*International Journal of Plant & Soil Science*

***Volume 36, Issue 5, Page 54-65, 2024; Article no.IJPSS.114320***
***ISSN: 2320-7035***

# Impact of Biochar on Pathogenic Bacteria and Bermuda Grass (*Cynodon dactylon*) Growth in Soil Treated with Chicken Manure

**Adzraku H-V [a], Blay M [a] and Tandoh PK [a*]**

[a] *Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors AHV and BM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author TPK managed the analyses of the study. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/IJPSS/2024/v36i54501

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/114320

***Original Research Article***

***Received: 04/01/2024***
***Accepted: 08/03/2024***
***Published: 16/03/2024***

## ABSTRACT

Chicken manure plays an important role in soil amendment by improving soil properties for plant growth. However, the high levels of pathogens can have an adverse effect on humans when used for sports turf. This study was conducted to 1) determine the pathogenic bacteria in chicken manure-amended soil, 2) identify the appropriate decomposition stage of manure associated with reduced number of pathogenic bacteria, 3) assess the effect of different rates of biochar on pathogenic bacteria in the amended soil and 4) determine the effect of biochar on the growth of Bermuda grass. The design used for the laboratory experiment was 3×3 factorial in Completely Randomised Design (CRD) and it was replicated three times. The factors involved were: decomposition stages of chicken manure (3 levels) and the different rates of biochar (3 levels). The best combination of biochar and chicken manure at the percentages of 0, 5, 10, and 15 were then

**Corresponding author: E-mail: pktandoh.canr@knust.edu.gh;*

used together with soil and sand mix at a ratio of 100:00 and 70:30 to plant Bermuda grass. This experiment showed that *E. coli* was present in chicken manure and that increasing the decomposition period had a significant effect on the *E. coli* by reducing its load. Also, the addition of biochar to the chicken manure resulted in a significant reduction of *E. coli* count ($p<0.01$). The soil amended with the biochar and manure also supported very well the growth of Bermuda grass with the 10% and 15% biochar manure mix in 70 to 30 ratio of topsoil and sand giving the best grass growth in terms of spread, thickness, height, and color. A regression analysis given by the equation $Y_{(coverage)}=176.857-23.0402_{(sprouting)}$ ($R^2=0.99$), indicated that sprouting significantly affected grass coverage such that 99% variation in the grass coverage was attributed to the sprouting. At the end of the study, it was concluded that well-composted chicken manure should be used together with biochar on sports fields to help remediate the problem of *E. coli* infestation and also improve the growth of grass on fields. Furthermore, biochar with chicken manure-amended soil could be ideal for vegetable garden to help reduce foodborne diseases caused by *E. coli* infestation.

*Keywords: Biochar; pathogenic bacteria; decomposition; infection; sprouting; colour; E. coli infestation; vegetable garden; chicken manure; amended soil.*

## 1. INTRODUCTION

Biochar is material rich in carbon that is produced when biomass such as wood, leaves or manure is heated in an enclosed container under anaerobic or oxygen- limited conditions for use specifically to improve environmental and soil health [1]. *Cynodon dactylon* commonly known as Bermuda grass is a perennial warm-season, fast growing and tough turfgrass which is dark green in colour which spreads by rhizome and stolons [2]. Bermuda grass is widely adapted in the tropical and the subtropical countries worldwide [2]. Bermuda grass is a major turf for sports fields because of its ability to withstand exceptional drought, strong traffic tolerance and quick recovery [2] Bermuda grass requires high maintenance and nutrients [2]. For this reason, a lot of inorganic fertilizers are used on turf grass sports fields to maintain the field's verdant appearance. Organic fertiliser could have been the best fertiliser to be applied on sports fields as a replacement to inorganic fertilizer. Also, there is a lot of compaction on sport fields due to the heavy trampling that goes with the game and this affects the soil structure. Organic fertiliser could have been used to remediate this problem. Football officials prefer inorganic fertilizers over organic fertilizers despite the potential benefits of organic fertilizers, partly because organic fertilizers like chicken dung may harbor harmful microorganisms like *E. coli* that are harmful to human health. These pathogenic bacteria have the potential to cause sickness in humans. On football fields, the football players can easily be infected if the soil contains these pathogenic bacteria because they come into direct contact with the soil most often during play. Also, they can easily ingest these pathogenic bacteria through drinking water because most often water is thrown on the ground to players who usually handle the container with dirty hands thus possibly introducing pathogenic bacteria into their system. This study therefore seeks to assess the extent to which biochar could be effective in controlling pathogenic soil bacteria on sport fields with specific focus on football fields.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The experiment was conducted at the Microbiology Laboratory of the Water and Sanitation Section of the Faculty of Engineering and the Department of Horticulture, Faculty of Agriculture, KNUST.

### 2.2 Experimental Design

#### 2.2.1 Experimental design for laboratory work

The design used for the laboratory work was 3×3 factorial in Completely Randomised Design (CRD) and it was replicated three times. The factors were: decomposition stages of chicken manure and the different rates of biochar as shown in the Fig. 1.

### 2.3 Experimental Design for Fieldwork

The design that was used for the fieldwork was 2*4 factorial Randomised Complete Block Design (RCBD). The set up was replicated three times.

