



## Influence of Physicochemical Parameters on the Distribution of Soil Mycoflora from Lowland and Upland Soils

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### ABSTRACT

From the research, a total of 132 isolates of 14 different fungal species belonging to 7 genera were isolated using dilution plate technique on Potato dextrose agar incorporated with 1% streptomycin, from the upland and lowland agricultural fields of Usmanu Danfodiyo University, Sokoto. *Aspergillus*, *Fusarium*, *Rhizopus*, *Alternaria*, *Saccharomyces*, *Trichoderma* and *Trichophyllum* were identified microscopically using lacto-phenol cotton blue method and macroscopically by comparing the cultural and morphological features with the help of authentic fungal manual and taxonomic key. Highest occurrence was obtained in *Aspergillus* species (34.85%), followed by *Fusarium* spp. (20.45%) and the least was obtained in *Alternaria* spp. having only 2.27%. It was found that, the soil pH and Mg (pH= 8.3, Mg= 1.33cmol/kg) were significantly higher ( $P < 0.05$ ) in lowland cultivated soil sample (C) when compared with upland cultivated (A), upland (B) and lowland uncultivated (D). No significant difference was observed in soil pH and Mg among the Upland cultivated (A), Upland (B) and lowland uncultivated soil samples. Organic carbon (OC), Nitrogen (N) and potassium (K) values differ significantly ( $P < 0.05$ ) among the treatments. A strong negative significant correlation between fungal abundance and pH (-0.63) was observed at  $P < 0.05$ , and also a negative, moderate and non-significant correlation observed with sand availability (-0.43), while a weak and negative correlation exists between the fungi, magnesium (Mg) and clay availability with -0.31 and -0.35 respectively. However, a very strong positive correlation exists between the moisture contents (MC) 0.62, sodium (Na) 0.62 and the fungal abundance, with a moderate one between the fungi and organic carbon (OC) 0.55, potassium (K) and phosphorus (P) with 0.51 all at  $P < 0.05$  significant level.

**Keywords:** Upland, Lowland, Cultivated, Correlation, Identification, Danfodiyo

### INTRODUCTION

Fungi are major components of biodiversity, essential for the survival of other organisms and are crucial in global ecological processes, ubiquitous in all types of habitats and are the most adaptable organisms (Agrios, 2005). Soil is one of the most important habitats for microorganisms (Nilima *et al.*, 2011). Forming complex environment that creates numerous barriers in the isolation, identification and quantification of soil-borne organisms, particularly fungi, some of which are beneficial or otherwise (Kowalchuk, 1999). Soil is the most precious natural resource and contains the most diverse assemblages of living organisms. Indigenous microbial populations in soil are of fundamental importance for ecosystem functioning in both natural and managed agricultural soils, because of their involvement in key processes like soil structure formation, organic matter decomposition, nutrient cycling and toxic removal. Microbial communities, particularly bacteria and fungi constitute an essential component of biological characteristics in soil ecosystems. It has been estimated that 1.5 million fungal species are present in natural ecosystems; about 10% have been described formally in the literature (Wang *et al.*, 2008). Similar to bacteria, fungi are important for immobilizing, or retaining, nutrients in the soil. In addition, many of the secondary metabolites of fungi are organic acids, so they help increase the accumulation of humic-acid rich organic matter that is resistant to degradation and may stay in the soil for hundreds of years (Elaine, 2015).

Some fungi form mycorrhizal associations with plant roots, the plants provide the fungi with food (sugars and other root exudates) and the mycorrhizae provide the plants with enhanced availability of plant nutrients (P, Zn, Ca, Mg, Mn, Fe, and Cu). Several studies which surveyed fungal diversity in different forest ecosystems, suggested a strong correlation between fungal and plant diversity, due to fungal host specificity (Peay *et al.*, 2013). Further, considerable evidence indicates that changes in the composition of a microbial community can be used to predict and dictate alteration in soil quality (Van Bruggen and Semenov, 2000; Breure, 2005).

