## ORIGINAL PAPER

# A 10-year survey of allergenic airborne pollen in the city of Porto (Portugal)

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Abstract Airborne pollen calendars are useful to estimate the flowering season of the different plants as well as to indicate the allergenic potential present in the atmosphere at a given time. In this study, it is presented a 10-year survey of the atmospheric concentration of allergenic pollen types. Airborne pollen was performed, from 2003 to 2012, using a 7-day Hirst-type volumetric trap. The interannual variation of the daily mean concentration of the number of pollen grains and the main pollen season was determined as well as the hourly variations and correlation with meteorological parameters. During the study period, 18 different allergenic pollen types were considered based on its representativeness on the total annual airborne pollen concentration. The lowest annual concentrations were sampled in 2006 and the highest in 2007. The highest airborne pollen concentration was found during early spring and early summer. On the contrary, December was the month with the lowest pollen concentration. The major pollen sampled belongs to trees followed by weeds and grasses, being the most representative pollen types in

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the atmosphere: Urticaceae, Platanus, Poaceae, Pinaceae, Cupressaceae, Acer, Quercus, Castanea, Plantago, Alnus, Olea europaea, Betula, Myrtaceae and Populus. Intradiurnal distribution patterns of the pollen types studied presented differences with some taxa being predominantly sampled in the morning (9–11 a.m.) while others in first night hours (between 9 and 12 p.m.). Significantly correlations were found between the airborne pollen concentration and meteorological parameters.

Keywords Aerobiology - Main pollen season - Meteorological parameters · Hourly distribution · Pollen spectrum

## 1 Introduction

Pollen grains are biological structures produced as part of the reproductive cycle of the higher plants. Pollen primary function is to perform the vital task of sexual reproduction; however, in consequence of the pollination process, the pollen becomes part of the atmospheric aerosol that can enter people's airways. This ''biopollutant'' could be innocuous if it was not the possibility of pollen to induce respiratory allergic reactions on susceptible individuals. Particularly in pollen from anemophilous plants, the existence of allergens in both inner part of the pollen (intine and

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cytoplasm) and pollen outer wall (submicroscopical sites of the exine and orbicules) (Linskens and Cresti [2000\)](#page-11-0) is the main cause triggering respiratory allergic reactions.

Pollen-related respiratory allergies, or as common known pollinosis, is a general health problem worldwide, affecting life quality of earth inhabitants (Ring [2012\)](#page-11-0). They are associated with an allergic response of patients to the pollen of several anemophilous trees, grasses and weeds that are released into the atmosphere during plant flowering season.

Sensitization to a determine pollen allergen deeply depends on intrinsic characteristics of a given population and variable between different regions of the world, being closely related to plant distribution, climatic conditions and urbanization levels (D'Amato et al. [2007](#page-10-0); Peden and Reed [2010](#page-11-0)). So, the development of aerobiological studies is important for the elaboration of pollen calendars indicatives of the presence and prevalence of allergenic pollen in the atmosphere of a sampling area and also allows, after several years of monitoring, to estimate the date for the flowering season of plants (Scheifinger et al. [2013](#page-11-0)). This knowledge is useful for clinicians, in order to adequate the standard skin prick test battery or the intradermal tests with extracts to the regional pollen spectrum, for effective immunotherapy or other treatment procedure. It can also be helpful for patients in taking prophylactic measures such as the planning of outdoor activities, contributing to an improvement on their life quality. Also, airborne pollen monitoring can contribute to support architectural landscape planning, for a more balanced selection of plant species that meets the quality of life of population living in urbanized areas (Cariñanos and Casares-Porcel [2011](#page-10-0)).

