Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 13-24
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IMPACT OF CO-APPLICATION OF BIOCHAR AND *PSEUDOMONAS AERUGINOSA* ON MICROBIAL PARAMETERS IN HEAVY METAL CONTAMINATED SOIL

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Received: 15-01-2024
Accepted: 06-02-2024

https://dx.doi.org/10.4314/sa.v23i2.2

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: *Faculty of Science, University of Port Harcourt.*

ABSTRACT

The use of biochar in remediation of heavy metal contaminated soil has gained global attention in the last decade. However, there is a need for more studies on the effects and interaction of biochar and functional microbes on the resident soil microorganisms and enzyme activities in the soil. This study, therefore investigated the effects of co-application of Adenopus breviflorus (Christmas melon) derived biochar and a heavy metal tolerant Pseudomonas aeruginosa on microbial population, bacterial diversity and enzyme activities in soil artificially spiked with cadmium, copper and lead in a pot experiment. Christmas melon seeds were collected from farms in Ago-Iwoye and subjected to pyrolysis to produce biochar which were modified with acid and base. Treatments included control, acidic biochar (B_A), co-application of acidic biochar and Pseudomonas aeruginosa (B_AP_S), basic biochar (B_B), co-application of basic biochar and Pseudomonas aeruginosa (B_BP_S), and Pseudomonas aeruginosa (P) alone. Microbial parameters were analyzed before and after treatments. The results obtained showed that treatments, particularly B_BP_S and B_APs, showed significant increases in microbial populations compared to the control. The predominant bacteria isolated were Pseudomonas spp.and Bacillus spp. Catalase and urease activities varied across treatments, with B_B treatment demonstrating the highest catalase activity (94.80 ± 4.90 $mgKMnO_4$ kg^{-1}). Urease activity was highest in the B_APs treatment (0.410 ± 0.091 $mgNH_4^+$ kg^{-1} h^{-1}).In conclusion, the co-application of biochar and Pseudomonas aeruginosa reduced heavy metals, boosted microbial populations, and increased enzyme activities in the soil. This strategy holds promise for mitigating soil contamination and promoting sustainable agriculture.

Keywords: Biochar; *Pseudomonas aeruginosa*; microorganisms enzyme activity, soil health

INTRODUCTION

Agricultural soils play a pivotal role in sustaining food production and ensuring global food security. However, the health and productivity of these soils are increasingly compromised by various factors, including the accumulation of heavy metals and the deterioration of soil microbial communities. Heavy metal contamination, stemming from industrial activities and improper waste disposal, poses serious threats to both

ecosystem integrity and human health (Sonone *et al*., 2020). Concomitant with this challenge, the degradation of soil microbial parameters diminishes nutrient cycling efficiency and weakens soil resilience, undermining agricultural productivity (Bai *et al*., 2023).

To address these pressing issues, the integration of sustainable agricultural practices becomes imperative. One approach that has gained considerable attention is the co-application of biochar and beneficial microorganisms. Biochar, a carbon-rich material derived from the pyrolysis of organic matter, has been recognized for its potential to improve soil structure, retain nutrients, and mitigate heavy metal availability (Xu *et al*., 2023). Complementing this, the utilization of beneficial microorganisms, such as *Pseudomonas aeruginosa*, holds promise for enhancing soil microbial activity, promoting nutrient cycling, and aiding the bioremediation of heavy metal-contaminated soils (Mishra *et al*., 2020).

Adenopus breviflorus, commonly known as Christmas melon or the short-flowered adenopus, is a tropical leguminous plant. In Nigeria's middle belt, *Adenopus breviflorus* (ADB) (family Curcubitaceae) is cultivated. It has climbing stems, much like other members of the curcubitacae family do. When it reaches maturity, it produces fruits with different numbers of seeds. The seeds are protected by a thin shell that may be readily removed by hand after drying. Although the anti-implantation action of the fruit has been demonstrated, the value of the seeds is uncertain (Oyedeji and Olorunsogo, 2020). Although numerous studies have been conducted using various types of biochar with various modifications, none have yet been done using any part of the *Adenopus breviflorus* plant, which is cost-effective given that it is a common plant in west Africa and the seeds are typically discarded as waste after the fruit is used.

