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Principle and Process of Biofiltration of Cd, Cr, Co, Ni & Pb from Tropical Opencast Coalmine Effluent

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Abstract Opencast coalmine effluent contains higher concentrations of Cd, Cr, Co, Ni and Pb. Biofiltration of these metals has been demonstrated successfully with the help of aquatic macrophytes i.e., *E. crassipes*, *L. minor* and *A. pinnata*. Experiments revealed *E. crassipes* reduced highest concentration of heavy metals followed by *L. minor* and *A. pinnata* on 20th days retention period. Plant tissue analysis revealed higher accumulation of metals in roots than leaves. Highly significant correlations have been noted between removal of heavy metals in effluent and their accumulation in roots and leaves of the experimental sets. Translocation factor also revealed lower transportation of metals from root to leaves. Reduction in chlorophyll and protein content was noted with the accumulation of heavy metals. N, P and K analysis in plant tissues indicated continuous decrease in their concentration with increasing metal concentration. Negative and significant correlations between metal accumulation and N, P and K concentrations in plant tissues showed adverse effects of heavy metals. Analysis of variance (Dunnett *t*-test) showed significant results ($p<0.001$) for all the metals in different durations.

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Keywords opencast coalmine · heavy metal · biofiltration, aquatic macrophytes · accumulation · translocation

1 Introduction

The presence of heavy metals in the environment is of major concern because of their toxicity and threat to plant and animal life. Heavy metals have been recognized as highly toxic due to their persistent nature and magnification properties through the food chain in the ecosystem. Chromium exists in hexavalent and trivalent forms. Its hexavalent form is more toxic than trivalent (Smith & Lec, 1972). Cr causes cancer in digestive tract and lungs (Kaufaman, 1970). Cadmium is considered as one of the five most toxic metals in aquatic environments (Nalewajko, 1995). Cd has toxic effects on biological systems and affects plant growth (Das, Samantary, & Rout, 1997, Mendelssohn, McKee, & Kong, 2001). Cd replaces Zn biochemically, causes high blood pressure, kidney damage, destruction of testicular tissue and red blood cells. Nickel causes dermatitis and bronchial problems (Axtell, Sternberg, Steven, & Claussen, 2003). Lead is an important ecotoxic element with chronic or latent toxicity effects (Breckle & Kahle, 1992). Pb effects kidney, nervous disorder and mental deficiency (De, 2004; Mo, Choi, & Robinson, 1988). Cobalt induces vomiting and rises in blood pressure. Co is

carcinogenic and causes destruction of red blood cells (D. K. Asthana & M. Asthana, 2003).

During the process of opencast coal mining, a variety of rock types with different compositions are exposed to atmospheric condition and undergo accelerated weathering (Upadhyay, Alka, Mishra, & Rai, 2006). Seepage of water from overburden dumps, exposed overburden and coal processing etc. comprises mining effluent. Mining effluent contain heavy metals (Azcue & Nriagu, 1994; Wong, 2003). Traditional effluent treatment plants do not remove heavy metals from the wastewater (Spearot & Peck, 1984.). Major heavy metal pollutants in open cast coalmines are Cd, Cr, Co, Ni and Pb. In majority of cases since after treatment of mining effluent the water is used either for irrigation of cropfields or discharged to surface water bodies. In both the cases presence of heavy metals in the treated wastewater create pollution problems. Removal of heavy metals by chemical precipitation, ion exchange, electrolysis and reverse osmosis with lime and caustic soda generates volumetric sludge and increases the cost of treatment (Spearot & Peck, 1984).

Phytoremediation includes rhizofilteration (absorption, concentration, and precipitation of heavy metals by plant roots), phytoextraction (extraction and accumulation of contaminants in harvestable plant tissue such as roots and shoots), and phytostabilization (absorption and precipitation of contaminants by plants) (Prasad & de Oliveira Freitas, 2003). Certain aquatic plants can tolerate, uptake and translocate high levels of certain heavy metals that would be toxic to most organisms. Accumulation of metals by plants has attracted considerable interest in recent years (Antunes, Watkins, & Duncan, 2001; Cohen-Shoel, Barkay, Ilzycer, Gilath, & Tel-Or, 2002; Shen & Liu, 1998). Plants that are tolerant to metals of high concentrations have been found useful for reclamation of mining effluent containing elevated levels of metals and other elements (Tordoff, Baker, & Willis, 2000).

