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UPTAKE AND DISTRIBUTION OF SELENIUM IN DIFFERENT FERN SPECIES

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There has been an interest in using hyperaccumulating plants for the removal of heavy metals and metalloids. High selenium (Se) concentrations in the environment are detrimental to animals, humans, and sustainable agriculture, yet selenium is also an essential nutrient for humans. This experiment was conducted to screen fern plants for their potential to accumulate selenium. Eleven fern species,* **Pteris vittata, P. quadriaurita, P. dentata, P. ensiformis, P. cretica, Dryopteris erythrosora, Didymochlaena truncatula, Adiantum hispidulum, Actiniopteris radiata, Davallia griffithiana,** *and* **Cyrtomium fulcatum,** *were grown under hydroponic conditions for one week at 20 mg L^{-1} selenate or selenite. Root Se concentrations reached 245–731 and 516–1082 mg kg^{-1} when treated with selenate and selenite, respectively. The corresponding numbers in the fronds were 153–745 and 74–1,028 mg kg^{-1} with no visible toxicity symptoms. Only three fern species were able to accumulate more Se in the fronds than the roots, which were* **D. griffithiana** *when treated with selenate,* **P. vittata** *when treated with selenite, and* **A. radiata** *regardless of the forms of Se.* **A. radiata** *was the best species overall for Se accumulation. More research is needed to further determine the potential of the fern species identified in this study for phytoremediation of the Se contaminated soils and water.

KEY WORDS: selenium, ferns, accumulation, phytoremediation

INTRODUCTION

Selenium (Se) is a naturally occurring metalloid found primarily in sedimentary rock formations in dry areas of the world (Frankenberger and Karlson, 1994). As a required trace element in the diet of animals and humans (Lewis, 1976), it is, however, a potential toxicant for human beings, livestock, plants, water fowl, wild fowl, and bacteria (Frankenberger and Karlson, 1994; Ohelendorf *et al.*, 1986). Se is essential to the animal diet in the range of 0.1 to 0.3 ppm. The recommended daily allowance (RDA) for adult humans ranges from 55 to 70 μg/day (NAS, 2000). High Se intake may be defined as 750 μg per person per day (Haygarth, 1994). Excess Se has been implicated in birth defects and sterility in fish and other wildlife. Also, in humans excessive exposure to Se may cause fatigue: the loss

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of hair, teeth, and nails; and sometimes even death (AMA, 1989). The U.S. Environmental Protection Agency (EPA) drinking water standard for selenium is 0.050 mg L^{-1} and the World Health Organization (WHO) guideline is 0.010 mg L^{-1}. The Ambient Water Quality Criterion for Se is 0.005 mg L^{-1}.

Recently, Se has gained attention as the element responsible for water fowl toxicity attributed to agricultural irrigation practices in the Se-rich soils of the San Joaquin Valley in California (Läuchli, 1993) and the Kendrick Irrigation District near Casper, Wyoming (See and Nafiz, 1992). The average Se concentration of the inflow to the Kesterson Reservoir (San Joaquin Valley) is about 300 μg L^{-1} (Tanji *et al.*, 1986). The maximum shallow groundwater Se concentration in the San Luis Drain service area of the San Joaquin Valley is 3800 μg L^{-1} (Deveral *et al.*, 1984). The Salinity Drainage Task Force Committee in California (UC Salinity/Drainage Program, 1993) has evaluated several strategies for reducing the load of Se from entering the effluents. One principle strategy is to improve water-management practices and irrigation efficiency of agriculture lands to reduce effluent volume.

In connection with irrigation and drainage management, phytoremediation has been evaluated as an alternative to remediate soil with high Se concentration. In this approach, plants, which accumulate and volatilize Se from contaminated soils and sediments, are cultivated (Bañuelos *et al.*, 2002; Berken *et al.*, 2002; Pickering *et al.*, 2003; Pilon Smits *et al.*, 1999; Wu *et al.*, 2003; Zayed *et al.*, 1998). The Se could then be extracted from soil/water, translocated to shoot tissue, and removed as Se-laden plant material.

