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EFFECTS OF INORGANIC SOIL AMENDMENTS ON THE ARSENIC UPTAKE AND GROWTH OF THREE EARLY STAGED LEGUMINOUS TREE SPECIES IN ARSENIC CONTAMINATED SOILS

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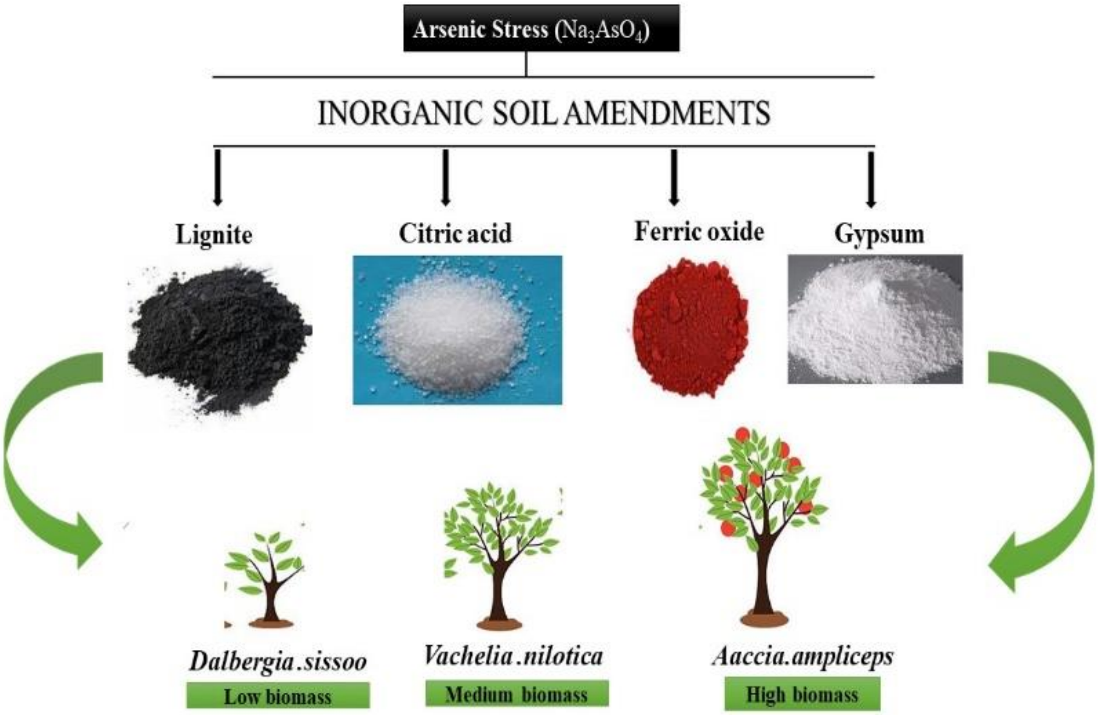
Abstract

Arsenic (As) pollution is a worldwide environmental issue due to the lethal and cancer-causing nature of as compounds. Inorganic amendments are widely reported to ameliorate heavy metal polluted soils by reducing the heavy metal mobility and enhancing the plant growth. The objectives of this study were to examine the effects of four soil inorganic amendments (Lignite (L), citric acid (CA), ferric oxide (FO) and gypsum (G)) on arsenic (As) bioavailability, As uptake and growth of selected three agroforestry leguminous trees species (*Acacia ampliceps, Vachellia nilotica* and *Dalbergia sissoo)* in As contaminated soil. A pot experiment was carried out in controlled conditions under two factor factorial completely randomized design. Pysico-chemical properties of soils along with several morphological, physiological and biochemical plant traits were examined to understand the growth of trees and As behavior under diverse conditions. Results indicated that gypsum was best inorganic amendment for all three species. The maximum shoot length (51.90 ± 0.49 cm), shoot diameter (9.84 ± 0.095 mm), , shoot dry weight (24.72 ± 0.16 g), branch dry weight (14.31 ± 0.09 g), root dry weight (27.88 ± 0.26 g), photosynthetic rate (7.24 ± 0.038 μ moles CO_2 m^{-2} s^{-1}), stomatal conductance (0.34 ± 0.003 mol H_2Om^{-2} s^{-1}), and the minimum arsenic (As) concentration in roots, shoots and leaves (20.34 ± 0.13, 7.48 ± 0.04 and 0.100 ± 0.001 mg/kg DW) were found in the plants of *Acacia ampliceps* treated with gypsum. The physiological attributes and growth trends for the tree species under all the treatments was noted as *A. ampliceps* > *V. nilotica* > *D. sisso*o while for the treatments was G > FO > L > CA > control. From this study, we concluded that arsenic (As) negatively affected plant's growth. Inorganic amendments, especially, gypsum was more useful to enhance growth of selected agroforestry tree species in arsenic contaminated soils.

