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UPTAKE ANALYSIS OF AS(III) AND AS(V) BY *CASSIA FISTULA* SEEDLINGS

A Thesis

by

VALERIA VELAZQUEZ

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2021

Major Subject: Chemistry

UPTAKE ANALYSIS OF AS(III) AND AS(V) BY *CASSIA FISTULA* SEEDLINGS

A Thesis
by
VALERIA VELAZQUEZ

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August 2021

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ABSTRACT

Velazquez, Valeria, Uptake Analysis of As(III) and As(V) by *Cassia fistula* Seedlings. Master of Science (MS), August, 2021, 43pp, 3 tables, 20 figures, 39 references, 11 titles.

The natural and anthropogenic contamination of water by arsenic has become a major concern to the environment and public health. Phytoremediation is an attractive alternative and ecologically friendly to remove contaminants as a potential high efficiency and low cost. Absorption of As(III) and As(V) was evaluated by the measuring the content of arsenic in the roots, leaves and stems *Cassia fistula* grown under controlled conditions in a hydroponics system. After seeds were germinated, the plants were grown in Hoagland nutrient solution individually supplemented with 1, 2, 5 ppm of As(III) and As(V). After two weeks of exposure, samples of leaves, roots, and stems were analyzed by ICP-OES. The results show the highest average amount arsenic was present in the roots and leaves in the plant samples; the presence of arsenic is favored in a range of concentrations of 1-5 ppm. *Cassia fistula* shows a promising alternative for the removal of arsenic in contaminated water. As well as to further understand arsenic toxicity in plants and effects on its biochemical processes.

DEDICATION

A mis padres, Sr. Ruperto y Sra. Luz Maria Velasquez.

Gracias a su sacrificio, trabajo, y amor incondicional, muchos de mis logros se han cumplido entre lo que se incluye este. Gracias papa por siempre ser franco conmigo, enseñarme a ser humilde, y andar con la sonrisa en la boca. Gracias mama por enseñarme a ser una mujer fuerte y motivarme en seguir adelante a pesar de las dificultades. Ustedes son mi pilar fundamental y apoyo en mi formación académica, mis valores, mis principios, mi perseverancia, y forjar la persona que ahora soy.

For my brothers, Juan, Manuel, and Pepe.

I am forever thankful for your supporting advice, my shoulders to lean on, and big brother love. All of you are great role models in my life. I wouldn't change you brothers.

For my nieces and nephew, Ximena, Ariana, Zuria, and Jose Jr.

Who always put a smile on my face and spread joy with their laugh.

To my instructors and friends who shared their knowledge, advice, patience, and words of encouragement to finish this study.

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CHAPTER I

BACKGROUND

Sources of arsenic

One of the most important natural resources of life is water, and only about 2.5% of water on earth is freshwater. Of that 2.5% approximately, 1% of water can be used as drinking water. A rapidly increasing population and increasing industrial activity have been associated with both the mobilization of heavy metals and release into the environment. Anthropogenic activities such as burning fossil fuels, industrial waste processes, and application of agrochemicals has led to high concentrations of heavy metals being found in both soils and groundwater³³. Components of industrial effluents have resulted in the accumulation of heavy metals in soils and the further contamination of aquifers¹⁹. These concentrations and speciation of toxic elements are controlled by many complex chemical conditions and biogeochemical processes²¹.

The most common toxic heavy metals identified by the US Environmental Protection Agency (EPA) at 65% of all Superfund sites: are lead, arsenic, chromium, cadmium, nickel, and zinc¹². Among the aforementioned metals, arsenic (As) has received increasing attention since the 1990s, due to the health effects and well-publicized crises in South-East Asia (West Bengal, Bangladesh, and Vietnam)²⁶. Millions of people living around these large areas rely on arsenic-contaminated groundwater for irrigation of staple crops. Considering the extensive contamination of As in the environment and all living organisms of a particular ecosystem, the development of an appropriate and effective treatment of arsenic is imperative.

The mobilization of arsenic in drinking water, soils, and sediment has grown significantly and poses a global concern. Arsenic is classified as a metalloid. Metalloids exhibit the properties of both metals and non-metals and have various physiological roles in humans and plants depending on concentration¹¹. However, As has no known function in living organisms. Contaminated drinking water has been shown to pose the highest threat to public health from arsenic contamination⁴³. As is naturally present in water in different oxidation states, which has been shown to be dependent on both redox and pH conditions²⁰. Arsenic has been shown to enter groundwater naturally through leaching, rock weathering, volcanic eruption, geochemical reaction, and biological activity².

Arsenic toxicity and epidemiological effects

Arsenic (As) is a toxic and non-degradable metalloid existing in the environment in both organic and inorganic forms² (Fig. 1). The organic forms of As are primarily found in soil solutions as either monomethyl arsenic acid (MMA) or dimethyl arsenic acid (DMA). Organic arsenic compounds are abundant in seafood, and less harmful to human health and are typically eliminated by the body (WHO, 2010). On the other hand inorganic arsenic has four main oxidation states: As^{3+} (arsenite), As^{3-} (arsine), As^{5+} (arsenate), and As^0 (elemental arsenic). The most commonly observed oxidation states are As(III) and As(V). However, studies have shown that arsenite is more toxic than arsenate, due to its propensity to bind to sulfhydryl groups, which has detrimental effects on general protein function⁴⁰. Chronic arsenic poisoning or also known as arsenicosis is often accompanied by a keratosis, which is a painful skin disease with eczemas and ulcers (WHO, 2010). Acute toxicity is generally higher for inorganic arsenic compounds than for organic arsenic compounds⁹. Inorganic arsenic compounds originate the formation of the characteristic nodes on the hands and soles of the foot³¹.

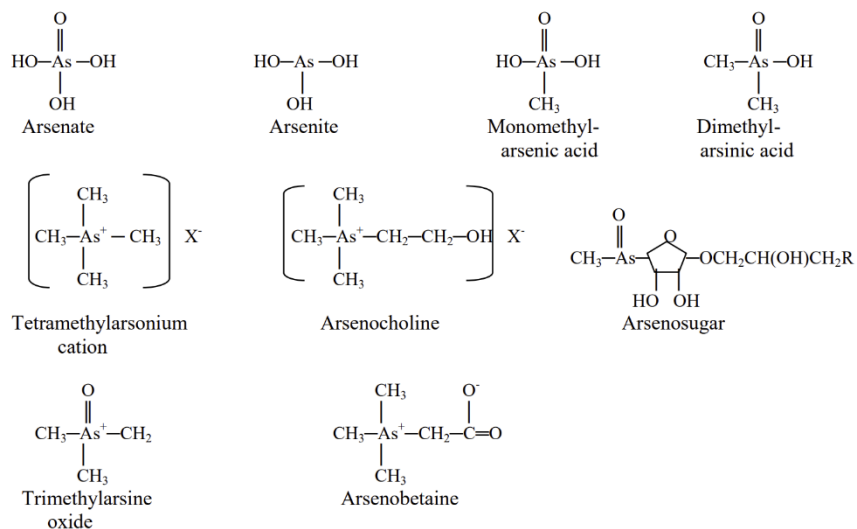


Figure 1: Chemical structures of arsenicals found in the environment.

Common anthropogenic uses of arsenic compounds included but were not limited to: wood preservatives, glass manufacture, electronics, alloys, catalysts, feed additives, fertilizers, and veterinary chemicals²³.

In some areas of India and Bangladesh, groundwater As concentration has exceeded 2000 $\mu\text{g l}^{-1}$ or 2ppm. Due to the increasing concentration of As in drinking water, it has been recognized by the World Health Organization (WHO) the permissible limit of As in drinking water as 10 $\mu\text{g l}^{-1}$. As contamination over long periods of time have been linked to skin lesions, peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular diseases and cancer (WHO, 2010). The most common external indicators of arsenic poisoning include black-foot disease, peripheral neuropathy, and encephalopathy³².

Arsenic accumulation by plants

Hyperaccumulating plants are defined as plants that accumulate heavy metals at a very high concentration in their tissues without affecting growth and metabolic function. Plants vary significantly in their ability to accumulate As, which is influenced by factors such as the concentration in soil, redox potential, and arsenic availability. In general, plants uptake As

through the roots, but submerged plants may also uptake arsenic from water by the leaves⁷. Approximately 12 species of arsenic hyperaccumulating plants have been identified which includes the Chinese brake fern within the *Pteridaceae* family⁴⁴. Plants with a low transfer factor (TF) are called excluders because of their restricted uptake and restricted translocation of As from roots to shoots. Radishes have shown a higher concentration of As in the shoots compared with the roots and identifying the plant as an excluder²⁰.

Techniques of Phytoremediation

Several methods have been documented for remediation of excessive metals/metalloids over the past few decades. The most common methods are soil washing, encapsulation, vitrification, precipitation, ion exchange, carbon adsorption, flocculation, reverse osmosis and coagulation¹⁹. These current techniques are rapid and effective but usually expensive, labor intensive, and often disrupt the environment.

Phytoremediation is a relatively new and environment-friendly technology that has been studied over the last 20 years. It is as an alternative or complementary *in situ* remediation technique to remove heavy metals from soil and water sources²⁵. It is an attractive option because can be used to treat several different pollutants and the diversity of mechanisms involved in the process. Table 1 summarizes the different techniques⁴.

Table 1: Techniques of Phytoremediation

Summary of the different techniques of phytoremediation.	
Technique	Description
Phytoextraction	Accumulation of pollutants in harvestable biomass i.e., shoots
Phytofiltration	Sequestration of pollutants from contaminated waters by plants
Phytostabilization	Limiting the mobility and bioavailability of pollutants in soil by plant roots
Phytovolatilization	Conversion of pollutants to volatile form and their subsequent release to the atmosphere
Phytodegradation	Degradation of organic xenobiotics by plant enzymes within plant tissues
Rhizodegradation	Degradation of organic xenobiotic in rhizosphere by rhizospheric microorganisms
Phytodesalination	Removal of excess salts from saline soils by halophytes

CHAPTER II

INTRODUCTION

Over the last few years, due to the rapid industrialization and urbanization, there is contamination of the environment with heavy metals, which has been responsible for the disturbance in biogeochemical cycle affecting all forms of life¹⁶. Heavy metals bioaccumulate in living organisms and their concentrations tend to increase as they are passed from lower trophic levels to higher trophic levels⁴. Among the various heavy metals, Arsenic (As) is a well-known toxic element that is considered a priority pollutant. Arsenic is a toxic metalloid element that is naturally present in the environment. Present in more than 200 minerals, arsenic is represented as the 20th most common basic element in the earth's crust⁴⁴. Arsenate (V) is the stable oxidation state in aerobic conditions. Under reducing conditions elemental arsenic, As(III) and arsine (III) exist. Arsenite (III) exists in moderately reducing conditions and is one of the most toxic arsenic compounds⁴¹. As the specific arsenic species in the environment is not normally determined in routine procedures, so the level and nature of arsenic exposure typically is not known.

