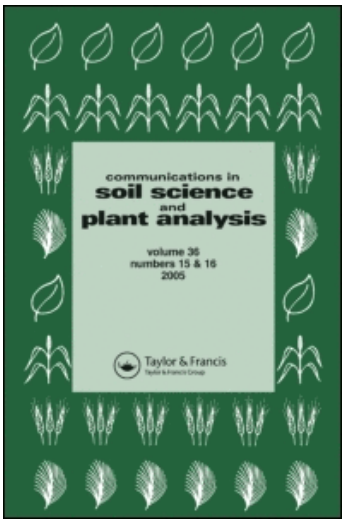

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Analysis of Arsenic Uptake by Plant Species Selected for Growth in Northwest Ohio by Inductively Coupled Plasma-Optical Emission Spectroscopy

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Analysis of Arsenic Uptake by Plant Species Selected for Growth in Northwest Ohio by Inductively Coupled Plasma–Optical Emission Spectroscopy

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Abstract: Arsenic (As) contamination is widespread in the industrial areas of northwest Ohio. Plant species that both take up As and are appropriate for the climate and growth conditions of the region are needed for phytoremediation to be successfully employed. Actively growing plants from 22 species of native genera were exposed to As in hydroponics systems (either 0, 10, or 50 mg As L^{-1}; 1 week) and commercially available potting mix (either 0, 10, 25, 100, or 250 mg As L^{-1}; 2 weeks), depending on their growth conditions. Aboveground plant tissues were harvested and digested, and concentrations of As were determined by inductively coupled plasma–optical emission spectrometry. The highest tissue concentrations of As (mg As kg^{-1} dw) were recorded in seven plant species: *Rudbeckia hirta* (661), *Helenium autumnale* (363 in tissues formed after exposure to As), *Lupinus perennis* (333), *Echinacea purpurea* (298), *Coreopsis lanceolata* (258), *Lepidium virginicum* (214), and *Linum lewisii* (214). These seven species are ecologically diverse, which suggests that phytoremediation of As using diverse assemblages of plants may be an option for a variety of environments.

Keywords: Greenhouse experiments, hydroponics, ICP, phytoremediation

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INTRODUCTION

Inhalation and ingestion of arsenic (As) can cause cancer as well as dermatological, respiratory, gastrointestinal, hepatic, renal, cardiovascular, and neurological problems (Abernathy 2001; Morton and Dunnette 1994). Because of these adverse health effects, the Occupational Safety and Health Administration (OSHA) set the permissible exposure limit for atmospheric As to 10 $\mu g\ m^{-3}$ (8-h workday; 40-h week) and the U.S. Environmental Protection Agency (USEPA) adopted a standard for As of 10 $\mu g\ L^{-1}$ for drinking water (USEPA 2001). Concern over As exposure was highlighted in 1997 when As topped the list of hazardous substances established by the Agency for Toxic Substances and Disease Registry (ATSDR) for the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). It has retained this position in each consecutive biannual review (U.S. Department of Health and Human Services 2005).

Arsenic is a natural component of numerous minerals and enters the environment as a component of pesticides, fertilizers, and industrial waste and through the combustion of fossil fuels (Cullen and Reimer 1989; Yan-Chu 1994). Traditional remediation practices use precipitation, filtration, and adsorption to remove As from water; stabilization, excavation, vitrification, and acid extraction are used to remove As from soil (USEPA 2002). Because As is a worldwide problem, it would be useful to develop less expensive, biological processes for the remediation of soils and waters in which it exists. Compared to the traditional approaches, bioremediation offers several economic and environmental benefits such as cost-effectiveness, lower end-point concentrations for pollutants, and less alteration and disruption of the affected environment (Schnoor et al. 1995).

Both microorganisms and plants can be used for the bioremediation of As. Microorganisms that oxidize arsenite (Santini 2000; Battaglia-Brunet et al. 2002) effectively reduce its solubility and may increase the rate of precipitation or filtration of As from contaminated water. Useful plant species can be exploited to extract, stabilize, or filter As from contaminated soil and water. However, it is becoming apparent that these plant species should be adapted to local environmental conditions (Krämer 2005) and accumulate relatively high tissue concentrations of As (Pivetz 2001) for phytoremediation to be successfully employed.

