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The Use of Soil Palynomorphs in Forensics

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ABSTRACT: The aim was to establish the forensic value of using palynomorphs in soil samples to link people or objects to crime scenes in order to establish or strengthen an association. This was done by determining the degree to which pollen assemblages of surface soil samples differ within the same area. Samples within the same localized area (the control site) showed a high degree of similarity, suggesting that pollen assemblages of surface soil samples from within a localized area are homogeneous. Standard methods were used for the collection and analysis of soil samples such as deflocculation, acetolysis for removal of cellulose and organic matter and silicate removal method to achieve better visualization and identification of pollen types. The results indicated that the cast of footprints and palm prints provided evidence of a two way transfer of materials between the palms and feet and the soil of the grassy area. Pollen analysis of the soil that had adhered to the palms and feet showed that the perpetrator of the imprint had been standing in that grassy area. The analysis of the interface between the body parts (palms and feet) and soil is therefore a potentially lucrative source of information for forensic reconstruction. This analysis shows that pollen can be used to associate perpetrators to crime scenes and should be seen as a useful tool in the analysis of hitherto unrecognized forensic materials in forensic palynology.

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Palynology is the science that studies, contemporary and fossil palynomorphs, including pollen, spores, orbicules, dinoflagellate cysts, acritarchs, chitinozoans and scolecodonts, together with particulate organic matter found in sedimentary rocks and sediments (Jansonius and McGregor, 1996). The study of pollen is called palynology and is highly useful in paleoecology, paleontology, archeology, and forensics. The usefulness of palynomorphs is due to their abundance, dispersal mechanisms, resistance to mechanical and chemical destruction, microscopic size and morphology. Their often complex morphology allows identification to an individual parent plant taxon, which can be related to a specific ecological habitat or a specific scene (Mildenhall *et al*, 2006). The main forensic application of palynology is to provide associative evidence, i.e. to establish or disprove links among people, places, and objects (Horrocks and Walsh, 1998). If one knows the composition of the pollen grain for a given area, then one will know what pollen assemblage should be found in samples collected from that area (Bryant *et al*., 1990). Pollen and spores are found everywhere. They are found in glacier ice, in the air, over the poles and over the ocean. It has been said that they have probably a wider distribution with regard to time and space than any other organism (Bryant *et al*., 1990).

Forensic palynology is the study of pollen and powdered minerals, their identification, and where and when they occur, to ascertain that a body or other object was in a certain place at a certain time. Pollen can tell a lot about where a person or object has been, because regions of the world, or even more particular locations such as a certain set of bushes, will have a distinctive collection of pollens. Pollen evidence can also reveal the season in which a particular object picked up the pollen. Its main forensic application is in providing associative evidence, i.e., assisting to prove or disprove a link between people and objects with places or with other people. For example, soil on a suspect's shoe or clothing can be analyzed for pollen and compared with control soil samples from the crime scene (Chambers, 1998).

Recently solved criminal cases show that the forensic use of pollen and spores can be used in many different crimes. A short list of these cases shows many ways pollen is now being used in the courtroom namely forgery, production and distribution of illegal drugs, assaults, robbery, rapes, homicide, genocide, terrorism, arson, hit and run crimes, counterfeiting of currency, identifying the origin of fake prescription drugs such as Viagra (Mildenhall *et al*,. 2006).

However, forensic palynology has not made much headway in Nigeria because of the difficulties and problems that are encountered in their isolation and identification (Sowunmi, 1973). The current study, therefore, is to elucidate the degree to which pollen assemblages of surface soil samples from within the

same localized area differ, and to determine the degree to which the pollen assemblage of a surface soil sample from within a localized area differs from those of other localized areas of similar vegetation type.

MATERIALS AND METHODS

Collection of samples: An open grassy area, measuring approximately 15 x 6 meters, was selected at a farmland beside the Asa River along Unity Road in Ilorin, Kwara State, North Central Nigeria, which is located between latitude and longitude 8 ° 28´0˝N to 4 ° 33` 0˝E. It forms a hollow approximately 2 meters below surrounding terrain and is surrounded by grasses and scattered shrubs and crops. Soil samples were collected on the 17th of February 2013.

