The Nucleus **50**, No. 3 (2013) 267-272

The Nucleus
A Quarterly Scientific Journal of Pakistan Atomic Energy Commission
NCLEAM, ISSN 0029-5698

PHYTOREMEDIATION USING *EICHHORNIA CRASSIPES* AQUATIC PLANT

A. MAJID and *N. SIDDIQUE[1]

Pakistan Institute of Engineering and Applied Sciences (PIEAS), P.O. Nilore, Islamabad, Pakistan

[1]Chemistry Division, Directorate of Science, PINSTECH, P.O. Nilore, Islamabad, Pakistan

(Received July 29, 2013 and accepted in revised form September 11, 2013)

Phytoremediation is a cost effective approach for the treatment of polluted soil and contaminated water. The effectiveness of one aquatic plant *Eichhornia crassipes* was evaluated for its capability in removing copper from copper solution using atomic absorption spectroscopy (AAS). The aquatic plant was placed in solutions containing 2 mg/L, 4 mg/L and 6 mg/L of copper, for a period of 18 days and the change in Cu concentration was measured. Results showed an increase of copper within the plants' root and shoot tissues and a decrease of copper concentration in the solution. It was found that roots tend to accumulate a higher amount of copper than shoots due to translocation process. The maximum growth of *Eichhornia crassipes* was in the 2 mg/L solution which shows that growth was affected by the presence of Cu in the water. The maximum removal of copper in the solutions containing *Eichhornia crassipes* was 87.5% from the 4 mg/L solution. *Eichhornia crassipes* accumulated upto 1265.0 mg/kg of Cu in its roots indicating that this aquatic plant species may be used as a hyper-accumulator for copper.

Keywords: Phytoremediation, *Eichhornia crassipes*, Copper, Industrial effluents, Hyper-accumulator, Translocation

1. Introduction

Metals, radionuclides and other inorganic and organic contaminants are among the most prevalent forms of environmental contaminants, and their removal from water bodies, soils and sediments is a rather difficult task [1]. Sources of anthropogenic metal contamination include smelting of metals or electroplating processes, energy and fuel production, the application of fertilizers and municipal sludge to agricultural land as well as numerous industrial manufacturing processes [1-3]. Heavy metal contamination of the biosphere has increased sharply since the 1900s [4] and poses major environmental and human health problems worldwide [5]. Unlike many organic contaminants, most metals cannot be eliminated from the environment by chemical or biological transformation [6,7]. Although it may be possible to reduce the toxicity of certain metals by influencing their speciation, they do not degrade and are persistent in the environment [8].

Various conventional remediation technologies that are used to clean heavy metal polluted soils are; soil *in-situ* vitrification, soil incineration, excavation and landfill, soil washing, soil flushing etc. while for liquid effluents processes such as solidification and stabilization, heavy metal removal by ion exchange processes, chemical precipitation, disinfection, adsorption by activated carbons and various other species such as sand, reverse osmosis and nano-filtration etc. may be used. Most of these methods are expensive, require high energy and have specific benefits and limitations [9-12].

Contrary to conventional methods, phytoremediation is a cost effective and environment friendly method. An aquatic plant-based treatment system requires less energy, it is a completely natural system and very easy to regenerate [13]. Several plant species, such as water lettuce (*Pistia stratiotes*) [14], water lilies (*Nymphaea spontanea*) [13] , parrot feather (*Myriophylhum aquaticum*), creeping primrose (*Ludwigina palustris*) and water mint (*Mentha aquatic*) [15] have been studied to determine their potential in accumulating heavy metals.

Among the various plant species, aquatic macrophytes are of great interest in the field of phytoremediation. These plants can accumulate heavy metals upto 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 [14]. Aquatic macrophytes, such as water hyacinth (*Eichhornia crassipes)*, is one of the most commonly used plants in constructed wetlands because it has fast growth rate, large uptake of nutrients and contaminants [16]. *Eichhornia crassipes* has also been found to be effective in removing trace elements from water and accumulates them in the plant bodies [17]. The aim of this study was to determine the potential of *Eichhornia crassipes* plant for use as a hyper accumulator for Cu.

