Bull. Environ. Contam. Toxicol. (2002) 68:540–548

DOI: 10.1007/s00128-001-0288-3

Bulletin of Environmental Contamination and Toxicology

Accumulation of Chromium and Scanning Electron Microscopic Studies in *Scirpus lacustris* L. Treated with Metal and Tannery Effluent

M. R. Suseela, S. Sinha, S. Singh, R. Saxena

National Botanical Research Institute, Rana Pratap Marg, P.B. No. 436, Lucknow-226 001 (U.P.), India

Received: 25 June 2001/Accepted: 15 December 2001

Chromium enters the aquatic environment from both natural and anthropogenic sources (Kimbrough et al, 1999). The major industrial sources of Cr are tanning, metallurgy, pigment, dye, textile manufacturing and electroplating. Cr contributes significantly towards the pollution of water resources. India is one of the largest leather exporting country which plays a vital role in the country's economy. However, the rapid growth of this industry is not without constraints. The tannery effluent, being discharged into water bodies after treatment from Common Effluent Treatment Plant, Unnao, UP, resulted in higher concentrations of Cr than recommended permissible limit (0.5 µg ml^{-1}). It has caused irreversible damage to the water resources, soil and agriculture due to constant deposition of metals. The tannery effluent contains variety of minerals that constitute a rich source of plant nutrients (Dhaneshwar,1990).

In view of the ability of aquatic and wetland plants to absorb, translocate and concentrate the metals (Sinha 1999, Qian et al. 1999, Naqvi and Rizvi 2000), there has been much interest in the use of constructed wetlands for the removal of pollutants from wastewater (Huddleston et al. 2000). The use of plants to improve water quality in municipal and more recently industrial wastewater treatment system is of great use and an emerging technology of phytoremediation (Brown et al. 1995, Salt et al. 1995). Hansen et al. (1998) also reported the use of two species of *Scirpus in* a constructed wetland for the removal of selenium from oil refining wastewater. The plant-based treatment systems are currently used in developed countries. However, in India these biological treatment systems are yet to be exploited on large scale.

The effects of metals on physiological and biochemical parameters in aquatic plants has been studied (Gupta et al. 1994, Sinha et al. 1996, 1997), however, the attention has not been paid on morphoanatomical changes induced by metals in aquatic plants. These changes may appear before any visible symptom of toxicity in the treated plants. The present study was planned to assess the Cr accumulation potential of *Scirpus lacustris* L. (Common name-Bulrush) and morphoanatomical changes

NBRI Research Publication No. (508) NS
Correspondence to: S. Sinha

induced by the metal. The plant was selected for the present study as it has a conspicuous root zone system which facilitates oxygen from aerial part to underground part which mobilizes metal from soil to the plant.

MATERIALS AND METHODS

The rooted emergent plant, *S. lacustris* was collected from water bodies of Lucknow and maintained in large hydroponic tubs in Aquatic Botany Laboratory, National Botanical Research Institute, Lucknow. For experimental studies, the plants of *S. lacustris* were detached from mother plant and grown in sand for 30 days in 10% Hoagland's solution. The plants with almost the same biomass (18-g fresh weight) were kept in all the treatment and control sets. The plants were subjected to two different concentrations (4, 8 µg ml^{-1}) of Cr (VI) under natural diffuse light (1000-1400 E m^{-2} s^{-1}) and temperature ranging 28-34°C during summer season. The test concentrations (4 & 8 µg ml^{-1}) for the treatment of Cr were selected as the concentration in influent (7.8 µg ml^{-1}) and effluent (4.5 µg ml^{-1}) of tannery wastewater approximates the same. The test concentrations were verified by analysis and the chemical used was of AR Grade. Two sets of each concentrations (three separate containers for each concentrations served as three replicates) were kept in one litre beaker (800 ml solution) containing different concentrations (4, 8 µg ml^{-1}) of Cr in 10% Hoagland's solution along with one set of control in 10% Hoagland's solutions. Gravel was used to hold the roots of emergent macrophytes. The solution was changed on every 3rd day and the total exposure was of 15 days as the plants have shown lethal symptoms beyond this exposure period.

