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- Land cover maps using GIS were created and compared with airborne pollen content.
- Circular statistics methods for studying pollen and land cover analysis were used.
- Hourly peaks were mostly observed at night or early in the morning.
- Local wind direction pattern influenced clearly to pollen distribution results.
- Pollen types showed different behaviour depending on their sources distribution.

Figure 1 consists of three maps of the Doñana wetland, labeled 'Don Benito', 'Píscis', and 'Zafra'. Each map shows a different distribution pattern of plant species across the wetland area. A legend on the left identifies the species: 'Sphagnum fuscum' (dark green), 'Sphagnum magellanicum' (light green), 'Sphagnum squarrosum' (yellow), 'Sphagnum subsecundum' (orange), 'Sphagnum squarrosum' (red), 'Sphagnum subsecundum' (purple), 'Sphagnum squarrosum' (brown), 'Sphagnum subsecundum' (pink), 'Sphagnum squarrosum' (grey), 'Sphagnum subsecundum' (white).

Pollen source area

Thus, the main land cover was different for each monitoring location, being irrigated crops, pastures and hardwood forests the main categories among 11 types described. Comparison of the pollen content with the predominant winds and land cover shows that the atmospheric pollen concentration is related to some source areas identified in the inventory. The study found that some pollen types (e.g. *Plantago*, *Fraxinus*-*Phillyrea*, *Alnus*) come from local sources but other pollen types (e.g. *Quercus*) are mostly coming from longer distances. As main conclusions, airborne particle concentrations can be effectively split by addressing wind with circular statistics. By combining circular statistics and GIS method with aerobiological data, we have created a useful

<http://dx.doi.org/10.1016/j.scitotenv.2017.01.085>
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tool for understanding pollen origin. Some pollen loads can be explained by immediate surrounding landscape and observed wind patterns for most of the time. However, other factors like medium or long-distance transport or even pollen trap location within a city, may occasionally affect the pollen load recorded using an air sampler.

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1. Introduction

Some biological airborne particles present in the air (mainly pollen grains and fungal spores) are widely known for being one of the most important allergy factors in humans (D'Amato et al., 2007; Docampo et al., 2007). The prevalence of allergic diseases increased in the last decade, especially in developed countries (Greiner et al., 2011), and has now reached 20% of the total world population (Armentia et al., 2007). Presence of pollen grains and fungal spores in the air depends on vegetation, available substrata (Arobba et al., 2000; Gonzalo-Garijo et al., 2006; Latorre, 1999; Raynor et al., 1975; Velasco-Jiménez et al., 2013) and environmental factors that enable their formation, release and transport (Frenguelli et al., 1989; Galán et al., 1989; Grinn-Gofroñ and Strzelczak, 2013; Mesa et al., 2003; O'Rourke, 1990; Tormo-Molina et al., 2015). Pollen transport between plants depends on a complex group of factors including features of the pollen grains, weather conditions suitable for pollen release, transportation, deposition and re-suspension phenomena (Fernández-Rodríguez et al., 2014b; Fernández-Rodríguez et al., 2014c; Sofiev et al., 2006). The air masses facilitate the transmission of important plant pathogens and pests from infected to non-infected areas (Isard et al., 2005), as well as dissemination of major allergens such as ragweed pollen from areas of high plant abundance to areas where ragweed plants have not yet been or are rarely observed (Šikoparija et al., 2013). Thus, in recent years more attention has been given to the analysis of the air mass origin, transport duration and travelled distance, which can explain sudden and increased levels in airborne concentrations (Fernández-Rodríguez et al., 2014c). Since the air mass and wind directions are variables of a non-linear character, thus circular statistics should be used to examine the overall distribution pattern as well as potential relationship between them and other variables of a linear and/or non-linear type. The application of circular statistics has become more evident in aerobiological surveys since it better addresses these dependences as well as enables analysis in relation to time (Albaladejo et al., 2012; Kasprzyk, 2006; Sadyś et al., 2015).

Use of Geographic Information Systems (GIS) software as analytical tool in aerobiology is increasing in recent years. This can be explained by factors such as the increase of the computational power and new technological developments. The data digitalisation and the understanding of these bio-aerosols, which are one element integrated with the environment, has been essential for its use as well. Consequently, GIS has been used as a decision support tool for applying preventive treatments in harvests (Merritt et al., 1999; Mitchell et al., 1996) and for selecting the optimal number of sampling points and their proper location (Alba et al., 2006), as well as an assessment tool in order to plan and design urban parks (Pauleit and Duhme, 2000) and the development of vegetation inventories (Skjøth et al., 2008). Analysis of potential sources of selected allergenic pollen types (Fernández-Rodríguez et al., 2014a; Rojo and Pérez-Badía, 2015; Skjøth et al., 2013) and spores (Fernández-Rodríguez et al., 2015) in the air was used as an instrument to estimate potential exposure risks to the high concentrations of those particles. Due to the link between pollen spectrum and plants distribution, land cover changes cause fluctuations in those pollen concentrations. Recent studies about the land cover influence and its relationships with aerobiological data have been developed (García-Mozo et al., 2016; Oteros et al., 2015; Rojo et al., 2015), being analysis of identification of the factors positively and negatively associated - a tool to know the behaviour of selected plants (Pinke et al., 2011; Skjøth et al., 2013; Skjøth et al., 2010). These studies have allowed us to understand the relationship between land

use and airborne pollen concentrations, and to identify their emission sources, e.g. the cases of *Olea* (Fernández-Rodríguez et al., 2014a; Oteros et al., 2015) or *Betula* (Skjøth et al., 2007) pollen. However, a big gap in knowledge still remains in regard to the connection between distribution of most pollen types and their sources of origin which can often be difficult to locate due to several causes. García-Mozo et al. (2016) studied changes provoked by human activities, finding a rise in *Olea europaea* pollen counts as a consequence of a growing surface occupied by olive crops. On the contrary, the authors observed a decrease in the pollen counts of some herbaceous plants which was explained as a result of reduction in their land covers, caused by a strong urban development. Climate change also affects plants distribution, and some species can spread into a new territory and reach higher concentrations than local plants (Hamaoui-Laguel et al., 2015; Ziello et al., 2012). Skjøth et al. (2013) stated that intense grazing and management (preventive cutting) are impediments for plants to achieve the maturity. These management strategies not only affect herbaceous plant phenology, but also make it more difficult to locate origin sources of herbaceous plants and trees, respectively. Sometimes, the use of wild plants being used as ornamental plants in cities (e.g. *Eucalyptus camaldulensis*, Pinaceae, *Olea europaea*, etc.) can make it more difficult to locate the origin of particles, especially if they grow near to the pollen trap (Maya-Manzano et al., 2017).

Hourly analysis of the most abundant five pollen types was also done in our work. In literature, factors such as the existence of pollen coming from different sources (Maya-Manzano et al., 2016), geographical barriers (Alba et al., 2000), different meteorological patterns (Alba et al., 2000), altitude (Alba et al., 2006) or differences attributed to species within a pollen type along the flowering season which can disturb hourly patterns (Peel et al., 2014), were cited as a possible explanation to understand differences between hourly patterns, even for the same pollen type.

Our study hypothesis is that whereas for some pollen types the dispersal behaviour is predominantly dependent on transport to medium-long distance, other pollen types seem to be more affected by presence of sources nearby pollen trap.

The aim of this study is to develop a method combining circular statistics and GIS for understanding the behaviour of pollen. With this method we want to describe the relationship between airborne pollen and land cover. This would be studied by means of the following tasks: 1) to analyse the behaviour of airborne pollen grains using Seasonal Pollen Index of the main 14 pollen taxa, 2) to discuss the hourly peaks in the distribution of the main 5 pollen types and their relationship with the predominant wind direction, and 3) to analyse the influence of land cover nearby the pollen traps, in areas of 10 km of radius, surrounding the three cities studied, in the Extremadura region of SW Spain.

