



THE DIVERSITY OF BACTERIA IN SOIL ORGANIC LAYER UNDER *SENNA SIAMEA* AND *AZADIRACHTA INDICA* STAND

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ABSTRACT: The study was carried within the campus of Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria, to assess the diversity of bacteria in soil organic layer under *S. siamea* and *A. indica* stand. The bacterial colony counts on *S. siamea* and *A. indica* soil samples varied significantly. Seven distinct strains of bacteria such as *Bacillus subtilius*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus feacalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were isolated. The distinct strains frequencies of bacteria occurrence were *Bacillus subtilius*, *Escherichia coli*, and *Staphylococcus aureus* had 8 times appearance (20%) each. *P. vulgaris* had 6 times appearance (15%), *S. feacalis* had 5 times appearance (12.5%), while *Pseudomonas aeruginosa* and *Klebsiella pneumonia* had 3 and 2 times appearance (7.5 and 5%) respectively in all studied plots. The *A. indica* soil showed significantly ($P < 0.05$) higher count of 14.30×10^5 cfu/g and least of 7.6×10^5 cfu/g while the *S. seamea* soil had a highest count 16.27×10^5 cfu/g and least of 5.83×10^5 cfu/g. The result of analysis of variance (ANOVA) carried out between the two species indicated that there exists a significant difference in bacterial population of the two species but their exist no significant difference in species location.

Keywords: Sample, Bacteria colony, Species, Frequency, Incubation

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1. INTRODUCTION

Soil is fundamental and increasable; it governs plant productivity of terrestrial ecosystems and it maintains biogeochemical cycles because microorganisms in the soil degrade, sooner or later, virtually all organic compounds including persistent xenobiotics and naturally occurring polyphenolic compounds. The living population inhabiting soil includes macro fauna, mesofauna, micro fauna and microflora (Nannipieri *et al.* 2003). These soil dwellers provide food or nutrients that support organisms that live above and below ground which are microbes. Soil microbes, bacteria, archaea, and fungi play diverse and often critical roles in the ecosystem.

The activities of soil microbes drive or contribute to the cycling of all major elements (Carbon, Nitrogen and Potassium) and this cycling affects the structure and the functions of soil ecosystems as well as the ability of soils to provide services to people. Soils however, play critical roles in buffering and filtering freshwater ecosystems and consequently are extremely important to human societies. We depend

on soils for the basis on which we and our buildings stand, and for the production of food, building materials, and other resources; indeed, soils influence most ecosystem services on which we depend (Dominati *et al.* 2010).

Soil bacteria are important contributors to primary productivity and nutrient cycling in arid land ecosystems and their populations may be greatly affected by changes in environmental conditions. Bacteria are some of the smallest and most abundant microbes in the soil. According to Dominati *et al.* (2010) that in a single gram of soil, there can be billions of bacteria. He further stated that there are an estimated 60,000 different bacteria species, most of which have not yet been named, and each has its own particular roles and capabilities. Most live in the top 0-20cm of soil where organic matter is present. Soil bacteria play a primary role in organic matter decomposition and are therefore central to soil ecosystem processes maintaining plant primary productivity. In light of recent concerns regarding the impact of agriculture and climate change on biodiversity and ecosystem functioning (Wardle *et al.*

1998; Tilman *et al.* 2001), it is imperative to gain a more detailed understanding of the bacterial community ecology in our remaining native areas. However, this task is arduous due to the enormous diversity of soil bacteria, together with the complexity of factors regulating their growth and survival. Moreover, the lack of suitable methods for effective qualitative and quantitative bacterial enumeration has prevented a satisfactory understanding of the primary drivers regulating soil bacterial community structure and functioning. However recent advances in molecular techniques are now beginning to open the soil 'black box' and are now commonly used to examine the ecology of soil bacteria (Ogram 2000).

The physico-chemical and structural characteristics of soil provide many microenvironments in which complex bacterial populations can evolve (Ranjard and Richaume 2001). Information on the types of bacteria that inhabit the rhizosphere of *A. indica* and indeed some other trees and the diversity of such bacteria is scanty. The level of hindrance and/ or reduction in the bacterial population and activity has not been adequately documented. A comparison of the bacterial diversity in soil organic layers of *A. indica* and *Senna siamea* therefore becomes paramount. The objective of this study is to assess the bacterial diversity in soil organic layer of *S. siamea*. Previous researches have indicated the presence of antimicrobial agents in some parts of *A. indica*. The level of inhibition or otherwise exhibited by these antimicrobial agents on bacteria is however uncertain.