In plants heavy metal concentrations surpassing the threshold limits have adverse health effects because they interfere with the normal functioning of plants⁴. Plants containing high concentrations of heavy metals have shown the oxidative stress via elevated accumulation of methylglyoxal and reactive oxygen species. The major reactive oxygen species (ROS) are singlet oxygen ($^1\text{O}_2$), superoxide ($\text{O}_2^{\bullet-}$), hydroxyl radicals ($\bullet\text{OH}$), and hydrogen peroxide (H_2O_2).

Heavy metals cause perturbations in the homeostasis between the generation and sequestration of ROS in the cellular compartments¹⁶. According to Gardea-Torresday(2005), permanence and mobility of heavy metals in soil are affected by several factors, which including soil conditions and metal species, the solubility in water, and the presence of humic substances. Essential metals such as manganese (Mn), copper (Cu), molybdenum (Mo), and zinc (Zn) take significant roles in physiological processes of living organisms and biochemical functions¹³. Non-essential heavy metals like arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), and chromium (Cr) have no known biological function and can have negative health consequences when ingested or absorbed in the human body the largest source of arsenic in the environment has been shown to be anthropogenic⁶. Useful hyper-accumulator plant species can extract, stabilize, or filter arsenic from contaminated soils and waters. Plant species can effectively reduce As solubility and may increase the rate of precipitation or filtration of As from contaminated waters²¹.

Arsenic in Water

Arsenic contamination in natural waters is a worldwide problem. As enters the environment through natural weathering process of arsenic-bearing rocks and minerals and also by industrial effluents, such as petroleum refining, fertilizers, pesticides, herbicides, glass ceramics, wood preservatives, and feed additives³⁷. Long term exposure to As contaminated drinking water has been linked to cardiovascular, lung, bladder, skin, and kidney cancer as well as pigmentation changes, neurological disorders, nausea and loss of appetite. The two most common forms of arsenic are found in natural waters are arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-})³⁹. Arsenic toxicity differs between various arsenic compounds, inorganic compounds have higher toxicity level than arsenic choline. Generally inorganic compounds have a higher toxicity

than organic arsenic compounds. However, the metalloid processes the ability to convert between inorganic and organic compounds by redox cycling¹³. As(III) arsenites are hard acids and have been shown to preferentially form complexes with oxides and nitrogen ligands. It predominates in moderately reducing anaerobic environments such as groundwater³⁹. Several studies have reported that arsenites are more toxic than arsenates due to arsenites are 4 to 10 times more soluble in water and thus more available for uptake. Although arsenate is considered less toxic, it is thermodynamically more stable due to it being prominent under normal conditions and becomes a major contaminant in ground water³⁹.

Most of the methods performed for arsenic removal from ground water are by physiochemical processes such as reverse osmosis, nanofiltration, coagulation, adsorption, and precipitation with lime, alum, or ferric sulfate. These commonly used methods typically require oxidation of As(III) to As(V) and one of the major drawbacks of these processes are the production of wet bulky sludge that requires a secondary treatment³⁷. Among these physical, biological, and chemical methods to remove As from water, phytoremediation is an efficient, viable and cost effective technology. The use of aquatic macrophytes such as poplar (*Populus spp.*) and willow (*Salix spp.*) are considered to be more suitable for wastewater treatment than terrestrial plants due to faster growth, production of more biomass and relative higher ability of heavy metal uptake⁴. According to Sood et al., 2012, they perform better purification due to direct contact with contaminated water. High concentration of As in the aquifer resulted from desorption of arsenic from Fe and Mn oxides. *C. fistula* has also been shown to be a potential hyperaccumulating plant with up to 90% removal of nickel(Ni) from an industrial wastewater, and even higher rate of removal for Ni(II) was observed with higher initial concentration of 25 mg/L¹⁴.

Arsenic in Soil

Arsenic contamination in soils limits agricultural productivity worldwide and can result in toxicity to soil biota. Mining poses one of the largest threats to the environment due to the large amounts of waste containing toxic compounds. Multiple treatments have been developed to remediate metal-contaminated soils, including chemical methods such as soil washing and immobilization using organic amendments, and physical methods such as electrokinetic remediation, soil replacement, vitrification, and soil isolation²⁷. These physical and chemical treatments are rapid and effective but often laborious and costly. In the last 20 years novel and more environment-friendly technologies have been developed, including phytoremediation, as an alternative or complementary *in situ* remediation technique to remove trace metals from soil. In the United States approximately 7-8 billion USD was spent on soil remediation, with about one third allocated to metal remediation^{27,37}. Phytoremediation of the same area would cost 10% less of the estimated cost of traditional remediation options²⁸. However, the disadvantages of this technique would be replanting and harvesting, longer time commitment, and limited contaminant remediation based on the plant's rooting zone²⁸. The efficiency of phytoremediation depends on soil characteristics and contaminant. Arsenic and phosphorous are chemically similar because these elements both form insoluble compounds with aluminum (Al) and iron(Fe) in soils. Fe-As and Al-As are the dominant forms of arsenic found in soils, although arsenic shows less affinity for aluminum oxides than does phosphates⁴¹. As(III) appears to adsorbed on iron (III) surfaces, and activated alumina has a higher affinity for As(V) than As(III) at pH 7⁴¹.

Both plants and microbes are currently being genetically engineered to be more efficient at contaminant uptake and breakdown. Examples of plants include *Arabidopsis thaliana* (L.)

Heynh, (Thale cress) and *Nicotiana tabacum* L. (tobacco) which have been modified to volatilize mercury (Hg) using organomecurial lyases (*merB*) and mercuric reductase (*merA*)¹⁸. There are numerous microorganisms that can methylate and demethylate As(V) and As(III) in soils, and transform inorganic arsenic species to organic species and vice versa²⁶. Bacteria, fungi, algae, and even humans have been shown to methylate arsenite [As(III)]⁴². Once arsenite is oxidized by the microbes As(III) and As(V) exhibit the potential to transform As into volatile arsine gases, namely arsine (AsH₃), monomethylarsine (MeAsH₂), dimethylarsine (Me₂AsH), and trimethylarsine (TMA)²⁹. Fungi including *Aspergillus*, *Candida*, *Scopulariopsis*, *Penicillium*, *Fusarium*, and *Trichoderma* can also methylate inorganic As species to organic species⁴². The potential of microbes for arsenic methylation and volatilization depends on the soil chemistry, As level and organic matter²⁷. To harness phytoremediation prospects, the efficiency of plants for removing the contaminant needs to be enhanced by As-resistant plant growth promoting microorganisms (PGPMs), it is envisioned as safe, promising, and sustainable biological tools for mitigating As toxicity in plants and for regulating As accumulation in phytoremediator plants⁴².

Arsenic Toxicity in Plants

Arsenic forms organic and inorganic complexes in the environment, however the two major biologically important species are As(III) and As(V), which are interconvertible depending on the redox status of the environment⁴⁰. Although As is a toxic element in either of its oxidation states, many organisms have developed defense systems towards As(V), making it to be less toxic than As(III)⁶. These mechanisms include the production of chelating agents by the plant roots, changes in the pH, and redox reaction which solubilize the microelements for natural

uptake. Several plants use phytochelatins to detoxify As(V), which have the chemical composition of (Glu-Cys-Gly)_n⁶. Phytochelatins are able to coordinate to As and allow this element to be transported and stored in the vacuole of the plant. Uptake of arsenic in plants adversely affects its metabolism leading to structural and functional disorders. Trivalent arsenic can similarly inhibit several metabolic processes by interacting with thiol (-SH) group inhibiting cellular function and cause plant death. Highly toxic trivalent arsenic affects the structure and catalytic function of many enzymes due to its higher affinity towards sulfhydryl groups of the enzymes⁴⁰. Several studies described the morphological changes, with reduced fresh and dry biomass of roots and shoots under As-rich growing medium⁴⁰. As a response to stress, plants have evolved to different detoxification strategies to maintain the cellular concentration of free metalloids, but also repair the damage caused by the free radicals due to oxidative stress⁴⁰. The suggested chemical groups have been proposed to contribute to bisorptive metal uptake: carboxyl, hydroxyl, carbonyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate, and phosphor-diester groups¹⁴. Although As(III) is considered to be more toxic than As(V), in living organisms including plants only, limited information on this higher toxicity exists and is still not fully understood. As well as the form As is translocated by phosphate transporters from roots to shoots¹⁰.

Cassia fistula

Cassia fistula or commonly known as the golden shower tree belongs to the family Fabaceae, genus: *Cassia*, species: *fistula* (Fig. 2). It is a widely used ornamental plant and has profusely yellow blooms in late spring. *C. fistula* trees grow between 6-9m tall in dry climates with full sun on well-drained soil³⁵. Studies have found high content chemically of ionizable groups present in *C. fistula* such as, carboxyl, carbonyl, alcoholic and amino groups which can

bind to toxic metals for the removal from aqueous solutions¹⁵. *C. fistula* has been shown to have high potential as a heavy metal bio-sorbent with up to 90% removal of nickel from industrial wastewater^{2,14}. Among the different types of hyper-accumulators, *Cassia fistula* seedlings have been used as a bio-sorbent to remove As(III) and As(V) from aqueous solution. Before selecting a plant, it must go under testability studies to confirm its hyperaccumulating plant potential.



Figure 2: *Cassia fistula* tree

Phytoremediation Characteristics

Research and development studies demonstrate plants can be used for metal retention (phytostabilization and phytofiltration) or removal (phytoextraction and phytostabilization) depending on the plant's ability to bioaccumulate or stabilize metals and their biomass production²⁵. Phytoremediation is an emerging technology that uses plants to reduce, extract, or immobilize heavy metals. Several plants have been investigated for various chemical substances,

including metals and organic contaminants, most commonly found in soil and water⁴³. Plants that can accumulate concentrations up to 100 times more than non-accumulator plants are considered hyperaccumulator plants. Due the ability to accumulate high concentrations of metal ions, hyper-accumulator plants are well suited to accumulate metals, which occurs preferably in their aerial parts, are facile to plant, and are plants that have fast growth as well as high biomass production²⁵.

