

Microbial Abundance of Waste Derived Biochar Incubated Acid Soil in Bangladesh

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Abstract

Due to climate change biochar is recently recommended as a control approach to increase crop productivity and global warming reduction. As biochar application changes the soil pH towards alkalinity, this effects acid soils nutrient cycles the same as microbial abundance. This research was conducted to investigate the microbial abundance as affected by waste-derived biochar application in two different rates on acidic soil of Bangladesh. Slow pyrolyzed (500±50°C) different waste-derived biochars viz. sewage sludge, sugarcane bagasse, potato peels, water hyacinth, and organic waste were applied at 10 tons ha⁻¹ and 15 tons ha⁻¹ on the acidic soil. An in-vitro incubation study was conducted on experimental soil applying all the biochar to understand how nutrient availability and carbon dynamics affect the microbial abundance of the acid soil. The incubation study was divided into two stages: submerged condition (up to 60 days) followed by a dry condition (61 to 120 days) and biochars were applied in two different rates such as 10 and 15 tons ha⁻¹. The viable count of bacteria significantly ($P<0.05$) increased with the variation of incubation periods and soil moisture content, although the rates did not make any difference. The beneficial Rhizobium spp. bacteria count ranged from 4.21 to 6.47 log CFU/g; Azotobacter spp. count ranged from 2.33 to 5.60 log CFU/g and Phosphate Solubilizing Bacterial count ranged from 2.30 to 3.74 log CFU/g. However, no sign of coliform bacteria or Escherichia coli was found in any sample, but also none of the biochar amended soil samples possessed Pseudomonas spp. and Trichoderma spp. In outcome, the waste-derived biochars treatments showed an insignificant impact on microbial parameters over the first 2 months after biochar incorporation which progressively increased with the course of time and the presence of oxygen.

Keywords: climate change, biochar incubation, microbial abundance, acid soil, Bangladesh

1. Introduction

Biochar is the product of thermal degradation of organic materials in the absence of air (pyrolysis) and distinguished from charcoal by its use as a soil amendment (Kambo and Dutta, 2015). Applying biochar in the soil can remediate the pollutants from the soil but also improve the soil properties. Biochar improves physical (e.g., water holding capacity, O₂ content and moisture level), chemical (e.g., pollutants immobilization and carbon sequestration), and biological (e.g., microbial abundance, diversity, and activity) properties of the soils (Gul *et al.*, 2015; Qi *et al.*, 2020 and 2021; Lozano *et al.*, 2021; Palansooriya *et al.*, 2022). It has individual nature to bind polar compounds through charged surface functional groups, which helps to immobilize rhizospheric heavy metals and agrochemicals on its surface and restricts their mobility into the crops (Bolan *et al.*, 2014). Biochar has been reported as a possible means to improve soil fertility as well as other ecosystem services and sequester carbon (C) to mitigate climate change (Sohi, 2013; Dhama *et al.*, 2021). The perceived influences on soil fertility have been described mainly by a pH increase in acid soils or improved nutrient retention through cation adsorption (Gul *et al.*, 2015). Nevertheless, biochar has also been shown to change soil biological community composition and abundance (Thies *et al.*, 2015). Biochar act as a soil conditioner and as a fertilizer that enhances plant growth by supplying and retaining nutrients, and by improving soil physical and biological properties (Kwapinski, 2019).

The nutrients (e.g., K, P, Ca and Mg) and the nutrient preservation capacity of biochars can promote the fertility of acidic soils and decrease the need for chemical fertilizers (Domingues *et al.* 2017; Al-Wabel *et al.* 2018). Biochar includes alkaline substances (carbonates and organic anions from acidic functional groups) and has high pH (as high as 10) (Fidel *et al.* 2017), and thus can be used as an alternative supplement for the improvement of soil

acidity (Berek and Hue 2016). This is the main purpose why the incorporation of biochar enhances crop yields, which is more apparent in acidic soils in tropical and subtropical regions than in temperate regions (Jeffery *et al.* 2017; Cornelissen *et al.* 2018). Concerning the liming impact, biochars derived from crop residues may inhibit the re-acidification of ameliorated acidic soils by increasing soil pH buffering capacity (Shi *et al.*, 2018). The alkaline character and ameliorating effects of biochars on acidic soils have attracted considerable research interest. Mainly, biochars derived from crop residues or other organic wastes that can improve acidic soils as well as mitigate climate change could benefit sustainable agriculture.

Microorganisms propagate in many manners, including soil organic matter (SOM) cycling and carbon (C) sequestration (Clemmensen *et al.*, 2013). The addition of biochar to soil is anticipated to feedback on ecosystem of SOM and nutrient cycling by modifying the formation and capacity of microorganisms (Lehmann *et al.*, 2011). Despite, the mechanisms by which biochar application transforms SOM dynamics via microorganisms inhabit is unclear (Gul *et al.*, 2015). The addition of biochar to the soil can enhance the SOM level (Lehmann *et al.*, 2006), affect C cycling (Farrell *et al.*, 2015), expedite nitrogen (N) dynamics (Nelissen *et al.*, 2012) or in some cases, even decrease organic N turnover (Prommer *et al.*, 2014). Ultimately, it can be expected that, microbial abundance will improve by the application of biochar in the soil. In the contrast, the biochar amendment did not alter the dissolved organic C (DOC) and dissolved organic N (DON) in one field investigation (Jones *et al.*, 2012), whereas it reduced the DOC concentration (Prommer *et al.*, 2014) in another field research. These opposing positive and negative influences of biochar application on SOM fractions may be connected to the specific methods directing C and N cycling under specific management practices. These processes alter with climate, crop rotation, fertilization, and soil biology characteristics and consequently result in inconsistent SOM dynamics and transformation (Demisie *et al.*, 2014; Prommer *et al.*, 2014).

Furthermore, rhizosphere bacteria and fungi also promote plant growth directly (Febriati and Rahayu, 2019). Biochar application may bring multiple benefits including mitigating climate change by sequestration of carbon and it can potentially enhance soil health. Biochar is also finding new applications as an animal feed, as a component of animal bedding and as a filter for affordable cleaning-up of industrial and wastewaters (Akoto-Danso *et al.*, 2018). These pyrolyzed feedstocks affect soil fertility in many ways, it can supplement nutrients by itself or make them more available for plant uptake by improving the decomposition of organic material or possibly, reduce decomposition rates of other organic material thereby increasing soil C concentration in the long run (Jung *et al.*, 2015). Microorganisms are actively associated with SOM dynamics and transformation in terms of mineralization and synthesis (Bowles *et al.*, 2014; Ng *et al.*, 2014). After biochar amended in soil, a greater breakdown of soil SOC was observed, accompanied by a higher microbial activity (Wardle *et al.*, 2008) or developments in the microbial community (Farrell *et al.*, 2013). Biochar addition contained SOC decomposition with modified microbial community structure by increasing Gram-positive bacteria in 30-day incubation research (Lu *et al.*, 2014). The enhancement of biochar reconstructed C sources use patterns of microorganisms, perhaps via changes in the microbial population (Tian *et al.*, 2016; Pietikäinen *et al.*, 2000). All these comparisons implied that SOM cycling might be modified via microorganisms after biochar addition. A number of investigations described variations in the microbial community after biochar amendment that were attributed to the physicochemical properties of biochar (e.g., aeration, sorption), as well as biochar-induced changes in soil properties such as pH (Xu *et al.*, 2014; Gul *et al.*, 2015). Furthermore, the majority of biochar studies have relied on short-time incubation experiments (Farrell *et al.*, 2013; Xu *et al.*, 2014). Long-term field trials studying organic C- and N-related consequences of biochar on microorganisms still remain to be investigated. As such considerations will help us understand the role of environmental factors in controlling biochar-induced changes in soil chemical and biological properties. However, in this research, we have investigated the microbial abundance change in the experimental acid soil after incubation of waste derived biochars. The specified objective of the study was to follow the microbial change occur in the soil that maybe positively or negatively impact the soil biodiversity.

2. Materials and Methods

2.1 Soil Sampling and Analysis

The researched soil sample, which was collected from a vegetable field of the village, Aukpara in Savar Union of Dhaka District, beside the dairy farm of Savar Cantonment. The soil sample collected represented the Tejgaon series. Tejgaon soil series belongs to Red Soil Tract or Modhupur tract, which consist of medium highland and well drained and pH ranges from 4.8 to 6.1, acidic in nature. The soils of this tract have clayey texture, and are deficient in organic matter, nitrogen, phosphorus and lime. The soil contains high quantity of iron and aluminum, which are highly aggregated. The structure of the soil is prismatic. It is highly sticky and plastic. After collection, soil sample was divided into two parts – one was used for required background analysis for various parameters

and the larger portion was kept separately for microbiological test and incubation study.

2.2 Feedstock Selection and Production of Biochar

Five different types of wastes were collected to produce different types of biochar. All the feedstock selected are potentially waste materials, and collected from different sources. Sewage Sludge was collected from Pagla Sewage Treatment Plant. Sugarcane bagasse was collected from the roadside sugarcane juice shop. Potato peels were arranged from the small agro-food processing industry in Pura Dhaka. Water hyacinths were collected from a pond, located in Keraniganj, Dhaka. Organic wastes were collected from home leftover vegetables and other municipal wastes.

Before the production of biochar, all the waste-derived feedstocks were well dried for a few days under the sunlight. After properly drying all the feedstocks, one by one all the feedstocks were processed and pyrolysis was done in a specially designed kiln. Individual feedstocks were placed in the bally of the cooker and then the head of the cooker is locked that no oxygen can enter inside the cooker. The cooker was then placed on the gas stove for burning. Approximate temperature 450-550°C was maintained after one hour. The feedstock was burnt for 3 hours to maintain the above-mentioned temperature.

Table 1. Types of waste used to produce biochar and symbols

Waste used for Biochar Production	Biochar Symbols
<i>Sewage Sludge</i>	SS
<i>Sugarcane Bagasse</i>	SB
<i>Potato Peel</i>	PP
<i>Water Hyacinth</i>	WH
<i>Organic Waste</i>	OW

2.3 Experimental Design and Incubation Study for Microbial Count

For this comprehensive study, the whole experiment was sub-divided into four parts. First, pre-soil and five different type of biochars were analyzed for their chemical properties and nutrient content. This was done to have a comparative study for their suitability to produce and incorporate in the agricultural system of Bangladesh. Second, the experimental soil was incubated in these ten biochars. Two soil moisture regimes used for 4 months- First 60 days was in submerged condition and last 60 days was in dry condition. These biochar incubated soils were analyzed to know their bioavailability of nutrients. Biochars were used at two different rates i.e. 10 tons ha⁻¹ and 15 tons ha⁻¹ in the incubated soils.

2.3.1 Experimental Design

Treatment combinations for microbial analysis are as follows Table 2:

Table 2. Treatment arrangements for microbial analysis

Rate of Biochar	Arrangement of experiments	Labeling on pots/containers
10 tons ha ⁻¹	Control Soil	C
	Soil + Biochar (Sewage Sludge)	T ₄
	Soil + Biochar (Sugarcane Bagasse)	T ₅
	Soil + Biochar (Potato Peel)	T ₈
	Soil + Biochar (Water Hyacinth)	T ₉
	Soil + Biochar (Organic Waste)	T ₁₀
15 tons ha ⁻¹	Soil + Biochar (Sewage Sludge)	T ₄₄
	Soil + Biochar (Sugarcane Bagasse)	T ₅₅
	Soil + Biochar (Potato Peel)	T ₈₈
	Soil + Biochar (Water Hyacinth)	T ₉₉
	Soil + Biochar (Organic Waste)	T ₁₀₀

The total viable count was taken before and after treatment (at different incubation periods and for two different biochar rates) as well as viable counts for specific bacteria viz., *Rhizobium* spp., *Azotobacter* spp., Phosphate Solubilizing Bacteria, Phosphate Solubilizing Fungi, and *Pseudomonas* spp., were also taken. All experiments were conducted in an aseptic condition i.e. in laminar airflow, which assured unwanted microorganisms from contaminating sterile materials. For the identification and microbial colony count of microorganism, five gm of each soil samples were weighted and poured into 150 ml conical flask containing 45 ml of sterile normal saline (Cappuccino and Sherman, 2007). Each soil sample was shaking in the shaker at 180 rpm (rotation per minute) for 30 minutes for homogenize individually and homogenized soil sample was serially diluted with sterile saline water. The serially diluted and non-diluted samples were then spread onto selective and non-selective medium containing petri plates with sterile spreaders and incubated at optimum condition for the observation of microorganisms. Each colony that appeared on the plates was considered as one colony-forming unit (CFU) (Sau *et al.*, 2017). All plates were incubated at their desired temperature for required hours and final counts of CFU taken after the completion of incubation period. However, the CFU was calculated as:

$$\text{CFU/gm} = (\text{number of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$$

Microorganisms were isolated from their specific selective media at different optimum condition based on colony characteristics. The isolated microorganisms were listed in Table 3.

Table 3. Isolated microorganisms growing on selective and non-selective media and their morphological characteristics on the petri-dish

Microorganisms	Media	Colony	Incubation condition
Total Aerobic Bacteria	Plate Count Agar	Creamy white, yellow, green color	37°C, 24 hours
Coliform	Chromocult Agar	Creamy white	
<i>Escherichia coli</i>	Chromocult Agar	Dark blue to violet	
<i>Pseudomonas</i> spp.	Cetrimide Agar	Yellow green to blue green colonies	(30-32) °C, 4-7 days
<i>Rhizobium</i> spp.	Congo Red Yeast Extract Mannitol Agar	Pink	
<i>Azotobacter</i> and Nitrogen Fixing Fungus	Nitrogen free Agar	Whitish or Cream Color	
Phosphate Solubilizing Bacteria and Phosphate Solubilizing fungi	National Botanical Research Institute Phosphate Bromo Phenol Blue (NBRI-PBPB) medium	Blue and White	
Total fungal count	Soya Dextrose Agar (SDA)	White, Creamy White colony	30°C, 24-48 hours

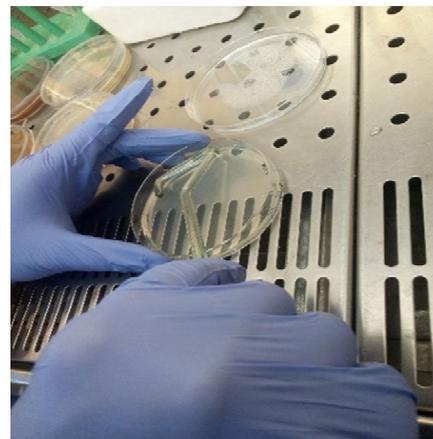
Samples from the different treated pots at the end of each incubation period were further subjected to serial dilution. One ml portions of different diluted (10⁻¹ to 10⁻⁵) samples were inoculated into selective and non-selective agar plates. Following the incubation, bacterial growth appeared after 24 hours-48 hours as before and for fungus, it took 2-4 days.

2.4 Identification of Microorganism

Microorganisms were identified by observing standard colony characterization on their specific selective culture media at different optimum incubation condition (Figure 1).



Serial Dilution



Sprad Plate Method

Figure 1. Microbial analysis procedure and used machine

2.5 Differentiation of Phosphate Solubilizing Microorganisms from Non-phosphate Solubilizes

Soil samples were inoculated on NBRIP-BPB culture media and incubated at standard condition for the observation of clear zone around colonies to differentiate phosphate solubilizes from non-phosphate solubilizes.

2.6 Statistical Analysis

The effect of biochar and microbial count was analyzed by one-way ANOVA, two-ways ANOVA and t-paired test with the help of Minitab 19.

3. Results and Discussions

3.1 Chemical Properties of Soil

The reddish-brown color of the soil indicated high oxidation and high percentage of clay content, although it had the texture class of silty clay. As soil had high content of clay, its water holding capacity was sufficient. Nevertheless, in dry condition, the soil became hard and in the wet condition, it was very sticky. The soil was strongly acidic in nature having pH ranging from 4.33 to 4.39. The soil was also very low in organic matter, which was much below 1%. Most of the total nutrients were present in limited concentration. Available nitrogen, phosphorus and sulphur was insufficient to support plant growth. On the other hand, high amount of iron and manganese present found in the soil. Accumulation of iron explains high acidity and high CEC in the soil. Other than iron, all the trace elements were in low in concentration. From the physio-chemical properties, it can be said for its acidity and low organic matter content, it is a problem soil for plant growth.

