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Removal of Arsenic (III) and Arsenic (V) from Aqueous Solution via Common Sunflower *Helianthus annuus* by Phytoremediation

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REMOVAL OF ARSENIC (III) AND ARSENIC (V) FROM AQUEOUS SOLUTION VIA COMMON SUNFLOWER *HELIANTHUS ANNUUS* BY PHYTOREMEDIATION

A Thesis

by

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ABSTRACT

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Phytoremediation provides a cost-effective, non-invasive process to remove contaminants from soils and groundwaters. Sunflowers are fast-growing species that have shown to be effective at removing various heavy metal pollutants from soils, including lead. Arsenic-related health issues are mainly attributed to exposure to arsenite (the As(III) anion), whereas Arsenate (the As(V) anion) is much less toxic. In the present work mammoth sunflower (*Helianthus annuus*) was the species investigated to remove both As(III) and As(V) ions from hydroponics solutions. The sunflower seeds were germinated for one week, placed under the sun for one day, and subsequently placed in a nutrient solution to improve the plant's growth before arsenic treatment. After one week of growth in the nutrient solution, the sunflowers were transferred to a nutrient solution contaminated with either arsenite or arsenate (at concentrations of either 1 ppm, 2 ppm, or 5 ppm of arsenic). After two weeks of growth in the arsenic treatments, the sunflowers were harvested and separated into three sections roots, stems, and leaves. The capacity of the sunflowers to uptake and store arsenic was determined by normalization in their biomass. The sunflower plants were dried, digested in nitric acid, and analyzed using a Perkin Elmer Optima 8300 DV Inducted-Coupled Plasma Optical Emission Spectroscopy (ICP-OES) for arsenic, macronutrient, and micronutrient accumulation.

DEDICATION

The accomplishment of my Master's degree is dedicated to my parents, Candelario Vazquez and Maria De Lourdes Hurtado Arias de Vazquez, my brothers Ivan Vazquez and Giovanni Vazquez, the unconditional support of my entire family, and the encouragement of my girlfriend Genesis Eng.

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A remarkable and respectful thanks to my Master's mentor and thesis committee chair, Dr. Jason Parsons, Professor in the Department of Chemistry, for giving me all the knowledge and research techniques for my thesis. The support they gave us as their students are unconditional to accomplish our academic objectives besides all the advice provided to succeed in life.

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CHAPTER I

BACKGROUND

Environmental Pollution

Pollution has been linked to severe environmental problems such as global warming, climatic change, ecosystem alterations, etc. As well pollution directly affects biodiversity, environmental and human health; studies have shown 9 million diseases in 2005 were linked to pollution (Landrigan et. al, 2017). Urbanization and industrialization are both significant contributors to environmental pollution; the increasing growth of population contributes to road construction, and motor vehicle traffic, directly affecting the ecosystem. Industries worldwide are introducing hazardous materials into soil and water through wastewater discharge containing heavy metals, that may cause damage to both the environment and human health. (Ukaogo et al., 2020). Heavy metals possess properties that can affect living organisms over time through increasing their normal biological and chemical concentrations in living tissues, which is known as bioaccumulation (Verma and Dwivedi et al., 2013).

Heavy Metals

Arsenic is a metalloid, semi-metal, however, it is commonly treated as heavy metal in biological studies, it is commonly found to be accumulated from daily routine sources such as water and foods and has been shown to cause several effects in humans. These metals groups are characterized by their high density and toxicity at low concentrations parts per billion levels

(ppb). Heavy metals are spread into the environment through industrial discharge, contaminated water, and natural processes that become approachable for human contact, resulting in fatal health complications; many of them are known to be potential carcinogens. (Yadav et al., 2019).

Arsenic Chemistry

Arsenic was discovered in the 1200s by the philosopher Albertus Magnus by heating orpiment with soap. Arsenic is part of element Group 15in the periodic table, considered part of the pnictogen family. Arsenic possesses an atomic number of 33 and an atomic weight of 74.921, forming part of the semi-metals. Arsenic-75 is considered the most stable and non-radioactive isotope in Arsenic contain 33 protons and 42 neutrons in a nucleus surrounded by 33 electrons in different energy shells,. Nonstable isotopes are also part of Arsenic, As (0), having an electron configuration of: $1s^2 2s^2\ 2p^6 3s^2 3p^6 34s^2 d^{10} 4p^3$ ascending order of orbital energies. Compared to elements like Phosphorus and Nitrogen, Arsenic possesses a higher ability to lose electrons, meaning that it has greater oxidation potential having the capability to combine with various elements forming covalent compounds. Arsenic has two common oxidation forms, arsenite (As(III)) and arsenate (As(V)); arsenite has a higher toxicity than arsenate and his molecules are 25-60 times more mobile in aqueous solutions (Dutré & Vandecasteele 1995). Arsenic, in nature is more commonly found in inorganic compounds, which are typically more toxic than the organic species (Flora et al., 2015).

Arsenic Exposure

Exposure to As(III) and As(V) is dangerous and can have deadly consequences for animals and humans, the element is commonly found in daily use instruments, and routine activities, and is generally ubiquitous in the environment. People can easily come into contact with Arsenic through the air, water, and food sources. In addition, humans are very susceptible to ingesting, inhaling, and skin absorbing arsenic entering directly into their organs, causing severe complications. Arsenic exposures can be considered natural or anthropogenic (Abdul et al., 2015). Recent studies demonstrated arsenic is a carcinogenic element (Bencko, 1977), invading different organs of the human body; when the inorganic form of arsenic enters the human body it primarily attacks the skin, lungs, and bladder. Other studies have also linked arsenic exposure to liver cancer (Chiu et al., 2004). As well as many other human diseases caused by arsenic (Hong et al., 2014). Arsenic inhalation is hazardous due to its transmission abilities directly affecting the respiratory and gastrointestinal systems. The usage of coal to keep warm in cool temperatures or to cook is a prevalent method to get exposure to arsenic, the arsenic levels that burning coal is capable of releasing are very alarming. Asian countries have experienced the highest levels of arsenic in daily sources due to the coal-burning contamination affecting entire regions (Li et al., 2006). Habitants affected by this problem manifested skin irritations, burns, and cancer, followed by internal organs toxicities

CHAPTER II

INTRODUCTION

Phytoremediation

Populations worldwide are increasing dramatically, developing industrialization and urbanization resulting in environmental destruction. Studies in mushrooms and plant samples have demonstrated efficient results in absorbing heavy metals from these environments (Tüzen, 2003). Unlike organic compounds, heavy metals are non-biodegradable and remain accumulated in the environment. Phytoremediation is a plant-based technique designed to take up chemicals from environments such as water and soil. Phytoremediation is a low-cost and non-invasive technique that is effectively used several times to reforest heavy metals contamination zones using a short-term process. In this method, plants are capable of accumulating heavy metals primarily in their roots. Roots system is extended to soil increasing their bioavailability being capable of changing the rhizosphere pH, increasing metal solubility, and extracting the chemical, regenerating the affected area, and not affecting the fertility of the soil (Yan et al., 2013).

Phytoremediation has the potential to be linked with further investigations references; recent investigations have demonstrated effective hyperaccumulators plants capable of uptake certain heavy metals by phytoremediation method (Peer et al., 2005). However, the environment possesses various possible effective plants available for future analysis.

Arsenic Metabolism in Plants

Arsenic is found around the environment in two forms As(V) and As(III) both forms are considered toxic for humans and disruptive to plants' metabolism, however, some plants (hyperaccumulators) have been demonstrated to be effective in taking up both forms from contaminated soils. One of the mechanisms plants use in absorbing As from soils, is the use of phosphate transporters that are able to transport the most abundant form of As; As(V), lowering the toxicity levels in the environment (Zhao et al., 2008). Whereas the second mechanism involve As(III) as a dithiol reactive compound that attaches enzymes containing dithiol co-factors and inactive it them (Finnegan et al., 2012). Pathways of arsenic interacting with plants are based on the nutrients that plants possess, while both forms affect plant metabolism, both possess different phytotoxic orders.

Arsenic and Phosphate Pathway

Plants with practical hyperaccumulation abilities primarily demonstrate their initial absorption stage in their roots. Phosphate interacts first with roots as an inorganic compound, converting into an organic compound in the stems; different from than roots stage, the amount carried by stems is generally lower than the initial root concentrations (Loughman et at.,1957). Roots are considered the most capable and faster part of the plant metabolism to absorb arsenate and transform it to arsenite; in a short-term process (Zhao et al., 2008).

CHAPTER III

METHODS

Plant Growth

Sunflower *Helianthus annus* seeds were germinated in sterilized paper towels. Rolls of paper towels were made with around 20-25 seeds per roll and incubated for five days at a temperature of 30°C, Approximately 10-15 seeds per roll did not incubate incubation. Forty-eight seedlings were placed in 4L container filled with nutrient solution for 1 week. One container for each concentration was used. The nutrient solution (a modified Hoagland nutrient solution) was prepared for 2L 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), and diluted to 2L with Deionized water. Ph was adjusted to 6.8 before getting in contact with the seeds. For better plant growth, seedlings were exposed to sunlight for 1 week in regular water and then 3 weeks in nutrient solution under UV light (290-320 nm) to improve plant photosynthesis (Escobar-Bravo et al., 2017). Air intake was supplied using an air compressor connected to the containers, which also aided in mixing the nutrient solution. After the growth process, containers were contaminated with Arsenic (III) (As_2O_3) and Arsenic (V) (Na_3AsO_4).