For phytoremediation to be successful, the selection of plant species that are efficient in Se accumulation is of primary importance. Several plants species classified as Se accumulators have the ability to extract and/or accumulate large amounts of Se, e.g., *Astragalus bisulcatus* (Byers, 1936) and *Stanleya pinnata* (Parker *et al.*, 2003). However, the uses of Se-accumulating plants are limited due to their low biomass and slow undefined growth requirements. Currently, field research on Se phytoremediation is in the experimental stage and several field experiments are ongoing in California (Bañuelos *et al.*, 2002; Terry and Bañuelos, 1999). Nearly all research on phytoremediation of Se-contaminated soils has been focused on angiospermic plants. Hence, little attention has been paid to the potential use of fern species as a tool for Se phytoremediation.

Recently, Komar *et al.* (1998) and Ma *et al.* (2001) reported an extremely efficient arsenic hyperaccumulator, *Pteris vittata* L., commonly known as Chinese Brake fern. This plant accumulates nearly 2.3% arsenic in its aboveground biomass and has many desirable attributes for use in the phytoremediation of contaminated soils, generating interest in using ferns for this purpose. Though several *Pteris* species have been identified as good accumulators of arsenic (Francesconi *et al.*, 2002; Meharg, 2003; Zhao *et al.*, 2002), little is known about their potential for the uptake of Se, which has a similar chemistry to arsenic. This lack of knowledge about fern plants with regard to their ability to accumulate Se has led to this laboratory study.

The objective of this research was to gain insight on the Se accumulation of ferns by comparing 11 different fern species (both *Pteris* and non-*Pteris*) under controlled environmental conditions. The specific objectives were to 1) determine Se distribution in the fern plants; 2) compare plant accumulation of two Se forms, selenate and selenite; and 3) evaluate Se accumulation between *Pteris* and non-*Pteris* ferns. After identifying the most efficient fern species, their potential can be further evaluated for phytoremediation of Se-contaminated water and soils.

MATERIALS AND METHODS

Five *Pteris* and six non-*Pteris* ferns species were screened for their potential for selenium accumulation. Included were *Pteris vittata* L., *Pteris quadriaurita* Retz., *Pteris dentata* Forssk., *Pteris ensiformis* Burm. F., *Pteris cretica* L., *Dryopteris erythrosora* (Eaton) Kuntze, *Didymochlaena truncatula* (Sw.) J. Sm., *Adiantum hispidulum* Sw., *Actiniopteris radiata* (Koenig ex Swartz) Link, *Davallia griffithiana* Hook., and *Cyrtomium fulcatum* (L.f) K. Presl. All fern plants were procured from a nursery (Milestone Agriculture, Inc., FL, USA) and were selected based mostly on their availability.

The fern plants with five or six pinnae were transferred to 500-mL opaque plastic containers with 0.2-strength Hoagland solution (Hoagland and Arnon, 1938) as the growth medium. The solution was aerated vigorously and replenished every three days. The ferns were grown hydroponically in a greenhouse with a temperature range of 20–26°C and humidity of ~70%. An 8-h photoperiod with a daily photosynthetic photon flux of 350 μmo m^{-2} s^{-1} at plant canopy was supplied by cool-white fluorescent lamps.

After 2 wk of equilibrium, 20-mg L^{-1} selenate as Na_2SeO_4 and selenite as Na_2SeO_3 were added to the solution, replicating the two main forms of Se found in soil and water. Deionized water was added as a control. Three replicates were used for each treatment. The plants were harvested after 1 wk of growth and separated into above- and below-ground biomass. The plant material was washed with tap water followed by deionized water, dried in an oven at 65°C, weighed, and ground for Se determination. The plant tissues were digested and prepared for Se analyses using EPA method 3050A. Included with each batch of digestions, one blank, one The National Institute of Standards and Technology (NIST) Standard Reference Material, one duplicate, and one sample spike were included for every 20 samples. Se in the plant digestate was analyzed by graphite furnace atomic absorption spectroscopy (Perkin Elmer SIMMA 6000, Norwalk, CT).

