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Effect of pH and Heavy Metal Concentration on Phytoaccumulation of Zinc by Three Duckweeds Species

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Abstract: Biosorption is an effective means of removal of heavy metals from wastewater. In this work the biosorption behavior of *Lemna* spp. of the family Lemnaceae was investigated as a function of pH, amount of biosorbent and initial Zn concentration. The aim of this study was to investigate the capacity of small free floating aquatic macrophytes namely *L. minuta, L. minor* and *L. trisulca* L. to purify waters polluted by Zn. Metal bioaccumulation process was affected by various values of pH and concentration of zinc solution. Plants treated with 15 mg/L of Zn accumulated 18366.4±2614 mg/kg DW of Zn. The highest bioconcentration potential of Zn was observed by *L. trisulca* (97 %), *L. minuta* (89%) and *L. minor* (83 %). The metal was accumulated in higher amount in dry biomass with increasing Zn levels. Bioconcentration Factor (BCF) ranged between 391 and 2955 for the three species. *L. trisulca* showed the highest bioconcentration potential. The results indicated that the biomass of *Lemna* spp. Is suitable for development of efficient biosorbent for the removal of zinc from wastewater of chemical and allied process industries.

Key words: *Lemna* · Heavy metal · Phytoremediation · Bioconcentration · Wastewaters

INTRODUCTION

Increasing industrialization and urbanization has given an increasing problem of heavy metals which are listed as priority pollutants by the US Environmental Protection Agency [1]. Soil and water pollution by toxic heavy metals is a major environmental concern worldwide. Heavy metals can readily enter bio-geochemical cycles and so major efforts should be made to prevent or minimize their distribution and damage. Phytoremediation is a novel technology for environmental clean-up whereby metal-accumulating plants remove, detoxify or stabilize pollutants from contaminated sites [2]. All plants, terrestrial and aquatic, have the ability to acquire and accumulate metal ions such as Pb, Cu, Mn and Zn, which are essential for plant growth and development. Some plants can also accumulate nonessential toxic metal ions. Consequently, the concept has emerged that plants can be used to remove toxic metals from soil and water thereby contributing to the remediation of polluted sites. Some aquatic plants have been investigated for their potential to improve wastewater quality because of their ability to grow in water polluted by heavy metals [3].

Aquatic plants play an important role in maintaining the purification capability of water and the entire aquatic ecosystem [4]. In the field of ecotoxicology, *Lemna* spp. (duckweed) have been used for the removal of heavy metals from wastewater and constructed wetlands [5,6]. These species present the additional advantage of growing under varied climatic conditions with rapid growth rates [7,8]. Because duckweed is easily raised even in the laboratory, the possible culturing for use as animal feed and for human consumption was also studied [9,10].

Duckweeds (*Lemna*, *Spirodela*, *Wolffia* and *Wolfiella*), are worldwide distributed in freshwater to brackish estuaries. These are free-floating, easy to culture in laboratory and are a convenient plant material for ecotoxicological investigations [11,12]. In particular, species of *Lemna* are reported to accumulate toxic metals and therefore are being used as experimental model systems to investigate heavy metal induced responses [13,11]. The accumulation of metals and metalloids in *Lemna* takes advantage of quality biomass for biosorption on the cell surface and high metabolic mediated incorporation of contaminants into the cells.

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The metabolic mediated incurporation is regarded as a more permanent sink of pollutants, while biosorption can be either temporary or permanent depending on the biosorption mechanism process involved [14].

There are several studies that have shown that most *Lemna* spp. show an exceptional capability and potential for the uptake and accumulation of heavy metals, radionuclides as well as metalloids, surpassing that of algae and other aquatic macrophytes [15,16]. Several comprehensive reviews have been written, summarizing many important aspects of this novel plant based technology [17-20]. These reviews give general guidance and recommendations for applying phytoremediation, highlighting the processes associated with applications and underlying biological mechanisms. There has been a tremendous amount of attention given to the use of biological systems for removal of radionuclides and heavy metals from solutions [21,22]. More recently, phytoremediation has emerged as one of the alternative technologies for removing pollutants from the environment [23,24].

