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Effectiveness of Bacterial Consortium and Biochar in Remediation of Heavy Metals in Polluted Soil

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Abstract

The remediation of heavy metal-contaminated soil is a crucial step in environmental recovery. This study evaluated the effectiveness of bacteria and biochar in reducing lead (Pb) and copper (Cu) contamination in agricultural soil surrounding the Medan Industrial Area. A Factorial Randomized Block Design (RBD) was employed, incorporating two factors: bacteria (Co*rynebacterium glutamicum* and *Lactobacillus sp.*) at three levels (0 g, 5 g, and 10 g per plant) and biochar application at three levels (0 g, 10 g, and 20 g per plant), using mustard greens (Brassica juncea) as the indicator plant. The observation parameters included biomass and Pb and Cu content in both soil and plant tissue. Data analysis was conducted using ANOVA and the Honestly Significant Difference Test (HSD) at the 5% significance level The results indicated that bacterial treatment at a level of 5 g per plant and biochar at 20 g per plant significantly increased the wet weight per plant, the wet weight per plot, and the wet weight of plant roots when biochar was administered. The concentrations of the heavy metals lead (Pb) and copper (Cu) in soil and plants decreased with increasing levels of bacteria and biochar. The average reduction in Pb in soil and plants due to bacterial treatment was 1.28% and 0.29%, respectively, while the average decrease in Cu content in soil and plants was 1.03% and 0.17%. Biochar treatment reduced Pb in soil and plants by 0.44% and 0.07%, respectively, and Cu by 0.34% and 0.08%. This study demonstrates that bacteria and biochar stabilize heavy metals in contaminated soil.

Keywords: Bacteria, Biochar, Contaminated Soil, Effectiveness, Remediation

1. Introduction

Land exposed to industrial waste or subjected to intensive fertilization with inorganic fertilizers, particularly SP-36, can accumulate heavy metals such as lead (Pb) and copper (Cu). High concentrations of heavy metal contamination can infiltrate the soil and irrigation systems, ultimately polluting the food chain and endangering the health of humans and other living organisms (Zhou, Y., Zeng, M., & Chen, 2021). Agricultural land in the communities surrounding the Medan Industrial Area, where vegetables are cultivated, is also at risk of accumulating heavy metals, including Pb and cadmium (Cd), in the tissues of edible plants (Wibowo, Sari & Santoso, 2022).

Heavy metal pollution mitigation efforts are crucial before levels exceed established thresholds, given the toxic nature of lead (Pb) and copper (Cu), which can accumulate in plant tissues and pose risks to consumer health (WHO, 2021). One effective approach to reducing heavy metal concentrations in soil is remediation. Remediation refers to cleaning or restoring contaminated soil to ensure it can be reused safely and productively.

Biological remediation methods, including bioremediation and phytoremediation, are effective and environmentally friendly alternatives for addressing pollution. Bioremediation employs microorganisms to degrade or stabilize pollutants, whereas phytoremediation leverages plants' physiological and biochemical capabilities to absorb, accumulate, and detoxify heavy metals from the environment (Glick, 2020).

This study utilized two types of bacteria, namely *Corynebacterium glutamicum* and *Lactobacillus sp.*, which were combined in a consortium to enhance the effectiveness of contaminant degradation. The synergistic interactions within the bacterial community produce specific enzymes that modify the chemical structure of pollutants into harmless forms (Chandran, 2011; Ma et al.,

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2020). Phytoremediation, another biological method, leverages the ability of plants to extract, accumulate, and detoxify heavy metals from contaminated environments. Plants are considered efficient natural remediation agents due to their unique biochemical and physiological mechanisms that facilitate remediation (Glick, 2020).

One additional approach in the remediation of contaminated soil is using biochar. Biochar is a carbon material resulting from biomass pyrolysis under oxygen-limited conditions with a high surface area and abundant organic carbon content. The application of biochar to the soil not only improves the physical and chemical properties of the soil, such as increasing the cation exchange capacity (CEC) and soil pH but also functions to stabilize heavy metals through the immobilization process (Dittakit et al ., 2023; Lehmann & Rondon, 2006; Shen et al ., 2016).