Table 4. Chemical properties of the soil

Parameters	pH	OC	OM	Total N	Total P (%)	Total K (%)	Total S (%)	Total Fe (ppm)	Total Zn (ppm)	Total Mn (ppm)	C:N ratio	CEC (Cmolc/kg)
	Range	(%)	(%)	(%)		(%)	(%)	(ppm)	(ppm)	(ppm)		
Value	4.33-4.39	0.23	0.40	0.20	0.03	0.17	0.26	919	3	12	2:1	26.45

3.2 Nutrient Content of Different Biochars

Most of the biochar found to be alkaline in nature (pH 6.4 to 10.02) due to high dissolution of base cations (Table 5). Due to the production methods and high temperature increases the pH value of biochars probably in consequence of the relative concentration of non-pyrolyzed inorganic elements that are already present in the original feedstocks (Novak *et al.*, 2009).

Table 5. Chemical properties (pH, EC (mS/cm), CEC (Cmol_c/kg) and OC %) of biochars

Parameters	Biochar Types				
	SS	SB	PP	WH	OW
pH	6.42±0.05	7.12±0.07	9.92±0.09	8.17±0.04	10.01±0.02
EC	0.70±0.03	0.05±0.002	9.77±0.04	0.05±0.006	9.90±0.07
CEC	27.6±0.07	271.4±0.26	181.1±0.24	300.1±0.36	125.3±0.11
OC	28.3±1.58	26.0±2.06	49.4±1.18	19.8±0.23	18.27±1.25

‘±’ standard deviation

Results indicated that potato peels biochar (49.4%) possessed the highest organic C content, respectively whereas organic waste biochar holds the lowest (18.27%; Table 5). This stable form of organic C would extensively affect physicochemical properties of soil. High-temperature biochar exhibits a high degree of aromatic C structures that are resistant to degradation, as they do not provide labile fraction of C to soil microbes (Novak *et al.*, 2009). Biochar is generally regarded as relatively inert when compared to their feedstocks. The carbon of biochars tends to be present in the soils for hundreds to thousands of years, depending on the feedstock and type of pyrolysis (Thies *et al.*, 2015).

The increase in soil C and nutrient status is due to thermal humiliation which means loss of volatile compounds (H and O mainly) of the original material and comparatively small losses of alkali nutrients by volatilization (Chan and Xu, 2009). Pyrolysis alters the nutrient content in the resulting biochar, which therefore affects nutrient availability to plants. Nutrient content mostly N, P, K and S in total content can vary according to the variation in feedstock (Figure 2).

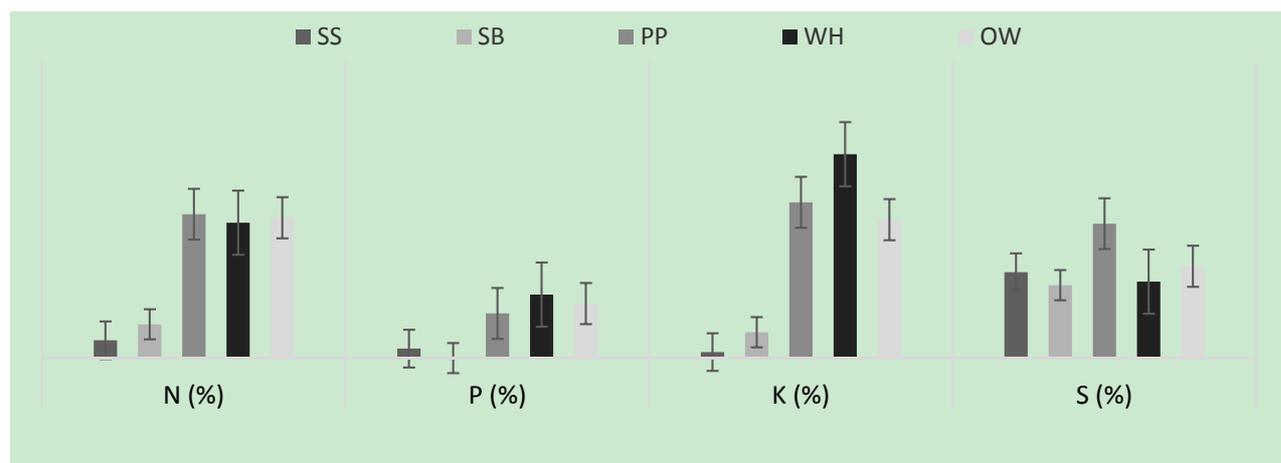


Figure 2. Variation in total NPKS nutrient content in biochars

Mainly the influence of feedstock is particularly evident in the case of total P. In the study, high concentration of phosphorus resulted in biochar produced from feedstock of water hyacinth (1.29%) whereas other biochars like potato peel and organic waste showed lower than 1%. Potato peel biochar and water hyacinth biochar had higher total K content than other biochars. All the biochars showed similar concentration of total S. Biochar produced from potato peel (2.72%) had the higher percentage of total K than other biochar (Figure 2). Biochars are variable materials in terms of total nutrient content and nutrient availability can vary in response to plant and soil.

3.3 Analysis of the Initial Soil and Biochar

3.3.1 Determination of pH

In this experiment, pH of initial soil sample and five biochars were measured by pH meter. The values of the pH are presented in the Figure 3.

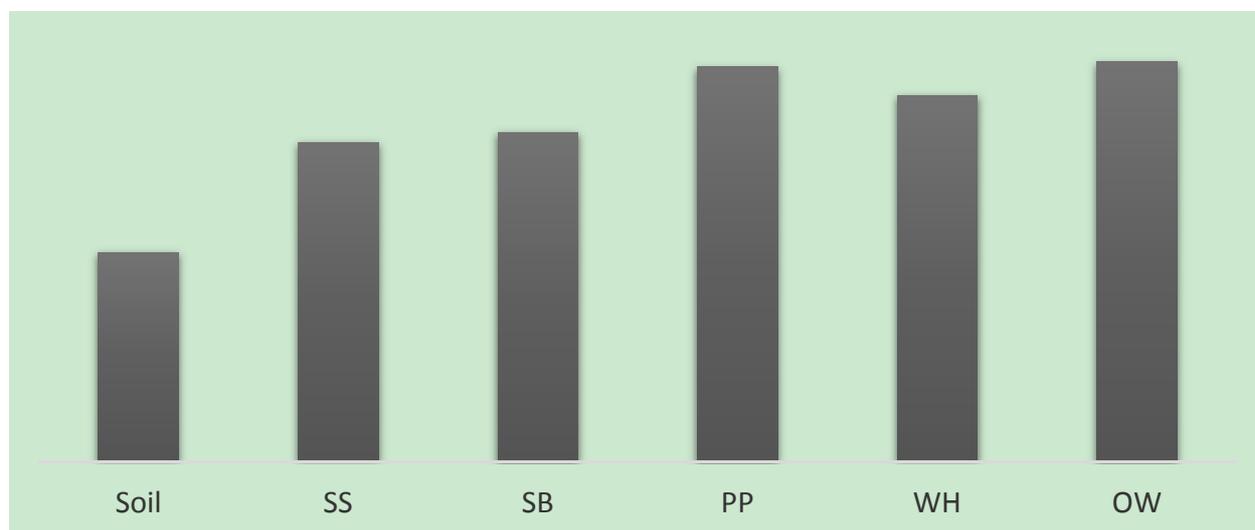


Figure 3. Comparison of pH value of soil and biochar samples

3.4 Total Viable Count

A serial dilution of the soil and waste derived biochars was executed to observe the bacterial and fungus growth and abundance. 1 ml portion of different diluted (10^{-1} to 10^{-5}) samples were inoculated into agar plates. Bacterial colonies started to appear in the petri-dishes after 24 hours and fungus appeared after 48 hours to 4 days depending on media. These appearances indicated the presence of bacteria and fungus in these materials. However, no growth observed in biochar samples. The results are presented in Table 6.

Table 6. Total viable count of initial soil and selected biochar samples

Types of Bacteria	Name of organisms	Microbial Medium used	Mean log value of CFU/g					
			Initial soil	Biochar Types				
				SS	SB	PP	WH	OW
Soil quality indicator	TABC	PCA	4.18±0.08	<1.0	<1.0	<1.0	<1.0	<1.0
	TCC	CHR	<1.0	<1.0	<1.0	<1.0	<1.0	
	TFC	SDA	4.30±0.02	<1.0	<1.0	<1.0	<1.0	
Foodborne Pathogen indicator	E.coli	CHR	<1.0	<1.0	<1.0	<1.0	<1.0	
Soil beneficial bacteria	<i>Rhizobium spp.</i>	YECRA	4.30±0.12	<1.0	<1.0	<1.0	<1.0	<1.0
	<i>Azotobacter spp.</i>	Ashby	2.40±0.07	<1.0	<1.0	<1.0	<1.0	<1.0
	PSB	NBRIP	<1.0	<1.0	<1.0	<1.0	<1.0	
	PSF	NBRIP	<1.0	<1.0	<1.0	<1.0	<1.0	
	<i>Pseudomonas spp.</i>	CTD	<1.0	<1.0	<1.0	<1.0	<1.0	
Plant pathogen inhibitor	<i>Trichoderma spp.</i>	SDA	<1.0	<1.0	<1.0	<1.0	<1.0	

'±' standard deviation

The soil quality indicator bacteria was found in initial soil sample and no foodborne bacteria found in the sample.

Soil beneficial bacteria was observed except Phosphobacter, Phosphorus solubilizing fungus and *Pseudomonas spp.* in the initial soil sample. As *Trichoderma spp.* is one of the potential plant pathogens inhibitors, thus presence of *Trichoderma spp.* will provide important information on the soil sample. Conversely, no microbial count was discovered in the biochars. The reason could be that the high temperature used for the pyrolysis process might have killed the microbes that were present in the corresponding biomass.

3.5 Microbial Study of the Biochar Amended Soils at Different Incubation Days

3.5.1 Determination of pH

For this analysis, four incubation periods were used i.e. 30, 60, 90 and 120 days; and two treatment of biochar were used in the soil i.e. 10 and 15 tons ha^{-1} (Figure 4). For microbial analysis, pH of these samples was measured.

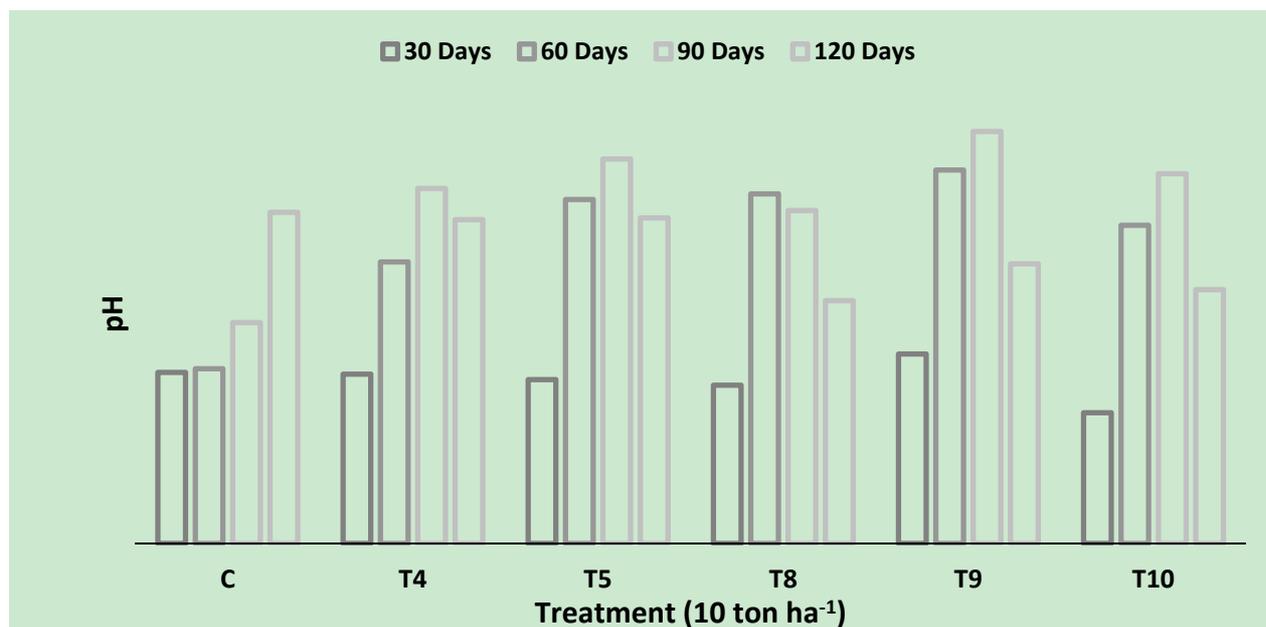


Figure 4. pH values of control and five different biochar incubated soils at different incubation periods

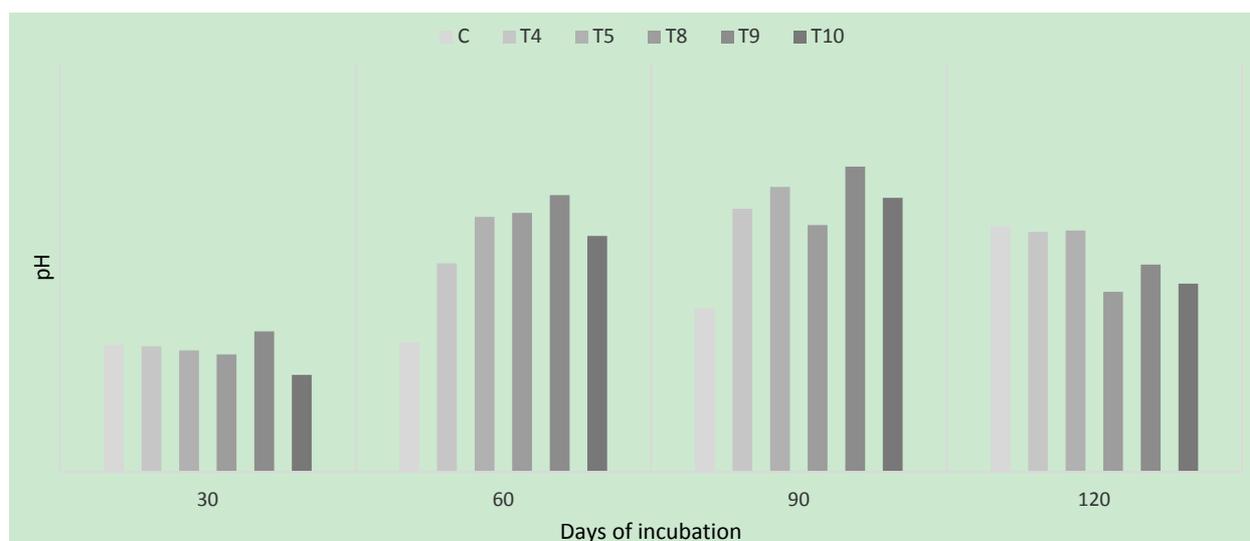


Figure 5. Comparison between days of incubated and pH for different treatment of biochar and control

The pH of the control soil and biochar incubated soils had difference in value with incubation days (Figure 5). For different biochar amended soils it is observed there are significant ($P < 0.05$) differences due to different biochar types. When biochar was added at a rate of 10 tons ha^{-1} , pH of water hyacinth biochar amended soil found to be

highest (pH 6.74 after 90 days of incubation). Although, after 120 days of incubation pH decreased to 6.02. On the contrary, the lowest pH was observed in 30 days incubation period.