In Portugal, aerobiological studies started in the 50th and 60th (Pinto da Silva [1955](#page-11-0), [1960,](#page-11-0) [1964](#page-11-0)) and along the years, some studies have been conducted on the type and concentration of pollen present in the atmosphere of several cities (Abreu and Ribeiro [2005](#page-10-0); Caeiro et al. [2007](#page-10-0); Câmara [2008](#page-10-0); Fernandes et al. [2010;](#page-10-0) Ribeiro et al. [2005\)](#page-11-0) as well as its relationship with meteorological factors (Ribeiro et al. [2003](#page-11-0)). Since 2003, the Portuguese aerobiology network has been performing the airborne pollen and fungal spores monitoring in some cities of Portugal (Caeiro et al. [2007;](#page-10-0) Nunes et al. [2008](#page-11-0)). Also, the classification of allergenic pollen types conjugating airborne pollen data with immunological studies (Ribeiro et al. [2009](#page-11-0); Sousa et al. [2011](#page-11-0)) and studies relating the regional pollen exposure with allergic respiratory hospital admissions (Ribeiro et al. [2009](#page-11-0)) have been performed. All these studies point out to the primary importance of long-term airborne pollen monitoring as a necessary tool for air quality measurements and implications in the public health assessment. Therefore, the aim of this study was to report for the first time a long-term survey of allergenic airborne pollen in the city of Porto.

## 2 Materials and methods

The aerobiological study was performed in the city of Porto, the second largest Portuguese city, located in the northwest Portugal. This city is limited at west by the Atlantic Ocean and at south by the Douro River. The annual average temperature is around 15  $\degree$ C, and the difference between warmer and colder monthly averages is below 10  $\degree$ C. Annual air humidity ranges between 75 and 80 %, and the total annual mean precipitation varies between 1,000 and 1,200 mm, with about 40 % in the winter season. Prevailing winds are from the W and NW in summer and from the E and SE in winter (Miranda et al. [2001](#page-11-0)).

The landscape surrounding the sampling point comprises two large public parks, the Botanical Garden and several other smaller cared and maintenance gardens. Also, the roundabouts, streets and avenue walks surrounding the sampler are usually ornamented with trees. The most common tree species found are Acer, Fraxinus, Liquidambar, Pinus, Platanus, Populus, Prunus, Quercus, Tilia, among others. At the herbaceous strata, plants like Plantago, Poaceae, Rumex or Urticaceae, dominate, being these frequently cut by government authorities.

Airborne pollen was continuously monitored, from January 2003 to December 2012, using a 7-day Hirsttype volumetric spore trap (model Lanzoni VPPS-2000; Lanzoni s.r.l., Italy), calibrated to sample air at ten liters per minute. This sampler was set on the roof of the Faculty of Sciences in Porto (41°11'N, 8°39'W), approximately 20 m above ground level, and has a  $2 \times 14$  mm intake orifice through which the sampled air is impacted onto a drum, covered with a Melinex tape, rotating once every 7 days. Pollen grains were trapped on this tape, coated with silicone oil, which was cut into seven daily segments and mounted on the slides with a mounting media of glycerol jelly. The daily and hourly mean concentration of the number of pollen grains was determined using an optical microscope at a magnification of  $400 \times$  along 4 full lengthwise traverses, divided into latitudinal sections of 2-mm (representing 1 h) intervals. Pollen counts were transformed into daily and hourly number of pollen grains per cubic meter of air.

The main pollen season was determined using a nonlinear logistic regression model fitted to the values of the accumulated sum of the daily airborne pollen concentration. After adjusting the model to each pollen type and year, a one-sided t test was used at the 5 % level, in order to estimate the beginning and ending dates of the main pollen season. These dates correspond to the thresholds where the daily difference between the pollen emission model and its superior and inferior asymptotes was significant. A more detailed description of this methodology can be found in Ribeiro et al. [\(2007](#page-11-0)).

The intradiurnal airborne pollen concentration was determined, for each year and pollen type studied, from the hourly values of atmospheric concentration registered during the main pollen season, according to the method described by Galán et al. [\(1991](#page-11-0)). For this, the days (1) where atmospheric pollen concentrations were superior to the third quartile (for Urticaceae and Poaceae) or to the average of the main pollen season concentration and (2) without precipitation occurrence were selected. Once the main pollen season of Urticaceae and Poaceae is much extended, comprising several peaks along the year interspersed with some null values, a more accurate daily distribution pattern can be achieved by using the third quartile of the main pollen season concentration.

Since the hourly counts vary interdaily and interannually, its values were expressed in percentage rather than in absolute numbers.