The effectiveness of biochar in heavy metal remediation is highly dependent on soil characteristics, the presence of minerals such as carbonate and phosphate, and the ph of biochar, which is generally alkaline. Biochar can reduce the bioavailability of heavy metals through complexation formation and precipitation of metals into insoluble compounds, such as hydroxides and metal carbonates. The increase in pH due to biochar application also plays an essential role in reducing the mobility of heavy metals such as Pb and Cd in contaminated soil (Chen et al, 2022; Ren et al, 2024). Integrating biochar and bioremediation by bacteria can accelerate the reduction of Pb and Cu content in soil, while improving the agronomic quality of agricultural land (Shen et al, 2019).

The mechanism of biochar in remediating soil contaminated with heavy metals is greatly influenced by the type of soil, the presence of cations in the soil, and the composition of the biochar itself. Previous studies have shown that biochar plays a role in stabilizing heavy metals by increasing the adsorption capacity of the soil and binding metal ions through interactions with minerals such as phosphate and carbonate (Panjaitan, E., & Sidauruk, 2019; Panjaitan, 2024) confirming that biochar can reduce the solubility and bioavailability of heavy metals, thereby reducing the potential for accumulation in plants. In addition, research by Sianipar et al. (2025) shows that the effectiveness of biochar is highly dependent on its chemical properties, mineral content, and acidity level (pH). Increasing the pH of biochar and the pyrolysis temperature is also an important factor in soil remediation, because most studies only focus on using biochar or microbes separately.

This study presents a novelty by combining biochar and inoculation of a specific bacterial consortium (*Corynebacterium glutamicum* and *Lactobacillus sp.*) in one remediation system. This combination is expected to increase the effectiveness of heavy metal stabilization in soil with two main mechanisms: provision of microbial habitat in biochar pores that support pollutant degradation and increasing the adsorption capacity of biochar against heavy metals. In addition, this study was conducted on active agricultural land around the Medan Mabar Industrial Area, making it more applicable than previous studies that generally focused on ex-mining land or heavy industrial waste. In addition, mustard plants (*Brassica juncea*) are used as phytoremediation agents, which are more relevant to local agricultural systems than other hyperaccumulator plants often used in remediation studies.

This study aims to evaluate the effectiveness of the combination of a consortium of bacteria *Corynebacterium glutamicum* and *Lactobacillus sp.* and rice biochar in reducing the concentration of heavy metals Pb and Cu in contaminated soil, using mustard greens (*Brassica juncea*) as an indicator plant.

2. Material and Methods

The research was conducted on the land of the Faculty of Agriculture, Methodist University of Indonesia, Jl. Harmonika Baru Pasar II, Tanjung Sari Medan, the geographical coordinates of this location are around 3.5750° North Latitude and 98.6200° East Longitude, with an altitude of around 30 meters above sea level. The soil samples used for this study were taken from residents' land next to the Medan Mabar Industrial Area, at a 0-40 cm depth.

The research design used a factorial randomized block design with three replications. The first factor of bacterial inoculant included no inoculant (I0), 5 g/plant (I1), 10 g/plant (I2), and biochar included: no biochar (B0), 10 g/plant (B1), 20 g/plant (B2). Mustard plants were maintained for 2 months, with watering until they reached field capacity. The parameters observed were wet weight per plant, wet weight per plot, plant root weight, and concentration of heavy metals Pb and Cu absorbed by plants and in the soil. Examination of several chemical properties of the soil and the concentration of Pb and Cu in the soil was carried out before and after the study. Soil pH: Measured using a pH meter with a soil and water ratio of 1:2.5; Cation Exchange Capacity (CEC): Determined using the extraction method with ammonium acetate at pH 7.0: Heavy Metal Content (Pb and Cu): Measured using Atomic Absorption Spectrophotometry (AAS) after the wet destruction process of soil samples with a mixture of nitric acid (HNO_3) and perchloric acid ($HclO_4$) (Pratama, 2021; Sulaeman; et al, 2005).

The collected data were processed and analyzed statistically using analysis of variance (ANOVA); if there were a significant difference, it would be further tested using Honestly Significant Difference (HSD) at the 5% level to see the differences between treatments. For analysis of variance (ANOVA) and further testing of Honestly Significant Difference (HSD) at the 5% significance level, SPSS Statistics version 26 was used. For initial data processing, table creation, and graphs, Microsoft

Excel 2019 was used.

