A PRELIMINARY AERO-PALYNOLOGICAL SURVEY OF UNIVERSITY OF UYO CAMPUSES, UYO, SOUTH SOUTH NIGERIA.

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ABSTRACT

Atmospheric pollen grains/spores are diverse and are major biological particles that trigger immune cells to release inflammatory chemical mediators, inducing respiratory-linked allergic conditions. More so pollen grains/spores have unique identifiable architectural structure relevant in taxonomical analysis of plant families and specific epithet. Based on this relevance, a preliminary investigation of airborne palynomorphs was conducted within two campuses; University of Uyo main campus and University of Uyo Town campus using a modified Tauber pollen and spore trap, stationed in four locations for 3 months (October - December 2019). A total of 107 species comprising 7 pollen types belonging to 5 plant families were recovered from the study area. Sixty-eight (68) fungi spores belonging to 5 families were dominant; other palynomorphs included1Pteridophyte spore, 1bacterial spore, 4 Indeterminate palynomorphs and 4 Monolete spores. Results show that spores were higher and more abundant than pollen types from the sampling locations. Recovered abundant pollen types in University of Uyo main campus include *Elaeis guineensis, Cyperus* sp., *Alchornea cordifolia* and *Amaranthus* sp. Also recovered were pollen grains of Poaceae, Arecaceae, Asteraceae and Euphorbiaceae. Fungi spores recovered include species of *Alternaria, Nigrospora, Curvularia* and *Aspergillus*. Pollen taxa of Poaceae and Arecaceae were dominant while *Nigrospora* species and *Aspergillus* species were the abundant occurring fungal spores' genera. The recovered abundant pollen types in University of Uyo Town campus includes *Aspilia africana* and *Phyllanthus* spp. Common fungal spores include those of genera*Alternaria*, *Curvularia*, *Gliomastix*, *Nigrospora*, *Pithomyces*, *Tetraploa*, *Exosporium*, *Meliola* and *Torula*. Pollen taxa of Asteraceae were dominant whereas *Curvularia* and *Nigrospora* were the abundant occurring fungal spores. The relevance of fungal spores as aeroallergens is commonly cited in the literature, hence, further investigation and molecular characterization of these spores and pollen grains recovered within the study area might be necessary for future pollen forecast and public health purposes.

INTRODUCTION

Pollen and spores of anemophilous plants are widely dispersed across large landscapes, dependent on prevailing climatic conditions. Other factors that help their spread include size, shape and density of the pollen and spores. However, wind-borne pollens can cause allergic reactions in both humans and animals, and in the past three decades pollen allergies have significantly increased (Bousquet *et al.,* 2001). Thus, the analysis of atmospheric pollens informs the public about levels of allergenic pollen (D'Amato *et al.,* 2007). It also, provides information on the phenology exhibited by anemophilous plants. Additionally, it helps to predict crop yields and aids the evaluation of climate change effects on floras at both local and regional scales (Gage *et al.,* 1999; Garcıa-Mozo *et al.,* 2006; Galan *et al.,* 2008). Yet less than 100 of the over 250,000 pollen producing plants are potent sources of allergens (Ziello *et al.*, 2012). In terms of potency, pollen allergens belong to various protein families, with profilins and EF-hand containing calcium-binding proteins being the most abundant group (Di Felice *et al.,* 2001; Andersson and Lidholm, 2003; Mothes *et al.,* 2004; Radauer and Breiteneder, 2006; Asam *et al.,* 2015). Generally, allergies are a heavy socio-economic burden worldwide (Gilles and Traidl-Hoffmann, 2014), as its prevalence is reported to be in the range of 20-30% (Pawankar *et al*., 2008). Furthermore, allergic disorders are so great that they can result into significant morbidity, employee absenteeism, loss of quality of life and in some instances, fatal outcomes (Potter *et al.,* 2009).

