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Tree Allergen Pollen-Related Content as Pollution Source in the City of Ourense (NW Spain)

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Received: 21 September 2020; Accepted: 22 October 2020; Published: 23 October 2020

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Abstract: Allergies became a major public health problem, identified as an important global pandemic with a considerable impact on the worldwide economy. In addition, a higher prevalence of pollen Type I sensitization cases in urban environments in comparison with the rural territories was detected. Our survey sought to assess the main biological pollution episodes caused by the aeroallergens of the major allergenic tree species in urban environments. A Hirst-type volumetric device was used for pollen sampling and a Burkard Cyclone sampler for the detection of tree atmospheric allergens over two years. The main allergens of *Alnus*, *Fraxinus*, *Betula*, *Platanus* and *Olea*, were detected in the atmosphere. Three peaks of important pollen concentrations were recorded throughout the year. The developed regression equations between pollen counts and allergen proteins registered great R^2 values. The number of days with probability of allergenic symptoms was higher when the pollen and allergen data were assessed altogether. *Fraxinus* allergens in the atmosphere were detected using Ole e 1 antibodies and the Aln g 1 allergens with Bet v 1 antibodies, demonstrating the cross-reaction processes between the principal allergenic proteins of the Oleaceae and Betulaceae families. Long Distance Transport processes (LDT) showed that pollen from *Betula* populations located in mountainous areas increased the secondary peaks of pollen and allergen concentrations, and air masses from extensive olive orchards of North-Eastern Portugal triggered the highest concentrations in the atmosphere of *Olea* pollen and Ole e 1 allergens.

Keywords: aeroallergens; tree; pollen; ELISA; allergy hazard days

1. Introduction

An ongoing global intensification of the incidence of pollen allergy diseases over the last half century was observed [1]. Allergies became a major public health problem in the urban atmosphere of the industrialized and emergent countries, recognized as an important global pandemic with a considerable impact on the worldwide economy [1,2]. In Europe, it was estimated that 20% of the citizens suffer from pollen Type I sensitive reactions [3], which increased in the most developed countries with incidences above 30% [4,5]. In addition, a higher prevalence of pollen-related sensitization cases in urban environments in comparison with rural spaces was detected [6,7]. Among the possible causes that have enlarged the allergenic content in the air of the urbans areas, the "heat island effect" of the cities prompts an increase of plants' pollen production and shifts pollen seasons to an earlier onset and lengthier durations [8]. Furthermore, urban atmospheric chemical contaminants favor a higher vegetal biomass growth and the increase of the allergenic protein content on pollen grains [9–11]. The greater occurrence and intensity of pollen allergic symptomatology in hypersensitized people during recent years was related with the increase of pollen production by plants [1,10]. Additionally, this situation is aggravated by the allergy problems derived from an inappropriate ornamental vegetation planning and design of green areas in cities [12]. The ornamental flora planted at gardens, parks or streets present a low biodiversity with a massive use of few species from the same families, mainly Oleaceae and Betulaceae in the case of Ourense, which can lead to cross-reaction processes between their allergens in sensitized people [12,13]. Besides the abovementioned, the assessment of pollen emissions from forestry or cultivations surrounding the urban environment should be also considered [14].

The most important allergenic taxa of the Ourense city, which constitute the 50% of the total of airborne pollen, are arboreal species from *Alnus*, *Fraxinus*, *Platanus*, *Betula* and *Olea* genus, which flower in winter and spring. *Alnus glutinosa* (L.) Gaertner is a widely represented tree in the riverside forests of Northern Spain. The pollen from alder causes the first occurrence of allergy symptoms along the year due to their major allergen Aln g 1 [15]. *Alnus* pollen type has been reported to be one of the main causes of pollinosis in Central and Northern Europe [16], with an increased rate of sensitization against their main allergen Aln g 1 during recent years [17]. In Northwestern Spain between 9% and 20% of hay fever sufferers are allergic to *Alnus* pollen [18]. *Fraxinus angustifolia* Vahl belongs to the Oleaceae family and is a tree largely extended in the North-Western Spain as natural species of river-bank vegetation [19] and as ornamental plants in green urban spaces [20]. In temperate zones of North and Central Europe the sensitization to ash pollen is a recognized problem, even more important than birch in some areas [21]. Around 18–34% of allergy sensitization rates can be attributed to the ash tree in Central Europe [22]. Fra e 1 is the main allergen for the ash sensible people [23]. *Platanus hispanica* Miller ex Münchh is the main source of the low biodiversity in urban areas since it is broadly planted as ornamental species in urban green areas and streets of the South European cities [24]. The plane pollen allergenic capacity has been attributed to the expression of their major allergen Pla a 1 [24]. Plane trees have an important allergological interest in Central and Southern European cities [16,25,26]. In Spain the prevalence of positive skin pick test varies from the 8–9% sensitized people in the Northern cities [27] to the 52–56% in Madrid [28]. Pollen from *Betula pendula* Roth represents one of the main sources of spring hay fever and asthma in Europe [29]. A study conducted in the Northwestern Spain noted that of 41.89% patients with a positive SPT (skin-prick-test) for *Betula alba* L. allergens, 10.75% were monosensitized [28]. Increases in allergen-specific therapy demand have been observed in years with *Betula* pollen in high concentrations [30]. The principal allergen of the birch pollen is the Bet v 1 protein [31]. In addition, it is demonstrated that people suffering oral allergy syndrome (OAS) showed oral symptoms before *Betula* pollinosis symptoms [32]. A large number of children are polysensitized to birch, ash and grass pollen in central Europe [33]. Important rates of olive pollen sensitization up to 29.7% of the allergic patients are recorded along the Mediterranean basin [34]. The northern limit of the olive tree distribution in the Iberian Peninsula is the Eurosiberian bioclimatic region where only 8% of the pollinosis people show positive effects to *Olea* pollen [35]. Common olive group 1 was the major allergen with 16-kDa [36]. The urban allergic population to the *Betula* and *Olea* pollen could display allergy responses in the winter months (as consequence of ash and alder pollen allergens), in the early spring because the Bet v 1 allergens or late spring due to Ole e 1 tree pollen related allergens.

