Phytoremediation Potential of *Pistia stratiotes* to Reduce High Concentration of Copper (Cu) in Acid Mine Drainage

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Abstract. Acid mine drainage (AMD) contains high concentration of various heavy metals and low pH levels, ranging between 2-4. The water samples used for this research are an artificial AMD, with varying concentration levels of copper (5 and 7 mg/l). The pH level is designed to be 4, where the designed pH level illustrates the actual characteristics of an AMD. The data for this research—pH and copper levels—are taken for 14 days. This research shows that exposure of the artificial AMD causes physiological effects to *Pistia stratiotes*, indicated by chlorosis of the plant starting from day 3 of the research. Furthermore, the result of this research illustrates that *Pistia stratiotes* is able to alter the artificial AMD’s pH level from 4 to 7.3 in 14 days. This plant is also capable of reducing the copper content as much as 92.45% and 88.00% with initial concentrations of 5.3 mg/l and 7.5 mg/l respectively in 14 days, with peak removal at day 3. It can be concluded that *Pistia stratiotes* is able to neutralize pH level and decrease copper contents in artificial AMD.

1. Introduction

Acid mine drainage (AMD) is formed when mineral sulphides, such as iron pyrites and iron disulphides (FeS2), oxidize. In general, acid mine drainage contains high level of acidity with pH levels ranging between 2-5. Acid mine drainage also contains various heavy metals, such as Cd, Co, Cr, Mn, Pb, Cu, Ni, and Zn. This high level of acidity and heavy metals can cause negative impacts to the environment, and furthermore can affect human health, water ecosystems, plant growths, and soil ecosystems [1]. According [2], acid mine drainage studied at several coal and mineral mining sites in Indonesia contain high concentration of heavy metals. One of the heavy metals studied there is copper (Cu), which concentration level ranges between 7-13 mg/l. These concentration levels surpass the water quality standard stated in Minister of Environment Decree No. 202 Year 2004 Regarding Wastewater Standard for Gold and Copper Mine Activities and/or Bussinesses, where the limit for copper content in water is 2 mg/l. The purpose of this study is to analyse the capabilities of *Pistia stratiotes* in neutralizing the pH level and reducing the copper (Cu) contents in artificial acid mine drainage using phytoremediation method.

One of the passive treatments used to treat acid mine drainage is phytoremediation. [3] stated that the most significant factors in phytoremediation are selection of suitable plants, uptake potential to absorb high levels of pollutants, and also can survive in polluted waters. According to [4], floating macrophytes are suitable for phytoremediation because of their rapid growth, high potential of heavy metal accumulation, and high tolerance towards toxic substances. *Pistia stratiotes* or water lettuce is a good bioaccumulative
agent to reduce copper (Cu) contents in water [5]. Based on a research obtained by [6], *Pistia stratiotes* was able to reduce copper contents in water as much as 90–94%. Therefore, utilization of *Pistia stratiotes* as a bioaccumulative agent towards heavy metals can be an alternative method to reduce high concentration of copper (Cu) in acid mine drainage.

2. Methodology

100 grams of fresh *Pistia stratiotes* plants were acclimatized in a plastic basin filled with 10 litres of ground water for 7 days. The collected plants were acclimatized to adapt with the surroundings and to eliminate dirt from the plants. The water samples used for this research are an artificial AMD, with varying concentration levels of copper (5 and 7 mg/l). The pH level is designed to be 4, where the designed pH level illustrates the actual characteristics of an AMD. The artificial acid mine drainage is prepared for each variation by dissolving CuSO₄.5H₂O in 2 litres of ground water until the designed initial concentration of Cu is obtained. The designed pH level is obtained by adding HNO₃ to the solution. The mass of CuSO₄.5H₂O needed to prepare artificial acid mine drainage for each variation can be measured using this equation:

\[
CuSO_4\cdot 5H_2O (\text{gr}) = \frac{\text{designed concentration mg Cu} \times M_{CuSO_4\cdot 5H_2O}}{A_{Cu} \times 1000}
\] (1)

4 grams of acclimatized *Pistia stratiotes* is placed in a cylindrical glass container containing 2 litres of artificial acid mine drainage for each concentration. The data for this research—pH and copper levels—is taken every 24 hours for 7 days, with an additional data taken 14 days after the beginning of the research. 100 ml of artificial acid mine drainage for each concentration is taken and placed in a glass bottle to determine pH and copper levels. The measurement of copper contents and pH levels in artificial AMD every 24 hours is done with the purpose to analyse the removal of copper contents and changes in pH levels, which can represent bioaccumulation potential of *Pistia stratiotes* in purifying Cu contaminated waters. Morphological changes in each *Pistia stratiotes* plants are also observed every 24 hours for 7 days, and at day 14 of the research, to determine the effect of acidity and copper contents in the solution against *Pistia stratiotes* plants.

