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Studies on soil nitrogen in relation to bacterial microflora in soil samples from agricultural farmland in Naraguta, Plateau State

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Abstract

A study was conducted to determine the relationship between soil bacterial microfloral and organic Nitrogen content including soil pH. Two study agricultural farmlands were chosen. Ten soil samples were collected from each of the two agricultural farmlands and analysed quantitatively for total bacteria and soil nitrogen content. Total bacteria count was determined using the standard plate count while soil nitrogen content was determined using Kjeldahl method. Bacterial count ranged from 3.5 x 10^6 to 10.0 x 10^6 cfu/g in site -1 while in site -2, the bacterial counts ranges from 4.5 x 10^6 to 13.0 x 10^6 cfu/g. The corresponding soil nitrogen for site 1 and 2 was 0.30 - 0.50 $mgkg^{-1}$ and 0.50 - 0.71 $mgkg^{-1}$ respectively. Bacterial count was found to be directly proportional to soil nitrogen. The dominant bacteria identified in order of significance were; *Bacillus spp* (23.0%) >*Klebsiella spp* (21.3%) >*Pseudomonas spp* (16.3%) >*Azotobacter spp.* (13.3%) > S. *aureus* (11.3%) >*E. coli* (7.5%) >*Streptococcus spp* (6.6%). The pH for both sites ranged between 6.0-7.6. Correlation analysis was determined between the soil bacterial load and organic nitrogen. There was positive correlation between soil nitrogen and the corresponding bacterial load even though it was not statistically significant (P>0.05). It can be concluded that there is a relationship between soil bacterial micro flora and nitrogen content and this can be used as an index of soil health and fertility.

Keywords: Bacteria; Nitrogen; Soil fertility; Agriculture; soil samples

1. Introduction

Bacteria are responsible for most organic changes that consequently lead to the formation of plants nutrients in the soil [1]. Soil microbes are responsible for the fixation of Nitrogen, conversions of organic component from one form to another. They affect numerous soil functions and are important indicators of soil facility. These bacteria maintain the status and consistently sustain the presence of nutrients in our farmland. Managing these nutrients through supportable use of soil properties is necessary for effective farming [2]. The bacteria genera that are either especially common or have attracted particular interest include: *Bacillus, Clostridium, Pseudomonas, Staphylococcus, Azotobacter and Klebsiella spp.* Identifying and quantifying these bacteria in the soil may be necessary for determining the status soil nutrient for a particular farmland [3]. This may perhaps aid in the conservation of nutrients in the soil for enhanced crop yield. Numerous factors including the physical and chemical properties of soils can influence the type of species, number and activities of bacteria in a soil [4]. Studies have shown that in addition to environmental factors such as temperature, moisture and CO_2 levels, soil structure, soil pH and other chemical properties are major determinants of soil microbial

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community [5, 6, 7]. Land use and soil management practices can influence soil nutrients through processes such as mineralization, oxidation, leaching and erosion [8, 9]. This may affect the presence and activities of soil microorganisms and hence soil fertility. For instance, intensive tillage operations common in continuously cultivated soils may lead to increased decomposition / demineralization of available nutrients which may result to loss of nutrients from these soils [10]. Studies examining how bacterial microflora in relation to soil chemical content influence the contributions of soil microbes (bacteria) to soil health and fertility are uncommon in tropical soil studies. There has been standing interest in enumerating the number and types of soil microorganisms. This arises from the desire to use such measurements to indicate the health and efficiency of a soil in agriculture [10]. Hence, the main objective of this research was to examine how soil bacterial microflora in relation to the soil organic nitrogen content served as an indicator of soil health and fertility in Naraguta, Plateau State. Interrelationships between soil chemical and microbial properties were also compared.

2. Material and methods

2.1. Description of Study Area

Series of soil samples collected from two agricultural farmlands in Naraguta Village located two miles north-east of the university of Jos permanent site. Two study sites were chosen in the area. Site – 1 was humic and there was no history of pesticides application records on the site and is always used for farming. Site - 2 was situated at about 20m from site – 1 and appeared to be non-humic. There was also no history of pesticides application in the area.

2.2. History of the sampling site

Various crops had been continuously cultivated on the lands for over 20 years. Some of the crops cultivated during the previous years were cocoyam (*Colocasia esculenta*), eggplant (*Solanum melongena*) pepper (*Capsicum* sp.), sweet potato, (*Ipomoea batatas*), Irish potato (*Salanum tuberosum*) and yam (*Dioscorea* sp.). At the time of sampling, both cassava and maize were cultivated on the lands.

