Chapter 1
Forensic Palynology: Checking Value of Pollen Analysis as a Tool to Identify Crime Scene in Semiarid Environments

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Abstract Taphonomic variables affecting pollen content of soil are especially relevant in semiarid localities, which could limit the potential of palynology as a source of evidence in courts. A number of positive experiences have so far been carried out in humid climates, but not in semiarid environments. Here we aim at comparing pollen spectra from soil surface samples and footwear sediment infill in order to evaluate the possibility of using palynology as associative evidence in a theoretical crime scene occurring in a semiarid environment. To check if any "handy forensic correspondence" can be found, five areas of the region of Murcia in south-eastern Spain, different in flora, vegetation and biogeography, were selected.

1.1 Introduction

Plants release pollen grains that mostly settle on the ground, where, if appropriate conditions, they can persist even for millennia; those pollen grains can be extracted from soil and analyzed, showing particular assemblages and giving precise information about the vegetation in the surrounding areas (Erdtman 1969; Moore et al. 1991). As a consequence, palynology has a potential as a source of evidences in solving legal issues, as was firstly proposed by Locard (1930) and evidenced by Wilhelm Klaus in 1959 (Erdtman 1969).

The theoretical principles of forensic palynology have been amply described by different authors and a number of methods and examples have been displayed, showing that palynology can be a valuable forensic tool at least for over 50 years and emphasizing potentiality of this "blooming science" (Palenik 1982; Mildenhall 1988; Bryant and Mildenhall 1990; Mildenhall 1990; Brown and Llewellyn 1991;

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Mildenhall 1992; Stanley 1992; Szibor et al. 1998; Bruce and Dettmann 1996; Eyring 1997; Bryant and Mildenhall 1998; Horrocks and Walsh 1999, 2001; Mildenhall 2004; Milne 2004; Bryant and Jones 2006; Mildenhall 2006; Mildenhall et al. 2006; Wiltshire and Black 2006; Bertino 2008; Bryant 2009; Dobrescu et al. 2011). Unfortunately, the full potential of forensic palynology remains neglected in most countries in spite of its proved versatility in many kinds of criminal inquiries.

Forensic palynology is not an exact science due to the diversity of factors that control whether pollen grains and spores are or not finally present in a given place, and in which proportions they occur, that is, because the existence of diverse taphonomic variables (Mildenhall et al. 2006; Wiltshire and Black 2006). Precisely because of the taphonomic variability affecting palynomorphs' presence in soils (and other surfaces too), it must be assumed a certain unpredictability of the spatial patterning of pollen spectra as well as great heterogeneity of pollen and spore assemblages (Wiltshire and Black 2006), but, even so, strong correlations have been shown between soil samples obtained from footwear or clothes and soil surface samples from a precise site (Bruce and Dettmann 1996; Horrocks et al. 1998, 1999; Brown et al. 2002; Bull et al. 2006; Riding et al. 2007).

Regardless its undeniable validity and with relations to those taphonomic questions above-referred must be considered that reported examples connecting soil surface samples and soil from footwear/clothes by their palynological assemblages are mostly related with mud in more or less humid climates (Horrocks et al. 1998, 1999; Bull et al. 2006; Mildenhall et al. 2006; Riding et al. 2007), but no experiences in forensic palynology have been carried out in arid or semiarid, Mediterranean environments. That is significant because mud and wet soils effectively trap pollen and easily stick to footwear and clothes in considerable amounts, unlike dry sediments, which easily lose pollen and hardly stick to surfaces.

1.1.1 Why Semiarid Sites Are Special?

In richly vegetated regions transport of pollen by winds, rivers and other factors has a subordinate effect on pollen spectra from soil samples but are of prime importance in arid areas (Horowitz 1992), and can lead to an over-representation of anemophilous taxa and even to a scarce presence of pollen grains and types. In addition, the oxic conditions in those dry environments usually involve a poor preservation or even complete disintegration of pollen grains, specially those having thin walls. For instance, modern surface samples from the arid south-western USA generally record less than 40 pollen types of which only five, namely *Pinus*, *Juniperus*, Poaceae, Chenopodiaceae, and Asteraceae, may account for 90 % of the pollen counts (Hall 1985). In these habitats, the anemophilous pollen percentages can be considerably higher than zoophilous ones even when anemophilous elements are less represented than zoophilous (El Ghazali and Moore 1998).