The tannery wastewater (influent and effluent) was collected manually in acid washed containers from Common Effluent Treatment Plant (CETP), Unnao, UP and brought to the field laboratory. Cr concentrations in the influent and effluent tannery wastewater were 7.8 and 4.5 µg ml^{-1} respectively. In tannery wastewater, Cr is present in the form of Cr (III) but it is taken up by the plant only in the form of Cr (VI). The plants of *S. lacustris* were treated with different concentrations (25, 50, 75 & 100%) of tannery wastewater (influent and effluent) along with one set of control in tap water. Three sets of each effluent concentrations served as triplicates and kept in one litre beaker (800 ml effluent) containing different effluent concentrations (25, 50, 75, 100%) and each set was harvested after 10, 15 and 30 days of exposure. The gravel was used to support the root of the plant and water level was maintained throughout the experiments with tap water. Harvested plants treated with Cr and tannery effluent were washed thoroughly with water. The roots and shoots of the plants were separated manually, dried in an oven at 80°C and kept for metal analysis. The roots and shoots were digested in HNO_3 (70%) using Microwave digestion system MDS 2000 and chromium was estimated by Atomic Absorption Spectrophotometer using air-acetylene flame gases at the

357.9 nm wave length. Cr content in control plants was below detection limit. Analysis of variance was performed for calculating the statistical significance of the results. Students t –test (two-tailed) was applied to see the significance level of the data. (Gomez and Gomez, 1984).

The roots and shoots of the plant treated with 8 μg ml^{-1} of Cr for one month were used for scanning electron microscopic studies, the plants treated with 4 μg ml^{-1} have shown no apparent change. The plant material was kept in 2.5% glutaraldehyde for fixation and left overnight. The material was passed through alcohol series for dehydration and drying was done in BAL-TEC CPD-030 critical point drier, using liquid CO_2 as carrier gas. The mounted specimens (six samples from each category) were coated with 15 nm thick gold and studied with Philips XL-20 Scanning Electron Microscope.

RESULTS AND DISCUSSION

The results of Cr accumulation in the roots and shoots of *S. lacustris* treated with 4 and 8 μg ml^{-1} of Cr for 7 and 15 days and concentration factors are shown in Table 1. The accumulation of Cr in the roots was

Table 1. Accumulation (μg g^{-1} dw) of Cr in the shoots and roots of *S. lacustris* at 7th and 15th day at different Cr (μg ml^{-1}) concentrations.

Conc.	Exposure Periods			
	7 d		15 d	
	Shoots	Roots	Shoots	Roots
4.0	25.19 ± 1.20 (6.29)	37.95 ± 1.17^{A} (9.48)	76.00 ± 1.59^{F} (19.0)	509 ± 35.2^{AE} (127.25)
8.0	54.62 ± 2.0^{C} (6.82)	377.76 ± 8.9^{BD} (47.22)	224.09 ± 1.25^{CF} (28.01)	708 ± 10.5^{BCF} (88.5)

Values in parentheses are the concentration factors. All the values are mean of three replicates ±SD. Root F (conc.) = 14.642^{a}. F (Exp.)=32.38^{a}; Shoot F (conc.)=2.24^{c}, F (Exp.)=3.45^{c}; ^{a} =p<0.01 ^{c} =p= Non significant. t-test ^{A}=p<0.01, ^{B}=p<0.001 compared to shoot; ^{C}=p<0.01, ^{D}=p<0.001 compared to 4 μg ml^{-1};^{E}=p<0.01, ^{F}=p<0.001 compared to 7 days.

