

# Microbial Characterization of Mangrove and Rainforest Soils, and Cowpea (*Vigna Unguiculata* L. Walp) Cultivation in Rivers State, Nigeria

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**Abstract-** Different groups of microorganisms are present in rainforest and mangrove vegetation soil types, but not much is known about their population and diversity, hence, this research was conducted to evaluate and compare seasonal variations in microbial population, and diversity in two vegetation soil types in Port Harcourt, Rivers State, Nigeria. Samples of top soil (0-15cm) and subsoil (15-30cm) were collected during the dry and rainy seasons, and subjected to standard analysis. Cowpea was also cultivated in the different soils and microbial population before and after cultivation were also investigated. Results showed that microbial population was significantly ( $P \leq 0.05$ ) highest during the dry season for both mangrove and rainforest vegetation types than during the other seasons. Ranges of microbial populations were: total heterothrophic bacteria  $7.8-25.0 \times 10^5$ cfu/g and  $6.6-22.1 \times 10^5$ cfu/g; total heterotrophic fungi  $2.0-5.4 \times 10^3$ cfu/g and  $0.3-0.9 \times 10^3$  cfu/g; Actinomycetes  $0.2-3.7 \times 10^3$ cfu/g and  $0.2-0.9 \times 10^3$ cfu/g; Nitrifying bacteria  $0.2-6.9 \times 10^2$  cfu/g and  $0.2-5.0 \times 10^2$ cfu/g; Nitrogen fixing bacteria ( $0.2-1.3 \times 10^2$ cfu/g and  $0.2-1.5 \times 10^2$ cfu/g) for rainforest and mangrove soils respectively. A total of 33 bacteria, 2 actinomycetes and 15 fungi species were isolated and identified from the two vegetation types across all seasons. *Bacillus* sp was the most predominant bacterium present, while *Aspergillus* was the most predominant fungus in both vegetation types and during all seasons. Microbial population increased in the different soils after the cultivation of cowpea and seasonal variations affected microbial diversity and populations.

**Indexed Terms-** Vegetation, Soil, Characterization, Bacteria, Fungi

## I. INTRODUCTION

Around and close to the equator are tropical rainforests, which experience a climate characterized by high rainfall and warm average temperatures (Dounias, 2018). Over hundreds of millions of years, they underwent multiple fragmentations that led to their current distribution across tropical America, Africa, Madagascar, Southeast Asia, New Guinea, and, to a lesser extent, Australia (Dounias, 2018; Lawal *et al.*, 2020). Their cover is estimated to be roughly 16.5 million square kilometers, or around 3% of the world's land surface, while being in perpetual decline ((Dounias, 2018).

Four different forest types make up these ecosystems: the first is lowland equatorial evergreen rainforests, which experience constant, heavy rainfall; the second, although they receive a lot of rainfall overall, moist deciduous and semi-evergreen seasonal forests experience alternating warm wet and cool dry seasons, which causes trees with null and void foliage to lose their leaves; the third, known as montane rainforests, can be found at elevations between 1,500 and 3,300 meters in highland regions with a milder temperature; and flooding subdues the fourth type of forest, known as flooded forests, which is made up of wetlands and floodplains (Ndarake & Umunna, 2015; Dounias, 2018; Lawal *et al.*, 2020). Tropical rainforests are home to between 40 and 75 percent of all biotic species, as well as two-thirds of all blooming plants and half of all living animal and plant species worldwide, though there are still millions of uncharacterized species of plants, invertebrates, and microorganisms in rainforests (Lawal *et al.*, 2020).

Mangroves are typically described as swampy places that are influenced by tides and found along the shore

of tropical and subtropical countries, they cover around 25% of the world's tropical coastline, and they serve as a barrier against wind, waves, water currents, and numerous other harsh weather phenomena, such as soil erosion and ocean swell (Palit *et al.*, 2022). Mangrove forests as a unique habitat connecting freshwater and marine ecosystems, and a nursery for aquatic organisms, have the capacity to sequester up to 25.5 million tons of carbon dioxide annually, which is four times more than inland terrestrial plants (Paingankar & Deobagkar 2018).

Mangrove forests are therefore distinctive and rich in biodiversity, they rank second among coastal wetlands in terms of productivity, after coral reefs, and the marine food chain is formed by the movement of organic matter from land to sea and the flow of energy between terrestrial and marine ecosystems (Dattatreya *et al.* 2018). Mangroves are found in two regions of the world: the east and the west; the African regions of the Atlantic, North, and South American mangroves, including the Galapagos Islands, make up the West Zone, while South Asian nations like India, Bangladesh, and Pakistan; Southeast Asian nations like Australia, New Zealand, and the Indonesian archipelago; and the eastern region of the African coast make up the east zone (Palit *et al.*, 2022).

The majority of mangroves' ecological significance has been discovered to be associated with the existence of a diverse and healthy microbiota, which includes protists, fungus, bacteria, and archaea (Lin *et al.*, 2019; Allard *et al.*, 2020). According to Allard *et al.* (2020), microbes, which inhabit a variety of microniches in mangrove ecosystems, particularly the rhizosphere, seem to be key players in the ecological functions of mangroves. These species may also be very important for the renewal and restoration of damaged mangroves, and Rhizosphere bacteria, for example, participate in the biogeochemical cycles of elements, particularly those of sulphur, iron, carbon, and nitrogen (Lin *et al.*, 2019) which describes the mangrove ecosystems (Baker *et al.*, 2021), aids in their high output (Haldar & Nazareth 2018) and helps them survive in intertidal zones (Mai *et al.*, 2021).

Furthermore, the microbial cycling of nitrogen and carbon has a significant role in mitigating climate change, and rhizosphere bacteria play a crucial role in

the breakdown of xenobiotics and pollutants, such as pesticides, solid waste, spilt crude oil, and the fixation of heavy metals in sediment (Mai *et al.*, 2021). Since a significant portion of the biosphere is made up of microbes found in marine and coastal sediments, knowledge of their diversity and ecology is essential to comprehending ocean processes worldwide (Baker *et al.*, 2021).

