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ORIGINAL ARTICLE

Attenuation of Chromium toxicity in mine waste water using water hyacinth

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The mine waste water at South Kaliapani chromite mining area of Orissa (India) showed high levels of toxic hexavalent chromium (Cr^{+6}). Cr^{+6} contaminated mine waste water poses potential threats for biotic community in the vicinity. The current field based phytoremediation study is an *in situ* approach for attenuation of Cr^{+6} from mine waste water using water hyacinth (*Eichhornia crassipes*) weeds by rhizofiltration method. The weeds significantly reduced (up to 54%) toxic concentrations of Cr^{+6} from contaminated mine waste water when passed through succeeding water hyacinth ponds. The reduction of toxic chromium level varied with the plant age and passage distance of waste water. Chromium phytoaccumulation and Bio-Concentration Factor (BCF) was maximum at growing stage of plant i.e. 75 days old plant. High BCF (10,924) and Transportation Index (32.09) for water hyacinth indicated that the weeds can be used as a tool of phytoremediation to combat the problem of *in situ* Cr contamination in mining areas.

Key words: *Chromium stress / Mine waste water / Phytoremediation / Water hyacinth*

Abbreviations: WH: Water hyacinth ponds. DAT: Days After Transplantation

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Chromium exists in two stable states, i.e. hexavalent chromium (Cr^{+6}) and trivalent chromium (Cr^{+3}) of which Cr^{+6} is the most toxic form. Open cast chromite mining activity leads to various environmental problems due to released Cr^{+6}.Contamination of soil and water in chromite mining areas is a serious environmental problem (ATSDR 1998; USEPA 1998; Misra *et al.* 2004). India is the second largest producer of chromites in the world. The state of Orissa in India accounts for 98% of total chromite reserves of the country and the South Kaliapani chromite mine area of Orissa contributes about 97% of the total chromite reserve of the state (IBM, 2004). As a result of growing open cast mining activities in the area, the environment in the vicinity is under threat.

Chromium toxicity affects the plant growth and metabolism to a considerable extent, which includes stunted growth, chlorosis, reduced crop yield, delayed germination, senescence, premature leaf fall, biochemical lesions, enzymatic changes and reduced biosynthesis (Panda and Patra 1997a; Panda and Patra 1997b; Panda and Patra 1998; Zayed *et al.* 1998; Srivastava *et al.* 1999; Zayed and Terry 2003; Mohanty *et al*. 2005a; Mohanty *et al.* 2009; Mohanty *et al.* 2010a; Mohanty *et al.* 2010b). The Chromium contaminated mine waters and soils are nutrient deficient and exhibit stress impacts on plant growth and microbial populations. Due to extreme poor regeneration capacity of the soil, nutrient recycling is affected which becomes the constraint for plant growth (Zayed and Terry 2003; Mohanty *et al*. 2005a; Mohanty *et al.* 2009; Mohanty *et al.* 2010b).

Plant based bioremediation technologies have been collectively termed as phytoremediation, this refers to the use of green plants and their associated micro biota for the in-situ treatment of contaminated soil and ground water (Sadowsky 1999; EPA 2000). The use of green plants to remove pollutants from the environment or to render them harmless is phytoremediation (Salt *et. al.* 1998). This technique is a cost effective plant based approach for removal of heavy metals from soil (Chaney *et. al.* 1997; Jena *et. al.*, 2004; Ghosh and Singh 2005a) and water (Terry and Banuelos 2000; Mohanty *et al*. 2005b; Mohanty and Patra 2011). The technique of phytoremediation can be achieved through different methods like phytoextraction, rhizofiltration, phytostabilisation, phytovolatilisation and phytodetoxification.

Water hyacinth growing luxuriantly in ponds and channels has been considered as a worst weed that chokes the flowing water system. The weed is a free floating tropical perennial aquatic herb with a developing root system and has been shown useful for effective rhizofiltration of toxic elements in natural and constructed wet lands (Zhu *et al.* 1999).

