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Diversity of Airborne Pollen and Fungal Spores in Nsukka, South-East Nigeria

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Abstract
Airborne pollen and fungal spores are major triggers of allergies and their abundance and seasonality depend on plant responses to climatic and meteorological variables. Studies on diversity and seasonal prevalence of airborne pollen and fungal spores in Nsukka (South- east Nigeria) and their relationship to climatic variables were carried out from June 2011-May 2012. The aim of the study was to determine the diversity of airborne pollen and fungal spores, ascertain their seasonal variations and the impact of weather variables on their dispersal. Airborne samples were collected using a Tauber-like pollen sampler , aerosamplers were collected monthly and acetolysed. Dominant pollen were those dispersed from Poaceae, Elaeis guineensis, Aspilia africana, Olax subscorpoides, Alchornea cordiforlia , Pentaclethra macrophylla and Casuarina equisetifolia . Total pollen count correlated positively with temperature and wind, though not significant and negatively with rainfall, relative humidity and wind. Spores dispersed by Fusarium, Nigrospora, Puccinia, Spadicoides, Alternaria and Helminthosporium were more prepoderant. A positive correlation occurred between total spores count and monthly rainfall. Rainy season (May – August) had lower records of pollen whereas the principal pollination period was during the dry season (September – November). The allergenic and pathogenic fungal spores were more abundant in rainy season (June –August) and late rainy season (September –November). The study revealed a strong influence of meteorological factors on abundance and spatial distribution of pollen and fungal spores

Key words; *Allergy; fungal spores; pollen, weather parameters*

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I. Introduction

Airborne pollen and fungal spores are major sources of inhalant allergens in a large number of allergic cases (Ho *et al*.,1995). They are easily filtered by the respiratory system where they trigger and exacerbate respiratory/inhalation allergies (Jae-Won *et al*.,1998). They have high aerodynamic properties, which increase their propensity to reach out to the public. People are exposed throughout life to their allergens directly or after the allergen bearing particles penetrate interiors (Harriet and Rogers, 2000). Aerobiological investigations have revealed that pollen and spores are the most dominant, pervasive, respirable and potent sources of allergen present in the atmosphere (Shahali *et al*., 2007)). They are ubiquitous and widely distributed in time and space than any other representatives of living matter (Shahali *et al*., 2007). Pollen and spores are usually within the size range of 10 µm-50 µm and as a result, they are easily filtered by the airways, those less than 10 µm penetrate the lower airways, where allergic reaction tend to manifest as asthma (Dankaart *et al*.,1991; Luo,1991).

The latter part of the 20^{th} century has experienced an increase in the prevalence of allergic diseases, implicating changing environment as significant causes (Tosunoglu *et al*., 2014). With the alarming increase in allergic disorders, such as allergic rhinitis, bronchial asthma and atopic dermatitis covering as high as 30 % of the population world over (Singh and Shahi, 2006). There has been a substantial increase in the prevalence of allergic diseases within Nigeria. Allergic diseases have a large impact on human health globally, with 10–30 % of the population affected by allergic rhinitis and more than 300 million affected by asthma (Albertine *et al*.,2014). It has been hypothesized that global climate change could alter the concentrations, distributions, dispersion patterns, and allergenicities of pollen and spores in the environment in ways that could further increase the prevalence of allergic diseases (Ziska *et al.*, 2007). Asthma is a chronic respiratory disease that are exacerbated by inhalation of airborne pollen and spores. Pollen and spores liberation are passive process and depends on such agents as rain, wind, humidity, etc (Giovanni *et al*., 2009). Their quantity and seasonality depend in large part on plant responses to climatic and meteorological variables (Ziska *et al.*, 2007). Pollen production varies however from individual- to individual, site-to-site, year-to-year, species-to species (Giovanni *et al*., 2009).

Studies on seasonal distribution pattern of pollen and fungal spores is an important aid for the effective management, treatment and diagnosis of pollen and fungal spores allergies(Jae-Won *et al.*, 1998:Tosunoglu *et al*.,2014). The use of suitable medications before the expected increase in atmospheric pollen counts will reduce the severity of allergic reactions (Ho *et al*., 1995). The present study was undertaken in Nsukka (South-east Nigeria) from June 2011-May 2012, to examine the spatial distribution and abundance of airborne pollen and fungal spores, the correlation of abundant pollen and spores with meteorological data was also studied.

