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Phytoremediation potential of *Eichhornia crassipes* for the treatment of cadmium in relation with biochemical and water parameters

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Abstract

The present investigation was aimed to assess the effect of cadmium chloride on some biochemical parameters and the potential of cadmium uptake of *Eichhornia crassipes*. The effects of increasing concentrations of cadmium chloride on growth characteristics of *E. crassipes* including root length, leaf area and biomass production was studied. The root growth of *E. crassipes* was severely affected at all concentrations of cadmium in the trays where plants were grown and in general it was serially increased. Similar trend was observed in average leaf area with 11.28 cm2 on 12th day in control while with only 7.05 cm2 at 75 ppm concentration. Chlorophyll contents were also observed to be decreased serially with the increasing concentrations of CdCl2. The lowest total chlorophylls were 118.56 mg/100g in comparison with 239.09 mg/100g in control set at the end of treatment. Polyphenol and proline contents were increased indicating the stress conditions due to toxicity of cadmium. Highest polyphenol and proline were 303.27 mg/100g and 8.14 mg/100g respectively at 50 ppm set of the treatment. Moreover, Electrical conductivity and total dissolved solids content of the various dilutions were decreased remarkably after 12 days of treatment. pH of the solution tend to become neutral while increase in turbidity might be related with root exudates and dead organics by the plants growing in the tray whereas decrease in hardness, acidity, sodium and potassium content was observed with increase in concentration. Values for BOD and COD were slightly increased at the end of treatment. Estimation of bioconcentration factor is very much important. It indicates that the species is more favourable to tolerate higher concentrations of heavy metals and also helps a lot in decontamination of the land, water etc. In this study increasing concentration of cadmium showed higher accumulation capacities and may be better treatment option for cadmium by means of phytoremediation

Key words: Phytoremediation, Bioconcentration factor, Biochemical parameters, Physico-chemical parameters, Heavy metals, Wastewater

Introduction

In nature land and water are precious natural resources for the sustainability of agriculture and the civilization of mankind. However, rapid industrialization and urbanization shows maximum exploitation and severe pollution in these resources. Land and water pollution by heavy metals is a worldwide issue (Gade, 2000). Worldwide all countries have been affected, though the area and severity of heavy metal pollution vary enormously so there has been an increasing concern with regard to accumulation of heavy metals in environment as they pose big threat to both human health and natural environment (McKeehan, 2000). In Central and Eastern European countries about 1.7 million sites were affected by heavy metals moreover in USA, there are 600 000 brown fields which are contaminated with heavy metals and need reclamation due to which 1,00,000 ha of cropland, 55,000 ha of pasture and 50,000 ha of forest have been lost (McKeehan, 2000; McGrath et al., 2001). In developing countries particularly in India, China, Pakistan and Bangladesh soil and water pollution is also severe where small industrial units are pouring their untreated effluents in the surface drains, which spread over near agricultural fields. In these countries, raw sewage is often used for producing

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vegetables near big cities (Ragnarsdottir and Hawkins, 2005). Cadmium salts are considered to be significant water pollutants not only because of their direct toxicity in water but also due to their ability to concentrate and incorporate into the food chain by aquatic plants and organisms with the natural process of bioaccumulation and biomagnification (Cearley et al., 1973). The annual worldwide release of cadmium is about 9,39,000 tones (Singh et al. 2003). Mobilization of heavy metals in environment due to industrial activities is of serious concern due to toxicity of these metals in human and other forms of life (Vieira and Volesky, 2000). Removal of toxic heavy metals from industrial wastewater is essential from the standpoint of environmental pollution control (Yuan et al., 2001).

In order to maintain good quality of soil and water there are continuous efforts have been made to develop technologies that are easy to use, sustainable and economically feasible. Physicochemical approaches have been widely used for remedying polluted soil and water. However, they experienced more difficulties for a large scale of remediation because of high initial capital cost, larger volume of chemicals, continuous maintenance, skilled technicians and even generate large amount of sludge which adds into the secondary waste generation, posing threats to aquatic life and minimizes the acceptability of the treatment technique (Rai, 2008).

The use of plant species for cleaning polluted soils and waters named as phytoremediation has gained increasing attention since last decade, as an emerging cheaper technology. Rhizofiltration is the removal of pollutants from the contaminated waters by accumulation into plant biomass. Literature survey suggests that several aquatic species have been identified and tested for the phytoremediation of heavy metals from the polluted water such as, sharp dock (*Polygonum amphibium* L.), duck weed (*Lemna minor* L.), water hyacinth (*Eichhornia crassipes*), water lettuce (*P. stratiotes*), water dropwort [*Oenathe javanica* (BL) DC], calamus (*Lepironia articulate*), pennywort (*Hydrocotyle umbellate* L.) (Prasad and Freitas, 2003). Recently the roots of Indian mustard and sunflower are found to be effective in the removal of Cd, Cr, Cu, Ni, Pb, Zn, and Pb, U, Cs-137, Sr-90 from hydroponic solutions, respectively (Zaranyika and Ndapwadza, 1995; Wang et al., 2002; Prasad and Freitas, 2003).

Aquatic macrophytes play an important role in structural and functional aspects of aquatic ecosystems by numerous ways. The ability of aquatic macrophytes to take up heavy metals make them acceptable research applicants especially for the treatment of effluents having medium concentration level pollutants and city sewage waters (Sood et al., 2012). Water hyacinth possesses a well-developed fibrous root system and large biomass and has been successfully used in wastewater treatment systems to improve water quality by reducing the levels of organic and inorganic nutrients. This plant can also reduce the concentrations of heavy metals in acid mine water while exhibiting few signs of toxicity. Dos Santos and Lenzi (2000) tested *Eichhornia crassipes* in the elimination of Pb from industrial effluents in a green house study and found it useful for Pb removal. Water hyacinth accumulates trace elements such as Ag, Pb, Cd, etc. and is efficient for phytoremediation of wastewater polluted with Cd, Cr, Cu and Se (Zhu et al., 1999). Tolerance to metals in plants can be achieved by purposeful accumulation into the tissues which favors uptake of metals or in such tissues which use them in non-functional parts as an indicator of tolerance or remain sturdy under adverse natural conditions like wind, rainfall, temperature fluctuations etc. showing greater ability of accumulation (Mane et al., 2010).

