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Hyperaccumulation of Copper by Two Species of Aquatic Plants

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Abstract— **Phytoremediation is a relatively new approach to treat wastewater contaminated by organic and inorganic substances including heavy metals. The effectiveness of two aquatic plants, *Centella asiatica* and *Eichhornia crassipes,* were evaluated for their capabilities in removing copper from copper solution. The aim of this study is to determine the potential of these aquatic plants to act as hyperaccumulators. The aquatic plants were put in 8 liters of solution containing of 1.5 mg/L, 2.5 mg/L and 5.5 mg/L of copper, for a period of 21 days. Analyses of heavy metals contents were conducted using an Atomic Adsorption Spectrometer. Results showed an increase of copper within the plants' roots and shoots tissues and a decrease of copper concentration in the solution. The maximum removal of copper in the solutions containing *Centella asiatica* was 99.6 as compared to 97.3 % in solutions containing *Eichhornia crassipes. Centella asiatica* accumulated a maximum amount of copper of 1353.0 mg/kg while *Eichhornia crassipes* accumulated 1147.5 mg/kg of copper in their roots. The accumulation of copper of more than 1000 mg/L in plants tissues indicate that both aquatic plants can be considered as hyperaccumulators of copper. Roots tend to accumulate a higher amount of copper than shoots due to translocation process. Significant removal of copper were obtained at p<0.01 for containers containing both aquatic plants, indicating that *Centella asiatica* and *Eichhornia crassipes* can be utilized in the phytoremediation method to remove copper from wastewater.**

Keywords - hyperaccumulation; copper; Centella asiatica; Eichhornia crassipes; phytoremediation

I. Introduction

The rapid pace of industrialization and urbanization activities has become a major environmental concern due to dispersal of wastes generated on the ecosystem. Heavy metals are one of the most hazardous contaminants that may be present in the aquatic ecosystem. These heavy metals are highly toxic to the aquatic biodiversity, and drinking contaminated water containing heavy metal poses severe health hazards in humans. Contaminations of copper, nickel and zinc are normally due to releases from a variety of sources including electroplating, mining, urban sewage, smelters, tanneries, textile industry and chemical industries [1]. According to Ministry of Health of Malaysia, the limit for drinking water quality is 0.01 mg/L for copper. The industrial effluent discharge standard for Malaysian inland water is 0.2 mg/L for copper, according to Environmental Quality Act, 1974 with latest amendment in 2000. Even though the industries may employ treatment systems to treat their effluents, sudden changes in their manufacturing processes or breakdown of wastewater equipment may cause wastewater containing heavy metals that exceed the discharge limits to be inadvertently released into the environment. Various methods have been employed to clean-up the effluents. These methods include ion exchange, chemical precipitation, disinfection, adsorption by activated carbons, reverse osmosis and nanofiltration. Most of these methods are expensive, require high energy and are not able to completely remove the heavy metals [2]. Contrary to this conventional method, phytoremediation, i.e. the use of trees of plants to remediate contaminated soil or water, is a relatively new approach which is considered more cost-effective and environment-friendly. The major benefits of using aquatic plant-based treatment system are that it requires much lesser energy, it is a completely natural system and very easy to regenerate [3].

Several plants species, such as water lettuce (*Pistia stratiotes*) [4], water lilies (*Nymphaea spontanea*) [3], parrot feather (*Myriophylhum aquaticum*), creeping primrose (*Ludwigina palustris*), and watermint (*Mentha aquatic*) [5] have been studied to determine their potential in accumulating heavy metals. Among the various plants species group, aquatic macrophytes attain greatest interest in the field of phytoremediation. Aquatic macrophytes have great potential to accumulate heavy metals inside their plants bodies. These plants can accumulate heavy metals up to 100,000 times greater than the amount in the associated water. Therefore, these macrophytes have been used for heavy metal removal from a variety of sources [4]. Aquatic macrophytes such as water hyacinth, is one of the most commonly used plants in constructed wetlands because of its fast growth rate and large uptake of nutrients and contaminants [1]. *Eichhornia crassipes* and *Centella asiatica* have also been shown to be effective in removing trace elements from water and accumulate them in their bodies [6].

In this study, two aquatic plants*, Eichhornia crassipes* and *Centella asiatica,* were used to determine their potential in removing Cu of various concentrations from copper solutions. *Eichhornia crassipes* and *Centella asiatica* are two of the most abundant aquatic plants in Malaysia and have been found to thrive in canals, rivers and man-made lakes. The effectiveness of the plants in removing copper from the solution as well as the potential of the plants to accumulate copper were determined based on copper content analyses in the solution, roots and shoots of the plants.

