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Effects of Arsenic on Nutrient Accumulation and Distribution in Selected Ornamental Plants

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Abstract

In Miami, Florida, 95% of residential and 33% commercial soils exceed the Florida Department of Environmental Protection goals for cleanup of arsenic contamination. Ornamental plants have not been fully investigated as a mechanism for phytoremediation of low level As contaminated soil. This study evaluates nutrient uptake by ornamental plants grown in a hydroponic system containing concentrations of 0, 10, 20, 30, 40, 50 or 70 uM As (0.0, 0.75, 1.5, 3.0, 3.75, 5.25 mg·L^{-1} As, respectively). Uptake of Ca, K, Mg and Mo was likely influenced by the toxic effect of As on root functions. Arsenic had little effect on Ca, K and Mg transportation to the shoot at any but the highest As exposure rate. Tissue P concentration was similar to or higher than that found in controls and As competition with P uptake occurred at 70 uM As only. Tissue sulfur initially increased then subsequently decreased at 70 uM As where uptake could no longer supply enough S for both detoxification and normal metabolic needs. The effect of As on plant B was likely a result of membrane leakage and overall tissue damage leading to a reduction in transpiration. Arsenic induced Fe deficiency was likely the primary cause of chlorosis; however, As induced reduction in Zn, Mn or Mg contributed to chlorosis. Copper use in cellular functions was very efficient; nevertheless, Cu deficiency was one of the initial effects of As toxicity. Differences in mineral uptake reflect the plant's attempt to detoxify As (*i.e.* increase in S for S-containing As chelators), mitigate damage to the cell (*i.e.* Ca to repair leaky menbranes) or continue cellular functions through alternative pathways (*i.e.* Fe superoxide dismutases to replace the function of Cu/ZnSOD).

Keywords

Arsenic, Micronutrients, Secondary Nutrients, Iris, Marigold, Sunflower, Switchgrass

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1. Introduction

Arsenic-based rodenticides, herbicides, insecticides and irrigation with water high in As have resulted in As contamination on and around turf-farms, orchards, greenhouses, golf courses and residential lawns and gardens [1]-[3]. An average As concentration (AC) of 13.7 mg·kg^{-1} was found in the fine clay fraction of 14 South Florida golf courses associated with high AC in groundwater [4]. Urban soils from Gainesville and Miami, Florida, have a range of 0.21 to 660 mg As kg^{-1} soil [5]. The Florida Department of Environmental Protection goals for cleanup of residential and industrial soils are 0.8 and 3.7 mg As kg^{-1} soil, respectively. In Miami 95% of soils sampled exceeded the Florida residential goal and 33% exceeded the commercial goal. Soil contaminated with As levels above regulatory goals is a problem for people living in South Florida.

Arsenic is not essential for plants [6] and has no known metabolic function. Plant species vary in their tolerance to As [7] with toxicity threshold levels ranging from 5 to 100 mg As kg^{-1} dry weight for most plants [8]. At low concentrations the oxidized form of As, arsenate, can act as an analogue of phosphate in which they share the same transport pathway [3] [9]. Arsenic may compete with P for uptake by high-affinity phosphate transporters in root cells [10]-[12]. Reduced As, arsenite, is likely taken up by aquaporin channels in plant roots [13]. 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 [3]. 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. In rice (*Oryza sativa*) [14] [15], cucumber (*Cucumis sativus*) [16], *Brassica juncea* [17], tomato (*Solanum lycopersicum*) [18], *Spartina patens* and *Spartina alterniflora* [19], As is reduced to arsenite in the root with only a small portion transported to the shoot. In contrast, plants that accumulate As will translocate a large portion of it to the shoot. The As hyperaccumulator *Pterisvittata* translocated 8x more As from root to shoot than the non-hyperaccumulator *P. tremula* [20] and 2.8x more than *P. ensiformis* [21].

The effect of As on micronutrient allocation between roots and shoot is in part a function of the concentration of soil As and the plant species sensitivity to As [22]. In addition, adsorption by roots, translocation, plant tissue in question, growth stage, and metabolic interactions with other elements will influence the distribution of micronutrients in plants. Arsenic toxicity to root membranes can limit transport of elements to shoot tissue [23] [24]. For example, As damage in tomato roots reduced transpiration and thus reduced B concentration in stem tissue at fruiting [24]. Calculations of leaf-to-root concentration ratios in bean plants at a late vegetative stage showed lower Cu, Fe and Mn concentrations with higher non-lethal As content in the growing media [25]. In tomato, higher non-lethal As resulted in lower Mn and higher Zn concentrations [22]. These differences were attributed to As induced interactions between various ions during uptake and translocation. For example, P-Cu interactions [26] [27] were believed to be influenced by As acting as an analog to P in metabolic reactions.

Arsenic in plant tissue rarely reaches levels toxic to humans because of its high toxicity to plants. Allocation between different plant tissue generally results in the lowest AC in fruit [22] [28]. The same As distribution should hold true for other reproductive parts.

Ornamental plants have not been fully investigated as a mechanism for phytoremediation of low level As contaminated soil. Ornamentals can partially offset the cost of contaminated land taken out of production through production of cut flowers and other marketable commodities. In addition, these plants can provide an aesthetic quality to buildings located on contaminated sites. A study was conducted to evaluate nutrient uptake by ornamental plants grown in a hydroponic system containing As. This report describes micronutrient distribution between root and shoot tissue in several ornamental plants.

2. Materials and Methods

2.1. Plant Species

Methods were previously described in [29]. The plants used in this study were iris (*Iris savannarum*), switchgrass (*Panicum virgatum*), *Tithonia rotundiflora*, *Coreopsis lanceolata*, sunflower (*Helianthus annuus*), and marigold (*Tagetes erecta*). 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 28 × 53 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 seedlings with at least two fully developed leaves, and iris and coreopsis plants,10 cm tall with ≥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**.

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 $Ca(NO_3)_2$, 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 $mg \cdot L^{-1}$ As (0.448 mg per reservoir tank). Plants were acclimated to hydroponic feeding for a minimum of one week before beginning As treatments.

An As solution concentration of 2 - 8 uM equates to a soil AC of 700 - 3000 $mg \cdot kg^{-1}$ [30]. Based on this and the levels of contaminated urban soils reported above, a range of 10 - 70 uM As solution concentration was selected to cover the range of low level As contamination found in south Florida's urban soils. Enough Na_2HAsO_4, dissolved in 1.0 L water, was added to different reservoirs to make a final tank concentration of 0, 10, 20, 30, 40, 50 or 70 uM As (0.0, 0.75, 1.5, 3.0, 3.75, 5.25 $mg \cdot L^{-1}$ As, respectively). Reservoir pH was adjusted daily to pH 6.5 with either NaOH or H_2SO_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 washed with a gentle spray to remove debris, agitated in a pool of water then washed a second time. 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. 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 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. A translocation factor (TF) was calculated as:

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

for each species and each treatment.

2.4. Statistical Analysis

Each plant species was analyzed separately. The data represent means calculated from six replicated pots for

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
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

each As treatment. Analysis of variance was performed using the Proc Mixed procedure of Statistical Analysis System [31]. Tukey adjusted least square means were used for comparison at $P < 0.05$ unless stated otherwise. Arithmetic means were used to calculate translocation factors.

3. Results

3.1. Dry Weight

Both coreopsis root and shoot dry weights declined with increasing solution AC (**Figure 1(a)**). Coreopsis 0.0 As control treatment produced at mean dry weight of 6.9 g with a shoot-to-root ratio of 2.52. Healthy roots could support 2.5x their weight in above ground dry matter. At 0.75 mg As L^{-1} solution there was a 32% reduction in dry weight; reductions for 2.25, and 5.25 mg As L^{-1} were 65% and 84%, respectively. A significant drop in dry weight began at 2.25 mg As L^{-1}; however, up to that point the allocation of dry matter between shoot and root tissue (shoot-to-root ratio) remained in a range from 2.5 and 2.3. As plants became smaller the loss in dry weight was equally shared by shoot and root tissue. A concentration of 5.25 mg As L^{-1} resulted in a drop in shoot-to root ration to 1.5. At this level of exposure, roots damaged by As had dropped below a critical level and a proportional shoot weight similar to that produced in the control could no longer be maintained.

Tithonia produced a mean dry weight in the control of 34.2 g (**Figure 1(b)**). Addition of as little as 0.75 mg As L^{-1} resulted in a 79% reduction in dry weight. In contrast to coreopsis, the dry weight shoot-to-root ratio for tithonia change very little with an increase in solution As, ranging from 1.11 to 0.98 decreasing steadily from 0.0 to 3.75 mg As L^{-1}, respectively.