2. Material and methods

2.1. Study area

The study was carried out from March 2011 to March 2014 in three localities of Extremadura (SW Iberian Peninsula), in Plasencia - PL (40° 2' N, 6° 5' W, 450 m a.s.l.), Don Benito - DB (38° 57' N, 5° 52' W, 276 m a.s.l.) and Zafra - ZA (38° 25' N, 6° 25' W, 509 m a.s.l.). Extremadura (Fig. 1) is an agricultural region (41 635 km²) with a 290 km spread from the northern border to the southern. Extremadura has a Mediterranean climate with hot summers (26.1 °C and 26.0 °C as mean temperatures in July and August during the period 1981–2010) and mild

winters (8.2 °C in January during the same period). Its annual mean temperature is 16.7 °C with an annual summation of rainfall of 499 mm (AEMET, 2016). Two main rivers (Guadiana and Tajo) separated by a central medium-high mountain range, run along the two provinces, Badajoz and Cáceres. Natural vegetation includes forests of holm oaks (*Quercus rotundifolia*), cork oaks (*Q. suber*) and Pyrenean oaks (*Q. pyrenaica*), the last one occurring in higher altitudes, between 600 and 900 m.a.s.l., together with other hardwood forests like chestnut (*Castanea sativa*), pine (*Pinus pinea*, *P. pinaster*) and eucalyptus (*Eucalyptus camaldulensis*, *E. globulus*). There are also important surfaces occupied by *Retama sphaerocarpa* and *Cistus ladanifer*. This landscape represents well the surroundings of Plasencia, situated in the North of Cáceres province. In most cases shrublands were eliminated to create dehesas – an agroforestry system of holm oaks and cork oaks. The area is for the most part, used for livestock production (associated with herbaceous plants like *Amaranthaceae* sp., *Asteraceae* sp., *Echium plantagineum*, *Rumex bucephalophorus*, *Plantago lanceolata*, *Poaceae* sp., etc.) and free-range grazing and sparsely for cereal, olive and grapevine cultivation, like in Don Benito and Zafra, both of them situated in the Badajoz province. Riparian forests are made up of *Ulmus minor*, *Fraxinus angustifolia*, *Salix atrocinerea*, *Celtis australis*, *Tamarix* sp., *Nerium oleander*, *Alnus glutinosa*, *Betula pendula*, *Populus alba* and *P. nigra* (Charco et al., 2008; Devesa, 1995).

2.2. Pollen data

Hirst type traps (Hirst, 1952) were placed at roofs of two local hospitals (in Plasencia and Zafra, at 17 and 6 m above ground level, respectively) and at a secondary school (in Don Benito, 6 m above ground level). The methodology developed by the Spanish Aerobiology Network (Galán et al., 2007) was used to obtain pollen data following the minimum European recommendations (Galán et al., 2014). Pollen data were expressed as daily pollen concentrations (pollen grains/m³) and the Seasonal Pollen Index (SPI) defined as the summation of daily pollen concentrations in days with the same prevalent wind direction, during the period studied (from March 2011 to March 2014). Daily pollen concentrations for each pollen type were assigned to each one of the eight octants depending on the prevalent wind direction. The summation of daily pollen concentrations associated to each octant during the period studied conformed the SPI (expressed as number of pollen grains because it is a sum) for each city studied.

This includes the 14 most abundant pollen types, excluding pollen types from ornamental trees (*Platanus*, *Cupressaceae*, *Pinaceae*, *Ulmus*). Those taxa are shown in Table 1. *Urticaceae* p.p. (*pro parte*) includes all the species of this family, except *Urtica membranacea*, which is studied as another pollen type.

2.3. Weather data

Daily and hourly meteorological data were acquired from the AEMET (Agencia Estatal de Meteorología) for PL, DB (2011–2014) and ZA (2011–2013). For each day, a prevalent wind direction was calculated and analysed using circular statistics.

2.4. Statistical analysis

Preliminary pollen data analysis and their relationships with selected meteorological variables were investigated using Shapiro-Wilk test for normality. The preliminary results revealed the non-normal distribution of the pollen data and according to these results, non-parametric statistics tests were applied. Scatter plots representing relationships between pollen distribution and wind direction patterns, and number of days with a particular wind direction were used.

Circular statistics was used in order to examine (1) circular-linear correlation between wind direction and daily mean pollen concentrations (results of this test could help in determination of pollen source location); (2) the mean time of the maximum hourly pollen concentration (for selected taxa) recorded in all three cities studied; and (3) wind direction pattern characteristic for each study site using GenStat v.17 (“cdescribe” and “cassociation” packages).

In the second studied case, pollen data measured with an hourly resolution were used. The five main pollen types (*Olea*, *Plantago*, *Poaceae*, *Quercus* and *Rumex*) were selected for the circular analysis, as only for these pollen types hourly data distributions were available. Hours with the maximum pollen concentration were selected. The number of selected hours varied between taxa and study sites, i.e., *Olea* ($n = 9–12$), *Plantago* ($n = 12–19$), *Poaceae* and *Quercus* ($n = 9–11$), and *Rumex* ($n = 14–19$). If the highest value occurred two or three times throughout the same day, then all these hours were included in the analysis. Then hours were converted into a corresponding angle, considering a 24 h period as one full cycle (360°). Finally, the mean angle (mean time of peak occurrence) was calculated using several circular descriptive tests described in Borycka and Kasprzyk (2014).

In the third studied case, the descriptive statistics for wind direction distribution measured in DB, PL and ZA were computed. This analysis was based on daily average pollen values and it was comprised of the following outputs: (1) mean direction (mean of the individual angles); (2) circular standard deviation (variation in the angles about the mean direction; the value of variation varies between 0 and 1); (3) mean resultant length (length of the average of random vectors on the unit circle. It is used to measure the concentration of unimodal circular distributions. The mean resultant length varies between 0 and 1 and that a value of the mean resultant length near 1 implies that there was a little variation in values of the angles.); (4) skewness (lack of

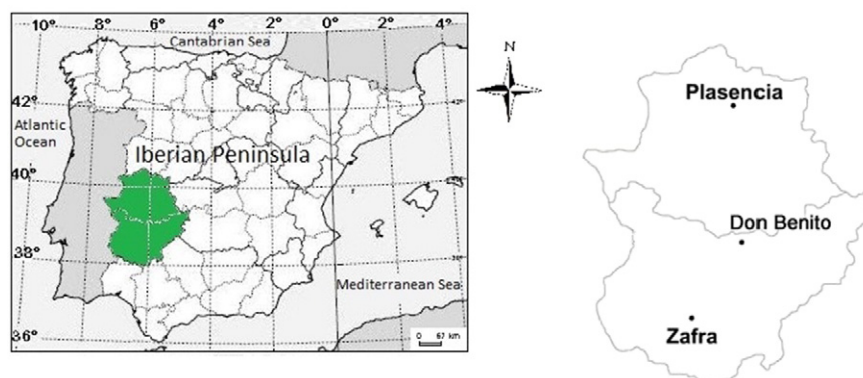


Fig. 1. Location of the Extremadura region in the Iberian Peninsula and location of Don Benito, Plasencia and Zafra in Extremadura.

Table 1

Results of the circular-linear correlation (r_c) that examined relationship between wind direction and average airborne pollen concentration in Don Benito, Plasencia and Zafrá (2011–2014). Statistically significant correlations ($p < 0.05$) were marked in bold.

