Physiological responses of water hyacinth, *Eichhornia crassipes* (Mart.) Solms, to cadmium and its phytoremediation potential

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Received: 27.11.2014 • Accepted/Published Online: 09.05.2015 • Final Version: 05.01.2016

Abstract: *Eichhornia crassipes* is an abundant floating aquatic weed that has great potential for cadmium (Cd) remediation owing to its large biomass and relatively high tolerance and accumulation capabilities. This study was conducted with *Eichhornia* in 5, 10, 15, and 20 mg L\(^{-1}\) CdCl\(_2\) in a hydroponic system for 21 days, and the Cd concentrations in the roots, shoots, and leaves were estimated. The plant showed tolerance, but at high Cd concentrations declines in biomass, root length, and leaf area were observed. Leaves showed a progressive decline in chlorophyll, carotenoid, and soluble protein and a significant elevation in lipid peroxidation. Cd uptake gradually increased in all the plant tissues up to 15 mg L\(^{-1}\) exposure, but at 20 mg L\(^{-1}\) the accumulation declined. Shoot tissues accumulated more Cd than root and leaf tissues. The highest accumulation by the plant was 1927.83 µg g\(^{-1}\) dry wt at 15 mg L\(^{-1}\) Cd. The maximum leaf, shoot, and root bioconcentration factors were 179.05, 187.59, and 169.3, respectively, and the maximum translocation factor of 1.003 was observed at 5 mg L\(^{-1}\) Cd. The root-to-leaf translocation of Cd was 100% efficient for all the doses of Cd exposure, except for 20 mg L\(^{-1}\). The results of this study suggested that water hyacinth tolerated phytotoxic concentrations of up to 15 mg L\(^{-1}\) and efficiently hyperaccumulated Cd in its above-ground tissues.

Key words: Metal, rhizofiltration, macrophyte, hyperaccumulator, MDA, plant pigment

1. Introduction

*Eichhornia crassipes* (Mart.) Solms, commonly known as water hyacinth, is a flowering monocot and an aquatic weed species of family Pontederiaceae, originally native to tropical and subtropical South America. It has a cosmopolitan distribution but is found mostly in the warmer regions of the world (Agunbiade et al., 2009). The plant has fast growth, large biomass, and tolerance for many metals/metalloids such as arsenic (Alvarado et al., 2008), silver (Pinto et al., 1987), cadmium (Agunbiade et al., 2009), chromium (Mishra and Tripathi, 2009), copper (So et al., 2003), iron (Jayaweera et al., 2008), nickel (Hadad et al., 2011), lead (Smolyakov, 2012), and zinc (Lu et al., 2004) as well as organics such as naphthalene (Nesterenko-Malkovskaya et al., 2012), ethion (Xia and Ma, 2006), and phenol (Nora and Jesus, 1997). Water hyacinth has also been used to improve the water quality of pulp and paper mill effluent (Yedla et al., 2002), wastewater treatment systems (Delgado et al., 1995), and dairy waste water (Trivedy and Patanshetti, 2002). It can also reduce the level of heavy metals from acid mine drainage water (Falbo and Weak, 1990) and petroleum refinery effluents (Ismail and Beddri, 2009), which makes it a good candidate for phytoremediation. Phytoremediation is a cost effective clean-up technique that uses plants as remediators of pollutants (Pilon-Smits and Freeman, 2006). Use of *E. crassipes* in remediation of polluted water bodies is gaining popularity due to its cost effectiveness and high capability to accumulate toxic heavy elements (Chua, 1998). Among the heavy metals, cadmium (Cd) is known to be highly toxic to both animals and plants (Kay, 1985; Deckert, 2005). A good Cd accumulator can concentrate >100 µg g\(^{-1}\) dry weight of metal (Baker and Brooks, 1989), has high biomass, rapid growth, and has bioconcentration factor (BCF) and translocation factor (TF) values >1 (Garbisu and Alkorta, 2001). BCF is the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate (Zayed et al., 1998), and TF is the leaf/root concentration ratio (Luo et al., 2005). Although several studies have removed a variety of metals from aquatic media, few attempts have been made to link the physiological responses of *E. crassipes* to its metal tolerance capabilities. In fact, Cd is known to impose phytotoxicity in plants by causing oxidative damage, which consequently leads to lipid peroxidation and impaired growth and photosynthesis (Mishra et

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al., 2006). Therefore, in this study an attempt has been made to observe the metal accumulation properties of *E. crassipes* in a 21-day hydroponic culture experiment while studying its detoxifying responses to Cd stress. Effects on biomass, root length, leaf area, photosynthetic pigments, and protein levels are evaluated as gross effect, while lipid peroxidation reflects oxidative stress. Moreover, this investigation provides a better understanding of the biological mechanisms adopted by water hyacinth in response to physiologically toxic concentrations of Cd.