In iris, a solution AC of 0.75 mg·L^{-1} reduced total plant dry weight accumulation to 50% of the control (**Figure 1(c)**). However, higher ACs up to 5.25 mg·L^{-1}, increased plant dry weight. This was true for both root and shoot tissue. Whole plant dry weight increased over controls by 1.8% and 2.2% with solution ACs of 3.0 and 5.25 mg as L^{-1}, respectively. The initial decrease in dry weight at low AC and subsequent increase in dry weight at higher ACs may result from complex interactions with other elements (Fe and Mn) during uptake and translocation. These interactions at low AC might inhibit growth but all defensive mechanisms may not be fully mobilized until solution AC increases. This will be discussed in more detail below. In addition to the reduction in dry weight, the shoot-to-root ratio slightly decreased from 3.0 in the 0.0 As control to 2.5 in the 0.75 mg As L^{-1} solution treatment. As dry weight increased with higher solution AC, shoot-to-root ratio returned to levels near those found in control plants, 2.8 and 3.0 for the 3.0 and 5.25 mg As L^{-1} solution treatments, respectively.

Marigold's dry weight was statistically similar in all treatments for both shoot and root tissue (**Figure 1(d)**). Shoot-to-root ratios were higher in As treatments than that in the control. Sunflower performed similar to marigold in that there were no differences in dry weight between treatments (**Figure 1(e)**). Sunflower dry weights tended to decline with increasing solution AC. Shoot-to-root ratios were higher in plant treated with 0.75 and 3.75 mg As L^{-1} solution As. Switchgrass dry weight decreased with increasing solution AC (**Figure 1(f)**). The drop in shoot dry weight was greater than that in roots resulting in a constant drop in shoot-to-root ratio with an increase in solution AC.

3.2. Arsenic Content

Arsenic accumulated by coreopsis plants tended to remain in root tissue (**Figure 2(a)**). As solution As increased, the shoot-to-root ratio of As accumulation went down. Maximum As uptake occurred at 2.25 mg As L^{-1} solution; above that level shoot As began to decline faster than root As. Switchgrass also accumulated As mostly in root tissue and the shoot-to-root ratio decreased with increasing solution As (**Figure 2(f)**). Very little As was taken up by iris plants (**Figure 2(c)**). More of the As taken up by iris was translocated to the shoot in all but the 3.0 mg As L^{-1} solution treatment. A high sensitivity in tithonia to As, as seen in the reduction in dry weight at 0.75 mg·L^{-1} solution concentration resulted in low As accumulation in plant tissue (**Figure 2(b)**). The shoot-to-root ratio of As accumulation increased from 0.3 mg in controls to 0.9 and 1.0 in the 0.75 and 2.2 mg As L^{-1} solution treatments. At 3.75 mg·L^{-1} plant uptake dropped to 0.4 mg As. Although there were no statistical differences in marigold root As, uptake tended to increase with increasing solution concentration. Marigold shoot As was higher than that in controls, however, less As was partitioned to shoot tissue with increasing solution concentration (**Figure 2(d)**). Sunflower root and shoot As content increased with increasing solution AC (**Figure 2(e)**). The shoot-to-root ratio for As was 0.9, 0.7, 0.8 and 0.8 in the 0.0, 0.75, 3.75 and 5.25 mg·L^{-1} treatments, respectively.

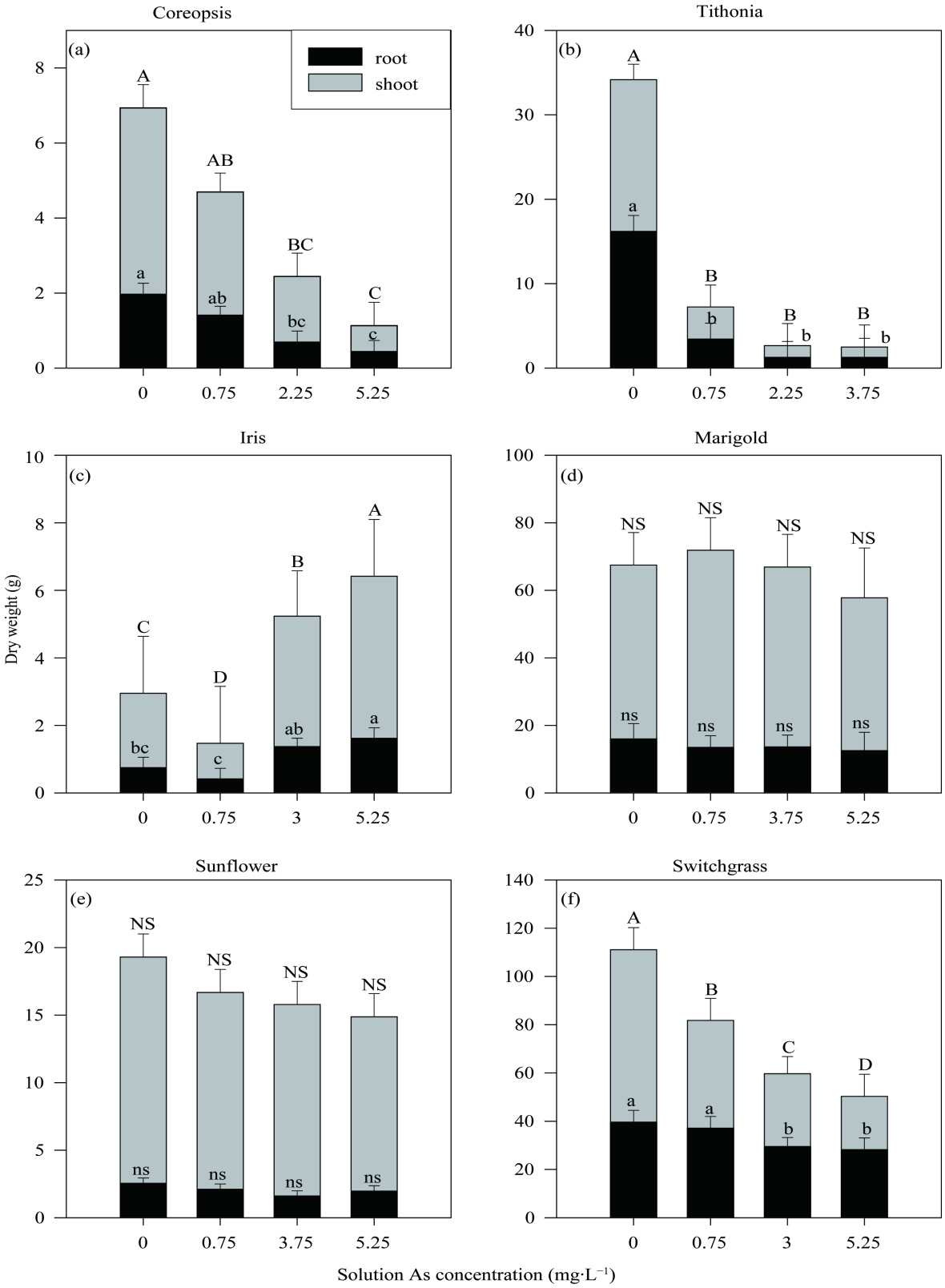

Figure 1. Effects of As concentration on mean root (black bar) and shoot (grey bar) tissue dry weights (g). Bar sections with the same letter are not significantly different at $P = 0.05$; capital letters designate shoot and lower case letters root weights. ns = not significantly different.

