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Removal of Arsenic (Iii) and Arsenic (V) from Aqueous Solution via Solanum lycopersicum Phytoremediation and Essential Nutrient Analysis

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REMOVAL OF ARSENIC (III) AND ARSENIC (V) FROM AQUEOUS SOLUTION

VIA *SOLANUM LYCOPERSICUM PHYTOREMEDIATION*

AND ESSENTIAL NUTRIENT ANALYSIS

A Thesis

by

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ABSTRACT

Garcia, Grecia, Removal of Arsenic (III) and Arsenic (III) from Aqueous Solution via *Solanum Lycopersicum* Phytoremediation and Essential Nutrient Analysis. Master of Science (MS), May, 2021, 69 pp, 26 figures, 25 tables, 81 references, 62 titles.

ICP-OES was used to investigate the uptake of arsenic (V) and arsenic (III) from aqueous solution using tomato seedlings. The effect of arsenic on essential nutrient uptake by tomato was analyzed. The experiment was performed at three levels of arsenic: 1ppm, 2 ppm and 5 ppm [added as sodium arsenate (Na3AsO4) and arsenic trioxide (As2O3)], in nutrient solution. Plants remained in solution for two weeks. Arsenic accumulation depended on arsenic concentration in solution, when exposed to higher arsenic concentrations the plant uptake increased. The highest arsenic accumulation was found in roots, followed by stems and leaves in that order. Essential nutrient uptake decreased from control values when plants were exposed to low arsenic levels (1ppm) and increased at high arsenic concentrations (2 ppm and 5 ppm). The arsenite level of 5 ppm damaged roots membranes decreasing arsenic and essential nutrient uptake.

DEDICATION

The completion of my master's degree would have not been possible without the support of my parents, Gerardo Garcia Gorena and Wendy Salazar de Garcia; the motivation of my grandparents Flavia Salazar, Elena Gorena, and Arnoldo Garcia; the playfulness of my brothers Gerry and Gonzalo; the inspiration of my aunts, Alma, Silvia, Elsa, and Flavia Salazar; and the patience of my fiancée Daniel Moreno. Thank you all for your love and support.

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CHAPTER I

BACKGROUND

Arsenic Pollution to the Environment

Arsenic is present in the environment due to geological and anthropogenic sources. The primary natural source of arsenic is volcanic eruptions. However, natural processes such as dust storms, forest fires, and geothermal activities (Garelick et. al, 2008), have largely contributed to the spread of this toxic element through water systems and residential soils. Nonetheless, anthropogenic sources are more prevalent and unfortunately more hazardous to the environment than natural sources. The most common anthropogenic causes for arsenic contamination include power plants that burn As-rich coals, disposal sites from As-processing plants, mining, increased erosion of land, and use of arsenic-based pesticides and herbicides (Ferguson and Gavis, 1972; Nriagu et. al, 2007; Parsons et. al, 2008).

Generally, the health effects of arsenic are caused by the ingestion of contaminated water and food (Mohammad et. al, 2009). In some cases, arsenic exposure is due to the organoarsenical compounds found in many seafoods, which are considered to be non-toxic (Hall, 2002; Gochfeld, 1995). However, the toxic effects of arsenic can also be related to the ingestion of crops/vegetables grown in arsenic contaminated soils. Unlike seafood, contaminated agricultural soils contain inorganic arsenic species, which are toxic. Arsenic in soils can be attributed to the traditional use of arsenic-based pesticides and contaminated irrigation water (Samal et. al, 2011). Therefore, due to the high toxicological

significance of the inorganic arsenic compounds, which include the +3 and +5 oxidation states, it is of great importance to analyze efficient soil remediation techniques and plant accumulation.

Arsenic Throughout History

Arsenic has been used for various purposes throughout history. Arsenic uses have ranged from being a cure for any possible illness to being known as a poison. During ancient times, arsenic treatments were employed to treat multiple health problems such as blood diseases, nervous and rheumatic conditions, malaria, diabetes, heart disease, respiration problems, among others (William T. Frankenberg Jr, 2002). The most common arsenical compounds utilized back then were orpiment (As_2S_3), realgar (As_4S_4), sodium cacodylate $(CH_3)_2AsO_2H$, and sodium arsalinate ($C_6H_7AsNNaO_3$) (Liu et al. 2008; Schafer, 1955; Garattini et al. 1973; Jollife, 1993). However, arsenic based treatments were banned after the late 1940s when increased doses of this metalloid were causing the patients more suffering than the actual disease. Nonetheless, arsenic is still used today as the therapeutic agent in the treatment of acute promyelocytic leukemia (Jian-Xiang, 2012; Shen et al. 1996; Evens et al. 2004).

Arsenic has also been known as the king of poisons since immemorial times. During the fifteenth century, the Borgia family employed arsenic as the main ingredient in their favorite poison called La Cantarella (Marianna et al. 2019). In the sixteenth century wives employed arsenic to get rid of their husbands by utilizing a substance called Aqua Toffana sold by the poisoner Giulia Toffana (Parascandola, 2012). By the nineteenth century arsenic became such a cheap and available product, due to its efficacy to kill pests and its uses as a drug such as in the Fowler's solution (1% potassium arsenite $KAsO_2$) (Waxman, 2001), that anyone could obtain the

toxic element. Due to the high availability arsenic became a popular poison during the Victorian era (Ian A. Burney, 2002; Haller, 1975; Haslam, 2013).

Arsenic in Soil

Throughout the years, arsenic has been employed in ore smelting (Jones, 2007), mining (Straskaba and Moran, 1990), and agriculture (Chou and De Rosa, 2003). The use of arsenic in these activities has led to a high concentration of arsenic in soil that is still observed today. The smelting and mining industry of gold led to high amounts of arsenic in the environment, since arsenopyrite and gold ores are commonly found together. In fact, to extract pure gold from the arsenopyrite ores, smelters would oxidize arsenic and sulfur by roasting (Hutchinson et al. 2007), which left traces of arsenic in soil. Arsenic has also been associated with the smelting of copper and lead ores (SME, 2015). More recently, however, the mining industry and the emission of arsenic have been regulated by the Environmental Protection Agency (Williams, 2001).

Agriculture, on the other hand, has led to high concentrations of arsenic in soil from the constant use of arsenic-based pesticides and herbicides. Arsenic became a popular pesticide and herbicide around 1867 with the use of Paris green (copper arsenate), which was a green color paint that was first used by a desperate farmer wanting to get rid of the beetle bugs infesting his potato plants (Frankenberg, 2002). The Paris green compound turned out to be an excellent insecticide and did not damage the crops and thus Paris green became famous among the American farmers. Over the years, other inorganic arsenic pesticides such as lead arsenate ($PbHAsO_4$), and calcium arsenate [$Ca_3(AsO_4)_2$] were employed in many countries around the world including the USA. It was not until 1970 that arsenic based pesticides, herbicides,

fungicides, and insecticides were banned (Rowe, 2014; Stoycheva, 2011). Due to lack of restrictions in the past, large arsenic concentrations have accumulated in the soil, which unfortunately are not easily removed.

Chemistry of Arsenic

Arsenic is a metalloid located in group VA of the periodic table. It is usually recognized as a semi-metal and it has the atomic number 33, and a molar mass of 74.922 g/mol. Arsenic present in the environment is typically found in the form of inorganic salts, organic salts, and gas. However, inorganic arsenic is considered to be the most toxic form for living organisms (Roy, 2002). The valence states of arsenic are -3, 0, +3, and +5. Arsine gas (-3) is the most toxic form of arsenic (Kuivenhoven & Mason, 2020), but due to its instability under oxygen conditions, its distribution is very limited (Panstar and Korpela, 2000). Elemental arsenic [As(0)], is considered to be non-toxic for human health because is poorly absorbed and easily eliminated by the body (Jolliffe, 1993; Duker, 2005). Arsenite (+3) and arsenate (+5) are the predominant species of arsenic in the environment under reducing and oxidizing conditions respectively (WHO, 2001). However, trivalent arsenic is considered to be more toxic than pentavalent arsenic compounds (Levy et al., 2012).

Inorganic arsenite (As^{+3}) and arsenate (As^{+5}) species are present in soil and interact with other elements, such interaction depends on soil pH and oxygen availability. In the most common soil pH values, arsenate is usually found as an anion (pK_{a1}=2.3; pK_{a2}=6.8; pK_{a3}=11.3), while arsenite is mainly found undissociated (pK_{a1}=9.2; pK_{a2}=12.7) (Martin et al., 2014). The organic compounds monomethylatarsonate (MMA) and dimethylarsinate (DMA) have also been

detected in soils (Garcia et al., 2002). Organic arsenicals are present in soil due to microbial activity that transforms inorganic arsenate and arsenite species into MMA and DMA compounds and vice versa (Pongratz, 1998; Turpeinen et al., 1999). The correlation between arsenic with iron (Fe), aluminum (Al), and manganese (Mn) has also been investigated and it has been suggested that arsenite and arsenate mobility in soil is mainly affected by these three elements (Szakova et al., 2009; Cai et al., 2002). However, the trivalent state of arsenic has shown higher mobility in soil than the pentavalent state (Bissen and Frimmel, 2003). The presence of arsenate species in aerobic soil composition has been detected to be higher than arsenite (Meharg and Hartley, 2002).

Arsenic Toxicity

According to the Federal Food, Drug, and Cosmetic Act, the limit for arsenic content in food has been set to 0.01 ppm (FDA, 2021). The same level has been set by the Environmental Protection Agency to regulate the amount of arsenic in drinking water (EPA, 2021). Beyond those limits, the ingestion of arsenic-contaminated food and water may lead to adverse health effects. Arsenite compounds are considered to be human carcinogens (Hall, 2002). Symptoms of acute arsenic poisoning include nausea, vomiting, convulsions, hypoxic encephalopathy, mental status changes, electrocardiographic abnormalities, respiratory failure, and even death (Fowler et al., 2015; Kyle et al., 1965; Alvarez, 1989). Chronic arsenic poisoning, on the other hand, leads to multisystemic diseases that involve the accumulation of this element in the liver, kidneys, heart, lungs, nervous system, gastrointestinal tract, skin, nails, and hair (Gleir et al., 2020; Kapaj et al., 2007).

Arsenic concentration in soil due to normal ecological conditions ranges between 0.1 to 40 ppm (EPA, 2001). Which is not considered to be a risk for human health. However, arsenic concentrations in soil have already exceeded the safe levels in some industrial, mining, and agricultural areas in the USA (Yang et al., 2007; Yokel and Delistraty, 2003). Due to the arsenic mobility, arsenic concentrations can reach the soil surface and groundwater systems. Ground water serves as the drinking source of many animal species. It is sometimes used for irrigation purposes during dry periods. Therefore, an efficient remediation technique must be employed to avoid arsenic toxicity in animals, and the accumulation of this toxic element in crops and vegetables. Otherwise, the wellbeing of multiple species of living organisms and human beings will be endangered.

CHAPTER II

INTRODUCTION

Phytoremediation

Phytoremediation is a technique that involves the removal of contaminants from soil and water systems by using plants. This technique uses the natural ability of plants to concentrate elements and compounds found in their surroundings and the ability to metabolize them and form different molecules within their tissues. Unlike physical and chemical remediation processes, that involve volatilization, leaching, vitrification, thermal treatment, and chemical extraction, phytoremediation techniques are considered to be cost-effective and noninvasive.

Phytoremediation has been performed for several heavy metals such as Zn, Cd, Cu, Pb, Cr, Hg including As. The technique has been analyzed in-depth since it was discovered that some plants were natural hyperaccumulators of certain metals. For instance, it has been reported that the Chinese brake fern *(Pteris vittata L.)* is an important hyperaccumulator of arsenic (Cao et al., 2004). The study of this remediation technique has increased during the last years hoping to find other plant species that can function as good arsenic accumulators and can grow in different environmental conditions. Moreover, phytoremediation has been linked to other scientific fields such as molecular biology and chemistry to determine how plants metabolize toxic elements such as arsenic within their tissues, and how the absorption of different essential nutrients varies after the uptake of the contaminant.

Arsenic Metabolism in Plant Tissues

Arsenic metabolism within plant tissues varies widely and has been found to be dependent on the plant species. For example, there are arsenic-resistant and non-resistant plants that have been investigated for phytoremediation purposes. Resistant plants have shown several mechanisms by which they can naturally accumulate arsenic, metabolize it, and store the toxic element within the tissues. On the other hand, small amounts of arsenic can be fatal for non-resistant plants. The mechanisms employed by arsenic-resistant plants consist of arsenate/phosphate suppression, phytochelatins synthesis, Mycorrhizal symbiosis, and inorganic to organic arsenic metabolism (Abbas et al., 2018; Meharg and Hartley, 2002).

Arsenate/Phosphate Suppression

Arsenate is a competitive inhibitor of macronutrient phosphate. Inside the plant, arsenic is transported across the plasma membrane via phosphate cotransport systems (Ullrich-Eberius et al., 1989). Due to this reason, plants absorb high arsenate concentrations and attempt to utilize it instead of phosphate in the synthesis of ATP. That leads to the formation of unstable ADP-As which eventually leads to the disruption of energy flow in the plant's cells (Meharg, 1994). It has been proposed that nutrition high in phosphate would help nonresistant plants become more resistant to arsenic because less arsenate would be absorbed by the plant. However, it has also been established that plant resistance occurs within the plant where arsenate and phosphate compete for ATP (Meharg, 1994). Therefore, when the phosphate concentrations are high within plant tissues the cells become insensitive to arsenate toxicity (Hung-Chi et al., 2012). It has also been observed that resistant plants typically possess high shoot phosphorus concentrations even

when the arsenate/phosphorus uptake has been suppressed by the plant. Thus, it has been suggested that the Arsenate/Phosphorus suppression might be due to high shoot P status (Wright et al., 2000).

Complexation of Arsenic

Another mechanism employed by arsenic-resistant plants consists of the synthesis of phytochelatins (PCs). Inside the plant tissues, arsenate is reduced to arsenite, which leads to the formation of reactive oxygen species (ROS) (Hartley-Whitaker et al., 2001). The generation of reactive oxygen species eventually leads to the formation of glutathione ([Y-glutamate-cysteine]n-glycine), which besides acting as an antioxidant, is also known for its role as the precursor of PCs (Meharg & Hartley-Whitaker, 2002). Complexation of arsenic by phytochelatins has been observed in cell suspension cultures of *Rauvolfia serpentina*, seedlings of *Arabidopsis* and preparations of *Silene Vulgaris* (Schmoger et al., 2000). Other kinetic studies include *A. thaliana, H. lanatus* and *Rubia tinctorum* (Sneller et al., Maitani et al., 1996). Phytochelatins are synthesized from reduced glutathione in a transpeptidation reaction catalyzed by the enzyme phytochelatin synthase (PCS), (SK Yadav, 2010). PCs complexation has been observed for other metals besides arsenic which include Cu, Hg, Pb, Cd, among others (Grill et al., 1985). For arsenic chelation to occur, arsenate must be reduced to arsenite by two glutathione molecules leading to the formation of a disulfide bond by the oxidized glutathione (Delnomdedieu et al., 1994; Cobbet, 2000). Phytochelatin binding to the arsenate anion in vitro and in vivo is still under investigation ((Schmoger et al., 2000). Although the location of As-Pc complexes inside the plant is still unknown, it is believed that they remain inside the acidic environment present at the root vacuoles (Schmoger et al., 2000, Meharg 2002).

Other Mechanisms

It has been suggested that the symbiosis with Mycorrhizal fungi increases the arsenic resistance in several plant species. Symbiotic resistance may be due to the main role of Mycorrhizal organisms which is obtaining phosphorus for their hosts (Li et al., 2006). It has been observed that symbiotic associations within Mycorrhizal fungi and nonresistant arsenic plants are less common than for arsenic resistant plants (Meharg et al., 1994).

In addition, plant metabolism from inorganic to organic arsenicals such as DMA and MMA has been suggested as a possible mechanism utilized by arsenic resistant-plants. However, methylation has not been found to occur within plant tissues currently (Pickering et al., 2000).

Transportation systems within plant tissues is another mechanism for arsenic resistance. It has been observed in many plant species that arsenic mobility within plant tissues is very limited (Carbonell et al., 1995). Low arsenic mobility within the plant has been related to the toxicity of protein sulfhydryl groups of proteins disrupting root function, and formation of iron and manganese plaques on the root surfaces, which causes decreased mobility of arsenic within the plant (Carbonell et al., 1995; Blute et al., 2004; Liu & Zhao, 2005). These factors, in turn, cause arsenic accumulation to be higher in roots of the plants and only small concentrations of arsenic can translocate to stem and leaves.

CHAPTER III

METHODS

Plant Growth

Beefsteak tomato seeds (The Seed Plant) were germinated by placing the seeds on sterilized paper towels which were damped and placed in an incubator for five days at 30 °C. Two weeks after germination, four seedlings were placed on 400 mL jars filled with sterilized nutrient solution and three replicates were prepared for each concentration. The nutrient solution was prepared as following: $CuSO_4$ ($5.0x10^{-7}$ mol/L); MoO_3 ($5.0\ x10^{-7}$ mol/L); KH_2PO_4 ($2.0\ x10^{-3}$ mol/L); $MgSO_4$ ($1.0\ x10^{-3}$ mol/L); $MnSO_4$ ($2.0\ x10^{-6}$ mol/L); H_3BO_3 ($2.5x10^{-5}$ mol/L); KCl ($0.05x10^{-3}$ mol/L); $ZnSO_4$ ($2.0x10^{-6}$ mol/L); KNO_3 ($6.0\ x10^{-3}$ mol/L); $Ca(NO_3)_2$ ($4.0\ x10^{-3}$ mol/L); $Fe(II)SO_4$ ($6.4\ x10^{-5}$ mol/L). The nutrient solution was pH adjusted to 6.8 before transplanting seedlings to containers.

