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Evaluation of Pollen Grains and Spores Potential in Revealing Vehicle Travel History on the Ife-Ondo Road, Southwestern Nigeria

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ABSTRACT: This study focuses on the significance of airborne pollen grains and spores as markers for tracking vehicles along specific routes. Using standard methods, the research examined monthly pollen distribution and its correlation with meteorological data. Dust and soot samples were collected monthly from the car air filters of commercial vehicles traveling on the Ife-Ondo road and subjected to palynological analysis for a period of 24 months. The findings revealed a total of 393 pollen grains and 131 spores, representing 40 pollen and 22 spore types from 27 and 17 families, respectively. Notable markers among these include *Hagenia abyssinica, Podocarpus milanjianus, Theobroma cacao, Cannabis sativa,* and *Myrianthus arboreus*, among others. These markers serve as crucial identifiers for vehicle-related investigations. The study revealed a statistically significant positive correlation between monthly total airborne pollen concentrations and relative humidity, rainfall, and wind speed. In a practical context, the study has created a valuable dataset and monthly atlas of pollen and spore types. This resource can be instrumental for security agencies in their efforts to investigate vehicle-related crimes and monitor vehicular movements along the Ife-Ondo road.

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The advent of vehicles has transformed modern society by providing independence and freedom of mobility. Vehicles have become of central importance both as objects of theft and as essential tools for offenders. Unfortunately, terrorists and criminals use vehicles to commit crimes such as kidnapping, arms dealing, robbery, fraud, as well as human and drug trafficking (INTERPOL, 2014). Hence, it is crucial to track the movement of vehicles as a means to apprehend the criminals involved. The security agencies are constantly burdened with the enormous task of obtaining evidence to link a suspect and their vehicles to certain routes. Unfortunately, they are still neophytes in the use of biological evidence for criminal investigations. As a result, during prosecutions, almost all confessional statements are challenged on the basis of police coercion. A significant challenge facing law enforcement agencies has been identifying vehicles associated with crime scenes and suspects (Orijemie and Israel, 2019; Adekanmbi *et al.,* 2021). To address the challenge, there is a need to generate data sets of ecological marker species that will be used to associate vehicles with specific routes. Car air filters (CAFs) are installed in ventilation systems and motor engines to prevent particulate matter from tampering with engine mechanics. They have also been used as low-cost "active-passive" air sampling media for hydrophobic organic compounds and palynomorphs for forensic palynological studies (Katsoyiannis *et al.*, 2012; Cai *et al.,* 2014; More *et al.*, 2013; Orijemie and Israel, 2019). The parts of the car, particularly the air filters, hold a large amount of aerosol, which contains pollen grains, spores, fungi spores, insects, and other constituents (More *et al.*, 2013). Pollen and spores from plants are dispersed and can get attached to objects within the surrounding environment during descent, which could assist in identifying source plant communities, source environments, and source areas for evidence (Mildenhall *et al.,* 2006). Most pollen grains are unlucky and end up as components of soil and dust, falling onto any surface exposed to the air

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and away from the parent plants, forming the aerospora. Those that end up in the atmosphere reflect its local vegetation (Adekanmbi and Ogundipe, 2010), and objects recovered from such an environment can be associated with it depending on the constituent palynomorphs. Pollen and spores are excellent proxy indicators of place. A particular pollen type can provide a locality of origin based on the plants that produced the pollen. This may allow people or objects of interest to be linked through their movements from one location to another (Horrocks and Walsh, 1998). Thus, the objective of this study is to evaluate the potential of pollen grains and spores in revealing vehicle travel history along the Ife-Ondo Road, Southwestern Nigeria.

MATERIALS AND METHODS

Description of the study area: Ife-Ondo Road (Trunk A) is a major highway in Nigeria that connects the cities of Ife and Ondo. It is a busy road that sees a lot of traffic, including both private and commercial vehicles. The study route comprises of 76.0 km and is located between (Latitude: 07.491.87ºN, Longitude: 004.552.11ºE, to Latitude: 07.104.09ºN, Longitude: 004.820.59ºE) as shown in Figure 1.

