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BIOTRANSFER OF AS(III) AND AS(V) IN *RAPHANUS SATIVUS*, *SOLANUM LYCOPERSICUM* AND *LACTUCA SATIVA*

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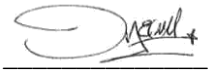
Unterschrift / *Firma*: 

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"Throw your dreams into space like a kite,

and you do not know what it will bring back,

a new life, a new friend, a new love, a new country."

— Anais Nin

 *For my friends and my family*

Who endured my ongoing stress during this process,

And who sat with me at the table

even though I could have poisoned them with arsenic.

ABSTRACT

Heavy metals are metal (or metalloid) elements with a high density and, according to the oxidation state, exposure time and route of exposure, with toxic effects on organisms (1). To this group belongs the Arsenic (As), widely distributed in the earth's crust, making it a natural contaminant of groundwater and soil. On the other hand, anthropogenic activities also contribute to arsenic pollution in air, water, and soil (2). Mexico is among the countries with the highest concentration of As in water; in several states of the republic, levels higher than those established by Mexican standards for water for human consumption (0.025 mg/L) and agricultural irrigation.

CONAGUA (National Water Commission in Mexico), in its regulations, establishes that the maximum concentration of As in the water used for agricultural irrigation is 0.1 mg/L. However, states such as Coahuila (0.74 mg/L), Durango (0.59 mg/L), and Chihuahua (0.27 mg/L) exceed this, and therefore the human consumption level (3).

The problem of As in water has led to the use of different As removal strategies from water. In some cases, wastewater is just diluted and used for the irrigation of agricultural fields.

Arsenic in organic and inorganic species has been related to different degrees of toxicity, the inorganic being the most toxics and with the most significant presence in the environment.

One of the primary sources of exposure to As is the contact with polluted water and soil; however, lately, this problem has been increased by the bio transfer of As by plants, becoming a significant entryway of the metalloid to food chains(1). The environmental crisis, the health risk, and the limited information about the bio transfer of As in crops in Mexico are the basis of this research.

To characterize the effect of As (III and V) on the soil-plant system, we evaluated the physicochemical analysis (conductivity, pH, humidity) of the soil, and the microbial population by bacteria culture in LB, B King, Nutritive, and NBRIP culture media. The established soil-plant system consisted on radish seeds, lettuce and tomato seedlings that were planted, and exposed to As through irrigation water with concentrations of 0.1. 0.3, 0.6 ppm independently. The analysis of the phenological development of crops was registered during and post-harvest. The concentration of total As (AsT) adsorbed on vegetative parts, in soil and water was determined through atomic absorption. The bio transfer concentration of As increased proportionally to the amount of irrigation water. The growth did not present affectation. The edible parts of radishes and tomatoes did not present As. The concentration in vegetative parts were roots>leaves>stems. Bacteria population reacted to As in different ways in each vegetable, with a general tendency to decrease.

KEYWORDS: arsenic, crops, pollution, radish, lettuce, tomato, biotransfer.

RESUMEN

Los metales pesados son elementos metálicos (o metaloides) con alta densidad y, dependiendo de su estado de oxidación, tiempo de exposición y ruta de exposición, con efectos tóxicos en los organismos (1). A este grupo pertenece el Arsénico (As), el cual se encuentra extensamente distribuido en la corteza Terrestre, convirtiéndolo en un contaminante natural de agua subterránea y suelos. Por otro lado, diversas actividades antropogénicas también contribuyen a esta contaminación de suelo, agua y aire (2). México se encuentra entre los países con mayor concentración de As en agua; en diversos estados de la república, las concentraciones son mayores que aquellas establecidas por las regulaciones mexicanas para consumo humano (0.025 mg/L) y para uso en agricultura.

CONAGUA, en sus regulaciones establece que la concentración máxima de As en agua para uso agrícola es 0.1 mg/L. Sin embargo, estados como Coahuila (0.74 mg/L), Durango (0.59 mg/L) y Chihuahua (0.27 mg/L) exceden ese máximo, y, por lo tanto, excediendo también el nivel para consumo humano (3).

El problema del As en agua ha llevado al uso de diferentes estrategias de remoción. En algunos casos las aguas residuales solo son diluidas y usadas para la irrigación agrícola.

El arsénico, tanto en sus especies orgánicas como en las inorgánicas, ha sido relacionado con diferentes grados de toxicidad; las especies inorgánicas tienden a ser más tóxicas y con mayor presencia en el medioambiente.

Una de las principales fuentes de exposición a As es agua y suelo contaminado; sin embargo, en tiempos recientes este problema se ha visto incrementado por la biotransferencia de As a través de plantas, convirtiéndose en una importante entrada del metaloide a las cadenas tróficas (1). La actual crisis ambiental, el riesgo a la salud y la limitada información sobre la bio transferencia de cultivos agrícolas en México son la base de esta investigación.

Para caracterizar los efectos del As (III y V) en el sistema planta-suelo se llevó a cabo un análisis fisicoquímico (pH, conductividad, humedad) del suelo y un análisis microbiano a través de cultivo de bacterias en medios de cultivo LB, B King, Nutritivo y NBRIP. Los sistemas establecidos consistías en lechugas, rábanos y jitomates, los cuales fueron expuestos a arsénico a través del agua de irrigación con concentraciones de 0.1, 0.3 y 0.6 ppm. El desarrollo fenológico de los cultivos fue evaluado durante y después de la cosecha. La concentración total de As (AsT) adsorbida en las partes vegetales, en agua y suelo fueron determinado a través de absorción atómica. La bio transferencia de As incremento de manera proporcional al tratamiento de agua recibido. El crecimiento vegetal no se vio afectado. Las partes comestibles de rábanos y jitomates no presentaron As. La acumulación de As fue en raíces>hojas>tallos. Las bacterias reaccionaron al As de manera diferente en cada vegetal, con una tendencia de disminución generalizada.

PALABRAS CLAVE: arsénico, cultivo, contaminación, rábano, lechuga, tomate, biotransferencia.

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0. ABBREVIATIONS AND SYMBOLS

AECOSAN – Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (Spanish Agency for Consumption, Food Safety and Nutrition)

As – Arsenic

AsB – arsenobetaine

AsC - arsenocholine

AsT – Total arsenic concentration

As III – Arsenic with oxidation number +3

As V – Arsenic with oxidation number +5

ATP - Adenosine triphosphate

ATSDR – Agency for Toxic Substances and Disease Registry (from United States of America)

Cd – cadmium

cm – centimeter

CO_2 – carbon dioxide

CONAGUA – Comisión Nacional del Agua

COOH - carboxyl group

Cr – chrome

Cu – copper

DMAs - dimethylarsinic acid

EPA – Environmental Protection Agency (from United States of America)

EU – European Union

Fe – iron

g - grams

H_3AsO_3 – arsenious acid, trioxoarsenic acid

$HAsO_4$ – hydrogen arsenate

Hg – mercury

LD_{50} – median lethal dose

MMAs(III) – Monomethylarsonic acid

MRL – minimal risk level

Ni – nickel

OH - hydroxide

P – phosphorus

pH – potential of hydrogen

Pb – lead

PGPB - plant growth promoting bacteria

PGPR – plant growth promoting rhizobacteria

PO^{-4} – phosphate

ppb – parts per billion (µg/l or µg/kg)

ppm – parts per million (mg/l or mg/kg)

redox – reduction-oxidation process

SEMARNAT – Secretaría de Medio Ambiente y Recursos Naturales (Mexican Ministry of the Environment and Natural Resources)

Si - silicon

SSA – Secretaría de Salud (Mexican Health Ministry)

TETRA - tetramethyl diarsine

TMAO - Trimethylamine N-oxide

UAdeC – Universidad Autónoma de Coahuila (Autonomous University of Coahuila)

UASLP – Universidad Autónoma de San Luis Potosí (Autonomous University of San Luis Potosi)

US – United States of America

WHO – World Health Organization

Zn - zinc

1. INTRODUCTION

Heavy metals are metallic (or metalloids) elements with a high density and toxic effects on human health at low concentrations. The toxicity of heavy metal depends on the oxidation state, exposure time, and route of exposure (2). Examples of heavy metals are: Mercury (Hg), chromium (Cr), lead (Pb), cadmium (Cd) and arsenic (As). This group has played a leading role in research in recent years due to its effects on toxicity (3).

Arsenic is an element of high toxicity and distribution in the environment, the Agency of Toxic Substances and Registry of Diseases' fund [(ATSDR), of the government from the United States of America] considers this element as a top priority. Acute and chronic exposures to As may cause different health effects, including cancer, development effects, reproductive effects, neurological effects, immunological-lymphoreticular effects, systemic effects, and death (4).

This metalloid distributed in the earth's crust, is considered a natural contaminant in groundwater and soil. Arsenic in the water is the response of different diseases in countries as Bangladesh (50 µg/L), India, China, Taiwan, Mongolia, Chile, Argentina, Mexico, and the United States (5–8). The anthropogenic activities such as mining, agriculture (use of pesticides or herbicides), glass, ceramic manufacturing, electronic industry, pigmentation, and fossil fuels also contribute to air, water, and soil contamination (9–12).

This problem has led to the use of different As removal strategies and processes that give rise to highly concentrated water remnants with As, which not in all cases receive the appropriate treatment and confinement. Therefore, we have identified that some wastewater is used as a diluent medium and subsequently reused for different activities, including irrigation of plant crops(13,14).

In Mexico, several states of the republic have reported concentrations higher than those established by Mexican standards for As in water for human consumption (0.025 mg/L). According to CONAGUA, the maximum content of As in the water used for agricultural irrigation must be 0.1 mg/L. However, states such as Coahuila (0.74 mg/L), Durango (0.59 mg/L) and Chihuahua (0.27 mg/L) exceed this specification(15,16).

Arsenic can be uptake by crops and become a source of additional exposure by the consumption of contaminated food. This bio transfer of As to the plants (by irrigation water or contaminated soil) is through the roots and rises to different parts of a plant (roots, stems, leaves, and fruit), and As can accumulate in varying amounts in the different parts.

The descending order of this deposition is the root-stem, leaf and fruit. This deposition can vary between different species of plants (17–19).

Health risk and the limited information about the bio transfer of As in crops in Mexico are the basis of this research. In this study, we tested the bio transference of As in plant crops of tomatoes, lettuces, and radishes with three different As concentrations reported in water in northern states of Mexico. The analysis of arsenic was carried out through acid digestion of the vegetal parts, followed by atomic absorption. The results were treated accordingly through descriptive statistics.

2. BACKGROUND

2.1 As Generalities

Arsenic occupies the 33rd place in the periodic table, belonging to the metalloid (or semimetals) group. It forms compounds with other elements; those compounds are of two natures: organic (carbon-linked) and inorganic (metal and non-metal linked).

There are two main inorganic compounds associated with this element. The difference lies in the oxidation number: arsenate, with an oxidation number of (V), more common in aerobic environments (e.g., soil and surface water), and arsenite, with oxidation number (III), most found in anaerobic environments (e.g., groundwater). Of these two inorganic compounds: the arsenite is the most stable, toxic, soluble, and mobile. Therefore, it is easier absorbed by organisms (20–22).

The transformation and presence of inorganic compounds depends mainly on environmental conditions, specifically of, redox conditions and the pH of the environment (a basic pH and reduction environment prevails *As (III),* as H_3AsO_3; while in an oxidizing, and a pH close to neutrality prevails *As (V),* as $HAsO_4$) (Figure 1)(23,24).

The organic compounds formed by biotransformation are based in *As (III)* and vary in the number of methyl molecules linked. These compounds have been proven toxic and easily absorbed, but they are less common than their inorganic counterparts; i.e., in the case of soils, only about 5% of the total arsenic quantity corresponds to organic species (25).

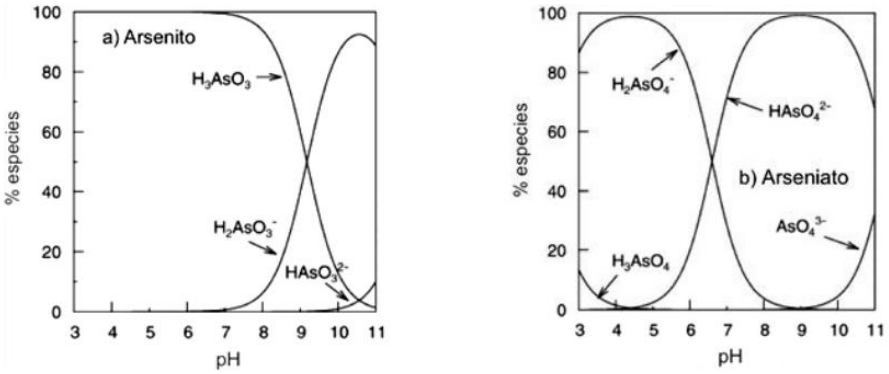


Figure 1. *pH-dependent As speciation (Smedley y Kinniburgh, 2002, taken from (19))*

Arsenic mobility is related to the content of iron, manganese, and aluminum oxides or hydroxides, due to the strength of absorption that these components have over As or how much they can trap through a crystallization. Iron oxide is vital when talking about the mobility of As in the soil. Another factor affecting the mobility of As is the organic matter (24,26–28). The presence of As in natural waters is also related to the presence of iron oxides in reductive environments, in these environments the iron hydroxides solubilize. Therefore, an increment of concentration of iron solubilized in water can be accompanied by an increase of As (29,30).

Another physicochemical characteristic of the soil that affects the mobility of the metalloid is the presence of phosphorus (P), and Silicon (Si) (e.g., *As(V)* competes with phosphorus, essential mineral for plant nutrition, for the adsorption sites on soil particles and transportation through the plastic membrane of the roots. At the same time, *As(III)* competes with Silicon for absorption sites on iron oxide surfaces, and it is also absorbed by root through Silicon path) (1,26).

2.2 Biotransference of As

Biotransference refers to the ability of a living organism to transfer certain chemicals through its inside.

This process depends on the oxidation state of the metalloid and causes different responses in the exposed organism, which may include, reduction, methylation, chelation, exudation, exclusion, accumulation, or translocation of the element chemistry (31–33).

A wide diversity of plant species has the ability to bio transfer *As* present in water and soil. And their response will depend on their own characteristics (i.e. detoxification mechanisms against metal accumulation) and their tolerance to heavy metals (31).

Several plant species can accumulate As after being bio transferred from the soil, e.g., inorganic *As* commonly accumulates mainly in the roots.

Once absorbed through the roots, *As* can be stored, expelled (exudation), or transferred to other parts of the plant through the xylem.

The mechanisms of tolerance of plants are showing in Table 1 (34,35).

Table 1. *Tolerance mechanisms of plants.*

Internal / External	Mechanism	Explanation
Internal	Radical exudates	They influence the solubility of essential and non-essential elements, directly, through acidification, chelation, precipitation and oxidation-reduction processes in the rhizosphere, and indirectly through effects on microbial activity physical properties of the rhizosphere and in the dynamics of root growth.
Internal	Cell wall link	The structural arrangement of cellulose and lignin allows them to form covalent bonds through their oxygen atoms with metals, sequestering them in the apoplast.
Internal	Chelation of the metals by various ligand in the cytosol (phytochelatin)	Formation of non-toxic complexes with metal ions through the interaction with the thiol groups of cysteine (Cys), forming a low molecular weight metal-CF (CBPM) complex, which subsequently binds to ions. Sulfur (S2- in the cytosol, stabilizing the CBPM) forms complex molecules of high molecular weight (CAPM). Consequently, these molecules cross the tonoplast; once inside the vacuole, the organic acids present (malate, citrate, oxalate) retain the ions of the metals and dissociate the FQ-metal complex.
Internal	Chelation of the metals by various ligand in the cytosol (metallothionein)	Mechanism of action homeostatic regulation and tolerance to metals in plants not fully established. Its participation in different plant species has been recognized, as is the case of *Oryza sativa* and *Arabidopsis thaliana* when treated with Cu, Cd, and Zn.
Internal	Chelation of the metals by various ligand in the cytosol (amino acids)	Histidine (Hi) - in hyper accumulative plants, can sequester metal through its carboxylate, amino, and imidazole groups. Proline - Can act as an osmoprotectant or as an inhibitor of lipo-peroxidation, serving in the sequestration of reactive oxygen species.
Internal	Presence of heat stress proteins	They are related to the protection of the photosynthetic system. And stability and repair of proteins during exposure to metal.

