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Effect of EDTA, Phosphate, pH and Metal Species on Cadmium and Nickel Uptake by Aquatic Macrophyte *Spirodela Polyrhiza*

Antaryami Singh*, P. Malodia, M. Kachhawaha, N. Ansari, S.K. Jain and P.K. Khatri

Defence Laboratory, Defence Research and Development Organization
Jodhpur 342 011, India
✉ antaryamisingh@yahoo.com

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Abstract: The phytoremediation potential of an aquatic macrophyte, *Spirodela polyrhiza* for cadmium (Cd) and nickel (Ni) removal was studied. Effect of different concentrations (1–10 mg/L) of Cd and Ni on multiplication rate and photosynthetic pigments were determined to evaluate the tolerance of the plants and the toxicity of Cd and Ni. Presence of EDTA (ethylene diamine tetraacetic acid) exerted a remarkable inhibition on the uptake of both Cd and Ni by the plants. Phosphate in the medium was found to favour the growth of the plants. However, the uptake Cd and Ni by the plant decreased significantly on the addition of phosphate. *Spirodela* exhibited a pH dependent phenomenon of metal accumulation. As compared to uptake at pH 7, Cd and Ni uptake was increased by 29% and 60% at pH 5. However, decline in Cd and Ni uptake was observed at alkaline pH 9 and pH 11. Accumulation of Cd and Ni by plants in the presence of single metal and combination of metal species (Cd+Ni+Zn+Cu) at different concentrations was evaluated. In the presence of other metal species, Cd and Ni uptake was reduced by 21–35% and 21–27%, respectively, as compared to plants exposed to Cd or Ni singly.

Key words: Cadmium, nickel, *Spirodela,* EDTA, phosphate, uptake.

Introduction

Heavy metals are important environmental pollutants that influence the quality of the surface water, and threaten the health of humans upon entering the food chain. Sources of heavy metal contaminants include metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries (Alloway, 1995). Heavy metals pose a critical concern to human health and environmental issues due to their high occurrence as a contaminant, low solubility in biota, and the classification of several heavy metals as carcinogenic and mutagenic (Diels et al., 2002). Toxic heavy metals cause DNA damage, and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Baudouin et al., 2002). Exposure to high levels of these metals has been linked to adverse effects on human health and wildlife. Heavy metal contamination in drinking and non-drinking water poses a major environmental and human health problem, which is still in need of an effective and affordable technological solution. Conventional technologies for the removal of heavy metals, such as chemical precipitation, ion exchange, electrochemical treatment and membrane technology are largely ineffective or extremely expensive, especially when metal concentration in the solution is extremely low (Lamai et al., 2005). Moreover,

*Corresponding Author

these methods are specific to each metal ion. New technologies are required that can reduce heavy metal concentrations to environmentally acceptable levels at affordable costs.

Bioremoval offers a potential alternative to existing methods and is defined as the accumulation and concentration of pollutants from aqueous solutions by the use of biological materials. Bioremoval of heavy metals is one of the most promising technologies involved in the removal of toxic metal from industrial waste streams and natural waters. The major advantages of the bioremoval technology are its effectiveness in reducing the concentration of heavy metal ions to very low levels, and its use of inexpensive biosorption materials and environmentally friendly technologies. Application of microbial cells (bacteria, fungi and microalgae) for metal remediation has been elaborately studied. Plants also have the ability to accumulate nonessential metals and this ability could be harnessed to remove pollutant metals from the environment (Rogers et al., 2000). Plant based bioremediation technologies have received recent attention as strategies to clean-up contaminated soil and water. Aquatic macrophytes also take up metals from the water, producing an internal concentration several fold greater than their surroundings. Many of the aquatic macrophytes have been found to be the potential scavengers of heavy metals from aquatic environment. Duckweeds (*Spirodela*, *Lemna*, *Wolffia* and *Wolfiella*) are a variety of aquatic plant floating at the water surface, distributed worldwide in freshwater to brackish estuaries and easy to culture in the laboratory. There is much evidence that duckweed could accumulate heavy metals in their tissues when grown in polluted waters (Boonyapookana et al., 2002; Kara and Kara, 2005).

High concentrations of toxic metal ions are being constantly poured into the environment, which results in the long lasting biohazards in the aquatic ecosystem. Of all the toxic heavy metals, cadmium (Cd) ranks the highest in terms of damage to plant growth and human health. Toxicity of cadmium is reported to be 2–20 times higher than many other heavy metals (Vassilev et al., 1998). Cadmium has been recognized for its negative effect on the environment and is accumulated throughout the food chain posing a serious threat to human health. Cadmium accumulated in the kidneys implicates a range of kidney diseases (WHO, 1997). Heavy metal ion such as Nickel (Ni) is an essential micronutrient for plant metabolism but when present in excess, can become extremely toxic (Williams et al., 2000). The metals like Cd and Ni not only cause serious health hazards but also disturb the ecological status of biota (Malik and Ahmad, 1995). Bioaccumulation of heavy metals by aquatic plants has promising potential for the ex situ and in situ clean-up of contaminated waters (Robinson et al., 2006).

