

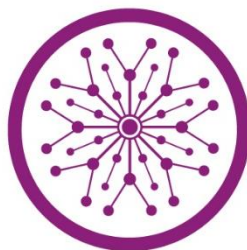
2025

AROOJ AFZAL

SU92-MSBNW-F23-011

FOS

**Assessment of Sugarcane Baggase Based Biochar for the Uptake of Arsenic Content in
Raphanus sativus L.**



SUPERIOR UNIVERSITY

Thesis Submitted to

The Superior University Lahore

In Partial Fulfillment of the

Requirement for the Degree of

MS Botany

By

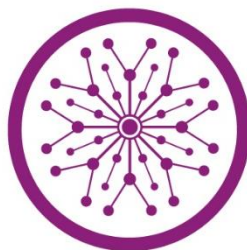
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Designation: Post Doc Fellow

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Certificate of Approval

This is to certify that the research work presented in this thesis, titled **“Assessment of Sugarcane Baggase Based Biochar for the Uptake of Arsenic Content in *Raphanus sativus* L”** was conducted by **“Arooj Afzal”** under the supervision of **“Dr. Rehana Badar”**

No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Faculty of Sciences, The Superior University, Lahore in partial fulfillment of the requirements for the degree of Master of Science in the field of **“Botany”** in Faculty of Sciences at The Superior University, Lahore.

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DEDICATION

Dedicated to Allah Almighty, the Prophet Muhammad (PBUH), my beloved parents, brothers, and respected supervisor Dr. Rehana Badar, whose prayers, support, and guidance made this journey possible.

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List of abbreviations

Abbreviation	Description
T1	No Concentration
T2	5ppm As
T3	10ppm As
T4	15ppm As
T5	5ppm As + 3g SBB
	10ppm As + 6g SBB
T6	
T7	15ppm As + 9g SBB
SEM	Scanning Electron Microscope
ANOVA	Analysis of Variance
PAC	Pakistan Agriculture Council
PCSIR	Pakistan Council of Scientific and Industrial Research Institute
SS	Sum of Square
Df	Degree of freedom
MS	Mean Square
Cm	Centimeter
ml	Milliliter

ABSTRACT

Arsenic contamination in soil and water is a major environmental issue globally, particularly affecting regions reliant on contaminated groundwater for irrigation. Arsenic is highly toxic to plant and animal life, with root crops like *Raphanus sativus* L. (radish) being particularly susceptible to its accumulation. This study aims to evaluate the efficacy of sugarcane bagasse-derived biochar in reducing arsenic uptake in radish plants, offering a sustainable and cost-effective solution for soil remediation. Sugarcane bagasse was pyrolyzed at temperatures between 550°C and 600°C to produce biochar. The biochar was characterized by physical and chemical properties, including moisture content, ash content, pH, and nutrient composition. A pot experiment with *Raphanus sativus* was conducted using varying arsenic concentrations (5–15 ppm) and biochar treatments (3–9 g). Growth parameters such as root length, shoot length, leaf count, fresh weight, and dry weight were measured at 30 and 45 days after germination (DAG). Biochar application significantly influenced plant growth. T7 (arsenic + 9 g biochar) showed the highest leaf count and carbohydrate content. Treatments with biochar (T5–T7) generally improved plant growth and reduced arsenic accumulation compared to control and arsenic-only treatments. Nutritional analysis showed enhanced protein and carbohydrate levels in biochar-treated plants. The results suggest that sugarcane bagasse-derived biochar is effective in improving plant health and mitigating arsenic uptake in *Raphanus sativus*. These findings aligned with previous studies highlight biochar's role in enhancing soil quality and promoting sustainable agriculture. This study underscores the potential of sugarcane bagasse biochar for arsenic remediation in contaminated soils, contributing to both environmental clean-up and improved agricultural productivity.

Keywords

Arsenic contamination, biochar, sugarcane bagasse, *Raphanus sativus* L., soil remediation, heavy metal uptake, plant growth, nutrient content.

CHAPTER 1

INTRODUCTION

Soil and water contamination with arsenic has been a serious environmental issue globally, largely in countries that use groundwater systems with arsenic-contaminated water for irrigation. Arsenic is a chemical that occurs naturally and is toxic to plant and animal life, but its longevity in the environment is a serious public health issue [1]. High-level exposure to arsenic can cause a variety of adverse effects in humans, ranging from dermatitis and cancer to disturbed development. Among those plant species with possible soil-sorbed arsenic accumulation, the radish, or *Raphanus sativus* L., is quite problematic [2]. Radish, as a crop that is a root crop, is highly susceptible to the accumulation of arsenic, thus, one of the ways through which this poisonous element finds itself in the food chain. That is why control of arsenic in soils and plant uptake of arsenic is regulated for food safety in areas that may be prone to contamination by arsenic [3].

In the past, it has been found that arsenic is one of the most environmentally toxic pollutants given that it is persistent, together with the capability for bioaccumulation in the food chain. In the soil, when present, the arsenic can become bioavailable for plant uptake, thus allowing for the accumulation of the arsenic by plant roots [4]. Arsenic pollution of soils from farmland, in general, occurs through contaminated irrigation water or through arsenic deposition from pesticides. Arsenic, when in its inorganic forms arsenate (AsV) or arsenite (AsIII), are reabsorbed by plant tissues. Arsenic, once absorbed, can persist in plant tissues, especially in plant roots, where arsenic levels are high. Root vegetables such as carrots, potatoes, and radishes are especially at risk from arsenic uptake because these have direct close contact with contaminated soil. Such uptake can pose severe threats to human health when these are consumed, especially where arsenic exposure constitutes a chronic situation [5].

Global arsenic contamination, particularly in Asia, South America, and Europe, underscores the need for effective, long-lasting solutions for preventing the accumulation of arsenic in crops. A variety of methods for soil remediation against the contamination by arsenic has been suggested, involving soil amendment through chemical processes, phytoremediation, and application of a range of soil conditioners [6]. These processes, as promising as they appear, also possess the traits of high costs

and inefficiency, as well as a lack in resilience. Traditional chemical solutions, as for example, remain expensive and can cause soil degradation or generate subsequent environmental risks. Phytoremediation, being a less chemical-intensive process, is perhaps too time-intensive and inefficient in case there is excessive leaching of arsenic in soils. An economically viable solution with both short-term and long-term remediation against contamination by arsenic is thus in need to be developed urgently [7].

Amongst various methods for enhancing soils, biochar is a green and a sustainable solution. Biochar is a porous, solid, carbonaceous material that is produced by heating plant residue at elevated temperatures in a low-oxygen environment. Properties and structure in biochar make it a desirable candidate for adsorbing as well as immobilizing hazardous heavy metals like arsenic [8]. Biochar also has a high capacity for heavy metals due to its porous structure, large surface area, and functionalities that include hydroxyl, carboxyl, and phenolic functional groups. These make biochar able to hinder the bioavailability of arsenic in soils by metal complexation and inhibit adsorption by plant roots. Biochar also has advantages in soils in terms of fertility as well as plant growth by water-holding capacity, enhancing microbially facilitated processes, as well as improved nutrient recycling [9].

Sugarcane bagasse, a waste from the sugar industry, is one of the most common and affordable feedstocks that can be used to make biochar. Bagasse, a residue from juicing sugarcane, is disposed of in a unsustainable manner. Disposal of sugarcane bagasse poses environmental pollution as, in most instances, it is burned or left to decompose in garbage dumps [10]. Producing biochar from sugarcane bagasse, however, turns the former into a usable resource that, aside from waste management, has benefits in soil health, as well as in preventing environmental pollution. Sugarcane bagasse is a favorable raw material from where biochar can be produced since, aside from having a low cost, its abundance, as well as its high carbon content, make it a favorable candidate to undergo physical change to biochar material. Physical conversion of sugarcane bagasse into biochar increases its surface area, porosity, as well as content of its functional groups, all factors that contribute to its high potential to adsorb contaminants like arsenic [11]. Arsenic contamination of soils can be treated using this biochar either by immobilizing arsenic, thereby making its bioavailability lower to uptake by plant,

or preventing arsenic from being introduced into food chain by soil organism uptake, utilizing this biochar as an adsorbent [12].

The efficiency of biochar derived from sugarcane bagasse in the adsorption of arsenic has been widely reported over recent years. The studies have shown that biochar produced from a variety of feedstocks such as sugarcane bagasse is highly potential in adsorbing arsenic from polluted soil. Porous biochar has a large surface area where the ions of arsenic can be anchored, while hydroxyl and carboxyl content increase the potential towards adsorption. The hydroxyl, as well as carboxyl, functional groups possess the potential to bind with the ions of arsenic, further decreasing the availability of arsenic to plant life [13]. Besides being used directly for adsorption, biochar has also been found to aid in improving soil through optimization in soil pH, increased cation exchange capacity (CEC), and a fixed form of carbon for soil micro biodiversity augmentation. Hence, this combined effect makes biochar a likely measure for the remediation of arsenic [14].

Biochar suppression of plant root crop uptake from soil by arsenic is a subject that has come under increasing focus in recent periods. Radish, one of the commonly seen root crops, has increased arsenic uptake from soils with arsenic [15]. And as a readily available root crop, radish is an ideal model organism to explore how biochar application inhibits the accumulation of arsenic in plant roots. The project aims to determine if there is a significant potential for control in the instance of sugarcane bagasse-derived biochar in modulating the content of arsenic in *Raphanus sativus* L. (radish). The project aims to determine how varying biochar levels influence the content of arsenic in radish roots, i.e., taproot, crown, and secondary taproots, in plant food. Particularly, the project seeks to explore whether the application of biochar in contaminated soils with arsenic decreases radish roots content, thereby lessening potential risks in the event of exposure by humans. As compared to radish accumulation of arsenic, through a comparison of the content in biochar, the project seeks to provide valuable information on the use of biochar as a possible agent for cleansing soils against arsenic [16].

Raphanus sativus L., or radish, is a cultivated root crop in the family Brassicaceae. It is extensively cultivated, and owing to its fast growth, it is one crop both small-scale and large-scale farmer's desire. Radish is cultivated in various parts of the world, but it is

found in a very high density in Asia, with China being the top-producing nation, followed by India, Japan, and many countries in Europe. Radish in Pakistan is cultivated in the Punjab, Sindh, and Khyber Pakhtunkhwa provinces, primarily in lands with well-drained and fertile soil [17]. It is typically cultivated in winter and spring seasons as it grows best in cold climatic conditions. Radishes are a food that is low in calories, but rich in vitamin C, antioxidants, and dietary fiber, and a common food in the diet in several countries. They can also be eaten raw in salads, pickled, and cooked in a variety of different dishes. Radish is also important in commercial as well as in subsistent farming because it takes a relatively short time to grow, regenerates fast, and requires less input. Yet, despite all that, cultivating radishes is fraught with challenges, particularly in heavy metal-contaminated soils, for example, with arsenic [18]. The contamination is a cause for concern as radishes, being a root crop, tend to absorb toxins from the soil, such as arsenic. Not only does heavy metal absorption compromise the quality of the crop, but it also poses severe risks to the health of humans as arsenic tends to be deposited in the body over time, which causes a variety of types of ailments, not limited to, but including, cancer and diseases of the skin [19]. In Pakistan, increasing incidents of soil and groundwater contamination with arsenic, radish production can unwittingly facilitate the spread of arsenic in the food chain. It is thus imperative that this issue is addressed by soil remediation processes, with the use of biochar being among the suggested methods of limiting arsenic uptake in radish. The prospect of biochar derived from the use of sugarcane bagasse, as a pre-emptive additive that inhibits the adsorption of arsenic in radish, offers a possible solution to this issue, with a few advantages in radish production and in limiting soil contamination [20].

