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ORIGINAL ARTICLE

Lead and cadmium removal from water using duckweed – *Lemna gibba* L.: Impact of pH and initial metal load

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Abstract The aim of this study was to investigate the potential of duckweed (*Lemna gibba*) in heavy metal (Pb and Cd) from water under different pH and metal loads. A total of three (2, 5 and 10 mg/L) strengths of Pb and Cd were used with varying pH (5, 7 and 9) and changes in metal concentration and metal uptake yield of system were recorded. The Pb and Cd removal ranged between 60.1% (2 mg/L at 9 pH) and 98.1% (10 mg/L at 7 pH) and 41.6% (10 mg/L at pH 9) and 84.8% (2 mg/L at pH 7), respectively. The duckweed set-up with pH 7 showed the optimum metal removal. The metal removal rate showed an inverse relationship with pH ($r^2 > 0.60$, for all). Bioconcentration factor (BCF) and metal uptake yield per unit of dry biomass (q_m) were recorded: 403–738 and 445–616, respectively for BCF_{Pb} and BCF_{Cd}. The q_m suggest the dose (mg/L) 5 and 10 at pH 5 as the best combinations for the optimum removal. Results, thus suggest that *L. gibba* can be a suitable candidate for removal of heavy metals from pollutant water bodies.

1. Introduction

The contamination of heavy metals in terrestrial and aquatic ecosystem has been appeared as a global environmental problem. The mining and unsafe disposal of industrial solid/liquid wastes is the prime source of heavy metals in the environment. In the urban areas the load of heavy metals in freshwater resources is at alarming level probably due to disposal of untreated or partially treated sewerage and industrial wastewaters. Due to acute toxicity associated with heavy metals, these are considered as environmental priority pollutants and are targeted for cleanup processes. The conventional metal remediation technologies involve the following: chemical precipitation (hydroxide precipitation and sulfide precipitation), ion-exchange, adsorption (activated carbon adsorbents, carbon nanotubes adsorbents, bioadsorbents), membrane filtration (ultrafiltration, reverse osmosis, nanofiltration and electrodialysis), coagulation–flocculation, flotation and electrochemical methods [1]. These technologies offer several advantages such as flexibility in design and operation, huge treatment capacity, high removal efficiency, and fast kinetics but also showcases limitations such as, generation of toxic sludge or other by-products, high operation and maintenance cost and high energy requirements [2,3]. Therefore, there is an urgent need to adopt technology with optimum efficacy

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and low capital investment and can be acceptable for wide range of metal contamination [3].

Phytoremediation is a plant-based cleanup process of any contaminated environment [4]. It is designated as quite simple and versatile technology to achieve specific remedial goals. There are several advantages of this process, such as technologically feasible, low operating costs, least possible sludge generation, and competitive performance [5]. The plenty of plant species (e.g., water hyacinth – *Eichhornia* sp., duckweeds - *Lemna* sp. and *Spirodella* sp., small water fern – *Azolla* sp., water lettuce – *Pistia* sp.) is known for heavy metal removal from aquatic media and for producing an internal concentration of metal several times greater than the surroundings [6]. *Lemna gibba,* belonging to the family – Lemnaceae, is a rooted free-floating aquatic plant consisting of small fronds. Due to the high growth rate and large uptake metal potential, members of Genus *Lemna* have been appeared as potential candidates for designing a duckweed-based heavy metal phytoremediation set-up. Few earlier workers have demonstrated high potency of *L. gibba* in heavy metals removal from the aquatic environment [7–9]. In metal uptake and chemical kinetic process the role of initial metal load and pH of medium are very critical factors. Such parameters need to be optimized in order to design an industrial-scale duckweed pond system for wastewater treatment process designing. As pH deemed to offer a very decisive role in bio-remediation process, there is an urgent need to address this research issue. After reviewing the available scientific literature it was realized that studies on role of metal loads and pH of media are not well undertaken by previous researchers. The contributory effect of metal load and pH performance on achieving maximum removal will further help to target metal pollution problem efficiently. Therefore, the aim of this study was to investigate the impact of pH and concentration of metals in aquatic media on removal efficiency of the duckweed system containing *L. gibba* as test species. The role of such parameters in plant growth and metal uptake yield was also studied using laboratory-based batch set-ups.