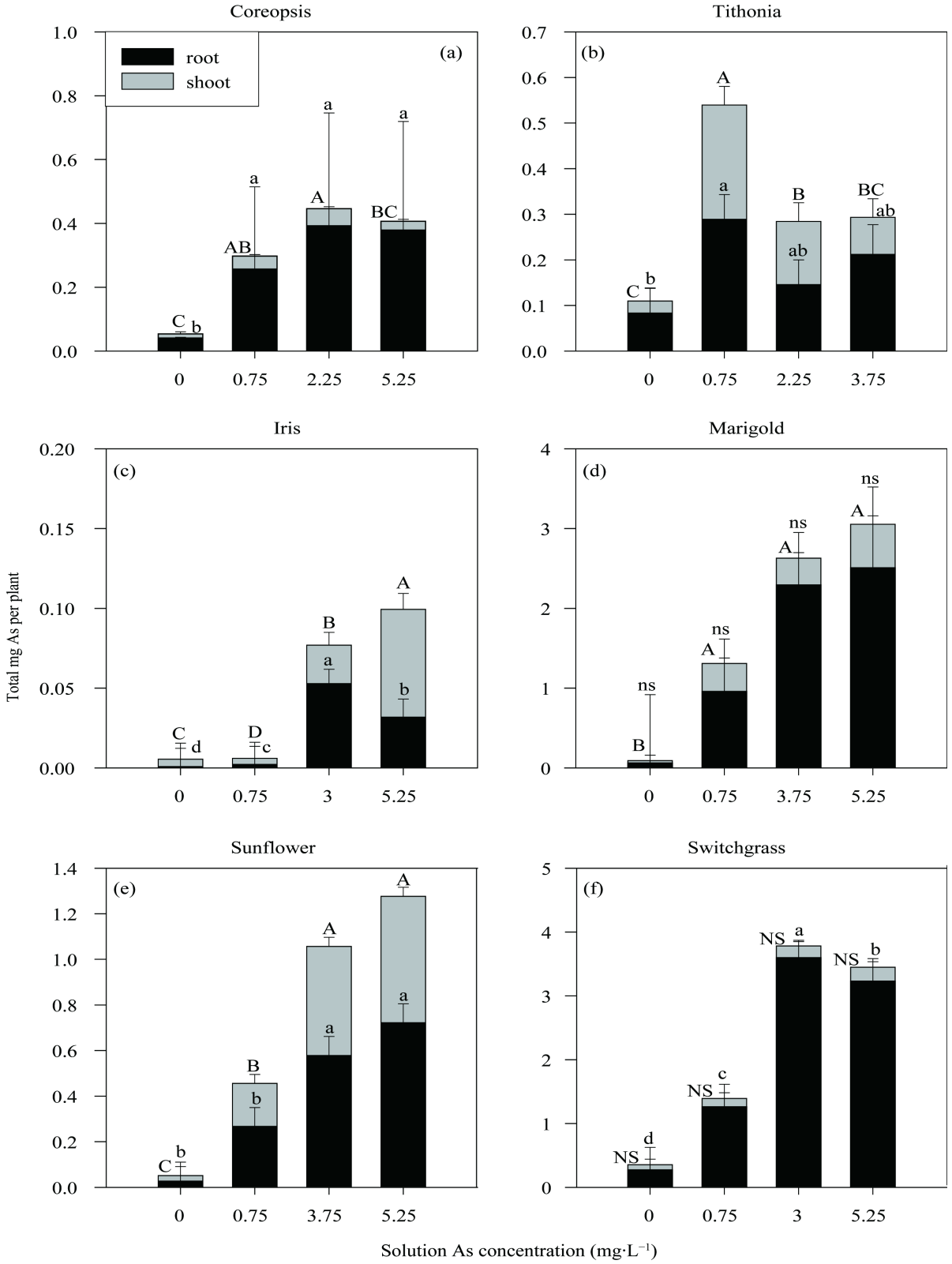

Figure 2. Effect of solution As concentration on root (black bar) and shoot (grey bar) tissue arsenic content in accumulated mg. Bar sections with the same letter are not significantly different at $P = 0.05$; capital letters designate shoot and lower case letters root weights. ns = not significantly different.

3.3. Plant as Concentration

The AC in coreopsis root and shoot tissue increased with increasing hydroponic solution AC (**Figure 3(a)**). Considering the continuous decrease in dry weight combined with an increase in As uptake to a solution concentration of 2.25 mg As L^{-1}, coreopsis plant protective mechanism against As were likely overwhelmed at this point. A higher AC was found in coreopsis root than shoot tissue. Maximum tithonia tissue AC was reached at 2.25 mg As L^{-1} (**Figure 3(b)**). Tithonia maintained a high shoot AC in all treatments.

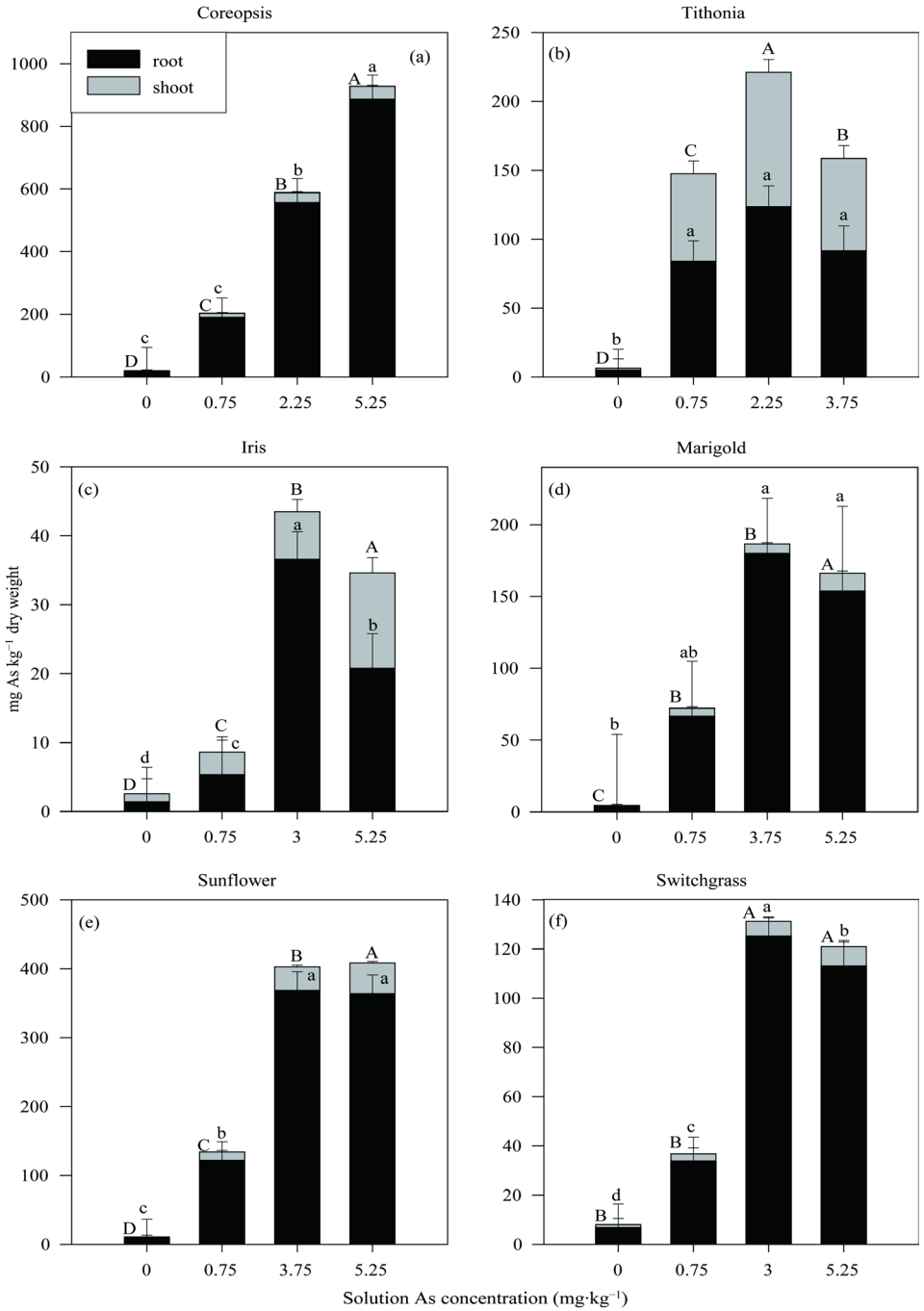

Figure 3. Effect of solution As concentration on root (black bar) and shoot (grey bar) tissue As concentration (mg As kg^{-1} dry weight). Bar sections with the same letter are not significantly different at P = 0.05; capital letters designate shoot and lower case letters root weights. ns = not significantly different.

Iris whole plant AC increased at 0.75 mg·L^{-1} solution concentration due to an initial drop in plant dry weight (**Figure 3(c)**). Subsequent increases in tissue AC were due to increased As uptake. Shoot AC increased with increasing solution As. At 5.25 mg As L^{-1} solution plant uptake was highest; however, AC declined due to a relatively small increase in root dry weight accompanied by greater transport of As to shoot tissue.

Marigold whole plant tissue AC peaked at 3.75 mg As L^{-1} solution (**Figure 3(d)**). At a hydroponic solution concentration of 5.25 mg As L^{-1}, shoot tissue AC dropped off due to a lower dry weight produced by that treatment. Switchgrass had a similar peak at 3.0 mg As L^{-1}; however, above this level a decrease in both As uptake and dry weight reduced tissue AC (**Figure 3(f)**). Sunflower AC peaked at 3.75 mg·L^{-1} solution As; however, there was a slight increase in shoot AC with an increase in solution As to 5.25 mg·L^{-1} (**Figure 3(e)**).

3.4. Plant Element Uptake

In coreopsis all elements trended to a decrease in root content with an increase in solution As (**Table 2**). Differences between zero As controls and 5.25 mg As L^{-1} solution were significant for all elements except Fe and Mo. For Ca, Cu, K and P significant differences appeared at 0.75 mg·L^{-1} solution As. A similar decrease in shoot element content with increasing solution As was found in shoot tissue. Shoot tissue had higher element content than root tissue for all elements except Fe and Mo (**Table 2**).

Table 2. The effect of solution As concentration on element distribution between shoot and root tissue (mg per plant).

