ASSESSMENT AND IMPACT OF ARSENIC CONTAMINATION ON GROWTH OF NITROGEN FIXING BACTERIA

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A thesis submitted in fulfillment of requirements for the award of the degree of Masters of Science (Environmental Science)

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DEDICATION

I dedicate this thesis to my beloved parents who have offered unconditional love and support and have always been there for me.

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I am very grateful to Almighty Allah, who is the most merciful and beneficent, who has blessed me with all His blessings and helped me and gave me strength to carry out this dissertation work.

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ABSTRACT

Arsenic is a poison that causes a serious threat to human and other organisms. Numerous living things, including humans, wildlife, and crops, were affected by the usage of these groundwater supplies for drinking and irrigation purposes.It is now commonly known that the plant rhizo microbes directly or indirectly is involved to plant fitness through the production of phytohormones, nutrient solubility, nitrogen (N2) fixation, establishment of phytopathogen biological control, and neutralization of metallic ion.Arsenic has a negative effect on nitrogen fixing bacteria because it reduces there enzymaticactivity, it also effects the root nodules that results in reduced growth.The results showed that with the increase in concentration of arsenic there was an decrease in growth of nitrogen fixing bacteria.Furthermore the increase in the concentration of arsenic the growth of root and shoot slowed down.

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CHAPTER 1

INTRODUCTION

1.1Arsenic containment:

Arsenic is a poison that causes a serious threat to huge human and other organisms. Prior to its use as a murder weapon, it was a cosmetic and medical remedy for treating skin ailments.(Abdulsalam et al., 2011). Numerous living things, including humans, wildlife, and crops, were affected by the usage of these groundwater supplies for drinking and irrigation purposes.(Hopenhayn, 2006), As-contaminated groundwater affects more than twenty million people in 7 seventy different nations, according to past studies. (Sun et al., 2010).

Arsenic contamination in soil and water resources poses a severe hazard to human health globally. As concentrations in normal soil range from one to forty mg/kg, but they may rise above the threshold level as a result of human activities including mineral extraction, the use of pesticides and herbicides, and irrigation of contaminated groundwater. In addition to drinking water that has been contaminated, one of the primary ways that individuals are exposed to as is through the soil to plant components. Higher concentration of Arsenic in soil not only increases the possibility that it will be absorbed by plants but also interfere with agriculture growth by reducing plant yield.

1.2Arsenic containment in soil:

Since expanding agricultural acreage is impractical, an FAO report (FAO, 2009) predicts that higher yields and enhanced cropping intensity will provide 90% of the necessary increase in crop production globally (Chi et al., 2018). This is a result of the limited amount of land that is accessible and the competition that urbanization and other land uses pose to agriculture. Additionally, deteriorating soil quality inhibits agricultural usage of lands. Additionally, the interaction of more frequent droughts, climate change, variability, and conflicting needs from other sectors has led to greater pressure on natural resources (Iizumi, 2014). They would have a significant impact because agriculture depends on productive soils and the availability of water sources.

The resident microbial population, which is influenced by edaphic variables and defined by species richness, community structure, and microbial biomass, maintains a careful check on the fertility of the soil. The diversity of soil bacteria regulates the soil nutrients and promotes plant development to keep agroecosystems in a homeostatic state. It is common knowledge that microbial communities with the proper kind of structure, variety, and function are crucial for regulating the productivity and sustainability of the environment. Given this, microbial community management is given emphasis in modern agricultural practices to maintain soil fertility(Shaji et al., 2021a). Therefore, any change in its composition leads to a reduction in the microbial activity, which affects the soil's fertility.

1.3 Arsenic containment in plants:

. Terrestrial plants can occasionally take up as through the soil through their roots or through airborne as that settles on their leaves. Higher quantities of arsenic are toxic to the majority of plants. Arsenate has similar structure to phosphate and capacity to compete with other carriers in the roots. Arsenic interrupts metabolic pathways and hinders plant growth. Inhibiting seed germination, reducing plant length, reducing shoot and root growth , and occasionally even mortality are possible signs of toxicity.(Marin et al., 1993), Reduced chlorophyll and protein concentrations, together with yellow, withered, and stunted leaves and roots.