A pair of clean feet and palms which is disinfected using 70% ethanol was walked once back and forth along the same line of travel (approximately 3.5m) across a muddy part of the grassy area. The resulting tracks consisted of ten pairs of footprints and palm prints, with pair member side by side and 2cm to 5cm apart. Each member of a pair was made in an opposite direction to the other. From the heel of each footprint and palm print, a soil sample (1mm surface scrapping) was taken with a sterilized scalpel blade. Further samples were taken from each footprint and palm print. The first of these consisted of a 1mm surface scraping, the second a gouge was made with the thumb and forefinger in the same place to a depth of approximately 20mm. The deeper samples were taken to determine whether or not pollen assemblages in surface samples change significantly within this depth. A total of nine samples was collected for analysis (Table 1). Each sample was collected using sterilized polyethylene bags.

Table 1: Sample collection and labelling

Study sample	Sample number
Right palm print	1
Left palm print	2
Right palm	3
Left palm	4
Right foot print	5
Left foot print	6
Left foot	7
Right foot	8
20mm soil depth	9

Isolation and analysis of pollens from soil: The soil samples were oven dried at 105ºC for 20 minutes soon after collection to inhibit any possible microbial activity. The soil samples were then ground in a mortar and passed through a 1mm sieve to remove coarse particles.

The method of Erdtman (1969) was adopted with little modification. One gram of soil of all samples was weighed and poured into different labelled beakers. Digestion was done by adding 10% NaOH to each of the tubes. Then the heat (80°C – 90°C for 2 to 3 minutes) was applied until there is no longer evidence of humic aggregates. The samples were then poured into labelled test tubes to allow for separation of residue from solvent. The separation is done by allowing the solvent settle over a period of time and the supernatant is decanted. The samples are then washed and centrifuged (1000ppm) several times to remove all caustic soda. Washing is done by adding water to the samples, centrifuging and then decanting the supernatant. The samples are then ready for hydrofluoric acid treatment.

A small amount of distilled water was added to the residue and mix thoroughly, and approximately 10 ml of 40 % hydrofluoric acid (HF) was added to centrifuge tube and place in a beaker of boiling water in water bath for about 30 minutes. The content was then centrifuged at 1000ppm and carefully decant into the labelled collector vessel and not down sinks. Dilute HCl was added, mixed and put in a simmering water bath for 3–5 minutes (this removes colloidal silica etc.). It was centrifuged again and decant. Lastly, distilled water was added, mixed, centrifuged and decant (Faegri and Iversen, 1989).

A small drop of the soil sample was smeared on a clean slide using a rod. A small drop of isopropyl (isopropanol) was added to the smear and mounted in glycerine. A cover slip was placed over the slide and the edges were permanent with nail polish. Observations were made using light microscope.

Determination of pollen frequency: The frequency of each pollen type was expressed as the percentage occurrence of all pollen types based on all occurrences using the formula:

$$\frac{P}{Y} \times 100$$

Where: P = the occurrence of each pollen type in the field of view, and

Y = total occurrence of all pollen types per sample.

Statistical analysis: All data were reported and analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Computer software was used. A probability value 0.05 was used as benchmark for significant difference between parameters.

RESULTS AND DISCUSSION

The descriptive microphotography of the pollen and spore types is compiled in Fig. 1. A total of 8 pollen types was discovered in all samples (Table 2; Fig. 1). The most frequent pollens are those of *Sida acuta* occurring in all the nine samples while those of *Tacca leontopetaloides* and *Terminalia catappa* occurred in only one sample each (Table 3). This gives credence to the high occurrence of pollens from the family Malvaceae and lower pollens from the families Dioscoreaceae and Combretaceae where the above

species belongs (Table 4). Pollens are morphologically diagnostic as most often they are species specific. The fact that the pollens can be identified to the species level is wholly supported by many researchers on polynomorphs to unravel some evidences in forensics, biostratigraphy, climatology, medicine-alleviation of pollinosis (hay fever-allergenic disease), forensic studies, mellisopalynology, plant evolution, taxonomy and environmental restoration activities (Adekanmbi, 2009; Ige, 2009 Adeonipekun and Ige, 2007; Palazzesi *et al*., 2007; Bayer and Kubitzki, 2003)