The concentrations used were 1ppm, 2 ppm, and 5 ppm. In addition, controls plants were grown in the absence of As. After two weeks of exposure the seedlings were harvested, and the plants were separated into roots, stems, and leaves. The plant samples were subsequently frozen and lyophilized for further steps.

Sample Preparation ICP-OES

Frozen samples were lyophilized using a Labconco freezone 4.5 f freeze dryer system at -55 °C for 72 hours. After drying the samples were ground, and acid digested to extract the elements. Nitric acid digestion was performed for each plant part and concentration, 0.1050 grams of ground dried plants were placed into 50 ml conical vials and 2.5mL of Nitric acid was added to each vile. Samples were heated at 110 C for 1 hour. After cooling, 2mL of 30% hydrogen peroxide (H_2O_2) was added to each vile, and the samples were heated for an additional 45 minutes. Finally, samples were diluted to 50mL with 18MΩ deionized water analysis.

ICP-OES Analysis

To analyze any alteration of macro and micronutrients depending on plant tissue and concentration based on control plant results, calibration standards were made containing As, Na, Mg, S, K, Ca, Mn, Fe, Ni, and P. Samples were analyzed by using ICP-OES. The operating condition of ICP-OES is summarized in Table 1. Wavelengths analyzed for each metal element are summarized in Table 2.

Table 1. ICP-OES Operating conditions for determination of elemental absorption.

Parameter	**Setting**
RF Power	1500 W
Nebulizer	GemCone LowFlow
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 treatments.

Element	Wavelength (nm)
As	188.979
Na	330.237
Mg	285.213
S	182.563
K	404.72
Ca	317.933
Mn	260.568
Fe	238.204
Ni	341.476
P	214.914
Mo	203.845

CHAPTER IV

RESULTS

ICP-OES Data for Arsenic Samples

Arsenic (III)

Data for the arsenic accumulation in roots stems, and leaves of the sunflower plants with 1ppm, 2 ppm, and 5 ppm of As(III) treatments are summarized in Table 3 and shown in Fig 1, 2, and 3 (each value is the mean for ten plants, three repetitions for sample). Arsenic accumulation increases for roots, stems, and leaves as concentrations of As(III) treatment increase. As seen in Figure 1 roots, As(III) levels are higher compared to the stem and leave portions of the plants.

Table 3. Arsenic content in sunflower roots, stems, and leaves.

Arsenic (III) accumulation by Roots, Stems and Leaves, mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	0	0	0	0	0	0
1ppm	2767.03	5.70	148.70	5.12	25.80	10.24
2ppm	2874.40	7.97	58.15	20.99	1.80	0.12
5ppm	4664.44	24.90	333.03	1.40	80.73	5.21

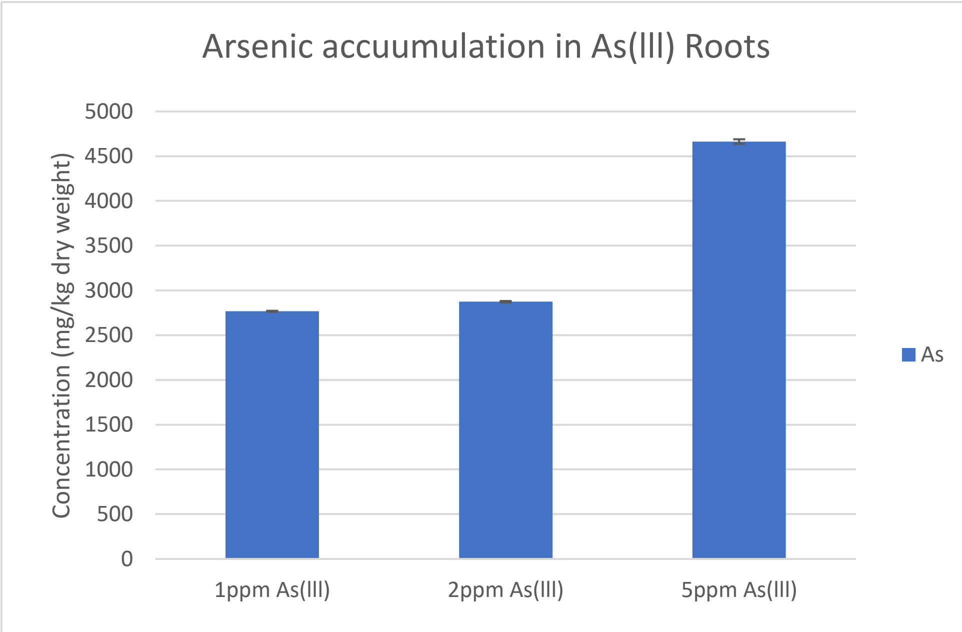

Figure 1. Arsenic accumulation in roots contaminated with As(III) treatments

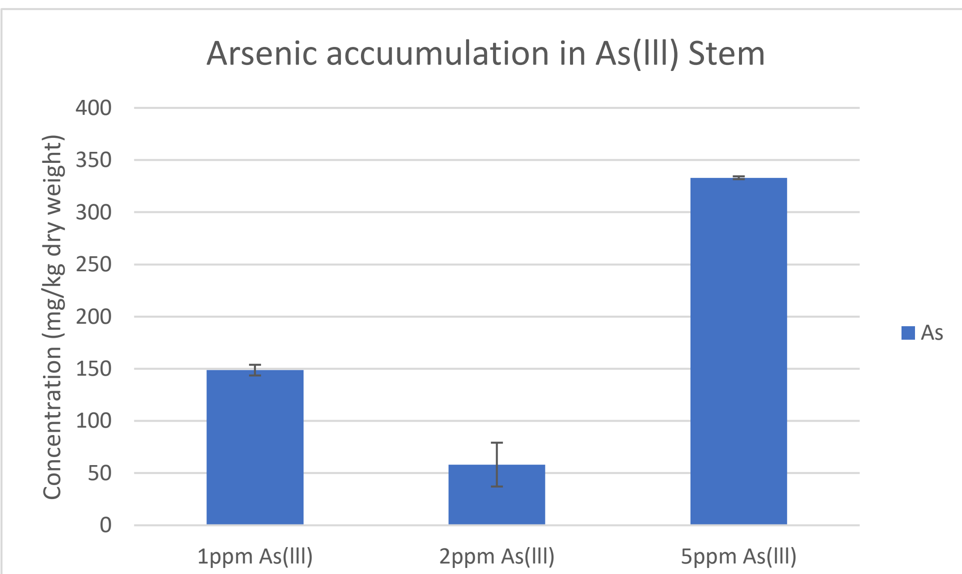

Figure 2. Arsenic accumulation in stems contaminated with As(III) treatments.

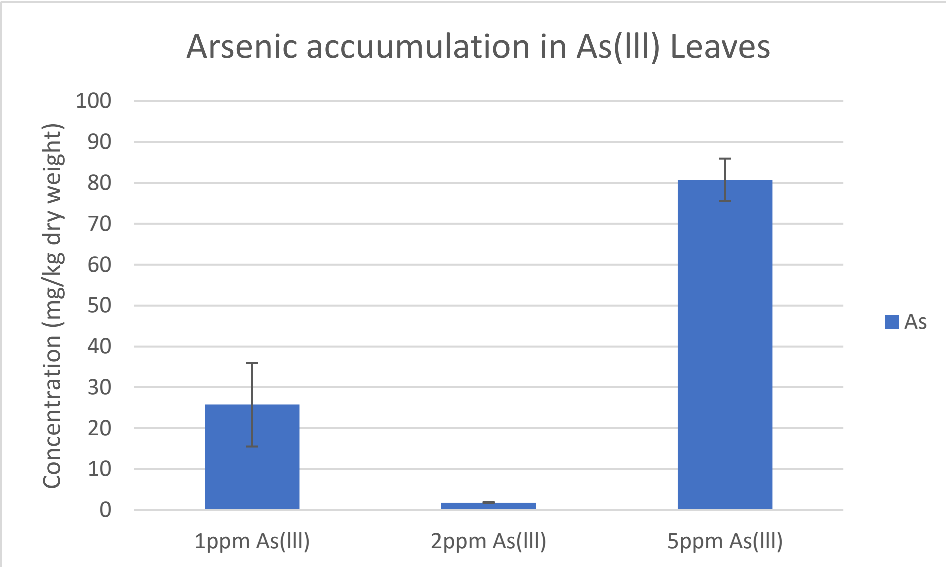

Figure 3. Arsenic accumulation in leaves contaminated with As(III) treatments.

Arsenic (V)

Data for the arsenic accumulation in roots, stems, and leaves of the sunflower plants with 1 ppm, 2 ppm, and 5 ppm of As(V) treatments are summarized in Table 4 and shown in Fig. 4, 5, and 6 (each value is the mean for ten plants, three repetitions for sample). Arsenic accumulation increases for roots, stems, and leaves as concentrations of As(III) treatment increase. As seen in Fig. 4 roots As(V) levels are higher compared to the stem and leave portions of the plants

Table 4. Arsenic content in sunflower roots, stems, and leaves.