2. Methodology

2.1. Plant material and growth conditions

L. gibba L. was collected from a freshwater body located nearby to the campus of the Doon University, Dehradun (India). The plant material was collected in a plastic circular container and brought to the laboratory. In laboratory the plant material was washed carefully to remove dirt, sludge and other adhesive debris from it. To avoid any contamination the second generation of *L. gibba* was obtained by culturing original individual in 1/10 diluted Hoagland solution for 10 days as per standard methodology described by Penningsfeld and Kurzman [10] and Eliasson [11]. The composition of Hoagland's solution was as (all in mg/L): KNO_3, 1515.0; KH_2PO_4, 680.0; $Ca(NO_3)_2–4H_2O$, 1180.0; $MgSO_4–7H_2O$, 492.0; $ZnSO_4–7H2O$, 0.22; H_3BO_3, 2.85; $Na_2MoO_4–2H_2O$, 0.12; $CuSO_4–5H2O$, 0.08; $MnCl_2–4H_2O$, 3.62; $FeCl_3–6H_2O$, 5.4; tartaric acid, 3.0 [12]. Nutrient solution was renewed twice every week. Prior to the experiment, containers were disinfected by immersion in 1% (v/v) NaClO for 3–5 min. The prominent and healthy plants were screened out to be used in further experimentations. All cultures, stock and experimental set-ups were kept at a temperature of 26 ± 2 °C, with a light intensity of 1120 Lx and a day–night cycle of 16:8 h.

2.2. Experimental design

The batch scale experimentation set-ups were designed in triplicates and the average results were reported. The pre-cleaned beakers of 500 ml capacity were used as experimental set-up. A total of three strengths (2, 5 and 10 mg/L) of cadmium and lead were prepared in double deionized water. The stock solution of cadmium and lead was prepared using cadmium (II) sulfate ($3CdSO_4{\cdot}8H_2O$) and lead (II) nitrate [$Pb\ (NO_3)_2$], salts respectively. AR grade chemical was used for stock preparation. The selected metal concentrations were considered to be sublethal for *L. gibba*. In the literature the LC_{50} (Lethal concentration 50) for *L. gibba* is 500 ± 23.4 mg/l for lead [13] and 50 ± 31.5 mg/l for cadmium [14]. To investigate the effect of pH on Cd and Pb removal by duckweed, three pH ranges, i.e. 5, 7 and 9 (slightly acidic to alkaline) were taken into account. The selection of pH range was done on the basis of the survival potential of duckweed for on different pH as reported in earlier literature (1; 13). The selection of pH was done on the basis of competitive growth dynamics of duckweed plant [15–17]. The initial pH of the solution was adjusted with 1 N HCl and 1 N NaOH solutions. For experimentation, 2.5 g live plant material was inoculated in 250 ml solution of metal in glass beaker (500 ml capacity) under the aforementioned conditions for period of 7 days. The load of inoculation biomass was calculated on the basis of total plant biomass required to cover the whole surface of the reactor (with approximately a single layer of fronds). The duckweed plant biomass was rinsed with distilled water before inoculation in experimental set-ups. In order to see the removal efficiency of duckweed live biomass the residual concentration of Pb and Cd was determined in inoculation media of all set-ups at the end of experimentation. The plant biomass was also analyzed in order to see the biological accumulation of concern metals in tissues of inoculated duckweed biomass. For that live specimens of duckweed were harvested from each experimental set-up and further processed for heavy metal load estimation. The plant samples were dried at 70 °C to determine the dry weight (X_m).

The metal solutions without plants acted as experimental control. Duplicates of all experimental set-ups were kept in triplicate as experimental control. The control set-up media were also analyzed for metal concentration changes by assuming that whether there was any adsorption of metals on flask wall.

2.3. Plant growth and BCF estimation

To measure the changes in the total biomass of *L. gibba* in experimental set-ups the plant biomass (mg) was measured at end of experimentation. The growth rate was measured using following formula (1)

$$\text{Plant growth rate(in \%)} = \frac{\text{Final biomass} - \text{initial Biomass}}{\text{Final biomass}} \times 100 \tag{1}$$

Bioconcentration factor (BCF) factor is an indicator of the metal accumulation ability of plants in respect of metal concentration in the medium, and allows for a comparison of the results [18]. BCF expressed as the ratio of the final metal ion concentration in *L. minor* biomass to the initial metal ion concentration in the experimental medium was calculated using following Eq. (2)

$$\text{Bioaccumulation factor(BCF)} = \frac{\text{Metal } C\text{biota}}{\text{Metal } C\text{media}} \quad (2)$$

where *C*biota and *C*media were the total metal concentrations in *L. gibba* biomass and culture media, respectively in mg kg^{-1}.