II. Materials And Methods

Study Area and sample collection

The study conducted in University of Nigeria Nsukka, (South –east Nigeria). Modified Tauber- like pollen trap was employed for the collection of the aero samples. The trap was placed at the height of 5 ft above the ground level (Figure 2). A solution made of glycerol (50 ml), formaldehyde (10 ml) and phenol (5 ml) were prepared and poured into the trap. The recipient solutions were collected monthly for the period of one year. Samples were sieved through 200 µm mesh wire gauze to filter off large organic particles. The liquid with suspended palynomorphs were centrifuged at 2500 revolution per minute for 5 minutes to recover the palynomorphs residues. The residues were washed three times with water and were acetolyzed according to a modified version of Erdtman (1971) procedures; acetolysis mixture which consist of concentrated sulphuric acid and acetic anhydride in the ratio of 9: 1 was prepared, 5 ml of the acetolysis mixture was poured into each sample and placed in water bath for 10 minutes at 100 ^{o}C. They were centrifuged, decanted and washed twice with distilled water. The recovered residues were stored in vials with two drops of glycerine. Temporary slides were prepared and examined using light Olympus CH Trinocular microscope (LM), equipped with 650 IS cannon Digital camera at x 400 and x 100 magnifications. Identification was based on comparison with reference collection of pollen slides, description and photomicrographs of pollen and spores using books and journals (Agwu and Akanbi, 1985: Y'bert 1979). Agwu (1997), Ezike *et al.* (2016), Agwu and Osibe (1992) also used Tauber-like pollen traps similar to the trap employed in this study and also the same methodology.

Pollen and fungal spores obtained were counted monthly and expressed in cubic meter of air. The data obtained were analyzed using the SPSS statistical package version 20 (SPSS Inc. Chicago, Illinois USA). Correlation coefficients were generated to examine the relationship between pollen, fungal spores frequency and meteorological data. Meteorological data were obtained from Nigerian Meteorological Centre, Enugu, Nigeria.