In this article, we report on the identification of plant species native to Ohio that have the ability to take up As at concentrations that are greater than soil levels. The present study was initiated because of the widespread occurrence of As in northwest Ohio that resulted primarily from the manufacturing industry in existence since the late 19th century. Arsenic was detected in soil (>5 mg kg^{-1} soil) from each of seven contaminated sites in Toledo during a recent survey; two of the sites contained As at levels of 60 to 100 mg kg^{-1} soil (unpublished data). Once identified, native As-accumulating

plant species will be tested for development of large-scale remediation technologies to treat regional brownfields, landfills, and wetlands.

MATERIALS AND METHODS

Experimental Conditions

Experiments with plants to be used in brownfields were done in a greenhouse at the Toledo Botanical Gardens (25–35°C day, 15–25°C night; 14 h of light was maintained using high-pressure sodium lamps). Seeds of 18 plant species (Table 1) were germinated with tap water in Oasis Horticubes® (Smithers-Oasis, Kent, Ohio). Seedlings with one or two true leaves and roots protruding from the cubes were transferred to 500-cm^3 plastic pots containing Sunshine Mix 1 (Sun Gro Horticulture, Bellevue, Wash.) and irrigated with deionized water (18 MΩ). Seedlings that attained a minimum fresh biomass of 1 g were irrigated thereafter with solutions (10 mL) of deionized water and As [0, 10, 25, 100, or 250 mg L^{-1} as sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$)] every other day for 2 weeks. To maintain adequate soil moisture, deionized water was added on days without As treatment. Aboveground plant tissues were harvested 24 h after the final treatment, oven dried (48–72 h, 55°C, Isotemp Oven, Fisher Scientific, Hampton, N.H.), and ground using an analytical mill (model A 11, IKA, Staufen, Germany). Replicates of plant species with a small collective biomass were pooled to obtain samples of at least 0.1 g dry weight (dw) for chemical analysis. Plants with biomass greater than 0.1 g dw were analyzed individually. Arsenic concentrations (mg As kg^{-1} dw) were reported for each species as mean ± standard deviation.

Experiments with plants to be used in areas with wet soil were done using hydroponics in July 2005 (25–35°C day, 15–25°C night; unsupplemented light regime). The roots of seedlings of four plant species (Table 2) were washed with tap water to remove soil particles, and the seedlings were placed into plastic net pots filled with clay beads. The net pots were transferred to nutrient film hydroponics systems (Aerojet, American Agritech, Tempe, Ariz.) with recirculating modified Hoagland's solution [pH = 6.0; deionized water, 2 mM calcium nitrate [$Ca(NO_3)_2$], 3 mM potassium nitrate (KNO_3), 0.5 mM monopotassium phosphate (KH_2PO_4), 1 mM magnesium sulfate ($MgSO_4$), 0.1 mM potassium sulfate (K_2SO_4), 5 μM iron nitrate [$Fe(NO_3)_3$], 25 μM sodium iron ethylenediaminetetraacetic acid (NaFeEDTA), 6 μM manganese chloride ($MnCl_2$), 6 μM zinc sulfate ($ZnSO_4$), 20 μM boric acid (H_3BO_3), 2 μM copper sulfate ($CuSO_4$)].

After 1 week of acclimation, the nutrient solution was replaced with modified Hoagland's solution containing either 0, 10, or 50 mg As L^{-1} as $Na_2HAsO_4 \cdot 7H_2O$. Five plants of each species were grown per As treatment; hydrogen peroxide (H_2O_2) was added to the recirculating

Table 1. Mean As concentrations (mg kg^{-1} dw $\pm$ standard deviation) in plant tissues

