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Palynomorphs: Associating footwear users with objects and environment using pollen and spores

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Abstract

Soil and water samples from a clay vessel (artefact) recovered from a site, along with control and soil samples from the soles of four pairs of shoes were analysed for palynomorphs. The analyses were carried out with a view of associating the clay-vessel with the site on the one hand, and the shoes with the site and vessel on the other. A comparison of pollen and spores from the four pairs of shoes with those of the vessel and soil surface samples obtained from the same environment gave a concurrence of 51-79.6% and 34.4-65.6% respectively. These results indicate variation in the palynomorph content of the three-sets of samples. However, the occurrence of the pollen of plants peculiar to the environment in the vessel and shoes sufficiently links the shoes to the object, thereby strongly associating the users with the scene.

Keywords: Forensic science; palynology; pollen spectra object; environment; Nigeria.

Introduction

Forensic science is fast becoming a more readily used tool in fighting crime. Although the general use of forensic science has been more accepted in New Zealand [1] and the United Kingdom [2] and to a comparatively less extent in the United States of America [3] than elsewhere, it is gradually gaining grounds the world over. Forensic investigations are often interdisciplinary and require several lines of evidence. One aspect of forensic science that offers promise is forensic palynology which is the "study of modern and fossil pollen, spores and other acid-resistant micro-plant remains in a legal context" [4]. That is, it is the application of palynology in detecting and arresting crime [5]. Palynology, as is well known, is the study of cells collectively referred to as palynomorphs (5 μm and 200 μm in size [6]), such as pollen grains, spores, acritarchs and dinocysts.

Forensic science, more often than not, attempts to link a suspect or group of suspects to crime scenes and/or objects. In the absence of physical witnesses, pollen and spore evidence from a forensic material can be used to link the suspect to the crime site [2, 7] and environment [8, 9] or at least trim down the list of suspects [4, 10]. This is largely based on the principle of "every contact leaves a trace'' first advocated by E. Locard in the 1930s [11]. That is to say; materials are transferred from one source to another upon contact; such materials are used as evidence to support the occurrence of contact between two or more objects. Palynomorphs, particularly pollen and spores are dispersed into the atmosphere, upon production, and eventually deposited on soils or other materials within or near their immediate surroundings [2] forming what is referred to as pollen signature.

Palynomorphs, being microscopic in nature, stick to clothing, soils, shoes, hair, skin, animals, materials and other objects without notice. Thus in the occurrence of crimes, criminals or suspects inadvertently carry the palynomorphs of that environment. Palynomorphs are therefore "unseen witnesses of crimes" [11]. Most importantly, particular environments, generally speaking, produce unique palynomorph signature. Hence soil samples from such environment naturally reflect this signature. Thus pollen signature (assemblage) provides useful information necessary to link individuals or criminals to a particular environment [6, 12, 13]. In cases where forensic materials such as dirt (soil samples or mud) from the shoes of suspects are available, a comparison of the pollen-data from them with those of a crime object or scene is pertinent. Similarities in the two sets of pollen assemblages recovered from the same locality will be crucial to arresting and convicting the suspect(s) as was the case with the Danube murder [6]. Indeed, such evidence proved a strong basis for associating a male suspect with the place where a lady was alleged to have been sexually assaulted by the former [13]. Horrocks and Walsh [13] found that the pollen evidence from the suspect's clothing and shoes was very similar to that from the alleged crime scene. Similarly, pollen evidence was also used to link a murderer to the victim and site of murder [14].

However, it has been found that the pollen content of several samples from one locality in the USA was found to be generally similar [15]. This suggests that pollen data from crime scenes and/or objects in an area will most probably show strong or high similarities with one another. If this can be proved in court, then in the absence of any physical witnesses, a criminal might escape prosecution. Forensic palynology is new in Nigeria; at an experimental stage hence, it is yet to be applied in the country's criminal justice system. The aims of this work are firstly, to ascertain if an object could be linked to a 'crime' or recovery site; secondly, if palynomorphs recovered from soil samples collected from shoes of suspects/criminals match those from a 'crime' site, and thirdly, if the object could be traced to the shoes and the person(s) that wore them. In addition, it seeks to determine if there are variations in the surface pollen assemblages from the same locality as well as the possible challenges such variations pose to forensic investigations.