MATERIALS AND METHODS

Sample Collection: *Lemna* species namely *L. minuta, L. minor* and *L. trisulca* were obtained from the surface of wastewater pond using a long handed mesh sampler. *Lemna* species were hydroponically grown for 20 days in 1 l of standard N free Espinas and Watanabe medium [25] in 20×25×5 cm plastic trays at 30 ± 2 °C in a polyhouse. They multiplied vegetatively and produced healthy biomass. The fresh biomass was harvested, thoroughly washed with distilled water, gently blot dried and used as inoculum for further experiment. 0.5 g of healthy fronds of *Lemna* species were inoculated into 200 mL of E and W medium supplemented with 1-20 mg/L of Zn in 500 ml glass beakers and incubated under same conditions as described above. Zn was provided as $Zn(NO_3)_2.6H_2O$). All solutions were prepared in double distilled water. *Lemna* fronds grown in unamended E and W medium served as controls. Fresh medium was added to compensate for evaporation losses through out the incubation period. Experiment was performed in triplicate. After 10 days the fronds were harvested, blot dried and weighed.

Heavy Metal Analysis: Heavy metal content in water samples was measured in filtrates after a preconcentration complexation step using (2%) ammonium pyrolodin dithiocarbamate (APDC), solvent extraction into methyl isobutyl keton (MIBK) followed by back extraction in nitric acid [26]. For plant materials, 0.2 g of fronds powder was digested according to the method described by [27]. Determinations of heavy metals in all samples were carried out by Varian inductively coupled plasma atomic emission spectroscopy (ICP-AES). The preference for bioaccumulation of heavy metals by *Lemna* spp. was estimated using the bioaccumulation factor (BF, ratio of heavy metal content in plant tissue compared to that in water) according to Shin *et al.* [28].

Removal of Heavy Metals by *Lemna* spp.: Water samples of 2 mL were taken daily from the vessels in order to measure Zn concentration removed from the nutrient solution. The percentage metal efficiency was calculated according to Tanhan *et al.* [29].

$$\%\ efficiency = \frac{C_0 - C_1}{C_0} \times 100$$

C_0 and C_1 are initial and remaining concentrations of metal in medium (mg/L). After 10 days of exposing, *Lemna* fronds to 1, 5, 10, 15 and 20 mg/L of Zn, the plants were rinsed with distilled water, filtered and dried for 48 h at 70 °C; dry mass was then determined [30]. Dry plant samples were digested with 10 ml nitric acid (69% HNO_3), diluted to 25 ml with distilled water and impurities were removed by filtration [31]. The metal concentration in water and in duckweed biomass was carried out with a flame atomic absorption spectrophotometer (Shimadzu AA 6601 F). A series of standard solutions (0.01, 0.25, 0.50 and 1.00 mg/L) were measured as check standards. Each sample was measured in duplicate.

Bioconcentration Factor: BCF is an index of the ability of a plant to accumulate metal ions with respect to the metal ion concentration in the growth environment. It is defined as the ratio of metal concentration in the dry biomass to the initial concentration of metal ion in feed solution [32]. BCF was calculated as described by Hawker and Connell [32].

$$BCF = C_{org} / C_w$$

where:
BCF = Bioconcentration factor (L/kg)
C_{org} = Content of chemical in organism (ìg/kg)
C_w = Concentration of chemical in the water (ìg/L)

RESULTS AND DISCUSSION

Effect of pH on Zn Accumulation by *Lemna* spp.: Accumulation of Zn by *Lemna* spp. at different pH and Zn concentration is shown in Table 1-3. With the passage of time accumulation of Zn by three species increased. Accumulation of Zn decreased when *Lemna* spp. was treated with higher concentration of Zn (15 and 20 mg/L). Plants treated with 20 mg/L of Zn at pH 6 accumulated the highest level of metal (23465±8735 mg/kg), while in alkaline pH the highest Zn accumulation was (14970±3678 mg/kg) for *L. trisulca* treated with 20 mg/L of Zn at pH 8. The lowest Zn accumulation was found to be (480±112 mg/kg) for *L. minor* treated with 1 mg/L of Zn at pH 4. The lowest Zn accumulation in alkaline pH medium was (523±161 mg/kg) for *L. minuta* treated with 1 mg/L of Zn at pH 10. It showed better accumulation at pH 6 as compared to pH 8. Puranik and Paknikar [33] found an increase in metal accumulation with increasing pH and initial metal concentration for Pb, Cd and Zn by *Citobacter* strain MCM B-181.

