Aerobiological investigation, meteorological impacts on pollen distribution and allergenicity of major pollen types in the atmosphere of Southeastern Nigeria

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Abstract

Airborne palynomorphs are regarded as important organic granules causing human allergic reactions such as pollinosis and asthma. In the present study, monthly depositional amount of airborne pollen and fern spores, impact of the surrounding plants on pollen distribution and allergenicity of common airborne pollen types, were investigated at selected sampling stations in Ebonyi and Anambra States, Southeastern Nigeria. Bioaerosols were collected with Tauber-like pollen samplers and subjected to conventional palynological treatment processes, microscopy and photomicrography. Enumeration of plants within the immediate vegetation showed that local plants produced some atmospheric pollen types at the study locations. Among other meteorological parameters, only relative humidity was found to yield statistically significant and positive correlation with monthly total airborne pollen concentrations in Anambra State. A positive but not significant correlation was also found between total airborne pollen counts and relative humidity in Ebonyi State. *Nephrolepis* sp., *Pteris* sp. and a trilete fern produced spores, while diatoms were also recovered. Some common pollen types including those of *Syzygium guineense* (Willd.) DC. and *Senna siamea* (Lam.) Irwin et Barneby in Ebonyi State; *Mariscus alternifolius* Vahl. and *Zea mays* L. in Anambra State, were collected in order to quantify and extract their crude protein contents for *Mus musculus* sensitization to achieve serology (ELISA) and haematology (differential and total white blood cell counts). Statistical significance was tested and observed in the correlation between amounts of haematological and serological parameters yielded by each test group; variations between amounts of these parameters yielded by each test group and those of the control, as well as at differing periods of sensitization. In the *Senna siamea* test group of *Mus musculus*, histopathological morbid characteristics were observed, including areas of necrosis (presence of dead cells) in the bronchi of a mortality; areas of necrosis and congestion in the bronchi and lungs of another mortality, respectively, after second sensitization. The study confirmed all the selected pollen types as potential sources of allergy at the study areas and revealed a multiplex of interplay among triggers of pollen allergy at different intervals of administration in mice, establishing a model of immunological reactions to varied pollen antigens in humans.

1.0 Introduction

Allergies to pollen and spores are more difficult to diagnose and treat than other allergies (Gonzalo-Garijo et al. 1996). According to Bousquet et al. (2011), pollen and spores are atmospheric allergens causing illnesses including hay fever and asthma in hundreds of millions of people around the world. The distribution of pollen grains has recently been monitored in several regions of the globe, in a bid to study the relationship between pollen concentrations and meteorological data (Pace et al., 2018); surrounding vegetation in the urban study areas (Charalampopoulos et al., 2018); public health data (Al-Dousari et al., 2018).

Aeropalynological study has been carried out in various parts of Nigeria by some workers. Ajikah et al. (2021a) provided a summary of earlier aeropalynological research work in the country, giving an account of the workers involved, study areas, method and duration of sampling, research aim, problems and constraints, approach to collecting reliable airborne pollen data, link between aeropalynologists, medical personnel and environmental assessors, as well as possibilities for further research studies. Previous aeropalynological studies in Southwest, Nigeria include Adekanmbi and Ogundipe (2010) who collected the aerospora data of four different sites at the University of Lagos, Lagos, Nigeria and observed a significant prepondrance of pollen types produced by Poaceae, including Mimosaceae, Arecaceae, Asteraceae, and Euphorbiaceae, among others. Adeonipekun (2012) stated that savanna plants were not represented in his recovered pollen spectra at Aiyetoro, Ogun State, Nigeria, due to the late harmattan season linked with unusual dust particles, compared to the previous year with a recovery of savanna pollen at the same location. Adeniyi et al. (2014) highlighted predominant pollen grains, including those of Poaceae, Cyperaceae, and *Alchornea cordifolia*. They deduced safe-risk periods of pollen allergy, connections between pollen levels, meteorological data, and public health records in Lagos, Nigeria. Ajikah et al. (2017) investigated the aerospora of three different locations in Lagos, Nigeria and found no significant but positive relationship between monthly airborne pollen levels and meteorological records, suggesting that pollen distribution may have been influenced by some other factors. Adeniyi et al. (2018) observed common airborne pollen types of *Casuarina equisetifolia*, *Alchornea cordifolia*, Cyperaceae, Poaceae, and Amaranthaceae in Lagos, Nigeria. From their findings, they discovered a major correlation between the common pollen grains and wheeze cases generated for two successive years, while Cyperaceae and Poaceae yielded a significant relationship with wheezes in the first sampling year. Adekanmbi et al. (2018b) reported an abundance of atmospheric pollen dispersed by *Tridax procumbens*, *Elaeis guineensis*, *Alchornea cordifolia*, *Senna* sp. and Poaceae at their study areas in Osun and Ogun States. They confirmed the important association between atmospheric pollen grains and the surrounding plants at their sampling locations. The work of Ibigbami and Adeonipekun (2020) highlighted *Alchornea cordifolia*, Euphorbiaceae, Poaceae and Amaranthaceae, as major contributors to airborne pollen spectra of their sampling sites in Lagos, Nigeria. Findings from their study revealed that relative humidity and temperature impact the distribution of pollen at their study locations. Ajikah et al. (2021b) discovered the preponderance of airborne pollen types dispersed by Convolvulaceae, Euphorbiaceae, Poaceae and Cyperaceae at their sampling sites in Lagos, Nigeria. They observed that aerospora corresponded with angiosperms in the surrounding flora of their sampling locations. Past aeropalynological findings in South-eastern Nigeria, include that of Njokuocha (2006) who recorded that *Alchornea cordifolia*, *Elaeis guineensis*, Asteraceae and Poaceae, dispersed copious atmospheric pollen types in Nsukka, Enugu State. Aeropalynological work previously done in South-southern Nigeria, include Adekanmbi et al. (2018a) who revealed that atmospheric pollen grains of *Tridax procumbens*, *Elaeis guineensis*, *Alchornea cordifolia*, *Terminalia* sp., Poaceae and Cyperaceae, dominated their study areas in Cross River and Delta States. They also confirmed that airborne pollen data corresponded significantly with relative humidity. In North-Central Nigeria, past

aeropalynological findings include those by: Essien and Agwu (2013) who recorded the dominance of airborne pollen dispersed by *Syzygium guineense*, *Elaeis guineensis*, *Alchornea cordifolia* and Poaceae, among others in Kogi State. The atmospheric pollen of *Elaeis guineensis*, *Alchornea cordifolia*, *Cassia* sp. and Poaceae, among others, were reported to be abundant in Garki, Abuja, by Ezike et al. (2016). The predominance of airborne pollen types dispersed by Poaceae and Chenopodiaceae/Amaranthaceae among others, at two study sites in Nasarawa State and Abuja, was reported by Ezikanyi et al. (2018). In North-western Nigeria, past aeropalynological work include Adekanmbi and Alebiosu (2018) who emphasized that *Syzygium guineense*, *Alchornea laxiflora*, *Pinus caribaea*, *Alchornea* sp., *Terminalia* sp., Poaceae and Sapotaceae produced abundant airborne pollen types. They also noted that the immediate vegetation contributed significantly to atmospheric pollen spectra at the study locations.

From past studies on allergenicity of pollen grains, Ye et al. (1988) employed the use of skin tests to ascertain the pollen allergenic potentials of certain common plants including, *Trema orientalis*, *Casuarina equisetifolia*, *Broussonetia* sp., *Humulus scandens* and Poaceae in their study locations. The similarity between sensitization using mutual boosting activities of pollen and latex in-vivo, and that of initially polysensitized allergic individuals, was affirmed by Mahler et al. (2000). The considerably low production level of IgG2b, IgG2a and SBP-specific IgG1, was enhanced by allergic challenges with Japanese cedar pollen combined with cholera toxin (JCP/CT), and this was earlier reported by Hirai et al. (2000). They observed that IgE was elicited only in mice serum previously combined with JCP/CT. They further revealed the prominent infiltration of eosinophils in the nasal mucosa of the mice initially challenged with JCP/CT-sensitized. Stern et al. (2005) carried out a successive exposure of BALB/C wild-type mice to topical applications of short ragweed pollen on daily basis. They recorded allergic symptoms in the conjunctivae, such as redness, chemosis, tearing and lid edema. They stressed that eosinophils and neutrophils were the polymorphonuclear cells responsible for severe inflammation in the conjunctivae. The successive sensitization with ragweed through nasal routes, triggered a rise in sneezing episodes for a duration of time in mice, as previously observed by Kato et al. (2014). They reported a notable rise in the level of polymorphonuclear tissue, which stimulated inflammation in the broncho-alveolar lavage fluid of mice, following an intratracheal sensitization, in comparison with the unchallenged group. They noted considerable multilayered epithelium, goblet cell hyperplasia and eosinophil infiltration in the lungs of experimental mice. Scevkova et al. (2015) carried out immunological trials and observed that the subjects were mostly sensitized to pollen types of Ambrosia (21.3% in 2002 and 13.9% in 2003) and Poaceae (25.5% in 2002 and 11.3% in 2003). Bastl et al. (2016) undertook a study to establish the relationship between airborne pollen concentrations, estimated allergen content of grass and birch pollen, burden of allergic symptoms in pollen hypersensitive individuals during periods of pollination in France, Australia, Finland and Germany. They affirmed there were variations in the allergen content of these flowering plants, based on seasonality and study locations, although with a positive relationship with frequency of allergic symptoms. Alebiosu et al. (2018) reported a significant correlation among the levels of some key allergy mediators in *Mus musculus*, after it was challenged with *Tridax procumbens* and *Alchornea cordifolia* pollen protein extracts. They also reported hair loss following second administration with protein extracts of *Alchornea cordifolia* pollen.

The great anemophilous nature of many angiosperms in Nigeria, has led to the spatial dispersal of pollen grains over time in various regions of the country. However, there is yet a paucity of reliable data on the pattern of pollen distribution at different periods or seasons of the year, as well as allergenic effects of pollen types. Our present study investigated the monthly rate of deposition of airborne pollen and spores, the impact of meteorological conditions and local vegetation on atmospheric pollen distribution, and allergic tendencies of common atmospheric pollen types at selected study locations in Southeastern Nigeria.

2.0 Materials And Methods

2.1 Description of the study area

The sampling locations were situated in Ebonyi and Anambra States of Southeastern Nigeria. The vegetation of Ebonyi state is a derived savannah being influenced by a climate that is characteristic of an average monthly temperature is 27^{o}C (Uguru et al., 2015). A bimodal rainfall with two peaks in the months of July and September, is experienced; rains begin considerably in April and ends in October, followed by an appreciable period of dryness between November and April; total annual rainfall falls within the range between 1500 to 2000 mm, having an average of 1800 mm (Uguru et al., 2015). A high humidity is also experienced ranging between 60% and 80% during the dry and wet seasons respectively (Uguru et al., 2015). In Anambra state, the vegetation is typically grassland together with woodland and scattered forests, surrounded by a tropical rainforest having tall trees, several climbers and dense undergrowth (Okigbo and Nwatu, 2015). It experiences a high annual rainfall ranging from 1400 mm in the northern zone to 2500 mm in the southern zone; it is seasonal and followed by a period of dryness lasting from November to February (Okigbo and Nwatu, 2015).