Consequently, efficient prevention strategies must evolve based on empirical evidence of pollen calendar and influences of pollinosis across urban and rural landscapes. Meteorological parameters such as temperature, rainfall, relative humidity, wind direction and velocity are linked to fluctuations in pollen concentrations (Adeniyi *et. al.,* 2014). However, rainfall and relative humidity usually yield negative correlations with pollen concentration in the air (Angosto *et al*., 2005; Piotrowska, and Kaszewski, 2011; Aboulaich *et al.,* 2012; Adeonipekun *et al.,* 2016). Recent concerns involve the trending global warming associated with elevated carbon dioxide (CO_2) levels. Effects include prolonged flowering seasons, which likely increases the load of allergenic pollen. For instance, the aggressive spreading of allergenic neophytes associated with abnormal flowering seasons, such as *Ambrosia artemisiifolia*, in Europe (Ziello *et al.,* 2012) and *Casuarina equisetifolia* in Nigeria(Njokuocha, 2006; Adeniyi *et al.,* 2014; Ezike, 2015; Ezike *et al.,* 2016). Against this backdrop, the present study was designed to develop a pollen calendar for Uyo metropolis with the following objectives: (i) characterize the pollen type within Uyo, and (ii) assess the most dominant/prevalent group of pollens or spores within the study area.

METHODOLOGY

Study Area:

Akwa Ibom state located at the south-south region of Nigeria, is characterized by tropical climate with two distinct seasons: wet and dry seasons. The wet season lasts between eight to nine months starting from mid-March till the end of November. The dry season has a short duration, from late November and lasts till early march. Despite the seasonal variations, by the nature and location of the state along the coast which exposes it to hot maritime air mass, rainfall is expected every month of the year. The State currently covers a total land area of 7,249 square kilometers. It is the 10th largest state in Nigeria in terms of landmass (Akwa Ibom State Government, AKSG, 2021). About 13.4 percent of the 960km of Nigeria's Atlantic Ocean coastline runs through the State (AKSG, 2021).

Sampling Methodology:

Pollen data were collected purposefully from four sites within University of Uyo campuses including: Two sites at the Main Campus;

1. University of Uyo Botanic Garden, UUBG (Latitude 05º02'20.4" N; Longitude 07º58'53.7" E),
2. Outer perimeter of UUBG (Latitude 05º02'21.8" N; Longitude 07º58'52.0" E).
   Also, two sites within Town campus,
3. Convocation Park (Latitude 05°02'20.2"N; Longitude 07°55'27.9"E) and,
4. Meteorological Synoptic Station (Latitude 05°02'16.3"N; Longitude 07°55'26.1"E).

Modified Tauber pollen trappers according to the method prescribed by Tauber (1974) and adapted after Agwu and Osibe (1992) were mounted on select trees within each sampling location. At the University of Uyo (UU) main campus, one Tauber trapper was mounted at the topmost branch of a 5 feet African Oil bean tree, *Pentaclethra macrophylla* (Figure 1). A similar trapper was mounted on 7 feet high *Ficus* tree species. at the convocation park of the town campus. For the two remaining sites, i.e., UUBG and the synoptic station; an improvised pollen buckets (measuring; 25 cm high and 17 cm wide) was mounted on tripod stand to trap palynomorphs (Figure 2).

A mixture of 50 ml of glycerol to provide stickiness, 10 ml of formaldehyde to preserve the trapped palynomorph and 5 ml of phenol to scare insects away were poured into the improvised samplers, placed in the locations (Essien and Agwu, 2013). The pollen traps were left for the period of three months (October – December 2019). But were periodically harvested monthly for acetolysis and microscopy of recovered palynomorphs.

Figure 1: Modified Tauber Trap Figure 2: Pollen Bucket

Pollen and Spore Isolation and Identification:
After the aero samples were collected from the selected location, they were taken to the laboratory and allowed to settle properly for about 24 hours. Thereafter the supernatant was decanted, and the residue preserved for acetolysis (Erdtman, 1969). The mixture was poured into plastic centrifuge vials, and then centrifuged at 2000 revolution per minute (rpm) for five minutes after which the supernatant was decanted and then a mixture of concentrated sulphuric acid and acetic anhydride at a ratio of 1:9 (1ml of H_2SO_4 and 9ml of $C_4H_6O_3$) is added to the residue in the centrifuge vial and then agitated (shaken). The centrifuge vials containing the mixture was then positioned in a rack and placed inside a water bath machine containing water and heated at 100ºC for ten (10) minutes. After the interval of ten minutes the mixture from the water bath was placed in the centrifuge machine and then centrifuged at 2000rpm for ten (10) minutes. The

supernatant (the acid) was decanted, and the residue was washed by adding distilled water, agitated (shaken), placed in the centrifuge machine and centrifuged at 2000rpm for five (5) minutes. The process of washing was done thrice and each time the supernatant was decanted till traces of the acetolysis mixture were gone and the residues were stored in vial bottles and labeled according to location and month of collection.

