



Characterisation of particulate matter on airborne pollen grains



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ABSTRACT

A characterization of the physical–chemical composition of the atmospheric PM adsorbed to airborne pollen was performed. Airborne pollen was sampled using a Hirst-type volumetric spore sampler and observed using a Field Emission Electron Probe Microanalyser for PM analysis. A secondary electron image was taken of each pollen grain and EDS spectra were obtained for individually adsorbed particles. All images were analysed and the size parameters of the particles adsorbed to pollen was determined. The measured particles' equivalent diameter varied between 0.1 and 25.8 μm , mostly in the fine fraction. The dominant particulates identified were Si-rich, Organic-rich, SO-rich, Metals & Oxides and Cl-rich. Significant daily differences were observed in the physical–chemical characteristics of particles adsorbed to the airborne pollen wall. These differences were correlated with weather parameters and atmospheric PM concentration. Airborne pollen has the ability to adsorb fine particles that may enhance its allergenicity.

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1. Introduction

Particulate matter (PM) is a mixture of solid and liquid particles found in the air from diverse natural and anthropogenic sources (WHO, 2013a). This group includes the primary biogenic aerosols that originate in the biosphere such as pollen (Calvo et al., 2013).

Pollen grains are biological structures produced by the higher plants in order to reproduce. Anemophilous plants, during the flowering season, release large amounts of aerodynamic pollen grains into the atmosphere that trigger respiratory allergic reactions. An epidemic increase in the prevalence of these pollen related respiratory allergic diseases is reported with 10–30% of the population being currently affected (Ring, 2012). This phenomenon has been linked to the interaction between the airborne pollen allergens, the pollutants, and the immune system (Ring, 2012).

It is known that allergy results from the interplay between the genetic predisposition and the exposure to allergens and adjuvant environmental cofactors that lead to allergic sensitization (Traidl-

Hoffmann et al., 2009). A growing attention has been devoted to the inhalable PM (PM_{10}) adverse effects on respiratory health (WHO, 2013b). Some studies have shown that the PM can attach to airborne pollen grains and induce modifications on its morphology (Duque et al., 2013; Guedes et al., 2009; Guimarães et al., 2012; Okuyama et al., 2007). Duque et al. (2013) showed that airborne pollen had mineral particles adhered to its wall and observed statistically significant differences in the airborne pollen wall's elemental composition compared to the pollen directly collected from the flower. However a complete understanding of the complex interaction between PM and airborne pollen grains and its influence on pollinosis is still missing. Particles adsorbed to pollen grains will likely act as adjuvants to modify the microenvironment in which allergens are released from pollen and facilitate the access of allergens to antigen-presenting cells, enhancing inflammatory processes (Le Souef, 2009; Traidl-Hoffmann et al., 2009). The degree of these reactions may be dependent on the physical characteristics of the PM and on the toxicity of its chemical composition.

This study describes the physical–chemical characteristics of the atmospheric particulate matter adsorbed to airborne pollen grains of *Pinus*, *Platanus* and *Acer* in the city of Porto, Portugal. These pollen types present concomitant flowering periods and are

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among the most representative allergenic pollen types present in the atmosphere of the city (Ribeiro and Abreu, 2014).

2. Material and methods

2.1. Study site

Porto (41° 8' 58 N and 8° 36' 39 W), the second largest Portuguese city with about 238 thousand inhabitants and a population density of 5736 inhabitants/km², is limited on the west by the Atlantic Ocean and on the south by the Douro River. It is quite urbanized, with stationary sources of anthropogenic atmospheric pollutants like oil, petrochemicals, incineration units and shipping port activities, presenting also a high level of road traffic. The most common species of trees found are *Acer*, *Fraxinus*, *Liquidambar*, *Pinus*, *Platanus*, *Populus*, *Prunus*, *Quercus* and *Tilia*. At the herbaceous strata, plants like *Plantago*, *Poaceae*, *Rumex* or *Urticaceae*, dominate.

