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Screening Ornamentals for Their Potential as As Accumulator Plants

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Abstract

Arsenic-based pesticides, herbicides and insecticides are used in horticultural operations resulting in soil contamination around greenhouse structures. Phytoremediation and phytostabilization are two techniques for treating arsenic (As) contaminated soil. Several ornamental plant species, Iris (*Iris savannarum*), switchgrass (*Panicum virgatum*), *Tithonia rotundiflora*, *Coreopsis lanceolata*, sunflower (*Helianthus annuus*), and marigold (*Tagetes erecta*), were evaluated for their potential use as accumulator plants. Based on dry weight, tithonia and coreopsis were most sensitive to As. Tithonia had an 85% reduction in dry weight at 0.75 mg As L^{-1} and coreopsis a 65% reduction at 2.25 mg As L^{-1} solution concentration. Iris dry weight increased with increasing solution concentrations but As did not accumulate in tissue. At the high As rate, marigold and sunflower had uptake ratios of 7.4 and 16.6, respectively, and translocation factors near one. Both show little effect of As toxicity on dry weights production, therefore, are appealing candidates for phytoremediation and phytostabilization. Switchgrass and iris can be harvested multiple times a year, making them candidates for phytostabilization.

Keywords: arsenic, iris, marigold, sunflower, switchgrass, translocation factor, uptake ratio

Abbreviations Translocation factor: TF = shoot As / root As in mg As kg^{-1} plant dry weight; uptake ratio: UR = mg As kg^{-1} plant dry weight/ solution As concentration in mg L^{-1}

1. Introduction

Arsenic is a metalloid that can accumulate in soil as a result of mining and industrial activities; application of As based herbicides, insecticides, rodenticides and wood preservatives; and irrigation with water contaminated with As (Zhao, Ma, Meharg, & McGrath, 2009). Naturally occurring soil As can range from 1-40 mg As kg^{-1} soil (Walsh, Sumner, & Keeney, 1977), however, contaminated levels can reach as high as 2600 mg As kg^{-1} soil (Meharg, Naylor, & Macnair, 1994). In horticultural crop production systems, arsenic-based pesticides, herbicides and insecticides have led to considerable contamination on turf, orchards, and around greenhouses, shadehouses and other structures (Woolson, Axley, & Kearney, 1971; Murphy & Aucott, 1998).

Arsenic is not essential for plants (Marin, Masscheleyn, & Patrick, 1992) and has no known metabolic function. Plant species vary in their ability to tolerate As (Meharg, 1994) with toxicity threshold levels ranging from 5 to 100 mg As kg^{-1} dry weight for most plants (Kabata-Pendias & Pendias, 1992).

Brake fern (*Pteris vittata*) found growing on an As contaminated site in Florida was the first arsenic hyperaccumulator described in the literature (Ma et al., 2001). Ma reported that brake fern fronds growing in 1500 mg kg^{-1} As contaminated soil accumulated up to 15861 ug As g^{-1} plant dry weight over a two week growing period. Since that time several other fern species have been identified as As-hyperaccumulators: *Pteris cretica*, *Pteris longifolia*, *P. vittata*, *Pteris umbrosa* (Zhao, Dunham, & McGrath, 2002) and *Pityrogramma calamelanos* (Visoottiviseth, Francesconi, & Sridokchan, 2002). However, not all ferns have the ability to hyperaccumulate As. Meharg (2003) reported that *Pteris straminea* and *Pteris tremula* did not hyperaccumulate As in their fronds. Plants that accumulate As translocate a large portion of it to their shoot. The As hyperaccumulator *P. vittata* shipped 8 x more As from root to shoot than the nonhyperaccumulator *P. tremula* (Caille, Zhao, & McGrath,

2005) and 2.8 x more than *Pteris ensiformis* (Singh & Ma, 2006).

Several investigators have identified a variety of species other than ferns that hyperaccumulate or are tolerant to high levels of soil As. Ansari et al. (2013) screened ten Indian mustard (*Brassica juncea* L.) genotypes an reported that at 50 um As, mustard shoots contained from 1.84 to 3.65 mg As g^{-1} dry weight. As accumulation was reported in *Chilopsis linearis* (desert willow) by Castillo-Michel et al. (2009), in the flowering plant *Cytisus striatus* by Bleeker, Schat, Vooijs, Verkleij, and Ernst (2003), and in *Isatis capadocica*, a brassica, by Karimi, Ghaderian, Raab, Feldmann, and Meharg (2009).