Solution As concentration (mg As L^{-1})	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
					Coreopsis lanceolata						
					Shoot						
0.00	0.2082 a[‡]	80.15 a	0.003889 a	0.3323 a	329.02 a	32.10 a	0.2232 a	0.0028 a	27.2460 a	13.57 ab	0.1305 a
0.75	0.1337 ab	54.68 ab	0.000372 b	0.1645 b	242.69 ab	17.32 b	0.2254 a	0.0027 a	17.4340 b	18.59 a	0.1515 a
2.25	0.0758 bc	29.85 bc	0.000103 b	0.1044 b	110.13 bc	11.48 bc	0.1318 ab	0.0015 ab	7.7110 c	5.82 bc	0.0432 b
5.25	0.0263 c	13.88 c	0.000038 b	0.0401 b	36.74 c	5.31 c	0.0610 b	0.0011 b	2.5680 c	2.36 c	0.0162 b
					Root						
0.00	0.0237 a	13.63 a	0.0005490 a	0.5380 ns	82.68 a	4.88 a	0.0307 a	0.0041 ns	11.51 a	8.12 a	0.0549 a
0.75	0.0229 a	6.72 b	0.0000208 b	0.4329 ns	52.20 b	3.05 ab	0.0318 a	0.0029 ns	6.93 b	7.27 ab	0.0401 a
2.25	0.0115 b	3.50 b	0.0000040 b	0.3321 ns	35.33 bc	2.09 b	0.0164 ab	0.0024 ns	4.81 bc	4.20 ab	0.0207 b
5.25	0.0086 b	2.68 b	0.0000012 b	0.1078 ns	24.36 c	1.20 b	0.0078 b	0.0010 ns	2.48 c	2.99 b	0.0171 b
					Iris (*Iris savannarum*)						
					Shoot						
0.00	0.1029 ab	21.17 ab	0.00664 ns	0.1629 bc	149.9 ns	8.69 ab	0.0249 ns	0.0017 b	15.6690 ns	6.74 b	0.0509 ns
0.75	0.0437 b	8.75 b	0.01087 ns	0.0780 c	70.2 ns	3.71 b	0.0068 ns	0.0010 b	7.1220 ns	4.92 b	0.0236 ns
3.00	0.1656 ab	31.54 ab	0.00100 ns	0.2906 ab	252.0 ns	13.23 ab	0.0350 ns	0.0053 a	24.5440 ns	15.52 ab	0.0846 ns
5.25	0.2170 a	39.92 a	0.00044 ns	0.4377 a	303.2 ns	17.31 a	0.0378 ns	0.0033 ab	29.4390 ns	20.48 a	0.0870 ns
					Root						
0.00	0.0190 b	2.60 b	0.00123 a	0.0703 ns	15.97 b	1.34 b	0.0058 ns	0.0006 b	2.9821 b	1.24 c	0.0236 ab
0.75	0.0137 b	2.37 b	0.00021 ab	0.0413 ns	8.58 b	0.87 b	0.0049 ns	0.0005 b	1.7966 b	1.32 c	0.0181 b
3.00	0.0398 a	8.76 a	0.00007 b	1.3009 ns	38.18 a	4.00 a	0.0428 ns	0.0012 a	6.5892 a	4.67 b	0.0392 ab
5.25	0.0552 a	11.72 a	0.00051 ab	1.7084 ns	52.87 a	4.85 a	0.0534 ns	0.0013 a	7.2494 a	5.84 a	0.0459 a

Continued

Marigold (*Tagetes erecta*)											
Shoot											
0.00	1.6424 ns	759.4 ns	0.8375 ns	3.11 ns	2003.1 ns	126.57 ns	2.8894 ns	0.0200 ns	309.8600 ns	144.31 ns	6.698 ns
0.75	2.0378 ns	745.2 ns	0.0323 ns	5.31 ns	1907.5 ns	132.78 ns	3.0097 ns	0.0274 ns	317.7700 ns	186.95 ns	5.911 ns
3.75	1.7653 ns	754.8 ns	0.0274 ns	3.57 ns	1976.6 ns	135.66 ns	3.2861 ns	0.0172 ns	290.5600 ns	165.78 ns	4.838 ns
5.25	1.4036 ns	856.5 ns	0.0170 ns	2.96 ns	1725.1 ns	122.55 ns	3.3772 ns	0.0235 ns	317.2100 ns	178.32 ns	3.984 ns
Root											
0.00	0.2842 ns	133.38 ns	0.0818 a	23.87 ns	212.84 ns	28.60 ns	3.3070 ns	0.0608 ns	18.6710 ns	49.65 ns	2.4165 ns
0.75	0.2770 ns	103.13 ns	0.0045 b	19.78 ns	202.08 ns	20.73 ns	2.6458 ns	0.0478 ns	17.5780 ns	40.26 ns	2.3926 ns
3.75	0.2486 ns	118.90 ns	0.0020 b	20.46 ns	181.31 ns	25.95 ns	2.9634 ns	0.0521 ns	21.3600 ns	60.16 ns	2.8357 ns
5.25	0.2720 ns	134.90 ns	0.0040 b	28.14 ns	143.09 ns	23.60 ns	2.2950 ns	0.0598 ns	21.5990 ns	45.70 ns	1.9958 ns
Sunflower (*Helianthus annuus* L.)											
Shoot											
0.00	1.0808 ns	242.93 ns	0.1110 a	1.16 ns	674.60 ns	69.27 ns	0.5007 ns	0.0132 ns	107.6400 ns	60.23 ns	1.2283 ns
0.75	1.2906 ns	277.70 ns	0.0082 b	0.90 ns	714.30 ns	73.05 ns	0.6209 ns	0.0157 ns	118.7900 ns	89.90 ns	1.7752 ns
3.75	1.0321 ns	258.70 ns	0.0018 b	1.30 ns	778.50 ns	70.42 ns	1.0815 ns	0.0152 ns	136.5300 ns	85.31 ns	1.7795 ns
5.25	0.8877 ns	227.63 ns	0.0017 b	0.71 ns	549.20 ns	61.67 ns	0.5687 ns	0.0102 ns	92.9500 ns	59.40 ns	1.0679 ns
Root											
0.00	0.0483 ns	17.22 ns	0.004104 a	1.40 ns	68.95 ns	3.30 ns	0.2974 ns	0.0058 ns	8.2870 ns	10.20 a	0.2294 ns
0.75	0.0477 ns	14.17 ns	0.000306 b	1.71 ns	57.40 ns	2.52 ns	0.3581 ns	0.0057 ns	9.8070 ns	6.65 ab	0.2076 ns
3.75	0.0289 ns	10.00 ns	0.000055 b	0.72 ns	46.34 ns	2.04 ns	0.1352 ns	0.0035 ns	8.1670 ns	5.88 ab	0.2627 ns
5.25	0.0372 ns	12.93 ns	0.000087 b	1.37 ns	53.30 ns	2.37 ns	0.2587 ns	0.0054 ns	9.2220 ns	4.11 b	0.3129 ns
Switchgrass (*Panicum virgatum*)											
Shoot											
0.00	1.0028 a	329.13 a	0.1062 ns	9.19 ns	2394.40 a	369.10 a	0.9095 a	0.0339 a	390.0200 a	138.68 a	1.4759 a
0.75	0.6768 b	224.37 b	0.0192 ns	3.33 ns	1547.20 b	247.72 b	0.7926 a	0.0194 b	257.2100 b	160.86 a	0.9271 b
3.00	0.3997 bc	161.17 bc	0.0050 ns	2.30 ns	999.20 c	167.31 c	0.7596 a	0.0166 b	177.2200 bc	137.44 a	0.6454 b
5.25	0.2971 c	96.47 c	0.0033 ns	2.29 ns	563.60 c	80.96 d	0.3969 b	0.0114 b	107.1500 c	65.40 b	0.3010 c
Root											
0.00	0.7386 ns	692.2 ns	0.0249 ns	145.51 ns	770.97 a	244.15 ns	4.2170 ns	0.0407 ab	113.1300 a	73.73 ns	1.0507 a
0.75	0.6282 ns	605.5 ns	0.0122 ns	124.72 ns	566.68 b	188.22 ns	3.3480 ns	0.0608 a	113.2000 a	91.02 ns	0.3889 b
3.00	0.4056 ns	421.4 ns	0.0007 ns	74.70 ns	549.71 b	168.50 ns	2.3100 ns	0.0253 b	93.1000 ab	84.98 ns	0.4974 b
5.25	0.6113 ns	498.9 ns	0.0061 ns	87.86 ns	321.16 c	127.35 ns	2.5050 ns	0.0421 ab	45.0500 b	73.63 ns	0.5357 b
Tithonia rotundiflora											
Shoot											
0.00	1.0084 a	448.7 a	0.01919 a	2.90 a	989.4 a	145.95 a	0.3776 a	0.0150 a	225.0700 a	61.90 a	0.3676 a
0.75	0.2256 b	97.4 b	0.00012 b	0.42 b	156.7 b	34.42 b	0.1199 b	0.0025 b	55.6200 b	22.34 b	0.0961 b
2.25	0.0520 b	17.6 b	0.00002 b	0.11 b	37.2 b	8.23 b	0.0250 b	0.0011 b	7.9900 b	4.32 b	0.0148 b
3.75	0.0381 b	15.0 b	0.00002 b	0.15 b	31.2 b	6.87 b	0.0234 b	0.0011 b	7.3400 b	4.64 b	0.0082 b
Root											
0.00	0.1788 a	62.28 a	0.02842 ns	35.02 a	281.95 a	19.86 a	0.4611 a	0.2635 a	25.5350 a	21.22 a	0.3041 a
0.75	0.0355 b	10.03 b	0.00015 ns	1.98 b	37.78 b	2.70 b	0.0870 b	0.0097 b	5.9890 b	5.19 b	0.0751 b
2.25	0.0109 b	2.85 b	0.00002 ns	0.46 b	6.03 b	0.79 b	0.0307 b	0.0023 b	1.4270 b	0.95 b	0.0121 c
3.75	0.0070 b	3.33 b	0.00003 ns	1.91 b	11.25 b	1.06 b	0.0367 b	0.0044 b	1.9050 b	1.98 b	0.0193 c

