PHYTOREMEDIATION OF HEAVY METAL

Sufia Irfan, Aishah AlAtawi

Biology Department, College of Science, Faculty of Science, University of Tabuk,
Tabuk 71491, Saudi Arabia

Abstract

Group of higher plants have an important role in the mobilization and removal of heavy metal toxicity from the aquatic ecosystem. Metal bioaccumulation dynamics is also affected by metal studied and environmental condition. Lemna is an ubiquitous aquatic macrophyte with potential for heavy metal removal. Though, biomass disposal and seasonal growth of aquatic macrophytes are some problems faced by the phytoremediation technology in its application from laboratory to the field. However, the disposed biomass of macrophytes can be used for several remarkable applications. Lemna minor, an experimental organism was grown in various concentration of Cr (VI) solution for 30 days. Cr (VI) toxicity in the plant showed decrease in phytomass along with inhibition in chlorophyll, protein, and carbohydrate and nitrate uptake. Metal residual in the experimental basins were also measured to evaluate the bioaccumulation ability of the experimental plant. Lemna minor was found extremely suitable for Cr removal in the laboratory studies.

Key words: *phytoremediation, heavy metal, toxicity*

1. INTRODUCTION

Heavy metals are toxic and ubiquitous in the environment, and their entry into the aquatic system is the outcome of natural and anthropogenic sources. These inflows are detrimental both to aquatic biota and humans suggesting an increasingly important role for this form of pollution. Such an adverse impact on the society is due to combined effects of population increase, affluence, and technology. Their presence in the environment is natural and several of them are an essential component of global ecosystem with toxicity depending upon their oxidation states and coordination numbers. Restoration of diminishing water resources by removing metals input through bioremediation is accepted technology of the century. Natural and artificially constructed wetlands provide passive technologies (Tam and Wong, 1994) for remediation of metals. Availability of the metal in soil–plant system can easily enter into food chain and also cause risk for humans, animals, plants and whole environment of our modern society (Farouk et al., 2011). Availability of the metal in soil and plant system allows them to enter into the ecological pyramid posing a risk for biota, humans, and the whole environment of contemporary developed community (Farouk et al., 2011). Water and soil quality is significantly affected by the toxicity of heavy metal and is a serious environmental issue, and most conventional remediation approaches do not provide acceptable solutions. Living organisms require varying amounts of few heavy metals. All metals produce toxicity at higher concentrations (Singh et al.,2011), some of the metals like Fe, Co, Cu, Mn, Mb and Zn are required by humans in trace quantities. Phytoremediation is a promising technology using plants and microbes to clean up contaminated air, soil, and water (Behera, 2014). Wetland Plants are being used successfully for the phytoremediation of trace elements in natural and constructed wetlands. The United States Environmental Protection Agency (UEPA) lists heavy metals as priority pollutants. Over, 70,000 chemicals are in application in the world in different industries and technologies (Cairns et al., 1988). Heavy metal pollution is continually deteriorating the ecosystem due to anthropogenic activities; therefore encouraging deep interests in natural and simulated model research investigating the mechanism used by plants to oppose their detrimental effects (Rascio and Navari-Izzo, 2011). Biomagnification of toxic elements to human food chain is a real danger threatening the humankind. To reduce the concentration of metal from the polluted system using biological organisms are cheaper, efficient and a more beneficial for the environment (Lone et al. 2008; Jing et al. 2007). Phytoremediation technique, extracts the metals from the polluted water and soil (Lombi et al. 2001; Bennett et al. 2003). Heavy metals pollution continuously contaminates aquatic ecosystems and causing severe damage to freshwater quality and

habitat. Heavy metals do not have chemical or biological processes to degrade like organic compounds. Therefore, their availability in the environment for a long time causing a detrimental health hazard on living organisms. Chromium metal has application in dyes and pigments, fabric making, electroplating and wood processing industry, from where it is released into the environment as a significant water and soil contaminant. Chromium has two stable oxidation states, trivalent and hexavalent. Chromium (III) is biologically inactive in the metallic state and absorbed by the organisms in very low quantity but Cr (VI) is more active and toxic and highly soluble in water (Chandra and Kulshreshtha, 2004) and it is very harmful to human health as it is carcinogenic. Chronic chromium toxicity causes cancer in the respiratory tract and lungs (Zayed and Terry, 2003).