Plant species			Concentrations of As in irrigation water			
Scientific name	Common name	*n*	10 mg As L^{-1}	25 mg As L^{-1}	100 mg As L^{-1}	250 mg As L^{-1}
Ammi majus L.	Large bullwort	3	20.5 $\pm$ 10.1	46.8 $\pm$ 27.0	81.3 $\pm$ 11.1	178 $\pm$ 31.1[b]
Andropogon gerardii Vitman	Big bluestem	3	BLD	BLD	21.1 $\pm$ 6.5	78.8 $\pm$ 40.0[b]
Andropogon scoparius Michx.	Little bluestem	3	7.8 $\pm$ 2.7	BLD	BLD	43.1 $\pm$ 25.3[a]
Asclepias tuberosa L.	Butterfly milkweed	7	11.3 $\pm$ 4.3	16.3 $\pm$ 7.6	71.8 $\pm$ 42.7	98.0 $\pm$ 45.1[b]
Asclepias syriaca L.	Common milkweed	5	BLD	21.1 $\pm$ 3.5	42.5 $\pm$ 18.5	102 $\pm$ 33.1[b]
Coreopsis lanceolata L.[d]	Lance-leaved coreopsis	1	23.2	60.3	150	258[c]
Dalea candida Michx. ex Willd.	Prairie clover	3	BLD	BLD	BLD	BLD
Elymus virginicus L.	Virginia wild rye	3	14.5 $\pm$ 2.9	13.9 $\pm$ 4.1	32.4 $\pm$ 10.3	70.0 $\pm$ 6.9[b]
Echinacea purpurea (L.) Moench[d]	Purple coneflower	1	7.6	27.5	89.4	298[c]
Hesperis matronalis L.	Dame's rocket	3	BLD	17.4 $\pm$ 10.5	19.4 $\pm$ 7.1	105 $\pm$ 20.4[b]
Hibiscus militaris All.	Rosemallow	3	BLD	16.9 $\pm$ 8.7	57.6 $\pm$ 36.3	161 $\pm$ 41.4[b]
Lepidium virginicum L.	Virginia pepperweed	3	BLD	34.3 $\pm$ 0.9	155 $\pm$ 60.0	214 $\pm$ 20.4[c]
Linum lewisii Pursh	Flax	4	BLD	18.0 $\pm$ 7.7	99.2 $\pm$ 58.8	214 $\pm$ 133.5[c]
Lupinus perennis L.[e]	Wild lupine	2	6.3 $\pm$ 0.0	11.4 $\pm$ 6.3	26.0 $\pm$ 9.7	333 $\pm$ 273.0[c]
Oenothera grandiflora L'Her	Evening primrose	3	BLD	18.0 $\pm$ 6.3	42.0 $\pm$ 5.3	67.9 $\pm$ 24.4[b]
Panicum virgatum L.	Switchgrass	3	11.3 $\pm$ 2.5	20.7 $\pm$ 4.4	31.9 $\pm$ 9.4	64.6 $\pm$ 13.1[b]
Rudbeckia hirta L.[d]	Black-eyed susan	1	34.6	54.4	261	661[c]
Verbena hastata L.	Swamp verbena	3	BLD	20.4 $\pm$ 4.1	35.8 $\pm$ 8.7	65.6 $\pm$ 32.6[b]

Notes: Seeds of *L. virginicum* and *V. hastata* were obtained from the USDA-NPGS in Ames, Iowa. Seeds of all other species were obtained from Wildseed Farms in Fredericksburg, Tex.

[a]Low; [b]Elevated; [c]High levels of As accumulation. Concentrations of As in all control plants (0 mg As L^{-1}) were below the lower limit of determination (BLD;$<$5 mg As kg^{-1} dw); [d]Value was obtained from a composite of four or more plants; [e]Value was obtained as mean of two plants.

Table 2. Mean As concentrations (mg As kg^{-1} dw $\pm$ standard deviation) in plant tissues

Plant species			Concentrations of As in nutrient solution	
Scientific name	Common name	*n*	10 mg As L^{-1}	50 mg As L^{-1}
Asclepias incarnata L.	Swamp milkweed	2	32.7 $\pm$ 5.6	—[a]
Aster novae-angliae (L.) Nesom	New England aster	5	22.8 $\pm$ 9.2	132 $\pm$ 69.0
Helenium autumnale L.	Sneezeweed	4	31.7 $\pm$ 12.7	88.7 $\pm$ 49.0
Silphium perfoliatum L.	Cup plant	5	21.2 $\pm$ 11.3	BLD

Notes: All seedlings were obtained from Naturally Native Nursery in Bowling Green, Ohio.

