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Effect of pH and Lead Concentration on Phytoremoval of Lead from Lead Contaminated Water by *Lemna minor*

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Abstract: Heavy metal pollution in the environment is a major global concern which has provoked the emergence of phytoremediation technologies for cleaning soils, aqueous streams, mine wastes and sewage by use of plants. Water contamination with heavy metals is a very important problem in the current world. To investigate the effect of pH on the removal of lead from contaminated water, an experiment was carried out by using duckweed (*Lemna minor*). *Lemna minor* has been shown to be very effective in phytoremediation of heavy metals from waters. *L. minor* was cultured in tap water. Initial lead (Pb) concentrations were 1, 5, 10, 15 and 20 mg/L in the experiment. Harvesting was done after 7, 14, 21 and 28 days. Metal bioaccumulation process was affected by various parameters such as exposure time, pH and concentration of lead solution. The accumulation of Pb by plant increased with the passage of time. Plants treated with 20 mg/L of Pb accumulated 21675 mg/Kg DW of Pb after 28 days at pH 5 (highest). However those treated with 1 mg/L of Pb at pH 10 accumulated the lowest (514 mg/Kg DW of Pb) after 7 days. These results suggested that pH played an important role in counteracting Pb stress in *L. minor*.

Key words: *Lemna minor* · Phytoremediation · Lead · pH · Bioconcentration factor

INTRODUCTION

Heavy metal pollution represents an important environmental problem due to toxic effects of metals and their accumulation throughout the food chain leading to serious ecological and health problems [1]. Environmental risk and damage occurs when the metals are available to living organisms [2]. Unlike organic pollutants, heavy metals are not degraded through biological processes, so require removal for water decontamination [3].

Heavy metals discharged into the environment from textile, printed circuit boards and electroplating industries constitute one of the major causes of water pollution. The effluents of electroplating factories and the printed circuit board factories are usually acidic and contain high concentrations of heavy metals including lead. Discharges of liquid effluents from chemical manufacturing facilities, pulp and paper mills and municipal wastewater treatment plants also results in the release of lead to aquatic ecosystems.

Lead (Pb) is considered as a strategic metal belonging to the "big three" toxic heavy metals and is being widely used in many important industrial applications [4]. It is one of the most abundant, ubiquitous toxic elements posing a critical concern to human and environmental health due to being persistent contaminant, low solubility and the classification as carcinogenic and mutagenic [5]. The permissible limit of lead in drinking water is 0.005 mg/L. It causes diseases such as anaemia, encephalopathy, hepatitis and nephritic syndrome. Thus, it is imperative that lead is removed before being discharged into the sewage system or into the aquatic environment.

Higher awareness of the ecological effects of toxic metals and their accumulation through the food chain has prompted a demand for purification of industrial waste waters prior to their discharge into the natural water bodies. Conventional cleanup technologies are generally too costly hence attention has been paid to environmentally friendly technique i.e., phytoremediation. It is the utilization of plants accumulation capabilities to remove contaminants from water, soil or air.

The capacity of aquatic plants such as duckweed (*Lemna* sp.) to remove potentially toxic heavy metals from

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water is well documented. Duckweeds were shown to take up heavy metals and survive in highly stressed aquatic environments [6]. *Lemna minor* known as common duckweed is fast-growing, adapt easily to various aquatic conditions and play an important role in extraction and accumulation of metals from waters.

Metal bioaccumulation by plants depends upon numerous biotic and abiotic factors, such as temperature, pH and dissolved ions in water [7] and the nature and amount of aquatic biomass. Thus, in this study we investigated the effect of pH and lead concentration on the growth potential and phytoaccumulation capacity of *L. minor* in lead contaminated water.

MATERIALS AND METHODS

Experimental Setup: Duckweed plants were picked up from a stream of water from the main campus. They were identified as *Lemna minor* and were cultured in a water tank in micro model IIT Delhi (the experimental site). Plastic containers of ten litre capacity were filled with water. A known quantity of *L. minor* was taken out of culturing tank and treated with different concentrations of Pb under alkaline pH conditions (8, 9 and 10) and also in acidic pH conditions (4, 5 and 6). A black line was drawn on the containers so a six litre water level could be kept constant. A stock solution was prepared by using lead nitrate salt. Different concentrations of lead as lead nitrate were prepared (i.e., 20, 15, 10, 5 and 1 mg/L). Every 1-2 days, the plants were checked and tap water was added to each container so the six litre water level line remained constant. Experimental pH was manually maintained at the defined value with 0.1 N NaOH and HNO_3. Samples were collected in an interval of 7, 14, 21 and 28 days.

