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# Spatial and temporal environmental pollen analysis of footwear worn in the area of Barcelos, North-West Portugal, in a forensic context

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**Abstract** In this work, we performed a palynological analysis of soil sampled from the footwear in order to provide their spatial and temporal classification. The sampling was performed in the shoes of 20 volunteers, in two different periods: autumn–winter and spring 2016/2017. The volunteers belonged to the same geographic region within a radius of 500 m. During the laboratorial treatment, the pollinic content present in the shoes sole was concentrated applying several treatments to destroy the exogenous material. A hierarchical cluster analysis was performed to verify whether it was possible to distinguish the samples based on their pollen assemblages. All the samples showed pollinic content, even when the shoes were apparently clean. The pollen types found were similar across the sampling period although with different pollen assemblages. In the winter samples, there was a prevalence of *Fraxinus*, Myrtaceae, Pinaceae (mostly *Cedrus*) and Poaceae pollen, while in the spring pollen of *Olea*, Poaceae, Pinaceae (namely *Pinus*) and *Quercus* was observed. Our results also demonstrated the discrimination between close sites (approximately 200 m), showing that the use of palynological analysis in footwear allows the collection of temporal and spatial information that can be used in forensic investigations.

**Keywords** Forensic sciences · Palynology · Soil

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## 1 Introduction

In the forensic context, pollen and other palynomorphs are 'silent proofs', since their small size allows them to easily adhere to various media without being noted by the naked eye (Mildenhall 2006). In association with this, other pollen characteristics such as resistance and morphology, which allow taxonomic identification, make palynomorphs a strong evidence in various crimes occurred in indoor (Nguyen and Weber 2016) and outdoor environments (Mildenhall 2006; Horrocks and Walsh 2001).

Soil works as a natural pollinic storage (Adams-Groom 2012), being able to provide investigative intelligence and/or evidential value in forensic cases. So, the contact of footwear with pollen-releasing plants or soil may provide information through palynological analysis and its comparison with control samples (Adams-Groom 2018; Mildenhall 2006), enabling the reconstitution of the possible crime scene flora, and help link a suspect to a specific location. The literature reports forensic cases of rape (Mildenhall 2006; Horrocks and Walsh 2001) and robbery (Mildenhall 1990) where this type of trace evidence provided useful information linking suspects and/or victims with a crime scene, and verifying testimonies and alibies.

Since the literature describing the discrimination of different places and/or pollen linking footwear to a specific crime scene is still scarce, limiting the number of references citable in court (Adams-Groom 2018), studies performed by Adams-Groom (2018) and Ochando et al. (2018) are essential to provide an empirical and statistical evidence of a link between a crime scene and a suspect. In this context, the main aim of this work was to test the capability of, statistically, grouping individuals by the palynological analysis of their footwear.

## 2 Materials and methods

### 2.1 Study area

This work was performed in Alvelos (41°31′44 N and 8°37′19 W), located in the north-west of Portugal. It is a rural area characterized by oak, pine and eucalyptus forests, shrubby areas (e.g. Asteraceae, Cupressaceae, Amaranthaceae, Ericaceae and *Ligustrum*) and agricultural fields, composed by small areas with temporal crops (e.g. vineyards, maize and fodder cultures).

The region has an Atlantic wet climate, with strong exposure to maritime winds, high annual precipitation (1200–2400 mm) and mild summers (Miranda et al. 2001). Regarding the type of soil, the study region is dominated by Cambisols (Cardoso et al. 1971).

### 2.2 Sampling

The sampling was performed in the shoes of 20 volunteers, of both genders, between 16 and 60 years old in two periods: autumn–winter (November 2016 to January 2017) and spring (March to May 2017). The volunteers belonged to the geographic region within a radius of 500 m in order to maintain a similar vegetation across the sampling area, to test whether even in close locations presenting the same vegetation the differentiation of samples through the palynological analysis is possible. The footwear of all the volunteers were 'athletic' type, in daily use, without being cleaned before the sampling took place.

The shoes' sole was scraped with a spatula into a petri plate placed under the shoe. Afterwards, the removal of the remaining material was obtained by brushing the sole with triton, followed by washing with distilled water.