The present investigation demonstrates phytoremediation potential of *Eichhornia crassipes* exposed to increasing concentration of cadmium chloride. The effect and accumulation was studied with reference to selected biochemical parameters and physico-chemical parameters of the water. Growth characteristics, chlorophyll, carotenoid, polyphenol and proline were studied so as to know the physiological changes associated with experimental species while water quality analysis was carried out to judge the natural ability to treat cadmium containing water. This was specially assessed with reference to electrical conductivity (EC), pH, total dissolved solids (TDS), turbidity, free CO_2, chemical oxygen demand (COD), biological oxygen demand (BOD), acidity, hardness, sodium and potassium content. Finally aaccumulation of cadmium (Cd^{++}) in *E. crassipes* was estimated to find out the tolerance capacity and toxicity from liquid medium.

Materials and Methods

Materials used in the study

Eicchornia crassipes **(**Mart.) Solms plants were collected from Mula-Mutha river of Pune city, and acclimatized in laboratory condition. A stock solution of analytical grade cadmium chloride $CdCl_2$. $2H_2O$ (1000 mg/l) was prepared in tap water and was later diluted as required. The plants were subjected to various concentrations of cadmium chloride i.e. 25, 50 and 75 ppm along with a control set in four plastic tubs of ten-liter of capacity each. Uniform sized plants with similar shoot area and root length were selected for the study. They were washed thoroughly under a running tap water, propagated hydroponically for twelve days and subjected to heavy metal treatments. Whole experiment was run with the initial concentrations of cadmium chloride in hydroponics and all the parameters were estimated at 0 (two hours), 4, 8 and 12 days. The chemicals used in this study were of the highest purity available and of analytical grade.

Study of the growth characteristics:

Ten plants from each treatment tray were carefully removed and washed thoroughly with water to remove any dirt and dust particles on the surface of the plant parts and blotted to surface dry. This plant material was analyzed for their growth characteristics. Various growth parameters namely root length, average leaf area, fresh weight biomass, dry weight biomass and moisture content of the randomly sampled leaves were recorded.

Estimation of photosynthetic pigments

Chlorophyll content

The chlorophylls of the mature leaves were estimated by following the method of (Mane et al., 2011). Fresh plant material (1g) was roughly homogenized in mortar by keeping the temperature at 2^{o}C in dark condition and extraction was carried out using 90% acetone, with the addition of pinch of magnesium carbonate, to protect and stabilize the chlorophylls. This extract was filtered through Whatman No.1 filter paper under suction using Buchner's funnel. The residue was washed thoroughly 2-3 times with 90% acetone, collecting all the washings in the same filtrate and final volume of the filtrate was made to 100 ml with 90% acetone. Absorbance of chlorophyll 'a' and 'b' was recorded using double beam UV-Visible spectrophotometer (Elico SL-159, India), at 663 and 645 nm using 90% acetone as blank. Following formulae were used to determine the chlorophyll content.

Chlorophyll 'a' = X = 12.7 x A_{663} - 2.69 x A_{645}

Chlorophyll 'b' = Y= 22.9 x A_{645} - 4.68 x A_{663}

Total chlorophyll (a + b) = Z = 8.02 x A_{663} + 20.20 x A_{645}

$$\text{Chlorophyll a / b / total (mg/100g fresh leaves)} = \frac{\text{X/Y/ Z x volume of extract x 100}}{\text{1000x weight of plant material (g)}}$$

Carotenoid content

The carotenoid content of the leaves was determined from the same extract used for chlorophyll estimation, by recording the absorbance at 480 nm using spectrophotometer by putting 90% acetone as the control. The carotenoids content was calculated by using the following formula (Borkar et al., 2011).

$$\text{Carotenoids content} = \frac{\text{A480 x volume of extract x 10 x 100}}{\text{2500 x weight of plant material (g)}}$$

Polyphenol content

The polyphenol content of the leaves was estimated following the method suggested by (Vesely et al., 2012). 2 ml of acetone extract used for chlorophyll was mixed with 10 ml, 20% sodium carbonate and adjust the volume to 35 ml with distilled water to this mixture 2 ml of Folin-Denis reagent was added, mixed thoroughly and final volume was adjusted to 50 ml with distilled water. The standard tannic acid solution (0.1 mg ml^{-1}) was used for the preparation of standard polyphenol curve by measuring the absorbance at 660 nm using double beam UV-Visible spectrophotometer.

Zinc (Zn++) concentration:

Zn^{++} concentration was estimated from the dried plant material (shoot and root combined). 0.5 g oven dried plant material was acid digested as per the standard method suggested by (Rivelli et al., 2012). Plant material was taken in a 150 ml clean beaker and to that 10 ml concentrated nitric acid was added. It was covered with a watch glass and kept for an hour till the primary reactions subsided. It was then heated on hot plate until all the material was completely dissolved. It was allowed to cool to room temperature and then 10 ml of perchloric acid (60%) were added to it and mixed thoroughly. It was then heated strongly on the hot plate until the solution became colorless and reduced to about 2-3 ml. While heating, the solution was not allowed to dry. After cooling, it was transferred quantitatively to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day it was filtered through Whatman No. 44 filter paper. The filtrate was stored properly and used for analysis of Zn^{++} by using Atomic Absorption Spectrophotometer (Perkin-Elmer, 3030A).

Bioconcentration Factor (BCF)

The bioconcentration factor provides an index of the ability of plant to accumulate the metal with respect to metal concentration in substrate. The bioconcentration factor was calculated as the ratio of the trace element concentration in the plant tissues at harvest to the concentration of the element in the external environment (Vesely et al., 2012; Rivelli et al., 2012). The BCF was calculated by using formula: BCF = *P/E*, Where, *P* represents the trace element concentration in plant tissues (ppm), *E* represents the residual concentration in water (ppm) or in sediment (ppm dry wt).

Water quality analysis

Following parameters were determined from the liquid solution of the plastic trays after twelve days of period. The methods of analysis were in consistent with the standard methods mentioned in 'Handbook of Water Analysis' (Borkar et al., 2011; Maiti, 2004).