Fig 1: Map of Nigeria showing the study area ⯎ Study area

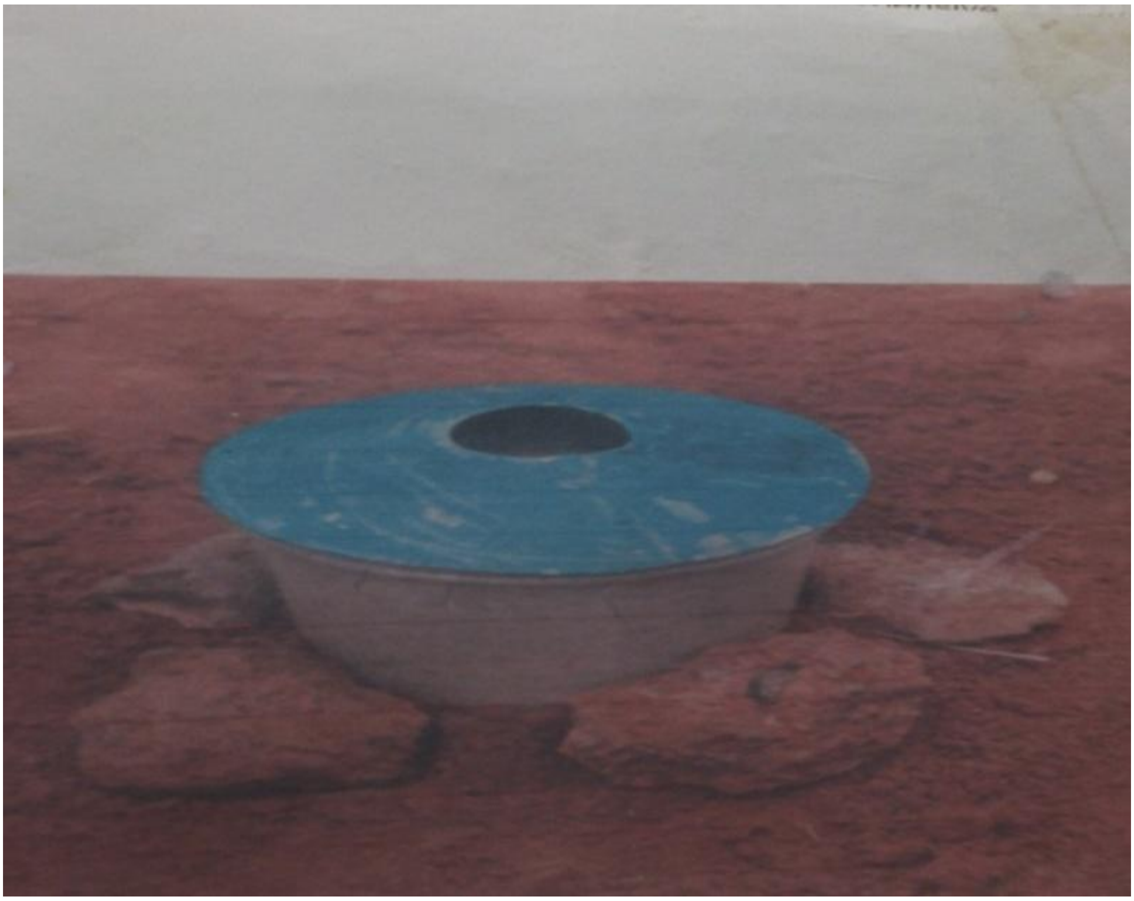

Figure 2: Modified Tauber-like Pollen trap in the field

III. Result

Sixty-nine pollen types which belong to 33 plant families were recorded from the aeroflora. Among these Forty –five, eighteen and five were identified to species, generic and family levels respectively. Major pollen contributors were; Poaceae 668(27.35 %), *Alchornea cordiforlia* 338 (13.84), *Elaeis guineensis* 321 (13.13), *Aspilia africana* 228(9.33 %) , *Olax subscorpoides* 178(7.29 %), , *Pentaclethra macrophylla* 134 (5.49 %) *and Casuarina equisetifoliai* 74 93.03 %) (Table 1). *Olax subscorpioides* pollen were present from September to February. Poaceae pollen were predominant from June to November. *Alchornea cordifolia* pollen was present from July to May, achieved anthesis in December.

The annual sum of fungal spores (2209) was less than pollen (2639). The months of May (31), August (81) and January (64) had the lowest record of pollen. There was a quantitative pollen record in April and was dominated by pollen of *Aspilia africana, Ageratum conyzoides and Bombax buonopozense*. The month of May had the lowest pollen record among the rainy season. The atmospheric pollen record started increasing from the month of September (Fig. 3). The pattern of pollen dispersal displayed three distinct periods: the period from May to August,2which corresponded with rainy season and had (470) lower atmospheric pollen content. Another period from September to November, was dry season, which was dominated by pollen (752). December –February, the harmattan period recorded 640 pollen.

Twenty eight fungi spores types were recorded (Table 2). The most dominant and prevalent fungi spores include those of *Fusarium* 541 (42.19 %), *Alternaria* 168 (13.10), *Nigrospora* 122(9.52 %), *Puccinia* 112(8.74) and Spadicoides 92(7.18 %). Some spore types were only present during the rainy season such as *Alternaria, Fusarium, Venturia, Triposporium* and *Helminthosporium.* The abundance of the spores became reduced during the late rainy season. Fungi which belong to Deuteromycotina quantitatively dominated (Table 3). The Spores of *Nigrospora* and *Puccinia* were more persistence occurring almost throughout the year. The lowest monthly spores was recorded in February (17).

Total pollen count correlated positively with temperature but negatively with rainfall, relative humidity and wind. Poaceae and *Elaeis guineensis* correlated positively with monthly rainfall whereas *Casuarina equisetiforlia*, *Olax subscoipoides* and *Aspilia africana* had a negative correlation with monthly rainfall. All dominant pollen with the exception of *Aspilia africana* had a negative correlation with monthly temperature. *Olax subscopoides* was significantly correlated with relative humidity and wind (Table 4). Total spores and all dominant spores recorded had a positive correlation with monthly rainfall and a negative correlation with temperature. All fungal spores with the exception of *Nigrospora* correlated positively with relative humidity and negatively with the wind (Table 5).