RESULTS AND DISCUSSION

Se Concentrations in the Roots and Fronds

Se accumulation was determined in the fronds and roots of 11 fern species grown in a nutrient solution containing selenate (SeO_4) or selenite (SeO_3) for 1 wk. Though the fern plants were exposed to relatively high concentrations of Se, i.e., 20 mg L^{-1} in a hydroponic system, no visible symptoms of phytotoxicity were observed in any of the plants. This indicates that the ferns were tolerant to Se. All 11-plant species showed considerable variation in Se accumulation (Figure 1). Root Se concentrations were 245–731 and 516–1082 mg kg^{-1} DW when treated with selenate and selenite, respectively. Similarly, frond Se concentrations were 153–745 and 74–1028 mg kg^{-1} DW. The plant species exhibiting the highest root Se concentration supplied with selenate and selenite were *D. truncatula* and *C. fulcatum*, respectively (Table 1).

The fern species varied widely in their ability to transport Se to the fronds. For selenate-treated plants (Figure 1a), the highest frond Se concentration was measured in *D. griffithiana* (745 mg kg^{-1}). For selenite-supplied plants, *P. vittata* had the highest frond Se concentration (1028 mg kg^{-1}, Figure 1b). Among all fern species tested, *A. radiata* was the overall most efficient in selenate and selenite accumulation and translocation. On the other hand, *D. erythrosora* contained relatively low levels of Se. It seems that *Pteris* ferns are no better than non-*Pteris* ferns in their ability to accumulate Se, which is both plant- and selenium-species dependent.

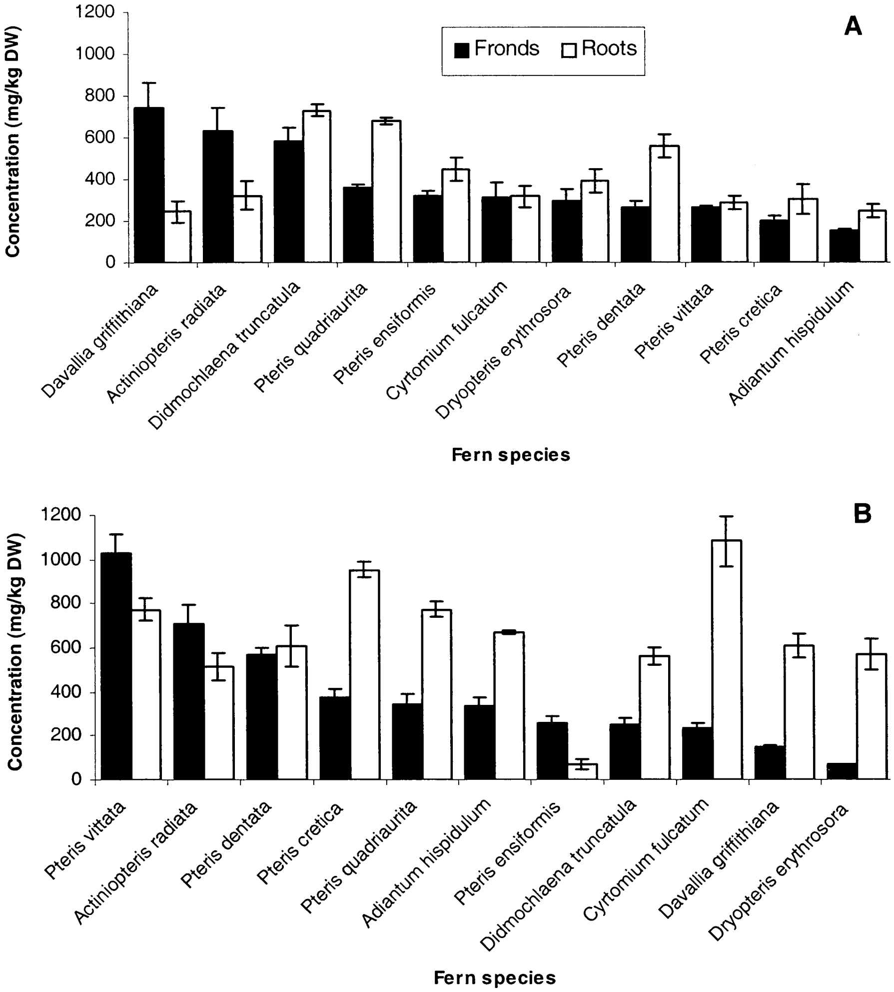

Figure 1 Se concentration in the fronds and roots of the 11 fern species supplied with selenate (A) and selenite (B). The fern plants were cultured hydroponically for 2 wk before exposing to 20 mg L^{-1} selenium for 7 d. The bars are standard error from three replicates.