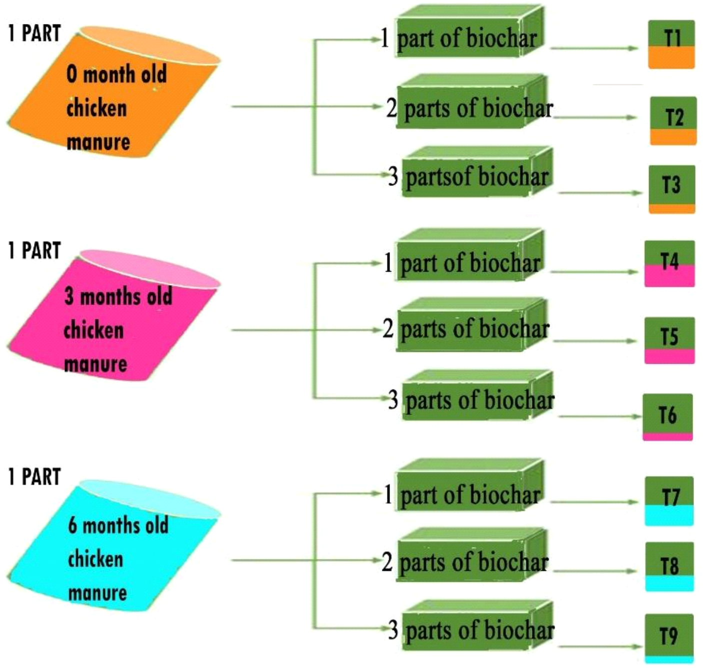


**Fig. 1. Various treatments for the experiment**

**Table 1. Treatments for fieldwork**

| Soil to Sand Ratio | Chicken Manure and Biochar Mix Percentage (%) | | | |
|---|---|---|---|---|
| | **0** | **5** | **10** | **15** |
| 100:0 | $T_{100:0}$ + 0% $T_{Bm}$ | $T_{100:0}$ + 5% $T_{Bm}$ | $T_{100:0}$ + 10% $T_{Bm}$ | $T_{100:0}$ + 15% $T_{Bm}$ |
| 70:30 | $T_{70:30}$ + 0% $T_{Bm}$ | $T_{70:30}$ + 5% $T_{Bm}$ | $T_{70:30}$ + 10% $T_{Bm}$ | $T_{70:30}$ + 15% $T_{Bm}$ |

* *$T_{100:0}$ = 100% of topsoil to 0% 0f sand * $T_{70:30}$ = 70% of topsoil to 30% of sand*
** $T_{Bm}$ = Biochar plus Chicken manure*

**Table 2. Various Ratios of Topsoil and Sand Mixes**

| Topsoil | Sand |
|---|---|
| 100 | 0 |
| 70 | 30 |

## 2.4 Experimental Procedure

The experiment was in two folds namely laboratory work and fieldwork.

## 2.5 Laboratory Work

Fresh Chicken manure was collected from Genesis Farm, a suburb of Kumasi, Ghana. Some of the fresh chicken manure (0 months old) were allowed to undergo decomposition for three to six months. These represented the various stages of decomposition of the chicken manure. Samples of the various decomposition levels of manure were put in zip lock bags and labelled accordingly. They were sent to the Microbiology Laboratory to be tested for presence of pathogenic bacteria. The pathogenic bacteria and their load, especially *E. coli* in the manure was determined using the Membrane Filtration Technique. Biochar at different rates (1, 2 and 3 parts) were added to the manure to determine what effect it could have on the pathogenic load, especially destruction of harmful bacteria such as *E. coli.* The different biochar rates and the manure at the different stages of decomposition were mixed and bagged in zip lock bags. These samples were kept in the laboratory for seven days and thereafter tested for the pathogenic bacteria using the Membrane Filtration Technique.

## 2.6 Fieldwork

The best result with less E. coli infestation from the laboratory work was mixed with topsoil and sand to grow Bermuda grass. The soil and the sand were mixed at different ratios and the various mixes were amended with the best biochar and chicken manure mix from the laboratory work. Different percentages by weight of the topsoil and the sand mix were used.

Different percentages (0%, 5%, 10% and 15%) of the best result from the laboratory (with less *E. coli* infestation) work were then added to the topsoil and sand mixes.

Topsoil was sieved to remove any lumps and debris. The various mixes of the topsoil, sand and the best result for the laboratory (with less *E. coli* infestation) work were prepared by weighing them in accordance with their respective ratio and percentages and were placed in containers with drainage holes underneath. Samples of each of the mixes were then taken and sent to the laboratory for the assessment of soil properties. All the mixes were replicated three times. Bermuda grass was then planted on each of the mixes using the sprigging method (using sprigs from dug grass) and were watered on daily bases till they were established. Basic maintenance practices such as removal of weeds were also done.

## 2.7 Data Collection

### 2.7.1 Laboratory work

For the laboratory work, data was collected on *E. coli* load in each of the various decomposition stages of chicken manure and the biochar and the chicken manure mixes.