Electrical conductivity and total dissolved solids

Electrical conductivity (EC) and Total dissolved solids (TDS) of the water containing zinc chloride was determined after every 4 days by using ELICO EC-TDS meter (CM 183, Make-India) where the electrode was directly dipped into the well diluted and filtered solutions to get direct digital display of the result.

Turbidity and pH

Turbidity of the sample was measured by Nephalometric method using CL 52D model of ELICO. Sample was poured into the cell and turbidity was read directly from digital display of the instrument. pH of the water samples was determined by using ELICO LI 127 pH meter after every 4 days of incubation.

Hardness

The total hardness of the water samples was determined by EDTA titration methods. In which 50 ml of well mixed sample was taken in a conical flask and pH of solution was adjusted up to 10. A pinch of Eriochrome black-T was added and titrated with 0.01M EDTA till wine red solution changes to blue. The volume of EDTA consumed was noted and similarly a reagent blank with distilled water was run to calculate the hardness of the water samples.

$$\text{Hardness (mg/L)} = \frac{\text{C x D x 1000}}{\text{ml of Sample}}$$

Alkalinity

Alkalinity of the samples was determined of 100 ml sample with the addition of 2-3 drops of phenolphthalein indicator. Pink solution developed was titrated with 0.02N H_2SO_4 till it disappears. Again the 2-3 drops of methyl orange were added to the same flask and titration was continued till pH is down to 4.5 or orange color changes to pink. The volume of H_2SO_4 added was noted as for each time and the total alkalinity was calculated. The values obtained were expressed in mg l^{-1}.

Sodium and potassium

Sodium and potassium of the water samples were estimated with the use of Flame photometer (Systronics-128) by preparing standard concentrations of sodium and potassium. The air pressure was kept at 0.5 kg/cm² and the gas feeder knob was adjusted so as to obtain a blue sharp flame.

Chemical Oxygen Demand (COD)

COD determination was carried out with dichromate reflux method (Saratale et al., 2010) with the addition of 10 ml of 0.25N potassium dichromate ($K_2Cr_2O_7$) and 30 ml H_2SO_4+Ag_2SO_4 reagent in 20 ml diluted sample. The mixture was refluxed for 2 h and was cooled to room temperature. The solution was then diluted to 150 ml by using distilled water and excess $K_2Cr_2O_7$ remained was titrated with ferrous ammonium sulphate (FAS) using ferroin indicator.

$$\text{COD (mg/L)} = \frac{\text{(A - B) x N x 1000 x 8}}{\text{Volume of Sample}}$$

Where, A is the ml of FAS used for blank; B is the ml of FAS used for sample, N is the normality of FAS and 8 is milliequivalent weight of oxygen.

Biological Oxygen Demand (BOD)

The dilution method was followed to determine the BOD after three days. For the same dilution water was prepared with the addition of nutrients namely phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride. The diluted sample was transferred to BOD bottles. After determining initial dissolved oxygen (DO), final DO was estimated from the bottles kept for incubation period of three days.

Statistical analysis

Statistical analysis of the data was carried out by using Graph Pad software. Mean and standard deviation (SD) was calculated.

Results and Discussion

Growth analysis

Growth changes are often first and most obvious reactions of plants under heavy metal stress (Hagemeyer, 1999). In present study plant growth was measured in terms of root length, leaf area and biomass production in terms of fresh and dry weight. The causes of growth reduction differ (Munns et al., 1995), but it is not clear which mechanisms plants employ to maintain residual growth for short and long term responses.

Root length

From the results, it can be seen that the root length of *Eichhornia crassipes* responded differentially with respect to Cadmium chloride treatment. Maximum root length was observed in control (12.5 cm) on 12^{th} day while minimum root length was observed as 5.2 cm at 75 ppm concentration on the 12^{th} day (Table 1). On the same day root length was observed to be decreased with an increase in concentrations showing perfect negative correlation. Literature survey showed that the heavy metals accumulations in water hyacinth increased linearly with the solution concentration in the order of leaves<stems<roots (Mane et al., 2011; Stratford et al. 1984). Munns (2003) concluded that the reduction might be attributed to the inhibition of hydrolysis of reserved foods and their translocation to the growing shoots. Growth changes are often the first and most obvious reactions of plants under heavy metal stress (Hagemeyer, 1999). Stratford et al. (1984) reported that Cd was toxic and caused substantial reduction in water hyacinth mainly by suppressing development of newly developing roots. It is known that Cd is non-essential heavy metal and has inhibitory effects on plant growth (Greger et al., 1991). Greger et al. (1991) reported that the uptake of Cd, both by roots and shoots, increased with the increasing metal concentration in the external medium. Stratford et al. (1984) found that the metal accumulation in water hyacinth increased linearly with the solution concentration in the order of leaves<stems<roots in water hyacinth. In general, most studies reported the higher concentrations of metals in roots than in shoots. Cd concentrations were reported to be higher in roots in most studies (Borkar et al., 2011; Rivelli et al., 2012). Some metals are accumulated in roots, probably due to some physiological barriers against metal transport to the aerial parts, while others are easily transported in plants. Translocation of trace elements from roots to shoots could be a limiting factor for the bioconcentration of elements in shoots (Zhu, et al., 1999). From the present investigation, it is clear that the root growth of *Eichhornia crassipes* was more sensitive and severely affected at higher concentrations of Cadmium in the water medium at the end of experiment. It might be due to higher uptake of Cd^{++} (Figure 1).

Leaf area

Maximum average leaf area was 13.06 cm^2 at 25 ppm on the 0 day and the minimum average leaf area was 7.05 cm^2 on the 12^{th} day observed at 75 ppm concentration (Table 1). On the 12^{th} day the leaf area of *Eichhornia* crassipes decreased with the increasing concentrations of $ZnCl_2$ and $CdCl_2$ (Table 1). From the present results, it is clear that leaf area per plant decreased when exposed to 25, 50 and 75 ppm concentration of Cadmium chloride. The mechanism of plants behind for growth reduction is not clear to maintain residual growth for short and long term responses (Munns, 2003). From the present results, it is clear that leaf area in *E. crassipes* affected at higher concentration of cadmium and might be associated with the toxic nature of the plant (Figure 1).

