



Assessment of the potential real pollen related allergenic load on the atmosphere of Porto city

M. Fernández-González^{a,b,*}, H. Ribeiro^{a,c}, J.R.S. Pereira^{a,d}, F.J. Rodríguez-Rajo^b, I. Abreu^{a,d}

^a Earth Sciences Institute (ICT), Pole of the Faculty of Sciences University of Porto, Porto, Portugal

^b Department of Plant Biology and Soil Sciences, University of Vigo, Vigo, Spain

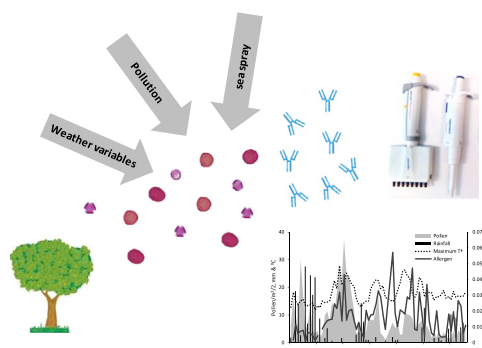
^c Department of Geosciences, Environment and Spatial Plannings, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal

^d Department of Biology of the Faculty of Sciences, University of Porto, Porto, Portugal

HIGHLIGHTS

- This paper informs about the aeroallergen concentrations in Porto city.
- We applied ELISA method to detect Bet v 1, Ole e 1, Lol p 1 and Par j 1/2 allergens.
- We detected high Pollen Allergen Potency with low levels of airborne pollen.
- The episodes of high allergenic risk are higher when we use allergen information.
- The sea air masses can induce higher aeroallergen concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

The knowledge of the allergen content in the atmosphere is a useful tool to establish the risk allergy warnings for the sensitive people. In Portugal the main airborne allergenic pollen come from trees (such as *Betula* or *Olea*), grasses or weeds (mainly *Urticaceae*). The present study sought the quantification of the Bet v 1, Ole e 1, Lol p 1 and Par j 1–2 aeroallergen concentration as well as how weather variables influence in the pollen and allergen concentration in Porto city. Aerobiological study was carried out by a Hirst-type volumetric sampler for pollen collection and a Burkard Cyclone sampler for the aeroallergens. A regression analysis between pollen and allergens was conducted for the identification the allergenic risk days. High Pollen Allergen Potency in the atmosphere was observed considering the low levels of airborne pollen detected. A significant and positive correlation has been obtained between pollen and aeroallergen values with the temperatures whereas the correlation was negative with relative humidity, rainfall and wind speed. Back trajectory methodology was applied in order to analyse the discordances between pollen and allergen maximum concentrations. The analysis showed that when the pollen and allergen peaks were registered on the same day, air masses always comes from the continent. However, when the peaks do not coincide, the air mass comes from the continent in the case of the pollen peak and from the sea for the allergen peak. This behaviour can be a consequence of the high humidity in the air masses from the sea, which can benefit the allergen release from pollen grains. In our study it was observed that the available traditional

* Corresponding author at: Earth Sciences Institute (ICT), Pole of the Faculty of Sciences, University of Porto, Porto, Portugal.
E-mail address: mfgonzalez@uvigo.es (M. Fernández-González).

information for allergenic Type I patients, corresponding to the amount of pollen grains in the bioaerosol, do not accurately identify the real allergenic load in the air.

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1. Introduction

The World Health Organization (WHO) reported that 30–40% of the global population shows allergic sensitization (Pawankar et al., 2011). In Portugal the prevalence of asthma reaches the 6.8% among the general population (Sa-Sousa et al., 2012), being the grass and birch pollen causing more allergy cases, inducing a sensitization rate of 40% of the sensible patients (Asam et al., 2015; Burbach et al., 2009).

The main airborne allergenic pollen types come from anemophilous plants, and in Porto is mostly represented by *Betula*, *Olea*, Poaceae and Urticaceae (mainly *Parietaria*) (Camacho, 2017). *Parietaria* pollen has a long persistence in the atmosphere with two long flowering periods, beginning in early spring and persists into the summer (D'Amato and Liccardi, 1994; D'Amato, 2007). In Portugal, in the northern-central regions and in the islands, *Parietaria* pollen occurs during spring and summer (Camacho, 2015; Todo-Bom et al., 2006; Caeiro et al., 2015) but in Porto city the *Parietaria* pollen is detected from mid-August until mid-September (Caeiro et al., 2014).

Birch represent one of the most important allergenic pollen that causes hay fever and asthma during the spring (Gomes, 2007). *Betula* pollen typically peaks in May in Northern Europe, whereas in the Southern Europe area their maximum pollen concentration mainly takes place in April (Corden et al., 2002). The responsible proteins of reactions to birch pollen belong to the Bet v family. The major allergen is Bet v 1, a 17-kDa protein with membrane binding and permeating capacities (Mogensen et al., 2007).

Olea trees bloom from May to June (Aira et al., 2011; Ribeiro and Abreu, 2014). Nowadays 13 allergens have been characterized in the pollen and fruits of *Olea europaea*; they have been so-called Ole e 1 to Ole e 13, although the last, Ole e 13, is only present in natural olives and does not in pollen (Castro et al., 2015; Esteve et al., 2012; Jiménez-López et al., 2013, 2016; Torres et al., 2014). The most important and prevalent olive allergen is Ole e 1 of 18.5–20 kDa and an IgE binding around the 80% among patients that suffer olive pollinosis (Quiralte et al., 2007).

Poaceae pollen is the most important cause of pollinosis throughout Europe (D'Amato et al., 2007b). Grass pollen is the second most abundant type in the atmosphere of Porto (Abreu et al., 2008) peaking from May to July (Ribeiro and Abreu, 2014) and September in Northern, Central and Eastern Europe (D'Amato et al., 2007b; Puc, 2011). Several genera belonging to the subfamily Pooideae are considered the main source of pollinosis, but the higher allergenic genera are *Lolium*, *Phleum* and *Dactylis*. In *Lolium perenne* there is 7 aeroallergens identified. The major allergen is Lol p 1 (De Linares et al., 2010), an acid protein of 35 kDa with intramolecular disulphide bridges (Johnson and Marsh, 1965), corresponding up to 95% of the prevalence in sensitized people (Kahn and Marsh, 1986). Lol p 1 presents a high cross reactivity with aeroallergens of other Poaceae, such as Phl p 1 of *Phleum* or Dac g 1 of *Dactylis* (Standring et al., 1987).