Pollen type	Statistic	DB	PL	ZA
<i>Alnus glutinosa</i>	r_c	0.095	0.113	0.067
	p	0.009	0.008	0.122
Amaranthaceae	r_c	0.142	0.087	0.078
	p	0.001	0.061	0.054
Anthemideae	r_c	0.026	0.015	0.117
	p	0.742	0.911	0.003
<i>Castanea sativa</i>	r_c	0.095	0.115	0.109
	p	0.018	0.004	0.016
<i>Echium</i>	r_c	0.081	0.035	0.138
	p	0.058	0.652	0.001
<i>Eucalyptus</i>	r_c	0.108	0.127	0.038
	p	0.002	0.002	0.492
Fraxinus-Phillyrea	r_c	0.026	0.073	0.134
	p	0.752	0.158	0.001
<i>Olea</i>	r_c	0.016	0.064	0.103
	p	0.904	0.231	0.008
<i>Plantago</i>	r_c	0.086	0.048	0.061
	p	0.020	0.443	0.153
Poaceae	r_c	0.028	0.020	0.068
	p	0.696	0.873	0.096
<i>Quercus</i>	r_c	0.026	0.011	0.057
	p	0.753	0.960	0.200
<i>Rumex</i>	r_c	0.046	0.060	0.086
	p	0.378	0.255	0.034
<i>Urtica membranacea</i>	r_c	0.087	0.069	0.061
	p	0.026	0.160	0.179
Urticaceae p.p. ^a	r_c	0.115	0.044	0.162
	p	0.002	0.471	0.001

^a This pollen type includes all species from the family except *Urtica membranacea*.

symmetry about the mean; symmetric, unimodal datasets have a skewness value of 0); (5) kappa estimate (this parameter provides the concentration parameter of the von Mises distribution for circular data and it is estimated from the observations. $k = 0$ means that the distribution is uniform, $k \rightarrow \infty$ the distribution becomes increasingly concentrated around the mean direction. When $k < 2$ Fisher (1993) claims that it is difficult to identify a von Mises distribution.); (6) probability test of randomness (the null hypothesis: observations come from a uniform distribution around the circle ($p > 0.05$)); (7) probability Rayleigh test of uniformity (the null hypothesis: observations come from a uniform distribution around the circle = there is no sample mean direction ($p > 0.05$)); (8) Chi-square von Mises and probability Chi-square von Mises (the von Mises distribution is the equivalent test of a normal distribution for circular data; the null hypothesis: observations follow a von Mises distribution ($p > 0.05$)). The theory grounds based upon which “cdescribe” and “cassociation” modules work, as well as the description of all circular tests, can be found in Fisher (1993).

2.5. Land cover data

Orthophotos of each city (dated in 2011 for DB and ZA, and dated in 2012 for PL) were used for mapping the main land cover categories. These orthophotos were obtained from the website of the Instituto Geográfico Nacional© (IGN). Buffers (influence area) of 10 km in radius surrounding the pollen trap for each city were created using a GIS software. Different layers (11) of the main land cover were designed for comparing with meteorological (predominant wind directions) and aerobiological data (SPI). Using a photographic software, a colour analysis was done to calculate surfaces inside the buffer, considering that the sum of all pixels for each layer is the total area of the buffer (314.72 km²), and converting the pixels for each layer into square kilometres. Different layers were separated and classified depending on the land cover in eight octants of directions (N-NE, NE-E, E-SE, SE-

Table 2

Mean time of the peak concentration calculated for selected taxa using circular statistics.

Pollen type	Variable	DB	PL	ZA
<i>Olea</i>	Mean time	01:52	09:48	04:00
	Angular dispersion	5 h 4 min	4 h 48 min	4 h 44 min
<i>Plantago</i>	Mean time	00:12	01:56	10:32
	angular dispersion	4 h 28 min	4 h 48 min	4 h 40 min
Poaceae	Mean time	00:44	02:52	10:16
	Angular dispersion	4 h 52 min	3 h 24 min	4 h 12 min
<i>Quercus</i>	Mean time	01:32	01:04	02:04
	Angular dispersion	4 h 56 min	4 h 36 min	4 h 12 min
<i>Rumex</i>	Mean time	10:44	08:44	06:48
	Angular dispersion	4 h 40 min	4 h 24 min	4 h 36 min

S, S-SW, SW-W, W-NW, NW-N) for correlating with the pollen sources. Following land cover categories were analysed: hardwood forests, olive crops, pastures, irrigated crops, urban areas, riparian forests, broom, vineyards, other uses and water bodies. A description of the main land cover at each location is shown in the supplementary Table S1.

3. Results

3.1. Pollen distribution analysis

Results of the circular-linear correlation analysis showed that presence of the various pollen taxa recorded in the air of DB, PL (2011–2014) and ZA (2011–2013) could not be explained by the changes in local wind direction (Table 1). The highest value of the correlation was $r_c = 0.162$ and it was found for Urticaceae p.p. in ZA (Table 1). Overall, 7 pollen types were statistically significantly correlated (although their values were low) with the wind direction both in DB and ZA, while only three similar relationships were detected in PL. *Castanea sativa* was the only pollen type that reached a level of statistical significance in all three studied sites. In contrast, pollen produced by grasses (Poaceae) and oak trees (*Quercus*) was not statistically significantly correlated with the wind direction in none of the sampling localities.

3.2. Mean time of the maximum hourly pollen concentration

Further application of circular statistics enabled analysis of the average time when the maximum pollen concentration occurred in the air of study sites. Examined pollen types were *Olea*, *Plantago*, Poaceae, *Quercus* and *Rumex* (Table 2). The majority of the peaks were observed at night or early in the morning before the dawn. *Quercus* pollen was found to reach the maximum concentration between 01:00 and 02:00 at all study sites. Other taxa showed more variability in the peak time and for example, *Plantago* reached the highest concentration in DB just after midnight, while in ZA at 10:30 (Table 2). Pollen of *Olea* peaked at night hours at DB and ZA, but in the morning for PL. Poaceae peaked in the morning only in ZA, while *Rumex* peaked in the morning in the three cities (Table 2). The angular standard deviation of the mean time was found within the range of three to 5 h between the maximum concentration recorded for the same taxa, but during different days (Table 2).

3.3. Wind direction analysis

The number of days in the year is shown in Fig. 2a b, c, when wind blew in different directions for each city. DB was the only city that showed a clear pattern with wind directions blowing from one main direction, from the W during all the period studied (Fig. 2a), whereas in PL the wind was coming from the SE in 2011, W in 2012 and S-SW in 2013 (Fig. 2b). Finally, in ZA predominant winds were coming from the N and in 2013 mostly from the SW (Fig. 2c).

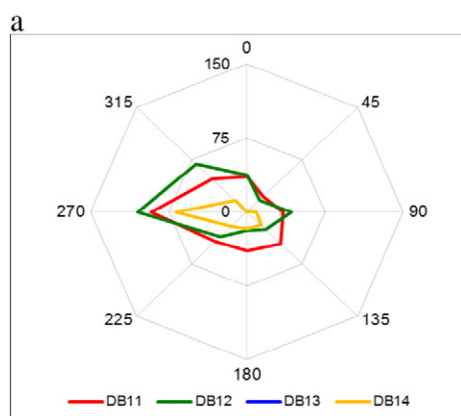


Fig. 2a. Wind Rose of Don Benito. No. of days along the year in that wind blows in each direction.

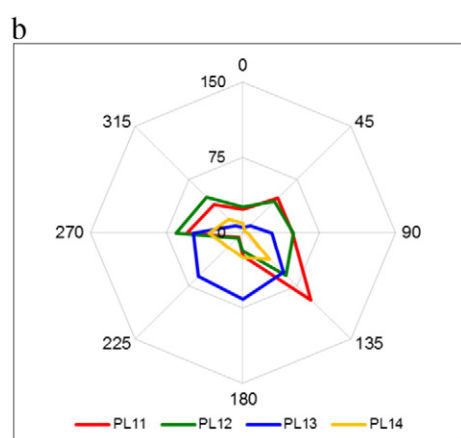


Fig. 2b. Wind Rose of Plasencia. No. of days along the year in that wind blows in each direction.

