Accompanying effects of sewage sludge and pine needle biochar with selected organic additives on the soil and plant variables

Bushra Fatima [1], Farhana Bibi [1], Muhammad Ishtiaq Ali [1], Jeremy Woods [2], Mushtaq Ahmad [3], Muhammad Mubashir [4], Mohd Shariq Khan [5], Awais Bokhari [6, 7], Kuan Shiong Khoo [8, *]

[1] Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.

[2] Centre for Environmental Policy, Imperial College London, United Kingdom.

[3] Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

[4] Department of Petroleum Engineering, School of Engineering, Asia Pacific University of Technology and Innovation, 57000 Kuala Lumpur, Malaysia.

[5] Department of Chemical Engineering, College of Engineering, Dhofar University, Salalah, Sultanate of Oman.

[6] Sustainable Process Integration Laboratory, SPIL, NETME Centre, Faculty of Mechanical Engineering, Brno University of Technology, VUT Brno, Technická 2896/2, 616 00, Brno, Czech Republic.

[7] Department of Chemical Engineering, COMSATS University Islamabad, Lahore, Pakistan.

[8] Faculty of Applied Sciences, UCSI University, UCSI Heights, 56000 Cheras, Kuala Lumpur, Malaysia.

*** Corresponding author:**

Dr. Kuan Shiong Khoo, Ph. D

Faculty of Applied Sciences, UCSI University, UCSI Heights, 56000 Cheras, Kuala Lumpur, Malaysia.

Email: kuanshiong.khoo@hotmail.com / kuanshiong.khoo@ucsiuniversity.edu.my

Abstract

Effects of synthetic fertilizer and nutrient leaching are serious problems impacting soil function and its fertility. Mitigation of nutrient leaching and use of chemical fertilizer is crucial if agricultural land is to meet sustainability and climate challenges. Biochar produced from agricultural bio-waste and municipal solid waste has been used for crop production and when applied in combination with organic nutrients may support mitigation of nutrient loss and adverse effects of chemical fertilizers. Different types of biochar and their application for soil enhancement have been observed, pine needle and sewage sludge derived low-temperature biochar along with compost, organic fertilizer in the form of manure and microalgal biomass may interact with soil chemistry and plant growth to impact nutrient loss and compensate the hazardous effect of chemical fertilizer, but it has not been investigated yet. This present study elaborates application of sewage sludge and pine needle biochar produced at 400^{o}C in an application rate of 5% w/w and 10 t h^{-1} in combination with compost, manure and microalgal biomasses of *Closteriopsis acicularis* (BM1) and *Tetradesmus nygaardi* (BM2) on the growth of Chickpea (*Cicer arietinum*) and Fenugreek (*Trigonella foenum-graecum*) crop assessed in a pot experiment over a two crop (Chickpea - Fenugreek) cycle in Pakistan. Results depict that the pine needle biochar with additives has increased plant height by 104.1±2.76 cm and fresh biomass by 49.9±1.02g, buffered the soil pH to 6.5 for optimum growth of crops and enhance carbon retention by 36%. This study highlights the valorization of sewage sludge and pine needle into biochar and the effect of biochar augmentation, its impact on soil nutrients and plant biomass enhancement. The greener approach also mitigates and helps in the sustainable management of solid wastes.

Keywords: Biochar; Microalgae; Chemical and structural characterization; Crop rotation; Soil; Organic additives

Table of abbreviations:

Sr. No.	Abbreviation	Definition
1	BPN_{400}	Pine needle biochar produced at 400^{o}C
2	BSS_{400}	Sewage sludge biochar produced at 400^{o}C
3	W2	Biochar weight in g
4	W1	Biomass weight in g
5	$C_{biomass}$	Carbon content of biomass
6	$C_{biochar}$	Carbon content of biochar
7	BM1	Biomass of *Closteriopsis acicularis*
8	BM2	Biomass of *Tetradesmus nygaardi*
9	TDS	Total dissolve solids
10	EC	Electrical conductivity
11	HHV	High heating value
12	SL	Shoot length
13	RL	Root length
14	FL	Full length
15	FW	Fresh weight
16	WHC	Water holding capacity

1. Introduction

The continuous use of synthetic fertilizer and cultivation practices results in soil nutrient loss and makes it unfertile (Suhag, 2016)(Turan *et al*., 2019)(Wang *et al*., 2018). Unfertile soil can be compensated for by applying fertilizer however the hazardous effects of chemical and synthetic fertilizer are long-lasting as it decreases the organic content and is not eco-friendly (Li *et al.*, 2022)(Turan, 2022). Synthetic fertilizer contains a high amount of nitrogen which damages green leaves to turn yellow or sometime pale-brownish color causing permanent wilting and death of plants thus reducing the overall crop production (Kolmanic *et al.*, 2022)(Bilen and Turan, 2022). Nitrogen and phosphate-based fertilizer cause groundwater contamination, pH disturbance of soil, and increased phosphorus content in the soil thus highly resulting in phosphorous contaminated agricultural runoff. There are various synthetic fertilizers being used that include urea, ammonium sulphate, di-ammonium phosphate, ammonium chloride, calcium ammonium nitrate and anhydrous ammonia (Hossain *et al.*, 2022)(Sonmez *et al* 2016)(Sönmez *et al.*, 2018). They are reported to contain lead, mercury and dioxin which spread in the atmosphere and accumulate in water and soil thus disturbing the whole ecosystem (Abdulsalam *et al.*, 2022).

To avoid losses of soil function, infertility, and crop diseases, there is a need to opt for organic farming as it is more eco-friendly in creating a sustainable and economical environment. Soil fertility problems can be addressed by using compositing, green manure, crop rotation, inter-cropping, and vermi-compositing (Crystal-Ornelas *et al*, 2021). With the increase in the human population, opting for these strategies can bring sustainability to the food system by increasing the crop yield up to the required levels.

To attain socio-economic sustainability, underdeveloped countries need proper guidelines and a model of production and consumption that is covered by the term “circular economy” (Lampridi *et al,* 2019). Natural resources can be preserved and increase dependency on planetary resources in a way that an eco-friendly environment can be created along with socioeconomic profits, long-span value construction, and waste management through end-use of products (Ashraf *et al*, 2020).

Availability of biomass is unlimited with an annual rate worldwide which is estimated at 146 billion metric t a year and comparatively in Pakistan, specifically in its rural region, the capacity of biomass reserves like manure, sludge, and agricultural waste is approximately 12 cubic metric tons energy production that is sufficient for 0.028 billion rural population (Turan, 2021) (Tareen *et al*., 2019). Availability of required land is problematic to account for higher feedstock production. However, in Pakistan, there are abundant lands to grow biomass and use its waste for energy and other byproducts production amongest them is black charcoal material known as biochar (Munir et al. 2021; Mumtaz et al. 2019; Selvarajoo et al., 2022). The physical and chemical nature of feedstock significantly affects the properties of biochar (Li *et al*., 2016; Mubashir et al. 2015). There exist several categories of waste residue that are potential feedstocks for biochar production through pyrolysis.

Sewage sludge is generally left unattended as residual waste coming from wastewater treatment streams as it needs expansive discarding strategies, better to be considered as a sustainable energy resource (Khoo *et al*., 2021). It would be in acquiescence with the European Union environmental plan to implement a circular economy supported by waste to energy production. Disposal strategies are badly needed for environmental safety because otherwise, they may result in the accumulation of hazardous pollutants aggregation, horizontal gene transfer and release of carcinogenic heavy metals (Shiels *et al*., 2019).

To address greenhouse gas emissions, agricultural biomass is potentially the best option to be used as feedstock for its carbon storage capacity and bulk nutrient release when applied as a soil conditioner (Khanmohammadi *et al*., 2015; Rajendran *et al*., 2022). In Himalayan timberlands, waste like pine needles is abundantly found with a production rate of 6.3 tonnes per year in Asia (Kala and Subbarao, 2018). The decay process of pine needles is very slow ,during decomposition most of times when they dries out openly in forest results in wildfires on large scales which are major contributors to environmental pollution. Sidewise, soil erosion, stunted growth of crops and soil pH alteration are major demerits of forest fires caused by pine needles. Loss of soil water retention, fertile soil and seed germination are also linked with huge fires caused by solid and heavy sheets of pine needles (Brantley *et al*., 2015).