2.4. Analytical procedure

The pH was measured using digital pH meter (Metrohm, Swiss made). Electrical conductivity was measured through digital conductivity meter (Remi, India). The residual level of Cd and Pb in experimental media and inoculated plant biomass was quantified using Atomic absorption spectrophotometer (Thermo Fisher. Model iCE 3000 Series AA System). The plant samples were dried at 70 °C and then digested with a HNO_3 and H_2SO_4 solution mixture according to the method as described by Mountouris and Voutsas [18]. Digested samples were diluted with Millipore water and filtered with Whatman no.42 filter paper. Then the sample was made up to 20 ml. The ready sample was then analyzed using AAS. All chemicals were used of AG grade while preparing reagents and standards during chemical analysis.

2.5. Statistical analysis

Statistical analysis was performed using rough data sets to conclude the result of Pb and Cd, removal and other biological parameters studied in all set-ups. Two-way ANOVA was used to analyze the impact of concentration of a particular metal and pH of media on metal removal rate in all experimental set-ups. The concentrations of metal and pH range were taken as fixed factor and removal rate as dependent parameter. SPSS® statistical package (Window Version 13.0) and STATISTICA® (Window Version 7) were used for data analysis. All statements reported in this study are at the $p < 0.05$ levels.

3. Result and discussion

3.1. Removal of Pb and Cd under different loads of metal ions and pH in set-ups

There was significant difference among different strengths of metals for removal rate during the experimentation. Table 1 gives an overview of the final concentration and removal rate (%) of Pb and Cd at different pH scales. Pb removal (%) was in the ranges of 60.1–98.1% at different pH in all experimental set-ups of duckweed. The maximum Pb removal (as compared to initial metal load) was recorded 98.1% (10 mg/L) followed, by 97.7% (5 mg/L) and 97.2% (5 mg/L). In 2 mg/L set-up, the maximum removal efficiency of *L. gibba* was recorded at pH 7 while in set-up with 5 mg/L and 10 mg/L load the maximum Pb removal was obtained at pH 5 and 7, respectively (Table 1). All experimental set-ups with different pH range can be arranged as in terms of removal efficacy: 7 pH > 5 pH > 9 pH. The impact of initial concentration of metal solution on removal rate was not very clear in this study. But overall the maximum removal was recorded at pH 7.

Two-way ANOVA results revealed that both pH and concentration of metal in duckweed media affect the Pb removal rate in experimental set-ups (Two-way ANOVA: $F = 22.06$; $P < 0.005$). The Cd removal in duckweed system ranged between 41.6% and 84.8% among different experimental set-ups (Table 2). The maximum removal (as compared to initial metal load) of Cd was 84.8% in 2 mg/L set-up with 7 pH followed by 79.8% (2 mg/L set-up) at 5 pH and 74.2% (5 mg/L set-up) at 7 pH. The terms of initial concentration of Cd in duckweed media for the removal order were 2 mg/L set-up > 5 mg/L set-up > 10 mg/L set-up (Table 1).

The results of two-way ANOVA clearly suggest that both factors: concentration (*C*) and pH (*P*) individually (two-way ANOVA: $F = 1027.91$, $p < 0.005$ and $F = 2104.09$, $p < 0.005$, respectively) and cumulatively affects ($C \times P$: $F = 103.8$, $p < 0.005$) the removal of Cd in duckweed system. The results of Cd removal are in accordance with that reported by earlier authors [19–22]. Few earlier researchers have also reported similar finding that duckweed can be a potential material to removal heavy metals from water and in this process the pH of water plays an important role in removal process [23–25]. The pH affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions [9].

In natural conditions, the members of Lemnaceae family are known to withstand wide range of pH starting from 3.5 to 10 [16]. The pH above 10 and below 3.5 can delimit the growth potential of plant [27]. The pH range of 4.5–7.5 was reported to be best suited range for the growth of duckweed species [25,26]. There are a variety of processes (e.g. adsorption, desorption, precipitation and co-precipitation process of metallic ions) involved in the removal of metal in duckweed system, which are directly or indirectly affected by the pH of the aquatic media [23]. Gothberg et al. [28] measured the Pb tolerance in *L. minor* and *S. polyrhiza* and validated that metal enrichment enhanced the tolerance of plants to metal contamination. A study conducted by Abdallah [7] found *L. gibba* potential candidate in removing Cr and Pb about 95% and 84%, respectively after 12 days of incubation.