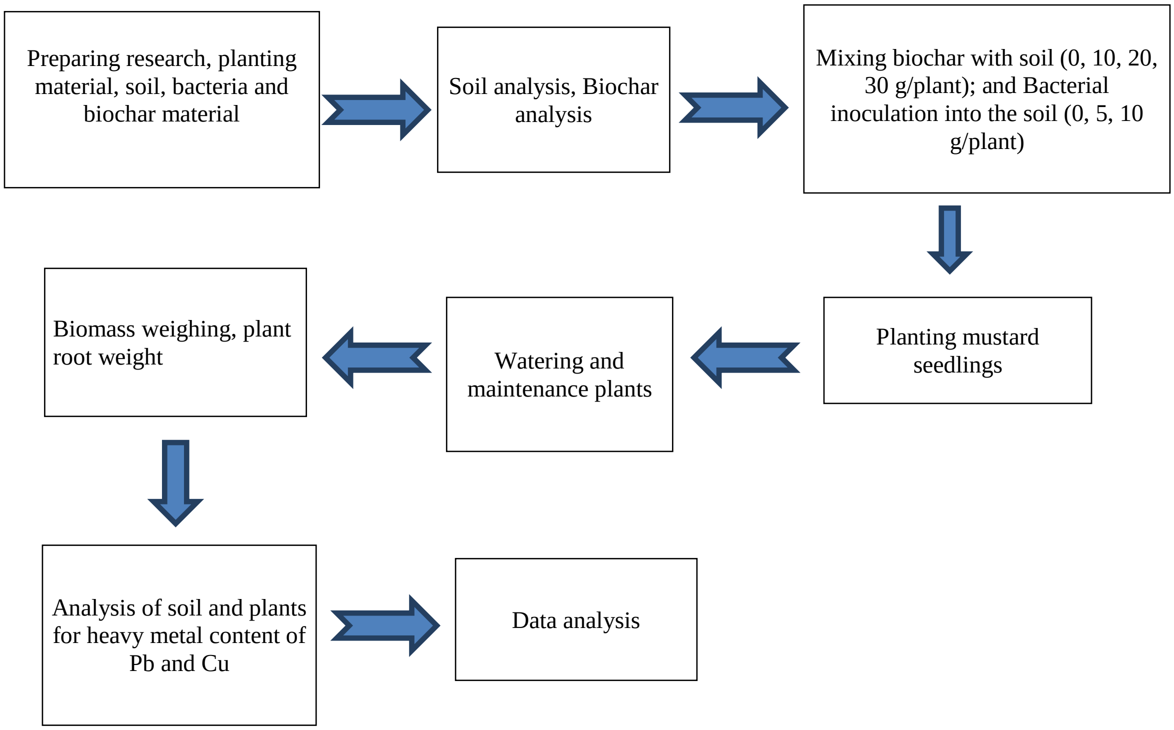

Figure 1. Research flow diagram

3. Results and Discussion

3.1. Wet Weight per Plant (g)

The heaviest wet weight per plant was found in plants with 20 g/plant biochar, followed successively by plants with 10 g/plant biochar and without biochar (Table 1). Table 1 shows no interaction between the administration of bacteria and biochar at each treatment level on the wet weight of mustard greens. Bacterial inoculation treatment did not significantly affect the wet weight of mustard greens at all treatment levels.

Corynebacterium and lactobacillus bacteria, and adding biochar at specific doses, can improve the chemical properties of the soil so that the vegetative growth and yield of mustard plants are better. With the addition of biochar applied to the soil, the availability of both macro and micronutrients can increase due to the mineralization of organic matter contained in the biochar.

Table 1. Average Wet Weight per Plant

Treatment	Wet Weight per Plant (g)
I_0	20.12 ±0.49
I_1	17.75 ±0.79
I_2	16.28 ±0.37
$BNJ_{0.05}$	-
B_0	16.35 ±0.48 ab
B_1	12.77 ±0.38 a
B_2	25.03 ±0.49 b
$BNJ_{0.05}$	11.12

Description: Numbers followed by the same letter in the same column and treatment group are not significantly different in the BNJ test at the 5% test level.