In spite of this, palynological study of surface soil samples is a suitable tool to register vegetation differences in arid environments (Carrión 2002), and seems to have a potential in forensic sciences (Guedes et al. 2011). Certainly, because the influence of a number of factors the pollen content of a soil could show not an "exact/correct picture" of the surrounding vegetation. Nonetheless, its particular pollen spectrum could be useful for comparison purposes (linking persons/objects with possible crime scenes), making necessary to test the existing correspondence in pollen content between soil surface samples and soil forensic samples from clothes, fabrics and footwear. This work is aimed to check if any "handy forensic correspondence" can be found between soil pollen spectra and pollen content of soil samples from shoes in a semiarid environment as southeastern Spain.

1.2 Materials and Methods

Five localities showing a diversity of plant communities were selected within the region of Murcia (Fig. 1.1). Details about location, climate, bioclimatic belt and vegetation of the sites are shown in Table 1.1. At each locality, clean outdoor boots

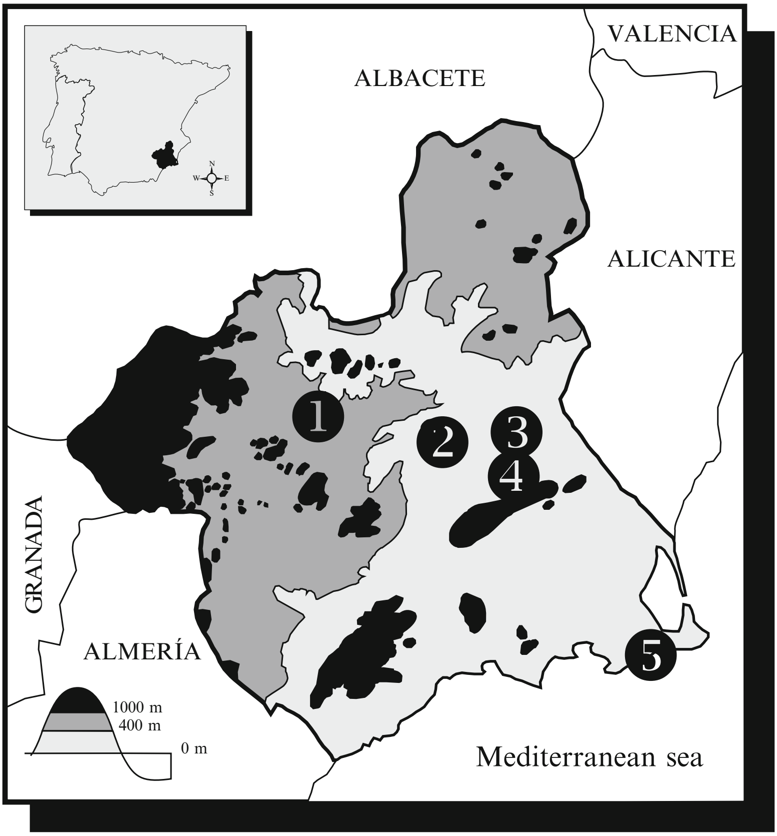

Fig. 1.1 Location of sampling sites in Murcia Region (Spain). *1* Carrascalejo; *2* Albudeite; *3* Espinardo; *4* La Alberca; *5* Cartagena

Table 1.1 Main characteristics of the sampling sites

Site	Altitude m.a.s.l.	Coordinates	Bioclimatic belt	Ombro-climate	Short description	Predominate species
Carrascalejo	620	38° 03′ 38″ N 01° 42′ 40″ W	Meso-Mediterranean	Dry	Small stream with special deciduous/ evergreen forest gallery	Trees: *Quercus faginea*, *Populus nigra* and *Populus alba* near the stream and *Pinus halepensis*, *Quercus rotundifolia*, *Fraxinus angustifolia* and *Olea europaea* nearby
						Shrubs: *Quercus coccifera*, *Daphne gnidium*, *Pistacia terebinthus*, *Pistacia lentiscus*, *Rhamnus lycioides*, *Genista scorpius*, *Ulex parviflorus*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Sideritis leucantha*, *Satureja obovata*
Albudeite	193	38° 01′ 24″ N 01° 23′ 42″ W	Upper thermo-Mediterranean	Semiarid	Stream (usually dry) with marly-saline-nitrified soils rich in Chenopodiaceae	Shrubs: *Suaeda vera*, *Anabasis hispanica*, *Atriplex halimus*, *A. glauca*, *Salsola genistoides*, *Tamarix boveana*, *T. canariensis*, *Limonium caesium*, *Capparis spinosa*, *Anthyllis cytisoides*, *Lygeum spartum*, *Stipa capensis*, *Helianthemum squamatum*