Materials and methods

A clay vessel (artefact) (Plate 1) 'was removed stolen' from its location and placed on the ground in a site located on the campus of the University of Ibadan, Ibadan, Nigeria. This site represents a hypothetical crime scene. The clay vessel was left in the site for a period of three weeks after which it was retrieved. Soil and water found in and on it were collected and taken to the laboratory for analysis. The samples were mixed to give a single sample and processed for palynomorphs. A vegetation study of the site was conducted while several pinches of surface soil samples were also collected [2]. These surface samples were combined into two sub-samples. Four volunteers, representing hypothetical suspected thieves, wearing plastic pairs of shoes (shoes 1, 2, 3 and 4), Plate 2 were asked to walk in four directions (north, south, east and west) around the site, after which soil samples were collected from the soles of their shoes; their location was recorded with a GPS device.

Ten grammes of each sample were subjected to standard pollen analyses procedure [16]. Ten millimetres of the final residue were mounted on a pair of slides and studied under x40 and x100 objectives of a light microscope. Pollen and spores were identified to family, genus and species levels but mainly to the latter two. The identifications were based on reference slides collection, and photomicrograph albums in the Palynology Laboratory, Department of Archaeology and Anthropology, University of Ibadan, Ibadan, the author's personal collection, as well as the following publications: [17-20]. Pollen diagrams were constructed using TILIA and TILIA software [21]. Lithological and pH analyses of the samples were carried out. Lithology (soil colour and grain sizes) of the samples were determined using the Munsell colour chart and soil grain-size chart respectively, while the pH analyses were carried out using an electronic pH meter.

Plate 1. The clay vessel (artefact) placed in the site.

Plate 2. The four shoes used in the study; left to right: Shoes 1, 2, 3 and 4.

Results

The locations of the vessel and shoes are as follows: vessel: N07^{o} 26′, 37.7′, E003 53′, 36.2′; Shoe 1: N07^{o} 26′, 35.0′, E003^{o} 53′, 37.9′; Shoe 2: N07^{o} 26′, 37.7′, E003^{o} 53′, 36. 2′; Shoe 3: N07^{o} 26′, 35.2′, E003^{o} 53′, 37.8′ and Shoe 4: N007^{o} 26′, 37.2′, E003 53′, 37.0′.

Vegetation study

The vegetation of the area is a mosaic of freshwater swamp, lowland secondary and riverine forests part which is cultivated annually. The vegetation study of the area was explored along three lines namely: (a) those within the immediate area of the stream, (b) on drier soil near the stream and (c) those located some 10-50 m away.

Most of the plants located within the immediate area of the stream were crops such as *Manihot ultissima, Dioscorea bulbifera, Carica papaya, Musa sapientum, M. paradisiaca, Sorghum bicolor, Zea mays, Curcubita pepo* and *Colocasia esculenta.* Herbs identified include *Amaranthus spinosus, Paullinia pinnata, Talinum triangulare, Oldenlandia corymbosa, Panicum maximum, Cassia rotundifolia,* *Phyllanthus pentandrus, Mimosa pigra, Lipocarpha chinensis, Coix lacryma-jobi, Tridax procumbens, Mormodica charantia, Ludwigia hyssopifolia, Cyperus* sp.*, Ageratum conyzoides, Synedrella nodiflora* and *Chromolaena odorata.*

Trees and shrubs within this area but on drier soil include *Spondianthus preussii, Bombax buonopozense, Spondias mombin, Newbouldia laevis, Delonix regia, Peltophorum pterocarpum, Terminalia superba, Raphia vinifera, Elaeis guineensis, Azadiractha indica, Alstonia boonei, Parkia biglobosa* and *Alchornea cordifolia.* Plants located 10-50 m away are *Adansonia digitata, Ceiba pentandra, Nauclea diderrichii, Milicia excelsa, Triplochiton scleroxylon, Polyscias* sp.*, Ficus exasperata, Ficus* sp.*, Elaeis guineensis, Terminalia superba, Alchornea cordifolia, Musa sapientum, M. paradisiaca,* and *Napoleona imperialis.*

Pollen results

One-hundred palynomorph types were recovered for this study. Forty-nine and 32 were recovered from the clay vessel and soil surface samples respectively, while 33-55 were recovered from the shoes (Table 1). Photographs of some of the recovered palynomorphs are presented in Plate 3 while pollen types are plotted on a pollen diagram (Figure 1).