Miretzky *et al.* [34] used *Spirodela intermedia* and *L. minor* for the simultaneous removal of heavy metals in laboratory experiences along 15 days. When added Zn concentration was 1 mg/L, the metal removed percentages were, respectively 95.73% and 97.56%. The trend of increased intake or adsorption of cationic metals on cell walls with pH increase has been reported for aquatic organisms and plants grown in hydroponic culture [35-37].

Several studies have demonstrated that duckweed species were able to accumulate and remove Zn from contaminated water. According to the study investigated by Sharma and Gaur [38], Zn accumulated in *L. polyrrhiza* was 27 μg/mg; the plants were exposed to 10 mg/L of Zn during 4 days. Jain *et al.* [39] used *L. minor* for Zn removal from polluted water. The aquatic plants were exposed to 1, 2, 4 and 8 mg/L of Zn supplied as zinc nitrate. After 14 days of treatment, Zn amount accumulated by tissues was 717, 1284, 2227 and 3698 mg/kg DM, respectively. In the study investigated by Mishra and Tripathi [40], *S. polyrrhiza* was tested for the removal of Zn (and Fe, Cu, Cd and Cr). The metals were added simultaneously at initial concentrations of 1, 2 and 5 mg/L. Accumulation of Zn in duckweed ranged between 0.782 and 1.5 mg/g DM.

Accumulation of Zn in Dry Biomass and BCF: A good heavy metal accumulator should have BCF of more than 1,000 [30]. Zn accumulation by *Lemna* spp. at different Zn concentrations and BCF are shown in (Table 4). All the three species showed increased accumulation of Zn, when the concentration of Zn in the medium was increased. Among the three species, *L. trisulca* showed highest levels of Zn in dry biomass (18366.4±2614 mg/kg) and highest BCF (2955) as compared to *L. minuta and L. minor*. Although *L. minor* showed lowest levels of Zn in dry biomass (818.4±34.4 mg/kg) and lowest BCF (391). In this study, it was seen that *L. trisulca*, whose growth was affected to greater extent than *L. minuta* and *L. minor*,

Table 1: Effect of pH on Zn accumulation after 10 days incubation (mg/kg) by *L. minuta*

Initial Zn Concentration (mg/L)	pH 2	4	6	8	10
1	612±121	821±362	3089±1269	2215±561	523±161
5	1841±291	4162±397	8620±2136	5325±842	1233±391
10	4621±341	8424±294	9540±697	4632±941	3462±412
15	8906±584	1036±962	11640±872	7632±496	4982±531
20	1249±674	12545±1720	13640±1321	8654±1321	5879±863

Table 2: Effect of pH on Zn accumulation after 10 days incubation (mg/kg) by *L. minor*

Initial Zn Concentration (mg/L)	pH 2	4	6	8	10
1	1023±224	480±112	3589±987	2791±761	529±231
5	3940±389	4062±361	8231±3214	4321±1042	1560±841
10	7520±1361	9210±633	13630±3892	7113±1230	2681±941
15	8932±1544	1336±1623	15360±4365	99210±3140	1858±631
20	9723±1050	15745±4221	16530±4863	13061±2987	4870±799

Table 3: Effect of pH on Zn accumulation after 10 days incubation (mg/kg) by *L. trisulca*

Initial Zn Concentration (mg/L)	pH 2	4	6	8	10
1	1811±321	1201±431	5412±736	2145±653	914±214
5	5931±860	6120±936	10230±2980	5623±1647	2630±769
10	9125±1863	11200±2121	15782±4892	8798±2364	4362±1234
15	11642±3264	15630±2561	17260±7963	10478±4214	4985±984
20	1282±6420	16952±4690	23465±8735	14970±3678	5321±812