### 2.7.2 Fieldwork

Samples of the various media mixes were sent to the laboratory for the following parameter to be determined:

- Nitrogen, using the Kjeldahl method (This method consists of transforming all nitrogen in a weighed sample into ammonium sulphate by digesting it with sulphuric acid, alkalizing it and determining the resulting ammonia by distilling it into a measured volume of standard acid) [3].
- Phosphorous, using the Bray's method (This method is used to determine available phosphorus in soil) [4].
- Soil Organic Carbon and Organic Matter Content using Walkley-Black method (Is a method used to determine the amount of oxidizable organic matter in which organic matter is oxidised with a known amount of chromate in the presence of sulphuric acid) [5].
- Cation Exchange Capacity using the Ammonium acetate method (Is used to displace exchangeable cations in a soil. This method gives an estimation of plant available base cations) [6].
- pH using the pH metre. Also, data was collected on the growth of Bermuda grass by considering the following parameters.
- Number of days for the grass to sprout after planting. This was done by counting the number of days it took the grass to sprout after planting.
- Colour of grass leaves. The colour was determined by observation with the help of a colour chart.
- Percentage coverage of the grass on the surface. This was determined by placing a circular quadrat on each of the setup and the number of grass that was covered by the quadrat was counted.
- The length of the grass. This was achieved by measuring the height of the grass in each setup using a ruler. The longest, the medium and the shortest length of grass in each setup was measured and the average was calculated. The measurement was done in cm.

## 2.8 Statistical Analysis of Data

Data collected from the experiments were subjected to Analysis of Variance (ANOVA), using Statistix Version 10. Treatment means for the laboratory work were compared at $p<0.01$ probability level while the treatment means for the fieldwork were compared at $p<0.05$.

# 3. RESULTS

## 3.1 Decomposition Period and *E. coli* Count IN Poultry Manure

*E. coli* counts in the different decomposition periods of chicken manure are shown in Table 3. After the experiment, fresh chicken manure recorded the highest *E. coli* count (134 cfu/100mL) while 6 months old composted chicken manure recorded the lowest *E. coli* count (19 cfu/100mL).

**Table 3. Effect of decomposition period on *E. coli* count in chicken manure**

| Decomposition Period | *E. coli* count (cfu/100mL) |
|---|---|
| 0 Month Old | 134 |
| 3 Months Old | 100 |
| 6 Months Old | 19 |

**Table 4. Effect of different rates of biochar and decomposition stage association on *E. coli***

| Biochar Rate | Decomposition Stage | | | Mean |
|---|---|---|---|---|
| | 0 Months Old | 3 Months Old Manure | 6 Months Old Manure | |
| 1 | $13^{b}$ | $11^{bc}$ | $9^{cd}$ | $11^{b}$ |
| 2 | $26^{a}$ | $7^{de}$ | $5^{ef}$ | $12.7^{a}$ |
| 3 | $10^{bcd}$ | $9^{cd}$ | $3^{f}$ | $7.3^{c}$ |
| Mean | $16.3^{a}$ | $9^{b}$ | $5.7^{c}$ | |

*LSD (0.01) Biochar = 1.56 Decomposition Stage=1.56 Biochar rate*Decomposition stage=3.51*

### 3.2 Effect of the Association between biochar Rate and Decomposition Stage on *E. coli*

Statistically, there were significant differences between biochar rates applied and decomposition stage of chicken manure interactions (Table 4.). Significantly, highest *E. coli* count (26 cfu/100mL) was recorded in two parts of biochar and fresh chicken manure while the least *E. coli* count (3 cfu/100mL) was recorded in the mix with three parts of biochar and six months old composted chicken manure. Across the biochar rates, significantly highest *E. coli* load (12.7 cfu/100mL) was recorded in two parts of biochar while the least *E. coli* count (7.3 cfu/100mL) was found in the three parts of biochar. Among the decomposition stages of chicken manure, significantly highest *E. coli* load (16.3 cfu/100mL) was noticed in fresh chicken manure while least *E. coli* load (5.7 cfu/100mL) was recorded in 6 months old manure.

### 3.3 Physiochemical Properties of the Various Media Samples

Significant differences were observed for total Nitrogen (%) among the various samples (Table 5). Highest Nitrogen percentage (0.165%) was found in $T_{100:0}$ plus 0% $T_{Bm}$ mix while the least total Nitrogen percentage (0.06%) was recorded in $T_{100:0}$ plus 10% $T_{Bm}$ mix.

Considering available Phosphorus, there were significant differences in all the various samples. Significantly highest available Phosphorus (1106.5mg/kg) was noticed in $T_{70:30}$ plus 15% $T_{Bm}$ mix while the least available Phosphorus (24.4mg/kg) was noticed in $T_{70:30}$ plus 0% $T_{Bm}$ mix.

Exchangeable Potassium showed significant differences in all the various samples. Significantly highest exchangeable Potassium (1.01cmol/kg) was found in $T_{70:30}$ plus 15% $T_{Bm}$ mix while the lowest exchangeable Potassium (0.28cmol/kg) was observed in $T_{100:0}$ plus 0% $T_{Bm}$ mix.

In the case of exchangeable Calcium, there were significant differences in all the various samples. Highest exchangeable Calcium (15.7cmol/kg) was observed in $T_{70:30}$ plus 15% $T_{Bm}$ mix while the least exchangeable Calcium (6.1cmol/kg) was found in $T_{70:30}$ plus 0% $T_{Bm}$ mix.

Considering exchangeable Magnesium, there were no significant difference between all the various samples ($p>0.01$).

Organic Carbon showed significant difference among all samples. Significantly, the highest Organic Carbon percentage (4.24%) was recorded in $T_{70:30}$ plus 15% $T_{Bm}$ mix while the lowest Organic Carbon percentage (1.45%) was noticed in $T_{70:30}$ plus 0% $T_{Bm}$ mix and $T_{100:0}$ + 0% $T_{Bm}$ mix.

