

## **Studies on removal of Zinc and Chromium from aqueous solutions using water Hyacinth**

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**Abstract:** Phytoremediation is an eco-friendly method for removal of pollutants, which can be relied upon as a sustainable technology, if implemented under optimum conditions of plant growth. The effectiveness of water hyacinth, a tropical weed, for the removal of Zinc (Zn) and Chromium (Cr) ions from aqueous solutions has been presented in this article. The potential of this plant in removing metals by phytoremediation was explored under various environmental factors such as pH, salinity, metal concentrations, available nutrients, and so on. The efficiency of metal removal was observed by varying the different parameters. It was found that the maximum removal of metals occurred at a neutral pH, low amount of salinity, lower metal ion concentrations, and lack of nutrients. The stress induced in a plant by metal absorption was visible from the health and growth pattern of the plants. The stress on water hyacinth due to metals was also assessed, by observing the changes in its chlorophyll and protein content.

**Keywords:** bioaccumulation, chlorophyll, heavy metal, phytoremediation, protein content, translocation ability, water hyacinth.

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### **INTRODUCTION**

Environment pollution due to heavy metals has been attracting attention worldwide over the past few decades due to its peculiar properties such as persistence, non-degradability, toxicity, and the like (Swarnalatha et al., 2013a; Swarnalatha et al., 2013b; Swarnalatha et al., 2014). Many processes have already been experimented by researchers for the removal of these toxic metals from wastewater, such as, chemical precipitation, reverse osmosis, electrolysis, and ion exchange (Jindal et al., 2005; Hasan et al., 2006; Miretzky et al., 2006). These methods involve one or more

disadvantages, such as, being expensive, difficult to implement, creating sludge disposal problems, power requirement, problems of maintenance, and so forth. Hence, researchers are always interested in devising an inexpensive and simple system for metal remediation from contaminated wastewaters. Also, traditional methods for heavy metal removal from wastewaters are usually inappropriate or very expensive, if applied to effluents containing low levels of contaminants (Gardea et al., 2005). Some aquatic plants have a high capability of accumulating heavy metals by different mechanisms, thereby purifying the contaminated soil and water (Badr et al., 2012; Miretzky et al., 2006; Sandra et al.,

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2008). This capability of plants can be made use of in removing toxic heavy metals and trace elements from the contaminated soil and water by a process referred to as phytoremediation.

Zinc (Zn) is a widely used metal in our day-to-day life, and hence, is usually seen in wastewater effluents. Zn is used in tires of vehicles, galvanizing processes, sewage, paints and so on. Zinc, if consumed in excess, is harmful to human beings, as it can affect the nervous and reproductive systems. Zinc is an essential micronutrient for plants, but can be toxic at higher concentrations. Chromium (Cr) is a highly toxic element and is potentially dangerous for man and environment. It can cause lung, kidney, and liver cancers, gastric damages, dermatitis, and the like. The various sources of Cr include effluents from tanneries, electroplating, mining, paper manufacture, batteries, paints, and so on. Chromium is toxic to humans, animals, and even plants.

Different mechanisms such as adsorption, precipitation, absorption, and the like are involved during the uptake of metals by aquatic plants (Marchand et al., 2011). The efficiency of metal removal by phytoremediation can be greatly enhanced by selection of the appropriate plant species. The selection may depend on the type of element to be remediated, its geographic location, the microclimate, hydrological conditions, soil properties, and accumulation capacity of the species (Badr et al., 2011; Shao et al., 2003). One such metal accumulating plant is the water hyacinth (*Eichhornia crassipes*), which is commonly found flourishing in the tropical and subtropical regions of the world. Water hyacinth is a fast growing, floating, aquatic plant, with a well-developed fibrous root system and a large biomass. Growth of the water hyacinth is mainly dependent on the ability of the plant to use solar energy, the nutrient composition of water, cultural methods, and environmental factors (US