Microscopic Analysis:
Two drops of the prepared residue were pipetted gently into a labeled microscopic slide using a micropipette and covered with a 22 x 22 mm size cover slip. A slide sealant was used to seal the slide and left to air-dry for 2 minutes. Thereafter, the prepared slides were viewed under the microscope. Photomicrographs of pollen grains and spores were taken using an Olympus light microscope (X40 magnification) mounted with a Moticam® 2.0 camera.

RESULT

Tables 1a-1d shows recovered palynomorphs and their distribution across the two campuses; while figure 3, are photomicrographs of some recovered species from the study area. A total of one hundred and seven (107) species comprising of seven (7) pollen types belonging to five (5) plant families were recovered from the pollen trappers. Sixty-eight (68) fungal spores belonging to five (5) families were dominant, other palynomorphs included, one (1) Pteridophyte spore, one (1) bacterial spore, four (4) Indeterminate palynomorphs and four (4) Monolete spore (undefined).

Asteraceae species had the greatest number of the pollen group, whereas Trichospaeriaceae were the most abundant spore species.Other plant families found in lower amounts within the recovered palynomorphs included Poaceae, Euphorbiaceae and Cyperaceae.

Table 1a: Palynomorphs from University of Uyo, Main Campus Botanic Garden

Site 1: UUBG					
Family	Name of Species	Oct	Nov	Dec	Total
A. Pollen					
ARECACEAE	*Elaeis guineensis*	-	1	-	1
CYPERACEAE	*Cyperus* sp.	1	-	-	1
B. Fungal Spores					
PLEOSPORACEAE	*Curvularia* sp.	1	-	-	1
SACCHAROMYCETACEAE	*Torulla* sp.	2	-	4	6
TRICHOSPAERIACEAE	*Nigrospora* sp.	2	5	3	10
VENTURIACEAE	*Venturia* sp.	-	1	1	2
Monolete spores	Monolete	4	-	-	4
Undefined spores	-	1	-	-	1
TOTAL		11	7	8	26

Table 1b: Palynomorphs from University of Uyo, Main Campus Botanic Garden Perimeter Fence

Site 2: UUBG Perimeter Fence					
Family	Name of Species	Oct	Nov	Dec	Total
A. Pollen					
ARECACEAE	Indeterminate	1	-	-	1
EUPHORBIACEAE	*Alchornea cordifolia*	-	1	-	1
POACEAE	Indeterminate	1	-	-	1
ARECACEAE	Indeterminate	1	-	-	1
B. Pteridophyte/Fungi spores					

PTERIDACEAE	*Pteris* sp.	-	1	-	1
BIONECTRIACEAE	*Gliomastrix* sp.	-	-	2	2
PLEOSPORACEAE	*Curvularia* sp.	3	-	2	5
	Alternaria sp.	1	1	2	4
	Pithomyces sp.	1	1	-	2
SACCHAROMYCETACEAE	*Torulla* sp.	1	-	-	1
TRICHOSPAERIACEAE	*Nigrospora* sp.	8	4	3	15
Monolete spores	-	7	-	-	7
Indeterminate	-	4	-	-	4
TOTAL		27	8	9	44

Table 1c: Palynomorphs from University of Uyo, Town Campus Convocation Park

Site: 3. Convocation Park					
Family	Name of Species	Oct	Nov	Dec	Total
A. Pollen	-	-	-	-	-
B. Spores					
BIONECTRIACEAE	*Gliomastix* sp.	-	1	-	1
PLEOSPORACEAE	*Alternaria* sp.	1	-	-	1
	Curvularia sp.	3	2	2	7
	Pithomyces sp.	1	-	1	2
TETRAPLOSPHAERIACEAE	*Tetraploa* sp.	1	1	1	3
TRICHOSPHAERIACEAE	*Nigrospora* sp.	2	1	1	4
TOTAL		8	5	5	18