2. Materials and methods

2.1. Plant material

Water hyacinth (*E. crassipes*) of uniform length and weight was collected from a freshwater pond situated in a village near the Assam University, Silchar campus. The pollution load of this pond was determined by analyzing water for common heavy metals; 250 mL of water samples were filtered and digested with 10 mL of concentrated analytical-grade nitric acid. The solutions were evaporated in a crucible to approximately 5 mL, then filtered into a 20 mL standard flask, and made up to the mark with distilled water. The water extract was analyzed for metals such as As, Cd, Cr, Cu, Fe, Pb, and Zn. Analyses of the heavy metal content of water samples were carried out with atomic absorption spectrometry (AAS, PerkinElmer). The results showed that all the metals were below detection limits of the instrument. The physicochemical properties of this pond were within the permissible limits and fit for human consumption (WHO, 2004). The collected plants were put in a hydroponic system containing tap water for a 1-week acclimatization period before being exposed to (CdCl₂). For quality control and quality assurance, the standard reference material of metals (Merck, Germany) was used for calibration and quality assurance in each analytical batch. The analytical data quality of metals was ensured with repeated analysis of quality control samples (n = 3), and the results were within the certified values. The detection limits of the instrument (AAS, PerkinElmer, model 3110) for As, Cd, Cr, Cu, Pb, Zn, and Se were 0.4, 0.5, 0.1, 0.19, 0.08, 0.002, and 0.02 µg L⁻¹, respectively.

2.2. Experimental set-up (hydroponic system)

Synthetic solutions of CdCl₂ (Merck, Germany) at 5, 10, 15, and 20 mg L⁻¹ concentrations were prepared using double-distilled water. The plants were put into containers containing 2 L of 30% Hoagland solution containing the desired Cd concentrations. The Hoagland solution provided the necessary nutrients to the plants during 21 days of growth. The plants were kept in the solution for 21 days. Plants of uniform size were used for experiments. There was one plant in each replicate, and each treatment contained three replicates. The control sets were similarly maintained in containers containing 2 L of 30% Hoagland solution only, without metal treatments. The volume of water in each tank was kept constant, and change in volume due to evaporation and transpiration was compensated for by the addition of deionized water. The pH, total hardness (as CaCO₃), nitrite (as NaN₂), chloride, and free chlorine were tested by their respective test kits (Himedia) and found to be 6.4–6.7 ppm, 225–282 ppm, 385–410 ppm, 120–145 ppm, and nil, respectively.

2.3. Plant growth, harvesting, and morphological parameters

Plants were harvested after 21 days without damaging the roots. They were rinsed in distilled water and separated into leaves, shoots, and roots. Shoot and root length and dry biomass (oven dried at 85 °C for 36 h) of different plant parts were measured. The root length and shoot length of water hyacinth were measured on the initial day and after 21 days of growth using a centimeter scale and expressed as cm/plant. The length and breadth of each leaf were measured initially and after 21 days, and the total leaf area was calculated using the formula L x B x K and expressed in cm²/plant, where L and B are the length and breadth, respectively, and K is the Kemp’s constant. For monocot, K is 0.9 (Kemp, 1960). The dry weight of roots, shoots, and leaves was measured using an electrical single-pan balance.