The annual average temperature is 15 °C, the total annual precipitation is of 1200 mm, mostly in winter, and the annual average air humidity is of 80%. Prevailing winds are from W and NW in the summer and from E and SE in the winter (Miranda et al., 2001).

The sampling point was set on the roof of the Faculty of Sciences in Porto, 20 m above ground level (Fig. 1). It is located within a residential area, near the ocean and the Douro River and close to a highway road that is one of the main entrances of the city, with frequent traffic congestion and jams during the rush hours.

2.2. Data sampling

Airborne pollen sampling was performed using a Hirst-type volumetric spore sampler (Lanzoni s.r.l.). This is a one-stage impactor designed to trap airborne biological particles composed

by a vane tail, which orientates the sampler according to the prevailing wind direction, and a built-in vacuum pump that samples air at a rate of 10 L min⁻¹, simulating human inhalation. By passing through a narrow intake orifice (2 × 14 mm) the sampled air impacts into a clock-driven drum covered with a double-sided Melinex tape where airborne pollen is retained. The drum rotates at an angular velocity of 2 mm h⁻¹, taking 7 days to perform a complete turn. After exposure, the tape is cut into 48 mm long segments, each representing one day of sample (Galán et al., 2007). In our study, the Melinex tape was coated with a double-sided adhesive carbon tape to allow the following analysis.

Seven consecutive days were sampled in March 2012 (14th to 20th), which corresponded to the peak pollination period of *Acer*, *Platanus* and *Pinus* when pollen trapped was enough for an adequate analysis (Ribeiro and Abreu, 2014).

The meteorological conditions were continuously monitored by a meteorological station located 7 m apart from the pollen sampler at approximately the same altitude. During the study period the temperature ranged between 6.3 and 20.9 °C, with an average of 12.8 °C. The average relative humidity was of 69.2%, with a minimum of 25.4% and a maximum of 94.5%. Precipitation was present on March 17th and 18th but in a low amount (Fig. 2A). The prevailing wind direction presented differences depending on the day (Fig. 2B).

The mass concentrations of PM₁₀ and PM_{2.5} were obtained from the monitoring station Sobreiras (type: urban; main pollution source: background) integrated in the Air Quality Monitoring Network of Porto-MA, managed by the Regional Commission of Coordination and Development of Northern Portugal, under the responsibility of the Ministry of Environment (<http://qualar.apambiente.pt/>). This station is approximately 2 km apart from the pollen sampling site. During the study period PM₁₀ values ranged from 0 to 121 µg/m³, with a daily average between 86 µg/m³