The laboratory analysis of several chemical properties of the soil showed that the soil fertility level at the location was moderate to high. Based on the results of the analysis before and after the study, it can be concluded that there was an increase in the nutrients N, P, K and C-organic, and pH respectively from 1.86%; 1.17 ppm; 0.38 me / 100; 4.6%, and 7.3 to 2.32%; 1.25 ppm; 0.43% and 5.12% and 7.5. Bacterial treatment with special strains, namely corynebacterium and lactobacillus, in addition to improving soil properties, biological properties and chemical properties of the soil to increase soil fertility, can also function as a bioremediator agent.

Increasing doses of biochar can increase the wet weight per plant. Biochar can improve permeability, porosity and soil structure. Looser soil will allow plant roots to grow and develop better, allowing plants to absorb more water and nutrients. The provision of biochar can also increase the water holding capacity of the soil and soil CEC and can neutralize soil pH, increasing the supply of nutrients to plants. Increased water and nutrient absorption by plants will increase wet weight (Sismiyanti et al., 2018). Activated charcoal is an alternative material for improving soil fertility, including chemical, physical, and biological properties. Mela Anggraini et al (2025) state that higher biochar can improve soil and increase plant productivity.

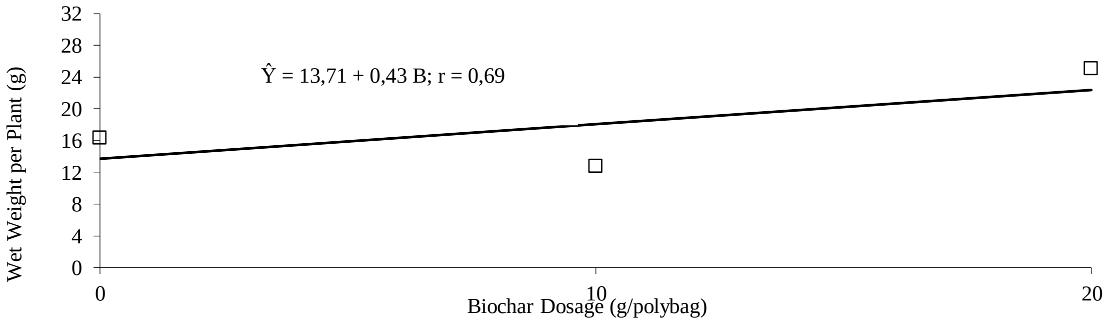

Figure 2. Effect of Biochar on Wet Weight per Plant

3.2. Wet Weight per Plot (g)

Table 2 shows that the bacterial treatment and the interaction between the two treatments had no significant effect on the wet weight per plot. In contrast, the biochar treatment significantly affected the wet weight per plot.

The heaviest wet weight per plot was found in plants given 20 g/plant of biochar, significantly heavier than those given 10 g/plant of biochar and without.

Increasing doses of biochar can increase the wet weight per plot. Increasing the dose of biochar can increase the concentration of available P in the soil. The maximum availability of P is usually at a pH of around 6 and 7. The availability of P nutrients is highly dependent on soil pH. Biochar can be used to increase the availability of P in acidic soils. Biochar can also increase the availability of K. According to Nguyen et al. (2009), potassium and phosphorus are essential for plant yields because these nutrients have the greatest effect on plant yields. Potassium activates several enzymes and plays an essential role in water balance in plants for carbohydrate transformation. The potassium element helps the formation of protein and photosynthesis and increases production, while the P element functions in photosynthesis and respiration.

Table 2. Average Wet Weight per Plot

Treatment	Wet Weight per Plot (g)
I_0	188.30 ±0.72
I_1	186.51 ±0.51
I_2	183.87 ±0.16
$BNJ_{0.05}$	-
B_0	169.47 ±0.49 c
B_1	182.57 ±0.95 b
B_2	206.60 ±0.58 a
$BNJ_{0.05}$	5.72

Description: Numbers followed by the same letter in the same column and treatment group are not significantly different in the BNJ test at the 5% test level.

