



## Phytofiltration of cadmium from water by *Limnocharis flava* (L.) Buchenau grown in free-floating culture system

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### ARTICLE INFO

#### Article history:

Received 20 December 2008

Received in revised form 16 April 2009

Accepted 8 May 2009

Available online 18 May 2009

#### Keywords:

Phytofiltration

Cadmium

*Limnocharis flava* (L.) Buchenau

Free-floating support

### ABSTRACT

A hydroponics experiment was conducted to examine the phytofiltration of Cd by *Limnocharis flava* (L.) Buchenau grown in low-level Cd-contaminated water. For this, 45 d old seedlings of *L. flava* were transferred to a floating-support culture system containing nutrient solution spiked with four levels of Cd (0.5, 1, 2 and 4 mg l<sup>-1</sup>) and were separately harvested after 3, 7, 21 and 30 d. After 30 d harvesting, the percentage removal of Cd from the above four treatments reached up to 98, 96, 95 and 93%, respectively. Interestingly, all treatments had higher growth rate than control at 95% confidence level and plants still remained healthy at 4 mg l<sup>-1</sup> Cd exposure. The bioaccumulation study showed a linear relationship of Cd ( $R^2 = 0.896\text{--}0.999$ ) in all plant parts with the exposure time (3–30 d) and Cd concentrations in hydroponics system (0.5–4 mg l<sup>-1</sup>). Although, the root of *L. flava* had higher Cd concentration than leaf and peduncles, the total Cd concentrations in aerial plant parts were higher than the roots. The maximum bioconcentration factor (BCF) and translocation factor (TF) value of *L. flava* were calculated as 984.42 and 1.43, respectively. Estimated Cd accumulation capacity of *L. flava* per unit area (m<sup>2</sup>) was found to be in the range of 218.35–1698.92 mg m<sup>-2</sup>. The experimental results demonstrated that *L. flava* is a suitable candidate for the phytofiltration (>93%) of Cd from low-level Cd-contaminated water.

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### 1. Introduction

In urban areas the demand for storm water treatment, to prevent the anthropogenic release of heavy metals into local water bodies, is increasing rapidly [1]. One of the highly toxic heavy metal present in storm water is Cd, which may cause kidney damage to human beings even at low exposures [2]. Anthropogenic pathways by which Cd release in to environment are through industrial waste from processes such as electroplating, manufacturing of plastics, paint pigments, alloy preparation and batteries that contain cadmium. House hold appliances, automobiles and trucks, agricultural implement, airplane parts, industrial tools, hand tools and fasteners of all kinds (e.g. nuts, bolts, screws, and nails) are commonly Cd coated. Cadmium is also used for luminescent dials, in photography, rubber curing, and as fungicides [3].

A variety of technologies (including chemical physical and biological treatment methods) are available for storm water treatment. However, these methods present different efficiencies for different

metals and they can be very expensive for the treatment of low-level metal contaminated water. In contrast, the phytofiltration has been proposed as a promising, environmentally friendly technology for removing the heavy metal concentration of the contaminated water. In phytofiltration, high metal-accumulating plants function as biofilters, which can be remarkably effective in sequestering metals from polluted waters [4]. Developing nations that lack financial support and incentives to implement remedial procedures could benefit from such cost-effective plant-based filtration systems to remove hazardous metals from storm water. The success of phytofiltration depends on plant growth rate and the ability to uptake high metal concentrations in plant biomass. Plant must produce sufficient biomass while accumulating high concentrations of heavy metals.

In recent years, many aquatic plants, usually those found in polluted water bodies have been suggested for waste water treatment, i.e., they have the ability to accumulate unusually high concentration of heavy metals, without impact on their growth and development. However, most species identified so far are not suitable for onsite phytofiltration due to their small root and shoot biomass and slow growth rate. In contrast, plants with good growth usually show low metal accumulation as well as low tolerance to heavy metals [3]. An ideal plant for phytofiltration is one with high

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biomass production, easily harvestable and with superior capacity for heavy metal tolerance and accumulation. So there is a need to screen still more plants and assess their function in storm water treatment facilities. An extensive Scopus (<http://www.scopus.com>) based survey revealed that there is no work reported so far using *Limnocharis flava* in phytofiltration of heavy metals. The paucity of data on the effectiveness of *L. flava* in heavy metal filtration has prompted us to undertake this study. The aim of this study was to examine the feasibility of using *L. flava* for the phytofiltration of low-level Cd-contaminated water.