significantly higher (p<0.01 at 4 μg ml^{-1}, p<0.001 at 8 μg ml^{-1}) than the shoots at both the exposure periods. The metal accumulation increased significantly with increase in concentrations in roots and shoots of the plant at 7 and 15 days. At both the concentrations, the accumulation of Cr was significantly higher at 15 days of exposure as compared to 7 days in both parts of the plants. The tissue concentration has been used as criteria for identifying hyperaccumulators plants (Reeves et al. 1996). In the present study, the concentration factor increased with increase in

Table 2. Accumulation ($\mu g\ g^{-1}$ dw) of Cr in the shoots and roots of *S. lacustris* at different concentrations of tannery wastewater (effluent) at different exposure periods collected from CETP, Unnao. Cr concentrations in effluent = 4.5 ± 0.40 $\mu g\ ml^{-1}$).

Conc. (%)	Exposure Period					
	10 d		15 d		30 d	
	Shoots	Roots	Shoots	Roots	Shoots	Roots
25	21.5 ± 1.5 (11.03)	27.3 ± 1.0 (13.99)	7.4 ± 1.5^{G} (3.81)	79 ± 2.6^{CH} (40.51)	27.6 ± 2.0^{G} (14.14)	78.5 ± 0.6^{C} (40.24)
50	26.3 ± 1.2 (6.75)	31.5 ± 1.6 (8.08)	13.8 ± 2.7^{F} (3.54)	78 ± 1.5^{CG} (20.00)	39.2 ± 1.6^{G} (10.04)	91.1 ± 8.6^{B} (23.36)
75	31.2 ± 3.5^{D} (5.33)	55.2 ± 5.9^{AE} (9.43)	13.9 ± 1.6^{F} (2.38)	78 ± 5.2^{BF} (13.33)	35.5 ± 1.8^{G} (6.08)	82.1 ± 4.8^{B} (14.04)
100	24.3 ± 0.4 (3.12)	34.5 ± 3.9 (4.42)	18.4 ± 2.6^{D} (2.35)	144 ± 10^{EHB} (18.46)	47.8 ± 1.2^{EG} (6.12)	99.6 ± 2.4^{EGC} (12.76)

Values in parentheses are the concentration factors. All the values are mean of 3 replicates ±SD. Root F (concentrations) = 1.45^{c}. F (Expo.) = 10.36^{b}. Shoot F (concentrations) = 3.60^{c} F (Expo.) = 30.52^{a}. $^{a}p<0.01$ $^{b}p<0.05$ ^{c}p= Non significant. t-test ^{A}=p<0.05, ^{B}=p<0.01, ^{C}=p<0.001 compared to shoot; ^{D}=p<0.05, ^{E}=p<0.01, compared to 25% effluent concentration; ^{F}=p<0.05, ^{G}=p<0.01, ^{H}=p<0.001 compared to 10 days.

exposure period and maximum (127.5) was found at 4 $\mu g\ ml^{-1}$ after 15 days of exposure. However, concentration factor decreased from 127.25 to 88.5 in the roots of the plant with increase in metal concentration (8 $\mu g\ ml^{-1}$). Similar results were also reported by Sinha and Chandra (1990).It has been reported that accumulation of metals varies from plant to plant and from one part to another. The accumulation of Cr in the roots of *S. lacustris* was much higher than the shoots. This fact suggests that translocation of Cr from root to shoot could be a limiting factor for the bioconcentration of the metal in shoot. Gupta et al. (1994) reported the accumulation (739 $\mu g\ g^{-1}$ dw) of Cr in the roots of the plant-lets of *S. lacustris* grown in Hoagland's solution and treated with 5 $\mu g\ ml^{-1}$ Cr for 7 days, however, the accumulation in shoots was 163 $\mu g\ g^{-1}$ dw. The ability of fine roots to accumulate high quantities of metal has also been reported (Sinicrope et al. 1992; Sinha, 1999). The results of the present study are in agreement with the findings of these authors showing higher accumulation of Cr in the roots of *S. lacustris*. Gupta et al. (1994) reported higher accumulation of Cr which is due to luxuriant growth of fine roots in Hoagland's solution.

