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The biochar-improved growth-characteristics of corn (*Zea mays* L.) in a 22-years old heavy-metal contaminated tropical soil

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Abstract. Biochar was suggested to lower the concentrations of heavy metals in contaminated soils and therefore may improve plant growth. This research was to evaluate the growth of corn (*Zea mays* L.) in a biochar-amended heavy-metal-contaminated tropical soil. Soil samples were collected from well-maintained experimental plots 22 years after amendment with heavy-metal containing industrial waste at 0 – 60 Mg ha⁻¹. Corn plants were grown for 4 weeks in the soil samples amended with biochar at 0 – 10 Mg ha⁻¹. The corn plant height and dry masses (roots, shoots, and the whole plants) were lowered by waste in relation to the increase in the soil Cu and Zn concentrations. The corn plant dry –weight masses (roots, shoots, whole plant) were well and negatively correlated with the soil Cu and Zn concentrations. The corn plant uptake of Cu and Zn decreased with the increase in the soil Cu and Zn concentration. Biochar improved the corn plant height and dry-weight masses, related to the decrease in the soil Cu and Zn concentrations. Biochar also increased the Zn uptake at waste level of ≥ 15 Mg ha⁻¹ and increased the Cu uptake at waste level ≤ 15 Mg ha⁻¹. The corn plant Cu uptake was linearly and positively correlated with plant dry-weight masses of roots, shoot, and whole plant masses.

1. Introduction

Heavy-metal contaminated soils are continuously becoming important problem in the environment [1-13]. The growth, development, and production of several plants were reported to be disturbed in the increasing heavy metals in polluted soils [3, 6, 9, 12, 14-19]. Our previous research showed that the growth of corn (*Zea mays*) [20], water spinach (*Ipomoea aquatica*), caisim (*Brassica chinensis* var. *Parachinensis*) and lettuce (*Lactuca sativa*) [20-21] were retarded by the presence of Cu and Zn originated from metal-wares industrial waste amended in tropical soils. For examples, [21] reported that the dry weight of caisim decreased by 38.1%, while those of water spinach and lettuce decreased by 52% and 100%, respectively, in soil amended with Cu-containing waste at 60 Mg ha⁻¹. Several other soil workers also reported the negative effects of heavy metals on the growth and production of agricultural commodities and grasses like napier grass (*Penisetum purpureum*) [12,17, 22-24].



The above problem must be solved. A number of physical, chemical, and biological methods had been recently offered [16, 25-37]. Among the promising methods were the use of organic matters particularly biochar [25, 27, 31, 38-42]. Biochar was reported to have relatively high adsorption capacity towards metal cations, ability to enhance soil pH, and most importantly durability in the soil environment [27,31, 39, 41-45]. [45] summarized that biochar was widely used to manage heavy metals in soil environment. It was due to its large specific surface area and high organic matter content and therefore it may significantly increase the soil pH, expand the soil cation exchange capacity and soil exchangeability. It may also decrease the soil soluble as well as mobility and availability of heavy metals in soils. The addition of biochar into the soil system may enhance among which the soil exchange capacity. The increase in the soil colloid negative charges may enhance the adsorption sites for heavy metals and lower their mobility and bioavailability and thus decrease the plant absorption on heavy metals and improve plant growth, development, and production. The increase in soil pH by biochar may also simultaneously stimulate the enhancement of heavy metal adsorption by soil colloids and plant growth, development and production.

This research was to evaluate the growth characteristics of corn plant that include plant growth (plant height and dry-weight of biomasses) and plant Cu and Zn uptakes in tropical soils amended with industrial waste high in Cu and Zn in 1998 (22 years ago) treated with biochar of rice husk.

2. Materials and Methods

2.1 Soil and biochar samples

This research was a glass-house experiment using soil samples contaminated with Cu and Zn collected from a well-maintained experimental field in Natar, Lampung, Indonesia, treated with industrial waste high in Cu and Zn in 1998. Soil was sampled from topsoils (0-15 cm) only from plots treated with 0, 15, and 60 Mg waste ha⁻¹, each was taken separately from the three replicates in the field. Soil samples were air-dried, ground to pass a-2-mm sieve, and mixed thoroughly before being used in the glass-house experiment. As reported by [20], the soil sample and industrial waste were initially characterized by some properties listed in Table 1. Biochar was prepared from rice husk.

Table 1. Selected initial properties of soil, compost, and industrial waste used in the experiment^a.