Phytoremediation employs plants to remove toxic substances from the soil. An ideal candidate for phytoremediation would have vigorous growth to help prevent transportation of the substance off site by erosion. The plant should accumulate the toxic substance in large amounts and sequester it in easily harvestable plant parts. Plants defined as As hyperaccumulators have a Bioaccumulation Factor (BF) > 1000 ug As g^{-1} plant dry weight (Machado-Estrada, Calderón, Moreno-Sánchez, & Rodríguez-Zavala, 2012). They have a shoot-to-root As concentration ratio (Translocation Factor (TF)) higher than 1. However, plants with a BF > 1 can be useful in phytoremediation (McGrath & Zhao, 2003), especially if they have a TF >1. Ornamental plants generally, have not been investigated as a potential source for phytoremediation and stabilization. Ornamentals can provide a marketable commodity, for example cut flowers, to somewhat offset the cost of land taken out of production. In addition, these plants can provide an aesthetic quality to buildings located on contaminated sites. The purpose of this study was to screen several ornamentals for their potential use as As accumulator plants.

2. Materials and Methods

2.1 Plant Species

Six plant species were used in this study: Iris (*Iris savannarum*), switchgrass (*Panicum virgatum*), *Tithonia rotundiflora, Coreopsis lanceolata*, sunflower (*Helianthus annuus*), and marigold (*Tagetes erecta*). Iris rhizomes, approximately 10 cm in length were collected from a single clonal plant. To stabilize rhizomes until a root system began to develop, iris plants were set in rockwool. All other species were planted in a 25% perlite, 37% pine bark, 8% sand, 30% coir potting mixture. Switchgrass seed was evenly sowed in 28x53 cm trays. Once plants reached 10 cm in height, 30 18-cm sections of turf were cutout and placed into 26-cm diameter pots (3.8 L). Switchgrass was trimmed to a uniform 15 cm height before initiation of treatments. Tithonia, coreopsis, sunflower and marigold were planted four seeds to a pot and thinned to one plant per pot shortly after emergence. The study was conducted over three different time periods with two plant species growing during each period. Dates for each time period are given in Table 1. Tithonia, sunflower and marigold had at least two fully developed leaves before pots were attached to a hydroponic system. Iris, switchgrass and coreopsis were at least 10 cm tall and iris and coreopsis had ≥ 3 leaves when hydroponic systems were activated.

Table 1. Planting, initiation of arsenic treatments and harvest dates for six plant species: Iris (*Iris savannarum*), switchgrass (*Panicum virgatum*), *Tithonia rotundiflora*, *Coreopsis lanceolata*, sunflower (*Helianthus annuus*) and Marigold marigold (*Tagetes erecta*)

Species	Planting	Treatment	Harvest
Iris (*Iris savannarum*)	21-Dec-09	10-Mar-10	13-May-10
Switchgrass (*Panicum virgatum*)	21-Dec-09	10-Mar-10	13-May-10
Tithonia rotundiflora	6-Oct-10	20-Oct-10	16-Nov-10
Coreopsis lanceolata	6-Oct-10	20-Oct-10	23-Nov-10
Sunflower (*Helianthus annuus*)	1-Feb-11	16-Feb-11	22-Mar-11
Marigold (*Tagetes erecta*)	1-Feb-11	16-Feb-11	22-Mar-11

2.2 Hydroponic System

Six ebb-and-flow type hydroponic plant maintenance systems were setup. Each system contained a 132 L reservoir tank connected to 12, 3.8-L pots. A timer allowed the system to cycle between 30 min. wet and 4 hr. drain periods, beginning at 8:00 A.M., ending at 4:00 P.M. followed by a 12 hr. drain period. Each 132 L tank contained a modified Hoagland solution with 2.0 mM $Ca(NO_3)_2$, 3 mM KNO_3, 1.0 mM $MgSO_4$, 0.25 mM $Ca(H_2PO_4)_2$, 12.5 uM H_3BO_3, 1.0 uM $MnSO_4$, 1.0 uM $ZnSO_4$, 0.25 uM $CuSO_4$, 0.2 uM $(NH_4)_6Mo_7O_{24}$, and 10

