Phytoremedial potential of aquatic macrophytes for the removal of copper in contaminated waters

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Abstract. The aim of this study is to identify the potential aquatic macrophytes to be used for phytoremediation and to compare the reduction of copper by two species of aquatic plants. Two aquatic plants; Centella asiatica and Eichhornia crassipes, the effectiveness were evaluated for their capabilities of copper removal from copper solution. The aquatic macrophytes were placed for 7 days in 6 liters of solution that contained 1 mg/L, 3 mg/L and 5 mg/L of copper. Atomic absorption spectrometer (AAS) was used to conduct analyses of heavy metals contents. It was shown in the results that Centella asiatica achieved complete removal of 1 mg/L copper concentration after the third day of treatment. Centella asiatica also displayed maximum removal of 92% of 3 mg/L copper concentration and 62% for 5 mg/L copper concentration. The peak efficiency at the end of the experiments were at almost similar for Eichhornia crassipes, 83% for the 1 mg/L concentration, 80% for the 3 mg/L concentration and 83% for the 5 mg/L concentration. Overall, Centella asiatica exhibit faster removal rate of copper compared to Eichhornia crassipes. In terms of effectiveness, the percentage removal in solution was higher using Centella asiatica at lower concentration of copper. However, at higher concentration of copper (5 mg/L), Eichhornia crassipes was found to be more effective in removing copper compared to Centella asiatica.

1. Introduction
The increasing rate of urbanization all over the world has led to the increase of economic and industrial activities, which in turn would most certainly increase the amount of harmful contaminants and pollutants discharged into the surrounding environment of those expanding urban areas. Depending on how the contaminants and pollutants are being handled, the level of pollution on the surrounding environment may vary and discharged into various mediums namely the air, water and soil. Heavy metals contaminant, in particular, is the by-product of mostly industrial and agriculture activities, which could seep into the soil and may make its way into nearby water bodies such as rivers, lakes and underground water wells. Contamination of agricultural soils with several heavy metals including lead (Pb), cadmium (Cd), copper (Cu) and chromium (Cr) has been widely reported [1,2].

Contamination of heavy metals in water source used for human consumption would have a detrimental effect to humans. For the sake of human safety and health in the area of contamination of heavy metals, the presence of heavy metals need to be reduced or better, removed to ensure the water on the site of contamination would not be a health hazard to the people who are living in and around the vicinity of that area. There are a number of ways to remediate a contaminated water body, which
can be categorized into three methods namely, biological treatment, chemical treatment and physical treatment. Each method has its own advantages and disadvantages in terms of practicality, costs of treatment process and its effect on the surrounding landscape. Therefore, the selection of method for remediating a water body are determined by many factors such as the scale of the pollution, the size of water body, the type of water body, the funding for the remediation process and also the cause of pollution [2,3].

Phytoremediation is an approach using aquatic macrophytes to treat water contaminated by organic and inorganic substances including heavy metals. Currently, phytoremediation using the aquatic macrophytes are used to overcome the environmental issues especially water and soil pollution. Phytoremediation takes advantage of the natural process in which the aquatic macrophytes undergo a process of breaking down inorganic compounds to less potent compounds [3,4]. Inorganic compounds such as heavy metal and synthetic substance cannot be biodegraded but they can be transformed through the body of the macrophytes where it is transformed into compounds, which have less mobility or toxicity than their original form. Aquatic macrophytes have great potential to accumulate heavy metals inside the plant bodies. In this study, two aquatic macrophytes, pennywort (*Centella asiatica*) and water hyacinth (*Eichhornia crassipes*) were used to determine their potential in removing copper (Cu) of various concentrations. The effectiveness of the plants was determined based on copper content analyses in the solution.

2. Materials and Methods

2.1. Selection of aquatic macrophytes

*Centella asiatica* and *Eichhornia crassipes* were collected from Sungai Kelang Ubi, Bukit Mertajam, Pulau Pinang. These aquatic macrophytes were placed in a tank containing tap water for two-weeks, before using the macrophytes for the removal of copper.