The seedlings were exposed to sunlight for 1 week, to force the development of chlorophyll, and then exposed to metal halide growth lamps for 3 weeks of growth in the hydroponics solutions. Air was supplied to the roots of the plants using an air compressor to avoid nutrient sedimentation and the development of an anoxic root environment. After the growth period, seedlings were contaminated with arsenic (III) and arsenic (V) (from As_2O_3 and Na_3AsO_4 respectively) at 1 ppm, 2 ppm, and 5 ppm. Control samples were included for each arsenic treatment and plant tissue.

Seedlings were exposed to arsenic treatments for two weeks. After removal, plants were separated into roots, stems, and leaves. Root and stem growth was measured and recorded to analyze physical changes. The samples were frozen and stored for further analysis.

ICP-OES sample preparation

Samples were lyophilized by using a Labconco Free Zone 4.5 freeze dryer system at -48 °C for 72 hours. Freeze-dried samples were homogenized and acid digested. An open-vessel digestion was performed for each plant tissue (root/stem/leave) and concentration (control/1ppm/2ppm/5ppm). Samples of arsenic (III), arsenic (V) and controls were acid digested in triplicates. Approximately 0.3 grams of dried plant tissue was deposited into 50 mL beakers. To the powdered plant materials, 10 mL of trace pure (plasma pure) concentrated nitric acid (HNO_3) was added to each beaker and samples were covered with watch glasses to avoid cross contamination and slow evaporation. The temperature was increased to 121 °C by using hotplates (Fisher Scientific). Samples were gently boiled for 4 hours. Afterward, 2 mL of 30% hydrogen peroxide (H_2O_2) was added to each beaker, and samples were heated to boiling for 20 min. Finally, 20 mL of 5% analytical grade nitric acid was added to each beaker. Samples were diluted to 8 mL with 18MΩ deionized water and stored in 50 mL conical vials.

ICP-OES sample Analysis

Acid digested samples were analyzed in triplicate using a Perkin Elmer, Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Table 1 summarizes the operating conditions of the ICP-OES. Calibration standards containing As, Na, Mg, S, K, Ca, Mn, Fe, Ni, Cu, and P were prepared to analyze changes in concentration of these macro and micronutrients depending on plant tissue and concentration. Wavelengths analyzed for each metal are summarized in table 2.

Table 1. ICP-OES Operating conditions for determination of elemental absorption by *Solanum Lycopersicum* under Arsenic (III) and Arsenic (V) treatments

Parameter	**Setting**
RF Power	1500 W
Nebulizer	Gemcone (low Flow)
Plasma Flow	15 L/min
Auxiliary Flow	0.2 L/min
Nebulizer Flow	0.55 L/min
Sample Flow	1.50 L/min
Injector	2.0 mm Alumina
Spray Chamber	Cyclonic
Integration Time	10-20 seconds
Replicates	3

Table 2. ICP-OES Wavelength selection for elemental uptake by Solanum Lycopersicum under As (III) and As(V) treatments

Element	Wavelength (nm)
As	193.70
Na	589.60
Mg	279.08
S	181.98
K	404.72
Ca	315.90
Mn	259.40
Fe	239.60
Ni	231.60
Cu	324.75
P	178.22

CHAPTER IV

RESULTS

ICP-OES Data for Arsenic (V) Treatments

Arsenic, As

ICP-OES data for the arsenic accumulation in the roots, shoots, and leaves of the tomato plants contaminated with 1ppm, 2 ppm, and 5 ppm of As(V) treatments are shown in Fig 1, 2 and 3 and summarized in table 3. Arsenic accumulation increased for roots, stems, and leaves as the concentration of the As(V) treatment increased. As seen in figure 1, arsenic accumulation in roots of tomato plants is much larger when compared to the stem and leave portion of the plants.

Table 3. Arsenic concentration (mg/Kg dry weight) in tomato roots, stems, and leaves. Each value is the mean for twelve plants (three repetitions for sample)

Arsenic accumulation by Roots, Stems, and Leaves						
Treatment	**Roots**	**Error**	**Stems**	**Error**	**Leaves**	**Error**
Control	0	0	0	0	0	0
1ppm	5.909333	0.129766	4.536703	0.147873	0	0.035939
2 ppm	33.85742	0.325099	11.32669	0.269705	0.294985	0.015609
5 ppm	134.6457	0.532573	31.11111	0.210602	2.600862	0.012534

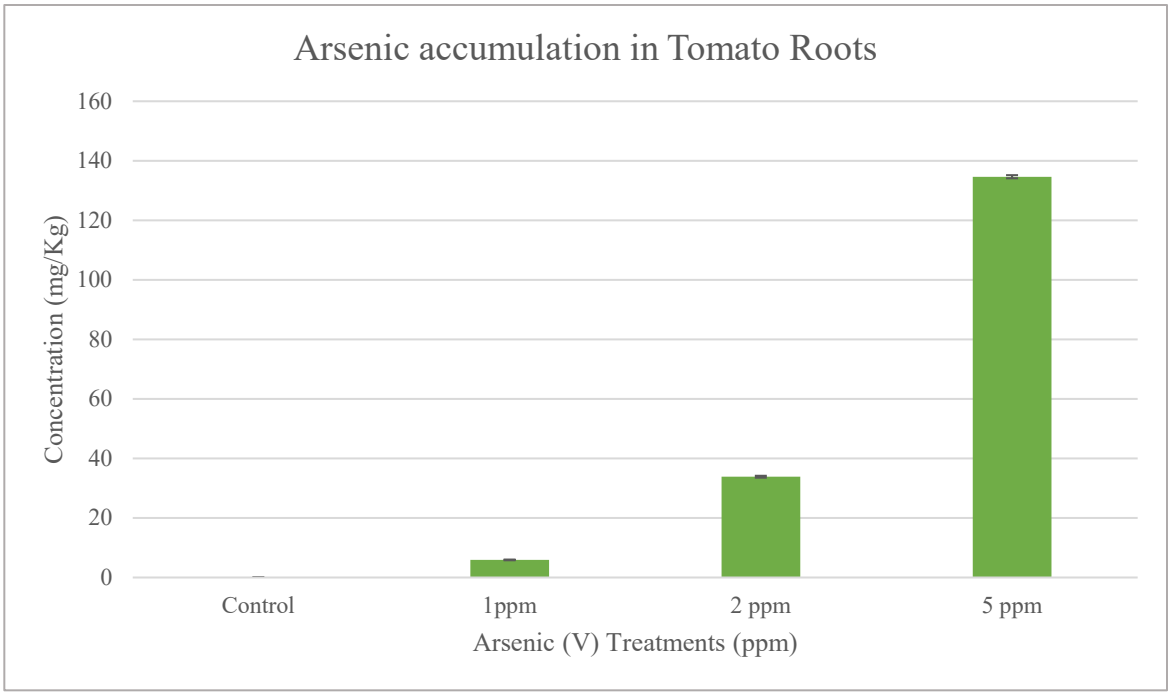

Figure 1. Arsenic accumulation in roots contaminated with arsenic (V) treatments. Each value is the mean for twelve plants (three repetitions for sample)

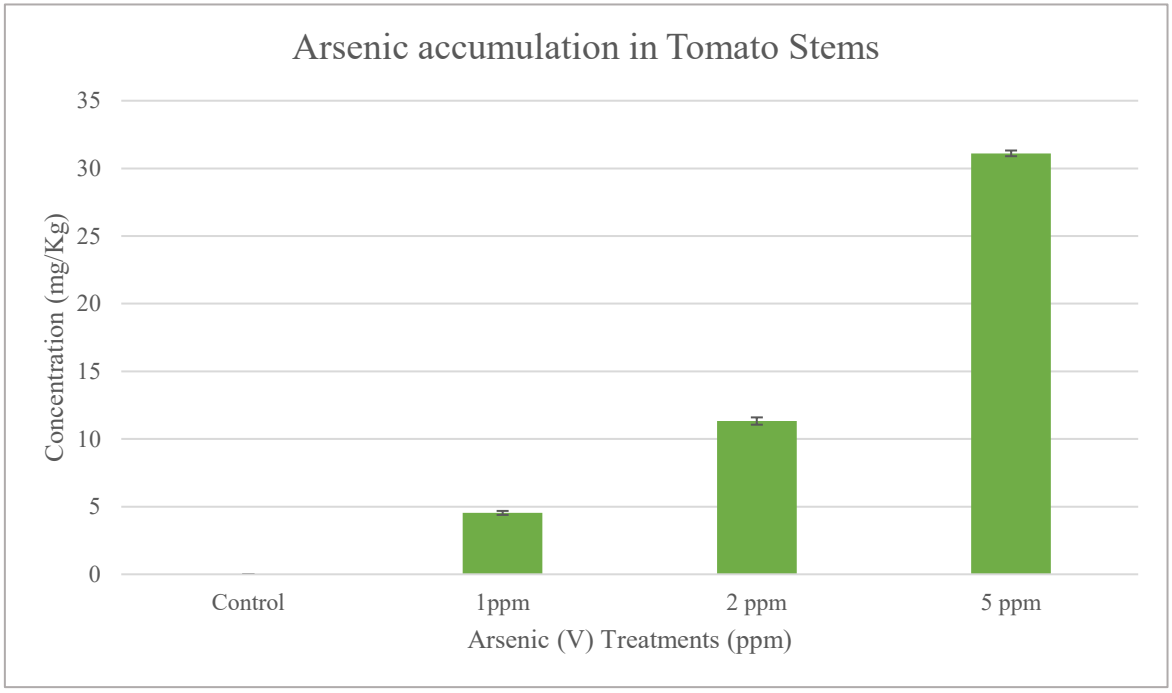

Figure 2. Arsenic accumulation in stems contaminated with arsenic (V) treatments. Each value is the mean for twelve plants (three repetitions for sample)

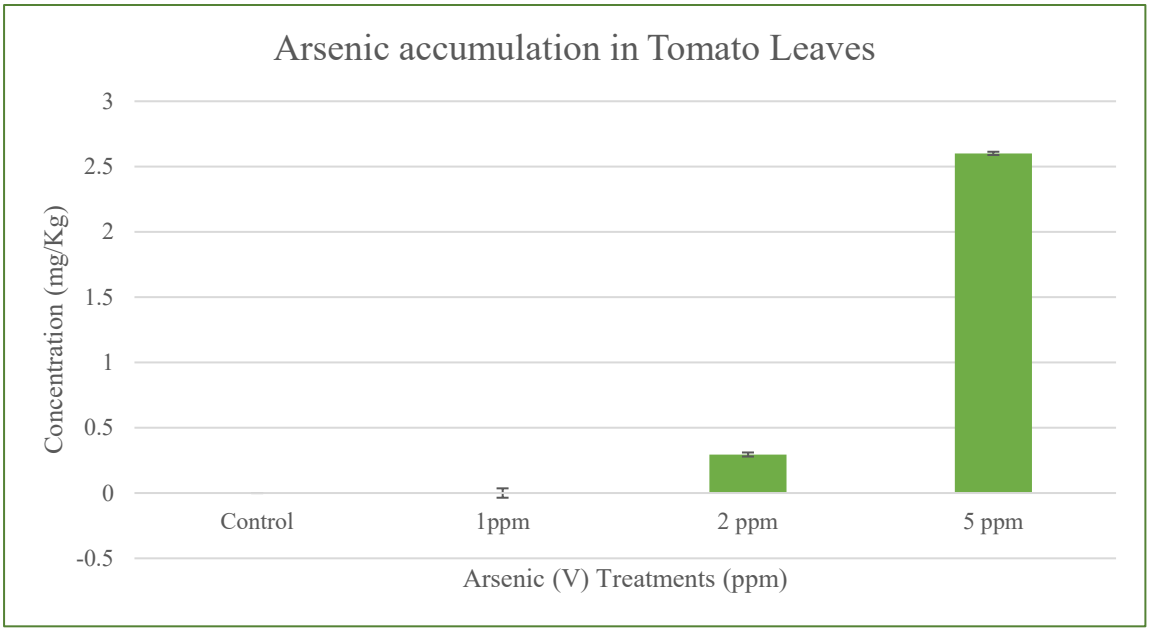

Figure 3. Arsenic accumulation in leaves contaminated with arsenic (V) treatments. Each value is the mean for twelve plants (three repetitions for sample)

Potassium, K

ICP-OES data for potassium accumulation in roots, shoots and leaves of tomato plants contaminated with 1 ppm, 2 ppm, and 5 ppm of As(V) treatments are shown in figure 4 and summarized in table 4. Potassium concentration decreases in roots and stems when plants are contaminated with 1 ppm of As(V). Potassium concentration in plants increased as the concentration of the arsenic treatment increased. Potassium accumulation in leaves followed a similar trend to roots and stems. However, potassium accumulation in leaves did not surpass control values.

Table 4. Potassium Accumulation (mg/Kg dry weight) in tomato roots, stems, and leaves. Each value is the mean for twelve plants (three repetitions for sample)

Potassium (K) accumulation in Roots, Stems, and Leaves						
Treatments	**Roots**	**Error**	**Stems**	**Error**	**Leaves**	**Error**
Control	17040.6	37.6	51604.8	1213.9	24526.7	153.2
1 ppm	13819.7	37.9	49602.9	583.6	21809.8	32.1
2 ppm	22221.5	27.3	45894.1	944.6	22342.2	205.5
5 ppm	23806.0	85.8	57148.1	386.7	22793.6	80.0

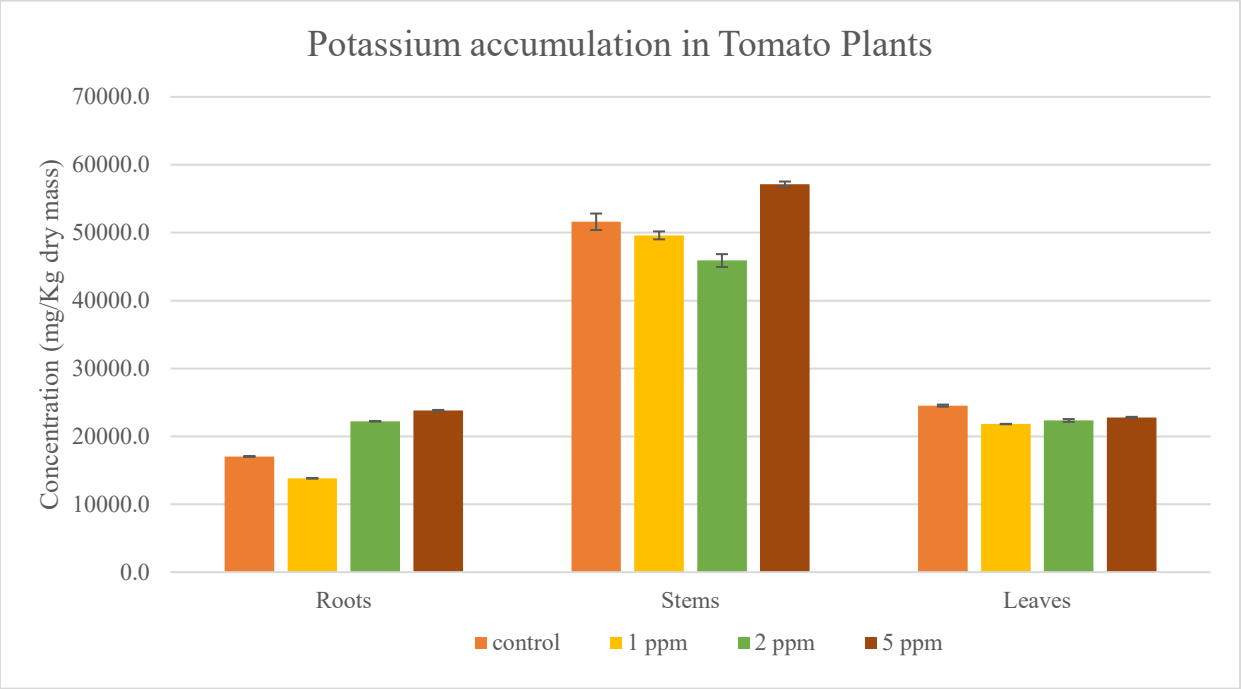

Figure 4. Effect of arsenic accumulation in potassium uptake by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

Phosphorus, P

Data for phosphorus accumulation is shown in figure 5 and summarized in table 5. Phosphorus concentration in roots shows a decrease at 1 ppm of As(V), but it starts increasing as the level of arsenic in the treatment increases. For the stem portion of the plant, phosphorus translocation remains relatively constant despite the increase in the arsenic treatment. However, leaves show an increasing trend in phosphorus translocation as the arsenic level in the treatment increases.