2.4. Analysis of plant leaves for chlorophyll and carotenoid contents

For estimation of plant pigments, circular pieces from young leaves were cut and extracted with 80% acetone using a mortar. The pigment extracts were centrifuged at 5000 tours/min/rotor for 10 min at 4 °C until the extract became fully transparent. The chlorophyll and carotenoid contents were determined using the following equations (Lichtenthaler, 1987):

\[
C_a = 12.25A_{663} - 2.79A_{645} \text{ (mg L}^{-1} \text{ FW)},
\]

\[
C_b = 21.50A_{665} - 5.10A_{645} \text{ (mg L}^{-1} \text{ FW)},
\]

\[
C_{a+b} = 7.15A_{663} + 18.71A_{645} \text{ (mg L}^{-1} \text{ FW)},
\]

\[
C_{a+c} = 1000A_{436} - 1.82C_a - 85.02C_b/198 \text{ (mg L}^{-1} \text{ FW)},
\]

where \(C_a\) = chlorophyll a; \(C_b\) = chlorophyll b; \(C_{a+b}\) = total chlorophyll; \(C_{a+c}\) = carotenoids; \(A_x\) = absorbance at x nm.

2.5. Analysis of plant leaves for lipid peroxidation (MDA) and soluble protein contents

Lipid peroxidation was determined by estimation of the malondialdehyde (MDA) content, following Heath and Packer (1968). Plant material (0.5 g) was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 × g for 5 min. For every 1 mL of aliquot, 4 mL of 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was heated at 95 °C for 30 min and then cooled quickly in an ice bath. The resulting mixture was centrifuged at 10,000 × g for 15 min, and the absorbance of the supernatant was taken at 532 and 600
nm. The nonspecific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM⁻¹ cm⁻¹.

Leaf soluble protein content of the fresh sample was determined after precipitation with TCA by the method of Lowry et al. (1951) using BSA as a standard.

2.6. Analysis of plant metal uptake capacity
Cadmium analysis was performed after digesting the dried samples in an acidic mixture of nitric and perchloric acids (HNO₃:HClO₄), as per APHA method (APHA, 1992), with an atomic absorption spectrophotometer (PerkinElmer). Cadmium uptake, depicted by a bioconcentration factor (BCF), is the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate (Zayed et al., 1998). It is calculated as follows: BCF = metal concentration in plant tissue at harvest (mg kg⁻¹)/initial concentration of the metal added in water (mg kg⁻¹). The ratio of heavy metal concentration in the leaf to concentration in the root of the plant is calculated as the translocation factor (TF) (Luo et al., 2005): TF = metal in leaf (mg kg⁻¹)/metal in root (mg kg⁻¹). The efficiency of translocation (in %) depicts the ability of the plant to translocate metal species from roots to leaves at different concentrations (Das et al., 2014). It is calculated as follows: efficiency (%) = metal in leaf (mg kg⁻¹)/metal in roots (mg kg⁻¹) × 100.

2.7. Quality control and quality assurance
For quality control and quality assurance, the standard reference material of Cd (Merck, Germany) was used for calibration and quality assurance with each analytical batch. Analytical data quality of metal was ensured with repeated analysis of quality control samples (n = 3), and the results were within the certified values. The detection limit for Cd was 0.5 µg L⁻¹.

2.8. Data analysis
Statistical comparison of means was done by analysis of variance (ANOVA). Multiple comparisons were done by Tukey’s test and using SPSS for Windows. Differences were considered significant at P < 0.05.

3. Results and discussion

3.1. Plant growth parameters and changes in biomass
E. crassipes without Cd treatments (control) showed an increase in root, shoot, and leaf biomass, whereas Cd treated groups showed progressive decline in biomass with increasing doses (Table 1). E. crassipes could tolerate high concentrations of Cd for 21 days in the hydroponic system. However, signs of toxicity in the form of chlorosis were evident at 5, 10, and 15 mg Cd L⁻¹, and at 20 mg Cd L⁻¹ gross necrosis and wilting of the older leaves occurred (Figure 1). When compared to control, there