Preparation of Samples for Analysis: The biomass weight was taken by drying duckweed plants on filter paper for 10 minutes (fresh weight). Plants were dried and their dried weights were noted in the lab notebook. Plants were analyzed for relative growth, metal accumulation and bioconcentration factor (BCF). In addition, all water samples were filtered by 0.45 μm membrane filters, acidified and stored at 4°C prior to analysis to analyze the Pb remained in the solution and hence to assess the removal potential of *L. minor*.

Quantification of Lead: The dried plant samples were heated in a muffle furnace at 500° C for 6 hours. The ash of each sample was dissolved in 5 ml of 20% HCl to dissolve the residue. Samples were heated on a hot plate to boiling. Required amount of HCl (20%) was added to avoid sample drying. The resulting solutions were filtered and diluted to 50 ml with deionized water in volumetric flasks. The Pb content of these plant samples and water samples were determined using flame atomic absorption spectrophotometry (AAS4129) with the following settings: wavelength 217 nm, lamp current 5 mA, slit 1 nm, fuel - acetylene and oxidant air. Pb concentration in plant samples was calculated using the following equation:

$$\text{Pb concentration (mg/kg)} = \frac{\text{Reading of Pb in sample (mg/L)*} \\ \text{Total volume of the sample(mL)}}{\text{Dry weight of the sample (g)}}$$

Calculations: Relative growth of control and treated plants were calculated as follows [8]:

Relative growth = [Final fresh weight]/ [Initial fresh weight]

Bioconcentration factor (BCF) provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate. The BCF was calculated as follow [9]:

BCF = [Concentration of metal in plant tissues]/ [Initial concentration of metal in external solution]

Removal of lead was calculated using the following equation [10]:

$$R (\%) = [(C_0\text{-}C_t)/C_0]*100$$

Where C_0 and C_t represent the residual concentration of metal at the beginning of the experiment and at time t, respectively.

RESULTS AND DISCUSSION

The results obtained, out of the experiments, have been shown in figures and tables. The *Lemna* biomass was harvested to get its yield and the metal analysis after the stipulated days of experiments. All these parameters have been discussed here.

Effect of pH on the Growth of *L. minor*: The effect of pH on relative growth of *L. minor* with different Pb concentrations and exposure time are shown in Figures. 1-4. Here, the highest value of relative growth was 5.901±0.23 for plants treated with 1 mg/L of Pb at

