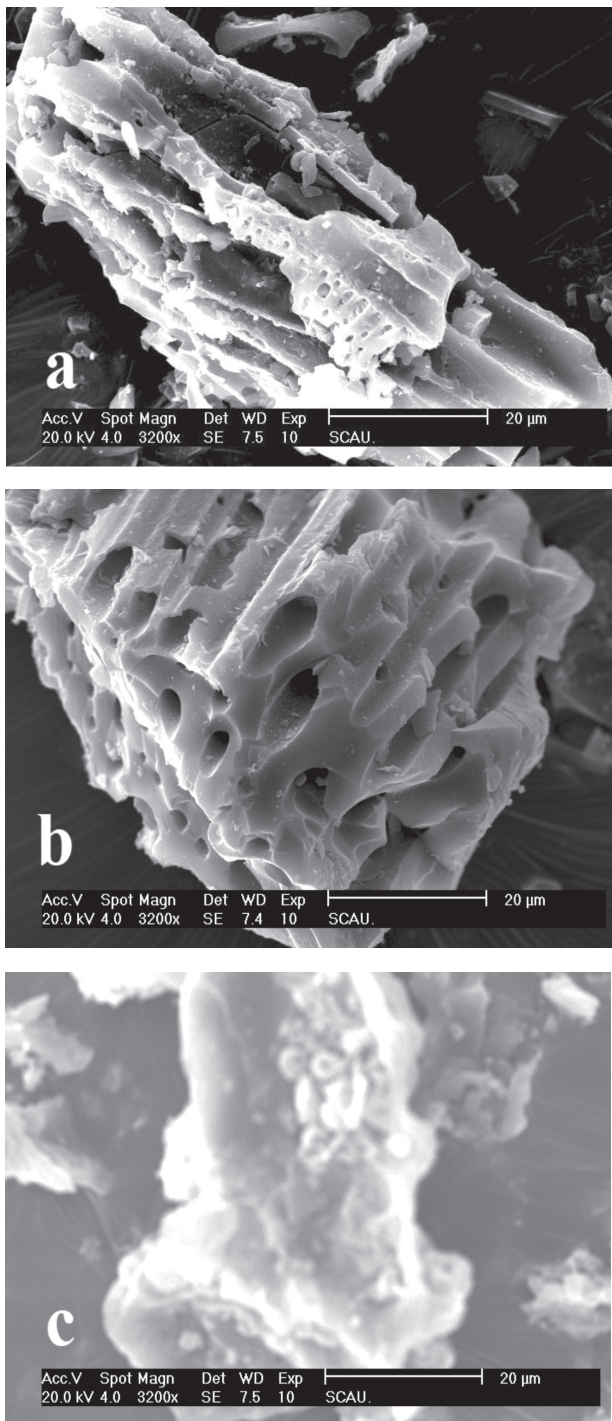
Fig. 1. Scanning electron microscope (SEM) images of *Wedelia trilobata* biochar (WB, a), *Pennisetum sinese* Roxb biochar (PB, b) and coffee grounds biochar (CB, c) pyrolyzed at 500ºC.

As shown in Fig. 2b), the biochar application increased SOM content in soil. For NL(TH), the 2% CB amendment brought the biggest rise in SOM content (48.01 g kg^{-1}), 42.38% higher than that in control, while SOM content increased significantly by 55.94% at the addition of 5% CB for NLGS (HK) as compared with control.

