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Phytoremediation of Trace Elements by Wetland Plants

Technical Report

Phytoremediation of Trace Elements by Wetland Plants

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REPORT SUMMARY

Some plants naturally absorb and hyperaccumulate trace elements in their tissues. In a process known as phytoremediation, scientists are harnessing this ability to remove toxic heavy metals and trace elements from contaminated soils and waters. This screening program quantified the capacity of various wetland plant species for removing trace elements from polluted water.

Background

Wetland plants differ in the extent to which they can accumulate different elements. Judicious selection of appropriate wetland plant species can greatly enhance trace element removal. Selection is based on the type of element(s) to be remediated, the geographical location, environmental conditions, and the known accumulation capacities of the species. For this reason, it is important to understand the abilities of different wetland plant species to absorb and transport trace elements under varying conditions. This study established trace element uptake curves for a large number of wetland plant species in order to determine their efficiency in removing potentially toxic elements from contaminated water.

Objective

- To quantify the capacity of water hyacinth for removing different concentrations of As, Cd, Cr, Ni, Cu, and Se from contaminated water.
- To quantify the capacity of brass buttons for removing different concentrations of As, Cd, Cr, Ni, Cu, Pb, and Se from contaminated water.
- To identify the best aquatic plant species for the removal of Se from contaminated water via plant Se volatilization and accumulation in harvestable biomass.
- *To identify the best aquatic plant species for the removal of Mn, Cd, Cu, Ni, Cr, Pb, Hg, B, As, and Se from contaminated water.*

Approach

Investigators collected plant species from wetlands and nurseries, grew them hydroponically in a greenhouse, and supplied them with trace elements for a fixed period of time. They measured the amount of trace elements removed into plant tissue, the amount of Se volatilized, and plant biomass, which determines any effects on plant growth. In order to separate the effect of environment versus the effect of plant species on uptake, they conducted tests to determine trace element uptake curves under carefully controlled environmental conditions. Finally, they identified the plant species that removed the most trace elements from solution without showing significant toxicity.

Results

This study of phytoremediation of trace elements revealed that

- Water hyacinth, duckweed, brass buttons, cattail, saltmarsh bulrush, parrot's feather, iris-leaved rush, and smartweed were excellent candidates for the phytoremediation of wastewater contaminated with trace elements such as Mn, Cd, Cu, Ni, Cr, Pb, Hg, B, As, and Se.
- Water hyacinth, an aquatic floating plant that is easily harvested, most efficiently accumulated Cd and Cr; it accumulated Se and Cu at moderate levels
- Brass buttons, a wetland plant, proved an excellent choice for the remediation of Cr-contaminated water, accumulating substantial amounts of Cr in the harvestable shoots. This is a very interesting find because most plants retain Cr in the roots. Brass buttons also effectively removed Pb.
- Of the 20 aquatic plant species screened, cattail, saltmarsh bulrush, parrot's feather, and iris-leaved rush were identified as the best candidates for the removal of selenate and selenite from contaminated water. These plants removed Se most effectively by accumulating it in harvestable biomass (up to 1.5 g Se m^{-2} removed) and by volatilization (up to 3.5 mg Se m^{-2} d^{-1} removed).
- Of the 12 wetland plant species screened, smartweed proved most effective for the phytoremediation of Mn, Cd, Cu, Ni, Cr, Pb, Hg, B, As, and Se.
- Duckweed was shown to be a good accumulator of Cd, Se, Cu, and Cr.

EPRI Perspective

Plants grown in the field absorb only soluble forms of trace elements, even though many insoluble forms may exist in the substrate as well. Plant uptake curves determined hydroponically are directly relevant to field applications, because hydroponically grown plants are supplied with soluble forms of the trace element under study. The use of hydroponics makes it possible to precisely define the amount of soluble trace element each plant species is exposed to as well as the composition and pH of the nutrient medium. This approach enabled investigators to directly compare different plant species under fixed conditions. Since the plant species mentioned above were highly efficient in removing specific trace elements under controlled environmental conditions, they will most likely prove effective for trace element removal under field conditions.

Keywords

Contructed wetlands
Phytoremediation
Trace elements

ABSTRACT

Trace element removal by wetland vegetation can be greatly enhanced by the judicious selection of appropriate wetland plant species. Selection is based on the type of element(s) to be remediated, the geographical location, environmental conditions and the known accumulation capacities of the species. For this reason, it is important to understand the abilities of different wetland plant species to absorb and transport trace elements under varying conditions. The goal of the screening research program described in this report was to quantify the capacity of various wetland plant species for removing trace elements from contaminated water.

Plant species were collected from wetlands and nurseries, grown hydroponically in the greenhouse, and supplied with trace elements for a fixed period of time. Tests to determine trace element uptake curves were conducted under carefully controlled environmental conditions in order to separate the effect of environment versus the effect of plant species on uptake.

This study identified plant species that removed the most trace elements from solution without showing significant toxicity for application in constructed wetlands. Water hyacinth (*Eichhornia crassipes*), duckweed (*Lemna minor*), brass buttons (*Cotula coronopifolia*), cattail (*Typha latifolia*), saltmarsh bulrush (*Scirpus robustus*), parrot's feather (*Myriophyllum brasiliense* var. Camb), iris-leaved rush (*Juncus ziphiodes*), and smartweed (*Polygonum hydropiperoides*) were excellent candidates for the phytoremediation of wastewater contaminated with trace elements such as Mn, Cd, Cu, Ni, Cr, Pb, Hg, B, As, and Se.

Plants grown in the field absorb only soluble forms of trace elements, even though many insoluble forms may exist in the substrate as well. Plant uptake curves determined hydroponically are directly relevant to field applications, because hydroponically grown plants are supplied with soluble forms of the trace element under study. The use of hydroponics makes it possible to precisely define the amount of soluble trace element each plant species is exposed to as well as the composition and pH of the nutrient medium. This approach enabled investigators to directly compare different plant species under fixed conditions. Since the plant species mentioned above were highly efficient in removing specific trace elements under controlled environmental conditions, they will most likely prove effective for trace element removal under field conditions.

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1
INTRODUCTION

Some plants have a natural ability to absorb and hyperaccumulate trace elements in their tissues (Baker, 1981; Rosenfeld and Beath, 1964). This ability is being harnessed to remove toxic heavy metals and trace elements from contaminated soils and waters in a process referred to as phytoremediation. Several terrestrial plants that are highly effective in absorbing and accumulating various toxic trace elements have been identified in the last two decades and are being evaluated for the phytoremediation of soils polluted with trace elements (Baker et al., 1994). There is evidence that wetland plants can also hyperaccumulate trace elements in their tissues. The exceptional accumulation of several heavy metals and trace elements (i.e., Ag, Fe, Zn, Cu, Pb, Cd) by several species of wetland plants was demonstrated by Larsen and Schierup (1981), Jain et al. (1989), Pinto et al. (1987), and Dunbabin and Bowmer (1992).

Wetland plants differ in the extent to which they can accumulate different elements. While some plants were reported to be accumulators of specific metals (e.g., *Salvinia natans* for Hg and *Lemna plyrrhiza* for Zn; Sen and Mondal, 1987; Sharma and Gaur, 1995), others were found to be unspecific collectors of various metals. For example, Rai et al. (1995a) found that wetland plant species like *Ceratophyllum demersum, Spirodela polyrhiza, Bacopa monnieri* and *Hygrorrhiza aristata* were able to accumulate, unspecifically, appreciable amounts of various metals including Cu, Cr, Fe, Mn, Cd and Pb. In the same study, other wetland plant species such as *Vallisneria spiralis* and *Alternanthera sessilis* were found to accumulate these metals to a lesser extent (Rai et al., 1995a).

Trace element removal by wetland vegetation can be greatly enhanced by the judicious selection of appropriate wetland plant species. Selection is based on the type of element(s) to be remediated, the geographical location, environmental conditions and the known accumulation capacities of the species. For this reason, it was important to develop knowledge about the abilities of different wetland plant species to absorb and transport trace elements under different conditions. The goal of this part of the research program was to quantify the capacity of various wetland plant species at removing trace elements from contaminated wastewaters. The specific objective was to establish trace element uptake curves for different wetland plant species under controlled environmental conditions. Trace element uptake curves, i.e., *the quantitative relationship between the concentration of a specific trace element in leaf tissues with increase in the external (substrate) concentration of that trace element,* may then be used to identify hyper-accumulating wetland plants. Hyperaccumulators transport substantial amounts of trace elements from the substrate medium (where the trace element may be present in low concentrations) through roots to shoots so that shoots have higher concentrations of trace elements than the roots. Thus, by determining trace element uptake curves we can identify those wetland plant species which are most efficient at removing toxic trace elements.

Trace element uptake curves were carried out under carefully controlled environmental conditions in order to separate the effect of environment versus the effect of plant species on uptake. Thus the wetland plant species were grown hydroponically (nutrient solution) in greenhouses or growth chambers, thereby controlling both aerial and root environments. This enabled different plant species to be directly compared under fixed conditions. If a plant species proved to be a hyper-accumulator of a specific trace element under controlled environment conditions, it was also highly likely to hyper-accumulate the element under field conditions. Although this did not closely simulate what happens in a real wetland, it was an effective way of quantifying the capacity of any plant species at accumulating various trace elements under standard conditions. This procedure can then be used to test other plant species and effectively compare among them because all plant species are grown under constant environmental conditions.

To achieve this objective we carried out laboratory studies in combination with wetland field studies (see other parts of this report). In our laboratory study, we have completed a series of five investigations in which we determined the efficiency of large number of wetland plant species at removing various potentially toxic trace elements from polluted waters as described below.

2
WATER HYACINTH (*EICHHORNIA CRASSIPES*)

2.1 Introduction

Water hyacinth, a floating plant with a developed fibrous root system and large biomass, is one of the most prolific and productive plants in the world (Tchobanolous, 1987). These properties make it an ideal candidate for rhizofiltration (i.e., the removal of pollutants from contaminated waters by accumulation into plant biomass, Dushenkov et al., 1995) of toxic trace elements from wastewater. In fact, water hyacinth has been used successfully in wastewater treatment systems to improve water quality by reducing the levels of organic and inorganic nutrients (Brix, 1993; Delgado et al., 1995). Water hyacinth also readily reduces levels of heavy metals in acid-mine water while exhibiting few signs of toxicity (Falbo and Weaks, 1990; O'Keefe et al., 1996). Furthermore, the plant can serve as a biological indicator because 1) the plant has been shown to accumulate trace elements such as Ag, Pb, Cd, etc., (Pinto et al., 1987; Delgado et al., 1993; Fett et al., 1994; Zaranyika and Ndapwadza, 1995), and 2) the plant trace element concentration has been shown to correlate very well with water concentration (Ismail et al., 1996). We have recently shown that water hyacinth is extremely efficient at taking up Cr(VI) from solution, and efficiently reduces Cr(VI), a suspected carcinogen, to Cr(III), a less toxic form of the element (Lytle et al., 1998). These recent data further illustrate the potential of water hyacinth for phytoremediation. The objective of this study was to determine the suitability of this plant for the phytoextraction of six trace elements (As, Cd, Cr, Cu, Ni, and Se) commonly found in wastewater from oil refineries and electric power plants, and in agricultural drainage water.

2.2 Materials and Methods

Plant materials: Water hyacinth (*Eichhornia crassipes*) was collected from a wetland located in Richmond, CA. Plants were grown and propagated hydroponically in buckets containing quarter strength Hoagland's solution (Hoagland and Arnon, 1938; Terry 1980). The plants were maintained in a greenhouse with a 16-hour daylength, temperature of 25-28 °C, and humidity of 50%. Small plants were grown for about five weeks to allow good root development before each experiment.

To determine the ability of water hyacinth to take up trace elements, plants were grown in a standard Nutrient-Film Technique (Zayed, 1987) with quarter-strength Hoagland's nutrient solution to which one of the following trace elements was added: As ($Na_2HAsO_4.7H_2O$), Cd ($CdSO_4$), Cr ($K_2Cr_2O_7$), Ni ($NiSO_4.6H_2O$), Cu ($CuSO_4.5H_2O$), or Se (Na_2SeO_4). Each trace element was supplied to a set of plants over a range of concentrations, i.e. 0, 0.1, 0.5, 1, 2, 5, and 10 mg L^{-1}. Three plants representing three replicates were used for each trace element concentration. Nutrient solutions were replaced every two days. The nutrient-film setup was

maintained under the same greenhouse conditions as described earlier. Plants were harvested after 14 days and thoroughly washed under running water to remove any trace element adhering to the tissue. Total fresh weights of the plants were measured before and after the experiment to determine the effect of different concentrations of trace elements on growth. Shoot and root tissues were separated from each other and dried at 70°C for 3 days. The dried tissues were weighed and then ground in a Wiley mill.

Trace element analyses were carried out by acid digestion of dried and ground tissue samples as described by Zarcinas et al. (1987), followed by measurement of total concentrations of trace elements in the acid digest by Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) or Atomic Absorption-hydride generation Spectrometry (AAS). Copper, Cr, Ni, and Cd were measured using ICP-AES (Fassel, 1978), while Se and As were measured using AAS (Mikkelsen 1987; Keith, 1992). The detection limits for the analytical methods for Cu, Ni, Cd, Cr, Se, and As were 6, 15, 4, 7, 1, and 2 μg L^{-1}, respectively. Standards (NIST) and blanks were run with all samples to ensure good quality control. Plants which had not been supplied with trace elements were also analyzed for total trace element concentration as a negative control. The bioconcentration factor (BCF) was calculated by dividing the trace element concentration in plant tissues (mg kg^{-1}) at harvest by the initial concentration of the element in the external nutrient solution (mg L^{-1}). Total accumulation rate (μg $g^{-1}d^{-1}$) of trace elements was calculated as following (concentration in μg g^{-1} DW, biomass in g DW):

$$\frac{\text{(shoot concentration x shoot biomass) + (root concentration x root biomass)}}{\text{(shoot biomass + root biomass) x 14}}$$

2.3 Results

Effect of trace elements on plant growth: All six elements had toxic effects on water hyacinth plants. Water hyacinth growth was inhibited by all elements at high external concentrations (e.g., Figure 2-1). The typical effect on water hyacinth growth as influenced by the trace element supply is illustrated for Ni (Figure 2-1A), with similar patterns resulting for Cd, Cr, Cu and As. Growth rate showed a progressive decrease when external concentrations of these elements were higher than 0.1 mg L^{-1}. At very high concentrations, i.e., 5 and 10 mg L^{-1}, water hyacinth showed a net fresh weight loss. Selenium had a different effect on growth (Figure 2-1B). In fact, it stimulated water hyacinth development at low concentrations. Growth rate decreased progressively only when external concentrations of Se were higher than 1 mg L^{-1}. Furthermore, net fresh weight loss occurred at only 10 mg L^{-1} of Se, but not at 5 mg L^{-1}. Besides growth inhibition, Cd caused chlorosis and As caused leaf death when supplied to plants at 5 and 10 mg L^{-1}.

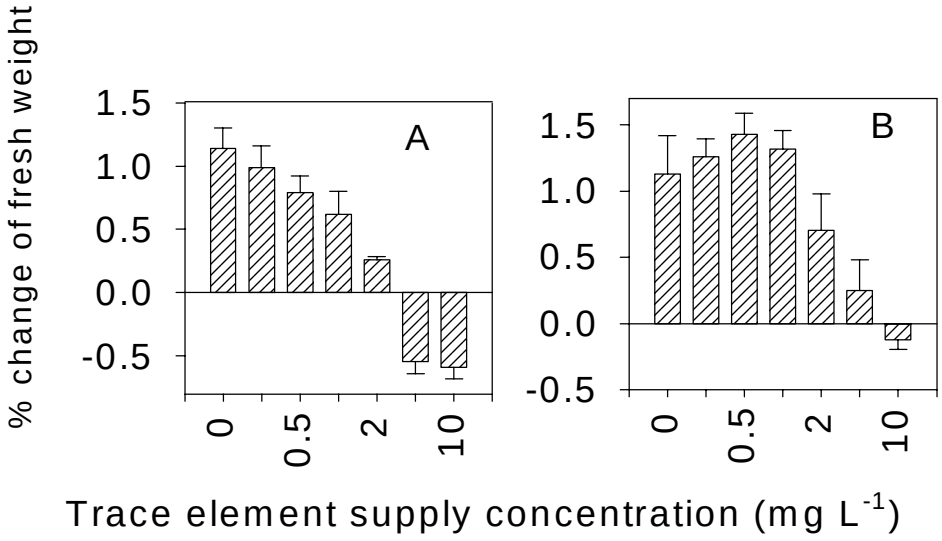

Figure 2-1
Percent change of fresh weight with Ni (A) and Se (B) treatment. Percent change of fresh weight is calculated as the difference in the fresh weight (g) of entire plants before and after trace element treatment divided by the fresh weight before treatment. Each value represents the mean and standard error of 3 replicates.

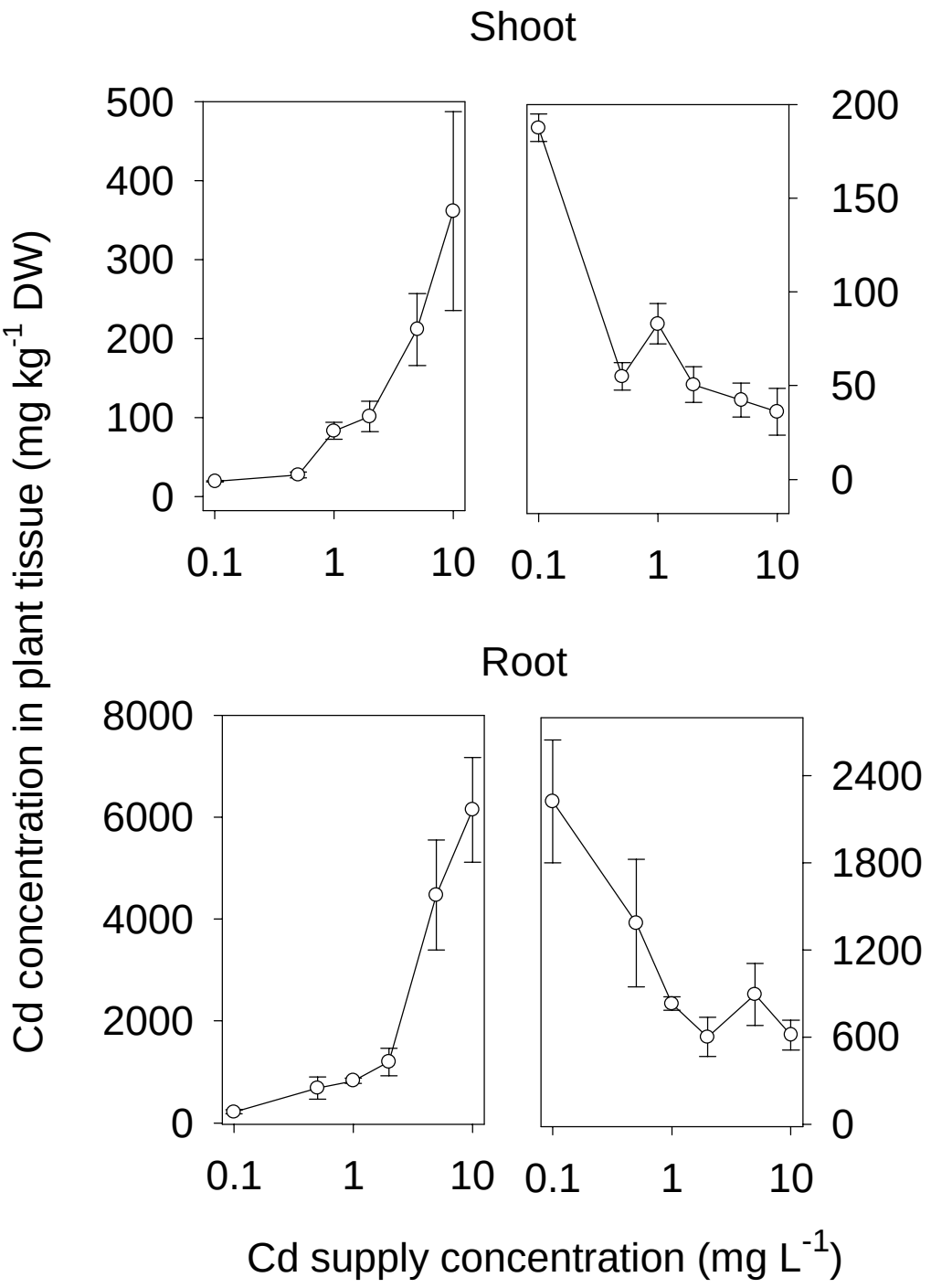

Figure 2-2
Cd concentration (left) and bioconcentration factor (right) in shoots (upper) and roots (lower). Each value represents the mean and standard error of 3 replicates.

Trace element concentration: Water hyacinth accumulated Cd to the greatest level compared to the other five elements tested. Cadmium concentration in plant tissue increased with external concentration. The highest levels of Cd found in roots and in shoots were 6,103 mg kg^{-1} DW and

371 mg kg^{-1} DW, respectively, when 10 mg L^{-1} of Cd was supplied. At all concentrations, Cd concentrations in roots were about 10 to 20 times higher than in shoots (Figure 2-2). The bioconcentration factor (BCF), which measures the ratio of trace element concentration in plant tissue to the concentration in the nutrient solution, was highest at low Cd concentration. The maximum BCFs for roots and shoots were 2,150 and 185, respectively (when 0.1 mg Cd L^{-1} was supplied). BCF decreased sharply as external Cd increased from 0.1 to 0.5 mg L^{-1}.

The accumulation pattern for Se differed from that of Cd. Selenium concentration in roots increased with the external Se concentration (Figure 2-3). However, unlike Cd, Se concentration in shoots increased more rapidly at low concentrations, resulting in an almost linear pattern (Figure 2-3). Furthermore, unlike all other elements under study, Se concentrations in roots were lower than in shoots at low external concentrations (0.1 to 2 mg L^{-1}). At high concentrations Se concentrations in roots exceeded those in shoots. The highest Se concentration in roots was only 319 mg kg^{-1} DW. On the other hand, Se was the second highest trace element accumulated in shoots. The highest concentration in shoots was 165 mg kg^{-1} DW (when the external Se was 10 mg L^{-1}). Also, unlike other elements, water hyacinth shoots showed a bell-shaped BCF curve for Se, i.e., the BCF for Se increased with the increase in Se levels at low external concentrations, then decreased at high concentrations. The highest shoot BCF for Se was 77 when external Se was 1 mg L^{-1}.

Concentration and BCF curves for Cr, Cu, Ni, and As were similar to those for Cd. However, concentrations of these elements in plant tissue were lower than those of Cd. The highest concentration in roots decreased in the order of Cr, Cu, Ni, and As (Figure 2-4), and the highest concentration in shoots decreased in the order of Cr, Ni, Cu, and As (Figure 2-5). These elements, like Cd, were accumulated primarily in roots rather than in shoots (Figure 2-4 and Figure 2-5). The highest concentrations of Cr were 3,951 mg kg^{-1} DW in roots and 119 mg kg^{-1} DW in shoots, and those of As were only 510 mg kg^{-1} DW and 44 mg kg^{-1} DW, respectively. Copper and Ni concentrations in shoots and roots were higher than As but lower than Cr (Figure 2-4 and Figure 2-5). Root BCF for Cr was very high (1,823 at 0.1 mg L^{-1} of external Cr), comparable to that of Cd (low As concentration in roots at 10 mg L^{-1} was probably due to the severe leaf damage and plant death caused by As toxicity).