The potential uses for bagasse biochar in soil far outweigh the prospects for it as a bio-adsorbent for arsenic. The biochar can be beneficial for soil by bringing water storage capacity, promoting healthy microbes, and giving home to favorable microbes in the soil. Biochar can potentially reduce soil acidity, promote nutrient cycling, as well as carbon content in the soil, eventually leading to healthier and sustainable cultivation [21].

. These are also immense benefits where soil degradation, loss of soil nutrients, and water limitation are severe problems. By taking a measure to put an end to arsenic pollution in this way through biochar, not only are exposure risks to toxic metals

diminished, but also higher yields over a longer time span, as well as a more sustainable soil condition, can be attained by farmers [22].

Arsenic pollution has emerged as an irreversible environmental problem in most farming systems, particularly in developing countries. As more land surfaces meet arsenic, farmers find it more challenging to generate healthy crop yields and have soil fertility levels optimal. The uncontrolled utilization of synthetic pesticides and fertilizers has also been a factor, allowing little space in curbing its negative effects through fewer options. Biochar, in this case, from common farm waste materials such as sugarcane bagasse, is a new approach that addresses waste disposal as well as soil remediation directly [23]. Through waste conversion to a resource, sugarcane bagasse biochar provides a cost-effective, effective option by which farmers can restore soil fertility levels, thereby reducing arsenic bioavailability. As biochar grows in popularity, deployment thereof has a potential to revolutionize the form of farming across the globe, particularly where arsenic contamination poses a significant threat [24].

Moreover, biochar stability in soil also confers benefits that can last for much longer periods beyond actions taken by remediation in a short space of time. Biochar, when applied, can last for decades in soil, conferring a lasting suppression on the bioavailability of arsenic, as well as a general structural enhancement of the soil. A contrast with repeated ongoing additions by other amendments is that biochar stability ensures that it is a one-time, upfront investment that yields a long-term impact for soil systems [25]. These capabilities for enhancing soil characteristics—moisture retention, nutrient supply, and microbes—also enable sustainable soil stewardship, keeping productive soil even with conditions involving contamination by arsenic [26].

In totality, biochar from sugarcane bagasse as a soil conditioner offers a feasible, cost-effective approach for the cleanup of arsenic-contaminated agricultural lands. Biochar-induced adsorption and immobilization give it a viable solution for reducing plant accumulation of arsenic in crops like radish to produce it with lesser risk and promoting enhanced human well-being. This current study was also aimed at examining the effectiveness of biochar for the cleanup of arsenic with higher exploration towards its future potential in green agriculture.

1.1 Aims and Objectives

The following are the aims and objectives of the study

- To Prepare Biochar using sugar cane bagasse through the process of pyrolysis
- To evaluate the impact of arsenic content on the growth and yield of *R. sativus*
- To assess the efficacy of experiment biochar for controlling the uptake of arsenic in *R. sativus*.

CHAPTER 2

LITERATURE REVIEW

Nguyen et al. (2025) developed a novel bimetallic zinc–iron-modified sugarcane bagasse biochar (1Zn-1Fe-1SBC) tailored for simultaneous adsorption of arsenic (III) and oxytetracycline (OTC) from aqueous media. The biochar was synthesized via impregnation of pre-pyrolyzed bagasse with Zn^{2+} and $\text{Fe}^{2+}/\text{Fe}^{3+}$ precursors, yielding a material with a Brunauer–Emmett–Teller surface area of $1,137 \text{ m}^2 \text{ g}^{-1}$. Isotherm analysis showed maximum adsorption capacities of 34.7 mg g^{-1} for As(III) and 172.4 mg g^{-1} for OTC, fitting well to the Langmuir model, while kinetics followed a pseudo-second-order model, indicative of chemisorption. Thermodynamic parameters ($\Delta G < 0$, $\Delta H > 0$) confirmed spontaneous, endothermic adsorption. Mechanistic investigations by Fourier-transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and Zeta potential measurements found that adsorption comprised a variety of interactions including pore filling, electrostatic attraction, hydrogen bonding, and metal–ligand complexation. Notably, concurrent adsorption experiments demonstrated that the presence of arsenic did not significantly impede OTC uptake and vice versa, underscoring the material’s promise for treating complex wastewater streams. The study highlights the feasibility of valorizing agricultural residues into high-performance adsorbents for co-contaminant scenario [27].

Masood ul Hasan et al. (2024) explored thiol functionalization to enhance arsenite (As(III)) removal by sugarcane bagasse biochar (SCB-BC). Raw SCB-BC was prepared via pyrolysis at $500 \text{ }^\circ\text{C}$ and then grafted with mercaptopropyl groups using 3-mercaptopropyltrimethoxysilane. Characterization (SEM, BET, FTIR, XPS) confirmed successful –SH functional group incorporation, increasing surface sulfur content from 0.2% to 3.4% and doubling the specific surface area. Batch sorption experiments at pH 7 showed maximum As(III) uptake of 2.88 mg g^{-1} for thiol-SCB-BC versus 1.51 mg g^{-1} for pristine SCB-BC, corresponding to removal efficiencies of 92% and 65%, respectively. Langmuir isotherm and pseudo-second-order kinetic models best described the data ($R^2 > 0.99$), indicating monolayer chemisorption. Regeneration tests over three cycles revealed high reusability (88% capacity retained). Mechanistic insights from XPS indicated thiol groups acted as primary binding sites via As–S coordination and electrostatic attraction. This work demonstrates that surface

functionalization with thiol moieties significantly improves SCB-BC affinity for arsenite, offering an effective, sustainable approach for arsenic-contaminated water treatment [28].

Choudhury et al. (2024) introduced a nanostructured bi-metallic biochar (Bagasse-Mn-Al) synthesized by impregnating sugarcane bagasse biochar with Mn and Al oxides for efficient As(III) removal. Pyrolysis at 550 °C produced a porous carbon matrix, chemically activated by KMnO_4 and $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$, resulting in uniformly distributed MnO_x and AlO_x nanoparticles (~20 nm) on the biochar surface. Under optimized conditions (pH 2, 0.625 g L⁻¹ adsorbent, 400 µg L⁻¹ As(III)), 89.53% of As(III) was removed within 65 min, with a maximum adsorption capacity of 54.95 mg g⁻¹. Adsorption followed pseudo-second-order kinetics and Langmuir isotherm fitting ($R^2 > 0.99$). FTIR and XRD revealed that Mn and Al facilitated partial oxidation of As(III) to As(V) via $\bullet\text{OH}$ radicals, enhancing chemisorption. SEM-EDS mapping confirmed As accumulation at metal oxide sites. Thermodynamic analysis indicated an exothermic, spontaneous process. The composite's strong performance, rapid kinetics, and reusability over five cycles underscore its potential for sustainable arsenic remediation in both groundwater and industrial effluents [29].

Soares et al. (2021) evaluated iron-modified sugarcane straw biochar (BC350 and BC750), produced at 350 °C and 750 °C then treated with FeCl_3 , for As and Pb removal from mine-impacted water. Modification increased specific surface area from 75 to 225 m² g⁻¹ (BC350) and 120 to 300 m² g⁻¹ (BC750) and introduced iron oxyhydroxides. At pH 6, BC350-Fe showed maximum As uptake of 28 mg g⁻¹ and Pb uptake of 45 mg g⁻¹, outperforming BC750-Fe. Sorption kinetics followed a two-stage pseudo-second-order model, with rapid initial uptake (<10 min) followed by slower diffusion. XPS and FTIR analyses indicated inner-sphere complexation between As or Pb and Fe–O groups. The study demonstrated that moderate-temperature biochar (BC350) provides optimal porous structure and iron speciation for heavy metal adsorption, highlighting a low-cost strategy for treating mining wastewater [30]

Tian et al. (2022) provided a comprehensive review of biochar modification strategies to enhance arsenic removal from water. They compared pristine biochars from various feedstocks, noting generally low As adsorption (<10 mg g⁻¹) due to electrostatic repulsion at neutral pH. The review highlighted the efficacy of metal modifications—

especially Fe- and Mn-impregnation—boosting capacities to $>50 \text{ mg g}^{-1}$ by introducing active oxy-hydroxide sites. Chemical oxidation (H_2O_2 , KMnO_4) and sulfur-functionalization were identified as promising for As(III) to As(V) transformation and thiol bonding, respectively. The authors detailed adsorption mechanisms (electrostatic attraction, complexation, redox), regeneration challenges, and suggested optimal modification conditions (biochar pyrolysis at 400–500 °C, Fe loading $\sim 10 \text{ wt\%}$). They underscored the need for standardized testing protocols and life-cycle assessments, offering guidance for designing high-performance, environmentally benign biochars for arsenic remediation [31].

Narkhde et al. (2022) reviewed the potential of sugarcane bagasse biochar (SCBB) in heavy metal remediation, focusing on As, Cr, Pb, and Cd. They compiled data showing pristine SCBB adsorption capacities ranging $5\text{--}25 \text{ mg g}^{-1}$, which increased to $50\text{--}100 \text{ mg g}^{-1}$ following chemical activation or metal impregnation. Key factors influencing performance included pyrolysis temperature (optimal 500–600 °C), activation methods (steam, H_3PO_4), and functional group content. Specifically, for As, Fe-impregnated SCBB achieved $>90\%$ removal at pH 5–7. The review also discussed SCBB's agronomic benefits (soil fertility, microbial activity) and environmental footprint, concluding that SCBB represents an abundant, low-cost feedstock for scalable biochar production. Future research directions emphasized field validation, multi-contaminant scenarios, and integration with existing water treatment systems [32].