The Urticaceae pollen is significantly represented in the pollen spectrum of Portugal. *Parietaria*, the main allergic pollen type of the Urticaceae, is commonly found in the countryside and urban areas of the city, growing on walls and soils with a high nitrogen content (Colombo et al., 2003). In the Porto region *Parietaria* pollen is responsible for many cases of severe pollinosis as sensible people showed 34.2% of specific IgE levels to *Parietaria* spp. pollen extracts (Sousa et al., 2014). The most important prevalent allergens are Par j 1 and Par j 2 with 14.7 kDa and 11.3 kDa respectively (Arilla et al., 2006). Both allergens belong to the non-specific lipid transfer protein (LTP) family

(Colombo et al., 1998). This family of proteins has been recently described as a new panallergen with a higher degree of cross-reactivity among vegetable and foods (Asero et al., 2000).

Several aerobiological studies conducted in many countries noticed that the monitoring of pollen and allergen content in the atmosphere could be a useful tool for improving the prevention of respiratory allergic diseases (Marchetti et al., 2017; Plaza et al., 2016a; Vara et al., 2016a). In fact, the combined study of the pollen and pollen related allergenic proteins have been used as a tool to know the potential real allergenic load in the air as several studies reported that the allergy symptoms might arise even at low pollen concentrations (Buters et al., 2012, 2015; Damialis et al., 2019; Fernández-González et al., 2010; Pei-Chih et al., 2019). In this way, aeroallergens can be also found as “free allergens” in the atmosphere (Wang et al., 2011).

The aeroallergen concentration in a given place depend on the presence and abundance of any anemophilous plant and their ability to produce pollen grains as well as other variables, such as the climatic conditions or the urbanization levels which can induce an increase of the pollen allergen potency (D'Amato et al., 2007b; Peden and Reed, 2010).

So, the aim of our study was to combine the traditional pollen information data with the aeroallergen content in the atmosphere of Porto in order to ascertain the real allergenic load in the atmosphere and the amount of days with potential allergy risk episodes for *Betula*, *Olea*, Poaceae and Urticaceae. The airborne pollen concentration of these taxa as well as its major aeroallergens, Bet v 1, Ole e 1, Lol p1 and Par j 1–2, were monitored. The influence of the weather-related variables in the airborne allergen and pollen concentrations, as well as the contribution of air masses in the highest peak concentrations were also assessed. This information can be used as a useful tool to prevent pollinosis in sensitized patients to different pollen types.

2. Material and methods

The present study was carried out in Porto (41°9'7, 20°N, 8°38'13, 20°W) (Portugal) during 2018. Porto is the second largest Portuguese city, surrounded by the Atlantic Ocean and the Douro River, with a total annual rainfall ranging between 1000 and 1200 mm, relative humidity between 75 and 80% and annual average temperature around 15 °C (Miranda et al., 2001). In the year 2018 mean annual temperature was of 15.7 °C, mean relative humidity of 78.7% and total rainfall of 1069 mm. During the study period, the higher maximum temperature was registered on 18th June (34.0 °C) coinciding with the day with lowest relative humidity 47.1% on 19th June. On the other hand, high rainfall levels were registered on the first study days (from 31 March to 10 April) and so the wind speed (3 April). These values were accompanied with high relative humidity percentages, although the highest value was registered on 10th June coinciding with the highest rainfall (7.16 mm) (Fig. 1).

The aerobiological study to monitor pollen and allergens was carried out by means of two volumetric traps placed on the roof top of the Faculty of Sciences of the University of Porto building. For the pollen sampling we used a Lanzoni (Lanzoni s.r.l.) volumetric 2000 Hirst type device (Hirst, 1952) following the method of the Spanish Aerobiology Network (Galán et al., 2007). Pollen data were expressed as pollen grains for total pollen or annual pollen integral (pollen per cubic meter of air) when referring to daily mean values (Galán et al., 2017). Aeroallergen content was sampled using a Multi Vial Cyclone Sampler (Burkard Manufacturing Co Ltd. Hertfordshire, UK). The proteins were

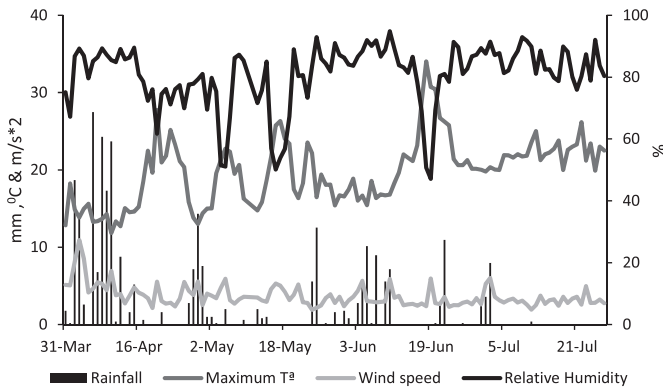


Fig. 1. Meteorological variables, rainfall (black bars), maximum temperature, wind speed and rainfall (lines) during the study period.

collected directly into a 1.5 mL Eppendorf (dry condition) vial during the flowering period of each taxa and stored at -20°C until use. The daily samples were quantified by means an Enzyme-Linked Immuno-Sorbent Assay (ELISA) following the protocol proposed by Takahashi et al. (2001) and Moreno-Grau et al. (2006). The samples were extracted with 120 μL buffer for 2 h, centrifuged at 4000 rpm during 10 min. to separate the particulate matter, and stored at -20°C . The proteins were quantified using specific 2-site antibody ELISA as previously described (Arilla et al., 2002, 2006). Microtiter plates were coated with monoclonal antibody over night at 4°C . The plates were blocked with PBS (pH 7.4), containing 1% bovine serum albumin and 0.05% Tween 20. Next, we incubated the plates with natural antibody allergens, or extracted airborne samples. Then we add a secondary biotinylated antibody, and 0.25 $\mu\text{g}/\text{mL}$ streptavidin-peroxidase (Sigma). All incubations were performed at 37°C for 1 h, and we make 3 washes with PBS-T between successive steps. Finally, the plates were incubated at room temperature in the dark with *o*-phenylenediamine (OPD) (Sigma), and stopped the reaction at 30 min. with H_2SO_4 (3 M) and measure the absorbance at 492 nm. The extracts were assayed as dilution series, and the daily concentrations were interpolated from the linear portion of the standard curve in ng/m^3 of air.

