SPATIAL DISTRIBUTION OF ATMOSPHERIC POLLEN AND FUNGI SPORES AND THEIR RELATION TO ALLERGY IN NIGERIA

BY

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June, 2015.

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SCHOOL OF POSTGRADUATE STUDIES

UNIVERSITY OF LAGOS

CERTIFICATION

This is to certify that the thesis

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Submitted to the School of Postgraduate Studies,
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is a record of Original Research

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DEDICATION

This work is dedicated to my father, late Associate Prof. J. N. Ezike.

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TABLE OF CONTENTS

		Pages
Cover page		i
Title Page		ii
Certification		iii
Dedication		iv
Acknowledgements		v
Table of Contents		vii
List of Tables		xi
List of Figures		xii
List of Plates		xiv
Abstract		xv
CHAPTER ONE: INTRODUCTION		
1.1	Background of the Study	1
1.2	Statement of the Problems	9
1.3	Objectives of the Study	11
1.4	Significance of the Study	12
1.5	Operational Definition of Terms	12
1.6	Abbreviations	13
CHAPTER TWO: LITERATURE REVIEW		

2.1 Aeropalynology 14

2.2 Allergy Survey 23

2.3 Allergic Diseases 24

2.3.1 Types of Asthma 24

2.3.2 Asthma in Pregnancy 25

2.3.3 Complication of Asthma 25

2.4 Prevalence of Asthma and Allergy 26

2.5 Rhinitis 26

2.5.1 Classification of Rhinitis 27

2.6 Asthma and Allergy Prevalence Worldwide 28

2.7 Conjuctivitis 31

2.7.1 Types of Allergic Conjunctivitis 33

2.8 Pollen and Fungal Spores Allergenicity 34

CHAPTER THREE: MATERIALS AND METHODS

3.1 Collection of Atmospheric Pollen and Fungal Spores 39

3.2 Setting up the Trap for Collection of Atmospheric Pollen and Fungal Spores 39

3.3 Pollen Extraction and Concentration 42

3.4 Digestion of Inorganic Materials 42

3.5 Acetolysis 42

3.6 Temporary Slides 43

3.7 Identification 43

3.8 Extraction of Pollen and Fungal Spores' Protein 43

3.9 Inoculation of Pollen and Fungal Spores' Protein into Mice 44

3.10 Immunoperoxidase Assay for determination of IgE in Mice Sera 45

3.11 Histopathology Procedures of the Respiratory organ 46

3.12 Statistical Analysis 46

3.13 Collection of Weather Parameters Data 46

CHAPTER FOUR: RESULTS

4.1 Seasonal Prevalence of Pollen and Fungal Spores in Nsukka, Enugu State (South East) Nigeria from June 2011 to May 2012 47

4.2 Seasonal Prevalence of Pollen and Fungal Spores in Obiakor, Rivers State (South South) Nigeria from June 2011 to May 2012 53

4.3 Seasonal Prevalence of Pollen and Fungal Spores in Akoka, Lagos State (South West) Nigeria from June 2011 to May 2012 58

4.4 Seasonal Prevalence of Pollen and Fungal Spores in Garki, Abuja (North Central) Nigeria from June 2011 to May 2012 62

4.5 Seasonal Prevalence of Pollen and Fungal Spores in Zaria, Kaduna State (North West) Nigeria from June 2011 to May 2012 67

4.6 Seasonal Prevalence of Pollen and Fungal Spores in Dukku, Gombe State (North East) Nigeria from June 2011 to May 2012 70

4.7 Seasonal variations of pollen, fungal spore types and charred Poaceae epidermis

among the six geo-political zones of Nigeria 73

4.8 Relationship between atmospheric Pollen, Fungal Spores and Weather Parameters 82

4.9 Protein Contents of Pollen and Fungal Spores 91

4.10 Clinical Features of Allergy 94

4.10.1 Mice Skin 94

4.10.2 Trachea of Mice 94

4.10.3 Lung of Mice 94

4.10.4 Bronchiole 94

4.11 Evaluation of Immunoglobulin E(IgE) in Mice Sera 97

4.12 Immune Cells 101

CHAPTER FIVE

5.1 Discussion 110

5.1.1 Pollen and Fungal Spores Distribution 110

5.1.2 Pollen, Fungal Spores Load and Meteorological Factors 117

5.1.3 Immunological Result 120

5.2 Conclusion 124

CHAPTER SIX

6.1 Summary of Findings 125

6.2 Contributions to Knowledge 127

REFERENCES 128

APPENDICES 139

LIST OF TABLES

		Pages
Table 4.1:	Atmospheric pollen count in Nsukka, Enugu State (South-East) Nigeria	49
Table 4.2:	Atmospheric fungal spores count in Nsukka, Enugu State (South-East) Nigeria	52
Table 4.3:	Atmospheric pollen count in Obiakor, Rivers State (South-South) Nigeria	55
Table 4.4:	Atmospheric fungal spores count in Obiakor, Rivers State (South-South) Nigeria	57
Table 4.5:	Atmospheric pollen count in Akoka, Lagos State (South-West) Nigeria	59
Table 4.6:	Atmospheric fungal spores count in Akoka, Lagos State (South-West) Nigeria	61
Table 4.7:	Atmospheric pollen count in Garki, Abuja (North-Central) Nigeria	64
Table 4.8:	Atmospheric fungal spores count in Garki, Abuja (North-Central) Nigeria	66
Table 4.9:	Atmospheric pollen count in Zaria, Kaduna State (North-West) Nigeria	68
Table 4.10:	Atmospheric fungal spores count in Zaria, Kaduna State (North-West) Nigeria	69
Table 4.11:	Atmospheric pollen count in Dukku, Gombe State (North-East) Nigeria	71
Table 4.12:	Atmospheric fungal spores count in Dukku, Gombe (North-East) Nigeria	72
Table 4.13	Total atmospheric pollen in the six geo-political zones of Nigeria	79
Table 4.14:	Total atmospheric fungal spores in the six Geo-political zones of Nigeria	80
Table 4.15	Distribution of Charred Poaceae cuticles in the six Geo-political Zones	81
Table 4.16:	Protein contents of some selected pollen and fungal spores	92
Table 4.17:	Relationship between protein contents and Immunoglobulin E levels (ng/ml) elicited after last sensitization.	93

LIST OF FIGURES

		Pages
Fig. 3.1:	Map of Nigeria, showing the six geo-political zones of Nigeria and study locations	40
Fig. 4.1:	Annual Percentage contributions of Poaceae pollen in the six geo-political zones of Nigeria from June 2011- May 2012	75
Fig. 4.2:	Percentage contributions of trees/ shrubs, grasses and herbaceous pollen in six geopolitical zones of Nigeria	76
Fig. 4.3:	Annual contributions in degree of anemophilous pollen in the six geo-political zones of Nigeria	77
Fig. 4.4:	Annual contributions in degree of entomophilous pollen in six geo-political zones of Nigeria	78
Fig. 4.5:	Relationship between atmospheric pollen and monthly rainfall (mm) in South East, South South and South West Nigeria	84
Fig. 4.6:	Relationship between atmospheric fungal spores and monthly rainfall (mm), temperature ^{o}C, humidity(%) and wind (knot) in South East, South South and South West Nigeria	85
Fig. 4.7:	Relationship between atmospheric pollen and monthly rainfall (mm) in North Central, North West and North East Nigeria	86
Fig. 4.8:	Relationship between atmospheric fungal spores and monthly rainfall (mm) in North Central, North West and North East Nigeria	87
Fig. 4.9:	Immunoglobulin E elicited by protein of pollen and fungal spores in mice	99
Fig. 4.10:	Mean values of Immunoglobulin E elicited by protein of pollen and fungal	

spores in mice 100

Fig. 4.11: Mean values of mice Lymphocytes elicited by protein of pollen and fungal spores 102

Fig. 4.12: Mean values of mice Basophil elicited by protein of pollen and fungal spores 104

Fig. 4.13: Mean values of mice Eosinophil elicited by protein of pollen and fungal spores 105

Fig. 4.14: Mean values of mice Monocyte elicited by protein of pollen and fungal spores 107

Fig. 4.15: Mean values of mice Neutrophil elicited by protein of pollen and fungal spores 108

LIST OF PLATES

		Pages
Plate 3.1:	Modified Tauber-like pollen trap in the field	41
Plate 4.1:	Photomicrographs of some representative pollen	89
Plate 4.2:	Photomicrographs of some representative fungi spores and charred Poaceae epidermis	90
Plate 4.3:	Physical manifestation of allergy provoked by pollen protein of *Oreodoxa oleracea* in mice	95
Plate 4.4:	Photomicrographs of respiratory organ of mice showing allergic symptoms	96
Plate 4.5:	Immune cell types predominant in allergic mice	109

ABSTRACT

The socioeconomic burden of pollen and fungi spores allergy are very high in terms of hospitalization, treatment, lethargy, poor concentration and behavioral changes which impact negatively on adults and children. The study of atmospheric pollen and fungal spores in the six geopolitical zones of Nigeria was carried out from June 2011 to May 2012. The aims of the study are to determine the seasonal prevalence of atmospheric pollen and fungal spores, examine the impacts of weather parameters on them and assess their allergenic potentials in mice. Airborne pollen and fungal spores were trapped with Tauber-like pollen traps and subjected to acetolysis, temporary slides were for microscopical examination and identification. Mature anthers which have not undergone anthesis were procured and vaccum dried at 35 ^{o}C. Both anthers and cultured fungal spores were subjected to protein extraction using 100 ml of 0.02 M phosphate buffered saline at pH 7.4. The protein content was assayed by Bradford procedures and the extracted proteins were inoculated into mice weekly by subcutaneous and intranasal injections. Blood samples were obtained by retro-orbital bleedings for sera and used for analysis of mice immunoglobulin E using enzyme linked immunoperoxidase assay. Mice were sacrificed at the end of the 5th week, the trachea, bronchi, bronchioles and lungs were subjected to histological staining and examination. Results from this work revealed seasonal variations of atmospheric pollen and fungal spores contents in the six geopolitical zones. They are rainy season, two major seasons of pollen and fungal spores abundance were noted in Southern and North Central zones; rainy seasons were characterized by higher fungal load and late rainy/harmattan season characterized by higher airborne pollen load. The North West and North East zones has one defined season of more allergenic exposure in terms of floral components. Poaceae pollen dominated North West and North East Nigeria during the rainy season and was the most dominant anemophilous pollen recorded in the atmosphere of Nigeria. While there was a clear abundance of entomophilous pollen in Southern zones, anemophilous pollen prevailed over entomophilous in Northern zones. Spores were more abundant in the South than the North. There was a positive correlation between the atmospheric aeroallergens and weather parameter. The pollen grains of anemophilous plants such as Poaceae, *Elaeis guineensis, Casuarina equisetifolia*, Cyperaceae, known to trigger allergic rhinitis were commonly recorded from the atmosphere of the six studied geopolitical zones. Immunological results using mice revealed an increase in immunoglobulin E levels and infiltration of lymphocytes. Pollen protein of *Oreodoxa oleracea* induced both intrinsic and extrinsic allergenic reactions which manifested dermatophytic and inflammatory reactions on the skin and within the lung parenchyma respectively. Spore protein of *Aspergillus niger*, pollen protein of *Mariscus ligularis* and *Terminalia catappa* elicited hypertrophy of the mucous gland, over pseudostratification of tracheal epithelium and inflammation within the lung parenchyma respectively. A direct relationship as recorded between allergen protein and immunoglobulin E elicited in some mice. *Oreodoxa oleracea* pollen protein skewed basophil production, which promoted inflammatory reactions in mice. The results indicated that the production index of pollen and fungi spores are highly sensitive to weather parameters, it also established that pollen and spores protein orchestrate immunological stimulation leading to lymphocyte infiltration and proliferation of other immune cells.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The transfer of pollen from anther to recipient stigma is the critical reproductive event among higher plants which leads to their dispersal into the atmosphere by various mechanisms. The atmosphere is laden with many kinds of suspended particles of organic and inorganic origin having great diversity in size, shape, and density and from diverse sources (Essien and Agwu, 2013). Aerobiological investigations have revealed that pollen and fungal spores are the most dominant, pervasive, respirable and potent sources of allergen present in the atmosphere. Pollen and spores are also more ubiquitous and widely distributed in time and space than any other representatives of living matter (Shahali *et al*., 2007). Their atmospheric concentration are frequently high enough to present a substantial antigenic load to exposed hypersensitive individuals (Horner *et al*., 2000). Their abundance depend generally on the degree of turbulence of the air, time of the day, season, local sources and climatic factors such as temperature, humidity, rainfall, wind direction and strength (Laycer, 2007). Airborne pollen and fungi spores are ubiquitous in indoors and outdoors environment due to their sizes, predominance in nature and aerodynamic properties which enhance their distribution (Bryce, 1995; Mesa, *et al.*, 2003). Knowledge of the kind and type of pollen or spores in the air has medical implications (Essien and Agwu, 2013).

Fungi grow almost everywhere, even as lichens inside Antarctic rocks (Horner *et al*., 2000). They grow over a wide temperature range (-5 to 50 ^{0}C and greater) (Gravesen, 1979), although individual species usually grow within a much narrower range. One of the most important physical parameters affecting fungal growth is moisture. Airborne fungal spores are usually

present in outdoor air throughout the year in high numbers and frequently exceed pollen concentrations (Horner *et al*., 2000), depending on environmental factors such as water and nutrient availability, temperature, and wind. Most fungi commonly considered allergenic, such as *Alternaria* spp., *Cladosporium* spp., *Epicoccum nigrum, Fusarium* spp. and *Ganoderma* spp., display a seasonal spore release pattern, but this is less well defined than it is for pollen (Horner *et al*., 2000). Aerobiologic surveys show that fungal spores are present in the atmosphere worldwide, nearly in all environments. They are universal atmospheric components indoors and outdoors and are now generally recognized as important causes of respiratory allergies. Allergic reactions associated with fungi involve the lower respiratory tract more frequently than do pollen allergies (Horner *et al*., 2000). More than 80 genera of fungi have been associated with symptoms of respiratory tract allergy (Horner *et al*., 2000), however multiple species may be observed at any time of the year. Fungi reproduce by releasing spores into the air, their inhalation is through respiration of dust particles contaminated with fungal spores. The ubiquitous presence of fungi in both indoors and outdoors environment is a potential health threat that is poorly understood and almost ignored in community medicine. Airborne fungal spores exposure frequently cause adverse human health effect with injury and dysfunction of multiple organs and systems including; respiratory, nervous, immune, haematological system and skin (Kothari *et al.,* 1993). Exposure to some fungal spores can also trigger infectious diseases for immune compromised persons. Despite the clinical importance and abundant release of fungal spores, relatively few investigations have focused on the relationship between airborne spores and allergic diseases (Kothari *et al.,* 1993).

The term allergy was coined in 1906 by von Pirquet to describe an altered reactivity in living beings (i.e., an IgE mediated hypersensitivity) caused by a foreign substance (Horner *et al*., 2000). Allergens, therefore, are the subset of antigens that stimulate an IgE-mediated response. Genetic factors are known to influence the ability to IgE-mediated reaction and those individuals with sustained elevated IgE levels are referred to as atopic. Type I allergic disease of fungal spores and pollen allergens typically manifests as rhinitis (hay fever), conjunctivitis, dermatitis, asthma etc. Allergic reactions, including respiratory allergy, may occur in two phases. The early-phase reaction occurs within minutes as a result of the release of preformed mediators. Late-phase responses occur 3 to 4 h after allergen exposure as a result of cellular infiltrates responding to early-phase mediators. A dual reaction involves both early- and late-phase reactions. Emerging evidence indicates that a significant, persistent inflammatory component in addition to IgE-triggered effects underlies the etiology of asthma.

Pollen and fungal spores allergen belong to type one hypersensitivities. Their proteins are immunomodulatory substances, which play crucial roles in the sensitization and/or exacerbation of allergies such as seasonal rhinitis, conjunctivitis, asthma, bronchial constriction and obstruction, pollinosis and atopic dermatitis (Phanichyakarn *et al*., 1989; D'Amato *et al*., 2002; Kamijo *et al.*, 2013). On an immunological response to allergen, the immune system makes immunoglobulin E antibodies which attach to immune cells. These undergo degranulation and trigger the cells to release histamine and other inflammatory chemical mediators such as cytokines, interleukins and prostaglandins. These chemical mediators elicit symptoms of allergy including wheezing, runny nose, itching, rashes, sneezing etc. These symptoms range from mild to life threatening symptoms and could either be localized or systemic (Singh and Kumar, 2004).

Allergic reactions from pollen or spores normally occur at the site of allergen deposition. Most inhaled particles greater than 10 µm are deposited in the nasopharynx and are associated with nasal and or ocular symptoms, reffered to as hayfever (Horner *et al*., 2000). Conversely particles less than 10 µm especially those of 5 µm can penetrate the lower airways. Pollen and fungal spores differ in sizes and are associated with both upper and lower respiratory systems (Horner *et al*., 2000). Additionally, there is now evidence that secondary dispersal of allergens, i.e., on other, smaller particles, possibly spore fragments, may serve as a vehicle for allergens. This would permit the deposition of allergens even from large spores into the lower airways (Horner *et al*., 2000).

World-wide, it is estimated that 300 million people are affected with bronchial asthma (Masoli *et al*., 2004). The prevalence of asthma is variable. It is a disease that has been observed to be more prevalent in developed countries with higher rates seen in Australia, UK, and New Zealand (Masoli *et al*., 2004). In Nigeria, the prevalence of asthma ranges from 7% to 18% in the general population. Sex ratio varies according to age (Masoli *et al*., 2004). In childhood, asthma affects more boys than girls for unknown reasons, but by the third decade, the prevalence becomes equal and subsequently, more women than men are affected. (Masoli *et al*., 2004; Ibe and Ele, 2002) Asthma prevalence is increasing despite recent advances being made in its management (Oni *et al*., 2010)

Pollen and fungal spores allergy are more difficult to diagnose and treat than other allergies because they are far more numerous and antigenically variable than other allergies and are exceedingly difficult to avoid (Garijo, 1996). The knowledge of qualitative and quantitative prevalance and their seasonal and annual variations are of paramount importance in effective

diagnosis and management of pollen related allergens (Singh and Kumar, 2004; D'Amato *et al*., 2007).

Garijo (1996) remarked that risk factors for allergy could be placed in two general categories, namely host and environmental factors. Host factors include heredity, gender, race and age, the most significant being the host factors. Allergic diseases are strongly familial, identical twins are likely to have the same allergic diseases about 70 % of the time while the same allergy occurs about 40 % of the time in non-identical twins (Tamari *et al*., 2013). Allergic parents are more likely to have allergic children and their allergies are more severe than those from non-allergic parents. Some allergies, however, are not consistent along genealogies. Allergic sensitization is inherited and related to an irregularity in the immune system (Docampo *et al*., 2007). However, there have been recent increase in the incidence of allergic disorders that cannot be explained by genetic factors alone. Four major environmental risk factors are recognized. They are : alterations in exposure to infectious diseases during early childhood, exposure to allergen load, environmental pollution, and dietary changes.

The risk of allergic sensitization and the development of allergies vary with age, with young children mostly at risk (Onyedum *et al*., 2013). Several studies have shown that immunoglobulin E (IgE) levels are highest in childhood and fall rapidly between the ages of 10 and 30 years. The peak prevalence of hay fever is highest in children and young adults and the incidence of asthma is highest in children under 10. Aderele (1979) reported that boys have a higher risk of developing allergy than girls. Although for some diseases, such as asthma in young adults, females are more likely to be affected with sex differences tending to decrease in adulthood. Ethnicity may also play a role in some allergies; however, racial factors have been

difficult to separate from environmental influences and changes due to migration. It has been suggested that different genetic loci are responsible for asthma, to be specific, in people of European, Hispanic, Asian, and African origins (Aderele, 1979).

International differences have been associated with the number of individuals within a population that suffer from allergy. Allergic diseases are more common in industrialized countries than in countries that are more traditional or agricultural, and there is a higher rate of allergic disease in urban populations than rural populations, although these differences are becoming less defined (Onyedum *et al*., 2013).

Airborne fungal spore concentration is however an indicator of pathogen development in agriculture and could be useful when infection levels are initially determined by source of inoculums rather than the weather. Monitoring of airborne inoculums integrated with meteorological data provides a valuable tool for establishing the basis for an accurate modern integrated pest –management strategy (Kasprzyk , 2004; Escuredo *et al*., 2010). The awareness of the potentially allergenic pollen and fungal spores counts and its changes throughout the period of release in a given area is of great importance in prophylaxis of allergic respiratory diseases which has become a social problem in all continents. Pollen and fungal spore counts are estimates of the antigenic challenge confronting allergic individuals. The nature of this challenge depends on the particular pollen and fungal spores types found in the atmosphere and also their airborne concentrations (Laycer, 2007). Horner *et al*. (2000) stated that aerobiology of pollen and fungal spores is of direct relevance to the medical community due to their implication in precipitating respiratory diseases (pollinosis), conjunctivitis, rhinoconjuctivitis, eczema /dermatitis and asthmatic attacks.

Oni *et al*. (2010) asserted that elicitation of allergic disease is multifactorial and dependent on the interaction between genetic predisposition and environmental factors, characterized by dysregulation of immunity. This dysregulation leads to a strongly polarized T helper type 2 (Th2) immune response and a chronic inflammation in the airways in response to innocuous antigens. In asthmatic patients, the penetration of allergens into the lungs leads to airway inflammation consisting of a peribronchial infiltration of $CD4^+$ T cells, macrophages, eosinophils and neutrophils and the presence of these cells in Bronchoalveolar Lavage Fluid (BALF). Asthmatic patients also present a goblet cell metaplasia/hyperplasia and characteristic modifications of the airway wall including epithelial hyperplasia, thickening of the basement membrane, subepithelial fibrosis, increased airway smooth muscle mass and, finally, airway hyper reactivity (AHR) to specific and non-specific stimuli (Oni *et al*., 2010).

The burden of these allergies to health care systems, families and patients increases worldwide (Onyedum *et al*., 2013). Despite the ubiquitous nature of pollen and spores, their great aerodynamic properties and implications in allergic reactions, virtually no previous attention has been given to survey their pattern of spatial distribution in atmosphere of different geopolitical zones of Nigeria, as documented in this work, though the South East and South West have been worked upon.

Some Aeropalynological studies in South West Nigeria include; Aeropalynological studies of the University of Lagos Campus, Nigeria was carried out by Adekanmbi and Ogundipe (2010). The result of their study reveals that palynomorphs abundance and diversity achieved its peak in May which corresponds to the wettest month of the sampled period. Their work did not extend to the two seasons in Nigeria. Adeonipekun and John (2011) carried out a research on

investigation of haze dust in Ayetoro-Itele Ota, South West Nigeria. They showed that the hazy dust studied contain high proportion of palynomorphs for Sudan/Guinea and derived Savanna as well as lowland rainforest ecozones. Adeonipekun and Olowokudejo (2012), verified the pollen rain in offshore location where there is no vegetation, the study confirmed pollen rain as the important contributor to marine sediments in Niger Delta. Adeniyi *et al*., 2014 studied the pollen records of Shomolu Local Government Area of Lagos State. Their study revealed that the most dangerous period for inhabitants of Shomolu occurred between October and September when the highest level of grass and herbaceous pollen grains were recorded. They also found that total pollen concentration correlates positively with the temperature and negatively with wind, rainfall and relative humidity. In their work the allergenicity of the pollen were not tested in any animal model. In South East, Agwu *et al*. (2004) carried out a study on airborne pollen and spores circulating at head level in Nsukka environment. The frequent and abundant pollen types they found include those of Poaceae(grasses), *Elaeis guineensis*, *Casuarina equisetifolia*, *Alchornea cordifolia*, *Milicia excelsa* and Amarathaceae / Chenopodiaceae. Their work however did not cover the two seasons in Nigeria. Njokuocha (2006) studied airborne pollen grains in Nsukka, Nigeria. He recorded the following predominant pollen and fern spores; *Casuarina equisetifolia*, *Milicia excelsa*, *Elaeis guineensis*, *Celtis integrifolia*, *Alchornea cordifolia*, Amaranthaceae / Chenopodiaceae, Combretaceae / Melastomataceae, *Nephrolepis biserrata*, *Thelypteris totta*, and *Dryopteris* spp. Njokuocha (2006) did not carry out the allegenicity study of pollen identified from his study area.

The present work is more broader than earlier studies carried out in Nigeria, it cut across different geo-political zones and vegetation types. Result from this work will form the basis for future works towards creating pollen calendars for forecasting of allergic incidences as well as

providing the basics for management approach to respiratory allergy. Pollen and fungi spores allergenicity of Nigerian plants has not been previously documented to the best of the researcher knowledge. The present work is the first to test the allergenicity of pollen and fungal spores on mice in Nigeria.

1.2 Statement of the Problem

Pollen and fungal spores (collectively referred to as 'aeroallergens') exacerbate allergies e.g. hay fever/allergic rhinitis and other asthmatic conditions, affecting hundreds of millions of people worldwide (Bousquet *et al*., 2011). In the United Kingdom, approximately 20% of the population suffer from hay fever and approximately 95 % of hay fever sufferers are allergic to grass pollen and 25% to tree pollen. There are approximately 150 species of grass in the UK, 12 of which are important with respect to atmospheric pollen load (Emberlin *et al*., 1999).

The increasing prevalence of allergic diseases over past decade is well established and accepted by most health authorities including the world Health Organization (WHO, 2003). The evidence from patients and stakeholders agreed that access to diagnosis, treatment, education, information and continued research should be a priority for health authorities and educationist. However, the absence of large scale epidemiological studies on prevalence has prevented most authorities from understanding the extent and taking the steps necessary to commission the requisite services (WHO, 2003).

Allergic diseases require expensive short term treatment. The real public health cost is the drain on resources over a prolonged period, as lives of the sufferer, may be impaired for several decades. To fully evaluate the socio-economic costs of allergic diseases, it is important to consider the direct costs of hospitalization, physical consultations and treatments and the indirect cost of days lost in work and education (D'Amato *et.al*., 2007). Also patients are

restricted in their social and physical activities, so their productivity and professional life will be affected. Lethargy, poor concentration and behavioral changes may arise as a result of persistent symptoms and poor sleep which can impact negatively on adults and children (D'Amato *et al*., 2007). When symptoms are severe and effective treatment is not available to patients, work days may be compromised and even lost.

Asthma is one of the most chronic allergic diseases which is estimated to affect as many as 300 million people worldwide and could increase by another 100 million by the year 2025(WHO, 2003). Exposure to allergens represents a key factor among the environmental determinants of asthma (D'Amato *et al*., 2007). The prevalence and incidence for asthma throughout Africa had increased remarkably in recent years, the condition was previously uncommon over most parts of the continent (WHO, 2003). Fifty years ago asthma was uncommon in Nigeria, however recent reports from different parts of Nigeria have shown a prevalence of adolescent and adult asthma in excess of 10 % and a rising trend in the prevalence of asthma (Desalu *et al*., 2013). The increase in burden the asthma has been attributed to environmental factors such as urbanization, industrialization and adoption western life style (Desalu *et al*., 2013). ISAAC study conducted among children in Nigeria, the prevalence of asthma was 18.4 % (Desalu *et al.*, 2011). In a hospital-based study in Southern region of Nigeria, the prevalence of asthma was 6.6 % among patients attending a respiratory clinic. Today, more than 30 % of the population is known to suffer from one or the other allergic ailment, bronchial asthma, allergic rhinitis while atopic dermatitis are dramatically increasing all over the world including developing countries (Singh and Kumar, 2004). The risk factors for asthma identified in Nigeria were family history of asthma, allergic rhinitis, outdoor and indoor allergens, tobacco smoking and obesity (Desalu *et al.*, 2011).

Many Nigerians suffer from other allergic diseases related to airborne pollen and spores. In the last 20-30 years, the prevalence of allergic diseases has escalated significantly, a trend that shows no signs of abating and the urban dwellers are mainly affected. Erhabor *et al*. (2002), remarked that asthma and other allergic diseases had not only become a national problem but international. According to him, the Nigerian government was not doing enough in curbing its spread, diagnosis and treatment, stressing that past administrations in the country only concerned themselves with eradication of communicable diseases while neglecting non-communicable diseases.

1.3 Objectives of the Study

Overall Aim

The overall aim of this study was to determine the seasonal prevalence and spatial distribution of airborne pollen and fungal spores in Nigeria and assess their allergenic potentials in mice.

The specific objectives were to;

1. Determine the seasonal prevalence of airborne pollen and fungal spores in the atmosphere of Nigeria and impacts of weather parameters on them.
2. Determine the concentration of protein in some selected pollen and fungal spores.
3. Establish the clinical features of allergy provoked by some selected pollen and fungal spores in mice.
4. Evaluate the immunoglobulin E antibodies and immune cells elicited by some selected pollen and fungal spores in mice

1.4 Significance of the Study

This study will lead to the development of pollen and fungal spores calendar which will display the flowering periods of plants and seasonal occurences of fungal spores in each studied locations in geopolitical zones. This will enable forecasting/predicting of their future occurrences and serve as a preliminary approach to the problems of allergic diseases caused by pollen and fungal spores. The recognition of recovered pollen and fungal spores allergenic potential will add to the knowledge about other identified allergies; such as drug allergy, food allergy, chemical sensitivity e.t.c., among which pollen and fungal spores have received little attention in Nigeria. The establishment of their clinical importance will aid in the diagnosis and treatment of allergenic conditions due to pollen and fungal spores.

1.5 Operational Definition of Terms

a. **Allergen** = is a substance that binds specifically to the antibody/substance that stimulate an immune response
b. **Anthesis** = is a period during which a flower is fully open and functional
c. **Antigen** = A substance that binds specifically to the antibody
d. **Charred Poaceae cuticles**= burnt epidermis of grasses
e. **Histamine** = Chemical found in some body cells which causes symptoms of allergy
f. **Intranasal** = Within the nose
g. **Pollen** = is a fine to coarse powder containing the microgametophyte of seed plant
h. **Spore** = A unit of asexual reproduction that may be adapted for dispersal and for survival
i. **Subcutaneous** = Beneath the skin
j. **Immunosobent Assay** = is a test that uses antibodies and colour change to identify a substance

k. **Sera** = is a clear pale yellow liquid that separate from the clot in the coagulation of blood.

l. **Immunoglobulin E** = Is an antibody that plays an essential role in type one hypersensitivities which manifests various allergic diseases.

m. **Antibody** = is a protein produced by the body's immune system when it detects harmful substances called antigen.

n. **SDS-PAGE =** Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis.

o. **ISSAC =** International Study of Asthma and Allergies in Childhood

p. **Wheeze ever =** Wheezing from birth.

q. **Current wheeze =** Wheezing at present.

1.6 ABBREVIATIONS

APC – Airborne pollen concentration

h- hours

Mins-minutes

CHAPTER TWO
LITERATURE REVIEW

2.1 Aeropalynology

The term palynology was coined by Hyde and Williams (1944). The term was originally defined as the study of pollen and other plant spores and their dispersal and application thereof. In a wider and a more contemporary sense, Palynology comprises the study of pollen and other plant – like microfossils. Aeropalynology is a branch of Palynology that studies pollen grains and spores that are dispersed into the atmosphere. According to Gregory (1992), aerobiology is usually understood to be the study of passively airborne microorganisms, their identity, behaviour, movements and survival. This field of science includes: identification, morphology, physiology, viability, longevity, sampling, concentrations, diurnal and seasonal patterns, phenology, emission, transport, dispersion, pollination, pollinosis and a host of other subjects. Aerobiology involves study of airborne particles of plant and animal origin. Some aerobiologists segregate the study of airborne pollen into a branch of aerobiology termed as 'aeropalynology. Pollen is nature's gift to mankind as it is responsible for pollination, fertilization, seed and fruit setting and multiplication of plants. However, some of the pollen after getting into air stream remain floating in the atmosphere before settling down on the ground. Some of these pollen on coming in contact with human beings induce allergic manifestations.

The primary objectives of aerobiological studies are to monitor, determine and detect the occurrence of pollen and spores and their relative representation in the atmosphere. On account of the tremendous applications of aeropalynology in public health and medicines, a new term has been added recently known as 'Medical Palynology'. This branch is concerned with the

study of airborne pollen and fungal spores, which are responsible for causing allergic manifestations including the triggering effect leading to asthmatic attacks. In addition, various aspects of immunotherapy are investigated involving hyposensitization of allergy patients by using pollen and fungal aeroallergen extracts. According to a recent trend, the scope of aerobiology has been widened to incorporate different kinds of biological particles (air spora) for example, viruses, bacteria, microalgae, microfungi, lichen fragments, soredia, seeds, protozoan cysts, insects and insect parts, spiders (Gregory 1992). Abiotic particles or gases affecting living organisms are also included currently in the concept of aerobiology. Thus, the aerobiological pathway involves at least five major steps, which are: source, liberation, passive transport, deposition and impact on vegetation, water bodies and various substrates. It is obvious that these different steps are intertwined and they are affected by environmental factors, such as, meteorology, physics and atmospheric chemistry (Gregory, 1992). Aerobiology has become an interdisciplinary science of great significance and applications in different fields, such as, ecology, medicine, pathology, agriculture, forestry and meteorology. There are various ways of dispersal of pollen in the atmosphere, however, the most important factor is wind which transmits pollen grains and spores from their sources to the target areas. Hence, windborne pollen both of flowering plants or the angiosperms and gymnospermous plants are significant in aerobiological studies.

Agashe and Alfadil (1989) carried out the aeropalynological survey of the atmosphere at Bangalore for six years. This survey was conducted by trapping airborne bioparticles such as pollen and fungal spores by operating vertical cylinder pollen traps. The traps were installed at different ecogeographical sites in Bangalore City. The results of qualitative and quantitative analysis of the atmospheric biopollutants were correlated with meteorological parameters such

as temperature, relative humidity, wind speed and cloud cover. It was shown that generally high temperature and low relative humidity enhanced the liberation and distribution of pollen in the atmosphere; whereas high humidity and low temperatures triggered the liberation and distribution of fungal spores in the atmosphere. Atmospheric pollen count was drastically reduced during the rainfall.

Teranishi *et al*. (2000) conducted an atmospheric pollen survey using a Durham sampler from 1983 through 1998 in Toyama City, Japan. They investigated yearly changes in the pollen season of Japanese cedar *Cryptomeria japonica* and analyzed the relationships between climatic factors and changes in the pollen counts. This revealed that the first day of the Japanese cedar pollen season advanced from mid-March to late February and the yearly change in the first day was significantly associated with the mean temperature in February. Again increase in total pollen count was significantly associated with the mean temperature in the previous July and the duration of the pollen season was suggested to be associated with the total pollen count. These results further indicated that climate change, especially increasing global warming, influences the early pollen scatter and increase in pollen count as well as elongation of pollen season of Japanese cedar.

The effect of temperature, relative humidity and rainfall on airborne *Ambrosia artemisifolia* pollen concentrations was examined by Barnes *et al*. (2000). During the ragweed (RW) season for the years 1997 and 1998. The ragweed season for this region begins in mid-August and ends by mid-October. Temperature patterns for the period demonstrated usual daily fluctuations with highs 13 ^{o}C to 35 ^{o}C and lows 8 ^{o}C to 24 ^{o}C. Relative humidity readings for the period varied between 25 and 100 %. Highest RW values were seen after seasonal cooling in September.

Daily rainfall for the period varied between 0 and 100 mm. Airborne RW always declined sharply after strong rainfall events (> 10 mm/day). Peak airborne RW concentrations were often associated with the passing of frontal boundaries and the change in wind direction and velocity that accompanies that passing. Factors influencing airborne RW concentrations were multiple and complex, but atmospheric forces had greater influence. The passing of major weather fronts and the associated temperature drops, wind disturbances and rainfall are the major factors.

The variation in airborne pollen concentration of the Braga region (Portugal) was studied by Riberio *et al*. (2003) in spring time, during the flowering of *Vitis vinifera*. Recorded data set was obtained for two consecutive years (1999 and 2000), using a Cour-type sampler. During this period, 36 taxa were observed in a total of 3,200 pollen grains m^{-3} of air (APC). The main pollen types observed were *Olea*, Poaceae, and *Castanea* sp., representing 74 % of the pollen spectrum. The airborne pollen concentration (APC) was significantly correlated with certain meteorological parameters. Pollen concentration was positively correlated with temperature and wind direction (East and Northeast) and negatively correlated with rainfall and number of rainy days.

Pollen flow of cultivated rice measured under experimental conditions was studied by Zhiping *et al*. (2004). The pollen flow pattern of a cultivated rice variety, Minghui-63, was studied at horizontal and vertical levels under experimental conditions. Data obtained from pollen traps for six designed populations (as pollen sources) at different intervals showed that the dispersal of rice pollen decreased with the increase of distance from pollen sources and that the rice pollen flow was significantly influenced by weather conditions, particularly by wind direction and speed. For a mean wind speed of 2.52 m/s in a downwind direction, the observed distance

of rice pollen dispersal was 38.4 m, indicating that rice pollen grains normally disperse at a relatively small range. However, the maximum distance of rice pollen flow could be up to 110 m, using regression analysis of pollen flow and wind speed, when the wind speed reached 10 m/ s in this study. The frequency of pollen flow was positively correlated with pollen source size within a given range, suggesting that pollen flow will occur effectively at a considerable rate in rice fields with sufficiently large pollen sources. In addition, many more pollen grains were detected at the height of 1.0–1.5 m than at 2.0 m, indicating that rice pollen mainly disperses at relatively low heights. Results from this study were useful both for minimizing transgene escape from transgenic rice and *in situ* conservation of wild relatives of rice, as well as for hybrid seed production, where an effective isolation buffer zone needed to be established.

The terms and major criteria used to define and limit pollen season were reviewed by Jato *et al*. (2006). Pollen data from Cordoba (Spain), Ourense (Spain) and Bologna (Italy) were used to ascertain the extent to which aerobiological results and pollen curves were modified by the criteria selected. Results were analysed using Spearmans correlation test. Phenological observations were also used to determine synchronization between pollen curves and plant phenology. The criteria for limiting the shortest and longest pollen season periods, as well as the earliest and latest start and end dates, varied according to the city and the taxon under study; in many cases, results for a given taxon also depended on the year. The smallest differences were obtained for *Platanus* and the greatest for Poaceae.

Malgorzatar (2007) determined the effect of meteorological conditions on hazel (*Corylus* spp.) and alder (*Alnus* spp.) pollen concentration in the air of Szczecin. The aim of the study was to determine seasonal variations in concentrations of hazel and alder pollen count due to

meteorological parameters. Measurements were performed using the volumetric method. The analysed meteorological parameters were the maximum temperature, relative humidity, rainfall and wind speed. The beginning and end of a season were established by the 95 % method. During seven years of study, the highest concentration of hazel pollen in the air was noted in 2003 (the total number was two - three times higher than in the other years), with the pollen season starting in most years in the beginning of January and lasting till the end of March or beginning of April. The highest concentration of alder pollen in the air was noted in 2003, similarly as hazel pollen. The pollen season started in the beginning of January (in 2003 and 2006 in the beginning of March) and lasted till the turn of the March and April. The highest pollen count of 674 grains/m^3 was observed in the end of March. A positive and statistically significant correlation (Pearson's coefficient and multiple regression) was found between the hazel and alder pollen concentration and air temperature and wind speed. A negative correlation was found in case of the relative humidity. A lot of analysed correlations were significant (significance level of P=0.05), although the percentage of explained variation (R2) was very low. Besides the individual rhythm of pollination, the meteorological conditions were the most important factors (mainly air temperature and wind speed) influencing the analysed pollen concentration in the air.

An aeropalynological study was carried out in the atmosphere of the city of Nerja (Southern Spain) by Docampo *et al*. (2007), during a period of 4 years (2000–2003), using a Hirst type volumetric pollen trap. An annual pollen index of 59,750 grains, on average, was obtained with 80–85 % of the total pollen recorded from February to May, with *Pinus*, *Olea*, Urticaceae, Cupressaceae, *Quercus* and Poaceae being the principal pollen producers in abundant order. A total of 29 pollen types that reached a 10-day mean, equal to or greater than 1 grain of pollen

per m^3 of air was reflected in a pollen calendar. The results were compared with those obtained for nearby localities and a correlation analysis was made between the daily fluctuations of the main pollen types and total pollen, and meteorological parameters (temperature, rainfall and hours of sun). The daily, monthly and annual values reached by the most important pollen types from an allergenic point of view (*Olea*, Urticaceae and Poaceae) confirms Nerja as a high-risk locality for the residents and the numerous tourists who visit the area.

David, 2009 carried out the effects of meteorological factors on airborne bracken (*Pteridium aquilinum* (L.) Kuhn.) spores in Salamanca (middle-west Spain) were carried out by. Temporal variation of airborne *Pteridium quilinum* spores concentration in Salamanca during 10 years from January 1998 to December 2007 were studied using a Burkard spore trap, and correlations with some meteorological parameters were analyzed. The number of spores that were counted was very low, due probably to the distance between the spore trap and the main bracken populations which were located 70 km away from the city. Long-range transport caused by winds coming from the Second Quadrant (IIQ) was supposed to be responsible for the appearance of bracken spores in Salamanca. The period from August to late October showed the most intense spore dispersal process, with an early morning distribution along the day. Years 2002 and 2007 with a low quantity of airborne spores were also characterized by low mean temperatures, always under 18 °C from May to June. Daily spore concentration shows positive correlation with temperature and sun hours but negative with fourth Quadrant (IVQ) winds and with relative humidity. No correlation between daily spore concentration and rainfall was found. Also, a positive correlation between number of spores and IIQ winds was observed during the main spore season (MSS) and prepeak period (PRE)

Gonzalez *et al*. (2010) carried out a work on atmospheric pollen count in Monterrey, Mexico. The study was designed to describe the prevalence of pollen in the city of Monterrey, Mexico, during the year 2004. Atmospheric pollen was collected with a Hirst air sampler, with an airflow of 10 L/min during the year 2004. Pollen was identified with light microscope; the average monthly pollen count as well as total was calculated from January 2004 to January 2005. The months with the highest concentration of pollen were February and March (289) and (142) grains/m^3 per day, respectively and July and November had the lowest concentration (20 and 11 grains/m^3 per day, respectively). Most of the pollen recorded corresponded to tree pollen (72 %). *Fraxinus* spp. had the highest concentration during the year (19 grains/m^3 per day; 27.5 % of the total concentration of pollen). Tree pollen predominated from January through March; with *Fraxinus* spp., *Morus* spp., *Celtis* spp., *Cupressus* spp., and *Pinus* spp. as the most important. Weed pollen predominated in May, June, and December and the most frequently identified, were Amaranthaceae/Chenopodiaceae, *Ambrosia* spp., and *Parietaria* spp. The highest concentration of grass pollen was reported during the months of May, June, September, October, and December with Gramineae/Poaceae predominating. Tree pollen was the most abundant during the year, with the *Fraxinus americana* having the highest concentration. Weed and grass pollen were perennial with peaks during the year.

The effect of recent climatic trends on Urticaceae pollination in two bioclimatically different areas in the Iberian Peninsula: Malaga and Vigo was carried out by Recio et *al*. (2009). The study covers the period 1991–2006 for Malaga and 1995–2005 for Vigo, and compares the differences in climate and phenological behaviour observed at both localities. The sampling of atmospheric pollen was performed with Hirst volumetric pollen traps. The two localities presented different tendencies as far as temperature was concerned, while the mean annual

temperature in the Mediterranean region had increased by 0.06 ^{o}C/year, the same parameter had decreased in the Atlantic area by 0.1 ^{o}C/year. This tendency was even more pronounced as far as the minimum temperatures were concerned, especially during spring in Malaga and autumn in Vigo. On the other hand, wind speed had tended to increase, periods of calm had diminished and winds blowing off the sea had increased in both places. These changes in meteorological parameters have advanced the end of the pollen season in Malaga and delayed its start in Vigo. Total annual pollen counts decreased in Vigo, while the number of pollen-free days had increased in both areas.

General trends in airborne pollen production and pollination periods at a Mediterranean Site (Badajoz, Southwest Spain) were examined by Molina *et al*. (2010). The aim of the study was to determine trends in the airborne pollen concentration and pollination period for the principal sources of pollen in Badajoz (Southwest Spain) over 15 years of monitoring (1994-2008). Airborne pollen was monitored by continuous sampling with a Hirst volumetric sampler. Pollen trends were investigated by linear regression and correlation analysis using mean annual and monthly pollen concentrations. The aerobiological results were compared with meteorological data (temperature and rainfall). During the study period, the mean total annual rainfall was 66.2 mm lower than normal and the mean annual temperature 0.8 °C higher than normal. No temporal trend was found for total airborne pollen concentration, but differences were observed for monthly data, namely, an increase in January, February, and May and a decrease in March and June. For the different pollen types studied, there was a general trend toward increased values in the month with the highest values, and this trend seemed to be related to temperature. The beginning of the main pollen season occurred later, and the end occurred sooner; therefore, the main pollen season seemed to be shorter. The result reflected trends in the response of

plants to changing rainfall stress patterns in Mediterranean countries, and these trends seem to be different from those of temperate countries.

Gonzalez *et al*. (2010) surveyed the incidence of fungals in a vineyard of origin Ribeiro (Ourence-North-western Spain). The study was carried out in a vineyard of the Ribeiro district during the year 2007. Their result showed that *Botrytis* reached the highest annual total value of spore production with 16,145 spores, followed by *Plasmopara* with 747 spores and *Uncinula* with 578 spores. In order to forecast the concentration of the phytopathogenic fungal spores, equations of lineal regression were elaborated including as estimators, variables with high correlation coefficient. For *Botrytis* the regression equation revealed 42.4 % of the variability of spore concentration, 26.1 % for *Uncinula* and 24.7 % for *Plasmopara*.

2.2 Allergy Survey

Oni *et al*. (2010), examined the prevalence, management and burden of asthma in Nigeria. The study was a cross sectional design involving clinical and lung function assessment. The diagnosis of asthma was made using the clinical features of asthma and lung function parameters (Forced expiratory volume in one second, Peak expiratory flow rate, Reversibility tests). In total, 120 asthma patients participated in the study. All subjects completed the clinical asthma control questionnaires. All items were rated with the calculation of their mean and percentages. Student t-test was used to calculate the difference between the mean of the lung function tests for subjects and control. The prevalence of asthma among respiratory unit patients was 6.6 % and higher in the first three decades of life with female preponderance (F:M=1.5-1).There was a strong family history of asthma (81.7 %). Associated allergies include rhinitis (75 %), pharyngitis (54 %), conjunctivitis (54 %) and dermatitis (30 %). Percentage of asthma

patients treated with bronchodilators alone (70 %), combined inhaled bronchodilators and steroid (28.3 %). Impaired daily activities include sports (84 %), job career (60 %), Physical activity (55 %), Social activity (54 %), household chores (61 %) and disturbed sleep (53 %). Subjects had significant low lung function values when compared with control ($P < 0.05$). The burden of asthma was very high despite the advanced knowledge of its pathophysiology and management of asthma.

Aderele, (1979) carried out the clinical and laboratory studies on bronchial asthma in 200 Nigerian children who were seen during a 2000 year period in Ibadan. He found, contrary to reports, that the condition was rare in African children, that after pulmonary tuberculosis, asthma was the next most common chronic chest disease in Ibadan.

2.3 Allergic Diseases

Asthma is one of the chronic allergic respiratory diseases characterized by episodes of attacks or inflammation and narrowing of airways. Asthma attacks involve shortness of breath, cough, wheezing, chest pain or tightness, or a combination of these symptoms. Many factors can trigger an asthmatic attack, they include allergens, infections, exercise, abrupt changes in the weather, or exposure to airway irritants, such as tobacco smoke and diseases such as gastroesophageal reflux disease. The pattern, duration, severity and frequency of symptoms vary (Erhabor *et al*., 2002).

2.3.1 Types of Asthma

1. Work – Related Asthma

Occupational asthma is defined as a disease characterized by variable airflow limitation and or bronchial hyper-responsiveness due to causes and conditions attributable to a particular working

environment and not to stimuli encountered outside the work place. Work – aggravated asthma is defined as concurrent asthma worsened by nontoxic or physical stimuli in the work place (Annesi *et al.,* 2001).

2. Exercise–Induced Asthma (EIA), or Exercise–Induced Bronchospasm

Defined as a condition in which exercise or vigorous physical activity triggers acute bronchospasm in persons with heightened airway reactivity. It is observed primarily in persons who are asthmatic but can also be found in atopic patients, allergic rhinitis, or cystic fibrosis, and even in healthy persons. Exercise-induced asthma is often a neglected diagnosis, and the underlying asthma may be silent in as many as 50 % of patient, except with exercise. Exercise, particularly running and cold weather exercise, induces asthmatic reactions in about 17 million Americans (Annesi *et al.,* 2001).

2.3.2 Asthma in Pregnancy

Asthma is the most common condition that affects the lung during pregnancy and are substantially increased risk for several adverse infant and maternal outcomes (Liu *et al*., 2001). With good asthma treatment during pregnancy, most women can breathe easily, stay healthy, have a normal pregnancy, and give birth to healthy baby (Annesi *et al.,* 2001).

2.3.3 Complications of Asthma

In most stages, asthma is a reversible condition, which means symptoms and airway flow obstruction significantly improve with treatment. Conversely, in a small percentage of asthmatics, the airway obstruction does not reverse, and these patients end up with Chronic Obstructive Pulmonary Disease (COPD), Chronic Bronchitis (CB) or Emphysema (Djukanovic *et al*., 1992). Complications associated with most medications used for asthma are relatively

rare, however, in those patients requiring long -term corticosteroid use, complications may include osteoporosis, immuno suppression, cataracts, weight gains, psychiatric disorders, diabetes, a vascular necrosis, (Djukanovic *et al*.,1992). The risk of these complications is far less with inhaled corticosteroids than with oral corticosteroids. Nevertheless, in patients with moderate or severe asthma whose disease has been well controlled with high – dose inhaled corticosteroids, every effort should be made to reduce the dose to as low as possible while maintaining good asthma control and minimizing the risk of exacerbations (Djukanovic *et al*., 1992).

2.4 Prevalence of Asthma and Allergy

Asthma mortality is associated with multiple factors, including delay in care, poor compliance, and lack of access to health care, theophylline toxicity, and overuse of B – agonist medications, (Spitzer, 1992). Speculation about the recent decline in asthma deaths has pointed to the more judicious use of prophylactic treatment, particularly inhaled steroids, (Siptzer, 1992). On average, 1,400 people die from asthma each year in the UK according to Piccard (2002). The asthma mortality rate in Israel during the years 1980 to 1997 was low, stable, and there was no difference in the asthma death rate between Jews and Arabs, suggesting that in this population, genetic predisposition was not likely to be a risk factor for mortality (Picard, 2002).

2.5 Rhinitis

Rhinitis is inflammation of the membrane tissue in the nose, causing sneezing, a runny nose, and sense of nasal obstruction. There are two major causes of rhinitis: an allergy called "allergic rhinitis", and an over activity of the nerves in the nasal tissue called "vasomotor rhinitis" (Scoppa, 1996).

2.5.1 Classification of Rhinitis

(a) **Atopic Rhinitis**

There are three types of atopic rhinitis. (a) Seasonal allergic rhinitis (also known as hay fever). This is triggered by allergy due to pollen, including trees in rainy season, grasses and herbaceous in dry season. Symptoms include sneezing, itching, tickling in the nose, runny or stuffy nose, and watery or itchy eyes. Seasonal rhinitis is diagnosed primarily by medical history (Ono and Abelson, 2005).

(b) Perennial Rhinitis (Year – Round) with Allergic Triggers

These triggers include indoor allergens such as mold, house dust mite, cockroach and animal dander. Foods commonly eggs, cows milk and peanut can be triggers. Symptoms are the same as seasonal allergic rhinitis but are experienced throughout the year (Tamari *et al*., 2013).

c) Perennial Rhinitis with non – Allergic Triggers

Although not triggered by allergy, it's an allergic-like condition with increased eosinophils (a special type of white blood cell associated with allergy) in the lining and secretions of the nose. Symptoms are the same as perennial rhinitis with allergic triggers, diagnosis is determined from negative skin tests and a nasal smear test positive for eosinophils and nasal polyps can be a complication of this condition (Tamari *et al*., 2013).

2. Vasomotor Rhinitis

Vasomotor rhinitis is caused by over activity of nerves in the nasal tissue. This can occur when emotionally upset, irritated by certain air temperature and humidity conditions (chilly weather, dry air from air – conditioning, sudden changes in temperature or humidity), during pregnancy, and during bacterial and viral infections. It can also be induced by drugs such as alcohol, anti-

hypertensive agents, aspirin, oral contraceptives, chemicals (cosmetics, smoke, noxious fumes) and from over use of decongestant nasal drops or sprays. Food induced rhinitis (gustatory rhinorrhea) may occur during consumption of hot and spicy foods (MacKay and Durham, 1998). The National Institute of Allergy and Infectious Disease (NIAID) estimates that ‘‘the number of people suffering from allergic rhinitis may be as high as 35 million’’. Allergic rhinitis may not seem dangerous in itself, but it can play a role in other diseases like asthma and sinusitis (Specter, 1999).

Complications of Allergic Rhinitis

Allergic rhinitis has a strong association with asthma (Specter, 1999). Another commonly associated condition is nasal polyps, which are growths of skin in the nasal tract that can cause obstruction and loss of smell and sinus and ear infection. Allergic rhinitis results in bad breath, a husky voice and sore throats, it worsens snoring and the tendency to sleep apnea in adults. It causes abnormal development of the mouth and teeth from chronic mouth breathing (Specter, 1999).

2.6 Asthma and Allergy Prevalence Worldwide

In 1998, International Study of Asthma and Allergies in Childhood (ISAAC) steering committee conducted a study to investigate the worldwide prevalence of asthma, allergic rhino, conjunctivitis, and atopic eczema(ISSAC,1998). A total of 463,801 children aged 13-14 years in 155 collaborating centers in 56 countries were used. Result showed differences of between 20 fold and 60 fold between centers in the prevalence of symptoms of allergy. For asthma symptoms, the highest 12-month prevalence were from centers in the UK, Australia, New Zealand and republic of Ireland, followed by centers in North, central and South America. The

lowest prevalence was from centers in several eastern European countries, Indonesia, Greece, China, Taiwan, Uzbekistan, India, and Ethiopia. For allergic rhino conjunctivitis, the centers with the highest prevalence were scattered across the world. The centers with lowest prevalence were similar to those for asthma symptoms. For atopic eczema, the highest prevalence came from scattered centers including some from Scandinavia and Africa that were not among centers with the highest asthma prevalence, the lowest prevalence rates of atopic eczema were similar in centers as for asthma symptoms (ISAAC, 1998).

According to a study on the prevalence of asthma in children living in villages, cities and refugee camps in Palestine, in autumn of 2000, the crude prevalence rate for wheezing –ever, wheezing in the previous 12 month, and physician –diagnosed asthma were 17.1 %, 8.8 %, 9.4 % respectively, with urban areas having higher prevalence rate than rural areas(Al-Shehri, 2000). Within urban areas, refugee camps had higher prevalence rate than cities, the prevalence of asthma and asthma symptoms in Palestine appears to be close to that of Jordan, but was much lower than Israel (Al- shehri, 2000). Another study was carried out on the differences in the prevalence of asthma and current wheeze between Jews and Arabs. This showed that the prevalence of asthma and current wheeze was significantly higher in Jewish children compared with Arab children. The asthma prevalence was 7.8 % for Jewish children and 4.9 % for Arab children (Al-Shehri, 2000).

A study of prevalence of self – reported allergic conditions in adult population in Israel showed that allergic conditions were higher in the Israeli Arab population and those with low income and low education level (Shahar and Lober, 1996). Screening for asthma and associated risk factors among urban school children in Abha city, Saudi Arabia showed that the prevalence of

asthma in school children in Abha was greater than that reported from most developing countries and closer to the rates reported in developed Countries, (Al -Shehri, 2000).

A study on the prevalence of asthma and other atopic diseases in Australian children showed that the prevalence of wheeze was significantly higher in boys (27.4 %) than girls (21.7 %). Children born in Australia were more likely to report current wheeze than those born elsewhere (Robertson, 1998).

An ISAAC study on prevalence of asthma and allergy in Hong Kong school children at age 13-14 years old showed that the prevalence rates of asthma ever, wheeze ever, and current wheeze were 11, 20 and 12 %, respectively, and were greater in boys. Rhinitis affected slightly over half of the subjects (52 %) and eczema was reported by a sixth (15 %), while current rhinitis and current eczema were presented in 44 % and 3.6 % of children respectively. Parental education and passive smoking were not important factors when compared to previous epidemiology data obtained in 1992. The prevalence rates for asthma ever and wheeze ever had increased by 71 and 255 %, respectively, in Hong Kong school children. Similar increasing trend was showed by the severity of asthma and respiratory symptoms (Leung, 2004).

According to Ige and Sogaolu (2004) recent hospital survey conducted in Nigeria over a seven-year period, which evaluated the changes in prevalence of symptoms of asthma, found that cases of wheezing had increased significantly in the 13 to 14-year age group. He remarked that although there was no clear explanation for the apparent surge in cases of asthma and its severity, the trend could possibly be ascribed to a general improvement in living standard. According to this hypothesis, “the decrease in the incidence of childhood infections following improvement in hygiene and standard of living would stimulate the immune system in the

direction that would enhance the development of asthma and other allergic states, rather than in fighting one infection or the other." (Ige and Sogaolu, 2004).

2.7 Conjunctivitis

Conjunctivitis is an inflammation of the conjunctiva characterized by cellular infiltration and exudation. The exudates may be purulent, mucopurulent, foamy, pseudo membranous or catarrhal. Conjunctivitis may be infectious; caused by micro-organism or pollen, spores, drug and devices such as hard and soft contact lens. Many Nigerians suffer from allergic conjunctivitis, which has become one of the most common allergic condition of the eye. Conjunctivitis is often referred to as "pinkeye". The viral or bacterial forms of conjunctivitis are contagious, while allergic conjunctivitis is not (Adeyeba *et al*., 2010).

Allergic conjunctivitis (AC) is typically a type 1 IgE-mediated hypersensitivity reaction with cell-mediated Th-2 involvement in some types. The ocular allergic response is a cascade of events that are coordinated by mast cells. The presence of an allergen makes the body to mount an antigen specific response with T-helper cells-2 (Th2), releasing cytokines and also producing antigen-specific immunoglobulin E (IgE). IgE then binds to mast cells with release of histamine and further release of cytokines, prostaglandins and platelet-activating factor with other intermediaries. These intermediaries cause an allergic inflammation and symptoms through the activation of inflammatory cells. Histamine binds to H1 receptors on nerve endings and causes the ocular symptom of itching and binds to H2 receptors of the conjunctival vasculature and produces vasodilatation and lacrimation. These mast cell-derived cytokines also recruit neutrophils and TH2 cytokines recruit eosinophils promoting increased sensitivity. This process could then progress to chronic allergic inflammation where there is proliferation of

fibroblast in the conjunctiva with resultant development of papillae in some patients (Tamari *et al*., 2013).

These events are responsible for the usual presentations in patients, with bilateral severe itching, watery discharge, acute or chronic redness, swollen eyelids and burning or foreign body sensation, with photophobia. Allergic Conjuctivitis (AC) may occur on a yearly basis in a particular season, giving rise to seasonal form or throughout the year as in perennial AC. Most of the hypersensitivity reactions are to specific allergens. Pollens are responsible for seasonal conjunctivitis associated with hay fever and tend to recur at the same time each year in those with atopy. Perennial conjunctivitis occurs as a result of several allergens such as house mites, animal dander and cosmetics. Symptoms occur all year round and may be worse in the mornings. Other allergens such as contact lenses, sutures and prostheses following eye surgery could give giant papillary AC. Reactions to eye drops, preservatives in the eye drops and cosmetics could give rise to contact dermatoconjunctivitis. These tend to resolve once the irritant is removed. Allergic conjuctivitis can follow seasonal variations at the early onset of the disorder but may become perennial as time goes on. Seasonal and perennial AC are said to occur in association with a history of other body allergies like asthma, hay fever, rhinitis, eczema or atopic dermatitis and or family history of the same. AC could seriously affect the patient's quality of life especially during the acute episodes. It could lead to children missing school for some days as a result of acute conjunctival inflammation with attendant discomfort. Most of the AC that affect children occur from the age of 5 to adolescence and rarely proceed beyond the age of 25. Before puberty more boys than girls are affected but beyond puberty there is no gender bias (Tamari *et al*., 2013). There are also climatic and racial risk factors involved in some types of AC. It is more prevalent in warm climatic conditions and more among Afro-

Caribbeans, Arabs and Asians and less among the white population. The prevalence seems to be declining among the Caucasians (Tamari *et al*., 2013). Among the Caucasians atopy in patients or family history of atopy is present in over 80% of those with AC and strong association of keratoconus. In African continent such associations have not been frequent. AC constitutes the highest group of eye problems seen in most out-patient eye consultations in the developing world, including Nigeria (Adeyeba *et al*., 2010).

Usually clinical diagnosis of AC is straightforward. A conjunctival swab excludes other forms or causes of conjunctivitis of infective means or associations. Serum immunoglobulin E (IgE) may be raised, radioallergosorbent test (RAST) skin prick testing may be negative or nonspecific. Conjunctival scrapings for eosinophils may help determine the cause of the allergy (Kari and Saari, 2010).

2.7.1 Types of Allergic Conjunctivitis

There are two forms of allergic conjunctivitis, seasonal and perennial types (Ono and Abelson, 2005). The perennial form of allergic conjunctivitis is usually caused by animal hair or dander, feathers, dust mites, pollen and spores. The most common form of allergic conjunctivitis is seasonal, which is triggered by mold spores and pollen from flowering trees, grass, and herbaceous. Allergic conjunctivitis may also result from the presence of a foreign body in the eye, such as a contact lens or glass eye. This type of allergic conjunctivitis is known as giant papillary conjunctivitis, and typically occurs in a person who wears hard or rigid contact lenses. Other substances that may cause an allergic reaction in the eyes include air pollutants, perfumes, cosmetics, chemicals, and smoke (Ono and Abelson, 2005). The most obvious symptoms of allergic conjunctivitis are redness and puffiness around the eyes, which affect the quality of life

of the sufferer. Other symptoms include; Itching or burning eyes, blurred vision, increased sensitivity to light. Since avoidance of the allergen is the best solution for allergic conjunctivitis, the steps for reducing or eliminating exposure to eye allergens are; remain indoors during peak pollen counts, wash hair and eyelids daily using a baby shampoo, wear sunglasses when outdoors, especially on windy days, to block pollen from the eyes, reduce dust mite exposure by keeping air ducts clean and using mattress covers or mite-proof bedding.