[1]Numbers in an individual column followed by the same letter are not significantly different at $P = 0.05$; ns = no significant difference.

Due to a small amount of sample available from the tithonia 5.25 mg As L^{-1} treatment, the highest As treatment level reported in **Table 2** for tithonia is from 3.75 mg As L^{-1} treatment. Arsenic significantly reduced tissue element content below that of the control for all elements in shoot and root tissue except for Cu. Four elements, Cu, Fe, Mn and Mo had higher element content in root than shoot tissue (**Table 2**).

The pattern of element accumulation in iris mirrored that of dry weight and As accumulation. For all elements except Cu, Fe and Mn there was an initial reduction in root content at 0.75 mg As L^{-1} solution concentration (**Table 2**). Iron and Mn had a similar initial reduction in root content but the differences were not significant. At higher solution ACs there was a ≥ increase in nutrient element content above that of the control. Root Cu content initially dropped below that in the control and remained low with increased solution As (significantly lower at 3.0 mg As L^{-1} solution only). There were no significant differences in shoot Cu between the control and As treatments; however, values were highest at 0.75 mg·L^{-1} solution As then continuously decreased from 0.001 to 0.0004 mg shoot Cu with 3.00 and 5.25 mg·L^{-1} solution As, respectively. There were no significant differences found in iris shoot K, Mn, P and Zn. Content of all shoot elements (except Cu) tended to decrease at 0.75 mg·L^{-1} solution As then increase at higher solution AC to 5.25 mg As L^{-1}.

With the exception of Cu there were no significant differences in marigold root element content between the control and As treatments (**Table 2**). For all elements, no differences in As treatments were found in marigold shoot tissue. Root Cu content declined by >94% with As treatments. Although difference were not significant in shoot tissue, Cu declined by >96% with As treatments. The response to As by sunflower was similar to that of marigold in that significant differences between control and As treatments were found only for root Cu and S and shoot Cu.

No significant differences were found between control and treatments in switchgrass root tissue for all elements except K, Mo, P and Zn (**Table 2**). In switchgrass shoot tissue, a steady decline in element content was found with increasing solution AC. With Cu, K and P the reduction at 5.25 mg·L^{-1} solution As was to <50% of that found in the zero As control.

3.5. Translocation Percentage and Tissue Concentration

As mentioned above an increase in solution AC led to an increase in As uptake and a decrease in total plant uptake of all elements by coreopsis (**Figure 2**, **Table 2**). Translocation from coreopsis root to shoot tissue either decreased or insignificantly decreased with increasing solution As for all elements except Cu and Mo (**Table 3**). An increase in Cu translocation was observed with as little as 0.75 mg·L^{-1} solution As, however, with Mo the increase was insignificant. Boron, Mn and S concentration in root tissue increased and Cu decreased with increasing Solution As (**Table 4**). Generally, shoot element concentrations were not dramatically different from that found in the control. Several elements Ca, Mg, Mn, Mo and S had an increase in concentration at 5.25 mg·L^{-1} solution As over that in the control. Given the large reduction in plant dry weight due to the increased As uptake, a higher tissue concentration of some elements would be expected. Chlorosis was observed on coreopsis leaves with 0.75 mg·L^{-1} solution As which is consistent with the reduction in Fe concentration. Copper translocation increased as copper concentration in both root and shoot tissue decreased. Copper is a component of enzymes associated with electron transport and protection against superoxide-free radicals [32]. Arsenic toxicity has been shown to cause an increase in reactive oxygen species leading to membrane leakage [33]. In the current study shoot Cu was reduced by ≥83% and root Cu by 94%. It is possible that reduced production of Cu containing enzymes used to detoxify oxygen-free radicals was a contributing factor in As toxicity at low solution As levels in coreopsis.

In tithonia, As translocation was greater at 0.75 and 2.25 mg·L^{-1} solution As than with the control (**Table 3**). Root element concentration trended downward for all elements to the point where at 5.25 mg As L^{-1} all element concentrations except P, S and Zn were significantly lower than in the control (**Table 3**). There was an increase in shoot B, Ca, Mg, Mn, P, S and Zn at 0.75 mg·L^{-1} solution AC followed by a drop in concentration as solution As increased. Both root and shoot Cu were significantly reduced by ≥97%, even at the low solution AC.

In iris there were no differences in translocation percent for any element except As and Fe (**Table 3**). Translocation of Fe was reduced at 3.0 mg·L^{-1} As and above. There were no visible signs of chlorosis and shoot Fe concentrations were similar to the control at all As treatments levels (**Table 4**). Root tissue Ca, K and Mg concentration increased with increasing solution As; however, there were no differences in shoot concentration of these elements. Root B concentration increased at higher solution ACs whereas, shoot As did not differ from the

Table 3. Translocation percent from root to shoot tissue for different elements in *Coreopsis lanceolata*, *Iris savannarum*, *Tagetes erecta* (marigold), *Helianthusannuus* L. (Sunflower), *Panicum virgatum* (Switchgrass) and *Tithonia rotundiflora* grown in hydroponic solution containing As concentrations from 0.0 to 5.25 mg·L^{-1}.