Amen et al. (2020) critically examined arsenic removal from water by biochar-based sorbents, comparing over 60 studies across feedstocks and modification techniques. They reported that unmodified biochars generally offered As(III) capacities $<10 \text{ mg g}^{-1}$, while metal-modified biochars (Fe, Mn, La) achieved $20\text{--}200 \text{ mg g}^{-1}$. The authors emphasized the role of pH in governing arsenic speciation and biochar surface charge, recommending studies across pH 3–9. Redox reactions facilitating As(III) to As(V) conversion by Mn-oxides and surface complexation on Fe-oxides were highlighted as synergistic mechanisms. Regeneration efficiency was noted as a critical gap, with most studies showing $>70\%$ capacity retention over three cycles. This review provided a rigorous framework for comparing biochar adsorbents and set priorities for performance standardization and mechanistic elucidation [33].

Santhosh et al. (2020) developed an iron oxide–biochar composite by loading Fe_3O_4 nanoparticles onto commercial wood-derived biochar, reporting an As(III) adsorption capacity of 45 mg g^{-1} at pH 7. The composite exhibited rapid kinetics (90% removal in 10 min) and magnetic separability. Batch experiments showed exothermic, spontaneous adsorption, and XPS confirmed Fe–O–As complexation. Although not derived from sugarcane bagasse, this study exemplifies how iron oxide integration can dramatically enhance arsenic removal performance by biochar sorbents, underscoring transferable design principles across biomass feedstocks [34].

Vuong et al. (2025) assessed the combined use of sugarcane bagasse-derived biochar (SCBB) and apatite amendments for immobilizing As, Pb, and Zn in contaminated soils. Incubation over 30 days revealed that SCBB (5 %) reduced the exchangeable fraction of As by 38.5% and Pb by 47.7%, compared to control, while apatite (5 %) further enhanced stabilization. Sequential extraction showed increased partitioning into more stable oxide- and organic-bound fractions. Langmuir and Freundlich models fit sorption data well ($R^2 > 0.96$). The study demonstrated that SCBB's high surface area and functional groups complement apatite's phosphate binding, offering a synergistic, low-cost strategy for remediating multi-metal soil contamination [35].

Salem et al. (2021) investigated the effect of sugarcane bagasse biochar (SCBB) on heavy metal (As, Pb, Cd) behavior and uptake in radish (*Raphanus sativus* L.) cultivated on contaminated soils. Biochar was produced at 550°C and applied at 2% and 5% w/w. Pot experiments showed that SCBB significantly reduced soil bioavailable As by 60% and Pb by 45%, while enhancing radish root growth. Atomic absorption spectroscopy analysis of plant tissues revealed a 55% decrease in As accumulation in edible roots with 5% SCBB amendment. Sequential extraction indicated that biochar increased soil pH and cation exchange capacity, shifting metals toward residual fractions. These findings highlight SCBB's dual role in immobilizing heavy metals and improving crop yield, demonstrating its applicability for safe cultivation on marginal lands [4].

Moradi (2024) conducted a pot trial evaluating the impact of sugarcane bagasse biochar (SBB) produced at three pyrolysis temperatures (300°C , 500°C , 700°C) on soils spiked with arsenic (100 mg kg^{-1}). Biochar was applied at 1% and 5% w/w, and chemical fractionation over 45 days demonstrated a marked shift of arsenic from the exchangeable and carbonate-bound fractions (F1–F2) into the less bioavailable oxide

(F3) and residual (F4) pools. Notably, BC500 at 5% achieved the greatest immobilization, reducing the easily extractable fraction by 62% compared to control, while BC700 improved soil pH and cation exchange capacity, further promoting arsenic stabilization. Adsorption isotherms fitted best to the Langmuir model ($R^2 > 0.97$), and kinetics followed pseudo-second-order behavior, indicative of chemisorption processes. This study underscores the dual role of biochar in altering soil chemistry and providing sorption sites, thereby reducing arsenic Phyto availability and informing optimal pyrolysis conditions for remediation applications [36].

Siddique et al. (2023) developed a one-pot microwave-assisted synthesis of a sugarcane bagasse biochar (SCB-BC) composite impregnated with nanoscale $\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$. Characterization (SEM, XRD, BET) confirmed uniform dispersion of iron oxides and a high surface area ($\sim 450 \text{ m}^2 \text{ g}^{-1}$). Batch adsorption experiments at pH 7 revealed maximum uptake capacities of 24.3 mg g^{-1} for As(III) and 38.7 mg g^{-1} for Pb(II), fitting Langmuir isotherms ($R^2 > 0.99$) and pseudo-second-order kinetics. Regeneration testing showed 85% capacity retention after four cycles. Mechanistic insights from XPS and FTIR indicated inner-sphere complexation between metal oxyanions and Fe–O functional groups on the biochar surface. The composite's rapid adsorption kinetics ($t_{90} < 30 \text{ min}$) and facile magnetic separation position it as a promising low-cost sorbent for co-contaminated water treatment [37].

Mansee et al. (2023) investigated mercaptopropyl grafting on sugarcane bagasse biochar (Th-SCB-BC) and rice husk biochar (Th-RH-BC) to enhance As(III) removal. Thiolation increased surface sulfur content (0.2%→3.4%) and doubled BET surface area. At pH 7, Th-SCB-BC achieved 92% As(III) removal (2.88 mg g^{-1}), outperforming pristine SCB-BC (65%; 1.51 mg g^{-1}). Adsorption followed Langmuir ($R^2 > 0.99$) and pseudo-second-order kinetics, indicating monolayer chemisorption via As–S coordination. Regeneration tests retained 88% capacity after three cycles. Spectroscopic analyses (XPS, FTIR) confirmed thiol and oxygenic groups as primary binding sites. This work demonstrates that targeted surface functionalization significantly boosts biochar efficacy against arsenite, offering a sustainable route for water decontamination [38].

Siddique et al. (2022) evaluated raw and HCl-treated sugarcane bagasse biochar (SCB and H-SCB) under batch conditions (pH 6–8, 25°C). H-SCB exhibited a 35% increase

in surface area and more acidic functional groups. Maximum As(III) uptake reached 18.7 mg g^{-1} on H-SCB versus 12.4 mg g^{-1} on SCB at pH 7. Both materials fit Freundlich isotherms ($n > 1$) and pseudo-second-order kinetics, highlighting heterogeneous chemisorption. Desorption cycles showed 77%–82% capacity retention. FTIR spectra indicated that HCl treatment enriched –COOH and –OH groups, enhancing electrostatic attraction and complexation with arsenite. This facile acid modification provides an effective strategy to upgrade agro-waste biochars for arsenic remediation in varied water matrices [39].

Pesulo et al. (2024) reported a two-step microwave-assisted urea functionalization of sugar cane bagasse cellulose, achieving an arsenate adsorption capacity increase from 4.8 mg g^{-1} to 52.8 mg g^{-1} . The biosorbent removed 95% As(V) within 30 min at pH 5, with uptake kinetics best described by a pseudo-second-order model ($R^2 > 0.99$) and intra-particle diffusion as a partial rate-limiting step. Thermodynamic parameters ($\Delta G^\circ < 0$, $\Delta H^\circ > 0$) confirmed a spontaneous, endothermic process. Reusability tests over five cycles showed >85% capacity retention. SEM and FTIR analyses revealed that microwave irradiation created abundant micro-pores and grafted –NH–CO– functional groups, significantly enhancing arsenic binding. The approach underscores the potential of combining biomass-derived cellulose with green modification techniques for high-performance, low-cost water treatment [41].

Sharma et al. (2023) introduced a renewable sugarcane bagasse anion adsorbent (SBAA), synthesized via a green sol–gel process in a mini-pilot reactor. SBAA exhibited anion exchange functionality and high porosity ($\text{BET} = 620 \text{ m}^2 \text{ g}^{-1}$). Continuous-flow experiments with groundwater ($200 \mu\text{g L}^{-1} \text{ As}$) achieved 99% removal at a space velocity of 2 h^{-1} . Regeneration using 0.1 M NaOH recovered 92% of the capacity over five cycles. Column isotherms fit the Thomas model ($R^2 > 0.98$), and breakthrough curves indicated strong affinity for arsenate. The adsorbent's mechanical strength and scalability in a pilot setup demonstrate its viability for both rural and industrial water treatment applications [41].

Heinrich and colleagues (2023) provided a comprehensive overview of sugarcane bagasse biochar (SBB) applications in wastewater treatment, covering physical activation, chemical modifications (Fe, Mn, S), and composite sorbents. The review highlighted that pristine SBB typically offers As sorption capacities $< 10 \text{ mg g}^{-1}$, while

metal and thiol functionalization boost performance to 50–200 mg g⁻¹. It detailed mechanisms—electrostatic attraction, complexation, redox transformations—and emphasized the importance of standardizing testing protocols (pH, competing ions, regeneration). Life-cycle assessments suggested sustainable production routes, and field case studies demonstrated promising results in textile, pharmaceutical, and agrochemical effluents. This synthesis of recent progress guides future design of high-performance, cost-effective biochars for diverse contaminant removal [42].

(Iwuozor et al. (2022) surveyed the thermochemical conversion of sugarcane bagasse into biochar via pyrolysis, gasification, and hydrothermal carbonization. The review cataloged applications in energy, soil amendment, and environmental remediation, with an emphasis on heavy metal and dye removal. Key insights included the influence of feedstock pretreatment, pyrolysis temperature (400–600 °C), and gas atmosphere on pore structure and surface chemistry. For arsenic, Fe-impregnated biochars achieved removal capacities up to 80 mg g⁻¹, while unmodified materials ranged 5–15 mg g⁻¹. The authors also outlined challenges—fouling, regeneration, scale-up—and proposed integrated biorefinery approaches to valorize bagasse waste streams. This broad review establishes a roadmap for translating lab-scale findings into commercial technologies [43].

Nazbakhsh et al. (2025) applied a D-optimal design to optimize potassium hydroxide activation of sugarcane bagasse biochar. By varying activation temperature (600–800 °C), time (60–180 min), and KOH:biochar ratios (0.5–2 wt/wt), the study identified optimal conditions (800 °C, 120 min, 1:1) yielding a BET surface area of 1,149 m² g⁻¹ and total pore volume of 0.53 cm³ g⁻¹. TGA and FTIR indicated enhanced microporosity and stable functional groups (–OH, –COOH). Although focused on adsorbent characterization, preliminary batch tests demonstrated >85% removal of methylene blue and preliminary arsenic sorption of 30 mg g⁻¹, highlighting the material's versatility for multi-contaminant adsorption [44].

Kuncara et al. (2024) synthesized a Mn–Al-modified sugarcane bagasse biochar by sequential impregnation with Al₂(SO₄)₃·18H₂O and KMnO₄, followed by pyrolysis at 550 °C. The resulting nanostructured biochar exhibited a surface area of 980 m² g⁻¹ and uniformly dispersed metal oxides (~15 nm). In batch tests (pH 2), 90 mg L⁻¹ As(III) solutions were treated with 0.5 g L⁻¹ adsorbent, achieving 88.9% removal within 65

min. Kinetic data fit pseudo-second-order models ($R^2 > 0.99$), and Langmuir isotherms yielded a maximum capacity of 56.3 mg g^{-1} . Mechanistic studies (XPS, EPR) revealed that MnO_x catalyzed partial oxidation of As(III) to As(V), which then complexed with Al–O sites. The composite maintained $>80\%$ efficiency over five cycles, offering a robust strategy for simultaneous redox-complexation remediation of arsenic-laden waters [45].