Fortunately, anyone with a history of allergic conjunctivitis may prevent future outbreaks or lessen their severity by identifying the specific allergens causing their symptoms and being diligent to avoid exposure to them (Adeyeba *et al*., 2010).

2.8 Pollen and Spores Allergenicity

Mutual boosting effects of sensitization with *Phleum pratense* pollen and latex glove extract on IgE Antibody responses in a Mouse Model was studied by Mahler *et al.*(2000). Groups of 10 BALB/C Mice were immunized with $Al(OH)_3$-adsorbed pollen extracts from *Phleum pratense*, *Ambrosia artemisifolia, Artemisia vulgaris* or *Betula pendula*. For control purposes, one additional group received adjuvant only and another group was not immunized. Half of the Mice of each group were subsequently immunized with $Al(OH)_3$-adsorbed latex glove extract, the other half with adjuvant only. Pollen and latex-specific IgE- and IgG1-antibody responses were analyzed by enzyme-linked immunosorbent assay and statistically evaluated by analysis of variance. Antibody responses to cross-reactive antigens were investigated by immunoblotting. They found significantly increased IgE reactivities to latex after pollen sensitization and vice versa. Moreover, Mice immunized with *Phleum pratense* pollen extract alone – without subsequent latex immunization – displayed IgE reactivity to latex. Cross-reactive antibodies

were directed against pollen antigens of approximately 60 kDa molecular weight. The results thus demonstrate a mutual boosting effect of pollen and latex sensitization in vivo which may be also operative in polysensitized pollen allergic patients.

Induction of IgE antibodies in mice and rhesus monkeys with recombinant birch pollen allergen was carried out by Vitalis *et al*. (1996). In this study an attempt was made to determine whether the different allergenicity of the major birch pollen allergen, rBet v 1, and a minor birch pollen allergen, rBet v 2, might be related to a different immunogenicity of the proteins as evaluated in experimental animal systems (Mice and rhesus monkeys). Purified recombinant allergens were injected into mice and rhesus monkeys with aluminum hydroxide as adjuvant for elicitation of specific IgE responses. Antibody responses to the allergens were detected by immunoblotting, and time courses of immune responses were measured by Elisa. In both animal models, more than the 10-fold dose or rBet v 2 was required to induce IgE antibodies, and even then, the amount of specific IgE antibodies elicited with rBet v 1 was substantially higher than that induced by rBet v 2 which formed stable polymers through disulfide bonds. They concluded that in the two different animal models (Mice and rhesus monkeys) the major birch pollen allergen, rBet v 1, induced substantially higher levels of IgE than rBet v 2. A reduced allergenicity of Bet v 2 caused by polymer formation would be in agreement with previous studies indicating reduced allergenicity of proteins on chemical polymerization.

Diesel exhaust particles (DEP) and allergenicity of pollen grains of *Lilium martagon* were studied by Chehregani and Kouhkan (2007). In this research, pollen grains of *Lilium martagon* that are known as a non-allergic substance were collected and exposed to DEP 5 . The allergy potency of different pollen extracts were compared by means of skin test, as well as analyses of

blood eosinophil numbers and IgE levels in the treated animals. Normal and DEP-exposed pollen grains were examined by scanning electron microscopy. Pollen extracts were also studied by SDS–PAGE for DEP-induced changes in protein profiles. Allergic bands were also studied and checked by using immunoblotting method. The results of the investigated allergy tests showed that DEP-exposed pollen grains are effective in inducing allergic symptoms. According to the microscopic observations, organic substances that existed in the DEP, mediated agglomeration of particles on the pollen surface. In appropriate conditions, water-soluble components of DEP may induce changes that affect the release of pollen proteins. SDS–PAGE showed that protein profiles of pollen grains were changed and some new bands appeared in DEP-exposed pollen grains. Immunoblotting studies showed a new band in DEP-exposed pollen grains that reacted strongly with anti-IgE, but there was no allergenic band in normal pollen grains. On the other hand, diesel exhaust particles can carry pollen allergen molecules, induce new proteins (allergens), and also act as adjuvant for allergens (Chehregani and Kouhkan, 2007).

The role of major olive pollen allergens Ole e 1, Ole e 9, and Ole e 10 on mice sensitization was studied by Barral *et al*. (2006). BALB/c mice were sensitized by 4 intraperitoneal injections of olive pollen extract in aluminum hydroxide. The allergic state was proved by measuring serum specific IgG1 and total IgE antibody levels. The IgG1 responses to olive pollen allergens were assayed by immunoblotting and enzyme-linked immunosorbent assay. Competition experiments between human IgE and mouse IgG1 binding to olive pollen allergens were performed. The result revealed that sensitization with olive pollen extract induced high levels of specific IgG1 and total IgE in all tested animals. Immunoblotting experiments showed that the mouse IgG1 binding pattern to pollen extract was complex and heterogeneous, as occurs with human IgE.

High IgG1 antibody levels to the major olive pollen allergens described for humans were detected in serum samples from sensitized mice, whereas minor olive pollen allergens induced no significant IgG1 response. Co- incubation of mouse serum samples with a cocktail of Ole e 1, Ole e 9, and Ole e 10 resulted in a significant decrease (60 %) in IgG1 binding to olive pollen extract. Specific mouse IgG1 strongly inhibited human IgE binding to olive pollen allergens.

Prevalence of sensitization to Aeroallergens in California patients with respiratory allergy was studied by Galant *et al*. (2010) utilizing aeroallergens thought to be relevant from recent aerobiologic and botanic data, 141 allergic and 17 asymptomatic control subjects were tested for the prevalence of 103 allergens.

A standardized prick puncture technique and standardized interpretation of wheal/flare responses were utilized using the same lot of allergen for 13 allergy practices distributed throughout California. Frequency curves based on prevalence were established to determine the number of tests required to give up to 90 % of positive responses for tree, weed and grass pollen, mold spores, and miscellaneous allergens which included house dust mite, cat, dog, and cockroach allergens. Positive responses in allergic subjects for grasses ranged from 46 % to 54 %, for herbaceous 19 % to 37 %, and for trees 10 % to 42 %. For molds the range was 11 % to 22 %. The response rate for *Dermatophagoides pteronyssinus* was 53 %, for *Dermatophagoides farinae* 42 %, for cat pelt 39 % and cat hair 37 %, for cockroach 23 % and dog dander 19 %. Asymptomatic control subjects responded to only 4 % of all allergens tested. Ninety percent of all positive tests required three miscellaneous allergens (house dust mite, cat, and cockroach), 9 molds, 2 grasses, 16 herbaceous, and 27 trees for a total of 57 allergens (56 % of total tested). There was no clear relationship between local and specific allergen response, probably related to the limited number of subjects tested and variability within the same geographic region.

Several seldom tested tree species and weed allergens showed a higher prevalence rate than several commonly tested for allergens (Galant *et al*., 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Atmospheric Pollen and Fungi Spores

Modified Tauber- like pollen traps were employed for the collection of pollen and fungi spores. The instrument was designed by Tauber (I974). The sampler is a non-volumetric sedimentary sampler that rely on gravity to access the composition of the atmosphere. It has a collar around it, the shape and the capacity of the receptacle beneath the collar was made large enough to accommodate the expected monthly rainfall. The material of the trap is sufficiently resistant to rust and can remain in the field for a full calendar year without cracking (Agwu, 1997). Pollen and spores are incorporated into the trap in a process analogous to incorporation of pollen and spores into sediment. The sampling locations were the six geopolitical zones of Nigeria; South East (Nsukka, Enugu State: 06^{o} 69^{1} N; 007^{o} 33^{1} E), South South (Obiakor, Rivers State: 06^{o} 77^{1} N; 006^{o} 34^{1} E), South West (Akoka, Lagos State: 06^{o} 30^{1} N; 003^{o} 31^{1} E), North Central (Garki, Abuja: 09^{o} 34^{1} N; 7^{o} 51^{1} E), North West (Zaria, Kaduna State: 11^{o} 0^{11} N:7^{o} 74^{1} E) and North East (Dukku, Gombe State: 11^{o} 48^{1} N; 11^{o} 53^{1} E) (Fig. 3.1). The recipient solutions were collected monthly for a period of one year, from June 2011 - May 2012.

3.2 Setting Up of the Trap for the Collection of Atmospheric Pollen and Spores

Each modified Tauber like pollen trap was placed at the height of 5 ft above the ground surface (Plate 3.1). The sampler was mounted at two sampling sites per study location . A solution made of glycerol (50 ml), formaldehyde (10 ml) and phenol (5 ml) was prepared and poured into each trap. The recipient solutions were collected monthly for the period of one year. The solution in the trap was replaced after each monthly harvest. The collected samples were stored in the refrigerator to stop any ongoing oxidation of plant materials.

Fig. 1: Map of Nigeria, showing the six geopolitical zones of Nigeria and study locations

Source: Rewaju, (2012)

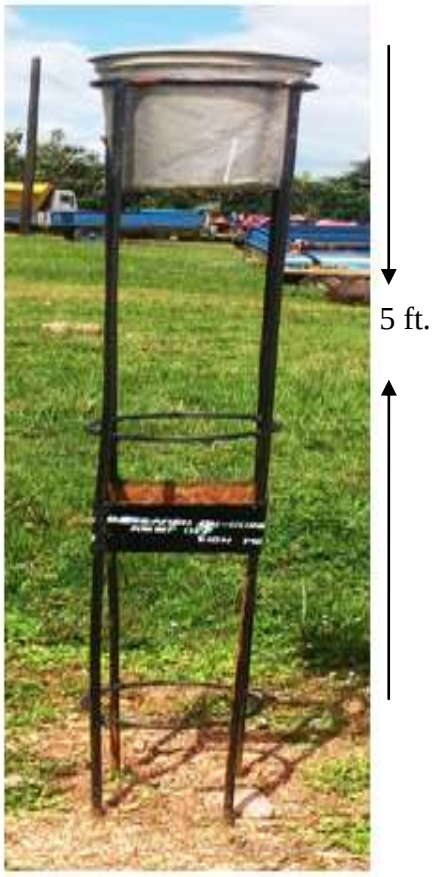


Plate 3.1: Modified Tauber-like Pollen Trap in the Field

3.3 Pollen Extraction and Concentration

The samples were sieved through 200 µ mesh wire gauze to filter off large organic and soil particles. The liquid with suspended palynomorphs was centrifuged at 2000 revolutions per minutes (rpm) for 5 mins. The supernatants were decanted and the residues retained. The precipitates were washed three times with water and centrifuged in order to recover the polleniferous residues.

3.4 Digestion of Inorganic Materials

The residue (precipitate) recovered from processes above were placed in plastic test tubes and 45 % hydrofluoric acid was added to each. The suspension was allowed to stand for 15 mins, after which it was stirred regularly to increase the rate of dissolution of solid inorganic compounds. At the end of the period the samples were centrifuged and the supernatant decanted to retain the residues. The samples were washed two times with water and once with glacial acetic acid. Each wash was followed by centrifugation and decantation in order to recover the polleniferous residue.

3.5 Acetolysis

The acetolysis solution was prepared from nine parts of Acetic anhydride and one part of tetraoxosulphate (vi) acid. Two to three (2-3) ml of the acetolysis solution was added to each of the samples. The centrifuge tubes containing the acetolysis mixture (palynomorphs and acetolysis solution) were placed in a water bath at 100 ^{o}C and allowed to stand for 5mins. The tubes were removed from the water bath, centrifuged for five minutes at 1500 r.p.m. and then decanted. The precipitates (pollen and spores) recovered were treated once with 2-3 ml of glacial acetic acid, centrifuged and decanted. The recovered sediment were further washed

twice with water, centrifugation and decantation followed each wash. Ten (10) ml of glycerol/alcohol solution in the ratio of 2:1 was added to each precipitate and transferred into plastic vial bottles for storage.

3.6 Slides Preparation

The content of each vial bottle was properly shaken and 2 drop (0.2 ml) of the suspension mounted on a slide and covered with 22 mm x 22 mm cover slip. Two slides were prepared from each residue sample and mount was covered with colourless nail varnish at the edges of the coverslips to prevent drying up. In this state the specimen could stay as long as 4 weeks without completely drying up. Microscopic examination was made with a light microscope. Pollen count and fine morphological studies were made at X 400.

3.7 Identification

Identification of pollen grains was based on comparism with reference collection of pollen slides and with description and photomicrographs of pollen and spores in books and journals such as Agwu and Akanbi (1985), Ybert (1979), Agwu and Ahize (1987) etc. The photomicrographs of some pollen, fungi spores, charred Poaceae epidermis were taken using motic camera MC 2000.

3.8 Extraction of Pollen and Fungal Spores Protein

Mature anthers from flower which have not undergone anthesis were procured and dried at room temperature for 3 days. Pure cultures of spores were produced using potato dextrose agar (PDA) and allowed to mature for three weeks. Spores were scooped out of the petri dishes prior to extraction. Both anthers and spores were defatted using diethyl ether for three times and allowed to dry under room temperature. They were extracted in 100 ml of 0.02 M phosphate

buffered saline (PBS) at pH-7.4. The mixtures were stirred overnight with magnetic stirrer at 4 ^{o}C, filtered with a muslin cloth, centrifuged at 3000 rpm for 10 mins and supernatant retained and sediment discarded. The extracts were precipitated with ammonium sulphate. The precipitates were retained and dialysed against PBS overnight. Protein content was assayed according to Bradford procedures. The crude pollen and spores protein were stored at -80 ^{0}C for later use.

3.9 Inoculation of Pollen and Fungal Spores Protein

Swiss Albino Mice (4-6 wks old) were purchased from the Nigerian Institute of Medical Research, Yaba. Maintained under a 12-hours light-dark cycle with free access to water and standards laboratory food. All experimental procedures conformed to international standards of animal welfare and were approved by the Animal Experimentation Ethics Committee of the Nigerian Institute of Medical research (Appendix 1). Crude protein extract (0.4 ml) obtained from six different pollen and two fungi spores was inoculated into mice by two subcutaneous injections and one intranasal injection. Blood samples were obtained by retro-orbital bleedings using heparinze capillary tubes and sera stored at -80 ^{o}C for later use in detecting immunoglobulin (IgE) levels. Blood smears were also obtained from the tail, the thin blood smear were fixed with methanol for 2-3 mins. One in three dilutions of Leishman stain and buffered water was prepared and cover the slide with the stain for 7-10 mins. The stain was washed off in a stream of buffered water. Distillled water was added on a slide and left for 2-3 mins to differentiate the film. The slides were allowed to dry on a rack. Using X 100 objective lens with oil emersion, the blood smears were examined and the different immune cells identified and quantified.

3.10 Immunoperoxidase Assay for Determination of IgE in Mice Sera

Immunoperoxidase assay for the determination of IgE level in the mice sera was employed, Mouse IgE Elisa kitt GWB 626057 purchased from GenWay Laboratory Technologies United State of America was employed. The kit is a highly sensitive two site enzyme linked immunoassay for measuring immunoglobulin E in biological samples of mice. The reagents in the kit were; diluent concentrate, wash solution concentrate, enzyme antibody conjugate, chromogen substrate solution, stop solution, anti- mouse IgE Elisa micro plate, mouse IgE calibrator. Manufacturer's specification on usage was strictly followed.

Procedure

All manufacturer's instructions on reagent dilution with distilled water were strictly adhered to and prepared prior to use. One hundrend (100) µL of the Mice sera were measured using micro pipette into predesignated microtitre wells and incubated for 30 mins. After removal of unbound protein by washing with Elisa machine, the wells were blotted to remove residual buffer. Anti IgE antibodies conjugated with Horseradish peroxidase (HRP) were added and incubated for 30 mins. These enzyme labeled antibodies form complexes with the previously bound IgE, this was followed by another washing. The enzyme bound to the immunosorbent was assayed by the use of 3,3,5,5- Tetramethyl benzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgE in the tested samples, absorbance at 450 nm was determined, which was a measure of the concentration of IgE in the tested samples. The quantities of IgE in the samples were interpolated from the standard curve constructed from the standards and corrected for sample dilution. Rating of the IgE level was done following Nigerian Institute of Health (NIH) (Appendix 8).

3.11 Histopathology Procedures of the Respiratory Organs

Processing of tissue samples for histological assessment followed established procedures of Kuo (2007). In brief, the tissue samples were rinsed with 0.9 % saline solution, fixed in 10 % formalin. Then the transverse sections of the trachea, bronchi, bronchiole and lung were obtained and treated as follows: (1) 10 % neutral buffered formalin for 1 hrs, twice; (2) 70 % alcohol for 1.5 hrs; (3) 80 % alcohol for 1.5 hrs; (4) 90 % alcohol for 1.5 hrs; (5) absolute alcohol for 1.5 hrs, twice; (6) xylene for 1.5 hrs, twice; (7) in molten wax at 65 °C for 2.5 hrs two changes. The processed tissues were embedded in paraffin and sectioned at 4 µ thickness, placed on frosted glass slides and dried on a 70 °C hot plate for 30 mins. The tissues were stained using the hematoxylin and eosin (H&E) stains. The sections were dewaxed in two changes of xylene (3 mins each), hydrated in two changes of 100 % ethanol, followed by 90 % ethanol and 70 % ethanol, for 3 mins each, rinsed with water (3 mins) and stained. The stained tissues were dehydrated with 70% ethanol followed by 90 % ethanol, placed in two changes of 100 % ethanol for 3 minutes each and cleared with two changes of xylene (3 mins each). Histopathology changes were observed and their photomicrographs taken with the aid of a Motic camera MC 2000.

3.12 Statistical Analysis

The data obtained were analyzed using the SPSS statistical package version 20 (SPSS Inc. Chicago, Illinois USA). Descriptive and frequency statistics were generated to examine the means of basophil, eosinophil, lymphocyte, monocyte, neutrophil and lmmunoglobulin E (IgE) (Appendix 9).

3.13 Collection of Weather Parameters Data

Weather parameters data were obtained from Nigerian Meteorological Centre Oshodi, Lagos.

CHAPTER FOUR

RESULTS

4.1 Seasonal Prevalence of Pollen and Fungi Spores in Nsukka, Enugu State (South East) Nigeria from June 2011 to May 2012

In Nsukka, Enugu State Nigeria, 69 pollen types which belong to 33 plant families were recorded for the aeroflora. Among these 45, 18 and five were identified to species, generic and family levels respectively. Major pollen contributors were; Poaceae, *Elaeis guineensis, Olax subscorpioidea, Alchornea cordifolia* (Schum and Thonn) among others (Table 4.1). *Olax subscorpioides* (Oliv) pollen were present from September to February. Poaceae pollen were predominant from June to November. *Alchornea cordifolia* (Schum and Thonn) 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* (,Pers.) C.D. *Ageratum conyzoides* (L.) *and Bombax buonopozense* (P. Beav). The month of May had the lowest pollen record among the rainy season. The atmospheric pollen record started increasing from the month of September (Table 4.1). The pattern of pollen dispersal displayed three distinct periods: the period from May to August, which corresponded with rainy season and had (470) lower atmospheric pollen content. Another period from September to December, was the late rainy/harmattan season, which was dominated by pollen (1242). January to April, the dry period recorded 927 pollen. The period of September- December 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 qualitatively and quantitatively dominated by anemophilous

pollen, majorly Poaceae and *Elaeis guineensis* (Jacq) etc and enthomophilous pollen. This period also was the major and principal pollination period.

Twenty eight fungi spores types were recorded from the traps (Table 4.2). The most dominant and prevalent fungi spores included those of *Nigrospora* sp., *Fusarium* spp., *Puccinia* sp. and *Alternaria* spp. Some spore types were only present during the rainy season such as *Alternaria* spp., *Fusarium* spp., *Venturia* sp., *Triposporium* sp. and *Helminthosporium* sp. The abundance of the spores reduced during the dry season from the month of November to March. Spores of fungi present throughout the year were those of *Nigrospora* sp. and *Puccinia* sp. The lowest monthly spores was recorded in February.

Table 4.1: Atmospheric pollen count of Nsukka, Enugu State (South East) Nigeria from June 2011 - May 2012

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Acacia* sp.	2	4	0	0	8	0	0	0	0	0	0	0
2	*Acacia* sp.	0	0	0	0	4	0	0	0	0	0	0	0
3	*Adansonia digitata* (l.)	0	0	0	0	0	0	0	0	0	2	0	0
4	*Afzelia africana (*SM).	0	0	0	4	2	0	0	0	0	0	0	0
5	*Ageratum conyzoides* (L.)	0	0	0	0	0	0	0	0	0	0	42	0
6	*Albizia* sp.	0	2	0	0	4	0	0	4	12	0	0	0
7	*Alchornea cordifolia* (Schum and Thonn)	0	12	0	2	20	20	104	8	120	28	23	1
8	Amarathaceae/Chenopodiaceae	0	2	2	1	4	0	0	0	0	0	0	0
9	*Anacardium occidentale* (L.	0	0	0	0	0	0	0	2	6	0	0	4
10	*Aneilema* sp.	2	0	0	0	0	4	0	0	0	0	0	0
11	*Anthocleista djalonensis* (A.chev.)	2	0	0	0	0	0	0	0	0	0	0	0
12	*Anthocleista vogali* (Planch)	2	4	0	0	4	0	0	0	0	0	0	0
13	*Antidesmis* sp.	0	0	0	0	0	0	2	4	0	0	0	0
14	*Aspilia africana (*Pers C.D. Adams)	0	0	0	6	18	0	6	0	6	0	192	0
15	*Barleria* sp.	0	0	2	0	0	0	0	0	0	0	0	0
16	*Berlinia grandiflora* (Vahl)	0	0	0	4	0	0	0	0	0	0	0	0
17	*Blighia sapida* (Lovett)	0	0	0	0	0	0	8	0	4		2	0
18	*Bombax buonopozense* P.Beav.	0	0	0	8	0	0	0	0	0	0	42	0
19	*Bridelia ferruginea* (L.)	0	12	0	2	4	0	0	0	0	0	0	0
20	*Canarium schweinfurthii (*Engl.)	0	2	0	2	0	0	0	0	0	0	0	0
21	*Carica papaya (*L).	0	0	0	10	0	0	0	0	0	1	0	0
22	*Cassia senna (*L.)	0	0	0	0	0	2	8	0	0	0	0	0
23	*Cassia mimosoides* (L.)	4	0	0	0	2	12	2		4	0	0	0
24	*Cassia* sp. (L.)	0	0	1	0	0	0	4	2	0	0	0	0
25	*Casuarina equisetifolia* (L.)	0	36	0	2	0	8	0	0	4	8	6	0
26	*Ceiba pentandra* (L.	0	0	0	10	0	0	0	0	0	0	4	0

Table 4.1: Atmospheric pollen count of Nsukka, Enugu State (South East) Nigeria Cont'd

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
27	*Celtis* sp.		8	0	0	8	0	0	0	0	0	0	0
28	*Celtis* sp.	0	0	0	0	8	0	0	0	0	0	0	0
29	*Chromolaena odorata* (L.)	0	0	0	0	0	0	0	0	0	0	14	
30	*Cissus* sp.	0	0	0	0	0	2	10	0	0	0	0	0
31	*Cochlospermum tinctorum* (A. Rich)	0	0	0	0	0	0	2	0	0	4	0	0
32	*Cocos nucifera* (L.)	0	0	0	0	0	0	0	0	6		16	16
33	Combretaceae	0	6	0	4	4	0	0	0	0	2	0	0
34	*Cucumis* sp.	0	0	0	0	0	0	2	0	0	0	0	0
35	Curcumbitaceae	0	0	0	2	0	0	0	0	0	0	0	0
36	*Cussonia bateri* (Seem.)	0	0	0	1	2	8	0	0	0	0	0	0
37	Cyperus spp.	0	0	0	10	4	0	0	0	0	0	0	0
38	*Daniella oliveri (*L.)	16	0	0	16	0	0	0	0	0	0	0	0
39	*Detarium senegalensis* (JF Gmelin)	0	4	0	0	0	0	0	0	0	0	0	0
40	*Dichrostachys cinerima (*L.)	0	0	1	0	0	0	0	0	0	0	0	0
41	*Dracaena arborea* (L.)	2	6	0	2	0	4	4	0	10	4	8	2
42	*Elaeis guineensis* (Jacq.)	16	20	2	88	12	28	80	20	24	28	1	2
43	*Eugenia nodiflora* (Aubl.)	2	0	0	2	2	0	0	0	0	0	7	0
44	*Gloriosa superba* (L.)	0	2	0	0	2	0	0	0	0	0	0	0
45	*Hymenocardia acida* (Tul.)	0	2	0	0	4	0	12	0	0	0	0	0
46	*Hymenocardia acida* (Tul.)	0		0	0	2	0	0	0	0	0	0	0
47	*Ipomoea* sp.	0	2	0	0	8	0	0	0	0	0	0	0
48	*Isoberlinia doka* (Craib and Stapf)	0	0	0	0	0	2	6	0	12	0	0	0
49	*Jatropha curcus* (L.)	0	0	0	0	0	0	0	0	0	0	16	
50	*Justicia* sp.	0	0	0	0	0	0	0	0	0	0	3	0
51	*Kigelia africana* (Lam)	0	0	0	6	0	0	0	0	0	0	0	0
52	*Lannea welwitchii* (Hiern)	0	0	0	0	2	0	0	0	0	0	0	0

Table 4.1: Atmospheric pollen count of Nsukka, Enugu State (South East) Nigeria from June 2011- May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
53	*Lophira* sp.	6	0	0	4	0	0	0	0	0	0	1	0
54	*Luffa aegyptica* (Mill.)	0	0	0	4	0	0	0	0	0	0	0	0
55	*Mangifera indica*(L.)	2	0	0	0	0	0	2	16	24	1	0	0
56	*Microdesmis sp*	0	0	1	0	0	0	0	0	0	0	0	0
57	*Nauclea latifolia* (Smith)	0	2	0	2	0	0	2	0	0	0	2	0
58	*Newbouldia laevis*(Seem)	0	0	0	0	0	0	0	0	0	0	18	0
59	*Olax subscorpioidea* (Oliv.)	0	0	0	112	32	2	0	8	24	0	0	0
60	*Pentaclethra macrophylla* (Benth)	0	0	0	0	12	36	0	0	4	0	15	0
61	*Phyllanthus* sp.	0	0	0	0	20	4	2	0	8	8	0	0
62	*Piliostima sp*	0	0	0	0	0	0	0	0	0	0	4	0
63	Poaceae	72	104	72	74	160	56	32	0	32	56	10	0
64	Portulacaceae	0	0	0	0	0	2	0	0	0	0	0	0
65	*Spathodea campanulata* (P. Beauv)	0	0	0	0	4	4	2	0	0	0	0	0
66	*Spondias mombin* (L.)	0	0	0	2	0	0	0	0	0	0	0	0
67	*Syzygium guineense*(Willd) DC.	0	0	0	0	0	4	0	0	0	0	0	1
68	*Tapinanthus* sp.	0	0	0	0	12	0	2	0	0	0	0	0
69	*Terminalia catappa* (L.)	0	0	0	0	4	0	0	0	0	0	0	0
	TOTAL POLLEN	**128**	**230**	**81**	**380**	**372**	**198**	**292**	**64**	**284**	**130**	**449**	**31**

Table 4.2: Atmospheric fungi spores counts of Nsukka, Enugu State (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	*Alternaria* spp.	0	136	24	0	0	0	0	0	0	0	4	4
2	*Bastrodesmium* sp.	0	0	0	0	102	0	0	0	0	0	0	0
3	*Beltrania* sp.	0	6	0	0	4	0	0	0	0	0	0	0
4	*Cephalosporium* sp.	0	0	0	0	0	0	0	0	4	0	0	0
5	*Cercospora* sp.	0	0	0	16	0	0	0	0	0	0	0	0
6	*Cladosporium* spp	0	0	0	0	0	3	0	6	0	0	0	0
7	*Curvularia* spp.	0	0	0	2	6	0	0	2	0	0	0	0
8	*Curvularia* spp.	0	2	0	0	0	0	0	0	0	0	0	0
9	*Diplocladiella* sp.	0	2	0	0	0	0	0	0	0	0	0	0
10	*Dreschlera* sp.	0	0	0	0	0	0	0	0	2	0	0	0
11	*Flagellospora* sp.	0	0	0	2	0	0	0	0	0	0	0	0
12	*Fusarium* spp.	6	13	0	122	400	0	0	0	0	0	0	0
13	*Helminthosporium* sp.	6	28	0	8	36	0	0	0	0	0	0	0
14	*Helminthosporium* sp.	0	0	4	0	0	0	0	0	0	0	0	0
15	Indeterminate	220	0	66	0	0	1503	0	99	0	0	6	6
16	*Leptothyrium* sp.	0	0	0	0	27	0	2	0	0	0	0	0
17	*Monilia* sp.	0	0	0	0	2	0	0	0	0	0	0	0
18	*Murogenella* sp.	0	0	0	0	0	0	0	0	2	0	0	0
19	*Nigrospora* sp.	16	0	16	16	14	4	16	14	6	20	0	0
20	*Pithomyces* sp.	0	0	1	0	2	0	0	0	0	2	0	0
21	*Pseudotorulla* sp.	0	1	0	0	0	0	0	0	0	0	0	0
22	*Puccinia* sp.	6	74	0	8	0	6	6	0	2	0	0	10
23	*Spadicoides* spp.	18	62	0	2	0	0	6	0	0	4	0	0
24	*Sporidesmium* sp.	0	0	0	0	0	0	0	0	1	0	0	0
25	*Stemphyluim* sp.	0	0	2	0	0	0	0	0	0	0	0	0
26	*Torulla* sp.	6	4	0	4	0	4	2	0	0	0	0	0
27	*Triposporium* sp.	10	0	1	8	14	0	0	0	0	0	0	0
28	*Venturia* sp.	1	0	4	24	0	0	0	0	0	0	0	0
	TOTAL SPORES	**295**	**579**	**118**	**214**	**607**	**170**	**32**	**121**	**17**	**26**	**10**	**20**

4.2 Seasonal Prevalence of Pollen and Fungi Spores in Obiakor, River State (South South) Nigeria from June 2011 to May 2012

In Obiakor, Rivers state, there was a decline of atmospheric pollen content from the month of March and throughout the rainy season (Table 4.3). Unlike the South East which showed three distinct patterns of pollen dispersal, there was a progressive increase in the atmospheric pollen content as the dry season approached from September through October, November, December, January, February to March. This corresponded however to decrease of atmospheric fungi spores (Table 4.4). Forty-seven (47) pollen types, which comprised of 31 families were identified from the aeroflora samples. Twenty-four, ninteen and four were identified to generic, species and family levels respectively. Dominant pollen included those of *Rhizophora* spp., *Cassipourea* sp., *Acrostichum aureum, Alchornea cordifolia*, Cyperaceae, Poaceae and Amarathaceae/Chenopodiaceae among others. *Rhizophora* spp. pollen were present from September to March,, achieved anthesis in December, *Cassipourea* sp. occurred from September to March. *Acrostichum aureum* spore was present from August to May. *Alchornea cordifolia* pollen grains were present from October to March. Poaceae pollen were present in June, October to May, achieved anthesis in December. Amarathaceae/ Chenopodiaceae pollen were present from October to March. *Elaeis guineensis* pollen occurred almost throughout the year. *Cassia* spp. pollen occurred from June to July and also from October to April.

Airborne pollen became more dominant during the dry season, from September to March. December (741), January (713) and February (610) had the highest records of pollen, whereas June (38), July (24) and August (27) had lowest record of pollen (Table 4.3).

Sixteen fungal spores types were identified from the aeroflora samples (Table 4.4). Months of June, July and August had higher records of fungi spores than other rainy months. The spores of Torulla sp., *Microsporium* sp., *Venturia* sp., *Alternaria* sp., *Spilocea* sp., *Murogenella* sp., *Diplosporium* sp. were very sporadically distributed over the months whereas the spores of *Spadicoides* spp., *Cladosporium* spp., *Curvularia* spp. and *Puccinia* spp. were more abundant during the rainy season from June to August. *Nigrospora* spp. occurred throughout the year.

Table 4.3: Atmospheric pollen count of Obiakor, River State (South South) Nigeria from June 2011-May 2012

S/N	POLLEN/FERN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	*Acrostichum aureum* (L.)	0	0	0	4	5	0	21	23	24	35	20	8
2	*Albizia* spp.	0	0	0	0	0	1	2		1	1	2	0
3	*Alchornea cordifolia* (Schum and Thonn)	0	0	0	0	40	61	125	445	333	41	22	31
4	Amarathaceae/Chenopodiaceae	0	0	0	0	1	1	1	22	18	5	0	0
5	*Anthocleista* sp.	0	0	0	0	0	1	0	9	0	0	0	2
6	*Antrocaryon micraster* (A. Chev.)	0	0	0	0	1	0	0	0	0	0	0	0
7	*Avicennia* sp.	0	0	0	0	0	0	0	0	0	16	0	0
8	*Berlinia tomentella* (keay)	0	0	0	0	1	1	0	0	0	1	0	0
9	*Cassia* spp.	2	1	0	0	9	12	5	3	18	2	5	0
10	*Cassipourea* sp.	0	0	0	7	12	6	22	9	10	13	0	0
11	*Casuarina equisetifolia* (L)	0	0	0	0	7	2	0	1	2	3	0	0
12	*Celtis zenkeri* (Engl.)	0	0	0	0	2	0	0	0	0	0	0	0
13	*Celtis zenkeri* (Engl.)	7	1	0	0	0	0	0	0	0	0	0	0
14	*Ceriops* sp.	0	0	0	0	0	0	0	0	0	1	0	0
15	*Citrus* sp.	0	0	0	0	0	1	0	0	0	0	0	0
16	*Coccinia grandis* (L.)	17	0	0	0	0	0	0	0	0	0	0	0
17	*Cocos nucifera* (L.)	0	0	0	0	5	3	0	0	0	0	3	0
18	Combretaceae	0	0	0	0	12	8	13	8	6	3	2	0
19	Cyperaceae	0	0	0	0	32	18	3	16	6	7	0	2
20	Diospyros sp.	0	0	0	0	0	0	2	0	0	0	0	0
21	*Dracaena arborea*	0	0	0	0	0	1	2	1	0	2	0	0
22	*Elaeis guineensis* (Jacq.)	15	17	20	0	13	17	20	32	14	11	10	2
23	*Eugenia* sp.	0	3	0	0	0	0	0	0	0	0	2	1
24	*Khaya ivorensis (*A. Chev.)	0	0	0	0	0	0	1	0	0	0	0	0
25	*Lannea welwitschii* (Hiern)	1	0	0	0	0	0	0	0	0	0	0	0

Table 4.3: Atmospheric pollen count of Obiakor, River State (South South) Nigeria from June 2011-May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
26	*Lonchocarpus* sp.	0	0	0	0	1	0	0	0	0	0	0	0
27	*Lophira sp*	0	0	0	0	0	0	0	2	0	1	0	0
28	*Ludwigia* sp.	0	0	0	0	0	1	0	0	0	0	0	0
29	*Mangifera indica* (L.)	0	0	0	0	0	2	0	0	0	0	1	0
30	*Maytenus* sp.	0	0	0	0	0	2	20	2	0	0	0	0
31	*Milicia excelsa* (L.)	0	0	0	0	1	5	3	14	7	1	0	0
32	*Mitragyna* sp.	0	0	0	0	0	2	0	0	0	0	0	0
33	*Monodora* sp.	0	0	0	0	0	0	0	2	0	0	0	0
34	*Montandra* sp.	0	0	0	0	0	1	0	0	0	0	0	0
35	*Nypa* sp	0	0	0	0	0		0	0	0	5	0	0
36	*Olax* sp.	0	0	0	0	0	8	5	0	0	0	0	0
37	*Pentaclethra macrophylla* (Benth)	0	0	0	0	10	0	0	0	0	0	0	0
38	*Phyllanthus* sp.	0	1	0	0	0	0	0	0	0	0	0	0
39	Poaceae	12	0	0	0	70	72	91	65	12	8	7	15
40	*Portulaca* sp.	0	0	0	0	0	1	0	0	0	0	0	0
41	*Psidium guajava* (L.)	0	0	0	0	1	0	0	0	0	0	0	0
42	*Pterocarpus* sp.	0	0	0	0		0	9	0	0	0	0	0
43	*Rhizophora* spp.	0	0	0	50	76	73	382	75	174	279	0	0
44	*Spondias mombin*	0	0	0	0	1	0	0	0	0	0	0	0
45	*Syzygium guineense*(Willd) D.C	0	1	0	0	1	0	3	1	1	3	0	0
46	*Tetrorchidium* sp.	0	0	0	0	2	0	0	1	0	0	0	0
47	*Uapaca* sp.	0	0	0	0	1	0	0	0	0	0	0	0
	TOTAL POLLEN	**38**	**24**	**27**	**61**	**283**	**294**	**741**	**713**	**610**	**411**	**74**	**69**

Table 4.4: Atmospheric fungi spores counts of Obiakor, River State (South South), 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	*Alternaria* spp.	1	0	0	0	0	0	0	0	0	0	0	0
2	*Cladosporium* spp.	96	93	110	0	0	0	0	0	0	0	0	0
3	*Curvularia* spp.	3	3	12	0	0	0	0	0	0	0	0	2
4	*Diplosporium* sp.	0	1	0	0	0	0	0	0	0	0	0	0
5	*Helminthosporium*	0	0	0	0	0	0	0	0	0	0	0	1
6	Indeterminate	0	0	0	0	3	0	0	0	0	0	0	0
7	*Microsporum* sp.	1	0	0	0	0	0	0	0	0	0	0	0
8	*Murogenella* sp.	0	3	0	0	0	0	0	0	0	0	0	0
9	*Nigrospora* spp.	16	1	15	5	26	17	18	30	42	52	60	38
10	*Pithiomyces* spp.	0	16	24	30	32	20	29	0	0	0	0	0
11	*Puccinia* spp.	11	35	0	0	1	0	0	0	0	4	0	5
12	*Spadicoides* spp.	75	31	40	20	4	0	0	0	0	0	0	6
13	*Spilocea* sp.	0	0	0	0	1	0	0	0	0	0	0	0
14	*Stemphylium* sp.	0	0	0	0	3	0	0	0	0	0	0	0
15	*Tetraploa* sp.	1	0	0	0	0	0	0	0	0	0	0	0
16	*Torulla* sp.	0	0	0	0	0	0	0	0	0	0	0	1
17	*Venturia* sp.	0	0	0	0	1	0	0	0	0	0	0	0
	TOTAL SPORES	**204**	**184**	**201**	**53**	**71**	**38**	**49**	**30**	**42**	**56**	**60**	**53**

4.3 Seasonal Prevalence of Pollen and Fungi Spores in Akoka, Lagos State (South West) Nigeria from June 2011 to May 2012

Forty- six pollen types were identified from aeroflora of Akoka, Lagos State. Twenty-nine, fifteen and two were identified to species, generic and family levels respectively. Dominant pollen include those of *Jatropha curcas*, *Lannea floccosa*, Amarathaceae/Chenopodiaceae, *Adansonia digitata, Delonix regia, Pentaclethra macrophylla, Terminalia catappa and Coccinia grandis. Jatropha curcas* pollen dominated in the months of August and September (Table 4.5). The months of August, September and October recorded higher number of pollen than the other months of the year.

Ten fungal spores types were identified, from the aerosamples (Table 4.6). The spores of *Nigrospora* sp., *Spadicoides* spp., *Torulla* sp.and *Curvularia* spp. were the most abundant. The spores of *Spadicoides* sp. and *Torulla* sp. were higher during the rainy season. There was a reduction in the quantity of spores during the dry season.

Table 4.5: Atmospheric pollen count of Akoka, Lagos State (South West) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	*Adansonia digitata*	0	0	6	2	0	0	0	0	0	32	1	5
2	*Ageratum conyzoides* (L.)	0	0	0	0	0	0	1	5	7	0	0	0
3	*Albizia* sp.	0	0	0	0	0	5	0	0	0	0	2	6
4	*Alchornea cordifolia* (schum and thonn)	0	0	1	1	0	0	0	0	0	0	8	7
5	Amaranthaceae / Chenopodiaceae	**4**	4	12	12	10	6	0	0	0	0	0	6
6	*Anacardium occidentale* (L.)	0	0	0	0	0	0	1	0	0	0	0	0
7	*Anthocleista djalonensis (*A. Chev.)	0	0	0	0	*7*	*6*	0	0	0	0	0	0
8	*Barleria* sp.	0	0	0	0	0	0	4	2	3	0	0	0
9	*Cassia* sp.	4	1	0	0	0	0	0	0	0	0	0	0
10	*Casuarina equisetifolia* (L.)	0	0	8	9	12	6	0	0	0	0	5	6
11	*Ceiba pentadra* (L.)	0	0	0	0	0	0	3	8	9	0	0	0
12	*Celtis* sp.	0	0	0	0	0	0	0	0	0	8	0	0
13	*Celtis zenkeri* (L.)	0	0	0	0	8	0	0	0	0	0	0	0
14	*Cissus* sp.	0	0	0	0	0	0	2	7	5	0	0	0
15	*Coccinia grandis*(L.	0	0	0	0	0	0	44	7	9	0	0	0
16	*Cocos nucifera* (L.)	0	0	0	0	1	0	0	0	0	0	0	0
17	*Combretum* sp.	0	0	0	0	0	0	2	0	0	0	0	0
18	*Cyperus* sp.	0	0	0	1	1	4	6	0	0	2	10	4
19	*Delonix regia*	0	0	1	1	0	0	0	0	0	32	1	0
20	*Dracaena arborea* (L.)	0	0	0	0	0	1	4	3	0	0	0	0
21	*Eugenia nodiflora* (Aubl)	0	0	0	0	10	0	0	0	0	4	0	0
22	*Eugenia* sp.	0	0	0	1	1	0	0	0	0	4	0	0
23	*Gloriosa superba* (L.)	0	0	1	1	1	0	0	0	0	0	0	0
24	*Hymenocardia acida* (Tul.)	0	0	0	0	8	9	7	0	0	0	0	0
25	*Ipomoea* sp.	0	0	20	0	0	0	1	0	0	0	0	0
26	*Jatropha curcas* (L.)	0	0	280	540	16	0	0	0	0	1	0	0
27	*Lannea floccose* (S.)	10	10	0	0	0	0	0	0	0	0	0	0

Table 4.5: Atmospheric pollen count of Akoka, Lagos State (South West) Nigeria from June 2011-May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
28	*Lannea welwitshii* (Hiern)	0	0	0	0	0	0	8	9	10	0	0	0
29	*Mangifera indica* (L.)	0	0	0	0	4	6	7	0	0	0	0	14
30	*Olax* sp.	0	0	0	0	32	0	0	0	0	0	0	0
31	*Olax gambecola* (Oliv.)	0	0	0	0	22	0	8	11	15	0	0	0
32	*Olax subscopioidea* (Oliv.)	0	0	0	0	22	0	0	0	0	28	13	0
33	*Parkia* sp.	0	0	1	0	0	0	0	0	0	1	0	0
34	*Paullinia pinnata* (L.)	0	0	0	0	0	0	1	0	0	0	0	0
35	*Pentaclethra macrophylla* (Benth)	0	0	0	0	32	12	0	0	0	0	0	0
36	*Phyllanthus* sp.	0	0	0	0	1	0	0	0	0	0	0	0
37	*Phyllanthus discoides* (L.)	0	34	32	20	1	8	0	0	0	0	0	0
38	Poaceae	14	10	10	8	12	16	12	0	0	0	0	0
39	*Polygala* sp.	0	0	4	1	0	0	0	0	0	0	0	0
40	*Rhizophora* sp.	0	0	0	0	0	0	10	0	0	0	0	0
41	*Rungia* sp.	0	0	0	0	6	0	0	0	0	0	0	0
42	*Securinga virosa* (Roxb). Baill	0	0	0	0	2	0	0	0	0	0	0	0
43	*Spondias mombin* (L.)	0	0	0	0	0	0	2	0	0	0	0	0
44	*Syzygium guinense* (Willd) D.C	6	8	0	0	0	2	0	0	0	0	0	0
45	*Terminalia catappa* (L.)	0	0	0	0	0	14	16	18	20	0	6	10
46	*Vernonia* sp.	0	0	1	1	7	8	0	0	0	4	5	0
	TOTAL	**38**	**40**	**349**	**598**	**187**	**74**	**121**	**70**	**78**	**116**	**55**	**54**

Table 4.6: Atmospheric fungi spores counts of Akoka, Lagos State (South West) Nigeria from June 2011- May 2012

S/N	FUNGI SPORES	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mch**	**Apl**	**May**
1	*Cladosporium* sp.	0	0	1	0	0	0	0	0	0	0	0	0
2	*Curvularia* sp.	0	0	10	9	6	2	0	0	0	0	13	1
3	*Diplocladiella* sp.	0	0	0	0	0	1	0	0	0	0	0	0
4	*Helminthosporium* sp.	5	3	0	0	0	1	0	0	0	0	0	0
5	*Nigrospora* sp.	5	3	10	10	6	13	0	0	0	0	1	5
6	*Puccinia* sp.	0	0	0	0	0	5	0	0	0	0	0	0
7	*Spadicoides* sp.	7	45	56	0	7	0	0	0	0	0	9	0
8	*Tetraploa* sp.	0	0	0	0	1	1	0	0	0	0	0	0
9	*Torulla* sp.	60	5	0	0	0	6	0	0	0	0	0	0
	TOTAL	**77**	**56**	**77**	**19**	**20**	**29**	**5**	**08**	**10**	**05**	**23**	**06**

4.4 Seasonal Prevalence of Pollen and Fungi Spores in Garki, Abuja (North Central) Nigeria from June 2011 to May 2012

Fifty eight (58) pollen types which comprised of thirty- six families were identified, three, twenty- six and twenty-sixpollen were identified to familial, generic and specie levels respectively (Table 4.7).

The annual contribution of fungal spores 3164 (54.98 %) to the aeroflora was found to be greater than pollen 2897 (45.01%). Higher number of pollen were recorded in the months of October 518 (17.65 %), November 472 (16.08 %) and December 354 (12.07 %) while February 124 (4.22 %) and May 96 (3.27 %) had the lowest records (Table 4.7). The major pollen contributors were *Elaeis guineensis, Lannea acida, Cassia* spp. and Poaceae, *Cochlospermum tinctorum,* Amarathaceae/Chenopodiaceae, *Luffa* sp., *Alchornea cordifolia, Khaya senegalensis* and *Pentaclethra macrophylla*. The anemophilous pollen recorded included those of Poaceae, *Cyperus esculenta,* Amarathaceae/Chenopodiaceae, *Casuarina equisetifolia, Cocos nucifera* and *Dracaena arborea*. In the months of October, through November, December and January, Pollen grains became dominant, with those not previously present in the atmosphere being found at this period. There was reduction in the number of fungal spores in the air from January to May (Table 4.8).

In the month of June, total pollen encountered was 295 whereas fungal spores were 310 (Tables 4.7 and 4.8). Dominant pollen grains were those of *Elaeis guineensis, Lannea acida*, *Cassia* spp. and Poaceae. The spores of *Tetraploa* sp. (111) and *Nigrospora* sp. (51) were more abundant during this month.

In the month of July, there was a drastic reduction of the atmospheric pollen (186) and an increase in the quantity of fungal spores (312). Eighteen fungal spore types were identified (Table 4.8). The aeroflora in August was dominated by fungal spores (510) compared to pollen (142) (Table 4.7 and 4.8). Several species of fungi spores which were not previously present were also recorded in these months. These include *Erysiphe graminis*, *Aspergillus* sp. and *Fusarium* sp.

Table 4.7: Atmospheric pollen count of Garki, Abuja (North Central) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	*Acacia* sp.	2		2	2	0	0	0	0	0	0	0	0
2	*Adansonia digitata*	0	0	0	0	0	12	4	0	0	0	0	0
3	*Ageratum conyzoides* (L.)	0	0	0	0	12	0	0	16	0	0	0	0
4	*Albizia* sp.	0	2	0	0	0	0	0	0	0	0	0	0
5	*Alchornea cordifolia* (Schum and Thonn)	1	0	0	2	12	52	8	2	16	4	6	0
6	*Aloe bateri*	0	0	0	3	0	0	0	0	0	0	0	0
7	Amarathaceae / Chenopodiaceae	0	0	44	0	8	16	0	4	0	0	0	0
8	*Aneilema beninlense*	0	2	0	0	0	0	0	0	0	0	0	0
9	*Anthocleista djalonensis* (A. Chev.)	0	0	0	4	0	0	0	20	0	0	0	0
10	*Aspilia africana* (Pers CD. Adam)	0	0	6	2	22	8	0	0	0	0	1	6
11	Asteraceae	4	0	0	0	0	0	0	0	0	0	0	0
12	*Bridelia ferruginea* (Benth)	0	0	0	0	24	0	0	0	0	0	0	0
13	*Bulbostylis* sp.	0	0	0	0	0	0	4	0	0	0	0	0
14	*Cassia* sp.	28	0	0	4	10	8	0	0	8	30	16	56
15	*Casuarina equisetifolia* (L.)	1	6	2	0	0	0	0	0	0	8	6	4
16	*Ceiba pentandra* (L.)	0	2	0	0	0	0	0	0	0	0	0	0
17	*Ceiba Pentandra* (L.)	0	2	0	0	0	0	0	0	0	0	0	0
18	*Celtis* sp.	0	0	0	0	0	16	20	0	0	0	0	0
19	*Cissus* sp.	0	0	0	2	0	0	0	0	0	0	0	0
20	*Cochlospermum tinctorum* A. Rich	0	16	0	0	0	0	42	0	0	0	0	0
21	*Cocos nucifera* (L.)	4	0	0	0	0	0	0	0	0	0	0	0
22	*Crotalaria* sp.	0	0	0	0	0	0	0	6	4	0	0	0
23	*Cyperus* sp.	0	0	0	0	4	44	4	1	10	6	2	2
24	*Dichrostachys* sp.	0	0	0	0	0	0	2	8	0	0	0	0
25	*Dracaena arborea* (L.)	0	0	8	2	0	0	0	0	0	0	0	0
26	*Drypetes* sp.	0	0	0	0	0	0	2	0	0	0	0	0
27	*Eichornia* sp.	2		0	0		0	0	0	0	0	0	0
28	*Elaeis guineensis* (Jacq)	118	6	8	5	6	206	8	8	0	0	0	0
29	*Eugenia* sp.	0	0	0	0	2	0	0	0	0	0	0	0
30	*Gardenia* sp.	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.7: Atmospheric pollen count of Garki, Abuja (North Central) Nigeria from June 2011-May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
31	*Gloriosa superba* (L.)	0	0	2	32	0	0	2	6	0	0	0	0
32	*Hexalobus* sp.	0	0	0	0	0	0	8	0	0	0	0	0
33	*Hymenocardia acida* (Tul.)	0	2	4	12	12	0	0	0	6	0	20	0
34	*Ipomoea* sp.	0	0	0	0	0	0	0	8	40	0	0	0
35	*Justicia extensa* (T. Anders)	0	0	2	0	0	0	0	0	0	0	0	0
36	*Justicia* sp.	0	8	6	0	0	0	0	0	0	0	0	0
37	*Justicia* sp.	0	0	0	0	0	2	4	13	18	134	14	12
38	*Khaya senegalensis* (Desr.) A. Juss	0	0	2	18	30	48	0	0	0	0	0	0
39	*Lannea acida* (A. Rich)	63	6	2	0	0	0	0	0	0	0	0	0
40	*Lannea welwitschii* (Hiern)	0	0	2	0	0	0	0	0	0	0	0	0
41	*Lophira* sp.	0	0	0	0	2	0	0	0	0	0	0	0
42	*Luffa* sp.	0	0	0	21	0	2	0	6	12	26	73	2
43	*Marantochloa* sp.	0	0	2	0	0	0	0	0	0	0	0	0
44	*Milletia* sp.	2	0	0	0	0	0	0	0	0	0	0	0
45	*Olax subscorpioides* (Oliv.)	0	0	0	0	0	0	12	12	0	0	0	0
46	*Parkia* sp.	2	0	0	0	4	0	0	16	0	0	0	0
47	*Parkia biglobosa* (Benth)	0	0	0	0	2	0	0	0	0	0	0	0
48	*Pentaclethra macrophyla* (Benth)	0	0	4	0	120	36	188	54	0	0	0	0
49	*Phyllanthus discoides (*Meull.)	2	0	0	0	0	0	0	0	0	0	0	0
50	Poaceae	48	44	44	68	248	20	28	16	8	0	0	0
51	*Polygala* sp.	0	0	2	0	0	0	2	0	0	0	0	0
52	*Solenostemon* sp.	0	0	0	0	0	0		2	0	0	0	0
53	*Syzygium* sp	0	0	0	0	0	0	12	2	0	0	0	0
54	*Terminalia* sp.	4	0	0	0	0	0	2	0	0	0	0	0
55	*Uapaca togoensis* (Pax)	14	0	0	4	0	0	0	0	2	2	8	14
56	*Vernonia* sp.	0	0	0	0	0	0	0	12	0	0	0	0
57	*Vigna multinervis* (Hutch and Daziel)	0	0	0	0	0	2	0	0	0	0	0	0
	TOTAL	**295**	**186**	**142**	**179**	**518**	**472**	**354**	**212**	**124**	**210**	**146**	**96**

Table 4.8: Atmospheric fungi spores counts of Garki, Abuja (North Central) Nigeria from June 2011 to May 2012

S/N	FUNGAL SPORES	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Alternaria* sp.	14	4	0	6	0	0	0	0	0	0	18	28
2	*Apiosporina* sp.	0	0	0	0	2	0	0	0	0	0	0	0
3	*Aspergillus* sp.	0	0	2	0	0	0	1	0	0	0	0	0
4	*Cereosporella* sp.	3	0	0	0	0	0	0	0	0	0	0	0
5	*Cladosporium* sp.	15	24	0	0	45		8	4	0	0	0	0
6	*Curvularia* sp.	0	4	12	9	96	2	0	18	9	0	0	0
7	*Erysiphe graminis*	0	0	2	0	0	1200	24	9	0	0	0	0
8	*Fusarium* spp.	0	0	6	0	0	20	0	0	0	0	0	0
9	*Hansfordiella* sp.	37	123	90	6	64	0	0	0	0	0	0	0
10	*Helminthosporium* sp.	6	0	6	30	0	6	8	0	0	0	0	0
11	Indeterminate	0	2	0	21	0	0	0	0	0	0	0	0
12	*Nigrospora* sp.	51	6	48	12	45	12	0	6	0	24	10	10
13	*Pithiomyces* sp.	12	15	20	20	54	6	36	0	0	0	0	0
14	*Puccinia* sp.	14	28	309	60	43	38	86	4	3	0	0	0
15	*Spadicoides* sp.	0	75	0	30	46	0	0	0	0	20	0	0
16	*Sporidesmium* sp.	32	0	0	0	165	0	0	0	0	0	0	0
17	*Tetraploa* sp.	111	3	9	3	2	0	12	0	0	0	0	0
18	*Torulla* sp.	17	28	6	12	3	1	1	0	0	0	0	0
19	*Venturia* sp.	0	0	0	1	8	0	0	0	0	0	0	0
	TOTAL	**310**	**312**	**510**	**210**	**573**	**1285**	**176**	**41**	**12**	**44**	**28**	**38**

4.5 Seasonal Prevalence of Pollen and Fungi Spores in Zaria, Kaduna State (North West) Nigeria from June 2011 to May 2012

Pollen were more dominant in the atmosphere in the months of May through June, July, August, September to October than in the months of November, December, January and February. Poaceae pollen were the major contributors in the months of June, July, August, September and October but they decreased in quantity in the subsequent months. *Syzygium guineense*, *Hymenocardia acida, Combretum* sp., *Albizia* sp. and *Khaya senegalensis* were next to Poaceae in abundance (Table 4.9).

Thirty two pollen types were recorded for the period of one year and these belong to twenty (20) families. *Elaeis guineensis* pollen were very sporadic, like those of *Tetrapleura tetraptera* and *Lannea* spp. Herbaceous pollen recorded were mainly those of *Cyperus* spp., *Ipomoea* sp. and Amarathaceae/Chenopodiaceae (Table 4.9).

Twenty fungi spore types were recorded (Table 4.10). Most dominant spores were those of *Nigrospora* sp., *Microsporium* sp. and *Cercospora* sp. Other spores which were sporadic include those of *Spadicoides* sp., *Heminthosporium* sp. and *Puccinia* sp. these were also found to be dominant in aero samples from the study locations of Southern Nigeria.

Table 4.9: Atmospheric pollen counts of Zaria, Kaduna (North West) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	Acacia sp.	4	0	0	2	4	9	8	0	6	0	0	0
2	*Ageratum conyzoides* (L.)	4	2	0	0	2	7	3	2	0	0	2	2
3	*Albizia* sp.	12	152	6	0	2	0	0	0	0	0	58	4
4	Amarathaceae /Chenopodiaceae	14	0	1	2	0	0	0	1	6	7	21	23
5	*Anacardium occidentale* (L.)	0	2	0	7	8	0	0	0	0	0	0	0
6	*Cassia hirsute* (L.)	0	0	8	25	12	0	0	0	0	0	0	0
7	*Cassia mimosoides* (L.)	0	0	0	0	0	0	0	0	0	0	0	0
8	*Cochlospermum planchonii* (Hook)	0	0	0	4	0	0	0	0	0	0	0	0
9	*Combretum grandiflorum* (G.Don.)	0	0	6	7	9	10	21	0	0	0	0	0
10	*Costus* sp.	0	0	0	2	0	0	0	0	0	0	0	0
11	*Cyperus* sp.	0	0	0	25	5	24	10	10	0	4	0	0
12	*Dracaena arborea* (L.)	0	0	4	4	2	5	0	0	0	0	0	0
13	*Elaeis guinensis* (Jacq)	0	0	0	0	2	0	0	0	0	0	0	0
14	*Hymenocardia acida* (Tul.)	99	38	11	0	2	7	8	0	0	5	10	
15	*Ipomoea* sp.	0	0	0	6	7	5	0	0	0	0	0	0
16	*Justicia extensa* (T.)	0	0	5	0	7	6	0	0	0	0	0	0
17	*Justicia* sp.	0	0	0	12	0	0	0	0	0	0	0	0
18	*Khaya senegalensis* (Ders.) A. Juss	0	0	0	0	27	15	11	0	0	0	4	5
19	*Lannea acida* (A. Rich.)	30	12	0	0	0	0	0	0	0	0	2	
20	*Lannea* sp.	0	0	0	3	3	0	0	0	0	0	0	0
21	*Lophira lanceolata* (Tiegh)	0	0	0	0	4	0	0	0	0	0	0	0
22	*Parkia bicolor* (A. Chev.)	0	0	0	0	0	0	0	0	0	5	0	0
23	*Parkia* sp.	0	0	5	6	0	0	0	0	0		0	0
24	*Parkia* sp.	0	0	0	0	0	0	0	4	0	2	0	0
25	*Pentaclethra macrophylla* (Benth)	0	8	4	4	0	0	0	2	0	4	10	2
26	*Phyllanthus* sp.	167	38	3	0	0	0	0	0	0	27	26	1
27	Poaceae	1982	1230	1056	71	27	2	1	1	3	0	0	1200
28	*Podocarpus* sp.	0	0	0	0	0	0	2	0	0	0	0	0
29	*Syzygium* sp.	28	46	36	0	0	0	6	0	0	6	42	6
30	*Terminalia glaucescens* (Planch)	0	0	0	0	0	0	0	0	0	5	0	0
31	*Tetrapleura tetraptera* (Schumach and Thonn0	0	10	2	0	0	0	0	0	0	0	0	0
32	*Trichilia roka* (Chiov.)	0	0	0	0	12	12	32	0	0	1	0	0
	TOTAL POLLEN	**2538**	**1526**	**1147**	**180**	**135**	**102**	**110**	**20**	**15**	**66**	**175**	**1243**

Table 4.10: Atmospheric fungi spores counts of Zaria, Kaduna State (North West) 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	*Cecospora* sp.	5	30	0	0	2	0	0	1	0	3	2	0
2	*Cladosporium* sp.	0	0	0	0	0	0	0	0	0	1	0	0
3	*Curvularia* sp.	0	0	1	4	0	0	0	0	0	0	0	0
4	*Curvularia* sp.	0	0	0	0	0	0	0	0	0	0	9	0
5	*Diplocladiella* sp.	0	1	0	0	1	0	0	0	0	0	0	0
6	*Diplocladiella* sp.	0	0	0	0	0	0	0	0	0	1	0	0
7	*Dreschlera* sp.	0	0	0	0	0	0	0	0	0	1	0	0
8	*Helminthosporium* sp.	1	2	2	0	1	0	0	0	0	1	0	0
9	*Microsporium* sp.	26	33	3	0	1		1	0	0	0	0	0
10	*Murogenella* sp.	0	1	0	0	0	0	0	0	0	0	0	0
11	*Nigrospora* sp.	20	43	6	7	7	2	0	0	0	3	0	3
12	*Phragmidium* sp.	0	0	0	0	4	0	0	0	0	0	0	0
13	*Pithiomyces* sp.	0	0	0	2	3	0	0	0	0	0	4	0
14	*Puccinia* sp	2	2	0	7	2	0	0	0	0	3	7	0
15	*Spadicoides* sp.	0	1	2	2	5	0	0	0	0	5	0	0
16	*Sporidesmium* sp.	0	0	0	4	0	0	0	0	2	4	0	0
17	*Therry fulkelii*	0	24	0	0	3	0	0	0	0	0	0	0
18	*Torulla* sp.	0	0	0	1	1	0	0	0	0	0	4	0
	TOTAL	**54**	**137**	**14**	**27**	**30**	**2**	**1**	**1**	**2**	**22**	**26**	**3**

4.6 Seasonal Prevalence of Pollen and Fungi Spores in Dukku, Gombe State (North East) Nigeria from June 2011-May 2012

Twenty seven pollen types belonging to eighteen families were recorded at Gombe. Ten, fifteen and two were identified to specific, generic and familial levels respectively. The major contributors of the pollen rain include Poaceae, *Lannea welwitschii*, *Ceiba pentandra*, *Parkia bicolor and Vitex doniana* among others (Table 4.11).