The process of uptake of metals by water hyacinth may take place through the cell membrane via diffusion and osmosis (Haider, Malik, Rahman, & Ali, 1983; Muramoto & Oki, 1983; Yahya, 1990). *Lemna minor* and *Azolla pinnata* possess excellent heavy metal removal capabilities (Gaur, Noraho, & Chauhan, 1994; Noraho & Gaur, 1996; Rahmani & Sternberg, 1999). Dushenkov, Kumar, Motto, and Raskin (1995) showed that aquatic plant roots could absorb 50% of 500 mg/l lead in 45 h. The depth to which plant roots can penetrate is limited and this restricts the uptake of contaminants and rhizosphere actions to shallower levels (William, 2002). The larger root systems and increased number of fine roots oxidize the rhizosphere to a greater extent, increasing the availability of metals. Vesk, Nockolds, and Allaway (1999) analysed the location of metals within roots of the water hyacinth using energy dispersive X-ray microanalysis, and found that copper, lead and zinc were not localized at the root surface, but were more highly concentrated in the innermost tissues. Peverly, Surface, and Wang (1995) studied a constructed marsh receiving landfill leachate and found that most metals (Cu, Pb, Cd & Fe) accumulated only within roots, although Zn did accumulate in shoots. Seasonal variations were noted by Cacador, Vale, and Catarino (2000) in root concentrations of Zn, Pb, Cu & Cd in *Spartina maritime* and *Halimone portulacoides* where lowest concentrations were observed in roots in January and increased during the growth period.

ATPase plays a significant role in the adaptation of heavy metal conditions and it is regulated at the molecular and biochemical level (Dietz et al., 2001). Padinha, Santos, and Brown (2000) reported thiolic protein concentration, adenylate energy charge (AEC) and photosynthetic efficiency in plants growing at sites with differing degrees of metal contamination. Thiolic protein (which binds metals) was higher in plants growing in metal contaminated environments, while leaf AEC ratio and photosynthetic efficiencies were lower in plants from the polluted sites.

There are more than 15 opencast coalmine projects of the Northern Coal Field Ltd. located in Sonbhadra and Singrauli districts of Uttar Pradesh and Madhya Pradesh states of India. Huge quantities of coal mining effluents are released from these open cast coalmine projects. These mine effluents are directly discharged without any treatment into the G.B. Pant Sagar reservoir acquiring the 450 km^2 area. Coalmine authorities in India have tried to remove heavy metals present into the coalmine water with the help of traditional treatment plants. However, removal of these metals was not found up to satisfactory level (EMP Bina, 2004; Technical Report of Khadia Coal Mine, 2005). Physico-chemical analysis of coalmine effluent contains higher levels of Cd, Cr, Co, Ni and

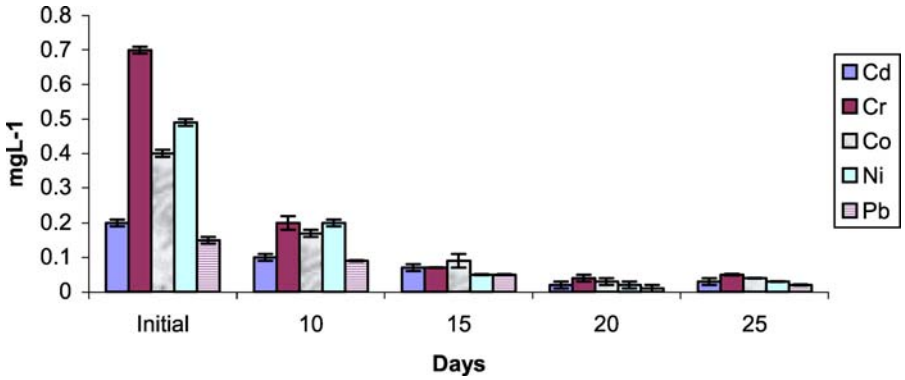

Figure 1 Reduction in concentration of heavy metals in coalmine effluent growing *E. crassipes*.

Pb. Henceforth, the present work aimed to remove Cd, Cr, Co, Ni and Pb from the mining effluent with the help of hydrophytes and to demonstrate the translocation and accumulation of metals in the plant tissues.