2. MATERIAL AND METHODS

Fresh stocks of *Lemna minor* was collected from local pond, fronds were washed carefully with tap water to remove any attached epiphytes and larval growth on the plants. Plants were acclimatized in the original culture tank (7.29 m^2) for a week, and maintained as stock culture. Experiments were performed in normal laboratory conditions. To study the effect of chromium *Lemna minor* plants of approximately same weight were taken, and allowed to grow in the 10 liter water of experimental tray of surface area 0.24 m^2. Treated plants were harvested after interval of 1st, 2nd, 5th, 10th, 20th and 25th days. Various concentrations of chromium (10, 20, 35, 50 µ m) were prepared by adding required aliquots of 1000 µm stock solution using $K_2Cr_2O_7$ to 5 % Hoagland solution Hoagland and Arnon (1950). Plants were thoroughly washed with deionized water and oven dried at 80 ºC for 48 hours. The dried sample was grinded with electric grinder. 5 g of powdered sample was digested with concentrated solution of $HNO_3/HClO_4$ (3:1, v/v) at 145 ºC for 2 hour. Total chromium accumulation in the plants ash was determined by using Perkin Elmer AAS 700 (Jones, 2001). Chlorophyll was calculated by the method of Mackinney (1941). Protein content of the plant tissue was estimated by the method adopted by Lowry et al. (1951). The uptake of NO_3-N from the medium was estimated calorimetrically by Brucine Sulphuric acid method (Nicholas and Nason, 1957) by measuring the depletion of nitrate from the external medium. Carbohydrate content of the plant was measured using the method of Dubois et al., (1956). Statistical tests have been performed using Statistical package for social sciences (SPSS v 22.0, IBM). Line weaver burk plot for uptake versus loads concentration in the external medium Maximum uptake rate (Vmax) and Km was calculated using MS Office excel sheet (v. 2010) with additional statistical package.

3. RESULT

The storage of chromium in the test plant was found to increase with increasing concentrations and time interval. Fig. 1 shows the rate of uptake in *Lemna minor* at different concentrations and intervals of time. At 10 µM Cr supply uptake was 1.53 mg/g for the day one, which reached up to 15 mg/g at the end of 25th days. At Cr supply of 20 µM uptake ranged from 2.09 to 19.42 mg/g. The rate of uptake for day one was 2.09 mg/g, thereafter it showed rapid rate of uptake. The amount of uptake measured was 17.42 mg/g at 25th day of the experiment. The last two concentrations (35, 50 µM) of Cr showed only difference of about 1.34 mg/g accumulation in tissues of *Lemna* between 20th and 25th experimental exposure.