The pollen concentration in the aerosol and their time-based sequence are the most traditional information offered for pollinosis patients [37]. Nevertheless, mismatches between the symptoms' appearance and the period of pollen presence in the atmosphere were detected in recent years at different regions [38]. Allergy symptoms can be triggered even at low pollen concentrations, so that several investigations highlighted the assessment of pollen and aeroallergens as a necessary instrument to establish the actual airborne allergenic load [10,39].

Our study sought to evaluate the tree-related aeroallergen content as a source of pollution in urban atmospheres with the aim to assess the real load of allergens in the air and the causes of potential allergenic hazard episodes for sensitized people.

2. Materials and Methods

The research was conducted in the city of Ourense located in the North-Western part of the Iberian Peninsula, an altitude of 454 m a.s.l. and a geographical location 42°20′ N–7°52′ W. The climate of this area is described as Oceanic with Mediterranean features, with an annual average temperature of 14 °C and a total precipitation of 772 mm in a year [40].

Aerobiological sampling of tree pollen and allergens during the years 2017 and 2018 was conducted using two volumetric traps placed on the roof of the Science Faculty building, approximately at 15 m above the ground level and near to the town center. Pollen was monitored using a Hirst-type Lanzoni VPPS-2000 volumetric sampler (Lanzoni s.r.l., Bologna, Italy) [41] with a pressure flow rate of 10 L/min, simulating the human breathing. Melinex adhesive tape was used as a pollen grain capture surface. Pollen quantification was conducted applying the methodology proposed by the Spanish Aerobiological Network (REA) [42], based on four longitudinal transects along the slides. The Main Pollen Season (MPS) was stablished using the Andersen method [43], which defines the MPS as the period from the day 2.5% of total annual pollen concentrations were reached to the date when 97.5% is accomplished. The classification recommended by the REA [30] was followed to categorize the pollen concentrations, as well as for the calculation of the thresholds of allergy hazard. For the quantification of the allergenic fraction, a Burkard Multi-Vial Cyclone Sampler (Hertfordshire, UK) with 16.5 L/min of aspiration flow rate was used. The bioaerosol particles were sampled into Eppendorf vials every 24 h and analyzed with the Takahashi et al. method [44] modified by the Moreno-Grau et al. method [45]. The 2-site ELISA methodology was used for the quantification of the aeroallergen content in the bioaerosol samples in four steps [12,46]. The antibodies Ole e 1 and Pla a 1 (Roxall S.A) were used for the determination of the allergen content of *Fraxinus*, *Olea* and *Platanus* allergens, and the Bet v 1 specific monoclonal antibody (ALK-Abelló) was used to quantify the *Betula* and *Alnus* allergen content in the aerosol. The absorbance was measured at 492 nm.

Meteorological data were acquired from the Galician Institute for Meteorology and Oceanography METEOGALICIA "Ourense" station, placed at 300 m of the pollen and allergen samplers. The measured parameters were temperature (°C), relative humidity (%), precipitation (mm) and wind speed (km/h) (Figure 1).

Spearman's non-parametric correlation test and Principal Component analysis (PCFA) were applied to evaluate the association between the pollen and the allergen concentrations in the air with the main weather parameters. A regression equation between the pollen and allergen data was conducted in order to obtain the aeroallergens thresholds and the amount of days with potential hazard of allergy prompted, both for pollen and allergens. The STATISTICA 7 program was used for the statistical analysis.

HYSPLIT back trajectories were assessed to study the daily pollen and allergen higher concentrations. The models led us to stablish the provenance (latitude, longitude and elevation) in the selected days of air masses using meteorological data at the 250, 500 and 700 m heights from the earth surface [47].

