

## Nutrient Uptake of Ornamental Plants Exposed to Arsenic in Hydroponic Solution

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### Abstract

Arsenic-based agro-chemicals have contaminated considerable acreage on turf-farms, orchards, and around horticultural production structures. A study was undertaken to evaluate iris (*Iris savannarum*), switchgrass (*Panicum virgatum*), *Tithonia rotundiflora*, *Coreopsis lanceolata*, sunflower (*Helianthus annuus*), and marigold (*Tagetes erecta*) for their potential use as arsenic (As) accumulator plants. Plants were grown hydroponically with a modified Hoagland solution containing either 0, 10, 50 or 70  $\mu\text{M}$  As (0.0, 0.75, 3.75, 5.25  $\text{mg L}^{-1}$ , respectively). At 5.25  $\text{mg As L}^{-1}$  solution there were no significant reductions in dry weight below that of the controls for iris marigold and sunflower. Maximum shoot As content (mg) for coreopsis and tithonia was reached at 0.75 and for switchgrass at 3.75  $\text{mg As L}^{-1}$  solution. Iris marigold and sunflower maximum shoot As levels occurred at a solution concentration above 5.25  $\text{mg As L}^{-1}$  solution, the high level used in this study. In general P decreased and S increased with increasing solution As. Marigold, switchgrass and sunflower, species that tolerated As at the levels used in this study, had a weak negative correlation between As and Cu concentrations in common. In these species As in hydroponic solution had no effect, or even slightly enhanced, P uptake compared to controls. Arsenic sensitive species coreopsis and tithonia had weak negative correlations between As and K and P in common. Coreopsis and tithonia appears to have a competitive uptake mechanism between arsenate with phosphate. Arsenic tolerance in iris appears to be a result of prohibiting As accumulation in root tissue.

### 1. Introduction

Arsenic-based pesticides, herbicides and insecticides have contaminated a large amount of acreage on turf-farms, orchards, and around greenhouses, shadehouses and other horticultural production structures (Woolson Axley, & Kearney, 1971; Murphy & Aucott, 1998). Naturally occurring soil As can range from 1-40  $\text{mg As kg}^{-1}$  soil (Walsh Sumner, & Keeney, 1977), however, contaminated levels can reach as high as 2600  $\text{mg As kg}^{-1}$  soil (Meharg Naylor, & Macnair, 1994).

Arsenic is not an essential element for plant nutrition (Marin Masscheleyn, & Patrick, 1993) and conducts no known metabolic function. Plants vary in their ability to tolerate As (Meharg, 1994). Toxicity threshold levels range from 5 to 100  $\text{mg As kg}^{-1}$  dry weight for most plants (Kabata-Pendias & Pendias, 1992). At low concentrations As in the oxidized form, arsenate, can act as an analogue of phosphate (Meharg et al., 1994; Zhao Ma, Meharg, & McGrath, 2009) and may compete with P for uptake by high-affinity phosphate transporters in root cells (Asher & Reay, 1979; Ullrich-Eberius, Sanz, & Novacky, 1989; Bleeker, Schat, Vooijs, Verkleij & Ernst, 2003). Arsenite, the reduced form of As, is likely taken up by aquaporin channels in plant roots (Meharg & Jardine, 2003). Once taken up by the roots, plants that accumulate As in aboveground tissue must detoxify it. Rice (*Oryza sativa*, L.) (Marin, Masscheleyn, & Patrick, 1992; Ma et al., 2008), cucumber (*Cucumis sativus* L.) (Mihucz et al., 2005), *Brassica juncea* (Pickering et al., 2000), tomato (*Lycopersicon esculentum*) (Burló Guijarro, Carbonell-Barrachina, Valero, & Martinez-Sa'nchez, 1999), *Spartina patens* and *Spartina alterniflora* (Carbonell-Barrachina, Aarabi, DeLaune, Gambrell, & Patrick, 1998), reduce arsenate to arsenite in the root, however, very little As is translocated from root to shoot.

Plants that accumulate As at a rate  $>1000 \text{ ug As g}^{-1}$  plant dry weight are known as hyperaccumulators (Machado-Estrada Calderón, Moreno-Sánchez & Rodríguez-Zavala, 2012). These plants translocate a large portion of As to the shoot. 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  $1500 \text{ mg kg}^{-1}$  As contaminated soil accumulated up to  $15861 \text{ ug As g}^{-1}$  plant dry weight over a two week growing period. Since that time several fern species have been identified as As-hyperaccumulators: *Pteris cretica*, *Pteris longifolia*, *Pteris vittata* and *Pteris umbrosa* (Zhao, Dunham, & McGrath, 2002) and *Pityrogramma calamitanos* (Visoottiviset et al., 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. The As hyperaccumulator *Pteris vittata* shipped 8 x more As from root to shoot than the nonhyperaccumulator *Pteris tremula* (Caille, Zhao, & McGrath, 2005) and 2.8 x more than *Pteris ensiformis* (Singh & Ma, 2006).

A variety of species, other than ferns, have been identified that hyperaccumulate or are tolerant to high levels of As. Ansari et al. (2013) reported that at  $50 \text{ um As}$ , mustard shoots (*Brassica juncea* L.) contained from 1.84 to 3.65 mg As  $\text{g}^{-1}$  dry weight. Arsenic accumulation was reported by Castillo-Michel et al. (2009) in *Chilopsis linearis* (Desert willow), by Bleeker et al. (2003) in the flowering plant *Cytisus striatus*, and by Karimi, Ghaderian, Raab, Feldmann, and Meharg (2009) in a brassica, *Isatis capadocica*.