Overall, the pH 7 and Cd dose 2 mg/L in duckweed media appeared as the best combinations for metal removal efficiency of the duckweed-based phytoremediation system. The metal removal from water could be the result of a combination of absorption and adsorption phenomena [22] in duckweed system. The regression analysis results clearly support the trend that increasing pH of media causes reduction in metal removal in duckweed-based phytoremediation system. Except to Cd 10 ppm set-ups, in experimental trials the removal rate showed significant ($r^2 > 0.60$ in all set-ups) inverse relationship with pH (Fig. 1).

Uysal and Taner [21] demonstrated the effect of pH, temperature and initial load of metal on removal efficiency of *L. minor*. They have found that the duckweed shows the maximum Cd removal at pH 6 and 25 °C temperature. Chawla et al., [23] concluded that the influence of dose of metal on uptake process could be attributed to the physicochemical aspects of cation transport and physiological status of the plant age. The low removal efficacy of duckweed at higher doses of metal in aquatic media attributed to the toxic impact

Table 1 Removal (%) of metals in duckweed set-ups under different metal and pH loads (mean ± SD, n = 3).

Strength of initial solution		pH			Strength of initial solution		pH		
		5	7	9			5	7	9
Pb (2 mg/L)	Final	0.18 ± 0.00	0.12 ± 0.02	0.79 ± 0.01	Cd (2 mg/L)	Final	0.40 ± 0.01	0.30 ± 0.01	0.99 ± 0.01
	Removal (%)	90.8	93.8	60.1		Removal (%)	80.00	84.8	50.1
Pb (5 mg/L)	Final	0.11 ± 0.01	0.13 ± 0.00	1.56 ± 0.01	Cd (5 mg/L)	Final	1.29 ± 0.03	1.28 ± 0.04	2.63 ± 0.04
	Removal (%)	97.7	97.2	68.7		Removal (%)	74.2	74.2	47.3
Pb (10 mg/L)	Final	0.32 ± 0.04	0.18 ± 0.01	3.55 ± 0.09	Cd (10 mg/L)	Final	4.79 ± 0.05	3.84 ± 0.07	5.83 ± 0.18
	Removal (%)	96.7	98.1	64.4		Removal (%)	52.1	61.5	41.6

ANOVA (two way)[b]	df[a]	F-value	P value	ANOVA (two way)[b]	df[a]	F-value	P value
Pb concentration (C)	2	290.68	< 0.005	Cd concentration (C)	2	1027.91	< 0.005
pH (P)	2	8824.22	< 0.005	pH (P)	2	2104.09	< 0.005
$C \times P$	4	22.06	< 0.005	$C \times P$	4	103.8	< 0.005
Total	27			Total	27		

[a] Error of df = 24.
[b] Considering pb concentration and pH as factors in removal rate.

Table 2 Biomass gain (%) in duckweed under different metal loads and pH conditions (mean ± SD, n = 3).

Strength of initial solution		pH			Strength of initial solution		pH		
		5	7	9			5	7	9
Pb (2 mg/L)	Final	2.81 ± 0.01	3.17 ± 0.05	2.86 ± 0.03	Cd (2 mg/L)	Final	2.87 ± 0.04	3.17 ± 0.05	2.86 ± 0.03
	Biomass gain (%)	12.4	26.8	14.5		Biomass gain (%)	15.1	22.6	27.1
Pb (5 mg/L)	Final	3.35 ± 0.04	3.73 ± 0.15	3.37 ± 0.11	Cd (5 mg/L)	Final	2.92 ± 0.02	2.94 ± 0.07	2.67 ± 0.11
	Biomass gain (%)	34.0	49.4	34.8		Biomass gain (%)	17.1	17.7	7.0
Pb (10 mg/L)	Final	3.30 ± 0.02	3.55 ± 0.08	3.22 ± 0.02	Cd (10 mg/L)	Final	2.67 ± 0.03	3.61 ± 0.14	3.24 ± 0.03
	Biomass gain (%)	32.1	42.2	28.8		Biomass gain (%)	7.2	44.4	29.7

ANOVA (two way)[b]	df[a]	F-value	P value	ANOVA (two way)[b]	df[a]	F-value	P value
Pb concentration (C)	2	120.64	< 0.005	Cd concentration (C)	2	340.64	< 0.005
pH (P)	2	56.98	< 0.005	pH (P)	2	112.98	< 0.005
$C \times P$	4	0.908	< 0.005	$C \times P$	4	2.118	< 0.005
Total	27			Total	27		

[a] Error of df = 24.
[b] Considering pb concentration and pH as factors in removal rate.