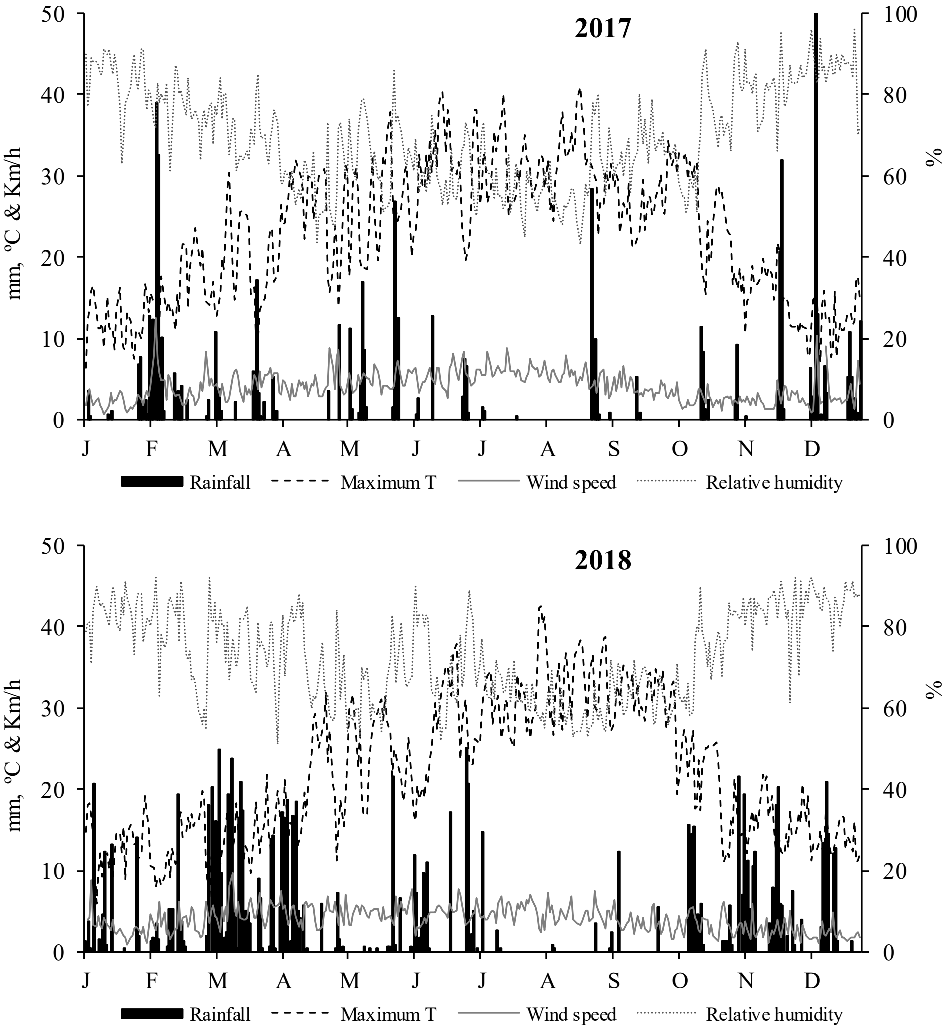


Figure 1. Meteorological variables: Maximum Temperature (black lines), Rainfall (black bars), Relative humidity (grey lines) and Wind speed (grey lines).

3. Results

Two periods of important tree pollen concentrations were recorded throughout the studied year (Figure 2). The first was consequence of the *Alnus* and *Fraxinus* blooms during January and February. The second, the quantitatively most important period, was mainly due to the pollination of *Betula*, *Platanus* and *Olea* during the spring months. The last period had a great impact on sensitization processes due to the flowering of trees with a high recognized allergy potential mainly in urban environments.

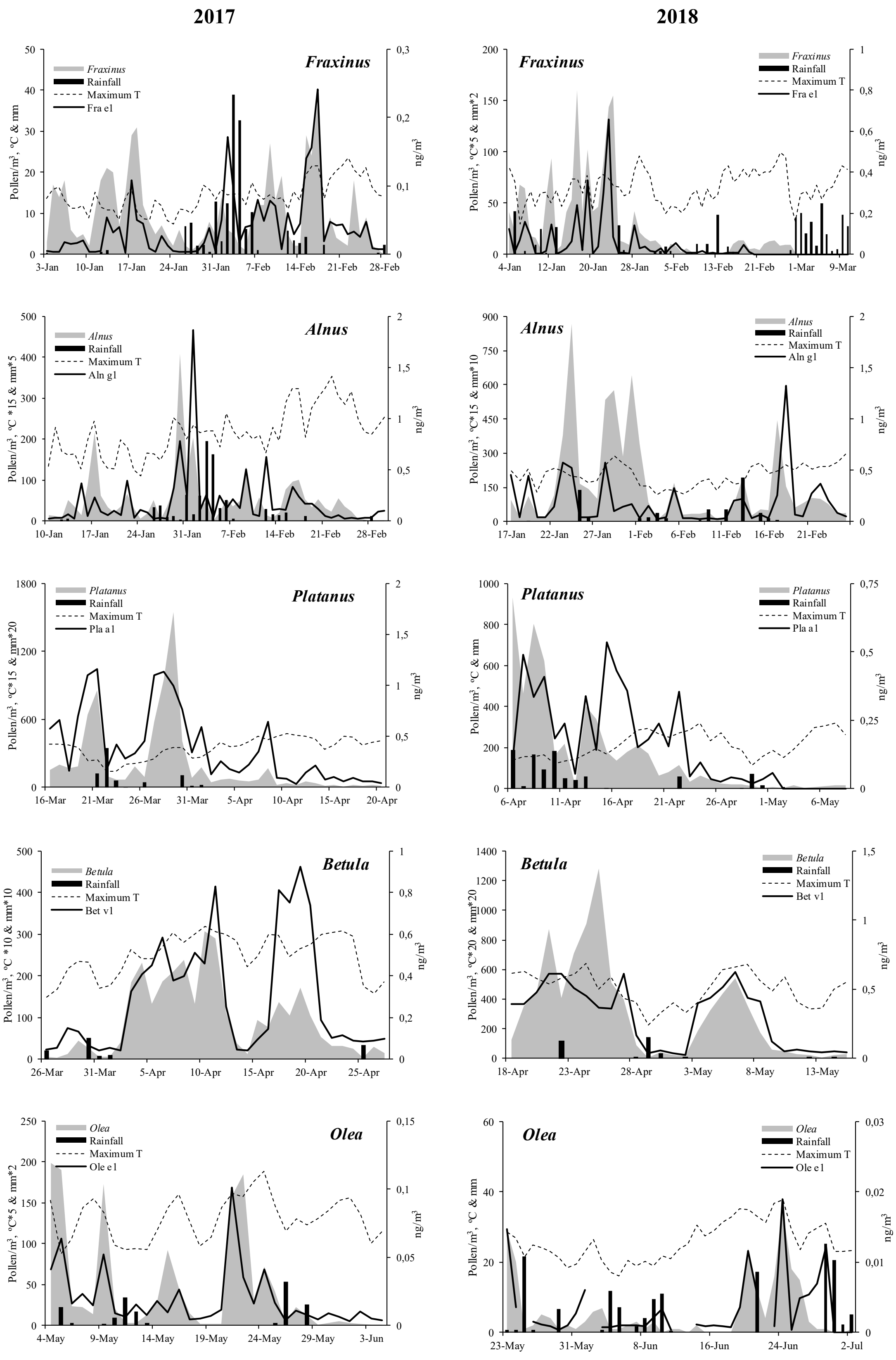


Figure 2. Pollen grain concentrations (grey area), allergen concentrations (black line), maximum temperature (points line) and rainfall (bar) for *Alnus*, *Betula*, *Fraxinus*, *Olea* and *Platanus* during the MPS.