2.2 Study sites

Sampling stations in Ebonyi State University, Ebonyi State (Latitude: 6^{o}19'33"N, Longitude: 8^{o}4'45"E) and Nnamdi Azikwe University, Anambra State (Latitude: 6^{o}15'34.6"N, Longitude: 007^{o}6'45.8"E) were randomly selected for the investigation. Security of the pollen traps and open characteristic of the sampling sites for unhindered air flow permitting adequate distribution of pollen and spores, were considered in the selection

of sampling stations (Ajikah et al., 2017; Adekanmbi and Alebiosu, 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Alebiosu and Adekanmbi, 2022). Arc GIS 10.3.1 software was used for the mapping of sampling points (Fig. 1).

2.3 Collection of meteorological data

The values of meteorological parameters including rainfall, relative humidity, wind speed and air temperature (July 2020 – June 2021) for both study locations in Ebonyi and Anambra States, were procured from the Nigerian Meteorological Agency, Oshodi, Lagos, Nigeria.

2.4 Plant enumeration

Enumeration of plants was conducted with quadrats of 25 x 25 m and 1.0 x 1.0 m for trees and herbs/grasses, respectively in the immediate vegetation (Adekanmbi and Alebiosu, 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Alebiosu and Adekanmbi, 2022). Plant samples were collected randomly in each plot; identified using keys, following Dalziel (1937), Hutchinson and Dalziel (1954), Keay (1959), Keay et al. (1964) and Keay (1989). The deposition of the samples was done in the University of Lagos Herbarium, Lagos, Nigeria.

2.5 Construction of Tauber-modified pollen samplers

At the sampling stations, Tauber-like pollen samplers were constructed, each comprising a pollen trap and an iron sampling stand, for the collection of bioaerosols consisting trapped atmospheric pollen and spores (Ajikah et al., 2015; Ajikah et al., 2017; Ezike et al., 2016; Adekanmbi and Alebiosu, 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Ajikah et al., 2021b; Alebiosu and Adekanmbi, 2022). The samplers were buried with one foot below ground level and positioned at head level (5 feet above ground level). A solution comprising 5 ml of phenol, 10 ml of formaldehyde and 50 ml of glycerol, was transferred into each pollen trap, covered with mesh and placed on the sampling stand (Ajikah et al., 2015; Ajikah et al., 2017; Ezike et al., 2016; Adekanmbi and Alebiosu, 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Ajikah et al., 2021b; Alebiosu and Adekanmbi, 2022) (Fig. 2).

2.6 Bioaerosol collection

Monthly collection of bioaerosols was done from July 2020 to June 2021. After each collection, the traps were thoroughly rinsed with distilled water, to prevent contamination of subsequent monthly sampling at the study sites (Ajikah et al., 2015; Ajikah et al., 2017; Ezike et al., 2016; Adekanmbi and Alebiosu, 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Ajikah et al., 2021b; Alebiosu and Adekanmbi, 2022).

2.7 Treatment, microscopy and photomicrography of bioaerosols

The bioaerosols were centrifuged at 2500 r.p.m for 5 minutes, in order to ensure separation of residues comprising palynomorphs from the supernatant fluids (Ajikah et al., 2015; Ajikah et al., 2017; Ezike et al., 2016; Adekanmbi and Alebiosu, 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Ajikah et al., 2021b; Alebiosu and Adekanmbi, 2022). The preparation of acetolysis mixture was done using concentrated sulphuric acid and acetic anhydride in a volume ratio of 1: 9; each sample residue was treated with five (5) ml of the prepared mixture and subjected to heating inside a water bath at 100 ^{o}C for 10 minutes (Erdtman, 1969). The supernatant residues were subjected to centrifugation, decanting and repeatedly washed twice with distilled water, followed by another centrifugation; the residues were preserved with two drops of glycerine (Erdtman, 1969), after which slides were prepared for light and scanning electron microscopy of the residues (Alebiosu and Adekanmbi, 2022). Scanning electron microscopy of the residues was carried out at the Central Analytical Facilities of Stellenbosch University, South Africa, in a bid to further reveal morphological properties of airborne pollen and spores (Sowunmi, 1995; Adekanmbi and Ogundipe, 2009; Alebiosu and Adekanmbi, 2022). Centrifugation of each supernatant residue (1ml) was done at 5,000 r.p.m for 5 mins, after which it was immersed in 50% acetone in water, washed and centrifuged again (twice) in 100% ethanol for dehydration (Alebiosu and Adekanmbi, 2022). Hexamethyldisilizane (HMDS) was then used in rinsing the samples after which they were dehydrated. There was an addition of pollen residue (100 µl) in ethanol, to aluminium foil pieces (10 mm x 10 mm) and these were dehydrated inside an incubator at 40 ^{o}C for 24 hrs. Standard aluminium scanning electron microscopic stubs (12 mm each) were used for the mounting of dry samples on foil and these were sputter-coated using a thin layer of gold to permit conductivity (Alebiosu and Adekanmbi, 2022). Identification of pollen and spores was achieved with the aid of photomicrographs from reference journals such as Sowunmi (1973, 1995), Adekanmbi (2009), Adekanmbi and Ogundipe (2009), Gosling et al. (2013) and unpublished reference albums. Pollen calendar for the study period at the sampling locations were constructed using Tilia 2.0.41 software, as previously employed by Alebiosu and Adekanmbi (2022) (Figs. 3–4).

2.8 Collection of pollen samples for allergenicity studies in mice

Some of the common atmospheric pollen types were produced by *Syzygium guineense* (Willd.) DC. and *Senna siamea* (Lam.) Irwin et Barneby, at the study location in Ebonyi State; *Mariscus alternifolius* Vahl. (Cyperaceae) and *Zea mays* L. (Poaceae) at the study location in Anambra State, hence their polliniferous specimens were collected for crude protein extraction (Adekanmbi et al. 2018b; Alebiosu and Adekanmbi, 2022).

2.9 Crude pollen protein extraction and assay

Crude protein extraction of selected pollen types was conducted by employing Folin Lowry methods (Association of Official Analytical Chemists, 1990). Polliniferous specimens were crushed using laboratory acidified sand sprinkled on the pollen grains, with the aid of a mortar and pestle, while 2 ml of buffer solution (phosphate buffered saline at pH 7.4) was added to ensure homogeneity of the specimens in the centrifuge tubes. Another 8 ml of buffer was used to rinse the remaining homogenate and was further introduced into the same set of centrifuge tubes (Adekanmbi et al. 2018b; Alebiosu and Adekanmbi, 2022). Sample centrifugation was done at 5000 r.p.m for 10 minutes. To obtain protein precipitates, 5 ml of 70% ammonium sulphate was introduced to the supernatant, followed by the collection of residues which were further suspended in 3 ml of phosphate buffer saline. Precipitates were later retained and subjected to dialysis for 12hrs against phosphate buffer saline (Adekanmbi et al. 2018b; Alebiosu and Adekanmbi, 2022).

To quantify the concentration of pollen protein in each plant, crude protein extracts of the pollen types were stored at -80°C. The estimation of crude protein concentration of the pollen types, was achieved using the procedures of Bradford (1976), Adekanmbi et al. (2018b), Alebiosu and Adekanmbi (2022).

The residual bovine serum albumin was serially diluted: 1 ml (200 g/ml), 0.8 ml (160 g/ml), 0.6 ml (120 g/ml), 0.4 ml (80 g/ml), and 0.2 ml (40 g/ml). A wavelength of 595 µm was considered in the calibration of the spectrophotometer and also blanked with a tube free of protein. After 5 minutes, the reading of absorbance values of both the standards and samples, was done at 595 µm wavelength. The absorbance values of the standards were graphically plotted against their concentrations. The concentrations and extinction coefficient of the unknown samples were estimated (Bradford, 1976; Adekanmbi et al. 2018b; Alebiosu and Adekanmbi, 2022).

2.10 Identification and sensitization of experimental mice

The *Mus musculus* is a suitable mouse in the study of diseases in humans, because it is a genetically sensitive animal and has also been observed to share close similarities with humans, based on its tissue structure and organization (Nguyen and Xu, 2008). Consequently, the *Mus musculus* mice were used, while their nomenclature was authenticated by Dr. Nnamdi Amaeze of the Department of Zoology, University of Lagos, Lagos, Nigeria. Female *Mus musculus* PWK/PhJ (6 weeks old), were sourced and maintained under laboratory conditions in the Animal House, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria, following the procedure of Conejero et al. (2007), Adekanmbi et al. (2018b), Alebiosu and Adekanmbi (2022).

The mice were subjected to all experimental protocols, according to international standards of animal welfare; research ethical approval (Protocol number IRB/15/302) was collected from the Institutional Review Board of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria (Adekanmbi et al. 2018b; Alebiosu and Adekanmbi, 2022).

A group of five mice were housed in a cage and used to sensitize with crude protein extracts of the four selected pollen types, as well as the control, giving a total of twenty-five mice. Constant feed and accessibility to portable water were provided for the experimental and control mice (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022). Subcutaneous injection and nasal administration of the experimental mice with crude pollen protein extract (0.4 ml) was carried out, in order to formulate a paradigm of atmospheric pollen inhalation in each experimental *Mus musculus*, following the procedures of Conejero et al. (2007), Adekanmbi et al. (2018b), Alebiosu and Adekanmbi (2022).

The administration of experimental *Mus musculus* with these pollen extracts, was repeated bi-weekly (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

2.11 Haematological studies

Haematological experiments including differential and total white blood cell counts, were performed in *Mus musculus* and this was conducted at the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

2.11.1 Differential counts of white blood cells

Prior to pollen sensitization, blood specimens of *Mus musculus* were collected from the pre-orbital vein, with the aid of a plain capillary tube to obtain blood smear on the slides, and this was repeatedly done a week after each administration (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).. The thin blood smear was fixed on each slide with methanol for 2–3 minutes, after which staining with Leishman stain was done. In order to flood each slide, 3% of the stock solution was used and then left for 45 minutes, so as to analyze the differential white blood cells. The fluid was then rinsed off the slide and differential white blood cells were microscopically examined, using x100 objective lens with oil immersion to estimate haematological parameters in numbers; differential white blood cells were identified, while hundred immune cells were counted on each slide, in accordance with the procedure of Conejero et al., 2007; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

2.11.2 Total white blood cell counts

For the total white blood cells (TWBC), 380 µl of the diluting fluid (Tursk solution) was first injected into clean lithium heparinized tubes. Each blood sample was introduced into 20 µl of diluting fluid in each tube and stirred thoroughly, leading to blood lysis and enhancing the release and counting of white blood cells. Counting of total white blood cells was done microscopically using x10 objective lens, with the aid of an haematocytometer and a coverslip (World Health Organization, 1980; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