The discovery of plants that hyperaccumulate As has renewed an interest in phytoremediation to treat contaminated sites. An ideal plant for phytoremediation would have vigorous growth to help prevent As from moving off site through erosion. It should take-up and store As in above ground plant parts for eventual harvest. Plants defined as As hyperaccumulators have a Bioaccumulation Factor (BF)  $> 1000 \text{ ug As g}^{-1}$  plant dry weight (Machado-Estrada et al., 2012). They have an As shoot-to-root ratio (Translocation Factor (TF))  $> 1$ . It has been found that plants with a BF  $> 1$  can be useful in phytoremediation if they store most of their As in the shoot leading to a TF  $> 1$  (McGrath & Zhao, 2003).

Ornamental plants have not been fully investigated as a mechanism for phytoremediation of As contaminated soil. Ornamentals can partially offset the cost of land taken out of production through the sale of cut flowers and other marketable commodities. In addition, these plants can provide an aesthetic quality to buildings located on contaminated sites.

The Florida Department of Environmental Protection goals for cleanup of residential and industrial soils are 0.8 and  $3.7 \text{ mg As kg}^{-1}$  soil, respectively. On 14 South Florida golf courses associated with high groundwater As concentrations, an average As concentration of  $13.7 \text{ mg kg}^{-1}$  was found in the fine clay fraction (Cai, Cabrera, Georgiadis, & Jayachandran, 2002). This indicates a possible impact from applications of arsenic-containing herbicides. In urban soils from Gainesville and Miami, Florida, a range of 0.21 to  $660 \text{ mg As kg}^{-1}$  soil was found (Chirenje et al., 2003). In the Miami soils 95% of these samples exceeded the Florida residential goal and 33% exceeded the commercial goal. Soil contaminated with As levels above regulatory goals is a problem in South Florida. The purpose of this study was to evaluate nutrient uptake by ornamental plants grown in a hydroponic system containing As.

## 2. Materials and Methods

### 2.1 Plant Species

Iris (*Iris savannarum*), switchgrass (*Panicum virgatum*), *Tithonia rotundiflora*, *Coreopsis lanceolata*, sunflower (*Helianthus annuus*), and marigold (*Tagetes erecta*) were used in this study. A 25% perlite, 37% pine bark, 8% sand, 30% coir potting mixture was used for all plants except iris. Ten cm iris rhizomes, collected from a single plant were set in rockwool to help maintain rhizome orientation during ebb and flow cycles in the hydroponic system. 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 treatments began. *Tithonia*, sunflower and marigold seedling with at least two fully developed leaves and iris and coreopsis 10 cm tall with  $\geq 3$  leaves were placed in 26-cm pots. 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.

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

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

## 2.2 Hydroponic System

Six ebb-and-flow type hydroponic plant maintenance systems were used for the study. Each system contained a 208 L reservoir tank filled with 132 L water. Each tank was 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 A.M., ending at 4 P.M. followed by a 12 hr. drain period. A modified Hoagland solution was used to supply plant nutrients. Nutrients were added in the form of concentrated stock solutions before tanks were brought to their final volume. Final nutrient concentrations in each tank were 2.0 mM  $\text{Ca}(\text{NO}_3)_2$ , 3 mM  $\text{KNO}_3$ , 1.0 mM  $\text{MgSO}_4$ , 0.25 mM  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , 12.5  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 1.0  $\mu\text{M}$   $\text{MnSO}_4$ , 1.0  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.25  $\mu\text{M}$   $\text{CuSO}_4$ , 0.2  $\mu\text{M}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ , and 10  $\mu\text{M}$  Fe-EDDHA. Tap water used to mix nutrient solutions averaged 0.0028 mg  $\text{L}^{-1}$  As (0.448 mg per reservoir tank). Plants acclimated to hydroponic feeding for a minimum of one week before beginning As treatments. Enough  $\text{Na}_2\text{HAsO}_4$ , dissolved in 1.0 L water, was added to different reservoirs to make a final tank concentration of 0, 10, 50 or 70  $\mu\text{M}$  As (0.0, 0.75, 3.75, 5.25 mg  $\text{L}^{-1}$  As, respectively). An As solution concentration of 2-8  $\mu\text{M}$  equates to a soil As concentration of 700-3000 mg  $\text{kg}^{-1}$  (Moreno-Jiménez, Esteban, Fresno, López de Egea, & Peñalosa, 2010). Based on this and the levels of contaminated urban soils reported above, a range of 10-70  $\mu\text{M}$  As solution concentration was selected to cover the range of low level As contamination found in south Florida's urban soils. Reservoir pH was adjusted daily to pH 6.5 with either NaOH or  $\text{H}_2\text{SO}_4$ . Nutrient and As solutions were replaced weekly. Plants were maintained in hydroponic solution until flowering.

## 2.3 Sample Analysis

Shoot and root tissue were harvested separately. Roots were agitated in a pool of water then washed with a gentle spray. Shoot and root tissue were 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

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

and translocation factor

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

were calculated for each species and each treatment.

Approximately 0.25 g of oven dried plant tissue was placed in 100-mL digestion tubes. Ten mL  $\text{HNO}_3$  was added and samples digested in a microwave digestion system 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. Element concentrations were 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.

## 2.4 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 performed 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

Results for plant growth and As uptake were reported in Reed, Ayala-Silva, Dunn, Gordon, and Meerow (2013). In summary, based on dry weight, tithonia and coreopsis did not produce enough biomass to merit consideration for bioremediation. 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 weight production at solution concentrations of 5.25 mg As L<sup>-1</sup>, therefore, are appealing candidates for phytoremediation and phytostabilization. Switchgrass and iris can be harvested multiple times a year, making them candidates for phytostabilization.

In the current report, there were significant differences at  $P \geq 0.05$  between As treatments and between shoot and root tissue As concentration in all plant species. In most species, dry weights decreased with increasing solution As, however, iris and marigold did not follow this trend. Arsenic uptake increased with successive harvests in switchgrass.