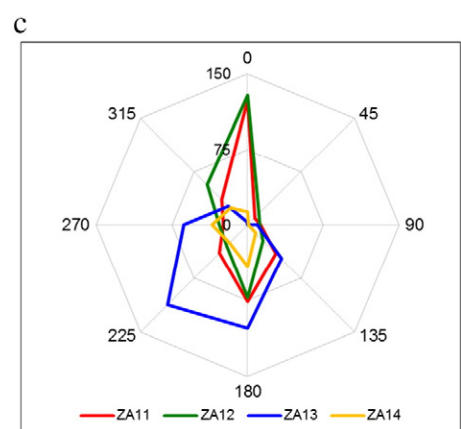


Fig. 2c. Wind Rose of Zafra. No. of days along the year in that wind blows in each direction.

Results of the wind direction analysis using descriptive circular statistics are given in Table 3. These are complementary to the information presented in radar charts (Fig. 2a, b, c). In DB and PL in all examined years wind direction did not follow a von Mises distribution. In contrast, in ZA wind direction followed the normal distribution in all examined years, as well as in joint 3-year period. The probability test of randomness revealed a non-uniform distribution in the wind direction recorded in DB and ZA throughout the entire period of study (2011–2014). In contrast, in PL, this test indicated a uniform distribution in 2013 and

for the joint 4-year period of time (Table 3). This was confirmed by the probability Rayleigh test of uniformity only for a single year, but not for the entire period under investigation. The mean wind direction varied between study sites; in DB, it was found to be oscillating around W-SW direction, in PL NNE-ESE with an exception in 2014, and in ZA NNE. The lowest variation in wind direction was found in ZA, where the angular dispersion reached a maximum of 10° in 2011. In contrast, more variability in wind direction was observed for DB with a maximum of 113° (2011) and in PL with a maximum of 133° (2013).

3.4. Land covers analysis

3.4.1. Don Benito

Table 4 shows a surface area of land covers for each octant and Fig. 3 shows the land cover map of DB. The most of the surface constitute irrigated crops and pastures, whereas other such as hardwood forests, olive crops, riparian forests, vineyards or other uses occupy a minority part of this buffer, with scattered spots. Water bodies are underrepresented in this area.

3.4.2. Plasencia

Fig. 4 exposes the land cover map of PL, while in Table 4 the surface area of land covers for each octant is shown. Hardwood forests (holm, cork oaks and chestnuts forests), located mostly in the N but well-represented in all directions and shrublands (concentrated in the SW) are the main land covers in this surface. Pastures, olive crops, riparian forests, other uses and water bodies also are well represented.

3.4.3. Zafra

The surface area occupied by the different land covers for each octant is shown in Table 4 and Fig. 5 shows the land cover map of ZA. In this city outstands the big number of scattered points surrounding the city, above all with respect to pastures, hardwood forests (holm and cork oak forests) and olive crops, which are concentrated almost homogeneously in all directions. There are also dispersed the riparian forests, water bodies, vineyards and other uses.

3.5. Pollen concentration and abundance in relation to predominant wind directions

Table 5 shows daily average pollen concentrations (pollen grains/ m^3) for the whole period studied in each pollen station and pollen type. The five most abundant pollen types were *Quercus*, *Poaceae*, *Olea*, *Plantago* and *Rumex*, being for the three most important pollen types the same order for the three cities studied. Seven pollen types include trees or shrubs and eight pollen types include herbaceous species. There is a general pattern if main wind direction and SPI are compared for each pollen station. Consequently, SPI recorded were the highest when the wind blew from the SW in DB, from the E in PL and from S and SE in ZA (Fig. 6). Some cases are exceptions to this general wind direction pattern such as *Alnus glutinosa* in PL and ZA, *Castanea sativa* in PL and ZA, *Eucalyptus* in PL and ZA, *Fraxinus-Phillyrea* in ZA, *Plantago* in DB and *Urtica membranacea* and *Urticaceae* p.p. in PL (S1).

4. Discussion

In aerobiological studies using GIS for forecasting purposes, there are mainly two different approaches. The “bottom-up approach” or traditional, in which initially we have vegetation cover, phenological and aerobiological data, and can use it to forecast or to analyse concentrations in intermediate points (Skjøth et al., 2008). The alternative one is called “top-down approach” which was developed by Skjøth et al. (2010). Land cover and aerobiological data obtained from a pollen monitoring network were used to produce a continuous grid of pollen concentrations by interpolation between available points. Therefore, vegetation inventory or land cover analysis should be necessary, not

Table 3

Results of descriptive circular statistics for wind direction measured simultaneously with the pollen concentration at Don Benito, Plasencia and Zafra (2011–2014).

Site	Statistic	2011	2012	2013	2014	2011–2014
Don Benito	Number of observations	184	359	334	70	947
	Width of equidistant sectors	45°	45°	45°	45°	45°
	Mean direction	207.24°	270.32°	197.74°	227.09°	226.38°
		(SSW)	(W)	(SSW)	(SW)	(SW)
	Circular standard deviation	113.33°	87.07°	75.50°	62.27°	90.94°
	Mean resultant length	0.14	0.32	0.42	0.55	0.28
	Skewness	0.27	−0.10	0.78	1.20	0.45
	Kappa estimate	0.29	0.66	0.93	1.34	0.59
	Prob. test of randomness	0.00	0.00	0.00	0.00	0.00
	Prob. Rayleigh test of uniformity	0.03	0.00	0.00	0.00	0.00
	Chi-square von Mises with 5 df	29.29	45.40	108.24	27.55	151.60
	Prob. Chi-square von Mises	0.00	0.00	0.00	0.00	0.00
Plasencia	Number of observations	297	366	346	77	1086
	Width of equidistant sectors	45°	45°	45°	45°	45°
	Mean direction	71.54°	15.69°	113.31°	200.32°	67.50°
		(ENE)	(NNE)	(ESE)	(SSW)	(ENE)
	Circular standard deviation	106.47°	121.32°	132.72°	80.81°	130.10°
	Mean resultant length	0.18	0.11	0.07	0.37	0.08
	Skewness	0.37	0.17	−0.08	0.24	0.29
	Kappa estimate	0.36	0.21	0.14	0.80	0.15
	Prob. test of randomness	0.00	0.00	1.00	0.00	1.00
	Prob. Rayleigh test of uniformity	0.00	0.02	0.20	0.00	0.00
	Chi-square von Mises with 5 df	100.70	113.67	242.92	21.96	250.79
	Prob. Chi-square von Mises	0.00	0.00	0.00	0.00	0.00
Zafra	Number of observations	281	347	318	–	946
	Width of equidistant sectors	45°	45°	45°	–	45°
	Mean direction	23.43°	24.31°	19.17°	–	22.31°
		(NNE)	(NNE)	(NNE)	–	(NNE)
	Circular standard deviation	9.59°	9.50°	5.50°	–	8.70°
	Mean resultant length	0.99	0.99	1.00	–	0.99
	Skewness	0.86	2.02	0.01	–	0.52
	Kappa estimate	36.20	36.85	109.16	–	43.88
	Prob. test of randomness	0.00	0.00	0.00	–	0.00
	Prob. Rayleigh test of uniformity	0.00	0.00	0.00	–	0.00
	Chi-square von Mises with 5 df	1.40	1.14	0.00	–	3.00
	Prob. Chi-square von Mises	0.93	0.95	1.00	–	0.70

df – degrees of freedom.

