EFFECT OF CO-APPLICATION OF RICE HUSK BIOCHAR AND UREA IN SOIL ON TOTAL NITROGEN AND GROWTH OF MAIZE *(Zea mays)*

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(2024)

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Supervisor: Dr. Hira Amjad

Co-Supervisor: Dr Imran Hashmi

Institute of Environmental Sciences & Engineering

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Haleema Ayaz

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LIST OF ABBREVIATIONS

BC	Biochar
C/N	Carbon-nitrogen
CCI	Chlorophyll content index
CEC	Cation exchange capacity
H/C	Hydrogen-carbon
NUE	Nitrogen uptake efficiency
O/C	Oxygen-carbon
PCA	Principal component analysis
RH	Rice husk
RHB	Rice husk biochar
TKN	Total kjeldahl nitrogen
U	Urea
WHC	Water holding capacity

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ABSTRACT

Urea is the most used nitrogen fertilizer in the world. Nitrogen losses due to volatilization and leaching are caused by overapplication. One of the best ways to maximize plant nitrogen uptake and reduce losses is to apply urea along with biochar. The purpose of this study was to evaluate the effects of rice husk biochar (RHB), together with urea, on maize development, the chlorophyll content index (CCI), plant total nitrogen (N), soil N, phosphorus (P), potassium (K), and nitrate nitrogen (NO_3-N). Two soil textures (sandy loam and silty clay loam) and two biochar concentrations (1% and 2% w/w) were used in a pot experiment along with 120 kg N/ha of urea. To compare the experiment's outcomes, a control group (one without biochar or urea) was kept. The characterization of biochar verified the existence of aromatic functional groups, high ash content, high Si concentration, thermal stability, macropores, and amorphous nature. In silty clay loam soil, therefore, combined application of biochar and urea resulted in maximum plant growth and soil nutrient concentrations, including root dry weight, leaf length, leaf fresh weight, leaf CCI, soil K, P, and NO_3-N. Biochar has a variety of functional groups and gaps that enhance soil health and nutrient availability, which in turn promotes maize development. Thus, the study concludes that applying urea and biochar together promotes soil growth and nutrient availability.

Keywords: Biochar, Chemical characterization, Total nitrogen, Maize growth, Nitrate retention, Macropores

CHAPTER 1

INTRODUCTION

1.1 Background

Worldwide, a large amount of inorganic nitrogen fertilizers are sprayed on agricultural fields in order to maintain crop production and increase yield. According to Heffer and Prudhomme (2014), urea is the most widely utilized nitrogen fertilizer in developing nations. Since nitrogen is a nutrient that plants need to flourish, farmers all over the world mostly rely on urea to produce crops (Rehman and Razzaq, 2017). Intensive farming methods have led to changes in the structure of the soil, which lowers nutrient retention and eventually impacts plant development and yield (Hartmann and Six, 2023). Reduced fertilizer uptake efficiency is the result of about half of the applied nitrogen being lost from the soil due to nitrate leaching, ammonia volatilization, and atmospheric release of N_2O (Sarkar *et al.,* 2012). According to Dawar *et al.* (2021) these losses result in detrimental environmental effects such eutrophication, lake acidification, global warming, and biodiversity loss. Reducing nitrogen losses is necessary to increase plant growth and soil fertility.

Every year, a lot of agricultural waste is produced, and since it is renewable and affordable, it can be used for a variety of applications (Chen *et al.,* 2015; Gao *et al.,* 2019). Though enormous amounts of rice crop wastes, including rice husk, are produced, there is no sustainable agricultural waste management plan available to reuse these residues. Most of them are burned in the open to prevent excessive buildup. The process of turning agricultural waste into biochar has gained popularity recently and is being used extensively as a soil conditioner in agricultural fields (Mandal *et al.,* 2016). Biochar production from rice husks may simultaneously lessen waste and environmental issues.

According to Faloye *et al.* (2019), biochar is a stable, carbon-rich substance that is produced by carefully burning material without oxygen. As an increasingly popular soil agronomic technique, biochar improves plant growth, soil fertility, and water retention (Das *et al.,* 2020). According to earlier research, the addition of biochar increases plant development because it improves soil nutrient retention (Sorrenti *et* *al.*, 2016) and availability when needed (Wang *et al.*, 2016). There have also been reports of improvements in the physical and biological properties of the soil, including its pH, bulk density, water-holding capacity, permeability, and microbiological health (Sohi *et al.,* 2010). Furthermore, biochar modifies nutrient dynamics, influences N cycling, and enhances soil nutrient content. By adsorption, ion exchange, and immobilization, it reduces N losses from soil (Clough *et al.*, 2013). It is crucial to analyze biochar analytically in order to fully comprehend the potential mechanisms underlying these effects. The various physical, chemical, and morphological properties of biochar are what promote plant development and soil nutrient availability. According to earlier reports, the biochar surface's porous structure (Selvarajh and Ch'ng, 2021) and aromatic functional groups (Ghorbani *et al.,* 2022) are what promote plant development and nutrient availability.

Pakistan cultivates maize (*Zea mays* L., family Poaceae) intensively as a short-day kharif crop. Food and animal feed are made from it (Khaliq *et al.*, 2004). It is possible to grow maize in both irrigated and rain-fed fields. Sand and clay loams are the ideal soil textures for sustaining productivity (Dawar *et al.,* 2022). After wheat, rice, and cotton, it is the fourth most grown crop in Pakistan and the third most used cereal crop overall. According to Ali *et al.* (2017), fresh maize has a high nutritional value. For every 100 grammes of fresh maize, there are 361 calories, 9.4 g of protein, 4.3 g of lipids, 74.4 g of carbs, 1.8 g of fiber, and 1.3 g of ash.

1.2 Significance of the study

Different organic and inorganic fertilizers are being used to improve maize growth and nutrient uptake ability. Biochar combined with urea is also used for increase in crop production. Hence there is a need to know the effect of biochar on soil nutrients and plant growth parameters. The study conducted further explored this combined application on maize in two different agriculture soils. The objective of this study was to explore different RHB properties responsible for improved soil health and crop production and to determine the effect of combined urea and biochar application on maize growth and soil nutrient availability.

1.3 Objectives

The objectives of this study were:

i. Prepare and determine the properties of rice husk biochar.
ii. Determine the effect of biochar combined with urea on total nitrogen.
iii. Determine the effect of biochar combined with urea on maize growth.

CHAPTER 2

LITERATURE REVIEW

2.1 Biochar production and characterization

The predominant share of global rice husk (RH) production is concentrated in Asian region, primarily due to extensive scale of rice milling industry in this area. The mean weight of paddy rice accounts for approximately 20% (Zou & Yang, 2019).

The successful application of RH as an agricultural waste is hindered by several key characteristics. These include its rigid surface, elevated silicon concentration, limited nutritional value, and notable resistance to breakdown by soil microbes (Pode, 2016). The consumption of RH is primarily limited to agricultural and bioenergy industries. Farmers frequently employ practice of burning agricultural residue on exposed fields as a method of land management. Consequently, RH would experience a substantial depletion of carbon (C), with an over 80% reduction in both sulfur (S) and nitrogen (N) content.

Additionally, RH would lose approximately 10% to 20% of phosphorus (P) and potassium (K) content. Moreover, it is widely acknowledged that this phenomenon serves as a significant contributor to presence of atmospheric aerosols, greenhouse gases, and hazardous substances, including carbon dioxide (CO_2), methane (CH_4), nitrogen oxides (NO_x), and sulfur dioxide (SO_2) (Singh & Sidhu, 2014). Despite longstanding recommendation to integrate RH directly into soil over the past forty years and demonstrated benefits of enhancing soil quality and productivity, not all agricultural practitioners have embraced this approach (Asadi *et al.,* 2021). According to Haefele *et al*. (2011), this method of carbon sequestration is deemed unsustainable due to its contribution to overall increase in greenhouse gas emissions.

Biochar production from underutilized waste materials has been increasingly popular in recent years as a viable approach to achieving sustainable agriculture and environmental objectives. Using biochar as soil amendments is gaining popularity, and researchers have recognized potential of RH as a valuable resource for this application (Karam *et al.,* 2022).

Shackley *et al.* (2012) observe that utilization of RH as a fuel in rural areas is increasing because of its abundance. The significant ash content resulting from combustion process with a high amount of reducing agents leads to incomplete oxidation of the remaining components. Silicon oxide, the main constituent of ash, functions as a protective barrier for organic molecules. Hence, burning of RH may be seen as a pyrolysis reaction. The biochar generated by process of RH combustion exhibits a yield, also known as the char-to-feedstock ratio, and a carbon content of approximately 35% (Premalatha *et al.*, 2023).

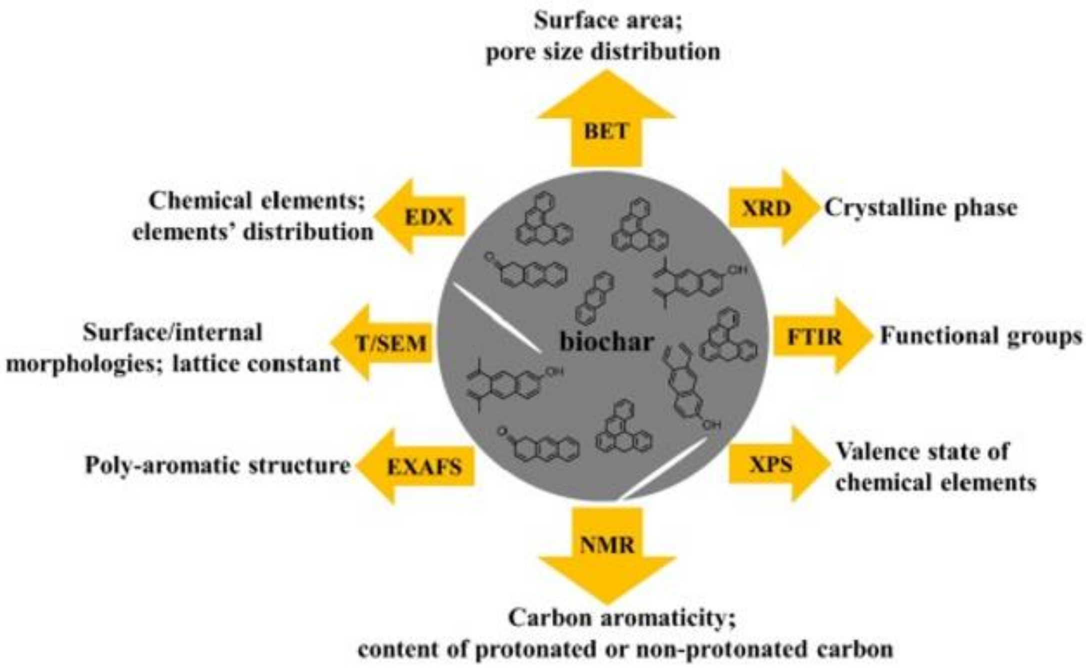


Figure 2.1: Biochar characterization methods (Adapted from Ghorbani *et al.*, 2019).

Several studies have been undertaken in recent years by Ghorbani *et al.* (2019), Huang *et al.* (2019), and Oladele (2019) to investigate the production and characterization of RHB. The pyrolysis method has been employed to synthesize RHB within a temperature range of 250 to 750°C. The structural properties of RHB, including surface area, structure, and pore sizes, may be analyzed using scanning electron microscopy (SEM). Additionally, the elemental compositions of the compound, such as carbon and nitrogen, may be determined using an Elemental Analyzer. Furthermore, the pH, electrical conductivity (EC), cation exchange capacity (CEC), and bulk density may be measured. The pyrolysis conditions and feedstock type influence particle size of biochar and its various (Wei *et al.,* 2017). Previous studies have indicated that the pyrolysis peak temperature primarily influences the biochar characteristics (Phuong *et al.*, 2015).

RHB exhibits several distinct characteristics (Armynah *et al.*, 2018):

1. RHB possesses greater silicon content.
2. Its pH value is comparatively lower than that of most other biochar.
3. RHB exhibits a significantly higher ash content when compared to other biochar.
4. RHB demonstrates a lower carbon (C) content than other biochar.

2.2 Physicochemical properties of biochar

2.2.1 pH

Based on a study by Abrishamkesh *et al*. (2015), RH pH range falls between 6.5 to 6.8. The pH values of RHBs are generated at various pyrolysis temperatures, spanning from 250 to 300°C up to 600 to 750°C. The pH positively correlates with the pyrolysis temperature. El-Naggar *et al.* (2019) have reported removing acidic functional groups, further supporting the notion of an increased pH value in RHBs. According to the study conducted by Wei *et al.* (2017), it was shown that RHBs produced at elevated pyrolysis temperatures exhibit a higher abundance of primary functional groups and a reduced presence of acidic functional groups. According to Suliman *et al.* (2016), an increase in pyrolysis temperature leads to an augmented presence of fundamental functional groups within resulting ash.

2.2.2 Elemental composition

Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), and silicon (Si) are predominant elements found in RHB. A strong positive relationship exists between temperature at which pyrolysis occurs and carbon content in RHB. Additionally, a significant negative correlation is observed between temperature of pyrolysis and hydrogen concentration in RHB. According to a study conducted by Eduah *et al.* (2019), it has been observed that elevating the pyrolysis temperature tends to lead to an increased carbon content in RHB. Furthermore, previous research conducted by Abrishamkesh *et al.* (2015) has demonstrated that RHB carbon content remains unaffected by pyrolysis temperature variations. The presence of RHB's C is primarily observed in comparatively more stable forms than RH. The carbon content of RHBs is more significantly influenced by pyrolysis temperature and relative humidity than hydrogen content.

According to Crombie *et al.* (2013), ratios of H/C and O/C may serve as valuable indicators to determine the extent of biomass carbonization and conversion into biochar. According to Xiao *et al.* (2016), H/C and O/C ratios decrease with increasing pyrolysis temperature during dehydration and decarboxylation reactions. According to Spokas (2010), there is a positive correlation between strength of C structure in biochar and formation of fused aromatic rings. Additionally, the process results in a more significant loss of hydrogen and oxygen. The estimation of aromaticity and polarity of biochar is conducted by evaluating H/C and O/C molar ratios, as described by Ray *et al.* (2020).

2.2.3 Chemical functional groups

Fourier transform infrared spectroscopy (FTIR) analysis has been employed to evaluate presence and growth of functional groups in RHBs. The RHBs synthesized under low pyrolysis temperatures have abundant silica functional groups, including Si-OH, Si-O-Si, and Si-H. According to Wei *et al.* (2017), as pyrolysis temperature is increased to 750°C, high-intensity regions associated with stretching C = C ring were no longer observed, coupled with aliphatic C-H stretching vibration. The FTIR spectrum of RHBs exhibit a notable peak linked to aromatic C-H out-of-plane bending vibration (Abrishamkesh *et al.*, 2015). The RHB synthesized at a pyrolysis temperature of 500°C exhibits a more pronounced peak compared to the RHB synthesized at a pyrolysis temperature of 300°C.

2.2.4 Physical structure

According to Shackley *et al.* (2012), during the process of pyrolysis, the outer layer of rice husk undergoes a transformation where it maintains a silica structure in the form of a rectangular shield. The research conducted by Singh *et al.* (2018) demonstrates that RHB has a highly porous structure. The carbonization process forms pores and channels with a geometric structure by burning organic components such as lignin and cellulose (Tomczyk *et al.*, 2020). RHB's porosity and surface area are significantly increased following the pyrolysis process, as evidenced by the SEM images presented in the study by Abrishamkesh *et al.* (2015). The structural similarity to feedstock and particle size of RHB increases with the decrease in pyrolysis temperature.

Nevertheless, increasing the pyrolysis temperature will increase the porosity of biochar. According to Hossain *et al.* (2020), pyrolysis process induces a significant alteration in particle size of RHB within temperature range of 200 to 400°C. However, particle size experiences only minor variations within temperature range of 400 to 800°C.

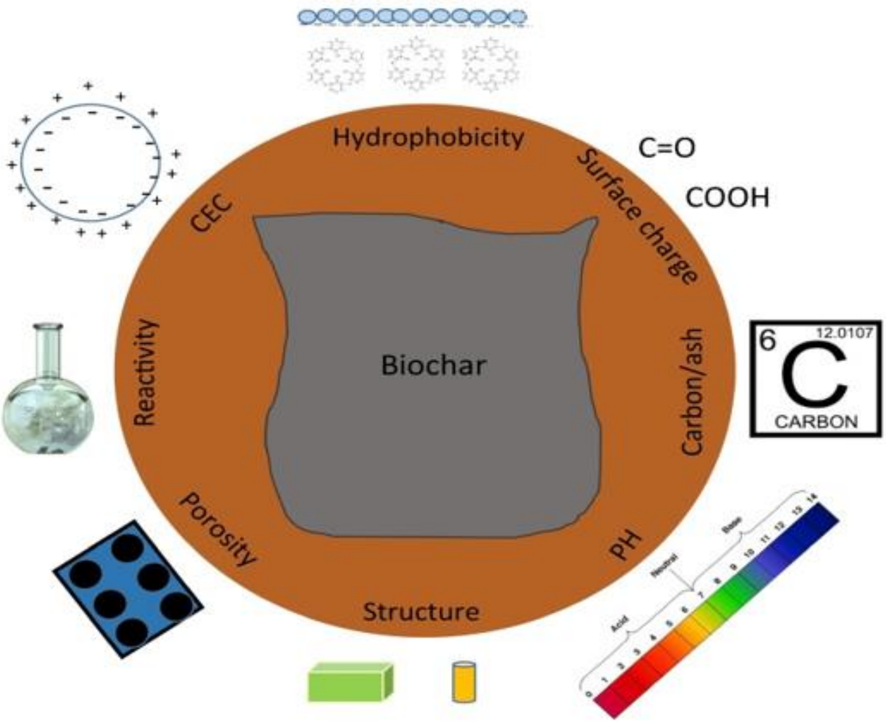


Figure 2.2: Physicochemical properties of biochar (Adapted from Das *et al.*, 2021)

2.3 Biochar as plant growth promotor

A study conducted by Jin *et al.* (2020) observed extensive long-term research to investigate impacts of incorporating rice straw directly into soil. This process facilitates nutrient release in soil (Naeem *et al.*, 2017). Furthermore, previous experiments have proposed that bioavailability of N in soil may experience a temporary decrease following direct application of RH, hence requiring addition of nitrogen fertilizers (Reichel *et al.*, 2018). Hence, using biochar derived from agricultural residues is a feasible alternative, as supported by the findings of Naeem *et al.* (2017).

