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Research Article

The potential use of humic acid-coated biochar for reducing Pb and Cu in the soil to improve plant growth

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Abstract

The use of biochar for remediating heavy metal-polluted soils is still partial. Various methods of controlling soil pollution are currently being implemented by combining several methods. One of which is coating the biochar with humic acid to increase the effectiveness of nutrient uptake by plants. This study aimed to elucidate the effect of humic acid-coated biochar (HCB) on reducing Pb and Cu in the soil to improve plant growth. Treatments tested were combinations of two factors. The first factor was the dose of HCB, namely D0 = control (without HCB), D1 = 15 t HCB ha^{-1}, D2 = 30 t HCB ha^{-1}, and D3 = 45 t HCB ha^{-1}. The second factor was the type of plants, namely spinach (P1), water spinach (P2) and mustard green (P3). Twelve treatments were arranged in a randomized block design with three replications. The parameters observed were plant height, plant leaf area, plant stem diameter, and plant fresh weight. The results showed that the best plant growth was achieved at a dose of 30 t HCB ha^{-1}. The mustard green had the highest Pb uptake (0.025 g pot^{-1}), and the lowest Pb uptake (0.014 g pot^{-1}) was observed for water spinach. The highest Cu uptake (0.443 g pot^{-1}) was observed in water spinach, followed by spinach (0.282 g pot^{-1}) and mustard green (0.143 g pot^{-1}). In general, the amount of Pb reduced by plants ranged from 40.04 to 87.28%, and the amount of Cu by plants ranged from 8.63 to 40.23%.

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Introduction

Heavy metal contamination will affect agricultural production, human health, environmental problems, and food quality throughout the food chain. Several countries, such as Japan, China, Korea, Thailand, and Indonesia, have been contaminated by heavy metals (Hamzah et al., 2017). Some metals are essential, but certain metals, such as Cd and Pb, are toxic and dangerous for human health. Some countries have exceeded the threshold value of heavy metals level. Soil Pb level in China is more than 0.2 mg kg^{-1} (Shifaw, 2018), while the Cd level in horticultural centres in East Java has reached 2.26 mg kg^{-1} (Hamzah et al., 2016; 2017). Heavy metal accumulation under agricultural land will affect the soil physical, biological, and chemical properties (Satpathy et al., 2014); the heavy metal will enter the plant root, leaves, and grain (Singh et al., 2010). Wang, 2005).

Plants have different ways of absorbing and accumulating heavy metals in their edible parts; that can directly transfer the heavy metals to the human body. The soil-to-plant transfer of heavy metals is of great importance (Kalavrouziotis et al., 2011). Heavy metals uptake by plant roots and their transfer and accumulation in soil rice have become interesting to study. Among agricultural products, rice is widely consumed as staple food worldwide, especially in Asia. In Asia, rice was the most source of soil Cd and Pb contaminants for human health (Zhang and Ke, 2004), especially in Japan (Tsukahara et al., 2003). Other agricultural products like horticulture products are also threatened with heavy metal contamination. Therefore, how to manage, stabilize, and immobilize the heavy metal is being concerned.

Biochar is carbon-rich material produced through the pyrolysis process at high temperatures with limited oxygen supply (Ahmad et al., 2013; Lu et al., 2014). Biochar was proved very effective in improving soil properties and crop yield (Jeffery et al., 2011). Biochar has good adsorption capacity and the potential as a carrier for slow-release fertilizer (Qin et al., 2016). Biochar is also known to be a reasonably effective solution to improve soil physical properties in the short term, but new chemical properties will be seen in a long time (Lehman et al., 2011). Many studies showed the capability of biochar in immobilizing trace elements (e.g. Cu), minimizing the phytotoxicity and detrimental effects of Cu (Park et al., 2016). According to Lu et al. (2014), the addition of biochar could increase the above-ground biomass and soil pH but lower the solubility of heavy metals. This is in line with the reduction of Cu accumulated in plant biomass. Biochar has good adsorption capacity and the potential as a carrier for slow-release fertilizer (Qin et al., 2016). The utilization of biochar is very effective for improving soil physical characteristics in a short period, and in the long term, the soil chemical characteristics will also improve (Lehman et al., 2011).