Arsenic (V) accumulation by Roots, Stems and Leaves, mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	0	0	0	0	0	0
1ppm	287.12	6.15	51.61	0.85	46.20	1.61
2ppm	1244.71	22.35	269.20	1.90	98.60	1.94
5ppm	3035.70	163.71	1332.70	479.10	359.14	16.72

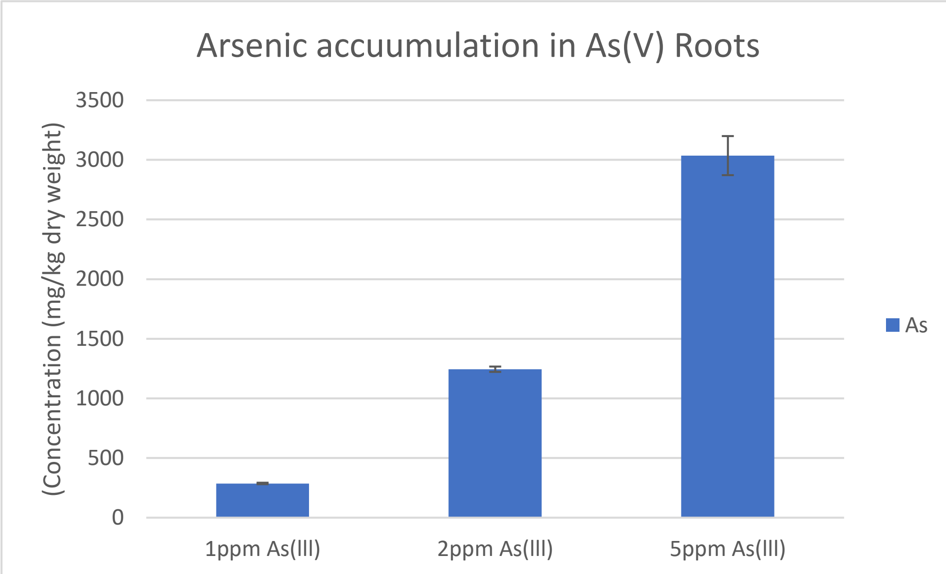

Figure 4. Arsenic accumulation in roots contaminated with As(V) treatments.

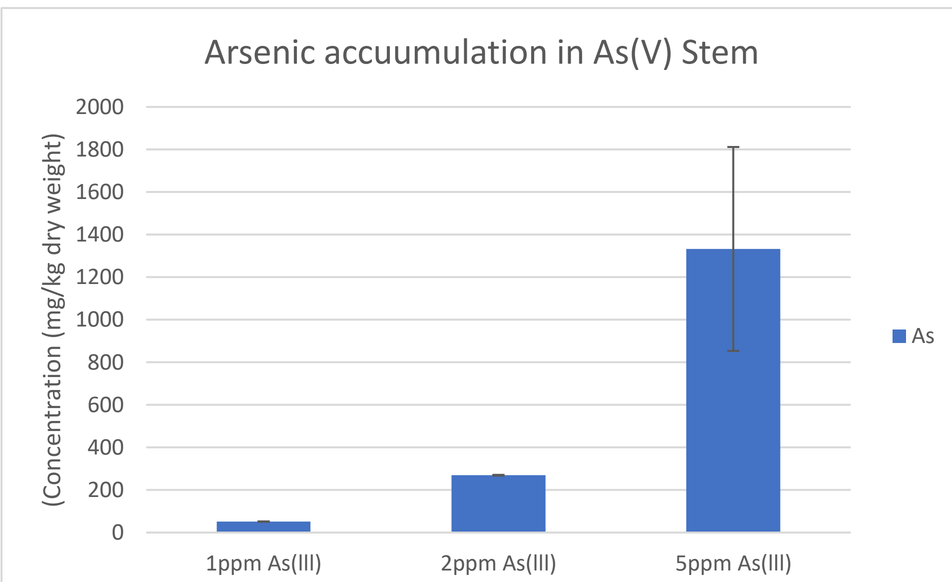

Figure 5. Arsenic accumulation in stem contaminated with As(V) treatments.

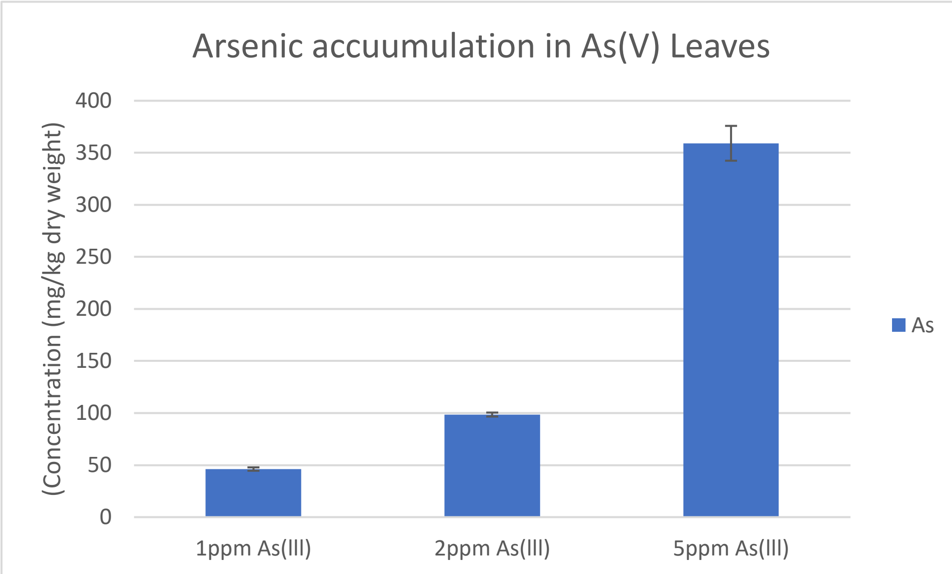

Figure 6. Arsenic accumulation in Leaves contaminated with As(V) treatments.

Macronutrients in the samples after As(III) treatment

Sodium. Data for the macronutrient sodium accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 5 and shown in Fig. 7, 8, and 9 (each value is the mean for eight plants, three repetitions for sample). Sodium showed an increase in 1 ppm roots and 1 ppm stem samples, followed by a decrease as concentration increased compared to control data for roots, stems, and leaves data, except for 5ppm stem showing a lightly increase in sodium concentration.

Table 5. Sodium content in sunflower roots, stems, and leaves treated by As(III).

Sodium accumulation by Roots, Stems and Leaves in As(III), mg/kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	49328.7	10746.7	16525.7	48.6	3349.8	455.2
1ppm	142706.9	1692.6	34058.3	263.4	32287.8	920.9
2ppm	86210.5	1282.7	10654.4	1050.8	140.5	2.1
5ppm	40059.1	2927.7	8001.7	47.9	3270.5	97.6

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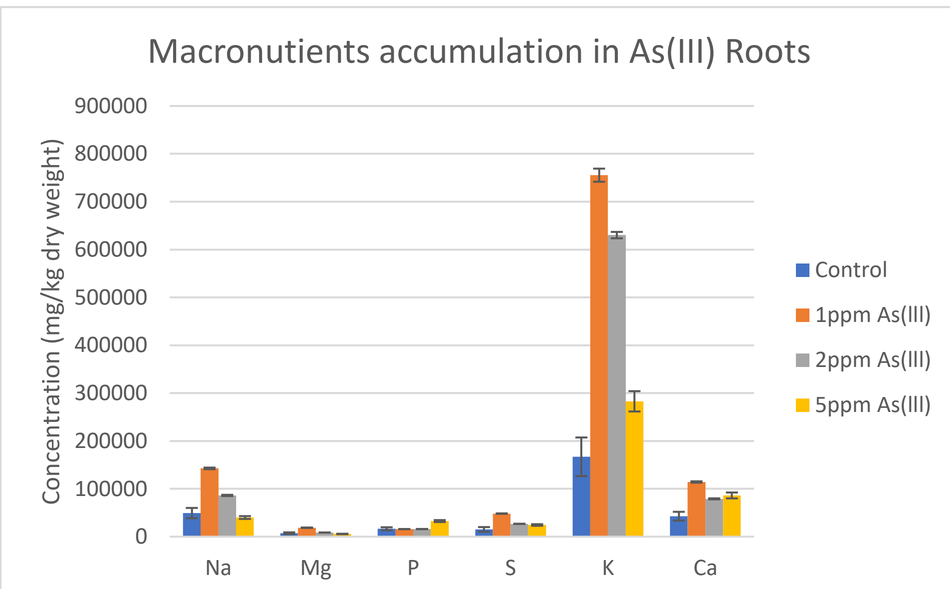

Figure 7. Macronutrient accumulation in Roots contaminated with As(III) treatments.

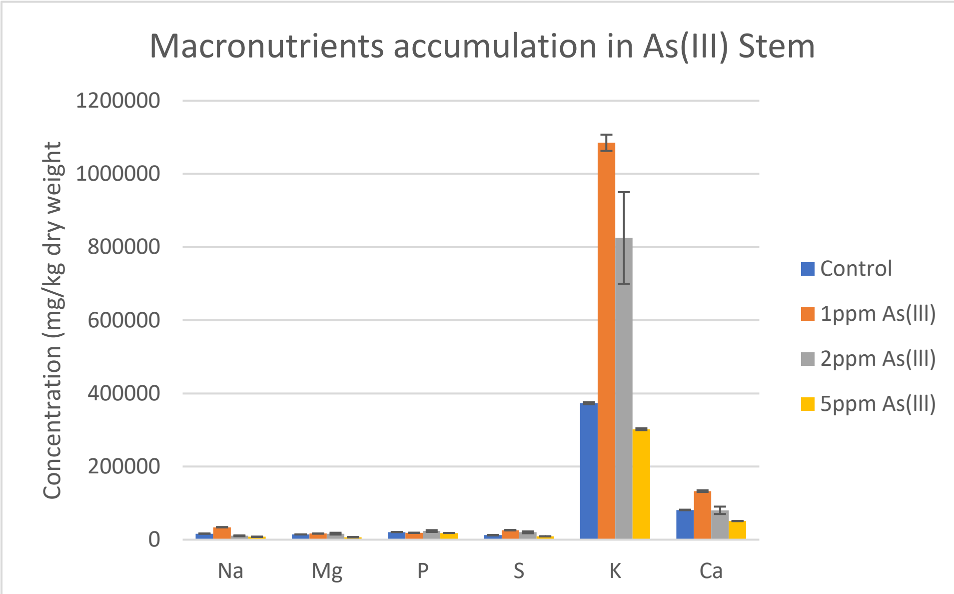

Figure 8. Macronutrient accumulation in Stems contaminated with As(III) treatments.