Pollen grains in Arecaceae family have less variation exhibited in the pollen morphology. They are mostly rounded, triangular to square, sometimes circular in polar view. Apertures of Combretaceae are triporate. Pollen grains in this family are characteristically echinate in ornamentation. Earlier, Sowunmi (1973) gave a similar morphological description. *Sida linfolia*, *S. acuta* and *Gossypium hirsutum* of Malvaceae share similar echinate characteristics. The echinate nature of the species of Malvaceae was also reported by Perveen and Qaiser (2009). The Fabaceae species, namely *Delonix regia, Acacia nilotica* and *Acacia albida* are characteristically panporate and quantity disparity was noted by Sowunmi (1973). *Ixora coccinea* of family Rubiaceae are bicolpate, panporate, participate. Walker (1976) suggested that elaborate exine sculpturing seems to be associated with entomophily, while pollen grains with smooth surfaces are largely characteristic of anemophilous plants. The slight size disparity is however noted in pollen grains of species of Dioscoreaceae and Euphorbiaceae by some researchers. This may not be unconnected with the fact that the pollen grain size is affected by acetolysis (Faegri and Iverson, 1989) and a few other chemicals used for grain isolation.

In order for palynological evidence to be accepted in a court of law, investigators must establish that correlation between the pollen profile of a forensic sample and its purported area of origin is causal and not merely coincidental. The similarities in sample 3 (right palm), 4 (left palm), 7 (left foot) and 8 (right foot), show that the body parts (palms and feet) had come in contact with the pollens in samples 1 (right hand print), 2 (left hand print), 5 (right foot print), 6 (left foot print) and 9 (20mm soil depth). Similarity and differences observed in the presence of pollens in the soil collected from the same area are an indication of good forensic evidence.

The laboratory of Mark Horrocks and associates at the University of Auckland has undertaken several studies that demonstrate the veracity of pollen evidence. They have shown, for instance, that sample of hash oil originating from the same marijuana crop, but subjected to different common filtering materials will show significantly similar pollen assemblages, a result of which is of great use to law enforcement agencies who often seek to determine a link between illicit drug samples found on different people or at different places (Horrocks and Walsh, 1997).

It may be argued that the pollen assemblage of a particular scene, for example an open grassy area, could be similar to the pollen assemblage of any other similar open grassy area. Horrocks and Walsh (1998) have also shown that this is not the case; while different soil samples collected from within a localized region (up to 15m) show similar pollen assemblages, there were significant differences among soil samples collected from different localized regions of similar vegetation type (up to 1km). This is again a demonstration that pollen evidence is a useful tool in associating suspects and objects with crime scenes.

Table 2: Plant species and pollen types

Species	Common name	Families	Pollen
Elaeis guineensis	African oil palm	Arecaceae	Panporate
Terminalia catappa	Bengal almond	Combretaceae	Triporate
Tacca leontopetaloides	Polynesian arrow foot	Dioscoreaceae	Monosulcate
Excoecaria agallocha	Excoecaria agallocha	Euphorbiaceae	Tricolporate
Delonix regia	Flamboyant or flame tree	Fabaceae	Panporate
Acacia nilotica	Gum Arabic tree	Fabaceae	Panporate
Acacia albida	Apple-ring acacia	Fabaceae	Panporate
Sida linifolia	Balai grand	Malvaceae	Bicolpate
Sida acuta	Common wireweed	Malvaceae	Triporate and panporate
Gossypium hirsutum	Upland cotton	Malvaceae	Pericolpate
Ixora coccinea	Flame of the woods or jungle flame	Rubiaceae	Bicolpate, panporate and pericolpate
Citrus medica	Citron	Rutaceae	Bicolpate

