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Assessment of pH and microbial properties of limestone and basement complex soils derived from Akamkpa Local Government Area, Cross River State.

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Abstract

Soil from various kinds parent materials differ from each other in physical, chemical, biological, mineralogical, and morphological properties. However, the evaluation of microbial properties of limestone and basement complex soil in Akamkpa Local Government Area of Cross River State will serve as a guide to farmers and government in educating them on the proper use of the land for agricultural productivity. Samples for the study were randomly collected and bulk at each of the locations at the depth of 0-15 and 16 – 30 cm for the upper, middle, and lower slope. The valley bottom with 44.50 x 10^6cfu/g had the highest bacterial count which was significantly ($P \leq 0.05$) different from those of upper slope (17.50 x 10^6cfu/g) and middle slope with 26.0 x 10^6cfu/g. Fungal count also had the highest microbial count at the valley bottom with 34.58 x 10^3cfu/g > upper slope (20.75 x 10^3cfu/g) > middle slope (14.25 x 10^3cfu/g) soil. The parent materials did not significantly ($P > 0.05$) influence bacteria count but had significant ($P \leq 0.05$) difference on fungal count with limestone (28.2 x 10^3cfu/g) and basement complex (18.2 x 10^3cfu/g). The interactive effect between the landscape position and parent materials showed that soils overlying limestone in the valley bottom significantly ($P \leq 0.05$) had the highest bacterial population (52.0 x 10^6cfu/g) compared to other parent material and landscape positions. Similarly, soils overlying limestone in the valley bottom significantly ($P \leq 0.05$) had the highest fungal count (53.0 x 10^3cfu/g) compared to other parent materials and landscape positions. It can be deduced from this research that parent materials, landscape position, and soil depth directly affect the distribution of bacteria and fungi population in soils.

Keywords: Limestone, Basement complex; Landscape position; Bacterial and Fungal count.
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1.0 Introduction

Soils are developed from different parent materials and are different from the original materials in which they originate. They differ in physical, chemical, microbial, morphological, and mineralogical properties. The inorganic parent materials can be formed as residual weathered rock materials (in-situ) or they can be transported from one location to another (Esu, 2010). The soils in Akampka Local Government Area are derived from basement complex rocks consisting of Granite gneiss. Basement complex rocks are known to occur at Mfamosing, Aningeje, Oban, and Obudu areas of Cross River State, Nigeria (Aki *et al*.,2014). The high rainfall of the study area (>3,500 mm) and high soil temperature (>27-31^{o}C) can enhance hydrolytic weathering by predisposing the gneiss to ferralitic pedogenesis (Akpan-idiok, 2012). Soils developed from the Basement Complex are generally acidic in nature, with low CEC, base saturation, and fertility level, usually suffering from multiple nutrients deficiencies. Soils derived from Basement complex rocks occupy most of Akampka, Biase, Obudu, and Odukpani Local Government Area of Cross River State (Esu, 2010). Limestone is a type of sedimentary rock that consists of more than 50% of carbonate minerals such as Calcium carbonate ($CaCO_3$) which are always in the form of aragonite, calcite, and dolomite plus a small amount of iron-bearing carbonates (Aki et al.,2012). Limestone deposits are also found in these areas which are sedimentary rocks

composed largely of the mineral of Calcite ($CaCo_3$) formed by either organic or inorganic process. Consolidated products of calcareous sand, limy mud, and crushed shells also constitute limestone (Srivastava *et al.,* 2015). The basement complex soils have a pH of 4.1-5.5 with high organic carbon. Low pH induces the accumulations of high levels of dissolved Fe, Cu, Mn, Al, and H in the soil with most major nutrients (N, P, K, S, and C) at limiting levels (Akpan-Idiok, 2009). Soils formed over limestone tend to have an alkaline reaction, high concentration of cations (Ca, Mg, Na, and K), low nitrogen, moderate phosphorus, abundant clay, and significant surface accumulations of black organic matter (Akpan-Idiok *et al.,* 2004). It was reported by Akinbola (2001) that soils developed on limestone had pH of 5.9-6.8 for surface soils and 5.8-6.3 for subsurface soils at the upper slope, middle slope, and lower slope. Landscape position; upper slope, middle slope, and lower slope significantly influence the chemical properties in the soil which led to variation in pH values within the toposequence. Soils developed from limestone had pH from 6.9 to 7.9 for surface and subsurface respectively (Aki, 2012). The soils developed on the basement complex are strongly acidic in reaction with mean values of 4.9 and 4.8 in surface and subsurface soils respectively (Aki, *et al.,* 2014).