Table 5. Phosphorus Accumulation (mg/Kg dry weight) in tomato roots, stems, and leaves. Each value is the mean for twelve plants (three repetitions for sample)

Phosphorus accumulation in Roots, Stems, and Leaves						
Treatment	Roots (ppm)	Error	Stems	Error	Leaves	Error
Control	14674.0	64.7	2070.0	34.5	2964.5	8.0
1 ppm	7336.5	26.2	2060.2	31.1	2802.7	18.0
2ppm	13459.6	59.3	2093.5	40.8	3041.3	23.0
5 ppm	14299.6	46.7	2214.3	13.0	3478.9	9.2

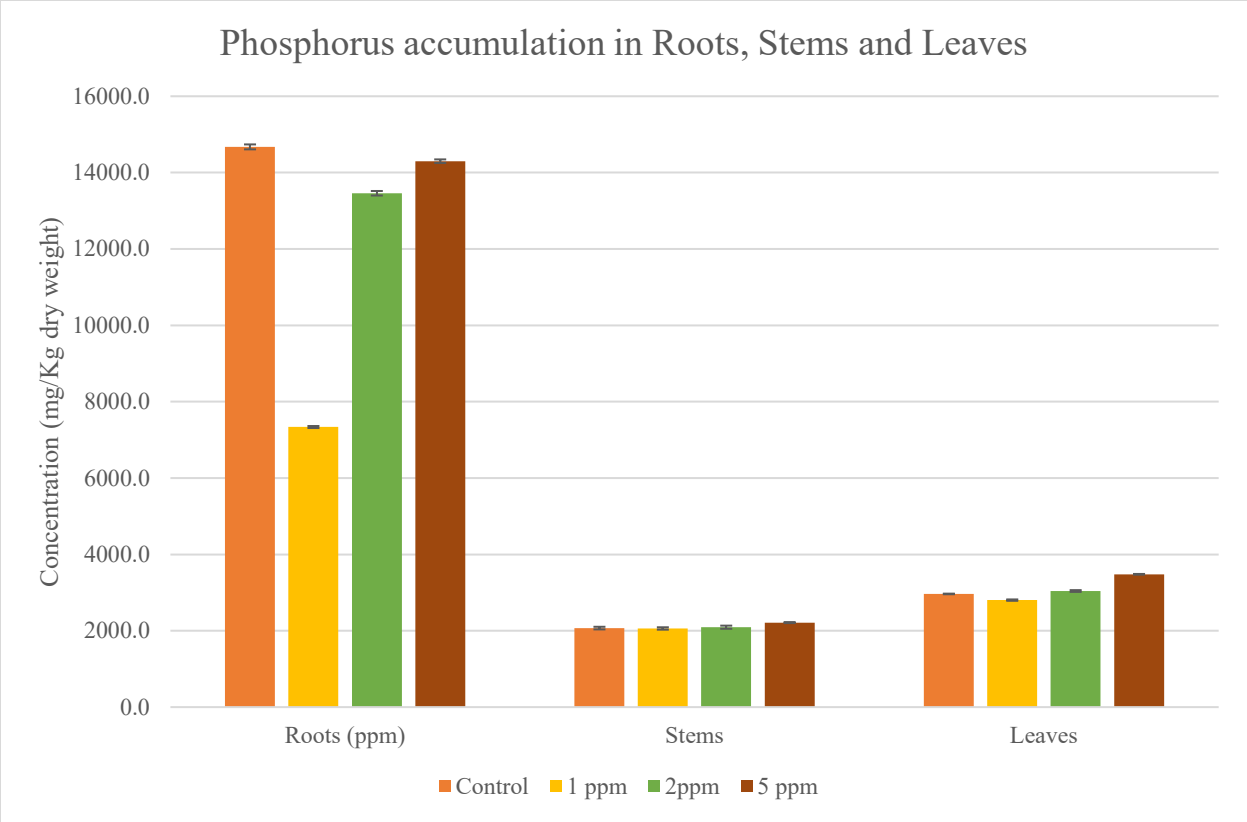

Figure 5. Effect of arsenic in phosphorus accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample).

Calcium, Ca

Calcium accumulation in roots is much larger than in stems and leaves as can be seen in figure 6. The accumulation of Ca in the plants is summarized in table 6. As can be seen in the data there was a slight change in the trend for stems since calcium accumulation at 5ppm of As(V) was lower than observed in the 2 ppm treatment. Overall, the data shows an initial decrease at 1 ppm but as the arsenic treatment concentration increases, calcium accumulation increases as well.

Table 6. Calcium Accumulation (mg/Kg dry weight) in tomato roots, stems, and leaves.
Each value is the mean for twelve plants (three repetitions for sample)

Calcium accumulation in Roots, Stems, and Leaves						
Treatment	Roots (ppm)	Error	Stems (ppm)	Error	Leaves (ppm)	Error
Control	25728.8	120.0	7417.3	211.6	9488.3	30.9
1 ppm	10734.9	21.0	6299.6	107.6	8943.1	9.3
2ppm	20251.3	247.2	6962.4	127.3	9208.8	82.8
5 ppm	21204.0	64.3	6506.2	47.0	10764.4	38.2

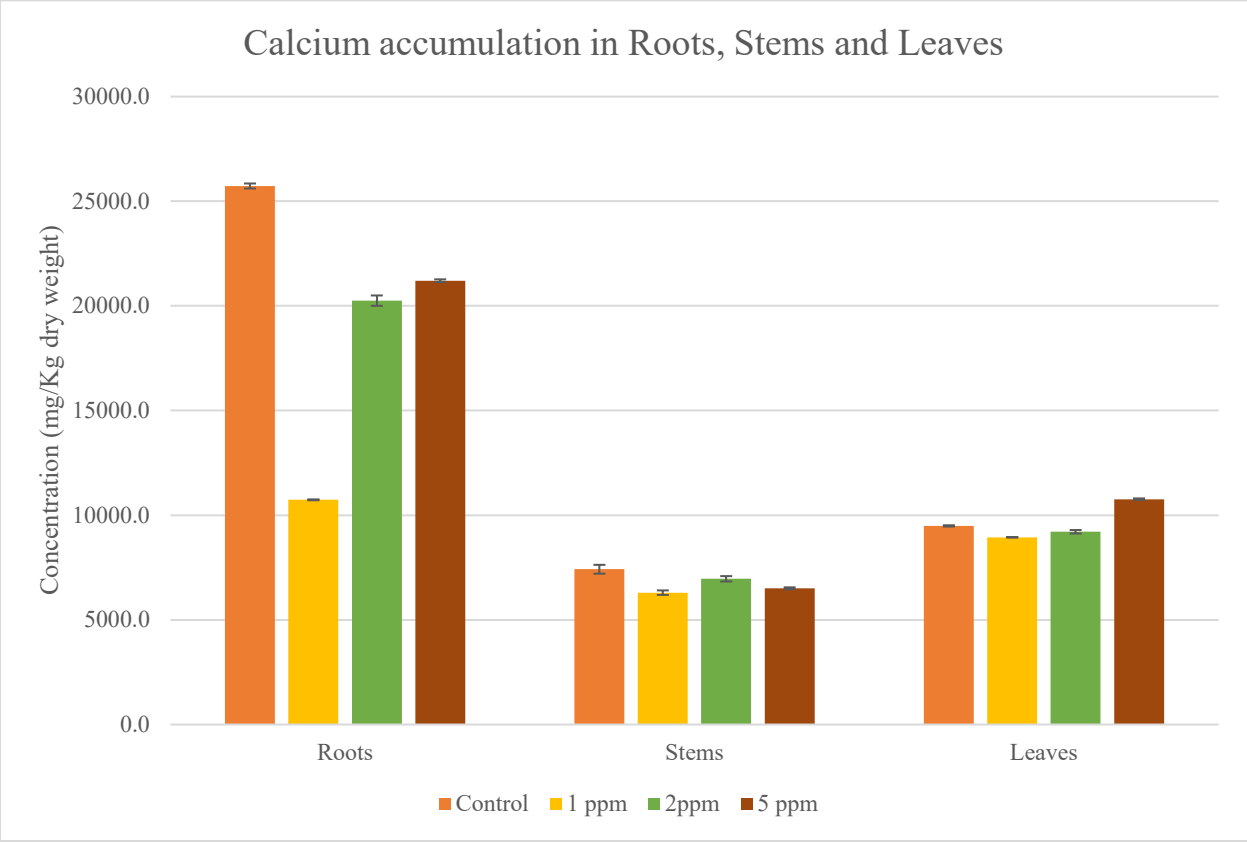

Figure 6. Effect of arsenic in calcium accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

Sulfur, S

Inside beefsteak tomato plants, there is a higher amount of sulfur accumulated in leaves when compared to roots and stems. Data is shown in figure 7 and summarized in table 7. Sulfur accumulation in roots increased as the concentration of arsenic (V) treatment increased. Sulfur translocation to stems decreased at 2ppm of As(V) treatment, while the sulfur concentration in leaves increased for all the arsenic treatments.

Table 7. Sulfur accumulation (mg/Kg dry weight) in roots, stems, and leaves. Each value is the mean for twelve plants (three repetitions for sample)

Sulfur Accumulation in Roots, Stems, and Leaves						
Treatment	Roots (ppm)	Error	Stems (ppm)	Error	Leaves (ppm)	Error
Control	1386.3	7.0	933.2	17.9	3832.5	15.1
1 ppm	1298.6	4.4	867.0	12.9	3653.9	8.9
2 ppm	1956.4	11.3	814.4	17.2	4253.1	34.2
5 ppm	2131.4	7.9	948.1	6.4	4003.4	9.8

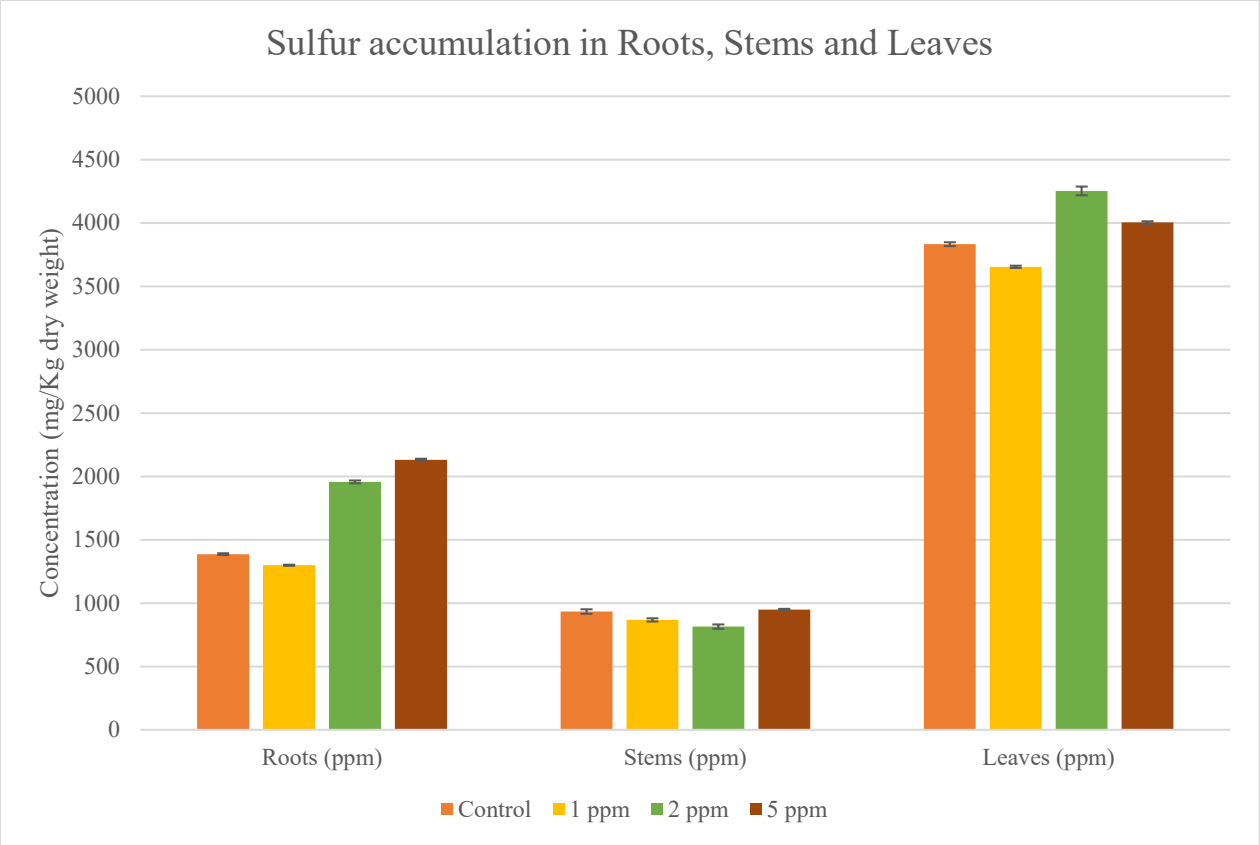

Figure 7. Effect of arsenic in Sulfur accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

Magnesium, Mg

ICP-OES data for magnesium accumulation is shown in figure 8 and the information is summarized in table 8. As can be seen from the figure, magnesium concentration is highest in leaves when compared to roots and stems. Concentrations of magnesium in stems and leaves were higher when tomato plants were subjected to 5 ppm of As(V). Magnesium concentration in roots of tomato plants was higher when they are subjected to 2ppm of arsenic.

Table 8. Magnesium accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Magnesium accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	1161.6	1.0	1225.6	27.6	1861.5	8.5
1 ppm	1336.1	4.3	1163.5	15.2	1748.2	1.8
2 ppm	1994.3	6.2	1152.5	22.0	2060.8	17.2
5 ppm	1909.3	4.9	1297.7	6.8	2266.8	4.9

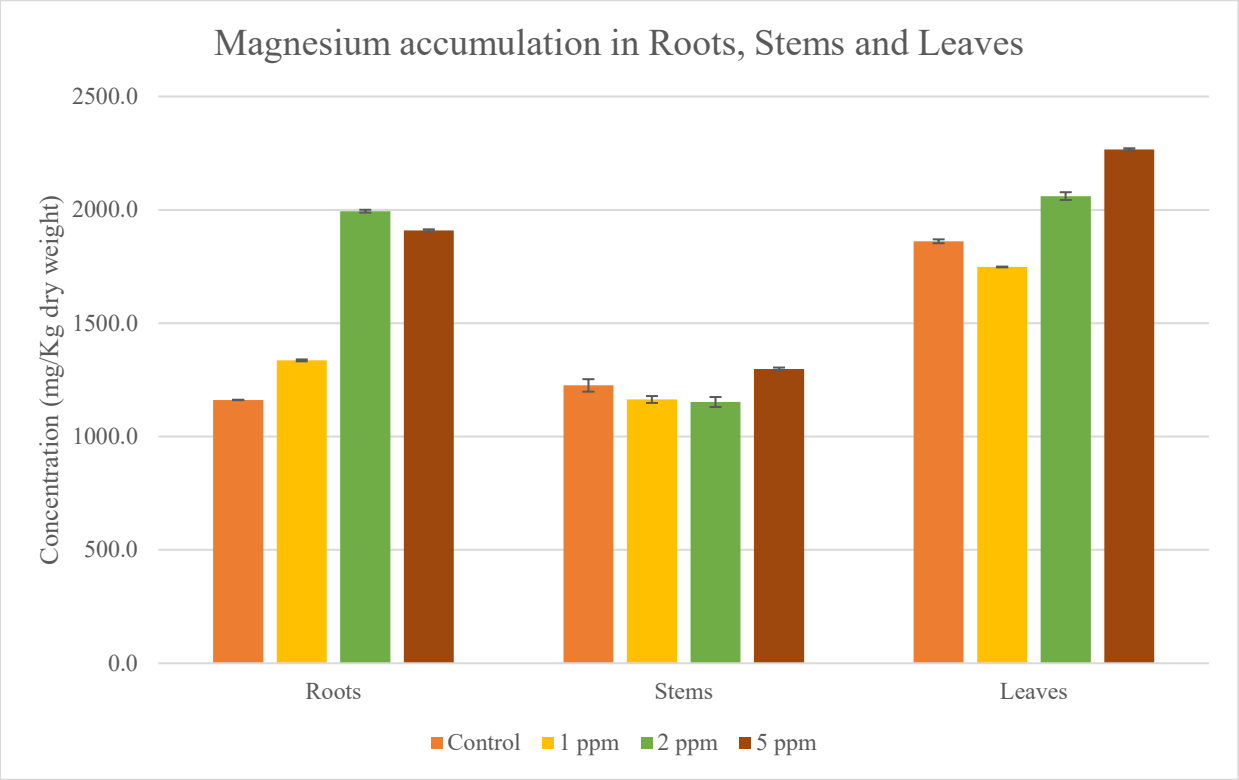

Figure 8. Effect of arsenic in magnesium accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample).

Sodium, Na

Data for sodium accumulation in roots, stems, and leaves of tomato plants is summarized in table 9 and shown in figure 9. As shown in the figure, sodium concentration in the root portion of the plant shows a sharp decrease when contaminated with arsenic. After a decrease at 1 ppm of As treatment, the Na concentration increased as the arsenic concentration increased in the roots. Sodium accumulation in stems and leaves showed a decrease as the concentration in the arsenic treatment was increased. Na levels accumulated by arsenic-contaminated plants never surpassed control values.

T**able 9. Sodium accumulation (mg/kg dry weight) in Roots, Stems, and Leaves.** Each value is the mean for twelve plants (three repetitions for sample).