The occurrence of *Alnus* pollen in the Ourense atmosphere was observed from the second fortnight of January to the end of February. We registered a total airborne pollen of 2692 and 6368 pollen grains in 2017 and 2018, respectively, during a MPS with a length of 40 and 52 days. The highest alder pollen value was recorded on 24 January with 867 pollen/m^3. The total annual integral of Aln g 1 was 9.200 ng and 7.386 ng in 2017 and 2018, respectively, with a peak observed on 1 February 2017 with 1.868 g/m^3, one day after the pollen peak. Although both peaks were recorded during a period of lack of rainfall, the Aln g 1 peak was observed during an increase (2.5 °C) in maximum temperatures (Table 1, Figure 2). The *Fraxinus* flowering occurred from January to early March. A total amount of 563 and 1575 pollen grains were recorded in 2017 and 2018, respectively, during the flowering period, with a duration of 57 and 66 days. The peak pollen concentration occurred on 17 January 2018 with 160 pollen and the allergen peak was recorded on 23 January 2018 with 0.660 ng/m^3, 6 days after the pollen maximum values. A period of rainfall absence and low temperatures coincided with the pollen and allergen maximums around 14.7 °C (Table 1, Figure 2).

The *Betula* pollen was detected in the atmosphere from the second fortnight of April until the first of May with a short MPS of 33 and 28 days in 2017 and 2018, respectively. The total airborne pollen was 3103 and 8397 pollen grains in 2017 and 2018, respectively, recording the pollen peak on 25 April 2018 with 1283 pollen/m^3. The annual integral of Bet v 1 allergen was 9.513 ng and 8.354 ng in 2017 and 2018, respectively, with a maximum concentration of 0.925 ng/m^3 on 19 April 2017. Both peaks are coincident with a period of rainfall absence; however, the Bet v 1 peak also coincided with a maximum temperature rise (from 22 °C on 14 April to 27 °C on Bet v 1 peak day) (Table 1, Figure 2). The *Platanus* MPS was also short from early April to early May. The seasonal plane pollen integral varies from 7290 in 2017 to 5399 in 2018, with a pollen peak registered on March 29th where 1549 grains/m^3 were registered in 2017. A total of 13.927 ng and 5.072 ng of Pla a 1 were detected during 2017 and 2018, respectively, in the atmosphere, and the maximum concentration was identified on 21 March 2017 with 1.157 ng/m^3. Both peaks coincided with the presence of precipitations and a decline of the maximum temperature around of 17 °C (Table 1, Figure 2).

The *Olea* flowering took place from the latest May until early July with a MPS length of 32 and 41 days in the two years of study. Wide variations in the annual pollen and allergen integral were detected between the two years of study with an amount of 1389 and 233 pollen grains and 0.604 ng 0.117 ng in 2017 and 2018, respectively. During the year 2018 both maximum allergen and pollen diurnal peaks were detected in the same day, whereas in 2017 with a difference of 15 days (Table 1, Figure 2).

Table 1. Date of the start, end and length of the main pollen season (MPS) (days), mean pollen (pollen/m^3), date of the pollen peak (day), pollen (pollen) and allergen, allergen peak of the MPS (ng/m^3), date of the allergen peak (day) and Pollen Allergen Potency (AP) (ng/pollen).

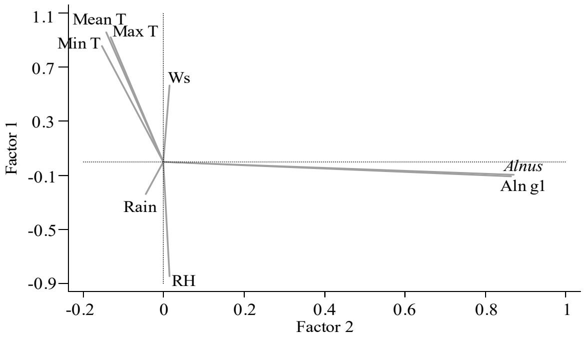
	2017					**2018**				
	Fraxinus	***Alnus***	***Platanus***	***Betula***	***Olea***	***Fraxinus***	***Alnus***	***Platanus***	***Betula***	***Olea***
MPS start	3-January	10-January	16-March	26-March	4-May	4-January	17-January	6-April	18-April	23-May
MPS end	28-February	2-March	20-April	27-April	4-June	10-March	25-February	8-May	15-May	2-July
MPS length	57	52	36	33	32	66	40	33	28	41
Mean pollen	10	52	203	94	43	24	159	164	300	6
Pollen peak	37	408	1549	307	199	160	867	932	1283	35
Peak date	17-February	31-January	29-March	10-April	4-May	17-January	24-January	6-April	25-April	24-June
Pollen	563	2692	7290	3103	1389	1575	6368	5399	8397	233
	Fra e 1	Aln g 1	Pla a 1	Bet v 1	Ole e 1	Fra e 1	Aln g 1	Pla a 1	Bet v 1	Ole e 1
Mean allergen	0.039	0.177	0.387	0.288	0.019	0.045	0.185	0.195	0.298	0.003
Allergen peak	0.241	1.868	1.157	0.925	0.101	0.660	1.320	0.535	0.624	0.019
Peak date	17-February	1-February	21-March	19-April	21-May	23-January	18-February	15-April	6-May	24-June
Allergen	2.237	9.200	13.927	9.513	0.604	2.970	7.386	5.072	8.354	0.117
AP (ng/pollen)	0.0040	0.0034	0.0019	0.0031	0.0004	0.0019	0.0012	0.0009	0.0010	0.0005

With the aim of determining the effect of the meteorological parameters in the pollen and allergen airborne content, a non-parametric Spearman's correlation test was conducted. Generally, spring-flowering trees showed positive correlations between airborne pollen and allergen concentrations and temperature, and negative with relative humidity ($p < 0.01$) (Table 2). Overall, the highest significant correlation coefficients were obtained among the allergen or the pollen and the average temperatures, with the greatest positive degree of association for the *Olea* pollen and the Ole e 1 allergen ($p < 0.01$). In addition, negative significant correlations between pollen concentrations with mean, maximum and minimum temperatures and positive with relative humidity were recorded with *Alnus* and *Fraxinus* pollen, as well as with Aln g 1 and Fra e 1 allergens, the winter bloom trees. For the rest of the studied parameters, we obtained positive significant correlations between pollen and allergens of *Betula*, *Olea* and *Platanus* and wind speed (Table 2).