Soil microorganisms, comprising of bacteria, fungi, archaea, viruses, and other microscopic organisms, form a diverse and complex community that plays a vital role in soil health and ecosystem functioning (De Vries *et al*., 2023). These soil organisms are responsible for various essential processes, including nutrient cycling, organic matter decomposition, disease suppression, and plant-microbe interactions (Fan *et al.*, 2018). Soil microorganisms are instrumental in the cycling of essential nutrients, such as carbon, nitrogen, phosphorus, and sulphur. They participate in the decomposition of organic matter, releasing nutrients that are then made available for plant uptake. Bacteria and fungi, in particular, are key players in organic matter decomposition. For instance, bacteria belonging to the genus *Bacillus* have been found to efficiently degrade plant residues and contribute to carbon mineralization in the soil (Schloter *et al*., 2018). Similarly, arbuscular mycorrhizal fungi form mutualistic associations with plant roots, aiding in the uptake of nutrients, especially phosphorus (van der Heijden *et al.,* 2015). Soil microorganisms also play a crucial role in disease suppression. Some bacteria and fungi produce antimicrobial compounds that inhibit the growth of pathogens. For example, certain strains of the bacterium *Pseudomonas fluorescens* produce antibiotics, such as pyoluteorin, which suppress the growth of soil-borne pathogens like *Pythium* and *Fusarium* (Raaijmakers and Mazzola, 2012).

Soil enzyme activity is a crucial indicator of soil health and plays a fundamental role in the functioning of terrestrial ecosystems. Enzymes are proteins produced by soil microorganisms and plants that facilitate biochemical reactions involved in nutrient cycling, organic matter decomposition, and overall soil functioning (García *et al*., 2016). High enzyme activity is indicative of a fertile soil with efficient nutrient cycling processes (Sahu *et al.*, 2017). Soil enzyme activity is closely linked to soil fertility. Enzymes involved in nutrient cycling, such as phosphatases, improve the availability of phosphorus by breaking down organic phosphorus compounds into plant-available forms (Wang *et al*., 2023). Similarly, the activity of urease enzymes influences nitrogen

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 13-24
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availability by catalyzing the hydrolysis of urea, a common nitrogen fertilizer, into ammonium (NH_4^+), which can be taken up by plants (Tabatabai, 1994). Among the diverse group of microorganisms used in bioremediation, *Pseudomonas,* a genus of Gram-negative bacteria widely distributed in soil environments have gained significant attention due to their versatility and robust degradation capabilities (Alkorta *et al*., 2019).

Most of the previous researches on the use of biochar had focused on the potential of this remediation strategy on heavy metal contamination in soil. However, too little study has been reported on the synergetic effects of co- application of biochar and functional microbes on microbial parameters in soil that are responsible for soil fertility and soil health. This present research therefore aimed to investigate the combined impact of biochar and *Pseudomonas aeruginosa o*n microbial parameters in soil.

MATERIALS AND METHODS

Preparation and modification of *Adenopus breviflorus* seed biochar

Christmas melon (*Adenopus beviflorus)* seeds were chosen for biochar preparation because it is rich in carbon and readilyavailable in local farms in Ago-Iwoye town. The plant material was washed under running water and carefully opened to obtain the seeds. The seeds were further washed with de-ionized water and dried at 30^{o}C for pyrolysis into biochar. *Adenopus breviflorus* seeds were placed in the furnace and heated at a rate of 20°C per minute until reaching a temperature of 500°C. The seeds were then held at this temperature for 5 hours. The resulting biochar was subsequently ground and sieved through a 2-mm mesh sieve (Huang *et al*., 2019; Ji *et al*., 2019). The biochar was modified in order to enhance its adsorption efficiency. An acidic and alkali modification of biochar was applied in this study; the produced biochar was placed in a beaker containing 2.0M of H_2SO_4 and agitated for 5 hours then allowed to dry at room temperature to obtain the acidic biochar (Hemavathy *et al*., 2020) while for the alkali, the same process was repeated using NaOH (Liu *et al*., 2020).