Humic substances are well known as one of the amendments used for the phytoextraction of heavy metals from soil. The humic substance has a complex molecular structure and many functional groups that are useful in stabilizing and immobilizing metal ions, especially cation. Humic acid will reduce the metal bioavailability and increase its capability in chelating metal ions with their functional groups. Humic acid affects heavy metal availability and mobility of heavy metals (Rong et al., 2020). Humic acid and biochar will transform the exchangeable fraction into the non-exchangeable fraction (Zhou et al., 2018) due to their large area and the high content of organic functional groups (Liu et al., 2022).

The purpose of the remediation method is to suppress the availability of heavy metals so that they would be difficult to absorb by plants. Humic acid stimulates and activates biological and physiological processes for microbes that live and nest in the soil and act as ameliorants (Ouni et al., 2014). Humic acid will also stimulate soil microbial activity in providing soil nutrients. Liu et al. (2022) observed that functional groups of humic substances require a suitable pH for binding heavy metals. The humic substance shows a good effect on the complexion. It decreases heavy metal bioavailability, but a single application of humic substances has a poor impact on available heavy metals in paddy plant tissues. Therefore, it is necessary to develop a new remediation scheme for heavy metal remediation by coupling humic substance and biochar.

Materials and Methods

Preparation of humic acid-coated biochar

Humic acid-coated biochar (HCB) is a mixture of biochar, humic acid, and adhesive polymer. The biochar was made from the rice straw by pyrolysis combustion at a temperature of 400 °C, while the humic acid was extracted from the organic matter using a modified method by Gayathri et al. (2019). Ten grams of organic matter was extracted with 100 ml of 0.5 N NaOH (1:10), shaken for 24 hours, cooled for 16 hours, centrifuged at 1500 rpm, and then filtered again to obtain humic compounds. These substances were added with 6 N HCl until the pH of the solution reached 2 and separated again with filter paper. The precipitate obtained was rinsed with distilled water to remove residual chloride. Then, it was oven-dried at 105 °C to characterize and determine the percentage of humic acid. The HCB was made by thoroughly mixing biochar and humic acid in a balanced ratio. One gram of adhesive polymer was added to the mixture.

Pot experiment

A pot experiment was conducted from April to July 2021, in Malang, East Java, at an altitude of ± 450 m above sea level. Treatments tested were combinations of two factors. The first factor was the dose of HCB, i.e. D0 = Control (without HCB), D1 = 15 t HCB ha^{-1}, D2 = 30 t HCB ha^{-1}, and D3 = 45 t HCB ha^{-1}. The second factor was the type of plants, i.e. spinach (P1), water spinach (P2) and mustard green (P3). Twelve treatments were arranged in a randomized block design with three replications. The HCB was applied to 5 kg of soil and incubated for one week before planting. The soil used in this experiment was air-dried topsoil from the rice field at Karangploso village, Malang Regency, East Java. The soil has the following characteristics: pH = 6.34 (neutral), organic C = 2.66% (medium), total N = 0.27% (medium), K_2O = 0.725 cmol kg^{-1} (very low), P_2O_5 = 58.77 mg kg^{-1} (high), Pb = 13.73 mg kg^{-1} (critical value), and Cu = 80.23 mg kg^{-1} (critical value). Soil and plant analysis were carried out in the laboratory of the Faculty of Agriculture, Tribhuwana Tunggadewi University, and the laboratory of the Soil Department, University of Pembangunan Nasional Veteran, East Java.

Plant height, plant leaf area, and plant stem diameter were measured every week after transplanting (WAT) until the harvesting time of 8 WAT. The plant growth measurement was made on three samples per treatment. The leaf area was measured using the constant method (Susilo, 2016). At the end of the experiment (8 WAT), the plant parts were separated into roots, stems, and leaves. The plant parts were washed with running water to remove impurities, and they were then dried in an oven at 60 °C for 72 hours for Pb and Cu analyses.

Pb and Cu uptake by plants

Pb and Cu uptake by plants was calculated by multiplying the percentage of Pb and Cu concentrations in plant parts with the dry weight of the plant parts. Pb and Cu contents in the plant parts were determined by weighing 2 g of the ground plant sample and then dissolving the sample with 10 mL of HNO_3 and $HClO_4$ and heating until the remaining volume was 2 mL. It was gradually reheated with distilled water until the liquid became clear (clean white). The clear liquid was then mixed with distilled water and filtered by the 3050 EPA method. The results of the filtering were then measured for the Cu and Pb content using an atomic absorption spectrophotometer based on light absorption with a wavelength range of 190-800 nm (Saputo et al., 2016; Maurya et al., 2018).