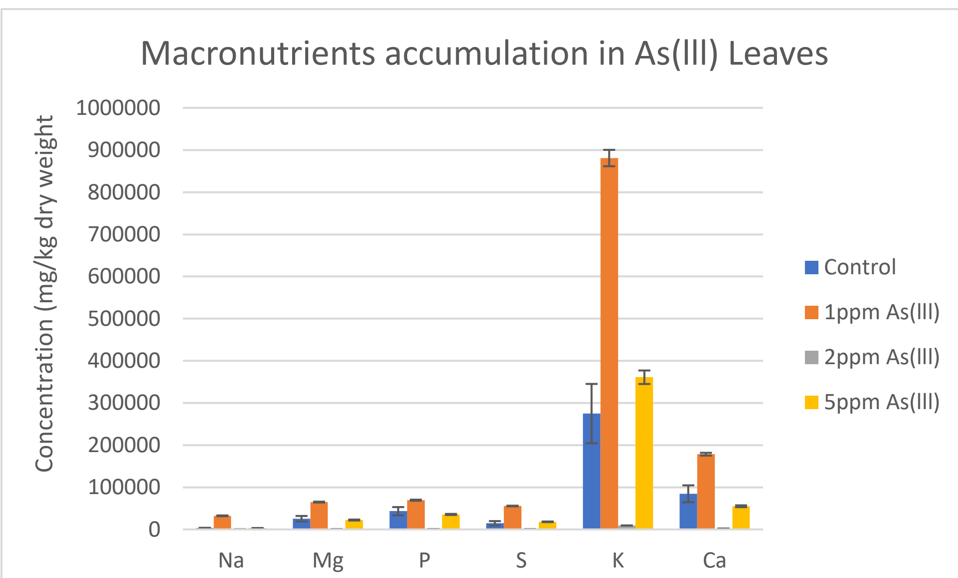

Figure 9. Macronutrient accumulation in Leaves contaminated with As(III) treatments.

Magnesium. Data for the Macronutrient Sodium accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 6 and shown in Figs. 7, 8, and 9 (each value is the mean for eight plants, three repetitions for sample). Magnesium showed an increase in 1 ppm roots and 1 ppm leaves treatment followed by a decrease as concentration increased compared to control data for roots, stems, and leaves data, except for the 1ppm stem sample which showed a slight decrease in magnesium concentration compared to control data.

Table 6. Magnesium content in sunflower roots, stems, and leaves treated by As(III)

Magnesium accumulation by Roots, Stems and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	7219.6	1836.6	14386.2	124.8	25368.9	6534.1
1ppm	18736.9	203.8	16675.5	245.6	64782.3	1064.5
2ppm	8749.5	119.8	16485.8	2494.	615.3	9.5
5ppm	5705.9	501.9	6857.6	39.2	22248.7	1145.2

Phosphorus. Data for the Macronutrient Phosphorus accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 7 and shown in Figs. 7, 8, and 9 (each value is the mean for ten plants, three repetitions for sample). Phosphorus showed an increase in 1 ppm leaves and a slight decrease for 5ppm leaves treatment followed by a decrease as the arsenic concentration increased, 5 ppm roots and 5ppm stem showed an increase in concentration not reaching the control levels, all this data compared to control data for roots, stems, and leaves data.

Table 7. Phosphorus content in sunflower roots, stems, and leaves treated by As(III).

Phosphorus accumulation by Roots, Stems, and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	16578.1	3130.4	20902.9	114.8	43226.1	9735.1
1ppm	15860.2	138.2	18848.7	290.9	69273.9	1298.9
2ppm	15977.8	215.4	23646.8	2474.9	897.4	12.3
5ppm	32624.7	2048.0	18307.1	70.6	35469.6	1281.8

Sulfur. Data for the Macronutrient Sulfur accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 8 and shown in Figs. 7, 8, and 9 (each value is the mean for ten plants, three repetitions for sample). Sulfur shows an increase in 1ppm roots and leaves and decreases as concentration increases for steam data, sulfur concentration decreases as the concentration of As(III) increases, all this data compared to control data for roots, stems, and leaves data.

Table 8. Sulfur content in sunflower roots, stems, and leaves treated by As(III).

Sulfur accumulation by Roots, Stems, and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	15174.7	4931.5	12303.9	142.7	14522.0	5220.8
1ppm	48246.1	468.4	25971.3	320.8	55492.6	791.9
2ppm	26851.5	220.0	20148.6	2573.1	551.7	6.6
5ppm	24437.4	1697.2	9195.3	43.0	18031.2	727.3

Potassium. Data for the Macronutrient Potassium accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 9 and shown in Figs. 7, 8, and 9 (each value is the mean for ten plants, three repetitions for sample). Potassium showed an increase in 1ppm roots, stems, and leaves. Potassium concentration decreases as the concentration of As(III) increases, all this data is compared to control data for roots, stems, and leaves data.

Table 9. Potassium content in sunflower roots, stems, and leaves treated by As(III).

Potassium accumulation by Roots, Stems, and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	166963.8	40429.1	373121.5	2838.6	274700.6	70390.0
1ppm	755406.8	13710.4	1085086.0	22265.9	880997.3	19425.7
2ppm	630244.8	6668.7	824786.1	125313.7	9324.4	156.4
5ppm	282870.5	21222.6	301977.3	2527.8	360992.6	16024.2

Calcium. Data for the Macronutrient Calcium accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 10 and shown in Fig. 7, 8, and 9 (each value is the mean for ten plants, three repetitions for sample). Calcium shows a tendency; to increase 1 ppm > 2 ppm decrease > 5ppm increase for roots, stems, and leaves as the concentration of As(III) increases, all this data compared to control data for roots, stems, and leaves data.

Table 10. Calcium content in sunflower roots, stems, and leaves treated by As(III).

Calcium accumulation by Roots, Stems, and Leaves in As(III) , mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	42823.7	9171.6	81310.9	705.94	84491.1	20033.4
1ppm	114318.3	1414.7	132826.2	2339.0	178356	3263.9
2ppm	79102.8	1043.9	80253.7	10152.1	1853.9	42.6
5ppm	86210.9	6137.7	50986.6	164.6	54935.8	2191.6

Micronutrients in the samples after As(III) treatment

Manganese. Data for the Micronutrient Manganese accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 11 and shown in Figs. 10, 11, and 12 (each value is the mean for ten plants, three repetitions for sample). Manganese shows a tendency; decrease to 1 ppm> 2 ppm decrease > increase 5 ppm for roots as concentration increases, increases, increase 1 ppm> 2 ppm decrease > increase 5 ppm for stem following by a decrease as concentration increase for leaves except for an increase in 5 ppm all this data compared to control roots, stems, and leaves.

Table 11. Manganese content in sunflower roots, stems, and leaves treated by As(III).

Manganese accumulation by Roots, Stems, and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	162.7	54.4	298.8	29.9	260.9	45.9
1ppm	115.1	2.9	1355.5	27.6	346.6	8.6
2ppm	91.9	2.1	510.3	60.9	4.4	0.13
5ppm	679.6	67.3	334.8	0.87	1086.8	48.4

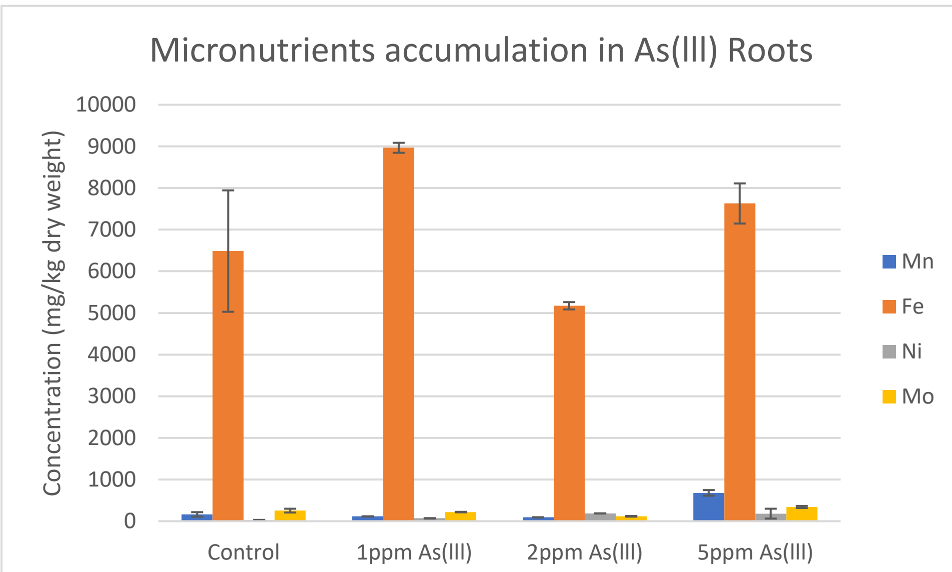

Figure 10. Micronutrients accumulation in Roots contaminated with As(III) treatments.