Soil sample collection

The soil used for the experiment was collected from Olabisi Onabanjo University College of Agricultural Sciences Tree Crop Nursery Development Project located in Ago-Iwoye, Ogun State, Nigeria between Latitudes 6°55' and 7°00N and between Longitudes 3°45 and 4°05E. Soil samples were taken at random using soil auger from 8-10 places at a depth of 0-15 cm. The soils were bulked put into sterile polythene bags and transported to the laboratory and greenhouse for laboratory analysis and pot experiment respectively. Plant materials and other debris present were removed by hand and the soil was air-dried and sieved using a 2.0 mm mesh to remove other smaller plant debris and stones.

Physicochemical properties analysis of soil and biochar

The physicochemical properties of the soil before treatment and biochar were analyzed using standard procedures (Association of Official Analytical Chemists, 2016).

Determination of pH of soil and biochar

Ten (10) gram of the sample was dissolved into 250 ml beaker containing distilled water, with a calibrated pH meter (HANNA Multimeter I1398 MODEL), the pH of the solution was then taken (AOAC, 2016)

Determination of Moisture Contents

To determine the moisture content, 1 g of the sample was weighed into a clean beaker which was labeled W1 and placed in an oven, for about 2 hrs at 105^{o}C to a constant weight, it was then placed in a desiccator to cool and prevent it from being exposed to moisture. The beaker was then reweighed and labeled W2. The difference in weight indicates the amount of water loss contained in the sample.

$$\% \text{ Moisture Content} = \frac{W1 - W2}{W1} \times 100$$

WI – Weight of the original sample

W2 – Weight of the sample after oven dry (Harris, 2010)

Determination of Ash Content

An empty crucible was weighed and labeled W1, Two (2) g of the sample was then weighed into the crucible. This was then placed in a muffle furnace at 450^{o}C for 4 hrs. The crucible was then removed, placed in a desiccator and reweigh as W2.

$$\%\,\text{Ash Content} = \frac{W2 - W1}{\text{Weight of sample(2g)}} \times 100$$

WI – Weight of empty crucible

W2 – Weight of crucible after ash (AOAC, 2016)

Determination of Nitrogen and Crude Protein

This involves three stages: digestion, distillation and titration

Digestion Stage

A 0.2 g of sample was weighed into a filter paper and gently transferred into a round bottom Kjeldahl flask. Then 25 ml of Conc. H_2SO_4, was added. On addition, a dark brown solution was observed. Further, 0.3 g of Kjeldahl tablet ($CuSO_4$ + Na_2SO_4 (1:1)) was added. The mixture was then digested for 1hr until a clear colorless solution is obtained. This was then made up to 100 ml with distilled water in a standard flask (Christian, and O'Reilly, 2013).

Distillation Stage

After the digestion stage, 10 ml of the aliquot (digest) was pipette and introduced into a round bottom 250 ml distill flask, 0.5g of NaOH was added, plus anti- bumping agent. In another flask, 50 ml of boric acid was prepared and screen methyl red indicator was added. The distillation was set up as with the outlet of tube inserted into the conical flask containing the boric acid for the collection of NH_3 through the condenser. As the nitrogen gas is being given off as NH_3, the color changes from red to green (Skoog *et al*., 2017).

Titration Stage

The distillate was titrated with 0.1M HCl to give the percentage of Nitrogen

$$\%\,\text{Nitrogen} = \frac{\text{TV} \times 0.1\text{M} \times 0.0014 \times 100}{\text{Weight of sample}}$$

TV = Titre Value; 0.0014 = Molarmass of Nitrogen/ 100 (Miller and Miller, 2010).