Biochar origin has been reported over 2000 years ago in the Amazonian Forest of Brazil and since then it is in use for soil nutrition and water retention (Turan, 2022) (Gonzaga *et al*., 2019) (Sizmur *et al*., 2016). It is a black charcoal-like substance that can be produced from pyrolysis of organic waste including agricultural, domestic and municipal solid waste with a temperature range from 300 to 1000 ^{o}C and above utilizing natural flames and innovative pyrolysis techniques. Pyrolysis is a process that results in designed biochar production which is specifically designed for its application (Granatstein *et al*., 2009) (Turan, 2021). Biochar can sequester carbon and neutralize greenhouse gas emissions (Selvarajoo *et al*., 2022)(Turan, 2020). The generation of side products like bio-oils is beneficial for providing socioeconomic renewable energy resources.

Certain additives have been added along with biochar to supplement nutritional sources for soil conditioning and plant growth (Irshad *et al*., 2022)(Turan, 2019). Biochar can act as a sink to store nutrients coming from the source which are organic fertilizer, compost, and microalgae in the present research. The additives to be used for crop production are termed ‘slow-release’ fertilizers (Colla and Rouphael, 2020). The degraded, organic, and stable material produced by the microbiological breakdown of organic substances under proper oxygen supply is termed compost

(Di *et al*., 2019; Pandit *et al*., 2019). These noxious sources can be converted into valuable and stabilized forms like compost that can enhance crop yield (Rasa *et al*., 2018). Large-scale production of crops and improvement in healthy agricultural practices can be achieved by opting for compost application to soil's health profile depends upon organic contents for being fertile by sustaining all nutrients in it (Toan *et al*., 2021). Microalgal biomass has been reported for soil conditioning and promoting plant growth-promoting hormones, antibacterial composites, plant growth-enhancing metabolites (Bibi *et al*., 2021; Giorcelli *et al*., 2019). The adaptable nature of unicellular microalgae, being photosynthetic, heterotrophic in origin, feasibility to adopt in waste watercourses along with yielding significant byproducts makes it the focus of research in the agricultural sector (Alvarez *et al*., 2021; Chew *et al*., 2021).

The objective of this research article is to estimate the impact of sewage sludge and pine needle-based biochar produced by thermal decomposition at 400°C, with the combination of organic additives that comprised of compost, manure fertilizer and microalgal biomass on chickpea-fenugreek 10 months crop rotation experiment. Plant height, fresh weight and soil parameters such as pH, total dissolved solids, water holding capacity and nutrients were estimated throughout the experiment. The novelty of this work lies in avoiding inorganic chemical fertilizer, co-application of two different low temperature produced biochar and use of microalgal biomass in addition to other additives. Soil nutrients and plant biomass production vary with soil texture, crop type, nature of feedstock selected for biochar production and biochar application rate. To best of authors knowledge, no studies have reported the combined impacts of pine needle-derived and sewage sludge-derived biochar along with compost, organic fertilizer and microalgal biomass on soil-plant system.

2. Materials and methods

2.1 Sample collection

2.1.1 Collection of feedstock, soil and additives.

The pine needles and sewage sludge as feedstock for biochar production were collected from matured pine trees and a nearby wastewater treatment plant at Quaid-I-Azam University located at 33°44’50” N 73°08’20” E Islamabad, Pakistan. It receives 94.76 millimeters (3.73 inches) of precipitation and has 125.05 rainy days (34.26% of the time) annually. The soil type of this area is majorly clay loam. The microalgal biomass of *Closteriopsis acicularis* (BM1 accession no. MT858355) and *Tetradesmus nygaardi* (BM2 accession no. MT858750) were collected from the already established microalgal bulk production unit in Bold Basal media with pH 7, temperature 25 to 30°C and compost made by animal manure and kitchen waste in composting reactor installed at a greenhouse station at Environmental microbiology Lab, Quaid-I-Azam University, Islamabad. Organic fertilizer with poultry and cow manure source of origin, produced by mixing cow and poultry manure with lukewarm water in a jar and experimental clay soil sample was collected using a sharp spade from nearby farmland in the residential area of the university.

2.1.2 Production of biochar

Biochar was prepared from sewage sludge and pine needles by pyrolysis in a biochar Retort kiln gasifier at 400°C for 1 hour shown in **Figure 1**. Biochar produced by this gasifier was designated as sewage sludge biochar or BSS_{400} and pine needle biochar or BPN_{400}.

The biochar was ground and passed through a sieve to save samples with particle size less than 2 mm for further use. Biochar yield was calculated by **Equation 1**:

$$\text{Biochar yield, BY (\%)} = \frac{W2}{W1} \times 100\% \qquad \text{Eqn (1)}$$

W1 is dry biomass weight in grams and W2 is biochar weight in grams.

Carbon retention (%) of biomass to biochar production is calculated by following formula in **Equation 2**:

$$\text{Carbon retention (\%)} = \frac{C_{biochar}}{C_{biomass}} \times BY \qquad \text{Eqn (2)}$$

$C_{biochar}$ is carbon content in biochar, $C_{biomass}$ is carbon content in biomass used and BY is biochar yield.

2.2 Characterization of biochar, additives and soil samples.

The biochar samples were characterized through proximate and ultimate analysis. The pH and electrical conductivity were determined by mixing the samples with distilled water at a ratio of 1:5. The proximate analysis was carried out using a thermogravimetric analyzer (TGA analyzer prepASH229). For heavy metals and micro-macro nutrients in biochar and crop rotation pot soil, samples were digested by aqua regia and hyper-chloric acid and evaluated by atomic absorption spectroscopy (AA240FS Fast Sequential Atomic Absorption Spectrophotometer). XRD imaging for aromaticity of biochar has been detected using X'Pert^3 MRD. Thermogravimetric analysis was done to observe the thermal stability of the biochar in N_2 with a thermogravimetric analyzer (TGA analyzer prepASH229).

The nitrogen, phosphorous and potassium content of organic fertilizer, compost and microalgal biomass was measured by using an agricultural test kit (HI-3896). Total dissolved solids and electrical conductivity of soil were estimated using gravimetric analysis. Water holding capacity of soil samples was calculated using the following formula

Equation 3:

$$\text{Water holding capacity (WHC)} = Y\text{-}Z/\text{initial soil weight} \times 100 \qquad \text{(Eqn) 3}$$

Y is the quantity of water in ml, Z is the weight of collected water and (Y-Z) is the amount of water retained in the soil.

Soil texture was determined using the Jar method and percentages of particles were calculated individually through United States Department of Agriculture (USDA). Soil texture triangle was used to determine soil type as shown in **Figure 2**.

Individual layer percentage is calculated as follow:

- % Sand = Sand layer/ overall height × 100 (Eqn 4)
- % Silt = Silt layer/ overall height × 100 (Eqn 5)
- % Clay = Clay layer/ overall height × 100 (Eqn 6)

The elemental composition of the biochar and additives were determined with a Micro Elemental Analyzer UNICUBE. BET surface area and pore size analysis were conducted with TriStar 3000 V6.07 A.