#### 3.2 Dry Weight

Differences in root dry weight from the 0.0 As control to 5.25 mg As L<sup>-1</sup> treatment were small or not significant in iris, marigold and sunflower (Figure 1). Significant differences in root dry weight appeared at 5.25 mg As L<sup>-1</sup> solution in switchgrass. Even at the high solution As rate, switchgrass produced a high root dry weight, an important consideration in selection of a species appropriate for limiting erosion of contaminated material. Reductions in root dry weight from toxic concentrations of As affected tithonia at the lowest concentration (0.75 As mg L<sup>-1</sup>) and coreopsis at 3.75 mg As L<sup>-1</sup>. Tithonia and coreopsis exhibited a similar sensitivity to As with shoot dry weight (Figure 2).

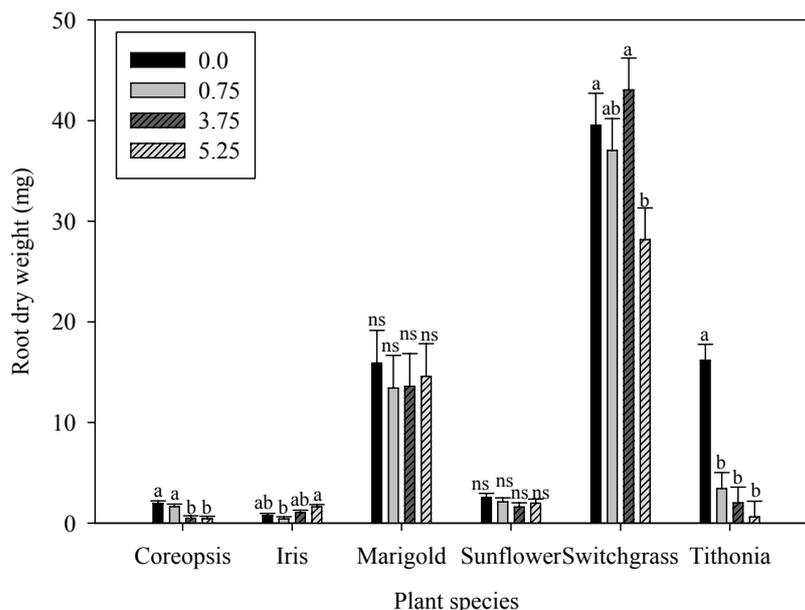


Figure 1. Root dry weight (mg) from plants grown in 0.0, 0.75, 3.75, or 5.25 mg As L<sup>-1</sup> solution concentration. Bars represent least squares means estimate with standard error. For each species similar letters atop bars indicates no significant difference by Tukey-Kramer at P 0.05 level

Treatment differences from the control in shoot dry weight for iris, marigold and sunflower were either non-significant or very small (Figure 2). Both iris and marigold had a slight tendency to increase dry weight with increasing solution As up to 5.25 mg L<sup>-1</sup>. Other researchers have reported similar findings at low As concentrations with potato (*Solanum tuberosum* L.) (Jacobs, Keeney, & Walsh, 1970), maize (Woolson et al., 1971), rice (Marin et al., 1992), *Spartina patens* and *S. alterniflora* (Carbonell-Barrachina et al., 1998), and Japanese mustard spinach (*Brassica rapa* L. var. *pervirdis*) (Shaibur & Kawai, 2009).

Significant reductions in switchgrass shoot dry weight began at the lowest level of solution As (Figure 2). However, despite decreasing dry weight production with increasing exposure to As, switchgrass growth remained

relatively vigorous. During this study, switchgrass was harvested three times and with each successive harvest dry weights increased. Warmer spring temperatures possibly influenced this increase in growth. After 76 d the control and high As treated plants increased dry weight at a rate of 0.22 and 0.19  $\text{cm d}^{-1}$ , respectively. At final harvest, 121 d, dry weight increased at a rate of 2.4 and 0.74  $\text{cm d}^{-1}$  for control and high As treatment, respectively.

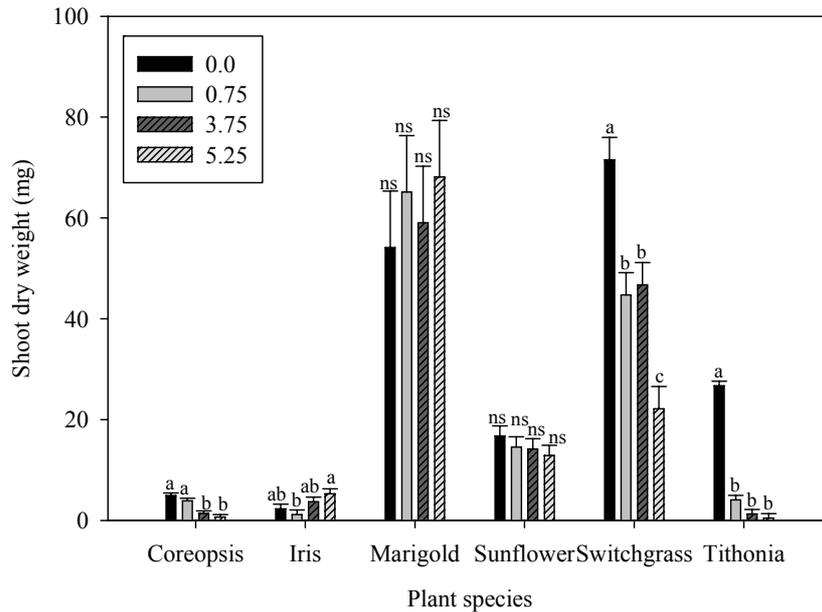


Figure 2. Shoot dry weight (mg) from plants grown in 0.0, 0.75, 3.75, or 5.25  $\text{mg L}^{-1}$  solution concentration. Bars represent least squares means estimate with standard error. For each species similar letters atop bars indicates no significant difference by Tukey-Kramer at P 0.05 level