Research on fungal diversity provides a basis for estimating the functional role of fungi in ecosystems. Soil fungal population is favored largely by organic farming systems (Drinkwater *et al.*, 1995; Girvan *et al.*, 2004). Acidic nature of soil is usually influenced by rainfall, this is because heavy rainfall causes leaching out the basic forming cations (Calcium, Magnesium, Potassium and Sodium), leaving mostly hydrogen and aluminium which are largely responsible for soil acidity. Conversely, farm yard manure amendment plays an important role in improving the soil quality by buffering the pH of the soil where, they increase basic cations (Swier *et al.*, 2011). Parham *et al.* (2002) showed that the soil pH increased significantly in the plots treated with cattle manure while application of chemical fertilizer resulted in slightly lowering pH values. Farm yard manure is a potentially important source of nitrogen (N), phosphorus (P), potassium (K). Significant increase in total N, available P and K contents with Farm yard manure

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addition is directly related to the large contents of these nutrients in the compost (Plaza *et al.*, 2004; Sadej and Przekwas, 2008). A gram of garden soil can contain around one million fungi, (both yeast and mould), which cannot use atmospheric carbon dioxide as a source of carbon; and are therefore chemo-heterotrophic (Sadej and Przekwas, 2008). The aim of the research was to assess the effects of physicochemical parameters on lowland and upland mycofloral distribution.

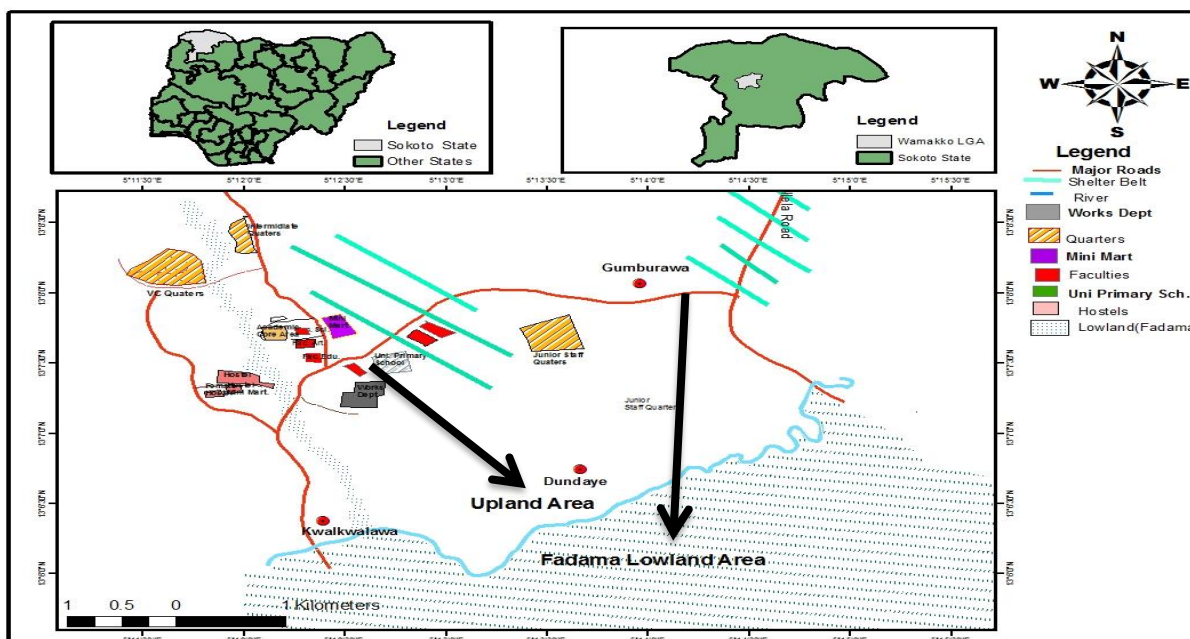
**MATERIALS AND METHODS**

**Study Site and Soil Sample Collection**

The study was conducted at Usmanu Danfodiyo University, Sokoto, main campus which is located at longitude 5° 11' 30" E and 5° 14' 30" E and latitudes 13° 8' 30" N and 13° 7' 0" N [9].

