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Bioaccumulation of Water Spinach (*Ipomoea reptans* Poir) in Phytoremediation of Heavy Metal Cu in Soil Contaminated by Lapindo Mud and Its Impact on Plant Growth

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Abstract

Lapindo mud (LL) contains various heavy metals, including copper (Cu), with concentrations exceeding environmental quality standards, thereby posing a risk of soil and ecosystem contamination. One effective method to mitigate this pollution is phytoremediation using hyperaccumulator plants. This study aims to (1) assess the effect of mixing Lapindo mud with soil on Cu levels in the planting medium and (2) determine the optimal planting medium composition for Cu uptake by land spinach (Ipomoea reptans Poir). The experiment involved four planting medium treatments: 100% mud, soil:mud = 1:1, soil:mud = 1:2, and soil:mud = 1:3, each weighing 600 grams per polybag. Plants were harvested at 10 and 20 days after planting, and Cu levels in the soil and plants, bioaccumulation, and growth parameters such as plant height and dry weight were analyzed. Data were subjected to ANOVA at a 5% significance level, followed by the Least Significant Difference (LSD) test. Results indicated that mixing Lapindo mud with soil reduced Cu content in the planting medium, with the lowest Cu concentration in the 1:1 soil:mud treatment (25 ppm) and the highest in the 1:3 treatment (29 ppm). The 1:1 treatment also resulted in the highest Cu uptake (10.17 ppm) and a bioaccumulation index of 0.40, identifying it as the most effective composition for Cu phytoremediation. These findings highlight the potential of land spinach as a phytoremediator to reduce Cu pollution in soils contaminated with Lapindo mud.

Keywords: Bioaccumulation Index, Plant Biomass, Plant Growth, Plant Cu Uptake, Soil Cu Content

1. Introduction

Lapindo Mud (LL), also known as Sidoarjo Mud (Lusi), is a hot mudflow that emerged due to drilling errors at a gas exploration well owned by Lapindo Brantas Inc. (Samudro, 2016). The mud contains heavy metals such as manganese (Mn), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), cobalt (Co), nickel (Ni), mercury (Hg), and arsenic (As), which have the potential to pollute the surrounding environment (Zannah, 2021). Lapindo mud is classified as hazardous and toxic waste (B3) because it contains heavy metals, including lead (Pb) at 35.41 ppm and copper (Cu) at 21.9 ppm (Samudro, 2016). Studies have shown that copper levels in the mud exceed the established threshold limits specified in the Minister of Health Decree No. 907 of 2002 and Government Regulation No. 82 of 2001, with total copper content reaching 60 mg/kg. This pollution has caused significant damage to the surrounding ecosystem. Continuous mudflows have destroyed agricultural land and wetlands, polluted water sources, reduced water quality, and devastated aquatic habitats (Alfina, 2024). Therefore, effective methods are needed to remediate soil contaminated with these heavy metals.

One method to address heavy metal pollution from Lapindo mud is phytoremediation. Phytoremediation is an inexpensive and environmentally friendly way to improve the quality of polluted environments. This technique has no significant negative impacts, is low-cost, has a positive impact on the environment and society, and provides direct benefits to public health. Hyperaccumulator plants are

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essential for phytoremediation because they can absorb heavy metals and store them in plant tissues. The land spinach plant (*Ipomoea reptans* Poir.) is one of many hyperaccumulator plants that can be used in phytoremediation (Hapsari, 2018).

The land spinach plant (*Ipomoea reptans* Poir.) is a species of the Ipomoea genus that can grow well on riverbanks, lakes, and even ditches or bodies of water that are not too deep (Hapsari, 2018). This plant grows quickly and can be harvested in four to six weeks. The *Ipomoea genus* reaches 400 species that grow from lowlands to highlands. For a long time, spinach has been consumed, especially as a vegetable (Shafira, 2024). Spinach can absorb inorganic substances through the tips of its roots. Once absorbed, inorganic substances are channelled to the stem through the vascular tissue, then distributed to all parts of the plant. In this process, inorganic substances undergo biological reactions and accumulate in the stem, then continue until they reach the leaves (Jundana, 2016). Water spinach (*Ipomoea aquatica*) has been shown to reduce Pb levels in batik wastewater, with effectiveness increasing with the number of plants used, although the final results remain above environmental quality standards (Hapsari, 2018).

