Spatial and temporal variations in the annual pollen index recorded by sites belonging to the Portuguese Aerobiology Network

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Abstract

Background: This study presents the findings of a 10-year survey carried out by the Portuguese Aerobiology Network (RPA) at 7 pollen-monitoring stations; 5 mainland stations (Oporto, Coimbra, Lisbon, Évora and Portimão) and 2 insular stations (Funchal (Madeira archipelago) and Ponta Delgada (Azores archipelago). The main aim of the study was to examine spatial and temporal variations in the annual pollen index with particular focus on the most frequently recorded pollen types.

Methods: Pollen monitoring (2003–2012) was carried out using Hirst-type volumetric spore traps, following the Minimum Recommendations proposed by the European Aerobiology Society Working Group on Quality Control**.** Daily pollen data were examined for similarities using the Kruskal-Wallis non-parametric test and Multivariate Regression Trees. Simple linear regression analysis was used to describe trends in Annual Pollen Index (API).

Results: The airborne pollen spectrum at RPA stations is dominated by important allergenic pollen types such as Poaceae, *Olea*, and Urticaceae. Statistically significant differences were witnessed in the API recorded at the 7 stations**.** Mean API is higher in the southern mainland cities, e.g. Évora, Lisbon and Portimão, and lower in insular and littoral cities. There were also a number of significant trends in API during the 10-year study.

Conclusion: This report identifies spatial and temporal variations in the amount of airborne pollen recorded annually in the Portuguese territory. There were also a number of significant changes in API, but no general increases in the amount of airborne pollen.

Keywords Aerobiology, Airborne Pollen, Multivariate Regression Tree, Trends, Portugal

1. Introduction

IgE-associated allergic diseases are increasing in prevalence and severity globally (Bousquet et al. 2011), and are becoming the most frequent chronic disease in the European Union (Zuberbier et al. 2014). In Portugal, the International Study of Asthma and Allergies in Childhood (ISAAC) showed an increase in the prevalence of allergic rhinoconjunctivitis and asthma symptoms in children, particularly in the 13–14 years age-group (Asher et al. 2006). Sa-Sousa et al. (2012) found the prevalence of "current asthma" (i.e. a positive answer to the question "Have you ever had asthma?" and at least one of 3 symptoms in the last 12 months) was 6.8% among the general Portuguese population.

Airborne pollen grains are considered to be one of the most important sources of aeroallergens and a risk to environmental health. The inhalation of pollen allergens induces respiratory allergy symptoms in sensitized individuals, clinically manifested as rhinitis, rhinoconjunctivitis and asthma (Gioulekas et al. 2004). In Portugal, the majority of patients sensitised to allergenic pollen are sensitised to grass pollen allergens. Sensitisation rates to pollen allergens have been reported to range between 5.1% (Cypress pollen) to 34.4% (grass pollen) (Burbach et al. 2009).

Knowledge of spatial and temporal variations in airborne allergenic pollen is important for health care professionals and allergic persons to manage treatment and avoid exposure to pollen allergens. As a result, regional and national networks routinely monitor the aerobiological content of the atmosphere and provide information in the form of alerts and forecasts (Karatzas et al. 2013). The Portuguese Aerobiology Network (RPA) was created in 2002, promoted by the Portuguese Society of Allergology and Clinical Immunology, and currently has 9 pollen-monitoring stations.

The first aerobiological studies in Portugal were conducted in the 50s and 60s by Pinto da Silva in the regions of Oporto, Lisbon and Sacavém. Following on from this, the Botanical Institute of Coimbra has, since 1978, conducted aeropalynological studies in cities such as Aveiro, Coimbra, Lisbon, and Oporto (Abreu et al. 2003). Other aeropalynological investigations included sporadic volumetric studies carried out in Lisbon during the 80s and 90s (Clode et al. 1992), and work conducted in the main urban centers in southern Portugal that started in the late 80s using gravimetric (Durham-modified) and later by volumetric (Hirst) methods (Brandão and Lopes 1990; Brandão et al. 2004). In addition, a network of pollen-monitoring stations based on the Cour method covered almost all of the Portuguese district capitals during the years 1999 and 2000 (Abreu et al. 2003).

Studies have been published for several Portuguese regions in regard to atmospheric pollen (Todo-Bom et al. 2006; Caeiro et al. 2007; Ribeiro et al. 2008; Câmara et al. 2009; Fernandes et al. 2010; Ribeiro and Abreu 2014; Camacho 2015) and airborne fungal spores (Abreu et al. 2003; Nunes et al. 2005; Oliveira et al. 2005; Sousa et al. 2015). Comparisons between airborne pollen concentrations recorded at sites in Portugal and other countries have been made (Fernández-Rodríguez et al. 2015), as well as immunochemical studies with allergenic pollen (Ribeiro et al. 2009; Sousa et al. 2014). In addition, studies relating to pollen exposure and hospital admissions for respiratory disease have been performed (Ribeiro et al. 2009; Camacho et al. 2016).

The main aim of the study was to examine spatial and temporal variations in the annual pollen index with particular focus on the most frequently recorded pollen types. This was accomplished by: (1) Establishing the pollen spectrum of each pollen-monitoring station; (2) Assessing the similarity of sites in respect to taxa and airborne pollen concentrations; (3) Determining whether changes had occurred in the amount of pollen recorded annually.

2. Material and Methods

2.1 Study areas

Airborne pollen monitoring during the period 2003–2012 was performed using volumetric spore traps of the Hirst (1952) design at all 7 stations belonging to the RPA; 5 mainland stations (Oporto, Coimbra, Lisbon, Évora and Portimão) and 2 insular stations (Funchal (Madeira) and Ponta Delgada (Azores), the later which started operating in 2006) (Fig. 1, Table 1).

The climate in the Portuguese territory varies considerably due to its location. It is the westernmost country of the Eurasian supercontinent, and is bordered by the Atlantic Ocean and Spain. There is therefore a strong contrast between coastal (western) and mainland (eastern) areas (Mesquita and Sousa 2009). Portugal has a warm temperate climate, mostly Mediterranean, characterized by hot, dry summers and cool, wet winters (Nunes et al. 2005). Mainland Portugal includes both temperate and Mediterranean territories (Mesquita and Sousa 2009). The north western half of Portugal, which includes the study areas of Oporto and Coimbra, reveals Meso-temperate and Meso-Mediterranean termoclimates. The city of Évora located in Alentejo in the central eastern region of Portugal, also exhibits Meso-Mediterranean features. In contrast, the central and southwestern part of Portugal, which includes the cities of Lisbon and Portimão, shows a Termo-Mediterranean termoclimate.