It is expedient to indicate the basis for identifying some pollen to species level. *Tridax procumbens* was differentiated from other members of the Asteraceae mainly on the basis of its 4-colporate nature; others which are common weeds in Nigeria are 3-colporate. *Terminalia* cf. *superba* is similar to other *Terminalia* species in terms of size (P/E, 18.9/17.6 ìm) but have characteristic transversely elongated ora (length 3.5 ìm, width 5.25 ìm); this feature is in line with the descriptions of [17]. *Peltophorum pterocarpum* was differentiated from *D. regia* in that there are a maximum of 7 to 11 bacules bordering each lumen in the former but 12-15 in the latter [22].

Furthermore, the exine is thinner in *D. regia* (6.0-7.0 μm) and thick (8.0-9.0 μm) in *P. pterocarpum*. *Zea mays* was differentiated from other pollen of the Poaceae family on the basis of its large size; (P x E, 75 x 92.5 ìm) and thick annulus of each pore [23]. The most abundant palynomorphs included *Alchornea* cf. *cordifolia, Terminalia* cf. *superba, L. hypossifolia, A. spinosus, C. papaya, E. guineensis, M. indica,* Poaceae, Asteraceae, Cyperaceae*, Phyllanthus* cf. *pentandrus, A. cissampeloides, S. preussii* and *Z. mays.* These palynomorphs were all present in the samples obtained from the shoes though they occurred in varying amounts (Tables 1 and 2; Plate 3); the pH values and lithology of the soil samples are presented in Table 3.

Table 1. Palynomorphs in surface samples and object as well as on pairs of shoes.

Sample/ Percentage occurrence on shoes	Shoe 1	Shoe 2	Shoe 3	Shoe 4
Palynomorph types in control samples (32)	11/ 34.4%	21/65.6%	17/53.1%	14/43.7%
Palynomorph types in clay vessel (49)	25/51.0%	39/79.6%	25/51.0%	25/51.0%
Palynomorph types on shoes	33	55	40	44
Total pollen counts	214	632	183	367

Table 2. Percentage representation of vegetation on shoes.

Vegetation/Shoes	Shoe 1	Shoe 2	Shoe 3	Shoe 4	Control samples	Clay vessel
Plants in vegetation (48)	27	36	25	25	20	33
% Occurrence	56.3	75.0	52.1	52.1	41.6	68.8

Table 3. pH values and lithology of soil samples.

Sample	pH value	Soil colour	Soil type
Control Sample 1	5.2	Dark brown	Sandy silt
Control Sample 2	5.4	Very dark greyish brown	Sand
Shoe 1	6.9	Dark greyish brown	Peaty silt
Shoe 2	4.9	Very dark brown	Peaty silt
Shoe 3	5.6	Very dark greyish brown	Sandy silt
Shoe 4	5.4	Dark reddish brown	Silt

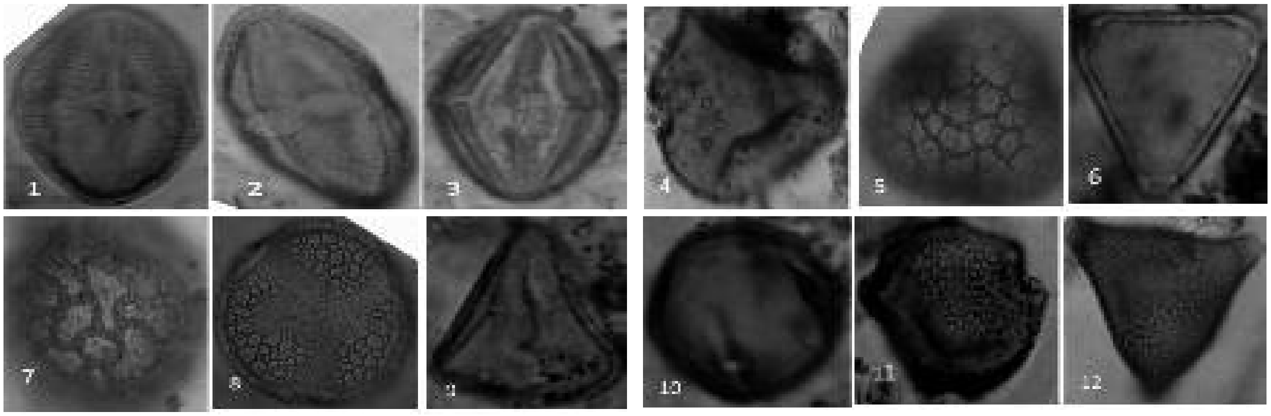

Plate 3. Palynomorphs recovered from the samples. **1-2.** *Triumfetta* sp., **3.** *Cissus quadrangularis*, **4.** *Adansonia digitata,* **5.** *Ceiba pentandra*, **6.** Protea sp., **7.** *Peltophorum pterocarpum*, **8.** *Cucurbita pepo*, **9.** Cyperaceae, **10.** *Meliaceae,* **11.** *Canthium setosum,* **12.** *Cardiospermum halicacabum.* **5** µm.

