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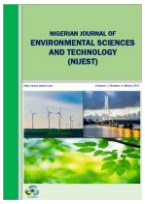

Impact of Seasonal Variations on the Colonial Populations of Bacteria and Fungi in Soil and on Buried Plant Stems

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ABSTRACT

Forest soils and stems buried in them usually have varying degrees of colonization and abundance of bacteria and fungi. This study was undertaken to determine the effects of seasonal variations on the population of bacteria and fungi isolated from forest soil and on plant stems buried in the soil. Soil sampling and stem burial studies were conducted over a 12-month period in 2019. Serially diluted soil samples were plated on suitable media for bacterial and fungal growth and thereafter counted after incubation. Buried stems were removed from the soil, rinsed and placed in flasks containing suitable media for fungal and bacterial cultivation. Colonial growth was counted after incubation. Soil moisture was highest during the wet season months of July (27.7%), August (23.5 %), September 26.1 %) and October (29 %) whereas the average soil moisture content was lowest in the dry season. Seasonal pH did not significantly affect microbial population levels in the various months. Colony counts for Pseudomonas spp. during the dry season months (January, February, March and April) were very low. Growth of the bacterium showed peaks in the May through October during which counts reached 10^9 cells per gram of soil except in August with counts of 10^8 cells per gram of soil. Micrococcus spp. and Bacillus spp. also showed similar trends in colony counts with little variations. Fungi were generally fewer in number than bacteria and only one peak which reached 10^7 cells/g soil was obtained for Fusarium spp. and Rhizopus spp. in September and October respectively. The density of Trichoderma spp. per gram of soil peaked at 10^6 cells in June, July, September and October. Counts for Aspergillus spp. was negligible in January, February, March and April but reached 10^6 cells per gram of soil in June, July and August. The colonization of Pseudomonas spp. on buried plant stem varied between 62% in June to 76% in October while Micrococcus spp. had levels which varied from 65% in May to 84% in June and 72% in October. Fusarium species were found most frequently on the stem every month except in February, March and April. Low colonization of Aspergillus spp. on stems occurred in January, February, March, November and December. Highest numbers of this organism was found in August, September and October. Rhizopus spp. was observed in 85 and 80% of the stem in September and October respectively but lower percentages of colonization occurred in January, February, March and April. In all the dry season months (January-April), all bacterial and fungal populations had low densities but their counts increased in the rainy season. Fungi were generally fewer in number than bacteria in both soil and stem burial experiments.

Keywords: Microbial population, Soil sampling, Stem burial, Forest soil, Colonization rate

1.0. Introduction

Forest represents one of the largest and most important ecosystems on earth covering more than 40 million km^2 representing 30% of the global land and forest ecosystems are found in most of earth's biomes and harbour a large population of global diversity (Liado *et al*., 2007). Soil microorganisms constitute nearly 1% of the soil mass and they have a major impact on soil properties and processes (Cenciani *et al*., 2009). Nutrient cycles and functioning of the ecosystem are influenced by seasonal changes of soil microbial biomass (Lipson and Schmidt, 2004). Many biological activities which influence the growth of plants, animals and microbial populations occur in the soil. Microorganisms found in forest soil have been shown to perform various beneficial roles such as decomposition of soil

organic matter, nitrogen cycling and increase of soil fertility (Symochko *et al*., 2015). The activities and numbers of soil microorganisms determine soil fertility and its environmental status in the ecosystems as reflected on the level of soil biological activities (Cenciani *et al*., 2009). Microbial diversity in the forest soil occupying a particular location within the soil or on forest ground have been discovered to be affected by various environmental and climatic conditions. The soil microbial inhabitants could be referred to as the dominant organisms in contrast to transient organisms which do not occur most frequently throughout the months. The determination of the impact of environmental change on soil microbial function requires an understanding of how environmental factors shape microbial community and composition in the soil.