The effective accumulation of heavy metals by aquatic plants and the effectiveness in phytoremediation technology have been widely studied. However, various factors may influence the accumulation of heavy metals by aquatic floating macrophytes. In the present investigation, the influence of anionic species, EDTA (ethylene diamine tetraacetic acid) and phosphate, pH, and the presence of other metal species on the accumulation of cadmium and nickel in an aquatic macrophyte, *Spirodela polyrhiza* was studied.

Materials and methods

Plant material

Spirodela polyrhiza plants growing in local pond water were collected for the study. Medium (Bonner and Devirian, 1939) containing all the essential micronutrients were used to maintain stock and experimental cultures. Healthy plants grown in BD medium for 10–12 days were selected for evaluating tolerance of the plants to cadmium and nickel and accumulation studies.

Growth and multiplication of plants

Tolerance of *Spirodela polyrhiza* to two heavy metals – cadmium and nickel was studied. Multiplication rate (MR) and fresh weight (FW) of the plants exposed to different concentrations of Cd and Ni was determined. Nine fronds (2–3 colonies) of 12 days old stock of *Spirodela polyrhiza* were inoculated in the medium supplemented with different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/L) of Cd prepared from cadmium chloride (Merck, Germany) or Ni prepared from nickel chloride (Merck, Germany). Multiplication rate of the untreated and treated plants was calculated using the formula as described by Muhonen et al. (1983).

$$MR = Fd - Fo \,/\, Fo\ (n) \times 100$$

where Fd =frond number on day of count; Fo = frond number on first day; n = number of culture days.

Total fresh weight was determined from the fronds multiplied up to 7 days of exposure to different metal concentrations. The treated and control plants were gently blotted and weighed. Cd and Ni accumulation by plants at different metal concentrations was also determined.

Estimation of chlorophyll and carotenoid content

Effect of different concentrations of Cd and Ni (1–10 mg/L) on the photosynthetic pigments – chlorophyll *a*,

chlorophyll *b* and carotenoid of *Spirodela polyrhiza* was evaluated. For determination of chlorophyll *a,* chlorophyll *b* and carotenoid, the control and treated plant material (250 mg fresh weight) in 20 ml of 80% acetone was kept overnight in stoppered conical flask at 4° C. On subsequent day, the plant sample was homogenized and centrifuged at 4000 rpm for 10 minutes. The final volume of the supernatant was made to 25 ml with 80% acetone. The optical density of the extract was measured at 480, 510, 645 and 663 nm using UV–VIS Spectrophotometer Shimadzu 1601.

The chlorophyll content was calculated according to the formula of Witham et al. (1971):

Chlorophyll *a* (mg g^{-1} FW) = 12.7 (D663) – 2.69 (D645) × V/1000 × W

Chlorophyll *b* (mg g^{-1} FW) = 22.9 (D645) – 4.68 (D663) × V/1000 × W

The carotenoid content was determined using the formula by Duxbury and Yentsch (1956):

Carotenoids (mg g^{-1} FW) = 7.9 (D480) – 3.6 (D510) × V/1000 × W

where V = volume of the chlorophyll extract and W = fresh weight of the plant

Effect of EDTA, phosphate and pH on accumulation

Effect of chelating agent EDTA, phosphate and different pH conditions on accumulation of Cd and Ni by *Spirodela polyrhiza* plants was studied. Plants were exposed to 2.0 mg/L of Cd or Ni in the BD medium supplemented with 0, 2.5, 5.0 and 7.5 mg/L of EDTA (disodium salt). Cd and Ni content of the plants were analysed for the accumulation in the presence and absence of EDTA. For evaluating the effect of phosphate on growth of the plants and accumulation of Cd and Ni, 9-frond plant of 10–12 days old stock was inoculated in BD medium supplemented with 0, 10, 20, 30, 40, 50, 100 and 200 mg/L of phosphate (potassium dihydrogen phosphate). Effect of different pH on accumulation of Cd and Ni by plants was also studied. 9-frond plants were exposed to 2.0 mg/L of Cd and Ni at pH 3, 5, 7, 9 and 11 for 7 days and analysed for metal content.