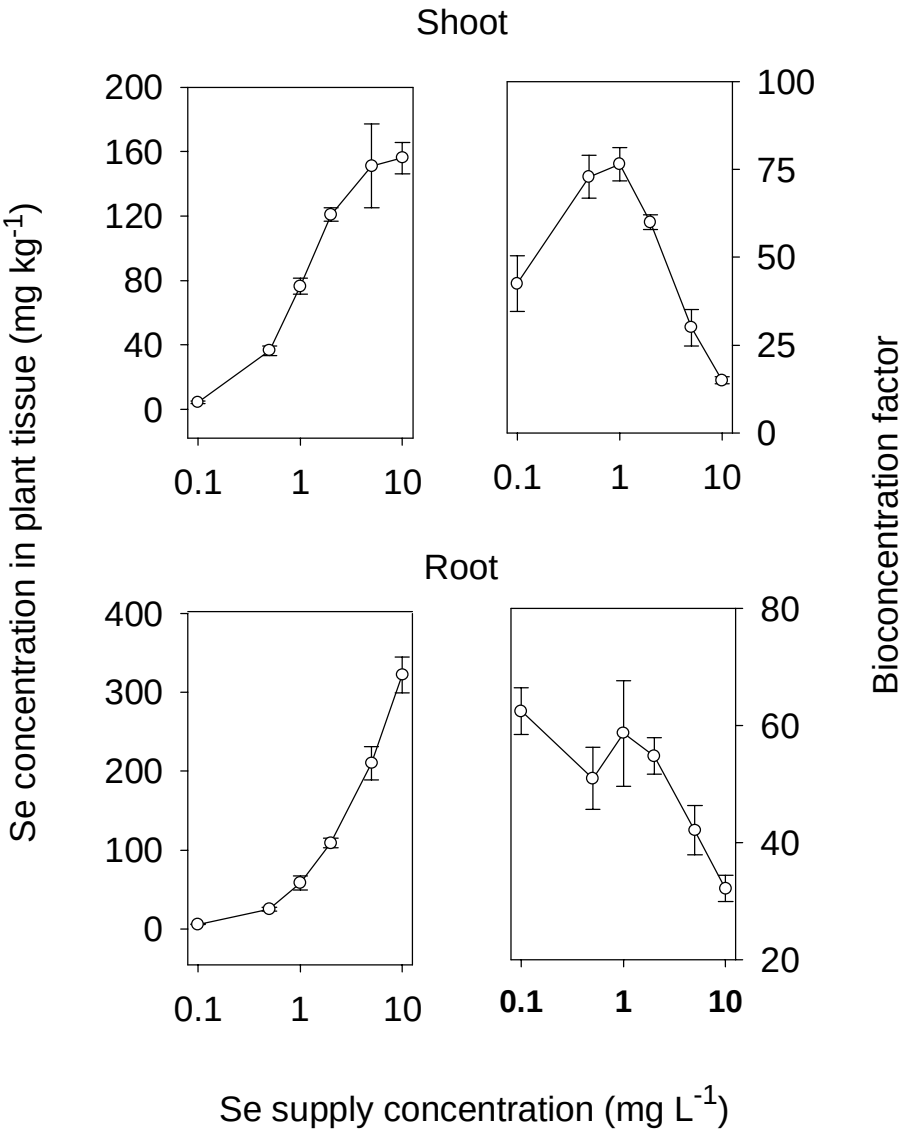

Figure 2-3
Se concentrations (left) and bioconcentration factors (right) in shoots (upper) and roots (lower). Each value represents the mean and standard error of 3 replicates.

Trace element accumulation: The rate of trace element accumulation, which indicates element concentration per unit dry biomass per day (whole plant basis), increased with external concentrations for all six elements (Table 2-1). The highest accumulation rate of 61.7 $\mu g\ g^{-1}\ d^{-1}$ was observed for Cd and it decreased in the order of Cr (26.5), Se (20.7), Cu (11.5), Ni (10.7) and As (4.6). The accumulation rate was highly dependent on the supply level of the trace element and increased substantially with each increase in external trace element concentration. Thus, the rate of Cd accumulation varied from 1.8 to 61.7 $\mu g\ g^{-1}\ d^{-1}$ with similar variations occurring for all other trace elements.

2.4 Discussion

Bioconcentration and bioconcentration factor: Tissue concentration has been used as a criteria for identifying hyperaccumulators (Reeves et al., 1996), but bioconcentration factors may sometimes be better indicators because the use of element

Table 2-1
Rate of total element accumulation of six trace elements by water hyacinth plants.

	Rate of element accumulation					
Element supply	Cd	Cu	Cr	Se	Ni	As
$mg\ L^{-1}$	---------- $\mu g\ g^{-1}$ DW d^{-1} ----------					
0.1	1.85 ± 0.46	1.15 ± 0.46	0.53 ± 0.23	0.58 ± 0.35	1.04 ± 0.12	0.35 ± 0.12
0.5	12.0 ± 4.83	4.26 ± 1.50	5.99 ± 1.96	2.88 ± 0.69	3.22 ± 0.65	0.46 ± 0.25
1.0	16.6 ± 5.07	11.7 ± 6.22	6.71 ± 2.33	6.89 ± 4.83	4.95 ± 1.15	0.69 ± 0.27
2.0	18.0 ± 4.26	11.1 ± 0.51	7.35 ± 1.50	11.3 ± 4.15	4.84 ± 1.54	3.22 ± 0.85
5.0	55.0 ± 5.99	22.8 ± 8.99	14.7 ± 2.88	12.2 ± 5.30	6.91 ± 3.69	7.49 ± 1.18
10.0	61.7 ± 13.4	26.5 ± 7.37	20.7 ± 4.84	11.5 ± 5.76	10.7 ± 5.07	4.61 ± 0.94

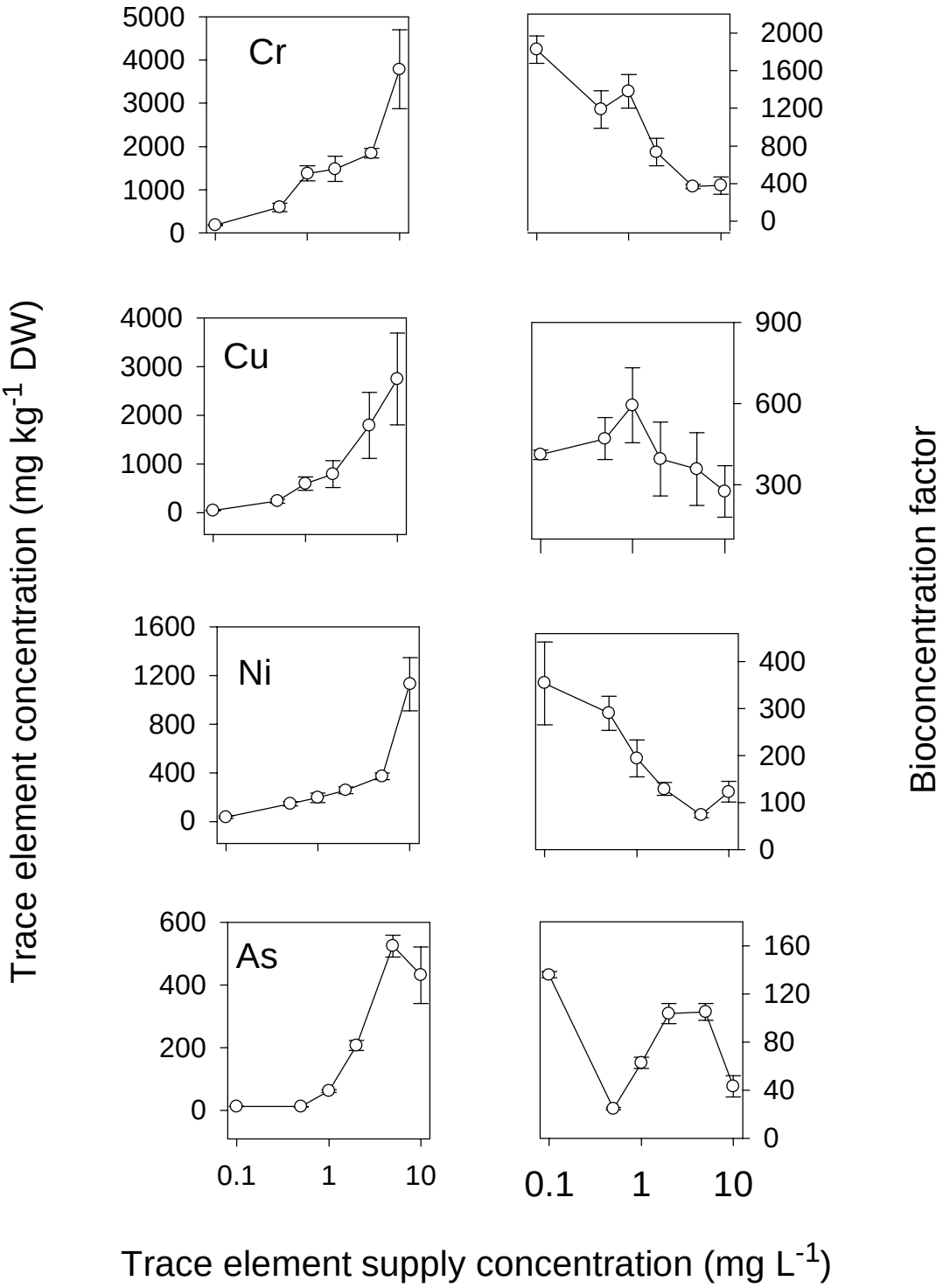

Figure 2-4
Root concentrations and bioconcentration factors of Cr, Cu, Ni, and As. Each value represents the mean and standard error of 3 replicates.

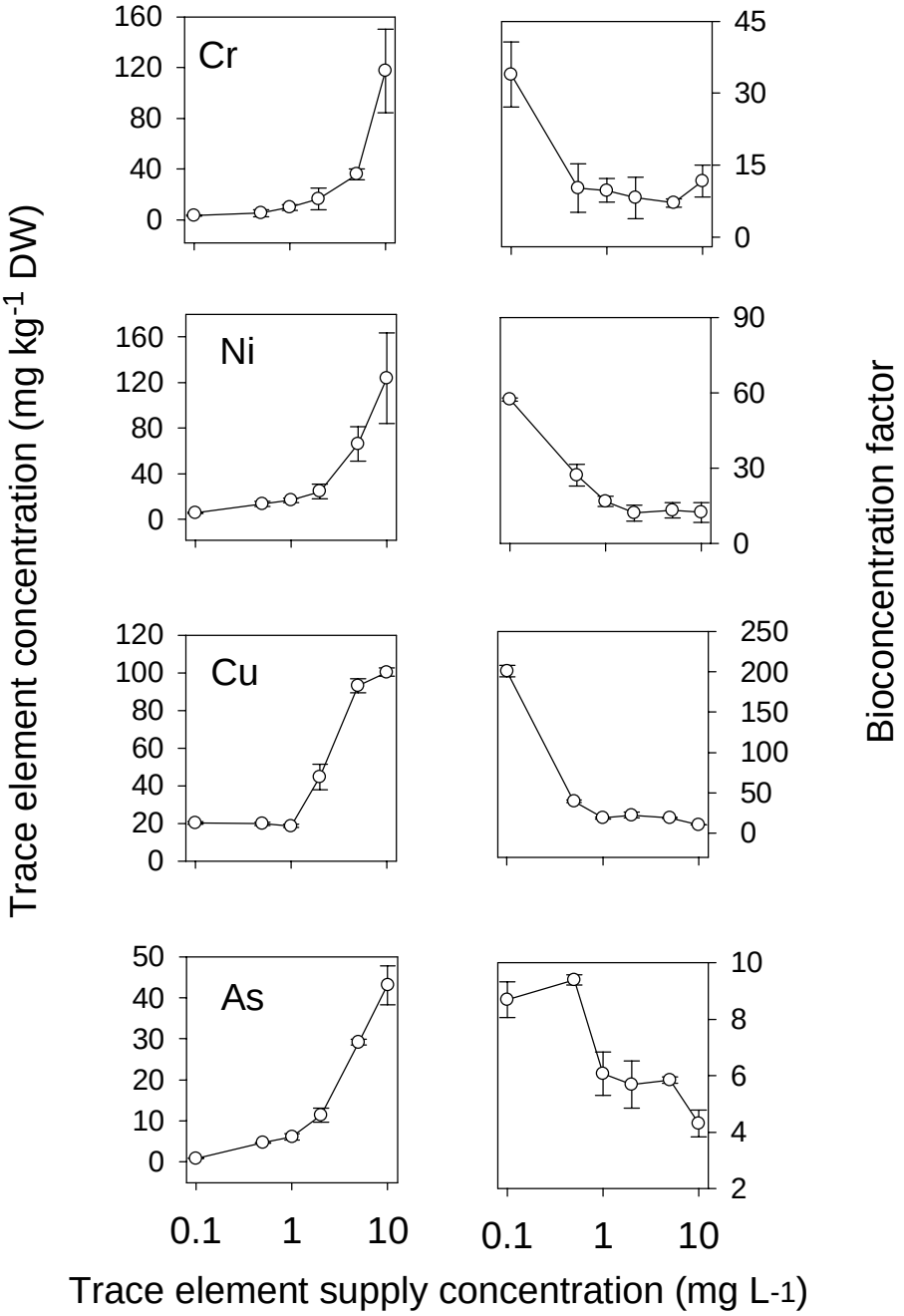

Figure 2-5
Shoot concentrations and bioconcentration factors of Cr, Cu, Ni, and As. Each value represents the mean and standard error of 3 replicates.

concentration does not take into account the trace element concentration in the substrate. The data show that BCFs were very high for Cd, Cu, Cr, and Se at low external concentrations, decreasing as the external concentration increased. The fact that water hyacinth had high BCFs for most elements at low external concentrations is also important for phytoremediation because, to its advantage, the process is more cost-efficient than other conventional techniques in treating large volumes of wastewater with low concentrations of pollutants. From the view of phytoremediation, a good accumulator should have: 1) the ability to take up more than 0.5% DW of a given element; and 2) the ability to bioconcentrate the elements in its tissue, e.g., a BCF of over 1000 (a 100-fold compared on a fresh weight, or in vivo basis) (Zayed et al., 1998).

Based on these criteria, our results show that water hyacinth is a good accumulator of Cd (Cd concentration = 0.63 %, BCF = 2,150, in roots). In fact, much higher concentrations, 10,600 and 36,000 mg kg^{-1} have been measured in water hyacinth plants treated with Cd, (Muramoto and Oki, 1983; Muramoto et al., 1989). Compared to *Lemna trisulca*, which has the highest Cd bioconcentration factor reported, i.e., 3,594-fold at a supply level of 0.64 mg L^{-1} (Huebert and Shay, 1993), water hyacinth showed a root BCF of ~1,400-fold when supplied with 0.5 mg L^{-1}. In our previous study (Zayed et al., 1998), we showed that Lemna minor also accumulates Cd best compared to Cr, Ni, Cu, Pb, and Se with the highest concentration of Cd being 13,300 mg kg^{-1} DW. In contrast, very low Cd concentrations and BCFs were observed for many other species (Zayed et al., 1998).

Water hyacinth is also a good accumulator of Cr, as the highest BCF for Cr was 1,823. Furthermore, the plant rapidly converts toxic Cr (VI) to relatively non-toxic Cr (III) (Lytle et al., 1996). Therefore, water hyacinth may be useful for the phytoremediation of waters containing low concentrations of Cr by removing the element into its tissues and storing it in a non-toxic form.

Selenium attained the second highest concentration in shoots (after Cd) although it was one of the elements least accumulated in roots. The highest concentration of Se in water hyacinth was 320 mg kg^{-1} DW, which was lower than that in *L. minor*. *Lemna minor* was shown to be a good accumulator of Se, with shoot concentrations as high as 4,270 mg Se kg^{-1} DW observed in a previous study (Zayed et al., 1998). There is limited information in the literature on the uptake and accumulation of Se by other aquatic plants commonly used for wastewater treatment. However, it has been shown that most terrestrial plants accumulate low amounts of Se in the order of 1 mg kg^{-1} DW or less (Lauchli, 1993).

For other elements, water hyacinth did not meet the criteria of a good accumulator. The highest concentration of Cu in water hyacinth was 2,655 mg kg^{-1} DW in roots and 106 mg kg^{-1} in shoots with a maximum BCF of 595. However, Low et al. (1994), reported that the dry root mass of water hyacinth accumulated 7,200 mg kg^{-1} DW of Cu when treated with 10 mg L^{-1} of Cu. Water hyacinth accumulated the lowest concentration of Ni in its roots among the four metals (i.e., Cd, Cr, Cu, and Ni) (Figure 2-4). Other studies have also showed that water hyacinth accumulates only little amounts of Ni (Turnquist et al., 1990; Zaranyika and Ndapwadza, 1995).

Differences in accumulation and distribution pattern: Water hyacinth bioaccumulated Cd, Cr, Cu, Ni, and As in roots to a much higher extent than in shoots (Compare Figure 2-4 and 2-5). This fact suggests that translocation of trace elements from roots to shoots could be a limiting

factor for the bioconcentration of these elements in shoots. Chromium, for example, had the lowest ratio of shoot to root concentrations in this study. Chromium is probably one of the most difficult elements for plants to translocate from root to shoot (Mishra et al., 1995, Kiekens et al., 1988, Jana, 1988).

The distribution pattern of Se was different from that of other elements tested. Selenium was more readily translocated to the shoot from the root at low external concentrations from 0.5 to 2.0 mg L^{-1} (Figure 2-3). It was the only element which had higher concentrations in shoots than in roots, with the ratio of shoot to root concentration reaching a maximum of about 1.6 at Se external concentration of 1 mg L^{-1}. It is interesting to note that efficient translocation of Se from roots to shoots may be only true when selenate, rather than selenite is supplied (Arvy, 1993).

Cadmium concentrations in water hyacinth shoots were 10 to 20 times lower than in roots (Figure 2-2). High Cd accumulation in plant roots is partially due to the binding of the cationic Cd to the anionic sites in the cell wall and inefficient transport to the shoot. In water hyacinth, Cd is thought to be accumulated in water hyacinth roots by adsorption to charged residues in the Donnan free space and by sequestration by phytochelatins (Jenatte et al., 1994). Copper, Cr, Ni, and As have accumulation and distribution patterns similar to Cd. Like Cd, the binding of Cu and Ni cations to the root cell wall can contribute to their higher concentrations in roots than in shoots. However, this binding property seems unlikely to be significant for Cr and As since both elements were supplied as anions. Furthermore, As is a metalloid like Se, but has a translocation pattern different from Se. Arsenic was also highly toxic to water hyacinth and caused leaf necrosis and plant death. In contrast, Se stimulated plant growth at Se levels as high as 1 mg L^{-1}.

Accumulation rate and environmental perspectives: Water hyacinth has a plant density of 14 ± 3.3 plants m^{-2} and a biomass of 1,494 ± 369.4 g m^{-2} (Sharma and Edem, 1988). Under our experimental conditions, water hyacinth accumulated as much as 61.7 µg Cd g^{-1} DW d^{-1} (whole plant basis, Table 2-1). Thus, water hyacinth has the potential to remove 922 g Cd per day per hectare of standing water hyacinth crop, assuming that plants are grown on wastewater containing 10 mg Cd L^{-1}. On average, a constructed wetland may receive 66.2 m^3 wastewater ha^{-1} d^{-1} (Knight et al., 1993). At a Cd concentration in the inlet water of 10 mg L^{-1}, a Cd loading rate of 662 g Cd ha^{-1} d^{-1} to a constructed wetland is expected. Thus, the potential Cd removal efficiency of water hyacinth substantially exceeds the expected Cd loading rate into a constructed wetland treatment system. However, under actual field conditions, several other factors may limit water hyacinth ability to remove Cd from water, e.g., presence of competing ions, temperature, humidity, competition with other plant species, etc. It should also be noted that 10 mg L^{-1} of Cd in the water could be toxic to water hyacinth over a long exposure period, which may limit its Cd removal potential. Nonetheless, water hyacinth can still bioaccumulate 27.6 g Cd (based on tissue concentrations found in this study, Table 2-1, and published biomass information) from one hectare of wetland surface area every day if the water contains 0.1 mg L^{-1} (the corresponding estimated Cd loading rate = 6.62 g Cd ha^{-1} d^{-1}).

With regard to Se, toxic effects on water hyacinth growth are less significant, as low concentrations of Se in the substrate do not inhibit plant growth (Figure 2-1). Furthermore, the Se concentration in agriculture drainage water in the San Joaquin Valley ranged from 0.3 to 1.4 mg L^{-1} (Presser and Barnes, 1984). This suggests that water hyacinth has the potential to phytoaccumulate approximately 43, 103, and 169 g Se per day per hectare of standing water

hyacinth crop at inlet wastewater Se concentration of 0.5, 1.0, and 2.0 mg L^{-1}, respectively (this is equivalent to an average Se loading rate of 33.1, 66.2, and 132.4 g ha^{-1} d^{-1}).

The phytoremediation efficiency of water hyacinth may be improved by periodically harvesting plants (Falbo and Weaks, 1990), which removes trace elements from the site and minimizes toxicity of phytoaccumulated trace elements to wildlife. The trace elements accumulated in plant tissue may then be recovered for commercial uses by ashing the plant biomass and recovering trace elements of interest (Raskin et al., 1994). For example, water hyacinth was used to remove a large amount of silver from industrial wastewater, and the silver in the ashed plants could be recovered very efficiently (Pinto et al. 1987). This study showed that water hyacinth accumulated high levels of Cd and Cr, and moderate levels of Cu, and Se. Therefore water hyacinth is a promising candidate for the phytoremediation of wastewater polluted with these elements.

3
BRASS BUTTONS (*COTULA CORONOPIFOLIA*)

3.1 Introduction

In the previous section we investigated the capacity of a floating aquatic plant to accumulate trace elements from the external growth medium. The current study focuses on a rooted emergent macrophyte common to fresh-brackish water, brass buttons (*Cotula coronopifolia*), which has the additional advantage of its roots being able to proliferate through the wetland sediments and phytoextract trace elements accumulated in the sediments. This species was selected due to its demonstrated ability to tolerate and grow in trace element contaminated wastewater at the San Francisco Bay constructed wetland in Richmond, CA (Hansen et al., 1998). Brass button was one of the three most common plant species in the Chevron constructed wetland, especially in Pass 1.

3.2 Materials and Methods

Mature brass button plants were collected from a constructed wetland at Chevron oil refinery facility in Richmond, California. The plants were cleaned thoroughly under gentle running water to remove adhering algae and insect larvae. Cuttings of 5-7 cm (cut from the nodes) were obtained and placed in 20-L containers containing half-strength Hoagland's solution at pH 5.0. All cuttings were maintained in growth chambers at 25 °C and at an irradiance of 400 μmol photon flux density (PFD) m^{-2} s^{-1} supplied over a 16-hour day length. The culture solutions were aerated and were replaced every three days. The cuttings were allowed to grow for one months. At the end of the one month period, the shoots from these mature plants were cut again to restart the process of multiplication of plants. At this time, a different treatment was developed in order to root the cuttings more rapidly. The 5-7 cm long cuttings were placed in half-Hoagland's solution supplemented with Indole Butyric Acid (IBA) at 0.2 mg L^{-1} to induce rooting. With this treatment the cuttings rooted well within a period of 3-5 days and rooting was uniform in all cuttings. All cuttings had 2-3 shoots and they were all uniform in height.

The experiments were carried out in plastic containers holding 1,500 ml quarter-strength Hoagland's solution containing (mM): 1.25 $Ca(NO_3)_2$, 0.5 KH_2PO_4, 1.5 KNO_3, 0.5 $MgSO_4$, and 0.25 NaCl, and (μM) 11.5 H_3BO_3, 2.3 $MnCl_2$, 0.19 $ZnSO_4$, 0.08 CuS_4, 0.026 H_2MoO_4, and 22.4 $FeSO_4$ (as ferric-sodium EDTA complex). The plants (one plant per container) were exposed to individual trace element at 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 mg L^{-1} concentrations, which were added to quarter-strength Hoagland's solutions. Plants without added trace elements served as controls. The six trace elements under study (cadmium, chromium, copper, lead, nickel, arsenic, and selenium) were supplied as cadmium sulfate, potassium chromate, copper sulfate, lead nitrate, nickel sulfate, sodium arsenate, and sodium selenite, respectively.