## 2. Materials and methods

### 2.1. Plant characteristics

*L. flava* is an emergent aquatic weed in the Limnocharitaceae family (Fig. 1). The plant inhabits shallow swamps, ditches, pools and wet rice fields, occurring in more or less stagnant fresh water. It is a perennial, erect, glabrous, lactiferous herb with a short stout rhizome with numerous fibrous roots. Aerial stems (peduncles) are flattened at the base, triangular up to 120 cm tall, bearing at the apex a cluster of flowers or a vegetative plant (ramet). Leaves are pale green, arising in clusters and rising above the water [5].

### 2.2. Experimental design

Seeds of *L. flava* were collected from the wetland field (Thalavady, Alleppey District, Kerala) and air dried for 24 h. The seeds were disinfected with 15% (v/v)  $H_2O_2$  for 30 min and washed thoroughly with deionized water. The seeds were then dipped in saturated  $CaSO_4$  solution for 3 h and then germinated in quarts sand for 15 days. Seedlings were transferred to 2 l glass tank and grown in half strength Hoagland's solution of pH = 7 for 15 days and subsequently in full strength nutrient solution for further 15 days as a pretreatment before experiments. The plants were kept in a controlled culture room at  $25 \pm 2^\circ C$ , under illumination provided by fluorescent lamps with a light to dark (LD) cycle of 14:10 h. A stock solution of Cd was prepared by dissolving AR grade of  $Cd(NO_3)_2$  at an initial concentration of  $1 \text{ mg l}^{-1}$ .

Acclimatized plant of *L. flava* (~20 g fresh weight) were placed in each experimental glass tank, which contained 2 l of full strength Hoagland's solution and 0.5, 1, 2 and  $4 \text{ mg l}^{-1}$  of Cd solution. The pH of the solution was adjusted to 7. One control group of plants was also prepared where Cd treatments were not provided. The plants were inserted into a thermo col substrate so that it could float freely and prevent the direct contact of aerial parts (peduncle sheath and leaf) with treated solution. Plant samples from each glass tank were separately harvested after 3, 7, 21 and 30 d to analyze for growth rate and metal content. The experiments were set up in triplicate for each concentration and exposure days.

### 2.3. Analyses

Growth variables measured included fresh weights, dry weights, biomass (as total dry weights of all plant parts), total growth (biomass-initial dry weight) and relative growth rate (g dry weight increase/g initial dry weight per day). Harvested plants were divided into three parts: roots, peduncles and leaves. Each part was oven dried for 24 h at  $100^\circ C$  and weighed. Dried plant tissues were ground with a mill. Powdered samples weighing 0.5 g were digested with 5 ml of  $HNO_3-HClO_4$  (5:1; v/v) and diluted to 100 ml with deionized double distilled water [1]. Water samples and digested plant samples were analyzed for Cd by an Atomic Absorption Spectrophotometer (8500; PerkinElmer, USA). The total accumulation and partitioning of the metals by the plants were calculated.

In order to ensure the quality control, Cd-spiked ( $2 \mu\text{g g}^{-1}$ ) *L. flava* control plant parts (root, petiole and leaf) were digested and analyzed separately. The percentage recoveries of Cd from three different plant parts were found in the range of 94–97%. Blanks sample were run after every 20 samples to check the precision of the method and sensitivity of the instrument.

The fresh parts of the plants were immediately used for the estimation of chlorophyll and protein contents. Chlorophyll content in leaves was extracted in 80% chilled acetone and estimated by the method of Arnon [6]. Similarly, carotenoid concentration in the same extract was calculated by the formula given by Duxbury and Yentsch [7]. Protein content in the leaves was estimated by the