The main pollen season (MPS) was determined using a pollen emission model (Cunha et al., 2003; Ribeiro et al., 2007) which fits a non-linear logistic regression model to the values of the accumulated sum of daily airborne pollen concentration sampled along a year (Cunha et al., 2003). The Levenberg–Marquardt method was used to fit the model by applying the “regression non-linear” function of the software IBM SPSS Statistics 23.0. A one-sided *t*-test was used at the 5% for Poaceae and Urticaceae and 10% for *Betula* and *Olea* level to delimit the MPS begin and end dates.

Following the classification suggested by the REA (Galán et al., 2007) we categorize the pollen concentrations in to four groups (Table 1).

A categorization of the aeroallergens thresholds was proposed in this work, which corresponds with the aforementioned pollen thresholds, by using a regression equation estimated correlating the daily allergen load vs pollen data.

Table 1
Pollen concentration classes in the four groups proposed by the REA (Galán et al., 2007).

Pollen type	Groups	Pollen-count classes (grains/ m^3)		
		Low	Moderate	High
<i>Betula</i>	3	1–30	31–50	>50
<i>Olea</i>	4	1–50	51–200	>200
Poaceae	2	1–25	26–50	>50
Urticaceae	1	1–15	16–30	>30

Meteorological data (maximum, minimum, average temperatures, rainfall and relative humidity) were continuously monitored by the meteorological station of the Faculty of Sciences of the University of Porto, located 7 m apart from the pollen and allergen samplers and at the same height.

Statistical analysis was performed by means of a non-parametric Spearman's correlation test, because the data did not adjust to a normal distribution, in order to analyse the relationship between daily pollen and allergen concentrations and main meteorological variables. The software IBM SPSS Statistics 23.0 was used for the statistical analysis.

Furthermore, daily pollen and allergen peak episodes have been studied by means the application of back trajectory analysis. The Hybrid Single Particle Lagrangian Integrated Trajectory model (HYSPLIT) developed by the National Oceanic and Atmospheric Administration (NOAA) was considered. These models allow us to determine hourly 24 h back trajectories in 3D (latitude, longitude and elevation) using the GDAS (Global Data Analysis System) meteorological files. Following this model (Rolph, 2016) the isentropic back-trajectories of air masses arriving in Porto at 20:00 UTC were calculated during the peak episodes of pollen and allergen concentrations. The backward trajectories were calculated for different heights from the earth surface: 300, 500 and 750 m (Plaza et al., 2016a, 2016b; Moreno-Grau et al., 2016; Izquierdo et al., 2017).

3. Results

The *Betula* flowering took place during April and early May in Porto, with a pollen season length of 19 days. During the main pollen season (MPS) a total of 518 *Betula* pollen grains were registered and a pollen peak observed on April 24th with 167 pollen/ m^3 . Likewise, the total amount of Bet v1 allergen was 4.333 ng with an allergen peak of 1.506 ng/m^3 on the same day than the pollen peak (April 24th). Both maximum peaks, pollen and allergen, coincide with a period of rainfall absence and an increase of maximum temperature (Table 2, Fig. 2).

The *Olea* flowering took place during June with a length of 17 days. The total airborne pollen was 220 pollen grains with a maximum daily pollen peak in June 19th with 57 pollen/ m^3 . The annual Ole e1 integral amount was 0.868 ng with its maximum daily peak of 0.141 ng/m^3 reached in the same day of the pollen one. Both peaks coincide with atmospheric conditions of maximum temperature increase and lack of rainfall (Table 2, Fig. 2).

The main pollen season of the Poaceae took place from June to July with a length of 56 days. The total Poaceae pollen amount was 466 pollen grains with a pollen peak of 36 pollen/ m^3 registered on July 6th. The results of analysis showed the presence of Lol p1 aeroallergen during the whole Poaceae pollination period. The annual allergen integral

Table 2

Date of the start and end of the main pollen season (MPS), length of the main pollen season (MPS) (days), pollen during the MPS (total pollen (pollen), pollen peak (pollen/ m^3)), date of the pollen peak (day) and allergen during the main pollen season (total allergen (ng), allergen peak (ng/ m^3)), date of the allergen peak (day). Pollen Allergen Potency (AP) (ng/pollen).

	<i>Betula</i>	<i>Olea</i>	Poaceae	Urticaceae
MPS start (day)	17-Apr	12-Jun	2-Jun	31-Mar
MPS end (day)	5-May	28-Jun	27-Jul	11-Jun
MPS lenght (days)	19	17	56	71
Mean (pollen/ m^3)	14	8	6	11
Peak (pollen/ m^3)	167	57	36	75
Peak date	24-Apr	19-Jun	6-Jul	22-Apr
Pollen (pollen)	518	220	466	1181
	Bet v1	Ole e1	Lol p1	Par j1–2
Mean (ng/ m^3)	0.155	0.031	0.128	0.017
Peak (ng/ m^3)	1.506	0.141	0.781	0.057
Peak date	24-Apr	19-Jun	1-Jul	12-May
Allergen (ng)	4.333	0.868	6.782	1.065
Pollen allergen potency (ng/pollen)	0.008	0.004	0.015	0.001