<table>
<thead>
<tr>
<th>CdCl₂ (mg L⁻¹)</th>
<th>Day (d)</th>
<th>Root (g/plant)</th>
<th>Shoot (g/plant)</th>
<th>Leaf (g/plant)</th>
<th>Root length (cm)</th>
<th>Total leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 d</td>
<td>0.44 ± 0.002</td>
<td>0.51 ± 0.003</td>
<td>0.62 ± 0.009</td>
<td>9.9 ± 0.264</td>
<td>165.0 ± 8.88</td>
</tr>
<tr>
<td>21 d</td>
<td>1.58 ± 0.36</td>
<td>2.13 ± 0.19</td>
<td>2.35 ± 0.22</td>
<td>20.3 ± 0.45</td>
<td>311.4 ± 4.20</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0 d</td>
<td>0.44 ± 0.002</td>
<td>0.51 ± 0.003</td>
<td>0.62 ± 0.003</td>
<td>9.9 ± 0.173</td>
<td>165.6 ± 1.52</td>
</tr>
<tr>
<td>21 d</td>
<td>0.86 ± 0.02' (−45.56%)</td>
<td>1.25 ± 0.25' (−41.31%)</td>
<td>1.22 ± 0.19' (−48%)</td>
<td>18.2 ± 0.50' (−10.34%)</td>
<td>276.5 ± 7.31' (−11.21%)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0 d</td>
<td>0.44 ± 0.003</td>
<td>0.51 ± 0.003</td>
<td>0.62 ± 0.003</td>
<td>9.9 ± 0.20</td>
<td>165.6 ± 3.21</td>
</tr>
<tr>
<td>21 d</td>
<td>0.67 ± 0.01' (−57.34%)</td>
<td>0.76 ± 0.02' (−64.08%)</td>
<td>0.83 ± 0.008' (−64.46%)</td>
<td>17.2 ± 0.37' (−15.27%)</td>
<td>254.7 ± 10.14' (−18.21%)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0 d</td>
<td>0.44 ± 0.003</td>
<td>0.50 ± 0.002</td>
<td>0.62 ± 0.006</td>
<td>9.96 ± 0.251</td>
<td>165.3 ± 3.20</td>
</tr>
<tr>
<td>21 d</td>
<td>0.55 ± 0.01' (−64.6%)</td>
<td>0.61 ± 0.01' (−71.12%)</td>
<td>0.72 ± 0.008' (−69.19%)</td>
<td>15.4 ± 0.40' (−24.13%)</td>
<td>225.9 ± 12.15' (−27.45%)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0 d</td>
<td>0.44 ± 0.001</td>
<td>0.50 ± 0.003</td>
<td>0.62 ± 0.009</td>
<td>9.9 ± 0.057</td>
<td>164.66 ± 4.5</td>
</tr>
<tr>
<td>21 d</td>
<td>0.46 ± 0.01' (−70.75%)</td>
<td>0.53 ± 0.01' (−75.16%)</td>
<td>0.65 ± 0.01' (−72.17%)</td>
<td>14.5 ± 0.20' (−28.57%)</td>
<td>205.8 ± 4.32' (−33.91%)</td>
<td></td>
</tr>
</tbody>
</table>

* = significantly different from control at P < 0.05; values are mean ± SD of 3 replicates; values in the parentheses include percent decrease in mean values as compared to the corresponding control values.
was a significant decline in root, shoot, and leaf biomass. The dry biomass (g/plant) of different plant tissues and root length (cm) and total leaf area (cm²) of *E. crassipes* grown in different cadmium concentrations for 21 days are presented in Table 1. The root lengths of water hyacinth treated with various concentrations of Cd for 21 days are shown in Figure 2. The results indicated that the root length of plants progressively declined with increases in Cd concentrations in water. At 5, 10, 15, and 20 mg L⁻¹ Cd exposure, respectively, the root biomass decline by 45.56%, 57.34%, 64.62%, and 70.75%; the shoot biomass declined by 41.31%, 64.08%, 71.12%, and 75.16%; and the leaf biomass declined by 48.08%, 64.46%, 69.19%, and 72.17% (Figure 3). Similarly, when compared to control, there were 10.34%, 15.27%, 24.13%, and 28.57% declines in root length and 11.21%, 18.21%, 27.45%, and 33.91% declines in total leaf area after 5, 10, 15, and 20 mg L⁻¹ Cd exposure for 21 days, respectively (Figure 4).

