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Full Length Research Paper

Bioaccumulation of metals from tannery sludge by *Typha angustifolia* L.

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The metal bioaccumulation capability of a common anchored hydrophyte, *Typha angustifolia* L. was studied in a green house trial. The plants could absorb significant amounts of the heavy metals like chromium, copper and zinc from tannery sludge. Different concentrations of tannery sludge were prepared in water and plants of *T. angustifolia* were exposed to the sludge for 30, 60 and 90 days. A significant reduction in sodium chloride percentage, chlorides and chemical oxygen demand (COD) was observed. The percentage reduction in all metals was significant. Cadmium and lead were found to be totally absent. A greater reduction of metals was observed in 30% concentration of sludge. A maximum reduction of 62% for Na, 42% for Cr, 38% for Cu and 36% for Zn was observed in 30% sludge after 90 days exposure of *T. angustifolia*. The bioaccumulation potential of *T. angustifolia* was greater for the heavy metals especially for Cr. The maximum metal uptake, observed after 90 days exposure of *T. angustifolia*, was 6,698 mg kg^{-1} for sodium, 20,210 mg kg^{-1} for chromium, 16,325 mg kg^{-1} for zinc and 7,022 mg kg^{-1} for copper in the roots. In shoots, the uptake was 3,745 mg kg^{-1} for sodium, 10,150 mg kg^{-1} for chromium, 3,509 mg kg^{-1}.for copper and 7,025 mg kg^{-1} for zinc. Aerial parts of *T. angustifolia* accumulated less heavy metal than the corresponding roots. *T. angustifolia* is suitable for the decontamination of most of the harmful metals from tannery sludge.

Key words: Bioaccumulation, tannery sludge, heavy metals, *Typha angustifolia*.

INTRODUCTION

There are reports on wetland plants like *Typha, Phragmites, Scirpus, Leersia, Juncus* and *Spartina* in reducing the levels of heavy metals in polluted waters (Vajpayee et al. 1995; Neralla et al., 1999; Deng et al., 2004; Weis and Weis, 2004; Shankers et al., 2005; Zhang et al., 2007). Such hyperaccumulator plants can be exploited for treatment of metals-containing wastes (Ensley, 2000). Until now such emergent hydrophytes have been used in phytoremediation of effluents in constructed wetland systems but have never been used to reduce heavy metal pollution in sludge. These wetland plants can be used for reducing heavy metals in toxic sludge such as the tannery sludge.

The sludge is considered a source of potentially toxic elements and its disposal is problematic especially if it contains several heavy metals. The production of sludge is increasing day by day as a result of wastewater treatment. It is generally bulky with a high moisture content and its composition may range from highly organic to mineral depending on its origin. Sewage sludge is being used as fertilizer in many countries. A potential strategy to remediate sludge is the use of plants to remove pollutants from the habitat or to render them harmless (Lasat, 2002). A wastewater treatment plant has been set up in Kasur, Pakistan, in order to minimize pollution before throwing the effluents of leather industry into Rohi Nullah, falling into the River Sutlej. In this treatment unit, the Kasur Tannery Waste Management Agency (KTWMA), a large quantity of toxic sludge is obtained due to a high amount of metals contained in it. It is being dumped into a landfill site after drying.

Metal bioaccumulation in hydrophytes depends upon numerous biotic and abiotic factors, such as temperature, pH and dissolved ions in water (Demerizen and Aksoy, 2004). There are reports indicating that some species may accumulate specific heavy metals, for example *Spirodela polyrhiza* accumulating Zn (Markert, 1993).

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The present study was carried out to determine the reduction in the metal content of tannery sludge by growing *Typha angustifolia* (Narrow Leaf Cattail) and the accumulation of metals in different parts of the plant.

MATERIALS AND METHODS

Samples of sludge were collected from the Kasur Tannery Waste Management Agency (KTWMA) located at the Depalpur Road, Kasur, Pakistan. Sludge samples were collected in plastic drums and transported to the Department of Botany, Punjab University, Lahore, Pakistan.

