

Accumulation of metals in selected macrophytes grown in mixture of drain water and tannery effluent and their phytoremediation potential

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Abstract

Phytoremediation is an emerging, ecofriendly and economically feasible technique for the restoration of heavy metals contaminated environment. In the present investigation, five native macrophytes growing naturally in a drain receiving tannery effluent viz *Bacopa monnieri*, *Eichhornia crassipes*, *Hydrilla verticillata*, *Ipomoea aquatica* and *Marsilea minuta* were evaluated for their heavy metal (Cr, Cu, Ni and Pb) accumulation potential in field conditions at Unnao, U.P., India. The results showed that metal accumulation by these macrophytes differed among species and tissue parts. The concentration of Cr, Cu, Ni and Pb in the root tissues were estimated in the range 3.38-45.59, 1.01-16.85, 1.81-4.43 and 1.02-4.24 $\mu\text{g g}^{-1}$ d.wt., whereas the corresponding shoot values were 8.79-48.81, 1.01-8.67, 0.84-2.89 and 1.02-2.84 for Cr, Cu, Ni and Pb respectively. Among the studied plants the translocation factor (TF) ranged between 1.07-2.60, 0.75-3.83, 1.44-2.57 and 0.49-3.76 for Cr, Cu, Ni and Pb, respectively. The highest metal TF was found in *M. minuta* (2.60, 3.83 and 2.57) for Cr, Cu and Ni respectively, whereas Pb was best translocated (3.76) by *B. monnieri*. Roots and shoots of the studied macrophytes showed a value of greater than 1 for metal enrichment coefficient. Findings suggest that *E. crassipes* can be used for phytoremediation of Cu and Ni whereas *M. minuta* and *H. verticillata* can be applied for the removal of Cr and Pb respectively from the contaminated water bodies.

Publication Data

Paper received:
30 March 2011

Revised received:
10 October 2011

Accepted:
18 October 2011

Key words

Enrichment coefficient, Heavy metals, Macrophytes, Phytoremediation, Translocation factor

Introduction

Contamination of the aquatic environment by the heavy metals is a serious environmental problem, which threatens aquatic ecosystems, agriculture and human health (Sasmaz et al., 2008). Heavy metals are persistent in nature and have the potential to accumulate in various sediments of the environment (Mishra and Tripathi, 2008). Conventional metal removal and mobilization techniques include sedimentation, adsorption, complexation, reverse osmosis, ion exchange, electrodialysis etc. (Dunbabin and Bowmer, 1992). Most of these technologies are quite costly, energy intensive and metal specific (Mishra and Tripathi, 2008). Contrary to this, use of plants for heavy metal removal from waste water offers a promising technology (Miretzky et al., 2004; Mishra and Tripathi, 2009). Aquatic

macrophytes have enormous potential to accumulate heavy metals inside their body from the liquid environments (Rai et al., 1995; Maine et al., 2006; Phetsombat et al., 2006; Singh and Singh, 2006; Sasmaz et al., 2008; Kousar and Puttalah, 2009). Therefore these macrophytes have been used for heavy metal removal from contaminated water bodies (Mishra et al., 2009; Rolli et al., 2010). A plant is said to be a hyperaccumulator; if it can concentrate the pollutants in a minimum percentage which varies according to the pollutant involved (for example: more than 1000 mg kg^{-1} of dry weight for nickel, copper, cobalt, chromium or lead; or more than 10,000 mg kg^{-1} for zinc or manganese) (Backer et al., 1994). This capacity for accumulation is due to the adaptative evolution of the plants to hostile environments through many generations. Many

scientists have focused on accumulation of heavy metals by aquatic macrophytes (Fritioff and Gregor, 2006; Radic *et al.*, 2010). In addition some have also studied the phytoremediation potential of aquatic macrophytes for contaminated sediment and water environment (Hinchman, 1998; Gratao *et al.*, 2005; Chehregani *et al.*, 2009). Still ample scope is available regarding the heavy metal removal/accumulation potential of different plant groups. Therefore present study was performed to evaluate the metal accumulation potential of selected macrophytes and to determine their large scale applicability for treatment/restoration of polluted water bodies.