Determinations of numbers of microorganisms in soil as well as their isolations, morphological and physiological characteristics are important because soil is a huge reservoir of many biologically active agents. An active microorganism is capable of colonizing and growing on substrates and should be present in large numbers as to alter its environment. The existence of microorganisms in a particular environment including humid or warm environment usually develops through the ability of that organism to grow and multiply on the nutrients available in that locality or which have been transmitted to that locality.

Forest soil clearly represents the most important habitat for soil microorganisms especially fungi and bacteria with their activities being supported by the decomposition of organic matter, dead plant and animal remains. Microorganisms are responsible for the decomposition of soil organic matter thereby releasing nutrients that are absorbed by plants. These microorganisms are of importance in maintaining the fertility of the soil and factors which alter the rate of microbial processes in the soil are of importance for the functioning of the forest ecosystem. Although fungi are known to be the most dominant in forest soil but some bacteria are also abundant in most forest soils and results from recent studies have demonstrated an active role of bacteria in litter transformation (Liado *et al*., 2007).

Temperature of the tropical forest soil is always warm and moisture is abundant in the wet season and the relative humidity is high. Plants occasionally shed their leaves and provide organic matter on which microorganisms grow on and derive their energy. The spatial heterogeneity of forest top soils determines the composition of microbial communities mainly through two sets of drivers which include soil and litter chemistry which affect both bacteria and fungal population and diversities, although to variable degrees (Baldrian, 2017). Leaves of living plants provide shade for protection of microorganisms from direct sunlight. This makes biological activity intense and results in the abundance of different microbial forms in the forest soil. Dead plant biomass including fallen leaves and deadwood are the sources of most carbon compounds for forest microorganisms. Tons of fallen leaf matter which accumulate yearly on forest floor surface and their transformations is of great importance for the cycling of carbon and other nutrients. Litter habitat is composed of a diverse group of fungi and bacteria which play important roles in decomposition and transformation of forest soil organic matter.

Woody biomass of trees is a large resource in forest ecosystem which is reach in nutrients. Many and diverse species of microorganisms are well adapted to living on woods (Blanchette and Shaw, 1978). Fungi and bacteria are important in the soil ecosystem because they function in the decomposition mineralization and help in the movement of soil mineral elements to plant roots (Widawati and Suliash 2001). Species of fungi are important components of biodiversity in tropical forest soils and fungi perform some activities on which larger organisms including humans depend. Reported values of soil fungal diversity and population are a reflection of the sampling methods which differ from one organism to the other (Brock, 1987). Saprotrophic basidiophytes and white-rot wood-decomposing fungi often act as major litter decomposers together with ascomycetous fungi (Eichlerova *et al*., 2015). Deadwoods are also a source of carbon compounds in the forest. Decomposition of dead woods is influenced by diversities and types of fungi and bacterial species and also on the environmental conditions prevalent in that habitat (Gessner, 2010). Microorganisms produce a wide range of extracellular enzymes, which allows them to effectively degrade recalcitrant fractions of dead plant biomass (Eichlerova *et al*., 2015). Bacterial strains are among the dominant group of organisms in the forest soil. The ability of bacteria to survive ecological conditions includes the formation of endospores which have thick strong walls which make it easy for them to survive in extremes of environmental conditions (Kundu *et al*., 2009). Forests provide a wide range of habitats

for bacteria and they are abundant on forest soils and litter. Forest ecosystem provides a broad range of habitats for bacteria, including soil and plant roots but bacteria seems to be especially abundant on the forest floor, in soil and litter. Soil bacteria are the primary drivers of these ecological habitats (Bardgett and Leemas 1995). Currently, bacteria community composition is an important determinant of ecosystem process rates, and identifying bacteria community composition has become an essential component for predicting ecosystem responses to environmental changes (Baldrian *et al*., 2012). However, before we can predict the ecosystem response to environmental change, we must first understand how the environment shapes bacteria community composition. For example, soil moisture can influence bacterial composition along topographic gradients as well as in multiple forest ecosystems (Brockett *et al*., 2012).