Soil microorganisms within these areas affect organic matter decomposition and transformation of nutrients, therefore the soils developed from Basement complex rocks and Limestone in Akamkpa support a lot of crops such as tree crop plantations (oil palm, rubber, Gmelina tree) and food crops production such as cassava, yam, cocoyam, plantain, and vegetables. Industrial activities that are ongoing in the study area include quarrying of limestone ($CaCO_3$) for cement production and mining of granite which could be used for road construction. The values of microbial populations were significantly lower in quarry mines than in fallow lands. The lower microbial biomass is attributed to low organic carbon in soils, Uquetan *et al.,* (2017). The following bacterial isolates were identified in limestone soils; *Azospirillum spp., Bacillus spp., Thiobacillus spp., Flavobacterium brevi, Nitrosomonas spp., Escherichia coli,* and *Pseudomonas aeruginosa.* The number of fungi in the soil is fewer than those of bacteria. All fungi have mycelium threads which are organized from individual hypha. (Haili *et al.,* 2008). Two strains of fungi were identified to dominate limestone soils, namely; *Aspergillus niger* and *Aspergillus flavus* (Panaiyadiyan and Chellaia,2011). A total of eight (8) different genera of bacteria were isolated and identified from basement complex soil samples from Alimosho Local Government Area, Lagos State. They were; *Staphylococcus aureus., Pseudomonas aeruginosa., Escherichia coli., Bacillus spp., Klebsiella pneumonia., Streptococcus spp., Proteus spp.,* and *Azotobacter spp.* and five (5) fungal species were isolated; *Aspergillus spp., Penicillium spp., Rhizopus spp., Mucor spp. and Fusarium spp.* (Funmilayo *et al.,*2015). It was observed that a basement complex soil had a higher number of bacteria isolates than fungi. The occurrence of more bacteria could be attributed to the tolerance of these microbes to wide variations of the soil properties, climatic condition, and availability of nutrients (Funmilayo *et al.,* 2015). It was observed that a basement complex soil had a

Table 1: Selected physical and chemical properties of the soil used for planting

Property	**Value**
pH(H_2O)	7.0
EC (dS/m)	0.19
OC (g/kg)	6.40
Total N (g/kg)	1.10
Available P (mg/kg)	10.20
Exchangeable cations (cmol/kg)	
K	0.64
Na	0.36
Ca	4.41
Mg	1.73
Exchangeable acidity (cmol/kg)	0.50
CEC (cmol/kg)	20.20
Particle size distribution (%)	
Sand	68.00
Silt	20.00
Clay	12.00
Textural class	Sandy loam

Table 2: NPK content of the bioslurry

Nutrient (%)	**Value**
N	1.90
P	0.32
K	6.50

higher number of bacteria isolates than fungi. The occurrence of more bacteria could be attributed to the tolerance of these microbes to wide variations of the soil properties, climatic condition, and availability of nutrients. The high fungal count could be attributed to the acidic nature of these soils since fungal growth is enhanced by the acid nature of an environment (Akpan et al., 2011). Furthermore, it was observed that based on the landscape positioning, the lower slope had the highest bacteria count while the upper slope had the least bacteria count. This is because the soils in the lower slope were rich in humus which led to high decomposition of organic matter (Uquetan *et al.,* 2017). This report is similar to the result of Udotong, *et al.*, (2008) and Fumilayo *et al., (*2015) who stated that soils in the basement complex were rich in nutrients due to the high rate of mineralization of organic matter. In limestone soil, the bacteria isolates, *Bacillus spp,* and *Cyanobacterium spp* occurred more frequently as heterotrophic bacteria and hydrocarbon utilizing bacteria respectively, while the fungi isolates; *Aspergillus spp.* and *Rhizopus spp.* were predominant as heterotrophic fungi and hydrocarbon utilizing fungi. (Fumilayo, *et al.*, 2015).

