



## Metal absorption by pigweed and Napier grass in biochar-treated soils

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### ABSTRACT

Heavy metal absorption by plants is suggested to be affected by soil treatment with different types of biochar. Due to the various types of available biochar materials in the environment, the effects of three representative biochar types were evaluated in a greenhouse experiment using polluted soils planted with pigweed (*Amaranthus spinosus* L.) and Napier grass (*Pennisetum purpureum* Schumach). Soil treatments were conducted with biochar of rice (*Oryza sativa*)-husk, corn (*Zea mays*)-cob, and cassava (*Manihot utilissima*)-stem at 10 Mg ha<sup>-1</sup>. Soils and plants were analyzed for Cu and Zn after 4 weeks of plant growth. The results showed that Cu and Zn accumulation by pigweed and Napier grass were higher in soils polluted with more Cu and Zn. Pigweed, in general, acted as a phytoextractor, accumulating more Cu and Zn in shoots, while Napier grass acted as a phyto-stabilizer, accumulating more Cu and Zn in roots. Pigweed accumulated Cu more effectively than Napier grass, while Napier grass accumulated Zn more effectively. In contrast to rice-husk or corn-cob biochar, cassava-stem biochar increased the concentrations of Cu and Zn in the soil. Rice-husk and corn-cob biochar enhanced soil Cu and Zn accumulation by pigweed and Napier grass, while cassava-stem biochar decreased it. Rice-husk and corn-cob biochar exhibited greater potential than cassava biochar for the phytoremediation of soil Cu and Zn by pigweed and Napier grass in heavily metal-polluted tropical soils.

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## 1. INTRODUCTION

The threat caused by heavy metals in the soil environment is now greater due to pollution and human negligence towards their safety (Asati et al., 2016; Chen et al., 2018; Miao et al., 2020; Salam, Hidayatullah, et al., 2021; Sun et al., 2023). For example, according to Sun et al. (2023), anthropogenic sources may contribute up to 80.2% of the heavy metal concentrations in soils, indicating a significant influence on the accumulation of heavy metals in soils. Some possible anthropogenic sources of heavy metals were coal-related industrial activities, sewage irrigation, the application of agrochemicals such as chemical fertilizers and pesticides, and vehicle emissions. As pollution levels continue to rise, the need for effective strategies to remediate heavy metal-contaminated soils has become more urgent than ever. Current approaches typically fall into three main categories: physical, chemical, and biological methods. Of these techniques, the use of the biological method called phytoremediation is reported to be promising (Guerra Sierra

et al., 2021; Ishii et al., 2015; Juel et al., 2021; Khodijah et al., 2019; Sarwar et al., 2017). Combining this approach with the use of biochar—a chemical remediation method—may significantly improve its effectiveness in addressing heavy metal contamination in soils (Afriyani et al., 2024; Chen et al., 2018; Guo et al., 2020; Hayyat et al., 2016; Liu et al., 2022; Salam et al., 2022; Wang et al., 2018).

Beyond its low cost, biochar offers several promising advantages thanks to its unique properties. It can help raise soil pH and increase the number of negatively charged surfaces in the soil, thereby enhancing the soil's overall capacity to immobilize heavy metal cations through adsorption (Chen et al., 2018; Guo et al., 2020; Król et al., 2020; Lahori et al., 2020; Olalekan et al., 2016; Salam et al., 2022; Ugwu et al., 2020; Zhang et al., 2018). Previous studies have shown that an increase in soil pH generally enhances the soil's adsorption capacity, which in turn reduces the levels of extractable heavy metals—such as those in soluble,

exchangeable, and other labile forms (Król et al., 2020; Olalekan et al., 2016; Zhang et al., 2018). Thus, biochar may make heavy metals available at suitable concentrations for plant-root absorption. Biochar may also retain some of the soil heavy metals while providing heavy metals at suitable concentrations for rapid absorption by plants. The magnitude of the effect of biochar also theoretically depends greatly on the type of biochar due to the nature of biochar origin (dos Santos Martins et al., 2019; Ghori et al., 2019; Huang et al., 2017; Liu et al., 2022).

Unlike biochar, which clearly affects the soil properties and metal absorption, plants affect soil heavy metals differently (Chen et al., 2019; Dietz et al., 2020; Juel et al., 2021; Khodijah et al., 2019; Ohta & Hiura, 2016; Silva et al., 2021). While biochar increased the soil pH, plant roots decreased the soil pH through the excretion of  $H^+$  ions and organic acids, which may decrease the soil pH (Dietz et al., 2020; More et al., 2020; Ohta & Hiura, 2016; Wu et al., 2018; Yang et al., 2019). The decrease in soil pH may decrease the soil adsorption capacity or the soil CEC, leading to the dissolution of some of the adsorbed and precipitated heavy metals, increasing the extractable heavy metals. These relationships may ultimately enhance the absorption of heavy metals by plant roots from soils contaminated with heavy metals. The biochar–plant interactions are dependent on the types of biochar and plant species.