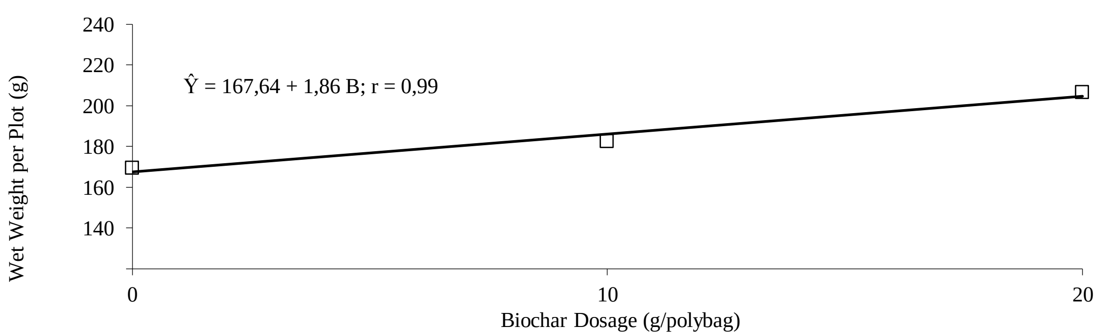

Figure 3. Effect of Biochar on Wet Weight per Plot

3.3. Wet Root Weight per Plant (g)

Table 3 shows that the bacterial inoculation treatment and the interaction between the two treatments had no significant effect on the wet weight of roots per plant. In contrast, the biochar treatment significantly affected the wet weight of roots per plant.

The heaviest wet root weight per plant was found in plants given 20 g/plant of biochar, which was significantly heavier than without biochar, but was not significantly different from those given 10 g/plant of biochar.

Increasing doses of biochar can increase the wet weight of plant roots. Biochar can improve the soil's ability to retain exchangeable cations so that nutrients are in a form that is more available to plants than those without biochar. Biochar has a greater ability than other forms of organic matter to absorb cations per unit carbon due to its greater surface area, surface charge, and charge density. After being applied to the soil, the surface of the biochar gradually becomes oxidized and thus the cation exchange capacity of the biochar increases (Daulay et al., 2022). This will improve root growth and development, increasing the plant roots' wet weight.

Table 3. Average Wet Root Weight per Plant

Treatment	Wet Root Weight per Plant (g)
I_0	2.39 ±0.06
I_1	2.74 ±0.07
I_2	2.84 ±0.07
$BNJ_{0.05}$	-
B_0	1.80 ±0.06 b
B_1	2.69 ±0.04 b
B_2	3.49 ±0.07 a
$BNJ_{0.05}$	1.31

Description: Numbers followed by the same letter in the same column and treatment group are not significantly different in the BNJ test at the 5% test level.

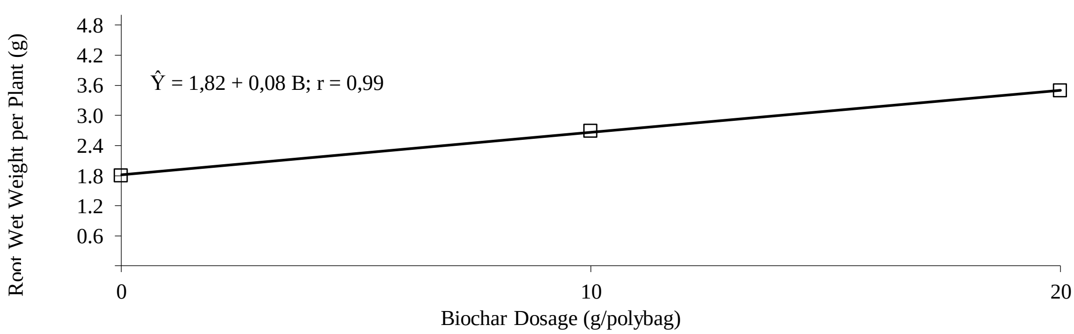

Figure 4. Effect of Biochar on Wet Weight of Plant Roots

3.4. Total Pb and Cd Content in Soil and Plants

Remediation using symbiotic mustard accumulator plants with Corynebacterium, *Lactobacillus sp.* and biochar can reduce heavy metals in the soil. The combination of mustard plants with bacteria or with biochar at a greater level will result in a decrease in the Pb content in the soil (Figures 4 and 5). The initial soil analysis showed that the Pb and Cu concentrations were 0.05 ppm and 0.12 ppm. The average decrease in Pb and Cu in soil with bacterial treatment was 1.28% and 1.03%. At the same time, the decrease in Pb and Cu in soil with biochar was 0.44% and 0.34%. The activity of corynebacterium and *Lactobacillus sp.* bacteria inoculated into the soil and around the roots of mustard plants function as a decomposer of contaminants or heavy metals (rhizodegradation). Metal contaminants in the soil are absorbed by bacteria attached to the roots of mustard plants and then broken down into parts with exudates in the plant root system (Mela Anggraini et al, 2025). Biochar can stabilize heavy metals in contaminated soil by significantly reducing the absorption of heavy metals by plants. It can improve its quality by improving the soil's physical, chemical and biological properties (Komárek, M., Vaněk, A., & Ettler, 2013).

