

# Removal of Cadmium and Zinc by Water Hyacinth, *Eichhornia crassipes*

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**ABSTRACT:** Toxic heavy metal pollution of water and soil is a major environmental problem, and most conventional remediation approaches do not provide acceptable solutions. Wetland plants are being used successfully for the phytoremediation of trace elements in natural and constructed wetlands. This study demonstrates the phytoremediation potential of water hyacinth *Eichhornia crassipes*, for the removal of cadmium (Cd) and zinc (Zn). The phytoaccumulation of heavy metals, Cd and Zn, by water hyacinth *E. crassipes*, was studied. Water hyacinths were cultured in tap water, which was supplemented with 0.5, 1, 2 and 4 mg/L of Cd and 5, 10, 20, and 40 mg/L of Zn, and were separately harvested after 0, 4, 8 and 12 days. The experiment showed that both Cd and Zn had effects on plant relative growth. Removal of metals from solution was fast especially in the first four days. The accumulation of Cd and Zn in shoots and roots increased with the initial concentration and also with the passage of time. Plants treated with 4 mg/L of Cd accumulated the highest concentration of metal in roots (2044 mg/kg) and shoots (113.2 mg/kg) after 8 days; while those treated with 40 mg/L of Zn accumulated the highest concentration of metal in roots (9652.1 mg/kg) and shoots (1926.7 mg/kg) after 4 days. The maximum values of bioconcentration factor (BCF) for Cd and Zn were 622.3 and 788.9, respectively, suggesting that water hyacinth was a moderate accumulator of Cd and Zn and could be used to treat water contaminated with low Cd and Zn concentrations.

**KEYWORDS:** water hyacinth (*Eichhornia crassipes*), accumulation, cadmium, zinc, removal, bioconcentration factor (BCF).

## INTRODUCTION

Rapid industrialization and urbanization have resulted in elevated emission of toxic heavy metals entering the biosphere.<sup>1</sup> Activities such as mining and agriculture have polluted extensive areas throughout the world.<sup>2,3</sup> The release of heavy metals in biologically available forms by human activity, may damage or alter both natural and man-made ecosystems.<sup>4</sup> Heavy metal ions such as Cu<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>, are essential micronutrients for plant metabolism but when present in excess, can become extremely toxic.<sup>5</sup>

Cadmium (Cd) is one of the most toxic heavy metals and is considered non-essential for living organisms.<sup>6</sup> Cd has been recognized for its negative effect on the environment where it accumulates throughout the food chain posing a serious threat to human health.<sup>7</sup> Cd pollution has induced extremely severe effects on plants.<sup>8</sup> Unlike Cd, zinc (Zn) is an essential and beneficial element for human bodies and plants. Complete exclusion of Zn is not possible due to its dual role, an

essential microelement on the one hand and a toxic environmental factor on the other.<sup>9</sup> However, Zn can cause nonfatal fume fever, pneumonitis, and is a potential hazard as an environmental pollutant.<sup>10</sup>

Recently there is a considerable interest in developing cost effective and environmentally friendly technologies for the remediation of soil and wastewater polluted with toxic trace elements.<sup>11</sup> Plants have the ability to accumulate nonessential metals such as Cd and Pb, and this ability could be harnessed to remove pollutant metals from the environment.<sup>12-14</sup> Plants-based bioremediation technologies have received recent attention as strategies to clean-up contaminated soil and water.<sup>15</sup> The submerged macrophytes are particularly useful in the abatement and monitoring of heavy metals.<sup>16</sup>

Water hyacinth, *Eichhornia crassipes*, is a floating macrophyte whose appetite for nutrients and explosive growth rate has been put to use in cleaning up municipal and agriculture wastewater.<sup>17</sup> It has been discovered that water hyacinth's quest for nutrients can be turned

in a more useful direction. The plant has been shown to accumulate trace elements such as Ag, Pb, Cd and Zn.<sup>18-21</sup> The focus on water hyacinth as a key step in wastewater recycling is due to the fact that it forms the central unit of a recycling engine driven by photosynthesis and therefore the process is sustainable, energy efficient and cost efficient under a wide variety of rural and urban conditions.<sup>22</sup> The aim of the present study was to demonstrate the phytoremediation potential of water hyacinth, *E. crassipes* for the removal of Cd and Zn.

## MATERIALS AND METHODS

### Experimental Procedures

*E. crassipes* was collected from a ditch in the suburb of Bangkok, Thailand and rinsed with tap water to remove any epiphytes and insect larvae grown on plants. The plants were placed in cement tanks with tap water under natural sunlight for one week to let them adapt to the new environment, then the plants of the same size were selected for further experiment. A stock solution (1000 mg/L) each was prepared in distilled water with analytical grade  $\text{CdCl}_2 \cdot 2\frac{1}{2} \text{H}_2\text{O}$  and  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  which was later diluted as required. The plants were maintained in tap water supplemented with 0.5, 1, 2, 4 mg/L of Cd and 5, 10, 20, 40 mg/L of Zn. Plants that were not exposed to metals served as controls. All experiments were performed in triplicate. The test durations were 0 (two hours), 4, 8 and 12 days. Tap water was added daily to compensate for water loss through plant transpiration, sampling and evaporation. After each test duration, plants were harvested. They were separated into shoots and roots, and were analyzed for relative growth, metals accumulation, and bioconcentration factor (BCF). In addition, the metals remained in the solution were measured to assess the removal potential of water hyacinth.