Empirical data on the impact of different biochar types in phytoremediation for contaminated tropical soils were not fully available. No accessible data related to this issue was available in the current literature, especially for Indonesian tropical soils. One of the probabilities was related to the scarcity of a single type of heavy metal contaminated soil with distinct and increasing concentrations for particular heavy metals like Cu, Zn, and other heavy metals to conduct this research. The impact of phytoremediation plants in the presence of various types of biochar on soil properties, plant growth, and heavy metal absorption was unknown and thus evaluated in this research using soil samples with varying concentrations of Cu and Zn. These kinds of soil samples were available in an experimental field located in Lampung, Indonesia, preserved naturally since 1998, or about 24 years. The soil concentrations of heavy metals ranged from 40 to 80 for Cu and 30 to 70  $mg\ kg^{-1}$  for Zn. Gaining a clear understanding of these relationships is essential for evaluating the effectiveness of combining phytoremediation plants with biochar in treating heavy metal-contaminated soils.

## 2. MATERIALS AND METHODS

### 2.1. Soil samples and experimental design

To achieve the research objective, soil samples with varying concentrations of heavy metals, specifically Cu and Zn, were utilized. Soils historically amended with high heavy metal-containing industrial waste, in Sidosari, Natar, South Lampung (Salam, Pakpahan, et al., 2021) were sampled at the  $A_p$  horizon (0–20 cm) in October 2022, or about 24 years after amendment. Soil samples consisted of Control ( $S_0$ ), soils historically treated with low concentrations of heavy metals ( $S_1$ ) and soils historically treated with high concentrations of heavy metals ( $S_2$ ). Analysis showed that  $S_1$  and  $S_2$  contained significant amounts of Cu, Zn and Ni (Salam et al., 2022; Salam, Pakpahan, et al., 2021).



Figure 1. The production of biochar by a simple pyrolyzer.

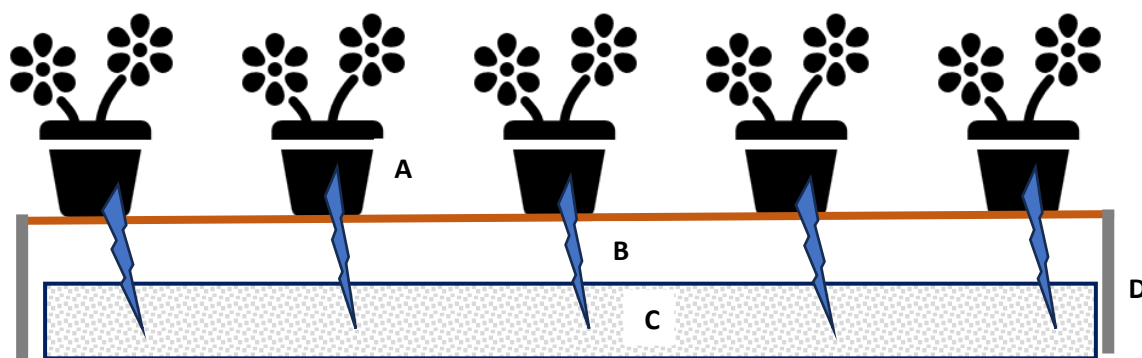
Air-dried soil samples were thoroughly mixed, ground, and passed through a 2-mm sieve before being used in the greenhouse experiment. Each experimental unit consisted of 400 grams of oven-dry equivalent soil placed in a 500-gram pot. The experiment was arranged in a completely randomized design (CRD) with 2 factors replicated three times. The first factor was soil types  $S_0$ ,  $S_1$ , and  $S_2$ , and the second factor was biochar types, each derived from the biomass of different plants: rice husk ( $B_1$ ), corn cob ( $B_2$ ), and cassava stem ( $B_3$ ). Biochar was given at  $10\ Mg\ ha^{-1}$  or 2 grams oven-dry equivalent biochar per pot containing 400 grams oven-dry equivalent soil sample.

Biochar was prepared using a conventional method by employing a simple pyrolyzer (Afriyani et al., 2024). Finely chopped plant materials such as rice husks, corn cobs, or cassava stems were placed in a pyrolyzer (Fig. 1) and burned for 20–30 minutes, until the top layer turned black. The charred material from the bottom was then gradually brought to the top, allowing the entire pile to blacken evenly. The charring process continued for 4–5 hours before being extinguished with water. The resulting biochar was air-dried, passed through a 4-mm sieve, and analyzed for moisture content.

After being thoroughly mixed on a clean plastic sheet, the soil–biochar mixtures were placed into pots. These pots were then brought to field water capacity using capillary action, facilitated by a wick that connected the soil in each pot to a water reservoir positioned underneath (Fig. 2). Water molecules from the Water Reservoir (C) moved capillary through the Connecting Wick (B) to reach the Soil Mass in Pot (A). The movement of water in the connecting wick stopped when the water content in the soil–biochar mixture reached its field capacity. Seedlings of pigweed (or amaranth, *Amaranthus spinosus* L.) or Napier grass (or elephant grass, *Pennisetum purpureum* Schumacher) were planted in the pot soils after one week of soil–mixture incubation in a greenhouse at open temperature and allowed to grow for 4 weeks at the soil–field water capacity. No fertilizers were added, and no pesticide was used.