2.12 Immunoperoxidase assay in plasma samples

In order to generate plasma samples, blood specimens were placed in eppendorf tubes and spun at 10,000 r.p.m for 10 minutes on an eppendorf refrigerator centrifuge machine, at the Nigerian Institute of Medical Research in Yaba, Lagos, Nigeria (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022). The Enzyme-Linked Immunosorbent Assay (ELISA) kits of serological parameters including Immunoglobulin-E (IgE) and cytokines; Interleukin-5 (IL-5), Interleukin-13 (IL-13) and Tumor Necrotic Factor-α (TNF-α), were supplied by Elabscience Biotechnology Inc. U.S.A and used with strict adherence to manufacturer's instructions. ELISA technique was adopted in estimating the amount of IgE and cytokines produced in plasma specimens, based on standard procedures of Kato et al. (2014), Dearman et al. (1993, 1994), Adekanmbi et al. (2018b), Alebiosu and Adekanmbi (2022). An ELISA/microplate reader was set to 450 nm for the simultaneous determination of optical density values of wells for both the standard and blank. The value of optical density for each standard (y-axis) was plotted versus the concentration (x-axis), using the curve expert version 1.4 software and a standard best fit curve was drawn through the graph points. Concentrations of IgE, IL-5, IL-13 and TNF-α were calculated using the values of optical density in the experimental specimens (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

2.13 Test of statistical significance

Pearson correlation test ($p < 0.05$) was used to examine the statistical significance in the correlation between monthly total pollen concentrations and values of meteorological parameters including rainfall, relative humidity, wind speed and air temperature; correlation among the amounts of haematological and serological parameters elicited by each test group at different periods of sensitization. Statistical significance in the differences among the amounts of haematological and serological parameters produced by each test group and those of the control group, as well as at varied periods of sensitization, was examined using a two-way ANOVA test ($p < 0.05$) (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

2.14 Histopathological examination of respiratory organs in dead *Mus musculus*

During the experiment, two mortalities were noted in the *Mus musculus* test group of *Senna siamea* and were opened for post mortem. Bronchi and lungs of these mice were harvested and submitted at the Histopathology unit in the Department of Anatomy and Molecular Pathology, College of Medicine, University of Lagos, Lagos, Nigeria (Alebiosu and Adekanmbi, 2022). Bronchi and lung tissues were processed while mounting of slides was done with D.P.X for microscopic examination of histopathological changes, according to the procedures of Adeline et al. (2010) and Alebiosu and Adekanmbi (2022). Histopathological morbid features in the dead mice were microscopically observed (x1000 objectives) by a Consultant histopathologist at the Federal Medical Centre, Lokoja, Kogi State, Nigeria (Alebiosu and Adekanmbi, 2022).

2.15 Photomicrography

Photomicrographs of certain identified pollen and fern spore types, differential and total white blood cells, and histologic morbid features, were captured with the aid an Olympus camera mounted on an Olympus CX31 photomicroscope (Plates 1–4).

3.0 Results

3.1 Relationship between atmospheric pollen types and enumerated local plants

In the immediate vegetation of the study site at Ebonyi State University, Abakaliki, Ebonyi State, some airborne pollen types were produced by local plants and these include *Tridax procumbens*, *Sida acuta*, *Senna siamea*, *Bombax buonopozense*, *Casuarina equisetifolia*, *Elaeis guineensis*, *Syzygium guineense*, *Alchornea cordifolia*, *Amaranthus* sp., *Terminalia* sp. and Poaceae (Table 1). In the immediate vegetation of the study site at Nnamdi Azikwe University, Awka, Anambra State, some airborne pollen types were produced by local plants and these include *Tridax procumbens*, *Alchornea cordifolia*, *Casuarina equisetifolia*, *Elaeis guineensis*, *Bombax buonopozense*, *Sida acuta*, *Terminalia* sp., *Albizia* sp., Asteraceae, Cyperaceae and Poaceae (Table 2).

Table 1
Relationship between enumerated local plants and airborne pollen recovered from the study location at Ebonyi State University, Abakaliki, Ebonyi State

S.no	Scientific name of local plants	Family name	Habit	Airborne pollen recovered
1.	*Aspilia africana* (Pers.) C.D. Adams	Asteraceae	Herb	
2.	*Tridax procumbens* L.	Asteraceae	Herb	*Tridax procumbens*
3.	*Senna siamea*	Fabaceae	Tree	*Senna siamea*
4.	*Sida acuta* Burm. f.	Malvaceae	Herb	*Sida acuta*
5.	*Amaranthus* sp.	Amaranthaceae	Herb	*Amaranthus* sp.
6.	*Gmelina arborea* Roxb.	Verbenaceae	Tree	
7.	*Bombax buonopozense* P.Beauv.	Bombacaceae	Tree	*Bombax buonopozense*
8.	*Elaeis guineensis* Jacq.	Arecaceae	Tree	*Elaeis guineensis*
9.	*Casuarina equisetifolia* L.	Casuarinaceae	Tree	*Casuarina equisetifolia*
10.	*Mangifera indica* L.	Anacardiaceae	Tree	
11.	*Terminalia* sp.	Combretaceae	Tree	*Terminalia* sp.
12.	*Syzygium guineense* (Willd.) DC.	Myrtaceae	Tree	*Syzygium guineense*
13.	*Manihot esculenta* Cranz.	Euphorbiaceae	Shrub	
14.	*Alchornea cordifolia* Schum. & Thonn.) Mull. Arg.	Euphorbiaceae	Shrub	*Alchornea cordifolia*
15.	*Zea mays* L.	Poaceae	Grass	Poaceae
16.	*Panicum maximum* Jacq.	Poaceae	Grass	Poaceae
17.	*Imperata cylindrica* (L.) P.Beauv.	Poaceae	Grass	Poaceae
18.	*Oryza sativa* L.	Poaceae	Grass	Poaceae

Table 2
Relationship between enumerated local plants and airborne pollen recovered from the study location at Nnamdi Azikwe University, Awka, Anambra state

S.no	Scientific name of local plants	Family name	Habit	Airborne pollen recovered
1.	*Aspilia africana* (Pers.) C.D. Adams	Asteraceae	Herb	Asteraceae
2.	*Chromolaena odorata* (L.) R.M. King & H. Rob.	Asteraceae	Herb	Asteraceae
3.	*Sida acuta* Burm. f.	Malvaceae	Herb	*Sida acuta*
4.	*Tridax procumbens* L.	Asteraceae	Herb	*Tridax procumbens*
5.	*Treculia africana* Decne. ex Trécul	Moraceae	Tree	
6.	*Vitex doniana* Sweet.	Lamiaceae	Tree	
7.	*Eucalyptus globulus* Labill.	Myrtaceae	Tree	
8.	*Mangifera indica* L.	Anacardiaceae	Tree	
9.	*Canarium schweinfurthii* Engl.	Burseraceae	Tree	
10.	*Draceana arborea* (Willd.) Link.	Dracaenaceae	Tree	
11.	*Anacardium occidentale* L.	Anacardiaceae	Tree	
12.	*Casuarina equisetifolia* L.	Casuarinaceae	Tree	*Casuarina equisetifolia*
13.	*Acacia* spp.	Mimosaceae	Tree	
14.	*Gmelina arborea* Roxb.	Verbenaceae	Tree	
15.	*Bombax buonopozense* P.Beauv.	Bombacaceae	Tree	*Bombax buonopozense*
16.	*Elaeis guineensis* Jacq.	Arecaceae	Tree	*Elaeis guineensis*
17.	*Terminalia* sp.	Combretaceae	Tree	*Terminalia* sp.
18.	*Albizia* sp.	Mimosaceae	Tree	*Albizia* sp.
19.	*Alchornea cordifolia* Schum. & Thonn.) Mull. Arg.	Euphorbiaceae	Shrub	*Alchornea cordifolia*
20.	*Panicum maximum* Jacq.	Poaceae	Grass	Poaceae
21.	*Imperata cylindrica* (L.) P.Beauv.	Poaceae	Grass	Poaceae
22.	*Pennisetum purpureum* Schumach.	Poaceae	Grass	Poaceae
23.	*Mariscus alternifolius* Vahl.	Cyperaceae	Sedge	Cyperaceae

3.2 Meteorological data and their statistical correlation with airborne pollen counts

There were variations in the monthly levels of rainfall, relative humidity, air temperature and wind speed at the study locations. In Ebonyi State, air temperature levels ranged from 26.7–32.3 ^{o}C, rainfall levels ranged from 27–84 mm, relative humidity ranged from 0–421.6% and wind speed ranged from 4.3–8.9 knots (Table 3). In Anambra State, air temperature levels varied from 27.1–32.1^{o}C, rainfall levels varied from 43–83 mm, relative humidity varied from 0–552.0% and wind speed varied from 0.3–4.8 knots (Table 3). The statistical significance test revealed that monthly total pollen counts showed significantly positive correlation ($P < 0.05$) with only monthly levels of relative humidity in Anambra State (Table 4). In Ebonyi State, a negative but not significant correlation was recorded between monthly total pollen counts and meteorological parameters including rainfall, air temperature and wind speed, while a positive but not significant correlation was found between monthly total pollen counts and relative humidity (Table 4). In Anambra State, a positive but not significant correlation was recorded between monthly total pollen counts and monthly levels of air temperature, while a negative correlation was found between monthly total pollen counts and meteorological parameters including rainfall and wind speed. (Table 5).

Table 3
Monthly mean values of meteorological parameters at the study locations (July 2020 – June 2021) Key: TR = Trace of Rain (Not measurable) Source: Nigerian Meteorological Agency, Lagos.

Meteorological parameters	Study locations	July 2020	Aug. 2020	Sep. 2020	Oct. 2020	Nov. 2020	Dec. 2020	Jan. 2021	Feb. 2021	Mar. 2021	Apr. 2021	May 2021	June 2021
Air temperature (°C)	Ebonyi state	27.3	26.7	27.5	28.7	30.1	30.1	31.1	32.3	30.9	30.7	30.1	29.0
	AnambraState	27.1	26.9	27.1	27.7	27.8	27.6	30.0	32.1	30.4	30.4	29.2	27.7
Relative humidity (%)	Ebonyi state	82	84	82	78	69	27	28	39	70	72	73	75
	AnambraState	81	83	83	83	80	68	43	53	73	73	77	80
Rainfall (mm)	Ebonyi state	393.7	365.8	375.0	99.2	183.0	0.0	0.0	0.0	96.1	135.0	421.6	369.0
	AnambraState	551.8	260.4	552.0	195.0	66.0	0.0	0.0	26.1	124.0	186.0	229.4	282.0
Wind speed (knot)	Ebonyi state	4.8	4.7	4.8	4.5	4.9	8.9	5.9	5.4	4.9	4.3	4.8	4.5
	AnambraState	3.3	3.5	3.1	2.2	2.3	2.5	0.3	2.2	3.5	4.8	4.3	4.2

Table 4. Correlation coefficientsa showing the degree of significance in the correlation between monthly pollen concentrations and values of mean monthly meteorological parameters at the study site in Ebonyi State at 95% confidence interval (P < 0.05)

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	Correlations		
		B	Std. Error	Beta			Zero-order	Partial	Part
1	(Constant)	13809.201	32392.380		.426	.685			
	Air temperature	-428.727	790.587	-.317	-.542	.607	-.002	-.216	-.170
	Wind speed	-293.812	1188.408	-.155	-.247	.813	-.208	-.100	-.078
	Relative humidity	72.507	98.694	.652	.735	.490	.145	.287	.231
	Rainfall	-15.385	8.045	-1.079	-1.912	.104	-.259	-.615	-.601

a. Dependent variable: Pollen count

Table 5. Correlation coefficientsa showing the degree of significance in the correlation between monthly pollen concentrations and values of mean monthly meteorological parameters at the study site in Anambra State at 95% confidence interval (P < 0.05)