Three replicate plants were exposed to trace element treatments for 10 days. The plants were then collected from the solution, rinsed thoroughly with distilled water, blotted dry and weighed for fresh weight. Plants were then oven-dried at 70 °C for 48 hours before plant dry weight was determined. The dry plant materials were ground to powder and representative samples were taken for chemical analysis. Elemental analysis was carried out by acid digestion of dry samples (Zarcinas et al., 1987), followed by measurement of total concentrations of all elements of interest in the acid digest using a Plasma 40 ICP-AES (Fassel, 1978). Selenium and arsenic was measured using the Atomic Absorption-Hydride generation technique as described by Terry et al. (1992). Unless otherwise is indicated, all tissue trace element concentrations are reported on dry weight basis throughout the report. The bioconcentration factor (BCF) was calculated as described in Section 2.

3.3 Results

***Trace element uptake*:** Of all seven elements tested for brass button, Cr accumulated in above ground tissues to the highest level (Fig 3.1). Concentration of Cr in brass button shoot tissues as high as 1,055 µg Cr g^{-1} was attained at the 10 mg L^{-1} supply level. This was extremely higher than the concentration of any other element tested for brass button; the next highest concentration obtained at the same supply level being 293 µg g^{-1} for Cd (Figure 3-2). The highest concentration measured for Ni, Cu, and Pb in brass button shoot tissues amounted to 175, 166 and 60 µg g^{-1}, respectively, at the 10 mg L^{-1} supply level (Figures 3-2 and 3-3). Se and As attained the least concentrations in brass button shoot tissues; i.e., concentrations of Se and As in shoot tissues never exceeded 30 µg g^{-1} at all supply levels (Figure 3-4). The uptake curves for Cr, Ni and Cu in brass button shoot were of increasing slope, while those of Cd, As, Se and Pb had a decreasing slope (Figures 3-1, 3-2, 3-3 and 3-4).

Trace element accumulation in root tissues differed substantially from that of shoot tissues. All trace elements tested accumulated to much higher levels in roots than in shoots. In brass button root tissues, Pb was the most acquired trace element, attaining levels as high as 4,530 µg Pb g^{-1} dry weight (Figure 3-4). Lead concentrations in roots increased progressively with each increase in Pb supply concentration up to 5 mg L^{-1}, after which no significant changes in root Pb concentrations were observed. Copper was the second most acquired element by brass button roots in this study, with a maximum concentration of 3,135 µg g^{-1} obtained at the highest Cu supply level (Figure 3-2). Cadmium and Cr accumulated in root tissues to approximately the same level of 1,500 to 1,800 µg g^{-1}, while Ni concentration in roots did not exceed 1115 µg g^{-1} (Figures 3-1 and 3-2). Arsenic and Se were the least absorbed trace elements by brass button roots in this study, attaining maximum concentration of 265 and 400 µg g^{-1}, repectively, at the 10 mg L^{-1} supply level (Figure 3-4).

Examination of the uptake curves of all seven elements studied for brass button indicates that both shoot and root tissues were saturated by As at 2 mg L^{-1} and by Pb at 5 mg L^{-1} (Figures 3-3 and 3-4). This is implied by the fact that any increase in trace element supply above those levels resulted in no or insignificant increase in tissue concentrations of the element.

Bioconcentration factors: Trace element BCF in brass button shoot tissues seemed to be very low (Figure 3-5). Root BCF was substantially higher than that of shoot for all trace elements and at all supply levels tested (Figure 3-5). With the exception of Pb and Ni, all tested elements

exhibited higher BCF at the low supply levels than at the higher levels. Root BCF of Ni and Pb increased markedly with each increase in external concentration up to 5 mg L^{-1} supply level after which Ni BCF did not change significantly while Pb BCF dropped considerably. The greatest root BCF of 1,850 was attained for Cr at 0.2 mg L^{-1} supply level; the next greatest root BCF being 906 observed for Pb at 5 mg L^{-1} supply level. The maximum root BCF for other tested trace elements was as follows: 441 at 0.1 mg L^{-1} for Cu, 304 at 0.5 mg L^{-1} for Se, 287 at 0.1 mg L^{-1} for Cd, 174 at 0.2 mg L^{-1} for As and 111 at 10 mg L^{-1} for Ni.

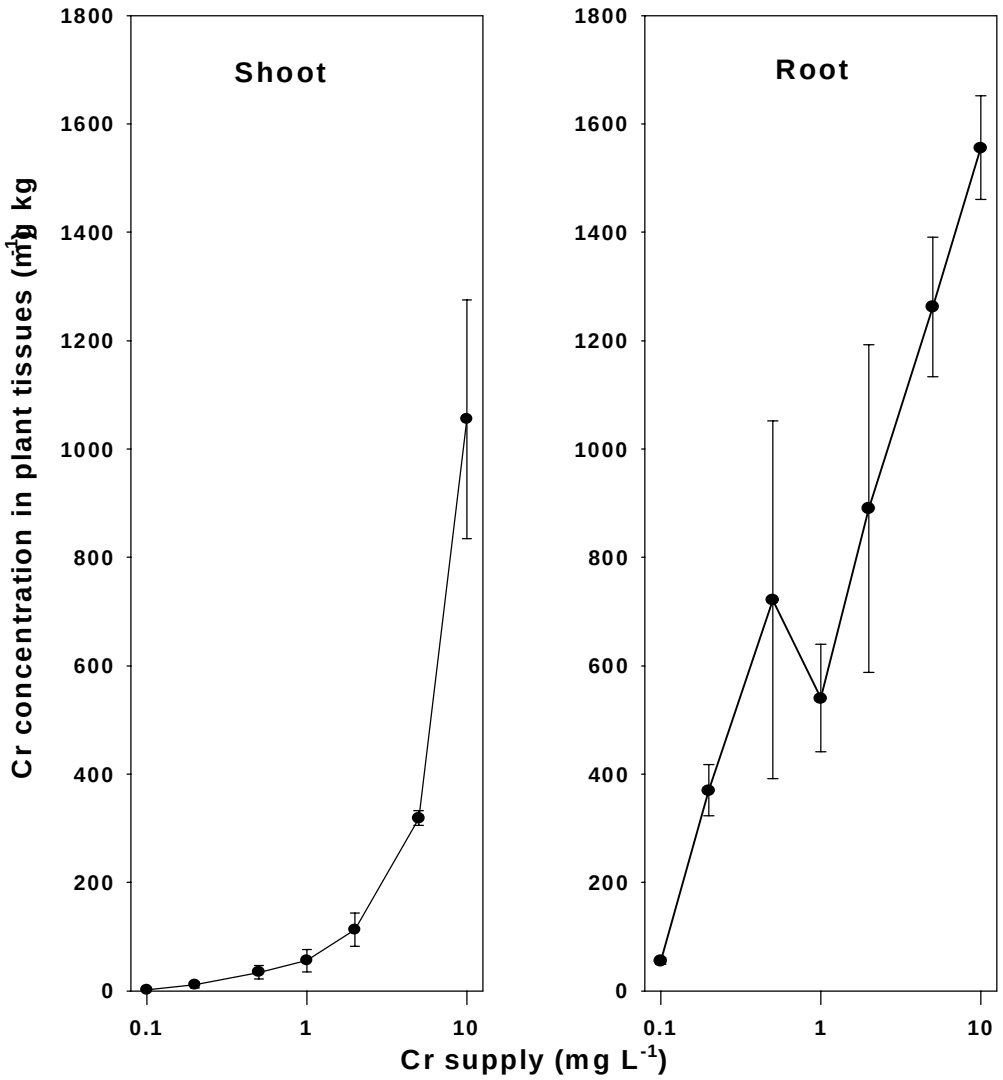

Figure 3-1
Influence of Cr concentration in the nutrient solution on Cr concentration in brass button shoot (left) and root (right) tissues. Vertical bars indicate standard deviations.

3.4 Discussion

All trace elements were accumulated in brass button roots to substantially higher levels than in shoots. Lead is a particularly interesting example of this since brass button accumulates up to 4000 µg g^{-1} in the roots and only 60 µg g^{-1} in the shoots. In shoots, Cr accumulated to the greatest level, while Pb was the most acquired trace element by roots. Chromium translocation from roots to shoots increased considerably with each increase in external Cr concentration. Arsenic was the least accumulated trace element by brass button shoots and roots in this study.

Brass button differs from duckweed and water hyacinth in that it has an established root system typical of a higher plant. It has the advantage that it can remove trace elements from the sediments as well as the water column. Brass button shows much promise in being an appropriate species to remove substantial amounts of Cr to the shoot, which could then be harvested. It is surprising that brass button probably accumulates more Cr in the shoot than it does in the root.

Chromium is an element that is difficult to remove from soil or water. Even after Cr is absorbed by roots from soil or water it is poorly translocated elsewhere and largely retained in the roots of most plants. Some elements are easily absorbed and translocated to above-ground plant tissues (e.g., B. Cd. Mn, Mo, Se, Zn), while others are less mobile due to their strong binding to soil components or root cell walls (e.g., Al, Ag, Cr, Fe, Hg, Pb) (Chaney, 1983). In the case of Cr, more than 95% of the Cr absorbed by nearly all plants is retained in the roots (Zayed et al., 1998). Even when soluble chromate is supplied, it is reduced to Cr(III) in plant roots and kept there as chelates, precipitates, or adsorbed Cr (Zayed et al., 1998; Lytle et al., 1998). Generally, Cr is not a required nutrient for any physiological or biochemical process in plants. However, some upland plant species (especially those growing on serpentine soils) can accumulate in their shoots relatively large amounts of the element. These are termed "chromium accumulators." Leaf contents in certain accumulator plants, were reported to be as high as 48,000 mg kg^{-1} (Lyon et al., 1968; Peterson, 1975). Unfortunately, there is little information regarding Cr accumulation in wetland plants. Another study (described in Section 5) showed little accumulation of Cr in shoots of most wetland plant species. This study is the first to report a wetland plant species that has the capacity to absorb large amounts of Cr from water and translocate more than 60% of the absorbed Cr to above ground tissues. Brass button is, therefore, an excellent wetland plant species that can be used for the phytoremediation of Cr-contaminated wastewaters.

Brass button is also suitable for the phytostabilization of other potentially toxic trace elements. It immobilizes a substantial amount of Pb and Cu in the root system, which would help in the wetland removal of these elements from wastewater.

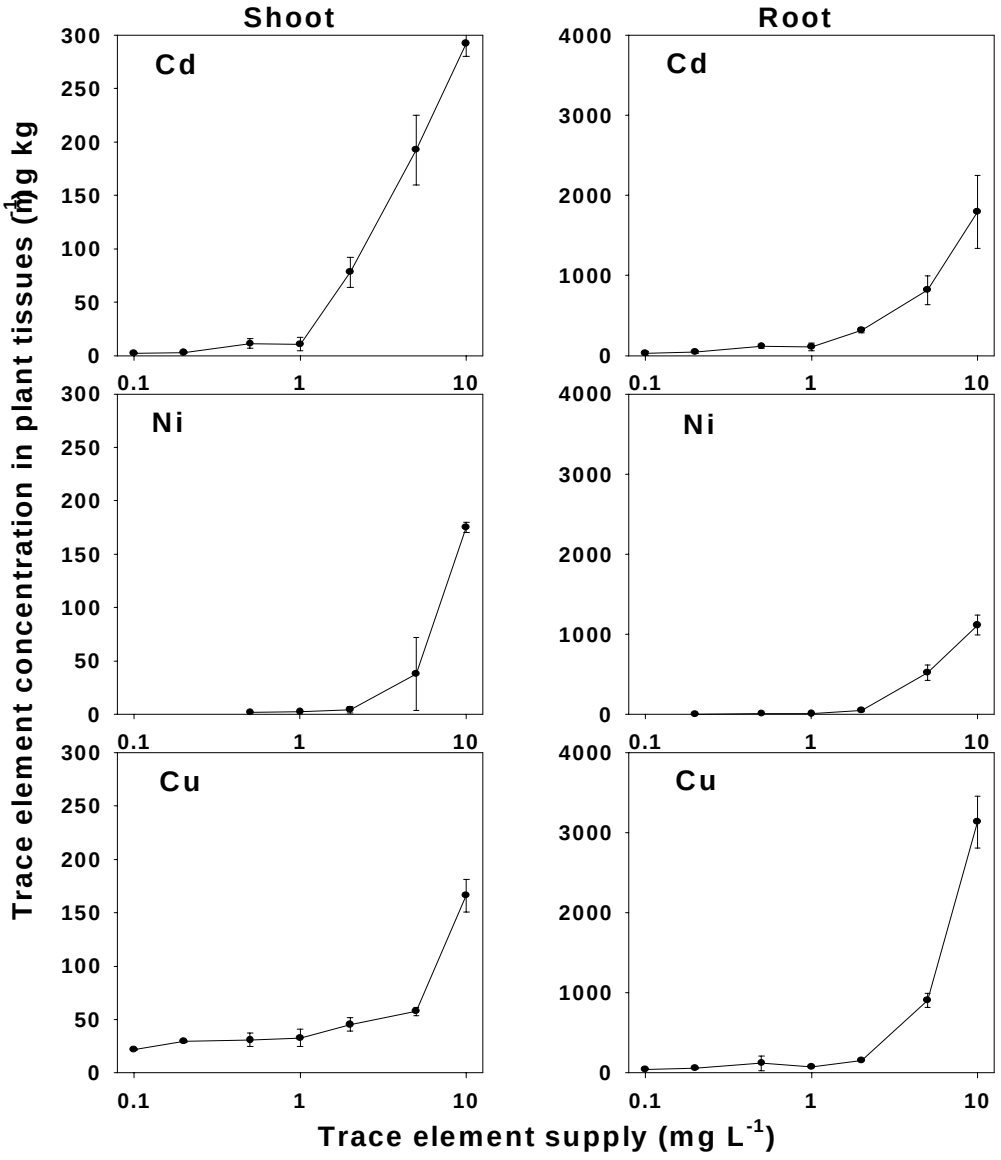

Figure 3-2
Influence of trace element supply concentration of Cd (top), Ni (middle) and Cu (bottom) (each trace element supplied individually in the nutrient solution) on the trace element concentration in brass button shoot (left) and root (right) tissues. Vertical bars indicate standard deviations.

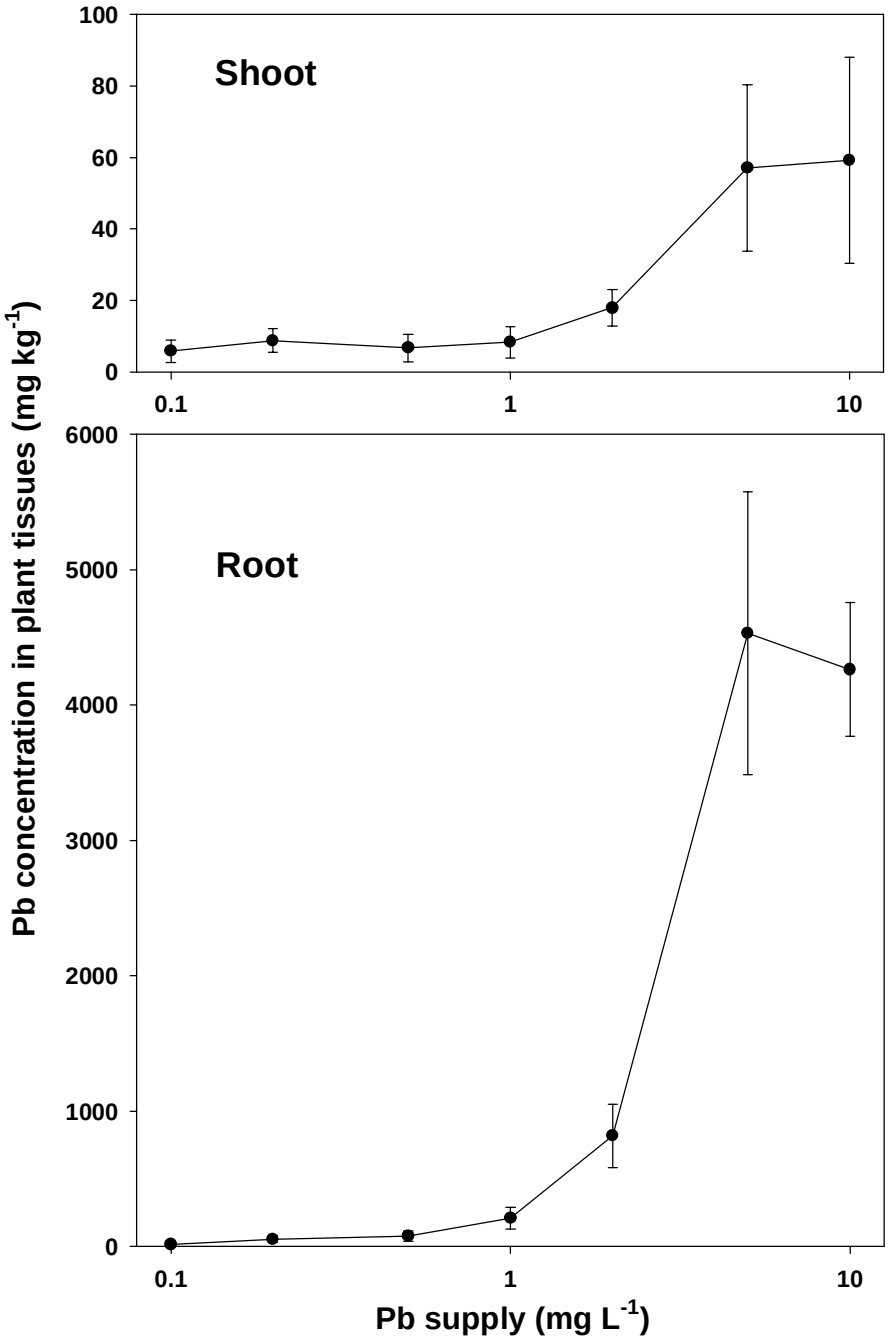

Figure 3-3
Influence of Pb concentration in the nutrient solution on Pb concentration in brass button shoot (top) and root (bottom) tissues. Vertical bars indicate standard deviations.

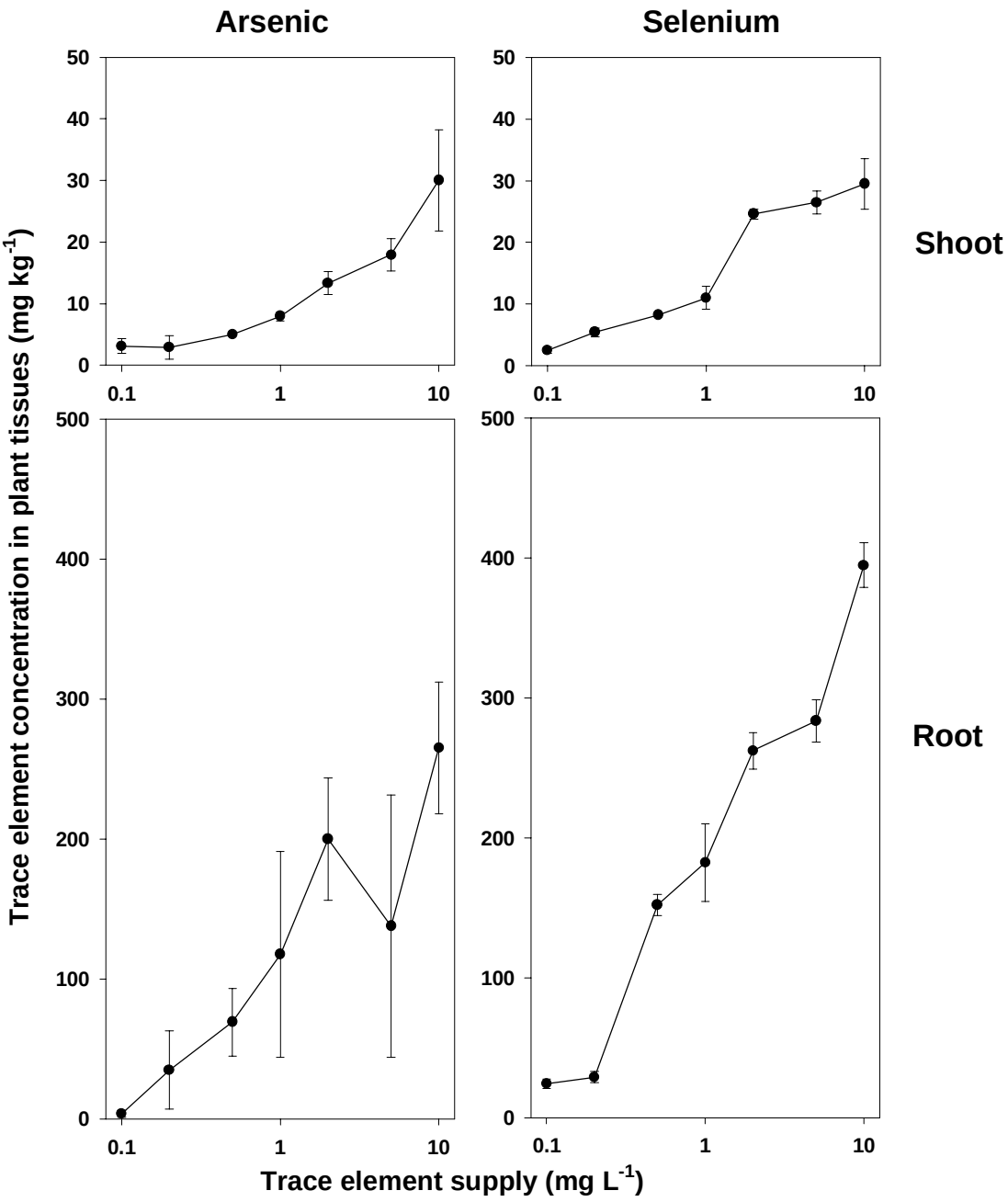

Figure 3-4
Influence of trace element supply concentration of As (left) and Se (right) (each trace element supplied individually in the nutrient solution) on the trace element concentration in brass button shoot (top) and root (bottom) tissues. Vertical bars indicate standard deviations.

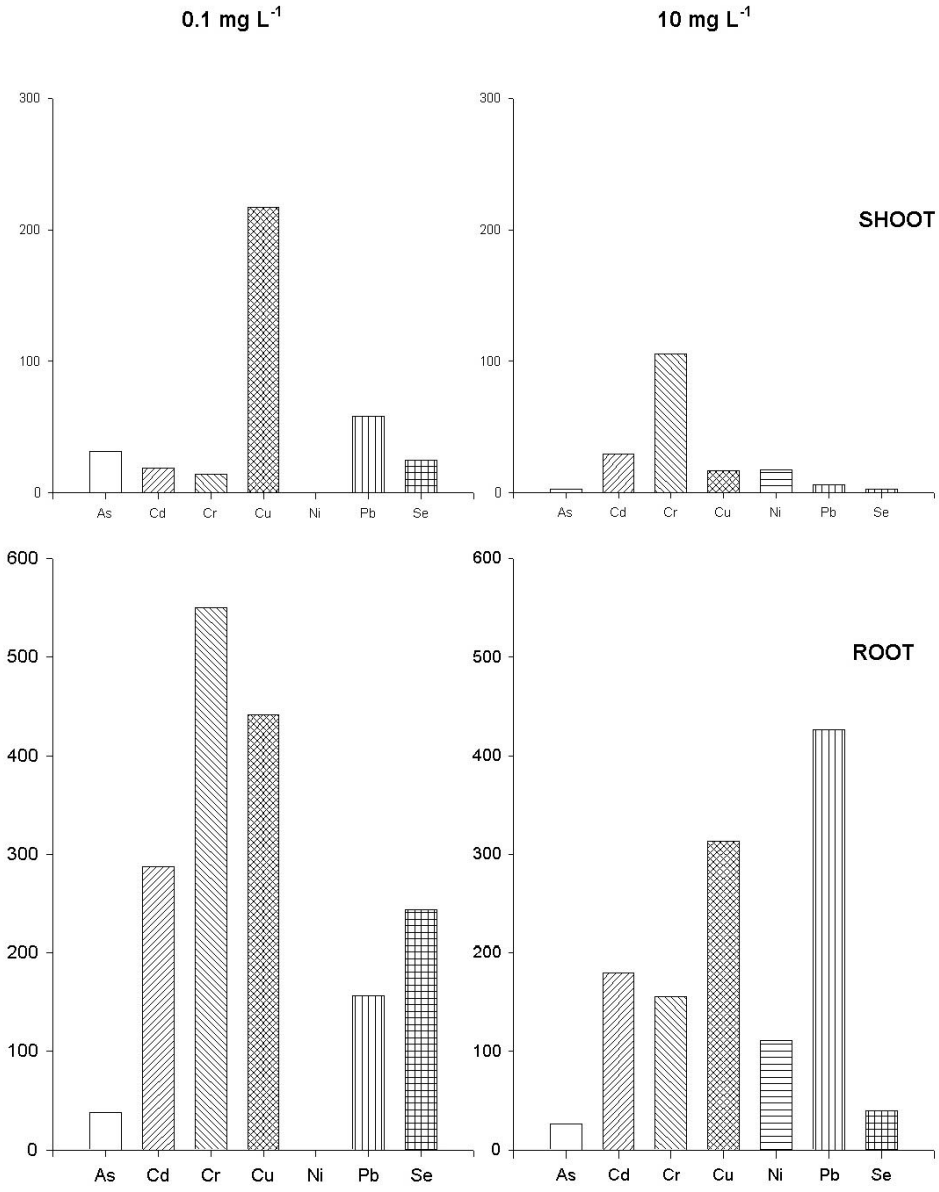

Figure 3-5
Trace element bioconcentration factors in brass buttons shoot (top) and root (bottom) tissues at the lowest (0.1 mg L^{-1}, left) and highest (10 mg L^{-1}, right) trace element supply concentration.