There is evidence that most tropical forest and mangrove soils may harbor novel microbial communities, however, these soils are an especially understudied microbial environment, and very few studies have explored the immense diversity of microbial communities, and activities in these soils; and also the effects of seasonal influences of precipitation and temperature on these soils microbial communities. This study there aimed to examine soil microbial community composition and diversity of the two vegetation types, and assess the impact of rainfall and temperature regimes on microbial community.

## II. MATERIALS AND METHODS

### Study Area

The study area was Port Harcourt metropolis; Port Harcourt is situated in the humid rainforest region of Southern Nigeria. It lies between latitude 4.5°N and longitude 7.0°E on an elevation of 18m above sea level. The mean annual rainfall in Port Harcourt ranges from about 3,000mm to 4,500mm. Annual maximum temperature ranges from 22°C to 29°C while relative humidity varies between 75% and 95%.

### Collection of Soil Samples

Soil samples were collected in two seasons: rainy season and dry season, and at two depths: 0-15cm and 15-30cm using a soil auger at three randomly selected sampling points, each within an area of 1m x m, at a distance of 30m apart in each location. Soil samples were collected from the three sampling points in each location, the soil samples were collected, composited and carefully placed into well labeled fresh polythene bags, soil samples for microbial analysis were collected using well labelled sterile sample bottles, which were placed into ice cooled containers. Soil core samplers were used to collect undisturbed soil from the mapped out areas for physical analysis. All the

samples were then transported to the laboratory for analysis.

Sampling was done monthly, for a period of one year (June 2018- May 2019). A total of twelve representative composite soil samples were collected for microbiological analysis from each location in each month of sampling during dry and rainy seasons.

#### Microbial Analysis

Microbial analysis of soils involved isolation and enumeration of microorganisms (culturable bacteria, fungi, actinomycetes, nitrifying bacteria and nitrogen fixing bacteria) from soil, which was done by viable plate count methods. Biochemical Characterization and Identification of isolates was also done.

#### Isolation, Purification and Characterization of Bacteria

Serial dilution of soil samples was done, up to  $10^{-3}$  dilution. Appropriate dilutions were plated onto nutrient agar medium and other agar media depending on the physiological bacterial type. The plates were incubated at  $37^{\circ}\text{C}$  for 24- 48hrs. The pure cultures were obtained based on the colony characteristics and the pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the bacterial isolates. Individual nitrogen fixing bacteria and nitrifying bacteria was isolated by spread –plating on the solid medium. 0.1ml portion of each sample was pipetted and plated out on the solid medium. A glass spreader sterilized with alcohol and flame was used to spread the inoculums evenly on the plates. The plates were incubated at  $37^{\circ}\text{C}$  for 24-48hrs. Purity of isolates was achieved by sub-culturing discrete colonies to freshly prepared sterile nutrient agar plates by dissolving 2.9g of nutrient agar in 100ml of distilled water and autoclaved at  $121^{\circ}\text{C}$  for 15 min. Nutrient agar was thoroughly shaken and then poured into washed bijou bottles which were tightly closed. The bottles were sterilized in the autoclave at  $121^{\circ}\text{C}$  for 15min. After autoclaving they were slanted and allowed to solidify. Slant culture medium was inoculated with the purified bacterial culture obtained by the isolation and purification processes. Characterization of isolates was done by observing morphological and colonial characteristics of the isolates on the nutrient agar plates.

The morphological tests were: Gram reaction, cell shape and size, endospores, position of endospores, motility, while the biochemical tests were catalase, oxidase, urease and coagulase tests, for the identification of isolates.

#### Isolation of Fungi

This was performed by the pour plate method using potato dextrose agar medium. The plates were incubated at  $25^{\circ}\text{C}$  for 2-3 days. Pure fungal colonies were obtained and identified by morphological structures observed by lacto phenol staining fewer than 100X lens.

#### Isolation of Actinomycetes

Actinomycetes were isolated from soil using Kenknight's Agar.

#### Media for Nitrogen Fixing Bacteria

Azospirillum Medium: Each of the components were measured with the weighing balance and put into a conical flask; containing 100ml distilled water and covered with aluminum foil. The pH of the medium was adjusted to 6.5, using a pH meter. The medium was autoclaved at a temperature of  $121^{\circ}\text{C}$  for 15 min and allowed to cool, then poured in petri dishes. Azotobacter Medium, and Rhizobium Medium (Yeast Mannitol Agar) were also used.

#### Statistical Analysis

Data collected from the various parameters was subjected to analysis of variance (ANOVA) at  $P \leq 0.05$ , and means were separated using Tukey's Pair Wise Comparison at 95% confidence intervals.

### III. RESULTS

#### Soil Microbial Population

Total heterothrophic bacteria population ranged from  $7.8 - 25.0 \times 10^5 \text{cfu/g}$  in the rainforest vegetation and  $6.6 - 18.2 \times 10^5 \text{cfu/g}$  in the mangrove vegetation, for 0-15cm and 15-30cm depths respectively (Table 1). Highest bacterial population of  $25.0 \times 10^5 \text{cfu/g}$  and  $22.1 \times 10^5 \text{cfu/g}$  was recorded at 0-15cm depth during the dry season in the rainforest and mangrove vegetation respectively while the lowest heterothrophic bacterial population of  $6.6 \times 10^5 \text{cfu/g}$  and  $6.8 \times 10^5 \text{cfu/g}$  were recorded at 15-30cm depth in

the mangrove vegetation during the peak of dry season and the peak of rainy season.