Statistical analysis

The data obtained were subjected to the one-way analysis of variance (ANOVA). The Least Significant Difference (LSD) test at a significant level of $p<0.05$ was performed to see the effect of humic acid-coated biochar doses and plant types on Pb and Cu uptake by the plants and plant growth.

Results and Discussion

Plant growth

Spinach, water spinach, and mustard green plants responded positively to the application of humic acid-coated biochar (HCB) in the soil containing critical levels of Pb and Cu. This can be seen from the increase in plant height presented in Figure 1. The application of 30 t HCB ha^{-1} gave the tallest plant throughout, while the pot experiment with no HCB applied gave the shortest plant. The average plant weight was 36-45 cm, 35-46 cm, and 21-28 cm for water spinach, spinach, and mustard green, respectively. This indicates that the HCB treatment is effective for improving plant growth.

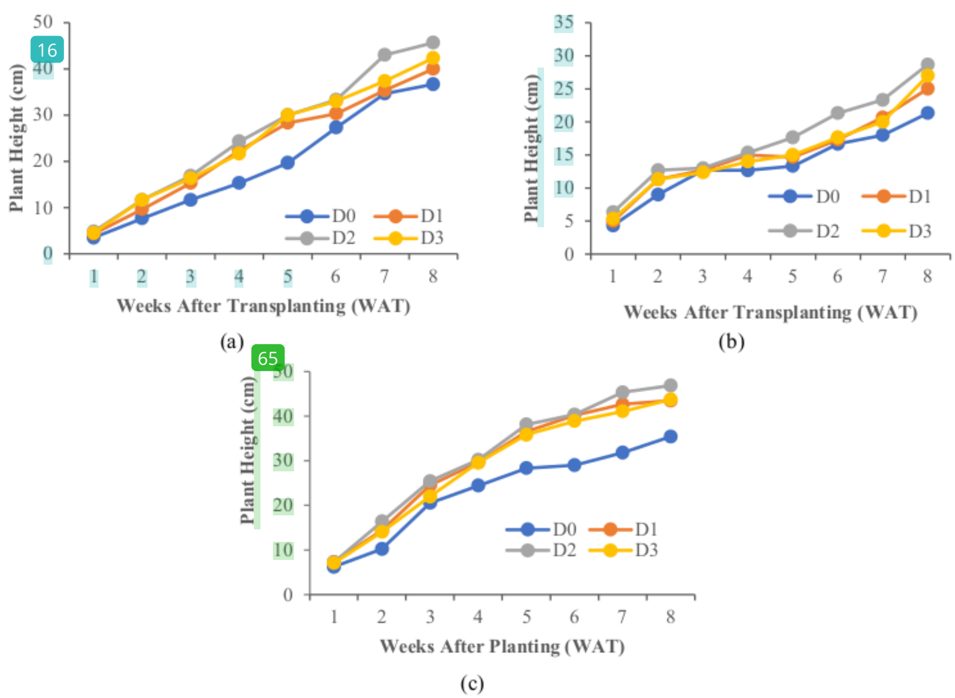

Figure 1. Effect of HCB application on plant height: (a) water spinach, (b) spinach, and (c) mustard green. D0 = control, D1 = 15 t HCB ha^{-1}, D2 = 30 t HCB ha^{-1}, D3 = 45 t HCB ha^{-1}.

Plant and shoot growth are affected by functional groups of humic acid, i.e. carboxylic (COOH) and phenolic (OH) groups (De Melo et al., 2016). Humic acid is important in enhancing nutrient uptake; thus, plant height increases. Improvement of nutrient uptake (especially N, P, and S) by the humic acid activity has been reported by Atiyeh et al. (2002) and Arancon et al. (2003). Acceleration of nutrient uptake enhances nitrogen metabolism and protein production, which ultimately increases the chlorophyll contents (Haghighi et al., 2012). This is also related to the humic acid function in increasing membrane cell permeability, photosynthesis, phosphate uptake, and root elongation (Ertani et al., 2015). The application of

30 t HCB ha^{-1} also significantly increased the leaf area of water spinach, mustard green, and spinach plants (Figure 2). The leaf area of the plant with no HCB added (D0) area was the smallest for the plant height (Figure 1). Humic compounds can increase vegetative growth presumably due to their capability to increase both the soil chemical properties, the cell permeability, and the growth hormone that will finally promote plant growth (Ampong et al., 2022). Humic acid will increase the permeability of membrane cells, respiration, photosynthesis, and elongation of roots (Akhmad et al., 2013) and improve the water holding capacity, soil structure, and microbial population (Shah et al., 2018) by increasing the soil biochemical activities; and increase soil nutrients availability (Yang et al., 2021).