Table 1d: Palynomorphs from University of Uyo, Town Campus Synoptic Station

Site 4: Synoptic Station					
Family	Name of Species	Oct	Nov	Dec	Total
A. Pollen					
ASTERACEAE	*Aspilia africana*	2	4	-	6
EUPHORBIACEAE	*Phyllanthus* sp.	3	-	-	3
B. Bacteria/Fungi Spores					
BACILLACEAE	*Exosporium* sp.	1	-	-	1
MELIOLACEAE	*Meliola* sp.	1	-	-	1
SACCHAROMYCETACEAE	*Torula* sp.	1	-	-	1
Monolete spore	-	-	1	2	3
Indeterminate	-	2	1	1	4
TOTAL		10	6	3	19

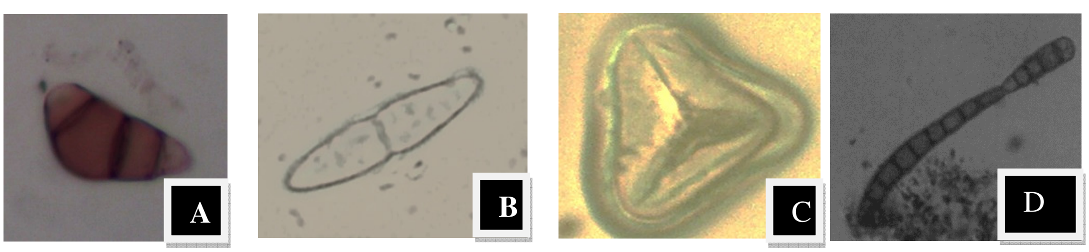

Figure 3: Selected Photomicrographs, (Magnification X40)

A: *Curvularia* sp., B: *Venturia* sp., C: *Elaeis guineensis*, D: *Torula* sp.

DISCUSSION

The results show clearly the presence of palynomorphs within the atmosphere of Uyo, a fast-cosmopolitan urban environment. However, the distribution pattern developed points to the flowering phenology of each taxon. In essence, availability of represented pollen or spore is associated with the time of flowering and maturity. Importantly however, is the spatial and daily variation that correlates with meteorological factors as stated in earlier sections from the onset. Jones and Harrison (2004) further confirm that the bonding of pollen to surfaces is affected by temperature and moisture. Although meteorological parameters were not assessed, yet, our inference of the high distribution patterns of spores is premised on their ability to adapt to increased temperatures that characterizes the onset of the dry seasons within the study area. This may determine the timing of the flowering season and release of fungi spores by way of photoperiodism, the rate of maturation of conidia, as well as the development of flowering organs through their physiology, or by affecting the dynamics of the air which the spores travel through (Lighthart *et al*., 1979; McDonald, 1980; Benninghoff, 1987). Furthermore, the amount of sunshine, rain or wind speed affect how much pollen/spore is released and how much the pollen/spore is spread around. On humid days, pollen/spore spreads slowly, during windy days, pollen/spore are transported over long distances (Gregory, 1978).

It is noteworthy that a considerable similarity exists in the array of pollen types recovered from the two locations, primarily based on their close latitudinal position. More so is the similarity in ecological zonation and vegetation. However, there is variation in the amount of palynomorphs recovered from the University of Uyo main campus, with a total of 44 species. This may be attributed to the level of extant vegetational cover within and around the vicinity of the campus, in comparison to the densely human and infrastructural presence at the town campus area.

CONCLUSION

The results of the present study have revealed the atmospheric distribution of different pollen/spore types recovered from the four-sampling location and quantitatively, the percentage composition of spores was higher and more abundant than the pollen type within the study area. However, the limitations of the available data, demands more research and an increased scoping area in tangent to seasonality. Thus, further longitudinal study of the entire state and molecular characterization of extant pollen/spore distribution will help define the rate of pollinosis within the study area. In addition, meteorological parameters will also aid better interpretation of climatic changes and its influence on the presence and distribution of these palynomorphs.

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