The research area (upland and lowland) are located at about ten kilometers north of Sokoto metropolis. The State lies on an altitude of 308 m above sea level, in Sudan savanna belt (Abdullahi *et al.*, 2011). The climate is hot semi-arid type and characterized by long dry season from October to May and short rainy season from June to September, with an annual mean rainfall of 724mm for a period of six years (Noma, 2005; Abdullahi *et al.*, 2011). Mean annual temperature fluctuates roughly between 43°C maximum and 18°C minimum (Anon, 2020).

Figure 1: Map Showing the Upland and Lowland areas of Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria. Source: Arc GIS, 2016



Soil samples were randomly collected using sterile soil auger at a depth of 0- 15cm from twenty-four (24) different locations, twelve (12) each from the lowland and upland areas (6- cultivated and uncultivated of each site respectively) As shown in Table 1. The soil samples were

placed into polythene bags, properly labeled and transported to Mycology laboratory, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto within one hour of collection for fungal analysis following modified techniques of Gaddeyya *et al.* (2012).

**Table 1: Locations of Soil Sample Collection**

Soil Samples	Location	Places
A	Usmanu Danfodiyo University, Sokoto, Nigeria	Upland Cultivated Soil
B		Upland Uncultivated Soil
C		Lowland Cultivated Soil
D		Lowland Uncultivated Soil

**Isolation of Soil Mycoflora**

Mycoflora were isolated from the composite samples using soil dilution plate techniques as used by Rohilla and Salar, (2012); Sajid and Bihar, (2014) with some modifications. Exactly, 1g of the soil sample was weighted and suspended in 10ml of sterile distilled water, in the preparation of serial dilution (10<sup>-1</sup> to 10<sup>-3</sup>), and diluent of 10<sup>-3</sup> was used for the fungal isolation. One milliliter (1ml) of the diluent (10<sup>-3</sup>) was

plated onto sterile petri dishes (in triplicate) each of which contained 15ml of sterile Potato Dextrose Agar (PDA) incorporated with 1% Streptomycin to prevent bacterial growth. The inoculum was then incubated at 28± 2°C for 5days. However, all the fungal isolates were continuously sub-cultured until the pure cultures were obtained.

### Identification of the Soil-borne Fungi

All the fungal pure cultures were studied macroscopically (morphologically) by observing their colony features (colour and texture) and then microscopically by staining with lactophenol cotton blue after inoculating the hyphal fragment onto a grease free sterile glass-slide and covered with a cover slip. The conidia, conidiophores and arrangement of spores of each, were observed through the microscope (X40 and X100 magnification) comparing with mycological atlas and

taxonomic key for their identification (David *et al.*, 2007; Sajid and Bihar, 2014).

### Frequency of Occurrences of the Isolated Fungi

Number of colonies per plate in a single gram (1g) of soil was calculated and the frequency of occurrences of each individual isolate as well as the genus were also calculated using the formula

$$\text{Frequency of occurrence} = \frac{\text{Number of individual species isolated}}{\text{Total number of all the species isolated}} \times 100$$

### Determination of Physico-chemical Parameters of the Soil Samples

The physico-chemical parameters of the collected soil analyzed includes; moisture contents, organic carbon, pH, nitrogen, phosphorus, magnesium, and potassium were analyzed using the procedures described by (Saravanakumar and Kaviyarsan, 2010; Rohilla and Salar, 2012).

### Statistical Analysis

Correlation analysis was done to compare the soil physico-chemical parameters to the fungal diversity and abundance. Variations due to different ecological type of soils were determined by analysis of variance (ANOVA). Duncan range was used for comparing the significant difference(s)

between means at  $P \leq 0.05$ . All the statistical analysis was evaluated using IBM SPSS software (Versions 21)

### RESULTS AND DISCUSSION

Exactly fourteen (14) different fungal species were identified belonging to 7 genera as follows; *Aspergillus*, *Fusarium*, *Rhizopus*, *Alternaria*, *Saccharomyces*, *Trichoderma* and *Trichophytum*. The highest occurrence frequency of the out of the seven genera was obtained in *Aspergillus species* (34.85%), followed by *Fusarium spp.* (20.45%), then *Rhizopus spp.* (18.94%), *Trichoderma spp.* and *Trichophytum spp.* all having 8.33% each, then *Saccharomyces* 6.82% and the least occurrence was obtained in *Alternaria spp.* which is having only 2.27% (Table 2).