Sodium accumulation in Roots, Stems, and Leaves						
Conc.	Roots	Error	Stems	Error	Leaves	Error
Control	2228.6	26.1	1273.8	46.4	723.4	1.5
1 ppm	841.0	2.0	1249.5	25.1	702.6	0.7
2 ppm	1002.9	1.0	1013.7	31.7	678.8	9.0
5 ppm	1382.2	4.6	1148.4	6.8	560.2	0.6

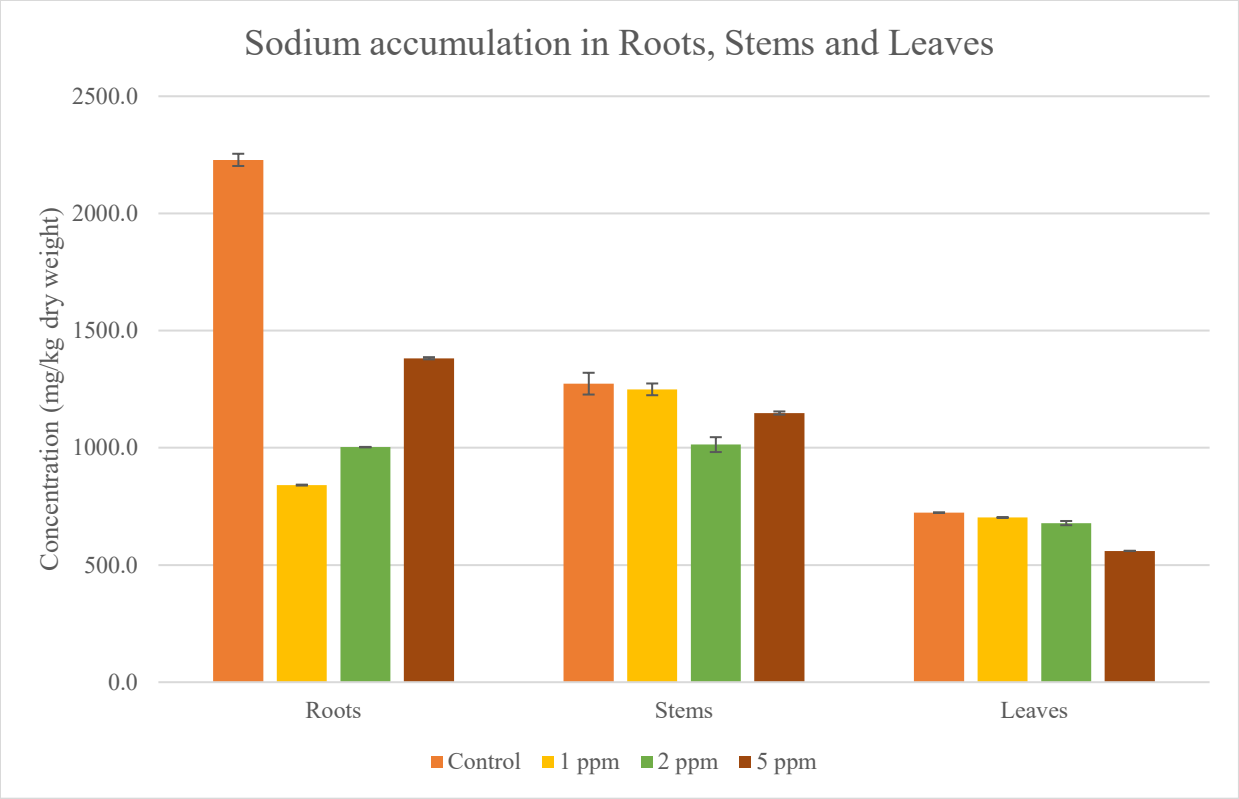

Figure 9. Effect of arsenic in sodium accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

Iron, Fe

ICP-OES data for iron accumulation in roots, stems and leaves of tomato plants is shown in figure 10 and summarized in table 10. As can be seen from figure 10, iron concentration in roots is much larger when compared to stems and leaves. Iron accumulation in the root portion of tomato plants shows a huge increase when tomato plants are subjected to arsenic treatments. Fe accumulation in stems and leaves remains relatively unchanged despite arsenic treatments.

Table 10. Iron accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Iron accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
control	728.4	1.5	57.6	1.3	57.9	3.1
1 ppm	1449.0	2.8	85.1	5.4	44.3	0.4
2 ppm	1351.4	19.2	117.7	2.4	48.2	0.7
5 ppm	2266.9	3.1	83.6	1.1	57.1	0.3

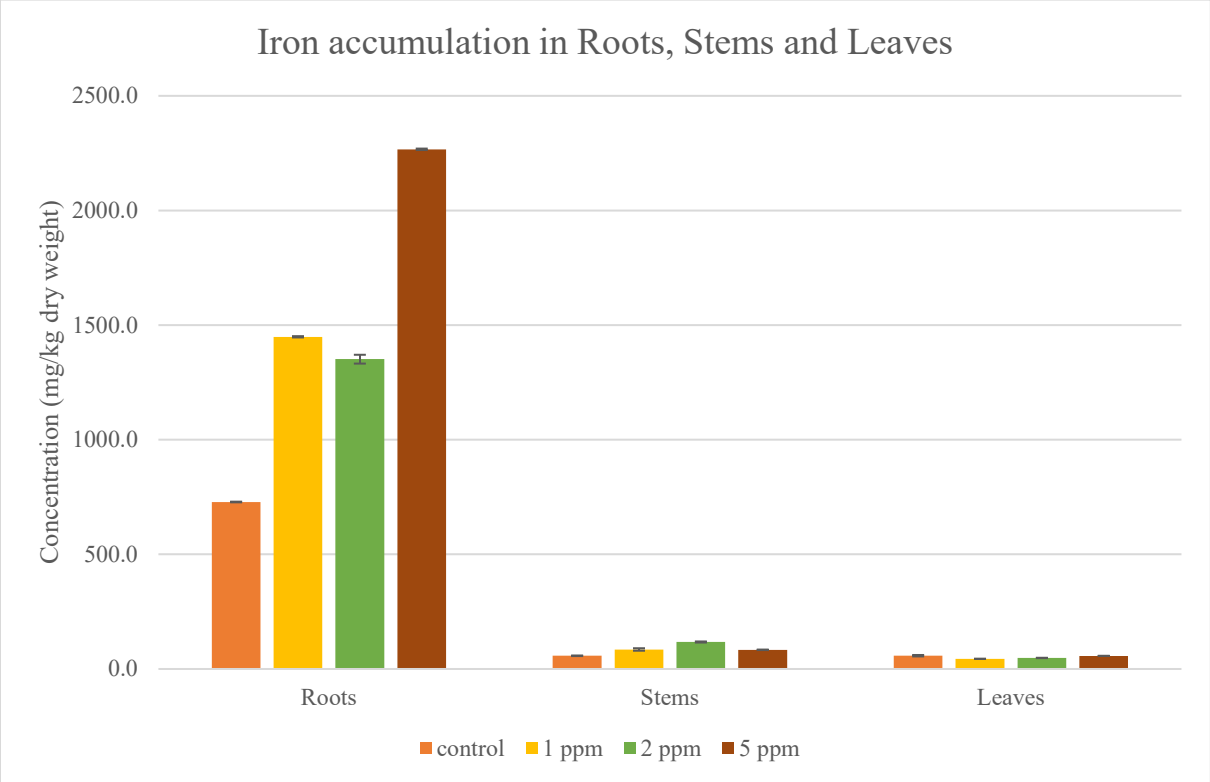

Figure 10. Effect of arsenic in iron accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

Manganese, Mn

As can be seen from figure 11 and the summarized data in table 11, the manganese concentration in tomato plants increased as the concentration of the arsenic treatment increased. Manganese accumulation in the root portion of the plant was much higher when compared to stems and leaves. Although the trend for manganese accumulation differs for each plant tissue, it is possible to observe that manganese concentration increases when the plant is exposed to higher arsenic concentrations. The trend is especially noticeable in roots and leaves.

Table 11. Manganese accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Manganese accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	126.5	0.2	8.3	3.6	40.7	0.3
1 ppm	109.4	0.2	16.3	0.3	47.9	0.0
2 ppm	289.3	4.9	13.0	0.4	58.6	0.5
5 ppm	197.3	0.5	16.6	0.2	61.3	0.2

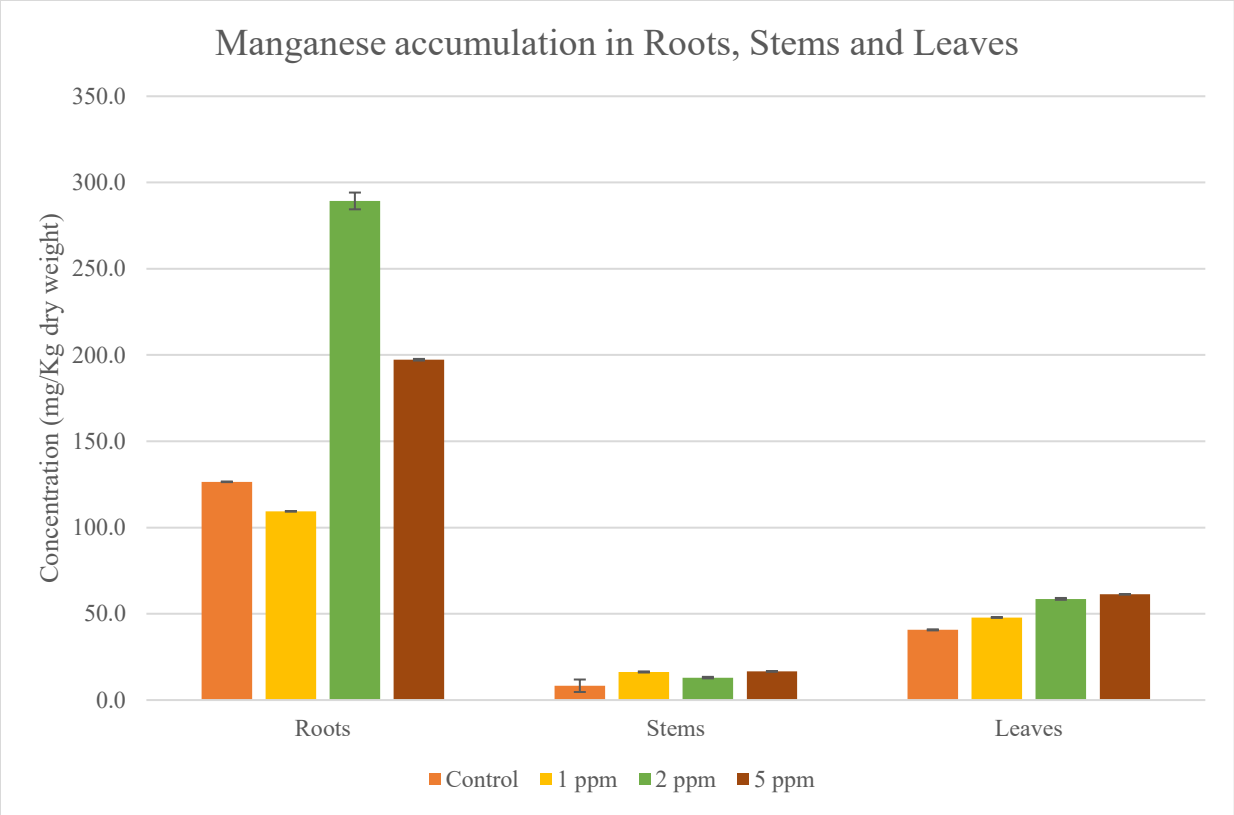

Figure 11. Effect of arsenic in manganese accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

Nickel, Ni

Data for nickel accumulation in tomato roots is shown in Figure 12 and is summarized in table 12. The data shows an increase in Ni concentration in roots and stems when plants are subjected to arsenic treatments. On the other hand, nickel accumulation in leaves of tomato plants remains unaffected independently of arsenic treatments, but slightly lower than the control plants.

Table 12. Nickel accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Nickel accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	2.33	0.02	1.02	0.10	2.67	0.02
1 ppm	7.91	0.46	0.35	0.04	2.08	0.01
2 ppm	6.33	0.06	3.03	0.12	2.08	0.02
5 ppm	5.15	0.02	88.15	0.49	2.01	0.01

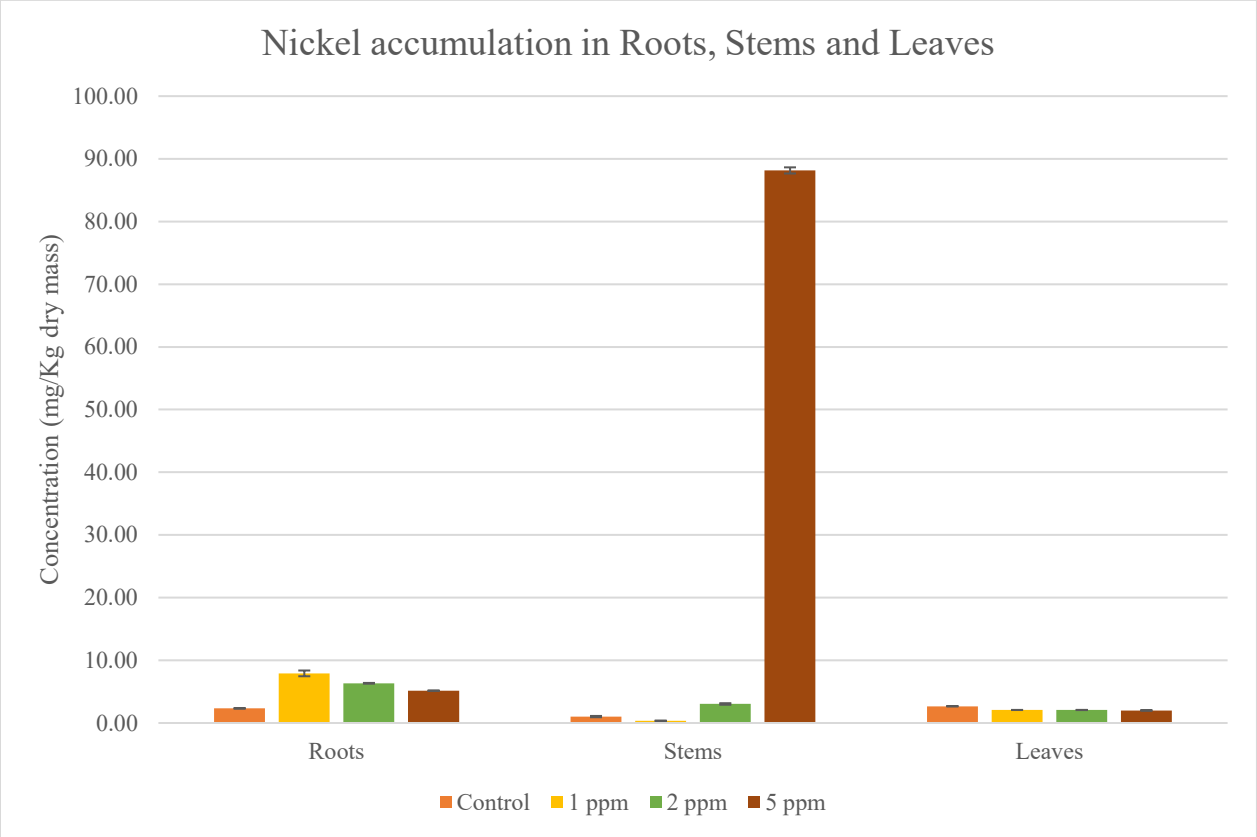

Figure 12. Effect of arsenic in nickel accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

Copper, Cu

Figure 13, shows ICP-OES data for copper accumulation in roots, stems and leaves of beefsteak tomato plants. It can be seen from the figure that copper accumulation is much larger in roots when compared to stems and leaves. Overall, copper concentration increased as the concentration of the arsenic treatment increased.