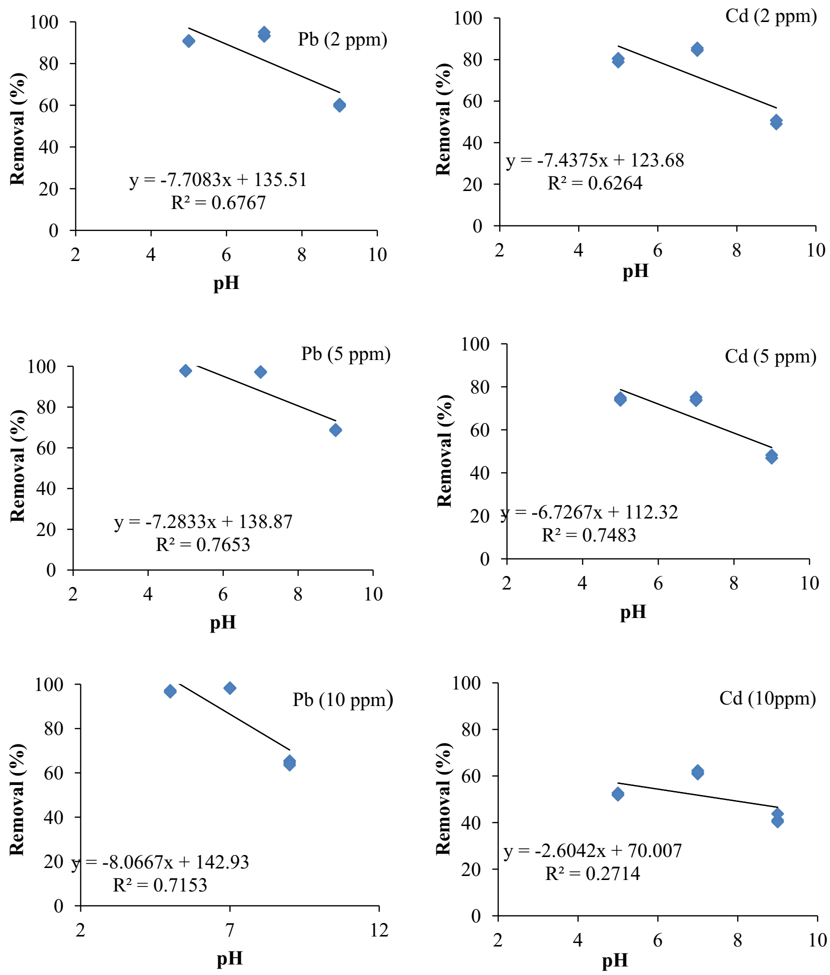

Figure 1 Relationships between metal removal rate and pH of culture media in duckweed set-ups.

of heavy metals in duckweed [29]. The physiological alternation and inhibition of enzymatic pathways in plant exposed to the high metal load is also reported by few earlier workers [30] that suggest the adverse impact of high metal contents on plan functioning. The metal load in duckweed-based phytoremediation system should be optimized in order to run the system at optimum scale.

3.2. Growth of L. gibba under experimental set-ups with different metal loads and pH

During the whole period, all fronds in the experimental cultures appeared green and vigorous and no signs of senescence were observed. However, Garnczarska and Ratajczak [31] have reported pigment degradation and photosynthesis inhibition in *Lemna trisulca* after inoculating in culture media having concentration of Cd > 1.12 mg/L. The trend of biomass yield in the different experimental set-ups is described in Table 2. In Cd containing set-ups, the *L. gibba* showed the growth (biomass gain) in the ranges of 7 (5 mg/L set-up at 9 pH) – 44.4% (10 mg/L set-up at 7 pH). The growth in plant biomass was 7.2–17.1% at pH 5, 17.7–44.4 at pH 7 and 7.0–29.7 at pH 9 for Cd containing set-ups. In terms of average growth trends observed in *L. gibba* under different strengths of Cd the set-ups can be arranged as follows: pH 7 > pH 9 > pH 5 (Table 2). The biomass gain in set-ups with different loads of Pb was in the order: 5 mg/L set-up > 2 mg/L set-up > 10 mg/L set-up at 5 pH and 10 mg/L set-up > 2 mg/L set-up > 5 mg/L set-up at pH 7 and 9 (Table 2). In Pb containing duckweed growth media the growth rate (% biomass gain) ranged between 12.4 (2 mg/L set-up with 5 pH) and 49.4 (5 mg/L set-up with 7 pH). For Pb containing set-ups the growth in plant biomass was 12.4–34% at pH 5, 26.8–49.4 at pH 7 and 14.5–34.8 at pH 9. The maximum biomass growth in duckweed was observed at pH 7 in all experimental set-ups with different loads of Pb. The trend of growth was in the order: 5 mg/L set-up > 10 mg/L set-up > 2 mg/L set-up at 5 pH and 5 mg/L set-up > 10 mg/L set-up > 2 mg/L set-up at pH 7 & 9 (Table 2). It is clear from the result that duckweed shows better growth rate at higher concentrations of Pb with pH scale from neutral to acidic range (see Table 3).

