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# Identification and Distribution of Bacteria and Fungi in Soils of Rivers State University Teaching and Research Farm

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***Abstract***

*Bacteria and fungi identification is very crucial in other to understand their roles in soil structure formation and plant growth. The distribution of bacteria and fungi is diverse, and varies greatly across different environment, such as soil and water. However, the presence of various diversities of these microbes can be either harmful or beneficial to agriculture. Therefore, this work was conducted to identify the various diversity of bacteria and fungi in the soils of the study area. The microbial count ranged from $3.2\times10^6$ to $6.7\times10^6$ for bacteria (0-15cm) and $1.7\times10^4$ to $4.3\times10^4$ for fungi (0-15cm). For 15-30cm, bacteria ranged from $1.2\times10^6$ to $3.0\times10^6$ and fungi ranged from $0.2\times10^4$ to $2.7\times10^4$. It was observed that the microbial population was greater in top soil (0-15cm depth) and reduces down the soil profile (15-30cm depth). The diversity of bacteria species identified incudes: Pseudomonas sp, Bacillus sp, Nocardia sp, Clostridium sp, Proteus sp, Micrococcus sp, Staphylococcus sp and Klebsiella sp. While the diversity of fungi species identified includes: Cladosporium sp, Aspergillus sp, Pichia sp, Fusarium sp and Penicillium sp.*

***Keywords:*** *Distribution, Bacteria, Fungi, Land use and Identification*

## I. INTRODUCTION

Soil microorganisms are an important part of the forest ecosystem. Their community structure, biological activity, and physiological and ecological adaptations to environmental changes play an important role in the mineralization, humification, and nutrient cycle of soil, the conversion of soil organic compounds and nutrient release, and regulation of the functional diversity of soil (Nkongolo and Narendrula-Kotha, 2020). Owing to the diversity of microorganisms, complementary, synergistic, redundant, and selective interactions among species can effectively enhance the ecosystem activity and the buffering capacity of soil against external disturbances (Xia *et al.*, 2016). Soil microorganisms control many processes in the soil ecosystem; therefore, an in-depth understanding of the composition and functional diversity of soil microorganisms is important for elucidating their important role in regulating key ecosystem processes and developing a healthy ecosystem (Griffiths *et al.*, 2004). The structure and diversity of soil microbial communities are affected by environmental gradients. Soil microorganisms have

significant differential sensitivity to many environmental factors such as climate change, soil properties, and plant growth (Wang *et al.*, 2017; Zhang *et al.*, 2018).

As a means for understanding mechanisms of biogeographic patterns, range size lacks the resolution of detailed distribution maps. No standard methodology exists for measuring the area over which a species is found and determining the area of occupancy implies an understanding of the species habitat breadth (Gaston and Fuller, 2009). For habitat specialists this may be somewhat straightforward because field sampling can focus on mapping species distributions within a targeted habitat. Fungi are problematic in this regard because species can be recovered from multiple distinct habitats. Over 60% of the fungi isolated as endophytes can also be isolated from leaf litter (Osono, 2006), implying that a researcher would need to consider the linked distributions among all potential habitats to effectively measure a specie range. Linking species distribution maps from more than one habitat provides a novel understanding of how fungi are distributed within and between habitats.

The objective of this study is to identify the species distribution of bacteria and fungi across the different land use area in Rivers State University Teaching and Research farm.

## II. MATERIALS AND METHODS

This experiment was carried out in Rivers State University Teaching and Research farm, main campus.

### *Soil sample collection*

Materials used for soil sampling are black polythene bags, Labeling materials and auger.

Samples were collected from six different land use type, at two depth (0-15 and 15-30) which were replicated three times and bulked into two samples for each land use type. The samples were taken, wrapped in a foil and stored in the refrigerator for microbial analysis

### *Microbial analysis*

Nutrient Agar (28g) was weighed (according to manufacturer's instruction) into a conical flask containing 1000ml of distilled water. The conical flask was put in an autoclave at a pressure of 15psi (121°c) for 15mins to sterilize.