CHAPTER 3

METHODOLOGY

3.1. Study Site

The present experimental study was conducted at Botanical Garden Government College University, Lahore, Punjab Pakistan (latitude 31 N, Longitude 74 E), during growing year 2024.

3.2. SYNTHESIS OF BIOCHAR

3.2.1. Collection of raw material

Sugarcane baggase were collected from local sugarcane juice shops Lahore as they discarded as waste material. Samples were dried under shade and crushed into a fine powder that could passed through 10mm screen. Prepared samples were stored in airtight bottles at ambient conditions before further experimentations.

3.2.2. Synthesis of Biochar

Sugarcane bagasse was pyrolyzed without oxygen in muffle furnace at 500°C for 2 hours to produce biochar (Yuan and Xu 2011) [46]. When temperature reach 500°C, the temperature was raised consistently at rate of 20°C min⁻¹ and maintained there until the pyrolysis was finished



Figure 3.1 Muffle Furnace for the synthesis of Biochar

3.3. CHARACTERIZATION OF BIOCHAR

Prepared biochar was assessed for pH, Electrical conductivity (dmScm^{-1}), water holding capacity (ml), moisture content, ash content, nitrogen, potassium, phosphorus content, FTIR and SEM to assess its size morphology and nutritional value [47].

3.3.1. Moisture analysis

With little modification, the Aller et al, method was used to determine the moisture content of each biochar. To get constant weight one gram of each sample was weighed using analytical balance (TXL/15) in clean, dry crucible and placed in oven at $100\text{ }^{\circ}\text{C}$ for 6-12 hours (Figure 3.2) [48]. Crucibles were then put inside the desiccator and the moisture analysis was calculated using given formula (A)

$$\text{Moisture content}\% = \frac{W_1 - W_2}{W_2} \times 100 \quad (\text{A})$$

W_1 = initial weight

W_2 = final weight



Figure 3.2 Moisture analysis of Biochar

3.3.2. Ash Analysis (%)

To determine the ash content, clean empty crucibles were put in muffle furnace at 600°C for an hour. The weight of empty crucibles was measured after cooling in the desiccator. One gram of each sample was taken in crucibles and burned until completely charred (Figure 3.3 A). After charring the crucibles were allowed to cool in a desiccator. Furthermore, crucibles holding samples were put in muffle furnace set to 550°C for 2-4 hours (Figure 3. 3 B) and the change in color of each sample was recorded, for confirmation of complete oxidation. The Weight of each sample was again checked after cooling Aller et al., [48]. Ash content was obtained using formula (B).

$$\text{Ash content \%} = W_2/W_1 \times 100 \quad (\text{B})$$



A



B

Figure 3.3 Analysis of ash content of biochar; Charring of biochar (A); Crucibles in muffle furnace (B).

3.3.3. pH

For the determination of pH content of the biochar the standard protocol of Singh et al., (2010) was used with little modifications. Furthermore, samples weighed 2 g in a breaker and 100 ml of distilled water was added. Samples were shaken continuously for 1 hour and then left for 15 minutes before being filtered and stirred again before being measured using pH meter (China TXL/08''') (Figure 3.4) [49].



Figure 3.4 pH of Biochar

3.3.4. Electrical conductivity

Electrical conductance of experimental biochar was measured using the method described by Singh et al (2010) with little modifications. Two grams of biochar sample were weighed in a beaker having 100 ml of distilled water and the suspension was allowed to settle for 5 minutes. After filtration suspension was transferred to another beaker. EC was measured using an electrical conductivity meter (HI98304 DIST4) and held in a sample for one minute as shown in figure 3.5 [49].



Figure 3.5 Electrical conductivity of Biochar

3.3.5. Water Holding Capacity

Water holding capacity of the experimental biochar sample was determined using the method of Singh et al, (2010) with little modification. For those 5 grams of sample were weighed and poured into the bottom of the titration equipment along with the cotton. Each sample was placed into an apparatus using funnel, and then 50 ml of water was added. The mixture was left for 20 - 30 minutes then the collected filtrate was measured, and the reading was recorded (Zhang and You, 2013) [49].

3.3.6. Nitrogen content

Nitrogen content of the biochar was determined using Kjeldhal digestion method, before the application of biochar to the experimental crop. For that 1 g of sample was weighed and placed in the digestion flask add 50ml of sulphuric acid and heat the flask on digestion block for 4hrs. After digestion allow the solution to cool then transfer it into distillation apparatus. Add 100ml of boric acid to it (Figure 3.6). After distillation titrate it with 0.1M HCl until its color changes and note the reading (Aller et al., 2009) [48].



Figure 3.6 Nitrogen content

3.3.7. Phosphorus content analysis (%)

The phosphorous content of the experimental biochar and soil was determined using the standard protocol of Glaser et al., (2019) with minor modifications. Each sample was weighed at 0.5g, followed by the addition of 3 mL of 6N HCl and 10 mL of water. The mixture was heated for 5 minutes and then filtered. The volume was adjusted to 50 mL with water. The resulting solution was then analyzed for phosphorous content using a UV Spectrophotometer [50] (Figure 3.7).



Figure 3.7 UV Spectrophotometer for analyzing phosphorus content

3.3.7. Potassium content analysis (%)

The potassium content of the experimental biochar and soil was determined using the standard protocol of Glaser et al., (2019) with minor modifications. A 0.5g sample from ash was weighed, followed by the addition of 3 mL of 6N HCl and 10 mL of water for biochar. Similarly, 0.5g of soil was weighed, followed by the addition of 3mL of 6N HCl and 10 mL of water for the soil sample. The mixture was heated for 5 minutes and then filtered. The volume was adjusted to 50 mL with water. The resulting solution was then analyzed for potassium content using a flame photometer [50].

3.3.8. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of the sample was performed to study the structural composition and presence of functional groups present on biochar surfaces using spectroscopy (Model Prestige-21 Shimadzu, Japan) (Lam et al., 2018) [51].

3.3.9. Scanning Electron Microscope

The structural morphology (size and shape) of prepared biochar was examined by Scanning electron microscope (JEOL Model JSM -5910 SEM) at an accelerating

voltage of 20 kV (Amin et al., 2016). SEM was used for surface morphology analysis and the SEM with EDX capability was used to investigate the localized carbon content on the biochar [52].

3.4. APPLICATION OF BIOCHAR

Certified seeds of *R. sativus* (Figure 3.8) were sown in late December 2024 in using pots (14 inch) in seven treatments (T1-T7), each with three replications, to evaluate the effect of applied biochar at different concentrations (0.3%, 0.6%, and 0.9% w/v). solutions of biochar were prepared at three different concentrations by dissolving biochar (3, 6, and 9) g in 1000 ml distilled water. The pH of solution was measured using pH meter and was adjusted by adding ammonia dropwise in it. Then the prepared experimental pots were irrigated with solutions of biochar and the seeds were sown subsequently.



Figure 3.8 Certified seeds of *Raphanus sativus*

3.4.1. Soil Preparation

Soil was collected from the Botanical Garden, Lahore, and air-dried. It was then passed through a 2 mm sieve to remove large debris and ensure uniformity in particle size for the pot experiment. and the sample from that solution was submitted to soil and

water testing Laboratory Thoker Niaz Beig Lahore for testing while some tests were performed at Pakistan Council of Scientific and Industrial Research Institute, Lahore.



Figure 3.9 Cultivation of *Raphanus sativus* in replications

3.4.2 Soil Testing

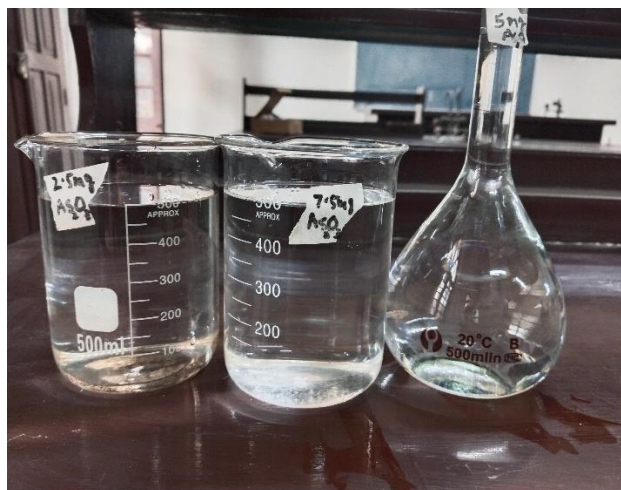
The collected soil sample was assessed for pH, electrical conductivity, water holding capacity, nitrogen, phosphorus, potassium, organic matter, soil texture and arsenic content. Similarly, soil was collected again from post-treated crops to assess its arsenic content, fluorine, and nitrate.

3.4.3. Pot Experiment

A total of 21 pots were used for the present experimental study; experiment was conducted in Complete Randomized Design (CRD) with three replications of each treatment. Each pot was filled with 10 Kg of soil. There were seven treatments such as; T1(Control left untreated) while T2 to T4 were also kept as control as arsenic was induced at different concentrations such as (5, 10, and 15) ppm, three out of seven were experimental treatments (T5 to T7) treated with biochar (3, 6, and 9)g and arsenic was also induced at different concentrations (5, 10, and 15) ppm (Figure 3.9). All the treatments were irrigated according to guidelines of Pakistan Agriculture Council (PAC).

3.4.4. Artificial Induction of Arsenic

Arsenic contamination was artificially induced by dissolving arsenic oxide in distilled water to create solutions with concentrations of 5 ppm, 10 ppm, and 15 ppm (Figure 3.10 A). These solutions were injected into the soil in all pots. In treatments T2 to T4, the arsenic solution was applied before sowing seeds, while in treatments T5 to T7, the solution was added after the application of biochar (Figure 3.10 B).



A



B

Figure 3.10 Arsenic solution (A); Insertion of Arsenic solution in the soil (B)

3.5 Data collection

Data regarding growth and yield attributes of *R. sativus* were collected by manual counting the number of leaves after 30 and 45 days of cultivation, data regarding root, and shoot length was also collected after 30 and 45 days. Similarly, the

data of fresh and dry weight of crops was also collected on the 30th and 45th day of cultivation.

3.6 ASSESSMENT OF POST TREATED SOIL AND CROP

Soil samples and fruits were collected from all the treatments following crop maturity, to evaluate the uptake of arsenic and the effect of biochar on arsenic uptake. Similarly, both samples of soil and crops were tested for fluorine and nitrates while *R. sativus* was tested for calcium and magnesium as this crop is a rich source of these minerals, so the impact of arsenic was calculated on these minerals, and the impact of biochar was also assessed.

