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RESEARCH ARTICLE

Uptake and distribution of metals by water lettuce (*Pistia stratiotes* L.)

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Abstract

Background, aim and scope Water quality impairment by heavy metal contamination is on the rise worldwide. Phytoremediation technology has been increasingly applied to remediate wastewater and stormwater polluted by heavy metals.

Materials and methods Laboratory analysis and field trials were conducted to evaluate the uptake of metals (Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) by an aquatic plant, water lettuce (*Pistia stratiotes* L.), and metal distribution in the plant.

Results The growth of water lettuce reduced Al, Fe, and Mn concentrations in water by >20%, K and Cu by >10%, and Ca, Mg, Zn, and Na to a lesser extent. A larger proportion of Ca, Cd, Co, Fe, Mg, Mn, and Zn was adsorbed or deposited on the external root surfaces while more Al, Cr, Cu, Ni, and Pb were absorbed and accumulated within the roots.

Discussion Water lettuce has a great ability in concentrating metals from its surrounding water with a concentration factor (CF) $\geq 10^2$. The bio-concentration factor (BCF), which excludes the part on the root surfaces, is a more appropriate index than the CF for the differentiation of hyperaccumulation, accumulation, or non-accumulation plants for metals.

Conclusions Water lettuce is a hyperaccumulator for Cr, Cu, Fe, Mn, Ni, Pb, and Zn and can be applied for the remediation of surface waters.

Recommendations and perspectives Further study on the bioavailability of metals in the water lettuce is needed for the beneficial use of metal-enriched plant biomass.

Keywords Dithionite–citrate–bicarbonate (DCB) extraction · Metal accumulation · Stormwater · Water lettuce (*Pistia stratiotes* L.)

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1 Background, aims and scope

Extensive studies have been conducted on metal accumulation by aquatic plants. The aquatic plants include floating plants, such as *Salvinia herzogii* (Maine et al. 2004), water hyacinth (*Eichhornia crassipes*; Mishra et al. 2008; Muramoto and Oki 1983), duckweed (including *Lemna polyrrhiza* L., *Lemna minor*, and *Spirodela polyrrhiza* W. Koch; John et al. 2008; Mishra and Tripathi 2008), mosquito fern (*Azolla pinnata* R. Brown; Mishra et al. 2008), and water lettuce (*Pistia stratiotes* L.; Maine et al. 2004; Mishra et al. 2008), emergent plants such as common cattail (*Typha latifolia*; Manab Das and Maiti 2008), and submerged plants such as pondweed (*Potamogeton pectinatus* or *Potamogeton crispus*; Badr and Fawzy 2008; Mishra et al. 2008), hydrilla (*Hydrilla verticillata*; Bunluesin et al. 2004; Mishra et al.

2008), and coontail (*Ceratophyllum demersum* L.; Badr and Fawzy 2008; Bunluesin et al. 2004). Interesting metals accumulated by these aquatic plants were mainly micronutrients or heavy metals, namely, Fe (Almeida et al. 2006; Manab Das and Maiti 2008), Mn (Mishra et al. 2008; Vardanyan and Ingole 2006), Cu (Almeida et al. 2006; Badr and Fawzy 2008), Ni (Manab Das and Maiti 2008; Vardanyan and Ingole 2006), Co (Vardanyan and Ingole 2006), Zn (Manab Das and Maiti 2008; Vardanyan and Ingole 2006), Cd (Badr and Fawzy 2008; Bunluesin et al. 2004; Mishra et al. 2008), Hg (Mishra et al. 2008; Molisani et al. 2006), Cr (Almeida et al. 2006; Mishra and Tripathi 2008), Ti (Vardanyan and Ingole 2006), Ba (Vardanyan and Ingole 2006), and Pb (Almeida et al. 2006; Badr and Fawzy 2008; John et al. 2008).