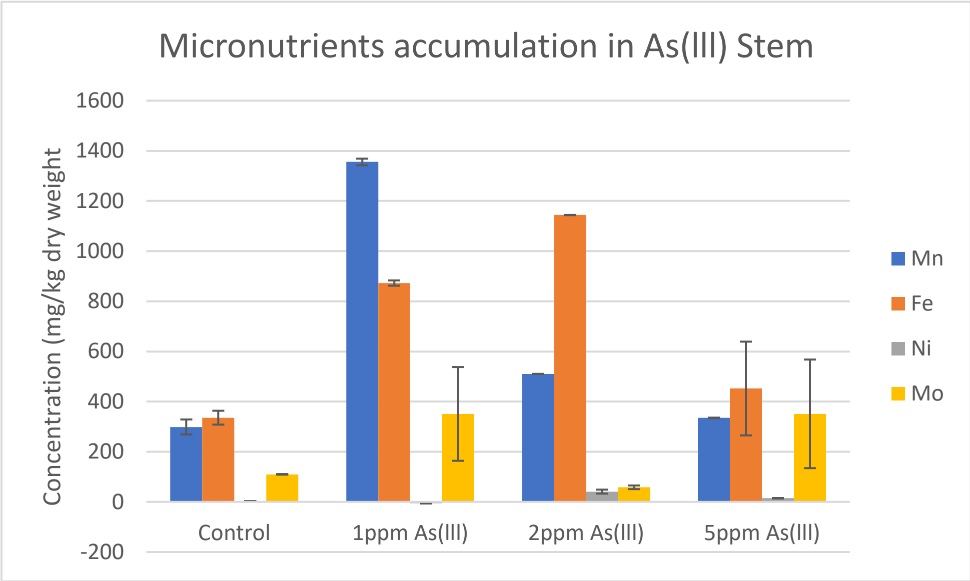

Figure 11. Micronutrients accumulation in Stem contaminated with As(III) treatments.

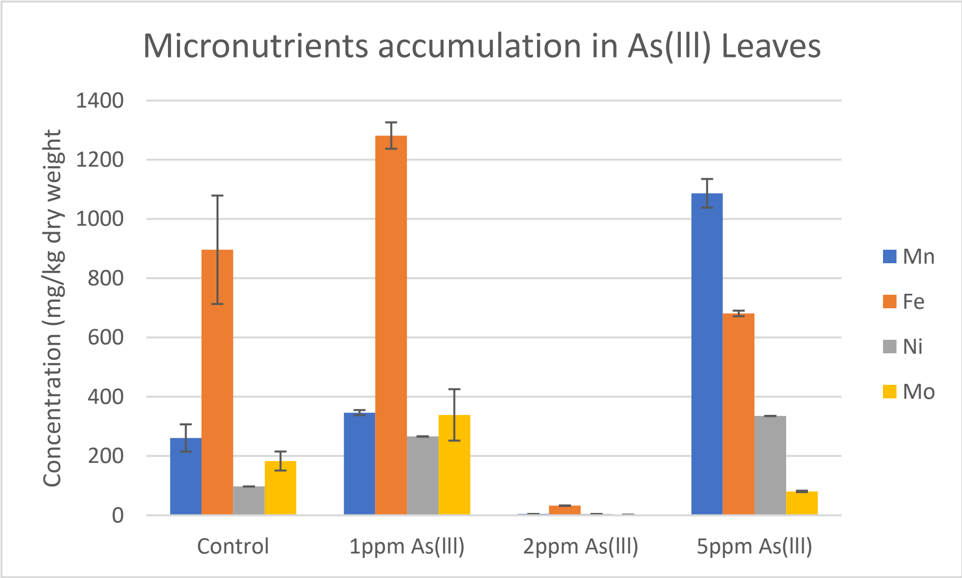

Figure 12. Micronutrients accumulation in leaves contaminated with As(III) treatments.

Iron. Data for the Micronutrient Iron accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 12 and shown in Figs. 10, 11, and 12 (each value is the mean for ten plants, three repetitions for sample). Iron showed a decrease in concentration for roots, an increase in the stem, an increase for leaves 1 ppm and 2 ppm but a decrease for 5 ppm. All data is compared to control roots, stem, and leaves treatments.

Table 12. Iron content in sunflower roots, stems, and leaves treated by As(III).

Iron accumulation by Roots, Stems, and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	6486.5	1458.3	335.7	13.2	896.3	182.9
1ppm	8966.1	120.0	872.6	10.6	1281.7	44.6
2ppm	5173.2	87.6	1143.9	56.5	32.5	0.74
5ppm	7629.6	482.1	452.0	8.9	681.3	9.4

Nickel. Data for the Micronutrient Nickel accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 13 and shown in Figs. 10, 11, and 12 (each value is the mean for ten plants, three repetitions for sample). All data is compared to control roots, stem, and leaves treatments. Nickel shows an increase in concentration for roots and leaves as the concentration of As(III) increases. For stem shown to decrease for 1 ppm and 5 ppm but increase for 2ppm.

Table 13. Nickel content in sunflower roots, stems, and leaves treated by As(III).

Nickel accumulation by Roots, Stems, and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	21.6	9.4	3.0	0.30	97.2	21.3
1ppm	69.1	4.6	5.6	0.4	266.1	124.4
2ppm	187.1	1.5	40.1	7.9	4.2	0.05
5ppm	181.1	118.3	14.5	0.12	335.1	210.9

Molybdenum. Data for the Micronutrient Molybdenum accumulation in roots, stem, and leaves for As(III) of the sunflower plants are summarized in Table 14 and shown in Figs. 10, 11, and 12 (each value is the mean for ten plants, three repetitions for sample). All data is compared to control roots, stem, and leaves treatments. Molybdenum showed increased concentration for 5 ppm roots, stem, and 1 ppm stem and leaves. For roots 1 ppm and 2 ppm molybdenum, a decrease is shown; 2 ppm stem, 2 ppm, and 5 ppm leaves show a decrease in molybdenum.

Table 14. Molybdenum content in sunflower roots, stems, and leaves treated by As(III).

Molybdenum accumulation by Roots, Stems, and Leaves in As(III), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	253.9	45.2	109.7	1.2	183.2	32.02
1ppm	218.7	5.7	350.8	1.87	338.7	86.8
2ppm	116.4	7.9	58.1	7.2	1.8	0.2
5ppm	341.1	24.9	351.1	21.6	80.7	2.4

Macronutrients in the samples after As(V) treatment

Sodium. Data for the Macronutrient Sodium accumulation in roots, stem, and leaves for As(V) of the sunflower plants are summarized in Table 15 and shown in Fig. 13, 14, and 15 (each value is the mean for ten plants, three repetitions for sample). Sodium showed an increase in 1 ppm, 2 ppm, and 5 ppm roots, for stem decrease in 1 ppm and 2 ppm followed by a decrease in 5 ppm, for leaves 1 ppm, 5 ppm show an increase and 2ppm a decrease in sodium concentration treatment, all data compared to control plants.

Table 15. Sodium content in sunflower roots, stems, and leaves treated by As(V).

Sodium accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	49328.7	10746.0	16525.7	48.5	3349.8	455.2
1ppm	57343.1	1138.7	9987.4	133.6	2841.7	79.8
2ppm	81706.7	1474.4	16106.9	224.3	1628.8	7.3
5ppm	64023.4	2806.8	32571.1	10038.9	4478.7	125.9

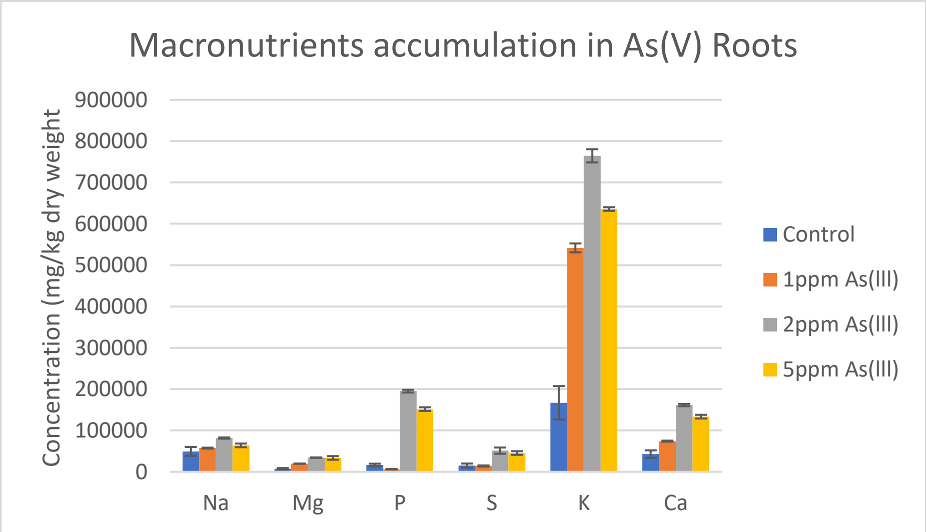

Figure 13. Macronutrients accumulation in Roots contaminated with As(V) treatments.