With a view to manage the problem of Cr^{+6} contamination in mine waste water, the present work was undertaken to attenuate toxic Cr^{+6} by phytoremediation technology. The investigation includes a preliminary assessment of physico-chemical parameters of contaminated soils and mine waste waters in the study sites. The periodic attenuation of toxic Cr^{+6} levels in mine waste water was monitored using water hyacinth weeds as a tool of phytoremediation. The water hyacinth weeds (*Eichhornia crassipes* Mart. Solms-Laub) were grown in experimental ponds with running untreated Cr^{+6} contaminated mine waste water. The study encompasses the phytoaccumulation ability of the weeds for attenuation of Cr^{+6} from mine waste water with reference to Bio Concentration Factor (BCF), Total Accumulation Rate (TAR) and Transportation index (Ti). The present *in situ* aquatic phytoremediation protocol emphasizes the rhizofiltration and phytoextraction ability of water hyacinth in attenuating the toxicity load of Cr^{+6} from irrigated mine waste water.

The paper provides the information on existing sources of chromium in the study sites, its remediation through bioaccumulation in root and shoots of the aquatic weed (Water hyacinth). The study was an effort to know the severity of chromium pollution in the environment and its attenuation through a designed *in situ* aquatic phytoremediation programme.

MATERIALS AND METHODS

Study Site

The study was undertaken at South Kaliapani chromite mine area of Orissa, India. It is located within latitudes 20^0 53’ and 21^0 05’ and longitudes 85^0 40’ and 85^0 53’ (Fig. 1). The experimental ponds of 2,000 sq ft. were selected for cultivation of water hyacinth plantlets near the mining site of Orissa Mining Corporation (OMC). The plot design was made showing the passage of irrigated untreated mine waste water through successive experimental ponds of water hyacinth (Table 1.). Cr^{+6} contaminated mine waste water (untreated) was passed through each experimental pond (size: 25′ x 20′ x 2′) in a zigzag manner with a coverage of an area of 500 sq. ft passage route to 2000 sq. ft. (for 4 consecutive ponds).

Plant Materials

The study was conducted by using water hyacinth weeds (*Eichhornia crassipes* (Mart.) Solms-Laub) which served as tool of phytoremediation. These aquatic weeds were used for attenuation of Cr^{+6} from contaminated untreated mine waste water.

Sampling of water, soil and plants

The samplings of soil, water and aquatic weed from the experimental site (excavated pond) were carried out as follows. The soil was air-dried for 5 days, and ground to the desired soil size (<2 mm). The sieved soils were analyzed for physico-chemical parameters. The mine waste water samples were used for filling the water hyacinth ponds. The sampling of mine waste water was conducted before and after its passage through different experimental water hyacinth ponds after 75 and 100 days of plant growth. The mine waste water analysis was carried out for estimation of physico-chemical parameters before and after its passage through different ponds. Water hyacinth weeds growing in different experimental ponds were sampled during 75 and 100 days after transplantation. Plantlets before transplantation and after 75 and 100 days of growth were collected from different experimental ponds and analyzed for total Cr content in root and shoots (Bonet *et al.* 1991). Before analysis of total Cr and total Fe content, the roots were rinsed with 0.01N HCl followed by washing with distilled water for removing mixed Fe and Cr hydrous oxides, which may have precipitated on the root surfaces.

Analysis of physico-chemical parameters:

The untreated mine waste water used for filling the experimental ponds was analyzed for pH, Electrical Conductivity (E.C.), Total Dissolved Solids (TDS), $PO4^{-3}$, P, NO_3-N, NO_3^-, Ca, Mg, Na and Cr^{+6} (APHA 1995). Soil analyses were conducted for pH, E.C. and other chemical parameters, i.e., TDS, $PO4^{-3}$, P, NO_3-N, NO_3^-, Cr, Ca and Mg (APHA 1995). The above analyses were made as per Soil and Irrigation Water (SIW) Analysis Manual (HACH 1992) using the SIW kit. Untreated mine waste water after its passage through different ponds of water hyacinth was analyzed for its Cr^{+6}content using HACH-DR-890 colorimeter (APHA 1995). The analyses were performed for waste water collected at different passage distances i.e., 500, 1000, 1500 and 2000 sq. ft. and also with different age of the plants i.e., 75 and 100 Days after transplantation (DAT). The flow of water to experimental ponds was slow (900 ml/min) and continuous. The amount of Cr^{+6} levels in mine waste water reduced significantly which is positively correlated with the biomass of the growing plants. The harvested plants were separated into shoot and root parts, oven-dried at 70 ^{0}C for 72 h, powdered and digested using a solution of HNO3:HClO4 (10:1 v/v) for Cr analysis in a Inductively Coupled Plasma Atomic Emission Spectrometer.