The absorption of heavy metals in mustard greens due to bacterial inoculation at 10 g/plant causes smaller absorption of Pb and Cu compared to 5 g/plant and without bacterial inoculation. In the provision of biochar with increasing doses, the content of heavy metals Pb and Cu in plant tissue will decrease with the increasing dose of biochar given. The lowest content of heavy metal Pb in mustard greens is in the provision of biochar of 20 g/plant, then followed by the provision with a dose of 10 g/plant and without the provision of biochar.

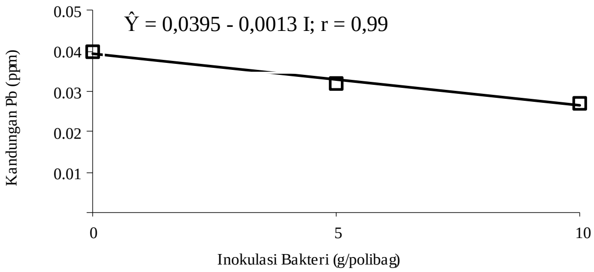

Figure 5. Effect of bacteria on Pb concentration in soil

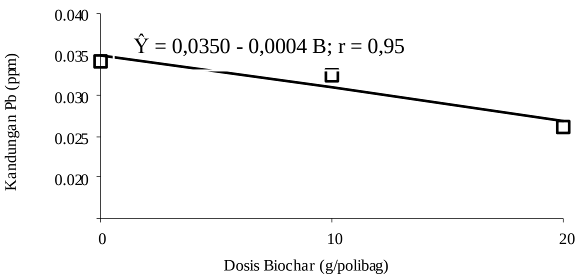

Figure 6. Effect of Biochar on Pb Concentration in Soil

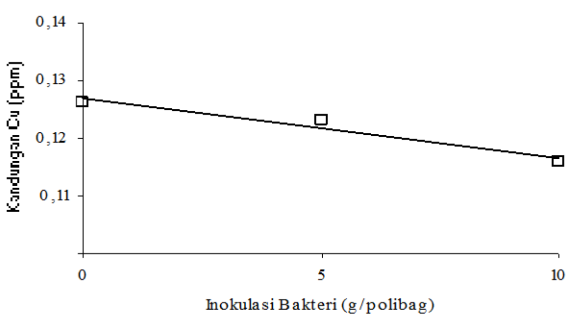

Figure 7. Effect of bacteria on Cu concentration in soil

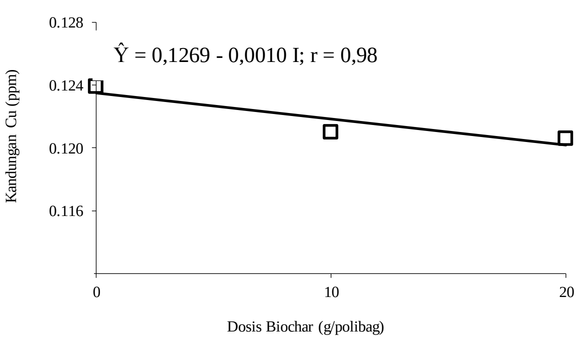

Figure 8. Effect of Biochar on Cu Concentration in Soil

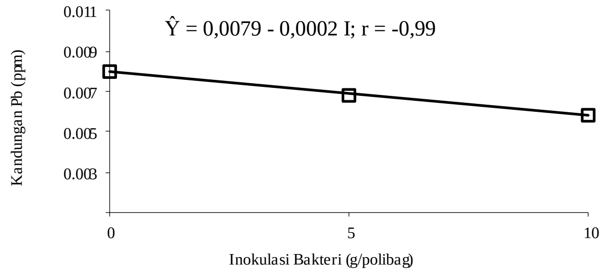

Figure 9. Effect of bacteria on Pb concentration in plants

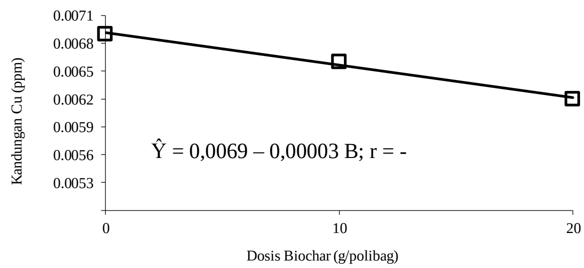

Figure 10. Effect of Biochar on Pb Concentration in Plants

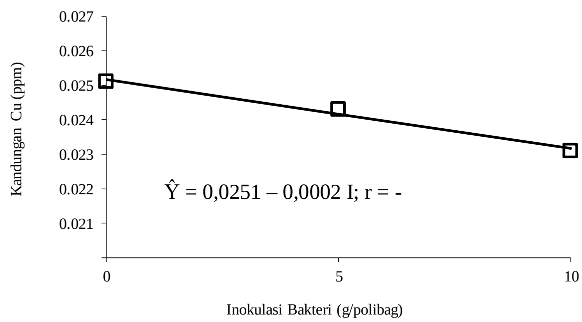

Figure 11. Effect of bacteria on Cu concentration in plants

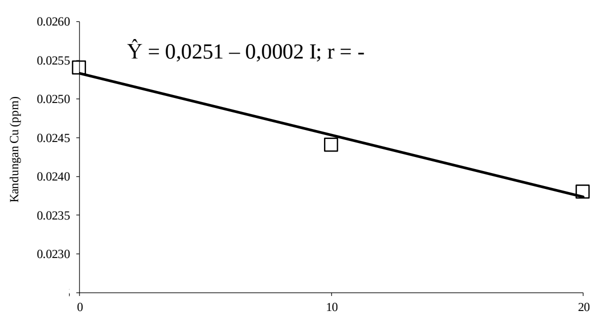

Figure 12. Effect of Biochar on Cu Concentration in Plants

Bacterial inoculation of mustard plants significantly influenced the concentrations of heavy metals, specifically lead (Pb) and copper (Cu), in both the soil and the plants. The activity of Corynebacterium and Lactobacillus bacteria, which were inoculated into the soil and around the roots of the mustard plants, acted as decomposers of contaminants and heavy metals through a process known as rhizodegradation. Metal contaminants in the soil were absorbed by bacteria that adhered to the roots of the mustard plants and subsequently broken down into less harmful components through exudates in the plant root system. The results indicated