Espinardo	95	38° 01′ 11″ N	Upper thermo-Mediterranean	Semiarid	Landscaped area in Campus of University of Murcia	Trees: in the selected garden *Morus alba, Phoenix dactylifera, Ph. canariensis, Schinus molle, Citrus limon, C. aurantium;* and *Acacia farnesiana, Robinia pseudoacacia, Pinus halepensis, Ceratonia siliqua* and *Ulmus pumila* nearby
		01° 10′ 06″ W				
La Alberca	58	37° 56′ 32″ N	Upper thermo-Mediterranean	Semiarid	*Eucalyptus* wood in anthropic area with nitrified soils	Trees: *Eucalyptus camaldulensis*
		01° 09° 07″ W				Shrubs: *Oxalis pes-caprae, Marrubium vulgare, Sisymbrium irio, Moricandia arvensis, Capsella bursa-pastoris, Piptatherum miliaceum, Hyparrhenia sinaica, Hordeum vulgare, Silybum marianum, Chrysanthemum coronarium, Malva parviflora*
Cartagena	40.	37° 34′ 33″ N	Lower thermo-Mediterranean	Semiarid	Coastal site with special *Periploca* thicket	Trees: *Periploca angustifolia, Chamaerops humilis*
		00° 57′ 53″ W				Shrubs: *Asparagus albus, Genista umbellata, Calicotome intermedia, Thymelaea hirsuta, Salsola oppositifolia*

with a maximum tread of 6 mm deep was "normally" walked around (that is, not using exaggerated force in order to deliberately entrain material into the boot tread) for 3 min, in random directions over an area of approximately 25 m^2. After that and by using a small clean spatula and a clean brush, all sediment in the boots soles was removed and saved in a new sterile plastic bag. Finally, a composite sample was collected as a control and consisting of 12–15 subsamples of soil taken at a depth of 1–2 mm with a clean spatula and put together in a sterile plastic bag to be thoroughly homogenized before pollen analysis.

After deflocculation by using sodium pyrophosphate, soil samples were prepared for pollen analysis according to the KOH, hydrofluoric acid and hydrochloric acid method, including flotation in zinc chloride (Dimbleby 1957, 1961; Frenzel 1964; Bastin and Couteaux 1966; Girard and Renault-Miskovsky 1969; Juvigné 1973). Pollen mounted in glycerol was identified and quantified at X400–X1000 by light microscopy.

1.3 Results and Discussion

As expected and with the exception of the locality at Cartagena, pollen spectra show relatively low diversity and dominance of anemophilous types (Hall 1985; El Ghazali and Moore 1998). Except for Carrascalejo, all selected sites are semiarid and, as expected, pollen spectra from soil samples (Fig. 1.2) depict five well-differentiated habitats and correlate quite well with main vegetation in their surrounding areas. After microscopic examination, a total of 57 pollen types (54 Magnoliophyta and 3 Pinophyta), 10 spore types (2 Bryophyta, 5 Algae and 3 Fungi) and one Oribatida species (moss mites) were identified.

For each study case, the pollen diagram (Fig. 1.2) shows a close resemblance between soil surface samples and those from footwear dust, not only in main pollen types but also in rare types and fungal and algal spores. Between 8 and 37 different types were identified in sites (Table 1.2). Maximum diversity was found in Cartagena and Espinardo but relative diversity in soil surface samples was higher than in shoe samples in Espinardo and Albudeite, and lower in samples from Carrascalejo, La Alberca and Cartagena. The proportion of taxa present both in soil and shoe samples moves around 45 % in Albudeite, Espinardo and Cartagena, reaching 61 % in Carrascalejo and almost 90 % in La Alberca (Table 1.2). Even though results are summarized in Figs. 1.2, 1.3 and Table 1.2 a short analysis for every site is done.