2.3 Greenhouse experiment

For agricultural purposes, the biochar was applied at a rate of 50g/kg to soil in pots placed at a greenhouse work station situated 33°44’50” N 73°08’20” E in Islamabad, Pakistan. Two leguminous crops were grown in a Chickpea-Fenugreek rotation manner from November 2019 to August 2020 to determine the long-term impact on soil texture. Chickpea (*Desi channa*) and fenugreek (*Kasuri meethi*) crops are leguminous plants, major pulse crops and protein-rich sources of Pakistan, they are cultivated at about 2.2 million ha. These crops need an optimized dose of fertilizers and nutrients for optimum growth. Chemical fertilizers have hazardous impacts on fertile land affecting the production rate of these major crops. Microalgal biomass was selected in this study as a source of nutrients for soil due to its high carbohydrate content. A study has been designed to avoid the impact of synthetic fertilizer using biochar in combination with certain additives. Garden soil was taken and mechanically homogenized using a hammer. For the pot experiment, organic fertilizer, compost and microalgal biomass were collected and prepared by grinding them into fine powder form. Microalgal biomass was selected due to its abundant availability and being a source of nitrogen, carbon and potassium, plant growth-promoting hormone, tested in combination with biochar to soil to estimate its impact on plant growth. A novel combination of sewage sludge and pine needles biochar along with organic fertilizer, compost and microalgae were organized in a specific ratio and mixed with soil in replicates. A total of 13 treatments of soil including 1 control (without amendments) were run in triplicates as presented in **Table 1**

Chickpea seeds were soaked in a hydrogen peroxide solution (3% v/v) for 20 minutes. The seeds were sequentially washed with 3% ethanol and deionized water before drying in an oven at 60°C for 8 h. In each pot, 4 seeds were sown 3 cm below the soil surface. The pots were placed in the greenhouse workstation under natural conditions. Weather conditions were monitored during the f6 months. The smaller and weak plants were removed from pots to allow the optimized growth of healthy ones for chickpea plants. Soil samples were collected at a depth of 5 cm to determine EC, pH, TDS, micro-nutrient, and macronutrients. Heavy metals estimation were performed upon harvest. Chickpeas were harvested at the end of the season. The fenugreek was planted in the same manner, except for a period of 3 months. During the greenhouse experiment, the height and weight of fresh biomass of both crops were measured after every 5 days.

2.4 Statistical analysis

The effect of biochar and additives on plant parameters of two crops was evaluated by using two-way ANOVA. Data were collected and subjected to statistics by using R version 4.1.3 and compared with the least significant difference (LSD) at a value of 0.05.

3. Results and discussions

3.1 Characterization of biochar

The physicochemical characterization of BSS_{400} and BPN_{400} has shown significant differences because they were affected by the nature of feedstocks used in biochar production as shown in **Table 2**. The ash content of BSS_{400} was recorded at 60% which is slightly higher because of less weight loss observed in TGA proximate analysis as compared to BPN_{400} which is 9%. Woody biomass is reported to contain low ash content and low moisture content while non-woody biomass like animal waste and industrial solid waste has high moisture content and high ash content

(Tomczyk *et al*., 2020). The ash content of sewage sludge biochar is higher because it contains higher inorganic compounds. Higher ash content in manure, poultry and industrial waste is due to the presence of silica, sewage sludge-based biochar is categorized by its higher ash content range from 64.2 to 79.5 %. (Zielinska and Oleszczuk, 2015) Sewage sludge biochar is alkaline in nature and pine needle is slightly acidic. The pH of biochar is usually alkaline with few exceptions and ranges from 7.0 to 10.2 (Inyang *et al*., 2010). Biochar shows differences in pH depending upon the nature of feedstock used. For wood-based biochar has an average pH of 2 units lower than other feedstocks-based biochar produced at the same temperature. (Tag *et al*., 2016). The pH value of biochar depends directly on carbonates and inorganic alkalis formation during pyrolysis (Ding, 2014). Sewage sludge biochar has carbonates and alkali which are responsible for alkaline pH (Yuan *et al*., 2011). Higher ash content and increased formation of oxygen functional groups are related to higher pH (Ronsse *et al*., 2013). The carbon content percentage of BPN_{400} is higher than BSS_{400} which is recorded to be 67.75% as compared to 15.97%, because of higher lignin content in pine needle feedstock which determines the carbon content of biochar (Shariff *et al*., 2016). Biochar derived from pine needles contains a higher percentage of carbon, has a longer life span and is more stable in the soil as compared to sewage sludge derived biochar due to the lignocellulosic composition of pine needle feedstock carbonization, carbon content and ash content of biochar is co-related with a higher lignin content of feedstock chosen (Sohi *et al*., 2010) Nitrogen values are recorded to me almost same in both samples of biochar. The C/N ratio indicates that BPN_{400} has a higher value of 26.7:1 as compared to BSS_{400} which is 6.8:1. Mineral elements concentration in biochar varies with the nature of feedstock and pyrolysis temperature (De la rosa *et al*., 2016) The surface area and pore size of BSS_{400} and BPN_{400} are 1.43 M^2g, 10.61 nm and 429.99 M^2g, 2.16 nm, respectively, shows that BPN has a higher surface area and lower pore size (Raj *et al*., 2021). Biochar surface area and porosity increased at higher pyrolytic temperatures (Bonelli *et al*., 2012). Development of porosity is related to organic matter decomposition in the substrate (Katyal *et al*., 2003). Biochar produced at 400°C is reported to have a high surface area and porosity responsible for significant contaminant sorption. (Uchimiya *et al*., 2011) The heavy metals including Mg, Ca, Cu, Cr, Mn, Ni, values observed by atomic adsorption spectroscopy have shown a higher concentration in BSS_{400} mg/L as shown in **Table 2**. Heavy metal content is higher in sewage sludge biochar because of its origin in a wastewater treatment plant that allows the mixing of several waste lines making it less organic (Liu *et al*., 2014). Biochar-derived from different feedstocks have abundant mineral composition like sodium, calcium and magnesium and iron (Jha *et al*., 2010). Electrical conductivity is higher in BSS_{400} depicts the salinity in the substance, pH of sewage sludge biochar is alkaline showing co-relation with its high salinity and ash content. It can be observed that pine needle biochar is more carbonaceous, less alkaline, high BET area and have less heavy metal toxicity as found in the literature (Askeland *et al*., 2019). The yield of biochar is 52.4 % in the case of BSS_{400} and 38.7% for $BPN_{400.}$

3.2 Carbon sequestration potential

Biomass-derived biochar has the ability to sequester carbon in soil owing to its organic properties and thereby greenhouse gas emissions can be reduced. The carbon retention percentage according to **Equation 2** for BPN_{400} is observed to be higher at 56.38% as compared to BSS_{400} which is 26.37%. Pine needle based biochar is observed to be able to sequester 67.75% of carbon content of pine needle biomass feedstock to soil by avoiding its open burning. This carbon sequestration ability significantly minimizes CO_2 release of the high heating value containing pine needle feedstock which is 21.61Mj/kg. Higher carbon retention, thermal stability in soil, longevity, nutrient leaching reduction, and enhancement of soil aggregation abilities of BPN_{400} make it a favorable option for carbon sequestration (Varma and Mondal, 2018). Biochar has been proven to be efficient source of plant production sustainability, global warming mitigation, soil conditioning, and various applications in soil including chemical sorption. (Tauqeer *et al*., 2022). BSS_{400} carbon retention percentage is comparatively lower than the value of 26.37% and HHV of sewage sludge feedstock is 14.4 Mj/kg. The result indicates that BPN_{400} being potentially stable with a high heating value can retain more carbon in soil (Filipe dos Santos Viana *et al*., 2018).

3.3 Structural analysis

3.3.1 Thermogravimetric analysis (TGA)

Thermogravimetric analysis was performed to determine the thermal stability of BPN_{400} and BSS_{400} at a temperature ranging from 100-900°C at continuous heat flow from 100 – 400[mW] as shown in **Figure 3** and **Figure 4**. The graphs illustrating the first slope in the temperature range of 54 – 200°C for BPN_{400} in **Figure 3 (B)** and 48 – 127°C for BSS_{400}, from **Figure 4 (B)** determined thermal stability of biochar and moisture loss, which is favorable as it uplifts fixed carbon availability, ultimately enhance soil fertility. After that, the slope becomes downward at 350°C for BPN_{400} and 299°C for BSS_{400} showing secondary pyrolysis and decomposition phase, illustrating hemicellulose and cellulose degradation followed by a decline in slope with a total weight loss of 91% for BPN_{400} and 36% for BSS_{400} above 400°C followed by lignin degradation indicating thermostability of sample before this temperature (Varma and Mondal, 2018). The weight loss in BSS_{400} is less as compared to BPN_{400} because of its higher ash contents. It can be concluded that the low-temperature biochar tends to have more mass reduction during thermal analysis as they are unstable to a temperature above their pyrolysis conditions (Naqvi *et al*., 2018). A slope can be observed in the TGA curve until the temperature reaches up to 800°C and after which the rate of degradation gets slower and almost constant. This stage is passive pyrolysis and here very low percentage of biomass residue is left over. Previous literature shows that the temperature ranges from 200 °C to 400 °C is for cellulosic and hemicellulose degradation while 210 °C to 900 °C is for lignin decomposition (Ratnasari *et al*., 2019). By increase of pyrolysis temperature, biochar becomes more stable thermally due to the formation of a more carbonized product. So, the TGA clearly shows that mass reduction in biochar samples in 91% and 36% is due to high ash content and less stability in the case of sewage sludge biochar while pine needle produced at the same temperature would be more stable in soil comparatively (Ali *et al*., 2021).