2. Experimental

2.1. Plant Material

Eichhornia crassipes or water hyacinth cannot tolerate very high or low water temperatures and will not grow in water with salinity >15 % of sea water [18]. Therefore in order to be effectively used for phytoremediation it is used in effluent treatment plants where the environmental conditions may be controlled. *Eichhornia crassipes* plants were collected from the Zoology Department of the University of the Punjab, Lahore, Pakistan. These aquatic plants were placed in a hydroponic system containing tap water for a two-week acclimatization period before being exposed to different concentrations of Cu solutions.

2.2. Preparation of Cu Contaminated Solutions

Synthetic solutions of copper at 2 mg/L, 4mg/L and 6 mg/L concentrations were prepared using copper standard solutions from Merck and de-ionised water.

2.3. Sample Collection and Analysis

The *Eichhornia crassipes* plants, after acclimatization in tap water for two weeks, were taken out; excess water was allowed to drain off and weighed. Three replicate tanks were prepared for each Cu concentration (2 mg/L, 4 mg/L and, 6 mg/L). Approximately 405 g to 410 g of *Eichhornia crassipes* plants were placed in tanks containing 8 L of copper solution at the desired concentrations. The plants were kept in the copper solution for 18 days. The control set-up consisted of 405 g to 410 g *Eichhornia crassipes* plants in a tank containing only water. The volume of water in each tank was kept constant and the change in volume due to evapo-transpiration was compensated for by the addition of de-ionised water. After every 24 hours interval, 25.0 ml of water samples were collected for Cu analysis.

In order to determine the uptake of Cu by *Eichhornia crassipes*, plant samples were collected at the commencement of the study and at six days intervals and separated into roots and shoots. The plants were washed with de-ionised water and after excess water was drained off, weighed. The variation in plant weight was used to monitor the growth of the plants during the study period. The plants were then dried in an oven for 24 hours at 70°C. The dried plants were ground and digested using Leads Public Analyst Method [19]. An atomic absorption spectrometer (AAS) was used for Cu analyses of water and plant samples using manufacturer specified parameters and conditions. A Perkin Elmer Aanalyst 800 AAS was used for analysis. Standard calibration method was used to obtain the results as described in our earlier publication [20].

3. Results and Discussion

3.1. Copper Removal from the Solution

Of the 6643 registered industries in Pakistan 1228 are considered highly polluting [21]. These include textile, pharmaceutical, chemicals (organic and inorganic), food industries, ceramics, steel, oil mills and leather tanning industries. According to the Pakistan National Environmental Quality Standards (NEQS) for Municipal and Liquid Industrial Effluents the amount of Cu released into inland water, sewage treatment plants and sea should not exceed 1.0 mg/L [22].

As mentioned earlier, the Cu concentration of water tanks containing 0, 2, 4 and 6 mg/L initial Cu solutions and *Eichhornia crassipes* plants was recorded daily. The results are shown in Figure 1 where the mean concentrations of copper solutions as a function of time are plotted. In all tanks containing *Eichhornia crassipes*, the concentrations of copper decreased from 6 mg/L to 3.1 mg/L, 4 mg/L to 0.5 mg/L and 2 mg/L to 0.32 mg/L after 18 days. These observations are in agreement with published results using parrot feather, creeping primrose and water mint in copper contaminated water with initial concentration of 5.56 mg/L [15] where the copper concentration decreased to 3.19, 3.06 and 3.48 mg/L respectively, after 21 days.

Figure 2 describes the percentage removal of copper as a function of time for *Eichhornia crassipes*. This figure shows that *Eichhornia crassipes* plants removed 84%, 87.5% and 48% of copper from the tanks containing 2 mg/L, 4 mg/L and 6 mg/L Cu solutions respectively within 18 days of exposure to contaminant. As evident from this plot, the highest removal is obtained within 24 hours of exposure and the percentage removal for all concentrations reaches their maximum values after approximately 11 days. Figure 2 also shows that copper removal is the highest for concentrations of 2 mg/L and 4 mg/L. This result indicates that *Eichhornia crassipes* plant is capable of Cu removal at lower concentrations and becomes less effective when Cu concentration is increased. Other studies have also concluded that