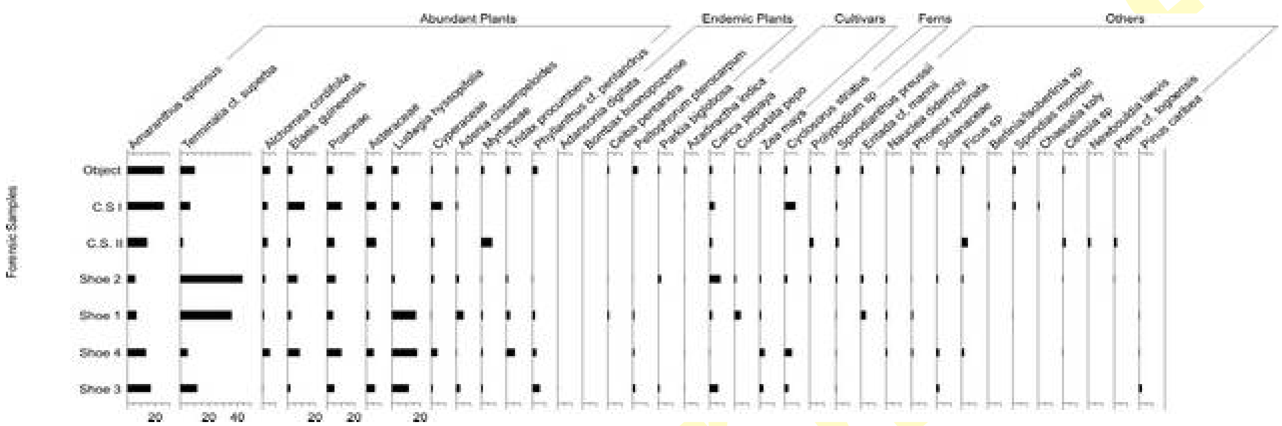

Figure 1. Pollen diagram of the most abundant taxa; Control sample.

Discussion

Of all the shoes, Shoe 2 which went east produced the highest number of pollen types (55), had the highest percentage of similarities with the clay vessel (79.6%) and control samples (65.6%) (Table 1). In addition, 75% of the plants in the study-area were represented in it (Table 2). Therefore, this pair of shoes were considered the best in associating its user with the site and vessel. The reasons for this similarity was attributed to its soil-type and pH value (Table 3) [24]. The soil-type (peaty silt) is known to be excellent for pollen preservation [24], although its pH of 4.9 (Table 3) was a bit higher than that above which palynomorphs are said to be susceptible to destruction, i.e. 4, [25], which is considerably acidic. Though the soil on Shoe 1, which went west, was also peaty silt its pH (6.9) was much higher. In addition, although fungal spores were recovered from all the samples, samples from Shoes 2 and 3 had paucity of fungal spores with 2 and 1 respectively. Dimbleby [24] stated that an abundance of fungal spores is an indication of high microbial activities which cause considerable disintegration of pollen. Therefore, pollen destruction, if it occurred, was greatly reduced in the sample on Shoe 2 than in others. Thus, these factors should be taken into consideration during forensic palynological investigations.

The pollen recovered from the shoes provided rather limited representations of the vegetation of the study-area and of the pollen in the clay-vessel. Consequently, these data, when considered on face value, might not strongly associate an individual with the crime-scene and object. The percentage values of the samples presented, when tendered in a legal context might not be regarded as constituting a strong enough evidence to link the 'suspects' to the crime scene. In other words, brilliant lawyers might argue that the evidence 'was not proved beyond reasonable doubt'. In the opinion of a non-forensic palynologist, the pollen content on the shoes appeared to differ significantly from those of the site and the clay-vessel. But in assessing the forensic significance of these results, it is important to have a look at aspects of the vegetation complex of the site. In this case, the most abundant and cultivated plants and those peculiar to the site shall be the main *foci.*