Forests represent a highly productive ecosystem that act as carbon sinks where soil organic matter is formed from residues after biomass decomposition as well as from rhizodeposited carbon (Baldrian, 2017). And factors such as pH, organic matter content, nutrient availability, climate conditions and biotic interactions affect the composition of bacterial communities in the soil is one of the important components and microbial activities and diversities in the soil help drive ecological and physicochemical reactions that occur in soil microenvironment. The determination of the impact of environmental change on soil microbial function requires an understanding of how environmental factors shape microbial composition (Allison *et al*., 2010). Microbial community composition is an important determinant of ecosystem process rates (Reed and Martiny 2007), and identifying microbial community composition has become an essential component for predicting ecosystem responses to environmental change (Baldrian, *et al*., 2012). Climatic change alters the relative abundance and function of soil communities because soil community members differ in their physiology, temperature sensitivity, and growth rates (Castro *et al*., 2010; Gray *et al*., 2011). The direct effects of climatic change on microbial composition and activities have been well studied (Castro *et al*., 2010).

Soil temperature is an important physical property that regulates most of the physical, chemical, and biological processes of the soil, and the physiological processes of soil organisms and forest plants (Zogg *et al*., 1997). Soil temperature has tremendous ecological impacts through evaporation, transpiration, organic matter decomposition, CO_2 emission due to soil respiration. In forest ecosystems, soil temperature regulates microbial transformations of nitrogen sulphur and other nutrients and controls decomposition of organic matter and formation of humus. Temperature is one of the most important factors influencing soil organic matter decomposition and microbial communities. Temperature, together with moisture content is among the most important environmental factors affecting microbial growth and activity in soils. The role of elevated temperature on microbial metabolism has received considerable recent attention (Bradford *et al*., 2008; Karhu, 2014).

Soil moisture is one the most important environmental factors influencing soil organic matter decomposition and production of greenhouse gases in terrestrial environments (Kirschboum, 2006). Soil water content is important in regulating oxygen diffusion, with maximum aerobic microbial activity occurring at moisture levels between 50% and 70% of water-holding capacity (WHC) (Linn and Doran, 1984). Seasonal changes in soil water content influence function–structure relationships of microbial communities and enzyme activities (Brockett *et al*., 2012). Soil moisture content, by altering conditions for soil microbiota, causes changes in the structural diversity and activity of microorganisms (Kim *et al*., 2008). Excess of water in the soil environment due to flooding or periodically heavy rainfalls is particularly threatening to aerobic bacteria (Walker *et al*., 2003). For soil microbiologist, it is important to determine the optimum moisture content of soil because it is the soil microbiota that is responsible for the rate of organic matter transformations in the soil microenvironment.

Soil pH is considered as one of the important factors that controls microbial community structure (Fierer and Jackson 2006; Lauber *et al*., 2009). pH influences abiotic factors, such as carbon availability, nutrient availability, and the solubility of metals etc. (Cho *et al*., 2016). In addition, soil pH also controls biotic factors, such as the biomass composition of microorganisms in both forest and agricultural soils (Rousk *et al*., 2010). This work is aimed to study the effects of seasonal variations on bacterial and fungal populations in forest soils and how these variations affect the colonization of buried plant stems over a 12-month examination period.

2.0. Materials and Methods

2.1. Study area and sampling

The topography of the study area is characterized by mean maximum and minimum temperatures which lie between 30-3°C and 20-25°C respectively. Rainy season in the area lasts for about 8 months from April to early November, with peaks in July, September and October. The dry season commences from mid-November to March. Soil samples were collected from 1-5cm of top soil and after passing the sample through sieve clothe, soil moisture was determined by placing soil in aluminium foil and the weights of the samples were measured. The soil was oven dried at 105°C for 24h to achieve a constant weight. Soil moisture was determined gravimetrically by weight deference and the values were converted to percentages.

Soil pHs were determined twice a month by placing 20g of air dried soil in glass beaker. Distilled water was added into the beaker and shaken. Soil pH was then determined with hand held pH meter (Hannah instrument).