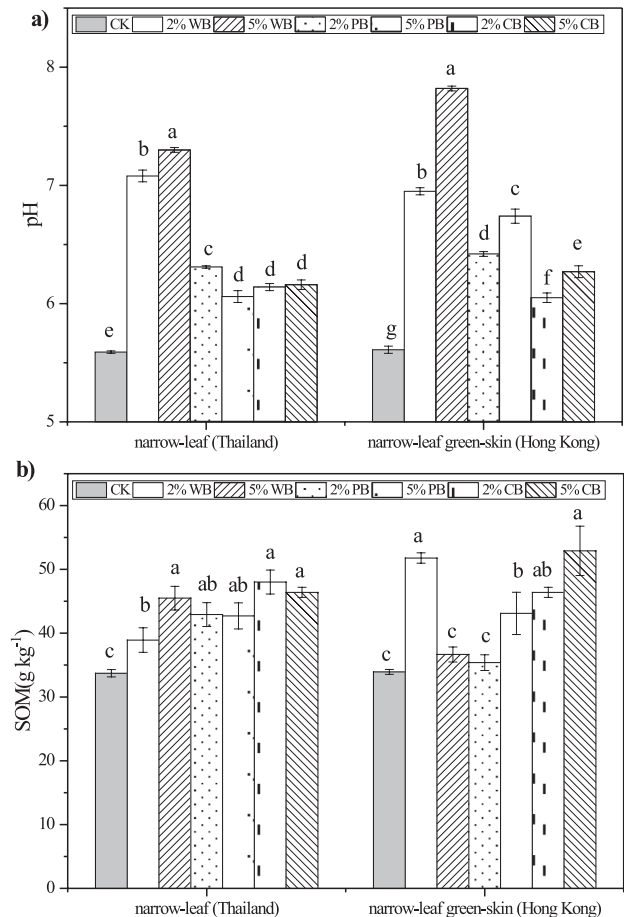In a word, after biochar application, soil pH and SOM tended to increase.

Fig. 2. Effect of different types and application rates of biochar on soil pH a) and SOM b) planted with two cultivars of *Ipomoea aquatica*; different letters indicate significant difference at the 5% level.

Effect of Biochar Application on the Concentration of Heavy Metals in Soil

Total Cd concentrations in rhizosphere soil and non-rhizosphere soil are shown in Fig. 3a). Compared with the control, the 2% CB amendment significantly increased the total Cd concentration in rhizosphere soil of NLGS (HK), the 5% WB and 5% PB amendment obviously decreased the total Cd concentration in rhizosphere soil of NL (TH). The 2% WB, 2% PB, 2% CB, and 5% CB significantly increased Cd in the non-rhizosphere soil of NL (TH). The 5% WB amendment decreased the concentration of Cd, while the 2% WB amendment and the other treatments (2% PB, 5% PB, 2% CB, and 5% CB) significantly increased the concentration of Cd in the non-rhizosphere soil of NLGS (HK) (Fig. 3a).

Compared with the control, 2% WB significantly increased the concentration of total Pb concentration in the NL (TH) rhizosphere soil, while 5% WB, 2% PB, and 5% CB amendments significantly decreased Pb concentration (Fig. 3b). Biochar significantly increased the concentration of Pb, except for the 5% CB amendments in the NLGS (HK) rhizosphere soil

(Fig. 3b). The 5% WB, 5% PB, and 5% CB treatments decreased Pb concentration in the non-rhizosphere soil of NL (TH) (Fig. 3b).

As shown in Fig. 3c), the 5% CB amendment significantly decreased total Cu concentration in the NL (TH) and NLGS (HK) rhizosphere soils. The 2%