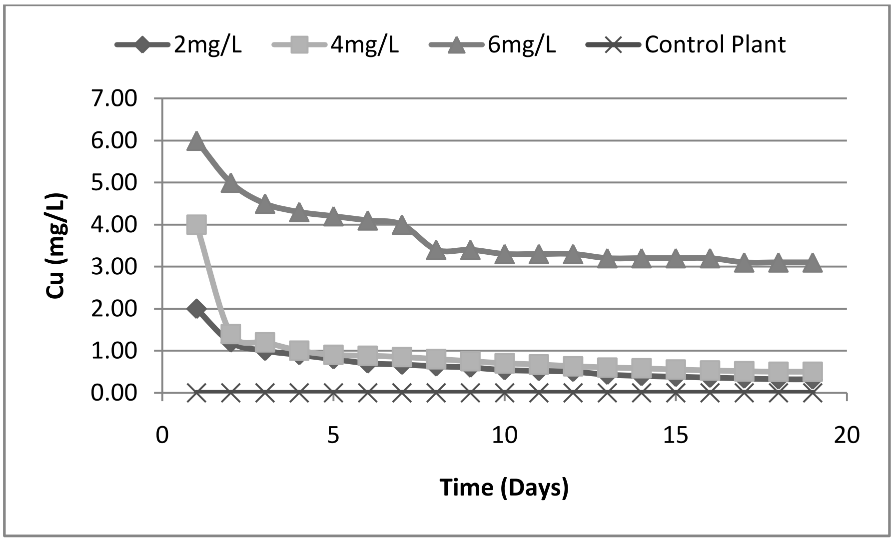

Figure 1. Removal of Cu using *Eichhornia crassipes* plant.

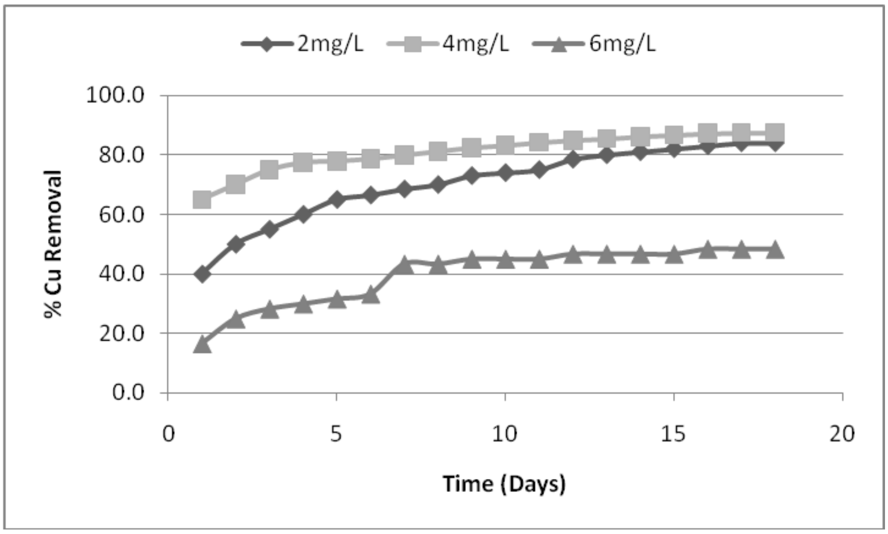

Figure 2. Percentage removal of Cu using *Eichhornia crassipes* plant.

Eichhornia crassipes plant is more efficient in the phytoremediation of dilute solutions of heavy metals [23]. Mishra et al. [14] reported that the plant performed extremely well in removing Cr and Zn from solution and was capable of removing up to 95% of Zn and 84% of Cr during 11 days period. Mokhtar et al. [24] found that *Centella asiatica* performed slightly better than *Eichhornia crassipes* in Cu removal from solution. The present results show that the lowest copper removal was obtained for copper concentration of 6 mg/L. This may be due to the saturation of sorption sites by copper ions at this high concentration.

3.2. Growth of Eichhornia crassipes in Cu Contaminated Solutions

In order for a plant to be effective for phytoremediation, its growth should not be adversely affected by the presence of the contaminant being removed. Therefore, the growth of *Eichhornia crassipes* plants in different Cu solutions was also studied over the study period to observe any adverse effects of Cu on the growth of the plant. The results obtained are given in Table 1 along with the results for the control sample. Growth was observed at all copper concentrations. However higher amounts of Cu did affect plant growth as can be seen from the trend of

Table 1. Growth of *Eichhornia crassipes* plant in different Cu concentration solutions.