Table 13. Copper accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Copper accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Roots
Control	126.74	0.51	6.61	0.19	13.96	0.08
1 ppm	115.88	0.17	7.34	0.14	15.94	0.02
2 ppm	210.65	1.77	15.37	0.41	14.02	0.12
5 ppm	208.39	0.55	8.16	0.44	17.50	0.04

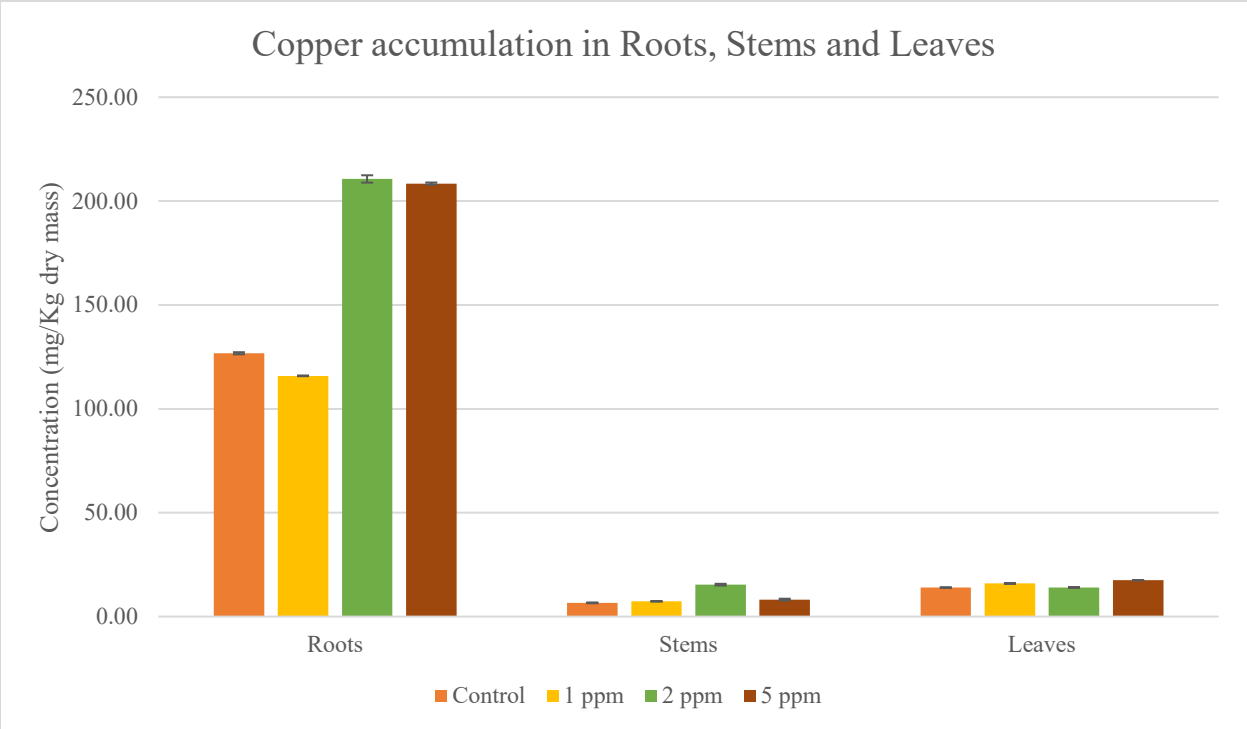

Figure 13. Effect of arsenic in Sulfur accumulation by tomato plants. Each value is the mean for twelve plants (three repetitions for sample)

ICP-OES Data for Arsenic (III) Treatments

Arsenic, As

ICP-OES data for arsenic accumulation in roots, stems, and leaves of tomato plants contaminated with 1ppm, 2 ppm and 5 ppm of As(III) treatments are shown in Fig 14, 15 and 16 and summarized in table 14. As seen in figure 14, arsenic accumulation in roots of tomato plants is much larger when compared to the stem and leave portion of the plant. The highest arsenic accumulation in roots is observed at 2 ppm of As(III) treatment. Arsenic translocation to stems and leaves increased as the concentration of arsenic treatment increased.

Table 14. Arsenic accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Arsenic accumulation in roots, stems and leaves of tomato plants						
	Roots	Error	Stems	Error	Leaves	Error
Control	0	0	0	0	0	0
1ppm	116.84	1.38	1.86	0.12	0.77	0.02
2 ppm	488.44	8.94	5.98	0.18	2.34	0.05
5 ppm	308.17	1.22	83.65	0.59	45.60	0.75

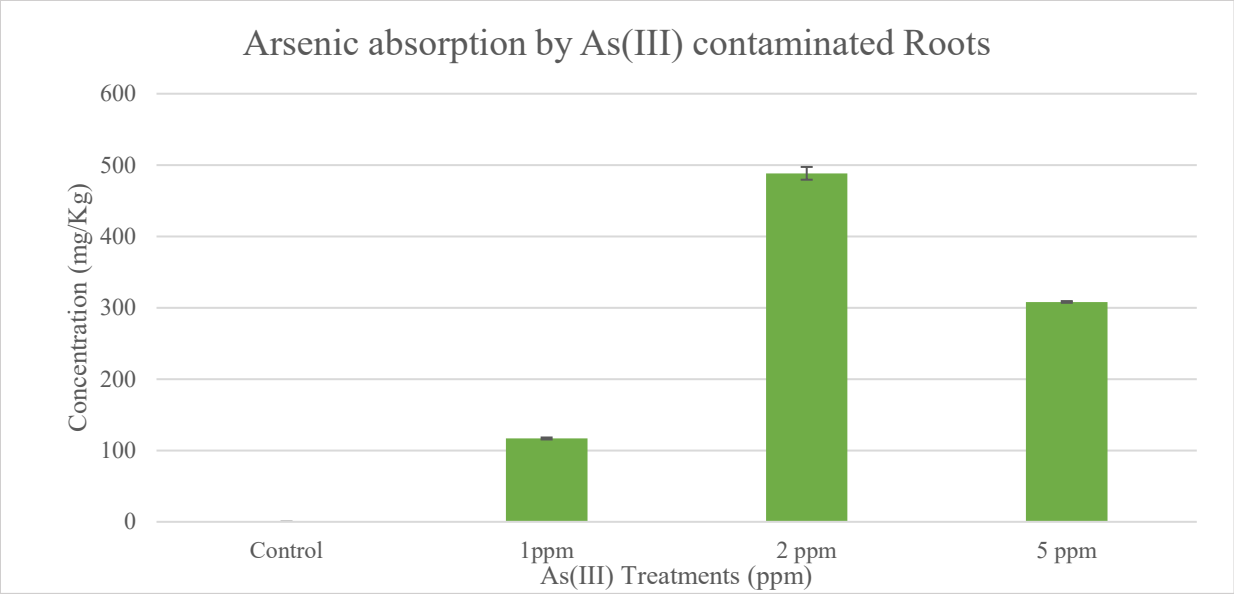

Figure 14. Arsenic accumulation in Roots of Tomato Seedlings. Each value is the mean for twelve plants (three repetitions for sample).

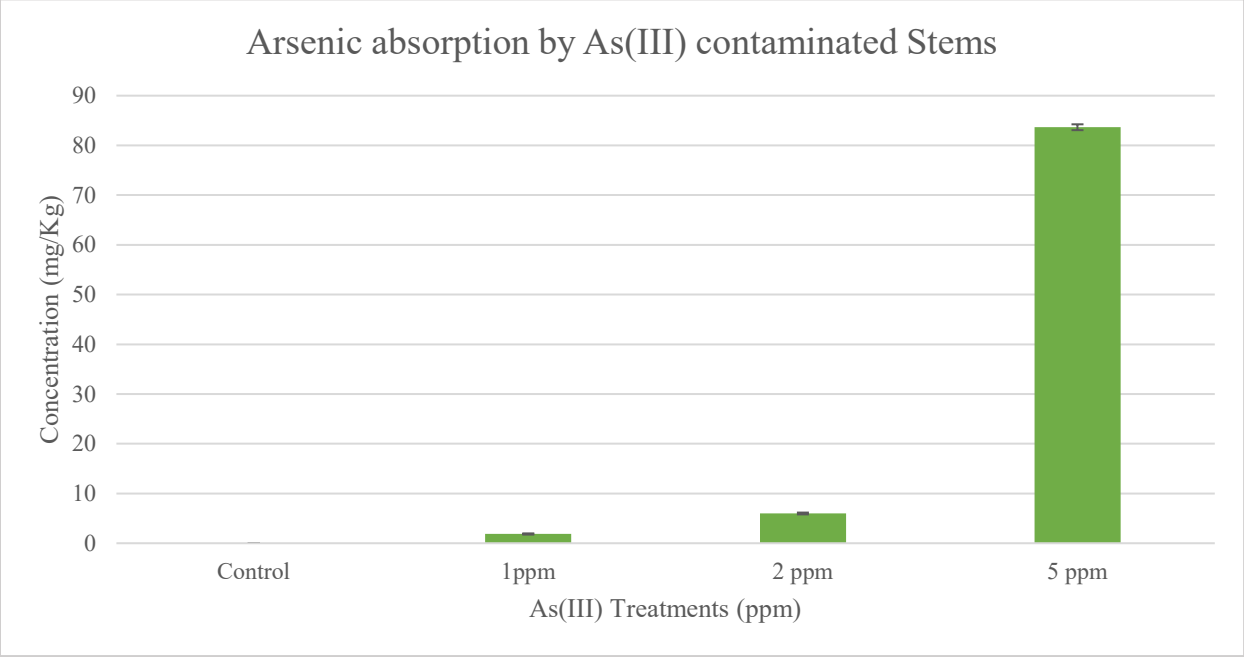

Figure 15. Arsenic accumulation in Stems of Tomato Seedlings. Each value is the mean for twelve plants (three repetitions for sample).

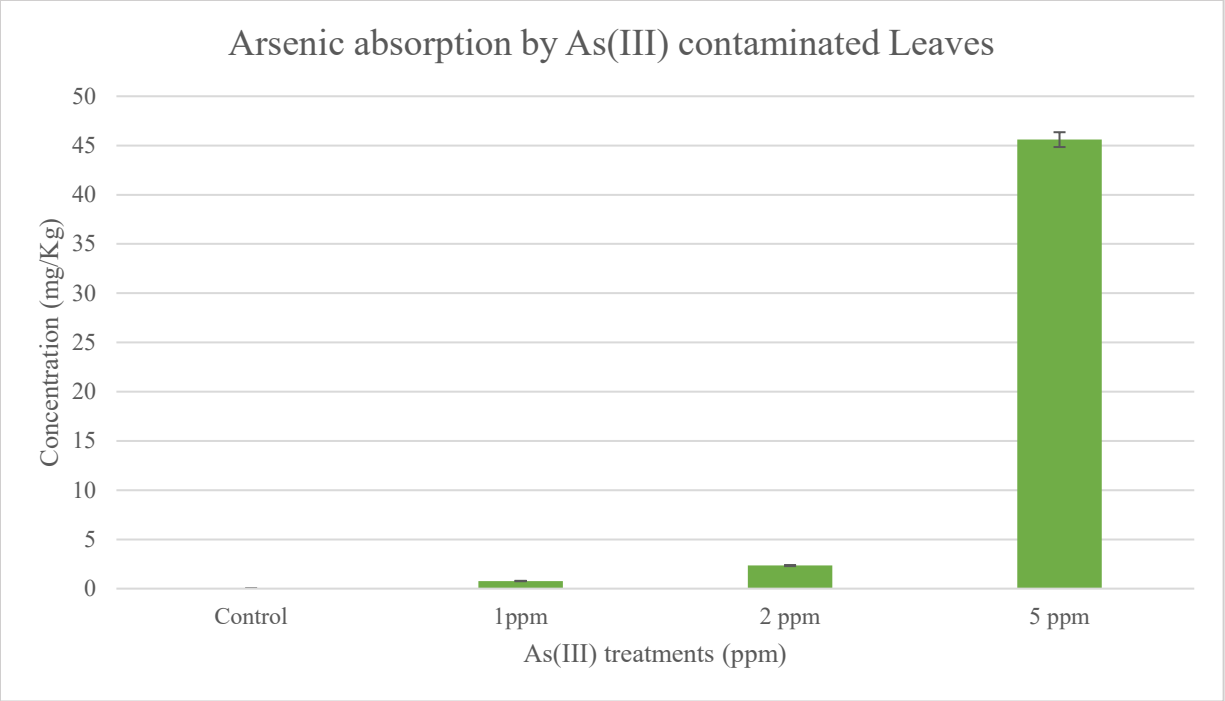

Figure 16. Arsenic accumulation in leaves of Tomato Seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Potassium, K

ICP-OES data for potassium accumulation is shown in figure 17 and is summarized in table 15. As can be seen in figure 17, potassium concentration in roots has an initial decrease in concentration at the 1 ppm treatment but starts increasing as the concentration in the arsenic treatment increases. Potassium translocation to stems showed an overall decrease compared to control plants. Potassium translocation to leaves increased as the concentration in the arsenic treatment increased and exceeded control values.

Table 15. Potassium accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Potassium accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
control	23253.9	2434.6	53748.2	1338.0	24105.5	341.1
1 ppm	18210.6	154.2	46646.3	573.0	26888.9	518.4
2 ppm	23246.8	500.5	51227.0	915.6	27737.5	246.7
5 ppm	28567.4	2642.8	46649.5	162.7	29641.2	264.0

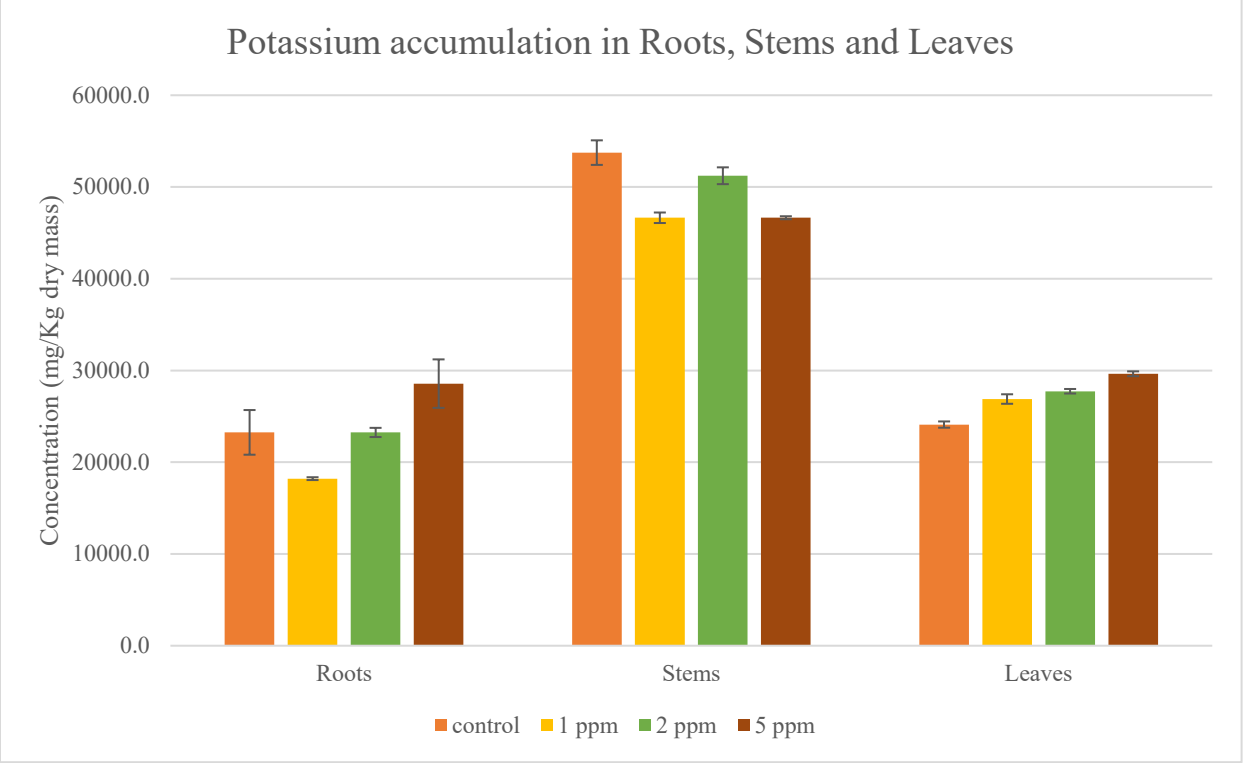

Figure 17. Effect of arsenic in potassium accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Phosphorus, P

Data for phosphorus accumulation is shown in figure 18 and is summarized in table 16. Phosphorus accumulation in roots tended to increase as the concentration in the arsenite treatment was increased. Potassium translocation to stems and leaves of tomato plants remained relatively unchanged despite arsenite treatments. Stems and leaves show the highest phosphorus accumulation when they are subjected to 2ppm of As(III) treatment.

Table 16. Phosphorus accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Phosphorus accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	10399.7	1358.4	1817.0	56.8	2512.0	39.5
1 ppm	9938.2	73.2	2070.5	33.5	2730.4	48.2
2ppm	11563.4	197.8	2254.7	70.2	3132.2	34.0
5 ppm	12080.8	290.3	2043.9	14.9	3004.6	43.2

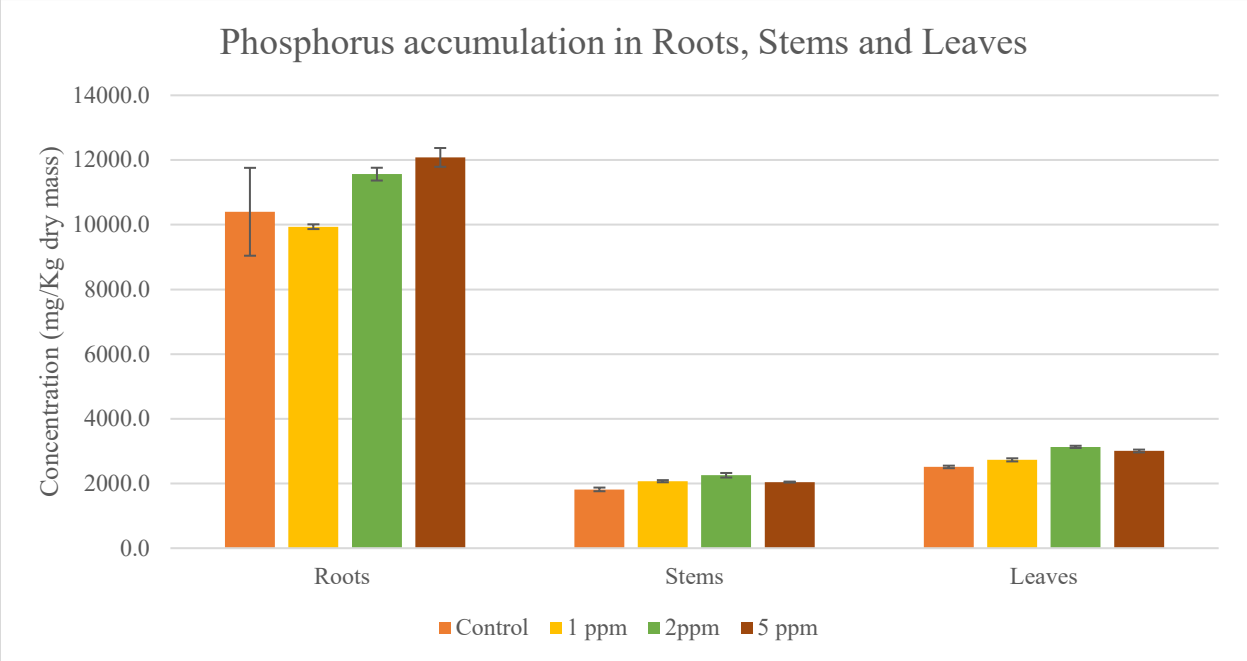

Figure 18. Effect of arsenic in phosphorus accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Calcium, Ca

Calcium accumulation in roots, stems, and leaves of beefsteak tomato plants is shown in figure 19 and summarized in table 17. Calcium accumulation increased as the arsenite concentration in the treatment increased. However, calcium concentration in roots was always below control values. Ca translocation to stems on the other hand, decreased with increasing arsenic concentration, while the calcium translocation to leaves was observed to increase with increasing As concentration.