Of the studies on metal accumulation by aquatic plants, most were conducted with laboratory or greenhouse environments using metal-enriched nutrient solutions (Bunluesin et al. 2004; John et al. 2008; Maine et al. 2004; Mishra and Tripathi 2008). Results from these studies were usually very impressive with high metal uptake or accumulation (>90%, (Mishra and Tripathi 2008)). However, it may be entirely different under field conditions such as lakes, reservoirs, and estuaries where both metals and nutrients are of much lower concentrations and other environmental factors are far less favorable. On the other hand, the performance of aquatic plants in the natural water bodies is more meaningful as degradation of natural aquatic ecosystem is a worldwide concern and yet conventional physical or chemical treatments are not feasible for the remediation of nonpoint source pollution at a large scale.

Investigations were conducted in natural water bodies such as lakes (Badr and Fawzy 2008; Vardanyan and Ingole 2006), reservoirs (Mishra et al. 2008; Molisani et al. 2006), and estuaries (Almeida et al. 2006). But related information on man-made water bodies and stormwater detention ponds is minimal. Stormwater carries with it nutrients, heavy metals, and other chemicals from urban area and agricultural fields and may contribute to the degradations of aquatic ecosystems (Casey et al. 2005; He et al. 2006). Stormwater detention ponds are constructed to collect and remediate eutrophic stormwater before it is discharged into estuaries. Aquatic plants are useful in enhancing the performance of man-made and natural wetland systems. Nutrient (N and P) removal by water lettuce was reported in a previous paper (Lu et al. 2010). Knowledge on metal removal potential is necessary for improved use of these plants for water quality improvement.

Due to the greater availability of soluble ferrous iron species in the anoxic conditions (Ponnamperuma 1972) and leakage of O_2 from the roots of aquatic plants (Armstrong 1979), Fe tends to precipitate in the oxidized zone of root surface, forming Fe oxyhydroxides as coatings on roots, which is termed iron plaque and has been widely observed in aquatic plants and terrestrial plants when subjected to flooding (Crowder and St-Cyr 1991; Hansel et al. 2001; Otte et al. 1989; Ye et al. 1997). Once formed, the large surface area of the iron plaque (which is often in excess of 200 $m^2\ g^{-1}$) provides a reactive substrate to sequester metals such as Zn, Cu, and Ni (Otte et al. 1989; Taylor and Crowder 1983b). As the partitioning of metals on the root surface, and within the roots and shoots has an important implication for predicting their potential bioavailability and/or movement under changed physicochemical conditions, it is of our interest to differentiate metals located outside and within the plant. In addition, such knowledge is necessary when making plant disposal decisions. Among the methods used to extract the metals located on the external surfaces of the roots, the dithionite–citrate–bicarbonate (DCB) extraction has proved to be the best for removing all the root external precipitates (McLaughlin et al. 1985; Taylor and Crowder 1983a). This technique involves the use of sodium dithionite as a strong reducing agent, sodium citrate as a chelating agent to maintain the extracted metals in solution and sodium bicarbonate as a buffer. The DCB method was reported to be very efficient in removing the iron oxyhydroxide coating without damaging root tissues (Bienfait et al. 1984; Otte et al. 1989) or leaving considerable amount of Fe on the surface of the washed roots as the other methods do. This method has been applied to rooted aquatic plants such as submerged and emergent aquatic plants (Chen et al. 2006; Liu et al. 2010). No attempt has been made to apply this method to such free floating aquatic plant as water lettuce. Also, previous studies mainly focused on a few metals, namely Fe, Mn, Zn, and Pb, the characterization of iron plaque and the interaction between iron plaque and metals. Since the DCB solution can not only extract Fe oxides and its associated metals but also metals adsorbed on the root surfaces, it is possible to apply this method for quantifying metals attaching to the roots. In this study, the DCB method was applied to differentiate metals on root surfaces from those within the roots, so that we could obtain a clearer view on the mechanisms and potential of metal removal by aquatic plants.

