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Forensic palynology revisited: Case studies from semi-arid Spain

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ABSTRACT

This article presents a palynological analysis of samples obtained in three different locations in the semiarid southeast Spain. Sampling was carried out on four surfaces (directly from the soil, then from textile samples that had been in direct contact with the ground, another set collected from clothing using adhesive and the final from sediment accumulated on footwear) after a forensic simulation. This study can be compared to previous work carried out in the same region. All of the samples were polleniferous, the pollen spectra giving relevant information about the vegetation of each location. We confirm the potential utility of palynology in forensic studies in order to establish the source of a sample and to be able to link people or objects to a crime scene. We show that samples from the same location may produce different results, depending on the sampling method, although they will show common taxa that will serve to identify the place where they were found. Moreover, it is shown that the use of glass microscope slides with adhesive is an adequate method to retrieve pollen from clothing surfaces.

1. Introduction

For 50 years, forensic palynology has been used as a source of police evidence, especially in countries such as New Zealand, the United Kingdom and the United States (Mildenhall 2010). Some of the works present in the bibliography of forensic palynology studies cover the resolution of cases where pollen evidence was crucial or even the only available proof (Mildenhall 1990, 2006a, 2006b, 2010; Brown 2006; Bryant and Jones 2006; McKinley and Ruffell 2007; Lippi and Mercuri 2009; Bryant et al. 1990; Brown et al. 2002; Wiltshire et al. 2015a, 2015b, 2015c). This shows how, given appropriate circumstances, it is possible to carry out a palynological study in the course of a police investigation and, at a relatively low cost, quickly produce results that can help the police, the victims, or even accuses suspects (Wiltshire 2015).

Some past studies include taphonomic remarks on how to interpret the differences in the pollen distribution in sediments (Wiltshire 2006; Horrocks and Walsh 1999; Horrocks et al. 1999; Riding et al. 2007; Petraco et al. 2008). Others provide general information of the field and its main methods (Bruce and Dettmann 1996; Bryant and Mildenhall 2011; Horrocks et al. 1998, 1999; Chazottes et al. 2004; Bull et al. 2006; Pye et al. 2007; Riding et al. 2007; Wiltshire et al. 2015b). Some authors have proposed methodologies for specific fields within forensic palynology (Horrocks 2004; Bryant 2011, 2013; Wiltshire et al. 2015a), but we are far from having reached a consensus with a detailed and standardized palynological protocol on the appropriate procedures in a forensic investigation.

The need to establish a theoretical framework unique to palynology has been acknowledged by forensic palynologists for a long time (Mildenhall 1982, 1988). Such a framework is considered essential for forensic palynologists, as it would enable them to present and defend credible arguments when interrogated in judicial headquarters (Wiltshire 2006). On an experimental basis, few works suffer from the problem of the equivalence between evidence from a shared location (Horrocks and Walsh 2001; Horrocks and Ogden 2003; Horrocks et al. 1998, 1999; Riding et al. 2007), but in most cases the examples shown are too difficult to compare with or link to real forensic situations (Bruce and Dettmann, 1996; Horrocks and Walsh 1999; McKinley and Ruffell, 2007; Horrocks et al. 1999; Bull et al. 2006; Riding et al. 2007). In addition, most of the published works with forensic palynology are geographically contextualised in temperate climates, adding even more difficulty to the search for experimental corollaries.

There is a lower level of tolerance to conjecture within this field than in other palyno-analytic areas. Even so, it cannot be contested that experimental works are certainly needed in forensic palynology. More work is yet needed to compare the pollen spectra recovered from

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different materials, and through different techniques, in the same geographical setting, as well as to explore locations that have not yet been researched. This article is motivated by these two concerns.

2. Materials and methods

2.1. Study area

This work began with the study of samples obtained on 18 September 2015 in the Escombreras Valley, in the municipality of Cartagena, Murcia (Fig. 1). This area belongs to the Baetic geologic zone, an area that runs from the coastal ranges to the mountains of Carrascoy and Espuña in the interior of Murcia. Bioclimatically speaking, this area is considered to be inside the thermo-Mediterranean belt, semi-arid ombroclimate (200–350 mm/yr) (Peinado et al. 1992).

2.2. Sampling sites and local vegetation

Samples were taken on 15 September 2015, from four different locations within an area of 8 km^2. These four locations share similar biogeographical features, including a predominance of endemic scrub in the area as well as the limited presence of trees.

Location A is sited at 138 m a.s.l. on a hill located by a road that connects Escombreras with the Nuestra Señora de los Remedios cemetery (37° 35′ 02.81″ N, 0° 56′ 38.65″ W). Among the local vegetation we found *Aristolochia baetica, Asparagus horridus, Atriplex glauca, Brachypodium retusum, Calicotome intermedia, Chamaerops humilis, Fagonia cretica, Genista umbellata, Helichrysum stoechas, Lithodora fruticosa, Lycium intricatum, Lygeum spartium, Periploca laevigata*, *Rosmarinus officinalis*, *Salsola oppositifolia* and *Stipa tenacissima*.

Location B is positioned at 148 m a.s.l. on a hill in front of Location A, around 20 m away from it (37° 35′ 02,54″ N, 0° 56′ 36,67″ W). At the time the samples were taken, the vegetation seen there was the same as in Location A, and therefore this location was not considered for the current study.

