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RESEARCH ARTICLE

PREFERENCE OF HEAVY METALS ACCUMULATION, TOLERANCE LIMIT AND BIOCHEMICAL RESPONSES OF *EICHHORNIA CRASSIPES* (MART.) EXPOSED TO INDUSTRIAL WASTE WATER

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ABSTRACT

Discharge of industrial waste water often poses serious threat to our environment. In the present study, heavy metals content in industrial waste water from common industrial effluent treatment plant in Unnao district (U.P.), India were quantified. The nickel (1.2 mg l^{-1}) and chromium (1.8 mg l^{-1}) content was high in industrial waste water and concentration was found in order Cr>Ni>Zn>Cu. *Eichhornia crassipes* Mart. plants were exposed to various concentrations of above evaluated industrial waste water (0, 25, 50, 75 and 100%) for 10 days and observed for their visible effects, tissue accumulation level and biochemical constituents (pigments, total protein, sugar and relative water contents and amylase activity) including antioxidants (catalase activity, peroxidase activity and Proline content) in *E. crassipes* leaves and roots. The accumulation of heavy metals was observed more in root than the leaves (Cr>Ni>Zn>Cu). Also inhibited biochemical constituents (pigments, protein and sugar contents and amylase activity) including antioxidants (catalase, peroxidase activity and proline content) in *E. crassipes.* Study concluded that, *E. crassipes* indicated accumulation of high content of Cr (Cr>Ni>Zn>Cu), exhibited visible symptoms of toxicity and altered biochemical constituents with exposure of industrial waste water. These findings may be helpful in phytoremedial approaches to minimize heavy metals content in surface water.

INTRODUCTION

Heavy metal as a water pollutant from discharge of industrial wastes is a global problem. Some industrial waste water often not treated properly and discharged into surface water bodies used for various purposes of human beings. These contamination pose hazardous effects on living organisms (**Pandey, 2006**). Excess concentrations of heavy metals in water pose adverse effects on aquatic organisms through changing their metabolic constituents. Tanneries effluents are mostly rich in chromium content along with other heavy metals. Effluent from electroplating, pigments and alloy industries also contribute high content of heavy metals (Ni, Zn, Cu, Pb etc.). More than 25 tanneries and other industries with various work nature discharge their effluents into the drain through a common effluent treatment plant (CETP) located in industrial area of Unnao district (U.P.), India. This waste water is used for irrigation purposes in nearby areas from drain, and finally discharged into Sai River, a significant tributary of river Gomati. Therefore, possibly a great risk to contaminate soil, water and plants.

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Heavy metals toxicity can induce severe cellular damage through oxidative stress, changes nutrient contents and metabolic activities in various crop and aquatic plants (**Sinha *et al.*, 2005 and Tiwari *et al.*, 2009**). Nickel, zinc and copper are essential micronutrients, play a vital role in the metabolism of plants (**Broadley *et al.*, 2007**). These metals act as a co-factor of enzymes and are beneficial for animals in trace quantities, but at higher concentrations pose physiological disorders in plant (**Tripathi *et al.*, 1981**). At elevated levels, the heavy metals (Ni, Zn, Cu and Cr etc.) induce nutrient imbalances and oxidative damage in plant cells (**Wang *et al.*, 2009**).

In view of the detrimental effects of heavy metals in plant, human health and a risk to contaminate our delicate food web, their removal from the fresh water sources has been challenge for a long time. Because, traditional cleanup process of heavy metal contaminated soils and water are not environment friendly, very expensive and practical only in small areas. The new cost effective technologies that include the use of organisms, biomass and live plants have been used very widely to extract heavy metals (**Gerendas *et al.*, 1999**). But, their phytoextraction capacity is metal specific and capabilities of tolerance through their cellular defense against heavy metals are variable. *Eichhornia crassipes* (Mart.) a widely grown aquatic plant in tropical and sub tropical regions have a characteristic rapid growth and well developed profuse root system, can potentially accumulate more heavy metals. Least information is available on preferential uptake and translocation of heavy metals and tolerance capacity of *E. crassipes* through changes their biochemical constituents under heavy metals- stress conditions.