### 2.2. Observation and statistical analysis

Soil and plant biomasses (roots and shoots) were harvested at the end of the 4-week plant growth. Soil samples were analysed for extracted Cu and Zn (using 1 *N*  $HNO_3$  extractant), pH (1:2 soil water using pH electrode), and CEC (using 1 *N*  $NH_4OAc$  pH 7.00 in batch method). Plant biomasses were analysed for oven-dry mass using an analytical balance and Cu and Zn contents using the method described in Silva et al. (2021).



**Figure 2.** Capillary watering system to maintain the soil field water capacity (A – pot with a hole at bottom, B – connecting wick from soil – biochar mixture with water, C – common water reservoir, D – Stand for pot holder).

Differences among treatments were compared using the standard error of the mean (SEM), which was predicted with Equation 1.

$$SEM = \frac{Std.P}{\sqrt{n}} \dots\dots\dots [1]$$

where Std.P was Population Standard Deviation and n was the population size. The standard deviation of the mean (Std. P) was calculated using Microsoft Excel at a significance level of  $\alpha = 0.05$ . Differences between two means were considered statistically significant when they exceeded twice the standard error of the mean ( $2 \times SEM$ ).

The relationships between each dependent variable and any independent variable were analyzed using single-variable simple linear regression employing the Microsoft Excel Application at  $\alpha = 0.05$ . The single-variable regression analysis for each independent variable was expressed with the following model with constants a and b, dvar = dependent variable, ivar = independent variable, and  $R^2$  = the related correlation coefficient:  $dvar = a + b \text{ ivar}$ ,  $R^2$ .

### 3. RESULTS

#### 3.1. Biochar effects on soil properties

The lowest pH in soils planted with pigweed was in the control soil treated with rice-husk, while the highest was in high-metal soils treated with corn cob and cassava stem biochar. Corn-cob and cassava-stem biochar in general enhanced the soil pH in all soil types ( $S_0, S_1, S_2$ ), all higher than the initial soil pH, i.e., 6.00, 6.01, and 6.08, respectively. On the contrary, rice husk biochar decreased the soil pH. Cassava-stem biochar had no noticeable effect on soil pH, whereas both rice-husk and corn-cob biochar slightly reduced the pH of soils planted with Napier grass (Table 1).

Soils with high heavy metal content exhibited higher pH levels than those with lower heavy metal content. Similar trends were also noted in Napier grass soils (Table 1). Nevertheless, the pH increases in soils planted with Napier grass were less pronounced. The soil pH decreased in order of treatment with biochar: cassava stem > corn-cob > rice husk, both in pigweed and Napier grass planted soils.

Soil copper and zinc levels showed a slight increase following biochar treatment, especially in low heavy metal soils planted with Napier grass and amended with cassava-stem biochar. Shown also in Table 1 that Cu and Zn decreased in order of high heavy metals soils > low heavy metals soils > control soils. The soil Cu and Zn decreased slightly in the order of cassava-stem > corn-cob > rice-husk treated soils. No pattern in the change in soil CEC with biochar and soil types.

#### 3.2. Biochar effects on plant growth

The height, root, and shoot of pigweed were stimulated by biochar treatment but inhibited by high levels of soil heavy metals. Cassava stem and corn cob biochar were more effective in stimulating plant growth compared to rice husk biochar (Table 2). A similar stimulating trend was observed with biochar treatment in Napier grass roots, but with higher plant biomass (Table 2). The root/shoot ratio of pigweed decreased in the order rice-husk > corn-cob > cassava-stem, and that of Napier grass increased with biochar of rice-husk < corn-cob < cassava-stem. The root-to-shoot ratio in heavy metal-polluted soils was also higher than in control soils.

#### 3.3. Biochar effects on metal absorption

The accumulation of Cu and Zn was, in general, highest in corn-cob and rice-husk, followed by cassava-stem biochar-treated soils, both in pigweed and Napier grass soils, particularly in high heavy-metal soils. This indicates that corn-cob and rice-husk resulted in higher Cu and Zn accumulation both in pigweed and Napier grass compared to cassava-stem biochar. Metal absorption in roots and shoots of pigweed was higher than in Napier grass (Table 3).

Nearly all translocation factor (TF) values were below 1.00. The highest TFs for copper and zinc in pigweed soils were observed in treatments with cassava-stem biochar. Similarly, most TF values in Napier grass were also under 1.00, with the highest being the zinc TF in low heavy metal soils treated with cassava-stem biochar. These findings indicate that cassava-stem biochar tends to increase TF values, suggesting it promotes greater translocation of metals to the shoots. By definition, TF represents the ratio of heavy metal accumulation in plant shoots to that in the roots (Table 3).

#### 3.4. Relations of metal absorption with soil properties and plant growth attributes

The presence of pigweed, Napier grass, and biochar led to changes in soils as evidenced by the relationships between soil properties and plant growth attributes (Table 4). As depicted in Table 4, that significant relationships were observed between the levels of soil Cu with pH ( $R^2 = 0.77^*$ ), soil Zn with pH ( $R^2 = 0.78^*$ ), and soil (Cu + Zn) with pH ( $R^2 = 0.79^*$ ) in pigweed planted soils. Additionally, a relationship was found between soil Cu and pH ( $R^2 = 0.43^*$ ) in Napier grass planted soils. The concentrations of Cu and Zn were evidently influenced by soil pH dynamics in the presence of plants, consistent with the general observation that soil heavy-metal concentrations are inversely related to soil pH.