Day	Control Plant Weight (g)	Plant Weight (in g) for Cu in mg/L		
		2	4	6
0	405.6	407.0	406.7	407.3
6	510.1	494.0	459.0	445.2
12	595.7	560.7	496.0	470.1
18	630.1	595.1	530.0	501.7

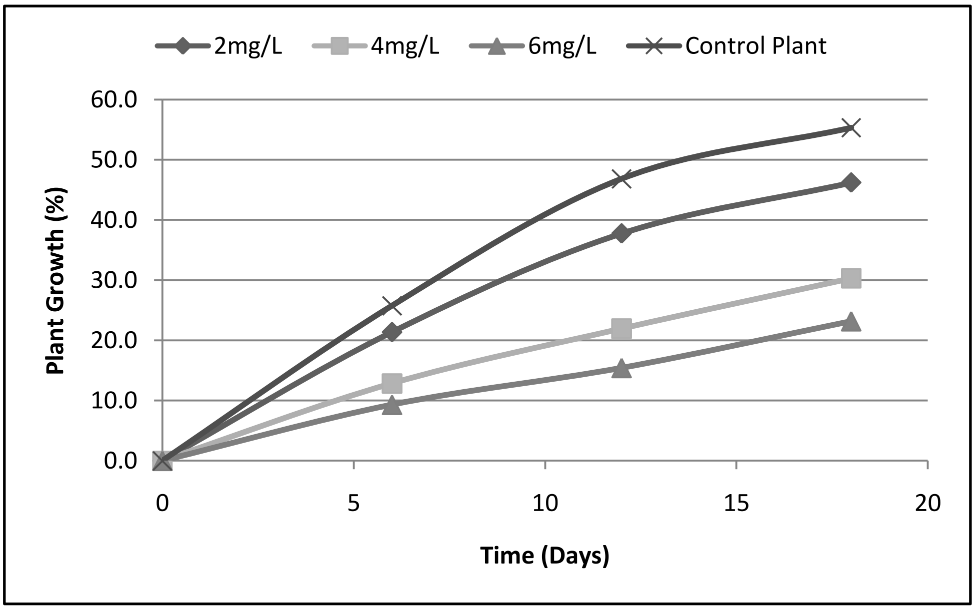

Figure 3. Percentage growth of *Eichhornia crassipes* plant in Cu contaminated water.

decreasing weight of plant with increase in Cu concentration. This trend is more apparent in Figure 3 in which the percentage growth has been plotted for plants grown in 0, 2, 4 and 6 mg/L of Cu solutions as a function of time. From this plot it can be seen that in the control sample the plants grew by 55.35% of their initial weight while in the 2, 4, and 6 mg/L of Cu solution samples the plants only grew by 46.22, 30.32 and 23.18% respectively. Once again these findings show that in order for *Eichhornia crassipes* plants to be effectively used for phytoremediation of Cu containing effluents the concentration of Cu should be low.

3.3. Copper Accumulation in Plant Tissues

Hyper-accumulation 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 plant system, either by accumulating in the roots or shoots [25]. Figure 4 shows copper accumulation in the roots and shoots of *Eichhornia crassipes* plants after the passage of 18 days. This figure shows that copper accumulation was mainly in the roots compared to the shoots. The highest accumulation of copper in *Eichhornia crassipes* plants was at 6 mg/L copper concentration. The measured copper concentrations in the roots and shoots of *Eichhornia crassipes* plants were found to be in the range of 1190.0 to 1265.0 mg/kg and 138.0 to 147.0 mg/kg respectively for the 6 mg/L samples. Similarly the Cu concentrations measured in the roots and shoots of *Eichhornia crassipes* plants were in the ranges 94.0 to 195.0 mg/kg and 24.5 to 28.0 mg/kg, respectively for the 2 mg/L samples. This indicates that at low concentration, copper is accumulated by specific sites while with increasing copper concentration these specific sites are saturated and the exchange sites are filled.