3.3.2 X-ray Diffraction analysis (XRD)

XRD result of BSS_{400} and BPN_{400} shows comparative peaks to depict the aromaticity and crystallinity in their structures as shown in **Figure 5 (A)** and **(B)**. By comparison of biochar from two different feedstocks, the degree of orientation can be observed. Peaks in the case of BPN_{400} are in the region between 15^o – 45^o represented numerous planes of (S) Struvite, (Q) Quartz, (C)

Calcite and (D) Dolomite. Whereas the condensed aromatic carbonized, and Quartz plane can be observed along 20^o to 30^o. orBSS$_{400}$ the region between 20^o to 65^o shows peaks of S, Q, C and D planes. The condensed aromaticity in this case lies in 25^o to 30^o. The difference in intensity of peak condensation in higher in case of BSS$_{400}$ biochar because it contains impurities as its origin is sewage sludge that contains impurities from all streams and secondly at temperature 400^{o}C the sewage sludge has not been converted into carbonaceous organic compound it is amorphous in that sense, but aromatic based on crystalline structure presented by impurities in its structure which gives it higher ash content as well.

Sewage sludge and pine needle-derived biochar show variation in aromaticity because of the different chemistry of feedstocks used. In the case of BPN$_{400}$ sharp peaks can be observed at 2theta around 20^o to 30^o which depicts the crystalline structure of the sample. The formation of peaks at 20-30^o are a result of pyrolytic treatment carried out at 400^o that has decreased the peak intensity in other regions, otherwise, these peaks can be observed at 16 to 20^o showing cellulose and hemicellulose in untreated feedstock as reported in the literature Jiang *et al*., 2007. The pine needle biochar XRD diffractogram has a prominent peak of quartz at 30^o while calcite and struvite can be observed in structure, but their peaks are comparatively less intense. In the case of sewage sludge biochar, a very sharp peak of calcite can be seen around 30^o, it also contains quartz and struvite abundantly justifying impurities in its structure linked to its origin from multiple waste streams. development of quartz and calcite structure in pine needle biochar and sewage sludge biochar can be evidenced by X-Ray diffraction analysis as reported in the literature (Ren *et al*., 2018).

3.4 Additives

The additives like microalgal biomass *of Closteriopsis acicularis* (BM1) and *Tetradesmus nygaardi* (BM2), organic fertilizer and compost has shown variation in their elemental compositions (refer to **Supporting Information Table SI-1)**. The value of C = 46 ± 1 % in microalgal biomass, 1.60 ± 0.03 % in organic fertilizer 10 ± 0.5 % in case of compost which shows that microalgal biomass is a rich source of carbon (Nappa *et al*., 2015). While the pH observed to be 7±0.2, N%= 5.2±0.2 and P% = 5.1±0.05 and K% = 0.6±0.05 in Microalgal biomass and pH = 6.5±0.05, N%= 1.60±0.01 %, K % = 1.76±0.005 in organic fertilizer. It can be concluded that Microalgal biomass has higher carbon and nitrogen contents as found in the literature that protein and phytochemical contents of microalgal biomass makes it a nutrient rich source (Ho *et al*., 2013) Growth condition alter the nitrogen and carbon content and overall chemical composition of microalgae (Shrestha *et al*., 2022), pH of three additives is in neutral range and K content of organic fertilizer and compost is almost similar. The trend of Carbon %, P % and pH is Microalgae > Compost> organic fertilizer, while for N% is Microalgae> Organic fertilizer> Compost and for K% is organic fertilizer> Compost> Microalgae.

Soil fertility and crop production depend upon the availability of nutrients as per required levels. Its important to maintain the suitable levels of organic matter in soils , that supports crop production and mantains total nutrient cycling in it (Tauqeer *et al*., 2022). Adding biochar solely is not significant for that purpose, organic fertilizer, compost and microalgal biomass. The microalgal biomass is an efficient source of nitrogen and carbon for the soil because it has a significant concentration of carbohydrates, lipids, proteins, vitamins, and essential minerals as found in the literature (Machado Sierra *et al*., 2021). Organic fertilizer comprises various sources of carbon, nitrogen, phosphorous and potassium for crop production as evidenced by literature. It has low K content and balance K and conductivity in soil (Chauhan, 2012) Manure has nitrogen, potassium and phosphorous for plant tissues and affects plant production. (Sakhonwasee, 2015) Manure applied in field studies showed that it has a source of ammonium ion most available form of nitrogen and contributes majorly to protein synthesis, ion transportation and osmoregulation in plants. (El-Sawy *et al* 2005). Compost supports plant production and soil structure by buffering soil pH, providing N, P and K for root and shoot development (M Al-Eraky *et al*., 2016). It can be concluded that the effects of organic fertilizer, microalgal biomass and compost depend upon the type of soil, crop, and physicochemical nature of these amendments themselves.

3.5 Soil Analyses

The soil texture is determined to be clay using soil textural table which shows that it is 10% sandy-loam, almost 47 % loam and 43% clay loam.

Parameters like electrical conductivity, pH, water holding capacity, TDS, macro-micro nutrients and heavy metals are explained below:

3.5.1 Electrical conductivity and pH

The salinity of soil varies with EC and pH value and with the type of crop being grown. The value of EC for soil as control is recorded to be 1.56 ppm which has increased up to 6.3 with crop rotation of chickpea and fenugreek plants and pH from 7 to 9 as given in Table 5. BPN400 and BSS400 have affected soil EC very significantly by 49.5 ppm and 27.6 ppm values in T3 and T4 and BPN400 decreased pH in start and kept it neutral at 7 while BSS400 being alkaline increased pH = 8.5 as compared to BPN400. Organic fertilizer and compost T1 and T2 have not much affected the EC value and it remained around 7.9 and 14.8 while Organic fertilizer increased and then neutralized soil pH to 7.3 and compost increased pH to 7.9. The collective effect of biochar with compost and organic fertilizer is observed to impact negatively the EC value to 3.8 ppm in T10 and pH 7.5. The trend of EC near harvesting time in treatments is observed as T3> T4> T2> T8>

T6> T1> S> TMB>T9>T10=TMS>T7>T5 and for pH isT9>T3>T5>T2>T10>T1>T4>TMB>TMS, T7>T8. The electrical conductivity of soil was significantly increased by BSS_{400} and BPN_{400}. The highest electrical conductivity value for BSS_{400}, BPN_{400} and compost added soil was because of salinity and pH value which lies in the optimum range in case of T8 which has BPN_{400} added to pot.

Previous study reported that pine needle biochar optimizes pH and EC value by increasing it to those levels because of Ca, Mg and Na in their structure and ability to release hydroxyl ion soil (Masto et al., 2013). The effect of biochar on pH and EC depends upon soil type (Kätterer et al., 2019), biochar can be used to buffer pH to avoid any synthetic buffer use in soil.