3. Results and Discussion

3.1. Morphological changes on *Pistia stratiotes*

Morphological changes on *Pistia stratiotes* plants in response to exposure to artificial acid mine drainage can be seen on figure 1. On the first day of the study (day 0), *Pistia stratiotes* plant was fresh and bright green in color, with white hairs covering the surface of the leaves, which could prevent the leaves from getting wet and help the plant to float. On the 3rd day of the study, it can be observed that some leaves of *Pistia stratiotes* in both variation of initial Cu concentrations (5 and 7 mg/l) had experienced chlorosis, which could be identified by several leaves turning yellow in color. In addition, there were also dead and detached leaves from the plant body on the 5th day of the study. On the 7th day, more leaves of the plant died but there were still a number of leaves that were still surviving, marked by the presence of chlorophyll on the leaves of the plants that were still attached to the plant body. On the final day of the research (day 14), most of the plant leaves had experienced necrosis and were detached from the plant body. Meanwhile, *Pistia stratiotes* plant, which was placed into a container filled with ground water as a control sample, did not show any signs of chlorosis or necrosis until the 7th day of the study, which was indicated by the plant leaves that were still fresh and bright green in color. Whereas on the 14th day of the study, *Pistia stratiotes* plant in the control container had shown symptoms of chlorosis which was marked by one of the leaves turning yellow in color.

According to [7], changes in leaf color and root growth are plant physiological responses to the content of heavy metals in the growth media, because this condition disrupts the activity in cells and plant metabolism. Exposure to heavy metals generally can cause leaves to experience chlorosis and necrosis. The results obtained also support the research which was conducted by [8], which stated that plant responses to toxicity from heavy metals include leaf discoloration, chlorosis, necrosis, dwarfism, gigantism, inhibition
of leaf expansion and inhibition of root growth. In plant cells, excessive heavy metal content can modify plasma membrane permeability, causing leakage of ions and dissolved substances. Some heavy metals such as copper (Cu) have high affinity for sulfidril and carboxyl categories, which will cause a decrease in plasma ATP-ase lemma activity. In addition, this heavy metal content will form free radicals that can damage several components of plant cells [9]. Excessive heavy metal contamination also has an impact on plant chlorophyll substances. Some metals from the sulfidril category that have high affinity can cause inhibition of enzyme and chlorophyll synthesis which can reduce chlorophyll contents in plants [10].

| Day | Cu Concentration 5 mg/l | Cu Concentration 7 mg/l | Control |
|-----|-------------------------|-------------------------|---------|
| 0   | ![Image](image1)         | ![Image](image2)        | ![Image](image3) |
| 3   | ![Image](image4)        | ![Image](image5)        | ![Image](image6) |
| 7   | ![Image](image7)        | ![Image](image8)        | ![Image](image9) |
| 14  | ![Image](image10)      | ![Image](image11)      | ![Image](image12) |

**Figure 1.** Morphological Changes on *Pistia stratiotes*.

### 3.2. pH level changes

![Graph](image13)  
**Figure 2.** pH changes in Artificial Acid Mine Drainage.
Figure 2a and 2b show the pH changes of artificial acid mine drainage in response to phytoremediation method using *Pistia stratiotes* plants. pH level in artificial acid mine drainage, with the initial copper contents of 5 mg/l, relatively increased from the beginning until the end of the research (see figure 2a). The pH level of the artificial acid mine drainage was altered to neutral condition, where the maximum increase occurred at the 3rd day of the research. This result agrees with the findings of [11] and [12] who observed that *Pistia stratiotes* is capable to neutralize solutions with acidic pH level. This study shows that pH level in artificial acid mine drainage was increased by 80.2%, with the final pH level at day 14 was as much as 7.3. Similarly, figure 2b also shows increased pH levels in artificial acid mine drainage with the initial copper contents of 7 mg/l. *Pistia stratiotes* plant is able to alter pH levels as much as 75%, with the initial pH level 4.0 to 7.0 in 14 days. Statistically, the pH changes in artificial acid mine drainage for initial concentration of 5 mg/l are better than the ones for concentration 7 mg/l (see figure 3). According to Minister of Environment Decree No. 202 Year 2004 Regarding Wastewater Standard for Gold and Copper Mine Activities and/or Bussinesses, the pH level standard that is permitted for acid mine drainage ranges between 6-9. Based on this standard, the median, average, 3rd quartile, and maximum value of pH in artificial acid mine drainage (initial concentration of 5mg/l) surpass the water quality standards stated above. The changes in pH level can be caused by photosynthesis process which involves dissolved CO2 in the form of H2CO3 [13]. The free OH- ions in water will eventually increase the pH levels in acid mine drainage [14]. The result of the study shows that the final pH levels in artificial acid mine drainage that were obtained had complied with the water quality standards stated above.