WB amendment significantly decreased the content of total Cu in the NL (TH) non-rhizosphere soil. However, Cu concentration in other treatments did not change significantly either in rhizosphere or non-rhizosphere soils.
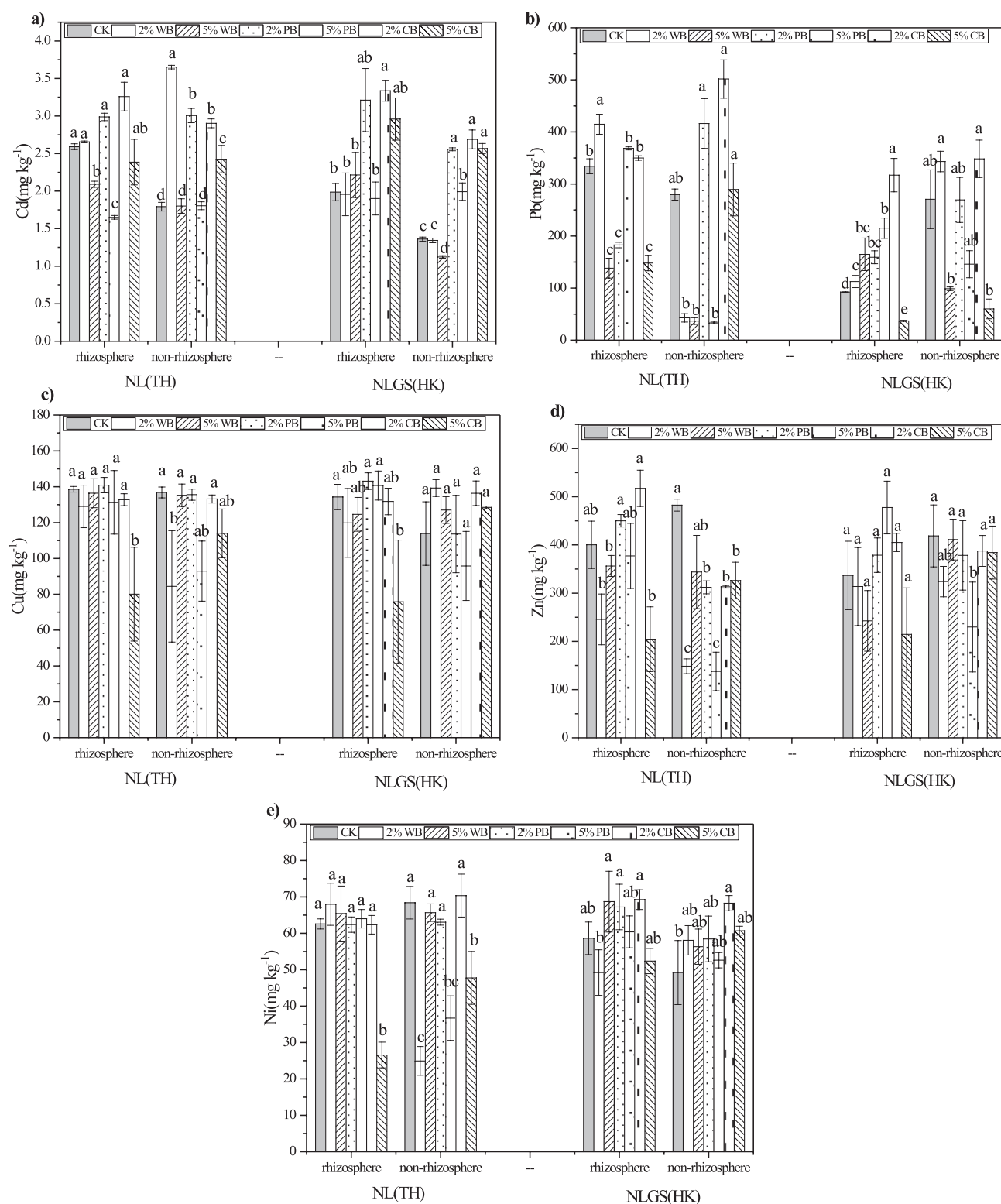

Fig. 3. Concentrations of Cd a), Pb b), Cu c), Zn d), and Ni e) in rhizosphere and non-rhizosphere soils; different letters above the bars indicate significant difference at the 5% level.

As shown in Fig. 3d), none of the biochar amendments significantly affected the total Zn concentration in the rhizosphere soils of NL (TH) and NLGS (HK). The 2% WB, 2% PB, 5% PB, 2% CB, and 5% CB treatments significantly decreased the Zn content in the non-rhizosphere soil of NL (TH). For the NLGS (HK) non-rhizosphere soils, only the 5% PB amendment significantly decreased the total Zn concentration.