1.4 Arsenic containment in root nodules:

Numerous studies have suggested that the presence of as in the soil may harm the morphological and biochemical processes of plants, reducing agricultural yields. As, for example, caused root cell plasmolysis, lignification, and discoloration in plants, which hampered plant growth. Additionally, as contamination severely reduced numerous plants germination rates, root and leaf biomass, shoot and root elongation, and seed vigor index. With as exposure, it has been shown that the number of leaves, the size of the leaves, the height of the plants, and the fresh and dry biomass of plants all decrease. As stress decreases the activity of the plant's photosynthetic mechanism, according to numerous studies. Reduced pigment photosynthesis, altered chloroplasts, and diminished photosystems I and II activity are all consequences of arsenic poisoning

Arsenic poisoning damages cell membranes, and it is well known that chloroplast membranes are especially susceptible to As-induced injury. In the arsenic-stressed leaves of P. vitiate and

Leucaena leucocephala, the chloroplast inner membranes were abnormal. The configuration of the chloroplast membranes changed as a result of as stress, according to the literature, which

also produced swelling and thylakoid membrane rupture. According to Upadhyaya et al., toxicity also reduced carotenoids and distorted the chloroplast membrane. Arsenic toxicity damages chloroplasts and changes their internal membranes, which has an adverse effect on the photosynthesis. (McArthur et al., 2008).

1.5 Impact of arsenic on Nitrogen Fixation:

It is now commonly knowing that the plant rhizo microbes directly or indirectly is involved to plant fitness through the production of phytohormones, nutrient solubility, nitrogen (N2) fixation, establishment of phytopathogen biological control, and neutralization of metallic ions. (Rilling et al., 2018).

In order for plants to absorb atmospheric N2, it must first be converted to ammonia by the nitrogen fixing bacteria. Therefore, by enlisting Nitrogen-fixing bacteria interaction with the environment, the host plant satisfies its nutritional requirement by absorbing it from the environment. Researches have also shown that some bacterial species, can colonies a range of plant niches, including the Endo sphere and rhizosphere, which are internal plant tissues that help non-leguminous plants meet their N needs.(Langer et al., 2008)

Many bacterial species are resistant to heavy metals due to the genetic composition. There are a number of ways bacteria deal with of arsenic, including decrease uptake, methylation, the adsorption by the cell walls. Arsenic is more harmful than chromium, and other toxic elements that rare present in the environment. Arsenate is detrimental to bacteria because it inhibits its enzymatic activity.

1.6 Impact of arsenic on Rhizome Bacteria:

Rhizobacteria have the potential to aggressively colonies plant roots and promote plant growth. PGPR can either stay outside of the plant cells or enter cells and remain there. PGPR
are intriguing organisms for the metal extraction by plants since they accelerate the rate at which plants acquire both metal and biomass. Examples include Rhizobium, and Pseudomonas. In order to prevent plants from growing in contaminated areas while simultaneously contributing significantly to heavy metal detoxification, farmers are urged to vaccinate them. These rhizobacterial microbes increase plant biomass while stabilizing, regenerating, and repairing heavy metal-contaminated soils.

It has been studied how plants and rhizobacteria cooperate to eliminate arsenic. According to a study, the application of a rhizobacterial decreased the negative impact of arsenic and improve crop productivity. Niell et al. assert that Enterobacter cloacae-infected canola plants developed more than other canila plants when arsenic was present.(Yang et al., 2020)

1.7 Economic loss in agriculture due to arsenic contamination:

Many countries around the globe are struggling with the arsenic issue. It is understood to be a significant threat to human health. Pakistan has accepted the need to assess the quality of drinking water for arsenic pollution in light of the problem with arsenic in Bangladesh and other surrounding countries. With UNICEF's assistance, the Government of Pakistan has started a number of initiatives in this regard since 1999. Water resources are being abused as the global population continues to increase. Water sources are frequently found to have extremely high levels of heavy metal and arsenic contamination. These pollutants enter surface and groundwater sources due to their high mobility. Surface water and groundwater can be found in many different places. Compared to surface water, groundwater is less likely to contain microbes and heavy metals. Although groundwater carrying heavy metals has been the focus of numerous research, the majority of people still drink it. A severe threat to approximately hundred million people in Southeast Asia is arsenic poisoning.

Anthropogenic and natural sources both contribute to groundwater pollution in Pakistan, according to multiple studies utilizing different univariate and multivariate statistical methodologies. There haven't been any previous assessments of the deep groundwater's long-term viability. Deeper pumping will cause the dissolved arsenic to go downstream, irreversibly diminishing deeper resources. Groundwater arsenic concentrations can be used to assess the cancer risk, and the average daily dosage (ADD) (CR). Several studies that evaluated the Toxicity of arsenic in drinking water employed these characteristics. Anthropogenic and natural sources both contribute to groundwater pollution in Pakistan, according to multiple studies utilizing different univariate and multivariate statistical methodologies.