The results of Cr accumulation from different concentrations (25, 50, 75, 100%) of tannery wastewater (influent & effluent) collected from CETP are shown in Table 2 & 3, respectively. The accumulation of Cr in the roots was found significantly higher than shoots at all the concentrations after 15 and 30 days and only in 75% concentration at 10 days. The comparison of different effluent concentrations showed no significant

Table 3. Accumulation ($\mu g\ g^{-1}$ dw) of Cr in the shoots and roots of *S. lacustris* at different concentrations of tannery wastewater (influent) at different exposure periods collected from CETP, Unnao. (Cr concentrations in the influent =7.8 ± 0.97 $\mu g\ ml^{-1}$).

Conc. (%)	Exposure Period					
	10 d		15 d		30 d	
	Shoots	Roots	Shoots	Roots	Shoots	Roots
25	48.5±0.0 (43.11)	30.0±1.2 (26.67)	6.0±0.9^{H} (5.33)	136±1.5^{CH} (120.89)	10.6±2.4^{G} (8.64)	52.1±0.3^{BH} (42.33)
50	44.8±2.8 (19.89)	49.5±4.3^{D} (22.00)	13.8±1.2^{G} (6.13)	351±15.2^{CFH} (156.0)	12.4±0.03^{G} (5.52)	65.4±1.1^{CH} (29.08)
75	47.5±4.5 (14.07)	62.2± 3.6^{ADB} (18.44)	12.8±1.3^{G} (3.79)	215±0.5^{CFH} (63.70)	15.7±0.6^{G} (4.64)	59.9±1.2^{CH} (17.74)
100	29.9±2.9 (6.66)	109.5±7.9^{F} (24.34)	10.9±1.0^{G} (2.42)	166±9.8^{BDG} (36.89)	12.9±1.3^{H} (2.88)	144±9.5^{BE} (31.95)

Values in parentheses are the concentration factors. All the values are mean of 3 replicates ±SD. Root F (concentrations) = 0.975^{c}. F (Expo.) = 7.064^{b}. Shoot F (concentrations) = 0.996^{c}. F (Expo.) = 42.002^{a}. $^{a}p<0.01$ $^{b}p<0.05$ ^{c}p= Non significant. **t-test** ^{A}=p<0.05, ^{B}=p<0.01, ^{C}=p<0.001 compared to shoot; ^{D}=p<0.05, ^{E}=p<0.01, ^{F}=p<0.001 compared to 25% influent concentration; ^{G}=p<0.01, ^{H}=p<0.001 compared to 10 days.

change in metal accumulation at lower effluent concentrations (upto 75%) at 15 and 30 days of exposure. At 100% effluent concentration, the metal accumulation showed significant increase in Cr content as compared to 25%. At 10 days, significant increase in metal accumulation was found at 75% effluent concentration as compared to 25% in both roots and shoots. The roots of the plant showed significant increase in metal content at 15 days as compared to 10 days in all the effluent concentrations, however, significant decrease at 30 days was found in 100% effluent concentration as compared to 15 days of exposure. In shoots, the accumulation of Cr decreased significantly in all the concentrations at 15 days compared to 10 days except at 100% concentration, followed by significant increase at 30 days, compared to 15 days. In the first 15 days, the plants showed the symptoms of wilting followed by regeneration. It may be the reason for less accumulation at 15 days of exposure and more at 30 days.

Table 3 showed the results of Cr accumulation in the plants treated with tannery wastewater (influent). The accumulation of Cr in the roots was found significantly higher than shoots at the exposure periods and except 25 and 50% at 10 days. In shoots, no significant change was noticed in metal accumulation with increase in effluent concentration at all the exposure periods. The metal accumulation in the roots increased significantly with increase in effluent concentration at 10 and 15 days of exposure, however, significant increase at 100% was noticed as compared to 25% at 30 days of exposure. The maximum accumulation of

Cr was found in the shoots of the plant at 10 days of exposure, followed by significant decrease. In roots, the metal accumulation increase significantly at 15 days, compared to 10 days followed by decrease except at 100%. Vajpayee et al. (1995) reported the accumulation (240 $\mu g\ g^{-1}$ dw) of Cr from tannery effluent (100%) by rooted emergent plant of *Bacopa monnieri* after 14 days of exposure. The accumulation of Cr in the roots of *S. lacustris* was found more due to luxuriant growth of roots than *Bacopa monnieri*.