Afterwards, the content of the petri plate was transferred to a tube, and in the laboratory, the pollen was extracted, the residue obtained was dried, glycerol phenolated was added and microscope slide were prepared as described in Guedes et al. (2011).

### 2.3 Data analysis

Pollen identification was carried out using an optical microscope (Leica DM/LS) at 400 × along ten regular traverse rows. Pollen grains were classified by comparison with a published atlas (Reille 1992, 1995, 1998). The percentage for each pollen taxa was calculated, in both seasons, for each individual. From these results, the mean was calculated, per season, for every taxon in order to analyse the dominant pollen present in each sampling period.

A hierarchical cluster analysis was performed to discriminate the samples based on their pollen assemblages. The cluster analysis was performed using the squared Euclidean distance as a distance measure and the Ward's method as a linking method, using the software IBM SPSS Statistics 24.

## 3 Results and discussion

Our study focused on the palynological analysis of footwear in order to establish a temporal and spatial identification of the collected samples. Pollen was observed in all the shoes, even when the footwear sole appeared clean immediately before the sampling. The presence of palynomorphs in footwear, without deposits of soil, was already reported by Adams-Groom (2018).

A total of 34 different taxa were identified in the samples. The results reveal a similar pollen diversity present in the samples collected in the spring (28 pollen taxa), when compared to the winter samples (26 pollen taxa), although with different pollen assemblages. Thus, the amount of airborne pollen in our region reaches its maximum between early spring and early summer, having March the higher average pollen values (Ribeiro and Abreu 2014). As footwear easily collects airborne pollen (Adams-Groom 2018), it was expected that spring samples would present a higher pollen diversity. However, soil works as a natural pollinic storage, retaining pollen from previous flowering seasons for long periods of time due to their physical and chemical resistance (Adams-Groom 2012), thus justifying the results obtained.

The dominant pollen present in each sampling period, with a frequency higher or equal than 2%, is presented in Fig. 1. A difference between the dominant pollen taxa found in the winter samples when compared to the spring samples is notorious. In the winter samples, a dominance of *Fraxinus,* Myrtaceae, Pinaceae (mostly *Cedrus*) and Poaceae pollen was observed, representing 70% of the pollen concentration, whereas in the spring samples pollen of *Olea*, Poaceae, Pinaceae (namely *Pinus*) and *Quercus*, representing 64% of the pollen concentration, was observed. We also observed pollen of Apiaceae, *Artemisia*, Asteraceae, *Betula*, Brassicaceae, Caryophyllaceae, Cupressaceae, *Echium*, *Juglans*, Lamiaceae, *Laurus*, *Ligustrum*, *Liquidambar* and *Rumex* in both sampling seasons, although in non-representative frequencies. It should be highlighted that in spite of its small representativeness, even in trace amounts, if their distribution, frequency and abundance are limited they could be the most useful and interesting taxa in a forensic context, since some taxa are produced in great quantity and may be of little relevance, while others occur in small amounts in samples but could be more site-specific (Adams-Groom 2015).

In each sampling season, a relationship between the dominant pollen taxa, the flowering season and the airborne pollen concentrations is evident, allowing the extraction of temporal information based on the palynological analysis of soil trapped by footwear. Temporal identification was possible due to the airborne pollen seasonality already demonstrated by several authors. In the region, this airborne pollen seasonality was demonstrated by Ribeiro and Abreu (2014), where the distribution of total airborne pollen concentration is characterized by well-demarked