The 5% CB amendment significantly decreased total Ni in the NL(TH) rhizosphere soil, while other amendments had no significant effects on total Ni
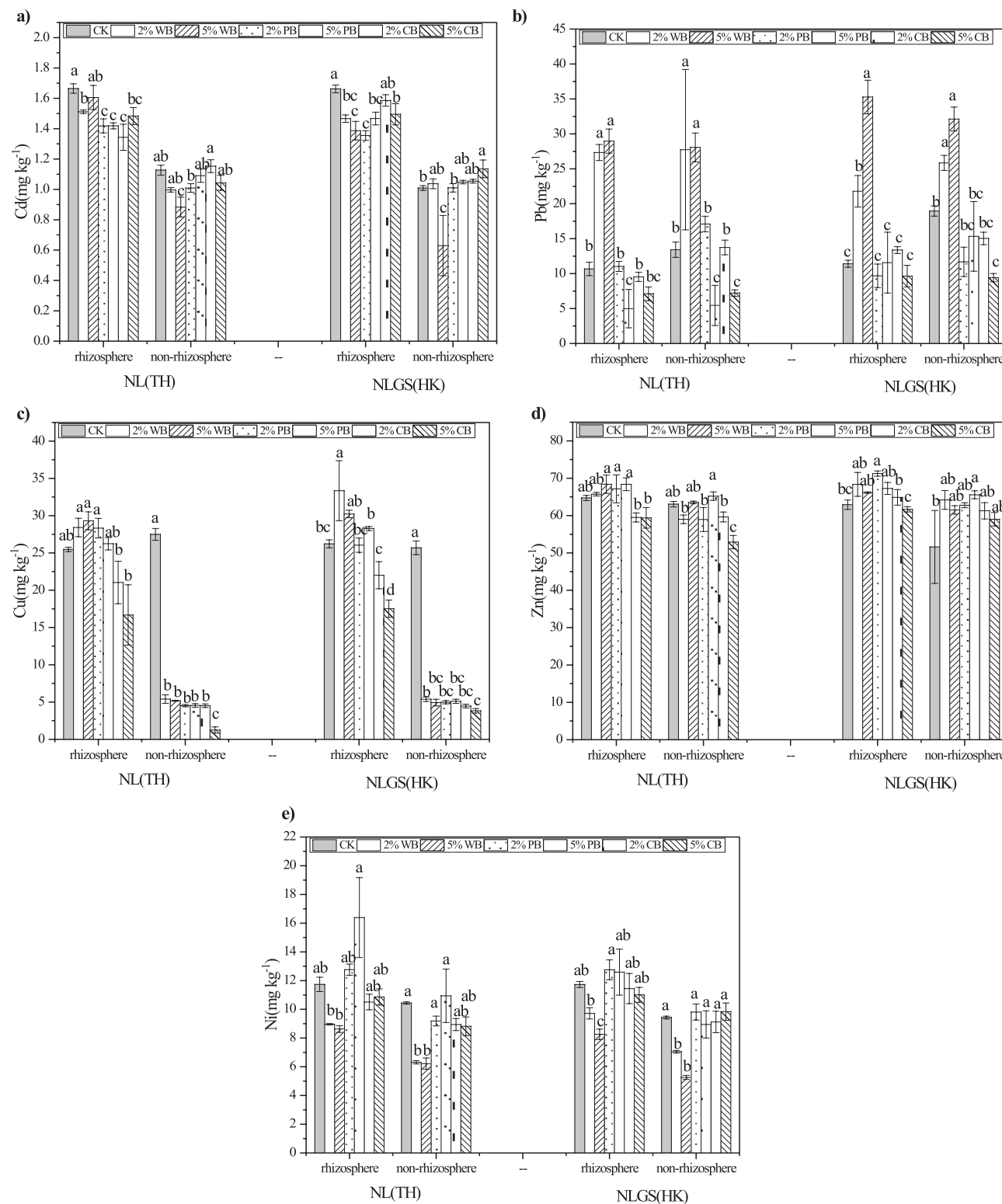

Fig. 4. Availability of Cd a), Pb b), Cu c), Zn d), and Ni e) in rhizosphere and non-rhizosphere soils; different letters above the bars indicate significant difference at the 5% level.

Table 2. Length and weight of *Ipomoea aquatica* with different biochar applications to the soil.

Part	Treatment		Dry weight(g)		Shoot height / Root length (cm)	
			N(TH)	NG(HK)	N(TH)	NG(HK)
Shoot	CK		0.82±0.09c	0.29±0.06b	20.04±0.95a	22.20±0.68a
	WB	2%	1.39±0.09a	0.58±0.08a	21.90±0.93a	21.39±1.56a
		5%	0.26±0.04d	0.19±0.07bc	13.69±0.64b	14.53±1.56b
	PB	2%	1.14±0.14ab	0.44±0.13ab	22.61±1.11a	20.68±1.18a
		5%	1.04±0.13bc	0.52±0.14a	14.58±2.88b	16.90±1.67b
	CB	2%	0.12±0.01d	0.06±0.01c	9.77±0.92c	10.16±0.52c
		5%	0.06±0.02d	0.05±0.00c	7.80±0.71c	8.50±1.27c
Root	CK		0.32±0.06b	0.14±0.02b	9.72±0.89a	9.90±0.99ab
	WB	2%	0.51±0.02a	0.25±0.01a	10.53±0.59a	9.82±0.45ab
		5%	0.09±0.02c	0.06±0.04b	9.70±0.99a	6.94±1.22bc
	PB	2%	0.34±0.04b	0.11±0.05b	11.38±1.12a	10.85±1.19a
		5%	0.39±0.08ab	0.13±0.04b	10.30±0.85a	9.95±1.04ab
	CB	2%	0.08±0.02c	0.04±0.01b	8.83±1.09a	8.89±0.85bc
		5%	0.06±0.01c	0.04±0.01b	9.22±0.71a	6.30±0.59c