3.5.2 Water holding capacity and TDS.

BPN_{400} in presence of compost has significantly elevated soil water holding capacity because both compost and biochar were highly porous thereby positively increasing the adsorption capacity of soil and enhancing the water holding capacity. BSS_{400} lowered this value that causes drought because of unavailability of water in pores as soil moisture for the roots while Compost in the presence of fertilizer has raised the water holding capacity to its maximum that is beyond the safe limit for healthy growth of crop as shown in **Table 3** . BSS_{400}, BPN_{400}, organic fertilizer and compost supported the overall increase in water holding capacity of experimental soil. Water holding capacity of soil with different treatments represents trend as T7> T6> T10> T4, T1> T3>T2, T9 > T8> T5> TMB, TMS. The water retention of soil has been determined by numerous field experimentations using different types of biochar (Tsuji *et al* 1975). The soil's chemical and physical texture has been reported to show maximized water holding capacity upon biochar addition to soil (Lefroy and Wijnhoud, 2001). Majorly, micro and mesopore take part in the water retention ability of soil. Inter and intra-pore space in biochar structure provides a cavity for water storage. Pine needle biochar in crop rotation experiment of 10m, with an application rate 10 t h^{-1} in treatment T7 is observed to show the highest water retention values which are justified by a study reported by Kattere et al. (2019) which claimed that longevity of biochar in soil significantly increase water retention of experimental soil (Kätterer et al., 2019).

Total dissolved solids (TDS) are defined as all inorganic and organic substances contained that can pass through a 2-micron filter. The trend followed in this experiment is T10>T9>s>T1>T2>T8>T5>T3>T7>T4 given in **Table 3** . Soil organic matter compounds such as humic/fulvic acids are also included in TDS, and levels between 30 and 60 ppm are considered optimum for most plants, Initial TDS of soil was 774 but at time of harvest it is observed to be 173 showing consumption of inorganic and organic matter (Yu et al., 2019). Compost lower TDS to 74 initially as compared to organic fertilizer and raised to 160 at the end similar as described by Lehmann et al in 2009. Organic fertilizer and compost-maintained TDS 111 initially and 102 near harvesting time. BPN_{400} balanced TDS to 91. BSS_{400} lower TDS initially to 77 and increased to 126. Compost, two types of biochar and organic fertilizer showed 133 TDS initially and 184. Compost and BSS_{400} increased TDS to 137. Compost manure and BPN_{400} increased TDS initially but lowered to 148. Compost, organic fertilizer and $BSS_{400\ \text{were}}$ lower initially but raised to 180. Microalgal biomass showed TDS to 86. Microalgal biomass in combination with biochar, fertilizer-maintained TDS to 162.

3.5.3 Macro – Micronutrients and Heavy metals

The macro and micronutrients have been altered significantly by different types of biochar and treatments with certain additives. **Table 4** shows that the concentration of Fe has decreased in all treatments as compared to control but increased among the group of treatments, highest values recorded using T5, TMS and T8 by values of 111.32 mg/kg, 104.68 mg/kg and 100.16 mg/kg as compared to other treatments. While increased concentrations of Mn (in T4 = 118.12 mg/kg), Zn (in TMB= 63.11 mg/kg, T8=63.06 mg/kg), Co (in T5 =7.08 mg/kg, T4=7.36 mg/kg) and Na (in TMS = 38.76 mg/kg) has been observed and decrease in values of Mn (in T8 =47.2 mg/kg), Zn (TMS=53.55 mg/kg and T3= 53.88 mg/kg), Co (in TMB=3.32 mg/kg) and Na (in T2= 28.96 mg/kg) has been recorded as compared to control. The Macronutrients including Ca increased in concentration by 874.24 mg/kg in T9 and decreased in T7 by 287.36 mg/kg value. K and Mg increased in their quantity with the higher value in T1 and T8 which are 95 mg/kg and 205.87mg/kg, Mg concentration increased within treatment but remain lower as compared to control which was 233.4 soil with organic fertilizer and BPN_{400} with compost and organic fertilizer supported highest possible concentration of K and Mg. Biochar formation directly controls Fe and Mn concentrations (M Al-Eraky *et al*., 2016). Study conducted in 2008 showed that the Iron concentration in pine needle biochar is lower than other biochar samples (Novak *et al.*, 2009*)*. Novak *et al* in 2009 suggested that biochar application increase Zn and Co and justify, Zn and Co increased concentration in pots contain Pine needle biochar added with organic additives (Di *et al*., 2019). It can be concluded that BPN_{400} has optimized Fe, Mn, Zn, Ca, K, Mg concentration solely and in combination with additives. Microalgal biomass has optimized Fe, Mn, Zn, Na concentrations only but its impact is lower than $BPN_{400.}$ While BSS_{400} decreased Zn, Ca and increased Co. Combination of two biochar with all additives have positively enhanced ca concentration which depicts that BPN_{400} concentration should be adjusted so to nullify effect of $BSS_{400.}$

The concentration of Cd and Cr is observed to be higher in control Zn in T8 is highest by 63.06 mg/kg and TMB by 63.10 mg/kg as given in **Table 4**. Ni increased supported by TMS by 28.28 mg/kg and Cu value recorded higher in T5 that is 77.64 mg/kg. Heavy metals like mercury are known to be removed completely during slow pyrolysis, while the concentration and bioavailability of overall heavy metals are reported to be increased (Yue *et al*., 2017). Sewage sludge biochar has increased Zn and Cu while decreased Cd concentration in soil which is justified by literature which shows that acid-soluble heavy metals like Zn and Cu concentration increase while Cd decreased on sewage sludge biochar application in soil. it makes Zn and Cu bioavailable to plant root uptake (Dong *et al*., 2011). The reason for such bioavailability enhancement might be negative charge of biochar, which allows electrostatic exchange of heavy metals. But there exists a dilemma regarding biochar effects on heavy metal release in soil (Beesley *et al*., 2014).

The consumption of vegetables containing heavy metals is one of the most pressing issues in recent years as it has a detrimental effect on human health. Toxic heavy metals accumulated in vegetables after being released into the ecosystem by various natural and man-made activities. Continued use of synthetic pesticides, irrigation of agricultural land with untreated urban and industrial wastewater, improper landfill of solid waste, and various other industrial activities are major causes of heavey metal accumulation in productive soils (Tauqeer *et al.*, 2022). In a study it is stated that precipitation of heavy metal is result of presence of functional groups like certain oxides and carbonates (Ameloot *et al.*, 2013). Though impact of negative charges presents on surface of biochar due to OH and -COOH groups, on heavy metal concentration is known but it needs further understanding of how heavy metals are kept bioavailable to soil microorganisms. The mobility of heavy metals in soil and its accumulation in vegetables are significantly affected by several soil and plant factors that control their bioavailability. The main symptoms of metal toxicity after being absorbed by vegetables are growth, biomass, low yield and low nutritional value. Human health risks from ingesting metal contaminated vegetables have been assessed by a variety of risk assessment equations (Tauqeer *et al.*, 2022)