Materials and Methods

The site selected for the study was Loamy drain adjacent to Leather Technology Park, Banthar, situated in Unnao district of Uttar Pradesh. Five different plant species collected from the vicinity were *Bacopa monnieri*, *Eichhornia crassipes*, *Hydrilla verticillata*, *Ipomoea aquatica* and *Marsilea minuta*. The plant samples were first washed with running tap water followed by distilled water to remove extraneous matter. After washing, the plant parts were separated, chopped and oven dried at 65°C for 24 hr. Effluent samples were collected, stored and analyzed for physicochemical characteristics following the standard method of APHA (2005).

Drain water and plants were analyzed for Cr, Cu, Ni and Pb on Atomic Absorption Spectrophotometer (AA 240 FS, Varian) after digesting the samples in the mixture of nitric and perchloric acid (5:1 v/v) following the method of Sahu *et al.* (2007).

Metal translocation factor (TF) and Enrichment coefficient (EC): The EC was calculated to derive the degree of contamination and heavy metal accumulation in growing medium (drain water) and in plants growing at the contaminated site (Kisku, 2000). The TF or mobilization ratio was calculated to determine relative translocation of metals from the growing medium to other parts (root and shoot) of the plant species (Barman *et al.*, 2000; Gupta *et al.*, 2008).

Results and Discussion

Physico-chemical analysis of drain water: The results of physicochemical parameters including heavy metal analysis of drain water are presented in Table 1. The drain water was slightly alkaline with pungent odor and dark brown color. The pH is generally acknowledged to be the principle factor governing concentration of soluble and plant available metals (Malviya and Rathore, 2007). The high turbidity may be because of dissolved solid and salts which may exert harmful effects on the aquatic life, not necessary due to their toxic effects but also due to the changes in the osmotic pressure (Hinchman *et al.*, 1998). The drain water was having high concentration of solids i.e. 7681.20, 1873.12 and 9554.32 mg l⁻¹ for TDS, TSS and TS respectively. Total alkalinity and hardness of water was recorded as 654.26 and 455.04 mg l⁻¹ respectively. As compared to other ions, carbonates and bicarbonates share the most part of the total alkalinity. Principle cations imparting hardness are Ca²⁺ and Mg²⁺ but other cations such as Fe²⁺ and Mn²⁺ may

also contribute to the hardness. The anions responsible for the hardness are mainly carbonate, bicarbonate, sulphate, fluoride, nitrate and silicate (Sahu *et al.*, 2007). Sulphate, chloride and phosphate were recorded as 86.30, 437.41 and 3.87 mg l⁻¹ respectively whereas potassium, sodium and calcium were recorded as 7.44, 98.63 and 67.63 mg l⁻¹ respectively. The ions may become part of drain water because of their interaction with the soil system and/or due to use of various inorganic chemicals in the industrial processes. BOD and COD of drain water were much higher than their respective prescribed standard i.e. 30 and 250 mg l⁻¹, respectively (Table 1) for the final discharge of tannery effluent in to the inland surface water (Malviya and Rathore, 2007).

The concentration of Cr, Cu, Ni and Pb were found to be 4.67, 0.26, 0.08 and 0.04 mg l⁻¹ respectively. The higher concentration of Cr in the drain water was may be because of the use of chromic acid during tanning process. The higher levels of Cr found is a matter of concern because this metal is known to be toxic (Mishra and Tripathi, 2009) Similar results in tannery waste water were reported by Sahu *et al.* (2007).

Table-1: Physico-chemical characteristics of drain water

Parameter	Effluent
pH	7.58 ± 0.06
Odour	Pungent
Colour	Dark brown
Turbidity	38.44 ± 5.13
TDS	7681.20±482.49
TSS	1873.12±195.88
TS	9554.32±613.11
Total Alkalinity	654.26 ± 50.83
Total hardness	455.04 ± 49.77
Sulphate	86.30 ± 5.60
Chloride	437.41 ± 21.87
DO	0.62 ± 0.04
BOD	342.89 ± 24.91
COD	1420.06 ± 98.61
Phosphate	3.87 ± 0.13
Potassium	7.44 ± 1.64
Sodium	98.63 ± 8.72
Calcium	67.63 ± 6.17
Metals	
Copper	0.26±0.01
Chromium	4.67±0.31
Nickle	0.08±0.01
Lead	0.04±0.01

All the values are in mg l⁻¹ (except pH, odour, colour and turbidity)