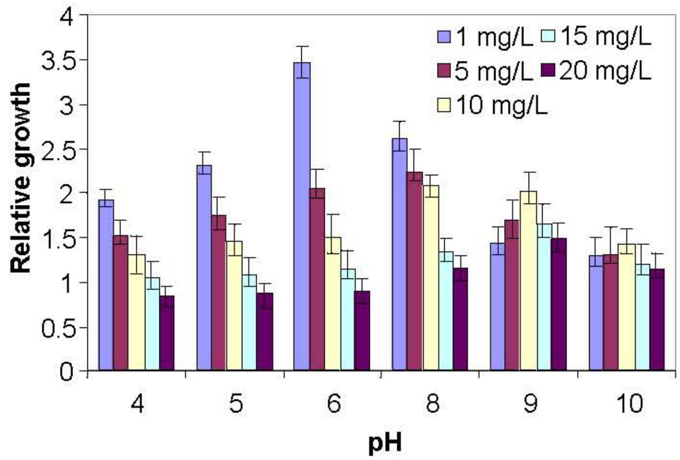

Fig. 1: Effect of pH on relative growth of *L. minor* after 7 days treatment

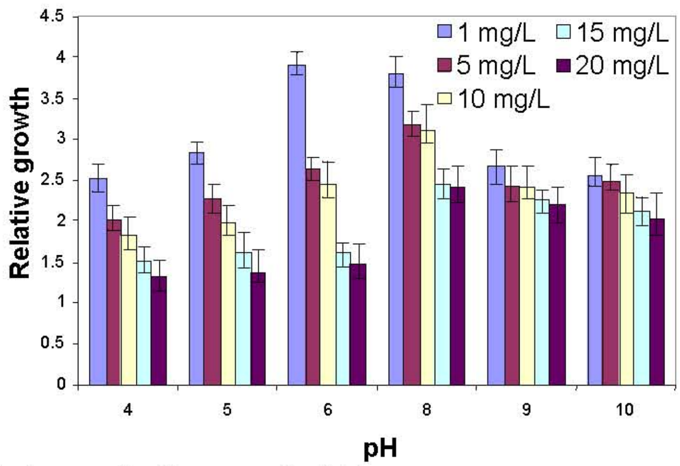

Fig. 2: Effect of pH on relative growth of *L. minor* after 14 days treatment

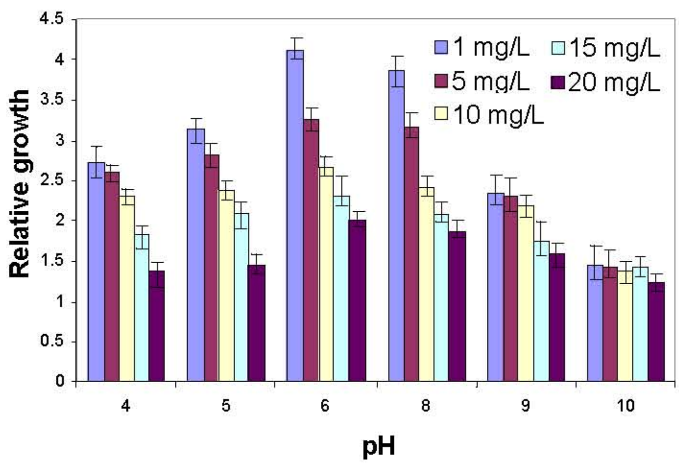

Fig. 3: Effect of pH on relative growth of *L. minor* after 21 days treatment

pH 6 for 28 days and the lowest value was 0.824±0.12 for plants treated with 20 mg/L of Pb at pH 4 for 7 days. From the figure obtained, it is clear that with increase in Pb concentration there is a decrease in growth of *L. minor* treated with acidic pH. It showed good growth at pH 6 as compared to pH 5 and pH 4. While in the alkaline pH, the highest value of relative growth was 4.085±0.3 for plants treated with 1 mg/L of Pb at pH 8 for 28 days. The lowest

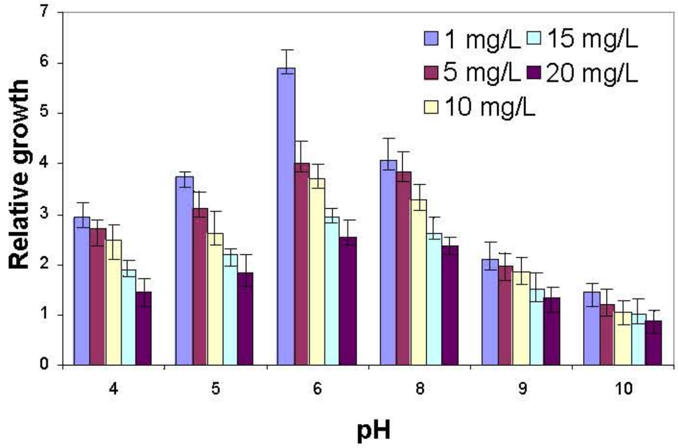

Fig. 4: Effect of pH on relative growth of *L. minor* after 28 days treatment

value was 0.901±0.22 for plants treated with 20 mg/L of Pb at pH 10 for 28 days. It showed good growth at pH 8 as compared to pH 9 and pH 10.

Relative growth of *L. minor* increased with the time in acidic pH treatments. It showed good growth up to 14 days and then there was a decrease in growth of plants in alkaline pH treatments. From the figures obtained, it is clear that pH 6 and pH 8 are good for the growth of *L. minor* and pH 6 is much better than pH 8.

L. minor showed two ''growth phases'' for almost all concentrations; the first till about day 5, the second from about day 5 to day 10. Apparently, growth rates finally decrease due to the progressive effects of shading and/or nutrient depletion in the lead contaminated water. In general, metal accumulation is independent of plant growth rate but depends on metal concentration.