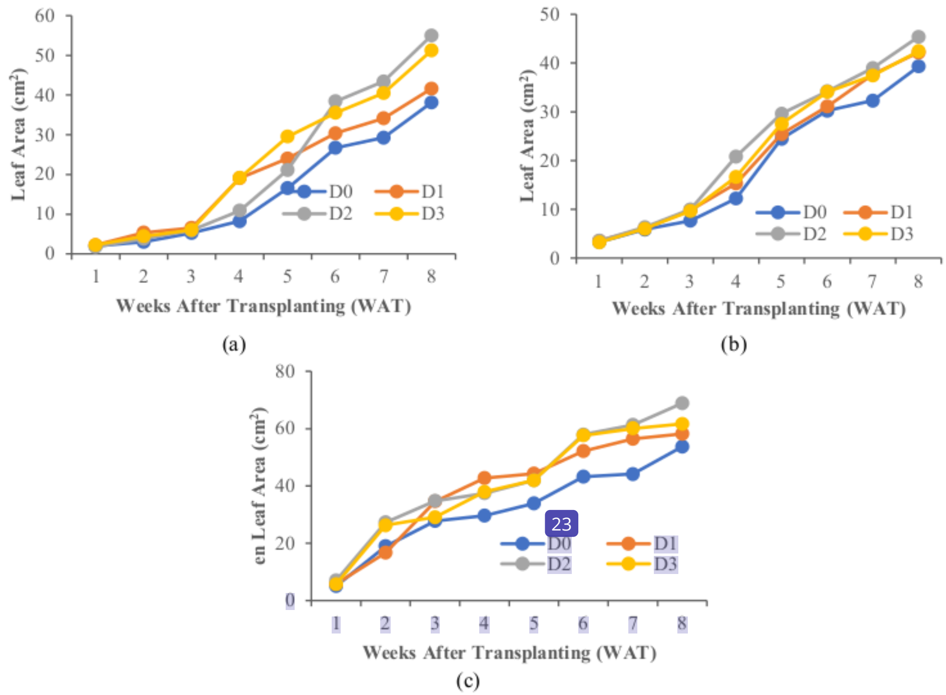

Figure 2. Effect of HCB application on plant leaf area: (a) water spinach, (b) spinach, and (c) mustard green. D0 = control, D1 = 15 t HCB ha^{-1}, D2 = 30 t HCB ha^{-1}, D3 = 45 t HCB ha^{-1}.

Humic acid also promotes several hormones, such as auxin and cytokinin, to enhance nutrient metabolism and photosynthesis (Nardi et al., 2021). Biochar was a habitat for beneficial microorganisms such as *Pseudomonas* sp. as P solubilizing and Acetobacter as N-fixing bacteria (Verdiana et al., 2016; Widowati et al., 2020). Biochar has a large number of micropores; hence, it functions as a habitat for microorganisms. A higher number of microorganisms in the soil will increase the availability of nutrients that plants in sufficient quantities easily absorb. The availability of sufficient nutrients in the soil will help the formation of the vegetative part of the plant. Also, the wider the leaf area, the more chlorophyll is formed, which increases photosynthesis. The process of photosynthesis will produce plant biomass in high quantities.

Biochar has the physical and chemical ability to supply several plant nutrients (Hidayat, 2015). Biochar can increase plant height and leaf area by increasing nutrient uptake (Sa'adah and Islami, 2019). Combining biochar with humic acid effectively enhanced plant height and leaf area. Humic acid results from organic matter extraction with a functional group capable of converting unavailable nutrients to available ones. Humic acid can maintain soil moisture, prevent nutrient leaching, improve soil structure, and increase microbial activity (Sembiring, 2016).