Accumulation of heavy metals by macrophytes: The heavy metal concentration in the roots and shoots of aquatic macrophytes collected from the drain receiving tannery effluent are shown in figure 1(A-D). In the present study, all the aquatic macrophytes have accumulated Cr, Cu, Ni and Pb at varied levels in their tissues. Cr is a non essential element and its compounds are highly toxic and detrimental to the growth and development of the plants (Mishra

and Tripathi, 2008). In the present study, the total Cr concentration ranged from 3.38 to 45.59 $\mu\text{g g}^{-1}$ d.wt. in root and 8.79 to 48.81 $\mu\text{g g}^{-1}$ d.wt. in shoots. The concentration of Cr was found more in shoots as compared to roots in all the macrophytes. Cr is easily absorbed by roots and then transported via the vascular system (Leghouchi *et al.*, 2009). The Cr concentration in all the plants was found above the standard given by SEPA (SEPA, 2005) *i.e.*, 0.5 $\mu\text{g g}^{-1}$ d.wt., FAO, WHO *i.e.* 5.0 $\mu\text{g g}^{-1}$ d.wt. and <0.2 $\mu\text{g g}^{-1}$ d.wt. (Codex Alimentarius Commission, 1984; Awasthi, 2000; IPCS, 1998), except in the roots of *M. minuta*. The roots and shoots of macrophytes accumulated Cr in the order of: *B. monnieri* > *E. crassipes* > *H. verticillata* > *I. aquatica* > *M. minuta*.

Although Cu is an essential micronutrient for normal plant metabolism; it has been reported to be toxic at high concentration (Li and Xiong, 2004). The highest Cu (16.85 $\mu\text{g g}^{-1}$ d.wt.) concentration was observed in the roots of *E. crassipes* and the lowest (1.01 $\mu\text{g g}^{-1}$ d.wt.) in *H. verticillata*, whereas in shoots, the highest Cu content (8.67 $\mu\text{g g}^{-1}$ d.wt.) and lowest (1.01 $\mu\text{g g}^{-1}$ d.wt.) in *I. aquatica* respectively. Excessive accumulation of Cu in plant tissue can be toxic affecting several physiological and biochemical processes and growth. Cu treatment brings changes in nitrogen metabolism with a reduction in total nitrogen (Llorens *et al.*, 2000). It results in increase of free amino acid (Mishra and Tripathi, 2008) Cu levels found in the roots and shoots of the aquatic macrophytes were recorded as 30 and 20 $\mu\text{g g}^{-1}$ d. wt. which are within the Indian standard (Awasthi, 2000; SEPA, 2005). The pattern of Cu accumulation in roots and shoots was as follows: *E. crassipes* > *M. minuta* > *B. monnieri* > *I.*

aquatica > *H. verticillata* and *E. crassipes* > *B. monnieri* > *H. verticillata* > *M. minuta* > *I. aquatica*, respectively.

The Ni concentration in normal plants ranges between 0.022 to 5.00 $\mu\text{g g}^{-1}$ d. wt. (Bowen *et al.*, 1966). The critical toxic concentration of Ni in plants has been found in range from 8 to 220 $\mu\text{g g}^{-1}$ d.wt. as suggested by McNichol and Beckett (1985) and 10 to 100 $\mu\text{g g}^{-1}$ d.wt. as suggested by Kabata-Pendias and Pendias (1992). Accumulation of Ni greater than 5.0 $\mu\text{g g}^{-1}$ d.wt. may induce toxic response in plants. The Ni concentration in the studied plants ranged between 1.81 to 4.23 and 0.84 to 2.89 $\mu\text{g g}^{-1}$ d.wt. in roots and shoots respectively. The Ni concentration in the roots of all the plants was beyond the safer limit as per Indian Standard (1.5 $\mu\text{g g}^{-1}$ d.wt., Awasthi, 2000) whereas in shoots, it was within the limit except for *B. monnieri* and *E. crassipes*. Results showed that Ni was poorly translocated to shoots. The Ni accumulation pattern was observed as *E. crassipes* > *B. monnieri* > *H. verticillata* > *M. minuta* > *I. aquatica* and *E. crassipes* > *B. monnieri* > *H. verticillata* > *I. aquatica* > *M. minuta* for roots and shoots respectively.