Means of these letters(a-d) followed after the data within columns indicate significant difference at the 5% level ($p<0.05$). WB, *Wedelia trilobata* biochar; PB, *Pennisetum sinese* Roxb biochar; CC, Coffee biochar; N(TH), narrow-leaf (Thailand) *I. aquatica*; NG(HK), narrow-leaf green-skin (Hong Kong) *I. aquatica*

concentration (Fig. 3e). For the NL(TH) non-rhizosphere soil, 2% WB, 5% PB, and 5% CB amendments significantly decreased the total concentration of Ni, while 5% WB, 2% PB, and 2% CB amendments had no significant effects on the total Ni concentration (Fig. 3e). The addition of biochar did not influence total Ni concentration no matter in the rhizosphere soil or non-rhizosphere soil of NLGS (HK) (Fig. 3e), except that the 2% CB treatment significantly increased the content of Ni in the NLGS (HK) non-rhizosphere soil.

The available Cd concentrations (Fig. 4a) were significantly decreased in the 2% WB, 2% PB, 5% PB, 2% CB, and 5% CB treatments in the NL (TH) rhizosphere soil and in the 2% WB, 5% WB, 2% PB, 5% PB, and 5% CB amendments in the NLGS (HK) rhizosphere soil. For non-rhizosphere soil, 5% WB and 2% PB treatments significantly decreased the available Cd when planted NL (TH). The 5% WB treatment significantly decreased the availability of Cd in NLGS (HK) soil, while 5% CB significantly increased the available-Cd concentration in the NLGS (HK) non-rhizosphere soil (Fig. 4a).

The 2% WB and 5% WB amendments significantly increased the available-Pb concentrations in both rhizosphere soil and non-rhizosphere soil of the two cultivars (Fig. 4b). 5% PB amendment significantly decreased the available Pb in NL (TH) rhizosphere soil, while 5% PB and 5% CB amendments significantly decreased the availability of Pb in NL (TH) non-rhizosphere soil. Moreover, 5% CB amendment also

significantly decreased the available Pb in NLGS (HK) non-rhizosphere soil.

The 5% CB amendment significantly decreased the available Cu in the NL (TH) rhizosphere soil (Fig. 4c). However, the 2% WB treatment increased the available Cu in NLGS (HK) rhizosphere soil. The addition of biochar significantly decreased the availability of Cu in the non-rhizosphere soils of NL (TH) and NLGS (HK).

None of the treatments had a significant effect on the available Zn concentration in the NL (TH) rhizosphere soil, while 2% PB significantly increased the available Zn in the NLGS (HK) rhizosphere soil (Fig. 4d). Only the 5% CB treatment decreased the available Zn in the NL (TH) non-rhizosphere soil and only 5% PB significantly increased the availability of Zn in the NLGS (HK) non-rhizosphere soils.

As can be seen in Fig. 4e), the addition of biochar did not significantly influence the availability of Ni in the NL(TH) rhizosphere soil. However, only 5% WB significantly decreased the available Ni in the NLGS(HK) rhizosphere soil. The 2% WB and 5% WB significantly decreased the available Ni concentrations in the non-rhizosphere soils of both cultivars, while the addition of PB and CB did not significantly affect the available Ni concentrations.

In summary, total heavy metal concentrations did not display significant differences between the rhizosphere and non-rhizosphere soils in the treatments with the application of the three types of biochar. In contrast, available heavy metal concentrations tended

to be higher in the rhizosphere soil than in the non-rhizosphere soil. In particular, available Cu and Cd concentrations were significantly higher in the rhizosphere soil than in the non-rhizosphere soil.