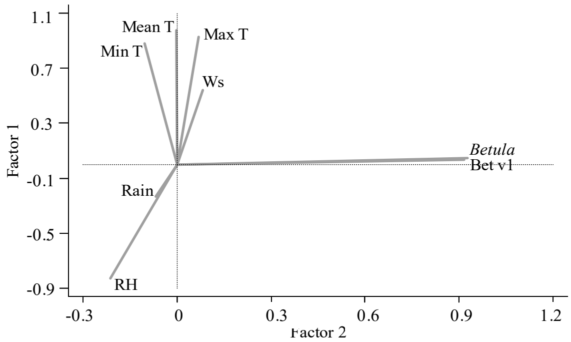
Table 2. Spearman correlations between pollen or allergen and the main meteorological variables (* $p < 0.05$; ** $p < 0.01$).

	Fraxinus	*Alnus*	*Platanus*	*Betula*	*Olea*
Average Temperature	−0.444 **	−0.416 **	0.120 **	0.250 **	0.306 **
Maximum Temperature	−0.383 **	−0.373 **	0.158 **	0.291 **	0.257 **
Minimum Temperature	−0.472 **	−0.432 **	ns	0.138 **	0.318 **
Relative Humidity	0.229 **	0.199 **	−0.277 **	−0.373 **	−0.161 **
Rainfall	ns	ns	ns	−0.108 **	ns
Wind Speed	−0.201 **	−0.129 **	0.278 **	0.279 **	0.245 **
Allergen	0.704 **	0.689 **	0.712 **	0.719 **	0.810 **
	Fra e 1	**Aln g 1**	**Pla a 1**	**Bet v 1**	**Ole e 1**
Average Temperature	−0.410 **	−0.387 **	ns	ns	0.202 **
Maximum Temperature	−0.405 **	−0.354 **	ns	0.107 **	0.170 **
Minimum Temperature	−0.376 **	−0.370 **	−0.142 **	−0.092 **	0.201 **
Relative Humidity	0.309 **	0.231 **	−0.182 **	−0.272 **	−0.131 **
Rainfall	ns	ns	ns	−0.073 *	ns
Wind Speed	−0.203 **	−0.187 **	0.175 **	0.168 **	0.205 **
Pollen	0.704 **	0.689 **	0.712 **	0.719 **	0.810 **

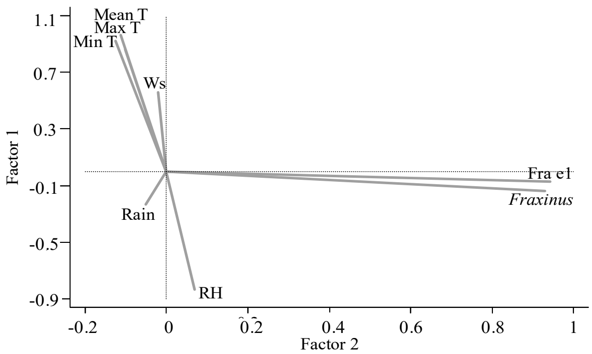
Furthermore, a principal component analysis (PCFA) was conducted with the aim to better understand the meteorological influence in the pollen and allergen airborne concentrations as PCFA showed the influence of all consider weather variables as a whole. The purpose of the analysis is to obtain a small number of linear combinations of the selected variables which account for most of the variability in the data. Three components have been extracted for each taxon, since they had eigenvalues greater than or equal to 1.0 and they account for together between 79% and 84% of the variability in the original data. The three PCs were correlated as follows: Component 1 temperatures and relative humidity, Component 2 pollen and allergen values and Component 3 wind speed and rainfall (Figure 3). To better understand the relationship between pollen/allergens and meteorological parameters, a plot with the PC explaining the higher variability PC1 vs. PC2 was conducted (Figure 3). The results obtained reinforced the correlation analysis results registering a high positive degree of association between the pollen counts and the allergen levels (Figure 3).



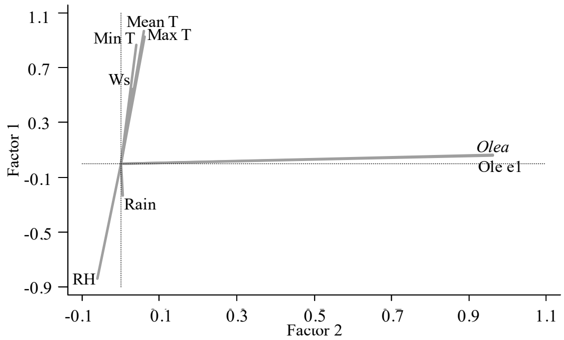
a	F1	F2	F3
Alnus	-0.107	**0.865**	-0.050
Aln g1	-0.096	**0.871**	0.012
Mean T	**0.958**	-0.143	-0.002
Max. T	**0.917**	-0.131	-0.204
Min. T	**0.854**	-0.154	0.190
RH%	**-0.843**	0.015	0.192
Rainfall	-0.237	-0.045	**0.875**
Wind speed	0.563	0.014	**0.567**



b	F1	F2	F3
Betula	0.034	**0.915**	0.002
Bet v1	0.047	**0.925**	-0.035
Mean T	**0.973**	-0.002	0.002
Max. T	**0.927**	0.069	-0.194
Min. T	**0.877**	-0.104	0.186
RH%	**-0.825**	-0.212	0.172
Rainfall	-0.232	-0.068	**0.873**
Wind speed	0.541	0.083	**0.577**