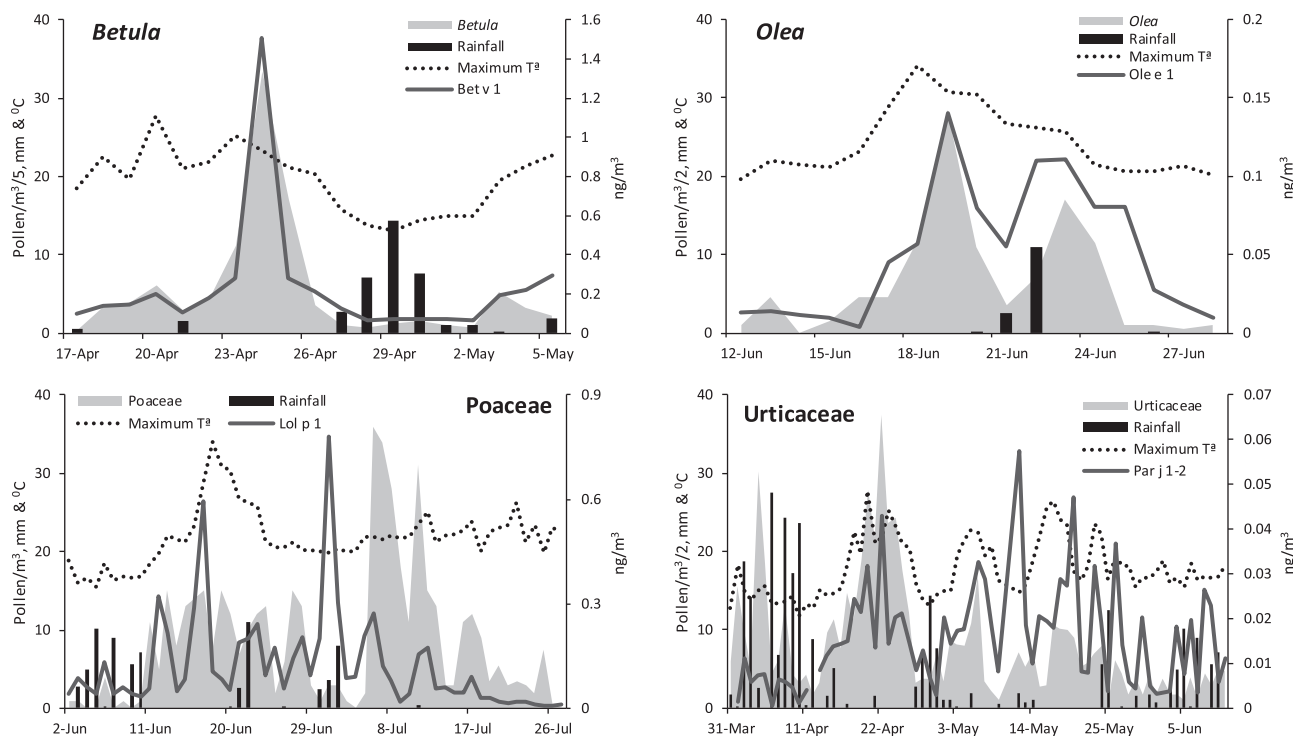


Fig. 2. Pollen grains concentrations (grey area), allergen concentrations (grey line), maximum temperature (black line) and rainfall (black bars) for *Betula*, *Olea*, Poaceae and Urticaceae during the main pollen season.

amount was 6.782 ng and a Lol p 1 daily peak of 0.781 ng/m³ was registered on July 1st, five days before the pollen peak. The aeroallergen peak coincides with rainfall and a decrease of the maximum temperature (Table 2, Fig. 2).

Urticaceae airborne pollen showed an annual pollen integral of 1181 pollen grains with a length of 71 days. The maximum daily airborne pollen in the air was reached on April 22th with 75 pollen/m³. The total amount of Par j 1–2 was 1.065 ng, and the allergen peak was observed on May 12th with 0.057 ng/m³, eighteen days before the pollen peak. The Par j 1–2 peak coincides with rainfall presence and a high decrease in maximum temperatures (Table 2, Fig. 2).

In addition, the Pollen Allergen Potency (AP) for each taxa, considered as the relationship between pollen and allergen concentrations, was calculated. The values varied between the taxa, the highest AP value was reached for Poaceae with 0.015 ng/pollen, following by *Betula* (0.008 ng/pollen) and *Olea* (0.004 ng/pollen), whereas the lower AP value was observed for Urticaceae with 0.001 ng/pollen (Table 2).

To know the number of days under potential risk of allergy in the Porto city, the threshold values established by the REA were considered. A regression equation was estimated (Fig. 3) in order to calculate the aeroallergens thresholds in the atmosphere considering the categorized pollen threshold values (Table 3). The adjusted R² values of the developed equations ranged from 0.92 for *Betula* to 0.18 for Poaceae. During the study period, 1 day with moderate potential risk and 3 days with high potential risk of allergy for the *Betula* pollen was observed whereas 3 days with moderate potential risk and 1 day with high sensitization potential risk by the Bet v 1 allergen concentrations were detected (Table 3, Fig. 3). *Olea* only showed one day with moderate potential risk of allergy when the pollen data were considered, even though the thresholds obtained for the Ole e 1 allergen noted 6 days of moderate potential risk of allergy and 3 days with high potential risk during the study period (Table 3, Fig. 3). Some discordances were also registered in the case of the grasses as the number of days with potential moderate risk of Poaceae pollen allergy were 4 (Table 3, Fig. 3). The days with moderate potential risk considering the threshold obtained for the Lol

p 1 allergen raised to 8 or to 6 days with moderate or high allergenic risk of sensitization (Table 3, Fig. 3). Finally, for the Urticaceae pollen, the observed days with potential moderate and high allergenic risk were 17 and 11, respectively. When the threshold for the allergen (Par j 1–2) was considered only 6 days with potential moderate risk were observed and the number of days with potential high risk increased to 24 (Table 3, Fig. 3).