The most abundant plants in the site were *Alchornea cordifolia, Amaranthus spinosus, Carica papaya, Elaeis guineensis, Ludwigia hyssopifolia, Oldenlandia* *corymbosa, Terminalia superba*, Cyperaceae, Poaceae and Asteraceae (*Synedrella nodiflora, Chromolaena odorata* and *Ageratum conyzoides*) and *Tridax procumbens*. It is very significant to state that the pollen-grains of these plants were recovered in abundance from the object and the shoes. This suggests, among other things, that these plants are high pollen producers with good dispersal mechanism(s). The pollen of cultivated plants on the site (*A. indica*, *C. papaya, C. pepo* and *Z. mays*) were also recovered. Although the pollen- grains of *Z. mays, C. pepo, A. indica* were not recovered from the control-samples they were present in the object and shoes. In addition, *Amaranthus* cf. *spinosus, A. cordifolia, C. papaya* and *E. guineensis* which were abundant in the site, were recovered from all the samples (control-samples, object and shoes). These pollen grains were the main link among the three forensic samples studied. On no other location within the boader area of the site do these plants grow together. The pollen of plants 'endemic' to the site (*P. pterocarpum, C. pentandra, A. digitata, B. buonopozense, C. pepo*, *P. biglobosa* and *Z. mays*) was also recovered in good amounts from the object and shoes. The recovery of the pollen of *Zea mays* from the shoes and object provided unequivocal precision that they were from that site [2]. In addition, the plants of *C. pentandra, B. buonopozense* and *C. pepo* were not flowering at the time the samples were collected. The recovery of their pollen, being deposited at least a season before the samples were collected, showed the potent value of pollen in forensic investigations. In the whole, the pollen evidence linked the shoes, and by implication the users, to the object.

Despite good representations of the pollen of most plants of the site in the control-samples, object and shoes, the pollen of some plants was not recovered. The most important of these were *D. bulbifera, M. ultissima, C. esculenta, M. sapientum* and *M. paradisiaca.* The absence of their pollen could be due to one or more of the following reasons: The formermost three are usually planted using tubers (asexual reproduction) while the latter two reproduce through suckers. These features make pollination (sexual reproduction), and by implication pollen production of less importance to these plants. In addition, the pollen of these cultivar are rarely recovered in palynological studies in Nigeria except that of *M. ultissima* which has been observed in honey samples so far [23]. Therefore these plants are poor pollen producers and/or their pollen probably have smaller amount of sporopollenin in their exine, and are less resistant to degradation [26]. The results also suggest that their mode of pollination and pollen dispersal are still not sufficiently known. Similarly, some pollens the parent plants of which were not within the site were recovered from the study. These include *Bridelia ferruginea, Isoberlinia* sp., *Canthium setosum* and *Pinus caribea*. The formermost three are all naturally found in open vegetation or savanna; their pollen might have been transported from elsewhere into the site. This was a possibility because heavy rains, accompanied by strong winds, occurred during the period when the artefact was in the site. The pollen of *P. caribea* might have been transported by wind into the site because stands of the tree are located some 250-300 m away.

Conclusion

Given the high similarities in the pollen and spore assemblages from the control-samples, object and the shoes, it could be stated that the forensic materials (object and boots) came from the same site that produced the control-samples. This fact was underscored by the recovery of pollen of cultivated and 'endemic' plants from the three forensic materials. It had also been established that a good number of pollen, parent plants of which were in the site, were present in the object. This indicated that the object was kept in the site for a period. It had also been revealed that, although there were overall similarities in the pollen signatures of samples from a locality; variations do exist; these variations may appear significant, but when the vegetation complex of the site is considered, the variations might not be significant for forensic purposes. A good knowledge of the appropriate pollen types and vegetation complexes is crucial for the successful application of palynology in crime detection.

Furthermore, the pollen assemblages of samples from the object and shoes were similar, sufficiently linking the latter to the former. Therefore, if the shoes were recovered from some suspects, they were *strongly* linked to the crime-site and the 'stolen' object. Hence levelling a criminal charge and securing a conviction would be a strong possibility.

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References

[1] Mildenhall, D. C. 1990. Forensic palynology in New Zealand, *Rev. Palaeobot. Palynol. 64,* 227-234.

[2] Bryant, V. M. and Mildenhall, D. C. 1998. Forensic palynology: A mew way to catch crooks. In: V. M., Bryant and J. W. Wrenn, Editors. *New Developments in Palynomorph Sampling, Extraction, and Analysis.* American Association of Stratigraphic Palynologists Foundation. *Contributions Series Number 33,* 145-155.

[3] Bryant V. M., Jones J. G. and Mildenhall, D. C. 1990. Forensic palynology in the United States of America, *Palynology*, 4, 193-208.

[4] Editorial. 2006. Forensic palynology: Why do it and how it works. *Forensic Science International, 163,* 163-172.