Properties	Units	Methods	Soil	Compost	Industrial Waste
Sand	%	Hydrometer	41.2		
Silt	%	Hydrometer	26.0		
Clay	%	Hydrometer	32.8		
pH (1:2)		Electrode	5.11		7.3
Organic C	g kg ⁻¹	Walkey and Black	1.28	275	
Total N	g kg ⁻¹	Kjeldahl		40.1	
C/N				6.85	
Cu	mg kg ⁻¹	DTPA	1.60		754
Zn	mg kg ⁻¹	DTPA	0.89		44.6
Pb	mg kg ⁻¹	DTPA	0.08		2.44
Cd	mg kg ⁻¹	DTPA	nd ^b		0.12

^aAfter [20]

2.2 Experimental design

Treatments were arranged in a randomized block design with 2 treatment factors and 3 blocks (replications). The first factor was soil samples including Control Soil (amended with 0 Mg waste ha⁻¹, S₀), soil sample with low heavy metals (amended with 15 Mg waste ha⁻¹, S₁), and soil with high heavy

metals (amended with 60 Mg waste ha⁻¹, S₂). The second factor was biochar, given at 0 (B₀), 5 (B₁), and 10 Mg ha⁻¹ (B₂).

Planting medium for the green-house experiment was prepared using a 200 g (oven-dry equivalent 105°C, 24 hours). Biochar (oven-dry equivalent 60°C 3 x 24 hours) was added and mixed thoroughly with the soil sample before the medium was put in plastic pots and capillary watered to the soil field-water capacity. Three corn seeds were planted in each pot and one seedling was left in each pot after one week. Corn plant was let to grow for 4 weeks. The soil water content was capillary maintained at the soil field water capacity during planting growth.

2.3 Soil and plant harvest and analyses

Plant and soil samples were harvested at the end of a four-week plant growth. Corn plant was cut at the soil surface. Plant biomasses (roots and shoots) were carefully cleaned from the soil masses using tap water. Plant biomasses were weighed for their dry-weight after being oven-dried at 60°C for 3 x 24 hours. Soil samples were also harvested after 4 weeks.

To analyze the concentration of Cu and Zn in plant roots and plant shoots, a 1 g of oven-dried and ground plant tissue was put in a porcelain crucible and placed in a furnace, heated at 300°C for 2 hours and then at 500°C for 4 hours, after which the plant sample was let to reach room temperature. The plant sample was wetted with several drops of distilled water and treated with 10 ml of 1 N HCl and put on a hot plate and let to gently boil. After cooling, the soluble plant tissue ash was filtered into a 100 ml volumetric flask. The crucible was then rinsed with 10 ml 1 N HCl and about 50 ml distilled water on the filter paper into the volumetric flask. Distilled water was added to dilute the filtrate to 100 ml. The filtrate was gently shaken before analyzed using flame AAS at $\lambda = 324.7$ nm for Cu and at $\lambda = 213.9$ nm for Zn.

The determination of the soil Cu and Zn used 1 N HNO₃ employing flame AAS at $\lambda = 324.7$ nm for Cu and $\lambda = 213.9$ nm for Zn. Soil pH (water 1:2) and Walkey and Black Organic C were also determined.

3. Results and Discussion

3.1 The growth and heavy metal uptake of corn plant in waste-amended soil

The growth of corn plant, as indicated by plant height and plant biomasses, was significantly depressed by waste (Figure 1 and Figure 2). The phenomena were related to the significant increase in the soil Cu and Zn concentration originated from waste amended about 22 years ago (Figure 3) which contained high amounts of Cu and Zn (Table 1). Analysis of variance (Anova) indicates that waste level significantly depressed the plant height and plant biomasses (roots, shoots, and the whole plant) and significantly enhanced the soil concentrations of Cu and Zn (Table 2). Our previous researches also showed that the waste borne Cu and Zn in the soils depressed the growth of several plants including corn plant, water spinach, caisim, lettuce, and napier grass [20-22]. The phenomena were also in accordance with various researches previously reported [14-17]. Elevated concentrations of heavy metals in soil system are detrimental to plants.