EPA, 1988). Water hyacinth is a member of pickerweed family (Foluso et al., 2009). It is one of the most productive plants on earth and is considered to be the world's worst aquatic plant. It forms dense mats that interfere with navigation, recreation, irrigation, and so forth, in the aquatic bodies. These mats will also compete with the native species of aquatic plants and slowly smother them. They can also impede the water flow and create unhygienic conditions, by becoming good breeding grounds for mosquitoes. The fibrous root system enhances its spread and the degree to which the contaminants discharged into the water system reach its roots. The broad leaves of the plant can assist in tolerating high metal concentrations. The water hyacinth has been seen to possess the ability for sorption of heavy metals (Foluso et al., 2009), such as, Cadmium (Cd), Zn (Hasan et al., 2006; Shao et al., 2004), Mercury (Hg) (Kathleen et al., 2007), Arsenic (As) (Sandra et al., 2008), Nickel (Ni), Phosphorus (P) (Hadad et al., 2006), and so on. The efficiency of the plant in removing metallic pollutants from aqueous solution depends on various environmental parameters, such as, pH, Oxidation reduction potential (ORP), salinity, time, initial metal concentrations, nutrient availability, and the like, all of which have not been appropriately studied before. The objectives of the present study are, therefore, to evaluate the potential of *E. crassipes* in phytoremediation, for removing heavy metals such as Zn and Cr from synthetic wastewater, under varying conditions of pH, salinity, time, initial metal concentrations, and nutrient availability. The bioaccumulative properties of the water hyacinth under these varying conditions were also studied.

## **MATERIALS & METHODS**

Young plants of the water hyacinth, collected from a eutrophied lake, were transported in big plastic bags to the

laboratory. They were rinsed with tap water, followed by distilled water, to remove the dirt and insect larvae that had grown on them. These plants were then placed in water tanks under natural sunlight, to allow them to adapt to the new environment. Later plants of the same shape, size, height, and weight were selected for further studies. The plants were grown in containers of six-liter capacity. The control, which is a plant grown in deionized water, was also established. The plants were monitored for a study period of 21 days or until complete wilting occurred. Growth of the plants under the period of study was noted by the visual changes that appeared on the aerial parts of the plants. The total volume of the solution in each container was kept constant by adding deionized water, to compensate for water lost through transpiration and evaporation.

Stock solutions of Zn and Cr were prepared for the study. The metals used were of analytical reagent (AR) grade and were added as Zinc nitrate ( $Zn(NO_3)_2 \cdot 6H_2O$ ) and Potassium chromate ( $K_2Cr_2O_7 \cdot 5H_2O$ ), respectively. The reagents were dissolved in deionized water, to get the desired heavy metal concentrations of Zn and Cr. All of these metals were added as single metal solutions, that is, one experimental set contained a single metal in a particular concentration. The pH was maintained using 0.1 N sulfuric acid or 0.1 N NaOH solution. Salinity was maintained using NaCl powder of AR grade. The study was conducted in the absence (referred as zero-fold) as well as presence (half-fold and one-fold) of nutrients. A nutrient solution, as shown in Table 1, was used as the standard nutrient solution. From this solution, a half-fold solution was also prepared.

At the end of each experiment, the plants were washed well using tap water and distilled water. All the plants were then

divided into roots and aerial parts, dried in an oven at  $105^{\circ}C$  to a constant weight, ground using an agate mortar, sieved through a  $150\mu m$  sieve, and stored in properly labeled polythene bags, for further analysis. The samples were digested with a  $HNO_3-HClO_4$  mixture in a 2:1 ratio (v/v) and diluted to 100 ml with deionized water. The digested samples were analyzed for heavy metal concentrations by means of an atomic absorption spectrophotometer. One gram of each sub-sample was then digested in concentrated Nitric acid until a clear solution was obtained. After cooling the solution was filtered through Whatman filter paper No. 42 and made up to 50 ml with deionized water. Effluent water samples were taken at different intervals for analysis of residual heavy metal concentrations. The growth of plants was observed under varying conditions of pH, salinity, nutrients, and initial metal ion concentrations. Results of the metal accumulation in plants were reported as the concentration (mg) of heavy metals in plants (kg).