Table 1. Effect of Cadmium chloride (ppm) on root length, leaf area, fresh weight and dry weight of leaves of *Eichhornia crassipes*.

Duration	Root Length				Leaf area			
	Control	25	50	75	Control	25	50	75
0 Day	7.6 (±1.93)	6.34 (±2.26)	6.62 (±1.86)	6.7 (±1.63)	12.71 (±4.18)	13.06 (±3.01)	12.56 (±5.91)	12.98 (±2.13)
4^{th} Day	9.7 (±2.48)	7.76 (±1.63)	5.78 (±0.50)	5.92 (±0.73)	11.49 (±3.56)	9.32 (±3.64)	7.88 (±0.67)	9.62 (±2.37)
8^{th} Day	12.1 (±1.04)	7.54 (±0.55)	6.1 (±0.39)	5.16 (±0.61)	11.54 (±1.78)	8.87 (±4.90)	8.66 (±3.10)	8.01 (±1.43)
12^{th} Day	12.5 (±0.84)	6.74 (±0.42)	5.9 (±0.35)	5.2 (±0.52)	11.28 (±2.38)	8.21 (±1.51)	7.23 (±2.35)	7.05 (±3.01)

	Fresh Weight				Dry weight			
Duration	Control	25	50	75	Control	25	50	75
0 Day	0.302 (±0.05)	0.305 (±0.09)	0.314 (±0.16)	0.325 (±0.12)	0.044 (±0.01)	0.042 (±0.02)	0.047 (±0.03)	0.047 (±0.02)
4^{th} Day	0.336 (±0.13)	0.309 (±0.12)	0.297 (±0.02)	0.293 (±0.07)	0.058 (±0.03)	0.036 (±0.02)	0.035 (±0.00)	0.037 (±0.01)
8^{th} Day	0.344 (±0.09)	0.320 (±0.17)	0.321 (±0.08)	0.305 (±0.08)	0.049 (±0.02)	0.051 (±0.23)	0.032 (±0.01)	0.031 (±0.01)
12^{th} Day	0.355 (±0.06)	0.303 (±0.06)	0.295 (±0.12)	0.285 (±0.08)	0.055 (±0.01)	0.052 (±0.00)	0.030 (±0.02)	0.029 (±0.02)

Each value is a mean of ten observations and values in parenthesis indicate standard deviation

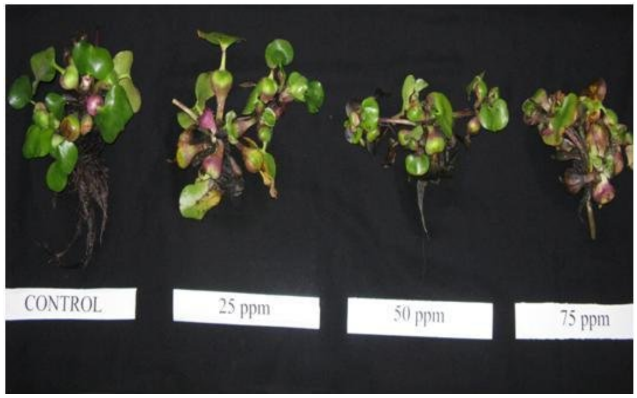

Figure 1. Effect of increasing cadmium chloride concentration on the growth parameters of *Eicchornia crassipes*.

Biomass

Fresh weight

It is evident from the results that the fresh weight of *E. crassipes* is sensitive parameter at various concentrations of Cadmium chloride. Fresh weight of the leaves was maximum with 0.355 g in control set on the 12th day and the minimum fresh weight was 0.285 g on 12th day at 75 ppm concentration (Table 1). On the 12th day the fresh weight decreased serially with the increase in concentration of cadmium. Though the plants showed slight increase in fresh weight on 4th and 8th day at 25, 50 ppm concentration over the control, it was adversely affected at higher dose at the end of treatment (Table 1). It is well known that aquatic biomass irrespective living or dead, exhibits capacity to remove heavy metals from wastewater. The reduction in shoot biomass production by the plant may be due to the chlorosis and necrosis of the leaves that reduce the photosynthetically active area (De Herralde et al., 1998). The decrease in fresh weight of the leaves of *E. crassipes* might be due toxic nature of $CdCl_2$ and the suppression of growth under such stress during the early developmental stages.

Dry weight

It is evident from the results that the dry weight of the plants is also another sensitive parameter like fresh weight. Dry weight of the leaves was maximum with 0.058 g on the 4th day in control set and minimum dry weight was 0.029 g on 12th day at 75 ppm concentration (Table 1). On the 12th day the dry weight decreased serially with increase in concentration showing a perfect negative correlation. The dry weight decreased with high concentration of $CdCl_2$ (Table 1). Munns (2003) concluded that the reduction might be attributed to the inhibition of hydrolysis of reserved foods and their translocation to the growing shoots. A decrease in dry weight of the leaves at the highest concentrations of $CdCl_2$ might be due to the inhibition in hydrolysis of reserved foods and their translocation to the growing shoots.

Moisture content

It is clear from the results that moisture percentage of plants slightly increased due to $CdCl_2$ in water medium but overall moisture content was not much affected. During Cadmium chloride treatment maximum moisture content was observed as 90.03% at 50 ppm concentration on 8th day and the minimum moisture content was observed as 82.73% in control set on 4th day (data not shown). On 12th day however there was increase in moisture content with the increase in the concentrations of cadmium. The decrease in moisture content in the leaves of *E. crassipes* at higher levels of $CdCl_2$ might be due to osmotic stress, which could not be maintained by plant while slight increase at higher levels might be the adaptation of plants to osmotic adjustment, which maintains water uptake and turgor with the accumulation of organic solutes (Li et al., 2005).