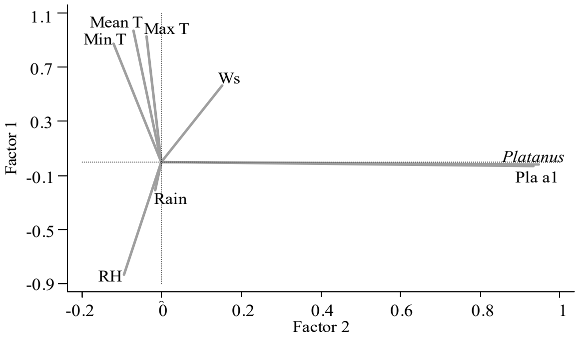


c	F1	F2	F3
Fraxinus	-0.140	**0.930**	-0.068
Fra e1	-0.075	**0.942**	0.004
Mean T	**0.963**	-0.112	-0.002
Max. T	**0.918**	-0.126	-0.205
Min. T	**0.863**	-0.102	0.192
RH%	**-0.834**	0.069	0.199
Rainfall	-0.235	-0.051	**0.877**
Wind speed	0.557	-0.021	**0.561**



d	F1	F2	F3
Olea	0.056	**0.962**	0.006
Ole e1	0.065	**0.962**	0.012
Mean T	**0.968**	0.060	0.003
Max. T	**0.926**	0.061	-0.198
Min. T	**0.868**	0.040	0.195
RH%	**-0.836**	-0.061	0.191
Rainfall	-0.233	0.004	**0.879**
Wind speed	0.548	0.029	**0.565**

Figure 3. *Cont.*



e	F1	F2	F3
Platanus	-0.031	**0.935**	0.062
Pla a1	-0.020	**0.947**	-0.023
Mean T	**0.969**	-0.070	-0.009
Max. T	**0.924**	-0.038	-0.213
Min. T	**0.872**	-0.122	0.188
RH%	**-0.834**	-0.094	0.225
Rainfall	-0.211	-0.017	**0.892**
Wind speed	**0.566**	0.152	0.530

Figure 3. Plot and Factors of principal components and factor analysis (PCFA) for each taxon, meteorological parameters: Mean, Maximum and Minimum temperatures (Mean T, Maximum T and Minimum T), Relative humidity (RH), Rainfall (Rain) and Wind speed (Ws) (a-*Fraxinus*. b-*Alnus*. c-*Platanus*. d-*Betula* and e-*Olea*).

The Pollen Allergen Potency (AP) index was calculated for each taxon, which represented the rate between the allergen and pollen grain concentrations. The highest value was 0.004 ng/pollen registered for *Fraxinus* and the lowest for *Olea* with 0.0004, both during the first year of study (Table 1).

Regression equations were performed to identify the aeroallergen thresholds for low, moderate and high hazard of symptomatology appearance on sensitized people (Table 3).

Table 3. Regression equations developed between the pollen concentrations (pollen/m^3) and allergen values (ng/m^3) during the main pollen season of the assessed taxa.

	Regression Equation	Adj. R^2	*p*
Fraxinus	Fra e 1 = 0.0002 + 0.002 × *Fraxinus* (pollen/m^3)	0.609	0.000
Alnus	Aln g 1 = 0.009 + 0.001 × *Alnus* (pollen/m^3)	0.289	0.000
Platanus	Pla a 1 = 0.011 + 0.001 × *Platanus* (pollen/m^3)	0.625	0.000
Betula	Bet v 1 = 0.011 + 0.001 × *Betula* (pollen/m^3)	0.512	0.000
Olea	Ole e 1 = 0.0003 + 0.0003 × *Olea* (pollen/m^3)	0.737	0.000

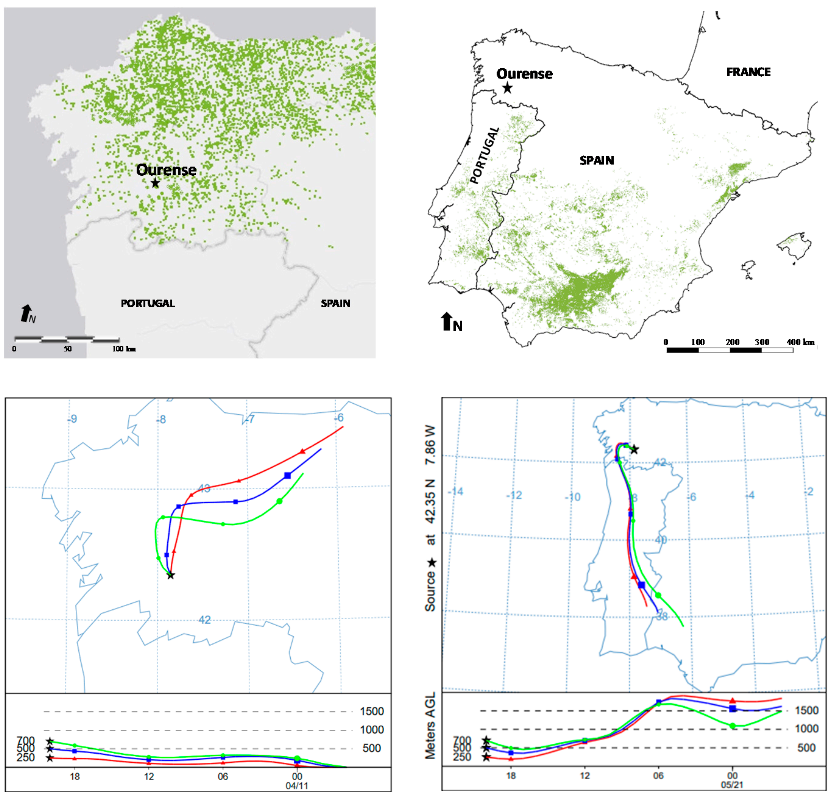
The pollen threshold concentrations suggested by the REA were followed to obtain the equivalent aeroallergens thresholds (Table 4). Values of the adjusted R^2 coefficients in the performed equations oscillated between 0.289 for *Alnus* and 0.737 for *Olea* (Table 3) The obtained thresholds were applied in order to ascertain the number of days with possible allergy hazard for sensitive patients. Considering the pollen data, the taxa that registered a higher quantity of days with moderate potential hazard for allergenic suffers were *Alnus* and *Platanus* with a sum of 22 and 27 days in both years, respectively (Table 4). In the case of the allergen airborne moderate levels, the great amount of days was registered for *Platanus* and *Betula* with a total of 30 and 31 days, respectively, during the two years of study (Table 4). Some discordances were also observed in the case of the episodes of high potential hazard on sensitive patients. *Alnus* and *Betula* registered the most important quantity with a sum of 55 and 58 days detected for the Aln g 1 and Bet v 1 allergen concentration during the period of the study (Table 4).