Total heterothrophic fungal population ranged from 0.9 -5.4 x10<sup>3</sup>cfu/g at both depths in the rainforest vegetation and 0.3 -0.9 x 10<sup>3</sup>cfu/g in the mangrove vegetation (Table 1). Highest fungal population of 5.4 x10<sup>3</sup>cfu/g was recorded at 0-15cm depth during the rainy season in the rainforest vegetation, this was followed by 4.5x10<sup>3</sup>cfu/g during the dry season, while lowest fungal population of 0.3 x10<sup>3</sup>cfu/g was recorded at 15-30cm depth during the dry season and in the peak of dry season (Table 1).

Actinomycetes population ranged from 0.2- 3.7 x10<sup>3</sup>cfu/g and 0.2- 2.9 x10<sup>3</sup>cfu/g at the two depths, in the rainforest and mangrove vegetation respectively (Table 1). Highest actinomycetes population counts of 3.7 x10<sup>3</sup>cfu/g was recorded at 0-15cm depth during the dry season in the rainforest vegetation, followed by 2.9 x10<sup>3</sup>cfu/g in the mangrove vegetation type at the same depth and season. Lowest actinomycetes population count of 0.2 x10<sup>3</sup>cfu/g was recorded at 15-30cm depth during the rainy season in the rainforest vegetation (Table 1).

Population counts for nitrifying bacteria (Nitrosomonas and Nitrobacter) ranged from 0.2 – 6.9 x10<sup>2</sup>cfu/g and 0.2-3.6x10<sup>2</sup>cfu/g at both depths in the

rainforest vegetation and 0.3-5.1 x10<sup>2</sup>cfu/g and 0.2-4.9x10<sup>2</sup> cfu/g at both depths in the mangrove vegetation, for nitrosomonas and nitrobacter respectively (Table 1). Highest counts for nitrosomonas (6.9cfu/g x10<sup>2</sup>) were recorded at 0-15cm depth during the dry season in the rainforest vegetation, while nitrobacter population was highest (4.9 x 10<sup>2</sup>cfu/g) at 15-30cm depth during the dry season in the mangrove vegetation. Lowest nitrosomonas and nitrobacter population counts of 2.0 x10<sup>2</sup>cfu/g were recorded at15-30cm depths during the peak of dry season in the rainforest and mangrove vegetation respectively (Table 1).

Population counts for nitrogen fixing bacteria (Azospirillum and Azotobacter) ranged from 0.3- 1.3 x 10<sup>2</sup>cfu/g and 0.2- 2.2 x 10<sup>2</sup>cfu/g at both depths in the rainforest vegetation and 0.2 - 1.1 x 10<sup>2</sup>cfu/g and 0.0-2.0 x 10<sup>2</sup>cfu/g at both depths in the mangrove vegetation, for Azospirillum and Azotobacter respectively (Table 1). Highest counts for Azospirillum (1.3 x 10<sup>2</sup>cfu/g) and Azotobacter (2.2 x 10<sup>2</sup>cfu/g) were recorded at 0-15cm depth during the dry season in the rainforest vegetation. The lowest counts for Azospirillum (0.2 x 10<sup>2</sup>cfu/g) and Azotobacter (0.0 x 10<sup>2</sup>cfu/g) and 0.1 x 10<sup>2</sup>cfu/g) were recorded at both depths during the rainy season in the mangrove vegetation (Table 1).

Table 1: Mean Seasonal Microbial Populations of Rainforest and Mangrove Vegetation Soils at the Two Depths

Vegetation	Season	Depth (cm)	Total Heterothrophic Bacteria (cfu x 10 <sup>5</sup> )	Total Heterothrophic Fungi (cfu/g x 10 <sup>3</sup> )	Actinomycetes (cfu/g x 10 <sup>3</sup> )	Nitrosomonas (cfu/g x 10 <sup>2</sup> )	Nitrobacter (cfu/g x 10 <sup>2</sup> )	Azospirillum (cfu/g x 10 <sup>2</sup> )	Azotobacter (cfu/g x 10 <sup>2</sup> )
Rainforest	Rainy	0-15	13.2cde	5.4a	0.3hi	1.5ef	1.0cd	0.5cde	0.4cd
		15-30	8.5fgh	3.2c	0.2hi	0.8fg	0.5e	0.3e	0.2cd
	Peak of Rain	0-15	14.7 <sup>c</sup>	4.5b	0.9ef	1.9de	1.2cd	0.7bcde	0.3cd
		15-30	9.3fgh	2.1d	0.5fghi	1.2efg	0.7cd	0.4e	0.6c
	Dry	0-15	25.0 <sup>a</sup>	4.0b	3.7a	6.9a	3.3b	1.3a	2.2a
		15-30	18.3 <sup>b</sup>	2.1d	2.3c	4.0c	3.6b	1.1abc	2.1a

	Peak of Dry	0-15	12.8cde	2.0d	1.1e	0.5fg	0.3cd	0.5e	0.5cd
		15-30	7.8gh	0.9ef	0.8efg	0.2g	0.2d	0.4e	0.5cd
Mangrove	Rainy	0-15	14.0cd	0.9e	0.4ghi	1.5ef	1.0cd	0.2e	0.1cd
		15-30	7.7gh	0.4efg	0.2i	0.7fg	0.4cd	0.2e	0.0d
	Peak of Rain	0-15	11.5def	0.8efg	0.5fghi	2.0de	1.4c	0.7abcde	0.4cd
		15-30	6.8h	0.5efg	0.3ghi	1.1efg	0.6cd	0.5e	0.2cd
	Dry	0-15	22.1 <sup>a</sup>	0.6efg	2.9b	5.1b	4.3ab	1.1ab	2.0ab
		15-30	15.7bc	0.3fg	1.6d	2.6d	4.9a	1.1abcd	1.5b
	Peak of dry	0-15	10.4efg	0.7efg	0.6fgh	0.7fg	0.5cd	0.5de	0.5cd
		15-30	6.6h	0.3g	0.5fghi	0.3g	0.2d	0.5e	0.2cd