The soils of the basement complex generally have sandy loam to clay on the surface and lateritic clay on the subsurface. The sand content varied from 80.17 - 85.20 %, silt 11.60 - 24.8 % and clay 4.2 - 15.4 % (Akpan-Idiok, 2012). This result is in line with the report of Ibanga et *al.*, (2005) who reported that limestone soil had sand content from 72.60-82.5 %, silt 8.49-13.4 %, and clay 6.0-19.0 %. The textures of the soil were between sandy loam, sandy clay, and sandy clay loam.

It is, however, very necessary to evaluate the microbial properties of limestone and Basement complex soils and to assess the effect of pH distribution in the Akampka Local Government Area of Cross River State.

2.0. Materials and methods

2.1 Location of the study area

The research was carried out in Mfamosing and Aningeje in Akamkpa Local Government Area of Cross River State. The area lies between latitude 05°7" North and latitude 008°31" East which was ascertained with a Global Positioning System (GPS).

The study area is characterized by high rainfall which starts from the month of April to October, reaching its climax in the month of June and September. Temperature ranges from 21°C - 23°C in the wet season and 24 - 27°C in the dry season. The area records relative humidity between 80-100 %. (CRBDA report, 2006).

2.2 Vegetation and Land Use of the Study Area

The study area falls within the tropical rainforest zone. The vegetation of the study area is a primary and mostly

