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Mycology in palaeoecology and forensic science

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ABSTRACT

Palynology (including mycology) is widely used in palaeoecological and bioarchaeological studies. Lake and mire sediments, soils, and the deposits accumulating in archaeological features, invariably contain plant and fungal remains, particularly pollen and spores. These serve as proxy indicators of ancient environmental conditions and events. Forensic palynology has been successfully employed in criminal investigations for more than two decades. In recent years, it has included fungal palynomorphs in profiling samples from crime scenes, and from exhibits obtained from suspects and victims. This contribution outlines the main features of palynology, and gives examples of case studies where fungal spores, pollen, and plant spores, have enhanced the interpretation of ancient landscapes and land-use, and provided pivotal intelligence, and probative evidence, in criminal investigations.

Introduction

Ecology is the interdisciplinary study of the distribution and abundance of organisms, their interactions with each other, and with their physico-chemical environment. The ecological literature involving fungi is vast, and only a selective and brief view of the growing appreciation of the importance of mycology in palaeoecology and forensic science can be presented here. Rather than whole plant and fungal remains, the main emphasis will be on palynomorphs[1] and palynological[2] profiles. Palynology provides the basic tool for a wide range of scientific studies, including those of: ancient environments (as in palaeoecology and bioarchaeology: Birks & West 1973; Dimbleby 1985; Huntley & Webb 1988; Edwards 2000; Innes *et al.* 2013) and contemporary ones (as in forensic investigations: Wiltshire 2016a).

Palaeoecology is the study of changes in the environment over time, and particularly those caused by the impact of human activity. Demonstration of these changes is generally achieved by studying the subfossil macro- and micro-remains of plants in sediments, and palaeosols.[3] Palynology has been established for over 100 y (von Post 1918) with internationally accepted conventions and analytical methods (Ertdman 1921, 1943, 1969; Faegri & Iversen 1964, Faegri *et al.* 1989; Nilsson & Praglowksi 1992; Jansonius & McGregor 1996; Brown 2008; Wiltshire 2016b).

Forensic science relates to, or deals with, the application of scientific knowledge to legal problems; to be termed 'forensic', any scientific information must be prepared for, and/or brought to, a court of law. Analysis of pollen and spores has been applied to forensic studies in a meaningful way for

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[1] Palynomorph: Any microscopic entity dispersed away from its origin.

[2] Palynology: The study of palynomorphs.

[3] Palaeosol: Ancient, usually buried, soil.

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approximately 30 y, and it has reached its most varied and highest level of development and utility in the UK over the last 22 y. GNS Science in New Zealand, and Texas A&M University (Department of Anthropology), are the only other centres that have a significant published record in forensic palynology (Mildenhall *et al.* 2006). Both specialise in provenancing illicit recreational and counterfeit medical drugs, and counterfeit honey. In the UK, palynology has been used in cases of murder, rape, missing persons, aggravated burglary, theft, and insurance fraud.

Palynology is also the basis for: aerobiology (allergens; Hyde 1969); melissopalynology (honey; Jones & Bryant 1992); oil prospection (Hopping 1967); palaeobotany (ancient plants and their evolution; Stuessy 2009); plant taxonomy (Harley & Ubara 2012); and climate change (Kutzbach & Guetter 1986; Chambers 1993).

Any pollen grain, plant spore, or fungal spore acts as a proxy indicator of the environment from which it was derived. If sufficient proxy indicators are identified and quantified, it is possible to reconstruct the nature of the past environments embedded during accumulation of the sediment over time. In a similar fashion, proxy indicators picked up from modern vegetation, soils, and other deposits, can allow the forensic practitioner to envisage the kind of environment from which the proxy indicator was produced. The value of reconstructing ancient and modern environments from proxy indicators is shown in the examples presented here.