The samples from Location C were gathered at 38 m a.s.l. by the road that connects Cartagena and Escombreras, specifically around the outskirts of Escombreras (37° 34′ 33.00″ N, 0° 57′ 53.00″ W). The local vegetation included *Arisarum vulgare, Aristolochia baetica, Artemisia herba-alba, Asparagus albus, Asphodelus fistulosus, Asteriscus maritimus, Ballota hirsuta, Calicotome intermedia*, *Carrichtera annua*, *Chamaerops humilis*, *Fagonia cretica, Foeniculum vulgare, Genista umbellata, Helictotrichon filifolium, Hyparrhenia sinaica, Lobularia maritima, Malva parviflora*, *Marrubium vulgare, Moricandia arvensis*, *Opuntia maxima, Periploca laevigata Phagnalon saxatile, Phillyrea angustifolia*, *Polygala rupestris, Rosmarinus officinalis, Salsola genistoides*, *Salsola oppositifolia*, *Sedum sediforme*, *Sonchus tenerrimus*, *Suaeda vera*, *Thymelaea hirsuta* and *Vallantia hispida*.

Location D samples were gathered at 54 m a.s.l., on a small slope located between the north entrance to Escombreras (Cartagena-Escombreras road) and the Escombreras ravine (37° 34′ 59.12″ N, 0° 55′ 39.94″ W). Among the local vegetation we found were *Asparagus albus, Chamaerops humilis, Launaea arborescens, Lavandula multifida, Lycium intricatum, Osyris quadripartita, Periploca laevigata*, *Salsola oppositifolia* and *Withania frutescens*.

2.3. Sampling materials and methods

A total of 17 samples were taken (Table 1). Three were gathered directly from the ground at each location, with the help of swabs. Five were retrieved from fabric samples that had been in direct contact with the ground. Three were found with the help of adhesive used on a trouser leg. Six more were gathered with swabs from sediment that accumulated on footwear worn by the scientists.

2.3.1. Soil samples

In order to gather samples from the soil surface, we used sterile swabs that had been previously labelled and dampened with

Fig. 1. Location map.

Table 1
Samples recovered from the different sites studied.

		Site A	Site B	Site C	Site D
Soil		AS1		CS1	DS1
Fabrics		AT17 AT18 AT19		CT3	DT3
Shoes	Right	AC23		CC9	DC9
	Left	AC24		CC10	DC10
Trousers		AP25		CP11	DP11

phenolated water (Fig. 2f). The sediments were gathered by touching the ground four or five times with each swab. Phenolated water was used in order to moisturise the swabs and facilitate the act of gathering the sediments, as well as to ensure its appropriate preservation until its arrival and handling back in the laboratory. For this research, one soil sample from each location has been used (Table 1).

2.3.2. Fabric samples

To gather fabric samples, we used a number of different 50 × 30 cm pieces taken from a cotton t-shirt (Fig. 2d). The fabric was placed in direct contact with the soil surface several times, applying pressure on it with our hands to emulate the pressure a body could apply, the idea being that this fabric would have belonged to a person found laying on the floor during a forensic investigation. For this study, three different fabric samples were used, each one unique to Location AB, C and D (Table 1).

2.3.3. Footwear samples

In every location, in order to simulate the sampling from footwear belonging to a suspect who had been on a forensic site, the researcher each wore a pair of plastic clogs that had not been used before and that had previously been cleaned in order to avoid any possible contamination (Fig. 2b). The researcher put on these plastic clogs before entering each of the three main sampling locations (AB, C and D). The shoes were used during the entire duration of sample gathering at each location, included the way travelled by foot from where the car was parked to the specific location, and on the way back. Once back at the laboratory, the samples were retrieved by washing the surface and the sole of each of the clogs with the help of swabs and phenolated water (Table 1).

2.3.4. Samples gathered with glass microscope slides and adhesive

In each of the locations, samples were also taken through the use of glass slides with adhesive on one of their side. The samples were gathered from the knee area of one of the researcher's trousers who was present during the sampling gathering (Fig. 2e). In some cases the knee area had not been in direct contact with the soil surface (AB zone), and in others it had (C and D zones). The samples were labelled and kept in hermetic cases designed to fit microscope slides.

2.4. Laboratory processing

For the extraction of palynomorphs we followed the conventional chemical method (Delcourt et al. 1959; Dimbleby 1985). This method has been adapted to meet the nature of our samples, but in every case

Fig. 2. Collection and sampling. (a) Equipment, material and instruments used for sampling. (b) Footwear weared during collection of samples. (c) Sampling of the soil surface. (d) Collection of fabrics samples. (e) Recovery of sediment from the surface of the trousers by using a slide with adhesive. (f) Labelling of one of the samples collected with swab.

the processes suggested by Wiltshire (2015) were also included. We added to each sample one or two tablets of *Lycopodium* spores, which helped to evaluate the "quality" of the chemical treatment, as well as to identify palynologically sterile samples. After laboratory processing, the samples were set on microscope slides with the use of glycerol gelatine. For counting, the under side of each slide was put on a template that had been divided into different small grid cells that had been numbered and identified (an England Finder slide). The purpose of this was to be able to place any palynomorph in the preparation and to be able to locate it in future reviews and verifications.

Palynological identification was made through conventional light microscopy (400× and 1000×) using a bridged comparison microscope and the reference palynomorph collection from the Department of Plant Biology of the University of Murcia. The data extracted from the pollen count were digitalised in Excel and were subsequently treated with Tilia Graph 1.7.16 in order to obtain pollen diagrams and group the samples according to cluster analysis.