Table 1: Dominant pollen in Nsukka (South-east Nigeria)

Pollen	Family	Total	
Albizia sp.	Fabaceae	25	1.02
Ageratum conyzoides	Asteraceae	42	1.72
Alchornea cordiforlia	Euphorbiaceae	338	13.84
Aspilia africana	Asterceae	228	9.33
Blighia sapinda	Sapindaceae	40	1.64
Bombax buonopozense	Bombacace	50	2.04
Cassia mimosoides	Fabaceae	26	1.06
Casuarina equisetifolia	Casuarinaceae	74	3.03
Cissus sp	Vitaceae	**18**	0.74
Cocos nucifera	Aracaceae	**42**	**1.72**
Cyperus spp.	Cyperaceae	**46**	**1.88**
Daniella oliveri	Fabaceae	**32**	**1.31**
Dracaena arborea	Asparagaceae	**42**	**1.72**
Elaeis guinensis	Aracaceae	**321**	**13.15**
Hymenocardia acida	Euphorbiaceae	**24**	**0.98**
Olax subscopoidea	olacaceae	**178**	**7.29**
Poaceae	Poaceae	**668**	**27.35**
Pentaclethra macrophylla	Mimosaceae	**134**	**5.49**
Mangifera indica	Anacardiaceae	**45**	**1.84**
Phyllanthus sp.	Euphorbiaceae	**42**	**1.72**
Isoberlinia doka	Fabaceae	**27**	**1.11**

Dominant pollen were those whose annual contribution exceeds 20 grains/m^3

Table 2: Monthly frequency of atmospheric fungi spores of Nsukka (South -east Nigeria) from June 2011 to May 2012.

S/N	FUNGI SPORES	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	*Torulla* sp.	6	4		4		4	2					
2	*Cladosporium* spp						3		6				
3	*Nigrospora* sp.	16		16	16	14	4	16	14	6	20		
4	*Fusarium* spp.	6	13		122	400							
5	*Helminthosporium* sp.	6	28	4	8	36							
6	*Curvularia* spp.				2	6			2				
7	*Pithomyces* sp.			1		2					2		
8	*Beltrania* sp.		6			4							
9	*Leptothyrium* sp.					27		2					
10	*Monilia* sp.					2							
11	*Puccinia* sp.	6	74		8		6	6		2			10
12	*Dreschlera* sp.									2			
13	*Venturia* sp.	1		4	24								
14	*Spadicoides* spp.	18	62		2			6			4		
15	*Pseudotorulla* sp.		1										
16	*Triposporium* sp.	10		1	8	14							
17	*Curvularia* spp.		2										
18	*Alternaria* spp.		136	24								4	4
19	*Diplocladiella* sp.		2										
20	*Stemphyluim* sp.			2									
21	*Helminthosporium* sp.			4									
22	*Cercospora* sp.				16								
23	*Flagellospora* sp.				2								
24	*Sporidesmium* sp.									1			
25	*Cephalosporium* sp.									4			
26	*Murogenella* sp.									2			
27	Indeterminata	220		66			1503		99			6	6
28	**TOTAL SPORES**	**295**	**579**	**118**	**214**	**607**	**170**	**32**	**121**	**17**	**26**	**10**	**20**

Table 3: Dominant Fungal spores in Nsukka (South-east Nigeria)

Fungal spores	Sub division	Total	%
Fusarium	Ascomycotina	541	42.19
Helminthosporium	Deuteromycotina	82	6.39
Nigrospora	Deuteromycotina	122	9.52
Puccinia	Basidiomycotina	112	8.74
Spadicoides	Deuteromycotina	92	7.18
Alternaria	Pezizomycotina	168	13.10
Triposporium	Ascomycotina	33	2.57
Venturia	Pezizomycotina	30	2.34

Dominant fungal spores were those whose annual contribution exceeds 30 spores/m^3