An ideal plant for phytoremediation must have the following characteristics: fast growing, deep roots, easy to harvest, high biomass, and tolerate and accumulate various metals in the aerial biomass²⁸. Suitable plants for As remediation are those capable to reduce As(V) to As(III), and can trap arsenic into complexes, and subsequently sequester the As-complexes into the vacuoles of the aerial parts of the plant²². A sub-process of phytoremediation known as phytoextraction is one of the few processes suitable for the remediation of arsenic. Phytoextraction is used for the removal of toxic metal ions from contaminated soils into the harvestable parts of the roots and above ground shoots of the plant⁴¹. The efflux of arsenic from the roots to shoots is demonstrated in plants such as *H. lanatus*, *Lycopersicon*, and also found that rice silicon transporter, Lsi (Low silicon rice 1; OsNIP2;1)³⁸. Lsi1 is an aquaporin and a major route for As(III) uptake into the rice roots, and is responsible for reducing As(V) to As(III)³⁸.

CHAPTER III

MATERIAL AND METHODS

Plant germination and cultivation

Cassia fistula seed pods were obtained from the trees on The University of Texas Rio Grande Valley-Brownsville, TX campus. The seeds were manually removed from the pods and washed with tap water and soap to completely remove the brown pulp. Next, the seeds were washed in 20% bleach solution for 15 minutes and rinsed five times with DI water. A water-impermeable seed coat formed after being sterilized and was removed from each seed. Each seed was scarified and left soaking overnight in 18M Ω distilled water, washed, and treated with PHYSAN 20 fungicide. The *C. fistula* seeds were germinated on sterilized paper towels dampened with water and placed in a LAB-LINE Imperial II Incubator at 26°C. After 2.5 weeks, seedlings were selected and placed in a hydroponic system using a modified Hoagland's nutrient solution for one week. The nutrient solutions consisted of the following: KNO₃, Ca(NO₃)₂ • 4H₂O, (2.5 × 10⁻⁴ M) CuSO₄ • 5H₂O, (1 × 10⁻³ M) ZnSO₄ • 7H₂O, (1.25 × 10⁻² M) H₃BO₃, (2.5 × 10⁻⁴ M) MoO₃, KH₂PO₄, MgSO₄, (1 × 10⁻³ M) MnSO₄, (2.5 × 10⁻² M) KCl, (6.4 × 10⁻² M) Fe(II)SO₄. After one week of growth the seedlings were transferred to a contaminated nutrient containing either As(III) or As(V) at 1ppm, 2ppm, and 5ppm for 2 weeks. After treatment, the golden shower seedlings were separated into leaves, stems, and roots and were stored in a freezer to be lyophilized.

ICP-OES sample preparation

Cassia fistula plant samples were lyophilized using a Labconoco Free Zone 4.5 freeze dryer at -48°C for 72hrs. Samples of leaves, stems, and roots were separately grounded into a fine powder. Between 0.2g and 0.5g of each powdered sample were placed into (50mL) beakers for digestion. The samples were digested in 10mL of pure trace metal nitric acid ($\text{HNO}_3=70\%$) and 2mL of H_2O_2 . The sample mixtures were covered with a watch-glass and placed on a hot plate under a fume hood to digest and evaporate to almost dryness for 4-6 hours. After samples were cooled to room temperature, they were diluted with 30mL of 5% nitric acid. Both micronutrients, macronutrients, and arsenic concentrations in the plant sample were determined using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer, Optima 8000 DV, Perkin Elmer, U.S.A.). Table 2 shows the selected wavelengths analyzed for each metal and Table 3 demonstrates the parameters used of the ICP-OES.

Elements	Wavelength(nm)
Na	589.592
Mg	279.077
P	213.617
S	181.975
K	404.721
Ca	315.887
Mn	259.372
Fe	238.205
Ni	231.604
Cu	327.39
As	188.979

Table 2: Selected wavelengths used for elemental analysis on ICP-OES

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 mL/min
Injector	2.0 mm Alumina
Spray Chamber	Cyclonic
Integration Time	10-20 seconds
Replicates	3

Table 3: ICP-OES parameters used for determination of elemental uptake by *Cassia f.* seedlings under As(III) and As(V) treatments

CHAPTER IV

RESULTS AND DISCUSSIONS

ICP-OES Results

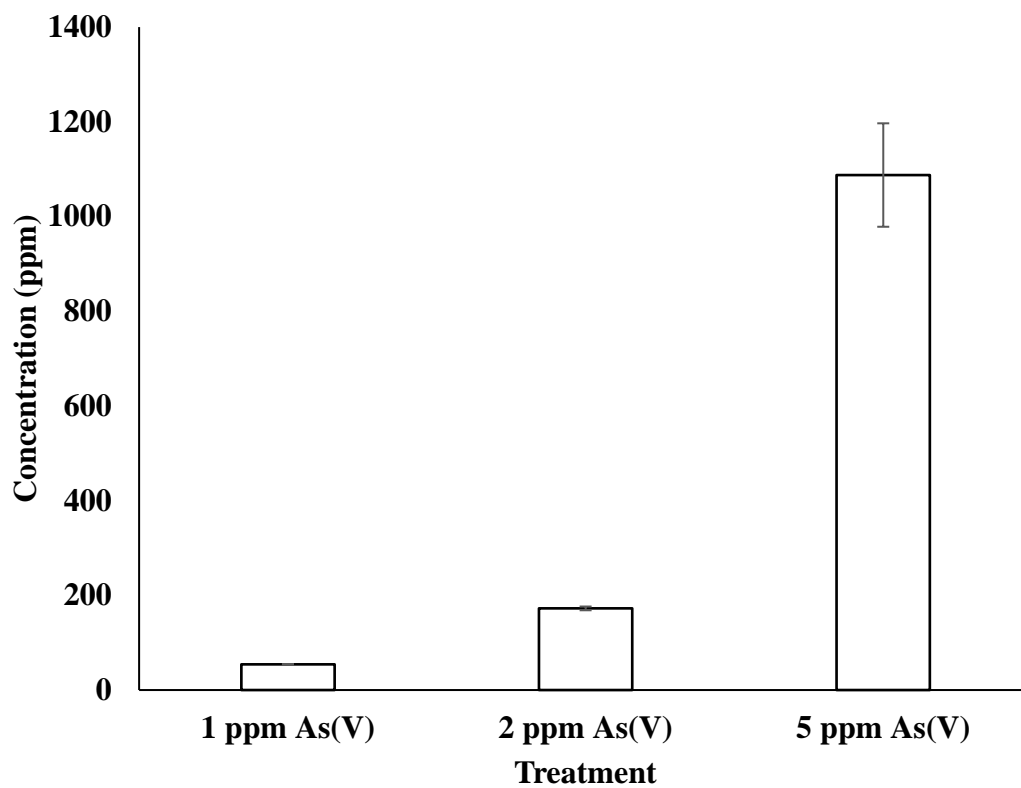


Figure 3: ICP-OES results of total arsenic accumulated of arsenic(V) treatments in *Cassia fistula* (golden shower) seedling roots.

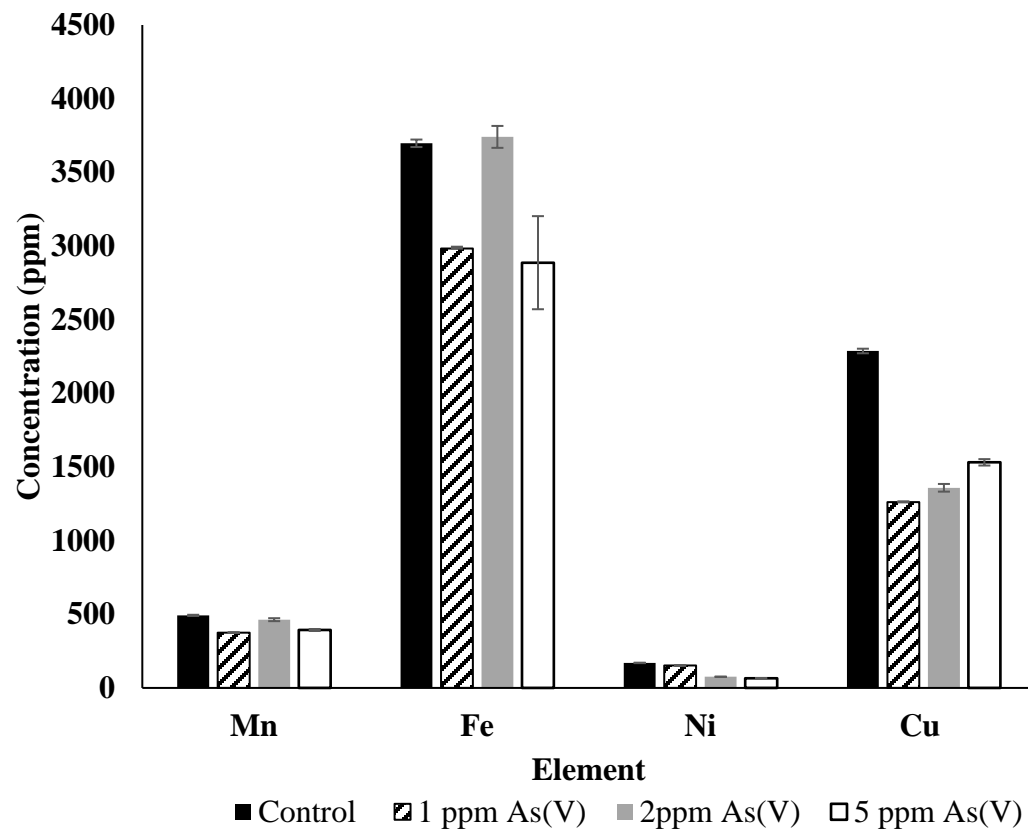


Figure 4: Concentration of micronutrients accumulated in *Cassia fistula* (golden shower) seedlings roots after treatment with As(V).