3. Results and discussion

All sampling methods have proven to be effective in terms of pollen-analytic results, with representative pollen and spore spectra and concentrations for each location identified (Table 2). Both at a qualitative and a quantitative levels, the pollen assemblages show relevant information about the local vegetation of each location studied. Taxa diversity in all samples exceeds 14 pollen types. Spore diversity reaches 11 distinct types, including fungi, bryophytes and pteridophytes (Table 2). Separate percentage pollen diagrams were constructed to include and exclude Chenopodiaceae from the pollen sum. Diagrams were also made including and excluding total pollen sum of the whole sample from each location (AB, C and D). For each of these diagrams, four different cluster analyses were conducted: one with pollen types excluding Chenopodiaceae, another one with all pollen types, another with all pollen types and spores, and lastly, one with pollen types excluding Chenopodiaceae but including all spore types. Each diagram showed similar groups of samples. Finally, the diagrams that best show similarity, or disparity, in the samples were selected (Figs. 3, 4 and 5). (See Fig. 6.)

Chenopodiaceae represents more than 20% of pollen grains identified in 13 out of 17 studied samples, is 55% of those identified in sample 9, and 80% in 6 (Fig. 4). In order to avoid the damping effect that all these occurrences could have on the percentages of the rest of the taxa, in Fig. 3 chenopods have been erased from the pollen sum used as the base for calculating the percentage of presence in each of the identified types. The calculation of the percentage of spores (Fig. 5) has been conducted separately from pollen and, in sum, all identified spore taxa have been taken into account.

To give a general idea of the pollen spectrum of each of the locations studied we placed a pollen sum in the upper part of the pollen diagram (Fig. 3), including all samples for each site, so that the differences between the three locations studied (AB, C and D) could be easily observed. In AB (once the Chenopodiaceae were excluded) there is a predominance of Poaceae, Brassicaceae, Caryophyllaceae, *Olea* and *Pinus*; in C, Genisteae, *Olea*, Cupressaceae, *Pinus*, Poaceae, *Aster* t. and Cichorioideae; in D, Cichorioideae, Cupressaceae, Genisteae and *Pinus* stand out, as expected due to the proximity (to the west) of a small

Table 2
Summary of the results. In the Count column, the number in parentheses represents the total pollen account including Chenopodiaceae.

Site	Samples	Pollen grains			Spores		
		Count (N°)	Diversity	Main taxa	Count (N°)	Diversity	Main taxa
A	AS1	280 (354)	21	Brassicaceae, Chenopodiaceae, *Olea*, Poaceae	165	9	*Multicellites*, *Polyadosporites*
	AT17	204 (269)	23	Chenopodiaceae, Poaceae, *Pinus*, Brassicaceae, Genisteae, *Olea*, Cupressaceae	487	9	*Monoporisporites*
	AT18	244 (349)	24	Chenopodiaceae, Poaceae, Genisteae, Cupressaceae, Fabaceae, Brassicaceae, *Olea*	437	9	*Polyadosporites*, fungal hyphae
	AT19	228 (316)	28	Chenopodiaceae, Poaceae, Cupressaceae, Genisteae	478	9	*Polyadosporites*, *Encalypta*
	AC23	46 (359)	16	Chenopodiaceae, Caryophyllaceae, Brassicaceae	911	9	*Alternaria*, *Monoporisporites*
	AC24	42 (360)	19	Chenopodiaceae, Poaceae, Caryophyllaceae	279	9	Fungal hyphae, *Monoporisporites*, *Polyadosporites*
	AP25	47 (342)	14	Chenopodiaceae, Cannabaceae, *Olea*, Caryophyllaceae, Genisteae, Fabaceae	1798	8	*Monoporisporites*
	A (summation samples)	1091 (2349)	39	Chenopodiaceae, Poaceae, Brassicaceae, Caryophyllaceae, *Olea*, *Pinus*	4555	10	Fungal hyphae, *Monoporisporites*, *Polyadosporites*
C	CS1	313 (361)	29	*Aster*, Cichorioideae	45	7	*Monoporisporites*, *Asplenium*
	CT3	255 (284)	29	Cupressaceae, Poaceae, *Pinus*	7087	7	*Hypoxylonites*
	CC9	61 (358)	17	Chenopodiaceae, Genisteae, *Olea*, Poaceae, *Artemisia*, *Pinus*	162	10	*Alternaria*, *Encalypta*
	CC10	49 (357)	18	Chenopodiaceae, *Olea*, *Aster*	233	10	*Alternaria*, *Encalypta*
	CP11	3 (21)	3	Chenopodiaceae, Cannabaceae	530	6	*Monoporisporites*, fungal hyphae, *Alternaria*
	C (summation samples)	681 (1381)	43	Chenopodiaceae, Genisteae, *Olea*, Cupressaceae, *Pinus*, Poaceae, *Aster*, Cichorioideae	8057	11	*Hypoxylonites*, *Monoporisporites*
D	DS1	363 (380)	29	Genisteae, Cichorioideae	60	7	*Monoporisporites*, *Encalypta*
	DT3	247 (288)	30	Cupressaceae, Chenopodiaceae	121	8	Fungal hyphae, *Polyadosporites*
	DC9	146 (353)	27	Chenopodiaceae, *Pinus*	142	9	*Alternaria*
	DC10	147 (360)	21	Chenopodiaceae, Cupressaceae	238	9	*Alternaria*
	DP11	11 (22)	9	Chenopodiaceae, *Pinus*	131	4	*Monoporisporites*
	D (summation samples)	914 (1403)	41	Chenopodiaceae, *Pinus*, Cupressaceae, Cichorioideae, Genisteae	692	11	*Alternaria*, *Monoporisporites*