The climate in the islands ranges from Temperate / Hyper-oceanic- sub-Mediterranean in Madeira, to Mediterranean pluviseasonal oceanic / Temperate hyper-oceanic sub-Mediterranean in the Azores (Table 1).

2.2 Vegetation landscape

Over one-third of the Portugal mainland is covered by forest and woodland. In the northern half of the country, *Pinus pinaster* (maritime pine) stands prevail. Whereas *Eucalyptus globulus* (blue gum) plantations are abundant along the western half of Portugal and interior parts of central and southern regions. In the southern half of Portugal, evergreen oak woodlands predominate. *Quercus suber* (cork oak) woodlands are the main forest land cover type in south-western Portugal and along the Tagus river valley, while *Quercus rotundifolia* (holm oak) predominates in the southeast (Nunes et al. 2005; Doody 2013; Batista et al. 2014).

Scrubland covers about one-quarter of the country, located mostly in the northern third and southeastern regions. These shrub formations are dominated by the species of the genus *Cytisus* (Fabaceae) and *Cistus* (Cistaceae), often occurring together in sedimentary sands and sandstones. In limestone areas, heather scrub composed by *Quercus coccifera* (garrigue-like scrubland) are also common (Nunes et al. 2005).

Agricultural areas cover one-third of the area of the Portuguese mainland and, although present throughout the entire country, are more abundant in the coastal plains of central Portugal along the main river valleys, as well as in the southeastern parts of the country. In central and northern Portugal, the agricultural landscape is a mosaic of small parcels of diverse crops, vineyards, and olive groves. The agricultural landscapes of southeastern Portugal are more extensive and homogeneous, dominated by dryland farming of cereal crops (Nunes et al. 2005).

In the Azores archipelago, a mixture of native, endemic and introduced exotic plant species occur. Much of the islands were covered by dense evergreen forest. Nowadays, the land has been cleared for agriculture and settlement. The native vegetation still persists in a number of isolated areas, dominated by *Myrica faia* (faya tree), *Myrsine africana* (Cape myrtle), *Erica azorica* (Azores heather), *Calluna vulgaris* (common heather), *Vaccinium cylindraceum* (whortleberry), and *Picconia azorica* (Azorean picconia). There are quite extensive plantations of the exotic conifer *Cryptomeria japonica*. Much of the forest is covered by *Ilex perado* ssp. *azorica*, *Juniperus brevifolia* (juniper) and other dominant species such *Erica azorica* (tree heather) and *Laurus azorica* (laurel) (Madeira et al. 2007).

In regards to Madeira, about two-thirds of the island is a natural park, in which the largest continuous and best preserved area of humid laurel forest is found. It originally covered the Mediterranean Basin and is nowadays restricted to Macaronesia. It contains a suite of unique plant species, with the most common trees belonging to the Lauraceae family, like the *Apollonias barbujana* (Canary Laurel), *Laurus novocanariensis* (Laurel), *Ocotea foetens* (fetid Laurel) and *Persea indica* (Madeira mahogany) (Borges et al. 2008). Funchal, in particularly, offers 17 public green spaces in which exotic plants from around the world thrive, the most common trees in the city being *Tipuana tipu*, *Jacaranda mimosifolia, Agathis robusta, Spathodea campanulata, Casuarina equisetifolia, Melia azedarach*, *Erythrina* spp., *Brachychiton* spp., besides several other species from families like Arecaceae, Cupressaceae and Cyatheaceae. In urban areas there are many patches of land used for agricultural purposes, mostly cultivated with *Musa acuminata, Vitis vinifera* and with horticultural production. Uncultivated land presents a variety of herbaceous plants dominated by *Parietaria* spp., *Arundo donax and Dactylis* spp. The outskirts of the city are dominated by exotic forest from the genera *Acacia*, *Eucalyptus* and *Pinus* (Quintal 2007; Borges et al. 2008).

2.3 Aerobiological data

Sampling and analysis was conducted following the Minimum Recommendations proposed by the European Aerobiology Society Working Group on Quality Control (Galán et al. 2014). All the samples were processed and analysed at the Palynology and Aerobiology Laboratory of the University of Évora, with the exception of samples from Funchal and Ponta Delgada, which part of the samples were analysed at Madeira University. The pollen-monitoring station in Ponta Delgada started functioning in 2006.

Each device is equipped with a vacuum pump drawing 10 L of air per minute, where particles are trapped on a Melinex tape coated with silicone solution (Lanzoni) and supported on a clockwork-driven drum moving at 2 mm/h. After sampling, the tapes were removed, cut in seven daily portions (each 48 mm in length), mounted on slides, and stained with glycerine gelatine solution with basic gel fuchsine (Lanzoni s.r.l., Italy). The identification and counting of pollen grains was performed by light microscopy (400x) along 4 longitudinal transects.

Pollen taxa were grouped in arboreal pollen types, which includes Arecaceae, Betulaceae, *Castanea*, Cupressaceae, Myrtaceae, *Olea*, *Salix,* Pinaceae, *Platanus*, *Quercus* and *Q. suber*. and Herbaceous pollen – Asteraceae, Chenopodiaceae, *Plantago*, Poaceae, *Rumex*, Urticaceae. Pollen grains of other taxa recorded recorded were grouped into a category called ‘Other pollen’. The unidentifiable pollen grains were grouped into an undetermined category represented as "NI".

For each station, the airborne pollen concentration was calculated and expressed as the number of pollen grains per cubic meter of air (p/m^3). The annual sum of daily average airborne pollen concentrations for all taxa combined was expressed as the Annual Pollen Index (API).

2.4 Statistical analysis

Normality of the dataset was assessed with the Kolmogorov-Smirnov and chi-square tests. Since the variables did not follow normal distribution ($p<0.05$), non-parametric tests and methods which do not require normality were applied. The Kruskal-Wallis test, used to determine whether there were significant differences in the total amount of pollen recorded annually at each station, was performed using SPSS 17.0. Similarity of sites in terms of pollen composition and concentrations was assessed with the help of Multivariate Regression Tree method (MRT). This method forms clusters of sites by repeating splitting of the data along axes of the explanatory variables. Each split is chosen to minimize the dissimilarity (sum of squared Euclidean distances, SSD) of data within the clusters (Breiman et al. 1984; De'ath and Fabricius 2000; De'ath 2002). The clusters and their dependence on input variables are presented graphically by a tree. The overall fit of the tree is specified as relative error (RE; SSD in the clusters divided by SSD of the undivided data) and the predictive accuracy is assessed by CV RE (cross-validated relative error) (Breiman et al. 1984; De'ath and Fabricius 2000). In this study, two predictors were used, i.e. longitude and latitude (coordinates WGS84), while the output variables were the concentrations of pollen present at all studied sites: Tree pollen types – Arecaceae, Betulaceae, *Castanea*, Cupressaceae, Myrtaceae, *Olea*, *Salix,* Pinaceae, *Platanus*, *Quercus* spp. and *Q. suber*; Herbaceous pollen –Asteraceae, Chenopodiaceae, *Plantago*, Poaceae, *Rumex*, Urticaceae. The finally selected tree was the most complex model within one standard error (1 SE) from the best predictive tree (Breiman et al. 1984), using 2000 multiple cross validations, to stabilise CV RE. Analyses were carried out in R 2.10.1 (R Development Core Team, 2011) using mvpart (Multivariate Partitioning) package (De'ath 2002).