Internal	Accumulation in the vacuoles	Fast storage to avoid damage in other tissues. There is a correlation between tolerance and increased vacuolization of root meristem cells in plants.
External	Mycorrhizal fungi (HEM)	1. Mobility of the metal in the apoplast. Retained by the Harting network. Preventing entrance to the root 2. Reduction of metal mobility in apoplast because of the hydrophobicity of fungus. 3. Secretion of chelating substances (organic acids and others). 4. Retention of metals in the other mycelium of the fungus.
External	Arbuscular mycorrhizal fungi (HMA)	1. Extracellular immobilization of metals by organic acids 2. Reduction of the ions transfer from the root system to the stem (intracellular precipitation of the metal by PO^{-4}) 3. Absorption of metal ions in the cell wall of different fungus structures and the retention of the metal in the mycorrhizosphere by the production of specific proteins (e.g., glomalin).

One of the most common responses to arsenic exposition is the accumulation (or deposition) in several tissues. Several authors have demonstrated this; the different parts of a plant (roots, stems, leaves, and fruit) accumulate different amounts of *As* in their tissues. The descending order of this deposition is root-stem and leaf-fruit (17–19).

This descending order is proof and allows us to analyze the journey of *As* once it has been taken by the roots from the water or the soil and then translocated to the aerial organs, decreasing the concentration in its way up (36,37).

Arsenic concentrations in each plant change according to their origin: agricultural area, presence/absence of foundries/mines/metallurgical areas and other industries nearby, compounds of *As* and the rate of contamination of the soil, nature and quality of water and irrigation intensity, as well as the composition of the soil, among others (36,37).

Some researchers have stablished that minimum concentrations of arsenic (as a non-essential element) could have positive effects in the plant development or at least not become phytotoxic(38,39). Nevertheless arsenic is mainly related to negative effects in the

plant development, some of those effects may include, low crop development, reduction in biomass, inhibit root extension, reduced height, reduced leaves number, among others effects (38).

2.3 Biotransformation of As

This biotransformation (transformation of *As* to an organic compound) is related to different biotic and abiotic factors.

In the biotic factor, we find the content of organic material in soils, microorganisms (bacteria and protozoan) in soils and rhizospheres, and some animals and plants, that through some biological process, i.e., excretion processes, can influence this transformation(21,26,40,41).

Among the abiotic factors involved in this transformation, we find the soil's geochemistry and mineralogical composition (21,40,41).

The process of biotransformation has an essential role in the increase of *As* in the environment(5,42).

The process of biotransformation starts with the mobilization of the metalloid from water or soil through absorption in the roots, this absorption in plants, is commonly involved in the energy generation process (*As (V)* substitutes and interfere with the formation of adenosine triphosphate (ATP)) (32,33,43,44).

While in bacteria, the mobilization and absorption process is linked, in some cases, to the respiration process (41,42).

Once an organism absorbs the metalloid, the *As* is biotransformed through reduction and oxidative methylation reactions and even volatilized.

The first step involves a reduction of *As (V)* to *As (III)*, followed by the addition of a first methyl group to obtain a monomethylarsonic acid (MMAsV); then, it could present a second reduction to monomethylarsenic acid (MMAsIII). A second methylation, would produce dimethylarsinic acid (DMAsV), which also can reduce to dimethylarsenic acid (DMAsIII), and so on, resulting in different organic compounds with three (TMAO), four (TETRA) or more methyl molecules (45).

This process has been identified and studied in microorganisms and plants, mainly. Some researchers have the hypothesis that this reduction (from *As(V)* to *As(III)*) is an attempt to reduce the toxicity of *As* in the cells, by the reduction of *As(V)* they can expel *As(III)* out of the cells, and in occasions, out of the organism(32,46). Arsenic biomethylation has been identified in cyanobacteria and algae, among other organisms (26,47–49). And some

protozoan, living in freshwater have been related to methylenation and even volatilization(50,51).

Zheng (2013) explained in a graphic way how the mobilization and transformation of As in a soil-plant system in a rice plant take place (Figure 2). We can appreciate the role of some minerals, such as Fe, and organic matter:

Depending on the nature of the soil, the predominance of arsenic species would be determined, in a well-aerated soil *As(V)* would be predominant, and it could be found adsorbed in some mineral as iron or aluminum, therefore not very mobile. Under reducing environment, the arsenate is reduced to *As(III)*, and becomes more mobile. Microbial species control these redox reactions and conditions in soils. These reduction processes change the pH in soil (due to consumption of protons and the increase pressure of CO_2)(51).

As it was stablished in figure 1, the change of pH causes changes in the availability of arsenic species, with a rise in pH *As(V)* releases from their adsorption sites in different minerals, this increase will also lower the adsorption of arsenic anions(34).

As for the role of organic matter, as some functional groups (COOH, phenol, catechol, OH) have a strong affinity of metal oxides, therefore they complete with As for these adsorption sites in metals. And that could be translated into more mobile arsenic (34).

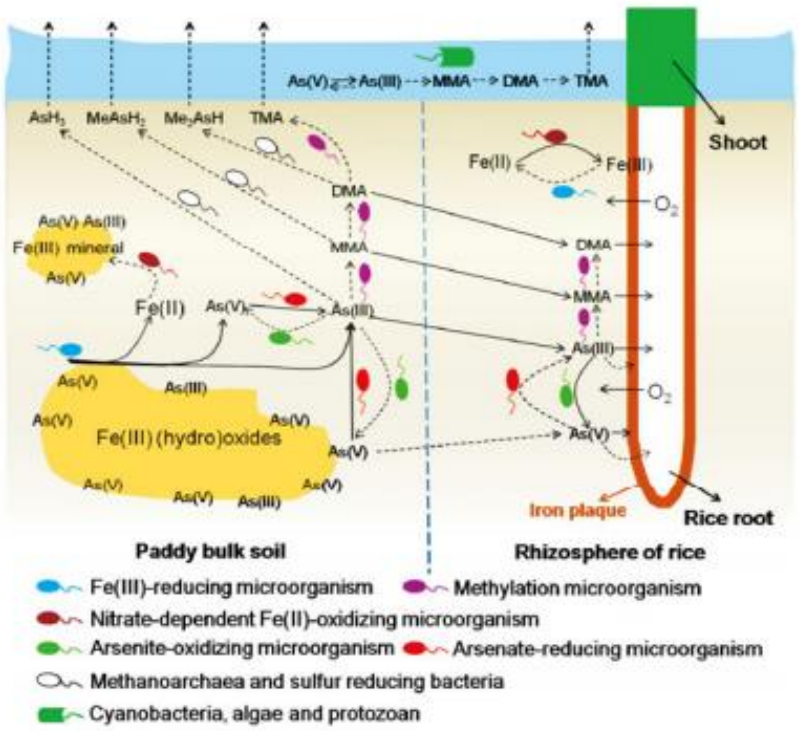


Figure 2. *Arsenic mobilization and transformation drove by microorganisms in flooded paddy soil. Solid lines identify dominant processes (34)*

2.4 Arsenic microbial biotransformation

The presence of pollutants in soil and its effects in the microbial communities has been study from many different point of views, in the case of heavy metals it has been stablished that biomass is significantly correlated to the stress and toxicity of heavy metals produce; other microbial activities seem to decrease as the phosphatase activities have also presented decreases (52–54). But there has been evidence that some microbial activities are stimulated with the presence on certain heavy metals, like, nitrogen-fixing and denitrifying(55).

In the other hand, the microorganisms play a main role in the transformation and transference of As has been known for a while now. The first bacteria with the capacity to reduce (*Bacterium arenreducens*) and oxidize (*B. arsenoxydans*) arsenic were identified in 1918 by Dr. Green, since then other researchers have been able to identify and isolate different bacteria (arsenic resistant, oxidizer or reducers) from soil samples.

The role of the bacteria has not been understood completely, some relations between the arsenic concentration and the number of colonies have been stablished, for instance, Huysmans and Frankenberger, noted a decline in colonies when *As(III)* concentrations were higher than 1 mg/L, while noted no effect with the increasing of concentrations of *As(V)* (56,57).

Diverse species of bacteria have been detected and isolated from various environments (soil, flooded soil, water). Some of these species are capable of synthesizing arsenite oxidase, oxidize *As(III)* into *As(V)* or reduce *As(V*) into As(III), or both. Some of these bacteria are tolerant to arsenate but not necessarily to arsenite. According to Jackson *et al.* (2005) and Han *et al.* (2016) arsenate resistant bacteria (As reducers) are more common in natural environments than arsenite resistant (As oxidizer) ones (46,58,59).

The identified species of arsenic resistant bacteria are distributed along more than 25 different bacteria genera. Some of the bacteria related in the reduction process are: *Bacillus arsenic, Geospirillum arsenophilus, Geospirillum barnesi, Crysiogenes arsenatis, Sulfurospirillum barnesii, Sulfurospirillum arsenophilum,* and *Oselenatis,* among others. In contrast, some of the bacteria related with the oxidation process are: *Thermus thermophiles, Thermus Aquaticus, P. arsenitoxidans, Crysiogenes arsenates,* and B*acillus arsenic oselenatis,* among others. The number of species of arsenic resistant bacteria keeps on growing (38,58,60–62).

It has been thoroughly discussed the known and the possible role of bacteria in a soil-plant system. One of the main roles known is carried out by the plant growth promoting rhizobacteria (PGPR), these bacteria provide phosphate for the plant and sequester Fe through the solubilization of it, this releases de As from the minerals, making it bioavailable(63).

Some researchers have studied the role of certain bacteria, like *pseudomonas sp*., in the enhancement of the growth of plants in the presence of arsenic, these bacteria seem to enhance the growth and development of roots, but do not affect the amount of arsenic adsorbed(64,65). Other researches try to identify the arsenic resistant bacteria so they can be used as part of bioremediation processes, as is the case of *Bacillus sp.* and *Aneurinibacillus aneurinilyticus*(61,66,67).

Other characteristics that's been related with most heavy metals resistant bacteria is that most of them seem to be Gram-positive. Biswas *et al.* identified isolated from severely arsenic contaminated groundwater in India, a series of reducing arsenate bacteria that turned out to be Gram-positive heterotrophic bacteria, they also present resistance to Hg, Zn, Cr, Cu, Cd, Ni (66,68).

Even though here our main focus is the behavior and possible role of the arsenic resistant bacteria in the process of biotransference and biomethylation, other considerations must be taken in account, for example, the role of other rhizospheric components, as iron(69).

One of the things that researchers have clear is that the bio transformation (reduction, oxidation or methylation) of arsenic, depends on a lot of different factors: characteristics of the soil, the microbial communities available, rhizospheric components, species of arsenic, among others (26). It is true that we have just begin to understand this process.

2.5 Toxic effects of As

The toxicity of *As (III)* relies on its ability to bind the sulfhydryl groups in proteins, causing its inactivation. It can also act as an endocrine disruptor by binding to hormonal receptors and interfering with intracellular signaling.

The toxicity of *As (V)* is due to phosphate substitution and inhibiting oxidative phosphorylation; Its mechanism of action is related to the competitive replacement of inorganic phosphate by an $(AsO_4)^{3-}$ in the formation of adenosine triphosphate (ATP) (32,33,43,44).

Studies carried out in different experimentation protocols have indicated *As (III)* as the main element involved in the various systemic alterations observed after exposure to this element. However, the trivalent organic compounds, as MMAs and DMAs (III), products of a biotransformation, present a higher degree of toxicity than that observed in some inorganic compounds (table 2) (70–73).

Table 2. *Toxicity levels of arsenic compounds*

Species	As (III)	TETRA	MMAs	DMAs	TMAO	AsB	AsC
LD_{50} (g kg^{-1})	0.0345	0.89	1.8	2.1	10.6	>10	>6.5

As exposure has been widely studied, mainly in humans, several carcinogenic effects have been related to it; However, non-carcinogenic effects continue to show new signs of toxicity, such as peripheral vascular disorders, liver, kidney, lung, bladder, neurotoxic and metabolic disorders, such as diabetes mellitus (74–76).

All health effects recognized are classified by ATSDR in seven categories: cancer, developmental effects, reproductive effects, neurological effects, immunological-lymphoreticular effects, systemic effects, and death. This US agency has established minimal risk levels (MRL: "estimate of the amount of a chemical a person can eat, drink or breathe each day without a detectable risk to health"), Table 3 (4).

Table 3. *Minimal risk levels reported by ATSDR for arsenic. Acute (<15 days) and chronic (>365 days) exposure.*

Exposition route	Duration of exposition	MRL	Endpoint
Oral	Acute	0.005 mg/kg día	Gastro.
Oral	Chronic	0.0003 mg/kg día	Dermal

Given the exposure problems, arsenic limits have been established by different organizations at national and international levels, Table 4.

Table 4. *International standards for arsenic exposure.*

Scope	Level	Set by
Food	0.5 – 2 mg/kg	EPA (77)
Water for human consumption	10 µg/kg	EPA (77)
Human general consumption (drink + food)	0.025-0.040 mg/ kg day	WHO (78)
Water for human consumption	0.025 mg/L	SSA (Mexico) (79)
Agricultural water	0.1 mg/L	CONAGUA (80)
White rice	0.2 mg/kg	AECOSAN (81)
Max. Daily human general consumption	0.3 – 8 mg/day	EU (82)
Land for agricultural, residential and commercial use	<22 mg/kg	SEMARNAT(83)

2.6 As in Mexico

The presence of *As* in the water of different regions in Mexico has been recorded and reported on several occasions.
In the north, the region known as Comarca Lagunera, which includes the states of Coahuila and Durango, has reported Arsenic in its waters since the 60's.

The northern region has been the most studied in the country because, even though the concentrations vary over time, the maximum levels detected in wells are up to 865 µg/L (Tlahualilo, San Francisco I. Madero and San Pedro) (Table 5) (14).

The Comarca Lagunera region has a chronic hydroarsenicism zone. Between the 1970s and 1980s in some rural populations of Coahuila and Durango, some epidemiological studies showed a high incidence of pathological conditions attributable to the presence of As such as skin lesions and peripheral vascular diseases. In 1983, an average concentration of *As* in the water of 0.411 mg/L, and a prevalence of cancerous skin lesions in the San Salvador de Arriba municipality of Francisco I. Madero, Coahuila (84). In 1986, the Autonomous University of Coahuila (UAdeC, by its acronym in spanish) evaluated the health status of 5,903 inhabitants of the Comarca Lagunera region, of which 204 (3.4%) had arsenic-related lesions, and 15 (0.2%) had cancerous lesions, all of them from the municipality of Francisco I. Madero (85).

In the north, in Sonora, concentrations of 2–305 µg/L were found in Hermosillo, Etchojoa, Magdalena, and Caborca. In the Sierra de Huautla the concentration of As is estimated between 0.5-0.7 mg/L(85).

A high concentration of arsenic (91 mg/L) recorded in Matehuala (San Luis Potosí, centric state), according to Martínez-Villegas (2013), is due to the presence of an old foundry plant nearby the river analyzed, this river supplies a craft complex, and the water does not commonly drink (86,87).

Inhabitants of Zimapán, Hidalgo exposure to highest arsenic concentrations on consumption water, with concentrations up to 1350 µg/L, presents hyperkeratosis ulcers and skin hyperchromies. In 1996, a group of Zimapán girls had severe vascular lesions related to chronic arsenic poisoning (88).

Also, in communities of Aguascalientes, Guanajuato, Jalisco, Sonora, and Zacatecas have concentrations above the limits (69). Nevertheless, the concentration of As in many other areas of high risk of hydrogeological contamination.