2.2. Preparation of copper contaminated solution

Synthetic copper solution at 1 mg/L, 3 mg/L and 5 mg/L concentration were prepared using the compound copper (II) sulphate, Cu (II) SO4 and distilled water. It was weighted accordingly using the analytical balance to determine the correct amount to obtain the desired concentration of copper (Cu). The calculation for the copper (Cu) concentrations is calculated using the dilution method formula, which is shown in equation 1.1.

\[
m_1v_1 = m_2v_2
\]

Where:

- \( m_1 \) = Concentration of the stock copper (Cu) solution (mg/mL)
- \( v_1 \) = Volume of the stock copper (Cu) solution needed to be added in to the container to achieve the required concentration (mL)
- \( m_2 \) = Required concentration of the copper (Cu) solution (mg/mL)
- \( v_2 \) = Required volume of copper (Cu) solution (mL)

2.3. Experimental set-up

The aquatic macrophytes, which were placed in tap water for two weeks, were washed with no tissue removed. To minimize the variations of the macrophytes tested, it is important that the plants have the same background, looked green and fresh, have approximately same size and have approximately the same number of leaves to avoid big surface differences. The criteria of the plant that were taken into account in choosing it for the experiment was its size, weight, root length and number of stems/leaves on the plant. On average, the weight of all the macrophytes were approximately 27 g, the roots length were about 8 cm and the number of stem/leaves were 5 or 6 for each plant. The criteria of the macrophytes would affect its functionality to absorb the copper (Cu) in the solution; therefore it is crucial to use plants of similar physical criteria to obtain a consistent and accurate data as possible. The experiment is set-up in 3 sets of varying copper (Cu) solution concentrations with 3 containers.
containing the same concentration of copper (Cu) solution. The aquatic macrophytes were placed in 6 liters of solution containing 1 mg/L, 3 mg/L, and 5 mg/L of copper. The macrophytes were placed in 2 out of the 3 containers in each set, where the container without any macrophytes inside of it acts as the control container. The study was conducted for 7 days where copper (Cu) solution is assessed by comparing the daily readings for copper (Cu) concentration in the solution where the macrophyte is placed in a container containing the copper (Cu) solution. Samples of the copper (Cu) solution are taken daily to check for the concentration of copper (Cu) using atomic adsorption spectrometer (AAS) throughout the period of the experiment. At the end of the experiment, the reduction of copper (Cu) in the solution is converted into efficiency in form of percentage.

3. Statistical Analysis
The data were presented as mean values of duplicates. Statistical analysis using the one-way ANOVA by Excel 2010 (Microsoft Office) was measured to assess significant differences between the two aquatic macrophytes. The comparisons of mean using the least significant different test were calculated for P-values. A value of P < 0.05 was considered significant.

4. Results and Discussions
Figure 1(a) and Figure 1(b) shows the mean final concentrations of copper solution. For containers containing *Centella asiatica*, the aquatic macrophytes was able to reduce the concentration of copper from 5 mg/L to 1.90 mg/L, 3 mg/L to 0.23 mg/L and complete removal for 1 mg/L copper concentration as shown in Figure 1(a). Comparable trend of removal was also observed for containers containing *Eichhornia crasipes*, where the concentration of copper decreases from 5 mg/L to 0.87 mg/L, 3 mg/L to 0.59 mg/L and 1 mg/L to 0.17 mg as shown in Figure 1(b). As reported by [3], *Centella asiatica* and *Eichhornia crassipes* used in the removal of copper was effective treating copper contaminated water with initial concentration of 5.5 mg/L where copper concentration was reduced to 0.92 mg/ and 2.1 mg/L by pennywort (*Centella asiatica*) and water hyacinth (*Eichhornia crasipes*), respectively. Similar study by [4], also reported effective removal of copper with initial concentration of 0.35 mg/L, which was reduced to 0.02 mg/L within 5 days.

**FIGURE 1.** Final concentration of copper by (a) *Centella asiatica* and (b) *Eichhornia crassipes*

Figure 2(a) and Figure 2(b) shows the percentage removal of copper within seven days for *Centella asiatica* and *Eichhornia crassipes*. Results showed that *Centella asiatica* achieved complete removal of 1 mg/L copper concentration after the third day of treatment as shown in Figure 2(a). *Centella asiatica* also exhibited maximum removal of 92% of 3 mg/L copper concentration and 62% for 5 mg/L copper concentration. Figure 2(b) showed that *Eichhornia crassipes* removed 83% of
copper at 1 mg/L, 80% of copper at 3 mg/L and 83% of copper at 5 mg/L within 7 days. Apparently as shown in Figure 2(a) and 2(b), the highest removal is obtained after the first day and the percentage removals for all concentrations reached the maximum after 5 days. Figure 2(b) also shows that copper removals by *Eichhornia crassipes* are the highest for the concentration of 1 mg/L and the least copper removals were at 83% for *Eichhornia crassipes*. *Centella asiatica* was able to removed 62% of copper at the concentration of 5 mg/L. As reported by [3], the reason why *Eichhornia crassipes* and *Centella asiatica* could perform extremely well at low concentration of copper and less effective at higher concentration of copper is due to that the loading effect where sorption sites were saturated by copper ions is at the highest concentration.