Effect of Biochar on Plant Growth

Plant shoot height was inhibited by biochar application except in the soils with 2% WB and 2% PB amendments (Table 2), and the 2% CB and 5% CB amendments exerted the strongest inhibiting effect on plant height, which decreased significantly by 48.8% and 38.9% in 2% and 5% CB, respectively, for NL(TH) and significantly by 38.2% and 45.8% in 2% and 5% CB, respectively for NLGS (HK) as compared with control.

Root length was inhibited significantly in the treatment of 5% WB (6.94 cm) and 5% CB (6.30 cm) for NLGS (HK), 64.6% and 63.6% shorter than in control, respectively. For NL(TH), the root length was not significantly different between the biochar amendment treatments and the control.

The addition of 2% CB, 5% CB, and 5% WB decreased significantly the dry weight of both shoots and roots of NL(TH) and NLGS (HK). Particularly in 2% CB and 5% CB, the dry weight was decreased more pronouncedly by 14.6% and 7.3% respectively for the shoots of NL(TH), by 25% and 18.8% respectively for the roots. Nevertheless, the dry weight increased significantly when added 5% PB and 2% WB, with 167.7% and 200.0% respectively for the aboveground part of NLGS (HK), and with 167.5% and 178.6% respectively for the underground part of NLGS (HK) as compared with control.

To sum up, the application of the three types of biochar to the soils tended to inhibit plant growth.

Effect of Biochar on the Contents of Heavy Metal in *I aquatica* Forsk

Biochar type had significant ($p<0.05$) influence on Cd, Pb, Cu, Zn, and Ni accumulation in shoots and roots; biochar application rate significantly influence Cd, Pb, and Zn accumulation in shoots and Pb, Cu, and Zn accumulation in roots (Tables 3 and 4). Pb and Ni accumulation in shoots differed significantly ($p<0.05$) between the two plant cultivars but not so in roots (Table 3). The interaction between plant cultivar and biochar type had a significant ($p<0.05$) effect on Cd and Ni accumulation in shoots, and Pb and Zn accumulation in roots (Table 4). The interaction between plant cultivar and biochar rate had a significant ($p<0.05$) effect on Cd and Zn accumulation in shoots (Table 3). The interaction between biochar type and biochar rate had a significant ($p<0.05$) effect on Cd and Ni accumulation in shoots and Pb accumulation in roots (Tables 3 and 4). The interaction between plant cultivar, biochar type, and biochar level had a significant ($p<0.05$) effect on Cd, Pb, Zn, and Ni accumulation in shoots (Table 3).

Table 3. Multi-factor variance analysis for shoot heavy metals content of *Ipomoea aquatica*.

Source	Cd	Pb	Cu	Zn	Ni
Significance of [a]					
P	NS	***	NS	NS	***
B	***	***	***	***	***
C	*	***	NS	**	NS
P×B	NS	**	NS	NS	***
P×C	**	NS	NS	**	NS
B×C	*	NS	NS	NS	**
P×B×C	**	*	NS	**	*

[a] By analysis of variance; ***$P<0.001$, **$P<0.01$; *$P<0.05$; NS, not significant. P, Plant type; B, Biochar type; C, Concentration of biochar

Table 4. Multi-factor variance analysis for root heavy metals content *Ipomoea aquatica*.

Source	Cd	Pb	Cu	Zn	Ni
Significance of [a]					
P	NS	NS	NS	NS	NS
B	***	***	***	***	***
C	NS	***	**	*	NS
P×B	NS	**	NS	*	NS
P×C	NS	NS	NS	NS	NS
B×C	NS	**	NS	NS	NS
P×B×C	*	NS	NS	NS	NS

[a] By analysis of variance; ***$P<0.001$, **$P<0.01$; *$P<0.05$; NS, not significant. P, Plant type; B, Biochar type; C, Concentration of biochar

The 2% PB amendment significantly reduced the contents of Pb and Ni in the shoots of NLGS (HK) and NL (TH) and that of Zn in the roots of NL (TH). The 5% PB did not significantly affect Cd, Cu, Zn, and Ni accumulation in the roots of the two cultivars. The 5% PB treatment significantly decreased shoot Pb, but significantly promoted root Pb in NLGS(HK).