Exposure to single and mixed metals species

The *Spirodela* plants were exposed to individual metals where Cd and Ni were added at concentrations of 1.0, 2.0 and 5.0 mg/L. Plants were also exposed to mixture of metals containing Cd 1.0 + Ni 1.0 + Zn 1.0 + Cu 1.0, Cd 2.0 + Ni 2.0 + Zn 2.0 + Cu 2.0 and Cd 5.0 + Ni 5.0 + Zn 5.0 + Cu 5.0 mg/L. Accumulation of Cd and Ni by plants in the presence of single metal and combination of metal species was determined.

Metal accumulation

The plant samples were digested using nitric acid and filtered. The filtrate was analysed for cadmium and nickel concentration using Atomic Absorption Spectrophotometer (Perkin Elmer, 2380). The metal content obtained was expressed as dry weight (DW).

Results

To evaluate the efficacy of *Spirodela polyrhiza* to treat cadmium or nickel contaminated water, the toxicity of these metals to the plants was determined. The effect of different concentrations of Cd and Ni on MR and FW of *Spirodela polyrhiza* is depicted in Table 1. Significant decrease in MR and FW was observed with increasing concentration of Cd up to 8.0 mg/L in the medium. However, no plant growth was observed in the presence of 9.0 mg/L and 10.0 mg/L Cd indicating that the plants were not tolerant to higher Cd concentrations. It was observed that 1.0 mg/L of Ni increased MR and FW at the end of 7 days to 27% and 14% of the control, respectively. However, the concentration of 9.0 and 10.0 mg/L of Ni proved to be toxic, affecting the plant growth severely. Fresh weight after 7 days decreased from 58.5 mg to 4.5 mg at 10.0 mg/L of Ni. MR and FW observed in the presence of Ni (1–10 mg/L) indicate considerably less toxicity to the plants as compared to the cadmium. MR was reduced by 46% in the presence of 5.0 mg/L Nickel. However, in the presence of 5.0 mg/L Cd, the MR was found to be reduced by 59%. The growth and multiplication ability of *S. polyrhiza* indicate that the plant is a suitable species for treating wide range of Cd and Ni contaminated water.

Photosynthetic pigments of *Spirodela* plants decreased significantly in the presence of Cd (Table 2). Chlorophyll *a* and chlorophyll *b* decreased by 15% and 16%, respectively, in the presence of 1.0 mg/L Cd in comparison to the control. Carotenoid content also exhibited reduction by 34% at 1.0 mg/L Cd. Photosynthetic pigments were further reduced at the higher concentrations of metal. It was observed that 1.0 mg/L of Ni marginally increased the chlorophyll *a*, chlorophyll *b* and carotenoid content (Table 3). The exposure to higher concentrations of 3.0 mg/L and 4.0 mg/L Ni reduced the chlorophyll *a* significantly to about 9% and 35% of the control. Similarly, chlorophyll *b* decreased from 0.410 to 0.358 and 0.322 mg/g fresh weight after 7 days of exposure to

Table 1: Effect of different concentrations of Cd and Ni on the growth of *Spirodela polyrhiza*

Metal (mg/L)	*Multiplication rate*		*Fresh weight (mg)*	
	Cd	*Ni*	*Cd*	*Ni*
Control	48.03 ± 1.59	50.00 ± 3.97	54.00 ± 2.00	58.50 ± 1.50
1.0	38.09 ± 1.58	63.49 ± 1.59	46.50 ± 1.50	66.50 ± 1.50
2.0	30.95 ± 0.79	51.58 ± 3.96	42.00 ± 1.00	59.00 ± 3.00
3.0	27.78 ± 2.38	41.26 ± 1.58	37.50 ± 5.50	50.00 ± 2.00
4.0	23.81 ± 1.59	35.71 ± 3.96	33.50 ± 1.50	45.50 ± 2.50
5.0	19.83 ± 0.79	26.98 ± 3.17	19.00 ± 1.00	36.50 ± 4.50
6.0	18.25 ± 0.79	22.22 ± 1.59	14.00 ± 0.00	30.50 ± 1.50
7.0	14.28 ± 0.00	20.63 ± 3.17	10.00 ± 0.00	17.50 ± 2.50
8.0	4.76 ± 0.00	15.07 ± 3.96	7.50 ± 0.00	11.00 ± 1.00
9.0	NS	4.75 ± 3.17	NS	6.50 ± 1.50
10.0	NS	3.17 ± 1.59	NS	4.50 ± 1.50

NS = No survival, plants did not multiply at these concentrations.