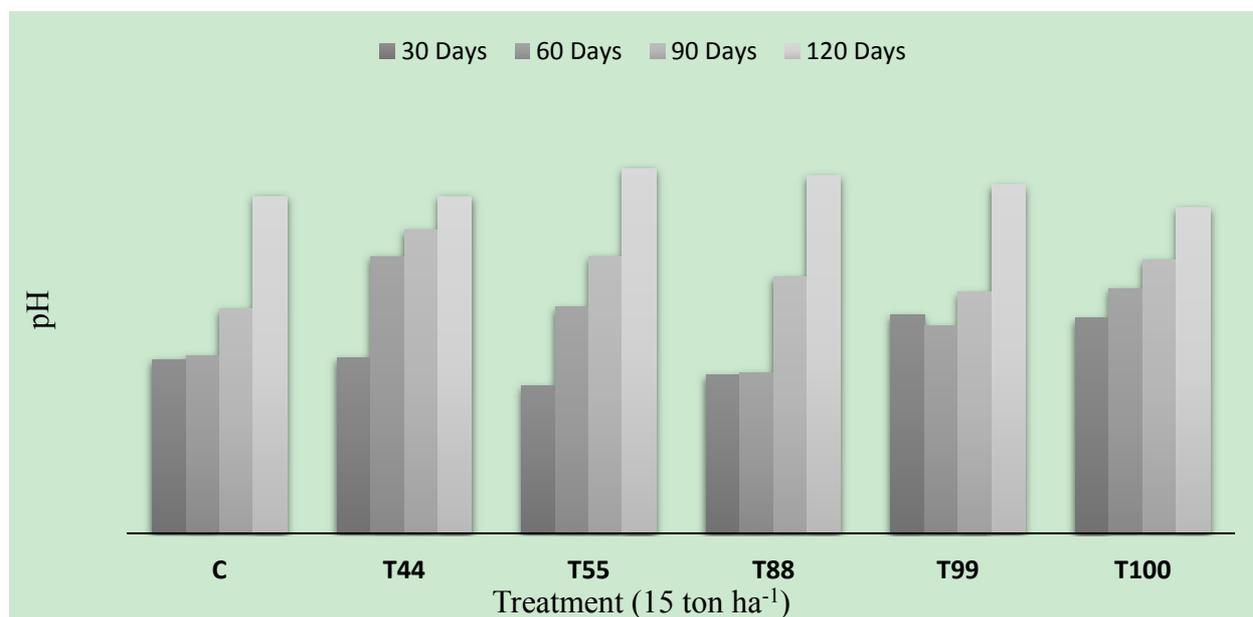


Figure 6. pH values of five different biochar applied soils and control soil incubated for different days

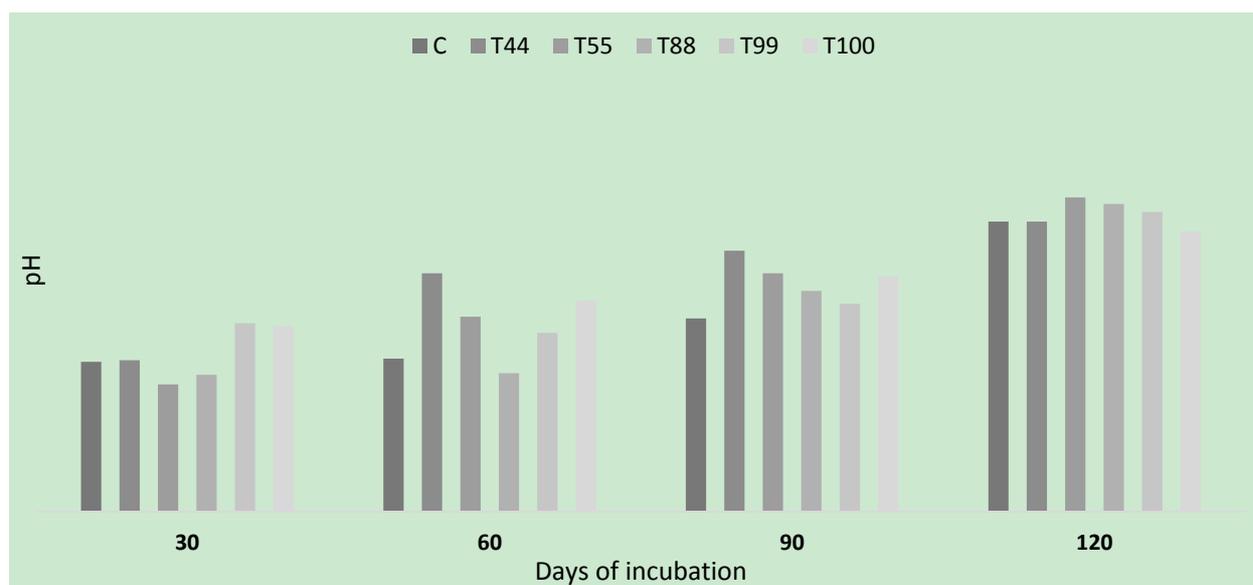


Figure 7. Comparison between days of incubated and pH values for different treatment of biochar (15 tons ha⁻¹) and control

In the case of biochars applied at 15 tons ha⁻¹ there was also significant ($P < 0.05$) differences between biochar amended soils. Control soil demonstrated the highest amount of pH 6.73 after 120 days of incubation (Figure 7). Between different biochar treatments, all the soil showed highest pH after 120 days of incubation. Every microorganism has a pH range within which growth is possible and typically shows a well-defined growth when pH optimum. Bacterial growth rates greatly influence by pH as they have limitation to their acidity tolerance. Fungus has less effect by pH than bacteria. Malik *et al.* (2019) showed that char increased pH, which positively influences total microbial abundance as well as survival capacity and reactivation of several oxygen-sensitive bacteria due to reduced surface tension, pH buffering and sorption of radicals. As observed from the experiment, 30 days biochar incubated soils had low pH than other incubation days. This can be a major factor for microbial abundance further experimented in this study.

3.5.2 Viable Count of Specific Bacteria and Fungi

3.5.2.1 Total Aerobic Bacterial Count (TABC)

The Aerobic Bacterial Count was used as an indicator of bacterial populations on the biochar amended samples. The test is based on an assumption that each cell will form a visible colony when mixed with agar containing the appropriate nutrients. It is a generic test for organisms that grow aerobically at mesophilic temperatures (25 to 40°C). In the study, total aerobic bacterial count has been used a soil quality indicator. The five different biochar amended soils show considerable variation both for different incubation days and according to the rate of biochar application. Results exhibited significant ($P < 0.001$) different from each of the treatments and in different incubation days. After 60 days of incubation bacterial count reduced from both initial and 30 days. This is due to 60 days of submerged condition that reduced the bacteria colony. Drastically, after one month of dry periods bacterial count increased both in 90 days and 120 days (Figure 8).

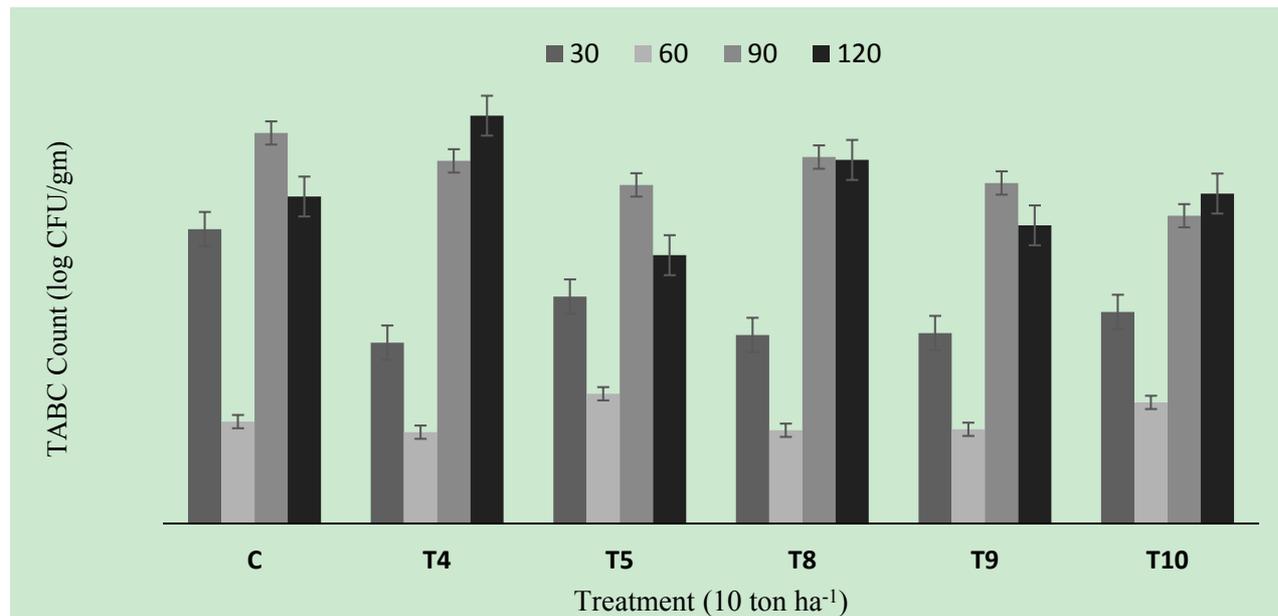


Figure 8. Total aerobic bacterial count (log CFU/g) for five different biochar applied and control soils

Among the five-biochar amended soil, highest amount of bacterial count displayed for sewage sludge biochar incubated soil (5.74 log CFU/g). All the amended soil showed lowest amount of bacterial count, which were between 2.45-2.76, log CFU/g. From the above Figure 8, the difference between different treatments with incubation days is exhibited. In the Figure 9, comparison between days of incubated and total aerobic bacterial count in log CFU/g for different treatment of biochar (10 tons ha⁻¹) and control has been showed.

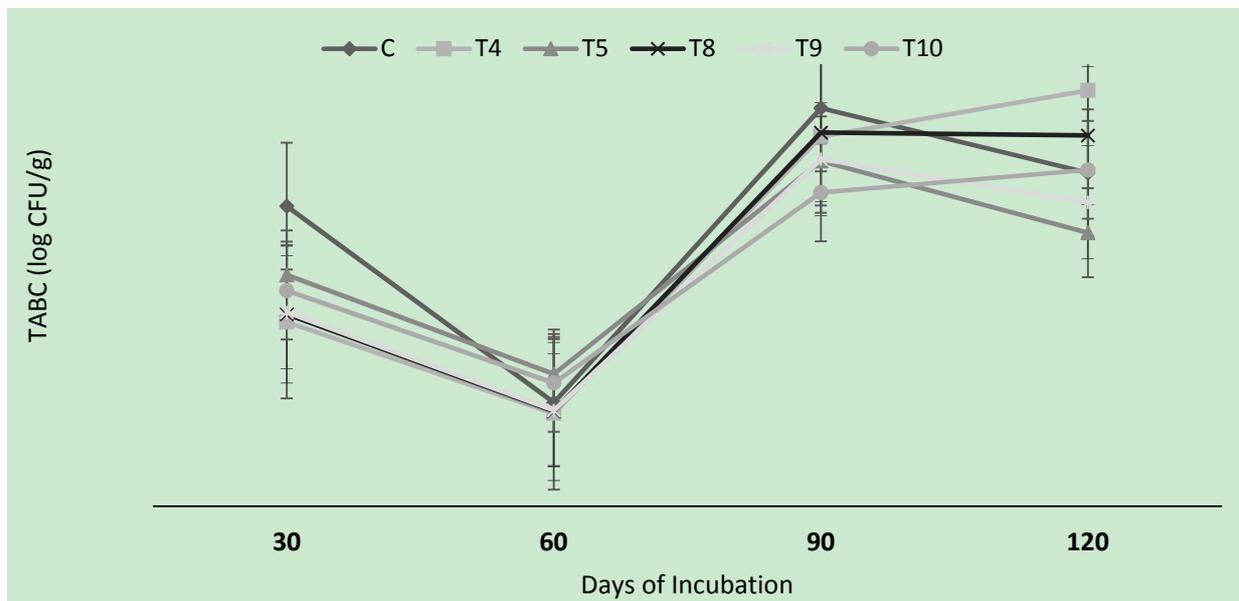


Figure 9. Comparison between days of incubated and total aerobic bacterial count in log CFU/g for different treatment of biochar (10 tons ha⁻¹) and control

In the following results of total aerobic bacterial count of biochar amended soils at a rate of 15 tons per hectare. It was observed that after 2 months of submergence bacterial count ranged 3.52-4.57 log CFU/g. In this case, sugarcane bagasse biochar amended soil was containing highest number of bacteria (6.08 log CFU/g) after 120 days of incubation. All the biochar amended soil expressed highest increase in aerobic bacteria after 2 months of dry periods.

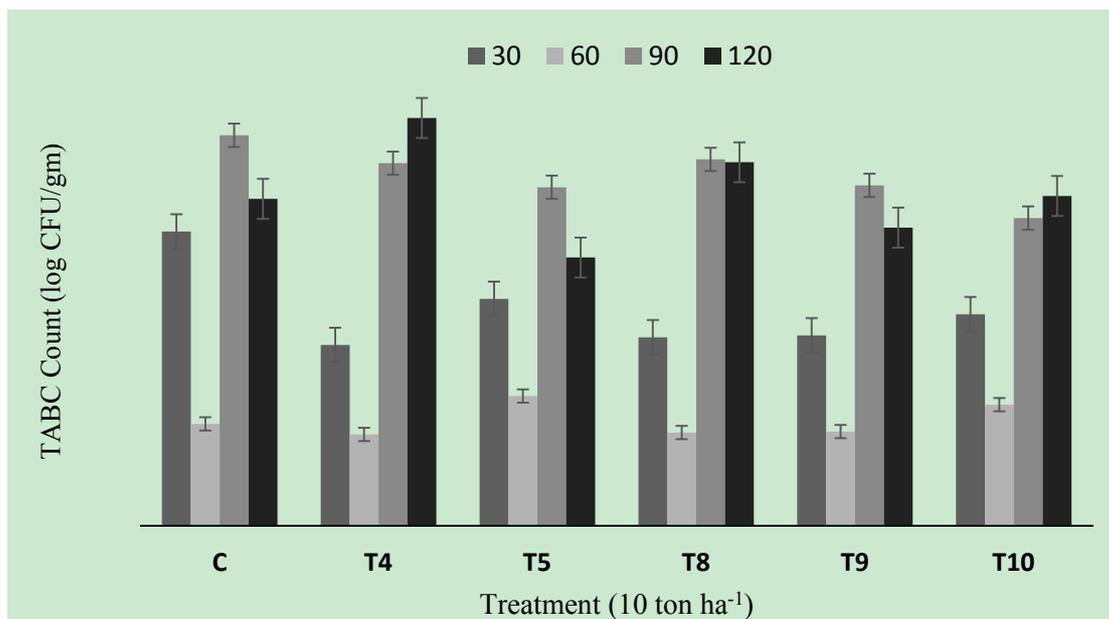


Figure 10. Total aerobic bacterial count (TABC) (log CFU/g) for five different biochar applied soils (15 tons ha⁻¹) and control soils

From Fig 10, it can be described that among five biochar amended soils sewage sludge biochar and sugarcane bagasse biochar showed highest amount of bacterial presence. Although, other biochar amended soil showed better response after 120 days of incubation and had consistence amount of bacteria through the 4 months.