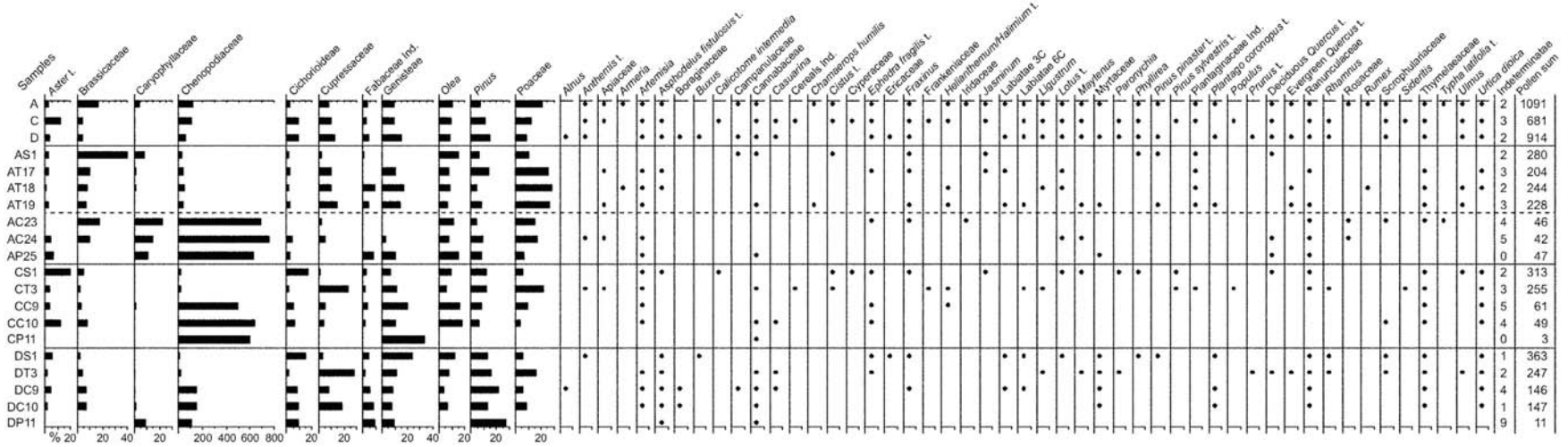

Fig. 3. Percentage pollen diagram. A, C and D samples are pollen sums including all pollen types from the different sites. Chenopodiaceae have been removed from the sum so that the low percentages of other taxa are visible. To decode samples names see Table 1.

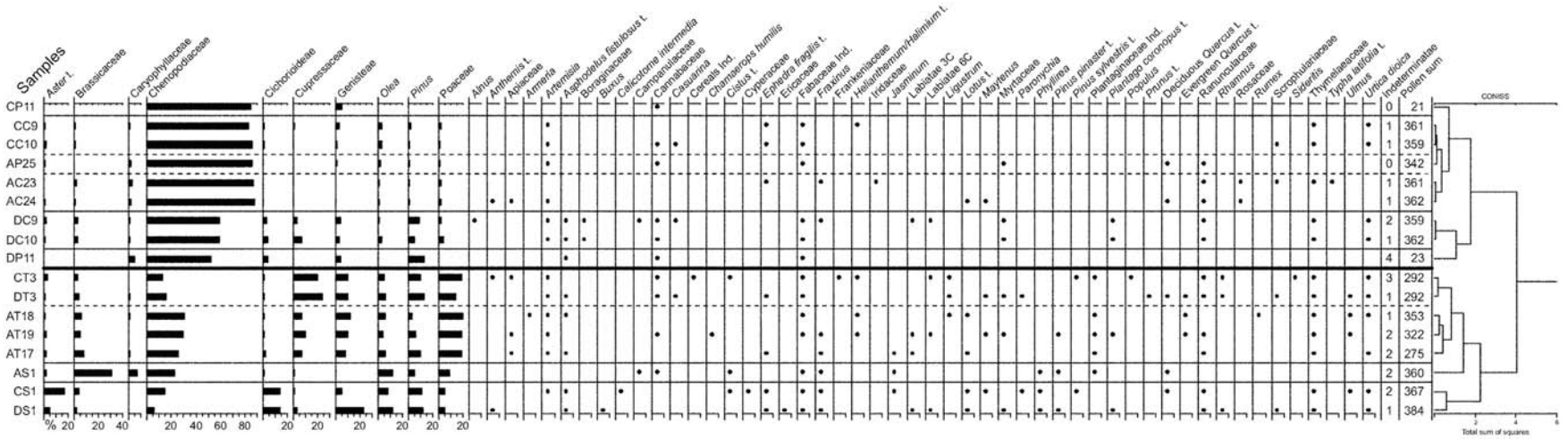

Fig. 4. Percentage pollen diagram with reordered samples after a cluster analysis. Chenopodiaceae were included in pollen sum and considered in reordination.