Table 5. *Arsenic concentration in water that exceed established standards in Mexico.*

Community	Concentration mg/L	State
Matehuala	91.05	San Luis Potosí
Zimapán	1.35	Hidalgo
Francisco I. Madero	0.86	Coahuila
Tlahualilo	0.59	Durango
San Pedro de las Colinas	0.49	Coahuila
Los Nogales 1	0.27	Chihuahua
La Trinidad	0.18	Chihuahua

The problem of arsenicism in Mexico became critical because the main exposure to this metalloid was, considered to be through the consumption of water. Based on this, different methodologies for treating water decrease the concentration of As in drinking water. However, now that has been proved that As can be incorporated through the consumption of contaminated food, after irrigation with water polluted with As.

2.7 As in agricultural plants

Among the vast diversity of plant species with the ability to bio transfer arsenic present in water and soil, we can find several agri-food species.

This biotransference by agri-food has been recorded in several studies conducted in various countries like Argentina, Australia, Bangladesh, Brazil, Belgium, Canada, China, Korea, Egypt, Spain, United States, France, Jamaica, Japan, Italy, Pakistan, Sweden, and Thailand (89).

Crop plants in which preexist evidence of inorganic compounds of *As* are diverse, Munish *et al.* (2019) recollected the data recorded in different studies from different countries, founding *As* in over 20 different crops, meats and other food products.

Potatoes, tubers, carrot, pepper, onion, cucumber, eggplant, lentils, and rice report higher concentrations of *As*; Despite this, there are also records of *As* in lettuce, apples, bananas, tomatoes, and edible mushrooms (89).

The tubers accumulate the highest levels of total arsenic (AsT), followed by leafy vegetables, fruits, and legumes, being fruit trees reporting the lowest levels of all (36,90,91).

Among the most relevant results are the total arsenic concentrations accumulated in crops, like, in descending order: tubers such as radish (674 ± 211 µg/kg) and potato (291 ± 176 µg/kg); vegetables such as cabbage (315 ± 69.7 µg/kg) and spinach (270 ± 182 µg/kg); leafy vegetables represented by amaranth leaves (265 ± 158 µg/kg); fruits such as bitter gourd

(262 ± 133 μg/kg), eggplant (217 ± 80 μg/kg) and tomato (84.4 ± 48.5 μg/kg) and finally legumes such as lentils (24.7 ± 16.7 μg/kg) and peas (69.2 ± 22.9 μg/kg).

Different experiments carried out in agri-food plants (such as those carried out by Samal *et al*. (2011), in Nadia, from the West Bengal district in India) have corroborated the metalloid's absorption from irrigation water; this was also corroborated and studied by Smith *et al.* (2009) in Australia, Smith hydroponically grew radishes, chard, mung beans and lettuces, the irrigation water was a mix of nutritional solution and 2 ppm of As(V), in this case the main concentration was found in root, while the radish adsorbed the highest arsenic concentration in its edible part(91).

These studies and many others are further proof that crops irrigated with water contaminated with As represent a health risk through the water-soil-food chain. Besides, it helps us realized that there is a direct relation between the amount of water used, the concentration of arsenic in it and the amount of *As* absorbed (92,93).

Knowing that *As* can be present in both soil and water, Biswas *et al.* (2012) analyzed different vegetables grown in contaminated soils; found that among all the vegetables analyzed, legumes had a higher concentration of AsT, especially peas (1300 ± 480 μg/kg) and lentils (1120 ± 144 μg/kg). Followed by onions (187 ± 77 μg/kg), spinach (910 ± 259 μg/kg), tomatoes (551 ± 262 μg/kg) and bitter gourd (529 ± 44 μg/kg) (90).

In Latin America, there are few works on the accumulation of *As* by agri-food plants after irrigation with contaminated water; A research with vegetables exposed to polluted soil and water was carried out in Chile, where they identified arsenic in lettuces, chards, potatoes, carrots, among others(94). In Mexico, AsT has been identified, so far, in epazote, coriander, marjoram, green tomato, chili, chilacayote, orange, loquat, banana, pomegranate, onion, and corn (95–97).

Another variable that we have to consider in this aspect is the arsenic compounds available for absorption by the crop; in the United States, the rice plant's availability for the uptake of *As (III)* is higher than that of *As (V)*, *MMAs* and *DMAs*. The rice accumulates the majority of inorganic As in its roots (98). While in an Australian experiment, radish, chards, lettuces and mug beans have reported less than 1% of the total arsenic as organic arsenic(91). An investigation with tomato, carried out in Spain and Korea, noted that most of the inorganic compounds stay stored in the roots. In contrast, organic compounds tend to be in the fruit and aerial parts (99,100).

In Mexico, the translocation of arsenic present in the soil (i.e., old mining places) in corn crops showed the highest concentration in the part of the crop area (101).

All these studies provide conclusive evidence that vegetables obtained from crops in agricultural areas contaminated with As, through water or soil, can significantly impact the toxic potential of agri-foods for human and animal consumption. It also gives evidence about

how different the plant's reaction to arsenic exposition can be, because it depends on many other factors that differ from country to country and from plant to plant. Knowing and understanding how the system soil-plant reacts to the presence of arsenic would help us to avoid overestimating the toxic risk they pose to health (36,73).

The lack that most research presents regarding the concentration of As in crops in Mexico, difficult the actions to establish proper laws that allow food security for the population.

3. JUSTIFICATION

A wide diversity of agri-food plant species has the capability of bio transfer As present in water and soil; the metalloid has been found in over 20 different plant species. The concentrations of As depend on the specific characteristics of each plant species, the pollution rate of the agricultural field, the source of the water, the intensity of irrigation, and the composition of the soil (36,37,102).

The bio transference of As to diverse vegetables is a worldwide problem. Various species of vegetables and fruits like tubers, carrots, chili, onion, cucumber, eggplant, lentils, rice, lettuce, apples, bananas, tomatoes, edible mushrooms and rice have present the metalloid. It has also been demonstrated that the different plant parts (roots, stems, leaves, and fruits) accumulate different amounts of As in their tissues, according to the concentration of As to which they are exposed (17–19).

The present records show that agri-food, for human or animal consumption, contaminated with As maybe a second and important source of exposition; this exposition which can biomagnifies the toxic effects of the metalloid.

Despite all the researches done on the concentration of *As* in plants for agricultural use, it is clear that the reaction of each vegetative species varies from one to another, we still need to explore the ecological capacity of plant species to survive and react to the exposure. The possible toxic effects and affectations in their development (height, weight general, or of specific parts), as well as the understanding of the distribution of As within the different parts of the plant, are essential parts of this analysis. The approach established for this research is the consideration of the whole, as a system, the water-soil-bacteria-plant system.

The bacterial population, in soil and rhizosphere, has an important role in the transformation, transference and, maybe, the resistance of plants to the exposition of arsenic.

The vegetable species selected for the present research are radish, tomato and lettuce. These represent some of the most common and consumed vegetables in Mexico, they also represent different types of vegetables: radish (tubers), lettuces (leafy) and tomato (aerial with fruit).

4. HYPOTHESIS

The crops of *Raphanus sativus* (radish), *Solanum lycopersicum* (tomato), and *Lactuca sativa* (lettuce) under greenhouse conditions perform the bio transfer of As to roots, stem, leaves and fruits.

5. GENERAL OBJECTIVE

To evaluate the capacity of biotransference of arsenic in crops of *Raphanus sativus* (radish), *Solanum lycopersicum* (tomato), and *Lactuca sativa* (lettuce) under greenhouse conditions.

5.1 Specific objectives

- Evaluate changes in the microbiological and physicochemical characteristics in soil polluted with As (III) and As (V) through irrigation water in the crop of *Raphanus sativus* (radish), *Solanum lycopersicum* (tomato) and *Lactuca sativa* (lettuce).
- Evaluate vegetative development characteristics (root density, stem length, number of fruits, number of leaves) of crops of *Raphanus sativus* (radish), *Solanum lycopersicum* (tomato), and *Lactuca sativa* (lettuce) after exposure to As (III) and As (V) through irrigation water.
- Quantify AsT concentration in soil, water, root, stem, leaves, and fruit of each crop through.

6. METHODOLOGY

6.1 Establishment of crops under greenhouse conditions

The vegetable crops selected for this project are *Raphanus sativus* (radish), *Solanum lycopersicum* (tomato) and *Lactuca sativa* (lettuce) (fig 2).

6.1.1 *Solanum lycopersicum* (*Lycopersicon esculentum* P.Mill)

Characteristics: It belongs to the *Solanaceae* family along with tobacco, chili, and potatoes. Its name comes from Nahuatl, Xictlitomatl ("belly button tomato"). It is an herbaceous plant with alternate leaves and flowers in the form of yellow or white stars. Red fruits weigh up to 750 grams. Tomato is an essential vegetable in the world after potatoes. Mexico is in the tenth producer top of this vegetable in the world (103).

The vegetative cycle of tomato is showing in table 6 and figure 3.

Table 6. *Vegetative cycle: Phenological stages of tomato*

Stage	Explanation	Days elapsed since planting
Seedling production	From the germination and emergence of the seedling	0-30
Vegetative development	The pant until the first flowering (formation of 5 to 10 leaves, height greater than 40 cm).	31-63
Flowering	Vegetative and reproductive growth. New leaves and flowers appear, and fruits.	59-98
Fruit filling	Production stage, the first fruits develop, and maturity begins.	114-158
Harvest	While harvesting the plant continues to develop new leaves and flower clusters	189-248

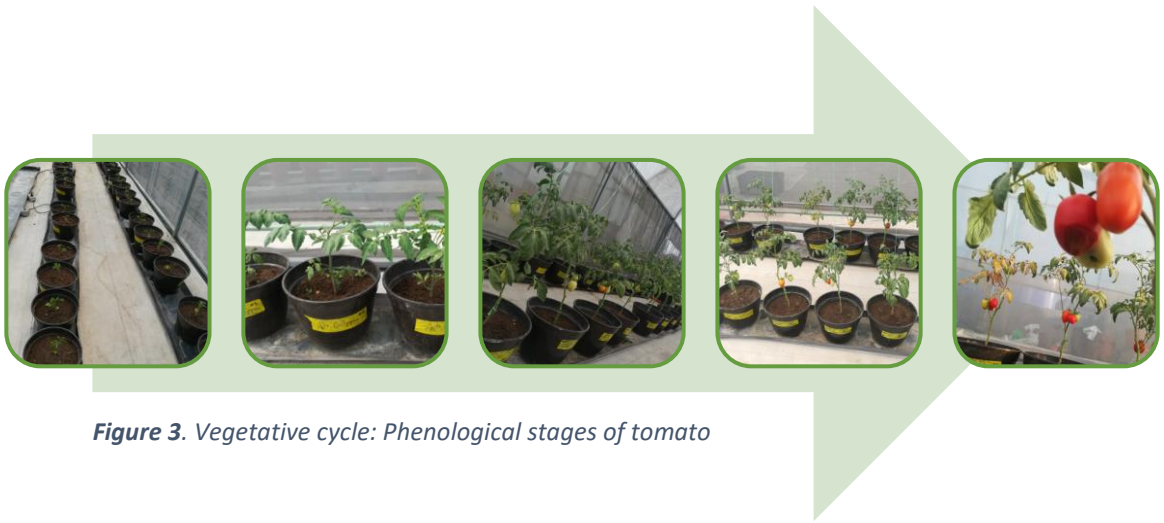


***Figure 3**. Vegetative cycle: Phenological stages of tomato*

For this project, the cultivation of this species started from seedling, into individual pots. The tomato seedlings were planted at enough depth to ensure total root coverage.

When the seedlings reached a height of 15 cm, a raffia guide to place at the top of the greenhouse, this guide prevented the plant's inclination and supported it throughout its growth.

6.1.2 *Raphanus sativus*

Characteristics: The plant height ranges from 0.5 to 1.20 meters, with a smooth and widely branched stem. Its leaves are finely pubescent with irregularly serrated edges. The root is edible (104).

Vegetative cycle (phenological stages): the crop cycle can go from 3-6 weeks, depending on the climatic conditions and the variety of radish. Commonly in the summer season, the average crop is of 30 days and in winter of 60. Below is a standard description of the stages of development (Table 7, figure 4).

***Table 7.** Vegetative cycle: Phenological stages of radish*

Stage	Explanation	Days elapsed since planting
Germination	Appearance of first cotyledons	0-15
Flowering	Appearance of true leaves	16-24
Flowering	Root and fruit development.	25-42
Harvesting	While harvesting, the plant continues to develop new leaves.	42-50

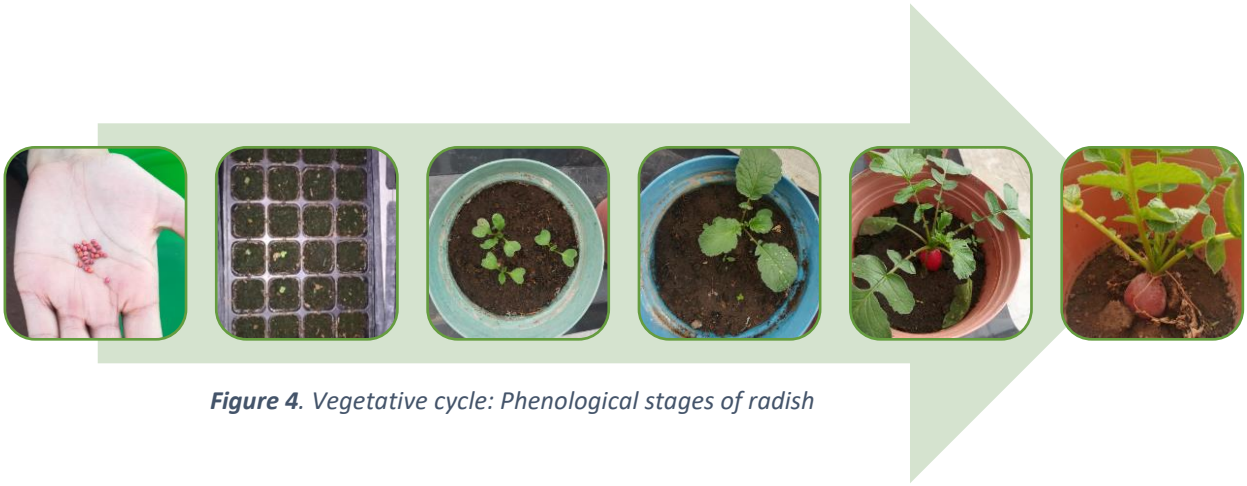


***Figure 4**. Vegetative cycle: Phenological stages of radish*

For this project, this specie was grown from seed. Three seeds were planted at a depth of 5 mm per pot to ensure growth. The radishes after the germination were transplanted in individual pots.

6.1.3 *Lactuca sativa* (variety capitata)

Characteristics: With sessile leaves forming a dense rosette around a short stem. There are considerable diversities of colors, and sometimes it can exhibit reddish spots.

Vegetative cycle (phenological stages): Its vegetative cycle is from 3 to 4 months. This cycle is affected by high temperatures and the variety of lettuce (Table 8, figure 5).

