Phytoremediation of heavy metal contaminated soil by *Chrysopogon zizanioides* L.

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**Abstract.** The electroplating industry is one of the industries producing various kinds of pollutant waste. One of the pollutants is Copper (Cu). It can cause environmental problems such as soil pollution that endanger microorganisms and other living beings and shift its ecological balance. Phytoremediation is a way to improve polluted land by using plants. This study aimed to investigate the reduction of Cu after the treatment of vetiver (*Chrysopogon zizanioides* (L.) Robercy). Plants were grown on contaminated soil for 28 days (absorption condition), then transferred to the soil without contamination and allowed to live for 28 days (elimination condition). In this study, the concentration of Cu heavy metal was analyzed in plants using Atomic Absorption Spectrophotometer (AAS). Phytoremediation potential was evaluated through absorption rate, elimination rate, along with Bioconcentration Factor (BCF), Biological Absorption Coefficient (BAC), and Translocation Factor (TF). The results showed that *C. zizanioides* could absorb Cu with the highest absorption rate of 1.45 mg.kg⁻¹.d⁻¹ and the highest elimination rate of 0.36 mg.kg⁻¹.d⁻¹. The absorption rate tends to be higher than the elimination rate. In this case, *C. zizanioides* can be used as an alternative for phytoremediation of Cu contaminated soil in the lightly to heavily polluted category.

**Keywords:** Cu²⁺; *Chrysopogon zizanioides* L.; heavy metal; phytoremediation.

1. Introduction
The electroplating industry is one industry that produces hazardous waste because it contains heavy metal compounds. Therefore, waste generated from electroplating industrial activities has heavy metal waste classified as Hazardous Toxic Materials [1]. As a result, efficient waste management is required to ensure that waste does not contaminate the environment. Plants that can absorb heavy metal contaminants from contaminated soil can reduce pollution from heavy metal contaminants in polluted soil. Phytoremediation is a method to improve contaminated land by using plants [2]. Hyperaccumulator plants having strong heavy metal absorption or accumulation characteristics in plant tissues are employed as phytoremediation agents. [3]. Research on vetiver (*C. zizanioides*) as a phytoremediation agent has been carried out, some of which state that vetiver (*C. zizanioides*) is very tolerant of various metals [4] such as As, Cd, Cu, Cr, and Ni [5][6][7][8].
The plant vetiver (C. zizanioides) was utilized in this investigation since it has been shown to improve soil that is contaminated by biogas effluent [9], tofu wastewater [10], antibiotics [11], palm oil waste [12], trichlorethylene (TCE) [13] [7], and remediate car wash wastewater [14]. Artificial electroplating waste with varied amounts of Cu was employed in this investigation. Cu metal was chosen because it is frequently found in electroplating industrial waste. Cu metal in electroplating waste can contaminate the environment and ecosystem. [15]. Based on the above mentioned context and the findings of literature studies, it appears that little information about the vetiver plant's activity has been discovered. (C. zizanioides) as a phytoremediation agent in absorbing heavy metal Cu. In this study, the roots and leaves of the vetiver (C. zizanioides) plant were used with soil media.

2. Methods

2.1. Tools and materials

Plant material: Vetiver (Chrysopogon zizanioides L.) used in the experiment was grown and cultivated in Greenhouse, part of Waste and Wastewater Management Laboratory-Research Unit for Clean Technology—National and Innovation Agency.

Research materials: Vetiver (Chrysopogon zizanioides L.), soil, urea, dan NPK, CuSO₄·5H₂O, HNO₃ 65%, H₂O₂ 33%, HCl 37%, distilled water

Research tools: Garden shovels, jars, sprayers, scissors, glass bottles, glass tools, analytical balance, oven, pestle, mortar, hotplate, soil tester, soil pH, Thermometer, Hygrometer, filter paper, AAS (Atomic Absorption Spectrophotometer), SEM (Scanning Electron Microscope).