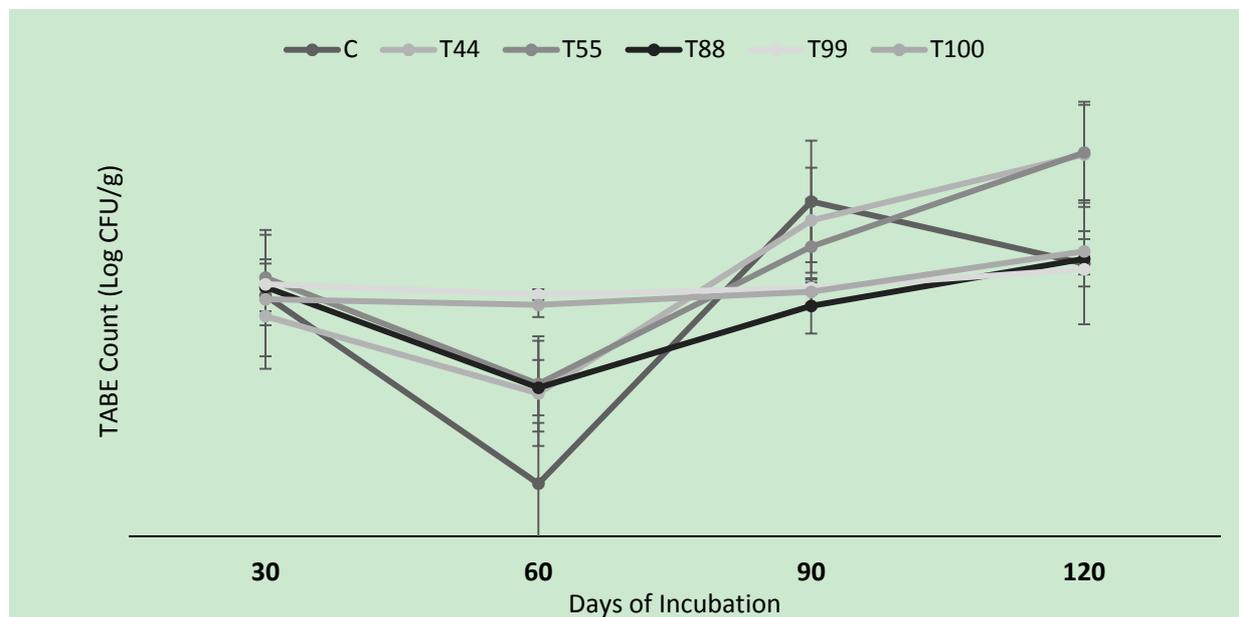


Figure 11. Comparison between days of incubated and total aerobic bacterial count in log CFU/g for different treatment of biochar (15 tons ha⁻¹) and control

Bacterial growth is higher for all of the biochar treated soils at the rate of 15 tons ha⁻¹ compared to that of the corresponding 10 tons per hectare biochar treated as well as the control soils as indicated higher number of total viable count (Figure 11). Although there was no colony in the biochars initially, however, when biochars were added to soils colonies appeared in the treated soils though the number was relatively smaller. Some scientists revealed that microbial biomass and growth increased significantly with increased addition of biochar (Rivera-Utilla *et al.*, 2001). They mentioned that bacteria sorb to biochar surfaces and become less susceptible to leaching which leads to increased bacterial abundance (Rivera-Utilla *et al.*, 2001). The ANOVA test indicates in the treatment and effects of incubation periods had highly significant ($P < 0.000$) effect on total viable count of aerobic bacteria.

3.5.2.2 Total Coliform Count (TCC) and Escherichia coli Count

No coliform or *Escherichia coli* was observed in any samples for both rates of biochar application. The coliform group of bacteria includes all the aerobic and facultatively anaerobic, Gram-negative, nonsporulating bacilli that produce acid and gas from the fermentation of lactose. The classical species of this group are *Escherichia coli* and *Enterobacter aerogenes*. Although, *E. coli* is a normal inhabitant of the intestinal tract of humans and other animals, *Ent. aerogenes* is most frequently found in grains and plants and may occur in human and animal faces. As from the study, incorporation of biochar in soil did not produce any coliform bacteria or *E. coli*, which is a positive sign for future application.

3.5.2.3 Total Fungal Colony (TFC)

The TFC gives a quantitative estimate of the concentration of microorganism such as yeast or mould spores in a sample. Fungi are abundant in soil and can survive both in acidic condition and in alkaline condition. Fungi are important in the soil as food source for other, larger organisms, pathogens, beneficial symbiotic relationships with plants and other organisms and soil health (Sylvia *et al.*, 2005). In this experiment, the effect of biochar on fungal colony was observed.

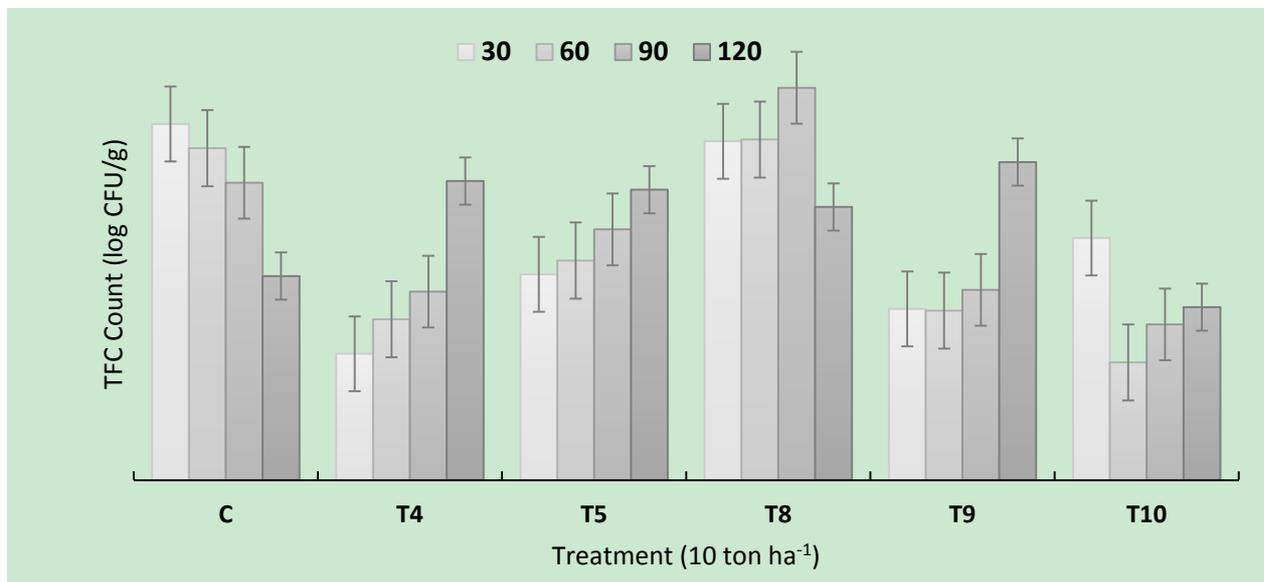


Figure 12. Total fungal colony (TFC) count (log CFU/g) for five different biochar applied soils (10 tons ha⁻¹) and control soil

It is observed from the figure that with the biochar difference total fungal colony operated highly significant ($P < 0.000$) with each other. It gives a demonstration that after 1 month of submergence control soil had the highest amount of colony 5.56 log CFU/g (Figure 12). Among the five biochar amended soils potato peel biochar showed highest amount of colony presence (5.77 log CFU/g). All through 120 days of incubation periods potato peel biochar showed consistent growth of fungal colony (Figure 15). Although, water hyacinth biochar amended soil showed highest increase in colony after 120 days of incubation (5.34 log CFU/g). Around the 120 days, incubation study the fungal colony ranged 4.18-5.77 log CFU/g (Figure 12).

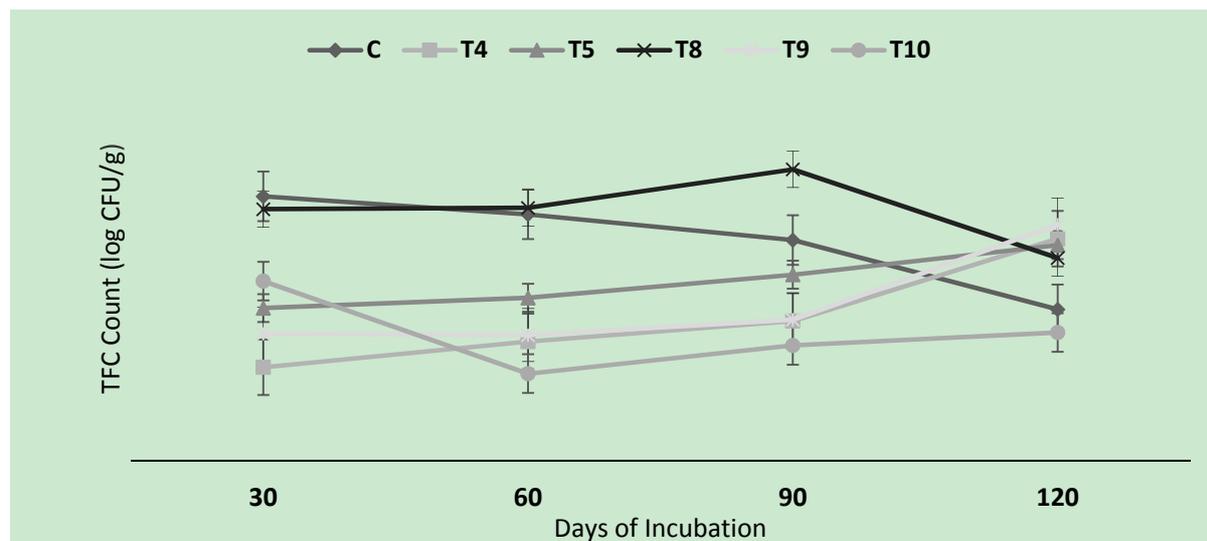


Figure 13. Comparison between days of incubated and total fungal colony in log CFU/g for different treatment of biochar (10 tons ha⁻¹) and control

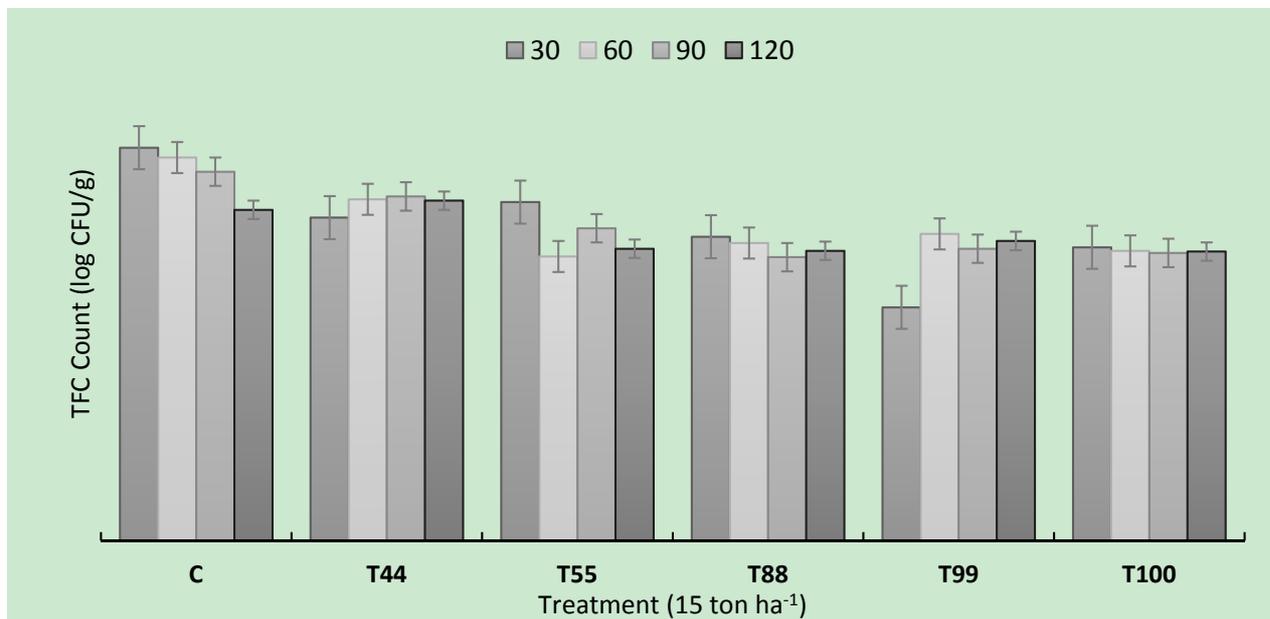


Figure 14. Total fungal colony (TFC) count (log CFU/g) for five different biochar applied soils (15 tons ha⁻¹) and control soil

In case of biochar applied at a rate of 15 tons per hectare it is observed that for total 120 days incubation period fungal colony ranged from 3.30 to 4.96 log CFU/g.

It is observed, control soil showed highest amount of colony presence after 30 days of incubation (4.96 log CFU/g; Figure 14). On the other hand, water hyacinth biochar incubated soils showed lowest amount of colony presence (3.30 log CFU/g) after 30 days of submergence. Among five biochars organic waste biochar showed lowest of colony count (4.09 log CFU/g) and it was altogether lower than other biochars.

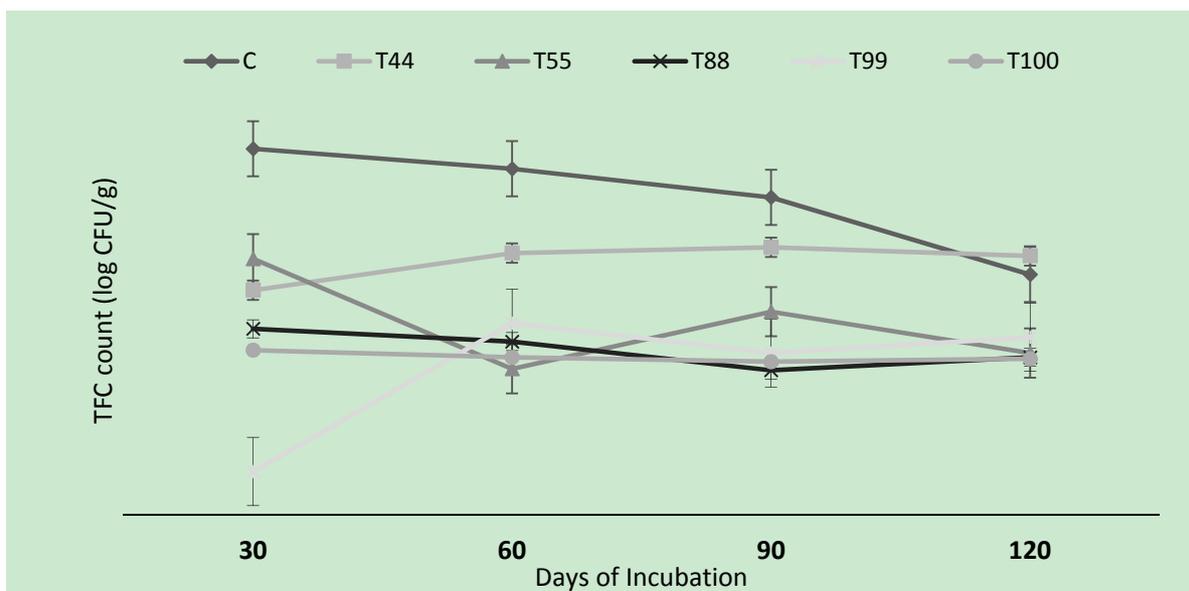


Figure 15. Comparison between days of incubated and total fungal colony in log CFU/g for different treatment of biochar (15 tons ha⁻¹) and control

Most of the environmental factors that influence the growth and distribution of bacteria and actinomycetes also influence fungi. The quality as well as quantity of organic matter in the soil has a direct correlation to the growth of fungi, because most fungi consume organic matter for nutrition (Subba Rao, 1999). In the recent study of

Verbruggen *et al.*, 2016 it was observed that biochar application to soils has potential to simultaneously improve soil fertility and store carbon that created synergistic relationship between biochar and plant growth promoting microbes, such as mycorrhizal fungi. It was also found that direct access of fungal hyphal contact to the biochar surfaces results in six time more phosphorus translocation to the host roots (Verbruggen *et al.*, 2016).

Between both the rates, it could be concluded that 10 tons ha⁻¹ showed more colonies among the biochar amended soils. However, 15 tons ha⁻¹ showed consistent growth of fungus. The ANOVA result showed that both rates are highly significant ($P < 0.000$) between biochar-amended soils.

3.5.2.4 Viable Count of *Rhizobium* spp.

The largest single contribution to biological N₂ fixation is carried out by rhizobia, which include a large group of both alpha and beta-proteobacteria, almost exclusively in association with legume (Terpolilli *et al.*, 2008). All rhizobia elicit the formation of root- or occasionally stem- nodules, plant organs dedicated to the fixation and assimilation of nitrogen. On the other hand, biochar has been used widely as a soil additive to increase soil fertility through improving the water holding capacity, soil cation exchange capacity (CEC), nutrient retention, and soil microbial and enzyme activities. Furthermore, in view of the facilitating *Rhizobium* survival, biochar can provide a habitat for microbes due to its porous structure, which has a high internal surface area and high ability to adhere to soluble organic matter (Lehmann *et al.*, 2011). The viable count of *Rhizobium* spp. in biochar-applied soils at a rate of 10 tons per hectare. It demonstrated that the treatment had highly significant effect on viable count ($P < 0.000$) and the effects of incubation periods ($P < 0.05$) was significant at a lower level (Figure 16). It is to be noted that, after incubation of 30, 60, 90 and 120 days under different field moisture condition, all soil receiving biochar treatment showed higher growth that control soil. The reason could be that the bacteria remained dormant initially in soil.