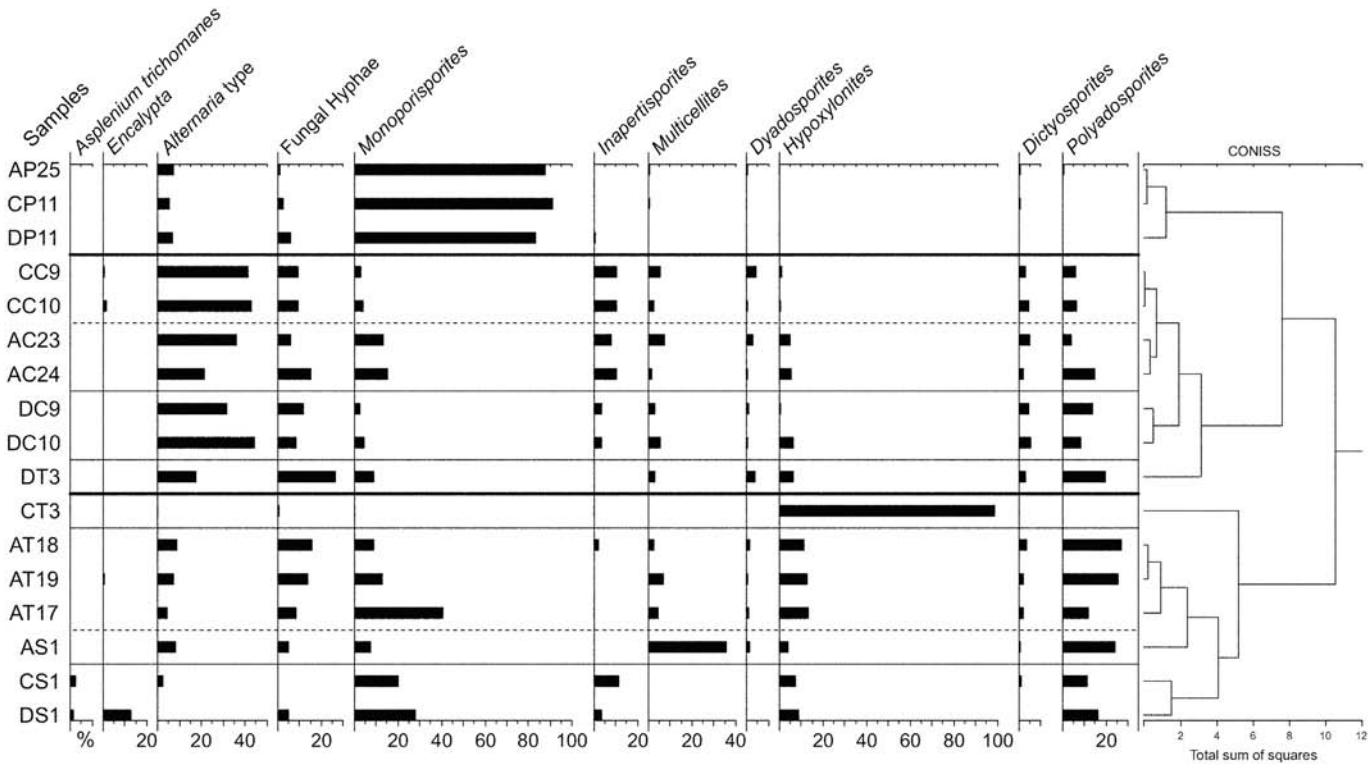

Fig. 5. Percentage spore diagram with reordered samples after a cluster analysis (Kalgutkar and Jansonius 2000).

population of pines, which are otherwise very scarce in the territory. Consequently, we can see all the samples grouped according to the location to which they belong.

The greatest differences in the pollen spectra are seen in relation to the type of sampling carried out; samples taken from the same location shown to offer different results. We should not be surprised at this since result. As other authors have pointed out, the differences in the pollen spectra of unique samples collected from the same location, separated by only a few meters, often reside in the quantities of pollen rather than in the number of different types of pollen (Horrocks et al. 1999). Many criminal scenes are usually restricted to only a few square meters,

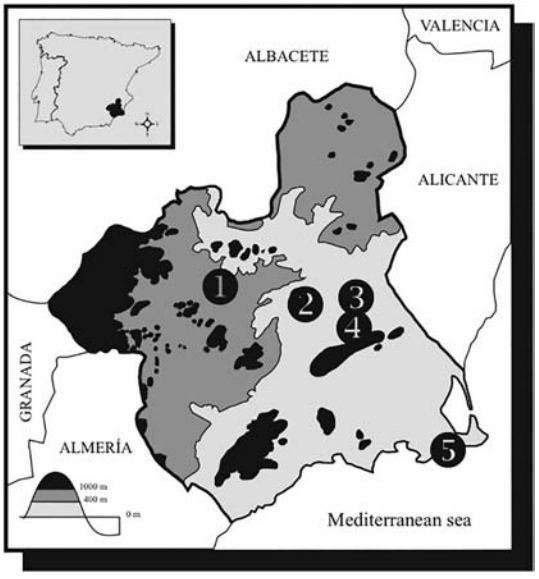

Fig. 6. Location of sampling sites in Murcia Region (Spain). 1, Carrascalejo; 2, Albudeite; 3, Espinardo; 4, La Alberca; 5, Cartagena (Martínez-Sánchez et al. 2008; Munuera and Carrión 2016).

meaning that they are "very localised areas" (Horrocks and Walsh 2001; Horrocks et al. 1999). These areas can show, on the ground, a particular and distinctive combination of pollen grains from species that make up the local and surrounding vegetation. Horrocks et al. (1998) argue that the pollen spectra of samples from a localised area can significantly differ from another, due to the variation of normal sampling of the soil surface and sampling of footwear (sampling randomly within a limited area). On the other hand, the pollen set of a given area could be similar to those of other zones with the same vegetation type. This taxonomic variation can be seen in the pollen types of the three studied locations, as well as in the different types of sampling carried out within each of the localities. The variation is significant in terms of the predominance of certain taxa and the concentration of certain pollen types.

The highest percentages of Chenopodiaceae (Fig. 3, Fig. 4) appear in the footwear samples, collected after washing them with wet swab (AC23, AC24, CC9, CC10, DC9 and DC10) and in those taken with adhesive tape from the surface of the researcher's trousers (AP25, CP11 and DP11), reaching, in some cases, 90% of the total pollen count. The smallest percentages are present in the samples taken directly from the soil with the swab (AS1, CS1 and DS1) and in those taken from the cotton fabric (AT17, AT18, AT19, CT3 and DT3).