Keywords: Phytoremediation, Agroforestry, Soil Pollution, Heavy Metal Contamination.

Graphical Abstract



1. INTRODUCTION

Globally, soil contamination of arsenic (As) is a very serious issue because of its highest toxicity, forming many lethal substance and vast distribution in the Earth's crust (Alam et al., 2021). The term Arsenic (As) originated from the Greek word arsenikon that means strong or powerful (Bakhat et al., 2021). The most abundant species in the soil environment are As^{V} and As^{III}. It can occur in different organic and inorganic forms. Inorganic forms are Arsenite (As^{III}) and arsenate (As^{V}) while organic forms are dimethyl and monomethyl arsenic compounds.

Inorganic form of arsenic (As) are highly toxic than organic species which are nearly less toxic (Zhai et al., 2020).

Exposure to As can cause skin, liver, lung and bladder disorders, and possibly even kidney and colon cancers (Vishnoi et al., 2018; Upadhyay et al., 2019). In drinking water, the tentative index value of arsenic concentration is 10 μg/L according to WHO; similarly, countries such as the United States and the European Union have also set a guideline value for As, because As contains toxic carcinogens in drinking water (Thiau-Fu et al., 2018). In Pakistan, only 23.5% rural and 30% urban population has access to clean drinkable water (Hassan et al., 2013; Waseem et al., 2014). It is estimated that about 20-40% of patients in Pakistan suffer from water-related diseases of arsenic and above 2.5

million children die in every year from diarrheal diseases alone. (WWF, 2007; PCRWR, 2010; Waseem et al., 2014).

Although Pakistan is the second largest export gross zone in leather and textile industries but only 725 registered tanneries are working in various cities such as Sahiwal, Faisalabad, Lahore, Kasur, Shaikhupura and Multan (Sanjrani et al., 2017).

Due to hazardous waste management practices and poor implementation of environmental laws, high concentrations of as are released in environment (Qurat-ul-Ain et al., 2017). Presence of toxic metals not only hinders the plant growth but also contaminates the soils. The bioavailability and lethality of as vary with soil types (Sheppard, 1992; Warren et al*.,* 2003; Das et al., 2004; Hossain, 2006).

Past observations demonstrate that the main factors to regulate the bioavailability of as in plants are chemical composition and quality of soil. For crops, arsenic has been found to be phytotoxic at an average accumulation of 20 mg kg^{-1}. It has been reported that as inhibits the metabolic pathways and thus hinders plant growth (Sheppard, 1992).

During photosynthesis, as inhibits the pentose-phosphate pathway which generally leads to plant death. In plants, the accumulation of as relies upon the elemental concentration, chemical form and presence of different particles in the soil (Tu and Ma, 2002).

Therefore, arsenic polluted soils are not very easy to remediate because technologies are very convoluted and expensive. In developing countries like Pakistan, many of the technologies used for the removal of heavy metals are uneconomical and expensive (Chowdhury et al., 2016). Arsenic contaminated soils can be cleaned by different soil amendments which absorb the pollutants by binding. This phenomenon is referred to as stabilization (Mladenov et al., 2010).

The major purpose of the inorganic amendments is to minimize the mobility of trace elements, thereby affecting their leaching capacity and bioavailability. The phytostablization and immobilization techniques are used as an effective method to remediate heavy metal contaminated soils (Nadeem et al., 2017; Amanullah et al*.,* 2016; Ashraf et al., 2019). Furthermore, use of annual/perennial plants in phyto-remediation has less advantages as compared to woody vegetations because such vegetation is highly productive, farm friendly and restricts the pollutants entry into the food chain (Asif et al., 2023 Hua et al., 2020).