Previous studies have demonstrated that application of biochar may enhance crop yield through many mechanisms, such as elevating soil pH, boosting cation exchange capacity (CEC), improving soil porosity, and enhancing soil-water interactions (Yuan *et al.*, 2019). The study conducted by Haefele *et al.* (2011) reveals that influence of

RHB on soil fertility and rice grain yield exhibits variability across different geographical locations. Ghorbani *et al.*, (2022) conducted a pot experiment on lentils and wheat to investigate effect of two rhizobial inoculants, applied at different rates in calcareous alkaline soil. Following the lentil harvest, the containers were then used to sow wheat without recycling RHB. Although both crops saw enhanced root development with increasing RHB treatment rates, it was observed that gains in above-ground biomass were only significant for wheat. Due to its influence on soil porosity, RHB has notable efficacy as a stimulant for root growth. When a crop is exposed to drought stress, RHB may increase above ground biomass and yield through subsequent extension of roots.

Varela Milla *et al.* (2013) conducted a field experiment to investigate growth of water spinach under different concentrations of RHB and wood biochar (WB). When RHB is administered at a 1.0 kg/m^3 rate, it optimizes leaf production and stem size. Nevertheless, the experimental data indicates that a 2.0 kg/m^3 density of RHB yields the highest average leaf width. All treatment rates of RHB result in longer leaves compared to the control. The observed differences in Si, K, ash content, and surface area between WB and RHB may explain the underlying reasons for WB comparatively lesser influence on spinach growth.

Singh Mavi *et al.* (2018) evaluated growth of wheat and maize crops in two soils characterized by distinct textures. The study aimed to assess the influence of RHB on crop productivity. After the cropping season, soils treated with RHB exhibit notable enhancements in oxidizable organic carbon and bioavailable nutrients, aligning with observed increase in maize biomass. The correlation between soil quality and increased maize biomass confirms this claim.

Singh *et al.* (2018) observed a significant increase in panicle length, tiller count, grain production, and straw yield of rice following application of 10 t/hm^2 RHB in a field trial. Nevertheless, the impact on grain yield is more evident. In their study, Huang *et al.* (2019) employed a consistent application of biochar across six consecutive growing seasons to assess the influence of RHB on the productivity of rice crops. The researchers observed a decline in grain yield during initial three seasons following RHB application. However, a significant rise in grain output was observed over the subsequent three seasons. This suggests that the duration and regularity of biochar application will play a crucial role in determining the beneficial effects of RHB treatment on rice productivity. The decrease in rice grain weight is the reason for decline in grain yield following biochar application over three growing seasons.

According to Ghorbani and Amirahmadi (2018), different biochar application rates (2 and 4%) on soil impact differently on maize development. In the ninth week of growth the plant height was found to be 85 cm with 4% biochar application rate. This measurement was considerably higher compared to plant height of 75 cm observed in control soil without any amendments. The dry weight of shoot in experimental groups with 2% and 4% RHB rates were significantly higher (154.7 and 156.8 g, respectively) than control group (148.8 g).

Numerous studies have provided evidence to support the notion that applying biochar with chemical fertilizers leads to a notable increase in crop yields, particularly in soils with low fertility. This increase may be attributed to the ability of biochar to directly supply nutrients to the crops or enhance the availability of nutrients Gandahi *et al.* (2015). Applying biochar with fertilizers has enhanced plants' capacity to absorb and utilize nitrogen (Mehmood *et al.*, 2018). The research conducted by Asadi *et al.* (2021) demonstrates that biochar with a high carbon (C) content may immobilize nitrogen and reduce the bioavailability of essential nutrients by adsorbing them onto the surface functional groups.

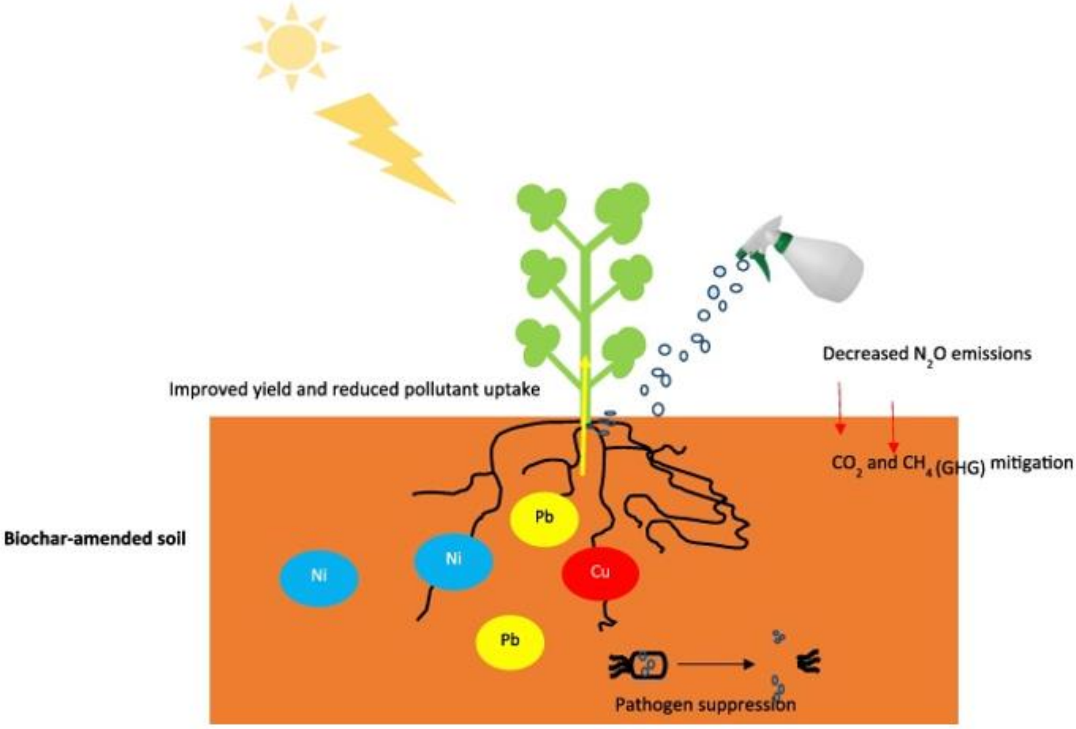


Figure 2.3: Impact of biochar application in soil (Adapted from Mehmood et al., 2018)

2.4 Effect of biochar on crop productivity in different textured soils

Application of RHB may enhance various aspects of soil quality and productivity. These include the status of soil nutrients, crop production, water retention, carbon sequestration, cation exchange capacity, nitrogen leaching, and the mitigation of toxicity in contaminated soil (Dejene and Tilahun, 2019). Using RHB as a substitute for conventional liming compounds in soil is feasible due to its high pH value. Specifically, soils with high acidity might experience beneficial effects when the pH increases, as this leads to a reduction in exchangeable aluminum (Al) and soluble iron (Fe) concentrations while simultaneously increasing the cation exchange capacity (CEC) (Karam *et al.*, 2022). Furthermore, crops may experience significant advantages from applying RHB owing to its elevated silicon concentration.

The application rate of RHB was impacted by factors such as the kind of crop, soil composition, and pyrolysis temperature of the RHB. Huang *et al.* (2019), noted that ongoing RHB treatment on paddy fields resulted in a significant increase in grain production ranging from 4 to 10% after four to six growing seasons. According to Hadiawati *et al.* (2019), the application of RHB at a rate of 5 t/ha resulted in a significant increase in above ground biomass and grain production of lowland rainfed rice in Indonesia, reaching up to 6.47 t/ha. Moreover, previous research has demonstrated that applying biochar at a rate of 2-8 t/ha is sufficient in investigating RHB effectiveness in enhancing crop productivity (Sandhya and Prakash, 2019).

Previous studies have indicated that using RHB generated through pyrolysis at elevated temperatures significantly enhances crop yield. According to Huang *et al.* (2019), applying RHB for 4-5 seasons resulted in an observed increase in size of rice panicles. Mahmoud *et al.* (2011), showed that using RHB in combination with NPK fertilizer for wheat cultivation exhibited a noteworthy capacity to mitigate the presence of cadmium (Cd). According to Fru *et al.* (2017), the use of RHB resulted in a more significant enhancement in the growth performance of *Talinum triangulare* compared to biochar derived from sawdust, cassava, or corncob. The nutrient retention capacity of biochar is subject to substantial influence from the specific soil type in which it is applied. According to Filho *et al.* (2019), using biochar may enhance effectiveness of phosphorus (P) fertilizers under acidic soil conditions.

According to Oladele (2019), the application of RHB in combination with N fertilizer resulted in increased rice grain yield for rain-fed rice grown on sandy clay loam and sandy loam soil. Nevertheless, a more significant proportion of clay in sandy clay soil decreased nutrient loss compared to sandy loam soil. In contrast to Alfisols, Ultisols typically exhibit a reduced cation exchange capacity due to extensive soil weathering. When RHB and fertilizer are co-applied, there is an observed increase in soil pH, although sandy loam soil exhibits a higher leaching rate compared to sandy clay loam soil. Moreover, the anti-caking properties exhibited by rice husk prove to be quite beneficial in paddy fields, as highlighted by (El-Gamal *et al.*, 2023).

According to Sarong and Orge (2015), cultivating water spinach and peanuts in acid sandy loam soils of the Philippines demonstrates a positive response to applying 30-40 g/kg of RHB. According to Ghorbani *et al.* (2019), the ash derived from RHB exhibits a significant abundance of alkaline carbonates, alkali earth metals, and organic anions, collectively contributing to its elevated pH level. In a pot experiment conducted by Manickam *et al.* (2015), the biomass of maize and rice was examined under different cultivation conditions. The experiment involved using sandy and acid sulfate soil, with varying rates of RHB at 2% and 5%. The results indicated an increase in biomass for both crops under these conditions. In their study, Koyama and

Hayashi, (2017) observed that RHB possesses a significant silicon content, leading to its potential utilization as a silicon-based fertilizer.

2.5 Availability of nutrients in biochar

The release of soil nutrients may not directly correlate with the overall nutrient concentration of biochar. According to El-Naggar *et al.* (2019), bioavailable nitrogen (N) forms present in biochar are comparatively lower than those found in the original feedstock used for its production. The effect of biochar on soil N availability is insignificant. However, it is worth noting that a high carbon-to-nitrogen (C/N) ratio has been associated with N immobilization, as suggested by Nguyen *et al.* (2017). Hence, scientists contended that the inclusion of biochar in soils necessitates the supplementary use of fertilizers containing this constituent (Nelson *et al.*, 2011). Mukherjee and Zimmerman (2013) conducted a batch extraction and column leaching experiment to investigate the release of inorganic nitrogen (N) from biochar derived from Laurel oak, Loblolly pine, and Gamma grass at temperatures of 400 and 650°C. The results indicated that the primary form of inorganic N released was ammonia. Nitrogen (N) and phosphorus (P) occurred predominantly in organic molecules, accounting for 61% and 93% of the total release, respectively. Furthermore, there exists a correlation between amount of volatile matter (VM) present in biochar and concentration of acidic functional groups about release of dissolved organic carbon, N, and P into surrounding water.

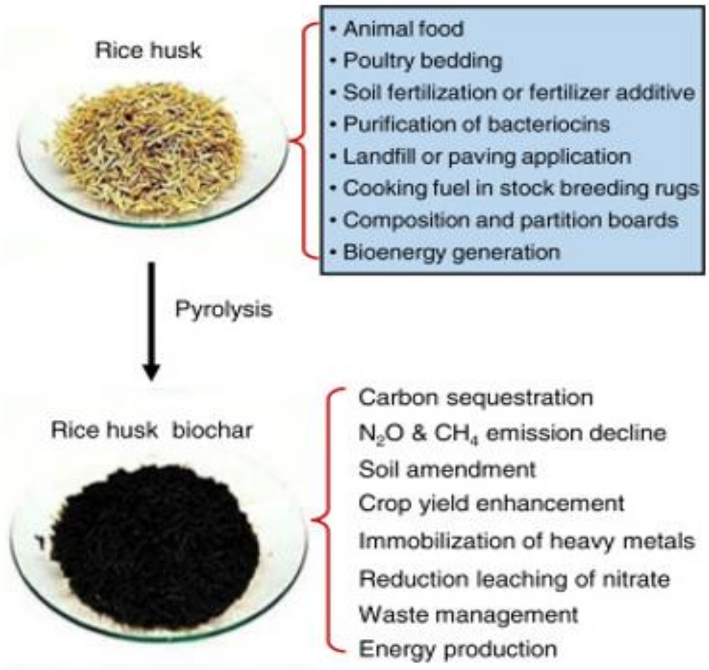


Figure 2.4: RH and RHB application (Adapted from Pode 2016)

2.6 Impact of biochar on nitrogen cycle

Ammonification, nitrification, ammonium volatilization, and emission of gaseous nitrogen and its oxides into atmosphere are integral components of the nitrogen cycle in natural ecosystems (Baiga and Rao, 2017). Additional processes encompass atmospheric nitrogen fixation, the absorption of ionic nitrogen by plants from water and soil.

The nitrification process is subject to notable influences from various environmental factors, including soil moisture content, temperature, pH, precipitation, human activities, and specific types of nitrogen fertilizers utilized (Rao *et al.,* 2017). During the subsequent phases of immobilization and mineralization, nitrate generated during nitrification process is assimilated into organic matter (Tanure *et al.,* 2019). Denitrification represents a further phase within the nitrogen cycle, when nitrates are converted into nitrogen, thereby removing bioavailable nitrogen, and releasing it into the atmosphere.

The denitrification ends in producing gaseous nitrogen in the form of N_2. However, during its progression, it also generates many additional gaseous nitrogen species, including N_2O and NO. The emission of nitrous oxide (N_2O), a greenhouse gas, contributes to atmospheric pollution by participating in reactions with ozone.

The absorption of biochar into soil has been found to impact the activity of soil microorganisms. This is primarily due to the provision of nutrients and the influence on soil pH and moisture content (Gul and Whalen, 2016). Soil bacteria play a significant role in regulating many activities within the nitrogen (N) cycle. According to Gul *et al.* (2015), soil bacteria may occupy a distinct and specialized ecological niche within biochar. Previous studies have reported that presence of BC in acidic soils with a pH of 5 significantly enhances nitrogen fixation in legumes, resulting in an average increase of 63% compared to control conditions. According to a study conducted by Mia *et al.* (2014), using grass-derived biochar (heated to a temperature of 400°C) at a concentration of 0.3% significantly enhanced nitrogen fixation by 56%. The nitrogen fixation of *Phaseolus vulgaris* was seen to exhibit a significant increase of 78% when subjected to *Eucalyptus deglupta* biochar, produced at a temperature of 350°C, at a rate of 0.6% (60 g/kg). Azeem *et al.* (2019) reported a comparable rise of 83% in N_2-fixation in mash bean plots that were supplemented with 0.3% of biochar derived from bagasse biomass at a temperature of 350°C, in comparison to plots without the addition of biochar. This increase was observed compared to legume crops that did not receive any biochar amendment. The provided examples illustrate that plant ability of nitrogen fixation is not dependent upon the rate of biochar application but instead on the properties of the biochar itself and the specific plant species. According to Mia *et al.* (2014), the pH of the soil is increased, resulting in enhanced bioavailability of phosphate. Additionally, inorganic nitrogen is immobilized, and the incorporation of macro and micronutrients from biochar occurs.