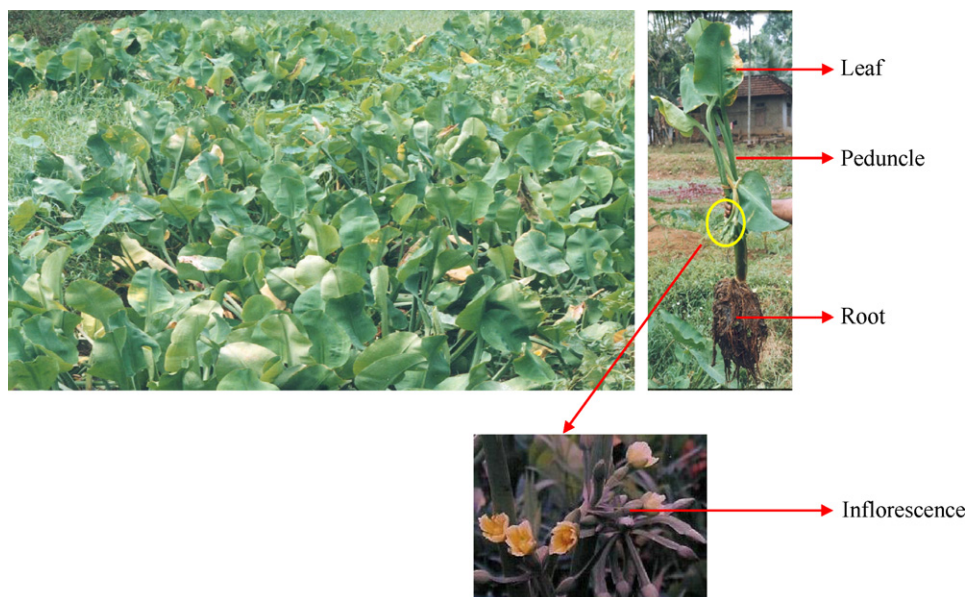


Fig. 1. Growth of *L. flava* (L.) Buchenau under natural conditions.

**Table 1**

Relative changes in the growth of *L. flava* on a fresh weight basis compared with initial fresh weight as 1.

Cd treatment (mg l <sup>-1</sup> )	Exposure days			
	3 d	7 d	21 d	30 d
0.5	1.02	1.11	1.28	1.35
1	1.09	1.21	1.29	1.39
2	1.14	1.18	1.25	1.30
4	1.23	1.16	1.21	1.28
Control	1.10	1.15	1.19	1.25

method of Lowry et al. [8] using bovine serum albumin (BSA) as a standard protein.

#### 2.4. Bioconcentration factor

Bioconcentration of heavy metal by aquatic organisms is described as the bioconcentration factor (BCF), which is the ratio of heavy metal accumulated by plants to that dissolved in the surrounding medium. For this, two bioconcentration factors were computed from the plant compartment concentrations as;