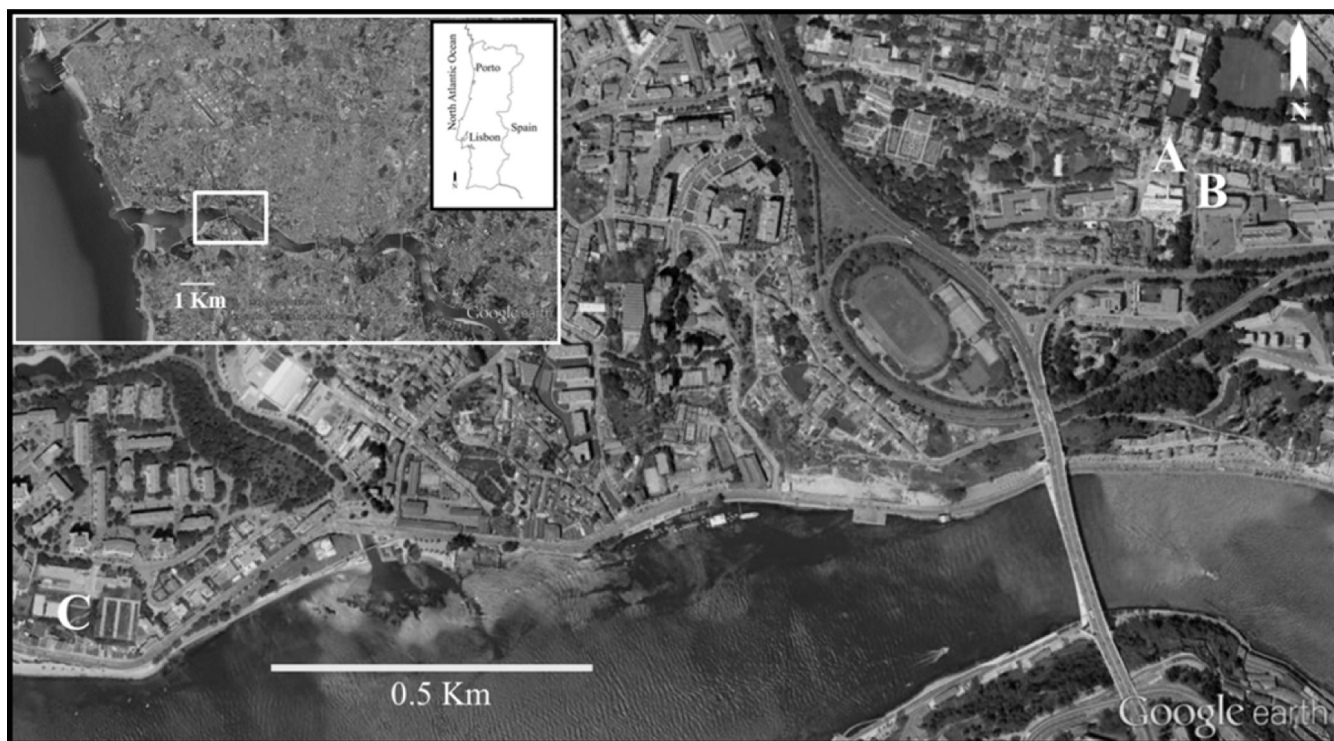


Fig. 1. Location of the spore sampler (A), the meteorological station (B) and the Sobreiras air quality monitoring station (C) (Google, Digital Globe images).