Determination of Total Carbon Content (Walkley-Black Wet Oxidation Method)

To determine the total carbon content, 0.5 g of the dry sample (powdered) was weighed into a 250 ml flask, 10 ml of 0.167 M $K_2Cr_2O_7$ was added to the sample; Twenty (20) ml of concentrated sulphuric acid was further added, agitated and allowed to attain equilibrium for 30 minutes. Thereafter, 200 ml of deionized water was added with 10 ml Phosphoric acid. To the solution 10 -15 drops of diphenylamine indicator was added and the colour changes from orange to violet and then titrated with 0.5 M Fe $(NH_4)_2(SO_4)_2$ to give a permanent green colour. A blank sample was also conducted.

$$\%\,\text{Carbon} = \frac{(B_{TV} - \text{TV}) \times 0.5\text{M} \times 0.003 \times 100}{\text{Weight of sample}}$$

B_{TV} – Blank Titre value; Tv – Titre value (Walkey and Black, 1934)

Heavy metal analysis of soil

Acid Digestion

Twenty-five (25) ml each of the samples was placed into a 250ml beaker (in duplicate) and 10 ml of Aqua regia (1:3 HNO_3 and HCl) was added. It was then placed on a heating mantle at 105^{o}C and heated for 45 minutes until the entire particle are completely digested and colorless. The sample was then filtered and makes up to 50 ml in a standard flask with distilled water (AOAC, 2016).The concentration of metals in the solution was analyzed using Buck scientific atomic absorption spectrophotometer (model 210A). Blank and standards were run after five

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 13-24
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determinations to calibrate the instrument (Haware and Pramond, 2011).

$$\text{Actual Concentration} = \frac{\text{Machine Concentrationx Volume makeup}}{\text{Volume of sample taken}}$$

Presented in Table 1 is the basic properties of the soil and biochar before the incubation experiment.

Soil incubation experiment and treatment design

A pot experiment was carried out in the green house of Department of Plant Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State. The dimension of each pot was (22cm x 25cm x18cm). Two kilogram (2 Kg) dried soil was used to fill each pot. The experimental soil was spiked artificially with 30 ppm per treatment using Cd $(NO_3)_2$, Pb $(NO_3)_2$ and $CuSO_4$ as cadmium, lead and copper sources, respectively. The spiked soils were left for 2 weeks to age before adding the different treatments (Haider *et al*., 2022).

Source and Preparation of Inoculum

The bacterium used in this study was isolated from Olabisi Onabanjo University College of Agricultural Sciences Tree Crop Nursery Development Project farm in Ago Iwoye ,Ogun State Nigeria Based phenotypic and molecular characterization, the strain was identified as *Pseudomonas aeruginosa* , The organism was screened for heavy metal tolerance and showed a high tolerance to in cadmium, copper and lead containing media.Pure culture of this bacteria was obtained from Microbiology Laboratory , Olabisi Onabanjo University, Ago-Iwoye. The organism was sub-cultured in an Erlenmeyer flask after which it was mixed thoroughly with the heavy metal contaminated soil in designated pots for co-application treatments and *Pseudomonas* treatment. Each treatment was carried out in triplicate making a total of 18 pots by following a completely randomized design; a pot without any remediation treatment (biochar and microorganism) was classified as the control.

EXPERIMENTAL DESIGN

Treatments were designated as follows:

Ctrl - Control
B_A – Acidic modified biochar
B_AP_S – Co-application of acidic modified biochar and *Pseudomonas aeruginosa*
B_B – Basic modified biochar
B_BP_S – Co-application of basic modified biochar and *Pseudomonasaeruginosa*
P_S – *Pseudomonas aeruginosa*

Microbiological analysis of soil samples

Bacterial population in the soil before treatment and after treatments were estimated using serial dilution and pour plate method on nutrient agar (Oxoid UK®) at 10^{-6} dilutions for bacteria and incubated at 35°C for 48 h. Similarly, fungal population in the soil were estimated using the same dilution (10^{-4}) inoculated on potato dextrose agar (Oxoid UK®) and incubated for 72 h at 35°C. Colonies on the culture plates were counted using a Gallenkamp colony counter. Bacterial isolates were characterized based on morphological, cellular and biochemical characteristics as described by the Bergey's manual of systematic bacteriology (Holt *et al.*, 1994).