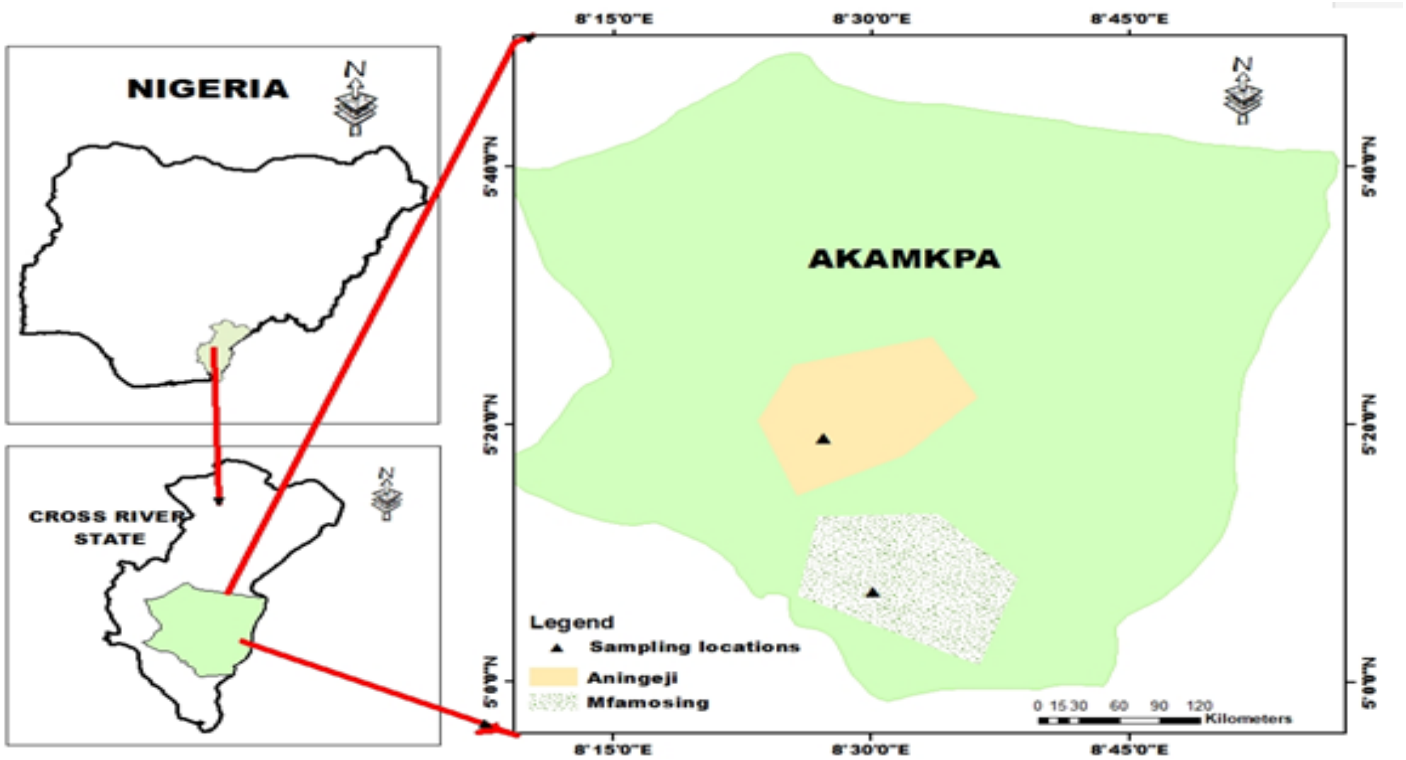

Fig. 1: Map of Akamkpa Local Government Areas, Cross River State Representing the Study Sites

secondary forest with plants such as yam, gmelina, maize, oil palm, and cassava. Human activities include quarrying and blasting of rocks.

2.3 Geology of the Study Area

The soils of the study area are derived from basement complex and limestone rocks. The area is strongly undulating and the soil overlying limestone consists mainly of carbonate which is usually in form of aragonite and dolomite (Ekweme, 2003).

2.4 Field study/description

Samples for the study were randomly collected based on the toposequence; Upper slope, middle slope, and lower slope from three different locations using soil auger at the depth of 0-15 cm and 15-30 cm. Samples were collected at 3 points in each of the locations following the toposequence of the sample areas; the upper slope, middle slope, and lower slope. Composite samples were collected from three points each at the different toposequence for microbial properties determination. The samples were put into well-labeled polythene bags for microbial determination and transported in a cool chest to Microbiological Laboratory, in the Department of the Soil Science University of Calabar for analysis.

2.2 Enumeration and identification of total viable bacteria

(TVB) and fungi (TVF) From Soil

Soil extract was used as diluents where 1000 g of the fresh garden soil was mixed with 1000 ml of distilled water in a conical flask. Then sterilized by autoclaving at 120^0 C and 15 psi for 15 minutes, filtered, and allowed to cool. Then 15 g of malt agar was added to the filtrate and sterilized agar using autoclave for serial dilution (Zuberer, 2004).

One gram (1 g) of each of the soil sample was suspended in 9 ml of sterile soil extract in a test tube. One ml aliquot from a dilution factor of 10^6 was used and the surface plating technique was used for the enumeration of bacteria. The enumeration of fungi isolates was also done using serial dilution for the soil sample prepared from 10^3 and 1 ml aliquot of the dilution factor. They were all incubated at 28^0C for 72 hours before colonies that developed were counted and expressed as colony-forming unit (cfu) per gram of soil sample (cfu/g).

2.3 Characterization and Identification of Bacterial and Fungal Isolates

The bacterial isolates were examined macroscopically by examining the incubated plates on nutrient agar for characteristics such as colour formation, nature of colonies (dry or moist), shape, nature of growth (pure or mixed), and degree of colonies (entire or lobate) characterized and identified by the use of standard methods of morphological and biochemical characterization was also carried out using the principles of Zuberer, (2004). Characterization and identification of fungal isolates were examined for colonial characteristics such as colour formation; aerial hyphae and odour (yeast cells) were also carried out. Microscopic examination of pure cultures of the bacterial isolates was carried out by observing cultures under a microscope with x100 objective lens using immersion oil after gram staining for characteristics such as gram reaction, the shape of arrangement of isolates for further identification (Akpan*et al.,* 2011).

2.4 Maintenance of Pure Culture

Discrete colonies were purified by repeated sub-culture unto appropriate agar media. Pure cultures were preserved on nutrient agar slants and stored in a refrigerator (4^oC) and at ambient temperature (28^oC) for further tests.