3.6.1 Assessment of Arsenic in soil and *R. sativus*

For the assessment of arsenic 1 g of the sieved soil and fruit sample was taken individually in separate beaker, and 10 mL of water with hydrochloric acid, was added mixture were kept on stirring till 30 min and the solutions were filtered using filter paper to obtain a clear extract. To process further all the steps were followed carefully as were mentioned on the kit a minute quantity of sample was taken the bottle and zinc dust, or sodium borohydride were added along with Mercuric bromide (HgBr₂) on test strip. This reaction usually generates arsine gas (AsH₃), which reacts with a test strip or pad (impregnated with mercuric bromide or another chromogenic agent). This mixture was left for about 40 min in a sealed bottle to get its color developed and the readings were recorded accordingly (Ngo et al., 2016) [53].

3.6.2. Assessment of macronutrients

As calcium, magnesium and nitrates are usually marked as macronutrients for most of the crops and soil, so the concentration of these nutrients was checked in all the experimental samples through spectrophotometrically (Singh et al., 2019) [54].

3.7 Statistical analysis

Collected data regarding growth and yield of the crop was assessed through applying analysis of variance to assess the level of significance. For statistical analyses Statistic program, version 8.1 was used, and data was analyzed for analysis of variance (ANOVA). Mean values were separated ($p=0.05$) and represented by different letters both in tables and figures along with $\pm$ standard error. Further all pair wise comparison was also check by applying Tukey HSD. Graphs were prepared using Graph pad Prism.

CHAPTER 4

RESULTS

4.1 Synthesis of Biochar

Biochar was successfully synthesized using sugarcane waste as raw material through pyrolysis. The process was carried out under limited oxygen conditions at a temperature suitable for carbonization, resulting in a fine, black, lightweight material with a porous structure. The biochar yield was satisfactory, and the final product was odorless. The synthesis process proved efficient and environmentally friendly, offering a sustainable way to convert waste into a valuable soil amendment (Figure 4.1).

4.1 CHARACTERIZATION OF BIOCHAR

The physiochemical characterization of the sample revealed insightful results regarding its composition and potential functional properties.

4.1.1 Moisture content

The moisture content of the experimental sample was 3.527% which indicated the moderate moisture retention which may affect the sample's microbial stability and shelf life (Table 4.1).

4.1.2 Ash content analysis

The ash content was seen low (0.032) %, which means a minimal presence of non-combustible inorganic matter in the sample (Table 4.1). Such a low ash percentage signifies a high purity of organic constituents, indicating that the material contains limited mineral impurities. Application of biochar with low ash content will be beneficial where a low inorganic load is essential for maintaining biocompatibility, chemical stability, or ensuring clean combustion profiles. Additionally, low ash content is often associated with better performance in pharmaceutical, agricultural, and biomedical materials, where inorganic residues could otherwise interfere with desired functional properties.

4.1.3 pH

Experimental samples of biochar displayed a high pH of 9.77, placing it well into the alkaline range (Table 4.1) which may enhance its antimicrobial potential but could also impact its compatibility depending on its intended application.

4.1.4 Electrical conductivity (EC)

The electrical conductivity (EC) was measured at 602 dS m⁻¹, a moderate value that implies the presence of soluble salts, potentially enhancing nutrient availability (Table 4.1).

4.1.5. Assessment of nutrients content of Biochar

In terms of nutrient composition, the sample contained 1.1 mg/kg of nitrogen, reflecting a modest amount of organic matter or residual nutrients. The phosphorus content, determined in two replicates, ranged from 0.074 mg/kg to 0.125 mg/kg, suggesting minimal but detectable levels essential for plant metabolic processes. The potassium content of the experimental biochar was found very low (0.034) mg/kg was not specified in the current dataset, potentially indicating either absence or a value below detectable limit. Lastly, the water holding capacity was noted to be 5 mL, a property that underscores the sample's potential to retain moisture, a critical attribute for soil conditioning or wound dressing materials, depending on the context of use (Table 4.1).

4.1.6. Fourier Transform Infrared Spectroscopy

FTIR identifies functional groups present in biochar, affecting their adsorption capacity, reactivity, and interaction with soil nutrients. The analysis revealed several key functional groups in the biochar sample such as C=C stretch at 1632.6 cm⁻¹ indicates the presence of alkenes or aromatic rings, which are common organic compounds in biochar. The C=C stretches observed at 1520.8 cm⁻¹ signify aromatic compounds, which contribute to the stability and longevity of biochar in the soil. Additionally, the C-F stretches found at 1416.4 cm⁻¹ and 1036.2 cm⁻¹ suggest the presence of fluorinated compounds, which are less commonly found in biochar. (Figure 4.1; Table 4.2). The detection of C=C aromatic structures suggests that the biochar has a highly stable structure, allowing it to persist in the soil for extended periods. This stability enhances carbon sequestration, making biochar an effective tool for improving

soil health and mitigating climate change by locking carbon in the soil for long-term storage (Table 4.2; Figure 4.2).



Figure 4.1: Synthesized biochar

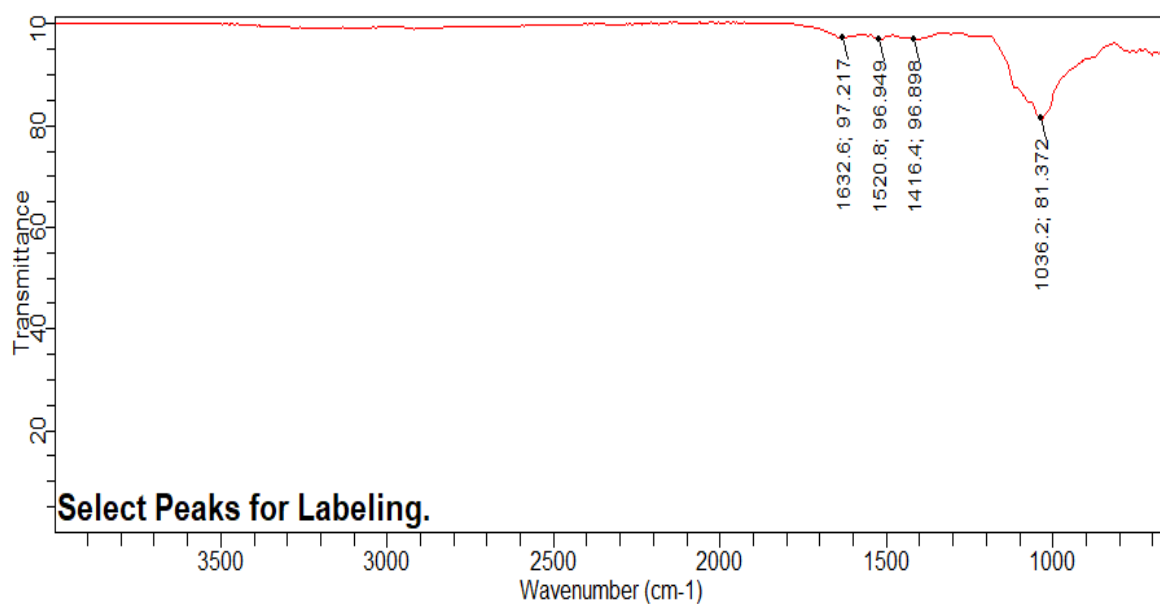


Figure 4.2 FTIR analysis of experimental biochar

Table 4.1 Assessment of nutrients content of Biochar

Parameter	Value
Moisture Content (%)	3.527
Ash Content (%)	0.032
pH	9.77
Electrical Conductivity (EC)	602
Nitrogen (mg/kg)	1.1
Potassium (mg/kg)	0.034
Phosphorus (mg/kg)	0.125
Water Holding Capacity (ml)	5 ml

Where, %: Percentage; EC: Electrical Conductivity; mg: milligram; kg: kilogram.

Table 4.2 FTIR Spectral Peaks and Corresponding Functional Groups Identified in Experimental Biochar

Peak (cm⁻¹)	Functional Group
1632.6	C=C
1520.8	C=C
1416.4	C-F Stretch
1036.2	C-F Stretch

4.1.7 SEM Analysis

The SEM analysis of sugarcane bagasse biochar reveals its highly porous nature, with distinct pore structures at different magnifications. At 500× magnification, macro-pores (~50 µm) dominate, providing a rough surface with visible cracks. These larger pores are beneficial for gas adsorption and facilitate larger molecule transport. At 2,000× magnification, mesopores (~2–10 µm) create a network of interconnected channels and cavities, enhancing the biochar's surface area for chemical reactions. At 5,000× magnification, micropores (<2 µm) form a fine, dense matrix, which improves the biochar's ability to adsorb smaller molecules, thereby increasing its surface reactivity.

Table 4.3 SEM Analysis of Sugarcane Bagasse Biochar

Magnification (×)	Pore Structure	Surface Morphology
500	Macro-pores (~50 µm)	Irregular, rough surface with visible cracks
2,000	Mesopores (~2–10 µm)	Network of interconnected channels and cavities
5,000	Micropores (<2 µm)	Highly porous matrix with fine pore distribution

4.2 APPLICATION OF BIOCHAR ON CROP

4.2.1 Soil health analysis

Physio-chemical Properties of Soil

The result of soil analysis revealed that particle size distributions of the soil surface (0-30cm) were 29% sand, 39.8% silt and 31% clay (Table 4.3). Soil health analysis clearly depicted that land is suitable for onion production, having optimum pH (7.3). Further, the soil organic carbon content was low (0.89%), which was due to continuous cultivation and removal of crop residue for animal feed and fuel. The total nitrogen and available phosphorous content were 0.29% and 11.66 ppm that represented moderate for nitrogen and low for available phosphorus (Table 4.3). However, the CEC value was found higher (29.42 cmol (+)/kg). while the extractable potassium was found medium 0.43 cmol (+)/kg.

Table 4.4 Selected Physio-chemical Properties of Soil at the Experimental Site

Physio-Chemical Property	Observed Value
Sand (%)	29%
Silt (%)	39.8%
Clay (%)	31%
Soil Texture	Clay Loam
pH (H ₂ O, 1:2.5)	7.3
Organic Carbon (%)	0.89%
Total Nitrogen (%)	0.29%
Available Phosphorus (ppm)	11.66 ppm
Cation Exchange Capacity (CEC)	29.42 cmol (+)/kg
Exchangeable Potassium	0.43 cmol (+)/kg

Where, centimoles of charge per kilogram of soil: cmol; %: percentage; CEC: Cation Exchange Capacity; ppm: parts per million

4.3 ASSESMENT OF IMPACT OF BIOCHAR ON CROP

4.3.1 Number of Leaves

Across the seven treatments, mean scores at 30 days after germination (DAG) range 5.333–6.000 with SDs ~0.577–1.000, showing consistent baselines. By 45 DAG, T7 showed the largest gain (5.333→7.333), indicating a strong response, while T1 and T6 rose modestly. T2 and T5 held at 6.000, with zero variability for T2 and no dispersion for T5 at 45 DAG. In contrast, T3 and T4 declined, notably T4 (5.333→4.000). These patterns highlight T7's superior effect, stability in T2/T5, and concern T3/T4. (Figure 4.4; Table 4.4).