only to prevent underestimation of the importance of some vegetation or avoid to ignore some vegetation patches in the middle points but also due to possible changes in the location of particles sources or land cover (García-Mozo et al., 2016; Skjøth et al., 2013). Aerodynamics of pollen grains is very important for the deposition and transport processes. Deposition process itself is not essential in aerobiology because traps are usually located above the emission level, so all trapped pollen is coming suspended by the wind. But slowly deposited pollen grains have more chances to be trapped by wind forces and transported until the trap. Differences between dispersion ability in pollen grains should be considered as well, being some pollen types recorded from long distances, such as *Ambrosia* sp. (Kasprzyk et al., 2011; Šikoparija et al., 2013), *Olea europaea* (Fernández-Rodríguez et al., 2014c) or *Betula* sp. (Skjøth et al., 2007). However, other have a more limited dispersion ability, and are mainly recorded near the source of emission, such as *Platanus* sp. (Bricchi et al., 2000; Nowak et al., 2012) or Poaceae (Peel et al., 2014). Finally, other frequent ornamental and wind-pollinated trees, such as *Ulmus minor*, *Acer negundo* and *Celtis australis*, usually do not show relevant airborne pollen records, even when they are situated near to the traps. The trunk diameter, branches development and the level of maturity in trees is also an important point and is able to establish differences between sampling points that show similar surrounding sources but different pollen records for some species (Maya-Manzano et al., 2017). The amount of pollen production per surface of emission source is another key factor for understanding airborne pollen (Sofiev et al., 2006). Moreover, other factors such as the presence of geographic features, changes in flowering depending on the weather (Tormo-Molina et al., 2015), high pollen concentrations in the atmosphere coming from other places (transport to medium-long distance), (Hernández-Ceballos et al., 2011; Kasprzyk, 2003; Maya-Manzano

et al., 2016) and re-suspension of particles (Jato et al., 2006) also should be taken into account for explaining the airborne pollen content.

According to Katelaris et al. (2004), pollen amount is considered as the representative from one place in a buffer of until 30 km from one point. Other authors suggest that pollen index at a place is representative for a bigger area depending on pollen features and sampling site (Oteros et al., 2015; Rojo et al., 2016). According to literature, it seems clear that there are neat differences between pollen types in their length of transport. In some of them, such as *Quercus* and *Olea*, a predominance of medium and long distance transport is observed (Fernández-Rodríguez et al., 2014a; Maya-Manzano et al., 2016; Rojo and Pérez-Badia, 2015; Rojo et al., 2015). However, here we described a method for studying relationship between airborne pollen and a wide range of specific landscapes at shorter distances. Moreover, when a scale is lower and the size of the buffer increase, details are shown in a worse way than in a smaller buffer, because layers of land cover are more dispersed, and the time spent on their analysis increases considerably. Similarly, Skjøth et al. (2010) analysed a buffer of 30 km in diameter and they underlined that for this approach is necessary to have an established network, outstanding the importance of the sources closer to the air samplers. For this reason, we decided to do our own layers based upon produced orthophotos with a high detail resolution. Rojo et al. (2015) analysed a buffer zone of 40 km in diameter and they highlighted that flora of urban green spaces had a marked impact on sampled pollen, which corresponded to the theory that surrounding vegetation and land cover at the edges of a city could be playing a more important role rather than other factors, such as transport of medium or long-distance. This explanation was also suggested by Bricchi et al. (2000) for *Platanus*, who argued that the highest particle deposition was recorded close to the sources and decreased quickly at distances

Table 4surface distribution (km²) in octants of main land cover for each city studied, calculated according to colour analysis.

Wind direction	N-NE	NE-E	E-SE	SE-S	S-SW	SW-W	W-NW	NW-N	Total	%
Don Benito										
Urban area	4.31	10.79	0.98	1.25	0.92	2.49	1.32	1.20	23.26	7.40
Irrigated crops	29.99	17.07	0.22	0.15	7.27	28.70	24.24	35.03	142.68	45.42
Hardwood forests	1.08	0.68	0.00	11.40	4.55	0.00	3.09	0.00	20.80	6.62
olive crops	0.03	1.02	1.20	2.66	1.02	0.21	0.15	0.00	6.28	2.00
pastures	1.18	7.13	36.61	23.28	24.82	7.00	5.51	0.66	106.18	33.80
Riparian forests	0.98	1.12	0.07	0.18	0.42	0.23	1.11	1.07	5.18	1.65
Other uses	1.46	1.06	0.19	0.22	0.26	0.63	2.90	1.25	7.96	2.54
Vineyards	0.00	0.00	0.00	0.00	0.00	0.00	0.51	0.00	0.51	0.16
Water bodies	0.23	0.39	0.00	0.13	0.00	0.00	0.46	0.05	1.26	0.40
Plasencia										
Urban area	0.00	0.06	0.90	3.05	3.95	0.03	0.01	0.03	8.02	2.55
Irrigated crops	0.20	0.15	0.00	0.00	0.11	0.29	5.02	1.18	6.94	2.21
Hardwood forests	36.08	36.02	27.11	16.27	20.58	26.25	32.86	35.30	230.48	73.38
Olive crops	0.05	0.00	3.42	6.24	0.97	0.11	0.03	0.07	10.89	3.47
Pastures	1.06	0.30	7.04	8.80	2.35	1.03	0.23	1.70	22.52	7.17
Riparian forests	0.00	0.13	0.23	0.26	0.07	0.29	0.64	0.03	1.65	0.53
Other uses	0.06	0.04	0.06	0.11	0.29	0.06	0.21	0.06	0.88	0.28
Shrublands	1.79	0.57	0.18	4.49	10.95	11.16	0.26	0.88	30.27	9.64
Water bodies	0.02	2.01	0.32	0.04	0.00	0.04	0.00	0.01	2.45	0.78
Zafra										
Urban area	0.06	4.94	4.46	4.56	0.22	0.05	0.17	0.22	14.68	4.67
Irrigated crops	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.67	0.21
Hardwood forests	15.66	3.15	2.30	1.10	12.00	28.18	22.31	19.04	103.75	33.03
Olive crops	13.87	11.58	9.00	5.52	12.26	4.10	3.56	9.50	69.39	22.09
Pastures	8.06	13.65	15.76	27.23	14.30	6.63	12.74	9.35	107.72	34.30
Riparian forests	0.09	0.16	0.08	0.07	0.06	0.07	0.21	0.25	0.99	0.32
Other uses	0.83	1.87	0.66	0.27	0.37	0.07	0.22	0.07	4.36	1.39
Vineyards	0.54	3.18	5.93	0.47	0.04	0.00	0.00	0.73	10.88	3.47
Water bodies	0.00	0.04	0.00	0.00	0.00	0.15	0.05	0.00	0.24	0.08
Vineyards-olive crops	0.14	0.70	0.40	0.04	0.00	0.00	0.00	0.10	1.38	0.44

greater than 400 m, following an exponential curve from the source. Despite the long-range transport (Fernández-Rodríguez et al., 2015; Smith et al., 2008), some papers considered that events produced by transport from long distances happen generally in an episodic way (Belmonte et al., 2008; Fernández-Rodríguez et al., 2015; Fernández-Rodríguez et al., 2014a; Rojo and Pérez-Badía, 2015).

Arguing that potential influence of pollen sources increases dramatically with the distance to the trap, some authors suggest that majority of the airborne pollen reaching a place is coming from long distances (Oteros et al., 2015; Rojo et al., 2015). An extreme case of this phenomenon is the pollen registered in the isolated islands or in the middle of the ocean (Darwin, 1846; Izquierdo et al., 2011). However, it is also known that the near source of a trap has a great power for disturbing pollen spectrum (Bricchi et al., 2000; Hofmann et al., 2014; Skjøth et al., 2013). For this reason, we chose a buffer of 10 km in radius as study area, concentrating our efforts on analysis of the closest surroundings of each city studied. Pollen sources have more influence on the aerobiological spectrum and their distribution can be used to explain the majority of local variations in the content of some airborne particles in the urban areas, where a population is mostly concentrated. Moreover, analyzing this size of study area, we are able to identify well local pollen sources with high disturbing effect, which should be related to different pollen types and different land uses.