![Figure 2](image)

**FIGURE 2.** Percentage removal of copper by (a) *Centella asiatica* and (b) *Eichhornia crassipes*

Table 1 shows the comparison of removal efficiencies of *Centella asiatica* and *Eichhornia crassipes* with previous studies done by other researcher using the same aquatic macrophytes. It is found that *Centella asiatica* in this study was slightly lower than the study conducted by [3]. Meanwhile for *Eichhornia crassipes* displayed better removal capability than the study conducted by [3]. This study also exhibited similar capability in removing copper when compared to [4] and [5] using *Eichhornia crassipes* where removal efficiency was between 83% to 89%.

| Reference | *Centella asiatica* | *Eichhornia crassipes* |
|-----------|---------------------|------------------------|
| This study | 62%                 | 83%                    |
| [3]        | 83%                 | 62%                    |
| [4]        | -                   | 83%                    |
| [5]        | -                   | 89%                    |

The analysis of variance (ANOVA) test done for the reduction percentage differences of the copper removal between *Centella asiatica* and *Eichhornia crassipes*. Table 2 shows the statistical analysis for the performance of *Centella asiatica* and *Eichhornia crassipes* treatment system. The ANOVA analysis showed that no significant difference (P > 0.05) at lower concentration of copper solution (1 mg/L & 3 mg/L) by both *Centella asiatica* and *Eichhornia crassipes* where both aquatic macrophytes
have similar capability in removing copper. The removal efficiency at 5 mg/L copper concentration for both aquatic macrophytes was proved to be statistically significant (P<0.05) where Eichhornia crassipes was found to be more effective in removing copper at the concentration of 5 mg/L compared to Centella asiatica.

TABLE 2. ANOVA table showing the performance of aquatic macrophytes in term of average percentage reduction of copper

| Source of Variance | df | SS            | MS            | F       | P       |
|--------------------|----|---------------|---------------|---------|---------|
| 1 mg/L             |    |               |               |         |         |
| Between macrophytes| 1  | 646.43063     | 646.43063     | 0.6558717 | > 0.05  |
| Error              | 14 | 13798.474     | 985.60527     | -       | -       |
| Total SS           | 15 | 14444.904     |               |         |         |
| 3 mg/L             |    |               |               |         |         |
| Between macrophytes| 1  | 16.382256     | 16.382256     | 0.0170599 | > 0.05  |
| Error              | 14 | 13443.881     | 960.27719     | -       | -       |
| Total SS           | 15 | 13460.263     |               |         |         |
| 5 mg/L             |    |               |               |         |         |
| Between macrophytes| 1  | 3026.1551     | 3026.151      | 4.951   | < 0.05  |
| Error              | 14 | 8556.86       | 611.20428     | -       | -       |
| Total SS           | 15 | 11583.015     |               |         |         |

5. Conclusions
The results of the present study showed that Centella asiatica and Eichhornia crassipes were effective in removing copper in contaminated water. In addition, Centella asiatica and Eichhornia crassipes successfully removed 1 mg/L copper concentration within the permissible amount allowed (0.2 mg/L) for industrial effluent discharge standard for Malaysia. The highest percentages of copper removal were 83% for Eichhornia crassipes and complete removal for Centella asiatica were obtained at concentrations of copper solution of 1 mg/L. Overall, Centella asiatica exhibit faster removal rate of copper compared to Eichhornia crassipes. In terms of effectiveness, the percentage removal in solution was higher using Centella asiatica at lower concentration of copper. However, at higher concentration of copper (5 mg/L), Eichhornia crassipes was found to be more effective in removing copper compared to Centella asiatica. Hence, it can be proposed that phytoremediation can be applied using both of the aquatic macrophytes for the removal of copper in contaminated water.

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