### 3.3 Tissue Arsenic Concentration

Root As was consistently higher than shoot As concentration for all species at all solution As levels (Figures 3 & 4). Figure 3 presents As concentration in root tissue. Coreopsis accumulated the largest root tissue As concentration of the species studied. There was a large increase in root As concentration between the 0.75  $\text{mg L}^{-1}$  treatment and higher solution As treatments (Figure 3). Total root As uptake did not vary much, ranging from 0.3 to 0.4  $\text{mg As}$  at 0.75 and 5.25  $\text{mg L}^{-1}$  solution As, respectively. Given total As content was similar in all treatments, the increase in root As concentration mostly was the result of declining root dry weight with increased exposure to As (Figure 1). Shoot As concentration increased and dry weight decreased with an increase in solution As (Figure 4). A similar pattern of large decreases in root and shoot dry weight controlling As concentration was observed with tithonia.

Marigold, sunflower and to a lesser extent switchgrass had relatively low decreases in root and shoot dry weights with increased exposure to As (Figures 1 & 2) and accumulation of As in root and shoot tissue drove increases in As concentration (Figure 3 and 4). At 3.75  $\text{mg L}^{-1}$  solution As, sunflower acquired the second largest root As concentration behind coreopsis (Figure 3). However, both coreopsis and sunflower produced comparatively little root dry matter (Figure 1). At 5.25  $\text{mg As L}^{-1}$  tithonia had the highest shoot As concentration followed by sunflower, coreopsis, iris, marigold, then switchgrass (Figure 4).

Arsenic concentration in iris root tissue was very low, even at the high solution As rate (Figure 3). Only 0.06  $\text{mg}$  total As was found in iris root and rhizome tissue. Despite a wide range in shoot As concentration there were no significant differences between the treatments and 0.0 As control (Figure 4).

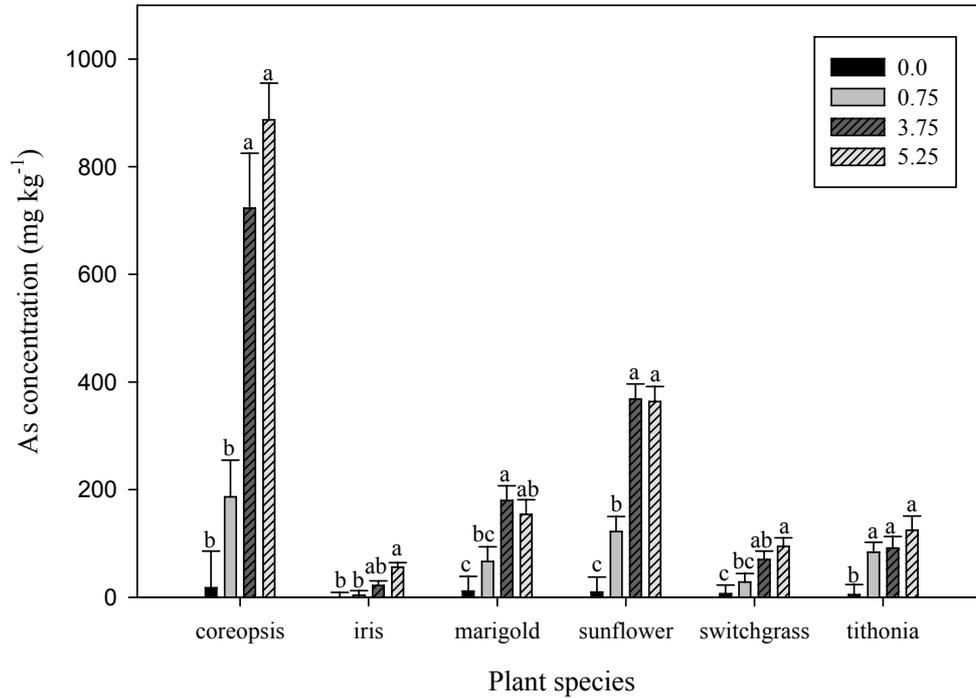


Figure 3. Root arsenic concentration (mg As kg<sup>-1</sup> dry weight) from plants grown in 0.0, 0.75, 3.75, or 5.25 mg As L<sup>-1</sup> solution concentration. Bars represent least squares means estimate with standard error. For each species similar letters atop bars indicates no significant difference by Tukey-Kramer at P 0.05 level