Solution concentration (mg·L^{-1})	As	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
						Coreopsis lanceolata						
0.00	29.2 a[I]	89.57 a	85.86 ab	87.60 b	39.65 ns	79.44 a	87.28 ns	87.87 ns	43.42 ns	70.52 a	63.43 ab	70.70 a
0.75	13.3 b	82.00 b	86.00 ab	93.32 a	29.32 ns	78.33 a	82.07 ns	85.14 ns	48.88 ns	68.11 a	71.05 a	75.18 a
2.25	13.3 b	86.69 ab	89.48 a	94.68 a	29.71 ns	75.72 a	84.00 ns	87.57 ns	45.63 ns	61.44 a	58.71 b	67.06 a
5.25	6.8 b	73.78 c	82.87 b	96.26 a	27.78 ns	59.08 b	80.75 ns	88.58 ns	53.58 ns	50.18 b	44.95 c	48.55 b
						Iris savannarum						
0.00	40.92 b	67.55 ns	75.23 ns	70.16 ns	56.13 a	75.67 ns	72.04 ns	61.22 ns	60.13 ns	68.56 ns	69.31 ns	52.67 ns
0.75	44.96 ab	70.04 ns	74.28 ns	68.42 ns	56.94 a	80.54 ns	75.47 ns	55.17 ns	56.03 ns	71.23 ns	69.53 ns	52.30 ns
2.25	33.74 b	76.99 ns	75.54 ns	88.46 ns	27.51 b	83.33 ns	73.40 ns	48.27 ns	76.12 ns	74.48 ns	73.75 ns	64.78 ns
5.25	69.25 a	77.25 ns	76.25 ns	60.17 ns	23.75 b	82.61 ns	75.87 ns	45.24 ns	67.06 ns	76.82 ns	74.14 ns	67.39 ns
						Tagetes erecta (marigold)						
0.00	17.69 ns	85.37 ab	85.77 ns	86.47 ns	11.54 b	91.24 ns	81.61 ns	48.02 ns	24.20 ns	94.00 ns	76.10 ns	71.04 ns
0.75	30.24 ns	88.89 a	88.10 ns	87.85 ns	23.77 a	92.06 ns	86.94 ns	54.51 ns	38.36 ns	94.77 ns	81.89 ns	70.89 ns
2.25	16.64 ns	87.75 ab	86.01 ns	92.78 ns	16.65 ab	89.80 ns	83.91 ns	52.34 ns	26.65 ns	92.79 ns	79.10 ns	61.46 ns
5.25	38.43 ns	84.47 b	87.02 ns	76.31 ns	14.69 ab	89.47 ns	84.22 ns	57.96 ns	31.37 ns	93.61 ns	80.10 ns	68.14 ns
						Helianthus annuus (sunflower)						
0.00	44.47 ns	95.54 ns	93.14 ns	94.24 ns	47.53 ab	90.68 ns	95.33 ns	62.30 ns	69.04 ns	92.83 ns	86.34 b	84.07 ab
0.75	43.92 ns	96.81 ns	95.84 ns	96.68 ns	45.62 ab	93.05 ns	97.06 ns	71.28 ns	77.03 ns	92.95 ns	93.88 a	90.52 a
2.25	45.41 ns	97.36 ns	95.67 ns	97.57 ns	65.78 a	93.09 ns	97.23 ns	76.15 ns	74.63 ns	93.35 ns	91.65 ab	83.83 ab
5.25	44.88 ns	95.65 ns	94.45 ns	93.94 ns	35.98 b	91.13 ns	96.26 ns	66.63 ns	65.49 ns	90.97 ns	93.52 a	78.01 b
						Panicum virgatum (switchgrass)						
0.00	23.58 ns	57.77 a	33.78 a	86.35 ns	9.29 ns	75.92 a	61.64 a	26.70 ns	43.59 a	77.00 a	65.02 a	59.27 b
0.75	20.69 ns	51.64 a	31.74 ab	79.37 ns	4.53 ns	73.37 a	59.54 a	26.00 ns	25.84 b	70.32 ab	64.86 a	70.81 a
2.25	5.00 ns	49.90 a	29.69 ab	87.64 ns	3.27 ns	64.50 b	49.85 ab	27.54 ns	40.38 a	65.37 b	61.57 a	56.58 b
5.25	18.13 ns	31.90 b	17.24 b	66.76 ns	3.25 ns	63.51 b	39.96 b	15.69 ns	22.05 b	71.04 ab	46.44 b	35.84 c
						Tithonia rotundiflora						
0.00	25.93 b	82.67 ns	84.66 ns	135.50 ns	22.28 ns	79.75 ns	86.20 ns	48.66 ns	18.70 ns	88.74 ns	78.00 ns	55.36 ns
0.75	45.84 a	86.42 ns	90.63 ns	44.96 ns	18.63 ns	80.26 ns	92.37 ns	58.60 ns	20.53 ns	89.86 ns	80.10 ns	56.54 ns
2.25	49.08 a	88.10 ns	86.51 ns	52.94 ns	26.60 ns	86.30 ns	91.52 ns	46.98 ns	34.38 ns	85.28 ns	82.66 ns	57.19 ns
5.25	31.80 ab	82.54 ns	85.96 ns	49.39 ns	11.12 ns	77.90 ns	89.52 ns	47.26 ns	31.40 ns	83.62 ns	76.29 ns	40.82 ns

[I]Numbers in any individual column followed by the same letter are not significantly different at $P = 0.05$; ns, no significant difference.

Table 4. The effect of solution As concentration on element concentration (mg·kg^{-1}) in shoot and root tissue.

Solution As concentration (mg·L^{-1})	B	Ca	Cu	Fe	K	Mg	Mn	Mo	P	S	Zn
					Coreopsis lanceolata						
					Shoot						
0.00	42.0 ns[1]	16291 b	0.623 a	65.42 a	66063 b	6536 b	46.09 c	0.5 b	5561 a	2744 d	26.80 b
0.75	39.4 ns	16922 b	0.108 b	51.27 b	72127 a	5498 c	71.43 b	0.8 b	5356 a	6145 a	47.65 a
2.25	43.7 ns	17289 b	0.040 c	45.26 b	63168 b	6537 b	60.87 b	0.9 b	4380 b	3380 c	24.47 b
5.25	37.8 ns	20470 a	0.023 c	49.43 b	53095 c	7779 a	110.97 a	1.7 a	3765 b	3536 b	25.49 b
					Root						
0.00	12.6 b	6992 ns	0.305 a	269.80 ns	44722 ns	2470 ns	16.83 b	2.0 ns	6018 ns	4114 b	29.00 ns
0.75	18.8 a	5393 ns	0.016 b	299.30 ns	44446 ns	2619 ns	26.42 a	2.0 ns	5800 ns	5590 ab	35.18 ns
2.25	16.8 a	5243 ns	0.007 b	439.70 ns	51346 ns	3109 ns	23.72 a	3.3 ns	6981 ns	5998 ab	30.03 ns
5.25	19.7 a	6241 ns	0.002 b	251.40 ns	54962 ns	2726 ns	17.87 b	2.1 ns	5608 ns	6568 a	40.15 ns
					Iris (*Iris savannarum*)						
					Shoot						
0.00	46.3 ns	10143 ns	6.758 a	94.03 ns	65272 ns	4189 ns	12.59 ns	0.9 ab	7355 ns	3384 b	26.02 ns
0.75	44.0 ns	9336 ns	1.157 b	71.67 ns	64668 ns	3978 ns	7.39 ns	0.9 ab	6745 ns	4634 a	22.35 ns
3.00	43.6 ns	8478 ns	0.218 b	80.67 ns	65369 ns	3608 ns	8.98 ns	1.3 a	6459 ns	4364 ab	22.34 ns
5.25	46.5 ns	8655 ns	0.138 b	69.67 ns	61873 ns	3758 ns	8.71 ns	0.7 b	6596 ns	4306 ab	20.27 ns
					Root						
0.00	26.8 b	3895 b	1.682 a	103.90 d	21294 d	1844 d	9.72 b	0.9 b	4168 ns	1856 b	39.55 a
0.75	34.0 a	4698 b	0.368 b	116.80 c	23012 c	2195 c	9.06 b	1.4 a	4863 ns	3717 a	43.27 a
3.00	29.1 ab	6033 a	0.054 d	817.00 a	28177 b	2819 b	27.49 a	0.9 b	4978 ns	3573 a	28.00 b
5.25	33.9 a	6703 a	0.065 c	680.50 b	33103 a	2989 a	24.29 a	0.8 b	4865 ns	3936 a	27.26 b
					Marigold (*Tagetes erecta*)						
					Shoot						
0.00	32.5 ns	14868 b	5.460 a	62.31 ns	39006 ns	2479 ab	57.15 ns	0.4 ns	6132 ab	2814 ns	135.88 ns
0.75	36.7 ns	12879 b	0.602 b	95.39 ns	32743 ns	2232 b	54.11 ns	0.5 ns	5761 b	3157 ns	113.72 ns
3.75	35.3 ns	14332 b	0.512 b	71.49 ns	35601 ns	2531 ab	65.33 ns	0.4 ns	5666 b	3401 ns	93.09 ns
5.25	30.6 ns	18767 a	0.353 b	63.81 ns	38559 ns	2709 a	70.92 ns	0.5 ns	7228 a	3872 ns	84.19 ns
					Root						
0.00	18.7 ns	8794 ns	5.455 a	1592.70 ns	12289 ns	1864 ab	222.23 ns	4.0 ns	1297 ns	3140 ns	161.46 ns
0.75	21.3 ns	8171 ns	0.393 b	1472.40 ns	14749 ns	1583 b	213.93 ns	3.8 ns	1600 ns	3401 ns	235.36 ns
3.75	18.9 ns	9211 ns	0.167 b	1488.90 ns	15482 ns	1885 a	231.41 ns	3.9 ns	1754 ns	4749 ns	240.64 ns
5.25	21.4 ns	10172 ns	0.427 b	1881.70 ns	17427 ns	1904 a	184.32 ns	4.6 ns	1853 ns	3705 ns	153.74 ns