Photosynthetic pigments

Chlorophylls

It is evident from the results that chlorophyll content of *E. crassipes* is severely affected by the toxicity up to 75 ppm salt concentration showing drastic changes in the chlorophyll content of the leaves with Cadmium chloride treatment. Higher salt concentration in the liquid medium is inhibitory for chlorophyll in the species studied. It is also evident that chl.‘a’:chl.‘b’ ratio was decreased at 75 ppm salt concentration and it is quite clear that highest salt concentration (75 ppm) is certainly negatively influential on chl. ‘a’: ’b’ ratio. *E. crassipes* appears to be rather sensitive in this respect and chl. ‘a’ appears to be more sensitive to toxicity than chl. ‘b’ (Table 2).

Table 2. Effect of Cadmium chloride (ppm) on chlorophyll content, photosynthetic pigments and their metal accumulation and antioxidant enzymes of *Eichhornia crassipes.*

	Cadmium Chloride (ppm)					Cadmium Chloride (ppm)			
Chlorophyll content	Control	25	50	75	Metal Accumulation	Control	25	50	75
Chl. 'a'	110.43 (±1.06)	88.01 (±0.36)	2.47 ±2.66)	2.04 (±1.36)	Residual concentration (mg/l)	0.084 (±0.004) 0.0[a]	0.36** (±0.15) +328	1.30* (±0.11)+ 1447	3.32 (±0.21) +3852
Chl. 'b'	128.73 (±3.83)	111.82 (±3.29)	73.35 (±3.27)	6.56 (±2.62)	*E. crassipes* (mg/kg)	11.42 (± 0.06) 0.0[a]	141.88* (±2.34) +1142	334.5* (±3.14 +2829	402.56 (±4.51 +3425
Total Chl.	239.09 (±3.63)	192.37 (±3.69)	145.77 (±3.04)	118.56 (±2.70)	Bioconcentration Factor	135.95 (± 0.09) 0.0[a]	394.11 (± 0.12) +189.9	257.3 (± 0.15) +89.26	121.25 (± 0.11) -10.81
Chl. 'a' : Chl. 'b' ratio	0.86 (±0.03)	0.79 (±0.02)	0.99 (±0.07)	0.78 (±0.04)					
Photosynthetic pigments	Control	25	50	75	Antioxidant enzymes	Control	25	50	75
Carotenoid	53.68 (±1.65)	52.72 (±1.89)	46.08 (±0.78)	31.52 (±1.01)	Catalase[b]	0.218 (±0.02)	0.225 (±0.02)	0.548 (±0.06)	0.184 (±0.01)
Proline	7.02 (±0.09)	7.33 (±0.1)	8.14 (±0.15)	4.39 ±0.21)	Peroxidase[b]	0.195 (±0.012)	0.245 (±0.02)	0.284 (±0.03)	0.175 (±0.01)
Polyphenol	151.63 (±2.31)	244.95 (±2.3)	303.27 (±4.26)	291.60 (±3.78)					

[a] The values indicates percentage variation relative to control values.
[b] Each value is expressed as Δ O.D. $min.^{-1}$ mg^{-1} protein
[c]Each value is expressed as mg 100^{-1} g fresh tissue
Each value is a mean of three determinations
Values in parenthesis indicate standard deviation

Chlorophyll content in plants correlates directly to the healthiness of plant (Li et al., 2005). Several cases of decreased chlorophyll content owing to metal toxicity have been reported in the plant kingdom growing in wetland ecosystems (Valavanidis et al., 2005). Chlorophyll degradation is the routinely observed response to stress or chiefly in elevated concentrations of various heavy metals (Chen and Djuric, 2001). Many deleterious environmental influences that inhibit plant growth, ranging from nutrient deficiencies to anthropogenic pollution, can result in decreased leaf chlorophyll and carotenoid contents (Chen and Djuric, 2001). Thus, changes in chlorophyll and carotenoid content and pigment ratios are important indicators of environmental stress and describes about the tolerance status of the species (Carter and Spiering, 2002). A decrease in chlorophyll content may be either due to inhibition of chlorophyll synthesis or its destruction or replacement of Mg ions (Chandra et al., 2009).

Carotenoids

The carotenoid content of the leaves of *E. crassipes* with Cadmium chloride treatment decreased with increasing concentrations of Cadmium chloride. Maximum carotenoid content was observed as 53.68 mg 100^{-1} in control set and a minimum of 31.52 mg 100^{-1} at 75 ppm on 12^{th} day of treatment (Table 2). Carotenoid content of the leaves decreased with increasing concentrations showing a negative correlation. Significant decrease in the carotenoid content implies decrease in protection action of carotenoid (Arora et al., 2006). According to (Vesely et al., 2012) carotenoids have two major functions in photosynthesis, they protect chloroplast from photo-oxidative damage and they also act as accessory light harvesting pigments because they absorb the light energy in the range of 400-500 nm (blue). Chen and Djuric (2001) were of the opinion that decrease in carotenoid content is a regular response to stress condition due to heavy metals. From the present investigation, it appears that toxic nature of cadmium might have caused a photo-oxidative damage in the leaves of *E. crasssipes*.

Proline

Proline content of leaves of *E. crassipes* subjected to Cadmium chloride increased maximally as 14.39 mg 100^{-1} at 75 ppm concentration and minimum as 7.02 mg 100^{-1} in control on 12^{th} day

(Table 2). Proline occurs widely in higher plants and accumulates in larger amounts than other amino acids, regulates the accumulation of usable nitrogen (Abraham et al., 2003). Number of authors as an adaptive trait concerned has considered proline accumulation in response to environmental stresses with stress tolerance and it is generally assumed that proline is acting as a compatible solute in osmotic adjustment (Lutts et al., 1999). It is evident that in general proline accumulates in plants exposed to various environmental stresses and is not an exception to toxicity stress of cadmium chloride in water medium. The increased levels of proline in the leaves showed a perfect positive correlation with increasing levels metal salt and might be to maintain osmoregulation, stabilization of proteins and conservation of nitrogen and energy for a post-stress period alongwith some other organic solutes in the species (Mane et al., 2011; Borkar et al., 2011).