Soil enzyme activity analysis

Soil enzyme activities before and after treatment were measure using the methods described by Tabatabai (1994). Urease activity (mg NH_4^+ kg^{-1} h^{-1}) was measured by quantifying the release of NH_4^+ during the hydrolysis reaction after incubating the samples with urea (1%) for 3 hours at 38°C. Catalase activity (mg $KMnO_4$ kg^{-1}) was determined by titrating the reduction of H_2O_2 using 0.1 M $KMnO_4$ after shaking a 5g soil sample in 100 ml distilled water for 30 minutes (Gu *et al*., 2019).

Statistical analysis

The data generated from the study were subjected to One-way analysis of Variance(ANOVA) and Paired-Samples T-test using SPSS 18.0 Statistical Package Program

(SPSS Institute, USA) The level of significance was set at p=0.05.

RESULTS

Physicochemical properties of the soil and biochar before experiment

Physicochemical properties of the soil and biochar before experiment began are shown in Table 1.

Table 1: Physico-chemical properties of biochar

Parameters	Soil	Biochar
pH	6.6	9.0
Texture	sandy-loam	-
% Ash	2.4	1.8
% Moisture	1.2	5.45
Pore Size (µm)	0.32	0.54
% Nitrogen	0.58	0.42
% Phosphorous	0.28	0.18
% Total Organic Compound	1.42	1.05
Copper (Cu) ppm	0.192	-
Cadmium (Cd) ppm	0.012	-
Lead (Pb) ppm	0.154	-

Effect of treatments on microbial population in the soil

Figure 1 and 2 show the impact of various treatments on soil bacterial and fungal loads. The control had a significantly lower mean bacterial load ($1.07x10^7$ CFU/g) compared to pre-experimental levels. Treatments involving various biochar applications and *Pseudomonas aeruginosa* showed higher bacterial counts ($3.07\ x10^7$ to $7.03\ x10^7$ CFU/g), with the *Pseudomonas aeruginosa* treatment exhibiting the highest count. Similarly, fungal counts increased in treated soils ($3.6\ x10^3$ to $8.2x10^3$ CFU/g) compared to the control ($1.1\ x10^3$ CFU/g). *Pseudomonas*-treated soil displayed the highest fungal count, while co-application of basic biochar and *Pseudomonas* had the lowest.