Sabouraud dextrose Agar (65g) was weighed (according to manufacturer's instruction) into a conical flask containing 1000ml of distilled water. The conical flask was autoclaved at a pressure of 15psi (121°c) for 15mins to sterilize.

### *Serial dilution protocol*

Glass wares were sterilized with an autoclave. A normal saline solution was prepared by dissolving 8.5g of NaCl in a conical flask containing 1000ml distilled water and autoclaved. Test-tubes were labelled according to number of samples, each sample having six test-tubes for a 6-fold serial

dilution. 9ml of normal saline was measured into each test-tube, and one gram of each soil sample put into the test-tube labelled $10^1$ respectively and diluted to 106. The plate pouring method of inoculation was used in the isolation of the microorganisms contained in the samples. A micro pipette was used to take 1ml each of the serial dilution prepared samples into the corresponding labelled petri dish. The petri dish was incubated at 380C for 24hrs for total heterotrophic count and at room temperature for 7days for fungi count.

***Determination of microbial load***

The samples were assessed visibly by counting the colony forming unit after 24hrs. The microbial load/ml was determined using the Cheesbrough (2004) formular:

$$\text{Count/ml} = \frac{\text{no of Colony Count}}{\text{Volume Plated}} \times \frac{\text{Dilution Factor}}{1}$$

***Identification of microbial isolates***

Freshly prepared NA and SDA were placed into already sterilized petri dish by pouring and allowing to solidify. From the old plates containing the microorganisms, the streaking method was used to isolate the individual bacteria cultures into a fresh plate for further study. The fungi cultures were sub cultured by carefully taking a fresh growing portion with a sterilized needle into a freshly prepared culture plates.

***Bacteria isolate identification***

The identification of bacteria isolate was determined by various methods of identification such as: morphology, gram staining, catalase test, indole test, glucose fermentation test etc

## III. RESULTS

**MICROBIAL COUNT AND IDENTIFICATION**

The result of the study as seen in table 4.1 shows that the microbial population of the study area is higher in the 0-15cm depth than in the 15-30cm depth which is due to the presence of higher amount of organic matter in the topsoil. Which is in line with (Brady and Weil 2008). It was observed that the bacteria count for 0-15cm depth ranged highest in P.P ($6.\times 10^6$) and lowest in H.H ($3.2\times 10^6$). While for the 15-30cm depth, the highest count was recorded in C.O ($3.0\times10^6$) and the lowest in C.F ($1.2\times10^6$).

The fungi count from the study in 0-15cm depth was observed to be highest in A.S ($4.3\times10^4$) and lowest in C.O ($1.7\times10^4$). While for the 15-30cm depth, the highest count was recorded in A.S ($2.7\times10^4$) and the lowest in P.P ($0.2\times10^4$).

The diversity of bacteria species identified across the study area as seen in table 1 include; *Pseudomonas sp, Bacillus sp, Nocardia sp, Clostridium sp, Proteus sp, Micrococcus sp, Staphylococcus sp, Klebsiella sp,*. Some of these bacteria species are beneficial to plant (*Pseudomonas sp;* promotes plant growth, *Bacillus sp;* capable of fixing nitrogen in soil, *Micrococcus sp;* capable of organic matter decomposition and nutrient cycling*)*, while some are pathogenic (Nocardia *sp* affects plant root, *Clostridium sp* produces toxins that are harmful to plant, *Proteus sp* causes plant diseases, *Staphylococcus sp* can contaminate soil though the bacteria specie is not typically soil-borne. *Klebsiella sp* causes plant diseases*)*
The diversity of fungi species identified as seen in table 1 include; *Cladosporium sp, Aspergillus sp, Pichia sp, Fusarium sp, Penicillium sp,*. These fungi species can be pathogenic (*Clasdosporium sp, Fusarium sp,* produces mycotoxins and causes fusariosis. *Aspergillus sp,* causes Aspergillosis) or beneficial (*Pichia sp,* used in food industries *and Penicillium sp,* produces antibiotics).