On the other hand, land spinach (*Ipomoea reptans*) has been reported to accumulate Cu from contaminated soil, with the highest accumulation in the roots, and metal concentrations increasing with increasing harvest age (Hapsari, 2012). Both studies suggest that water spinach, both land and water spinach, can absorb and accumulate heavy metals, but remain limited to a single medium (wastewater or contaminated soil). This study expands on these findings with a novel approach: a mixture of soil and Lapindo mud in several ratios. This strategy yields additional information on optimal media conditions to enhance Cu absorption while maintaining plant growth, thereby offering novelty in the context of utilizing Lapindo mud for phytoremediation.

The objectives of this research are as follows. First is to understanding the mixing ratio between mud and soil can affect the concentration of copper (Cu) in the planting medium. And second is to understanding the composition of land spinach (*Ipomoea reptans* Poir) that optimally absorbs the heavy metal copper (Cu).

2. Material and Methods

2.1. Place and Time of Research

This research was conducted in several locations. The mud sampling process was carried out in the area within the Lapindo mud embankment, Sidoarjo, East Java (7°31'20" S; 112°42'46" E) in August 2024 at an altitude of ± 8 m above sea level. Planting of kale and measurement of plant growth parameters (plant height and root dry weight) were carried out in the Green House of the Faculty of Agriculture, "Veteran" National Development University of East Java, Surabaya (7°20'25" S; 112°46'08" E) in September–October 2024, at an altitude of ± 5 m above sea level. Analysis of copper (Cu) content in soil was conducted at the Laboratory of the Agricultural Instrument Standards Implementation Agency (BPSIP), East Java, Karangploso, Malang (7°55'40" S; 112°37'12" E) in October–November 2024, at an altitude of ± 506 m above sea level.

2.2. Materials and tools

The equipment used in this experiment includes an analytical balance, a volumetric pipette, a 100 mL measuring flask, a 250 mL Erlenmeyer flask, a burette, a beaker, a dropper, and a spray bottle. In addition, the study employed tools such as a camera, a ruler, a polybag, a plastic bag, a sack, a shovel, an analytical balance, a 1.5-litre mineral water bottle, a hoe, and stationery. Meanwhile, the materials used in this study include land spinach seeds (*Ipomoea reptans Poir*), water, soil contaminated with Lapindo mud, 0.1 M HCl solution, glucose solution, 0.1 M NaOH solution, 1% starch solution, salt (for making sample solutions), a mixture of HNO_3 and $HClO_4$ solutions, and distilled water.

2.3. Research methods

This research is an experimental study with three treatments. The first treatment used 100% mud as a control planting medium. The second treatment used a planting medium with a 1:1 soil-to-mud ratio. The third treatment used a planting medium with a 1:2 soil-to-mud ratio. The fourth treatment used a planting medium with a 1:3 soil-to-mud ratio. Each polybag contained 600 g of planting medium, and harvesting was performed at 10 and 20 days after planting. Before the planting medium was used, a baseline analysis was conducted for each treatment. This baseline analysis will then be used for observation parameters at 0 days after planting.

2.4. Research Design

This study used a completely randomized design (CRD) with 4 treatments, each repeated 3 times. Harvests were conducted on the 10th and 20th days, with 12 experimental units at each harvest time, for a total of 24 experimental units. The mathematical model of the Completely Randomized Design is:

$$Yij = \mu + Ti + \varepsilon ij$$

with:

Yij: Observation value of the i-th treatment in the j-th replication.
μ: General average.
Ti: Effect of treatment i.
Ɛij: Effect of error in the i-th treatment and j-th replication

2.5. Basic Analysis

A preliminary analysis was conducted to determine the composition and condition of the planting medium prior to treatment. This analysis included mud, soil, and mud-soil mixtures in ratios of 1:1, 2:1, and 3:1. The mixed planting medium was air-dried and sieved to obtain a finer texture. The sieved material was then analyzed to determine its heavy metal content (Cu), organic carbon (C-organic), and pH. The results of this baseline analysis will be used as a reference for observation parameters at 0 Days After Planting (DAP).