The Least Significant Difference (LSD) test showed that there were no significant differences in fresh weight and dry weight in all three plant types (Table 1) due to the application of humic acid-coated biochar (HCB), but the humic acid-coated biochar (HCB) doses significantly affected the plant fresh and dry weight parameters (Table 1). The rate of HCB affected the yield parameters, even though there was no difference among doses of HCB on the plant weight. The application of 15 t HCB ha^{-1} seemed to show the best dry and fresh weight (Table 1). This result is in line with the review of Rosa et al. (2014), that humic acid doses significantly affected shoot growth, while the HCB source affected both the shoot and root growth. Humic acid will promote hormone production such as auxin, cytokinin, and metabolic enzymes; by improving nutrient uptake and leaf chlorophyll concentration, the roots, shoots, and yield will increase. The similar yields observed between the different doses of HCB happened because the application rates are determined by the environmental and soil conditions, source and composition of biochar and humic acid, and crop types. Rose et al. (2014) stated that combined humic acid and other substances such as mineral fertilizer and biochar would form nutrient-complex and subsequent crop uptake slowly, but their effect mostly depends on the humic acid source, application rate, and crop types.

Table 1. The effect of HCB on the fresh and dry weight of water spinach, spinach, and mustard green plants.

Treatments	Fresh weight (g)	Dry weight (g)
Water Spinach		
D0 (control)	48.98 a	11.55 a
D1 (15 t HCB ha^{-1})	79.67 b	15.59 a
D2 (30 t HCB ha^{-1})	82.33 b	17.27 a
D3 (45 t HCB ha^{-1})	64.67 ab	14.78 a
LSD 5%	24.71	6.72
Mustard Green		
D0 (control)	34.67 a	4.01 a
D1 (15 t HCB ha^{-1})	45.67 ab	5.76 b
D2 (30 t HCB ha^{-1})	53.00 b	6.92 c
D3 (45 t HCB ha^{-1})	51.33 b	5.33 b
LSD 5%	13.11	1.02
Spinach		
D0 (control)	31.67 a	6.53 a
D1 (15 t HCB ha^{-1})	49.33 b	11.30 b
D2 (30 t HCB ha^{-1})	46.33 b	13.82 b
D3 (45 t HCB ha^{-1})	46.00 b	8.66 ab
LSD 5%	13.09	4.46

Notes: Numbers followed by the same letters in the same column show that they are not significantly different. HCB = biochar coated with humic acid, LSD = least significant difference.

Humic acid is a nutrient supplement in the form of organic substances that have a complex molecular structure. It stimulates and activates biological and physiological processes for microbes in the soil (Ouni et al., 2014). According to Suwardi and Wijaya (2013), using humic acid will benefit agricultural soil because it can convert unavailable elements into available forms. In addition, it maintains moisture, resists the leaching of nutrients, and improves soil structure to promote microbial activity. Compared with biochar, it is suspected that humic acid has not played a role in the short term but will play an active role in the long term. Biochar is a nest for microorganisms that can increase soil microbial activity. As an amendment, biochar is quite effective in improving physical properties in the short term, but new chemical properties will be seen in the long term (Lehman et al., 2011). Widowati et al. (2017) reported that combining biochar with NPK fertilizer increased organic carbon by 40%. Organic matter is the most important component of soil fertility, which plays an important role in maintaining quality, including soil ecology (Gajda et al., 2013; Benbi et al., 2015).

Biochar combined with other ingredients such as nitrogen and phosphorus is also effective in improving plant growth. Widowati et al. (2020) reported that biochar combined with organic fertilizers improved soil chemical properties. The use of HCB in this study is considered effective because of the role of humic acid, which provides nutrients. Ammal et al. (2020) proved that biochar could increase soil fertility due to the improved status of macro and micronutrients. Dume et al. (2016) also revealed that biochar could increase soil pH, cation exchange capacity, organic carbon, total nitrogen, exchangeable cations, and available phosphorus.

Pb and Cu uptake by plants

Trace metal contents of Pb and Cu in plants part (root and leaves) at the end of the experiment are presented in Figure 3. Generally, the concentrations of Pb and Cu were significantly higher in below-ground biomass (roots) than in above-ground biomass (leaves). The roots tend to accumulate more heavy metals than other plant parts. The plants have difficulties in translocating the heavy metals from roots to shoots. This condition will enhance root damage due to the high concentration of heavy metals. The Pb uptake amounts in spinach, water spinach, and mustard green roots were 0.023 g pot^{-1}, 0.014 g pot^{-1}, and 0.025 g pot^{-1}, respectively. Pb contents in leaves of spinach, water spinach, and mustard green plants were 0.001 g pot^{-1}, 0.001 g pot^{-1}, and 0.001 g pot^{-1}, respectively.