Therefore, study was aimed to quantify some potentially toxic heavy metals in industrial waste water discharged into surface water bodies, preferential phytoextraction capacity of *Eichhornia crassipes* (Mart.) and tolerance limit with respect to some biochemical changes in their cells. The findings may be helpful in phytoremedial technology for removal of heavy metals from surface water.

MATERIALS AND METHODS

All reagents used to prepare the solutions were of analytical grade. High purity de-ionized (Milli Q system: resistivity 18.2 MΩcm. TOC < 10 μgl^{-1}) was used for all experiments. The industrial waste water was collected in pre-cleaned polyalkalene bottles from a common effluent treatment plant, situated in Unnao district (U.P.), India. The waste water was collected thrice from May to June (2013), which was pooled out using suitable preservative to make a composite sample. The composite waste water sample was analyzed for some potentially toxic heavy metals (Cr, Ni, Zn and Cu) following the standard methods (**APHA, 2005**).

The healthy young plants of *Eichhornia crassipes* (Mart.) were collected from a natural uncontaminated pond, washed with distilled water and maintained in large hydroponic tubs. For experimental studies, healthy plants of *E. crassipes* were further acclimatized in nutrient medium (10% Hoagland solution) for 4 weeks under laboratory conditions. Healthy plants were selected which were uniform in size and weight. Experiment was performed on known weight basis, and the plant of *E. crassipes* (3 plant per tub) were kept in tubs and filled with 3L capacity of different four concentrations of industrial waste water using tap water along with one set of control (tap water) both in triplicate. The plants were treated with four different concentrations of heavy metals (Cr, Cu, Ni and Zn) evaluated in industrial waste water (25, 50, 75 and 100%) under standard physiological conditions providing light dark 14:10h cycle, 115 µmol m^2s^1 illumination provided through day fluorescent tube light at 28±2°C for 8 days. The plants were refilled with initial metal concentration at every 2^{nd} day. The plant of *E. crassipes* (3 plants per tub) were kept in tubs and filled with 3L capacity of different four concentrations of waste water along with one set of control. Plants were harvested at day 10 of the treatment and the blotted roots and leaves were used for tissue concentration of heavy metals (Cr, Cu, Ni and Zn) and biochemical constituents were analyzed in leaves.

Tissue concentration in dried plant materials of *E. crassipes* were determined after the digestion in nitric acid (70%) and perchloric acid using hot plate (**Piper, 1966**) and metal content was estimated by Perkin Elmer 700 Atomic Absorption Spectrophotometer (AAS) using air-acetylene gases. The translocation ability was calculated by dividing the concentration of trace element accumulated in the root tissue by that accumulated in shoot tissues.

$$TA = (Ar/As)_i$$

Where *i* denote the heavy metal, *TA* is the translocation ability and is dimensionless. *Ar* represents the amount of trace element accumulated in the roots ($\mu g\ g^{-1}$ dry weight), and *As* represents the amount of trace element accumulated in the shoot ($\mu g\ g^{-1}$ dry weight). Some biochemical constituents were analyzed at day 10 of exposure of industrial effluent. The blotted fresh leaves were used for estimation of pigment, protein and sugar contents. Chlorophylls (Lichtenthaler and Well burn, 1983) and carotenoids content (**Duxbury and Yentish, 1956**) in the leaves (100 mg) of treated and control plants were extracted in 80% chilled acetone and estimated by using Perkin Elmer Spectrophotometer. Protein content (**Lowry *et al*., 1951**) in the leaves was measured by the method using bovine serum albumin as the standard protein. Sugar content was estimated (**Dubois *et al*., 1956**) by using spectrophotometer (Perkin Elmer). Amylase activity was determined by the method of **Katsuni and Fekuhara, (1969).** Catalase (**Euller and Von, 1959**) and peroxidase activity (**Luck, 1963**) was estimated by using spectrophotometer. Proline was assayed by the method of **Bates *et al*., (1973).** Relative Water Content (RWC) was measured using the method of **Weatherly (1950).**

All the data presented in the table are mean value (n=3). The student't' test method and LSD values were employed to test statistical significance of data (**Panse and Sukhatme, 1961**).

RESULTS AND DISCUSSION

The industrial waste water was determined for heavy metals (Cr, Cu, Ni and Zn) content (Table 1); it contained high content of chromium (1.8 mg l^{-1}) and nickel (1.2 mg l^{-1}) as compared to standard limits prescribed for industrial waste water discharged into inland surface waters (Table 1).

