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Performance of Aquatic Plant Species for Phytoremediation of Heavy Metals Contaminated Water

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Abstract

Heavy metals and organic pollutants are ubiquitous environmental pollutants affecting the quality of soil, water, and air. Over the past 5 decades, many strategies have been developed for the remediation of polluted water. Strategies involving aquatic plant use are preferable to conventional methods. The 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 duckweed *Spirodela polyrhiza* to remove heavy metals from wastewater and the biochemical effect of heavy metals on *Spirodela polyrhiza.* One-month laboratory experiments have been 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 *Spirodela*

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polyrhiza to ameliorate the wastewater contaminated with heavy metals.

Keywords: Phytoremediation, Heavy metals, Wastewater, Biochemical parameters, *Spirodela polyrhiza*

1. Introduction

The progress of urbanization and technologies led to the rise of anthropogenic activities, which consequently have high production of pollutants, affecting ecosystems, including aquatic biomes. One of the contaminating forms that cause environmental impact is heavy metals, which are produced in large quantities by inappropriate disposal of batteries, residential, industrial, agricultural, and mining waste. Such components generate bioaccumulative effects, classifying them as dangerous elements that must be removed from the environment. However, in species such as plants, this bioaccumulative effect can be exploited, aiming towards a biotechnological and bioengineering application to remove metals, called phytoremediation, by employing floating aquatic macrophytes, which have high potential due to their property of retaining contaminants.

Heavy metals are important environmental pollutants, and many of them are toxic even at very low concentrations. Biosphere pollution due to toxic metals has accelerated dramatically since 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 and Pendias, 1989). Toxic metal contamination of soil, aqueous waste streams, and groundwater pose a major environmental and human health problem, which is still in need of an effective and affordable technological solution. Despite 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, that exceed the soil levels (Baker and 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 and 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 and Grant, 1962; Baker and 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 several 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 possess the unique ability to adapt and evolve to tolerance to heavy metals (Antonovics, Bradshaw and Turner, 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 that take place in the plant system. These changes are due to the interaction of sulfahydryl groups of the enzymes. Aquatic plants growing in the polluted water absorb heavy metals, which enter into the food chain, posing a serious threat to human health (Rachel Isaksson et al., 2007).

The use of plant species for cleaning polluted waters named phytoremediation has gained great interest and is adopted by scientists, governmental and non-governmental organizations. However, the concept of using plants to clean up contaminated environments is not new. About 300 years ago, plants were proposed for use in the treatment of wastewater and have gained increasing attention for the last two decades, as an emerging and cheaper technology. 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, water hyacinth (*Eichhornia crassipes* C.F.P.Mart Solms) (Kay et al., 1984), pennywort (*Hydrocotyle umbellata* L.) (Dierberg, et al., 1987), duckweed (*Lemna minor* L.) and water velvet (*Azolla pinnata* R.Br.) (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* Miller were found to remove a wide variety of metal ions from solutions (Jackson et al., 1990; Jackson et al., 1993). Other species with phytoremediation abilities are *Myriophylum brasiliense*, *Salix* sp., and *Populus* sp. (Brown, 1994). *Spirodela polyrhiza* is an aquatic plant, which absorbs heavy metals in water and has been used as the research material for the present study.

2. Materials and Methods

2.1 Experimental Set up

The investigation was carried out using aquatic macrophyte *Spirodela polyrhiza* L. These were collected from a local freshwater pond, IEMPS, Vikram University, Ujjain, Madhya Pradesh. Different concentrations (10, 20, 30, 40, 50, and 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 (12g) were transferred in plastic troughs containing different percent solution of heavy metals (6 liters in each trough) for thirty days. The physiological and biochemical parameters of plants were analyzed on the 30th day. The total chlorophyll content (mg/g fresh weight) of fresh leaves of plants was analyzed by the standard method of Arnon (1949), total protein content (mg/g fresh weight) by the method of Lowery et al. (1951). Nitrate reductase activity (μ mol/min activity/g of fresh weight) was analyzed by the method of by Camm & Stein (1974), and heavy metal accumulation in plants were determined using Atomic Absorption Spectrophotometer (AAS make – Perkin Elmer, USA) at a wavelength and slit width recommended in the manual of Perkin Elmer (1981) following standard protocol proposed by APHA (1990) and total biomass (Dry weight basis).