Table 2: Effect of Cd on the photosynthetic pigments of *Spirodela polyrhiza*

Cadmium (mg/L)	*Chlorophyll a*	*Chlorophyll b*	*Carotenoids*
		mg/g FW	
Control	0.342 ± 0.016	0.410 ± 0.024	0.256 ± 0.009
1.0	0.291 ± 0.014	0.344 ± 0.013	0.171 ± 0.010
2.0	0.251 ± 0.015	0.344 ± 0.012	0.153 ± 0.006
3.0	0.203 ± 0.004	0.218 ± 0.010	0.133 ± 0.003
4.0	0.192 ± 0.017	0.198 ± 0.005	0.114 ± 0.002
5.0	0.148 ± 0.002	0.160 ± 0.006	0.109 ± 0.003
6.0	0.141 ± 0.004	0.159 ± 0.005	0.089 ± 0.004
7.0	0.093 ± 0.003	0.115 ± 0.004	0.108 ± 0.002

Table 3: Effect of Ni on the photosynthetic pigments of *Spirodela polyrhiza*

Nickel (mg/L)	*Chlorophyll a*	*Chlorophyll b*	*Carotenoids*
		mg/g FW	
Control	0.342 ± 0.016	0.410 ± 0.024	0.256 ± 0.009
1.0	0.357 ± 0.014	0.418 ± 0.013	0.274 ± 0.010
2.0	0.348 ± 0.010	0.400 ± 0.016	0.250 ± 0.007
3.0	0.310 ± 0.015	0.358 ± 0.006	0.180 ± 0.012
4.0	0.221 ± 0.013	0.322 ± 0.006	0.136 ± 0.010
5.0	0.179 ± 0.006	0.245 ± 0.017	0.120 ± 0.003
6.0	0.161 ± 0.011	0.221 ± 0.007	0.109 ± 0.003
7.0	0.117 ± 0.004	0.146 ± 0.005	0.098 ± 0.003
8.0	0.094 ± 0.003	0.115 ± 0.004	0.108 ± 0.003

3.0 mg/L and 4.0 mg/L of Ni, respectively. Carotenoid content was also effected at higher concentrations of Ni with 29% and 47% decrease at 3.0 mg/L and 4.0 mg/L Ni. With increasing concentrations of Ni, progressive decrease in the chlorophyll *a*, chlorophyll *b* and carotenoid contents was observed.

The uptake of cadmium was 533.79 ± 23.22 µg/g DW in the presence of 1.0 mg/L Cd and increased with increasing metal concentration in the medium (Table 4). Similar uptake pattern was observed for Ni. The uptake of Cd and Ni was proportional to the initial concentration and the plants treated with 7.0 mg/L of Cd accumulated the highest concentration of metal (1568.25 ± 35.16 µg/g DW), while those treated with 8 mg/L of Ni accumulated the highest concentration of metal in the plants (1608.60±15.90 µg/g DW) after 7 days. The results

Table 4: Uptake of Cd and Ni by *Spirodela polyrhiza* at different metal concentrations

Metal (mg/L)	*Cadmium*	*Nickel*
	µg/g DW	
1.0	533.79 ± 23.22	571.02 ± 30.42
2.0	1024.11 ± 35.46	1080.00 ± 28.20
3.0	1274.49 ± 42.18	1243.44 ± 34.47
4.0	1363.44 ± 14.64	1342.74 ± 11.67
5.0	1485.48 ± 11.70	1537.23 ± 38.32
6.0	1551.69 ± 67.02	1599.30 ± 38.37
7.0	1568.25 ± 35.16	1601.25 ± 15.52
8.0	1471.02 ± 43.29	1608.60 ± 15.90
9.0	ND	1147.68 ± 13.86
10.0	ND	ND

Note: ND = not determined.

indicate the increasing ability of *Spirodela* to accumulate Cd and Ni with increase in concentration of metals in the solution.

Effect of two anionic species, EDTA and phosphate in the medium on the quantity of metal uptake by the plants were assessed. Inhibition of Cd and Ni uptake was observed when EDTA was added in the medium. About 51% reduction in Cd accumulation was observed after 24 hours in the presence of 2.5 mg/L EDTA (Figure 1a). With increasing concentration of EDTA in the medium, accumulation of Cd by the plants was further reduced. The effect of EDTA on the uptake of Ni was more pronounced showing 59% to 69% reduction in accumulation in the presence of 2.5 to 7.5 mg/L EDTA after 24 hours (Figure 1b). The results indicate that the uptake levels of both Cd and Ni were lowered considerably when EDTA ions was present in the medium.