2.2. Artificial waste treatment

A 1000 ppm Cu metal stock solution was made by dissolving 3.93 g of CuSO₄·5H₂O crystals into 1000 mL of distilled water, then homogenized and put into a 1000 mL glass bottle. Cu solutions with concentrations of 25, 75, and 150 ppm were made by diluting the stock solution as much as 25, 75, and 150 mL, respectively, into a 1000 mL volumetric flask and filled with distilled water to the mark, then homogenized and put into receptacle 5 L.

2.3. Heavy metal exposure

Wastewater treatment is divided into control; Cu wastewater with a concentration of 25 ppm, 75 ppm, and 150 ppm. Each treatment was carried out in 3 repetitions. Treatment is carried out by pouring the solution onto the soil. This marks the 0th day of phytoremediation, and the plants were then grown for 28 days to absorb the heavy metals (uptake condition). The remaining plants were transferred to uncontaminated growing media and allowed to live for 28 days (elimination condition).

2.4. Sampling of soil, roots, and leaves

Soil samples were taken on days 0 and 28 for absorption conditions and days 0 and 28 for elimination conditions. The soil was taken from each jar for control and treatment, then weighed 5 g. Soil samples that have been sampled are dried to a constant weight. Soil samples were digested and tested for Cu concentration using AAS (Atomic Absorption Spectrophotometer).

A sampling of roots was carried out on days 0, 14, 28 for absorption conditions and on days 0, 14, and 28 for elimination conditions in both control and treatment. The roots were weighed to measure the wet weight with an analytical balance, then dried in a 50 °C oven to reach a constant weight. The absorption conditions of the roots for day 0 and 28 were determined using SEM-EDS for elemental content analysis.

2.5. Destruction and determination of Cu concentration

The soil samples destruction was carried out by the wet acid digestion method based on Turek et al., (2019) [16]. 12 ml HNO₃ and 4 ml HCl were added to 1 g of soil sample in an Erlenmeyer. After two
hours on a hotplate, the sample was transferred to a 100 mL volumetric flask, filtered, and diluted with distilled water.

The destruction of root and leaf samples was carried out using the wet digestion method based on Pequerul et al., (1993) [17]. Dry root and leaf samples weighing 0.1 g were crushed. 1 mL of 65% HNO₃ was added to the sample in a 100 mL Erlenmeyer and homogenized until the sample was moist. 0.8 mL of 33% H₂O₂ was added and homogenized. After that, the sample was heated on a hotplate until it became frothy. After 7-8 minutes, the brown tint faded, and the solution was cooled. The solution was then transferred to a 25 mL volumetric flask, filtered through 0.45 m filter paper. 1 mL 37% HCl was added, and distilled water was added up to the volumetric flask’s mark. Each sample is performed in threes.

Determination of the concentration of heavy metals in each sample was carried out by Atomic Absorption Spectrophotometer (AAS) Agilent Technology 200 Series AA. The results obtained were converted from mg·L⁻¹ to mg·kg⁻¹, DW.

2.6. Data analysis

The data obtained was used to determine Bioconcentration Factor (BCF) as the ratio of heavy metal content in roots to soil. Biological Absorption Coefficient (BAC) as the ratio of heavy metal content in leaves to soil, Translocation Factor (TF) as the ratio of heavy metal content in leaves to roots.

After the calculation is complete, the data is tabulated in Microsoft Excel to calculate the average value of each sample replication. The results are then presented in the form of tables and graphs, with the following formula:

\[
BCF = \frac{[\text{heavy metal}]_{\text{roots}}}{[\text{heavy metal}]_{\text{soil}}} \quad (1)
\]

\[
BAC = \frac{[\text{heavy metal}]_{\text{leaves}}}{[\text{heavy metal}]_{\text{soil}}} \quad (2)
\]

\[
TF = \frac{[\text{heavy metal}]_{\text{leaves}}}{[\text{heavy metal}]_{\text{roots}}} \quad (3)
\]

\[
\text{Uptake rate} = \frac{[\text{heavy metal}]_{\text{treatment}} - [\text{heavy metal}]_{\text{control}}}{\text{Day of metal exposure}} \quad (4)
\]

\[
\text{Elimination rate} = \frac{[\text{heavy metal}]_{\text{treatment}} - [\text{heavy metal}]_{\text{control}}}{\text{Day of metal exposure}} \quad (5)
\]

3. Results and Discussion

3.1. Cu concentration in soil

To determine the bioavailability of Cu in soil, the concentration of Cu in the soil is measured (Figure 1). The results showed that plants treated with 50 ppm, 100 ppm, and 150 ppm had lower Cu concentrations in the soil during the absorption period.