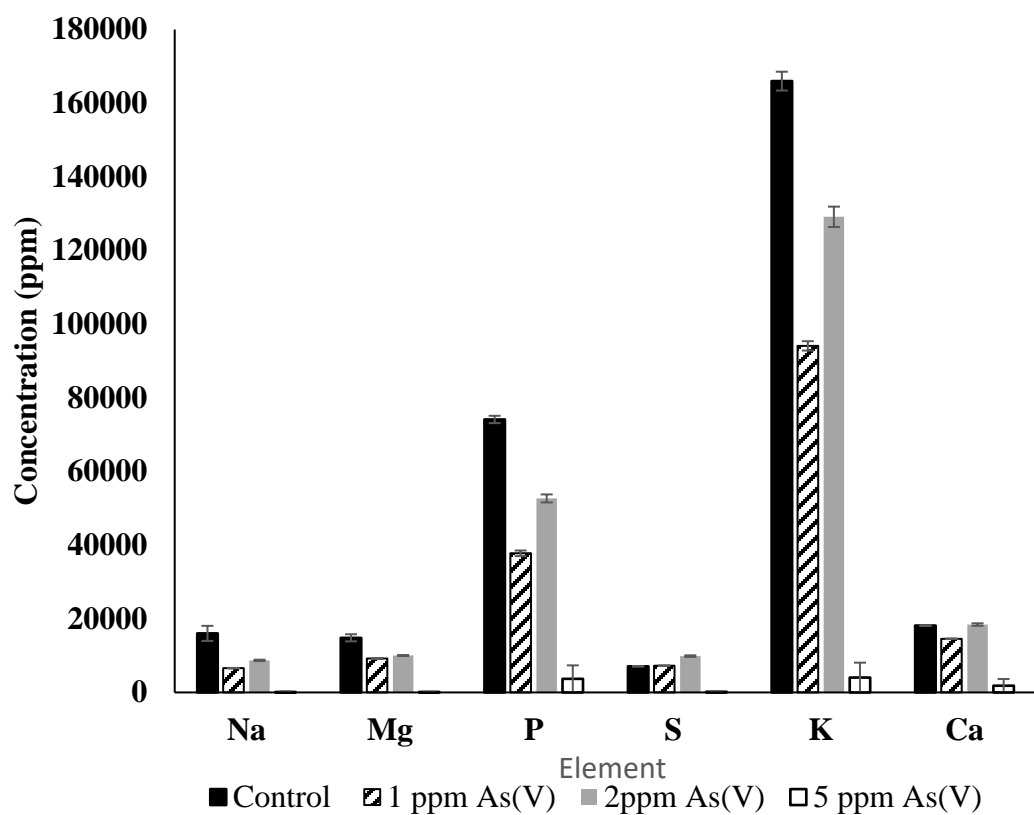


Figure 5: Macronutrients accumulated in *Cassia fistula* (golden shower) seedlings roots after treatment with As(V).

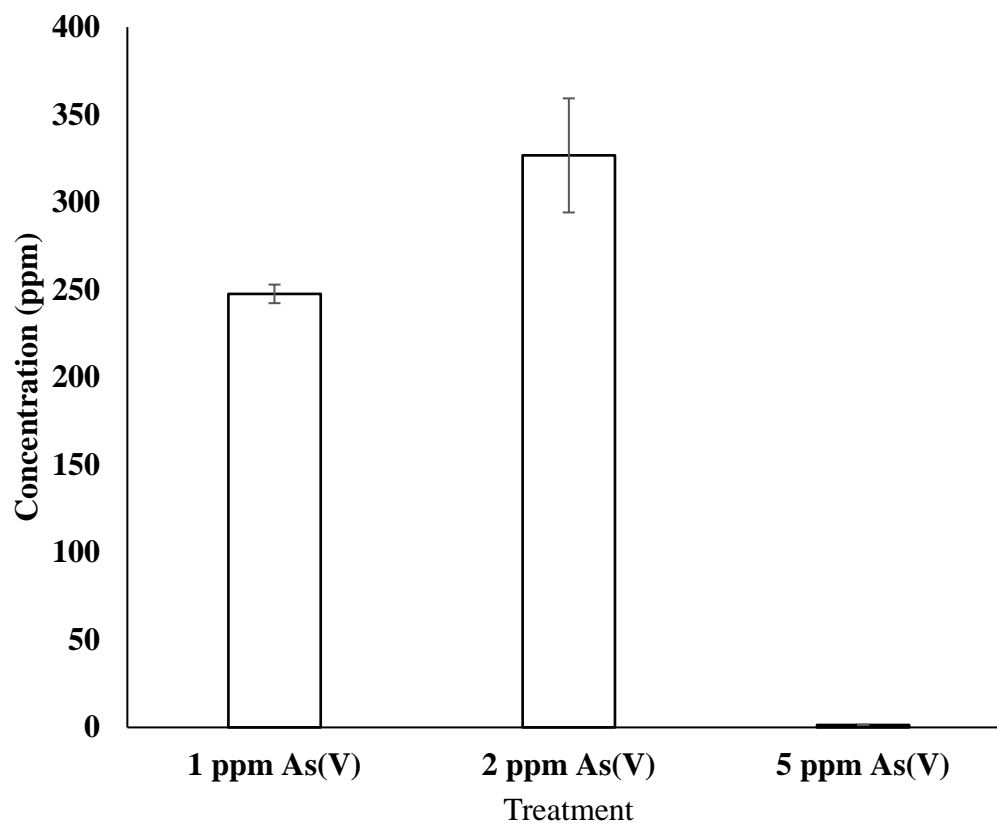


Figure 6: ICP-OES results of total arsenic accumulated of arsenic(V) treatments in *Cassia fistula* (golden shower) seedling shoots.

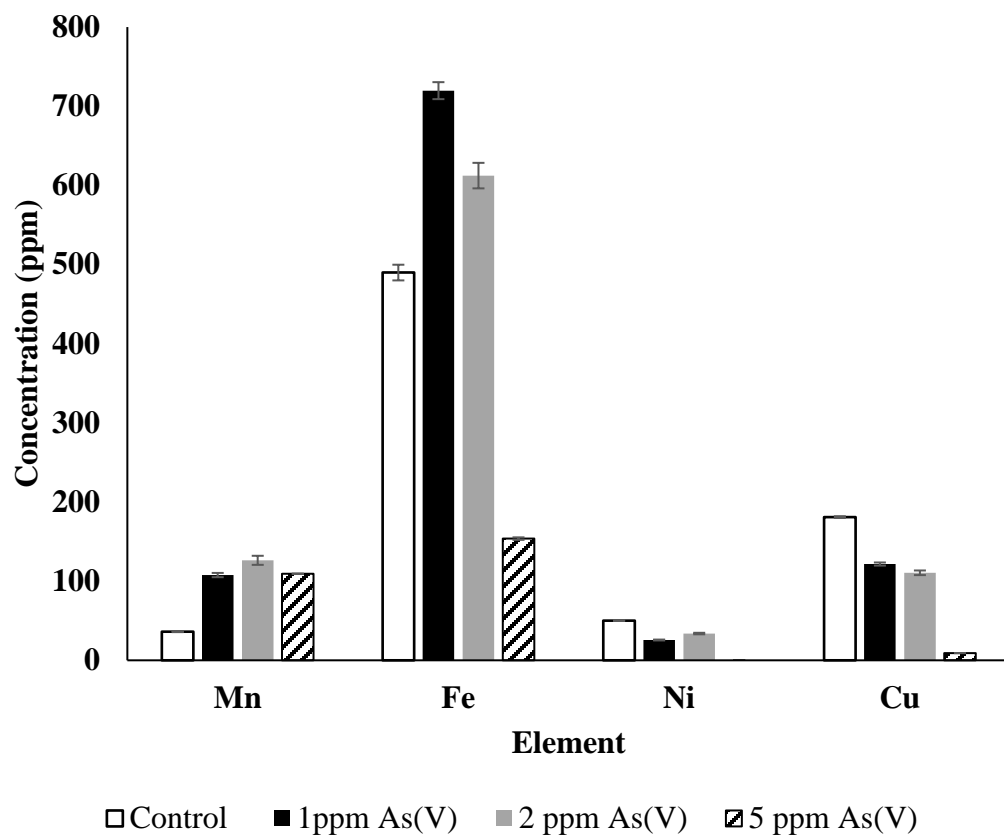


Figure 7: Concentration of micronutrients accumulated in *Cassia fistula* (golden shower) seedlings shoot after treatment with As(V).

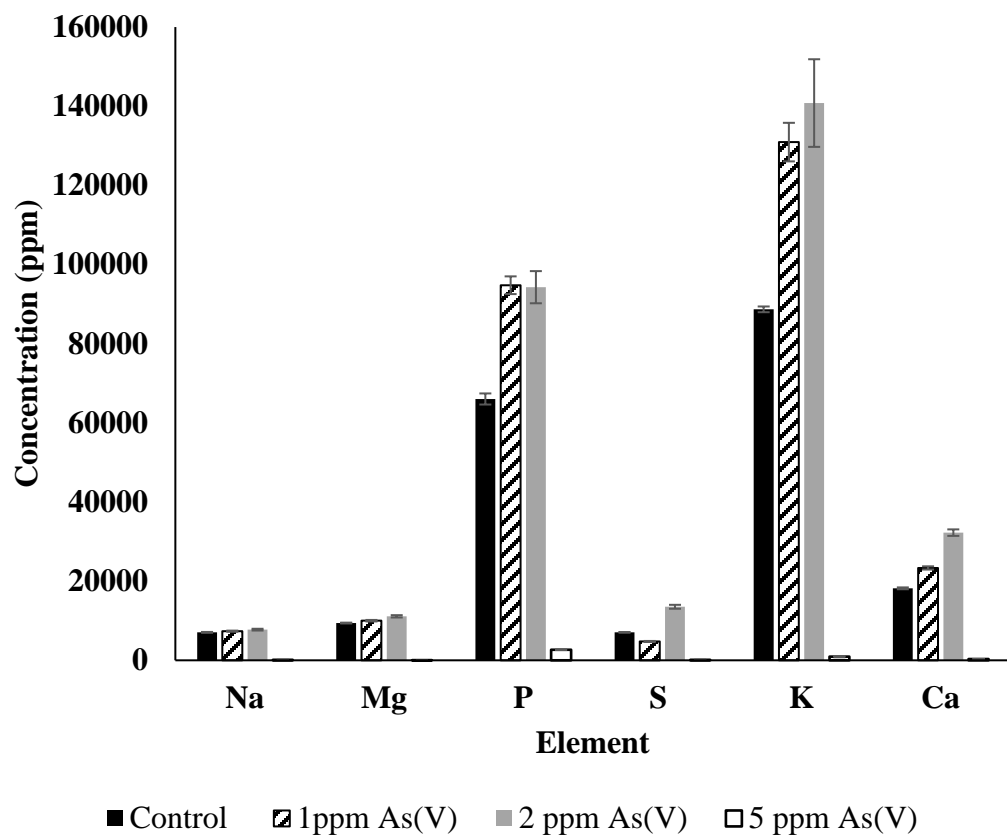


Figure 8: Macronutrients accumulated in *Cassia fistula* (golden shower) seedlings shoot after treatment with As(V).