### Sample Analyses

#### Relative Growth

Relative growth of control and treated plants was calculated as follows:

$$\text{Relative growth} = \frac{\text{Final fresh weight (FFW)}}{\text{Initial fresh weight (IFW)}}$$

#### Metals Accumulation

Metals accumulation in plant and water samples was measured. Digestion of samples in this study was performed according to the Standard Methods by APHA.<sup>7</sup> Plant samples were decomposed to dry matter by heating at 120°C for 24 hours in a hot air oven and the ash was digested with nitric acid and filtered into

**Table 1.** Maximum growth response of water hyacinth exposed to Cd and Zn.

Parameter	Cd	Zn
Relative growth	0.85 <sup>a</sup>	0.89 <sup>c</sup>
Metal accumulation (mg/kg)		
shoot	113.2 <sup>a</sup>	1926.7 <sup>c</sup>
root	2044 <sup>a</sup>	9652.1 <sup>c</sup>
Residual concentration (mg/L)	0.185 <sup>a</sup>	6.29 <sup>c</sup>
BCF	622.3 <sup>b</sup>	788.9 <sup>d</sup>

<sup>a</sup> 4 mg/L Cd, <sup>b</sup> 2 mg/L Cd, <sup>c</sup> 40 mg/L Zn, <sup>d</sup> 5 mg/L Zn.

a volumetric flask. The final volume was made up with deionized water and heavy metals analysis was done using a flame atomic absorption spectrophotometer (FAAS). The results of the accumulation were reported, as concentration (mg) of Cd and Zn in plants (kg). The concentration of metals remained in the residual solution was measured using FAAS.

#### BCF

The 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 follows:<sup>11</sup>

$$\text{BCF} = \frac{\text{Concentration of metal in plant tissue}}{\text{Initial concentration of metal in external solution}}$$

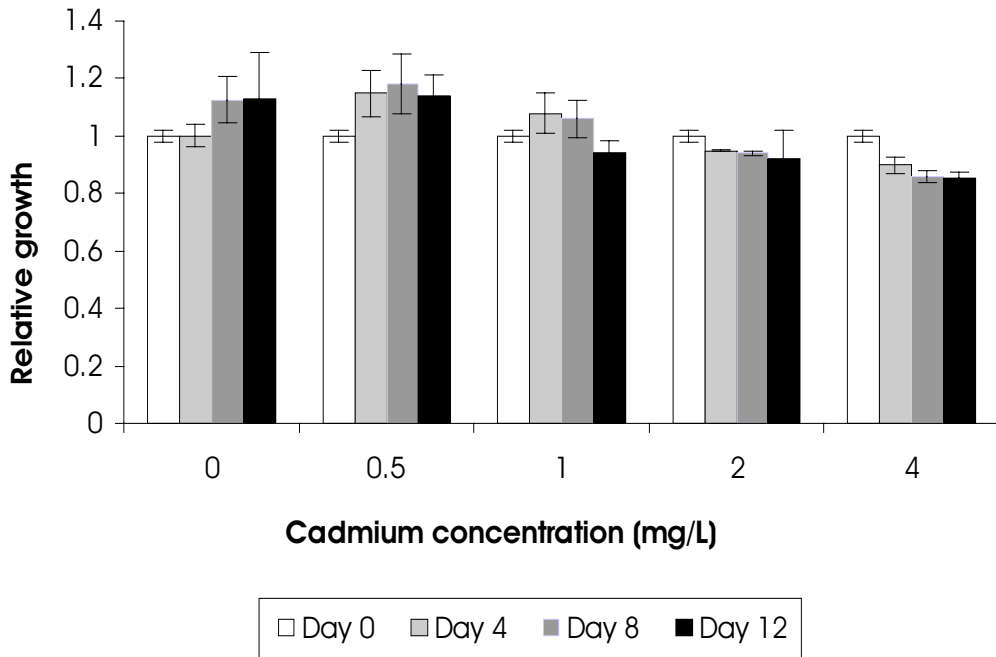
### Statistical Analysis

The mean numbers of relative growth, metal concentration and BCF were calculated and subjected to Analysis of Variance (ANOVA) using randomized block design and Least Significant Difference method (LSD) on the SPSS for windows program after analysis of the homogeneity of variance according to Cochran's test.<sup>23</sup>