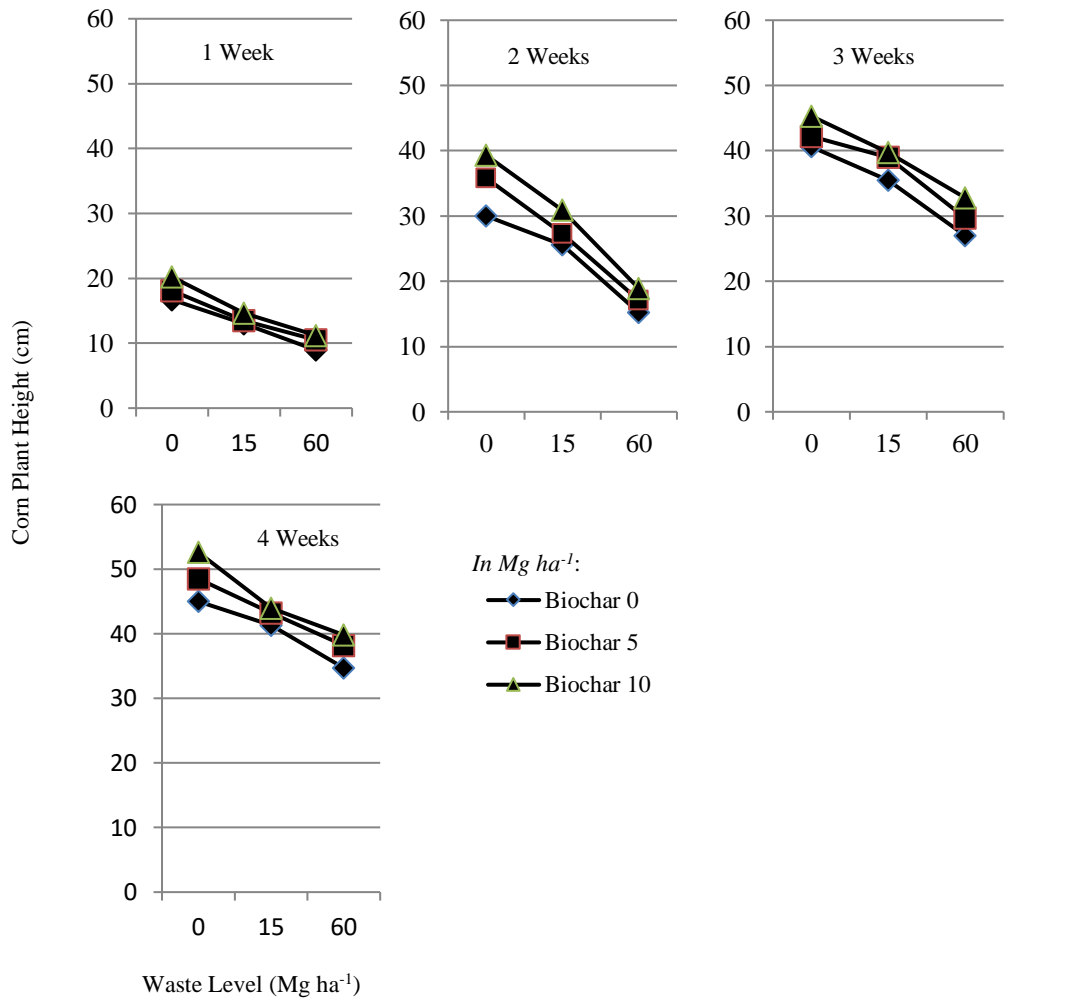


Figure 1. The improvement of corn plant height in waste-amended soil by biochar.