For a preliminary experiment, different percentage concentrations of dry sludge (w/v): 20, 40, 60, 80 and 100% were prepared in plastic pots of 4 L capacity by homogenously mixing the sludge with tap water. Tap water was used as control. Plants of the aquatic hydrophyte, *T. angustifolia* were collected from the Botanical Garden, University of the Punjab, Lahore, Pakistan. Three plants of uniform size and biomass were grown in each treatment with three replicates of each. The maximum percentage concentration that the plants could tolerate was observed.

Based upon the result of preliminary experiment, the sludge concentrations selected for the actual experiment were 30 and 60% along with control (0%). The experiment was set up in a wire house having a glass roof in the Department of Botany in "Completely Randomized Design" with factorial arrangement (Steel and Torrie, 1981). Plants were grown in different concentrations of sludge in triplicates. Three plants of uniform sizes were grown in each pot. The experiment comprised three treatments with three replicates each, to be analyzed at three monthly intervals.

A detailed physico-chemical analysis of the wet sludge was carried out to determine pH, conductivity, total dissolved solid (TDS), NaCl, chemical oxygen demand (COD), anions such as carbonates, bicarbonates and chlorides and metals. The pH, conductivity, TDS, and NaCl percentage was measured with the help of a multi-parameter Meter (Hanna, Model HI 98107) and a conductivity meter (Hanna Model HI 9835), respectively. COD was determined according to the procedure described by Greenberg et al. (1998). Chlorides were measured titrimetrically. The estimation of Ca^{++}, Na^+ and K^+ was done on a Flame photometer (PFP7 and PFP7/C JENWAY) while estimation of heavy metals was done on an Atomic Absorption Spectrophotometer (AA-1275/ VARIAN, Australia).

After retrieval, the plants were acid digested according to the method described by Greenberg et al. (1998). The digested samples were again subjected to metal analysis by flame photometry and atomic absorption spectrophotometry.

Statistical analyses

The statistical analyses of the data were carried out using Analyze-it-Free Software available on the web.

RESULTS

In the preliminary experiment, plants of *T. angustifolia* started to wither, with the leaves wilting, followed by chlorosis and decay in higher concentrations within a week. After some days, the plants completely lost their vigor, showing a disability to tolerate the 80 and 100% concentrations of sludge. Thus, these concentrations were not included in the actual experiment and further experiment was carried out with Control (0%), 30, and 60 concentrations.

The results of some parameters like pH, conductivity, TDS, NaCl percentage, chlorides and COD are shown in Figure 1. The pH and conductivity values of different sludge concentrations showed a slightly increasing trend after the growth of plants of *T. angustifolia* in the different percentages while increase in the TDS content was significantly more pronounced, increasing both with increasing concentration and increasing period of plant growth. On the other hand, a significant reduction in the NaCl content was observed for all sludge concentrations after growing plants of *T. angustifolia.* The reduction showed an increase with increasing period of growth of plants. The reduction was more pronounced with the maximum percentage level of sludge. The chlorides and COD values showed similar reduction with increasing growth period of plants.