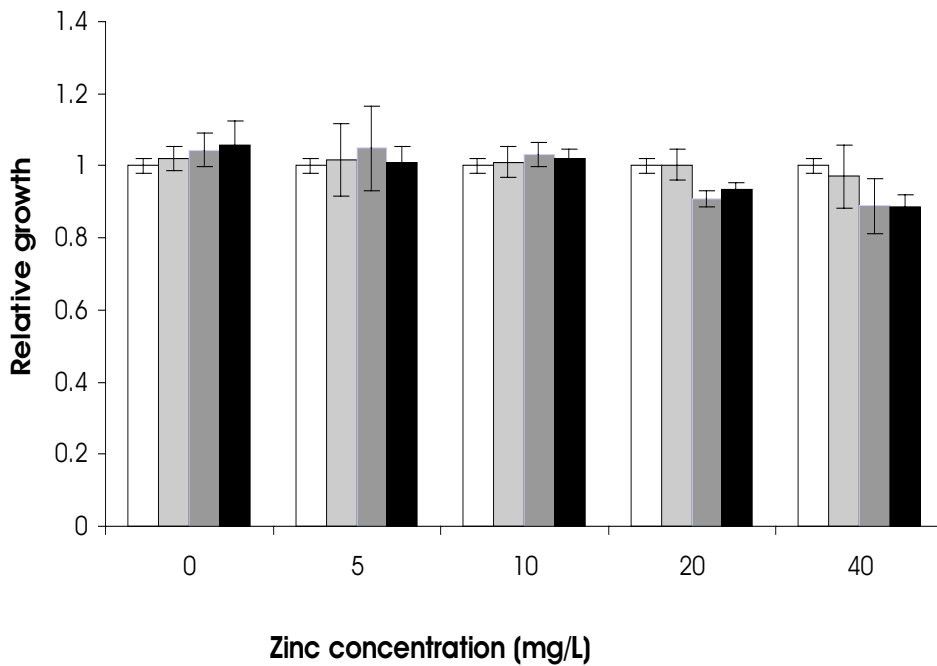
## RESULTS

### Relative Growth

The effects of Cd and Zn on relative growth of *E. crassipes* at different concentrations and exposure times were shown in Figure 1. The relative growth of control plants significantly increased ( $P \leq 0.05$ ) with the passage of time. In plants treated with Cd, the relative growth significantly increased ( $P \leq 0.05$ ) in 0.5 and 1 mg/L treatments, but decreased in 2 and 4 mg/L treatments. In plants treated with Zn, the relative growth significantly increased ( $P \leq 0.05$ ) in 5 and 10 mg/L treatments, but decreased in 20 and 40 mg/L treatments. The lowest values of relative growth were 0.85 and 0.89 for water hyacinth treated with Cd at 4 mg/L and Zn at 40 mg/L, respectively. The comparison of maximum relative growth of water hyacinth exposed



A



B

Fig 1. The effects of Cd (A) and Zn (B) on relative growth of *E. crassipes* at different metal concentrations and exposure times.

to Cd (4 mg/L) and Zn (40 mg/L) is shown in Table 1.

### Metals Accumulation

Cd and Zn accumulations by water hyacinth at different concentrations and exposure times were separately shown in Fig 2 and Fig 3, respectively. In

general, there were increases in metal accumulation in shoots and roots when metal concentration and exposure times were increased ( $P \leq 0.05$ ). For Cd, control and plants treated with 2 and 4 mg/L showed a significant difference ( $P \leq 0.05$ ) in metal accumulation (Fig 2). There was a significant difference ( $P \leq 0.05$ ) in