Compared to heavy metals such as Cd, Cu, Zn, and Pb, non-heavy metals such as K, Ca, Na, Mg, and Al are usually overlooked. Although they are not as deteriorating as heavy metals, they also affect water quality and contribute to algal bloom. In addition, for recycling purpose, it is necessary to monitor the concentrations of these metals in the plant. Therefore, the objectives of this study were to investigate the removal potential of both heavy metals (Fe, Mn, Zn, Cu, Cr, Ni, Pb, Cd, Co) and non-heavy metals (K, Ca, Na, Mg, Al) by water lettuce in stormwater detention ponds and to understand the mechanisms of metal removal by this plant.

2 Materials and methods

2.1 Experimental design

Two stormwater detention ponds (called the West Pond and the East Pond), located to the west and east side, respectively, of the University of Florida, Indian River Research and Education Center (IRREC) Facility in Fort Pierce, were selected for this study. The East Pond had an area of approximately 2,500 m^2 and the West Pond, approximately 5,000 m^2. The West Pond received stormwater from IRREC teaching gardens. The land surrounding the East Pond was used for citrus production but had been left fallow since the occurrence of canker 5 years ago. Besides receiving stormwater from the fallow land, the East Pond also received stormwater from the ditch along the Kings Highway.

For each pond there were two plots, i.e., the control (without plants) and the remediation plot (with water lettuce), which were separated from each other and from the rest part of the pond by a soft wall made of weather-resistant plastic material, which allows only water and dissolved ions to pass through. The bottom of the soft wall was inserted into the sediment by an impregnated stainless iron chain and its top was floated on water surface by means of a wrapped foam bump. The height of the soft wall was equal to the maximum water depth (2 and 3 m in the East and West Pond, respectively) of the plot site when the pond was full of water. Therefore, the height of the soft wall could change according to the water level. Each plot had an area of 72 m^2 (12 m×6 m).

Each of the remediation plots was initially transplanted with young lettuce plants in July, 2005, which covered about 1/20 of the total water surface area. Sampling of water and plant from both plots began after the full establishment of the plants in the remediation plots in September 2005, which was approximately 2 months after the experimental set up.

Water samples were collected weekly from the control and the remediation plots and analyzed for total dissolved metal concentrations. General water quality was also monitored by measuring N and P concentrations, pH, electrical conductivity (EC), suspended solids, and turbidity.

Water lettuce was sampled monthly from the remediation plots. Plant samples were randomly collected from each plot following a standard procedure. A plastic circle frame of 30 cm in diameter was used for collecting the plant samples. The circle frame was randomly thrown six times within each remediation plot. Plants within the targeted circle were collected and made into two composited samples for each plot. The plant samples were placed in plastic bags filled with some pond water to keep fresh, and transported to the laboratory in an ice chest. In the laboratory, after being thoroughly rinsed with deionized water to remove adhering materials and blotted dry, the roots and shoots were separated and their fresh weights were recorded. Plant samples were oven-dried at 70°C for 7 days, weighed, and pulverized to <1 mm using a four Canister Ball Mill (Kleco Model 4200, Kinetic Laboratory Equipment Company, Visalia, CA) prior to analysis for total metal concentrations.

Besides monthly sampling, plants were also periodically harvested to maintain three-fourths plant coverage of each remediation plot surface to avoid over-crowding of the plants. For each harvest, the total fresh weight of lettuce plant was recorded, plant moisture was determined, and total quantity of dry plant biomass yield was calculated for each plot. Harvested plant materials were applied to the field as an organic amendment. Total amounts of each metal removed from the water by the harvested plant were quantified by multiplying the amounts of plant biomass by the metal concentrations in the plant.

To differentiate metals that were absorbed into the roots from those attached to the external surfaces of the roots, a DCB extraction technique was applied (McLaughlin et al. 1985; Taylor and Crowder 1983a). Briefly, 25 g of fresh roots were soaked in 450 mL of DCB solution (containing 400 mL 0.3 mol L^{-1} sodium citrate, $Na_3C_6H_5O_7{\cdot}2H_2O$, 50 mL 1.0 mol L^{-1} sodium bicarbonate, $NaHCO_3$, and 3 g sodium dithionite, $Na_2S_2O_4$) at 60°C for 20 min. Then, the roots were removed, rinsed several times with deionized water and blotted dry before they were oven-dried and analyzed for the amounts of metals absorbed by the roots. The DCB extract was filtered through a 0.45-μm membrane filter and analyzed for metal concentrations. The amount of metals dissolved in the DCB solution is considered as attached to the external surfaces of the roots by adsorption or surface deposition.