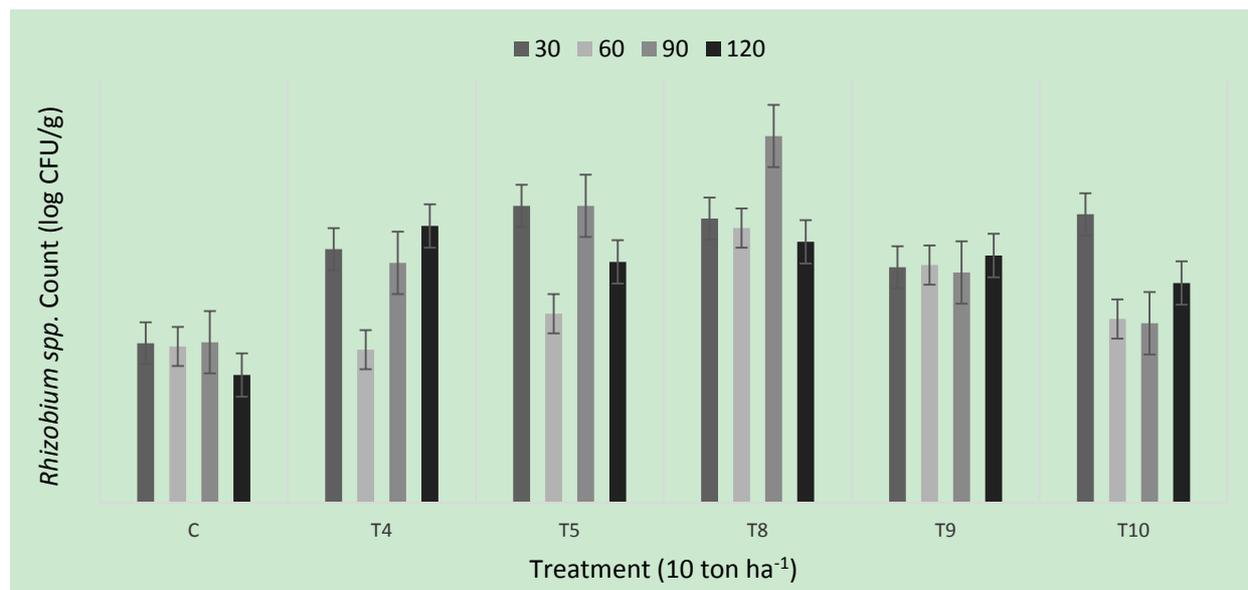


Figure 16. *Rhizobium* spp. (log CFU/g) for five different biochar applied (10 tons ha⁻¹) & control soils

The highest amount of *Rhizobium* colony counted (6.47 log CFU/g) for potato peel biochar amended soil after 90 days of incubation (Figure 19). Nevertheless, the colony count was drastically reduced after 1 month to 5.47 log CFU/g. It might be due to the fact that *Rhizobium* are able to use NH₄⁺ and NO₃⁻ ions as a nitrogen source but when char is added ion utilization is being hampered (Spokas *et al.*, 2012). Other biochar amended soil showed increase in colony after 120 days of incubation (Figure 17).

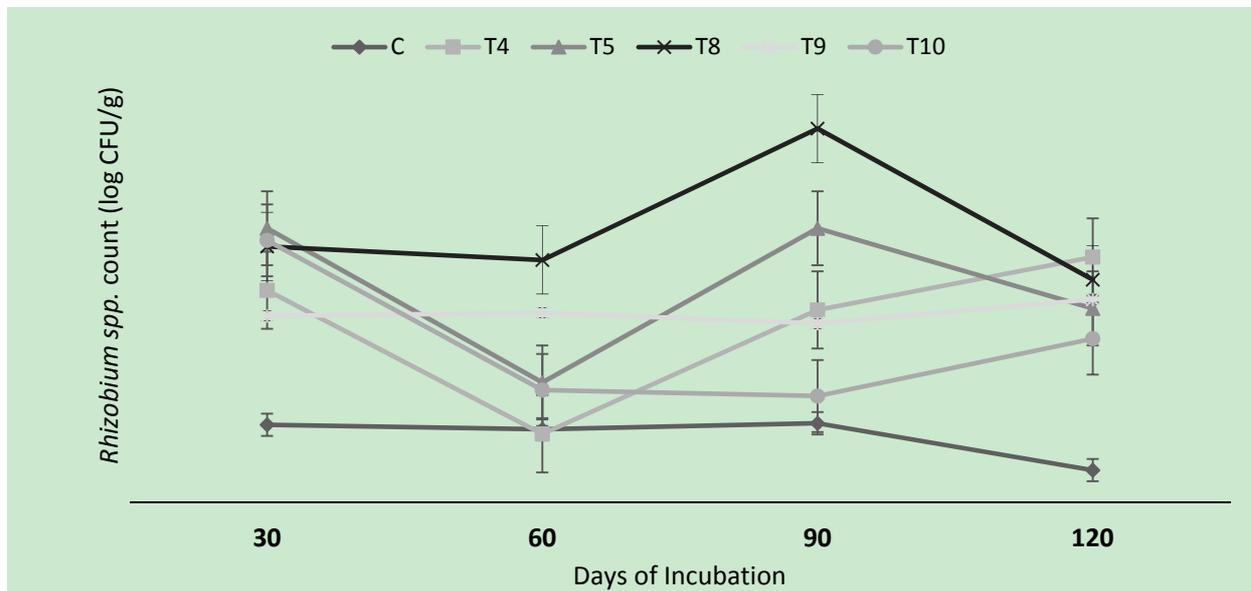


Figure 17. Comparison between days of incubated and *Rhizobium spp.* in log CFU/g for different treatment of biochar (10 tons ha⁻¹) and control

The colony count of *Rhizobium spp.* when biochar added at a rate of 15 tons ha⁻¹. It showed the treatment had highly significant effect on viable count ($P < 0.000$) and the effects of incubation periods ($P < 0.000$) was also highly significant (Figure 18).

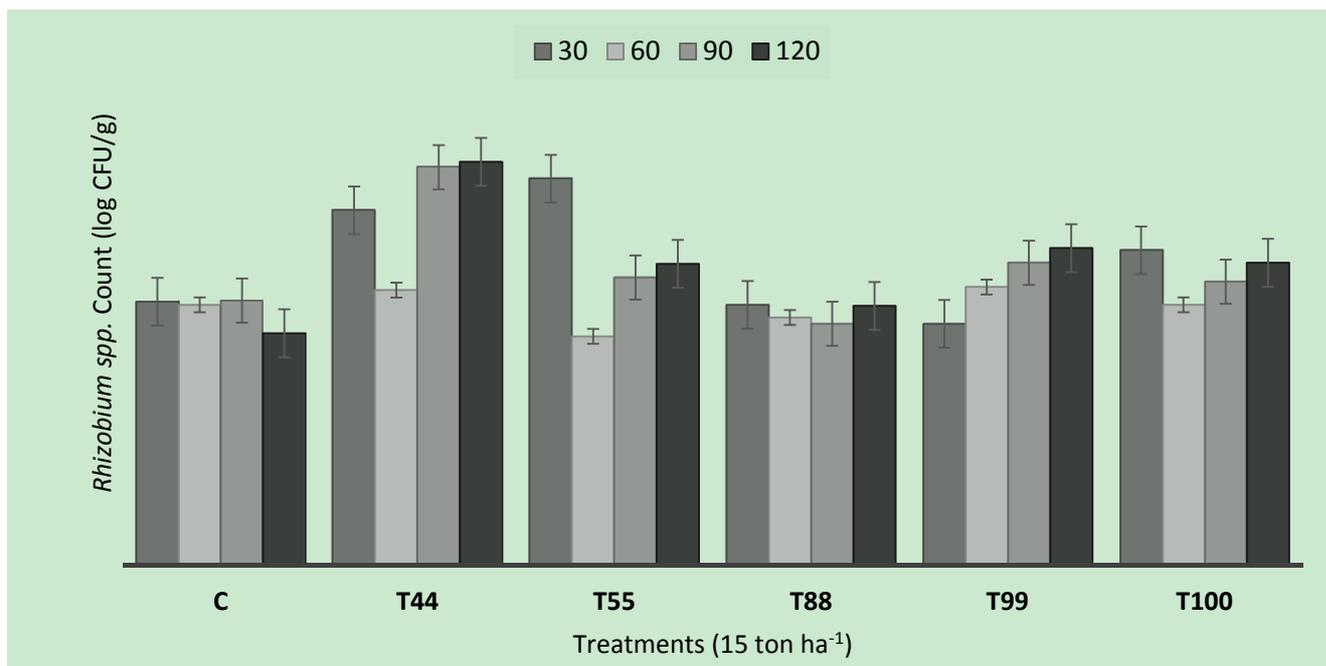


Figure 18. *Rhizobium spp.* (log CFU/g) for five different biochar applied (15 tons ha⁻¹) & control soils

It is clear that in both rates control soil had the lowest amount of *Rhizobium* colonies. On the contrary, sewage sludge biochar incubated soil showed highest amount (5.84 log CFU/g) of colony presence after 120 days of incubation. It was observed after 120 days that was 2 months of dry condition colony count increased both for control soil and biochar amended soil. All the soil ranged 4.47-5.84 log CFU/g colony (Figure 18 and 19).

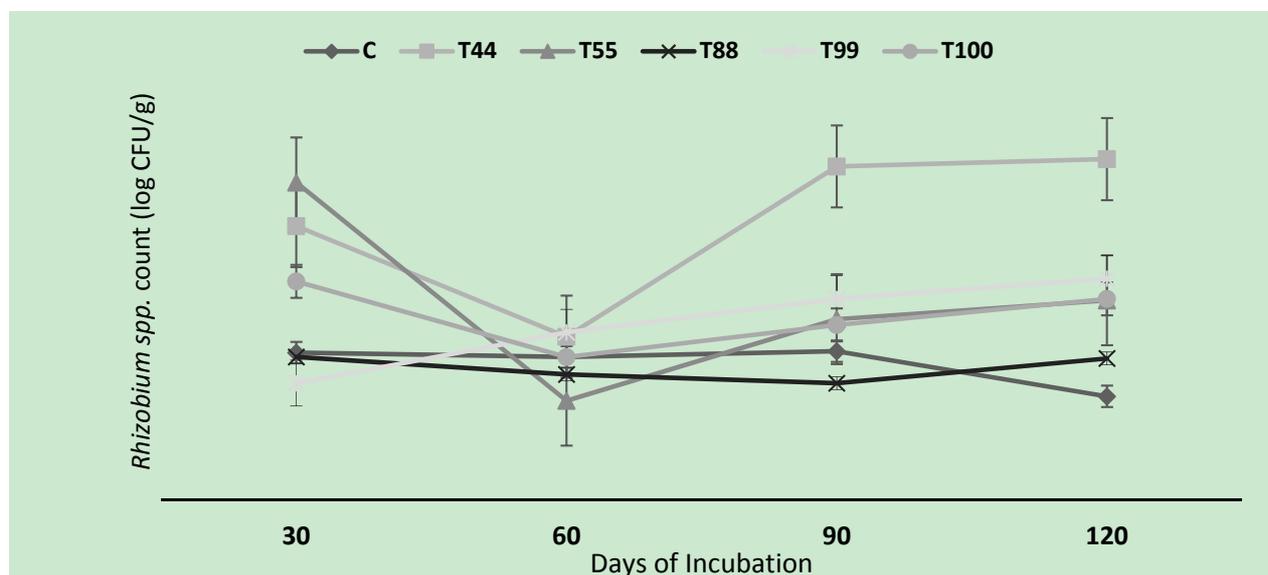


Figure 19. Comparison between days of incubated and *Rhizobium spp.* in log CFU/g for different treatment of biochar (15 tons ha⁻¹) and control

The main factors that affect the increase of *Rhizobium spp.* are available nitrogen and pH in neutral condition. Although biochar can create some unexpected changes in bacterial colony. Eckmeier *et al.* (2007) reported that BNF is negatively affected due to char as it adversely affects nitrogen-fixing bacteria particularly *Azotobacter* and *Bradyrhizobium*. Rillig *et al.* (2010) found char to drastically alter pH level and thereby directly affect chemical reaction of *Bradyrhizobium* that leads to reduced colony count. Nevertheless, it did not comply similarly with the experiment. For few biochar amended soil showed increase in no. of colony compare to control soil. The ANOVA test demonstrated significant ($P < 0.05$) effects for both rates and for four incubation days individually. The biochars had significant ($P < 0.000$) differential effect on the viable count of *Rhizobium spp.*

3.5.2.5 Viable Count of *Azotobacter spp.*

Aerobic bacteria belonging to the genus *Azotobacter* represent a diverse group of free-living diazotrophic (with the ability to use N₂ as the sole nitrogen source) microorganisms commonly occurring in soil (Martyniuk and Martyniuk, 2003). Their main property is the ability to fix nitrogen non-symbiotically, with a genomic content of G-C of 63-67.5% and distributed in the soil, water and sediment (Jiménez *et al.*, 2011). In the experiment, using biochar as a soil amendment promoting both N₂ fixation and growth of *Azotobacter*. As biochar is characterized by high porosity on the surface area, which may provide additional pore space for water and microbes for proliferation (Glodowska *et al.*, 2017). It has been reported that population of *Azotobacter* associated with chickpea was higher in a soil-charcoal mixture indicating the suitability of this material as an inoculant carrier (Egamberdieva *et al.*, 2018). In this research a wide range of beneficial bacterial species have been found in abundance among them *Azotobacter* is one of them. Biochar applied in the experimental soil at two different rates (10 and 15 ton per hectare). Five different wastes derived biochar's were used to amend the experimental soils.

The control soil showed lowest amount of colony count 2.08 log CFU/g after 30 days of incubation (Figure 20). Although, after 120 days of incubation colony count increased to 2.85 log CFU/g. It may be explained in terms that *Azotobacter* is sensitive to acidic pH (Jnawali *et al.*, 2015). It supported that the treatment had highly significant effect on viable count ($P < 0.000$) and the effects of incubation periods ($P < 0.000$) was also highly significant. Among the five biochar amended soils potato peel biochar showed highest amount of colony count (5.60 log CFU/g), but it was in the submerged condition (Figure 20). The pH increases due to submergence and biochar altogether increased growth of *Azotobacter*.