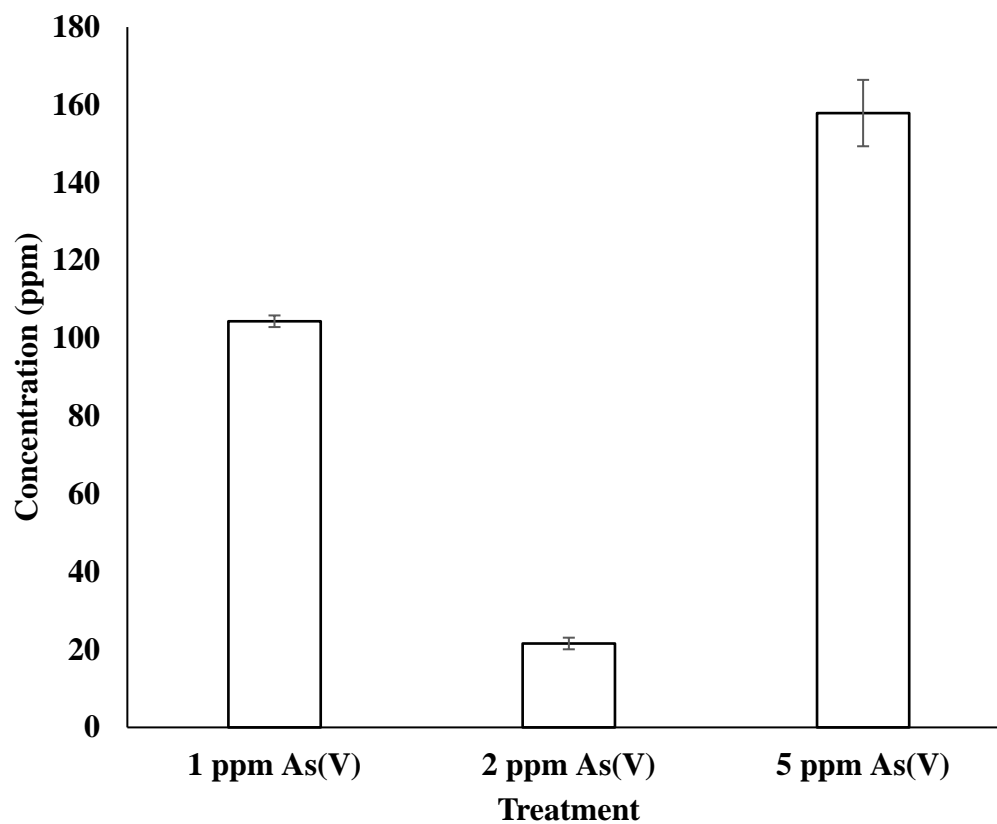


Figure 9: ICP-OES results of total arsenic accumulated of arsenic(V) treatments in *Cassia fistula* (golden shower) seedling leaves.

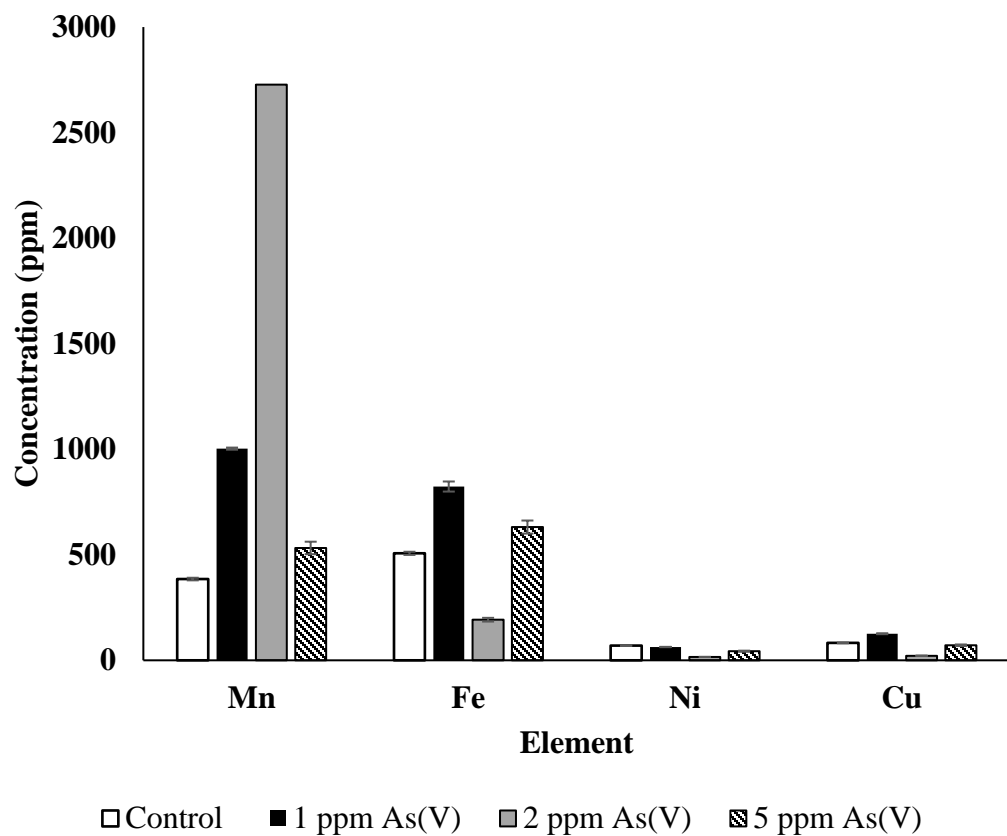


Figure 10: Concentration of micronutrients accumulated in *Cassia fistula* (golden shower) seedlings leaves after treatment with As(V).

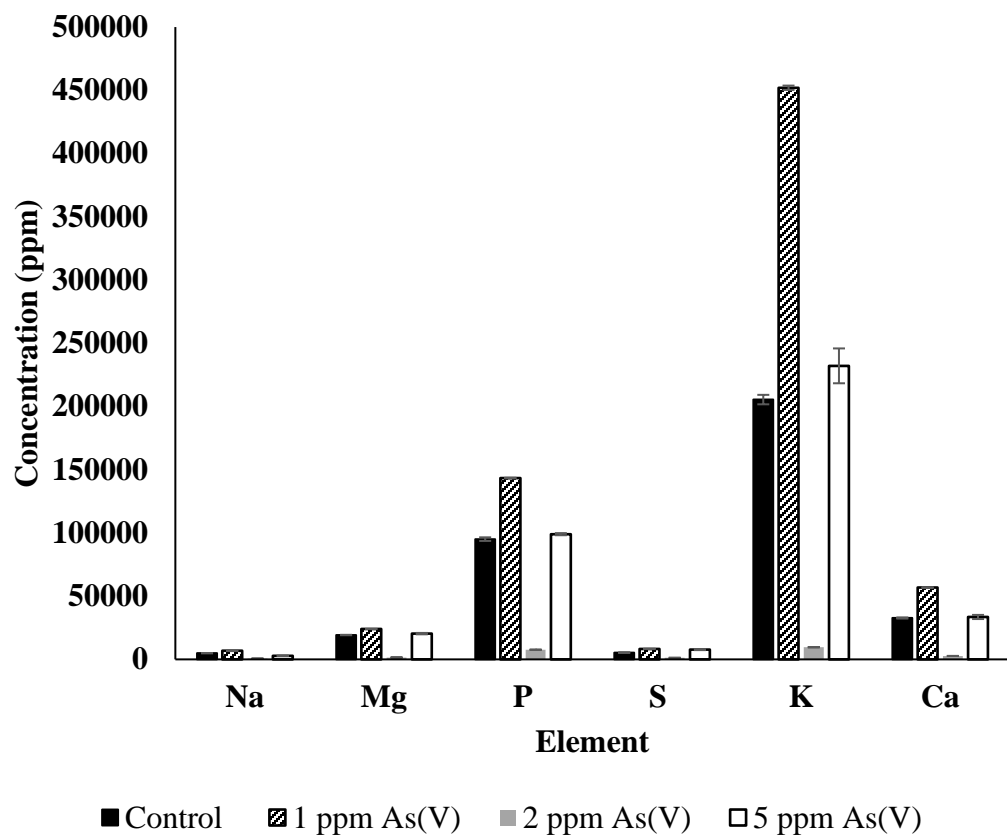


Figure 11: Macronutrients accumulated in *Cassia fistula* (golden shower) seedlings leaves after treatment with As(V).

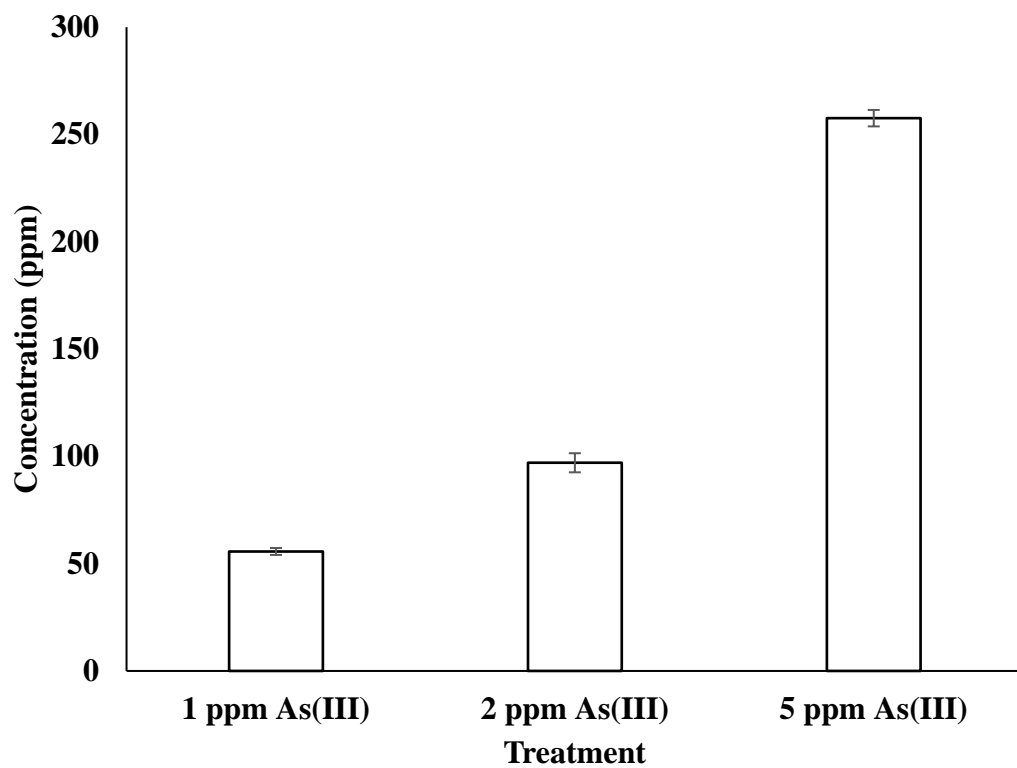


Figure 12: ICP-OES results of total arsenic accumulated of arsenic(III) treatments in *Cassia fistula* (golden shower) seedling roots.