Effect of different concentrations of phosphate on growth of *Spirodela polyrhiza* was studied. Multiplication rate and fresh weight in the presence of 10 to 200 mg/L phosphate after 7 days was determined. The presence of phosphate in the medium was found to favour the growth of the plants. Increase in MR (Figure 2a) and fresh weight (Figure 2b) was observed in the presence of up to 20 mg/L phosphate. No significant difference in MR and FW was observed with further addition of phosphate up to 50 mg/L. At higher concentration of 100 mg/L, decrease in MR and FW was observed. The concentration of 200 mg/L was, however, observed to be toxic to the plants with significant decrease in MR and FW as compared to the control plants. Similar effect of phosphate was observed when either Cd or Ni (2.0 mg/L) was present in the medium. Presence of phosphate was found to reduce the toxicity of Cd or Ni to plants. With the addition of phosphate to medium containing Ni or Cd, the growth parameters increased with increasing phosphate concentration. For evaluating the effect of phosphate ion on the uptake of cadmium and nickel by *Spirodela polyrhiza,* the plants were grown in media supplemented with Cd (2.0 mg/L) or Ni (2.0 mg/L) and different phosphate concentrations of 0–200 mg/L. The accumulation of both Cd and Ni by the plants decreased significantly on the addition of phosphate (Table 5). Phosphate at lowest concentration of 10.0 mg/L caused 22.42% and 28.70% reduction in the uptake of Cd and Ni, respectively. With further increase of phosphate, the metal accumulation was reduced.

As compared to uptake at pH 7, Cd and Ni uptake was increased at pH 5 (Table 6). Maximum accumulation of Cd and Ni after 7 days was 1071.72±43.08 µg/g DW and 1435.86±28.83 µg/g DW at pH 5. Acidic pH 3 had adverse effect on the growth of the plants both in the presence of Cd or Ni. At pH 9 and 11, decrease in uptake was observed as compared to uptake at pH 5.

Uptake of Cd by plants on exposure to single metal and combination of metal species is presented in Table

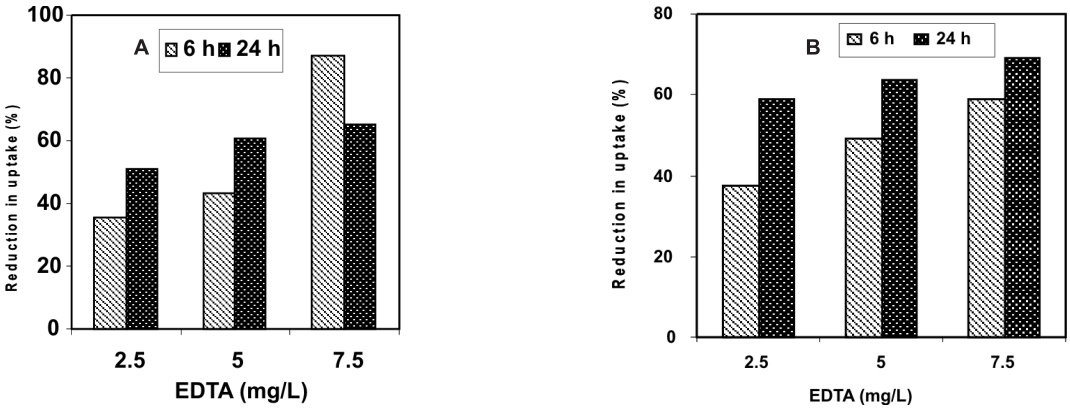

Figure 1: Effect of EDTA on uptake of (A) Cd and (B) Ni in *Spirodela polyrhiza*.