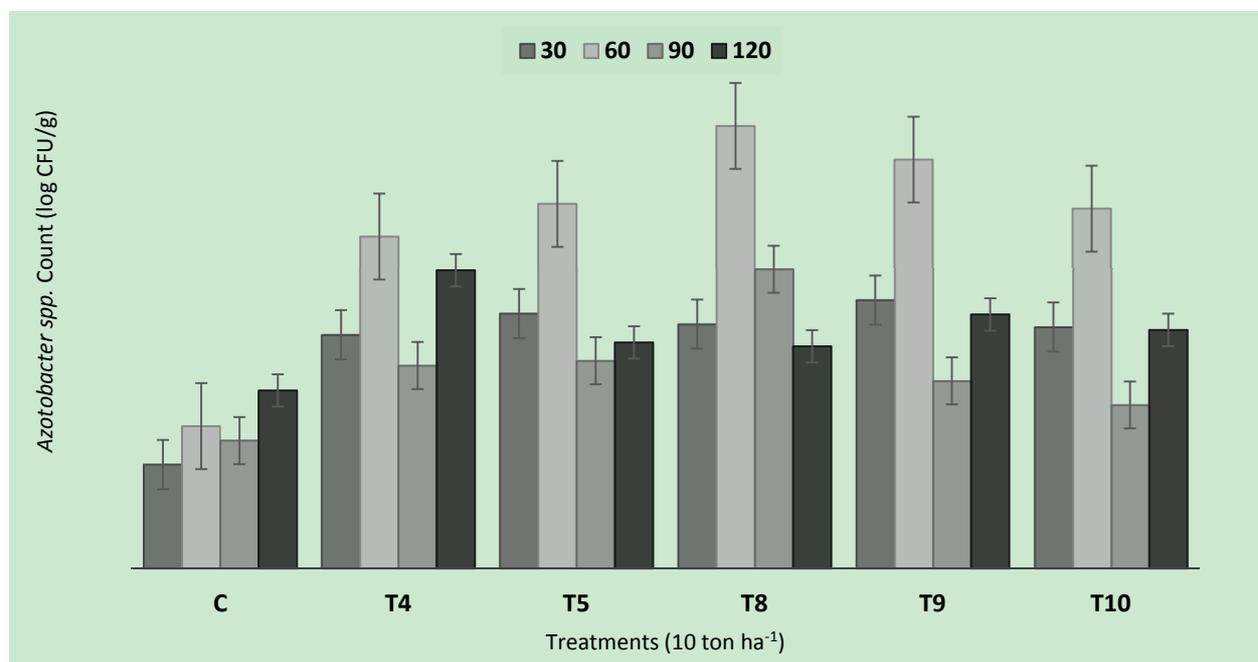


Figure 20. *Azotobacter spp.* (log CFU/g) for five different biochar applied (10 tons ha⁻¹) & control soil

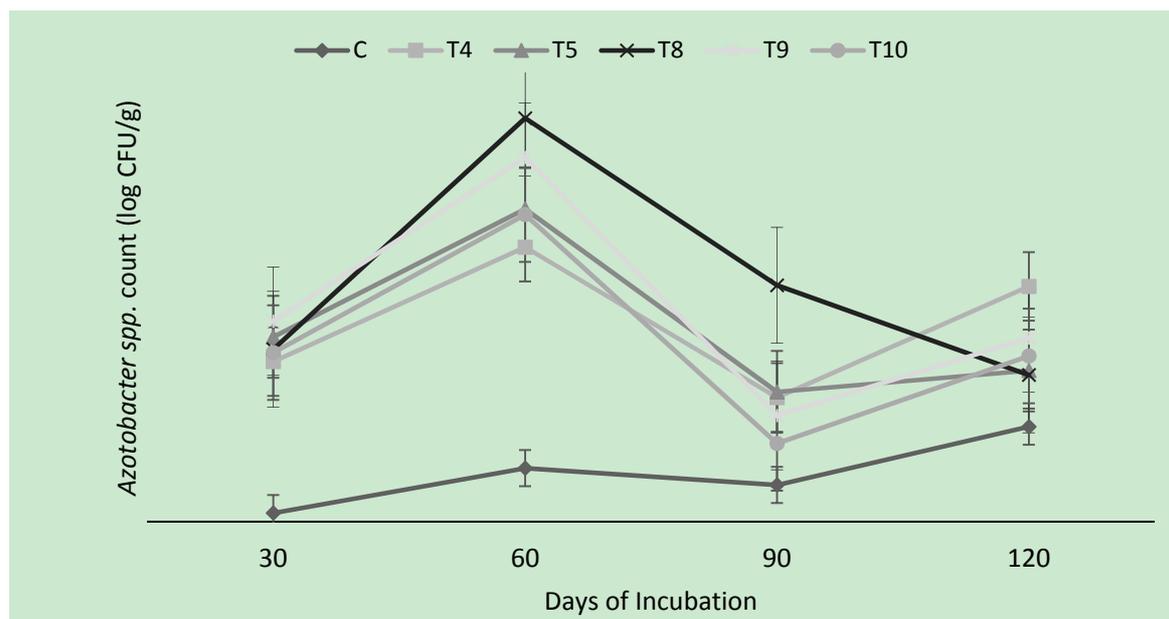


Figure 21. Comparison between days of incubated and *Azotobacter spp.* in log CFU/g for different treatment of biochar (10 tons ha⁻¹) and control

All the biochar amended soil had increased colony count after 60 days of incubation and 2 months of submerged under water ranging from 4.45-5.60 log CFU/g. On the other hand, after the dry condition began, the growth started to reduce. After 90 days of incubation, the colony count ranged 2.33-4.11 log CFU/g (Figure 21). Three biochar amended soils demonstrated increase in colony count after 120 days of incubation (Figure 24). Sewage sludge biochar, water hyacinth biochar and organic waste biochar were found higher growth after 2 months of dry periods. It can be explained in terms of biochar-induced stimulation of microbial activity, along with change in microbial community, composition and structure (Gomez *et al.*, 2014). Results showed a demonstration that the treatment had highly significant ($P < 0.000$) effect on viable count and the effects of incubation periods was also highly significant ($P < 0.001$).

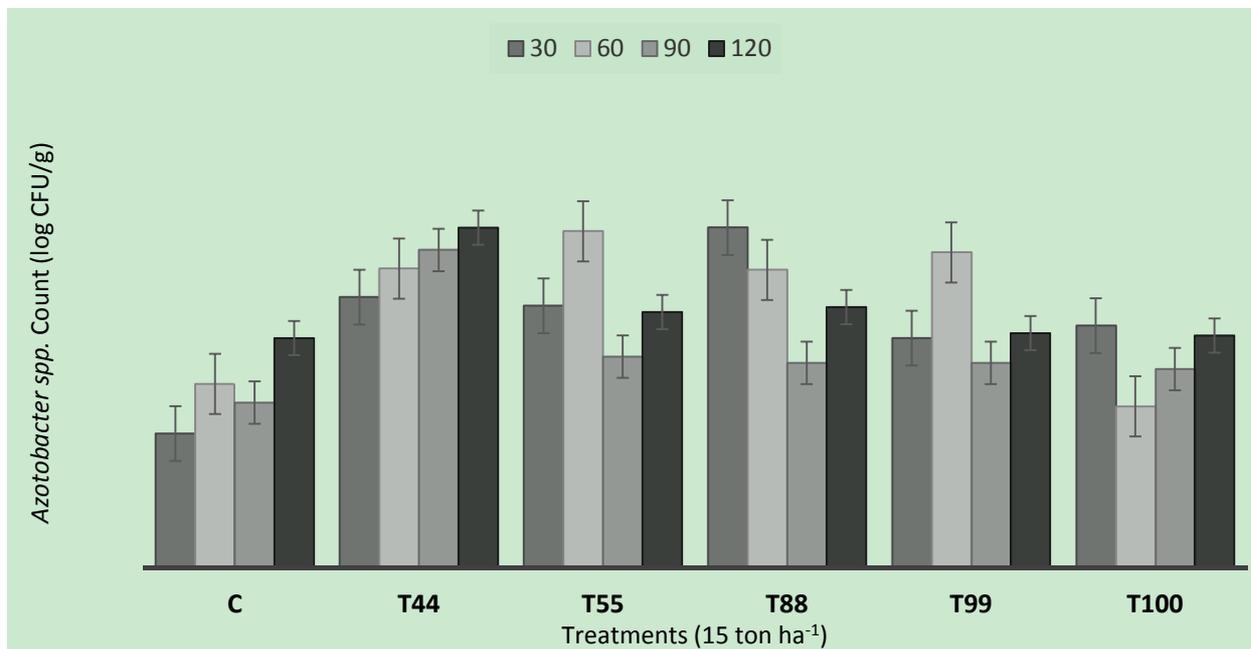


Figure 22. *Azotobacter* spp. (log CFU/g) for five different biochar applied (15 ton ha⁻¹) & control soils

All the biochar amended soil had higher amount of colony after 120 days than other incubation day (Figure 22). Count of *Azotobacter* increased in biochar treated soil after 120 days of incubation, might be due to the fact that *Azotobacter* are able to use NH₄⁺ or NO₃⁻ ions as a nitrogen source from added biochars. Although it is visual that after 90 days of incubation all the biochar amended soil showed lesser amount of colony, ranging 2.60-3.56 log CFU/g. Eckmeier *et al.* (2007) suggested that BNF is negatively affected due to char as it adversely affects nitrogen fixing bacteria particularly *Azotobacter* and *Bradyrhizobium*. Rilling *et al.* (2010) found char to drastically alter the pH level and thereby directly affect chemical reaction of *Azotobacter*, which leads to reduced colony count.

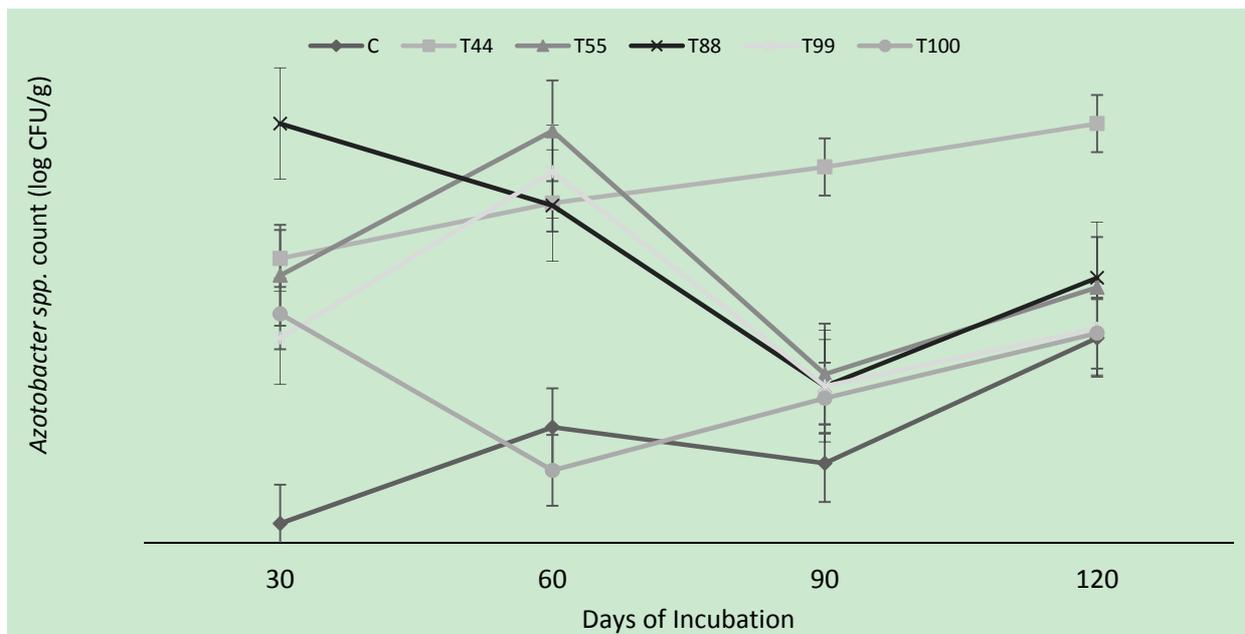


Figure 23. Comparison between days of incubated and *Azotobacter* spp. in log CFU/g for different treatment of biochar (15 tons ha⁻¹) and control

Research findings can be suggested that compare to potato peel biochar and organic waste biochar; sewage sludge

biochar had an excellent performance in promoting the growth of most bacterial strains (Figure 24), which might be due to the favorable physico-chemical properties of sewage sludge biochar (Yang *et al.*, 2019). Biochar as a pH-neutralizing agent can promote growth of *Azotobacter* by making soil neutral to alkaline pH. Although biochar reported to be positively or negatively affected soil microbial abundance (Lu *et al.*, 2017). The effect of biochar addition on the growth of *Azotobacter* showed differently for each case and variation seen between rates. Other than that, statistically both rates do not have significant difference from each other, which expressed that other factor like soil moisture, pH and type of biochar may alter the growth.

3.5.2.6 Viable Count of Phosphate Solubilizing Bacteria (PSB)

Phosphate solubilizing bacteria capable of solubilizing inorganic phosphorus from insoluble compounds (Chen *et al.*, 2006). P-solubilizing ability of rhizosphere microorganisms is considered one of the most important traits associated with plant phosphate nutrition. On the contrary, biochar is a potential phosphorus source for the mitigation of phosphorus depletion. However, the chemical and biological effects of the release of P from biochar is still unclear. The relationship between PSB and biochar as a P-source is still on verge of discovery. A number of factors, such as pH and ionic strength, influences the behavior of biochar P in soil (Silber *et al.*, 2010). However, no reports were found about the P solubilizing in biochar by various phosphorus solubilizing bacteria. The following study was carried out to find the count of colonies influencing and enhancing the solubilizing of slightly soluble or insoluble P, such as CaHPO_4 , FePO_4 and AlPO_4 , as well as their corresponding pyrophosphates, in pyrolytic biochar application in experiment soil.

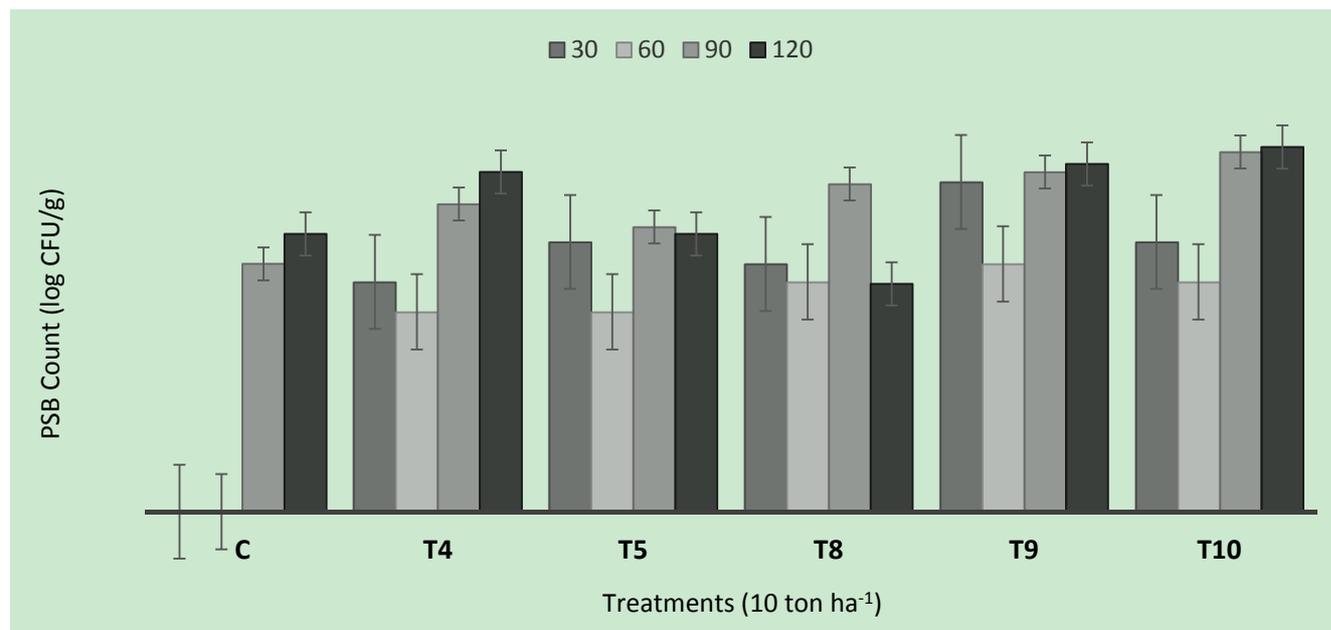


Figure 24. Viable count of PSB (log CFU/g) for five different biochar applied soils (10 tons ha^{-1}) and control soils

Research findings are exhibited that microbes significantly ($P < 0.05$) affected P leaching from the biochar amended soil (Figure 24). As for control soil it was revealed < 1.0 log CFU/g colony in the first 2 months of incubation. Although, after 1 month of dry period the colony growth was found and after 120 days of incubation it was 2.78 log CFU/g. All the five biochar-amended soil showed increase in growth after first 30 days but all of them reduced after 2 months of submerged condition. This phenomenon may be accounted for the discrepant effects on P solubilization from biochar (Xu *et al.*, 2019). The suppressing effects of other microorganism who used phosphate as oxygen source may also cause it.