2.6. Observation Variables

This study's observations covered several aspects. In plants, the parameters observed included Cu content, plant height, and root and plant dry weight. Content analysis was conducted using the AAS (Atomic Absorption Spectrophotometry) method. Meanwhile, observations on the growing media included pH and C-Organic, which were analysed using the electrometric method and the Walkley-Black method, respectively.

2.7. Research Framework

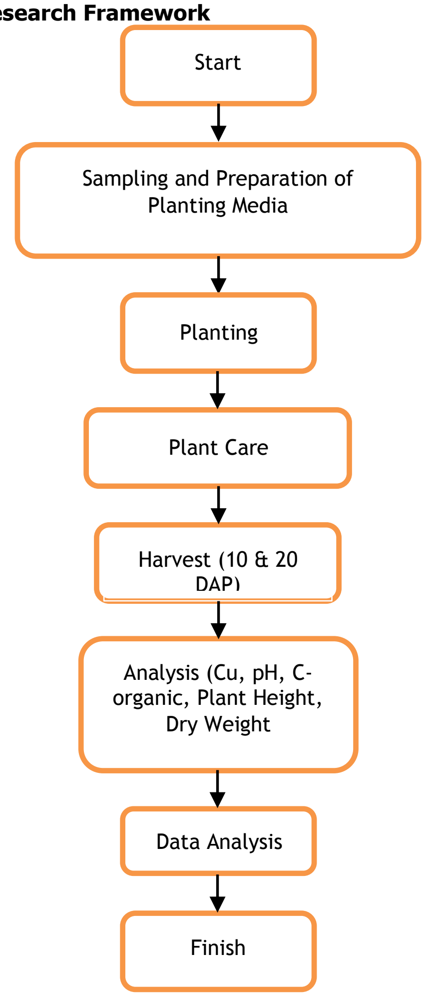

Figure 1. Research flow diagram

2.8. Data analysis

Observational data, collected both in the field and through laboratory testing, were further analyzed to gain a deeper understanding. Data related to Cu heavy metal uptake, plant height, pH, root dry weight, plant dry weight, and organic carbon content were analyzed using analysis of variance (ANOVA) at a 5% significance level to determine whether the given treatment had a significant effect. If the analysis of variance (ANOVA) showed a significant difference, a further test was conducted using the Least Significant Difference (LSD) at the 5% significance level to determine differences between treatments. The entire data analysis process was carried out using Microsoft Excel 2021.

3. Results and Discussion

3.1. Basic Analysis Results of Planting Media

A basic analysis of a growing medium is a crucial initial step in evaluating its quality and suitability for plant cultivation. The main parameters analyzed include heavy metal content (e.g., copper), organic carbon (C-organic), and soil acidity (pH). Each parameter provides important information about the physical and chemical conditions of the growing medium, which can affect plant growth, nutrient uptake, and potential plant toxicity.

Heavy metals such as Cu (copper) are micronutrients needed in small amounts; excessive concentrations can be toxic and disrupt plant physiological processes (Jundana, 2016). Meanwhile, organic carbon (C) content reflects the level of media fertility because it improves soil structure, increases microbial activity, and enhances nutrient availability (Kamisah, 2024). Meanwhile, pH determines nutrient availability and soil biological activity; media that are too acidic or too alkaline can inhibit plant growth.