The pollen taxa recorded showed a close resemblance with those of Kaduna. Atmospheric pollen load declined from the month of September through October, November, December, January and February and increased again in the months of April and October. The highest risk period for pollen hypersensitive individuals was April – August.

Fourteen fungi spore types were recorded (Table 4.12). Airborne fungi spores were more concentrated between the months of August and October. Spores of fungi were very sporadically distributed throughout the month. Compared to Southern study locations, the number of *Spadicoides* sp. was lower. *Alternaria* sp. whose presence has been noted to be frequently associated with the rainy season were not found in North West and North East study locations.

Table 4.11: Atmospheric pollen count of Dukku, Gombe (North East) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Acacia mellifera* (Benth)	2	0	0	0	0	2	0	3	0	1	1	6
2	*Adansonia digitata* (L.)	0	0	0	0	0	0	0	0	0	4	1	14
3	Amaranthaceae /Chenopodiaceae	0	0	0	1	0	0	0	0	0	0	0	0
4	*Anthocleista* sp.	0	0	0	1	1	1	0	0	0	0	0	0
5	*Berlinia* sp.	1	10	0	2	0	0	0	0	0	0	23	0
6	*Blepharis* sp.	0	0	0	0	0	0	0	0	0	2	1	0
7	*Borreria* sp.	0	0	0	1	0	0	0	0	0	0	0	0
8	*Cassia nigricans* (L.)	0	0	0	0	0	0	0	0	0	2	0	0
9	*Cassia* sp	0	0	1	0	0	0	0	0	0	0	0	0
10	*Ceiba pentandra* (L.)	12	14	4	1	1	1	0	1	1	0	2	7
11	*Gmelina* sp.	0	0	1	0	0	0	0	0	0	0	0	0
12	*Justicia* sp.	0	0	1	1	1	1	9	11	10	25	0	0
13	*Khaya grandifolia* (A. Chev.)	0	0	0	0	0	0	0	0	0	2	2	1
14	*Khaya senegalensis* (A. Chev.)	0	0	0	1	0	0	0	0	0	0	0	0
15	*Lannea acida* (A. Rich.)	50	36	18	1	0	0	0	0	0	19	22	28
16	*Lannea welwitschii* (A. Rich.)	28	17	0	0	0	0	0	0	0	19	7	19
17	*Milletia* sp.	0	0	0	0	1	0	0	0	0	0	0	0
18	*Ocimum* sp.	0	0	0	2	1	0	0	0	0	0	0	0
19	*Parinari* sp.	7	6	0	0	0	0	0	0	0	1	5	0
20	*Parkia bicolor* (Merr)	26	15	4	0	0	4	0	0	0	20	19	85
21	*Pentaclethra macrophylla* (Benth0	17	0	1	1	2	3	0	0	0	1	0	6
22	Poaceae	207	1195	115	95	204		1		5	11	3	0
23	*Podocarpus* sp.	0	0	0	0	0	0	0	0	0	1	0	0
24	*Protea* sp.	0	0	0	0	0	0	0	0	0	1	0	0
25	*Pterocarpus* sp.	7	19	0	0	0	0	0	0	0	1	2	3
26	*Vignia* sp.	0	0	0	0	0	0	0	0	0	1	0	0
27	*Vitex* sp.	18	14	15	14	8	8	2	18		25	0	2
	TOTAL POLLEN	**1375**	**1326**	**160**	**121**	**219**	**20**	**12**	**33**	**16**	**136**	**88**	**171**

Table 4.12: Atmospheric fungi spores counts of Gombe (North East) Nigeria from June 2011 to May 2012

S/N	FUNGAL SPORES	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Cercospora* sp.	0	0	0	0	2	0	0	0	0	0	0	0
2	*Curvularia* sp.	0	0	0	0	1	0	0	0	0	0	0	0
3	*Didymella* sp.	0	0	1	0	0	0	0	0	0	0	0	0
4	*Fusarium* sp.	0	0	0	14	0	0	0	0	0	0	0	0
5	*Helminthosporium* sp.	0	0	1	0	0	0	0	0	0	0	3	0
6	Indeterminate	0	0	1	0	20	0	0	0	0	0	0	0
7	*Microsporium* sp.	0	0	1	0	2	0	0	0	0	0	0	0
8	*Nigrospora* sp.	0	0	45	0	0	0	0	0	0	0	12	0
9	*Nigrospora* sp.	0	0	1	0	1	0	0	0	0	0	0	0
10	*Phragmidium* sp.	0	0	1	0	0	0	0	0	0	0	0	0
11	*Pithiomyces* sp.	0	0	15	1	0	0	0	0	0	0	0	0
12	*Puccinia* sp.	0	0	2	1	0	0	0	0	0	0	0	0
13	*Spadicoides* sp.	0	0	1	0	0	0	0	0	0	0	0	0
14	*Sporidesmium* sp.	0	0	0	0	4	0	0	0	0	0	0	0
15	*Torulla* sp..	0	0	0	1	5	0	0	0	0	0	0	0
	TOTAL SPORES	**0**	**0**	**69**	**17**	**35**	**0**	**0**	**0**	**0**	**0**	**15**	**0**

4.7 Seasonal Variations of Pollen, Fungal Spore Types and Charred Poaceae Epidermis Among the Six Geopolitical Zones of Nigeria

Poaceae pollen was the most abundant anemophilous pollen in all studied locations but were more abundant in North West and North East Nigeria (Fig. 4.1), during the rainy months than the drier months (Tables 4.9 and 4.11). In South East Nigeria, Poaceae pollen grains dominated from June to November. In South South, they dominated from October to January. They were present from June to December in South West study location.

The herbaceous pollen encountered in the atmosphere of Nigeria were largely majorly those of family Chenopodiaceae/Amaranthaceae, Cyperaceae, Asteraceae (Tubuliflorae and Liguliflorae complex) as well as those of *Crotalaria* sp. However, their annual contributions represented a smaller percentage of the pollen load (Fig. 4.2).

The recorded airborne pollen were placed in three categories, grasses, herbaceous and trees/ shrubs on the basis of their sources. Trees and shrubs pollen were more dominant in Southern than in Northern Nigeria and constituted the bulk of atmospheric pollen load. They were less dominant in North West and North East and constitutes half of the total pollen in North Central Nigeria (Table 4.2). Trees /shrubs pollen dominated from September-February, October-May and August to March in South East, South South and South West Nigeria respectively. The prevalence of trees/shrubs pollen was recorded between the months of September and April in North Central region. In the North West and North East, most trees/shrubs pollen made their appearance in the atmosphere from the month of August and were distributed throughout the year, though sporadic. The dominant trees/shrubs pollen in North West Nigeria include those of *Albizia* spp., *Syzygium guineense*, *Hymenocardia acida*, *Pentaclethra macrophylla* and

Phyllanthus sp. among others. In North East, *Lannea acida*, *Berlinia* sp., *Ceiba pentandra*, *Pterocarpus* sp. and *Parkia bicolor* were more abundant. In North Central Nigeria, *Elaeis guineensis*, *Ceiba pentandra*, *Alchornea cordifolia*, *Khaya senegalensis* and *Pentaclethra macrophylla* were recorded. In the South East, *Elaeis guineensis*, *Pentaclethra macrophylla*, *Alchornea cordifolia*, *Albizia* sp., *Mangifera indica* and *Casuarina equisetifolia* dominated. In the South South, *Rhizophora* spp., *Cassipourea* sp., *Alchornea cordifolia*, *Elaeis guineensis*, *Milicia excelsa* and *Cassia* spp. dominated. In South West, *Jatropha curcas*., *Lannea floccusa*, *Casuarina equisetifolia*, *Adansonia digitata*, *Pentaclethra macrophylla* and *Terminalia catappa* were in abundance.

Anemophilous pollen dominated the atmosphere of the Northern Nigeria and was mainly represented by Poaceae pollen, whereas enthomophilous pollen dominated in the Southern Nigeria (Fig 4.3 and 4.4).

Based on varied morphotypes assessment of flora component, the order of pollen predominance of the six geopolitical zones was; South East was greater than North Central, North Central greater than South South, South South was greater than South West, South West was greater than North West and North West greater than North East (Table 4.13).

Podocarpus sp. was recorded in North West in the month of November (Plate 4.1p)**.** The source of this bisaccate pollen is most likely the Cameroun mountain range or its extension to Nigeria.

Fungal spores were predominantly recorded in Southern Nigeria during the rainy season, their quantity declined in dry season. Fungal spore load in North- West and North- East were fewer and were sporadically distributed especially in North East (Table 4.14). Charred Poaceae epidermis was not recorded in studied location of South West, they were more dominant in Northern Nigeria especially in North West and North East (Table 4.15).

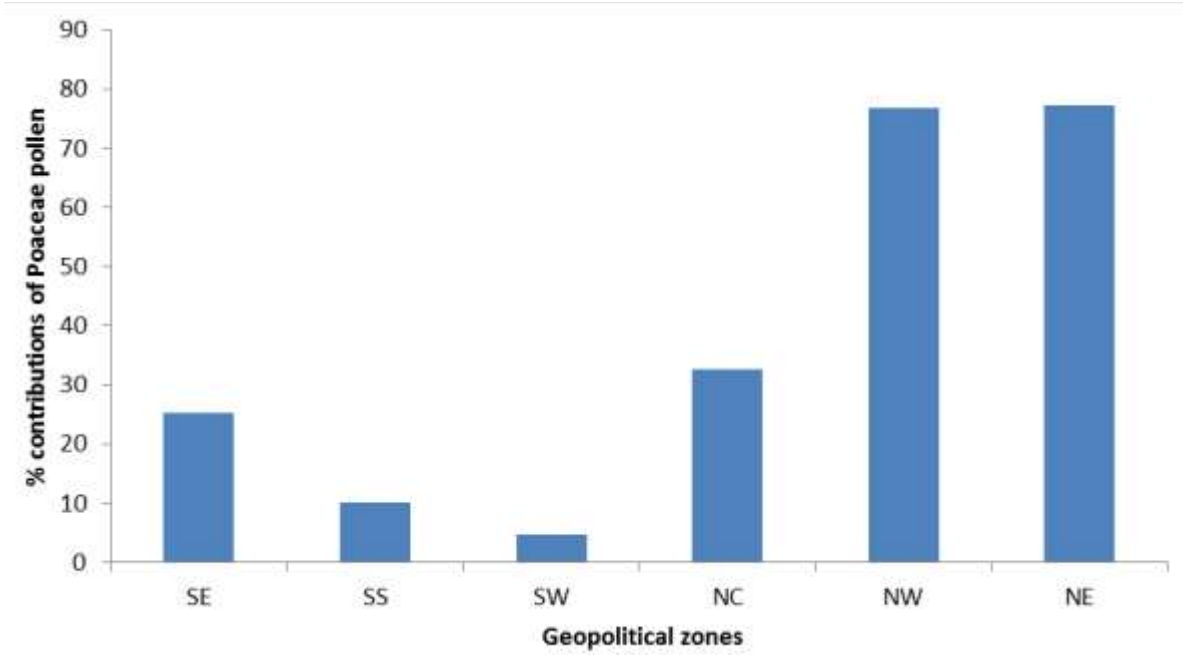


Fig. 4.1: Annual Percentage (%) contributions of Poaceae pollen in the six geo-political zones of Nigeria from June 2011- May 2012

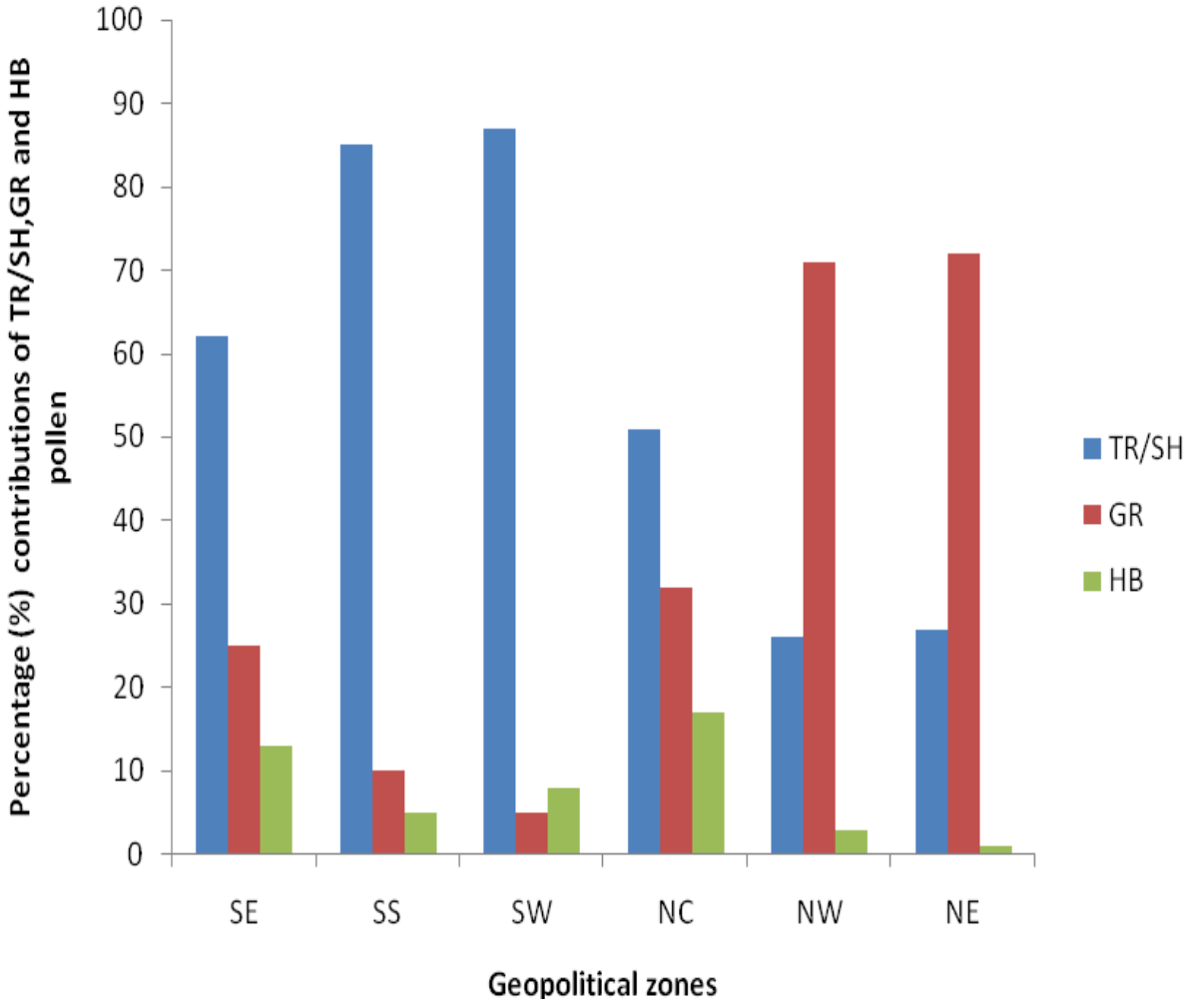


Fig . 4.2: Percentage contribution of trees/shrub, grasses and herbaceous pollen

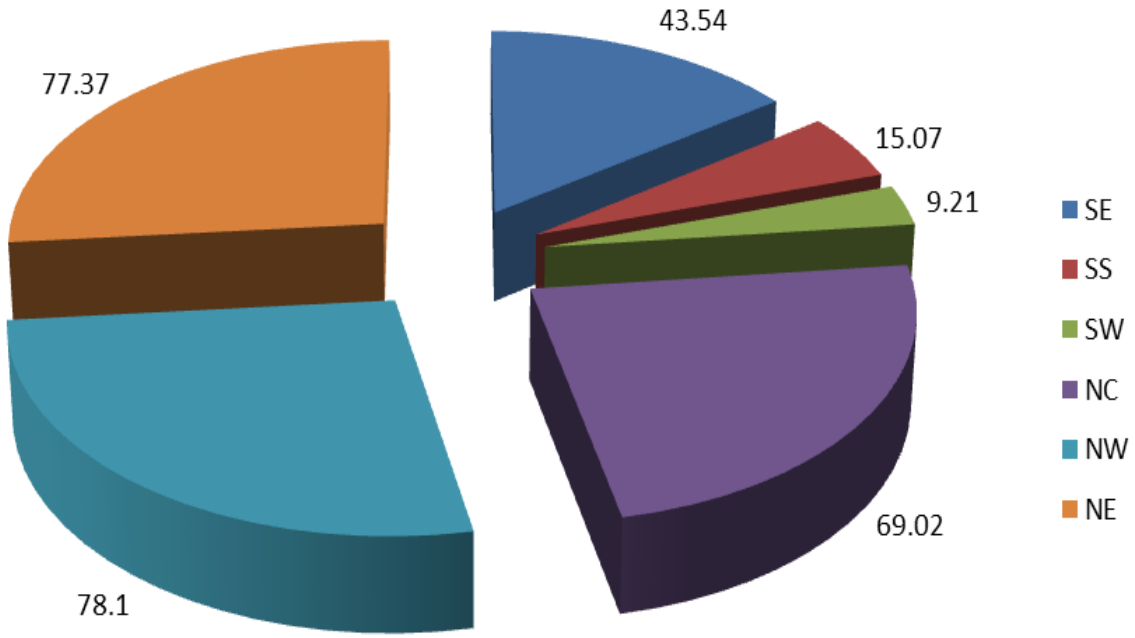


Fig. 4.3: Annual contributions in degree of anemophilous pollen in the six geo-political zones of Nigeria

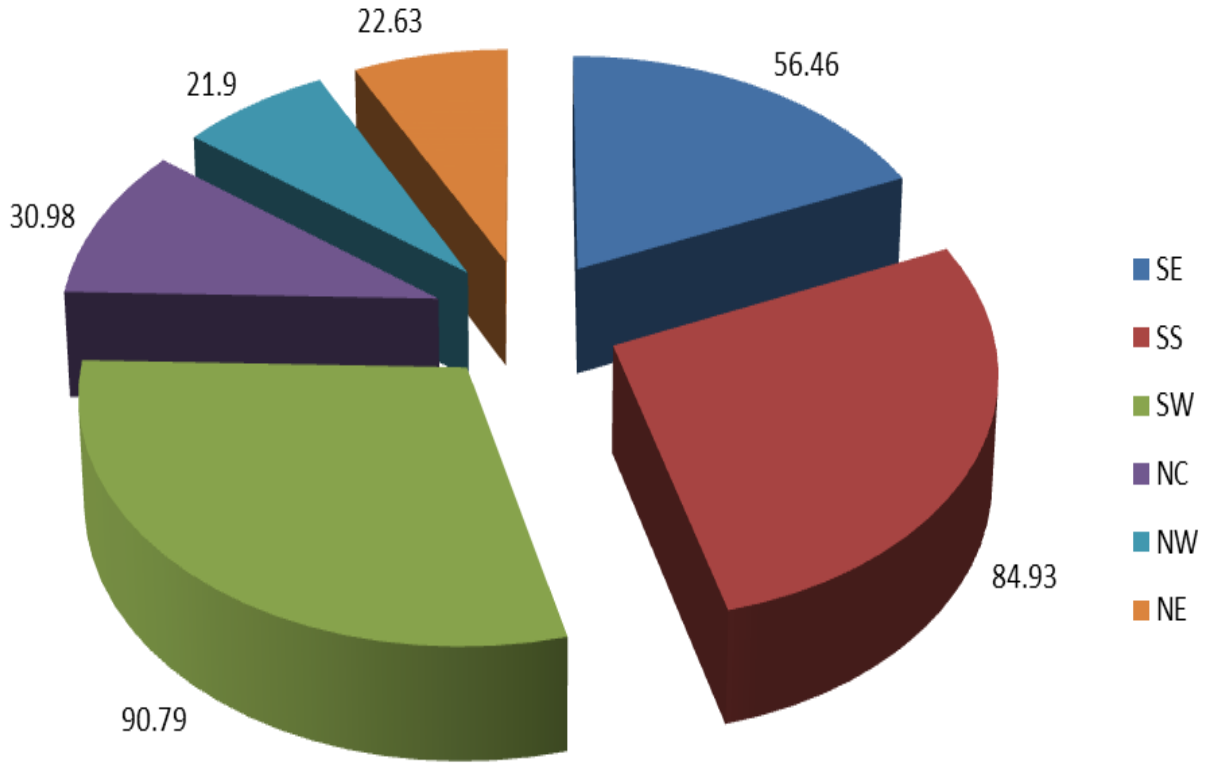


Fig. 4.4: Annual contributions in degree of entomophilous pollen in the six geo-political zones of Nigeria

Table 4. 13: **Total airborne pollen in the six geopolitical zones of Nigeria for one year (June 2011- May 2012)**

MONTHS / LOCATIONS	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
SE	128	230	81	380	372	198	292	64	284	130	449	31
SS.	38	24	27	61	283	294	741	713	610	411	74	61
SW	38	40	349	598	187	74	121	70	78	116	55	54
NC	295	186	142	179	518	472	354	212	124	210	146	96
NW	2538	1526	1147	180	135	102	110	20	15	66	175	1243
NE	1375	1326	160	121	216	14	12	33	16	136	88	171

SE- South- East

SS- South-South

SW- South West

NC- North -Central

NW- North West

NE- North East

Table 4. 14: **Total airborne fungal spores counts in the six geopolitical zones of Nigeria for one year (June 2011- May 2012)**

MONTHS / LOCATIONS	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
SE	**295**	**579**	**118**	**214**	**607**	**170**	**32**	**121**	**17**	**26**	**10**	**20**
SS.	**204**	**184**	**201**	**53**	**71**	**38**	**49**	**30**	**42**	**56**	**60**	**53**
SW	**77**	**56**	**77**	**19**	**20**	**29**	**5**	**08**	**10**	**05**	**23**	**06**
NC	**310**	**312**	**510**	**210**	**573**	**1285**	**176**	**41**	**12**	**44**	**28**	**38**
NW	**54**	**137**	**14**	**27**	**30**	**2**	**1**	**1**	**2**	**22**	**26**	**3**
NE			**69**	**17**	**35**						**15**	

SE- South- East

SS- South-South

SW- South West

NC- North -Central

NW- North West

NE- North East

Table 4. 15: Charred Poaceae cuticles recorded from the six geopolitical zones

MONTHS / LOCATIONS	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
SE	0	8	0	0	0	20	15	16	12	8	2	1
SS.	2	2	0	0	21	23	35	20	60	2	0	0
SW	0	0	0	0	0	0	0	0	0	0	0	0
NC	0	0	8	28	20	43	15	5	3	0	0	0
NW	0	12	10	75	50	0	0	0	0	52	14	0
NE	10	10	70	24	0	10	0	0	110	24	0	0

SE- South- East

SS- South-South

SW- South West

NC- North -Central

NW- North West

NE- North East

4.8 Relationship between Airborne Pollen, Fungi Spores and Weather Parameters

The distributional pattern of weather parameters vary throughout the year in the six geopolitical zones of Nigeria. Rainfall records were higher in Southern Nigeria, especially in South South which had rainfall records throughout the year. The months of November and December had no record of rainfall in South East Nigeria. The month of December also had no record of rainfall in South West Nigeria. Humidity was comparatively higher in Southern Nigeria, it varied between 55 % - 85 % in South East, 70.3 % - 91 % in South South, 81 % - 89 % in South West Nigeria. Average temperature ranges between 25.5 ^{0}C (August) -28.7 ^{0}C (February) in South East Nigeria, 25.2 ^{0}C (August) - 27.9 ^{0}C (March) in South South and 25.5 ^{0}C (August) - 28.5 ^{0}C (March) in South West Nigeria. Wind speed (knot) was higher in December (6.5) in South East, in South South wind speed was the same from January to May (2.4), other months had lower values. The month of August had the highest wind speed (7.5) in South West. In Northern Nigeria, more rainy months occurred in North Central than in North West and North East Nigeria. There was no record of rainfall in North West and North East Nigeria from the month of November to March . Humidity was higher in North Central than other Northern zones, a range of 38% - 88%. North West and North East had a range of 19 % - 82 % and 20 % - 64 % respectively. Average temperature was higher in Northern versus Southern Nigeria. In North Central, highest temperation was recorded in May (34.3 ^{0}C) and lowest recorded in August (24.7 ^{0}C). In North West highest temperature occcurred in April (28.8 ^{0}C) and lowest occurred in February (22.4 ^{0}C) and in North East Nigeria highest temperature was recorded in March (28.0^{0}C) and lowest recorded in September (24.0 ^{0}C).

The distribution and abundance of airborne pollen and fungi spores also varied over the six geopolitical zones and were highly modulated by weather parameters. There was a direct

relationship between the varied morphotypes of airborne fungi spores and monthly rainfall whereas airborne pollen had an indirect relationship with monthly rainfall in the studied locations of Southern Nigeria (Fig. 4.5). In the South East, pollen abundance correlated with a decrease in atmospheric rainfall whereas spores were more abundant during the rainy season from the month of June to October. In Obiakor (Rivers State), South South Nigeria, a gradual decrease in rainfall correlated with influx of pollen morphotypes and their quantity from October to March (Fig. 4.5). In Akoka, Lagos State, more fungi spores counts were recorded during the rainy period from the month of June to August. The quantitative abundance of airborne pollen corresponded with a decline in rainfall from August (the short dry season between the two rainfall maxima) to October. The high morphotypes and quantitative abundance of fungi spores were recorded during the rainy season in all studied locations of Southern Nigeria (Fig. 4.6). The most dominant spores during the rainy season were *Torulla* sp., *Fusarium* sp., *Cladosporium* sp. *Spadicoides* sp., *Puccinia* sp. among others.

In Garki, Abuja, North Central Nigeria, pollen dominated during the dry season when the monthly rainfall was lower. In the North West and North East there were records of high morphotypes and quantitative abundance of pollen during the more rainy months (Fig.4.7), which was majorly populated by Poaceae pollen. The decrease of the abundance of Poaceae pollen correlated with influx of other non poaceae pollen such as; *Albizia* sp., *Acacia* sp., *Syzygium* sp., *Khaya senegalensis* etc. in North West whereas *Lannea acida*, *Gmelina arborea*, *Lannea welwitschii*, *Ceiba petandra*, *Vitex doniana* correlated with decrease of Poaceae pollen in North East Nigeria.

Low fungal spores record correlated with low monthly rainfall and humidity in North West and North East(Fig. 4.8)

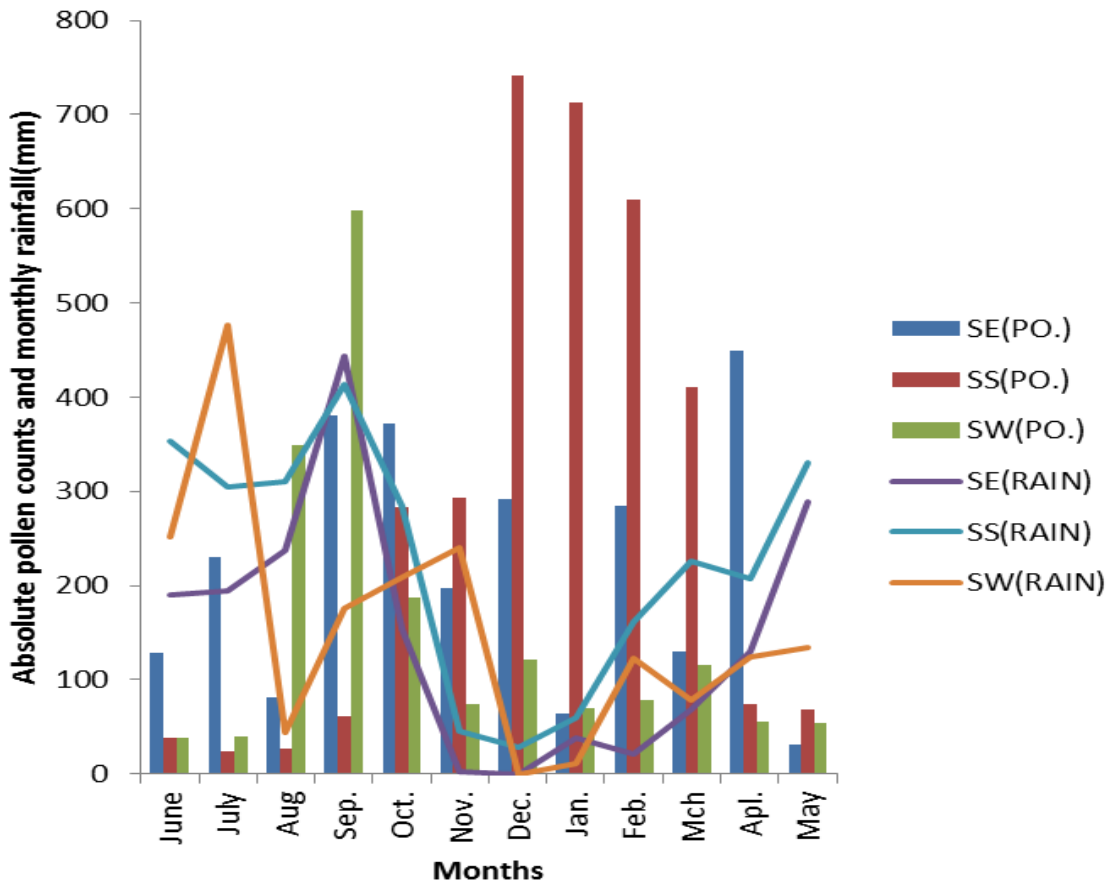


Fig 4.5: Relationship between atmospheric pollen and monthly rainfall (mm) in South East, South South and South West Nigeria

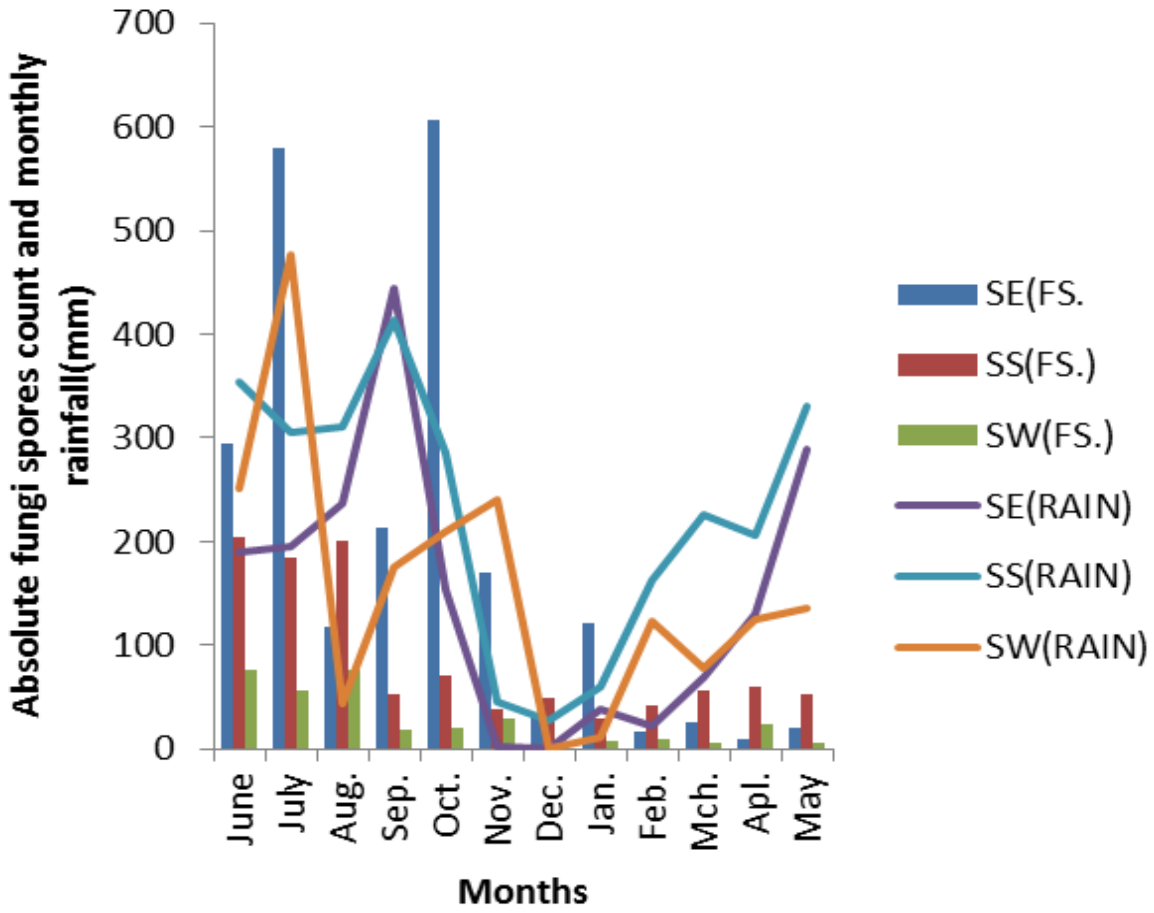


Fig 4.6: Relationship between atmospheric fungi spores and monthly rainfall (mm), temperature ^{o}C, humidity(%) and wind (knot) in South East, South South and South West Nigeria

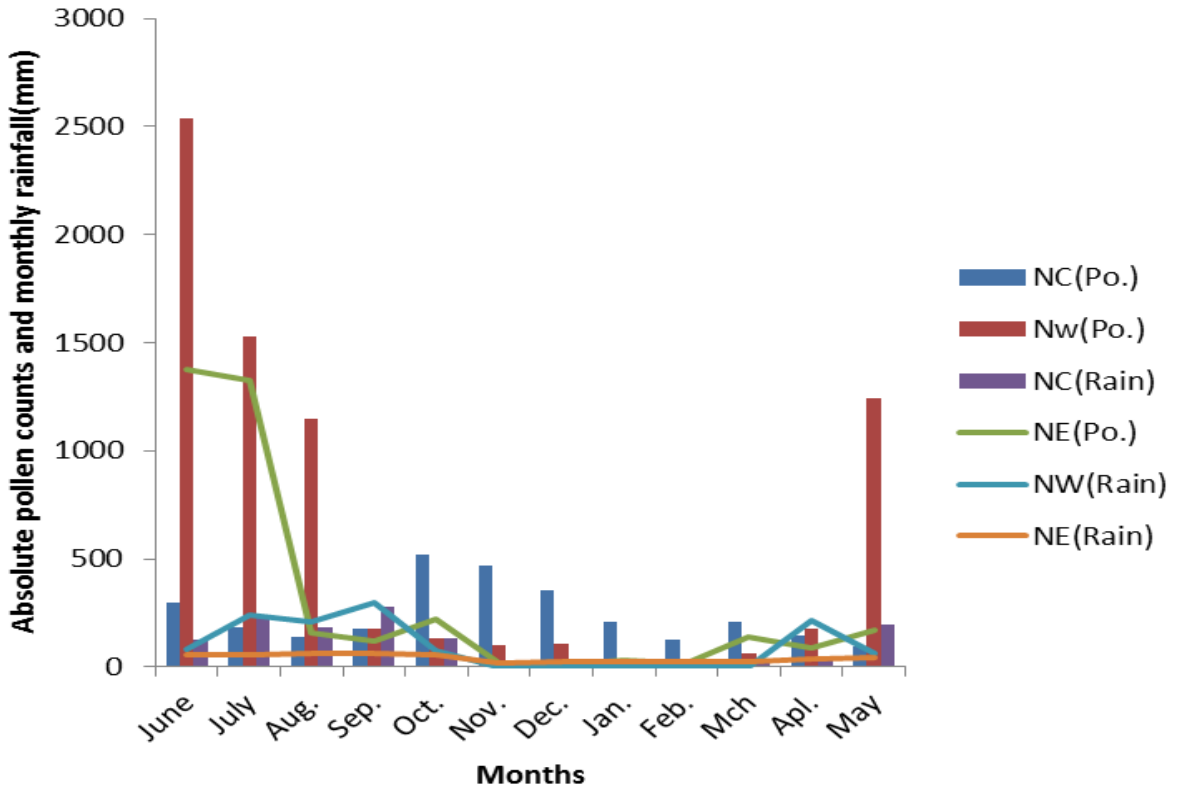


Fig 4.7: Relationship between atmospheric pollen and monthly rainfall (mm) in North Central, North West and North East Nigeria

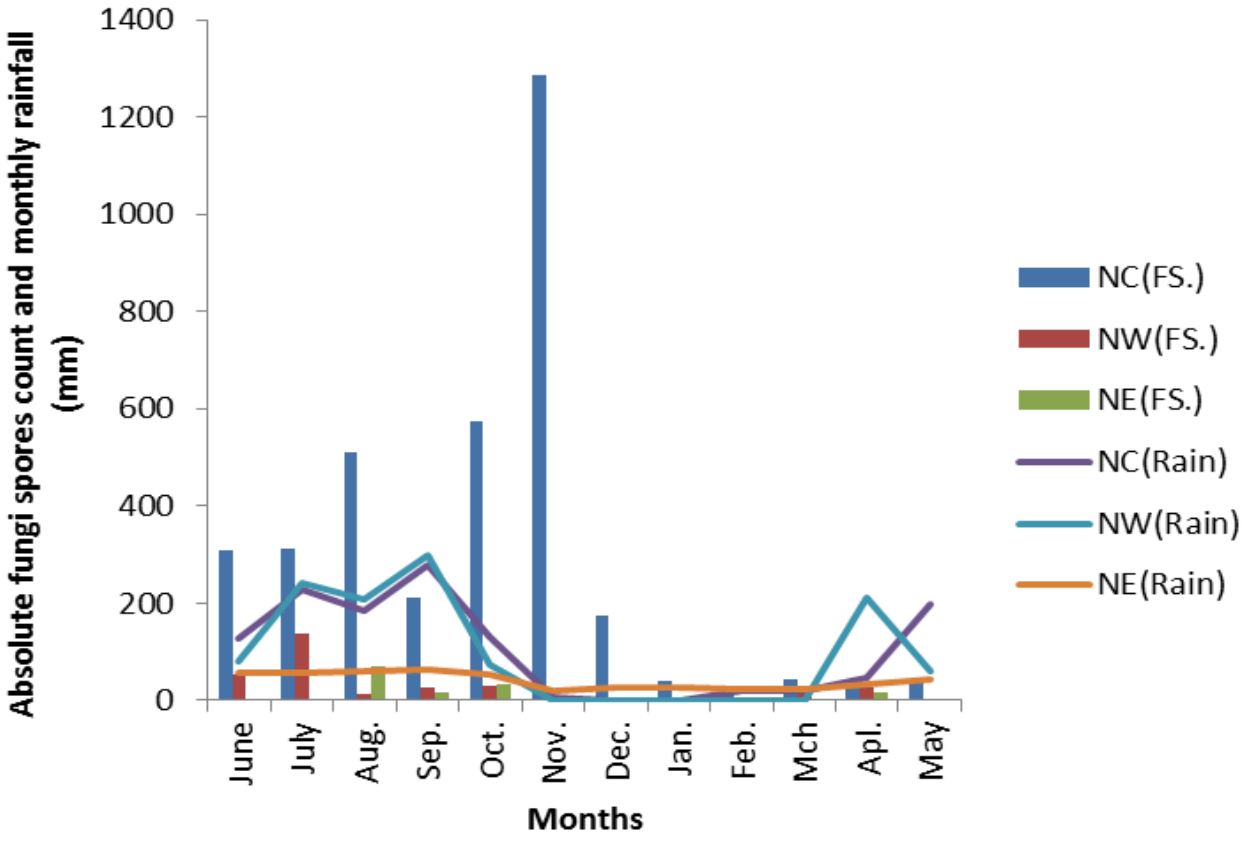


Fig 4.8: Relationship between atmospheric fungi spores and monthly rainfall (mm) in North Central, North West and North East Nigeria

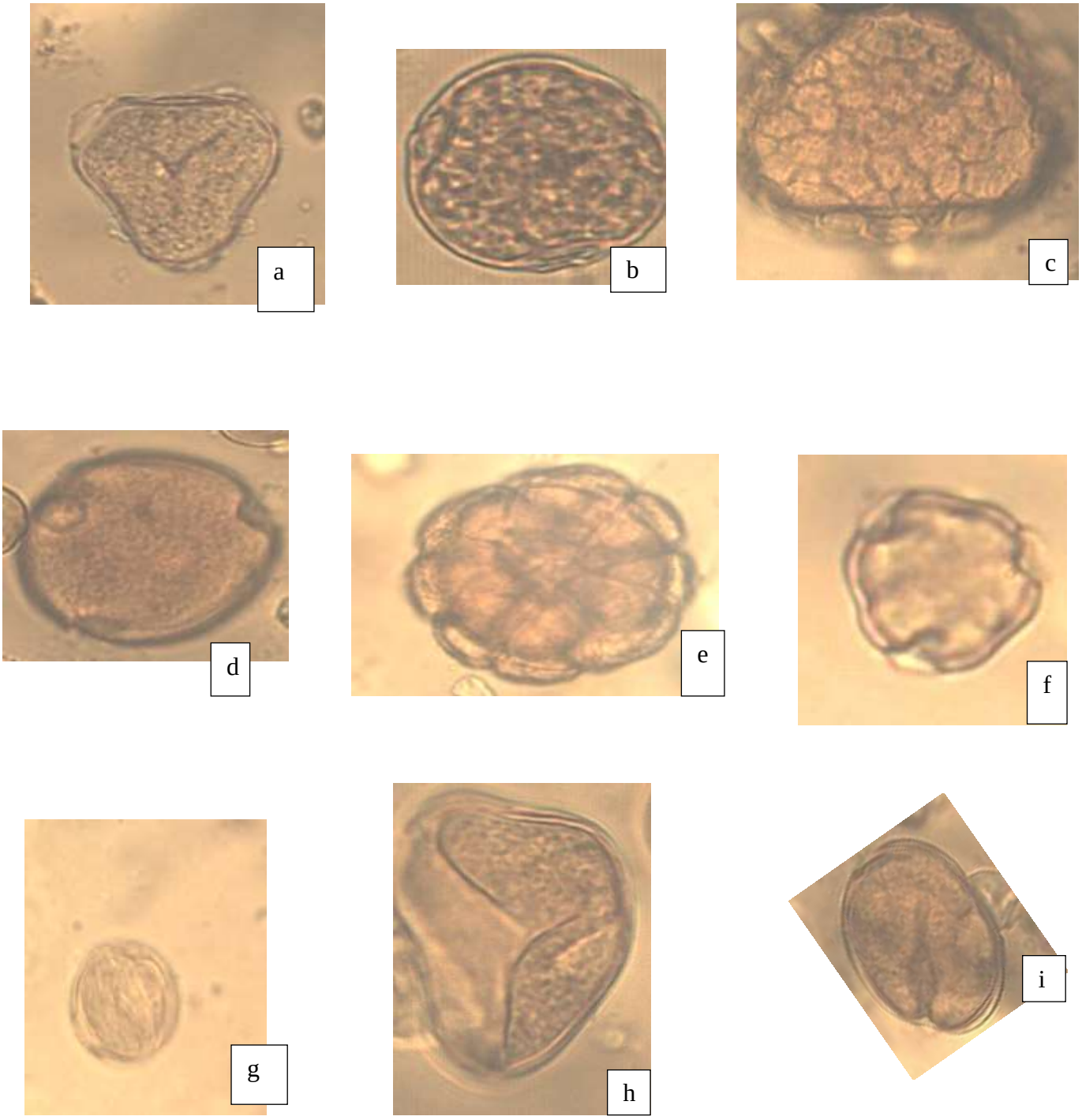


Plate 4. 1: Photomicrographs of some representative pollen

Mag X400

Plate 4.1: Photomicrographs of some representative pollen cont'd

Mag X 400

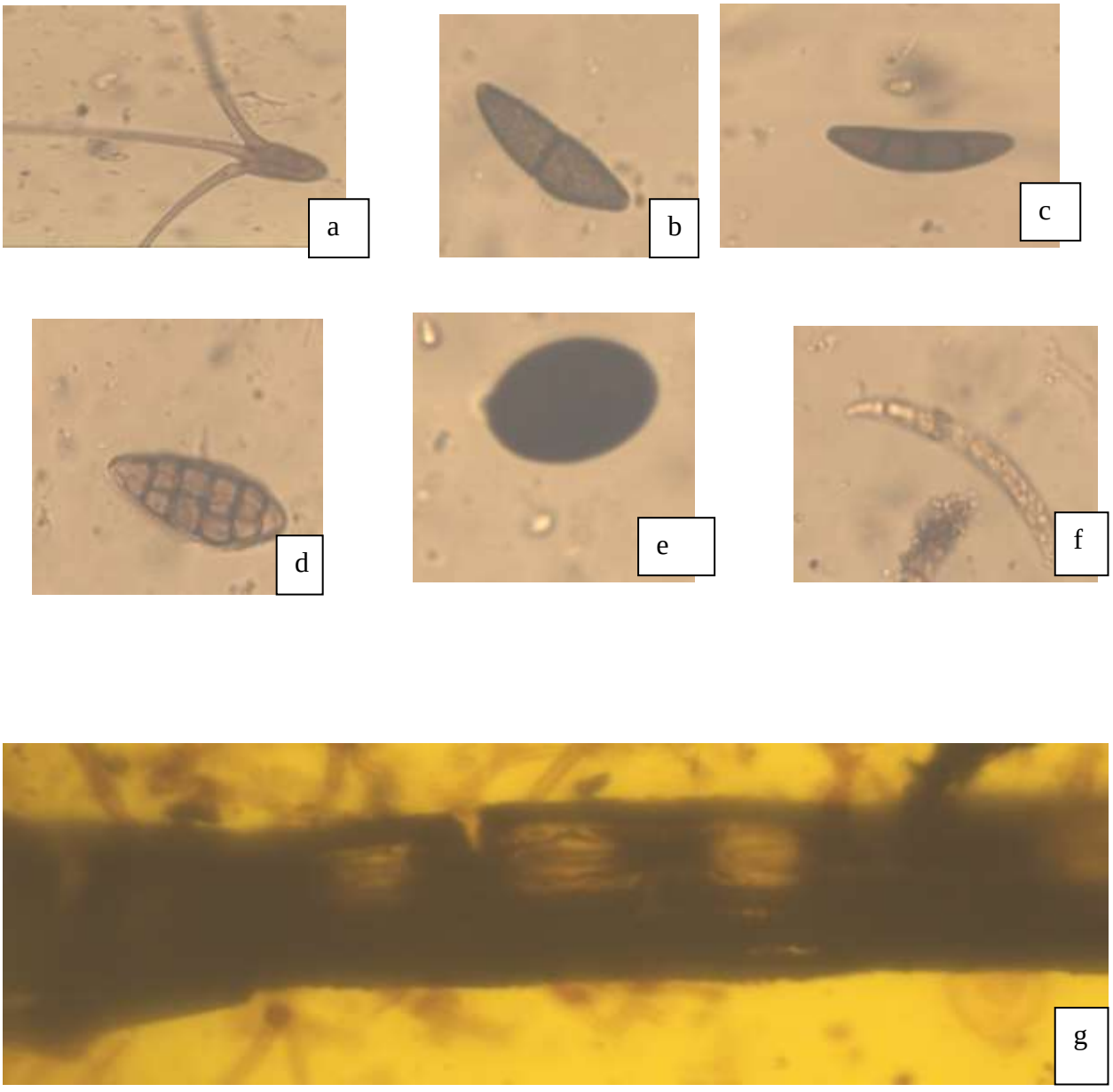


Plate 4.2: Photomicrographs of fungi spores and Charred Poaceae epidermis

Mag X400

4.9 Protein Contents of Some Selected Pollen and Fungi Spores

A total of six pollen and two fungal spores were studied for their protein content. (Table 4.16). *Mangifera indica* pollen yielded the highest value of protein (0.698 mg/ml) whereas *Oreodoxa oleracea* yielded the least (0.208 mg/ml). There was a relationship between the protein concentration and the elicited IgE titre values in some pollen. For example, *Mangifera indica* with the highest protein concentration (0.698 mg/ml) induced the highest IgE titre value (13.29 ng/ml) in mice after last sensitization, whereas *Oreodoxa oleracea* with the lowest protein (0.208 mg/ml) induced the lowest IgE titre value (3.38 ng/ml) (Table 4.17). This direct relationship was not found in all the pollen and spores protein. Among the spores, *Fusarium* sp. yielded a higher protein level than *Aspergillus niger*.

Table 4.16: Protein contents of some selected pollen and fungal spores

Pollen and fungi spores	Protein (µg/ml)
Aspergillus niger	236.49
Fusarium sp.	278.38
Mangifera indica	698.65
Mariscus ligularis	236.49
Oreodoxa oleracea	208.11
Panicum maximum	420.51
Sacciolepis africana	420.27
Terminalia catappa	420.27

Table 4.17: Relationship between the protein contents and IgE levels (ng/ml) elicited after the last sensitization

Pollen and fungi spores	IgE (ng/ml)	Protein (µg/ml)
Aspergillus niger	7.35	236.49
Fusarium sp.	9.60	278.38
Mangifera indica	13.29	698.65
Mariscus ligularis	13.10	236.49
Oreodoxa oleracea	3.38	413.51
Panicum maximum	7.19	420.27
Sacciolepis africana	13.13	278.37
Terminalia catappa	13.18	420.27

4.10 Clinical Features of Allergy

4. 10.1 Mice Skin

All Mice inoculated with *Oreodoxa oleracea* pollen protein exhibited a dermatophytic reaction, with physically features of swelling and rashes on the mice skin (Plate 4.3). The allergen also caused a higher mortality rate at the 3rd week and skewed the production of basophil (Plate 4.12)

4.10.2 Trachea of Mice

The negative control (Plate 4.4a) was not inoculated and the positive control (Plate 4.4b) received phosphate buffered saline, both controls showed a normal trachea. Mice inoculated with *Mariscus ligularis* pollen protein, showed over pseudostratification of epithelial layer. Mice sensitized with *Sacciolepis africana* pollen protein, showed proliferation of sub epithelial mucous gland. Mice which received *Aspergillus niger* spore protein, exhibited hypertrophy of the mucous gland (Plate 4.4).

4.10.3 Lung of Mice

Compared to positive and negative control, mice inoculated with *Oreodoxa oleracea* pollen protein, *Terminalia catappa*, *Fusarium* sp. protein showed inflammation within the lung parenchyma (Plate 4.4f, g, h, j, & k).

4.10.4 Bronchiole

Compared to positive and negative control, mice inoculated with *Aspergillus niger* showed inflammation around terminal bronchiole unlike in control and other mice which received other pollen and spore proteins (Plate 4.4i).

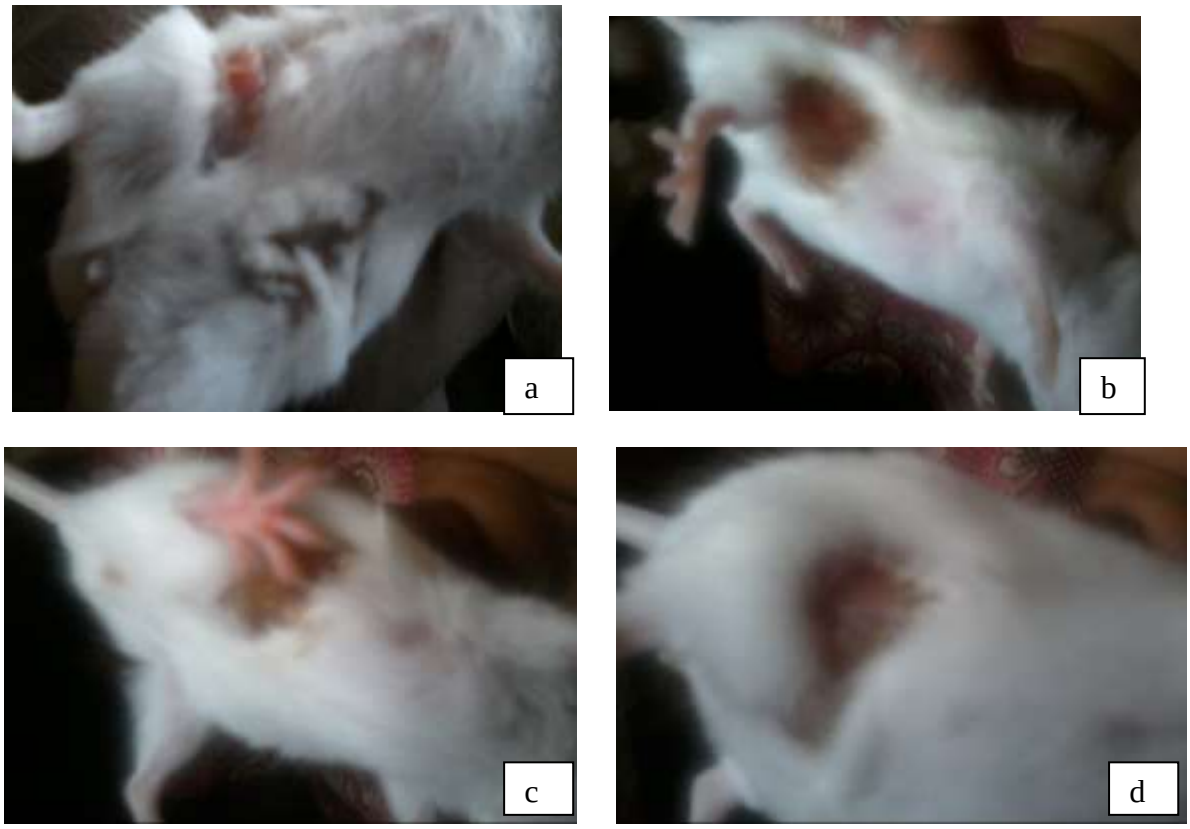


Plate 4.3 a, b, c & d: Physical manifestation of allergy provoked by *Oreodoxa oleracea* pollen in Mice

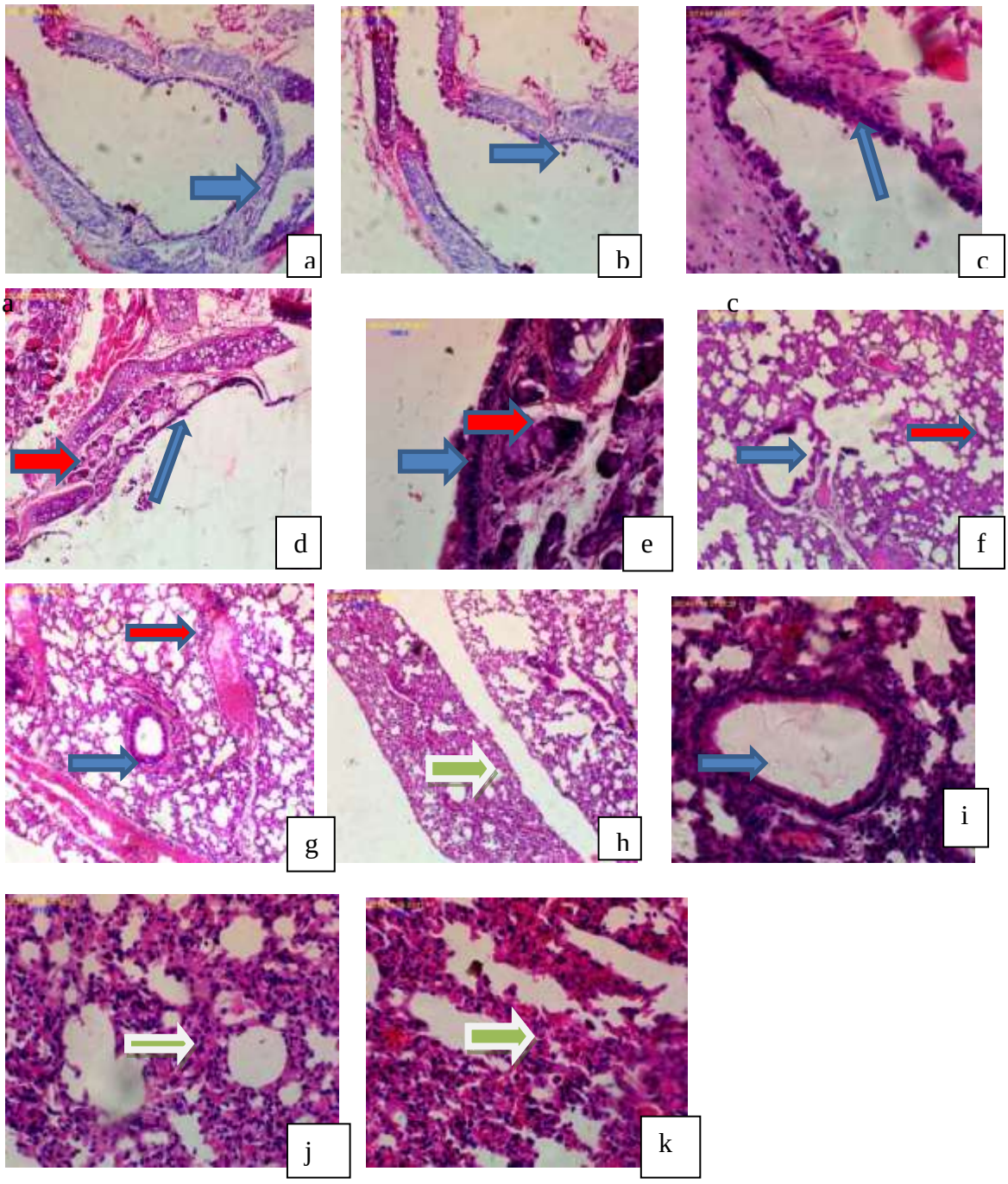


Plate 4.4: Photomicrographs of respiratory organs of mice
All except (i) -Mag X1000

(i)- Mag X2000

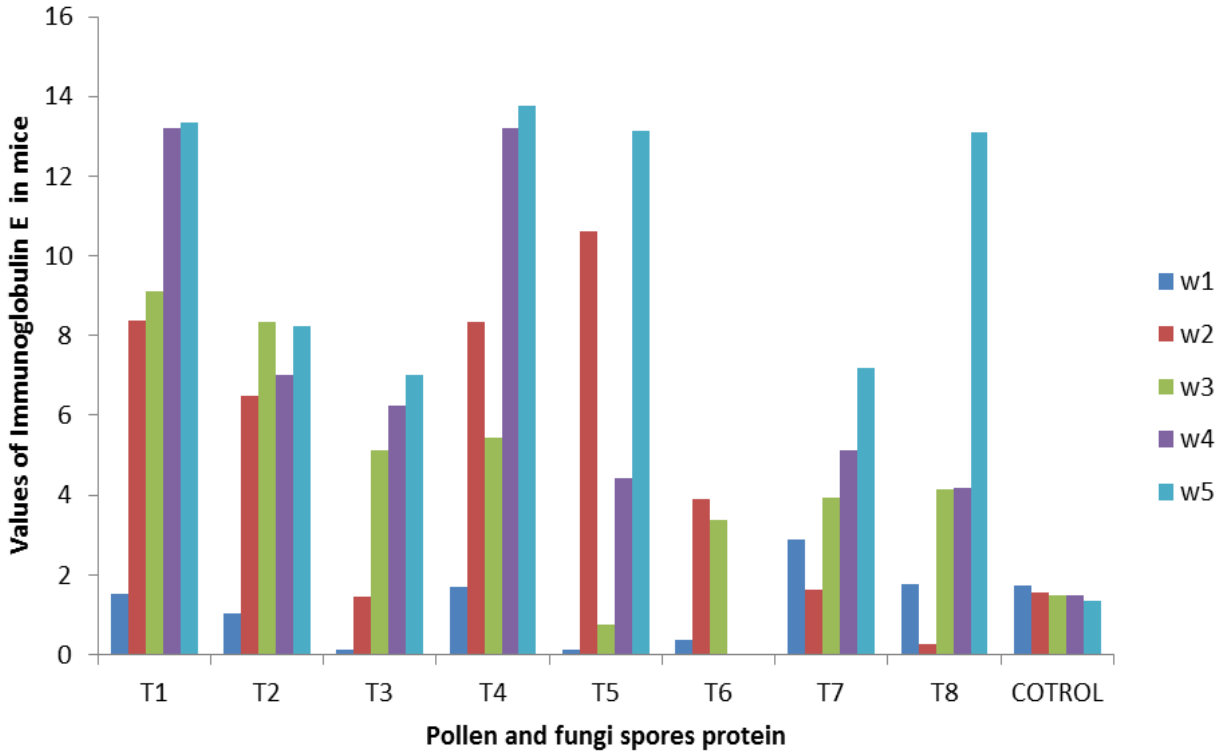
4.11 Evaluation of Immunoglobulin E (IgE) in Mice Sera

Immunoglobulin E antibodies were found present in all animals before the commencement of the experiment and ranged between (0.125 ng/ml - 2.87 ng/ml). At week two, the animals presented a higher IgE levels than the previous week one (w1) before inoculation, the rate of change however was not the same for all pollen and fungi spores protein over the weeks. *Terminalia catappa* protein produced a seven fold change in IgE than initially presented before inoculation. The elicitation of a high IgE by *Terminalia catappa* was closely related to *Mangifera indica,* which also elicited a seven fold change than was initially presented at week one (Fig.4.9). The induction of IgE values was found to be progressive in Mice that were inoculated with *Terminalia catappa, Aspergillus* sp.*, Panicum maximum* and *Mariscus ligularis.* Mice that received the protein of *Fusarium* sp., *Mangifera indica, Sacciolepis africana and Oreodoxa oleracea* had fluctuated IgE throughout the week. *Terminalia catappa, Magnifera indica, Sacciolepis africana* and *Mariscus ligularis* induced a higher IgE titre values; 13.34 ng/ml, 13.78 ng/ml, 13.13 ng/ml and 13.10 ng/ml respectively after the last sensitization. Mice that received *Magnifera indica* protein had a decrease in IgE value from week 2 (8.34 ng/ml) to week 3 (5.43 ng/ml), which built up to 13.19 ng/ml at 4^{th} week and 13.78 ng/ml at 5^{th} week.