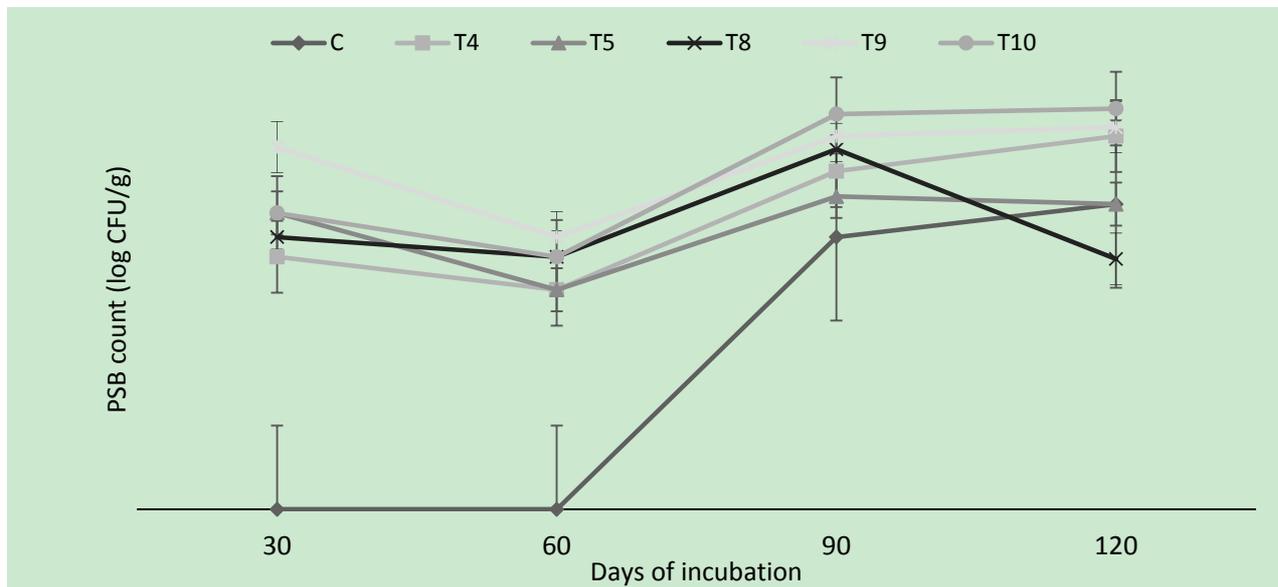


Figure 25. Comparison between days of incubated and viable count of PSB in log CFU/g for different treatment of biochar (10 tons ha⁻¹) and control

On the other hand, after 120 days of incubation all the treatment showed great increased in colony count. The highest growth was observed in organic waste biochar incubated soil (3.65 log CFU/g). The PSB were found higher when compared to phosphate solubilizing bacteria in control soil in the incubation days (Figure 25). The results expressed highly significant ($P < 0.000$) with the biochar types and also with the incubation days.

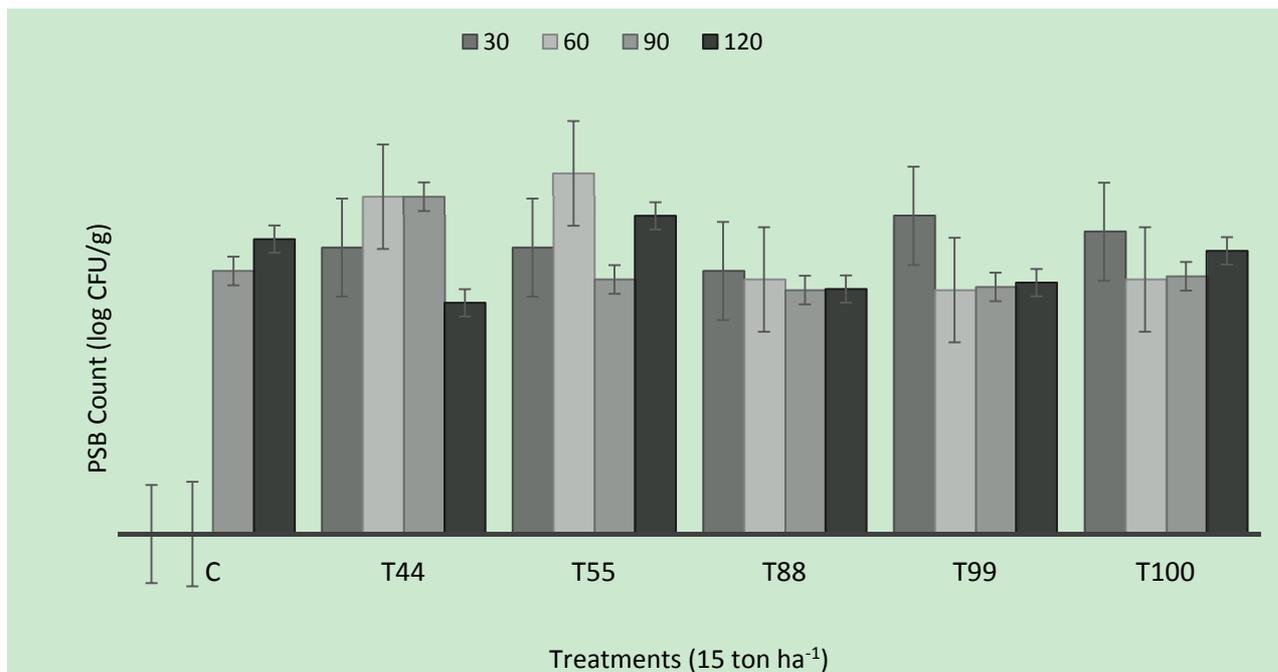


Figure 26. Viable count of PSB (log CFU/g) for five different biochar applied soils (15 tons ha⁻¹) and control soils

The PSB colony count displayed higher in sugarcane bagasse biochar treated soil after 60 days of incubation (3.4 log CFU/g; Figure 25). In case of 15 tons ha⁻¹ of biochar application after 30 days PSB colony count was showing higher than other incubation days. As phosphorus exists extensively as organic and inorganic forms in biochar

feedstocks (e.g. manure and sludge), PSB have been shown to enhance the dissolution of insoluble P compounds (Li *et al.*, 2018). PSB have the potential to assist biochar decomposition and biochar are able to facilitate their metabolism due to the improved surface functional groups, surface charge, and porosity (Chen *et al.*, 2019). Although from the present experiment, it was observed that after 120 days of incubation four treatment showed increase in colony ranging 2.31-3.0 log CFU/g. Only sewage sludge biochar treated soil showed reduced result as colony count reduced from 3.18 to 2.18 log CFU/g after 120 days of incubation (Fig 30). Previous studies showed that the stability of phosphate solubilizing bacteria in terms of biochar application are related to the type of the P-containing compounds, the number of Fe/Al-containing compounds, and the functional groups of biochar (Huang and Tang, 2016). Although reduction of colony could be due to the lowering of pH and subsequent increase in iron oxidizing bacteria.

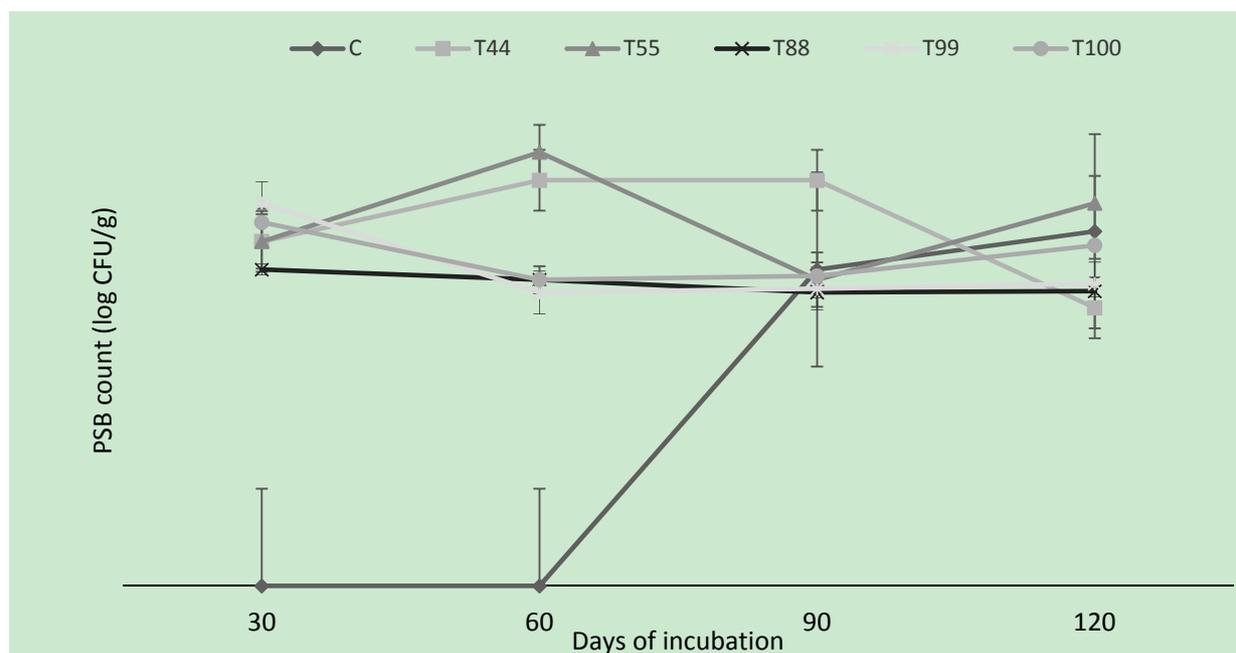


Figure 27. Comparison between days of incubated and viable count of PSB in log CFU/g for different treatment of biochar (15 tons ha⁻¹) and control

To apply biochar in soil as a P-fertilizer, it is crucial to understand the interaction between biochar-P and soil microorganisms. In this research, five biochars produced at different temperature were incubated at two different rates in soil. The result demonstrated that the phosphate solubilizing bacteria have increased after biochar application and it demonstrated that the biochars had highly significant ($p < 0.001$) effect on PSB growth. In research, it was revealed that the release of biochar-P was the main cause to increase in colony of PSB and the dissolution of the P-containing compounds helped them to regain. Metaphosphate and P-associated with C in biochar are the main P species in biochar, which was utilized by phosphate solubilizing bacteria (Goswami *et al.*, 2019). Although due to P-containing compounds with good crystal, structure and high polymerization degree which can affect the PSB to use the phosphorus within biochar and can reduce the colony count. In other words, phosphate solubilizing bacteria can play a regulating role on biochar-P. It could not only enhance the release of biochar-P and utilize all types of the released P (i.e. Ortho-P and Pyro-P) in biochar, but also release the P back to the environment.

3.5.2.7 Viable Count of Phosphate Solubilizing Fungi (PSF)

Phosphate-solubilizing microorganisms are ubiquitous, whose number vary from soil to soil. In soil, phosphate-solubilizing bacteria constitute 1-50 % and fungi 0.1-0.5 % of the total respective population. Generally, the phosphate-solubilizing bacteria outnumber phosphate-solubilizing fungi by 2-10 times (Khan *et al.*, 2010). Generally, the P-solubilizing fungi exhibit the greater P-solubilizing activity as they produce more acids than bacteria (Goswami *et al.*, 2019). The relation between biochar and PSF is not clear yet. Although, biochar-P is utilized by phosphate solubilizing microorganism but specific relationship between PSF and biochar is not observed yet. After 120 days of incubation periods and biochar types the colony count has showed no significant difference, which indicate that due to change in biochar type or rate do not effect on PSF growth.

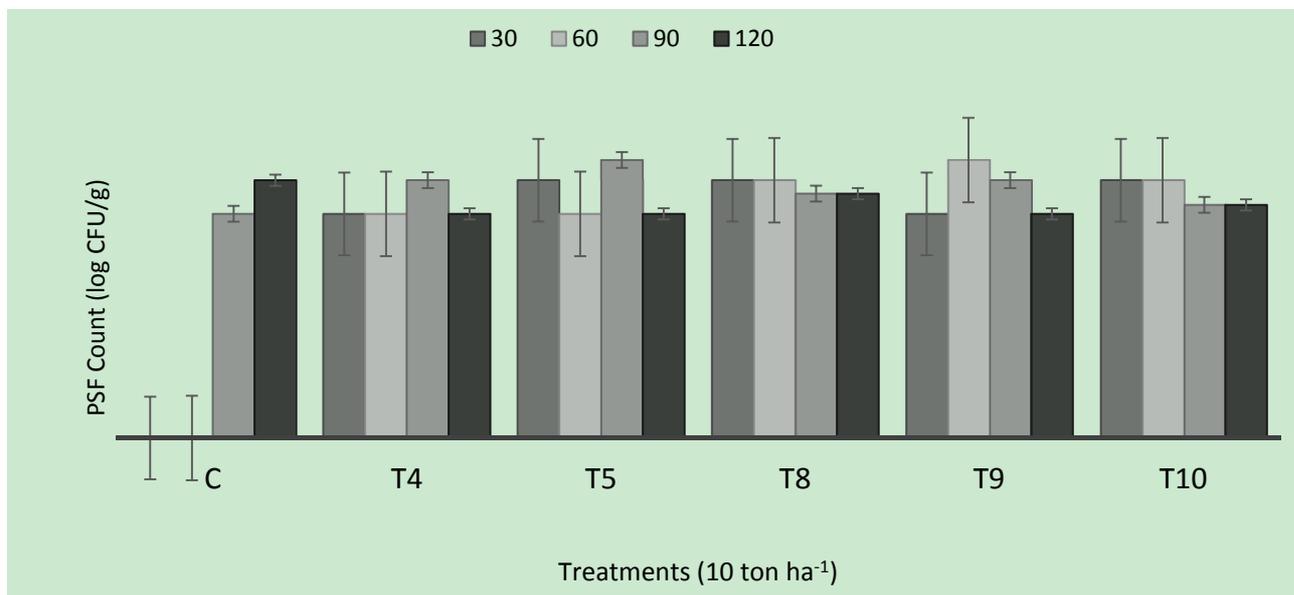


Figure 28. Viable count of PSF (log CFU/g) for five different biochar applied soils (10 tons ha⁻¹) and control soil

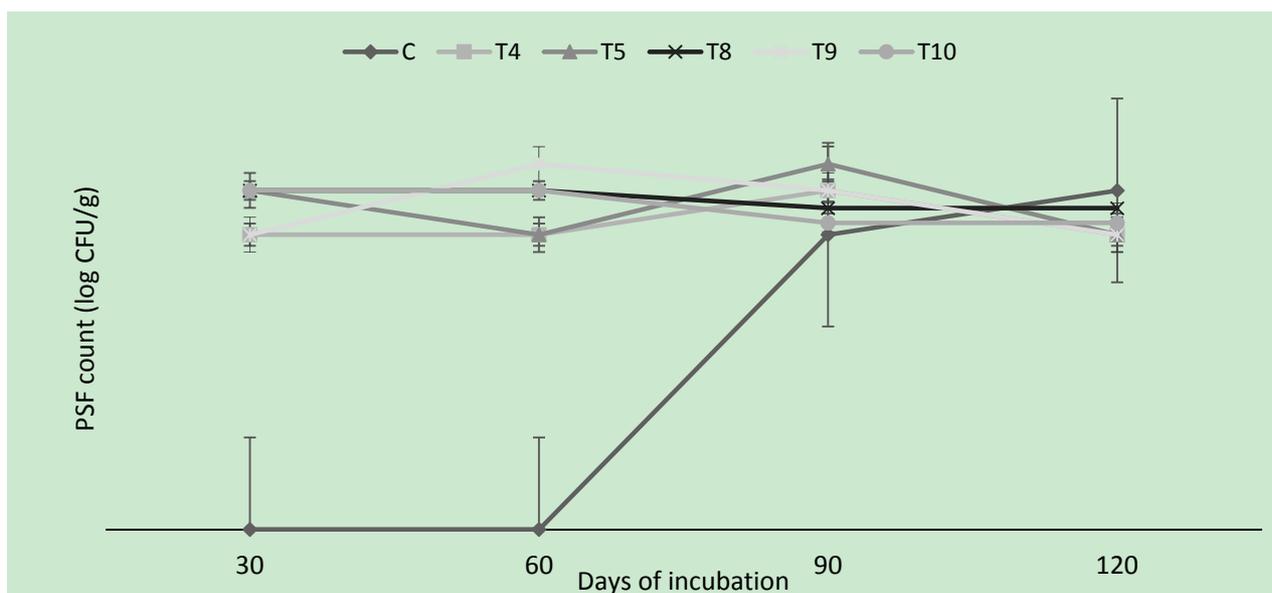


Figure 29. Comparison between days of incubated and viable count of PSF in log CFU/g for different treatment of biochar (10 tons ha⁻¹) and control

The highest colony count found after 60 days of incubation for water hyacinth biochar treated soil (2.48 log CFU/g; Figure 29). Most of the biochar showing similar result in terms of colony count. Control soil had no growth before 90 days of incubation. Only 120 days of incubation result showed significant ($P < 0.05$) difference in colony count with biochar type.