2.2. Isolation and culturing

The experimental plot located in a forest in Uzo-Uwani, Nsukka measured 10 x 5m and was mapped with wire gauze to avoid disturbance. Soil samples were collected twice per month from January to December 2019 from the surface (0 – 5cm). The samples were gently collected into sterile conical flasks containing either Nutrient broth for bacterial cultivation or Potato Dextrose broth and shaken at 50 x g in Gallenkamp shaker for 5h. The samples were serially diluted using normal saline solution and plated onto Nutrient agar for bacterial enumeration and Potato Dextrose agar (PDA) plates for the enumeration of fungi. Control uninoculated plates were separately prepared. Nutrient agar plates were incubated at 35°C in an incubator for 24h. The PDA plates were placed on the laboratory bench for 48 h at an approximate temperature of 30±2°C. Colony counts were obtained from the plates after the incubation periods. Pure bacterial cultures were obtained by streaking on fresh agar plates. Only plates containing 30 and less than 300 colonies were considered valid. Otherwise, they were not recorded (NR).

2.3. Stem burial studies

Sterile banana stems each measuring 10 x 3cm were placed inside the soil at a depth of about 12cm. Eight such stems were buried on the first day of every month. Soil was added to completely burn the stems. Two buried stems were each removed for either bacterial or fungal examination after 14th and 28th day of burial. Each stem was aseptically rinsed in sterile distilled water and placed in conical flasks containing Nutrient broth for bacterial cultivation or Potato Dextrose broth for fungal cultivation; then shaken at 50 x g in Gallenkamp shaker for 5h. The samples were serially diluted using normal saline solution and plated onto Nutrient agar for bacterial enumeration and Potato Dextrose agar (PDA) plates for the enumeration of fungi. Nutrient agar plates were incubated at 35°C in an incubator for 24h. The PDA plates were placed on the laboratory bench for 48h at an approximate temperature of 30±2°C. Pure bacterial cultures were obtained by streaking on fresh agar plates. Only plates containing 30 and less than 300 colonies were considered valid. Otherwise, they were not recorded (NR).

2.4. Identification of the isolates

Bacteria were identified based on their morphological, physiological and biochemical characteristics as described in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Fungal isolates were identified based on their morphological and cultural characteristics as outlined by Pitt and Hocking (1997).

3.0. Results and Discussions

Bacteria and fungi showed differences in their rates of occurrence in both the soil experiment and the stem burial studies. Four bacterial genera namely, *Bacillus* spp., *Pseudomonas* spp. and *Micrococcus* spp. were recovered from the soil in high numbers and they also showed best stem colonization.

Fungal strains namely *Aspergillus* spp., *Fusarium* spp, *Rhizopus* spp., and *Trichoderma* spp. were dominant in the soil samples and were isolated in high numbers but only three of these fungi namely *Aspergillus* spp., *Fusarium* spp, and *Rhizopus* spp. displayed best colonization of the buried plant stems.

Results in Figure 1 show the mean soil moisture contents of the study area. It is evident that soil moisture was highest during the wet season months of July, (27.7%); August, (23.5 %); September, 26.1 % and October (29 %) whereas the average soil moisture contents were lowest in January, (6.4%); February, (5.6 %); March, (3.1%); April, (17.6 %). Data in Figure 2 shows the average soil pH values for the various months and these changes did not give any significantly positive growth responses to microbial population levels.