Polyphenols

Very little attention has been paid towards the influence of metals on the polyphenol metabolism in plants. Parida et al. (2002) were of the opinion that accumulation of polyphenols played a key role in plants towards stress. In the present study, polyphenol content of the leaves increased with the increasing concentrations of $CdCl_2$ especially from 25 ppm to 50 ppm however, it was slightly decreased at 75 ppm concentration on 12th day. The maximum increase in polyphenol was observed as 303.27 mg 100^{-1} at 50 ppm concentration and minimum polyphenol content was observed as 151.63 mg 100^{-1} in control observed at the end of experiment (Table 2). It is clear that $CdCl_2$ induce accumulation of secondary metabolites in the experimental species in order to tolerate higher levels of toxicity stress and adverse conditions aroused.

Enzymes as antioxidants

In plant cell, antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) have been considered to act as a defensive team, whose combined purpose is to scavenge reactive oxygen species (ROS) and protect cells from oxidative damage (Odjegba et al., 2007; Mittler, 2002; Mittova et al., 2003). In plants, the links between ROS production and photosynthetic metabolism are particularly important (Rossel et al., 2002). However, an elaborate and highly redundant plant ROS network, composed of antioxidant enzymes and antioxidants, is responsible for maintaining the levels of ROS under tight control (Mittler, 2002).

Catalase (E.C. 1.11.1.6)

Catalase is a common enzyme found in nearly all living organisms, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. It is the most effective antioxidant enzyme preventing oxidative damage (Mittler, 2002). Hydrogen peroxide is a harmful byproduct of many normal metabolic processes and to prevent damage, it must be quickly converted into other less toxic substances. To end this, catalase is frequently used by cells to rapidly catalyse the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Mittler, 2002; Mane et al., 2011; Rivelli et al., 2012). The activity of this enzyme was observed to be decreased with Cadmium chloride treatment. Maximal activity of enzyme catalase was observed as 0.548 at 50 ppm concentration on the 12th day, and the minimum activity of the enzyme catalase was observed as 0.184 Δ O.D. min.$^{-1}$ mg^{-1} protein at 75 ppm concentration on the same day (Table 2). Initial increase at lower levels of $CdCl_2$ toxicity and then decrease at higher level indicates the strong adjustment developed by the leaves to tolerate higher toxic environment.

Peroxidase (E.C. 1.11.1.7)

It is well documented that in plants subjected to cadmium stress the activity of peroxidase increases (Saratale et al., 2011) an in the present study maximal activity of enzyme peroxidase (POD) was observed as 0.284 Δ O.D. min.$^{-1}$ mg^{-1} protein at 50 ppm concentration on 12th day and minimal activity was observed as 0.175 at 75 ppm concentration on the same day (Table 2). POD is widely distributed in higher plants where it is involved in various processes, including lignification, auxin metabolism, salt tolerance and heavy metal tolerance (Odjegba et al., 2007). The increase in the activity of peroxidase under $CdCl_2$ stress may be regarded as an inhibition of stimulated secondary metabolism. It may also be involved in scavenging the reactive oxygen species in plants grown under stress condition.

Inorganic constituents

Cadmium accumulation and bio-concentration factor (BCF)

The toxic effect of metals on physiological functioning within plants is connected to their accumulation in different plant tissues (Liu et al., 2005). Several cases of accumulation of heavy metals such as Zn, Cu, Pb, Cd, Ni and Cr, have been thoroughly studied in several wetland plant species, such as *E. crassipes*, *Typha latifolia, Spartina alterniflora* and *Phragmites australis* (Liu et al., 2005). Cadmium has no biological function and is extremely toxic, even at low concentrations and is easily assimilated by plants (Milone et al., 2003). Cadmium enters into the aquatic environment through the sources like electroplating industries, batteries, chemicals and various other applications. The accumulation of Cd^{++} by aquatic plants and their subsequent toxic effect has been reported by several investigators (Liu et al., 2005; Vesely et al., 2012). Cadmium affects various steps in plant metabolism particularly stimulation in activities of several enzymes has been also demonstrated (Rivelli et al., 2012; Borkar et al., 2011).

Bioconcentration factor (BCF) is a useful parameter to evaluate potential of plants in accumulating metals and this value is calculated on a dry weight basis. The appropriateness of a plant for phytoremediation potential is often judged by its BCF. The change in BCF of *E. crassipes* was studied to know capacity of *E. crassipes* to concentrate cadmium from varied effluent concentrations. An increase in cadmium (Cd^{++}) accumulation in *E. crassipes* leaves with increasing concentrations of cadmium in the hydroponics was observed (Table 2). The maximum accumulation of Cd^{++} by *E. crassipes* was 402.56 mg/kg at 75 ppm concentration of cadmium chloride after 12 days while it was minimum at control set with 11.42 mg/kg. In the present study, increase in concentration of cadmium in *E. crassipes* at elevated levels might be due to increasing levels already added in the liquid medium, which could also be related with Cd^{++} tolerance. The residual concentration was minimum by 0.084 mg/l in control set while the highest 3.32 mg/l was observed at 75 ppm of cadmium chloride (Table 2). Vesely et al. (2012) found that the metals accumulations in water hyacinth increased linearly with the solution concentration in the order of leaves<stems<roots in water hyacinth.

As a fact, larger BCF implies better phytoaccumulation capability and tissues with BCF greater than 1,000 are considered high, and less than 250 low, with those between classified as moderate (Rivelli et al., 2012; Borkar et al., 2011). The BCF values were increased at 25 (394.11) and 50 (257.30) ppm concentration of cadmium chloride and was lesser than control set at 75 (-10.81) ppm concentration. Only 25 ppm concentration showed highest BCF values which in equivalent with the previous study carried out by Xiaomei et al. (2004). This clearly shows that the 25 ppm concentration is the limiting concentration and later concentrations are harmful for the growth, development and contributes only as toxicity to the plant though higher accumulation is observed in dry tissues. BCF value at 25 ppm shows that *E. crassipes* has capacity to accumulate Cd^{++} through liquid medium after twelve days exposure period, which is also in accordance with other studies (Table 3) and it can be concluded that at 25 ppm concentration *E. crassipes* has moderate accumulating capacity. The sequestration of heavy metals in plants is achieved mainly by absorption and accumulation mechanisms (Dhir, 2010). The toxic effect of metals on physiological functioning within plants is connected to their accumulation in different plant tissues (Liu et al., 2005).