Continued

Sunflower (*Helianthus annuus* L.)											
Shoot											
0.00	66.9 b	14722 c	6.687 a	70.33 a	41071 c	4183 b	30.70 ns	0.8 ab	6613 b	3647 b	75.62 b
0.75	88.0 a	19063 a	0.618 b	60.61 ab	49657 a	5208 a	42.57 ns	1.0 a	8329 a	6282 a	131.08 a
3.75	78.4 ab	15714 bc	0.150 b	60.39 ab	45550 b	5174 a	32.06 ns	0.7 b	8439 a	4172 b	100.14 b
5.25	69.5 ab	17991 ab	0.128 b	54.40 b	42736 bc	4973 a	41.79 ns	0.8 ab	7206 ab	4675 b	88.72 b
Root											
0.00	18.8 ab	6728 ns	1.707 a	524.50 ns	27056 ns	1282 ns	115.66 ns	2.3 ns	3357 b	3761 ns	87.56 b
0.75	22.1 a	6130 ns	0.133 b	694.30 ns	32707 ns	1191 ns	133.56 ns	2.4 ns	5394 a	2787 ns	96.64 b
3.75	18.1 b	6172 ns	0.033 b	464.50 ns	30318 ns	1282 ns	89.85 ns	2.2 ns	5347 a	3468 ns	168.36 a
5.25	18.8 ab	6439 ns	0.042 b	700.80 ns	27887 ns	1193 ns	120.79 ns	3.1 ns	4655 ab	2106 ns	153.49 a
Switchgrass (*Panicum virgatum*)											
Shoot											
0.00	14.1 ns	4564 ns	2.670 a	81.79 ns	33942 b	5238 c	13.30 b	0.5 ns	5493 ns	1968 d	20.93 b
0.75	14.3 ns	5039 ns	0.447 b	75.42 ns	34860 a	5561 a	17.51 b	0.4 ns	5771 ns	3553 b	20.74 c
3.00	13.2 ns	5337 ns	0.165 b	77.15 ns	33184 c	5534 b	26.75 a	0.5 ns	5899 ns	4581 a	21.52 a
5.25	13.3 ns	4403 ns	0.162 b	73.15 ns	25552 d	3714 d	17.92 b	0.5 ns	4904 ns	2999 c	13.55 d
Root											
0.00	16.1 b	14095 ab	0.660 a	2216.60 ns	19557 a	4790 ab	67.52 ns	1.0 ab	2910 c	1845 b	26.94 a
0.75	14.4 b	11786 b	0.055 b	2018.40 ns	17152 c	3925 b	55.18 ns	1.4 a	3576 a	2382 ab	10.59 b
3.00	14.0 b	14426 ab	0.025 b	2699.10 ns	19271 b	6021 a	83.65 ns	0.8 b	3313 b	2948 a	17.87 ab
5.25	21.9 a	15755 a	0.022 b	2364.30 ns	11254 d	3874 b	71.79 ns	1.4 a	1603 d	2677 a	16.76 ab
Tithonia rotundiflora											
Shoot											
0.00	52.6 a	23271 a	1.817 a	147.88 ns	56961 a	7733 ab	22.15 b	0.9 ns	11580 a	3309 b	23.90 b
0.75	60.0 a	25929 a	0.030 b	107.34 ns	40956 b	9130 a	33.22 a	0.7 ns	14762 a	6047 a	25.62 a
2.25	37.3 b	12593 b	0.018 c	84.03 ns	26679 c	5926 bc	18.28 b	0.8 ns	5755 b	3157 b	10.08 c
3.75	28.6 b	10964 b	0.018 c	120.91 ns	24460 d	5148 c	18.00 b	0.8 ns	5404 b	3640 b	7.35 d
Root											
0.00	12.0 a	4693 a	1.725 a	1871.00 a	21414 a	1465 a	29.28 a	14.4 a	1955 ns	1632 ns	22.81 ns
0.75	10.8 a	2909 ab	0.045 b	430.30 b	10983 ab	814 ab	26.09 ab	2.9 b	1767 ns	1488 ns	22.09 ns
2.25	5.5 b	2242 b	0.015 b	346.50 b	5174 b	649 b	24.16 ab	1.8 b	1162 ns	772 ns	11.24 ns
3.75	4.7 b	1399 b	0.013 b	861.50 b	5054 b	476 b	15.21 b	1.5 b	867 ns	782 ns	6.99 ns

[I]Numbers in an individual column followed by the same letter are not significantly different at P = 0.05; ns = no significant difference.

control. Both root and shoot concentrations of S increased with an increase in solution As. Tissue Cu concentration was significantly lower than that in the control for all solution As levels. Considering the non-significant drop in total iris shoot Cu content (**Table 2**), Cu deficiency if present, may have been slight. Copper's biochemical function in plants is mainly as an enzyme activator [32] in oxidation/reduction reactions. Given that there was an increase in root Fe and Mn concentration and no differences in shoot Fe, Mn and Zn tissue concentrations (**Table 3**), there were likely adequate levels of these nutrients in the plant.

In marigold, a difference in translocation percent from that in the control was found only in the 0.75 solution As treatment for Fe (**Table 3**). Root and shoot Cu concentration decreased below the control level with addition of As (**Table 4**). For the remaining elements, there were no significant differences in tissue concentration between the controls and As treatments for all except shoot Ca in the 5.25 solution As treatment. Non-significant but higher root and shoot S concentrations were found in As treatments than in the control.

Sunflower translocation percent in the control was similar to treatments for all elements except S (**Table 3**). At 0.75 and 5.25 $mg \cdot L^{-1}$ As, higher S translocation was found between root to shoot tissue. There was no difference in root concentration of Ca, K and Mg (**Table 4**); these elements had higher shoot tissue concentrations in As treated than in control plants. Phosphorus and Zn root tissue concentrations in As treatments were ≥ that found in the control. Tissue Cu concentration decreased significantly below that found in the control in all root and shoot As treatments. Shoot element concentrations in As treatments were ≥ that found in the control for all elements except Cu and Fe. Most shoot tissue element concentrations were highest at a solution AC of 0.75 $mg \cdot L^{-1}$. At higher solution, As levels remained ≥ that found in the control. Shoot Fe levels gradually decreased at higher As levels until at 5.25 $mg \cdot L^{-1}$ As the Fe concentration became significantly lower than that in the control.

There were few differences in switchgrass translocation percent; the general trend was lower translocation in the high As treatment. Root concentrations of Ca, Fe, Mg, Mn and Mo in the control were similar to that found in As treatments (**Table 4**). Phosphorus concentration initially increased with As additions up to 5.25 mg As L^{-1}, at which point root tissue P levels dropped below that of the control. Most shoot element concentrations in As treated plants were similar to concentrations found in the control. Potassium and Mg had an initial increase in shoot concentration followed by a decline in concentration from that point with increasing solution As. Both root and shoot S concentrations were higher in As treated plants than in the control. Tissue S increased with exposure up to 5.25 $mg \cdot L^{-1}$ then it began to decline. Copper concentration was significantly below that of the control at all levels of As exposure.

4. Discussion

4.1. Ca, K and Mg

Calcium uptake is dependent on young root tips and is transported to the shoot by the transpiration stream [32]. Arsenic toxicity had a varying effect on Ca, K and Mg concentration. Root Ca concentration increased with exposure to As in cordgrass (*Spartina densiflora*), winter wheat (*Triticum aestivum*) and castor bean (*Ricinus communis*) but there was a decrease in tissue Ca with As exposure in Barley (*Hordeum vulgare*) and *Pterisvittata* [34]-[38]. Liu *et al*. [35] and Melo *et al*. [36], discovered an increase in shoot Ca in wheat and castor bean. The effect of As toxicity on plant Ca levels is generally thought to result from reduced transpiration affecting transport of Ca up through the plant [32]. A high amount of K and Mg in the growth medium can compete with Ca for plant uptake. However, in this study the Ca/Mg ratio in plant tissue did not vary much in any of the species. Potassium and Mg tissue concentrations generally followed that of Ca; an increase or decrease in concentration compared to the control was similar or not significantly different for all species and tissue type except for coreopsis shoot K and switchgrass root Ca. Uptake of Ca, K and Mg was likely influenced by the toxic effect of As on root functions. In addition, reduced growth limited the demand for Ca, K and Mg and contributed to low uptake [22]. As had little effect on Ca, K and Mg transportation to the shoot at any but the highest As exposure rate.

With the exception of tithonia, Ca, K and Mg tissue concentrations differed little with changes in tissue AC from that found in controls. In the As sensitive plant tithonia a reduction in Ca, K and Mg reflects a general disruption of metabolic functions. Singh *et al*. [39], reported a reduction in membrane stability with increased exposure to As. Reduced tissue Ca levels found may be related to As induced membrane stability; leaky membranes may be related to the low accumulation of K and Mg found in tithonia.

4.2. Phosphorus

Phosphate and arsenate are taken by a common carrier [40]. Phosphorous uptake concentrations are controlled by a high affinity carrier at low solution P and a low affinity carrier at high solution P. These carriers have a higher affinity for P than As, thus more P than As should enter the cell. Since As is similar to P it should be able to replace it in many cellular functions [37]. Arsenic however cannot replace the role of P in energy transfer [25] [41]. As As continues to replace P a plant may respond as if a P deficiency exists and increase uptake. In contrast, a defensive response to increasing As, is to suppress the P/As high affinity carrier when tissue P level are sufficient [42]. Based on this, the effect of As on P status may depend on plant species and the amount of As and P available for uptake. In this study tissue P concentration generally was similar to or higher than that found in controls and As competition with P uptake occurred at 5.25 mg As L^{-1} only. Reed *et al*. [43] reported that whole plant P accumulation tended to increase in iris and marigold with increasing tissue As. In these plants there appears to be no suppression of P/As uptake and P uptake is consistent with the scenario proposed by Cox [41].