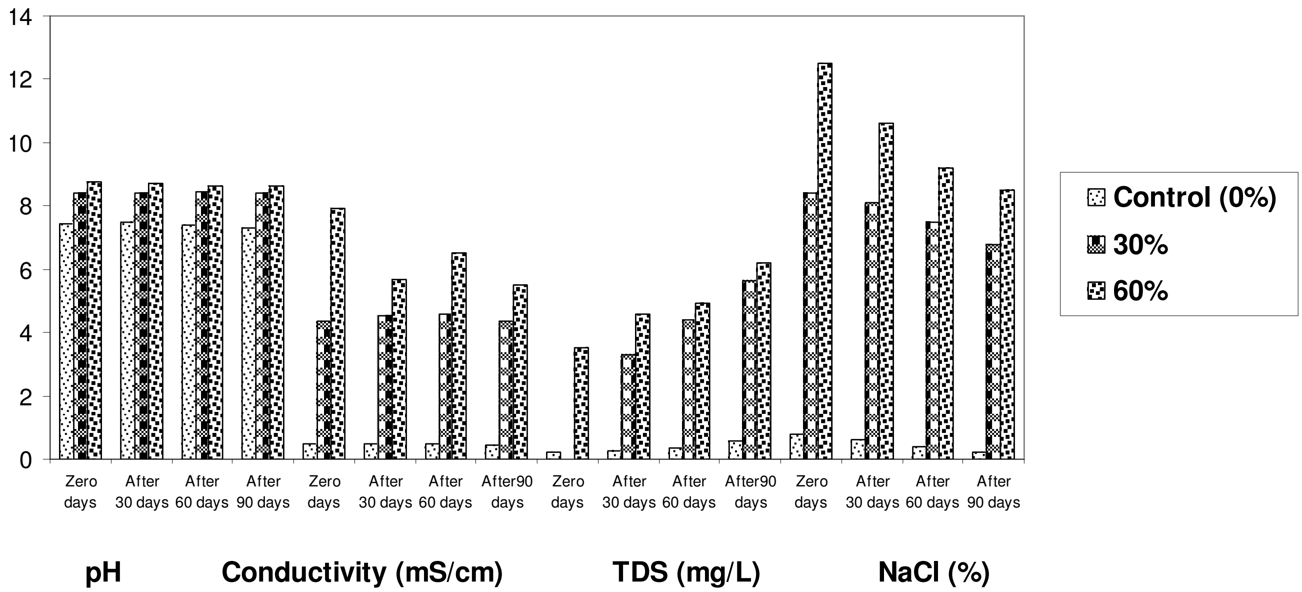
The metal contents of the different sludge percentages were determined before introducing plants of *T. angustifolia.* The amounts of metals were in the order sodium> chromium> zinc> copper, while cadmium and lead were totally absent. It was observed that metal contents of all sludge percentages were significantly reduced (Figure 2). The highest percentage reduction was observed for Na, while heavy metal reduction was maximum in case of Cr (Figure 3). On the whole greater percentage reductions occurred in 30% sludge concentration as compared to 60%, especially for heavy metals.

In all cases metal uptake was higher in the roots than shoots and was almost double the amount in case of sodium. The root to shoot difference was not so drastic in case of heavy metals showing a better translocation of heavy metals (Figures 4 and 5).

Among all metals, chromium showed the maximum uptake by plants of *T. angustifolia* (Figures 4 and 5). The general trend in the uptake of these metals showed a good correlation of their corresponding amounts in the sludge i.e. Cr> Zn> Cu.> Na. The amount of metals increased with increasing concentration of sludge and with increasing exposure period of plants in the sludge. The maximum metal uptake was observed after 90 days growth; 6,698 mg kg^{-1} for sodium, 20,210 mg kg^{-1} for chromium, 16,325 mg kg^{-1} for zinc and 7,022 mg kg^{-1} for copper in the roots. While the amount was almost reduced to half for sodium (3,745 mg kg^{-1}) and to two thirds in heavy metals; 20,210 mg kg^{-1} for chromium, 7,025 mg kg^{-1} for zinc and 3,509 mg kg^{-1} for copper in shoots. Translocation of heavy metals from roots to shoots was much better as compared to sodium. The values for bioaccumualtion of metals were quite high for Cr and Cu as compared to Zn (Table 1). The least bioaccumulation was observed for Na.

DISCUSSION

Metal uptake and bioaccumulation studies have been done by several workers in aquatic plants, both for hydrophytes in natural populations and in experiments.



L.S.D P=0.05	pH	Conductivity	TDS	NaCl
For time	0.107	1.290	1.320	3.228
For conc.	0.107	1.290	1.320	3.228

Figure 1. Changes in some physico-chemical parameters in different percentages of tannery sludge before and after growing *Typha angustifolia.*