2. MATERIALS AND METHODS

2.1 The Study Area

The study was carried within the campus of Modibbo Adama University of Technology Yola, Adamawa State, located between latitude 9° 24' and 9° 29' N and longitude 12° 33' and 12° 35' East. Modibbo Adama University of Technology covers an area of 43.38km². Adamawa State is inhabited by 3,920,101 people (National Bureau Statistic 2007). The wet or rainy season fall between April and November, which is characterized by single Maxima in August. During this season, the moisture laden South West trade wind from Atlantic Ocean blow over the area. Seventy percent of the total rainfall in the area happens to fall within four months of May- August (Adebayo and Tukur 1999). The area has an average of 62 rainy days, while average amount of rainfall recorded in the area is 972mm. The dry season which is the harmattan period is between December and March. The period is characterized to dry, dusty and hazy Northern trade wind that blows over the area from Sahara Desert. Temperature within the area varies with season. Although the temperatures are relatively high almost all the year round, temperature of the area ranges from 27°C- 40°C. December and January in the

coldest months with average temperature of 34°C (Adebayo and Tukur 1999).

2.2 Study Design and Data Collection

Preliminary study was carried out for the purpose of identifying and delineating the study sites in the portion of the plantation comprising of *A. indica* and *S. seamea*. Soil samples were collected at random using Complete Randomized Design (CRD) as described by Ogunleye (2002) Five trees each were selected from *A. indica* and *S. seamea* plantation. Soil samples were collected from the organic layer 0-20cm soil depth, 30cm from the base of the trees using hand trowel from each sample point. A composite sample were mixed thoroughly, passed through 2 mm sieve to remove extraneous materials as described by Akosim *et al* (2015) and into sterile polythene bags and carried to the laboratory within 24 hours of collection (Sanchez 1975). The types of bacteria present in the sample were identified and their diversity analysed.

The laboratory analyses involved adding 1g of soil into 9 ml of sterile water in a test tube. This was then shaken vigorously. 1ml from the diluted 1g of soil and 9 ml of sterile water was serially transferred into other test tubes. Also, 1ml of the serial dilution is poured into a Petri dish and molten agar/ media was then added into the Petri dish. Isolation of soil bacterial population was estimated by the method of using the nutrient agar medium at 10⁵ dilution described by (Zain and Naing 2002). The inoculated petri-dishes were incubated at 30± 10C for 24 h for 5 days for bacterial colonies to be identified, counted and compared. Representative isolates of bacteria were identified under the microscope with the help of standard manuals by (Gilman 1995).

3. RESULTS AND DISCUSSION

Colony count were lower in the *A. indica* soil which was the soil sample Az while there was a higher count on the *S. seamea* soil which was the soil sample Ss for the same growth media in 24 hours of incubation. The *A. indica* soil showed highest count of 14.30 × 10⁵ cfu/g and least of 7.6 × 10⁵ cfu/g (Table 1) while the *S. seamea* soil had a highest count 16.27 × 10⁵ cfu/g and least of 5.83 × 10⁵ cfu/g (Table 2). Colony count on the *A. indica* was lower than that of *S. seamea* in the Soil. The bacterial count on both soil samples varied significantly (P<0.05). Seven distinct strains of bacteria were isolated. These include; *Bacillus subtilius*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

The frequencies of occurrence of the distinct bacteria colonies showed that *Bacillus subtilius*, *Escherichia coli*, and *Staphylococcus aureus* had the most

frequency of occurrence with 8 times appearance which is (20%) each. *P. vulgaris* had 6 times (15%) frequency of occurrence; *S. feacalis* had 5 times (12.5%) frequency of occurrence, while *Pseudomonas aeruginosa* and *Klebsiella pneumonia* had 3 and 2 times which is (7.5 and 5%) frequency of occurrence respectively in all studied plots (Table 3).

The relative abundance of bacteria at different points and between the two-different species of trees indicates that bacterial population in cfu/g of *S. seamea* in almost all sampled points exhibited a higher microbial composition than that of *A. indica*. The result of analysis carried out to compare the bacterial population between the two species, location and the species to location of sample collection area indicates that there exist a significant ($P < 0.05$) difference in bacterial population of the two species but their exist no significant difference in location and species/ location.

Bacterial count in the soil organic layer under *A. indica* was shown to be lower than that of soils under *S. siamea*. This may not be unconnected with the presences of many antimicrobial constituents present in the leaves of *A. indica* as reported by (Subbarao and Anna 2015). This is particularly so because leaves are a component of organic soil and whatever is found in the leaves is most likely to be found in the organic soil. The lower bacterial count conforms to the findings of Subbarao and Anna (2015) who reported that leaf extract of Neem contained extracts that have antimicrobial functions.