1.8 Current situation of arsenic contamination in Pakistan:

In Pakistan, numerous research using various univariate and multivariate statistical techniques revealed that anthropogenic and natural causes both contribute to groundwater pollution (Farooqi et al., 2007). There haven't been any previous assessments of the deep groundwater's long-term viability. (Michael and Voss, 2008) Between twenty five and thirty six of the population in different provinces are impacted by as pollution. . The levels of Arsenic s and their potential sources have not been thoroughly investigated. Therefore, it is essential to evaluate the water in different areas of the nation. Arsenic is becoming a major health concern Pakistan, and groundwater poses serious health hazards.

Drinking water tainted with arsenic is harmful to human health, which decreases the socioeconomic status of the population. In addition to raising disease and mortality, inadequate access to safe drinking water also drives up health care costs, lowers workplace productivity, lowers school enrollment, and widens poverty. Therefore, having access to clean water is essential for both lowering poverty and providing basic healthcare.(Ahmad et al., 2004). Clean water is described as "water that is free from pathogenic agents, free from harmful chemicals, free from color and odor, and usable for home usage". Arsencosis is the a more dangerous derivate of arsenic and it was more longer adverse effects. Arsenic-rich water drinking over an extended period of time is harmful to human health, including skin disorders. Lesions that have already developed are irreversible, even after access to clean drinking water is restored for an extended period of time. The chance of developing kidney, bladder, lung, and blood vessel diseases and other comorbidities, is also increased by prolonged exposure. Increasing health concerns over arsenic contaminated water have mostly been observed in different areas of Pakistan. Districts that are most negatively impacted have union councils that are located quite near to the Indus river. The principal crops farmed in this well-known agricultural producing region are cotton, wheat, and sugarcane. These crops necessitate significant pesticide and fertilizer use, which is probably why the ground water is contaminated with arsenic. (Podgorski et al., 2017)

1.9 Objectives:

1. To evaluate the effect of arsenic containment on the growth of nitrogen-fixing bacteria.
2. To evaluate bioaccumulation of arsenic in roots.
3. To evaluate the effect of arsenic on root and shoot length.

CHAPTER 2

MATERIAL AND METHODS

2.1 Sampling site:

The samples were taken from Pea plant. It's scientific name is *Pisum sativum*.It belongs to the family known as Fabaceae. As a winter crop in the plains, pea is sown from mid-October to mid-November. Sowing takes place in temperate climates from October through March.7 roots were pick from NARC. Those roots were taken who have root modules because nitrogen fixing bacteria are present in plant nodules.

2.2 Effect of Arsenic on the growth of nitrogen fixing bacteria

Roots were chosen with loop inoculation in 15ml N.B. and were incubated after the nutrient broth had been poured in 5 different flasks, autoclaved, and filled with the broth. Bacterial growth was selected and placed in 5 flasks with varying quantities of arsenic. 5 beakers were obtained and filled with concentrations of 50ppm, 75ppm, 100ppm, and 125ppm of arsenic.

First, 2 roots were taken *Pisum Sativum* plant from fields. It was then rinse with abundant tap water. It was then washed by 15% ethanol for 3 minutes for surface disinfection. After sterilizing the roots, Mortar and pestle both were sterilized, and Root nodules were crushed in it. Sterilized forceps were used to pluck the nodules. Nutrient broth 9ml was made in the test tube. Then the broth was incubated for 24-36 hours on 36˚. It was then spread on N.Plates and then incubated again for 24-36 hours.

Roots were chosen with loop inoculation in 15ml N.B. and were incubated after the nutrient broth had been poured in 5 different flasks, autoclaved, and filled with the broth.