Pollen counts were classified using three pollen count classes according to the Portuguese Aerobiology Network (<http://www.rpaerobiologia.com>): low  $(1-30 \text{ pollen/m}^3)$ ; moderate  $(30-60 \text{ pollen/m}^3)$ ; high  $($ >60 pollen/m<sup>3</sup> $)$ . These thresholds can be indicatives of the clinical implication of the pollen concentration results in Porto city.

Finally meteorological data (rainfall, temperature, relative humidity and wind velocity) were correlated with the airborne pollen concentrations of the selected types by means of the Spearman rank correlation test, with a significance level of 99, 95 and 90 %.

### 3 Results

During the study period, 27 allergenic different pollen types were identified, of which only 18 were considered in this study based on its representativeness on the total annual airborne pollen concentration (Table [1](#page-3-0)). The pollen types were divided into Poaceae, trees (Acer, Alnus, Betula, Castanea, Cupressaceae, Fraxinus, Myrtaceae, Olea europaea, Platanus, Pinaceae, Populus, Quercus and Salix) and weeds (Chenopodiaceae–Amaranthaceae, Plantago, Rumex and Urticaceae). The major pollen sampled belongs to trees: 48 % followed by weeds: 34 % and Poaceae: 8 %.

The most frequent pollen types found in the atmosphere of Porto are shown in Fig. [1](#page-5-0). Pollen from Urticaceae (Parietaria plus Urtica) provided 30 % of the pollen concentration, more than the double of Platanus, the second most common, and approximately four times more than Poaceae. These three pollen types account for more than a half of the total annual airborne pollen concentration in Porto.

The other frequently observed pollen types belong mainly to trees such as Pinaceae, Acer, Quercus, Castanea, Alnus, Betula. Other representative weed pollen was from Plantago, Rumex and Chenopodiaceae–Amaranthaceae, but with considerable fewer incidences.

Overall, the total annual airborne pollen concentration was minimal in 2006, but very similar to the one observed in 2003 (4,500 and 4,634 pollen grains, respectively). The year 2007 registered the highest values attaining 12,096 pollen grains (Table [1](#page-3-0)). In four out of the 10 years studied (2004, 2005, 2009 and 2010), the total annual airborne pollen concentration presented similar values, around 7,000 pollen grains. Independently for each pollen type, there was no similar trend in the maximum and minimum values of annual pollen concentrations.

The highest average airborne pollen concentration was found from early spring until early summer, being March the month with the higher average pollen values  $(2,300 \pm 1,162)$ . On the contrary, the autumn months, particularly November and December, were the ones with the lowest airborne pollen concentration  $(107 \pm 35 \text{ and } 118 \pm 64, \text{ respectively})$  (Fig. [2](#page-5-0)).

However, throughout the year, the distribution of total airborne pollen concentration presents several annual peaks due to the diversity of taxa present in Porto atmosphere and its different flowering seasons (Table [1](#page-3-0); Fig. [3](#page-5-0)).

<span id="page-3-0"></span>

of annual airborne pollen concentration and main pollen season dates and duration of most abundant pollen types in the atmosphere of Porto Table 1 10-year median of annual airborne pollen concentration and main pollen season dates and duration of most abundant pollen types in the atmosphere of Porto median Table 1 10-year





<span id="page-5-0"></span>Rumex 0.9 Fraxinus 0.7 Populus 0.9 Cheno-Amar Myrtaceae 1.8  $0.5$ Betula 2.0 Salix 0.4 Olea europaea  $2.2$ Alnus 2.4 Plantago 2.5 Castanea 2.5 Ouercus 2.8 Urticaceae 30.1 **Million**  $Acer$  4.5 Cupressaceae 6.2 Pinaceae 7.3 Platanus 12.2 Poaceae 8.2

Fig. 1 Percentages of the most representative pollen types in atmosphere of Porto along the year the atmosphere of Porto from 2003 to 2012



Fig. 2 Monthly average pollen sampled in the atmosphere of Porto from 2003 to 2012

The airborne pollen concentrations started rising in the beginning of the year due to the flowering of Myrtaceae, Cupressaceae, Fraxinus, Alnus and Salix. Myrtaceae and Cupressaceae pollen can be found in the atmosphere in late December while Fraxinus and Alnus have its flowering period starting in the beginning of January. All of them present the flowering peak during February, although Myrtaceae pollen can be found sporadically along the year. Salix flowering period is the latest among this group, starting in the beginning of February and peaking in early March.