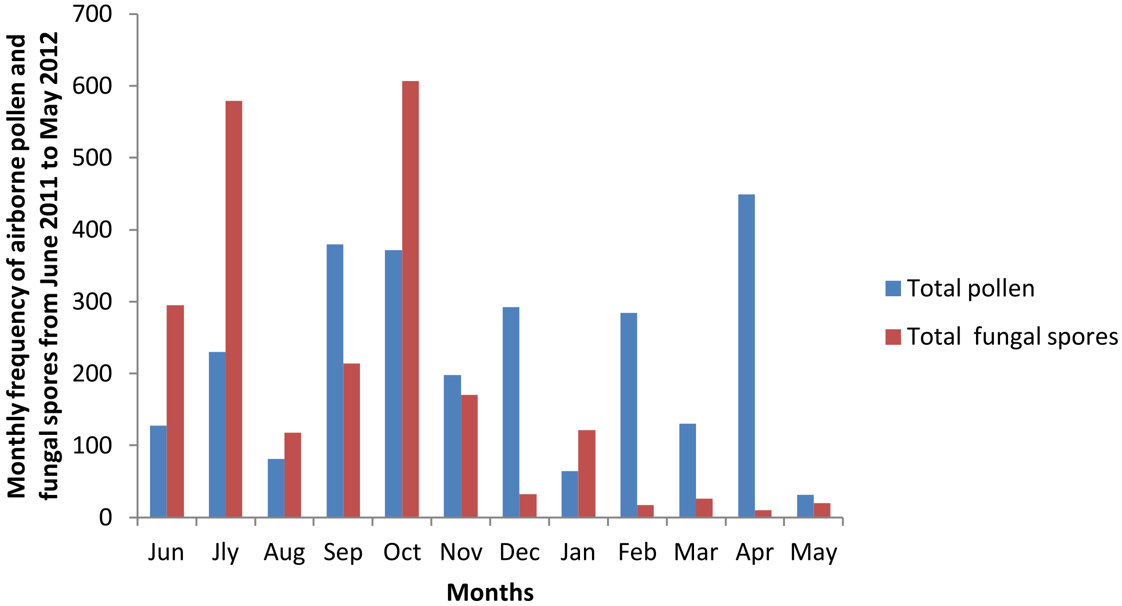

Fig. 2: Monthly variations of airborne pollen and fungal spores in Nsukka(South-east Nigeria) from June 2011 to May 2012

Table 4: Correlation coefficients between frequency of pollen and meteorological data

Pollen count	R	T	R.H	W
Aspilia africana	-.039	.256	0.051	-.473
Alchornea cordiforlia	-.596	.283	-.519	.195
Casuarina equisetiforlia	-.100	-.332	.259	.053
Elaeis guineensis	.118	-.220	-.297	-.051
Olax subscopoidea	-.355	-.168	-.677*	.586*
Poaceae	.234	-.357	.511	-.479
Total pollen	-.050	.026	-1.33	.020

*Correlation is significant at the *P*= 0.05 level (2- tailed)
R, Mean monthly rainfall (mm); T, Mean monthly temperature (^{o}C); R.H. Mean monthly relative humidity(%); Mean monthly wind speed (km/hr)

Table 5: Correlation coefficients between frequency of fungal spores and meteorological data

Spores count	R	T	R.H	W
Alternaria	.230	-.251	.319	-.374
Bastrodemium	.015	- .149	.220	-.278
Fusarium	.230	-.251	.319	-.374
Helminthosporium	.253	-503	.507	-.348
Nigrospora	. 014	-.031	-.273	.083
Puccinia	.182	-.345	.304	-.161
Spadicoides	.118	-.314	.283	-.091
Total spores	.238	-.503	.507	-.410

*Correlation is significant at the p = 0.05 level (2- tailed)
R, Mean monthly rainfall (mm); T, Mean monthly temperature (^oC); R.H. Mean monthly relative humidity(%); Mean monthly wind speed (km/hr)

IV. Discussion

The frequency of pollen and fungal spores varied from month to month and from season to season in the atmosphere of Nsukka (South-east Nigeria). Pollen from some enthomophilous plants including ornamental plants located in the study were seldom represented in the aerosamples probably because of their lower pollen production. Pollen dispersed from Poaceae, *Elaeis guineensis*, *Aspilia africana*, *Olax subscorpoides*, *Alchornea cordiforlia* , *Pentaclethra macrophylla and Casuarina equisetifolia* taxa were more dominant and are suspect of excitant of hay fever and other allergies in the study area and environs. Some studies have shown that if a tree is pollinated solely by insects, it can be ruled out as an important cause of hay fever and for a pollen to cause hay fever, it must be produced in large quantities, be disseminated by the wind and possess allergenic toxicity. The anemophilous pollen, which dispersed more pollen in the atmosphere among other dominant pollen were Poaceae, *Elaeis guinensis* and *Casuarina equisetifolia.* The period of September- February was designated as the higher risk period for pollen hypersensitive individuals who inhabit or frequently visit the area, because the atmosphere at this period was quantitatively dominated by anemophilous pollen, majorly Poaceae and *Elaeis guineensis*. This period was also the major and principal pollination period. In similar work carried out in Nsukka (Njokuocha, 2006), Poaceae and *Elaeis guineensis* dominated, pollen were more abundant in late rainy season – early dry season/Harmattan. In the present work, the concentration of pollen exceeded that of the fungal spores, this however is contrary to the view of Horner *et al*., 1995, who stated that airborne fungal spores occur widely and often in far greater concentrations than pollen grains..