Cadmium is well known for its phytotoxicity and causes changes in morphological, physiological, and biochemical parameters (Gallego et al., 2012). The presence of Cd in the external environment leads to alterations in the growth and development patterns of plants. The present study showed that, compared to the control, there was a significant decline in root, shoot, and leaf biomass in *E. crassipes*. The decrease in biomass might be due to rapid Cd-induced physiological damage such as lipid peroxidation, reduced photosynthesis, reduction of soluble protein, and altered composition and concentration of free amino acid and carbohydrate metabolism (Moya et al., 1984; Alfadul and Al-Fredan, 2013). A similar reduction in dry biomass was observed in Cd treated *Pistia stratoites* (Das et al., 2014). Kay et al. (1984) also reported a Cd related reduction in water hyacinth growth with relative growth rates reduced to about 10% of those of the control, mainly through the suppression of new root development. Cd exposure not only reduced dry weight, shoot and root length, chlorophyll levels in leaves, and levels of photosynthesis, but also enhanced the concentration of malondialdehyde (MDA, a lipid peroxidation product) in tomato plant parts (Cho and Park, 1999). A Cd-induced reduction in the length of shoots and roots of *Oryza sativa* was also observed (Moya et al., 1984).
3.2. Chlorophyll and carotenoid contents of plant leaves
One of the visible effects of Cd on water hyacinth at higher doses was the chlorosis and wilting of leaves. Concomitant with these, there was a significant reduction in chlorophyll and carotenoid contents (Table 2). For control plants, total chlorophyll contents were 6.15 ± 0.081 mg g⁻¹ FW (n = 3), while 10, 15, and 20 mg L⁻¹ Cd treatments registered significant reductions in total chlorophyll contents (5.38 ± 0.17, 3.04 ± 0.12, and 1.68 ± 0.138 mg g⁻¹ FW, respectively; n = 3). However, the plants seemed to be tolerant to 5 mg L⁻¹ Cd, as this dose failed to register significant reductions in total chlorophyll contents. Carotenoid content, on the
other hand, declined significantly in all the treatment groups. This drastic reduction of carotenoid may be the result of strong production of reactive oxygen species due to Cd stress (Ghnaya et al., 2009). The chlorosis of some lower leaves of \textit{E. crassipes} might be due to the Cd-induced inhibition of chlorophyll synthesis (Clijsters and Van Assche, 1985) and interference with photosystems (Siedlecka and Baszynski, 1993). In fact, Cd strongly binds to proteins, thereby decreasing the accumulation of pigment–lipoprotein complexes, including photosystem I (PSI) (Sárvári et al., 1999) and PSII (Küpper et al., 2007). Cd also decreases the contents of large and small subunits of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) as well as other enzymes of photosynthesis and chlorophyll biosynthesis (Gillet et al., 2006). This reduction in chlorophyll and carotenoid contents of \textit{E. crassipes} under Cd stress can be regarded as a specific response of the plants to metal stress, which resulted in chlorophyll degradation and inhibition of photosynthesis. A Cd related decrease in chlorophyll content was also reported in \textit{E. crassipes} at 25 µg mL$^{-1}$ of Cd (Mishra et al., 2007). The reduction in chlorophyll content might be due to increased cell or tissue damage or lipid peroxidation, as observed in this study and supported by Cho and Park (1999) in tomato plants. Wilting, which followed chlorosis, was also observed in the highest dose of Cd in this study. Wilting can be the consequence of cadmium-induced suppression of transpiration by stomatal closure and reduced stomatal conductance (Leita et al., 2001).

### 3.3. Lipid peroxidation and protein contents

Cd has been reported to increase lipid peroxidation via generation of reactive oxygen species (ROS) in plants (Chaoui et al., 1997). Lipid peroxidation has been shown to disrupt membrane organization and provoke functional losses and modifications of proteins and DNA bases (Pitzschke et al., 2006). MDA is the final product of peroxidation of membrane lipids and accumulates when the plants are subjected to oxidative stress. Therefore, MDA level is routinely used as an index of lipid peroxidation under stress conditions. In the present study, 5 mg L$^{-1}$ Cd exposure failed to cause any oxidative damage to this plant (Table 3). Generally, free radical generation and membrane damage would be low in tolerant plants and, thereby, form lower levels of MDA content. Therefore, lower MDA content in seedlings under Cd stress may support its tolerant nature. However, higher doses (10–20 mg L$^{-1}$ Cd) caused significant oxidative damage to the plant leaves. This underscores the highly toxic nature of Cd and its capability to cause oxidative damage to plants (Cho and Seo, 2004).