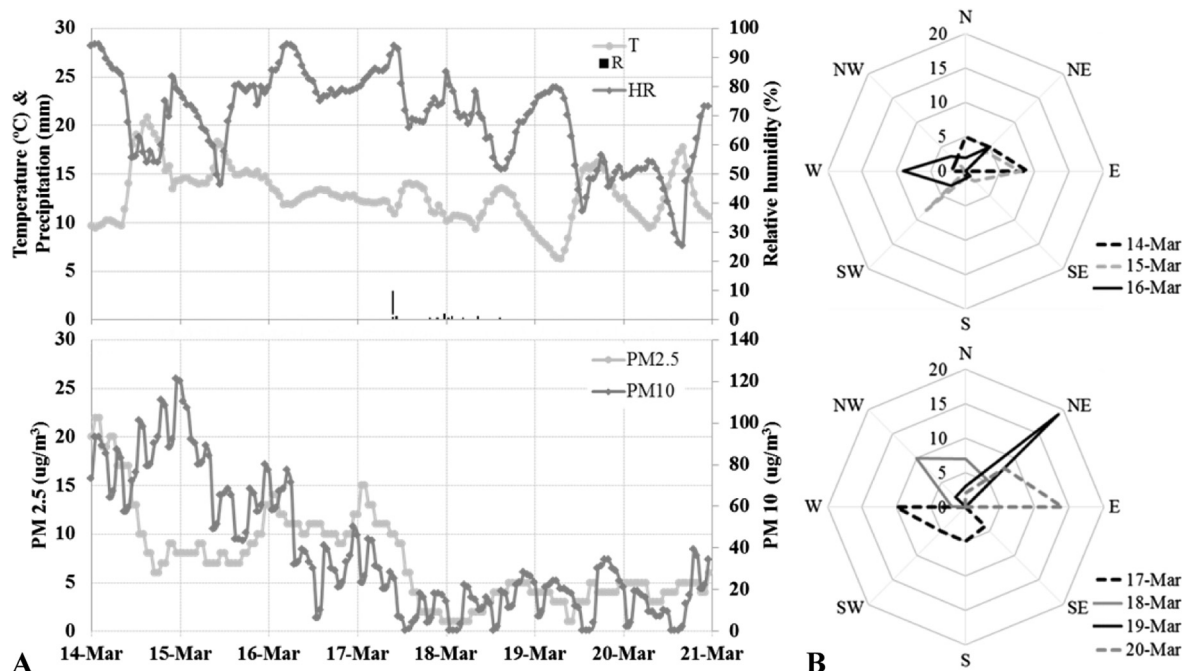


Fig. 2. Hourly temperature (T), precipitation (R), relative humidity (HR), PM₁₀ and PM_{2.5} (A) and frequency of hourly average wind direction (B) during the study period for the sampling area in the city of Porto.

(March 14th) and 14 µg/m³ (March 20th), while PM_{2.5} values ranged between 3 and 14 µg/m³, being 22 µg/m³ the highest value observed (March 14th) (Fig. 2A). The daily average of mass concentrations of PM₁₀ observed in the first two sampling days surpasses the limit fixed by the EU for protection of human health (50 µg/m³) while PM_{2.5} values were always lower than the annual average limit fixed for the protection of human health (25 µg/m³), according to the Directive 2008/EC/50.

2.3. Analysis of pollen

A Field Emission Electron Probe Microanalyser (JEOL JXA-8500F), equipped with an EDS spectrometer (Oxford INCA X-Act) was used to investigate the physical–chemical characteristics of the particulate matter attached to the surface of airborne pollen. The samples were kept on the carbon tape, mounted over microscope slides and were carbon coated with the same thickness to achieve conductivity and reduction of electron charge. Since analysed pollen size varies between 20 and 60 µm, and assuming a density of 1 for biological material (van Hout and Katz, 2004), EDS analyses were performed at 10 kV accelerating voltage and with a beam current of 10 nA during 15 s, which ensures that the volume of excitation caused by the beam is smaller than the pollen size. The magnifications of the collected images varied between 2300× and 3300×.

Slides were scanned along traverse lines and pollen types randomly selected were identified visually. A total of 419 pollen grains was observed. For each pollen a secondary electron image (SEI) was taken and an EDS spectrum for individual particles adsorbed to the airborne pollen surface was obtained. A total of 1914 EDS spectra was analysed and used to chemically classify the particles based on the most prominent element peak intensities.

All images were analysed to determine the pollen and particles' equivalent projected area diameter (Eq. (1)) (diameter of a circle having the same area as the projection area of the particle), number of particles counted, percentage of pollen area occupied by particles and particle size distribution by using AxioVision Rel.4.8 software from Carl Zeiss. A total of 7683 particles was counted.

$$\text{Particle equivalent diameter} = (\text{particle projected area} / \pi)^{1/2} \times 2 \quad (1)$$

2.4. Statistical analysis

We used the IBM SPSS statistics v.21 for statistical analysis. Box-plot diagrams were performed as well as histograms of the physical characteristics of the measured particles attached to pollen. The normality of the data was assessed by the Kolmogorov–Smirnov test.

An analysis of variance (ANOVA) was performed followed by a LSD test, to compare the pollen average equivalent diameter, the average number of particles and average percentage of area occupied by particles between the three types of pollen studied, or by a Tukey test to ascertain if the average pollen equivalent diameter, the average number of particles, their equivalent diameter and average percentage of area occupied by particles for each pollen-type studied did not vary daily.

The relationship between the average daily physical characteristics of PM attached to airborne pollen grains, the meteorological parameters and the PM₁₀ and PM_{2.5}, was calculated by means of Pearson/Spearman correlation coefficients at significance level 5% and 1%. Both correlation coefficients were calculated to ascertain the existence of linear and non-linear correlations between the tested variables. A principal components analysis was performed for the PM's chemical composition and weather parameters in order to ascertain the relationships between these variables.

3. Results

3.1. Physical characterization

A total of 140 pollen grains of *Acer*, 123 of *Pinus* and 156 of *Platanus* were analysed. Significant differences were observed between the average values of each pollen-types' equivalent

diameter, number of particles/pollen and percentage of area occupied by particles (Fig. 3).