Bio Concentration Factor (BCF), Total Accumulation Rate (TAR) and Transportation index (Ti) were analyzed as per following formulae (Zurayk *et al.* 2002; Ghosh and Singh 2005b).

$$BCF = \frac{\text{Cr concentration in plant tissue (mg/kg)}}{\text{Initial concentration of chromium in the external nutrient solution (mg/kg)}} \times 100$$

$$TAR = \frac{\text{(Shoot concentration x Shoot biomass + Root concentration x Root biomass)}}{\text{[(Shoot biomass+ Root biomass) x days of growth]}}$$

N.B.: TAR (mg/kg/day), Biomass (gm dry wt.) and Concentration (mg/kg dry matter)

$$Ti = \frac{\text{Cr concentration of leaves (mg/kg)}}{\text{Cr content of root (mg/kg)}} \times 100$$

Statistical Analysis and Presentation of Data:

Soil, water and plants were sampled in triplicates each and the data presented in the figures and tables are AM (arithmetic mean).

Table 1 Passage area coverage by mine waste water through different experimental ponds at South Kaliapni chromite mine area of Sukinda, Orissa, India

Treatments	Pond number	Area of passage (sq. ft)
Mine waste water	-	0
Water hyacinth ponds	1	500
	2	1000
	3	1500
	4	2000

Table 2 Physico-chemical parameters of the experimental excavated pond soil.

PH	7.2
E.C. (mS/cm)	0.05
W.H.C (%)	36.32
PO_4^{-3}-P (Kg/ha)	24.25
NO_3^{-}N (Kg/ha)	20.75
Organic C (mg/kg)	31.5
Exchangeable K (Kg/ha)	194
Total Cr (mg/kg dry soil)	11170
Total Fe (mg/kg dry soil)	223415

Table 3 Physico-chemical properties of Cr^{+6} contaminated mine waste water.

pH	E.C. (mS)	TDS ($mScm^{-1}$)	PO_4^{-3} (mgl^{-1})	P (mgl^{-1})	NO_3^--N (mgl^{-1})	NO_3^- (mgl^{-1})	Ca & Mg (mgl^{-1})	Na ($meql^{-1}$)	Cr^{+6} (mgl^{-1})
8.4	0.5	0.125	0.81	0.37	2.96	13.03	2.2	7.78	0.66

Table 4 Bio-Concentration Factor (BCF), Transportation index (Ti) and Total Accumulation Rate (TAR) of Water hyacinth weeds from different experimental Ponds.

Treatment ponds	Ti			Bio Concentration Factor of Total Plant			TAR ($mg\ kg^{-1}\ Day^{-1}$)
	Days After Transplantation			Days After Transplantation			
	75	100	125	75	100	125	
1st	10.25	74.66	32.09	17127.71	13804.18	10924.15	0.43
4th	3.76	36.48	12.84	11944.27	6162.54	5422.60	1.17

RESULTS

Physico-chemical parameters of the soil

The physico-chemical parameters of the soils of excavated ponds were presented in Table 2. Soils were slightly alkaline with low concentrations of N, P and K. The chromium (11170 mg/kg dry soil) and iron contents of the soil (2, 23, 415 mg/kg dry soil) were high as compared to normal soil whereas the NO_3-N content of experimental excavated pond soil was low.