Table 4: Bioconcentration of Zn by *Lemna* spp. after 10 days incubation

Concentration of Zn in	*L. minuta*		*L. minor*		*L. trisulca*	
the medium (mg/L)	Zn in dry mass (mg/kg)	BCF	Zn in dry mass (mg/kg)	BCF	Zn in dry mass (mg/kg)	BCF
Control	34.3±1.6		25.1±1.2		38.5±2.6	
1	3261.4±148.2	1974	818.4±34.4	412	1863.1±31.4	1897
5	6156.7±379.1	1436	5312.3±291.6	426	8226.4±1246.2	2955
10	9213.5±1043	764	7245.8±842.3	391	12435.7±1364.6	2894
15	12874.3±154.9	961	11462.4±1328.7	569	8366.4±1521.2	1614
20	14931.7±2006.2	839	12397.8±1987.4	378	2397.8±2112.6	1468

accumulated higher levels of Zn. This showed that dead or necrosed biomass is able to adsorb more metal. The BCF values decreased with increasing Zn concentration in growth medium in all the three species.

A similar effect of Zn on growth and chlorophyll content of *Wolffia globosa* was observed by Boonyapookana *et al*. [41]. Sela *et al*. [42] also reported that as compared to other toxic metals like Cd, Ni and Zn, diminishing effect of Cr on nitrogenase activity of *A. filiculoides* was less. This may be related to the fact that Zn is one of the micronutrients for plants but becomes toxic at higher concentrations. In the present study, *L. trisulca* accumulated the highest amounts of Zn at all concentrations provided in the growth medium. All three *Lemna* spp. used in this study showed the potential to hyperaccumulate Zn. Khellaf and Zerdaoui [43] have also reported removal and concentration of zinc by *Lemna gibba*, but the BCF values observed for Zn in case of three species in the present study are much higher as compared to the BCF value (100) for Zn by *Lemna gibba* reported by Khellaf and Zerdaoui [43]. Bioconcentration factor (BCF) is a useful parameter to evaluate the potential of the plants in accumulating metals and this value is calculated on a dry weight basis. Jain *et al*. [39] also found that the BCF value for water velvet (*Azolla pinnata*) and duckweed (*Lemna minor*) treated with Pb and Zn, gradually decreased with increasing metal concentration in the feed solution. Metal accumulations by macrophytes can be affected by metal concentrations in water and sediments. The ambient metal concentration in water was the major factor influencing the metal uptake efficiency of plants [44].

Percentage removal of *Lemna* spp. for 1, 5, 10, 15 and 20 mg/L Zn treatment is shown if Figure 1-3. Removal potential is good at pH 6 (95 %) as compared to pH 8 (40 %) for 1 mg/L of Zn concentration while at higher concentration of Zn (20 mg/L) removal potential in acidic medium is good as compared to alkaline pH medium. All the three species showed increased removal of Zn when the concentration of metal in the medium was increased. Among the three species, *L. trisulca* showed highest removal of Zn (97 %) as compared to *L. minuta* (89 %) and *L. minor* (83 %). It was found that *L. trisulca* could remove maximum quantity of Zn (97 %).

Lemna trisulca, L. minuta and L. minor removal the Zn efficiencies ranges from 49-97 %, 35-89 % and 40-83% respectively. The obtained results showed that the maximum concentration of zinc removal by *L. trisulca* (97 %) and minimum absorption of Zn concentration (35 %) was found by *L. minuta* (Figure1-3). Adsorbent materials (biosorbent) derived from suitable biomass both the living and dead can be used for the effective removal and recovery of heavy metal ions from wastewater streams [45]. The bioconcentration of heavy metal by aquatic plants such as *Lemna* has already demonstrated by Megateli *et al.* [46] and Jalali-Rad *et al*. [47].

The obtained results suggested that *L. trisulca* was more capable for bioconcentration of Zn as compared to and *L. minuta* and *L. minor*. Being a free floating macrophyte *L. trisulca* may be more useful to remove Zn from sediments in natural/field conditions. For the studied period, a significant difference was observed in the removal of metals by the different macrophytes.