**Table 1.** Nutrient composition of the standard nutrient solution (one-fold)

Nutrients	Concentration in mg/l
Ammonium dihydrogen phosphate	28.8
Potassium nitrate	75.8
Magnesium sulphate	184.9
Calcium chloride	73.5
Sodium nitrate	85

The plants were grown in varying conditions of pH, salinity, initial metal concentration, nutrients, and so on. The growth and health of plants were recorded during the study period. The plants were thus observed to be, Healthy with green leaves (H), Unhealthy with brown leaves (UB), Unhealthy with almost dead leaves (UD), and Dead (D). The residual concentrations of Zn and Cr in the samples

were determined at intervals of three days. The percentage of the efficiency of metal removal was then calculated.

To compare the relative amounts of accumulated heavy metal between plants and metal ions, the bioconcentration factor (BCF) was selected as an indicator for the tendency of a heavy metal to accumulate in plants. A larger factor implied better phytoaccumulation capability. The translocation ability (TA) was calculated by dividing the concentration of a trace element accumulated in the root tissues by that accumulated in the shoot tissues. A larger ratio implied poorer TA.

The change in the protein and chlorophyll content of the plant leaf were also analyzed by observing the respective contents before and after the experiment. The protein content was estimated by the Bradford Method (Sadasivam and Manickam, 2009), which uses a different content- the protein capacity to bind a dye quantitatively. Chlorophyll is the essential

component for photosynthesis and occurs in chloroplasts, as a green pigment, in all photosynthetic plant tissues. They are bound loosely to proteins, but are readily extracted in organic solvents such as acetone and ether. Chlorophyll is extracted in 80% acetone and the absorption coefficients at 663 nm and 645 nm are read on a spectrophotometer. Using the absorption coefficients the total amount of chlorophyll is calculated (APHA, 2005).

## RESULTS & DISCUSSIONS

To study the efficiency of the removal of metallic pollutants under various environmental conditions of an aquatic system, phytoremediation studies on the water hyacinth were done under changing parameters of pH, nutrients available, and initial metal ion concentrations. The treated samples were taken at an interval of three days to test for residual metal concentrations.

The visual changes observed during the study period are shown in Table 2.

**Table 2.** Visual changes observed for water hyacinth

Stress	Days								
	0	3	6	9	12	15	18	21	
Zn only	H	H	H	H	H	UB	UB	UD	
Zn +nutrients(1 fold)	H	H	H	H	H	H	UB	UD	
Zn + nutrients(1/2 fold)	H	H	H	H	H	UB	UB	D	
Cr only	H	H	H	H	UB	UB	UB	UD	
Cr+ nutrients (1 fold)	H	H	H	H	H	UB	UB	UD	
Cr + nutrients (1/2 fold)	H	H	H	H	UB	UB	UB	UD	

The plants grown in Zinc ions were found to be healthier and more tolerant than the plants grown in Chromium ions. Zinc is a known micronutrient for plants that can be toxic at excess concentrations, whereas, Cr is toxic even at lower concentrations. It was also observed that the plants grown in the presence of nutrients remained greener and healthier longer when compared to the plants grown

in a starved condition (without nutrients).

Hydrolysis, complexation, precipitation, and redox reactions are pH-dependent phenomena that will influence the speciation and availability of heavy metals for biosorption (Gardea Torresdy et al., 2005). The effect of pH on the removal of Zn from synthetic wastewater was studied by varying the pH from 2 to 10, with an initial Zn concentration of 10 mg/l (Fig. 1).