The addition of 2% CB significantly reduced shoot Cd and Pb contents for the two cultivars and Ni content for NLGS (HK). The 5% CB amendment significantly decreased Cd accumulation but significantly increased Cu accumulation in the shoots of NL(TH). For NLGS (HK), 5% CB amendment significantly decreased Cd accumulation in the shoots, with no significant influence on the other heavy metals. The 2% and 5% CB amendments significantly promoted Pb and Cu accumulation in the roots of the two cultivars. Cd and Zn contents in the roots of the 2% CB and 5% CB treatments were not significantly different from those
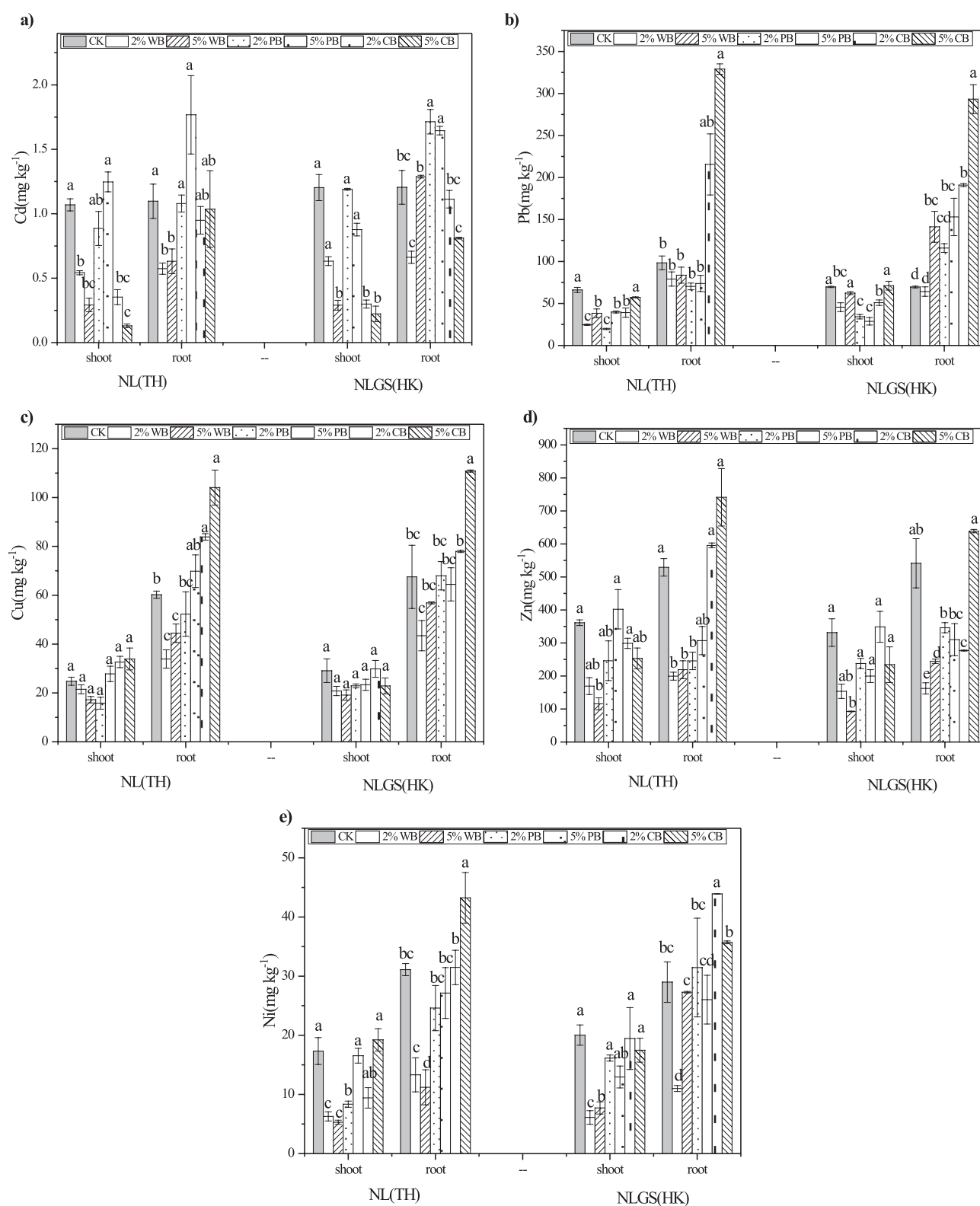
Fig. 5. Concentrations of Cd a), Pb b), Cu c), Zn d), and Ni e) in the shoots and roots of *Ipomoea aquatic*; different letters above the bars indicate significant difference at the 5% level.

in control. Ni content in the roots of NLGS (HK) but not NL(TH) was significantly increased by 2% CB. In the 5% CB treatment, Ni content in roots were not significantly different from those in control for both cultivars.