2.5 Determination of soil pH

Soil pH was determined using a glass electrode pH meter at 1:2.5 soil: solution ratio and 1 N KCl (IITA, 1979).

2.6 Statistical analysis

The data collected were analyzed using a two-way analysis of variance.

3.0 Results and Discussion

3.1 Effect of landscape position and type of parent material on soil microbial population

Landscape position (upper slope, middle slope, and valley bottom) significantly ($p<0.05$) influenced bacterial population with the valley bottom soil (44.50 x 10^6 cfu/g) as shown in Table 1 having the highest bacterial count which was significantly ($P<0.05$) different from those of upper slope (17.50 x 10^6 cfu/g) and middle slope soil with 26.0 x 10^6 cfu/g. This result is in line with the result of Ulrich and Becker (2006) and Uquetan *et al.,*(2017) who stated that the lower slope was rich in humus which was due to the high rate of organic matter decomposition by the microorganisms. However, parent materials (limestone and basement complex) did not significantly ($p>0.05$) influenced the bacterial population. Therefore, the study has indicated that different landscape positions can create remarkable differences in the bacterial populations.

Similarly, landscape position (upper slope, middle slope, and valley bottom) significantly ($p<0.05$) influence fungal population with the valley bottom soil (34.58 x 10^3cfu/g) having the highest fungal population which was significantly different from those of upper slope (20.75 x 10^3cfu/g) and middle slope (14.25 x 10^3cfu/g) soil. The result is in line with the report of Funmilayo *et al.,* (2015) and Uquetan *et al.,* (2017) who stated that the lower slopes had more fungal populations than upper and middle slopes. However, parent materials (limestone and basement complex) significantly ($p\leq0.05$) influences fungal population, where soil developed on limestone (28.2 x 10^3cfu/g) had significantly ($p>0.05$) higher fungal count than the soil developed on basement complex (18.2 x 10^3cfu/g). Therefore, the study has indicated that different landscape positions and parent materials can create remarkable differences in the fungal population.

The result further revealed that the bacterial count was slightly higher in soil developed on limestone than soil developed on the basement complex by 3.0 x 10^6. The maximum bacterial population was > in valley bottom soil > middle slope soil > upper slope soil. The bacterial count was higher in valley bottom than middle slope by 18.5 x 10^6. Similarly, the bacterial count was higher in valley bottom than upper slope by 27.0 x 10^6. The result also revealed that the fungal count was higher in soil developed on limestone than soil developed on the basement complex by 10 x 10^3. The maximum fungal population as observed was > in valley bottom soil > upper slope > then middle slope. Also, the fungal count was higher in valley bottom than middle slope by 20.33 x 10^3. Similarly, the fungal count was higher in valley bottom than upper slope by 13.85 x 10^3.

However, considering the landscape positions and the two-parent materials as a whole (i.e. interaction effect), the result showed that soils overlying limestone in the valley bottom significantly ($p<0.05$) had the highest bacterial population (52.0 x 10^6cfu/g) compare to other parent materials and landscape positions (Table 1). Basement complex in upper slope had the lowest bacterial population (13.0 x 10^6cfu/g). Similarly, soils overlying limestone in the valley bottom significantly ($p<0.05$) had the highest fungal count (53.0 x 10^3cfu/g) compare to other parent material and landscape positions. The fungal count under limestone parent material in the valley bottom soil was significantly different ($p<0.05$) from other landscape positions and parent materials and middle slope soil developed on limestone had the lowest fungal (7.5x 10^3cfu/g).

3.2 Effect of soil depth and landscape position on soil microbial population

Soil depth (0-15 and 15-30 cm) did not significantly ($p>0.05$) influence the bacterial and fungal population in this study (Table 2).