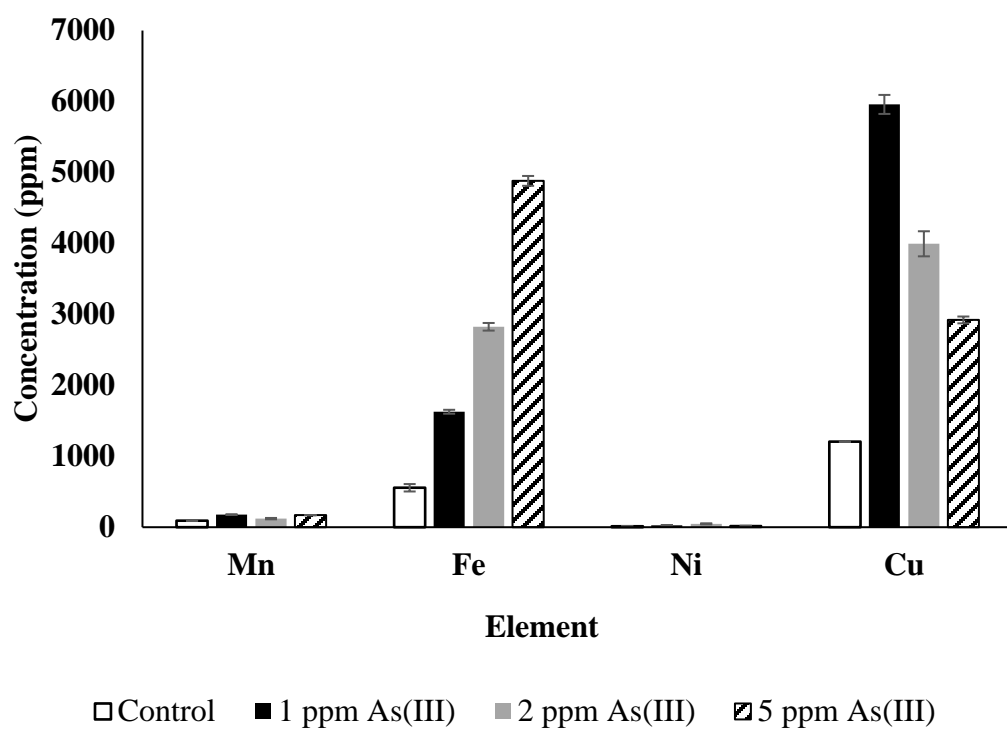


Figure 13: Concentration of micronutrients accumulated in *Cassia fistula* (golden shower) seedlings roots after treatment with As(III).

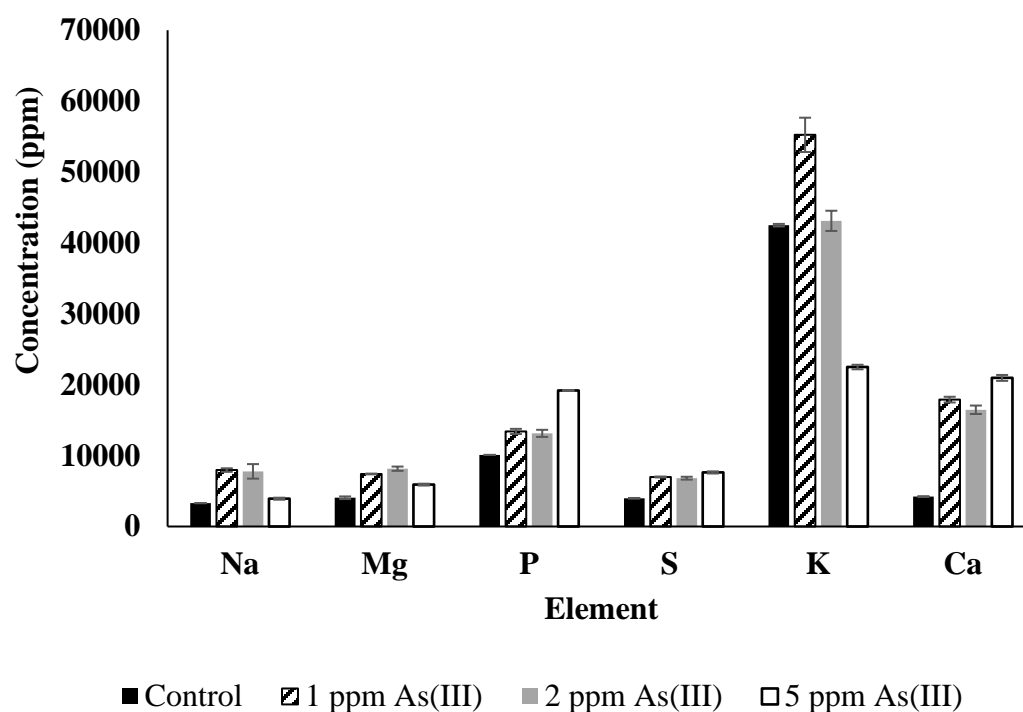


Figure 14: Macronutrients accumulated in *Cassia fistula* (golden shower) seedlings roots after treatment with As(III).

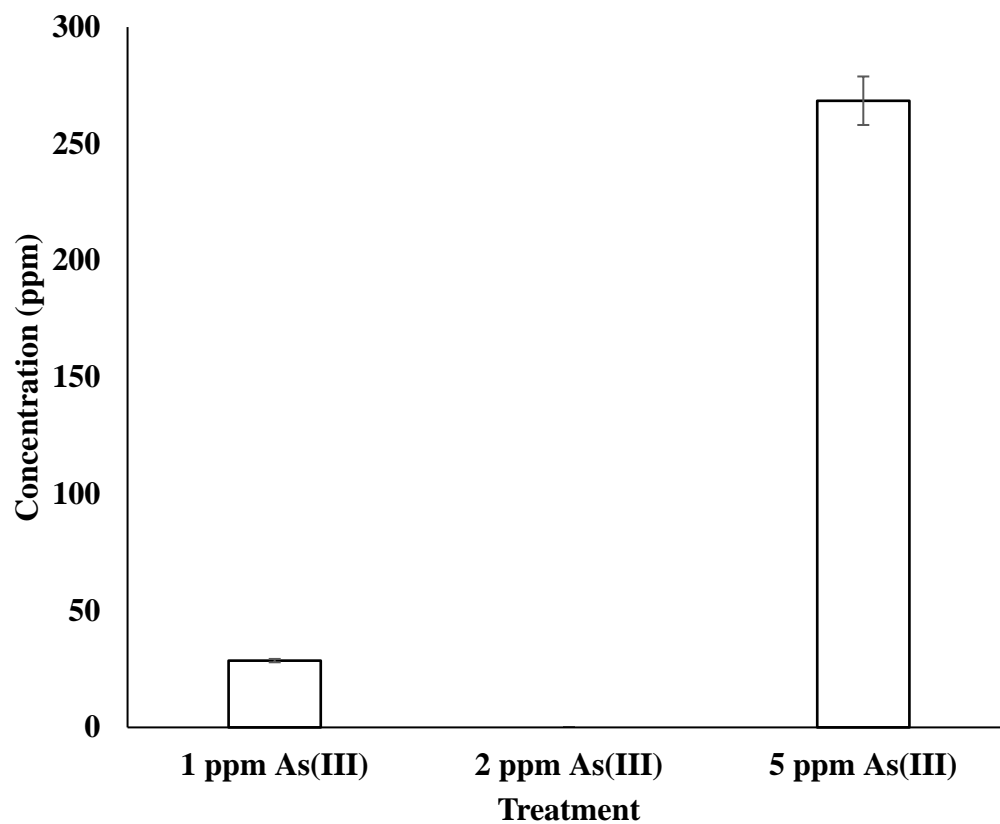


Figure 15: ICP-OES results of total arsenic accumulated of arsenic(III) treatments in *Cassia fistula* (golden shower) seedling shoots.