Bacterial growth was selected and placed in 5 flasks with varying quantities of arsenic. 5 beakers were obtained and filled with concentrations of 10ppm, 50ppm, 75ppm, 100ppm, and 125ppm of arsenic .Bacterial growth was assessed by O.D performed every third day for six week. O.D was performed on UV spectrophotometer on 260 wavelength

`2.3 Evaluating bioaccumulation of arsenic in roots:

5 beakers were obtained and filled with concentrations of 10ppm, 50ppm, 75ppm, 100ppm, and 125 ppm of arsenic. To observe the bioaccumulation of arsenic in roots its atomic absorption value was observed by the end of six weeks. Acid digestion was used to prepare the sample.2 gram of roots were taken in the beaker and then 5 to 10 ml of per chloric acid($HClO_4$) for digestion, was heated , then cooled down and 30ml distilled water was added in it. This mixture was filtered by Whatman filter paper and was then analyzed through atomic absorption at PCRWR.

2.4 Evaluating effect of arsenic on root and shoot length:

Five concentrations of Arsenic were taken i.e., 10ppm,50ppm,75ppm,100ppm, and 125ppm. 6 pots of plants were placed for every concentration. Each plant was extracted after every successive week to access the effect of Arsenic on root and shoot length with increasing time periods.

Figure 2.1 Nutrient Broth Flask in Incubator

Figure 2.2 Sample for Atomic Absorption Spectroscopy

Figure 2.3 Autoclave

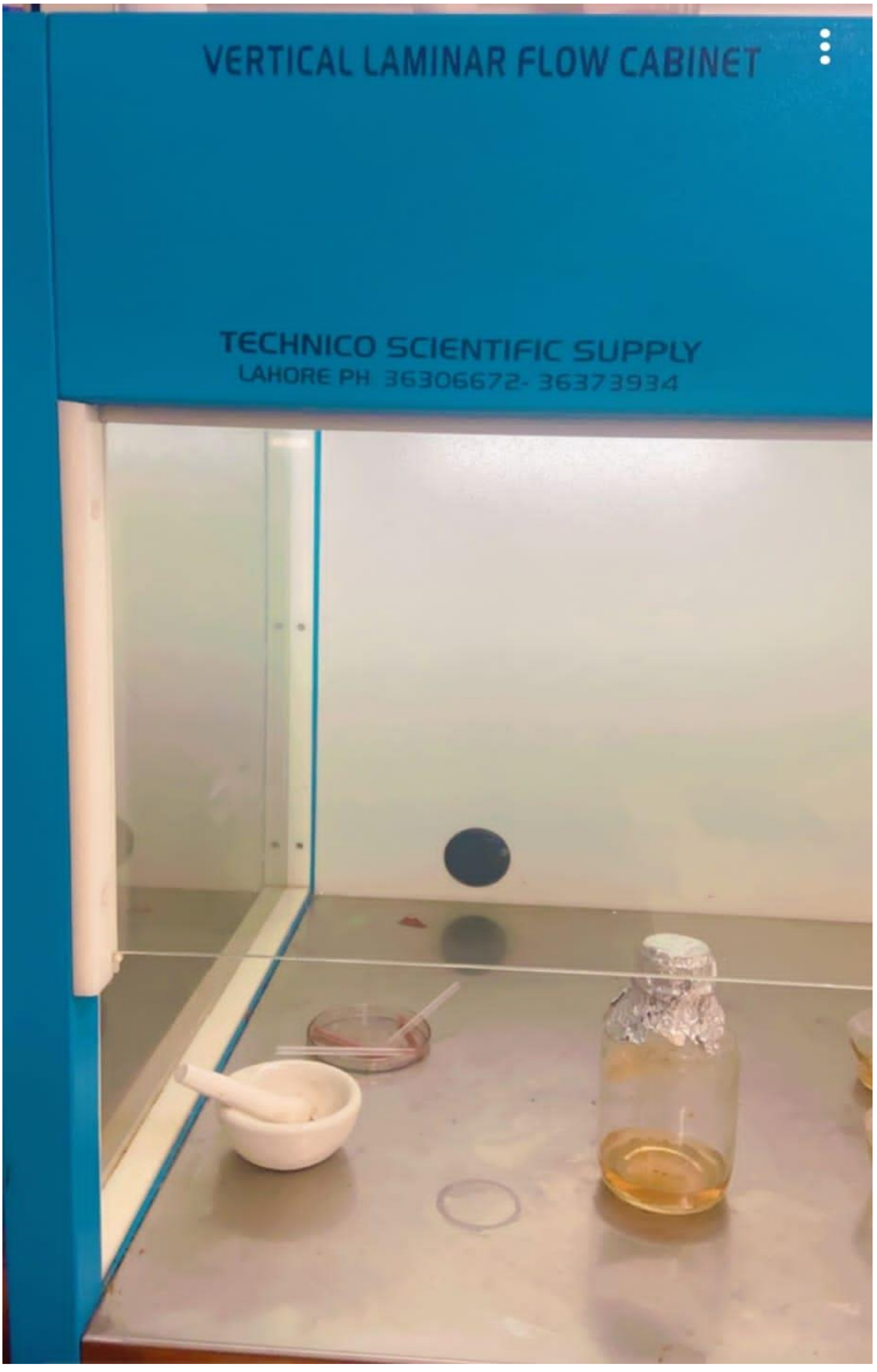

Figure 2.4 Vertical laminar flow cabinet

Figure 2.5 Roots Nodules crushed in Mortar and pestle

Figure 2.6 Roots of leguminous plant

CHAPTER 3

RESULTS AND DISCUSSION

Although it is considered that arsenic is not a necessary component for living organisms, plants absorb arsenic from their surroundings as they grow. According to studies, small amounts of arsenic l can promote plants' growth. Arsenic in excess prevents the production of chlorophyll and suppresses photosynthesis. The poisonous effects of arsenic on plant enzyme activity are another way in which it manifests itself. A number of enzymes are very obviously inhibited by arsenic. For instance, the roots' highly accumulated arsenic prevents catalase from working properly, which could otherwise shield plants from excessive hydrogen oxide poisoning. Reduced catalyze activity will have a negative impact on crop growth.