$$BCF^a = C_{\text{roots}}/C_{\text{water}} \quad (1)$$

$$BCF^b = C_{\text{aerial(peduncle+leaf)}}/C_{\text{water}} \quad (2)$$

#### 2.5. Translocation factor

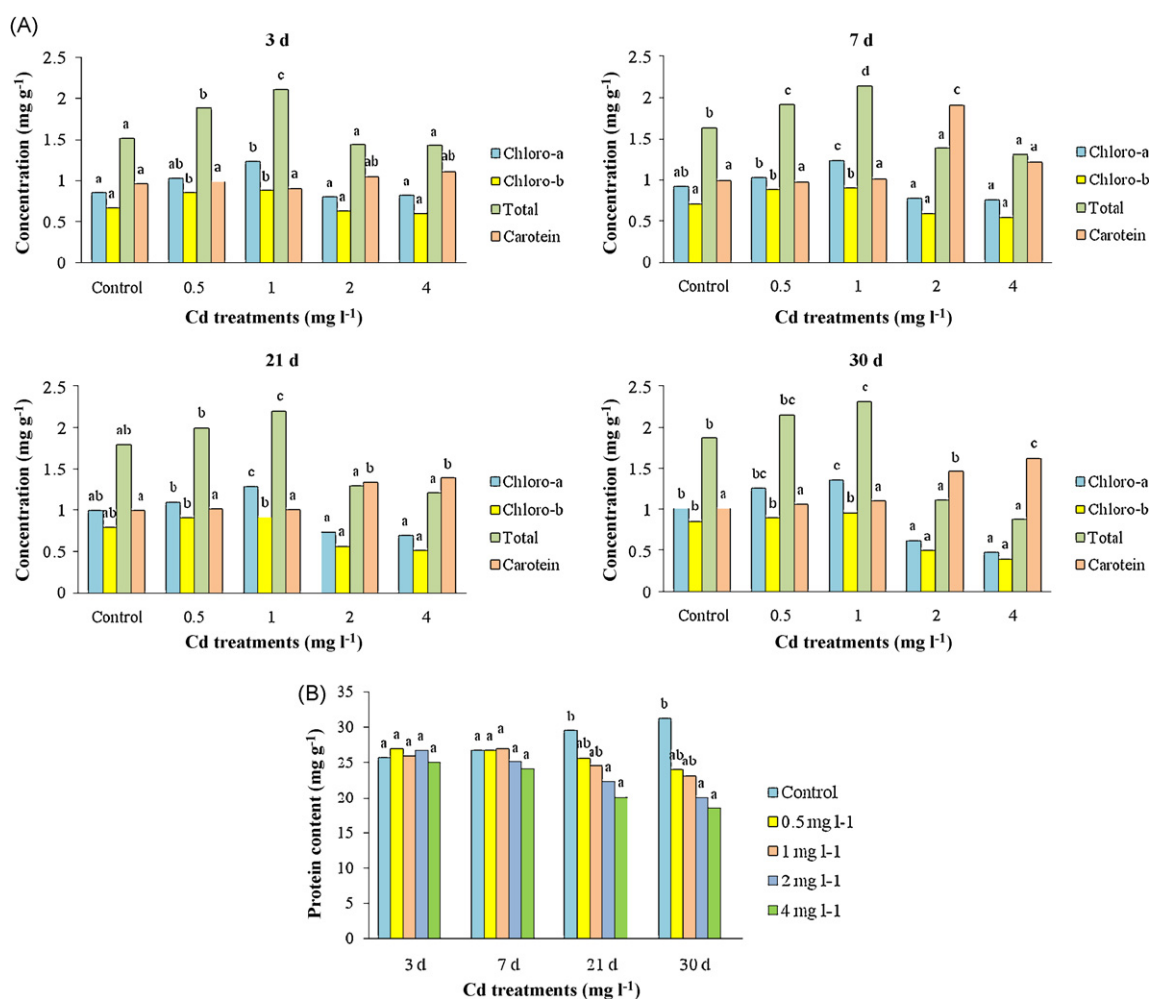
The translocation of heavy metal from the roots to harvestable aerial part is generally expressed as the translocation factor (TF). It was calculated on a dry weight basis by dividing the heavy metal concentration in aerial parts (peduncles + leaf) by the heavy metal concentration in root. Based on the above two equations (1) and (2), the translocation factor can be expressed as;

$$TF = BCF^b/BCF^a \quad (3)$$

### 3. Results and discussion

#### 3.1. Effect of Cd on plant growth

Growth changes are often the first and most obvious response of plants under heavy metal stress. The effect of different Cd concentrations on growth of *L. flava* is shown in Table 1. The growth difference during various exposure days (in terms of fresh matter production) was calculated in comparison with their initial fresh matter production as one. In all the four treatments, there was a noticeable increase of plant growth with the exposure periods. However, when the Cd treatments exceed 1 mg l<sup>-1</sup>, it was noted that the fresh weight of the plants slightly decreased. Similarly, the relative growth rate (RGR) of *L. flava* was higher in all the treatments



**Fig. 2.** (A) Changes in Chlorophyll-a, b, total-chlorophyll and carotenoids during various exposure days. Vertical concentration bars with different letters in a particular exposure day are significantly differ at 95% confidence level ( $p < 0.05$ ; ANOVA-DMRT). Fig. 1C. Changes in protein content during various exposure days. (B) Changes in protein content during various exposure days. Vertical concentration bars with different letters in a particular exposure day are significantly differ at 95% confidence level ( $p < 0.05$ ; ANOVA-DMRT).

than the control plants. As the Cd concentrations increased from 0.5 to 2 mg l<sup>-1</sup>, the RGR was also increasing and at 4 mg l<sup>-1</sup> concentration, RGR decreased slightly (figure not shown). Therefore, it appeared that low-level Cd concentration could stimulate the plant growth. Dou [9] found that although Cd is not generally considered as an essential element, it may stimulate the growth of some plants in small amounts. However, in an earlier study, Hasan et al. [10] reported that Cd has inhibitory effects on the growth of *Eichhornia crassipes* and the relative growth rate was negative at exposure of 4 mg l<sup>-1</sup> of Cd. Further, another study by Phetsombat et al. [11] found that *Salvinia cuculla* remained healthy at 2 mg l<sup>-1</sup> of Cd exposure. Similarly, Stratford et al. [12] reported that Cd was toxic and caused substantial reduction in *E. crassipes* growth mainly by suppressing development of new roots, and reducing relative growth rates to about 10% of those controls. This clearly suggests that Cd toxicity depend upon the plant species and external concentration.