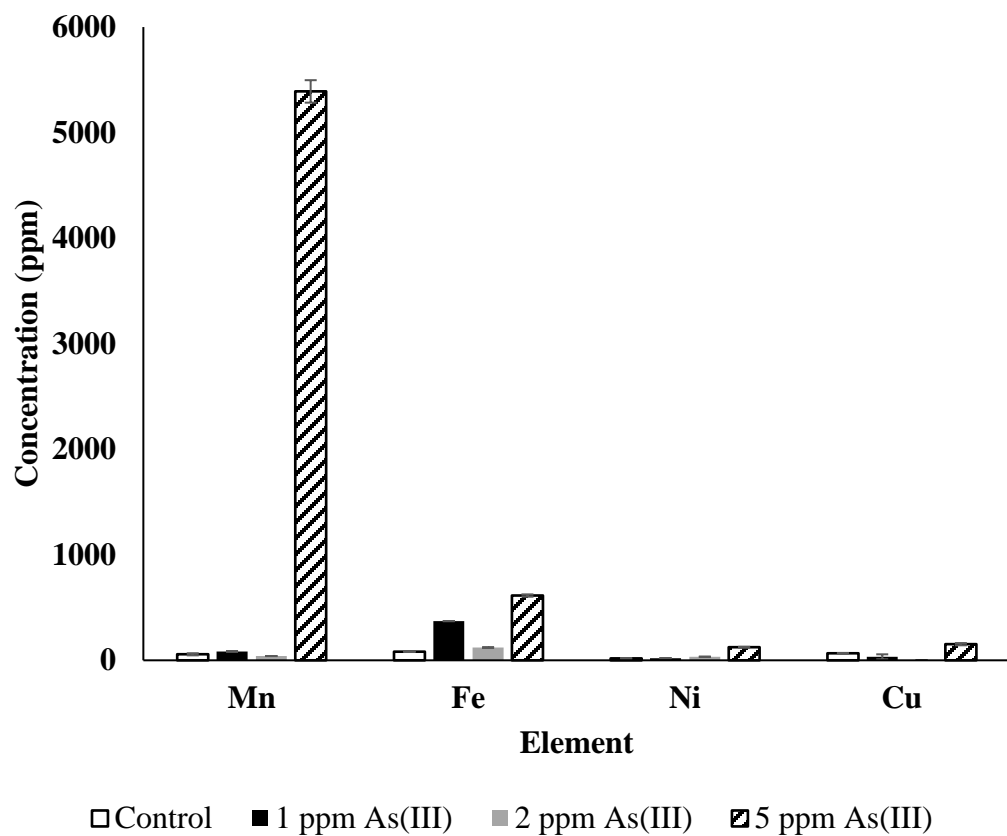


Figure 16: Concentration of micronutrients accumulated in *Cassia fistula* (golden shower) seedlings shoot after treatment with As(III).

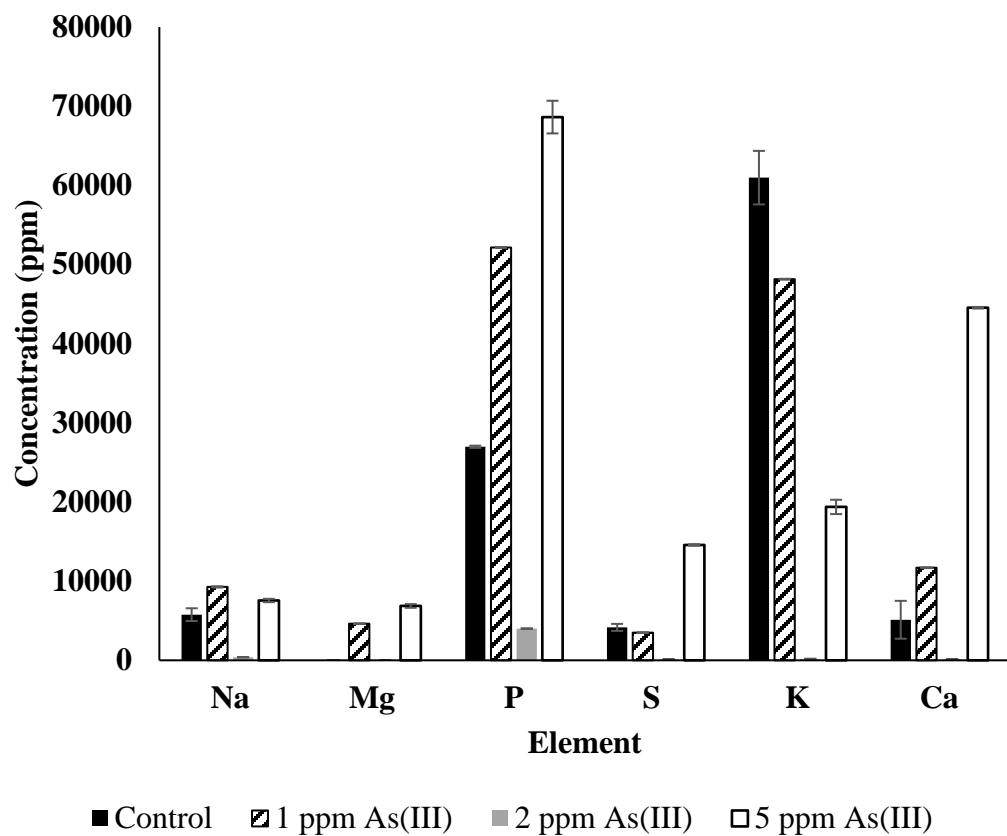


Figure 17: Macronutrients accumulated in *Cassia fistula* (golden shower) seedlings shoot after treatment with As(III).

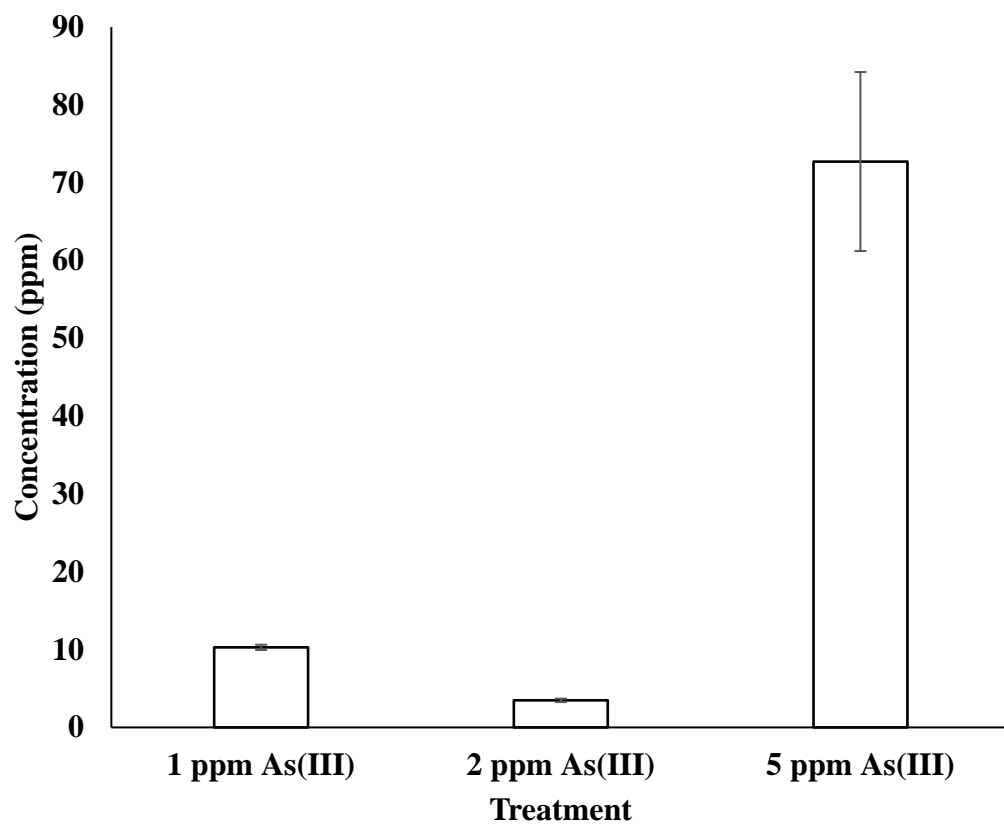


Figure 18: ICP-OES results of total arsenic accumulated of arsenic(III) treatments in *Cassia fistula* (golden shower) seedling leaves.

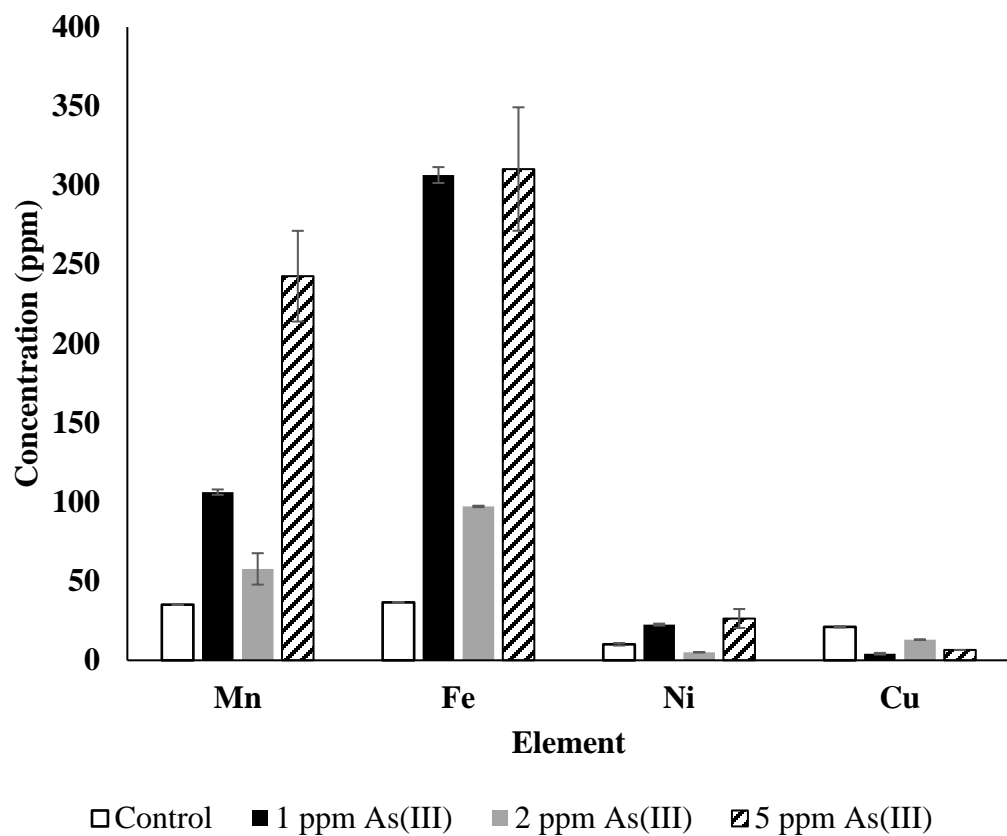


Figure 19: Concentration of micronutrients accumulated in *Cassia fistula* (golden shower) seedlings leaves after treatment with As(III).