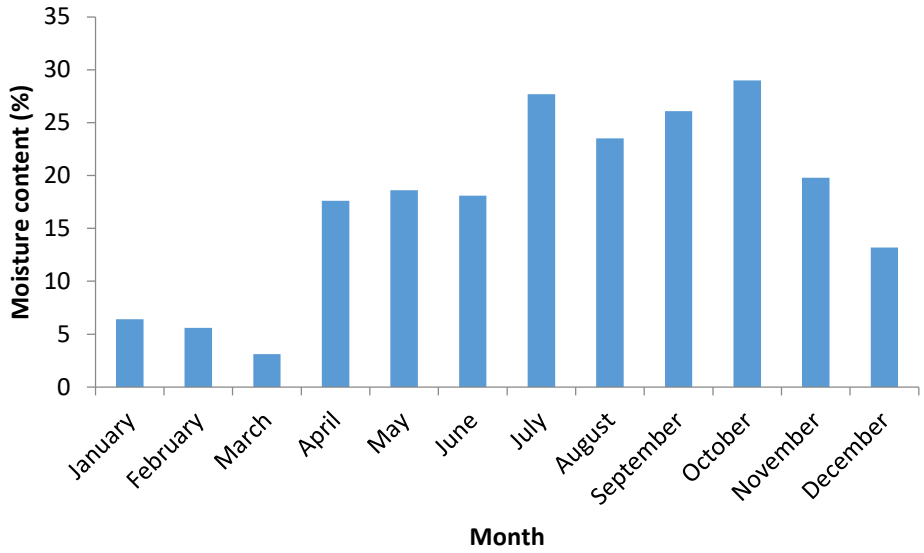

Figure 1: Moisture contents of soil at the study location

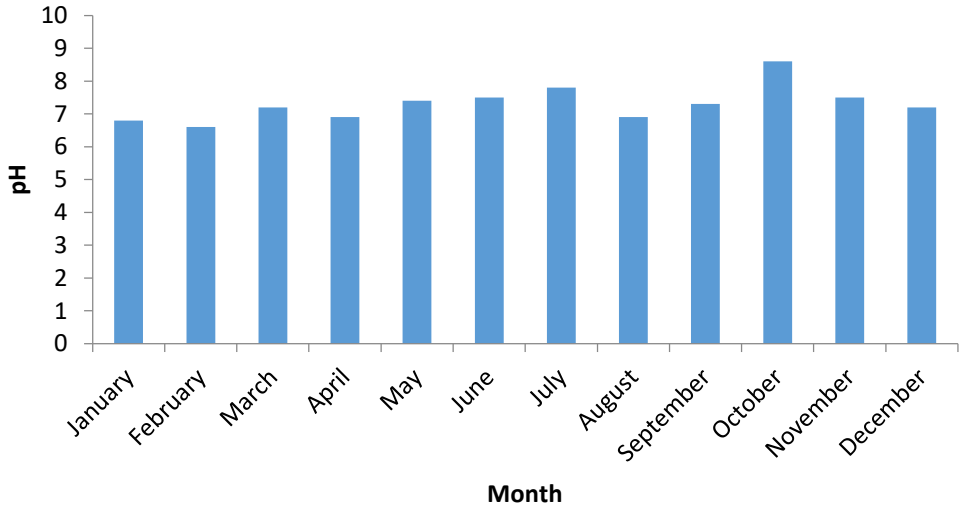

Figure 2: Soil pH at the study location

For *Pseudomonas* spp., colony counts during the dry season months (January, February, March and April) were very low (Table 1). Colonial growth showed peaks in May, June, July, September and October during which counts reached 10^9 cells per gram of soil. This level fell in November and December. *Bacillus* spp. and *Micrococcus* spp. counts also showed similar trends with little variations (Table 1). In all the dry season months (January-April), all bacterial populations did not reach densities of 10^6 cells/gram of soil (Table 1).