2.2 Chemical analysis

Water samples were filtered through a 0.45-μm membrane filter and preserved at $pH<2.0$ by adding concentrated HNO_3 prior to the analysis of total dissolved metal concentration using inductively coupled plasma optical emission spectrometry (ICP-OES, Ultima, JY Horiba Inc. Edison, N.J.) following EPA method 200.7 (U. S. Environmental Protection Agency 2001).

Pulverized plant samples (each 0.400 g) were digested with 5 mL of concentrated HNO_3 in digestion tube using a block digestion system (AIM 500-C, A.I. Scientific Inc., Australia) following a standard procedure provided by the manufacturer. The metal concentrations in the digester were determined using the ICP-OES.

The University of Florida Soil and Water Science Laboratory at the Indian River Research and Education

Center-Fort Pierce is NELAP (US National Environmental Laboratory Accreditation Program) certified for environmental tests (E76888). All the analyses were conducted following the standard operation procedures of the National Environmental Laboratory Accreditation Conference (NELAC 2003), with a recovery requirement of 90–110% for all the studied elements.

2.3 Data validation and analysis

When the concentration of a metal was below the metal's method detection limit (MDL), half of its MDL was used in graph or in calculation (U. S. Environmental Protection Agency 2006).

Differences in metal concentrations between the control and remediation plots were tested using the TTEST procedure in SAS software (SAS Institute 2001).

3 Results

3.1 General water quality improvement

The growth of water lettuce improved water quality in the remediation plots, which was evidenced by decreased total solids, turbidity, and N and P concentrations, as compared to the control plots. These results were reported and discussed in a previous paper (Lu et al. 2010).

3.2 Reduction of metal concentrations in water

Aluminum, Ca, Fe, K, Mg, and Na were the main metals detected in the water samples of both ponds (Table 1) with mean concentrations of 0.21, 44, 0.33, 7.8, 15, and 51 mg L^{-1}, respectively in the control plot of East Pond, and 0.25, 22, 0.15, 3.9, 2.8, and 16 mg L^{-1}, respectively in the control plot of West Pond. Copper, Mn, Ni, and Zn were of very low concentrations. The concentrations of As Cd, Co, Cr, and Pb were mostly below their MDLs, they were not shown in Table 1. The two ponds had similar concentrations in Al, Cu, Ni, and Zn, while the concentrations of Ca, Fe, K, Mg, Mn, and Na in the East Pond were much higher than those in the West Pond, which agreed with the EC measurement (Lu et al. 2010).

Aluminum, Ca, Fe, K, and Mn concentrations in the remediation plots of both ponds were significantly ($P<0.01$) reduced by the growth of water lettuce. Magnesium, Cu, Na and Ni concentrations were also significantly ($P<0.01$) reduced in the remediation plot of East Pond. Compared to the control plots, Fe, Mn, and Al concentrations in water were reduced by an average of more than 20% by the growth of water lettuce. Potassium and Cu were reduced by more than 10% in the remediation plots. Calcium, Mg, Zn, and Na concentrations in the water were also reduced in the remediation plots to certain extent.

3.3 Metal accumulation by the plant roots

Metal concentration factors (CFs; Fig. 1), which are calculated as the ratio of total metal concentration in the plant root (mg kg^{-1}) over that in the surrounding water (mg L^{-1}), indicates a plant's metal concentration capacity. All the investigated metals (Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn) had a CF higher than 10^2, with Al, Cd, Co, Cr, Fe, Mn, and Pb having a CF higher than 10^4. The CF values of the 14 metals changed in the following order for the East Pond: Cr>Mn>Co>Pb>Fe>Zn>Cd>Al>Ni>Cu>K>Ca>Mg>Na, whereas for the West Pond the order was: Cr>Fe>Mn>Co>Al>Pb>Cd>Ni>K>Zn>Cu>Mg>Ca>Na.