2.3. Samples collection

The soil samples were collected with a spatula which was sterilized in the field by dipping in alcohol. The depth of soil sampling was approximately 1 inch below the litter layer. There was a distance of ten meters between each sample taken to ensure that they were representative of soil being investigated. These soil samples were collected in sterile polythene bags. Total of 20 samples were taken, ten samples from each sampling sites (Cheesebrough, 2000).

2.4. Samples Analysis

The number of soil microorganisms was determined using the dilution spread plate technique. Nutrient Agar (NA) was used to culture bacteria. A dilution blank was prepared by weighing 1 g of fresh soil into 9 ml of sterile water. This was a 10^{-1} dilution. After shaking, 1 ml of the dilution was transferred aseptically into another 9 ml of sterile water to give a 10^{-2} dilution. This process was repeated 5 times (each new dilution made from the previous dilution) until 7fold dilutions were obtained (10^{-1} to 10^{-7}). Dilutions 10^{-4} to 10^{-7} was inoculated on the NA plates as follows: 0.1 ml of aliquot was dropped on a plate and spread over the plate with a sterile plastic spreader. After spreading, the lid was replaced, labeled and the plate inverted for incubation which was done at 26°C for 24 hours. Each sample inoculated was done in duplicates. After incubation, the plates were observed for colony morphologies and counting of colonies. Assuming each colony was produced by a single organism, the number of bacteria in the soil samples were calculated using a standard formula for calculating cfu/g [11].

2.5. Statistical Analysis

One-way Analysis of Variance (ANOVA) was used to test for differences between the two farmlands. Correlation analysis was used to determine the interrelationship between soil nitrogen and bacterial microflora. All analyses were carried out using the SPSS software-20.0 version.

3. Results and discussion

The table below shows the bacterial load and their corresponding organic nitrogen and soil pH. Site – 1 sample 7, had the highest total plate count ($10.0x10^6$ cfu/g), while site – 1, sample 1, had the lowest bacterial count ($3.5x10^6$ cfu/g), and the lowest organic nitrogen (0.30mgkg-1). Site 1 had the highest pH at sample 4 (7.6) and showed lowest pH at

sample 1 (6.0). Site -2, sample 1 had the highest bacteria count ($13.0x10^6$cfu/g) and highest organic nitrogen (N), ($0.71mgkg^{-1}$) at sample -2.

Table 1 Bacteria Biomass, Organic Nitrogen (N) and the Soil pH of Site 1 and 2.

Samples	TPC-1	TPC-2	Org.N-1	Org.N-2	pH-1	pH-2
1	3.5	13.0	0.3	0.7	6.0	6.5
2	6.2	6.5	0.4	0.5	6.3	7.3
3	6.2	6.7	0.4	0.6	7.2	6.1
4	4.7	8.2	0.4	0.7	7.6	6.6
5	7.2	6.7	0.5	0.6	6.8	6.4
6	7.0	7.7	0.4	0.6	6.6	7.2
7	10.0	9.7	0.5	0.7	7.3	7.1
8	9.2	10.5	0.5	0.7	7.0	7.1
9	4.5	4.5	0.3	0.5	6.5	6.5
10	6.5	10.5	0.4	0.7	7.1	6.1

TPC – Total plate count (cfu / mlx 10¬-6), Org-N – organic nitrogen (mg/kg)

Table 2 Microscopic and biochemical features of bacterial isolates and their percentage frequency.

GR	SH	AR	SF	COA	CAT	IND	OXI	MPO	% FREQ
-ve	Rod	Chain	-ve	-ve	-ve	-ve	+ve	*Pseudomonas spp.*	16.3
-ve	Bacilli	Chain	+ve	-ve	+ve	+ve	-ve	*Bacillus spp.*	23.0
-ve	Rod	Chain	-ve	-ve	+ve	-ve	+ve	*Azotobacter spp.*	13.3
+ve	Coccal	Chain	-ve	+ve	+ve	-ve	-ve	*S.aureus*	11.3
-ve	Rod	Chain	-ve	-ve	-ve	-ve	-ve	*Klebsiella spp.*	21.3
+ve	Coccal	Chain	-ve	-ve	-ve	-ve	-ve	*Streptococcus spp.*	6.6
-ve	Rod	Chain	-ve	-ve	-ve	+ve	-ve	*E. coli.*	7.5

Key: GR- Grain Reaction, SH- Shape, AR- Arrangement, SF- Spore Form, Cat- Catalase Test Coa- Coagulase Test, Ind- Indole Test, Oxi- Oxidases test, MPO- Most Probable Organisms, % Freq- Percentage Frequency, +ve = positive, -ve = negative