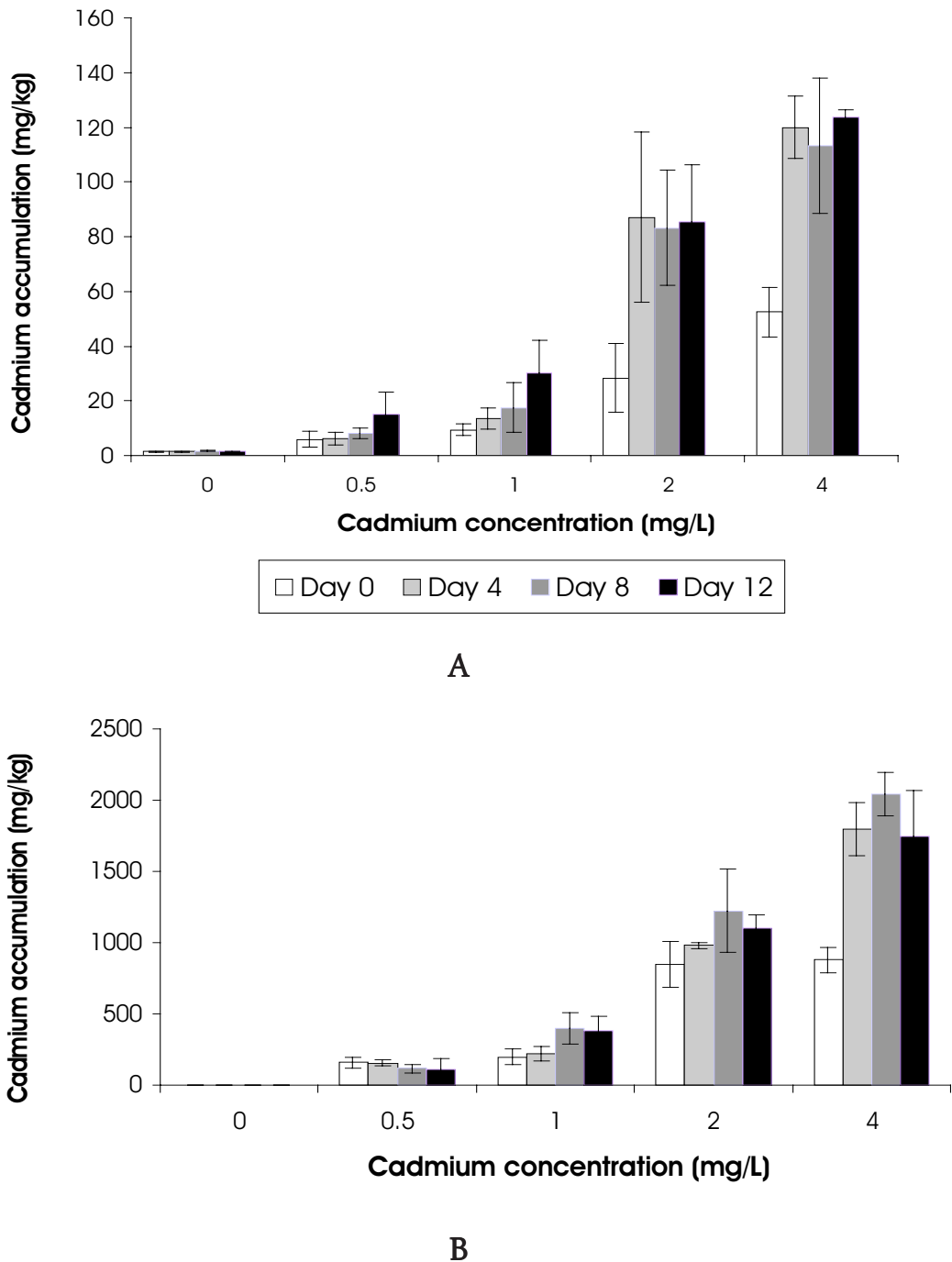


Fig 2. The accumulation of Cd in shoots (A) and roots (B) of *E. crassipes*.

Cd accumulation with the passage of time at all concentrations except for 0 and 0.5 mg/L. For Zn, significant differences ( $P \leq 0.05$ ) between control and treated plants were found at all metal concentrations (Fig 3). There was a significant difference ( $P \leq 0.05$ ) in accumulation with the passage of time at all

concentrations. Plants treated with 4 mg/L of Cd on day 8 accumulated the highest level of metal in shoots (113.2 mg/kg; Fig 2A) and in roots (2044 mg/kg; Fig 2B); while plants treated with 40 mg/L of Zn on day 4 accumulated the highest level of metal in shoots (1926.7 mg/kg; Fig 3A), and in roots (9652.1 mg/kg; Fig 3B).