4
SELENIUM

4.1 Introduction

Selenium (Se) remediation is of primary importance because it is a serious worldwide pollutant in agricultural and industrial wastewaters (Terry and Zayed, 1998). One notable example was the environmental disaster at the Kesterson reservoir in the San Joaquin Valley, California, where bioaccumulation of Se from contaminated agricultural drainage water by birds and fish resulted in deformity and death (Ohlendorf et al., 1986; Saiki and Lowe, 1987; Skorupa, 1998). The removal of Se from wastewater flowing through a constructed wetland occurs by uptake and accumulation in plants (phytoextraction), by reduction to insoluble forms that are retained in the sediments, and by biological volatilization of inorganic Se to the atmosphere as a relatively non-toxic gas, dimethylselenide. Many investigators have measured Se volatilization from soils, water, and sediments containing inorganic Se (Chau et al, 1976; Zieve and Peterson, 1984; Cooke and Bruland, 1987; Frankenberger and Karlson, 1994; Hansen et al., 1998). These studies showed that both plants and microbes can volatilize Se *in situ*, and that the rate of volatilization is dependent on Se concentration, Se speciation, temperature, organic carbon and other environmental factors. Se volatilization detoxifies inorganic forms of Se and removes it from the contaminated site where it may enter the food chain (Wilber, 1980; Ganther et al., 1966, McConnell and Portman, 1952; Atkinson et al., 1990).

Selenium accumulation and volatilization by plants has been shown to account for a significant portion of Se removed from contaminated wastewater, soils and sediments (Cooke and Bruland, 1987; Allen, 1991; Velinsky and Cutter, 1991; Zhang and Moore, 1997). Many plant species have been shown to volatilize and accumulate Se (Lewis et al., 1966; Asher et al., 1967; Zieve and Peterson, 1984; Duckart et al., 1992; Terry et al., 1992). Previous comparative studies with terrestrial plants and crop species revealed that there was substantial variation among species in the ability to phytoextract and volatilize Se (Duckart et al., 1992; Terry et al., 1992).

There is almost no information in the literature regarding which aquatic plant species accumulates and/or volatilizes Se at the fastest rates. The lack of knowledge about aquatic plant species, with regard to their ability to volatilize Se, led to the present laboratory study. The objective of the research reported here is to gain more information about which species are *potentially* the most efficient at accumulating and volatilizing Se by comparing twenty different aquatic plant species under uniform, controlled environmental conditions.

4.2 Materials and Methods

Aquatic plants were collected from the wild or purchased from nurseries. The following plant species were used: azolla (*Azolla caroliniana* Willd.), baltic rush (*Juncus balticus* L.),

cattail(*Typha latifolia* L.), fuzzy water clover *(Marsilea drummondii* Add. Brown), hardstem bulrush (*Scirpus acutus* Muhl.), iris-leaved rush (*Juncus xiphioides*), Louisiana iris (*Iris louisiana* hybr.), mare's tail (*Hippuris vulgaris* L.), Mexican sprangletop (*Leptochloa uninervia* Hichc. & Chase), monkeyflower (*Mimulus guttatus* Fisch. ex DC.), parrot's feather (*Myriophyllum brasiliense* Camb.), saltgrass (*Distichlis spicata* L. Greene), saltmarsh bulrush (*Scirpus robustus*), smartweed (*Polygonum hydropiperoides* Michx.), spikerush (*Eleocharis obtusa* Willd. Schult.), umbrella plant (*Cyperus alternifolius* L.), water forget-me-not (*Myosotis scorpioides* L.), water hyacinth (*Eichhornia crassipes* Mart. Solms.), water primrose (*Ludwigia repens* Forst.), and water zinnia (*Wedelia trilobata* Hitchc.).

The emergent plants were grown in 8-inch pots with soil [UC Mix, which consisted of fertilized peat moss and Colma sand (1:0.75)] in a greenhouse with a short daylength (8 h) and controlled temperature (25°C). Floating aquatic plants were maintained in plastic tubs containing half-strength Hoagland's solution (Hoagland and Arnon, 1938). All plants were watered with 0.5 Hoagland's solution once a week and with water once a week. The plants were maintained under these conditions for a year. Indian mustard plants (*Brassica juncea* , accession no. 173874), obtained from the North Central Regional Plant Introduction Station, Ames, IA, were grown from seeds to the onset of flowering on coarse sand and then treated the same way as the wetland plants.

For the experiments described here, 1 to 3 plants per replicate were removed from soil, gently washed in tap water, and placed in 4- liter plastic boxes containing unaerated 0.125 strength Hoagland's solution. Three replicates were used for all treatments. After one week in hydroponic solution, the plants were supplied with selenate or selenite (as the sodium salt) at 20 µM, i.e., 1.6 mg L^{-1} Se. This concentration was chosen because rates of Se volatilization and uptake were linear up to 20 µM for *Brassica juncea* (deSouza et al., 1998). After an additional week on hydroponic medium containing Se, individual plants were transferred into Magenta boxes (Sigma) containing 200 ml de-ionized water and selenate or selenite at 20 µM. The Magenta boxes were placed into airtight Se volatilization chambers kept in a plant growth chamber maintained at 25°C and 50% humidity, with a 24 h photoperiod and an irradiance of 400 µmol m^{-2} s^{-1} photosynthetic photon flux, mainly as fluorescent light with some incandescent light. Se volatilization was measured from plants placed in de-ionized water rather than in hydroponic solution, in order to minimize the contribution of Se volatilizing-bacteria in solution. Volatile Se produced by the plants was trapped for 24 h in alkaline peroxide traps as described previously (Zayed and Terry, 1992). After collection of volatile Se for 24 h, samples of trap solution were kept at 4°C until analysis, and the plants were carefully washed under flowing tap water and dried in an oven at 70°C for 3 days. The dried plant material was weighed, separated into roots and shoots and ground in a mill to a powder. The powdered plant tissues were acid-digested according to Martin (1975).

Volatile Se trapped in the alkaline peroxide trap solution was analyzed as follows: a 5 mL alkaline peroxide solution was heated at 95°C for 30 min, 5 mL concentrated HCl was added, and the solution was heated again at 95°C for 30 min. The volatile Se in the trap solution is transformed into selenite by this procedure. Vapor-generation atomic absorption spectroscopy was used to measure the Se concentration in the acid digested plant tissues and volatilization trap solutions (Mikkelsen, 1987). Se volatilization over the 24 h period was normalized to the dry weight of the plants used in the experiment. A wheat flour standard (NIST, 1.1 mg Se/kg) and blanks were used with all digestions. Three replicate plants were used for each treatment in all experiments.

4.3 Results

Selenium volatilization rates were measured from twenty aquatic plant species supplied with 20 µM selenate and selenite, and compared with Indian mustard (*Brassica juncea*., Figure 4-1). Indian mustard was used as a reference plant because it is one of the best terrestrial Se volatilizers known (Terry et al., 1992; Terry and Zayed, 1994) and a very effective species for Se phytoremediation (Banuelos and Meek, 1990; Terry and Zayed, 1998). The variation in Se volatilization among the aquatic plant species was around 50-fold (Figure 4-1A,B).

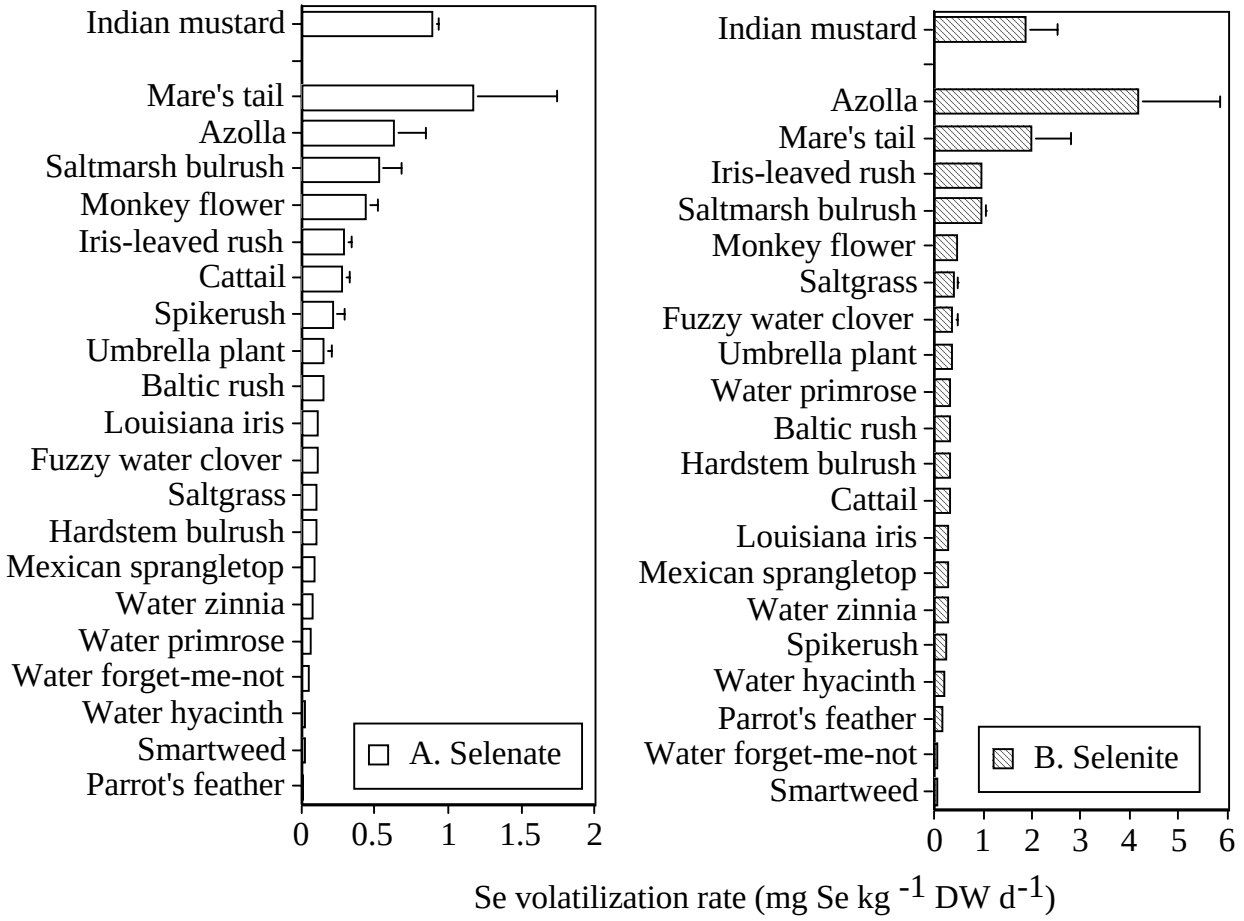

Figure 4-1
Rate of Se volatilization per unit dry weight for twenty wetland plant species and Indian mustard, supplied with 20 µM selenate (A) or selenite (B) for 7 days. Shown values are the average and standard error of three replicates.

The highest Se volatilization rate from selenate was attained by mare's tail, 1.0 mg Se kg^{-1} DW d^{-1}; for selenite the highest rate was attained by azolla, 4.0 mg Se kg^{-1} DW d^{-1}. Se volatilization rates from selenate and selenite were significantly correlated ($P<0.001$), i.e., the best plant species for Se volatilization from selenate were also the best species for selenite volatilization. Expressed per unit dry weight of plant tissue, Se volatilization rates were generally 2- to 3-fold higher when selenite was supplied (Figure 4-1B) than when selenate was supplied (Figure 4-1A). The best species for Se volatilization had rates that compared favorably, but were not significantly different to the rate attained by the best terrestrial Se volatilizer, Indian mustard.

The twenty aquatic species also showed considerable variation in the Se concentrations accumulated in roots and shoots (Figures 4-2 and 4-3). Root Se accumulation varied 20-25 fold (Figure 4-2A,B), and shoot tissue Se concentrations varied 50-85 fold (Figure 4-3A,B). The tissue Se concentrations in roots and shoots were significantly correlated with each other ($P<0.05$). The plant species exhibiting the highest root Se concentrations when supplied with selenate or selenite were water forget-me-not and monkey flower, respectively. The root Se concentrations were, in general, comparable when plants of a certain species were supplied with selenate or selenite. For selenate-treated plants (Figure 4-3A), the highest shoot Se concentration was measured in iris-leaved rush, and for selenite-supplied plants, azolla had a significantly ($P<0.001$) higher shoot Se concentration than the other aquatic species (Figure 4-3B); however, this was probably due to the fact that azolla, being a very small floating fern, could not be separated into root and shoot. Since selenite is poorly translocated to the shoot (Asher et al., 1967; Arvy, 1993), the root tissue present in the azolla sample may well have increased the "shoot" Se concentration substantially.

When supplied with selenate, the best aquatic species for Se accumulation in roots had comparable or higher root Se concentrations than Indian mustard (Figure 4-2A); however, in selenite-supplied plants, the best species for Se accumulation showed 1.5- to 2-fold lower root Se concentrations than Indian mustard (Figure 4-2B). Since these differences were not statistically significant, we conclude that the best aquatic plant species had root and shoot Se concentrations of Se comparable to Indian mustard.

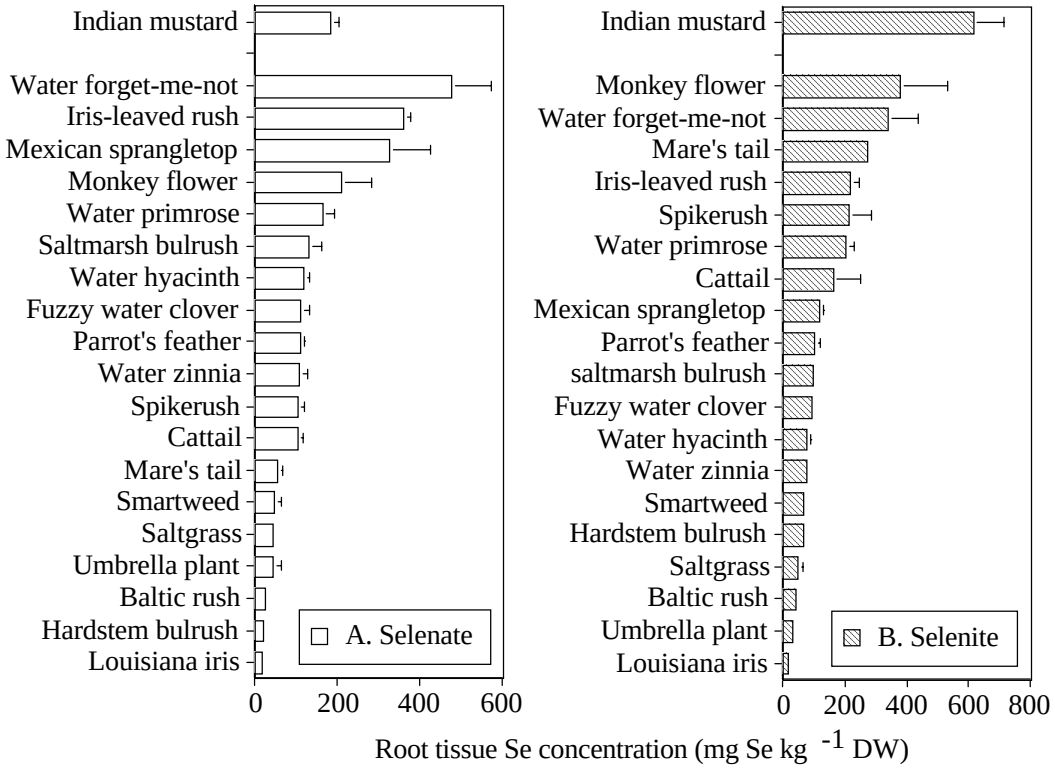

Figure 4-2
Se concentration in the roots of the 21 plant species shown in Figure 4-1, supplied with selenate (A) or selenite (B). Shown values are the average and standard error of three replicates. NB: Azolla is not shown because it had no distinguishable roots.

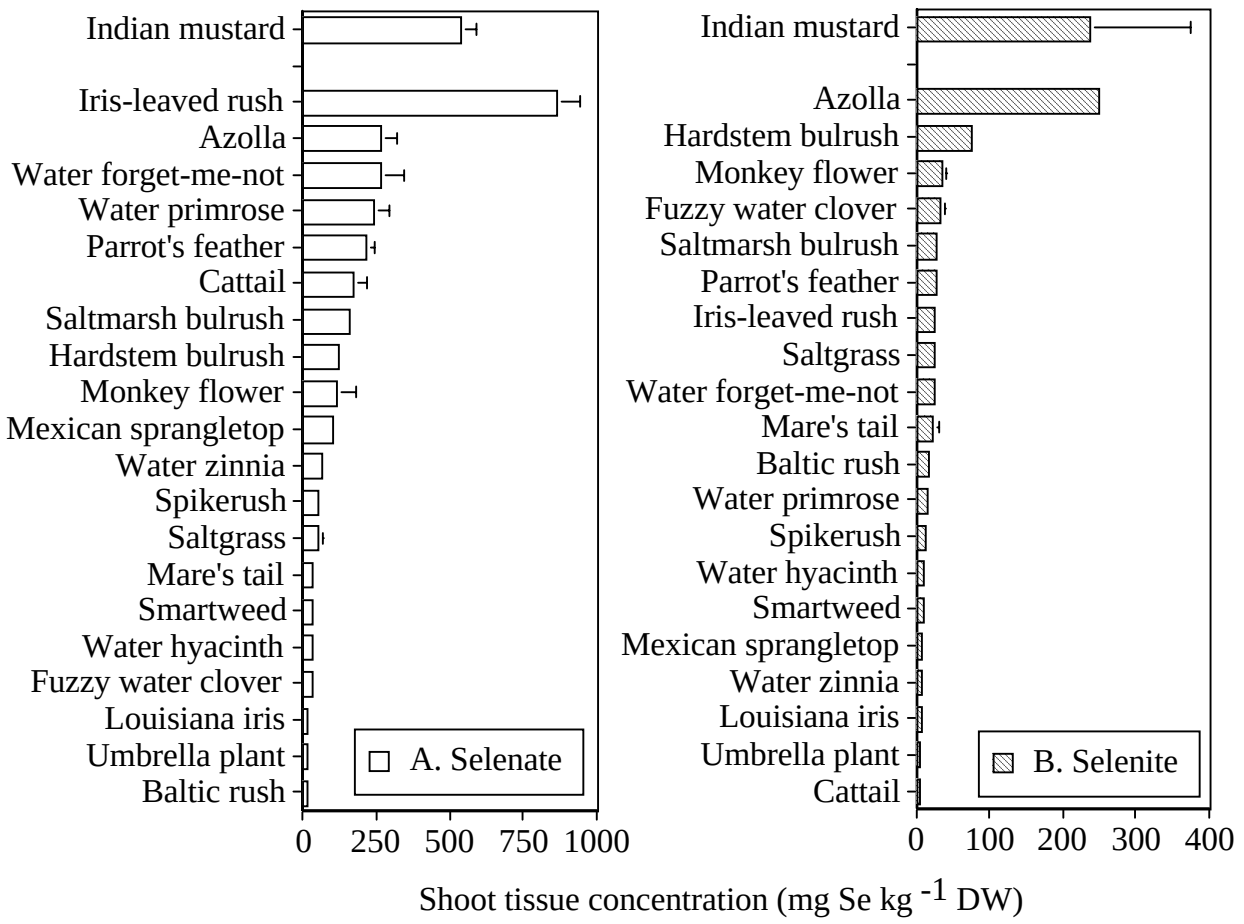

Figure 4-3
Se concentration in the shoots of the 21 plant species shown in Figure 4-1, supplied with selenate (A) or selenite (B). Shown are the average and standard error of three replicates.

The translocation of Se from root to shoot is of interest because it influences the harvestability of the extracted Se, and thus the phytoremediation efficiency. Shoot Se concentrations were generally several-fold higher in plants supplied with selenate, as compared to selenite, except for azolla, baltic rush, and fuzzy water clover (Figure 4-3A,B). The Se concentrations in selenite-treated plants were 2- to 10-fold lower in shoots than in roots, while in selenate-treated plants the Se concentrations in roots and shoots were comparable. Our results show that there was a clear difference in root-to-shoot translocation between selenate and selenite (Figure 4-4). In hardstem bulrush, for example, the ratio of shoot Se concentration to root Se concentration was 6.5 for selenate-supplied plants and only 1.2 for selenite supplied plants. Regardless of the form of Se supplied, hardstem bulrush was by far the best translocator of Se to the shoot.

In order to compare the Se phytoremediation potential of different plant species, it is important that, in addition to Se volatilization and accumulation per unit dry weight, we also measure the potential of each species to produce biomass. The biomass productivity potential of these aquatic species was estimated by measuring the harvestable biomass per unit (soil/water) surface area of the plants used for these experiments (Figure 4-5). There was 50-fold variation among the species; the best biomass producer was cattail.

By combining the data from Figures 4-1 and 4-5, it was possible to express the Se volatilization capacity of each plant species per unit surface area. As shown in Figure 4-6, the Se volatilization per unit surface area varied about 30-fold among the plant species. The plant species that volatilized the most Se per unit surface area when supplied with selenate or selenite were cattail and saltmarsh bulrush, respectively. The best Se volatilizing aquatic plant species showed rates of Se volatilization per unit soil surface area that were comparable or better than the terrestrial volatilizer, Indian mustard. Cattail and saltmarsh bulrush even volatilized 2 to 3-fold more Se per square meter per day than Indian mustard.

Similarly, when harvestable biomass productivity (Figure 4-5) and shoot tissue Se concentration (Figure 4-3) were combined, the potential harvestable Se per unit surface area could be calculated (Figure 4-7). The Se removal capacity varied among the aquatic species by 20- to 30-fold. When plants were supplied with selenate (Figure 4-7A) or selenite (Figure 4-7B) the best Se phytoextractors per unit surface area were cattail and hardstem bulrush, respectively. The potential Se removal per unit soil surface area was about five times higher when selenate was supplied, compared to selenite, since selenate was translocated better to the shoot. When compared to Indian mustard, Se removal per unit surface area by the aquatic plants was impressive: selenate removal to harvestable tissue was higher than Indian mustard for six aquatic plant species (Figure 4-7A), while selenite removal was comparable with Indian mustard in six aquatic species (Figure 4-7B).