The Lamnaceae family is capable to survive in a fairly wide pH range. In nature, plant species of Lamnaceae family can tolerate pH of 3.5 to 10. The optimal pH range is 4.5-7.5 [11]. The plant growth continues but decreases both in the lower or upper limits of this pH range. Relative growth of plants increased with initial pH especially in the pH 6-8 range. Relative growth was clearly decreased by Pb concentrations. These results show that Pb ions have quite a negative effect on plant growth.

Landolt and kandler [12] showed that duckweed *Lemna aequinoctialis* had the same growth rate at pH 4.8 to 7.0 ranges. However, it was found that plant biomass was generally more at high pH values (7.0-8.5) than at low pH values. In the present study, plant growth was higher at pH 6-8 range. The relative growth of 1 mg/L Pb was found higher than control plants. The relative growth of *L. minor* was decreased significantly by Pb ions at the 1-20 mg/L Pb concentration range. Relative growth decreased from 85% (1 mg/L) to a 2% (20 mg/L).

Salvinia rotundifolia was found to be affected significantly from toxic effects of Pb. The maximum growth reduction was observed from 80 to 72.3 mg dry wt. /gm fresh wt. during a treatment period of 6 days. The `xenotoxic effect' leading to the loss in plant weight reflects severe impairment of metabolic processes including the photosynthetic activity [13].

Results from an experimental study by Chong *et al.* [14] indicated that *L. minor* can grow well in pH from 6 to 9 while the lowest value of pH it can tolerate was in between pH 5-6. The growth rate of *L. minor* was inhibited gradually with increasing concentration of ammonia. However, nitrate had few inhibitory on the growth. Peroxidase activity of *L. minor* in ammonia culture was higher than that in nitrate culture because of the toxicity of ammonia.

Effect of pH on Lead Accumulation by *L. minor*: Accumulation of Pb by *L. minor* at different pH and Pb concentrations with exposure time is shown in Table 1. With the passage of time accumulation of Pb by *L. minor* increased. Accumulation of Pb decreased when *L. minor* was treated with higher concentrations of Pb (10, 15 and 20 mg/L). Plants treated with 20 mg/L of Pb at pH 5 for 28 days accumulated the highest level of metal (21675±8567 mg/Kg) while in alkaline pH the highest Pb accumulation was 14960±4628 mg/Kg for plants treated with 20 mg/L of Pb at pH 8 for 28 days. The lowest Pb accumulation was found to be 545±305 mg/Kg for *L. minor* treated with 1 mg/L of Pb at pH 4 for 7 days. The lowest Pb accumulation in alkaline pH medium was 514±781 mg/Kg for plants treated with 1 mg/L of Pb at pH 10 for 7 days. It showed better accumulation at pH 5 as compared to pH 8. A correlation study at pH 5 for 28 days treatments has

Table 1: Effect of pH on Pb accumulation (mg/Kg) of *Lemna minor*

		pH					
Days of treatment	Initial Pb concentration (mg/L)	4	5	6	8	9	10
7	1	545±305	846±435	832±421	682±602	532±639	514±781
	5	2290±394	4145±379	4060±514	3105±589	1572±694	1233±827
	10	4420±496	7674±622	7480±642	6580±645	2928±697	2262±945
	15	6570±589	11310±444	11130±614	9175±690	3952±728	3249±834
	20	8244±294	18452±399	13220±791	11720±821	4330±670	4153±799
14	1	847±471	984±219	956±311	867±359	690±431	620±385
	5	3620±502	4615±547	4485±647	4195±721	1785±829	1975±601
	10	6830±497	8950±621	8360±742	7280±391	3259±697	2954±724
	15	8805±623	12390±594	11775±570	9390±497	4158±754	4026±831
	20	9720±743	14960±543	14098±724	10240±687	4552±697	4879±998
21	1	911±520	2170±673	2073±585	1482±540	751±427	746±368
	5	4160±835	9480±1185	8616±1025	6635±976	2172±724	1752±621
	10	7520±1040	15020±5832	13190±4220	9820±1780	3361±876	4367±920
	15	10620±2545	18090±7288	15933±6430	11760±2570	4752±1015	4652±988
	20	11480±7491	17380±6856	16135±6598	13560±3764	5556±874	5236±798
28	1	1024±657	5631±723	2285±684	1604±585	958±468	916±412
	5	4930±878	10600±1820	10203±1784	7260±1085	2879±676	1966±620
	10	8250±1286	14540±5835	14140±4375	9910±1587	3436±734	3461±782
	15	11640±3275	17980±7492	16390±6552	13695±4082	4987±864	4895±836
	20	12480±8625	21675±8567	16482±6896	14960±4628	6282±1055	5987±987