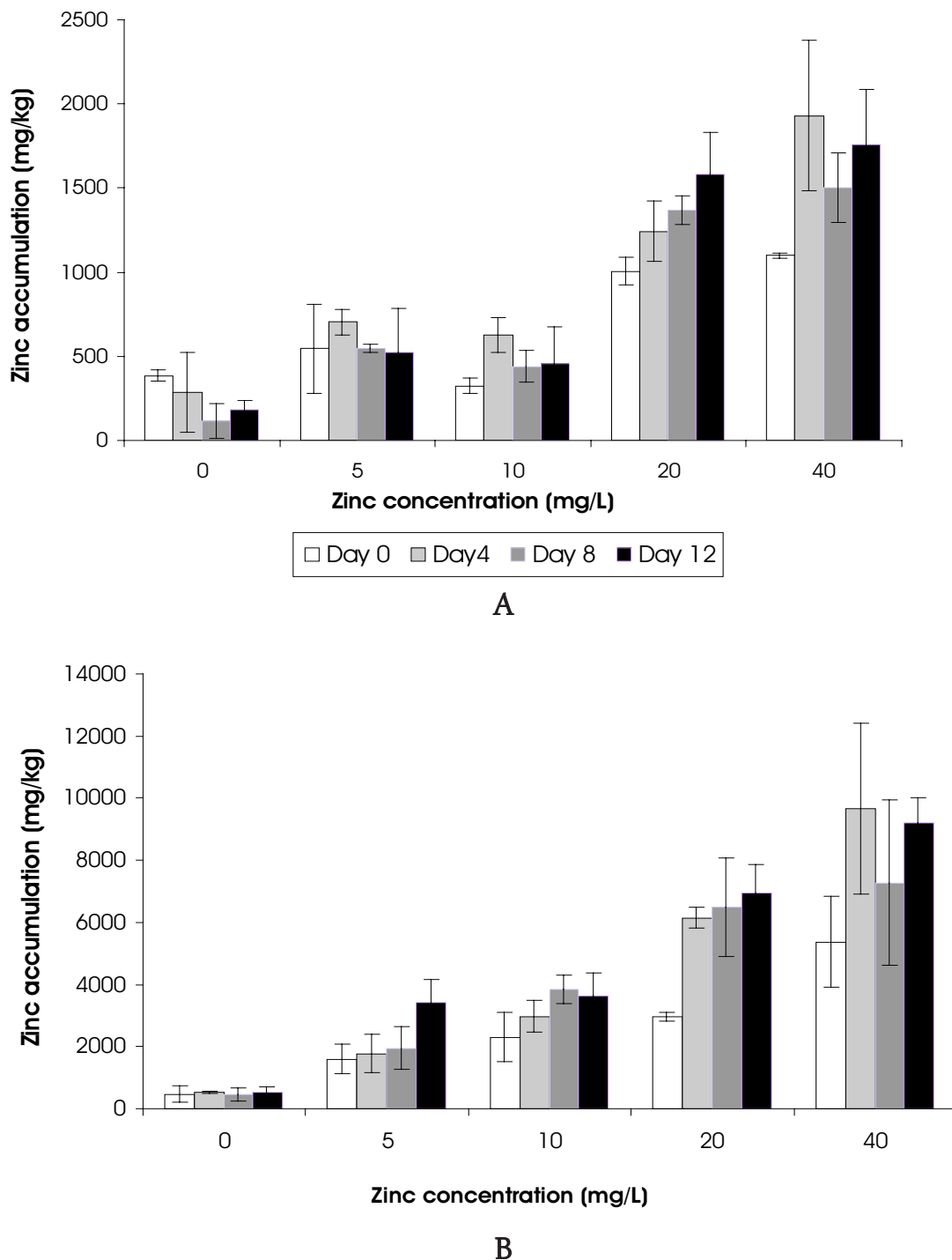


Fig 3. The accumulation of Zn in shoots (A) and roots (B) of *E. crassipes*.

The comparison of maximum accumulation of Cd and Zn in water hyacinth exposed to Cd (4 mg/L) and Zn (40 mg/L) is shown in Table 1.

#### Metals Remained in the Residual Solution

The concentrations of dissolved metals remained

in the residual solutions were shown in Fig 4. They were significantly decreased ( $P \leq 0.05$ ) when the exposure times were increased. The concentrations of dissolved Cd in the solutions at 0.5, 1, 2 and 4 mg/L were 0.003, 0.005, 0.088 and 0.185 mg/L, respectively on day 12 (Fig 4A). The concentrations of dissolved Zn in the