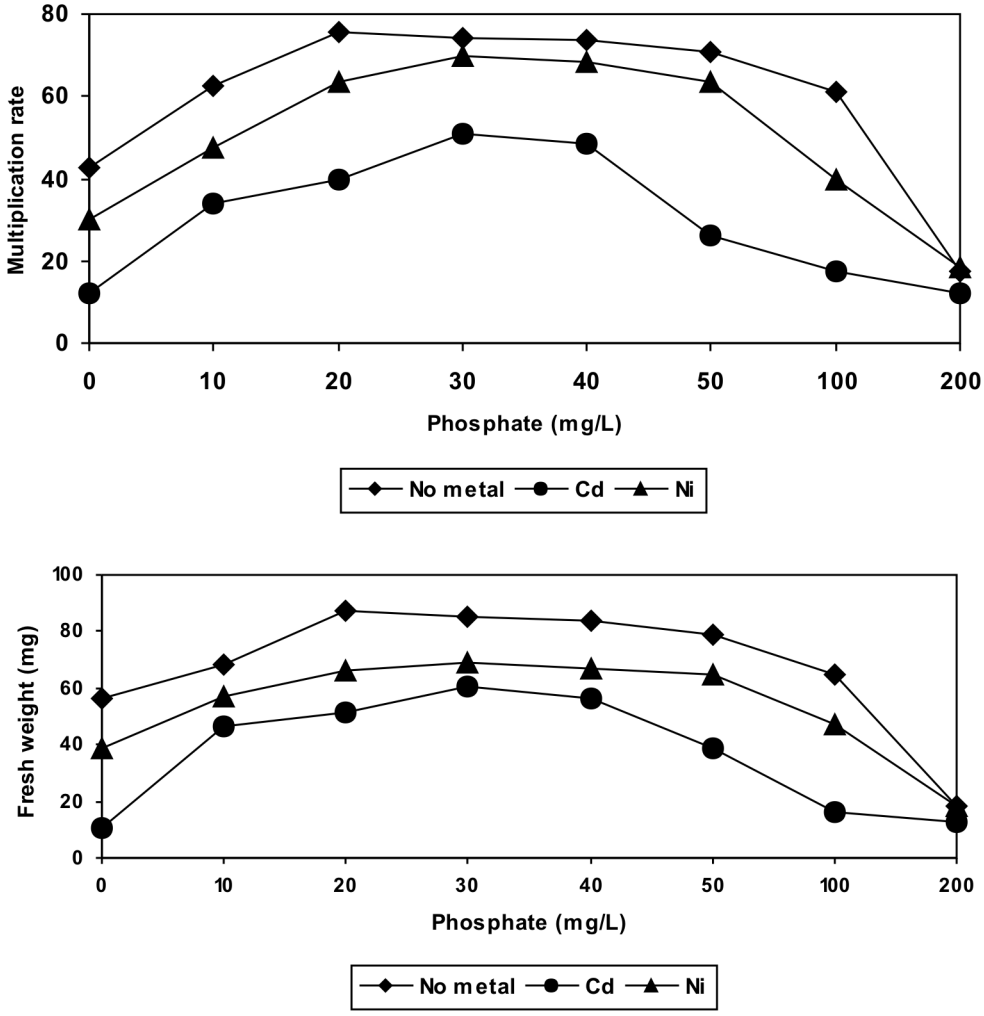

Figure 2: Effect of phosphate on (A) Multiplication rate (MR) and (B) Fresh weight (FW) of *Spirodela polyrhiza* exposed to Cd or Ni.

Table 5: Effect of phosphate on the uptake of Cd and Ni by *Spirodela polyrhiza*

Phosphate (mg/L)	*Cadmium*	*Nickel*
	µg/g DW	
0	1245.51 ± 40.95	1590.00 ± 72.42
10	966.21 ± 39.36	1133.55 ± 63.47
20	686.88 ± 47.91	844.29 ± 23.67
30	488.25 ± 43.11	655.71 ± 34.41
40	558.63 ± 26.85	737.13 ± 21.84
50	573.09 ± 15.48	816.42 ± 29.22
100	726.21 ± 28.20	1058.55 ± 44.64
200	926.91 ± 76.23	1138.92 ± 91.02

Table 6: Effect of pH on the uptake of Cd and Ni by *Spirodela polyrhiza*

pH	*Cadmium*	*Nickel*
	µg/g DW	
3	ND	ND
5	1071.72 ± 43.08	1434.86 ± 28.83
7	827.58 ± 22.86	893.79 ± 23.22
9	597.93 ± 17.29	670.35 ± 25.35
11	595.86 ± 28.26	616.53 ± 17.79

ND= not determined, plants did not multiply.

7. Cadmium content of the plants increased significantly with increasing metal concentration (1.0–5.0 mg/L) in the medium. Similar effect was also observed in the presence of mixed metals. Cd accumulation was dependent on the initial metal concentration. In the plants treated with Cd in presence of other metal species, Cd uptake reduced by 21% to 35% as compared to plants exposed to Cd singly. About 21–27% decrease in Ni uptake by the plants due to presence of other metal species was observed (Table 8). Cd and Ni content of the plants

was 280.35 µg/g DW and 437.58 µg/g DW, respectively, in the plants exposed to mixed metal species at concentration of 5.0 mg/L each. The comparison of Cd and Ni uptake in the plants exposed to mixture of metal species indicate higher uptake of Ni as compared to Cd. The results show preferential uptake of Ni over Cd by the *Spirodela* plant.