Table 4. Thresholds of allergenic risk for each taxon considering the pollen (Galán et al., 2007) and the allergens. Number of days with allergy risk periods for pollen or allergen. Total number of days during the period of study of high risk by means the combination of pollen and allergen data.

	Risk Level	Pollen	2017	2018	Total	Allergen	2017	2018	Total	Pollen + Allergen
Alnus	**Low**	1–30	89	97	186	0.011–0.039	28	17	45	64
	Moderate	31–50	13	9	22	0.040–0.059	5	6	11	
	High	>50	17	27	44	>0.059	32	23	55	
Fraxinus	**Low**	1–30	107	75	182	0.002–0.065	50	39	89	16
	Moderate	31–50	2	6	8	0.067–0.109	5	3	8	
	High	>50	0	10	10	>0.109	4	8	12	
Platanus	**Low**	1–50	67	78	145	0.012–0.058	13	12	25	34
	Moderate	51–200	18	9	27	0.059–0.201	22	8	30	
	High	>200	6	8	14	>0.201	21	10	31	
Betula	**Low**	1–30	57	96	153	0.013–0.038	0	3	3	59
	Moderate	31–50	5	3	8	0.039–0.055	13	18	31	
	High	>50	17	19	36	>0.055	36	22	58	
Olea	**Low**	1–50	50	39	89	0.001–0.0166	31	18	49	1
	Moderate	51–200	7	0	7	0.0169–0.065	10	1	11	
	High	>200	0	0	0	>0.065	1	0	1	

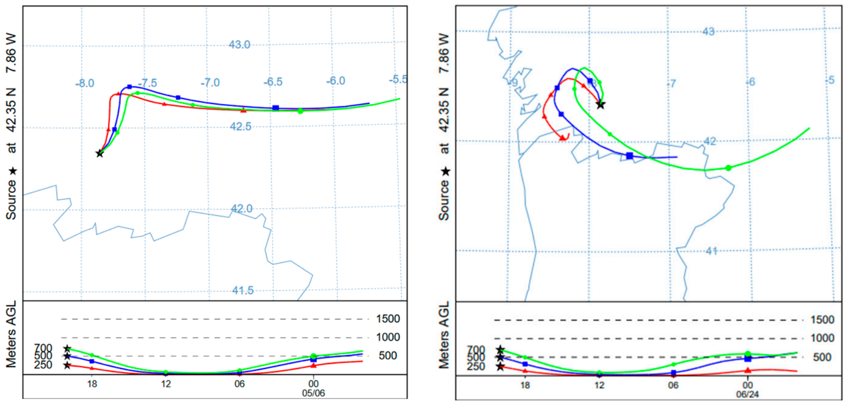
Furthermore, a back-trajectory analysis was conducted to explain the timing discrepancies observed between the pollen and allergen peaks for all taxa. Only special situations were observed in the case of *Betula* and *Olea* (Figure 4). The analysis led us to detect that the second pollen and allergen *Betula* peaks were coincident with air masses from the high mountainous areas around the city of Ourense. In the case of *Olea* and Ole e 1, the pollen and allergen maximum peaks were influenced by continental air masses coming from the North of Portugal (Figure 4).

Betula *Olea*



(a) (c)

Figure 4. *Cont.*



(**b**) (**d**)

Figure 4. Sampling location area, green zones indicate the *Betula* and *Olive* groves use Spanish Forest Species Inventory for *Betula* and CORINE land cover for *Olea.* Maps showing 24-h HYSPLIT backward trajectories at a final height of 250, 500 and 700 m agl. at considered episodes: (**a**) 17 April 2017; (**b**) 6 May 2018; (**c**) 21 May 2017; (**d**) 24 June 2018.

4. Discussion

The occurrence of pollen grains in the atmosphere was recognized as a cause of important pollution problems such as allergies to human health [48]. The most important tree allergenic taxa of the city, which constituted the 50% of the total of airborne pollen, were studied to determine their importance in the atmospheric allergenic load. Regarding the pollen season, *Alnus* and *Fraxinus* are arboreal species that flower in winter whereas *Betula*, *Olea* and *Platanus* with spring flowering. Several studies reported similar findings for *Alnus* in the same area and for other countries regarding the start, end date and duration of the MPS, but with differences in the total amount of annual pollen [10,49,50]. Some authors found that meteorological parameters, as mean temperature during the previous months to pollination, affect the annual airborne pollen sum [51]. We registered a shorter birch pollen season duration with higher total pollen amount than those reported by several studies for the same area [10,49] or in other European countries like Portugal [47], Poland [51], Sweden [52] and Romania [53]. In the case of *Olea*, a similar MPS duration was registered regarding the reported by studies carried out in the same area [54] or in Portugal [47,55]. However, longer MPS with higher pollen number was observed in Mediterranean areas because of the olive crops [56].

The classically information for hyper sensitized patients is the concentration of pollen grains in the atmosphere and their timing [24]. Nevertheless, in the last years the period of pollen exposure often did not coincide with the symptoms' appearance in different regions [16,25]. In the present study we detected a high atmospheric allergenic load [10] in the atmosphere coinciding with low levels of airborne pollen because the presence of pollen allergens in the air. Although pollen allergens are firstly carried in the atmosphere by pollen grains [37], they may also could be detected in the microaerosol suspension which could remain for longer periods in the atmosphere [57]. Our results reinforced the fact that pollen concentration data must be combined with the aeroallergen detection in order to determine the real load of atmospheric allergenic particles and the development of complete and lasting systems aimed to observe and gather environmental pollution information [11,47]. In spite of regression equations between pollen counts and allergen protein registered high R^2, several discordances were detected between the days of aeroallergen and pollen allergy risk, as in some cases did not coincide. Considering both together, the *Alnus* pollen and allergen data, the number of days with high allergenic hazard raised to 64 compared with their assessment separately. For *Fraxinus*, when the pollen and allergen concentrations were combined, the number of episodes with potential hazard raised to 16 for high risk of symptomatology appearance. Additionally, lower discrepancies were observed in the case of *Platanus* and *Betula* as the number of days with high potential hazard of allergy increased to 34 and 59, respectively, when the pollen and allergen data were considered together. No differences were observed in the case of *Olea*. The developed innovative tools for quantification of the atmospheric allergenic load are especially useful and necessary for complementing the classic pollen counts to attain an improvement and optimization of the clinical allergy treatments administration and a decrease in medication consumption by the sensitized-to-pollen population [11,47]. The establishment of new periods of allergen presence in the atmosphere will highlight novel perspectives in the epidemiological study of respiratory allergy-related disorders and the biological pollution.