Selenium Translocation and Bioconcentration

Selenium (Se) translocation from roots to shoots is of interest because it influences the harvestability of extracted Se; thus, phytoremediation efficiency. Generally, Se transport from the roots to the fronds is highly dependent on the chemical form of Se supplied. The frond/root ratios of Se in the plants were 0.62–3.0 for plants supplied with selenate (Figure 2a) and 0.13–1.3 for plants treated with selenite (Figure 2b). The bioaccumulation factors, Se concentration ratio in the plant to the media, were 7.65–37.2 when treated with selenate (Figure 3a) and 3.68–51.4 when treated with selenite (Figure 3b). The highest total Se accumulation (fronds + roots) was in *C. fulcatum* when supplied with selenate (Table 1), while *A. radiata* and *C. fulcatum* accumulated the most when supplied with selenite (Tables 1 and 2). When treated with selenate, *D. griffithiana* was the most efficient in translocating

Table 1 Selenium accumulation in fronds and roots (treated with selenate)

Fern species	Total frond boimass (g)	Total root boimass (g)	Total Se in a frond (mg)	Total Se in a root (mg)
Davallia griffithiana	1.66 ± 0.24	1.01 ± 0.20	1.23	0.25
Actiniopteris radiata	2.98 ± 0.30	1.00 ± 0.21	1.88	0.32
Didmochlaena truncatula	3.38 ± 0.23	0.71 ± 0.12	1.97	0.52
Pteris quadriaurita	2.04 ± 0.36	0.50 ± 0.06	0.74	0.34
Pteris ensiformis	2.40 ± 0.22	0.65 ± .0.13	0.76	0.29
Cyrtomium fulcatum	7.76 ± 1.21	3.91 ± 0.07	2.39	1.24
Dryopteris erythrosora	3.98 ± 0.31	1.08 ± 0.41	1.16	0.42
Pteris dentata	2.93 ± 0.33	0.81 ± 0.20	0.77	0.45
Pteris vittata	3.46 ± 0.52	0.86 ± 0.30	0.91	0.25
Pteris cretica	5.89 ± 1.33	1.21 ± 0.11	1.16	0.37
Adiantum hispidulum	4.40 ± 1.26	0.58 ± 0.13	0.67	0.14