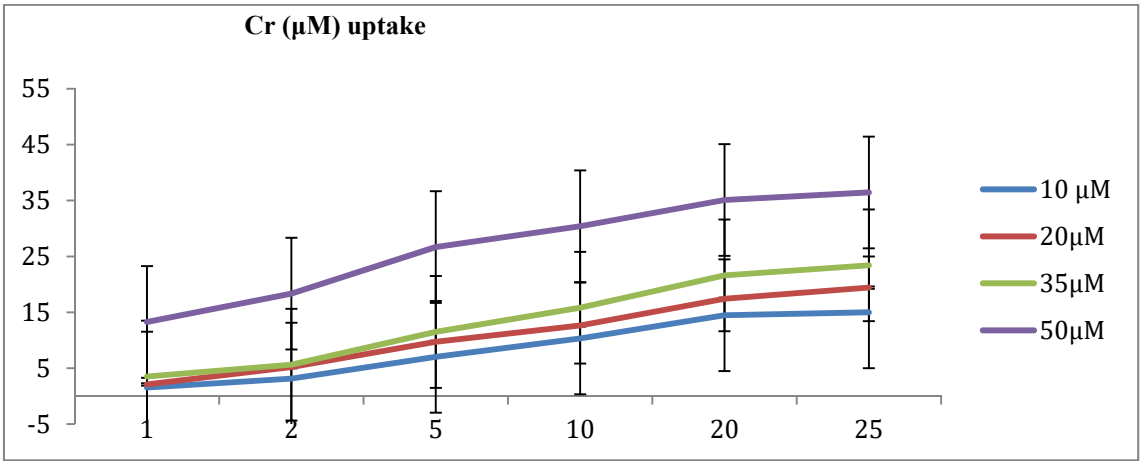

Fig. 1. Uptake of different Cr (VI) concentrations in *Lemna minor*

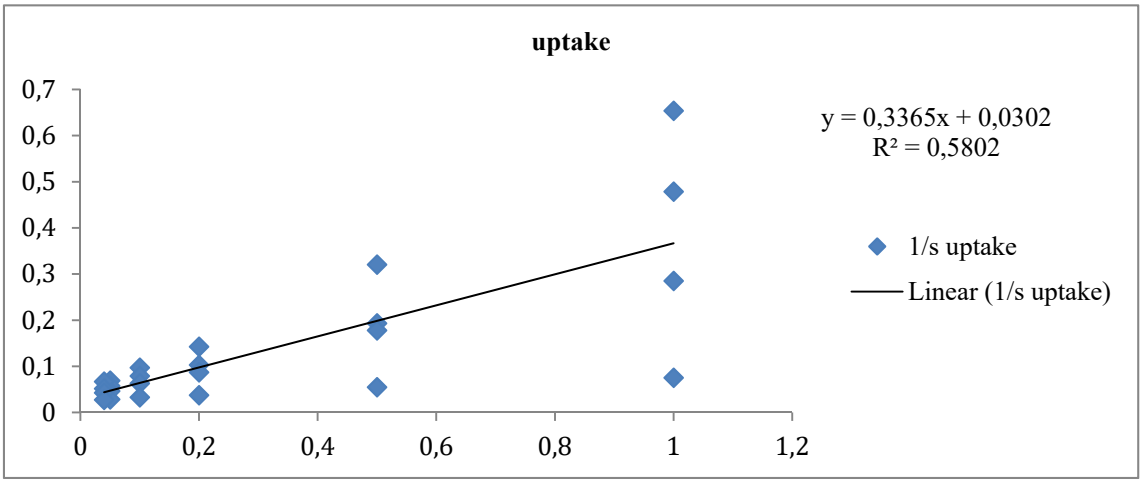

Fig. 2. Kinetics of uptake of different Cr (VI) concentrations in *Lemna minor*

In Fig.2, R2 value indicates that almost 58% of the variation in 1/Vo (Y) is due to variation in 1/S (X).

Y intercept= 0.0302 is equal to 1/Vmax, where, Vmax= 33.11

The slope regression line 0.3365 is equal to Km/Vmax where, Km= 11.4

The uptake of different Cr (VI) concentrations by *Lemna minor* followed Michaelis- Menten kinetics, there was an increase in the metal with increase in metals in the external solution. Km/Vmax was equal at slope regression of 0.3365.

Effect of Heavy metal toxicity on Lemna minor biomass, chlorophyll, protein, carbohydrate and nitrate uptake

Toxicity of metal depends on the concentration and exposure period. Effect of Cr treatment was clearly observed in fresh weight biomass measurement. At 10 µM and 20 µM of Cr concentration, there was a biomass increase. The last twenty-fifth days of experiment showed biomass increase of (203 ± 17.0 mg/g F.W) and (194 ± 16.1 mg/g F.W) at 10 and 20 µM of Cr concentration. The concentration of 50 µM showed the significant suppression in biomass. At 50 µM Cr, fresh weight of (184 ± 12.7 mg/g) after five days decreased by 6 mg/g F.W and after ten days and finally to (173 ± 15.0 mg/g F.W) at the end of the experiment (Table 1 shows the ANOVA value for biochemical parameters).