Among the recovered pollen, anemophilous pollen quantitatively dominated, because of their greater pollen production in compensation of inefficiency of wind in pollination ecology, they therefore have greater propensity to reach out to people. Though, hypersensitive individuals, children and immunocompromised individuals are usually more prone to pollen and spores allergies (Singh and Mathur, 2012). Beside the elicitation of allergenic reactions by protein contained in pollen sporoderm, there are factors which influence pollen allergenicities. Singh and Kumar, 2004 suggest that urbanization with its high levels of vehicle emissions, and westernized lifestyle are linked to the rising incidence of pollen-induced respiratory allergy seen in most industrialized countries. Zhao et al. (2015) found that pollen of the common ragweed (*Ambrosia artemisiifolia*) has higher concentrations of allergen when the plant is exposed to NO_2 exhaust gases. Recently the prevalence of allergic diseases especially asthma, conjuctivities, dermatitis etc has increased, usually attributed to increase in environmental pollution as environmental pollutants have been found to act as adjuvant of pollen allergenicity (Bartra *et al*., 2007). This implies that bioaccumulation of environmental pollutant by pollen could also creates differences in their allergenicities.

Among the non arboreal taxa, Poaceae pollen were the most dominant preceded by *Aspilia africana*. Tosunoğlu *et al*. (2014) also found Poaceae most dominant among the non arboreal taxa in the atmosphere of Büyükorhan. Ezike *et al*.(2016) found them dominant in aerosamples collected in the atmosphere Garki, Abuja(North-Central Nigeria). In their work Poaceae pollen dominated persistently form June to February. In the present work, Poaceae were almost persistently present thoroughout the year. Poaceae pollen could be the major perennial allergenic candidate in the study area. Poaceae pollen have been found to play a crucial role in allergy because of their copious, bulk and disproportionate amount of pollen they disperse into the atmosphere (Ezike *et al*.,2016). They are major cause of pollinosis in many parts of the world (D'Amato *et al*., 2007). Although their atmospheric frequency differ regionally. Grass-induced pollinosis is the most common pollen allergy also in Europe (Amato *et al*., 2007). The antigens of grass pollen, like those of the other allergenic pollen grains, are rapidly released, when pollen comes into contact with the oral, nasal, or eye mucosa, thereby inducing the appearance of hay-fever symptoms in sensitized patients. As a consequence, the concentration of airborne grass pollen influences the degree of symptoms in hypersensitive individuals. In London (UK), the lowest atmospheric concentration of grass pollen able to induce the appearance of hay-fever symptoms was shown to be 10–50 grains/m^3 (Amato *et al*., 2007).