***Table 8.** Vegetative cycle: Phenological stages of lettuce.*

Stage	Explanation	Days elapsed since planting
Seedling production	Appearance of the radicle, cotyledons emerge. Appearance of 3 - 4 real leaves.	1-30
Rosette development	Appearance of new leaves. Rosette formation with 12 - 14 leaves.	30-70
Head appearance	Leaves wider leaves curved along the axis of the central rib in an upright position.	70-95
Flowering	The head loses quality and acquires an elongated shape. Stem elongation. Height from 1 to 1.5 meters.	95-105
Harvesting	Harvesting	105

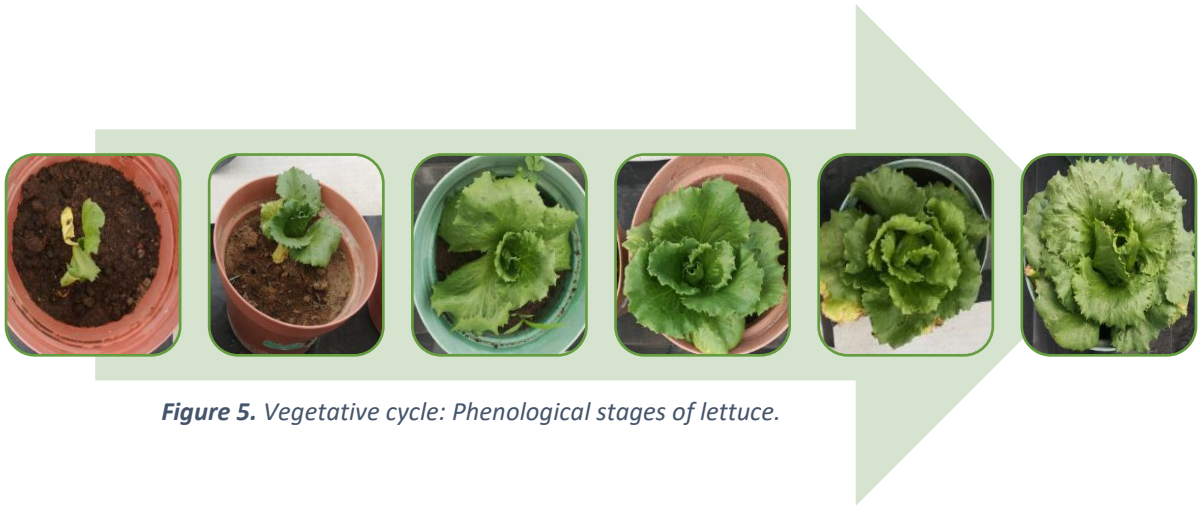


Figure 5. *Vegetative cycle: Phenological stages of lettuce.*

The seedlings of lettuce were planted at enough depth to ensure total root coverage.

6.1.4 Greenhouse Conditions

The plants under controlled conditions of temperature (20°C - 40°C) and humidity (30% - 60%), were placed at random (n = 4) in groups by treatment in the greenhouse of the Engineering Faculty of the Agroindustrial area of the Autonomous University of San Luis Potosí (UASLP, by its acronym in Spanish) (fig. 6).

Figure 6. *Greenhouse crops: A) tomatoes, B) lettuces, C) radishes, D) tomatoes.*

Before use all the pots were cleaned with water and disinfected with a 20% solution of commercial-grade sodium hypochlorite. They were left in the sun for 4 hours for drying.

A piece of cloth (pellon) is placed at the bottom of each pot to avoid the exit of the ground, and then they were filled with agricultural soil. The pots used have a diameter between 22-30 cm; each pot received between 2.5-3.0 kg of soil (a mixture of soil and sand). All pots had holes in the bottom as a means of drainage.

All the treatment groups were added with a nutrient solution to simulate the cultivation conditions in the open field and establish under these conditions, the influence of As(III) and As V on the biotransference of arsenicals towards plants.

The nutritive solution (SN) contains phosphoric acid, calcium nitrate, potassium nitrate, magnesium sulfate, and Multimicro®, in the concentrations shown in Table 9.

Table 9. *Composition of the nutritive solution.*

Component	Original concentration	Volume used in the SN
Phosphoric acid	22 mL/600 mL	20 mL
Calcium nitrate	114.8 g/600 mL	20 mL
Potassium nitrate	65 g/600 mL	20 mL
Magnesium sulfate	82 g/600 mL	20 mL
Potassium sulfate	15g/600 mL	20 mL
Multi micro	15 g/600 mL	20 mL

6.2 Arsenical Treatment

The arsenic irrigation was every three days, leaving a rest day between each arsenic irrigation. Depending on the environmental conditions and the humidity of the crops in the rest day sometimes they were watered with clean water. Additionally, they were supplied with a nutritive solution once a week. An example weekly schedule shown in Table 10.

Table 10. *Weekly Schedule simulation.*

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Arsenic	Water (if needed)	Arsenic	Water (if needed)	Arsenic	Nutritious solution

The appropriate volumes of irrigation were measured, to avoid leaking and loss of *As*, and to assure the appropriate humidity of soil; see Table 11.

***Table 11**. Irrigation volumes by species.*

VEGETALE	*QTY. WATER*
RADISH	250 mL
LETTUCE	300 mL
TOMATO	300 mL

Regarding arsenic solutions: 3 concentrations of arsenic were selected.

- 0.1 ppm – maximum concentration for irrigation water in Mexican standards.
- 0.3 ppm – approximate concentration reported in groundwater in Chihuahua.
- 0.6 ppm – average concentration of reports in agricultural areas in other northern states.

For the preparation of the arsenic solutions, $NaAsO_2$ was used for *As(III)* solution, considering the molecular weight of $NaAsO_2$ (129.91 g mol^{-1}) and the salt purity (99%). Na_2HAsO_4 heptahydrate was used for *As(V)* solutions, considering the molecular weight of Na_2HAsO_4 (312.01 mol^{-1}) and purity (99%). All calculations involved the molecular weight of arsenic as: 74.92 g mol^{-1}.

The vegetable groups were divided into the four groups of exposition randomly (Table 12). The arsenic irrigation time is of one month for radishes, two months for tomatoes and lettuces.

***Table 12.** Distribution of plant species according to the type of arsenic and concentration.*

Number of individuals exposed to arsenic								
Plant species	As III			As V			Water (control)	Total
	0.1 ppm	0.3 ppm	0.6 ppm	0.1 ppm	0.3 ppm	0.6 ppm		
Tomato	4	4	4	4	4	4	4	28
Radish	4	4	4	4	4	4	4	28
Lettuce	5	5	5	-	-	-	5	20

The exposition to *As(III)* of radishes and lettuces started in March. While the administration of *As(V)* of all vegetables and *As(III)* of tomatoes started in April. Unfortunately, the lettuces were only exposed to *As(III)*, because it was not possible to obtain more seedlings on time to *As(V).*

6.3 Soil Analysis

This analysis consisted of 2 parts: microbiological and physicochemical analysis.

6.3.1 Physicochemical Analysis during the phenological cycle.

The register of humidity, conductivity, and pH during the phenological cycle of the crops.

6.3.1.1 pH measurement during the phenological cycle

For experimentation purposes and to ensure the health of the plants during the experimentation, pH measures were carried out once a month per pot. For this purpose, a portable potentiometer of the brand Kelway, model HB-2 was used (Fig. 7). It has a measuring capacity from 3.5 to 8.

Measurement method: Before inserting the potentiometer, ensure that the needle points to 7. Then the equipment was firmly inserted into the ground, making sure to cover the electrode with earth.

Figure 7. *Equipment used for pH measurement in a tomato pot.*

6.3.1.2 Humidity measurement during the phenological cycle

For experimentation purposes and to ensure the health of the plants during the experimentation, humidity measures were also carried out once a month per pot. For this purpose, the equipment used was a Lustron brand soil moisture meter (fig. 8), model PMS-714. Which has a measuring capacity of 0% to 50%; if the humidity is higher than 50%, the screen shows three horizontal lines.

Measurement method: Before inserting the device, the screen should read zero. Then the equipment was firmly inserted into the ground, making sure that the electrode was covered entirely.

The stabilization of the device varies according to the earth's condition, so it was monitored until the numbers on the screen stop changing (<1 minute).

After removing the moisture meter, a cleaning with water was made to prevent the transfer of soil to another pot.

Figure 8. *Equipment used for humidity measurement in a radish pot*

6.3.1.3 Conductivity measurement during the phenological cycle

For experimentation purposes and to ensure the health of the plants during the experimentation, conductivity measures were carried out. The equipment used was a Soil Test™ HI 98331, brand TPM (fig.9). It has a measure capacity from 0,00 to 4,00 mS/cm.

Measurement method: Before inserting the device, the screen should read zero. The equipment was firmly inserted into the ground, making sure that the electrode was covered entirely.

The stabilization of the device varies according to the earth's condition, so it was monitored until the numbers on the screen stop changing.

After removing the moisture meter, a cleaning with water was made to prevent the transfer of soil to another pot.

The recommended interval for conductivity is from 0.5 - 1.9 mS/cm, depending on the development stage of the plant.

Figure 9. *Equipment used for conductivity measurement*

6.3.2 Physicochemical Analysis after harvest

6.3.2.1 Obtaining soil samples and pH measurement

The soil samples were obtained at the beginning and the end of the phenological cycle of each crop. In harvesting time, the plants were extracted and separated in their vegetative parts, and soil was sieved to extract any plant traces.

Homogenized soil was sundried and taken a sample in tubes of 30 ml of each pot of each group of treatment. The soil samples were divided into two portions: one part for microbiological analysis and the other for physicochemical analysis.

6.3.2.2 Soil physicochemical analysis

Following the methodology established by SEMARNAT (1 gram soil diluted in 10 ml of distillated water) the measurement of pH was carried out with a portable pHmeter Yieryi SA-0259 (105).

6.3.3 Soil Microbiological Analysis

The culture mediums used were: LB, B King, Nutritious, and NBRIP. According to the type bacteria group. The formulation and reference culture times used correspond to those reported by Ahmad (2006); Table 13.

Table 13. *Used culture media and its components (66).*

Medium	Type of bacteria/ fungus	Components	Culture times
LB	All	Peptone, yeast extract, NaCl, Agar	48-96 h
B King	Pseudomonas	Peptone protease No. 3, K_2HPO_4, $MgSO_4 \bullet 7H_2O$, Glycerol, Agar	48-72 h
Nutritious	Sporulated	Commercial medium	5-6 days
NBRIP	Phosphate solubilizers	Glucose, Agar, KCl, $MgSO_4 \bullet 7H_2O$, $MgCl_2 \bullet 6H_2O$ $Ca_3(PO_4)_2$, $(NH_4)_2SO_4$	48 -72h

Starting of dilution 1: 1000 (1 g of homogenized soil diluted in 1 mL of distilled water) were prepared dilutions consecutively with 100 microliters of each solution in 1 mL by two more times. 20 µL of these solutions were placed in the medium of culture in a sterile atmosphere, during 24 and 48 hours in an incubator at 28 °C.

The counted bacteria colonies were related at the end of this time. According to the bacteria colonies obtained, the culture was repeated at the same or a higher or lower dilution if needed, to corroborate the results. Each culture was repeated twice.

After the initial counting the colonies that were well defined, with a defined color and that were present in more than one exposition group were isolated and replanted in new culture media. After 48 hours they were tested for Gram positive and negative The Gram test was carried using methyl violet and a microscope to corroborate the stain color. The bacteria stained violet or dark blue are categorized as Gram-positive; meanwhile, the ones stained pink are Gram-negative (66,106,107). The values of bacteria colonies are reported in the number of colonies.

6.4 Physical analysis of vegetative parts

The following measurements were considered as part of the physical vegetative analysis: growth (development through time), final weight and length of the vegetative components (roots, stems, leaves and fruit). And in the specific case of tomatoes, the number of fruits.

6.4.1 Vegetative growth

As a part of this physical analysis the height or diameter is the main measurement considered. For each vegetable different considerations were taken.

On a general basis, all height measurements were done by using a commercial measuring tape of 60 cm. The measure was carried out individually for each pot and was done once a week. All recordings were done in cm and recorded in a virtual log. The measures were taken following what is explained below for each vegetable species.

6.4.1.1 Radish

For radishes the measure of the height was made from the soil level in the pot to the tip of its leaves, registering the higher result. Figure 10 exemplifies the process.

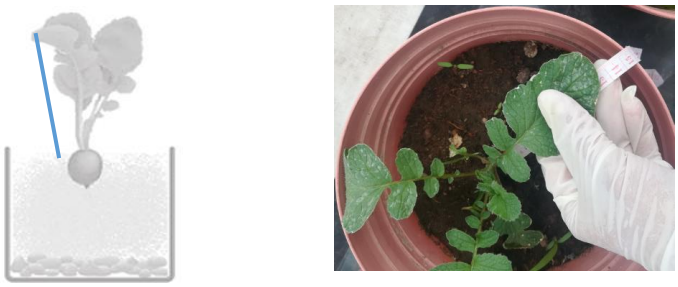


Figure 10. *Exemplification of the radish vegetative growth measurement (67)*

6.4.1.2 Lettuce

In the lettuce, the diameter of the growth was the measurement recorded. The recording involved two measurements as explained in the following in figure 11.

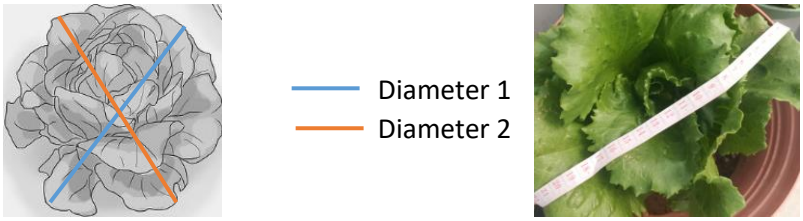


Figure 11. *Exemplification of the measurement of the vegetative growth of lettuce (68).*

6.4.1.3 Tomato

In the case of tomatoes, the measurement of the height was made from the soil level in the pot to the tip of its leaves, registering the higher growth; using the raffia guide as a guide for the placement of the measuring tape. The process is exemplified in the following figure 12. As part of this analysis the number, size and color of fruits is also registered.

Figure 12. *Exemplification of the measurement of the vegetative growth of tomato (69).*

6.4.2 Final vegetative measurements

When the harvest time was appropriate, the vegetables were carefully removed from the pots.

The different parts were separated (root, stem, leaves, fruit, and soil), photographed, measured with a common ruler, and weighed with a portable balance OHAUS, CS Series. The roots were recovered from the soil through sifting.

The different parts were placed in paper bags, they were exposed to indirect sunlight in the greenhouse to initiate the drying process. They were exposed to indirect sunlight 1-2 weeks until dry. Finally, they were taken to the laboratory and pulverized. Values for the physical vegetative analysis were reported in grams (g) and centimeters (cm).

6.5 Quantification of total As

6.5.1 Sample preparation

Each vegetative part (root, stem, leaves, fruit) of each exposition group was homogenized, meaning all vegetative individuals exposed to the same arsenic concentration were mixed.

To each sample (0.1 g) of pulverized dried vegetable or soil of each exposition group 3 mL of HNO_3 were added and left for four days under environmental conditions. After the four days, 10 mL of H_2O_2 were added (fig.13). The samples were left to rest one more day (108). The previous digested samples were diluted with deionized water until the acid concentration reaches 10%, meaning that these samples received 17 ml of deionized water. At the end process of digestion, these were filtered with Syringe micropore filters. For each vegetative part exposed two samples were taken.

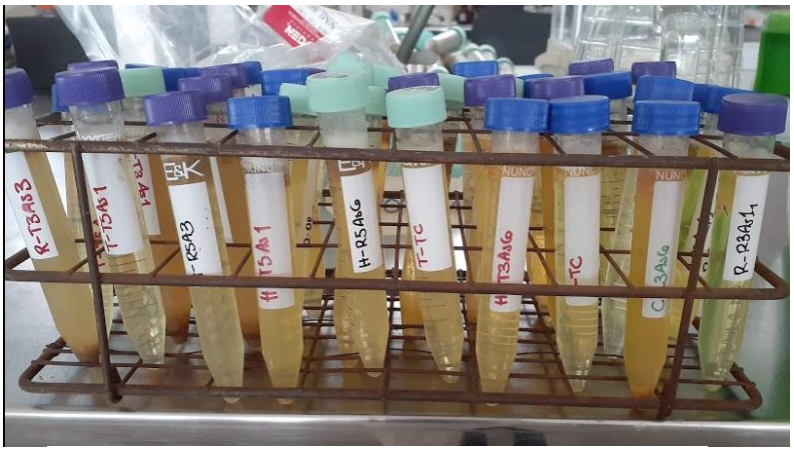


Figure 13. *Samples under digestion with HNO_3 and H_2O_2.*

6.5.2 Total Arsenic

For this measurement, a dilution 1:1 (0.5 ml of digested sample and 0.5 ml of water) was poured in each graphite cylinder. Each measurement is repeated 3 times by the equipment. Two samples for each vegetative part exposed were introduce into the equipment.