Various microorganisms can be inhibited by arsenic, with mostly nitrogen fixing bacteria showing the overt inhibition. The arsenic pollution may alter the population of these helpful microorganisms, which may then affect the soil's normal metabolic process. As can be absorbed by plants through their roots, where excessive accumulation has a dramatic impact on root lengthening and cellular proliferation (Sathishkumar et al., 2004). Additionally, due of the impairment of the photosynthetic system and increased functional damage in plants, crops are less productive.

3.1 Growth in different concentration of Arsenic for 6 weeks

To measure the effect of Arsenic on the growth of nitrogen fixing bacteria Bacterial growth was selected and placed in 5 flasks with varying quantities of arsenic. 5 beakers were obtained and filled with concentrations of 50ppm, 75ppm, 10ppm, 100ppm, and 125ppm of arsenic. The results showed that with the increase in concentration of arsenic there was a decrease in growth of nitrogen fixing bacteria. At 10ppm there was a slight growth up to 2.5 then the graph reached a steady pace. A similar pattern was seen at 50ppm.However the growth increases up to 3 at75ppm, 100ppm and125ppm.

In results in this study showed that there was a slight increase in growth of bacteria in the presence of arsenic contamination. The graphs showed a substantial rate growth. A experiment was conducted in laboratory microcosms to examine the impact of arsenic

(As) poisoning on the productivity of f nitrogen fixing bacteria in agricultural soil. Microcosm samples were periodically collected and examined using RT-qPCR. Even with a brief exposure to increase As concentrations, reduced bacterial activity was seen. The bacterial activity was reduced as the As exposure duration was extended.(Chakraborty et al., 2017)