2.2 Statistical Analysis

The data obtained was subjected to the DMRT test. Mean direct values provided no conclusive results. DMRT test compares mean values and expresses significant subsets. The last subset represents the highest significance, while the first subset represents the least significance. The Univariate analysis of variables in heavy metal concentrations against *Spirodela polyrhiza* was calculated.

3. Results and Discussion

The results of the heavy metal accumulation have been presented in Table –1. These results did not yield a clear picture of the effect of heavy metals.

3.1 Heavy Metal accumulation

The accumulation of all the heavy metals was higher at 10% concentration and was 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 *Spirodela polyrhiza* was Pb > Mn > Zn > Co > Cu. Similar observations have been made by Rai and Tripathi (2008) in *Azolla Pinnata.* They observed that Hg removal was high in *A. Pinnata* comparison to *Vallisneria spiralis,* ion R2 = 0.91) was obtained between applied Hg doses and accumulated amounts of biomass. 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 and. Kanmani 2009). These reports support the observation that *Spirodela polyrhiza* can be used as a heavy metal absorbent also.

3.2 Total Chlorophyll

Total Chlorophyll content in *Spirodela polyrhiza* 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 was maximum where Zn was treated. The hierarchy of heavy metal was Zn > Co > Pb > Cu > Mn. Mooreland and Nuvitzsky (1998) attribute of 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 and Sarma (2007) reported that fertilizer factory effluent containing heavy metals drastically decreases the chlorophyll content of plants. Heavy metal containing solutions have a deleterious effect on the chlorophyll content of *Spirodela polyrhiza* in the present study.

3.3 Total Protein content

Total protein content decreases in all heavy metal treated concentrations (Table 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 and Vijayarenagn (2006) observed reduced protein content at higher concentrations of Cobalt treatment in black gram. Devlin (1975) reported that Nitrogen is a precursor for the synthesis of amino acids. Mayz and 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 are one of the earliest indicators of heavy metal poisoning. Kim et al. (1978), Kastori et al. (1992), and Bhattacharjee and Mukherjee (1994) made similar observations. Therefore protein content is drastically reduced in *Spirodela polyrhiza* treated with heavy metals.

3.4 Nitrate reductase

Nitrate reductase activity decreases in all concentrations of heavy metal treatments (Table 5 & 10). Manganese had the maximum effect while Cobalt had the least effect and the order of toxicity is Mn > Pb > Cu > Zn > Co. The 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 a common target of the toxicants. Siddique (1982) and Bhandal and Kumar (1992) also noticed that inhibition of nitrate reductase activity indicates that this enzyme is sensitive to metal salts.

3.5 Biomass

Spirodela polyrhiza biomass decreased by uptake of heavy metals (Table 6 &11). The decrease was moderate at 50%, while it was minimum at 20% concentrations. Vasquez et al. (1994) are of the opinion that reduction in biomass is due to the energy expenditure in metal tolerance mechanisms 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.

Table: 1 Effect of different concentrations of different heavy metals on *Spirodela polyrhiza.*

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 - µ mol /min activity/g of fresh weight. For uniform calculation values converted into percent value.

Table: 2 Scheffe [a b] Post hoc test of *Spirodela polyrhiza* against different heavy metals.

	N	Subset				
GP		1	2	3	4	5
Mn	18	.4822				
Cu	18		3.2728			
Pb	18			6.9744		
Co	18				6.9833	
Zn	18					7.1433

a. Uses harmonic mean sample size = 18.000.

b. Alpha = .05

Table: 3 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Chlorophyll content against different heavy metals.