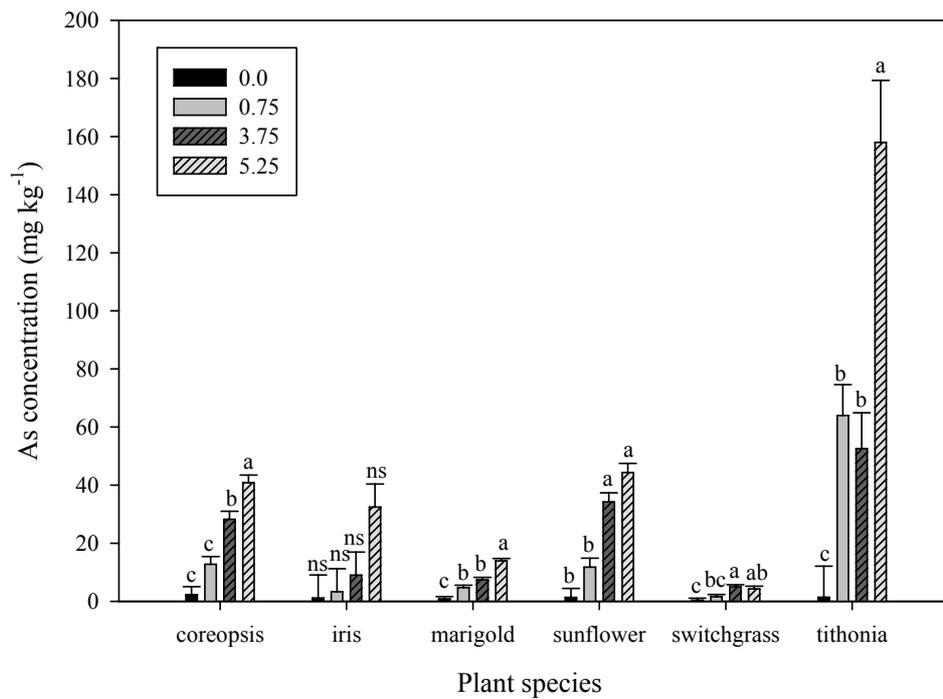


Figure 4. Shoot arsenic concentration (mg As kg<sup>-1</sup> dry weight) from plants grown in 0.0, 0.75, 3.75, or 5.25 mg As L<sup>-1</sup> solution concentration. Bars represent least squares means estimate with standard error. For each species similar letters atop bars indicates no significant difference by Tukey-Kramer at P 0.05 level

### 3.4 Total Shoot As

Figure 5 shows the mg As in shoot tissue for each species for each treatment. The maximum shoot As content in coreopsis, and tithonia occurred at 0.75 mg L<sup>-1</sup>. Marigold followed by sunflower contained the greatest amount of shoot As after 50 d growth at 0.85 and 0.56 mg As, respectively. Shoot As increased with increasing solution As concentration with the concentration for optimum removal above 5.25 mg L<sup>-1</sup>, the highest As concentration used in this study. Both species produced flowers in all treatments. Iris, grown for 160 d, did not reach its optimum As removal but contained less than half the total shoot content of sunflower. Switchgrass (160 d) had an optimum removal near 3.75 mg L<sup>-1</sup> representing As collected from two cuttings. Both switchgrass and iris plants can be repeatedly cut and new growth occurs. Additional harvests could substantially increase the amount of As removed. The uptake ratio and translocation factor at 5.25 mg As L<sup>-1</sup> solution for each species is as follows: coreopsis – 73.0 and 0.07, tithonia – 33.4 and 1.13, sunflower – 16.6 and 0.88, iris 6.7 and 9.21, marigold – 7.4 and 1.20, and switchgrass – 1.5 and 0.16 (Reed, Ayala-Silva, Dunn, Gordon, & Meerow, 2013).

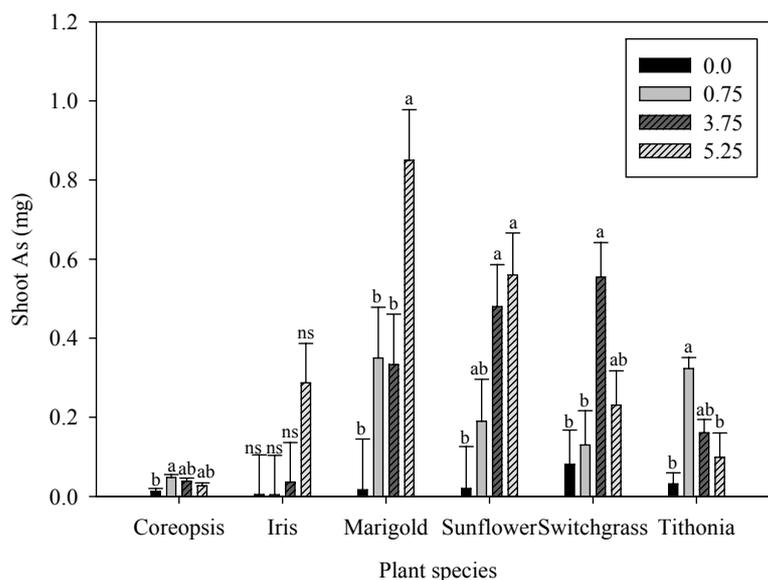


Figure 5. Shoot arsenic (mg) from plants grown in 0.0, 0.75, 3.75, or 5.25 mg As L<sup>-1</sup> solution concentration. Bars represent least squares means estimate with standard error. For each species similar letters atop bars indicates no significant difference by Tukey-Kramer at P 0.05 level

### 3.5 Arsenic and Plant Nutrition

Pearson Correlation Coefficients for plant As concentration matched with selected plant nutrient element concentration revealed no consistent trends among the different species. For example in iris, changes in plant As concentration were not related to changes in any plant anion or cation nutrient, whereas, As had a weak to moderate positive correlation with Fe in coreopsis and marigold and a weak negative correlation in tithonia (Table 2). There were few significant correlations between As and either P or S but in general P decreased and S increased with increasing As. Marigold, switchgrass and sunflower, species that tolerated As at the levels used in this study, had only a weak negative correlation between As and Cu concentrations in common. Arsenic sensitive species coreopsis and tithonia had weak negative correlations between As and K and P in common.

Total nutrient element content (mg) is given in Table 3. In general coreopsis element content at 5.25 mg As L<sup>-1</sup> solution was lower than that found in the control. Sulfur content increased at 0.75 mg L<sup>-1</sup> As solution concentration and began to decline thereafter. At 0.75 mg L<sup>-1</sup> solution As, plant dry weight reduction from As toxicity was not yet apparent. The same trend held for switchgrass and sunflower, although in sunflower differences were mostly non-significant. An increase in switchgrass S content was found at solution As treatments up to 3.75 mg L<sup>-1</sup>. In iris, nutrient element content was greater at 5.25 mg As L<sup>-1</sup> than in the control. An increase in S content did not appear until 3.75 mg L<sup>-1</sup> solution As. In marigold, there were few statistical differences in element content between the control and high As level. Significantly lower element content in tithonia first appeared at the lowest (0.75 mg L<sup>-1</sup>) solution As concentration for all elements except B and P. Also, tithonia had a significant drop in dry weight between the control and lowest As treatment.