Also, site -2, sample 9 had the lowest bacterial count ($4.5x10^6$cfu/g), lowest organic nitrogen (N) ($0.50mgkg^{-1}$). The pH range was between 6.1 and 7.3. Table 2, Shows the microscopic and biological features of bacterial isolates, the bacterial isolates from all the samples include *Pseudomonas spp. Bacillus spp. Azotobacter spp. Staphylococcus aureus, Klesbsiella spp. Streptococcus spp. and E. coli* are all representend in the table. The pattern distributed of isolates and their relative abundance observed is as follows, *Bacillus spp.*> (23.0%) >*Klebsiella Spp*. (21.3%) > *Pseudomonas spp* (16.3%) >*Azotobacter spp.* (13.3%) >*Staphylococcus aureus*, (11.3%) >*Escherichia coli* (7.5%) and *Streptococcus spp* been the lowest (6.6%). Table 3 show the percentage frequency of bacterial isolates with respect to sites. *Bacillus spp.* had the highest frequency of 26.6% while *Streptococcus spp.* showed the lowest frequency of 5.0% in both sites. At site -1, *Klebsiella Spp.* showed the highest frequency of 22.2% while *Escherichia coli* had the lowest frequency of 6.2%. The pattern of distribution of isolates observed in site -2 is as follows: *Bacillus spp.*> (26.6%) >*Klebsiella spp.* (20.8%) >*Pseudomonas spp.* (15.8%) > Azotobacter spp. (15.2%) >*Staphylococcus aureus, = Escherichia coli* (8.2%) >*Streptococcus spp.* (5.0%). The pattern of distribution of isolates at site 1 also follows: *Klebsiella spp.* (22.2%) >*Staphylococcus spp.* (18.5%) >*Pseudomonas spp.* (17.2%) >*Bacillus spp.* (16.*0%*) >*Azotobacter* equal to *Streptococcus spp.* (9.8%) >*Escherichia coli* (6.2%).

Table 3 Percentage Frequency of Bacterial isolates with Respect to Sites.

Organisms	**Site – 1 (%)**	**Site – 2 (%)**
Pseudomonas spp.	14(17.2)	25(15.8)
Bacillus spp.	13.(16.0)	24(15.2)
Azotobacter spp.	8(9.8)	42(26.6)
S. aureus	15.(18.5)	13(8.2)
Klebsiella spp.	18(22.2)	33(20.8)
Streptococcus spp.	8(9.8)	8(5.06)
Echerichia coli.	5(6.2)	13(8.2)
Total	81(100)	158(100)

4. Discussion

This study shows the distribution of bacterial micro floral assessed by colony count method and organic nitrogen content assessed by Kjeldahl method. The bacteria count was found to be directly proportional to soil nitrogen. This implied that increase in organic matter increase soil microbial load. [12], observed that in general, site high in nitrogenous materials and humus has larger numbers of micro-organisms than habitat poor in organic matter. In order of relative abundance, *Bacillus spp* shows the highest percentage frequency of 23.0 %. This implied that *Bacillus spp is* the dominant species which is in line with [13], who observed that *Bacillus spp* considerably abundant in very rich and productive soil. This shows that both sites are good for agricultural practice. Table 3 show the relationship between the percentage frequencies of bacterial isolates from both sites. *Bacillus spp* had the highest frequency of 20.6 % at site -2 which indicate that site -2 is more productive than site -1 and is also in line with the work of [5]. The soil pH of both sites ranged between 6.0 and 7.6 this is similar to work of [14] who observed that a soil pH of 6.6 – 7.3 is favorable for microbial activities. Correlation analysis carried out between the bacterial load and total organic nitrogen was positive with determination coefficient of 0.98 and 0.92 for site -1 and site -2 respectively; although the result was not statistically significant ($p>0.05$). These results agree with the work of [15]. This may be due to the abundance of nitrogen fixing bacteria *(pseudomonas spp, Azotobacter spp* and *Klebsiella spp)* in the soil. [16] observed that the Gram – Negative rod notably *Pseudomonas fluorescence* are active in organic matter decomposition. [17], revealed that inoculation of soil with *Azotobacter spp.* results in increase in nitrogen level implying that the presence of these organisms in the soil increase nitrogen level.

5. Conclusion

The results of this research work clearly showed a relationship between the soil nitrogen and the soil bacterial micro flora, this means that increase in number of microbial flora leads to increase in the organic nitrogen content of the soil and these can be exploited and used as an indicator of soil health and fertility.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors whose names are mentioned hereby declare that they have no conflict of interest in this research article and that in case any of such comes up, it will be resolved hitch-free. The authors also declare that this research is solely sponsored by them without any external intervention.

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