3.4 Metal distribution in the shoots and roots of water lettuce

Metal distribution between the shoots and roots of water lettuce can be characterized by the root/shoot (R/S) ratio, which is metal concentration in the roots over that in the shoots (Table 2). Most of the studied metals were not

Table 1 Metal concentration reduction by growing water lettuce in East and West Pond

Location	Plot	Ca mg L^{-1}	K	Mg	Na	Al	Cu	Fe	Mn	Ni	Zn
East Pond	Control	44	7.8	15	51	0.21	0.0073	0.33	0.019	0.0053	0.016
	Remediation	40	7.0	14	46	0.17	0.0057	0.23	0.013	0.0031	0.015
	Significance	**	**	**	**	**	**	**	**	**	NS
West Pond	Control	22	3.9	2.8	16	0.25	0.0064	0.15	0.0044	0.0043	0.011
	Remediation	21	3.3	2.7	16	0.13	0.0055	0.08	0.0023	0.0043	0.011
	Significance	**	**	NS	NS	**	NS	**	**	NS	NS

NS not significant

**$P<0.01$ denotes significant difference between the means of control and treatment; sample number $n=123$

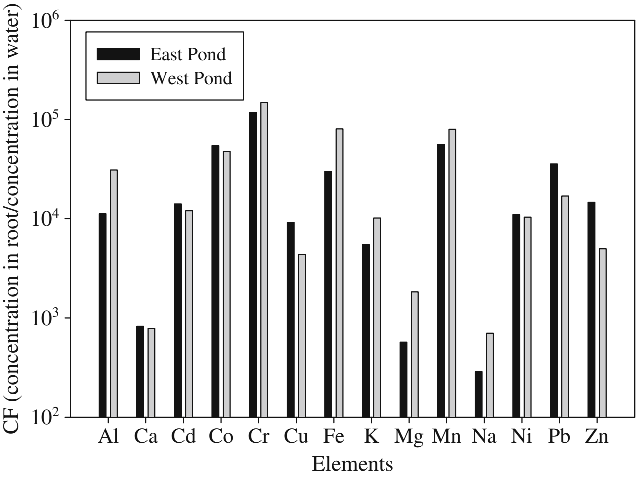

Fig. 1 Plant metal concentration factors (CF, metal concentration (mg kg^{-1}) in plant roots divided by metal concentration in the surrounding water (mg L^{-1})) in East and West Pond

effectively transported from the roots to the shoots, with a (R/S) ratio higher than one. Of the 14 metals, only Ca had an R/S ratio less than one, which means higher Ca concentration was found in the shoots than in the roots. Potassium, Mg, and Na had an R/S ratio close to one. For Cr, Cu, Fe, and Ni, more than 80% of their accumulation occurred in the roots, with an R/S ratio close or higher than 6. This was most prominent in the case of Fe with an R/S ratio>17. Much higher concentrations of the above four elements in the roots than in the shoots was also observed by Jayaweera et al. (2008) and other studies (Maine et al. 2004; Manab Das and Maiti 2008; Qian et al. 1999). Some physiological barriers are believed to play a role in preventing their transport from root to the aerial tissues (Zhu et al. 1999), which is one of the mechanisms of protecting the aerial part from being damaged by excessive metals (Fe, Cu, Ni, and Cr) where photosynthesis takes place. Although Fe, Cu, and Ni are essential for plant growth, when at excessive levels, they are toxic to plants. For heavy metals, such as Cd, Co, and Pb, which are not essential and toxic to plants, they were only detected in the roots.