A similar result was also observed in the Cu content in the leaves, but the amount of Cu taken up by roots and leaves was relatively high compared with Pb. The amounts of Cu uptake in the roots of spinach, water spinach, and mustard green were 0.282 g pot^{-1}, 0.443 g pot^{-1}, and 0.143 g pot^{-1} for spinach, water spinach, and mustard green, respectively. In comparison, the amounts of Cu uptake in the leaves were 0.020 g pot^{-1}, 0.066 g pot^{-1}, and 0.041 g pot^{-1} for spinach, water spinach, and mustard green, respectively (Figure 3). The results showed that humic acid-coated biochar effectively reduced Pb and Cu uptake by spinach, water spinach, and mustard green by accumulating the Pb and Cu in their root parts.

Types of organic amendments and doses rate affect the heavy metal present in the shoots of a plant (Li et al. (2021). The highest Pb uptake (0.025 g pot^{-1}) was observed in mustard green, and the lowest was in spinach at 0.0014 g pot^{-1}. According to FAO (2014), the Pb level did not exceed the critical level (0.3 mg kg^{-1}), while the Cu content exceeded the critical level

(10 mg kg^{-1}). The highest Cu uptake in the plant roots was found in the water spinach (0.443 g pot^{-1}), while on spinach and mustard green, Cu uptake was 0.282 g pot^{-1} and 0.143 g pot^{-1}. The difference in the uptake of Cu and Pb was because each plant had different morphological properties, although all plants were categorized as moderate heavy metal accumulators. The Bio Concentration Factor (BCF) value of each plant for Pb was 0.29, 0.16, and 0.63 for spinach, water spinach, and mustard green, respectively; while the BCF value for Cu was 0.67, 1.01, and 0.98 for spinach, water spinach, and mustard green. This means that the BCF moderately transferred the heavy metals from the soil into the plant. BCF is the ratio of the concentration of heavy metals in roots and the concentration of heavy metals in the soil (Yoon et al., 2006).

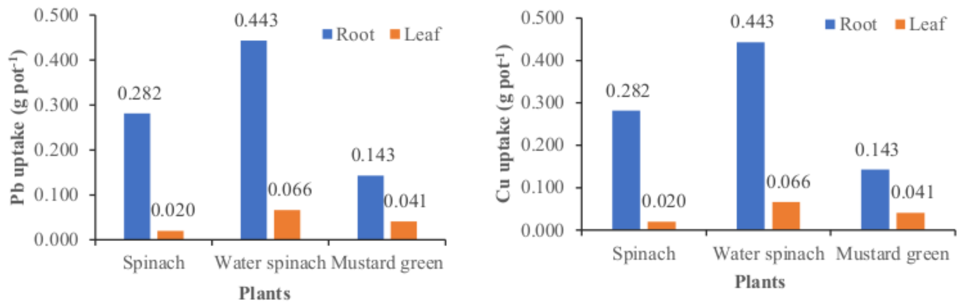

Figure 3. Pb and Cu uptake by plants.

Results of this study also showed that mustard green was more effective than the other plants in stabilizing soil Pb, while the water spinach was more effective than the other plants in immobilizing Cu. The results of this study also showed that spinach, water spinach, and mustard green planted on heavy metal-contaminated soil could absorb relatively high heavy metals. Water spinach, mustard green, and spinach are remediation plants that are possible to absorb and reduce heavy metal content in the soil. However, most heavy metals accumulate in the roots, while the heavy metal content in leaves and stems is relatively low.

Heavy metals taken up by the plants will be transferred into the roots, stems, and leaves. Results of this study showed that the contents of heavy metals in roots, stems, and leaves were quite high (Figure 4). When the Cu is present in the soil and absorbed in large quantities, it will be dangerous to plants and humans because it is carcinogenic (Hardiana, 2009). Water spinach, mustard green, and spinach are accumulator plants that absorb heavy metals and transfer them to the roots, stems, and leaves. In general, plants have a root structure consisting of vascular tissue, including phloem, xylem, endodermis, and epidermis. The endodermis has a very large role in the deepest layer of the root because it has a Casparian band (Casparian strip). This controls the transfer of water and solutions, including those containing heavy metals. According to Alberto et al. (2013), plants can decontaminate heavy metals through roots transported to the top. Some heavy metals will be inhibited or prevented from spreading in this process. However, this depends on the number of contaminants available for uptake. Heavy metal accumulation is a determinant of its translocation to plant parts. Detoxification of heavy metals by plants can improve quality by increasing soil physical, chemical, and biological properties. The absorbed heavy metals (non-essential) will enter plant cells through competition with other essential metals by taking over the binding site. This condition results in an interaction between the uptake of essential and non-essential metals that are toxic to plants. After entering the cell, heavy metals bind to phytochelatins and form a stable complex, which is then transported to the vacuole. This process will further detoxify (Komarek et al., 2013). The detoxification of heavy metals affects the soil, which will produce healthy plants.