The pH of media again plays vital role in nutrient uptakes and functioning of plant physiology. Probably, duckweed shows better growth patterns at moderate pH in water media because of high metabolic activities. It has been observed that in majority of experimental set-ups the plant survived up to 7 days and thereafter, necrosis in inoculated duckweed was observed. Both metal concentration and pH directly affect

Table 3 Bio-concentration factor (BCF) (mean ± SD, $n = 3$) for different metals in set-ups.

Strength of initial solution	pH – 5	pH – 7	pH – 9	Strength of initial solution	pH – 5	pH – 7	pH – 9
Pb (2 mg/L)	491.66 ± 16.07	438.33 ± 5.77	403.33 ± 32.53	Cd (2 mg/L)	472.5 ± 6.24	463.5 ± 5.07	445.0 ± 6.76
Pb (5 mg/L)	738.00 ± 21.63	455.33 ± 7.57	452.66 ± 29.95	Cd (5 mg/L)	513.46 ± 19.74	587.66 ± 10.27	482.13 ± 27.01
Pb (10 mg/L)	666.33 ± 11.01	567.66 ± 4.16	480.66 ± 16.56	Cd (10 mg/L)	616.60 ± 7.59	513.43 ± 5.88	507.23 ± 7.58
ANOVA (two way)[b]	*df*[a]	*F*-value	*P* value	ANOVA (two way)[b]	*df*[a]	*F*-value	*P* value
Pb concentration (*C*)	2	116.72	< 0.005	Cd concentration (*C*)	2	110.56	< 0.005
pH (*P*)	2	243.59	< 0.005	pH (*P*)	2	47.14	< 0.005
$C \times P$	4	37.1	< 0.005	$C \times P$	4	39.201	< 0.005
Total	27			Total	27		

[a] Error of $df = 24$.
[b] Considering pb concentration and pH as factors in removal rate.

the growth patterns in duckweed based bioremediation system as two-way ANOVA results clearly support this statement ($p < 0.005$, for all). The stress conditions at high metal load in plants lead to activation of metabolic synthesis. This results in the excess biomass gain through rapid synthesis of new protein and carbohydrates in plant tissues. Under chemical stress conditions an increase in plant biomass occurs mainly due to loading of chloroplasts with starch granules [16]. Few earlier workers have also reported synthesis of metal-binding peptides in aquatic macrophytes (*Eicchornia*, *Pistia* and *Hydrilla*) exposed to heavy metals [32,33]. The high growth (biomass gain) in set-ups with 10 mg/L metal load was observed for Cd containing *L. gibba* set-ups. Garnczarska and Ratajczak [31] also observed dose-dependent accumulation of two polypeptides in Cd-treated *Lemna* fronds. The results are in accordance with earlier reports that Cd-induced stress in ambient environmental leads to biomass gain in duckweed although, and further detailed studies on biochemical aspects are required to support the evidence. No uniform trend in biomass gain in respect of load of Pb and Cd in media was observed. The biomass gain under different metal loads was in the order: 5 mg/L > 10 mg/L > 2 mg/L for Pb and 10 mg/L > 2 mg/L > 5 mg/L for Cd (at pH 7 and 9). Cd set-ups showed slightly different growth pattern at pH 5 (i.e. 5 mg/L > 2 mg/L > 10 mg/L). Probably, the high doses of Cd in duckweed cause synthesis of few metabolites (starch, protein, polypeptices, etc.) that could contribute excess biomass in such set-ups. The further biochemical aspects of Cd-induced biomass changes need to be investigated in order to trace the effect of high Cd doses on cellular and molecular levels in plants. Therefore, phytoremediation potential of duckweed plant was well understood with added benefit that safe disposal of plant biomass is not a problem. The lignocellulosic duckweed was suggested to be an excellent feedstock for bio-energy production; therefore, the end-product utilization of harvested plant is not issue to concern. The harvested biomass can be further processed into energy products such as biogas, bioethanol or biochar.