Simple linear regression analysis was used to describe trends in the API of selected pollen seasons following the methodology described in literature (e.g. Thackeray et al. (2010), Ziello et al. (2012), Smith et al. (2014) Ugolotti et al. (2015)). The following statistics are presented: the slope of the regression; coefficient of determination (R^2); probability level (p). Regression calculations were carried out using software Statistica 10 (StatSoft 2011). Results were considered significant with probability levels <0.05.

3. Results

3.1 Spatial variations in the airborne pollen spectrum recorded at monitoring stations in Portugal

The airborne pollen spectrum recorded at each of the 7 pollen-monitoring stations belonging to the RPA is described (Figs 2 A-G). In general, airborne herbaceous pollen (especially Urticaceae and Poaceae) dominated coastal (Oporto and Lisbon) and insular (Funchal and Ponta Delgada) monitoring stations. Further inland, tree pollen types such as Cupressaceae (Coimbra) and *Quercus* spp. (Évora) were predominant. The southwestern coastal city of Portimão was an exception, with airborne *Olea* being the most frequently recorded.

3.2 Relationships in the amount of airborne pollen recorded at monitoring stations in Portugal

Statistically significant differences ($p < 0.05$) were witnessed in the API of all pollen taxa combined at the 7 pollen-monitoring stations included in this study during the period 2003–2012. The API from Funchal and Ponta Delgada were similar but differed significantly from those of Lisbon, Évora and Portimão. The API from Évora differed significantly from the API recorded in Oporto and Coimbra. It was noted that higher concentrations of atmospheric pollen were observed at stations in the south, and Évora has the highest API (mean API=77259). The insular cities of Funchal (mean API=2695) and Ponta Delgada (mean API=5238) recorded the lowest API (Fig. 3).

MRT models were performed in order to reveal the similarities between sampling sites. Performance of the model for the whole data set (Fig. 4 A) was rather low (RE=0.98, CV RE=0.98). However, it indicated much higher pollen concentrations for longitude >= -0.27, i.e. in Évora, particularly for Cupressaceae, *Olea, Platanus*, Poaceae, *Quercus*, *Q. suber* and Urticaceae (Node 3) (Table 2 A).

MRT analysis for the data set excluding Évora resulted in a tree with three terminal nodes based on geographical location (Table 1 and Fig. 4B). The first split related to longitude. Sites located to the west of -13.03 (i.e. Ponta Delgada and Funchal - Node 2) were separated from Lisbon, Oporto, Portimão, and Coimbra to the east (Node 3). The second split was related to latitude, with Lisbon, Oporto and Coimbra to the north (latitude ≥ 37.92, Node 4) being separated from Portimão in the south (latitude < 37.92, Node 5). At Funchal and

Ponta Delgada the concentrations of all pollen were the lowest. In turn, Portimão was characterized by much higher abundance of *Olea* and *Quercus* spp. than in Lisbon, Oporto and Coimbra (Table 2 B).

3.3 Temporal variations in the amount of airborne pollen recorded at monitoring stations in Portugal

The monthly distribution of airborne pollen for all sites belonging to the RPA (2003-2012) are presented (Fig. 5). The majority or airborne pollen in Portugal were observed from March to June (73% of the pollen total), with the exception of Évora and Portimão where the main peaks occurred from March to May. Airborne pollen was mainly detected from March to July on the insula sites of Funchal (Madeira) and Ponta Delgada (Azores) and the mainland coastal city of Oporto. The most airborne pollen was detected in the air of Oporto and Lisbon in April (20% and 24% of the total pollen collected, respectively), whereas the highest airborne pollen concentrations were recorded during May in Coimbra (22%) Évora (36%) Portimão (44%) and Funchal (17%). In Ponta Delgada, the pollen peak was detected in June (19%). In general, only small amounts of pollen (≤2% of total pollen) were recorded in the air during autumn.

There were a number of significant trends ($p < 0.05$) in the API of tree (Table 3) and herbaceous (Table 4) taxa during the 10-year period of pollen monitoring. For tree pollen, the most significant trends were recorded in Ponta Delgada. These were significant decreases in the amount of airborne Arecaceae, Betulaceae*, Castanea, Quercus,* and *Salix* pollen. There were several other significant negative decreases in airborne tree pollen observed in this study at Lisbon (Arecaceae and Myrtaceae) and Évora (Pinaceae). The other significant trends were towards increases in atmospheric tree pollen in Oporto (*Castanea*), Coimbra (*Olea* and *Quercus*), Lisbon (*Castanea*), Évora (Arecaceae) and Funchal (Cupressaceae). There were no significant changes in the amount of tree pollen in the air in Portimão (Table 3).

There were also a number of significant negative trends in airborne herbaceous pollen recorded in Ponta Delgada, these were for Chenopodiaceae, *Plantago, Rumex* and Urticaceae. Significant decreases in airborne Chenopodiaceae pollen were recorded in Oporto and Portimão. Significant increases in airborne herbaceous pollen were recorded in Coimbra (Urticaceae*, Plantago, Rumex*), while Urticaceae in Portimão revealed a negative trend.

4. Discussion

The results of a decade of pollen monitoring performed by the Portuguese Aerobiology Network have shown that there are notable differences in the spectrum of airborne pollen recorded at the 7 stations. The pollen spectrum recorded at the RPA stations is made up by a smaller number of pollen types than observed in other studies conducted on the Iberian Peninsula (e.g. Perez-Badia et al. (2011)). This is, in part, due to the insular and coastal nature of many of the sites. In general, the pollen spectrum has Mediterranean characteristics, with Cupressaceae, *Olea*, Poaceae, *Quercus* spp. and Urticaceae pollen featuring prominently.