Table 1: Relative frequency of bacteria per gram forest soil measured over a 12-month period

Month	Number of organisms		
	Pseudomonas spp.	*Bacillus* spp.	*Micrococcus* spp.
January	NR	NR	NR
February	NR	$2.6x10^2$	NR
March	$6.3x10^2$	$3.1x10^2$	NR
April	$6.5x10^3$	$3.4x10^3$	$3.2x10^3$
May	$8.2x10^9$	$6.2x10^8$	$4.9x10^4$
June	$7.4x10^9$	$6.5x10^9$	$6.9x10^8$
July	$7.9x10^9$	$7.3x10^9$	$8.8x10^8$
August	$8.1x10^8$	$8.1x10^9$	$7.4x10^9$
September	$7.8x10^9$	$7.4x10^9$	$7.9x10^9$
October	$6.5x10^9$	$8.6x10^8$	$4.9x10^9$
November	$5.1x10^8$	$5.4x10^7$	$8.2x10^7$
December	$4.8x10^3$	$5.2x10^5$	$2.1x10^2$

NR: Not recorded

During the months of January, February, March, April and December 2019, there was very little rainfall and the numbers of fungi were very low (Table 2). Fungi were fewer in number than bacteria and only one peak which reached 10^7 cells/g soil was obtained for *Fusarium* spp. in September and for *Rhizopus* spp. in October. The density of *Trichoderma* spp. per gram of soil started to increase from April and peaked at 10^6 cells in June, July, September and October. Colony counts for *Aspergillus* spp. was negligible in January, February, March and April but reached 10^6 cells per gram of soil in June, July and August. Colony counts of *Aspergillus* spp. reduced in September and October with further reductions in November and December (Table 2).

Table 2: Relative frequency of fungi per gram forest soil measured over a 12-month period

Month		Number of organisms		
	Aspergillus spp.	*Fusarium* spp.	*Rhizopus* spp.	*Trichoderma* spp.
January	$6.2x10^2$	$2.8x10^3$	NR	NR
February	$2.8x10^2$	NR	NR	NR
March	NR	NR	NR	NR
April	$1.0x10^4$	$4.0x10^3$	$3.9x10^3$	$5.1x10^5$
May	$2.1x10^5$	$6.4x10^5$	$3.6x10^5$	$1.6x10^5$
June	$6.5x10^6$	$5.5x10^5$	$4.4x10^7$	$3.9x10^6$
July	$5.3x10^6$	$4.9x10^6$	$5.9x10^7$	$4.1x10^6$
August	$4.9x10^6$	$4.2x10^6$	$5.8x10^6$	$4.0x10^5$
September	$6.9x10^5$	$4.1x10^7$	$4.2x10^6$	$6.6x10^6$
October	$9.2x10^5$	$8.5x10^6$	$2.6x10^7$	$7.5x10^6$
November	$9.5x10^3$	$7.4x10^5$	$7.5x10^5$	$4.9x10^5$
December	$6.6x10^2$	$3.8x10^4$	$4.3x10^5$	NC

NR: Not recorded

A number of previous studies have explored the influence of multiple environmental factors on the distribution of soil moisture (Nyberg, 1996; Crave and Gascuel-Odoux, 1997). Marked seasonal changes in vegetative cover were also thought partially to explain differences in soil moisture variability observed on different sampling dates (Reynolds, 1970). Reynolds (1970) examined the relationship between soil moisture variability, amount of rainfall and insolation received in the week preceding the sampling, and the moisture content and vegetation cover at the time of sampling. Although no attempt was made to infer the relative influence of each of these factors, trends were identified that were consistent with the notion that soil moisture variability increases with increasing mean moisture content. Specifically, it was noted that low variance was associated with dry periods i.e. low mean moisture content; and that high variance was associated with wet periods.

Bacteria grew in small colonies from the stems with percent colonization higher than the fungi (Table 3). The highest colonization for all three bacteria occurred between the months of May through October. The occurrence of *Pseudomonas* varied between 62% in June to 76% in October while *Micrococcus* spp. had levels which varied from 65% in May to 84% in June and 72% in October. *Bacillus* spp. had highest colonization rates in May with percent colonization at 61%. Highest reductions in the percent colonization of stems for all three bacteria occurred in the dry season months such that in January, February and March almost all the stems had very low percent colonization rates (Table 3).

