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Accumulation of Heavy Metals from Contaminated Wastewater by Aquatic Plant *Lemna minor* and Their Biochemical Effects on it

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ABSTRACT

Toxic metal contamination of soil, aqueous waste streams and groundwater causes major environmental and human health problems. The most commonly used methods for dealing with heavy metal pollution are still extremely costly. Use of aquatic plants to extract, sequester and/or detoxify pollutants is a new and powerful technique for environmental cleanup. Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological properties. The aim of this work is to evaluate the potential of free floating duck weed *Lemna minor* to remove heavy metals from wastewater and their biochemical effect on it. One-month laboratory experiments were conducted to mark the percentage removal of different heavy metals at different concentrations and the effect of heavy metals on nitrate reductase activity, total chlorophyll and protein contents of the plant. Approximately 93% of total heavy metal induced-toxicity appears resulting in the reduced activities of nitrate reductase, total chlorophyll and protein content of the plant. The results recommended the use of *Lemna minor* to ameliorate the wastewaters contaminated with heavy metals.

INTRODUCTION

Heavy metals are important environmental pollutants and many of them are toxic even at very low concentration. Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution (Nriogo 1979). The primary sources of this pollution are the burning of fossil fuels, the mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides and sewage (Kabata-Pendias & Pendias 1989). Toxic metal contamination of soil, aqueous waste streams and groundwater poses a major environmental and human health problem, which is still in need of an effective and affordable technological solution. In spite of the ever-growing number of toxic metal-contaminated sites, the most commonly used methods of dealing with heavy metal pollution are still either the extremely costly process of removal and burial or simply isolation of the contaminated sites. In addition to sites contaminated by human activity, natural mineral deposits containing particularly large quantities of heavy metals are present in many regions of the globe. These areas often support characteristic plant species that thrive in these metal enriched environments. Some of these species can accumulate very high concentrations of toxic metals to levels, which far exceed the soil levels (Baker & Brooks 1989). In many ways, living plants can be compared to solar driven pumps, which can extract and concentrate several elements from their environment. From soil and water, all plants have the ability to accumulate heavy metals, which are essential for their growth and development. These metals include Mg, Fe, Mn, Zn, Cu, Mo and Ni (Langille & MacLean 1976). Certain plants also have the ability to accumulate heavy metals, which have no known biological function. These include Cd, Cr, Pb, Co, Ag, Se and Hg (Hanna & Grant 1962, Baker & Brooks 1989). However, excessive accumulation of these heavy metals can be toxic to most plants. The ability to both tolerate elevated levels of heavy metals and accumulate them in very high concentrations has evolved both independently and together in a number of different plant species (Ernst et al. 1992). Plant growth is generally restricted by heavy metals. Some plant species however, particularly the ones inhabiting areas with chronically high metal concentrations, possesses unique ability to adopt and evolve to tolerance heavy metals (Antonovics et al. 1971, Woolhouse 1983).

The ability of aquatic plants to accumulate heavy metals from water is well documented. Many free floating, emergent and submerged species have been identified as potential accumulators of heavy metals. Such plants could be utilized for the improvement of the water quality and for reducing the pollution load in the water bodies. Toxic metals also cause a high level of phytotoxicity in plants as a result of several physiological and biochemical changes, which take place in the plant system. These changes are due to interaction of sulfahydryl groups of the enzymes. Aquatic plants growing in polluted water absorb heavy metals, which enter into food chains, posing serious threat to human health (Rachel Isaksson et al. 2007).

To prevent the hazards of toxic metal pollution, aquatic plants are being used in recent years as functional intent for wastewater treatment successfully. Recently, there has been growing interest in the use of metal-accumulating roots and rhizomes of aquatic or semi aquatic vascular plants for the removal of heavy metals from contaminated aqueous streams. For example, *Eichhornia crassipes* (Kay et al. 1984), *Hydrocotyle umbellata* (Dierberg et al. 1987), and *Lemna minor* and *Azolla pinnata*

Table 1: Effect of various concentrations of different heavy metals on *Lemna minor*.