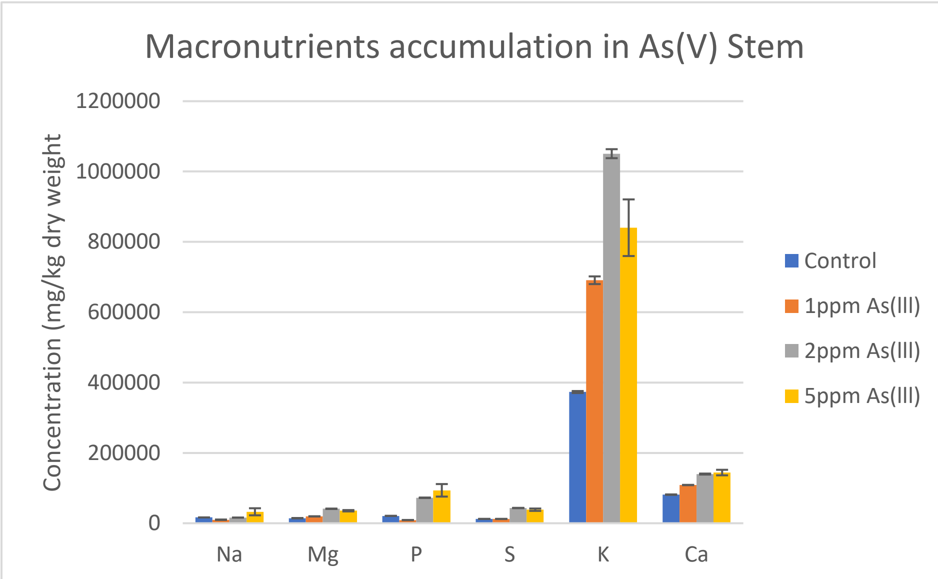

Figure 14. Macronutrients accumulation in Stems contaminated with As(V) treatments.

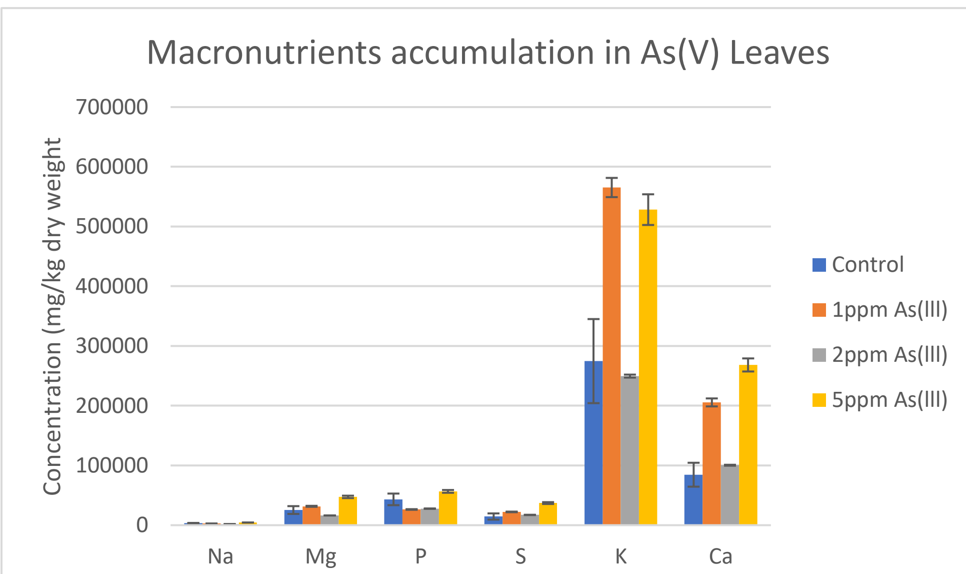

Figure 15. Macronutrients accumulation in Leaves contaminated with As(V) treatments.

Magnesium. Data for the Macronutrient Magnesium accumulation in roots, stem, and leaves for As(V) of the sunflower plants are summarized in Table 16 and shown in Figs. 13, 14, and 15 (each value is the mean for ten plants, three repetitions for sample). Magnesium showed an increase in roots, stems, and leaves as concentrations of As(V) increased, all data compared to control plants.

Table 16. Magnesium content in sunflower roots, stems, and leaves treated by As(V).

Magnesium accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	7219.5	1836.6	14386.2	124.8	25368.9	6534.1
1ppm	19758.3	325.8	19555.3	125.9	31620.9	823.7
2ppm	34312.7	615.6	41097.07	454.8	16289.4	125.2
5ppm	33622.4	1215.5	35555.1	1959.8	47390.3	2025.5

Phosphorus. Data for the Macronutrient Phosphorus accumulation in roots, stem, and leaves for As(V) of the sunflower plants are summarized in Table 17 and shown in Fig. 13, 14, and 15 (each value is the mean for ten plants, three repetitions for sample). Phosphorus showed an increase in roots, stems, and leaves as concentrations of As(V) increases, except for 1 ppm roots, stems, and leaves that showed a slight decrease in comparison with control treatments.

Table 17. Phosphorus content in sunflower roots, stems, and leaves treated by As(V).

Phosphorus accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	16578.1	3130.3	20902.9	114.8	43226.1	9735.1
1ppm	6201.9	88.7	9036.7	324.1	26323.1	525.7
2ppm	195153.8	3252.7	72448.9	665.6	27690.5	312.4
5ppm	151681.6	6722.6	93587.3	17852.5	56583.5	2347.9

Sulfur. Data for the Macronutrient Sulfur accumulation in roots, stem, and leaves for As(V) of the sunflower plants is summarized in Table 18 and shown in Fig. 13, 14, and 15 (each value is the mean for ten plants, three repetitions for sample). Sulfur showed an increase in roots, stems, and leaves as concentrations of As(V) increased compared to control treatments.

Table 18. Sulfur content in sunflower roots, stems, and leaves treated by As(V).

Sulfur accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	15174.7	3130.3	12303.9	142.7	14522.04	5220.8
1ppm	14107.6	1432.7	12203.9	305.9	22210.1	508.9
2ppm	51286.5	7545.1	43342.4	444.1	17213.6	263.7
5ppm	45459.9	2046.02	38518.3	3378.1	37029.9	1563.8

Potassium. Data for the Macronutrient Potassium accumulation in roots, stem, and leaves for As(V) of the sunflower plants are summarized in Table 19 and shown in Figs. 13, 14, and 15 (each value is the mean for ten plants, three repetitions for sample). Potassium shows a higher increase in roots, stem, and leaves as concentrations of As(V) increase compared to control treatments.

Table 19. Potassium content in sunflower roots, stems, and leaves treated by As(V).

Potassium accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	166963.8	40429.09	373121.5	2838.6	274700.6	70390.05
1ppm	541810.6	10764.4	690957.6	10970.2	565307.3	16077.5
2ppm	764489.1	15970.7	1050826.3	12760.3	249608.6	2506.6
5ppm	635739.7	29580.03	840309.3	80527.3	528292.5	25693.22

Calcium. Data for the Macronutrient Calcium accumulation in roots, stem, and leaves for As(V) of the sunflower plants is summarized in Table 20 and shown in Fig. 13, 14, and 15 (each value is the mean for ten plants, three repetitions for sample). Calcium showed a higher increase in roots, stem, and leaves as concentrations of As(V) increased compared to control treatments.

Table 20. Calcium content in sunflower roots, stems, and leaves treated by As(V).

Calcium accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	42823.7	9171.6	81310.9	705.9	84491.1	20033.4
1ppm	74218.06	1304.7	108678.7	486.2	205556.5	6680.9
2ppm	161524.7	2750.1	139833.8	1456.5	100369.8	785.9
5ppm	133436.7	4436.5	144118.2	7828.8	268267.4	10926.9

Micronutrients in the samples after As(V) treatment

Manganese. Data for the Micronutrient Manganese accumulation in roots, stem, and leaves for As(V) in the sunflower plants are summarized in Table 21and shown in Fig. 16, 17, and 18 (each value is the mean for ten plants, three repetitions for sample). Manganese showed an increase in roots, stem, and leaves, as the concentration of As(V) increased, except for 2 ppm and 5 ppm stem where concentration decreased compared with control treatments.

Table 21. Manganese content in sunflower roots, stems, and leaves treated by As(V).