uM Fe-EDDHA. Tap water used to mix nutrient solutions averaged 0.0028 mg L^{-1} As (0.448 mg per reservoir tank). Plants were allowed to acclimate to hydroponic feeding for a minimum of one week before beginning As treatments. Enough Na_2HAsO_4 was added to different reservoirs to make 0, 10, 20, 30, 40, 50 or 70 uM As as a final solution concentration. Iris, switchgrass, sunflower and marigold were grown in 0, 10, 20, 40, 50 or 70 uM As (0.0, 0.75, 1.5, 3.0, 3.75, 5.25 mg L^{-1} As, respectively) while coreopsis and tithonia were grown in 0, 10, 30, 50, or 70 uM As. Reservoir pH was adjusted daily to pH 6.5 with either NaOH or H_2SO_4. Nutrient and As solutions were replaced weekly. Arsenic waste water was collected, filtered to remove As, and filters properly disposed of as a hazardous waste. Plants were maintained in hydroponic solution until flowering, 40 to 121 days depending on the species. A portion of iris rhizome from the 0.0 and 5.25 As treatments were maintained in 0.0 and 133 uM (10 mg L^{-1}) As solution, respectively, for 366 days before plants flowered.

2.3 Sampling

Plant height was measured from the soil surface along the main stem to the upper most node for tithonia, sunflower and marigold or with coreopsis to the uppermost point when leaves were held upright. For iris the combined length of each leaf substituted for plant height. Prior to initial As treatment switchgrass was clipped to 10 cm height. Canopy height was measured at 76, 91 and 121 days and following each measurement the canopy was clipped back to a 15 cm height. Plant growth was calculated as height at harvest (accumulated height for switchgrass) minus initial height. Prior to applying treatments, and again at harvest the number of leaves was recorded for all species except switchgrass. The initial leaf number was subtracted from the final count to determine new leaf growth. Shoot and root tissue were harvested separately, oven dried at 45 °C until there was no longer a weight change with additional drying and the dry weights recorded. Dried tissue was stored for analysis. An uptake ratio

$$\text{UR} = \text{mg As kg}^{-1}\text{ plant dry weight/ solution As concentration in mg L}^{-1} \quad (1)$$

and translocation factor

$$\text{TF} = \text{shoot As / root As in mg As kg-1 plant dry weight} \quad (2)$$

were calculated for each species and each treatment.

2.4 Sample Analysis

Approximately 0.25 g of oven dried plant tissue was placed in 100 mL digestion tubes. Ten mL HNO_3 was added and samples digested in a Start D microwave digestion system (Milestone Inc., Shelton, Connecticut) for 15 min to reach 200 °C then kept at this temperature for an additional 15 min. Digests were diluted to 100 mL and stored at 4 °C prior to analysis. Arsenic concentration was determined by inductively coupled plasma – optical emission spectrometry with an iCAP 6300 Duo View (ThermoFisher Scientific, West Palm Beach, Florida). Data were analyzed and concentrations determined using ThermoFisher Scientific iCAP 6300 iTEVA software. Arsenic was determined at the most intense atomic emission.

2.5 Statistical Analysis

Each plant species was analyzed separately. The data represent means calculated from six replicated pots for each As treatment. Analysis of variance was preformed using the Proc Mixed procedure of Statistical Analysis System (SAS Inst., 1999). Tukey adjusted lsmeans were used for comparison at $P < 0.05$ unless stated otherwise. Arithmetic means were used to calculate uptake ratio and translocation factor.

3. Results

3.1 General Comments

Statistical analysis revealed significant differences in As concentration at $P \geq 0.05$ between treatments for all plants species used in this study. Significant differences in As concentration at $P \geq 0.05$ also were found between root and shoot tissue for all species. An increase in solution As lead to an increase in As tissue concentration, however, this was not the case with iris leaf and flower tissues. There were no differences for As between treatments. Iris and marigold dry weights tended to increase with increasing solution As up to 5.25 mg L^{-1}. In *Panicum virgatum* As uptake increased with successive harvests (data not shown).

3.2 Plant Physical Characteristics

Compared to the control, significant treatment dry weight differences in root and shoot tissue for coreopsis and tithonia first appeared at 2.25 and 0.75 mg L^{-1} solution As concentration, respectively (Table 2). At 5.25 mg L^{-1} coreopsis had an 84% reduction in total plant dry weight producing 29 fewer leaves and 15 cm less growth than the 0.0 mg As L^{-1} control. The overall inhibition of growth seen in coreopsis was even more striking in tithonia.