Apparently, the plants of *S. lacustris* treated with 8 $\mu g\ ml^{-1}$ Cr for a period of 30 days were short and stunted as compared to control. The length of root, shoots and plant-let raised under Cr treatment were small than in control. Inhibition of root elongation has been a sensitive parameter for assessing the toxicity of heavy metals. This may be related to decrease in cell water content (Barcelo & Poschenrieder, 1990).

Scanning electron microscopic (SEM) studies revealed that the plants grown under Cr treatment exhibited morphoanatomical changes in their roots and shoots as compared to the control (Plate. 1a, b, c, d, e, f) after 30 days of exposure. In treated plants, the roots developed luxuriant growth of root hairs and the length of root hairs was also increased significantly (Plate 1b), whereas the control plants had only few root hairs (Plate 1a). It has been reported that the number of root hairs may increase in the plants under stress conditions (Kahle, 1993). This indicates that the metal accumulation was more in roots through root hair, which is also correlated by the higher accumulation of Cr in the roots than shoots. The study of Scanning electron micrographs showed that the relative proportion of pith and cortical tissue layers increased significantly in the root of metal treated plants (Plate 1 b) as compared to control (Plate 1a). In control plants, there were 12 layers of cortex (Plate 1a) in the root whereas the number increased to 15-18 layers of cortex in treated plants (Plate 1b). In shoot, the vascular bundles were scattered, large bundles were few at the central region and smaller ones were at the periphery in the control plants (Plate 1c). The numbers of vascular bundles were increased in Cr treated plants (Plate 1d), however, most of the vascular bundles were closed due to which translocation of water and solutes get disturbed. It has been reported that the number of vascular bundles may alter in the root and shoot of the plant treated with heavy metals (Setia and Bala, 1994). Prasad and Hagemayer (1999), reported insufficient supply of nutrients and hormones from the roots, adversely influence the differentiation of tissues in the stems. In the present study, it could be the reason for stunted growth in metal treated plants.

The transverse section of shoot revealed that the cortical tissue was parenchymatous network with meshes represented by abundant, irregularly shaped air-cavities both in control and treated plants (Plate 1e, f). The vessel density, the dimension of vessel elements and number of