Concentrations of As in all control plants (0 mg As L^{-1}) were below the lower limit of determination (BLD; <5 mg As kg^{-1}).

[a]All individuals of *A. incarnata* died in 50 mg As L^{-1}, and tissues were not analyzed by ICP-OES.

solutions as needed to control pH and minimize algal growth. Aboveground tissues of living plants were harvested after 7 days, oven dried (48–72 h, 55°C), and ground using the analytical mill. In the instances where substantial biomass formed during the treatment period, new shoots were analyzed separately from the remainder of the plant tissue. Each plant grown in hydroponics was analyzed individually, and tissue concentrations were reported as mean $\pm$ standard deviation.

Plant-Tissue Analyses

Plant tissue samples, to a maximum of 0.5 g dw, were mixed with analytical-grade nitric acid (HNO_3) (10 mL; Fisher Scientific, Pittsburgh, Penn.) in Teflon® digestion vessels (120 mL) and digested in a microwave (Mars Xpress, CEM, Matthews, NC) according to a modification of USEPA method 3051 (15 min ramp time, 200°C, 105 min hold time). After cooling, deionized water (20 mL) was added to each vessel. Resultant solutions were filtered (No. 2 Whatman filter paper) and refrigerated. Solutions were diluted to 3.5% HNO_3 prior to analysis. Aliquots (7.2 mL) were analyzed using an inductively coupled plasma–optical emission spectrometer (ICP-OES; IRIS Intrepid II, Thermo Electron Corporation, Waltham, Mass.) fitted with an autosampler (Cetac ASX-10, Thermo Electron Corporation). Resulting data were used for statistical analyses. Significant differences in concentrations of As in plant tissues were identified by analysis of variance (Tukey test; $\alpha = 0.05$) on log-transformed data using SAS (version 9; SAS Institute Inc., Cary, N.C.).

Standardization of the ICP-OES

Quantification of As by ICP-OES required appropriate instrument standardization. Plant tissue standards from the National Institute of Standards and Technology (NIST) with sufficient concentrations of As to determine limits of detection and appropriate wavelengths for As analysis were not available. Therefore, peach leaves (NIST No. 1547) were digested and spiked with known amounts of As. The wavelengths initially selected were 189.042 nm (order 1 and 2), 193.759 nm, and 197.262 nm. From this test, it was determined that the mean of orders 1 and 2 at wavelength 189.042 nm provided acceptable accuracy across all As concentrations encountered. This wavelength also maximized the signal-to-noise ratio in a variety of solution matrices and minimized elemental interferences. Following this procedure, the calculated limit of detection was 10 μg As L^{-1} in the ICP-OES solution matrix, which corresponds to 5 mg As kg^{-1} dw in plant tissue (Figure 1). Our limit of detection is comparable to those reported by the Utah State University Analysis Laboratory (10 mg As kg^{-1} for plant tissue) and the Service Testing and Research Laboratory at the Ohio State University (4.5 mg As kg^{-1}). It should be noted that the limit of detection for the ICP-OES (5 mg As kg^{-1} dw) was low enough to detect As concentrations in plant tissues that were slightly more than background levels encountered in the environment. Plants growing in uncontaminated environments typically have As concentrations of less than 2 mg As kg^{-1} dw (Brooks, Holzbecher, and Ryan, 1981).

RESULTS

Experiments with Plant Species to be Used at Brownfields

In preliminary experiments that were designed to determine the concentrations of As at which plants would grow in the test systems, *L. perennis* did not survive when treated with 500 and 1000 mg As L^{-1}. For this reason, 250 mg As L^{-1} was used as the highest As concentration in all subsequent experiments. Some of the plants exposed to the higher concentrations of As exhibited discoloration and/or stunted growth (Figure 2); however, only living plant tissues were harvested and analyzed. Each plant species took up the greatest amount of As at treatment concentrations of 250 mg As L^{-1} (Table 1).