2 Methodology

Aquaculture experiments were conducted using pollution tolerant plant species i.e., *Eichhornia crassipes, Lemna minor* and *Azolla pinnata* (Axtell et al., 2003; Fogarty et al., 1999; Kelly, Mielke, Dimaquabo, Curtis, & Dewitt, 1999; Mallick, Shardendu, & Rai, 1996; Wolverton & McDonald, 1976). Hydrophytes were collected from the Agrofarm pond of the Banaras Hindu University, Varanasi, India. Plants were cultured in 150 l capacities of glass aquariums (100×50×30 cm) containing 95 l of mining effluent collected from open cast coal mine of the NCL, Singrauli.

Roots of plant were washed thoroughly with distilled water before they were placed in separate glass aquariums. Monocultures were prepared with 100% coverage of the total surface area of the aquarium used for aquaculture. Control experimental sets contained only mining effluent without any macrophyte. Five replicates of each experimental set were prepared i.e., total 20 sets.

Effluent used for aquaculture experiments were analysed at initial level, 10th, 15th, 20th and 25th day. Plant tissues (root and leaves) were also analysed on similar intervals. A constant water level was maintained in the aquariums with the addition of distilled water. Mining effluent was analysed using Standard Methods for Examination of Water and Wastewater (1995). Plant tissues were oven dried on 80°C. The dried tissues were weighed and ground to powder for analysis. The plants were analysed for total nitrogen-N by microKjeldahl method (Peach & Tracey, 1956) and total phosphorus-P by wet oxidation method

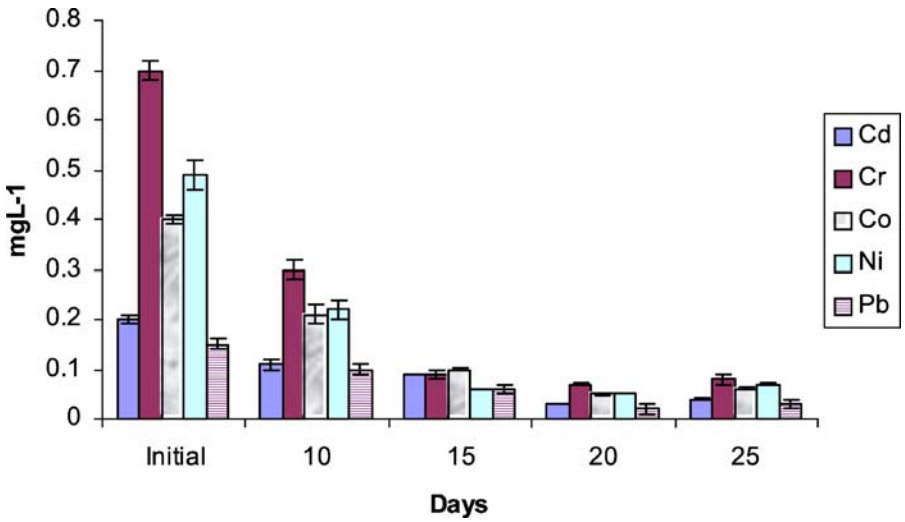

Figure 2 Reduction in concentration of heavy metals in coalmine effluent growing *L. minor*.

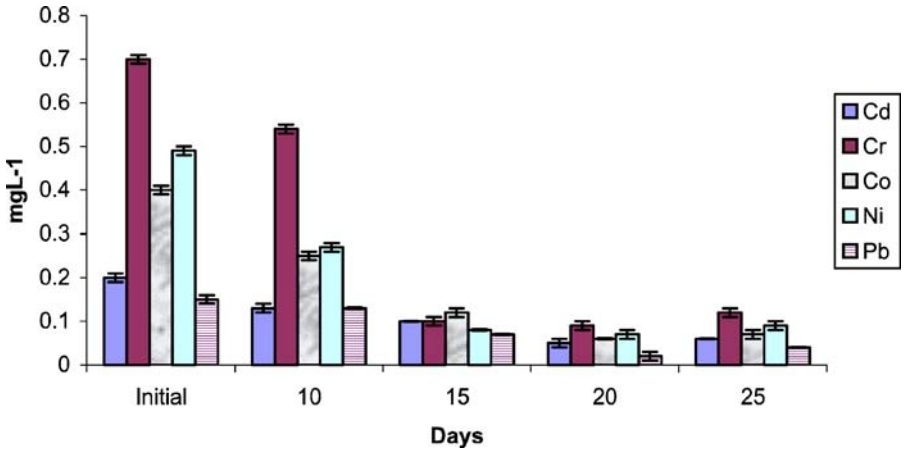

Figure 3 Reduction in concentration of heavy metals in coalmine effluent growing *A. pinnata*.