### Table 2. Effect of cadmium treatments on leaf pigment contents of \textit{Eichhornia crassipes} after 21 days.

<table>
<thead>
<tr>
<th>CdCl$_2$ (mg L$^{-1}$)</th>
<th>Chlorophyll (mg g$^{-1}$ fresh weight)</th>
<th>Carotenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_a$</td>
<td>$C_b$</td>
</tr>
<tr>
<td>0</td>
<td>6.15 ± 0.081</td>
<td>1.67 ± 0.143</td>
</tr>
<tr>
<td>5</td>
<td>5.69 ± 0.09'</td>
<td>1.86 ± 0.072''</td>
</tr>
<tr>
<td>10</td>
<td>4.07 ± 0.042'</td>
<td>1.30 ± 0.132'</td>
</tr>
<tr>
<td>15</td>
<td>2.27 ± 0.218'</td>
<td>0.767 ± 0.1'</td>
</tr>
<tr>
<td>20</td>
<td>1.48 ± 0.117'</td>
<td>0.202 ± 0.096'</td>
</tr>
</tbody>
</table>

$C_a$ = chlorophyll a; $C_b$ = chlorophyll b; $C_a + b$ = total chlorophyll; $C_x + c$ = carotenoid. Values are mean ± SD (n = 3); * = significantly different and ** = not significantly different at P < 0.05 at various doses of Cd for a particular plant pigment as compared to control values.

### Table 3. Effect of cadmium treatments on leaf MDA and protein contents of \textit{Eichhornia crassipes} after 21 days.

<table>
<thead>
<tr>
<th>CdCl$_2$ (mg L$^{-1}$)</th>
<th>Control</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol g$^{-1}$ FW)</td>
<td>5.69 ± 0.463</td>
<td>8.3 ± 0.325''</td>
<td>20.51 ± 2.79'</td>
<td>25.98 ± 2.26'</td>
<td>33.55 ± 1.63'</td>
</tr>
<tr>
<td>Protein (mg g$^{-1}$ FW)</td>
<td>24.32 ± 0.58</td>
<td>20.0 ± 1.0'</td>
<td>17.89 ± 0.84'</td>
<td>13.46 ± 0.46'</td>
<td>9.43 ± 0.51'</td>
</tr>
</tbody>
</table>

* = significantly different and ** = not significantly different from control at P < 0.05; values are mean ± SD of 3 replicates.
In this study the leaf soluble protein contents declined from 24.32 ± 0.58 mg g⁻¹ FW in the control to 20.0 ± 1.0, 17.89 ± 0.84, 13.46 ± 0.46, and 9.34 ± 0.51 mg g⁻¹ FW (n = 3 in each case), respectively, in 5, 10, 15, and 20 mg L⁻¹ Cd treatment groups (Table 3). This reduction may be due to degradation by proteases due to Cd, as observed in pea plants, (Romero-Puertas et al., 2002), or it may be a mark of general toxic stress due to Cd (Clijsters and Assche, 1985). Metal stress can also act at different sites to inhibit a large number of enzymes having functional sulphydryl groups. It results in a deleterious effect on the normal protein by disrupting pathways and protein synthesis.

3.4. Metal uptake capacity
The cadmium concentrations in roots, shoots, and leaves of *Eichhornia crassipes* plants after exposure to different solutions of CdCl₂ are shown in Table 4 and Figure 5. The metal concentration gradually increased in all plant tissues up to 15 mg L⁻¹ Cd exposure, but at 20 mg L⁻¹ the accumulation started declining. However, this value is still higher than the corresponding accumulation at 5 mg L⁻¹. The magnitude of increase in Cd concentrations in plant parts seemed to be dependent on exogenous Cd concentration, which increased with increasing Cd concentrations in water. Therefore at 5, 10, and 15 mg L⁻¹ there were simultaneous increases in tissue Cd concentrations. Although the quantity of trace elements accumulated by water hyacinth had been shown to correlate well with the concentration of heavy metals in the water, the highest Cd concentration (20 mg L⁻¹) had a limiting effect on accumulation potential, probably due to phytotoxicity at a higher concentration.