Physico-chemical parameters of mine waste water

Cr^{+6} levels in mine waste water was 0.66 mg/l. High alkaline pH value (8.4) and Cr^{+6} content of mine waste water are presented in Table 3. Phosphorus (P) and NO_3-N concentration were much less as compared to normal irrigation water and found to be 0.37 mg/l and 2.96 mg/l respectively (Table 3). The Cr^{+6} level in the irrigated mine waste water was 0.65 mg/l which exceeds the toxic limit value of 0.008 mg/l. Na content (7.78 mg/l) was found high. Ca and Mg content (2.2 mg/l) was low. Irrigated mine waste water from different experimental ponds were sampled and analyzed for Cr^{+6} content. Cr^{+6} levels in mine waste water were found to decrease with increasing area of water passage through successive experimental water hyacinth ponds at 75 DAT (Fig. 2A) and 100 DAT (Fig. 2B). After 100 days of transplantation of the weeds, the reduction of Cr^{+6} level was from 21% to 54% in running mine waste water of successive ponds having 2000 Sq ft. passage distance. The reduction of Cr^{+6} level in mine waste water was higher when the weeds were grown in ponds for 100 days as compared to 75 days. It was also observed that the pond wise sequential reduction of Cr^{+6} level in the supplied mine waste water was significant as evident from percent reduction values of Cr^{+6} (Fig. 2A and Fig 2B). The reduction of Cr^{+6}concentration in running mine waste water indicated a change from toxic (0.65 mg/l) to non toxic (0.07 mg/l) state when passed through a distance of 2000 sq. ft. (area of four successive cultivated ponds) for 100 days-grown water hyacinth weeds. The level of Cr^{+6} of irrigated waste water reflects the value of available Chromium in growing plant parts and the data are presented in Fig 3.

Chromium bioavailability in water hyacinth weeds

Total Chromium content in roots and shoots showed significant differences in its distribution (Fig. 3). Cr accumulation was more in roots than shoots during all stages of plant growth. Maximum accumulation of total Cr was observed in roots of the plants on 100 DAT in comparison to 75 days after transplantation. Aerial parts of the plants showed 10 to 100 fold less Cr as compared to roots.

Cr accumulation and bio-concentration factor (BCF) generally increased with age of the plants as well as with increase in external Cr availability in water. BCF (10,924) and Transportation index (Ti) values (32.09) were very high for water hyacinth. Ti values of different plants indicated that root to shoot translocation of Cr was very high after 100 days of plant growth. All the plants showed a general trend of fall in Ti values for Cr in 125 days grown plants as compared with 100 days grown water hyacinth plants and varies with increase in chromium concentration. Bio-concentration factor (BCF) of Cr was maximum at highest growing period of water hyacinth plants (with high biomass) which depict Cr is accumulating in plants up to75 days of growth (Table 4).