In addition, a great prevalence of pollen related incidence of allergy in urban environments compared with the rural areas was detected [6]. It is noteworthy that the allergy incidence was prompted by an inaccurate planning and design of the urban tree vegetation, with several plants of the same family that can develop cross-reactions processes between their allergens, enhancing sensitizations in sensitive people [12]. One of the major results of our study was the detection of the *Fraxinus* and *Alnus* pollen related allergens in the air by using antibodies from another genus. This finding evidences the cross-reactivity between the principal allergens of the Oleaceae and Betulaceae tree families, referred as the capacity of several IgE antibodies to recognize diverse antigens [58]. Due to their resilience and tolerance to adapt to urban conditions, both tree families are broadly planted as tree ornamental vegetation in green areas of urban settings [59]. The urban allergic population to the *Betula* and *Olea* pollen could display allergy responses in the winter months (as a consequence of ash and alder pollen allergens), in the early spring because the Bet v 1 allergens or late spring due to Ole e 1 tree pollen related allergens. Some authors pointed out that the people with oral allergy syndrome (OAS) showed oral symptoms before *Betula* pollinosis symptoms [32], and this symptomatology may lead to people think that with a lower concentration of Bet v 1 allergen there are symptoms. Patients allergic to birch have previously suffered from the symptoms during the flowering of the alder, which is known as priming effect.

Moreover, Long Distance Transport processes (LDT) could also justify that airborne pollen concentrations are not always related with the actual exposure to their main allergens [60,61]. In the case of *Betula*, the first peak of the pollen curve matched with the flowering of the birch populations in the surroundings of the city, whilst the secondary was registered when the nearest *Betula* tree had finished its flowering period [62]. Topographical characteristics must also be taken into account when considering pollen transport. The HYSPLIT models showed that pollen originating from *Betula* populations located in mountainous areas even at some distance from the city, which flowers some weeks later due to its higher altitudinal distribution, could be transported through the channels formed by the river crossing the Ourense city, increasing the secondary peaks of pollen and allergen concentration. During the year 2017, the second peak pollen potency seems higher compared to the first peak, possibly related to a possible transport of high-potency birch pollen from the most elevated areas of the region, as it was observed for the olive pollen in South of Europe [60]. In addition, the back trajectories during the maximum concentrations of olive and Ole e 1 in the air noticed the influence of the air masses from the widespread olive orchards of North-Eastern Portugal in the amount of olive related bio particles in the atmosphere. *Betula* and *Olea* pollen morphology favors its transport over medium or long distances [60,63,64].

The analysis of the main meteorological variables, pollen and aeroallergen concentrations showed different results depending on the taxa. In the spring flowering trees, a statistically significant positive correlation between pollen or allergen occurrence and temperature [54] and wind speed was observed. On the contrary, the association degree was positive with relation to humidity in the case of the winter flowering trees and negative with temperatures, which presented negative correlations with temperatures. Therefore, weather-related factors, such as mild temperatures, influenced the dispersion of spring and fall pollen and allergens, as previously described by other authors [10,65,66].

5. Conclusions

The major allergens of *Alnus*, *Fraxinus*, *Betula*, *Platanus* and *Olea* were detected in the atmosphere of the Ourense city. Two peaks of important pollen concentrations were recorded throughout the year. One of the major findings of our study was the detection of the *Fraxinus* and *Alnus* pollen related allergen proteins in the air using antibodies from another genus, demonstrating the cross-reactivity processes between the principal allergenic proteins of the Oleaceae and Betulaceae families. The developed regression equations between pollen counts and allergen proteins registered high R^2 values. We observed high atmospheric allergenic load in the atmosphere coinciding with low levels of airborne pollen because of the presence of pollen allergens in the air. The number of days with a moderate and high hazard of allergy was higher when the pollen and allergen data were assessed together. Considering the pollen data individually, the number of episodes of high allergy symptomatology hazard were understated. The combination of pollen and allergen information should be evaluated to ascertain the real biological pollution in the atmosphere and the actual potential risk episodes for the sensitized population. Long Distance Transport processes (LDT) also explain that airborne pollen levels could not appropriately represent the exposure to their main allergens. The applied back trajectory analysis showed that pollen from *Betula* populations located in mountainous areas increased their secondary peaks of pollen and Bet v 1 concentrations and southern air masses from intensive plantations caused the highest airborne *Olea* concentrations.

Author Contributions: Conceptualization, F.J.R.-R. and M.F.-G.; methodology, F.J.R.-R. and M.F.-G.; software, E.G.-F. and A.G.; validation, S.Á.-L., F.J.R.-R. and M.F.-G.; formal analysis, S.Á.-L., F.J.R.-R. and M.F.-G.; investigation, S.Á.-L., F.J.R.-R. and M.F.-G.; resources, E.G.-F. and A.G.; data curation, S.Á.-L., E.G.-F. and A.G.; writing—original draft preparation, S.Á.-L., F.J.R.-R. and M.F.-G.; writing—review and editing, S.Á.-L., F.J.R.-R. and M.F.-G.; visualization, F.J.R.-R.; supervision, F.J.R.-R.; project administration, F.J.R.-R.; funding acquisition, F.J.R.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Partnership (ED431E 2018/07) and BV1 research group (ED431C 2017/62-GRC) and the grant number AGL2014-60412-R of the Economy and Competence Ministry of Spain Government. González-Fernández E. was supported by the FPU grant from the Ministry of Science, Innovation and Universities, Spain (FPU15/03343).

Conflicts of Interest: The authors declare no conflict of interest.

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