Organic matter percentage also recorded significant difference between all the various samples. Significantly highest Organic matter percentage (7.47%) was recorded in $T_{70:30}$ plus 15% $T_{Bm}$ mix whereas the least Organic Matter percentage (2.66%) was noticed in $T_{70:30}$ plus 0% $T_{Bm}$ mix and $T_{100:0}$ plus 10% $T_{Bm}$ mix.

There were no significant differences between pH values in the various samples.

**Table 5. Physiochemical properties of the various media samples**

| SAMPLES | N (%) | P (mg/kg) | K (cmol/kg) | Ca (cmol/kg) | Mg (cmol/kg) | OC (%) | OM (%) | pH |
|---|---|---|---|---|---|---|---|---|
| $T_{100:0}$ + 0% $T_{Bm}$ | 0.165[a] | 28.45[g] | 0.28[c] | 8.5[cde] | 1.8[a] | 2.64[b] | 4.72[b] | 7.15[a] |
| $T_{100:0}$ + 5% $T_{Bm}$ | 0.135[ab] | 812.9[e] | 0.71[ab] | 11.5[bc] | 4.4[a] | 2.64[b] | 4.72[b] | 6.79[a] |
| $T_{100:0}$ + 10% $T_{Bm}$ | 0.06[d] | 872.9[b] | 0.94[ab] | 13.3[ab] | 3.4[a] | 1.45[d] | 2.66[c] | 7.35[a] |
| $T_{100:0}$ + 15% $T_{Bm}$ | 0.11[bc] | 858.9[c] | 0.88[ab] | 12.7[ab] | 4.6[a] | 2.64[b] | 4.72[b] | 7.59[a] |
| $T_{70:30}$ + 0% $T_{Bm}$ | 0.125[b] | 24.4[h] | 0.58[bc] | 6.1[e] | 2.8[a] | 1.45[d] | 2.66[c] | 7.09[a] |
| $T_{70:30}$ + 5% $T_{Bm}$ | 0.12[b] | 687.9[f] | 0.61[bc] | 7.7[de] | 3.2[a] | 1.85[c] | 3.35b[c] | 7.16[a] |
| $T_{70:30}$ + 10% $T_{Bm}$ | 0.06[d] | 850.5[d] | 0.81[ab] | 11.3[bcd] | 3.8[a] | 2.64[b] | 4.72[b] | 7.55[a] |
| $T_{70:30}$ + 15% $T_{Bm}$ | 0.08[cd] | 1106.5a | 1.01[a] | 15.7[a] | 2.8[a] | 4.24[a] | 7.47[a] | 7.99[a] |
| CV (%) | 6.82 | 0.11 | 9.72 | 6.52 | 25.33 | 2.89 | 8.08 | 4.82 |
| LSD | 0.04 | 3.74 | 0.37 | 3.74 | 4.49 | 0.37 | 1.87 | 1.87 |

***** *$T_{100:0}$ = 100% of topsoil to 0% 0f sand * $T_{70:30}$ = 70% of topsoil to 30% of sand * $T_{Bm}$ = Biochar plus Chicken manure*

### 3.4 Effect of Biochar Plus Manure and Soil Mix on Bermuda Grass Coverage

Statistically, there were significant differences between the biochar plus manure combination and soil to sand ratio interactions (Table 6). Significantly, highest percentage of Bermuda coverage (76.6%) was recorded in 15% of biochar plus manure combination and soil only which was similar to those grown in 15% of biochar plus manure combination and 70 to 30 ratio of soil and sand respectively. The lowest percentage of Bermuda coverage (30.00%) was observed in 0% of biochar plus manure combination and topsoil interaction. Among the percentages of biochar plus manure combination, significantly highest percentage of Bermuda coverage (76.67%) was found in 15% of biochar plus manure combination whereas the least percentage of Bermuda coverage (30.83%) was recorded in 0% of biochar plus manure combination.

There were no significant differences between the soil to sand ratio.

### 3.5 Effect of Biochar Plus Manure and Soil Mix on the Average Hieght of Bermuda

The effect of biochar plus manure and soil mix applications is shown in Table 7. The result revealed significant differences between the biochar plus manure combination and soil to sand ratio interactions. The highest height of Bermuda grass (10.63cm) was recorded in 15% of biochar plus manure combination and soil interaction while the lowest height of Bermuda grass (7.63cm) was recorded in 0% of biochar plus manure combination and topsoil interaction.

Across soil to sand ratio, significantly highest height of Bermuda grass (9.53cm) was found in topsoil only whereas the least height of Bermuda grass (8.58cm) was recorded in 70 to 30 ratio of soil and sand respectively.

There were no significant differences between the percentages of biochar plus manure combination.

### 3.6 Effect of Biochar Plus Manure and soil mix on Sprouting of Bermuda Grass (day)

In the case of sprouting of Bermuda grass (Table 8), there were significant differences between the biochar plus manure combination and soil to sand ratio interaction. Significantly highest number of days (6.67) for Bermuda grass to sprout was noticed in 0% of biochar plus manure combination and topsoil interaction whereas the lowest number of days (4.00) for Bermuda grass to sprout was found in 15% of biochar plus manure combination and topsoil interaction.