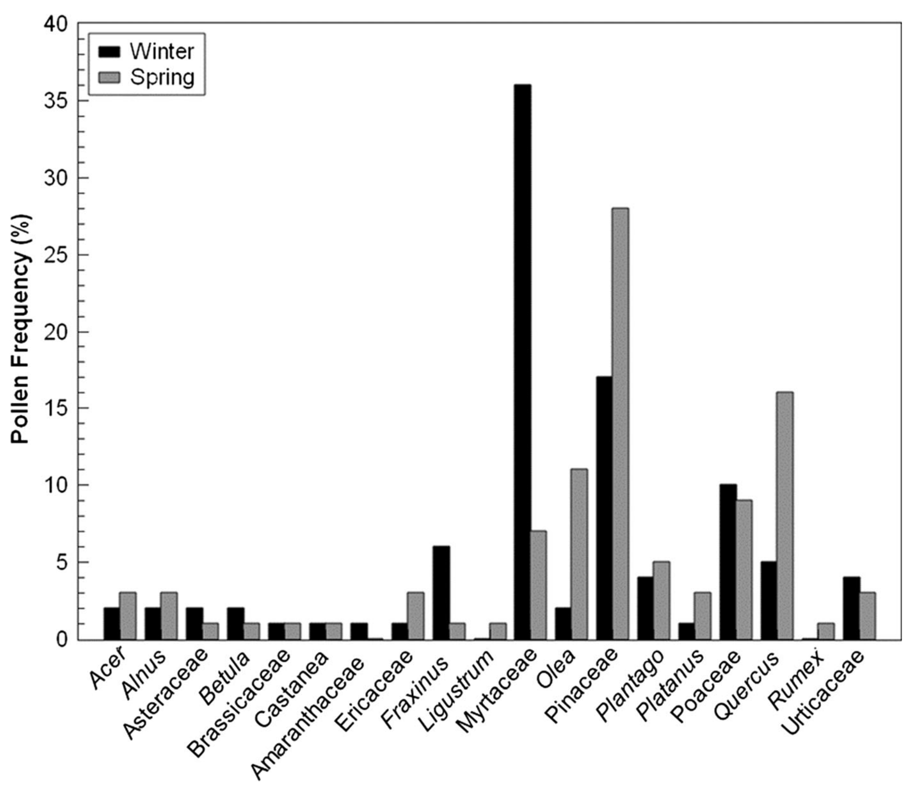


**Fig. 1** Frequency of the dominant pollen types (frequency greater than 2% in a given season) found in the winter and spring