However, the role of inorganic amendments in altering arsenic (As) mobility in soil, impacting its bioavailability and their interaction with leguminous woody perennials has not been widely studied. Therefore, it is yet unclear and conflicting how the inorganic amendments can affect As bioavailability and growth of the selected three nitrogen fixing tree species “*Acacia ampliceps, Vachellia nilotica* and *Dalbergia sissoo”* in As polluted soil.

In this study, we investigate the growth and potential of As accumulation of three different nitrogen fixing tree species under As-contaminated soil with the use of four different inorganic amendments. The results of this study would allow the farmers and scientific community to enhance the tree planting and designing phytoremediation program under as contaminated environments in a more sustainable way.

2. MATERIALS AND METHODS

The pot experiment was conducted in the post graduate research area of the Department of Forestry and Range Management (FRM), University of Agriculture Faisalabad (UAF) (31^o 25'57'' N, 73^o 04' 21'' E) during February 2019 to September 2019. Soil was collected from 0 to 15 cm depth from the research area of Forest Department (FRM), UAF.

Prior to conduct a pot experiment, soil samples were dried in sunlight, grind and pass through a 2 mm sieve and gradually mixed with to ensure homogeneity. After that, the soil was spiked with 20 mg/kg arsenic using sodium arsenate salt (Na_3AsO_4) and incubated for four weeks. Each pots about 7 kg soil was filled and internally lined with polythene sheet to avoid leaching of As.

Four different inorganic amendments including Lignite (L), Citric acid (CA), Ferric oxide (FO) and Gypsum (G) were also applied in soil. All amendments were mixed in the soil at the rate of 3% wt/wt prior to experiment.

Healthy, disease free and uniform sized about three months old seedlings of the three agroforestry trees species (*Dalbergia sissoo, Acacia ampliceps* and *Vachellia nilotica)* were procured from the nursery of FRM, UAF and grown in the pots. Each treatment was replicated thrice. The information about climatic conditions in the experiment area and Forest nursery during the year 2019 (Table 1).

Table 1: Climatic Data of Study Period

Month	Av. Max. Temp. (^{o} C)	Av. Min. Temp.(^{o} C)	Precipitation (mm)	Sunshine Hours (Hours)
January	19.2	07.0	18.0	05.4
February	20.3	09.1	64.2	01.6
March	26.0	13.8	55.7	03.0
April	35.0	20.6	31.2	05.4
May	39.0	23.9	39.1	06.8
June	42.4	27.4	35.5	08.5
July	38.0	28.0	102.8	05.5
August	38.0	28.5	80.9	04.5
September	37.7	27.8	21.8	05.0

Growth Parameters

Before harvesting, certain growth parameters of plants were determined. Measuring tape was used to determined shoot length and root length of plant. Secondly, Digital Vernier caliper was used to determined the collar diameter (mm) of shoots of standing plants. After harvesting dry weights were taken by drying plant samples in oven (101-1AB) at 75˚C for 5 days until a constant weight was obtained and dry weight was measured by using weigh balance (AND GULF).

Physiological Parameters

Physiological parameters: photosynthetic rate (m mol of CO_2 m^{-2} s^{-1}), transpiration rate (m mol O_2 m^{-2} s^{-1}) and stomatal conductance (m mol H_2O m^{-2} s^{-1}) were measured from leaves, by using the Infra-Red Gas Analyzer. Leaf gas exchange measurements were taken from second mature fully expanded leaf from 9.00 to 11.00 A.M using a portable gas exchange system, CIRAS-3 (PP-Systems, Amesbury, MA 01913 USA).

Estimation of Arsenic (As) Concentration

Roots, shoots and leaves were separately oven dried at 75°C till constant weight. In a conical flask, 0.5 g of oven-dried plant material was weighed and incubated with 2:1 mixture of concentrated nitric acid and perchloric acid and left for 12 hours at room temperature. Flasks were sealed with aluminum foil to avoid dust entry (Abbas et al., 2018).

The digestion flasks were digested on a hot plate up to 350°C until fumes become visible and the solution seems transparent. After digestion, flasks were cooled and added 25 mL of distilled water for dilution. The estimation of arsenic concentration in the soil and different plant parts by using a hydride generation-atomic absorption spectrometer (HG-AAS; Agilent AA 240 with VGA-77; Australia)

Statistical Analysis

The experiment was analyzed by using a completely randomized design (CRD) under two factor factorial using Statistix 8.1 Software. Mean values were compared by two-way ANOVA followed by the Tukey’s test.