Analytical parameters

Electrical conductivity (EC) and Total Dissolved Solids (TDS)

Electrical conductivity is the most common measure of water and is indicative of the ability of an aqueous solution to carry an electric current. It is a fact that excess salts in water detrimentally affect plants, both physically and chemically. It is obvious that EC of the water increases with the increasing levels of cadmium chloride in the solution. It was maximum by 693.67 µS on the 0 day at 75 ppm concentration and a minimum by 85.26 µS on the 12th day in control set. On 12th day the EC increased with increasing concentration and showed a perfect positive correlation (Table 4).

Table 3. Bioconcentration factors (BCF) for zinc in various plants used for phytoremediation.

Sr. No.	Plant species	Bioconcentration Factor	Reference
1)	*Eichhornia crassipes(Leaf)*	394.11	Present study
2)	*Eichhornia crassipes*	622.3	Xiaomei et al. (2004)
3)	*Azolla pinnata (root)*	24,000	Sela et al. (1989)
5)	*Elodea nuttalli*	1700	Nakada et al. (1979)
6)	*Lemna polyrrhiza*	650	Jain et al.(1990)

The varying levels of EC in water containing different concentrations of $CdCl_2$ might be due the external additions of salts and also might be due to different binding capacities of the roots, root excretions and competition by the roots to get nutrients from the liquid medium. This also clearly shows the capacity of *E. crassipes* to treat cadmium containing water. Similar increase in electrical conductivity in rooting medium has been reported earlier (Gokhale et al., 2008; Mane et al., 2011). Mahmood et al. (2005) also observed the reduction in conductivity due to absorption of pollutants by plants.

TDS is simply the sum of the cations and anions concentration expressed in mg/l. It is obvious that TDS in the water increases with the increasing levels of cadmium chloride. The maximum TDS content was observed as 349.33 mg/l on the 0 day at 75 ppm concentration and the minimum value was recorded on 12th day by a value of 43.0 mg/l in control set (Table 4). On the 12th day, the TDS content increased with the increase in concentration, showing a perfect positive correlation. Khosravi et al. (2005) reported the importance of TDS uptake by *Azolla filiculoides* for their growth in wetlands. Groudev et al. (2011) observed reduction of total dissolved solids from 2620 ppm to 1230 ppm in treatment of acid mine drainage from an uranium deposit by means of a natural wetland. A good reduction (90 %) of total suspended solids by constructed wetland plants with a retention time of 7 days was reported (Amelia, 2001). The varying levels of TDS in the cadmium containing water were obviously due to the external additions of $CdCl_2$ and it's interactions with root and organic-inorganic ions already present in water.

pH and turbidity

pH of the water is the measure of H^+ ion activity of the water system. It indicates whether the water is acidic, neutral or alkaline in nature. The maximum pH was recorded with 8.85 on 12th day in control on 0 day while it was minimum by a value of 5.48 on 12th day in control set (Table 4). In general, pH was observed to be slightly acidic with the addition of cadmium chloride in the hydroponic medium. The change in pH may be due to release of some root exudates in response to the stress to adapt itself with the existing environment. Wagner (1997) found that at water temperature of 25 ^oC, both *A. pinnata* and *A. filiculoides* showed maximum growth at pH values from 5-7. pH seems to be the most important parameter in the biosorptive process: it affects the solution chemistry of the metals, the activity of functional groups in biomass and competition of metallic ions (Li et al., 2005).

Table 4. Effect of Cadmium Chloride (ppm) on electrical conductivity, total dissolved solids, pH and turbidity of water after 12 days of incubation with *Eichhornia crassipes.*

Duration	EC				TDS			
	Control	25	50	75	Control	25	50	75
0 Day	144.22 (±1.51)	345.67 (±5.77)	518.33 (±6.35)	693.67 (±3.21)	73.13 (±0.06)	175.1 (±0.10)	260.00 (±0.14)	349.33 (±0.58)
4th Day	134.6 (±1.01)	308.33 (±0.58)	313.0 (±1.0)	378.67 (±0.58)	63.14 (±0.21)	155.24 (±0.25)	185.65 (±0.34)	223.61 (±0.21)
8th Day	102.37 (±0.21)	167.80 (±0.26)	202.0 (±1.00)	223.33 (±2.08)	51.40 (±0.10)	103.63 (±1.06)	164.92 (±0.92)	131.93 (±0.58)
12th Day	85.26 (±0.40)	129.45 (±0.62)	181.24 (±0.23)	189.25 (±0.28)	43.0 (±0.61)	96.67 (±1.89)	121.95 (±1.31)	124.10 (±1.02)
Duration	pH				Turbidity			
	Control	25	50	75	Control	25	50	75
0 Day	8.85 (±0.05)	6.57 (±0.02)	6.68 (±0.01)	6.74 (±0.01)	0.75 (±0.04)	2.11 (±0.06)	2.59 (±0.08)	2.85 (±0.07)
4th Day	7.75 (0.04)	6.25 (0.01)	6.35 (0.02)	6.51 (0.01)	3.98 (±0.07)	7.52 (±0.12)	8.42 (±0.15)	9.89 (±0.16)
8th Day	6.89 (0.01)	6.02 (0.00)	6.41 0.01)	6.42 (0.01)	4.47 (±0.06)	5.30 (±0.14)	5.27 (±0.12)	5.70 (±0.18)
12th Day	5.48 (±0.00)	6.25 (±0.01)	6.34 (±0.00)	6.28 (±0.01)	4.60 (±0.09)	7.10 (±0.11)	7.98 (±0.08)	10.60 (±0.19)

Each value is a mean of ten observations and values in parenthesis indicate standard deviation

Maximum turbidity was observed as 10.60 on the 12th day at 75 ppm concentration and a minimum turbidity was observed as 0.75 on the 0 day in control set. On the 12th day the turbidity increased with the increase in the concentration. It is definite that the increase in turbidity might be due to the external addition of salt and also due to the root exudates released to face the stress condition (Table 4).