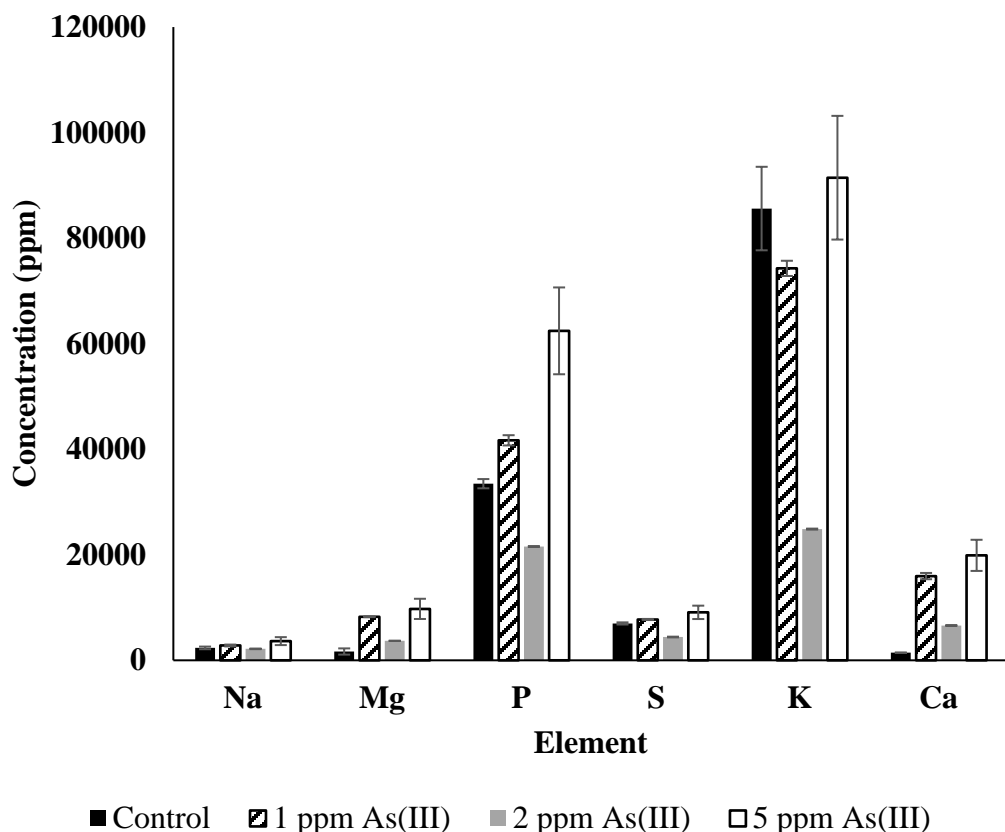


Figure 20: Macronutrients accumulated in *Cassia fistula* (golden shower) seedlings leaves after treatment with As(III).

The uptake results of As(III) and As(V) show an accumulation of arsenic in the leaves and roots of the *Cassia f.* seedlings. In Fig. 9 and 18 seedlings treated with As(V) and As(III) accumulated a similar concentration of approximately 3ppm in the leaves. The concentration observed in the seedlings roots treated with As(V) accumulated 61 ppm (Fig. 1), which was a much higher concentration compared to the roots treated with As(III) 4ppm (Fig.12). A concentration of approximately 10ppm was observed in the shoots of the plants treated with As(V), whereas in the shoots treated with As(III) only accumulated a concentration of approximately 3ppm. These differences in the concentrations observed in the accumulation of As(III) and As(V) may have been due to the differences in toxicity of As(III) and As(V). As(III)

is known to be more toxic than As(V), which typically triggers biochemical responses and typically lower accumulation of As(III) compared to As(V).

The *Cassia fistula* seedlings treated with As(III) and As(V) demonstrated a high amount of iron and copper throughout the shoot and root system. As(III) treated seedlings had an approximate concentration of 113 ppm of iron and 241 ppm of copper shown in Fig. 13 and 16. Plants treated with As(V) had a concentration found of 259 ppm of iron in the roots and 95 ppm found of copper (Fig.5 and Fig. 7). A iron concentrations of 11 ppm was observed in leaves, as can be seen in Fig. 10, and a smaller amount of 5 ppm Fe(iron) was found in the shoots. However, the shoots of the seedlings treated with As(V) had the highest concentration of manganese, which was approximately 36 ppm (Fig.7). The concentration observed in the leaves treated with As(III) was 12 ppm of Fe and 15 ppm of Mn(manganese) as can be seen in Fig.19.

Fig. 5 and Fig. 14 show the K concentration in the roots of As(V) and As(III) treated plants to be approximately 13218 ppm and 800ppm. As can be seen in Fig. 14, the root concentration for As(III) treated seedling also had a Ca concentration of 635 ppm and the shoots Ca concentration was 4461 ppm. Shoots treated with either of As(V) or As(III) were observed to have a 3421 ppm and 4290 ppm potassium concentration. Furthermore, the concentration of potassium found in the leaves treated with As(V) is shown in Fig. 11 and was approximately 4591ppm, and the concentration of Mg was about 7015 ppm. However, in the seedlings treated with As(III) in Fig. 20 the concentration of K is approximately 6985 ppm and a high concentration of P found to be 4322 ppm.

Seedlings treated with 2ppm As(III) showed a simultaneous increase in concentration of the roots of iron and copper in the roots (Fig. 12 & 13). Arsenate treated plants were found to have a high accumulation in the plant roots at 5ppm As(V) treatment, as well as an increase in Fe and Cu. The arsenic(V) treated plants observed to have a higher accumulation of Fe and Cu in the 2ppm As(V) treatment in the roots (Fig.3). Several researchers have reported similar results as Fe and Mn content increased in the roots of As stressed plants. Iron is known to make As less mobile due to the formation of iron plaques on the surface of the root²⁴. This could be the reason the *Cassia fistula* seedling demonstrated a simultaneous increase of iron in the roots for both As(III) and As(V) treatments. When the shoots were treated with 2ppm As(III) there was also an increase in iron and copper. The increase in concentration of Mn in the shoots can be seen in Fig. 7 as As(V) treatment increased.

For the As(III) and As(V) treated plants it was observed that a simultaneous increase in Fe and Mn in the leaves occurred. Similar effect has been observed in *Macadamia integrifolia* leaves³⁴. Manganese (Mn) is a key micronutrient for plant growth, enzyme activation, biotic redox reactions, splitting of water molecules, and decontamination of free radicals. Excessive manganese may be stored in vacuoles, cell walls, and different leaf tissues³. As well Mn can be chelated in Mn-P complexes and complexed by organic acids in leaves, which prevents the uptake and translocation of other elements. Few studies have provided direct evidence concerning growth of Mn cluster root and micronutrient effects on arsenic.

Potassium is a macronutrient that serves as a dominant cation that stimulates cell division, has a close relationship with protein synthesis and plant growth¹. In the shoots of the tree seedlings it was observed to have the highest accumulation of As(III) occurred in the shoots at 5ppm As(III) treatment. In Fig, 12 and 13 there is a simultaneous increase of As(III) and K⁺ concentration in the leaves. *Cassia fistula* seedlings showed that arsenic(III) exposure increased K⁺ contents in the leaves. The increase of K⁺ accumulated in the roots of As(V) treated seedlings is similarly to the study reported by Ahmad et al., 2016 in the chickpea plant (*Cicer arietinum*) using K⁺ and Ca²⁺ fertilizers to lower Cd toxicity. The increasing trend may be a mechanism of As resistance by maintaining growth of emerging leaves and roots which may be due to potassium being a stress resistant element in plants to adapt to stress⁸.

Phosphorous (P) in plants is key in energy transfer mechanisms and to drive the biochemical reaction for germination through the formation of plant maturity. Studies have reported that As(V) uses various phosphate channels for its entry into the plant cell because P is chemically analogous to arsenic. It is reported that under a high concentration of As(V) in rice roots, a P deficiency is reflected due to competitive inhibition of phosphate binding to the phosphate transporters in the roots^{13,24}. The controls in this study show a high concentration of P, but as the As(III) and As(V) concentration increases phosphorous decreases in the roots. The decrease of P uptake may be one of the factors affecting morphological changes and root function. The concentration of phosphorous within the plant shoots and leaves were found to increase as the concentration as arsenic increased. This accumulation of P in the shoots and leaves may be caused to alleviate As toxicity by reducing the translocation and the formation of nutrient stress²⁴.

CHAPTER V

CONCLUSION

The uptake results of arsenite and arsenate uptake by *Cassia fistula* or golden shower tree seedlings grew well under hydroponic conditions and 1ppm, 2ppm, and 5ppm arsenate and arsenite treatment. The accumulation and distribution of arsenic in different parts of the *Cassia fistula* seedlings support to determine the mechanism of arsenic detoxification and hyperaccumulator potential. *Cassia f.* seedlings treated with arsenite accumulated approximately 4ppm in the roots, 3ppm in stems, and 1ppm in the leaves. Arsenate treated plants absorbed approximately 61 ppm in the roots, 10ppm in the shoots, and 3ppm in the leaves. Golden shower tree seedlings were shown to have a higher uptake of As(V) in the roots than the roots of As(III) when seedlings were treated with As. As the arsenite concentration increased in the nutrient solution the content of macronutrients decreased in the seedlings roots. This may indicate a response from the arsenic toxicity leading K accumulation to increase in the roots of the plant to bind onto the negatively charged arsenic species in the roots. The decrease of K⁺ accumulation in the plant shoots of the arsenic treated plants could be related to the formation of Fe or Mn plaques on the roots preventing the translocation of nutrients throughout the plant. Overall, the results of this study demonstrate the uptake tolerance of As(III) and As(V) in the *Cassia fistula* seedlings as well as a potential measure for remediation. The objectives of this study were to determine the As(III) and As(V) accumulation and its effect on the absorption of micronutrients and macronutrients by the golden shower tree.

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BIOGRAPHICAL SKETCH

Valeria Velazquez was born and raised in Brownsville, Texas to Luz Maria Velasquez and Ruperto Velasquez. She attended Harmony Science Academy for her freshman and sophomore year of high school and completed the last two years of high school and the first two years of undergraduate studies at Mathematics and Science Academy in May 2017. After graduating high school Valeria continued her undergraduate studies at The University of Texas Rio Grande Valley and graduated with a Bachelor of Science in Chemistry May 2019 by the age 18. In the fall of 2019, she decided to continue her education and pursue her master's degree in Chemistry at the University of Texas Rio Grande Valley. While she attended school and worked on her thesis research she was employed as a Graduate Teaching Assistant. She was also granted the opportunity to work as a laboratory technician the summer of 2020 at a recycling of fats and oils plant, Fatty Chem By-Products in Los Fresnos, TX. Valeria graduated with her Master of Science in Chemistry in August of 2021. Valeria Velazquez's permanent address is 1245 W. Saint Francis Street Brownsville, Texas, 78520. Her personal email is vvelazz06@gmail.com