Among the percentages of biochar plus manure combination, highest number of days (6.33) for Bermuda grass to sprout was recorded in 0% of biochar plus manure combination whereas the least number of days (4.33) for Bermuda grass to sprout was found in 15% of biochar plus manure combination.

Across soil to sand ratio, highest number of days (5.50) for Bermuda grass to sprout was obtained in 70 to 30 ratio of soil and sand whereas the least number of days (4.92) for Bermuda grass to sprout was recorded in topsoil only.

### 3.7 Regression Relationship between Sprouting and Grass Coverage

Fig. 1 illustrates the regression relationship between sprouting and grass coverage, where sprouting significantly affected grass coverage such that 99% variation in the grass coverage was explained by the sprouting.

**Table 6. Effect of biochar plus manure and soil:sand mix on percentage coverage (%) bermuda grass**

| | Soil: Sand | | |
|---|---|---|---|
| **Biochar plus manure (%)** | **100:00** | **70:30** | **Means** |
| 0 | $30.00^{c}$ | $31.67^{c}$ | $30.83^{c}$ |
| 5 | $51.67^{bc}$ | $63.33^{ab}$ | $57.50^{b}$ |
| 10 | $61.67^{ab}$ | $63.33^{ab}$ | $62.50^{b}$ |
| 15 | $76.67^{a}$ | $76.67^{a}$ | $76.67^{a}$ |
| Means | $55.00^{a}$ | $58.75^{a}$ | |

*HSD(0.05) : biochar plus manure= 22.56, Soil:sand = 6.90, Biochar plus manure*soil:sand = 22.56*

**Table 7. Effect of biochar plus manure and soil mix on the average height of bermuda (cm)**

| | Soil: Sand Ratio | | |
|---|---|---|---|
| **Biochar plus manure (%)** | **100:00** | **70:30** | **Means** |
| 0 | 9.27[ab] | 7.63 [b] | 8.45[a] |
| 5 | 9.10[ab] | 9.23[ab] | 9.17[a] |
| 10 | 9.13[ab] | 8.90[ab] | 9.02[a] |
| 15 | 10.63[a] | 8.53[ab] | 9.58[a] |
| Means | 9.53[a] | 8.58[b] | |

*HSD (0.05): biochar plus manure= 1.47, Soil:Sand =0.77, Biochar plus manure*soil:sand = 2.51*

**Table 8. Effect of biochar plus manure and soil mix on sprouting of bermudagrass (day)**

| | Soil: Sand Ratio | | |
|---|---|---|---|
| **Biochar plus manure (%)** | **100:00** | **70:30** | **Means** |
| 0 | 6.67[a] | 6.00[ab] | 6.33[a] |
| 5 | 4.67[bc] | 5.67[abc] | 5.17[b] |
| 10 | 4.33[bc] | 5.67[abc] | 5.00[b] |
| 15 | 4.00[c] | 4.67[bc] | 4.33[b] |
| Means | 4.92[b] | 5.50[a] | |

*HSD (0.05) : biochar plus manure=1.01, Soil:Sand =0.53, Biochar plus manure*soil:sand = 1.73*

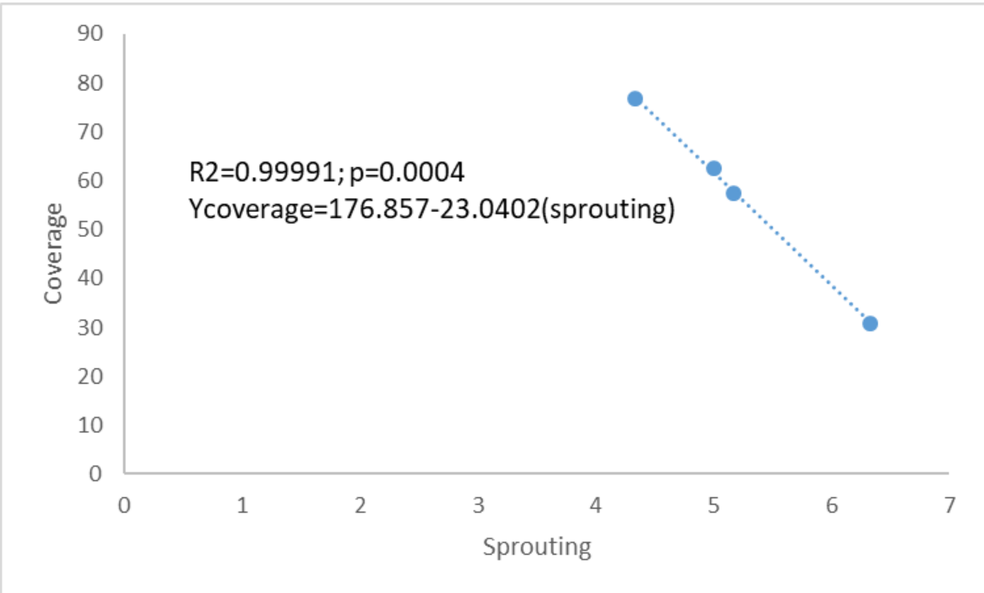


**Fig. 2. Regression relationship between sprouting and grass coverage**

**Table 9. Effect of biochar plus manure and soil mix on colour of bermuda grass**

| **Media** | **Colour** | **Colour Name** |
|---|---|---|
| $T_{100:0}$ + 0% $T_{Bm}$ | | Light green |
| $T_{100:0}$ + 5% $T_{Bm}$ | | Grass green |
| $T_{100:0}$ + 10% $T_{Bm}$ | | Dark green |
| $T_{100:0}$ + 15% $T_{Bm}$ | | Dark green |
| $T_{70:30}$ + 0% $T_{Bm}$ | | Light green |
| $T_{70:30}$ + 5% $T_{Bm}$ | | Dark green |
| $T_{70:30}$ + 10% $T_{Bm}$ | | Dark green |
| $T_{70:30}$ + 15% $T_{Bm}$ | | Dark green |