In order to know the influence of the main weather variables into the pollen and allergen atmospheric content a Spearman correlation test was applied. A significant positive correlation ($p < 0.01$) was observed between airborne pollen and allergen concentrations of the studied taxa and the average, maximum and minimum temperatures. The strongest correlation was recorded for Bet v1 vs maximum temperature ($R^2 = 0.821$, $p < 0.01$). In general the best correlations were observed between the allergen or the pollen and average temperature. Otherwise, a negative significant correlation between pollen and allergen concentrations and rainfall, relative humidity and wind speed was observed for the most studied taxa. The high significant negative correlation was registered between *Betula* pollen or Bet v 1 allergen and the rainfall of the same day and the day before (Table 4).

The discordances observed in the maximum peak pollen and allergen concentrations were analyzed by means of a back trajectory analysis. The interpretation of the analysis showed that the most important contributors of *Betula* pollen and Bet v1 allergens in the atmosphere of Porto are continental air masses. The *Olea* and Ole e 1 peaks are coincident with maritime air masses. In the case of Poaceae/Lol p 1 and Urticaceae/Par j1–2, for which the allergen and pollen maximum values are no coincident, a trend towards a pollen peak conditioned by continental air masses or an allergen peak coincident with maritime air masses presence was detected (Fig. 4).

4. Discussion

The major prevalence of pollen related respiratory allergies usually occurs during spring and early summer in Europe (D'Amato et al.,

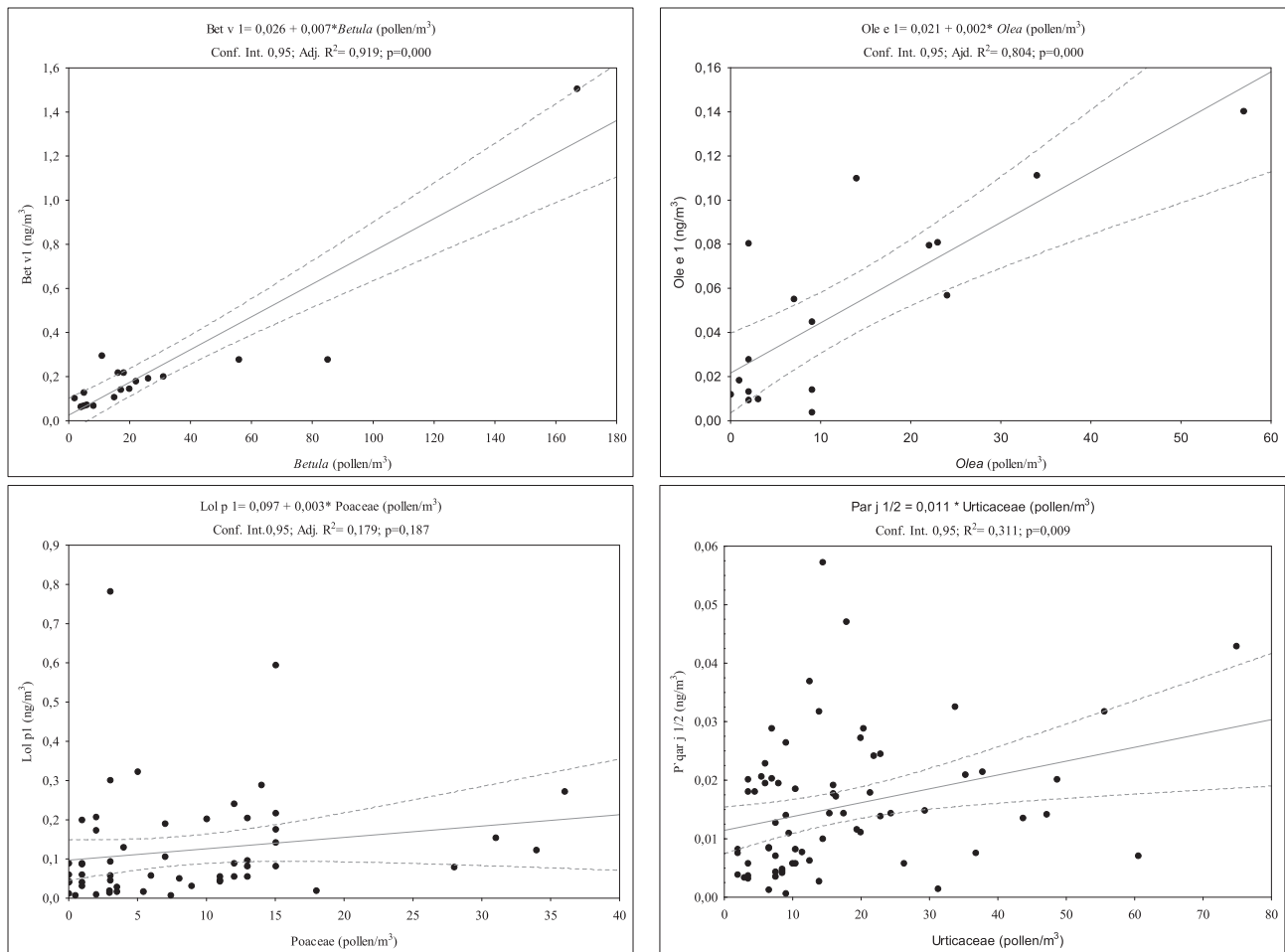


Fig. 3. Pollen concentrations (pollen/m³) versus allergen values (ng/m³) during the main pollen season for each taxa.

2015). In a long-term aerobiological study performed in Porto, it was reported that 50% of the total annual integral of airborne pollen sampled during this period belongs to *Betula*, *Olea*, *Urticaceae* and *Poaceae*, this last being most predominant during the summer (Ribeiro and Abreu, 2014). These data emphasizes the important contribution of the taxa monitored in our study to the allergenic load potency in the Porto atmosphere.