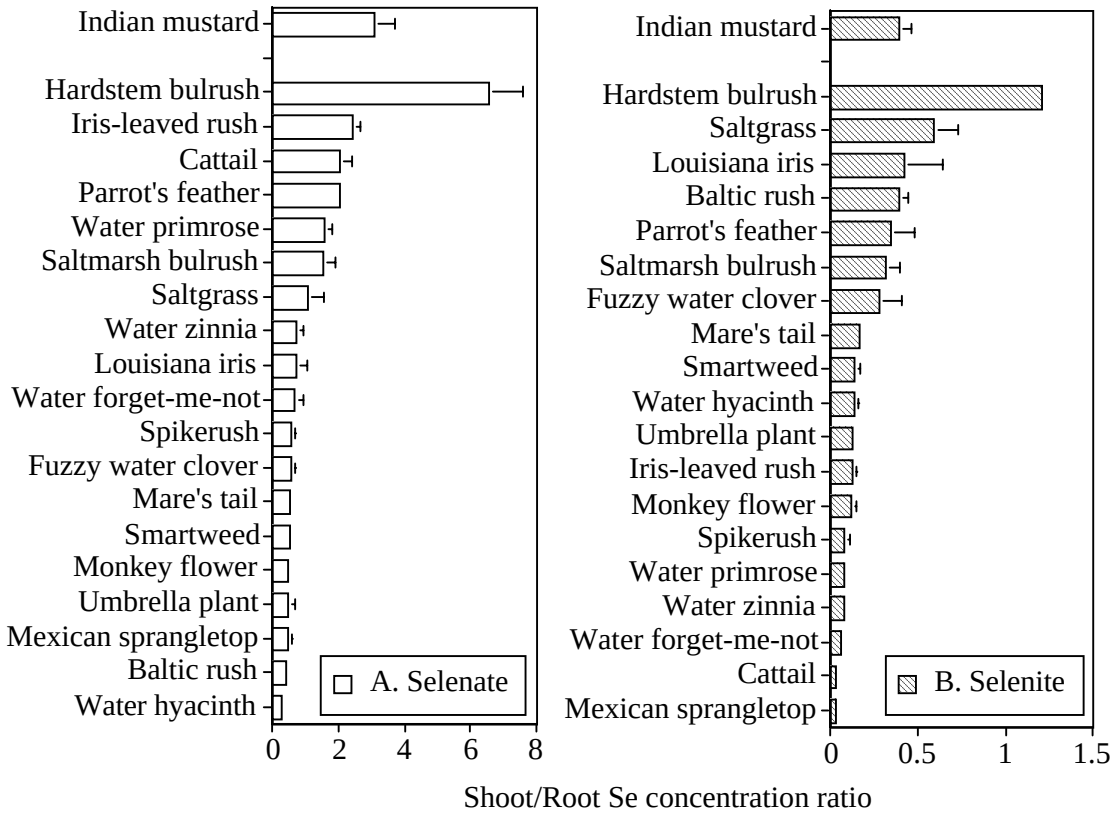

Figure 4-4
Ratio of shoot / root Se concentrations, as determined from Figures 4-2 and 4-3, in plants supplied with selenate (A) or selenite (B). Shown values are the average and standard error of three replicates. NB: Azolla is not shown because it had no distinguishable roots.

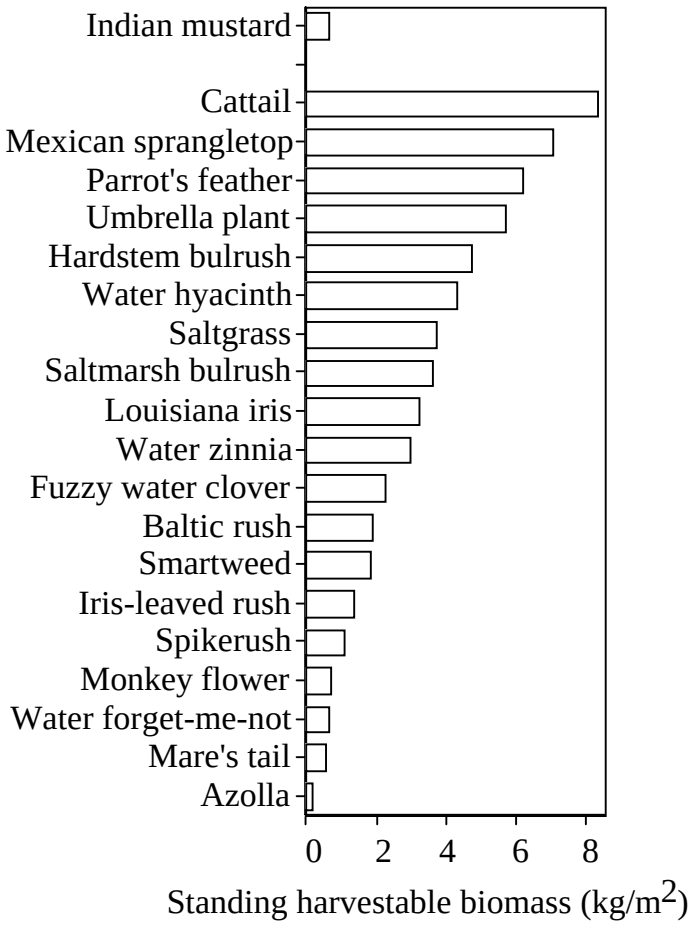

Figure 4-5
Harvestable standing biomass/unit surface area of the plant species used. NB: water primrose is not shown because it died before its biomass could be measured.

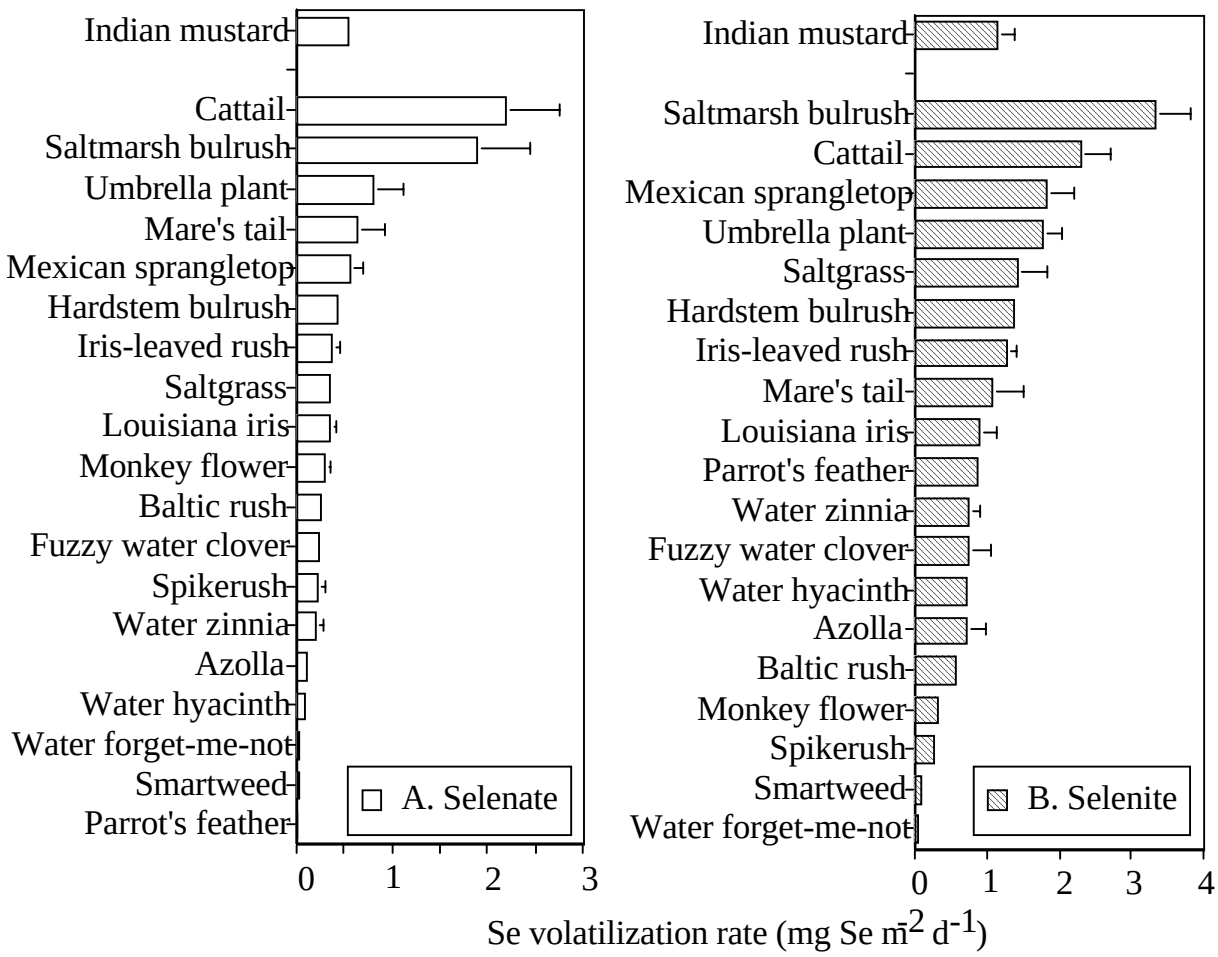

Figure 4-6
Rate of Se volatilization per unit surface area for the plant species used, supplied with selenate (A) or selenite (B). NB: water primrose is not shown because no biomass data were available. Shown are the average and standard error of three replicates.

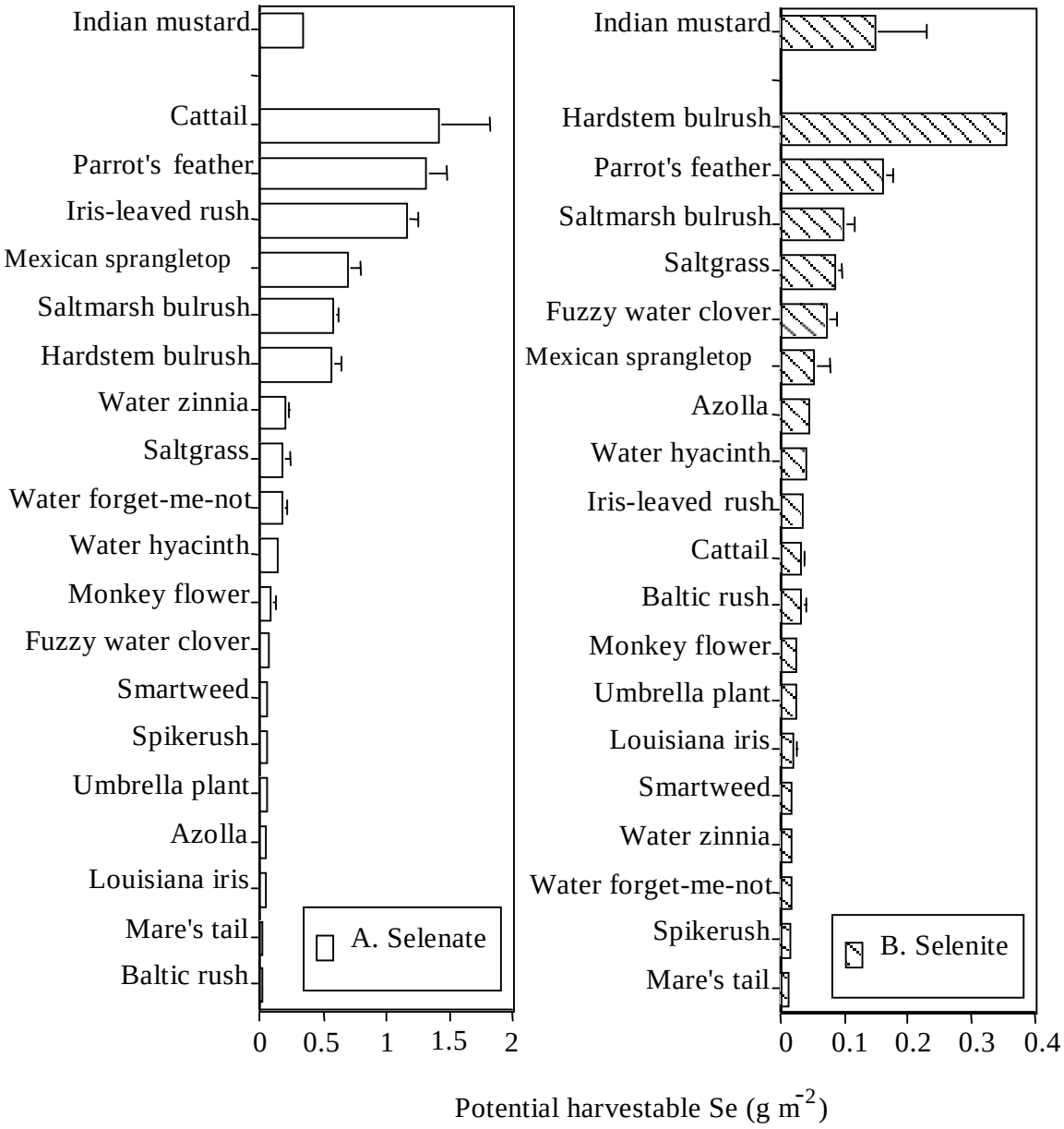

Figure 4-7
Potential harvestable Se per square meter for the plant species used, supplied with selenate (A) or selenite (B). NB: water primrose is not shown because no biomass data were available. Shown values are the average and standard error of three replicates.

4.4 Discussion

The results of this work clearly show that aquatic plant species vary greatly in their ability to accumulate Se in their tissues and volatilize it, when supplied with different forms of Se. These plants also varied in their biomass production. Regardless of the form of Se supplied, there was a 50-fold variation in Se volatilization rate (expressed per unit dry weight of plant tissues), a 50- to 85-fold variation in tissue Se concentration, and a 50-fold variation in plant biomass accumulation. Saltmarsh bulrush and mare's tail emerged as two aquatic plant species which volatilized Se at high rates, both on an area basis (which reflects the biomass productivity) as well as per unit plant tissue. The best aquatic plant species for Se volatilization compared well with the high rates exhibited by the terrestrial plant, Indian mustard, which has been shown to be a good candidate for Se phytoremediation (Banuelos and Meek, 1990; Terry and Zayed, 1998).

The best aquatic plant species for Se volatilization (per unit dry weight) were the same regardless of the form of Se supplied, i.e., selenate or selenite. However, Se volatilization from selenite was generally about 2-fold higher than volatilization from selenate by the same aquatic species. The same result was found for Indian mustard, and de Souza et al. (1998) concluded that the higher volatilization rate from selenite was due to the fact that when selenite is supplied, it is rapidly converted to a selenomethionine-like organic Se form which is easily volatilized; selenate on the other hand, is rapidly absorbed and translocated to the shoot but only very slowly metabolized to organic forms. Thus, selenate reduction appears to be a rate-limiting step for Se volatilization in plants.

Se accumulation in shoot tissues varied substantially with the oxidation state of supplied Se. With respect to selenate, cattail, parrot's feather and iris-leaved rush were the most effective species in accumulating Se (Figure 4-7). Cattail accumulated high Se levels mostly because of its high biomass, parrot's feather because of its high biomass and high shoot Se concentration, and iris-leaved rush because of its high shoot Se concentration. Thus, cattail, parrot's feather and iris-leaved rush are logical choices to field test in wetlands constructed for the cleanup of selenate-containing agricultural drainage water. One concern would be salinity: it is not known how parrot's feather and iris-leaved rush will grow at the salinity levels associated with agricultural drainage water. With respect to selenite accumulation, hardstem bulrush, parrot's feather and saltmarsh bulrush were best (Figure 4-7), owing to fairly high levels of both biomass and shoot Se concentration. These species may therefore be the species to test in constructed wetlands built for the treatment of oil refinery effluent or selenite-contaminated industrial wastewater. It is interesting that parrot's feather appears to be a good accumulator of both selenate and selenite, since the related aquatic species water milfoil, *Myriophyllum spicatum* was reported to be a good species for the remediation of heavy metals (Wang et al., 1996) as well as TNT (Hughes et al., 1997). Another aquatic species which is already being used successfully for the phytoremediation of heavy metals is azolla (Tel-Or, 1994). Azolla was also one of the best Se accumulators per unit dry weight in our studies, but its Se removal per unit surface area was limited by its low biomass productivity.

An important difference in how aquatic plants respond physiologically to selenate compared to selenite is that when selenate was supplied to the aquatic plants, it was translocated readily to the shoot. In contrast, selenite-Se accumulates mostly in the root. This observation, which has also been reported for terrestrial species (Asher et al., 1967; Arvy, 1993; deSouza et al., 1998), has

important consequences for Se phytoremediation. Because the root tissue Se concentrations in the aquatic plants were comparable in plants supplied with selenate or selenite, and the shoot tissue Se concentrations were about five-fold higher in plants supplied with selenate, the total amount of Se accumulated from selenate was about five-fold higher than from selenite. This means that per unit surface area, a wetland will be able to phytoextract five times more Se from selenate-contaminated wastewater (e.g. agricultural drainage water) than from selenite-contaminated wastewater (e.g. oil refinery wastewater).

Factors which affect the growth performance of wetland plant species include salinity, pH, temperature, levels of other pollutants, and competitive strength. The objective of this work was to determine the maximum potential of each plant species to accumulate and/or volatilize Se under optimum lab conditions. Our approach was to examine a large number of plant species in laboratory experiments. When we compare the lab results obtained here with actual data obtained in the field, there is good agreement. For example, cattail and saltmarsh bulrush were shown to have high rates of Se volatilization per unit area in lab experiments (Figure 4-6); these species were also effective volatilizers of selenite in the San Francisco Bay constructed wetland (Hansen et al., 1998) and of selenate in the Corcoran wetland (Terry, report in preparation for EPRI). With regard to biomass production, the potential biomass measured in the lab under optimal conditions was significantly correlated (R^2=0.71, P=0.035, n=6) with data available to us from the Allegheny Power Services constructed wetland at Springdale, PA (Terry, report in preparation for EPRI). Both examples lend confidence to the use of laboratory-obtained data to identify species which may be worth testing in the field.

While phytoextraction is an efficient technology for Se removal from contaminated wastewater, Se accumulation in plant tissues (i.e., shoots but also roots) may pose a threat to wildlife (Skorupa, 1998). One management approach to this problem is to periodically harvest Se-containing plant material from the site and dispose of it in a suitable way, e.g., as a Se-containing amendment to fertilize Se-deficient soils or as a biofuel (Terry and Zayed, 1998). Shoot removal has an added advantage that it greatly increases Se volatilization, thus further enhancing Se removal from the contaminated site (Zayed and Terry, 1994). Another way to remove Se from a contaminated site while minimizing toxicity to wildlife is to use plant species which are good Se volatilizers but poor Se accumulators. In this respect, umbrella plant may be a suitable species, since it was one of the five best Se volatilizing aquatic plants but was one of the lowest accumulators of harvestable Se in tissues.

In conclusion, the results presented here demonstrate the great potential of aquatic plants to volatilize and accumulate Se. We have shown that there is substantial variation in Se remediation potential among different aquatic species and we have identified those species with superior capacities for phytovolatilization and phytoextraction of selenate or selenite.

5
SCREENING TWELVE PLANT SPECIES AND TEN TRACE ELEMENTS

5.1 Introduction

One major observation for all the above tested plant species and trace elements is that an external supply concentration of 1 mg L^{-1} seems to be the critical level after which a major break through in the element uptake usually occurs. It seems logical, therefore, to focus on plant responses to trace elements at this level of supply. Therefore, in this study we made use of this phenomenon by screening a larger number (12 species) of plant species for several elements (ten elements) at one trace element supply level (1 mg L^{-1}). All the twelve wetland plant species were screened under identical aerial and root environmental conditions for their ability to remove ten potentially toxic trace elements. To deal with this large number of species and trace elements we used a modified approach, in which we exposed all plant species to the same concentration of each trace element using a recirculating-nutrient culture technique. This technique enabled all the plant species to be exposed to the same solution all the time. In addition, the nutrient solutions were changed every 48 hrs in order to ensure a continuous exposure to equal concentrations of the elements and to eliminate possible substrate limitation.

5.2 Materials and Methods

Twelve wetland plant species, listed in Table 5-1, were collected from wetlands in Richmond, Mendota, and Cocoran, California, and nursery gardens in Berkeley, Sebastopol, and Petaluma, California. Collected plants were grown and propagated hydroponically in the greenhouse with quarter-strength Hoagland's solution containing (mM): 1.25 $Ca(NO_3)_2$, 0.5 KH_2PO_4, 1.5 KNO_3, 0.5 $MgSO_4$, and 0.25 NaCl, and (µM) 11.5 H_3BO_3, 2.3 $MnCl_2$, 0.19 $ZnSO_4$, 0.08 $CuSO_4$, 0.026 H_2MoO_4, and 22.4 $FeSO_4$ (as ferric-sodium EDTA complex).

Ten trace elements were investigated in this study including manganese (Mn, $MnCl_2 \cdot 4H_2O$), cadmium (Cd, $CdSO_4 \cdot 8H_2O$), copper (Cu, $CuSO_4 \cdot 5H_2O$), nickel (Ni, $NiSO_4 \cdot 6H_2O$), chromium (Cr, K_2CrO_4), lead (Pb, $Pb(NO_3)_2$), mercury (Hg, $HgCl_2$), boron (B, $Na_2 \cdot B_4O_7 \cdot 10H_2O$), arsenic (As, $NaAsO_2$), and selenium (Se, Na_2SeO_3).

In order to determine the rate of trace element uptake and accumulation for each plant species, plants were grown hydroponically in the greenhouse with quarter-strength Hoagland's solution at pH 6.0 using Nutrient-Film Technique (NFT) as described by (Cooper, 1979). In NFT cropping system the plants are grown bare-rooted in long, narrow water-proof channels down which flows a very shallow stream of recirculating nutrient solution (Cooper, 1979). In this

Table 5-1
Wetland plant species tested in this study. The first column is their abbreviations being used in the figures.

	Common name	Scientific name
FW	Fuzzy Water Clover	*Marsilea drummondii*
IR	Iris-leaved Rush	*Juncus xiphioides*
MT	Mare's Tail	*Hippuris vulgaris* L.
MF	Monkeyflower	*Mimulus guttatus* Fisch.
PF	Parrot's Feather	*Myriophyllum brasiliense*
SG	Sedge	*Cyperus pseudovegetus*
SW	Smart Weed	*Polygonum hydropiperoides*
SC	Smooth Cordgrass	*Spartina alterniflora*
SR	Striped Rush	*Baumia rubiginosa*
UP	Umbrella Plant	*Cyperus alternifolius* L.
WL	Water Lettuce	*Pistia stratiotes* L.
WZ	Water Zinnia	*Wedelia trilobata* Hitchc.

study, plant roots were placed in 10-ft long rigid polyethylene troughs that were placed on an inclined surface with 1:100 slope. The nutrient solution was supplied to plant roots by means of a submersible pump that was placed in a 25-L nutrient holding tank. Each nutrient tank supplied the culture solution to two troughs, which were set up for one trace element treatment with six plant species. The six plant species with three replicates for each species (18 plants) were randomly distributed on the two troughs. Five identical NFT systems, each consisting of two troughs, were run side by side, with each system representing one trace element and containing three replicates of each of six plant species. Four separate runs were required to complete the screening of 12 plant species for their ability to take up 10 trace elements. In every run, plants were supplied with 1 mg L^{-1} concentration of the trace elements under study and uptake was determined after a treatment period of ten days. Choice of the trace element concentration of 1 mg L^{-1} was based on that this level is environmentally relevant and that it is the minimum concentration at which most trace elements may cause some effects on plant growth. Thus, it would be possible to identify those plant species that have sufficient metal tolerance to be considered for phytoremediation.

Plants with similar sizes and growth stages were selected for each experiment (Figure 5-1). Plant fresh weight (shoots and roots) was measured before transplanting into the NFT troughs. Nutrient solutions were changed every 48 hrs to ensure a relatively constant media concentration during the experimental period. The entire hydroponic setup was maintained under controlled greenhouse conditions with a 16-hour daylength, and a day and night temperature of 25°C ± 2.

Plants were harvested at the end of each culture period, thoroughly washed with running tap water, blotted dry on paper towels, and whole plant fresh weight determined. Shoot and root tissue were then separated from each other, dried at 70°C for 3 days, weighed, and ground using a Wiley mill for chemical analysis.

Elemental analysis was carried out by acid digestion of dry samples as described by Zarcinas et al. (1987), followed by measurement of total concentrations of all elements of interest in the acid digest using Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) or Atomic Absorption-Hydride Generation Spectrometry (AAS-HG). Manganese, Cd, Cu, Ni, Cr, Pb, and B were measured using ICP-AES according to Fassel (1978), while Hg, As, and Se were measured using AAS-HG (Terry et al., 1992; Keith, 1992). Standard reference materials (spinach, NIST 1570a; tomato, NIST 1573a; and San Joaquin soil, NIST 2709) and blanks were used with all digestions.