** $T_{100:0}$ = 100% of topsoil to 0% of sand * $T_{70:30}$ = 70% of topsoil to 30% of sand * $T_{Bm}$ = Biochar plus Chicken manure*

### 3.8 Effect of Biochar Plus Manure and Soil Mix on the Colour of Bermuda Grass

Bermuda grass on $T_{100:0}$ plus 10% $T_{Bm}$ mix, $T_{100:0}$ plus 15% $T_{Bm}$ mix , $T_{70:30}$ plus 5% $T_{Bm}$ mix, $T_{70:30}$ plus 10% $T_{Bm}$ mix and $T_{70:30}$ plus 15% $T_{Bm}$ mix were dark green in colour whereas the colour of Bermudagrass on $T_{100:0}$ plus 0% $T_{Bm}$ mix and $T_{70:30}$ plus 0% $T_{Bm}$ were light green (Table 9).

## 4. DISCUSSION

The results of this study highlighted the significance of decomposition time on chicken manure in reducing the number of *E. coli* count. The results indicated a significant($p<0.01$) decrease in *E. coli* count in the 3 months old and 6 months old composted chicken manure. Reduction in the number of *E. coli* count in 3 months old and 6 months old composted chicken manure can be attributed to high temperature and temperature fluctuations during the decomposition process of the chicken manure. According to Erickson et al. [7], temperatures reached in a well-managed compost operation are within 55 ºC to 65 °C and such temperatures are well above the thermal death points of mesophilic pathogens, such as *E. coli* O157:H7 and Salmonella spp. [8]. Semenov et al. [9] also indicated that the survival of *E. coli* O157:H7 in manure under fluctuating temperatures was generally lower than that under constant temperature. Talaro et al. [8] also found out that, reduction in survival of the organism was more pronounced when the amplitude in the temperature oscillations was larger (7 °C) than at smaller amplitudes (4 °C) and also, temperature increase might constitute greater stress and energy expenditure for the organism than decrease in temperature [8]. Besides high temperature, other mechanisms are also known to get involved in the reduction of *E. coli* count during composting, which includes microbial antagonism, production of organic acids, pH change, desiccation and starvation stresses, exposure to ammonia emission, and competition for nutrients [10].

From the results of this study, addition of biochar to the various decomposition stages of chicken manure resulted in significant ($p<0.01$) reduction in the number of *E. coli* count in all the various decomposition stages. This can be attributed to the liming potential of biochar. The initial pH value of the biochar used was 8.5, which is very alkaline. According to Bach et al. [11], *E. coli* count decline rate increases as pH increases. Another explanation for the effectiveness of biochar on the removal of *E. coli* is that *E. coli* may bind more efficiently to biochar possibly due to increase in overall attractive forces between bacteria surfaces and grain surfaces [12]. According to Derjaguin-Landua-Verwey-Overbeek theory (DLVO), an *E. coli* cell may experience a combination of attractive van der waal forces and repulsive electrostatic forces when it comes into close proximity to grain [13]. However, the effect of the different rates of biochar was not consistent as it moved from the smallest quantity to the highest quantity. The results obtained after the laboratory analysis of the various media samples showed that only topsoil without biochar plus manure mix had the highest percentage total Nitrogen (0.165) while the topsoil and sand mixes amended with biochar manure combinations recorded lower percentage total Nitrogen. This means that addition of biochar and manure to the soil and sand mixes resulted in the reduction of total nitrogen in the various media amended with biochar manure combinations. Reduction in total nitrogen concentration can be attributed to volatilization during heating of biochar and that structures such as amino acid, amines and amino sugar which contain nitrogen are condensed into recalcitrant form [14]. Other studies have also reported mobilisation of nitrogen upon addition of biochar to soil [15].

Available Phosphorus increased with an increase in the amount of biochar manure mix. According to Ch'ng et al. [16], soil amended with biochar or compost or a mixture of both increased total phosphorus, available phosphorus and inorganic phosphorus. Sasmita et al. [17] also found that application of biochar with or without organic fertiliser linearly increased the soil available P in Indonesian acidic soil medium during a 15-day incubation period. Exchangeable Potassium increased with increase in the amount of biochar plus manure mix. Nigussie et al. [18] found that biochar applied to chromium-polluted soils improved the soil exchangeable bases (K) in Ethiopia and they concluded that the increase in the exchangeable bases was as a result of the presence of ash in the biochar which helps in the immediate release of mineral nutrients like K for crop use. DeLuca et al. [19], also reported a greater soil available P content in biochar-amended soils compared to unamended soils and attributed the improvement to biochar's capacity to retain and exchange phosphate ions due to its positively charged surface sites.