At 5.25 mg As L^{-1} there was a 98% reduction in dry weight, virtually no new leaves produced and 30 cm less growth than the control. Coreopsis and tithonia had URs > 20 and a TF of 0.07 and 1.13, respectively.

Table 2. Selected physical characteristics, uptake ratio (UR) and Translocation Factor (TF) of plants grown in varying As solution concentrations

Solution As concentration (ppm)	Root dry wt (g)	shoot dry wt (g)	New leaf growth	Plant growth (cm)	UR	TF
	Coreopsis lanceolata					
0.00	1.97 a†	4.96 a	43.8 ab	20.4 a	0.0 c	0.44 a
0.75	1.64 ab	3.91 ab	48.5 a	13.9 ab	85.4 a	0.17 b
2.25	0.70 bc	1.75 bc	27.3 abc	11.0 b	79.9 a	0.16 b
3.75	0.48 c	1.39 c	24.8 bc	8.8 b	52.5 b	0.13 b
5.25	0.44 c	0.69 c	15.0 c	5.5 b	73.0 ab	0.07 b
	Tithonia rotundiflora					
0.00	16.19 a	26.72 a	101.8 a	36.8 a	0.0 c	0.00 b
0.75	3.44 b	4.10 b	33.8 b	16.3 b	102.3 a	1.27 ab
2.25	1.27 b	1.55 b	9.8 c	10.3 bc	47.3 b	2.38 a
3.75	2.01 b	1.31 b	8.1 c	7.0 c	20.2 c	1.06 ab
5.25	0.61 b	0.46 b	-0.5 c	6.2 c	33.4 bc	1.13 ab
	Iris savannarum					
0.00	0.75 ab	2.29 ab	29.0	1467.2	0.0 b	2.93 a
0.75	0.42 b	1.15 b	28.3	1354.8	5.0 ab	1.23 a
1.50	0.41 b	1.10 b	20.7	874.7	5.9 ab	0.82 a
3.00	1.40 a	3.95 ab	28.5	1703.4	5.5 ab	0.63 a
3.75	1.06 ab	3.69 ab	24.8	1333.0	3.3 ab	1.54 a
5.25	1.62 a	5.31 a	17.3	1060.1	6.7 a	9.21 a
	Tagetes erecta					
0.00	15.91	54.12	58.3 b††	59.9	0.0 c	0.23 a
0.75	13.42	65.13	59.0 b	61.3	20.3 a	0.50 a
3.00	16.45	58.31	74.0 ab	59.2	7.3 bc	0.34 a
3.75	13.60	59.05	65.7 ab	52.9	10.6 b	0.27 a
5.25	14.58	68.13	81.3 a	63.4	7.4 bc	1.20 a
	Helianthus annuus					
0.00	2.55	16.75	16.2 b	16.4	0.0 c	0.89 a
0.75	2.10	14.57	20.8 ab	15.2	32.1 a	0.84 a
3.00	2.47	17.23	23.0 a	18.2	25.7 ab	1.23 a
3.75	1.60	14.18	24.8 a	15.6	18.1 b	0.84 a
5.25	1.98	12.89	21.2 ab	16.2	16.6 b	0.88 a
	Panicum virgatum					
0.00	39.55 a	71.54 a	-	79.1 a	0.0 b	0.20 b
0.75	37.03 ab	44.74 bc	-	59.5 c	3.5 a	0.24 b
1.50	36.74 ab	57.26 ab	-	69.3 b	2.7 a	0.10 b
3.00	29.45 b	30.24 cd	-	55.8 cd	1.8 ab	0.56 b
3.75	43.05 a	46.73 bc	-	69.7 b	2.5 a	1.65 a
5.25	28.16 b	22.16 d	-	49.8 d	1.5 b	0.16 b

† means followed by the same letter are statistically similar at $P \geq 0.05$.

†† Lsmeans estimates not adjusted by Tukey's.

Marigold and sunflower had no significant differences in root and shoot dry weights and plant growth between treatments (Table 2). Averaged across As treatments, the UR for marigold was 11.4 and the TF 0.5. The UR in sunflower declined slightly with increasing As solution. Averaged across treatments the UR and TF were 23.1 and 0.9, respectively.