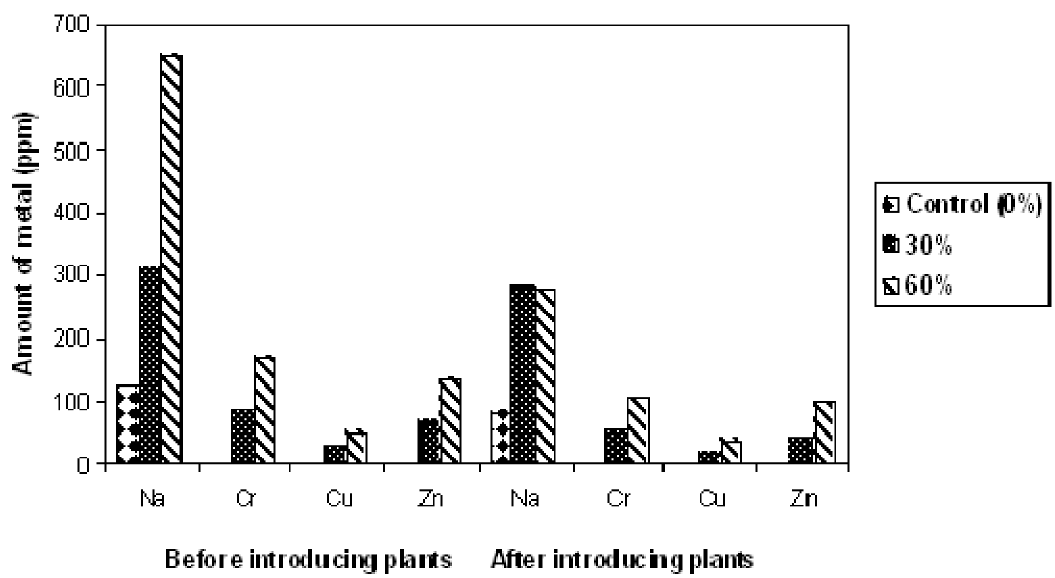


Figure 2. Amount of metals detected in different percentages of tannery sludge before and after introducing *Typha angustifolia.*

All kinds of hydrophytes like floating *Azolla rubra, Lemna gibba, Lemna minor, Lemna trisulca, Nelumbium speciosum, Pistia stratiotes, Salvinia molesta* and *Salvinia herjogii*; anchored floating *Nymphaea spontanea* and *Nymphaea stellata*; anchored submerged *Chara corallina,* Calluna *vulgaris, Ceratophyllum demersum, Hydrilla, verticillata, Mentha aquatica, Myriophyllum aquaticum, Myriophyllum spicatum, Najas indica, Potamogeton crispus, Potamogeton natans, Potamogeton pectinatus, Potamogeton pusillus* and *Vallisneria spiralis*; and anchored emergent *Alternanthera sessilis, Juncus articulatus, Ludwigia palustris, Persicaria* sp., *Phrag-*