The calibration curve was realized based on a 100-ppm standard solution, considering every 20-ppm an interval. Therefore, obtaining a curve with values of 0, 20, 40, 60, 80, and 100 ppm. This curve was done daily for as long as the measurement took place. Along the 5 days of measurement de correlation factors had an average of 0.9663±0.0057.

The equipment used to quantification arsenic total was an Atomic Absorption Spectrometer (SpectrAA 220Z) (fig. 14) (108,109). The values for total arsenic concentration are reported in parts-per-billions (ppb).

Figure 14. *Atomic Absorption equipment used for the AsT measurement*

6.6 Statistical analysis

For the bacterial cultivation, the analysis of variance through a Kruskal-Wallis test and Mann-Whitney test were carried out.

For the arsenic concentrations in vegetative parts, water and soil, the analysis of variance through a factorial arrangement 3x3x3 and Tukey's mean grouping was used to determine the significant difference between the different exposition groups that comply with a normal distribution.
The analysis of variance through a Kruskal-Wallis test and Tukey's mean grouping were used to determine significance difference between the different exposition groups that did not comply with a normal distribution.

The normal distribution was determined through Shapiro-Wilcox test.
All statistical analysis was performed using RStudio program. The statistical significance assumed was $p<0.05$. All experiments were conducted with two replicates by treatment.

7. Results & Discussion

In the following sections the results and theories formulated around said results are shown. The results and analysis correspond to those of soil analysis (7.1) during and after harvest and the microbiological analysis, followed by the Physical examination of vegetative parts (7.2) during and after harvest, and the chapter finishes with the total arsenic concentrations (7.3) registered in the different vegetative parts.

All the tables presented below represent the average data of the groups.

The data corresponding to pH, conductivity, humidity, as well as the gram test results, are not submitted to statistical analysis.

7.1 Soil Analysis

7.1.1 Physicochemical analysis during the phenological cycle

Exposed below are the average results for pH, humidity and conductivity for the different vegetative species.

All the treatment groups were added with a nutrient solution to simulate the cultivation conditions in the open field. The recommended interval for conductivity is from 0.5 - 1.9 mS/cm, depending on the development stage of the plant. While the recommended pH must be over 6.0 and over 8.0, and the humidity must be above 50% (110).

The average pH for all the treatment groups and vegetables was 6.7.

Respect to conditions of humidity. The average dates are reported in table 14. The humidity always reached more than 50% in all vegetables. These measurements helped us keep the optimal conditions for the growth of vegetables.

Table 14. *Average humidity registered before watering for vegetables during cropping time.*

Vegetable	Humidity before watering %
Radishes	38% ± 6
Lettuces	32% ± 8
Tomatoes	50% ± 2

The average conductivity in the exposition groups registered a greater interval, than those registered in the control groups. In control groups conductivities were more stable. The conductivities are reported in table 15.

Table 15. *Average conductivity for each group of exposition In all vegetables.*

Exposition group	Average conductivity (mS/cm)	Value *p
Control	1.4875 ± 0.15	
As(III) 0.1 ppm	**0.57 ± 0.29***	0.05
As(III) 0.3 ppm	**0.49 ± 0.16***	0.0001
As(III) 0.6 ppm	**0.48 ± 0.34***	0.0016
As(V) 0.1 ppm	1.022 ± 0.61	
As(V) 0.3 ppm	0.84 ± 0.5	
As(V) 0.6 ppm	0.83 ± 0.189	

*p= control vs As groups

Conductivity has been positively related to metal availability, being an important factor just after pH; Some researchers have reported no significant correlation between some heavy metals, especially As and Pb, and soil salts and EC(111,112), the soil of groups with exposition to arsenic presented an average of 0.831 mS/cm, that is lower than the control group.

Maybe the concentrations that were worked in this research were low, so the added arsenate or arsenite, did not elevate the conductivity as some researches establish (112).

The conductivity for the higher As(III) concentrations (0.3 and 0.6 ppm) are really close to that (0.465 mS cm^{-1})reported in Hidalgo in a soil heavily polluted with Pb, Cd and As(113).

Overall, the conditions were good for cropping.

Through all the experimentation phase these three properties were stable, true the phenological cycles there was no major change. The presence of arsenic does not affect the properties of the soil.

7.1.2 Physicochemical Analysis after harvest

The general interval for the pH measurements was 6.27 - 6.63. No significant differences were reported between the control groups and the exposition to arsenic groups in any vegetable (table 16).

The presence of arsenic does not seem to affect the properties of soil or its functionality as agricultural field.

Table 16. *pH measurements after harvesting*

Exposition group	*pH potentiometer*
Lettuce Control	6.355
Lettuce As(III) 0.1 ppm	6.275
Lettuce As(III) 0.3 ppm	6.275
Lettuce As(III) 0.6 ppm	6.63
Lettuce average	6.4 ± 0.2
Radish Control	6.32
Radish As(III) 0.1 ppm	6.325
Radish As(III) 0.3 ppm	6.365
Radish As(III) 0.6 ppm	6.51
Radish As(V) 0.1 ppm	6.435
Radish As(V) 0.3 ppm	6.29
Radish As(V) 0.6 ppm	6.36
Radish average	6.4 ± 0.1
Tomatoes Control	6.35
Tomatoes As(III) 0.1 ppm	6.27
Tomatoes As(III) 0.3 ppm	6.41
Tomatoes As(III) 0.6 ppm	6.435
Tomatoes As(V) 0.1 ppm	6.325
Tomatoes As(V) 0.3 ppm	6.355
Tomatoes As(V) 0.6 ppm	6.42
Tomato average	6.4 ± 0.09

(for lettuces n=16, for radishes and tomatoes n=28)

7.1.3 Soil Microbiological Analysis

After the bacteria cultivation and the proper cultivation time the counting of colonies was done. The complete (two repetitions for each exposition group) results were multiplied by its dilution factor.

Tables 17-20 show the results obtained for bacteria colonies for radishes soil in the different culture mediums.

RADISHES

In the **Nutritive medium**, the group of exposition As(III) 0.6 ppm presented a significant increment in the growth of colonies in comparison to the other groups. The groups of As(III)

0.1 and 0.3 ppm showed a decrease significant in the number de colonies of bacteria of 50 and 71%, respectively. In the case of treatments, with As(V) no diminution showed in the number of colonies respect to the control group.

Table 17. *Bacterial growth in* ***nutritive*** *culture for radishes soil (n=21)*

Group of exposition	***Bacteria***	***Value *p***
Control	3500±500	
As III 0.1 ppm	**1500±500***	0.05
As III 0.3 ppm	**1000±0***	0.03
As III 0.6 ppm	**9000±2000***	0.05
As V 0.1 ppm	3500±500	
As V 0.3 ppm	4000±0	
As V 0.6 ppm	2000±1000	

*p= control vs As(III) groups

Considering Nutritive grows sporulated, and one of the main genera of this type of bacteria is *Bacillus sp.*, ***Bacillus sp***. has been related with arsenic resistant bacteria, absorption/binding to metal and plant growth promoting bacteria (PGPB) that could explain the increase in the bacteria colonies(61,66,114,115). However, at low concentrations of As(III), it could be related to bactericidal effects in this group of microorganisms (sporulated). The higher population in the exposition of As(V) could confirm the theory that arsenate resistant bacteria are more common in natural environments than arsenite resistant ones (46,58).

The growth of bacteria in BK medium (table 18) showed a significant increase in the population of treatments As(III) 0.1 and 0.3 ppm compared with control, presenting significant difference. The treatments of Arsenic As(V) in 0.1 and 0,3 ppm show a decrease significantly.

The other groups did not show significative differences between them, presenting a stable growth of pseudomonas bacteria. These genera of bacteria have been related to the enhancement of a healthy growth of plants under toxic conditions. Pseudomonas have been related to PGPB, some of the activities related to them are fixing atmospheric nitrogen, solubilize and sequester iron, production of different plant growth substances (as hormones) to enhance development in different stages of the vegetative cycle, solubilize minerals (as phosphorus), increase efficiency of water-use, etc. Some of this enhancement activities have been tested in radishes under salinity stress and in chickpeas under As(V) stress, obtaining positive results (114,116).

The increment of bacteria colonies could mean that when the presence of the most toxic species of arsenic in low concentrations they help the plant before the "activation" of a different mechanism of defense, like the theory exposed in 7.3.1.

Table 18. *Bacterial growth in* ***BK*** *culture for radishes soil (n=21)*

Group of exposition	***Bacteria***	***Value *p***
control	10000±0	
As III 0.1 ppm	**240000±35000***	0.037
As III 0.3 ppm	**340000±60000***	0.037
As III 0.6 ppm	3000±1000	
As V 0.1 ppm	**5000±500***	0.037
As V 0.3 ppm	**4000±2500***	0.037
As V 0.6 ppm	1000±5000	

*p= control vs As groups

The growth of bacteria in LB medium (table 19) showed a significant increase in the population of treatments of As(III) 0.3 and 0.6 ppm compared with control. The group with As(V) 0.1 ppm showed un decrement significant respect to the group control.

The other groups (As(III) 0.1, As(V) 0.3 and As(V) 0.6 ppm) did not show significative differences between them, they seem to have a similar growth to the reported by the control group.

There are several theories that could explain the behavior of the bacteria, the increment and decrement could be related directly to the resistance to arsenic; the greater increase in As(III) could be directly related to the fact that in natural environments arsenite resistant are less common and in the long-term they are the surviving group and multiply to help the equilibrium of the plant (46,58,118,120).

Table 19. *Bacterial growth in* ***LB*** *culture for radishes soil (n=21)*

Group of exposition	***Bacteria***	***Value *p***
control	4000±1000	
As III 0.1 ppm	4000±1000	
As III 0.3 ppm	**9000±0***	0.037
As III 0.6 ppm	**7500±1500***	0.050
As V 0.1 ppm	**2000±0***	0.037
As V 0.3 ppm	3500±1500	
As V 0.6 ppm	4000±1000	

*p= control vs As groups

The results in the number of colonies with NBRIP medium showed in table 20. The group of exposition As(III) 0.6 ppm showed an increase in growth of phosphate solubilizers bacteria in a 200% compared to the control group. In the group of exposition to As(III) of 0.1 and 0.3 ppm, these showed an insignificant decrease in the growth of colonies of bacteria compared to the control group. The decreasing effect was also observed in the three exposition groups of As(V).

Table 20. *Bacterial growth in* ***NBRIP*** *culture for radishes soil (n=21)*

Group of exposition	***Bacteria***	***Value *p***
control	16500±2500	
As III 0.1 ppm	4500±2500*	0.050
As III 0.3 ppm	2100±1700*	0.050
As III 0.6 ppm	500000±20000*	0.050
As V 0.1 ppm	1500±500*	0.050
As V 0.3 ppm	1500±500*	0.050
As V 0.6 ppm	1500±500*	0.050

*p= control vs As groups

The relationship of soil arsenic (As V) with phosphorus (P) have a negative correlation(117), because it has been indicated that it competes directly for the same carrier molecules that uptake both elements in plant roots. It has also been established that P can reduced As toxicity. The decrease in bacteria under the influence of As(V) could respond to the idea that As(V) inhibits the exudation of roots and solubilization of more phosphorus, without phosphorus these bacteria could not proliferate (38).

LETTUCES

Tables 21-25 show the results obtained for colonies for lettuces soil in the different culture mediums.

The number of colonies of bacteria showed no differences significant between the groups in the Nutritive medium (Table 21). The soil-plant system created by lettuces does not seem to react significantly to the presence of arsenic in the population of sporulated bacteria.

Table 21. *Bacterial growth in* ***nutritive*** *culture for lettuces soil (n=12)*

Group of exposition	***Bacteria***
control	4500±1500
As III 0.1 ppm	6000±2000
As III 0.3 ppm	3000±1000
As III 0.6 ppm	2000±1000

The soil-plant system created by lettuces does not seem to react significantly to the presence of arsenic in the population of Sporulated bacteria. We do appreciate a tendency to decrease when the presence of arsenic increases, possibly of bacteria that are not resistant to arsenic. A downward tendency has been recorded in some other researches(118,119).

A decrease in this specific media could be explained through the changes of composition in the population, some researchers have conclude that with a long-term exposition population have a slight tendency of decrease but the main changes are in the composition (119)

The results in the number of colonies with BK medium showed in Table 22. The group de As (III) with 0.1 and 0.3 ppm showed a significant decrease in the number of colonies of bacteria compared with control.

Table 22*. Bacterial growth in* ***BK*** *culture for lettuces soil (n=12)*

Group of exposition	***Bacteria***	***Value *p***
control	17500±6500	
As III 0.1 ppm	**4000±2000***	0.035
As III 0.3 ppm	**3500±500***	0.035
As III 0.6 ppm	20000±0	

*p= control vs As groups

The soil-plant system created by lettuces does not seem to react significantly to the presence of arsenic in the population of sporulated bacteria. The decrease in the number of the colony population does not present a concentration-dependent relationship (118,120,121). Because the As (III) 0.6 ppm group does not show modifications, it seems that the higher arsenic concentration does not allow intracellular passage, which delimits bacterial growth,

having a bactericidal effect. The affectation to heavy metals has been stablished as greater than fungi but lesser than aetinomycetes (122,123).

Table 23 shows the results of the number of colonies of bacteria in LB medium. The concentration of As (III) 0.1 ppm showed an increment significantly statistic difference respect to group control.

***Table 23**. Bacterial growth in **LB** culture for lettuces soil (n=12)*

Group of exposition	*Bacteria*	*Value *p*
control	4500±500	
As III 0.1 ppm	**20000±1000***	0.050
As III 0.3 ppm	3500±500	
As III 0.6 ppm	5000±3000	

*p= control vs groups

In the NBRIP medium. The presence of *As(III)* 0.1 ppm incremented significantly the number de colonies of phosphorus solubilizer bacteria population (Table 24). We can consider an enhancement under the influence of a low concentration of As(III) in which the exudations of roots solubilized more phosphorus, producing an increment, when the concentration of arsenic increased, the bactericidal effect took over and affect the bacteria, after we could consider that a new equilibrium is being reached, maybe after a longer term exposition we could observe an increase in the quantity of bacteria and a new equilibrium reached with the help of those bacteria that are arsenic resistant. This idea of the affectation of certain genera and phyla, could apply to all the results reported in this investigation, and the idea of a new equilibrium being reached with the bacteria that resist the stress of exposition to certain toxic materials has been exposed before in other researches (119,121).

***Table 24.** Bacterial growth in **NBRIP** culture for lettuces soil (n=12)*

Group of exposition	*Bacteria*	*Value *p*
control	10000±5000	
As III 0.1 ppm	17500±7500	
As III 0.3 ppm	**300±100***	0.031
As III 0.6 ppm	**2300±800***	0.031

*p= control vs As groups

TOMATOES

The results in the number of colonies of bacteria with the Nutritive medium showed in Table 25. The group de As (III) with 0.1 ppm showed a significant increase in the number of colonies of bacteria compared with control. There is a noticeable increase of the bacteria population in the groups of exposition of As(V), compared to those presented in control group, we could think of an increase of bacteria resistant to arsenic, some of those bacteria have been identified in the genera *Bacillus sp.* and that genera belongs to the sporulated related the growth in this medium (61,66).