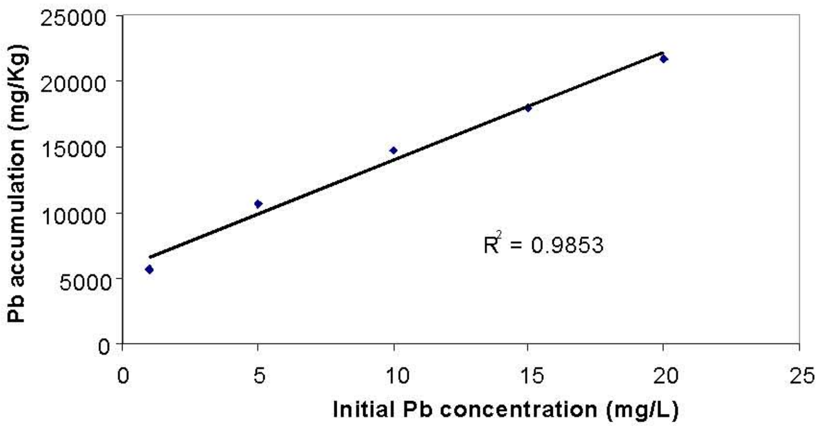

Fig. 5: Effect of concentration on lead accumulation capacity of *L. minor* at pH 5 for 28 days treatment

been done to find out the relation between lead accumulation and initial concentration of lead as shown in Figure 5. A positive correlation (0.9853) was obtained.

Wang *et al.* [15] obtained the maximum specific adsorption (13600±1900 mg/Kg) of *phormidium* at pH 5 solution for Pb. Puranik and Paknikar [16] found increase in metal accumulation with increase in pH and initial metal concentration for Pb, Cd and Zn by Citrobacter strain MCM B-181. Stepniewska *et al.* [17] obtained 53-416 mg/Kg DW of Pb from *Azolla caroliniana*. However, in the *H. lupulus* the leaves contained 72400 mg/Kg DW of Pb [18]. The common aquatic plant *L. minor* can remove up to 90% of soluble Pb from water [19]. Sorption of lead ions by dried *Azolla filiculoides* L. was highest (95%) at pH 4.5 and removed up to 93000 mg/Kg DW of Pb [20]. It is made possible by the plants defense mechanisms like the synthesis of detoxifying peptides (phytochelatins) or immobilization in the cell walls [21-24] as well as in the dried form as has been suggested by many experiments [20, 25-27].

Uysal and Taner [28] examined the ability of the *L. minor* to remove soluble lead under different pH values (4.5-8.0) and temperature (15-35°C) in presence of different Pb concentrations (0.1-10.0 mg/L) for 7 days. Their results show Pb accumulation was highest at pH 4.5 and then it decreased to pH 6, but it did not change at pH 6-8 range. The maximum lead accumulation was obtained at 30°C as