Sacciolepis africana, elicited the highest level of IgE at first sensitization (ten fold change), which decreased drastically in weeks w_2 (0.750 ng/ml), had a little increase in w_3 (4.43 ng/ml) and built up again at w_4 (13.13 ng/ml). Mice sensitized with *Oreodoxa oleracea* pollen protein induced a three fold change in IgE levels from w_1 (0.351 ng/ml) to w_2 (3.91 ng/ml) and was reduced at w_3 (3.38 ng/ml). Mice injected with *Panicum maximum* protein had a reduction in IgE level at w2 (1.625 ng/ml) from the initial value at w_1 (2.87 ng/ml), from w2 there was a built up of the IgE level which then increased progressively. *Mariscus ligularis* protein

induced a reduction of IgE from w_1 (1.75) to w2 (0.25), the level was however maintained at w_3 (4.13) and w_4 (4.19) and built up at the 5^{th} week (13.10). Conversely the IgE of the control Mice which received phosphate buffered saline decreased over the weeks, w_1 (2.87 ng/ml), w_2 (2.50 ng/ml), w_3 (1.80 ng/ml), w_4 (1.63 ng/ml) and w_5 (0.81 ng/ml).

The IgE induced by *Terminalia catappa* pollen protein in mice was not significantly from different from that induced by *Mangifera indica* and significantly different from other pollen and fungi spores protein and control (Fig.4.8). IgE elicited by *Fusarium* sp protein in mice differed significantly from those of *Terminalia catappa*, *Mangifera indica*, *Oreodoxa oleracea* and control (Fig. 4.10).



F.g 4.9: Immunoglobulin E elicited by protein of pollen and fungal spores in mice

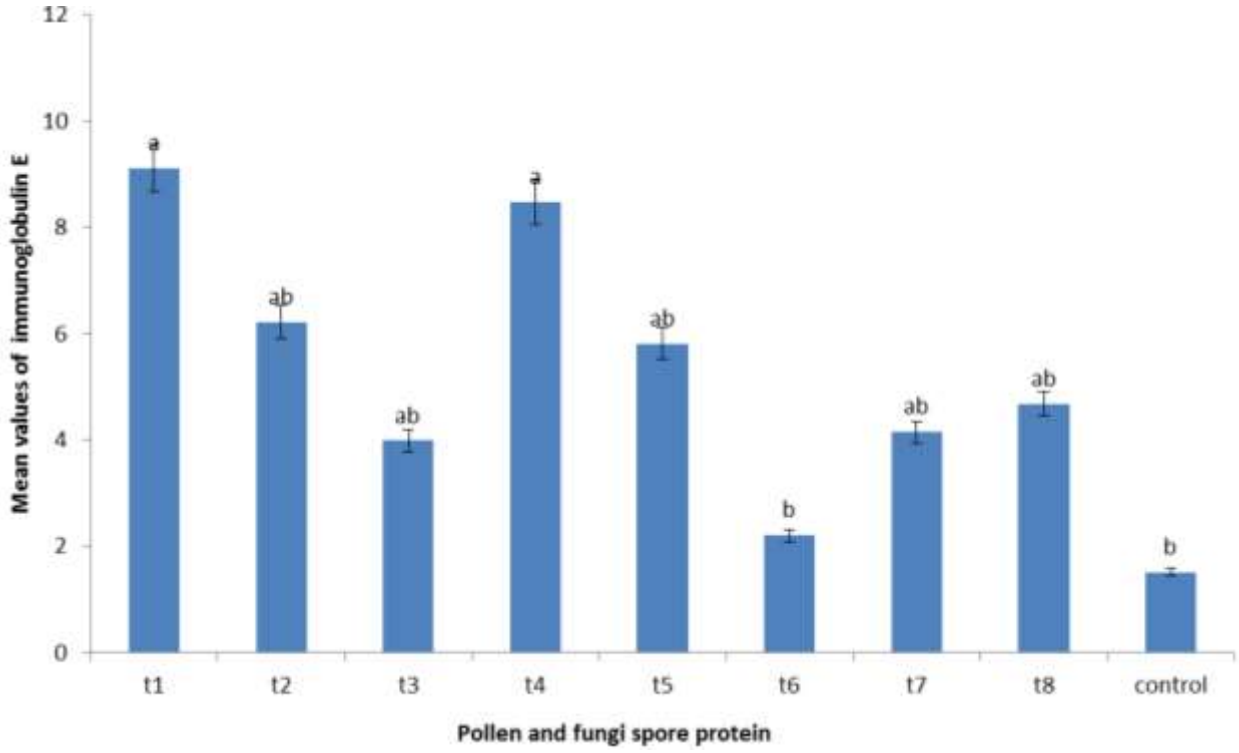


Fig. 4.10: Mean values of Immunoglobulin E elicited by protein of pollen and fungal spores in mice

Mean bars with different alphabets (a, b and ab) are significantly different (P< 0.05).

4.12 Immune Cells

The most predominant immune cells predominant in sensitized mice were lymphocyte, basophil and eosinophil (plate 4.5). Lymphocyte proliferated over the weeks as mice were sensitized, this could be depicted by the number of fields that gave 100 % immune cell. In control mice, more than fifty (50) fields gave 100 % immune cells, which was predominated by lymphocyte. In mice sensitized with pollen and fungal spores protein, the number of fields decreased as mice were sensitized over the weeks. Less than twenty (20) fields gave 100 % immune cell after the last sensitization which depicted the infiltration of lymphocyte in sensitized mice. The lymphocyte elicited by *Terminalia catappa* pollen protein in mice differed from those elicited by *Fusarium* sp., *Aspergillus niger*, *Mangifera indica*, *Oreodoxa oleracea*, *Mariscus ligularis* and control (Fig.4. 11). Lymphocyte induced by *Fusarium* sp. spore protein differed from those induced by *Terminalia catappa*, *Sacciolepis africana*, *Oreodoxa oleracea* and *Panicum maximum*. Lymphocyte induced by *Oreodoxa oleracea* pollen protein differed from all pollen and fungal spores protein and control.

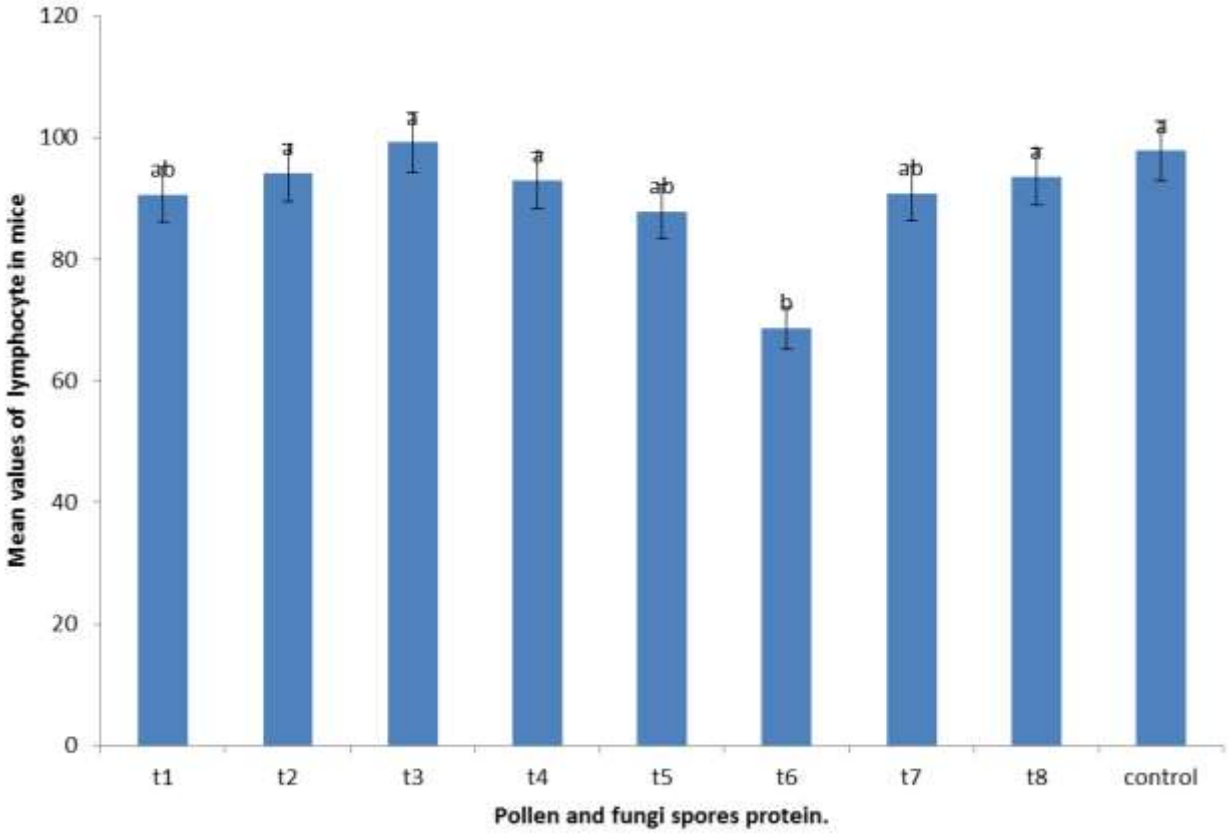


Fig. 4.11: Mean values of mice lymphocyte elicited by protein of pollen and fungal spores

Mean bars with different alphabet (a, b and ab) are significantly different ($P < 0.05$).

The pollen protein of *Oreodoxa oleracea* and *Sacciolepis africana* skewed basophil production than other pollen and spores protein (Fig. 4.12). *Mariscus ligularis, Panicum maximum, Magnifera indica, Terminalia catappa* also induced basophil production. *Terminalia catappa* and *Fusarium* sp elicited the highest values of eosinophil in mice, others include *Aspergillus niger, Mariscus ligularis, Panicum maximum* and control in decreasing order. Statistical analysis shows that the eosinophil induced by *Terminalia catappa* differed from those of *Mangifera indica, Sacciolepis africana* and *Oreodoxa oleracea* whereas the eosinophil induced by *Fusarium* sp. differed from those elicited by *Aspergillus niger, Mangifera indica, Sacciolepis africana, Oreodoxa oleracea, Panicum maximum, Mariscus ligularis* and control (Fig. 4.13).

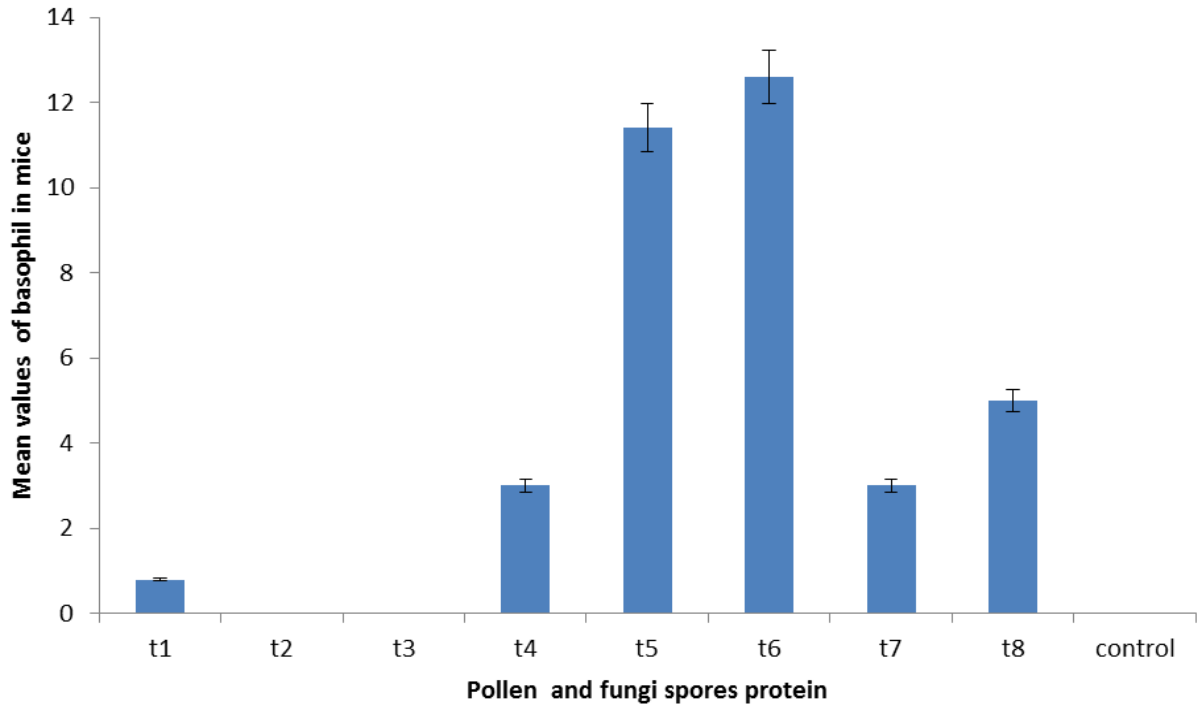


Fig. 4.12: Mean values of mice basophil elicited by protein of pollen and fungal spores

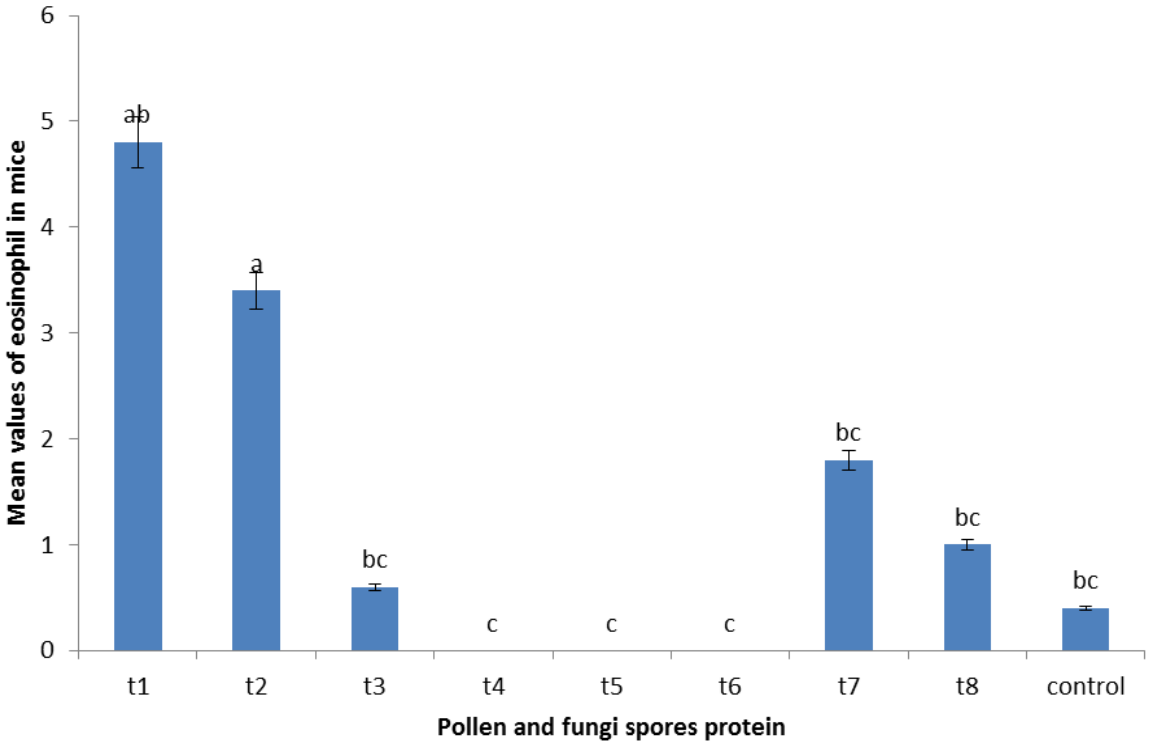


Fig. 4.13: Mean values of mice eosinophil elicited by protein of pollen and fungal spores

Mean bars with different alphabets (a, ab, bc and c) are significantly different (P< 0.05).

Monocyte recorded was higher proportion in mice that received *Mangifera indica* and *Panicum maximum* pollen protein than in mice which received other pollen and fungi spores protein and also in that control mice. There was no significant difference between the mean values of monocyte elicited by pollen and spores protein (Fig. 4.14). Neutrophil was present only in mice which received *Terminalia catappa* and *Mariscus ligularis* (Fig. 4.15).

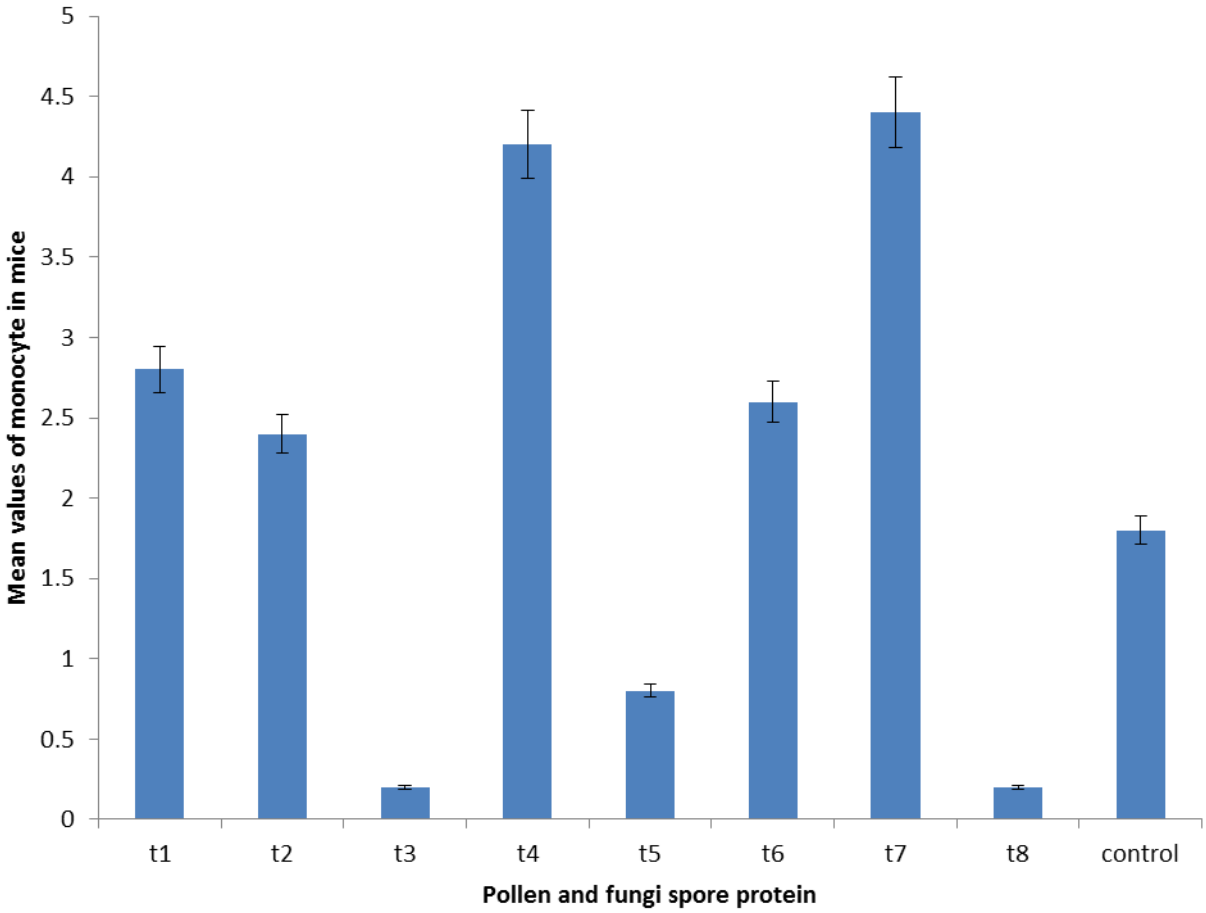


Fig. 4. 14: Mean values of mice monocyte elicited by protein of pollen and fungal spores

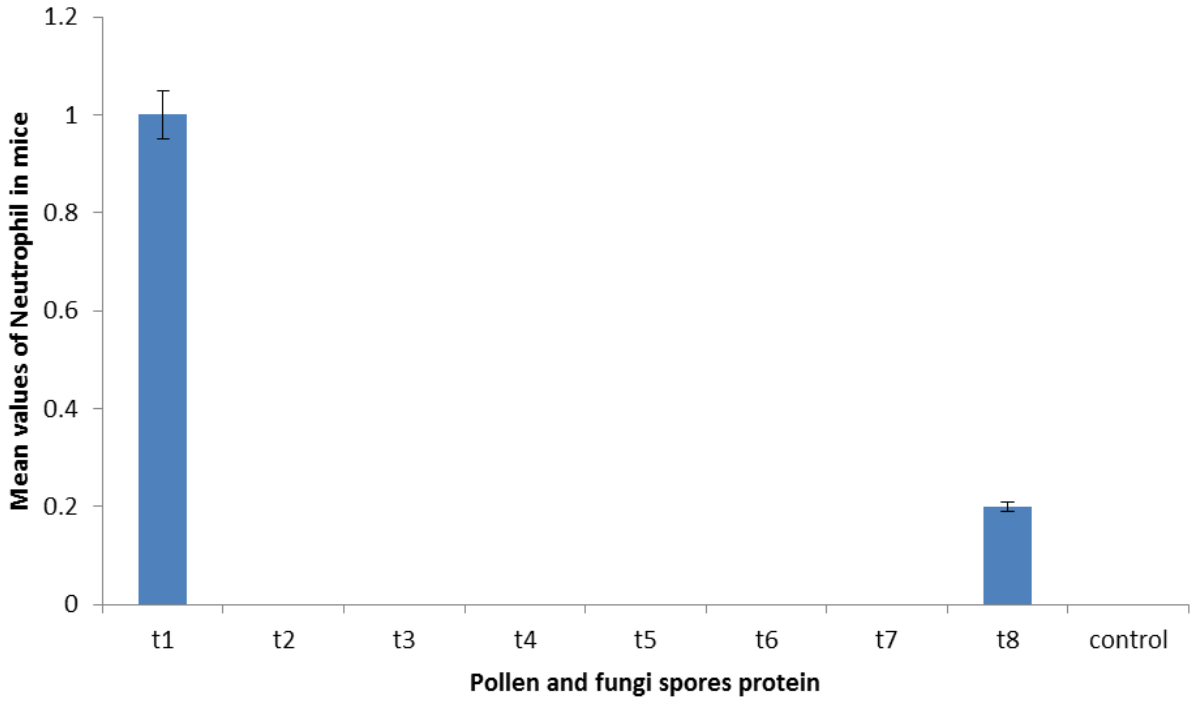


Fig. 4.15: Mean values of mice neutrophil elicited by protein of pollen and fungi spores

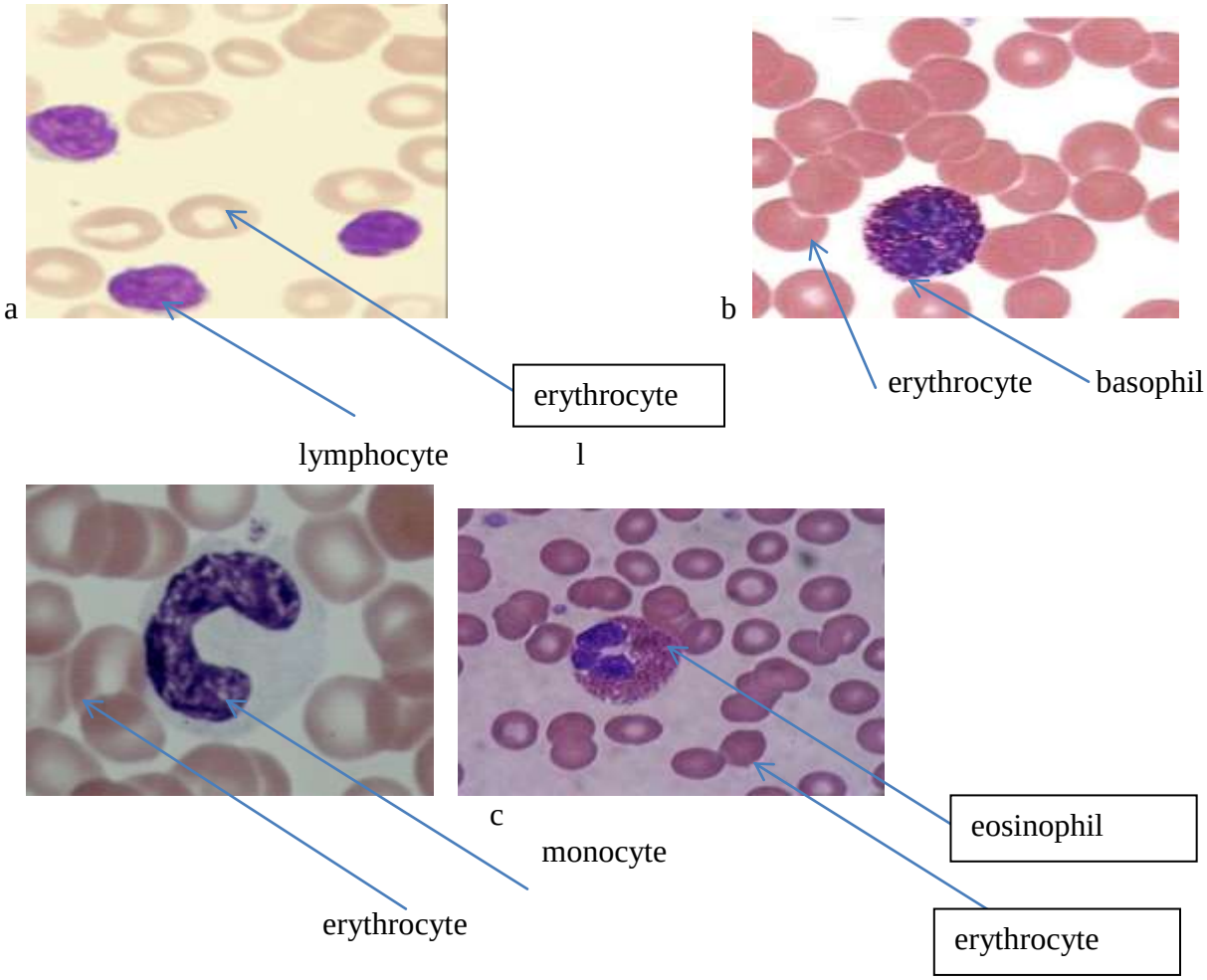


Plate 4.5: Immune cell types predominant in allergic mice (a). Lymphocyte (b) Basophil (c)monocyte (d) Eosinophil Mgx1000

CHAPTER FIVE

5.1 Discussion

5.1.1 Pollen and Fungal Spores Distribution

This study provided detailed information on the relative abundance of pollen and fungal spores present in the aeroflora of Nigeria. The pollen reflected the vegetation types in the studied areas and was found to be richer and more diverse in the Southern and North Central Nigeria, than in North West and North East Nigeria.

In South East (Enugu) Nigeria, the monthly pollen record showed three distinct periods; rainy season (May-August), late rainy/early dry season (Sep.- Dec.) and dry season (Jan.-April). Pollen counts were higher during the late rainy/dry season and this period was designated the risk period for pollen hypersensitive individual who frequently visit or inhabit the area. The period was denoted as Poaceae – *Elaeis guineensis* period. The most dominant fungal spores include those of *Fusarium* sp., *Alternaria* sp., *Spadicoides* sp., *Bastrodesmium* sp., and *Nigrospora* sp. In South South, pollen recorded showed a marked division between a rainy and dry season. Pollen increased progressively as the dry season approached. A lot of Pteridiophyte spores were recorded coupled with abundance of fungi spores morphotypes indicating a more humid environment than the South West and South East study areas. Risk period was October-March and was denoted as *Rhizophora* spp.- *Alchornea cordiforlia* period. The pollen which dominated include those of *Rhizophora* sp., *Alchornea cordiforlia*, *Acrostichum aureum*, *Elaeis guineensis* and Poaceae. The most preponderant fungal spores were *Nigrospora* sp., *Pithiomyces* sp., *Spadicoides* sp. and *Cladosporium* sp.. In South West, a short dry season which occurred between the two rainfall maxima and an increase in wind speed favoured high influx of *Jatropha curcas* pollen. *Nigrospora* sp., *Spadicoides* sp. and *Torulla* sp.

Pollen were more predominant from August –November in North-Central Nigeria. Qualitative abundance of pollen was noted more towards the onset of dry season in North Central Nigeria and was denoted as Poaceae-*Pentaclethra macrophylla* period. Most preponderant pollen include those of *Elaeis guineensis*, Poaceae, *Cassia* sp., *Alchornea cordifolia* and *Pentaclethra macrophylla*. Fungal spores most dorminant were *Tetraploa* sp., *Pithiomyces* sp., *Puccinia* sp. and *Erysiphe graminis.* North West and North East were dominated by Poaceae pollen during the rainy season. In dry season, the preponderance pollen in North-West were those of *Cyperus* sp., Amarathaceae/Chenopodiaceae, *Phyllanthus* sp., *Khaya senegalensis* and *Trichilia roka*. *Microsporium* sp., *Nigrospora* sp. and *Cecospora* sp. were the most dominant fungal spores. In North- East, *Lannea acida, Parkia bicolor* and *Vitex doniana* were the most dominant pollen whereas *Nigrospora* sp. and *Fusarium* sp. were the most dominant fungal spores.

Flowering occurred throughout the year but with variations from one month to the other. Grass pollen dominated the atmosphere of Northern Nigeria than Southern Nigeria, several species of grasses flowered successfully throughout the year and had their maximum flowering period between the month of June-October. Pollen allergies at this period (June- October) could be attributed to upsurge of Poaceae pollen in the atmosphere of Northern Nigeria, because of their higher antigenic load. The grasses contributed a high pollen load in North West, North East and North Central, constituting a higher percentage of the total pollen except in North Central Nigeria. In Southern Nigeria, Poaceae pollen grains were present in the atmosphere almost throughout the year. They were more abundant at rainy season; June and July and late rainy/hamattan period September to December. Njokuocha (2006), also reported the persistent dominance of grass pollen in the atmosphere of South East Nigeria. Obtulowiz *et al*. (1991) also reported that the grass pollen were the most important allergenic pollen in Poland. Poaceae

was the most abundant nonarboreal pollen type in the atmosphere of Büyükorhan, and this family represented 7.00 % of the annual pollen index (Tosunoglu *et al*., 2014). Poaceae pollen grains were initially recorded in the atmosphere of Büyükorhan in the 3rd week of January, reached maximum levels between the 20th and 31st weeks, and lasted until the 3rd week of June (23rd week) (Tosunoglu *et al*., 2014). Poaceae pollen grains were reported as the most common among non-arboreal pollen in studies conducted in İzmir (7.7%–6%), (Guvensen and Ozturk, 2003), Sakarya (18.95%) (Bicakci, 2006), and Didim (6.33%) (Bilisik *et al*., 2008). Grass pollen is quantitatively one of the most important aeroallergen vectors worldwide. It is a major cause of allergic reactions including conjunctivitis, rhinitis, and other upper and lower respiratory tracts problems occurring during the flowering season of different grasses.

Poaceae pollen are among the most dominant anemophilous pollen in Nigeria especially in Northern Nigeria. The plants play a crucial role in allergy because of their copious, bulk and disproportionate amount of pollen they disperse in the atmosphere . Poaceae pollen is the major cause of pollinosis in many parts of the world. Although its frequency differs regionally, grass-induced pollinosis is the most common pollen allergy also in Europe (D'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. Grass pollen grains are also quantitatively the most important aeroallergen vectors worldwide. 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).

In South East and Northern Nigeria study locations, a decrease of Poaceae correlated with an increase in charred Poaceae cuticles, which resulted from burnt epidermis of grasses. This actually shows that after grasses were burnt down by annual bush fires, their pollen decreased in the atmosphere and their burnt epidermis increased. Decrease in Poaceae pollen in Northern Nigeria from November to May, could be attributed to the normal nomadic life style of the people and frequent fire event (evident from charred Poaceae cuticles), which checked the growth of grasses, resulting in lower grass pollen content in the atmosphere. Dominant presence of charred Poaceae cuticles, was very peculiar to Northern Nigeria. There were lower records of charred Poaceae cuticles in Southern Nigeria than in Northern Nigeria. The absence of charred Poaceae cuticle in aeroflora of Lagos (South West) perhaps depicted absence of bush fires. Since Lagos is a built up urban area.

During the last 40 years, the frequency of symptoms of allergic diseases has increased dramatically, especially in children and people living in urban areas (Chakra *et al*., 2009). Several factors have contributed to this increase, among which are airborne pollutants from gaseous and particulate emissions. Airborne pollen grains can release hundreds of small particles called pollen cytoplasmic granules (PCGs). These may be present in atmospheric samples taken during the pollen season, and some studies show a 50-fold increase in their atmospheric concentration on days after rainfall. In the same way, airborne pollutants may modify pollen grains structurally, thereby increasing the release of PCGs in the atmosphere (Chakra *et al*., 2009).

The herbaceous pollen encountered in the atmosphere of the studied locations were majorly those of Chenopodiaceae/Amarathaceae species, Cyperaceae, Asteraceae (Tubuliflorae &

Liguliflorae complex), *Crotalaria* sp. etc. In Southern Nigeria, trees/shrubs pollen dominated, whereas grass pollen dominated the Northern Nigeria.

Based on pollen diversity assessment of floral component, the order of pollen predominance of the six geopolitical zones could also be a reflection of their biodiversity, however the assessment could have been influenced by, intensity of flowering, pollen productivity, limitations of sampler, wind direction and strength, geography, variations in microclimatic conditions and the movement of the Inter Tropical Convergent Zone (ITCZ) in 2011.

Over time, researchers have theorized that anemophilous pollen are more implicated in allergy, because of their higher antigenic load than the entomophilous pollen (Shahali *et al*.,2009) . Wind pollinated plants usually have inconspicuous flowers but can produce a vast amount of pollen. Their small size, smooth, dry and non-sticky nature of anemophilous pollen facilitates their easy dispersal in air. Anemophilous pollen has many adaptations to free dispersal:It is produced in large quantities, is light, dry, smooth or with reduced ornamentation (Faegri and Pijl, 1979), there is little pollenkitt on its surface (Hesse, 1981). Entomophilous plants on the other hand produce much less pollen. Pollen grains have rich ornaments (Faegri and Pijl, 1979) and they are covered by pollenkitt or viscin threads. Owing to this they become glued together and attach to an insect visiting the flower (Hesse, 1981). These features cause the pollen of entomophilous plants to occur usually in low concentrations or sporadically. People most prone to enthomophilous pollen allergens are usually the Mowers, Gardeners and Farmers who continuously come in closer contact with them. Anemophilous pollen dominate the pollen rain of any area, representing major seasonal carriers of allergens (Shahali *et al*.,2009). It is usually more implicated in public ill-health, because of their great aerodynamic properties which make

them to reach out to greater number of the population. Anemophilous pollen dominated the atmosphere of Northern Nigeria, whereas enthomophilous pollen dominated the Southern Nigeria. This finding agrees with the view of Dyakowska (1959), who found out that bulk airborne pollen comes from anemophilous plants. Kasprzyk (2004) showed that the dominance of enthomophilous pollen in the atmosphere of Rzeszow and its environs could be attributed to the wide variety of ornamental (herbs and trees) plants growing around the sampling site.

Three categories of trees/shrubs were recognized in this present study based on flowering period (1). Those with short, intense flowering period example; *Maytenus* sp, *Anacardium occidentale, Cussonia bateri, Eugenia nodiflora* etc. (2). Types without any definite flowering season, they were present almost throughout the year e.g; Poaceae, *Cassia spp,* Amarathaceae /Chenopodiaceae, *Olax subscopioides, Elaeis guineensis* etc. (3). Those with double flowering pattern of short duration, example; *Casuarina equisetifolia, Syzygium guineense* etc.

Podocarpus sp. pollen, whose plant is not indigenous in Nigeria was recorded in North West Nigeria at the month of November. Adeonipekun (2014) also recorded *Podocarpus* in South-West Nigeria, but could not conclude what genus the bisaccate he found belong to.

Northern Nigeria had one defined season of more allergenic exposure dominated by Poaceae pollen during the rainy season hence, hypersensitive individuals could be more monosensitized at that period. Southern Nigeria had two seasons; the season of higher risk period for pollen which corresponded to the late rainy/ dry season and the higher risk period of fungi exposure, which also corresponded to rainy season.

Allergenic reactions due to fungal spores were deduced to be more predominant during rainy and late rainy season (June-December) than in dry season January to May in the atmosphere of Nigeria. The months of the rainy season was proliferated with spores of fungi. A high

predominance of fungal spores was recorded in all Southern and North Central Nigeria. Some of the fungi spores recorded in this work have been confirmed allergenic in other countries for example; *Alternaria* sp., *Fusarium* sp., *Cladosporium* sp. *Curvularia* sp., *Pithiomyces* sp. etc. (Horner *et al*., 2000). They pose a great threat to hypersensitive individuals especially the immuno-compromise patients.

Erysiphe graminis, a pathogenic fungi on members of Poaceae family (both cultivated and wild species) were found sporadic in August, dominated the atmosphere in November and contributed to the bulk concentration of total atmospheric fungi spores 1200 (93.38%). Their dominance correlated with the decline in Poaceae pollen. The pathogenic spores recorded included; *Helminthosporium* sp., which is implicated in leaf spot of rice. The presence of *Alternaria* spores in the atmosphere and their impact on agriculture and human health have been studied by Escuredo *et al*. (2010). *Alternaria* spp is a potential source of allergic disorders in human. *Alternaria solani* produce an early blight in potato crops. The pathogen can infect all aerial parts of solanaceous crops including tomato, potato, eggplant, and pepper, as well as potato tubers (Escuredo *et al*., 2010). Other pathogenic fungi spores encountered include those of; *Torulla* sp., *Tetraploa* sp., *Nigrospora* sp., *Spadicoides* sp., *Puccinia* sp., etc. Most spores sporulated during the rainy season. The spores of *Nigrospora* sp., *Puccinia* sp. and *Spadicoides* sp. occurred throughout the year but more predominant during the rainy season. The persistent occurrence of some dominant fungi spores could be an indicator of pathogen development in the area and knowledge of their presence could assist the farmers and agriculturists to protect their crops from diseases.

5.1.2 Pollen, Fungal Spores Load and Meteorological Factors

The record of lower atmospheric pollen in the Southern part of the country corresponded with the periods of higher monthly rainfall, higher humidity and lower temperature, whereas higher atmospheric pollen load correlated with higher temperature, lower rainfall and humidity. Thus atmospheric pollen grains were more dominant at more drier and windy months than humid months. Higher monthly rainfall reduced airborne pollen concentration and favoured sporulation of fungal spores. This findings are related to the views of Barnes *et al*. (2000), Teranishi *et al. (*2000), Riberio *et al*. (2003), Njokuocha (2006), who found that airborne pollen concentration significantly correlated with temperature and wind direction and negatively correlated with rainfall and number of rainy days. The result of the present study agrees with Adeniyi *et al*. (2014) who also found a postive correlation between pollen concentration and temperature and a negative correlation with relative humidity and rainfall.

In Northern Nigeria especially North West and North East, abundant number of Poaceae pollen was recorded in rainy season, whereas higher varied morphotypes of pollen was recorded during the drier period, but their quantity was less compare to Poaceae pollen which highly influenced atmospheric pollen content during the rainy season. North East and North West with lowest rainfall and humidity have lower fungal spores load, among the Northern study locations, North- Central had a higher record of fungal spores which positively correlated during the rainy season. Peternel *et al*. (2006) found that low pollen concentrations correlated with high levels of rainfall and humidity. Also, Green *et al.* (2004) found that the major factors affecting airborne pollen counts were maximum and minimum temperatures.

A relationship between the pollen concentration and daily meteorological elements is of great practical importance. Applying simple statistical analyses, several studies found significant positive correlations between daily Poaceae pollen concentration and daily maximum temperature (Valencia-Barrera *et al*., 2001; Green *et al.*, 2004; Kasprzyk and Walanus, 2010), daily minimum temperature (Green *et al*., 2004), daily mean temperature (Puc and Puc, 2004; Peternel *et al*., 2006; Kasprzyk and Walanus, 2010), and daily global solar flux (Valencia-Barrera *et al*., 2001; Kasprzyk and Walanus, 2010), the relative humidity (Valencia- Barrera *et al*., 2001; Puc and Puc, 2004; Peternel *et al*., 2006; Kasprzyk and Walanus, 2010) and rainfall (Valencia-Barrera *et al*., 2001; Green *et al*., 2004; Puc and Puc, 2004; Peternel *et al*., 2006; Kasprzyk and Walanus, 2010). Subba *et al*. (1988) demonstrated that flowering season periods can differ by several months between genera and some Poaceae species have a circadian mechanism of pollen shedding, each species having their own temporal features of pollen release, with duration that may vary between 2 and 13 h. Minckley *et al*., (2012) showed that moisture anomalies accounted for 3 % to 24 % of the variation in arboreal pollen abundance for *Fagus*, *Pinus*, *Quercus* and *Tsuga*.

The atmospheric fungi spores had a direct relationship with rainfall in the present study. More fungi spores were recorded during the periods of higher rainfall. This finding agrees with those of Phanichyakarn *et al.* (1989), Agashe and Alfaldil (1989), who found higher number of fungal spores in the rainy season and lower number in dry season in the atmosphere of Bangkok and Bangalore respectively. In Northern Nigeria; North West and North East which received low rainfall and had a lower record of atmospheric fungal load. The amount of rainfall varied across the six geopolitical zones of Nigeria. This however created differences in

the variations of aeroallergens. Climate and vegetation differences also influenced the type, abundance and prevalence of airborne pollen and fungi spores in the studied locations.

In Southern Nigeria, which recorded a lot of rain, the effect on the pollen levels in the air was not the same with Northern Nigeria, which did not get much rain. This therefore created the differences between low pollen content in Southern and North Central Nigeria and higher pollen content in extreme North; North West and North Central Nigeria during the months of April, May, June and July.

The drier season favoured the release of pollen from the anther and influences the atmospheric pollen content positively. Wind, temperature, rainfall and sunshine play vital role in the amount of pollen dispersed in the atmosphere. Wind is a natural carrier of aeroallergens and plays a significant role in transport of aeroallergens (Shahali *et al*.,2009).

The actual start and severity of the season depends on several factors including temperature, rain, humidity and wind speed/direction. As a result of these variables, their atmospheric count vary from one season to the other resulting to a more allergenic exposure to pollen during the dry season especially during the harmattan period in Southern and North Central Nigeria, a more allergenic exposure to Poaceae pollen during the rainy season in North West and North East Nigeria and an allergenic exposure to fungal spores over the atmosphere of Nigeria during the rainy season.

5.1.3 Immunological Result

Result of the immunological aspect of this work showed that some entomophilous pollen could possess higher allergen protein content but because of their lower aerodynamic properties, they are found lower in the atmosphere than the anemophilous pollen.

Immunoglobulin type E (IgE) is one of the five classes of antibodies or an isotype of immunoglobulin, that has been found only in mammals. IgE plays a very essential role in type 1 hypersensitivity which manifest various allergic diseases such as asthma, allergic rhinitis etc (Niederberger *et al*., 2002; D'Amato *et al*., 2002). Analysis of IgE titre values indicated 0.125 ng/ml - 2.87 ng/ml in mice before sensitization. All pollen and spores allergen induced an alteration in immune system which resulted to a higher IgE titre values, especially at the last week of the experiment. The elevated value of IgE means that the protein of the pollen and spores were recognized by the antibodies as foreign bodies. A rising level of antibody to specific antigen indicated active infection and led to tissue inflammation. The result further confirmed that IgE plays important role in the development of allergy. The continuous change in IgE values of the animal as they were sensitized with allergen protein indicated that, on continuous exposure to allergenic triggers, the IgE will persistently increase. The result also shows that allergenic reaction presents itself with high IgE with or without physical features. Among the eight pollen and spores subjected to allergenicity investigation, only *Oreodoxa oleracea* presented a physical dermatophytic manifestation of allergy. Routine checks of the IgE level became imperative especially for the atopic individuals.

In human, IgE level in a normal non atopic individual is 0.3 µg/ml and about 10 times this value or 12 µg/m in atopic individuals (Sudha *et al*., 2010). IgE level has been discovered to

gradually increase throughout childhood, with a peak at 10-15 years of age, it decreases throughout adulthood (Shahali *et al*., 2009). IgE levels are influenced by genetic makeup, immune status and environmental factors (allergen exposure) (Pyle, 2013). IgE also plays a critical role in mediating atopic diseases, significant correlation between serum IgE concentration and disease have been demonstrated in allergic asthma and atopic dermatiatis (Pyle, 2013).

High IgE leads to the production of free oxygen radicals (FORs), which are very reactive molecules because of their unpaired electrons (Yariktas *et al*., 2004). They are however neutralized by enzymatic and nonenzymatic defense systems such as superoxide dismutase (SOD oxygen), catalase (CAT), glutathione peroxidase (GSH-Px), vitamin E, glutathione and vitamin C (Yariktas *et al*., 2004). When the balance between free oxygen radical production and the antioxidative defence mechanism is disturbed, the level of free oxygen radicals increases which leads to tissue damage, impairs the membrane permeability and fluidity, and results in functional and structural disorders and even cell death (Yariktas *et al*., 2004). Vitamin E localized in cell membrane plays an important role to break the chain reaction. For this reason vitamins C and E administration in infections can be useful for preventing the FORs damage (Yariktas *et al*., 2004).

White blood cell (leukocyte) is an integral part of human protection against antigens. The body makes different types of white blood cells that work continously to keep us healthy. There are five types of immune cell types ; basophil, eosinophil, neutrophil, lymphocyte and monocyte were used to assess the allergenic potential of pollen and spores in this study.

Lymphocyte was the most dominant immune cells encountered in the hematological tests. Lymphocyte cells proliferated over the weeks in all mice sensitized with allergen protein, unlike in control mice, this however was depicted by a decrease in the number of fields that presented 100% immune cells. The infiltration of lymphocyte was found to correlate with a high IgE elicitation.

Basophils, along with eosinophils and neutrophils, constitute a group of white blood cells known as granulocytes (Franco *et al*., 2013). Basophil was highly elicited by *Oreodoxa oleracea* and *Sacciolepis africana* followed by *Mariscus ligularis, Panicum maximum* and *Mangifera indica* in decreasing order of dominance. The high level of basophil elicited by *Oreodoxa oleracea* caused a dermatophytic reaction on the skin of mice, which physically featured as swelling and rashes, it also caused an inflammation within the lung parenchyma. *Sacciolepis africana* also induced inflammation within the lung parenchyma. Research has shown that when immunoglobulin E binds to specialized receptor molecules on basophils, they release stores of inflammatory chemicals, including histamine, serotonin, and leukotrienes (Franco *et al*., 2013). These chemicals have a number of effects, including constriction of the smooth muscles, which leads to breathing difficulty; dilation of blood vessels, causing skin flush and hives; and an increase in vascular permeability, resulting in swelling and a decrease in blood pressure. Basophils also incite immediate hypersensitivity reactions in association with platelets macrophages and neutrophils (Franco *et al*., 2013).

The pollen protein of *Terminalia catappa, Panicum maximum, Mariscus ligularis,* spores protein of *Fusarium* sp. and *Aspergillus niger* elicited higher eosinophil compared to other pollen and fungi spores protein and also control mice. For over 100 years, the eosinophil has

been associated with allergic disease (Martin *et al.*,1996). At present, eosinophils appear to be associated pathologically with asthma, atopic dermatitis, allergic rhinitis, eosinophilic gastroenteritis, and certain eye diseases. The effector functions of eosinophils appear to be derived primarily from release of lipid mediators and proteins, including cytokines and granule proteins (Martin *et al.*,1996). Eosinophil degranulation results in the release of several cytotoxic cationic granule proteins. Furthermore, release of cytokines by eosinophils and other cells involved in inflammation amplifies and regulates localized immune responses. Altogether, the eosinophil's capacity to release and be influenced by a variety of mediators, including the granule proteins and cytokines, implicates this cell in the pathology of inflammation and in the perpetuation of the inflammatory response (Martin *et al.*,1996).

Pollen that elicited high level of neutrophil were *Terminalia catappa* and *Mariscus ligularis* . The role of neutrophil in allergic symptoms of the airways has received increasing attention recently. There is a robust correlation between air way neutrophils and human asthma (Little *et al*., 2002). The severity of airway disease appears to correlate with number of neutrophils. Neutrophils are over represented in patients with asthma exacerbation severly or poorly controlled asthma and in those who die of asthma (Wenzel *et al*., 2002). Neutrophils are abundant cellular components of the immune system. They discharge their arsenal of toxic agents against host tissues, resulting in oxidative damage and inflammation.

5.2 Conclusion

The present survey of airborne pollen and fungi spores has contributed to our existing knowledge of abundance and dispersal of pollen grains and fungal spores in the atmosphere of Nigeria. It is expected that the results of the present work will provide useful data to the allergologists of Nigeria for selecting pollen allergens during calendar months of the year in six geopolitical zones, which will facilitate proper diagnosis and treatment. Allergy sufferers can also use the information to plan their outdoor activities in order to avoid exposure to allergens. Hypersensitive individuals in Southern and North-Central Nigeria are more polysensitized especially during the drier period, whereas individuals in Northern Nigeria are more monosensitized especially during the rainy season.

The pollen and fungal spores protein elicite immunological changes, however their contents vary among plants even of the same family. The rate at which pollen and fungal spores induce the production of immunoglobulin type E (IgE) antibodies vary. *Terminalia catappa*, *Aspergillus niger*, *Panicum maximum*, *Mariscus ligularis* and *Oreodoxa oleracea* induced progressive production of IgE in mice. The IgE produced over the week by *Fusarium* sp., *Mangifera indica* and *Sacciolepis africana* fluctuated, but all showed a higher value of IgE after the last sensitization at 5^{th} week depicting an orchestrated immunological response involving lymphocyte, basophil, eosinophil and neutrophil cells. All pollen and fungal spores protein caused proliferation of lymphocyte whereas basophil and eosinophil were more implicated in inflammation in extrinsic and/ or intrinsic allergenic reaction.

CHAPTER SIX

6.1 Summary of Findings

OBJECTIVES	FINDINGS
1. To determine the spatial distribution of atmospheric pollen and spore and their relationship to weather parameters	1. Two major seasons of pollen and spores abundance were noted in Southern and North Central Nigeria; a rainy season dominated by fungal spores and late rainy/harmattan dominated by pollen 2. Quantitative abundance of pollen was recorded during the rainy season and majorly dominated by Poaceae in North West and North East Nigeria. 3. Pollen concentration in the air had a direct relationship with temperature and wind 4. Rainfall favoured sporulation of fungal spores.
2. Determination of allergen protein concentration	1.There was a relationship between the allergen protein and elicited IgE in some Mice .
3. Establishment of systemic and physical features of allergy provoked by pollen and spores	1. Pollen protein of *Oreodoxa oleracea* induced a characteristic dermatophytic reaction physically featuring as swelling, rashes and hair loss on mice skin 2. *Mariscus ligularis* and *Sacciolepis africana* pollen proteins and spore protein of *Aspergillus niger* showed inflammatory reaction on respiratory system.
4. Evaluation of immunoglobulin E(IgE) antibodies and immune cells of mice developed against pollen	1. Repeated low doses of pollen and spores protein resulted in persistent IgE production with and without physical features of allergy.

and spores protein.	2. Pollen protein of *Oreodoxa oleraceae* and *Sacciolepis africana* elicited high basophil cells which depicted their potency in inflammation. 3. Allergen protein of *Terminalia catappa, Panicum maximum, Mariscus ligularis* pollen and *Fusarium* sp. spore induced higher eosinophil cells than *Aspergillus niger, Mangifera indica*, *Sacciolepis africana, Oreodoxa oleracea* and control. 4. All studied pollen and spore proteins induced alteration in immune system which resulted in lymphocyte infiltration. 5. Mice inoculated with allergen protein had a greater number of neutrophils, lymphocytes, eosinophils and basophils than control mice.

6.2 Contributions to Knowledge

1. The result establishes the basis for future work towards creating Nigerian pollen and fungal spores calendar of the study locations which indicate two different risk periods for pollen hypersensitive individuals; late rainy/dry season in North Central and Southern Nigeria, rainy season for individuals in North West and North East Nigeria. Rainy season was also noted as a period for dominant fungi spores over the atmosphere of the six geopolitical zones. This however provides useful prophylactic and avoidance tool for allergic people.
2. The research work shows that persistent low doses of allergen result in increase in IgE level with or without physical features of allergy.
3. The work further establishes that serological analysis of immunoglobulin E integrated with immune cell count will assist in identification of allergen.

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APPENDIX 1

ETHICAL APPROVAL

INSTITUTIONAL REVIEW BOARD

NIGERIAN INSTITUTE OF MEDICAL RESEARCH

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Tel: 01-4823123, 01-7744723, 08050254484, 08033460947 *Fax:* 01-4823123, 234-1-3426171
E-mail: nimr_irb@yahoo.com *Website:* www.nimr-nig.org
Secretariat: Room 207, Biochemistry Division, Research Block, NIMR

21st Oct, 2014

PROJECT TITLE: **SPATIAL DISTRIBUTION OF ATMOSPHERIC POLLEN AND FUNGI SPORES AND THEIR RELATION TO ALLERGY IN NIGERIA.**

PROJECT №: **IRB/14/261**

APPROVAL LETTER

The above named proposal has been adequately reviewed; the protocol and safety guidelines satisfy the conditions of NIMR-IRB, policies regarding experiments that use human subjects.

Therefore the study under its reviewed state is hereby approved by Institutional Review Board, NIMR.

PROF. F. E. OKONOFUA
Name of IRB Chairman ..... *Signature of IRB Chairman & Date*

MRS. O. A. NWOGBE
Name of IRB Secretary ..... *Signature of IRB Secretary & Date*

This approval is given with the Investigator's Declaration as stated below;
By signing below I agree/certify that:

1. I have reviewed this protocol submission in its entirety and that I am fully cognizant of, and in agreement with, all submitted statements.
2. I will conduct this research study in strict accordance with all submitted statements except where a change may be necessary to eliminate an apparent immediate hazard to a given research subject.
   - I will notify the IRB promptly of any change in the research procedures necessitated in the interest of the safety of a given research subject.
   - I will request and obtain IRB approval of any proposed modification to the research protocol or informed consent document(s) prior to implementing such modifications.

APPENDIX 2

METEOROLOGICAL DATA OF ENUGU, SOUTH EAST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	26.4	189.8	85	4.9
2011	JULY	25.7	194.9	85	4.9
2011	AUGUST	25.5	237.4	85	5.9
2011	SEPTEMBER	25.6	443.6	86	4.3
2011	OCTOBER.	26.4	153.8	83	4.4
2011	NOVEMBER.	27.7	2.0	72	4.1
2011	DECEMBER.	26.2	0.0	52	6.5
2012	JANUARY.	27.1	39.0	55	6.1
2012	FEBRUARY.	28.7	21.2	72	4.7
2012	MARCH	30.8	68.1	61	4.9
2012	APRIL	28.5	129.5	76	6.5
2012	MAY	27.1	288.7	81	4.7

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 3

METEOROLOGICAL DATA OF RIVERS, SOUTH SOUTH NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	26.2	353.8	89	2.7
2011	JULY	25.3	304.8	70.3	2.3
2011	AUGUST	25.2	310.2	91	2.5
2011	SEPTEMBER	25.5	413.0	90	1.9
2011	OCTOBER	25.9	284.7	89	1.9
2011	NOVEMBER	26.7	45.3	86	1.6
2011	DECEMBER	26.7	28.3	74	1.8
2012	JANUARY	26.1	59.5	73	2.4
2012	FEBRUARY	26.8	162.0	84	2.4
2012	MARCH	27.9	225.3	82	2.4
2012	APRIL	26.8	206.7	86	2.4
2012	MAY	26.7	330.6	86	2.4

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 4

METEOROLOGICAL DATA OF LAGOS, SOUTH WEST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{0}C)	RAINFALL(MM)	HUMIDITY (%)	WIND(KNOTS)
2011	JUNE	26.5	251.9	89	3.7
2011	JULY	25.5	476.9	89	6.0
2011	AUGUST	25.5	43.7	88	7.5
2011	SEPTEMBER	26.0	175.3	86	3.7
2011	OCTOBER.	26.2	209.3	88	2.6
2011	NOVEMBER.	27.0	240.5	88	2.6
2011	DECEMBER.	27.5	0.0	80	3.0
2012	JANUARY.	27.1	10.5	81	3.2
2012	FEBRUARY	27.6	122.2	84	3.8
2012	MARCH	28.5	78.1	83	3.7
2012	APRIL	28.2	124.7	83	4.2
2012	MAY	27.3	134.9	86	3.6

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 5

METEOROLOGICAL DATA OF ABUJA NORTH CENTRAL NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP. (^{0}C)	RAINFALL(MM)	HUMIDITY (%)	WIND(KNOTS)
2011	JUNE	26.2	128.6	83	4.1
2011	JULY	25.7	227.6	87	4.1
2011	AUGUST	24.7	183.5	88	3.8
2011	SEPTEMBER	25.2	278.0	86	4.0
2011	OCTOBER	25.8	130.3	83	3.7
2011	NOVEMBER	26.3	6.8	64	4.0
2011	DECEMBER	25.3	0.0	40	4.5
2012	JANUARY	26.2	0.0	41	4.5
2012	FEBRUARY	29.2	20.6	52	4.2
2012	MARCH	30.9	19.0	38	5.0
2012	APRIL	32.6	45.2	65	6.6
2012	MAY	34.3	198.5	78	3.9

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 6

METEOROLOGICAL DATA OF KADUNA, NORTH WEST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP (^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	25.5	80.9	76	4.7
2011	JULY	24.6	240.3	78	4.6
2011	AUGUST	24.0	208.0	82	4.6
2011	SEPTEMBER	24.3	298.7	80	3.6
2011	OCTOBER	25.0	72.7	73	2.5
2011	NOVEMBER	24.0	0.0	37	4.7
2011	DECEMBER	22.4	0.0	26	7.0
2012	JANUARY	23.1	0.0	23	6.4
2012	FEBRUARY	27.4	0.0	23	4.8
2012	MARCH	28.2	0.0	19	7.0
2012	APRIL	28.8	212.8	58	9.7
2012	MAY	26.5	61.6	70	5.6

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 7

METEOROLOGICAL DATA OF GOMBE, NORTH EAST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	25.3	76.4	56	5.9
2011	JULY	24.5	183.0	57	5.2
2011	AUG.	24.4	157.2	60	4.8
2011	SEPTEMBER	24.0	119.5	64	4.4
2011	OCTOBER	25.7	70.2	54	4.5
2011	NOVEMBER	27.0	0.0	20	3.7
2011	DECEMBER	27.8	0.0	28	3.8
2012	JANUARY	27.9	0.0	26	3.7
2012	FEBRUARY	27.9	0.0	22	3.6
2012	MARCH	28.0	0.0	24	5.8
2012	APRIL	27.7	6.8	35	6.0
2012	MAY	27.5	93.0	45	5.3

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 8

NATIONAL INSTITUTE OF HEALTH (NIH) ON IMMUNOGLOBULIN E RATING SCALE

RAST rating	IgE level (ng/ml)	Comment
0	< 0.35	ABSENT OR UNDETECTABLE ALLERGEN SPECIFIC IgE
1	0.35 - 0.69	LOW LEVEL OF ALLERGEN SPECIFIC IgE
2	0.70 - 3.49	MODERATE LEVEL OF ALLERGEN SPECIFIC IgE
3	3.50 - 17.49	HIGH LEVEL OF ALLERGEN SPECIFIC IgE
4	17.50 - 49.99	VERY HIGH LEVEL OF ALLERGEN SPECIFIC IgE
5	50.0 - 100.00	VERY HIGH LEVEL OF ALLERGEN SPECIFIC IgE
6	> 100.00	EXTREMELY HIGH LEVEL OF ALLERGEN SPECIFIC IgE

The RAST test is scored on a scale from 0 to 6:

APPENDIX 9

SPSS ANALYSIS DATA GENERATED FROM BASOPHIL, EOSINOPHIL, LYMPHOCYTE, MONOCYTE, NEUTROPHIL AND IgE

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Basophil	t2	5	.8000	.83666	.37417	-.2389	1.8389	.00	2.00
	t2	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t3	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t4	5	3.0000	2.00000	.89443	.5167	5.4833	.00	5.00
	t5	5	11.4000	22.16529	9.91262	-16.1218	38.9218	.00	51.00
	t6	5	12.6000	27.61883	12.35152	-21.6933	46.8933	.00	62.00
	t7	5	3.0000	4.52769	2.02485	-2.6219	8.6219	.00	11.00
	t8	5	5.0000	8.97218	4.01248	-6.1404	16.1404	.00	21.00
	control	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	45	3.9778	12.05006	1.79632	.3575	7.5980	.00	62.00
Eosinophil	t2	5	4.8000	4.32435	1.93391	-.5694	10.1694	.00	10.00
	t2	5	3.4000	3.43511	1.53623	-.8653	7.6653	.00	8.00
	t3	5	.6000	.89443	.40000	-.5106	1.7106	.00	2.00
	t4	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t5	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t6	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t7	5	1.8000	2.68328	1.20000	-1.5317	5.1317	.00	6.00
	t8	5	1.0000	1.22474	.54772	-.5207	2.5207	.00	3.00
	control	5	.4000	.89443	.40000	-.7106	1.5106	.00	2.00
	Total	45	1.3333	2.52262	.37605	.5755	2.0912	.00	10.00
Lymphocyte	t2	5	90.6000	9.01665	4.03237	79.4043	101.7957	75.00	97.00
	t2	5	94.2000	3.11448	1.39284	90.3329	98.0671	90.00	97.00
	t3	5	99.2000	.83666	.37417	98.1611	100.2389	98.00	100.00
	t4	5	93.0000	9.69536	4.33590	80.9616	105.0384	76.00	100.00
	t5	5	87.8000	22.81885	10.20490	59.4667	116.1333	47.00	99.00

	t6	5	68.6000	41.84854	18.71523	16.6382	120.5618	.00	99.00
	t7	5	90.8000	7.62889	3.41174	81.3275	100.2725	83.00	99.00
	t8	5	93.6000	9.39681	4.20238	81.9323	105.2677	77.00	100.00
	control	5	97.8000	4.91935	2.20000	91.6918	103.9082	89.00	100.00
	Total	45	90.6222	17.67161	2.63433	85.3131	95.9314	.00	100.00
Monocyte	t2	5	2.8000	4.38178	1.95959	-2.6407	8.2407	.00	10.00
	t2	5	2.4000	.54772	.24495	1.7199	3.0801	2.00	3.00
	t3	5	.2000	.44721	.20000	-.3553	.7553	.00	1.00
	t4	5	4.2000	9.39149	4.20000	-7.4611	15.8611	.00	21.00
	t5	5	.8000	1.09545	.48990	-.5602	2.1602	.00	2.00
	t6	5	2.6000	5.81378	2.60000	-4.6188	9.8188	.00	13.00
	t7	5	4.4000	7.36885	3.29545	-4.7496	13.5496	.00	17.00
	t8	5	.2000	.44721	.20000	-.3553	.7553	.00	1.00
	control	5	1.8000	4.02492	1.80000	-3.1976	6.7976	.00	9.00
	Total	45	2.1556	4.65127	.69337	.7582	3.5530	.00	21.00
Neutrophil	t2	5	1.0000	2.23607	1.00000	-1.7764	3.7764	.00	5.00
	t2	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t3	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t4	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t5	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t6	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t7	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t8	5	.2000	.44721	.20000	-.3553	.7553	.00	1.00
	control	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	45	.1333	.75679	.11282	-.0940	.3607	.00	5.00
IgE	t2	5	9.1120	4.81354	2.15268	3.1352	15.0888	1.52	13.34
	t2	5	6.2202	3.01220	1.34710	2.4801	9.9603	1.02	8.35
	t3	5	3.9880	3.03713	1.35825	.2169	7.7591	.13	7.00
	t4	5	8.4700	5.11675	2.28828	2.1167	14.8233	1.70	13.78
	t5	5	5.8130	5.84470	2.61383	-1.4442	13.0702	.13	13.13
	t6	5	2.2042	1.86866	.83569	-.1161	4.5245	.00	3.91
	t7	5	4.1480	2.13563	.95508	1.4963	6.7997	1.63	7.19
	t8	5	4.6840	4.99121	2.23214	-1.5134	10.8814	.25	13.10
	control	5	1.5180	.13809	.06176	1.3465	1.6895	1.35	1.73
	Total	45	5.1286	4.27168	.63678	3.8452	6.4120	.00	13.78

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Basophil	Between Groups	949.778	8	118.722	.786	.618
	Within Groups	5439.200	36	151.089		
	Total	6388.978	44			
Eosinophil	Between Groups	116.800	8	14.600	3.221	.007
	Within Groups	163.200	36	4.533		
	Total	280.000	44			
Lymphocyte	Between Groups	3226.978	8	403.372	1.381	.238
	Within Groups	10513.600	36	292.044		
	Total	13740.578	44			
Monocyte	Between Groups	97.511	8	12.189	.514	.838
	Within Groups	854.400	36	23.733		
	Total	951.911	44			
Neutrophil	Between Groups	4.400	8	.550	.952	.488
	Within Groups	20.800	36	.578		
	Total	25.200	44			
IgE	Between Groups	263.706	8	32.963	2.201	.051
	Within Groups	539.173	36	14.977		
	Total	802.879	44			

Post Hoc Tests

Homogeneous Subsets

Basophil

Duncan

Treatment	N	Subset for alpha = 0.05
		1
t2	5	.0000
t3	5	.0000
control	5	.0000
t2	5	.8000
t4	5	3.0000
t7	5	3.0000
t8	5	5.0000
t5	5	11.4000
t6	5	12.6000
Sig.		.176

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Eosinophil

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
t4	5	.0000		
t5	5	.0000		
t6	5	.0000		
control	5	.4000	.4000	
t3	5	.6000	.6000	
t8	5	1.0000	1.0000	
t7	5	1.8000	1.8000	
t2	5		3.4000	3.4000
t2	5			4.8000
Sig.		.256	.052	.305

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Lymphocyte

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
t6	5	68.6000	
t5	5	87.8000	87.8000
t2	5	90.6000	90.6000
t7	5	90.8000	90.8000
t4	5		93.0000
t8	5		93.6000
t2	5		94.2000
control	5		97.8000
t3	5		99.2000
Sig.		.067	.373

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Monocyte

Duncan

Treatment	N	Subset for alpha = 0.05
		1
t3	5	.2000
t8	5	.2000
t5	5	.8000
control	5	1.8000
t2	5	2.4000
t6	5	2.6000
t2	5	2.8000
t4	5	4.2000
t7	5	4.4000
Sig.		.254

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Neutrophil

Duncan

Treatment	N	Subset for alpha = 0.05
		1
t2	5	.0000
t3	5	.0000
t4	5	.0000
t5	5	.0000
t6	5	.0000
t7	5	.0000
control	5	.0000
t8	5	.2000
t2	5	1.0000
Sig.		.083

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

IgE

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
control	5	1.5180	
t6	5	2.2042	
t3	5	3.9880	3.9880
t7	5	4.1480	4.1480
t8	5	4.6840	4.6840
t5	5	5.8130	5.8130
t2	5	6.2202	6.2202
t4	5		8.4700
t2	5		9.1120
Sig.		.103	.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The transfer of pollen from anther to recipient stigma is the critical reproductive event among higher plants which leads to their dispersal into the atmosphere by various mechanisms. The atmosphere is laden with many kinds of suspended particles of organic and inorganic origin having great diversity in size, shape, and density and from diverse sources (Essien and Agwu, 2013). Aerobiological investigations have revealed that pollen and fungal spores are the most dominant, pervasive, respirable and potent sources of allergen present in the atmosphere. Pollen and spores are also more ubiquitous and widely distributed in time and space than any other representatives of living matter (Shahali *et al*., 2007). Their atmospheric concentration are frequently high enough to present a substantial antigenic load to exposed hypersensitive individuals (Horner *et al*., 2000). Their abundance depend generally on the degree of turbulence of the air, time of the day, season, local sources and climatic factors such as temperature, humidity, rainfall, wind direction and strength (Laycer, 2007). Airborne pollen and fungi spores are ubiquitous in indoors and outdoors environment due to their sizes, predominance in nature and aerodynamic properties which enhance their distribution (Bryce, 1995; Mesa, *et al.*, 2003). Knowledge of the kind and type of pollen or spores in the air has medical implications (Essien and Agwu, 2013).