Table 17. Calcium accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Calcium accumulation in Roots, Stems, Leaves						
Conc.	Roots	Error	Stems	Error	Leaves	Error
Control	22111.54	376.8857	5299.98	176.0123	7253.044	145.1656
1 ppm	16273.19	36.39986	6698.877	110.7138	7230.793	103.0092
2ppm	18184.55	276.9037	5706.863	83.63236	8354.376	70.61989
5 ppm	19143.81	12.55641	5115.72	30.04569	8320.962	70.39727

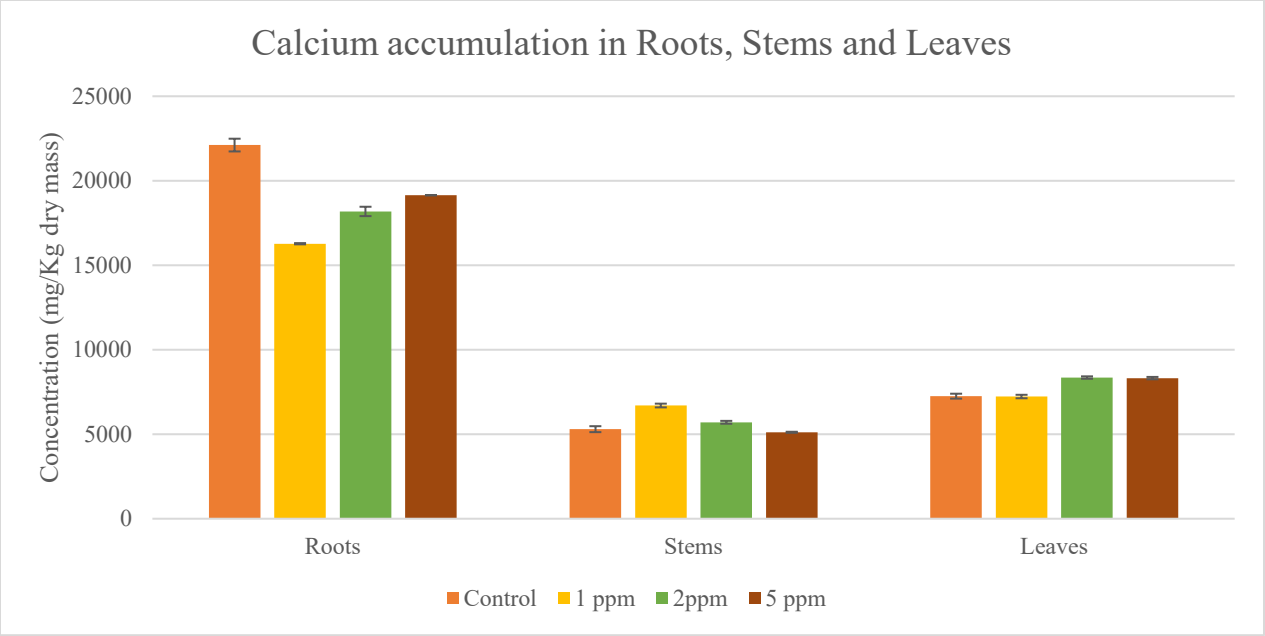

Figure 19. Effect of arsenic in calcium accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Sulfur, S

Sulfur accumulation data for roots, stems and leaves is shown in figure 20 and is summarized in table 18. Sulfur accumulation in roots and leaves was higher in the 2ppm treatment. Sulfur accumulation in roots increased as the arsenic concentration increased. However, sulfur concentration in stems remained relatively unaffected and independent of arsenic concentration.

Table 18. Sulfur accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample)

Sulfur accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	984.98	124.00	756.97	24.52	2207.34	34.40
1 ppm	1162.18	13.24	829.94	19.65	2220.70	41.54
2 ppm	2245.61	52.52	844.23	13.00	2674.38	25.42
5 ppm	2093.62	181.76	885.39	6.89	2241.82	22.12

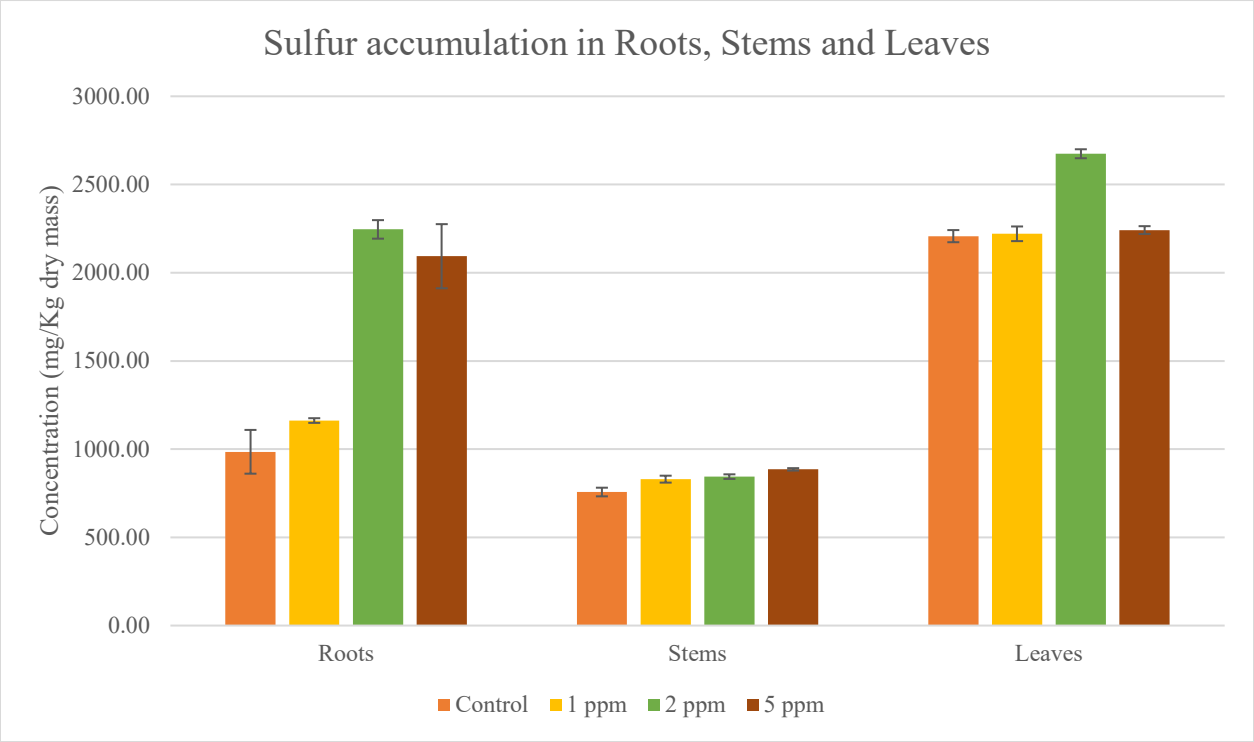

Figure 20. Effect of arsenic in sulfur accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Magnesium, Mg

ICP-OES data for magnesium accumulation is shown in figure 21 and summarized in table 19. Magnesium accumulation in roots, stems and leaves was highest when tomato plant was contaminated with 2 ppm of as As (III). Magnesium accumulation in roots shows an initial decrease in Mg accumulation when plant was subjected to 1ppm As(III).

Table 19. Magnesium accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Magnesium accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	1582.266	78.97346	1157.047	27.27812	1170.443	17.63324
1 ppm	1265.86	10.34128	1191.607	14.44578	1404.328	22.43048
2 ppm	1827.347	36.21314	1303.985	21.08384	1559.526	9.376203
5 ppm	1486.051	93.91489	1192.228	5.663088	1521.154	13.54337

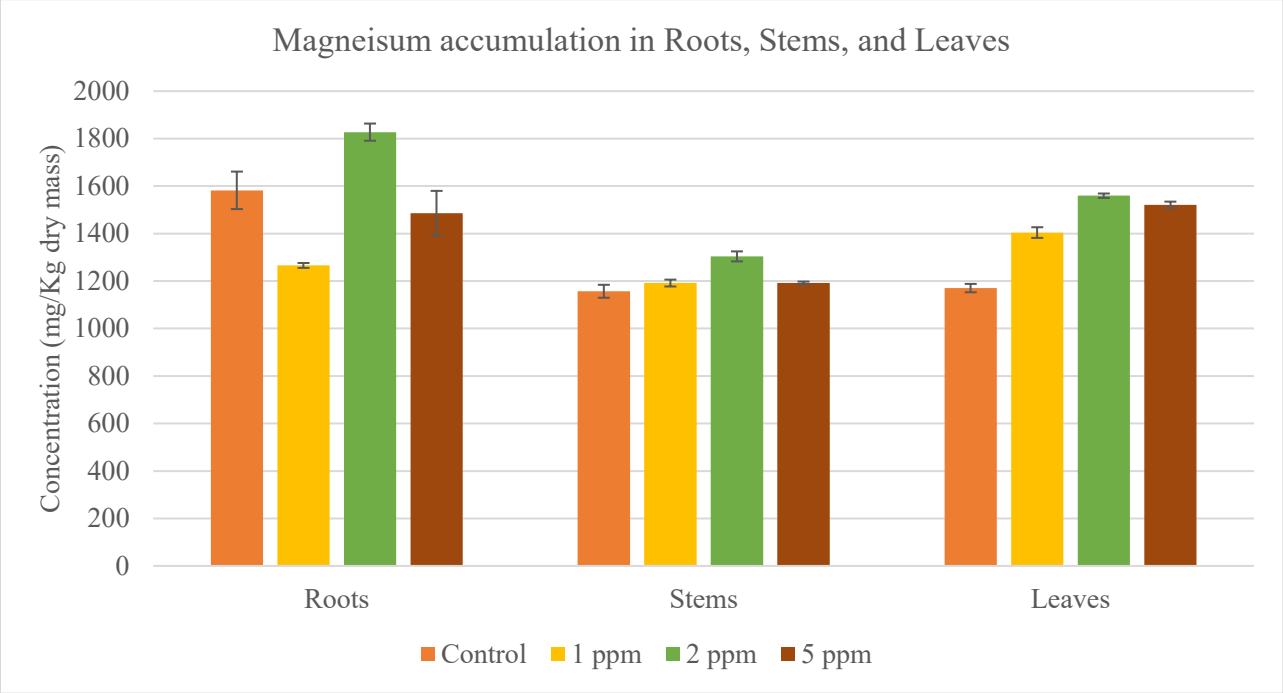

Figure 21. Effect of arsenic on magnesium accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Sodium, Na

Sodium accumulation data is shown in figure 22 and summarized in table 20. Sodium accumulation in roots of tomato plant showed an initial decrease when plants were treated with 1 ppm of arsenite. The highest sodium accumulation was observed in the roots when plant was subjected to 2 ppm of arsenite. The accumulation of sodium in stems and leaves was observed to increase with increasing arsenic concentration.

Table 20. Sodium accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample)

Sodium accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	452.78	60.42	908.20	25.31	474.59	7.29
1 ppm	384.98	4.40	864.51	10.04	508.24	8.32
2 ppm	766.57	19.76	1186.87	18.44	816.33	5.84
5 ppm	742.08	1.18	1206.56	3.43	1324.31	15.86

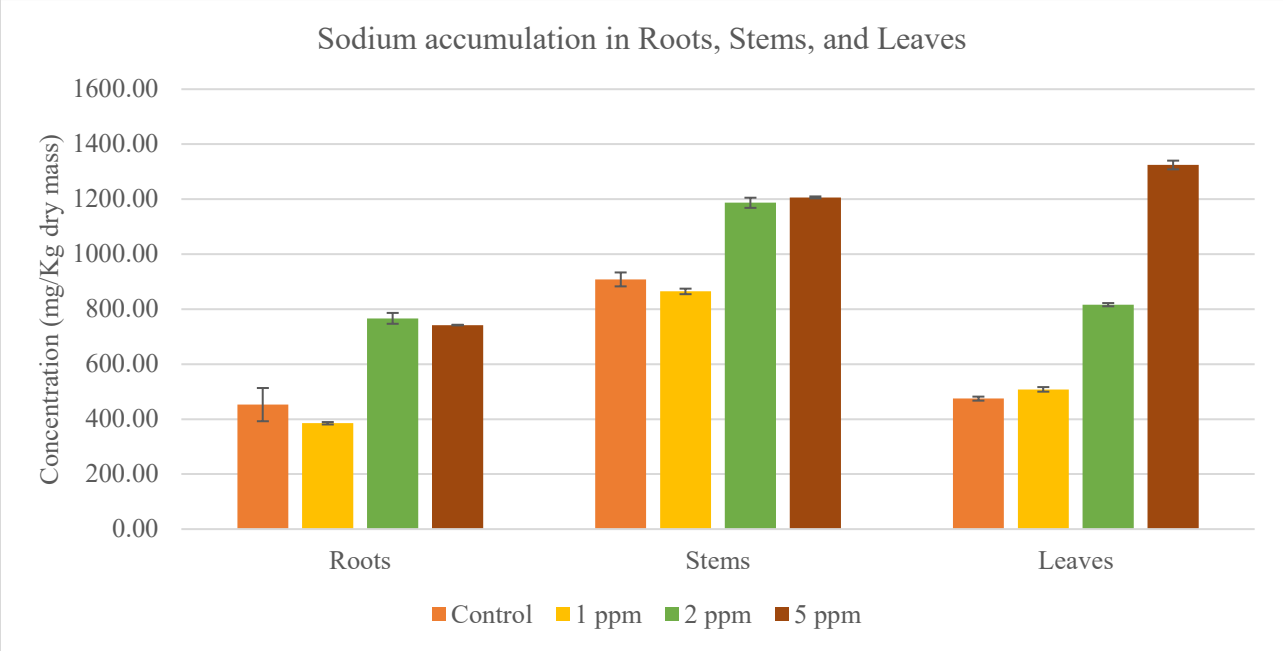

Figure 22. Effect of arsenic on sodium accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Iron, Fe

ICP-OES data for iron accumulation is shown in figure 23 as is summarized in table 21. Iron accumulation in roots increased as the arsenite concentration increased. However, iron accumulation in stems and leaves was also elevated with arsenic concentration compared to the control plants.

Table 21. Iron accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample)

Iron accumulation in Roots, Stems and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
control	674.54	33.16	34.26	0.78	31.87	0.91
1 ppm	627.62	5.22	164.10	5.35	33.81	1.56
2 ppm	1228.66	20.59	48.40	1.26	46.43	0.85
5 ppm	1422.17	65.59	68.74	3.46	46.16	0.43

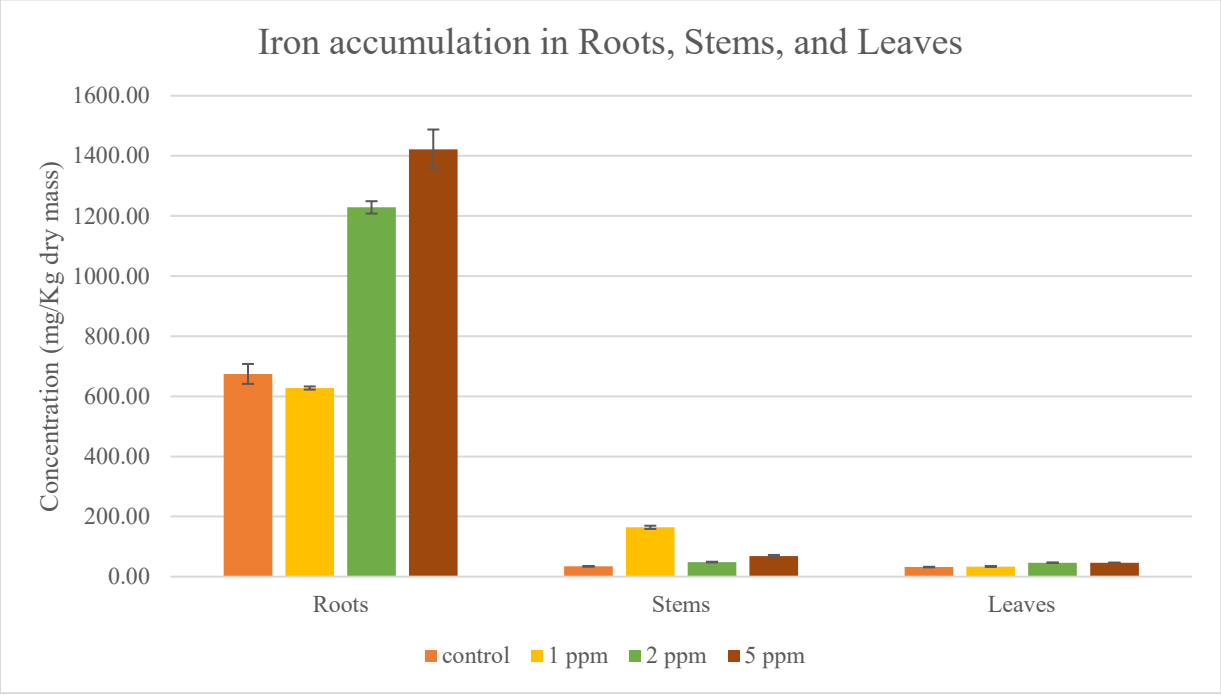

Figure 23. Effect of arsenic in iron accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Manganese, Mn

ICP-OES data for manganese accumulation is shown in figure 24 and summarized in table 22. Manganese accumulation in roots, stems and leaves was higher when tomato plant was exposed to 2 ppm of arsenite treatment.