For all exposure doses, shoot tissues accumulated more Cd than root and leaf tissues. Metal uptake by plants involves transport across the plasma membrane of root cells, loading in xylem tissues, translocation, detoxification, and subsequently, sequestration at cellular levels (Lombi et al., 2002). A good hyperaccumulator is recognized by its ability to amass metals primarily in the shoots, both at low and high exogenous metal concentrations (Antosiewicz, 1992). When entering the root, Cd moves by apoplast until it reaches the endodermis. Plants possess several functional barriers that curb the uptake of Cd into the cytoplasm. At the tissue level, the major barriers are the endodermis and the cell walls of the central cylinder that restrain the uptake of heavy metals into the stelar cells, thus providing for the normal initiation of lateral roots. At the cellular level, the major barrier is the plasmalemma, which restricts Cd uptake into the protoplast (Seregin and Ivanov, 2001). In the present study, the shoot and leaf Cd concentrations were higher than the root Cd concentrations, indicating efficient root-to-shoot transport. In addition, the plant accumulated 1927.8 ± 17.03 µg g⁻¹ dry wt of Cd at a 15 mg L⁻¹ exposure concentration, which reflected an ideal hyperaccumulation potential (concentration > 100 µg g⁻¹ dry wt is considered Cd hyperaccumulation; Baker and Brooks, 1989). Again, for the same dose, root, shoot, and leaf Cd accumulations were 1908.69 ± 18.8, 1966.18 ± 28.58, and 1908.61 ± 5.71 µg g⁻¹ dry wt, respectively.

Different findings were reported for the uptake of Cd by water hyacinth (Deng et al., 2004). The greatest amount

<table>
<thead>
<tr>
<th>CdCl₂ (mg L⁻¹)</th>
<th>Cadmium concentration (µg g⁻¹ dry wt) in plant parts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
</tr>
<tr>
<td>5</td>
<td>846.6 ± 43.22</td>
</tr>
<tr>
<td>10</td>
<td>956.0 ± 43.44</td>
</tr>
<tr>
<td>15</td>
<td>1908.6 ± 18.88 *</td>
</tr>
<tr>
<td>20</td>
<td>921.97 ± 38.13</td>
</tr>
</tbody>
</table>

Mean ± SD (n = 3); * indicates significance at P < 0.05 at different doses for a particular plant tissue.
of Cd accumulated by *Eichhornia crassipes* was 36 g kg⁻¹ (Muramoto and Oki, 1983). Zhu et al. (1999) reported that *E. crassipes* accumulated 6103 and 371 mg kg⁻¹ dry wt of Cd in roots and shoots, respectively, when 10 mg Cd L⁻¹ was supplied. In another study a Cd concentration of 0.31 mg g⁻¹ dry wt was observed in water hyacinth over 15 days (Mishra and Tripathi, 2008). Water hyacinth strongly accumulated Cd with a concentration of 462 mg kg⁻¹ (Wang et al., 2002). Liao and Chang (2004) found Cd accumulation in the roots to be 10.05 mg kg⁻¹ dry wt at 0.06 mg L⁻¹ Cd. In another study, water hyacinth accumulated a high concentration of metals in roots (2044 mg kg⁻¹); however, the same study found relatively little Cd accumulation in shoots (Luo et al., 2004). Zhu et al. (1999) reported a BCF value of 647.4 for 10 mg L⁻¹ Cd treatment, indicating the efficient translocation of Cd in the present study, we also found higher leaf concentrations of Cd across the endodermis, membrane integrity to allow symplastic movement might be important for continuous Cd accumulation in shoots (Cho and Park, 1999).

### 3.5. Bioconcentration factor (BCF) and translocation factor (TF) of cadmium

The bioconcentration factor (BCF), which was defined as the ratios between concentrations of metal in plant tissue/initial concentration of metal in external solution, was calculated and is presented in Table 5. There was a general decrease in the BCF with increasing Cd concentrations after 21 days of growth. *Eichhornia* showed a maximum leaf BCF of 179.05, shoot BCF of 187.59, and root BCF of 169.3 at 5 mg L⁻¹ Cd (Table 5). The leaf/root concentration ratios [translocation factor (TF)] of Cd in *E. crassipes* plants grown for 21 days are shown in Table 5. TF is the indicator of the capacity of the plant to transport Cd from roots to aerial parts. The data showed that the maximum TF observed in *Eichhornia* was at 5 mg L⁻¹ Cd (1.003 TF), achieved after 21 days of growth. The root-to-leaf translocation of Cd was 100% efficient for all doses of Cd exposure except 20 mg L⁻¹, which showed 91.89% efficient translocation (Table 5).