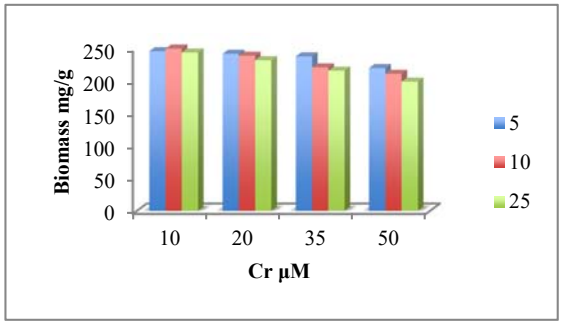

Fig. 3. Biomass (mg/g F.W) in the test plant tissue

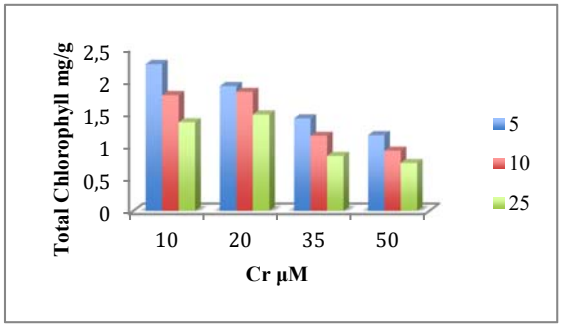

Fig. 4. Chlorophyll (mg/g F.W) in the test plant tissue

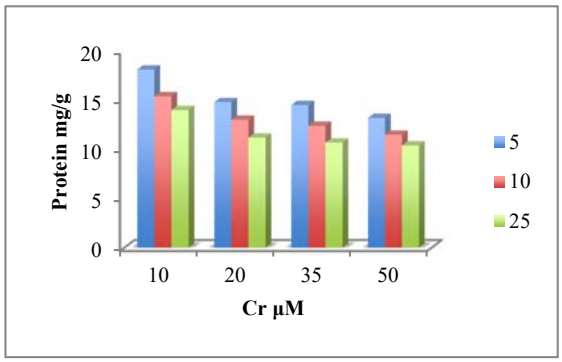

Fig. 5. Protein (mg/g F.W) in the test plant tissue

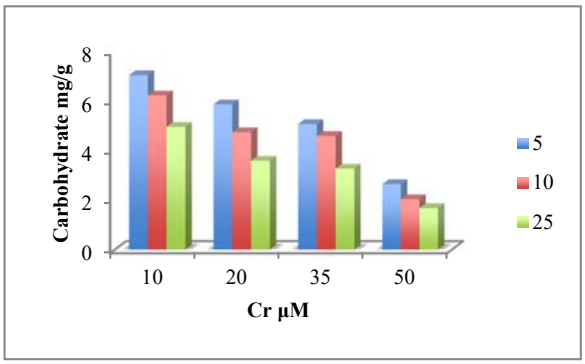

Fig. 6. Carbohydrate (mg/g F.W) in the test plant tissue

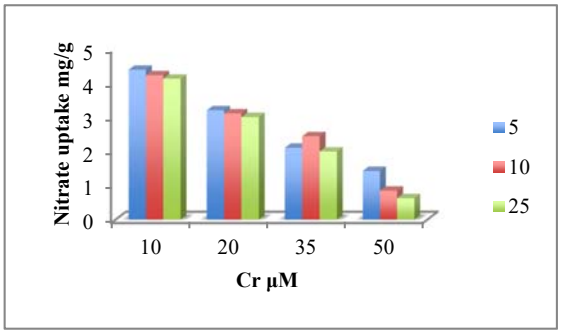

Fig. 7. Nitrate uptake (mg/g F.W) in the test plant tissue

There was a marked reduction in chlorophyll biosynthesis in *Lemna* (Fig. 4). Initial Cr supply (10 µ M) caused a reduction in chlorophyll pigment of the *Lemna* leaf tissues by (2.26 ± 0.09 mg/g F. W.) after five days treatment with metal. Thereafter, further reduction of about (1.36 ± 0.08 mg/g F. W.) was observed at the end of the experiment. The highest concentrations of Cr (35 and 50 µ M) resulted in reduction by 63 % and 54 % of chlorophyll respectively in comparison to initial Cr (10 µ M) concentration at the end of twenty-five days. The earlier intervals of five and ten days showed the uniformity in the reduction of biosynthesis of chlorophyll pigment (Fig. 4).