3.3. Bioconcentration factor (BCF) and metal uptake potential (q_m) of L. gibba

The ambient metal concentration in aquatic media is one of the critical factors that influence the metal uptake efficiency in aquatic plants [34,35]. Few earlier workers have suggested that BCF can be used as blueprint of metal uptake efficiency of aquatic weeds [21,28] in phytoremediation trials. The BCF values for Pb and Cd in set-ups with different pH and concentration are shown in Tables 4 and 5. The BCF values for Pb increased significantly ($p < 0.005$) with respect to increasing Pb concentrations in culture media at pH 7 and 9. The ranges of BCF_{Pb} ranged between 403 (at 9 pH) and 491.6 (at 5 pH) for 2 mg/L set-up, 452.6 (at 9 pH) and 738 (at 5 pH) for 5 mg/L set-up, and 480.6 (at 9 pH) and 666 (at 5 pH) for 10 mg/L set-up. It is clear from the trend that duckweed showed comparatively low BCF_{Pb} values in experimental set-up with pH 9. In terms of pH of media the set-ups for BCF_{pb} can be arranged in the order: pH 5 > pH 7 > pH 9. For Cd

Table 4 Bioaccumulation, Specific metal ion uptake (q_m) and uptake yield in set-ups with Pb (mean ± SD, $n = 3$).

pH	C_o (mg/L)	X_m (g)	C_{acc} (mg/g)	q_m (mg/g)	Uptake yield (%)
5	2	2.81 ± 0.01	0.98 ± 0.03	0.34 ± 0.01	49.1
5	5	3.35 ± 0.04	3.69 ± 0.10	1.10 ± 0.02	73.8
5	10	3.30 ± 0.02	6.66 ± 0.11	2.01 ± 0.04	66.3
7	2	3.17 ± 0.05	0.89 ± 0.02	0.28 ± 0.01	44.5
7	5	3.73 ± 0.15	2.27 ± 0.03	0.61 ± 0.03	45.5
7	10	3.55 ± 0.08	5.67 ± 0.04	1.60 ± 0.51	56.7
9	2	3.55 ± 0.08	0.80 ± 0.06	0.28 ± 0.02	40.3
9	5	3.37 ± 0.11	2.26 ± 0.14	0.67 ± 0.04	45.2
9	10	3.22 ± 0.02	4.80 ± 0.16	1.49 ± 0.04	48.0

X_m: dried biomass; C_o: initial metal ion concentration; C_{acc}: bioaccumulated metal ion concentration after seven days; q_m: specific metal ion uptake determined as the amount of metal per unit of dry biomass = $[C_{acc}/X_m]$; uptake yield = $[C_{acc}/C_o] \times 100$ (data are mean of three replicates; ± is the standard deviation).

Table 5 Bioaccumulation, Specific metal ion uptake (q_m) and uptake yield in set-ups with Cd (mean ± SD, $n = 3$).

pH	C_o (mg/L)	X_m (g)	C_{acc} (mg/g)	q_m (mg/g)	Uptake yield (%)
5	2	2.87 ± 0.04	0.94 ± 0.02	0.33 ± 0.01	47.2
5	5	2.92 ± 0.02	2.56 ± 0.09	0.87 ± 0.03	51.3
5	10	2.67 ± 0.03	6.16 ± 0.07	2.30 ± 0.06	61.6
7	2	3.17 ± 0.05	0.92 ± 0.01	0.30 ± 0.00	46.3
7	5	2.94 ± 0.07	2.93 ± 0.05	1.00 ± 0.04	58.7
7	10	3.61 ± 0.14	5.13 ± 0.05	1.42 ± 0.07	51.3
9	2	2.86 ± 0.03	0.89 ± 0.01	0.28 ± 0.01	44.5
9	5	2.67 ± 0.11	2.41 ± 0.13	0.90 ± 0.02	48.2
9	10	3.24 ± 0.03	5.07 ± 0.07	1.56 ± 0.03	50.7

X_m: dried biomass; C_o: initial metal ion concentration; C_{acc}: bioaccumulated metal ion concentration after seven days; q_m: specific metal ion uptake determined as the amount of metal per unit of dry biomass = $[C_{acc}/X_m]$; uptake yield = $[C_{acc}/C_o] \times 100$ (data are mean of three replicates; ± is the standard deviation).