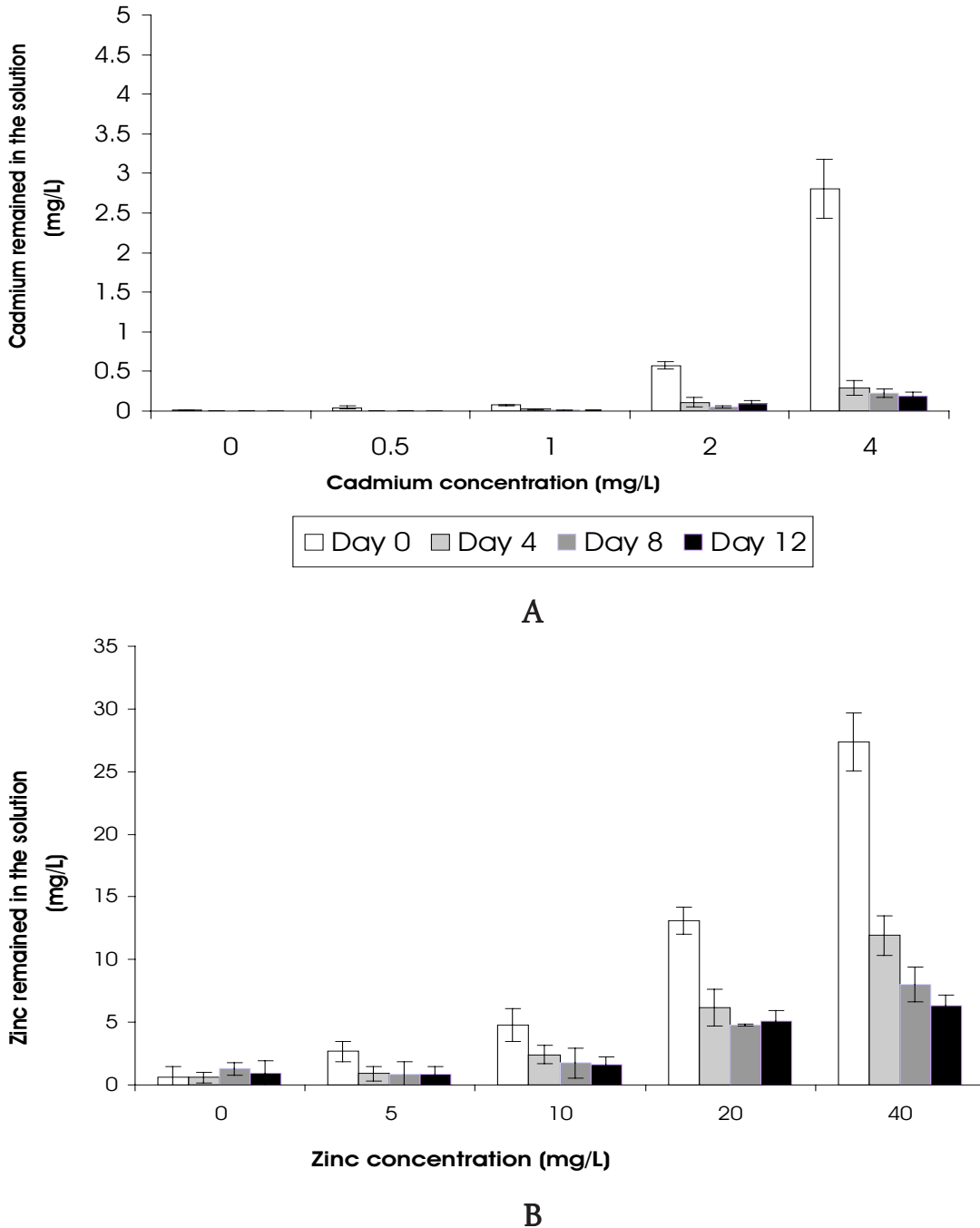


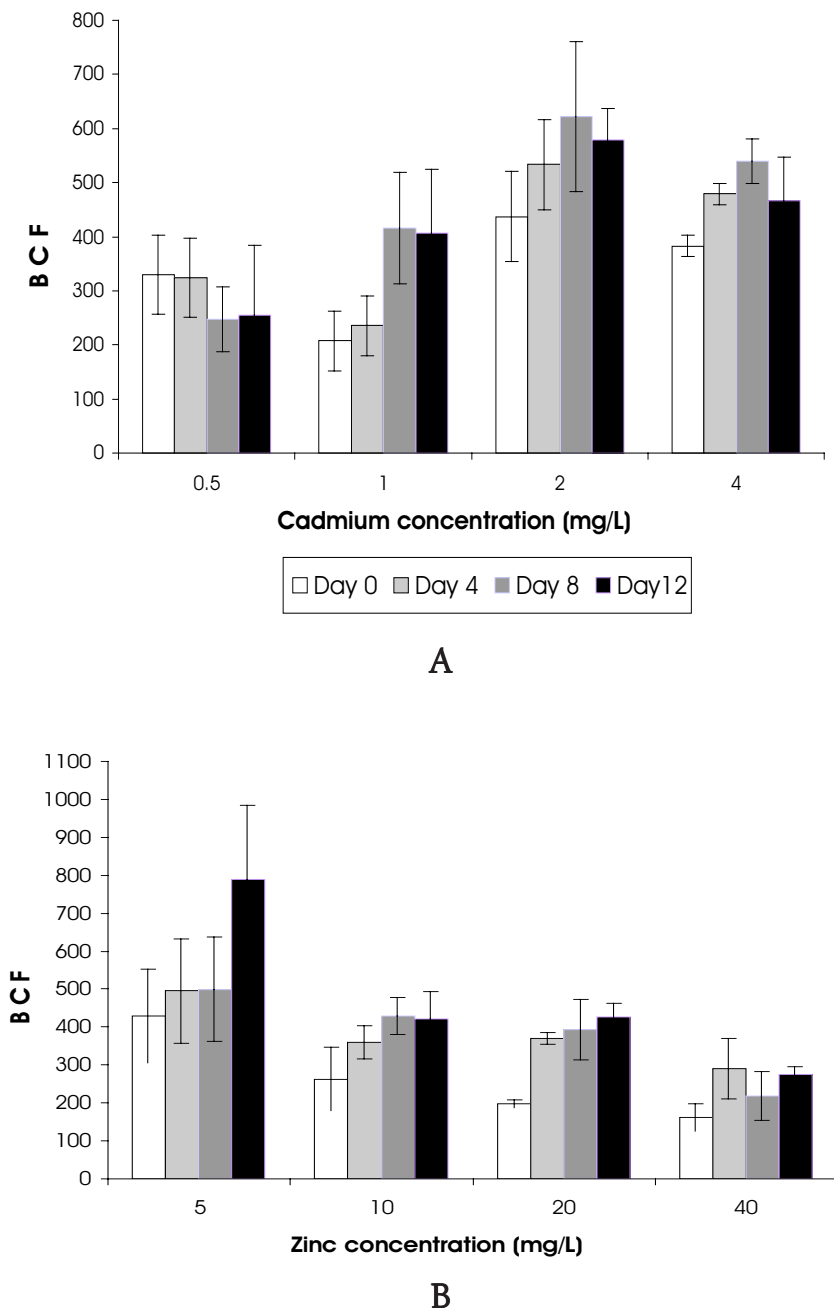
Fig 4. Cd (A) and Zn (B) remained in the residual solution after 12 days.

solutions at 5, 10, 20 and 40 mg/L were 0.82, 2.42, 5.06 and 6.29 mg/L, respectively on day 12 (Fig 4B). The comparison of maximum residual concentration of Cd and Zn is shown in Table 1.

**BCF**

The BCF values for Cd and Zn at different

concentrations and exposure times were shown in Fig 5. In general, the BCF values for Cd and Zn increased with the passage of time ( $P \leq 0.05$ ). The BCF values for Cd significantly increased ( $P \leq 0.05$ ) with Cd concentration in the feed solution at each exposure time and then decreased when the Cd concentration was over 2 mg/L (Fig 5A). The maximum BCF of 622.3



**Fig 5.**The bioconcentration factor (BCF) values of Cd (A) and Zn (B) in *E. crassipes* at different metal concentrations and exposure times.

was obtained in plants treated with 2 mg/L of Cd on day 8. The BCF values for Zn significantly decreased ( $P \leq 0.05$ ) with Zn concentrations in the feed solution at each exposure time and the maximum BCF of 788.9 was found in plants treated with 5 mg/L of Zn on day 12 (Fig 5B). The comparison of maximum BCF of water hyacinth exposed to Cd (2 mg/L) and Zn (5 mg/L) is shown in Table 1.