3. RESULTS

Growth Parameters

Results showed that gypsum (G) significantly increased shoot length, root length, shoot diameter, dry weight of shoot, root, branch and leaves as compared to control in all three selected agroforestry species (Fig.1).

All of the growth parameters showed better results in under all inorganic amendments especially Gypsum (G) as compared to control treatment. In gypsum (G) treatment, maximum height of plant was recorded in the plants of *A. ampliceps* (51.90±0.49), whereas minimum shoot length was recorded in *D. sissoo* plants (10.74±0.07) in control treatment. In this study, general trend for species was *Acacia. ampliceps* > *Vachellia. nilotica* > *Dalbergia. sissoo* and the treatments order was observed as G > FO > L > CA > control, respectively.

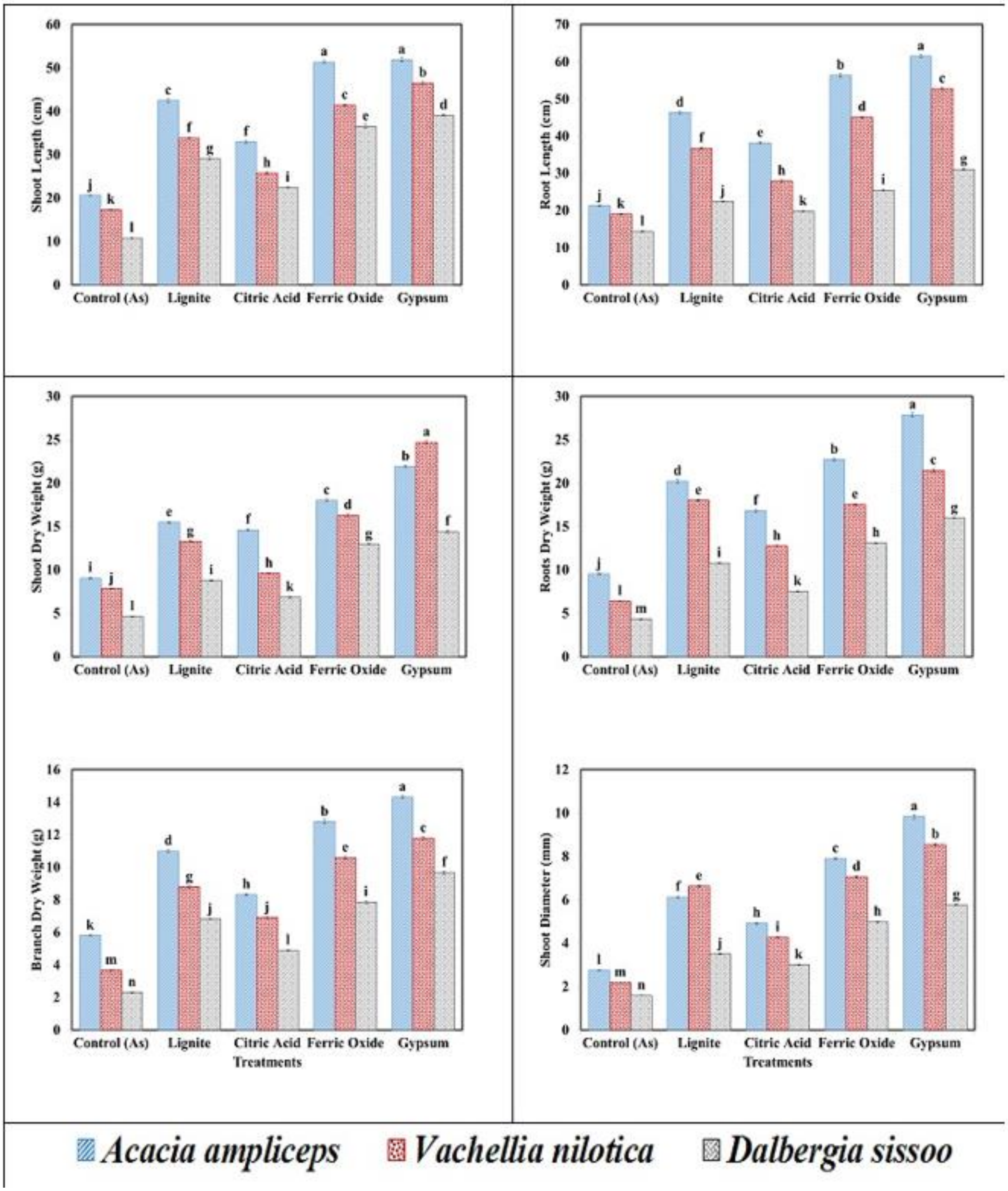


Figure 1: Growth Parameters of Selected Agroforestry Tree Species under Various Inorganic Amendments