In DB, according to land cover map and their subsequent study using colour analysis (Table 4), the main land cover categories in the cities studied were irrigated crops (45.4%) and pastures (33.8%). Therefore, the abundance of some types of herbaceous plants such as *Amaranthaceae*, *Plantago*, *Poaceae*, *Rumex*, *Urtica membranacea* and *Urticaceae* p.p. in their airborne contents in this city seems to be related with the surrounding land cover. This relationship was also highlighted by (Hruska and Staffolani, 2010). However, we cannot rule out the uncertainties related to the vegetation inventory of annual herbaceous plants, with their variability in annual distribution and strong dependence of the meteorological conditions, as was studied by other authors

(de La Guardia et al., 1998; Rizzi Longo et al., 2004; Sabariego et al., 2011; Sánchez Mesa et al., 2003).

Silva-Palacios et al. (2007) found different contents of relative humidity in the wind depending on its direction in the same region where the present work was carried out. This water load could affect the herbaceous plants pollination, although the found correlation coefficient with wind direction was low. In the case of *Urticaceae*, made up of synanthropic species showing frequent presence in the cities surroundings (Romano et al., 1991), it seems that heavy metals like copper (Cu) and lead (Pb), derived from high-traffic and pollution in the cities, stimulate pollen viability (Fotiou et al., 2011). We can indicate, in absence of different patterns from the usual one, for these pollen types, that herbaceous plants are influenced by the main wind direction pattern. There is a clear effect of prevalent wind direction (causing an indirect or random effect in the analysis of the pollen recorded) in the airborne content of the cities studied when we want to interpret the pollen concentrations by means of land cover analysis. This could be a source of error and it should be minimized.

Hardwood forests (73.4%) and shrublands (9.6%) are the main land cover in PL (Fig. 4 and Table 4), outstanding the importance of *Quercus* and *Castanea sativa*, which frequently appeared in surroundings of the urban area. Their pollen concentrations in the aerobiological study are quite representative as well. In spite of the little surface occupied by riparian forests (0.5%), the presence of Jerte River, which is close to the city, can be the explanation of the abundance of pollen types such as *Alnus glutinosa* or *Fraxinus-Phillyrea* in this city, associated to riparian vegetation. In the case of ZA, pastures (34.3%), holm-cork oak forests (33.0%) and olive crops (22.1%) occupy the majority of area studied, and pollen from herbaceous plants (*Poaceae*, *Plantago*, *Rumex*), *Quercus* and *Olea* play a key role.

Wind direction influence depends on the pollen types selected. *Alnus glutinosa* and *Fraxinus-Phillyrea* pollen types in PL and ZA, are more abundant with the wind from the NE in PL and NW in ZA. On the contrary happens in DB, with riparian forests located in the NW. Both

Land cover map in Don Benito

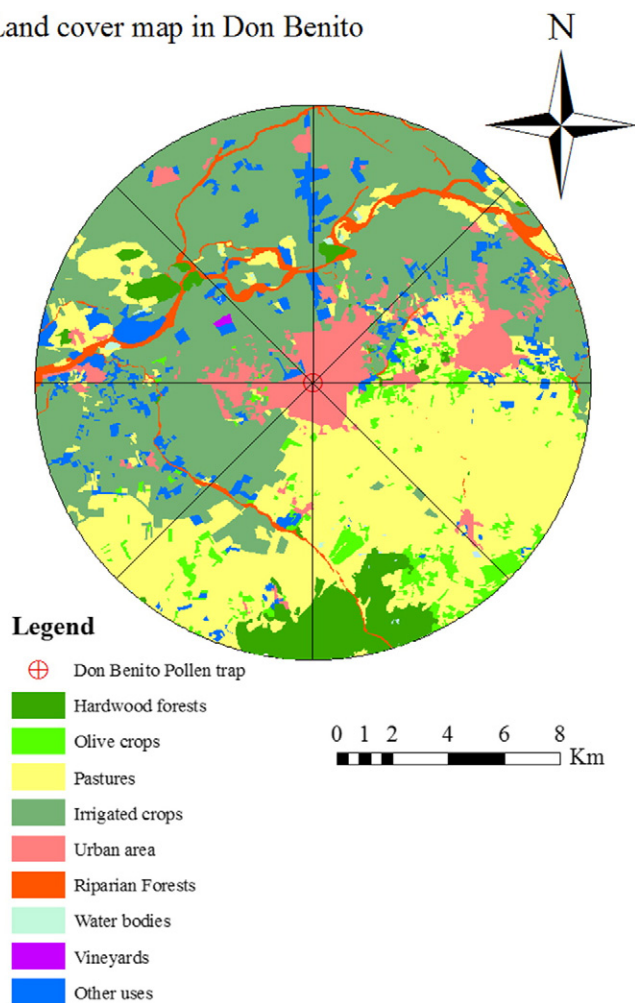


Fig. 3. Land cover map in Don Benito. Buffers of radius 10 km.

Land cover map in Plasencia

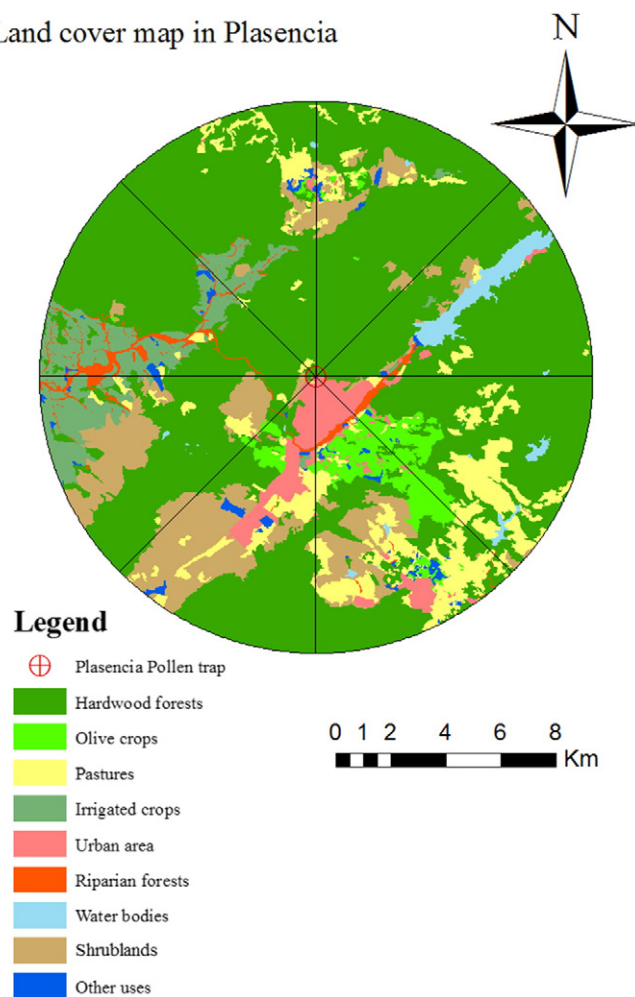


Fig. 4. Land cover map in Plasencia. Buffers of radius 10 km.

above mentioned pollen types showed higher SPI when the wind is coming from areas with riparian forests surfaces, because the presence of alder and ash forests.

Other significant cases happened in DB and ZA with pollen types from herbaceous plants (*Amaranthaceae*, *Anthemideae*, *Echium*, *Plantago*, *Poaceae*, *Rumex*, *Urticaceae* p.p. and *Urtica membranacea*) when the predominant winds blow from the S (SW in the case of DB), where many pastures are situated. High concentrations of *Castanea sativa* in PL are correlated with wind directions from the N, where forests of chestnut trees grow frequently.