3.6 Plant analyses

3.6.1 Effect on plant growth parameters

Chickpea plant shown variation in growth pattern on application of biochar in different combinations presented in **Figure 5** and **Table 6**. The highest value of plant total length and fresh weight was recorded in T8 containing (BPN_{400} + Compost + fertilizer) as compared to S (control) TL and FW and lowest values recorded in T6 that did not enhance plant growth at all (M Al-Eraky et al., 2016). Addition of biochar in soil has shown promoted seed growth, biomass yield, nutrients in soil and crops grown in conditioned soils. Alongwith these profits, biochar provides space and porosity for microbial growth and activity, nutrients for microbial populations (Tauqeer *et al*., 2022). This is because in T6 (BPN_{400} + Compost), porous nature and low N: P: K ratio of compost that is 0.7:1.02:1.37 along with BPN_{400} ability to absorb nutrients supplied by compost owing to its highly porous structure has caused unavailability of nutrients to plants root, and thereby damaged the crop. While trend of plant length and fresh weight followed by certain treatment was TMB> T3> T1> T5 possibly due to nitrogen fixing ability and production of certain plant promoting hormones from microalgal biomass in TMB that promoted plant growth (Khan et al., 2018), T3 contains BSS_{400} which is unstable in soil alkaline in nature and source of heavy metals T5 and T1. Meanwhile BSS_{400} proves best for plant growth when applied in combination with fertilizer and compost. Sewage sludge is source of heavy metal and minerals, but its adsorption efficiency gets enhanced in presence of nutrient source such as organic fertilizer and compost thereby increased crop productivity. The combination of two biochar proves favorable for crop growth in presence of all nutrient sources that were organic fertilizer and compost, because buffering property of biochar and their variable porosity along with adsorption capacity stabilized the rhizobium and remarkably optimized crop production (Saletnik et al., 2019). After sowing fenugreek plant seeds in already established soil experimental combinations, the behavior of biochar varied as shown in **Figure 5** and **Table 6**. T4 with just BPN_{400} has enhanced TL ad FW compared to control, most probably because biochar has initially absorbed nutrients on its surface during first crop growth and then slowly released into soil during crop rotation which enhanced soil fertility for long term and significantly increased crop production (de Araujo et al., 2019). TMS with microalgal biomass in soil showed increase in TL and FW and T1 with BSS_{400} and organic fertilizer have shown minimum values but almost same in impact. Possibly, previous crop of chickpea plant may have extracted all available minerals provided by BSS_{400} and organic fertilizer .Sewage sludge derived biochar in combination with fertilizer, compost and microalgae does not support plant growth that is because high nutrients supply created survival of fittest competition and made conditions non-feasible for microorganisms in the rhizosphere (Gajera et al., 2020). It can be observed from the above output of two-way ANOVA that both treatment and parameters are significantly different for Cicer plant and Trigonella plant growth ##LSD Test for different treatments

Conclusions

Application of sewage sludge and pine needles derived low temperature biochar to chickpea and fenugreek crops grown on infertile land has shown a significant increase.in crop productivity and soil nutrients. Applying biochar without synthetic fertilizer has greatly increased crop length and fresh weight as well as did soil nutrient optimisation. The use of organic fertilizers and compost has an impact on root development, aeration texture and water retention, but to optimize soil profile and texture, they need a sink to optimize and cushion the exchange of soil-plant root nutrients, which was well done by using Pine needle biochar. The biomass of microalgae acted as organic fertilizer because it is huge source of nitrogen, stabilising the pH at neutral, high carbon content and has optimized the levels of phosphorous and calcium in soil. Based on our information, this research is novel to explore the effect of agricultural and municipal waste derived biochar with compost, manure fertilizer and microalgae on chickpea-fenugreek crop rotation. In future, application of this novel combination should be considered on routine basis as improvement policy when exploring issues related to soil fertility and crop cultivation practices.

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Figure 5: X-Ray Diffraction diffractogram peaks for (A) BPN_{400} (B) BSS_{400}.

Figure 6: Two way ANOVA for *Cicer arietinum* (A) and *Trigonella foenum-graecum plants (B) showing* treatment and parameters are significantly different as observed in LSD test method

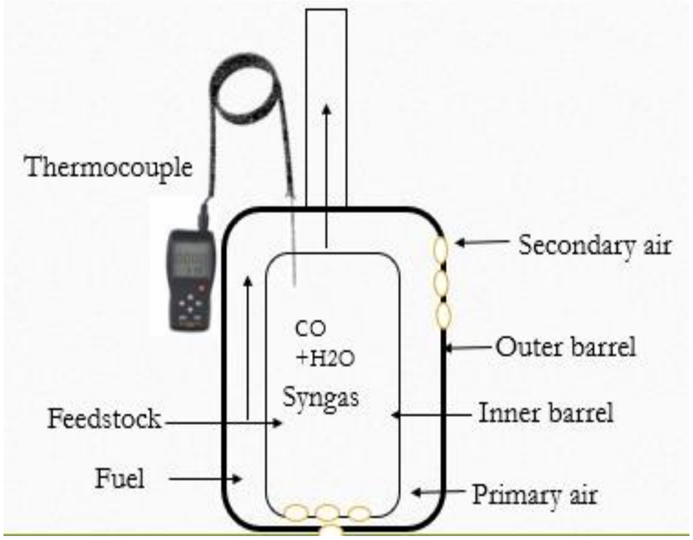


Figure 1: Retort kiln gasifier for biochar production operates at 400 °C.

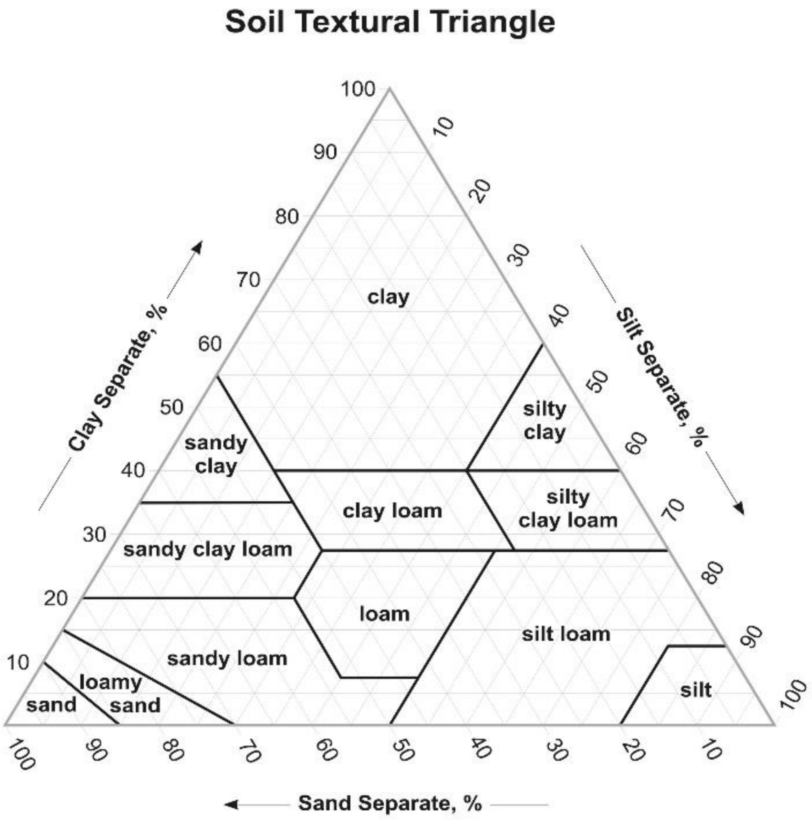


Figure 2: Soil texture triangle for soil texture analysis

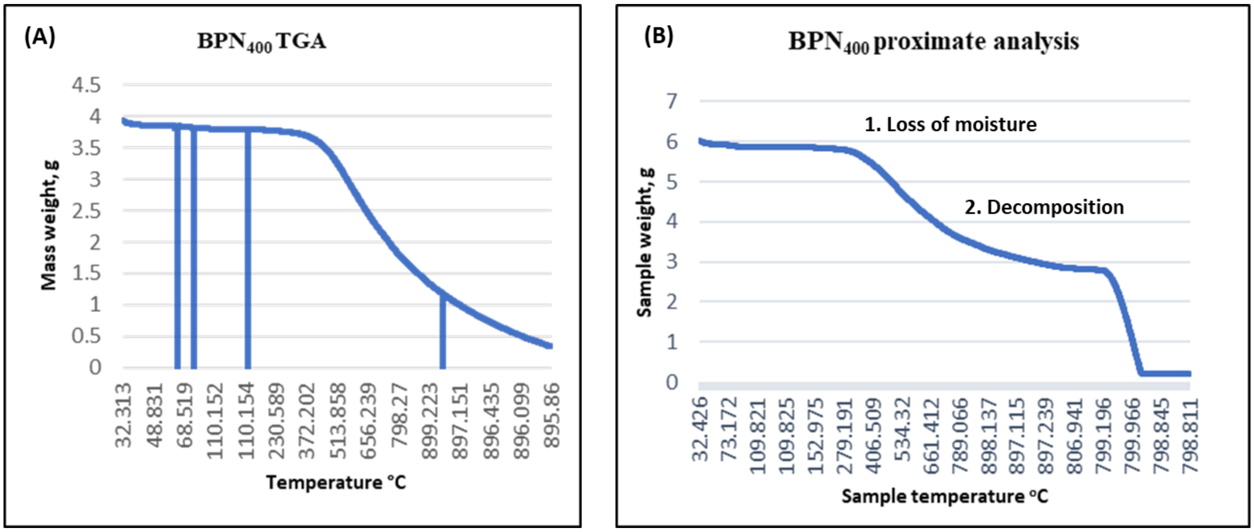


Figure 3: Thermogravimetric analysis of BPN$_{400}$ (A) TGA curve (B) Proximate analysis