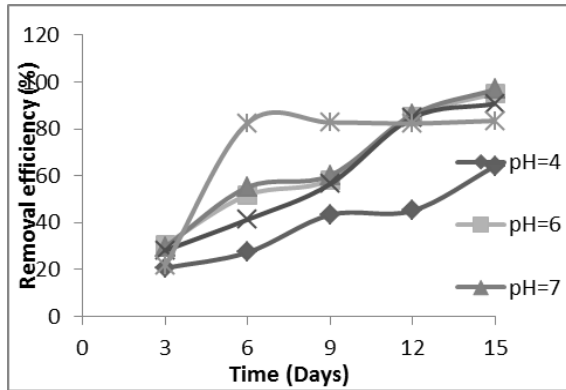


Fig. 1. Removal efficiency of Zinc for varying pH

All plants maintained at a lower pH died on the second day itself. At pH 4 the plants survived up to the twelfth day, but were unhealthy with yellow and brown leaves from the third day onward. At a higher pH also the plants survived, but were with ill health. The removal efficiency was found to be at a maximum at a pH of 7 (98%), at the end of 15 days. The removal efficiency was found to decrease with an increase and decrease in pH. At a higher pH, there could be a competition between the  $H^+$  ions and metal ions in the uptake sites of the roots and at a lower pH the decrease could be due to the binding of metal ions with organic acids, thus forming complexes that decreased the metal uptake by plants. A decrease in pH can also lead to an excess of hydronium ion formation, inhibiting the metal uptake. Similar results were observed in the case of Cr also. All plants were found to be in a good condition at a pH of 7. At an initial concentration of 3 mg/l, there was complete removal of Cr at the end of twelfth day (at pH 7) and the efficiency reduced to 60% (at pH 5) and 33% (at pH 9). The removal efficiency decreased with an increase (90% at pH 9) and decrease in pH (84% at pH 5) at the end of the fifteenth day.

Experiments were conducted by varying the initial concentrations of Zn and Cr in the solution. Thus, concentrations of 3, 6, 9, and 12 mg/l of Zn and Cr, respectively, were used for the study. The pH of the contents was maintained at 7. Partial

wilting of leaves was found in plants kept in containers of solutions 9 mg/l and 12 mg/l from the sixth day onward and plants were almost dead from the twelfth day onward. The plants grown in a concentration of 3 mg/l continued to be healthy throughout the experiment. It was found that Zn concentrations in all the containers decreased with time and were within permissible limits (CPCB, 2010) at the end of the experiment. The highest percentage of removal (99.3%) was found in the container with a Zn concentration of 3 mg/l (Fig. 2).

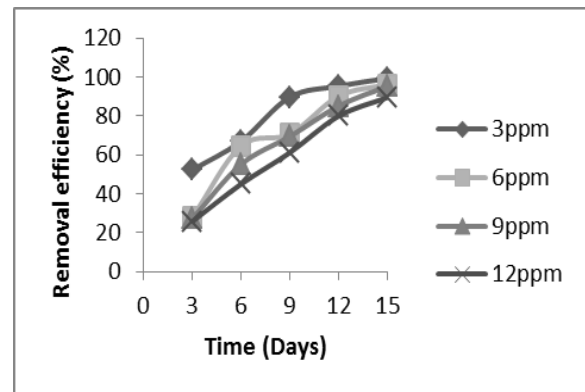


Fig. 2. Removal efficiency of Zinc under varying Initial concentrations

At lower metallic concentrations there was less competition between the ions at the uptake sites, while the opposite occurred at higher concentrations. Similar results were obtained for studies with Cr also. Complete removal of the metal ions was observed at lower concentrations. It was found that the metal ions were completely removed on the sixth day itself for a concentration of 1 mg/l of Cr and on the twelfth day for a concentration of 3 mg/l of Cr. Thus, the removal efficiency decreased with an increase in the metal ion concentrations in the solution.

Different salinity concentrations of 0, 0.25 ppt, 0.5 ppt, 1 ppt, 1.5 ppt, 2 ppt, and 3 ppt were tried for finding their effect on heavy metal removal by water hyacinth. The plants were seen to become unhealthy when the salinity concentrations were

greater than 2 ppt, leading to their death on the third day itself. At higher salinities, metals can form complexes with the chloride ion, which are difficult for the plants to take up. Higher salinity can dehydrate plants leading to their death. It was found that the plants grown at salinities of 0.25 ppt and 0.5 ppt remained healthier throughout the experiment compared to the plants grown in solutions lacking salinity. The removal efficiency increased with salinity increase up to 0.5 ppt and with a further increase in salinity, the removal efficiency decreased. It is also seen that plants grown in Zn solutions tolerated more salinity than plants grown in Cr solutions. For Zn, a maximum removal of 98% was observed at the end of 15 days, at a salinity of 0.5 ppt (Fig. 3), whereas, for Cr, the maximum removal efficiency was observed for a salinity of 0.25 ppt.