II. MATERIALS AND METHODS

A. Plant Material

Eichhornia crassipes and *Centella asiatica* were collected from Harapan Lake, at Universiti Sains Malaysia, Penang. These aquatic plants were put in a hydroponic system containing tap water, for a two-week acclimatization period, before being exposed to heavy metal contaminants.

B. Preparation of Cu contaminated solution

Synthetic solutions of copper at 1.5 mg/L, 2.5mg/L and 5.5 mg/L concentrations were prepared using the copper standard solution and water.

C. Experimental Set-Up

The aquatic plants, which were put in tap water for two weeks, were taken out; excess water was allowed to drain off and weighed. Approximately 250 g to 300 g of each aquatic plant were put into containers containing 8 L of copper solution at the desired concentrations. The plants were kept in the copper solution for 21 days. There were three replicates for each plant at each concentration (1.5 mg/L, 2.5 mg/L and, 5.5 mg/L). The control set-up consisted of plants put into containers containing water. The volume of water in each tank was kept constant and the change in volume due to evapotranspiration was compensated by the addition of deionised water.

D. Heavy metals analysis

For every 24 hours interval, 25.0 ml of water sample were collected for heavy metals analysis. The plants samples were collected at the start and at every five-day intervals of the experiment and separated into roots and shoots. The plants were washed with deionised water, allowed to drain off excess water and weighed. Subsequently, the plant was dried in the oven for 24 hours at 70.0 °C, for preparation to ascertain the accumulation of heavy metal of each sample. The dried plant was ground and digested by using Nitric-Perchloric Acid Digestion method as described by USEPA, 1995 (APHA,1995). An atomic adsorption spectrometer (AAS) was used for heavy metals analyses of water and plant samples.

E. Statistical Analysis

The data were presented as mean values of three replicates. Statistical analysis using the one-way ANOVA was used to assess significant differences among the various copper concentrations. The comparisons of mean using the least significant different tests were calculated for P-values. A value of $P<0.01$ was considered significant. Statistical analysis software used in this research was Minitab version 15.

III. RESULTS AND DISCUSSION

A. Copper removal from the solution

Fig. 1 and Fig. 2 show the mean final concentrations of copper solutions as a function of time. The figures show that as the exposure to contaminant increase, the concentrations of copper decrease. For containers containing *Eichhornia crassipes*, the concentrations of copper decrease from 5.5 mg/L to 2.1 mg/L, 2.5 mg/L to 0.11 mg/L and 1.5 mg/L to 0.04 mg/L as shown in Fig. 1. For containers containing *Centella asiatica,* the concentrations of copper decrease from 5.5 mg/L to 0.92 mg/L, 2.5 mg/L to 0.01 mg/L and 1.5 mg/L to 0.03 mg/L. These results indicate that treatment using *Centella asiatica* is able to meet the limit of industrial effluent discharge standard for Malaysian inland water which is 0.2 mg/L for copper for all three initial concentrations. Results obtained using both plants are better compared to published results using parrot feather, creeping primrose and water mint for treating copper contaminated water with initial concentration of 5.56 mg/L [5]. In the study, the copper concentration was reduced to 3.19, 3.06 and 3.48 mg/L for parrot feather, creeping primrose and water mint respectively, after 21 days.