4.3. Sulfur

Sulfur is an essential constituent in glutathione and phytochelatins [32]. These S containing compounds are capable of reducing As and chelating it for compartmentalization in a vacuole [32] [44] [45]. An increase in S or S containing compounds with an increase in As was reported for castor bean [36], *Chilopsis linearis* [46] and *Ceratophyllumdemersum* [45]. In this study S concentration increased with higher tissue As. Sulfur uptake tended to spike with exposure to low levels of As, and tissue S concentration was higher than, or in the case of sunflower roots, equal to that found in controls. This is consistent with the role of S in detoxification through reduction and chelation of As. However, S concentration tended to peak then decline at the highest solution AC. Rausch and Wachter [47] felt than increased glutathione and phytochelatin production used in As detoxification would limit S needed for normal metabolic functions. This could explain the initial increase followed by a subsequent reduction in tissue S when uptake could not supply enough S for both detoxification and normal metabolic needs.

4.4. Micronutrients

Boron is taken up by plants with the flow of water through the roots and is relatively immobile in plants [32]. Plant species in our study, where dry weight decreased with increasing exposure to As, B uptake and translocation percentage also were reduced. Older leaves on these plants tended to show signs of wilting (curled leaves and loss of turgor). Plant species that were not as sensitive to As had dry weights, uptake and tissue concentration levels similar to the controls. These plants showed no visual signs of water stress. Singh *et al*. [39] reported membrane stability decreased with increasing exposure to As. The effect of As on plant B status is likely a result of membrane leakage and overall tissue damage leading to a reduction in transpiration.

Molybdenum uptake is reduced by competition with sulfate ions and enhanced by the presence of phosphate ions [32]. In this study, Mo and S uptake move in unison; an increase or decrease in S uptake was mirrored by a similar change in Mo uptake. Plant species which maintained dry weights as the AC increased also maintained Mo status similar to that found in control treatments. Sensitive species had a reduction in uptake, but only with tithonia was tissue concentration lower than in the control. The effect of As on Mo uptake is attributed to As damage to roots that was observed at harvest.

Iron and Mn are constituents in a variety of enzymes [32]. The effect of As on dry weight likely limited demand for Fe and Mn in sensitive species. Leaf chlorosis was observed in coreopsis, tithonia and switchgrass. Chlorosis is a symptom for Mg, Mn and Fe deficiencies. Shoot Mg and Mn concentrations tended to increase with increasing As exposure, whereas Fe tended to decrease. Arsenic induced Fe deficiency was likely the primary cause of chlorosis. However, As toxicity causes a breakdown in membrane stability [39] and a general decline in mineral uptake; As induced reductions in Zn, Mn or Mg would contribute to chlorosis. Changes in root Fe concentration were not matched in shoot Fe concentration and As seemed to affect transport of Fe from root to shoot. This is in agreement with Carbonell-Barrachina *et al*. [22] and Shaibur *et al*. [37], who reported similar As effects on the translocation of Fe.

Zinc is used in a number of enzymes and functions similarly to Mn and Mg as an enzyme activator [32]. Arsenic induced dry weight reductions also reduced Zn uptake. Increased As exposure decreased Zn translocation

percent as well. Zinc is required for tryptophan synthesis as a precursor to the growth hormone auxin. Rosetting, a common Zn deficiency symptom, was observed in several species.

Copper is used as a constituent in electron transport proteins and oxidation/reduction reactions [32]. Most notably, Cu is used to neutralize reactive oxygen species through Cu/Zn superoxide dismutase (Cu/ZnSOD) [48]. This enzyme protects the cell membrane from attack by superoxide radicals. In this study, tissue Cu concentration declined greatly (≤20% of control) upon exposure to As at the lowest level. Graham *et al*. [49] reported that under Cu deficiency, efficient cultivars can produce normal yield at or near the critical concentration of Cu for growth. Iris, marigold and sunflower experienced normal growth despite a large reduction in tissue Cu concentration. Cu usage in cellular functions in these species may be very efficient. Low Cu status with low exposure to As implies that Cu deficiency is one of the initial effects of As toxicity. Photosynthesis, respiration, lignification, and superoxide radical neutralization can all be compromised due to this interaction. Copper is a strong competitor with other metals for protein binding sites, however, very little free Cu is found in the cytoplasm [50]-[52]. In yeast (*Saccharomyces cerevisiae*), a Cu chaperone is required for transport within the cell and formation of Cu/ZnSOD [53]. The Cu chaperone requires S rich proteins to function. Arsenic detoxification requires binding As to S-rich glutathione and phytochelatins [32] [44] [45]. High S use for As detoxification may be a factor limiting tissue Cu by suppressing production of the Cu chaperone.

4.5. Iris

The response of iris to As toxicicty was unique among the ornamentals studied. There was an initial drop in growth at low As exposure but enhanced growth at higher non-lethal exposure levels.

Arsenic is known to act as an analog to P in high-affinity uptake sites and substitute for P in certain metabolic processes [40]. Iris initial low growth with low As exposure maybe due to competition with P for high-affinity sites; in the process of acquiring P, As was also taken up. If As substituted for P in cellular functions, a P limitation would lead to a growth inhibition. At higher ACs a suppression of high-affinity P uptake may reduce As uptake. Meharg and Mcnair [42] reported As competition with P and a suppression of the high-affinity P uptake system as a mechanism of As tolerance in *Holcus lanatus*. Arsenic does not compete as effectively with P for low-affinity uptake sites allowing for P metabolism to occur with less interference from As. This may have led to increased growth over the control.

At low ACs Cu uptake is disrupted more than other elements. Translocation percent is similar to control and dry weight, after an initial decline, increased to levels higher than that of the control. This implies tissue Cu is used efficiently. One function of Cu is activating Cu/Zn superoxide dismutase (SOD) for removal of reactive oxygen species; however, Fe and MnSOD enzymes can perform the same function. At 0.75 $mg{\cdot}L^{-1}$ solution As, the need for Fe and MnSOD to take over a part of the function of Cu/ZnSOD may contribute to reduced growth. Above 3.0 $mg{\cdot}L^{-1}$ solution As, Fe and Mn accumulation increases and root concentrations increase dramatically; shoot concentrations are no different than that found in the control. If Cu uptake is indeed limiting growth at 0.75 $mg{\cdot}L^{-1}$ solution As, at higher As levels higher Fe and Mn uptake could result in adequate SOD activity. Perhaps some substitution of Fe for Cu in other enzymes occurs.

Increases in iris total plant S with increasing exposure to As implies S is available for phytochelatin production leading to As detoxification. Increases in root Ca with increasing exposure to As implies greater ability for repair of membrane leakage.

Markovska *et al*. [54] reported that in *Brassica juncea* different enzymatic and non-enzymatic antioxidants had their maximum response to Cd induced oxygen stress at different levels of exposure. For example, with exposures of 0.0, 10, 30, 50 and 100 µM, ascorbate peroxidase activity was lowest at 30 µM but highest at 50 µM Cd. Anjum *et al*. [55] reported increasing enzymatic activity and decreasing non-enzymatic activity of antioxidants to increases in Cd induced oxidative stress in mungbean. Srivastava *et al*. [56] reported phytochelatin and antioxidant production responded differentially to As stress in *Hydrillaverticillata*. Production of superoxide dismutase, ascorbate peroxidase and glutathione reductase, enzymes used to neutralize free radicals, each peaked at different As exposure levels. Plants had a small growth increase at low As levels. Srivastava *et al*. [56] attributed a decrease in photosynthetic pigments to impaired uptake of P, Fe, Cu and Mn.

Differences in the synchronization of mechanisms to adapt to low tissue Cu with mechanisms for As detoxification, such as, S metabolism for phytochelatan production may play a role in the growth response of iris. This may help explain the initial reduction in growth at low As exposure followed by the increase in growth at

higher non-lethal exposure. Arsenic exposure causes widespread damage to a number of essential plant functions *i.e*. electron transport, neutralizing free radicals, photosynthesis, and membrane integrity [33] [34] [39]. Offsets in the timing of when factors controlling detoxification, damage control, and the initiation of secondary systems as primary systems are damaged may result in a complex growth response.

5. Conclusion

Arsenic toxicity can influence uptake of soil nutrients and their redistribution inside plant tissue. Calcium, Fe, P and S concentrations tended to increase as the plant attempted to detoxify or neutralize the effects of As. Reduction in Cu concentration is an early effect of As toxicity. Differences in mineral uptake reflect the plant's attempt to detoxify As (*i.e*. increase in S for S-containing As chelators), mitigate damage to the cell (*i.e*. Ca to repair leaky membranes) or continue cellular functions through alternative pathways (*i.e*. Fe superoxide dismutases to replace the function of Cu/ZnSOD).

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