### 3.6 Plant P

There were no significant As differences between the control and As treatments in root P concentration for coreopsis, iris and marigold (Figure 6). In switchgrass, root P was lowered at the high rate only. With sunflower, the 0.75 and 3.75 mg As L<sup>-1</sup> treatments had a higher root and shoot P than the control (Figure 6 & 7). Root and shoot P decreased with increasing solution As in tithonia. Shoot P was similar in all As treatments and control for coreopsis, marigold and switchgrass (Figure 7) and in iris, only the 5.25 mg As L<sup>-1</sup> treatment had lower shoot P concentration than the control. Tithonia was the only species where solution As above 3.75 mg L<sup>-1</sup> appeared to significantly reduce P uptake below that of the control. Adequate P was supplied in the nutrient solution and As concentrations used were apparently not high enough to overwhelm P uptake. The lack of a connection between As availability in the growing medium and tissue P is in agreement with Meharg and Macnair (1992) who reported, increased plant phosphate status led to reduced arsenate influx.

Table 2. Correlation coefficient and *P* value for As tissue concentration with selected elements for *Coreopsis lanceolata*, iris, marigold (*Tagetes erecta*), sunflower (*Helianthus annuus*), switchgrass (*Panicum virgatum*) and *Tithonia rotundiflora*

<i>Coreopsis lanceolata</i>											
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
As	-0.4043	-0.2087	-0.4407	0.3572	-0.4475	-0.0765	0.2064	0.7048	-0.3471	0.0850	0.0650
	*		*	*	*			***	*		
<i>Iris savannarum</i>											
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
As	0.0366	-0.0433	-0.1804	0.1298	0.1059	0.0070	-0.0634	-0.1729	-0.1098	0.0693	-0.3181
											*
Marigold ( <i>Tagetes erecta</i> )											
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
As	-0.0306	0.1589	-0.2862	0.4350	-0.2491	-0.0037	0.3381	0.3258	-0.1812	-0.0150	-0.0159
			*	*			*	*			
Sunflower ( <i>Helianthus annuus</i> )											
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
As	-0.3645	-0.3749	-0.4247	0.0870	-0.1013	-0.4342	0.1501	0.0594	0.1042	0.0205	0.1528
	*	*	*			*					
Switchgrass ( <i>Panicum virgatum</i> )											
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
As	0.1639	0.1612	-0.2521	0.0636	-0.3755	-0.0277	0.1690	0.1185	-0.1017	0.4371	0.0763
			*		*					**	
<i>Tithonia rotundiflora</i>											
	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
As	-0.2884	-0.4086	0.1332	-0.4042	-0.6820	-0.3077	0.1431	-0.3696	-0.5029	0.1063	-0.2640
		*		*	***			*	*		

\*  $P \leq 0.1$ , > 0.01, \*\*  $P \leq 0.01$ , > 0.001, \*\*\*  $P \leq 0.001$ .

Table 3. The effect of solution As concentration (mg As L<sup>-1</sup> solution) on element content (mg pot<sup>-1</sup>) in plant tissue for *Coreopsis lanceolata*, iris, marigold (*Tagetes erecta*) sunflower (*Helianthus annuus*), switchgrass (*Panicum virgatum*) and *Tithonia rotundiflora*