The result revealed that topsoil (0-15 cm) bacterial and fungal population count was higher than subsoil bacterial and fungal count in limestone soils. This is in line with the result of Akpan *et al*., (2011) and Okonkwo (2010) who reported that microbial population decreased with increasing soil depth. The result also corresponds with the work of Wuczkowski *et al.* (2003) who reported that the abundance of fungi is on the topsoil (0-15cm) may be attributed to the fact that fungi are strict aerobes. The result indicated that the microbial populations were not influenced by soil depths. However, the bacteria count was higher than the fungi count. The bacterial count was higher in topsoil by 6.7x 10^6 cfu/g than subsoil. Similarly, fungal count in topsoil was higher

Table 2:
Mean bacterial (x 10^6cfu/g) and fungal (x 10^3cfu/g) count between landscape position and soil depth in limestone soil

	Bacterial	Fungi
Landscape position (LP)		
Upper slope	17.5b	20.8b
Middle slope	26.0b	14.2b
Valley bottom	44.5a	34.6a
LSD ($p<0.05$)	12.71	10.75
Soil depth (SD)		
0-15 cm	32.7	24.2
15-20 cm	26.0	22.2
LSD ($p<0.05$)	NS	NS
LPxSD		
Upper slope x 0-15 cm	20.5	20.8
Upper slope x 15-20 cm	14.5	20.7
Middle slope x 0-15 cm	31.2	16.3
Middle slope x 15-20 cm	20.8	12.2
Valley bottom x 0-15 cm	46.3	35.3
Valley bottom x 15-20 cm	42.7	33.7
LSD ($p<0.05$)	NS	NS

soil by 2.0x 106 than subsoil (Table 2).

Landscape position (upper slope, middle slope, and valley bottom) significantly ($p<0.05$) influenced bacterial population with the valley bottom soil (44.50 x 10^6cfu/g) having the highest bacterial count which was significantly different from those of upper slope (17.50 x 10^6cfu/g) and middle slope soil (26.0 x 10^6cfu/g). Similarly, landscape position (upper slope, middle slope, and valley bottom) significantly ($p<0.05$) influenced fungal population with the valley bottom soil (34.58 x 10^3cfu/g) having the highest fungal population which was significantly different from those of upper slope (20.75 x 10^3cfu/g) and middle slope (14.25 x 10^3cfu/g) soil.

However, the interactive effect between landscape positions and soil depth showed that there was significant ($p>0.05$) interaction between landscape position(upper slope, middle slope, and valley bottom) and soil depth (0-15 and 15-30 cm).

3.3 The number of soil bacteria and soil fungi isolates

A total of eight (8) distinct strains of bacteria were isolated. They were; *Acinetobacter spp., Microscoccusleutus., Calcoaceticus spp., Rhizobium spp., Pseudomonas spp., Azotobacter spp., Streptomyces spp.,* and *Enterobacter spp*. *Rhizobium spp.* Were 26.67 percent of occurrence being the most dominant while the least prevalent bacterial were *Calcoacetius spp* .with percentage occurrence of 2.22 percent (Table 3). *Micrococcus spp* and *Pseudomonas spp* were also among the bacterial isolates isolated by Panaiyan *et al.,* (2011) with *Bacillus spp* genera being the most dominant species whereas *Chromobacterium spp* and *Nocardia spp* were the least species. This shows similarity with the result obtained in this study. Also, throughout the different sampling locations, a total of ten (10) distinct types of fungi species were isolated with *Cladosporium spp* been the most dominant fungi, having 21.43 percent of occurrence while *Alternaria spp, Scopulariopsis ssp, a*nd *Pullulavia spp* were the least dominant fungi with percentage occurrence of 2.5 percent (Table 4). The result is similar to the result obtained by Fumilayo *et al.,* (2015).

3.4 Effect of parent materials and landscape position on pH

Parent materials (Basement complex and Limestone) significantly ($p\leq 0.05$) influenced the pH of the soils. The pH values for soils developed from the Basement complex were acidic in nature with values ranging from 4.6-4.9. The soils developed from limestone had values from 6.9 and 8.1 (Table 5 and appendix 11). This result corresponds with the results of Aki *et al.,*(2014) who stated that soils developed from the Basement complex and Limestone were alkaline and acidic in nature respectively. There was a significant ($p\leq 0.5$) difference among the parent materials in pH values. There was no significant ($p\leq 0.05$) difference in pH within the landscape position in both the Basement complex and Limestone (Table 6).