Our results showed short MPS for *Betula* and *Olea* (less than one month), within the average length reported by Ribeiro and Abreu (2014) for the same area. The MPS length, is closely dependent with the phenology and on the number of species representing in a pollen type in a specific region as well as the meteorological conditions during the flowering period. In Porto city birch is mainly represented by *Betula pendula* and therefore the MPS length tends to be shorter by comparison

with nearby areas of Spain or Italy (Perugia), where there is reported the presence of several different species of *Betula* (Jato et al., 2013; Rodríguez-Rajo et al., 2011a). In the case of *Olea*, larger MPS was observed in southern European areas (43–95 days), which may be related with the existence of super-intensive olive groves releasing high amounts of pollen (Galera et al., 2018; Puljak et al., 2016; Rojo et al., 2016; Ruiz-Valenzuela and Aguilera, 2018) that contribute to mid or long distance transport and therefore longer pollen residence times in the atmosphere (Fernández-Rodríguez et al., 2014; Hernández-Ceballos et al., 2014; Moreno-Grau et al., 2016).

Poaceae and Urticaceae were reported to account around 38% of the annual pollen spectrum of Porto (Ribeiro and Abreu, 2014). The Poaceae family comprise several species, however, during our study the length of its MPS was slightly shorter than the registered in Vienna, Berlin or Turku (Kmenta et al., 2016, 2017) or other Southern European places (Puljak et al., 2016; Rojo et al., 2016; Cebrino et al., 2018; Ruiz-Valenzuela and Aguilera, 2018; Tormo-Molina et al., 2010).

Traditionally, lower total annual pollen is observed for the studied taxa in the city of Porto (Ribeiro and Abreu, 2014; Caeiro et al., 2017) when compared with nearby Mediterranean areas (Aira et al., 2011; Jato et al., 2010, 2013; Moreno-Grau et al., 2016; Plaza et al., 2016a; Rodríguez-Rajo et al., 2011a, 2011b). The 2018 pollen behaviour was no exception. The Porto city location close to the Ocean and Douro River can justify the low airborne pollen concentration but this may not imply a similar behaviour for the aeroallergen content. High concentrations of pollen are usually related with higher total levels of airborne allergens (Bastl et al., 2016; Rodríguez-Rajo et al., 2011b). Although it has been frequently reported in the literature the occurrence of years

Table 3

Pollen and allergen Thresholds and risk days during the study period.

		Risk (days)		Thresholds (pollen/m ³ or ng/m ³)		
		Moderate	High	Low	Moderate	High
Pollen	<i>Betula</i>	1	3	0–30	31–50	>50
	<i>Olea</i>	1	0	0–50	51–200	>200
	<i>Poaceae</i>	4	0	0–25	26–50	>50
	<i>Urticaceae</i>	17	11	0–15	16–30	>30
Allergen	Bet v1	3	1	0–0.230	0.240–0.380	>0.380
	Ole e1	6	3	0–0.027	0.028–0.088	>0.088
	Lol p1	8	6	0–0.160	0.175–0.250	>0.250
	Par j1–2	6	24	0–0.013	0.014–0.018	>0.018

Table 4

Spearman correlations between pollen or allergen and the main meteorological variables.