Table 1. Heavy metals content in composite sample of industrial waste water used in the experiment

Heavy metals (mg/l)	Average value	ISI standards (1974) (Discharge limits of industrial waste waters into inland surface water)
Chromium	1.84±0.21	0.05-1.5
Nickel	1.18±0.22	0.05
Zinc	0.35±0.12	5.0-15.0
Copper	0.20±0.15	0.05-1.5

*ISI standard No. 2490 (1974)

The heavy metals content in industrial waste water was in the order Cr>Ni>Zn>Cu. Such range of heavy metals could cause toxicity to aquatic life after discharge into surface water bodies (Vajpayee *et al*., 2001). The test plant (*E. crassipes)* accumulated high content of Cr (root, 392.4 $\mu g\ g^{-1}$ dry weight and shoot, 80.5 $\mu g\ g^{1}$ dry weight) and Ni (root, 70 $\mu g\ g^{-1}$ dry weight and shoot, 30 $\mu g\ g^{-1}$ dry weight). Heavy metals uptake in the root was found in the order Cr>Ni>Zn>Cu. The accumulation of heavy metals in root and shoot was dose dependent (Table 2), their tissue accumulation was observed more in root than the shoot indicated high stabilization of metals in roots of *E. crassipes*. Translocation of Ni from root to shoot was faster than other quantified metals (Figure 1). Tissue content of Cr was higher than other estimated metals (Ni, Zn and Cu) but its rate of translocation from root to shoot portion was low, whereas Ni showed high value of translocation factor. Translocation factor was in the order Ni>Zn>Cu>Cr. Heavy metals in the growth medium, in combination, show antagonistic and synergistic effects for their uptake (**Liu *et al*., 2004**). The rate of translocation of Cr to aerial portion decreased with increase in heavy metals concentration in industrial waste water (Figure 1).

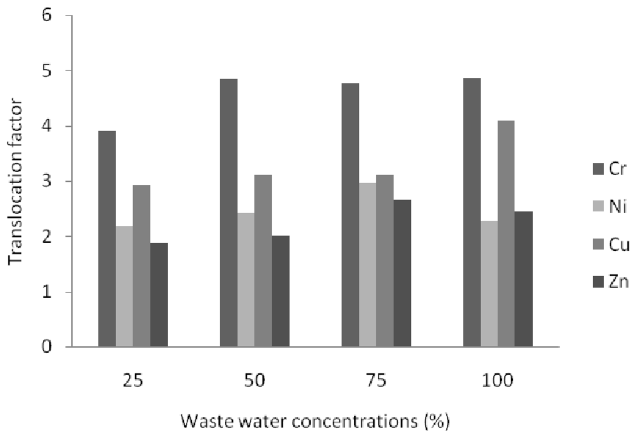

Fig. 1. Effect of industrial waste water concentrations on translocation factor in *Eichhornia crassipes* (Mart.) plants

It might be due to high phytostabilization of Cr in root tissues (**Tiwari *et al*., 2009**) or antagonistic effects with other metals in growth medium (**Liu *et al*., 2004**) or the conditions existed in the water-plant system (**Singh and Pandey, 2011**). Uptake in root and translocation in aerial parts may be supported with low translocation factor which showed great potential of phytostabilization of heavy metals in the root. The roots of plant act as a barrier against heavy metals translocation possibly due to potential of tolerance mechanism (**Ernst *et al*., 1992**).

Visible symptom appeared as necrosis and browning on upper margin of leaves, few leaves wilted and curled and fragmentation of root with exposure of in industrial effluent was observed, it could be due to elevated levels of heavy metals in water and their absorption in *E. crassipes* (**Pandey, 2006**). These symptoms could be developed due to adverse effects of excess heavy metals on chlorophyll synthesis and physiological disorders of metabolism in leaves of test plants (**Odjegba and Fasidi, 2007**). The severity of visible symptoms of toxicity in plants was less at diluted industrial waste water. The pigment contents (chlorophyll a, b and total chlorophyll and carotenoids) decreased in *E. crassipes* with exposure of undiluted industrial effluent (Table 3). Excess heavy metals, including chromium and nickel, inhibit synthesis of pigments in plants; decreased pigment contents may cause chlorosis in leaves (**Stobart *et al*., 1985**). Protein (Fig. 2) and sugar contents decreased by 87 and 89%, respectively in *E. crassipes* leaves at undiluted industrial waste water.