## DISCUSSION

Growth changes are often the first and most obvious reactions of plants under heavy metal stress.<sup>24</sup> In the present study, the relative growth increased in plants treated with low concentration of Cd (0.5 and 1 mg/L), but decreased with high concentration (2 and 4 mg/L). It appeared that low Cd concentration could stimulate plants' growth. Dou<sup>25</sup> found that although Cd is not generally considered an essential element, yet it may stimulate growth of some plants in small amounts. It is known that Cd is a non-essential heavy metal, and has inhibitory effects on plant growth.<sup>26</sup> Stratford *et al.*<sup>27</sup> reported that Cd was toxic and caused substantial reduction in water hyacinth growth mainly by suppressing development of new roots, and reducing relative growth rates to about 10% of those of controls. In plants treated with Zn, the relative growth increased in 5 and 10 mg/L treatments but decreased in 20 and 40 mg/L treatments. The addition of Zn at low concentration had a favorable effect on the growth of water hyacinth, which may be attributed to the fact that the plants utilize Zn as a micronutrient for their growth.<sup>28</sup> Delgado *et al.*<sup>29</sup> found that in long term experiment (24 days), water hyacinth exposed to 9 mg/L of Zn resulted in 30% reduction in weight. Schat *et al.*<sup>30</sup> reported that Zn toxicity was first expressed in reduced root growth. It has been proved by El-Ghamery *et al.*<sup>31</sup> who reported that the non-lethal concentration of  $Zn^{2+}$  showed an inhibitory effect on cell division in root tips of *Nigella*

*sativa* and *Triticum aestivum*.

In the present study, water hyacinth accumulated the highest concentration of metals in roots (2044 mg/kg for Cd and 9652.1 mg/kg for Zn). However, relatively little Cd (113.2 mg/kg) was translocated to the shoot, while Zn was translocated at a much higher concentration (1926.7 mg/kg). This result demonstrated that Zn was much more mobile than Cd. The accumulation of metals in the roots and shoots of water hyacinth has been shown in field studies in which water hyacinth was used as a biological monitor in metal pollution.<sup>32</sup> Greger<sup>33</sup> reported that the uptake of Cd, both by roots and shoots, increased with increasing metal concentration in the external medium but the uptake was not linear in correlation to the concentration increase. Stratford *et al.*<sup>27</sup> found that the metals' accumulations in water hyacinth increased linearly with the solution concentration in the order of leaves < stems < roots in water hyacinth. This study demonstrated a pattern of metal uptake similar to that of Stratford *et al.*<sup>27</sup> and we found that both Cd and Zn accumulated more in roots than in shoots. Qian *et al.*<sup>34</sup> treated 12 plant species (fuzzy water clover, iris-leaved rush, mare's tail, monkeyflower, parrot's feather, sedge, smart weed, smooth cordgrass, striped rush, umbrella plant, water lettuce and water zinnia) with 10 trace elements (As, B, Cd, Cr, Cu, Pb, Mn, Hg, Ni and Se) and reported that with the exception of B, all trace elements studied accumulated to substantially higher concentrations (from 5 to 60 folds) in roots than in shoots of all plant species. In general, most studies reported the higher concentration of metals in roots than in shoots. Cd concentrations were reported to be higher in the roots in most studies.<sup>35,36</sup> Normally Zn, Cd or Ni concentrations are 10 (or more) times higher in root than in shoot.<sup>37</sup> Soltan and Rashed<sup>38</sup> treated water hyacinth with several heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) and concluded that water hyacinth accumulated higher concentrations of heavy metals in the roots than in the aerial parts. However, there are a few studies that showed the higher accumulation of Cd in shoots than in roots.<sup>39,40</sup>

**Table 2.** Bioconcentration factors (BCF) for Cd and Zn in various plants.

Plant species	BCF		Reference
	Cd	Zn	
<i>Eichhornia crassipes</i>	622.3	788.9	Present study
<i>Lemna polyrrhiza</i>	650	44	28, 60
<i>Elodea nuttalli</i>	1,700	3,000	55
<i>Azolla pinnata</i> (root)	24,000	12,000	56
<i>Eriocaulon aquaticum</i> (root)	2.7	39	57
<i>Myriophyllum exalbescens</i>	-	1,640	59
<i>Bacopa monnieri</i>	400	-	61
<i>Ricciocarpus natans</i>	-	3,700-8,800	62
<i>Zostera marina</i> (aboveground)	0.62	78	58

Matagi *et al.*<sup>41</sup> have extensively reviewed on the heavy metal removal mechanisms in wetlands. Denny<sup>42,43</sup> noted that the main route of heavy metal uptake in wetland plants was through the roots in the case of emergent and surface-floating plants like water hyacinth. In locating the sites of mineral uptake in plants, Arisz<sup>44</sup> found that ions penetrated plants by passive process, mostly by exchange of cations which occurred in the cell wall. Denny<sup>42</sup> concluded that heavy metals were taken up by plants by absorption and translocation, and released by excretion. Sharpe and Denny<sup>45</sup> and Welsh<sup>46</sup> showed, however, that much of the metal uptake by plant tissue is by absorption to



anionic sites in the cell walls and the metals do not enter the living plant. This explains why wetland plants can have very high magnitude of heavy metal concentration in their tissues compared to their surrounding environment.<sup>47,48</sup>

Movement of metal-containing sap from the root to the shoot, termed translocation, is primarily controlled by two processes: root pressure and leaf transpiration.<sup>49</sup> Some metals are accumulated in roots, probably due to some physiological barriers against metal transport to the aerial parts, while others are easily transported in plants. In the present study, although Cd and Zn translocation to the plant aerial parts occurred and continued to go on during the whole experiment, it was slower than sorption by roots. Translocation of trace elements from roots to shoots could be a limiting factor for the bioconcentration of elements in shoots.<sup>50</sup> Additionally, Cd accumulation in 4 mg/L treated plants reached the highest level in roots and then decreased; while for Zn accumulation, 40 mg/L treated plants reached the highest level in shoots and roots, and also decreased. It can be proposed that the roots reached saturation during the period and there exists mechanism in roots that could detoxify heavy metals or transfer them to aerial parts.