BCF is an index of hyperaccumulation as well as efficiency of metal sequestration. A wide range of BCF values were documented in *E. crassipes*. Zhu et al. (1999) reported a BCF value of 647.4 for 10 mg L⁻¹ Cd treatment, and Lu et al. (2004) reported 622.3 for 2 mg L⁻¹ Cd treatment. Agunbiade et al. (2009) reported Cd BCF root and shoot values of 17 and 45, respectively, in *E. crassipes* collected from metal-contaminated coastal waters, while Liao and Chang (2004) reported BCF values of more than 1000 in *E. crassipes* shoots at low Cd concentrations. For an ideal hyperaccumulator, TF values should be >1 (Garbisu and Alkorta, 2001). Agunbiade et al. (2009) reported a Cd TF value of 2.63 in *E. crassipes* from metal-contaminated coastal waters. In the present study, the maximum TF was observed in *E. crassipes* at 5 mg L⁻¹ Cd (1.003 TF). It indicated *E. crassipes* is a good hyperaccumulating plant with efficient metal transport properties. Again, TF for 10 and 15 mg Cd L⁻¹ was also >1; however, the highest dose of Cd across the endodermis, membrane integrity to allow symplastic movement might be important for continuous Cd accumulation in shoots (Cho and Park, 1999).

### Table 5. Bioconcentration factor (BCF), translocation factor (TF), and translocation efficiency (%) of cadmium in different parts of *Eichhornia crassipes*.

<table>
<thead>
<tr>
<th>CdCl₂ (mg L⁻¹)</th>
<th>BCF_root</th>
<th>BCFShoot</th>
<th>BCFLLeaf</th>
<th>BCFWholeplant</th>
<th>TF</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>169.3 ± 8.64</td>
<td>187.5 ± 12.3</td>
<td>170 ± 10.49</td>
<td>526 ± 31.0</td>
<td>1.0 ± 0.017</td>
<td>100.4 ± 1.76</td>
</tr>
<tr>
<td>10</td>
<td>95.6 ± 4.34</td>
<td>98.6 ± 7.63</td>
<td>95.8 ± 6.8</td>
<td>290 ± 18.35</td>
<td>1.00 ± 0.03</td>
<td>100.2 ± 3.2</td>
</tr>
<tr>
<td>15</td>
<td>127.2 ± 1.25</td>
<td>131.07 ± 1.9</td>
<td>127.2 ± 0.38</td>
<td>385 ± 3.40</td>
<td>1.0 ± 0.007</td>
<td>100 ± 0.78</td>
</tr>
<tr>
<td>20</td>
<td>46.09 ± 1.90</td>
<td>48.36 ± 1.08</td>
<td>42.41 ± 3.83</td>
<td>121 ± 33.76</td>
<td>0.92 ± 0.05</td>
<td>91.8 ± 5.3</td>
</tr>
</tbody>
</table>
20 mg L⁻¹ caused a slight decline in TF (0.918). This decline in the TF value with increasing Cd concentrations after 21 days of growth signifies an exclusion strategy (Baker, 1981). Higher root metal concentrations and lower shoot metal concentrations at higher dose indicate restriction of Cd from the root to aerial parts via xylem sap flow. The present work, as well as Ertekin et al. (2015) on *Landoltia punctata* and Torbati (2015) on *Lemma minor*, indicated the usefulness of aquatic plants for phytoremediation purposes for various pollutants.

Thus, the present study showed that *E. crassipes* accumulated high amounts of Cd and responded well at physiologically phytotoxic Cd concentrations. Fast growth, large biomass, abundance, and easy harvesting of plants further suggested their suitability for phytoremediation purposes. The use of plants to remove cadmium from water would be suitable in an Indian context where the cost of other established remediation techniques is often too steep to afford. Moreover, due to the high accumulation of Cd in aerial parts (shoots and leaves), BCF and TF factors >1, and efficient translocation potential, *E. crassipes* can be used on a large scale to remediate Cd from water.

**Acknowledgments**

We thank the Biotech Hub, Life Science, Assam University, Silchar, for providing facilities for biochemistry work. We also thank the anonymous reviewers for their valuable suggestions.

**References**