Laboratory analysis: Twelve commercial vehicles were chosen at random for sampling on the Ife-Ondo road between January 2020 and December 2022. Dust and soot from a newly fitted automobile air filter were removed once a month for 24 months by shaking the car air filter and putting the dust and soot in a sterile 20 ml sample bottle (Figure 2). The sample bottles were labeled, carefully sealed, and stored in the freezer. After screening to remove small debris, dust and soot were macerated using hydrochloric acid (HCl) and hydrofluoric acid (HF) to dissolve the carbonate and silicic acid components. Furthermore, to remove labile organics, sediments were subjected to acidified zinc chloride before being treated with acetic anhydride and sulphuric acid. To keep the palynomorphs from drying out, the final residue was stored in 100% glycerine. Slides for quantitative microscopic investigation were prepared. A light microscope with a (*40) objective lens was used to scan and count the prepared slides, and photomicrographs of chosen palynomorphs were obtained using a Moticam 2300 (Plate 1).

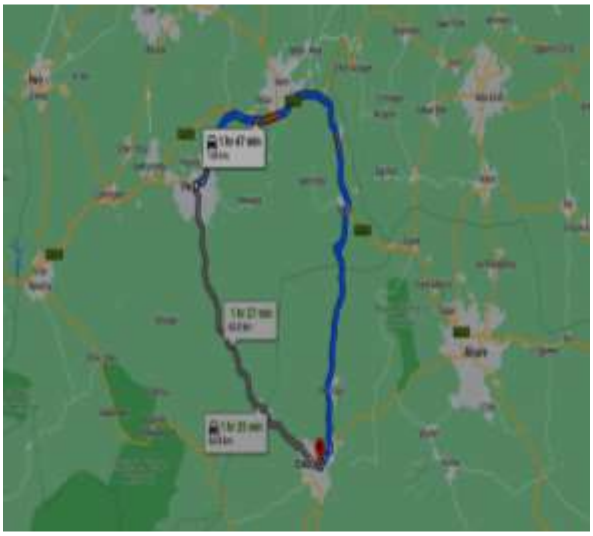

Fig 1. The travel route between Ife and Ondo town

Pollen Identification, Pollen Sum and Spectra Constitution: Palynomorphs were identified using a reference collection at the Palaeobotany and Palynology Laboratory, University of Lagos. Pollen types and keys from published journals and description keys of African pollen grains and spores (Demske et al., 2013; Cugny et al., 2010; Gelorini et al., 2011; Gosling et al., 2013; and Van Geel et al., 2003).

Development of Pollen Calendar: Pollen calendar data for each sample location was created by calculating the average monthly abundance of pollen and spores in each location over a 24-month period (Figure 3). To generate the pollen calendar, the pollen diagram was created using TILIA and TGView software (Grimm, 1991). The pollen calendar displays the following information: the months of the year, all pollen and spores recorded in the study area, and the mean abundance of each pollen and spore arranged by month (Figure 4).

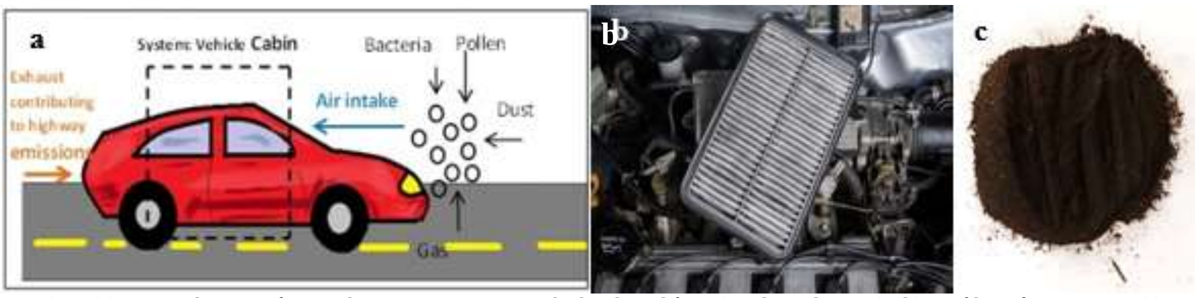

Fig 2. (a) System diagram of particulate matter entering a vehicle adapted from (Doyle et al., 2014); (b) Air filter of a motor car; (c) dust/soot

RESULTS AND DISCUSSION

A total of 393 pollen grains and 131 spores, representing 40 pollen and 22 spore types from 27 and 17 families, respectively, were recorded (Tables 1 and 2).