Sl. No.	Parameters	Heavy Metal	Concentrations (%) 10	20	30	40	50	100
1.	* Total heavy metal accumulation (%)	Cu	1.04	0.83	0.75	0.73	0.65	0.31
		Co	1.23	0.85	0.40	0.36	0.31	0.23
		Mn	12.23	11.14	10.89	4.08	0.84	0.57
		Pb	15.29	11.11	10.26	10.25	9.77	8.17
		Zn	0.86	0.52	0.49	0.44	0.40	0.33
2.	* Total Chlorophyll content (%)	Cu	0.177	0.135	0.128	0.119	0.106	0.80
		Co	0.221	0.148	0.144	0.110	0.104	0.87
		Mn	0.380	0.371	0.351	0.290	0.280	0.250
		Pb	0.371	0.358	0.318	0.308	0.285	0.241
		Zn	0.397	0.327	0.314	0.284	0.277	0.248
3.	* Total Protein content(%)	Cu	3.88	3.28	3.24	2.83	2.51	2.40
		Co	4.08	3.32	2.70	2.53	2.37	2.04
		Mn	4.67	4.27	4.18	4.05	4.02	3.91
		Pb	2.31	2.08	2.04	2.01	2.0	1.98
		Zn	5.67	5.41	5.32	4.64	4.50	3.88
4.	* Nitrate Reductase activity (%)	Cu	3.90	3.87	3.83	3.80	3.66	3.60
		Co	3.72	3.46	3.41	3.32	3.14	3.04
		Mn	3.87	3.82	3.76	3.74	3.70	3.61
		Pb	3.69	3.48	3.33	3.24	3.11	3.07
		Zn	3.65	3.54	3.20	3.15	3.06	3.02
5.	* Biomass Production ~(%)	Cu	0.808	0.737	0.653	0.640	0.504	0.430
		Co	0.913	0.838	0.686	0.497	0.441	0.344
		Mn	1.567	1.235	0.115	0.963	0.913	0.485
		Pb	1.150	1.498	1.424	1.138	1.274	0.872
		Zn	0.847	0.780	0.516	0.312	0.234	0.189

*Average values, heavy metal accumulation-ppm, Total chlorophyll content-mg/g fresh weight, Nitrate reductase activity-mmol/min activity/g of fresh weight. For uniform calculation values were converted into percent values.

(Jain et al. 1989) take up Pb, Cu, Cd, Fe and Hg from contaminated solutions. In a related development, cell suspension cultures of *Datura innoxia* were found to remove a wide variety of metal ions from solutions (Jackson et al. 1990, Jackson et al. 1993).

Other species with a phytoremediation abilities are: *Myriophylum brasiliense*, *Salix* sp. and *Populus* sp. (Brown 1995). *Lemna minor* is an aquatic plant, which absorbs heavy metals in water has been used as research material for the present study. Heavy metal absorption by this plant is in maximum levels at certain conditions. The explosion of *Lemna minor* within water pollution has been increased by factors like advancing of technology in industrial actions. Duckweed shows promise for the removal of Cd, Se, and Cu from contaminated wastewater since it accumulates high concentration of these metals (Jain et al. 1989).

MATERIALS AND METHODS

The investigation was carried out using aquatic macrophyte *Lemna minor* L. collected from a local freshwater pond, IEMPS, Vikram University, Ujjain, Madhya Pradesh. Different concentrations (10, 20, 30, 40, 50, 100%) of Cu, Co, Mn, Pb and Zn were prepared by diluting the stock solution in Hoagland's solution (Hoagland & Arnon 1938). The acclimated test plants (12 g) were transferred to plastic troughs containg different percent solution of heavy metals (6 liter in each trough) for thirty days. The physiological and biochemical parameters of plants were analyzed on 30th day.

Table 2: Scheffe^{ab} Post hoc test of *L. minor* against different-heavy metals.

GP	N	Subset 1	2	3	4	5
Mn	18	0.4822				
Cu	18		3.2728			
Pb	18			6.9744		
Co	18				6.9833	
Zn	18					7.1433

Table 3: Scheffe^{ab} Post hoc test of *L. minor*: Chlorophyll content against different-heavy metals.

GP	N	Subset 1	2	3	4	5
Cu	18	0.17733				
Co	18		0.20939			
Zn	18			0.23628		
Mn	18				0.26478	
Pb	18					0.29772

Table 4: Scheffe^{ab} post hoc test of *L. minor*: Protein content against different-heavy metals.

GP	N	Subset 1	2	3	4	5
Pb	18	2.7583				
Cu	18		2.9328			
Zn	18			2.9789		
Co	18				3.8367	
Mn	18					3.9922

Table 5: Scheffe^{ab} post hoc test of *L. minor*: Nitrate reductase activity against different-heavy metals.

GP	N	Subset 1	2	3	4	5
Co	18	3.2694				
Zn	18		3.250			
Cu	18			3.3417		
Pb	18				3.5522	
Mn	18					3.5789

The total chlorophyll content (mg/g fresh weight) of fresh leaves of plants was analyzed by standard method of Arnon (1949), total protein content (mg/g fresh weight) by Lowery et al. (1951), nitrate reductase activity (µmol/min activity/g of fresh weight) by Camm & Stein (1974), heavy metal accumulation in plants using Atomic Absorption Spectrophotometer (AAS make-Perkin Elmer, USA, Perkin Elmer 1981) following standard protocol proposed by APHA (1995), and total biomass on dry weight basis.