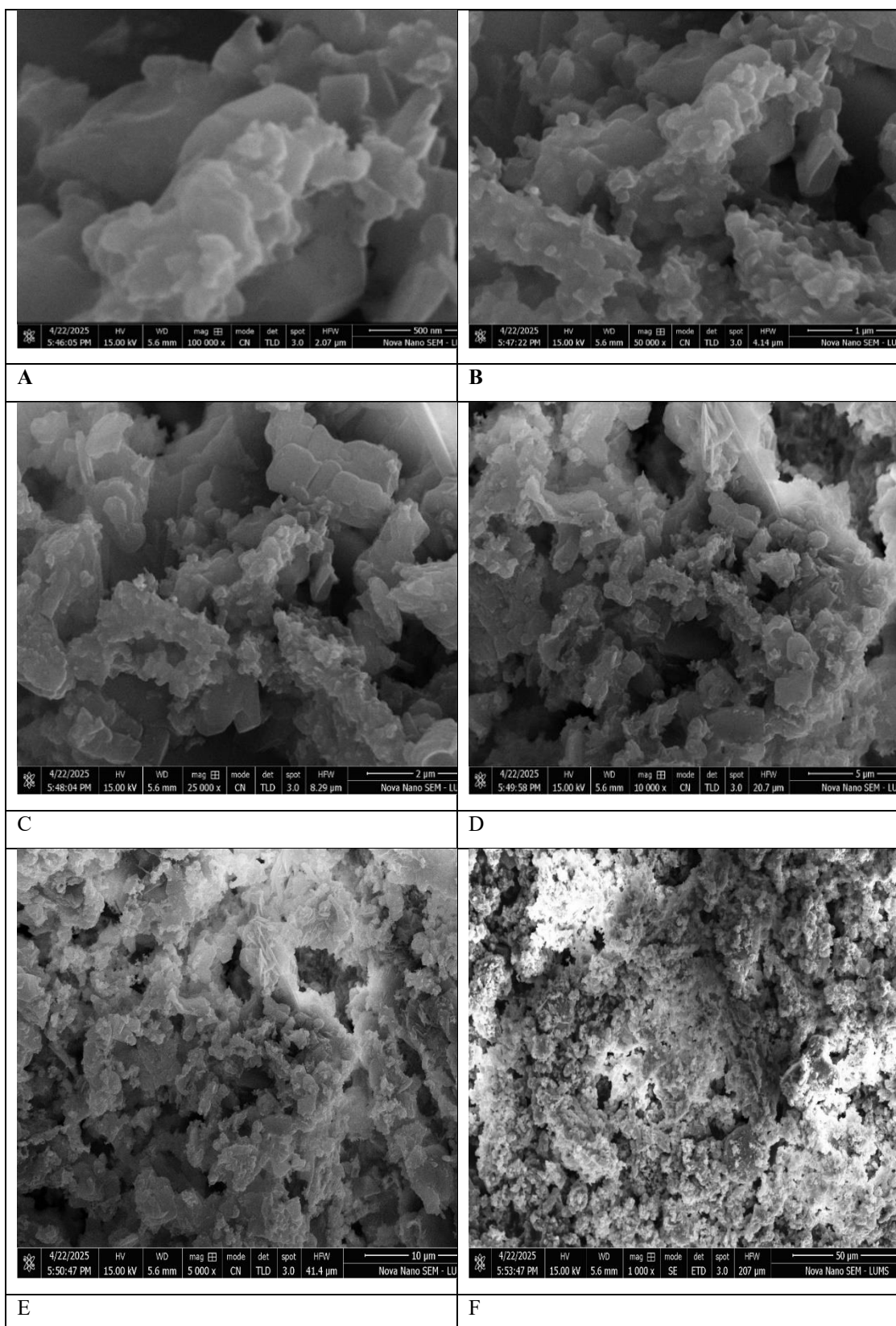


Figure 4.3 SEM analysis of experimental Biochar assessed at various magnification such as 1, 2, 5, 10 and 50 µm and at 500 nm (A-F).

Table 4.5 Effect of Treatments on Radish Growth Parameters

Treatment	No. of Leaves Mean $\pm$ SD		Root Length (cm) Mean $\pm$ SD		Shoot Length (cm) Mean $\pm$ SD		Fresh Weight (g) Mean $\pm$ SD		Dry Weight (g) Mean $\pm$ SD	
	(30 Days)	(45 Days)	(30 Days)	(45 Days)	(30 Days)	(45 Days)	(30 Days)	(45 Days)	(30 Days)	(45 Days)
T1	5.66 $\pm$ 0.57	6.0 $\pm$ 1.0	4.56 $\pm$ 0.40	11.9 $\pm$ 0.3	5.96 $\pm$ 0.89	11.1 $\pm$ 0.28	0.82 $\pm$ 0.05	3.60 $\pm$ 0.37	0.06 $\pm$ 0.02	1.94 $\pm$ 0.65
T2	6.0 $\pm$ 1.00	6.0 $\pm$ 0.0	2.66 $\pm$ 0.57	5.26 $\pm$ 0.4	3.56 $\pm$ 0.40	3.76 $\pm$ 0.25	0.63 $\pm$ 0.03	0.70 $\pm$ 0.73	0.10 $\pm$ 0.08	0.36 $\pm$ 0.44
T3	5.66 $\pm$ 0.57	5.33 $\pm$ 0.58	1.96 $\pm$ 0.50	4.80 $\pm$ 0.2	4.50 $\pm$ 0.3	5.0 $\pm$ 0.5	0.70 $\pm$ 0.06	1.90 $\pm$ 0.18	0.06 $\pm$ 0.02	0.96 $\pm$ 0.54
T4	5.33 $\pm$ 0.57	4.0 $\pm$ 1.0	3.26 $\pm$ 0.68	4.06 $\pm$ 0.5	4.73 $\pm$ 0.4	4.93 $\pm$ 0.4	1.38 $\pm$ 0.04	2.12 $\pm$ 0.11	0.53 $\pm$ 0.32	0.58 $\pm$ 0.75
T5	5.66 $\pm$ 0.57	6.00 $\pm$ 0.0	3.66 $\pm$ 0.28	8.6 $\pm$ 0.5	4.83 $\pm$ 0.28	9.66 $\pm$ 0.57	0.78 $\pm$ 0.36	3.82 $\pm$ 0.45	0.07 $\pm$ 0.006	2.14 $\pm$ 0.7
T6	5.66 $\pm$ 0.57	6.00 $\pm$ 1.0	3.60 $\pm$ 0.52	6.66 $\pm$ 0.28	7.56 $\pm$ 0.4	9.43 $\pm$ 0.40	0.81 $\pm$ 0.07	4.18 $\pm$ 0.3	0.05 $\pm$ 0.01	1.96 $\pm$ 0.86
T7	5.33 $\pm$ 0.57	7.33 $\pm$ 0.58	3.66 $\pm$ 0.57	5.13 $\pm$ 0.7	6.50 $\pm$ 0.5	7.93 $\pm$ 0.4	0.94 $\pm$ 0.09	2.53 $\pm$ 0.49	0.07 $\pm$ 0.006	1.46 $\pm$ 0.57

Where, mean values were separated ($p=0.05$) and represented in table along with $\pm$ standard error.

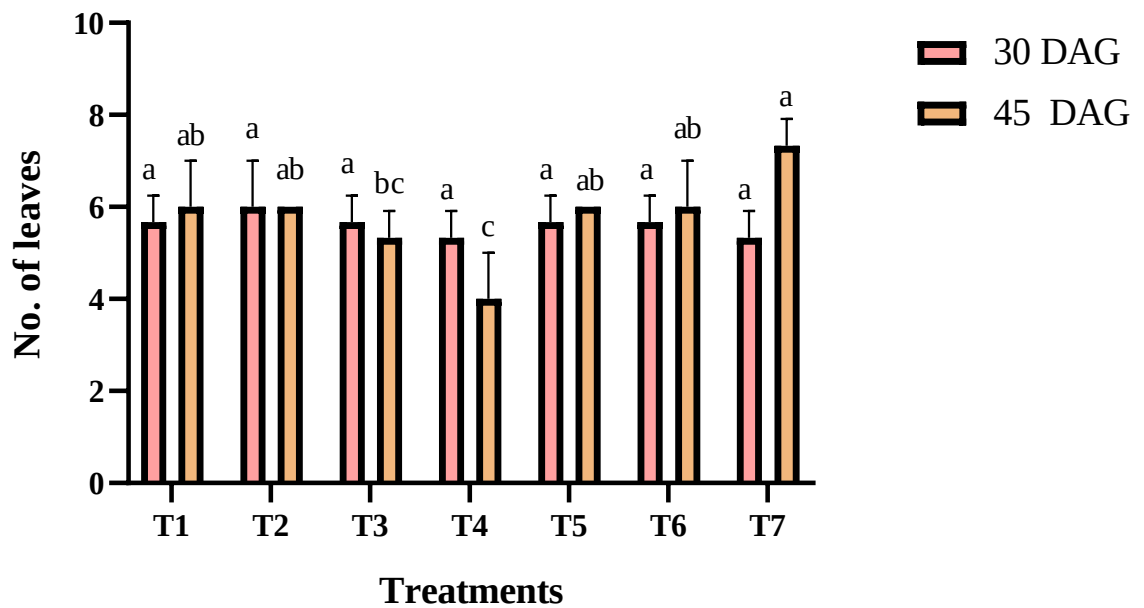


Figure 4.4 Number of leaves of *R. sativus* after the application of biochar

Where DAG: days after germination; T1: Control crop; T2-T4: negative control (with induction of arsenic); T5-T7: experimental crop treated with biochar. Results were analyzed through analysis of variance and were found significant lettering (a-c) represents highly significance to lowest at P (0.05).