**Table.1 BACTERIA POPULATION AND IDENTIFICATION**

| Location | Depth | Bacteria count (cfu/g) | Bacterial Identified | Fungi count (cfu/g) | Fungi Identified |
|---|---|---|---|---|---|
| Hoop House | 0-15cm | $3.2 \times 10^6$ | *Pseudomonas* sp, *Bacillus* sp, *Nocardia* sp, *Clostridium* sp, *Proteus* sp, | $3.6 \times 10^4$ | *Cladosporium sp, Aspergillus sp,* |
| | 15-30cm | $1.5 \times 10^6$ | *Pseudomonas* sp, *Bacillus* sp, *Nocardia* sp, *Proteus* sp, *Clostridium* sp | $1.4 \times 10^4$ | *Cladosporium sp, Aspergillus sp* |
| Coconut Plantation | 0-15cm | $3.7 \times 10^6$ | *Pseudomonas* sp, *Bacillus* sp, | $2.9 \times 10^4$ | *Cladosporium sp, Aspergillus sp, Pichia sp, Fusarium sp* |
| | 15-30cm | $1.2 \times 10^6$ | *Pseudomonas* sp, *Bacillus* sp, | $0.7 \times 10^4$ | *Cladosporium sp, Aspergillus sp,* |
| Apiary snailery (Forestry) | 0-15cm | $5.9 \times 10^6$ | *Pseudomonas* sp, *Bacillus* sp, *Micrococcus sp, Nocardia* sp, | $4.3 \times 10^4$ | *Cladosporium sp, Aspergillus sp, Pichia sp, Fusarium sp* |
| | 15-30cm | $2.8 \times 10^6$ | *Pseudomonas* sp, *Bacillus* sp, | $2.7 \times 10^4$ | *Cladosporium sp, Aspergillus sp, Pichia sp, Fusarium sp* |

| | | | | | |
|---|---|---|---|---|---|
| | | | *Micrococcus sp, Nocardia* sp, | | |
| Cattle Ranch | 0-15cm | $5.5 \times 10^6$ | *Pseudomonas* sp, *Bacillus* sp, *Proteus* sp, *Micrococcus sp, Nocardia* sp, *Clostridium* sp | $4.0 \times 10^4$ | *Cladosporium sp, Aspergillus sp* |
| | 15-30cm | $1.9 \times 10^6$ | *Nocardia* sp, *Clostridium* sp, *Pseudomonas* sp, *Proteus* sp, *Bacillus* sp, *Micrococcus sp,* | $2.3 \times 10^4$ | *Cladosporium sp, Aspergillus sp* |
| Citrus orchard | 0-15cm | $6.5 \times 10^6$ | *Klebsiella sp, Bacillus sp, staphylococcus sp* | $1.7 \times 10^4$ | *Aspergillus sp, Fusarium sp* |
| | 15-30 cm | $3.0 \times 10^6$ | *Pseudomonas sp, Staphylococcus sp, Micrococcus sp, Klebsiella sp* | $1.3 \times 10^4$ | *Penicillium sp, Fusarium sp* |
| Plantain plantation | 0-15cm | $6.7 \times 10^6$ | *Clostridium sp, Nocardia sp, Staphylococcus sp, Bacillus sp* | $3.9 \times 10^4$ | *Cladosporium sp, Fusarium sp Penicillium sp* |
| | 15-30cm | $2.8 \times 10^6$ | *Staphylococcus sp, Klebsiella sp, Pseudomonas sp,* | $0.2 \times 10^4$ | *Aspergillus sp, Penicillium sp, Fusarium sp* |

## Table.2 Biochemical characterization of bacteria isolate

| Isolate code | Grams reaction | Cell morphology | Oxidase | Catalase | Citrate | Starch hydrolyses | Spore test | $H_2S$ | MR | VP | Indole | Glucose | Sucrose | Lactose | Motility | Maltose | Mannitol | Probable genera |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HH, CR, FP, CP | - | Rods | + | + | + | - | - | - | - | - | + | A/G | A/G | A/G | + | A | - | *Pseudomonas* sp |
| HH, CR | + | Rods | - | - | - | - | + | - | - | - | + | A/G | A/G | - | + | - | A | *Clostridium* sp |
| HH, CR | - | Rods | - | + | - | - | - | + | - | - | Neg | A/G | A/G | - | - | - | - | *Proteus* sp |
| HH, CR, FP, CP | + | Rods | - | + | + | + | + | - | - | - | - | A/G | A/G | - | + | - | A | *Bacillus* sp |
| HH, CR, FP | + | Rods | - | + | + | + | - | - | - | - | - | A | A | A | - | A | A | *Nocardia* sp |
| CR, FP, | + | Cocci | - | + | + | - | - | - | - | - | + | - | A | - | - | A | A | *Micrococcus sp* |
| CO, PP | - | Cocci | - | + | - | - | - | - | - | - | - | - | A | - | - | - | - | *Klebsiella sp* |