Physiological Parameters

Some of the physiological parameters have shown significantly different results against different types of inorganic amendments under as stress (Fig.2). All the amendments showed better results and enhanced photosynthetic rate, stomatal conductance and transpiration rate as compared to control. In gypsum (G) treatment, *A. amplicpes* showed best results as compared to other amendment treatments. Therefore, addition of amendments, especially gypsum (G) was decreased the as stress and can improve the physiological attributes. In this study, general trend for species was *Acacia. ampliceps* > *Vachellia. nilotica* > *Dalbergia. sissoo* and the treatments order was observed as G > FO > L > CA > control, respectively.

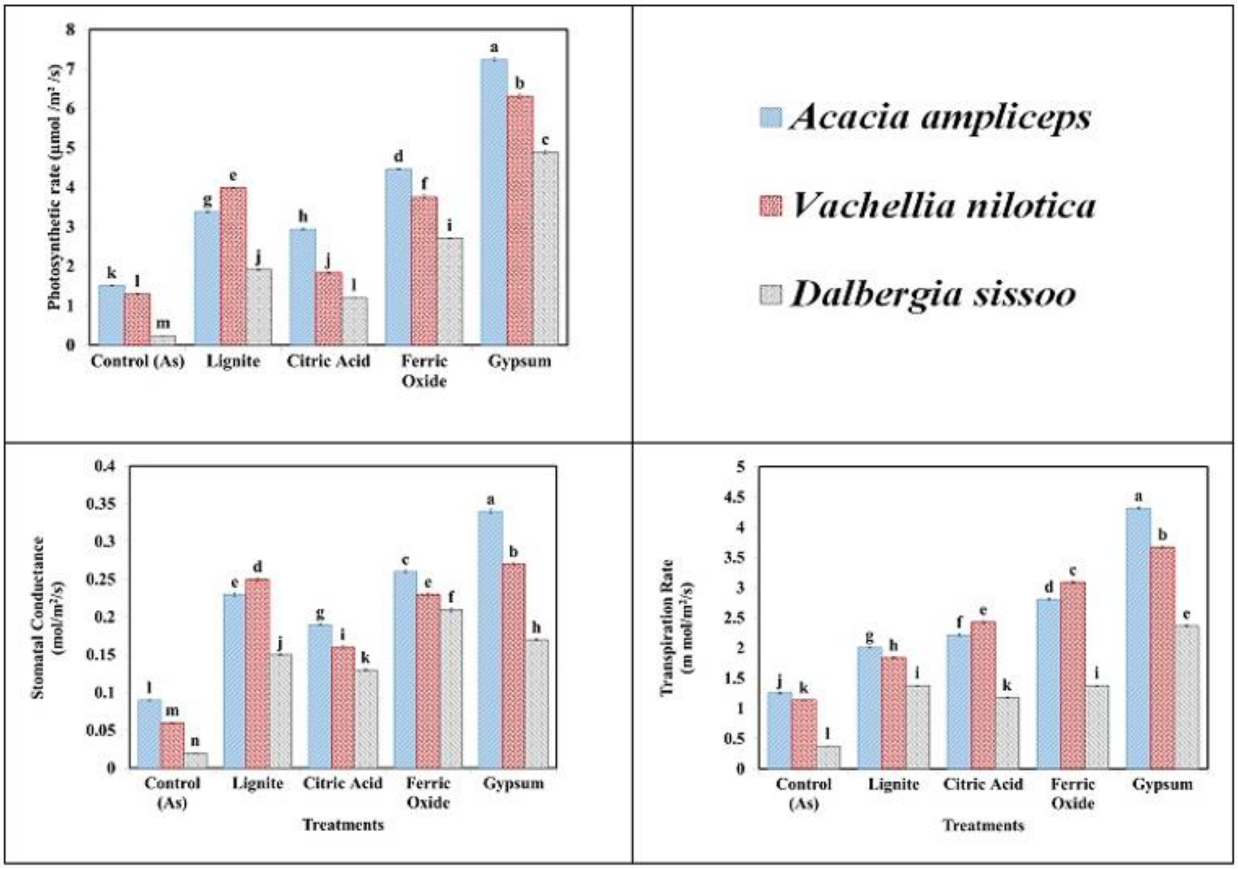


Figure 2: Physiological Parameters of Selected Agroforestry Tree Species under Various Inorganic Amendments