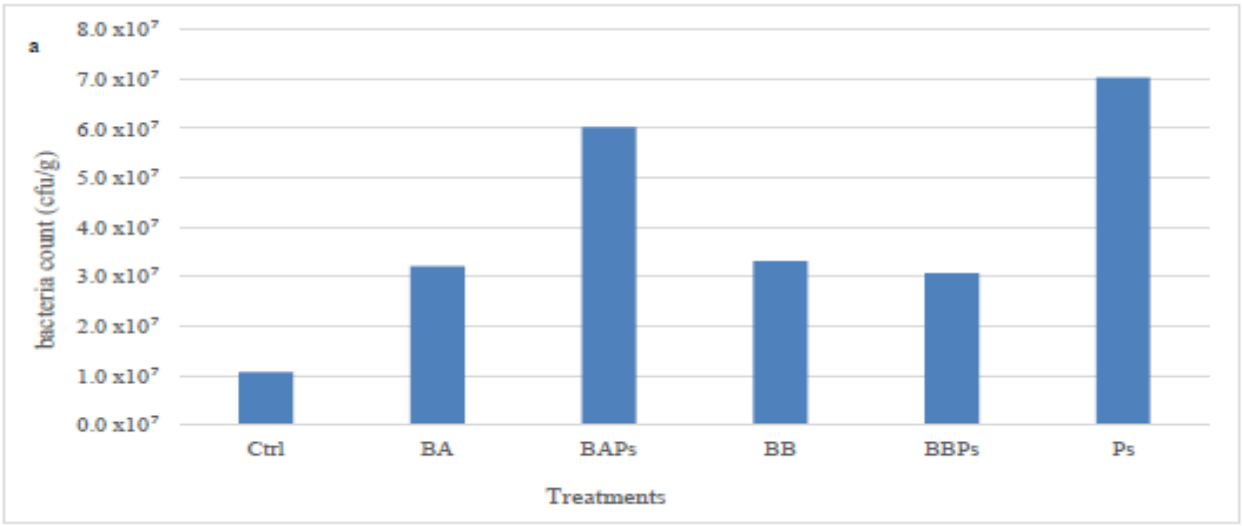

Fig. 1: Effect of treatments on bacteria load in the soil

KEY: Ctrl: Control, BA: Acidic Biochar, BAPS: Acidic Biochar and *Pseudomonas*, BB: Basic Biochar, BBPs: Basic Biochar and *Pseudomonas*, PS: *Pseudomonas.*

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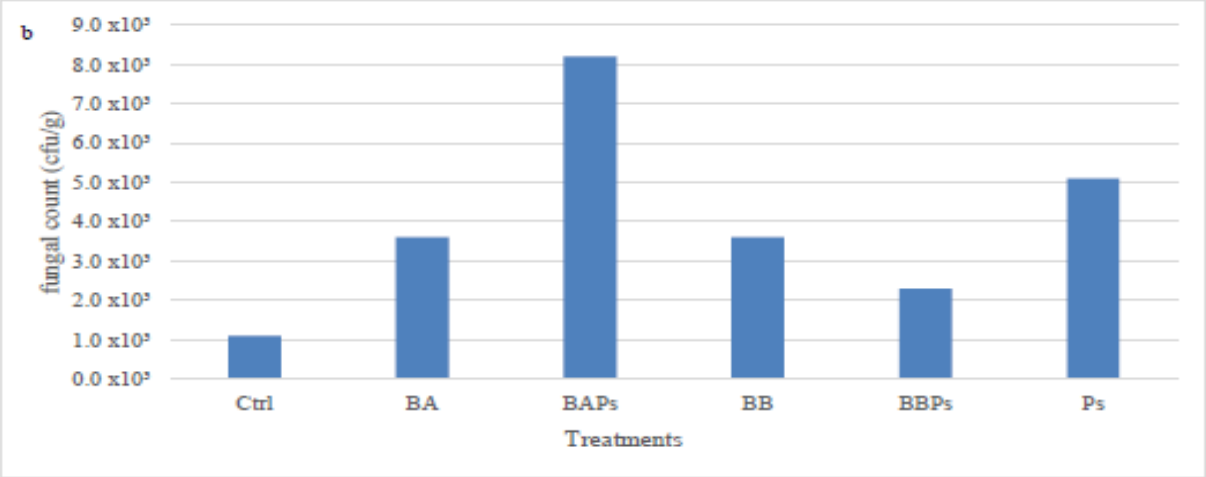

Fig 2: Effect of treatments on fungal load in the soil

KEY: Ctrl: Control, $B_{A:}$ Acidic Biochar, B_AP_S: Acidic Biochar and *Pseudomonas aeruginosa*, B_B: Basic Biochar, B_BP_S: Basic Biochar and *Pseudomonas aeruginosa.* P_S: *Pseudomonas aeruginosa*.

Occurrence of bacterial genera in the treated soil

Presented in Table 2 is the occurrence and distribution of bacterial genera in treated soils, including pre-experimental soil. *Bacillus* spp. and *Pseudomonas* spp. were consistently dominant across all treatments. Remarkably, pots treated with acidic and basic biochar showed a higher prevalence of bacterial genera compared to other conditions.

Table 2: Occurrence of bacterial genera in the treated soil

Bacteria	**Treatments**						
	P.E.	Ctrl	B_A	B_AP_S	B_B	B_BP_S	P_S
	No. of isolates						
Pseudomonas spp.	2	0	0	1	2	1	1
Enterobacter spp.	1	0	0	0	0	0	0
Enterococcus spp.	0	0	0	1	0	0	0
Proteus spp.	0	2	2	0	1	0	0
Bacillus spp.	2	0	2	0	1	1	1
Klebsiella spp.	0	1	0	0	0	0	0
Acinetobacter spp.	0	0	1	0	0	0	0
Corynebacterium spp.	0	0	1	0	0	0	0
Acetobacter spp.	0	0	0	1	1	0	0
Staphylococcus spp.	0	0	0	2	0	0	0
Streptococcus spp.	0	0	0	0	1	0	1
Listeria spp.	0	0	1	1	1	1	0
Total	5	3	7	6	7	3	3