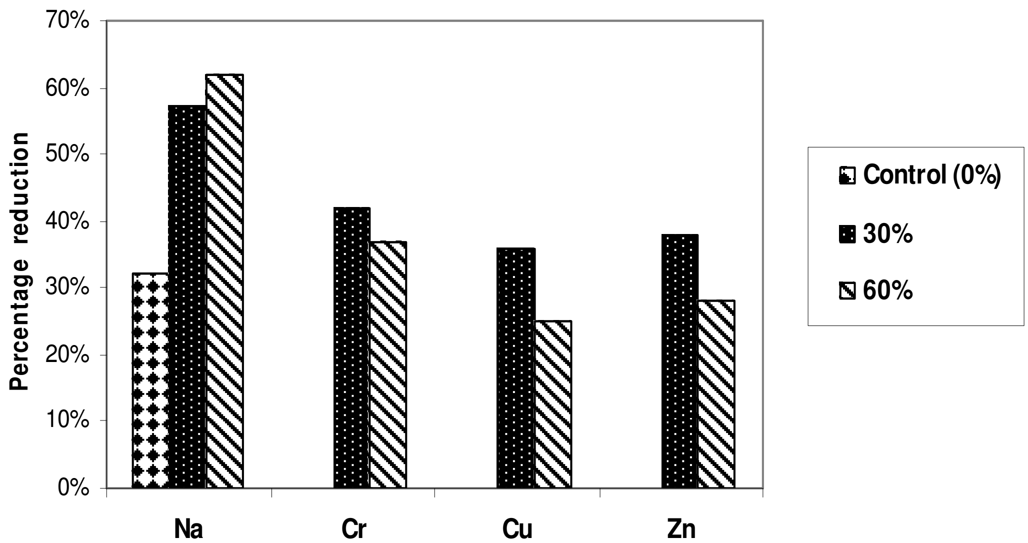
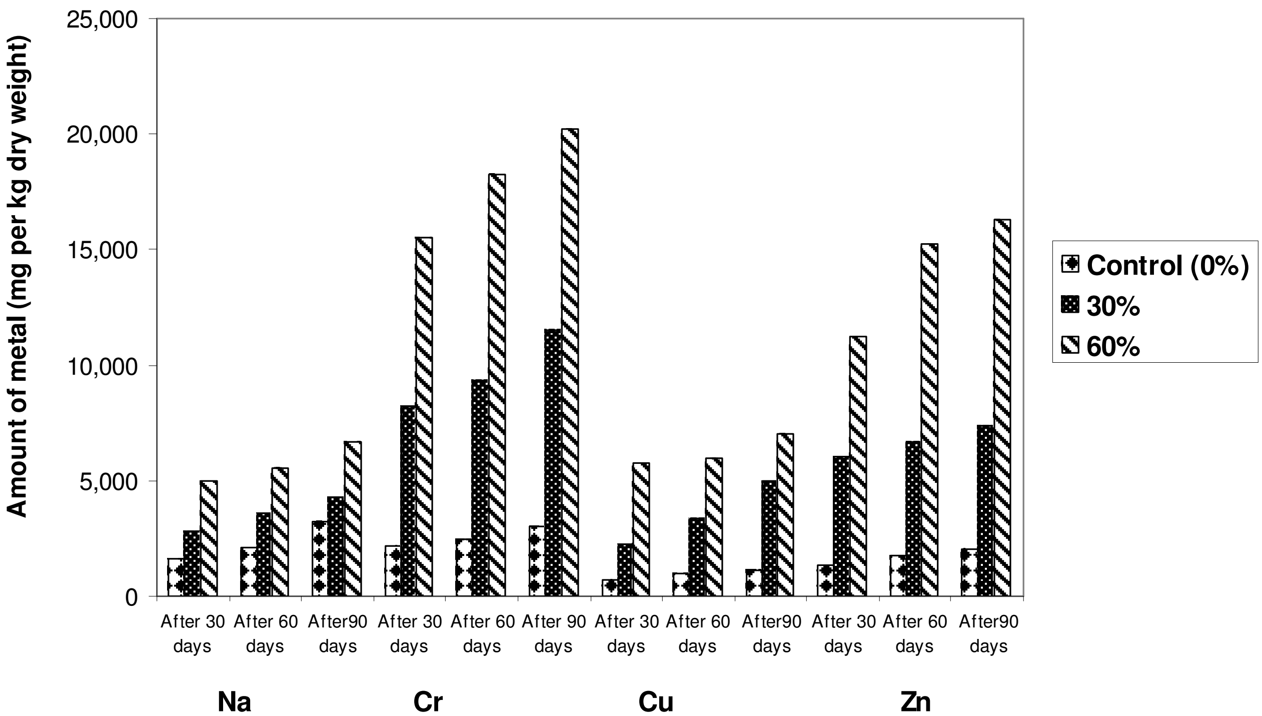