Microbial populations were more in soils developed from Limestone than in Basement complex and also within the lower slope of soils developed on limestone.

4.0 Summary

This research investigated the microbial population and pH variations of soils developed on basement complex and Limestone in Aningeje and Mfamosing in Akamkpa Local

Table 3:
Bacteria isolates, frequency and percentage of occurrence

Bacteria isolates	Frequency of occurrence	Percentage of occurrence (%)
Acinetobacter spp	5	11.11
Micrococcus luteus	4	8.89
Calcoaceticus spp	1	2.22
Rhizobium spp	12	26.67
Leucothricmucor spp	9	20.0
Azotobacter spp	4	8.89
Streptomyces spp	7	15.56
Arthrobacter spp	3	6.67
TOTAL	45	100

Table 4:
Fungi isolates, frequency and percentage occurrences

Fungi isolates	Frequency of Occurrence	Percentage of Occurrence (%)
Alternaria spp	1	2.50
Mucor spp	8	20.00
Scopulariopsis spp	1	2.50
Oospora spp	3	7.50
Cladosporium spp	14	35.00
Trichothecium spp	5	12.50
Helminthosporium spp	2	5.00
Rhizopus spp	2	5.00
Pullulavia spp	1	2.50
Aspergillus spp	3	7.50
TOTAL	40	100

Table 5:
Distribution and variation of pH on parent materials.

Parent materials	pH
Basement complex	4.9^{b}
Limestone	8.1^{a}
LSD(≤0.05)	0.533^{**}

**= significant at 5%

Table 6:
Distribution and variation of pH on landscape position for basement complex and Limestone soils.

Landscape position	Basement complex	Limestone
Upper slope	4.5^{a}	7.5^{a}
Middle slope	4.8^{a}	7.9^{a}
Valley bottom	4.9^{a}	8.1^{a}
LSD(≤0.05)	NS	NS

NS= not significant

Government Area, Cross River State. Soil samples were collected at two depths 0-15 and 15-30 cm for laboratory determination. The bacteria and fungi count were determined using standard procedure.

The results indicated that landscape position (upper slope, middle slope, and valley bottom) significantly ($p<0.05$) influenced bacterial population and fungal count with the valley bottom soil (44.50×10^6cfu/g) having the highest bacterial count which was significantly different from those of upper slope (17.50×10^6cfu/g) and middle slope soil (26.0×10^6 cfu/g). Similarly, landscape position (upper slope, middle slope, and valley bottom) significantly ($p<0.05$) influences the fungal population with the valley bottom soil (34.58×10^3cfu/g) having the highest fungal population which was significantly different from those of upper slope (20.75×10^3 cfu/g) and middle slope (14.25×10^3 cfu/g) soil. Conversely, soil depth (0-15 and 15-20 cm) did not significantly ($p>0.05$) influence the bacterial and fungal populations in the study. However, the result revealed that the topsoil bacterial and fungal population count was higher than the subsoil bacterial and fungal count. Different parent materials affect soil pH.

However, the interactive effect of landscape positions and the two-parent materials result showed that there was a significant ($p<0.05$) between landscape position (upper slope, middle slope, and valley bottom) on bacterial and fungal population and there was no significant difference ($p > 0.05$) in bacterial population within the parent materials in soils developed on basement complex rather there was a significant difference ($p < 0.05$)in fungal population within the same parent materials. Also, the landscape positions and soil depth (i.e. interaction effect), the result showed that there was significant ($p <0.05$) interaction between landscape position (upper slope, middle slope, and valley bottom) and soil depth (0-15 and 15-30 cm).

5.0 Conclusion

This study reveals that parent materials and soil depth did not have any significant difference in microbial population rather landscape position showed a significant difference in the microbial population. Increasing soil depth decreases the microbial population due to a certain limit of Physicochemical properties of soil. Valley bottom soil and soil developed on limestone were favorable for increasing population of bacteria and fungi than soil developed on basement complex and will support agricultural activities because it shows that the soil contains a high level of organic matter decomposition.

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