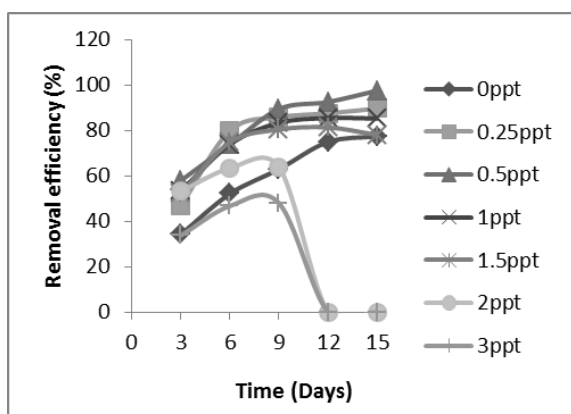


Fig. 3. Removal efficiency of Zinc under varying salinity

The pH was maintained at 7, with an initial concentration of 3 mg/l and salinity of 0.5 ppt for Zn and 0.25 for Cr, respectively, for studying the effect of heavy metal removal of Zn and Cr in the presence of nutrients. The nutrients were prepared in two concentrations one-fold and half-fold, as given in Table 1. The removal efficiency of metals Zn and Cr, using the water hyacinth, were studied under varying conditions of nutrient availability, with: i) metal alone, ii) metal

with one-fold nutrients, and iii) metal with half-fold nutrients.

When no nutrients were added, there was complete removal of Zn on the fifteenth day and Cr on the twelfth day from the respective solutions under optimum conditions. A removal efficiency of Zn of 67% on the sixth day, 96% on the twelfth day, and 100% on the fifteenth day was achieved. Removal of Cr was found to be much faster with Cr, as 100% removal was observed on the twelfth day itself. There was a removal of 63% of Cr on the third day itself and it increased to 80% on the ninth day.

On addition of nutrients to metal solutions, the rate of removal and removal efficiency of all metals was found to be decreasing (Table 3).

Table 3. Efficiency of metal removal in the presence of nutrients

Days	% efficiency 0 fold nutrients		% efficiency ½ fold nutrients		% efficiency 1 fold nutrients	
	Zn	Cr	Zn	Cr	Zn	Cr
3	37	30	49	59	24	26
6	67	45	57	63	48	47
9	77	69	62	70	52	54
12	85	78	68	79	66	58
15	92	81	86	81	69	63
18	95	87	89	84	74	79
21	97	90	91	87	86	82

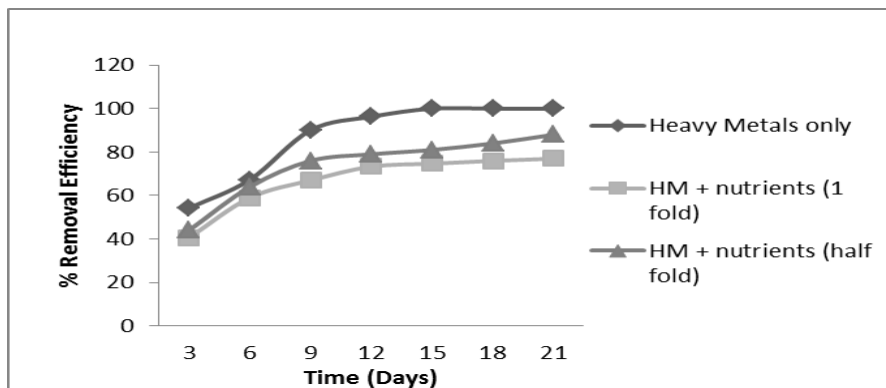
With half-fold of nutrients, it was observed that the removal of Zn by plants was at a slow pace. Hence, complete removal of Zn was not obtained during the study period of 21 days and there was a maximum removal of 88% only on the twenty-first day, under the optimum conditions. There was a removal of 64% of Zn on the sixth day and it increased only to 79% on the twelfth day, 81% on the fifteenth day, 84% on the eighteenth day, and 88% on the twenty-first day. Thus the percentage removal efficiency was found to decrease when nutrients were added. Similar results