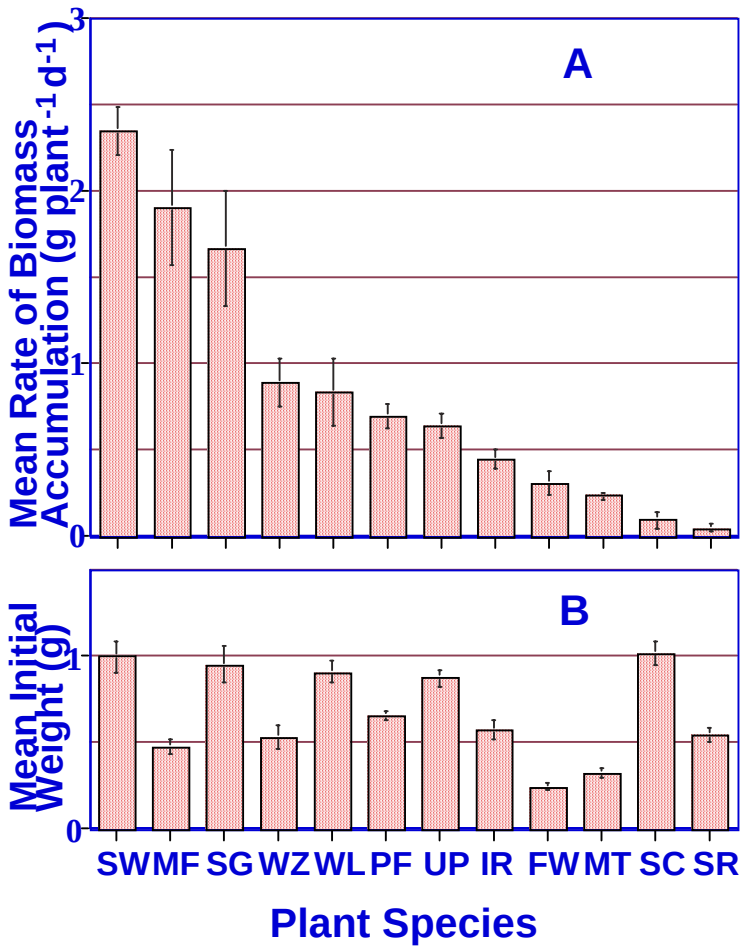

Figure 5-1
Average rate of plant biomass accumulation of twelve plant species treated with ten trace elements (A); and average initial fresh weight of plants before treatment (B). See Table 5-1 for description of plant species. Error bars indicate standard error, n = 3.

Rate of trace element removal by each plant species (μg plant^{-1} d^{-1}) was calculated as follows: [{Trace element concentration after treatment (μg g^{-1}) X final plant dry weight (g plant^{-1})} – {trace element concentration before treatment (μg g^{-1}) X initial plant dry weight (g plant^{-1})}] ÷ treatment period (days). Initial background trace element concentrations in plant tissues were determined on representative samples of various plant species before the experiment. Initial plant dry weight was estimated from measurements of plant fresh weight before treatment and water content determined on representative sample of similar size and growth stage.

5.3 Results and Discussion

Plant biomass accumulation: Ideally, all plant species should be at the same size and growth stage when exposed to trace element treatments in order to compare among them for their ability to remove various trace elements under study. However, different wetland plant species differ substantially in their growth rate, morphology, physiology, and size. In addition, in the current study some plants were propagated from seeds, others propagated vegetatively, and some obtained already propagated from local nurseries. To minimize variability among plant species, all individual plants were allowed to adapt to their new greenhouse environment for a period of at least four weeks. Plants with comparable sizes and growth stages were then selected for experimental work. As shown in Figure 5-1, initial fresh weights of all plants used in all trace element studies were mainly between 0.3 to 1 g per plant before transplanting to the NFT system. Plants with higher initial weights did not necessarily grow faster than those with lower initial weights. Thus, final plant weights did not correspond to initial weights due to differential rates of biomass accumulation of individual plant species (Figure 5-1A & B). Generally, the rate of biomass accumulation varied substantially (up to 60-fold) among various plant species under study (Figure 5-1A). Smartweed grew most rapidly and accumulated an average of 2.34 g plant^{-1}day^{-1} fresh weight, whereas striped rush accumulated only 0.04 g plant^{-1}day^{-1}. Other species were intermediate in their biomass accumulation. Apparently, the rate of plant biomass accumulation differed among species due to species effects and not due to differences in initial plant sizes.

Variation in trace element uptake and accumulation in plant tissues: With the exception of boron, all trace elements studied accumulated to substantially higher concentrations (from 5- to 60-fold) in roots than in shoots of all plant species (Figures 5-2 and 5-3). Boron concentrations in shoots and roots were comparable in all plants. This is because B moves into the plants during active transpiration across a concentration gradient and once in the plant, it moves readily through the xylem in the transpiration stream and accumulates at the point where water is lost through stomata in the leaf (Powell et al., 1997). Therefore, all plants tested showed a high degree of B accumulation in their shoots. Chromium exhibited the greatest difference (60 fold) between shoot and root element concentrations, whereas B and As had the least difference (1- to 5-fold) between shoot and root concentrations. Of all 10 elements studied, B accumulated to the highest concentration in plant shoots (up to ~1,150 μg g^{-1} DW in mare's tail) followed by Mn (up to 200 μg g^{-1} DW in umbrella plant) and Cd (up to 150 μg g^{-1} DW in water zinnia) (Figures 5-2 and 5-3). Copper, Ni, and Hg accumulated in shoots to a similar extent (up to ~90 μg g^{-1} DW), while shoot concentrations of Cr, Pb, Se, and As were the least (up to 35-60 μg g^{-1} DW). In roots, Cr attained the greatest concentration (up to 3000 μg g^{-1} DW in smartweed) followed by Pb (up to 1800 μg g^{-1} DW in smartweed), Mn (up to 1700 μg g^{-1} DW in stripped rush), and Cd (up to 1400 μg g^{-1} DW parrot's feather). Intermediate trace element concentrations in roots (up to 1000-

1200 μg g^{-1} DW) were attained for Cu, Ni, Hg, and B. Root concentrations of Se and As did not exceed 380 and 180 μg g^{-1} DW, respectively, in any plant species studied. Apparently, trace element uptake is completely independent from plant requirement for these elements since the nutrient elements Mn, Cu and B were taken up and accumulated in plant tissues to much lesser extent than that of the unneeded elements Cr and Pb.

Variation in plant species abilities to bioconcentrate various trace elements: The goal of the present study is to identify wetland plant species that are most efficient at removing trace elements from wastewaters polluted with potentially toxic trace elements. Previous research indicated that a good metal-accumulator wetland plant species will have the ability to take up better than 0.5% DW of a given element and bioconcentrate the element in its tissues to 1000-fold the initial element supply concentration (Zayed et al., 1998a).

Manganese

Manganese is one of the elements that received very little attention in the literature with respect to its uptake and accumulation by wetland plant species. Bur-reed (*Sparganium americanum*), bladderwort (*Utricularia* spp.) and the liverwort *Scapania undulata* are the top wetland species reported for Mn accumulation (Caines et al., 1985; Albers et al., 1993). They accumulated 1,590, 3,660, and 9,377 μg Mn g^{-1} in the shoots, respectively, when exposed to external Mn concentration of 2.75, 2.75, and 50 mg L^{-1}, respectively.

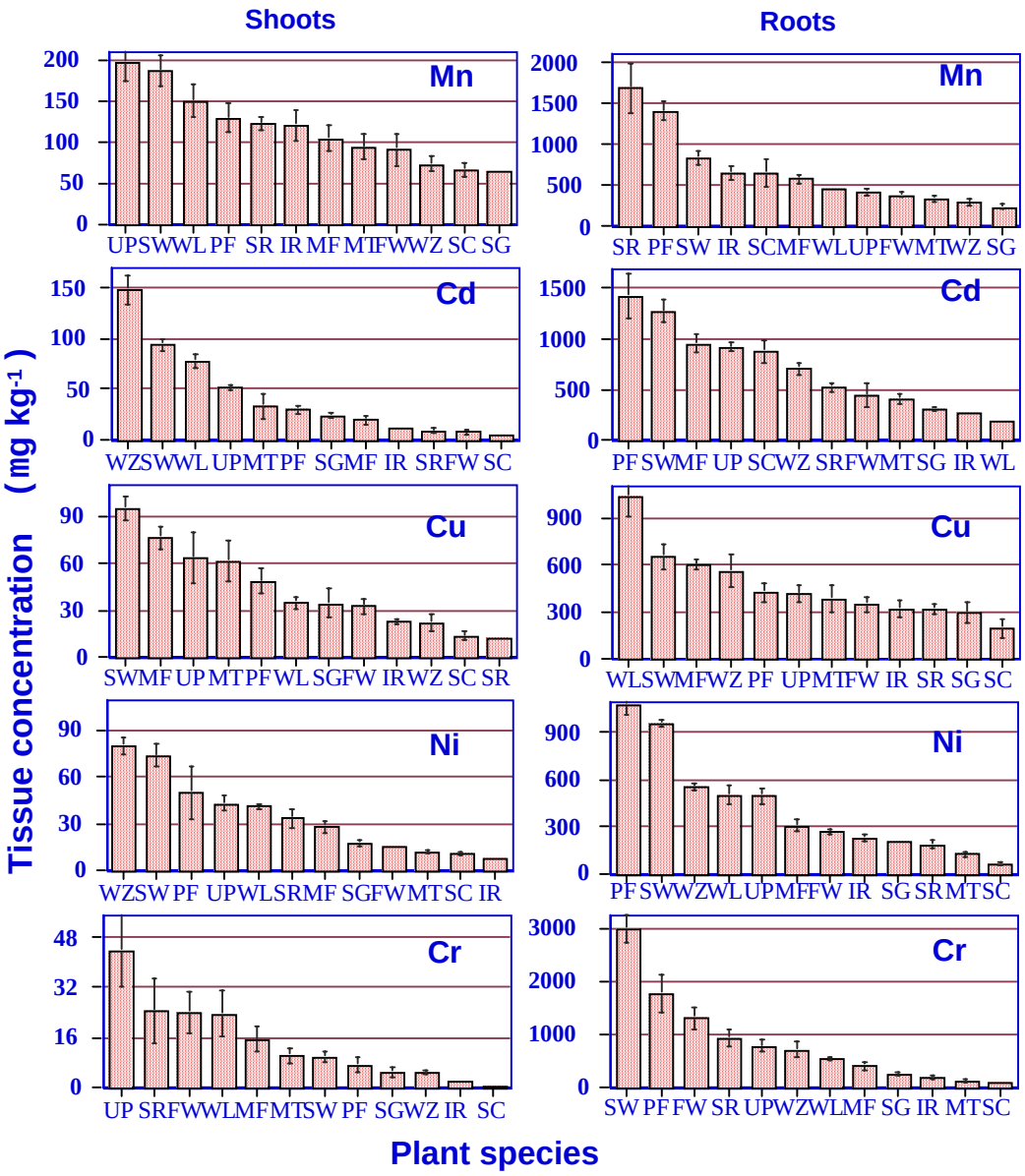

Figure 5-2a
Trace element concentration in shoot (left) and root (right) tissues of twelve wetland plant species. Trace elements are manganese (Mn), cadmium (Cd), copper (Cu), nickel (Ni), and chromium (Cr). See Table 5-1 for description of plant species. Error bars indicate standard error, n = 3.

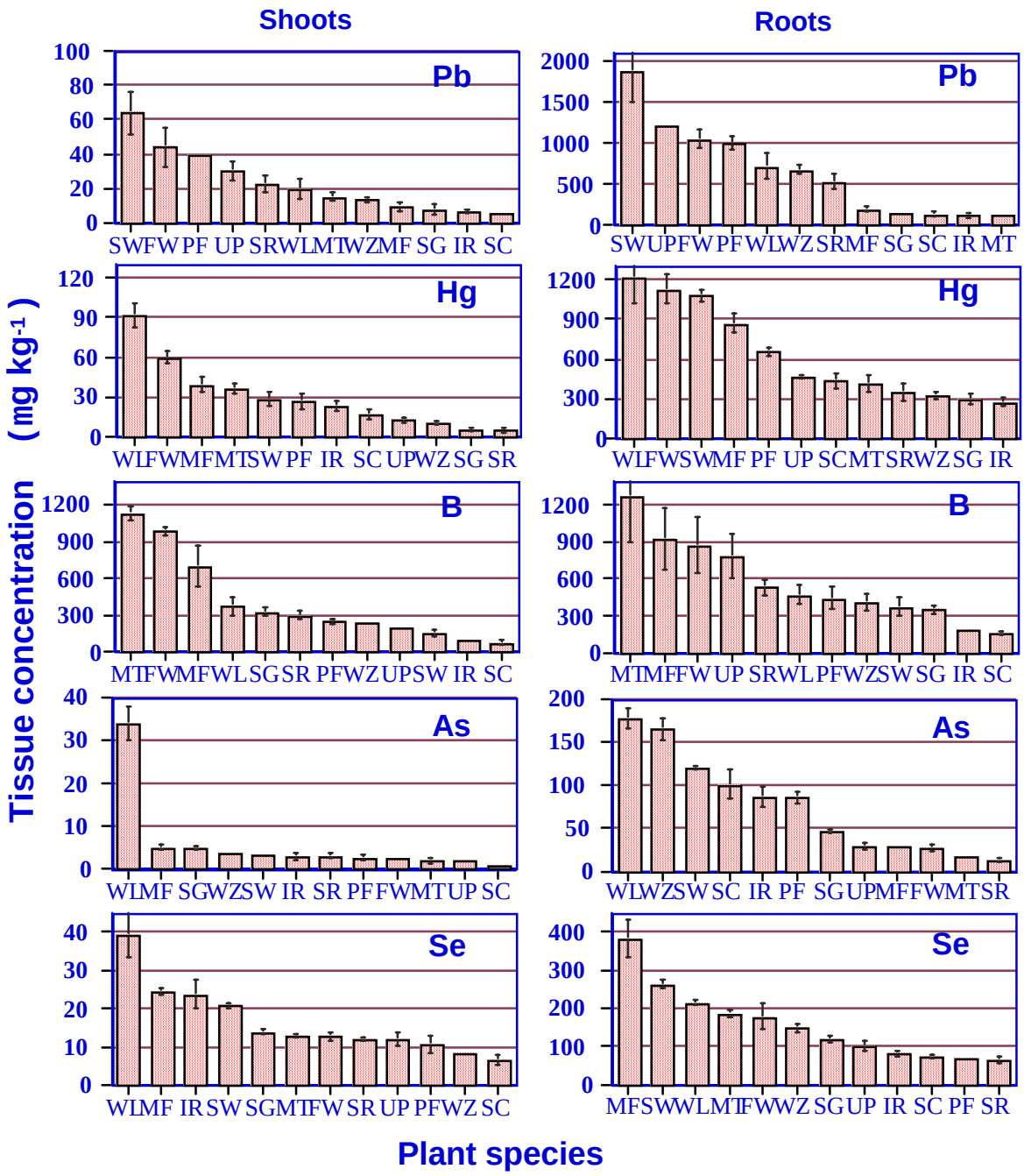

Figure 5-2b
Trace element concentration in shoot (left) and root (right) tissues of twelve wetland plant species. Trace elements are lead (Pb), mercury (Hg), boron (B), arsenic (As), and selenium (Se). See Table 5-1 for description of plant species. Error bars indicate standard error, n = 3.

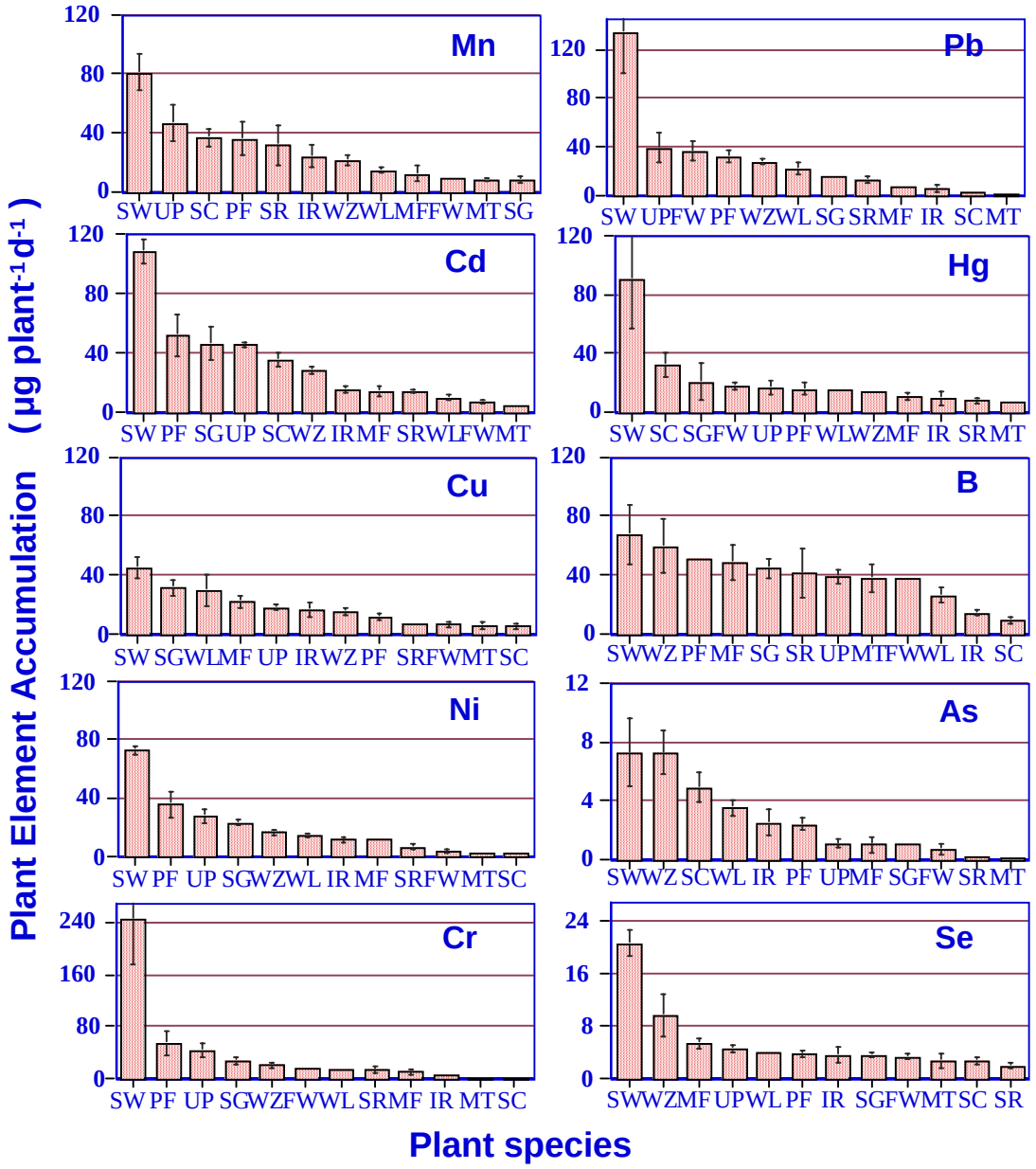

Figure 5-3
Rate of trace element accumulation by whole plant tissues (µg plant^{-1} day^{-1}) of twelve wetland plant species. Trace elements are manganese (Mn), cadmium (Cd), copper (Cu), nickel (Ni), chromium (Cr), lead (Pb), mercury (Hg), boron (B), arsenic (As), and selenium (Se). See Table 5-1 for illustrations of plant species. Error bars indicate SE, n = 3.

By comparison, the highest tissue manganese concentration recorded in this study was attained by striped rush, which accumulated 1,683 µg g^{-1} DW in its root tissues (Figure 5-2a). This is 7-fold higher than the lowest root tissue Mn concentration of 234 µg g^{-1} found in sedge roots. Among the twelve plant species under study, umbrella plant accumulated Mn to the greatest level (198 µg g^{-1} DW) in shoot tissues; smartweed was slightly lower (187 µg g^{-1} DW). Mn

concentrations in these two species were about three times the lowest Mn shoot concentration of 64 µg g^{-1} DW, which was detected also in sedge plants (Figure 5-2a).

Cadmium

In our previous studies, we reported that the floating plants water hyacinth and duckweed were very good accumulators of Cd. They accumulated up to 6 and 13 mg Cd g^{-1} DW, respectively, when supplied with 10 mg Cd L^{-1} (Zayed et al., 1998a; Zhu et al., 1999). These same species were also found to be the top species in Cd accumulation throughout the literature (Muramoto et al., 1989; Huebert and Shay, 1993). In this study, the greatest Cd concentration was attained by parrot's feather; it accumulated 1,426 µg Cd g^{-1} DW in root tissues (Figure 5-2a). While in shoot tissues, water zinnia accumulated the highest Cd concentration of 148 µg Cd g^{-1} DW. Smartweed attained the second highest concentration of Cd in both roots (~1300 µg g^{-1}) and shoots (~90 µg g^{-1}). Lowest Cd concentration in roots was found in water lettuce (193 µg g^{-1}), whereas lowest shoot Cd level was detected in smooth cordgrass with a concentration of 5 µg g^{-1} DW (Figure 5-2a).

Copper

Most species under study accumulated Cu to more or less the same degree in their roots (300-650 µg g^{-1}) except water lettuce (1,038 µg g^{-1}) and smooth cordgrass (194 µg g^{-1}). In shoot tissues, the highest Cu concentration of 95 µg g^{-1} was found in smartweed (Figure 5-2a) and the lowest Cu level of 12 µg g^{-1} was attained by striped rush. Previous studies have also shown that most other wetland plant species accumulate very little Cu in their tissues, especially above ground tissues (Taylor and Crowder, 1983; Luo and Reimmer, 1995; Smith, 1994; Gregor and Kautsky, 1991). Floating wetland plants seem to be an exception, however, they bioaccumulate Cu to higher levels of 300 to 15,000 µg g^{-1} in duckweed (Jain et al., 1989; Bassi and Sharma, 1993; Dirilgen and Inel, 1994; Zayed et al., 1998a), 6,000 to 7,000 µg g^{-1} in water hyacinth (Low et al., 1994; Zhu et al., 1999) and 2,500 to 3,000 µg g^{-1} in bacopa (Gupta et al., 1994).

Nickel

Highest Ni concentrations in roots (1,077 µg g^{-1} DW) and shoots (80 µg g^{-1} DW) were attained by parrot's feather and water zinnia, respectively (Figure 5-2a). When Ni concentrations in both roots and shoots were taken into account, smartweed was found to be the top Ni accumulator plant species among the species tested in this study with 953 and 74 µg Ni g^{-1} DW accumulated in root and shoot tissues, respectively. Smooth cordgrass was the poorest Ni accumulator with only 122 µg Ni g^{-1} DW accumulated in roots and 11 µg Ni g^{-1} DW in shoots. The greatest Ni accumulation by a wetland plant species of 9,000 µg g^{-1} DW was found in the waterfern *Azolla filiculoides* followed by *Salvinia natans* (6,295 µg g^{-1} DW) (Sela and Garty, 1989; Srivastav et al., 1994). Duckweed was also reported to accumulate Ni to high levels ranging from 2,000 to 5,500 µg g^{-1} DW (Jain et al., 1990; Sharma and Gaur, 1995; Zayed et al., 1998a). However, most emergent wetland plant species showed lower Ni accumulation than floating species: for example cattail had low root and shoot Ni concentrations of 1000 and 400 µg g^{-1}, respectively, (Taylor and Crowder, 1983) and bulrush had a root Ni concentration of 110 µg g^{-1} (Lee et al., 1981).