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	Correlations		
		B	Std. Error	Beta			Zero-order	Partial	Part
1	(Constant)	-3021.626	2699.704		-1.119	.300			
	Air temperature	65.196	74.392	.427	.876	.410	-.150	.314	.224
	Wind speed	-177.809	92.973	-.835	-1.912	.097	-.255	-.586	-.489
	Relative humidity	27.629	12.226	1.365	**2.260**	**.048**	.182	.649	.577
	Rainfall	-.744	.479	-.543	-1.554	.164	-.268	-.507	-.397

a. Dependent variable: Pollen count

3.3

Palynological findings

Annual total pollen counts of 14588 and 2117 were recorded in the atmosphere of the study sites in Ebonyi and Anambra States, respectively. At the study site in Ebonyi state, 23 atmospheric pollen types were recorded. Among these, six, seven and ten were identified to the familial, generic and species levels respectively. The unidentified pollen types were designated as pollen indeterminate. Total pollen counts were relatively higher in the months of February 1812 (12.42%), March 176 (1.21%), September 254 (1.74%), October 7,731 (53.00%), November 3,837 (26.30%) and

December 253 (1.73%). Relatively lower records were observed in the months of January 167 (1.15%), April 148 (1.02%), May 67 (0.46%), June 13 (0.09%), July 58 (0.40%) and August 72 (0.49%). *Alchornea cordifolia*, *Syzygium guineense*, *Elaeis guineensis*, *Senna* sp., *Borreria* sp. and Poaceae produced the common airborne pollen types (Fig. 3a).

At the study site in Anambra state, 16 airborne pollen types were recorded. Among these, four, five and seven were identified to familial, generic and species levels respectively. Total airborne pollen counts were higher in the months of February 194 (9.16%), May 110 (5.20%), August 165 (7.79%), September 354 (5.15%), October 284 (13.4%) and November 962 (45.44%). Lower records were observed in the months of January 75 (3.54%), March 22 (1.04%), April 69 (3.26%), June 52 (2.46%), July 17 (0.80%) and December 58 (2.74%). Common airborne pollen types were produced by Poaceae, *Alchornea cordifolia* and *Elaeis guineensis* (Fig. 4a).

The highest concentration of airborne Poaceae pollen was recorded in the month of October 7574 (55.70%) and lowest in the month of June 3 (0.02%) in Ebonyi state, while it was highest in the month of November 711 (58.42%) and lowest in the month of March 7 (0.58%) in Anambra state (Table 6). Ferns including *Pteris* sp., *Nephrolepis* sp. and a trilete fern produced spore types at the two study sites (Figs. 3b and 4b). More detailed morphological characteristics of some atmospheric pollen types at study sites, was revealed through scanning electron microscopy (Plates 1b and 2b). Pollen calendar representing monthly distribution of airborne pollen and fern spore types at both sites during the study period, are presented in Figs. 3–4.

3.4 Crude pollen protein quantification, haematological and serological analyses

Different crude protein concentrations were recorded in the pollen types of *Syzygium guineense*, *Senna siamea*, *Mariscus alternifolius* and *Zea mays* (Table 7); crude protein concentration was found higher in the pollen of *Zea mays* (72.74 mg/ml), while lower concentration was recorded in the pollen of *Senna siamea* (15.90 mg/ml).

Table 6
Distribution of Poaceae pollen in the atmosphere of the study locations (July 2020 – June 2021)

Study locations	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Total
Ebonyi state	20,	23,	201,	7574,	3772,	141,	39,	1778,	11,	19,	16,	3,	13597
	0.15%	0.17%	1.48%	55.70%	27.74%	1.04%	0.29%	13.08%	0.08%	0.14%	0.12%	0.02%	
Anambra state	8,	81,	88,	83,	711,	20,	26,	55,	7,	42,	66,	30,	1217
	0.66%	6.66%	7.23%	6.82%	58.42%	1.64%	2.14%	4.52%	0.58%	3.45%	5.42%	2.47%	

Table 7. Crude protein estimation of selected pollen types for allergenicity studies

Selected pollen types	Crude protein concentrations (mg/ml)
Zea mays	72.74
Senna siamea	15.90
Syzygium guineense	29.50
Mariscus alternifolius	61.46

Mean levels of haematological parameters including lymphocytes, neutrophils, eosinophils and monocytes (polymorphonuclear cells), TWBC, as well as serological parameters including IgE, IL-5, IL-13 and TNF-α, were observed to vary in the experimental groups at different periods of sensitization. In the experimental group of *Zea mays*, an increase in neutrophils and IgE corresponded with a decrease in lymphocytes, monocytes, eosinophils, TWBC, TNF-α, IL-5 and IL-13 after first sensitization; an increase in lymphocytes, monocytes, IgE and IL-13 corresponded with a decrease in neutrophils and TWBC after second sensitization. In the experimental group of *Senna siamea*, an increase in lymphocytes, monocytes, TWBC, IgE, TNF-α, IL-5 and IL-13 corresponded with a decrease in neutrophils and eosinophils after first sensitization; an increase in lymphocytes, eosinophils and TWBC corresponded with a decrease in neutrophils, IgE, TNF-α, IL-5 and IL-13 after second sensitization. In the experimental group of *Syzygium guineense*, an increase in lymphocytes, TWBC and IL-5 corresponded with a decrease in neutrophils, monocytes,

IgE and IL-13, after first sensitization; an increase in neutrophils, monocytes, eosinophils and TWBC corresponded with lymphocytes, IgE, IL-5 and IL-13; there were also scant records of atypical lymphocytes. In the experimental group of *Mariscus alternifolius*, an increase in lymphocytes, TWBC and IL-5 corresponded with a decrease in neutrophils, monocytes, eosinophils, IgE and IL-13 after first sensitization; an increase in neutrophils, monocytes, eosinophils and IL-13 corresponded with a decrease in lymphocytes, TWBC, IgE and IL-5; there were also scant records of atypical lymphocytes (Table 8).

Table 8
Mean levels of haematological and serological parameters elicited by each test group and those of the control

Test and control groups	Sensitization periods	Haematological parameters					Serological parameters			
		N (%)	L (%)	M (%)	E (%)	TWBC (mm^3)	IgE (µg/ml)	TNF-α (pg/ml)	IL-5 (pg/ml)	IL-13 (pg/ml)
		Mean levels								
Zea mays	PRE-POST	7.00 ± 1.53	92.00 ± 1.20	0.67 ± 0.67	0.67 ± 0.67	6466.00 ± 466.67	746.8 ± 0.02	1132.58 ± 0.05	6582.5 ± 0.23	4835.2 ± 0.06
	1st POST	58.00 ± 1.53	42.00 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	5333.20 ± 944.43	1051.8 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2nd POST	37.20 ± 2.73	62.00 ± 2.65	0.60 ± 0.33	0.00 ± 0.00	2983.20 ± 963.65	1170.4 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	5286.5 ± 0.17
Senna siamea	PRE-POST	37.00 ± 1.25	40.33 ± 19.19	1.00 ± 0.58	1.33 ± 0.33	5630.00 ± 202.76	1100.8 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	6516.5 ± 0.04
	1st POST	29.60 ± 0.88	68.60 ± 0.67	1.60 ± 0.33	0.00 ± 0.00	10660.00 ± 785.99	2796.8 ± 0.05	19.4 ± 0.02	242.9 ± 0.04	13930.6 ± 0.05
	2nd POST	29.20 ± 0.33	69.00 ± 0.58	1.60 ± 0.33	0.40 ± 0.33	16860.00 ± 2626.94	1441.5 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	5900.6 ± 0.04
Syzygium guineense	PRE-POST	38.60 ± 0.67	59.60 ± 0.88	1.60 ± 0.33	0.00 ± 0.00	5600 ± 264.58	2051.4 ± 0.02	67.71 ± 0.03	0.00 ± 0.00	7076.1 ± 0.04
	1st POST	30.00 ± 1.16	68.60 ± 0.33	1.40 ± 0.88	0.00 ± 0.00	7916.00 ± 192.21	1746.4 ± 0.05	0.00 ± 0.00	7772.5 ± 0.02	1712.6 ± 0.04
	2nd POST	45.60 ± 1.86	52.00 ± 1.53	1.60 ± 0.33	0.60 ± 0.33	12782.00 ± 808.46	424.9 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mariscus alternifolius	PRE-POST	47.60 ± 1.45	49.20 ± 0.88	2.00 ± 0.58	1.00 ± 0.58	4338.00 ± 338.30	4982.4 ± 0.03	0.00 ± 0.00	6862.5 ± 0.02	6323 ± 0.01
	1st POST	13.20 ± 2.85	86.60 ± 2.85	0.00 ± 0.00	0.00 ± 0.00	8034.00 ± 1284.63	1407.6 ± 0.04	0.00 ± 0.00	7300 ± 0.02	1584 ± 0.04
	2nd POST	47.60 ± 11.05	51.40 ± 11.98	0.60 ± 0.67	0.40 ± 0.33	5516.00 ± 2013.77	1001 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	5201.1 ± 0.02
Control group	PRE-POST	29.00 ± 0.50	68.50 ± 1.16	2.33 ± 0.44	0.00 ± 0.00	4991.67 ± 120.19	1661.70 ± 0.00	0.00 ± 0.00	3308.75 ± 0.02	8150.70 ± 0.01
	1st POST	28.67 ± 0.88	68.67 ± 0.88	2.67 ± 0.33	0.00 ± 0.00	5000.00 ± 132.29	814.60 ± 0.04	0.00 ± 0.00	6617.50 ± 0.04	9611.00 ± 0.05
	2nd POST	29.33 ± 0.33	68.33 ± 1.45	2.00 ± 0.58	0.00 ± 0.00	4983.33 ± 109.29	2508.80 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	6690.40 ± 0.02

KEY: N = Neutrophils; L = Lymphocytes; M = Monocytes; E = Eosinophils; TWBC = Total white blood cell; IgE = Immunoglobulin E; TNF-α = Tumor necrotic factor α; IL-5 = Interleukin-5; IL-13 = Interleukin-13; S = Significant difference; NS = Difference not significant; NF = Difference not found (where parameters were absent).

3.5 Test of statistical significance

Statistical significance was recorded in the variations between amount of some haematological and serological parameters produced by certain experimental groups and those of the control. Significant variations were found among the amounts of these parameters at different periods of sensitization in certain experimental groups (Table 9). A significant correlation was found between levels of some haematological and serological parameters produced by all experimental groups; IgE increased significantly with reduced TNF-α, IL-5 and IL-13 in the *Zea mays* experimental group; lymphocytes increased significantly with reduced monocytes, while neutrophils decreased significantly with elevated IgE,

TNF-α, IL-5 and IL-13 in the *Senna siamea* experimental group; IL-5 decreased significantly with elevated IgE, TNF-α and IL-13 in the *Syzygium guineense* experimental group; IgE increased significantly with elevated TNF-α and IL-13 in the *Mariscus alternifolius* experimental group (Table 10).