Fungi grow almost everywhere, even as lichens inside Antarctic rocks (Horner *et al*., 2000). They grow over a wide temperature range (-5 to 50 ^{0}C and greater) (Gravesen, 1979), although individual species usually grow within a much narrower range. One of the most important physical parameters affecting fungal growth is moisture. Airborne fungal spores are usually present in outdoor air throughout the year in high numbers and frequently exceed pollen concentrations (Horner *et al*., 2000), depending on environmental factors such as water and nutrient availability, temperature, and wind. Most fungi commonly considered allergenic, such as *Alternaria* spp., *Cladosporium* spp., *Epicoccum nigrum, Fusarium* spp. and *Ganoderma* spp., display a seasonal spore release pattern, but this is less well defined than it is for pollen (Horner *et al*., 2000). Aerobiologic surveys show that fungal spores are present in the atmosphere worldwide, nearly in all environments. They are universal atmospheric components indoors and outdoors and are now generally recognized as important causes of respiratory allergies. Allergic reactions associated with fungi involve the lower respiratory tract more frequently than do pollen allergies (Horner *et al*., 2000). More than 80 genera of fungi have been associated with symptoms of respiratory tract allergy (Horner *et al*., 2000), however multiple species may be observed at any time of the year. Fungi reproduce by releasing spores into the air, their inhalation is through respiration of dust particles contaminated with fungal spores. The ubiquitous presence of fungi in both indoors and outdoors environment is a potential health threat that is poorly understood and almost ignored in community medicine. Airborne fungal spores exposure frequently cause adverse human health effect with injury and dysfunction of multiple organs and systems including; respiratory, nervous, immune, haematological system and skin (Kothari *et al.,* 1993). Exposure to some fungal spores can also trigger infectious diseases for immune compromised persons. Despite the clinical importance and abundant

release of fungal spores, relatively few investigations have focused on the relationship between airborne spores and allergic diseases (Kothari *et al.,* 1993).

The term allergy was coined in 1906 by von Pirquet to describe an altered reactivity in living beings (i.e., an IgE mediated hypersensitivity) caused by a foreign substance (Horner *et al*., 2000). Allergens, therefore, are the subset of antigens that stimulate an IgE-mediated response. Genetic factors are known to influence the ability to IgE-mediated reaction and those individuals with sustained elevated IgE levels are referred to as atopic. Type I allergic disease of fungal spores and pollen allergens typically manifests as rhinitis (hay fever), conjunctivitis, dermatitis, asthma etc. Allergic reactions, including respiratory allergy, may occur in two phases. The early-phase reaction occurs within minutes as a result of the release of preformed mediators. Late-phase responses occur 3 to 4 h after allergen exposure as a result of cellular infiltrates responding to early-phase mediators. A dual reaction involves both early- and late-phase reactions. Emerging evidence indicates that a significant, persistent inflammatory component in addition to IgE-triggered effects underlies the etiology of asthma.

Pollen and fungal spores allergen belong to type one hypersensitivities. Their proteins are immunomodulatory substances, which play crucial roles in the sensitization and/or exacerbation of allergies such as seasonal rhinitis, conjunctivitis, asthma, bronchial constriction and obstruction, pollinosis and atopic dermatitis (Phanichyakarn *et al*., 1989; D'Amato *et al*., 2002; Kamijo *et al.*, 2013). On an immunological response to allergen, the immune system makes immunoglobulin E antibodies which attach to immune cells. These undergo degranulation and trigger the cells to release histamine and other inflammatory chemical mediators such as cytokines, interleukins and prostaglandins. These chemical mediators elicit symptoms of allergy including wheezing, runny nose, itching, rashes, sneezing etc. These symptoms range from mild to life threatening symptoms and could either be localized or systemic (Singh and Kumar, 2004).

Allergic reactions from pollen or spores normally occur at the site of allergen deposition. Most inhaled particles greater than 10 µm are deposited in the nasopharynx and are associated with nasal and or ocular symptoms, reffered to as hayfever (Horner *et al*., 2000). Conversely particles less than 10 µm especially those of 5 µm can penetrate the lower airways. Pollen and fungal spores differ in sizes and are associated with both upper and lower respiratory systems (Horner *et al*., 2000). Additionally, there is now evidence that secondary dispersal of allergens, i.e., on other, smaller particles, possibly spore fragments, may serve as a vehicle for allergens. This would permit the deposition of allergens even from large spores into the lower airways (Horner *et al*., 2000).

World-wide, it is estimated that 300 million people are affected with bronchial asthma (Masoli *et al*., 2004). The prevalence of asthma is variable. It is a disease that has been observed to be more prevalent in developed countries with higher rates seen in Australia, UK, and New Zealand (Masoli *et al*., 2004). In Nigeria, the prevalence of asthma ranges from 7% to 18% in the general population. Sex ratio varies according to age (Masoli *et al*., 2004). In childhood, asthma affects more boys than girls for unknown reasons, but by the third decade, the prevalence becomes equal and subsequently, more women than men are affected. (Masoli *et al*., 2004; Ibe and Ele, 2002) Asthma prevalence is increasing despite recent advances being made in its management (Oni *et al*., 2010)

Pollen and fungal spores allergy are more difficult to diagnose and treat than other allergies because they are far more numerous and antigenically variable than other allergies and are exceedingly difficult to avoid (Garijo, 1996). The knowledge of qualitative and quantitative prevalance and their seasonal and annual variations are of paramount importance in effective

diagnosis and management of pollen related allergens (Singh and Kumar, 2004; D'Amato *et al*., 2007).

Garijo (1996) remarked that risk factors for allergy could be placed in two general categories, namely host and environmental factors. Host factors include heredity, gender, race and age, the most significant being the host factors. Allergic diseases are strongly familial, identical twins are likely to have the same allergic diseases about 70 % of the time while the same allergy occurs about 40 % of the time in non-identical twins (Tamari *et al*., 2013). Allergic parents are more likely to have allergic children and their allergies are more severe than those from non-allergic parents. Some allergies, however, are not consistent along genealogies. Allergic sensitization is inherited and related to an irregularity in the immune system (Docampo *et al*., 2007). However, there have been recent increase in the incidence of allergic disorders that cannot be explained by genetic factors alone. Four major environmental risk factors are recognized. They are : alterations in exposure to infectious diseases during early childhood, exposure to allergen load, environmental pollution, and dietary changes.

The risk of allergic sensitization and the development of allergies vary with age, with young children mostly at risk (Onyedum *et al*., 2013). Several studies have shown that immunoglobulin E (IgE) levels are highest in childhood and fall rapidly between the ages of 10 and 30 years. The peak prevalence of hay fever is highest in children and young adults and the incidence of asthma is highest in children under 10. Aderele (1979) reported that boys have a higher risk of developing allergy than girls. Although for some diseases, such as asthma in young adults, females are more likely to be affected with sex differences tending to decrease in adulthood. Ethnicity may also play a role in some allergies; however, racial factors have been

difficult to separate from environmental influences and changes due to migration. It has been suggested that different genetic loci are responsible for asthma, to be specific, in people of European, Hispanic, Asian, and African origins (Aderele, 1979).

International differences have been associated with the number of individuals within a population that suffer from allergy. Allergic diseases are more common in industrialized countries than in countries that are more traditional or agricultural, and there is a higher rate of allergic disease in urban populations than rural populations, although these differences are becoming less defined (Onyedum *et al*., 2013).

Airborne fungal spore concentration is however an indicator of pathogen development in agriculture and could be useful when infection levels are initially determined by source of inoculums rather than the weather. Monitoring of airborne inoculums integrated with meteorological data provides a valuable tool for establishing the basis for an accurate modern integrated pest –management strategy (Kasprzyk , 2004; Escuredo *et al*., 2010). The awareness of the potentially allergenic pollen and fungal spores counts and its changes throughout the period of release in a given area is of great importance in prophylaxis of allergic respiratory diseases which has become a social problem in all continents. Pollen and fungal spore counts are estimates of the antigenic challenge confronting allergic individuals. The nature of this challenge depends on the particular pollen and fungal spores types found in the atmosphere and also their airborne concentrations (Laycer, 2007). Horner *et al*. (2000) stated that aerobiology of pollen and fungal spores is of direct relevance to the medical community due to their implication in precipitating respiratory diseases (pollinosis), conjunctivitis, rhinoconjuctivitis, eczema /dermatitis and asthmatic attacks.

Oni *et al*. (2010) asserted that elicitation of allergic disease is multifactorial and dependent on the interaction between genetic predisposition and environmental factors, characterized by dysregulation of immunity. This dysregulation leads to a strongly polarized T helper type 2 (Th2) immune response and a chronic inflammation in the airways in response to innocuous antigens. In asthmatic patients, the penetration of allergens into the lungs leads to airway inflammation consisting of a peribronchial infiltration of $CD4^+$ T cells, macrophages, eosinophils and neutrophils and the presence of these cells in Bronchoalveolar Lavage Fluid (BALF). Asthmatic patients also present a goblet cell metaplasia/hyperplasia and characteristic modifications of the airway wall including epithelial hyperplasia, thickening of the basement membrane, subepithelial fibrosis, increased airway smooth muscle mass and, finally, airway hyper reactivity (AHR) to specific and non-specific stimuli (Oni *et al*., 2010).

The burden of these allergies to health care systems, families and patients increases worldwide (Onyedum *et al*., 2013). Despite the ubiquitous nature of pollen and spores, their great aerodynamic properties and implications in allergic reactions, virtually no previous attention has been given to survey their pattern of spatial distribution in atmosphere of different geopolitical zones of Nigeria, as documented in this work, though the South East and South West have been worked upon.

Some Aeropalynological studies in South West Nigeria include; Aeropalynological studies of the University of Lagos Campus, Nigeria was carried out by Adekanmbi and Ogundipe (2010). The result of their study reveals that palynomorphs abundance and diversity achieved its peak in May which corresponds to the wettest month of the sampled period. Their work did not extend to the two seasons in Nigeria. Adeonipekun and John (2011) carried out a research on

investigation of haze dust in Ayetoro-Itele Ota, South West Nigeria. They showed that the hazy dust studied contain high proportion of palynomorphs for Sudan/Guinea and derived Savanna as well as lowland rainforest ecozones. Adeonipekun and Olowokudejo (2012), verified the pollen rain in offshore location where there is no vegetation, the study confirmed pollen rain as the important contributor to marine sediments in Niger Delta. Adeniyi *et al*., 2014 studied the pollen records of Shomolu Local Government Area of Lagos State. Their study revealed that the most dangerous period for inhabitants of Shomolu occurred between October and September when the highest level of grass and herbaceous pollen grains were recorded. They also found that total pollen concentration correlates positively with the temperature and negatively with wind, rainfall and relative humidity. In their work the allergenicity of the pollen were not tested in any animal model. In South East, Agwu *et al*. (2004) carried out a study on airborne pollen and spores circulating at head level in Nsukka environment. The frequent and abundant pollen types they found include those of Poaceae(grasses), *Elaeis guineensis*, *Casuarina equisetifolia*, *Alchornea cordifolia*, *Milicia excelsa* and Amarathaceae / Chenopodiaceae. Their work however did not cover the two seasons in Nigeria. Njokuocha (2006) studied airborne pollen grains in Nsukka, Nigeria. He recorded the following predominant pollen and fern spores; *Casuarina equisetifolia*, *Milicia excelsa*, *Elaeis guineensis*, *Celtis integrifolia*, *Alchornea cordifolia*, Amaranthaceae / Chenopodiaceae, Combretaceae / Melastomataceae, *Nephrolepis biserrata*, *Thelypteris totta*, and *Dryopteris* spp. Njokuocha (2006) did not carry out the allegenicity study of pollen identified from his study area.

The present work is more broader than earlier studies carried out in Nigeria, it cut across different geo-political zones and vegetation types. Result from this work will form the basis for future works towards creating pollen calendars for forecasting of allergic incidences as well as

providing the basics for management approach to respiratory allergy. Pollen and fungi spores allergenicity of Nigerian plants has not been previously documented to the best of the researcher knowledge. The present work is the first to test the allergenicity of pollen and fungal spores on mice in Nigeria.

1.2 Statement of the Problem

Pollen and fungal spores (collectively referred to as 'aeroallergens') exacerbate allergies e.g. hay fever/allergic rhinitis and other asthmatic conditions, affecting hundreds of millions of people worldwide (Bousquet *et al*., 2011). In the United Kingdom, approximately 20% of the population suffer from hay fever and approximately 95 % of hay fever sufferers are allergic to grass pollen and 25% to tree pollen. There are approximately 150 species of grass in the UK, 12 of which are important with respect to atmospheric pollen load (Emberlin *et al*., 1999).

The increasing prevalence of allergic diseases over past decade is well established and accepted by most health authorities including the world Health Organization (WHO, 2003). The evidence from patients and stakeholders agreed that access to diagnosis, treatment, education, information and continued research should be a priority for health authorities and educationist. However, the absence of large scale epidemiological studies on prevalence has prevented most authorities from understanding the extent and taking the steps necessary to commission the requisite services (WHO, 2003).

Allergic diseases require expensive short term treatment. The real public health cost is the drain on resources over a prolonged period, as lives of the sufferer, may be impaired for several decades. To fully evaluate the socio-economic costs of allergic diseases, it is important to consider the direct costs of hospitalization, physical consultations and treatments and the indirect cost of days lost in work and education (D'Amato *et.al*., 2007). Also patients are

restricted in their social and physical activities, so their productivity and professional life will be affected. Lethargy, poor concentration and behavioral changes may arise as a result of persistent symptoms and poor sleep which can impact negatively on adults and children (D'Amato *et al*., 2007). When symptoms are severe and effective treatment is not available to patients, work days may be compromised and even lost.

Asthma is one of the most chronic allergic diseases which is estimated to affect as many as 300 million people worldwide and could increase by another 100 million by the year 2025(WHO, 2003). Exposure to allergens represents a key factor among the environmental determinants of asthma (D'Amato *et al*., 2007). The prevalence and incidence for asthma throughout Africa had increased remarkably in recent years, the condition was previously uncommon over most parts of the continent (WHO, 2003). Fifty years ago asthma was uncommon in Nigeria, however recent reports from different parts of Nigeria have shown a prevalence of adolescent and adult asthma in excess of 10 % and a rising trend in the prevalence of asthma (Desalu *et al*., 2013). The increase in burden the asthma has been attributed to environmental factors such as urbanization, industrialization and adoption western life style (Desalu *et al*., 2013). ISAAC study conducted among children in Nigeria, the prevalence of asthma was 18.4 % (Desalu *et al.*, 2011). In a hospital-based study in Southern region of Nigeria, the prevalence of asthma was 6.6 % among patients attending a respiratory clinic. Today, more than 30 % of the population is known to suffer from one or the other allergic ailment, bronchial asthma, allergic rhinitis while atopic dermatitis are dramatically increasing all over the world including developing countries (Singh and Kumar, 2004). The risk factors for asthma identified in Nigeria were family history of asthma, allergic rhinitis, outdoor and indoor allergens, tobacco smoking and obesity (Desalu *et al.*, 2011).

Many Nigerians suffer from other allergic diseases related to airborne pollen and spores. In the last 20-30 years, the prevalence of allergic diseases has escalated significantly, a trend that shows no signs of abating and the urban dwellers are mainly affected. Erhabor *et al*. (2002), remarked that asthma and other allergic diseases had not only become a national problem but international. According to him, the Nigerian government was not doing enough in curbing its spread, diagnosis and treatment, stressing that past administrations in the country only concerned themselves with eradication of communicable diseases while neglecting non-communicable diseases.

1.3 Objectives of the Study

Overall Aim

The overall aim of this study was to determine the seasonal prevalence and spatial distribution of airborne pollen and fungal spores in Nigeria and assess their allergenic potentials in mice.

The specific objectives were to;

1. Determine the seasonal prevalence of airborne pollen and fungal spores in the atmosphere of Nigeria and impacts of weather parameters on them.
2. Determine the concentration of protein in some selected pollen and fungal spores.
3. Establish the clinical features of allergy provoked by some selected pollen and fungal spores in mice.
4. Evaluate the immunoglobulin E antibodies and immune cells elicited by some selected pollen and fungal spores in mice

1.4 Significance of the Study

This study will lead to the development of pollen and fungal spores calendar which will display the flowering periods of plants and seasonal occurences of fungal spores in each studied locations in geopolitical zones. This will enable forecasting/predicting of their future occurrences and serve as a preliminary approach to the problems of allergic diseases caused by pollen and fungal spores. The recognition of recovered pollen and fungal spores allergenic potential will add to the knowledge about other identified allergies; such as drug allergy, food allergy, chemical sensitivity e.t.c., among which pollen and fungal spores have received little attention in Nigeria. The establishment of their clinical importance will aid in the diagnosis and treatment of allergenic conditions due to pollen and fungal spores.

1.5 Operational Definition of Terms

a. **Allergen** = is a substance that binds specifically to the antibody/substance that stimulate an immune response
b. **Anthesis** = is a period during which a flower is fully open and functional
c. **Antigen** = A substance that binds specifically to the antibody
d. **Charred Poaceae cuticles**= burnt epidermis of grasses
e. **Histamine** = Chemical found in some body cells which causes symptoms of allergy
f. **Intranasal** = Within the nose
g. **Pollen** = is a fine to coarse powder containing the microgametophyte of seed plant
h. **Spore** = A unit of asexual reproduction that may be adapted for dispersal and for survival
i. **Subcutaneous** = Beneath the skin
j. **Immunosobent Assay** = is a test that uses antibodies and colour change to identify a substance

k. **Sera** = is a clear pale yellow liquid that separate from the clot in the coagulation of blood.

l. **Immunoglobulin E** = Is an antibody that plays an essential role in type one hypersensitivities which manifests various allergic diseases.

m. **Antibody** = is a protein produced by the body's immune system when it detects harmful substances called antigen.

n. **SDS-PAGE =** Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis.

o. **ISSAC =** International Study of Asthma and Allergies in Childhood

p. **Wheeze ever =** Wheezing from birth.

q. **Current wheeze =** Wheezing at present.

1.6 ABBREVIATIONS

APC – Airborne pollen concentration

h- hours

Mins-minutes

CHAPTER TWO
LITERATURE REVIEW

2.1 Aeropalynology

The term palynology was coined by Hyde and Williams (1944). The term was originally defined as the study of pollen and other plant spores and their dispersal and application thereof. In a wider and a more contemporary sense, Palynology comprises the study of pollen and other plant – like microfossils. Aeropalynology is a branch of Palynology that studies pollen grains and spores that are dispersed into the atmosphere. According to Gregory (1992), aerobiology is usually understood to be the study of passively airborne microorganisms, their identity, behaviour, movements and survival. This field of science includes: identification, morphology, physiology, viability, longevity, sampling, concentrations, diurnal and seasonal patterns, phenology, emission, transport, dispersion, pollination, pollinosis and a host of other subjects. Aerobiology involves study of airborne particles of plant and animal origin. Some aerobiologists segregate the study of airborne pollen into a branch of aerobiology termed as 'aeropalynology. Pollen is nature's gift to mankind as it is responsible for pollination, fertilization, seed and fruit setting and multiplication of plants. However, some of the pollen after getting into air stream remain floating in the atmosphere before settling down on the ground. Some of these pollen on coming in contact with human beings induce allergic manifestations.

The primary objectives of aerobiological studies are to monitor, determine and detect the occurrence of pollen and spores and their relative representation in the atmosphere. On account of the tremendous applications of aeropalynology in public health and medicines, a new term has been added recently known as 'Medical Palynology'. This branch is concerned with the

study of airborne pollen and fungal spores, which are responsible for causing allergic manifestations including the triggering effect leading to asthmatic attacks. In addition, various aspects of immunotherapy are investigated involving hyposensitization of allergy patients by using pollen and fungal aeroallergen extracts. According to a recent trend, the scope of aerobiology has been widened to incorporate different kinds of biological particles (air spora) for example, viruses, bacteria, microalgae, microfungi, lichen fragments, soredia, seeds, protozoan cysts, insects and insect parts, spiders (Gregory 1992). Abiotic particles or gases affecting living organisms are also included currently in the concept of aerobiology. Thus, the aerobiological pathway involves at least five major steps, which are: source, liberation, passive transport, deposition and impact on vegetation, water bodies and various substrates. It is obvious that these different steps are intertwined and they are affected by environmental factors, such as, meteorology, physics and atmospheric chemistry (Gregory, 1992). Aerobiology has become an interdisciplinary science of great significance and applications in different fields, such as, ecology, medicine, pathology, agriculture, forestry and meteorology. There are various ways of dispersal of pollen in the atmosphere, however, the most important factor is wind which transmits pollen grains and spores from their sources to the target areas. Hence, windborne pollen both of flowering plants or the angiosperms and gymnospermous plants are significant in aerobiological studies.

Agashe and Alfadil (1989) carried out the aeropalynological survey of the atmosphere at Bangalore for six years. This survey was conducted by trapping airborne bioparticles such as pollen and fungal spores by operating vertical cylinder pollen traps. The traps were installed at different ecogeographical sites in Bangalore City. The results of qualitative and quantitative analysis of the atmospheric biopollutants were correlated with meteorological parameters such

as temperature, relative humidity, wind speed and cloud cover. It was shown that generally high temperature and low relative humidity enhanced the liberation and distribution of pollen in the atmosphere; whereas high humidity and low temperatures triggered the liberation and distribution of fungal spores in the atmosphere. Atmospheric pollen count was drastically reduced during the rainfall.

Teranishi *et al*. (2000) conducted an atmospheric pollen survey using a Durham sampler from 1983 through 1998 in Toyama City, Japan. They investigated yearly changes in the pollen season of Japanese cedar *Cryptomeria japonica* and analyzed the relationships between climatic factors and changes in the pollen counts. This revealed that the first day of the Japanese cedar pollen season advanced from mid-March to late February and the yearly change in the first day was significantly associated with the mean temperature in February. Again increase in total pollen count was significantly associated with the mean temperature in the previous July and the duration of the pollen season was suggested to be associated with the total pollen count. These results further indicated that climate change, especially increasing global warming, influences the early pollen scatter and increase in pollen count as well as elongation of pollen season of Japanese cedar.

The effect of temperature, relative humidity and rainfall on airborne *Ambrosia artemisifolia* pollen concentrations was examined by Barnes *et al*. (2000). During the ragweed (RW) season for the years 1997 and 1998. The ragweed season for this region begins in mid-August and ends by mid-October. Temperature patterns for the period demonstrated usual daily fluctuations with highs 13 ^{o}C to 35 ^{o}C and lows 8 ^{o}C to 24 ^{o}C. Relative humidity readings for the period varied between 25 and 100 %. Highest RW values were seen after seasonal cooling in September.

Daily rainfall for the period varied between 0 and 100 mm. Airborne RW always declined sharply after strong rainfall events (> 10 mm/day). Peak airborne RW concentrations were often associated with the passing of frontal boundaries and the change in wind direction and velocity that accompanies that passing. Factors influencing airborne RW concentrations were multiple and complex, but atmospheric forces had greater influence. The passing of major weather fronts and the associated temperature drops, wind disturbances and rainfall are the major factors.

The variation in airborne pollen concentration of the Braga region (Portugal) was studied by Riberio *et al*. (2003) in spring time, during the flowering of *Vitis vinifera*. Recorded data set was obtained for two consecutive years (1999 and 2000), using a Cour-type sampler. During this period, 36 taxa were observed in a total of 3,200 pollen grains m^{-3} of air (APC). The main pollen types observed were *Olea*, Poaceae, and *Castanea* sp., representing 74 % of the pollen spectrum. The airborne pollen concentration (APC) was significantly correlated with certain meteorological parameters. Pollen concentration was positively correlated with temperature and wind direction (East and Northeast) and negatively correlated with rainfall and number of rainy days.

Pollen flow of cultivated rice measured under experimental conditions was studied by Zhiping *et al*. (2004). The pollen flow pattern of a cultivated rice variety, Minghui-63, was studied at horizontal and vertical levels under experimental conditions. Data obtained from pollen traps for six designed populations (as pollen sources) at different intervals showed that the dispersal of rice pollen decreased with the increase of distance from pollen sources and that the rice pollen flow was significantly influenced by weather conditions, particularly by wind direction and speed. For a mean wind speed of 2.52 m/s in a downwind direction, the observed distance

of rice pollen dispersal was 38.4 m, indicating that rice pollen grains normally disperse at a relatively small range. However, the maximum distance of rice pollen flow could be up to 110 m, using regression analysis of pollen flow and wind speed, when the wind speed reached 10 m/ s in this study. The frequency of pollen flow was positively correlated with pollen source size within a given range, suggesting that pollen flow will occur effectively at a considerable rate in rice fields with sufficiently large pollen sources. In addition, many more pollen grains were detected at the height of 1.0–1.5 m than at 2.0 m, indicating that rice pollen mainly disperses at relatively low heights. Results from this study were useful both for minimizing transgene escape from transgenic rice and *in situ* conservation of wild relatives of rice, as well as for hybrid seed production, where an effective isolation buffer zone needed to be established.

The terms and major criteria used to define and limit pollen season were reviewed by Jato *et al*. (2006). Pollen data from Cordoba (Spain), Ourense (Spain) and Bologna (Italy) were used to ascertain the extent to which aerobiological results and pollen curves were modified by the criteria selected. Results were analysed using Spearmans correlation test. Phenological observations were also used to determine synchronization between pollen curves and plant phenology. The criteria for limiting the shortest and longest pollen season periods, as well as the earliest and latest start and end dates, varied according to the city and the taxon under study; in many cases, results for a given taxon also depended on the year. The smallest differences were obtained for *Platanus* and the greatest for Poaceae.

Malgorzatar (2007) determined the effect of meteorological conditions on hazel (*Corylus* spp.) and alder (*Alnus* spp.) pollen concentration in the air of Szczecin. The aim of the study was to determine seasonal variations in concentrations of hazel and alder pollen count due to

meteorological parameters. Measurements were performed using the volumetric method. The analysed meteorological parameters were the maximum temperature, relative humidity, rainfall and wind speed. The beginning and end of a season were established by the 95 % method. During seven years of study, the highest concentration of hazel pollen in the air was noted in 2003 (the total number was two - three times higher than in the other years), with the pollen season starting in most years in the beginning of January and lasting till the end of March or beginning of April. The highest concentration of alder pollen in the air was noted in 2003, similarly as hazel pollen. The pollen season started in the beginning of January (in 2003 and 2006 in the beginning of March) and lasted till the turn of the March and April. The highest pollen count of 674 grains/m^3 was observed in the end of March. A positive and statistically significant correlation (Pearson's coefficient and multiple regression) was found between the hazel and alder pollen concentration and air temperature and wind speed. A negative correlation was found in case of the relative humidity. A lot of analysed correlations were significant (significance level of P=0.05), although the percentage of explained variation (R2) was very low. Besides the individual rhythm of pollination, the meteorological conditions were the most important factors (mainly air temperature and wind speed) influencing the analysed pollen concentration in the air.

An aeropalynological study was carried out in the atmosphere of the city of Nerja (Southern Spain) by Docampo *et al*. (2007), during a period of 4 years (2000–2003), using a Hirst type volumetric pollen trap. An annual pollen index of 59,750 grains, on average, was obtained with 80–85 % of the total pollen recorded from February to May, with *Pinus*, *Olea*, Urticaceae, Cupressaceae, *Quercus* and Poaceae being the principal pollen producers in abundant order. A total of 29 pollen types that reached a 10-day mean, equal to or greater than 1 grain of pollen

per m^3 of air was reflected in a pollen calendar. The results were compared with those obtained for nearby localities and a correlation analysis was made between the daily fluctuations of the main pollen types and total pollen, and meteorological parameters (temperature, rainfall and hours of sun). The daily, monthly and annual values reached by the most important pollen types from an allergenic point of view (*Olea*, Urticaceae and Poaceae) confirms Nerja as a high-risk locality for the residents and the numerous tourists who visit the area.

David, 2009 carried out the effects of meteorological factors on airborne bracken (*Pteridium aquilinum* (L.) Kuhn.) spores in Salamanca (middle-west Spain) were carried out by. Temporal variation of airborne *Pteridium quilinum* spores concentration in Salamanca during 10 years from January 1998 to December 2007 were studied using a Burkard spore trap, and correlations with some meteorological parameters were analyzed. The number of spores that were counted was very low, due probably to the distance between the spore trap and the main bracken populations which were located 70 km away from the city. Long-range transport caused by winds coming from the Second Quadrant (IIQ) was supposed to be responsible for the appearance of bracken spores in Salamanca. The period from August to late October showed the most intense spore dispersal process, with an early morning distribution along the day. Years 2002 and 2007 with a low quantity of airborne spores were also characterized by low mean temperatures, always under 18 °C from May to June. Daily spore concentration shows positive correlation with temperature and sun hours but negative with fourth Quadrant (IVQ) winds and with relative humidity. No correlation between daily spore concentration and rainfall was found. Also, a positive correlation between number of spores and IIQ winds was observed during the main spore season (MSS) and prepeak period (PRE)

Gonzalez *et al*. (2010) carried out a work on atmospheric pollen count in Monterrey, Mexico. The study was designed to describe the prevalence of pollen in the city of Monterrey, Mexico, during the year 2004. Atmospheric pollen was collected with a Hirst air sampler, with an airflow of 10 L/min during the year 2004. Pollen was identified with light microscope; the average monthly pollen count as well as total was calculated from January 2004 to January 2005. The months with the highest concentration of pollen were February and March (289) and (142) grains/m^3 per day, respectively and July and November had the lowest concentration (20 and 11 grains/m^3 per day, respectively). Most of the pollen recorded corresponded to tree pollen (72 %). *Fraxinus* spp. had the highest concentration during the year (19 grains/m^3 per day; 27.5 % of the total concentration of pollen). Tree pollen predominated from January through March; with *Fraxinus* spp., *Morus* spp., *Celtis* spp., *Cupressus* spp., and *Pinus* spp. as the most important. Weed pollen predominated in May, June, and December and the most frequently identified, were Amaranthaceae/Chenopodiaceae, *Ambrosia* spp., and *Parietaria* spp. The highest concentration of grass pollen was reported during the months of May, June, September, October, and December with Gramineae/Poaceae predominating. Tree pollen was the most abundant during the year, with the *Fraxinus americana* having the highest concentration. Weed and grass pollen were perennial with peaks during the year.

The effect of recent climatic trends on Urticaceae pollination in two bioclimatically different areas in the Iberian Peninsula: Malaga and Vigo was carried out by Recio et *al*. (2009). The study covers the period 1991–2006 for Malaga and 1995–2005 for Vigo, and compares the differences in climate and phenological behaviour observed at both localities. The sampling of atmospheric pollen was performed with Hirst volumetric pollen traps. The two localities presented different tendencies as far as temperature was concerned, while the mean annual

temperature in the Mediterranean region had increased by 0.06 ^{o}C/year, the same parameter had decreased in the Atlantic area by 0.1 ^{o}C/year. This tendency was even more pronounced as far as the minimum temperatures were concerned, especially during spring in Malaga and autumn in Vigo. On the other hand, wind speed had tended to increase, periods of calm had diminished and winds blowing off the sea had increased in both places. These changes in meteorological parameters have advanced the end of the pollen season in Malaga and delayed its start in Vigo. Total annual pollen counts decreased in Vigo, while the number of pollen-free days had increased in both areas.

General trends in airborne pollen production and pollination periods at a Mediterranean Site (Badajoz, Southwest Spain) were examined by Molina *et al*. (2010). The aim of the study was to determine trends in the airborne pollen concentration and pollination period for the principal sources of pollen in Badajoz (Southwest Spain) over 15 years of monitoring (1994-2008). Airborne pollen was monitored by continuous sampling with a Hirst volumetric sampler. Pollen trends were investigated by linear regression and correlation analysis using mean annual and monthly pollen concentrations. The aerobiological results were compared with meteorological data (temperature and rainfall). During the study period, the mean total annual rainfall was 66.2 mm lower than normal and the mean annual temperature 0.8 °C higher than normal. No temporal trend was found for total airborne pollen concentration, but differences were observed for monthly data, namely, an increase in January, February, and May and a decrease in March and June. For the different pollen types studied, there was a general trend toward increased values in the month with the highest values, and this trend seemed to be related to temperature. The beginning of the main pollen season occurred later, and the end occurred sooner; therefore, the main pollen season seemed to be shorter. The result reflected trends in the response of

plants to changing rainfall stress patterns in Mediterranean countries, and these trends seem to be different from those of temperate countries.

Gonzalez *et al*. (2010) surveyed the incidence of fungals in a vineyard of origin Ribeiro (Ourence-North-western Spain). The study was carried out in a vineyard of the Ribeiro district during the year 2007. Their result showed that *Botrytis* reached the highest annual total value of spore production with 16,145 spores, followed by *Plasmopara* with 747 spores and *Uncinula* with 578 spores. In order to forecast the concentration of the phytopathogenic fungal spores, equations of lineal regression were elaborated including as estimators, variables with high correlation coefficient. For *Botrytis* the regression equation revealed 42.4 % of the variability of spore concentration, 26.1 % for *Uncinula* and 24.7 % for *Plasmopara*.

2.2 Allergy Survey

Oni *et al*. (2010), examined the prevalence, management and burden of asthma in Nigeria. The study was a cross sectional design involving clinical and lung function assessment. The diagnosis of asthma was made using the clinical features of asthma and lung function parameters (Forced expiratory volume in one second, Peak expiratory flow rate, Reversibility tests). In total, 120 asthma patients participated in the study. All subjects completed the clinical asthma control questionnaires. All items were rated with the calculation of their mean and percentages. Student t-test was used to calculate the difference between the mean of the lung function tests for subjects and control. The prevalence of asthma among respiratory unit patients was 6.6 % and higher in the first three decades of life with female preponderance (F:M=1.5-1).There was a strong family history of asthma (81.7 %). Associated allergies include rhinitis (75 %), pharyngitis (54 %), conjunctivitis (54 %) and dermatitis (30 %). Percentage of asthma

patients treated with bronchodilators alone (70 %), combined inhaled bronchodilators and steroid (28.3 %). Impaired daily activities include sports (84 %), job career (60 %), Physical activity (55 %), Social activity (54 %), household chores (61 %) and disturbed sleep (53 %). Subjects had significant low lung function values when compared with control ($P < 0.05$). The burden of asthma was very high despite the advanced knowledge of its pathophysiology and management of asthma.

Aderele, (1979) carried out the clinical and laboratory studies on bronchial asthma in 200 Nigerian children who were seen during a 2000 year period in Ibadan. He found, contrary to reports, that the condition was rare in African children, that after pulmonary tuberculosis, asthma was the next most common chronic chest disease in Ibadan.

2.3 Allergic Diseases

Asthma is one of the chronic allergic respiratory diseases characterized by episodes of attacks or inflammation and narrowing of airways. Asthma attacks involve shortness of breath, cough, wheezing, chest pain or tightness, or a combination of these symptoms. Many factors can trigger an asthmatic attack, they include allergens, infections, exercise, abrupt changes in the weather, or exposure to airway irritants, such as tobacco smoke and diseases such as gastroesophageal reflux disease. The pattern, duration, severity and frequency of symptoms vary (Erhabor *et al*., 2002).

2.3.1 Types of Asthma

1. Work – Related Asthma

Occupational asthma is defined as a disease characterized by variable airflow limitation and or bronchial hyper-responsiveness due to causes and conditions attributable to a particular working

environment and not to stimuli encountered outside the work place. Work – aggravated asthma is defined as concurrent asthma worsened by nontoxic or physical stimuli in the work place (Annesi *et al.,* 2001).

2. Exercise–Induced Asthma (EIA), or Exercise–Induced Bronchospasm

Defined as a condition in which exercise or vigorous physical activity triggers acute bronchospasm in persons with heightened airway reactivity. It is observed primarily in persons who are asthmatic but can also be found in atopic patients, allergic rhinitis, or cystic fibrosis, and even in healthy persons. Exercise-induced asthma is often a neglected diagnosis, and the underlying asthma may be silent in as many as 50 % of patient, except with exercise. Exercise, particularly running and cold weather exercise, induces asthmatic reactions in about 17 million Americans (Annesi *et al.,* 2001).

2.3.2 Asthma in Pregnancy

Asthma is the most common condition that affects the lung during pregnancy and are substantially increased risk for several adverse infant and maternal outcomes (Liu *et al*., 2001). With good asthma treatment during pregnancy, most women can breathe easily, stay healthy, have a normal pregnancy, and give birth to healthy baby (Annesi *et al.,* 2001).

2.3.3 Complications of Asthma

In most stages, asthma is a reversible condition, which means symptoms and airway flow obstruction significantly improve with treatment. Conversely, in a small percentage of asthmatics, the airway obstruction does not reverse, and these patients end up with Chronic Obstructive Pulmonary Disease (COPD), Chronic Bronchitis (CB) or Emphysema (Djukanovic *et al*., 1992). Complications associated with most medications used for asthma are relatively

rare, however, in those patients requiring long -term corticosteroid use, complications may include osteoporosis, immuno suppression, cataracts, weight gains, psychiatric disorders, diabetes, a vascular necrosis, (Djukanovic *et al*.,1992). The risk of these complications is far less with inhaled corticosteroids than with oral corticosteroids. Nevertheless, in patients with moderate or severe asthma whose disease has been well controlled with high – dose inhaled corticosteroids, every effort should be made to reduce the dose to as low as possible while maintaining good asthma control and minimizing the risk of exacerbations (Djukanovic *et al*., 1992).

2.4 Prevalence of Asthma and Allergy

Asthma mortality is associated with multiple factors, including delay in care, poor compliance, and lack of access to health care, theophylline toxicity, and overuse of B – agonist medications, (Spitzer, 1992). Speculation about the recent decline in asthma deaths has pointed to the more judicious use of prophylactic treatment, particularly inhaled steroids, (Siptzer, 1992). On average, 1,400 people die from asthma each year in the UK according to Piccard (2002). The asthma mortality rate in Israel during the years 1980 to 1997 was low, stable, and there was no difference in the asthma death rate between Jews and Arabs, suggesting that in this population, genetic predisposition was not likely to be a risk factor for mortality (Picard, 2002).

2.5 Rhinitis

Rhinitis is inflammation of the membrane tissue in the nose, causing sneezing, a runny nose, and sense of nasal obstruction. There are two major causes of rhinitis: an allergy called "allergic rhinitis", and an over activity of the nerves in the nasal tissue called "vasomotor rhinitis" (Scoppa, 1996).

2.5.1 Classification of Rhinitis

(c) **Atopic Rhinitis**

There are three types of atopic rhinitis. (a) Seasonal allergic rhinitis (also known as hay fever). This is triggered by allergy due to pollen, including trees in rainy season, grasses and herbaceous in dry season. Symptoms include sneezing, itching, tickling in the nose, runny or stuffy nose, and watery or itchy eyes. Seasonal rhinitis is diagnosed primarily by medical history (Ono and Abelson, 2005).

(d) Perennial Rhinitis (Year – Round) with Allergic Triggers

These triggers include indoor allergens such as mold, house dust mite, cockroach and animal dander. Foods commonly eggs, cows milk and peanut can be triggers. Symptoms are the same as seasonal allergic rhinitis but are experienced throughout the year (Tamari *et al*., 2013).

c) Perennial Rhinitis with non – Allergic Triggers

Although not triggered by allergy, it's an allergic-like condition with increased eosinophils (a special type of white blood cell associated with allergy) in the lining and secretions of the nose. Symptoms are the same as perennial rhinitis with allergic triggers, diagnosis is determined from negative skin tests and a nasal smear test positive for eosinophils and nasal polyps can be a complication of this condition (Tamari *et al*., 2013).

2. Vasomotor Rhinitis

Vasomotor rhinitis is caused by over activity of nerves in the nasal tissue. This can occur when emotionally upset, irritated by certain air temperature and humidity conditions (chilly weather, dry air from air – conditioning, sudden changes in temperature or humidity), during pregnancy, and during bacterial and viral infections. It can also be induced by drugs such as alcohol, anti-

hypertensive agents, aspirin, oral contraceptives, chemicals (cosmetics, smoke, noxious fumes) and from over use of decongestant nasal drops or sprays. Food induced rhinitis (gustatory rhinorrhea) may occur during consumption of hot and spicy foods (MacKay and Durham, 1998). The National Institute of Allergy and Infectious Disease (NIAID) estimates that ‘‘the number of people suffering from allergic rhinitis may be as high as 35 million’’. Allergic rhinitis may not seem dangerous in itself, but it can play a role in other diseases like asthma and sinusitis (Specter, 1999).

Complications of Allergic Rhinitis

Allergic rhinitis has a strong association with asthma (Specter, 1999). Another commonly associated condition is nasal polyps, which are growths of skin in the nasal tract that can cause obstruction and loss of smell and sinus and ear infection. Allergic rhinitis results in bad breath, a husky voice and sore throats, it worsens snoring and the tendency to sleep apnea in adults. It causes abnormal development of the mouth and teeth from chronic mouth breathing (Specter, 1999).

2.6 Asthma and Allergy Prevalence Worldwide

In 1998, International Study of Asthma and Allergies in Childhood (ISAAC) steering committee conducted a study to investigate the worldwide prevalence of asthma, allergic rhino, conjunctivitis, and atopic eczema(ISSAC,1998). A total of 463,801 children aged 13-14 years in 155 collaborating centers in 56 countries were used. Result showed differences of between 20 fold and 60 fold between centers in the prevalence of symptoms of allergy. For asthma symptoms, the highest 12-month prevalence were from centers in the UK, Australia, New Zealand and republic of Ireland, followed by centers in North, central and South America. The

lowest prevalence was from centers in several eastern European countries, Indonesia, Greece, China, Taiwan, Uzbekistan, India, and Ethiopia. For allergic rhino conjunctivitis, the centers with the highest prevalence were scattered across the world. The centers with lowest prevalence were similar to those for asthma symptoms. For atopic eczema, the highest prevalence came from scattered centers including some from Scandinavia and Africa that were not among centers with the highest asthma prevalence, the lowest prevalence rates of atopic eczema were similar in centers as for asthma symptoms (ISAAC, 1998).

According to a study on the prevalence of asthma in children living in villages, cities and refugee camps in Palestine, in autumn of 2000, the crude prevalence rate for wheezing –ever, wheezing in the previous 12 month, and physician –diagnosed asthma were 17.1 %, 8.8 %, 9.4 % respectively, with urban areas having higher prevalence rate than rural areas(Al-Shehri, 2000). Within urban areas, refugee camps had higher prevalence rate than cities, the prevalence of asthma and asthma symptoms in Palestine appears to be close to that of Jordan, but was much lower than Israel (Al- shehri, 2000). Another study was carried out on the differences in the prevalence of asthma and current wheeze between Jews and Arabs. This showed that the prevalence of asthma and current wheeze was significantly higher in Jewish children compared with Arab children. The asthma prevalence was 7.8 % for Jewish children and 4.9 % for Arab children (Al-Shehri, 2000).

A study of prevalence of self – reported allergic conditions in adult population in Israel showed that allergic conditions were higher in the Israeli Arab population and those with low income and low education level (Shahar and Lober, 1996). Screening for asthma and associated risk factors among urban school children in Abha city, Saudi Arabia showed that the prevalence of

asthma in school children in Abha was greater than that reported from most developing countries and closer to the rates reported in developed Countries, (Al -Shehri, 2000).

A study on the prevalence of asthma and other atopic diseases in Australian children showed that the prevalence of wheeze was significantly higher in boys (27.4 %) than girls (21.7 %). Children born in Australia were more likely to report current wheeze than those born elsewhere (Robertson, 1998).

An ISAAC study on prevalence of asthma and allergy in Hong Kong school children at age 13-14 years old showed that the prevalence rates of asthma ever, wheeze ever, and current wheeze were 11, 20 and 12 %, respectively, and were greater in boys. Rhinitis affected slightly over half of the subjects (52 %) and eczema was reported by a sixth (15 %), while current rhinitis and current eczema were presented in 44 % and 3.6 % of children respectively. Parental education and passive smoking were not important factors when compared to previous epidemiology data obtained in 1992. The prevalence rates for asthma ever and wheeze ever had increased by 71 and 255 %, respectively, in Hong Kong school children. Similar increasing trend was showed by the severity of asthma and respiratory symptoms (Leung, 2004).

According to Ige and Sogaolu (2004) recent hospital survey conducted in Nigeria over a seven-year period, which evaluated the changes in prevalence of symptoms of asthma, found that cases of wheezing had increased significantly in the 13 to 14-year age group. He remarked that although there was no clear explanation for the apparent surge in cases of asthma and its severity, the trend could possibly be ascribed to a general improvement in living standard. According to this hypothesis, “the decrease in the incidence of childhood infections following improvement in hygiene and standard of living would stimulate the immune system in the

direction that would enhance the development of asthma and other allergic states, rather than in fighting one infection or the other." (Ige and Sogaolu, 2004).

2.7 Conjunctivitis

Conjunctivitis is an inflammation of the conjunctiva characterized by cellular infiltration and exudation. The exudates may be purulent, mucopurulent, foamy, pseudo membranous or catarrhal. Conjunctivitis may be infectious; caused by micro-organism or pollen, spores, drug and devices such as hard and soft contact lens. Many Nigerians suffer from allergic conjunctivitis, which has become one of the most common allergic condition of the eye. Conjunctivitis is often referred to as "pinkeye". The viral or bacterial forms of conjunctivitis are contagious, while allergic conjunctivitis is not (Adeyeba *et al*., 2010).

Allergic conjunctivitis (AC) is typically a type 1 IgE-mediated hypersensitivity reaction with cell-mediated Th-2 involvement in some types. The ocular allergic response is a cascade of events that are coordinated by mast cells. The presence of an allergen makes the body to mount an antigen specific response with T-helper cells-2 (Th2), releasing cytokines and also producing antigen-specific immunoglobulin E (IgE). IgE then binds to mast cells with release of histamine and further release of cytokines, prostaglandins and platelet-activating factor with other intermediaries. These intermediaries cause an allergic inflammation and symptoms through the activation of inflammatory cells. Histamine binds to H1 receptors on nerve endings and causes the ocular symptom of itching and binds to H2 receptors of the conjunctival vasculature and produces vasodilatation and lacrimation. These mast cell-derived cytokines also recruit neutrophils and TH2 cytokines recruit eosinophils promoting increased sensitivity. This process could then progress to chronic allergic inflammation where there is proliferation of

fibroblast in the conjunctiva with resultant development of papillae in some patients (Tamari *et al*., 2013).

These events are responsible for the usual presentations in patients, with bilateral severe itching, watery discharge, acute or chronic redness, swollen eyelids and burning or foreign body sensation, with photophobia. Allergic Conjuctivitis (AC) may occur on a yearly basis in a particular season, giving rise to seasonal form or throughout the year as in perennial AC. Most of the hypersensitivity reactions are to specific allergens. Pollens are responsible for seasonal conjunctivitis associated with hay fever and tend to recur at the same time each year in those with atopy. Perennial conjunctivitis occurs as a result of several allergens such as house mites, animal dander and cosmetics. Symptoms occur all year round and may be worse in the mornings. Other allergens such as contact lenses, sutures and prostheses following eye surgery could give giant papillary AC. Reactions to eye drops, preservatives in the eye drops and cosmetics could give rise to contact dermatoconjunctivitis. These tend to resolve once the irritant is removed. Allergic conjuctivitis can follow seasonal variations at the early onset of the disorder but may become perennial as time goes on. Seasonal and perennial AC are said to occur in association with a history of other body allergies like asthma, hay fever, rhinitis, eczema or atopic dermatitis and or family history of the same. AC could seriously affect the patient's quality of life especially during the acute episodes. It could lead to children missing school for some days as a result of acute conjunctival inflammation with attendant discomfort. Most of the AC that affect children occur from the age of 5 to adolescence and rarely proceed beyond the age of 25. Before puberty more boys than girls are affected but beyond puberty there is no gender bias (Tamari *et al*., 2013). There are also climatic and racial risk factors involved in some types of AC. It is more prevalent in warm climatic conditions and more among Afro-

Caribbeans, Arabs and Asians and less among the white population. The prevalence seems to be declining among the Caucasians (Tamari *et al*., 2013). Among the Caucasians atopy in patients or family history of atopy is present in over 80% of those with AC and strong association of keratoconus. In African continent such associations have not been frequent. AC constitutes the highest group of eye problems seen in most out-patient eye consultations in the developing world, including Nigeria (Adeyeba *et al*., 2010).

Usually clinical diagnosis of AC is straightforward. A conjunctival swab excludes other forms or causes of conjunctivitis of infective means or associations. Serum immunoglobulin E (IgE) may be raised, radioallergosorbent test (RAST) skin prick testing may be negative or nonspecific. Conjunctival scrapings for eosinophils may help determine the cause of the allergy (Kari and Saari, 2010).

2.7.1 Types of Allergic Conjunctivitis

There are two forms of allergic conjunctivitis, seasonal and perennial types (Ono and Abelson, 2005). The perennial form of allergic conjunctivitis is usually caused by animal hair or dander, feathers, dust mites, pollen and spores. The most common form of allergic conjunctivitis is seasonal, which is triggered by mold spores and pollen from flowering trees, grass, and herbaceous. Allergic conjunctivitis may also result from the presence of a foreign body in the eye, such as a contact lens or glass eye. This type of allergic conjunctivitis is known as giant papillary conjunctivitis, and typically occurs in a person who wears hard or rigid contact lenses. Other substances that may cause an allergic reaction in the eyes include air pollutants, perfumes, cosmetics, chemicals, and smoke (Ono and Abelson, 2005). The most obvious symptoms of allergic conjunctivitis are redness and puffiness around the eyes, which affect the quality of life

of the sufferer. Other symptoms include; Itching or burning eyes, blurred vision, increased sensitivity to light. Since avoidance of the allergen is the best solution for allergic conjunctivitis, the steps for reducing or eliminating exposure to eye allergens are; remain indoors during peak pollen counts, wash hair and eyelids daily using a baby shampoo, wear sunglasses when outdoors, especially on windy days, to block pollen from the eyes, reduce dust mite exposure by keeping air ducts clean and using mattress covers or mite-proof bedding.

Fortunately, anyone with a history of allergic conjunctivitis may prevent future outbreaks or lessen their severity by identifying the specific allergens causing their symptoms and being diligent to avoid exposure to them (Adeyeba *et al*., 2010).

2.8 Pollen and Spores Allergenicity

Mutual boosting effects of sensitization with *Phleum pratense* pollen and latex glove extract on IgE Antibody responses in a Mouse Model was studied by Mahler *et al.*(2000). Groups of 10 BALB/C Mice were immunized with $Al(OH)_3$-adsorbed pollen extracts from *Phleum pratense*, *Ambrosia artemisifolia, Artemisia vulgaris* or *Betula pendula*. For control purposes, one additional group received adjuvant only and another group was not immunized. Half of the Mice of each group were subsequently immunized with $Al(OH)_3$-adsorbed latex glove extract, the other half with adjuvant only. Pollen and latex-specific IgE- and IgG1-antibody responses were analyzed by enzyme-linked immunosorbent assay and statistically evaluated by analysis of variance. Antibody responses to cross-reactive antigens were investigated by immunoblotting. They found significantly increased IgE reactivities to latex after pollen sensitization and vice versa. Moreover, Mice immunized with *Phleum pratense* pollen extract alone – without subsequent latex immunization – displayed IgE reactivity to latex. Cross-reactive antibodies

were directed against pollen antigens of approximately 60 kDa molecular weight. The results thus demonstrate a mutual boosting effect of pollen and latex sensitization in vivo which may be also operative in polysensitized pollen allergic patients.

Induction of IgE antibodies in mice and rhesus monkeys with recombinant birch pollen allergen was carried out by Vitalis *et al*. (1996). In this study an attempt was made to determine whether the different allergenicity of the major birch pollen allergen, rBet v 1, and a minor birch pollen allergen, rBet v 2, might be related to a different immunogenicity of the proteins as evaluated in experimental animal systems (Mice and rhesus monkeys). Purified recombinant allergens were injected into mice and rhesus monkeys with aluminum hydroxide as adjuvant for elicitation of specific IgE responses. Antibody responses to the allergens were detected by immunoblotting, and time courses of immune responses were measured by Elisa. In both animal models, more than the 10-fold dose or rBet v 2 was required to induce IgE antibodies, and even then, the amount of specific IgE antibodies elicited with rBet v 1 was substantially higher than that induced by rBet v 2 which formed stable polymers through disulfide bonds. They concluded that in the two different animal models (Mice and rhesus monkeys) the major birch pollen allergen, rBet v 1, induced substantially higher levels of IgE than rBet v 2. A reduced allergenicity of Bet v 2 caused by polymer formation would be in agreement with previous studies indicating reduced allergenicity of proteins on chemical polymerization.

Diesel exhaust particles (DEP) and allergenicity of pollen grains of *Lilium martagon* were studied by Chehregani and Kouhkan (2007). In this research, pollen grains of *Lilium martagon* that are known as a non-allergic substance were collected and exposed to DEP 5 . The allergy potency of different pollen extracts were compared by means of skin test, as well as analyses of

blood eosinophil numbers and IgE levels in the treated animals. Normal and DEP-exposed pollen grains were examined by scanning electron microscopy. Pollen extracts were also studied by SDS–PAGE for DEP-induced changes in protein profiles. Allergic bands were also studied and checked by using immunoblotting method. The results of the investigated allergy tests showed that DEP-exposed pollen grains are effective in inducing allergic symptoms. According to the microscopic observations, organic substances that existed in the DEP, mediated agglomeration of particles on the pollen surface. In appropriate conditions, water-soluble components of DEP may induce changes that affect the release of pollen proteins. SDS–PAGE showed that protein profiles of pollen grains were changed and some new bands appeared in DEP-exposed pollen grains. Immunoblotting studies showed a new band in DEP-exposed pollen grains that reacted strongly with anti-IgE, but there was no allergenic band in normal pollen grains. On the other hand, diesel exhaust particles can carry pollen allergen molecules, induce new proteins (allergens), and also act as adjuvant for allergens (Chehregani and Kouhkan, 2007).

The role of major olive pollen allergens Ole e 1, Ole e 9, and Ole e 10 on mice sensitization was studied by Barral *et al*. (2006). BALB/c mice were sensitized by 4 intraperitoneal injections of olive pollen extract in aluminum hydroxide. The allergic state was proved by measuring serum specific IgG1 and total IgE antibody levels. The IgG1 responses to olive pollen allergens were assayed by immunoblotting and enzyme-linked immunosorbent assay. Competition experiments between human IgE and mouse IgG1 binding to olive pollen allergens were performed. The result revealed that sensitization with olive pollen extract induced high levels of specific IgG1 and total IgE in all tested animals. Immunoblotting experiments showed that the mouse IgG1 binding pattern to pollen extract was complex and heterogeneous, as occurs with human IgE.

High IgG1 antibody levels to the major olive pollen allergens described for humans were detected in serum samples from sensitized mice, whereas minor olive pollen allergens induced no significant IgG1 response. Co- incubation of mouse serum samples with a cocktail of Ole e 1, Ole e 9, and Ole e 10 resulted in a significant decrease (60 %) in IgG1 binding to olive pollen extract. Specific mouse IgG1 strongly inhibited human IgE binding to olive pollen allergens.

Prevalence of sensitization to Aeroallergens in California patients with respiratory allergy was studied by Galant *et al*. (2010) utilizing aeroallergens thought to be relevant from recent aerobiologic and botanic data, 141 allergic and 17 asymptomatic control subjects were tested for the prevalence of 103 allergens.