### 3.2. Effect of Cd on photosynthetic pigments and protein content

The changes in photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) and soluble proteins are shown in Fig. 2A and B, respectively. The experimental results indicated that there was a significant increase ( $p < 0.05$ ) in photosynthetic pigments (Chlorophyll a, b and total) of *L. flava* during low-level Cd treatments (0.5–1 mg l<sup>-1</sup>). However, exposure to 4 mg l<sup>-1</sup> of Cd up to 30 d reduces the concentration of chlorophyll a, b, and total chlorophyll in *L. flava*. After 30 day exposure, there was a noticeable increase in (23.43%) total chlorophyll content was occurred in *L. flava* grown in 1 mg l<sup>-1</sup> of Cd treatment. However, when the Cd concentration in feed solution was above 1 mg l<sup>-1</sup>, chlorophyll contents significantly reduced than their respective control. The reductions were more prominent on 30 d exposure. On the contrary to chlorophyll contents, increases in carotenoids contents in high Cd treatments (2–4 mg l<sup>-1</sup>) were observed in entire sampling periods as compared to their respective controls. The protein content during various treatments and exposure days are presented in Fig. 1C. From this figure; it is clear that protein con-

tent was slightly increased during initial exposure periods, however at longer duration and higher exposure doses (4 mg l<sup>-1</sup>) protein content was significantly reduced ( $p < 0.05$ ) than their respective controls.

The heavy metals generate reactive oxygen species in the plants grown under stress conditions which damage photosynthetic pigments and may also catalyze degradation of proteins through oxidative modification and increased proteolytic activity [13]. The increased carotenoid level in Cd treated *L. flava* is probably a part of strategy adopted by the plant to counteract the toxic effect of free radicals generated under Cd stress, which is an agreement with other reports in aquatic plants [14]. However, more studies are needed to establish the antioxidant defense system in *L. flava* to cope with the Cd toxicity, especially the strategy involving activation of various enzymatic and non-enzymatic antioxidants as important components of antioxidant defense mechanism.

### 3.3. Accumulation of Cd in plant tissues

Cd content in the roots, peduncles and leaves of *L. flava* was evaluated periodically. The metal content significantly increased when the exposure time and metal concentration were increased ( $p \leq 0.05$ ). At Cd concentration of 0.5, 1, 2 and 4 mg l<sup>-1</sup>, the Cd content in the leaves increased to the maximum concentrations of 159, 319, 470 and 1063  $\mu\text{g g}^{-1}$  dry weight, respectively, and 119, 245, 408 and 1086  $\mu\text{g g}^{-1}$  dry weight, respectively in the peduncles and 214, 395, 1033 and 1590  $\mu\text{g g}^{-1}$  dry weight in the roots respectively, after 30 d harvesting. Although, the root of *L. flava* had higher Cd concentration than leaf and peduncles, the total Cd concentrations in aerial plant parts (peduncle+leaf) were higher than the roots. The concentration of Cd in the control plants was found to be 6.5, 1.6 and 1.2  $\mu\text{g g}^{-1}$  in the roots, leaves and peduncles, respectively. The accumulation of Cd in various parts of aquatic macrophytes under laboratory conditions has been reported in several species of aquatic plants, for example *Ipomea aquatica* [15], *E. crassipes* [16], *Potamogeton natans* [17], *Myriophyllum aquaticum* [18], *Pistia stratiotes* [19], *Lemna minor* [20,21], *Elodea canadensis* [1] and *S. cuculla*