Table 1: Atmospheric pollen count of Ife - Ondo Road

Plant taxa	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total	%
Phyllanthaceae														
Amanoa strobilaceae	2	1	1	3	0	0	0	0	0	0	2	1	**10**	**2.5**
Cyperaceae														
Cyperus sp.	0	0	0	0	0	3	1	2	2	0	0	0	**8**	**2.0**
Kyllinga erecta	0	0	0	0	2	1	1	0	0	0	0	0	**4**	**1.0**
Arecaceae														
Elaeis guineensis	0	0	0	0	0	1	2	1	1	3	1	1	**10**	**2.5**
Asteraceae														
Tridax procumbens	0	0	0	0	0	1	1	0	3	0	0	0	**5**	**1.3**
Aspilia africana	1	2	1	0	0	0	0	0	0	2	1	3	**10**	**2.5**
Sapindaceae														
Chytranthus obliquinervis	2	1	0	0	0	0	0	0	0	0	1	1	**5**	**1.3**
Lecaniodiscus cupanoides	0	0	0	5	1	2	1	1	1	0	0	0	**11**	**2.8**
Paulinia pinnata	0	3	3	1	0	0	0	0	0	0	0	2	**9**	**2.3**
Convovulaceae														
Ipomea purpurea	0	0	0	0	0	0	0	2	2	0	0	0	**4**	**1.0**
Poaceae	1	2	1	9	7	1	0	0	2	1	1	5	3**0**	**7.6**
Panicum maximum	3	0	0	0	0	3	0	4	0	1	1	1	**13**	**3.3**
Pennisetum purpureum	3	1	1	4	1	2	4	1	0	0	1	2	**20**	**5.1**
Hyperthelia dissolute	0	4	2	2	0	0	0	0	0	0	0	0	**8**	**2.0**
Charred poceae cuticle	1	4	1	0	0	0	0	0	0	2	1	4	**13**	**3.3**
Rosaceae														
Hagenia abyssinica	0	0	0	0	0	0	0	0	1	2	4	2	**9**	**2.3**
Insects parts	1	0	0	0	2	0	0	1	0	0	0	0	**4**	**1.0**
Podocarpaceae														
Podocarpus milanjianus	0	0	0	0	0	0	1	1	1	0	0	1	**4**	**1.0**
Cucurbitaceae														
Cucumis sativus	0	0	0	0	0	0	1	2	0	0	0	0	**3**	**0.8**
Fabaceae														
Delonix regia	0	0	0	0	6	9	8	4	1	0	0	0	**28**	**7.1**
Pterocarpus abyssinicus	0	0	0	0	1	1	0	2	1	0	0	0	**5**	**1.3**
Caesalpinia decapetala	1	3	1	8	2	1	0	0	1	0	0	2	**19**	**4.8**
Baphia massaiensis	0	0	0	2	2	4	1	0	0	0	0	0	**9**	**2.3**
Tephrosia elata	0	0	0	0	0	3	1	2	4	0	0	0	**10**	**2.5**
Hymenophyllaceae														
Hymenophyllum capilare	1	1	0	0	0	0	0	0	0	0	0	1	**3**	**0.8**
Talinaceae														
Talinum triangulare	0	0	0	0	2	1	0	0	0	0	0	0	**3**	**0.8**
Rutaceae														
Calodendrum capense	0	0	0	0	0	0	0	0	2	1	1	1	**5**	**1.3**
Icacinaceae														
Lasianthera africana	0	0	1	1	0	0	0	0	0	0	0	0	**2**	**0.5**
Celastraceae														
Hippocratea affinis	0	0	0	0	0	3	2	1	1	0	0	0	**7**	**1.8**
Nyctaginaceae														
Boerhavia diffusa	2	1	1	0	0	0	0	0	0	1	0	1	**6**	**1.5**
Apocynaceae														
Baissea multiflora	0	0	0	1	3	1	5	1	0	0	0	0	**11**	**2.8**
Capparaceae														
Ritchiea capparoides	3	2	0	0	0	0	0	0	0	0	0	1	**6**	**1.5**
Dichapetalaceae														
Dichapetalum stuhlmannii	0	0	0	1	1	0	0	0	0	0	0	0	**2**	**0.5**
Hamamelidaceae														
Trichocladus ellipticus	0	1	2	1	1	0	0	0	0	0	0	0	**5**	**1.3**
Malvaceae														
Theobroma cacao	1	2	4	1	4	5	0	0	0	0	0	0	**17**	**4.3**
Mansonia altissima	0	0	0	0	0	2	1	4	0	0	0	0	**7**	**1.8**
Cannabaceae														
Cannabis sativa	0	0	0	0	0	3	6	7	3	1	0	0	**20**	**5.1**
Ulmaceae														
Celtis zenkeri	1	1	3	1	0	0	0	0	0	2	1	2	**11**	**2.8**
Celtis philippensis	1	1	0	0	0	0	0	0	0	0	0	2	**4**	**1.0**
Ebenaceae														
Diospyros dendo	0	0	0	0	0	0	3	3	4	1	0	0	**11**	**2.8**
Euphorbiaceae														
Ricinodendron heudelotii	0	0	1	5	4	1	0	0	0	0	0	0	**11**	**2.8**
Cecropiaceae														
Myrianthus arboreus	1	2	3	5	0	0	0	0	0	0	0	0	**11**	**2.8**
Total	**25**	**32**	**26**	**50**	**39**	**48**	**39**	**39**	**30**	**17**	**15**	**33**	**393**	
%	**6.4**	**8.1**	**6.6**	**12.7**	**9.9**	**12.2**	**9.9**	**9.9**	**7.6**	**4.3**	**3.8**	**8.4**		