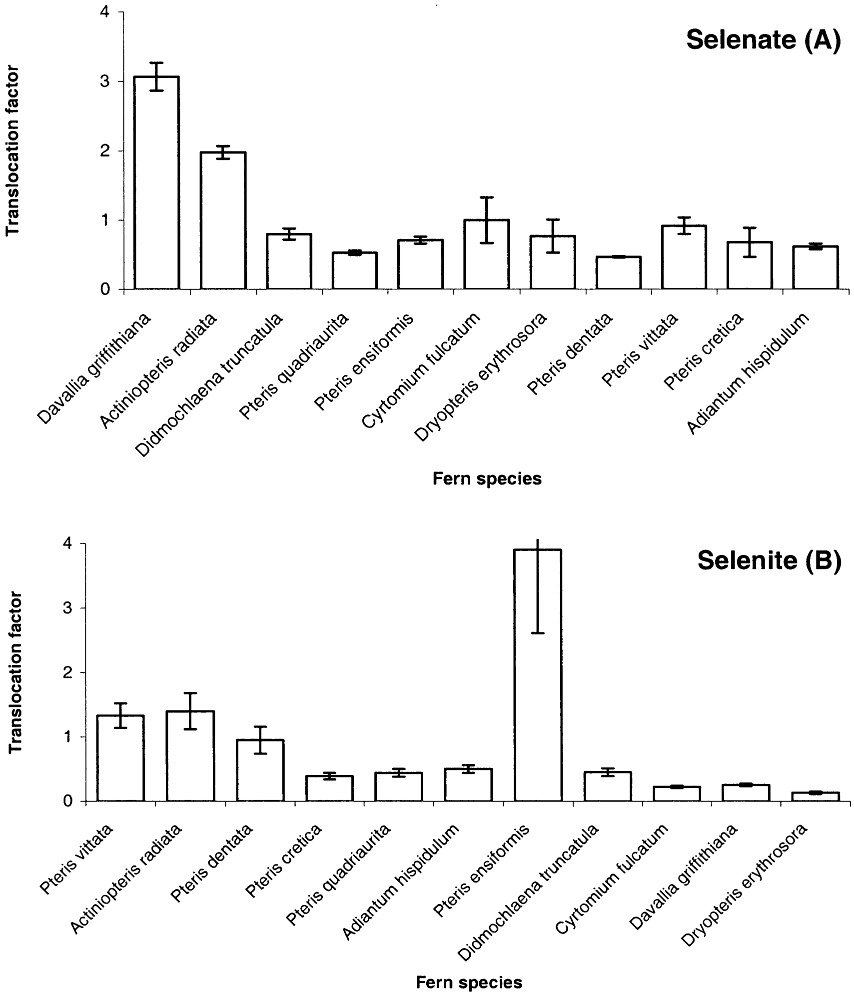

Figure 2 Se translocation factor (TF; concentration ratios in the fronds to roots) of the 11 fern species supplied with selenate (A) and selenite (B). The fern plants were exposing to 20 mg L^{-1} Se for 7 d. The bars are standard error from three replicates.