KEY: P.E: Pre-experimental sample, Ctrl: Control, $B_{A:}$ Acidic Biochar, B_AP_S: Acidic Biochar and *Pseudomonas aeruginosa*, B_B: Basic Biochar, B_BP_S: Basic Biochar and *Pseudomonas aeruginosa.* P_S: *Pseudomonas aeruginosa*

Effect of treatments on soil enzymatic activity

Table 3 shows the effect of different treatments on soil enzymatic activity (catalase activity and urease activity). The mean catalase activity varied from 62.15 ± 4.40 to 94.80 ± 4.90 $mgKMnO_4\ kg^{-1}$. The control group exhibited a mean catalase activity of 62.15 ± 4.40 $mgKMnO_4\ kg^{-1}$, providing a baseline for comparison with the treated soils. The mean values of urease activity of the soil samples ranged from 0.388 ± 0.046 to 0.410 ± 0.091 mg $NH_4^+\ kg^{-1}\ h^{-1}$ with the control group having an average urease activity of 0.406 ± 0.049 mg $NH_4^+\ kg^{-1}\ h^{-1}$.

Table 3: Effect of treatments on soil enzymatic activity

Treatment	Catalase ($mgKMnO_4\ kg^{-1}$)	Urease ($mgNH_4^+\ kg^{-1}\ h^{-1}$)
Ctrl	62.15 ± 4.40*	0.406 ± 0.049
B_A	69.86 ± 3.08*	0.406 ± 0.037
B_APs	92.52 ± 4.90	0.410 ± 0.091
B_B	94.80 ± 4.90*	0.405 ± 0.042
B_BPs	78.67 ± 3.41	0.409 ± 0.085
P_S	78.67 ± 3.41	0.388 ± 0.046

Note: Significant differences are indicated by asterisks (*). $p< 0.05$ (compared to Control). KEY: Ctrl: Control, B_A: Acidic Biochar, B_APS: Acidic Biochar and *Pseudomonas*, B_B: Basic Biochar, B_BP_S: Basic Biochar and *Pseudomonas*, P_S: *Pseudomonas.*

DISCUSSION

This study was carried out to investigate the effects of the co-application of Christmas melon derived biochar and a heavy metal tolerant *Pseudomonas aeruginosa* on microbial population, bacterial diversity and enzyme activities inasoil artificially spiked with heavy metals (Cadmium, Copper and Lead) usinga pot experiment. There were notable differences in the physicochemical properties of the soil and biochar in our study compared with previous findings by other researchers. The pH of soil in this study was slightly acidic (6.6) whereas Nie *et al.* (2018) reported an acidic topsoil (5.8). In contrast, the biochar used in our study had an alkaline pH of 9.0, differing from the alkalinity observed by Nie *et al*. (2018) with a pH of 11.3 in their acidic topsoil (pH 5.8). The total organic compound of 1.42 % is significantly lower than the organic carbon level reported by Nie *et al*. (2018) in topsoil (14.5 %) and biochar (532.0 %). These disparities in pH and organic carbon may have implications for nutrient availability and microbial activity in the soil. Furthermore, variations in nutrient levels, such as % nitrogen and % phosphorus, compared with the results reported in earlier studies may impact the overall fertility of the soil and nutrient cycling dynamics. Additionally, differences in metal concentrations, notably copper, cadmium, and lead, emphasize the importance of understanding the environmental implications associated with soil amendments.