that bacterial treatment at increasing levels reduced the concentrations of heavy metals Pb and Cu in both the soil and the plants. The results showed that the average content of Pb and Cu metals in mustard plants decreased with increasing levels of bacterial inoculation. Heavy metals Pb and Cu content were highest in the treatment without bacterial inoculation. Still, with increasing levels of bacterial inoculation, there was a decrease in the content of heavy metals Pb and Cu in mustard plants. Plant roots absorb metal waste from the soil and translocate it to the parts of the plant above the ground.

4. Conclusion

This study demonstrates that applying biochar and a bacterial consortium positively impacts the biomass production of mustard greens while reducing the concentrations of heavy metals, specifically lead (Pb) and copper (Cu), in both the soil and plant tissues.

1. A biochar application of 20 g per plant significantly enhanced the wet weight per plant (25.03 g) and per plot (206.63 g), correlating with improvements in soil structure and nutrient availability.
2. Root growth increased significantly in the treatment with 20 g of biochar per plant.
3. Bacterial inoculation at a dose of 10 g per plant effectively reduced the concentrations of lead (Pb) and copper (Cu) in the soil by 1.28% and 1.03%, respectively.
4. Biochar effectively reduces the accumulation of heavy metals in plant tissue, achieving average reductions of 0.07% for lead (Pb) and 0.08% for copper (Cu). The maximum residue limits (MRL) established for food consumption are 0.2 mg/kg (ppm) for cadmium (Cd) contamination in vegetables and 0.5 mg/kg for lead (Pb) as specified in SNI 7387:2009.

Suggestion

Integrating biochar and bacteria into the remediation program for heavy metal-contaminated soil is recommended. Further research is needed on a field scale and across different soil types to assess the consistency of the results.

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