![Figure 3. Distribution of pH Values in Artificial Acid Mine Drainage](image)

3.3. Copper content removal

The copper content removal in artificial acid mine drainage using *Pistia stratiotes* as a phytoremediation agent is illustrated by Figure 4a and 4b. The changes of copper contents in artificial acid mine drainage tend
to decrease until the end of the research for both of the varying initial copper contents (see figure 4a and 4b). *Pistia stratiotes* is competent in reducing copper contents from 5 mg/l to 0.4 mg/l in 14 days (see figure 4a), with the removal percentage as much as 92.45%. This result confirms that *Pistia stratiotes* can be a hyperaccumulative plant to remove copper contents in polluted water. Similar result is also shown in a research by [15], who stated that *Pistia stratiotes* can accumulate high levels of heavy metals such as Zn, Cr, Cu, Pb, and Cd. This reduction of copper contents also agrees with preceding studies which confirmed that *Pistia stratiotes* is capable in reducing copper contents in water with high removal percentage [6, 16, 17]. The maximum copper content removal occurred on the 3rd day of the research, removing 54.72% of the initial copper contents.

The results of this study also show that *Pistia stratiotes* can reduce copper contents in artificial acid mine drainage from 7 mg/l to 0.9 mg/l in 14 days (see figure 4b), with the removal efficiency as much as 88%. This removal efficiency is lower than the removal efficiency obtained by artificial acid mine drainage with the initial concentration of 5 mg/l. This result is also confirmed by statistics which shows that Cu concentration values for both variation of concentration are positively skewed (see figure 5), with the median, 1st quartile, and minimum values of artificial acid mine drainage, with the initial Cu concentration of 5 mg/l, met the copper content standard stated in Minister of Environment Decree No. 202 Year 2004 Regarding Wastewater Standard for Gold and Copper Mine Activities and/or Bussinesses. Similar results can be seen in the research done by [18] in which it is stated that *Pistia stratiotes* is capable of eliminating cadmium (Cd) contents with a higher removal efficiency when exposed to water with lower cadmium content— 82% removal in the 2 mg/l solution— than when exposed to water with higher cadmium content— 70% removal in the 5 mg/l solution. [19] reported that heavy metals removal depends on contact time and initial metal concentration. In his research using water hyacinths to remove cadmium content, it was stated that the initial absorption of metals was quickly obtained within 48 hours and the equilibrium was achieved gradually after 120 hours. [19] also stated that the ability of heavy metal removal by plants would be higher for lower initial metal concentrations and would decrease if the metal concentration increased. Some studies also prove a similar thing for the absorption of metals by water lettuce of *Pistia stratiotes*. [18] found that *Pistia stratiotes* were able to eliminate mercury concentrations in coal mining wastes from initial concentration of 10 μg/l to 2 μg/l – or around 80% of mercury contents – in 21 days. Mercury absorption by the *Pistia stratiotes* plant is about four times higher at lower concentrations [18, 20]. This result is related to the physiological conditions of the *Pistia stratiotes* plant. [21] stated that lower Cu concentrations in plants (5 mg/l) can improve the carbohydrate synthesis process. The carbohydrate content in plants will increase when the concentration of Cu contained is lower. This condition is caused by the detoxification process of free radicals by the enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase or gluthathione reductase [22]. Similar thing is stated in the study by [23], which explained that carbohydrate synthesis is inhibited as the concentration of copper (Cu) increases in plants, because the toxicity of copper will increase, thus the content of heavy metals can damage plant components involved in photosynthesis process. Plants absorb heavy metal elements and nutrients from their living media through osmosis method along with the evapotranspiration process. Water and metal elements will be transported through root cells by the absorption process. Then, dissolved substances will be transported vertically through xylem from roots to leaves [24]. Therefore, the fresh weight of the plant will increase and the concentration of copper (Cu) in the living media (water) will decrease along with the increasing copper concentration in the plants [6].

According to Minister of Environment Decree No. 202 Year 2004 Regarding Wastewater Standard for Gold and Copper Mine Activities and/or Bussinesses, the copper content standard that is permitted for acid mine drainage is 2 mg/l. Therefore, the final concentration of copper (Cu) in artificial acid mine drainage that were obtained in this research had complied with the water quality standards stated above.
4. Conclusion

Morphological changes in *Pistia stratiotes* plants in response to the exposure to artificial acid mine drainage can be determined from the color changes on the leaves, and also chlorosis and necrosis of the plants. This study shows that retention times have significant effects on pH level changes and removal of copper contents in artificial acid mine drainage. *Pistia stratiotes* is able to alter pH level in artificial acid mine drainage, with the first variation of Cu concentration, from 4.05 to 7.3 in 14 days, while pH level in the second variation of Cu concentration in artificial acid mine drainage, increased from 4 to 7 in 14 days. This plant is also capable to reduce the copper content as much as 92.45% and 88.00% with the initial concentration of 5.3 mg/l and 7.5 mg/l respectively in 14 days, with peak removal occurred at day 3. In conclusion, *Pistia stratiotes* plant can be used as a passive treatment solution for treating high level of acidity and copper contents in acid mine drainage.

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