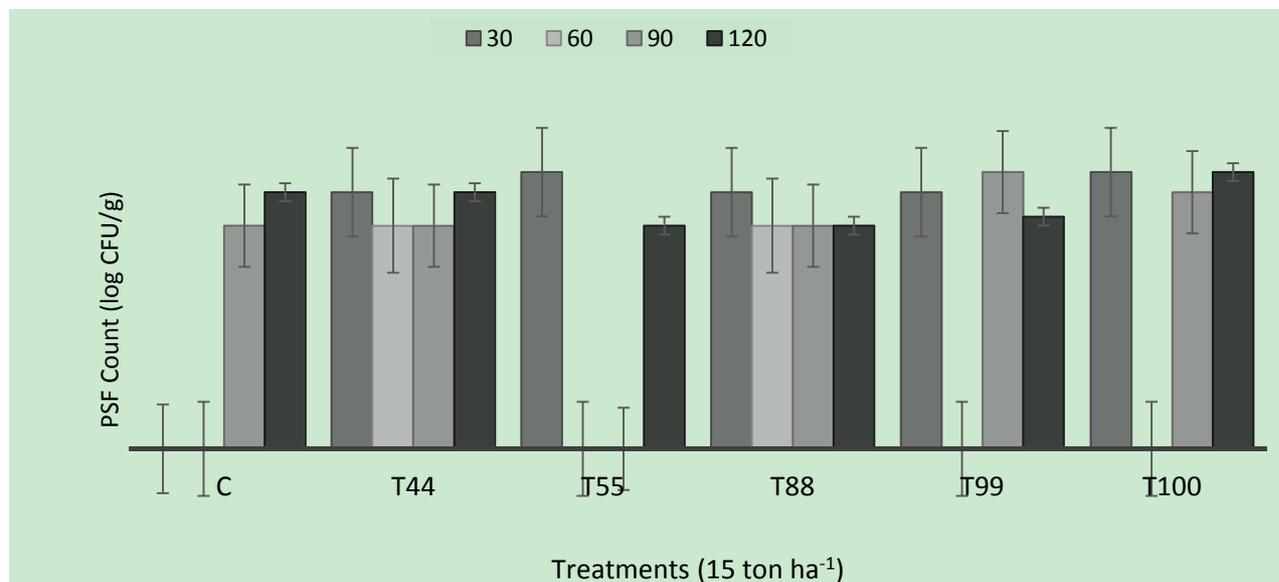


Figure 30. Viable count of PSF (log CFU/g) for five different biochar applied soils (15 tons ha⁻¹) and control soils

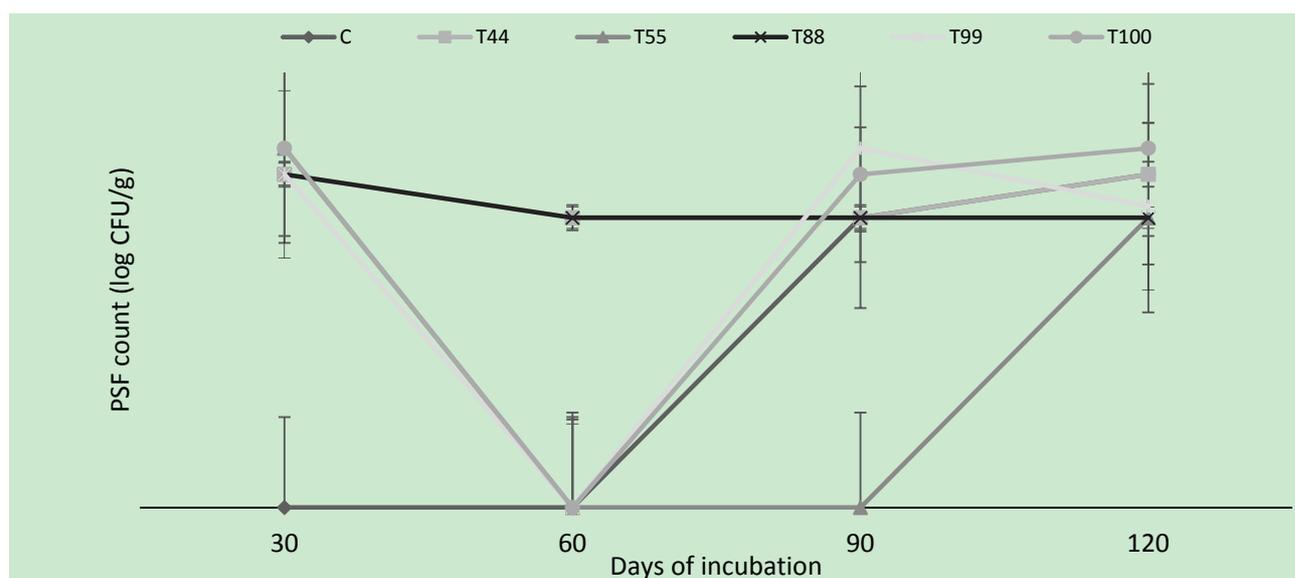


Figure 31. Comparison between days of incubated and viable count of PSF in log CFU/g for different treatment of biochar (15 tons ha⁻¹) and control

It was observed that control soil, sugarcane bagasse biochar, water hyacinth biochar and organic waste biochar showed <0.1 log CFU/g colony (Figure 30). Although, all of the treatment showed similar growth of PSF after 120 days of incubation. Between both rates there was no significant difference found after ANOVA test for PSF growth. In most of cases, biochar supplies a favorable habitat for bacteria growth through cell sorption (Palansooriya *et al.*, 2019) but for fungi, the direct relationship between cell adsorption rate and growth stimulation has not been investigated.

3.5.2.8 Viable Count of *Pseudomonas* spp. and *Trichoderma* spp.

Pathogen pressure is a major cause of yield loss in agricultural systems and an important factor affecting the structure and productivity of natural plant communities. Bacteria antagonistic to plant pathogens are known to reduce plant infection (Allan *et al.*, 2010). These bacteria have been extensively studied in agricultural systems where they significantly contribute to soil suppressiveness (Weller *et al.*, 2002). Many biocontrol strains of fluorescent *Pseudomonas* produce extracellular secondary metabolites that inhabit the growth of fungal pathogens

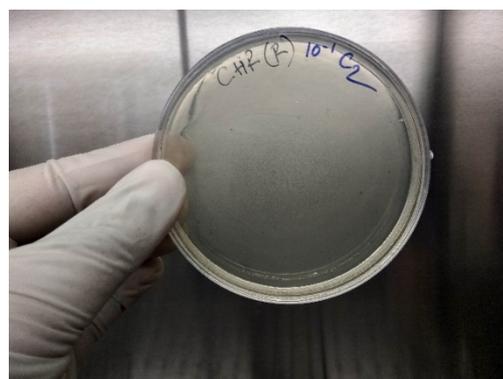
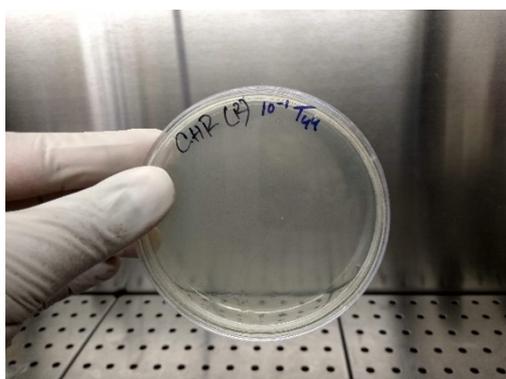
and account for part of the disease-suppressive activity. Although, its existence in the initial soil and in experimental soil had not been found. It indicates that these biochar-amended soils were somehow unable to protect the crop from plant pathogens. The occurrence of *Pseudomonas spp.* is necessary in utilizing phosphate by the plant, but none of the sample was found containing *Pseudomonas spp.* Therefore, *Pseudomonas spp.* should be increased on the soil for improving the soil microbial health.

Trichoderma spp. are free-living fungi that are common in the soil and root ecosystems. *Trichoderma spp.* is an important bio-control microorganism with *Trichoderma harzianum* protecting plants against many soil-borne pathogens- *Fusarium*, *Pythium*, *Rhizoctonia* promoting the growth of several crops including bean (Harman, 2011). In experiments made obvious that some isolates of *Trichoderma spp.* produce hydrolytic enzymes, which may destroy the cell wall components of many microorganisms (Chandra *et al.*, 2009). Nevertheless unfortunately, none of the biochar treated soils and control soil sample were detected with *Trichoderma spp.*, it would be a great concern to use as the *Trichoderma* and *Pseudomonas spp.* as a good soil health accelerator. As *Trichoderma spp.* is one of the potential plant pathogen inhibitors, thus presence of them will provide important information on the biochar application.

Representative photos of cultured petri dish of selective and non-selective media:



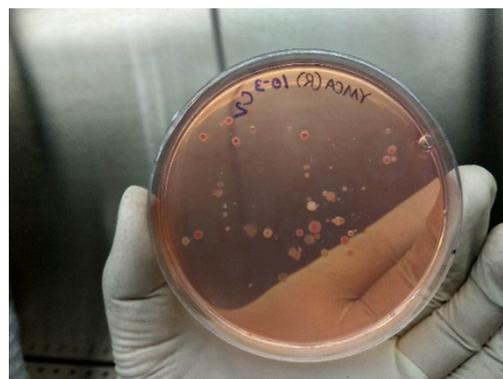
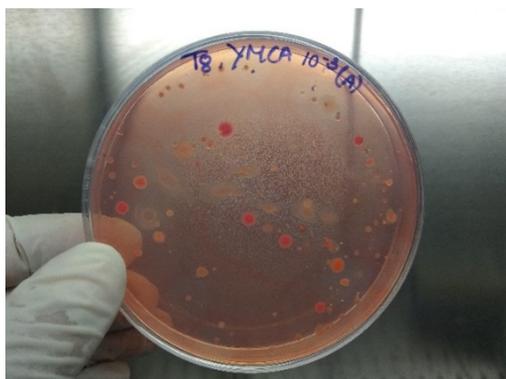
Total Aerobic Bacterial count (TABC) on PCA plate



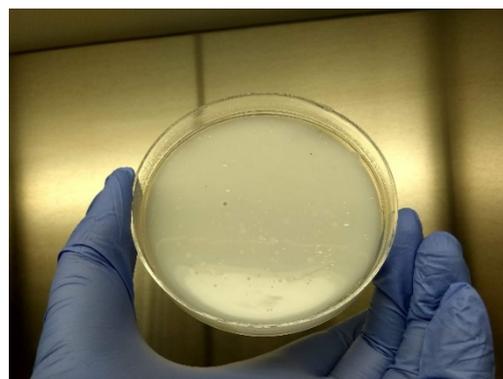
Total Coliform count (TCC) on Chromocult media



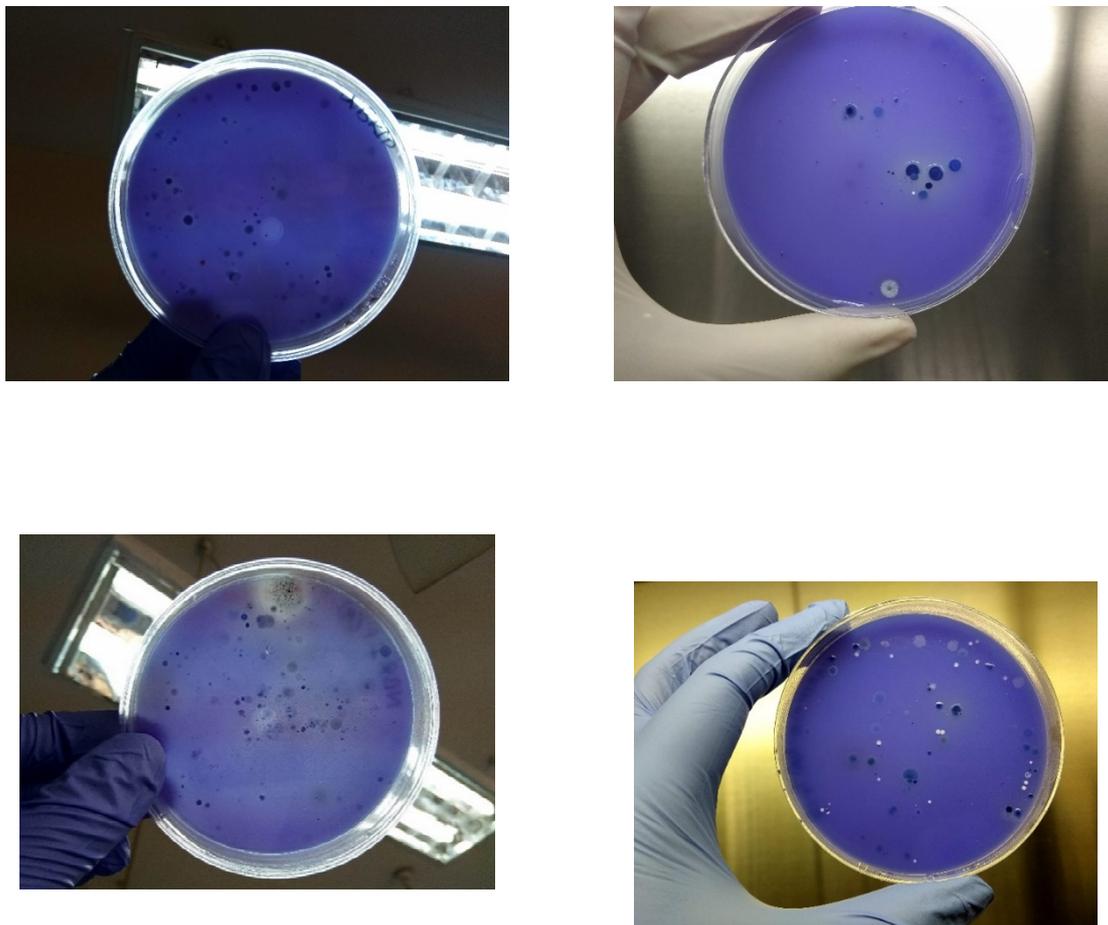
Total Fungal Count (TFC) on SDA media



Rhizobium spp. on the YERCA agar media



Azotobacter spp. on Ashby Mannitol Agar media



PSB and PSF on NBRIP Media

*Pseudomonas spp.* on Cetrinide Agar Media

Figure 32. Microbial analysis of control soil and biochar incubated soils

5. Conclusion

Research results revealed that waste-derived biochar treatment in the soil significantly ($P < 0.05$) improved the abundance of ammonification bacteria, aerobic nitrogen-fixing bacteria and phosphorus solubilizing bacteria and reduced harmful pathogenic bacteria as, *Escherichia coli*. In the initial biochar samples, no microbial count was observed. After incubation periods, there was a change in the total viable count even than the pre-soil itself. Nonetheless, soil moisture content operated a crucial part in the bacterial growth and significantly ($P < 0.001$) affected their colony counts. Total aerobic bacteria count deteriorated sharply during submergence but after 60

days of dry periods colony count improved. Sewage sludge biochar amended soil showed promising maximum increase of aerobic bacteria in both rates. The opposite image was seen in the case of total fungal colony count. As fungi can survive in all types of conditions, soil acidity did not cause any difference in their availability. On the other hand, only organic waste biochar caused a decrease in fungal count after 120 days of incubation, which can be used to suppress unnecessary fungal effects on plants. Rhizobium spp. is one of the most important bacteria whose presence can provide a beneficial effect on agricultural soil. The application of biochar demonstrated a significant ($P < 0.001$) increase in colony count than in the control soil. Soil moisture condition did not show any significant difference but dry condition provided improvement in bacterial colony count. Sewage sludge biochar amended soil resulted highest increase in colony after 120 days. Another nitrogen-fixing bacterium Azotobacter whose presence is also important for plants as they fix N_2 non-symbiotically. In the study, using biochar as a soil amendment promoted both N_2 fixation and increased the colony count of Azotobacter. The presence of Azotobacter spp. was found low in control soil but biochar application significantly ($P < 0.05$) increased colony count. Best treatment resulted when sewage sludge biochar was applied in the dry condition. A similar result was found for Phosphate Solubilizing Bacteria and Phosphate Solubilizing Fungi whereas soil moisture condition and biochar rate difference could not provide any extra benefit. It was unfortunate and a negative side that two important soil beneficial microorganisms such as Pseudomonas spp. and Trichoderma spp. not found in the biochar amended soils.

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