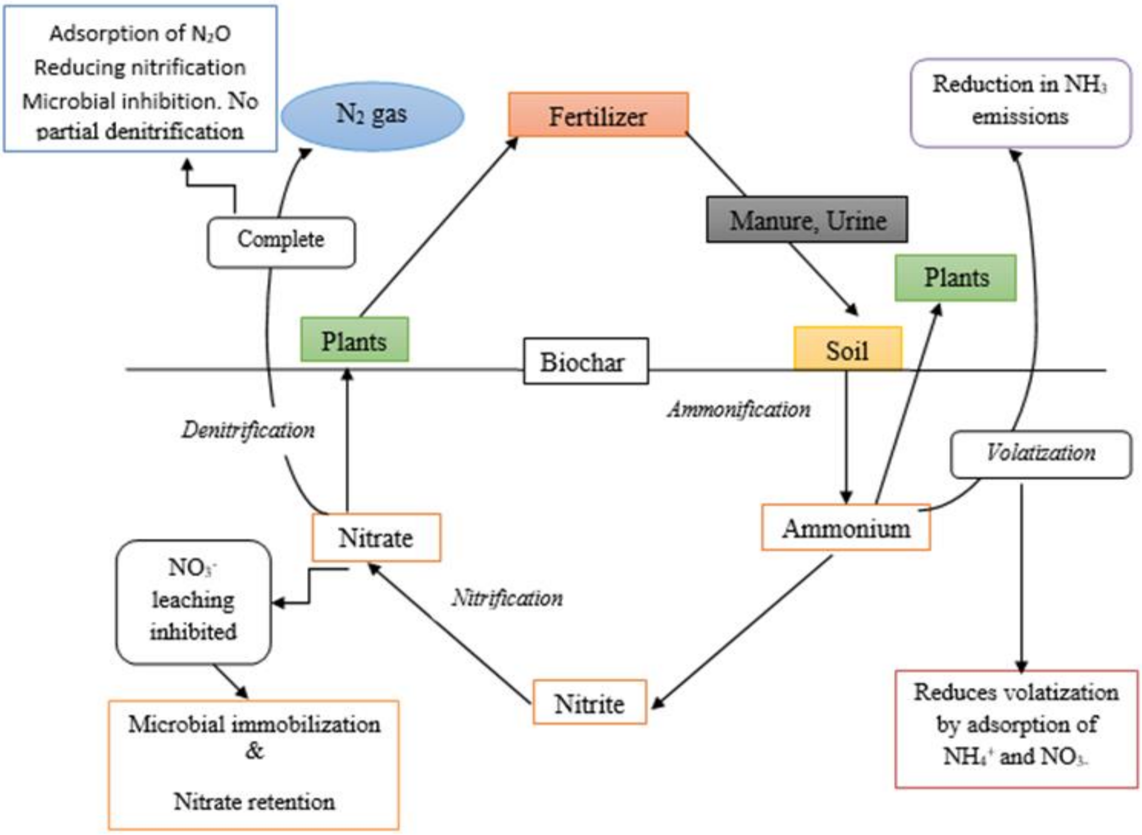


Figure 2.5: Biochar impact on nitrogen cycle (Adapted from Mandal *et al.*, 2016)

The biochar impact on nitrification has been presented in previous studies (Taghizadeh *et al.*, 2012). The ion-adsorbing characteristics of biochar have been found to have a consequential effect on NH_4^+ concentration in soil, as observed by Zhao *et al.* (2014). This, in turn, directly influences the functioning of ammonium oxidants and nitrifying bacteria. Furthermore, Zhao *et al.* (2014) revealed that applying biochar resulted in an enhancement of soil NH_3 nitrification treated with both inorganic and organic nitrogen fertilizers. According to Ulyett *et al.* (2014), biochar has been found to enhance the environmental conditions conducive to the growth and activity of nitrifying bacteria. This is achieved by the elevation of pH levels, improvement in aeration, and increased soil moisture. The capacity of biochar to adsorb NH_4^+ and inhibit its conversion into NH_3 has been substantiated by scientific research, indicating its potential to alleviate NH_3 loss in soil (Chen *et al.*, 2013). The high specific surface area of biochar facilitates the adsorption of NH_3 gas from the surrounding atmosphere. The study by Mandal *et al.* (2016) found that using poultry litter derived biochar at a temperature of 550°C resulted in a notable reduction in the volatilization of NH_3 from soils fertilized with urea. Chen *et al.* (2013) conducted research indicating that applying green waste biochar at a temperature of

450°C may effectively reduce the volatilization of ammonia from soil. The specific surface area of biochar and the presence of acidic functional groups responsible for the adsorption of NH_3 are significant determinants in its capacity to alleviate volatilization induced by nitrogen fertilizer (Gul and Whalen, 2016).

According to Cayuela *et al.* (2014), a meta-analysis examining the impact of biochar on denitrification, it was shown that the average reduction in N_2O emissions from soils supplemented with biochar is 54% compared to soils without biochar amendment. It identified several mechanisms by which biochar impacts the emissions of nitrous oxide (N_2O) from soil. These mechanisms include enhanced immobilization of NO_3 in microbial biomass, increased absorption of these ions by plants, elevation of pH levels, reduction in bulk density and augmentation of porosity.

2.7 Application and impact of nitrogenous fertilizers in soil

Nitrogen fertilizer efficiency may be assessed by nitrogen uptake efficiency (NUE). The statistic referred to in this context is the ratio of a plant's yield to the quantity of nitrogen it receives, as Puga *et al.* (2020) described. The most often utilized nitrogen fertilizers include ammonium nitrate, ammonium nitrate lime, urea and ammonium sulfate. The nitrogen content of these fertilizers exhibits variation, with specific formulations containing other components that serve to mitigate soil acidity.

Nevertheless, it has been observed that nitrogen fertilizers may experience a significant reduction in nutritional content, up to 50%, upon their application to soil, hence deviating from their primary purpose of nutrient provision (Dimkpa *et al.*, 2020). The elevation of greenhouse gas concentrations may be attributed to the emissions of N_2O, groundwater contamination, and the eutrophication of surface waters resulting from the depletion of nutrients produced by using nitrogen fertilizers (Coskun *et al.*, 2017). The fundamental reason for the low level of nitrogen fertilizer usage after its incorporation into the soil is attributed to these losses (Puga *et al.*, 2020). Consequently, this leads to suboptimal crop yields and increased expenditures associated with agricultural production. Nitrous oxide (N_2O), produced as a byproduct during the denitrification process, is widely acknowledged to constitute more than 50% of the total anthropogenic greenhouse gas emissions attributed to the agricultural sector. The creation of smog and the occurrence of air pollution resulting from particulate matter (PM) and aerosols may be intensified by the emissions of nitrogen oxides (Erisman *et al.*, 2013).

Based on a study conducted by Bednarek *et al.* (2014), it has been determined that nitrates (NO_3), which are the most easily transported ionic form of nitrogen compounds found in soil, play a significant role in the contamination of water bodies and the process of eutrophication.

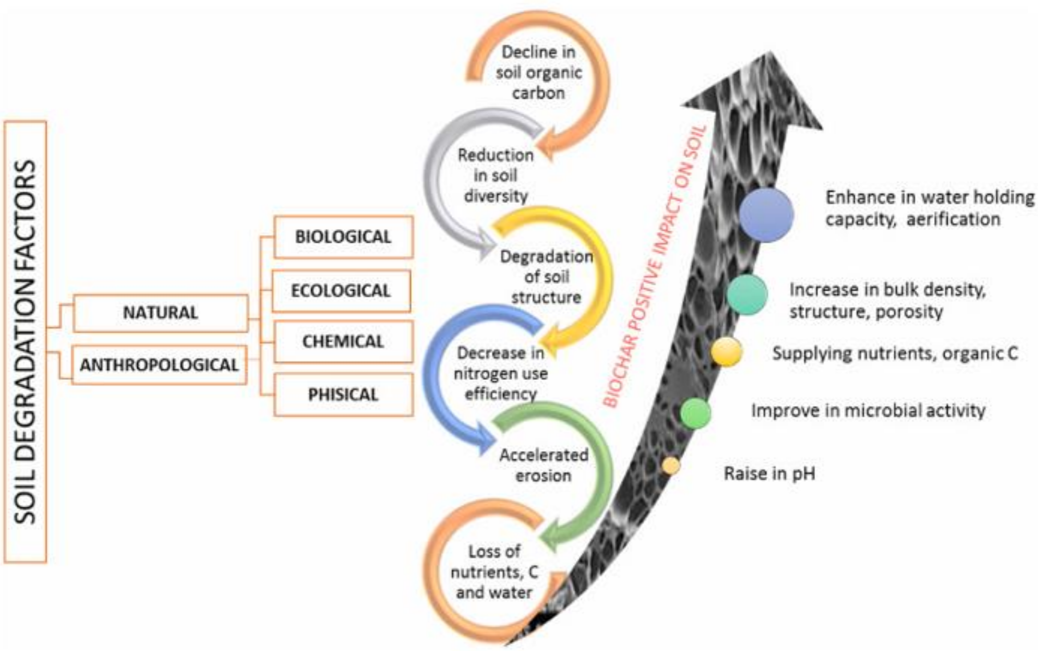


Figure 2.6: Soil degradation factors (Adapted from Premalatha *et al.*, 2023)

2.8 Combined application of biochar and synthetic fertilizer

Inorganic fertilizers present a distinct array of challenges, whereas biochar is limited by their insufficient nutritional content, rendering them ineffective as standalone fertilizers (Oladele 2019). The research conducted by Omara *et al.* (2020) provided evidence that the combination of biochar and inorganic nitrogen positively impacted the growth of maize crops grown on sandy soils with poor physicochemical properties. The utilization of urea, a commonly used fertilizer, in combination with biochar, at varying rates of 5 to 15 t/ha, resulting in an increase in nitrogen uptake efficiency (NUE) ranging from 25 to 45% compared to crops cultivated only with the fertilizer. The optimal outcome was observed when a biochar application rate of 10 t/ha was employed. In combination with using RHB at a temperature of 350°C, Oladele (2019) observed that efficacy of urea fertilizer was superior. Applying biochar at rates ranging from 3 to 6 t/ha, along with a nitrogen fertilizer rate of 30 kg/ha, resulted in a significant enhancement of 140% in the agronomic efficiency of *Oryza sativa*. Following two years of employing the combination of fertilizer and biochar, there was a substantial increase of around 100% in the retrieval of nutrients in grain. Applying biochar and nitrogen fertilizer resulted in notable enhancements in soil structure, as evidenced by reductions in bulk density and concurrent increases in water holding capacity, pH levels, total organic carbon content, and availability of calcium ions.

In their study, Zheng *et al.* (2017) observed that applying diammonium phosphate and potassium chloride in combination with biochar (wheat straw, 350 and 550°C) significantly improved various crop parameters. Specifically, compared to crops where synthetic fertilizer was used in isolation, the combined application of these substances led to an 11% increase in grain yield, a 43% enhancement in agronomic nitrogen use efficiency, and a 12% rise in net income. Numerous papers have highlighted several advantages of applying biochar, including a notable increase in crop output, improved accessibility of nutrients, and reduced leaching of nutrients.

The study conducted by Liao *et al.* (2021) showed a decrease in NO_3 leaching, N_2O emissions, and organic N mineralization in sandy loam soil after applying biochar in conjunction with nitrogen fertilizer. According to Phares *et al.* (2020), the application of triple superphosphate (TSP) at a rate of 60 kg P/kg and BC (rice husk, subjected to a temperature of 400°C) at a rate of 2.5 t/ha increased the availability of phosphorus in the rhizosphere. It promoted the growth of nodules in cowpea (*Vigna uguiculata*) plants. No significant changes in soil pH were seen in the tested system, and the pH of the soil remained constant at 6 throughout the study. The researchers also determined that enhancing the antioxidant properties of cowpea leaves and roots through the simultaneous application of biochar and TSP is a viable methodology. This entails augmenting the overall concentration of phenols, flavonoids, alkaloids, saponins, and tannins.

Yan *et al.* (2019) demonstrated synergistic effect of BC (sawdust, heated to 350°C) and NPK (a combination of urea, triple superphosphate, and potassium). The crop of soybeans (*Glycine max*) exhibited a substantial increase in both biomass and seed output, amounting to nearly four times the previous levels. In a recent study, Zhang *et al.* (2019) conducted a comprehensive examination of the topic concerning the availability and cycling of nutrients in conjunction with microbial activity and the simultaneous application of synthetic fertilizers and biochar. The measurement of soil bacterial activity was conducted both before and after the introduction of biochar and a mixture of inorganic fertilizers, consisting of 14 g/kg of KH_2PO_4, 0.51 g/kg of KNO_3, 0.80 g/kg of NH_4NO_3, and 0.95 g/kg of $Ca(NO_3)_2$ (Phares *et al.,* 2020). The immobilization of nitrogen occurred because of the application of biochar.

Rajkovich *et al.* (2012), examined the effects of biochar derived from dairy manure, paper sludge, and food waste produced at various temperatures (300, 400, 500 and 600°C), in comparison to a control group consisting of soil supplemented with synthetic fertilizers (10:20:20 NPK). The findings revealed that when the biochar application rate exceeded 2.0% (w/w), equivalent to 26 t/ha, it reduced corn growth. The increased levels of sodium (Na) in the soil may have impeded the growth of maize plants. This effect is believed to be mediated by the elevation of osmotic potential, which hampers the plant's ability to absorb water. The process of biochar synthesis at low temperatures (300-400°C) was influenced by nitrogen immobilization.

The combined use of biochar and synthetic fertilizers generally results in observable benefits, including increased crop yields, improved soil properties, and accelerated nitrogen cycling. Although this approach does not entirely substitute chemical fertilizers with organic alternatives, it does reduce fertilizer application rates by promoting nutrient retention within the soil. Elevated microbial activity is frequently regarded as an indicator of soil health. Moreover, the combined use of biochar and synthetic fertilizers has demonstrated supplementary advantages, such as decreased emissions of greenhouse gases resulting from the heightened immobilization of NO_3 in microbial biomass. Additionally, this co-application has been found to enhance the absorption of these ions by plants (Zheng *et al.,* 2012).

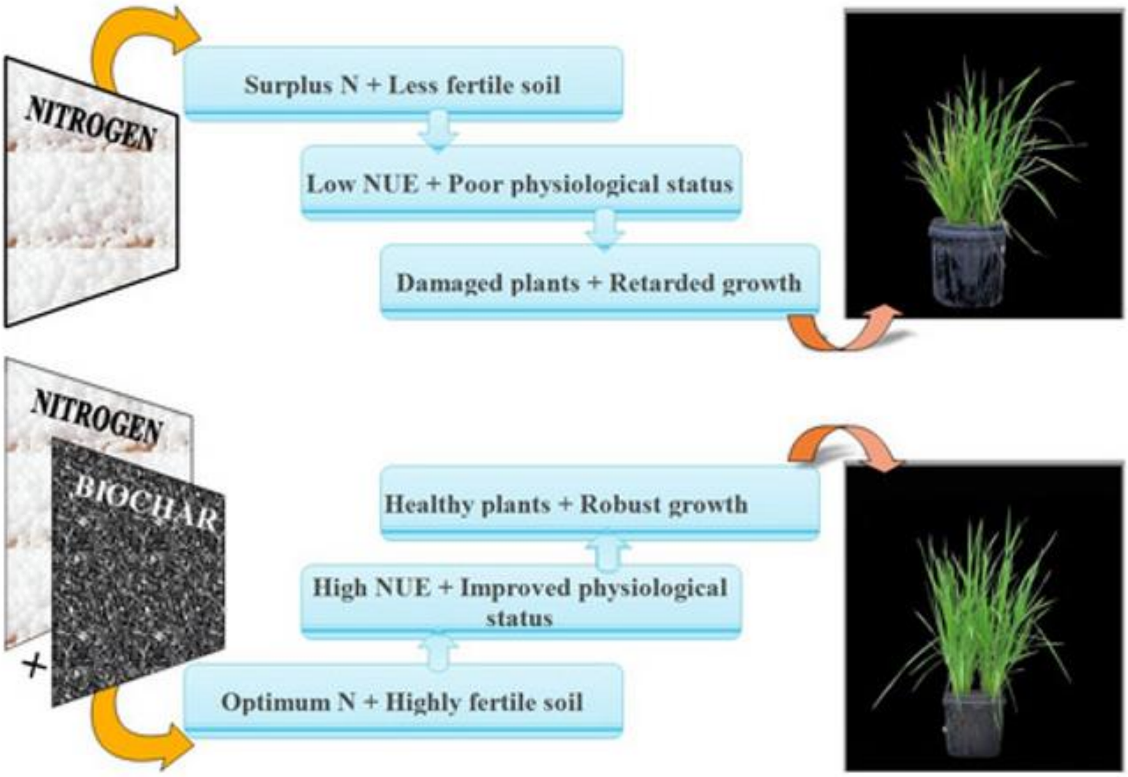


Figure 2.7: Effect of co-applied biochar and synthetic fertilizer on soil and plant (Selvarajh & Ch'ng, 2021)

CHAPTER 3

METHODOLOGY

The summary of the experimental process followed throughout this research is demonstrated in Figure 3.1.

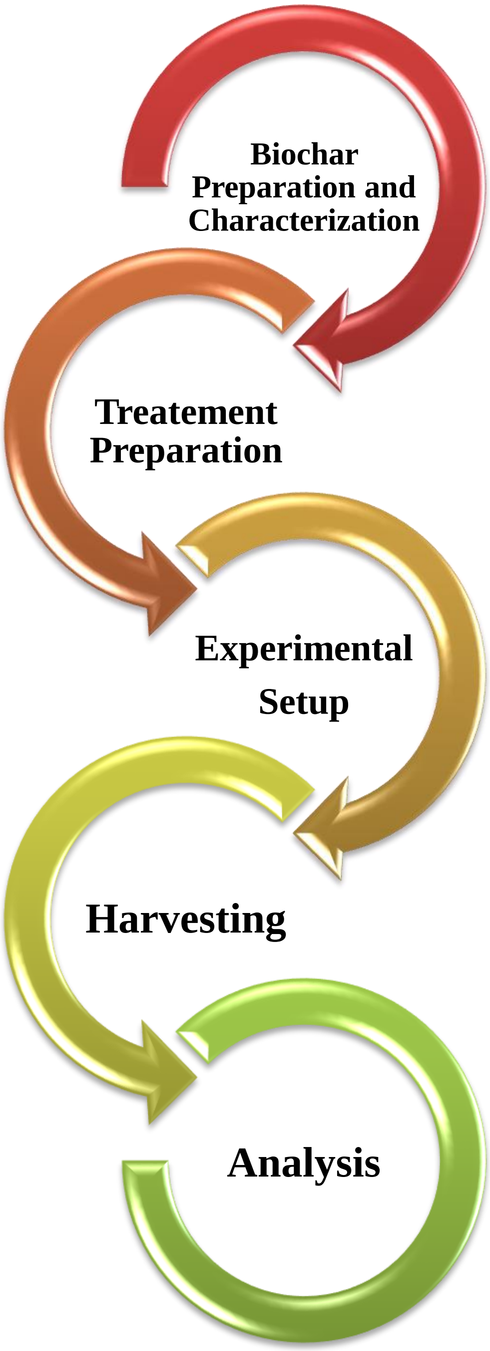


Figure 3.1: Summary of the process

3.1 Materials and methods

An experiment was set up in greenhouse at National University of Sciences and Technology (NUST). *Zea mays* L. commonly known as maize was selected because of its specific nutritional value and to tackle issues of food security. Maize seeds (OPV-3 NARC) were purchased from National Agriculture Research Center (NARC), Islamabad, Pakistan. Maize is planted bi-annually in Islamabad. Maximum and minimum mean air temperature during experiment was recorded to be 35 and 24 °C.

Figure 3.2: Greenhouse used for experiment.

3.2 Preparation of biochar

Feedstock of rice husk (RH) was collected from a local rice mill at Sialkot, Pakistan. RH was washed two times to remove any dust particles and impurities. After washing, it was sun dried for 48-72 hours. Pyrolysis was done in furnace (TF-1200X, Hefei Ke Jing Materials Technology Co., Ltd., Hefei, China). The temperature in furnace rises. Later it was maintained at 550°C for 5 hours. After preparation of biochar, it was stored in different storage boxes.