It was interesting that the plant species could be placed into one of three groups, based on maximum tissue concentrations of As: (a) plants with relatively low ($<$60 mg As kg^{-1}), (b) elevated (60 to 200 mg As kg^{-1}), and (c) high concentrations ($>$200 mg As kg^{-1}; Table 1). Plant species in group c (*C. lanceolata*, *R. hirta*, *E. purpurea, L. virginicum*, *L. lewisii*, and *L. perennis*) had significantly greater tissue concentrations of As than those

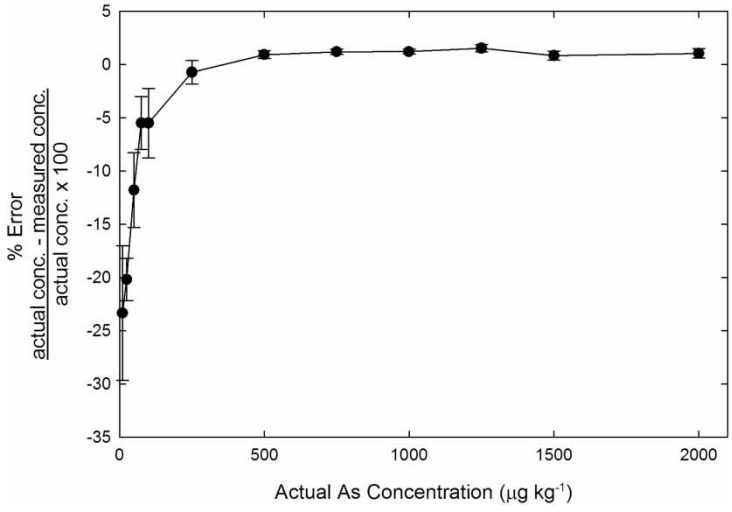

Figure 1. For ICP-OES standard solutions containing 10 μg As L^{-1}, the concentration was overestimated by 20% with 6.3% relative standard error. In standard solutions with 2 mg As L^{-1}, the concentration was underestimated by 1.0% with 0.4% relatve standard error.

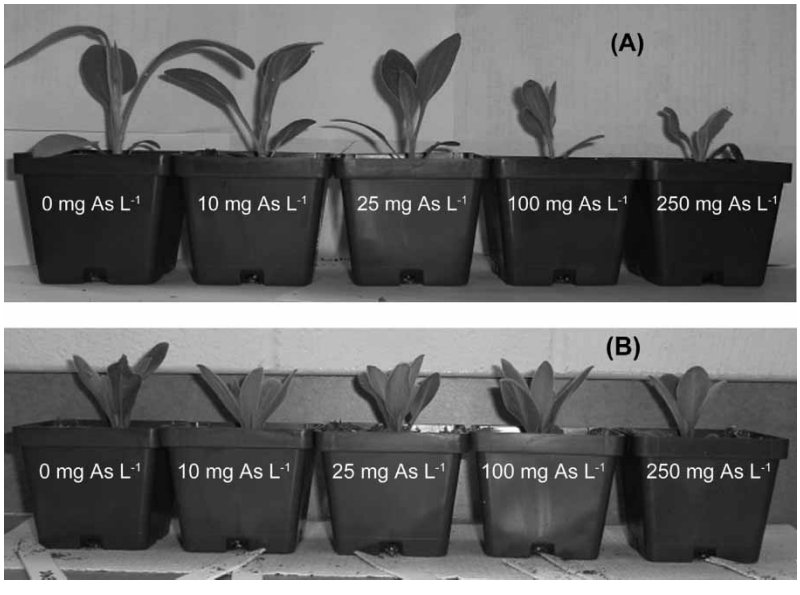

Figure 2. Some plant species exhibited discoloration or stunted growth with increasing treatment concentrations of As (A, *R. hirta*). Others exhibited no noticeable differences between control and treatment individuals (B, *H. matronalis*).