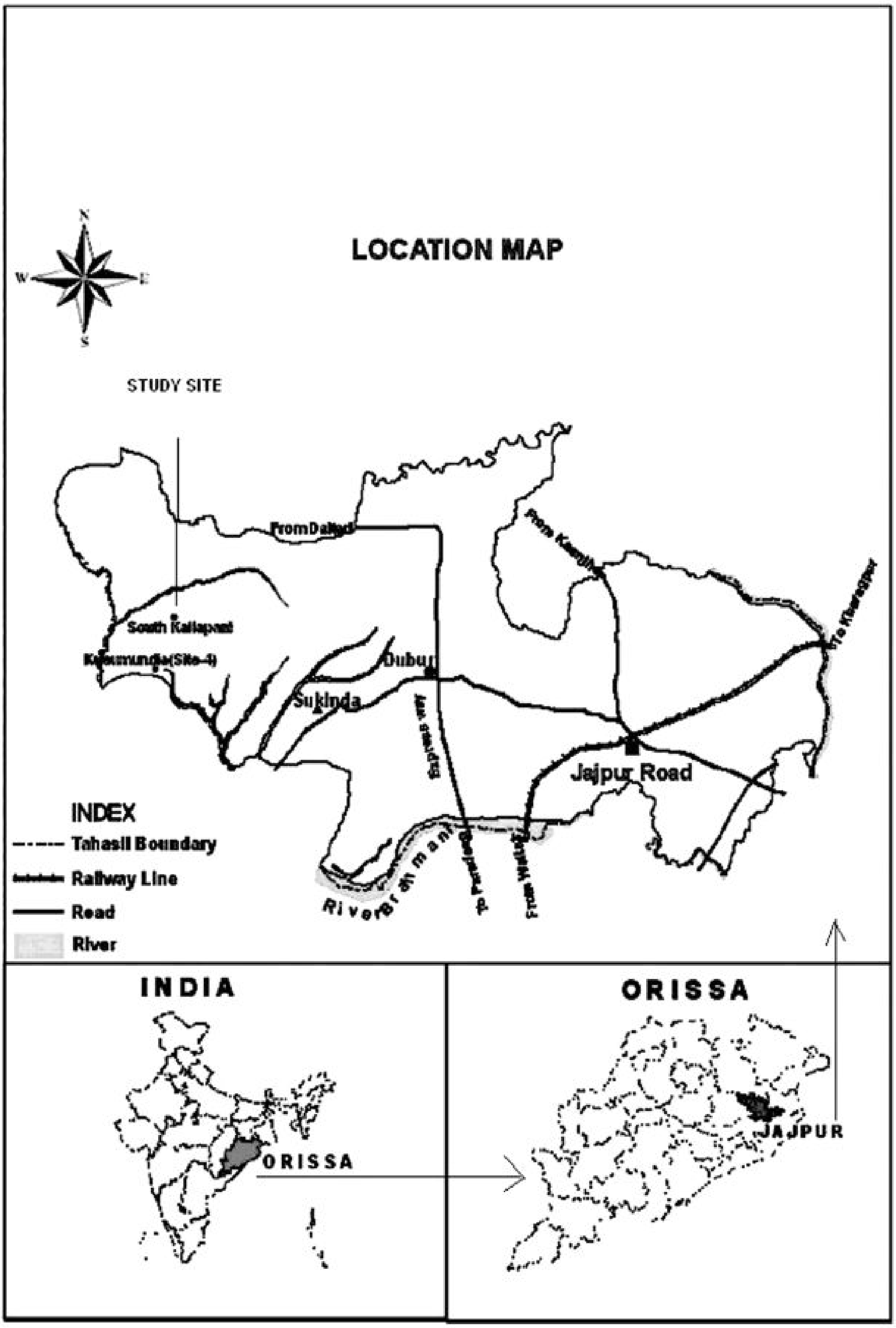

Figure 1 Map showing location of study site (South Kaliapani) in Jajpur district of Orissa.