Discussion

Aquatic plants have been identified as a potential useful group for accumulating and bioconcentrating heavy metals. The tolerance to Cd and Ni in the duckweed, *Spirodela polyrhiza* was studied as a first step to determine the use of this aquatic plant species to remove heavy metals from polluted water. Among the metals studied, Cd is classified as extremely toxic and Ni is classified as moderately toxic. It was found that both Cd and Ni concentrations in the medium have a great impact on the growth responses and the physiological processes in *Spirodela.* Excess metal concentration inhibited both frond growth and frond multiplication of *Spirodela polyrhiza.* Cadmium inhibited duckweed growth even at low concentration and plants showed significant reduction of the biomass at concentrations between 1 and 8 mg/L. At higher concentrations (9–10 mg/L) of Cd, no growth of the plants was observed. Ni when present at concentrations ≤1.0 mg/L was an essential element for the development of *Spirodela* fronds because of its important role in cellular metabolism. Concentrations higher than 3 mg/L of Ni were toxic for the macrophytes and decreased considerably the growth rate. The most common effect of Cd toxicity in plants is stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways (Arduini et al., 1996). In our study, varied concentrations of Cd and Ni affected fresh weight of *Spirodela polyrhiza*. Parameters such as MR and FW are used as useful indicators of metal toxicity in plants. In the present study, Cd stress showed a higher decline in these parameters as compared to Ni. Nickel is ubiquitous in nature and in some organisms it acts as an essential co-factor of some enzymes. On the other hand, it is reported that the higher concentration of nickel ions interact with many cellular components such as nucleotides, amino acids and phospholipids which probably results in the disorders in physiological and biochemical processes (Bhushan and Hoondal, 1997). Based on their chemical and physical properties, three different molecular mechanisms of heavy metal toxicity have been reported: (i) production of reactive species by autooxidation and Fenton reaction (Fe, Cu), (ii) blocking of essential functional groups in biomolecules (Cd, Hg), and (iii) displacement of essential metal ions from biomolecules (Schutzendubel and Polle, 2002).

Photosynthetic pigments being the most important biochemical parameter indicating the metabolic activity of the plant are considered as a suitable bioindicator of toxicity. Various abiotic stresses decrease the chlorophyll content in plants. Several reports show chlorophyll biosynthesis inhibition by metals in higher plants (Prasad and Prasad, 1987). In the present study, Cd and Ni exposure significantly decreased the total chlorophyll content of the test plant. Decrease in the carotenoid content in the presence of Cd and Ni was also detected. The decline in chlorophyll content in plants exposed to Cd stress is believed to be due to the inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALA dehydratase) and protochlorophyllide reductase (Van Assche and Clijsters, 1990) associated with chlorophyll biosynthesis; impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Van Assche and Clijsters, 1990) and the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule. Our results of decrease in chlorophyll content corroborate with the findings of Siedlecka and Krupa (1996) who also found

Table 7: Effect of metal species on the uptake of Cd in *Spirodela polyrhiza*

Metal species (mg/L)	*Cd uptake (µg/g DW)*
Cd 1.0	136.63 ± 10.14
Cd 2.0	235.83 ± 5.04
Cd 5.0	382.74 ± 27.90
Cd 1.0 + Ni 1.0 + Zn 1.0 + Cu 1.0	107.58 ± 12.75
Cd 2.0 + Ni 2.0 + Zn 2.0 + Cu 2.0	153.09 ± 16.29
Cd 5.0 + Ni 5.0 + Zn 5.0 + Cu 5.0	280.35 ± 68.04

Table 8: Effect of metal species on the uptake of Ni in *Spirodela polyrhiza*

Metal species (mg/L)	*Ni uptake (µg/g DW)*
Ni 1.0	180.00 ± 13.41
Ni 2.0	302.04 ± 20.46
Ni 5.0	597.93 ± 19.20
Ni 1.0 + Cd 1.0 + Zn 1.0 + Cu 1.0	142.74 ± 28.80
Ni 2.0 + Cd 2.0 + Zn 2.0 + Cu 2.0	225.51 ± 8.76
Ni 5.0 + Cd 5.0 + Zn 5.0 + Cu 5.0	437.58 ± 8.76

a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. Reduction of chlorophyll content by excess Cd has also been reported in *Riccia* sp. by Prasad et al. (2004) who concluded that high Cd inhibits the formation of chlorophyll by interfering with protochlorophyllide production.