- **Carrascalejo.** A total of 23 pollen types was identified, 61 % of them both in soil and shoe samples. In spite of some differences in percentages, pollen spectra from soil and shoes correlate quite well. According with its dominance in the surroundings, *Pinus* is the dominant type, being *Quercus*, Chenopodiaceae, Asteraceae, *Populus* and Cistaceae other important elements characterizing the site. Noteworthy is the presence of fungal spores (*Glomus*, Sordariaceae and *Tilletia*) and algae zygospores and aplanospores (*Zygnema, Rivularia,*

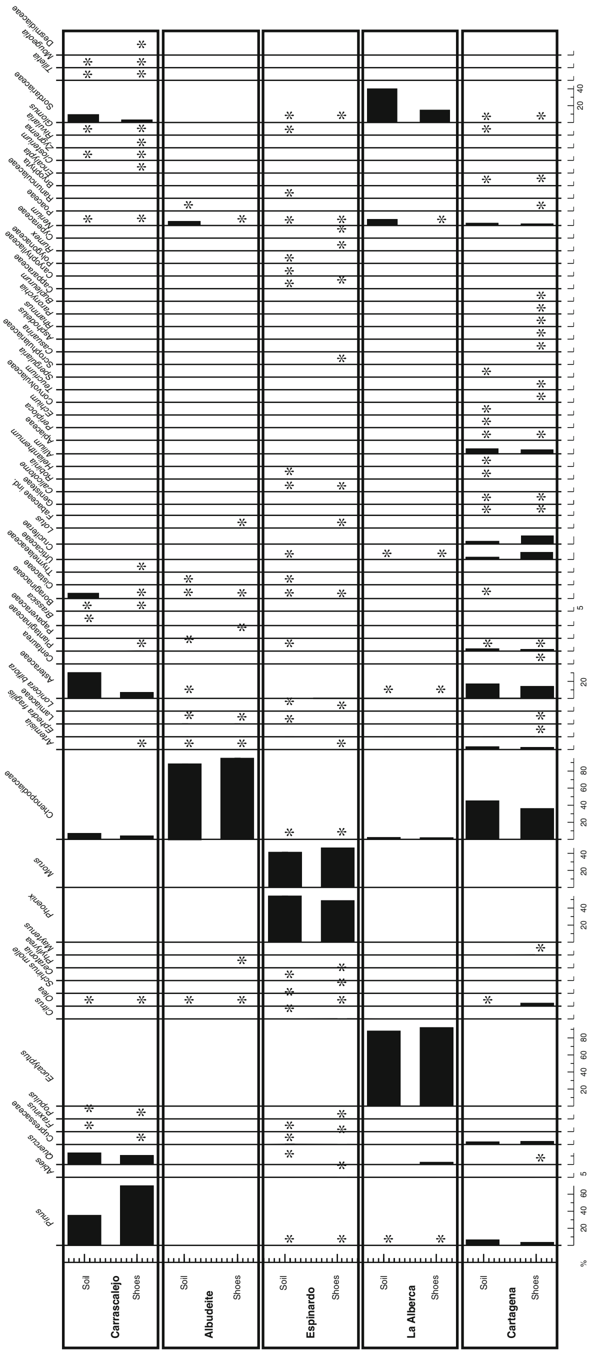

Fig. 1.2 Pollen diagram showing percentages for soil surface and shoe samples. Percentages below 2 % showed as *

Table 1.2 Number of pollen/spore types found in sites

	Total	Soil	Shoes	Both	Both
Carrascalejo	23	16	21	14	60.9 %
Albudeite	13	10	9	6	46.1 %
Espinardo	32	25	22	15	46.9 %
La Alberca	8	7	8	7	87.5 %
Cartagena	37	23	30	16	43.2 %