**Table 1.** Effect of biochar types on pH, CEC, and extracted Cu and Zn of heavy metal-polluted soils planted with pigweed or Napier grass.

No	Treatment		pH	CEC	Cu	Zn
	Symbol	cmol <sub>c</sub> kg <sup>-1</sup>		..... mg kg <sup>-1</sup> .....		
<i>Pigweed</i>						
T <sub>1</sub>	S <sub>0</sub> B <sub>1</sub>	5.28 a	8.08 bc	42.4 a	38.0 a	
T <sub>2</sub>	S <sub>0</sub> B <sub>2</sub>	5.72 b	7.73 bc	39.0 a	34.5 a	
T <sub>3</sub>	S <sub>0</sub> B <sub>3</sub>	5.89 b	9.96 d	46.7 a	42.2 a	
T <sub>4</sub>	S <sub>1</sub> B <sub>1</sub>	6.17 c	6.82 a	60.6 b	58.7 b	
T <sub>5</sub>	S <sub>1</sub> B <sub>2</sub>	6.14 c	6.97 a	69.0 bc	64.9 bc	
T <sub>6</sub>	S <sub>1</sub> B <sub>3</sub>	6.20 c	8.31 c	74.5 cd	70.8 c	
T <sub>7</sub>	S <sub>2</sub> B <sub>1</sub>	6.24 c	7.68 bc	79.8 d	69.7 c	
T <sub>8</sub>	S <sub>2</sub> B <sub>2</sub>	6.34 c	7.67 b	78.6 cd	70.0 c	
T <sub>9</sub>	S <sub>2</sub> B <sub>3</sub>	6.33 c	6.68 a	78.8 cd	71.6 c	
	SEM at $\alpha = 0.05$	± 0.11	± 0.31	± 5.23	± 4.80	
<i>Napier Grass</i>						
T <sub>1</sub>	S <sub>0</sub> B <sub>1</sub>	5.73 a	8.08 bc	40.7 a	36.3 a	
T <sub>2</sub>	S <sub>0</sub> B <sub>2</sub>	5.87 b	7.73 bc	37.2 a	31.6 a	
T <sub>3</sub>	S <sub>0</sub> B <sub>3</sub>	5.98 de	9.96 d	45.0 a	39.5 a	
T <sub>4</sub>	S <sub>1</sub> B <sub>1</sub>	5.74 a	6.82 a	58.8 b	56.9 b	
T <sub>5</sub>	S <sub>1</sub> B <sub>2</sub>	5.89 bc	6.97 a	67.5 bc	62.3 bc	
T <sub>6</sub>	S <sub>1</sub> B <sub>3</sub>	6.05 def	8.31 c	71.8 c	65.9 bc	
T <sub>7</sub>	S <sub>2</sub> B <sub>1</sub>	6.06 ef	7.68 bc	77.9 c	67.9 c	
T <sub>8</sub>	S <sub>2</sub> B <sub>2</sub>	5.97 cd	7.67 b	76.5 c	69.7 c	
T <sub>9</sub>	S <sub>2</sub> B <sub>3</sub>	6.08 f	6.68 a	77.1 c	71.3 c	
	SEM at $\alpha = 0.05$	± 0.04	± 0.31	± 5.20	± 4.92	

**Notes:** S is soil treated in 1998 with no waste (S<sub>0</sub>), with 15 Mg waste ha<sup>-1</sup> (S<sub>1</sub>), and with 60 Mg waste ha<sup>-1</sup> (S<sub>2</sub>), B is biochar types: rice husk (B<sub>1</sub>), corn cob (B<sub>2</sub>) cassava stem (B<sub>3</sub>); figures in one column with the same characters for the same plant do not vary significantly.

**Table 2.** Effect of biochar types on the vegetative attributes of pigweed or Napier grass in heavy metal polluted soils.