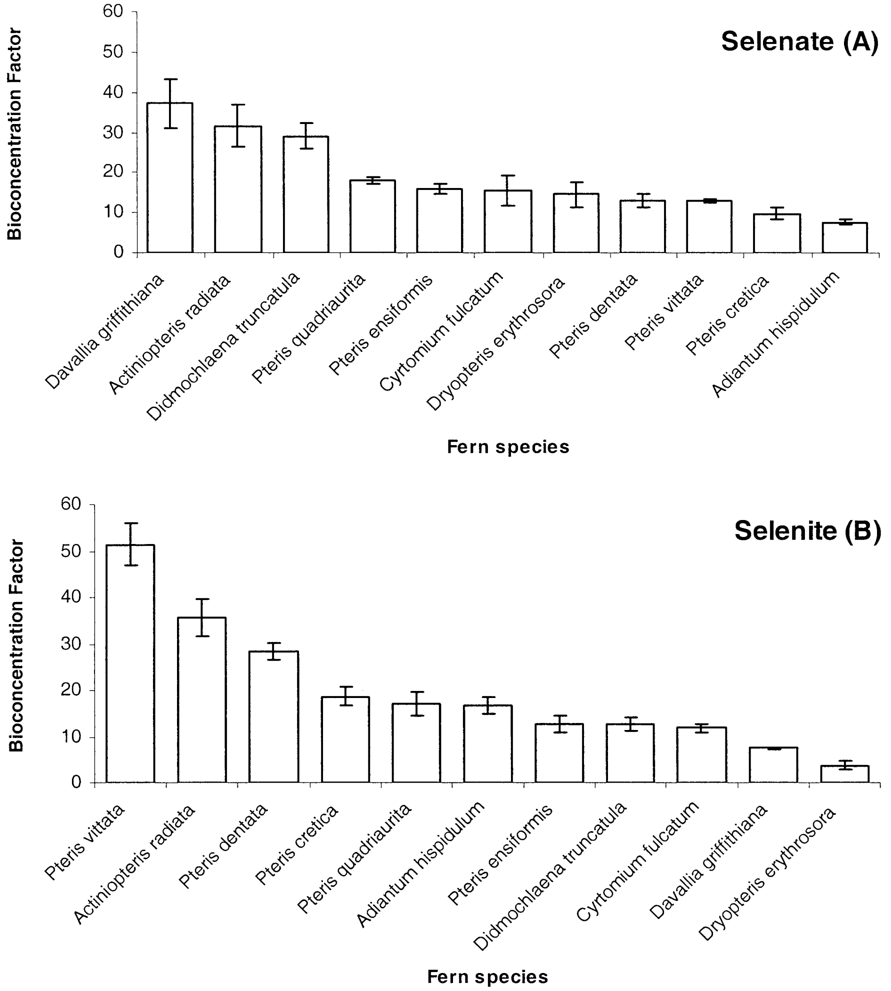

Figure 3 Se bioconcentration factor (concentration ratios in plant to the media) of the 11 fern species supplied with selenate (A) and selenite (B). The fern plants were cultured hydroponically with 20 mg L^{-1} selenium for 7 d. The bars are standard error from three replicates.

Se from roots to fronds. This was followed by *A. radiata*, which also was an efficient Se absorber (Figure 2a). However, when treated with selenite, the greatest transportation of Se to the fronds was observed in *P. ensiformis*, followed by *P. vittata* and *A. radiata* (Figure 2b).

Variation in the Se chemical form greatly influenced the ability of plants to accumulate and translocate Se from the roots to the fronds. In general, plant leaves accumulate Se to the highest levels when treated with selenate as opposed to selenite (Zayed *et al.*, 1998). The results of this work clearly established that the ferns studied varied greatly in their ability to accumulate Se within their tissues when supplied with different forms of Se. For some marine algae, Se inhibited growth only at high concentrations, i.e., 10 mM selenate or 1 mM selenite (Wong and Oliveira, 1991). These fern plants also seemed to a have high resistance to the Se toxicity, since no visual Se toxicity symptoms were observed in these fern plants, even at concentration of 20 mg L^{-1} Se. However, Hopper and Parker (1999)

Table 2 Selenium accumulation in fronds and roots (treated with selenite)

Fern species	Total frond boimass (g)	Total root boimass (g)	Total Se in a frond (mg)	Total Se in a root (mg)
Pteris vittata	1.95 ± 0.63	0.43 ± 0.06	2.00	0.33
Actiniopteris radiata	3.45 ± 0.17	1.13 ± 0.13	2.45	0.58
Pteris dentata	2.63 ± 0.51	0.36 ± 0.06	1.49	0.22
Pteris cretica	2.68 ± 1.18	0.36 ± 0.05	1.00	0.34
Pteris quadriaurita	3.16 ± 0.29	0.41 ± 0.06	1.08	0.31
Adiantum hispidulum	2.93 ± 0.54	0.49 ± 0.09	0.97	0.33
Pteris ensiformis	3.85 ± 0.75	0.38 ± 0.03	0.98	0.03
Didmochlaena truncatula	4.81 ± 0.85	1.20 ± 0.23	1.21	0.67
Cyrtomium fulcatum	7.07 ± 0.17	1.31 ± 0.25	1.68	1.42
Davallia griffithiana	2.56 ± 0.46	0.55 ± 0.07	0.39	0.33
Dryopteris erythrosora	6.12 ± 0.56	1.75 ± 0.33	0.45	0.99

reported phytotoxicity in ryegrass and strawberry clover when treated with selenate and selenite. According to them, selenite was more phytotoxic than selenate, especially for shoot growth. Se accumulation in the frond tissues varied substantially with the oxidation state of Se supplied. With respect to selenate, *D. griffithiana and A. radiata* were the most effective species in accumulating Se (Figure 1a). However, Se accumulation did not correlate with plant biomass. *C. fulcatum* had the largest biomass among the plants studied, but Se accumulation was not the highest. Similar results were also observed in *D. erythrosora.* However, plant Se uptake rate and plant biomass potential are important for efficient Se accumulation in ferns.

With respect to selenite accumulation, *P. vittata* and *A. radiata* were the most promising (Figure 1b), owing to fairly high levels of both plant biomass and frond Se concentration. These species could be further tested for treating selenite-contaminated water and wastewater. It is also interesting that *A. radiata* appeared to be a good accumulator of both selenate and selenite, while *P. vittata* is an accumulator of only selenite.