(Jackson, 1962). Potassium was analysed by flame photometer.

For chlorophyll analysis, chlorophyll was extracted in 80% chilled acetone by using Arnon's method (Arnon, 1949). The protein content of leaf material was estimated following Lowry, Rosebraugh, Farr, and Randall (1951). Bovine serum albumin was used as a standard.

3 Heavy Metal Analysis

Analysis of heavy metals through different methods reveals large deviation therefore, for greater efficacy particle induced X-ray emission (PIXE) has been used during present investigation. Particle induced X-ray emission (PIXE) has been proven as analytical tool capable of detecting elemental concentrations down to parts per million (Murozono, Ishii, Yamazaki, Matsuyama, & Iwasaki, 1999). PIXE was successfully used for heavy metal analysis because of its very high sensitivity for study of wastewater and plant tissue analysis (Mireles et al., 2004).

PIXE results indicating higher accuracy have been used for Samples were irradiated 3 to 10 min in a vacuum chamber by 3 MeV protons. LEGe detector was used to measure the concentrations of heavy metals in the samples. For PIXE spectrum analysis a least square fitting computer program based on the pattern analysis method (Murozono et al., 1999) was used.

Statistical comparisons of means were examined with one-way ANOVA. Correlations were also used for statistical significance. SPSS 10 statistical package was used for statistical analysis.

4 Results and Discussion

Concentrations of heavy metals in mine effluent (Figures 1, 2 and 3) revealed continuous decrease up to 20th day. Slight increase of heavy metals in

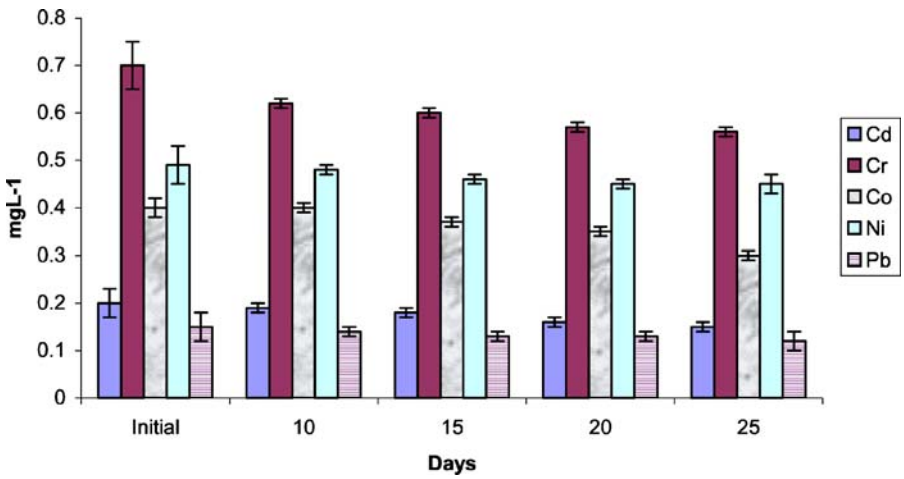

Figure 4 Reduction in concentration of heavy metals in coalmine effluent in control experimental sets.

Table I Accumulation of Cd, Cr, Co, Ni and Pb by hydrophytes grown in coal mining effluent (μg/g)