Methods

Pollen and spores may be embedded in various matrices such as soil, organic and inorganic sediments (e.g. peat, silt, mud, clays), and other palyniferous[4] materials (e.g. leaf litter, humus, food, gut contents). They may also be embedded in natural and synthetic fabrics, and may form films, or be included in debris on various surfaces (e.g. footwear, various parts of vehicles, skin, hair, furniture, weapons, luggage, paper, and vegetation). Palynomorphs are widely distributed and are routinely retrieved from many different kinds of material and objects (Wiltshire 2009).

In palaeoecology, a restricted number of materials are processed to obtain palynomorphs, and analysis is concentrated on organic and inorganic sediments, and palaeosols. The retrieval of palynomorphs from forensic soils or sediments uses the same procedures as in palaeoecology and archaeology, but when they need to be recovered from surfaces or fabrics, they must first be extracted from those items and then subjected to standardised chemical treatments. The aim is to obtain and present palynomorphs such that their gross and fine structure can be observed by light microscopy (with or without phase contrast), so as much background material as possible in the sample has to be removed. Palynomorphs are washed from the sample or specimen; the washings are then sieved and centrifuged to obtain a pellet of palyniferous material (Wiltshire 2016b).

[4] Palyniferous: Having palynomorphs coating the surface, or being embedded in, a sample.

The background materials needing removal are humic acids, cellulose, lignin, and silica, and this is achieved by passing the sample through a series of digestions. After an initial boiling in potassium or sodium hydroxide to eliminate humic acids, the sample is sieved through a mesh (apertures typically 120 μm diameter) to retrieve any macro-remains. It is then centrifuged to form a pellet to which treatments are applied sequentially by boiling in: glacial acetic acid, hydrochloric acid, acetolysis mixture (concentrated sulphuric acid and acetic anhydride), and hydrofluoric acid. In between each treatment, the sample is washed and centrifuged and finally neutralised, and stained. It is then embedded in a preferred mountant to make a permanent preparation. In palaeoecological samples it is sometimes possible to obtain very 'clean' preparations, where nothing impedes a clear view of the various pollen grains and spores (Fig 1), but in archaeological or forensic samples, the background material is often impossible to remove completely. This makes palynomorph identification and quantification particularly difficult, especially where charred fragments, and other recalcitrant materials, are abundant (Fig 2). Details of the most appropriate methods of preparation for palaeoecological, archaeological, and forensic studies are outlined in Moore *et al.* (1992), Clarke (1994), Wood *et al.* (1996), and Wiltshire (2016b).

Pollen, plant, and many fungal palynomorphs are able to withstand the stringent preparation treatment because of the robust polymers embedded in their cell walls, sporopollenin in the case of plant palynomorphs, and chitin in fungal ones. Relatively few decomposer microorganisms possess enzymes capable of hydrolysing these complex polymers and, in hostile environments (those with extreme temperature, low water potential, and/or low oxygen tension) pollen, plant spores, and fungal spores can persist in rocks and certain sediments for millions of years and, in soils, for decades.

Palynological preparations are rarely processed for fungal remains alone, and this can cause problems where spores are thin-walled or have delicate appendages. Some pollen may disappear during processing because of the small amount of sporopollenin in the cell wall, and fungal spores with very thin walls, or thin-walled appendages, can be lost. Some pollen taxa may even have some degree of size alteration, although experience has shown that this does not appear to be the case with thick-walled fungal palynomorphs. A range of spores found in various criminal investigations is shown in Fig 3.

Analysis involves systematic scanning of prepared microscope slides, and identification of all palynomorphs encountered in equidistantly spaced traverses. Magnifications of ×400–×1000 are necessary, and phase contrast microscopy is favoured by many practitioners where appropriate. In many cases, a view of the interior structure of the pollen grain wall is necessary for identification, and thousands of palynomorphs may need to be identified and counted to obtain a representative profile of the contents of the sample. Thus, scanning electron microscopy (SEM) is not used routinely as it is impractical and unnecessary (Jones & Bryant 2007). The diversity in size, shape, structure, and wall sculpturing, aid the identification of pollen and plant spores (Fig 4).

A guide to useful literature for identification of fungal spores is given in Hawksworth & Wiltshire (2011, 2015).