Table 1. Basic Analysis of Planting Media

Treatment	Cu(ppm)	Corganik(%)	pH
Land	24	1.34	7.9
Mud	32	1.09	8.6

Based on the data in Table 1, the mud planting medium has a copper (Cu) content of 32 ppm, an organic C content of 1.09%, and a pH of 8.6. In terms of heavy metal content, a Cu value of 32 ppm is quite high and could pose a risk to the environment and plants if not managed properly. According to several soil quality standards, such as the WHO (1996), which sets the critical limit for copper (Cu) heavy metal content in soil at 36 mg/kg (Abrham & Gholap, 2021). Depending on the type of soil and its use. The Cu content in this mud is already close to the upper limit, so it needs to be monitored because it has the potential to cause heavy metal accumulation in plants and can have a negative impact on human health if consumed continuously through the food chain. Meanwhile, the soil planting medium has a copper (Cu) content of 24 ppm, an organic C content of 1.34%, and a pH of 7.9. In terms of heavy metal content, a Cu content of 24 ppm is considered moderate and within safe limits for plant growth according to most agricultural soil quality standards. This value is lower than that of mud, which can reach 32 ppm, thus lowering the risk of heavy metal toxicity to plants. However, long-term accumulation can occur, especially with high cropping intensity or additional contamination.

3.2. Observation of Planting Media After Mixing the Mud and Soil Combination

The mixing process between mud and soil was carried out in three treatments: K1 (mud:soil ratio = 1:1), K2 (2:1), and K3 (3:1). Each mixture was placed in a 15x15 cm polybag with a total media weight of 600 grams. After the mixing process, the media were left for two days (incubation process) to allow time for the planting media to interact and stabilise their composition before basic analysis was carried out. This incubation process aims to adjust the chemical properties of the materials, including the potential for ph-neutralization reactions, nutrient release, and changes in heavy-metal forms. After the 2-day incubation period, a basic analysis was performed for each treatment. The parameters analyzed included Cu content, organic C content, and pH.

Table 2. Cu, pH, and organic content of planting media

Treatment	Cu(ppm)	Corganik(%)	pH
(Mud 100%)	32	1.09	8.6
(1:1)	25	1.62	8.1
(2:1)	27	1.68	8.2
(3:1)	29	1.57	8.4

Description: The number of samples used in the basic analysis of planting media is one sample with one replication.

The K1 (1:1) treatment showed the most balanced results, with a Cu content of 25 ppm (moderate), an organic C content of 1.62%, and a pH of 8.1 (slightly alkaline), indicating improved growing medium and reduced potential for heavy metal toxicity. The K2 (1:2) treatment had 27 ppm Cu, the highest organic C of 1.68%, and a pH of 8.2, indicating an increase in soil fertility but also a slight

increase in Cu. The K3 (1:3) treatment had the highest Cu content (29 ppm), organic C (1.57%), and pH (8.4), indicating an increased risk of heavy metal accumulation, even though the media quality remained better than that of the single material. Overall, increasing the proportion of sludge improved organic matter, but also increased the Cu content and pH of the growing media.

3.3. Observation of pH of Planting Media

The results showed that there was no significant difference in pH between the 0- and 20-day treatments using the 5% BNJ test, but pH values changed in all treatments after planting. At 0 days after planting, the pH of the soil and mud were 7.9 and 8.6, respectively, while the mixtures of K1, K2, and K3 had an initial pH of 8.1, 8.2, and 8.4. After 20 days, the pH increased to 9.03 (K0), 9.10 (K1), 8.83 (K2), and 8.86 (K3).