3.3 Characterization of biochar

Prepared biochar was characterized by several analytical techniques to evaluate the effect of chemical addition with biochar. The following section describes the experimental procedure with some theoretical background for each technique.

3.3.1 Biochar yield

The product (RHB) was grinded with pastel mortar. The final weight of the produced biochar was noted to calculate the yield (Stella Mary *et al.,* 2016). Percentage yield was calculated using the following formula:

$$Yield_{biochar} = \frac{m_{biochar}}{m_{raw}} \times 100\%$$

where $Yield_{\text{biochar}}$ = mass yield of biochar, %; m_{biochar} = mass of biochar, kg; m_{raw} = mass of raw biomass, kg.

3.3.2 pH

The pH was measured by preparing a suspension of biochar and water at 1:20 (v/w). The suspension was shaken for 24 hours at 130 rpm (Zheng *et al.,* 2013).

3.3.3 SEM-EDX analysis

Scanning electron microscope-energy dispersive x-ray spectroscopy (SEM-EDX) images were captured at 20 kV for morphological characterization of RHB. EDX probe for SEM was used on selected target point for quantitative chemical analysis (SEM: JSM 6490A, Jeol, Japan; EDX: EDAX Brooker, Germany).

3.3.4 FTIR analysis

Functional groups on the surface of biochar were determined using Fourier transform infrared spectrometry (FTIR) (FTIR Spectrum 100, PerkinElmer, USA). The KBr disk method was used for this purpose. The wavenumber in a range of 4000 - 400 cm^{-1} was used having a resolution of 1 cm^{-1}.

3.3.5 XRD analysis

Crystalline structure of RHB was analyzed using X-ray diffractometer (XRD D2 Phaser, Bruker, Germany) with Cu K_α radiation. Diffractograms were obtained using a continuous scan from 10 to 80° (2θ), with a step size of 0.04° (2θ). XRD is a non-destructive analytical technique used to characterize crystalline solid materials. The degree of crystallinity was determined based on characteristic peak`s intensity at 2θ.

3.3.6 TGA analysis

Thermogravimetric analysis (SDT 650, TA instruments, USA) was conducted to determine the thermal stability of produced biochar. The sample was placed in the furnace where temperature was raised gradually up to 1200°C. Weight of the sample was simultaneously measured by analytical balance placed outside the furnace.

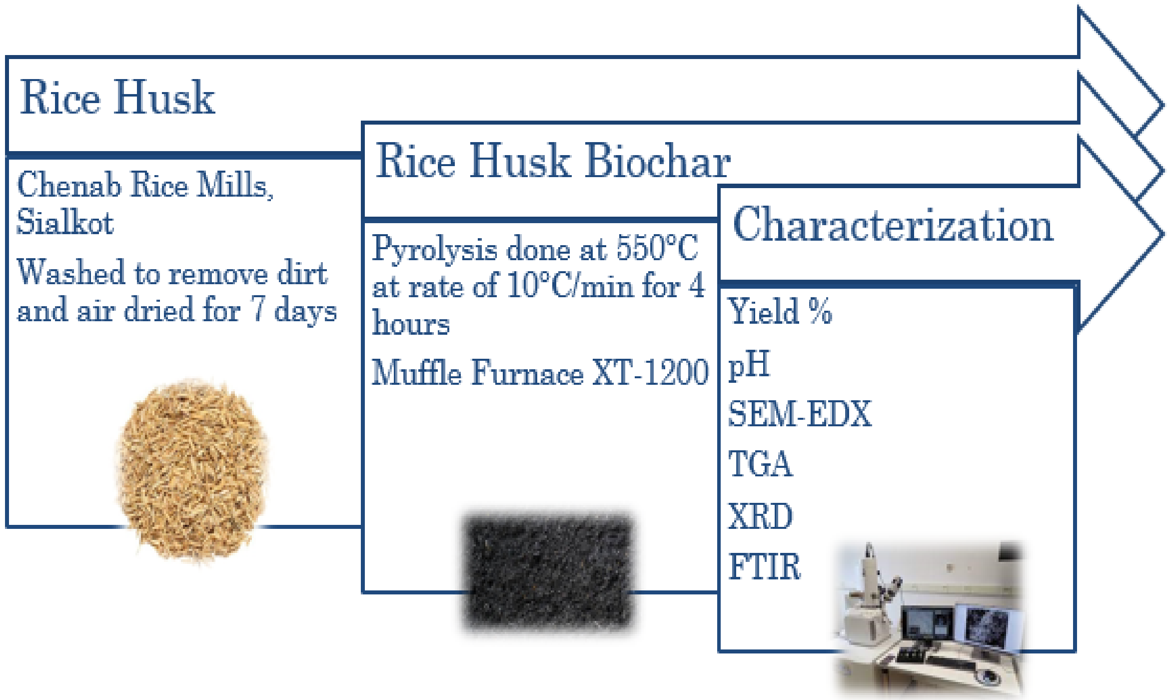


Figure 3.3: Biochar preparation and characterization

3.4 Experimental design

Two types of agricultural soils were selected based on the textural differences soil 1 (sandy loam) and soil 2 (silty clay loam). Soil 1 and soil 2 were collected from National University of Sciences and Technology (NUST) and a local nursery in Islamabad, respectively. A pot experiment was conducted in a greenhouse, well illuminated with natural light. Pots were filled with 2 kg soil and a total of six treatments were proposed to be applied on both sandy loam and silty clay loam soil as elaborated in Table 3.1.

Table 3.1: Treatments proposed in current study

	Treatments	**Replicates**
Sandy loam		
T1	Control	T1R1, T1R2, T1R3, T1R4
T2	BC 1%	T2R1, T2R2, T2R3, T2R4
T3	BC 2%	T3R1, T3R2, T3R3, T3R4
T4	U	T4R1, T4R2, T4R3, T4R4
T5	BC 1% + U	T5R1, T5R2, T5R3, T5R4
T6	BC 2 % + U	T6R1, T6R2, T6R3, T6R4
Silty clay loam		
T7	Control	T7R1, T7R2, T7R3, T7R4
T8	BC 1%	T8R1, T8R2, T8R3, T8R4
T9	BC 2%	T9R1, T9R2, T9R3, T9R4
T10	U	T10R1, T10R2, T10R3, T10R4
T11	BC 1% +U	T11R1, T11R2, T11R3, T11R4
T12	BC 2% +U	T12R1, T12R2, T12R3, T12R4

3.5 Preparation of experimental soil

The experimental soil was air dried to remove moisture before the experiment. After air drying soil was grinded in pastel and mortar. This step was performed to crush large soil particles into small uniform particles. Next, the soil was passed through a 2 mm stainless sieve. This step ensures the uniformity of soil particles. Prepared soil is further analyzed for physicochemical parameters.

3.6 Physicochemical analysis of soil

3.6.1 pH

Soil pH is an indicator of soil which can be defined as negative logarithm of Hydrogen ion concentrations. To determine soil pH, 50 g of soil was air dried and then put into a 100 ml glass beaker. Then 50 ml deionized water was added into the soil. Later we mixed this mixture by using glass rod for 30 minutes. The suspension was stirred three times after every 10 minutes. pH calibrated meter (HANNA Instruments HI 83141) was used and combined electrode was put in suspension. Readings were taken after every 30 seconds with one decimal point. Later combined electrode was removed from suspension and rinsed thoroughly with deionized water (Estefan *et al.*, 2013).

3.6.2 EC

Salinity of soil is the concentration of inorganic salts which are soluble in soil. It was measured by extraction of soil sample with water**.** Soil EC was measured by taking 50 g of soil. It was oven dried at 110 °C for 2 hours. This soil was collected in a 100 ml beaker and 50 ml of deionized water was added in it by using volumetric flask. The mixture was mixed well by using a glass rod and allowed it to stand for 30 minutes. Suspension was stirred after intervals of every 10 minutes. After one hour, the suspension is stirred and filtered by using a suction pump**.** A funnel was taken and covered with filter paper. Whatman No. 42 filter paper was attached tightly to the bottom of funnel in such a way that it was covered properly. The suction pump was then opened, and suspension was added in funnel. Filtration was continued until soil in funnel cracks. This procedure was repeated unless a clear solution was obtained**.** The conductivity meter (HANNA Instruments HI 83141) was calibrated, and filtrate was transferred into a 50 ml beaker. The conductivity meter was then immersed in solution and reading was measured. This conductivity cell was removed from mixture and rinsed thoroughly from deionized water (Estefan *et al.*, 2013).

3.6.3 Moisture content

Soil moisture can be a limiting factor as it affects growth of crops by effecting availability of nutrients. Moisture content of soil not only effects on transformation of nutrients but also controls biological behavior of soil. It is measured by taking 10 g of soil and oven drying it at 105°C for 24 hours. Next day dried soil is taken, container is removed, and lid is fixed. The sample is cooled in a desiccator for 30 minutes and weighted again. The moisture content of soil is then calculated by using the following formula:

$$Moisture\ content\ (\%) = \frac{wet\ soil - dry\ soil}{dry\ soil} \times 100$$

3.6.4 Soil texture

Soil texture was determined using saturated paste method. To characterize soil texture 100g of air-dried soil is collected in a 100 ml beaker. Distilled water is added to the soil gradually until a uniform paste is formed. The saturated paste equals the weight of the water required to saturate dry soil samples. Texture may be assessed using table below.

Table 3.2: Saturated paste moisture content and approximate soil texture range

SPE (%)	Soil texture
< 20	Sand or loamy sand
20-35	Sandy loam
35-50	Loam or silt loam
50-65	Clay loam
65-135	Clay
>80	Organic soils > 15 % soil organic matter

US Salinity Laboratory Staff, 1954.

3.6.5 Soil nutrient concentration

Potassium (K) and nitrate nitrogen (NO_3^--N) determination was performed by AB-DTPA extract method by using flame photometer and spectrophotometer respectively (Soltanpour and Schwab, 1977). Soil extractable phosphorus (P) was determined using Olsen method (Olsen, 1954). Soil nitrogen (N) was determined by Kjeldahl method (Bremner, 1965).

Table 3.3: Physicochemical properties of experimental soils (soil 1 and 2) and RHB

Properties	Soil 1	Soil 2
pH	7.81	8.23
EC (dS m^{-1})	0.26	0.44
Texture	Sandy loam	Silty clay loam
Moisture Content (%)	1.21	2.01
Phosphorus (mg kg^{-1})	43.56	58.24
Potassium (mg kg^{-1})	57.44	74.26
Nitrate nitrogen (mg kg^{-1})	10.04	13.96
Nitrogen (%)	0.07	0.08

3.7 Seed preparation

Maize seeds were washed with distilled water and then sterilized using sodium hypochlorite solution (NaClO). Seed sterilization is important to prevent pest attacks after sowing. Seeds were collected in a beaker and 5% (v/v) sodium hypochlorite solution was added. Seeds were soaked for 10 min in this solution. After 10 min, seeds were taken out and washed 2-3 times with distilled water.

3.8 Pot experiment

Urea was applied at the rate of 120 kg N/ha because laboratory or pot experiment require twice the amount of fertilizer applied in field experiments (Kundu *et al.*, 1996). Biochar application rates were selected after thorough literature review (Dey and Mavi, 2021; Manolikaki and Diamadopoulos, 2019). Pots were placed in completely randomized design with 4 replicates for all treatments. Four maize seeds were sown in each pot. Moisture content was maintained throughout the experiment by irrigating the pots with tap water.

3.8.1 Treatment application

After seedling emergence phosphorus and potassium fertilizers were applied at the rate of 60 kg P/ha and 50 kg K/ha respectively. Urea was applied in two split doses each of 60 kg N/ha. The first dose was applied at 8 days after seedling emergence and the second dose at 18 days after seedling emergence. There was no sign of pest or disease attack hence no herbicide or pesticide was applied.

3.9 Plants harvesting

Plants were harvested 45 days after seedling emergence. Above ground (leaves and shoots) and below ground (roots) biomass was carefully separated and removed from each pot to minimize damage. Plants were washed with deionized water to remove soil. Plant length (roots, shoots, and leaves) and fresh weight (root, shoot and leaves) were measured. For dry weight (roots, shoots, and leaves) plants were oven dried for 48 hours at 68°C. For further analysis dried plant samples were stored in polythene bags. Harvested soil was air dried for soil analysis.

Figure 3.4: Maize pots before harvesting

3.10 Plant and soil analysis

Soil and plant N was analyzed using Kjeldahl method (Bremner, 1965). To determine N content in grounded sample acid digestion was performed at high temperature in semi-automatic DK-6 digestion unit (VELP Scientifica, Italy) with copper sulphate ($CuSO_4$) and potassium sulphate as a catalyst. After digestion, distillation (semi-automatic Kjeldahl unit, VELP Scientifica, Italy) of samples was carried out followed by titration with 0.02 N H_2SO_4.

For the measurement of K and NO_3^--N soil extract was prepared following AB-DTPA method by mixing 10 g of air-dried soil with extracting solution and then shaken for 15 min at 180 rpm. The extract was then filtered using Whatman no. 42 filter paper. The K concentration in samples was analyzed using flame photometer at 767-nm wavelength (BK-FP 6450, BIOBASE, China). For NO_3-N sample extract was mixed with copper sulphate, hydrazine sulphate and sodium hydroxide solution and was heated in water bath at 38°C for 20 min. Color developing reagent was mixed well with the above sample and after 20 min absorbance was noted using spectrophotometer (Specord 200, Analytik Jena, Germany) at 540-nm wavelength. Sample concentration was calculated from calibration curve (Soltanpour and Schwab, 1977).

Soil extractable P concentration was calculated using Olsen P method (Olsen, 1954). Soil was extracted using 0.5 M sodium bicarbonate solution shaken at 200 rpm for 30 min and filtered with Whatman no. 42 filter paper. Absorbance of extracted samples were measured at 882-nm using spectrophotometer (Specord 200, Analytik Jena, Germany). The P concentration was obtained from calibration curve.

2.3 Statistical analysis

Statistix 8.1® (Analytical software, Tallahassee, FL, USA) was used for data processing. The dataset was statistically analyzed using two-way analysis of variance (ANOVA) with treatments and soil type as two factors. Tuckey HSD test was performed for pair wise comparisons at significance value $P<0.05$. Principal Component Analysis (PCA) was carried out using XLSTAT version 2021. Expressed results were the mean of four replicates with standard deviation (SD).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterization of biochar

4.1.1 Scanning electron microscope

SEM pictures were captured of the RHB surface at two different magnifications: 2,500 and 10,000. The surfaces of the biochar are spotted with pores in both photos. (Fig 4.1 a). the second pore was more evident at 10,000 magnification (Fig 4.1 b). The fractured structure of the biochar can be seen with pores at 1,000 and 2,500 X (Fig 4.1 c and d).

The porosity of the soil reveals its quality. The porous nature of biochar was revealed by scanning electron microscopy pictures of rice husk biochar obtained at 2,500 and 10,000 X. The International Union of Pure and Applied Chemistry (IUPAC) classified spore size, and biochar showed macro-porous structure (pore size > 50 nm) (Sing, 1991; Downie *et al.*, 2009). Plant water availability is increased by the macropores in biochar (de Jesus Duarte *et al.,* 2023). According to Głąb *et al.* (2016), biochar increases soil aggregates and porosity, which both enhance soil health. Soil porosity is ultimately improved by the "expansion effect" that biochar's macropores demonstrated, which produced more porous space between soil pores (Blanco-Canqui, 2017). According to Aslam *et al.* (2014), applying biochar led to increases in soil porosity, nutrient penetration, and granular structure. This is explained by the fact that the pores in biochar serve as a haven for beneficial soil fungus and microorganisms as well as a binding surface for nutritional anions and cations. Plant growth and nitrogen uptake are enhanced by this (Atkinson *et al.*, 2010).

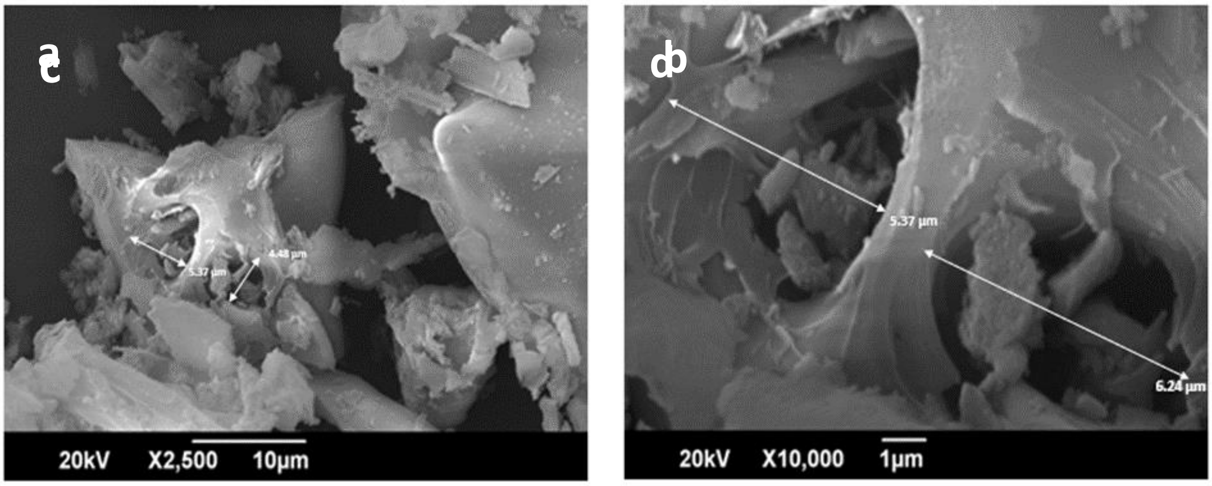


Figure 4.1: SEM image **a)** 2,500 X **b)** 10,000X of RHB prepared at 550°C

4.1.2 Energy dispersive spectroscopy

Using SEM-EDX elemental analysis of RHB, it was possible to identify various components on the surface of the biochar. The prepared biochar's EDX spectrum is shown in Fig. 2. The biochar sample was found to include silicon (Si), carbon (C), and potassium (K). Si, C, and K were abundant in the rice husk biochar, according to the EDX study. Saeed *et al.* (2019) found in another investigation that biochar made from rice husk has a lower carbon content and a higher silica content than biochar made from other biomasses. A valuable addition to soil is biochar, and potassium is a significant macronutrient (Farrar *et al.,* 2021). Although silica is found in all plants and is categorized as a functional nutrient, it is not currently regarded as a key plant nutrient (Ali *et al.,* 2020). A study on water spinach found that adding rice husk charcoal rich in silica enhanced crop quality and yield, boosted resilience to pathogens and pests, and showed a high tolerance for drought and heavy metal stress (Varela Milla *et al.,* 2013).