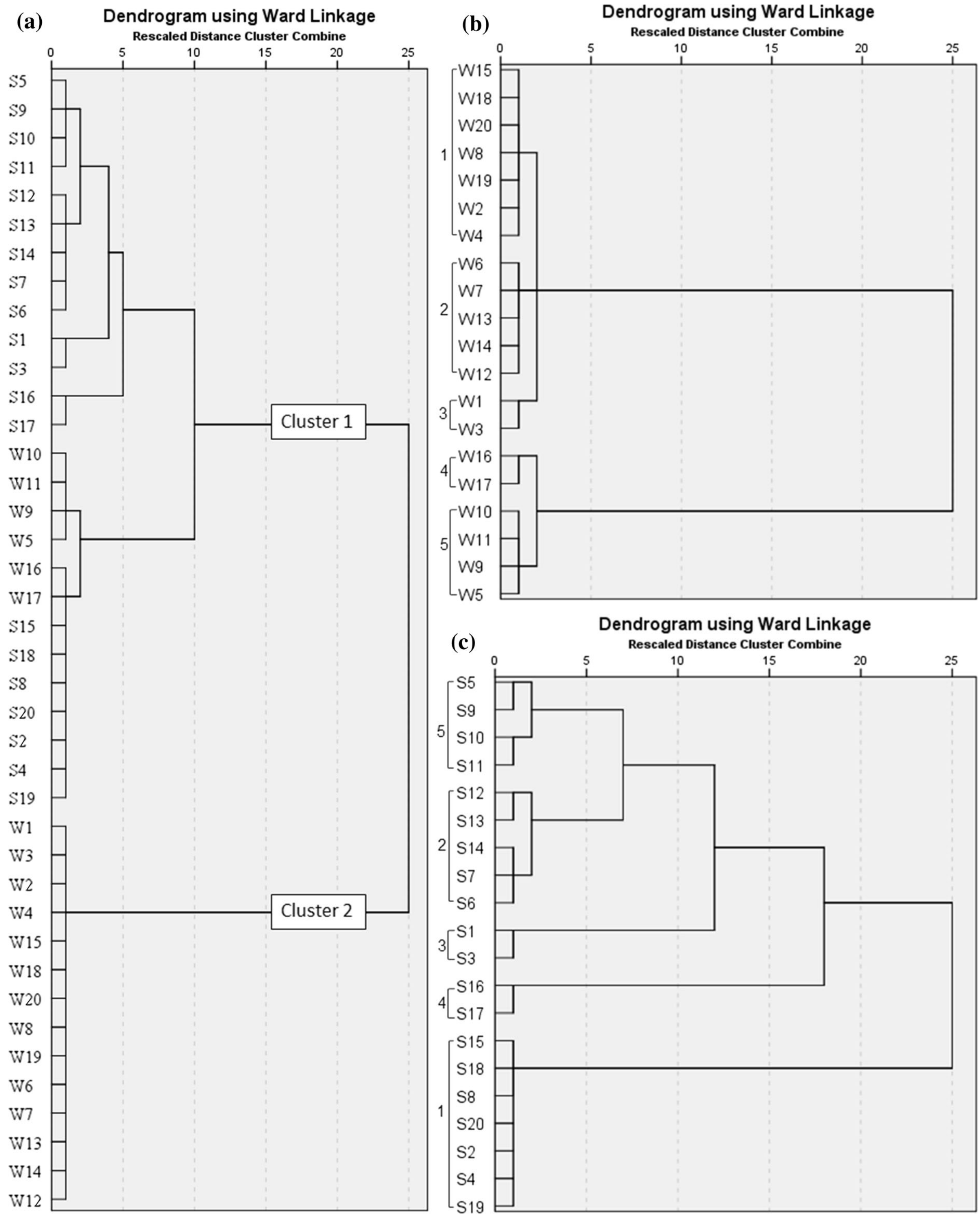


**Fig. 2** Dendogram of cluster analysis performed using footwear samples from **a** both seasons, **b** winter and **c** spring. *S* spring, *W* winter


**Fig. 3** Individuals' residential location, also corresponding to their last frequented location in both sampling seasons. Different cluster groups are highlighted in the figure (1–5 groups)

peaks by the diversity of pollen taxa in the atmosphere and its different flowering seasons. Even though our results allowed a temporal discrimination, in crime cases this may not be possible, since the presence of pollen on footwear can be dependent on other factors besides seasonal exposure or direct collection from the habitat. An example of such factors is the interval of time between the collection of the footwear by the police and the crime, since its continuous use allows the collection of new material at different times and locations, hindering the correlation between the pollen assemblage retrieved from the footwear and that of the local of the crime at the time (Wiltshire 2016). The types of soil are also an important factor that may influence the results (Guedes et al. 2011). Item cleaning by the suspects or the homogeneity of the habitat across areas or regions may also be factors that have to be considered in crime cases.

Figure 2 depicts a hierarchical cluster analysis performed to test the pollen assemblages for any forensic interest discrimination. When all collected samples were considered, two major clusters are visible (Fig. 2a). In cluster 2, fourteen (out of twenty samples) collected in the winter clustered together, while in cluster 1 all twenty spring samples plus six, closely related, winter samples are joined. These results show that temporal discrimination of soil samples is possible, but Newsome and Adams-Groom (2017) did not find them in temperate British woodland soil.

Figure 2b, c details a cluster analysis performed separately for both sampling seasons. Discrimination between samples was observed, with five groups emerging in both seasons. There was a correspondence between the groups formed in both seasons by the samples from the same individuals.

The individuals' residence, also corresponding to their last frequented location in both seasons, is plotted in Fig. 3. The existence of five location clusters is observed, each formed by the same individuals aggregated in the cluster analysis. These results demonstrate that, in the study region, spatially close sites (approximately 200 m) have characteristic pollen spectrums, allowing individuals to be grouped based on their last frequented location. The negligible influence of past frequented locations on the results meets the conclusions obtained by Riding et al. (2007), where the palynological signature reflects the last site visited and past sites become more diluted.

## 4 Conclusions

The use of palynological analysis in footwear allowed the collection of temporal and spatial information that can be used in forensic investigations. The dominant pollen types are related to the seasonality of the airborne pollen, enabling the sampling season to be determined. The pollen assemblage was different between samples of the same season, since its composition is dependent on the flora surrounding the individuals last frequented location. This allowed the individuals to be spatially grouped without resorting to the collection of control samples.

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