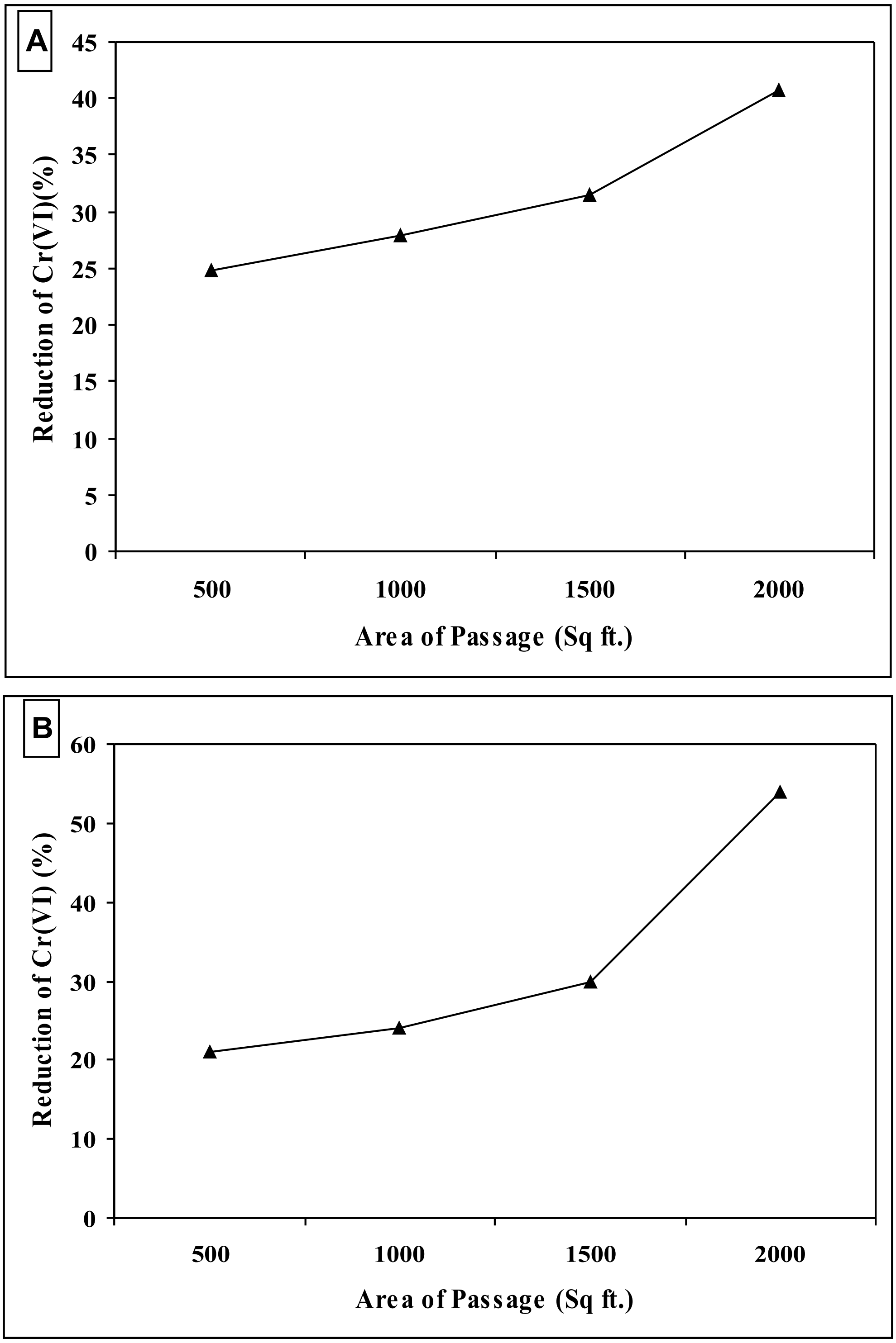

Figure 2 (A&B). Reduction percent of Cr+6 content from irrigated mine waste water after passage through ponds filled with water hyacinth. (**A**: 75 DAT; **B**: 100 DAT)