No	Treatment		Height	Root	Shoot	Root + Shoot	Root/Shoot
	Symbol	... cm ...	..... g plant <sup>-1</sup> .....				
<i>Pigweed</i>							
T <sub>1</sub>	S <sub>0</sub> B <sub>1</sub>	10.8 b	0.69 ef	0.33 cd	1.02	2.10	
T <sub>2</sub>	S <sub>0</sub> B <sub>2</sub>	12.0 d	0.65 de	0.42 e	1.07	1.50	
T <sub>3</sub>	S <sub>0</sub> B <sub>3</sub>	13.2 e	0.74 f	0.55 f	1.29	1.35	
T <sub>4</sub>	S <sub>1</sub> B <sub>1</sub>	9.66 a	0.57 bc	0.19 a	0.76	3.00	
T <sub>5</sub>	S <sub>1</sub> B <sub>2</sub>	11.2 bc	0.61 cd	0.29 bc	0.90	2.10	
T <sub>6</sub>	S <sub>1</sub> B <sub>3</sub>	11.0 b	0.62 cd	0.35 cde	0.97	1.77	
T <sub>7</sub>	S <sub>2</sub> B <sub>1</sub>	10,0 a	0.48 a	0.18 a	0.66	2.67	
T <sub>8</sub>	S <sub>2</sub> B <sub>2</sub>	11.7 cd	0.58 bc	0.37 de	0.95	1.57	
T <sub>9</sub>	S <sub>2</sub> B <sub>3</sub>	11.7 bc	0.55 b	0.25 ab	0.80	2.20	
	SEM at $\alpha = 0.05$	± 0.34	± 0.02	± 0.04	± 0.06		
<i>Napier Grass</i>							
T <sub>1</sub>	S <sub>0</sub> B <sub>1</sub>		2.40 e	1.17 ab	3.57 c	2.05	
T <sub>2</sub>	S <sub>0</sub> B <sub>2</sub>		0.88 a	1.56 cd	2.44 a	0.56	
T <sub>3</sub>	S <sub>0</sub> B <sub>3</sub>		1.46 bcd	2.25 e	3.71 c	0.65	
T <sub>4</sub>	S <sub>1</sub> B <sub>1</sub>		1.24 b	1.06 ab	2.30 a	1.17	
T <sub>5</sub>	S <sub>1</sub> B <sub>2</sub>		1.44 bc	1.00 a	2.44 a	1.44	
T <sub>6</sub>	S <sub>1</sub> B <sub>3</sub>		1.72 d	1.12 ab	2.84 b	1.54	
T <sub>7</sub>	S <sub>2</sub> B <sub>1</sub>		1.35 bc	1.65 d	3.00 b	0.82	
T <sub>8</sub>	S <sub>2</sub> B <sub>2</sub>		1.55 cd	2.12 e	3.67 c	0.65	
T <sub>9</sub>	S <sub>2</sub> B <sub>3</sub>		1.51 cd	1.33 bc	2.84 b	1.14	
	SEM at $\alpha = 0.05$		± 0.13	± 0.14	± 0.17		

**Notes:** S is soil treated in 1998 with no waste (S<sub>0</sub>), with 15 Mg waste ha<sup>-1</sup> (S<sub>1</sub>), and with 60 Mg waste ha<sup>-1</sup> (S<sub>2</sub>), B is biochar types: rice husk (B<sub>1</sub>), corn cob (B<sub>2</sub>) cassava stem (B<sub>3</sub>); figures in one column with same characters for the same plant do not vary significantly.

Furthermore, significant relationships were observed between soil Zn and Cu ( $R^2 = 0.98^*$ ) and soil (Cu + Zn) and Cu ( $R^2 = 0.99^*$ ) in pigweed-planted soils. The significant relationships in Napier grass planted soil appeared between the soil Zn with Cu ( $R^2 = 0.99^*$ ), soil (Cu + Zn) with Cu ( $R^2 = 1.00^*$ ), and soil (Cu + Zn) with Zn ( $R^2 = 1.00^*$ ) (Table 4). There was no significant relationship between soil heavy metal concentrations and cation exchange capacity (CEC) in either pigweed or Napier grass soils (Table 4).

Pigweed growth was influenced by a combination of soil characteristics, other plant growth traits, and levels of heavy metal accumulation. This is supported by the significant correlation coefficients observed between growth attributes and various soil properties. Significant relationships appeared between pigweed root-weight with soil Cu ( $R^2 = 0.62^*$ ), with soil Zn ( $R^2 = 0.57^*$ ), with soil (Cu + Zn) ( $R^2 = 0.60^*$ ), and with plant height ( $R^2 = 0.42^*$ ). The significant relationships also appeared between the pigweed shoot weight and CEC ( $R^2 = 0.66^*$ ), with plant height ( $R^2 = 0.79^*$ ) and root weight ( $R^2 = 0.72^*$ ). Significant relationships also appeared between plant total-weight with soil CEC ( $R^2 = 0.63^*$ ), with soil (Cu + Zn) ( $R^2 = 0.45^*$ ), with plant height ( $R^2 = 0.68^*$ ), plant root-weight ( $R^2 = 0.89^*$ ), and with shoot weight ( $R^2 = 0.95^*$ ). Plant root accumulation of Cu was well correlated with plant total weight ( $R^2 = 0.95^*$ ), shoot Cu with root Cu ( $R^2 = 0.43^*$ ), total accumulation of Cu with root Cu ( $R^2 = 0.90^*$ ), and with shoot Cu ( $0.65^*$ ). Significant relationships also appeared between shoot Zn with root Zn ( $R^2 = 0.50^*$ ) and total Zn with root Zn

( $R^2 = 0.99^*$ ) (Table 4). The data clearly demonstrate that soil properties—particularly copper (Cu), zinc (Zn), and cation exchange capacity (CEC)—had a significant influence on the growth characteristics of pigweed, which play a crucial role in the plant's ability to accumulate heavy metals.

These phenomena were also observed clearly for Napier grass. The Napier grass growth attributes were also affected by several soil properties, other plant attributes, and heavy metal accumulation. The significant relationships appeared between plant total-weight with soil CEC ( $R^2 = 0.43^*$ ) and with root weight ( $R^2 = 0.40^*$ ). Accumulation of root Cu was well correlated with root weight ( $R^2 = 0.82^*$ ) and with shoot weight ( $R^2 = 0.82^*$ ). Total accumulation of Cu was well correlated with root weight ( $R^2 = 0.92^*$ ), with shoot weight ( $R^2 = 0.92^*$ ), and with root Cu ( $R^2 = 0.92^*$ ). Total accumulation of Zn was well correlated with root Zn ( $R^2 = 0.83^*$ ) and with shoot Zn ( $R^2 = 0.71^*$ ) (Table 4).