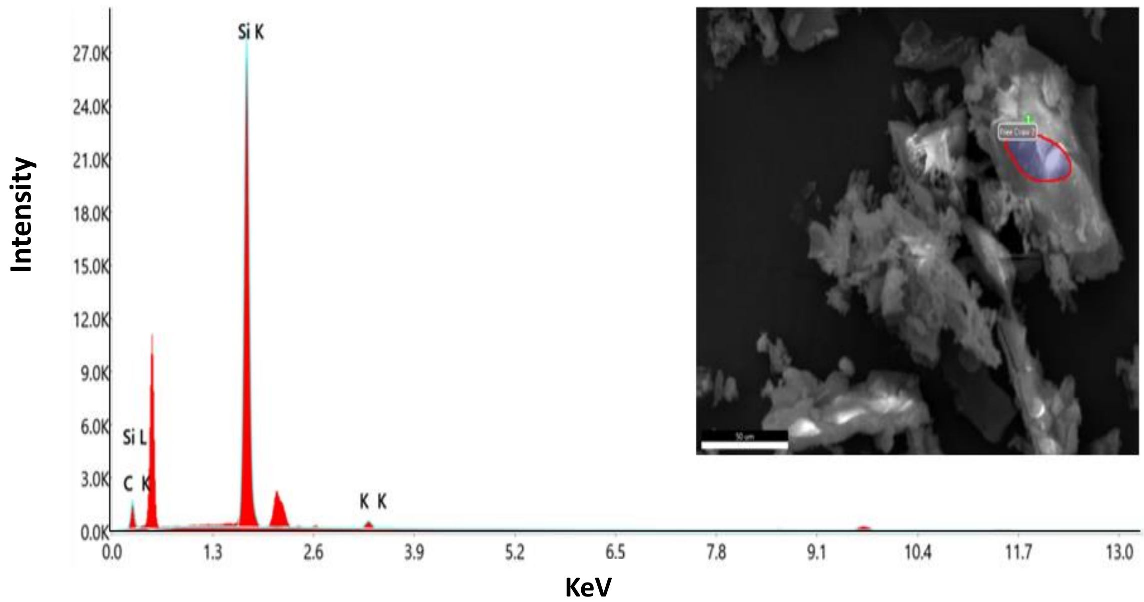


Figure 4.2: EDX spectrum of RHB, Si, K, and C represents silicon, potassium, and carbon elements respectively

4.1.3 Fourier transform infrared spectroscopy

The detected spectrum peaks indicated that the surface of the biochar exhibited stable and aromatic functional groups. Together with other elements, the existence of double and single bonded carbon molecules confirms the aromatic structure of biochar. The previously mentioned SEM-EDX results further support the presence of both symmetric and asymmetric silica in biochar. According to several studies (Claoston *et al.*, 2014; Tomczyk *et al.*, 2020; Ray *et al.*, 2020; Armynah *et al.*, 2018; Liu *et al.*, 2015), rice husk biochar usually contains all these bands. Overall, as the temperature of pyrolysis rises, so does the presence of fundamental functional groups. This biochar enhances soil pH and fertility when added to sandy soil, which in turn promotes plant growth and nutrient retention (Shaaban *et al.*, 2013).

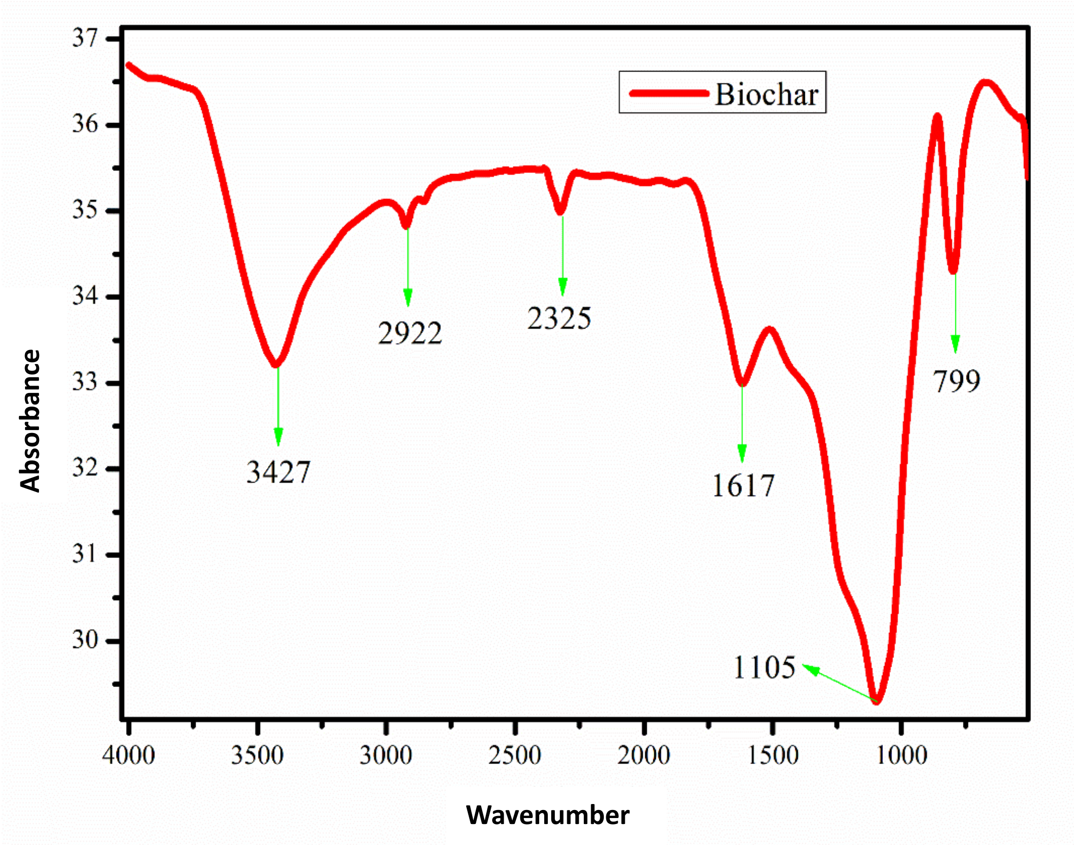


Figure 4.3: FTIR spectrum of RHB prepared at 550 C

4.1.4 X-ray diffraction

RHB's crystallinity was assessed using the x-ray diffraction (XRD) method. As demonstrated in Figure 4.4, the heterogeneous nature of RHB was revealed by the XRD pattern recorded. Numerous tiny peaks seen across the spectrum suggested the presence of various inorganic chemicals. An was detected as a single peak between 20° and 30°. The amorphous nature of RHB was demonstrated by the large region perpendicular to the graphite layer. Biochar's heterogeneity was demonstrated by small dispersive peaks (Zahra *et al.*, 2022). According to Zhang *et al.* (2017), thermal degradation of cellulose changes previously crystalline graphite into amorphous graphite at pyrolysis temperatures above 400° C. Hossain *et al.* (2020) provided additional confirmation that biochar produced at low temperatures has crisp, narrow XRD peaks and strong crystallinity. Another study validated the stable nature of biochar by observing its heterogeneity and amorphous character when created at varying temperatures (Das *et al.*, 2021). By increasing soil stability and soil aggregates, adding this biochar to the soil improves water and nutrient retention, which in turn promotes root penetration, plant growth, and crop yield (Jeffery *et al.*, 2011; Githinji, 2014).

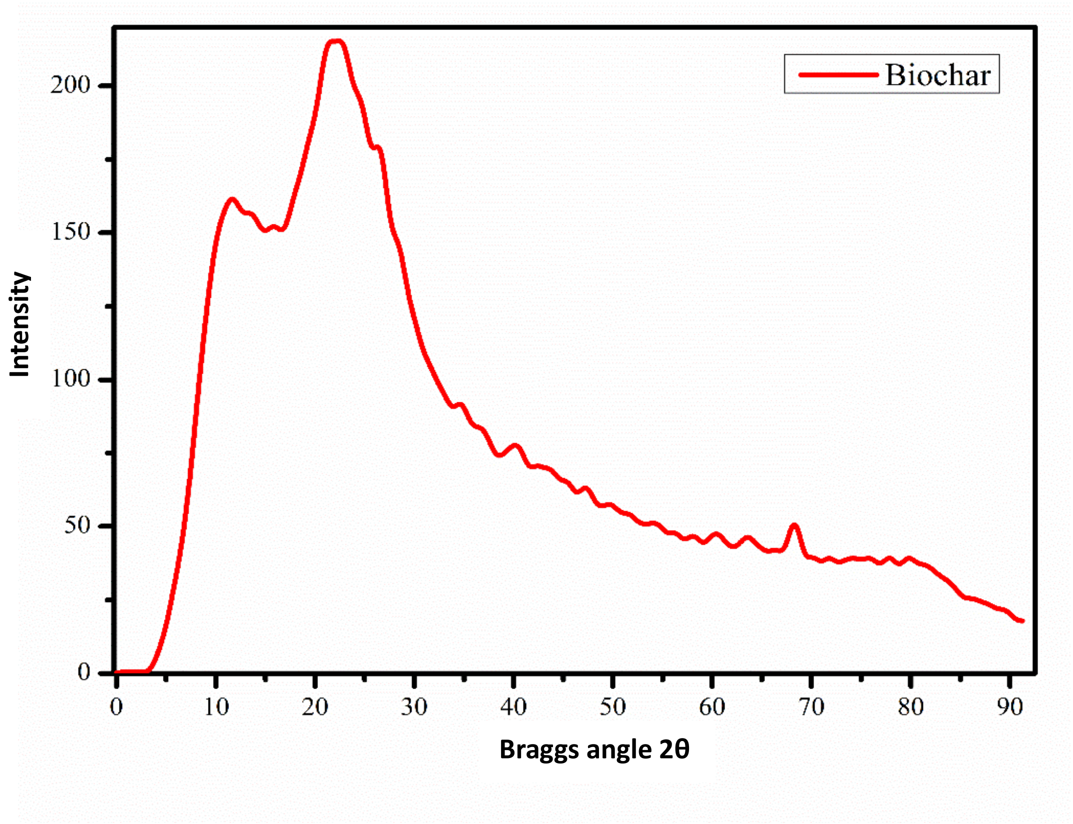


Figure 4.4: XRD diffractogram of RHB prepared at 550 °C

4.1.5 Thermogravimetric analysis

RHB's TGA revealed that it was thermally stable. Just the sample's starting weight was lost in total. This was separated into three stages according to how components deteriorated as the temperature rose. A slight deterioration was noted. This resulted from the sample's early loss of low molecular weight chemicals. Major degradation was observed between in the second weight loss. This thermal breakdown took place with an inert gas present. Following this, oxidative deterioration in the presence of air occurred. At this point, all that was present was ash and fixed carbon. At this point, a little amount of fixed carbon and ash were shown to be lost.

The amount of ash increases as the temperature of pyrolysis rises. Compared to biochar made from other biomasses, the RHB contains higher ash (Asadi *et al.*, 2021). It was shown in a study using pine wood biochar that high ash content biochar gives soil more mineral nutrients (Kim *et al.*, 2012). Thermogravimetric analysis (TGA) in this work demonstrated that the biochar generated had a low volatile matter content and was highly stable.

Higher pyrolysis temperatures result in biochar that has fewer organic molecules that degrade (cellulose, lignin, and hemicellulose). This is since huge, difficult-to-break polyaromatic molecules are left behind after the carbonation process of raw materials breaks down small molecular components. As a result, compared to biochar produced at low temperature, the overall weight loss is relatively little (Zhang *et al.,* 2017). A Thermally stable biochar with a low weight loss rate and a high solid residue content is produced by the high pyrolysis temperature (Chen *et al.,* 2016). Plant development was enhanced by the application of low volatile matter biochar combined with fertilizer (Deenik *et al.,* 2009).

According to the findings of the analyses carried out for this work, biochar made from RH feedstock at 550 °C is an amorphous, silica-rich, porous, and thermally stable material that contains aromatic carbon compounds. The feedstock's carbohydrates are totally broken down by high pyrolysis temperatures, and the only thing left over in the biochar is aromatic chemicals. Because of these qualities, biochar is very advantageous for use in agriculture (Asadi *et al.,* 2021).

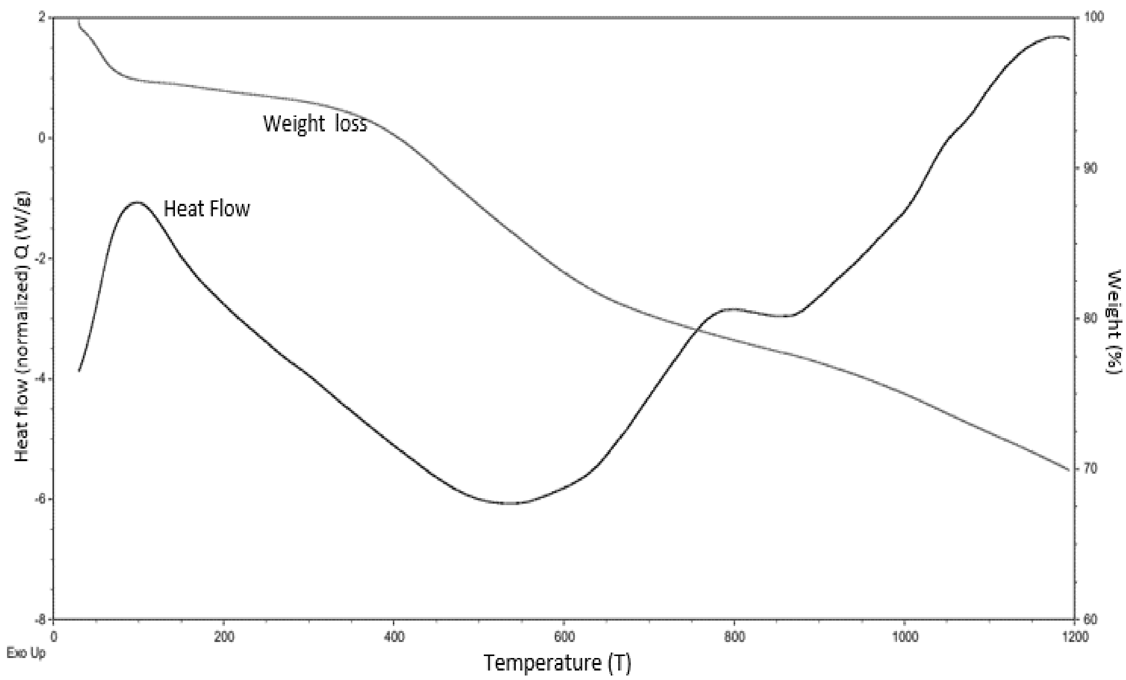


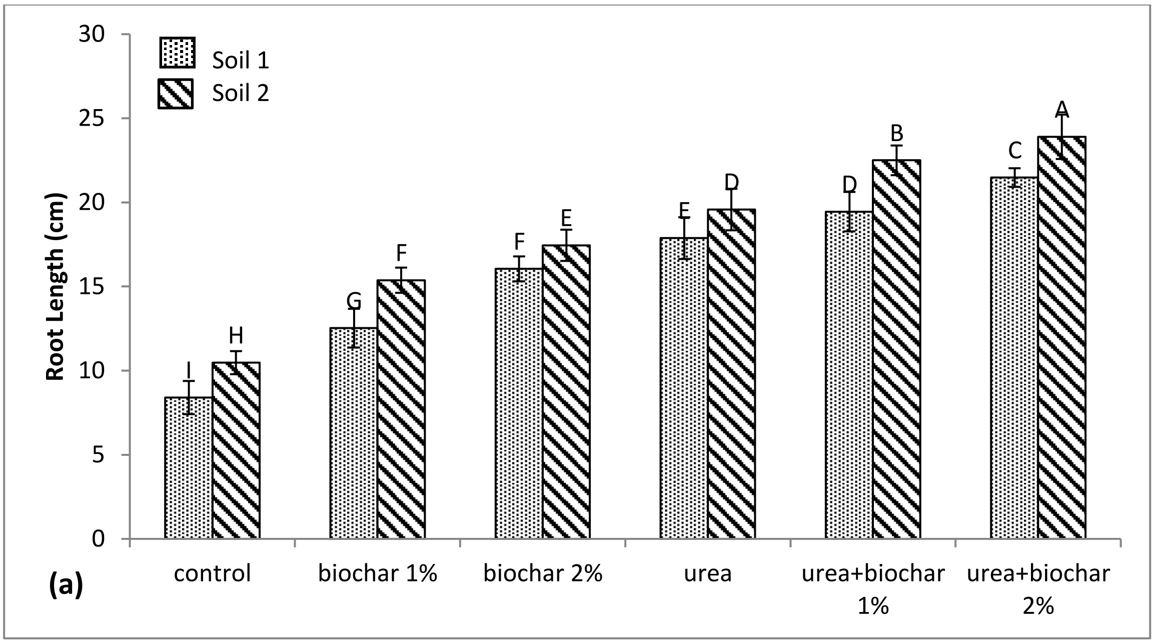
Figure 4.5: TGA of RHB prepared at 550°C