From the middle of March, the flowering period of Acer, Platanus, Populus and Pinus follow one another ending in early April.



Fig. 3 Distribution of the most abundant pollen types in the

In the beginning of April was observed the start of Betula and Quercus pollination period, with higher pollen concentration being attained usually in the middle of April, although for Quercus, its peak pollination period usually occurs slightly later and persists until late May.

The start of pollination period of O. europaea occurs in early May, peaking during this month. The Castanea flowering period occurs just after, in early June ending in July.

The Rumex pollen was sampled from middle of April until late June with higher concentrations found in the end of May, while Plantago was sampled from middle of May until late July with the higher concentrations found in the beginning of June. In the beginning of July started the flowering period of the Chenopodiaceae–Amaranthaceae, peaking in August and being present in the atmosphere until November.

Urticaceae and Poaceae have a significant presence during all year long, although the highest concentrations were recorded in March until early June for the first one and in May until July for the second one (Fig. 3).

Concerning the duration of the main pollen season of the different pollen types identified, it was much shorter for the majority of the tree species studied (between 15 days for Platanus to 35 days for Quercus), with exception to Myrtaceae and Cupressaceae pollen. The weed pollen presented larger main pollen seasons (between 42 days for Rumex to 92 days for Urticaceae) (Table [1](#page-3-0)).



Fig. 4 Average hourly distribution, expressed in percentage, of the most abundant pollen types in the atmosphere of Porto

Considering the intradiurnal distribution patterns of the pollen types studied, some differences were observed. Five groups with similar hourly distribution patterns could be determined (Fig. 4). Urticaceae, Cupressaceae, Plantago, Acer and Chenopodiaceae–Amaranthaceae pollen presented one well-defined daily peak count. Its pollen concentration started to increase at first morning hours reaching a maximum value between 9 and 11 a.m. Acer and Chenopodiaceae-Amaranthaceae also presented a slight increase in pollen later in the afternoon (4–10 p.m.).

<span id="page-7-0"></span>

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min minimum; avg average

Another group comprising Quercus, O. europaea, Poaceae, Pinaceae, Betula and Populus pollen presented higher airborne concentrations during the first night hours (between 9 and 12 p.m.). After this night peak, the airborne concentration decreases abruptly to increase progressively between 8 and 12 a.m. The lowest concentrations were found in the first afternoon hours.

Platanus pollen was present in the atmosphere in similar concentrations along the day, although a slight increase from 9 to 11 a.m. was observed.

Fraxinus, Salix, Myrtaceae and Rumex pollen presented several daily peak counts particularly around 2–6, 9–10 p.m. and 6 a.m.

Finally, Alnus and Castanea pollen presented particular intradiurnal airborne distribution patterns, with the first one reaching highest concentrations from middle afternoon to early night hours and the second one tended to be recorded in higher concentrations from 12 p.m. to 2 a.m.

Considering the pollen counts according to the threshold classes defined, it was observed that very seldom values of airborne pollen concentration surpassed the high pollen level. Along the 10 years of monitoring, only Urticaceae, Platanus, Poaceae, Pinaceae, Cupressaceae, Acer and O. europaea exceeded daily values of 60 pollen/ $m<sup>3</sup>$  (Table [2\)](#page-7-0).

Overall airborne pollen of the studied taxa was positively correlated with temperature and negatively correlated with the rain, relative humidity and wind speed. Correlation coefficients were in most of the cases very highly significant ( $p$  value <0.01).