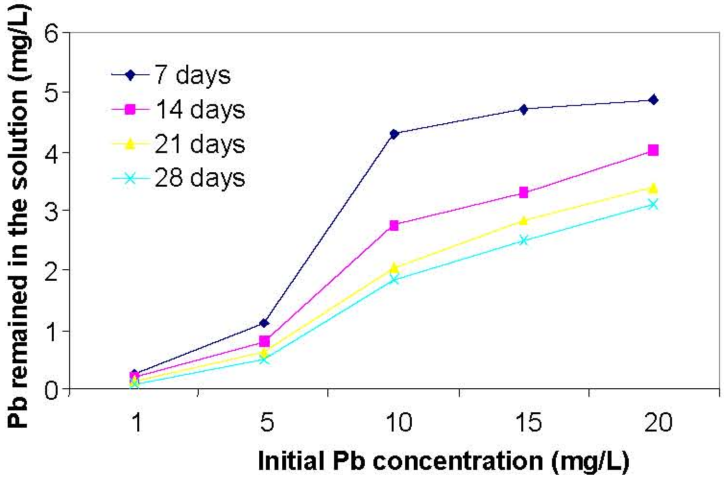

Fig. 6: Lead remained in the residual solution at pH 5

Table 2: Effect of pH on BCF values of *Lemna minor*

		pH					
Days of treatment	Initial Pb concentration (mg/L)	4	5	6	8	9	10
7	1	545±30	846±43	832±51	682±62	532±104	514±39
	5	458±49	829±37	812±58	621±58	314±96	246±27
	10	442±78	767±62	748±42	658±65	293±69	226±45
	15	438±89	754±44	742±54	612±69	263±78	217±34
	20	412±94	723±64	661±91	586±40	217±67	208±42
14	1	847±48	984±57	956±50	867±44	690±35	620±27
	5	724±77	923±91	897±82	839±78	357±56	395±65
	10	683±68	895±86	836±74	728±62	326±48	295±43
	15	587±54	826±78	785±67	626±53	277±46	268±32
	20	486±47	748±72	705±64	512±57	228±39	244±25
21	1	911±128	2170±154	2073±135	1482±107	751±95	746±73
	5	832±87	1896±144	1723±135	1327±122	434±87	350±70
	10	752±78	1502±128	1319±114	982±92	336±66	309±54
	15	708±65	1206±106	1062±97	784±85	317±50	291±46
	20	574±52	869±93	807±84	678±67	278±38	262±32
28	1	1024±190	5631±235	2285±211	1604±200	958±175	916±150
	5	986±182	2120±215	2041±203	1452±166	576±142	393±120
	10	825±134	1454±187	1414±165	991±140	344±78	346±84
	15	776±107	1199±140	1093±127	913±125	332±57	326±68
	20	624±84	1084±95	824±88	748±75	314±50	299±34

8.622 mg/g Pb in 10 mg/L and the minimum was at 15°C as 0.291 mg/g in 0.1 mg/L at pH 5.

Effect of Initial Lead Concentration on the Removal of Lead by *L. minor*: Study on the effect of initial lead concentration on the removal of lead by *L. minor* has been done at pH 5 as *L. minor* showed highest lead accumulation at pH 5. Graphical representation of concentration of Pb (mg/L) remained in the water samples at pH 5 with respect to the exposure time are shown in Figure 6. Pb content in water was decreased with the passage of time. The lowest value of Pb remained in the residual solution was 0.090±0.099 mg/L for 28 days treated with 1 mg/L of Pb while the highest value was shown by 20 mg/L of Pb for 7 days (4.8±0.41) treatment.

Banerjee and Sarkar [13] found within a span of 5 days treatment period, maximum uptake (96.5 %) of Pb ions by *Salvinia* (50 g) at pH of 5.5 from a lead-based synthetic solution (1.5 L). At extreme conditions of either acidic or alkaline, decrease in metal uptake may be considered as an effect of ion competition for adsorption sites, or it could lead to hydroxide precipitation of Pb which prevents uptake [29].

Hurd and Sternberg [30] exposed *L. minor* to concentrations of 0.0, 5.0 and 10.0 mg/L of Pb for 7 days in laboratory-scale (700 mL) batch reactors containing soil-based sediments. They observed overall removal amounts of 95 %, with 85 % removal occurring within the first day.