mg As L <sup>-1</sup> solution	As	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
<i>Coreopsis lanceolata</i>												
0	0.05 b†	0.24 a	93.76 a	0.07 a	0.87 a	411.7 a	37.0 a	0.25 a	0.007 a	38.8 a	20.3 ab	0.19 a
0.75	0.35 a	0.18 ab	72.39 a	0.04 ab	0.69 ab	347.9 a	23.8 a	0.30 a	0.007 a	28.7 a	25.9 a	0.22 a
3.75	0.35 a	0.07 bc	26.35 b	0.01 b	0.29 ab	100.0 b	9.8 b	0.08 b	0.003 ab	9.1 b	7.0 b	0.04 b
5.25	0.41 a	0.04 c	16.56 b	0.01 b	0.15 b	61.1 b	6.5 b	0.07 b	0.002 b	5.1 b	3.9 b	0.03 b
SE	0.06	0.03	9.62	0.01	0.15	43.4	3.2	0.03	0.001	3.4	4.4	0.02
<i>Iris savannarum</i>												
0	0.0059 b	0.12 a	23.78 ab	0.02 ab	0.23 b	165.9 ab	10.0 ab	0.03 ab	0.002 a	186.5	8.0 b	0.07
0.75	0.0060 b	0.06 b	11.09 b	0.01 b	0.12 b	78.7 b	4.6 b	0.01 b	0.001 a	89.2	6.3 b	0.04
3.75	0.0631 ab	0.21 a	36.98 ab	0.02 ab	0.41 ab	278.1 ab	17.4 ab	0.04 ab	0.004 a	315.7	20.4 a	0.11
5.25	0.3506 a	0.27 a	51.62 a	0.03 a	2.12 a	356.1 a	22.2 a	0.09 a	0.005 a	366.9	26.3 a	0.13
SE	0.1	0.04	9.5	0.01	0.42	66.4	3.4	0.01	0.001	7.5	5.3	0.02
<i>Tagetes erecta</i> (marigold)												
0	0.2170 b	2.01	932.65	2.17	27.69	2310.4	163.0	6.42	0.083	343.6	177.4	9.49
0.75	1.3338 ab	2.55	931.9	1.91	25.50	2327.1	172.7	6.10	0.078	372.7	227.2	8.94
3.75	2.6745 a	2.25	954.25	1.92	24.47	2438.4	180.5	6.69	0.072	348.5	224.1	8.21
5.25	3.1513 a	2.86	1305.3	2.23	31.31	2891.7	219.4	7.06	0.090	454.3	252.7	8.23
SE	0.5	0.4	160.9	0.3	5.8	434.5	33.6	1.0	0.01	53.4	37.1	1.1
<i>Helianthus annuus</i> (sunflower)												
0	0.0517 b	1.56 a	341.82	0.55 a	2.76	911.2	90.5	1.09	0.025	153.9	70.4	2.41 a
0.75	0.4567 ab	1.13 ab	287.68	0.41 a	2.18	748.3	76.4	0.97	0.017	131.6	96.6	1.67 ab
3.75	1.0567 a	1.01 ab	262.63	0.41 a	1.82	653.2	70.5	0.80	0.018	108.9	64.5	1.44 ab
5.25	1.2783 a	0.88 b	196.70	0.32 b	1.87	579.6	55.7	0.62	0.018	92.5	63.5	1.15 b
SE	0.2	0.2	40.3	0.05	4.1	105.9	11.4	0.18	0.002	20.4	11.8	0.2
<i>Panicum virgatum</i> (switchgrass)												
0	0.3532 b	1.74 a	1021.3	0.73	154.70	3165.4 a	613.3 a	5.13	0.075	503.2 a	221.3 bc	2.53 a
0.75	1.1918 ab	1.31 ab	829.9	0.43	128.05	2113.9 bc	435.9 b	4.11	0.080	370.4 a	260.8 b	1.32 bc
3.75	3.6261 a	1.31 ab	685.4	1.16	62.29	2236.1 bc	420.0 b	3.41	0.077	387.9 a	340.8 a	1.96 ab
5.25	2.9822 ab	0.88 b	622.4	0.35	90.35	885.4 d	203.1 c	2.87	0.055	150.3 b	130.1 c	0.84 c
SE	0.7	0.1	121.7	0.2	37.5	137.3	40.6	1.0	0.01	34.4	25.0	0.2
<i>Tithonia rotundiflora</i>												
0	0.1150 b	1.80 a	792.98 a	0.45 a	39.61 a	1697.4 a	249.8 a	1.01a	0.298 a	388.5 a	76.0 a	0.77 a
0.75	0.6500 a	1.48 b	708.56 a	0.21 b	5.10 b	989.9 b	214.3 b	0.64 b	0.023 b	327.1 a	27.5 b	0.44 b
3.75	0.3733 ab	0.10 c	35.83 b	0.02 c	2.16 b	69.7 c	15.6 c	0.09 c	0.003 b	18.1 b	6.1 b	0.04 c
††5.25	0.2100	0.004	1.53	0.0005	0.007	1.4	0.5	0.004	0.0005	0.3	3.0	0.003
SE	0.003	0.002	316.5	0.0002	12.0	2075.3	20.3	0.002	0.001	85.6	8.2	0.0003

† Values followed by the same letter are not significantly different at  $P < 0.05$ .

†† Insufficient tissue was available for inclusion in statistical analysis. Values given are a composite from one root and two shoot samples.

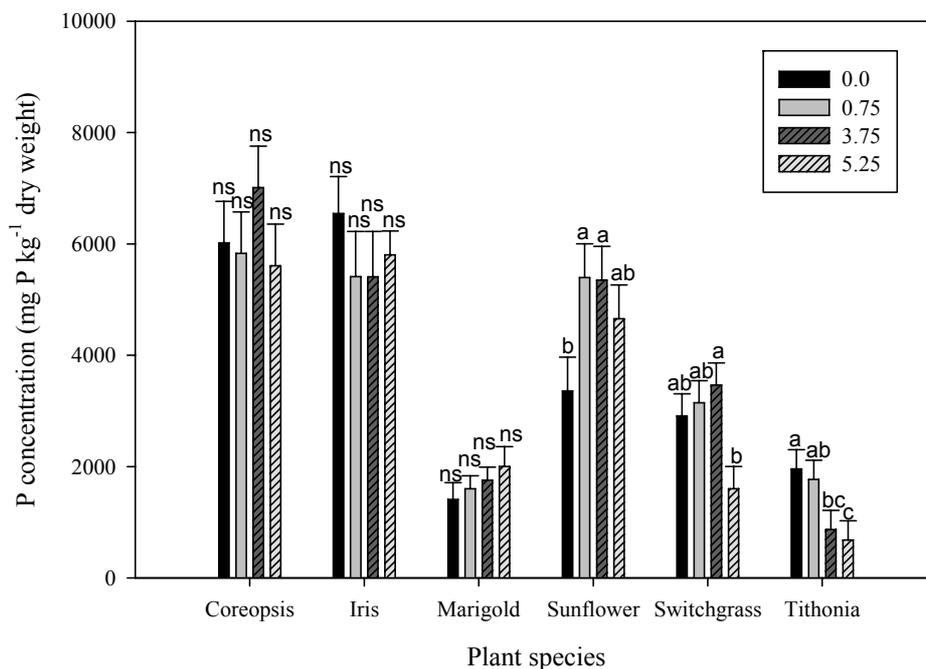


Figure 6. Root phosphorus concentration ( $\text{mg P kg}^{-1}$  dry weight) from plants grown in 0.0, 0.75, 3.75, or 5.25  $\text{mg As L}^{-1}$  solution concentration. Bars represent least squares means estimate with standard error. For each species similar letters atop bars indicates no significant difference by Tukey-Kramer at P 0.05 level