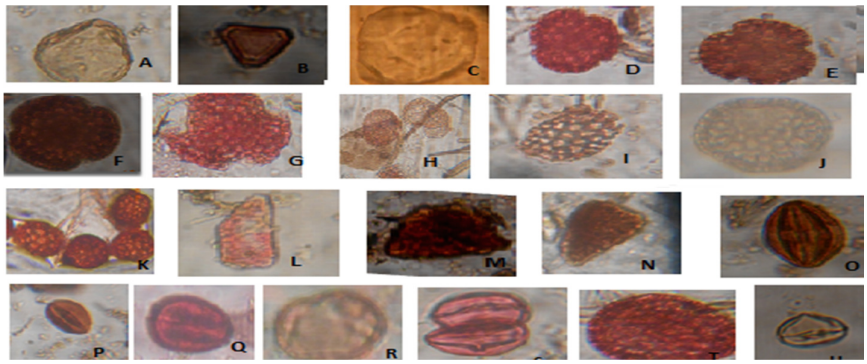

Fig. 1: Pollens recovered from the study site: a – panporate pollen of *Elaeis guineensis*, b – triporate pollen of *Terminalia catappa*, c – monosulcate pollen of *Tacca leontopetaloides*, d, e, f – tricolporate pollens of *Excoecaria agallocha*, g – panporate pollen of *Acacia albida*, h, i – panporate pollens of *Acacia nolitica*, j, k – panporate pollen of *Delonix regia*, l – triporate pollen of *Sida acuta*, m, n – panporate pollens of *Sida acuta*, o, p – bicolpate pollen of *Sida linfolia*, q – pericolpate pollen of *Gossypium hirsutum*, r – pericolpate pollen of *Ixora coccinea*, s – bicolpate pollen of *Ixora coccinea*, t – panporate pollen of *Ixora coccinea*, and u – bicolpate pollen of *Citrus medica*

Table 3: Occurrence of pollens in soil samples

Study samples	Total pollen per species	Frequency (%)	Species	Family
Right palm print	22	10.58	*Citrus medica*	Rutaceae
	24	11.53	*Delonix regia*	Fabaceae
	51	24.51	*Ixora coccinea*	Rubiaceae
	17	8.17	*Tacca leontopetaloides*	Dioscoreaceae
	39	18.75	*Sida linfolia*	Malvaceae
	55	26.44	*Sida acuta*	Malvaceae
Left palm print	40	42.10	*Ixora coccinea*	Rubiaceae
	32	33.68	*Sida acuta*	Malvaceae
	12	12.63	*Acacia nilotica*	Fabaceae
	11	11.57	*Excoecaria agallocha*	Euphorbiaceae
	14	11.20	*Citrus medica*	Rutaceae
Right palm	8	6.40	*Delonix regia*	Fabaceae
	13	10.40	*Acacia nilotica*	Fabaceae
	37	29.60	*Ixora coccinea*	Rubiaceae
	22	17.6	*Sida linfolia*	Malvaceae
	31	24.80	*Sida acuta*	Malvaceae
	11	12.08	*Gossypium hirsutum*	Malvaceae
Left palm	40	43.95	*Ixora coccinea*	Rubiaceae
	23	25.27	*Sida acuta*	Malvaceae
	17	18.68	*Sida linfolia*	Malvaceae
Right foot print	8	6.10	*Elaeis guineensis*	Arecaceae
	52	39.69	*Ixora coccinea*	Rubiaceae
	45	34.35	*Sida acuta*	Malvaceae
	16	12.21	*Delonix regia*	Fabaceae
	10	7.63	*Excoecaria agallocha*	Euphorbiaceae
Left foot print	9	11.25	*Delonix regia*	Fabaceae
	24	30.00	*Ixora coccinea*	Rubiaceae
	31	38.75	*Sida acuta*	Malvaceae
	8	10.00	*Terminalia catappa*	Combretaceae
	8	10.00	*Excoecaria agallocha*	Euphorbiaceae
Left foot	11	13.09	*Acacia albida*	Fabaceae
	6	7.50	*Gossypium hirsutum*	Malvaceae
	28	33.33	*Ixora coccinea*	Rubiaceae
	14	16.66	*Sida linfolia*	Malvaceae
	19	22.61	*Sida acuta*	Malvaceae
	6	7.14	*Delonix regia*	Fabaceae
Right foot	8	10.66	*Elaeis guineensis*	Arecaceae
	22	29.33	*Ixora coccinea*	Rubiaceae
	27	36.00	*Sida linfolia*	Malvaceae
	18	24.00	*Sida acuta*	Malvaceae
20mm depth	18	46.15	*Ixora coccinea*	Rubiaceae
	21	53.85	*Sida acuta*	Malvaceae