Table 3: Colonization of buried stem by bacteria measured over a 12-month period

Month	Percent colonization		
	Pseudomonas spp.	*Bacillus spp.*	*Micrococcus spp.*
January	NR	30	NR
February	NR	NR	NR
March	NR	NR	NR
April	40	45	NR
May	50	61	65
June	62	49	80
July	77	36	84
August	72	40	79
September	79	46	80
October	76	48	72
November	49	45	39
December	35	31	31

NR: Not recorded

The numbers of fungi were examined as they grew and colonized the buried sterile banana stems. Colony totals of fungi which grew from the buried stems are summarized in Table 4. Only three fungal species namely *Aspergillus, Fusarium, Rhizopus* were predominant during the stem burial studies. The stem burial experiment showed a similar pattern as the soil experiment for instance, during the dry season months, there were generally low microbial counts as compared to counts obtained in wet season months (Table 4). *Fusarium* species were found most frequently on the stem every month except in February, March and April. Representative members of this genus appeared most frequently and more consistently than the other fungi especially from May to November and its occurrence varied between 75% in June and July; 81% in August and 73 and 79% in September and October respectively. Variations were found in January, February, March and April. Suppression of *Aspergillus* spp. occurred in January, February, March, November and December. Highest numbers of this organism was found in August, September and October. *Rhizopus* spp. was observed in 85 and 80% of the stem in September and October respectively but lower percentages of colonization was recorded in January, February, March and April. All fungi were more prevalent in August, September and October and decreased in January, February, March, April and December. Changes in soil nutrient content and some other environmental conditions more especially the availability of rainfall must have played a role in the pattern of microbial composition in soil and stem as reported in this work.

Table 4: Colonization of buried stem by fungi measured over a 12-month period

Month	Percent colonization		
	Aspergillus spp.	*Fusarium* spp.	*Rhizopus* spp.
January	30	32	NR
February	NR	NR	NR
March	NR	NR	NR
April	44	NR	NR
May	69	64	62
June	52	75	71
July	50	75	69
August	62	81	77
September	68	73	85
October	72	79	80
November	38	53	60
December	32	39	53

NR: Not recorded

Numbers of both fungi and bacteria varied from time to time and from month to month. This tends to confirm that some elements of the microenvironment influence the types and numbers of microorganisms in the forest soil microenvironment (Girvan, 2003). It was reported that species of microorganisms must reach at least 10^6 cells /g of soil to be of ecological importance in the soil (Symochko *et al*., 2015), but findings from this work revealed that for both soil experiment and stem burial studies, this level was not attained in the dry season months. The soil pH did not significantly influence microbial population levels as reported in this work. Cho *et al*. (2016) stated that soil pH was critical to microbial community diversities and growth and these responses differed between a naturally acidic conifer forest soil with low pH and a sub urban forest soil with neutral pH but was

loaded with several contaminants. Soil characteristics such as moisture, composition and diversity of substrates positively affected microbial population in the soil (Loeppmann *et al*., 2016). In the forest environment, large amounts of litter fall in the dry season and microorganisms derive energy by the metabolism of these organic matters, but this increased addition of organic matter did not result to increased fungal and bacterial densities observed in this study. Highest reductions in percent microbial colonization of the stem occurred in dry season months especially in January, February, March and April contrary to high percent colonization observed in the rainy season. It is evident from this work that soil water content optimally determined microbial population and Zogg *et al*. (1997) suggested that free water connecting soil particles optimally influenced microbial population and diversity patterns by controlling nutrient availability and cell movement while Brockett *et al*. (2012) reported that soil moisture was the major factor that influenced microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada.

4.0. Conclusions

This investigation was conducted to observe the populations of bacteria and fungi in forest soil determined over a 12-month period. Microbial colonization of buried straw was also evaluated with a view to finding how seasonal variations affect the rates of plant stem colonization by the organisms. Different levels of growth responses in terms of bacterial and fungal populations occurred due to changes in some environmental conditions like moisture and pH. Microbial population was highest during the wet season and the lowest fungal and bacterial populations occurred in the dry season. Positive growth responses of the organisms were largely dependent on the soil moisture but did not significantly depend on soil pH.

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