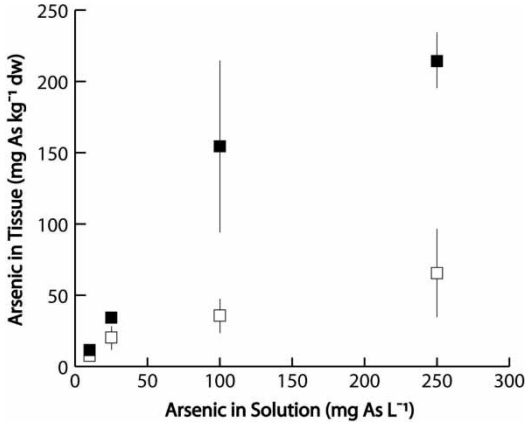

Figure 3. Plant species accumulated more As in the presence of increasing concentrations of As. This relationship was observed for species with greater [e.g., *Lepidium virginicum* (group c; closed squares)] and lesser [e.g., *Verbena hastata* (group a; open squares)] abilities to accumulate arsenic. Error bars represent standard deviations ($n = 3$).

in group a ($P < 0.05$; Figure 3); however, the concentrations for species in groups b and c were not significantly different. Plant species in group c will be used to develop large-scale remediation technologies useful in remediating As-contaminated brownfields and landfills.

Experiments with Plant Species to be Used in Areas with Wet Soil

There was no difference in tissue concentration at the 10 mg As L^{-1} treatment between any of the plant species selected for wet soils. At the greatest concentration of As used (50 mg As L^{-1}), individual plants of *A. incarnata* died during the treatment period, and As uptake by *S. perfoliatum* was inhibited (Table 2). In contrast, *A. novae-angliae* and *H. autumnale* had tissue concentrations of 132 and 88.7 mg As kg^{-1}, respectively, and significantly greater tissue concentrations of As than *S. perfoliatum*. Interestingly, newly formed stems and leaves of *H. autumnale* and *S. perfoliatum* had higher concentrations of As (363 mg and 191 mg As kg^{-1}, respectively) than older plant tissues (88.7 mg and 20.2 mg As kg^{-1}, respectively).

DISCUSSION

An important aspect of this study was to use plant species that would be applicable for phytoremediation efforts in northwest Ohio. Nineteen of the 22 plant

species tested are native to Ohio; the remaining three species (*A. majus*, *O. lamarckiana*, and *D. candida*) belong to native genera (USDA 2004). The selected species were chosen to span five groups: (i) *D. candida* and *L. perennis* are legumes, generally adapted to poor soil conditions and perhaps useful in the remediation of brownfields; (ii) *A. majus*, *A. tuberosa*, *A. syriaca*, *C. lanceolata*, *E. purpurea*, *H. militaris*, *L. lewisii*, *O. lamarckiana*, *R. hirta*, and *V. hastata* are prairie plants selected for potential use in restoring brownfields to native ecosystems; (iii) *A. incarnata*, *A. novae-angliae*, *H. autumnale*, and *S. perfoliatum* are plants adapted to moist soil conditions that could be used to remediate hydric soils and As-contaminated water; (iv) *H. matronalis* and *L. virginicum* are mustards; (v) *A. gerardii*, *A. scoparius*, *E. virginicus*, and *P. virgatum* are grasses. Several mustards (Anderson et al. 1999; Banuelos, Zambrzuski, and Mackey 2000; Salido et al. 2003) and grasses (Xia 2004; Gulz, Gupta, and Schulin 2005) are known to accumulate metals and metalloids. Based on their growth characteristics, plant species in groups i, ii, iv, and v were screened for As uptake in experiments with potting mix; those in group iii were screened in hydroponics systems.

On a comparative basis, the following plant species had significantly greater end-point tissue concentrations of As (mg As kg^{-1} dw): *R. hirta* (661), *H. autumnale* (363 in tissues formed during exposure to As), *L. perennis* (333), *E. purpurea* (298), *C. lanceolata* (258), *L. virginicum* (214), and *L. lewisii* (214). These seven species are ecologically diverse, suggesting that phytoremediation using these species may be an option for environments that include both wetlands and brownfields. Of the five ecological groupings of plants, only the grasses did not take up significant levels of As.