Iris root and shoot dry weights increased with increasing solution concentrations above 3.00 mg As L^{-1} (Table 2). Although not significantly different, total dry weight from the 5.25 mg L^{-1} treatment was ≥ 2 x that found in the control. Although there were no differences in the number of new leaves developed and total leaf length between the control and all As treatments, with the 5.25 mg L^{-1} treatment 12 fewer new leaves were produced, and the new leaves that were produced were longer than new leaves produced by the control. An increase in solution As led to an increase in individual leaf lengths (data not shown). Iris produced a 5.3 average UR that varied little with increases in solution As concentration. There were no significant differences in iris TF, although the values varied from 0.82 to 9.21. High TF values tended to occur in plants that produced a large number of leaves.

Switchgrass control had significantly greater root dry weight than that found in 3.0 and 5.25 mg L^{-1} As treatments and greater total shoot dry weight than all but the 1.5 mg L^{-1} treatment (Table 2). Shoot dry weights increased with successive cuttings in all treatments (data not shown). An increase in greenhouse temperature occurred as spring approached and likely contributed to the increase in growth. The control had greater total growth, combined over three cuttings, than any As treatment. Growth showed a weak trend to decrease with increasing solution As concentration. Switchgrass had a low UR, 2.4 averaged over treatments and a low TF, 0.5.

3.3 Plant Arsenic

During the course of this study, several tanks failed and therefore data reported in Figure 1 represent only treatments common to all plant species (0.0, 0.75, 3.75 and 5.25 mg As L^{-1}). Statistical groupings were adjusted accordingly. In this study As uptake patterns by plants could be separated into three groups: 1) sensitive plants that at low As concentrations had reduced growth (coreopsis and tithonia), plants that tolerated As in the growing medium by preventing its accumulation in tissue (iris), and plants that took-up As but could moderate As toxic effects (marigold, sunflower and switchgrass). The average concentration in control tanks was 0.0028 mg As L^{-1} and coreopsis readily accumulated As at this low level. Both coreopsis and tithonia had maximum As accumulation at low solution levels 2.25 (not shown) and 0.75 mg As L^{-1}, respectively (Figure 1). This was accompanied by a 65% reduction in dry weight for coreopsis and an 85% reduction for tithonia at maximum plant As levels (Table 2).