A standardized prick puncture technique and standardized interpretation of wheal/flare responses were utilized using the same lot of allergen for 13 allergy practices distributed throughout California. Frequency curves based on prevalence were established to determine the number of tests required to give up to 90 % of positive responses for tree, weed and grass pollen, mold spores, and miscellaneous allergens which included house dust mite, cat, dog, and cockroach allergens. Positive responses in allergic subjects for grasses ranged from 46 % to 54 %, for herbaceous 19 % to 37 %, and for trees 10 % to 42 %. For molds the range was 11 % to 22 %. The response rate for *Dermatophagoides pteronyssinus* was 53 %, for *Dermatophagoides farinae* 42 %, for cat pelt 39 % and cat hair 37 %, for cockroach 23 % and dog dander 19 %. Asymptomatic control subjects responded to only 4 % of all allergens tested. Ninety percent of all positive tests required three miscellaneous allergens (house dust mite, cat, and cockroach), 9 molds, 2 grasses, 16 herbaceous, and 27 trees for a total of 57 allergens (56 % of total tested). There was no clear relationship between local and specific allergen response, probably related to the limited number of subjects tested and variability within the same geographic region.

Several seldom tested tree species and weed allergens showed a higher prevalence rate than several commonly tested for allergens (Galant *et al*., 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Atmospheric Pollen and Fungi Spores

Modified Tauber- like pollen traps were employed for the collection of pollen and fungi spores. The instrument was designed by Tauber (I974). The sampler is a non-volumetric sedimentary sampler that rely on gravity to access the composition of the atmosphere. It has a collar around it, the shape and the capacity of the receptacle beneath the collar was made large enough to accommodate the expected monthly rainfall. The material of the trap is sufficiently resistant to rust and can remain in the field for a full calendar year without cracking (Agwu, 1997). Pollen and spores are incorporated into the trap in a process analogous to incorporation of pollen and spores into sediment. The sampling locations were the six geopolitical zones of Nigeria; South East (Nsukka, Enugu State: 06^{o} 69^{1} N; 007^{o} 33^{1} E), South South (Obiakor, Rivers State: 06^{o} 77^{1} N; 006^{o} 34^{1} E), South West (Akoka, Lagos State: 06^{o} 30^{1} N; 003^{o} 31^{1} E), North Central (Garki, Abuja: 09^{o} 34^{1} N; 7^{o} 51^{1} E), North West (Zaria, Kaduna State: 11^{o} 0^{11} N:7^{o} 74^{1} E) and North East (Dukku, Gombe State: 11^{o} 48^{1} N; 11^{o} 53^{1} E) (Fig. 3.1). The recipient solutions were collected monthly for a period of one year, from June 2011 - May 2012.

3.2 Setting Up of the Trap for the Collection of Atmospheric Pollen and Spores

Each modified Tauber like pollen trap was placed at the height of 5 ft above the ground surface (Plate 3.1). The sampler was mounted at two sampling sites per study location . A solution made of glycerol (50 ml), formaldehyde (10 ml) and phenol (5 ml) was prepared and poured into each trap. The recipient solutions were collected monthly for the period of one year. The solution in the trap was replaced after each monthly harvest. The collected samples were stored in the refrigerator to stop any ongoing oxidation of plant materials.

Fig. 1: Map of Nigeria, showing the six geopolitical zones of Nigeria and study locations

Source: Rewaju, (2012)

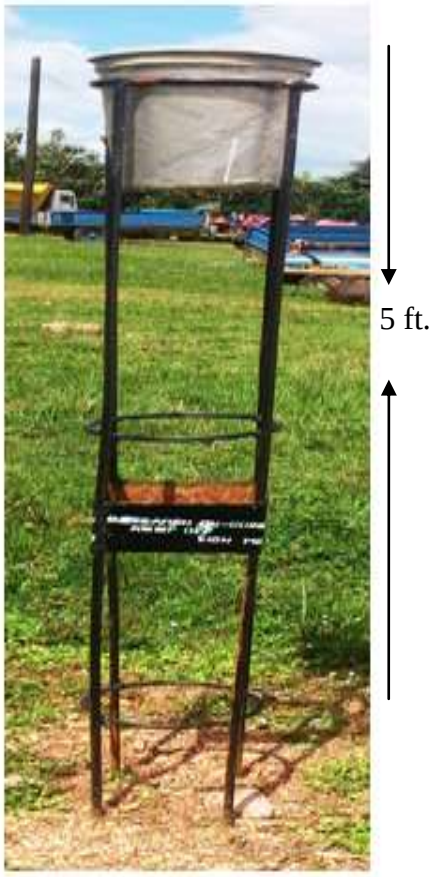


Plate 3.1: Modified Tauber-like Pollen Trap in the Field

3.3 Pollen Extraction and Concentration

The samples were sieved through 200 µ mesh wire gauze to filter off large organic and soil particles. The liquid with suspended palynomorphs was centrifuged at 2000 revolutions per minutes (rpm) for 5 mins. The supernatants were decanted and the residues retained. The precipitates were washed three times with water and centrifuged in order to recover the polleniferous residues.

3.4 Digestion of Inorganic Materials

The residue (precipitate) recovered from processes above were placed in plastic test tubes and 45 % hydrofluoric acid was added to each. The suspension was allowed to stand for 15 mins, after which it was stirred regularly to increase the rate of dissolution of solid inorganic compounds. At the end of the period the samples were centrifuged and the supernatant decanted to retain the residues. The samples were washed two times with water and once with glacial acetic acid. Each wash was followed by centrifugation and decantation in order to recover the polleniferous residue.

3.5 Acetolysis

The acetolysis solution was prepared from nine parts of Acetic anhydride and one part of tetraoxosulphate (vi) acid. Two to three (2-3) ml of the acetolysis solution was added to each of the samples. The centrifuge tubes containing the acetolysis mixture (palynomorphs and acetolysis solution) were placed in a water bath at 100 ^{o}C and allowed to stand for 5mins. The tubes were removed from the water bath, centrifuged for five minutes at 1500 r.p.m. and then decanted. The precipitates (pollen and spores) recovered were treated once with 2-3 ml of glacial acetic acid, centrifuged and decanted. The recovered sediment were further washed

twice with water, centrifugation and decantation followed each wash. Ten (10) ml of glycerol/alcohol solution in the ratio of 2:1 was added to each precipitate and transferred into plastic vial bottles for storage.

3.6 Slides Preparation

The content of each vial bottle was properly shaken and 2 drop (0.2 ml) of the suspension mounted on a slide and covered with 22 mm x 22 mm cover slip. Two slides were prepared from each residue sample and mount was covered with colourless nail varnish at the edges of the coverslips to prevent drying up. In this state the specimen could stay as long as 4 weeks without completely drying up. Microscopic examination was made with a light microscope. Pollen count and fine morphological studies were made at X 400.

3.7 Identification

Identification of pollen grains was based on comparism with reference collection of pollen slides and with description and photomicrographs of pollen and spores in books and journals such as Agwu and Akanbi (1985), Ybert (1979), Agwu and Ahize (1987) etc. The photomicrographs of some pollen, fungi spores, charred Poaceae epidermis were taken using motic camera MC 2000.

3.8 Extraction of Pollen and Fungal Spores Protein

Mature anthers from flower which have not undergone anthesis were procured and dried at room temperature for 3 days. Pure cultures of spores were produced using potato dextrose agar (PDA) and allowed to mature for three weeks. Spores were scooped out of the petri dishes prior to extraction. Both anthers and spores were defatted using diethyl ether for three times and allowed to dry under room temperature. They were extracted in 100 ml of 0.02 M phosphate

buffered saline (PBS) at pH-7.4. The mixtures were stirred overnight with magnetic stirrer at 4 ^{o}C, filtered with a muslin cloth, centrifuged at 3000 rpm for 10 mins and supernatant retained and sediment discarded. The extracts were precipitated with ammonium sulphate. The precipitates were retained and dialysed against PBS overnight. Protein content was assayed according to Bradford procedures. The crude pollen and spores protein were stored at -80 ^{0}C for later use.

3.9 Inoculation of Pollen and Fungal Spores Protein

Swiss Albino Mice (4-6 wks old) were purchased from the Nigerian Institute of Medical Research, Yaba. Maintained under a 12-hours light-dark cycle with free access to water and standards laboratory food. All experimental procedures conformed to international standards of animal welfare and were approved by the Animal Experimentation Ethics Committee of the Nigerian Institute of Medical research (Appendix 1). Crude protein extract (0.4 ml) obtained from six different pollen and two fungi spores was inoculated into mice by two subcutaneous injections and one intranasal injection. Blood samples were obtained by retro-orbital bleedings using heparinze capillary tubes and sera stored at -80 ^{o}C for later use in detecting immunoglobulin (IgE) levels. Blood smears were also obtained from the tail, the thin blood smear were fixed with methanol for 2-3 mins. One in three dilutions of Leishman stain and buffered water was prepared and cover the slide with the stain for 7-10 mins. The stain was washed off in a stream of buffered water. Distillled water was added on a slide and left for 2-3 mins to differentiate the film. The slides were allowed to dry on a rack. Using X 100 objective lens with oil emersion, the blood smears were examined and the different immune cells identified and quantified.

3.10 Immunoperoxidase Assay for Determination of IgE in Mice Sera

Immunoperoxidase assay for the determination of IgE level in the mice sera was employed, Mouse IgE Elisa kitt GWB 626057 purchased from GenWay Laboratory Technologies United State of America was employed. The kit is a highly sensitive two site enzyme linked immunoassay for measuring immunoglobulin E in biological samples of mice. The reagents in the kit were; diluent concentrate, wash solution concentrate, enzyme antibody conjugate, chromogen substrate solution, stop solution, anti- mouse IgE Elisa micro plate, mouse IgE calibrator. Manufacturer's specification on usage was strictly followed.

Procedure

All manufacturer's instructions on reagent dilution with distilled water were strictly adhered to and prepared prior to use. One hundrend (100) µL of the Mice sera were measured using micro pipette into predesignated microtitre wells and incubated for 30 mins. After removal of unbound protein by washing with Elisa machine, the wells were blotted to remove residual buffer. Anti IgE antibodies conjugated with Horseradish peroxidase (HRP) were added and incubated for 30 mins. These enzyme labeled antibodies form complexes with the previously bound IgE, this was followed by another washing. The enzyme bound to the immunosorbent was assayed by the use of 3,3,5,5- Tetramethyl benzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgE in the tested samples, absorbance at 450 nm was determined, which was a measure of the concentration of IgE in the tested samples. The quantities of IgE in the samples were interpolated from the standard curve constructed from the standards and corrected for sample dilution. Rating of the IgE level was done following Nigerian Institute of Health (NIH) (Appendix 8).

3.11 Histopathology Procedures of the Respiratory Organs

Processing of tissue samples for histological assessment followed established procedures of Kuo (2007). In brief, the tissue samples were rinsed with 0.9 % saline solution, fixed in 10 % formalin. Then the transverse sections of the trachea, bronchi, bronchiole and lung were obtained and treated as follows: (1) 10 % neutral buffered formalin for 1 hrs, twice; (2) 70 % alcohol for 1.5 hrs; (3) 80 % alcohol for 1.5 hrs; (4) 90 % alcohol for 1.5 hrs; (5) absolute alcohol for 1.5 hrs, twice; (6) xylene for 1.5 hrs, twice; (7) in molten wax at 65 °C for 2.5 hrs two changes. The processed tissues were embedded in paraffin and sectioned at 4 µ thickness, placed on frosted glass slides and dried on a 70 °C hot plate for 30 mins. The tissues were stained using the hematoxylin and eosin (H&E) stains. The sections were dewaxed in two changes of xylene (3 mins each), hydrated in two changes of 100 % ethanol, followed by 90 % ethanol and 70 % ethanol, for 3 mins each, rinsed with water (3 mins) and stained. The stained tissues were dehydrated with 70% ethanol followed by 90 % ethanol, placed in two changes of 100 % ethanol for 3 minutes each and cleared with two changes of xylene (3 mins each). Histopathology changes were observed and their photomicrographs taken with the aid of a Motic camera MC 2000.

3.12 Statistical Analysis

The data obtained were analyzed using the SPSS statistical package version 20 (SPSS Inc. Chicago, Illinois USA). Descriptive and frequency statistics were generated to examine the means of basophil, eosinophil, lymphocyte, monocyte, neutrophil and lmmunoglobulin E (IgE) (Appendix 9).

3.13 Collection of Weather Parameters Data

Weather parameters data were obtained from Nigerian Meteorological Centre Oshodi, Lagos.

CHAPTER FOUR

RESULTS

4.1 Seasonal Prevalence of Pollen and Fungi Spores in Nsukka, Enugu State (South East) Nigeria from June 2011 to May 2012

In Nsukka, Enugu State Nigeria, 69 pollen types which belong to 33 plant families were recorded for the aeroflora. Among these 45, 18 and five were identified to species, generic and family levels respectively. Major pollen contributors were; Poaceae, *Elaeis guineensis, Olax subscorpioidea, Alchornea cordifolia* (Schum and Thonn) among others (Table 4.1). *Olax subscorpioides* (Oliv) pollen were present from September to February. Poaceae pollen were predominant from June to November. *Alchornea cordifolia* (Schum and Thonn) 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* (,Pers.) C.D. *Ageratum conyzoides* (L.) *and Bombax buonopozense* (P. Beav). The month of May had the lowest pollen record among the rainy season. The atmospheric pollen record started increasing from the month of September (Table 4.1). The pattern of pollen dispersal displayed three distinct periods: the period from May to August, which corresponded with rainy season and had (470) lower atmospheric pollen content. Another period from September to December, was the late rainy/harmattan season, which was dominated by pollen (1242). January to April, the dry period recorded 927 pollen. The period of September- December 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 qualitatively and quantitatively dominated by anemophilous

pollen, majorly Poaceae and *Elaeis guineensis* (Jacq) etc and enthomophilous pollen. This period also was the major and principal pollination period.

Twenty eight fungi spores types were recorded from the traps (Table 4.2). The most dominant and prevalent fungi spores included those of *Nigrospora* sp., *Fusarium* spp., *Puccinia* sp. and *Alternaria* spp. Some spore types were only present during the rainy season such as *Alternaria* spp., *Fusarium* spp., *Venturia* sp., *Triposporium* sp. and *Helminthosporium* sp. The abundance of the spores reduced during the dry season from the month of November to March. Spores of fungi present throughout the year were those of *Nigrospora* sp. and *Puccinia* sp. The lowest monthly spores was recorded in February.

Table 4.1: Atmospheric pollen count of Nsukka, Enugu State (South East) Nigeria from June 2011 - May 2012

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Acacia* sp.	2	4	0	0	8	0	0	0	0	0	0	0
2	*Acacia* sp.	0	0	0	0	4	0	0	0	0	0	0	0
3	*Adansonia digitata* (l.)	0	0	0	0	0	0	0	0	0	2	0	0
4	*Afzelia africana (*SM).	0	0	0	4	2	0	0	0	0	0	0	0
5	*Ageratum conyzoides* (L.)	0	0	0	0	0	0	0	0	0	0	42	0
6	*Albizia* sp.	0	2	0	0	4	0	0	4	12	0	0	0
7	*Alchornea cordifolia* (Schum and Thonn)	0	12	0	2	20	20	104	8	120	28	23	1
8	Amarathaceae/Chenopodiaceae	0	2	2	1	4	0	0	0	0	0	0	0
9	*Anacardium occidentale* (L.	0	0	0	0	0	0	0	2	6	0	0	4
10	*Aneilema* sp.	2	0	0	0	0	4	0	0	0	0	0	0
11	*Anthocleista djalonensis* (A.chev.)	2	0	0	0	0	0	0	0	0	0	0	0
12	*Anthocleista vogali* (Planch)	2	4	0	0	4	0	0	0	0	0	0	0
13	*Antidesmis* sp.	0	0	0	0	0	0	2	4	0	0	0	0
14	*Aspilia africana (*Pers C.D. Adams)	0	0	0	6	18	0	6	0	6	0	192	0
15	*Barleria* sp.	0	0	2	0	0	0	0	0	0	0	0	0
16	*Berlinia grandiflora* (Vahl)	0	0	0	4	0	0	0	0	0	0	0	0
17	*Blighia sapida* (Lovett)	0	0	0	0	0	0	8	0	4		2	0
18	*Bombax buonopozense* P.Beav.	0	0	0	8	0	0	0	0	0	0	42	0
19	*Bridelia ferruginea* (L.)	0	12	0	2	4	0	0	0	0	0	0	0
20	*Canarium schweinfurthii (*Engl.)	0	2	0	2	0	0	0	0	0	0	0	0
21	*Carica papaya (*L).	0	0	0	10	0	0	0	0	0	1	0	0
22	*Cassia senna (*L.)	0	0	0	0	0	2	8	0	0	0	0	0
23	*Cassia mimosoides* (L.)	4	0	0	0	2	12	2		4	0	0	0
24	*Cassia* sp. (L.)	0	0	1	0	0	0	4	2	0	0	0	0
25	*Casuarina equisetifolia* (L.)	0	36	0	2	0	8	0	0	4	8	6	0
26	*Ceiba pentandra* (L.	0	0	0	10	0	0	0	0	0	0	4	0

Table 4.1: Atmospheric pollen count of Nsukka, Enugu State (South East) Nigeria Cont'd

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
27	*Celtis* sp.		8	0	0	8	0	0	0	0	0	0	0
28	*Celtis* sp.	0	0	0	0	8	0	0	0	0	0	0	0
29	*Chromolaena odorata* (L.)	0	0	0	0	0	0	0	0	0	0	14	
30	*Cissus* sp.	0	0	0	0	0	2	10	0	0	0	0	0
31	*Cochlospermum tinctorum* (A. Rich)	0	0	0	0	0	0	2	0	0	4	0	0
32	*Cocos nucifera* (L.)	0	0	0	0	0	0	0	0	6		16	16
33	Combretaceae	0	6	0	4	4	0	0	0	0	2	0	0
34	*Cucumis* sp.	0	0	0	0	0	0	2	0	0	0	0	0
35	Curcumbitaceae	0	0	0	2	0	0	0	0	0	0	0	0
36	*Cussonia bateri* (Seem.)	0	0	0	1	2	8	0	0	0	0	0	0
37	Cyperus spp.	0	0	0	10	4	0	0	0	0	0	0	0
38	*Daniella oliveri (*L.)	16	0	0	16	0	0	0	0	0	0	0	0
39	*Detarium senegalensis* (JF Gmelin)	0	4	0	0	0	0	0	0	0	0	0	0
40	*Dichrostachys cinerima (*L.)	0	0	1	0	0	0	0	0	0	0	0	0
41	*Dracaena arborea* (L.)	2	6	0	2	0	4	4	0	10	4	8	2
42	*Elaeis guineensis* (Jacq.)	16	20	2	88	12	28	80	20	24	28	1	2
43	*Eugenia nodiflora* (Aubl.)	2	0	0	2	2	0	0	0	0	0	7	0
44	*Gloriosa superba* (L.)	0	2	0	0	2	0	0	0	0	0	0	0
45	*Hymenocardia acida* (Tul.)	0	2	0	0	4	0	12	0	0	0	0	0
46	*Hymenocardia acida* (Tul.)	0		0	0	2	0	0	0	0	0	0	0
47	*Ipomoea* sp.	0	2	0	0	8	0	0	0	0	0	0	0
48	*Isoberlinia doka* (Craib and Stapf)	0	0	0	0	0	2	6	0	12	0	0	0
49	*Jatropha curcus* (L.)	0	0	0	0	0	0	0	0	0	0	16	
50	*Justicia* sp.	0	0	0	0	0	0	0	0	0	0	3	0
51	*Kigelia africana* (Lam)	0	0	0	6	0	0	0	0	0	0	0	0
52	*Lannea welwitchii* (Hiern)	0	0	0	0	2	0	0	0	0	0	0	0

Table 4.1: Atmospheric pollen count of Nsukka, Enugu State (South East) Nigeria from June 2011- May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
53	*Lophira* sp.	6	0	0	4	0	0	0	0	0	0	1	0
54	*Luffa aegyptica* (Mill.)	0	0	0	4	0	0	0	0	0	0	0	0
55	*Mangifera indica*(L.)	2	0	0	0	0	0	2	16	24	1	0	0
56	*Microdesmis sp*	0	0	1	0	0	0	0	0	0	0	0	0
57	*Nauclea latifolia* (Smith)	0	2	0	2	0	0	2	0	0	0	2	0
58	*Newbouldia laevis*(Seem)	0	0	0	0	0	0	0	0	0	0	18	0
59	*Olax subscorpioidea* (Oliv.)	0	0	0	112	32	2	0	8	24	0	0	0
60	*Pentaclethra macrophylla* (Benth)	0	0	0	0	12	36	0	0	4	0	15	0
61	*Phyllanthus* sp.	0	0	0	0	20	4	2	0	8	8	0	0
62	*Piliostima sp*	0	0	0	0	0	0	0	0	0	0	4	0
63	Poaceae	72	104	72	74	160	56	32	0	32	56	10	0
64	Portulacaceae	0	0	0	0	0	2	0	0	0	0	0	0
65	*Spathodea campanulata* (P. Beauv)	0	0	0	0	4	4	2	0	0	0	0	0
66	*Spondias mombin* (L.)	0	0	0	2	0	0	0	0	0	0	0	0
67	*Syzygium guineense*(Willd) DC.	0	0	0	0	0	4	0	0	0	0	0	1
68	*Tapinanthus* sp.	0	0	0	0	12	0	2	0	0	0	0	0
69	*Terminalia catappa* (L.)	0	0	0	0	4	0	0	0	0	0	0	0
	TOTAL POLLEN	**128**	**230**	**81**	**380**	**372**	**198**	**292**	**64**	**284**	**130**	**449**	**31**

Table 4.2: Atmospheric fungi spores counts of Nsukka, Enugu State (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	*Alternaria* spp.	0	136	24	0	0	0	0	0	0	0	4	4
2	*Bastrodesmium* sp.	0	0	0	0	102	0	0	0	0	0	0	0
3	*Beltrania* sp.	0	6	0	0	4	0	0	0	0	0	0	0
4	*Cephalosporium* sp.	0	0	0	0	0	0	0	0	4	0	0	0
5	*Cercospora* sp.	0	0	0	16	0	0	0	0	0	0	0	0
6	*Cladosporium* spp	0	0	0	0	0	3	0	6	0	0	0	0
7	*Curvularia* spp.	0	0	0	2	6	0	0	2	0	0	0	0
8	*Curvularia* spp.	0	2	0	0	0	0	0	0	0	0	0	0
9	*Diplocladiella* sp.	0	2	0	0	0	0	0	0	0	0	0	0
10	*Dreschlera* sp.	0	0	0	0	0	0	0	0	2	0	0	0
11	*Flagellospora* sp.	0	0	0	2	0	0	0	0	0	0	0	0
12	*Fusarium* spp.	6	13	0	122	400	0	0	0	0	0	0	0
13	*Helminthosporium* sp.	6	28	0	8	36	0	0	0	0	0	0	0
14	*Helminthosporium* sp.	0	0	4	0	0	0	0	0	0	0	0	0
15	Indeterminate	220	0	66	0	0	1503	0	99	0	0	6	6
16	*Leptothyrium* sp.	0	0	0	0	27	0	2	0	0	0	0	0
17	*Monilia* sp.	0	0	0	0	2	0	0	0	0	0	0	0
18	*Murogenella* sp.	0	0	0	0	0	0	0	0	2	0	0	0
19	*Nigrospora* sp.	16	0	16	16	14	4	16	14	6	20	0	0
20	*Pithomyces* sp.	0	0	1	0	2	0	0	0	0	2	0	0
21	*Pseudotorulla* sp.	0	1	0	0	0	0	0	0	0	0	0	0
22	*Puccinia* sp.	6	74	0	8	0	6	6	0	2	0	0	10
23	*Spadicoides* spp.	18	62	0	2	0	0	6	0	0	4	0	0
24	*Sporidesmium* sp.	0	0	0	0	0	0	0	0	1	0	0	0
25	*Stemphyluim* sp.	0	0	2	0	0	0	0	0	0	0	0	0
26	*Torulla* sp.	6	4	0	4	0	4	2	0	0	0	0	0
27	*Triposporium* sp.	10	0	1	8	14	0	0	0	0	0	0	0
28	*Venturia* sp.	1	0	4	24	0	0	0	0	0	0	0	0
	TOTAL SPORES	**295**	**579**	**118**	**214**	**607**	**170**	**32**	**121**	**17**	**26**	**10**	**20**

4.2 Seasonal Prevalence of Pollen and Fungi Spores in Obiakor, River State (South South) Nigeria from June 2011 to May 2012

In Obiakor, Rivers state, there was a decline of atmospheric pollen content from the month of March and throughout the rainy season (Table 4.3). Unlike the South East which showed three distinct patterns of pollen dispersal, there was a progressive increase in the atmospheric pollen content as the dry season approached from September through October, November, December, January, February to March. This corresponded however to decrease of atmospheric fungi spores (Table 4.4). Forty-seven (47) pollen types, which comprised of 31 families were identified from the aeroflora samples. Twenty-four, ninteen and four were identified to generic, species and family levels respectively. Dominant pollen included those of *Rhizophora* spp., *Cassipourea* sp., *Acrostichum aureum, Alchornea cordifolia*, Cyperaceae, Poaceae and Amarathaceae/Chenopodiaceae among others. *Rhizophora* spp. pollen were present from September to March,, achieved anthesis in December, *Cassipourea* sp. occurred from September to March. *Acrostichum aureum* spore was present from August to May. *Alchornea cordifolia* pollen grains were present from October to March. Poaceae pollen were present in June, October to May, achieved anthesis in December. Amarathaceae/ Chenopodiaceae pollen were present from October to March. *Elaeis guineensis* pollen occurred almost throughout the year. *Cassia* spp. pollen occurred from June to July and also from October to April.

Airborne pollen became more dominant during the dry season, from September to March. December (741), January (713) and February (610) had the highest records of pollen, whereas June (38), July (24) and August (27) had lowest record of pollen (Table 4.3).

Sixteen fungal spores types were identified from the aeroflora samples (Table 4.4). Months of June, July and August had higher records of fungi spores than other rainy months. The spores of Torulla sp., *Microsporium* sp., *Venturia* sp., *Alternaria* sp., *Spilocea* sp., *Murogenella* sp., *Diplosporium* sp. were very sporadically distributed over the months whereas the spores of *Spadicoides* spp., *Cladosporium* spp., *Curvularia* spp. and *Puccinia* spp. were more abundant during the rainy season from June to August. *Nigrospora* spp. occurred throughout the year.

Table 4.3: Atmospheric pollen count of Obiakor, River State (South South) Nigeria from June 2011-May 2012

S/N	POLLEN/FERN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	*Acrostichum aureum* (L.)	0	0	0	4	5	0	21	23	24	35	20	8
2	*Albizia* spp.	0	0	0	0	0	1	2		1	1	2	0
3	*Alchornea cordifolia* (Schum and Thonn)	0	0	0	0	40	61	125	445	333	41	22	31
4	Amarathaceae/Chenopodiaceae	0	0	0	0	1	1	1	22	18	5	0	0
5	*Anthocleista* sp.	0	0	0	0	0	1	0	9	0	0	0	2
6	*Antrocaryon micraster* (A. Chev.)	0	0	0	0	1	0	0	0	0	0	0	0
7	*Avicennia* sp.	0	0	0	0	0	0	0	0	0	16	0	0
8	*Berlinia tomentella* (keay)	0	0	0	0	1	1	0	0	0	1	0	0
9	*Cassia* spp.	2	1	0	0	9	12	5	3	18	2	5	0
10	*Cassipourea* sp.	0	0	0	7	12	6	22	9	10	13	0	0
11	*Casuarina equisetifolia* (L)	0	0	0	0	7	2	0	1	2	3	0	0
12	*Celtis zenkeri* (Engl.)	0	0	0	0	2	0	0	0	0	0	0	0
13	*Celtis zenkeri* (Engl.)	7	1	0	0	0	0	0	0	0	0	0	0
14	*Ceriops* sp.	0	0	0	0	0	0	0	0	0	1	0	0
15	*Citrus* sp.	0	0	0	0	0	1	0	0	0	0	0	0
16	*Coccinia grandis* (L.)	17	0	0	0	0	0	0	0	0	0	0	0
17	*Cocos nucifera* (L.)	0	0	0	0	5	3	0	0	0	0	3	0
18	Combretaceae	0	0	0	0	12	8	13	8	6	3	2	0
19	Cyperaceae	0	0	0	0	32	18	3	16	6	7	0	2
20	Diospyros sp.	0	0	0	0	0	0	2	0	0	0	0	0
21	*Dracaena arborea*	0	0	0	0	0	1	2	1	0	2	0	0
22	*Elaeis guineensis* (Jacq.)	15	17	20	0	13	17	20	32	14	11	10	2
23	*Eugenia* sp.	0	3	0	0	0	0	0	0	0	0	2	1
24	*Khaya ivorensis (*A. Chev.)	0	0	0	0	0	0	1	0	0	0	0	0
25	*Lannea welwitschii* (Hiern)	1	0	0	0	0	0	0	0	0	0	0	0

Table 4.3: Atmospheric pollen count of Obiakor, River State (South South) Nigeria from June 2011-May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
26	*Lonchocarpus* sp.	0	0	0	0	1	0	0	0	0	0	0	0
27	*Lophira sp*	0	0	0	0	0	0	0	2	0	1	0	0
28	*Ludwigia* sp.	0	0	0	0	0	1	0	0	0	0	0	0
29	*Mangifera indica* (L.)	0	0	0	0	0	2	0	0	0	0	1	0
30	*Maytenus* sp.	0	0	0	0	0	2	20	2	0	0	0	0
31	*Milicia excelsa* (L.)	0	0	0	0	1	5	3	14	7	1	0	0
32	*Mitragyna* sp.	0	0	0	0	0	2	0	0	0	0	0	0
33	*Monodora* sp.	0	0	0	0	0	0	0	2	0	0	0	0
34	*Montandra* sp.	0	0	0	0	0	1	0	0	0	0	0	0
35	*Nypa* sp	0	0	0	0	0		0	0	0	5	0	0
36	*Olax* sp.	0	0	0	0	0	8	5	0	0	0	0	0
37	*Pentaclethra macrophylla* (Benth)	0	0	0	0	10	0	0	0	0	0	0	0
38	*Phyllanthus* sp.	0	1	0	0	0	0	0	0	0	0	0	0
39	Poaceae	12	0	0	0	70	72	91	65	12	8	7	15
40	*Portulaca* sp.	0	0	0	0	0	1	0	0	0	0	0	0
41	*Psidium guajava* (L.)	0	0	0	0	1	0	0	0	0	0	0	0
42	*Pterocarpus* sp.	0	0	0	0		0	9	0	0	0	0	0
43	*Rhizophora* spp.	0	0	0	50	76	73	382	75	174	279	0	0
44	*Spondias mombin*	0	0	0	0	1	0	0	0	0	0	0	0
45	*Syzygium guineense*(Willd) D.C	0	1	0	0	1	0	3	1	1	3	0	0
46	*Tetrorchidium* sp.	0	0	0	0	2	0	0	1	0	0	0	0
47	*Uapaca* sp.	0	0	0	0	1	0	0	0	0	0	0	0
	TOTAL POLLEN	**38**	**24**	**27**	**61**	**283**	**294**	**741**	**713**	**610**	**411**	**74**	**69**

Table 4.4: Atmospheric fungi spores counts of Obiakor, River State (South South), 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	*Alternaria* spp.	1	0	0	0	0	0	0	0	0	0	0	0
2	*Cladosporium* spp.	96	93	110	0	0	0	0	0	0	0	0	0
3	*Curvularia* spp.	3	3	12	0	0	0	0	0	0	0	0	2
4	*Diplosporium* sp.	0	1	0	0	0	0	0	0	0	0	0	0
5	*Helminthosporium*	0	0	0	0	0	0	0	0	0	0	0	1
6	Indeterminate	0	0	0	0	3	0	0	0	0	0	0	0
7	*Microsporum* sp.	1	0	0	0	0	0	0	0	0	0	0	0
8	*Murogenella* sp.	0	3	0	0	0	0	0	0	0	0	0	0
9	*Nigrospora* spp.	16	1	15	5	26	17	18	30	42	52	60	38
10	*Pithiomyces* spp.	0	16	24	30	32	20	29	0	0	0	0	0
11	*Puccinia* spp.	11	35	0	0	1	0	0	0	0	4	0	5
12	*Spadicoides* spp.	75	31	40	20	4	0	0	0	0	0	0	6
13	*Spilocea* sp.	0	0	0	0	1	0	0	0	0	0	0	0
14	*Stemphylium* sp.	0	0	0	0	3	0	0	0	0	0	0	0
15	*Tetraploa* sp.	1	0	0	0	0	0	0	0	0	0	0	0
16	*Torulla* sp.	0	0	0	0	0	0	0	0	0	0	0	1
17	*Venturia* sp.	0	0	0	0	1	0	0	0	0	0	0	0
	TOTAL SPORES	**204**	**184**	**201**	**53**	**71**	**38**	**49**	**30**	**42**	**56**	**60**	**53**

4.3 Seasonal Prevalence of Pollen and Fungi Spores in Akoka, Lagos State (South West) Nigeria from June 2011 to May 2012

Forty- six pollen types were identified from aeroflora of Akoka, Lagos State. Twenty-nine, fifteen and two were identified to species, generic and family levels respectively. Dominant pollen include those of *Jatropha curcas*, *Lannea floccosa*, Amarathaceae/Chenopodiaceae, *Adansonia digitata, Delonix regia, Pentaclethra macrophylla, Terminalia catappa and Coccinia grandis. Jatropha curcas* pollen dominated in the months of August and September (Table 4.5). The months of August, September and October recorded higher number of pollen than the other months of the year.

Ten fungal spores types were identified, from the aerosamples (Table 4.6). The spores of *Nigrospora* sp., *Spadicoides* spp., *Torulla* sp.and *Curvularia* spp. were the most abundant. The spores of *Spadicoides* sp. and *Torulla* sp. were higher during the rainy season. There was a reduction in the quantity of spores during the dry season.

Table 4.5: Atmospheric pollen count of Akoka, Lagos State (South West) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	*Adansonia digitata*	0	0	6	2	0	0	0	0	0	32	1	5
2	*Ageratum conyzoides* (L.)	0	0	0	0	0	0	1	5	7	0	0	0
3	*Albizia* sp.	0	0	0	0	0	5	0	0	0	0	2	6
4	*Alchornea cordifolia* (schum and thonn)	0	0	1	1	0	0	0	0	0	0	8	7
5	Amaranthaceae / Chenopodiaceae	**4**	4	12	12	10	6	0	0	0	0	0	6
6	*Anacardium occidentale* (L.)	0	0	0	0	0	0	1	0	0	0	0	0
7	*Anthocleista djalonensis (*A. Chev.)	0	0	0	0	*7*	*6*	0	0	0	0	0	0
8	*Barleria* sp.	0	0	0	0	0	0	4	2	3	0	0	0
9	*Cassia* sp.	4	1	0	0	0	0	0	0	0	0	0	0
10	*Casuarina equisetifolia* (L.)	0	0	8	9	12	6	0	0	0	0	5	6
11	*Ceiba pentadra* (L.)	0	0	0	0	0	0	3	8	9	0	0	0
12	*Celtis* sp.	0	0	0	0	0	0	0	0	0	8	0	0
13	*Celtis zenkeri* (L.)	0	0	0	0	8	0	0	0	0	0	0	0
14	*Cissus* sp.	0	0	0	0	0	0	2	7	5	0	0	0
15	*Coccinia grandis*(L.	0	0	0	0	0	0	44	7	9	0	0	0
16	*Cocos nucifera* (L.)	0	0	0	0	1	0	0	0	0	0	0	0
17	*Combretum* sp.	0	0	0	0	0	0	2	0	0	0	0	0
18	*Cyperus* sp.	0	0	0	1	1	4	6	0	0	2	10	4
19	*Delonix regia*	0	0	1	1	0	0	0	0	0	32	1	0
20	*Dracaena arborea* (L.)	0	0	0	0	0	1	4	3	0	0	0	0
21	*Eugenia nodiflora* (Aubl)	0	0	0	0	10	0	0	0	0	4	0	0
22	*Eugenia* sp.	0	0	0	1	1	0	0	0	0	4	0	0
23	*Gloriosa superba* (L.)	0	0	1	1	1	0	0	0	0	0	0	0
24	*Hymenocardia acida* (Tul.)	0	0	0	0	8	9	7	0	0	0	0	0
25	*Ipomoea* sp.	0	0	20	0	0	0	1	0	0	0	0	0
26	*Jatropha curcas* (L.)	0	0	280	540	16	0	0	0	0	1	0	0
27	*Lannea floccose* (S.)	10	10	0	0	0	0	0	0	0	0	0	0

Table 4.5: Atmospheric pollen count of Akoka, Lagos State (South West) Nigeria from June 2011-May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
28	*Lannea welwitshii* (Hiern)	0	0	0	0	0	0	8	9	10	0	0	0
29	*Mangifera indica* (L.)	0	0	0	0	4	6	7	0	0	0	0	14
30	*Olax* sp.	0	0	0	0	32	0	0	0	0	0	0	0
31	*Olax gambecola* (Oliv.)	0	0	0	0	22	0	8	11	15	0	0	0
32	*Olax subscopioidea* (Oliv.)	0	0	0	0	22	0	0	0	0	28	13	0
33	*Parkia* sp.	0	0	1	0	0	0	0	0	0	1	0	0
34	*Paullinia pinnata* (L.)	0	0	0	0	0	0	1	0	0	0	0	0
35	*Pentaclethra macrophylla* (Benth)	0	0	0	0	32	12	0	0	0	0	0	0
36	*Phyllanthus* sp.	0	0	0	0	1	0	0	0	0	0	0	0
37	*Phyllanthus discoides* (L.)	0	34	32	20	1	8	0	0	0	0	0	0
38	Poaceae	14	10	10	8	12	16	12	0	0	0	0	0
39	*Polygala* sp.	0	0	4	1	0	0	0	0	0	0	0	0
40	*Rhizophora* sp.	0	0	0	0	0	0	10	0	0	0	0	0
41	*Rungia* sp.	0	0	0	0	6	0	0	0	0	0	0	0
42	*Securinga virosa* (Roxb). Baill	0	0	0	0	2	0	0	0	0	0	0	0
43	*Spondias mombin* (L.)	0	0	0	0	0	0	2	0	0	0	0	0
44	*Syzygium guinense* (Willd) D.C	6	8	0	0	0	2	0	0	0	0	0	0
45	*Terminalia catappa* (L.)	0	0	0	0	0	14	16	18	20	0	6	10
46	*Vernonia* sp.	0	0	1	1	7	8	0	0	0	4	5	0
	TOTAL	**38**	**40**	**349**	**598**	**187**	**74**	**121**	**70**	**78**	**116**	**55**	**54**

Table 4.6: Atmospheric fungi spores counts of Akoka, Lagos State (South West) Nigeria from June 2011- May 2012

S/N	FUNGI SPORES	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mch**	**Apl**	**May**
1	*Cladosporium* sp.	0	0	1	0	0	0	0	0	0	0	0	0
2	*Curvularia* sp.	0	0	10	9	6	2	0	0	0	0	13	1
3	*Diplocladiella* sp.	0	0	0	0	0	1	0	0	0	0	0	0
4	*Helminthosporium* sp.	5	3	0	0	0	1	0	0	0	0	0	0
5	*Nigrospora* sp.	5	3	10	10	6	13	0	0	0	0	1	5
6	*Puccinia* sp.	0	0	0	0	0	5	0	0	0	0	0	0
7	*Spadicoides* sp.	7	45	56	0	7	0	0	0	0	0	9	0
8	*Tetraploa* sp.	0	0	0	0	1	1	0	0	0	0	0	0
9	*Torulla* sp.	60	5	0	0	0	6	0	0	0	0	0	0
	TOTAL	**77**	**56**	**77**	**19**	**20**	**29**	**5**	**08**	**10**	**05**	**23**	**06**

4.4 Seasonal Prevalence of Pollen and Fungi Spores in Garki, Abuja (North Central) Nigeria from June 2011 to May 2012

Fifty eight (58) pollen types which comprised of thirty- six families were identified, three, twenty- six and twenty-sixpollen were identified to familial, generic and specie levels respectively (Table 4.7).

The annual contribution of fungal spores 3164 (54.98 %) to the aeroflora was found to be greater than pollen 2897 (45.01%). Higher number of pollen were recorded in the months of October 518 (17.65 %), November 472 (16.08 %) and December 354 (12.07 %) while February 124 (4.22 %) and May 96 (3.27 %) had the lowest records (Table 4.7). The major pollen contributors were *Elaeis guineensis, Lannea acida, Cassia* spp. and Poaceae, *Cochlospermum tinctorum,* Amarathaceae/Chenopodiaceae, *Luffa* sp., *Alchornea cordifolia, Khaya senegalensis* and *Pentaclethra macrophylla*. The anemophilous pollen recorded included those of Poaceae, *Cyperus esculenta,* Amarathaceae/Chenopodiaceae, *Casuarina equisetifolia, Cocos nucifera* and *Dracaena arborea*. In the months of October, through November, December and January, Pollen grains became dominant, with those not previously present in the atmosphere being found at this period. There was reduction in the number of fungal spores in the air from January to May (Table 4.8).

In the month of June, total pollen encountered was 295 whereas fungal spores were 310 (Tables 4.7 and 4.8). Dominant pollen grains were those of *Elaeis guineensis, Lannea acida*, *Cassia* spp. and Poaceae. The spores of *Tetraploa* sp. (111) and *Nigrospora* sp. (51) were more abundant during this month.

In the month of July, there was a drastic reduction of the atmospheric pollen (186) and an increase in the quantity of fungal spores (312). Eighteen fungal spore types were identified (Table 4.8). The aeroflora in August was dominated by fungal spores (510) compared to pollen (142) (Table 4.7 and 4.8). Several species of fungi spores which were not previously present were also recorded in these months. These include *Erysiphe graminis*, *Aspergillus* sp. and *Fusarium* sp.

Table 4.7: Atmospheric pollen count of Garki, Abuja (North Central) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
1	*Acacia* sp.	2		2	2	0	0	0	0	0	0	0	0
2	*Adansonia digitata*	0	0	0	0	0	12	4	0	0	0	0	0
3	*Ageratum conyzoides* (L.)	0	0	0	0	12	0	0	16	0	0	0	0
4	*Albizia* sp.	0	2	0	0	0	0	0	0	0	0	0	0
5	*Alchornea cordifolia* (Schum and Thonn)	1	0	0	2	12	52	8	2	16	4	6	0
6	*Aloe bateri*	0	0	0	3	0	0	0	0	0	0	0	0
7	Amarathaceae / Chenopodiaceae	0	0	44	0	8	16	0	4	0	0	0	0
8	*Aneilema beninlense*	0	2	0	0	0	0	0	0	0	0	0	0
9	*Anthocleista djalonensis* (A. Chev.)	0	0	0	4	0	0	0	20	0	0	0	0
10	*Aspilia africana* (Pers CD. Adam)	0	0	6	2	22	8	0	0	0	0	1	6
11	Asteraceae	4	0	0	0	0	0	0	0	0	0	0	0
12	*Bridelia ferruginea* (Benth)	0	0	0	0	24	0	0	0	0	0	0	0
13	*Bulbostylis* sp.	0	0	0	0	0	0	4	0	0	0	0	0
14	*Cassia* sp.	28	0	0	4	10	8	0	0	8	30	16	56
15	*Casuarina equisetifolia* (L.)	1	6	2	0	0	0	0	0	0	8	6	4
16	*Ceiba pentandra* (L.)	0	2	0	0	0	0	0	0	0	0	0	0
17	*Ceiba Pentandra* (L.)	0	2	0	0	0	0	0	0	0	0	0	0
18	*Celtis* sp.	0	0	0	0	0	16	20	0	0	0	0	0
19	*Cissus* sp.	0	0	0	2	0	0	0	0	0	0	0	0
20	*Cochlospermum tinctorum* A. Rich	0	16	0	0	0	0	42	0	0	0	0	0
21	*Cocos nucifera* (L.)	4	0	0	0	0	0	0	0	0	0	0	0
22	*Crotalaria* sp.	0	0	0	0	0	0	0	6	4	0	0	0
23	*Cyperus* sp.	0	0	0	0	4	44	4	1	10	6	2	2
24	*Dichrostachys* sp.	0	0	0	0	0	0	2	8	0	0	0	0
25	*Dracaena arborea* (L.)	0	0	8	2	0	0	0	0	0	0	0	0
26	*Drypetes* sp.	0	0	0	0	0	0	2	0	0	0	0	0
27	*Eichornia* sp.	2		0	0		0	0	0	0	0	0	0
28	*Elaeis guineensis* (Jacq)	118	6	8	5	6	206	8	8	0	0	0	0
29	*Eugenia* sp.	0	0	0	0	2	0	0	0	0	0	0	0
30	*Gardenia* sp.	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.7: Atmospheric pollen count of Garki, Abuja (North Central) Nigeria from June 2011-May 2012 Cont'd

S/N	POLLEN	MONTHS											
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
31	*Gloriosa superba* (L.)	0	0	2	32	0	0	2	6	0	0	0	0
32	*Hexalobus* sp.	0	0	0	0	0	0	8	0	0	0	0	0
33	*Hymenocardia acida* (Tul.)	0	2	4	12	12	0	0	0	6	0	20	0
34	*Ipomoea* sp.	0	0	0	0	0	0	0	8	40	0	0	0
35	*Justicia extensa* (T. Anders)	0	0	2	0	0	0	0	0	0	0	0	0
36	*Justicia* sp.	0	8	6	0	0	0	0	0	0	0	0	0
37	*Justicia* sp.	0	0	0	0	0	2	4	13	18	134	14	12
38	*Khaya senegalensis* (Desr.) A. Juss	0	0	2	18	30	48	0	0	0	0	0	0
39	*Lannea acida* (A. Rich)	63	6	2	0	0	0	0	0	0	0	0	0
40	*Lannea welwitschii* (Hiern)	0	0	2	0	0	0	0	0	0	0	0	0
41	*Lophira* sp.	0	0	0	0	2	0	0	0	0	0	0	0
42	*Luffa* sp.	0	0	0	21	0	2	0	6	12	26	73	2
43	*Marantochloa* sp.	0	0	2	0	0	0	0	0	0	0	0	0
44	*Milletia* sp.	2	0	0	0	0	0	0	0	0	0	0	0
45	*Olax subscorpioides* (Oliv.)	0	0	0	0	0	0	12	12	0	0	0	0
46	*Parkia* sp.	2	0	0	0	4	0	0	16	0	0	0	0
47	*Parkia biglobosa* (Benth)	0	0	0	0	2	0	0	0	0	0	0	0
48	*Pentaclethra macrophyla* (Benth)	0	0	4	0	120	36	188	54	0	0	0	0
49	*Phyllanthus discoides (*Meull.)	2	0	0	0	0	0	0	0	0	0	0	0
50	Poaceae	48	44	44	68	248	20	28	16	8	0	0	0
51	*Polygala* sp.	0	0	2	0	0	0	2	0	0	0	0	0
52	*Solenostemon* sp.	0	0	0	0	0	0		2	0	0	0	0
53	*Syzygium* sp	0	0	0	0	0	0	12	2	0	0	0	0
54	*Terminalia* sp.	4	0	0	0	0	0	2	0	0	0	0	0
55	*Uapaca togoensis* (Pax)	14	0	0	4	0	0	0	0	2	2	8	14
56	*Vernonia* sp.	0	0	0	0	0	0	0	12	0	0	0	0
57	*Vigna multinervis* (Hutch and Daziel)	0	0	0	0	0	2	0	0	0	0	0	0
	TOTAL	**295**	**186**	**142**	**179**	**518**	**472**	**354**	**212**	**124**	**210**	**146**	**96**

Table 4.8: Atmospheric fungi spores counts of Garki, Abuja (North Central) Nigeria from June 2011 to May 2012

S/N	FUNGAL SPORES	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Alternaria* sp.	14	4	0	6	0	0	0	0	0	0	18	28
2	*Apiosporina* sp.	0	0	0	0	2	0	0	0	0	0	0	0
3	*Aspergillus* sp.	0	0	2	0	0	0	1	0	0	0	0	0
4	*Cereosporella* sp.	3	0	0	0	0	0	0	0	0	0	0	0
5	*Cladosporium* sp.	15	24	0	0	45		8	4	0	0	0	0
6	*Curvularia* sp.	0	4	12	9	96	2	0	18	9	0	0	0
7	*Erysiphe graminis*	0	0	2	0	0	1200	24	9	0	0	0	0
8	*Fusarium* spp.	0	0	6	0	0	20	0	0	0	0	0	0
9	*Hansfordiella* sp.	37	123	90	6	64	0	0	0	0	0	0	0
10	*Helminthosporium* sp.	6	0	6	30	0	6	8	0	0	0	0	0
11	Indeterminate	0	2	0	21	0	0	0	0	0	0	0	0
12	*Nigrospora* sp.	51	6	48	12	45	12	0	6	0	24	10	10
13	*Pithiomyces* sp.	12	15	20	20	54	6	36	0	0	0	0	0
14	*Puccinia* sp.	14	28	309	60	43	38	86	4	3	0	0	0
15	*Spadicoides* sp.	0	75	0	30	46	0	0	0	0	20	0	0
16	*Sporidesmium* sp.	32	0	0	0	165	0	0	0	0	0	0	0
17	*Tetraploa* sp.	111	3	9	3	2	0	12	0	0	0	0	0
18	*Torulla* sp.	17	28	6	12	3	1	1	0	0	0	0	0
19	*Venturia* sp.	0	0	0	1	8	0	0	0	0	0	0	0
	TOTAL	**310**	**312**	**510**	**210**	**573**	**1285**	**176**	**41**	**12**	**44**	**28**	**38**

4.5 Seasonal Prevalence of Pollen and Fungi Spores in Zaria, Kaduna State (North West) Nigeria from June 2011 to May 2012

Pollen were more dominant in the atmosphere in the months of May through June, July, August, September to October than in the months of November, December, January and February. Poaceae pollen were the major contributors in the months of June, July, August, September and October but they decreased in quantity in the subsequent months. *Syzygium guineense*, *Hymenocardia acida, Combretum* sp., *Albizia* sp. and *Khaya senegalensis* were next to Poaceae in abundance (Table 4.9).

Thirty two pollen types were recorded for the period of one year and these belong to twenty (20) families. *Elaeis guineensis* pollen were very sporadic, like those of *Tetrapleura tetraptera* and *Lannea* spp. Herbaceous pollen recorded were mainly those of *Cyperus* spp., *Ipomoea* sp. and Amarathaceae/Chenopodiaceae (Table 4.9).

Twenty fungi spore types were recorded (Table 4.10). Most dominant spores were those of *Nigrospora* sp., *Microsporium* sp. and *Cercospora* sp. Other spores which were sporadic include those of *Spadicoides* sp., *Heminthosporium* sp. and *Puccinia* sp. these were also found to be dominant in aero samples from the study locations of Southern Nigeria.

Table 4.9: Atmospheric pollen counts of Zaria, Kaduna (North West) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	Acacia sp.	4	0	0	2	4	9	8	0	6	0	0	0
2	*Ageratum conyzoides* (L.)	4	2	0	0	2	7	3	2	0	0	2	2
3	*Albizia* sp.	12	152	6	0	2	0	0	0	0	0	58	4
4	Amarathaceae /Chenopodiaceae	14	0	1	2	0	0	0	1	6	7	21	23
5	*Anacardium occidentale* (L.)	0	2	0	7	8	0	0	0	0	0	0	0
6	*Cassia hirsute* (L.)	0	0	8	25	12	0	0	0	0	0	0	0
7	*Cassia mimosoides* (L.)	0	0	0	0	0	0	0	0	0	0	0	0
8	*Cochlospermum planchonii* (Hook)	0	0	0	4	0	0	0	0	0	0	0	0
9	*Combretum grandiflorum* (G.Don.)	0	0	6	7	9	10	21	0	0	0	0	0
10	*Costus* sp.	0	0	0	2	0	0	0	0	0	0	0	0
11	*Cyperus* sp.	0	0	0	25	5	24	10	10	0	4	0	0
12	*Dracaena arborea* (L.)	0	0	4	4	2	5	0	0	0	0	0	0
13	*Elaeis guinensis* (Jacq)	0	0	0	0	2	0	0	0	0	0	0	0
14	*Hymenocardia acida* (Tul.)	99	38	11	0	2	7	8	0	0	5	10	
15	*Ipomoea* sp.	0	0	0	6	7	5	0	0	0	0	0	0
16	*Justicia extensa* (T.)	0	0	5	0	7	6	0	0	0	0	0	0
17	*Justicia* sp.	0	0	0	12	0	0	0	0	0	0	0	0
18	*Khaya senegalensis* (Ders.) A. Juss	0	0	0	0	27	15	11	0	0	0	4	5
19	*Lannea acida* (A. Rich.)	30	12	0	0	0	0	0	0	0	0	2	
20	*Lannea* sp.	0	0	0	3	3	0	0	0	0	0	0	0
21	*Lophira lanceolata* (Tiegh)	0	0	0	0	4	0	0	0	0	0	0	0
22	*Parkia bicolor* (A. Chev.)	0	0	0	0	0	0	0	0	0	5	0	0
23	*Parkia* sp.	0	0	5	6	0	0	0	0	0		0	0
24	*Parkia* sp.	0	0	0	0	0	0	0	4	0	2	0	0
25	*Pentaclethra macrophylla* (Benth)	0	8	4	4	0	0	0	2	0	4	10	2
26	*Phyllanthus* sp.	167	38	3	0	0	0	0	0	0	27	26	1
27	Poaceae	1982	1230	1056	71	27	2	1	1	3	0	0	1200
28	*Podocarpus* sp.	0	0	0	0	0	0	2	0	0	0	0	0
29	*Syzygium* sp.	28	46	36	0	0	0	6	0	0	6	42	6
30	*Terminalia glaucescens* (Planch)	0	0	0	0	0	0	0	0	0	5	0	0
31	*Tetrapleura tetraptera* (Schumach and Thonn0	0	10	2	0	0	0	0	0	0	0	0	0
32	*Trichilia roka* (Chiov.)	0	0	0	0	12	12	32	0	0	1	0	0
	TOTAL POLLEN	**2538**	**1526**	**1147**	**180**	**135**	**102**	**110**	**20**	**15**	**66**	**175**	**1243**

Table 4.10: Atmospheric fungi spores counts of Zaria, Kaduna State (North West) 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	*Cecospora* sp.	5	30	0	0	2	0	0	1	0	3	2	0
2	*Cladosporium* sp.	0	0	0	0	0	0	0	0	0	1	0	0
3	*Curvularia* sp.	0	0	1	4	0	0	0	0	0	0	0	0
4	*Curvularia* sp.	0	0	0	0	0	0	0	0	0	0	9	0
5	*Diplocladiella* sp.	0	1	0	0	1	0	0	0	0	0	0	0
6	*Diplocladiella* sp.	0	0	0	0	0	0	0	0	0	1	0	0
7	*Dreschlera* sp.	0	0	0	0	0	0	0	0	0	1	0	0
8	*Helminthosporium* sp.	1	2	2	0	1	0	0	0	0	1	0	0
9	*Microsporium* sp.	26	33	3	0	1		1	0	0	0	0	0
10	*Murogenella* sp.	0	1	0	0	0	0	0	0	0	0	0	0
11	*Nigrospora* sp.	20	43	6	7	7	2	0	0	0	3	0	3
12	*Phragmidium* sp.	0	0	0	0	4	0	0	0	0	0	0	0
13	*Pithiomyces* sp.	0	0	0	2	3	0	0	0	0	0	4	0
14	*Puccinia* sp	2	2	0	7	2	0	0	0	0	3	7	0
15	*Spadicoides* sp.	0	1	2	2	5	0	0	0	0	5	0	0
16	*Sporidesmium* sp.	0	0	0	4	0	0	0	0	2	4	0	0
17	*Therry fulkelii*	0	24	0	0	3	0	0	0	0	0	0	0
18	*Torulla* sp.	0	0	0	1	1	0	0	0	0	0	4	0
	TOTAL	**54**	**137**	**14**	**27**	**30**	**2**	**1**	**1**	**2**	**22**	**26**	**3**

4.6 Seasonal Prevalence of Pollen and Fungi Spores in Dukku, Gombe State (North East) Nigeria from June 2011-May 2012

Twenty seven pollen types belonging to eighteen families were recorded at Gombe. Ten, fifteen and two were identified to specific, generic and familial levels respectively. The major contributors of the pollen rain include Poaceae, *Lannea welwitschii*, *Ceiba pentandra*, *Parkia bicolor and Vitex doniana* among others (Table 4.11).

The pollen taxa recorded showed a close resemblance with those of Kaduna. Atmospheric pollen load declined from the month of September through October, November, December, January and February and increased again in the months of April and October. The highest risk period for pollen hypersensitive individuals was April – August.

Fourteen fungi spore types were recorded (Table 4.12). Airborne fungi spores were more concentrated between the months of August and October. Spores of fungi were very sporadically distributed throughout the month. Compared to Southern study locations, the number of *Spadicoides* sp. was lower. *Alternaria* sp. whose presence has been noted to be frequently associated with the rainy season were not found in North West and North East study locations.

Table 4.11: Atmospheric pollen count of Dukku, Gombe (North East) Nigeria from June 2011 to May 2012

S/N	POLLEN	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Acacia mellifera* (Benth)	2	0	0	0	0	2	0	3	0	1	1	6
2	*Adansonia digitata* (L.)	0	0	0	0	0	0	0	0	0	4	1	14
3	Amaranthaceae /Chenopodiaceae	0	0	0	1	0	0	0	0	0	0	0	0
4	*Anthocleista* sp.	0	0	0	1	1	1	0	0	0	0	0	0
5	*Berlinia* sp.	1	10	0	2	0	0	0	0	0	0	23	0
6	*Blepharis* sp.	0	0	0	0	0	0	0	0	0	2	1	0
7	*Borreria* sp.	0	0	0	1	0	0	0	0	0	0	0	0
8	*Cassia nigricans* (L.)	0	0	0	0	0	0	0	0	0	2	0	0
9	*Cassia* sp	0	0	1	0	0	0	0	0	0	0	0	0
10	*Ceiba pentandra* (L.)	12	14	4	1	1	1	0	1	1	0	2	7
11	*Gmelina* sp.	0	0	1	0	0	0	0	0	0	0	0	0
12	*Justicia* sp.	0	0	1	1	1	1	9	11	10	25	0	0
13	*Khaya grandifolia* (A. Chev.)	0	0	0	0	0	0	0	0	0	2	2	1
14	*Khaya senegalensis* (A. Chev.)	0	0	0	1	0	0	0	0	0	0	0	0
15	*Lannea acida* (A. Rich.)	50	36	18	1	0	0	0	0	0	19	22	28
16	*Lannea welwitschii* (A. Rich.)	28	17	0	0	0	0	0	0	0	19	7	19
17	*Milletia* sp.	0	0	0	0	1	0	0	0	0	0	0	0
18	*Ocimum* sp.	0	0	0	2	1	0	0	0	0	0	0	0
19	*Parinari* sp.	7	6	0	0	0	0	0	0	0	1	5	0
20	*Parkia bicolor* (Merr)	26	15	4	0	0	4	0	0	0	20	19	85
21	*Pentaclethra macrophylla* (Benth0	17	0	1	1	2	3	0	0	0	1	0	6
22	Poaceae	207	1195	115	95	204		1		5	11	3	0
23	*Podocarpus* sp.	0	0	0	0	0	0	0	0	0	1	0	0
24	*Protea* sp.	0	0	0	0	0	0	0	0	0	1	0	0
25	*Pterocarpus* sp.	7	19	0	0	0	0	0	0	0	1	2	3
26	*Vignia* sp.	0	0	0	0	0	0	0	0	0	1	0	0
27	*Vitex* sp.	18	14	15	14	8	8	2	18		25	0	2
	TOTAL POLLEN	**1375**	**1326**	**160**	**121**	**219**	**20**	**12**	**33**	**16**	**136**	**88**	**171**

Table 4.12: Atmospheric fungi spores counts of Gombe (North East) Nigeria from June 2011 to May 2012

S/N	FUNGAL SPORES	MONTHS											
		Jun	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**
1	*Cercospora* sp.	0	0	0	0	2	0	0	0	0	0	0	0
2	*Curvularia* sp.	0	0	0	0	1	0	0	0	0	0	0	0
3	*Didymella* sp.	0	0	1	0	0	0	0	0	0	0	0	0
4	*Fusarium* sp.	0	0	0	14	0	0	0	0	0	0	0	0
5	*Helminthosporium* sp.	0	0	1	0	0	0	0	0	0	0	3	0
6	Indeterminate	0	0	1	0	20	0	0	0	0	0	0	0
7	*Microsporium* sp.	0	0	1	0	2	0	0	0	0	0	0	0
8	*Nigrospora* sp.	0	0	45	0	0	0	0	0	0	0	12	0
9	*Nigrospora* sp.	0	0	1	0	1	0	0	0	0	0	0	0
10	*Phragmidium* sp.	0	0	1	0	0	0	0	0	0	0	0	0
11	*Pithiomyces* sp.	0	0	15	1	0	0	0	0	0	0	0	0
12	*Puccinia* sp.	0	0	2	1	0	0	0	0	0	0	0	0
13	*Spadicoides* sp.	0	0	1	0	0	0	0	0	0	0	0	0
14	*Sporidesmium* sp.	0	0	0	0	4	0	0	0	0	0	0	0
15	*Torulla* sp..	0	0	0	1	5	0	0	0	0	0	0	0
	TOTAL SPORES	**0**	**0**	**69**	**17**	**35**	**0**	**0**	**0**	**0**	**0**	**15**	**0**

4.7 Seasonal Variations of Pollen, Fungal Spore Types and Charred Poaceae Epidermis Among the Six Geopolitical Zones of Nigeria

Poaceae pollen was the most abundant anemophilous pollen in all studied locations but were more abundant in North West and North East Nigeria (Fig. 4.1), during the rainy months than the drier months (Tables 4.9 and 4.11). In South East Nigeria, Poaceae pollen grains dominated from June to November. In South South, they dominated from October to January. They were present from June to December in South West study location.