were observed for plants grown in Cr also. A maximum removal of 86% was only got on the twenty-first day under optimum conditions. There was a removal of 42% of Cr on the third day and it hardly increased to 71% on the twelfth day, 79% on the fifteenth day, 81% on the eighteenth day, and 86% on the twenty-first day. A significant increase in percentage removal was not found after the fifteenth day.

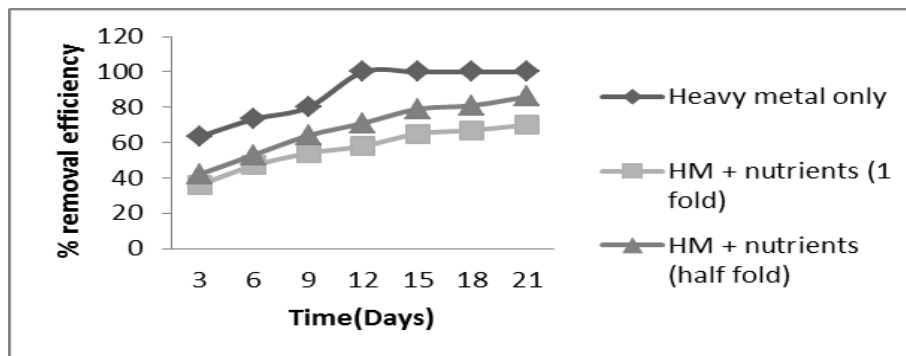
When one-fold nutrients were added, complete removal of Zn was not obtained during the study period of 21 days and there was a maximum removal of 77% only on the twenty-first day, under the optimum conditions. There was a removal of 59% of Zn on the sixth day and it slowly increased to 73% on the twelfth day, 74% on the fifteenth day, 76% on the eighteenth day, and 77% on the twenty-first day. It was found that the percentage removal efficiency was decreasing as the nutrient concentration increased (Fig. 4).

The same trend was observed for removal of Cr also. A maximum removal of 70% of Cr was only obtained at the end of the twenty-first day. There was a removal of 36% of Cr on the third day and it slowly increased to 58% on the twelfth day, 65% on the fifteenth day, 67% on the eighteenth day, and 70% on the twenty-first day (Fig. 5).

This could be due to the fact that with no nutrients available, the plants were using metals as nutrients for their metabolism, and hence, the removal of metals was at a faster rate. On addition of nutrients, the plants preferred nutrients to metals for their metabolism, and hence, the uptake of metals decreased. Thus, with the addition of more nutrients in the solution (one-fold) lesser removal of metals was observed than from a solution with less nutrients (half-fold).