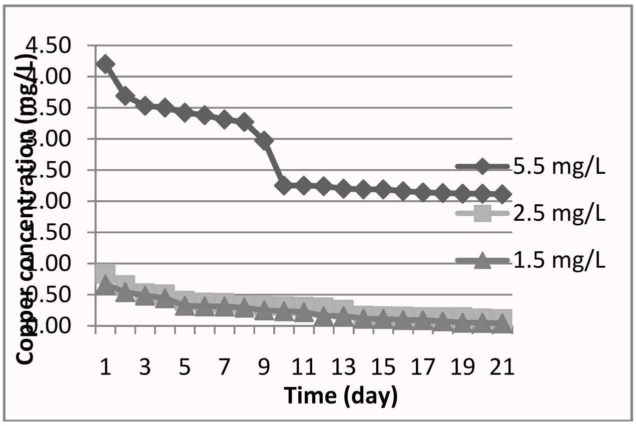

Figure 1. Copper concentration in solution containing *Eichhornia crassipes*

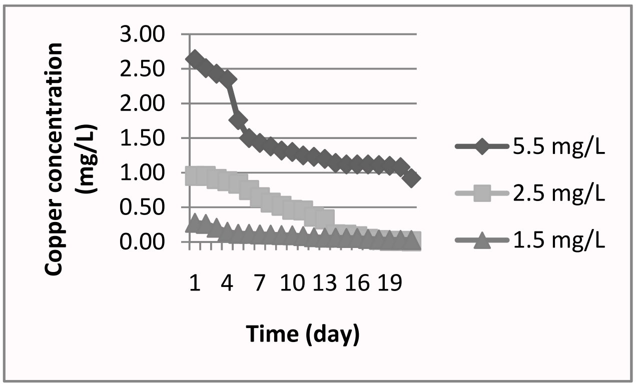

Figure 2. Copper concentration in solution containing *Centella asiatica*

Fig. 3 and Fig. 4 show the percentage removal of copper as a function of time for *Eichhornia crassipes* and *Centella* *asiatica*, respectively. Fig. 3 showed that *Eichhornia crassipes* removed 97.3% of copper at 1.5 mg/L, 95.6 % at 2.5mg/L and 61.6 % at 1.5 mg/L within 21 days of exposure to contaminant. As evident in the figure, the highest removal is obtained within 24 of exposure and the percentage removals for all concentrations are maximized at approximately 14 days. Figure 3 also shows that copper removals are the highest for concentrations of 1.5 mg/L and 2.5 mg/L. This result indicates that *Eichhornia crassipes* is capable to remove copper at lower concentrations. Other studies have also concluded that *Eichhornia crassipes* are more efficient in the phytoremediation of dilute concentration of heavy metals [7]. Fig. 4 shows that *Centella asiatica* gives a higher copper removal, 99.6 %, within 21 days of exposure at concentration of 2.5 mg/L. The results show that the least copper removals were at 61.4% for *Eichhornia crassipes* and at 83.3% for *Centella asiatica* for copper concentration of 5.5 mg/L. This is due to the loading effect where the sorption sites were saturated by copper ions at the highest concentration. A similar trend was reported by Mishra et. al [4], where the plant performed extremely well in removing the Cr and Zn from their solution and was capable of removing up to 95% of zinc and 84% of chromium during 11 days incubation period.

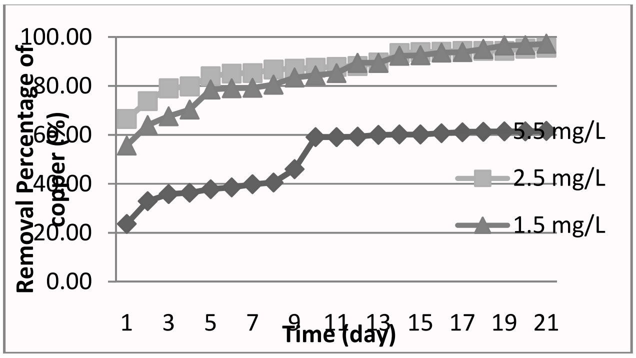

Figure 3. Percentage removal of copper by *Eichhornia crassipes*

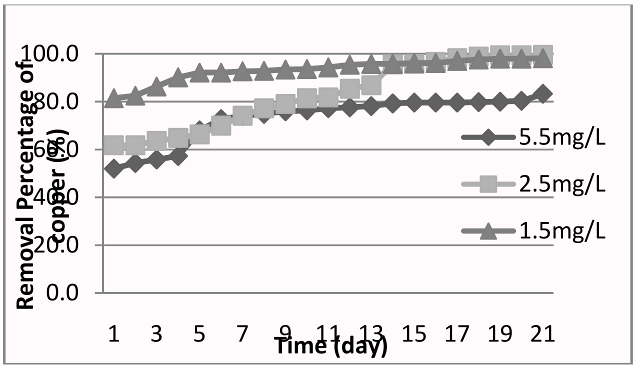

Figure 4. Removal percentage of copper by *Centella asiatica*

B. Copper accumulation in the plants tissues.

Hyperaccumulation of heavy metals is said to occur when the plants are able to accumulate more than 1000 mg/L of the heavy metal into the plants system, either by accumulating in the roots or shoots [8]. Table I shows that copper accumulation in *Centella asiatica* is higher than that in *Eichhornia crassipes.* The accumulations of copper in the roots of *Centella asiatica* (1105.5 to 1353.0 mg/kg) is higher compared to *Eichhornia crassipes* (997.5 to 1147.5 mg/kg). The greatest accumulation of copper in two aquatic plants was at 5.5 mg/L concentration of copper solution. This indicates that at low concentration, copper accumulated by specific sites while with increasing copper concentration the specific sites are saturated and the exchange sites are filled. *Centella asiatica* and *Eichhornia crassipes* can be considered as hyperaccumulator since the amount of copper that can be accumulated are more than 1000 mg/kg.