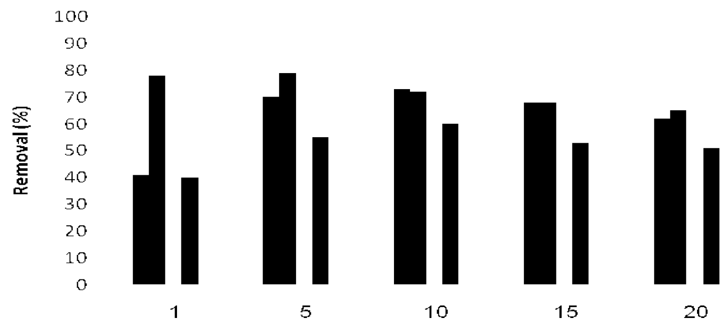

Fig. 1: Percentage removel of Zn by *L. minor* at different pH treatment

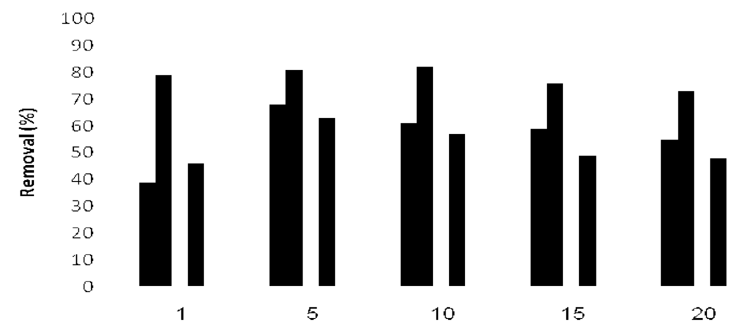

Fig. 2: Percentage removel of Zn by *L. minuta* at different pH treatment

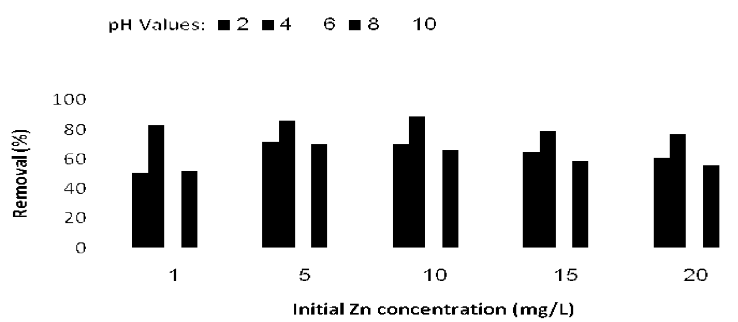

Fig. 3: Percentage removel of Zn by *L. trisulca* at different pH treatment

It could be proposed that all species were highly efficient in Zn uptake. Metals are neither biodegradable in the environment nor completely removed by treatment. Currently used water treatment technologies for metal removal involve chemical precipitation, evaporation; electrochemical treatment and the use of ion exchange resine that are expensive and sometimes ineffective, especially when metal are present in solution at very low concentrations.

At high concentrations, metals are toxic but some plants have developed physiological mechanisms to cope with metals. These include the cellular exclusion of heavy metals, adsorption of those metal insensitive enzymes chelation of the metal ions by chelators [48]. Organic acids and some amino acids, particularly histidines have roles in chelation of metal ions. Peptide ligands include metallothioneins, small gene encoded cysteine rich polypeptides and phytochelatins, which are produced in plants by enzymes that are expressed in response to heavy metals [49,50]. The absorption of metals without much effect on the normal growth of *Lemna* could be attributed to the above explanations. Although many biosorbent materials like treated sawdust [51], beech sawdust (*Fagus orientalis*) [52] and seaweed *Sargassum wightii* [53] have been used to remove heavy metal by passive adsorption, all the adsorbents need some kind of pretreatment to increase adsorption.

CONCLUSION

From the above discussed results, it was concluded that *L. trisulca* is a better option than *L. minor* and *L. minuta* for treating high strength heavy metal wastewater. Comparing the effect of pH, the effect of pH between 5 and 6 are more marked in zinc accumulation than other pH. It is clear that there is removal at lower pH values, but the rate of the removal is quite slow. Hence, it can be concluded that at lower pH values, other mechanisms like physical adsorption could have taken an important role and ion exchange mechanism might have reduced. The results indicated that the biomass of *Lemna* spp. is suitable for development of efficient biosorbent for the removal of Zn from waste water. Further studies on the recovery of metals from the *Lemna* spp. by employing improve methods will be useful for extending the use of the laboratory level experiment with aquatic plant to large scale and field level removal systems.

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