Table 9
Degree of statistical significance in the differences between haematological and serological levels elicited by each test group and those of the control, as well as at varied periods of sensitization in each test group ($P < 0.05$)

Test groups		Haematological parameters					Serological parameters			
		N (%)	L (%)	M (%)	E (%)	TWBC (mm^3)	IgE (µg/ml)	TNF-α (pg/ml)	IL-5 (pg/ml)	IL-13 (pg/ml)
Zea mays	Differences between test group and control	S (*P* value: 3.1E-0)	S (*P* value: 1.3E-05)	S (*P* value: 0.000672)	NF (*P* value: 0.00)	NS (*P* value: 0.25529)	S (*P* value: 4.74E-31)	NF (*P* value: 0.00)	S (*P* value: 2.17E-39)	S (*P* value: 5.89E-36)
	Differences between sensitization periods	S (*P* value: 0.000282)	S (*P* value: 0.000495)	NF (*P* value: 0.00)	NF (*P* value: 0.00)	NS (*P* value: 0.120043)	S (*P* value: 8.79E-33)	NF (*P* value: 0.00)	S (*P* value: 2.17E-39)	S (*P* value: 1.3E-30)
Senna siamea	Differences between test group and control	NS (*P* value: 0.474731)	NS (*P* value: 0.736707)	NS (*P* value: 0.141113)	NS (*P* value: 0.346594)	S (*P* value: 0.000213)	S (*P* value: 4.33E-30)	S (*P* value: 4.52E-21)	S (*P* value: 2.65E-38)	S (*P* value: 6.02E-35)
	Differences between sensitization periods	NS (*P* value: 0.808887)	NS (*P* value: 1)	NS (*P* value: 0.437852)	NS (*P* value: 0.346594)	NS (*P* value: 0.055032)	S (*P* value: 1.22E-26)	S (*P* value: 4.52E-21)	S (*P* value: 1.47E-38)	S (*P* value: 7.02E-39)
Syzygium guineense	Differences between test group and control	S (*P* value: 7.46E-05)	S (*P* value: 7.91E-05)	NS (*P* value: 0.186905)	NS (*P* value: 0.080516)	S (*P* value: 1.45E-06)	S (*P* value: 3.3E-31)	NF (*P* value: 0.00)	S (*P* value: 4.94E-33)	S (*P* value: 1.22E-40)
	Differences between sensitization periods	S (*P* value: 0.000129)	S (*P* value: 0.000105)	NS (*P* value: 0.780169)	NS (*P* value: 0.080516)	S (*P* value: 0.000446)	S (*P* value: 2.75E-27)	NF (*P* value: 0.00)	S (*P* value: 8.51E-42)	S (*P* value: 1.18E-36)
Mariscus alternifolius	Differences between test group and control	NS (*P* value: 0.799941)	NS (*P* value: 0.937843)	S (*P* value: 0.002827)	NS (*P* value: 0.346594)	NS (*P* value: 0.174723)	S (*P* value: 2.35E-30)	NF (*P* value: 0.00)	S (*P* value: 5.24E-31)	S (*P* value: 6.9E-39)
	Differences between sensitization periods	S (*P* value: 0.015662)	S (*P* value: 0.02083)	NF (*P* value: 0.00)	NS (*P* value: 0.346594)	NS (*P* value: 0.321016)	NS (*P* value: 1.53E-31)	NF (*P* value: 0.00)	S (*P* value: 1.75E-41)	S (*P* value: 8.37E-30)

Table 10. Correlation matrix of haematological and serological parameters in Zea mays test group

		N	L	M	E	TWBC	IgE	TNF	IL-5	IL-13
N	Pearson Correlation	1	− .974	− .854	− .977	.158	− .741	.741	.741	.741
	Sig. (2-tailed)		.145	.348	.136	.899	.469	.469	.469	.469
	N	5	5	5	5	5	5	5	5	5
L	Pearson Correlation	− .974	1	.715	.904	− .377	.569	− .569	− .569	− .569
	Sig. (2-tailed)	.145		.493	.281	.754	.614	.614	.614	.614
	N	5	5	5	5	5	5	5	5	5
M	Pearson Correlation	− .854	.715	1	.945	.378	.982	− .982	− .982	− .982
	Sig. (2-tailed)	.348	.493		.212	.753	.121	.121	.121	.121
	N	5	5	5	5	5	5	5	5	5
E	Pearson Correlation	− .977	.904	.945	1	.054	.866	− .866	− .866	− .866
	Sig. (2-tailed)	.136	.281	.212		.965	.333	.333	.333	.333
	N	5	5	5	5	5	5	5	5	5
TWBC	Pearson Correlation	.158	− .377	.378	.054	1	.546	− .546	− .546	− .546
	Sig. (2-tailed)	.899	.754	.753	.965		.632	.632	.632	.632
	N	5	5	5	5	5	5	5	5	5
IgE	Pearson Correlation	− .741	.569	.982	.866	.546	1	-1.000	-1.000	-1.000
	Sig. (2-tailed)	.469	.614	.121	.333	.632		.000	.000	.000
	N	5	5	5	5	5	5	5	5	5
TNF	Pearson Correlation	.741	− .569	− .982	− .866	− .546	-1.000	1	1.000	1.000
	Sig. (2-tailed)	.469	.614	.121	.333	.632	.000		.000	.000
	N	5	5	5	5	5	5	5	5	5
IL-5	Pearson Correlation	.741	− .569	− .982	− .866	− .546	-1.000	1.000	1	1.000
	Sig. (2-tailed)	.469	.614	.121	.333	.632	.000	.000		.000
	N	5	5	5	5	5	5	5	5	5
IL-13	Pearson Correlation	.741	− .569	− .982	− .866	− .546	-1.000	1.000	1.000	1
	Sig. (2-tailed)	.469	.614	.121	.333	.632	.000	.000	.000	
	N	5	5	5	5	5	5	5	5	5

Table 11
Correlation matrix of haematological and serological parameters in *Senna siamea* test group.

		N	L	M	E	TWBC	IgE	TNF	IL-5	IL-13
N	Pearson Correlation	1	.818	.543	.[a]	.947	.890	.890	.[a]	.890
	Sig. (2-tailed)		.390	.635	.	.207	.301	.301	.	.301
	N	5	5	5	5	5	5	5	5	5
L	Pearson Correlation	.818	1	.927	.[a]	.959	.990	.990	.[a]	.990
	Sig. (2-tailed)	.390		.244	.	.183	.089	.089	.	.089
	N	5	5	5	5	5	5	5	5	5
M	Pearson Correlation	.543	.927	1	.[a]	.783	.866	.866	.[a]	.866
	Sig. (2-tailed)	.635	.244		.	.428	.333	.334	.	.333
	N	5	5	5	5	5	5	5	5	5
E	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.		.	.	.	.	.
	N	5	5	5	5	5	5	5	5	5
TWBC	Pearson Correlation	.947	.959	.783	.[a]	1	.989	.989	.[a]	.989
	Sig. (2-tailed)	.207	.183	.428	.		.094	.094	.	.094
	N	5	5	5	5	5	5	5	5	5
IgE	Pearson Correlation	.890	.990	.866	.[a]	.989	1	1.000	.[a]	1.000
	Sig. (2-tailed)	.301	.089	.333	.	.094		.000	.	.000
	N	5	5	5	5	5	5	5	5	5
TNF	Pearson Correlation	.890	.990	.866	.[a]	.989	1.000	1	.[a]	1.000
	Sig. (2-tailed)	.301	.089	.334	.	.094	.000		.	.000
	N	5	5	5	5	5	5	5	5	5
IL-5	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.	.	.	.	.		.
	N	5	5	5	5	5	5	5	5	5
IL-13	Pearson Correlation	.890	.990	.866	.[a]	.989	1.000	1.000	.[a]	1
	Sig. (2-tailed)	.301	.089	.333	.	.094	.000	.000	.	
	N	5	5	5	5	5	5	5	5	5

a. Cannot be computed because at least one of the variables is constant.

Table 12
Correlation matrix of haematological and serological parameters in Syzygium guineense test group

		N	L	M	E	TWBC	IgE	TNF	IL-5	IL-13
N	Pearson Correlation	1	− .901	− .125	.359	− .356	.778	.778	− .778	.778
	Sig. (2-tailed)		.286	.921	.766	.768	.433	.433	.433	.433
	N	5	5	5	5	5	5	5	5	5
L	Pearson Correlation	− .901	1	− .319	.082	.727	− .427	− .427	.427	− .427
	Sig. (2-tailed)	.286		.793	.948	.482	.719	.719	.719	.719
	N	5	5	5	5	5	5	5	5	5
M	Pearson Correlation	− .125	− .319	1	− .971	− .883	− .721	− .721	.721	− .721
	Sig. (2-tailed)	.921	.793		.154	.311	.488	.488	.488	.488
	N	5	5	5	5	5	5	5	5	5
E	Pearson Correlation	.359	.082	− .971	1	.744	.866	.866	− .866	.866
	Sig. (2-tailed)	.766	.948	.154		.466	.333	.333	.333	.333
	N	5	5	5	5	5	5	5	5	5
TWBC	Pearson Correlation	− .356	.727	− .883	.744	1	.310	.310	− .310	.310
	Sig. (2-tailed)	.768	.482	.311	.466		.799	.799	.799	.799
	N	5	5	5	5	5	5	5	5	5
IgE	Pearson Correlation	.778	− .427	− .721	.866	.310	1	1.000	-1.000	1.000
	Sig. (2-tailed)	.433	.719	.488	.333	.799		.000	.000	.000
	N	5	5	5	5	5	5	5	5	5
TNF	Pearson Correlation	.778	− .427	− .721	.866	.310	1.000	1	-1.000	1.000
	Sig. (2-tailed)	.433	.719	.488	.333	.799	.000		.000	.000
	N	5	5	5	5	5	5	5	5	5
IL-5	Pearson Correlation	− .778	.427	.721	− .866	− .310	-1.000	-1.000	1	-1.000
	Sig. (2-tailed)	.433	.719	.488	.333	.799	.000	.000		.000
	N	5	5	5	5	5	5	5	5	5
IL-13	Pearson Correlation	.778	− .427	− .721	.866	.310	1.000	1.000	-1.000	1
	Sig. (2-tailed)	.433	.719	.488	.333	.799	.000	.000	.000	
	N	5	5	5	5	5	5	5	5	5

Table 13. Correlation matrix of haematological and serological parameters in *Mariscus alternifolius* test group

		N	L	M	E	TWBC	IgE	TNF	IL-5	IL-13
N	Pearson Correlation	1	.818	.543	.[a]	.947	.890	.890	.[a]	.890
	Sig. (2-tailed)		.390	.635	.	.207	.301	.301	.	.301
	N	5	5	5	5	5	5	5	5	5
L										
	Pearson Correlation	.818	1	.927	.[a]	.959	.990	.990	.[a]	.990
	Sig. (2-tailed)	.390		.244	.	.183	.089	.089	.	.089
	N	5	5	5	5	5	5	5	5	5
M										
	Pearson Correlation	.543	.927	1	.[a]	.783	.866	.866	.[a]	.866
	Sig. (2-tailed)	.635	.244		.	.428	.333	.334	.	.333
	N	5	5	5	5	5	5	5	5	5
E										
	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.		.	.	.	.	.
	N	5	5	5	5	5	5	5	5	5
TWBC										
	Pearson Correlation	.947	.959	.783	.[a]	1	.989	.989	.[a]	.989
	Sig. (2-tailed)	.207	.183	.428	.		.094	.094	.	.094
	N	5	5	5	5	5	5	5	5	5
IgE										
	Pearson Correlation	.890	.990	.866	.[a]	.989	1	1.000	.[a]	1.000
	Sig. (2-tailed)	.301	.089	.333	.	.094		.000	.	.000
	N	5	5	5	5	5	5	5	5	5
TNF										
	Pearson Correlation	.890	.990	.866	.[a]	.989	1.000	1	.[a]	1.000
	Sig. (2-tailed)	.301	.089	.334	.	.094	.000		.	.000
	N	5	5	5	5	5	5	5	5	5
IL-5										
	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.	.	.	.	.		.
	N	5	5	5	5	5	5	5	5	5
IL-13										
	Pearson Correlation	.890	.990	.866	.[a]	.989	1.000	1.000	.[a]	1
	Sig. (2-tailed)	.301	.089	.333	.	.094	.000	.000	.	
	N	5	5	5	5	5	5	5	5	5

a. Cannot be computed because at least one of the variables is constant.