Generally, higher concentrations of the heavy metals were found in the plant roots than in the plant shoots, which was especially true for Pb and Cu.

Discussion

Biochar can improve soil porosity and specific surface area because of the macropores and micropores in biochar [36-38]. These characteristics may influence soil biological community [39, 40], nutrient cycles [41, 42], and soil structure and quality [38], and in turn promote plant growth. In the present study, the WB and PB amendments significantly increased the growth of *I. aquatica* Forsk. Among these treatments, the 2% WB had the most significant impact on plant growth (Table 2). The results indicated that the growth of *I aquatica* Forsk varied depending on biochar type and application rate. Kumar et al. [24] documented significantly improved plant growth and physiology. However, in this study, the CB amendment significantly inhibited the growth of *I. aquatica* Forsk. The results might be due to different physicochemical properties of the biocharss and the heavy metals they contained.

Biochar has a large specific surface area, surface energy, and strong tendency to combine with heavy metal ions, thus it can be used to remove and passify heavy metals in wastewater and soil [24, 43]. The properties of the modified are conducive to promoting the degradation of harmful substances and the deactivation, changing the form of heavy metal ions in soil [44]. SOM influences the availability of soil heavy metals due to its abundant functional groups [45]. The specific surface area and adsorption capacity of SOM are far larger than any mineral gel [45]. The binding of heavy metals on SOM reduces the absorption of Cd and Zn by plants [46]. Lots of researchers evidenced that biochar can increase soil pH and SOM, and further reduced heavy metals bioavailability [47]. In the present study, the addition of biochar significantly increased soil pH value and SOM content and decreased the availability of some heavy metals in soil. The 5% CB brought the biggest increase in SOM and the availability of heavy metals in soil. However, the SOM content was significantly higher in the 2% WB treatment than in the 5% WB treatment, which may be related to the carbon release ability of biochar or microbial utilization, indicating that a higher rate of biochar may not necessarily always bring a larger increase in SOM content. Long et al. [48] documented that water-soluble Cd and the organic-bound Cd increases gradually with the increase of DOM, but the iron/manganese oxide-bound Cd does not change significantly.

Numerous studies have shown that the effect of biochar on the bioavailability of heavy metal varied with soil properties, biochar types, plant species, and metal contaminants [23]. In our research (Tables 3 and 4), plant varieties significantly affected Pb and Ni accumulation in the plant shoot, while significant variations in all tested metals (Cd, Pb, Cu, Zn, Ni) accumulation in plant shoots and roots under the influence of different biochar types. For biochar level, it significantly affected Cd, Pb, and Zn accumulation in shoot and Pb, Cu, and Zn accumulation in roots. The interactions between plant variety, biochar type, and biochar level also affected heavy metal accumulation in the plants to varying degrees. As shown in Fig. 5, effects on the absorption of heavy metals differed with biochar composition and level, the 2% WB had the most significant promoting effect on the restriction of absorption by plant. Furthermore, the treatments of 2% and 5% WB, 2% and 5% PB, and 2% CB did not have significant effects on the total concentration of Pb, Cu, Zn, and Ni in the soils, although in some treatments, heavy metal concentrations in the soils were increased. On the one hand, this may be related to the biochar character (such as pH and the microstructure) and biochar organic composition [25, 49]. On the other hand, such result might be caused by the heavy metals on the biochar, which were released into the soil. Thus, the raw materials of biochar should be ecologically friendly.

Conclusion

In conclusion, the three types of biochar significantly increased soil pH and SOM content. However, the influence on the bioavailability of heavy metals was biochar type-dependent. The 2% and 5% WB amendment significantly decreased the plant uptake of Cd, Pb, Cu, Zn, and Ni. Part treatments of PB and CB amendments increased total heavy metals and their availability in the soils, indicating that the heavy metals contained in the biochars might have been released into the soil. Thus, the raw materials of biochar should be ecologically friendly. The findings from this work provide theoretical support for pollution regulation in vegetable fields.

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Conflict of Interest

The authors declare no conflict of interest.

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