Chromium

Among the ten elements studied, Cr accumulated to the greatest concentrations in plant roots and to the least levels in plant shoots. Smartweed acquired the highest concentrations of Cr of 2,980 µg g^{-1} in its roots (Figure 5-2a). By comparison, the next highest root Cr concentrations observed in this study of 1,767 and 1300 µg g^{-1} were attained by parrot's feather and fuzzy water clover, respectively. All other plant species accumulated Cr in their roots to much lower levels (90 to 900 µg g^{-1}). Similarly, the highest Cr concentration in shoots of 44 µg g^{-1} was attained by umbrella plants with the next highest Cr concentration of 24 µg g^{-1} observed in three plant species including striped rush, fuzzy water clover, and water lettuce (Figure 5-2a). All other species accumulated less than 16 µg g^{-1} Cr in their shoot tissues. Smooth cordgrass accumulated the least amounts of Cr in roots (87 µg Cr g^{-1}) and shoots (0.83 µg Cr g^{-1}). Our previous research identified water hyacinth as a good accumulator (3,951 µg Cr g^{-1}) and duckweed as a modest accumulator (2,870 µg Cr g^{-1}) of Cr (Zayed et al., 1998a; Zhu et al., 1999). The greatest value of 3,200 µg Cr g^{-1} DW reported by other researchers for Cr accumulation by wetland plant species was observed in bacopa and bulrush plants supplied with 5 mg L^{-1} Cr in solution culture (Sinha et al., 1993; Guptal et al., 1994). Like Cu and Ni, floating and submerged plant species were more efficient than rooted emergent species in accumulating Cr from contaminated waters (Srivastav et al., 1994; Jana, 1988; Vaipayee et al, 1995).

Lead

Lead uptake and accumulation by most wetland plant species (e.g., *Potamogeton crispus, P. perfoliatus, Myriophyllum alterniflorum, Littorella uniflora, Isoetes lacustris, Distichilis spicata, Deschampsia cespitosa, Salicornia virginica Elodea canadensis*) is very limited and does not exceed 40-fold the external Pb supply level (Welsh and Denny, 1980; Chigbo et al., 1982; Gallagher and Kibby, 1980). Several floating wetland plant species, however, were shown to accumulate appreciable amounts of Pb. For instance, water hyacinth treated with 8 mg Pb L^{-1} accumulated 25,800 µg g^{-1} Pb (Muramoto and Oki, 1983). Giant duckweed (*Lemna polyrhiza*) accumulated 10,000 µg g^{-1} (whole plant tissue basis) when supplied with 10 mg L^{-1} Pb in solution culture (Sharma and Gaur, 1995) and water velvet accumulated 1,200 µg Pb g^{-1} after 14 days of exposure to 1 mg Pb L^{-1} in water (Jain et al., 1990). In this study the highest Pb concentrations in both shoots (64 µg g^{-1}) and roots (1,882 µg g^{-1}) were attained by smartweed (Figure 5-2b). Umbrella plant, fuzzy water clover and parrot's feather accumulated intermediate Pb levels in both shoots (30-45 µg g^{-1}) and roots (~1000-1200 µg g^{-1}). Monkey flower, sedge, smooth cordgrass, iris-leaved rush and mare's tail were the poorest plant species in Pb uptake and accumulation at both the root and shoot levels with Pb concentration not exceeding 18 µg g^{-1} in shoots and 200 µg g^{-1} in roots (Figure 5-2b).

Mercury

Like Pb, Hg accumulation by wetland plants is highly limited. Most plant species tested in this study were poor accumulators of Hg including umbrella plant, smooth cordgrass, mare's tail, striped rush, water zinnia, sedge, and iris-leaved rush; they accumulated less than 500 µg Hg g^{-1} in roots and less than 30 µg Hg g^{-1} in shoots. The highest Hg concentrations in both roots (1,217 µg g^{-1}) and shoots (92 µg g^{-1}) were reached by water lettuce followed by fuzzy water clover which

accumulated 1,127 µg g^{-1} and 60 µg g^{-1} in roots and shoots, respectively (Figure 5-2b). Water lettuce was also reported to bioaccumulate Hg to 5,000-fold its initial concentration in the external medium (Lenka et al., 1992). By comparison, water hyacinth exposed to Hg contaminated effluent containing 0.13 mg Hg L^{-1} accumulated 946 µg Hg g^{-1} in root tissues (Lenka et al., 1990).

Boron

Boron is a micronutrient that is essential for normal growth and development of plants. However, little information is available about B uptake and accumulation by wetland plants. In the current study we found that Mare's tail attained the greatest B concentration in both shoots (1,132 µg g^{-1}) and roots (1,276 µg g^{-1}) (Figure 5-2b). Monkey flower and fuzzy water clover exhibited intermediate B accumulation in both shoots (700-1000 µg g^{-1}) and roots (~900 µg g^{-1}). All other plant species had B concentrations below 500 µg g^{-1} in both shoots and roots tissues. In an early study by Glandon and McNabb (1978) *Lemna minor* was found to be a good accumulator of B with B concentrations reaching up to 3,200 µg B g^{-1} in plants growing in a sewage oxidation pond. Other plant species collected from the same pond (e.g., *Myriophyllum spicatum* and *Elodea canadensis*) accumulated only 38 to 61 µg B g^{-1} DW (Glandon and McNabb, 1978). In a recent study on B risk assessment in wetlands, it was shown that the longer lived, woody *Taxodium distichum* plants, which were collected from a wetland with a surface water B level of 0.02 to 27.8 mg L^{-1}, have higher shoot B concentrations (645 µg g^{-1}) than seasonal, herbaceous species (142 µg g^{-1} for *Peltandra virginica*, 118 µg g^{-1} for *Saururus cernuus*, 105 µg g^{-1} for *Pontederia cordata*, and 80 µg g^{-1} for *Typha latifolia*) (Powell et al., 1997).

Arsenic

Generally, all the twelve plant species tested in this study were poor accumulators of As. With the exception of water lettuce, all plant species tested bioconcentrated As in their shoots to less than 5-fold the initial As supply concentration (i.e., <5 µg As g^{-1} DW) (Figure 5-2b). Like Hg, water lettuce accumulated the highest concentration of As in both roots (177 µg g^{-1}) and shoots (34 µg g^{-1}). In an earlier study, we demonstrated that water hyacinth was also a poor accumulator of As; it accumulated 44 µg As g^{-1} in shoot tissues when exposed to 10 mg L^{-1} in solution culture (Zhu et al., 1999). Higher levels of As accumulation in plant tissues (200 to 500 µg As g^{-1} DW) were previously reported for several submerged and floating aquatic species growing in pond water containing 4 to 46 µg As L^{-1} including water lettuce, *Nettlia* spp., water gentian (*Nymphoides indica*), bladderwort (*Ultricularia* spp.), hydrilla (*Hydrilla verticillata*), muskgrass (*Chara* spp.), and water spinach (*Ipomoea aquatica*) (Lee et al., 1991). Similarly, submerged species (e.g., *Potamogeton pectinatus)* exposed to As-contaminated gold-mine effluent accumulated much more As (~5000 µg As g^{-1}) than emergent species (e.g., *Typha latifolia* accumulated 41 µg g^{-1} and 98 µg g^{-1} in shoots and roots, respectively) (Dushenko et al., 1995). Submerged and floating aquatic species differ markedly from rooted emergent species in that their foliage is exposed to the surrounding water. Leaf uptake of trace elements from water may be a significant pathway in submerged and floating species and may account for the higher As levels detected in these species. In the current study, however, only plant roots were in contact with the nutrient solution and no element uptake occurred through leaf tissues.

Selenium

Like As, all species studied were found to be poor accumulators of Se because Se concentrations in shoot and root tissues did not exceed 40 µg g^{-1} and 400 µg g^{-1}, respectively, in any plant species tested (Figure 5-2b). Among all species under study, monkey flower accumulated the highest Se concentration in roots (384 µg g^{-1}), while water lettuce accumulated the highest Se concentration in the shoots (39 µg g^{-1}). The low Se concentration observed in this study may be partly due to the fact that we supplied Se as SeO_3^{2-}, the form of Se that is mostly found in industrial wastewaters, which is known to be taken up by plants at lower rates than other soluble forms of Se (e.g., SeO_4^{2-}) and is hardly transported from root to shoot tissues (Zayed et al., 1998b). However, using the same form of Se, we have recently noted that the floating species duckweed was a good accumulator of Se with tissue concentrations reaching 4.27 mg Se g^{-1} when supplied with 10 mg Se L^{-1} as Na_2SeO_3 for 8 days in solution culture (Zayed et al., 1998a). Water hyacinth, on the other hand, was not a good Se accumulator even when supplied with Se as SeO_4^{2-}; it attained low Se levels in both shoot and root tissues (<165 µg g^{-1} and <319 µg g^{-1}, respectively) when exposed to 0.1 to 10 mg Se L^{-1} as Na_2SeO_4 in solution culture for 14 days (Zhu et al., 1999). Similar results were obtained by Ornes et al. (1991) who showed that while duckweed accumulated 0.50 mg Se g^{-1} from water, water hyacinth accumulated only 0.30 mg Se g^{-1}, whereas azolla and salvinia accumulated 1.00 and 0.70 mg Se g^{-1} DW, respectively.

Table 5-2
Top plant species identified for accumulating trace elements

Element	Shoot Concentration mg kg^{-1}	Root Concentration mg kg^{-1}	Harvestable Tissue Accumulation µg $plant^{-1}$ d^{-1}
Mn	Umbrella plant 198	Striped rush 1683	Smartweed 36
Cd	Water zinnia 148	Parrot's feather 1426	Smartweed 16
Cu	Smartweed 95	Water lettuce 1038	Water lettuce 30
Ni	Water zinnia 80	Parrot's feather 1077	Water lettuce 14
Cr	Umbrella plant 44	Smartweed 2980	Water lettuce 15
Pb	Smartweed 64	Smartweed 1882	Water lettuce 22
Hg	Water lettuce 92	Water lettuce 1217	Water lettuce 15
B	Mare's tail 1132	Mare's tail 1277	Water zinnia 46
As	Water lettuce 34	Water lettuce 177	Water lettuce 3.5
Se	Water lettuce 39	Monkey flower 384	Smartweed 4.41

Rate of trace element accumulation by wetland plants: Due to the large variation in the rate of biomass accumulation of the various plant species tested as discussed above, it is important to take into account plant biomass accumulation when different plant species are compared for their trace element removal efficiency. For this reason, we calculated the rate of trace element accumulation for various plant species as the amount of each trace element accumulated per plant per day (see equation in Materials and Methods) (Figure 5-4). Rate of element accumulation by plants varied significantly among various species and for different trace elements, i.e., Mn ranged from (μg $plant^{-1}$ day^{-1}) 8 to 81; Cd from 5 to 108; Cu from 5.62 to 45; Ni from 2 to 73; Cr from 2 to 246; Pb from 1 to 134; Hg from 5.6 to 90; B from 9 to 68; As from 0.11 to 7; and Se from 2 to 21 (Figure 5-4). Highest rates of trace element accumulation of all 10 trace element studied were attained by the same plant species, smartweed. This is attributed to the fact that smartweed exhibited the highest rate of biomass accumulation and attained high tissue concentrations of all elements studied. Compared to other species, smartweed had substantially higher rates (2- to 130-fold higher) of trace element accumulation for most elements studied including Pb, Cr, Mn, Hg, Cd, Ni, and Se. Water zinnia achieved similar rates of As and B accumulation to those obtained for smartweed. In addition to smartweed, species such as umbrella plant, parrot's feather, and water zinnia were among the top four accumulators for the ten trace elements tested (Figure 5-4). Smartweed was especially effective for accumulating Cr, Pb, and Cd, and to some extent Hg, and Mn. Mare's tail and smooth cordgrass were the poorest accumulators for most trace elements studied mainly due to their low rate of biomass accumulation in addition to their low trace element uptake capabilities for most elements.

Phytoremediation perspective*:* Our data show that smartweed was the best plant species for the accumulation of all ten elements under study based on the rate of element accumulation by whole plant tissues (see above). However, when only the rate of trace element accumulation in harvestable tissues is considered, other plant species may become more effective than smartweed(Table 5-2). A good example of this discrepancy is B uptake and accumulation in different plant parts of various species. Mare's tail accumulated the highest B concentrations in both shoots and roots (Figure 5-2b), whereas smartweed attained the greatest rate of B accumulation per whole plant per day (Figure 5-4) due to its highest rate of plant biomass accumulation (Figure 5-1A). However, water zinnia was found to be the best species in accumulating B based on the rate of accumulation in harvestable tissues only (Table 5-2), though B concentration in water zinnia shoots was much lower (240 μg g^{-1}) than that of mare's tail (1,132 μg g^{-1}). Thus, using trace element accumulation in harvestable plant tissues as a criterion for comparison, we found that water lettuce, a floating plant that can be wholly harvested, was the best species (among those studied) for the removal of most trace elements, i.e., Cu, Ni, Cr, Pb, Hg, and As (Table 5-2). Smartweed was still the best species in accumulating only Mn, Cd, and Se. Boron was best accumulated in the harvestable tissues of water zinnia as discussed above.

Nevertheless, from a phytoremediation standpoint the actual plant removal efficiency of any given trace element is the product of plant density and the rate of element accumulation in harvestable plant parts. Unfortunately, there is a significant lack of information in the literature with regard to wetland plant densities. Considering the available information, smartweed, in addition to being a fast growing wetland plant species that has a high efficiency to accumulate several trace elements in its root and shoot tissues as shown in this study, seems to have a high plant density of up to 850 plants m^{-2} (Thomas and Bazzaz, 1993). This substantiates the importance of this plant species in removing large volumes of potentially toxic trace elements

from water entering constructed wetland treatment systems. Based on the results of this study, the removal potential of various trace elements by the harvestable tissues of smartweed is estimated as follows (g ha^{-1} d^{-1}): 306 Mn, 300 B, 136 Cd, 108 Ni, 108 Pb, 102 Cu, 71 Hg, 37.4 Se, 13.6 Cr, and 5.11 As. By comparison, water lettuce, which is identified as the best plant species to accumulate Cu, Ni, Cr, Pb, Hg, and As in its harvestable tissues, has a low plant density of less than 100 plant m^{-2}. Assuming a maximum plant density of 100 plant m^{-2} for water lettuce, its estimated trace element removal efficiency would be as follows (g ha^{-1} d^{-1}): 29.6 Cu, 26.2 B, 22 Pb, 15 Hg, 14.5 Cr, 14.4 Ni, 14.4 Mn, 10.2 Cd, 4.13 Se, and 3.51 As.

However, it should be pointed out that these potential rates of element removal are merely estimates based on data obtained for hydroponically grown plants for 10 days. In our calculations, it was assumed that the rate of uptake and growth were linear during the experimental period. They must be, therefore, interpreted with caution when extrapolated to plants growing under natural conditions due to the differences in environmental conditions, length of exposure to the trace element, element concentration, chemical species of the element, growth stage, root media, concentrations of other elements, etc. One advantage of our work is that we compared the uptake and accumulation of ten trace elements by twelve plant species under identical root and shoot conditions, something that is not generally available in the literature. Table 5-2 lists the top plant species observed in our study based on element concentration in shoots, element concentration in roots, and element accumulation in harvestable tissues. Thus, Table 5-2 provides some of the available choices of appropriate plant species that may be used for the removal of the ten elements studied based on their accumulation in plant tissues or their rate of removal by harvestable plant parts. Selection of the appropriate plant species should be based on element concentration in plant tissues if element recovery from harvested plant materials is an important goal of the phytoremediation process. But, if the major goal of the phytoremediation process is to remove the maximum amount of element in the shortest time possible, then the selection should be based on the rate of element accumulation in harvestable tissues as well as known information on plant densities.

6
DUCKWEED (*LEMNA MINOR*)

6.1 Introduction

Duckweed (*Lemna minor*), a fast-growing floating plant, is commonly found in wetlands. It adapts easily to various aquatic conditions, and plays an important role in the extraction and accumulation of metals from waters. Several studies have shown that duckweed can accumulate high concentrations of various heavy metals and trace elements. This has been shown for Zn and Cu (Dirilgen and Inel, 1994); Cd, Pb, Zn, Mn, Co, Cu, Ni and Fe (Jain et al., 1988); Fe and Cu (Jain et al., 1989); Cd, Cu, Zn, As and Se (Jenner and Janssen-Mommen, 1993); Hg (Mo et al., 1989); and Cr (Bassi et al., 1990). We compared the bioaccumulation of six elements that are of great environmental concern due to their known toxicities to animals and humans and because of their wide-spread occurrence in the environment: cadmium, chromium, copper, lead, nickel, and selenium.

6.2 Materials and Methods

Duckweed plants were collected from the San Francisco Bay constructed wetland (Hansen et al., 1998) in Richmond, California. The plants were cleaned thoroughly under gentle running water to remove adhering algae and insect larvae. The plants were transferred to plastic aquaria of 3-L capacity containing quarter-strength Hoagland's solution at pH 6.0. The bottom parts of the aquaria (up to the height of the nutrient solution) were covered with aluminum foil to prevent algal growth. All the aquaria were maintained in growth chambers at 25 °C and at an irradiance of 400 μmol photon flux density (PFD) m^{-2} s^{-1} supplied over a 16-hour day length. The culture solutions were replaced every two days.

The experiments were carried out in Magenta boxes (Sigma) containing 250 ml quarter-strength Hoagland's solution containing (mM): 1.25 $Ca(NO_3)_2$, 0.5 KH_2PO_4, 1.5 KNO_3, 0.5 $MgSO_4$, and 0.25 NaCl, and (μM) 11.5 H_3BO_3, 2.3 $MnCl_2$, 0.19 $ZnSO_4$, 0.08 CuS_4, 0.026 H_2MoO_4, and 22.4 $FeSO_4$ (as ferric-sodium EDTA complex). The plants (2 g fresh weight) were exposed to individual trace element at 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 mg L^{-1} concentrations, which were added to quarter-strength Hoagland's solutions. Plants without added trace elements served as controls. The six trace elements under study (cadmium, chromium, copper, lead, nickel and selenium) were supplied as cadmium sulfate, potassium chromate, copper sulfate, lead nitrate, nickel sulfate, and sodium selenite, respectively.

Three replicate groups of plants were exposed to trace element treatments for 8 days. The plants were then collected from the solution, rinsed thoroughly with distilled water, blotted dry and weighed for fresh weight. Plants were then oven-dried at 70 °C for 48 hours before plant dry

weight was determined. In general, average dry weight of each group of plants ranged from 5 to 10 % depending on trace element treatment. The dry plant materials were ground to powder and representative samples were taken for chemical analysis. Elemental analysis was carried out by acid digestion of dry samples (Zarcinas et al., 1987), followed by measurement of total concentrations of all elements of interest in the acid digest using a Plasma 40 ICP-AES (Fassel, 1978). Selenium was measured using the Atomic Absorption-Hydride generation technique as described by Terry et al. (1992). Unless otherwise is indicated, all tissue trace element concentrations are reported on dry weight basis throughout the manuscript. The bioconcentration factor (BCF) was calculated as follows:

$$BCF = \frac{\text{trace element concentration in plant tissues (mg kg}^{-1}\text{) at harvest}}{\text{initial concentration of the element in the external nutrient solution (mg L}^{-1}\text{)}}$$

6.3 Results

Cadmium was accumulated to the greatest levels compared to the other five trace elements supplied to duckweed (Figure 6-1). The Cd concentration in duckweed (whole plant tissue) supplied with 10 mg Cd L^{-1} was 13.3 mg Cd g^{-1} dry plant materials. By comparison, the next highest concentration attained was 4.27 mg g^{-1} for Se. The slope of the uptake curve of tissue Cd versus Cd supply (Figure 6-1) increased slightly with increasing supply concentration up to 1 mg L^{-1}. Increasing supply concentration above 1 L^{-1} sharply increased the slope of the uptake curve. Supplying Cd to duckweed plants at 5 mg L^{-1} or higher concentrations caused chlorosis in the plants. The extent of chlorosis was more severe in plants treated with 10 mg L^{-1}, where all the fronds had turned yellow. This was accompanied with ca 25% decrease in growth relative to the control plants (data not shown).

The bioconcentration factor provides an index of the ability of the plant to accumulate the trace element with respect to the trace element concentration in the substrate. Among all tested trace elements, Cd attained the highest BCF of 1,333 at the 10 mg L^{-1} supply level compared to all other elements tested (Figure 6-1). At the lowest Cd supply concentration (0.1 mg L^{-1}), Cd attained the second greatest BCF (after Cu) of ~550. Cadmium BCF decreased as the Cd supply increased from 0.1 to 0.2 mg L^{-1} after which any increase in Cd supply resulted in an increase in Cd BCF (Figure 6-1).

Selenium, Cu and Cr were accumulated in duckweed plant tissues to lower concentrations (3 to 4 mg g^{-1} at 10 mg L^{-1} supply level) than Cd (Figure 6-2), but higher than Pb and Ni (Figure 6-3). Tissue concentrations of Se, Cu and Cr increased slightly as the supply level increased from 0.1 to 1 mg L^{-1}. Increasing the supply level above 1 mg L^{-1} resulted in sharper increase in plant tissue concentrations of the trace element supplied, i.e., Se, Cu or Cr. The greatest tissue concentrations of 4.27, 3.36 and 2.87 mg g^{-1} were all reached at the highest supply level for Se, Cu and Cr, respectively. Copper and Se had greater impact on plant growth than Cr and chlorosis was observed on some plants treated with 5 or 10 mg L^{-1} Cu or Se but not Cr.

Although the uptake curves of these three trace elements were of similar pattern (Figure 6-2), the BCF curves were not similar. While Se BCF increased only when Se supply was increased from 5 to 10 mg L^{-1} and remained more or less constant with increasing supply level up to 5 mg L^{-1}, Cu

BCF continued to decrease with each increase in the Cu supply concentration. Chromium BCF increased with increasing the supply level from 0.1 to 0.5 mg L^{-1}, then remained constant up to 2 mg L^{-1} after which, it decreased sharply with each increase in Cr concentration in the nutrient solution (Figure 6-2).

Lead and Ni were the two elements which were least acquired by duckweed in this study (Figure 6-3). The highest concentrations attained in duckweed tissue for these elements were 1.79 mg Ni g^{-1} and 0.63 mg Pb g^{-1} (the supply level in both cases was 10 mg L^{-1}). Very low concentrations of Pb and Ni were found in tissues of plants supplied with trace element in a concentration below 2 mg L^{-1}. Above 2 mg L^{-1} supply level, tissue Pb and Ni increased considerably. Lead had some deleterious effect on plant growth similar to that of Cd with chlorosis appearing on plants at the highest two supply concentrations. Plants were more resistant to Ni toxicity since the growth was not affected to a great extent even at the 10 mg L^{-1} supply level (data not shown).

Table 6-1
Incidence of toxicity symptoms and percentage of growth reduction of duckweed (*Lemna minor*) plants exposed to 10 mg L^{-1} of the various trace elements under study.