Manganese accumulation by Roots, Stems and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	162.7	54.4	298.8	29.9	260.9	45.9
1ppm	270.9	5.5	318.2	4.1	1197.1	44.5
2ppm	354.9	6.6	381.03	12.5	636.8	4.9
5ppm	253.7	5.9	265.8	19.07	1020.1	39.9

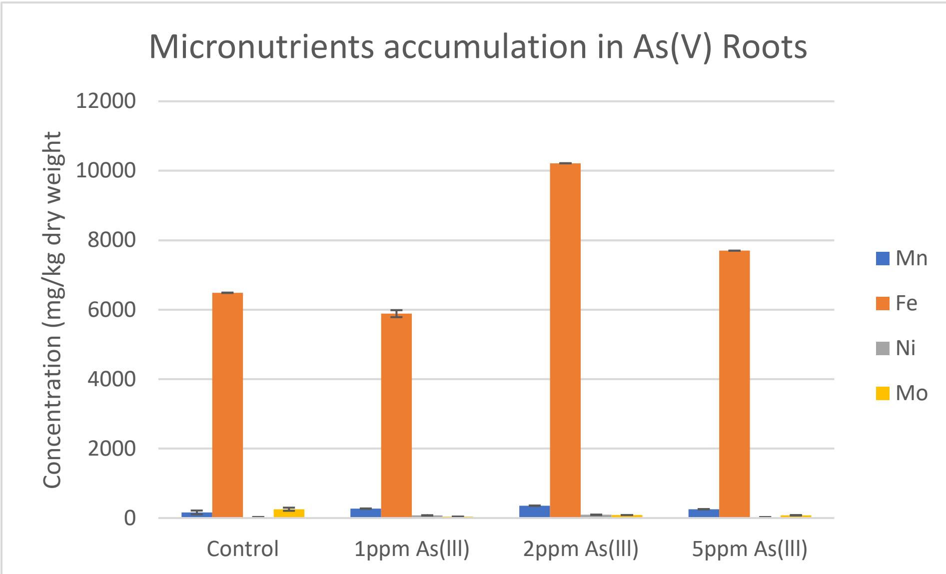

Figure 16. Micronutrients accumulation in Roots contaminated with As(V) treatments.

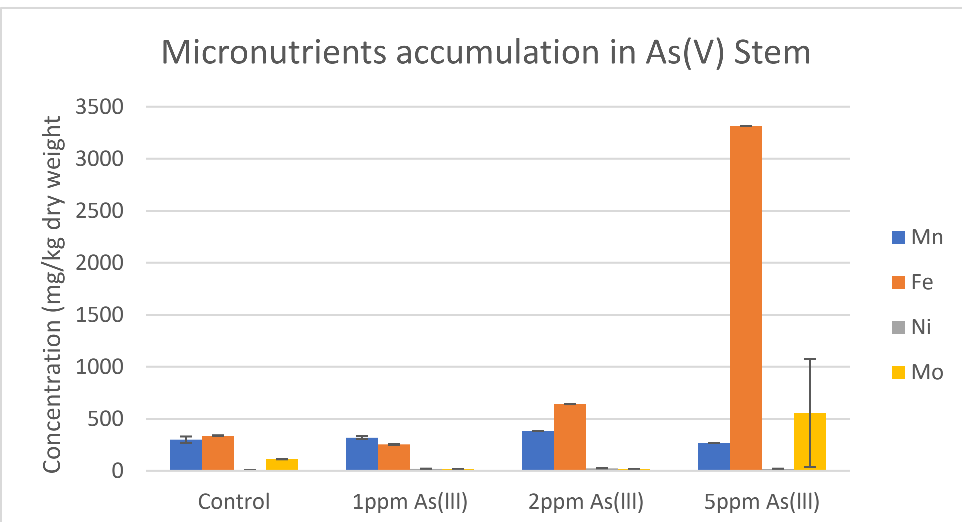

Figure 17. Micronutrients accumulation in Stem contaminated with As(V) treatments.

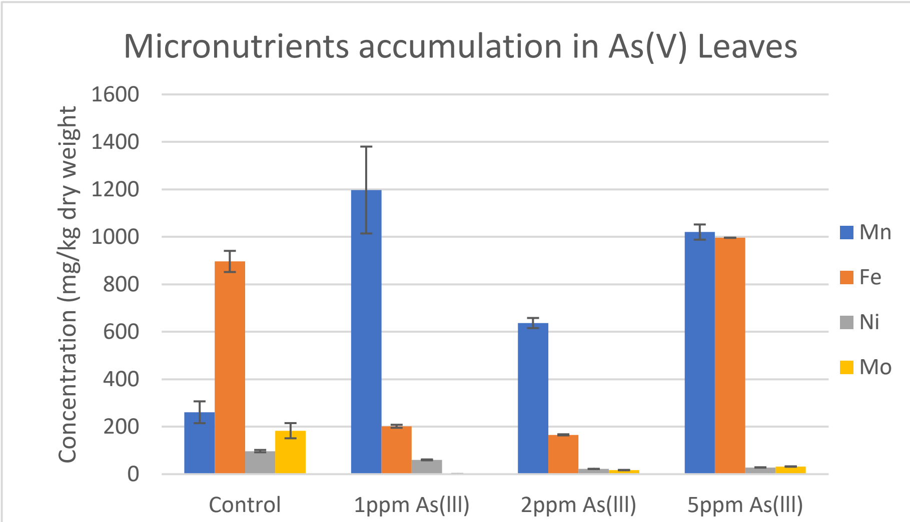

Figure 18. Micronutrients accumulation in leaves contaminated with As(V) treatments.

Iron. Data for the Micronutrient Iron accumulation in roots, stem, and leaves for As(V) of the sunflower plants are summarized in Table 22 and shown in Figs. 16, 17, and 18 (each value is the mean for ten plants, three repetitions for sample). Iron shows an increase in roots and stem as the concentration of As(V) increases, except for 2 ppm stem and 1 ppm, 2 ppm leaves, where concentration decreases compared with control treatments. Iron recorded the highest uptake at 2 ppm rots; 10216 (mg/kg) compared to control.

Table 22. Iron content in sunflower roots, stems, and leaves treated by As(V).

Iron accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	6486.5	1458.3	335.7	13.1	896.3	182.9
1ppm	5885.4	100.4	251.7	4.00	201.9	6.4
2ppm	10216.4	158.7	638.9	9.9	165.8	1.9
5ppm	7699.3	356.9	3313.5	1350.8	996.1	23.9

Nickel. Data for the Micronutrient Nickel accumulation in roots, stem, and leaves for As(V) of the sunflower plants are summarized in Table 23 and shown in Figs. 16, 17, and 18 (each value is the mean for ten plants, three repetitions for sample). Nickel showed an increase in roots, stems, and leaves as the As(V) concentration increased, except for 2 ppm and 5 ppm leaves, where concentration decreased compared with control treatments.

Table 23. Nickel content in sunflower roots, stems, and leaves treated by As(V).

Nickel accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	21.6	9.4	3.01	0.30	97.2	21.3
1ppm	77.00	1.5	19.3	0.5	60.3	2.3
2ppm	93.2	1.9	23.04	0.95	22.2	0.31
5ppm	22.2	1.7	16.6	3.3	28.4	1.7

Molybdenum. Data for the Micronutrient Molybdenum accumulation in roots, stem, and leaves for As(V) of the sunflower plants are summarized in Table 24 and shown in Figs. 16, 17, and 18 (each value is the mean for ten plants, three repetitions for sample). Molybdenum showed a decrease in roots, stem, and leaves as the concentration of As(V) increases, except for 5 ppm stem, where concentration increases compared with control treatments.

Table 24. Molybdenum content in sunflower roots, stems, and leaves treated by As(V).

Molybdenum accumulation by Roots, Stems, and Leaves in As(V), mg/Kg dry weight.						
Treatment	Roots	Error	Stem	Error	Leaves	Error
Control	253.9	45.2	109.7	1.2	183.2	32.02
1ppm	42.9	0.90	14.8	0.34	0.46	0.46
2ppm	86.4	1.13	15.2	1.3	17.3	0.81
5ppm	79.7	2.2	554.3	520.00	32.22	0.86

CHAPTER V

DISCUSSION

Arsenic

According to the results, As(III) and As(V) accumulation are higher in root seedlings. Both concentrations showed higher accumulation in the roots of Sunflowers *Helianthus annus*. Stem and leaves for both concentrations showed a tendency for higher accumulation of arsenic as concentration increases 1ppm> 2ppm> 5ppm. However, As(III) stem and As(III) leaves show a decrease as concentration increases. In addition, the majority of As(III) samples, demonstrated lower levels of arsenic. On the other hand, the majority of As(V) samples demonstrated only accumulation increments.

Higher chemical accumulation in roots has been shown before in other investigations compared to lower concentrations present in stems and leaves (Parsons, et al., 2007). Reviewing the plant systems studied and the results obtained, studies have shown that roots are the principal As(III) and As(V) absorption part also As(III) contains the higher percentages of arsenic absorbed, demonstrating concentrations between 2000-4700 as ppm concentrations increase.

Sodium

Higher sodium concentrations are beneficial for plants, not affecting their metabolism, even more, when potassium is in low concentration due to their similarity in structure (Maathuis et al .,2013) For the sunflower plants contaminated with As(III), roots were demonstrated to be

the higher sodium accumulators. Stems showed a higher increase of sodium for 1 ppm and 5ppm. However, for 2 ppm treatments, the concentration appears to be lower than the control concentration. For As(V) contaminated plant's accumulation tendency appears similar. Roots uptake higher Sodium accumulation than stem and leaves that recorded higher concentration than controls but not higher than the As(III) roots samples.

Magnesium

The magnesium concentration in plants is significant for their development because this element is an indispensable component for an essential plant metabolite. Studies have demonstrated that magnesium concentrations are less than potassium but similar to sulfur and phosphorus (Wilkinson et al., 1990). As(V), showed higher concentrations as arsenic concentration increased; similar to arsenic (III), roots possess the higher concentrations for magnesium in both arsenic concentrations showing some similarities in magnesium concentration with sulfur and potassium as in previous studies.

Phosphorus

Phosphorus is a vital element for plants; this chemical is present in the soil in lower amounts but in forms that are only available out of the rhizosphere. However, plants require a higher concentration of phosphorus to survive. As mentioned, phosphorus enters plants by transporters that transfer phosphorus across membranes (Schachtman et al.,). Variation in phosphorus was obtained for roots, stems, and leaves in all concentrations. These variations may be due to a failure in the phosphate mechanism related to the abiotic stress. Concentrations above the control concentration were reflected in 5ppm As(V) roots and leaves.