Table 1: Colony Forming Unit and Isolated Bacteria in *A. indica* ($\times 10^5$ CfU/g)

Sample	Location	Isolated Bacteria	(Cfu/g)
Az.1	A	<i>Streptococcus feacalis</i>	14.03
Az.1	B	<i>Staphylococcus aureus</i>	9.4
Az.1	C	<i>Staphylococcus aureus</i>	9.1
Az.1	D	<i>Staphylococcus aureus</i>	8.73
Az.2	A	<i>Proteus vulgaris</i>	7.97
Az 2	B	<i>Bacillus subtilus</i>	7.67
Az 2	C	<i>Streptococcus feacalis</i>	8.97
Az 2	D	<i>Pseudomonas aeruginosa</i>	10.37
Az3	A	<i>Eschrachea coli</i>	8.9
Az 3	B	<i>Bacillus subtilus</i>	9.2
Az 3	C	<i>Eschrachea coli</i>	8.5
Az 3	D	<i>Proteus Vulgaris</i>	11.17
Az 4	A	<i>Staphylococcus aureus</i>	10.87
Az 4	B	<i>Proteus vulgaris</i>	8.5
Az 4	C	<i>Proteus vulgaris</i>	9.13
Az 4	D	<i>Proteus Vulgaris</i>	7.23
Az 5	A	<i>Bacillus Subtilus</i>	8.43
Az 5	B	<i>Eschrachea coli</i>	7.63
Az 5	C	<i>Eschrachea coli</i>	9.1
Az 5	D	<i>Klebsiella Pneumonia</i>	7.7

Source: Field Survey, 2016

The bacterial count in the organic soil under *S. siamea* was found to be much higher than that of soils under *A. indica*. That is to be expected since research has not shown any part of *S. siamea* to contain antimicrobial constituents that may inhibit the growth and multiplication of bacteria (Arbonnier 2004).

Table 2: Colony Forming Unit and Isolated Bacteria in *S. seamea* ($\times 10^5$ CfU/g)

Specie	Location	Isolated Bacteria	Cfu/g
Ss 1	A	<i>Bacillus Subtilus</i>	15.4
Ss 1	B	<i>Bacillus Subtilus</i>	16.27
Ss 1	C	<i>Escherichia Coli</i>	15.53
Ss 1	D	<i>EscherichiaColi</i>	13.13
Ss 2	A	<i>Klebsiella Pneumonia</i>	13.43
Ss 2	B	<i>Bacillus Subtilus</i>	14.00
Ss 2	C	<i>Staphylococcus Aureus</i>	12.33
Ss 2	D	<i>StaphylococcusAureus</i>	13.60
Ss 3	A	<i>Streptococcus Feacalis</i>	10.37
Ss 3	B	<i>Proteus Vulgaris</i>	8.93
Ss 3	C	<i>Bacillus Subtilus</i>	11.67
Ss 3	D	<i>Escherichia Coli</i>	6.37
Ss 4	A	<i>Pseudomonas Aeruginosa</i>	6.00
Ss 4	B	<i>Pseudomonas Aeruginosa</i>	11.87
Ss 4	C	<i>Bacillus Subtilus</i>	7.87
Ss 4	D	<i>Streptococcus Feacalis</i>	6.17
Ss 5	A	<i>Staphylococcus Aureus</i>	5.83
Ss 5	B	<i>Staphylococcus Aureus</i>	13.63
Ss 5	C	<i>Bacillus Subtilus</i>	10.13
Ss 5	D	<i>StreptococcusFeacalis</i>	8.37

Source: Field Survey, 2016

Table 3: Frequency of Identified Bacterial Population in the Entire Site

Identified Bacterial Species	Frequenc y	Percent%
<i>Bacillus subtilus</i>	8	20.0
<i>Escherichia coli</i>	8	20.0
<i>Klebsiella pneumonia</i>	2	5.0
<i>Pseudomonas aurogenosa</i>	3	7.5
<i>Proteus vulgaris</i>	6	15.0
<i>Staphylococcus aureus</i>	8	20.0
<i>Streptococcus feacalis</i>	5	12.5
Total	40	100.0

Source: Field Survey, 2016.

Analysis of variance at ($P > 0.05$) level of probability showed a significant difference in the bacterial count in the soil organic layer of *A. indica* and that of *S. siamea*. The frequency of occurrences of these bacteria was also observed to be more in the soil samples of *S. siamea* than *A. indica*. The presences of about 140 chemical compounds in the leaves, seeds and bark of *A. indica* as reported by Biwas et al., (2002) and the antimicrobial properties of some of these chemical compounds may perhaps account for this difference.

Neem is a very valuable N-fixing tree with multipurpose uses. It was observed that the colony forming unit of *A. indica* soil samples were less when

compared to that of *S. siamea*; since leaf extract of *A. indica* is reported to have strong antimicrobial activity against bacteria and fungi, since leaves are a component of soil organic layer, the presence of these antimicrobial agents in the soil organic layer of *A. indica* will not be out of place. This perhaps accounts for the lower bacterial colony count in the soil samples collected under *A. indica* than those collected in soils under *S. siamea*.

4. CONCLUSION

In conclusion, study of the diversity of bacteria in soil organic layer under *S. siamea* and *A. indica* stand within the campus of Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria, indicated that:

1. Neem is a very valuable N-fixing tree with multipurpose uses.
2. It was observed that the colony forming unit of *A. indica* soil samples were less when compared to that of *S. siamea*.
3. The leaf extract of *A. indica* is reported to have strong antimicrobial activity against bacteria and fungi.

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