	Bet v1		<i>Betula</i>		Ole e1		<i>Olea</i>		Lol p1		Poaceae		Par j1–2		Urticaceae	
	R ²	p	R ²	p	R ²	p	R ²	p	R ²	p	R ²	p	R ²	p	R ²	p
Allergen			0.801**	0.000			0.668**	0.003	1.000		0.372**	0.005	1.000		0.350**	0.003
Allergen_1	0.678**	0.002	0.415	0.087	0.715**	0.002	0.334	0.206	0.645**	0.000	0.321*	0.017	0.320**	0.008	0.242*	0.047
Allergen_2	0.250	0.333	0.099	0.704	0.368	0.177	−0.156	0.579	0.392**	0.003	0.195	0.158	0.299*	0.015	0.203	0.099
Allergen_3	−0.138	0.610	−0.057	0.833	−0.007	0.982	−0.555*	0.040	0.365**	0.007	0.184	0.188	0.406**	0.001	0.149	0.234
Pollen	0.801**	0.000			0.668**	0.003			0.372**	0.005	1.000		0.350**	0.003	1.000	
Pollen_1	0.628**	0.005	0.512*	0.030	0.737**	0.001	0.527*	0.036	0.175	0.202	0.628**	0.000	0.212	0.081	0.582**	0.000
Pollen_2	0.341	0.180	0.152	0.559	0.532*	0.041	0.181	0.519	0.039	0.782	0.400**	0.003	0.132	0.285	0.448**	0.000
Pollen_3	−0.047	0.862	−0.014	0.959	0.296	0.304	−0.140	0.633	0.054	0.699	0.287*	0.038	0.127	0.307	0.379**	0.001
Tmax	0.821**	0.000	0.738**	0.000	0.277	0.282	0.057	0.827	0.076	0.579	0.487**	0.000	0.602**	0.000	0.355**	0.002
Tmax_1	0.651**	0.003	0.601**	0.008	0.366	0.164	0.105	0.699	−0.070	0.610	0.504**	0.000	0.466**	0.000	0.190	0.116
Tmax_2	0.512*	0.036	0.550*	0.022	0.356	0.193	0.327	0.234	−0.080	0.565	0.308*	0.023	0.467**	0.000	0.114	0.349
Tmax_3	0.274	0.305	0.303	0.253	0.474	0.087	0.132	0.652	−0.125	0.371	0.190	0.173	0.293*	0.016	0.084	0.497
Tmin	0.718**	0.001	0.635**	0.004	0.502*	0.040	0.734**	0.001	−0.078	0.570	0.371**	0.005	0.416**	0.000	0.102	0.396
Tmin_1	0.688**	0.002	0.630**	0.005	0.682**	0.004	0.654**	0.006	−0.077	0.577	0.355**	0.008	0.386**	0.001	0.053	0.664
Tmin_2	0.603*	0.010	0.526*	0.030	0.718**	0.003	0.685**	0.005	−0.116	0.404	0.229	0.096	0.274*	0.024	−0.030	0.804
Tmin_3	0.353	0.180	0.392	0.133	0.626*	0.017	0.318	0.267	−0.084	0.550	0.121	0.389	0.089	0.474	−0.119	0.335
Tavg	0.784**	0.000	0.720**	0.001	0.782**	0.000	0.659**	0.004	0.002	0.990	0.406**	0.002	0.573**	0.000	0.296*	0.012
Tavg_1	0.690**	0.002	0.656**	0.003	0.750**	0.001	0.435	0.092	−0.096	0.486	0.423**	0.001	0.490**	0.000	0.197	0.102
Tavg_2	0.605*	0.010	0.628**	0.007	0.389	0.152	−0.029	0.919	−0.120	0.387	0.254	0.064	0.411**	0.001	0.115	0.347
Tavg_3	0.362	0.169	0.383	0.143	0.279	0.334	−0.270	0.351	−0.205	0.141	0.138	0.324	0.239	0.051	0.004	0.977
RH	−0.588**	0.008	−0.401	0.089	0.738**	0.001	0.753**	0.000	−0.133	0.328	−0.220	0.103	−0.426**	0.000	−0.458**	0.000
RH_1	−0.294	0.236	−0.022	0.932	0.829**	0.000	0.666**	0.005	−0.034	0.805	−0.361**	0.007	−0.344**	0.004	−0.440**	0.000
RH_2	−0.309	0.228	−0.459	0.064	0.675**	0.006	0.364	0.182	0.041	0.771	−0.129	0.351	−0.398**	0.001	−0.300*	0.012
RH_3	−0.229	0.393	−0.222	0.408	0.503	0.067	−0.013	0.964	0.130	0.354	−0.062	0.661	−0.294*	0.016	−0.249*	0.041
Rain	−0.652**	0.002	−0.745**	0.000	−0.297	0.248	−0.534*	0.027	0.133	0.329	−0.345**	0.009	−0.558**	0.000	−0.280*	0.018
Rain_1	−0.686**	0.002	−0.533*	0.023	−0.506*	0.046	−0.615*	0.011	0.211	0.122	−0.214	0.117	−0.298*	0.013	−0.177	0.143
Rain_2	−0.369	0.145	−0.372	0.141	−0.571*	0.026	−0.565*	0.028	0.157	0.255	−0.233	0.090	−0.329**	0.006	−0.109	0.374
Rain_3	−0.116	0.668	−0.132	0.625	−0.662**	0.010	−0.446	0.110	0.200	0.151	−0.387**	0.004	−0.256*	0.036	−0.157	0.200
Ws	−0.033	0.892	−0.393	0.096	0.081	0.758	−0.174	0.503	−0.183	0.177	−0.296*	0.027	−0.211	0.082	0.051	0.675
Ws_1	−0.476*	0.046	−0.658**	0.003	−0.197	0.464	0.006	0.983	−0.098	0.475	−0.350**	0.009	−0.325**	0.006	0.148	0.221
Ws_2	−0.375	0.138	−0.260	0.313	0.000	1.000	−0.007	0.980	−0.045	0.744	−0.195	0.158	0.025	0.838	0.107	0.382
Ws_3	−0.226	0.399	−0.267	0.318	0.319	0.267	0.192	0.510	−0.078	0.580	−0.238	0.087	−0.150	0.225	0.105	0.392

* $p < 0.05$.** $p < 0.01$.

with high pollen values but low allergen concentrations and vice-versa (Galán et al., 2013; Buters et al., 2012; Moreno-Grau et al., 2016; Plaza et al., 2016b).

Several studies point discordances between the date of pollen and aeroallergen appearance in the atmosphere (D'Amato et al., 2008; Rodríguez-Rajo et al., 2011b; Vara et al., 2016b). In our study for both trees, the timing of the pollen and allergen peaks coincide, corresponding to an increase of maximum temperature and absence of rainfall. But for the weeds the pollen and allergen peaks were observed in different dates. In the case of the allergens, the peak coincides with a decrease in maximum temperature and the presence of precipitations as well as high relative humidity. Several authors noted that the dispersion of aeroallergens can be related with high temperatures and rainfall episodes or humidity increase during the previous days (D'Amato et al., 2007a; Fernández-González et al., 2011; Grote et al., 2003; Knox, 1993). After short periods of rainfall, pollen is deposited on different surfaces, and under wet conditions can suffer hydration inducing the release of the allergenic proteins to the atmosphere (Moreno-Grau et al., 2006).

The categorization of the aeroallergens thresholds based on the pollen thresholds of allergenic risk for *Betula*, *Olea*, Poaceae and Urticaceae (Galán et al., 2007), and estimated correlating the daily allergen load vs pollen data, showed that the days of aeroallergen allergenic risk do not coincide with the registered for the airborne pollen thresholds. For Urticaceae, in only 6 days possible high risk of allergies coincide for airborne pollen and aeroallergens, which makes 29 days of possible potential allergy risk either attributed to pollen or aeroallergens alone. Also there was observed the appearance of a first possible moderate aeroallergen risk event detected 3 and 24 days before the first pollen peak for *Olea* and Poaceae respectively, while for *Betula* and Urticaceae were observed 3 and 15 days after the first pollen peak. These results

show the importance of the combination of pollen and aeroallergen quantification to know the total allergenic load in order to improve the allergenic risk alert models for the sensitized people.

The correlation analysis between pollen or aeroallergen concentrations with the main meteorological parameters varies with the taxa. Generally, high positive spearman correlation coefficient was observed between pollen/allergen and temperature, but negative with relative humidity, rainfall and wind speed, with the exception of *Olea* pollen and the Ole e 1 allergens for which the correlation was positive with the relative humidity. Similar findings are reported by other authors (De Linares et al., 2010; Plaza et al., 2016b), being observed that the weather conditions directly affect pollen release but the aeroallergen presence can be modulated namely by humidity, atmospheric pollutants (Singer et al., 2005).