#### 4. DISCUSSION

This research clearly revealed that both pigweed and Napier grass absorbed Cu and Zn, with pigweed showing a higher affinity for Cu and Napier grass for Zn, demonstrating distinct preferences for these heavy metals. The uptake of heavy metals increased with higher levels of heavy metal pollution in soils, as evidenced by the greater accumulation of Cu and Zn in heavily polluted soils compared to low metal and control soils (Table 3).

**Table 3.** Effect of biochar types on Cu and Zn absorption by pigweed or Napier grass in heavy metal-polluted soil.

Treatment No	Symbol	Cu				Zn				
		R	S	R + S	TF	R	S	R + S	TF	
		..... $\mu\text{g plant}^{-1}$ .....				..... $\mu\text{g plant}^{-1}$ .....				
<i>Pigweed</i>										
T <sub>1</sub>	S <sub>0</sub> B <sub>1</sub>	0.77 e	0.66 d	1.43 e	0.86	0.55 ab	1.05abc	1.60ab	1.91	
T <sub>2</sub>	S <sub>0</sub> B <sub>2</sub>	0.44 a	0.60 bc	1.04 a	1.36	1.19 cd	0.99 a	2.18cd	0.83	
T <sub>3</sub>	S <sub>0</sub> B <sub>3</sub>	0.59 bc	0.58 abc	1.17 b	0.98	0.45 a	1.02 ab	1.47a	2.27	
T <sub>4</sub>	S <sub>1</sub> B <sub>1</sub>	0.55 b	0.61 c	1.16 b	1.11	0.87 bc	1.03 ab	1.90bc	1.18	
T <sub>5</sub>	S <sub>1</sub> B <sub>2</sub>	0.66 cd	0.61 c	1.27 c	0.92	1.51 de	1.04 abc	2.55de	0.69	
T <sub>6</sub>	S <sub>1</sub> B <sub>3</sub>	0.55 b	0.55 a	1.10 ab	1.00	0.59 ab	1.10cd	1.69ab	1.86	
T <sub>7</sub>	S <sub>2</sub> B <sub>1</sub>	0.72 de	0.60 bc	1.32 cd	0.83	1.72 e	1.16d	2.88e	0.67	
T <sub>8</sub>	S <sub>2</sub> B <sub>2</sub>	0.71 de	0.68 d	1.39 de	0.96	2.17 f	1.28e	3.45f	0.59	
T <sub>9</sub>	S <sub>2</sub> B <sub>3</sub>	0.52 b	0.57 ab	1.09 ab	1.10	0.70 ab	1.06 bc	1.76abc	1.51	
SEM at $\alpha = 0.05$		$\pm 0.03$	$\pm 0.01$	$\pm 0.04$		$\pm 0.19$	$\pm 0.03$	$\pm 0.21$		
<i>Napier Grass</i>										
T <sub>1</sub>	S <sub>0</sub> B <sub>1</sub>	0.24 ab	0.14 c	0.38 ab	0.58	0.92 b	0.62bc	1.54 b	0.67	
T <sub>2</sub>	S <sub>0</sub> B <sub>2</sub>	0.32 cd	0.13 bc	0.45 c	0.40	0.97 b	0.39 a	1.36 b	0.40	
T <sub>3</sub>	S <sub>0</sub> B <sub>3</sub>	0.45 e	0.19 d	0.64 e	0.42	1.09 bc	0.42 a	1.51 b	0.39	
T <sub>4</sub>	S <sub>1</sub> B <sub>1</sub>	0.31 c	0.13 bc	0.44 bc	0.42	1.36 d	1.05 ef	2.41 cd	0.77	
T <sub>5</sub>	S <sub>1</sub> B <sub>2</sub>	0.23 a	0.14 c	0.37 a	0.61	1.72 e	0.89de	2.61 d	0.52	
T <sub>6</sub>	S <sub>1</sub> B <sub>3</sub>	0.30 bc	0.12 bc	0.42abc	0.40	0.43 a	0.48ab	0.91 a	1.12	
T <sub>7</sub>	S <sub>2</sub> B <sub>1</sub>	0.38 d	0.14 c	0.52 d	0.37	1.37 d	0.75cd	2.12 c	0.55	
T <sub>8</sub>	S <sub>2</sub> B <sub>2</sub>	0.51 e	0.11 ab	0.62 e	0.22	1.24 cd	1.23 f	2.47 cd	0.99	
T <sub>9</sub>	S <sub>2</sub> B <sub>3</sub>	0.36 cd	0.09 a	0.45 c	0.25	1.45 d	0.68 c	2.13 c	0.47	
SEM at $\alpha = 0.05$		$\pm 0.03$	$\pm 0.01$	$\pm 0.03$		$\pm 0.12$	$\pm 0.09$	$\pm 0.18$		

**Notes:** S is soil treated in 1998 with no waste (S<sub>0</sub>), with 15 Mg waste ha<sup>-1</sup> (S<sub>1</sub>), and with 60 Mg waste ha<sup>-1</sup> (S<sub>2</sub>), B is biochar types: rice husk (B<sub>1</sub>), corn cob (B<sub>2</sub>) cassava stem (B<sub>3</sub>); figures in one column with the same characters for the same plant do not vary significantly; TF translocation factor.