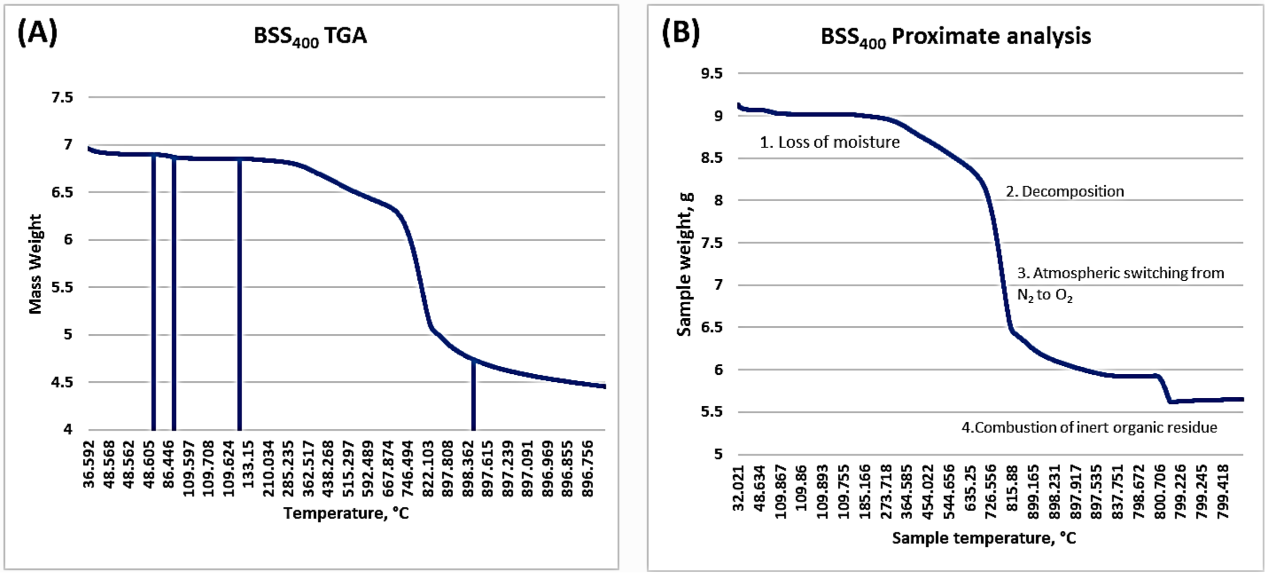
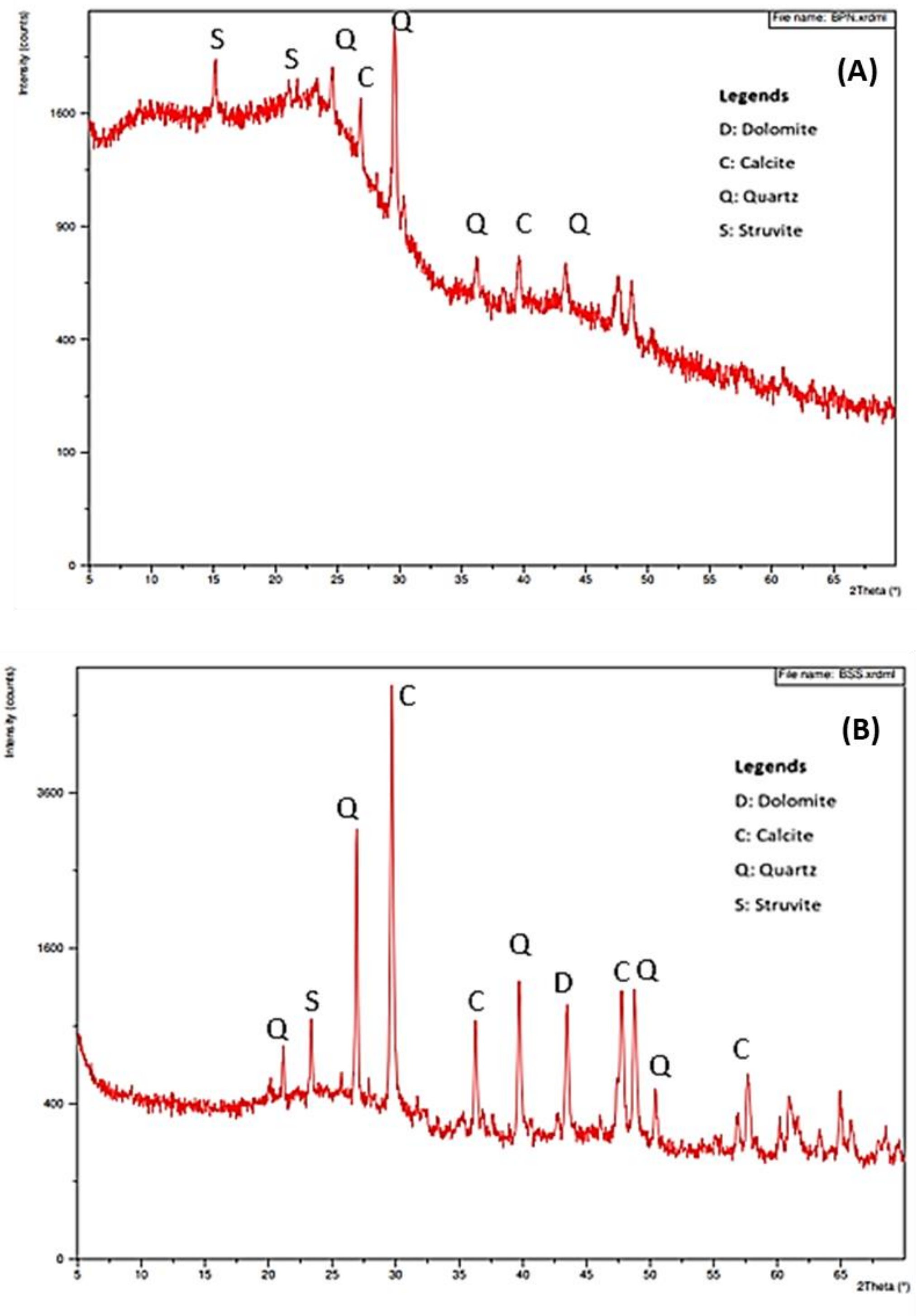


Figure 4: Thermogravimetric analysis of BSS_{400} (A) TGA curve (B) Proximate analysis