A backward trajectory analysis was conducted to obtain a deeper understanding about the discordances between the pollen and allergen timing in the atmosphere. The retro-trajectories during the maximum allergen and pollen peak episodes were calculated. Our findings showed that when the pollen peak matches with the allergen peak the air mass always comes from the continent. However, when the pollen and allergen peaks do not coincide, the air mass comes from the continent during pollen peaks and from the sea in the allergen peak days. Additionally, when the air comes from the sea the level of pollen decreases and the allergen values increase. This fact might indicate that aeroallergen concentration can be favoured by the sea breeze. These air masses are more humid and transport sea spray particles rich in salts that can facilitate allergens release. Also, Solomon et al. (1983) described the intense expulsion of proteins resulting from the osmotic pressure caused by humidity increase and the subsequent strong environmental evaporation during the following hours that lead to a rapid atmospheric dispersion of submicronic particles. Less pollen concentration and higher allergen

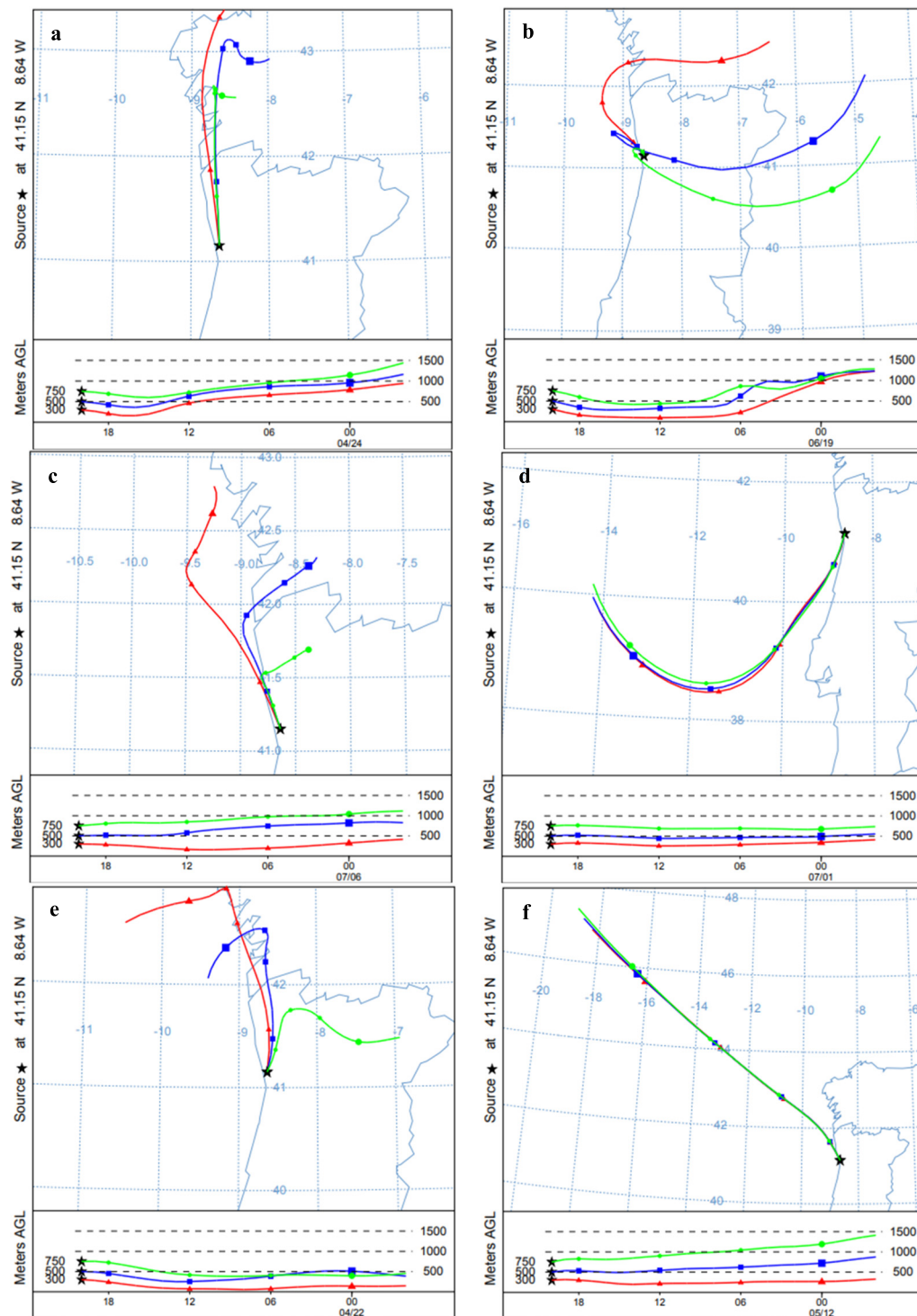


Fig. 4. Maps showing 24-h HYSPLIT backward trajectories at a final height of 300, 500 and 750 m agl at considered episodes: a) *Betula* and *Bet v 1* peak days 24th April; b) *Olea* and *Ole e 1* peak days 19th June; c) *Poaceae* peak day 6th July; d) *Lol p 1* peak day 1st July; e) *Urticaceae* peak day 22th April; f) *Par j 1/2* peak day 12th May.

levels in coastal areas than in inland cities have been verified by others authors (Jato et al., 2010; Rodríguez-Rajo et al., 2010).

5. Conclusions

Our study showed the presence of High Pollen Allergen Potency from the main important allergenic pollen in the atmosphere of Porto, in spite of low levels of airborne pollen. Using only the pollen data,

episodes of high allergenic risk of pollinosis were underestimated when compared with the information about aeroallergens. Only the combination of both information, pollen and allergens data, allow us to know the total number of possible risk days for the sensitized people.

Some discordances between the dynamic of airborne pollen and aeroallergens were also detected. In the case of the trees, both pollen and allergen maximum peaks coincide. For *Poaceae*/*Lol p 1* and *Urticaceae*/*Par j1–2*, the allergen and pollen maximum values are no

coincident as a trend towards a pollen peak conditioned by continental air masses and an allergen peak coincident with maritime air masses presence was detected.

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