Arsenic Contents

The results showed that the concentration of arsenic in roots, shoots and branch + leaves were significantly different in all three nitrogen fixing tree species as compared to control. Arsenic (As) concentration in roots, shoots and branch + leaves of selected nitrogen fixing tree species have been shown in Fig. 3. Maximum concentration of arsenic (92.47±0.55) was recorded in control treatment while on the other hand minimum concentration of arsenic (20.34±0.13) was present in the plants where gypsum was applied.

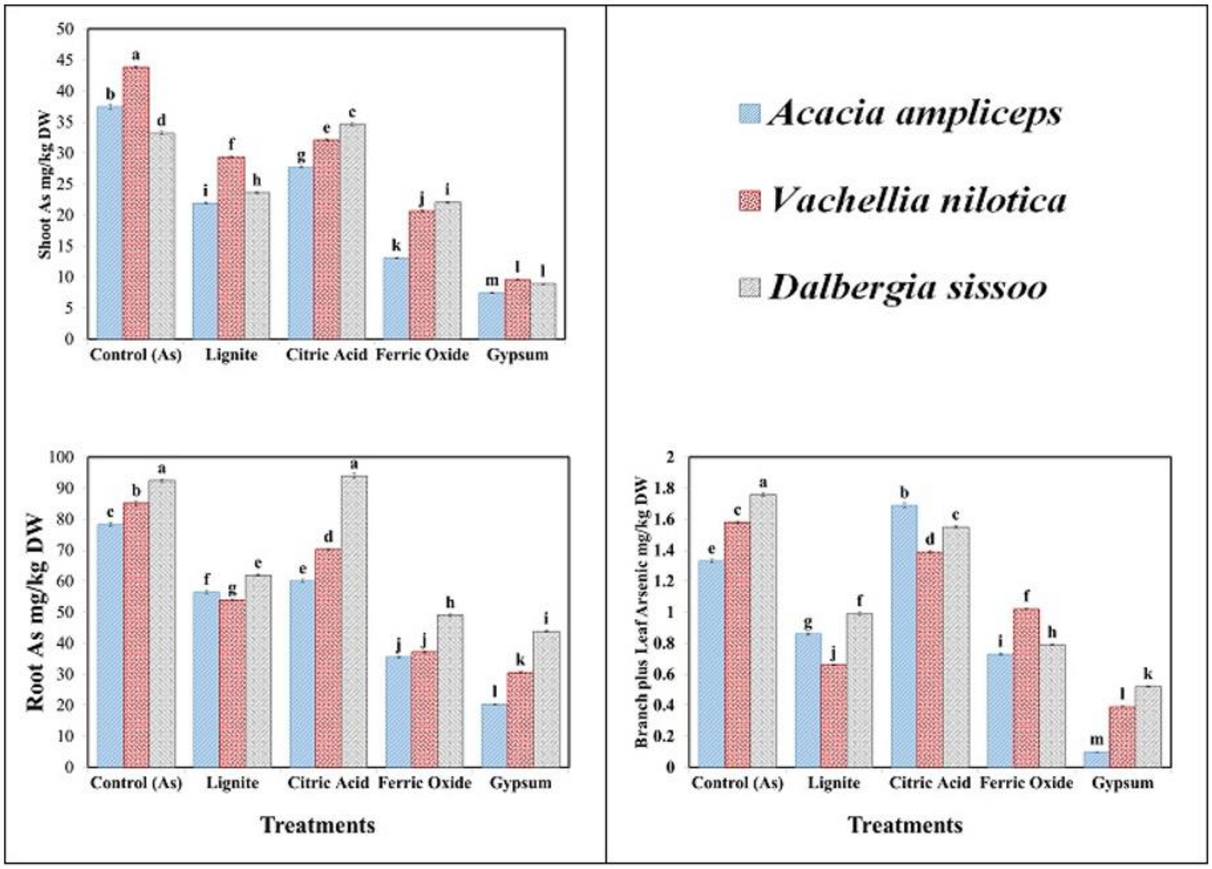


Figure 3: Concentration of Arsenic in Various Plant Parts of Selected Leguminous Tree Species under Various Organic Amendments