Eucalyptus pollen is more common in PL when the wind blows from the W and SW. This case is interesting because of presence of riparian forests in the E of the city and because of being this direction the predominant wind direction. Nevertheless, the highest SPI were recorded when wind blew from the W. The same happened in ZA, with the wind blowing from the NW and W. Therefore, *Eucalyptus* pollen type content could be due to nearby sources to the air sampler having some specimens growing as ornamental trees in these cities (Maya-Manzano et al., 2017), while were observed in the riparian forests surrounding the city, growing of naturalised way as well. *Olea europaea* SPI is related in DB and ZA with southerly winds and for PL with winds from the east direction, according to maps of land cover and supplementary material (Fig. S1). *Quercus* was the pollen type whose relationships with land cover distribution are the worst because high SPI are correlated with SW winds in DB, with winds from the E and SW bearings in PL and SE for ZA. These wind directions do not coincide with the surface occupied mostly by holm-cork oak forests. It is true that

these types of trees appeared in these quadrants but in vegetation spots, and the surface occupied is lower. A possible explanation for this fact is that *Quercus* pollen is moved to medium or long-range distance (Hernández-Ceballos et al., 2011; Maya-Manzano et al., 2016). In the last article, authors found that the minimum distances between these three aerobiological stations and *Quercus* woodlands were estimated for 40 km for PL, 66 km for DB and 62 km for ZA. This coincides with the non-statistically significant correlation with the wind direction in none of the sampling localities.

In the hourly analysis of the five most abundant pollen types, night peaks were frequent, which could be due to the presence of turbulences phenomena, provoked for differences between air temperature and ground temperature at night, causing a resuspension of particles (Grewling et al., 2016). This contrast with findings in the same area with traditional hourly analysis (*Olea* in Fernández-Rodríguez et al., 2014c; *Quercus* in Maya-Manzano et al., 2016) and previous report of Kasprzyk (2006) who analysed the mean time of pollen peak concentrations for five taxa (*Ambrosia*, *Artemisia*, *Poaceae*, *Rumex*, *Urtica*) using circular statistics. With an exception of *Ambrosia* which peaked earlier (07:30), remaining taxa achieved their maximum concentration between 11:00 and 12:00 in an urban area, while *Ambrosia*, *Artemisia* and *Rumex* peaked from 08:00 to 10:00 and *Urtica* with *Poaceae* from 11:00 to 11:30 in a rural area (Kasprzyk, 2006). The hourly peaks reported in this study do not reflect the average pattern of hourly pollen presence but eventual atmospheric phenomena that may have relevant incidence in some days for the total amount of pollen recorded. This eventually is manifested because they are rarely constant between

Land cover map in Zafra

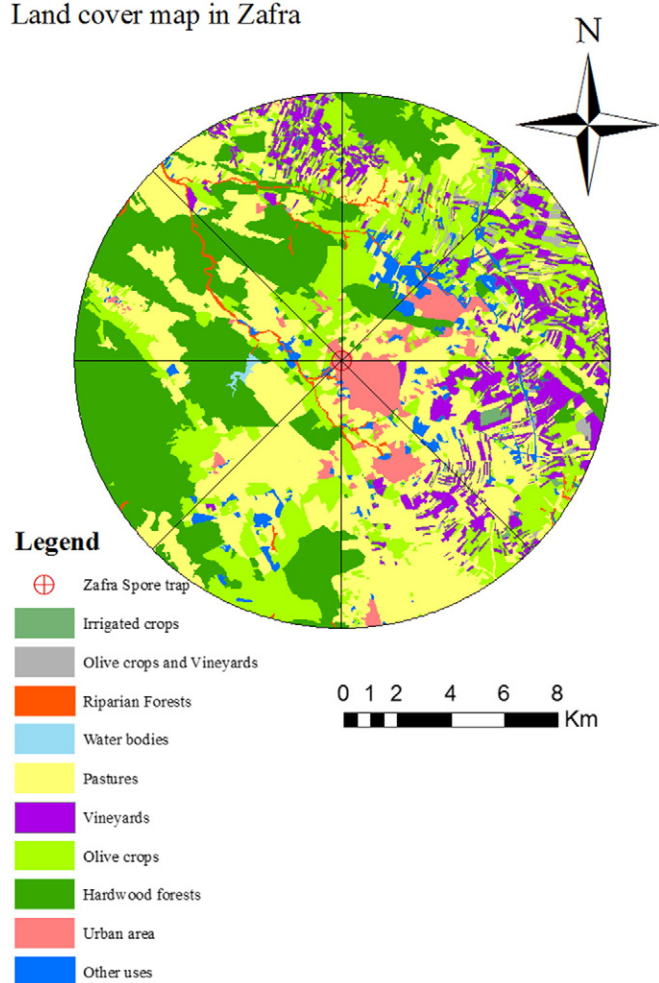


Fig. 5. Land cover map in Zafra. Buffers of radius 10 km.

pollen stations and because of their wide angular dispersion. Often, pollen types are composed by different species, such as *Quercus* or *Poaceae*, with different flowering seasons and hourly patterns along this whole period, and due to the tendency to group them in an only and artificial group, it could disturb the mean hourly peaks, according to findings of Peel et al. (2014) for *Poaceae* species.

Other possible explanations can be the presence of different hourly patterns depending on the weather conditions or considering also the

Table 5

Mean daily pollen concentration (pollen grains/m³) for pollen types studied during the whole period of study (DB Don Benito, PL Plasencia, ZA Zafra).

Pollen type	DB	PL	ZA
<i>Alnus glutinosa</i>	0.3	0.6	0.2
Amaranthaceae	0.9	0.4	0.7
Anthemideae	0.3	0.3	0.2
<i>Castanea sativa</i>	0.2	1.6	0.2
<i>Echium</i>	0.5	0.5	0.5
<i>Eucalyptus</i>	0.7	0.1	0.2
<i>Fraxinus-Phillyrea</i>	0.5	1.6	0.4
<i>Olea</i>	13.9	8.0	24.1
<i>Plantago</i>	3.1	3.0	2.8
<i>Poaceae</i>	15.3	16.5	16.4
<i>Quercus</i>	32.0	43.5	39.7
<i>Rumex</i>	1.2	2.2	1.1
<i>Urtica membranacea</i>	0.5	0.4	0.4
Urticaceae p.p. ^a	1.0	0.6	0.6

^a This pollen type includes all species from the family except *Urtica membranacea*.

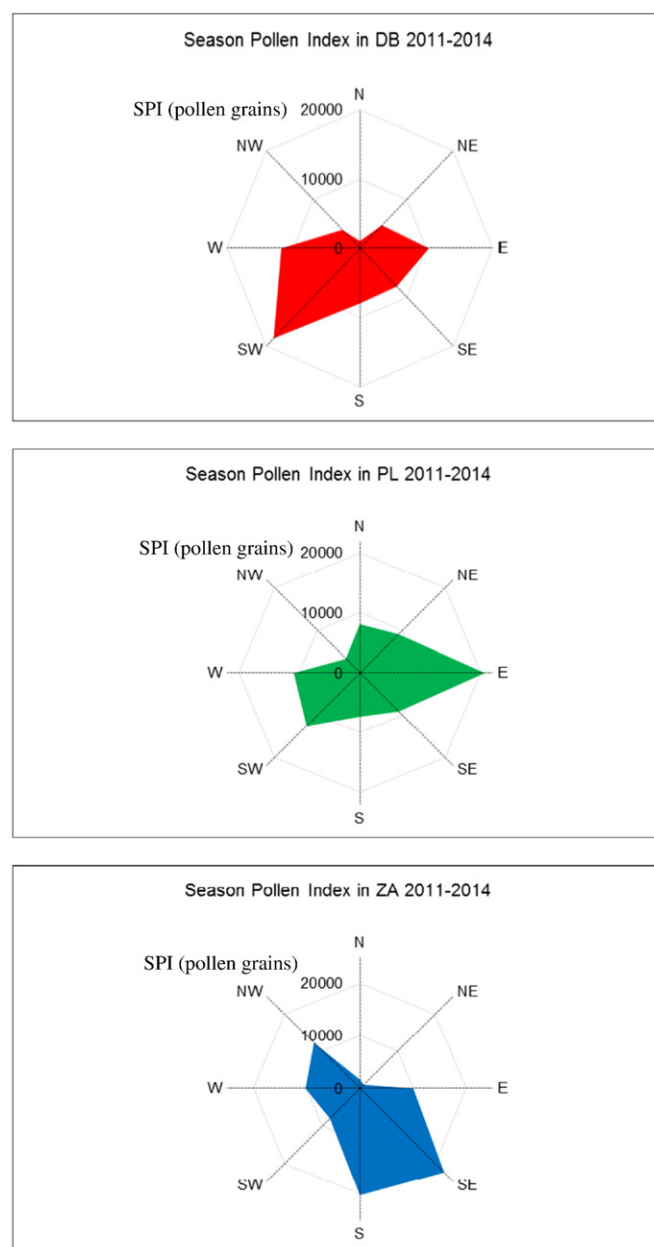


Fig. 6. Total Seasonal Pollen index (SPI) from each city studied, for each octant, during the season 2011–2014.