Hardness and alkalinity

Maximum hardness was observed as 101.33 mg l^{-1} on 0 day at 75 ppm concentration and the minimum hardness was observed as 333.33 mg l^{-1} on 12th day in control (Table 11). On 12th day hardness of water containing Cadmium chloride increased with increase in concentration showing a perfect positive correlation and such an increase is definitely due to added salt to the water medium. Maximum alkalinity was observed as 268.0 mg l^{-1} on 12th day at 75 ppm concentration and the minimum was 87.33 mg l^{-1} on 0th day in control (Table 5). At the end of experiment alkalinity also increased similar to that of hardness of water with the increase in the cadmium chloride concentration.

Sodium and potassium

Maximum sodium content was observed as 17.19 mg l^{-1} on 0 day at 50 ppm concentration and minimum sodium content observed by a value of 12.34 mg l^{-1} on 12th day of the experiment at 25 ppm concentration of cadmium chloride (Table 5). On the same day 12th day sodium content slightly decreased with increase in concentration which might be used by plant for development and to minimize metal stress. It decreased from 50 to 75 ppm concentrations of Cadmium chloride treatments. Potassium is essential in nearly all the processes needed to sustain plant growth and reproduction. It plays a vital role in photosynthesis, translocation of photosynthates, protein synthesis, activation of plant enzymes, control of ionic balance and regulation of plant stomata. Potassium is essential in nearly all the processes needed to sustain plant growth and reproduction. Maximum potassium content observed with a value of 26.92 mg l^{-1} on 0 day in control while minimum potassium content observed was 11.12 mgl^{-1} on 12th day of experiment at 75 ppm (Table 5). It was also observed that potassium content of water also decreased with increase in concentration. Such a decrease in potassium content at the end of the experiment with the increase in concentration of cadmium chloride might be due to uptake by *Eichhornia* for development and to minimize the stress condition aroused.

COD and BOD

Chemical oxygen Demand determines the oxygen required for the chemical oxidation of most organic matter and oxidisable inorganic substances with the help of a strong chemical oxidant. Highest value for COD was observed 28.65 mg l^{-1} on 12th day at 75 ppm concentration while the minimum COD content was observed as 15.24 mg l^{-1} on 0 day in control. At the end of experiment COD content of water increased in all the sets with increase in the concentration of cadmium chloride.

Table 5. Effect of Cadmium Chloride (ppm) on selected water quality parameters.

Sr. No.	Duration	Hardness				Alkalinity			
		Control	25	50	75	Control	25	50	75
1	0 Day	42.67 (±1.15)	46.67 (±1.15)	95.33 (±1.15)	101.33 (±2.31)	87.33 (±1.41)	97.33 (±3.06)	126.67 (±1.15)	151.00 (±2.31)
2	12th Day	33.33 (±1.15)	41.67 (±2.31)	80.67 (±1.15)	84.67 (±1.15)	154.00 (±3.46)	242.67 (±1.15)	260.0 (±3.46)	268.0 (±0.00)
		Sodium				Potassium			
3	0 Day	15.16 (±0.26)	16.25 (±0.15)	17.19 (±0.18)	16.54 (±0.19)	26.92 (±0.45)	25.13 (±0.36)	22.82 (±0.32)	15.13 (±0.26)
4	12th Day	14.23 (±0.22)	12.34 (±0.12)	13.21 (±0.14)	13.01 (±0.11)	24.56 (±0.54)	22.31 (±0.31)	18.62 (±0.28)	11.12 (±0.25)
		COD				BOD			
5	0 Day	15.24 (±1.57)	16.25 (±1.68)	19.24 (±1.71)	26.21 (±2.01)	4.95 (±0.19)	5.64 (±0.13)	5.49 (±0.18)	6.54 (±0.19)
6	12th Day	16.25 (±1.51)	18.95 (±1.89)	23.54 (±0.95)	28.65 (±1.01)	5.46 (±0.11)	5.78 (±0.25)	7.65 (±0.24)	7.45 (±0.89)

Each value is a mean of ten observations and values in parenthesis indicate standard deviation

The increase in the COD content is definitely due to the external addition of salt and root secretions over time. Dissolved oxygen (DO) measures are vital for maintaining aerobic conditions in natural waters that receive polluted matter. Maximum BOD content was observed as 7.65 mg l^{-1} on the 12th day at 50 ppm concentration while a minimum BOD content was observed as 4.95 mgl^{-1} on 0 day in control (Table 5). It is definite that the degrading plant parts and root exudates are the main reasons behind increase in BOD content at the end of the experiment.

Conclusions

The water hyacinth is available in Indian water bodies and where it is possible to use it in wastewater treatment by setting wastewater treatment ponds with the application of this ecofriendly technique. From the present study, the floating hygrophyte *E. crassipes* based treatment has proved as a promising tool for the treatment of cadmium containing effluents especially below 25 ppm. It is clear that the growth characteristics of Eichhornia *crassipes* were more sensitive and severely affected at higher concentrations of Cadmium in the water medium at the end of experiment and might be due to higher uptake of Cd^{++}. Decreased levels of chlorophyll and carotenoid might be related with the cadmium as a metal stress indicator and describes about the tolerance status of the species. The increased levels of proline in the leaves might be to maintain osmoregulation and face the metal stress. Cadmium chloride might induce accumulation of secondary metabolites in the experimental species in order to tolerate higher levels of toxicity stress and adverse conditions aroused. The increase in the activity of peroxidase under $CdCl_2$ stress may be regarded as an inhibition of stimulated secondary metabolism at higher levels. We found that the 25 ppm concentration is the limiting concentration and later concentrations are harmful for the growth, development and contributes only to toxicity in the plant though higher accumulation is observed in dry tissues. The varying levels of TDS in the cadmium containing water were obviously due to the external additions of $CdCl_2$. It is definite that the increase in turbidity might be due to the external addition of salt and also due to the root exudates released to face the stress condition. The degrading plant parts and root exudates could also be the main reasons behind increase in BOD. Bioconcentration factor showed a clear conclusion and supportive proof about the species as a tool for phytoremediation of cadmium containing wastewaters. We also suggest the need to understand processes that affect metal availability, metal uptake and translocation in E. crassipes. Detail investigations and understandings of various aquatic species, accumulation of different metals under influence of various effluents should be studied with respect to physiological changes associated with the plants and their ability to treat the industrial and domestic wastewaters.

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