***Table 25**. Bacterial growth in **Nutritive** culture for tomatoes soil (n=21)*

Group of exposition	*Bacteria*	*Value *p*
control	9000±3000	
As III 0.1 ppm	8500±2500	
As III 0.3 ppm	7500±1500	
As III 0.6 ppm	5000±2000	
As V 0.1 ppm	**16000±2000***	0.050
As V 0.3 ppm	10000±3000	
As V 0.6 ppm	10000±0	

*p= control vs As groups

In the BK medium, the presence of *As(III)* 0.1, 0.3, and 0.6 ppm showed a decrement significantly the number de colonies. This effect also showed with As (V) to 0.3 ppm (Table 26).

***Table 26.** Bacterial growth in **BK** culture for tomatoes soil (n=21)*

Group of exposition	*Bacteria*	*Value *p*
control	57500±2500	
As III 0.1 ppm	**7500±500***	0.050
As III 0.3 ppm	**10500±500***	0.050
As III 0.6 ppm	5000±0*	0.037
As V 0.1 ppm	**295000±105000***	0.015
As V 0.3 ppm	**13000±3000***	0.050
As V 0.6 ppm	**10000±0***	0.037

*p= control vs As groups

The decrease in certain bacteria phyla population has been recorded in other researches, commonly in long term expositions the tendency is to decrease and then population recovers

because microbial population adapts to the presence of the metal/metalloid (118,119,123,124).

Table 27 showed the results with the LB medium. The presence of arsenic causes a decrease in the bacteria general population, in the groups As (III) 0.01 and 0.6 ppm, this decrease tendency has been reported in other researches(118,119).

***Table 27**. Bacterial growth in **LB** culture for tomatoes soil (n=21)*

Group of exposition	*Bacteria*	*Value *p*
control	8500±1500	
As III 0.1 ppm	**5000±0***	0.037
As III 0.3 ppm	6000±1000	
As III 0.6 ppm	**3000±2000***	0.050
As V 0.1 ppm	8000±1000	
As V 0.3 ppm	8000±3000	
As V 0.6 ppm	6500±1500	

*p= control vs As groups

The results in the medium NBRIP showed in Table 29. The increase of phosphorus solubilizer bacteria in the exposition to *As(V)* 0.6 ppm could be a direct reaction to the competition phosphorus, and As must enter the plant parts. The increase in phosphorus intake has been reported in plants exposed to arsenic. There is also an increase in the group exposition of *As(III),* but this is a lower increase, it could be explained knowing that the main competition for *As(III)* is silicon (1,51,65).

***Table 28.** Bacterial growth in **NBRIP** culture for tomatoes soil (n=21)*

Group of exposition	*Bacteria*	*Value *p*
control	3000±2000	
As III 0.1 ppm	6000±0	
As III 0.3 ppm	4000±1000	
As III 0.6 ppm	1000±0	
As V 0.1 ppm	6500±500	
As V 0.3 ppm	5500±3500	
As V 0.6 ppm	**9500±1500***	0.050

*p= Control vs As groups

After the registration of number of colonies, were selected groups of bacterias predominance by their shape, color, and macroscopic characteristics (figure 15).

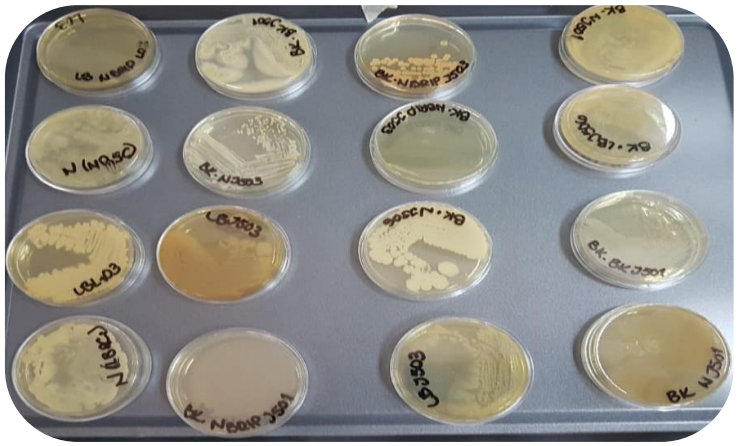


***Figure 15**. Example of bacteria re-cultivation*

Colonies obtained for microscopic identification were stained with gram stain *(Figure 28).* The results showed a vast majority of positive Gam bacteria (93%). Between the isolated bacteria sporulated, pseudomonas and phosphates solubilizers were detected. We can assume that all these bacteria are arsenic resistant.

The few Gam negative bacteria detected are those with less exposition to *As(III),* which is known to be more toxic.

The presence of phosphate solubilizers, even in the highest concentrations, talks to us about the existent competition between phosphate and arsenic.

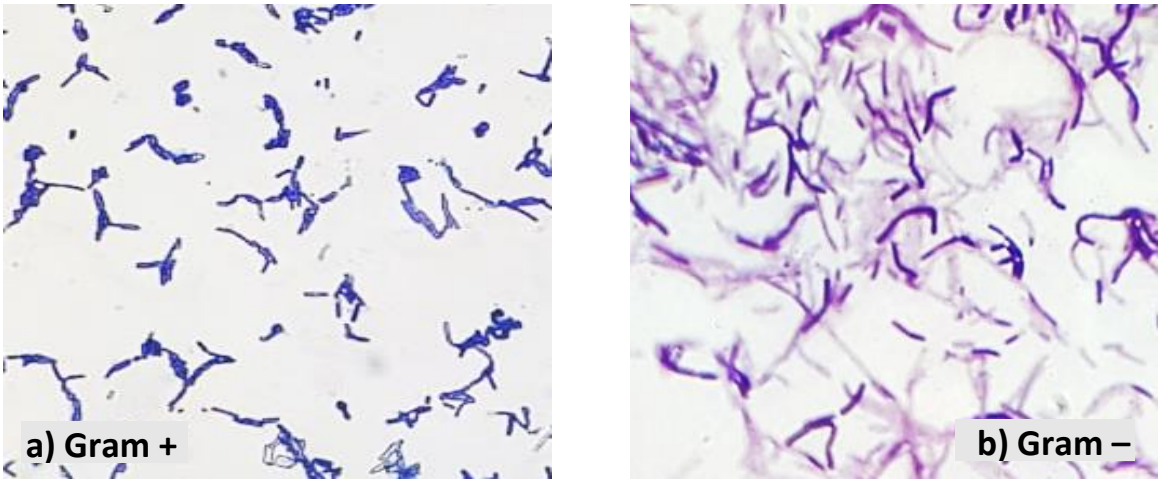


***Figure 16.** Example of Gram tinction of the different colonies evaluated 10X*

7.2 Physical analysis of vegetative parts

The physical analysis results are divided accordingly to its vegetative species, and in each species first is reported the growing through time, the average final growth, and the weight of the parts.

7.2.1 Radishes

In table 29, accordingly, the final height or average growth of radishes was determined with a mathematic average of all the individuals of the corresponding exposition group considering the last day of measurement, corresponding to the day before the harvest; this measure will be considered as the maximum growth of the radishes.

The difference is between the group control and the group of exposition *As(III)* 0.6 ppm; the arsenic concentration seems to have affected the vegetable development of the group, they developed several cm less, this could be the first sign of phytotoxicity, that some researchers have reported when the presence of arsenic is detected(38).

Table 29. *Average growth of radishes*

Average growth of radishes exposed to arsenic (n=28)		
Type of exposition	Conc. (ppm)	Average growth (cm)
Control	H_2O	24.1 ± 1.4
As III	0.1	23.4 ± 4.5
As III	0.3	20.3 ± 2.6
As III	0.6	**18.7 ± 0.9***
As V	0.1	22.6 ± 3.6
As V	0.3	22.8 ± 3.4
As V	0.6	22.6 ± 3.9

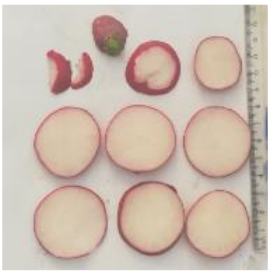
*p= 0.03 control vs As(III) 0.6 ppm.

After the harvest, separation and weighed of the parts, the results obtained for radishes were also processed through a mathematical average (figure 17). The results (table 30) on the weight of stem, roots, and fruits showed no significant difference between the groups. The treatment with As(III) 0.03 ppm showed a decrease significant in the weight of leaves in comparison with the control group.

a) Radish leaves and stems

b) Radish and roots

c) Radish



d) Complete radish

e) Weighing of radishes' leaves

***Figure 17**. Separation of radishes' parts*

***Table 30.** Average weights of different parts of radishes exposed to arsenic*

Average weights (g) of different parts of radishes exposed to arsenic					
Type of exposition	Concentration (ppm)	Weight of Stems (g) n= 28	Weight of Leaves (g) n=28	Weight of Roots (g) n=28	Weight of Fruit (g) n=28
Control	H_2O	8.3 ± 2.1	13.0 ± 2.5	2.75 ± .68	36.8 ± 18.5
As III	0.1	7.3 ± 1.3	13.5 ± 2.1	2.0 ± 3.5	27.3 ± 9.3
	0.3	6.3 ± 3.1	**8.0 ± 0.8***	2.8 ± 1.3	40.5 ± 12.3
	0.6	6.3 ± 0.7	10.8 ± 0.8	3.8 ± 2.0	35.8 ± 7.4
As V	0.1	6.5 ± 1.4	11.0 ± 1.0	3.5 ± 1.7	43.0 ± 21.4
	0.3	7.3 ± 2.8	9.3 ± 0.8	4.0 ± 3.3	36.8 ± 16.3
	0.6	7.8 ± 1.7	11 ± 1.0	6.5 ± 2.6	41.8 ± 4.1

*p= 0.0057 As(III) 0.3 ppm vs control

7.2.2 Lettuces

Accordingly, the final diameter or average growth (table 31) of lettuces was determined with a mathematic average of all the individuals of the corresponding exposition group considering the last day of measurement, corresponding to the day before the harvest; this measure will be considered as the maximum growth of the lettuces. The results showed no significant differences between the groups of arsenic compared to control group.

Table 31. *Average growth of lettuces.*

Average growth of lettuces exposed to arsenic (n=20)		
Type of exposition	Conc. (ppm)	Diameter (cm)
Control	H_2O	29.7 ± 4.7
As III	0.1	28.0 ± 3.0
	0.3	29.0 ± 1.6
	0.6	27.2 ± 2.5

After the harvest, separation and weighed of the parts, the results obtained for lettuces were also processed through a mathematical average (figure 22).

While assessing the weight of leaves and roots (Table 32), no significant difference between the weight of leaves was detected, so the development of leaves does not seem to be affected by arsenic. In the case of roots, the treatment with As(III) 0.1 ppm showed an increment (compared to control group), we could consider an enhancement(93). However, following groups presented a decrement significant in the weight of roots in comparison with the control group.

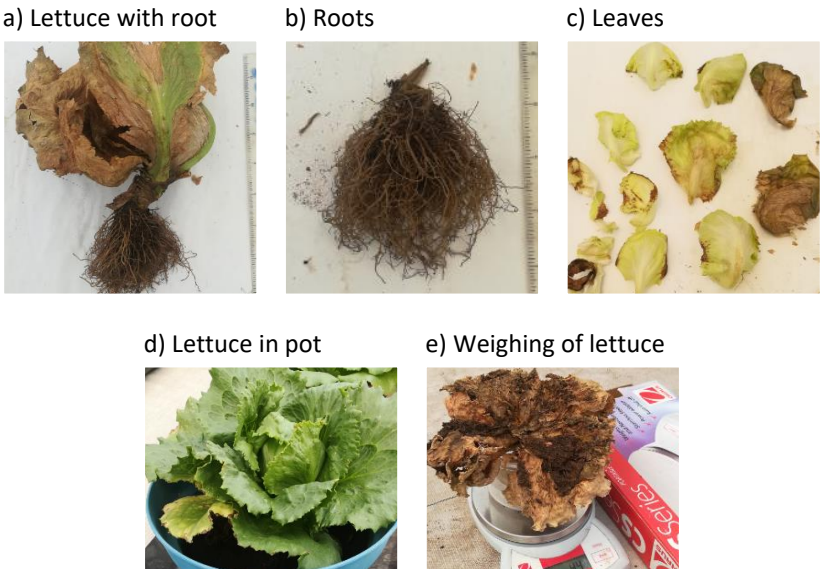


Figure 18. *Separation of lettuces' parts*

Table 32. *Average weights of different parts of lettuces exposed to arsenic.*

Average weights (g) of different parts of lettuces exposed to arsenic			
Type of exposition	Conc. (ppm)	Weight of Leaves (g) (N=20)	Weight of Roots (g) (N=20)
Control	H_2O	11.2 ±3.4	10.4 ± 4.0
As III	0.1	13.8 ±5.5	**15.6 ± 1.5***
As III	0.3	10.6 ±4.0	8.6 ± 2.4
As III	0.6	7.8 ±2.2	**2.2 ± 0.9***

*p= 0.01 As(III) 0.1 ppm vs control; p=0.0001 As(III) 0.6 ppm vs control
p=0.001 As(III) 0.6 ppm vs As(III) 0.3 ppm; p= 0.000003 As(III) 0.6 ppm vs As(III) 0.1 ppm
p=0.0018 As(III) 0.1 vs As(III) 0.3 ppm

7.2.3 Tomatoes

Accordingly, the final height or average growth (table 33) of tomatoes was determined with a mathematic average of all the individuals of the corresponding exposition group considering the last day of measurement, corresponding to the day before the harvest; this measure will be considered as the maximum growth of the tomatoes. The results showed no significant differences between the groups of arsenic compared to control group.

Table 33. *Average growth of tomatoes.*

Average growth of tomatoes exposed to arsenic		
Type of exposition	Conc. (ppm)	Average growth (cm)
Control	H_2O	74.4 ± 11.2
As III	0.1	74.2 ± 12.6
	0.3	81.0 ± 13.5
	0.6	86.2 ± 16.2
As V	0.1	78.1 ± 10.2
	0.3	77.7 ± 12.5
	0.6	67.9 ± 17

The presence of arsenic apparently enhanced the growing of tomatoes plants, resulting in almost all the groups being taller than the control group. The theory of enhancement by the presence of arsenic could be tested in tomatoes, it has been proven that tomatoes growth is more affected by organic arsenic species(38,39,125).

Below the results for assessment of development according to quantity of fruits (table 34), where no significant differences were detected.

***Table 34**. Quantity of tomatoes (fruits) harvested by group.*

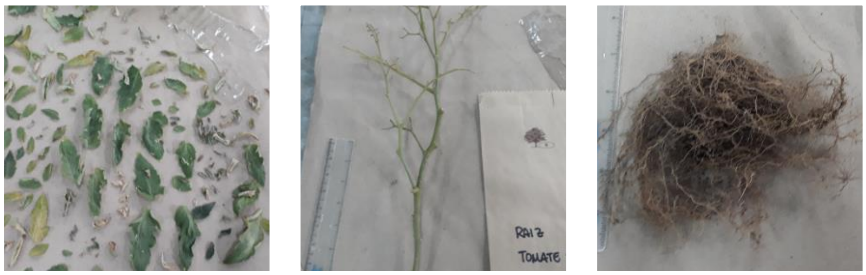
Total tomatoes (fruits)				
Type of exposition	Red Tomatoes	Orange Tomatoes	Green Tomatoes	Total Tomatoes
Control	6	2	5	13
As III 0.1 ppm	7	6	8	21
As III 0.3 ppm	13	2	1	16
As III 0.6 ppm	6	2	1	9
As V 0.1 ppm	7	2	5	14
As V 0.3 ppm	9	0	3	12
As V 0.6 ppm	17	0	3	20

The group of exposition of ***As(III)*** has a clear tendency of decrease in the number of tomatoes produced by the group. While the group of exposition of *As(V)* does not seem to have a clear tendency. In a first approach the lower concentrations (0.3 and 0.6 ppm) of ***As(III)*** and the higher of *As(V)* enhance the production of fruits. But this analysis is a first approach, because at the time of harvesting there were several green fruits and flowers, so maybe the production would be different if left for more time.

After the harvest, separation and weighed of the parts, the results obtained for tomatoes were also processed through a mathematical average (figure 19).