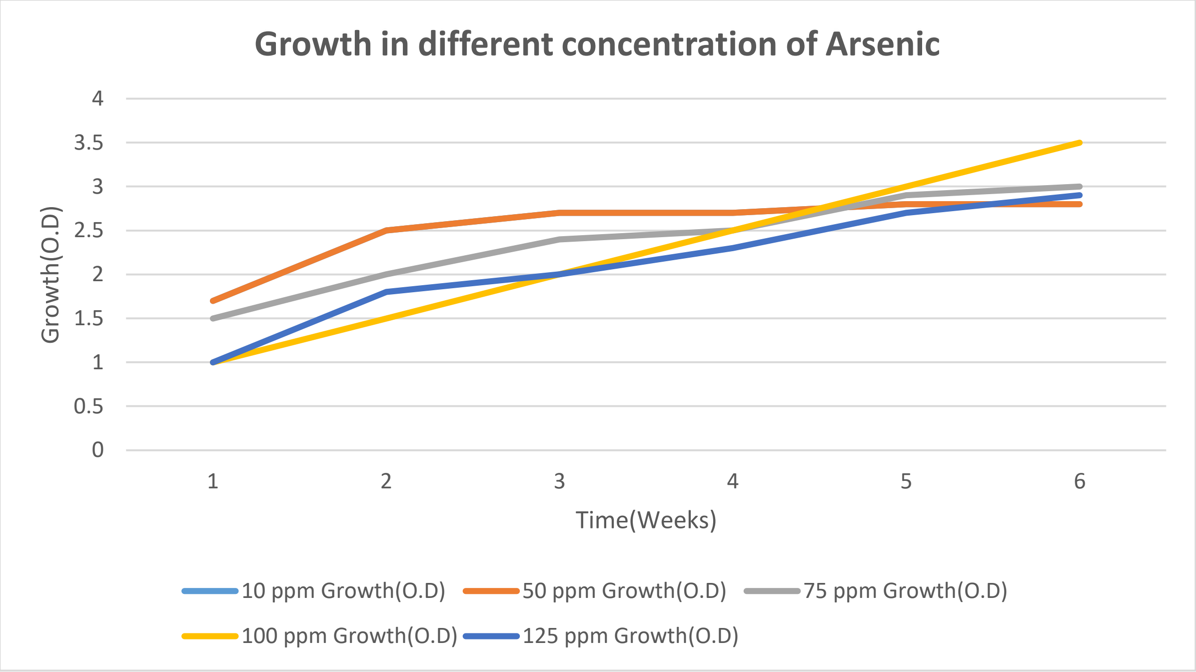

Figure 3.1: Growth of nitrogen fixing bacteria

3.2 Root length

Since germination of the seeds is one of the processes that is susceptible to contamination, it is crucial to take this into account when examining how heavy metals affect seedling growth. A study investigated how arsenic affected seed germination and discovered that arsenic prevented the germination of both types of examined plants. The seedlings' root and shoot lengths were measured to show that arsenic impeded their growth. Both Satabdi and Nayanmani's root and shoot lengths were dropped substantially as compared to the control.(Gupta et al., 2008)There was a decrease in root and shoot length with the increase in the concentration of arsenic. More growth reduction was observing at 125ppm as compare to the other lower readings. The total arsenic concentration of the roots and root nodules was evaluated by digesting them in all flask containing different concentration of arsenic. (50ppm, 75ppm, 100ppm, and 125pm). plant roots were dipped in each flask for

8 weeks to evaluate arsenic effect on root and shoot length. The graphs showed that with the increase in the concentration of arsenic the growth or root slowed down. The growth was more in the first 3 weeks then it slowed down. At 50ppm the growth was more but with increase in concentration the growth slowed down. The growth was least at 125ppm.