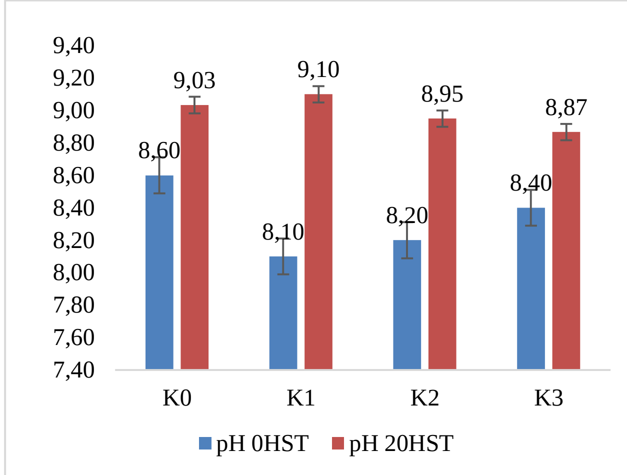

Figure 2. Graph of pH Value of Planting Media 0HST and 20HST

The increase in pH is thought to be due to the decomposition of organic materials releases base ions such as Ca^{2+}, Mg^{2+}, and K^{+}, as well as the activity of microorganisms that produce OH^{-} ions, thereby increasing soil pH (Syachroni, 2017). Treatments with soil-to-mud ratios of 1:2 and 1:3 showed lower increases in pH, possibly due to root exudates and microbial activity that neutralize some of the base. An increase in pH above 9, such as in a 1:1 soil-to-mud ratio, can reduce the availability of microelements (Fe, Zn, Mn, and Cu). Thus, although Lapindo mud can increase macronutrients and organic matter, its high alkaline nature still needs to be controlled to avoid chemical stress on plants.

3.4. Observation of Organic Planting Media

The results showed that organic C levels decreased in all treatments between 0 and 20 days after planting, reflecting the decomposition of organic matter and its utilization by plants and microorganisms during growth. The K2 treatment had the highest levels (decreased from 1.68% to 1.52%), while the K0 treatment had the lowest levels (decreased from 1.09% to 1.05%). The 5% LSD test (0.09) showed significant differences between treatments, with K2 significantly different from K0. The addition of soil to the mud significantly increased the organic C content of the growing medium, supported microbial activity, improved soil structure, and reduced the mobility of heavy metals through complex formation (Jundana, 2016). The mixture of soil and mud also provided readily biodegradable organic matter, accelerating decomposition and increasing the availability of organic C (Risma, 2023). Conversely, the use of mud alone (K0) was less effective at providing organic matter, so the soil-mud mixture, especially K2, was more effective at improving the quality of the growing medium and supporting the phytoremediation system.

Table 3. Corganik Results 20 HST

Treatment	Corganik(%)	
	0HST	20 HST
100% Mud	1.09	1.05 ± 0.01 a
1:1	1.62	1.37 ± 0.03 b
1:2	1.68	1.52 ± 0.04 cd
1:3	1.57	1.46 ± 0.03 c
BNT 5%	-	0.09

Description: Numbers followed by the same letter in the same treatment indicate no significant difference in the 5% BNT test. tn = no significant effect; DAP: Days After Planting; K0 (100% Mud); K1 (Soil and Mud 1:1); K2 (Soil and Mud 1:2); K3 (Soil and Mud 1:3.).

3.5. Plant Height Observation

The results showed that the height of water spinach plants at 10 and 20 days after planting was not statistically significantly different according to the 5% LSD test, but the trend indicated an effect of planting medium composition on vegetative growth. At 10 days after planting, the K0 treatment (100% mud) produced the lowest plant height (4.87 cm), presumably due to the mud's dense physical properties and its high pH (8.6), which inhibited nutrient absorption (Hartati, 2021). Conversely, K1 (soil:mud 1:1) recorded the highest plant height (8.93 cm), followed by K2 (7.73 cm) and K3 (7.56 cm), indicating the role of soil in improving the structure and aeration of the medium (Erwiyansyah, 2015). At 20 days after planting, a similar pattern was observed, with the highest K1 (20.43 cm) and the lowest K0 (11.46 cm). The soil mixture increases nutrient availability and supports root development, both crucial factors in soil fertility (Lestari, 2019). Although not statistically significant, these results indicate that media with soil:mud ratios of 1:1 or 1:2 better support kale growth and heavy metal uptake in the phytoremediation system.