Means with same letters are not significantly different  $P \leq 0.05$

#### Soil Microbial Diversity and Distribution

A total of thirty- one (31) bacterial species were isolated and identified during the seasons and in the vegetation types. The species of bacteria include: *Alcaligenes*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Corynebacterium*, *Edwardsiella*, *Enterobacter*, *Escheichia*, *Flavobacterium*, *Kluvera Morganella*, *Microbacterium*, *Micrococcus*, *Nitrosomonas*, *Nitrobacter*, *Providencia*, *Pseudomonas*, *Salmonella*, *Salinicoccus Serratia*, *Staphylococcus* and *Raoultella*. A total of two (2) actinomycetes were isolated and identified during the seasons and in the vegetation types. The species of actinomycetes include: *Actinomyces* and *Nocardia* (Tables 6 and 7). All bacterial and actinomycetes isolates present in the mangrove vegetation were also present in the rainforest, except for *Enterobacter sakazaki* which was present in the mangrove soils, but absent in the

rainforest (Table 4). *Bacillus spp* was the most predominant bacterial species isolated in all the months and in the vegetation types, while *Aspergillus species* was the most predominant fungi isolated (Tables 2 – 5).

A total of fifteen (15) fungal species were isolated and identified during the seasons and in the vegetation types, and they include: *Aspergillus*, *Arthroderma*, *Candida*, *Choanephora*, *Fusarium*, *Mucor*, *Penicillium*, *Trichoderma*, *Streptomyces*, *Saccharomyces*, *Rhizopus* and *Rhodotorula* (Tables 5 and 8).

The occurrence of bacteria and actinomycetes in soil was higher during the dry (March- May) and rainy season (September- November), than during the peak of rainy (June- August) and the peak of dry season (December- January) (Table 2).

Table 2: Monthly/Seasonal Distribution of Bacterial Isolates in Soils

ISOLATES	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
<i>Enterobacter sakazaki</i>	+	+	+	+	+	+	-	-	+	+	+	+
<i>Bacillus niacin</i>	+	+	+	+	+	+	+	+	+	+	+	+

<i>Staphylococcus epidermis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Microbacterium binotii</i>	-	+	+	+	+	+	+	+	+	+	+	-
<i>Escherichia hermanni</i>	-	-	+	+	+	-	-	-	+	+	+	-
<i>Bacillus firmus</i>	+	+	+	+	+	-	-	+	+	+	+	-
<i>Enterobacter aerogenes</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>Bacillus sporothermodurans</i>	+	-	+	+	+	+	+	+	+	+	+	+
<i>Bacillus tequilensis</i>	+	+	+	+	+	-	-	+	+	+	+	+
<i>Staphylococcus schleiferi</i>	+	-	+	+	+	+	+	+	+	+	+	+
<i>Kluvera sp</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>Escherichia coli</i>	+	-	+	+	+	+	+	+	+	+	+	-
<i>Providencia alcalifaciens</i>	-	-	+	+	+	+	+	+	+	+	+	+
<i>Micrococcus caseolyticus</i>	+	-	+	-	+	+	+	+	+	+	+	-
<i>Raoultella ornithinolytica</i>	-	-	+	+	+	-	-	+	+	+	+	-
<i>Staphylococcus lentus</i>	+	+	+	-	-	+	+	+	+	+	-	+
<i>Salinicoccus kuningensis</i>	+	-	+	-	-	-	-	+	+	-	-	+
<i>Morganella morganii</i>	-	-	-	+	+	+	+	+	+	+	-	+
<i>Bacillus subtilis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Bacillus cereus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Pseudomonas sp</i>	+	+	+	+	+	+	+	+	+	+	+	-
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	+	+	+	+	+	+	-
<i>Salmonella sp</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>Flavobacterium sp</i>	-	+	+	-	-	-	-	-	-	+	+	-
<i>Azospirillum sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Nitrosomonas sp</i>	+	+	+	+	+	+	+	+	+	+	-	+
<i>Nitrobacter sp</i>	+	+	+	+	+	+	+	+	-	-	+	+
<i>Actinomyces sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Serratia marcescens</i>	+	+	+	+	+	+	+	+	-	+	+	+
<i>Azotobacter sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Micrococcus sp</i>	+	+	+	+	+	+	+	+	+	+	+	-
<i>Nocardia sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Alcaligenes faecalis</i>	-	-	-	+	+	+	-	-	+	+	+	-
<i>Corynebacterium sp</i>	-	-	+	+	+	-	-	+	+	+	+	-

KEY: + = bacterial type isolated, - = bacterial type not Isolated

(September- November), than during the peak of rainy (June- August) and the peak of dry season (December- January) (Table 3).

The occurrence of fungal isolates in soil was higher during the dry (March- May) and rainy season

Table 3: Monthly/Seasonal Distribution of Fungal Isolates in Soils

ISOLATES	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
<i>Arthroderma sp</i>	-	-	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus lentulus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Trichophyton sp</i>	-	+	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Penicillium sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Fusarium sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Rhizopus sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Candida sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Sacchromyces sp</i>	-	-	-	+	+	+	+	+	+	+	+	-
<i>Rhodotorula sp</i>	-	-	+	+	+	+	-	-	+	-	+	+
<i>Mucor</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus flavus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Trichoderma sp</i>	-	+	+	+	+	-	-	-	+	+	+	+
<i>Streptomyces sp</i>	-	+	+	+	+	-	-	-	+	+	+	+
<i>Choanephora sp</i>	-	-	+	+	+	-	-	+	+	+	+	-

KEY: + = Fungal Type Isolated - = Fungal Type not Isolated

mangrove soils, in all the months of sampling (Table 4).