### 4 Discussion

Pollen is a natural biological source of particulate matter in the atmospheric aerosol. However, its contribution is seasonal as can be seen by our results and all other aerobiological studies performed around the world. In Porto, the main airborne pollen sampled in spring belongs to trees and Urticaceae. Also, during spring, the pollination period of some weeds begins. In the summer months, the majority of pollen sampled belongs to non-arboreal types with prevalence of Poaceae. In the winter, the presence in the atmosphere of Alnus, Cupressaceae, Fraxinus and Salix pollen was recorded. This seasonality, strongly dependent on several environmental features, is one of the main reasons why airborne monitoring and pollen alert systems are so important. The highest levels of airborne pollen concentration in Porto were attained in the late winter until early spring months (March– April); nonetheless, concentrations were high from February to July. Similar patterns were observed in other cities of Portugal and in Galicia (Spain) (Aira et al. [2011;](#page-10-0) Caeiro et al. [2007](#page-10-0)); however, from south to north, a trend in earlier beginning dates of the pollination period for the different pollen is observed (Caeiro et al. [2007;](#page-10-0) Fernandes et al. [2010\)](#page-10-0). This may have consequences in the long-distance pollen transport between the southernmost regions and the north, resulting in the occurrence of high airborne levels before or/and after the local flowering season (Fern-ández-Rodríguez et al. [2013](#page-11-0); Hernandez-Ceballos et al. [2013](#page-11-0)).

In Porto, it was observed a dominance of the arboreal contribution for the airborne pollen spectrum, with most of the main pollen types identified belonging to this category (around 48 %). This reflects an urbanization trend with the predominance of frequently cared and maintenance gardens by government authorities or homeowners and the plantation of trees, in some cases exotic, for ornamental purposes in city avenues, street-walks and in public parks. Nonetheless, the Urticaceae and Poaceae pollen are still a large proportion of the Porto city airborne pollen spectrum (around 38 %).

In Porto city, the main pollen types identified are similar to those found in other cities of Portugal (Caeiro et al. [2007](#page-10-0)). However, the total concentration of airborne pollen found is much less than the levels reported in the literature for other high urbanized locations. In fact, this is confirmed by the almost insignificant number of days with moderate and high concentration of a given airborne pollen among the 10-year study. This situation was somewhat expected due to the particular geographical position of the city of Porto. This city is facing the Atlantic Ocean and is bordered at south by the Douro River; it presents prevailing winds from the W and NW (seashore) and therefore transporting low or almost null pollen. In fact some regions close to the seashore are reported to have lower annual levels of airborne pollen (Boi and Llorens [2013](#page-10-0); Câmara 2008: Moreno-Grau et al. [1998](#page-11-0)) due to marine perturbed conditions. Nonetheless, the lower levels of pollen in the air could not necessarily imply less risk for allergies. In fact in a study performed in Porto for the elemental characterization of the pollen surface, it was reported that airborne pollen samples acquired an extraneous layer, exhibiting a more than tenfold increase in Na and Cl contents as well a significant increment on Mg contents that are related to the sea spray (Duque et al. [2013](#page-10-0)). These modifications that pollen undergoes in the air could induce changes for instance in the upper respiratory tract membranes ionic equilibrium or permeability facilitating respiratory allergic reactions.

The shorter main pollen seasons of the different pollen types identified were observed among trees, that usually produce a great amount of pollen and a rapidly release in the beginning of the main pollen season, being the maximum airborne concentrations achieved few days later. This characteristic diminishes the exposure period to the allergenic reaction inducer in opposite to the pollen of Poaceae and Urticaceae that is present in the atmosphere for many months. However, the sequence and overlapping of flowering seasons observed from January until July can lead to an exacerbation of allergenic respiratory symptoms. For instance, in a 3-year study performed in Porto, tree airborne pollen concentration and hospital admissions of patients with respiratory disease were highly significant positively correlated (Ribeiro et al. [2009](#page-11-0)).

Analysis of the intradiurnal distribution of pollen showed the existence of different patterns among the studied taxa. It is accepted that the different patterns in intradiurnal pollen distribution could be an indication of the distance between the pollen sources and the trap. But can also be related to specific traits of taxa such as minimum temperature for dehiscence or the existence of taxa having several species, such Poaceae, presenting different anthesis time (Dahl et al. [2013\)](#page-10-0). For anemophilous species, the anthesis and pollen dispersion occur during daylight, particularly during the first morning hours coinciding with a period of thermal inversion, increase in wind velocity, decrease in relative humidity and solar exposure (moisture-desiccation transition) that facilitate anther dehiscence, pollen emission and dispersion (Dahl et al. [2013\)](#page-10-0). This relation was demonstrated by the overall significantly positive correlation between high airborne pollen concentration and warm weather with absence of rain observed in our study.