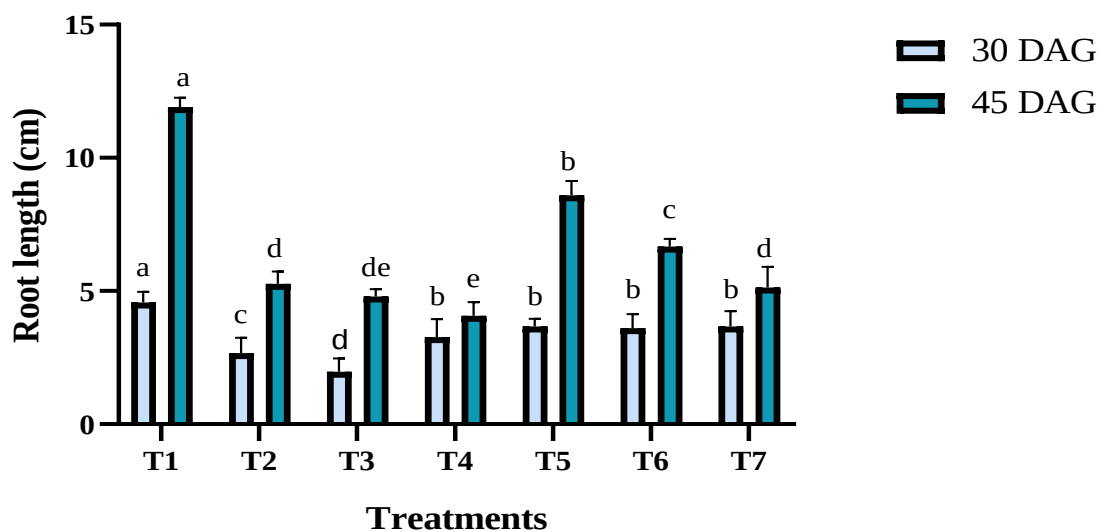


Figure 4.5 Root length of *R. sativus* after the application of biochar

Where DAG: days after germination and fresh weight was taken in grams (g); T1: Control crop; T2-T4: negative control (with induction of arsenic); T5-T7: experimental crop treated with biochar. Results were analyzed through analysis of variance and were found significant lettering (a-c) represents highly significance to lowest at P (0.05).

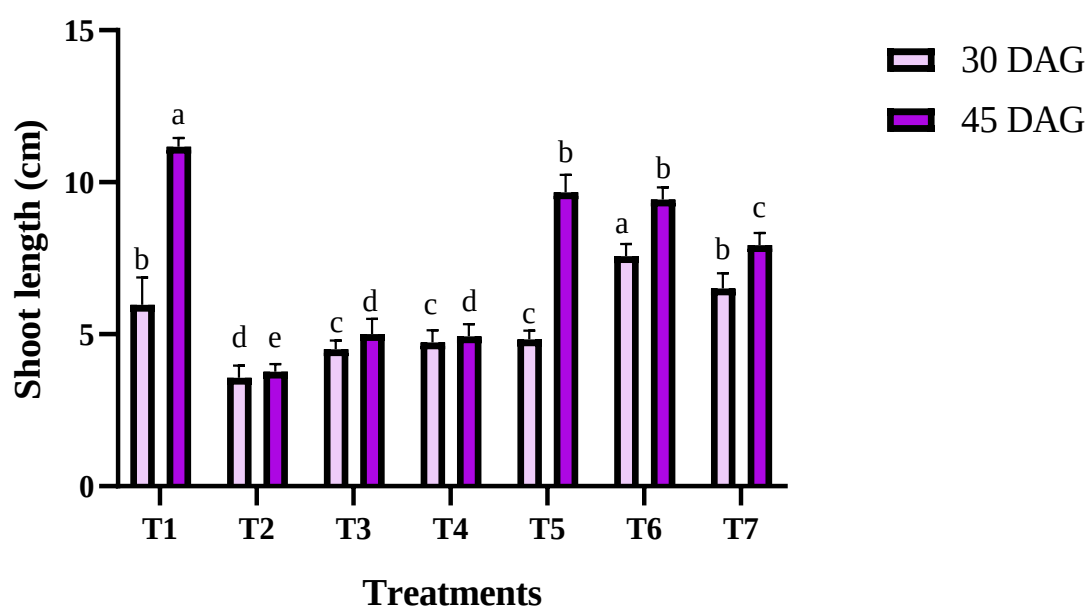


Figure 4.6 Shoot length of *R. sativus* after the application of biochar

Where DAG: days after germination and fresh weight was taken in grams (g); T1: Control crop; T2-T4: negative control (with induction of arsenic); T5-T7: experimental crop treated with biochar. Results were analyzed through analysis of variance and were found significant lettering (a-c) represents highly significance to lowest at P (0.05).

4.3.2. Root Length

The data shows mean values and standard deviations (SD) for seven treatments (T1–T7) at 30 and 45 days after germination (DAG). T1 had the highest growth at both stages (4.567 and 11.900), indicating strong early and sustained performance. T3 had the lowest growth, especially at 30 DAG. T5 showed a notable improvement by 45 DAG, rising to 8.600. Overall, treatments showed variable responses, with some (e.g.,

T4, T7) having moderate to low growth. Standard deviations suggest consistent measurements across most treatments. (Figure 4.5; Table 4.4).

4.3.3. Shoot Length

The shoot length data shows T1 and T6 performing best overall. T1 had the highest length at 45 DAG (11.167 cm), while T6 led at 30 DAG (7.567 cm) and maintained high growth. T5 also showed significant increase from 4.833 to 9.667 cm. In contrast, T2 consistently had the lowest shoot length. T3 and T4 showed modest growth with minimal changes. Standard deviations were generally low, indicating reliable measurements. Overall, T1, T5, and T6 were the most effective treatments for shoot growth. (Figure 4.6; Table 4.4).

4.3.4. Impact on fresh and dry weight

Fresh weight data reveals that T6 had the highest value at 45 DAG (4.180 g), indicating strong biomass accumulation. T5 also performed well, increasing significantly from 0.780 to 3.820 g. T1 showed consistent growth, while T4 started highest at 30 DAG but showed moderate gain. T2 had the lowest values overall, with minimal improvement. T3 and T7 had moderate increases. Standard deviations were generally low, ensuring data reliability, except for T2 at 45 DAG, indicating variability. T5 and T6 were the most effective treatments.

Dry weight data indicates that T5 had the highest value at 45 DAG (2.143 g), showing strong biomass development. T6 and T1 also performed well with 1.967 g and 1.947 g, respectively. T2 had the lowest increase, suggesting weak growth. While T4 had a high initial dry weight at 30 DAG, it showed minimal gain. T3 and T7 displayed moderate improvement. High standard deviations, especially at 45 DAG, suggest variability in responses. Overall, T5, T6, and T1 were the most effective treatments.

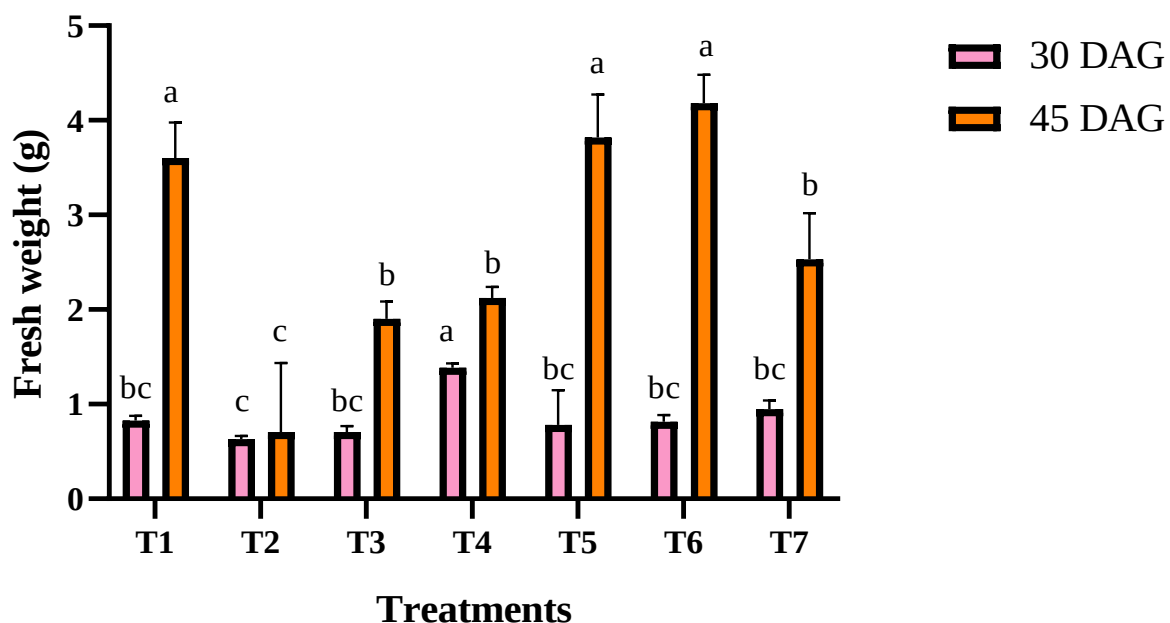


Figure 4.7 Fresh weight of *R. sativus* treated with biochar after the induction of arsenic

Where DAG: days after germination and fresh weight was taken in grams (g); T1: Control crop; T2-T4: negative control (with induction of arsenic); T5-T7: experimental crop treated with biochar. Results were analyzed through analysis of variance and were found significant lettering (a-c) represents highly significance to lowest at P (0.05).

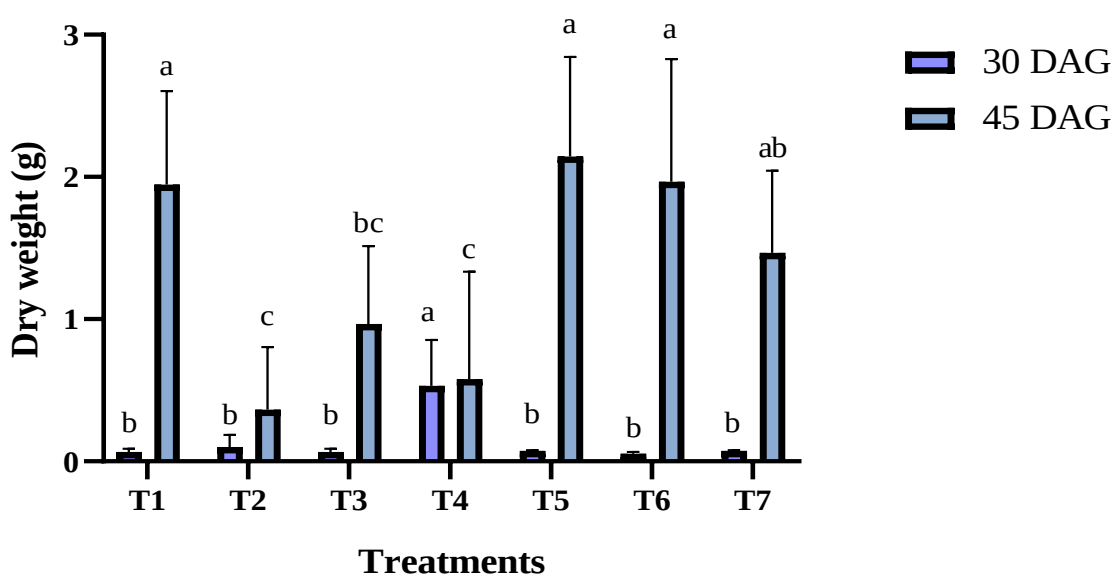


Figure 4.8 Impact of Arsenic and biochar on dry weight of *R. sativus*

Where DAG: days after germination and fresh weight was taken in grams (g); T1: Control crop; T2-T4: negative control (with induction of arsenic); T5-T7: experimental crop treated with biochar. Results were analyzed through analysis of variance and were found significant lettering (a-c) represents highly significance to lowest at P (0.05).