*Alkaligenes faecalis* and *Corynebacterium sp* were present in the rainforest soils, but absent in the

Table 4: Distribution of Bacterial isolates in the Mangrove and Rainforest Vegetation Types

Bacterial Isolates	Mangrove Swamp	Rainforest
<i>Enterobacter sakazaki</i>	+	-
<i>Bacillus niacin</i>	+	+
<i>Staphylococcus epidermis</i>	+	+
<i>Microbacterium binotti</i>	+	+
<i>Escherichia hermanni</i>	+	+
<i>Bacillus firmus</i>	+	+
<i>Enterobacter aerogenes</i>	+	+
<i>Bacillus sporothermadurans</i>	+	+
<i>Bacillus tequilensis</i>	-	+
<i>Staphylococcus schleiferi</i>	+	+
<i>Kluyvera sp</i>	+	+
<i>Escherichia coli</i>	+	+
<i>Providencia alcalifaciens</i>	+	+
<i>Micrococcus caseolyticus</i>	+	+

<i>Raoultella ornithinolytica</i>	+	+
<i>Staphylococcus lentus</i>	+	+
<i>Salinicoccus kunningensis</i>	+	+
<i>Morganella morgani</i>	+	+
<i>Bacillus subtilis</i>	+	+
<i>Bacillus cereus</i>	+	+
<i>Pseudomonas sp</i>	+	+
<i>Pseudomonas aeruginosa</i>	+	+
<i>Salmonella sp</i>	+	+
<i>Flavobacterium sp</i>	+	+
<i>Azospirillum sp</i>	+	+
<i>Nitrosomonas sp</i>	+	+
<i>Nitrobacter sp</i>	+	+
<i>Actinomyces sp</i>	+	+
<i>Serratia marcescens</i>	+	+
<i>Azotobacter sp</i>	+	+
<i>Micrococcus sp</i>	+	+
<i>Norcadia sp</i>	+	+
<i>Alkaligenes faecalis,</i>	-	+
<i>Corynebacterium sp</i>	-	+
<i>Edwardsiella sp</i>	-	+

KEY: + = Bacterial Type Isolated - = Bacterial Type not Isolated

All fungal isolates were present in the rainforest and mangrove vegetation (Table 5).

Table 5: Distribution of Fungal Isolates at Mangrove and Rainforest vegetation Types

Fungal isolates	Mangrove Swamp	Rainforest
<i>Arthroderma sp</i>	+	+
<i>Aspergillus lentulus</i>	+	+
<i>Trichophyton sp</i>	+	+
<i>Aspergillus niger</i>	+	+
<i>Penicillium sp</i>	+	+
<i>Fusarium sp</i>	+	+
<i>Rhizopus sp</i>	+	+
<i>Candida sp</i>	+	+
<i>Aspergillus fumigatus</i>	+	+
<i>Sacchanomyces sp</i>	+	+
<i>Rhodotorula sp</i>	+	+
<i>Mucor</i>	+	+
<i>Streptomyces sp</i>	+	+
<i>Tricoherma sp</i>	+	+
<i>Choanephora sp</i>	-	+

KEY: + = Fungal Type Isolated, - = Fungal Type not Isolated



The biochemical characterization and identification of bacterial isolates is presented in Table 6.

Table 6: Biochemical Characterization and Identification of Bacterial Isolates

Isolate code	Microscopy	Catalase	Motility	MR	VP	Indole	Salt .T	Starch Hydrolysis	Urease	Citrate	Oxidase	Mannitol	Maltose	Lactose	Sucrose	Glucose	Macroscopy	Probable identity
A 1-20	-ve short rods	+	-	-	-	+	+	+	+	-	+	A	A	A	N	A	Pink round raised smooth	<i>Enterobacter sakazakii</i>
A 21-40	+cocobacillus	+	+	-	+	+	+	-	+	-	+	N		A	A	A	Cream round raised	<i>Bacillus niacin</i>
A 61-80	+small Cocci in chains	+	-	+	+	-	+	+	+	-	-	N	A	N	A	A	Deep yellow round raised	<i>Staphylococcus epidermidis</i>
B 1-20	+ve short rods	+	-	-	-	-	+	+	-	-	+	A	A	A	A	A	Round	<i>Microbacterium binoti</i>
B 21-40	+ve chained ellipsoidal rods with white centre	+	+	-	+	+	+	+	+	+	+	A	A	A	A	A	White large round serrated edge, flat surface	<i>Bacillus cereus</i>
C 1-20	+ve small short rods in pairs	+	+	-	+	+	+	+	+	+	+	A	A	A	A	A	Pale flat serrated/irregular raised flat	<i>Bacillus subtilis</i>
C 41-60	-ve slender rods	+	+	+	-	+	+	-	-	-	+	A	A	A	A	A	Red small round smooth	<i>Escherichia hermani</i>
D 1-20	+ve chained rods	+	+	-	-	+	+	+	-	-	-	A	A	N	N	A	White large round flat centered edge	<i>Bacillus firmus</i>
D21-40	-ve small slim straight rods			-	-	+	+	+	+	+	+	A	A	A	A	A	Pale small round raised smooth	<i>Salmonella Sp</i>
E 21-40	-ve short slim rods	+	+	-	+	+	+	-	-	-	+	A	A	A	N	A	Pink round smooth	<i>Enterobacter</i>