Kabata-Pendias and Pendias (2001) reported that Pb content of plants grown in uncontaminated areas varied in between 0.05 to 3.0 $\mu\text{g g}^{-1}$ d.wt. The highest Pb concentration was measured in the roots of *H. verticillata* (4.24 $\mu\text{g g}^{-1}$ d.wt.) whereas the lowest was found in shoots of *M. minuta* (1.02 $\mu\text{g g}^{-1}$ d.wt.). The concentration of Pb in plant roots ranged between 1.02 to 2.88 $\mu\text{g g}^{-1}$ d.wt. The Pb concentration in the roots of *B. monnieri*, *H. verticillata* and *I. aquatica* and in shoots of *E. crassipes* and *I. aquatica* was

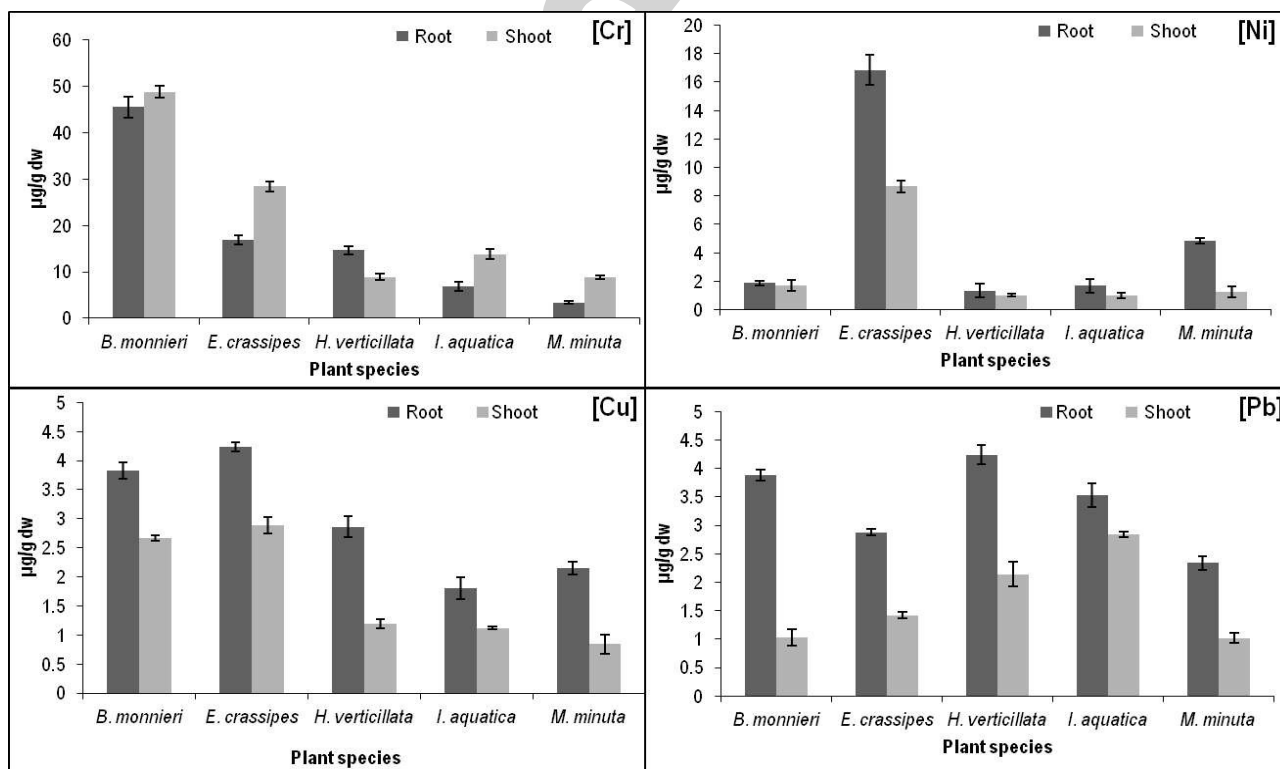


Fig. 1: Concentration of Cr, Cu, Ni and Pb in roots and shoots of different macrophytes grown in mixture of drain water and tannery effluent.