Sulfur

Sulfate transporters transport sulfur in the roots and then into the whole plant. When the plant has an excess of sulfur is either directed to sulfolipids or reduced and converted to sulfide and transferred to cystine, depositing the remainder excess in the vacuole (Hoefgen et al., 2007). For sulfur roots, stem, and leaves contaminated with As(lll), sulfur accumulation increases as arsenic concentration increases. For As(V) tendency of increase was also recorded, roots seem to be the higher accumulator of sulfur. However, sulfur 2 ppm As(III) leaves showed a decrease in concentration. A biological factor can cause this in their response to achieve sulfur by aqueous solution.

Potassium

Potassium is a cation related to sodium; when potassium is present in lower milligram concentrations in plants, sodium is present in higher concentrations levels. When this happens, plants could be affected. Potassium is indispensable for neutralizing sodium stress, while Sodium release potassium (Mäser et al., 2002). According to results obtained for roots, stem, and leaves, potassium seems to be higher than sodium, resulting in favor for plant metabolism because sodium remains lower than potassium demonstrating concentration levels at highest around 8000-milligram concentration for the stem. In contrast, potassium reflects at its highest around 300,000 milligrams concentrations in the stems.

Calcium

Roots hairs are the channels where calcium enters directly into roots cells when in contact with soil. These hair roots are specialized for absorbing nutrients by forming a surface area with absorption per unit of root length (Yang and Jie, 2005). Referring to data obtained in the results,

we can notice that roots channels from both concentrations did a prominent absorption process, demonstrating results in around 80,000 milligrams concentrations. Nonetheless leaves for As(V) showed higher levels of calcium, recording amounts of around 200,000 milligrams. All results obtained demonstrated an affective absorption by roots cell looking at the concentration increases compared with control concentrations.

Manganese.

Sunflower *Helianthus annus* treatments showed that manganese is different from most macronutrients, showing the highest concentration level in leaves compared to their control. This micronutrient shown 1197.185(mg/kg) in 1 ppm leaves As(V), an increase from control data shown; 260.96 (mg/kg). Like the other micro/macronutrients, manganese first enters the cell by roots cells. Once in the inner side of the plasma membrane, manganese is transported into the xylem, moves forward to the phloem, and finally be translocated into the plant tissues (Alejandro et al., 2020). When plants possess high toxicity of manganese, black spots may appear. However, results suggest that sunflower seedlings uptake a low affinity of manganese from both arsenic concentrations, not affecting their metabolite.

Iron

Data demonstrated that Iron presents the highest concentration uptake at 2ppm roots and a partial increase in all concentrations from As(III) stems. The As(V) leaves, were iron concentration shown to decrease when the experiment suggested an increase in all concentrations; however, leaves present an increase compared to control. However, these

differentiations in results could represent a proper stabilization in plant homeostasis, as ferrum is a free iron that is insoluble and toxic. It seems that sunflower seedlings neutralized Iron pH by a chelation reduction system at the roots and get a proper absorption result (Hell and Stephan, 2003).

Nickel

Previous studies on soybeans seedlings demonstrated that the absorption of nickel in soybean plants suggested that nickel absorbed depends on the amount of nickel in the soil also that the transfer of nickel throughout the plant is dependent on roots cells transporters and regulated by parenchyma cells (Cataldo et al., 1978). Nickel showed an increase in the majority of their concentrations, the only concentrations presenting decreases were As(III) 2 ppm, for As(V) 1 ppm, 2ppm, and 5ppm leaves. Suggesting that nickel in the nutrient solution was properly absorbed in these samples and different than studies as nickel was absorbed by water.

Molybdenum.

The Argentinean region has been suffering from an excess of molybdenum in its groundwaters. A previous study demonstrated that cress (Lepidium sativum L.) is an effective plant to uptake this chemical. Researchers conducted a phytoremediation system between cress growth in this region and hydroponically. Both samples demonstrated noticeable results ranging from <0.1 (LOD) to 7000 μg/L Mo at pH 7 (Lawson-Wood et al., 2021). Results obtained in sunflower seedlings showed a decrease in most concentrations compared to control treatments, except from 5 ppm roots and leaves from arsenic(lll) and Arsenic (V) stem. Similar to the Argentinean findings, sunflower seedlings showed lower concentrations than control treatments. These results might be due to a multivariate statistical evaluation of Molybdenum ions.

CHAPTER VI

CONCLUSIONS

Sunflower *Helianthus annus* seedlings uptake As(III) and As(V) from aqueous nutrient solutions and then translocate the arsenic to stems and leaves. It was found that the highest amount of both As(III) and As(V) that is accumulated by sunflowers seedlings are in the roots. Nonetheless, sunflowers' growth in As(III) recorded the highest accumulation amount. Lower amounts of arsenic are translocated in stems and leaves. These findings are referred to the variation of nutrients absorbed and the oxidation state of arsenic in an aqueous solution.

In conclusion, sunflower *Helianthus annus* accumulate a higher amount of As(III) than As(V). Both arsenic concentrations damage plant roots after the contamination process. This may be a factor in the chemical absorption and translocation through the plant. It was also concluded that sunflowers accumulate more arsenic as the concentration increases. This trend was observed in the roots of plants treated with As(III), the only exception for this trend was observed in 2 ppm stem and 2 ppm leaves of As(III). Since roots membranes got damaged due to high toxicity, transporters did not transfer an accurate amount to steam and leaves. For As(V) the highest accumulation amount was in roots, concluding that sunflowers accumulate more As(V) in their roots as the concentration in the aqueous solution increases. No exceptions were observed in these seedlings.

Sunflower seedlings were found to uptake less sodium in As(III) 5, ppm roots, 2ppm, 5ppm stem, 1 ppm, 2 ppm leaves, and 1 ppm stem and 5 ppm leaves for As(V), compared to control treatments. Higher values were recorded in roots 1ppm As(III). These variations shown in macronutrient sodium are linked to the oxidation state of arsenic.

Drastically high magnesium concentrations were found in roots for both As(III) and As(V). The same trend was obtained in the translocation of magnesium to steams, and leaves exposed to both arsenics. Exceptions were observed in magnesium decreases in 5 ppm roots, stem, and leaves and 2 ppm for sunflowers contaminated with As(III). As(V) decreases were shown only in 2 ppm leaves, which can be caused by root damage due to toxicity.

Phosphorus accumulation by sunflower roots is lower than control values when exposed to As(III) and higher in some concentrations such as 1ppm roots, 2ppm stem and 5ppm leaves. On the other hand, As(V) showed a decrease in all concentrations with the exception in 2 ppm roots, and leaves and 5 ppm stems and leaves.

Sulfur accumulation in roots is higher when sunflower is exposed to As(III) treatments. Only lower than control treatments in 2ppm leaves and 5ppm stem and leaves. Similar higher absorption trends are observed in As(V) for all concentrations except for 1ppm roots and stem, demonstrating a decrease in concentrations.

As(V) shows the same tendency of higher potassium absorption as arsenic concentration increases. Potassium uptakes show an increase in all concentrations for As(III) treatments, obtaining high accumulation amounts such as 755406.8 (mg/kg) for 1 ppm roots. Stem and leaves also demonstrated higher levels than control treatments except for 2ppm leaves, where analysis recorded a decrease in concentration. In these samples, the highest amount recorded was

on a 2ppm stem with 1050826.3 (mg/kg). Only one exception was observed in 2ppm leaves where a decrease was shown.

In conclusion, calcium demonstrates an increase as arsenic concentration increases for both As(III) and As(V). The translocation of calcium in each arsenic was demonstrated to have accurate results. Sunflower seedlings showed a higher calcium absorption in roots, and the tendency recorded the same for stems and leaves. Suggesting that sunflowers are effective calcium accumulators when higher arsenic concentrations get into a metabolite.

Manganese accumulation increases in roots, stem, and leaves as As(V) concentration increases. Only one exception was shown in the 5ppm stem, a slight decrease compared to control was observed, with the control record 298.78 (mg/kg) and 5 ppm stem 265.78(mg/kg). In As(lll) sunflower samples, decreases in concentration were present in 1ppm, 2ppm roots, and 2 ppm leaves.

Iron accumulation levels were highly present in 2ppm roots As(V) 10,216(mg/kg). However, decreases in concentration were present in 1ppm roots and 2ppm leaves. For As(III), in most sunflower treatments iron tended to increase as arsenic concentration increases. Some exceptions were shown in 2ppm As(lll) roots and leaves and 5ppm leaves.

Sunflower treatments showed Nickel accumulation for all concentrations. As arsenic concentration increases, nickel absorption increases. Few exceptions were noted in As(V) treatments only for 1ppm leaves, 2 ppm leaves, and 5 ppm leaves. For As(III) treatments, all samples showed an increase in accumulation for roots, stem, and leaves, except for 2ppm leaves that showed a decrease in nickel accumulation.

Molybdenum concentration suggests that in the presence of arsenic accumulation will decrease. Sunflower treatments showed a decrease in all roots, stem, and leaves samples for both arsenic concentrations in molybdenum accumulation. Few exceptions were shown with a slight positive increase for 5ppm roots and stem for As(III), and only in 5ppm stem for As(V).

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BIOGRAPHICAL SKETCH

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