		*Correlation coefficient	Initial	10th day	15th day	20th day	25th day
Cd							
E. crassipes	Root	−0.978	0.2±0.01	1.5±0.01	2.7±0.02	3.2±0.12	3.0±0.02
L. minor	Root	−0.974	0.1±0.01	1.3±0.02	2.2±0.03	2.7±0.11	2.4±0.02
A. pinnata	Root	−0.972	0.1±0.01	1.2±0.01	2.0±0.1	2.5±0.2	2.0±0.01
E. crassipes	Leaves	−0.894	BDL	0.35±0.01	0.7±0.1	1.0±0.10	0.7±0.02
L. minor	Leaves	−0.750	BDL	0.2±0.01	0.6±0.02	0.8±0.01	0.5±0.01
A. pinnata	Leaves	−0.630	BDL	0.2±0.01	0.5±0.01	0.7±0.01	0.3±0.02
Cr							
E. crassipes	Root	−0.989	1.6±0.2	98.7±5.3	120.9±8.5	132.7±8.5	127.5±6.7
L. minor	Root	−0.980	1.0±0.1	85.7±4.8	112.7±9.0	120.3±7.0	112.0±5.2
A. pinnata	Root	−0.939	0.7±0.01	80.7±6.1	97.8±5.0	103.7±6.3	95.2±3.7
E. crassipes	Leaves	−0.981	0.2±0.02	40.2±2.7	48.2±4.3	52.3±2.7	48.7±2.8
L. minor	Leaves	−0.978	0.05±0.01	27.8±1.5	42.9±3.2	45.5±3.0	39.3±1.7
A. pinnata	Leaves	−0.926	BDL	22.2±2.0	32.7±2.5	38.7±2.5	32.4±2.5
Co							
E. crassipes	Root	−0.976	2.0±0.2	35.7±2.1	40.7±2.5	45.7±2.9	40.3±2.0
L. minor	Root	−0.967	1.2±0.1	30.0±2.7	36.3±2.0	40.5±2.0	35.4±2.0
A. pinnata	Root	−0.906	1.0±0.1	28.7±2.0	30.2±1.9	35.3±2.0	30.7±2.5
E. crassipes	Leaves	−0.991	0.1±0.01	15.7±1.8	20.8±1.7	28.2±1.7	23.7±2.7
L. minor	Leaves	−0.648	BDL	14.2±1.0	17.5±1.8	21.6±1.9	15.2±1.2
A. pinnata	Leaves	−0.358	BDL	12.8±1.0	15.7±1.5	17.8±1.5	11.5±1.0
Ni							
E. crassipes	Root	−0.978	3.1±0.2	95.7±3.8	108.7±7.2	128.9±6.3	115.7±6.8
L. minor	Root	−0.956	1.2±0.1	81.2±2.9	97.5±5.6	109.9±6.0	115.7±5.7
A. pinnata	Root	−0.917	1.0±0.01	76.7±3.8	85.7±3.4	92.3±4.2	87.1±2.3
E. crassipes	Leaves	−0.988	0.1±0.01	32.5±2.0	41.7±2.0	52.7±2.7	45.6±3.2
L. minor	Leaves	−0.996	0.02±0.001	20.5±1.7	35.8±1.9	41.8±2.5	37.2±2.1
A. pinnata	Leaves	−0.979	0.01±0.001	18.5±1.0	28.5±1.7	32.7±1.8	27.0±2.0
Pb							
E. crassipes	Root	−0.928	5.2±0.3	180.7±4.3	192.2±9.8	215.7±6.5	206.2±7.2
L. minor	Root	−0.859	2.0±0.1	172.7±6.2	180.3±5.2	193.5±6.0	182.7±6.7
A. pinnata	Root	−0.740	1.2±0.01	163.2±6.7	172.8±8.2	185.2±5.8	173.5±5.2
E. crassipes	Leaves	−0.958	0.12±0.01	41.0±2.5	52.2±3.7	60.2±3.7	55.5±2.7
L. minor	Leaves	−0.936	0.05±0.001	30.9±2.0	46.8±2.7	51.3±2.0	42.0±2.2
A. pinnata	Leaves	−0.807	0.02±0.001	28.3±1.7	37.9±2.0	40.5±2.0	32.0±2.3

*Correlation coefficient between heavy metal in plants and effluent ($p<0.001$)

effluent on 25th day in experimental sets containing aquatic plants was also noted. This might be due to degradation of plant parts after accumulating higher concentration of heavy metals. Present finding supports the earlier reports of Beckett and Davis (1977); Borkert, Cox, and Tucker (1988); Davies, Puryear, Newton, Egilla, and Grossi (2002); Ewais (1997); Long et al. (2003); Windham, J. S. Weis, and P. Weis (2002); Winterbourn, McDiffet, and Eppley (2000). Similar investigations carried out on higher plants indicated the release of nutrients when the internal concentration was high (Gaur, Singhal, & Hasija, 1992). Experimental sets containing *E. crassipes* removed highest concentration of heavy metals followed by *L. minor* and *A. pinnata*. Principal component analysis revealed *E. crassipes* removed significantly higher content of all the heavy metals than *L. minor* and *A. pinnata*.