3.5 Estimation of annual metal removal

Periodic harvesting of water lettuce is necessary not only for maintaining an optimal growth density, but also for effective removal of nutrients (N and P; Lu et al. 2010) and metals from the waters, otherwise the nutrients and metals would be released back into the water system after the plant died and decomposed. Harvesting was mainly conducted in the summer when both temperature and rainfall are high and the plant growth rate is the highest during a year. Water lettuce removed a considerable amount of macroelements

Table 2 Metal concentrations in water lettuce roots and shoots and metal root/shoot (R/S) ratios from East and West Pond

Location	Tissue	Ca g kg^{-1}	K	Mg	Na	Al mg kg^{-1}	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
East Pond	Root	31.5	36.7	7.21	13.4	2750	0.614	0.909	20.9	39.4	6126	760	7.97	6.94	184
	Shoot	33.9	29.0	6.92	11.3	796	0.263[b]	0.125[b]	2.20	4.17	354	196	0.78	0.93[b]	71
	R/S[a]	0.9	1.3	1.0	1.2	3.5	2.3	7.3	9.5	9.4	17.3	3.9	10.2	7.5	2.6
West Pond	Root	15.8	39.1	4.98	12.3	3400	0.611	0.395	17.6	30.0	5295	230	6.14	6.02	40.6
	Shoot	40.1	32.0	3.85	10.8	623	0.263[b]	0.125[b]	3.12	2.93	243	145	0.95	0.93[b]	38.3
	R/S	0.4	1.2	1.3	1.1	5.5	2.3	3.2	5.6	10.2	21.8	1.6	6.5	6.5	1.1

[a] *R/S* Root/shoot ratio in metal content

[b] When plant concentration of an element was below method detection limit (MDL), one-half of the MDL value was shown and used for the all calculations

such as Ca, K, and Mg, and a sizable amount of microelements such as Fe and Mn from the stormwaters (Table 3). High metal contents in the roots of water lettuce have been reported elsewhere (1,038 mg Cu kg^{-1} (Qian et al. 1999), 9.43 mg Co kg^{-1}, 27.07 mg Pb kg^{-1}, 107.3 mg Cr kg^{-1} (Vardanyan and Ingole 2006)). In comparison, water lettuce in these two ponds were far from reaching its potential in removing trace metals, especially for Cd, Co, Ni, and Pb because of their low concentrations in the waters. For both dry matter and most elements, West Pond's annual removal rate was twice that of East Pond. The higher rate in the West Pond was related to higher biomass yield due to more favorable conditions, as high total organic carbon and EC in East Pond might have negatively affected the growth of water lettuce (Lu et al. 2010).

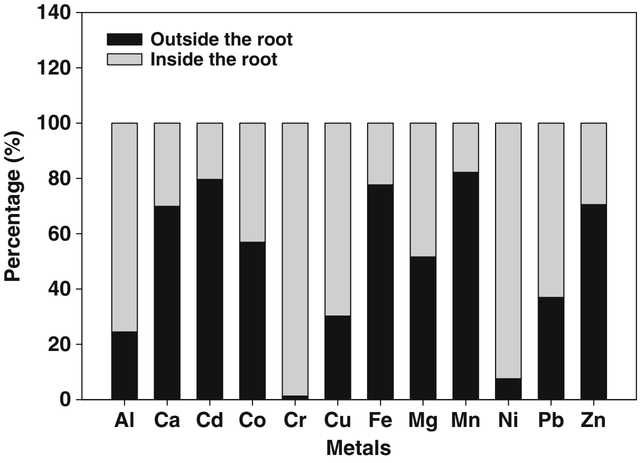

Fig. 2 Distribution of metals outside and inside of the water lettuce roots

3.6 Metal uptake and surface adsorption

According to their distribution between the outside and the inside the roots (Fig. 2), the 12 metals (as Na is a component of the DCB solution and the highly mobile nature of K in plant, these two elements were excluded) can be grouped into two categories: (1) a higher proportion was located on the external surfaces of the roots: Ca, Cd, Co, Fe, Mg, Mn, and Zn, and (2) a higher proportion was located inside the roots: Al, Cr, Cu, Ni, and Pb. Many studies have been conducted on elements such as Fe, Mn, Cd, Pb, Cu, and Zn (Hansel et al. 2001; Vesk et al. 1999). The distribution patterns of Fe, Mn, and Zn agree with those from St-Cyr and Campbell's research (St-Cyr and Campbell 1996). As a plant-essential nutrient, Ni was found mainly inside the roots (>90%). Although Cr is a non-essential element, more than 90% of it had entered the roots. This part of Cr could have been strongly bound by the cell wall to prevent possible damage to the plant (Maine et al. 2004). Magnesium was equally distributed outside and inside the root. About 80% of the Fe was located on the external surface of the roots as the main component of the iron plaque (St-Cyr and Campbell 1996).