3.6 Histopathological study of respiratory organs from some dead *Mus musculus*

In the control group, no abnormalities were seen in the bronchi and lungs. In the *Senna siamea* experimental group, areas of necrosis (presence of dead cells) and congestion were seen in the bronchi and lungs of a dead *Mus musculus* respectively after second sensitization. Areas of necrosis were observed in the bronchi of another dead *Mus musculus* in this group after second sensitization (Plate 4).

4.0 Discussion

4.1 Distribution of aeroflora and its relationship with the surrounding vegetation

The first one-year aeroflora data in both Ebonyi and Anambra states, Southeastern Nigeria, have been obtained, revealing the diversity of airborne pollen and fern spore types recovered at the study locations. The current study has provided a reliable basis for comparing pollen distribution in the two distally situated states, hence a significant disparity was noted between the annual total pollen records of these areas. This finding corresponds with Alebiosu et al. (2018); Adekanmbi and Alebiosu (2018); Adekanmbi et al. (2018a), Adekanmbi et al. (2018b) and Alebiosu and Adekanmbi (2022) who revealed a notable difference between the annual total pollen counts of 7229 and 2235; 3839 and 2723; 4305 and 1815; 8258 and 6682; 7080 and 3347, respectively at two distal study locations in their studies. In the present work, the considerable dissimilarity between annual total pollen counts of the study sites, may be linked with drivers such as varying pollen production and mechanisms of pollen dispersal, plant density in the surrounding vegetation, as well as weather conditions during the period of study (Alebiosu et al., 2018). Additionally, a total of 23 and 16 pollen types identified at the sampling stations for the all-year round study period, is similar to the findings of Alebiosu et al. (2018), Adekanmbi and Alebiosu (2018), Adekanmbi et al. (2018a), Adekanmbi et al. (2018b) and Alebiosu and Adekanmbi (2022) who reported a total number of 28 and 30; 29 and 30; 21 and 23; 29 and 26; 23 and 26 pollen types, respectively, at two distally located sites in their investigations.

Months with incidence of higher total airborne pollen counts (February to March and September to December) at the study location in Ebonyi State, is in accordance with previous records of Njokuocha (2006), who noted that pollen were abundant during the late rainy season to early dry season, starting from September to January in the atmosphere of Nsukka, South Eastern Nigeria. Adeniyi et al. (2018) designated October as the month of pollen peak at their study site in Lagos, Nigeria. Adekanmbi and Alebiosu (2018) regarded October to March as high-risk months for pollen hypersensitive individuals at study sites in Northeastern Nigeria. Adekanmbi et al. (2018b) reported greater airborne pollen concentrations from October to January at study sites in Southwestern Nigeria. Ezike et al. (2016) recorded highest pollen load from October to January; the harmattan months which they identified as a period of greater risk for pollen allergic individuals in Garki, Abuja, North-Central Nigeria. Adekanmbi et al. (2018a) also revealed greater atmospheric pollen counts in October and December-April in Cross River state, South-southern Nigeria. Months with incidence of lower total airborne pollen counts in Anambra state (January, March, April, June, July and December) agree with observations of Ajikah et al. (2021b) who discovered lowest atmospheric pollen levels between December and January, in Lagos, Nigeria. However, this finding is in contrast with Ajikah et al. (2021b) who recorded higher airborne pollen levels from April to July, in Lagos, Nigeria. This also deviates from Essien and Aina (2014) who reported higher total pollen counts in the month of May at their study sites.

In the study, the observation of relative humidity as an important meteorological factor controlling the distribution of airborne pollen at the study location in Anambra State, corresponds with the findings of Adeniyi et al. (2014) and Ibigbami and Adeonipekun (2020) who reported the significant impact of relative humidity in the aerofloral distribution of their study sites in Lagos, Nigeria. Adekanmbi et al. (2018a) also established the significant influence of relative humidity in the distribution of pollen grains at their study locations in Delta and Cross River States of Nigeria. However, this is in contrast with Ezike et al. (2016) who reported a negative correlation between pollen counts and relative humidity at their study location in Garki, Abuja, Nigeria.

Some plants within the immediate vegetation were represented in the atmospheric pollen spectra and this supports the previous work of Agwu (1997), Njokuocha (2006), Adeonipekun (2012), Adekanmbi and Alebiosu (2018), Alebiosu et al. (2018), Adekanmbi et al. (2018a, b), Ajikah et al. (2021b) and Alebiosu and Adekanmbi (2022) who emphasized a significant contribution to the airborne pollen load by plants within the surrounding vegetation of their respective study locations.

Some of the identified pollen types in the study, have been earlier reported and these include *Elaeis guineensis* (Ong et al., 2012; Ezikanyi et al., 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Ajikah et al., 2021b; Alebiosu and Adekanmbi, 2022). Cyperaceae (Anonymous, 2000; Singh and Kumar, 2002; Adeniyi et al., 2018; Adekanmbi et al., 2018a, b; Ajikah et al., 2021b), *Terminalia* sp. (Njokuocha, 2006; Essien and Agwu, 2013; Ajikah et al., 2015; Adekanmbi and Alebiosu, 2018; Adekanmbi et al., 2018a, b), *Senna* sp., Cyperaceae, Amaranthaceae and Asteraceae (Anonymous, 2000; Singh and Kumar, 2002) and Poaceae (Agwu and Osibe, 1992; Anonymous, 2000; Singh and Kumar, 2002; Njokuocha, 2006; Adeniyi et al., 2014; Ajikah et al., 2015; Ezike et al., 2016; Adekanmbi and Alebiosu, 2018; Ezikanyi et al., 2018; Adeniyi et al., 2018; Alebiosu et al., 2018; Adekanmbi et al., 2018a, b; Ibigbami and Adeonipekun, 2020; Ajikah et al., 2021b; Alebiosu and Adekanmbi, 2022). In the study, the recovery of *Tridax procumbens* and *Aspilia africana*; members of Asteraceae, is in congruence with findings of D'Amato (1998) who stated that this plant family is commonly allergenic, one of which is *Artemisia* sp. that is observed flowering between September and October in developed

and less developed areas, as well as in the Mediterranean. The representation of *Amaranthus* sp. in the study, is similar to the observations of Lombardero et al. (1992), in which many plant species belonging to Amaranthaceae have been associated with allergic reactions. Alebiosu et al. (2018) also reported the allergenic potential of pollen produced by *Tridax procumbens* at their study location in Ogun State, Southwest, Nigeria. According to Galan et al. (2016), pollen produced by Amaranthaceae are also widely dispersed in the Mediterranean. They stated that some of their species thrive under conditions lacking water availability and are more abundant in dry areas of the South-eastern, Iberian Peninsula.

Throughout the sampling period, the concentration of airborne Poaceae pollen was noted, even though there were considerable monthly variations at the study sites and this is in accordance with Latorre and Belmonte (2004) who reported that Poaceae pollen was observed all-year round in Catalonia, Spain, particularly during spring when many grasses blossom. This is corroborated by Essien and Aina (2014) who stated that Poaceae pollen grains are antigenic and can disperse microscopic particles into the atmosphere once released from the flowers. Pollen dispersed by Poaceae are major allergens in the atmosphere of Europe (Sanchez-Mesa et al., 2003; Garcia-Mozo et al., 2010) and they are one of the most common causes of pollinosis in various regions of the world (D'Amato et al., 2007). Pollen of Poaceae have been regarded as an important source of antigen associated with pollinosis (D'Amato et al., 2007 and Taketomi et al., 2008).

The recovery of spores dispersed by *Nephrolepis* sp., *Pteris* sp. and a trilete fern at both study locations, is in line with Adekanmbi and Alebiosu (2018), Alebiosu et al. (2018), Adekanmbi et al. (2018a, b) and Alebiosu and Adekanmbi (2022) who previously confirmed the predominance of these spores in the atmosphere of their study areas. *Nephrolepis* sp. has been discovered as the producer of dominant airborne spores in earlier findings of Njokuocha (2006) and Adeonipekun et al. (2016). Essien et al. (2014) observed *Pteris dentata*, *Pteris* sp., and a trilete fern, as main producers of spores in their study. Adeonipekun et al. (2016) revealed the dominance of spores produced by *Nephrolepis* sp., *Polypodium* sp., cf. *Cyclosorus afer*, *Adiantum* sp., *Lycopodium* sp. and a trilete fern among others. Ong et al. (2012) noted a considerable recovery of airborne *Nephrolepis auriculata* in Singapore. Guarin et al. (2015) affirmed that Polypodiaceae and Gleicheniaceae families produced three types of airborne fern spores in Medellin, Colombia. The representation of these ferns in the study, portends a fresh water swamp forest was well established around the sampling stations (Alebiosu and Adekanmbi, 2022)

There were fluctuations in the period of recovery of certain pollen types at the study locations and this may have been influenced by variations recorded in the level of meteorological conditions including wind speed, air temperature, rainfall and relative humidity that enhanced anthesis, dehiscence and atmospheric transport of the pollen grains during the study period (Huang, 1998; Kizilpinar et al., 2011; Alebiosu et al., 2018). This is also congruent with the observations of Ajikah et al. (2017), who cited that plant density and blossom periods pose an enormous effect on atmospheric pollen levels. This point of view is corroborated by Kaplan (2004) who emphasized important variations in airborne distribution of pollen types between countries, regions and cities.