Keys:
HH - Hoop house A/G - Acid and gas formed
CR - Cattle ranch. A - Only acid formed.
FP - Apiary snailery
CO - Citrus orchard
PP - Plantain plantation
CP - Coconut plantation

**Table.3 Morphological Characteristics and identity of fungal isolate**

| Isolate Code | Colour | Font | Elevation | Colony edges | Alcohol tolerance | | Identified genera |
|---|---|---|---|---|---|---|---|
| | | | | | 10% | 20% | |
| HH, CR, FP, CP, PP, CO | Dark brown | | Slightly raised | | No | No | *Cladosporium sp* |
| FP, CP, CO, PP | Cream | | | Oval edges | + | + | *Pichia sp* |
| HH, CR, FP, CP, PP | Cream | White mucoid | Convex | Fimbriate | No | No | *Aspergillus sp* |
| FP, CP | Pink | Loose | Low convex | Entire | No | No | *Fusarium sp* |
| CO, PP | Black | | Circular | | | | *Aspergillus niger* |
| PP, CO | Grey | | Slightly raised | | | | *Penicillium sp* |

## PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

**Soil pH:** The pH of the soil ranges from 2.85 in the Apiary snailery area, to 6.20 in the coconut plantation. Based on the results gotten, the soil in the Apiary snailery is said to be extremely acidic compared to the other land use areas, this is as a result of the presence of coniferous tree species found in that area (Li *et al.* 2022). The soils in hoop house, cattle ranch, plantain plantation, and citrus orchard is moderately acidic while the soil in coconut plantation is slightly acidic. According to FDALR (1985).

**Soil Organic carbon:** The percentage organic carbon ranges from 0.94% in the Apiary snailery, to 2.07% in the coconut plantation. This result in line with the work of (Oriakpono *et al.*, 2018) shows that coconut plantation has the highest percentage of organic carbon, apiary snailery has the lowest percentage while citrus orchard, plantain plantation, hoop house and cattle ranch have moderate percentage.

**Soil Organic matter:** The quality and quantity of organic matter present in a soil greatly determines the composition and abundance of microbial diversity in that soil. The organic matter content is highest in coconut plantation (3.57%), moderate in citrus orchard (2.02%), plantain plantation (1.96), hoop house (2.16%) and cattle ranch (2.70), and lowest in apiary snailery (1.62%). And this is in line with the work of (Oriakpono *et al.*, 2019).

**Soil Available phosphorus:** The available P content of the study area ranges from 38.61mg/kg in cattle ranch to 154.44mg/kg in apiary snailery. In line with EDALR (1985), the result shows that all the sampling areas have high content of available phosphorus.

**Soil Sodium:** The Na content ranged lowest at 0.43cmol/kg in coconut plantation, and highest at 0.83cmol/kg in cattle ranch. In line with (Ene *et al.*, 2018), the Na content in cattle ranch is high while the Na content in coconut plantation, citrus orchard, hoop house, apiary snailery and plantain plantation is moderate.

**Soil Magnesium:** The Mg content ranges from 1.8cmol/kg in hoop house to 5.4cmol/kg in plantain plantation. This shows that the citrus orchard (2.8cmol/kg) and hoop house (1.8cmol/kg) has moderate Mg content while plantain plantation (5.4cmol/kg), apiary snailery (4.4cmol/kg), coconut plantation (4.2cmol/kg), and cattle ranch (4.4cmol/kg) has high Mg content. This result is based of the work of (Oriakpono *et al.*, 2018).