3.7 Metal bio-concentrated by plant

As a portion of the metals taken up by plant from water was actually located on the external surfaces of the roots by adsorption or deposition instead of being absorbed into the plant, the CFs previously calculated based on the total amount of metal removed by plant may not accurately indicate the bio-accumulation capacity of a plant for certain metals. Therefore, it is necessary to make corrections. Another index, bio-concentration factor (BCF), the ratio of metal concentration within the plant roots (mg kg^{-1}) over that in the surrounding water (mg L^{-1}), which can more accurately reflect the plant's uptake potential, was calculated (Fig. 3). For metals such as Cd, Fe, and Mn with a large proportion being adsorbed on the external surfaces of the roots, their BCF values were much smaller than the respective CF values. For metals like Cr and Ni with a large proportion being absorbed into the roots, the difference between their BCF and CF value was small.

4 Discussion

Growing water lettuce in stormwater detention ponds not only improves water quality by decreasing the turbidity and nutrient concentrations such as N and P (Lu et al. 2010), but also by removing metals (Table 1). Better metal removal performance by aquatic plants was reported by several researchers with removal rates close or higher than 90% (Mishra and Tripathi 2008; Mungur et al. 1997). But these high removal rates were usually associated with laboratory or greenhouse experiments which provided more favorable environmental conditions for plant growth in

Table 3 Annual metal removal rates by periodic harvesting of water lettuce biomass

Location	Total dry matter kg ha^{-1}	Al	Ca	Fe	K	Mg	Mn	Na	Zn	Cd g ha^{-1}	Co	Cr	Cu	Ni	Pb
East Pond	10,455	16	357	29	344	70	5.3	138	1.3	4.0	4.9	92	107	31	51
West Pond	26,005	55	546	57	853	134	5.3	370	1.2	11	10	189	336	52	110

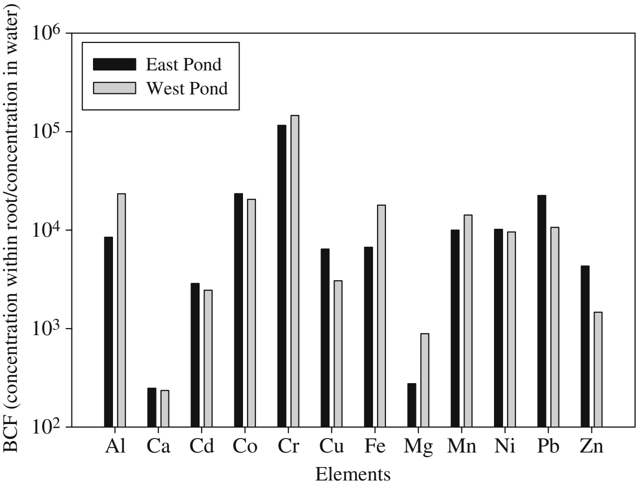

Fig. 3 Plant metal bio-concentration factors (BCFs), the ratio of metal concentration within plant root (mg kg^{-1}) over that in the surrounding water (mg L^{-1}), in the East and West Ponds

terms of light, temperature, and nutrients. In addition, high metal concentrations in the water were used in those studies (Ingole and Bhole 2003; Maine et al. 2001). High metal removal rates are also common when aquatic plants are used for the remediation of wastewater containing high concentrations of metals (Kao et al. 2001). In eutrophic stormwaters, contaminant concentrations are usually lower than those in wastewaters (point source of pollution), but may still pose a potential threat to the environment. Phytoremediation using aquatic plants with high metal removal capacity provides an energy-efficient (use of solar energy) and cost-effective approach for reducing nonpoint source pollution in surface waters at a large scale.