While assessing the weight of the vegetative parts (table 35), the leaves and fruits showed no significant difference between in respect to the control group. The development of roots

a) Leaves b) Stem c) Root



d) Fruits e) Weighing of fruit

***Figure 19**. Separation of tomatoes' parts*

and stems presented first an increase in treatment with As(III) 0.1 ppm, while the groups of As (III) 0.3 and 0.6 ppm, showed a decrease of 56% and 67 %, respectively. .

***Table 35**. Average weights of different parts of tomatoes exposed to arsenic.*

Average weights (g) of different parts of tomatoes exposed to arsenic					
Type of exposition	Conc. (ppm)	Weight of Stems (g)	Weight of Leaves (g)	Weight of Roots (g)	Weight of Fruit (g)
Control	H_2O	19.5 ± 8.0	5.0 ± 1.1	27.3 ± 11.9	73.2 ± 21.54
As III	0.1	16.0 ± 10.8	3.5 ± 2.4	23.5 ± 8.7	57.2 ± 35.0
As III	0.3	12.3 ± 6.7	4.3 ± 1.7	**12 ± 7.8****	65.5 ± 8.6
As III	0.6	9 ± 4.7	2.3 ± 2.0	**9.5 ± 2.6****	50.0 ± 23.3
As V	0.1	**20.3 ± 3.6***	4.5 ± 5.8	34.8 ± 4.6	76.9 ± 12.9
As V	0.3	20 ± 8.5	4.8 ± 7.4	**14.3 ± 6.8****	61.4 ± 24.9
As V	0.6	8 ± 6.5	2.5 ± 3.6	**11.3 ± 7.8****	75.8 ± 20.7

*p = 0.049 As(V) 0.1 ppm vs As(V) 0.6 ppm (n=28)

**p = groups of exposition vs control. p= 0.013 As(III) 0.3 ppm vs control; p =0.0031 As(III) 0.6 ppm vs control; p=0.045 As(V) 0.3 ppm vs control; p=0.0087 As(V) 0.6 ppm vs control (n=28)

7.3 Quantification of total As

The transfer of As from the soil to the plant was evaluated with the sum of the concentration of As detectable in soil + root + stem + leaves + fruit, according to the plant species, by treatment.

The concentrations are represented In parts per billion (µg kg-1 dry weight).

The water used for irrigation was drinking water for domestic use from the West Zone of the City of San Luis Potosí, whose arsenic concentration of 17.5 ppb; the presence of As in the soil of the control group is considered to be the source of exposure.

7.3.1 Radish

Table 36 shows the AsT results in soil, root, and leaves. In the stems and fruits of radish, the presence of As was not detected (data not shown). The lack of arsenic in the edible part of the radish, corresponds to that reported by Bhatti *et al.* (2013) where the least part that accumulated arsenic was the edible root. Despite compliance with the general trend detected in the accumulation of arsenic, the results reported here are lower than others previously reported under different circumstances (90,100).

Regarding the concentration of As in the soil, a significant increase was observed in the pots watered with As (III) 0.1 -0.6 ppm ($p = 0.05$), being higher in the pots watered with 0.3 ppm. In the case of As V treatments, only a significant increase in treatment of 0.6 ppm was observed.

In the results in roots, no significant differences were observed in the treatments of As with respect to the control group. In leaves, a significant increase was observed in the treatments with As (III) 0.3 -0.6 ppm, as well as in the As (V) group treated with 0.6 ppm.

In leaves, a concentration-dependent behavior was observed in the As (III) groups. In the case of As (V), the groups treated with 0.1 and 0.3 ppm, no significant differences were observed concerning the control group, unlike the treatment with 0.6 ppm where an increase was observed. This finding suggests that possibly at this concentration, there is significant conservation of the toxic metalloid in the leaves, in contrast to the concentrations of 0.1 and 0.3 ppb of As (III), which are low. This could be because the plant activates phyto detoxification mechanisms such as exudation (45). However, this must be studied in greater detail.

The arsenic transfer through the soil-plant system showed a significant increase in the As (III) treatments of 0.3-0.6 ppm and in As (V) in the group with 0.6 ppm.

Table 36. *Arsenic concentrations in different vegetative parts of radish according to exposition*

Group	Treatment	Soil (ppb)	Root (ppb)	Leaves (ppb)	Soil-Plant (ppb)
Control	H_2O	32.84	65.22	9.91	107.97
As III	0.1 ppm	**80.90***	39.67	12.03	132.6
	0.3 ppm	**138.81***	52.16	**43.52***	**234.49***
	0.6 ppm	**110.49***	57.61	**34.30***	**202.40***
As V	0.1 ppm	49.09	47.38	9.09	105.56
	0.3 ppm	19.76	60.57	12.75	93.08
	0.6 ppm	**144.00***	71.22	**45.78***	**261.00***

*p= 0.006 As(III) 0.3 ppm vs control; 0.0061 As(III) 0.6 ppm vs control; 0.004 As(V) vs control for soil. *p=0.011 As(III) 0.3 ppm vs control; 0.077 As(III)0.6 ppm vs control; 0.01 As(V) 0.6 ppm vs control for leaves. *p= 0.04 AsT vs control.

Figure 20 shows the As concentrations by treatment in the different plant parts, the AsT concentrations in the soil-plant system. In soil, the treatments with As (III) 0.3 and 0.6 ppm show an increase of 59.1% and 54.6% respectively concerning the concentration of observed in the total arsenic present in the soil-plant column; similarly, the disposition of As in the group with As (V) treated with 0.6 ppm, shows a significant increase of 55.2%.

The trend observed in all treatments is the inverse relationship that exists in the concentration of As present in the soil concerning that of the roots.

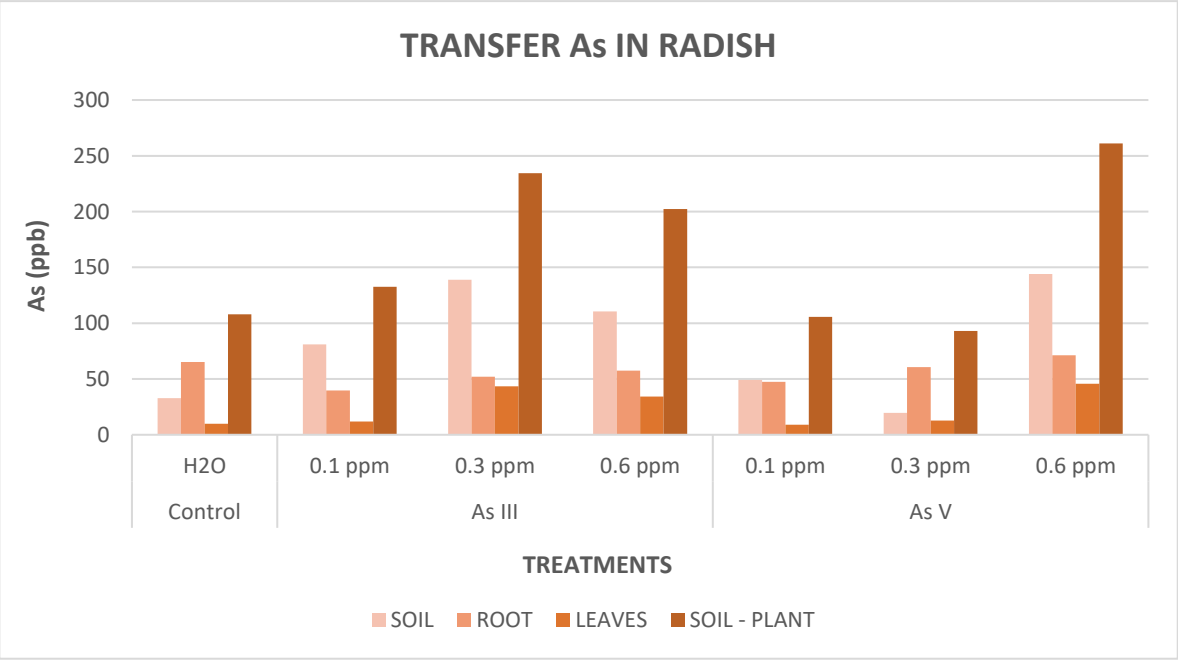


Figure 20. *Transfer of As in the radishes' soil-plant system*

The ratio of the availability of As was determined with the sum of the concentrations of As (III) and As (V) (Figure 21).

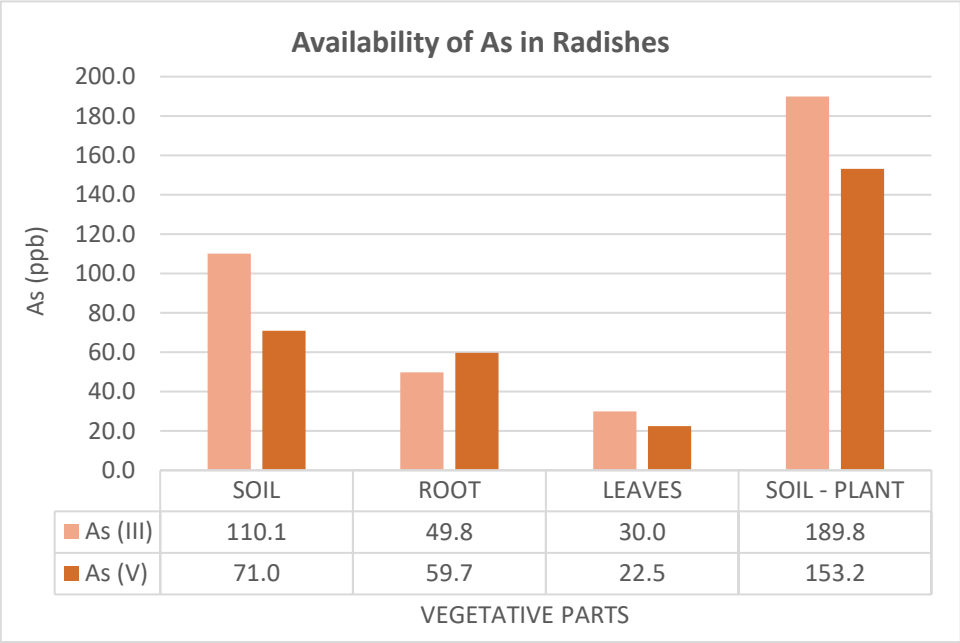


***Figure 21**. Availability of As(III) and As(V) in radishes*

The availability of arsenic in the radish plant shows an approximately homogeneous proportion of As (III) and As (V) in roots (49.8 and 59.7 ppb respectively), as well as in leaves of 30.0 and 22.5 ppb. The greater presence of arsenic in the As (III) exposure groups seems to support the Han et al. exposure (2016) theory that As (III) is more easily exuded by << plants, although it has higher toxicity; this can also explain the low concentrations in As (V) if we consider that As (V) must first become As (III) and then be expelled (45).

Carbonell-Barrachina *et al.* (1999) expressed a possible explanation for this, Possibly, radishes have developed a defense strategy called avoidance ("limited absorption by roots or transport limited to shoots"), which could explain the high accumulation of arsenic in the root system and the ground (38).

7.3.2 Lettuces

The result of the arsenic contents in different vegetative parts and soil of the lettuces are reported in the following table 38. The results reported are the average of all the repetitions done. All the concentrations of arsenic are expressed in parts per billions (µg/L).}

Table 37 shows the AsT results in soil, root, center, and leaves. The concentrations of As in the soil showed an increment significant decrease to higher As (III) 0.1 > 0.3 > 0.6 ppm. The group control showed 23.37 ppb of arsenic. This remnant comes from As in irrigation water.

In the center of lettuces, the presence of As was not detected in the group control, As (III) 0.1 and 0.3 ppm. However, in the group As (III), 0.6 ppm showed a concentration low (4.8 ppb).

***Table 37**. Arsenic concentrations in different vegetative parts of lettuce according to exposition.*

Group	Treatment	Soil (ppb)	Root (ppb)	Center (ppb)	Leaves (ppb)	Soil – Plant (ppb)
Control	H_2O	23.37	96.1	0	12.7	132.17
As III	0.1 ppm	**107.15***	**164.8***	0	10.4	**282.35***
	0.3 ppm	**83.19***	**182.2***	0	20.2	**285.59***
	0.6 ppm	**60.46***	128.3	**4.8***	**36.7***	**230.26***

*p=0.05 As (III) soil vs control *p=0.015 As(III) 0.3ppm vs control, p=0.0245 As(III) 0.1 ppm vs control for roots
*p= 0.0381 As(III) 0.6 ppm vs control for leaves. *p= 0.05 AsT vs control

The results in roots present a similar tendency of the group of exposition to As(III) presented in radishes' leaves, this is: increase in the concentration of arsenic in roots from 0.1 ppm to 0.3 ppm but then we registered a decrease when reaching the 0.6 ppm exposition. But in this case we also notice an increased in the concentration of arsenic present in leaves, so this stablishes two possibilities of what happened after the increase in the concentration of arsenic: a defense mechanism (like exudation through roots) or the saturation in roots moved more arsenic to the leaves.

The leaves present a continuous increasing tendency, corresponding to some reports that stablish that with higher exposition to arsenic higher is the absorption to the vegetative parts. And again, the tendency that the higher accumulation occurs in roots is present(17–19,93).

Figure 22 shows the As concentrations by treatment in the different plant parts, the AsT concentrations in the soil-plant system. In soil, the treatments with As (III) 0.1. 0.3 and 0.6 ppm show an increase concerning the concentration observed in the total arsenic present in the soil-plant column; the results indicate that As accumulates mainly in the roots (17–19,93).

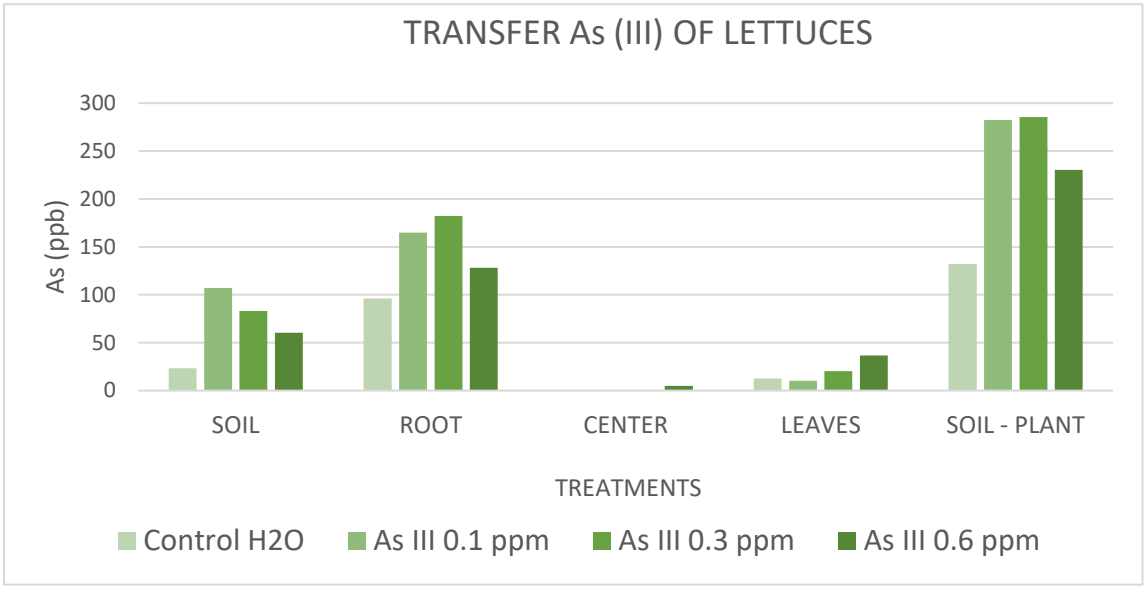


***Figure 22**. Arsenic concentration in Lettuces' soil-plant system*

The ratio of the availability of As was determined with the sum of the concentrations of As (III) and As (V) Figure 23.