The concentration of heavy metal Cu in the soil with treatments of I (25 ppm), II (75ppm), and III (150 ppm) was 44.33; 71.53; and 177.60 mg.kg⁻¹, then decreased to 22.18; 34.76; and 56.78 mg.kg⁻¹, respectively. Removal efficiency for treatments I, II and III were 50%, 51.4% and 68%, respectively. The presence of a decrease after 28 days of waste absorption at each concentration or treatment suggests that heavy metal absorption by Chrysopogon zizanioides (L.) Roberty is possible.
3.2. Cu accumulation in roots
Root samples were used to examine Cu accumulation in the roots of vetiver (*C. zizanioides*). In both the control and treatment groups, vetiver roots (*C. zizanioides*) were sampled on days 0, 14, and 28 for absorption and days 0, 14, and 28 for elimination. The following results were obtained from the tests as shown in Figures 2 and Figure 3.
On day 28, the amount of Cu deposited in the roots of each treatment increased. Cu metal had the greatest concentration on day 28 in each treatment of 25, 75, and 150 ppm, which was 39.94, 46.78, and 59.33 mg kg\(^{-1}\), DW. The Cu content in the control treatment was quite low, with sequential values of 0.38; 0.57; 0.66; 0.69; 0.75 mg kg\(^{-1}\), DW. This means that the higher the Cu concentration taken by plants, the longer the metal absorption time. According to Notodarmojo (2005) [19], Cu concentrations in roots and leaves range between 5 and 20 mg kg\(^{-1}\), DW, with a critical value of 60 to 120 mg kg\(^{-1}\), DW. The results show that the roots have Cu concentrations of 39.94, 46.78, and 59.33 mg kg\(^{-1}\), DW, for treatments of 25, 75, and 150 ppm, respectively. Cu concentrations in roots and leaves vary from 5 to 20 mg kg\(^{-1}\), DW, with 60 to 120 mg kg\(^{-1}\), DW being the critical value. For 25, 75, and 150 ppm treatments, the roots exhibit Cu concentrations of 39.94, 46.78, and 59.33 mg kg\(^{-1}\), DW, respectively. Cu concentrations were 2.38, 3.58, and 7.10 mg kg\(^{-1}\), DW, on day 28 for treatments 25, 75, and 150 ppm, respectively (Table 1).

### Table 1. Cu concentration in roots of *Chrysopogon zizanioides* L.

| Day | Treatment (ppm) | Uptake rate (mg kg\(^{-1}\), DW) | Elimination rate (mg kg\(^{-1}\), DW) |
|-----|-----------------|----------------------------------|-------------------------------------|
| 0   | 25              | 3.88                             | 5.29                                |
|     | 75              | 9.01                             | 6.85                                |
|     | 150             | 10.58                            | 18.32                               |
| 14  | 25              | 5.71                             | 3.63                                |
|     | 75              | 18.66                            | 5.23                                |
|     | 150             | 20.91                            | 9.00                                |
| 28  | 25              | 14.22                            | 2.38                                |
|     | 75              | 20.62                            | 3.58                                |
|     | 150             | 53.67                            | 7.10                                |
The Cu levels in the control treatment were 0.18, 0.14, and 0.07 mg.kg$^{-1}$, respectively, while the Cu levels in the experimental treatment were 0.18, 0.14, and 0.07 mg.kg$^{-1}$, respectively. Because there was a process of transferring plants to new soil without metal contaminants throughout time, there was a decline in Cu levels during the elimination circumstances.

3.3. Uptake rate and elimination rate Uptake rate and elimination rate

Cu absorption and elimination rates have a wide range of values (Table 2). On the other hand, the absorption rate is generally larger than the elimination rate. High and low Cu concentrations in the soil, plant metabolism, soil acidity, the presence of other metals, and the composition of organic matter all influence the rate of uptake or removal. Phytoremediation agents are typically chosen for their high absorption rate and ability to detoxify [18].