TABLE I. ACCUMULATION OF COPPER BY EICHHORNIA CRASSIPES AND CENTELLA ASIATICA

Exposure time (days)	Accumulation of Cu (mg/kg)					
	1.5mg/L		2.5mg/L		5.5mg/L	
Eichhornia crassipes	Shoots	Roots	Shoots	Roots	Shoots	Roots
5	19.5	87.5	121.5	477.5	115.5	997.5
10	21.5	90	124.5	532.5	119.5	1085
15	21	130	125.5	575	121	1130
20	25	165	127.5	597.5	127.5	1147.5
Centella asiatica						
5	19	85	110.5	490	109.5	1105
10	21.5	97.5	113.5	530	111.5	1168
15	23.5	135	125.5	575	120	1323
20	25	150	127	597	122.5	1353

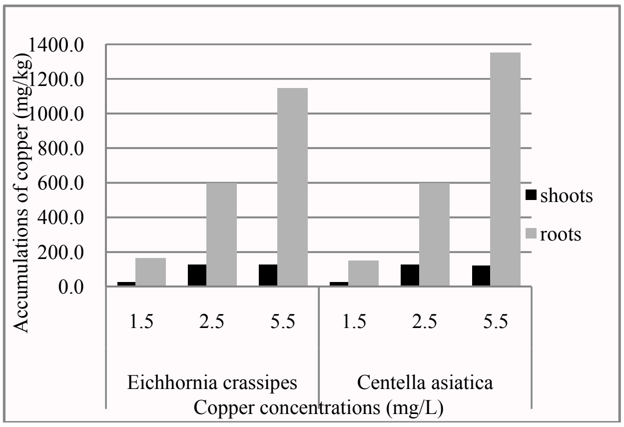

Figure 5. Total accumulation of copper in plants after 21 days of exposure to contaminant.

Fig. 5 shows that copper accumulation was mainly in the roots compared to the shoots. As copper is more localized in the aquatic plant roots, it indicates that rhizolfiltration may be the predominant mechanism for accumulation of copper [7]. Plants may accumulate higher concentration of metals in the roots since roots are usually at the base of the plant and removed from photosynthetic process for their own tolerance [5]. This result concurs with other researchers which obtained similar results [6]. Many researchers have concluded that accumulation of metals occur mainly in the roots of plants, due to the slow mobility of metal transport from root to shoot [9]. Chandra et al. [9] in 2004 have shown that the accumulation of most metals was higher in roots as compared to shoots. The study suggested that the least accumulation of metals in the shoots is due to the slow mobility of metal transport from root to shoot. The translocation and accumulation of metals in roots structure may be due to the process of rhizolfiltration [4]. The plants that were examined and reported to have better accumulation of metals in root portions include water hyacinth, water milfoil (*Myriophyllum aquaticum*) and water lettuce.

C. *Plant growth assessment*

In this study, the growth of the plants was assessed by monitoring the wet weight of the plants before and after exposure to contaminated wastewater.

TABLE II. WET WEIGHT OF PLANTS BEFORE AND AFTER EXPOSURE TO COPPER SOLUTION

	Wet Weight of plants (g)					
	1.5 mg/L		2.5 mg/L		5.5 mg/L	
	Initial	Final	Initial	Final	Initial	Final
Eichhornia crassipes	285.6	698.7	279.4	441.9	291.0	431.5
Centella asiatica	289.7	571.6	281.5	507.0	287.6	436.0

Table II showed an increase of wet weight for *Centella asiatica* and *Eichhornia crassipes* after exposure to contaminant. For *Eichhornia crassipes,* the wet weight increased from 291.0 g to 431.5 g while the wet weight of *Centella asiatica* increased from 287.6 g to 436.0 g at 5.5 mg/L of copper contaminant. This result indicated that concentration of copper contaminant did not significantly affect the growth of *Centella asiatica* and *Eichhornia crassipes.* In fact, both plants seemed healthy and produced new shoots. The growth of *Centella asiatica* was higher than the growth of *Eichhornia crassipes* based on their increased wet weights. This result suggests that *Centella asiatica* are able to grow better at longer exposure duration compared to *Eichhornia crassipes*. Both plants show potential to be used in the phytoremediation system which requires plants to be able to accumulate acceptable amount of metals and also survive in the contaminated condition.

IV. CONCLUSION

In this study, the effectiveness of two aquatic plants, *Centella asiatica* and *Eichhornia crassipes*, in removing copper from copper solutions were investigated. Results obtained indicated that both plants were very effective in removing copper. These plants were able to remove the heavy metal successfully without any physical signs of being affected by it. The highest percentages of removal of copper was 99.6 % for *Centella asiatica* and 97.3 % for *Eichhornia crassipes,* were obtained at concentrations of copper solution of 2.5 mg/L and 1.5 mg/L respectively *Centella asiatica*, a less studied plant in the field of phytoremediation, shows higher accumulation of copper in its roots compared to *Eichhornia crassipes*. The statistical analysis performed reveals a significant difference ($P < 0.01$) of copper removal for both aquatic plants. Overall results indicate that both, *Centella asiatica* and *Eichhornia crassipes,* can be used for phytoremediation of copper contamination in water.

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