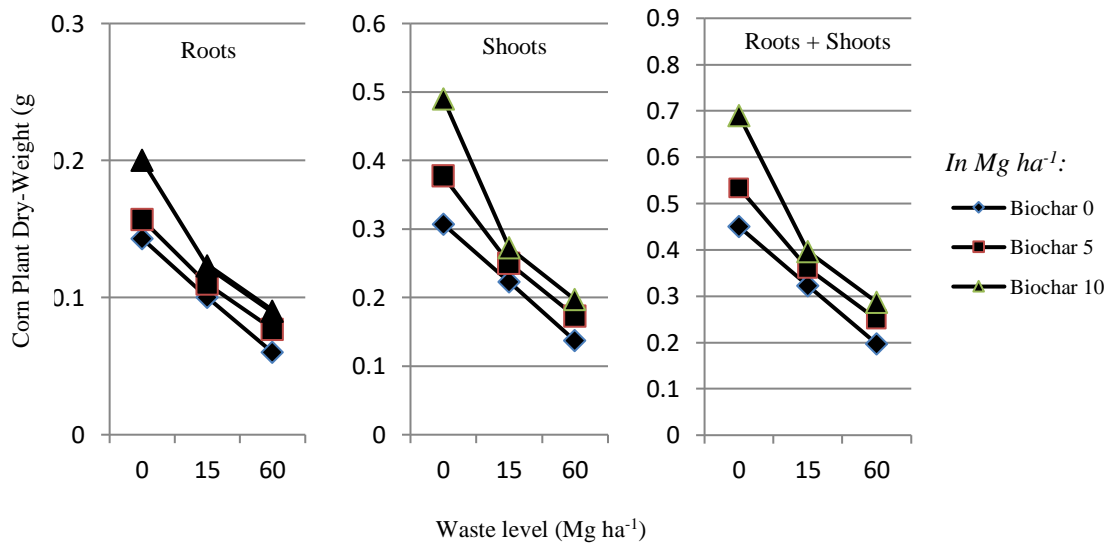


Figure 2. The improvement of plant biomasses in waste-amended soil by biochar.

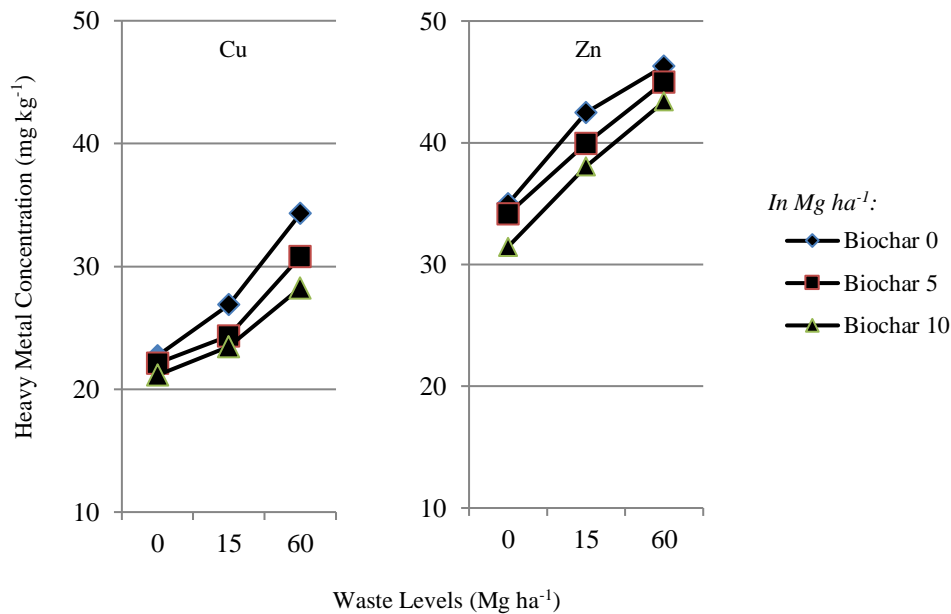


Figure 3. The effect of biochar on Cu and Zn concentrations in waste-amended soil.

Table 2. Analysis of variance of characteristics of soil and corn plant grown in heavy-metal contaminated soil treated by biochar.

Soil and Corn Plant Characteristics	Waste	Biochar	Waste Level vs Biochar
Plant Height	* ^a	*	ns
Root Dry-Weight	*	*	*
Shoot Dry-Weight	*	*	ns
Plant Dry-Weight	*	*	ns
Soil Cu	*	*	*
Soil Zn	*	*	ns
Soil Organic C	*	*	ns
Soil pH	*	*	ns
Root Cu	*	ns ^b	ns
Root Zn	ns	ns	ns
Shoot Cu	*	*	*
Shoot Zn	*	ns	ns

^aSignificant and ^bns non-significant at 5%

The corn plant uptake of Cu and Zn, which included those accumulated in roots and in shoots, was however significantly lowered by waste levels (Figure 4, Table 2). These observations indicate Cu and Zn in roots and shoots decreases with the increase in the soil concentrations of Cu and Zn. The presence of high Cu and Zn in soil inhibited the absorption of Cu and Zn by plants, showing Cu and Zn disturbance of corn-plant root effectiveness in absorbing Cu and Zn. The suggestion is supported by the fact that the corn-plant root biomass is negatively correlated with the soil Cu and Zn with relatively high correlation coefficients ($R^2 = 0.74^*$ with soil Cu and $R^2 = 0.88^*$ with soil Zn) (Table 3). Table 3 also shows that

corn plant shoot biomass is negatively correlated with soil Cu ($R^2 = 0.62^*$) and with the soil Zn ($R^2 = 0.82^*$). Therefore, high Cu and Zn is detrimental not only to roots but also to shoots of corn plant. However, it is clear that the translocation factor for Cu and Zn was in general > 1.00 , indicating that corn plant was a good phytoextractor for Cu and Zn. Corn was also previously suggested as a heavy-metal bio-accumulator [46].