4.2 Growth parameters

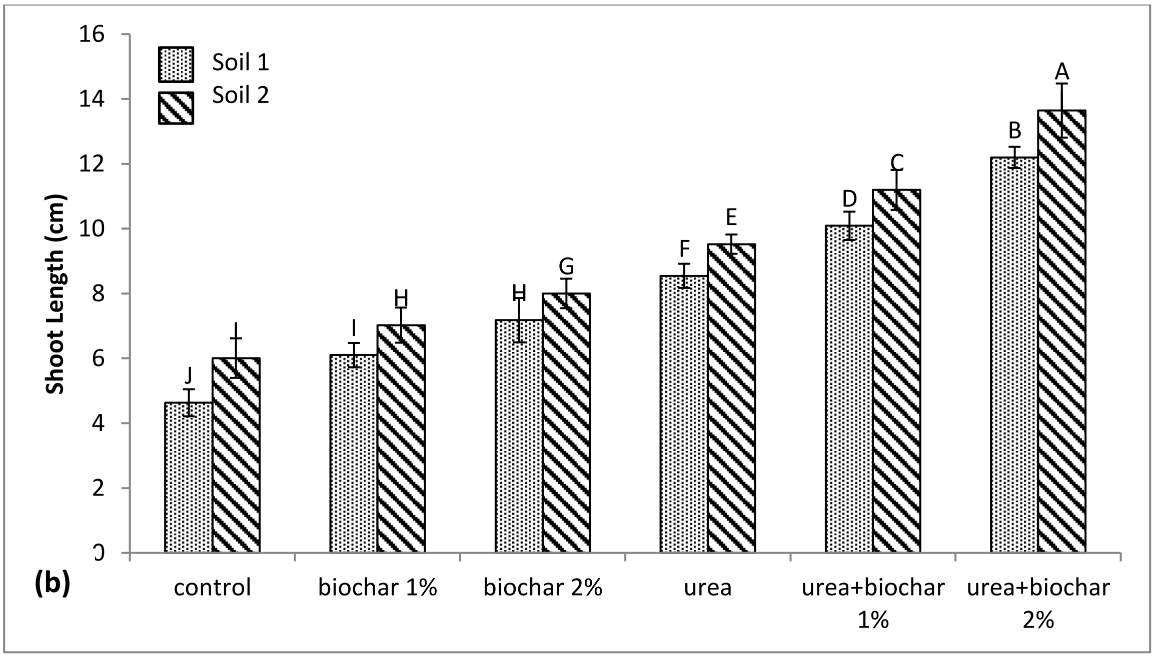
4.2.1 Plant length

The Fig. 4.6 shows the impact of co-applying urea and RHB on maize growth in various textured soils. The maximum lengths of the shoots leaves and roots were noted in soil 2 in the control group. The minimum lengths of the shoot leaf , and root were noted in soil 1. In comparison to BC 1% treatment rate, addition of BC 2% has demonstrated a rise in root shoot and leaf length in both soils.

In both soils, urea alone treatment (U) has increased the length of the roots, shoots, and leaves; however, adding urea with 2% BC has demonstrated a good response to plant length. In soil 2, the root length rose by 23% when BC 2% + U was used instead of U alone. The above-ground biomass in soil 2 showed the maximum length cm in shoot length, whereas soil 1 showed the least length with BC 2% +U. In all treatments, the largest increase in plant length was observed in soil 2 (silty clay loam). This increase can be the result of the soil's clay content, which offers more nutrients, a higher capacity to hold water, and a higher amount of soil organic matter than any other soil texture (Dou *et al.*, 2016). Urea greatly boosted maize growth response when added. Biochar on its own has not demonstrated a very strong growth response as compared to inorganic fertilizer (urea). These findings demonstrated that maize growth was maximized when urea and biochar were applied simultaneously. Coelho *et al.* (2018) have reported findings like this. This is because biochar increases the nutritional (N, P, and K) and physicochemical qualities of soil, making these nutrients more easily accessible for plant growth (Premalatha *et al.*, 2023)



Soil 1
Soil 2
Root Length (cm)
30
25
20
15
10
5
0
A
B
C
D
D
E
E
F
F
G
H
I
control
biochar 1%
biochar 2%
urea
urea+biochar 1%
urea+biochar 2%
(a)



Soil 1
Soil 2
Shoot Length (cm)
16
14
12
10
8
6
4
2
0
A
B
C
D
E
F
G
H
H
I
I
J
control
biochar 1%
biochar 2%
urea
urea+biochar 1%
urea+biochar 2%
(b)

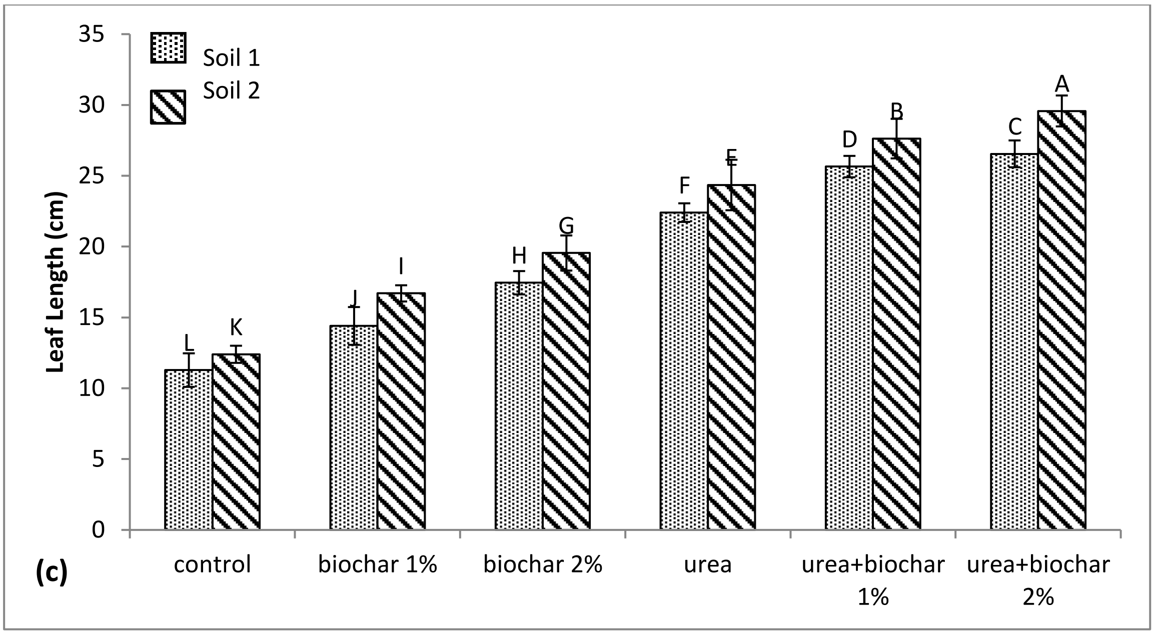


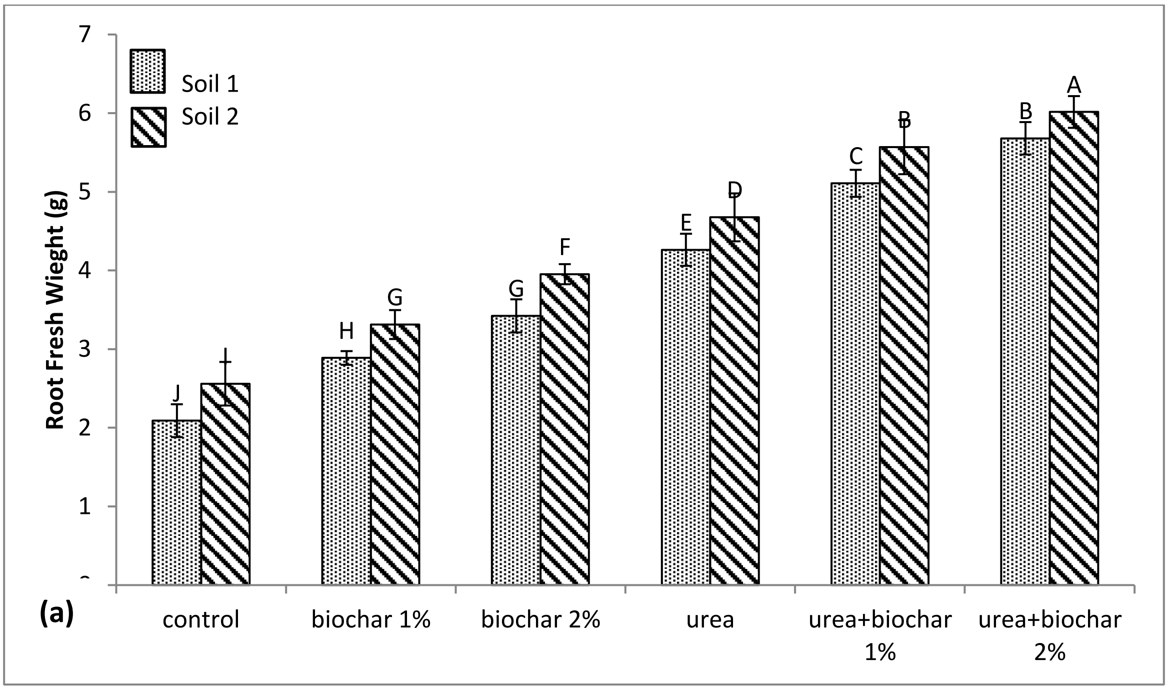
Figure 4.6: Effect of different treatments on length of **(a)** root, **(b)** shoot and **(c)** leaf. The results are mean of four replicates ± standard error (SE) bars carrying letters showed the significant difference declared by ANOVA two-way followed by Tuckey HSD test ($p<0.05$)

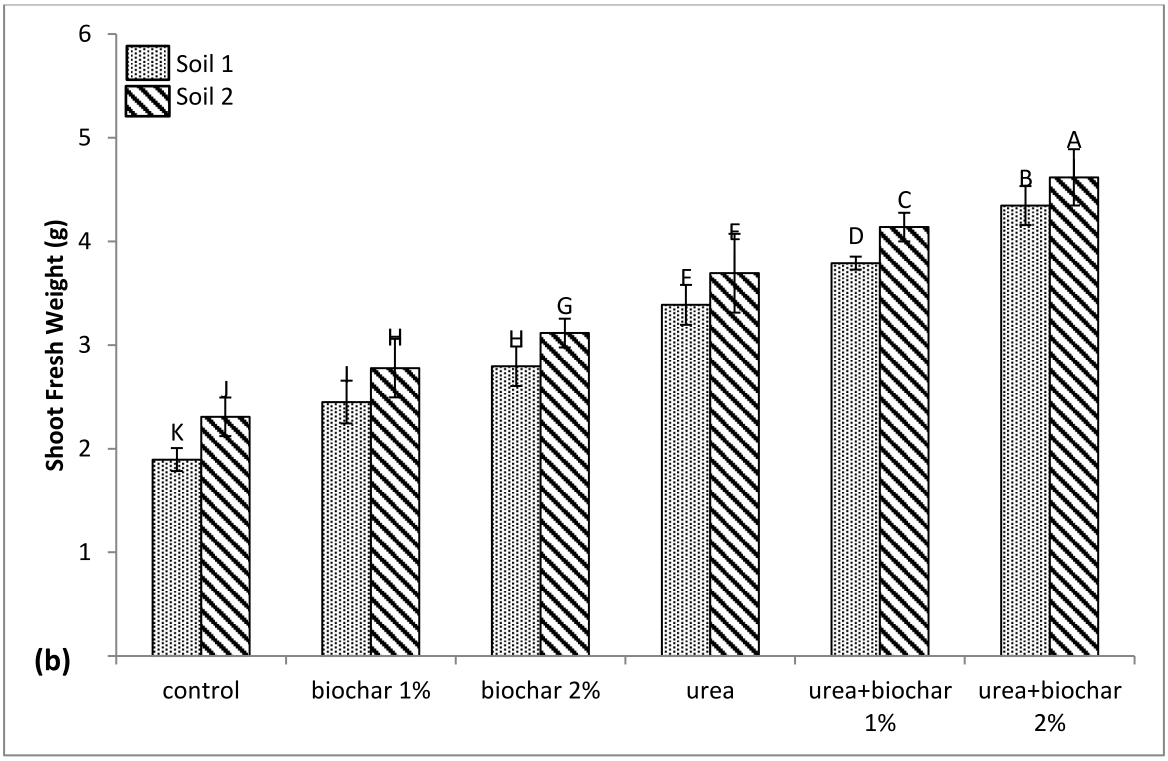
4.2.2 Plant fresh weight and dry weight

Soil 2 exhibited the highest overall fresh (Fig. 4.7) and dry (Fig. 4.8) weight biomass, accompanied by a rise in leaf fresh weight. Compared to a 1% biochar treatment, the root fresh weight in soil 1 increased by 21% with a 2% biochar application rate. When compared to treatments using only biochar, urea alone has demonstrated superior fresh weight of roots shoots and leaves in both textured soils. In soil 2, adding just urea enhanced shoot fresh weight compared to 2% biochar.

The fresh weight of the roots, shoots, and leaves increased in both soils when treated with BC 1% + U and BC 2% + U. However, soil 2 has outperformed soil 1 in terms of performance. The total dry weight of the roots, shoots, and leaves has increased when treated with a combination of urea and biochar. In soil 2, BC 2% + U led to a 21% increase in root dry weight compared to U. In a similar vein, applying BC 2% has resulted in a reduction in leaf dry weight compared to BC 2% + U. According to Tanure *et al.* (2019), increased soil nutrient retention, higher water-holding capacity, and enhanced fertility and soil structure may be responsible for the improvement in maize growth when biochar and urea are present.

The rate at which biochar is applied is also correlated with growth rate acceleration. According to Liu *et al.* (2017), ryegrass's dry weight increases as the rate of biochar application rises. Reduced growth rate is the result of reduced nutrient adsorption capability, which is caused by reduced biochar application rate (Selvarajh *et al.*, 2021). The results above are supported by a positive correlation found in the PCA biplot between plant growth indices and the co-application of biochar urea.





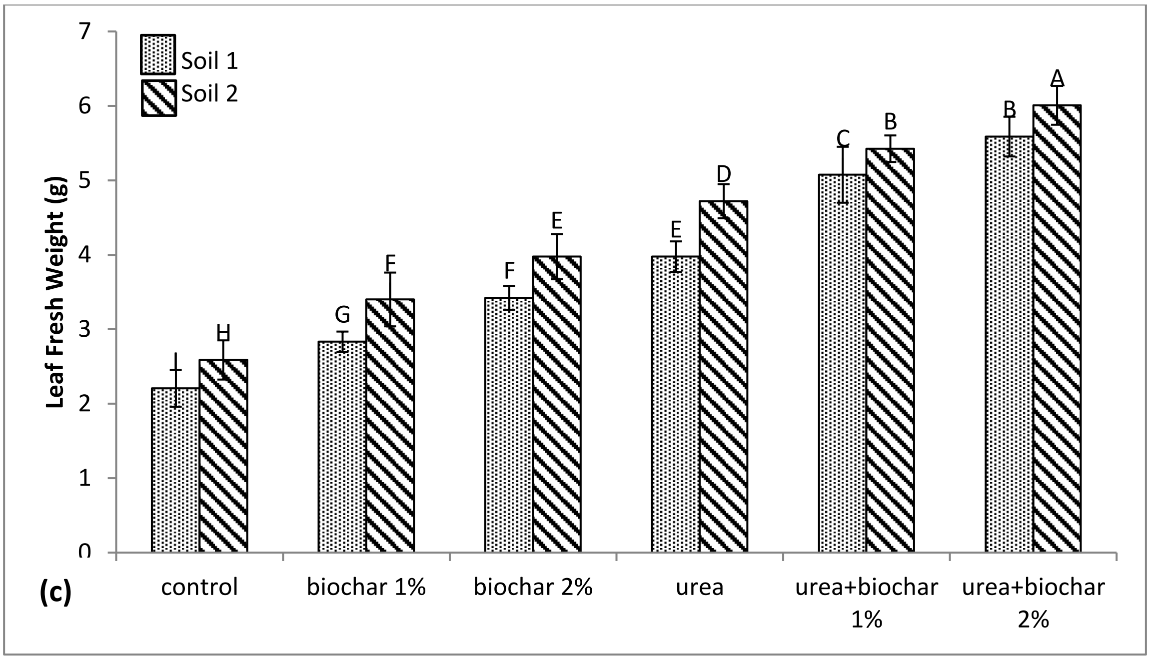
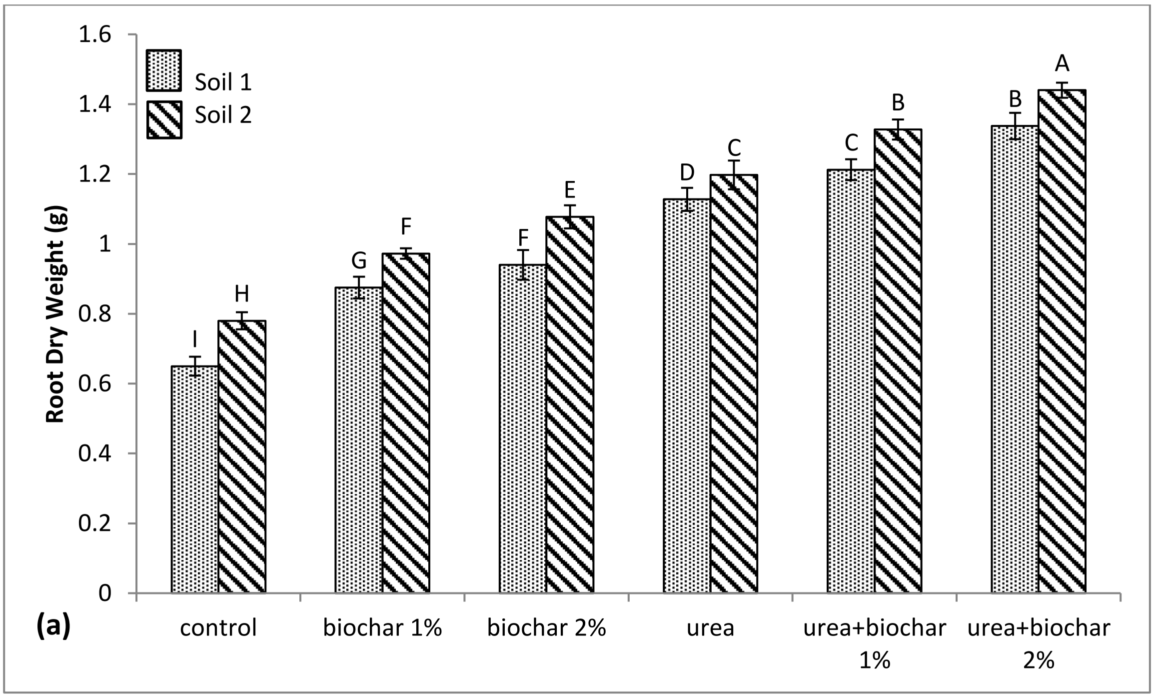