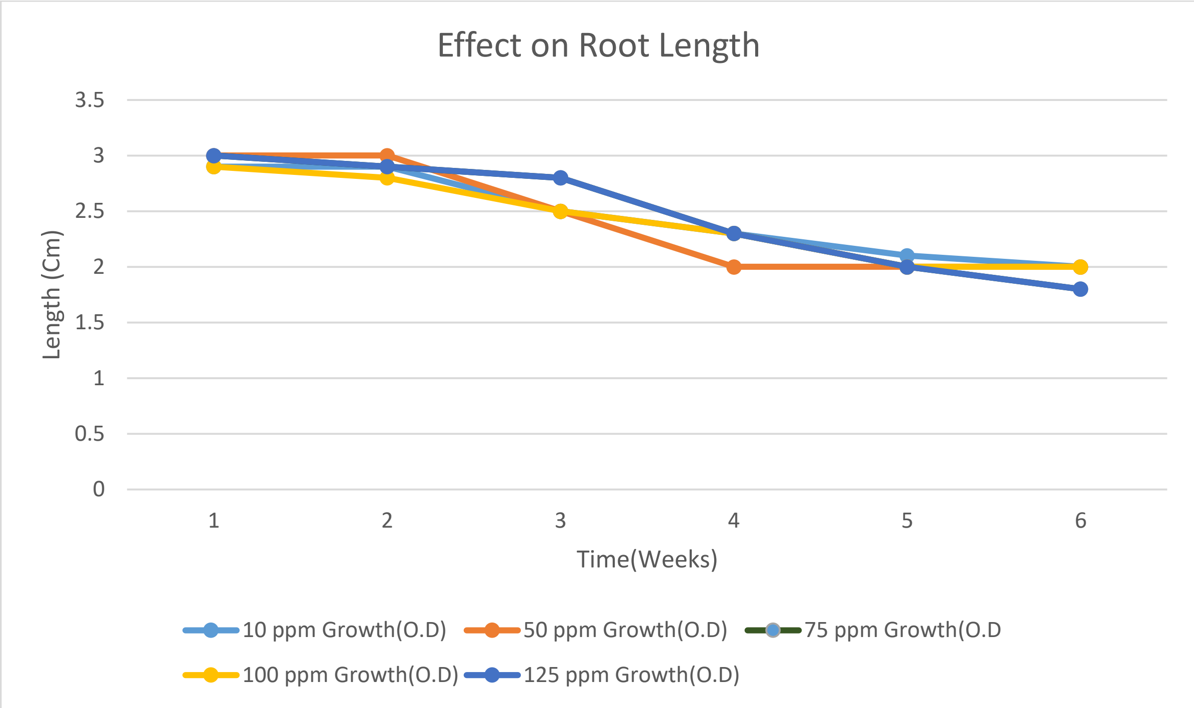

Figure 3.2: Effect on root length

3.3 Shoot length

Similar to root length the shoot length also showed a similar downward trend. The graphs showed that with the increase in the concentration of arsenic the growth of shoots slowed down. The growth was more in the first 3 weeks then it slowed down. At 50ppm the growth was more but with increase in concentration the growth slowed down. The growth was least at 125ppm.

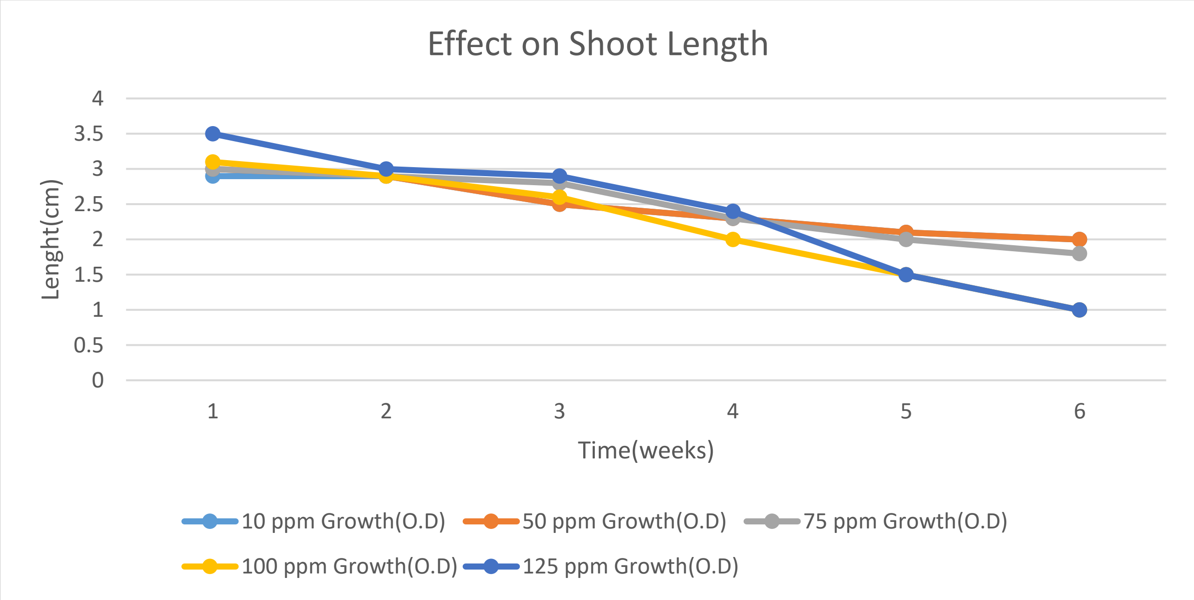

Figure 3.3: Effect on shoot length at 10ppm

3.4 Bioaccumulation of arsenic in roots

Arsenic levels in the soil and a plant's ability for accumulation and translocation determine how much arsenic is present in the plant's edible parts. Plants can absorb inorganic forms of arsenic with the aid of transporter proteins. Arsenic typically builds up in the roots of plants before moving to the shoots and grains. The results demonstrated that there was increase in bacteria activity with the increase in Arsenic concentration. A study was done to observe the root sequestration in legume plants. Results showed that the Plants were able to acquire arsenic in their roots with a poor transfer index to the shoot in an as contaminated soil experiment. This tendency was enhanced by inoculation with fungus (AMF), which also increased biomass production and shoot phosphorus concentration.(Bhattacharya et al., 2021)

To observe the bioaccumulation of arsenic in roots its atomic absorption value was observed by the end of six weeks. The prepared mixture was filtered by whatman filter paper and was then analyzed on atomic absorption on wavelength 1937 at PCRWR. The graphs showed that there was more accumulation of arsenic in roots at higher concentration. The growth was 1 at 10ppm,1.5 at 50ppm,2 at 75ppm,2.5 at 100ppm and about 3 at 125ppm.