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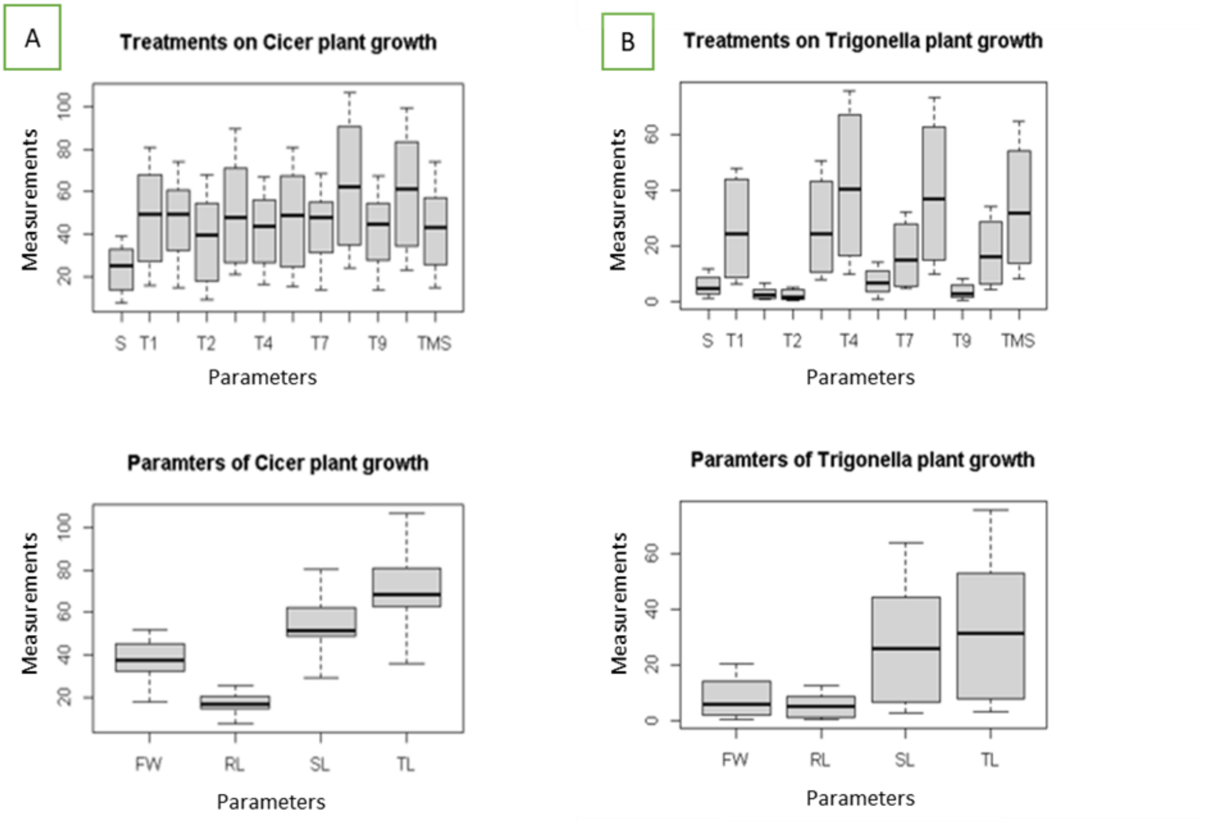

Figure 6: Two way ANOVA for *Cicer arietinum* (A) and *Trigonella foenum-graecum plants (B) showing* treatment and parameters are significantly different as observed in LSD test method

Table 1: Treatments applied to soil for crop rotation experiment

Treatments	**Names**	**Biochar g/kg**		**Compost g/kg**	**Organic fertilizer g/kg**	**Microalgae g/kg**
		BSS_{400}	**BPN_{400}**			
T1	**Soil+ Organic Fertilizer**	0	0	0	50	0
T2	**Soil + Compost**	0	0	25	0	0
T3	**Soil + BSS_{400}**	50	0	0	0	0
T4	**Soil + BPN_{400}**	0	50	0	0	0
T5	**Soil+ BSS_{400}+ Compost**	50	0	25	0	0
T6	**Soil+ BPN_{400} + Compost**	0	50	25		
T7	**Soil + Compost +Fertilizer**	0	0	25	50	0
T8	**Soil+ BPN_{400}+ Compost + Fertilizer**	0	50	25	50	0
T9	**Soil+ BSS_{400}+ Compost+ Fertilizer**	50	0	25	50	0
T10	**Soil+ BPN_{400} + BSS_{400} + Compost +Fertilizer**	50	50	25	50	0
TMB	**Soil+ BPN_{400} + BSS_{400} + Compost +Fertilizer + Microalgae**	50	50	25	50	10
TMS	**Soil +Microalgae**	0	0	0	0	10

Table 2: Physicochemical characterization of biochar produced from sewage sludge and pine needle at 400°C.

Variables	Pyrolyzed sludge biochar Dry base	Pine needles biochar Dry base
Total Carbon %	15.97	67.75
Ashes %	60	9
C/N ratio w/w	6.8:1	26.7:1
Total Nitrogen %	2.32	2.53
pH	10	6.1
BET Surface area (M^2g)	1.44	429.99
Pore size (nm)	10.61	2.16
Mg (mg/kg)	4.21	-
Ca (mg/kg)	3.13	-
Cu (mg/kg)	0.03	0.04
Cr (mg/kg)	1.75	1.53
Mn (mg/kg)	0.05	0.07
Ni (mg/kg)	0.09	0.07
EC (dSm^{-1})	1.9	1.1
yield	52.4	38.7

Table 3: Water holding capacity (WHC), Electrical conductivity (EC), Total dissolved solids (ppm) and pH of soil recorded before and after plant harvesting.

Treatments	**Water holding capacity of soil (%)**	**EC pS/m**		**TDS (ppm)**		**pH**	
		Before	**After**	**Before**	**After**	**Before**	**After**
S	50	1.56	6.3	774	173	7.0	9.0
T1	70	4.9	7.9	100	168	7.4	7.3
T2	60	14.9	14.8	74	160	6.8	7.9
T3	62	4.3	49.5	77	126	6.0	8.5
T4	70	8.6	27.6	91	91	4.6	7.0
T5	30	15.3	3.1	84	137	6.9	8.0
T6	82	1.4	10.4	0	0	0	0
T7	94	7.7	3.5	111	102	6.0	6.6
T8	50	1.7	14.5	160	148	7.0	6.5
T9	60	1.2	4.1	130	180	7.5	13
T10	78	13	3.8	133	184	5.3	7.5
TMB	25	0	4.5	0	162	0	6.7
TMS	25	0	3.8	0	86	0	6.6

Table 4: Effect on Micro and Macronutrients initially and after harvestation crop rotation

Treatments	Micronutrients (mg/kg)					Macronutrients(mg/kg)			Heavy metals (mg/kg)				
	Fe	**Mn**	**Zn**	**Co**	**Na**	**K**	**Ca**	**Mg**	**Zn**	**Cd**	**Cr**	**Ni**	**Cu**
S	129.08	60.24	55.78	5.60	32.02	48.57	422.52	233.40	55.78	19.28	32.84	27.80	65.32
T1	99.68	49.08	55.56	5.72	31.00	95.00	455.36	156.68	55.56	16.96	28.12	19.68	63.76
T2	99.40	50.60	57.08	6.24	28.96	94.68	491.56	155.8	57.08	17.04	27.72	22.84	63.72
T3	96.68	50.36	53.88	6.68	29.00	86.24	374.24	186.92	53.88	16.68	31.56	23.48	63.40
T4	97.04	118.12	51.76	7.36	33.00	71.32	409.80	127.40	51.76	17.08	30.44	19.92	68.52
T5	111.32	50.84	58.32	7.08	35.4	84.96	565.84	197.88	58.32	17.00	31.40	22.84	77.64
T6	0	0	0	0	0	0	0	0	0	0	0	0	0
T7	96.96	49.04	51.94	7.6	35.48	65.02	287.36	135.53	51.94	18.44	32.28	19.40	60.68
T8	100.16	47.20	63.06	6.88	29.70	81.24	372.84	205.87	63.06	17.12	28.44	27.08	60.36
T9	83.04	49.56	54.52	5.28	29.21	93.23	874.24	192.30	54.52	16.52	28.84	20.76	63.28
T10	98.68	49.64	51.12	6.96	31.56	86.60	518.08	189.12	51.12	16.84	31.32	20.92	59.04
TMB	99.20	49.24	63.11	3.32	29.57	42.85	461.68	115.57	63.11	16.44	31.76	24.84	64.96
TMS	104.68	54.36	53.55	6.48	38.76	65.62	498.16	38.76	53.55	14.72	29.24	28.28	65.32

Table 5 showing output of two way ANOVA for *Trigonella foenum-graecum* plant after 90 days of cultivation.

Fenugreek crop	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr (>F)**
Treatments	11	28323	2575	28.28	<2e – 16 ***
Parameters	3	20739	6913	75.92	< 2e – 16 ***

Table 6 showing output of two way ANOVA for *Cicer arietinum* plant after 182 days

Chickpea crop	**Df**	**Sum sq**	**Mean sq**	**F value**	**Pr (>F)**
Treatments	11	13722	1247	26.82	<2e-16***
Parameters	3	61548	20516	434.52	<2e-16***