Plants with higher biomass accumulate higher metals (Fritioff, Kautsky, & Greger, 2005). Fine lateral roots of the water hyacinth reduce highly toxic Cr (VI) to the less toxic Cr (III), and then translocate the relatively non-toxic Cr (III) to leaf tissues (Lytle et al., 1998). This process might increase the accumu-

Table II Translocation factor for Cd, Cr, Co, Ni and Pb accumulation in hydrophytes

	E. crassipes	*L. minor*	*A. pinnata*
Cd			
Initial	0	0	0
10th day	0.23	0.15	0.16
15th day	0.25	0.27	0.25
20th day	0.31	0.29	0.28
25th day	0.23	0.2	0.15
Cr			
Initial	0	0	0
10th day	0.40	0.32	0.27
15th day	0.39	0.38	0.33
20th day	0.40	0.38	0.50
25th day	0.38	0.35	0.34
Co			
Initial	0	0	0
10th day	0.43	0.47	0.44
15th day	0.51	0.48	0.52
20th day	0.61	0.53	0.50
25th day	0.58	0.42	0.37
Ni			
Initial	0	0	0
10th day	0.33	0.25	0.24
15th day	0.38	0.36	0.33
20th day	0.43	0.40	0.35
25th day	0.39	0.30	0.30
Pb			
Initial	0	0	0
10th day	0.22	0.17	0.17
15th day	0.27	0.25	0.21
20th day	0.27	0.26	0.21
25th day	0.25	0.22	0.18

lation capacity of the plant. Control experimental set (Figure 4) was also recorded with slight decrease in heavy metal content. Chemical precipitation and microbial degradation might have resulted in reducing the heavy metal content in control experimental set. Analysis of variance (Dunnett *t*-test) showed significant results ($p<0.001$) for all the metals in different durations.

Root and leaf tissue analysis of selected plants showed higher concentration of heavy metal in roots than leaf. Cd increased from 0.2±0.01 to 3.2± 0.12 μg/g in root of *E. crassipes*. Similarly *L. minor* and *A. pinnata* also showed increase in concentration of Cd from 0.1±0.01 to 2.7±0.11 and 0.1±0.01 to 2.5± 0.2 μg/g, respectively (Table I). Leaves also showed accumulation of 1.0±0.01, 0.8±0.01 and 0.7± 0.01 μg/g. Accumulation of Cd in root and leaves is clearly indicated by the results. Cadmium has a higher solubility than other heavy metals (Forstner & Wittmann, 1979). Analytical results (Table I) pertaining to the heavy metal concentrations in root and leaves of the plant revealed highest concentration of Cr as 132.7±8.5 in the root of *E. crassipes* followed by 120.3±7.0 in *L. minor* and 103.7±6.3 μg/g in the *A. pinnata*. Leaves of these plants showed highest concentration 52.3±2.7 in *E. crassipes* followed by 45.5±3.0 and 38.7±2.5 μg/g in *L. minor* and *A. pinnata*, respectively. Higher concentration of heavy metals in the root of plants as compared to leaves may be associated with its lower translocation from root to leaves. Plant species growing on metal contaminated water have restricted translocation of metals to the aerial parts (Zaranyika & Ndapwadza, 1995). Correlation between removal of heavy metals from the mining effluent and its accumulation in roots and leaves was found highly significant ($p<0.001$) for all the heavy metals tested (Table I).

Co accumulation was also highest in root (45.7± 2.9) and leaf (28.2±1.7) of *E. crassipes* (Table I). *L. minor* and *A. pinnata* also accumulated high concentration of Co from initial level 1.2±0.1 to 40.5±2.0 and 1.0±0.1 to 35.3±2.0 in their root. Correlations were highly significant between metal accumulation in plant tissues (root and leaf) and removal from mining effluent. Ni was also accumulated by selected plants in very high concentration in root as 128.9± 6.3, 109.9±6.0 and 92.3±4.2 μg/g by *E. crassipes*, *L. minor* and *A. pinnata*, respectively. Ni translocation was very low from root to leaves in all the selected plants. Pb concentration was very high in all the plants in roots (215.7±6.5, 193.5±6.0, 185.2± 5.8 μg/g). Ni was recorded in leaves as 60.2±3.7, 51.3±2.0, and 40.5±2.0 in *E. crassipes, L. minor* and *A. pinnata*, respectively, which shows very low translocation from root to leaves. Analysis of variance (Dunnett *t*-test) revealed significant ($p<0.05$) temporal variation for all the metals in plant tissues.