Sapindaceae, Poaceae, Fabaceae, Euphorbiaceae, Malvaceae, Cannabaceae, Trichosphariaceae, Telchosporaceae, Pleosporaceae, and Phaeosphariaceae are the families that predominated this route. The highest pollen and spore counts were recorded in April (12.7%) and May (17.5), while the lowest pollen and spore counts were recorded in November (3.8%) and October (0.8%), as shown in Tables 1 and 2.

Table 2: Atmospheric spore count of Ife - Ondo Road

Dactylosporaceace														
Bactrodesmium obovatum	2	1	1	2	0	0	0	0	0	0	0	0	**6**	**4.6**
Trichosphaeriaceae														
Brachysporium sp.	0	0	0	0	0	0	3	2	1	1	2	5	**14**	**10.7**
Dactylosporaceace														
Bactrodesmium obovatum	2	1	1	2	0	0	0	0	0	0	0	0	**6**	**4.6**
Teichosporaceae														
Byssothecium sp.	3	2	1	0	0	0	0	0	0	0	0	1	**7**	**5.3**
Cladosporium sp.	5	1	3	4	2	2	1	1	0	0	0	0	**19**	**14.5**
Coniochaetaceae														
Coniochaeta sp.	0	0	0	1	1	0	0	0	0	0	0	0	**2**	**1.5**
Caryosporaceae														
Caryospora callicarpa	0	0	0	1	0	0	0	0	0	0	0	0	**1**	**0.8**
Pleosporaceae														
Alternaria sp.	0	0	0	0	3	1	1	0	0	0	0	0	**5**	**3.8**
Stemphylium sp.	0	0	0	0	0	0	1	1	0	0	0	0	**2**	**1.5**
Curvularia sp.	0	0	0	2	1	3	3	1	0	0	0	1	**11**	**8.4**
Pithomyces sp.	0	0	0	0	1	1	0	0	0	0	0	0	**2**	**1.5**
Cyathaceae	1	0	0	0	0	0	0	0	0	0	0	0	**1**	**0.8**
Diporotheca sp.	0	0	0	1	0	0	0	0	0	0	0	0	**1**	**0.8**
Fungi mycelium	0	0	0	3	5	5	4	5	1	0	0	0	**23**	**17.5**
Glomeraceae														
Glomus sp.	0	0	2	2	0	0	0	0	0	0	0	0	**4**	**3.0**
Meliolaceae														
Meliola sp.	0	0	0	0	0	0	0	1	0	0	0	0	**1**	**0.8**
Apiosporaceae														
Nigrospora sphaerica	1	0	0	1	1	0	0	0	0	0	0	0	**3**	**2.3**
Phaeosphaeriaceae														
Phaeosphaeria sp.	0	0	0	2	6	0	2	1	3	0	0	0	**14**	**10.7**
Podosporaceae														
Podospora sp.	1	1	0	0	0	0	0	0	0	0	0	0	**2**	**1.5**
Sordariaceae														
Sordaria sp.	1	1	0	0	0	0	0	0	0	0	0	0	**2**	**1.5**
Didymosphariaceae														
Spegazzinia tesarthra	0	0	0	0	1	0	0	0	0	0	0	0	**1**	**0.8**
Sordariomycetes incertae sedis														
Sporidesmium Cf. *macrurum*	1	1	1	1	0	0	0	0	0	0	0	0	**4**	**3.0**
Tetraplosphaeriaceae														
Tetraploa aristata	0	0	0	0	0	0	0	0	0	0	0	1	**1**	**0.8**
Saccharomycetaceae														
Torula sp.	0	0	0	0	2	0	0	0	0	0	0	0	**2**	**1.5**
Total	**16**	**7**	**8**	**20**	**23**	**12**	**15**	**12**	**5**	**1**	**3**	**9**	**131**	
%	**12.2**	**5.3**	**6.1**	**15.3**	**17.5**	**9.2**	**11.4**	**9.2**	**3.8**	**0.8**	**2.3**	**6.9**		