[5] Bryant, V. 2013. *Pollen and spore use in forensics.* In: *Wiley Encyclopedia of Forensic Science (2nd*

Edition). A. Jamieson and A. Moenssens, Editors. John Wiley and Sons, Ltd. Chichester, U.K.

[6] Erdtman, G. 1966. *Pollen Morphology and Plant Taxonomy*. Hafner Publishing Company, New York and London, 553pp.

[7] Mildenhall, D. C. 2008. Civil and criminal investigations. The use of spores and pollen. *SIAK-Journal " Zeitschrift für Polizeiwissenschaft und polizeiliche Praxis 4,* 35-52.

[8] Rivera, B. H. and Rodriquez, M. G. 2016. Characterization of airborne particles collected from car engine air filters using SEM and EDX techniques. *International Journal of Environmental Research and Public Health, 13,* 985-1000.

[9] More, S., Thapa, K. K. and Bera, S. 2013. Potential of dust and soot from air-filters of motor vehicle engines as a forensic tool: first experimental palynological approach in India. *Journal of Forensic Research*, *4(1),* 177-183.

[10] Orijemie E. A. 2012. *Pollen and Spores: Unseen Witnesses of Crimes*. Seminar Paper presented in the Department of Archaeology and Anthropology, University of Ibadan, Ibadan, Nigeria, September 5th, 2012.

[11] Morgan, R., Freudiger-Bonzon, J., Nichols, K., J. T., Dunkerley, S., Zelazowski, P. and Bull, P. 2009. The forensic analysis of sediments recovered from footwear. In Ritz, K., Dawson, L. and Miller, D. (Eds.), *Criminal and Environmental Soil Forensics*, Springer Netherlands, pp. 253- 269.

[12] Bryant, V. M. 1989. Pollen: Nature's fingerprints of plants. 1990 Yearbook of Science and the Future, *Encyclopedia Britannica*, Chicago, Illinois, pp. 92-111.

[13] Horrocks, M. and Walsh, K. A. J. 2001. Pollen on grass clippings: putting the suspect at the scene of the crime. *J Forensic Sci. 46(4),* 947-949.

[14] Mildenhall, D. C. 2004. An example of the use of forensic palynology in assessing an alibi. *J Forensic Sci. 49(2),* 1-5.

[15] Horrocks, M., Walsh, K. A. J. and Coulson, S. A. 1998. Forensic palynology: variation in the pollen content of soil surface samples, *J. Forensic Sci. 43,* 320-323.

[16] Faegri, K. and Iversen, J. 1988. *Textbook of Pollen Analysis*. John Wiley and Sons Alden Press London, 328pp.

[17] Sowunmi, M. A. 1973. Pollen of Nigerian Plants I: Woody Species, *Grana 13,* 145-186.

[18] Sowunmi, M. A. 1995. Pollen of Nigerian Plants II: Woody Species, *Grana 34*, 120-141.

[19] Salard-Cheboldaeff, M. P. 1980., Palynologie Camerounaise I: Pollens de la mangrove et des fourrés Arbrustifs Côtiers. 105e Congrès National des Sociétés Savantes, *Caen*, *Sciences, Fasc. I,* 233-247.

[20] Salard-Cheboldaeff, M. P. 1985. Palynologie Camerounaise VI: Grains de pollen des Savanes Périforestières. 110e Congrès National des Sociétés Savantes, Montepellier, *Sciences, Fasc. V,* 231-248.

[21] Grimm, E. C. 2011. TILIA and TILIA *software [computer program], 1.7.16 version.* Illinios State Museum, Research and Collections Center, 1011 East Ash Street, Springfield, 62703, USA.

[22] Orijemie, E. A. (In Press). Pollen morphology of three Caesalpinioideae (Leguminosae) Ornamental species in Nigeria. *Nigerian Journal of Botany.*

[23] Orijemie, E. A. *Pollen Atlas of Nigerian Honeys* [In preparation].

[24] Dimbleby, G. W. 1985. *Palynology of Archaeological Sites*. Academy Press, 176pp.

[25] Sowunmi, M. A. and Awosina, E. O. 1991. Pollen analyses of Kariya Wuro Rock Shelter, Bauchi State. *The Nigerian Field, 56,* 163-170.

[26] Phuphumirat, W., Mildenhall, D. C. and Purintavaragul, C. 2009. Pollen deterioration in a tropical surface soil and its impact on forensic palynology. *The Open Forensic Science Journal*, *2,* 34-40.

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