A total of 2254 particles was analysed on *Acer* pollen, 4770 on *Pinus* and 659 on *Platanus* and the average number of particles per pollen were 16 ± 12 , 39 ± 32 and 4 ± 4 respectively. The *Pinus* pollen equivalent diameter presented an average size that was approximately double the *Acer*'s and triple the *Platanus*'s (Fig. 3). Despite similar ratios particles/pollen size found for *Acer* and *Pinus*, this last pollen-type presented an average area occupied by particles lower than the *Acer* pollen (5.7 ± 7.1 and 7.5 ± 8.7 respectively). Around 75% of the sampled pollen did not present more than 10% of the total area occupied by particles.

Similar particle size distributions were obtained in the three pollen-types studied, varying between 0.1 and $25.8 \mu\text{m}$ (Fig. 4). Ultrafine particles, although present, were not measured due to image resolution restrictions.

About 96% of the counted particles presented a diameter lower than $5 \mu\text{m}$; 84% had a diameter in the range of the fine particles

fraction and almost 50% were in the submicron particle size range (median values in Table 1).

Considering the daily variations in the parameters measured, no significant differences were observed in the average equivalent diameter of each studied type of pollen. In contrast, the physical characteristics of the particles adsorbed to the pollen wall presented significant daily differences (Table 1).

The lowest average number of particles counted per pollen grain in *Pinus* was observed on March 16th while in *Platanus* on the 20th and on the 14th in *Acer*. The average equivalent diameter of the measured particle attached to the pollen grains was the lowest on March 18th.

Significant correlations were found between the physical characteristics of the PM adsorbed to the pollen wall and the meteorological parameters (Table 2). In the *Acer* and *Pinus* airborne pollen, a positive correlation between the particles equivalent diameter and percentage of area occupied by particles with the temperature was observed. Precipitation showed no consistent

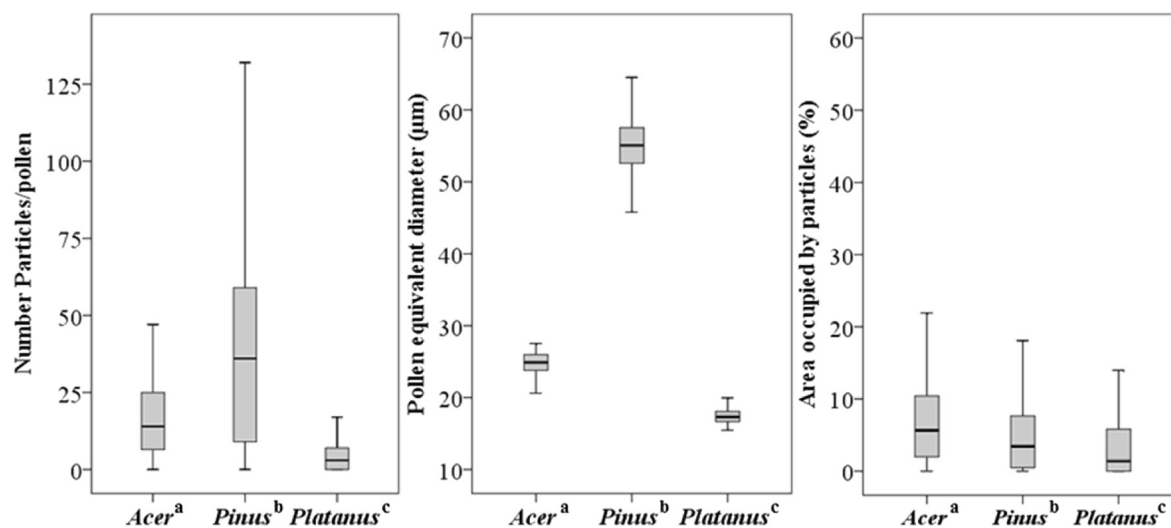


Fig. 3. Number of particles counted per pollen, airborne pollen equivalent diameter and percentage of occupied area by particles of *Acer*, *Pinus* and *Platanus* pollen. Different letters indicate statistically significant differences given by the ANOVA test followed by the LSD pos-hoc test (p -value < 0.05).