Water hyacinth effectively removes appreciable quantity of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) from freshwater especially at low concentrations.<sup>38</sup> Maine *et al.*<sup>21</sup> reported that remaining Cd concentration in water was inversely related with time and depended on the initial Cd concentration. In the present study, Cd and Zn were efficiently depleted from the solution with the greatest decrease observed during the first four days. The sharp decrease in Cd and Zn concentration remaining in the residual solutions is indicative of the fast attainment of a saturation state. As soon as the saturation state was reached, it seemed a little difficult for plants to further absorb Cd or Zn. Still the concentration decreased with the passage of time.

Bioconcentration factor (BCF) is a useful parameter to evaluate the potential of the plants in accumulating metals and this value was calculated on a dry weight basis. Metal accumulations by macrophytes can be affected by metal concentrations in water and sediments.<sup>51</sup> The ambient metal concentration in water was the major factor influencing the metal uptake efficiency.<sup>52</sup> In general, when the metal concentration in water increases, the amount of metal accumulation in plants increases, whereas the BCF values decrease.<sup>53</sup> It was also reported that in most cases, BCF values decreased with increasing metal concentrations in the soil.<sup>54</sup> Jain *et al.*<sup>28</sup> found the BCF values for water velvet (*Azolla pinnata*) and duckweed (*Lemna minor*) treated with Pb and Zn gradually decreased with increasing

metal concentration in feed solution. Zhu *et al.*<sup>50</sup> found that the BCFs of water hyacinth were very high for Cd, Cu, Cr and Se at low external concentration, and they were decreasing as the external concentration increased. In the present study, for plants treated with Zn, the BCF values decreased when Zn concentration was increased. For plants treated with Cd, the BCF values first increased with the increase of Cd concentration, and then decreased when Cd concentration was over 2 mg/L. It can be concluded that the best BCF value was obtained when the external solution concentrations were 2 mg/L for Cd and 5 mg/L for Zn.

In the present study. The BCF values for Zn were a little higher than those of Cd at the same duration in most cases, indicating that the accumulation potential of Zn by water hyacinth was slightly higher than that of Cd. The maximum BCF values for Cd and Zn were 622.3 and 788.9, respectively, indicating that *E. crassipes* is a moderate accumulator of Cd and Zn based on the arbitrary criteria by Zayed *et al.*<sup>11</sup> and Zhu *et al.*<sup>50</sup> Metal accumulation potential and BCF values can vary among submerged species of macrophytes (Table 2). Some other aquatic plant species have been shown to exhibit higher accumulation of Cd or Zn and therefore are considered excellent Cd or Zn accumulators. Nakada *et al.*<sup>55</sup> found high BCF values for Cd (1,700) and Zn (3,000) in *Elodea nuttallii*. Sela *et al.*<sup>56</sup> reported the very high BCF values for Cd (24,000) and Zn (12,000) in the roots of water fern, *Azolla filiculoides*. In comparison, other aquatic plant species were proven to be poor accumulators of Cd or Zn, and very low Cd or Zn BCF values were observed. For example, Miller *et al.*<sup>57</sup> reported that the BCF values for Cd and Zn in soft-water pipewort were only 2.7 and 39, respectively; while Brix *et al.*<sup>58</sup> found that the BCF values for Cd and Zn in the aboveground parts of eelgrass (*Zostera marina*) were only 0.62 and 78, respectively. Franzin and McFarlane<sup>59</sup> reported the BCF value for Cd of only 6 in *Myriophyllum exalbescens* in contaminated sites; while Jain *et al.*<sup>28</sup> reported the BCF value for Zn in *Azolla pinnata* of only 44.

The appropriateness of a plant for phytoremediation potential is often judged by its BCF. BCF values over 1000 are generally considered evidence of a useful plant for phytoremediation.<sup>11</sup> However, in this study, with the BCF values of water hyacinth a little under 1000, this plant can be considered as a moderate accumulator for Cd and Zn. Further studies are needed to extend the growth season of the plant for utilizing in Cd and Zn treatment in effluents. Effluents containing these metals at low concentration may be treated by continuously passing them through a bed of water hyacinth growing in ponds. The harvestable parts, rich in accumulated metals, can be easily and safely

processed by drying, ashing or composting for recycling.<sup>40</sup> It represents a cost-effective plant-based technology for the removal of metals from the environment and has great potential for future applications.

In conclusion, water hyacinth may be used in "Eco-technology" (environmental technology) in constructed wetlands. Wetlands help to prevent the spread of heavy metal contamination from land to the aquatic environment. High metal removal rates of close to 100% have been reported both in natural and artificial constructed wetlands.<sup>41</sup> The advantage of constructed wetlands being easy and cheap to construct and operate suggests they are a suitable alternative for wastewater purification.

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