4.2 Haematological characterization of sensitized mice

The five main immune cells in haematology are lymphocytes, monocytes, neutrophils, basophils and eosinophils (World Health Organization, 1980; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022). In the study, only basophils were not identified in the polymorphonuclear counts of *Mus musculus* experimental and control groups. This is because basophils are the scarcest circulatory granulocytes being only 0.3% of all nucleated cells in the bone marrow (Bosch et al., 2011) and also have the smallest diameter range of 11–13 µm (World Health Organization, 1980). The dominant polymorphonuclear cells were neutrophils and lymphocytes, followed by monocytes and eosinophils (Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022). The relatively lower amounts of monocytes and eosinophils elicited in the study, can be associated with their sources, since the formation of eosinophils takes place in the bone marrow and derived from pluripotent stem cells (Sanderson, 1992), while monocytes are often formed from promonocytes in the bone marrow (Eastham, 1992). Monocytes infiltrate the blood and proliferate, reaching the tissues as mature macrophages; they are seen in connective tissues but frequently appear in the liver, spleen, lymph nodes and lungs (Eastham, 1992). The most abundant leukocytes are neutrophils formed within a little duration of time, but are constantly present in the human immune system, because they are steadily produced by the bone marrow (Bosch and Ramos-Casals, 2014; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022). The atypical lymphocyte (lymphocyte anomaly) recorded in some *Mus musculus* in *Syzygium guineense* and *Mariscus alternifolius*, is a non-malignant aberrant lymphocyte that can be found in the blood. It is initiated by a non-specific reaction to stress from various stimuli, leading to manifestations of an array of clinical disorders (Karalyan et al. 2016; Alebiosu and Adekanmbi, 2022).

Certain differential white blood cells and total white blood cells were significantly elevated between sensitization periods in the experimental groups compared to the control and this aligns with Nadimi et al. (2008), Adekanmbi et al. (2018b), Alebiosu and Adekanmbi (2022) who recorded significant variations in mean levels of leukocytes elicited by the test and control groups of allergic individuals with coronary artery disease. According to Stone et al. (2000), an elevated level of peripheral blood and tissue eosinophils, is typically associated with to mild peripheral blood eosinophilia, resulting into varied allergic conditions such as atopic dermatitis, allergic rhinitis and atopic asthma.

4.3 Serological characterization of plasma from induced mice

In the serological investigation, elevated levels of IgE, IL-5 and IL-13 were significantly found in some *Mus musculus* test groups compared to the control, while no significant increase was recorded for TNF-α levels in the experimental groups, except after the first sensitization in the *Senna siamea* group. This corresponds with Alebiosu and Adekanmbi (2002) who recorded no significant difference between TNF-α levels in their test

groups and those of the control. This is also in accordance with Bettiol et al. (2000) who observed no significant variation in the levels of TNF-α among atopic, non-atopic and healthy individuals. They also discovered higher counts of serum IgE and blood eosinophils in atopic and non-atopic subjects, compared to the control group and this further corroborates the findings of significantly elevated levels of serum IgE in *Senna siamea*, *Zea mays* and *Mariscus alternifolius* in the present study. This is also in line with Conejero et al. (2007) who recorded considerably higher amount of IgE, IL-5, and IL-13 in allergen-specific serum, compared to control and mice administered with alum only, after experimental mice were subcutaneously injected with pollen of *Olea europaea*. Moverare et al. (2000) reported that cultures stimulated with birch pollen extracts yielded greater IL-5/ IFN- and IL-13/ IFN- ratios, compared to cells stimulated with tetanus toxoid. They also recorded that IL-5/ IFN- ratio in cultures stimulated with birch pollen extracts, was considerably greater than that of the control group. Abou-Chakra et al. (2009) found that administration of a rat group with pollen cytoplasmic granules of *Phleum pretense*, brought about elevated levels of IL-5 and IL-13 in broncho-alveolar lavage fluid, in comparison to the negative control group. They observed that those administered with only pollen grains from this plant elicited no considerable variation in IL-5 and IL-13, in comparison to the negative control group. They also noted that rat test groups challenged with both pollen grains and pollen cytoplasmic granules, yielded inconsiderably lower levels of IL-5 and IL-13 compared to the test groups administered with pollen cytoplasmic granules alone.

An elevated level of serum IgE is a marker of allergic inflammation and atopy, which potentially induces an asthmatic condition (Moverare et al., 2000; Razi and Moosavi, 2010; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022). Krishna et al. (2017) recorded an important relationship between levels of serum IgE and asthma. Ngoc et al. (2005) affirmed that allergen-specific T_H2 responses and consequent release of interleukins including IL-4, IL-5, and IL-13, leads to a cascade of events preceding allergic inflammation. The initiation, differentiation and persistence of allergy responses are influenced by major cytokines including IL-4, IL-5, and IL-13 (Conejero et al., 2007; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

4.4 Immunological interaction among allergy mediators

Mean levels of haematological and serological markers in the study, were controlled by the magnitude of immunological responses produced by the *Mus musculus* experimental groups during periods of pollen challenge and this portends varying limits of tolerance to their administration with different pollen allergens (Janeway et al., 2005; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022). Some haematological and serological markers produced by all *Mus musculus* experimental groups, revealed a substantially positive relationship and this reflects a multiplex of interaction among allergy triggers at different periods of sensitization. These findings are in congruence with Adekanmbi et al. (2018) who reported a significant association among immunological markers in *Mus musculus* previously sensitized with pollen of *Alchornea cordifolia* and *Tridax procumbens*. Alebiosu and Adekanmbi (2022) recorded an important correlation among allergy mediators in *Mus musculus*, earlier challenged with pollen of *Borreria verticillata*, *Leucaena leucocephala*, *Terminalia catappa* and *Panicum maximum*. Gabrielsson et al. (1998) recorded a correlation between the amounts of IL-13-producing cells and IgE levels in pollen-stimulated allergen-specific serum among pollen allergy sufferers. They emphasized the involvement of IL-13 and other T_H2 cytokines in the regulation of allergen-specific IgE levels in atopic individuals. Moverare et al. (2000) stressed the significance of examining IgE-mediated diseases through the estimation of IL-4 and IL-13 secretions in peripheral blood mononuclear cells. According to Corrigan and Kay (1992), normal allergic reactions to inhaled birch pollen allergens were stimulated by T_H2 cytokines such as IL-4, IL-5 and IL-13, as promoted by elevated release of allergen-specific IgE. In hypersensitive individuals, the release of IgE is initiated by many interactions in which T-cells, B-cells, eosinophils, mast cells, IL-4 and IL-13 are involved (Razi and Moosavi, 2010).

In the *Mus musculus* experimental groups, a considerably negative relationship was observed among some haematological and serological parameters; these findings are buttressed by Neaville et al. (2003) who noted a negative relationship between IL-5 and IL-13 production among subjects from birth to the age of one year; Van-Cauwenberg et al. (2006) affirmed that IL-5 and TNF-α enhance cell activation; recruitment of neutrophils, eosinophils and T-lymphocytes, and also facilitates the stability of eosinophils within the tissues of nasal mucosa; Corrigan and Kay (1992) pointed out the key role of IL-5 and IL-13 in elevating the level of activated eosinophils at regions of inflammation including the bronchial mucosa; Taube et al. (2002) posited that IL-5 is veritable for the differentiation, activation, persistence and recruitment of eosinophils; Luttman et al. (1996) affirmed that IL-13 further activates allergic reactions by enhancing the differentiation and persistence of eosinophils.

However, the levels of eosinophils and cytokines elicited by experimental groups in the study, yielded no significant association and this corresponds with Bettiol et al. (2000) who recorded no association between eosinophil counts and cytokine levels in non-atopic asthmatics; in addition, one reason for this correlation lag might have been as a result of longer cytokine production after allergen challenge (Jung et al., 1996); another plausible reason for the lack of correlation is eosinophil scarcity in the blood, as reflected by their levels of production, given that they are usually accumulated in the bone marrow (World Health Organization, 1980; Sanderson, 1992 Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022) and this differs from cytokines produced by T_H2 cells as an integral component of the peripheral blood cells (Moverare et al., 2000).

Necrotic areas and congestion were recorded in the bronchi and lung tissues of dead *Mus musculus* in *Senna siamea* test group. This is similar to the work of Alebiosu and Adekanmbi (2022) who reported histological morbid characteristics including extensive areas of inflammatory cells and alveoli filled with fluid in the lungs of a *Mus musculus* mortality. The accumulation of neutrophils, lymphocytes and cytokines in the blood of

Mus musculus perhaps stimulated hyperresponsiveness to the pollen allergen, leading to the incidence of inflammation (Wills-Karp, 1999 and Taube et al., 2002). This is also supported by Janeway et al. (2005) who stated that mice with a condition similar to asthma, elicit greater amounts of T_H2 cytokines including IL-4, IL-5, and IL-13, as well as the stimulation of airway inflammation with the involvement of lymphocytes and eosinophils. They posited that the degranulation of mast cell and T_H2 activation, induce accumulation of eosinophils which becomes stable. They also suggested that this stability is characteristic of severe allergic inflammation and may be pivotal in the damage of tissues. Vignola et al. (1999; 2000) submitted that the unique friability and shedding of the bronchial epithelium during asthmatic cases, could be associated with an alteration in the inflammatory processes stimulated by apoptotic cells in the respiratory routes. Gleich (1990) suggested that toxic compounds originating from eosinophils, may be involved in the rupture of tissues situated in bronchi-linked airways. Schneider et al. (1997) discovered that neutrophils, lymphocytes, eosinophils and macrophages concentrated in the lungs of Brown-Norway rats previously administered with ovalbumin and also ovalbumin infiltrated with the bacterium, *Bordetella pertussis*; they also observed eosinophil recruitment in bronchial submucosa, together with interstitial and submucosal inflammation comprising infiltrates of mononuclear cells and eosinophils; they submitted that neutrophils account for an integral composition of allergen-induced white blood cells and are dominant in lung tissues. Neutrophils and some other triggers of inflammation, have been associated with tissue damage and responses from respiratory pathways (Kay and Corrigan, 1992; Suzuki et al., 1996). Sur et al. (1993) stated that intense infiltration of neutrophils led to lethargic asthma. Bochenska-Marciniak et al. (2003) opined that chronic bronchitis and allergic rhinitis are some of the most predominant neutrophilic and eosinophilic inflammatory reactions, respectively; they also submitted that the presence of different cytokines may be useful either as a synergy or individually, in making an inference about the kind of prevailing inflammation. Severe respiratory inflammation is induced by inflammatory cells through the production of different cytokines and inflammatory triggers (Bochner et al., 1994 and Lukacs et al., 1995). According to Walsh (2000), inflammation of the respiratory pathways is basically involved in the development of asthma and is initiated by a great number of pro-inflammatory cells dominating the bronchial mucosa. Campbell et al. (1998) cited that several triggers of hyperresponsiveness in bronchi and asthmatic responses at both early and late phases in humans, are similar to those in mouse lungs. Tumas et al. (2001) stated that the histological, functional and anatomical features of lungs in mice are identical to those of humans. However, it is worthy of note that the subcutaneous method of pollen allergen challenge employed in this investigation, has been reported to be the most efficient for producing the highest amount of IgE and inflammation of the respiratory pathways in mice (Wills-Karp, 1999; Repa et al., 2004; Adekanmbi et al., 2018b; Alebiosu and Adekanmbi, 2022).