	Cu	Se	Pb	Cd	Ni	Cr
Chlorosis	Yes	Yes	Yes	Yes	No	No
Lowest supply level causing >10% growth reduction (mg L^{-1})	5	2	2	2	10	ND
% Growth Reduction (dry weight basis) at 10 mg L^{-1}	35.1	26.0	24.5	23.9	13.1	8.3

ND = Not detected

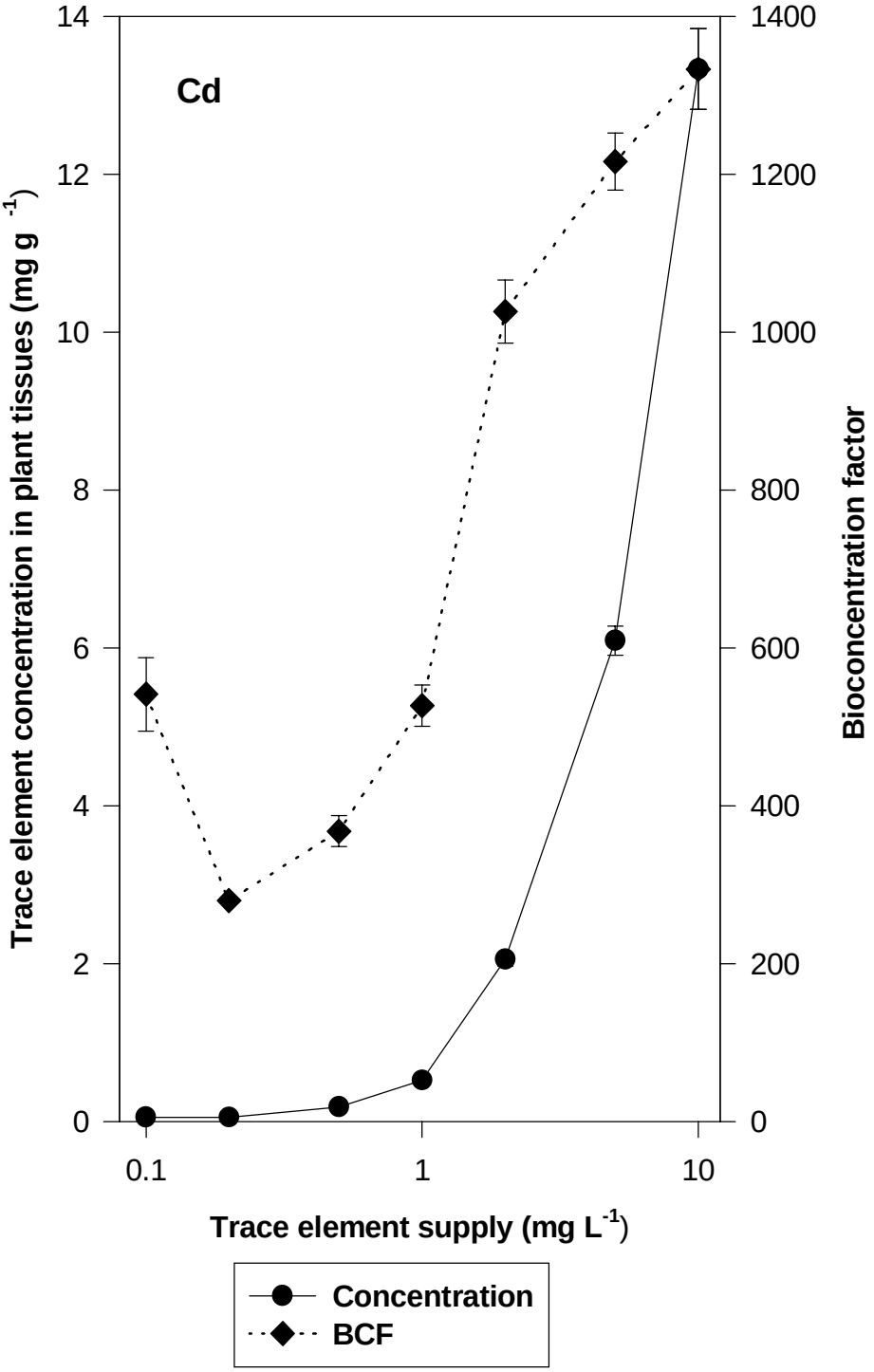

Figure 6-1
Influence of Cd concentration in the nutrient solution on Cd concentration in duckweed dry plant tissues and Cd bioconcentration factor. Vertical bars indicate standard deviations.

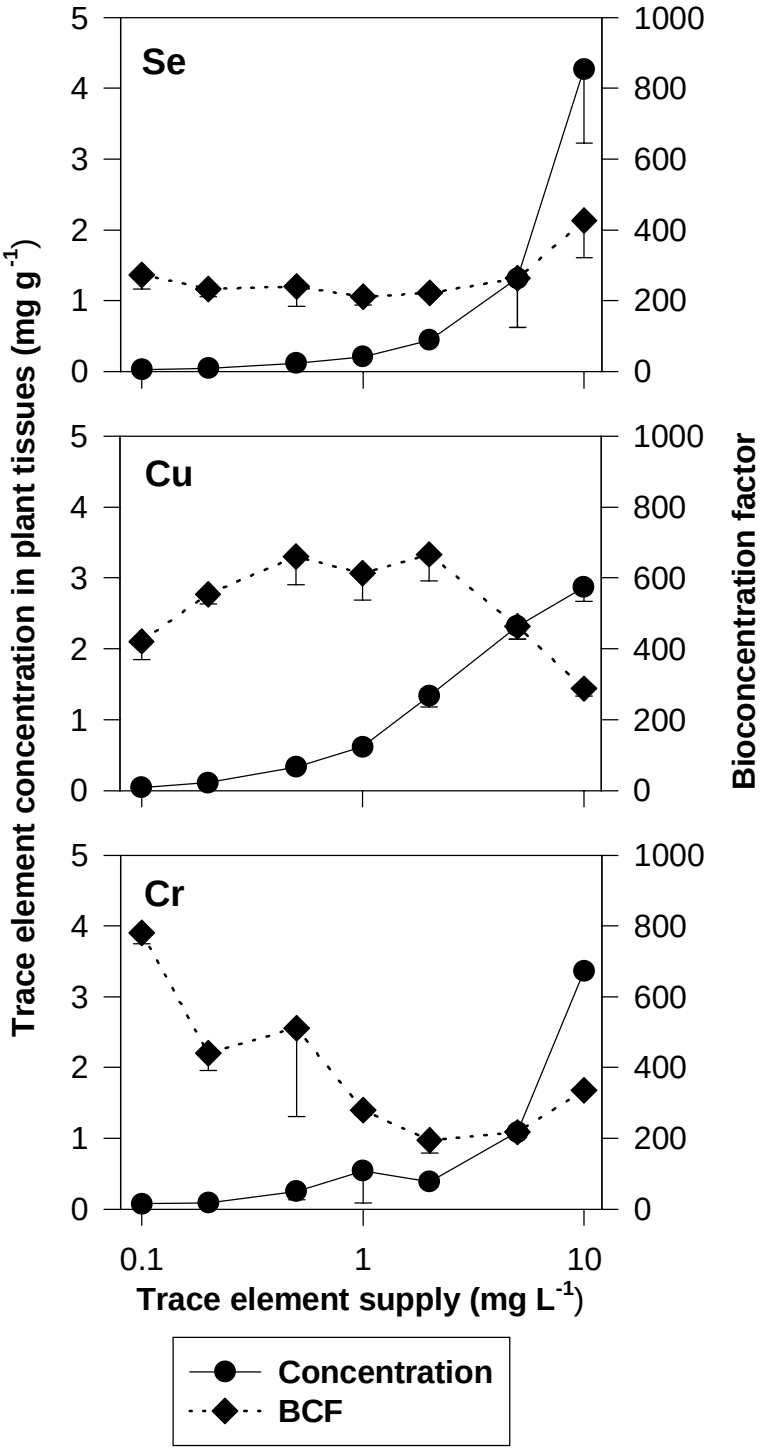

Figure 6-2
Influence of trace element supply concentration of Se (*top*), Cu (*middle*), and Cr (*bottom*) (each trace element supplied individually in the nutrient solution) on the trace element concentration in duckweed dry plant tissues and trace element bioconcentration factor. Vertical bars indicate standard deviations.

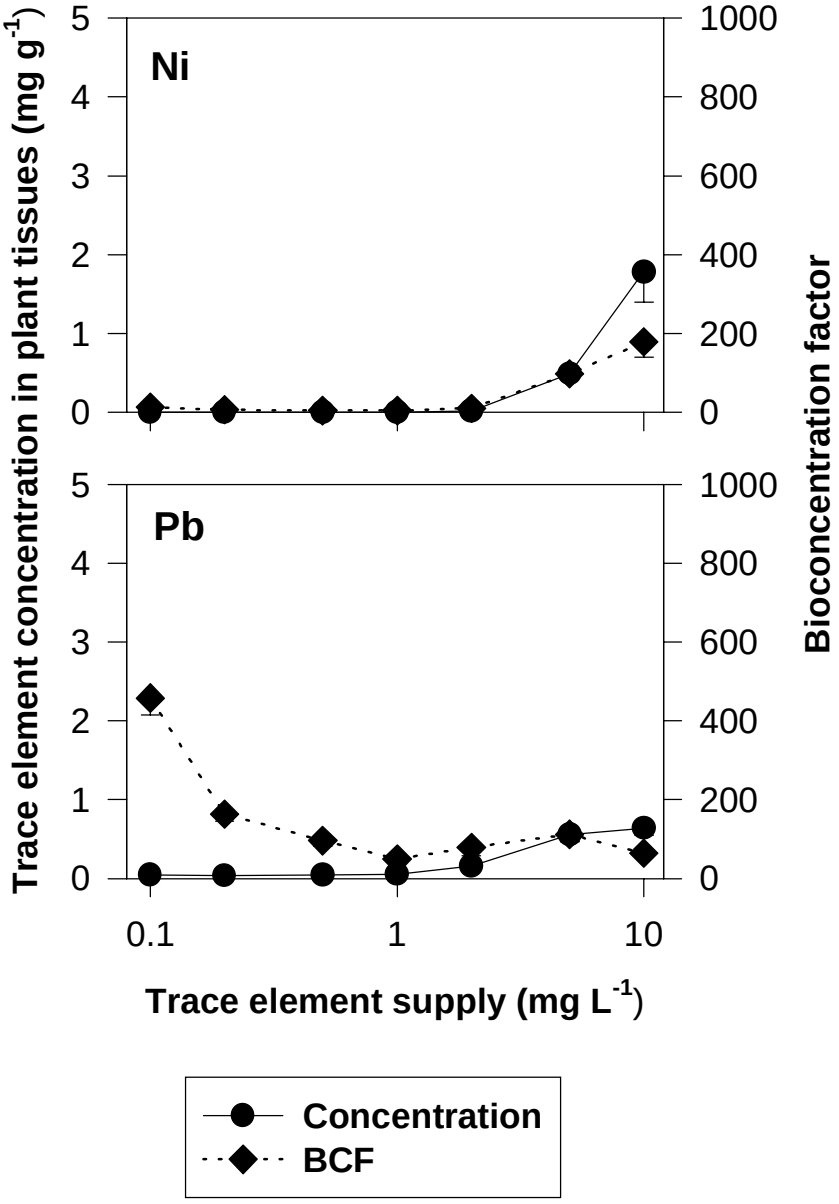

Figure 6-3
Influence of trace element supply concentration of Ni (*top*), and Pb (*bottom*) (each trace element supplied individually in the nutrient solution) on the trace element concentration in duckweed dry plant tissues and trace element bioconcentration factor. Vertical bars indicate standard deviations.

Nickel BCF did not change significantly as the supply concentration increased from 0.1 to 2 mg L^{-1}. Increasing the supply level above 2 mg L^{-1} caused a considerable rise in the Ni BCF.Contrary to Ni, Pb BCF decreased sharply with increasing the supply level from 0.1 to 1 mg L^{-1} after which Pb BCF remained mostly unchanged (Figure 6-3).

Generally, duckweed bioconcentrated the six elements under study to different levels at low supply concentrations compared to that at high supply concentrations. For example, at the lowest supply level (0.1 mg L^{-1}) the BCFs for Cr, Cu and Pb (420, 780 and 458, respectively) were higher than those obtained at the highest supply level (10 mg L^{-1}) (287, 336 and 63, respectively). On the other hand, BCF values of 1,333, 427 and 179 for Cd, Se and Ni, respectively, were higher at 10 mg L^{-1} than those (541, 273 and 13, respectively) obtained at 0.1 mg L^{-1} supply level.

6.4 Discussion

The results show that, under our experimental conditions, duckweed accumulated Cd most (i.e., to the highest concentration in plant tissue), then Se, Cu, Cr, Ni and Pb in descending order. Each of these elements is dealt with separately below. Examination of the relationship between the concentration of trace element in plant tissues and the supply concentration of the element shows that tissue concentrations were much higher at 5 and 10 mg L^{-1} than at concentrations of 1 mg L^{-1} or less (Figures 6-1, 6-2 and 6-3). At high supply concentrations, duckweed attained concentrations of some of the tested elements (e.g., Cd, Se) to levels which were comparable to those found in known hyperaccumulators of these elements (Rosenfeld and Beath, 1964; Reeves et al., 1996). Even at low supply concentrations (below 1 mg L^{-1}), duckweed effectively bioaccumulated most trace elements; the bioconcentration factors were 300-500 for Cd, 500-800 for Cu, 400-700 for Cr, 200-300 for Se, and 50-450 for Pb.

Duckweed exhibited some reduced growth and visual symptoms of toxicity (e.g., chlorosis) at the higher supply concentrations. The toxicity effect of each trace element on plant growth was, in descending order of damage, Cu > Se > Pb > Cd > Ni > Cr (Table 6-1). Nickel and Cr induced very little damage to duckweed, there being no visual manifestations of toxicity with either element and growth reduction only at 10 mg L^{-1} Ni.

Cadmium

Of the six trace elements investigated here, duckweed accumulated Cd to the highest concentrations of 13 mg g^{-1} with a BCF ranging from 500 to 1,300 at various Cd supply levels. The maximal limit of accumulation of a trace element that must be achieved by a plant species to be classified as a hyperaccumulator and hence a good phytoremediator of that element is not yet well defined. The following concentrations in the dry matter of any above-ground tissues have been suggested as thresholds to define hyperaccumulation in terrestrial plants: 10,000 mg kg^{-1} (1.0%) for Zn and Mn; 1,000 mg kg^{-1} (0.1%) for Ni, Co, Cu, Cr and Pb; 100 mg kg^{-1} (0.01%) for Cd and Se (Reeves et al., 1996). However, the use of element concentration in dry plant tissues as a criterion for identifying plant species that can be good phytoremediators does not take into account the trace element concentration in the root substrate. The bioconcentration factor may sometimes be a better indicator if the external trace element level is too low. Thus, in our treatment of what to be considered a good accumulator we will base our discussion on two arbitrary criteria: 1) its ability to take up better than 0.5% DW of a given element, and 2) its ability to bioconcentrate the element in its tissues, e.g., a BCF of over 1000 (a 100-fold compared on a fresh weight, or *in vivo* basis).

Based on the above criteria, our results show that *Lemna minor* is a very good accumulator of Cd (1.3% Cd, BCF = 1,300). However, some other wetland plant species have been shown to exhibit higher accumulation of Cd and, therefore, are considered excellent Cd accumulators. In two separate studies, Muramoto and Oki (1983) and Muramoto et al. (1989) showed that water hyacinth accumulated 36 and 10.6 mg Cd g^{-1}, respectively. Several wetland species attained higher BCF values than duckweed. Rai et al. (1995a) reported Cd BCF values ranging from 2,125 to 29,000 for six wetland plant species (*Ceratophyllum demersum, Spirodela polyrhiza, Bacopa monnieri, Hygrorrhiza aristata, Vallisneria spiralis* and *Alternanthera sessilis*) and two algae (*Hydrodictyon reticulatum* and *Chara corallina*). Other species of *Lemna* were also shown to be even better accumulators of Cd than *L. minor*. Researchers have found high levels of Cd accumulation in *L. giba* and *L. trisulca.* These species accumulated 12.8 and 2.3 mg g^{-1} Cd with a Cd BCF of 5,953 and 3,594, respectively (Huebert and Shay, 1993, Devi et al, 1996).

By comparison, other wetland plant species proved to be poor accumulators of Cd. *Scirpus robustus* and *Spartina patens* accumulated 0.2 and 0.25 mg g^{-1} Cd when exposed to 0.5 and 1.0 mg L^{-1} Cd, respectively (Lee et al., 1981). Very low Cd BCFs were observed for many wetland species, e.g., 73 for cattail (McNaughton et al., 1974), 400 for bulrush and *Bacopa monnieri* (Lee et al., 1981; Sinha and Chandra, 1990), 250 for *Spartina patens* (Lee et al., 1981).

Selenium

In terrestrial plants, most plants accumulate low amounts of Se in the order of 1 µg g^{-1} dry weight (DW) or less (Läuchli, 1993). However, there are several plant species, "selenium accumulators", which occur only on seleniferous soils and which accumulate as much as 4 mg g^{-1} DW (Läuchli, 1993). Unfortunately, little information is available on the uptake and accumulation of Se by aquatic plants. Ornes et al. (1991) examined the ability of four floating aquatic plants to accumulate Se from water. Their results show that *Azolla caroliniana* absorbed Se to the highest tissue concentration (1 mg Se g^{-1} DW), followed by *Salvinia rotundifolia* (0.7 mg g^{-1}), *Lemna minor* (0.5 mg g^{-1}), and *Eichhornia crassipes* (0.3 mg g^{-1}). In another study, hydroponically grown saltgrass accumulated 429 µg g^{-1} Se with a Se BCF of 429 (Wu and Huang, 1991).

In our study, Se was the second most accumulated element by duckweed; it reached a maximum of 4.27 mg Se g^{-1}. The greatest Se BCF attained by duckweed in our study was 850. Thus, duckweed may be considered as a modest accumulator of Se. However, our study indicates that duckweed compares well to Se accumulator species in that it accumulates Se to comparable concentrations. In additions, in an outdoor study conducted by Allen (1991) eight different wetland plant species were grown in an experimental wetland with a water Se concentration of 10 µg L^{-1}. Among all the tested wetland plant species it was found that duckweed attained the highest concentration of Se with a BCF of 11,500. Thus, duckweed shows considerable potential for the phytoremediation of Se-polluted waters.

Copper

Copper differs from all the other five elements tested in this study in that it is the only trace element essential for plant growth. Duckweed appears to be a good accumulator of Cu. Bassi and

Sharma (1993) found that *L. minor* accumulated as much as 15 mg Cu g^{-1} DW. In another study, Jain et al. (1989) reported that *L. minor* accumulated 5.23 mg g^{-1} Cu when exposed to 8 mg Cu L^{-1} for a period of 10 days. Tissue Cu concentrations for *Lemna* ranged from 0.25 to 4.5 mg g^{-1} under various experimental conditions and a Cu supply ranging from 0.2 to 10 mg L^{-1} (Jain et al., 1988 and 1989; Dirilgen and Inel, 1994). The highest Cu concentration attained in our study was 3.36 mg Cu g^{-1} at 10 mg Cu L^{-1} supply level. By comparison, water hyacinth supplied with 10 mg L^{-1} accumulated 7.2 mg g^{-1} in roots (Low et al., 1994). *Bacopa* (whole plant tissues), *Typha latifolia* (roots), and *Scirpus* (roots) accumulated Cu in the range of 2 to 3 mg g^{-1} when grown in solution culture (Sinha and Chandra, 1990; Taylor and Crowder, 1983b; Gupta et al., 1994; Sela et al., 1989).

In the present work, duckweed bioconcentrated Cu in its dry matter to approximately 200- to 800-fold the external concentration of Cu. BCF values of 3000 (Bassi and Sharma, 1993) and 696 to 1,655 (Jain et al., 1988; 1989) were reported by other researchers for *L. minor*. This range of BCF values is much higher than those reported for other wetland plant species, e.g., below 30 for cattails and cordgrass (Taylor and Crowder, 1983a; Alberts et al., 1990).

Chromium

Our results show that although duckweed attained greater Cr concentrations in its tissues compared to other wetland plant species, its BCF values were much lower than those reported by other researchers for other wetland plant species. In this study, Cr concentration in duckweed tissues ranged from 0.04 to 2.87 mg Cr g^{-1}, depending on supply concentration. By comparison, *Ceratophyllum demersum* accumulated 0.16 to 0.87 mg Cr g^{-1} (Garg and Chandra, 1990), *Chara* and *Hydrilla* accumulated 0.29 and 0.59 mg g^{-1} Cr, respectively (Rai et al., 1995b), water hyacinth accumulation capacity ranged from 0.05 to 0.15 mg g^{-1} in various treatments (Zaranyika and Ndapwadza, 1995; Jana, 1988), while *Scirpus validus* and *Cyperus esculentus* accumulated 0.55 and 0.73 mg g^{-1} Cr, respectively (Lee et al., 1981).

Greatest Cr BCF values were reported for *Ceratophyllum demersum* (15,330-31,400) (Rai et al., 1995a; Garg and Chandra, 1990) and for *Hydrodictyon reticulatum* (11,394) (Rai et al., 1995a). Chromium BCF values in this study ranged from 280 to 660. Thus, duckweed may be considered as a modest accumulator of Cr. For wastewaters contaminated mainly with Cr, other wetland plant species may remove Cr faster than duckweed. However, for wastewaters contaminated with Cd, Cu or Se in addition to Cr, duckweed will be an excellent choice since it will remove most of the Cd, Cu and/or Se as well as appreciable amounts of Cr.

Nickel

Among all wetland plant species that were tested so far for Ni accumulation, water hyacinth, *Azolla* and *Salvinia* proved to be the best accumulators of Ni. Water hyacinth attained a Ni BCF of 1601 (Zarankiya and Ndapwadza, 1995), while *Azolla* and *Salvinia* accumulated 9 and 6.3 mg g^{-1} Ni, respectively, and were recommended to be used as indicators of Ni pollution (Sela et al., 1989; Srivastava et al., 1994; Sen and Bhattacharyyra, 1994). Our results showed that *L. minor* accumulated 1.79 mg g^{-1} when supplied with 10 mg L^{-1} Ni in hydroponics. In an earlier study, Jain et al. (1988) reported that *L. minor* accumulated 0.46 mg g^{-1} Ni when supplied with 8-10 mg

L^{-1} Ni in solution culture. *Lemna minor* is clearly a poor accumulator of Ni. Other wetland plant species were also shown to be poor accumulators of Ni. For example, *Scirpus* and *Cyperus* accumulated 0.11 and 0.15 mg g^{-1} Ni, respectively (Lee et al., 1981).Cattails in a solution culture accumulated 1 and 0.4 mg g^{-1} Ni in their roots and shoots, respectively (Taylor and Crowder, 1983b).

Our results also indicate that the ability of *L. minor* to bioconcentrate Ni is very poor at low Ni concentrations (0.1 to 2.0 mg L^{-1}); it increased to near 200 as the Ni supply concentration exceeded 2 mg L^{-1}. In other studies, duckweed was reported to ha Ni BCF of 550 and 562 (Sharma and Gaur, 1995; Jain et al., 1990). Some other wetland plant species exhibited even lower BCF values than duckweed: for example *Cyperus* and *Scirpus* had a BCF of 110 and 150, respectively (Lee et al., 1981).

Lead

The greatest amount of Pb accumulated by a wetland plant species was 25.8 mg g^{-1} for water hyacinth (supplied with 8 mg Pb L^{-1}) (Muramoto and Oki, 1983). *Lemna polyrhiza* accumulated 10 mg Pb g^{-1} (supplied with 10 mg Pb L^{-1}) (Sharma and Gaur, 1995). These plant species are obviously very good accumulators of Pb and would be excellent for phytoremediation. Other species have exhibited very high BCFs, ranging from 2,133 to 8,064 (8,064 for *C. demersum*, 2,521 for *S. polyrhiza*, 2,133 for *Chara*, and 7,174 for *Hydrorrhiza*) which would also qualify them as good phytoremediators (Rai et al., 1995a). By comparison, our study with *L. minor* shows that it accumulated only 0.63 mg g^{-1} and attained BCFs ranging from only 50 to 460. Thus, *L. minor* appears to be a poor accumulator of Pb and not useful from a phytoremediation standpoint. Lead accumulation in the wetland plants *Cyperus, Typha* and *Myriophyllum* ranged from 0.44 to 1 mg g^{-1} (Lee et al., 1981; Welsh and Denny, 1980) and may also not be useful for Pb phytoremediation.

7
CONCLUSIONS AND ACKNOWLEDGMENTS

7.1 Conclusions

Several plant species (e.g., water hyacinth, duckweed, smartweed, brass buttons, cattail, and saltmarsh bulrush) were identified as superior candidates for the phytoremediation of trace elements in constructed wetlands. These plants were shown to be excellent candidates for the removal of specific trace elements under controlled environment (hydroponic) conditions. Plant uptake curves determined hydroponically were directly relevant to field situations because hydroponically-grown plants are supplied with soluble forms of the trace element under study. Plants grown in the field also absorb only soluble forms of trace elements, even though many insoluble forms may exist in the substrate as well. Therefore, by using hydroponics, it was possible to define precisely the amount of soluble trace element that each plant species was exposed to, and the composition and pH of the nutrient medium. This enabled different plant species to be directly compared under fixed conditions. The plant species mentioned above that efficiently removed trace elements under hydroponic conditions are therefore also very likely to be efficient for trace element removal under field conditions. Constructed wetlands planted with these superior plant species will be very effective at removing trace elements from contaminated wastewater.

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