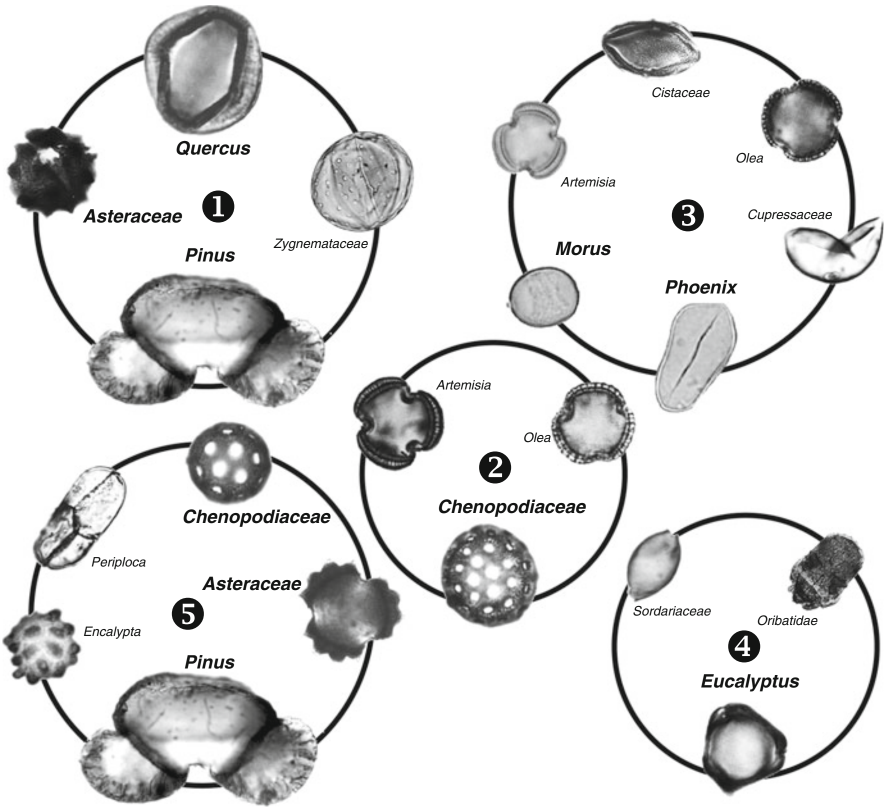

Fig. 1.3 Summary chart of the discrimination of the five sites on the basis of the pollen percentages both in shoes and soil samples. *1* Carrascalejo; *2* Albudeite; *3* Espinardo; *4* La Alberca; *5* Cartagena

Desmidiaceae, Closterium and *Mougeotia*). Such a number of particular occurrences are probably due to a footstep on a wet site near the stream.

- **Albudeite.** Chenopodiaceae, Poaceae and *Tamarix* are the more abundant plants on this site (Table 1.1), but in pollen counts Chenopodiaceae reach by itself 88.6 % in soil surface samples and 95.3 % in shoe samples. *Artemisia*, Lamiaceae, Cistaceae and Poaceae are other characteristic elements. Only 13 pollen types were identified in Albudeite, six of them both in soil and shoe samples.

- **Espinardo.** Although other trees are frequent in the selected area (*Citrus*, *Schinus*, *Fraxinus*, *Robinia*; Table 1.1), their pollen grains are scarce in samples while pollen from *Phoenix* and *Morus* exceeded 95 % of the pollen found both in soil surface and shoe samples, probably because *Morus* was just finishing blooming and *Phoenix* blooms through the whole year. Presence of *Lonicera* and low percentages of *Pinus* and *Cupressaceae* are noteworthy, especially the last ones, which are "highly under-represented" having in mind their significant presence in the vicinity of the garden, its high production of pollen and its anemophilous dispersal.
- **La Alberca.** With the only exception of *Quercus* (only found in shoes), the same taxa are found in soil surface and shoe samples. A low diversity characterizes samples from La Alberca, which are dominated by *Eucalyptus* (88–92 %), a pollen type totally absent in the other sites. The high presence of Sordariaceae agrees with the use of the area as grazing land.
- **Cartagena.** In spite of being the driest location, shows the highest diversity with a total of 37 taxa, 30 of them found in shoe samples. Correlation of taxa between soil surface and shoe samples reaches 43 %. Both spectra match very well and show the main elements of the surrounding area, including characteristic, entomophilous taxa like *Periploca*, *Maytenus*, *Calicotome* and *Rhamnus*

1.4 Conclusion

The forensic use of palynology is challenging when dealing with semiarid regions, principally due to the particularities of pollen taphonomy and, in addition, because of the limited possibilities of adherence of dry soil to footwear. Here we have compared pollen assemblages in soil surface samples with those from soil samples in footwear walked, and found remarkable correlation. However, this is a preliminary study and a more complete, wide-ranging research is still needed. This new study should be orientated towards a thorough investigation of the effect of time (weeks, months) on the pollen spectra so as to elucidate when the control samples will stop being valid as evidential samples due to the biases caused by differential preservation of pollen grains and spores.

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