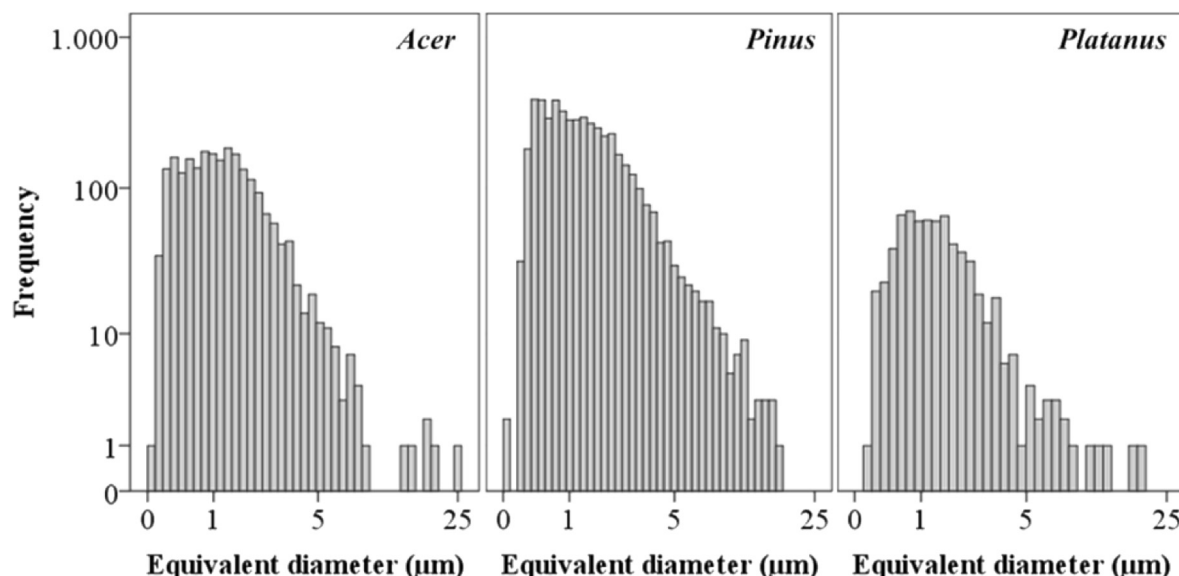


Fig. 4. Size distribution of equivalent diameter of the measured particles attached to airborne *Acer*, *Pinus* and *Platanus* pollen wall.

Table 1

Daily values of the airborne pollen equivalent diameter and measured physical characteristics of the particulate adsorbed to the pollen wall.