Figure 4.7: Effect of different treatments on fresh weight of **(a)** root, **(b)** shoot and **(c)** leaf. The results are mean of four replicates ± standard error (SE) bars carrying letters showed the significant difference declared by ANOVA two-way followed by Tuckey HSD test (p<0.05)



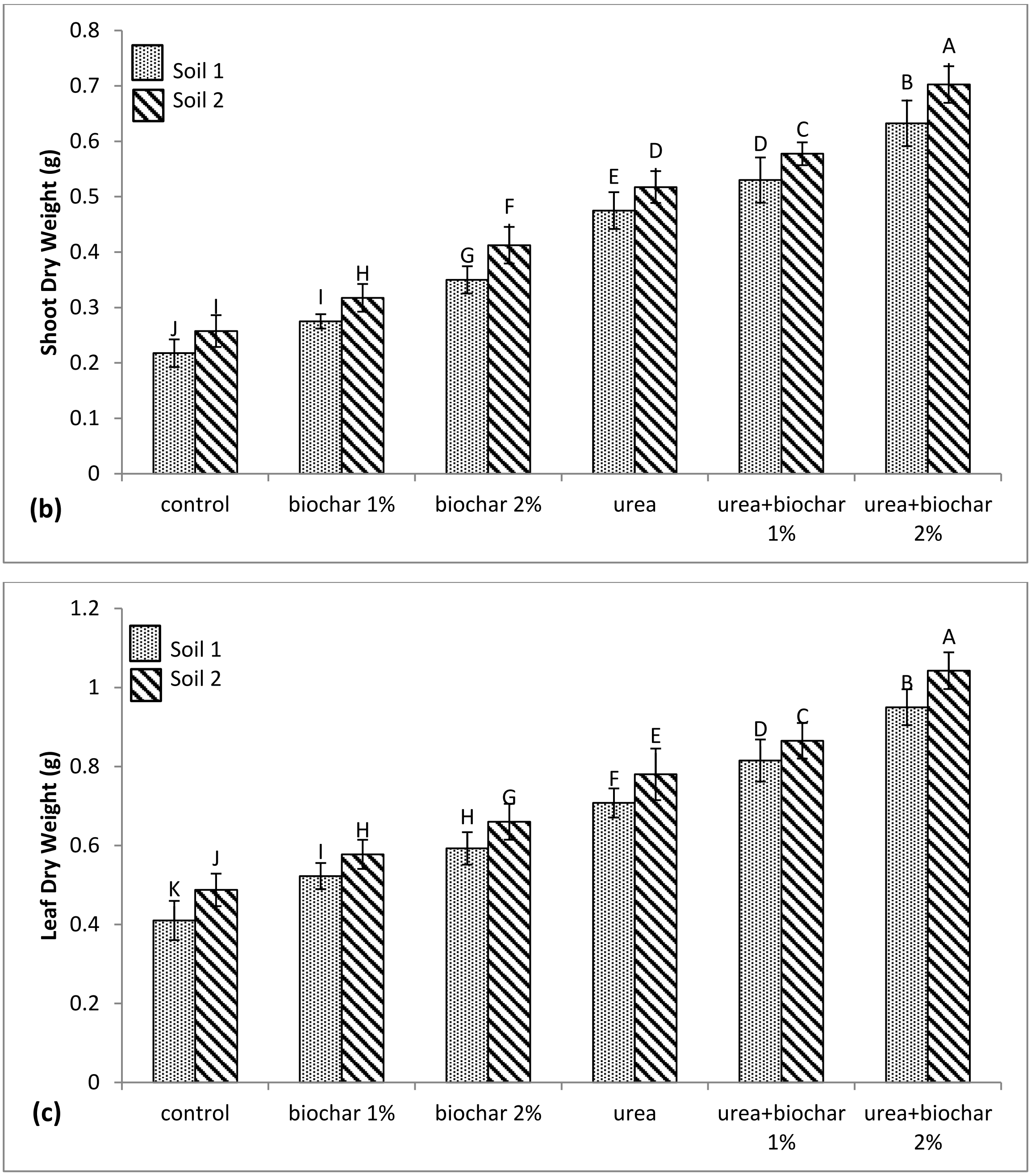


Figure 4.8: Effect of different treatments on dry weight of **(a)** root, **(b)** shoot and **(c)** leaf. The results are mean of four replicates ± standard error (SE) bars carrying letters showed the significant difference declared by ANOVA two-way followed by Tuckey HSD test ($p<0.05$)

4.3 Chlorophyll content index

Table 2 displays the combined impact of urea biochar treatments on leaf CCI in relation to soil textures. Overall, urea and biochar improve leaf CCI. When urea was added, the CCI in soil 1 increased by when compared to the control group when BC 2% was applied. Compared to soil 1, soil 2's CCI of leaves produced great results. Compared to soil 1, the CCI in soil 2 has increased by in BC 2% + U treatment. Biochar exhibited a favorable impact on the chlorophyll content index (CCI) of leaves. The primary photosynthetic pigment in charge of absorbing sunlight is chlorophyll (Croft *et al.,* 2017).

Both soil 1 (sandy loam) and soil 2 (silty clay loam) now have considerably higher chlorophyll content indices thanks to the addition of urea and rice husk biochar. Yet soil 2 had the highest degree of CCI. This is because the soil has more organic matter and clay, which enhance photosynthetic activity (Dou *et al.,* 2016). According to Lyu et al. (2016), biochar raises chlorophyll by promoting electron transport and photosystem II (PS II), which speeds up photosynthesis. The combination of biochar and urea may have increased the amount of chlorophyll, which could be attributed to increased plant development and N availability. Plants in all growth stages have higher levels of chlorophyll as a result (Ghorbani *et al.,* 2022; Liu *et al.,* 2022). Lai *et al.* (2017) and Suryanto *et al.* (2022) reported similar outcomes. High amounts of total nitrogen in leaves also contribute to the increase in the chlorophyll content index.

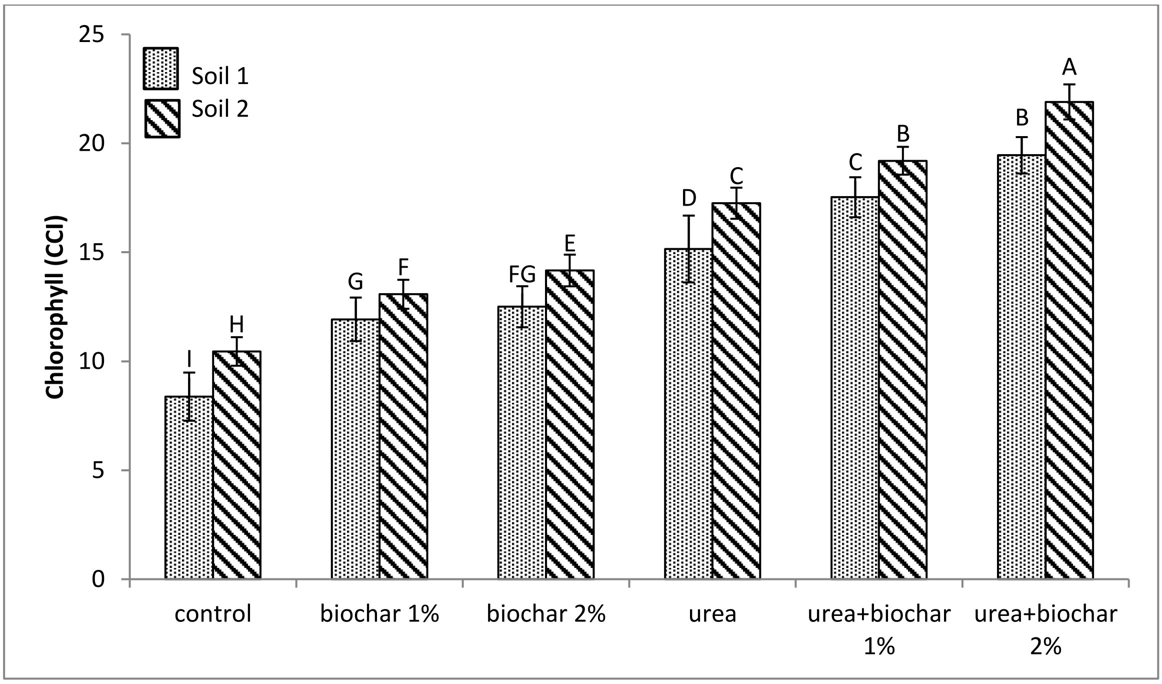


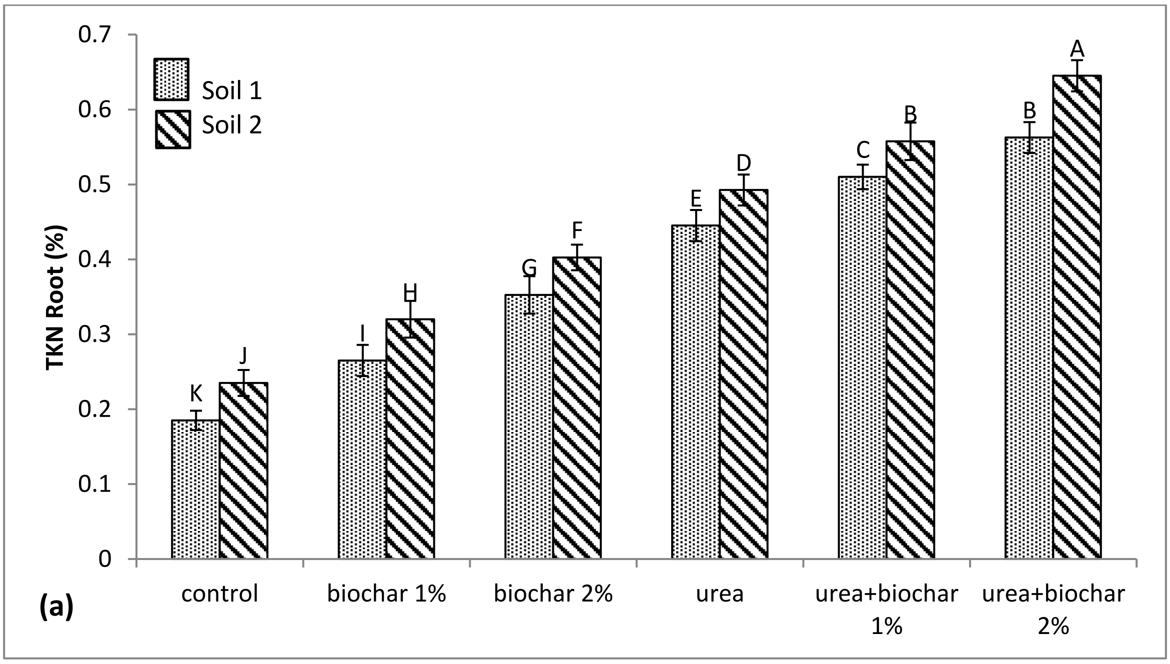
Figure 4.9: Effect of different treatments on CCI of leaf. The results are mean of four replicates ± standard error (SE) bars carrying letters showed the significant difference declared by ANOVA two-way followed by Tuckey HSD test ($p<0.05$)

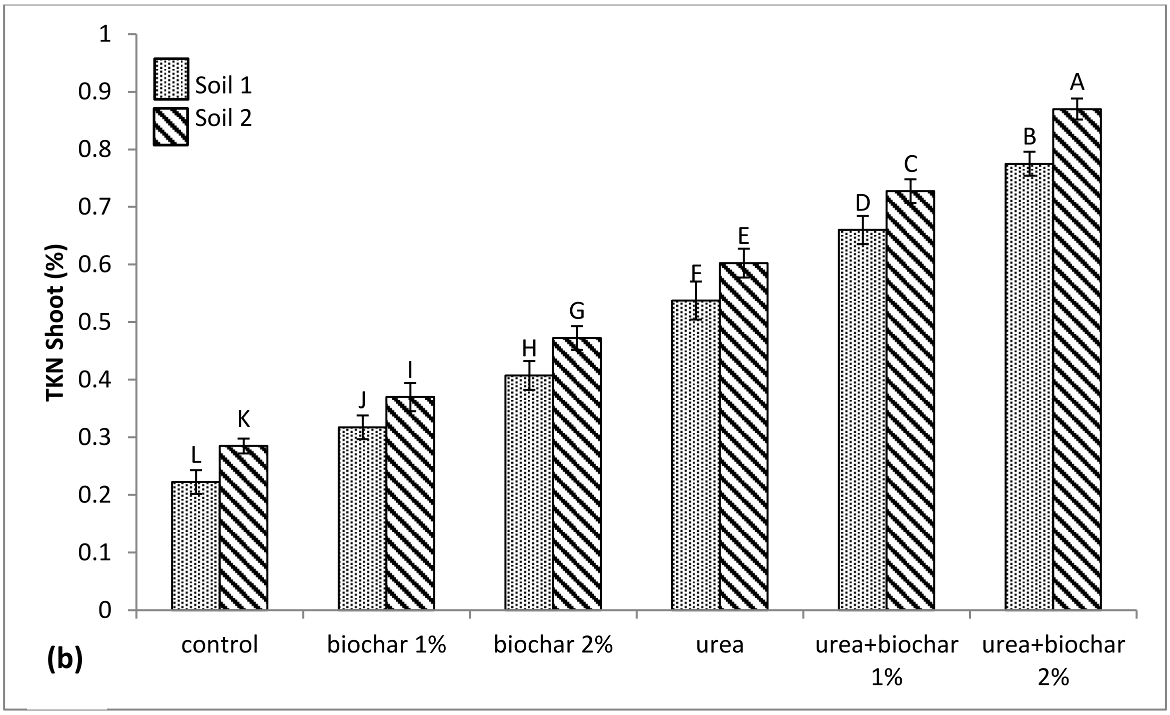
4.4 Soil and plants nutrient analysis

Figure 5 displays the total nitrogen content of soil samples, roots, shoots, and leaves in two distinct soil treatments. Soil 2 had a higher total N content than soil 1, as measured by both plants and soil. Compared to soil 2, the N content of roots in soil 1

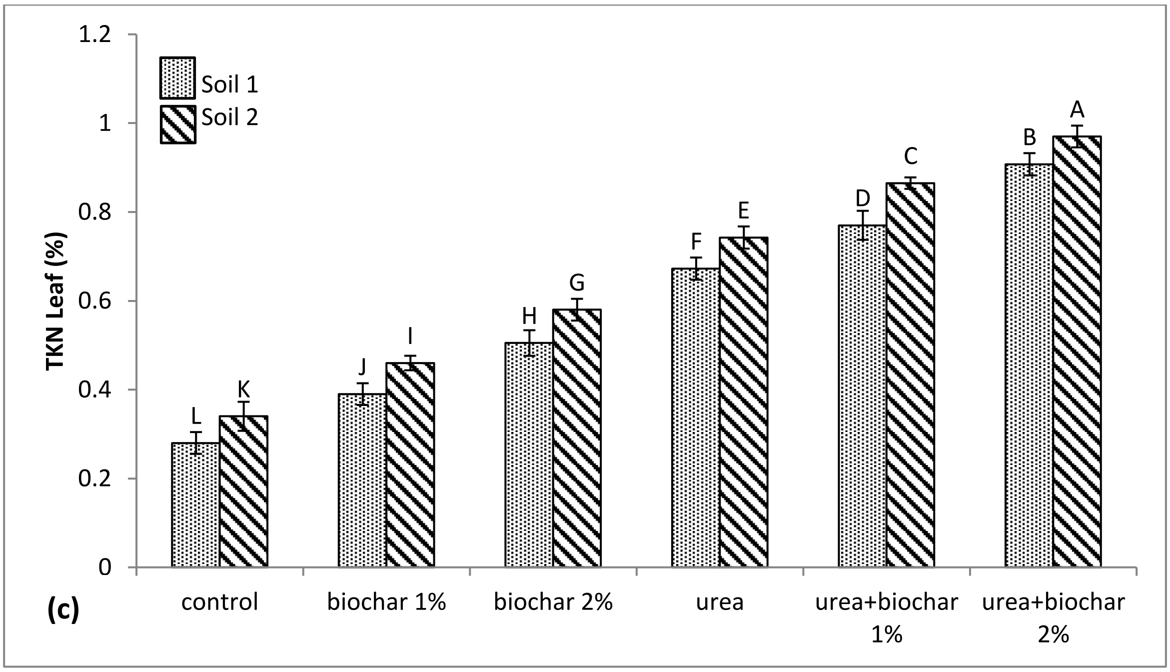
Overall, BC 2% did well for soil 2's leaf N content. In both soils, the trend for the N concentration of the soil was identical. Soil 2 showed the highest level of N (0.09%) in the control treatment.

On the other hand, the control soil, soil 1, had the lowest amount. In the plant tissues under investigation, the amount of total nitrogen buildup peaked in the leaves. In the current study, the order of total nitrogen content in maize is leaves > shoots > roots. Biochar also raises the total nitrogen concentration in leaves relative to the chlorophyll content index (Ran *et al.,* 2020). The high mineral N content in the soil, which is easily absorbed by plants, could be the cause of the high nitrogen concentration (Dawar *et al.,* 2021). These findings support the findings of Coelho *et al*. (2018), who found that applying biochar along with urea improves maize N uptake and overall nutritional status. In a similar vein, Selvarajh and Ch'ng's study from 2021 found that adding biochar along with urea improves the ability of plants to absorb nutrients from the soil. Additionally, urea-biochar treatments and plant total nitrogen showed a favorable correlation, according to the PCA analysis results.