Table 2: Frequency Occurrence of Soil-borne fungi of Usmanu Danfodiyo University, Sokoto Dry land farm and lowland farm

S/No	Identified Fungi	Occurrence Times	Frequency Occurrence (%)
	<i>Aspergillus species</i>	46	34.85
1.	<i>A. niger</i>	12	09.09
2.	<i>A. fumigates</i>	08	06.06
3.	<i>A. flavus</i>	07	05.30
4.	<i>A. nidulans</i>	08	06.06
5.	<i>A. orizae</i>	11	08.33
	<i>Alternaria species</i>	03	02.27
6.	<i>A. longifes</i>	03	02.27
	<i>Fusarium species</i>	27	20.45
7.	<i>F. oxysporum</i>	10	07.58
8.	<i>F. solani</i>	08	06.06
9.	<i>F. mangifera</i>	09	06.82
	<i>Rhizopus species</i>	25	18.94
10.	<i>R. stolonifa</i>	12	09.09
11.	<i>R. orizae</i>	13	09.85
	<i>Saccharomyces spp.</i>	09	06.82
12.	<i>S. cerevisae</i>	09	06.82
	<i>Trichophytum spp.</i>	11	08.33
13.	<i>T. quallinum</i>	11	08.33
	<i>Trchoderma spp.</i>	11	08.33
14.	<i>T. harzianum</i>	11	08.33
	<b>Total Isolates</b>	<b>132</b>	<b>100.00</b>

It was found that, the soil pH and Mg were significantly higher ( $P < 0.05$ ) in lowland cultivated soil sample (C) (pH= 8.3, Mg=1.33cmol/kg) when compared with upland cultivated (A), upland uncultivated (B) and lowland uncultivated (D). No significant difference was observed in soil pH and Mg among the Upland cultivated (A), Upland uncultivated (B) and lowland uncultivated soil samples. Organic carbon (OC), Nitrogen (N) and potassium (K) values differ significantly ( $P < 0.05$ ) among the treatments with lowland uncultivated (D) having higher values (OC= 1.24%, N= 0.11% and K= 0.95cmol/kg) than, upland cultivated (A), upland non-cultivated (B) and lowland cultivated (C) soil samples (Table 2). From the result, the total of 132 fungal isolates and 14 species slightly corresponds to the findings of Rakesh-Sharma and Raju (2013) who reported 137 fungal isolates and 12 species from different crop fields, *Trichoderma harzianum* have frequency of occurrence of 8.33% which is also closed to that of his findings (8.75) in the same research. However, *Aspergillus* species was found to be the most occurring and

abundant fungi with 34.84% frequency of occurrence out of the total population of all the isolated fungal species, followed by *Fusarium* spp. (20.45%), these findings also corroborates with that of Saravanakumar and Kaviyaran (2010) who reported *Aspergillus* species as the most abundant in the soil and accounted for 71.74% and also a little higher of *Aspergillus niger* (10.46%) than what was obtained (9.09%) in this research, and also domination of the community by *Aspergillus* species is somehow similar to the findings of Saravanakumar and Kaviyaran (2010) in the same research. The domination and abundance of species might be attributed to their ubiquitous distribution in nature and also to their ability to produce toxins and mycotoxins which may prevent the growth of other fungal species (Nakuleshwar *et al.*, 2013). Meanwhile, *Aspergillus* species's ability to withstand all sort of environment stress ranging from temperature, humidity, salt concentration and also considering the endemic nature of their spores (Agrios, 2005).

**Table 3:** Physico-chemical properties of the Soil collected in UDUS Dry land farm and lowland farm between October to December.