4. DISCUSSION

This study was originated to check the effects of four soil inorganic amendments on arsenic (As) bioavailability, As uptake and growth of selected three agroforestry leguminous trees species (*Acacia. ampliceps, Vachellia. nilotica* and *Dalbergia. sissoo*) under arsenic (As) contaminated soil. The Lignite (L), Citric acid (CA), Ferric oxide (FO) and Gypsum G) were selected for this study. Gypsum (G) was performed a very good and effective results in increasing plants growth against all three selected agroforestry tree species as compared to other inorganic amendments were used in this study (Figure 1). Growth and other physiological processes of plants with inorganic amendments based on species specific, gypsum (G) was showed effective and good results to enhancing the growth of *D. sissoo* when compared with other three amendments. On the other hand, ferric oxide (FO) was showed the effective results in enhancing the growth of *V. nilotica* and *A. ampliceps* as compared to citric acid (CA) and lignite (L). *A. ampliceps,* in this regard, showed a best results and great potential against As stress in all the treatments than *V. nilotica* and *A. ampliceps* as compared to control.

Toxic concentrations of arsenic triggered various disorders in plants at morpho-physiological and biochemical levels leading to retarded growth, necrosis, reduced photosynthetic activities (Abbas et al., 2018; Suriyagoda et al., 2018; Jiang et al., 2022), plant water status (Bhat et al., 2021) and reactive oxygen species metabolism (Siddiqui et al., 2020). Gunes et al. (2009) reported that arsenic increased its concentrations in plant organs, reduced seedling growth, and induced oxidative stress through increasing ROS and MDA concentrations in plant cells. Singh et al. (2021) reported impaired root morphology and reduced growth and development in Arabidopsis plants. Similarly, working with Cicer arietinum, Kapoor et al. (2021) demonstrated that higher uptake of arsenic resulted in retarded plant growth and development, and affected water potential and physio-biochemical events. Recently, Mishra et al. (2021) recorded that the toxic levels of arsenic hindered plant height, root hair density, seedling growth, and photosynthetic pigments. Working with the wheat crop, Saeed et al. (2021) reported that arsenic toxicity wholly affected seedling germination, root growth, and biomass production, seed productivity, and thus persuaded the oxidative stress. According to Khan et al. (2020), higher uptake of heavy metal ions by plant roots alters the synthesis of photosynthetic pigments. Similar was reported by Rahman et al. (2015) where authors stated that high arsenic concentrations resulted in a substantial reduction in chlorophyll pigments and photo assimilates production. In the same context, Chaves et al. (2009) reported reduced seedling growth, photosynthesis, gasses exchange parameters. Singh et al. (2019) studied the effects of two concentrations of arsenic viz. 100 and 200 µM and reported that both levels resulted in a prominent reduction in photosynthetic and transpiration rates, and stomatal conductance with a genotypic dependent response.

Arsenic stress causes oxidative stress through the excessive accumulation of ROS in plant cells that cause injury to the cells and disrupt the metabolic events (Ahammed and Yang, 2022; Nahar et al., 2022). Under arsenic stress, alterations in antioxidative enzymes have been well reported in different crop species (Rai et al., 2011; Suhel et al., 2022; Dolui et al., 2022). A similar work was reported in which tree plants under arsenic-contaminated sites recorded a significant reduction in growth, physiological and biochemical traits. Higher uptake of arsenic by plant roots causes disorders in primary (such as fundamental chemical processes that control plant growth) and secondary (i.e., non-essential chemical compounds) metabolisms. For example, working with Ceratophyllum demersum (L.), Mishra et al. (2008) recorded a significant increase in amino acids (i.e., Gys) activity under arsenic stress conditions. Srivastava et al. (2011) reported a significant increase in amino acids in Hydrilla verticillate seedlings when exposed to arsenic stress. A significant decrease in protein content in arsenic-treated seedlings was also demonstrated by various authors (Singh et al. 2006; Gupta and Ahmad, 2014).