The most frequently recorded pollen types include those that could potentially impact on the environmental health of the allergic population in Portugal. The GA^2LEN skin test study shows that pollen allergens from Poaceae (the grass family) have the highest prevalence rates of all the pollen types included in skin prick test in Portugal (Burbach et al. 2009). Poaceae pollen, because of its ubiquitous nature and the allergenic capacity, is considered to be one of the most important aeroallergens in Europe (Sánchez-Mesa et al. 2003) including the Iberian Peninsula (Jato et al. 2009; Rodríguez-Rajo et al. 2010). Poaceae pollen featured prominently at all the sites included in this study, Évora in particular.

Prevalence rates to *Olea* (olive) pollen allergens are ranked second in Portugal (21.3 %) (Burbach et al. 2009). *Olea* pollen was recorded at all sites in Portugal, but less frequent in Douro littoral (the region of Oporto) and scarce in the islands. *Olea* was by far the most frequently recorded pollen type at Portimão and featured prominently in the pollen spectrum of Coimbra, Lisbon, and Évora. The highest amounts of airborne *Olea* in Portugal were recorded in Évora, but the API for airborne *Olea* pollen in RPA stations are comparatively lower than those recorded in Spanish regions, like Toledo and Ciudad Real and Córdoba, which are important centres for olive oil production (García-Mozo et al. 2008). The relatively high levels of *Olea* pollen recorded in the Alentejo (Évora) and Algarve (Portimão) regions are due to local sources, and augmented by the atmospheric transport from Andalusia in Southern Spain (Galán et al. 2013; Fernández-Rodríguez et al. 2014) where olive trees are widely grown (Recio et al. 2002; Recio et al. 2006).

Urticaceae pollen appeared among the most frequently recorded pollen types at all sites and *Parietaria* pollen allergens were ranked third in regard to prevalence rates in Portugal (17.5%) (Burbach et al. 2009).

Plants belonging to the Betulaceae (which comprises *Alnus* and *Betula* pollen types) and the Pinaceae families occur naturally in forests. Betulaceae mostly occur in Northern regions of Portugal. On the other hand, the appearance of Cupressaceae and *Platanus* in the airborne pollen spectrum is largely due to the introduction of ornamental species into the urban landscape. Some of these pollen types, such as Betulaceae, Cupressaceae and

Platanus, are known aeroallergens. For this reason, Cariñanos and Casares-Porcel (2011) suggested there is a need for guidelines regarding the design and planning of urban green spaces with a low allergy impact.

There are significant spatial variations in the amount of airborne pollen recorded at each of the pollen-monitoring stations included in this study. The mean API is higher in inland sites such as the southern city of Évora. In contrast, coastal and insular sites generally record the lowest API. The exception is the monitoring station in Portimão, which is considered be have inland characteristics and records high pollen levels due to north and north-easterly winds that transport pollen across the Algarve region (Caeiro 2004).

MRT analysis of similarity confirms the presence of considerable spatial variations in pollen composition, and separates insular pollen-monitoring stations that record lower airborne pollen concentrations (Ponta Delgada and Funchal) from those on the mainland in the east (Portimão, Lisbon, Oporto and Coimbra). Évora has a notable impact on the results because the site records the highest pollen concentrations. Removing Évora from the MRT analysis has the result of separating Portimão in the south from Lisbon, Oporto and Coimbra in the north. Portimão records higher levels of airborne *Olea* and *Quercus* pollen than the other mainland sites (excluding Évora), as evergreen oak woodlands are the main forest type in the region and olive groves are part of the agricultural landscape (Nunes et al. 2005).

Évora, Portimão and Lisbon are cities with Mediterranean characteristics with pollen levels comparable to those reported in Toledo (Perez-Badia et al. 2011) and Madrid (Bustillo et al. 2002; Gutiérrez et al. 2006) in Central Spain, and Ciudad Real in Southern Spain (Prieto et al. 2002). On the other hand, pollen levels observed in Oporto and Coimbra are lower compared to other similar bio-climatic regions from the Iberian Peninsula (Perez-Badia et al. 2011). In the case of most inland stations, like Évora, the pollen spectrum is dominated by airborne tree pollen (e.g. *Quercus*, *Olea*, Cupressaceae and *Platanus*), which are also predominant pollen types in Badajoz (Extremadura region, SW Spain), the nearest Spanish city (Gonzalo-Garijo et al. 2006). Insular and coastal stations tend to have lower pollen concentrations than those in the mainland due to a lower potential for pollen resuspension and less land mass with plant coverage (Belmonte et al. 1995). For this reason, the mean API detected in Funchal (2695) and Ponta Delgada (5238) are lower than other bioclimatically related areas on the mainland, but were also lower than those reported for similar geographical areas such as the Canary Islands (API=6309) (Belmonte et al. 2008) and Mallorca (PI = 20027) in Spain (Boi and Llorens 2013). The pollen types most frequently recorded at insular and coastal pollen-monitoring stations were from herbaceous plants, Poaceae and Urticaceae (*Parietaria* sp., *Urtica* sp. and *Urtica membranacea*), which originate from several species that tend to bloom year-round (Camacho 2015).

In all RPA stations, with the exception of Oporto, the highest atmospheric concentrations of pollen were observed from late winter until the end of spring (March to June), as observed by Boi and Lloren (2013), Docampo et al. (2007) and Ruffoni et al. (2013). Peak concentrations occurred earlier in the South of the Portuguese territory and later in the North. In the coastal cities such as Funchal, Ponta Delgada and Oporto, the highest airborne pollen concentrations were detected slightly later (March to July). Airborne pollen concentrations remained at lower levels during summer and particularly in autumn months.

Previous work concerned with changes in airborne pollen levels across Europe found trends towards increased amounts of airborne pollen recorded annually for many taxa (Ziello et al. 2012). This study did not find evidence of a clear tendency towards an increase in atmospheric pollen. For both tree and herbaceous pollen, there was a combination of significant increases and significant decreases in the API. In general, there were more significant increases in airborne pollen recorded at northerly sites, particularly Coimbra. Whereas, there were more significant negative trends at sites in the south. Ponta Delgada, the most southerly site included in this study, had 9 significant negative trends. It is not known why there is a tendency for pollen levels on the Azores archipelago to decrease, but it could be related to land use (Cariñanos et al. 2004; Galán et al. 2016) or meteorological conditions during the period of study period.

5. Conclusions

This 10-year aeropalynological survey identifies spatial and temporal variations in the main airborne allergenic pollen types that occur in the Portuguese territory. The pollen spectrum is dominated by important allergenic anemophilous pollen types such as Poaceae, *Olea*, and Urticaceae (i.e. *Parietaria*) pollen. Statistically significant differences were witnessed in the API recorded at the 7 RPA pollen-monitoring stations. The API is higher in the southern mainland cities such as Évora, Lisbon and Portimão and lower in insular and coastal cities. There were a number of significant trends in the API of tree and herbaceous taxa during the 10-year study, but there were no general increases in the amount of airborne pollen recorded annually.