During the course of this investigation, we noted that special attention should be given to the method used in sampling plant tissue. For example, stems and leaves obtained from *H. autumnale* that formed during the exposure period had an average As concentration (363 mg As kg^{-1}) that was four-fold greater than the average concentration (88.7 mg As kg^{-1}) in older tissue from the same plants. Thus, it would be judicious in similar types of experiments to measure As concentrations in the newest plant material separately from older tissues, as was done by Meharg (2003).

Most researchers screen plants for As uptake based on the criteria that the species (i) belong to genera known to accumulate As (Zhao, Dunham, and McGrath 2002), (ii) are closely related to species that accumulate As (Meharg 2003), (iii) generally tolerate As (Schmidt et al. 2004), and (iv) are prevalent in environments contaminated with As (Carbonell-Barrachina et al. 1998). The approach used in this study was somewhat different, in that plant species with no previously known potential for As uptake were tested; their selection was based primarily on the ability to grow in a targeted environment and climate. It was encouraging that this approach yielded positive results and that several plant species are suitable for further testing.

Another approach to screening for uptake of As involves the collection and assay of plants growing at As-contaminated sites. A variety of plant genera and species that take up As have been identified using this approach (Reay 1972; Bech et al. 1997; Visoottiviseth, Francesconi, and Sridokchan 2002; Flores-Tavizon et al. 2003; Baroni et al. 2004). However, there are some fundamental drawbacks: (i) sampling may occur in sites with concentrations of As that are relatively low, so that the limits of a plant species' potential are not known; (ii) soil heterogeneity may lead to inaccurate estimates of As exposure (Meharg 2003); and (iii) dust particles may externally contaminate plant tissues, resulting in overestimates of As accumulation (Meharg 2003). The results from the current study are not influenced by these drawbacks.

Although the species in the current study took up As, none could be classified as a hyperaccumulator. Plant species that are hyperaccumulators of As are defined by the ability to attain tissue levels that are at least 1000 mg As kg^{-1} dw and exceed As concentrations in the substrate (Meharg 2003). In this study, the highest concentration of As (660.6 mg As kg^{-1} dw) was attained by *R. hirta*. In comparison, *Pteris vittata*, accumulates more than 5,000 mg As kg^{-1} dw when exposed to soil containing only 50 mg As kg^{-1} (Ma et al. 2001) and *Pityrogramma calomelanos* accumulates more than 8,000 mg As kg^{-1} dw in soil contaminated with 135 mg As kg^{-1} dw (Francesconi et al. 2002). Other hyperaccumulators of As include four species of fern in the genus *Pteris* (Ma et al. 2001; Zhao, Dunham, and McGrath 2002), *Lemna gibba* (Mkandawire and Dudel 2005), and *Lepidium sativum* (Robinson et al. 2003). *Jasione montana*, *Calluna vulgaris*, *Agrostis spp.* (Porter and Peterson 1975), *Silene vulgaris* (Schmidt et al. 2004), and *Lolium temulentum* (Bagga and Peterson 2001) accumulate high concentrations of As (greater than 1000 mg As kg^{-1} dw), but tissue concentrations do not exceed substrate concentrations.

It seems obvious that hyperaccumulators such as *P. vittata* and *P. calomelanos* offer the potential for rapid remediation, but they are not necessarily the best choices for every environment. Both plants are subtropical ferns that may not be suited for growth in more temperate areas. Instead, it would seem prudent to select plants that are native and adapted to the region in which As contamination exists for potential use in phytoremediation applications.

CONCLUSIONS

One of the ultimate goals is to develop a phytoremediation system for As-contaminated soil and water. This development is an ongoing process with the first step reported here: plant species for varied environmental conditions are screened for the ability to take up As. The remaining steps include field testing those plant species with the greatest remediation potential to

determine optimum environmental conditions and rates of As accumulation using field lysimeters and wetland mesocosms. Finally, the most appropriate plants and environmental conditions will then be incorporated into a phytoremediation system that will be tested at a contaminated site. Of the 22 plant species that were screened in this study, 7 were chosen for further testing: *R. hirta*, *L. perennis*, *L. virginicum*, *L. lewisii*, *C. lanceolata*, *E. purpurea*, and *H. autumnale*. The process of screening plants for their abilities to take up As will continue in an effort to expand the variety and selection of species appropriate for field testing.

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