	N	Subset				
GP		1	2	3	4	5
Cu	18	.17733				
Co	18		.20939			
Zn	18			.23628		
Mn	18				.26478	
Pb	18					.29772

a. Uses harmonic mean sample size = 18.000.
b. Alpha = .05

Table: 4 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Protein content against different heavy metals.

	N	Subset				
GP		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

a. Uses harmonic mean sample size = 18.000.
b. Alpha = .05

Table: 5 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Nitrate reductase activity against different heavy metals.

	N	Subset				
GP		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

a. Uses harmonic mean sample size = 18.000.

b. Alpha = .05

Table: 6 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Biomass production against different heavy metals.

	N	Subset				
GP		1	2	3	4	5
Cu	18	.6419				
Co	18		.7031			
Pb	18			.9913		
Zn	18				1.1843	
Mn	18					1.3587

a. Uses harmonic mean sample size = 18.000
b. Alpha = .05

Table: 7 Scheffe [a b] Post hoc test of *Spirodela polyrhiza* against different concentrations of heavy metals

	N	Subset					
CONC		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

a. Uses harmonic mean sample size = 18.000.
b. Alpha = .05

Table: 8 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Chlorophyll content in various concentrations of heavy metals.

	N	Subset					
CONC		1	2	3	4	5	6
100	15	.19870					
50	15		.22647				
40	15			.23367			
30	15				.24067		
20	15					.26120	
10	15						.26253

a. Uses harmonic mean sample size = 18.000.
b. Alpha = .05

Table: 9 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Protein content in various concentrations of heavy metals.

	N	Subset					
CONC		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

a. Uses harmonic mean sample size = 18.000.

b. Alpha = .05

Table: 10 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Nitrate Reductase activity in various concentrations of heavy metals.

	N	Subset					
CONC		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

a. Uses harmonic mean sample size = 18.000.
b. Alpha = .05

Table: 11 Scheffe [a b] Post hoc test of *Spirodela polyrhiza*: Biomass Production in various concentrations of heavy metals.

	N	Subset					
CONC		1	2	3	4	5	6
100	15	.7741					
50	15		.8969				
10	15			.9292			
40	15				1.0339		
30	15					1.0529	
20	15						1.1681

a. Uses harmonic mean sample size = 18.000.
b. Alpha = .05

Table: 12 Arbitrary values obtained from DMRT Post hoc tests – Effect of various heavy metals on *Spirodela polyrhiza.*

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

Table: 13 Arbitrary values obtained from DMRT Post hoc tests – Effect of various concentrations on *Spirodela polyrhiza.*

Sl. No	Concentration (%)	Inter action	Chloro phyll	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

4. Conclusion

On comparison of the Scheffe Post hoc test, the following results are obtained in the tolerance capacity of *Spirodela polyrhiza* 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 > C

The highest effect of heavy metal in *Spirodela polyrhiza* 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 *Spirodela polyrhiza*. Except for biomass, all concentrations had a similar effect in *Spirodela polyrhiza*. 20% and 30% concentrations had a significant effect in increasing biomass, while all other concentrations of heavy metal had no significant effect on *Spirodela polyrhiza*. Heavy metals like Manganese, Zinc, and lead have a greater effect in reducing the physiological properties of *Spirodela polyrhiza*, while 20-30% concentrations of all heavy metals have an influence on the biomass production of *Spirodela polyrhiza.*

Spirodela polyrhiza 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.

Based on the results obtained during the present study, the use of macrophyte such as *Spirodela polyrhiza* is recommended for the phytoremediation of different kinds of effluents. The high percentage of heavy metal accumulation in plant biomass proves that *Spirodela polyrhiza* species as very good hyperaccumulators for Zn, Cd, Pb, Ni, Fe, and Cu. The use of such plant species for mitigation of contaminated sites can surely be a cost effective, simple, and economically better option. These plants provide very promising output for industrial waste for a state like Madhya Pradesh.

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