**Soil Calcium:** The Ca content ranges from 1.8cmol/kg in citrus orchard to 6.0cmol/kg in coconut plantation. This shows that the calcium content in citrus orchard, hoop house, plantain plantation, apiary snailery and cattle ranch is low while the Ca content in coconut plantation is moderate. This result is in line with (Orji and Oko-jaja 2016).

**Soil Potassium**: The K content ranges from 0.07cmol/kg in plantain plantation to 0.21cmol/kg in cattle ranch. This result entails that plantain plantation (0.07cmol/kg) and citeus orchard (0.08cmol/kg) has very low Potassium content while hoop house (0.15cmol/kg), apiary snailery (0.12cmol/kg), coconut plantation (0.11cmol/kg) and cattle ranch (0.12cmol/kg) has low potassium content. This result is in line with (Orji and Oko-Jaja 2016).

| Sample code | pH (1:2.5) | Total exchangeable acidity (cmol/kg) | organic carbon (%) | organic matter (%) | Available phosphorus (mg/kg) | K (cmol/kg) | Ca (cmol/kg) | Na (cmol/kg) | Mg (cmol/kg) |
|---|---|---|---|---|---|---|---|---|---|
| C.O | 4.79 | 2.48 | 1.17 | 2.02 | 56.16 | 0.08 | 1.8 | 0.5 | 2.8 |
| H.H | 4.77 | 2.00 | 1.25 | 2.16 | 49.14 | 0.15 | 2.6 | 0.5 | 1.8 |
| P.P | 4.31 | 2.96 | 1.13 | 1.95 | 70.2 | 0.07 | 1.8 | 0.5 | 5.4 |
| F.R | 2.85 | 2.08 | 0.94 | 1.62 | 154.44 | 0.12 | 2.4 | 0.54 | 4.4 |
| C.P | 6.20 | 1.92 | 2.07 | 3.57 | 91.26 | 0.11 | 6.0 | 0.43 | 4.2 |
| C.R | 5.25 | 1.92 | 1.68 | 2.70 | 38.61 | 0.21 | 5.0 | 0.83 | 4.4 |

**Table.4 SOIL CHEMICAL PROPERTIES**

**Table.5 Particle size distribution**

| Sample name | Sand % | Silt % | Clay % | Textural class |
|---|---|---|---|---|
| CO | 84.2 | 1.8 | 14.0 | Loamy sand |
| HH | 88.2 | 1.8 | 10 | Loamy sand |
| PP | 86.2 | 1.8 | 12 | Loamy sand |
| AS | 87.2 | 2.8 | 10.0 | Loamy sand |
| CP | 82.2 | 4.8 | 13.0 | Loamy sand |
| CR | 89.2 | 2.8 | 8.0 | Loamy sand |

## IV. DISCUSSION

There was a greater population of bacteria and fungi in the top soil compared to deeper depth. Some of these bacteria species are beneficial to plant (Pseudomonas sp; promotes plant growth, Bacillus sp; capable of fixing nitrogen in soil, Micrococcus sp; capable of organic matter decomposition and nutrient cycling), while some are pathogenic (Nocardia sp affects plant root, Clostridium sp produces toxins that are harmful to plant, Proteus sp causes plant diseases, Staphylococcus sp can contaminate soil though the bacteria specie is not typically soil-borne. Klebsiella sp causes plant diseases)

These fungi species can be pathogenic (Clasdosporium sp, Fusarium sp, produces mycotoxins and causes fusariosis. Aspergillus sp, causes Aspergillosis) or beneficial (Pichia sp, used in food industries and Penicillium sp, produces antibiotics).

## V. CONCLUSION

From the results obtained, it was observed that Bacteria and fungi were isolated from all sampled environments, indicating their ubiquitous presence. Identification of isolates revealed the presence of both beneficial (e.g., Pseudomonas, Bacillus) and pathogenic (e.g., Aspergillus, Klebsiella) microorganisms. The result gotten also shows that microbial population cam be affected by land use type.

The result of the physical and chemical properties of the soil shows that generally the soil in the study area is an acidic loamy sand.

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