**Table 4.** The relationships of soil and plant attributes in polluted soils.

	S pH	S CEC	S Cu	S Zn	S (Cu+Zn)	h	r (W)	s (W)	r+s (W)	r Cu	s Cu	r+s Cu	r Zn	s Zn	r+s Zn
S-pH	1.00	0.14	0.77*	0.78*	0.79*	0.02	0.48	0.14	0.27	0.03	0.08	0.05	0.20	0.25	0.22
S CEC	<b>0.02</b>	1.00	0.19	0.21	0.20	0.36	0.49	0.66*	0.63*	0.01	0.02	0.01	0.13	0.01	0.11
S Cu	<b>0.43*</b>	<b>0.20</b>	1.00	0.98*	0.99*	0.11	0.62*	0.31	0.45	0.04	0.02	0.13	0.01	0.22	0.45
S Zn	<b>0.35</b>	<b>0.24</b>	<b>0.99*</b>	1.00	0.99*	0.13	0.57*	0.32	0.44	0.02	0.03	0.01	0.01	0.38	0.20
S Cu+Zn	<b>0.39</b>	<b>0.22</b>	<b>1.00*</b>	<b>1.00*</b>	1.00	0.12	0.60*	0.31	0.45*	0.03	0.02	0.01	0.20	0.42	0.24
h	-	-	-	-	-	1.00	0.42*	0.79*	0.68*	0.08	0.02	0.01	0.07	0.03	0.03
r (W)	<b>0.04</b>	<b>0.04</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	-	1.00	0.72*	0.89*	0.01	0.01	0.01	0.32	0.23	0.33
s (W)	<b>0.13</b>	<b>0.38</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	-	<b>0.04</b>	1.00	0.95*	0.04	0.01	0.03	0.07	0.03	0.07
r+s (W)	<b>0.03</b>	<b>0.43*</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	-	<b>0.40*</b>	<b>0.47</b>	1.00	0.95*	0.01	0.02	0.07	0.03	0.07
r Cu	<b>0.23</b>	<b>0.13</b>	<b>0.07</b>	<b>0.05</b>	<b>0.06</b>	-	<b>0.82*</b>	<b>0.82*</b>	<b>0.33</b>	1.00	0.43*	0.90*	0.13	0.29	0.15
s Cu	<b>0.05</b>	<b>0.58</b>	<b>0.31</b>	<b>0.34</b>	<b>0.32</b>	-	<b>0.14</b>	<b>0.14</b>	<b>0.10</b>	<b>0.01</b>	1.00	0.65*	0.28	0.23	0.30
r+s Cu	<b>0.10</b>	<b>0.31</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	-	<b>0.92*</b>	<b>0.92*</b>	<b>0.40</b>	<b>0.92*</b>	<b>0.11</b>	1.00	0.19	0.32	0.22
r Zn	<b>0.01</b>	<b>0.29</b>	<b>0.10</b>	<b>0.10</b>	<b>0.10</b>	-	<b>0.01</b>	<b>0.01</b>	<b>0.06</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	1.00	0.50*	0.99*
s Zn	<b>0.03</b>	<b>0.31</b>	<b>0.28</b>	<b>0.33</b>	<b>0.30</b>	-	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.06</b>	<b>0.14</b>	<b>0.02</b>	<b>0.31</b>	1.00	0.30
r+s Zn	<b>0.01</b>	<b>0.38</b>	<b>0.21</b>	<b>0.24</b>	<b>0.22</b>	-	<b>0.01</b>	<b>0.01</b>	<b>0.03</b>	<b>0.02</b>	<b>0.05</b>	<b>0.01</b>	<b>0.83*</b>	<b>0.71*</b>	1.00

**Notes:** S is soil, h plant height, r plant root, s plant shoot, W dry-weight; \* significant at  $\alpha=0.05$ ; top part for pigweed and bottom part (**bold face**) for Napier grass.

These findings suggest that both pigweed and Napier grass can be utilized for remediating soils contaminated with low concentrations of Cu and Zn, as well as other heavy metals like Fe, Mn, Ni, and others sharing similar characteristics with Cu and Zn. This suggests that these plants could be used more broadly on contaminated soils—not just in Lampung, but also in other tropical regions with similar soil characteristics, particularly those classified as Ultisols.

This behavior aligns with findings from previous studies, which reported that both plants function as effective heavy metal bioaccumulators (Afriyani et al., 2024; Ishii et al., 2015; Salam, Hidayatullah, et al., 2021; Silva et al., 2021). Afriyani et al. (2024) previously reported that the soil heavy metals increased with the industrial waste levels but were decreased by Thorny amaranth (*Amaranthus spinosus*) or pigweed. The plant growth, as well as root and shoot dry weights, were lowered by the high soil Cu and Zn. The accumulation of Cu and Zn was higher in plant shoots than in roots, correlating well with their concentrations in the soil. Pigweed proved to be an effective Cu and Zn phytoextractor in heavily metal-polluted tropical soils, showing enhanced performance in the presence of biochar.