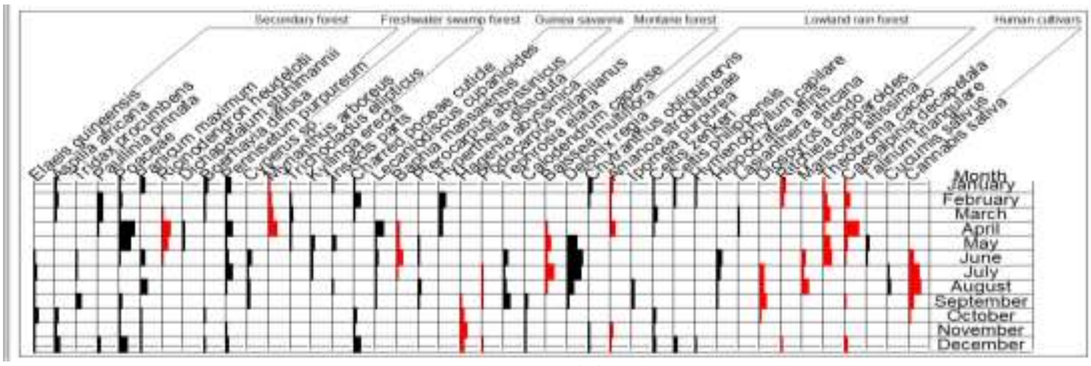

Fig 3. Pollen calendar of Ife - Ondo Road

Theobroma cacao (4.3%), *Cannabis sativa* (5.1%), *Caesalipinia decapetala* (4.8%), *Delonix regia* (7.1%), and *Pennisetum purpureum* (5.1%) were found to be prevalent (Figure 3). This route was dominated by fungal spores like *Brachysporium* sp. (10.7%), *Curvularia* sp. (8.4%), fungi mycelium

(17.5%), *Cladosporium* sp. (14.5%), and *Phaeosphaeria* sp. (10.7%), as shown in Figure 4. A weak positive correlation was observed between the mean monthly values of relative humidity, rainfall, and wind speed, which correlated significantly with the total palynomorph count on Ife-Ondo Road (Figure 5). Two years of aeroflora data along the Ife-Ondo road have been obtained, revealing the diversity of airborne pollen and fern spore types recovered at the study location.