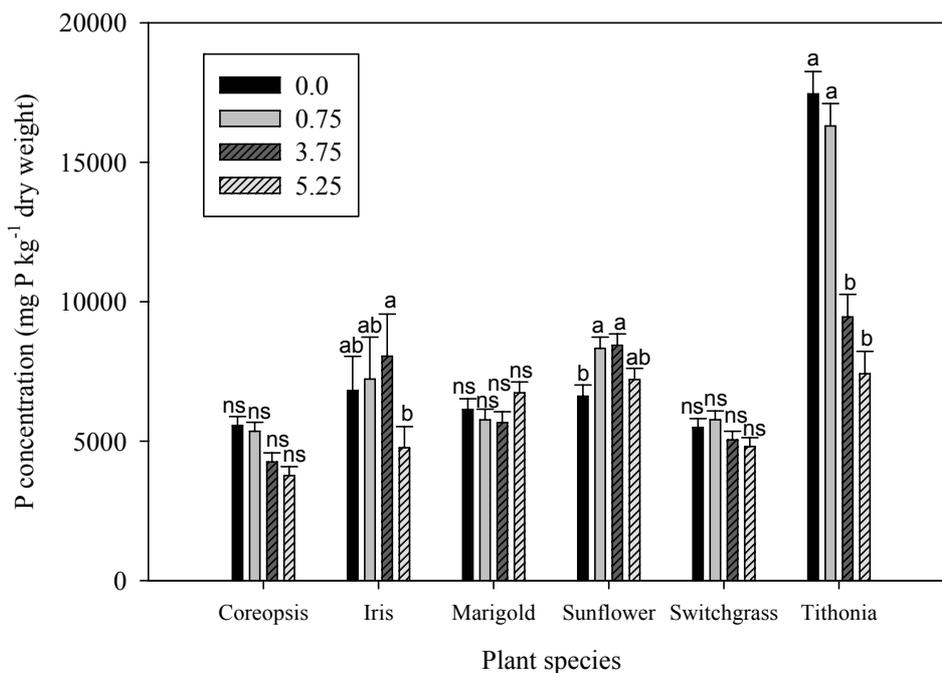


Figure 7. Shoot phosphorus concentration ( $\text{mg P kg}^{-1}$  dry weight) from plants grown in 0.0, 0.75, 3.75, or 5.25  $\text{mg As L}^{-1}$  solution concentration. Bars represent least squares means estimate with standard error. For each species similar letters atop bars indicates no significant difference by Tukey-Kramer at P 0.05 level

#### 4. Discussion

Arsenate uptake by plants can be considered an analogue of phosphate in that they share the same transport pathway (Meharg et al., 1994). At low soil P a mechanism with high affinity for phosphate operates and competition from As for uptake sites is weak (Meharg & Macnair, 1992). As soil P levels increase this system

becomes increasingly inactive. Once arsenate is taken up by a root cells, a small amount may be transported to the xylem but the majority is reduced to arsenite (Zhao et al., 2009). Arsenite is either exported back into soil, transported in the xylem to stem and leaves, or complexed with an organic compound for storage in a vacuole. Arsenic non-hyperaccumulators tend to store most of the arsenite in the root with little transported to stem or leaf tissue.

All plants used in this study were As non-hyperaccumulators, however, there were different patterns in As accumulation, tolerance/avoidance among the plant species. Only a small amount of As was found in iris root and shoot tissue. Increasing solution As had little affect of tissue P concentration. In iris, either root cells were very efficient at As efflux back into soil or As did not compete effectively with P for uptake sites. Arsenic tolerance in iris appears to be a result of prohibiting any accumulation in root tissue.

Based on reduction in dry weight, tithonia was most sensitive to As exposure with an 85% reduction in weight at the lowest ( $0.75 \text{ mg As L}^{-1}$ ) solution concentration. Root and shoot As concentrations were roughly equal and As appeared to reduce P uptake. In this study the highest solution As concentration was  $70 \mu\text{M}$  ( $5.25 \text{ mg L}^{-1}$ ). Baldwin and Butcher (2007) reported significant differences in tissue P concentration with *Pteris cretica* (Moonlight fern) plants between zero As controls and plants exposed to  $500 \mu\text{M}$  arsenic. This observation held for root, stem, and leaf tissue. *Pteris cretica* is an As hyperaccumulator where As was selectively accumulated in stem and root tissue. Castillo-Michel et al. (2009) reported similar results with *Chilopsis linearis* (Desert willow) suggesting competition between arsenate with phosphate for carriers in the plasma membrane. Coreopsis and tithonia appears to have a similar competitive uptake mechanism.

In marigold and switchgrass there were no differences between controls and As treatments for root and shoot P. Sunflower  $0.75$  and  $3.75 \text{ mg L}^{-1}$  As treatments had higher root and shoot P than controls. Arsenic in root tissue increased with increasing solution As in these species. An As concentration less than 9% of that in root tissue was found in shoots of marigold, switchgrass and sunflower. The presence of As in hydroponic solution had no effect, or even slightly enhanced, P uptake compared to controls.

There were no differences in root or shoot P concentration in marigold and only at the high As rate in iris was there a reduction in shoot P concentration below that of the control. Although there were no statistical differences between the high As rate and the control, secondary and micronutrient contents all generally increased with increasing solution As concentration. Improved mineral nutrition at  $5.25 \text{ mg L}^{-1}$  As could account for the higher dry weights in iris and marigold.

## 5. Conclusions

Based on a rapid reduction in dry weight with exposure to As, coreopsis and tithonia were plants that proved to be very sensitive to As. Total As in plant tissue reached high levels even at low solution As concentrations. Plant nutrient uptake declined with increasing solution As concentration. Iris took up very little As at the two lower solution concentrations and dry weights actually increased. This resulted in either no difference or an increase in nutrient uptake. In contrast with iris, switchgrass readily took up As. There was a decrease in dry weight but even at the high solution As concentration a high amount of biomass was produced. There was a decline in plant nutrient content with increasing tissue As. In marigold and sunflower there were no differences in nutrient content with increasing tissue As up to the highest solution concentration used in this study. Iris, switchgrass, sunflower and marigold, candidates for phytostabilization or phytoremediation maintained adequate plant nutrient uptake at solution As concentration of at least  $5.25 \text{ mg L}^{-1}$ .

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