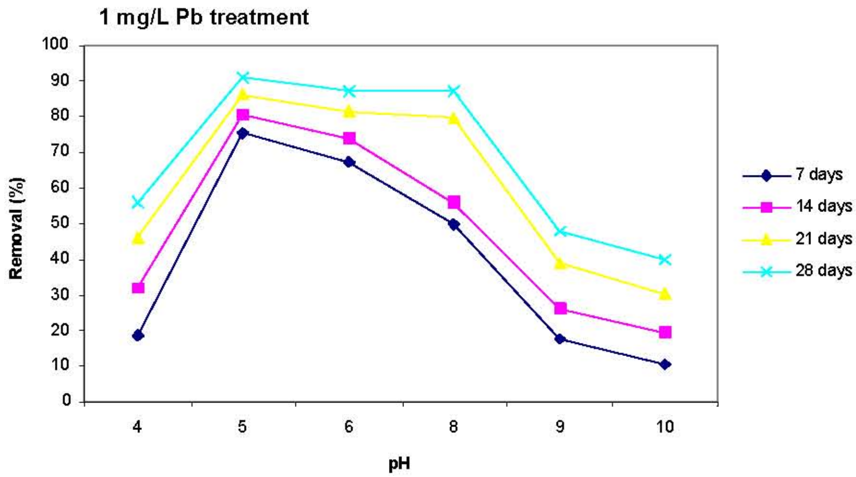

Fig. 7: Percentage removal of lead by *L. minor* at 1 mg/L of Pb treatment

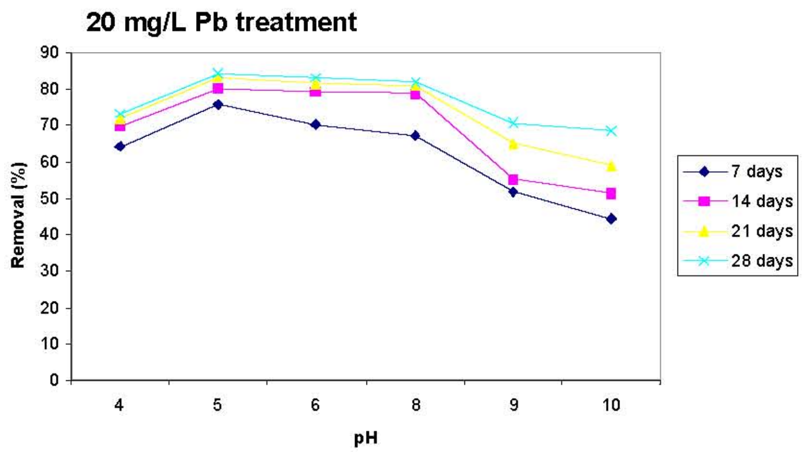

Fig. 8: Percentage removal of lead by *L. minor* at 20 mg/L of Pb treatment

Bioconcentration Factor (BCF): Bioconcentration factor is defined as the ratio of metal concentration in the biomass to the initial concentration of metal in the feed solution (reported as L/Kg). BCF was calculated for quantifying the Pb removal potential of the *L. minor*. BCF values over 1000 are generally considered evidence of a useful plant for phytoremediation.

The BCF values for Pb at different pH and Pb concentrations with exposure times are shown in Table 2. From table it was found that BCF values decreased with increase in Pb concentration in the feed solution. BCF values increased with the passage of time. The maximum BCF of 5631±235 was obtained in plants treated with 1 mg/L of Pb at pH 5 for 28 days and the minimum of 412±94 in plants treated with 20 mg/L of Pb at pH 4 for 7 days respectively in acidic pH treatments.

While in alkaline pH treatments, the maximum BCF was 1604±200 of plants treated with 1 mg/L of Pb at pH 8 for 28 days and the minimum BCF was 208±42 of plants treated with 20 mg/L of Pb at pH 10 for 7 days.

Percentage removal of *L. minor* for 1 mg/L and 20 mg/L Pb treatment is shown in Figure 7 and Figure 8 respectively. Removal potential is good at pH 5 (91 %) as compared to pH 8 (87 %) for 1 mg/L of Pb concentration while at higher concentration of Pb (20 mg/L) removal potential in acidic medium is good as compared to alkaline pH medium.

Saygideger *et al.* [31] studied effect of Pb and pH on Pb uptake, chlorophyll and nitrogen content of *Typha latifolia* L. and *Ceratophyllum demersum* L. They found the highest metal concentration at pH 9 in the macrophyte tissues. The lowest Pb^{2+} values were found at pH 5. This might be due to competition with hydrogen ions on the cell membrane surface. While solubility of Pb^{2+} increased at pH 5, the metal uptake by the macrophytes decreased probably due to negative effect of low pH.

CONCLUSION

The results showed that 1 mg/L of Pb acts as a nutrient for the plant showing very good relative growth, whereas when the concentration of Pb goes to 20 mg/L the growth is hampered.

Comparing the effect of pH, the effect of pH 5 and pH 6 are more marked in lead accumulation than other pH. The order of removal potential of Pb by *L. minor* at different pH in descending order is as follows: pH 5> pH 6> pH 8> pH 4> pH 9> pH 10. This study can lead to a practical method of Pb contaminated wastewater treatment.

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