Soil Pb and Cu reduction

Soil amendments such as biochar, humic acid, or biochar enriched with humic acid effectively decreased the availability of Cd, Cu, Pb, and As (Li et al., 2021). These amendments reduce the exchangeable fraction and increase the oxidation and residual fraction. Figure 4 shows spinach, water spinach, and mustard green plants reduced soil Pb between 40.04 and 87.28%, while Cu was between 8.63% and 40.23%. This is in line with the research result of other researchers that biochar can remediate Pb between 18.8% and 77.0% and Cu around 20% (Sizmur et al., 2011; Jiang et al., 2012). These results showed that applying humic acid-coated biochar is effective in immobilizing the heavy metals in polluted soils. The decline of Pb and Cu uptake in plant tissue was probably due to the immobilization process (physisorption, chemisorption, and precipitation) of the heavy metals by biochar and humic acid. Biochar effectively inhibited the activation of plants to metals and reduced the uptake of metals by plants. The immobilization of heavy metal by biochar will convert the heavy metal into more stable and less toxic forms.

But, the presence of humic acid promoted the formation of humic acid Pb and Cu water complex and prevented the formation of Pb and Cu hydroxide, thus increasing the availability and mobility of soil Pb and Cu.

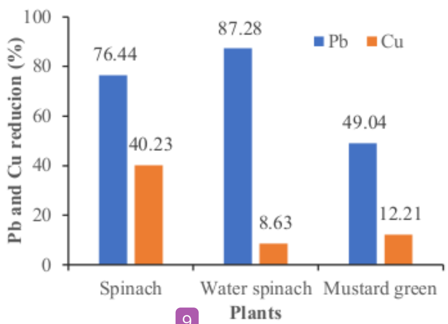

Figure 4. Reduction of Pb and Cu contents in the soil studied.

The results showed that humic acid-coated biochar consistently reduced soil Pb and Cu concentrations. The reduction of soil Pb was greater than soil Cu. This means that humic acid-coated biochar is more efficient in stabilizing the Pb than the soil Cu. Biochar characteristics affect the immobilization mechanisms. Biochar characteristics, such as high surface area, specific group, and alkaline character, are essential in heavy metal sorption. Biochar characteristics such as surface heterogeneity, functional groups, and large surface area will stabilize heavy metals by absorbing their soil surface and enhancing the precipitation and surface sorption due to their microporous structure.

The bioavailability of metals can be reduced by humic acid due to their strong affinity and their ability to form a stable chelate with metal ions through their carboxylic groups and phenolic-OH. Metals have different affinities for binding humic acid, depending on their stability constant, and from this point of view, Pb (14.8) have a stronger affinity than Cu (13.3). This emphasizes that the binding order of metals to humic acid in contaminated soil is Pb>Zn~Cu>Cd (Gusiatin and Kulikowska, 2016). Rong et al. (2020) reported that Cu and Pb have two binding sites; thus, the complexing capacity order is Pb>Cu (Abate and Massini, 2001). Rong et al. (2020) found that a reduction of available Pb (20% to 39%) in the soil was higher than Cd (23% to 37%). The characteristics and behavior of Pb and Cu reflected the results obtained in this study, where less Pb was taken up by plants than Cu (Figure 4). This means that less Cu is immobilized by humic acid-coated biochar than Pb so that more Cu is in available form and can be taken up by plants.

Conclusion

Applying humic acid-coated biochar (HCB) at a dose of 30 t ha^{-1} increased the growth of water spinach, spinach, and mustard green plants that were grown on soil containing the critical levels of Pb and Cu. The three plants took up Pb and Cu in the roots and leaves in the range of 1.85-8.02 mg kg^{-1} and 45.60-59.82 mg kg^{-1}. The application of HCB to the soil water spinach, mustard green, and spinach reduced soil Pb content from 40.04 to 87.28% and soil Cu from 8.63 to 40.23%.

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