Taxa such as *Aster*, Brassicaceae, Cichorioideae, Cupressaceae, Genisteae, *Olea, Pinus* and Poaceae make an appearance in the three locations, and do not appear to differ between the various techniques of sampling used. However, there are slight differences between each locality, with greater presence of Brassicaceae, Caryophyllaceae and Poaceae in Location A, and a lower presence of Cichorioideae. On the other hand, in Location C there is a greater presence of *Aster T. pinus* is slightly more abundant in Location D, as one would expect due to the proximity of a small pine population about 600 m northwest of the sampling site. The sum of the counts of all the samples from each locality (A, C and D) give a general idea of the most outstanding characteristics of each (Fig. 3).

In Fig. 4, samples appear ordered according to the conglomerates of homogeneity grouped according to the TILIA program, considering the

pollen taxa present in each sample and their relative proportions (including Chenopodiaceae).

The diagram (Fig. 4) can be divided into two well-defined parts. On one hand, we can see samples of footwear and trousers, and on the other hand, samples of soil and fabric from the three locations. Two of these zones are particularly interesting, because of their smaller but well-defined groupings.

In relation to the samples from footwear and trousers, a great similarity can be observed in the pollen spectra registered for zones AB and C differing from Location D due to their greater content of Chenopodiaceae and a lower presence of pollen from *Pinus,* Poaceae, *Olea,* Genisteae and Cupressaceae. For the localities AB and D, there is a strong association between the samples from footwear and those from the trousers. The sample from trousers of zone C are separated from those of footwear from the same locality, probably as a consequence of the deviation that occurred due to the low quantity of pollens identified (only 21, of which 20 were Chenopodiaceae). On the other hand, the degree of similarity between the elements (right and left) of each pair of footwear for each location is, as expected, considerably high in all cases.

The differences observed between the groups footwear/trousers and fabric/soil could indicate that the amount and diversity of pollen types that are deposited on upright plant materials (leaves, stems, branches, etc.) and on the surface of the soil are different, as Wiltshire suggested (2006). Studies in aerobiology conducted by Munuera et al. (1995) in the southeast of the Iberian Peninsula reinforce this idea, showing a high concentration of Chenopodiaceae in the atmosphere during the period in which the samples were taken. The direct contact of footwear (upper face of the shoe) and the trousers during the sampling of vegetation within each localities could explain the overrepresentation of Chenopodiaceae in comparison with the samples that come directly from the soil (fabric and soil samples), therefore highlighting the level of effectiveness of this type of sampling (footwear/trousers) when it comes to recovering palynomorphs from the plant environment. Thus, in future studies, it would be interesting to differentiate between the process of recovery of samples (and therefore of palynomorphs) from the sole of the footwear (in direct contact with the ground surface) and the upper part thereof (which would come in contact with the local vegetation of the area). In this way, the existence of differences between these two surfaces could be confirmed and quantified, and the corresponding study methods corrected for possible deviations.

Another possible explanation for the greater presence of Chenopodiaceae in the samples of footwear and trousers could be the fact that both the shoes and the trousers show the pollen spectrum of a wider area than the samples obtained from the soil with the swab or the cotton fabric. The reason is that shoes and trousers were worn not solely in the exact place of the sampling, but also during the short walk to and from the research vehicle.

In view of the results, it can be stated that the use of adhesive tape on microscope glass slide (as in the case of the trouser samples) is a more than adequate method to recover pollen and spores from the surface of garments. The adhesive tape method could very well be one of the best methods to collect pollen from a suspects' clothing. It is simpler, faster and less expensive than other methods, as other authors have shown in the recovery of pollens and fibres (Flinn 1992; Wu et al. 2006).

One of the possible limitations of the application of forensic palynology is the dilution or mixing of the pollen set in a criminal scene with those of other sites visited by suspect and investigator, both before, during and after the alleged crime. In this regard, Wiltshire (2006) notes that "it must be remembered that the assemblage obtained from the sole of a shoe (or any other object) will never match any crime scene perfectly. By its very nature, footwear may pick up palynomorphs from more than one place. However, the experience has shown that unless the offender walks on soil, vegetation, or on organic debris, shoes tend to pick up small (often insignificant) amounts of pollen during general wear. […] Nevertheless, the popularity of training shoes with highly structured soles has facilitated the use of forensic palynology in criminal investigations. It is very difficult indeed to remove palynomorphs completely from such footwear, even when it has been put through the washing machine." Therefore "[…] footwear is such an interesting and valuable resource in forensic palynology that it warrants further consideration elsewhere".

The samples of the soil surface taken from the three localities (AS1, CS1 and DS1) have been placed together after cluster analysis, highlighting the fact that the localities share a large number of pollen groups (Fig. 4). However, fabric samples from AB appear distinct from those obtained in C and D. We should not forget that these samples were obtained by pressing the cotton fabric against the ground by hand, simulating the effect of a person who had fallen. This method could, perhaps, explain the distinction in the pattern of the grouping of pollen between the soils of AB and CD.

The results of this work confirm that the pollen set of a sample of soil surface within a localised area can differ significantly from that of other nearby areas, even those with similar vegetation (Horrocks and Walsh 2001; Horrocks et al. 1998; Mildenhall 2006a; Mildenhall et al. 2006). The differences between the sediment samples of Location AB and Locations C and D can be explained by the type of soil and the plant density present in each of the zones. While the soil of Location AB is characterised by a greater presence of organic matter to a certain depth (1–3 cm), in the other two locations, the soil was observed as very poor in organic matter and of much shallower depth. From a strictly pedological point of view, one could say that Locations C and D are essentially empty soil above an outcrop of bedrock. The vegetation of Location AB is more grouped and denser than in locations C and D, in which the vegetation is more dispersed. The three locations are also affected by more abiotic factors such as: temperature (similar in all three locations), light (very similar in the three areas), humidity (similar for the three locations, although somewhat more intense in Location C, due to its greater proximity to the sea), oxygen and nutrients (greater disposition in Location AB since it has a soil richer in organic components). In the third place, the samples (AT17, AT18, AT19, CT3 and DT3), which present smaller amounts of Chenopodiaceae, are well grouped, although they are still the dominant taxon, alongside Poaceae (Fig. 4). In this group, samples of fabric from Location AB appear to be well grouped and differentiated from fabric samples from localities C and D.