Figure 4.9 Morphological differences among all treatments (T1-T7)

4.4. Effect of Biochar on Arsenic Immobilization and Soil Nutrient Dynamics

The analysis of treatments (T1–T7) highlights the important influence of arsenic contamination and the mitigating role of biochar on arsenic (As), fluoride (F), and nitrate (NO_3^-) levels (Table 4.5). In the control treatment (T1), where neither arsenic or biochar was applied, baseline values for arsenic were (0.05 ppm), fluoride (1.1 mg/L), and nitrate (95 mg/L) recorded. While the crop exposed to arsenic without biochar amendment (T2–T4) showed a clear increase in arsenic concentration (5.0–14.56) ppm alongside variable increases in fluoride (1.7–4.2 mg/L) and substantial reductions in nitrate (82.3–32.7) mg/L, indicating the detrimental effects of arsenic stress on soil and nutrient stability. However, the introduction of biochar in treatments T5–T7 significantly altered this trend. In comparison to arsenic only treatments, biochar application lowered arsenic concentration (3.9–13.2ppm), regulated fluoride levels, and somewhat improved nitrate retention. Notably, T7, which combined 15 ppm As with 9 g SBB, showed effective fluoride

reduction (1.2 mg/L, nearly returning to control levels) and an increase in nitrate content (51.2 mg/L), highlighting the superior efficacy of higher biochar doses. These results underscore the potential of biochar as a sustainable amendment to mitigate heavy metal contamination and nutrient loss, offering a promising approach for improving soil quality and plant health in arsenic-affected agricultural systems.

4.5 Impact of Biochar on Arsenic Uptake and Nutritional Profile of *R. sativus*

Arsenic exposure at increasing concentrations (5, 10, and 15 ppm) had significant effect arsenic accumulation in the crops, resulting in arsenic contents 4.2, 8.6, and 14.0 ppm, respectively (Table 4.6). The use of biochar at respective rates of 3 g, 6 g, and 9 g in arsenic-contaminated soils resulted significant decrease in arsenic absorption with tissue concentrations dropping to 2.7, 7.2, and 12.6 ppm, respectively (Table 4.6). This illustrates the biochar's capacity to encapsulate arsenic and lower its bioavailability. Fluoride (F) levels were generally steady across all treatments, indicating that arsenic stress and biochar amendment had limited effect on fluoride uptake. In contrast, nitrate levels were significantly altered with arsenic stress alone producing a substantial drop, particularly at 15 ppm (47.8 ppm nitrate compared to 12.8 ppm in the control), indicating poor nitrogen metabolism. However, biochar treatments improved nitrate accumulation, with the greatest recovery shown at 5 ppm arsenic plus biochar (218.8 ppm nitrate). Calcium and magnesium contents also varied under arsenic stress. Calcium level declined considerably at 10 ppm arsenic but were stabilized or increased after biochar application. Magnesium content, which was originally decreased under arsenic stress, increased significantly with biochar amendments, especially at lower arsenic concentrations, with the maximum value (500 mg/L) recorded in the 5 ppm arsenic plus 3 g biochar treatment. These results collectively indicate that sugarcane baggase based biochar not only mitigates arsenic uptake but also enhances nutrient uptake and improves the overall nutritional quality of *R. sativus* under arsenic-contaminated conditions.

In short, the data highlights the impact of arsenic (As) on both soil and plant tissue, along with its effects on nutrient levels. T1, the control, had minimal arsenic levels in both soil and plant tissue, with high nitrate (95 mg/L), calcium (640 mg/L), and magnesium (400 mg/L). As arsenic concentration increased in the soil (T2-T4), plant tissue arsenic levels and the reduction in nitrate, calcium, and magnesium were observed. Biochar-treated treatments (T5-T7) showed a decrease in arsenic uptake in plant tissue, especially in T5,

which had the lowest As in plant tissue (2.7 ppm) and the highest magnesium (500 mg/L), suggesting biochar's potential in mitigating arsenic toxicity.

The sulfate content in radish was greatest at 10 ppm arsenic (1400 mg/l) and somewhat lower in control. The addition of SBB (T5-T7) dramatically lowered SO₄ levels in radish, with T7 having the lowest concentration of sulphate (7000mg/l). This suggests that SBB is efficient in lowering radish sulfate absorption during arsenic stress, particularly at higher biochar levels. Whereas the sulfate concentration in soil rose considerably with arsenic treatment alone, peaking at T2 (50 mg/l). The addition of SBB (T5-T7) had conflicting effects on soil, whereas T6 and T7 remained high (50 and 40 mg/l). SBB may decrease soil SO₄ in lower arsenic circumstances but is less effective at higher level.

Table 4.6 **Effect of Arsenic and Biochar Applications on Soil**

Sr. No.	Treatments	As (ppm)	F (mg/l)	Nitrate	SO₄ (mg/l)
1.	T1	0.05	1.1	95	20
2.	T2	5.0	1.7	82.3	50
3.	T3	9.4	4.2	32.7	40
4.	T4	14.56	2.7	40	30
5.	T5	3.9	0.5	42.6	20
6.	T6	7.7	4.8	51.5	50
7.	T7	13.2	9.8	38.3	40

Where T1: No Concentration; T2: 5ppm As; T3:10ppm As; T4:15ppm As; T5:5ppm As + 3g SBB; T6:10ppm As + 6g SBB; T7:15ppm As + 9g SBB

Table 4.7 Impact of Biochar on Arsenic Uptake and Nutritional Profile of *R. sativus*

Sr no.	Treatments	As (ppm)	Fluorine	Nitrate	Ca (200mg/l)	Mg (150mg/l)	SO₄ (200mg/l)
1.	T1	0	40.0	12.8	640	400	13500
2.	T2	4.2	38.0	187.8	560	240	13250
3.	T3	8.6	37.8	199.2	400	250	14000
4.	T4	14	38.4	47.8	800	300	12500
5.	T5	2.7	38.4	42.6	1040	100	10000
6.	T6	7.2	39.0	45.0	560	350	10000
7.	T7	12.6	39.6	207.4	640	400	7000

Where T1: No Concentration; T2: 5ppm As; T3:10ppm As; T4:15ppm As; T5:5ppm As + 3g SBB; T6:10ppm As + 6g SBB; T7:15ppm As + 9g SBB

CHAPTER 5

DISCUSSION

The current study explored the role of sugarcane bagasse-derived biochar in mitigating arsenic toxicity in plants and improving their growth in contaminated soils. Biochar amendments were shown to significantly influence various growth parameters such as root length, shoot length, and leaf count, with treatment T1 (control) exhibiting the most substantial growth. T1's root length at 45 days was 11.9 cm, and its shoot length was 11.17 cm, indicating strong growth potential. These results align with previous studies on biochar's effectiveness in improving plant growth. Nguyen et al. (2025) found that biochar modifications can substantially enhance the adsorption of contaminants, including arsenic, in aquatic systems, demonstrating the versatility of biochar in mitigating the effects of environmental pollutants [27]. In this study, the biochar's ability to improve plant growth while reducing arsenic uptake aligns with findings by Narkhde et al. (2025), who reported that biochar effectively mitigates arsenic contamination in soil, improving plant health and growth [32].

Moreover, the biochar's impact on arsenic uptake was significant. Treatment T1 exhibited minimal arsenic concentrations in both soil and plant tissue, with the lowest levels observed in the plant tissue. This is consistent with Masood ul Hasan et al. (2024), who demonstrated that surface modifications, such as thiol-functionalization of biochar, increased its arsenic removal efficiency [28]. In our study, T5, a biochar-amended treatment, showed a reduced arsenic concentration of 2.7 ppm in plant tissue, supporting biochar's ability to immobilize arsenic in the soil and prevent its uptake by plants. This result corroborates the work of Siddique et al. (2023), who found that iron-modified biochar composites significantly reduced arsenic uptake in plants [37].

Nutritional analysis revealed that biochar treatments, particularly T1 and T7, showed a boost in protein and carbohydrate content, suggesting that biochar not only mitigates arsenic toxicity but also enhances the nutritional quality of plants. This mirrors the findings of Vuong et al. (2025), who reported that biochar enhanced nutrient availability in the soil, leading to better crop yields and nutritional quality [35]. Similarly, Choudhury et al. (2024) showed that metal-impregnated biochars, like Mn-Al modified

biochar, significantly enhanced arsenic removal efficiency and improved plant growth, emphasizing biochar's role in improving both soil fertility and plant health [29].

The soil analysis revealed changes in soil properties due to biochar amendments. Biochar improved soil pH increased organic matter content and influenced nutrient profile. These findings are consistent with studies by Salem et al. (2021), which showed that biochar applications enhanced soil pH and shifted metals to more stable fractions, thereby reducing bioavailability and improving soil fertility [3]. Additionally, the biochar's porous structure, confirmed by SEM analysis, likely facilitated enhanced water retention and nutrient adsorption, supporting the results of Soares et al. (2021), who found that biochar amendments significantly improved soil structure and nutrient availability [30].

In summary, this study supports the findings of previous research that biochar derived from sugarcane bagasse has considerable potential as an effective soil amendment for both improving plant growth and reducing arsenic toxicity. The results corroborate earlier work, demonstrating biochar's ability to enhance soil properties, promote plant growth, and reduce arsenic uptake. Furthermore, biochar's surface modifications, particularly with metals and thiols, significantly improve its arsenic-removal capacity, as shown in studies by Masood ul Hasan et al. (2024) and Siddique et al. (2023). This highlights biochar's versatility and effectiveness as a sustainable solution for managing arsenic-contaminated soils and enhancing agricultural productivity [28,37].

CHAPTER 6

CONCLUSION

In conclusion, this study demonstrates the significant potential of sugarcane bagasse-derived biochar in mitigating arsenic toxicity in plants and enhancing growth parameters in contaminated soils. The results show that biochar amendments, especially in treatments like T1, significantly improved plant growth, with root and shoot lengths reaching their highest values at 45 days. Soil analysis revealed improved soil properties, including increased pH and organic matter content, further supporting biochar's efficacy in enhancing soil fertility and structure. These findings are consistent with previous studies on biochar's ability to reduce arsenic bioavailability, improve soil health, and promote plant growth. The study highlights the promise of using biochar as a sustainable, cost-effective solution for managing arsenic-contaminated soils and improving agricultural productivity, aligning with global efforts to address environmental pollution and enhance crop yields.

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