Table- 2: Translocation of heavy metals from soil to different parts of macrophytes grown in mixture of drain water and tannery effluent

Plants	Cr			Cu			Ni			Pb		
	ECS	ECR	TF	ECS	ECR	TF	ECS	ECR	TF	ECS	ECR	TF
<i>Bacopa monnieri</i>	10.42	9.74	1.07	7.39	6.66	1.11	48.68	33.91	1.44	94.40	25.11	3.76
<i>Eichhornia crassipes</i>	6.07	3.60	1.69	66.53	34.24	1.94	53.79	36.77	1.46	70.02	34.57	0.49
<i>Hydrilla verticillata</i>	1.89	0.10	1.66	5.32	3.99	0.75	36.37	15.23	2.39	103.19	52.07	1.98
<i>Ipomoea aquatica</i>	2.96	1.46	2.02	6.66	3.99	1.67	22.99	14.26	1.61	85.92	69.11	1.24
<i>Marsilea minuta</i>	1.88	0.72	2.60	19.22	5.02	3.83	27.29	10.64	2.57	56.93	24.88	2.29

Values are means of three replicates. ECS: Enrichment coefficient for shoot, ECR: Enrichment coefficient for root, TF: Translocation factor

found to be more than the safer limits as per Indian Standard (2.5 $\mu\text{g g}^{-1}$ d.wt., Awasthi, 2000). The Pb accumulation pattern was observed as *H. verticillata* > *B. monnieri* > *I. aquatica* > *M. minuta* > *E. crassipes* with respect to root and *E. crassipes* > *I. aquatica* > *H. verticillata* > *B. monnieri* > *M. minuta* with respect to shoot. The metal accumulation results observed in the present study are in good agreement with other workers (Dwivedi et al., 2008; Mishra and Tripathi, 2009; Galletti et al., 2010; Xue et al., 2010).

Translocation factor may be used to assess a plants potential for phytoremediation purpose. For rooted plants partitioning of metals between roots and shoots becomes an important factor to consider them suitable for phytoremediation purpose (Gratao et al., 2005). TF of metals in the studied macrophytes are presented in Table 2. It ranged between 1.07 to 2.60, 0.75 to 3.83, 1.44 to 2.57 and 0.49 to 3.76 for Cr, Cu, Ni and Pb respectively. The difference in TF indicates the preferential accumulation/uptake and translocation of metals. Translocation factors higher than 1 were determined in metal accumulator species whereas TF was typically lower than 1 in metal excluder species. TF greater than 1, indicates an efficient ability to transport metal from root to shoot, most likely due to efficient metal transporter system and probably sequestration of metals in leaf vacuoles and apoplast (Lasat et al., 2000). In the present study, all the plants showed a root to shoot translocation factor of >1 for all the metals except for Cu and Pb in case of *H. verticillata* and *E. crassipes*, respectively. Average translocation factor studied for these plants showed that *M. minuta* (2.82) effectively transported metals from roots to shoots followed by *B. monnieri* (1.84) and *H. verticillata* (1.69). These macrophytes can be used for the phytoremediation of aquatic water bodies contaminated with heavy metals. Potential of aquatic plants for phytoremediation purpose is well recognized (Rai et al., 1995). Some aquatic plants including *Hydrilla*, *Eichhornia* and *Bacopa* have been extensively studied by various authors and have been found to accumulate high levels of Cr, Cu, Ni and Pb (Dwivedi et al., 2008; Xue et al., 2010). Various other studies demonstrated waste water treatment and phytoremediation potential of plants like *Eichhornia* and *Marsilea* sps (Gothberg and Gregor, 2006).

Enrichment coefficient is a very important factor which indicates phytoremediation potential of a given species (Zhao et al., 2003) An enrichment coefficient factor greater than 1 indicates special

ability of plants to absorb and transport metals from media and stored them in their body parts (Sasmaz et al., 2008). In the present study, the EC ranged between 1.88 to 10.42, 5.32 to 66.53, 22.99 to 53.79 and 56.93 to 103.19 for Cr, Cu, Ni and Pb, respectively with respect to shoot whereas corresponding root values ranged between 0.10 to 9.74, 3.99 to 34.24, 10.64 to 36.77 and 24.88 to 69.11, respectively. The metal concentration and the EC in shoots were invariably higher than in the roots.

Results demonstrate that these macrophytes have accumulated several times higher concentration of Cr, Cu, Ni and Pb as compared to waste water concentration of these metals. Based on the finding, *E. crassipes* may be recommended for phytoremediation of aquatic bodies contaminated by Cu and Ni whereas *B. monnieri* and *H. verticillata* can be used for the removal of Cr and Pb respectively.

Acknowledgments

The authors are highly thankful to the Director, IITR and Head, Department of Environmental Science, BBA University, Lucknow for providing infrastructure facilities.

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