topography, something discussed by Alba et al. (2000), who found four different patterns for *Olea europaea*, or the altitude (Alba et al., 2006). The presence of episodes with pollen loads coming from different sources, each one with a different hourly pattern, can also be taken into consideration, because of the distance amongst the three cities in the present work. It has been studied in the same three locations by Maya-Manzano et al. (2016) for *Quercus*, who recorded that airborne pollen in PL and ZA comes from two sources, whereas in only one for DB.

Finally, it should be noted that number of days with an hourly data resolution was limited. Thus, the degrees of freedom in analysed data sets (*Olea*, *Poaceae* and *Quercus*) varied between 8 and 10, while generally recommended range is 11–20. However, this is not the case of *Plantago* and *Rumex*, where this condition has been fulfilled.

5. Conclusions

Some pollen grain concentrations recorded in the three cities studied are explained by the immediate surrounding vegetation and non-

homogeneous distribution of land cover nearby pollen traps. Consequently, some pollen types seem to act of a local way. We can see a clear effect in herbaceous plants such as *Plantago*, *Urtica membranacea* and *Urticaceae* p.p., moreover in trees as *Alnus glutinosa*, *Castanea sativa*, *Eucalyptus*, *Fraxinus* and *Olea europaea*.

Nevertheless, in some pollen types, e.g. *Quercus* in all locations, or herbaceous plants such as *Amaranthaceae*, *Anthemideae*, *Echium*, *Poaceae* and *Rumex*, this effect is not observed and transport of medium or long distance may be alleged; nevertheless, the sources of those pollen types are much more homogeneously distributed than the former ones, in spite of being more abundant too. Also the trap location within a city with respect to predominant wind directions should be taken into account for the presence or absence of records. When we want to interpret the pollen concentrations by means of land cover analysis, there is a clear effect of prevalent wind direction in the pollen spectrum of the cities studied. The most prevalent wind direction has an indirect or random effect with the pollen recorded. To interpret land cover influence properly, this factor must be reduced.

With respect to the hourly peaks analysis in the main five pollen types, the majority of the peaks were observed at night or early in the morning, probably due to turbulence phenomena or re-suspension episodes provoked by differences of temperature between air and ground. Differences in topography, altitude, meteorological conditions, or diverse phenological patterns or flowering season inside the selected pollen types could be an explanation as well.

As a rule of thumb, in order to interpret airborne pollen records from volumetric pollen traps accurately, it is necessary to identify big disturbing pollen sources near to the pollen trap and the predominant wind directions.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.01.085>.

Acknowledgements

This work was conducted thanks to the Regional Government of the Junta de Extremadura in Spain (Project Number PRI06A190) and the European Social Fund (Project number PRI BS10008).

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Table S1: surface distribution (km²) and percentage representation of main land cover for each city studied inside the 10km buffer.

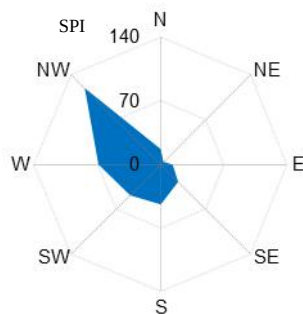
Land cover	Don Benito		Plasencia		Zafra	
	Surface (km ²)	(%) representation	Surface (km ²)	(%) representation	Surface (km ²)	(%) representation
Hardwood forests	20.80	6.62	230.48	73.38	103.74	33.03
Olive crops	6.28	2.00	10.89	3.47	69.39	22.09
Pastures	106.18	33.80	22.52	7.17	107.72	34.30
Irrigated crops	142.68	45.42	6.94	2.21	0.67	0.21
Urban areas	23.26	7.40	8.02	2.55	14.68	4.67
Riparian forests	5.18	1.65	1.65	0.53	0.99	0.32
Shrublands	0	0	30.27	9.64	0	0
Vineyards	0.51	0.16	0	0	10.88	3.47
Other uses	7.96	2.54	0.88	0.28	4.36	1.39
Water bodies	1.26	0.40	2.45	0.78	0.24	0.08
TOTAL	314.11	100	314.10	100	314.08	100

Figure S1: Seasonal pollen Indexes from pollen types with different results from the usual wind direction pattern and their relationships with predominant wind directions in Don Benito (DB), Plasencia (PL) and Zafra (ZA), during the period analyzed (2011-2014).

Alnus glutinosa in PL 2011-2014



Alnus glutinosa in ZA in 2011-2014



Castanea sativa in PL 2011-2014



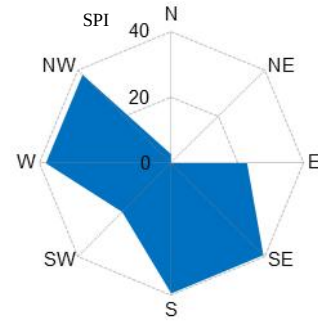
Castanea sativa in ZA in 2011-2014



Eucalyptus in PL 2011-2014



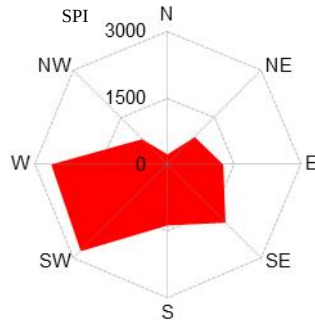
Eucalyptus in ZA in 2011-2014



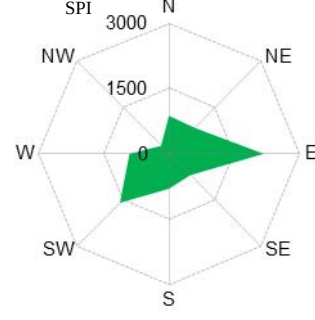
Fraxinus-Phillyrea in ZA in 2011-2014



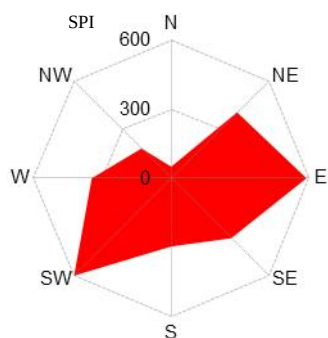
Olea europaea in DB 2011-2014



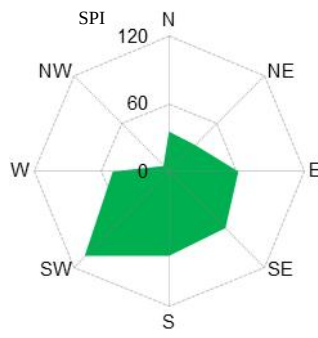
Olea europaea in PL 2011-2014



Plantago in DB 2011-2014



Urtica mb in PL 2011-2014



Urticaceae p.p. in PL 2011-2014