Addition of biochar manure mix resulted in the increment of exchangeable calcium concentration. As the dose of biochar plus manure mix increased from 5% to 10% and 15% there was a significant increase of exchangeable calcium in all the media. Biochar applied to chromium-polluted soils improved the soil exchangeable bases (K) in Ethiopia and was concluded that increase in the exchangeable bases was as a result of the presence of ash in the biochar which helps in the immediate release of mineral nutrients like K for crop use [18].

Although exchangeable Magnesium was statistically the same in all the various media, they had different values. Results indicated that addition of biochar manure to the various media resulted in the increment of exchangeable Magnesium. According to Jha et al. [20], addition of biochar to an acidic Alfisol at 2%, 4%, and 6% (w/w) increased mean soil exchangeable Ca and Mg concentration by 50%, 92%, and 138% respectively. The results showed that application of biochar manure increased soil organic carbon concentration in the various media [21], found that application of compost and biochar, alone or in combination, increased soil organic carbon content than that in the unamended soils, which is indicative that biochar and/or compost applications to soils can enhance carbon accumulation and sequestration. Organic matter content increased in all the media that was amended with biochar manure mix. This can be attributed to addition of biochar manure mix to the various soil combinations. Soil organic matter content significantly increased with the increasing rates of the chicken manure [22]. The results showed that application of biochar and manure increased soil pH in all the various media (Table 5). However, no significant differences were found among the pH values of all the media. The increases in soil pH due to the application of biochar could be attributed to the high pH of the biochar (8.5) used in the experiment. According to Lehmann et al. [23], biochar has been shown to have a significant liming ability due to its intrinsically high pH and concentration of basic cations retained from the initial feedstock. [18], also attributed increased soil pH in biochar-amended soil to ash accretion and further explained that ash residues were highly dominated by carbonates alkali and alkali earth metals.

The media combination which had biochar and manure caused the Bermuda grass to sprout within a very short time as compared to the media without biochar and manure. Early emergence of Bermuda grass can be attributed to the high concentration of phosphorus in the biochar manure amended media as phosphorus is an essential nutrient for early vigorous root and shoot development [24]. It is no wonder that sprouting significantly affected grass coverage such that 99% variation in the grass coverage was explained by the sprouting. This suggests that sprouting is needed for good grass coverage according to this study. The height of Bermuda grass increased remarkably in the media amended with biochar plus manure mix compared to the unamended media. Increase of Bermuda grass could be attributed to the high concentration of Phosphorus and high content of soil organic carbon present in the media. According to Bigelow et al. [24], Phosphorus is associated with the growth of leaves. The results indicated that application of biochar plus manure to the various media had a remarkable effect on the rate at which Bermuda grass spread. This can be attributed to high concentration of available phosphorus, exchangeable potassium, calcium, magnesium, high content of soil organic carbon and high pH value in the biochar manure amended media. According to [24], phosphorus is associated with healthy root formation and it also increases the growth of leaf and stem dramatically. Potassium assists in better water and nutrient uptake while helping synthesize proteins and starches by plants. Potassium also helps the grass to build thicker cell walls [25]. Bermuda grass performs well at a pH range of 6.5 to 7.6 and McKenzie et al. [26] stated that soil pH influences nutrient absorption and plant growth. Almost all the pH values (within 7.15 to 7.99) for the soil mixes were within the required pH range for Bermuda grass growth.

Genetically, Bermuda grass is dark green in colour. Application of biochar plus manure mix at all levels resulted in improving the colour of Bermuda grass compared to the media without biochar plus manure. The Biochar plus manure amended media produced Bermuda grass with dark green colour while the media without biochar manure produced Bermuda grass with light green colour. This is as a result of high content of plant nutrients such as phosphorus, potassium, magnesium and calcium in the biochar plus manure amended media. Biochar under normal conditions contains nitrogen (N), phosphorus (P) and basic cations like calcium (Ca), magnesium (Mg) and potassium (K) [27]. Amanullah et al. [28] stated that, application of chicken manure to soil helps to increase soil retention and uptake of nutrients by plant.