Table 22. Manganese accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Manganese accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	442.1177	23.11395	10.90096	0.415445	44.23342	2.507025
1 ppm	409.2139	0.915454	20.49677	0.415795	66.16662	2.014721
2 ppm	773.7512	10.75391	28.36581	0.544044	125.2158	0.26329
5 ppm	592.9763	2.682049	7.448902	0.152406	61.40934	0.599371

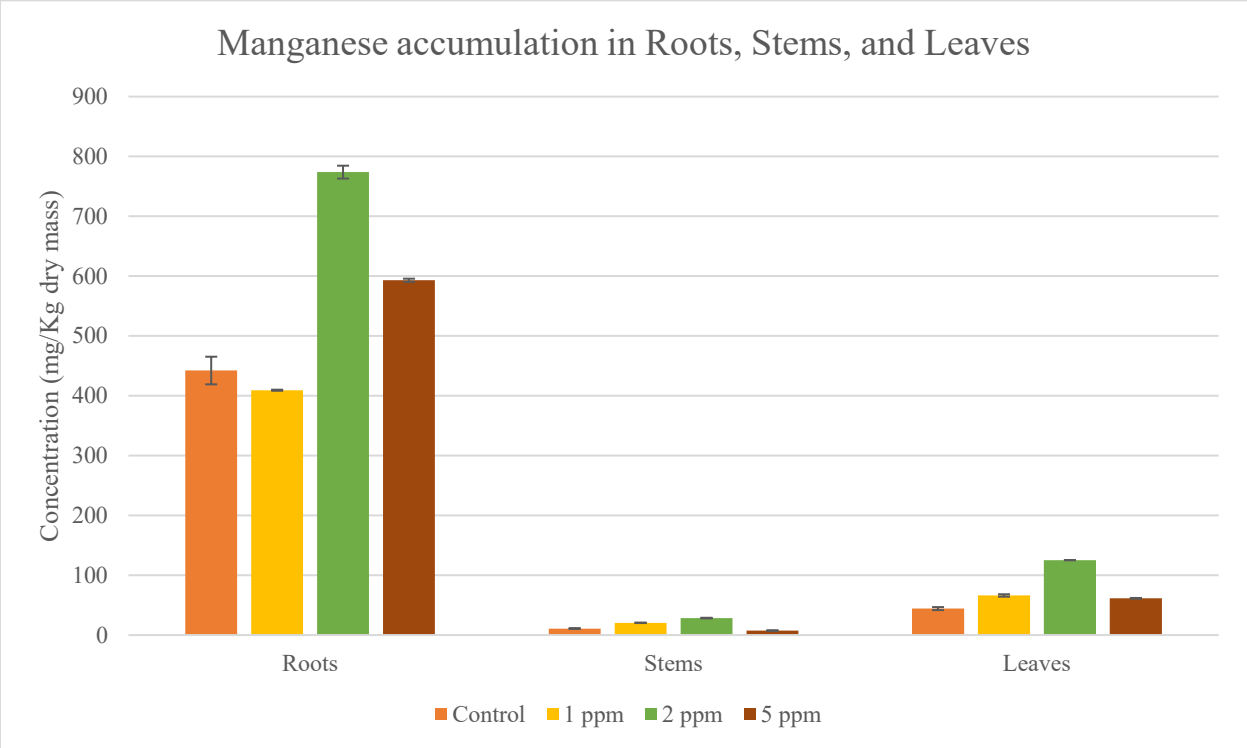

Figure 24. Effect of arsenic in manganese accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Nickel, Ni

Data for nickel accumulation is shown in figure 25 and is summarized in table 23. Nickel accumulation showed a large increase when exposed to 5ppm of As(III) treatment. However, the stems accumulated more nickel when exposed to the 2 ppm treatment. Nickel accumulation in leaves was observed to decrease as the arsenic concentration in the treatment increased.

Table 23. Nickel accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample)

Nickel accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	2.92	0.52	0.88	0.13	1.85	0.14
1 ppm	3.94	0.06	1.45	0.04	1.96	0.06
2 ppm	5.67	0.11	8.43	0.73	1.47	0.02
5 ppm	18.68	0.10	2.60	0.36	1.72	0.07

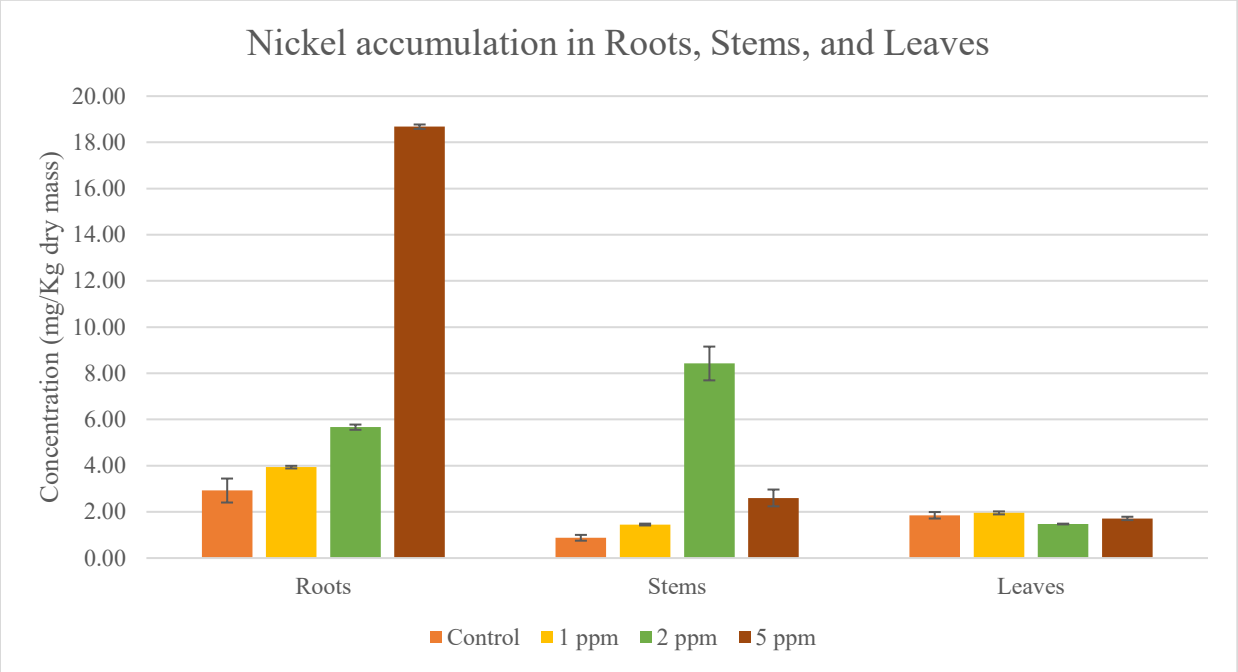

Figure 25. Effect of arsenic in nickel accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Copper, Cu

Data for copper accumulation in roots, stems, and leaves of tomato plant is shown in figure 26 and summarized in table 24. The highest copper accumulation in roots was observed at 2ppm of arsenite treatment. Copper concentration in stems and leaves remains relatively unaffected and independent of arsenic treatment.

Table 24. Copper accumulation (mg/Kg dry weight) in Roots, Stems, and Leaves. Each value is the mean for twelve plants (three repetitions for sample).

Copper accumulation in Roots, Stems, and Leaves						
Treatment	Roots	Error	Stems	Error	Leaves	Error
Control	56.22	2.50	6.29	0.21	8.93	0.43
1 ppm	49.46	0.40	6.91	0.09	9.37	0.19
2 ppm	118.83	1.75	8.12	0.10	9.84	0.07
5 ppm	92.53	0.70	10.54	0.13	8.09	0.03

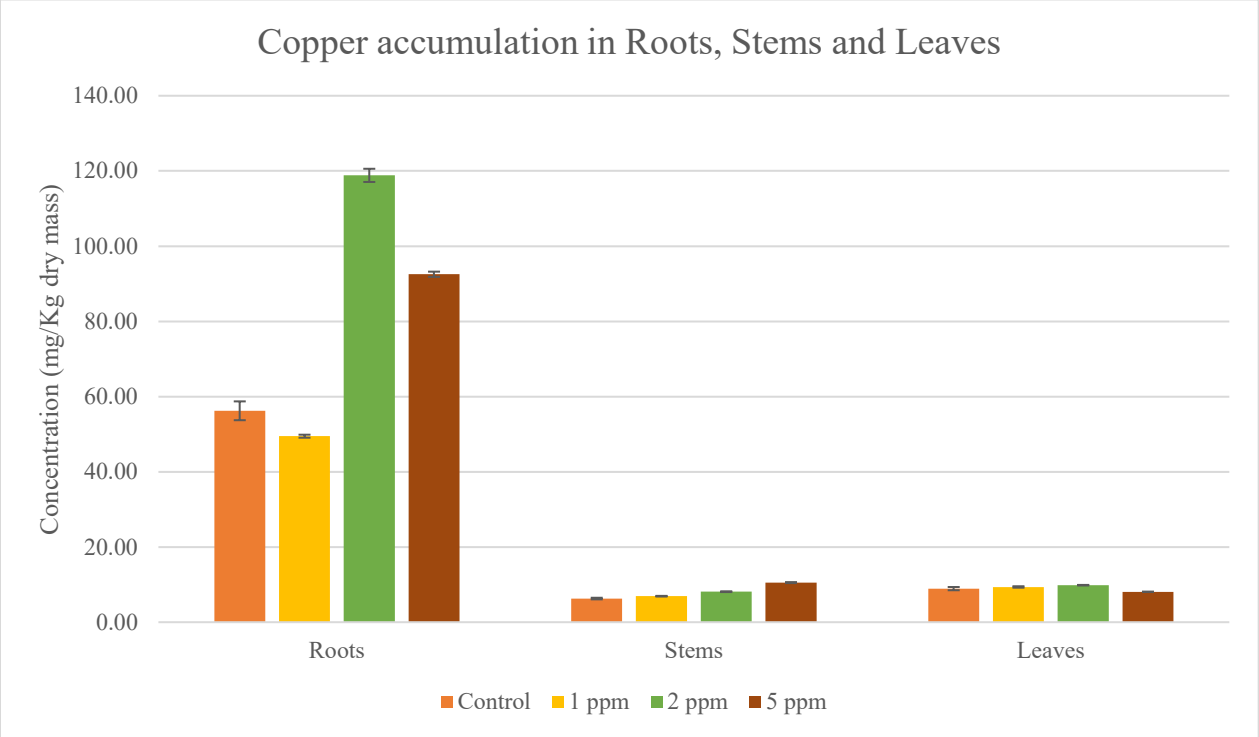

Figure 26. Effect of arsenic in copper accumulation in tomato seedlings. Each value is the mean for twelve plants (three repetitions for sample).

Table 25. Beefsteak Tomato Seedlings Average Growth

Beefsteak Tomato seedlings average growth (cm)								
	Arsenic (V)				Arsenic (III)			
Tissue	**Control**	**1 ppm**	**2 ppm**	**5 ppm**	**Control**	**1 ppm**	**2 ppm**	**5 ppm**
Roots	13.00	16.00	14.00	12.50	18.00	16.00	17.00	14.00
	22.00	15.00	14.00	20.00	17.00	15.00	15.00	13.00
	13.50	17.00	13.50	19.00	18.00	16.00	16.00	13.00
	13.00	19.00	13.50	12.00	20.00	17.00	16.00	12.00
	11.00	16.00	19.00	15.00	14.00	18.00	9.00	15.00
	15.50	18.00	12.00	14.00	16.00	17.00	17.00	16.00
	12.00	18.00	10.00	10.00	16.00	19.00	16.00	13.00
	11.50	22.00	15.00	15.00	14.00	16.00	17.00	12.00
	13.00	10.00	10.00	13.00	14.00	15.00	16.00	12.00
	15.00	11.00	20.00	16.00	10.00	13.00	17.00	13.00
	15.00	5.00	9.00	15.00	16.00	13.00	15.00	12.00
	11.00	7.00	11.00	14.00	14.00	14.00	13.00	14.00
Average	**13.79± 0.86**	**14.50 ± 1.48**	**13.42± 0.98**	**14.63±0 .80**	**15.58± 0.75**	**15.75±0 .54**	**15.33± 0.66**	**13.25± 0.37**
Stems	17.00	15.00	15.00	17.50	23.00	15.50	13.00	14.00
	15.50	21.00	7.00	14.00	16.00	14.00	13.00	12.00
	22.50	14.80	13.00	17.50	16.00	13.50	8.50	10.00
	12.00	18.00	12.50	17.50	15.00	13.50	9.50	12.50
	13.50	15.00	13.00	16.50	19.00	12.00	12.50	14.00
	14.50	14.00	16.00	13.00	24.00	13.00	14.00	14.00
	15.00	10.00	13.50	16.00	17.00	14.50	10.00	13.50
	17.50	14.00	12.50	12.00	13.50	17.50	10.00	13.00
	17.50	9.50	12.50	7.50	10.00	15.00	12.50	8.00
	17.50	12.00	12.00	8.90	10.00	13.50	17.00	7.00
	9.50	11.00	11.80	9.50	14.00	12.00	16.00	9.50
	5.70	11.00	11.50	3.00	14.00	9.50	17.00	8.00
Average	**14.81 ± 1.25**	**13.78 ± 0.97**	**12.53 ± 0.62**	**12.74 ± 1.35**	**15.96 ± 1.262**	**13.63 ± 0.578**	**12.75 ± 0.83**	**11.29 ±0.76**

CHAPTER V

DISCUSSION

Arsenic, As

According to the results, arsenic accumulation in seedlings of tomato plants was highest in roots, followed by stems and leaves in that order. Similar results have been observed in other plants showing high arsenic concentration in roots and much lower arsenic concentrations in stems and leaves (Parsons, et al., 2008; Carbonell et al., 1995). Such distribution of arsenic accumulation in roots, stems and leaves indicates low mobility of this metalloid within the plant tissues. Low mobility of arsenic species in tomato seedlings has been linked to arsenic having high toxicity in radicular membranes and the formation of iron and manganese plaques on the root surface (Carbonell et al., 1995; Liu and Zhu, 2005).

Arsenic uptake by tomato plants has been linked to arsenic availability in the nutrient solution. Therefore, the higher the arsenic concentration in the treatment, the higher the arsenic accumulation in the roots. However, that was not the case for roots of tomato seedlings contaminated with 5 ppm of arsenic (III) since root membranes were damaged before the end of the second week of treatment (as determined from visual inspection color of the roots changed). In addition, the uptake of some essential nutrients ceased, there was an evident reduction of growth in roots and stems (14.9%), and plants showed chlorosis in leaves.

Overall, in the present study, the seedlings of beefsteak tomato plants accumulated more arsenic when contaminated with arsenic (III) than when treated with arsenic (V). The highest amount of arsenic in tomato seedlings (488 ppm) was accumulated in the roots and was observed at 2ppm of As(III) treatment. However, the toxic effects of As (III) on the tomato seedlings were observed with the 5 ppm treatment.

Potassium, K

A decrease in the concentration of potassium in the roots of tomato plants contaminated with 1 ppm of arsenic (III) and arsenic (V) was observed. The decrease in potassium concentration may be due to abiotic stress which led the plants to a reduced intake of potassium, which has been observed in other plants under stress conditions (Syed, 1999). However, as the arsenic level in the treatment increased, potassium accumulation was observed to increase as well. This trend in potassium absorption has been observed in other plants such as corn seedlings (Parsons et al., 2005) and *Spartina alterniflora Loisel* (Carbonell et al., 1998). Such behavior has been linked to potassium as a common counter ion for the As(III) and As (V) anions (Clarkson and Hanson, 1980). Therefore, due to the absorption of the negatively charged arsenic species in the roots, the plants increased the intake of a positive cation to counteract the adverse effect and produce a charge balance (Parsons et al., 2005).