Increase in Cr concentrations caused a decrease in tissue protein content of *Lemna minor*. The first concentration (10 µm) of Cr affected the protein composition (18.1 ± 0.83 mg/g F.W) after first five days. Thereby reduction in protein content was found to be (15.4 ± 0.29 mg/g F.W) after ten days, which was finally measured to be (14.0 ± 0.20 mg/g F.W) at the end of the experiment. At 20 µm of Cr exposure level, there was a reduction by 82 % during exposure period of five days (Table 1, 2). Thereby, degradation of tissue protein reached to 11.2 0.28 mg/g F.W. Carbohydrate in the test plant tissue was more in the fifth day of metal exposure in all the Cr supply. At Cr supply of 10 µM,

carbohydrate content was similar for all the experimental exposure duration, while at 50 µM, value of sugar in the plant tissue extract was 1.68 mg/g F.W at 25th experimental day (Fig. 6). There was a total reduction of total sugar by 89.9 % after twenty-five days at 50 µ M Cr. The trend of NO_3- N uptake by the plant tissue was directly dependent on an increase in chromium concentration as shown in Fig. 7. The initial nitrate uptake was (7.04 ± 0.11 mg g-1 F. W) at 10 µ M Cr, which finally reduced to about 52.3 % at the end of the experiment. The two intermediate concentrations of Cr 20 and 35 µ M produced similar effects because there was not very significant difference in the uptake reduction. The high Cr (50 µ M) showed the total inhibition by 83.8 % from uptake in control sample (Table 1, 2).

4. DISCUSSION

The use of aquatic plants in phytoremediation depends on their ability to bear toxic treatments. The biotoxicity of Cr (IV) is the function of its capacity to cross biological membranes, its oxidizing capabilities (Losi et al., 1994) and its influence in photosynthesis and respiration (Larcher, 1995). Free floating plants such as *Lemna* can take up elements from the nutrient water (Sculthorpe, 1967). Metals are bound to a variety of biochemical sites in the tissues and maintain their concentration gradient in plant from the uptake of an external environment. The reason of storage of metals in plant tissues may be through passive absorption occurring down a concentration gradient (Simkiss and Taylor, 1989). Free floating *Lemna* plants can efficiently absorb and adsorb metal from contaminated water and aqueous wastes streams. Heavy metals have a negative impact on the growth of the plant but on the other hand they have different various mechanisms to protect against detoxification. Excessive heavy metal exposure toxicity has variations of interactions at the cellular /molecular level. It may be due to binding of metals to Sulphydryl groups associated with protein resulting in the hindrance of activity or structure deformity or from the displacement of an essential element causing a deficiency in the cell (Van Assche and Clijsters, 1990). Heavy metal exposures to the plant tissue also suppose to produce free radicals and reactive oxygen species causing oxidative stress in cell (Dietz et al., 1999). Plants have the ability to maintain various potential mechanisms at a cellular level that can play an important function in detoxification and tolerance to heavy metal exposure. Plant tissues have multiple strategies for the defense against heavy metal build up, starting from the plasma membrane level, either by reducing the uptake of heavy metals or my accelerating the efflux pumping of metals that have already established in the cytosol (Hall, 2002). Inside the cell protoplast, self-fixation procedure is available for the repair of stress-damaged proteins involving metallothioneins (heat shock proteins) and chelation of metals by organic acids, amino acids or peptide molecules or through their exclusion away from metabolic processes by transportation into the vacuole.

The accumulation of Cr in *Lemna* was found to be increasing with their respective concentrations. A linear increase in the chromium uptake in plant was noticed with increased metal supply. In *Salvinia*, a free-floating plant metal accumulation was rapid and involved passive uptake via absorption of metal ion on to plant surface (Sune et al., 2007). The storage pattern in aquatic plants also depends on their growing season. The Cr (VI) uptake at low concentration (10 µM) may be due to metabolism-dependent uptake by the plant tissues.

There was no evident effect on morphological growth and fresh weight biomass of *Lemna* colony at low Cr (10 and 20 µ M) concentrations (Fig-3). But at high Cr (35 and 50 µ M) reduction in biomass was observed clearly. Free-floating macrophytes have a greater capacity to store high concentration of metals in their body mass without showing an adverse effect on the growth (Matagi et al., 1998). Increased photosynthetic process is basis for the building up of organic compounds, which accounts for 80-90% of total dry matter of the plant (Bishnoi et al., 1993). Heavy metals like Cr, showed reduction in biomass production in *Bacopa monnieri* (Tokalioglu and Kartal, 2006). There was a direct relation between Cr uptake and chlorophyll reduction in the macrophyte. The maximum suppression of chlorophyll biosynthesis was observed at (50 µM) Cr concentration. This result was in agreement with findings of other reports on another free floating species *P.stratiotes* (Nichols et al., 2000; Bassi et al. 1990) and *Azolla* (Wilson and Al-Hamdani, 1997) and *L. minor* (Panda and Khan, 2002). The decline in chlorophyll content would be due to either reduction in Fe content or replacement of Mg^{2+} molecule in chlorophyll by heavy metals (Hu et al., 2007). Decrease in chlorophyll due to chromium

toxicity results in iron and zinc deficiency causing damage to chlorophyll biosynthesis (Barcelo et al. 1985) and also posed inhibitory role in sugar formation in *Lemna minor*. The loss in protein content may also be contributed to leakage and diffusion of soluble protein out of the plant or catabolic enzymes induction destroying the protein (Jana and Bhattacharjee, 1988).