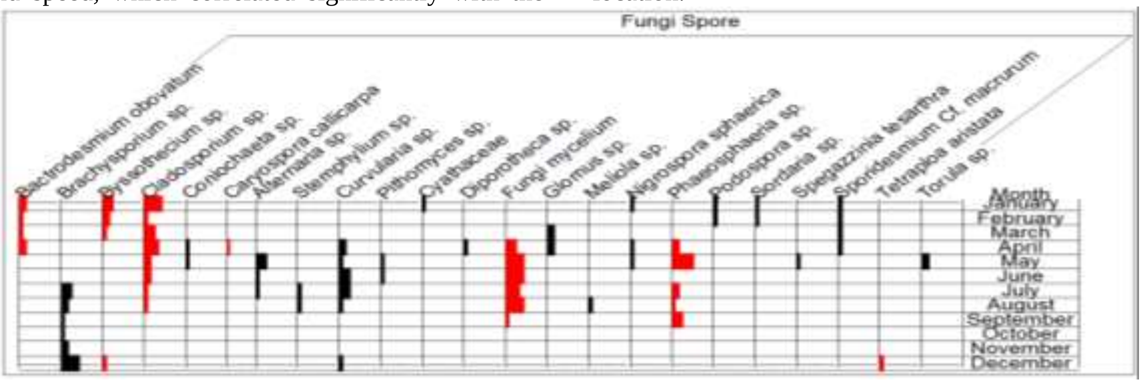

Fig 4. Spore calendar of Ife - Ondo Road

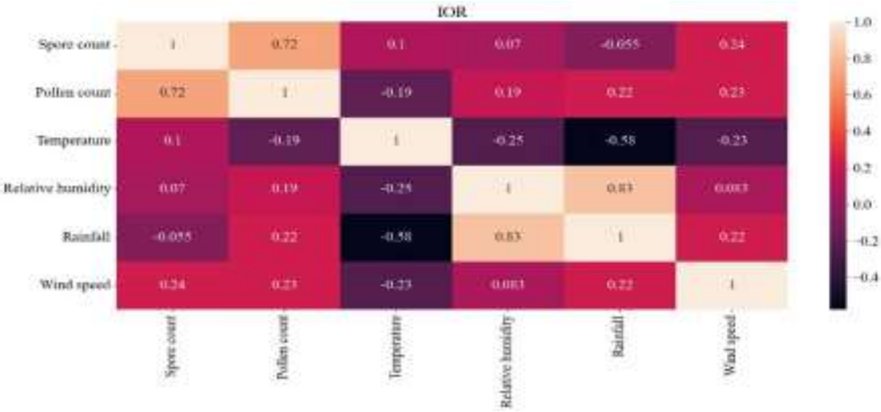

Fig 5. Degree of significance between total monthly pollen and spore count in Ife-Ondo road and meteorological parameters