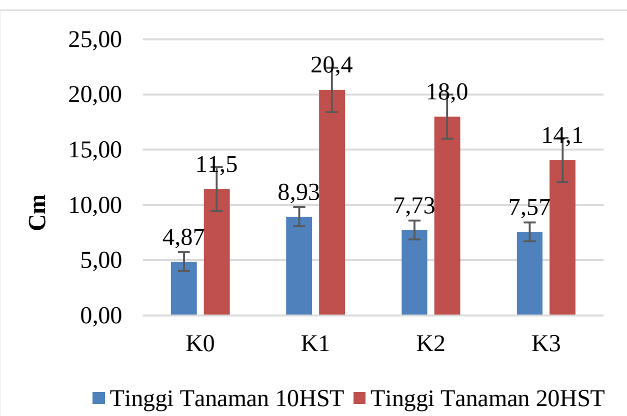

Figure 3. Graph of Water Spinach Plant Height

3.6. Plant Dry Weight Observation

Dry weight is a key indicator of photosynthetic productivity in plants, reflected in the amount of biomass. Plant dry weight reflects the pattern of photosynthetic accumulation. Because dry weight results from photosynthetic activity, the higher the dry weight, the better the plant's growth.

Table 4. Plant Dry Weight

Treatment	Plant Dry Weight(g)	
	10 HST	20 HST
100% Mud	0.10 ± 0.01 a	0.26 ± 0.01 a
1:1	0.17 ± 0.01 c	0.48 ± 0.03 b
1:2	0.15 ± 0.01 bc	0.50 ± 0.04 c
1:3	0.14 ± 0.00 b	0.29 ± 0.01 a
BNT 5%	0.02	0.09

Description: Numbers followed by the same letter in the same treatment indicate no significant difference in the 5% BNT test. tn = no significant effect; DAP: Days After Planting; K0 (100% Mud); K1 (Soil and Mud 1:1); K2 (Soil and Mud 1:2); K3 (Soil and Mud 1:3.).

The results showed that the dry weight of water spinach plants increased in all treatments from 10 to 20 days after planting, reflecting continued biomass growth. At 10 days after planting, K1 recorded the highest dry weight (0.17 g), while K0 recorded the lowest (0.10 g). A similar pattern was observed at 20 days after planting, with K2 the highest (0.49 g) and K0 the lowest (0.10 g) remained the lowest (0.26 g). The 5% BNT test showed significant differences between treatments, with K1 and K2 providing the best results compared to K0 and K3. Factors such as nutrient availability, pH, soil structure, and toxic conditions, such as heavy metals, influence dry biomass formation (Galal, 2018).

3.7. Root Dry Weight Observation

The study showed an increase in the dry weight of water spinach roots from 10 HST to 20 HST across all treatments. At 10 HST, the values ranged from 0.03–0.05 g without significant differences, while at 20 HST the differences became significant, with the highest K1 (0.16 g) and K2 (0.15 g), indicating that the 1:1 and 2:1 planting media were more optimal in supporting root growth. This media composition provided good aeration and nutrient availability (Sembiring, 2023), whereas pure mud (K0) and K3 (1:3) had the lowest growth due to minimal aeration and the mud's dense physical properties (Ciptawati, 2022).

Table 5. Root Dry Weight

Treatment	Root Dry Weight(g)	
	10 HST	20 HST
100% Mud	0.02 ± 0.01	0.08 ± 0.01 a
1:1	0.04 ± 0.01	0.16 ± 0.02 bc
1:2	0.03 ± 0.01	0.15 ± 0.02 b
1:3	0.04 ± 0.00	0.08 ± 0.01 a
BNT 5%	tn.	0.05

Description: Numbers followed by the same letter in the same treatment indicate no significant difference in the 5% BNT test. tn = no significant effect; DAP: Days After Planting; K0 (100% Mud); K1

(Soil and Mud 1:1); K2 (Soil and Mud 1:2); K3 (Soil and Mud 1:3.).

3.8. Uptake of Heavy Metal Cu by Water Spinach Plants

The results showed an increase in Cu uptake in water spinach from 10 DAP to 20 DAP in all treatments. At 10 DAP, the difference between treatments was not significant, with the highest value in K1 at 3.88 ppm and the lowest in K0 at 1.26 ppm. However, at 20 DAP, the difference became significant, with K1 (10.17 ppm) and K2 (9.72 ppm) being the highest and significantly different from K3 (5.38 ppm) and K0 (3.76 ppm). This finding indicates that the 1:1 (K1) and 2:1 (K2) soil-mud mixtures were more effective at increasing Cu uptake than pure mud. Uptake is influenced by soil chemical and physical properties, pH, organic C content, and plant species (Juhri, 2017). The increase in uptake from 10 DAP to 20 DAP confirms the accumulation of Cu in plant tissues as growth progresses, especially in roots and leaves, although environmental conditions also influence the rate of accumulation.