## 5. CONCLUSIONS

This experiment showed that *E. coli* was present in chicken manure and that increasing the decomposition period had a significant effect on the *E. coli* by reducing its load. Also, the addition of biochar to the chicken manure resulted in a significant reduction of *E. coli* count ($p<0.01$). The soil amended with the biochar and manure also supported very well the growth of Bermuda grass with the 10% and 15% biochar manure mix in 70 to 30 ratio of topsoil and sand giving the best grass growth in terms of spread, thickness, height, and color. A regression analysis given by the equation $Y_{(coverage)}=176.857-23.0402_{(sprouting)}$ ($R^2=0.99$), indicated that sprouting significantly affected grass coverage such that 99% variation in the grass coverage was attributed to the sprouting. It was concluded that well-composted chicken manure should be used together with biochar on sports fields to help remediate the problem of *E. coli* infestation and also improve the growth of grass on fields.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Lehmann J, Rondon M. Bio-char soil management on highly weathered soils in the humid tropics, Biological approaches to sustainable soil systems, CRC press, Boca Raton. 2006;517-530. Available:http://dx.doi.org/10.1201/9781420017113.ch36
2. Brosnan JT, Deputy J. Bermuda grass. Department of tropical plant and soil science. Turf management; 2008.
3. Rhee CK. Determination of total nitrogen; 2001. Available:http://doi.org/10.1002/0471142913.fab0102s00.
4. Bray RH, Kurtz LT. Determination of total, organic and available forms of phosphorus in soils. Soil Science. 1945;59:39-45.
5. Bernaldo GB, Bertsch F, Nilo PG, Suvannang N, Hayr DR. Soil organic carbon, Walklay-black method: Titration and colorimetric method. Version. 2019;22-25.
6. Chapman HD. Cation exchange capacity. In: C.A. Black (ed). Method of soil analysis-chemical and microbiological properties. Agronomy. 1965;9;891-901.
7. Erickson MC, Liao J, Ma L, Jiang X, Doyle MP. Inactivation of *Salmonella spp*. In cow manure composts formulated to different initial C:N ratios. Bioresour. Technol. 2009;100:5898–5903.
8. Talaro KP, Talaro A, Physical and chemical control of microbes. In foundations in microbiology, 4th ed.; The McGraw-Hill Companies: New York, NY, USA. 2002;325–326.
9. Semenov AV, van Bruggen AHC, van Overbeek L, Termorshuizen AJ, Semenov AM, Influence of temperature fluctuations on *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cow manure. FEMS Microbiol Ecol. 2007;60:419–428.
10. Wilkinson KG, Tee E, Tomkins RB, Hepworth G, Premier R, Effect of heating and aging of poultry litter on the persistence of enteric bacteria, Poult. Sci. 2011;90:10-18.
11. Bach SJ, Stanford K, McAllister TA. Survival of *Escherichia coli* O157:H7 in feces from corn-and barley-fed steers. FEMS Microbiol Lett. 2005;252:25–33.
12. Mohanty SK, Cantrell KB, Nelson KL, Boehm BA. Efficacy of biochar to remove *Escherichia coli* from Stormwater under Steady and Intermittent Flow; 2014. Available:www.sciencedirect.com
13. Malte H, The DLVO theory in microbial adhesion. Colloids Surf. 1999;(1-4):105-119.
14. Cao X, Harris W. Properties of dairy-manure-derived biochar pertinent to its potential use in remediation Technology. 2010;101. Available:https://doi.org/10.1016/j.biortech.2010.02.052
15. Deenik JL, Goro U, Yudai S, A. Sidibe A. McClellan, M. Antal. Charcoal volatile matter content and its effects on plant growth and biological properties of an infertile tropical soil. Joint Annual Meeting; 2008.
16. Ch'ng HY, Ahmed HO, Ab Majad NM, Biochar and compost influence the phosphorus availability, Nutrients uptake and growth of maize (*Zea mays* L.) in tropical acid soil, Pakistan Journal of Agricultural Sciences. 2014;51(4).
17. Sasmita KD, Iswandi A, Syaiful A, Sudirman Y, Gunawan D, Application of biochar and organic fertiliser on acid soil

as growing medium for Cacao (*Theobroma cacao* L.) seedlings, International Journal of Sciences Basic and Applied Research. 2017;36(5):261–273.

18. Nigussie A, Kissi E, Misaganaw M, Ambaw G. Effects of biochar application on soil properties and nutrient uptake of lettuces (*Lactuca sativa*) grown in polluted soils, American-Eurasian Journal of Agriculture and Environmental Science. 2012;12(3): 369–376.S218–S221.
19. DeLuca TH, MacKenzie MD, Gundale MJ, Biochar effects on soil nutrient transformations, in biochar for environmental management: Science and technology". Earthscan Publications, London, UK. 2009;251–270.
20. Jha P, Neenu S, Rashmi I, Meena BP, Jatav RC, Lakaria BL, Biswas AK, Singh M, Patra AK. Ameliorating effects of leucaena biochar on soil acidity and exchangeable ions, Communications in Soil Science and Plant Analysis. 2016;47: 10:1252-1262.
DOI: 10.1080/00103624.2016.1166380
21. Trupiano D, Cocozza C, Baronti S, The effects of biochar and its combination with compost on lettuce (*Lactuca sativa* L.) growth, soil properties, and soil microbial activity and abundance, Hindawi International Journal of Agronomy, 12 pages. Soil Biology and Biochemistry. 2017;62: 137-146.
Article ID 3158207
22. Ru SH, Zhang GY, Junfang N, Sun SY, Wang L, Geng N. Effect of chicken manure application on crop yields and soil nutrients accumulation in wheat-corn rotation system, Biomedical Engineering and Biotechnology; 2012.
23. Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D. Biochar effects on soil biota–a review, Soil Biology and Biochemistry. 2011;43(9):1812-1836.
24. Bigelow CA, Tudor TW, Nemitz RJ. Facts about phosphorus and lawns: Purdue agronomy. Turf Science. 2016;2.
25. Caliskan B, Caliskan CA. Potassium nutrient in plants and its interactions with other nutrients in hydroponic culture; 2017.
26. McKenzie HR. Soil pH and plant nutrients. Agri-facts: Practical Information for Alberta's Agriculture Industry; 2003.
27. Major J, Lehmann J, Rondon M, Goodale C, Fate of soil-applied black carbon: downward migration, leaching and soil respiration, Global Change Biology. 2010a;16(4):1366-1379.
28. Amanullah MM, Sekar S, Muthukrishnan P, Prospects and potential of poultry. manure: Journal of Plant Science. 2010;9(4):172-182.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*https://www.sdiarticle5.com/review-history/114320*