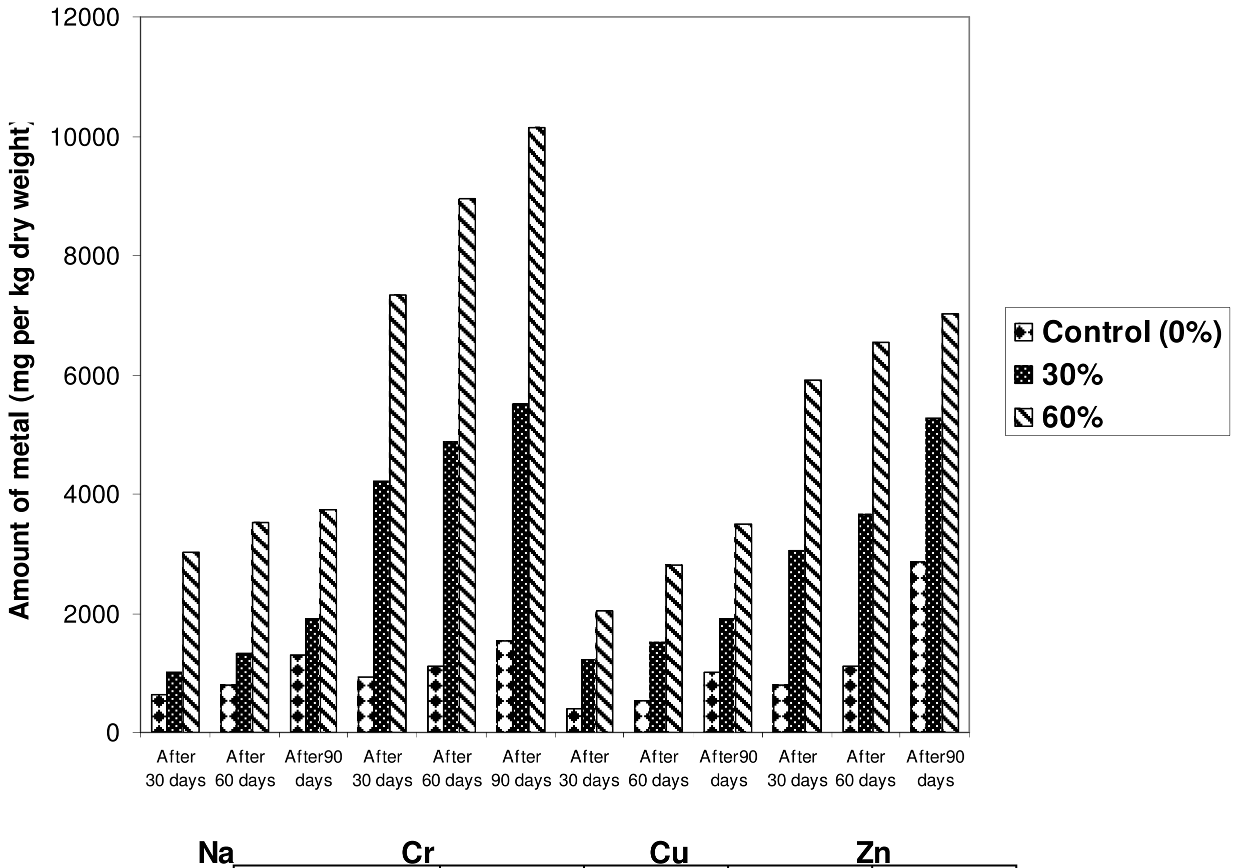
Figure 3. Reduction observed in different percentages of tannery sludge after growing *Typha angustifolia* for 90 days.



L.S.D P=(0.05)	Na	Cr	Cu	Zn
For time	50.02	71.92	40.97	59.78
For conc.	55.25	79.43	45.25	66.02

Figure 4. Amount of heavy metals in roots of *Typha angustifolia* growing in different percentage of tannery sludge.

mites sp. *Sagittaria sagittilia, Scirpus* sp. And *Spartina* sp. have been studied (Rai et al., 1995; Cardwell et al., 2002; Sinha et al., 2002; Kamal et al., 2004; Keskinkan et al., 2004; Maine et al., 2004; Shiny et al., 2004; Weis and Weis, 2004; Mkandawire et al., 2004; Mkandawire and Dudel, 2005; Shankers et al., 2005; Choo et al., 2006; Fritioff and Gregor, 2006; Vardanyan and Ingole, 2006; Demirezen et al., 2007).



L.S.D P=(0.05)	Na	Cr	Zn	Cu
For time	32.67	43.99	32.18	21.58
For conc.	36.08	48.59	35.54	23.83

Figure 5. Amount of heavy metals in shoots of *Typha angustifolia* growing in different percentage of tannery sludge.

Table 1. Coefficient of bioaccumulation in roots and shoots of *Typha angustifolia* after 90 days exposure in different concentrations of sludge.