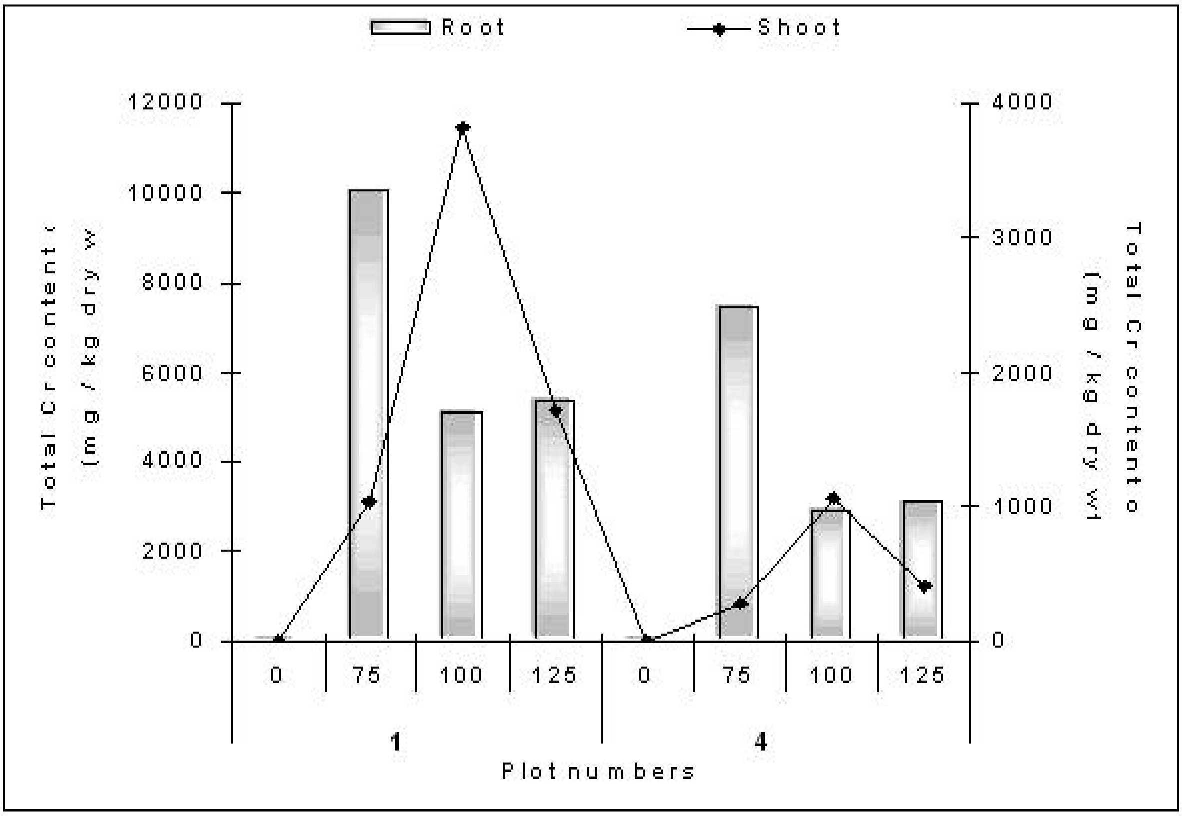

Figure 3 Total Cr content of Water hyacinth collected from different experimental plots on 0, 75, 100 and 125 DAT.

DISCUSSION

The soil chromium content at the experimental excavated ponds exceeds the recommended guideline as given for a natural soil (30-300 mg/kg as described by Katz and Salem 1994). Low NO_3-N content of soil may be due to negative correlation of N-Mineralization with contamination level (Bath 1989). Total Cr content of the pond soil was very high (11,170 mg/kg dry soil) in comparison to the natural soil which shows the range from 05 - 3000 mg/kg dry soil (Zayed and Terry 2003; Krishnamurthy and Wilkens 1994; Pawlisz 1997). The soil at the experimental pond can be categorized as serpentine one as it has the range of Cr concentration between 634 –125, 000 mg/kg dry soil (Adriano 1986).

Cr^{+6} concentrations in mine waste water exceeded the permissible limits as described for fresh water system: 0.001 mg/l; irrigation water: 0.008 mg/l and drinking water: 0.01 mg/l (Krishnamurthy and Wilkens 1994; Pawlisz 1997; Mohanty and Patra 2011). Less remediation of Cr^{+6} by 75 days old water hyacinth weeds as compared to 100 days old plants may be attributed to their low biomass yield.

Higher Cr bioaccumulation in roots in comparison to shoots was also reported earlier by several workers (Katz and Salem1994; Zayed and Terry 2003; Pulford and Watson 2003; Ghosh and Singh 2005a; Dong *et al.* 2007; Zhang *et al.* 2007; Erenoglu *et al.* 2007). High bioavailability of Cr in roots and its low translocation to shoots is a common phenomenon (Dickinson and Lepp 1997;

Zayed and Terry 2003). Chromium concentration in plants (grown in "normal" soil) is usually less than 1 mg/kg, rarely exceed 5 mg/kg, and is typically in the order of 0.02 – 0.2 mg Cr/kg dry weight (Zayed and Terry 2003). The high Cr accumulation in root cells was supported by Shanker *et al*., (2004) who suggested immobilization of chromium from the vacuoles. The amount of chromium increased from 0 to 75 days and from 75 to 100 days but the Cr content decreased from 100 to 125 days which depicts that the accumulation of chromium was non-linear and showed a negative correlation. The recovery of Cr by the weeds from mine waste water can be interpreted from the values of total accumulation rate (TAR). From BCF values it was also observed that root to shoot translocation has been increased up to the plant age of 100 days but after that there was an deterioration in plant growth and biomass which may be attributed to the toxic impacts of Cr^{+6} in mine waste water filled ponds. This indicates that the water hyacinth weeds have the ability to translocate Cr from root to shoot up to 100 days growth. Its subsequent compartmentalization in plant organelle was studied by Shanker *et al*. (2004). Better translocation is advantageous for phytoextraction as it can reduce Cr concentration and thus reduce toxicity potential to the root. However, Cr translocation to the shoot is one of the resistance mechanisms to overcome high Cr concentration in the soil (Ghosh and Singh, 2005b).

Thus, water hyacinth can be used as tools of rhizofiltration and phytoextraction to combat the problem of *in situ* Cr contamination. The extensive and massive fibrous root system of water hyacinth could be a helpful means for filtering out the metal pollutants at the miming sites to use the mine waste water for irrigation purpose. Further, future phytoremediation strategies will be planned to reduce chromium bioavailability in plants which will give a suggestive measure for the farmers growing crops at the chromium contaminated sites.

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