containing experimental set-up the BCF_{Cd} ranged between 445 (at 9 pH) and 472.5 (at 5 pH) for 2 mg/L set-up, 482.1 (at 5pH) and 587.6 (at 7 pH) for 5 mg/L set-up, and 507.2 (at 9 pH) and 616.6 (at 5 pH) for 10 mg/L set-up. In general, high BCF values denote the accumulative loads of metals in plant biomass which can be used as indicator parameter to select a plant or a treatment set-up for designing a phytoremediation system. The increasing BCFs with respect to increasing dose of metals in culture media were recorded in this study. But the experimental set-ups with pH 5 showed the high values of BCFs. Comparatively, the value of BCF_{Pb} was higher than BCF_{Cd} that suggests high accumulative efficiency of *L. gibba* for Pb as compared to Cd. The physiological need of metals in plant and uptake kinetics directly or indirectly affects the accumulative process for certain species of metals. BCF values over 1000 signify appropriateness of a plant (i.e. hyper-accumulative plant) for phytoremediation. In the current study, the BCF values of *L. gibba* for both metals were lower than 1000 in all experimental set-ups. On the basis of obtained results, *L. gibba* can be considered as a moderate accumulator for Cd and Pb under given conditions of current study.

However, *L. gibba* was found more efficient in lead removal from aquatic media. The results suggest that *L. gibba* can be used as harvestable plants for removal of heavy metals from pollutant water bodies through processes of separating, drying and ashing of the plant biomass after completion of the process of remediation [36,37]. This process deemed to be less-technical, cost-effective plant-based technology for the removal of metals from the environment. However, the large-scale performance and long-term efficiency of the system need should be optimized in order to develop a duckweed-based bioremediation system for industrial scale.

The metal up per unit of dry biomass (X_m) of *L. gibba* in all set-ups was also measured (Tables 4 and 5). There was no visible different between the set-ups for harvested dry biomass of duckweed at the end. The metal uptake (q_m) was measured as a ratio of C_{acc}/X_m, where C_{acc} was bioaccumulated concentration of metals and X_m was metal up per unit biomass. In set-ups with different loads of Pd the q_m ranged between 0.34 and 2.01 mg g^{-1} for pH 5, 0.28 and 1.60 mg g^{-1} for pH 7 and 0.28 and 1.49 mg g^{-1} for pH 9 (Table 4). Overall uptake yield in experimental set-up was optimum in set-up with 5 and 10 mg/L Pb strength at pH 5. The uptake yield of Pb was the maximum in 5 mg/L set-up with 5 pH followed by other set-ups. The uptake yield in set-ups was directly related to the metal loads in culture medium.

In Cd containing set-ups the per unit metal uptake (q_m) ranged between 0.33 and 2.30 mg g^{-1} for pH 5, 0.30 and 1.42 mg g^{-1} for pH 7 and 0.28–1.56 mg g^{-1} for pH 9 (Table 5). The maximum uptake yield (61.6%) was recorded in set-up with 5 pH and 10 mg/L strength of Cd. The average uptake yield in set-ups with Cd was 53.4% at pH 5, 52.1% at pH 7 and 47.8% at pH 9 (Table 5). The result of regression analysis suggests the role of initial metal loads in culture media in uptake yield of metals in duckweed. However, among all set-ups with different pH, the experimental set-ups with 10 mg/L strength of metal showed the better uptake results. Results clearly suggest that the load of metal ions and pH of aquatic media directly affects the uptake process of metals by plants in duckweed-based phytoremediation system. However, competition between metals and nutrients in the uptake by roots and in the plant translocation system should also be optimized [38] before designing the duckweed-based metal removal system.

4. Conclusions

The aim of this study was to investigate the effect of metal load and pH of aqueous media on metal removal rate and biomass productivity in a duckweed-based phytoremediation system. Results suggested that removal was directly related to the load of metal in media and pH 5 and 7 appeared as optimum range for better performance of the system. The pH showed inverse relationship with removal rate, in all set-ups. The metal uptake yield and BCFs suggested the pH 7 and medium and high metal loads of Pb and Cd for the desirable results of metal removals. Our study supports the candidature of *L. gibba* as biological agent in designing of a metal bioremediation system after optimizing the metal load and pH of media for better performance of the system. This can serve as an economical alternative treatment technique under a decentralized treatment opportunity.

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