The plant uptake of Cu and Zn are dependent of plant biomass; the uptake is greater with the increase in plant biomasses. The plant uptake of heavy metals particularly for Cu is highly correlated with plant, root, and shoot biomasses with high correlation coefficients (0.89^* for roots, 0.92^* for shoots, and 0.95^* for the whole plant (Table 4).

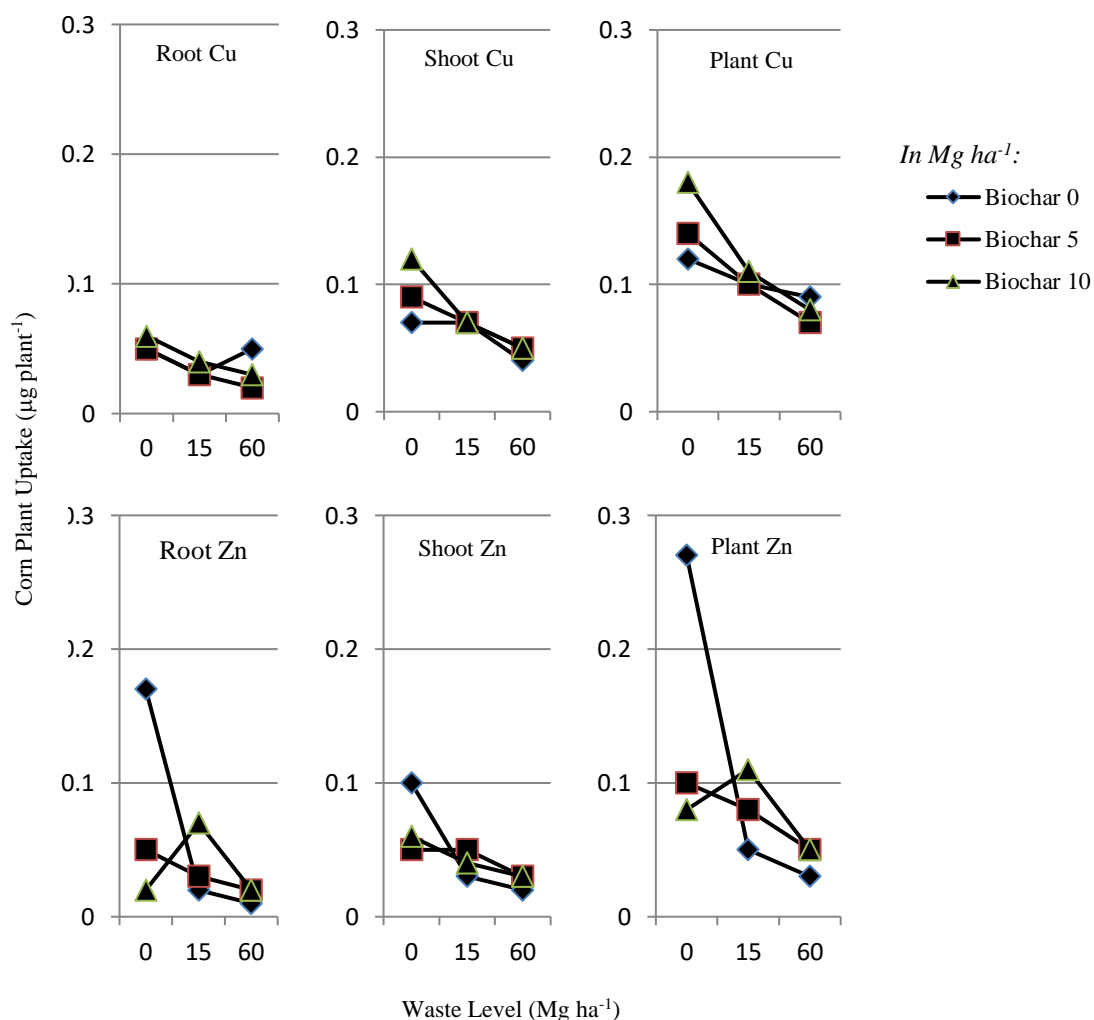


Figure 4. The effect of biochar on corn plant uptake of Cu and Zn from waste-amended soils.