Stems and leaves of tomato seedlings contaminated with arsenic (V) and leaves of tomato contaminated with arsenic (III) show a similar trend to that of roots. Potassium concentration increased as the arsenic concentration in the roots increased, indicating an efficient translocation system. However, that is not the case for stems contaminated with arsenite since plants died

when exposed to the 5ppm treatment of As(III). Nevertheless, it is important to highlight that even when plants died at 5 ppm, overall potassium uptake (for all arsenic treatments) in stems was lower than the control levels. This may have been due to the formation of plaques on the roots preventing the translocation of nutrients or the damage of potassium transporters when root membranes stop working correctly.

Phosphorus, P

Results for phosphorus concentration in roots of tomato seedlings showed a decrease of this nutrient when the plant was exposed to either 1 ppm of arsenate or arsenite. When the plant was exposed to 1 ppm of arsenic (V), phosphorus accumulation decreased approximately by 50% compared to that of the control plant. The reduction in phosphorus accumulation in roots contaminated with 1 ppm of arsenic (III) was approximately 16% compared to the control. This decrease in concentration may have been due to a damaging of the phosphate mechanisms within the plant due to the abiotic stress. However, the high decrease in concentration when plant was subjected to the arsenate treatment may be have been due to the competitive inhibition between phosphate and arsenate (Meharg and Hartley, 2002). Surprisingly, results suggest for both arsenic (III) and arsenic (V) treatments, that as the arsenic levels in treatment increased, phosphorus accumulation increased as well. The concentrations resemble those found in the control plants.

On the other hand, results for phosphorus accumulation in shoots of tomato seedlings suggest no change in the intake of this nutrient despite treatment with arsenic. This suppressed phosphorus intake by stems of tomato seedling has been observed in arsenic-resistant plants

which also show a high shoot phosphorus status (Meharg and Hartley, 2002;). It is believed that such down-regulation of the high affinity phosphate transporter turns out to be a coincidental benefit and aids in arsenic resistance (Fitter et al., 1998). The trend of phosphorus accumulation in leaves of tomato seedlings showed a similar trend to stems; however, at the same time it is possible to observe a subtle increase in the concentration of P as the arsenic level in the treatment increases.

Calcium, Ca

Results for calcium accumulation in roots of tomato seedlings showed values below the normal for all arsenic treatments. Calcium concentration in roots contaminated with 1ppm arsenic (III) and arsenic (V) showed an initial decrease like in the previous elements analyzed. This decrease may have been due to a stress response, when subjected to low concentrations of arsenic, especially in the pentavalent form.

As the levels of arsenic treatments increased, the calcium accumulation in roots increased as well. Possibly, resisting to the stress response since it has been observed that calcium alleviates toxic metal stress by reducing metal uptake (Hasanuzzaman and Fujita, 2015). However, those values do not surpass the calcium concentration levels in control plants. Such limitation may be strongly linked to the increase in potassium and magnesium accumulation in roots of tomato seedlings when plants are exposed to high levels of arsenic. It has been shown that high levels of potassium and magnesium reduce the intake of calcium in plants (Tuteja and Mahajan, 2007).

The calcium in stems was also affected by increased amounts of potassium and magnesium at high arsenic concentrations. However, overall calcium accumulation in stems remained relatively unchanged despite arsenic treatments. A similar trend was observed in the leaves of tomato seedling. However, it was shown that at higher arsenic levels, calcium in leaves tended to increase, which may possibly alleviate the arsenic stress.

Sulfur, S

Results of sulfur accumulation in roots and leaves of tomato seedlings indicated an increasing trend as the arsenic concentration in the treatments increased. For plants contaminated with 1 ppm of arsenic (V), an initial decrease in sulfur accumulation in roots and leaves was observed. However, as the arsenic concentration in the treatment was increased, the sulfur concentration in the plant increased as well. The same trend was observed in plants contaminated with arsenic (III). The initial decrease in sulfur accumulation might be a response to the stress caused by the toxic element. On the other hand, the increase in sulfur accumulation may be due to the formation of glutathione and low molecular weight thiols. It has been demonstrated that many plants employ these molecules to reduce arsenic toxicity and also for arsenic storage (Parsons et al., 2005; Pickering et al., 2000). It has been observed that arsenic-resistant plants synthesize phytochelatins (utilizing reduced glutathione molecules) to complex arsenite and make it less toxic (Delnomdedieu et al., 1994).

The sulfur accumulation in the stem portion of the plant remained relatively unchanged despite the increasing arsenic concentration in the treatments. The unchanging sulfur concentration inside the stem portion of the tomato seedlings indicates that there is low mobility

of sulfur from roots to stems. However, sulfur concentration in leaves continued to increase despite unchanging sulfur accumulation in stems. This tendency may have been due to increased production of low molecular weight thiols and phytochelatins protecting the leaves from arsenic intoxication and arsenic storage in vacuoles in the leaves.

Magnesium, Mg

ICP-OES results for magnesium showed an increasing accumulation of this essential nutrient in beefsteak tomato seedlings as the levels of arsenic increased. The leaves from the plants contaminated with arsenic (V) and roots contaminated with arsenic (III) indicated an initial decrease in magnesium concentration when the plant was subjected to 1ppm of arsenic. This behavior may have been due to the stress conditions caused by the toxicity of arsenic as it has been observed in the uptake of the elements previously discussed. Oxidative stress caused by arsenic has been shown to damage the membrane structures of plants, ultimately leading to electrolyte leakage and a nutrient imbalance (Chandrakar et al., 2018).

Overall, however, arsenic-treated plants showed an increase in magnesium accumulation in all their tissues. Such behavior can be part of a mechanism for alleviating arsenic intoxication produced in the tomato seedlings. Researchers have demonstrated the efficacy of magnesium at minimizing oxidative damage caused by abiotic stress and they have also showed that magnesium is also effective at decreasing heavy metal accumulation in body tissues (Meireles da Silva et al., 2017; Matovic et al., 2011). Moreover, magnesium plays an important role in antioxidant activity, as well as being the cofactor of several enzymes that decrease oxidative stress (Silva et al., 2016).

Sodium, Na

Plants contaminated with arsenic (III) and roots of tomato seedlings contaminated with arsenic (V) showed an increase in sodium accumulation as the arsenic level in the treatment increased. According to the concept of "essentiality" developed by Arnon and Stout in 1939, sodium is cataloged as a nonessential nutrient since it is not required by C3 plants to complete their life cycle. However, tomato seedlings accumulated sodium as a defense mechanism against the oxidative damage produced by arsenic, especially on those plants contaminated with arsenic (III). It has been observed that plants supplemented with exogenous sodium nitroprusside can decrease the oxidative damage caused by arsenic. Positive results have been observed in *Vicia faba*, wheat and rice seedlings (Ahmad et al., 2020; Hasanuzzaman and Fujita, 2013; Golam et al., 2014). It has been suggested that sodium nitroprusside can decrease heavy metal-induced oxidative damage since it acts as a donor of nitric oxide (NO) which improves antioxidant defense, the ascorbate-glutathione cycle and the glyoxalase cycle in stressed plants (Ahmad et al., 2020).

Stems and leaves of arsenic (V) treated plants showed relatively no change in sodium concentration as the levels of arsenic increased. These results may be attributable to the low arsenic concentration accumulated by stems and leaves subjected to arsenic (V) treatments.

Iron, Fe

Roots of tomato seedlings showed a large increase in iron accumulation when they were contaminated with arsenic (III) and arsenic (V) treatments. For both treatments, iron accumulation was larger when exposed to 5 ppm. Such an increase in iron concentration may

have been due to the formation of iron plaques on the roots of tomato seedlings. The formation of iron plaques to sequester arsenic from soil or aquatic environments has been observed in rice (Liu et al., 2006), corn seedlings (Parsons et al., 2005), Lupinus albus (Fresno et al., 2016), among other species.

The low arsenic accumulation present in the upper portion of the plants may be due in a great extent to the iron plaque formation in the roots of tomato seedlings. It has been shown that besides their role of sequestering arsenic, iron plaques also decrease arsenic uptake and mobility into stems and leaves (Liu et al, 2004). Despite the large iron concentrations found in the roots of the plants at high arsenic levels, accumulation in stems and leaves remained unchanged. The low amount of iron present in stems and leaves indicates very low mobility of this metal inside the seedlings. This fact provides more credibility to the formation of iron plaques on the roots of the plant.

The only exception to the unchanged iron concentration in stems occurred when the plant was subjected to 1ppm of arsenic (III). At this arsenic concentration, iron accumulation increased beyond the control values. This could be explained by the low amount of iron accumulated in the roots exposed to the same treatment. Low accumulation of iron in roots in the form of iron plaques lead to a translocation of this element to the stem portion of the plant.

Manganese, Mn

Manganese accumulation in beefsteak tomato seedlings increased as the arsenic levels in the treatment increased. The trend was present in roots, stems and leaves which indicated an efficient mobility of this metal within the plant. The roots, however, accumulated the largest

amount of manganese when compared to stems and leaves. This behavior may be an indicative of the formation of manganese plaques on the root surface. It has been observed that formation of manganese plaques is a defense mechanism against arsenic toxicity since it decreases the translocation ability of the heavy metal to the upper portions of the plant. This effect on manganese concentration has been observed in maize plants (Boisson et al., 1999), corn seedlings (Parsons et al., 2005), and rice seedlings (Liu and Zhu, 2005).

It is important to highlight that manganese translocation from roots to leaves was efficient. Despite large accumulation in roots, and possible formation of plaques, manganese moved freely within the plant. This indicates manganese is also important to decrease oxidative damage inside the leaves of tomato seedlings. The role of manganese in decreasing oxidative stress has been linked to the Mn-superoxide dismutase (SOD) activity. It has been observed in other plant species that Mn-SOD can reduce cellular damage caused by reactive oxygen species (Bowler et al., 1991; Shenker et al, 2004).

Nickel, Ni

Nickel accumulation in roots and stems of tomato seedlings contaminated with arsenic (III) increased as the level of arsenic in the treatment was increased. Nickel concentration in stems was higher at 2ppm since root membranes were damaged during the 5ppm treatment. Plants contaminated with arsenic (V) also exhibit a higher nickel accumulation when compared to the control plants. This increase in nickel accumulation may have been due to a defense mechanism by the plant against the arsenic-induced oxidative damage. It has been observed that nickel is a key activator of an isoform of the glyoxalase I enzyme that degrades cytotoxic

methylglyoxal (MG), which is overproduced during abiotic stress (Mustafiz et al., 2014). The essentiality of nickel as an activator of glyoxalase to degrade MG molecules and production of reduced glutathione to alleviate oxidative damage has also been suggested but it is still under investigation (Fabiano et al., 2015).

Leaves of tomato seedlings showed a decrease in nickel accumulation when plants were subjected to arsenic. However, there was relatively no change in nickel concentration as the arsenic levels increased. Such behavior implies that mobility of nickel from stems to leaves is low in the presence of arsenic.

Copper, Cu

Copper accumulation in roots of tomato seedlings increased as the level of arsenic was increased. When plant was subjected to 1 ppm of arsenic treatment, copper concentration in roots was less than in control plants. However, copper accumulation increased by approximately 50% when exposed to high arsenic levels. This means that copper is an essential element to alleviate the arsenic-induced oxidative damage inside plant tissues. It is known that an excess or a deficiency of copper in plant nutrition leads to free radical formation, threatening the plant well-being (Yamasaki et al., 2008). However, the essentiality of copper during photosynthetic electron transport, mitochondrial respiration and oxidative stress responses is also well known (Yruela, 2005). Increase in copper concentration in roots may have also been due to Cu/Zn superoxide dismutase formation which has been found to defend tomato plants exposed to stress (Perl-Treves and Galun, 1991).

Copper accumulation in stems and leaves remained relatively consistent despite increasing arsenic concentrations. This behavior suggests that copper is either not very mobile within plant tissues or the biologival pathways utilizing copper in the stem and leaves portions are not affected when plant is exposed to arsenic. It is important to highlight that roots subjected to 5 ppm of arsenic (III) accumulated less copper than in the 2 ppm treatment, which is due to a malfunction of root membranes during the 2 weeks of treatment

CHAPTER VI

CONCLUSIONS

Beefsteak tomato seedlings uptake arsenic from aqueous nutrient solutions and then translocate the arsenic to stems and leaves. It was found that the highest amount of arsenic that is accumulated by tomato seedlings was located inside the roots of this plant. Only small amounts of arsenic are translocated to the upper portions of the plant and this behavior is strongly linked to the variations of essential nutrient accumulation.

The essential nutrient uptake, as well as the arsenic translocation within the tissues of the plant are also related to the oxidation state of arsenic in the aqueous solution. It was concluded that tomato seedlings accumulate more arsenic (III) than arsenic (V) inside the roots. However, it was also observed that high arsenic (III) concentrations damage root membranes affecting the uptake and further translocation of arsenic and essential nutrients.

It was also observed that the highest amount of arsenic was accumulated by roots contaminated with 2ppm of arsenic in the trivalent form (489 ppm). The tomato seedlings accumulate more arsenic as the concentration of the element increases in the nutrient solution. This trend was observed in roots, stems, and leaves of plants treated with arsenic (III) as well as with arsenic (V). The only exception for this trend was observed in roots contaminated with 5 ppm of arsenic (III) since root membranes were damaged due to high toxicity.

The highest amount of arsenic accumulated in roots contaminated with arsenic (V) was 135 ppm and occurred during the 5ppm treatment. Unlike plants contaminated with arsenic (III), tomato seedlings were not damaged by high concentrations of arsenic (V). In fact, average growth increased when plants were exposed to arsenic (V) treatments. It was concluded that arsenic (III) is more toxic for tomato seedlings than arsenic (V).

Arsenic mobility within plant tissues was observed to be low. The low mobility was reflected by the low arsenic concentration in stems and leaves compared to the arsenic concentration in roots. This trend was observed for both oxidation states of arsenic. The only exception to this trend occurs in stems and leaves contaminated with 5 ppm of arsenic (III). However, due to the damaging of root membranes caused by arsenic toxicity, it was concluded that plant behavior does not follow the normal trend.

The potassium uptake was observed to decrease when plants were contaminated with low amounts of arsenic. However, when the arsenic concentration in the nutrient solution was high (5ppm), potassium uptake by tomato plants increased. A similar trend was observed for roots and stems contaminated with arsenic (III) and (V). However, potassium translocation to leaves was observed to depend on the oxidation state of arsenic. Potassium translocation to leaves contaminated with arsenic (V) was lower than control values and remained unchanged despite arsenic concentration. On the other hand, potassium translocation to leaves increased as the arsenic (III) concentration was increased.

It was concluded that phosphorus accumulation by roots of tomato seedlings was lower than control values when exposed to arsenic (V) and higher when exposed to arsenic (III). Phosphorus uptake by roots was strongly linked to arsenic concentration in hydroponics media. At low arsenic levels, phosphorus concentration decreased significantly, but the uptake increased

at high arsenic concentrations. On the other hand, arsenic translocation to stems remained unchanged under different arsenic treatments. This trend is more remarkable for arsenic (V) treated plants. Translocation of phosphorus to leaves slightly increased as the arsenic concentration in the media was increased.

Calcium uptake by tomato seedlings after exposure to arsenic treatments was lower than control values. It was found that calcium accumulation in tomato was drastically decreased when plants were exposed to low arsenic treatments. As the arsenic level in the treatment increased, calcium uptake increased but did not surpass control values. However, exceptions to this trend were observed in the leaves.

Magnesium uptake in the roots of tomato seedlings increased drastically when tomato seedlings are exposed to arsenic (V). The same behavior was observed in the translocation of magnesium to leaves when exposed to arsenic (V). However, no significant change was observed in translocation to stems. The same trend in magnesium uptake was observed for roots, stems, and leaves exposed to arsenic (III) treatments. However, due to root membrane damage, magnesium uptake decreased when exposed to 5 ppm of arsenic (III).

Sodium uptake by tomato plants was strongly linked to the oxidation state of arsenic in the treatment. It was found that tomato seedlings uptake less sodium compared to control plants when exposed to arsenic (V), and more sodium under arsenic (III) treatments. The same trend was observed in roots, stems, and leaves.

Sulfur accumulation in roots was higher when tomato seedlings were exposed to high arsenic treatments. Sulfur translocation to arsenic-contaminated stems remained unchanged,

while sulfur translocation to leaves was slightly increased. The same trends were observed for both arsenic (III) and arsenic (V) treatments.

It was found that iron accumulation in the tomato roots increased when plants were subjected to either arsenic (V) and arsenic (III) treatments. Iron accumulation was strongly dependent on the concentration of arsenic in the treatment. At higher arsenic concentrations, the iron uptake by plants was higher. Translocation to stems and leaves remained unaffected despite the concentration of arsenic in the treatment. Arsenic was observed to control the mobility of iron within plant tissues.

Manganese uptake in the roots of tomato seedlings was observed to be lower than control values when plants were exposed to 1ppm of arsenic treatments. The highest amount of manganese in roots was observed at 2 ppm of either As (III) and As(V). Manganese concentrations in the roots decreased at 5 ppm of arsenic treatments. Manganese translocation to stems and leaves increased as the concentration of arsenic in the treatment increased.

The accumulation of nickel in the roots of tomato tended to increase when exposed to arsenic (III) and arsenic (V). Nickel translocation to stems increased at high arsenic concentration but remained relatively unchanged in the leave portion of the plants. A similar trend was found for copper since the accumulation of this element inside the roots of the plant increased at high arsenic (III) and arsenic (V) concentrations. Copper translocation to stems and leaves remained unaffected despite arsenic concentration.

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BIOGRAPHICAL SKETCH

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