Table 2. Uptake rate and elimination rate of Cu in Chrysopogon zizanioides L.

| Day | Treatment (ppm) | Uptake rate (mg.kg$^{-1}$, DW) | Elimination rate (mg.kg$^{-1}$, DW) |
|-----|-----------------|--------------------------------|-----------------------------------|
| 14  | 25              | 0.37                           | 0.25                              |
|     | 75              | 1.29                           | 0.36                              |
|     | 150             | 1.45                           | 0.15                              |
| 28  | 25              | 0.48                           | 0.08                              |
|     | 75              | 0.71                           | 0.13                              |
|     | 150             | 0.43                           | 0.25                              |

This study found that the uptake rate is higher than the elimination rate, which could be beneficial for using vetiver (C. zizanioides) as a phytoremediation alternative. Furthermore, these plants have several advantages that are important for phytoremediation plants, such as being easy to grow and producing a large amount of biomass in a short amount of time, having a long root system, and being local plants that are adapted to climatic conditions and soils near polluted areas, requiring minimal maintenance. [2].

3.4. Phytoremediation potential in vetiver (C. zizanioides)

The BCF value was greater than 1, while the BAC and TF values were less than 1, in the absorption condition of 25, 75, and 150 ppm. The BCF and BAC values in the elimination condition were less than 25, 75, and 150 ppm, respectively, while the TF value is greater than 1 (Table 3). Based on these findings, vetiver (C. zizanioides) can be employed as phytostabilization in phytoremediation, as evidenced by the BCF value of more than one and TF less than one. The ability of plants to endure metal translocations is one of the most important qualities that a phytostabilizing agent must possess.

The ability to remediate soil (Table 1) and the relatively strong ability to be absorbed and accumulated by plant roots confirm this. According to Ramachandra et al., (2018) [19] in situations of elimination rather than absorption, a higher TF value can be beneficial in promoting the use of these plants as phytoremediation agents. According to these findings, the TF value was higher in the elimination condition in this investigation. In this regard, it may be stated that vetiver (C. zizanioides) can be used as a phytoremediation option and can be combined with other hyperaccumulators.
Table 3. Cu concentration in soil, roots, and leaves of vetiver (*C. zizanioides*).

| Cu conc. (ppm) | Condition | Soil (mg.kg⁻¹) | Root (mg.kg⁻¹) | Leaves (mg.kg⁻¹) | BCF | BAC | TF |
|---------------|-----------|----------------|----------------|------------------|-----|-----|----|
|               | Uptake    |                |                |                  |     |     |    |
| 25            | Control   | 0.00           | 0.75           | 0.18             | 1.80| 0.47| 0.26|
|               | Treatment | 22.18          | 39.94          | 10.36            |     |     |    |
|               | Elimination | 0.00        | 0.07           | 0.27             | 0.22| 0.52| 2.34|
|               | Treatment | 10.76          | 2.38           | 5.56             |     |     |    |
| 75            | Uptake    | 0.00           | 0.75           | 0.18             | 1.35| 0.56| 0.42|
|               | Treatment | 34.76          | 46.78          | 19.60            |     |     |    |
|               | Elimination | 0.00        | 0.07           | 0.27             | 0.25| 0.57| 2.25|
|               | Treatment | 14.16          | 2.38           | 8.04             |     |     |    |
| 150           | Uptake    | 0.00           | 0.75           | 0.18             | 1.04| 0.52| 0.50|
|               | Treatment | 56.78          | 59.33          | 29.78            |     |     |    |
|               | Elimination | 0.00        | 0.07           | 0.27             | 0.22| 0.41| 1.85|
|               | Treatment | 32.18          | 7.10           | 13.13            |     |     |    |

4. Conclusion

After 28 days of heavy metal absorption, the concentration of Cu in the soil was reduced. Cu accumulation in vetiver roots increased after 28 days of metal absorption and decreased when transferred to uncontaminated media. The uptake rate of Cu tends to be higher than the rate of elimination, it means that vetiver has the potential to be used as a phytoremediation agent.

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