The data obtained were subjected to DMRT test. Mean direct values provided no conclusive results. DMRT test compares mean values and expresses significant subsets. The last subset represent highest significance while the first subset represents least significance. The univariate analysis of variables in heavy metal concentrations against *Lemna minor* was conducted.

RESULTS AND DISCUSSION

The results of the heavy metal accumulation by *Lemna minor* are presented in Table 1.

Heavy metal accumulation: The accumulation of all the heavy metals was higher at 10% concentration, and lower at 100% concentration (Tables 2 & 7). Further, it is evident from Table 2 that the uptake was maximum for lead and minimum for copper. The order of accumulation of different heavy metals in *Lemna minor* was Pb > Mn > Zn > Co > Cu. Similar observations have been made by Rai & Tripathi (2008) in *Azolla pinnata.* They observed that Hg removal was high in *A. pinnata* in comparison to *Vallisneria spiralis.* High correlation was obtained between applied Hg doses and accumulated amounts of biomass ($R^2 = 0.91$). Sylas et al. (2007) found that heavy metal accumulation is high in submerged plant *Cabomba caroliniana, Eichhornia crassipes* and *Salvinia molesta*. Tewari et al. (2007) reported that the adsorption potential of dried biomass *E. crassipes* was excellent for the removal of cadmium and chromium. Ming-Cheng Shin et al. (2007) reported algae as a good source of absorbent for arsenite, which could oxidize over 80% of arsenite to arsenate. Sorption of Cd (II) by various aquatic plants has been studied (Cheung et al. 2001, Christophi & Axe 2000, Fraysse et al. 2000, Hasar et al. 2000). It is also reported that remediation of sites contaminated with heavy metals is particularly challenging (Chany et al. 1997, Baker 1981, Chaithanya & Kanmani 2009). These reports support to the observation that *Lemna minor* can be used as a heavy metal absorbent also.

Table 6: Scheffe^{ab} Post hoc test of *L. minor*: Biomass production against different heavy metals.

GP	N	Subset 1	2	3	4	5
Cu	18	0.6419				
Co	18		0.7031			
Pb	18			0.9913		
Zn	18				1.1843	
Mn	18					1.3587

Table 7: Scheffe^{ab} Post hoc test of *L. minor* against different concentrations of heavy metals.

Conc	N	Subset 1	2	3	4	5	6
100	15	3.4113					
50	15		3.8920				
40	15			4.6193			
30	15				5.1533		
20	15					6.0310	
10	15						6.7200

Table 8: Scheffe^{ab} Post hoc test of *L. minor*: Chlorophyll content in various concentrations of heavy metals.

Conc	N	Subset 1	2	3	4	5	6
100	15	0.19870					
50	15		0.22647				
40	15			0.23367			
30	15				0.24067		
20	15					0.26120	
10	15						0.26253

Table 9: Scheffe^{ab} Post hoc test of *L. minor*: protein content in various concentrations of heavy metals.

Conc	N	Subset 1	2	3	4	5	6
100	15	3.0360					
50	15		3.2040				
40	15			3.2393			
30	15				3.3373		
20	15					3.3733	
10	15						3.6087

Total chlorophyll: Total chlorophyll content in *Lemna minor* decreased in all concentrations of heavy metal treatments (Tables 3 & 8). It was lowered in high concentrations and was normal in low concentrations. Chlorophyll was low where Mn was treated and maximum where Zn was treated. The hierarchy of heavy metal was Zn > Co > Pb > Cu > Mn. Mooreland & Nuvitzsky (1998) attributed inhibition of chlorophyll synthesis to the presence of phytotoxins in the solution or inhibition of light activated Mg^{2+} and ATPase activity. Vajpayee et al. (2000) reported that chromium accumulation reduces chlorophyll biosynthesis in *Nymphaea alba*. Sarma & Sarma (2007) reported that fertilizer factory effluent containg heavy metals drastically decreases chlorophyll content of plants. Heavy metal containg solutions have a deleterious effect on the chlorophyll content of *L. minor* in the present study.

Table 10: Scheffe^{ab} Post hoc test of *L. minor*: Nitrate reductase activity in various concentrations of heavy metals.

Conc	N	Subset 1	2	3	4	5	6
100	15	3.2227					
50	15		3.3360				
40	15			3.3587			
30	15				3.4540		
20	15					3.4973	
10	15						3.6120

Table 11: Scheffe^{ab} Post hoc test of *L. minor*: Biomass production in various concentrations of heavy metals.