The impact of various treatments on the microbial population in the soil, particularly the bacterial and fungal loads was determined (Fig. 1 and Fig. 2). In a similar study, Nie *et al*. (2018) investigated the influence of biochar on soil microbial populations. They observed a significant increase in bacterial and actinomycetes populations with increasing biochar application rates. In contrast, the fungal population decreased with biochar application, indicating a potential inhibition of the fungal community. The microbial responses to biochar were attributed to the complex interactions involving physical, chemical, and biological processes. The pH of the biochar, which was alkaline (pH 11.3), played a crucial role in microbial responses. Leta (2018) also noted that the influence of biochar on microorganisms in the rhizosphere was associated with the soil type, with varying effects on microbial activity observed in different studies. While some studies reported an increase in microbial biomass and activity, others observed a decrease, emphasizing the complexity of biochar-soil interactions. Furthermore, the microbial replication rate increased in some biochar-amended soils, highlighting the potential for biochar to positively impact microbial abundance and activity (Pietikäinen *et al*., 2000; Steiner *et al*., 2009). Our findings align with existing literature, suggesting that biochar applications can influence microbial populations in soil, with effects varying based on factors such as

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp 13-24
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biochar type, application rate, and soil characteristics.

The occurrence and distribution of bacterial genera in treated soil were also examined, and the results are presented in Table 2. Among the bacteria, *Bacillus* spp. and *Pseudomonas* spp. were consistently the most dominant across all treatments. The total number of bacterial genera isolated was notably higher in biochar-treated soils, emphasizing the potential of biochar to act as a carbon source, promoting microbial activity and diversity (Laird *et al*., 2010). Leta (2018) had earlier reported an increase in microbial diversity in biochar-amended soils. Higher availability of nutrients or labile organic matter on the biochar surface and reduced competition among microorganisms are possible reasons for increase in microbial diversity.

The effect of treatment on the enzymatic activities in the soil is presented in Table 3. Among the treatment groups, the highest catalase activity was observed in the B_B treatment, which was significantly higher compared to the control. On the other hand, the B_A treatment exhibited a slightly higher catalase activity compared to the control, but the difference was significant. The highest urease activity was observed in the B_APs treatment, which was significantly higher compared to the control. The P_S treatment also showed a lower urease activity compared to the control, although the difference was not statistically significant. Our findings align with previous research indicating that biochar amendments can enhance the activities of enzymes like urease and phosphatase (Gomez-Eyles *et al*., 2013; Sun *et al*., 2017). Huang *et al*. (2023) discussed the variable response of soil enzymes to biochar, influenced by factors such as biochar amount, type, and soil characteristics. Their study found a significant increase in urease and sucrase activities with higher biochar doses, attributing this effect to increased organic matter and nutrient content, fostering conditions for microbial growth and enzyme activity. Similarly, Vithanage *et al*. (2018) observed elevated catalase and dehydrogenase activities with biochar application, emphasizing the influence on redox enzymes. The close relationship between soil microorganisms and enzymes is emphasized, as biochar application increased soil urease activity and microbial abundance, showing a significant correlation (Xu *et al*., 2020). Nie *et al*. (2018) conducted experiments with sugarcane bagasse biochar and found increased activities of urease, catalase, and invertase in heavy metal-contaminated soil. The activities rose significantly with higher biochar doses, indicating potential soil self-purification. The authors attributed the enhancement of enzyme activities to biochar's impact on soil organic matter, water retention, pore structure, and cation exchange capacity. Furthermore, they noted that changes in enzyme activity correlated with soil pH alterations induced by biochar. The study also highlighted the potential immobilization of heavy metals, affecting enzyme activity positively. Our study findings parallel these results, with increased enzyme activity corresponding to decreased bioavailability of heavy metals in biochar-amended soils. These findings, are in agreement with previous studies which demonstrated that biochar amendments can significantly influence soil enzymatic activities, providing valuable insights into the soil's metabolic processes and microbial interactions

CONCLUSION

The significant increases observed in microbial populations, particularly in response to the B_BPs and B_APs treatments shows the efficacy of biochar-*Pseudomonas* co-application in fostering a robust and diverse microbial community. Moreover, the notable enhancement of catalase and urease activities within the B_BPs and B_APs treatments signifies a marked improvement in soil nutrient cycling and organic matter decomposition. The success demonstrated in this experiment holds the promise of rejuvenating soil health, fostering resilient ecosystems, and ultimately contributing to sustainable food production.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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