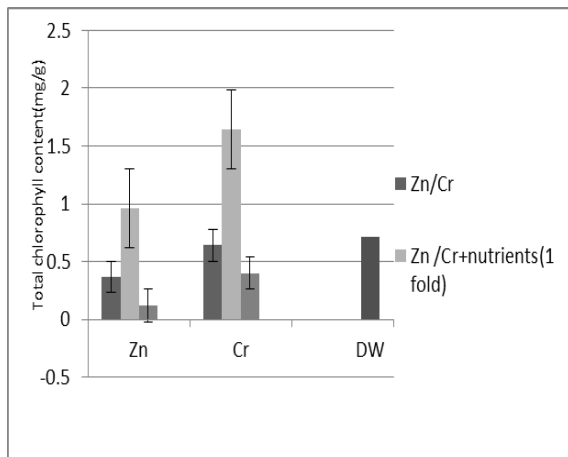


**Fig. 4.** Removal efficiency of Zinc under varying nutrient conditions

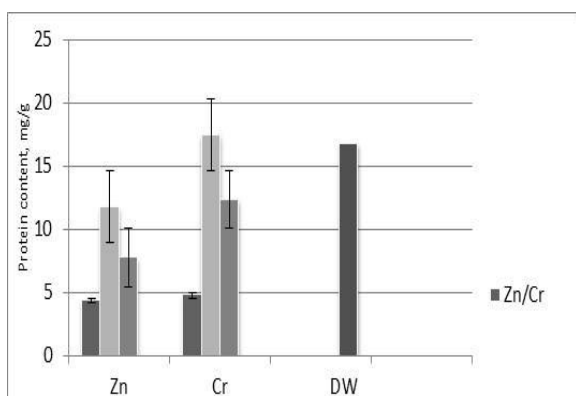


**Fig. 5.** Removal efficiency of Chromium under varying nutrient conditions

It can be seen from Fig. 6 that plants grown in metal solutions had lesser values of both chlorophyll and protein content when compared to plants grown in distilled water. This showed that heavy metals were inducing stress on the plants, reducing the chlorophyll and protein contents. The decrease in these contents could also be considered as a bio-indicator of pollution. Thus, plants grown in a Cr-containing solution was having a protein content of 4.8 mg/g and a chlorophyll content of 0.40 mg/g, whereas, the control values were 16.8 mg/g and 0.71 mg/g, respectively. On addition of the nutrients it was seen that the protein and chlorophyll contents increased (Fig. 7).



**Fig. 6.** Change in Total chlorophyll content (mg/g) under heavy metal stress



**Fig. 7.** Change in Protein content under heavy metal stress

Thus, protein and chlorophyll contents were higher when nutrients in one-fold were used with the metals. Thus, plants in Cr solution had a chlorophyll and protein content of 1.64 mg/g and 17.5 mg/g with one-fold nutrients and 0.64 mg/g and 12.4 mg/g with half-fold nutrients, respectively. Plants grown in metal solutions with nutrients had a low uptake of heavy metals. Similar results were observed in plants grown in Zn solutions also.

**Table 4.** Comparison of BCF, TA

Heavy metal	Bioconcentration factor(BCF)		Translocation Ability(TA)	
	Heavy metal only	With nutrients	Heavy metal only	With nutrients
Zn	154	199	0.241	1.54
Cr	14	23	0.67	2.72

The BCF was found to be higher for Zn, whereas, the TA values were higher for Cr (Table 4). This showed that Cr had a lesser phytoaccumulation capability than Zn.

Thus, it could be seen that the metals were more concentrated in the roots than in the aerial parts. This result of accumulation of metallic pollutants in the roots of plants was found to be consistent with the studies reported by Aisien et al. (2010), Badr et al. (2012), Dhote and Dixit (2009), Lu et al. (2004), Mahamadi (2011), Maine et al. (2006), Soltan and Rasheed (2003), and so on. The ability to translocate the metals to the upper parts depended on the TR values. Higher TR values showed poor translocation ability. The higher the translocation ratio, the poorer was the translocation ability. It was easy for the plant species with TR values greater than 1 to translocate metals from the roots to the shoots than those that restricted metals in their roots (Badr et al., 2012). In the study, Zn showed the maximum translocation ability.



## CONCLUSIONS

The effectiveness of water hyacinth in removing appreciable quantities of heavy metals such as Zn, and Cr from wastewater was seen in these studies. The removal efficiency of a 100% could be obtained by using water hyacinth and providing the optimum conditions of growth, such as, pH, salinity, and metal ion concentrations. It was found that the metal removal efficiency by plants decreased on addition of nutrients and the highest efficiency was observed in the absence of nutrients. However, the growth of the plant in terms of chlorophyll content, protein content, BCF, and TA were favored by the application of nutrients. Thus, water hyacinth, which was considered as a nuisance in the water bodies, could be effectively used for heavy metal removal, thereby, cleaning our precious resources in an economical and sustainable manner.

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