For efficient water treatment, it is necessary to remove metal- or nutrient-loaded plant biomass from water bodies to keep an optimum plant density (0.2–0.7 kg dry biomass m^{-2} was suggested by Reddy and DeBusk 1984). If not harvested, the majority of the elements would be returned to the water from the plants by the decomposition processes (Brix 1997). It has been shown that more intensive management with more frequent and timely harvest of plant biomass could lead to a higher removal rate (DeBusk and Reddy 1991). In Florida during the wet season when temperature is also favorable for water lettuce growth, plants should be harvested every other week to maintain about three-fourths coverage of the water surface (DeBusk and Reddy 1991).

Plants are one of the sinks for metals in the water column. As most metals (except Ca) are not effectively transported to shoots from the roots (Table 2), plant root is an important final destination for the metals. High concentrations of such metals as Cd, Co, Cr, and Pb in the roots can pose a hazard to the plant. Fortunately, only a portion of the total metal located in the root section moves into the root while a significant portion remains on the external surfaces of the roots, either complexed or adsorbed onto the root surfaces. This was confirmed qualitatively by Hansel et al. (2001) applying X-ray microprobe and X-ray fluorescence microtomography to freeze-dried root cross-sectional slices and quantitatively by the DCB extraction in this study (Fig. 2).

A plant is commonly defined as a hyperaccumulator for a metal if the CF of that metal is over 10^3 (Bunluesin et al. 2004). According to this definition, water lettuce can be considered a hyperaccumulator for such trace metals as Cr, Cu, Fe, Mn, Ni, Pb, and Zn. But for hyperaccumulation, we tend to emphasize the amount of metals accumulated within the plant by absorption. Therefore, the BCF, which excludes the portion of metals on the external surfaces of the roots, is a more appropriate index than CF for the differentiation of hyperaccumulation, accumulation, or non-accumulation plants for metals. Based on the BCF index of 10^3 as the criterion, water lettuce is a hyperaccumulator for Cr, Cu, Fe, Mn, Ni, Pb, and Zn. Many reported BCFs are actually CFs without excluding the portion of metals on the external surfaces of the roots (Bunluesin et al. 2004; Zayed et al. 1998). Although this may not change the conclusion regarding a hyperaccumulator for certain metals, as is the case in this study, it is important that a BCF is used for differentiating a hyperaccumulator from a regular plant based on plant physiology principle. In addition, this differentiation help understand the mechanisms of metal accumulation and detoxification by plants.

5 Conclusions

Growth of water lettuce in stormwater detention ponds reduced metal concentrations in the water. Water lettuce has great potential for removing metals from the surrounding water even when their concentration are extremely low, with the values of CFs ranging from 10^2 to 10^5. By periodic harvesting, considerable amounts of metals, including macro- and microelements, were removed from the stormwaters. All the 14 metals investigated except for Ca, had a metal R/S ratio >1, indicating that a higher proportion of the metals in the water lettuce plant remained in the roots rather than being transported to the shoots. The DCB extraction is useful for differentiating metals attached to the external surfaces from those absorbed inside the roots. More than 50% of the Ca, Cd, Co, Fe, Mg, Mn, and Zn recovered in the roots were actually attached to the external surfaces, while more than 50% of Al, Cr, Cu, Ni, and Pb was absorbed into the roots. Water lettuce is a hyperaccumulator for Cr, Cu, Fe, Mn, Ni, Pb, and Zn based on the BCF of 10^3 as a criterion.

6 Recommendations and perspectives

Metals taken up by water lettuce can be located within the plant or only attach on the external surfaces of the roots. When the harvested water lettuce is used as a soil amendment, these two parts of a metal may be very different in their release from the plant biomass, and subsequent availability or toxicity to plants. Therefore, further studies on their different behavior are necessary for proper recycling of metal-enriched plant biomass of water lettuce.

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