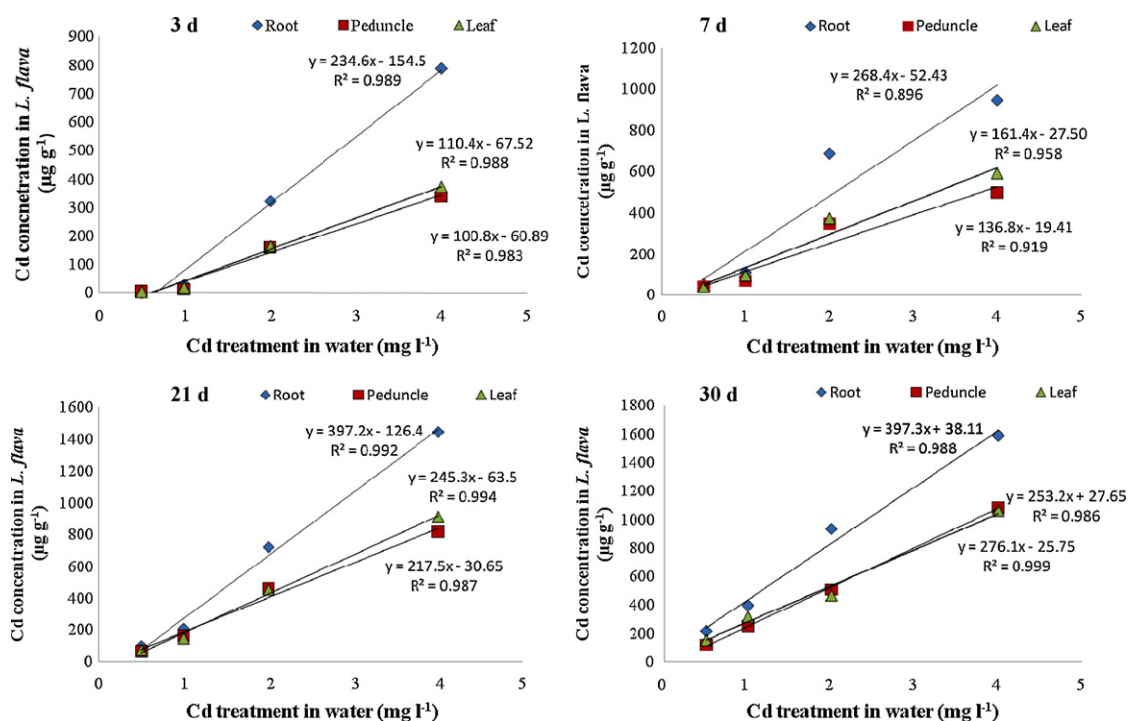


Fig. 3. Linear relationship of Cd concentration in water versus plant parts (root, peduncle and leaf) during various exposure days.



the aerial parts occurred and continued to go on during the whole experiment, it was slightly decreased in higher Cd treatments. It can be proposed that the aerial parts reached saturation during the period and translocation rate was slightly reduced or more or less static during the higher exposure doses (2 and 4 mg l<sup>-1</sup>).

Earlier studies proved that emergent species accumulated high concentrations of metals in their roots under natural conditions but much less so in their shoots, and the accumulation increased further with increased external concentration. The submersed and free-floating species accumulated high levels of metals in both their roots and shoots. Metals accumulated in the shoots of *E. canadensis* and *P. natans* were derived mostly from direct metal uptake from the water column [31–33].

Previous finding suggest [1,34] that Cd accumulates both extra and intracellular. The Cd accumulation in the extracellular compartment has been shown to increase with increasing external concentration [1]. Intracellular ion accumulation may increase with the increasing cation exchange capacity of cell walls due to the ion gradient established in the vicinity of plasma membrane [35–36]. Further, the process of intracellular Cd uptake in plants may be both active and passive and previous findings concerning various species are divergent [37–39]. To reach cytoplasm in plant tissue, Cd first has to pass the plasma membrane; this can happen, for example, via ion channels or specialized membrane proteins that mediate ion transport [40–46]. Translocation of ions is possible in the apoplast, in the phloem, and acropetally in the xylem [1]. In the present study, there was no direct contact of aerial plant parts with the Cd treated water phase and therefore, there was no chance for the direct accumulation of Cd via shoot (apoplastic translocation). Hence the higher Cd concentration found in aerial plant parts (peduncles and leaf) is due to acropetal translocation of Cd from water, via roots to peduncles to leaf.

### 3.7. Removal of Cd from surrounding water

The percentage removal of Cd from water by *L. flava* during various exposure days are presented in Table 3. The concentrations of spiked Cd remained in the residual solutions were significantly decreased ( $p \leq 0.05$ ) when the exposure time were increased. The concentrations of treated Cd in the solution at 0.5, 1, 2 and 4 mg l<sup>-1</sup> were 0.008, 0.041, 0.089 and 0.261 mg l<sup>-1</sup>, respectively, after 30 d harvesting. In other words, the percentage removal of Cd from the above four treatments reached up to 98, 96, 95, 93% respectively, after 30 d harvesting.