In the present study, Cd and Ni were efficiently depleted from the solution by *Spirodela polyrrhiza*. It was noticed that, increasing Cd and Ni concentration in the nutrient solution significantly increased uptake of Cd and Ni by the plants. The results indicate that *Spirodela* plants could be a good candidate for the phytoremediation of water polluted with 1–7 mg/L Cd or 1–8 mg/L Ni. Duckweeds effectively remove appreciable quantity of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) from freshwater especially at low concentrations. Maine et al. (2001) reported that remaining Cd concentration in water was inversely related with time and depended on the initial Cd concentration. Boonyapookana et al. (2002) have reported phytoaccumulation by duckweed *Wolffia globosa* in the presence of 1 to 8 mg/L of Cd and Cr. Cadmium is readily taken up and accumulated in trace quantities, but is not essential for plant growth. Accumulation of Cd has been reported for plants grown in hydroponics, pot culture and under field conditions (Benavides et al., 2005). Denny (1987) noted that the main route of heavy metal uptake in emergent and surface-floating plants like water hyacinth was through the roots. In locating the sites of mineral uptake in plants, it has been found that ions penetrated plants by passive process, mostly by exchange of cations which occurred in the cell wall. Much of the metal uptake by plant tissue is by absorption to anionic sites in the cell walls and the metals do not enter the living plant. This explains why plants can have very high magnitude of heavy metal concentration in their tissues compared to their surrounding environment (Hasan et al., 2009).

The success of phytoremediation depends upon the ability of a plant to accumulate and translocate heavy metals, a function of the specific phenotype and genotype (Chen et al., 2003). Several studies have documented that the addition of chelates to the soil such as ethylene-diamine-tetracetic acid and structural analogues increases the phytoavailability of heavy metals by forming water-soluble chelate-heavy metal complexes (Huang et al., 1997). However, EDTA exerted a remarkable inhibition on the uptake of both Cd and Ni by the plants in the present study. Our results corroborate with the findings of Liu and Wu (1993) who have reported similar effects of decreased metal accumulation in *Anacystis* in the presence of chelating agents. It is postulated that this inhibitory effect is due to the formation of a stable complex which then hinders the uptake of metals by plant biomass. The presence of EDTA has also been found to enhance desorption of heavy metals from biomass. This implies that EDTA might be a stronger competitor for heavy metal bonding than the binding sites on the plant biomass surfaces. Probably, the bond between heavy metals and the binding sites on cell surfaces is so weak that it is readily broken and replaced by EDTA-heavy metals. The results indicate that EDTA and other complex forming compounds should be eliminated from water in order to increase the recovery of heavy metals using plant biomass.

Significant reduction in Cd uptake by *Spirodela polyrhiza* was observed with addition of 10–200 mg/L of phosphate. Similar observation was recorded for Ni uptake at all the test concentrations of phosphate in the medium. This decrease in metal accumulation would be attributed to strong interaction among chemical components, and competition among ions in aquatic environment. The results are in corroboration with Wang and Duan (2009) who have reported significant reduction in arsenic accumulation in rice seedlings with increasing external phosphate concentrations.

Spirodela exhibited a pH dependent phenomenon of metal accumulation. As compared to accumulation at pH 7, Cd and Ni accumulation was increased by 29% and 60% at pH 5. However, decline in Cd and Ni accumulation by about 28% was observed at alkaline pH 9 and pH 11. Nickel uptake by plants was also reduced by 25% and 31% at pH 9 and pH 11 as compared to accumulation at pH 7. The results indicate that pH regulated Cd and Ni uptake in *Spirodela* plants. In the acidic pH, uptake was stimulated whereas at basic pH it was strongly inhibited. Several studies have indicated that maximum uptake occurs in acidic conditions and that the uptake of all the metals tested showed decrease at alkaline pH (Kukier et al., 2004).

In water bodies, metals may be present in combination. Consequently, metal pair interaction is a factor to be considered. However, there are few studies on the effect of metal pair interactions on metal accumulation by plants. In the present study, accumulation of Cd and Ni by *Spirodela* in the presence of single metal and combination of metal species (Cd+Ni+Zn+Cu) at different concentrations was evaluated. The Cd or Ni contents in the Cd+Ni+Zn+Cu group were lower than in the metals present individually. The presence of one metal ion in solution decreased the uptake of the other metal

ion. Similar results have been reported. The bioaccumulation of single metal is known to be influenced by the presence of other metals, resulting in inhibited or enhanced bioaccumulation of one metal in the mixture (Peralta-Videa et al., 2002). The comparison of Cd and Ni accumulation by the plants exposed to mixture of metal species in the present study indicated preferential accumulation of Ni as compared to Cd.

Conclusion

The growth and multiplication ability of *Spirodela polyrhiza* in the presence of Cd and Ni indicate that the plant is a suitable species for treating contaminated water containing up to 7.0 mg/L Cd and 8.0 mg/L Ni. Plants showed increasing ability to accumulate Cd and Ni with increase in concentration of metals. The uptake of Cd and Ni was negatively correlated with EDTA and phosphate. *Spirodela polyrhiza* showed accumulation potential at different pH ranges and also in the presence of other metal species suggesting its feasibility for use in phytoremediation of metals in diverse aquatic environment.

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