Table 2. Tissue concentration of *Eichhornia crassipes* (Mart.) exposed to various concentrations of industrial waste water at day 8 of exposure

Tissue concentration (µg g^{-1} dry weight)		Tap water	Waste water concentrations (%)				LSD P=0.05
			25	50	75	100	
Cr	Root	ND	115.6	195.7	277.3	392.4	164.1
	Leaves	ND	29.6	40.3	58.0	80.5	30.9
Ni	Root	ND	26.6	37.7	48.9	68.9	25.7
	Leaves	ND	12.2	15.6	16.5	30.2	11.0
Cu	Root	0.9	18.7	23.3	27.1	44.1	19.4
	Leaves	0.4	6.4	7.4	8.7	10.8	4.8
Zn	Root	1.0	20.4	31.6	42.7	50.6	24.3
	Leaves	0.4	10.8	15.7	16.0	20.7	6.2

ND: not detectable

Table 3. Effect of industrial waste water on some biomolecules content and enzymes activity in *Eichhornia crassipes* (Mart.)

Parameters	Tap water	Waste water concentrations (%)				LSD P=0.05
		25	50	75	100	
Chlorophyll a (mg g^{-1} f. w.)	1.42(0.0)	0.83(-41.5)	0.78(-45.0)	0.69(-51.4)	0.49(-65.4)	0.43
b	0.44(0.0)	0.27(-38.6)	0.19(-56.8)	0.15(-65.9)	0.13(-70.4)	0.15
Total chlorophyll (mg g^{-1} f. w.)	1.86(0.0)	1.10(-40.8)	0.97(-47.8)	0.84(-54.8)	0.62(-66.6)	0.58
Carotenoids (mg g^{-1} f. w.)	0.76(0.0)	0.49(-35.5)	0.42(-44.7)	0.36(-52.6)	0.25(-67.1)	0.23
Sugar (µg g^{-1} f. w.)	65.0(0.0)	27.5(-57.6)	24.5(-62.3)	22.5(-65.3)	7.50(-88.4)	26.50
Amylase (mg g^{-1} f. w.)	8.72(0.0)	2.33(-73.2)	1.53(-82.4)	1.32(-84.8)	1.20(-86.2)	4.00
Catalase (ml H_2O_2 hyd g^{-1} f. w.)	140(0.0)	136(-2.9)	122(-12.8)	82(-41.4)	44(-68.5)	51.04
Proline (µg g^{-1} f. w.)	1.56(0.0)	1.59(+1.9)	1.19(-23.7)	1.06(-32.0)	0.60(-61.5)	0.51
Peroxidase (mg g^{-1} f. w.)	22.32(0.0)	8.24(-63.0)	3.20(-85.6)	2.60(-88.3)	1.72(-92.2)	10.70

f. w.: fresh weight, Parenthesis indicate % decrease/increase over control

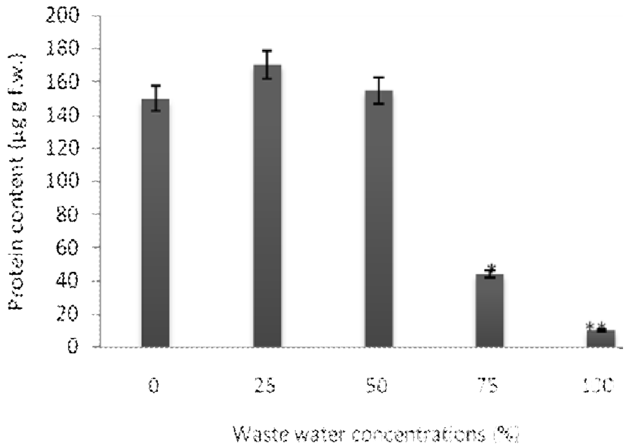

Fig 2. Effect of industrial waste water concentrations on protein content in *Eichornia crassipes* (Mart.) *- significant at P<0.05 and **- significant at P<0.01 levels (n=3)

Decrease in protein and carotenoids content is not favorable to strengthen defense system. Carotenoids content serve as accessory pigment for photosynthesis and are considered playing an important role in plant defense system against metal-stress conditions. It also acts as non-enzymatic antioxidants, quenches singlet oxygen rapidly and helps in protection of chlorophyll under stress conditions. Some proteins bind metals and form metallo-protein, which reduces the effect of metal toxicity in plant cells. Heavy metals accumulated in plant tissues in high concentration affect the metabolic activities in cells (**Sinha *et al*., 2006 and Mittler 2002**) have been reported.