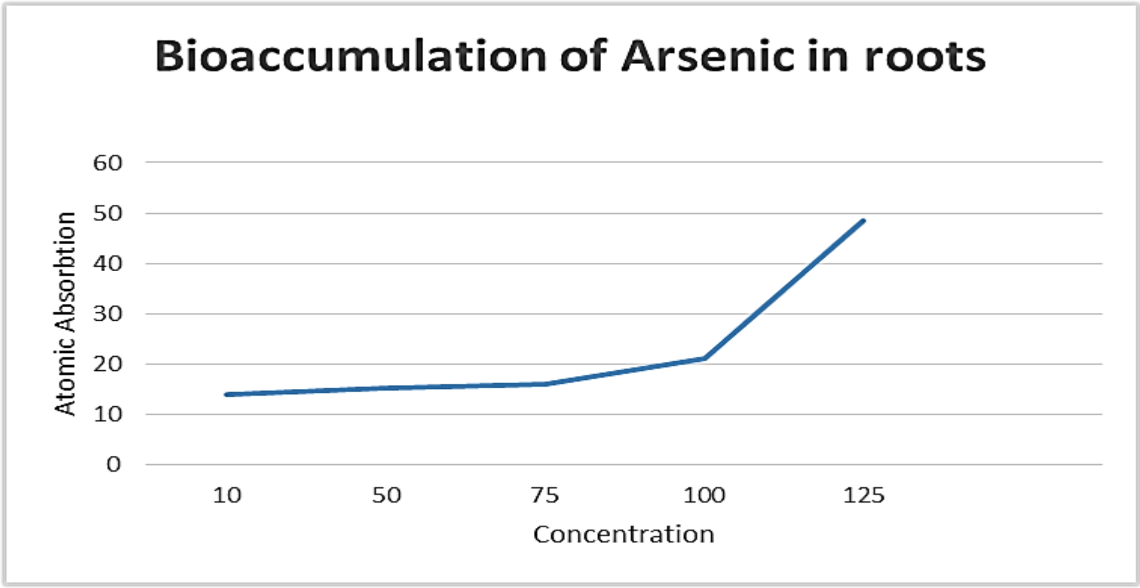

Figure 3.4: Bio accumulation of arsenic in root

3.5 Permissible Limits for Edible Plant in Arsenic

The permissible limits for arsenic in edible plants vary depending on the type of plant and the country or region in which it is grown. In the United States, the Food and Drug Administration (FDA) has set a limit of 10 parts per million (ppm) for inorganic arsenic in rice and rice products.

The European Union (EU) has set a limit of 2 ppm for inorganic arsenic in rice and rice products. These limits are based on the type of arsenic (inorganic vs organic) and the specific product.

CONCLUSION:

1. Contaminated soil effects the plant microbial activity. Higher concentration of arsenic inhibits the growth of nitrogen fixing bacteria, therefore, reduce plants growth. The study findings show that arsenic containment in soil can inhibit the growth of nitrogen fixing bacteria activity that are responsible for converting atmospheric nitrogen into the soil.
2. Arsenic is a dangerous toxic pollutant that can inhibit the growth of plant by effecting root and shoot growth. The findings of the study suggest that with the increase in the concentration of arsenic the growth or root and shoots slowed down. Arsenic analysis in the soil is important because arsenic is a threat to humans and plants.
3. The study observes the bioaccumulation of arsenic in the roots of the *Pisum sativum plant.* The results showed that there was more accumulation of arsenic in roots at higher concentrations

RECOMMENDATION:

1. One of the most prevalent environmental toxins, arsenic enters drinking water supplies and has a negative impact on millions of people globally. Arsenic must be removed from drinking water and wastewater immediately using innovative technologies like ion exchange process. The plants should be supplied with water that doesn't contain any harmful substances.
2. Since arsenic is a necessary component of both industrial and agricultural production, arsenic pollution is a widespread issue throughout the world. Preventive measures should be taken in order to limit the use of arsenic and to reduce the formation of these pollutants.
3. Different programs should be implemented for monitoring and assessing arsenic pollution in, agricultural land and water sources.

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