Table 6. Results of Cu Absorption in Plants

Treatment	Cu absorption (ppm)	
	10 HST	20 HST
100% Mud	1.26 ± 0.22	3.76 ± 0.63 a
1:1	3.88 ± 0.89	10.17 ± 0.95 bc
1:2	2.64 ± 0.89	9.72 ± 0.94 b
1:3	3.31 ± 0.73	5.38 ± 1.20 a
BNT 5%	tn.	3.22

Description: Numbers followed by the same letter in the same treatment indicate no significant difference in the 5% BNT test. tn = no significant effect; DAP: Days After Planting; K0 (100% Mud); K1 (Soil and Mud 1:1); K2 (Soil and Mud 1:2); K3 (Soil and Mud 1:3.).

3.9. Bioaccumulation of Cu in Water Spinach Plants

Bioaccumulation of heavy metals in plants is the process of accumulating metal elements from the environment (especially from water and soil) into plant tissue, both in the roots, stems, and leaves (Susilowati, 2021).

Table 7. Bioaccumulation of Cu

Treatment	10 HST	20 HST
100% Mud	0.04 ± 0.01	0.11 ± 0.02 a
1:1	0.15 ± 0.04	0.40 ± 0.04 bc
1:2	0.10 ± 0.03	0.36 ± 0.03 b
1:3	0.11 ± 0.02	0.18 ± 0.04 a
BNT 5%	tn.	0.11

Description: Numbers followed by the same letter in the same treatment indicate no significant difference in the 5% BNT test. tn = no significant effect; DAP: Days After Planting; K0 (100% Mud); K1 (Soil and Mud 1:1); K2 (Soil and Mud 1:2); K3 (Soil and Mud 1:3.).

The results showed that Cu bioaccumulation in water spinach increased from 10 DAP to 20 DAP across all treatments, confirming the accumulation of metals over time. At 10 DAP, bioaccumulation values ranged from 0.04–0.15, with K1 (1:1 soil and mud) the highest, while K0 (100% mud) was the lowest. At 20 DAP, K1 and K2 (2:1) remained the highest (0.40 and 0.36), significantly different from K0 (0.11) and K3 (0.18). Bioaccumulation is influenced by metal concentration, soil pH, organic matter content, and soil physical properties (Jaya, 2021). Low pH increases metal solubility, while organic matter can increase or decrease metal availability through chelation (Galal, 2018). The texture of Lapindo mud, which is dominated by clay fractions, reduces porosity and inhibits root growth, thereby affecting Cu absorption (Widaningrum, 2017). These findings indicate that optimal soil-mud ratios, especially K1 and K2, significantly enhance water spinach's ability to accumulate Cu.

Figure 4. K0 water spinach plant 10 days after planting

Figure 5. K1 water spinach plant 10 days after planting

Figure 6. K2 water spinach plant 10 days after planting

Figure 7. K3 water spinach plants 10 days after planting

Figure 8. K0 water spinach plants 20 days after planting

Figure 9. K1 water spinach plants 20 days after planting

Figure 10. K2 water spinach plant 20 days after planting

Figure 11. K3 water spinach plants 20 days after planting

4. Conclusion

Based on the research conducted, it can be concluded that a1:1 soil-to-mud planting media mixture is recommended as an effective medium for reducing copper (Cu) heavy metal pollution while promoting safer plant growth. Mixing soil and mud can reduce the levels of the heavy metal copper (Cu) compared to pure mud, while increasing Cu availability compared to soil alone. The 1:1 mixture proved to be the most effective, exhibiting the highest Cu absorption (10.17 ppm) and the greatest bioaccumulation factor (0.40).

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