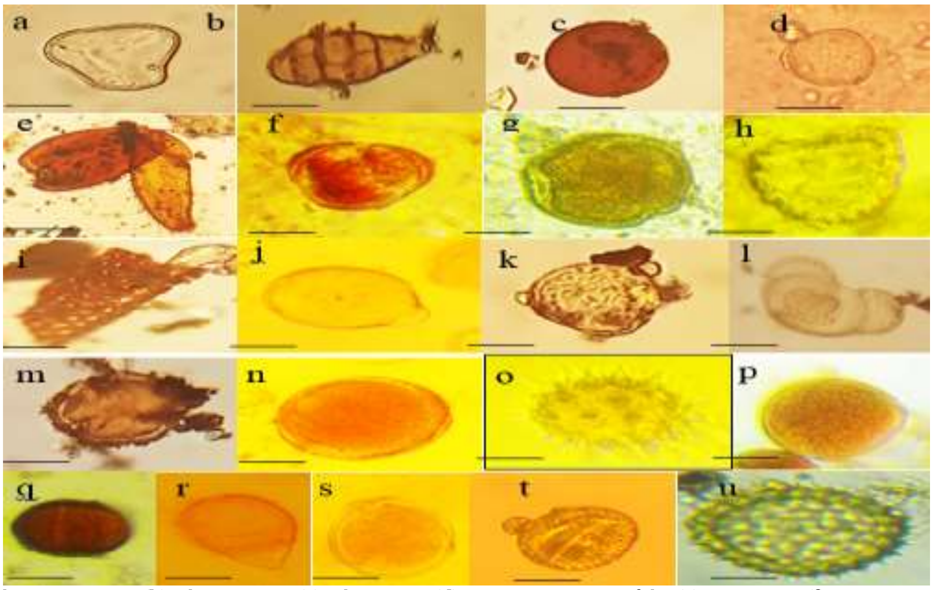

Plate 1: (a) *Elaeis guineensis* **(b)** *Alternaria* sp. **(c)** *Glomus* sp. **(d)** *Casuarina equisetifolia* **(e)** Insect part **(f)** *Tectona grandis* **(g)** *Luffa cylindrica* **(h)** *Pteris dentata* **(i)** Charred poaceae cuticle **(j)** *Solanum melongenea* **(k)** *Senna siamea* **(l)** *Pinus caribea* **(m)** *Alchornea cordifolia* **(n)** *Hyptis suaveolens* **(o)** *Aspilia africana* **(p)** *Senna occidentalis* **(q)** *Mangifera indica* **(r)** Fungi spore **(s)** *Milicia excelsa* **(t)** *Drepanocarpus lunatus* **(u)** *Sida veronicaefolia* **Mag*400, scale bar 10um.**

The recovery of *Hagenia abyssinica* along a 282-meter elevation gradient is due to its preference for higher altitudes and suitable climatic and topographic conditions, supporting earlier reports of their presence in upper altitudinal montane forests (Mengistu et al., 2023). *Hagenia abyssinica* and *Podocarpus milanjianus* were recorded in the fourth quarter, indicating increased wind speeds from the upper montane forest to the lowlands during the harmattan season. *Myrianthus arboreus* predominates and has been reported as a marker for early forest colonization stages and a potential bio-indicator for environmental degradation in forested ecosystems (Achoundong et al., 2000). *Theobroma cacao* recovery indicates potential activities related to cocoa plantations, transportation, and trade along the route. Its flowering intensity peaks between April and June, consistent with previous reports (Gordon, 1976). *Cannabis sativa* recovery from May to October suggests nearby cultivation, possibly indicating illegal cultivation, transportation, and consumption. *Cannabis sativa* psychoactive compounds, produced in trichomes on flower bracts (Andre et al., 2016), make it attractive to all smokers

Tetraploa aristata weak representation in December indicates water bodies may have dried up, causing aerospora representation. They are also found in coastal plain environments with high salinity, such as mangroves and salt-and-brackish-water marshes (Medeanic, 2006). *Cladosporium* sp., a moderately abundant plant pathogen, causes leaf spots and hyperparasites in other fungi (Bensch et al., 2012). It has also been isolated in unshelled Nigerian groundnut samples (Turner et al., 2003), suggesting groundnut farming activities along this route. The presence of dead wood indicators on the woodland floor, such as *Bactrodesmium obovatum* (Van Geel et al., 1981), which is associated with decaying wood of deciduous trees, indicates the presence of a local tree in the fungi spore record. The abundance of *Byssothecium* sp. increases progressively with elevation from April to September. The predominance of *Phaeosphaeria* sp. at high altitude along this route demonstrates that the number of spores increases as elevation increases (López-Vila et al., 2014). *Phaeosphaeria* sp. has been found on sub-Antarctic islands almost as frequently as it has on Victoria Land (Onofri et al., 1994). These taxa were found in relative abundance along both the central and coastal transects and are almost certainly endemic to Antarctica. *Phaeosphaeria* species are generally parasites of cereals, grasses, sedges, and rush plants (López-Vila et al., 2014). Mean monthly values of relative humidity, rainfall, and wind speed correlated significantly with the total palynomorph count in Ife-Ondo Road. This suggests that these environmental factors play a crucial role in influencing the presence and abundance of palynomorphs in the area.

Conclusions: The study reveals that higher plants of varying plant habits and other anthropogenic activities contributed to atmospheric pollen and spore spectra along the study route, regardless of height. Relative humidity, rainfall, and wind speed played an important role in influencing atmospheric pollen distribution along this route. Airborne pollen types were dispersed by some parent plants in the immediate vegetation at the study locations.

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