The presence of Cr (VI) concentration has caused suppression in nitrate uptake, and nitrate is an essential component of chlorophyll molecule (Sela et al., 1989). The two forms of nitrogen used up by plants are NO_3-N and NH_4^+ with different utilization by plants (Solomonson and Barber, 1990). Accumulation of plenty of metal shows the adverse effect on the absorption and transport of essential nutrient, causing disturbance to the plant metabolism and thereby affecting growth and reproduction (Xu and Shi, 2000). The inhibition of NO_3-N uptake by *Lemna* tissues was clearly reflected by the increase in chromium concentration. There was gradual decrease in N uptake may be due to binding of metals with enzyme which finally resulted in damage to catalytic functions (Nichols et al. 2000). The decrease in nutrient translocation and uptake due to chromium toxicity was also observed in Citrullus spp. (Dube et al. 2003). Nitrate reduction, catalyzed by nitrate reductase is supposed as an entry port for the incorporation of inorganic nitrogen in plant tissues (Singh et al. 1998). Once the heavy metals enter the aquatic system multiple dynamic transformations may occur (Jhonston, 1993). Metals may be trapped or transferred from one compartment to another from plants to water and wetlands sediment. The use of plants as an indicator of metal contamination may be one of the options in wastewater treatment in budget-limited water project of the nations.

Table 1. ANOVA for biochemical parameters with respect to time (5, 10, 25)

Parameters	Source	Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll	Time Between Groups	.686	2	.343	1.784	.222
	Error Within Groups	1.730	9	.192		
	Total	2.416	11			
Protein	Time Between Groups	25.782	2	12.891	3.924	.060
	Error Within Groups	29.565	9	3.285		
	Total	55.347	11			
Nitrate	Time Between Groups	6.345	2	3.172	1.153	.358
	Error Within Groups	24.771	9	2.752		
	Total	31.115	11			
Carbohydrate	Time Between Groups	.241	2	.120	.061	.941
	Error Within Groups	17.793	9	1.977		
	Total	18.034	11			
Biomass	Time Between Groups	379.167	2	189.583	.692	.525
	Error Within Groups	2464.500	9	273.833		
	Total	2843.667	11			

Table 2. ANOVA for biochemical parameters with respect to conc (10, 20, 35 & 50)

Parameters		Sum of Squares	df	Mean Square	F	Sig.
Chlorophyll	Conc Between Groups	1.647	3	.549	5.708	.022
	Error Within Groups	.769	8	.096		
	Total	2.416	11			
Protein	Conc Between Groups	28.953	3	9.651	2.925	.100
	Error Within Groups	26.393	8	3.299		
	Total	55.347	11			
Nitrate	Conc Between Groups	24.166	3	8.055	9.274	.006
	Error Within Groups	6.949	8	.869		
	Total	31.115	11			
Carbohydrate	Conc Between Groups	17.528	3	5.843	92.349	.000
	Error Within Groups	.506	8	.063		
	Total	18.034	11			
Biomass	Conc Between Groups	2284.333	3	761.444	10.891	.003
	Error Within Groups	559.333	8	69.917		
	Total	2843.667	11			

5. CONCLUSION

This study was focused on the laboratory evaluation of heavy metal (Chromium VI) uptake by free-floating macrophyte, *Lemna minor*. The macrophyte was found to accumulate higher concentration of the target heavy metal. The present study also tested the biological parameters of *Lemna minor* exposed to the metal toxicity, exhibiting negative effect on the morphology and biochemical functions of the macrophytes in presence of higher loads of Chromium metal. However, plant was physiologically active even at higher toxicity loads, demonstrating the ability of *Lemna minor* in stripping heavy metal contaminations from the fresh water resources.

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