In Fig. 5, a cluster analysis and reordination have been carried out by using spore concentrations. In relation to the diversity of non-pollen taxa, the presence of the majority of fungal morphotypes in practically all the samples analysed (Table 2 and Fig. 5) is noteworthy. This great diversity is observed throughout the spectrum, with a predominance of *Alternaria* type, Fungal Hyphae, *Monoporisporites*, *Hypoxylonites* and *Polyadosporites* (Kalgutkar and Jansonius 2000).

The groupings of samples according to the method used in the sampling (footwear, trousers, fabric and soil) that are seen in this diagram are similar to those in Fig. 4. However, in this case there is a closer relationship between the samples of trousers (AP25, CP11 and DP11), undoubtedly due to the high presence of Monoporisporites. Studies conducted by Munuera and Carrión (1995) and Munuera et al. (1995) in the southeast of the Iberian Peninsula corroborate the abundance of *Alternaria* spores, showing a high concentration in the atmosphere during the period in which the samples were taken. Appearance in the footwear samples is of significant interest.

A previous study provides data of interest in the context of the results described above. Martínez-Sánchez et al. (2008) and Munuera and Carrión (2016) took five different vegetation areas from the same region, the southeast of Spain, in which the collection and comparative study of two categories of samples was carried out, one from the surface sediment and another from the sediment deposited in the footwear sole. The palynological study of soil surface samples is an adequate tool for recording vegetation differences in arid environments (Carrión 2002), and it seems to have great potential for forensic sciences

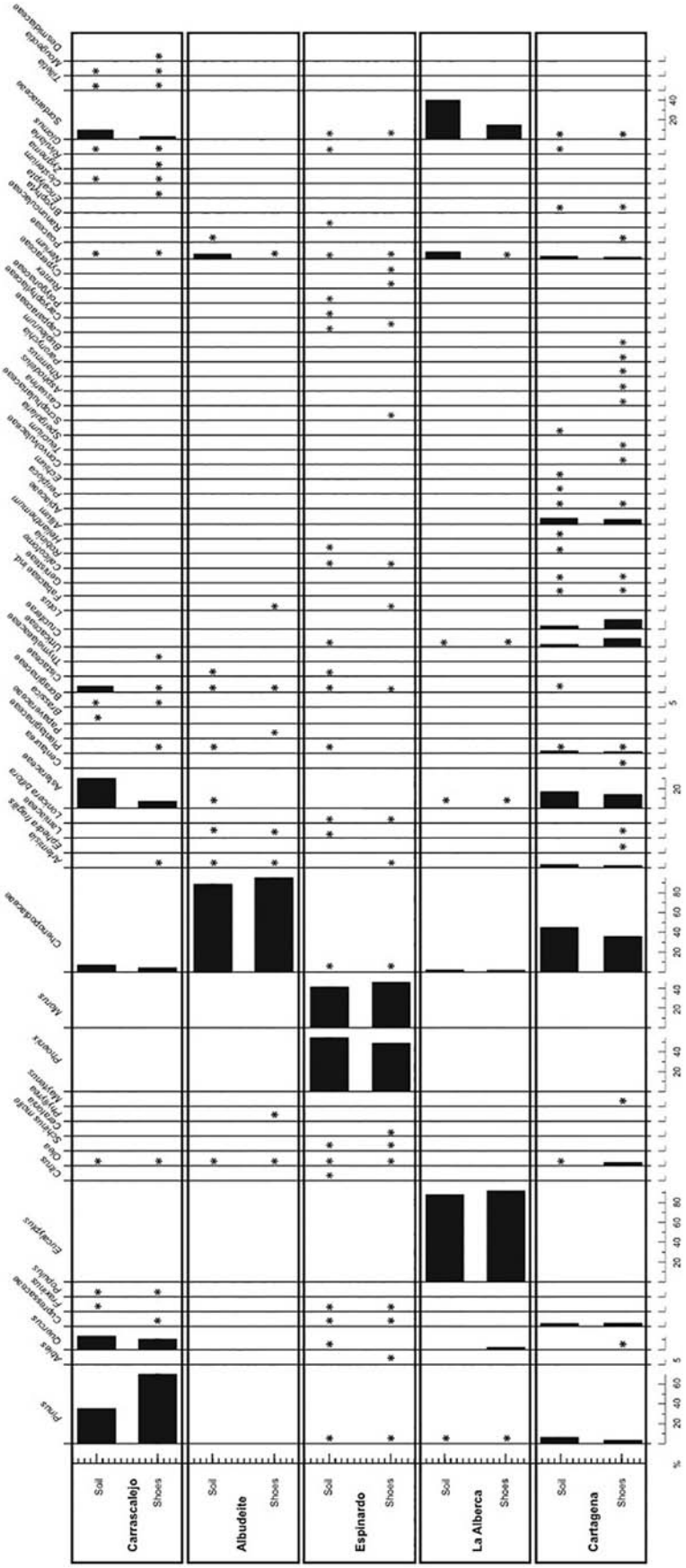

Fig. 7. Pollen diagram showing pollen and spore percentages for soil surface and shoe samples. Percentages below 2% showed as*.