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Figure Legends:

Figure 1**.** Location of 7 monitoring stations of the Portuguese Aerobiology Network (RPA).

Figure 2 A-G. Airborne pollen spectrum of the 7 RPA monitoring stations (2003–2012). Pollen types and percentages

Fig. 3. Temporal variations in airborne pollen from each monitored station during the study period (2003-2012): (A) Annual Pollen Index; (B) Monthly distribution of airborne pollen

Figs 4 A-B. Multivariate regression tree for daily average airborne pollen concentrations recorded during this study: (A) All sites; (B) Excluding Évora n = number of cases.

Fig. 5. The monthly distribution of airborne pollen recorded at each monitored station during the study period (2003-2012)

Table Legends:

Table 1. Bioclimatic characteristics of each monitoring station (Rivas-Martínez 2001).

Table 2. Daily average pollen concentrations at MRT terminal nodes: (A) Model for all sites; (B) Model excluding Évora.

Table 3. Trends in Annual Pollen Index of tree taxa at the sites studied. The following statistics are presented: slope of the regression over time, standard error of the regression slope (SE), coefficient of determination (R^2); probability level (*p*). Trends were considered significant ($p < 0.05$) are marked in bold.

Table 4. Trends in Annual Pollen Index of herbaceous taxa at the sites studied. The following statistics are presented: slope of the regression over time, standard error of the regression slope (SE), coefficient of determination (R^2); probability level (*p*). Trends were considered significant ($p < 0.05$) are marked in bold.

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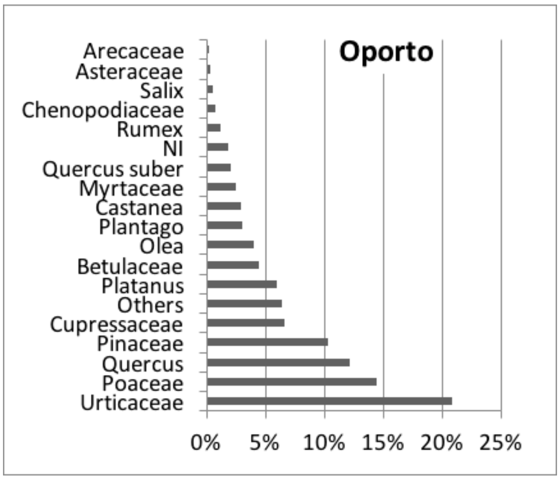
A



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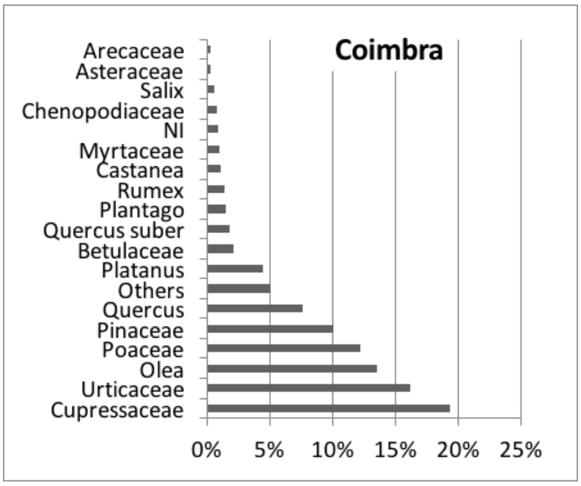

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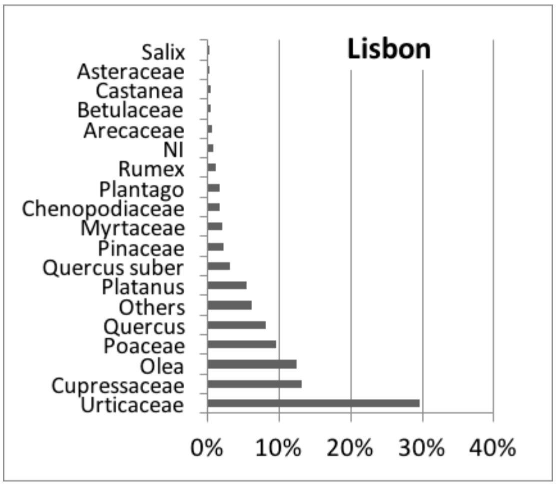

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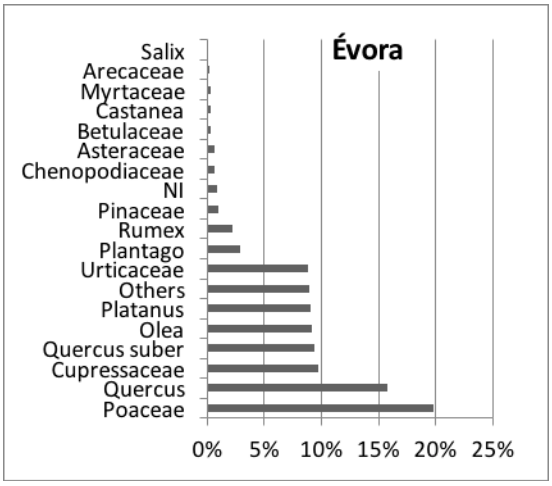

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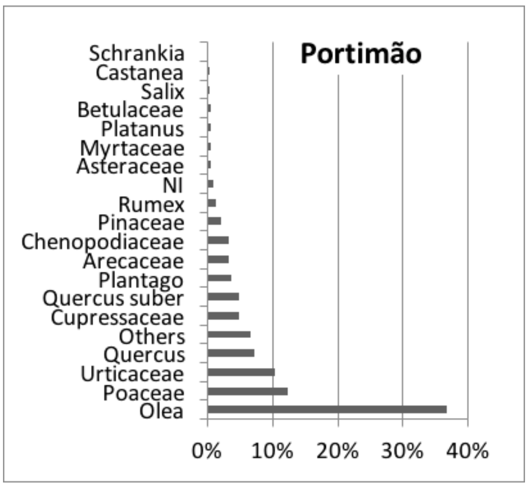

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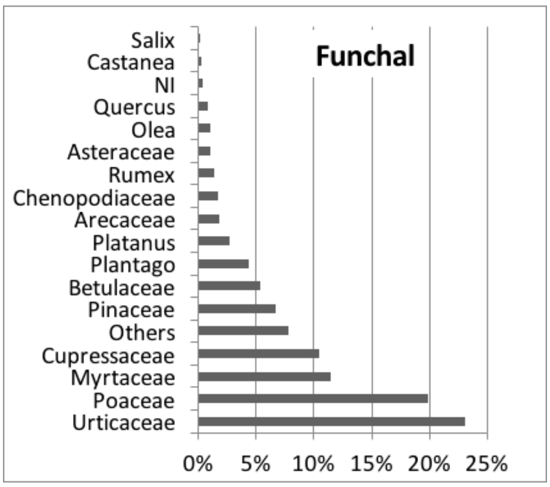