The herbaceous pollen encountered in the atmosphere of Nigeria were largely majorly those of family Chenopodiaceae/Amaranthaceae, Cyperaceae, Asteraceae (Tubuliflorae and Liguliflorae complex) as well as those of *Crotalaria* sp. However, their annual contributions represented a smaller percentage of the pollen load (Fig. 4.2).

The recorded airborne pollen were placed in three categories, grasses, herbaceous and trees/ shrubs on the basis of their sources. Trees and shrubs pollen were more dominant in Southern than in Northern Nigeria and constituted the bulk of atmospheric pollen load. They were less dominant in North West and North East and constitutes half of the total pollen in North Central Nigeria (Table 4.2). Trees /shrubs pollen dominated from September-February, October-May and August to March in South East, South South and South West Nigeria respectively. The prevalence of trees/shrubs pollen was recorded between the months of September and April in North Central region. In the North West and North East, most trees/shrubs pollen made their appearance in the atmosphere from the month of August and were distributed throughout the year, though sporadic. The dominant trees/shrubs pollen in North West Nigeria include those of *Albizia* spp., *Syzygium guineense*, *Hymenocardia acida*, *Pentaclethra macrophylla* and

Phyllanthus sp. among others. In North East, *Lannea acida*, *Berlinia* sp., *Ceiba pentandra*, *Pterocarpus* sp. and *Parkia bicolor* were more abundant. In North Central Nigeria, *Elaeis guineensis*, *Ceiba pentandra*, *Alchornea cordifolia*, *Khaya senegalensis* and *Pentaclethra macrophylla* were recorded. In the South East, *Elaeis guineensis*, *Pentaclethra macrophylla*, *Alchornea cordifolia*, *Albizia* sp., *Mangifera indica* and *Casuarina equisetifolia* dominated. In the South South, *Rhizophora* spp., *Cassipourea* sp., *Alchornea cordifolia*, *Elaeis guineensis*, *Milicia excelsa* and *Cassia* spp. dominated. In South West, *Jatropha curcas*., *Lannea floccusa*, *Casuarina equisetifolia*, *Adansonia digitata*, *Pentaclethra macrophylla* and *Terminalia catappa* were in abundance.

Anemophilous pollen dominated the atmosphere of the Northern Nigeria and was mainly represented by Poaceae pollen, whereas enthomophilous pollen dominated in the Southern Nigeria (Fig 4.3 and 4.4).

Based on varied morphotypes assessment of flora component, the order of pollen predominance of the six geopolitical zones was; South East was greater than North Central, North Central greater than South South, South South was greater than South West, South West was greater than North West and North West greater than North East (Table 4.13).

Podocarpus sp. was recorded in North West in the month of November (Plate 4.1p)**.** The source of this bisaccate pollen is most likely the Cameroun mountain range or its extension to Nigeria.

Fungal spores were predominantly recorded in Southern Nigeria during the rainy season, their quantity declined in dry season. Fungal spore load in North- West and North- East were fewer and were sporadically distributed especially in North East (Table 4.14). Charred Poaceae epidermis was not recorded in studied location of South West, they were more dominant in Northern Nigeria especially in North West and North East (Table 4.15).

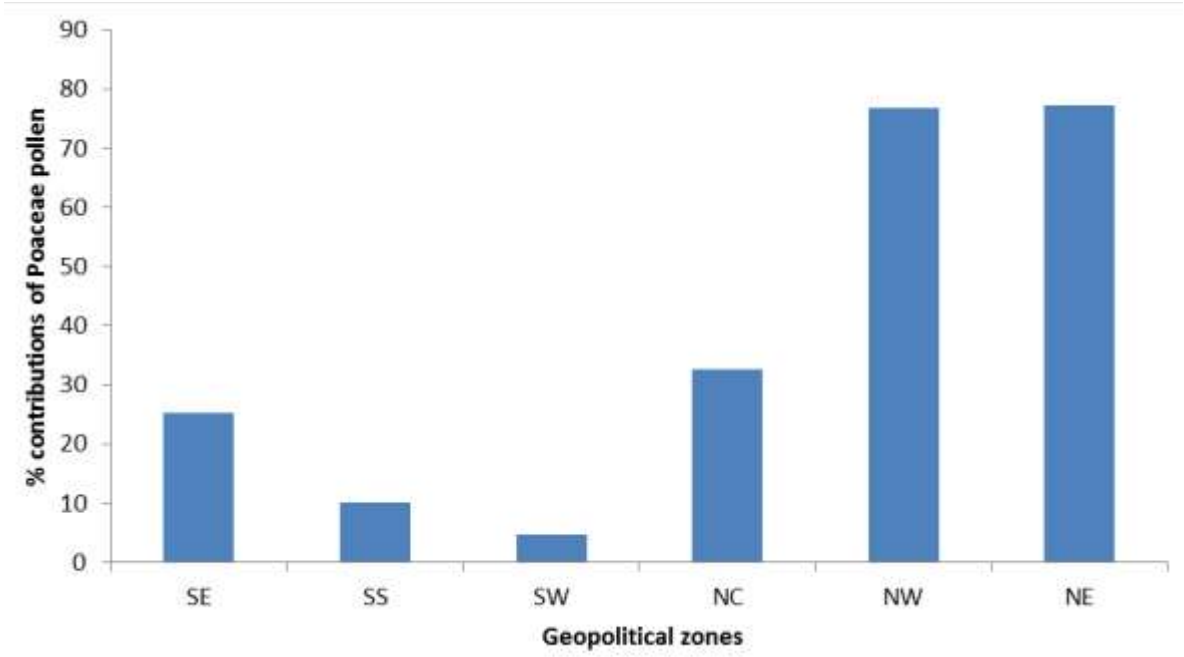


Fig. 4.1: Annual Percentage (%) contributions of Poaceae pollen in the six geo-political zones of Nigeria from June 2011- May 2012

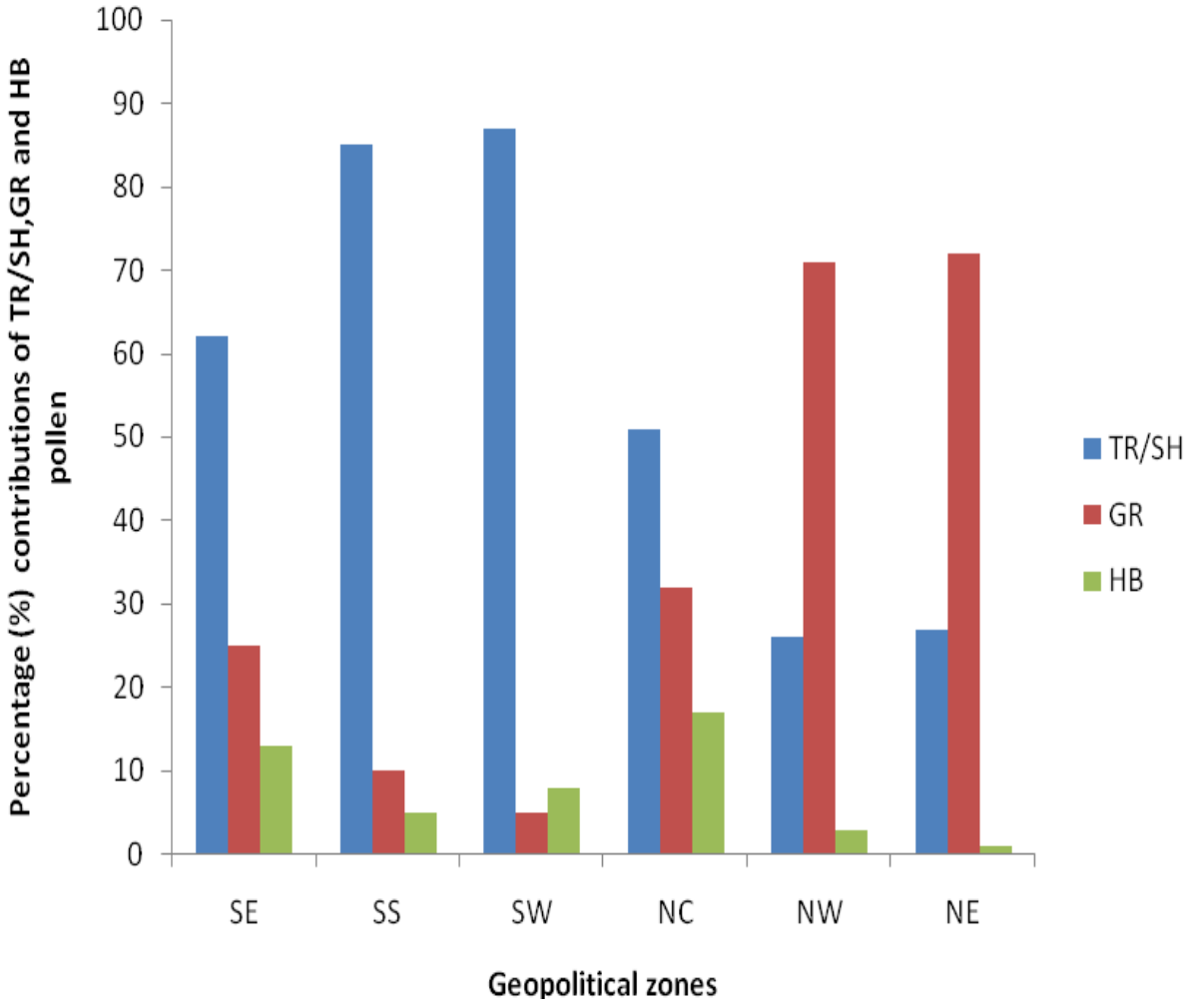


Fig . 4.2: Percentage contribution of trees/shrub, grasses and herbaceous pollen

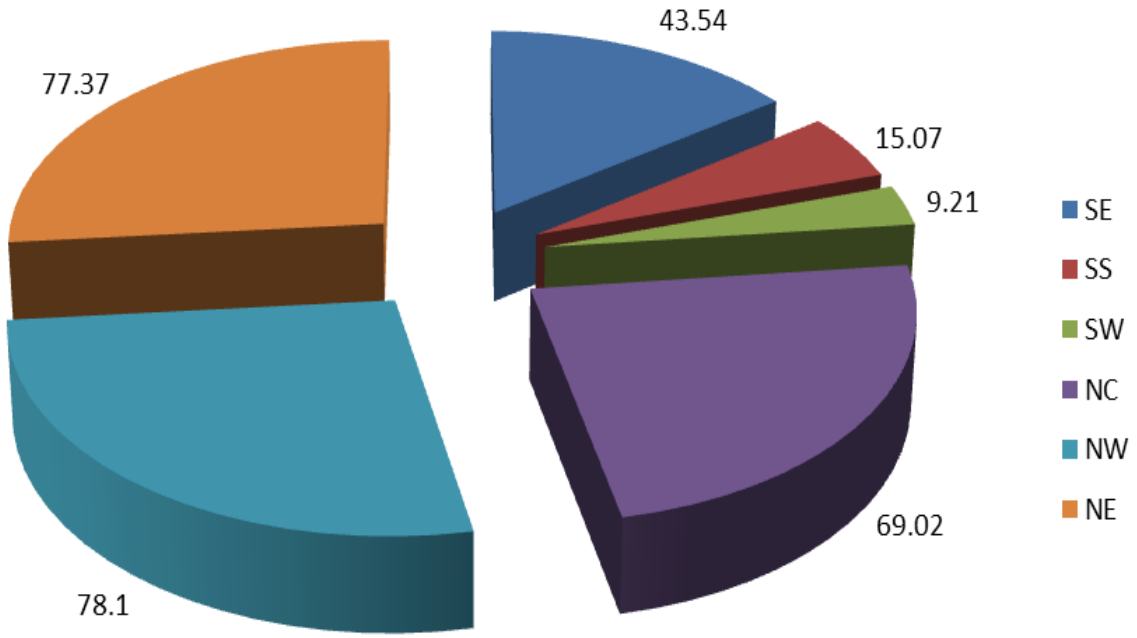


Fig. 4.3: Annual contributions in degree of anemophilous pollen in the six geo-political zones of Nigeria

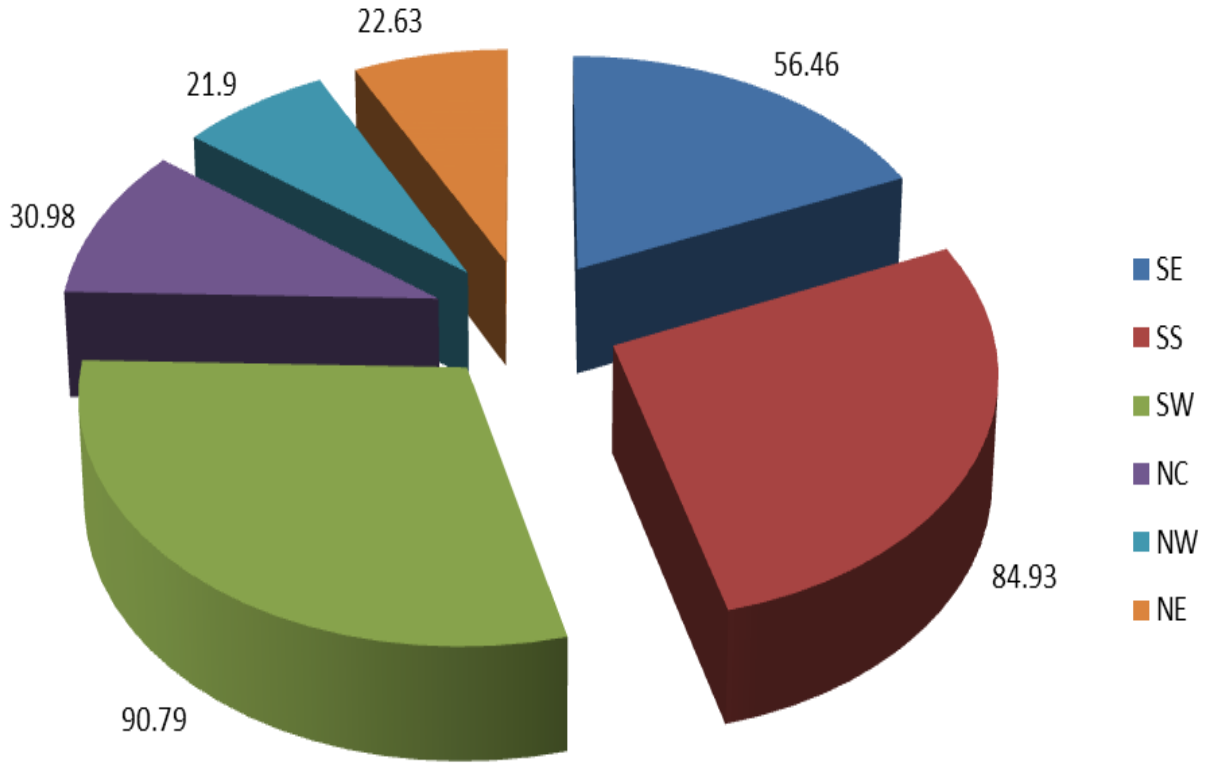


Fig. 4.4: Annual contributions in degree of entomophilous pollen in the six geo-political zones of Nigeria

Table 4. 13: **Total airborne pollen in the six geopolitical zones of Nigeria for one year (June 2011- May 2012)**

MONTHS / LOCATIONS	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
SE	128	230	81	380	372	198	292	64	284	130	449	31
SS.	38	24	27	61	283	294	741	713	610	411	74	61
SW	38	40	349	598	187	74	121	70	78	116	55	54
NC	295	186	142	179	518	472	354	212	124	210	146	96
NW	2538	1526	1147	180	135	102	110	20	15	66	175	1243
NE	1375	1326	160	121	216	14	12	33	16	136	88	171

SE- South- East

SS- South-South

SW- South West

NC- North -Central

NW- North West

NE- North East

Table 4. 14: **Total airborne fungal spores counts in the six geopolitical zones of Nigeria for one year (June 2011- May 2012)**

MONTHS / LOCATIONS	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
SE	**295**	**579**	**118**	**214**	**607**	**170**	**32**	**121**	**17**	**26**	**10**	**20**
SS.	**204**	**184**	**201**	**53**	**71**	**38**	**49**	**30**	**42**	**56**	**60**	**53**
SW	**77**	**56**	**77**	**19**	**20**	**29**	**5**	**08**	**10**	**05**	**23**	**06**
NC	**310**	**312**	**510**	**210**	**573**	**1285**	**176**	**41**	**12**	**44**	**28**	**38**
NW	**54**	**137**	**14**	**27**	**30**	**2**	**1**	**1**	**2**	**22**	**26**	**3**
NE			**69**	**17**	**35**						**15**	

SE- South- East

SS- South-South

SW- South West

NC- North -Central

NW- North West

NE- North East

Table 4. 15: Charred Poaceae cuticles recorded from the six geopolitical zones

MONTHS / LOCATIONS	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
SE	0	8	0	0	0	20	15	16	12	8	2	1
SS.	2	2	0	0	21	23	35	20	60	2	0	0
SW	0	0	0	0	0	0	0	0	0	0	0	0
NC	0	0	8	28	20	43	15	5	3	0	0	0
NW	0	12	10	75	50	0	0	0	0	52	14	0
NE	10	10	70	24	0	10	0	0	110	24	0	0

SE- South- East

SS- South-South

SW- South West

NC- North -Central

NW- North West

NE- North East

4.8 Relationship between Airborne Pollen, Fungi Spores and Weather Parameters

The distributional pattern of weather parameters vary throughout the year in the six geopolitical zones of Nigeria. Rainfall records were higher in Southern Nigeria, especially in South South which had rainfall records throughout the year. The months of November and December had no record of rainfall in South East Nigeria. The month of December also had no record of rainfall in South West Nigeria. Humidity was comparatively higher in Southern Nigeria, it varied between 55 % - 85 % in South East, 70.3 % - 91 % in South South, 81 % - 89 % in South West Nigeria. Average temperature ranges between 25.5 ^{0}C (August) -28.7 ^{0}C (February) in South East Nigeria, 25.2 ^{0}C (August) - 27.9 ^{0}C (March) in South South and 25.5 ^{0}C (August) - 28.5 ^{0}C (March) in South West Nigeria. Wind speed (knot) was higher in December (6.5) in South East, in South South wind speed was the same from January to May (2.4), other months had lower values. The month of August had the highest wind speed (7.5) in South West. In Northern Nigeria, more rainy months occurred in North Central than in North West and North East Nigeria. There was no record of rainfall in North West and North East Nigeria from the month of November to March . Humidity was higher in North Central than other Northern zones, a range of 38% - 88%. North West and North East had a range of 19 % - 82 % and 20 % - 64 % respectively. Average temperature was higher in Northern versus Southern Nigeria. In North Central, highest temperation was recorded in May (34.3 ^{0}C) and lowest recorded in August (24.7 ^{0}C). In North West highest temperature occcurred in April (28.8 ^{0}C) and lowest occurred in February (22.4 ^{0}C) and in North East Nigeria highest temperature was recorded in March (28.0^{0}C) and lowest recorded in September (24.0 ^{0}C).

The distribution and abundance of airborne pollen and fungi spores also varied over the six geopolitical zones and were highly modulated by weather parameters. There was a direct

relationship between the varied morphotypes of airborne fungi spores and monthly rainfall whereas airborne pollen had an indirect relationship with monthly rainfall in the studied locations of Southern Nigeria (Fig. 4.5). In the South East, pollen abundance correlated with a decrease in atmospheric rainfall whereas spores were more abundant during the rainy season from the month of June to October. In Obiakor (Rivers State), South South Nigeria, a gradual decrease in rainfall correlated with influx of pollen morphotypes and their quantity from October to March (Fig. 4.5). In Akoka, Lagos State, more fungi spores counts were recorded during the rainy period from the month of June to August. The quantitative abundance of airborne pollen corresponded with a decline in rainfall from August (the short dry season between the two rainfall maxima) to October. The high morphotypes and quantitative abundance of fungi spores were recorded during the rainy season in all studied locations of Southern Nigeria (Fig. 4.6). The most dominant spores during the rainy season were *Torulla* sp., *Fusarium* sp., *Cladosporium* sp. *Spadicoides* sp., *Puccinia* sp. among others.

In Garki, Abuja, North Central Nigeria, pollen dominated during the dry season when the monthly rainfall was lower. In the North West and North East there were records of high morphotypes and quantitative abundance of pollen during the more rainy months (Fig.4.7), which was majorly populated by Poaceae pollen. The decrease of the abundance of Poaceae pollen correlated with influx of other non poaceae pollen such as; *Albizia* sp., *Acacia* sp., *Syzygium* sp., *Khaya senegalensis* etc. in North West whereas *Lannea acida*, *Gmelina arborea*, *Lannea welwitschii*, *Ceiba petandra*, *Vitex doniana* correlated with decrease of Poaceae pollen in North East Nigeria.

Low fungal spores record correlated with low monthly rainfall and humidity in North West and North East(Fig. 4.8)

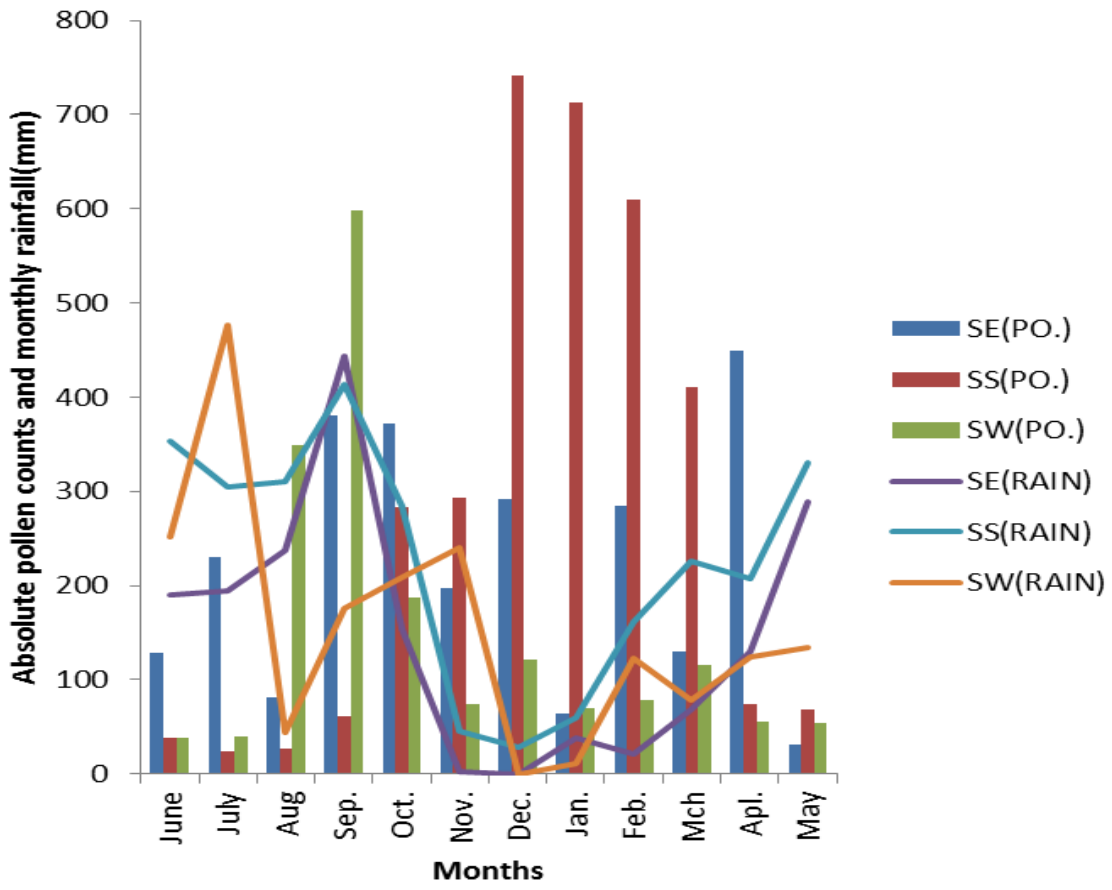


Fig 4.5: Relationship between atmospheric pollen and monthly rainfall (mm) in South East, South South and South West Nigeria

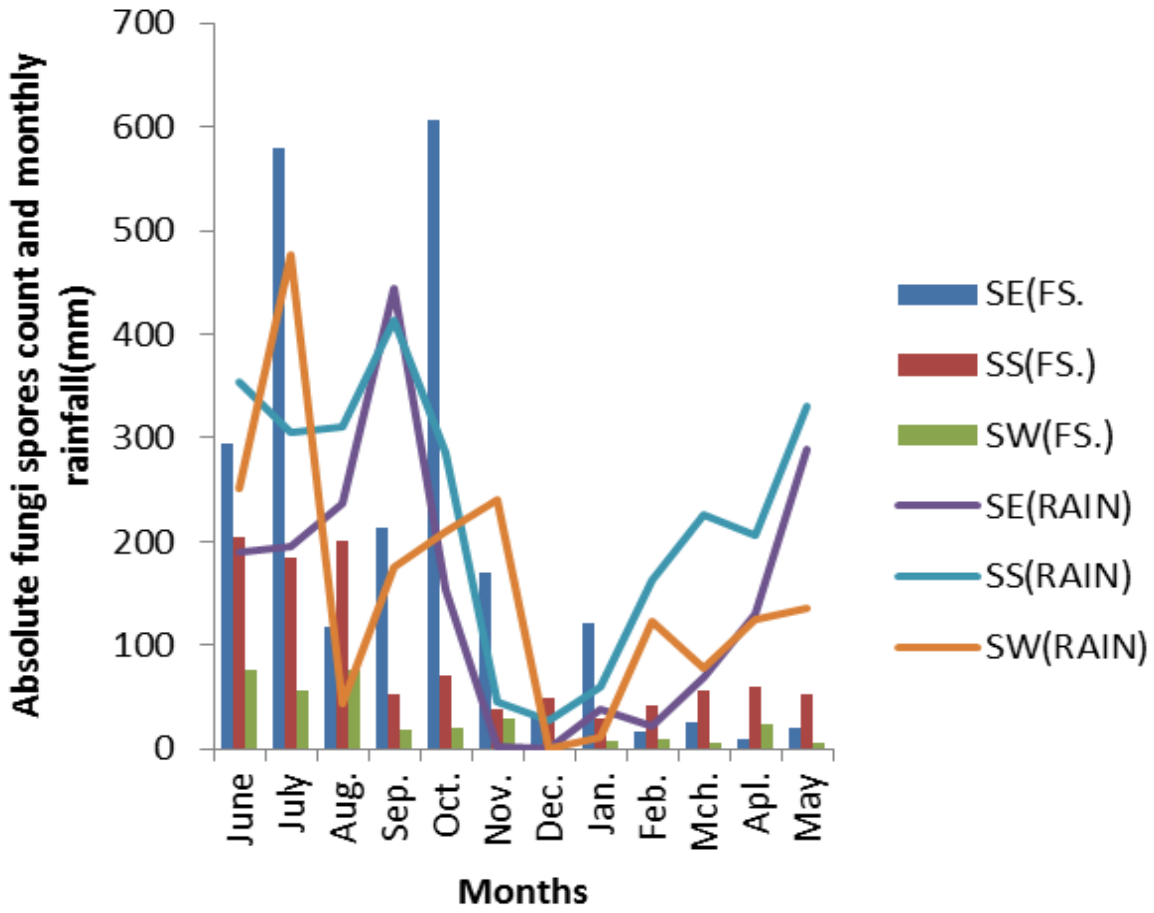


Fig 4.6: Relationship between atmospheric fungi spores and monthly rainfall (mm), temperature ^{o}C, humidity(%) and wind (knot) in South East, South South and South West Nigeria

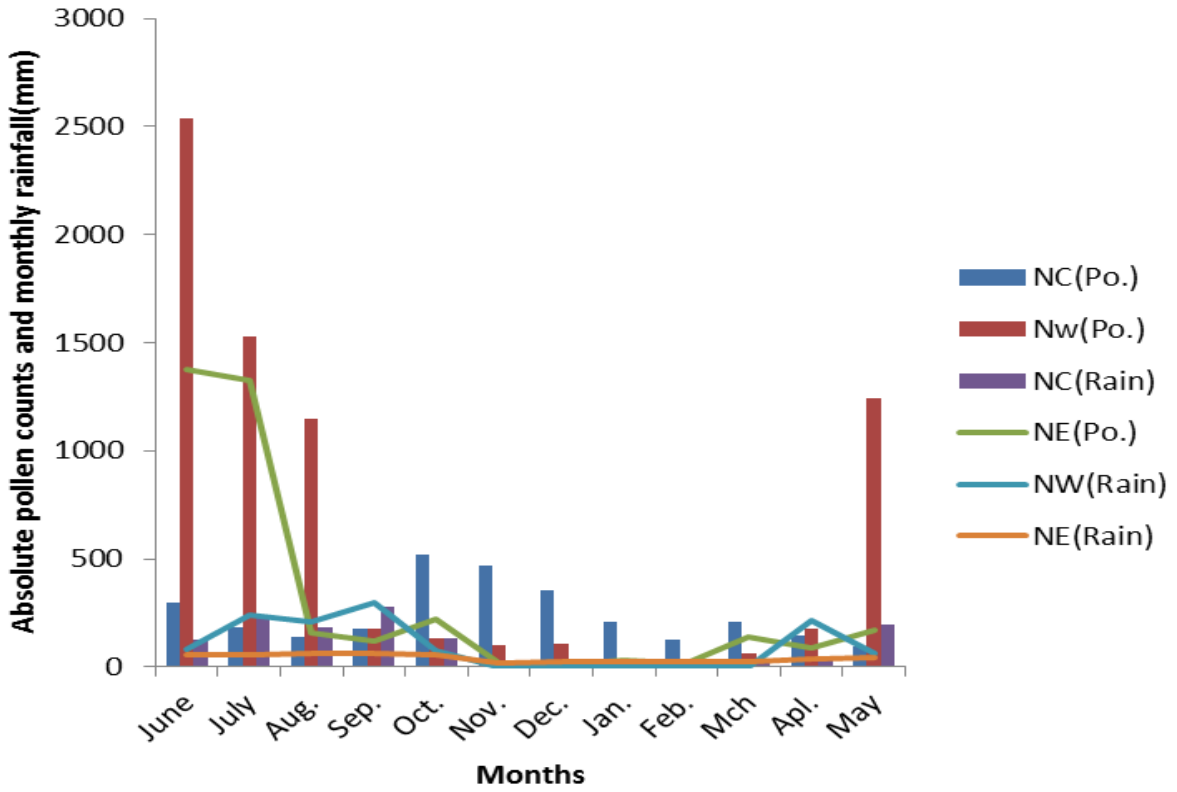


Fig 4.7: Relationship between atmospheric pollen and monthly rainfall (mm) in North Central, North West and North East Nigeria

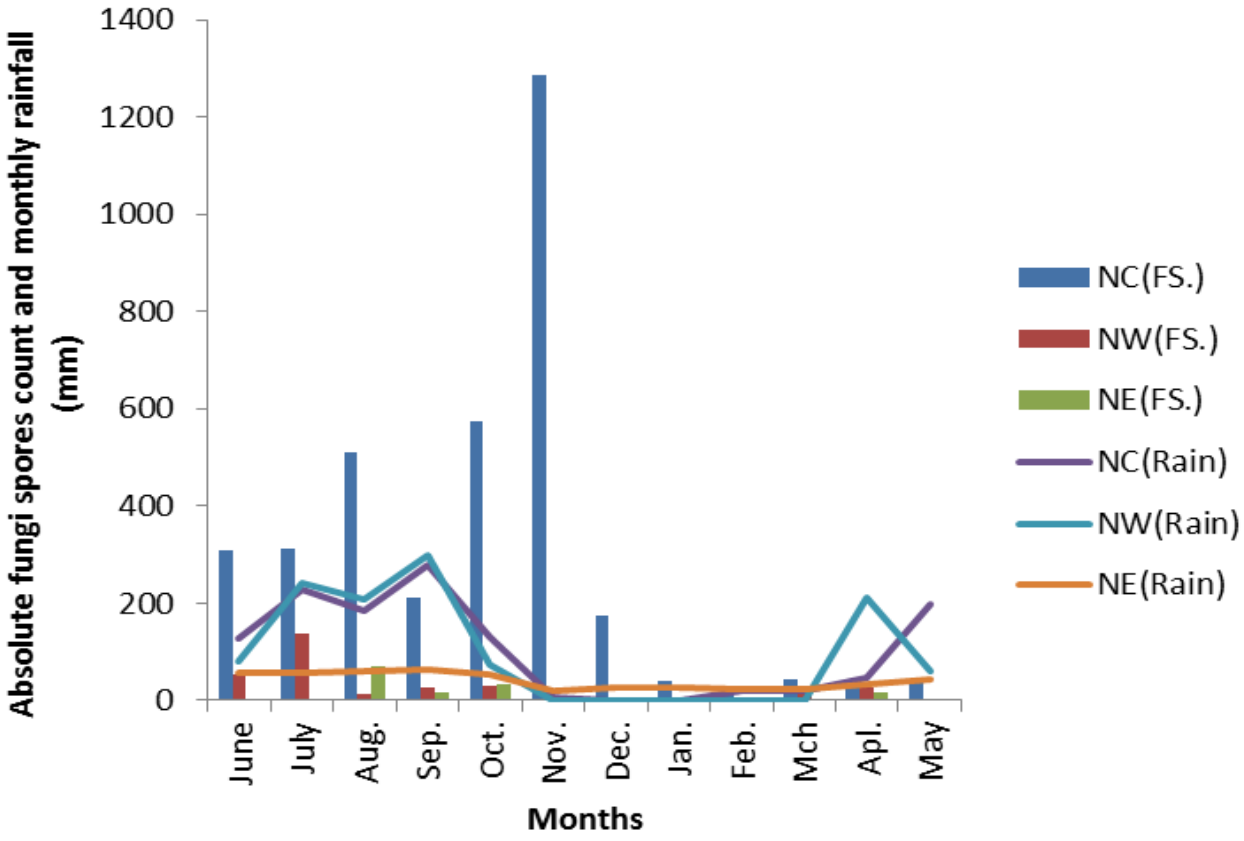


Fig 4.8: Relationship between atmospheric fungi spores and monthly rainfall (mm) in North Central, North West and North East Nigeria

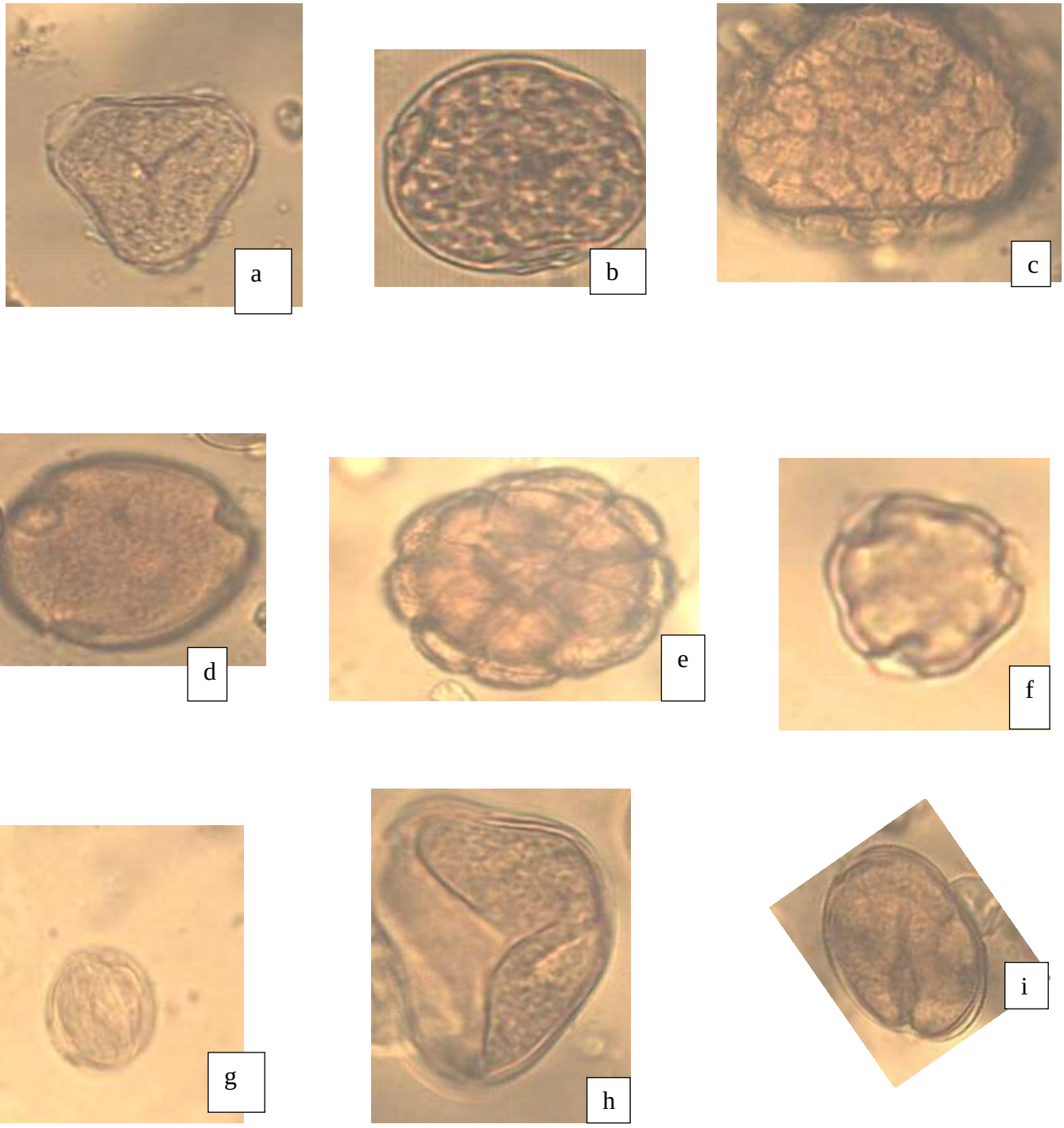


Plate 4. 1: Photomicrographs of some representative pollen

Mag X400

Plate 4.1: Photomicrographs of some representative pollen cont'd

Mag X 400

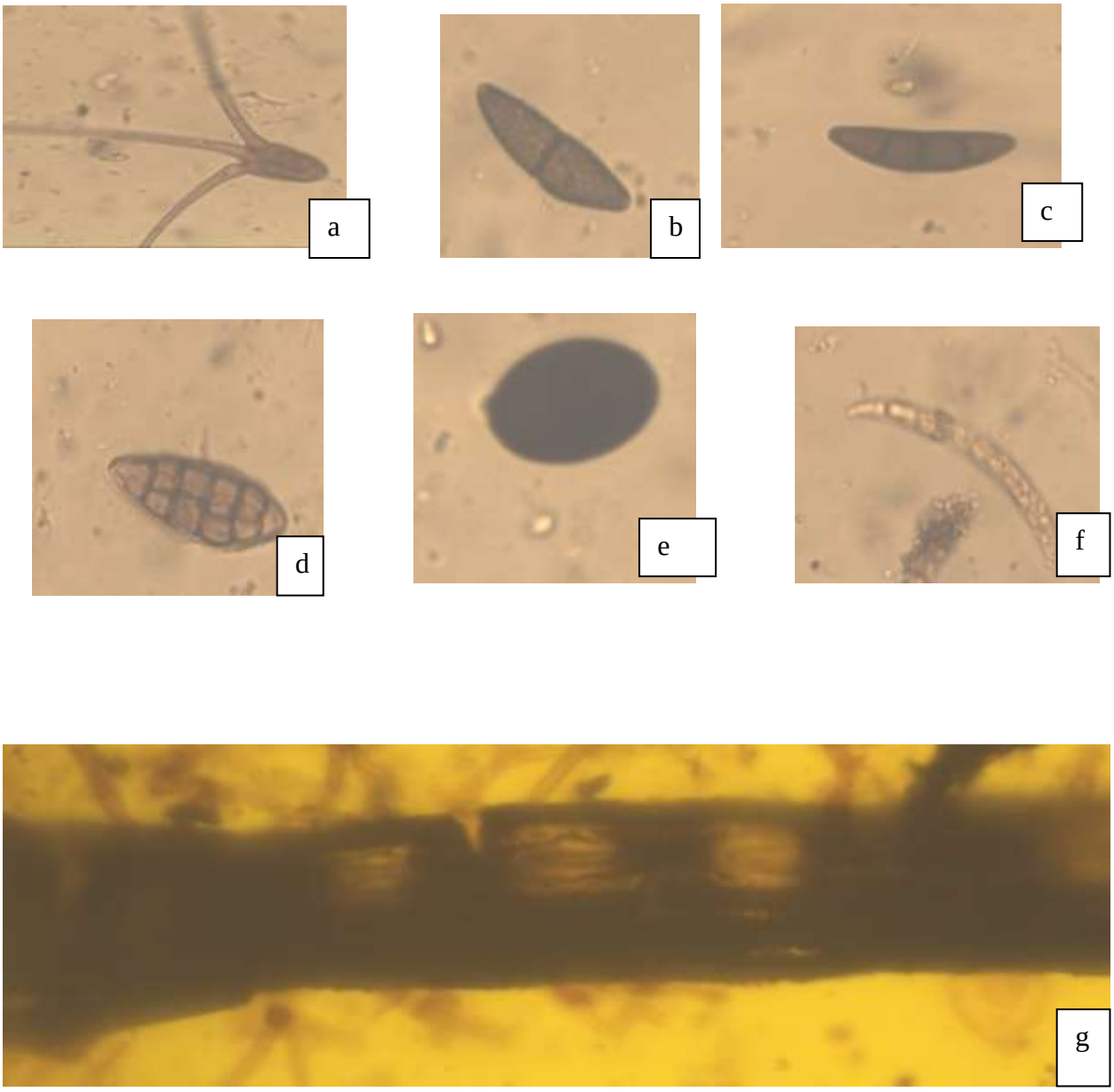


Plate 4.2: Photomicrographs of fungi spores and Charred Poaceae epidermis

Mag X400

4.9 Protein Contents of Some Selected Pollen and Fungi Spores

A total of six pollen and two fungal spores were studied for their protein content. (Table 4.16). *Mangifera indica* pollen yielded the highest value of protein (0.698 mg/ml) whereas *Oreodoxa oleracea* yielded the least (0.208 mg/ml). There was a relationship between the protein concentration and the elicited IgE titre values in some pollen. For example, *Mangifera indica* with the highest protein concentration (0.698 mg/ml) induced the highest IgE titre value (13.29 ng/ml) in mice after last sensitization, whereas *Oreodoxa oleracea* with the lowest protein (0.208 mg/ml) induced the lowest IgE titre value (3.38 ng/ml) (Table 4.17). This direct relationship was not found in all the pollen and spores protein. Among the spores, *Fusarium* sp. yielded a higher protein level than *Aspergillus niger*.

Table 4.16: Protein contents of some selected pollen and fungal spores

Pollen and fungi spores	Protein (µg/ml)
Aspergillus niger	236.49
Fusarium sp.	278.38
Mangifera indica	698.65
Mariscus ligularis	236.49
Oreodoxa oleracea	208.11
Panicum maximum	420.51
Sacciolepis africana	420.27
Terminalia catappa	420.27

Table 4.17: Relationship between the protein contents and IgE levels (ng/ml) elicited after the last sensitization

Pollen and fungi spores	IgE (ng/ml)	Protein (µg/ml)
Aspergillus niger	7.35	236.49
Fusarium sp.	9.60	278.38
Mangifera indica	13.29	698.65
Mariscus ligularis	13.10	236.49
Oreodoxa oleracea	3.38	413.51
Panicum maximum	7.19	420.27
Sacciolepis africana	13.13	278.37
Terminalia catappa	13.18	420.27

4.10 Clinical Features of Allergy

4. 10.1 Mice Skin

All Mice inoculated with *Oreodoxa oleracea* pollen protein exhibited a dermatophytic reaction, with physically features of swelling and rashes on the mice skin (Plate 4.3). The allergen also caused a higher mortality rate at the 3rd week and skewed the production of basophil (Plate 4.12)

4.10.2 Trachea of Mice

The negative control (Plate 4.4a) was not inoculated and the positive control (Plate 4.4b) received phosphate buffered saline, both controls showed a normal trachea. Mice inoculated with *Mariscus ligularis* pollen protein, showed over pseudostratification of epithelial layer. Mice sensitized with *Sacciolepis africana* pollen protein, showed proliferation of sub epithelial mucous gland. Mice which received *Aspergillus niger* spore protein, exhibited hypertrophy of the mucous gland (Plate 4.4).

4.10.3 Lung of Mice

Compared to positive and negative control, mice inoculated with *Oreodoxa oleracea* pollen protein, *Terminalia catappa*, *Fusarium* sp. protein showed inflammation within the lung parenchyma (Plate 4.4f, g, h, j, & k).

4.10.4 Bronchiole

Compared to positive and negative control, mice inoculated with *Aspergillus niger* showed inflammation around terminal bronchiole unlike in control and other mice which received other pollen and spore proteins (Plate 4.4i).

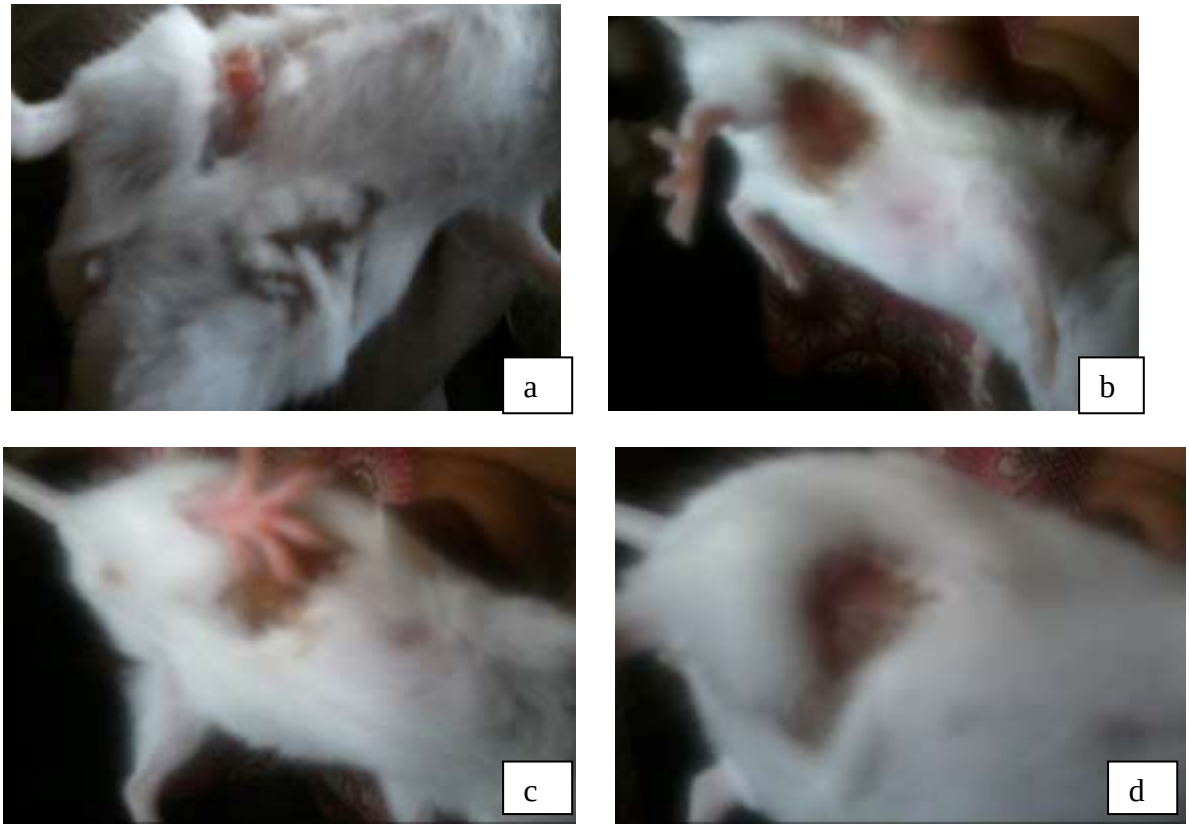


Plate 4.3 a, b, c & d: Physical manifestation of allergy provoked by *Oreodoxa oleracea* pollen in Mice

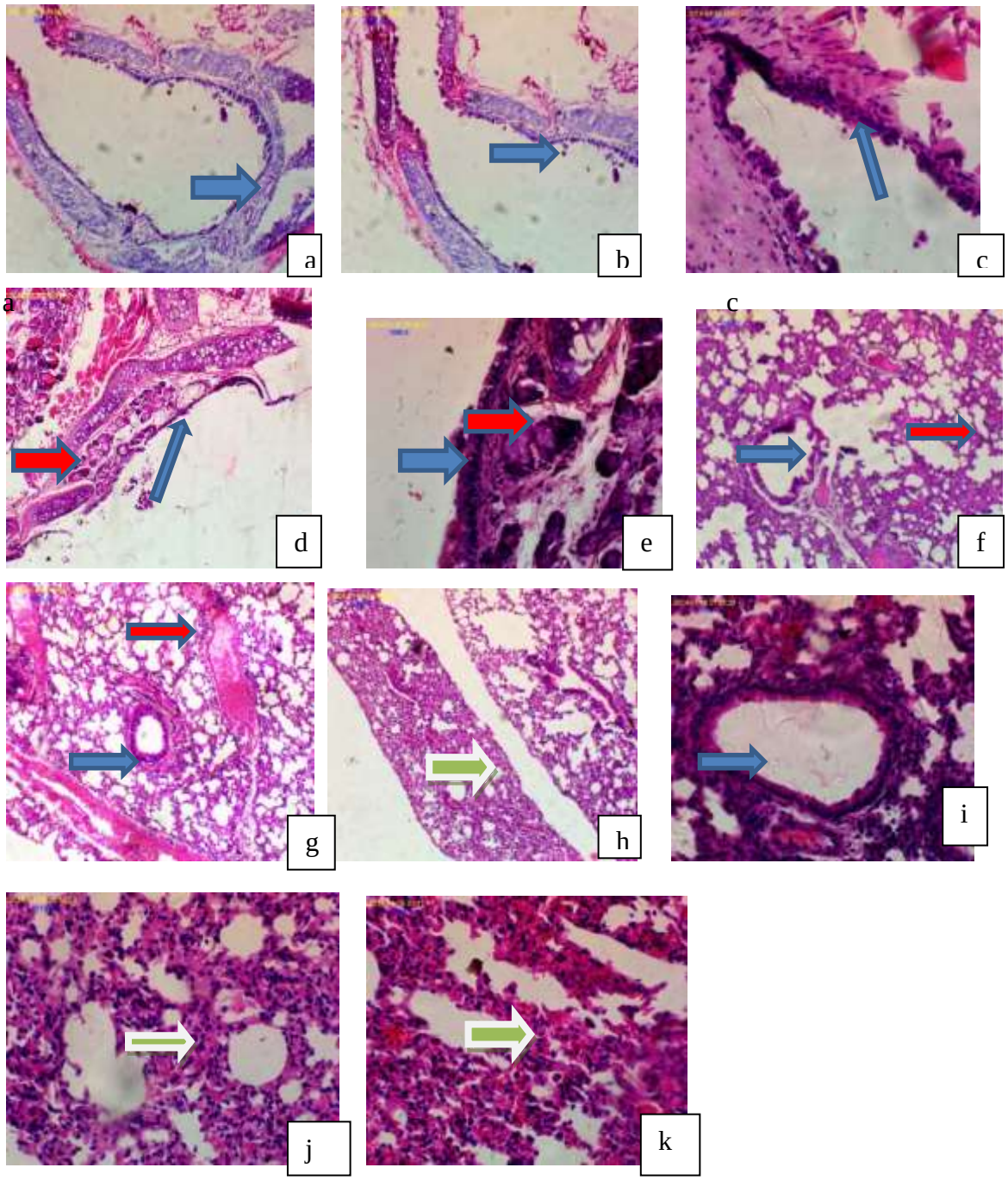


Plate 4.4: Photomicrographs of respiratory organs of mice
All except (i) -Mag X1000

(i)- Mag X2000

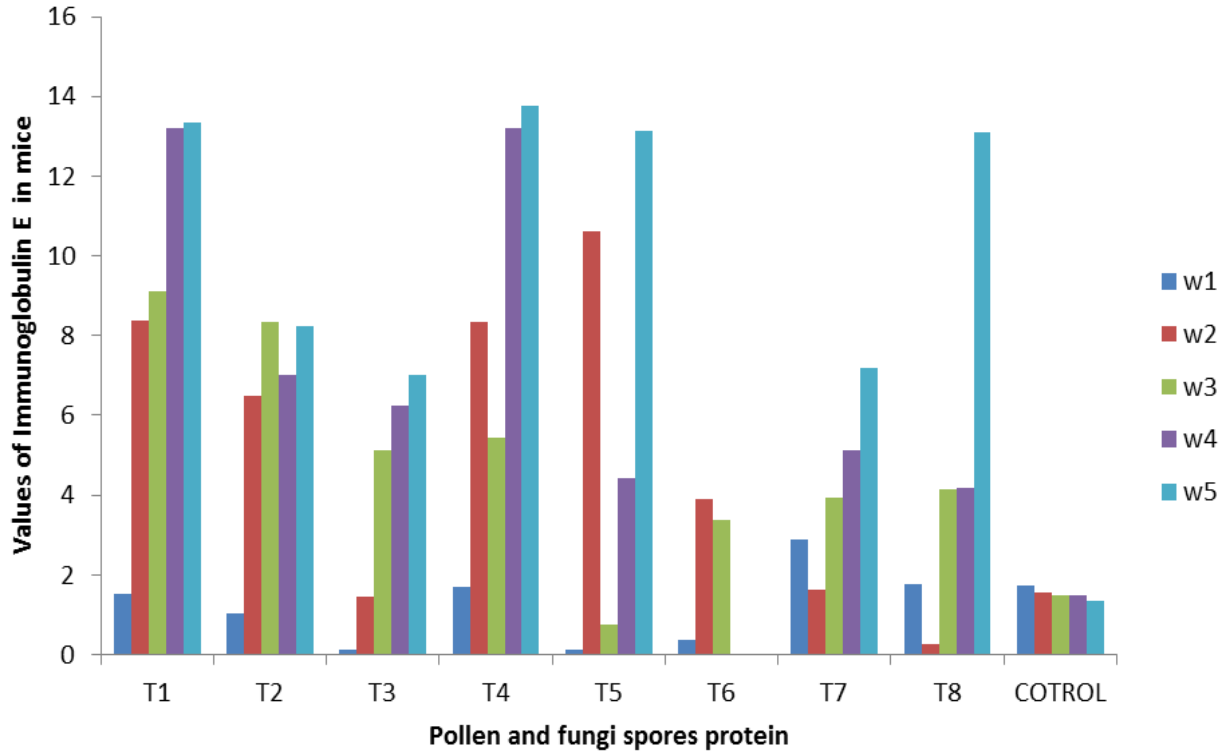
4.11 Evaluation of Immunoglobulin E (IgE) in Mice Sera

Immunoglobulin E antibodies were found present in all animals before the commencement of the experiment and ranged between (0.125 ng/ml - 2.87 ng/ml). At week two, the animals presented a higher IgE levels than the previous week one (w1) before inoculation, the rate of change however was not the same for all pollen and fungi spores protein over the weeks. *Terminalia catappa* protein produced a seven fold change in IgE than initially presented before inoculation. The elicitation of a high IgE by *Terminalia catappa* was closely related to *Mangifera indica,* which also elicited a seven fold change than was initially presented at week one (Fig.4.9). The induction of IgE values was found to be progressive in Mice that were inoculated with *Terminalia catappa, Aspergillus* sp., *Panicum maximum* and *Mariscus ligularis.* Mice that received the protein of *Fusarium* sp., *Mangifera indica, Sacciolepis africana and Oreodoxa oleracea* had fluctuated IgE throughout the week. *Terminalia catappa, Magnifera indica, Sacciolepis africana* and *Mariscus ligularis* induced a higher IgE titre values; 13.34 ng/ml, 13.78 ng/ml, 13.13 ng/ml and 13.10 ng/ml respectively after the last sensitization. Mice that received *Magnifera indica* protein had a decrease in IgE value from week 2 (8.34 ng/ml) to week 3 (5.43 ng/ml), which built up to 13.19 ng/ml at 4^{th} week and 13.78 ng/ml at 5^{th} week.

Sacciolepis africana, elicited the highest level of IgE at first sensitization (ten fold change), which decreased drastically in weeks w_2 (0.750 ng/ml), had a little increase in w_3 (4.43 ng/ml) and built up again at w_4 (13.13 ng/ml). Mice sensitized with *Oreodoxa oleracea* pollen protein induced a three fold change in IgE levels from w_1 (0.351 ng/ml) to w_2 (3.91 ng/ml) and was reduced at w_3 (3.38 ng/ml). Mice injected with *Panicum maximum* protein had a reduction in IgE level at w2 (1.625 ng/ml) from the initial value at w_1 (2.87 ng/ml), from w2 there was a built up of the IgE level which then increased progressively. *Mariscus ligularis* protein induced a

reduction of IgE from w_1 (1.75) to w2 (0.25), the level was however maintained at w_3 (4.13) and w_4 (4.19) and built up at the 5^{th} week (13.10). Conversely the IgE of the control Mice which received phosphate buffered saline decreased over the weeks, w_1 (2.87 ng/ml), w_2 (2.50 ng/ml), w_3 (1.80 ng/ml), w_4 (1.63 ng/ml) and w_5 (0.81 ng/ml).

The IgE induced by *Terminalia catappa* pollen protein in mice was not significantly from different from that induced by *Mangifera indica* and significantly different from other pollen and fungi spores protein and control (Fig.4.8). IgE elicited by *Fusarium* sp protein in mice differed significantly from those of *Terminalia catappa*, *Mangifera indica*, *Oreodoxa oleracea* and control (Fig. 4.10).



F.g 4.9: Immunoglobulin E elicited by protein of pollen and fungal spores in mice

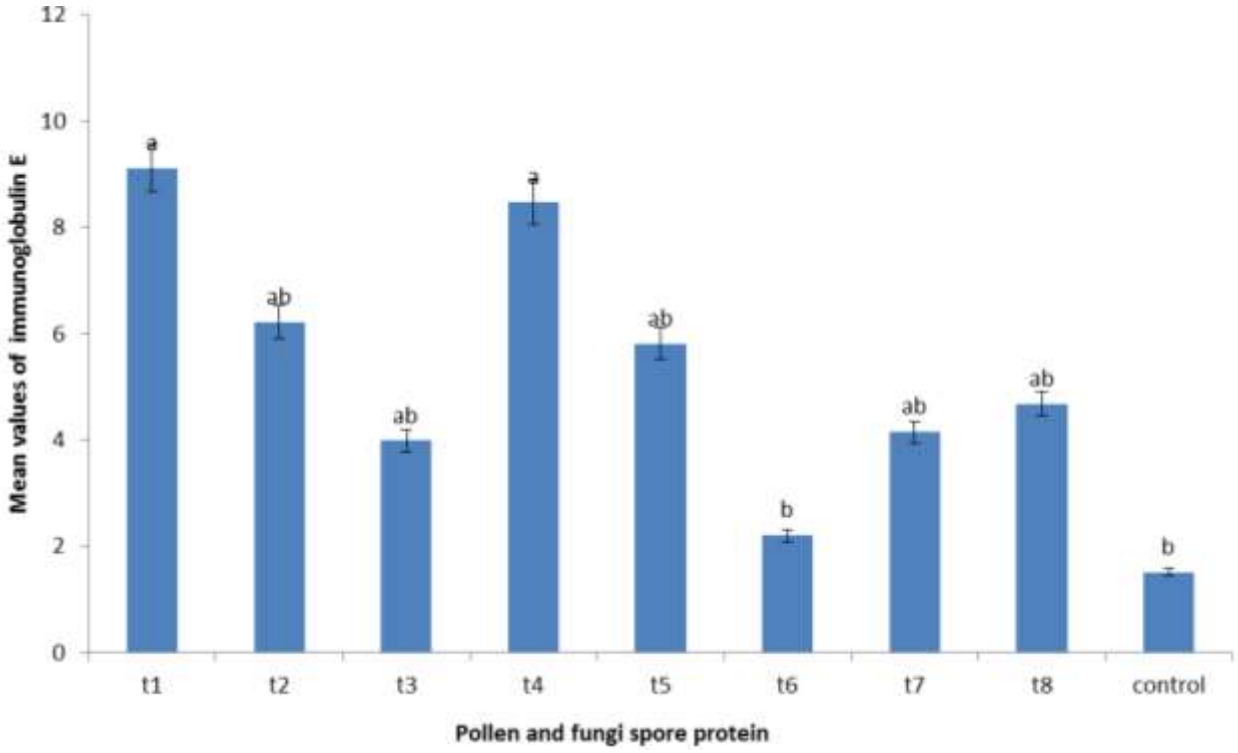


Fig. 4.10: Mean values of Immunoglobulin E elicited by protein of pollen and fungal spores in mice

Mean bars with different alphabets (a, b and ab) are significantly different (P< 0.05).

4.12 Immune Cells

The most predominant immune cells predominant in sensitized mice were lymphocyte, basophil and eosinophil (plate 4.5). Lymphocyte proliferated over the weeks as mice were sensitized, this could be depicted by the number of fields that gave 100 % immune cell. In control mice, more than fifty (50) fields gave 100 % immune cells, which was predominated by lymphocyte. In mice sensitized with pollen and fungal spores protein, the number of fields decreased as mice were sensitized over the weeks. Less than twenty (20) fields gave 100 % immune cell after the last sensitization which depicted the infiltration of lymphocyte in sensitized mice. The lymphocyte elicited by *Terminalia catappa* pollen protein in mice differed from those elicited by *Fusarium* sp., *Aspergillus niger*, *Mangifera indica*, *Oreodoxa oleracea*, *Mariscus ligularis* and control (Fig.4. 11). Lymphocyte induced by *Fusarium* sp. spore protein differed from those induced by *Terminalia catappa*, *Sacciolepis africana, Oreodoxa oleracea* and *Panicum maximum*. Lymphocyte induced by *Oreodoxa oleracea* pollen protein differed from all pollen and fungal spores protein and control.