Fitzgerald, Caffrey, Neasaratnam, and McLoughlim (2003) and Cordwell, Hawker, and Greenway (2002) reported plants differ widely in their ability to accumulate heavy metals. Root tissues accumulate significantly greater concentrations of metals than shoot indicating higher plant availability of the substrate metals as well as its limited mobility once inside plants. Plants immobilize metal in roots and in the oxygenated rhizosphere (Ye et al., 2001).

Table III Mineral composition of aquatic plants grown in coalmine effluent (mg/g)

	N		P		K	
	Root	Leaves	Root	Leaves	Root	Leaves
E. crassipes						
Initial	41.5±2.3	41.2±2.1	5.9±0.5	4.7±0.2	47.5±2.7	45.4±2.5
10th day	37.3±1.9	32.4±2.0	4.7±0.4	4.3±0.7	42.5±2.0	39.4±2.1
15th day	32.8±2.0	29.4±1.7	4.2±0.2	3.7±0.5	39.5±1.9	37.8±2.0
20th day	30.7±1.7	28.7±1.8	3.9±0.2	3.1±0.3	37.2±1.7	36.4±1.9
25th day	26.2±1.2	24.3±1.0	3.2±0.3	2.7±0.2	31.5±2.0	28.8±1.7
L. minor						
Initial	37.2±2.0	35.4±1.5	5.2±0.4	4.8±0.5	46.2±2.6	44.4±2.0
10th day	32.2±1.9	31.8±2.0	4.2±0.7	3.9±0.2	39.7±1.9	37.2±2.1
15th day	30.5±1.7	29.7±1.7	3.8±0.2	3.5±0.3	36.3±1.7	34.5±1.5
20th day	29.4±1.2	27.4±1.6	3.5±0.2	3.3±0.4	35.4±1.5	32.8±1.4
25th day	24.0±1.2	22.2±1.5	3.0±0.3	2.6±0.2	30.2±1.7	27.4±1.5
A. pinnata						
Initial	37.8±1.9	33.8±1.5	5.2±0.5	4.3±0.5	46.0±1.7	42.4±2.0
10th day	33.5±1.8	30.4±1.7	4.6±0.3	3.7±0.3	40.5±1.9	36.5±1.7
15th day	30.5±1.2	27.6±1.3	3.9±0.5	3.7±0.2	38.5±1.5	32.8±1.2
20th day	28.7±1.5	25.4±1.0	3.2±0.3	2.7±0.6	32.7±1.8	28.4±1.5
25th day	22.1±1.0	20.9±1.2	2.8±0.2	2.3±0.2	28.4±1.5	25.7±1.7

Translocation factor (root to leaf ratio) showed lower values for all the plants (Table II). Translocation was noted highest on 20th day analysis. Translocation factor showed lower transportation of heavy metals from roots to leaves. Lower accumulation of metals in leaves than root can be associated with protection of photosynthesis from toxic levels of trace elements (Baker, 1981; Landberg & Greger, 1996;

Table IV Correlations between heavy metal accumulation and N, P, and K content in roots and leaves of aquatic plants grown in coalmine effluent*

	N		P		K	
	Root	Leaf	Root	Leaf	Root	Leaf
E. crassipes						
Cd	−0.920	−0.955	−0.952	−0.906	−0.894	−0.842
Cr	−0.852	−0.944	−0.917	−0.806	−0.831	−0.796
Co	−0.908	−0.961	−0.950	−0.883	−0.885	−0.839
Ni	−0.897	−0.954	−0.938	−0.863	−0.866	−0.818
Pb	−0.940	−0.939	−0.949	−0.942	−0.909	−0.845
L. minor						
Cd	−0.892	−0.884	−0.962	−0.931	−0.926	−0.933
Cr	−0.858	−0.820	−0.942	−0.911	−0.917	−0.910
Co	−0.880	−0.858	−0.959	−0.930	−0.933	−0.928
Ni	−0.838	−0.800	−0.928	−0.895	−0.902	−0.893
Pb	−0.880	−0.885	−0.954	−0.925	−0.924	−0.923
A. pinnata						
Cd	−0.880	−0.910	−0.958	−0.948	−0.936	−0.970
Cr	−0.820	−0.851	−0.907	−0.852	−0.831	−0.90
Co	−0.877	−0.905	−0.950	−0.923	−0.919	−0.959
Ni	−0.821	−0.846	−0.891	−0.853	−0.838	−0.908
Pb	−0.841	−0.882	−0.954	−0.922	−0.908	−0.937