Table 4: Frequency of pollens per plant family

Families	Pollen	Frequency (%)
Arecaceae	31	2.12
Combretaceae	25	1.73
Dioscoreaceae	18	1.23
Euphorbiaceae	143	9.78
Fabaceae	308	21.06
Malvaceae	527	36.04
Rubiaceae	361	24.69
Rutaceae	49	3.35
Total	1462	100

Conclusion: Results from this study suggest that pollen assemblages of surface soil samples from within the same localized area are homogeneous. This illustrates that pollen analysis of soil samples is a valuable forensic tool in associating suspects and objects with crime scenes.

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REFERENCES

Adekanmbi, OH (2009). Pollen grains of Asteraceae and analogous echinate grains. *International Journal of Botany, 5: 295-300.*

Adeonipekun, AP; Ige, EO (2007). The refugia forest phenomenon: Implications on the palaepalynology of the Neogene Niger/Cross River Delta. *Science Focus*, 12: 17-21.

Bayer, C; Kubitzki, K (2003). Malvaceae. In: The Families and Genera of Vascular Plants, Kubitzki, K (Ed.). Vol. 5. Capparales and Non-

Betalain Caryophyllales, Malvales, Springer, Berlin. pp. 225-311.

Bryant, VM Jr; Mildenhall, DC; Jones, JG (1990). *Forensic Palynology in the United States of America*, 14: 193-208.

Chambers, FM (1998). The Palynological Investigations. In: Fasham, P.J., Kelly, R.S., Mason, MA; White, RB (eds) (1998). The Graeanog Ridge: the Evolution of a Farming Landscape and its Settlements in North-West Wales. Cambrian Archaeological Monographs, No. 6. Cambrian Archaeological Association, Aberystwyth, 52–63.

Erdtman, G (1969). Handbook of Palynology. Munksgaard, Copenhagen. pp. 486.

Faegri, K; Iversen, J (1989). Textbook of Pollen Analysis, 4th Edition. Wiley and Sons, Inc. Chichester, New York. pp. 328.

Horrocks, M; Walsh, KAJ (1998). Forensic palynology: assessing the value of the evidence, V.A. (Eds) Review of Paleobotany and Palynology, Special Issue, New Frontiers and Applications in Palynology- IX IPC.103, 69-74.

Horrocks, M; Walsh, KAJ (1997). A Bayesian approach to interpreting forensic pollen evidence. Review of Paleobotany and Palynology, pp. 947.

Ige, OE (2009). A late tertiary pollen record from Niger Delta, Nigeria. *Int. J. Bot*., 5: 203-215.

Jansonius, J; McGregor, DC (1996). Introduction, Palynology: Principles and pplications. *American Association of Stratigraphic Palynologists Foundation,* 1: 1 – 10.

Mildenhall, DC; Wiltshire, PE; Bryant, VM Jr (2006). Hypericum pollen determines the presence of burglars at the scene of a crime: An example of forensic palynology. *Forensic Science International*, 163(3): 231–235.

Palazzesi, L; Pujana, RR; Burrieza, HP; Steinhardt, AP (2007). Pollen grain morphology of selected allergenic species native to Southern South America. *J. Torrey Botanic. Soc.*, 134: 527-533.

Perveen, A; Qaiser, M (2009). Pollen flora of Pakistan-Malvaceae: Dombeyoideae-Lxii. *Pak. J. Bot*., 41: 491-494.

Sowunmi, MA (1973). Pollen grains of Nigeria plants 1. Woody species. *Grana*, 13: 145-186.