4.5 Allergenicity of Poaceae pollen

Among the selected pollen types, a comparatively higher crude protein concentration was recorded in the pollen of *Zea mays* belonging to the family Poaceae. This aligns with Alebiosu and Adekanmbi (2022) who observed greater crude protein content in the pollen of *Panicum maximum*, compared to other selected pollen types in their study. This is also supported by Bastl et al. (2016) who emphasized the importance of allergen content as a considerable factor for allergy sufferers and may pose a larger impact on these individuals than atmospheric pollen levels. This further corroborates the pollen allergenic activities of this plant family, as previously reported by several workers: Suphioglu et al. (1992) cited that pollen of Poaceae consists of antigens being dispersed into the air once dehiscence occurs. Pollinosis has been associated with the predominance of atmospheric Poaceae pollen initiating allergic responses (D'Amato et al., 2007; Taketomi et al., 2008). According to Singh and Dahiya (2008) who undertook an immunological survey in some parts of India, Poaceae was noted as one of the common provenances of pollen allergens in Kanpur and Central India. Galant et al. (1998) observed a higher incidence of positive reactions to grasses among allergic individuals, compared to trees, molds, weeds and some other producers of allergens in the atmosphere of California, USA. Pollen of *Pennisetum* sp., *Imperata* sp. and *Cynodon* sp. belonging to the Poaceae family, has earlier been proven to be the major aeroallergens in Delhi (Singh et al., 1987; Rajkumar, 2003). Ahlawat et al. (2013) established the allergenicity of some grasses such as *Imperata cylindrica*, *Cynodon dactylon*, *Pennisetum typhoides* and *Sorghum vulgare*, using skin prick test and ELISA.

Pollen of Poaceae has been regarded as the dominant airborne allergen inducing pollinosis in many parts of the world (Sanchez-Mesa et al., 2003; D'Amato et al., 2007; Garcia-Mozo et al., 2010; Scevkova et al., 2015). Abou-Chakra et al. (2009) affirmed that allergenic pollen produced by Poaceae, triggers respiratory diseases in several industrialized parts of the globe. According to D'Amato et al. (2017), atmospheric pollution can induce allergenicity in pollen grains by combining with pollen surfaces, enhancing their allergenic activities and stimulating airway inflammation in allergic sufferers. Pollen hypersensitive people may experience increased reactivity to inhaled allergens in respiratory pathways, resulting from atmospheric pollution and hyper-responsiveness in the bronchi (D'Amato et al., 2002).

5.0 Conclusion

This is the first scanning electron microscopy of aerospora in the southern region of Nigeria. Angiosperms of varying plant habits contributed to atmospheric pollen spectra at the sampling sites, regardless of height. Relative humidity played an important role in influencing atmospheric pollen distribution in Anambra State. Airborne pollen types were dispersed by some parent plants in the immediate vegetation at the study locations. The current investigation provides information about safe-risk periods for pollen allergic sufferers at the study sites, through the formulation of pollen calendar. The airborne spore distribution of represented ferns has provided an additional information about the pteridology of these locations.

The allergenic activities of some dominant atmospheric pollen types have been revealed in female *Mus musculus* through a multiplex of interaction among allergy triggers at different periods of sensitization in mice, establishing a model of immune response to varied pollen allergens in humans. However, immunotherapeutic administration in hypersensitive people will be ameliorated by a more adequate knowledge of interaction among triggers of inflammation associated with allergy posed by predominant atmospheric pollen types in the study. Allergenicity of these pollen types should also be investigated using pollen allergic individuals by means of skin prick test.

Data from pollen monitoring over successive years in several locations of Nigeria, should be documented for a better understanding of long-term dynamics in their atmospheric distribution. This step will enable prophylaxis by pollen allergic sufferers to mitigate the exacerbating burden impacted by pollen allergens. This will also guide allergy experts and other health-care providers in Nigeria, to achieving more accurate diagnoses and treatment episodes for pollinosis patients.

Declarations

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Compliance with Ethical Standards: The research study involved using animals (*Mus musculus* mice) subjected to international ethical standards of animal welfare.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors have read, understood, and have complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors.

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Figures

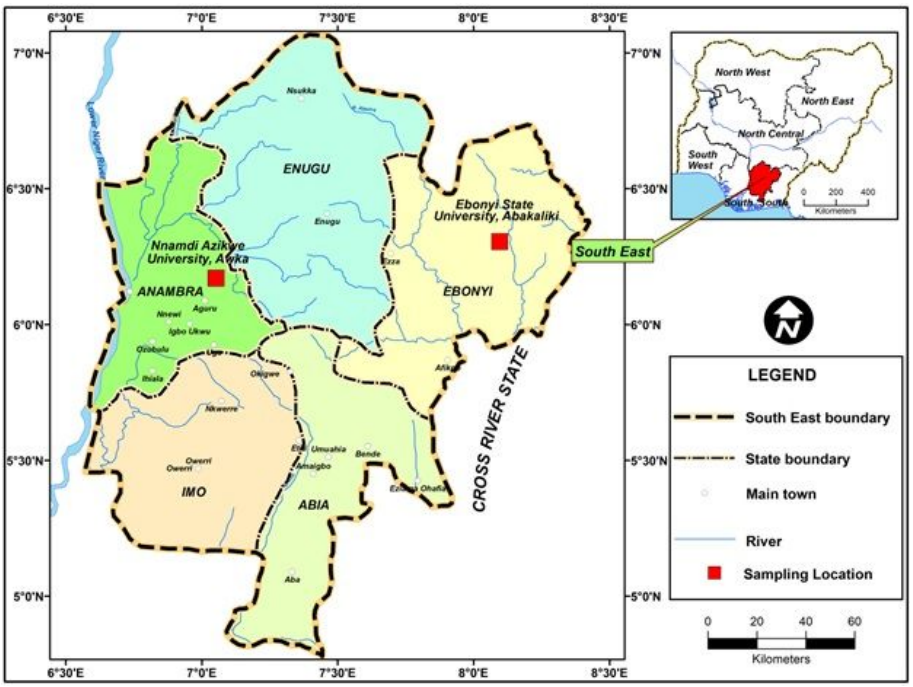
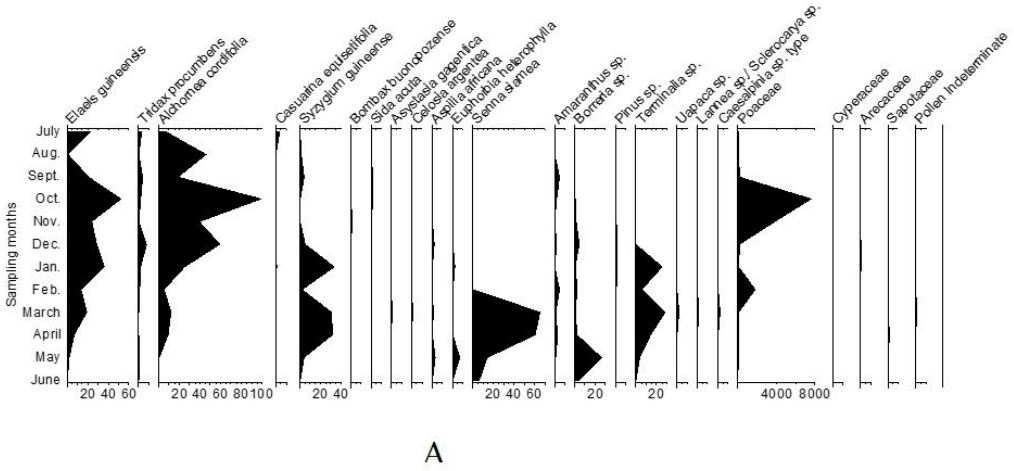


Figure 1

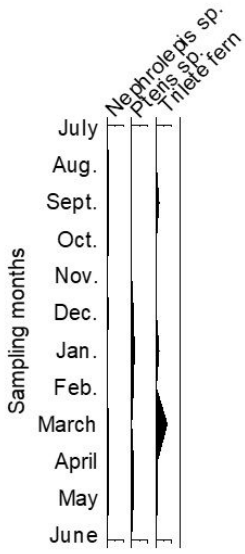
Map showing the sampling stations in Ebonyi and Anambra states of South-Eastern Nigeria.

Figure 2

A mounted pollen sampler at the sampling station in Ebonyi State University, Ebonyi State, Nigeria.



A



B

Figure 3

a. Pollen calendar showing the distribution of pollen types in the atmosphere of the study site in Ebonyi State University, Ebonyi State. b. Fern spore calendar showing the distribution of fern spore types in the atmosphere of the study site in Ebonyi State University, Ebonyi State.

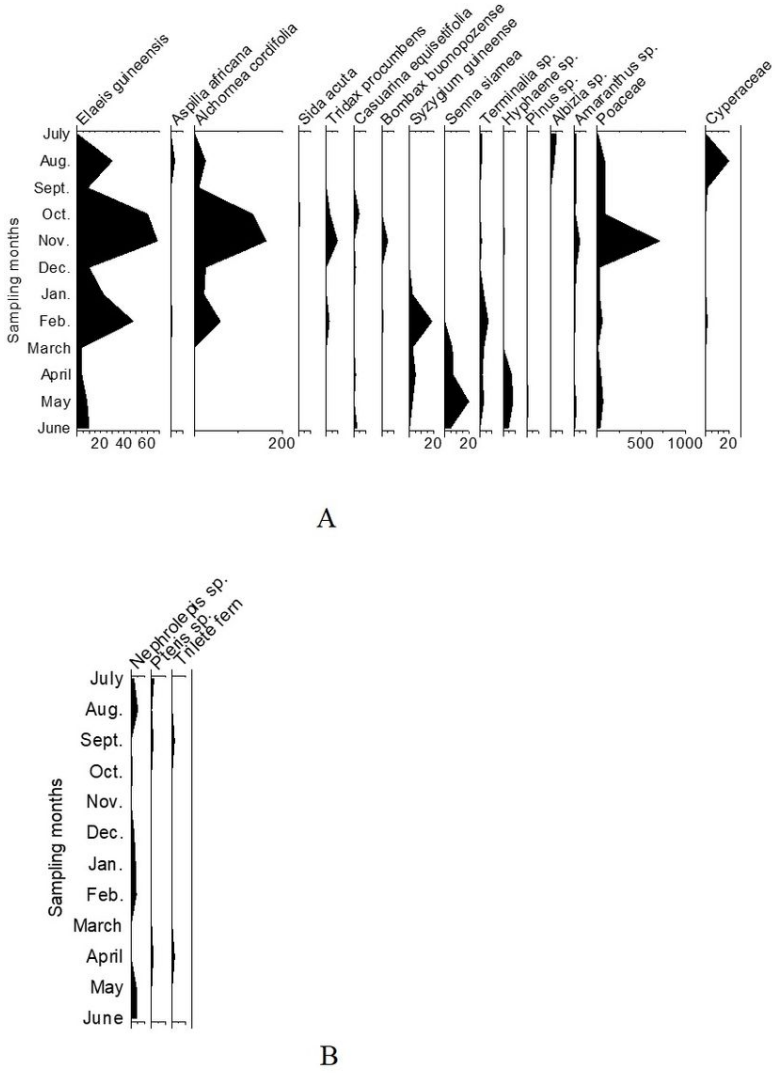


Figure 4

a. Pollen calendar showing the distribution of pollen types in the atmosphere of the study site in Nnamdi Azikwe University, Anambra State. b. Fern spore calendar showing the distribution of fern spore types in the atmosphere of the study site in Nnamdi Azikwe University, Anambra State.

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