Day	<i>Acer</i>					<i>Pinus</i>					<i>Platanus</i>				
	Equivalent diameter (μm)														
	NP	Mean ± SD	Median	Min	Max	NP	Mean ± SD	Median	Min	Max	NP	Mean ± SD	Median	Min	Max
14th	21	24.1±1.8	24.6	20.6	26.4	17	54.7±5.0	53.7	45.8	63.7	23	17.5±0.7	17.6	16.5	18.7
15th	18	24.8±1.6	25.4	21.8	26.7	18	55.7±3.9	55.7	48.6	63.2	19	17.0±0.9	16.9	15.7	19.5
16th	29	25.0±1.7	25	22.1	27.5	16	53.5±4.9	53.8	45.9	62.2	20	17.2±1.0	17.3	15.5	19.1
17th	20	25.3±1.3	25.3	22.7	27.3	16	56.9±3.6	57.3	48.8	64.5	31	17.2±1.0	17.3	15.6	19.2
18th	18	24.8±1.3	25	22.9	26.7	19	55.4±3.1	55.4	47.8	61.7	20	17.5±0.8	17.5	15.9	18.8
19th	17	24.8±1.5	24.8	21.3	27	18	54.3±4.1	54	47.5	62.2	23	17.8±1.2	17.9	15.9	20
20th	17	24.3±1.3	24.4	20.8	26.6	19	54.6±3.4	55	46.5	60.1	20	17.4±0.9	17.7	15.5	18.8
p-value		0.248					0.304					0.144			
<i>Acer</i>					<i>Pinus</i>					<i>Platanus</i>					
Particles															
Day	N	Mean±SD	Median	Min	Max	N	Mean±SD	Median	Min	Max	N	Mean±SD	Median	Min	Max
Number of particles															
14th	190	9±12 ^a	3	0	38	782	46±42 ^{ab}	59	0	132	46	2±4 ^{ac}	0	0	13
15th	401	22±12 ^b	21	7	47	886	49±22 ^a	54	12	92	106	6±4 ^{ab}	6	0	13
16th	435	15±11 ^{ab}	11	0	40	287	18±25 ^b	7	0	84	132	7±5 ^b	7	0	17
17th	413	21±8 ^b	22	4	35	654	41±27 ^{ab}	36	0	115	153	5±3 ^{ab}	4	1	14
18th	309	17±13 ^{ab}	14	0	45	1051	55±34 ^a	45	4	123	103	5±4 ^{ab}	6	0	13
19th	270	16±13 ^{ab}	13	0	44	751	42±31 ^{ab}	43	1	103	99	4±5 ^{abc}	1	0	17
20th	236	14±12 ^{ab}	12	0	34	359	19±21 ^b	12	0	81	20	1±2 ^c	0	0	8
p-value		0.013					0.001					0.00			
Equivalent diameter (μm)															
14th	190	1.3±1.8 ^{ab}	0.9	0.1	19.9	782	2.0±1.7 ^a	1.6	0.3	15.4	46	1.2±0.9 ^{ab}	0.9	0.2	3.3
15th	401	1.4±1.5 ^{ab}	0.9	0.2	14.3	886	2.0±1.9 ^a	1.4	0.2	15.9	106	1.5±1.0 ^{abc}	1.4	0.2	5.6
16th	435	1.6±1.5 ^a	1.4	0.2	25.8	287	1.8±1.9 ^a	1.4	0.3	16.5	132	1.9±2.4 ^{ac}	1.4	0.3	18.2
17th	413	1.6±1.5 ^a	1.4	0.1	18.1	654	1.3±1.2 ^b	1.0	0.2	14.7	153	2.0±1.9 ^c	1.5	0.2	12.2
18th	309	1.2±1.1 ^b	1.0	0.2	14.6	1051	1.2±1.1 ^b	0.9	0.2	14.3	103	1.0±0.5 ^b	0.9	0.2	2.4
19th	270	1.2±1.2 ^b	0.9	0.2	8.4	751	1.4±1.6 ^b	0.9	0.2	11.9	99	1.3±1.1 ^{ab}	0.9	0.2	5.4
20th	236	1.4±1.7 ^{ab}	0.9	0.1	17.5	359	1.4±1.5 ^b	1.0	0.2	11.0	20	1.4±0.8 ^{abc}	1.4	0.3	4.2
p-value		0.000					0.000					0.000			
Area occupied by particles (%)															
14th		6.2±12.8 ^a	0.7	0	56.4		11.1±11.5 ^a	7.2	0	30.7		1.0±2.1 ^a	0	0	7.4
15th		11.5±12.3 ^a	7.6	2	51		10.4±8.3 ^{ab}	7.7	1.3	31.5		4.8±4.9 ^{ab}	3.9	0	15.9
16th		6.6±6.9 ^a	5.8	0	35.8		3.7±6.0 ^c	0.4	0	21.9		7.4±7.7 ^b	4.9	0	25.7
17th		12.1±4.5 ^a	12.3	4.7	20.4		3.6±3.7 ^c	2.4	0	14.8		7.1±6.5 ^b	5.3	0.2	26.8
18th		5.1±4.2 ^a	3.6	0	13.9		4.5±3.2 ^{bc}	3.6	0.7	10.9		1.8±1.5 ^a	1.5	0	5
19th		5.6±6.9 ^a	4.1	0	27.3		4.8±5.0 ^{abc}	3.6	0	18.1		2.9±4.3 ^a	0.2	0	14
20th		5.9±7.3 ^a	2.9	0	23.7		2.0±3.2 ^c	0.2	0	10.6		0.8±1.8 ^a	0	0	6.6
p-value		0.041						0.000						0.000	