Regardless of the Se form supplied, *A. radiata* was by far the most efficient Se accumulator in and translocator to the fronds. It was an efficient absorber and stored a relatively large amount of Se. Since no visible Se toxicity symptoms were observed in these fern plants, greater accumulation of Se can be assumed given a longer growing period. At this (20 mg L^{-1}) level, Se is toxic to Indian mustard, a plant that has been used to study Se uptake (Table 3). Compared to the plants tested for Se accumulation in the literature, the ferns species tested in this experiment were comparable in terms of selenate accumulation and more efficient in terms of selenite accumulation (Table 3). Another difference discussed previously is that the ferns seemed to be much more tolerant to Se than those plants tested in the literature.

An important difference in how fern plants respond physiologically to selenate compared to selenite is that when selenate was supplied to the fern plants, it was translocated more readily to the fronds (Figure 2a). By contrast, selenite primarily accumulated in the roots with the exception of *P. ensiformis* (Figure 2b). This observation has also been reported for terrestrial plants (Pilon-Smits *et al.*, 1999) and has important consequences for Se phytoremediation. The differences in plant Se uptake and movement are attributable to the Se form supplied. Previous research has shown that while selenate uptake is driven metabolically, selenite uptake has a major passive component (Zayed *et al.*, 1998).

Table 3 Comparison of plant accumulation of selenate and selenite

Plant name	Treatment	Duration (week)	Accumulation (mg/kg) Roots	Accumulation (mg/kg) Shoots	Reference
Selenate					
Ferns	20 mg/L	1	245–730	153–745	Current research
Festuca arundinacea	4 mg/L	5	384	883	Wu *et al.*, 1988
Brassica napus	40 mg/kg	19	25–103	288-470	Bañuelos *et al.*, 1987A
Festuca arundinacea	40 mg/kg	19	NA	50	Bañuelos *et al.*, 1987A
Brassica oleracea	5 mg/kg	7		377	Bañuelos and Meek, 1989
Beta vulgaris	5 mg/kg	7		735	Bañuelos and Meek, 1989
Brassica juncea	3 mg/L	1	177	150	De Souza *et al.*, 2002
Brassica juncea	3 mg/L	1	10	550	Zayed *et al.*, 1998
Brassica juncea	2 mg/L	9	197–470	501–1092	Bañuelos *et al.*, 1987B
Brassica juncea	2 mg/kg	9	152–332	407–769	
Selenite					
Ferns	20 mg/L	1	70–1081	73–1028	Current research
Festuca arundinacea	4 mg/L	5	433	142	Wu *et al*, 1988
Brassica oleracea	5 mg/kg	7		49	Bañuelos and Meek, 1989
Beta vulgaris	5 mg/kg	7		29	Bañuelos and Meek, 1989
Brassica juncea	3 mg/L	1	700	100	Zayed *et al.*, 1998

While phytoextraction is an efficient technology for Se removal from contaminated water, Se accumulation in plant tissue may pose a threat to wildlife (Skorupa, 1998). However, fern plants are not typically eaten by wildlife due to their bitterness. A phytoremediation project would only require periodic harvesting of Se contaminated plant material from the site and suitable disposal there of, e.g., as a Se-containing amendment to fertilize Se-deficient soils or as a biofuel (Terry and Zayed, 1998). These results clearly show that ferns have potential for the phytoremediation of Se-contaminated soils and water. This is because they were not only tolerant, but also reasonably efficient in taking up Se.

CONCLUSION

The results presented here demonstrate the potential of fern plants to accumulate Se. We have shown that there was substantial variation in Se-accumulation potential among different fern species and we have identified some species with potential for the phytoremediation of Se-contaminated sites. The fact that little phytotoxicity was observed in the Se-laden plants coupled with relatively high Se accumulation suggests that it should be possible to use ferns to phytoremediate Se-contaminated water and soils. However, additional research is still needed to further explore this potential. Specifically, the following research is suggested, including further screening for more efficient Se hyperaccumulators: 1) optimization of Se uptake and translocation and 2) understanding the mechanisms of Se hyperaccumulation and tolerance.

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