Among the fungal spores, most predominant were *Fusarium*, *Alternaria*, *Nigrospora and Puccinia*. Quantitatively members of Ascomycotina dominated, but qualitatively fungal spores morphotypes belonging to Deuteromycotina prevailed. Ianovici *et al*. (2011) also found the most important fungal allergen sources among the fungi from the class of the *Deuteromycetes*. Airborne Fungal spores, like pollen induce type I hypersensitivity (allergic) respiratory reactions in sensitized atopic subjects, causing rhinitis and/or asthma. Many studies have shown that exposure to airborne fungal spores may be associated with acute toxic effects, allergic rhinitis, rhino-conjunctivitis, allergic broncho-pulmonary aspergillosis, and bronchial asthma in both adults and children (Hasnain *et al*., 2012). More quantitative abundance of fungal spores were recorded in July and October dominated by *Alternaria* and *Fusarium* respectively. *Alternaria* is plant pathogenic and also causes respiratory allergies, accompanied by bronchial asthma (Infante *et al*.,1999). *Alternaria* spores have been recorded in the atmosphere of other areas of the world. It was found persistently present throughout the year in the atmosphere of Tetouan (Filali *et al*., 2014).. Stennett and Beggs (2004) found them year round in Sydney, though they peak in spring. The spores of *Alternaria* were the most dominant among other fungal spores population in the atmosphere of Kuwait. The record of spores of *Alternaria* in July and their significant correlation with rainfall and relative humidity shows that their spores could be perennial but highly hydrophilic. *Fusarium* species are important plant pathogens. Nelson *et al*.(1994) outlined some diseases they cause such as crown rot, head blight, and scab on cereal grains. They also cause infection in human such as keratitis, onychomycosis and respiratory allergies (Evans *et al*., 2004). In the study area *Fusarium* spores peaked in late rainy season (October). Their spores were found associated with reduced rainfall. In Nigeria, numerous people suffer from conjuctivitis, dermatitis and other allergies. In the case of vernal kerato conjunctivitis which is one of the leading allergic conjunctivitis, hospital prevalence was found to be higher in the rainy season and declined in the dry season (Keziah , 2014). This disease and other allergenic diseases could be elicited by preponderance of fungal spores during the rainy season.

In overall, the sum total of fungal spores were higher in rainy season than in the dry season. Ezike *et al*. (2016) found fungal spores more predominant in rainy season than dry season with the exception of *Erysiphe graminis* in the atmosphere of Garki, Abuja. Essien and Oluwagbemiga 2014, recorded fungal spores more in the month of December followed by march, in the atmosphere of Anyigba, kogi State, Nigeria. This however depicted dissimilarity in airspora components as the vegetation and geography differ and especially as the local weather parameter also differs not only from place to place but even from day to day, month to month and season to season.

Fungal spores are widely distributed in an outdoors environment, studies have shown that they are highly modulated by the prevailing weather condition (Essien and Aina, 2014, Filali *et al.*,2014; Hasnain *et al*.,2012; Stennett and Beggs, 2004). The negative correlation of total pollen counts and 71 % of dominant pollen with rainfall, shows that rainfall washes down suspended pollen in the atmosphere, reducing their antigenic load. The correlation of 88 % of dominant fungal spores with relative humidity, shows their sporulation being favoured by moisture content in the atmosphere. This finding agrees with Ezike et al.(2016) and further explains the reason why greatest number of fungal spores have been found in the tropical than in temperate regions (Hyde and Gol, 2003). Teranishi *et al*.,2000 found that first day of pollen dispersal was closely correlated with the mean temperature in Japan. Lyon et al. (1984) and Jae-Won et al.(1998) found a positive correlation between humidity, precipitation and ascospores. Contrary to the finding, Li and Kendrick, 1994 indicated higher spore counts of conidial fungi associated with higher temperature and lower relative humidity, while those for Ascomycetes were greatest at higher relative humidity and lower temperature. Ezike at al. (2016), noted high infux of *Erysiphe graminis* in dry season (November). This shows that inspite of the majority of the fungal spores which sporulate in rainy season, some could also be found dominant in an outdoors environment during the dry season. However, in this study, humidity was noted as a strong weather parameter that greatly influenced fungal spores dispersal, this was further confirmed by Li and Kendrich, (1994),Bamba *et al*.(2014), Alberto *et al*. (2006) etc.

V. Conclusion

The present study reveals dominant pollen and fungal spores in the atmosphere of Nsukka (South-east Nigeria). The dominant pollen and fungal spores morphotypes indicated in the work could be major excitant of allergenic diseases in the study area. However further studies on their allergenicities needs to be carried out, before establishing them as allergenic candidates. This work therefore establishes a platform for a clinical research on airborne pollen and fungal spores of the study area. Their Spatial distribution and abundance were influenced by weather parameters. More humid months had higher dominance of fungal spores whereas drier months had dominance of pollen. Hypersensitive individuals to airborne pollen should adopt an adaptive measures such as using nose mask, to reduce their exposure to allergenic pollen especially during the harmattan period. Reduction of wetness will reduce the sporulation of fungal spores in an ambient environment.

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