Amylase activity was reduced by 86% at undiluted waste water, consequently, decreased sugar content in *E. crassipes* leaves (Table 3). The activity of amylase is important for sugar formation from starch in plants. Some antioxidants (proline content and activities of catalase and peroxidase) determined in leaves were inhibited at each dilution level of industrial effluent (Table 3). Maximum inhibitory effect in biochemical constituents including antioxidants estimated in test plants found at undiluted industrial waste water. At this stage, tissue concentration of heavy metals in root (Cr, 392; Ni, 70; Zn 50.6 and Cu, 34.4 µg g^{-1} dry weight) and shoot (Cr, 80.5; Ni, 30; Zn. 20.7 and Cu 10.8 µg g^{-1} dry weight) was quantified. The reduction in biochemical constituents in test plants could be attributed due to suppressed antioxidants (catalase and peroxidase activities and protein, proline and carotenoid contents) possibly an indication of failure in defense system of plants (**Mittler, 2002**).

The decline in proline content in *E. crassipes* was might be due to toxic effects of Cr and Zn in industrial waste water on enzymes and metabolism involved in proline synthesis (**Rout and Shaw, 1998**). Due to the activity of catalase and peroxidase, H_2O_2 produced in response to heavy metals - stresses gets converted into non toxic H_2O in cells. Decline in catalase and peroxidase activities also regarded as a general response to many stresses and it is supposedly due to inhibition of enzyme synthesis or a change in assembly unit (**Gerendas *et al*., 1999**). The reduction in biochemical constituents estimated in plants was less at diluted industrial waste waters.

Relative water content was decreased with increase in waste water concentrations in *E. crassipes* (Figure 3). Due to richness of organic and inorganic elements in waste water the ratio of water intake inside the cell reduces. Higher concentrations of heavy metals like Cr and Ni damage the cell membrane constituents, leakage the cell sap and reduce the water content in cells has been reported (**Sinha *et al*., 2005**).

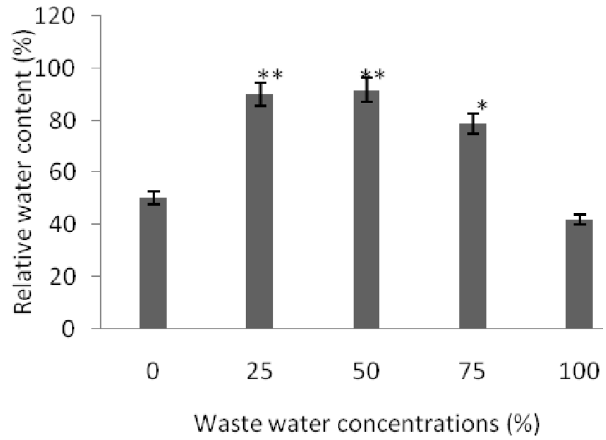

Fig. 3. Effect of industrial waste water concentrations on relative water content (RWC) in *Eichornia crassipes* (Mart.) plants. *- significant at P<0.05 and **- significant at P<0.01 levels. T- show ±S.E. value (n=3)

Industrial waste water (Unnao district, U.P., India) contained high content of heavy metals (Cr, Cu, Ni and Zn). *E. crassipes* accumulated higher content of Cr in order Cr>Ni>Zn>Cu. The accumulation of heavy metals was dose dependent and more in root than the shoot but their translocation from root to shoot was variable and affected with concentration of heavy metals in industrial waste water. Test plants exhibited visible symptoms of toxicity and altered biochemical constituents including antioxidative enzymes and biomolecules in response to excess heavy metals in industrial waste water. Thus, *E. crassipes* may be used for phytoextraction of Cr in contaminated water. These finding may be helpful in phytoremedial approaches to excess heavy metals particularly Cr and Ni in surface water bodies.

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