The results to this research do not comply with some of other researches that stablished that tubers are the higher accumulators of arsenic, lettuces seems to be a greater accumulator under the circumstances in which it was carried out, some studies have already detected that some leafy vegetables can accumulate more arsenic than some tubers(94,102).

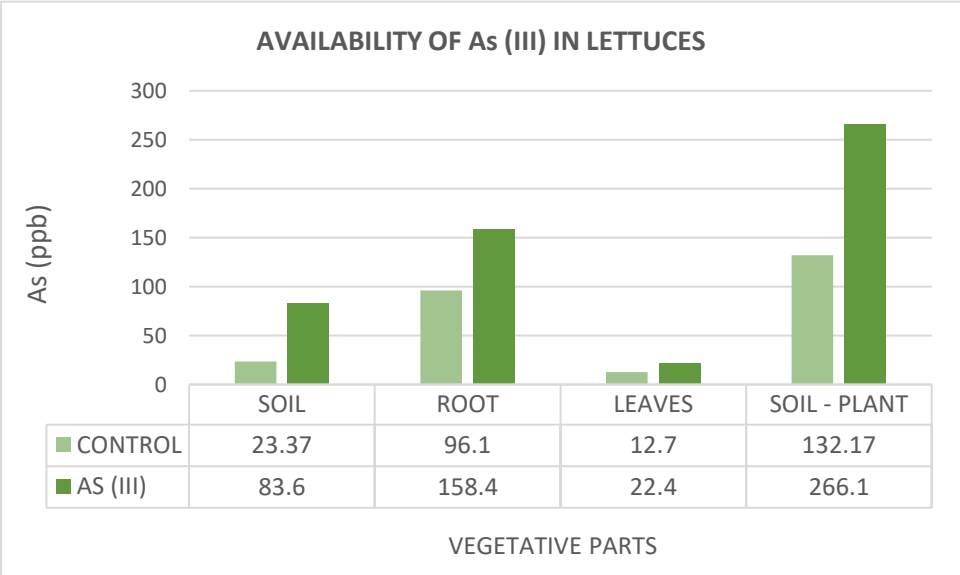


***Figure 23.** Availability of As (III) in Lettuces.*

The concentrations of arsenic here reported are lower to other concentrations reported 68 to 448 ppb. The behavior of the absorption of arsenic by lettuces was similar to those found in this study in the accumulated soil-plant (93,94).

Finally, despite the presence of As in the control group, exposure to As in all its concentrations doubles its concentration and specifically that there is this biotransference.

7.3.3 Tomatoes

The result of the arsenic contents in different vegetative parts and soil of the tomatoes are reported in the following table 38. The results reported are the average of all the repetitions done. All the concentrations of arsenic are expressed in parts per billions (µg/L). In the fruits of tomatoes, the presence of As was not detected (data not shown).

The concentrations of arsenic in the soil increase proportionally to the amount of arsenic of the exposure and increases accordingly with the accumulation of arsenic in roots and leaves. The bioavailable arsenic in the soil seems to be statistical the same, so we can assume that the rate of absorption of the plant does not vary with the concentration of exposition.

In stems, the main statistic difference of accumulation belongs to the exposition to *As(III)* 0.6 and As (V) 0.6 ppm. Those could be proof of the defense mechanism of tomatoes (translocation) after exposition to a higher concentration.

All groups presented a higher accumulation of arsenic in its roots, followed by the leaves, which corresponds to previous researches done under different circumstances (17–19).

Groups with exposition to *As(III)* presented higher concentrations of arsenic in the soil-plant system, and the tendency of increase. The tendency of arsenic in roots the group with exposition to *As(V)* is also to increase. This increase of concentrations in the plant while the exposition to arsenic increases is as expected and has been proven in other researches(93).

Table 38. *Arsenic concentrations in different vegetative parts of tomato according to exposition.*

Group	Treatment	Soil (ppb)	Root (ppb)	Stem (ppb)	Leaves (ppb)	Soil-Plant (ppb)
Control	H_2O	60.44	66.7	2.25	16.7	146.09
As III	0.1 ppm	108	**125.6***	0.5	19.2	**253.3***
	0.3 ppm	**119.62***	**126.1***	1.7	23.7	**271.12***
	0.6 ppm	**117.62***	**146.4***	**14.5***	**34.8***	**313.32***
As V	0.1 ppm	74.32	**104.3***	0	11.5	**190.12***
	0.3 ppm	**102.87***	**113.7***	0.7	7.6	**224.87***
	0.6 ppm	**117.3***	**124.9***	7.1	**36.9***	**286.2***

*p=0.02 As(III) 0.1 ppm and As(III) 0.3 ppm vs control; 0.002 As(III) 0.6 ppm vs control; p= 0.031 As(V) 0.6 ppm vs control for root. *p= 0.04 As(III) 0.6 ppm vs control. * p=0.021 As(III) 0.3; 0.009 As(V) 0.6 ppm vs control. *p= 0.025 As(III) 0.1 ppm vs control; 0.004 As(III) 0.3 ppm vs control; 0.006 As(III) 0.6 ppm vs control; 0.052 As(V) 0.3 ppm vs control; 0.006 As(V) 0.6 ppm vs control for soil

In stems, the main statistic difference of accumulation belongs to the exposition to *As(III)* 0.6 ppm. This could be a prove of the defense mechanism of tomatoes (translocation).

The Figure 24 shows the transference of arsenic in the parts presented before (soil, roots, stems, and leaves) complies with a tendency and gives some ideas as to the behavior of the plant.

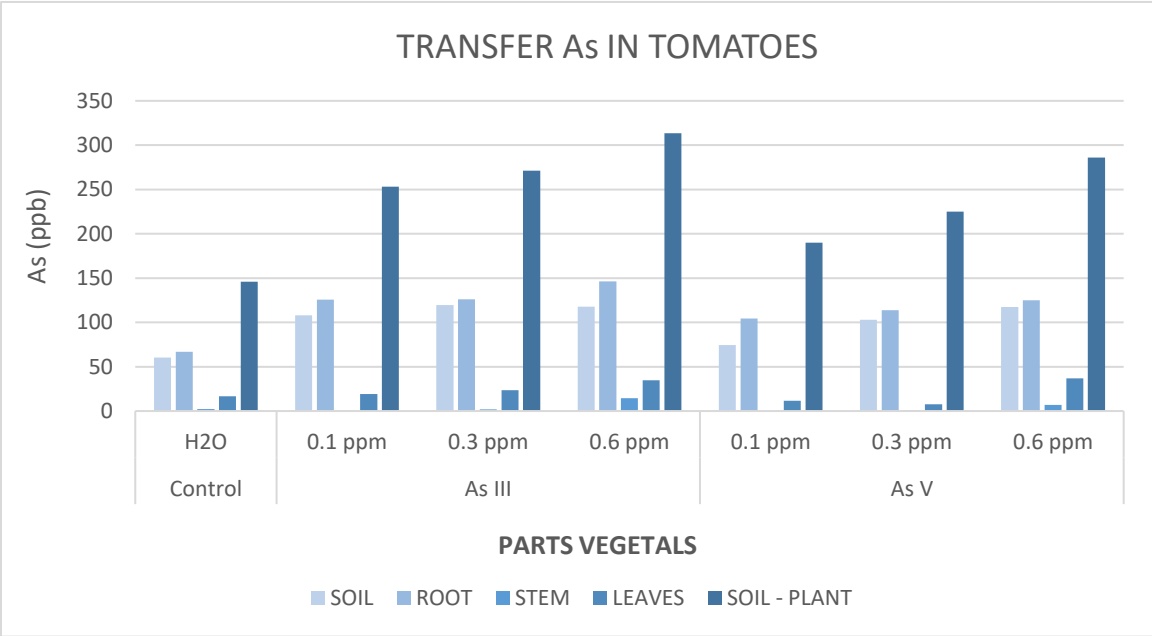


Figure 24. *Arsenic concentration in tomatoes' soil-plant system*

The ratio of the availability of As was determined with the sum of the concentrations of As (III) and As (V) Figure 25.

The availability of arsenic in the tomatoes plant shows an approximately homogeneous proportion of As (III) and As (V) in soil and roots, as well as in leaves of 25.90 and 18.67 ppb. The more significant presence of arsenic in the As (III) exposure groups seems to support the Han *et al.* exposure (2016) theory that As (III) is more easily exuded by << plants, although it has higher toxicity; this can also explain the low concentrations in As (V) if we consider that As (V) must first become As (III) and then be expelled (45,46).

The lack or low concentration of arsenic in eatable fruits like tomatoes has been stablished in different studies(36,90,91,99). The high accumulation of arsenic in its roots, even higher than in radishes was also reported by Bhatti *et al.* (2013) who compared accumulation in carrots, radish, spinach and tomatoes (102).

Tomatoes present higher affinity for As(III), that is backup by the fact that tomatoes roots have a quick transformation of As(V) to As(III), and considering that arsenite is afterwards efflux to environment this could explain the high presence of arsenic in soil too(126).

Tomatoes reports a higher affinity for arsenic than radishes, but not higher than lettuces.

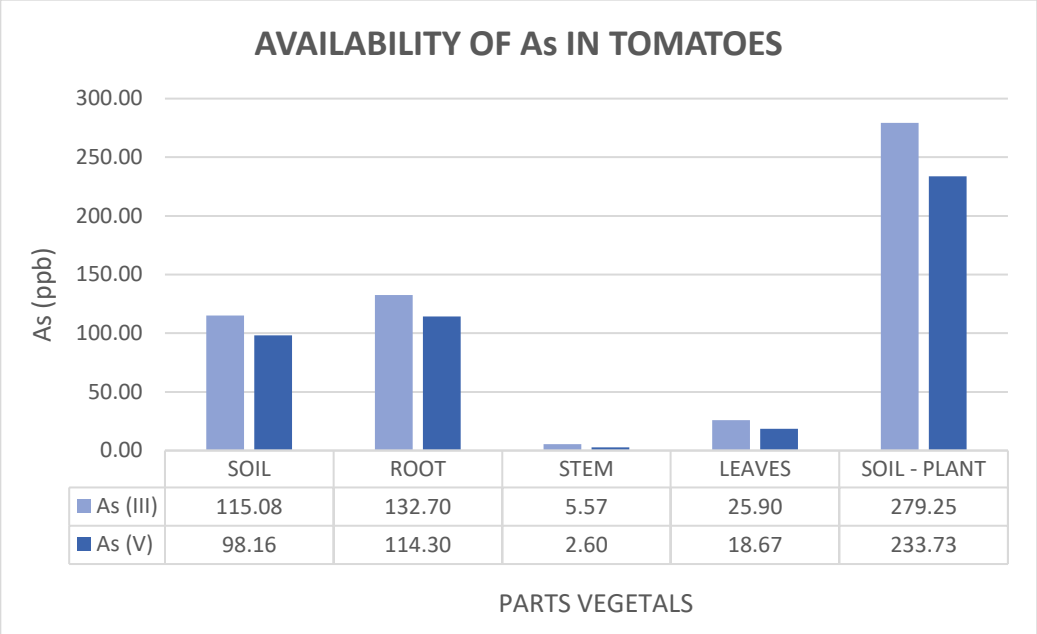


Figure 25. *Availability of As (III) and As (V) in tomatoes*

8. Conclusion

It is interesting to observe and analyze the different responses and behaviors of the three soil-plant systems established when exposed to arsenic in different concentrations.

The accumulation of total arsenic in vegetables resulted in lettuce>tomatoes>radish. Lettuce seems to have a bigger affinity to arsenic, which was corroborated with the lower concentrations of arsenic in soil.

According to the results of this research lettuce is the edible vegetable that more toxic and dangerous for humans and animals could be, the concentration reported in its leaves is classified as not safe considering the Chinese maximum permissible level of As in food (0.05 $\mu g\ g^{-1}$ fresh weight)(102).

The accumulation of arsenic in vegetative parts can be resume as follows roots>>leaves>stems>fruits.

In all cases an increase in the arsenic concentration of exposition means an increase in the arsenic accumulated in the different part of the plants. The translocation to other components, mainly leaves, is higher in the groups of exposition to *As(III).*

The overall development and growth of the vegetables were not affected by the presence of arsenic. The first signs of under development were seen in the weight of roots of lettuces and tomatoes.

In radishes, the presence of arsenic affects the physical development of the plant, in this investigation represented by weight of parts and growth, the development decreases while the concentration of exposition of arsenic increases. The presence of arsenic in the concentrations used does not affect the quality of the edible part, being that they do not reported adsorption of arsenic. Radish seems to answer to the presence of arsenic with a defense mechanism called avoidance, resulting in the translocation to specific parts, like roots and leaves, or maybe exudation. The mechanisms of defense of radish should be explored deeply in future researches.

In the case of lettuce, the lower concentrations seem to enhance the growing and development of the parts (here represented by roots and leaves), with the increase of the arsenic concentrations the development decreased, but in a low rate. Lettuce accumulates a higher concentration in roots; after an exposition to a higher concentration of arsenic it has developed a mechanism of defense, according to the results it could be translocation to other parts (leaves) or exudation. This investigation does not give enough information the mechanism of defense of lettuce to arsenic.

In the case of tomatoes, the affectations related to the presence of arsenic are divided, arsenic enhances height, leaves and roots development, apparently does not affected stems;

this could be because is the part that least arsenic adsorbed. Does not involve in the production or quality of fruits, because no arsenic was measured in them. Tomatoes were the vegetables that reported arsenic in more vegetative parts, so with further information maybe it would be possible to corroborate that the mechanism of defense used by tomatoes is the translocation.

Regarding the soil and the bacteria population, most of the colonies isolated corresponded to Gram positive bacteria.

The presence of arsenic did not modify the physical properties (pH, conductivity or humidity) of the soil, so at these concentrations the functionality of the agriculture soil is not affected.

Along with further analysis, we could observe the relation between bacteria and the presence of arsenic, the general population of bacteria tendency is to decrease. It is stablished that the presence of bacteria is indispensable for the transformation and uptake of As, since the redox of As is mainly attributed to them. The present investigation that the different genera of bacteria are involved in complex processes and these processes seem to be modified by the presence of arsenic in its different oxidative states, since the exposition groups are reacted differently.

In the other hand there seems to exist evidence to support other researches relating to the resistance to arsenic from some sporulated bacteria. Still, in the studied systems, the major tendency was to decrease, a further investigation respecting the genera of said bacteria would help to understand said behavior.

Other genera of bacteria that got interesting tendencies are the pseudomonas, these bacteria related in some researches to the enhancement and well-being of the plant, presented several ups and downs in its tendencies. Further study of these bacteria could explain why none of the vegetables represent a big development fail.

Finally, the phosphorus solubilizers bacteria presented ups and downs according to the oxidation number of the arsenic, giving evidence of the direct competition that phosphorus has with arsenic.

9. Limitations and Recommendations

Due to the worldwide pandemic, the full original target was not achieved; the missing part consisted of an analysis of the biomethylation of the arsenic absorbed by the plants. This analysis would help to fully understand the plant-soil system behaviors and reactions to the presence of arsenic. Those would also help in the determination of the real toxicity of vegetables, in the present case, the edible part of lettuce.

According to the results here obtained another important approach would be the development of new experiments with main target the understanding of the defense mechanism of each type of vegetable and assessing if this changes according to the toxic material they are exposed to. Following this, it is important to understand that each vegetable species reacts in different ways to toxic elements, and these reactions should not be generalized.

Another future approach is the isolation of the bacteria to identify the species involved, to assess if they change between vegetable species with and without exposition to a certain toxic substance.

One important consideration during this type experiments is keeping full control in the environmental variables, like temperature; some vegetable species are more sensible to said changes. In the present investigation, lettuce was the most susceptible to rise of temperatures in San Luis Potosí, even though we tried to avoid hydric stress this could have been present in minimal percentage, this raises the question if this could have affected the absorption of arsenic, resulting in the lettuce with higher arsenic concentrations.

10. References

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