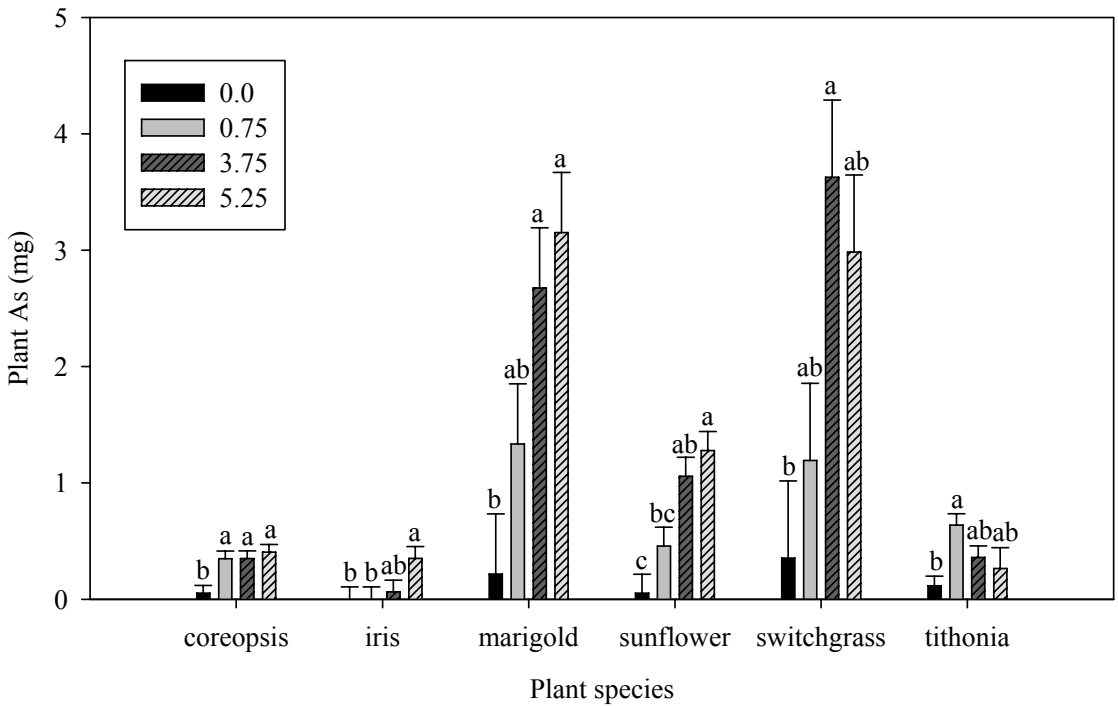

Figure 1. Whole plant arsenic content (mg) from plants grown in 0.0, 0.75, 3.75, or 5.25 mg As L^{-1} solution concentration

Iris accumulated very little As in its tissue at solution levels up to 5 mg As L^{-1} with no negative effects on growth (Figure 1). Maximum As accumulation in iris was above the high level used in this study. It is worth mentioning that as long as the rhizome remains healthy iris leaves can be removed and will be replaced by new growth. A portion of the rhizome remains above the surface and can be readily harvested increasing total As removal from soil.

Switchgrass had a maximum plant As content at 3.00 mg L^{-1} of 3.8 mg As kg^{-1} dry weight, the highest amount removed by any species at any solution concentration. Switchgrass can be harvested multiple times throughout a growing season. Marigold and sunflower continued to accumulate As in plant tissue through the highest solution concentration used in this study with no significant effect on shoot or root dry weights (Table 2). The solution concentration where maximum As accumulation occurred was above the highest concentration used in the study. Given no apparent affects on plant dry weights these plants may remove As from soils considerably more contaminated.

4. Discussion

All plants used in this study were As non-hyperaccumulators, however, there were different patterns in As accumulation among the plant species. Only a small amount of As was found in iris root and shoot tissue. Based on reduction in dry weight, tithonia and coreopsis were most sensitive to As exposure and did not produce sufficient dry weight for remediation purposes. Iris and marigold dry weights at the 5.25 mg L^{-1} treatments increased over controls by 2.3 and 1.3 x, respectively. Similar increases in dry weight at low As concentrations were reported by Carbonell-Barrachina, Aarabi, DeLaune, Gambrell, and Patrick (1998) with *Spartina patens* and *Spartina alterniflora*, Shaibura and Kawai (2009) with *Brassica rapa*, Srivastava et al. (2007) with *Hydrilla verticillata*, Woolson et al. (1971) with maize, Marin et al. (1992) with rice and Jacobs, Keeney, and Walsh (1970) with peas, wheat and potatoes. Jacobs et al. (1970) attributed this to improved plant P status as a result of arsenate displacing phosphate from soil increasing P availability. However, in the present study plants were grown hydroponically and this mechanism would not be in effect. Improved mineral nutrition at low As concentration could account for the higher dry weights.

Phytoremediation of As usually requires phytoextraction and/or phytostabilization. Phytoextraction involves As uptake from soil by plant roots and subsequent transport from root to shoot tissue where it can be harvested. Phytostabilization requires production of enough plant biomass to prevent soil As movement from the contaminated site by erosion and As sequestration in plant roots to limit leaching. An appropriate candidate for phytoremediation should accumulate As at a rate equal to or greater than that found in the soil, i.e., it should have an uptake ratio (UR) ≥ 1. In the present study coreopsis and tithonia had the highest UR but even at the lowest solution As concentration biomass production was too low for use in As removal or soil stabilization. Marigold and sunflower have UR values at 5.25 mg L^{-1} solution As concentration of 7.4 and 16.6, respectively. Their TFs (translocation factor) are near one, and at the high As rate both produce dry weights similar to their controls. Therefore, marigold and sunflower are appealing candidates for phytoremediation and phytostabilization. Switchgrass and iris are plants that can be harvested multiple times a year. This can partially offset the low UR and TF in switchgrass making it a candidate for phytostabilization. Iris has a UR of 6.7 and a TF of 9.2. The TF of iris and marigold continued to increase with increasing solution As up to the high rate used in this study. The maximum tissue As concentration for these species was not determined. Iris is an attractive candidates for phytostabilization and perhaps even phytoremediation. The fact that both marigold and iris produced flowers at all As rates may provide an added advantage in that cut flowers may provide small amounts of revenue to help offset the cost of remediation.

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