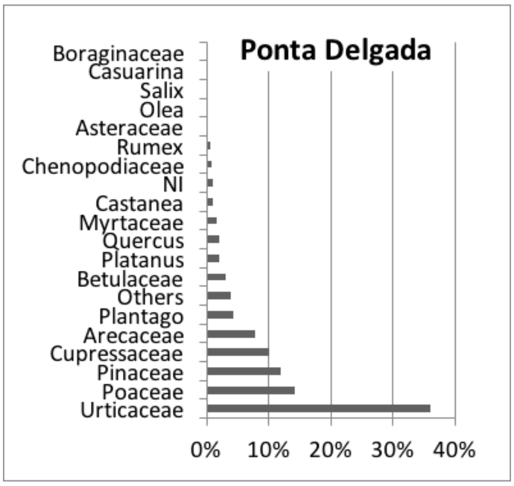
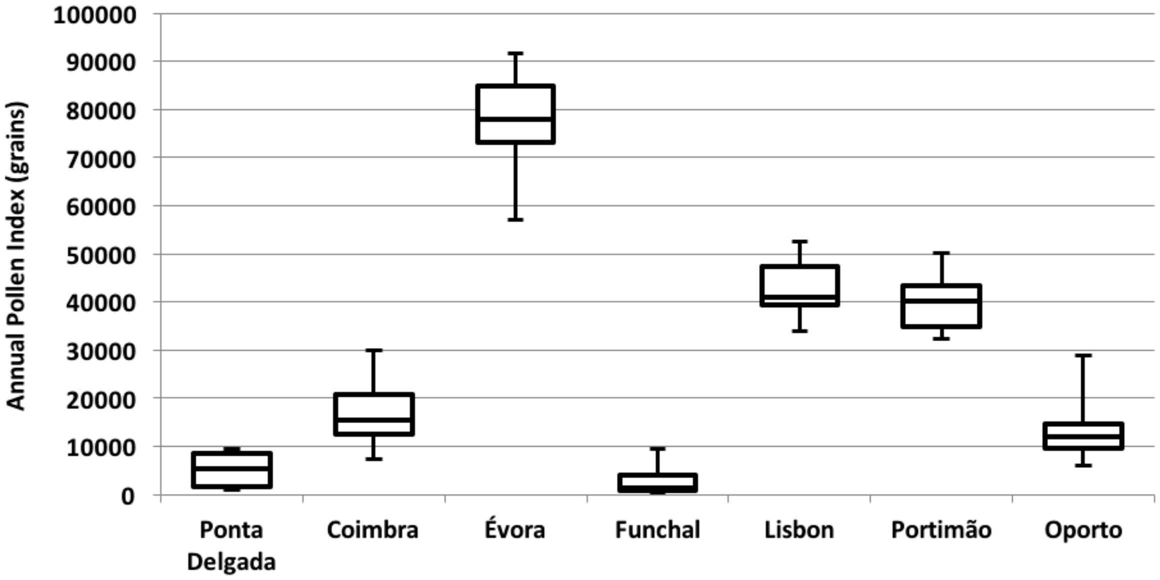
G  Ponta Delgada Boraginaceae Casuarina Salix Olea Asteraceae Rumex Chenopodiaceae NI Castanea Myrtaceae Quercus Platanus Betulaceae Others Plantago Arecaceae Cupressaceae Pinaceae Poaceae Urticaceae 0% 10% 20% 30% 40%	

Fig. 3. Temporal variations in the Annual Pollen Index recorded at each monitored station during the study period (2003-2012)