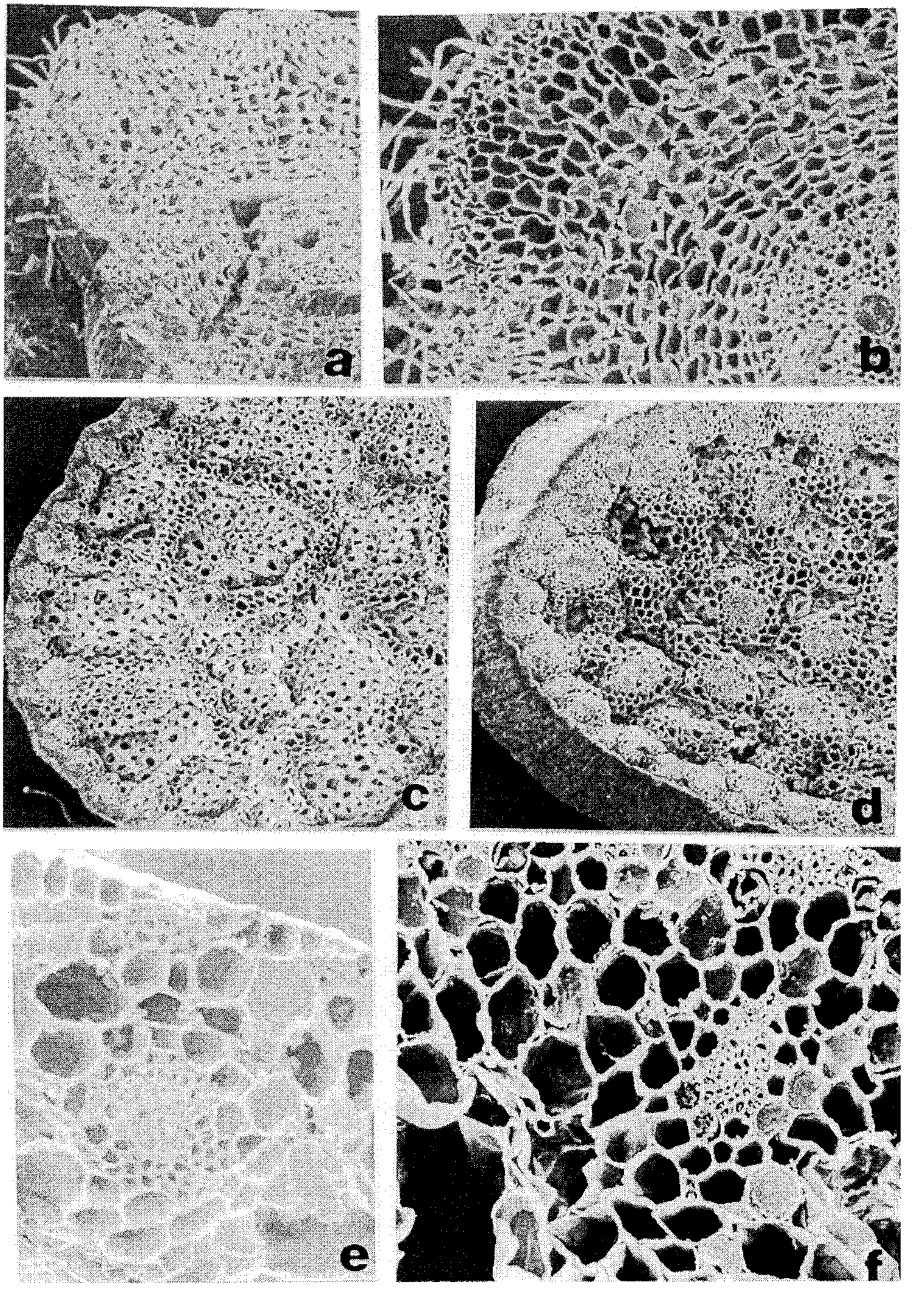

Plate 1. Scanning electron micrographs showing anatomical changes in different plant parts of *S.lacustris*, transverse sections of **a**: Control root, 121x, **b**: Treated root, 200x **c:** Central vascular region of control plant shoot, 63x **d**: Central vascular region of treated plant shoot, 63x **e**: peripheral cortical region of control plant shoot, 400x **f**: Peripheral cortical region of treated plant shoot, 400x.

fibers decreased significantly in metal treated plants (Plate 1f). Heavy metals have been shown to affect both cell division and cell elongation in plants (Setia & Bala, 1994) which is found to cause a considerable decrease in the girth and length of roots and shoots. Nishizono et al, (1987) reported that the metal ions seem to attack various cellular components, including cell wall and membranes resulting in differential alterations that ultimately lead to their disorganization. Structural changes in the plant axis may be correlated to disfunctioning of root system due to metal treatments.

The results of the present study suggest that the increased number of root hairs and closure of vascular bundles may be used as indicator of Cr pollution. The plants of *S. lacustris* seem to be efficient in the removal of Cr as it showed high accumulation of Cr both from solution culture and tannery effluent in its roots than the other plants (Gupta et al., 1994). In view of the above, the plants of *S. lacustris* may be used for the removal of Cr from the wastewater.