Soil 1
Soil 2
TKN Shoot (%)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
L
K
J
I
H
G
F
E
D
C
B
A
control
biochar 1%
biochar 2%
urea
urea+biochar 1%
urea+biochar 2%
(b)



Soil 1
Soil 2
TKN Leaf (%)
1.2
1
0.8
0.6
0.4
0.2
0
L
K
J
I
H
G
F
E
D
C
B
A
control
biochar 1%
biochar 2%
urea
urea+biochar 1%
urea+biochar 2%
(c)

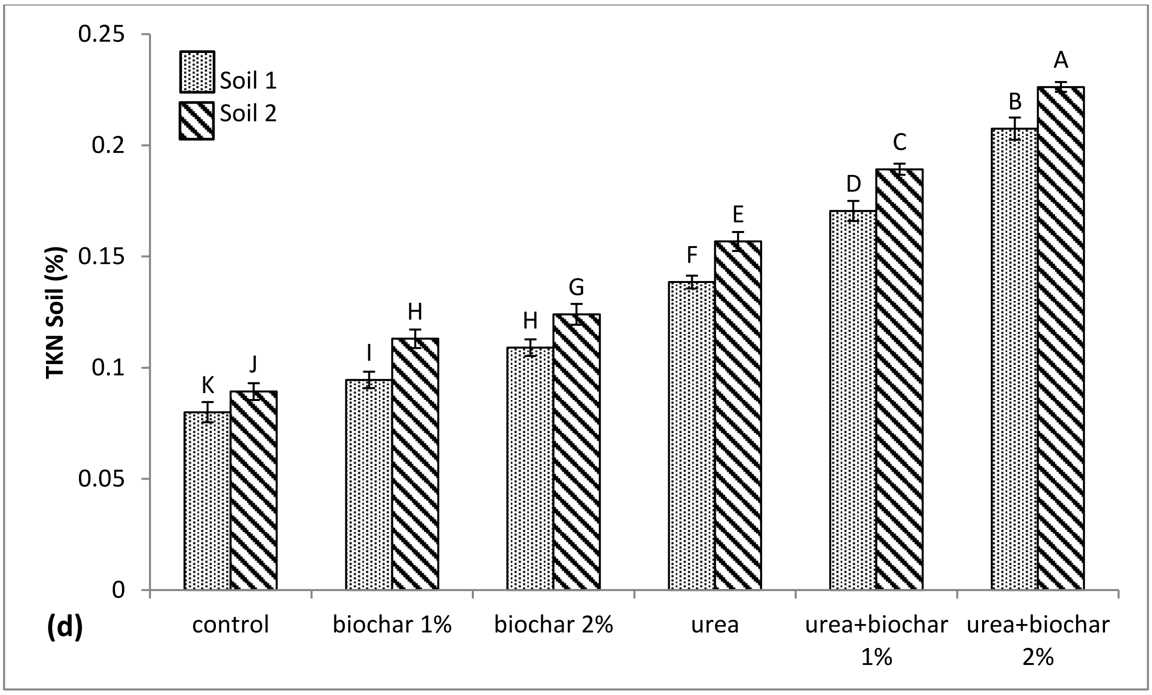


Figure 4.10: Effect of different treatments on Total Kjeldahl Nitrogen (TKN) in **(a)** root, **(b)** shoot, **(c)** leaf, and **(d)** soil. The results are mean of four replicates ± standard error (SE) bars carrying letters showed the significant difference declared by ANOVA two-way followed by Tuckey HSD test (p<0.05)

When biochar was present, the amount of soil accessible P increased (Table 1). P doubled in BC 2% compared to the control treatment in both soils. The amount of P and K in the soil was barely affected by either urea or biochar in either of the two soils. In silty clay loam soil (soil 2), the maximum soil K was noted. In BC 1 and BC 2%, the soil K concentration exhibited a beneficial reaction. The application rate of biochar resulted in a rise in K concentration in soil 1 and a increase in soil 2. In both soil textures, the concentration of soil NO_3^--N rose as the amount of biochar increased (Table 1).

The results of the physicochemical study of both soils indicate that soil 2 has higher amounts of N, P, K, and NO_3^--N than soil 1. This could be the cause of soil 2's higher nutrient concentration in the study's post-harvest nutrient analysis. Furthermore, whereas sandy soils contain big particles that are unable to bind nutrients, clay-rich soils often have a greater grip on nutrients that are available for plant absorption.

The soil's nutrient concentration has increased with the addition of charcoal and urea. This increase in nutrients in the soil can be explained by a few different methods. First off, according to Yan *et al.* (2019), biochar's high porosity and surface area aid in the growth of nitrogen-fixing bacteria in soil.

The SEM data show that the biochar utilised in this experiment has a high porous structure. Biogeochemical interfaces (BGI) could occur because of this (Yaashikaa *et al.,* 2020). Finally, the likelihood of absorbing nitrogen and ammonium is increased by the negative charge on the surface of biochar, the presence of functional groups, and its high cation exchange capacity (Ghorbani *et al.,* 2021). FTIR spectrum data amply demonstrated the abundance of negative functional groups, particularly hydroxyl groups. The application of urea combined with biochar in the current study has significantly increased the concentration of NO_3^--N and total nitrogen in the soil. The rise in NO_3^--N concentration is caused by the addition of biochar, which increases the mineral N rate (Baiga and Rao, 2017; Dey and Mavi, 2021). Additionally, when biochar decreases nitrate leaching, soil nitrate retention rises (Liu *et al.,* 2017; Egyir *et al.,* 2023). Furthermore, it has been demonstrated that biochar can reduce nitrate losses in loamy soils by improving nitrogen utilization efficiency (Liao *et al.,* 2020). Peng et al. (2021) found similar outcomes, reporting that applying biochar in addition to urea enhanced soil N retention and maize N uptake. In a different study, biochar and inorganic fertiliser were found to enhance soil total nitrogen by respectively, and to result in a nett increase in NO_3^--N (Ullah *et al.*, 2020). These findings aligned with the results of the PCA biplot, which indicated a strong positive correlation between the co-application of biochar and urea.

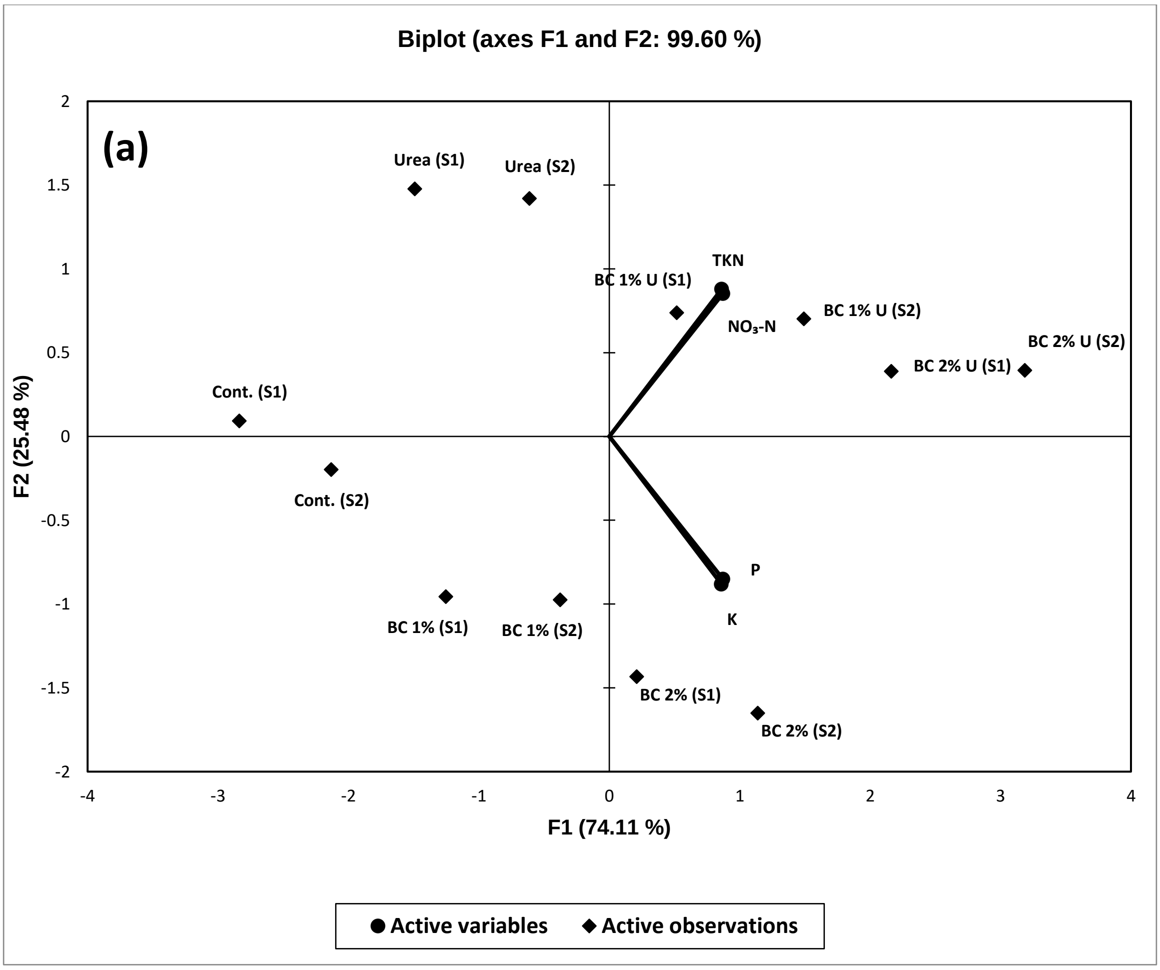
According to Sparks *et al.* (2022), clayey soils have a propensity to retain P in the soil through both non-electrostatic and electrostatic processes. Therefore, silty clay loam soil has the highest accessible P concentration. The concentration of P in soil treated with biochar has increased due to the hydroxyl group found in rice husk charcoal. Sarkhot et al. (2013) also verified that P sorption occurs on the biochar surface when hydroxyl groups are present. P fixation limits the amount of accessible P in soil. The addition of biochar to soil helps raise the amount of phosphorus that is readily available for plant uptake. This is explained by the fact that biochar's alkaline properties enhance the soil's environment, which reduces P fixation by Al and Fe. Numerous research has also demonstrated an increase in P concentration in the presence of biochar (DeLuca *et al.,* 2015; Gandahi *et al.,* 2015; Gao *et al.,* 2018; Selvarajh *et al.,* 2021). The increased potassium content in the experimental soil may have contributed to the overall increase in the concentration of potassium (K) in soil. Biochar made from rice husks could be another source of potassium.

The presence of potassium in biochar was revealed by the SEM-EDX analysis of RHB. Therefore, it may be said that incorporating biochar into soil raises the concentration of K in the soil. Oram *et al.* (2014) and Vamvuka *et al.* (2020) reported similar outcomes. On a PCA biplot, the beneficial impact of biochar on soil P and K concentration can be observed.

The result is mean of four replicates ± standard error (SE). Letters shows the significant difference declared by ANOVA two-way followed by Tuckey HSD test ($p<0.05$)

4.5 Principal component analysis

PCA was used to statistically analyze growth metrics, nitrogen concentrations in plants, and nutrient concentrations in soil samples. In response to the study parameters, biplots were created for the most effective treatment evaluation (Fig 4.11). In the plant and soil biplots, the PCA accounts for 99.48 and 99.60% of the variation, respectively. According to the findings, BC 1% + U and BC 2% + U performed exceptionally well in terms of plant and soil parameters. In plants, there was a negative correlation between the growth parameters of the roots and the growth of the leaves and shoots, but a strong correlation between the two. Potassium and phosphorus concentrations in soil showed a positive correlation with one another, but a negative correlation with total N.



Biplot (axes F1 and F2: 99.60 %)
(a)
Urea (S1)
Urea (S2)
TKN
BC 1% U (S1)
NO₃-N
BC 1% U (S2)
BC 2% U (S2)
BC 2% U (S1)
Cont. (S1)
Cont. (S2)
P
K
BC 1% (S1)
BC 1% (S2)
BC 2% (S1)
BC 2% (S2)
F2 (25.48 %)
F1 (74.11 %)
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-4
-3
-2
-1
0
1
2
3
4
Active variables
Active observations

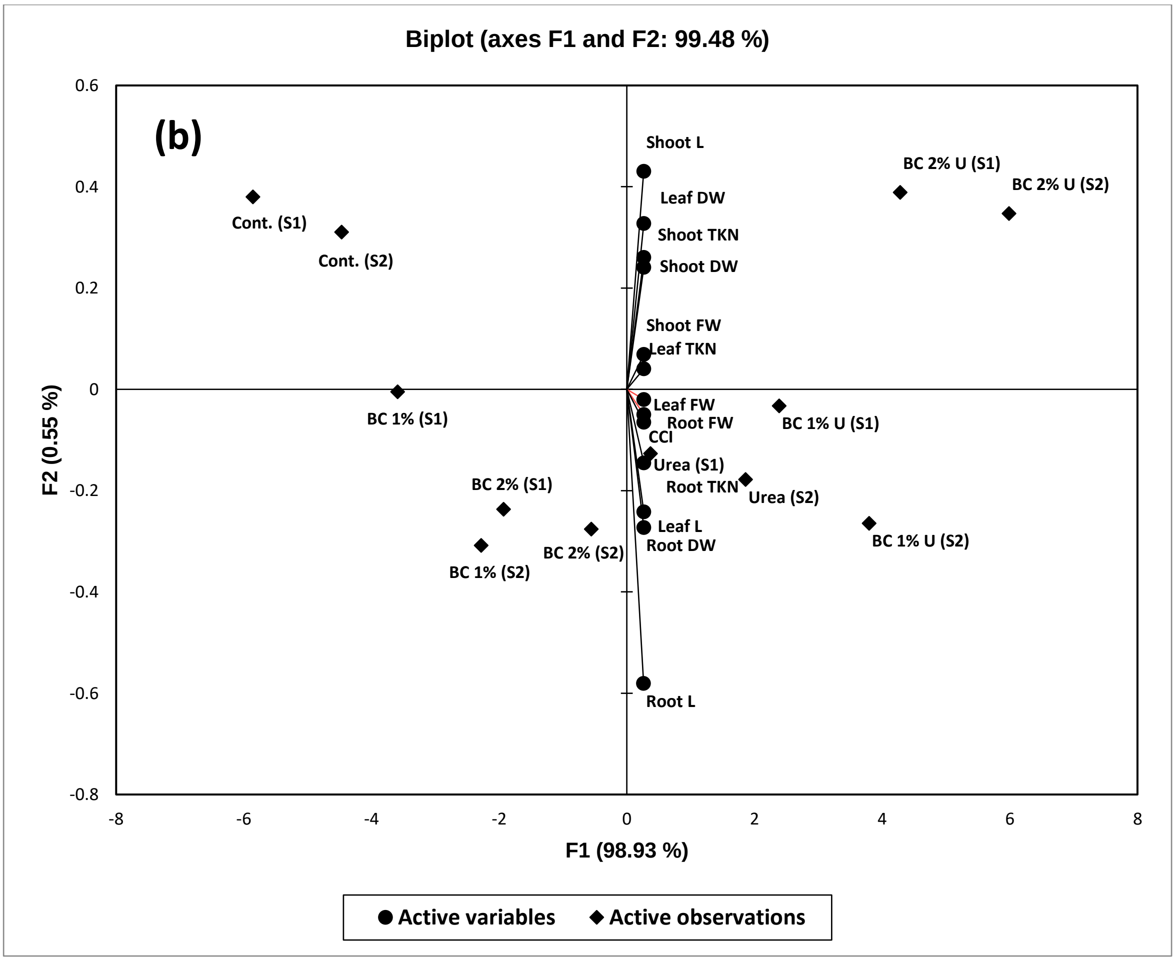


Figure 4.11: Principal Component Analysis (PCA) of plant and soil parameters. The Biplots are generated with significant contributions from the main factors of PCA (F1 and F2). Active variables are **(a)** Total Kjeldahl Nitrogen (TKN), Nitrate Nitrogen (NO_3-N), Potassium (K) and Phosphorus (P) and **(b)** parameters of plant such as Length (L), Fresh Weight (FW), Dry Weight (DW), Total Kjeldahl Nitrogen (TKN) and Chlorophyll Content Index (CCI); however, R, S and L is for root, shoot and leaf respectively. Active observations for both Soil 1 (S1) and Soil 2 (S2) are control, Biochar 1% (BC 1%), Biochar 2% (BC 2%), Urea, Biochar 1%, and Urea (BC 1% U), Biochar 2% and Urea (BC 2% U)

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study's findings suggest that applying charcoal and urea alone is insufficient to boost maize growth and improve the soil's nutritional status. The co-application of urea and biochar resulted in a notable increase in soil nutrient concentration, total nitrogen, chlorophyll content index, and maize growth. The various properties of rice husk biochar, including its pore size, functional groups, ash content, chemical composition, and thermal stability, all have a role in improving soil nutrient availability and plant growth. Compared to sandy loam, the silty clay loam soil performed better in all treatments. In conclusion, there is a great chance that applying 2% biochar along with 120 kg N/ha of urea will increase crop productivity.

6.2 Recommendations

The following recommendations are proposed after thorough research and literature review.

- Carry out long-term research (for instance, spanning several growing seasons) to determine the long-term effects of co-applying urea and biochar on soil nutrients and maize growth, considering any possible changes in soil microbial populations and nutrient cycling.
- Evaluate the dose-response relationship between various applications of biochar and urea to determine the ideal ratio that enhances availability of nutrients and maize growth while limiting impacts on the environment.
- Examine the composition and operation of the soil's microbial communities, which are essential for the cycling of nutrients and interactions between plants and microbes.

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