This research further confirms the role of pigweed as a Cu and Zn phytoextractor. Table 3 displays high TF values for pigweed, suggesting its effectiveness as a heavy-metal phytoextractor. This plant characteristic may, in fact, indicate that it is easier to remove heavy metals from the harvestable parts of pigweed. On the other hand, Napier grass consistently exhibited translocation factor (TF) values below 1.00, suggesting that heavy metals tend to remain in the roots rather than being transferred to the shoots. While this makes it harder to extract heavy metals from the soil, it also reduces the risk of environmental contamination through erosion. In this sense, both pigweed and Napier grass offer distinct yet valuable benefits for heavy-metal remediation—pigweed by facilitating removal, and Napier grass by stabilizing contaminants in place.

As expected, the addition of biochar altered the equilibrium between the soil and plant roots. Although plant

roots may have contributed to a drop in soil pH, as noted by Salam et al. (2022), the application of cassava-stem and corn-cob biochar generally increased soil pH across all soil types, regardless of their heavy metal content. In plots planted with pigweed, the resulting pH levels were consistently higher than the baseline values measured before any biochar or planting treatments—specifically, 6.00, 6.01, and 6.08, respectively (Table 1). Conversely, rice husk biochar decreased the soil pH. The probable increase in soil pH by this biochar may have been surpassed by plant root organic acid and H<sup>+</sup> secretion. However, the cassava-stem biochar had no effect, while rice-husk and corn-cob biochar decreased the pH of soils planted with Napier grass. This suggests that the pH-enhancing impact of cassava-stem biochar was overshadowed by the organic acid and H<sup>+</sup> root secretion of Napier grass. The pH increases in soils planted with Napier grass were lower than those in soils planted with pigweed.

Soil concentrations of copper (Cu) and zinc (Zn) followed a decreasing trend from highly contaminated soils to moderately contaminated soils, and were lowest in the control plots. This pattern reflects the legacy of waste disposal practices dating back to 1998. As expected, biochar treatments influenced these metal concentrations. Overall, the reductions were modest and followed the order: cassava stem > corn cob > rice husk biochar (Table 1). These effects are likely due to the enhanced adsorption of heavy metals by the biochar, which increases the soil's negative charge and improves its binding capacity. This sequence indicated that cassava-stem biochar was not effective in remediating heavily metal-polluted soils because it left high heavy metal concentrations in soils, while corn-cob and rice-husk were better remediators because they left lower Cu and Zn concentrations in soils. Part of the heavy metals were absorbed by plant roots, which must have been higher in soil treated with corn-cob or rice-husk biochar.

Overall, the accumulation of copper (Cu) and zinc (Zn) was generally highest in soils treated with corn cob and rice husk biochars, followed by those treated with cassava stem biochar. This pattern was consistent in both pigweed and

Napier grass, especially in soils with high levels of heavy metal contamination. This indicates that corn-cob and rice-husk resulted in higher Cu and Zn accumulation both in pigweed and Napier grass compared to cassava-stem biochar. Metal absorption in roots and shoots was higher in pigweed than in Napier grass. However, pigweed showed greater accumulation of Cu and Zn in roots, while Napier grass showed greater accumulation in roots than in shoots. Therefore, pigweed was identified as a phytoextractor and Napier grass as a phyto-stabilizer.

The data showed that soil heavy metals were absorbed by pigweed and Napier grass, with their performance greatly determined by soil and plant growth attributes. The important soil properties were soil Cu, soil Zn, soil (Cu + Zn), and soil CEC. The plant attributes for both pigweed and Napier grass included plant height, total plant weight, root weight, shoot weight, and accumulation of heavy metals. The accumulation of heavy metals in plants can differ between roots, shoots, and overall uptake. These variations are influenced by a range of plant characteristics that affect how metals are absorbed from the soil and distributed within plant tissues. In this study, pigweed and Napier grass exhibited distinct patterns of heavy metal absorption and accumulation, highlighting species-specific responses. Such differences are crucial to consider when selecting plants for phytoremediation, as their effectiveness can vary significantly depending on the species and soil type. Understanding these dynamics is essential for developing effective remediation strategies across diverse global soil conditions.

## 5. CONCLUSIONS

The accumulation of copper (Cu) and zinc (Zn) in plants increased in response to higher levels of heavy metal contamination in the soil. Overall, pigweed functioned as a phytoextractor, absorbing greater amounts of Cu and Zn into its shoots, whereas Napier grass acted more as a phytostabilizer, concentrating these metals primarily in its roots. Pigweed proved more efficient at accumulating Cu, while Napier grass was more effective at accumulating Zn. The addition of rice husk or corn cob biochar enhanced the uptake of Cu and Zn by both plants, whereas cassava stem biochar reduced their accumulation. In contrast to rice husk and corn cob, cassava stem biochar led to an increase in soil concentrations of copper (Cu) and zinc (Zn). Both rice husk and corn cob biochars demonstrated greater potential than cassava stem biochar in supporting the phytoremediation of Cu and Zn in heavy metal-contaminated tropical soils using pigweed or Napier grass. Future research exploring the effects of different biochar particle sizes may offer deeper insights and help refine these findings.

## Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear to influence the work reported in this paper.

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