Treatment	pH	Moisture (%)	OC (%)	N (%)	PMg/Kg	Mg (Cmol/Kg)	K (Cmol/Kg)
A	7.4±0.10 <sup>b</sup>	1.0±0.02 <sup>c</sup>	0.32±0.02 <sup>d</sup>	0.06±0.00 <sup>d</sup>	0.79±0.12 <sup>c</sup>	0.60±0.07 <sup>b</sup>	0.46±0.02 <sup>d</sup>
B	7.5±0.20 <sup>b</sup>	1.0±0.02 <sup>c</sup>	0.56±0.02 <sup>c</sup>	0.07±0.00 <sup>c</sup>	0.94±0.04 <sup>b</sup>	0.60±0.03 <sup>b</sup>	0.56±0.04 <sup>c</sup>
C	8.3±0.20 <sup>a</sup>	1.5±0.03 <sup>b</sup>	1.08±0.02 <sup>b</sup>	0.10±0.00 <sup>b</sup>	1.12±0.06 <sup>a</sup>	1.33±0.55 <sup>a</sup>	0.87±0.04 <sup>b</sup>
D	7.2±0.26 <sup>b</sup>	2.0±0.06 <sup>a</sup>	1.24±0.05 <sup>a</sup>	0.11±0.00 <sup>a</sup>	1.16±0.03 <sup>a</sup>	0.70±0.06 <sup>b</sup>	0.95±0.04 <sup>a</sup>
(SE)	0.14	0.05	0.11	0.00	0.05	0.12	0.06
Sig.	*	*	*	*	*	*	*

Values are expressed as means ± SE of n=3, values with the same letter(s) along the column are statistically similar ( $P < 0.05$ )  
KEY: **A**= Upland Cultivated Soil; **B**= Upland uncultivated; **C**= Lowland Cultivated; **D**= Lowland uncultivated

**Table 4:** Pearson Correlation between the Fungal Abundance and Soil Physico-Chemical Parameters of the Soils of Usmanu Danfodiyo University, Sokoto

Variables	Correlation (r)	Significance (P<0.05)
Fungal abundance by pH	-0.63	0.028*
Fungal abundance by MC	0.62	0.031*
Fungal abundance by OC	0.55	0.066 <sup>ns</sup>
Fungal abundance by N	0.56	0.061 <sup>ns</sup>
Fungal abundance by P	0.51	0.088 <sup>ns</sup>
Fungal abundance by Ca	0.06	0.856 <sup>ns</sup>
Fungal abundance by Mg	-0.31	0.032 <sup>ns</sup>
Fungal abundance by K	0.51	0.089 <sup>ns</sup>
Fungal abundance by Na	0.62	0.032*
Fungal abundance by CEC	0.45	0.145 <sup>ns</sup>
Fungal abundance by Sand	-0.43	0.159 <sup>ns</sup>
Fungal abundance by Silt	0.43	0.160 <sup>ns</sup>
Fungal abundance by Clay	-0.35	0.913*

Key: OC= Organic carbon, N= Nitrogen, P= Phosphorus, Ca= Calcium, Mg= Magnesium, K= Potassium, Na= Sodium, CEC= Cat ion exchange capacity  
\* Correlation is significant at the 0.05 Confidence level

Total of one hundred and thirty-two (132) fungal species isolated in this study, belonged to seven (7) genera which are a bit lower than the findings of Saravanakumar and Kaviyaran (2010), who isolated thirteen (13) fungal genera

from his study. Probably because of the climatic conditions and soil fertility of their study area that varies.

There is strong negative significant correlation between fungal abundance and pH (-0.63) at  $P < 0.05$ , also a

moderately negative and non-significant correlation observed with sand availability (-0.43), with a weak and negative correlation exists between the fungi, magnesium (Mg) and clay availability, which implies that any slight increase in the pH, the fungal population will decrease. However, strong positive correlation was observed in the clay, moisture contents, sodium and the fungal abundance, with a moderate one between the fungi and organic carbon (OC), potassium (K) and phosphorus (P) at  $P < 0.05$  significant level, all of which corresponded to the findings of Saravanakumar and Kaviyarasan (2010); Smith *et al.* (2007) and Nakuleshwar *et al.* (2013) that both reported inverse relation between fungal abundance, pH, Nitrogen and Magnesium content and direct relation with organic carbon and potassium.

### CONCLUSION

The study revealed that most of the physicochemical parameters (pH, Moisture Contents, Sodium and Soil type) of the study area influence the distribution and abundance of soil mycoflora in both the upland and lowland soils whether cultivated or uncultivated soils type.

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