Metals	Concentration	Amount of metal in sludge concentrations	Amount of metal in roots after 90 days	Amount of metal in shoots after 90 days	Coefficient of bioaccumulation in roots	Coefficient of bioaccumulation in shoots
Na	**30%**	312.5	4,306	1,902	13.77	6.08
	60%	652.3	6,698	3,745	10.26	5.74
Cr	**30%**	85.3	11,520	5,512	135.0	64.61
	60%	172.3	20,210	10,150	117.29	58.90
Cu	**30%**	30.6	4,954	1,908	161.89	62.35
	60%	51.3	7,022	3,509	136.88	68.40
Zn	**30%**	68.2	7,406	5,280	108.59	77.41
	60%	135.1	16,325	7,025	120.84	51.99

In Pakistan, tanning is one of the most prominent and potentially polluting industry in terms of waste generation, containing rather high concentrations of metals, particularly Cr. Chromium merits a special reference for its extreme toxicity due to the interaction of its compounds with living cells (Costa, 1997). In chromium uptake and bioaccumulation studies, prepared concentrations of chromium have been used in experiments (Sinha et al., 2002; Maine et al., 2004; Shiny et al., 2004; Choo et al., 2006). In a few cases, hydrophytes have been used to remove metals from tannery effluent concentrations (Sinha et al., 2002). This is the first study of removal of metals from toxic tannery sludge concentrations using an anchored emergent hydrophyte, *T. angustifolia* without any obvious symptoms of toxicity. It can be employed as an on-site remediation strategy at the treatment plant, so that the sludge can be used as a fertilizer in the cultivated fields.

Metals in tannery waste occur in complex form and vary widely in their availability to the plants. Plants that hyperaccumulate metals have a tremendous potential for application in remediation of metals in the environment. *T. angustifolia* has proved to be one such hydrophyte with good hyperaccumulation capabilities. In the hyper-accumulator plant, *Leersia hexandra*, chromium bioaccumulation in the leaves has been observed to be 5,005 mg kg $^{-1}$ DW while in the roots it was higher 18,656 mg kg $^{-1}$ from prepared culture solutions of Cr. *T. angustifolia* showed a better bioaccumulation of the same metal up to 10,150 mg kg $^{-1}$.

Significant metal uptake was observed in *T. angustifolia* at higher concentrations of sludge and increased with increasing concentration of sludge. This ability to tolerate high amount of metal levels is apparently associated with the ability of the plant to limit translocation of metals to the shoot. Increasing zinc concentration in sediments caused an increase in plant parts in *Typha* and *Persicaria* (Cardwell et al., 2002). This is in line with the present finding showing lesser concentration of metals in sludge as compared to a higher concentration in plant roots. Demirezen and Aksoy (2004) have made similar observations in *T. angustifolia*, where a higher amount of metals in roots was observed as compared to the sediments in which they were growing. Moreover, they think that the short life cycle of *T. angustifolia* is the main reason for more metals in roots than in shoots.

In the present study a higher amount of metals was observed in the roots than in shoots. Even though, the amount of metals was less in the leaves, but it was as high as 2,215mg kg^{-1} of K in case of essential metals and as high as 10,150 mg kg^{-1} of Cr in case of heavy metals. Whereas in case of roots, maximum metal uptake was observed as 6,698 mg kg^{-1} for Na and 20,210 for Cr after 90 days of exposure. Similar observations have been made by many workers for many other plants; in *Typha* and *Persicaria* by Cardwell et al. (2002), in *N. indica*, *V. spiralis* and *A. sessilis* by Sinha et al. (2002), in *P. stratiotes* and *S. herzogii* by Maine at al. (2004), in many aquatic macrophytes from lakes by Vandanyan and Ingole (2006), in *N. spontanea* by Choo et al. (2006), in *P. natans* by Fritioff and Greger (2006) and in *Leersia hexandra* by Zhang et al. (2007).

In the hyperaccumulator plant, *L. hexandra*, chromium bioaccumulation in the leaves has been observed to be 5,005 mg kg^{-1} dw while in roots it was as high as 18,656 mg kg^{-1} from prepared culture solutions of Cr. *T. angustifolia* even showed a better bioaccumulation of the same metal up to 10,150 mg kg^{-1} in the leaves and 20,210 mg kg^{-1} in the roots from the concentrated metal content present in the sludge. Thus, this plant can qualify as a hyper-accumulator according to the criterion given by Baker and Brooks (1989).

The percentage reduction in the metal content of the sludge after a 90 day exposure of *T. angustifolia*, typically in case of chromium, signifies the fact that regular growth and harvesting of these plants in specially prepared treatment ponds with a 30 - 40% sludge concentration for three such cycles will completely remove most of the metals from tannery sludge whereby it can be applied as fertilizer in fields.

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