*($p<0.05$)

Table V Chlorophyll and Protein content in foliage of aquatic plants grown in mining effluent (mg/g fresh weight)

	Initial	10th day	15th day	20th day	25th day
E. crassipes					
Chlorophyll	0.7±0.01	0.6±0.02	0.43±0.01	0.3±0.01	0.2±0.01
Protein	6.0±0.2	4.5±0.2	4.0±0.1	2.5±0.1	2.1±0.02
L. minor					
Chlorophyll	0.67±0.04	0.55±0.03	0.38±0.02	0.2±0.01	0.1±0.01
Protein	5.3±0.1	3.8±0.1	3.1±0.2	2.4±0.1	1.9±0.01
A. pinnata					
Chlorophyll	0.58±0.02	0.49±0.02	0.32±0.01	0.1±0.01	0.08±0.001
Protein	5.2±0.1	3.7±0.1	3.0±0.1	1.8±0.06	1.5±0.01

Peverly et al., 1995). Heavy metal concentrations in plant tissues of experimental sets showed different capacities of metal accumulation by the plants. Data obtained indicate that roots mainly retain accumulated metals. Roots are dipped in effluent, which allowed higher area for absorption whereas, in leaves as such absorption was rare and accumulation was only through transportation from roots. Heavy metal concentrations in plants varied with plant species and heavy metal concentrations of effluent.

Mineral composition of aquatic plants grown in coalmine effluent (Table III) revealed regular decrease of N, P and K content in all the plants. Metal accelerates the growth of plants, which needs nutrients for growth. Poor nutrient condition of effluent resulted in decay of plants after 20th day of experiment. Correlations (Table IV) between N, P and K in root and leaves of plants and heavy metal accumulation in plants were found negatively significant ($p<0.05$). Interactive effect of heavy metals and their effect on nutrient status of plant might have decreased N, P and K content in plant tissues. Similar findings were observed by Ait Ali, Bernal, and Alter (2002) in their study on reeds. Samecka-Cymerman and Kempers (2001) observed in their experiments that investigated plants characterize significant negative correlations between contents of heavy metals and nutritional elements in plants, water and bottom sediments.

Chlorophyll content reduced from 0.7±0.01 to 0.2± 0.01 and protein 6.0±0.2 to 2.1±0.02 mg/g fresh weight in *E. crassipes* (Table V). Similarly *L. minor* and *A. pinnata* also showed reduction in chlorophyll and protein after accumulation of heavy metals. Correlations between chlorophyll and protein content in plant foliage and heavy metal content in foliage was also negatively significant (Table VI). Abdel-Basset, Issa, and Adam (1995) also observed decrease in total chlorophyll concentration due to faster hydrolysis when plants are under stress due to heavy metal accumulation. Total chlorophyll concentration is a unifying parameter for indicating the effect of specific interventions. Heavy metals could affect each component at a different level creating changes in

Table VI Correlations between chlorophyll and protein content in foliage and heavy metals in foliage of aquatic plants*

	Cd	Cr	Co	Ni	Pb
E. crassipes					
Chlorophyll	−0.875	−0.887	−0.862	−0.840	−0.825
Protein	−0.912	−0.923	−0.926	−0.910	−0.894
L. minor					
Chlorophyll	−0.925	−0.915	−0.741	−0.892	−0.808
Protein	−0.957	−0.952	−0.854	−0.952	−0.904
A. pinnata					
Chlorophyll	−0.937	−0.873	−0.654	−0.838	−0.740
Protein	−0.975	−0.897	−0.767	−0.910	−0.844

*($p<0.05$)

some part of plants physiology and not in others (Manios, Stentiford, & Millner, 2003). Reduction in chlorophyll and protein content in plants indicates heavy metals' deleterious effects.

5 Conclusion

Present investigation revealed higher concentrations of heavy metals such as Cd, Cr, Co, Ni and Pb in the coalmine effluent. Translocation factor study showed highest accumulation of heavy metals in plant roots and its lower translocation from root to leaves. It was observed that *E. crassipes* removed higher quantity of heavy metals from the effluent as compared to *L. minor* and *A. pinnata*. Maximum removal of heavy metals by the aquatic plants was observed on 20th day of retention period. In tropical environmental conditions these aquatic macrophytes grow luxuriantly and found promising accumulator of heavy metals. These aquatic macrophytes may be recommended for the biofiltration of heavy metals from the coalmine effluents.

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