### 3.8. Phytofiltration efficiency of *L. flava*

Under natural growing conditions, 443.62–454.33 g dry weight of *L. flava* per square meter can be produced in one growing season [5]. In the present study the total accumulation of Cd by *L. flava* (root + peduncle + leaf) in four different Cd treatments were found to be in the range of 492.21–3739.43 μg g<sup>-1</sup> (Table 4). From this estimate, the absorption and accumulation per unit area of Cd can be calculated. The values were found to be in the range of 218.35–1698.92 mg m<sup>-2</sup>. Although, the laboratory investigation differs from an on-site investigation in many ways (because of impact of various factors such as microclimate, hydrobiology, hydrochemistry etc.), our study provides quantitative information using *L. flava* to remove Cd from low-level Cd-contaminated water and lays the foundation for more detailed field trialing.

Proper disposal of the harvested plant parts is the final and most important step in any kind of plant-based remediation technology. The higher BCF and TF values of tested species enable them to accumulate large amount of hazardous metals in their harvested parts and if not disposed properly; the accumulated heavy metals may back to the system or can enter into the food chain through brows-

**Table 4**  
Uptake potential of *L. flava* exposed to different Cd treatments.

Variables	Values
Maximum relative growth (on a fresh weight basis compared with initial fresh weight as 1) after 30 d exposure	1.39
Maximum dry matter production after 30 d exposure (per plant)	10.58 g
Root	3.71 g
Peduncle	4.95 g
Leaf	1.92 g
Maximum dry matter production in <i>L. flava</i> under natural conditions (per plant)	53.45 g*
Maximum weed density of <i>L. flava</i> (number of weeds per square meter)	8.50*
Maximum dry matter production in <i>L. flava</i> per square meter (weed density per square meter × dry matter production per plant)	454.33 g
Maximum Cd accumulation in whole plant (Cd concentration per gram dry weight × total dry matter) after 30 d exposure	12,669.84 μg
Root (1590.45 μg g <sup>-1</sup> dry weight × 3.60 g dry matter)	5725.62 μg
Peduncle (1085.90 μg g <sup>-1</sup> dry weight × 4.76 g dry matter)	5168.88 μg
Leaf (1063.08 μg g <sup>-1</sup> dry weight × 1.67 g dry matter)	1775.34 μg
Percentage removal of Cd from spiked water	98%
Maximum residual Cd concentration in spiked water after 30 d harvesting	0.261 mg l <sup>-1</sup>
Estimated Cd accumulation potential of <i>L. flava</i> per unit area	218.35–1698.92 mg m <sup>-2</sup> †

\* reported by Abhilash et al. [7].

† Estimated Cd accumulation potential of *L. flava* per unit area was calculated by multiplying the total Cd accumulation in *L. flava* (μg g<sup>-1</sup>) with the maximum dry matter production (weed density per square meter × dry matter production per plant). It is expressed as mg m<sup>-2</sup>.

ing animals. At the end of the growth period, plant biomass must be harvested, dried or incinerated, and the contaminant-enriched material should be deposited in a special dump or added into a suitable smelter. Recovery of Cd from harvested plant parts is another technological option. However, the cost benefit analysis of the recovery process should be conducted in order to evaluate the feasibility of various disposal techniques.

## 4. Conclusions

Heavy metal pollution seriously threatens the health of aquatic ecosystem worldwide and requires novel, low-cost, flexible and effective phytofiltration technologies. Despite considerable and rapid progress in recent years, lack of most suitable plants are still limiting the effectiveness of phytoremediation approaches. In the present study, we employed a hydroponics system with a free-floating support to evaluate the phytofiltration potential of *L. flava* against low-level Cd contamination in water. The advantage of this free-floating culture system is its easy harvesting. The results of the present study indicated that *L. flava* is a suitable plant for the phytofiltration of low-level Cd contamination from water (>93%) because of its (i) higher bioconcentration factor (BCF; ~934.86 after 30 d), (ii) translocation factor of >1, (iii) higher relative growth rate and biomass, and (iv) easy culture. However, a suitable harvesting and hazardous materials disposal system will be required for meaningful results. Further, more studies are needed to evaluate the on-site application of this free-floating phytofiltration technique.

## Acknowledgements

The authors are thankful to Director, National Botanical Research Institute, for providing facilities. Thanks are also due to Mr. Kalesh

Kumar for seed collection of *L. flava* and Dr. M.P. Nayar, Ex-Director, Botanical Survey of India for encouragements.

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