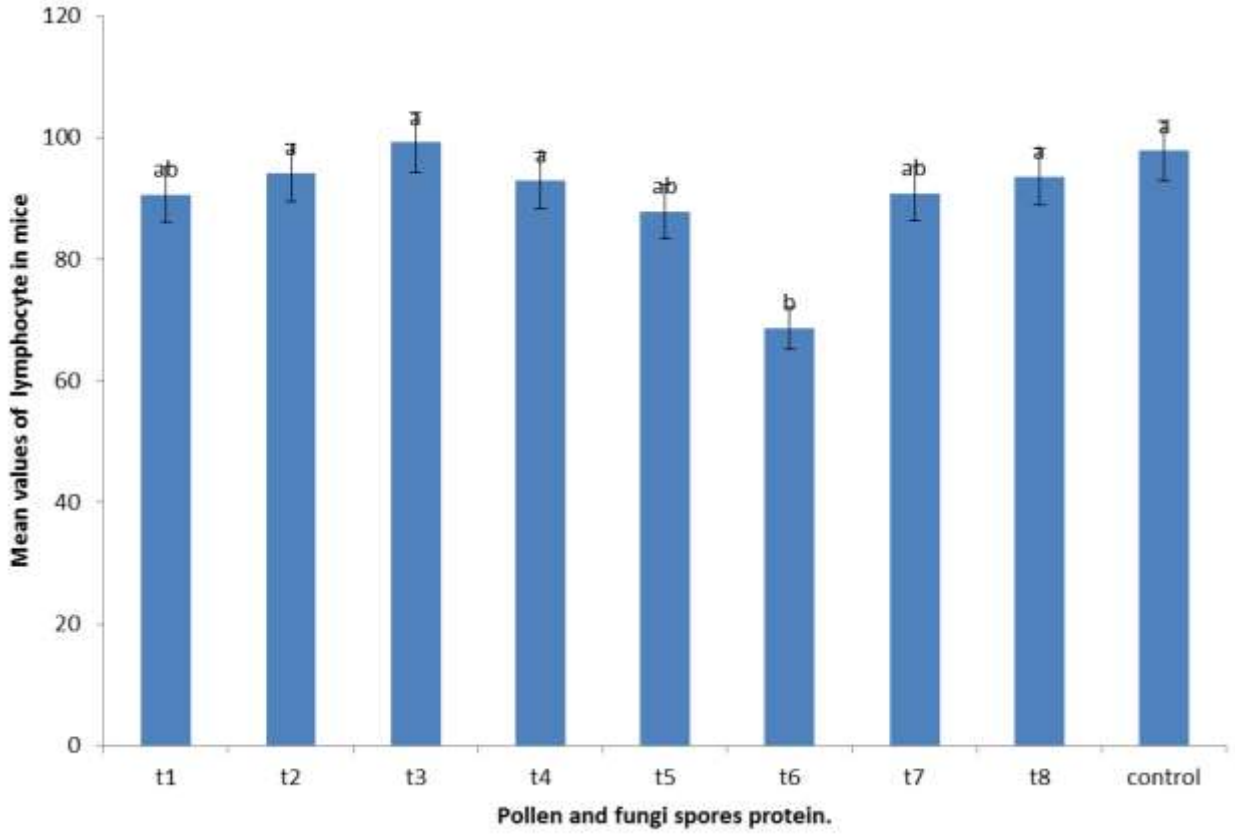


Fig. 4.11: Mean values of mice lymphocyte elicited by protein of pollen and fungal spores

Mean bars with different alphabet (a, b and ab) are significantly different ($P < 0.05$).

The pollen protein of *Oreodoxa oleracea* and *Sacciolepis africana* skewed basophil production than other pollen and spores protein (Fig. 4.12). *Mariscus ligularis, Panicum maximum, Magnifera indica, Terminalia catappa* also induced basophil production. *Terminalia catappa* and *Fusarium* sp elicited the highest values of eosinophil in mice, others include *Aspergillus niger, Mariscus ligularis, Panicum maximum* and control in decreasing order. Statistical analysis shows that the eosinophil induced by *Terminalia catappa* differed from those of *Mangifera indica, Sacciolepis africana* and *Oreodoxa oleracea* whereas the eosinophil induced by *Fusarium* sp. differed from those elicited by *Aspergillus niger, Mangifera indica, Sacciolepis africana, Oreodoxa oleracea, Panicum maximum, Mariscus ligularis* and control (Fig. 4.13).

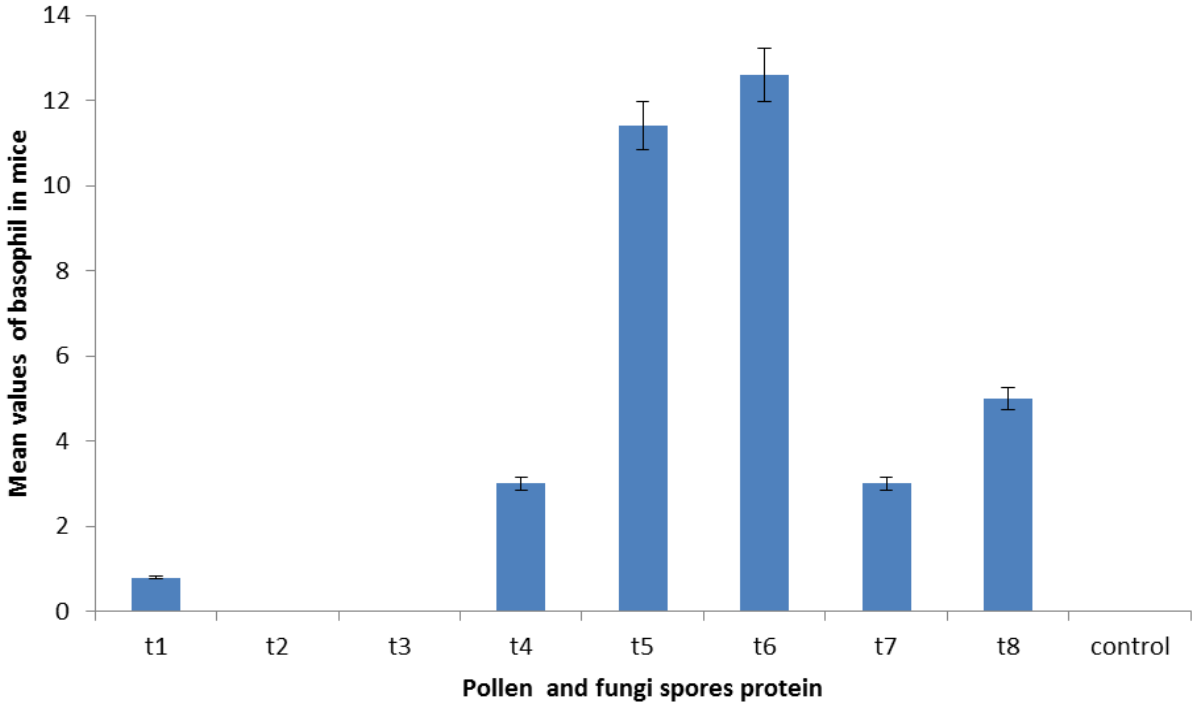


Fig. 4.12: Mean values of mice basophil elicited by protein of pollen and fungal spores

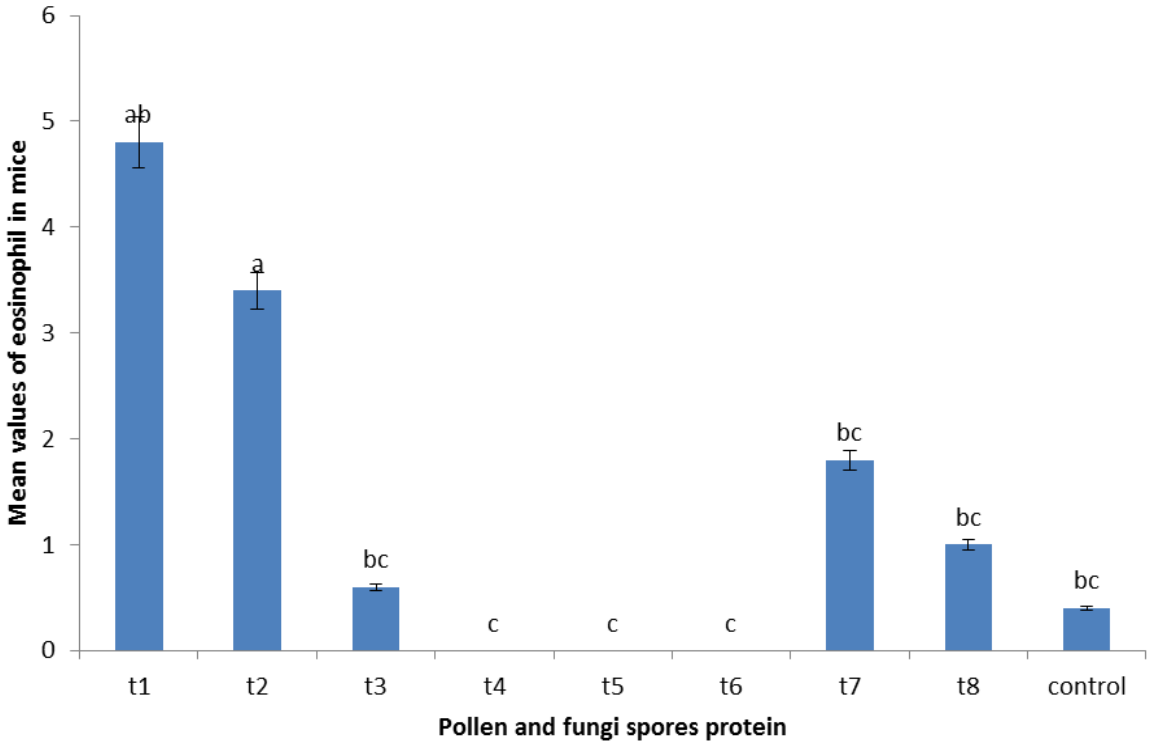


Fig. 4.13: Mean values of mice eosinophil elicited by protein of pollen and fungal spores

Mean bars with different alphabets (a, ab, bc and c) are significantly different (P< 0.05).

Monocyte recorded was higher proportion in mice that received *Mangifera indica* and *Panicum maximum* pollen protein than in mice which received other pollen and fungi spores protein and also in that control mice. There was no significant difference between the mean values of monocyte elicited by pollen and spores protein (Fig. 4.14). Neutrophil was present only in mice which received *Terminalia catappa* and *Mariscus ligularis* (Fig. 4.15).

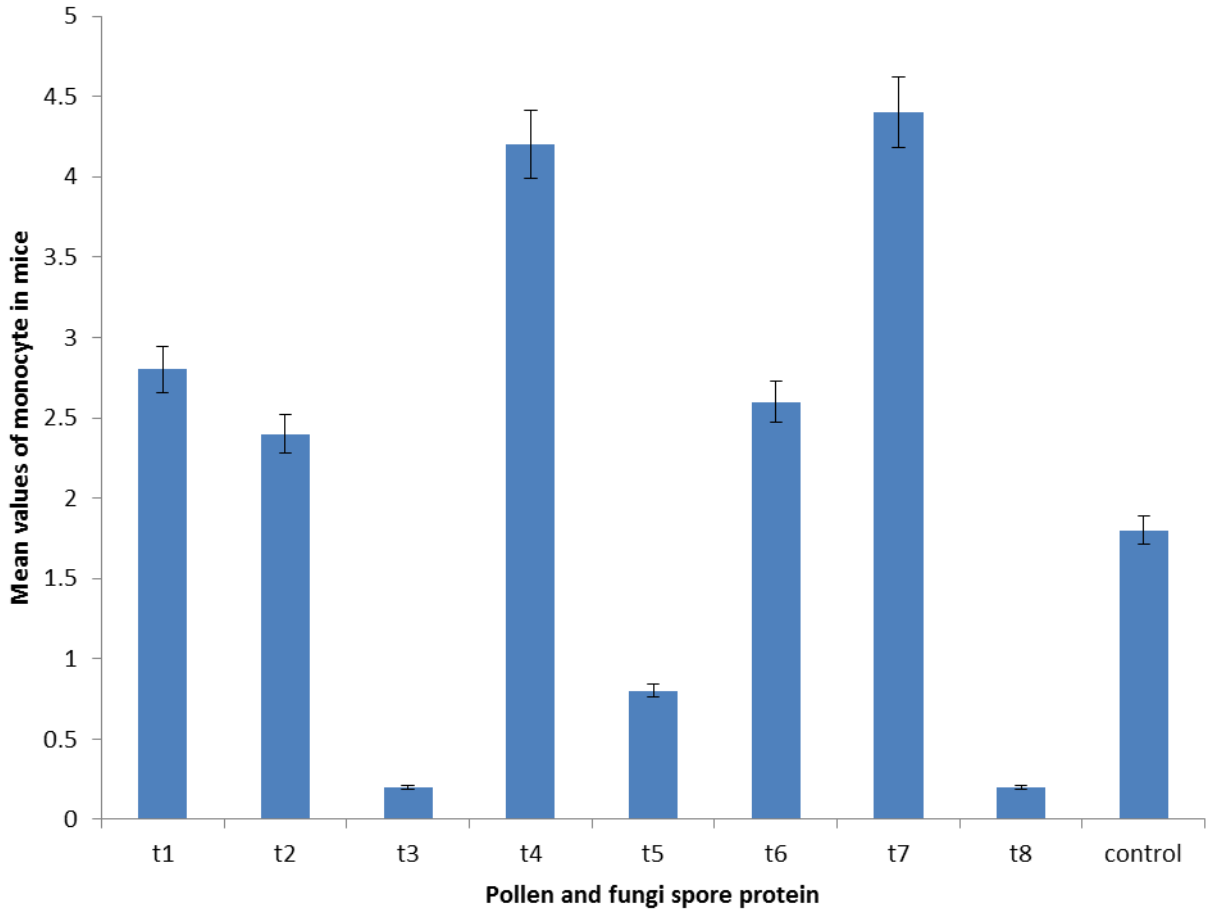


Fig. 4. 14: Mean values of mice monocyte elicited by protein of pollen and fungal spores

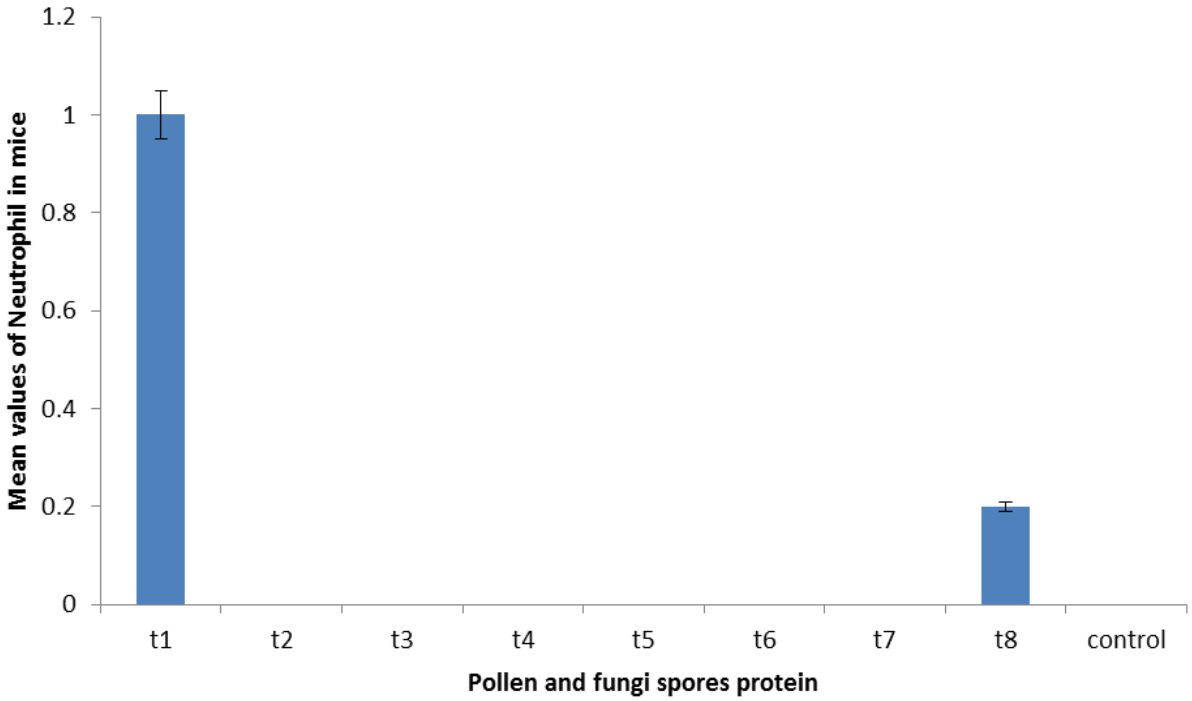


Fig. 4.15: Mean values of mice neutrophil elicited by protein of pollen and fungi spores

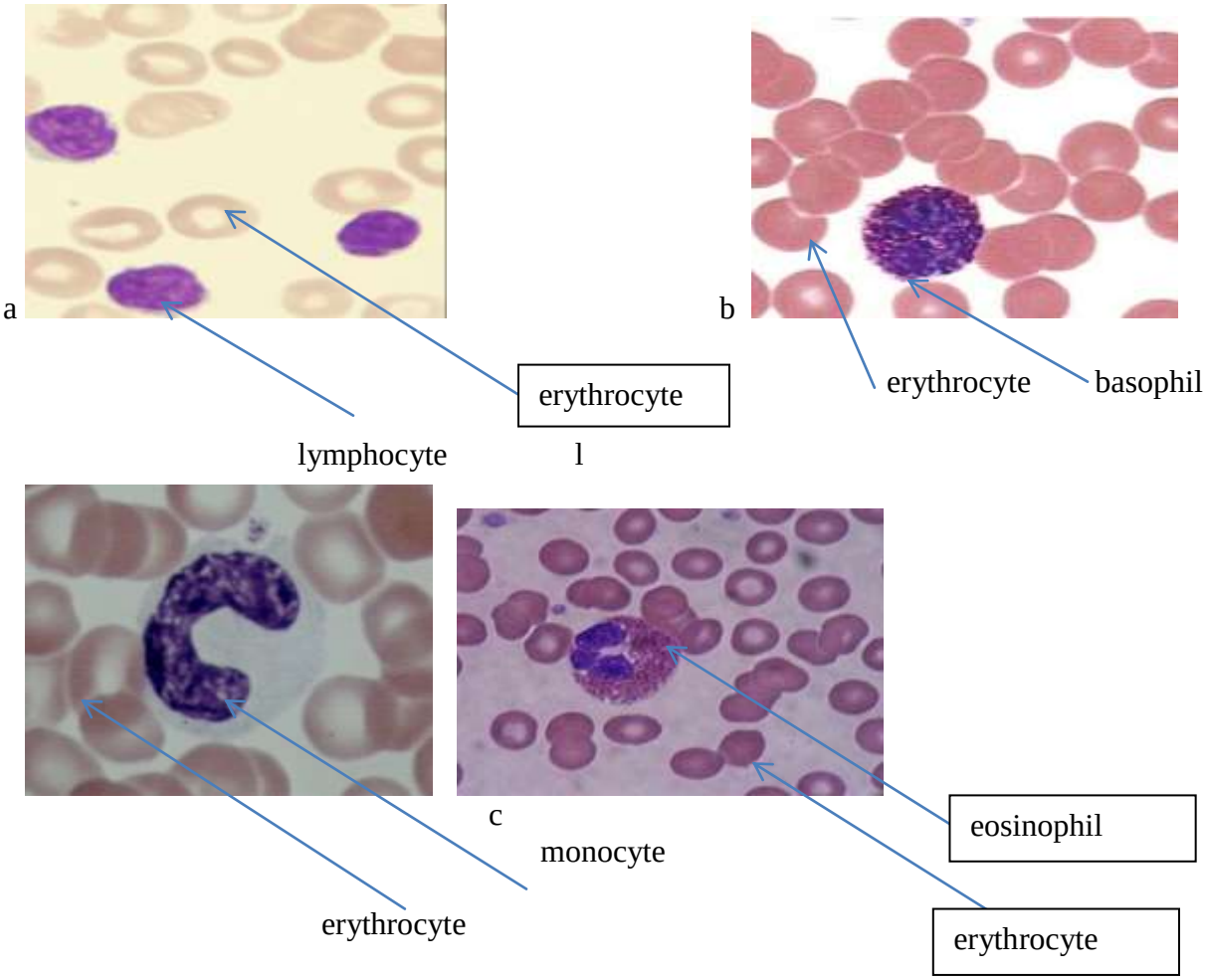


Plate 4.5: Immune cell types predominant in allergic mice (a). Lymphocyte (b) Basophil (c)monocyte (d) Eosinophil Mgx1000

CHAPTER FIVE

5.1 Discussion

5.1.1 Pollen and Fungal Spores Distribution

This study provided detailed information on the relative abundance of pollen and fungal spores present in the aeroflora of Nigeria. The pollen reflected the vegetation types in the studied areas and was found to be richer and more diverse in the Southern and North Central Nigeria, than in North West and North East Nigeria.

In South East (Enugu) Nigeria, the monthly pollen record showed three distinct periods; rainy season (May-August), late rainy/early dry season (Sep.- Dec.) and dry season (Jan.-April). Pollen counts were higher during the late rainy/dry season and this period was designated the risk period for pollen hypersensitive individual who frequently visit or inhabit the area. The period was denoted as Poaceae – *Elaeis guineensis* period. The most dominant fungal spores include those of *Fusarium* sp., *Alternaria* sp., *Spadicoides* sp., *Bastrodesmium* sp., and *Nigrospora* sp. In South South, pollen recorded showed a marked division between a rainy and dry season. Pollen increased progressively as the dry season approached. A lot of Pteridiophyte spores were recorded coupled with abundance of fungi spores morphotypes indicating a more humid environment than the South West and South East study areas. Risk period was October- March and was denoted as *Rhizophora* spp.- *Alchornea cordiforlia* period. The pollen which dominated include those of *Rhizophora* sp., *Alchornea cordiforlia*, *Acrostichum aureum*, *Elaeis guineensis* and Poaceae. The most preponderant fungal spores were *Nigrospora* sp., *Pithiomyces* sp., *Spadicoides* sp. and *Cladosporium* sp.. In South West, a short dry season which occurred between the two rainfall maxima and an increase in wind speed favoured high influx of *Jatropha curcas* pollen. *Nigrospora* sp., *Spadicoides* sp. and *Torulla* sp.

Pollen were more predominant from August –November in North-Central Nigeria. Qualitative abundance of pollen was noted more towards the onset of dry season in North Central Nigeria and was denoted as Poaceae-*Pentaclethra macrophylla* period. Most preponderant pollen include those of *Elaeis guineensis*, Poaceae, *Cassia* sp., *Alchornea cordifolia* and *Pentaclethra macrophylla*. Fungal spores most dorminant were *Tetraploa* sp., *Pithiomyces* sp., *Puccinia* sp. and *Erysiphe graminis.* North West and North East were dominated by Poaceae pollen during the rainy season. In dry season, the preponderance pollen in North-West were those of *Cyperus* sp., Amarathaceae/Chenopodiaceae, *Phyllanthus* sp., *Khaya senegalensis* and *Trichilia roka*. *Microsporium* sp., *Nigrospora* sp. and *Cecospora* sp. were the most dominant fungal spores. In North- East, *Lannea acida*, *Parkia bicolor* and *Vitex doniana* were the most dominant pollen whereas *Nigrospora* sp. and *Fusarium* sp. were the most dominant fungal spores.

Flowering occurred throughout the year but with variations from one month to the other. Grass pollen dominated the atmosphere of Northern Nigeria than Southern Nigeria, several species of grasses flowered successfully throughout the year and had their maximum flowering period between the month of June-October. Pollen allergies at this period (June- October) could be attributed to upsurge of Poaceae pollen in the atmosphere of Northern Nigeria, because of their higher antigenic load. The grasses contributed a high pollen load in North West, North East and North Central, constituting a higher percentage of the total pollen except in North Central Nigeria. In Southern Nigeria, Poaceae pollen grains were present in the atmosphere almost throughout the year. They were more abundant at rainy season; June and July and late rainy/hamattan period September to December. Njokuocha (2006), also reported the persistent dominance of grass pollen in the atmosphere of South East Nigeria. Obtulowiz *et al*. (1991) also reported that the grass pollen were the most important allergenic pollen in Poland. Poaceae

was the most abundant nonarboreal pollen type in the atmosphere of Büyükorhan, and this family represented 7.00 % of the annual pollen index (Tosunoglu *et al*., 2014). Poaceae pollen grains were initially recorded in the atmosphere of Büyükorhan in the 3rd week of January, reached maximum levels between the 20th and 31st weeks, and lasted until the 3rd week of June (23rd week) (Tosunoglu *et al*., 2014). Poaceae pollen grains were reported as the most common among non-arboreal pollen in studies conducted in İzmir (7.7%–6%), (Guvensen and Ozturk, 2003), Sakarya (18.95%) (Bicakci, 2006), and Didim (6.33%) (Bilisik *et al*., 2008). Grass pollen is quantitatively one of the most important aeroallergen vectors worldwide. It is a major cause of allergic reactions including conjunctivitis, rhinitis, and other upper and lower respiratory tracts problems occurring during the flowering season of different grasses.

Poaceae pollen are among the most dominant anemophilous pollen in Nigeria especially in Northern Nigeria. The plants play a crucial role in allergy because of their copious, bulk and disproportionate amount of pollen they disperse in the atmosphere . Poaceae pollen is the major cause of pollinosis in many parts of the world. Although its frequency differs regionally, grass-induced pollinosis is the most common pollen allergy also in Europe (D'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. Grass pollen grains are also quantitatively the most important aeroallergen vectors worldwide. 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).

In South East and Northern Nigeria study locations, a decrease of Poaceae correlated with an increase in charred Poaceae cuticles, which resulted from burnt epidermis of grasses. This actually shows that after grasses were burnt down by annual bush fires, their pollen decreased in the atmosphere and their burnt epidermis increased. Decrease in Poaceae pollen in Northern Nigeria from November to May, could be attributed to the normal nomadic life style of the people and frequent fire event (evident from charred Poaceae cuticles), which checked the growth of grasses, resulting in lower grass pollen content in the atmosphere. Dominant presence of charred Poaceae cuticles, was very peculiar to Northern Nigeria. There were lower records of charred Poaceae cuticles in Southern Nigeria than in Northern Nigeria. The absence of charred Poaceae cuticle in aeroflora of Lagos (South West) perhaps depicted absence of bush fires. Since Lagos is a built up urban area.

During the last 40 years, the frequency of symptoms of allergic diseases has increased dramatically, especially in children and people living in urban areas (Chakra *et al*., 2009). Several factors have contributed to this increase, among which are airborne pollutants from gaseous and particulate emissions. Airborne pollen grains can release hundreds of small particles called pollen cytoplasmic granules (PCGs). These may be present in atmospheric samples taken during the pollen season, and some studies show a 50-fold increase in their atmospheric concentration on days after rainfall. In the same way, airborne pollutants may modify pollen grains structurally, thereby increasing the release of PCGs in the atmosphere (Chakra *et al*., 2009).

The herbaceous pollen encountered in the atmosphere of the studied locations were majorly those of Chenopodiaceae/Amarathaceae species, Cyperaceae, Asteraceae (Tubuliflorae &

Liguliflorae complex), *Crotalaria* sp. etc. In Southern Nigeria, trees/shrubs pollen dominated, whereas grass pollen dominated the Northern Nigeria.

Based on pollen diversity assessment of floral component, the order of pollen predominance of the six geopolitical zones could also be a reflection of their biodiversity, however the assessment could have been influenced by, intensity of flowering, pollen productivity, limitations of sampler, wind direction and strength, geography, variations in microclimatic conditions and the movement of the Inter Tropical Convergent Zone (ITCZ) in 2011.

Over time, researchers have theorized that anemophilous pollen are more implicated in allergy, because of their higher antigenic load than the entomophilous pollen (Shahali *et al*.,2009) . Wind pollinated plants usually have inconspicuous flowers but can produce a vast amount of pollen. Their small size, smooth, dry and non-sticky nature of anemophilous pollen facilitates their easy dispersal in air. Anemophilous pollen has many adaptations to free dispersal:It is produced in large quantities, is light, dry, smooth or with reduced ornamentation (Faegri and Pijl, 1979), there is little pollenkitt on its surface (Hesse, 1981). Entomophilous plants on the other hand produce much less pollen. Pollen grains have rich ornaments (Faegri and Pijl, 1979) and they are covered by pollenkitt or viscin threads. Owing to this they become glued together and attach to an insect visiting the flower (Hesse, 1981). These features cause the pollen of entomophilous plants to occur usually in low concentrations or sporadically. People most prone to enthomophilous pollen allergens are usually the Mowers, Gardeners and Farmers who continuously come in closer contact with them. Anemophilous pollen dominate the pollen rain of any area, representing major seasonal carriers of allergens (Shahali *et al*.,2009). It is usually more implicated in public ill-health, because of their great aerodynamic properties which make

them to reach out to greater number of the population. Anemophilous pollen dominated the atmosphere of Northern Nigeria, whereas enthomophilous pollen dominated the Southern Nigeria. This finding agrees with the view of Dyakowska (1959), who found out that bulk airborne pollen comes from anemophilous plants. Kasprzyk (2004) showed that the dominance of enthomophilous pollen in the atmosphere of Rzeszow and its environs could be attributed to the wide variety of ornamental (herbs and trees) plants growing around the sampling site.

Three categories of trees/shrubs were recognized in this present study based on flowering period (1). Those with short, intense flowering period example; *Maytenus* sp, *Anacardium occidentale, Cussonia bateri, Eugenia nodiflora* etc. (2). Types without any definite flowering season, they were present almost throughout the year e.g; Poaceae, *Cassia spp,* Amarathaceae /Chenopodiaceae, *Olax subscopioides, Elaeis guineensis* etc. (3). Those with double flowering pattern of short duration, example; *Casuarina equisetifolia, Syzygium guineense* etc.

Podocarpus sp. pollen, whose plant is not indigenous in Nigeria was recorded in North West Nigeria at the month of November. Adeonipekun (2014) also recorded *Podocarpus* in South-West Nigeria, but could not conclude what genus the bisaccate he found belong to.

Northern Nigeria had one defined season of more allergenic exposure dominated by Poaceae pollen during the rainy season hence, hypersensitive individuals could be more monosensitized at that period. Southern Nigeria had two seasons; the season of higher risk period for pollen which corresponded to the late rainy/ dry season and the higher risk period of fungi exposure, which also corresponded to rainy season.

Allergenic reactions due to fungal spores were deduced to be more predominant during rainy and late rainy season (June-December) than in dry season January to May in the atmosphere of Nigeria. The months of the rainy season was proliferated with spores of fungi. A high

predominance of fungal spores was recorded in all Southern and North Central Nigeria. Some of the fungi spores recorded in this work have been confirmed allergenic in other countries for example; *Alternaria* sp., *Fusarium* sp., *Cladosporium* sp. *Curvularia* sp., *Pithiomyces* sp. etc. (Horner *et al*., 2000). They pose a great threat to hypersensitive individuals especially the immuno-compromise patients.

Erysiphe graminis, a pathogenic fungi on members of Poaceae family (both cultivated and wild species) were found sporadic in August, dominated the atmosphere in November and contributed to the bulk concentration of total atmospheric fungi spores 1200 (93.38%). Their dominance correlated with the decline in Poaceae pollen. The pathogenic spores recorded included; *Helminthosporium* sp., which is implicated in leaf spot of rice. The presence of *Alternaria* spores in the atmosphere and their impact on agriculture and human health have been studied by Escuredo *et al*. (2010). *Alternaria* spp is a potential source of allergic disorders in human. *Alternaria solani* produce an early blight in potato crops. The pathogen can infect all aerial parts of solanaceous crops including tomato, potato, eggplant, and pepper, as well as potato tubers (Escuredo *et al*., 2010). Other pathogenic fungi spores encountered include those of; *Torulla* sp., *Tetraploa* sp., *Nigrospora* sp., *Spadicoides* sp., *Puccinia* sp., etc. Most spores sporulated during the rainy season. The spores of *Nigrospora* sp., *Puccinia* sp. and *Spadicoides* sp. occurred throughout the year but more predominant during the rainy season. The persistent occurrence of some dominant fungi spores could be an indicator of pathogen development in the area and knowledge of their presence could assist the farmers and agriculturists to protect their crops from diseases.

5.1.2 Pollen, Fungal Spores Load and Meteorological Factors

The record of lower atmospheric pollen in the Southern part of the country corresponded with the periods of higher monthly rainfall, higher humidity and lower temperature, whereas higher atmospheric pollen load correlated with higher temperature, lower rainfall and humidity. Thus atmospheric pollen grains were more dominant at more drier and windy months than humid months. Higher monthly rainfall reduced airborne pollen concentration and favoured sporulation of fungal spores. This findings are related to the views of Barnes *et al*. (2000), Teranishi *et al. (*2000), Riberio *et al*. (2003), Njokuocha (2006), who found that airborne pollen concentration significantly correlated with temperature and wind direction and negatively correlated with rainfall and number of rainy days. The result of the present study agrees with Adeniyi *et al*. (2014) who also found a postive correlation between pollen concentration and temperature and a negative correlation with relative humidity and rainfall.

In Northern Nigeria especially North West and North East, abundant number of Poaceae pollen was recorded in rainy season, whereas higher varied morphotypes of pollen was recorded during the drier period, but their quantity was less compare to Poaceae pollen which highly influenced atmospheric pollen content during the rainy season. North East and North West with lowest rainfall and humidity have lower fungal spores load, among the Northern study locations, North-Central had a higher record of fungal spores which positively correlated during the rainy season. Peternel *et al*. (2006) found that low pollen concentrations correlated with high levels of rainfall and humidity. Also, Green *et al.* (2004) found that the major factors affecting airborne pollen counts were maximum and minimum temperatures.

A relationship between the pollen concentration and daily meteorological elements is of great practical importance. Applying simple statistical analyses, several studies found significant positive correlations between daily Poaceae pollen concentration and daily maximum temperature (Valencia-Barrera *et al*., 2001; Green *et al*., 2004; Kasprzyk and Walanus, 2010), daily minimum temperature (Green *et al*., 2004), daily mean temperature (Puc and Puc, 2004; Peternel *et al*., 2006; Kasprzyk and Walanus, 2010), and daily global solar flux (Valencia-Barrera *et al*., 2001; Kasprzyk and Walanus, 2010), the relative humidity (Valencia- Barrera *et al*., 2001; Puc and Puc, 2004; Peternel *et al*., 2006; Kasprzyk and Walanus, 2010) and rainfall (Valencia-Barrera *et al*., 2001; Green *et al*., 2004; Puc and Puc, 2004; Peternel *et al*., 2006; Kasprzyk and Walanus, 2010). Subba *et al*. (1988) demonstrated that flowering season periods can differ by several months between genera and some Poaceae species have a circadian mechanism of pollen shedding, each species having their own temporal features of pollen release, with duration that may vary between 2 and 13 h. Minckley *et al*., (2012) showed that moisture anomalies accounted for 3 % to 24 % of the variation in arboreal pollen abundance for *Fagus*, *Pinus*, *Quercus* and *Tsuga*.

The atmospheric fungi spores had a direct relationship with rainfall in the present study. More fungi spores were recorded during the periods of higher rainfall. This finding agrees with those of Phanichyakarn *et al.* (1989), Agashe and Alfaldil (1989), who found higher number of fungal spores in the rainy season and lower number in dry season in the atmosphere of Bangkok and Bangalore respectively. In Northern Nigeria; North West and North East which received low rainfall and had a lower record of atmospheric fungal load. The amount of rainfall varied across the six geopolitical zones of Nigeria. This however created differences in the variations of

aeroallergens. Climate and vegetation differences also influenced the type, abundance and prevalence of airborne pollen and fungi spores in the studied locations.

In Southern Nigeria, which recorded a lot of rain, the effect on the pollen levels in the air was not the same with Northern Nigeria, which did not get much rain. This therefore created the differences between low pollen content in Southern and North Central Nigeria and higher pollen content in extreme North; North West and North Central Nigeria during the months of April, May, June and July.

The drier season favoured the release of pollen from the anther and influences the atmospheric pollen content positively. Wind, temperature, rainfall and sunshine play vital role in the amount of pollen dispersed in the atmosphere. Wind is a natural carrier of aeroallergens and plays a significant role in transport of aeroallergens (Shahali *et al*.,2009).

The actual start and severity of the season depends on several factors including temperature, rain, humidity and wind speed/direction. As a result of these variables, their atmospheric count vary from one season to the other resulting to a more allergenic exposure to pollen during the dry season especially during the harmattan period in Southern and North Central Nigeria, a more allergenic exposure to Poaceae pollen during the rainy season in North West and North East Nigeria and an allergenic exposure to fungal spores over the atmosphere of Nigeria during the rainy season.

5.1.3 Immunological Result

Result of the immunological aspect of this work showed that some entomophilous pollen could possess higher allergen protein content but because of their lower aerodynamic properties, they are found lower in the atmosphere than the anemophilous pollen.

Immunoglobulin type E (IgE) is one of the five classes of antibodies or an isotype of immunoglobulin, that has been found only in mammals. IgE plays a very essential role in type 1 hypersensitivity which manifest various allergic diseases such as asthma, allergic rhinitis etc (Niederberger *et al*., 2002; D'Amato *et al*., 2002). Analysis of IgE titre values indicated 0.125 ng/ml - 2.87 ng/ml in mice before sensitization. All pollen and spores allergen induced an alteration in immune system which resulted to a higher IgE titre values, especially at the last week of the experiment. The elevated value of IgE means that the protein of the pollen and spores were recognized by the antibodies as foreign bodies. A rising level of antibody to specific antigen indicated active infection and led to tissue inflammation. The result further confirmed that IgE plays important role in the development of allergy. The continuous change in IgE values of the animal as they were sensitized with allergen protein indicated that, on continuous exposure to allergenic triggers, the IgE will persistently increase. The result also shows that allergenic reaction presents itself with high IgE with or without physical features. Among the eight pollen and spores subjected to allergenicity investigation, only *Oreodoxa oleracea* presented a physical dermatophytic manifestation of allergy. Routine checks of the IgE level became imperative especially for the atopic individuals.

In human, IgE level in a normal non atopic individual is 0.3 µg/ml and about 10 times this value or 12 µg/m in atopic individuals (Sudha *et al*., 2010). IgE level has been discovered to gradually

increase throughout childhood, with a peak at 10-15 years of age, it decreases throughout adulthood (Shahali *et al*., 2009). IgE levels are influenced by genetic makeup, immune status and environmental factors (allergen exposure) (Pyle, 2013). IgE also plays a critical role in mediating atopic diseases, significant correlation between serum IgE concentration and disease have been demonstrated in allergic asthma and atopic dermatiatis (Pyle, 2013).

High IgE leads to the production of free oxygen radicals (FORs), which are very reactive molecules because of their unpaired electrons (Yariktas *et al*., 2004). They are however neutralized by enzymatic and nonenzymatic defense systems such as superoxide dismutase (SOD oxygen), catalase (CAT), glutathione peroxidase (GSH-Px), vitamin E, glutathione and vitamin C (Yariktas *et al*., 2004). When the balance between free oxygen radical production and the antioxidative defence mechanism is disturbed, the level of free oxygen radicals increases which leads to tissue damage, impairs the membrane permeability and fluidity, and results in functional and structural disorders and even cell death (Yariktas *et al*., 2004). Vitamin E localized in cell membrane plays an important role to break the chain reaction. For this reason vitamins C and E administration in infections can be useful for preventing the FORs damage (Yariktas *et al*., 2004).

White blood cell (leukocyte) is an integral part of human protection against antigens. The body makes different types of white blood cells that work continously to keep us healthy. There are five types of immune cell types ; basophil, eosinophil, neutrophil, lymphocyte and monocyte were used to assess the allergenic potential of pollen and spores in this study.

Lymphocyte was the most dominant immune cells encountered in the hematological tests. Lymphocyte cells proliferated over the weeks in all mice sensitized with allergen protein, unlike in control mice, this however was depicted by a decrease in the number of fields that presented 100% immune cells. The infiltration of lymphocyte was found to correlate with a high IgE elicitation.

Basophils, along with eosinophils and neutrophils, constitute a group of white blood cells known as granulocytes (Franco *et al*., 2013). Basophil was highly elicited by *Oreodoxa oleracea* and *Sacciolepis africana* followed by *Mariscus ligularis, Panicum maximum* and *Mangifera indica* in decreasing order of dominance. The high level of basophil elicited by *Oreodoxa oleracea* caused a dermatophytic reaction on the skin of mice, which physically featured as swelling and rashes, it also caused an inflammation within the lung parenchyma. *Sacciolepis africana* also induced inflammation within the lung parenchyma. Research has shown that when immunoglobulin E binds to specialized receptor molecules on basophils, they release stores of inflammatory chemicals, including histamine, serotonin, and leukotrienes (Franco *et al*., 2013). These chemicals have a number of effects, including constriction of the smooth muscles, which leads to breathing difficulty; dilation of blood vessels, causing skin flush and hives; and an increase in vascular permeability, resulting in swelling and a decrease in blood pressure. Basophils also incite immediate hypersensitivity reactions in association with platelets macrophages and neutrophils (Franco *et al*., 2013).

The pollen protein of *Terminalia catappa, Panicum maximum, Mariscus ligularis,* spores protein of *Fusarium* sp. and *Aspergillus niger* elicited higher eosinophil compared to other pollen and fungi spores protein and also control mice. For over 100 years, the eosinophil has

been associated with allergic disease (Martin *et al.*,1996). At present, eosinophils appear to be associated pathologically with asthma, atopic dermatitis, allergic rhinitis, eosinophilic gastroenteritis, and certain eye diseases. The effector functions of eosinophils appear to be derived primarily from release of lipid mediators and proteins, including cytokines and granule proteins (Martin *et al.*,1996). Eosinophil degranulation results in the release of several cytotoxic cationic granule proteins. Furthermore, release of cytokines by eosinophils and other cells involved in inflammation amplifies and regulates localized immune responses. Altogether, the eosinophil's capacity to release and be influenced by a variety of mediators, including the granule proteins and cytokines, implicates this cell in the pathology of inflammation and in the perpetuation of the inflammatory response (Martin *et al.*,1996).

Pollen that elicited high level of neutrophil were *Terminalia catappa* and *Mariscus ligularis* . The role of neutrophil in allergic symptoms of the airways has received increasing attention recently. There is a robust correlation between air way neutrophils and human asthma (Little *et al*., 2002). The severity of airway disease appears to correlate with number of neutrophils. Neutrophils are over represented in patients with asthma exacerbation severly or poorly controlled asthma and in those who die of asthma (Wenzel *et al*., 2002). Neutrophils are abundant cellular components of the immune system. They discharge their arsenal of toxic agents against host tissues, resulting in oxidative damage and inflammation.

5.2 Conclusion

The present survey of airborne pollen and fungi spores has contributed to our existing knowledge of abundance and dispersal of pollen grains and fungal spores in the atmosphere of Nigeria. It is expected that the results of the present work will provide useful data to the allergologists of Nigeria for selecting pollen allergens during calendar months of the year in six geopolitical zones, which will facilitate proper diagnosis and treatment. Allergy sufferers can also use the information to plan their outdoor activities in order to avoid exposure to allergens. Hypersensitive individuals in Southern and North-Central Nigeria are more polysensitized especially during the drier period, whereas individuals in Northern Nigeria are more monosensitized especially during the rainy season.

The pollen and fungal spores protein elicite immunological changes, however their contents vary among plants even of the same family. The rate at which pollen and fungal spores induce the production of immunoglobulin type E (IgE) antibodies vary. *Terminalia catappa*, *Aspergillus niger, Panicum maximum, Mariscus ligularis* and *Oreodoxa oleracea* induced progressive production of IgE in mice. The IgE produced over the week by *Fusarium* sp., *Mangifera indica* and *Sacciolepis africana* fluctuated, but all showed a higher value of IgE after the last sensitization at 5^{th} week depicting an orchestrated immunological response involving lymphocyte, basophil, eosinophil and neutrophil cells. All pollen and fungal spores protein caused proliferation of lymphocyte whereas basophil and eosinophil were more implicated in inflammation in extrinsic and/ or intrinsic allergenic reaction.

CHAPTER SIX

6.1 Summary of Findings

OBJECTIVES	FINDINGS
1. To determine the spatial distribution of atmospheric pollen and spore and their relationship to weather parameters	1. Two major seasons of pollen and spores abundance were noted in Southern and North Central Nigeria; a rainy season dominated by fungal spores and late rainy/harmattan dominated by pollen 2. Quantitative abundance of pollen was recorded during the rainy season and majorly dominated by Poaceae in North West and North East Nigeria. 3. Pollen concentration in the air had a direct relationship with temperature and wind 4. Rainfall favoured sporulation of fungal spores.
2. Determination of allergen protein concentration	1.There was a relationship between the allergen protein and elicited IgE in some Mice .
3. Establishment of systemic and physical features of allergy provoked by pollen and spores	1. Pollen protein of *Oreodoxa oleracea* induced a characteristic dermatophytic reaction physically featuring as swelling, rashes and hair loss on mice skin 2. *Mariscus ligularis* and *Sacciolepis africana* pollen proteins and spore protein of *Aspergillus niger* showed inflammatory reaction on respiratory system.
4. Evaluation of immunoglobulin E(IgE) antibodies and immune cells of mice developed against pollen	1. Repeated low doses of pollen and spores protein resulted in persistent IgE production with and without physical features of allergy.

and spores protein.	2. Pollen protein of *Oreodoxa oleraceae* and *Sacciolepis africana* elicited high basophil cells which depicted their potency in inflammation. 3. Allergen protein of *Terminalia catappa, Panicum maximum*, *Mariscus ligularis* pollen and *Fusarium* sp. spore induced higher eosinophil cells than *Aspergillus niger*, *Mangifera indica*, *Sacciolepis africana, Oreodoxa oleracea* and control. 4. All studied pollen and spore proteins induced alteration in immune system which resulted in lymphocyte infiltration. 5. Mice inoculated with allergen protein had a greater number of neutrophils, lymphocytes, eosinophils and basophils than control mice.

6.2 Contributions to Knowledge

4. The result establishes the basis for future work towards creating Nigerian pollen and fungal spores calendar of the study locations which indicate two different risk periods for pollen hypersensitive individuals; late rainy/dry season in North Central and Southern Nigeria, rainy season for individuals in North West and North East Nigeria. Rainy season was also noted as a period for dominant fungi spores over the atmosphere of the six geopolitical zones. This however provides useful prophylactic and avoidance tool for allergic people.
5. The research work shows that persistent low doses of allergen result in increase in IgE level with or without physical features of allergy.
6. The work further establishes that serological analysis of immunoglobulin E integrated with immune cell count will assist in identification of allergen.

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APPENDIX 1

ETHICAL APPROVAL

INSTITUTIONAL REVIEW BOARD

NIGERIAN INSTITUTE OF MEDICAL RESEARCH

6, Edmond Crescent Off Murtala Muhammed Way, P.M.B. 2013 Yaba, Lagos.
Tel: 01-4823123, 01-7744723, 08050254484, 08033460847 Fax: 01-4823123, 234-1-3425171
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Secretariat: Room 207, Biochemistry Division, Research Block, NIMR

21st Oct, 2014

PROJECT TITLE: **SPATIAL DISTRIBUTION OF ATMOSPHERIC POLLEN AND FUNGI SPORES AND THEIR RELATION TO ALLERGY IN NIGERIA.**

PROJECT №: **IRB/14/261**

APPROVAL LETTER

The above named proposal has been adequately reviewed; the protocol and safety guidelines satisfy the conditions of NIMR-IRB, policies regarding experiments that use human subjects.

Therefore the study under its reviewed state is hereby approved by Institutional Review Board, NIMR.

PROF. F. E. OKONOFUA
Name of IRB Chairman

Signature of IRB Chairman & Date

MRS. O. A. NWOGBE
Name of IRB Secretary

Signature of IRB Secretary & Date

This approval is given with the investigator's Declaration as stated below;
By signing below I agree/certify that:

1. I have reviewed this protocol submission in its entirety and that I am fully cognizant of, and in agreement with, all submitted statements.
2. I will conduct this research study in strict accordance with all submitted statements except where a change may be necessary to eliminate an apparent immediate hazard to a given research subject.
   - I will notify the IRB promptly of any change in the research procedures necessitated in the interest of the safety of a given research subject.
   - I will request and obtain IRB approval of any proposed modification to the research protocol or informed consent document(s) prior to implementing such modifications.

APPENDIX 2

METEOROLOGICAL DATA OF ENUGU, SOUTH EAST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	26.4	189.8	85	4.9
2011	JULY	25.7	194.9	85	4.9
2011	AUGUST	25.5	237.4	85	5.9
2011	SEPTEMBER	25.6	443.6	86	4.3
2011	OCTOBER.	26.4	153.8	83	4.4
2011	NOVEMBER.	27.7	2.0	72	4.1
2011	DECEMBER.	26.2	0.0	52	6.5
2012	JANUARY.	27.1	39.0	55	6.1
2012	FEBRUARY.	28.7	21.2	72	4.7
2012	MARCH	30.8	68.1	61	4.9
2012	APRIL	28.5	129.5	76	6.5
2012	MAY	27.1	288.7	81	4.7

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 3

METEOROLOGICAL DATA OF RIVERS, SOUTH SOUTH NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	26.2	353.8	89	2.7
2011	JULY	25.3	304.8	70.3	2.3
2011	AUGUST	25.2	310.2	91	2.5
2011	SEPTEMBER	25.5	413.0	90	1.9
2011	OCTOBER	25.9	284.7	89	1.9
2011	NOVEMBER	26.7	45.3	86	1.6
2011	DECEMBER	26.7	28.3	74	1.8
2012	JANUARY	26.1	59.5	73	2.4
2012	FEBRUARY	26.8	162.0	84	2.4
2012	MARCH	27.9	225.3	82	2.4
2012	APRIL	26.8	206.7	86	2.4
2012	MAY	26.7	330.6	86	2.4

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 4

METEOROLOGICAL DATA OF LAGOS, SOUTH WEST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{0}C)	RAINFALL(MM)	HUMIDITY (%)	WIND(KNOTS)
2011	JUNE	26.5	251.9	89	3.7
2011	JULY	25.5	476.9	89	6.0
2011	AUGUST	25.5	43.7	88	7.5
2011	SEPTEMBER	26.0	175.3	86	3.7
2011	OCTOBER.	26.2	209.3	88	2.6
2011	NOVEMBER.	27.0	240.5	88	2.6
2011	DECEMBER.	27.5	0.0	80	3.0
2012	JANUARY.	27.1	10.5	81	3.2
2012	FEBRUARY	27.6	122.2	84	3.8
2012	MARCH	28.5	78.1	83	3.7
2012	APRIL	28.2	124.7	83	4.2
2012	MAY	27.3	134.9	86	3.6

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 5

METEOROLOGICAL DATA OF ABUJA NORTH CENTRAL NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP. (^{0}C)	RAINFALL(MM)	HUMIDITY (%)	WIND(KNOTS)
2011	JUNE	26.2	128.6	83	4.1
2011	JULY	25.7	227.6	87	4.1
2011	AUGUST	24.7	183.5	88	3.8
2011	SEPTEMBER	25.2	278.0	86	4.0
2011	OCTOBER	25.8	130.3	83	3.7
2011	NOVEMBER	26.3	6.8	64	4.0
2011	DECEMBER	25.3	0.0	40	4.5
2012	JANUARY	26.2	0.0	41	4.5
2012	FEBRUARY	29.2	20.6	52	4.2
2012	MARCH	30.9	19.0	38	5.0
2012	APRIL	32.6	45.2	65	6.6
2012	MAY	34.3	198.5	78	3.9

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 6

METEOROLOGICAL DATA OF KADUNA, NORTH WEST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP (^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	25.5	80.9	76	4.7
2011	JULY	24.6	240.3	78	4.6
2011	AUGUST	24.0	208.0	82	4.6
2011	SEPTEMBER	24.3	298.7	80	3.6
2011	OCTOBER	25.0	72.7	73	2.5
2011	NOVEMBER	24.0	0.0	37	4.7
2011	DECEMBER	22.4	0.0	26	7.0
2012	JANUARY	23.1	0.0	23	6.4
2012	FEBRUARY	27.4	0.0	23	4.8
2012	MARCH	28.2	0.0	19	7.0
2012	APRIL	28.8	212.8	58	9.7
2012	MAY	26.5	61.6	70	5.6

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 7

METEOROLOGICAL DATA OF GOMBE, NORTH EAST NIGERIA FROM JUNE 2011 TO MAY 2012

YEAR	MONTHS	AV.TEMP.(^{O}C)	RAINFALL(MM)	HUMIDITY(%)	WIND(KNOTS)
2011	JUNE	25.3	76.4	56	5.9
2011	JULY	24.5	183.0	57	5.2
2011	AUG.	24.4	157.2	60	4.8
2011	SEPTEMBER	24.0	119.5	64	4.4
2011	OCTOBER	25.7	70.2	54	4.5
2011	NOVEMBER	27.0	0.0	20	3.7
2011	DECEMBER	27.8	0.0	28	3.8
2012	JANUARY	27.9	0.0	26	3.7
2012	FEBRUARY	27.9	0.0	22	3.6
2012	MARCH	28.0	0.0	24	5.8
2012	APRIL	27.7	6.8	35	6.0
2012	MAY	27.5	93.0	45	5.3

SOURCE-NIGERIAN METEOROLOGICAL CENTRE OSHODI, LAGOS

APPENDIX 8

NATIONAL INSTITUTE OF HEALTH (NIH) ON IMMUNOGLOBULIN E RATING SCALE

RAST rating	IgE level (ng/ml)	Comment
0	< 0.35	ABSENT OR UNDETECTABLE ALLERGEN SPECIFIC IgE
1	0.35 - 0.69	LOW LEVEL OF ALLERGEN SPECIFIC IgE
2	0.70 - 3.49	MODERATE LEVEL OF ALLERGEN SPECIFIC IgE
3	3.50 - 17.49	HIGH LEVEL OF ALLERGEN SPECIFIC IgE
4	17.50 - 49.99	VERY HIGH LEVEL OF ALLERGEN SPECIFIC IgE
5	50.0 - 100.00	VERY HIGH LEVEL OF ALLERGEN SPECIFIC IgE
6	> 100.00	EXTREMELY HIGH LEVEL OF ALLERGEN SPECIFIC IgE

The RAST test is scored on a scale from 0 to 6:

APPENDIX 9

SPSS ANALYSIS DATA GENERATED FROM BASOPHIL, EOSINOPHIL, LYMPHOCYTE, MONOCYTE, NEUTROPHIL AND IgE

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Basophil	t2	5	.8000	.83666	.37417	-.2389	1.8389	.00	2.00
	t2	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t3	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t4	5	3.0000	2.00000	.89443	.5167	5.4833	.00	5.00
	t5	5	11.4000	22.16529	9.91262	-16.1218	38.9218	.00	51.00
	t6	5	12.6000	27.61883	12.35152	-21.6933	46.8933	.00	62.00
	t7	5	3.0000	4.52769	2.02485	-2.6219	8.6219	.00	11.00
	t8	5	5.0000	8.97218	4.01248	-6.1404	16.1404	.00	21.00
	control	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	45	3.9778	12.05006	1.79632	.3575	7.5980	.00	62.00
Eosinophil	t2	5	4.8000	4.32435	1.93391	-.5694	10.1694	.00	10.00
	t2	5	3.4000	3.43511	1.53623	-.8653	7.6653	.00	8.00
	t3	5	.6000	.89443	.40000	-.5106	1.7106	.00	2.00
	t4	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t5	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t6	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t7	5	1.8000	2.68328	1.20000	-1.5317	5.1317	.00	6.00
	t8	5	1.0000	1.22474	.54772	-.5207	2.5207	.00	3.00
	control	5	.4000	.89443	.40000	-.7106	1.5106	.00	2.00
	Total	45	1.3333	2.52262	.37605	.5755	2.0912	.00	10.00
Lymphocyte	t2	5	90.6000	9.01665	4.03237	79.4043	101.7957	75.00	97.00
	t2	5	94.2000	3.11448	1.39284	90.3329	98.0671	90.00	97.00
	t3	5	99.2000	.83666	.37417	98.1611	100.2389	98.00	100.00
	t4	5	93.0000	9.69536	4.33590	80.9616	105.0384	76.00	100.00
	t5	5	87.8000	22.81885	10.20490	59.4667	116.1333	47.00	99.00

	t6	5	68.6000	41.84854	18.71523	16.6382	120.5618	.00	99.00
	t7	5	90.8000	7.62889	3.41174	81.3275	100.2725	83.00	99.00
	t8	5	93.6000	9.39681	4.20238	81.9323	105.2677	77.00	100.00
	control	5	97.8000	4.91935	2.20000	91.6918	103.9082	89.00	100.00
	Total	45	90.6222	17.67161	2.63433	85.3131	95.9314	.00	100.00
Monocyte	t2	5	2.8000	4.38178	1.95959	-2.6407	8.2407	.00	10.00
	t2	5	2.4000	.54772	.24495	1.7199	3.0801	2.00	3.00
	t3	5	.2000	.44721	.20000	-.3553	.7553	.00	1.00
	t4	5	4.2000	9.39149	4.20000	-7.4611	15.8611	.00	21.00
	t5	5	.8000	1.09545	.48990	-.5602	2.1602	.00	2.00
	t6	5	2.6000	5.81378	2.60000	-4.6188	9.8188	.00	13.00
	t7	5	4.4000	7.36885	3.29545	-4.7496	13.5496	.00	17.00
	t8	5	.2000	.44721	.20000	-.3553	.7553	.00	1.00
	control	5	1.8000	4.02492	1.80000	-3.1976	6.7976	.00	9.00
	Total	45	2.1556	4.65127	.69337	.7582	3.5530	.00	21.00
Neutrophil	t2	5	1.0000	2.23607	1.00000	-1.7764	3.7764	.00	5.00
	t2	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t3	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t4	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t5	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t6	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t7	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	t8	5	.2000	.44721	.20000	-.3553	.7553	.00	1.00
	control	5	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	45	.1333	.75679	.11282	-.0940	.3607	.00	5.00
IgE	t2	5	9.1120	4.81354	2.15268	3.1352	15.0888	1.52	13.34
	t2	5	6.2202	3.01220	1.34710	2.4801	9.9603	1.02	8.35
	t3	5	3.9880	3.03713	1.35825	.2169	7.7591	.13	7.00
	t4	5	8.4700	5.11675	2.28828	2.1167	14.8233	1.70	13.78
	t5	5	5.8130	5.84470	2.61383	-1.4442	13.0702	.13	13.13
	t6	5	2.2042	1.86866	.83569	-.1161	4.5245	.00	3.91
	t7	5	4.1480	2.13563	.95508	1.4963	6.7997	1.63	7.19
	t8	5	4.6840	4.99121	2.23214	-1.5134	10.8814	.25	13.10
	control	5	1.5180	.13809	.06176	1.3465	1.6895	1.35	1.73
	Total	45	5.1286	4.27168	.63678	3.8452	6.4120	.00	13.78

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Basophil	Between Groups	949.778	8	118.722	.786	.618
	Within Groups	5439.200	36	151.089		
	Total	6388.978	44			
Eosinophil	Between Groups	116.800	8	14.600	3.221	.007
	Within Groups	163.200	36	4.533		
	Total	280.000	44			
Lymphocyte	Between Groups	3226.978	8	403.372	1.381	.238
	Within Groups	10513.600	36	292.044		
	Total	13740.578	44			
Monocyte	Between Groups	97.511	8	12.189	.514	.838
	Within Groups	854.400	36	23.733		
	Total	951.911	44			
Neutrophil	Between Groups	4.400	8	.550	.952	.488
	Within Groups	20.800	36	.578		
	Total	25.200	44			
IgE	Between Groups	263.706	8	32.963	2.201	.051
	Within Groups	539.173	36	14.977		
	Total	802.879	44			

Post Hoc Tests

Homogeneous Subsets

Basophil

Duncan

Treatment	N	Subset for alpha = 0.05
		1
t2	5	.0000
t3	5	.0000
control	5	.0000
t2	5	.8000
t4	5	3.0000
t7	5	3.0000
t8	5	5.0000
t5	5	11.4000
t6	5	12.6000
Sig.		.176

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Eosinophil

Duncan

Treatment	N	Subset for alpha = 0.05		
		1	2	3
t4	5	.0000		
t5	5	.0000		
t6	5	.0000		
control	5	.4000	.4000	
t3	5	.6000	.6000	
t8	5	1.0000	1.0000	
t7	5	1.8000	1.8000	
t2	5		3.4000	3.4000
t2	5			4.8000
Sig.		.256	.052	.305

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Lymphocyte

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
t6	5	68.6000	
t5	5	87.8000	87.8000
t2	5	90.6000	90.6000
t7	5	90.8000	90.8000
t4	5		93.0000
t8	5		93.6000
t2	5		94.2000
control	5		97.8000
t3	5		99.2000
Sig.		.067	.373

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Monocyte

Duncan

Treatment	N	Subset for alpha = 0.05
		1
t3	5	.2000
t8	5	.2000
t5	5	.8000
control	5	1.8000
t2	5	2.4000
t6	5	2.6000
t2	5	2.8000
t4	5	4.2000
t7	5	4.4000
Sig.		.254

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Neutrophil

Duncan

Treatment	N	Subset for alpha = 0.05
		1
t2	5	.0000
t3	5	.0000
t4	5	.0000
t5	5	.0000
t6	5	.0000
t7	5	.0000
control	5	.0000
t8	5	.2000
t2	5	1.0000
Sig.		.083

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

IgE

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
control	5	1.5180	
t6	5	2.2042	
t3	5	3.9880	3.9880
t7	5	4.1480	4.1480
t8	5	4.6840	4.6840
t5	5	5.8130	5.8130
t2	5	6.2202	6.2202
t4	5		8.4700
t2	5		9.1120
Sig.		.103	.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.