Numerous studies reported the potential of organic and inorganic amendments for ameliorating the adverse effects of arsenic phytotoxicity in plants (Qiao et al., 2018; Stazi et al., 2022). In this work, our results clearly demonstrated that inorganic gypsum improved the seedling growth, physiology, and antioxidant activities and decreased the accumulation of arsenic ions in different tree organs. Kang et al. (2018) also confirmed the same that soil amendment with gypsum (as an inorganic amendment) showed a considerable effect on arsenic uptake and translocation in different plant organs. Working with rice crops, Irem et al. (2022) evaluated the performance of various organic and inorganic amendments and demonstrated that soil amended with iron sulfate recorded significantly reduced accumulation of arsenic content in different plant organs. In the same study, authors further stated that iron-based fertilizers increased the development of root-Fe-plaque that decreases arsenic uptake through confiscating the arsenic molecules (Irem et al., 2022). Similar was reported by Farrow et al. (2015) in which author's revealed reduced arsenic uptake when soil was amended with 2% Fe-oxide. According to Yu et al. (2017), soil amendments with iron-based fertilizers resulted in reduced uptake of arsenic in different plant organs. In this work, we recorded that the inorganic amendments with gypsum when applied as sole significantly decreased arsenic accumulation in different organs of all tested tree species. According to Tripathi et al. (2012), plants can tolerate arsenic stress through an antioxidant defense system by increasing the activities of antioxidant enzymes such as glutathione and reducing the activity of ROS. According to Pandey et al. (2017), some secondary metabolites such as alkaloids, and phenolics are produced under arsenic stress. High production of these compounds improved plants' tolerance to heavy metal stress (Isah, 2019). A similar was reported in this work where organic treatments significantly increased the production of secondary metabolites in all tested tree species.

Furthermore, it is also well demonstrated that the selection of woody species is an important strategy for enhancing plant performance under stressful conditions. Nawaz et al. (2016) demonstrated that both trees and shrubs plants normally showed high phytoremediation potential against arsenic stress associated with longer and strong root systems and fast growth rates. Longer and thicker roots help plants maintain moisture uptake even under high concentrations of heavy metals (Gąsecka et al., 2021). Our work reported the same that all tree species showed a variable response to arsenic stress. Among the studied tree species, *A. ampliceps* recorded significantly higher growth indices, and physiological and biochemical attributes when compared with other species under arsenic stress. High tolerance of *A. ampliceps* was associated with increased photosynthetic pigment contents and better antioxidant enzyme activities than other species. In line with our results, Abbas et al. (2018) reported similar findings that species respond differentially to heavy metal stress conditions in terms of growth and physiological indices. Furthermore, Harrison et al. (2013) demonstrated that environmental conditions also determine the plant tolerance potential. This might be another reason for the variable response of species in this work. Hussain et al. (2017) studied the response of tree species to arsenic stress and reported that arsenic stress significantly decreased seedling-height tolerance index and increased the root length stress tolerance index of all tested tree species.

5. CONCLUSION

Arsenic (As) contamination in soil is a global threat to food safety and human health. Intake of As-contaminated water through irrigation of soil is one of the major pathways of as exposure to humans. Inorganic amendments can play a major role to reclamation and amelioration of as contaminated soil which absorbs the pollutants by binding and reduce the mobility of trace elements. This phenomenon is referred to as stabilization. It is observed that use of inorganic amendments can enhance plant growth and reduce as phytoavailable in plant parts.

In conclusion, the addition of four different inorganic amendments showed good result as compared to control but gypsum (G) reduced as translocation and Phytoavailability and give the best growth in all agroforestry species under As-contaminated soil. On the other side, *A. ampliceps* showed a best result among all the three species and significant reduction in as uptake with gypsum (G) application as compared to other amendments, which can be grown in arsenic contaminated soils. In future, stake holders can use inorganic amendments as an additive to improve the phytoremediation of As-contaminated soil by agroforestry tree species. Moreover, they should consider phytoremediation to remediate as contaminated sites with the help of cheap on site operations and byproducts of mines and industries.

Conflicts Of Interest

The authors declare no conflicts of interest.

Authors Contributions

F.E., M.F.N. and N.K.N. M.I.A. conceived the idea and designed the experiment. F.E and M.F.N performed the experiment and data analysis. F.E and M.F.N. prepared the original draft of this work. M.I.A. and N.A. contributed to the revision and proofreading of this manuscript.

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