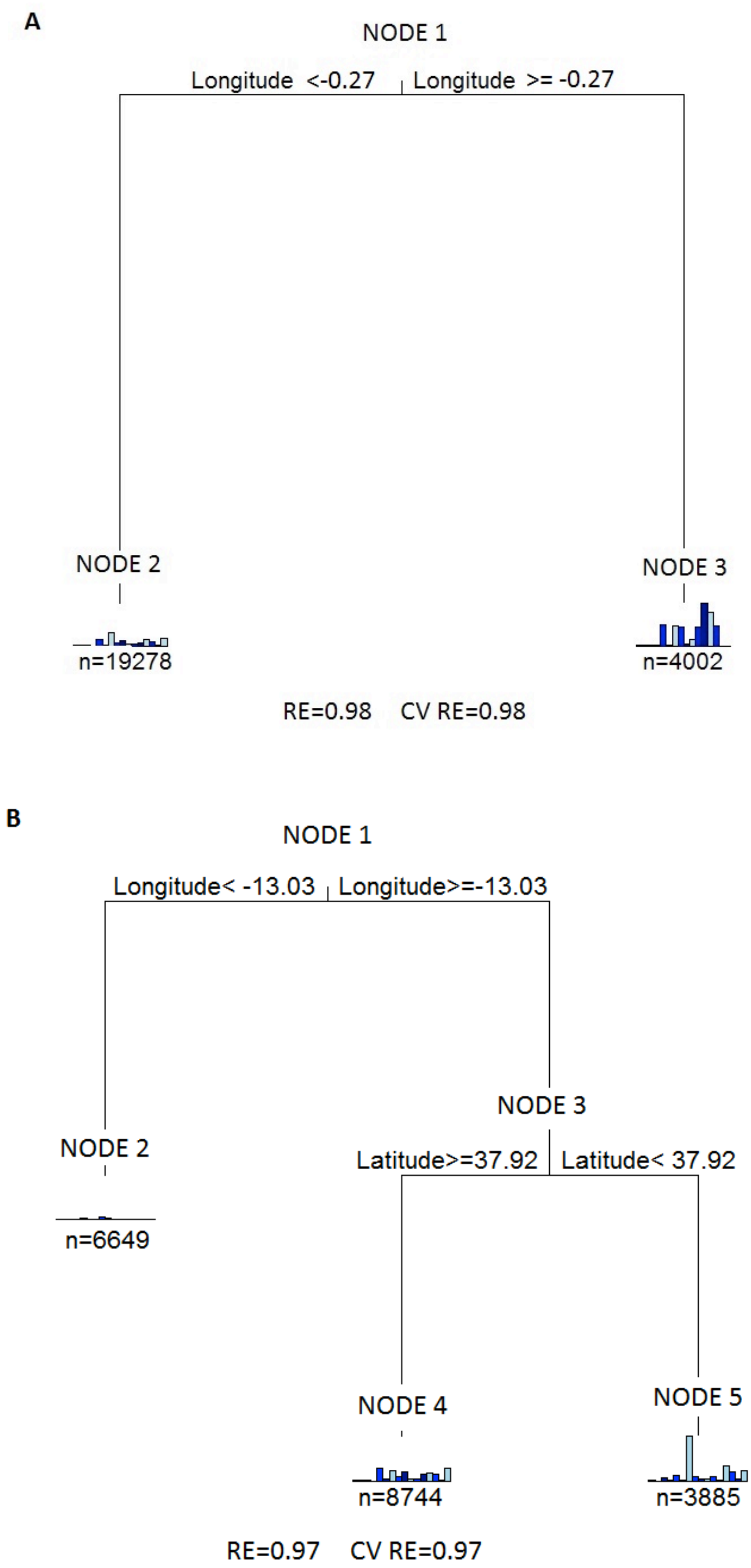


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Fig. 5. The monthly distribution of airborne pollen recorded at each monitored station during the study period (2003-2012)

(B)

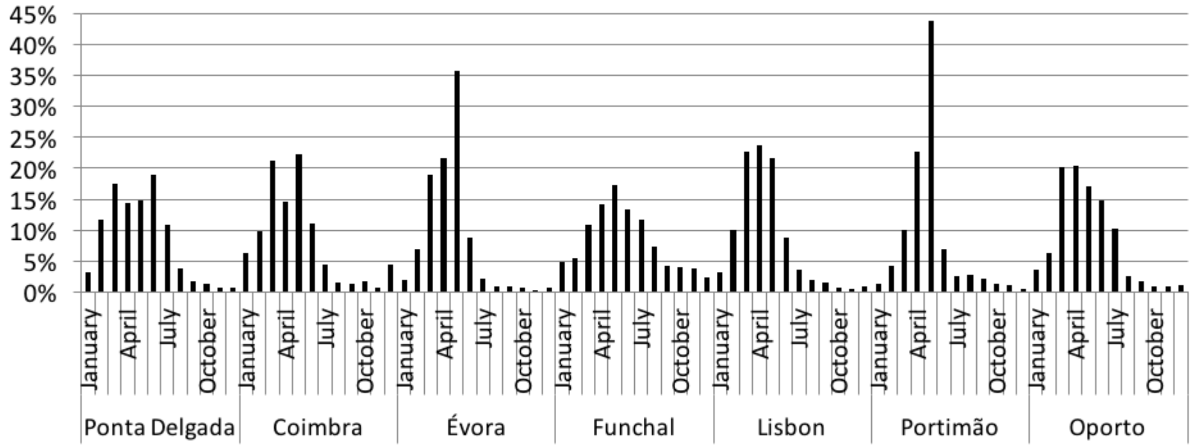

Table 1. Bioclimatic characteristics of each monitoring station (Rivas-Martínez 2001).

Station	Latitude & Longitude	Termoclimate	Altitude (m)	Average Annual Temperature (°C)	Total Annual Precipitation (mm)
Oporto	41.13 N -8.63 W	Mesotemperate Meso-submediterranean	80	14.7	1253.5
Coimbra	40.2 N -8.42 W	Mesomediterranean	140	15.5	905.1
Lisbon	38.72 N -9.13 W	Termomediterranean	55	17.0	725.8
Évora	38.57 N 7.9 E	Mesomediterranean	300	15.9	609.4
Portimão	37.13 N -8.53 W	Termomediterranean	34	16.8	466.0
Funchal	32.65 N -16.9 W	Temperate Hyperoceanic-submediterranean	400	19.7	627.2
Ponta Delgada	37.73 N -25.67 W	Mediterranean pluviseasonal oceanic / Temperate hyperoceanic submediterranean	50	17.3	1027.1

Table 2. Daily average average pollen concentrations at MRT terminal nodes: (A) Model for all sites; (B) Model excluding Évora.

Pollen types	(A) All sites Node		(B) Excluding Évora Node		
	2	**3**	**2**	**4**	**5**
Arecaceae	7.44	0.28	0.57	11.66	9.72
Asteraceae	0.27	1.23	0.06	0.27	0.63
Betulaceae	0.78	0.67	0.42	1.17	0.53
Castanea	0.45	0.56	0.08	0.78	0.33
Chenopodiaceae	1.31	1.24	0.12	1.16	3.68
Cupressaceae	6.94	20.65	1.10	12.01	5.54
Myrtaceae	1.04	0.55	0.60	1.59	0.57
Olea	12.89	19.55	0.05	9.98	41.43
Pinaceae	4.92	2.15	1.69	8.69	1.97
Plantago	1.75	6.08	0.56	2.42	2.27
Platanus	1.93	19.29	0.33	2.15	4.17
Poaceae	3.30	42.10	1.01	6.25	0.57
Quercus	6.80	33.59	1.01	8.07	13.84
Q. suber	4.46	19.83	0.04	6.18	8.14
Salix	0.74	0.25	0.06	0.91	1.51
Urticaceae	3.49	18.83	2.35	4.06	4.16

Table 3. Trends in Annual Pollen Index of tree taxa at the sites studied. The following statistics are presented: slope of the regression over time, standard error of the regression slope (SE), coefficient of determination (R^2); probability level (*p*). Trends were considered significant ($p < 0.05$) are marked in bold.