																		<i>aerogene s</i>
F 21-40	-ve short rods	+	+	+	-	+	+	-	-	+	+	A	A	N	A	A	Cream Yellow circular flat	<i>Bacillus</i>
																	undulated dry	<i>sporothromodurans</i>
F 41-60	-ve rods scatterd																Red small round shiny	<i>Serratia marcescens</i>
G 1-20	+ve short rods	+	+	-	+	+	+	+	-	-	-	A	A	A	A	A	Cream, round raised concentric surface	<i>Bacillus tequilensis</i>
G 61-80	+ve cocci in chains	+	-	-	+	+	+	+	+	-	-	A	N	N	A	A	Cream round raised with dotted surface	<i>Staphylococcus schleiferi</i>
H 21-40	-ve short rods	+	-	-	-	+	+	-	-	+	+	A	N	A	N	A	Brown flat dry	<i>Klugvera sp</i>
J 1-20	-ve short rods			+	+	-	+	+	+	+	+	A	A	A	A	A	Light yellow flat smooth	<i>Flavobacterium sp</i>
J 21-40	-ve single short rods	+	-	+	-	+	+	-	-	-	-	A	N	A	A	A	White raised rough surface	<i>Escherichia coli</i>
J 41-60	-ve short rods	+	-	+	-	+	+	+	-	-	+	N	A	A	N	A		<i>Ewardsiella sp</i>
J 61-80	-ve slim short Rods	+	-	+	-	-	+	+	-	-	-	N	A	A	N	A	White with pink centre irregular edge raised surface.	<i>Providencia alcalifaciens</i>
J81-100	-ve small rods	+	+	+	-	+	+	+	+	+	+	A	A	A	A	A	Green small round raised	<i>Pseudomonas aeroginosa</i>
K 21-40	+vecoci in pairs	+	+	-	-	+	+	+	-	-	+	N	A	A	A	A	Orange round, raised smooth	<i>Micrococcus caseolytus</i>
K 41-60	-ve short rods	+	-	+	-	+	+	+	+	+	+	A	A	A	A	A	Light brown shiny	<i>Raoultella ornithinolytica</i>
L 1-20	+small clustered cocci	+	+	+	-	+	+	+	-	-	+	A	A	A	A	A	Milk, round ,raised moist	<i>Staphylococcus lentus</i>

N 21-40	+ve small cocci	+	+	+	+	-	+	-	-	-	-	-	A	N	A	A	N	Cream circular raised dotted surface	<i>Salinicoccus kunmingensis</i>
Q 41-60	-ve short rods	+	+	+	-	+	+	-	-	-	-	-	A	N	A	A	A	Yellow raised, smooth	<i>Morganelia morganii</i>

The biochemical characterization of isolates on special media (actinomycetes, nitrifying and nitrogen fixing bacterial species) is presented in Table 7.

Table 7: Characterization and Identification of Bacterial Isolates from the Study Soils

Isolate code	Macroscopy	Microscopy	Probable identity
A	White Large Fluffy Growth, White Reverse	Long Pink Branched, Filaments Are Septate	<i>Actinomyces Sp</i>
B	Deep Red/Rose Red, Reverse, White Fluffy, Small Colony	Long Branched Pink Septate	<i>Norcadia Sp</i>
C	White/Milky Small Round, Raised Colories	-Ve Small Cocci In Chains	<i>Azospirillum Sp</i>
D	Milky Small Round Raised Colony		<i>Azospirillum Sp</i>
E	Small Round Moist	-Ve Short Rods	<i>Nitrobacter Sp</i>
F	Small Round Shinny	-Ve Short Rods	<i>Nitrobacter</i>
G	Milky Small Round, Raised Mucrid	-Ve Small Rods	<i>Nitrosozonas Sp</i>
I	Milky Small Round Raised, Dry/Smooth	-Ve Small Dry Rode	<i>Nitrosozonas Sp</i>
J		-Ve Small Skin Rode	<i>Nitrosozonas Sp</i>

The characterization and identification of fungal isolates is presented in Table 8.

Table 8: Characterization and Identification of Fungal Isolates

Isolate Code	Macroscopy	Microscopy	Probable Identity
A	White fluffy spores, white reverse	Erect sporangiospheres forming large erminal, globose sporangia	<i>Mucor sp</i>
B	Cream coloured, leafy projections, brown reverse	Hyaline macroconidia, multiseptate with thick walls	<i>Microsporium Sp</i>

C	White sudelike, white reverse	One celled conidia	<i>Choanephora sp</i>
D	Small cream colonies	Oral budding yeast	<i>Candida Sp</i>
E	White background, red centre, deep pink reverse	Smooth thin walled macroconidia with numerous chlamydo spores	<i>Trichophyton Sp</i>
F	White background, blackspores brown reverse	Septate hyphae bearing Conidia	<i>Aspergillus Sp</i>
G	White flat fluffy, white reverse	Hyphae are aseptate bearing Conida	<i>Mucor Sp</i>
H	White spores, white reverse	Hyphae are aseptate	<i>Mucor Sp</i>
I	Flat to cottony with a suede-like to granular texture, deep rose red reverse	Septate hyphae bearing conidiophore which are Laterally attached to hyphae	<i>Arthroderma Sp</i>
J	Suedelike white with interspersed grey-green patches of spores	Septate hypae bearing conidiospores and conidia	<i>Aspergillu lentulus</i>
K	White flat fluffy colony with a brilliant lemon yellow reverse	Spherical microconida	<i>Trichophyton Sp</i>
L	Dark brown black spores surrounded with white edge, brown reverse	Biserate Coridia Head With phialides borne On septate metulae	<i>Aspergillus niger</i>
M	Green suede/velvety growth with white periphery cream to yellow reverse	Septate hyphae with brached conidiophores bearing phialides coridia are arranged in chains on phialides	<i>Penicillium Sp</i>
N	White colony growth with greyish to black spores, yellow reverse	Non septate branched sporangiophores, with round head sporangia and shrouds	<i>Rhizopus Sp</i>
O	Large cream smooth round shiny	Oval shaped cells	<i>Candida Sp</i>
P	Brown fluffy with white spores and deep green-black reverse	Erect apically branched elongate conidiophores producing acropetal chains of conidia	<i>Choanephora sp</i>
Q	Flat white fluffy colony with depressed centre light-pink reverse	Septate hyphae bearing conidia laterally to the hyphae	<i>Apergillus Sp</i>
R	White suedelike, with creamy centre and spikelike edge, white reverse	Aseptate hyphae bearing large sporangiophore	<i>Mucor SP</i>