Conc	N	Subset 1	2	3	4	5	6
100	15	0.7741					
50	15		0.8969				
10	15			0.9292			
40	15				1.0339		
30	15					1.0529	
20	15						1.1681

‘a’ uses harmonic mean sample size = 18.000; ‘b’ Alpha = 0.05, for Table 2 to Table 11

Total protein content: Total protein content decreases in all heavy metal treated concentrations (Tables 4 & 9). The decreases were maximum at higher concentrations and had the maximum effect followed by lead, copper, zinc and cobalt, while it was minimum for manganese. The descending order of toxicity was Mn > Co > Zn > Cu > Pb. Kumar & Vijayarenagn (2006) observed reduced protein content at higher concentrations of cobalt treatment in black gram. Mayz & Cartwright (1984) observed that heavy metal treated plants showed limited availability of nitrogen because amino acid synthesis was reduced. Jacobs et al. (1977) observed that proteins are highly sensitive to heavy metals and one of the earliest indicators of heavy metal poisoning. Kim et al. (1978), Kastori et al. (1992) and Bhattacharjee & Mukherjee (1994) made similar observations. Therefore, protein content is drastically reduced in *Lemna minor* treated with heavy metals.

Nitrate reductase: Nitrate reductase activity decreases in all concentrations of heavy metal treatments (Tables 5 & 10). Manganese had the maximum effect, while cobalt had the least effect and the order of toxicity was Mn > Pb > Cu > Zn > Co. Phototoxic effects of heavy metals are widely reported (Woolhouse 1983). Srivastava (1980) noticed that nitrate reductase activity in plants gives a good estimate of the nitrogen status of the plant. Lehninger (1984) observed that as enzymes are the functional units of metabolism, toxicological studies pointed out that the enzymes are common target of the toxicants. Siddique (1982) and Bhandal & Kumar (1992) also noticed that inhibition of nitrate reductase activity indicates that this enzyme is sensitive to metal salts.

Biomass: *Lemna minor* biomass decreased by uptake of heavy metals (Tables 6 &11). The decrease was moderate at 50%, while it was minimum at 20% concentrations. Vasquez et al. (1994) is of the

Table 12: Arbitrary values obtained from DMRT Post hoc tests-Effect of various heavy metals on *L. minor*.

S.No	Heavy Metal	Interaction	Chlorophyll	Protein	Nitrate reductase	Biomass	Total
1.	Cu	1	2	2	3	1	9
2.	Co	2	4	4	1	2	13
3.	Zn	3	5	3	2	4	17
4.	Mn	4	1	5	5	5	20
5.	Pb	5	3	1	4	3	16

Table 13: Arbitrary values obtained from DMRT Post hoc tests-Effect of various concentrations on *L. minor*.

S.No	Concentration(%)	Interaction	Chlorophyll	Protein	Nitrate reductase	Biomass	Total
1.	100	1	1	1	1	1	5
2.	50	2	2	2	2	2	10
3.	40	3	3	3	3	4	16
4.	30	4	4	4	4	5	21
5.	20	5	5	5	5	6	26
6.	10	6	6	6	6	3	33

opinion that reduction in biomass is due to the energy expenditure in metal tolerance mechanism such as compartmentalization of metals in intracellular compartments. Varshney et al. (2007) reported that aquatic weeds have great potential for biomass production. The order of tolerance was Mn > Zn > Pb > Co > Cu.

CONCLUSION

On comparison of the Scheffe Post hoc test the following results were obtained in the tolerance capacity of *Lemna minor* to various heavy metals studied.

Heavy metal accumulation:	Pb > Mn >Zn > Co > Cu
Total chlorophyll:	Zn > Co > Pb > Cu > Mn
Protein content:	Mn > Co > Zn > Cu > Pb
Nitrate reductase:	Mn > Pb > Cu > Zn > Co
Biomass:	Mn > Zn > Pb > Co > Cu

The highest effect of heavy metal in *Lemna minor* is by manganese followed by zinc and lead, while the effect of cobalt stands next with copper showing the least effect. Similarly, a table of values was obtained for the effect of various concentrations, in general, in *Lemna minor*.

Except biomass, all concentrations had similar effect in *Lemna minor*. 20% and 30% concentrations have significant effect in increasing biomass, while all other concentrations of heavy metal have no significant effect on *Lemna minor*.

Heavy metals like manganese, zinc and lead have greater effect in reducing the physiological properties of *Lemna minor*, while 20-30% concentrations of all heavy metals have an influence on the biomass production of *Lemna minor*.

Lemna minor can be used as a remediation for removal of manganese, zinc and lead and its biomass can also be increased. Concentrations up to 10% in aquatic ecosystems are tolerable and the tolerance capacity decreases with increasing concentrations of heavy metals.

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