Acknowledgments. We thank the Director, National Botanical Research Institute Lucknow, for necessary facilities. S. Singh and R. Saxena are indebted to Ministry of Environment and Forest, New Delhi for financial Assistance.

REFERENCES

Brown SL, Channy R, Angle JS, Baker AJM (1995) Zinc and cadmium uptake by hyperaccumulator Thalspi caerulescens and metal tolerant *Silene vulgaris* grown on sludge-amended soils. Environ Sci Technol 29: 1581-1585.

Barcelo J, Poschenrieder C (1990) Plant water relations as affected by heavy metal stress. A review J Plant Nutr 13: 1-37.

Dhaneshwar RS (1990) Tannery wasterwater treatment and disposal-State of art. Ind Jour Env Protect 10: 134-141.

Gupta M, Sinha S, Chandra P (1994) Uptake and toxicity of metals in *Scirpus lacustris* L and *Bacopa monnieri* L. J Environ Sci Health A 29 (10): 2185-2202.

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research. John. Wiley & Sons, New York .

Hansen D, Duda PJ, Zayed A, Norman T (1998) Selenium removal by constructed wetlands: role of biological utilization. Environ Sci Tech 32: 591-597.

Huddleston GM, Gilespie WB, Rodgers JH (2000) Using constructed wetlands to treat biochemical oxygen demand and ammonia associated with a refinery effluent. Ecotoxicol Environ Safety 45: 188-193.

Kahle H. (1993) Response of roots of trees to heavy metals. Environ and Experi Botany 33: 99-119.

Kimbrough DE, Cohen Y, Winer AM, Creelman L, Mabuni C (1999) A critical assessment of chromium in the environment. Critical reviews in Environ Sci and Technol 29:1-46

Naqvi SM, Rizvi SA (2000) Accumulation of chromium and copper in three different soils and bioaccumulation in an aquatic plant, *Alternanthera philoxiroides*. Bull Environ Contam Toxiol 65: 55-61.

Nishizono H, Ichikawa H, Suziki S, Ishi F (1987) The role of root cell wall in the heavy metal tolerence of *Athyrium yokoscense*. Plant and soil 101: 15-20.

Prasad MNV, Hagemayer J (1999) Heavy metal stress in plants: From molecules to ecosystems (Springer-Verlag; Berlin, Heidel-burg, Germany).

Qian JH, Zayed A, Zhu YL, Yu M, Norman T (1999) Phytoaccumulation of trace elements by wetland plants: |||.Uptake and accumulation of ten trace elements by twelve plant species. J Environ Qual 28: 1448-1457.

Reeves RD, Baker AJM, Brooks R (1996) Abnormal accumulation of trace metals by plants. Mining Environ Manage 3: 4-8.

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation : A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13: 468-474.

Setia RC, Bala R (1994) Anatomical changes in root and stem of wheat (*Triticum astivum* L.) in response to different heavy metals. Phytomorphology 44: 95-104.

Sinha S (1999) Accumulation of Cu, Cd, Cr, Mn and Pb from artificially contaminated soil by *Bacopa monnieri*. Environ Monit and Assess 57: 253-264.

Sinha S, Gupta M, Chandra P (1997) Oxidative stress induced by Iron in *Hydrilla verticillata* (l.f.) Royle: Response of Antioxidants. Ecotoxicol Environ Safety 38: 286-291.

Sinha S, Gupta, M, Chandra P (1996) Bioaccumulation and biochemical effects of mercury in the plant *Bacopa monnieri* (L.). Environ Toxicol & Water quality 11 : 105-112.

Sinicrope T, Langis R, Gergberg RM, Busnardo MJ, Zedler JB (1992) Removal of metals by wetlands Mesocosm subjected to different hydroperiods. Ecol Engg 1: 309-322.

Vajpayee P, Rai UN, Sinha S, Tripathi RD, Chandra P (1995) Bioremediation of tannery effluent by aquatic macrophytes. Bull Environ Contam Toxiol 54 : 546-553.