(Guedes et al. 2011). As might be expected, and with the exception of coastal Cartagena, the pollen spectra show a relatively low diversity with a dominance of wind-pollinated taxa (Hall 1985; El Ghazali and Moore 1998). Through microscopic examination, a total of 57 pollen types (54 Magnoliophyta and 3 Pinophyta), 10 spore types (2 Bryophyta, 5 Algae and 3 Fungi) and 1 Oribatidae acari type were identified. For each case study, the pollen diagram (Fig. 7) showed a close similarity between soil surface samples and sediment retrieved from footwear, not only in the main pollen types, but also in rare types and fungal spores and seaweed. The proportion of matching taxa in soil and footwear samples is around 45% in Albudeite, Espinardo and Cartagena, reaching 61% in Carrascalejo and almost 90% in La Alberca. In Carrascalejo a total of 23 pollen types were identified, 61% of them in both soil and footwear samples. According to its presence in the environment, *Pinus* is the dominant type, with *Quercus*, Chenopodiaceae, Asteraceae, *Populus* and Cistaceae other important elements that characterise the site. In Albudeite, Chenopodiaceae, Poaceae and *Tamarix* are the most abundant types, but in the pollen count the Chenopodiaceae reach 88.6% in the samples of the surface of the soil and 95.3% in the samples of footwear. In Espinardo, although some trees exist in the selected area (*Citrus, Schinus, Fraxinus* and *Robinia*), their pollen grains were scarce in the samples, while the pollen of *Phoenix* and *Morus* exceeded 95% of the pollen found both on the surface of the ground and in the samples of the footwear. In La Alberca, with the sole exception of *Quercus* (only found in shoes), the same taxa are found on the ground surface and shoe samples. Finally, the greatest diversity is observed in Cartagena, with a total of 37 taxa, 30 of them present in the footwear samples. The correlation of taxa between the soil surface and the footwear samples reaches 43%.

The pollen spectra studied by Martínez-Sánchez et al. (2008) show five well differentiated habitats which are quite well correlated with the main vegetation present in their surrounding areas (Fig. 7). Despite the presence of coincident pollen taxa in some of them, differences marked by the concentrations of the major taxa are noted.

The results from Cartagena site (Martínez-Sánchez et al. 2008) show a high similarity to the data obtained in our samplings in each of the three locations studied in Escombreras. Although the three locations seem similar, from a biogeographic and vegetational point of view, the cluster reveals that the three localities (AB, C and D) are statistically different. In both studies, the predominant taxon corresponds to Chenopodiaceae pollen and even the proportions of other taxa like Asteraceae and *Pinus* are similar. The recovered footwear samples in the two studies, not only offer optimal results but also show a high number of coincident taxa, such as *Pinus*, Chenopodiacae, Asteraceae, *Quercus, Olea, Maytenus, Artemisia, Ephedra fragilis* and Cupressaceae. For both studies, the identification of the spore of the moss *Encalypta* (accidentally present in three of our samples), marks a differentiation between this most recently studied location and others in the southeast of the Spain.

The palynomorphs that could help to trace evidence in a crime scene (mainly pollen grains and spores) will have their origin mainly in soil (the remains of more or less important sediments can be deposited in footwear, skin, clothing, etc.), in plants where friction or other contacts may occur and, to a lesser extent, the atmosphere itself.

The few experimental works already published on the use of soil as a source of pollen evidence generally refer to small areas from a single location (Horrocks and Walsh 1999; Horrocks et al. 1998, 1999; Bull et al. 2006) but are still important and necessary studies, both in terms of the variation of the pollen footprint between the different types of sampling within a specific area, and of the variations existing between more separated localities.

4. Final remarks

To our knowledge, no previous work of forensic palynology presents an experimental study of the possible variation in the spatial distribution of pollen assemblages within the same environment or given area, or the differences that may arise due to different types of sampling within the same. This is a fundamental limitation on the applicability of the forensic analysis carried out to date, and probably marks the research route to be followed in the coming years.

The body of results accumulated in recent years has made it clear that the only truly predictable aspect of the spatial pattern of pollen spectra is its unpredictability (Wiltshire 2006, 2015), and that the diversity of pollen morphotypes can vary significantly from one sample to another (Horrocks et al. 1998, 1999). Future experimental studies will allow the establishment of new models to help interpret the processes of dispersion and the presence of palynomorphs within a specific crime scene.

Studies such as this one contribute to a better knowledge of the processes that may be occurring in semiarid environments, where (besides the taphonomic differences that affect the distribution and deposition of pollens) the recovery of samples from forensic scenarios can be compromised. Forensic investigations in semiarid locations present unique challenges, at least in comparison to areas of higher precipitation. In wetter climates, the very nature of the soil and its low dryness facilitates the adhesion processes on the elements present in a forensic scenario and enables the recovery of larger amounts of samples.

After the study conducted we can conclude that:

1. Samples from the same forensic scenario may present different pollen spectra depending on the method used for their collection, so the establishment of correlations between objects or people that may have been present in that place must be done on the basis of samples from the same type.
2. Despite the possible disparity in the percentages found in the samples, depending on the sampling method used to take them, there is a palynological signal in the background that allows the establishment of relations between samples with a certain degree of reliability.
3. Here, we confirm the potential of pollen analysis in forensic scenarios of arid lands.
4. Under very defined circumstances, geographical or forensic scenarios can be determined based on the palynological contingent.
5. It is necessary to carry out more experimental studies that recreate the real and diverse conditions that can occur in a forensic scenario and the difficulties that may arise in obtaining valid samples, as well as establishing a protocol of action that can be carried out by an agent even without the presence of a palynologist (although this will always desirable).

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