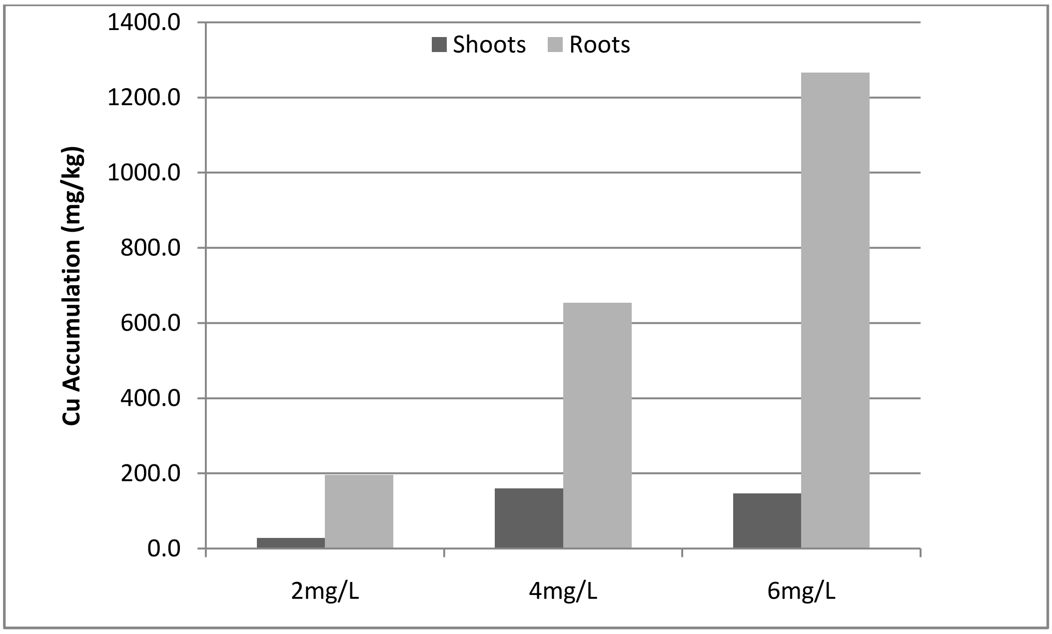

Figure 4. Cu accmulation in *Eichhornia crassipes* plant shoots and roots after 18 days.

Table 2. Translocation Ability using *Eichhornia crassipes* plant in various concentrations of Cu contaminated water. (Translocation Ability = $[Cu]_{roots}/[Cu]_{shoots}$).

Day	Cu in mg/L		
	2	4	6
6	3.84	3.80	8.62
12	5.99	4.08	8.83
18	6.96	4.09	8.61

The translocation ability as calculated and presented in Table 2 provides an estimate of how a contaminant is distributed in the plant roots and shoots. The values of this parameter are higher for 2 mg/L and 6 mg/L concentrations. Moreover this parameter increases in the initial part of the study and then evens out especially at higher Cu concentrations.

Eichhornia crassipes can be considered as a hyper-accumulator as the amount of copper that can be accumulated is more than 1000 mg/kg. As copper is more localized in the aquatic plant roots, it indicates that rhizol-filtration may be the predominant mechanism for accumulation of copper [14, 25]. Plants may accumulate higher concentration of metals in the roots as roots being at the base of the plant are removed from the photosynthetic process [15]. This is in agreement with previous findings [17] which conclude that accumulation of metals occur mainly in the roots of plants, due to the slow mobility of metal transport from root to shoot [26]. 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 [27].

4. Conclusions

The results obtained in this study show that *Eichhornia crassipes* plant may be used for phytoremediation of Cu containing effluents especially at low Cu concentrations without adverse effects on plant growth. However due to its invasive nature when planted outside its native range, i.e. the Amazon basin it may be more productive to use local plants such as Indian mustard which has proved effective in the removal of divalent cations of toxic metals. Moreover as *Eichhornia crassipes* plant is most effective at low metal concentrations it may be used once the toxic metal content of effluents has been reduced due to other treatment processes such as adsorption by activated carbon. Furthermore young plant seedlings grown in aerated water have been found to be more effective in removing heavy metals from water due to the dramatic increase in surface to volume ratio that occurs after germination and as germinating seedlings are capable of ab/adsorbing large quantities of toxic metal ions [9].

Acknowledgements

The authors would like to thank the Zoology Department of the University of the Punjab, Lahore, Pakistan for the provision of *Eichhornia crassipes* plants used in this study.

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