Therefore, it is expected an increase in airborne pollen in the first morning hours especially coming from pollen released in the vicinity of the pollen trap while the pollen transported from more distant areas would have an irregular pattern. Apart from Salix, all pollen types studied presented an increase in airborne concentration between 9 and 11 a.m. Urticaceae, Cupressaceae, Plantago, Acer and Chenopodiaceae– Amaranthaceae pollen, presenting one well-defined morning peak count, Fraxinus, Populus, Pinus, Quercus and Rumex pollen, with one peak also in the morning, and Platanus pollen, present in similar concentrations along the day, have probably their sources nearer the sampling point. All these species can be found in the vicinity of the sampling site.

Quercus, O. europaea, Poaceae, Pinaceae, Betula and Populus pollen also presented highest airborne concentrations during the first night hours. Also Fraxinus, Salix and Rumex pollen presented a peak during these hours. This increase can be related not only to more distant pollen sources from the sampling site but also to the meteorological conditions associated with the atmospheric boundary layer instability. In the city of Porto, there is an increase in air relative humidity and decrease in wind velocity during the first night hours. This can accelerate the airborne pollen hydration, making them heavier and facilitate its gravitational settling allowing its capture by the sampler. However, in the case of Poaceae, it is well documented the inexistence of a pollen diurnal emission pattern. The different species comprising this family have specific timing of anthesis along the day, even during night (Dahl et al. [2013\)](#page-10-0). The bimodal peak observed in Poaceae was already reported for other regions of Portugal (Caeiro et al. [2013](#page-10-0); Ribeiro et al. [2008\)](#page-11-0).

The significant correlations between the airborne pollen content and the meteorological parameters have been widely reported in aerobiological literature. Meteorological parameters such as temperature, relative humidity and rainfall can have a primordial influence on the main pollen season, affecting the continuous emission and dispersion of pollen grains. This is why the relation pollen weather is the ground base for several forecasting models (Scheifinger et al. [2013\)](#page-11-0). For instance, consecutive rainy days lead to a reduced pollen emission rate and dispersion as well as an extended main pollen season and reduced amount of pollen. In our study, this negative influence of rain on airborne pollen levels was observed with almost pollen types presenting significant correlation coefficients. However, in the absence of rain and with favorable temperatures during the flowering period,

<span id="page-10-0"></span>the number of open flowers with dehiscent anthers at the same time is higher, leading to greater airborne pollen levels as well as a main pollen season with a shorter duration. In Porto, temperature had a positive influence on airborne pollen levels, with maximum temperature presenting, in most of the cases, the highest correlation coefficients. High temperatures favor dehydration and bursting of the anther and can lead also to a synchronous pollen emission (Dahl et al., 2013).

Relative humidity had a negative correlation with airborne pollen levels, being the most influence parameter in more of the half pollen types studied. In fact, lower humidity levels facilitate anther locular fluid disappearance by evaporation (Pacini and Hesse [2004;](#page-11-0) Dahl et al. 2013). However, the relative importance of this process for pollen release can be species dependent (Dahl et al. 2013), and this may justify the lower influence of relative humidity compared with temperature in some of the pollen types studied.

Pérez-Badia et al.  $(2011)$  $(2011)$  reported that the positive influence of wind speed indicates pollen transport from plants growing some distance away from the sampling point. In our study, wind speed presented overall negative or null influence on airborne pollen levels which can indicate that pollen comes from the vicinity of the pollen trap. This negative correlation with wind speed can also indicate that the highest airborne concentrations during the first night hours observed for some pollen types can be more related to the atmospheric boundary layer instability rather than to long-distance transport.

#### 5 Conclusions

A study of the airborne pollen of the Porto city during 10 years (2003–2012) is presented. Throughout the year, total airborne pollen concentration presents some peaks due to the diversity of taxa with different flowering seasons. The most abundant pollen types in the atmosphere were Urticaceae, Platanus and Poaceae. Airborne pollen concentrations start to increase progressively in late December beginning of January, peaking in March but continuing high until July. Interannual and interdiurnal variations in pollen concentrations were observed as well as significant

The airborne pollen content of Porto city is qualitatively similar to other regions of Portugal. However, the differences observed in the main pollen season dates and the pollen amount recorded of the different taxa can have important implications in public health.

meteorological parameters.

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