Site/Pollen		***Betulaceae***	***Castanea***	**Cupressaceae**	**Myrtaceae**	***Olea***	**Pinaceae**	***Platanus***	***Quercus***	***Quercus suber***	***Salix***	***Arecaceae***
Oporto	Mean	607.23	399.24	912.86	337.21	560.04	1396.12	422.64	1997.72	1693.59	168.23	1492.36
	Slope	60.00	49.20	118.00	0.27	46.90	90.00	31.20	2.00	225.00	2.06	144.00
	R^2	0.15	0.40	0.32	0.00	0.13	0.16	0.31	0.00	0.30	0.01	0.25
	SE	47.97	20.22	57.00	18.94	41.15	70.00	15.71	78.4	113.50	8.10	82.80
	p	0.24	**0.04**	0.07	0.99	0.28	0.23	0.08	0.98	0.08	0.80	0.12
Coimbra	Mean	354.31	191.67	3277.11	168.55	2282.51	1701.00	751.74	1293.06	297.76	90.64	40.99
	Slope	12.60	24.80	-109.00	2.38	207.00	-57.50	107.00	228.00	22.80	13.70	7.80
	R^2	0.04	0.23	0.02	0.01	0.49	0.02	0.16	0.76	0.05	0.21	0.26
	SE	20.37	15.01	257.20	8.90	69.80	126.00	83.10	43.03	33.66	8.73	4.36
	p	0.55	0.13	0.68	0.79	**0.02**	0.66	0.23	**0.00**	0.51	0.15	0.11
Lisbon	Mean	227.09	170.52	5669.02	925.45	5321.12	4397.59	706.16	4153.43	3493.97	548.64	8362.08
	Slope	-4.97	27.70	-96.20	-117.80	-51.40	-9.27	4.06	-47.53	204.00	1.59	-620.00
	R^2	0.03	0.56	0.02	0.40	0.01	0.01	0.01	0.01	0.20	0.00	0.40
	SE	9.57	8.22	211.70	48.24	172.50	74.70	17.44	176.50	137.50	17.03	254.60
	p	0.62	**0.01**	0.66	**0.04**	0.77	0.90	0.82	0.79	0.17	0.93	**0.04**
Évora	Mean	243.84	202.28	7511.54	201.38	7112.38	783.27	7019.04	12220.18	7214.12	90.97	102.82
	Slope	-1.52	21.10	-69.30	3.29	160.00	-46.38	471.00	554.00	364.00	-2.18	28.10
	R^2	0.01	0.30	0.01	0.01	0.06	0.40	0.16	0.10	0.18	0.09	0.59
	SE	8.13	10.75	285.40	9.44	205.000	18.99	354.90	548.00	255.40	2.33	7.84
	p	0.86	0.08	0.81	0.74	0.45	**0.04**	0.22	0.34	0.19	0.38	**0.01**
Portimão	Mean	186.42	115.27	1958.25	202.92	14629.61	694.97	1471.89	4889.96	2874.03	534.66	3438.02
	Slope	-7.56	9.90	74.00	-4.99	-45.90	-18.14	-71.30	-170.80	48.20	-12.30	-81.00
	R^2	0.14	0.30	0.07	0.09	0.00	0.09	0.18	0.08	0.03	0.05	0.06
	SE	6.27	5.07	86.4	5.29	342.20	19.25	50.60	194.50	89.60	18.73	110.40
	p	0.26	0.08	0.42	0.37	0.90	0.37	0.19	0.40	0.60	0.53	0.48
Funchal	Mean	143.33	7.12	280.93	307.65	26.51	568.28	118.76	545.22	21.91	36.43	52.33
	Slope	16.50	-0.21	54.00	69.00	0.99	91.10	26.40	108.00	5.20	5.90	4.81
	R^2	0.12	0.00	0.36	0.30	0.01	0.21	0.18	0.30	0.35	0.06	0.06

	SE	15.04	1.62	23.88	35.73	3.87	58.60	19.03	55.60	2.30	7.70	6.25
	p	0.30	0.90	**0.04**	0.08	0.80	0.16	0.20	0.08	0.05	0.47	0.46
Ponta Delgada	Mean	154.53	55.55	528.26	82.22	3.76	621.34	107.82	106.54	-	3.31	403.38
	Slope	-42.99	-18.29	-82.50	-3.23	0.08	-125.70	-22.14	-29.26	-	-1.24	-119.80
	R^2	0.71	0.58	0.19	0.02	0.01	0.47	0.26	0.51	-	0.58	0.66
	SE	11.27	6.40	69.500	9.53	0.467	54.40	15.05	11.63	-	0.43	34.65
	p	**0.01**	**0.03**	0.28	0.74	0.86	0.06	0.19	**0.04**	-	**0.03**	**0.01**

Table 4. Trends in Annual Pollen Index of herbaceous taxa at the sites studied. The following statistics are presented: slope of the regression over time, standard error of the regression slope (SE), coefficient of determination (R^2); probability level (p). Trends were considered significant ($p < 0.05$) are marked in bold.

Site/Pollen		Asteraceae	Chenopodiaceae	*Plantago*	Poaceae	*Rumex*	Urticaceae
Oporto	Mean	43.81	103.77	1430.46	827.97	283.10	75.03
	Slope	-0.96	-11.73	-2.72	97.00	-27.08	16.64
	R^2	0.04	0.48	0.00	0.12	0.14	0.44
	SE	1.54	4.11	69.90	85.10	25.19	6.24
	p	0.55	**0.02**	0.97	0.28	0.31	**0.03**
Coimbra	Mean	51.58	124.92	255.90	2063.54	243.93	1297.75
	Slope	5.10	10.80	25.60	165.00	23.70	350.82
	R^2	0.23	0.16	0.52	0.23	0.55	0.59
	SE	2.90	8.08	8.25	100.80	7.11	98.1
	p	0.11	0.21	**0.01**	0.14	**0.01**	**0.01**
Lisbon	Mean	142.77	765.77	950.34	2366.07	1350.99	113.36
	Slope	-3.85	-25.16	-25.68	-3.50	-19.66	68.82
	R^2	0.08	0.22	0.10	0.00	0.01	0.35
	SE	4.44	15.83	25.55	83.10	73.30	31.42
	p	0.41	0.15	0.34	0.97	0.79	0.06
Évora	Mean	446.05	449.74	2212.01	15317.60	1742.57	4981.05
	Slope	-11.57	-22.02	71.00	-435.30	-34.61	24.34
	R^2	0.08	0.45	0.10	0.04	0.03	0.00
	SE	12.68	8.17	71.90	738.00	65.20	206.80
	p	0.39	**0.02**	0.35	0.57	0.61	0.91
Portimão	Mean	222.68	1301.10	803.17	202.81	1942.68	167.66
	Slope	-5.70	-56.00	8.00	12.00	-47.65	-221.43
	R^2	0.05	0.18	0.01	0.20	0.07	0.74
	SE	8.22	40.47	24.42	8.06	59.70	43.85
	p	0.51	0.20	0.75	0.17	0.44	**0.00**
Funchal	Mean	27.20	44.38	181.24	72.07	-	0.49
	Slope	3.06	6.60	30.90	14.00	-	13.92
	R^2	0.18	0.13	0.53	0.22	-	0.31

	SE	2.21	5.75	9.65	8.68	-	7.00
	p	0.20	0.28	**0.01**	0.14	-	0.08
Ponta Delgada	Mean	10.73	37.94	220.37	742.77	23.74	1349.75
	Slope	-0.46	-7.17	-65.8	-173.50	-5.85	-420.80
	R^2	0.04	0.48	0.61	0.45	0.55	0.54
	SE	0.94	3.02	21.31	78.60	2.17	158.40
	p	0.64	0.06	**0.02**	0.07	**0.04**	**0.04**