S	White periphery with greenish centered spores yellow reverse	Septate hyphae with conidiophores bearing conidia.	<i>Aspergillus Sp</i>
T	Small round glistening colonies	Oval budding cells	<i>Saccharomyces Sp</i>
U	White suedelike colony, raised centre and depressed, yellow	One celled conidia, smooth wall	<i>Choanephora sp</i>
V	reverse		<i>Rhodotorula sp</i>
W	Pink red glistening small round colony with moist surface	Oval shaped budding cells	<i>Fusarium sp</i>
X	Fluffy colony with a distinct rose red, white-yellow reverse White lawn like growth with yellow periphery in a spiky leafy form, yellow reverse	Septate hyphae with presence of long septate conidia Aseptate branched sporangiophores with round head conidia	<i>Rhizopus sp</i>

• Microbial Population in Soils Cultivated with Cowpea during the Rainy and Dry Season

The mean population counts of microorganisms in soils before and after planting of cowpea is presented in Table 9. All the physiological types of microorganisms isolated and identified had higher mean counts of  $25.9 \times 10^5$ cfu/g,  $6.7 \times 10^3$ cfu/g,  $3.8 \times 10^3$ cfu/g,  $6.3 \times 10^2$ cfu/g,  $3.5 \times 10^2$ cfu/g,  $2.3 \times 10^2$ cfu/g,  $2.6 \times 10^2$ cfu/g and  $3.9 \times 10^2$ cfu/g at 12 weeks, after the cultivation of cowpea than the mean counts of  $16.9 \times 10^5$ cfu/g,  $1.9 \times 10^3$ cfu/g,  $1.5 \times 10^3$ cfu/g,  $4.1 \times 10^2$ cfu/g,  $2.2 \times 10^2$ cfu/g,  $1.3 \times 10^2$ cfu/g,  $1.4 \times 10^2$ cfu/g and  $0.0 \times 10^2$ cfu/g in soils, recorded before cultivation, irrespective of seasons for total heterothrophic bacteria, total heterothrophic fungi, Actinomycetes, *Nitrosomonas*, *Nitrobacter*, *Azospirillum*, *Azotobacter*, and *Rhizobium* respectively (Table 9).

The mean counts of microorganisms in rhizosphere soils of cowpea during the rainy and dry season are presented in Table 4.14. All the types of microorganisms isolated and identified had higher counts of  $24.4 \times 10^5$ cfu/g,  $5.8 \times 10^3$ cfu/g,  $3.1 \times 10^3$ cfu/g,  $8.0 \times 10^2$ cfu/g,  $4.5 \times 10^2$ cfu/g,  $1.8 \times 10^2$ cfu/g,  $2.3 \times 10^2$ cfu/g, and  $2.6 \times 10^2$ cfu/g in the rainy season than population counts of  $17.4 \times 10^5$ cfu/g,  $2.8 \times 10^3$ cfu/g,  $2.2 \times 10^3$ cfu/g,  $2.4 \times 10^2$ cfu/g,  $1.2 \times 10^2$ cfu/g,  $1.7 \times 10^2$ cfu/g,  $1.7 \times 10^2$ cfu/g and  $1.3 \times 10^3$ cfu/g recorded in the dry season, for total heterothrophic bacteria, total heterothrophic *Fungi*, *Actinomycetes*, *Nitrosomonas*, *Nitrobacter*, *Azospirillum*, *Azotobacter* and *Rhizobium* respectively (Table 9)

Table 9: Mean Values of Microbial Population in Soils Cultivated with Cowpea during the Rainy and Dry Season

Season	Time of planting	Total Heterothrophic Bacteria (cfu/g x 10 <sup>5</sup> )	Total Heterothrophic Fungi (cfu/g x 10 <sup>3</sup> )	Actinomycetes (cfu/g x 10 <sup>3</sup> )	Nitrosomonas (cfu/g x 10 <sup>2</sup> )	Nitrobacter (cfu/g x 10 <sup>2</sup> )	Azospirillum (cfu/g x 10 <sup>2</sup> )	Azotobacter (cfu/g x 10 <sup>2</sup> )	Rhizobium (cfu/g x 10 <sup>2</sup> )
Rainy	Before	23.2 <sup>c</sup>	2.4 <sup>c</sup>	1.8 <sup>c</sup>	6.9 <sup>b</sup>	4.0 <sup>b</sup>	1.0 <sup>d</sup>	1.8 <sup>c</sup>	0.0 <sup>c</sup>
	After	27.5 <sup>a</sup>	9.1 <sup>a</sup>	4.4 <sup>a</sup>	9.0 <sup>a</sup>	5.0 <sup>a</sup>	2.6 <sup>a</sup>	2.7 <sup>a</sup>	5.2 <sup>a</sup>
Dry	Before	10.6 <sup>d</sup>	1.3 <sup>d</sup>	1.2 <sup>d</sup>	1.2 <sup>d</sup>	0.4 <sup>d</sup>	1.5 <sup>c</sup>	0.9 <sup>d</sup>	0.0 <sup>c</sup>

After	24.2 <sup>b</sup>	4.3 <sup>b</sup>	3.2 <sup>b</sup>	3.5 <sup>c</sup>	2.0 <sup>c</sup>	1.9 <sup>b</sup>	2.4 <sup>b</sup>	2.6 <sup>b</sup>
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#### IV. DISCUSSION

The results of the microbial population of soils showed that vegetation, season and depths had significant effects on the microbial populations of the soils. Population of identified microorganisms varied according to the vegetation types and the seasons studied. Total heterotrophic bacteria population was generally higher in the top soils than in the sub soils (Table 1). This could be due to variations in the number of colony forming units and with sampling depths due to reduced exposure to sunlight and the decreased organic matter content as well as reduced O<sub>2</sub> tensions in the lower layers of the soil. This position is supported by the assertions of Rocha *et al.* (2016), Behera *et al.* (2019), and Liu *et al.* (2019) who in addition to these factors, reported vegetation and salinity especially in mangrove forest as influencing bacterial community composition.