3.2 Effect of biochar

Biochar at 5-10 Mg ha⁻¹ was in general effective in affecting the soil and plant characteristics in heavy-metal containing-waste amended-soil (Table 2). In general biochar significantly affected plant height and biomass dry-weight, soil heavy metals, organic C, and pH, and also Cu accumulated in corn plant

shoots. These findings were in accordance to those reported by [47-48]. For example, [47] reported that biochar 5 and 10% enhanced the corn biomasses in an artificially Pb-Cd-Cr-contaminated soil. [48] reported that biochar treatment increased plant biomasses of green pepper and eggplant fruit as high as 16.0-519.9%.

The soil concentrations of Cu and Zn extracted by 1 N HNO₃ were consistently and significantly lowered by the presence of biochar (Figure 3). It was possible that the soil adsorption sites for heavy metals were enlarged by biochar which was reported to have high amounts of negative charges. The significant increase in soil pH by biochar (Table 2) may have also stimulated the increase in the soil capacity to adsorb heavy metals including Cu and Zn. This synergic effect of biochar may have finally lowered the soil concentrations of Cu and Zn in soil. This phenomenon was also shown by [47-48].

Table 3. The relationships between corn-plant dry-weight masses and Cu and Zn concentrations in heavy-metal contaminated soils.

Soil Heavy Metal	Plant Parts	Linear Equation	R ²
Cu	Roots	Root Dry-Weight = - 0.0083 Soil Cu + 0.3338	0.74* ^a
	Shoots	Shoot Dry-Weight = - 0.0200 Soil Cu + 0.7884	0.62*
	Whole Plants	Plant Dry-Weight = - 0.0283 Soil Cu + 1.1222	0.66*
Zn	Roots	Root Dry-Weight = - 0.0077 Soil Zn + 0.4217	0.88*
	Shoots	Shoot Dry-Weight = - 0.0194 Soil Zn + 1.0374	0.82*
	Whole Plants	Plant Dry-Weight = - 0.0271 Soil Zn + 1.4591	0.84*

^aSignificant at 5%

Table 4. The relationships between corn-plant dry-weight masses and uptake of Cu and Zn in heavy-metal contaminated soils.

Plant Heavy Metal	Plant Parts	Linear Equation	R ²
Cu	Roots	Root Dry-Weight = 3.0242 Plant Cu + 0.0096	0.89* ^a
	Shoots	Shoot Dry-Weight = 4.2425 Plant Cu - 0.0288	0.92*
	Whole Plants	Plant Dry-Weight = 3.9673 Plant Cu - 0.0332	0.95*
Zn	Roots	Root Dry-Weight = 0.1261 Plant Zn + 0.1118	0.05ns ^b
	Shoots	Shoot Dry-Weight = 0.2046 Plant Zn + 0.1775	0.27*
	Whole Plants	Plant Dry-Weight = 0.4381 Plant Zn + 0.3469	0.07ns

^aSignificant, ^bns non-significant at 5%

The lowering bio-availabilities of heavy metals may have lowered negative effects of heavy metals on the growth of corn plant. This suggestion is supported by Figure 1 and Figure 2 that show that the corn plant height and root and shoot biomass dry-weight in biochar-amended soils were significantly higher than those in soils not amended with biochar, which was also shown by [47-48]. Biochar mitigated the soil heavy metal contamination.

The corn plant uptake of heavy metals, particularly Cu, accumulated in shoot was significantly affected by biochar (Table 2). However, in general biochar increased the Zn uptake at waste level of $\geq 15 \text{ Mg ha}^{-1}$ and increased the Cu uptake at waste level $\leq 15 \text{ Mg ha}^{-1}$ (Figure 4). The corn plant Cu uptake was also linearly and positively correlated with plant dry masses of roots, shoot, and whole plant masses (Table 4)

4. Conclusions

The corn plant height and dry masses (roots, shoots, and the whole plants) were lowered by waste in relation to the increase in the soil Cu and Zn concentrations. The corn plant dry masses (roots, shoots, whole plant) were well and negatively correlated with the soil Cu and Zn concentrations. The corn plant uptake of Cu and Zn decreased with the increase in the soil Cu and Zn concentrations. Biochar improved the corn plant height and dry masses, related to the decrease in the soil Cu and Zn concentration. Biochar also increased the Zn uptake at waste level of $\geq 15 \text{ Mg ha}^{-1}$ and increased the Cu uptake at waste level $\leq 15 \text{ Mg ha}^{-1}$. The corn plant Cu uptake was linearly and positively correlated with plant dry masses of roots, shoot, and whole plant masses.

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