Also, populations of total heterotrophic bacteria from mangrove soils were lower than those found in the rainforest soils, though not significantly different (Table 1). This could be as a result of the acidic and waterlogged nature of the mangrove soils. Generally, microbial counts were found to be more in oxygen rich soils compared to carbondioxide rich soils (Goodluck, 2024). Lower bacteria population in mangrove forests soils, as compared to the rainforest soils could be as a result of the strongly acidic pH of the soils in the mangrove, as compared to the rainforest which had a moderately acid pH as reported in the study by Hamzah *et al.* (2018).

The total heterotrophic bacteria population was significantly higher than the population of other groups of microorganisms in both top and subsoil, and in both vegetation types, during all the seasons, especially at dry season which represents the beginning of rains (Table 1). This could be due to the unique environmental condition, making mangrove a hot area for microbial diversity, and nutrients and ideal circumstances for microbiological colonization are provided by mangrove vegetation (Palit *et al.*, 2022). Total heterotrophic fungal population was higher in the topsoil than in the subsoil. Fungal population was generally low, in both vegetation types and during all

the seasons as compared to the bacterial population (Table 1). This is consistent with findings by Lee *et al.* (2019) who reported low counts for fungi in mangrove forests. Despite the higher concentration of nutrients and moisture in the mangrove soils, fungal population was relatively low; this could be attributed to the frequent flooding of saline waters in the mangrove, which restricted the growth of fungi, and probably, the very strongly acidic property of the soils (Simões *et al.*, 2015; Luis *et al.*, 2019).

Additionally, Actinomycetes population were generally very low, corresponding to less than 0.2% of the bacterial population, were higher in the topsoils than in the subsoils, and the counts were higher in soils of the rainforest vegetation than in the mangrove soils. Also, the actinomycetes population was relatively low during all seasons, except during the dry season /beginning of rains, where the population increased significantly in topsoil and subsoil (Table 1). Large variations in actinomycetes population have been observed, depending on the ecosystem studied. In heavy-metal-contaminated rhizosphere soil in China, Zhang *et al.* (2017 and Yang *et al.* (2020) reported variations in their population.

Population counts of Nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) were higher in the topsoil than in the subsoil. Population counts for nitrosomonas were higher in soils of the rainforest vegetation, while population counts of nitrobacter were higher in the mangrove soils (Table 1). Also, population counts for Nitrogen fixing bacteria (*Azospirillum* and *Azotobacter*) were generally low, and no significant difference was observed in topsoil and subsoil, and in both vegetation types. Highest nitrogen fixing bacteria population was recorded at dry season, (beginning of rains) [Table 1]. Low population of Nitrogen fixing bacteria in soil could be postulated as one of the major reasons for corresponding low nitrogen levels in the soils, both in the mangrove and rainforest vegetation. Although, mangrove ecosystems are highly productive and rich in Carbon, they are considered low nutrient environments (De Santana *et al.* 2021). Nitrogen has been highlighted as one of the major nutrients limiting mangrove growth and major factors contributing to nitrogen loss in these

ecosystems are tidal export of nitrogen, denitrification and the soil type (Soares-Gomes *et al.*, 2016; Palit *et al.*, 2022).

Population and diversity of microorganisms can be influenced by changes in the physical and chemical characteristics of soil and also by the seasons (Liu *et al.* 2019; Ma *et al.* 2020). Majority of the bacterial and fungal isolates identified were present during the rainy and dry seasons (beginning of rains). While fewer diversities were recorded during the peak of rainy season and in the peak of dry season. Bacterial species like *Escheirichia hermani*, *Alkaligenes faecalis*, and *flavobacterium* were absent at both peak of dry season and peak of rainy season, while *Enterobacter aerogenes* and *Corynebacterium sp* were absent, only at peak of dry season (Tables 2, 6 and 7). These bacterial species have been reported in different studies (Zhou *et al.* 2017; Liu *et al.* 2019; Ma *et al.* 2020; Meng *et al.* 2021). Fungal isolates like *Streptomyces*, *Trichoderma*, *Choanephora sp* and *Boytritis cinerea* were absent at the peak of dry season, but present at every other season (Tables 5 and 8). Arguably, seasonal and temporal shifts in rainfall, especially in ecosystems where organisms may be at or near physiological tolerance can have a large impact on the diversity, abundance and responsiveness of soil microbial communities. This position is amplified by (Palit *et al.*, 2022).

The diversity of bacteria of bacteria and fungi isolated and identified in the rainforest and mangrove vegetation and their relative abundance, is a proof that the soil represents the most diverse and important ecosystem on the planet. The most predominant and frequently occurring genus of bacteria identified are the *Bacillus sp*, with over five different species isolated (Table 2). This was in agreement with findings by Lawal *et al.* (2020), in which bacteria is the predominant soil microbe followed by Actinobacteria and then Fungi. Similarly, Ohimain *et al.* (2024) in their study carried out in the Niger Delta region of Nigeria, found that *Bacillus sp* dominated the rhizosphere of all the mangrove plants, and the rhizosphere of the mangrove trees contained a variety of *Bacillus species*, including *Klebsiella quasipneumoniae*, *Photobacterium ganghwense*, *B. mycoides*, *B. paramycoides*, *B. pumilus*, and *B. siamensis*. Notably, bacterial species are able to

survive adverse environmental conditions, by producing extremely drought resistant endospores (Ndarake & Umunna, 2015).

## CONCLUSION

Soils of rainforest and mangrove contains an array of bacterial and fungal species with variations in their populations due to the significant effects of vegetation, season and depths on microbial communities.

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