p-value associated with the ANOVA test; different letters indicate statistically significant differences (Tukey HSD, $p < 0.05$). SD: standard deviation; Min: minimum; Max: maximum; NP: number of pollen; N: number of particles.

influence and relative humidity only influenced particles equivalent diameter counted in *Pinus* pollen. Significant correlations between the physical characteristics of the adsorbed particulate and the wind direction were only observed in the airborne pollen of *Acer* and *Platanus*. Furthermore, in the airborne pollen of *Acer* and *Pinus* the percentage of area occupied by particles was also positively correlated with the PM's mass concentration found in the atmosphere (Table 2).

3.2. Chemical characterization

The chemical composition of 1914 individual particles was analysed and classified as described in Table 3. Although present, the detection of N was not possible under this measurement conditions. The dominant particles identified were Si-rich (Si–O: 47.9%, aluminosilicates: 10.9%, Fe–Si: 2.0%) and Organic (rich in elemental and in organic carbon components). Other abundant particles were SO-rich (SO–Ca: 61.8%, SO–Ca–K: 13%, SO–Ca–Na: 11.8%), Metals & Oxides (Fe–O: 10.8%) and Cl-rich (particularly combined with K, Mg, Na: 6.4%). These five groups accounted for almost 90% of the adsorbed particulate matter chemical composition, presenting a size distribution mostly in the PM_{2.5} fraction. However the size

distribution of Si-rich, organic, SO-rich and Cl-rich particles also presented other secondary peaks in coarser size ranges, particularly 5–10 μm (Fig. 5).

Aluminum oxides, Pb-rich, Na-rich and Ca-rich particles were also present. Other complex particles constituted by microclusters for example of sea salt and Si, which could not be differentiated, were observed.

The particle size distribution of the five main chemical groups shows that most adsorbed particles were in the PM_{2.5} fraction. However there were also observed other secondary peaks in coarser size ranges, particularly 5–10 μm , for Si-rich, organic, SO-rich and Cl-rich particles (Fig. 5).

Daily variations were observed in the chemical composition of particles adsorbed to the pollen wall (Fig. 6A). For example, on March 18th a visible reduction in the number of Si-rich particles and an increase in the SO-rich, Cl-rich and sea salt misc. ones were registered. On March 17th a similar tendency for SO-rich and Cl-rich was observed.

Principal components analysis (PCA) applied to the particulate matter chemical composition and weather parameters showed that the data could be reduced into four dimensions (Table 4). These results show that a higher number of Si-rich particles tends to be

Table 2
Pearson (unshaded cells) and Spearman (shaded cells) significant correlation coefficients between daily average physical characteristics of particulate matter attached to airborne pollen of *Acer*, *Pinus* and *Platanus*, meteorological parameters and PM₁₀ and PM_{2.5}. Values presented correspond to the highest correlation coefficient obtained comparing the Pearson vs. Spearman results.

		<i>Acer</i>						<i>Pinus</i>						<i>Platanus</i>						
		N		ED	%Area occupied			ED			%Area occupied			N		ED		%Area occupied		
		Mean	Mean	Mean	Mean	Min	Max	Mean	Min	Max	Mean	Max	Min	Max	Mean	Max	Mean	min	Max	
T	Mean						0.91**	0.90**			0.81*	0.88**								
	Min			0.81*	0.85*					0.76*										
	Max						0.79*													
RH	Mean									0.91**								0.76*		
	Min									0.84*										
	Max									0.76*										
R	Mean						−0.76*	−0.77*					0.76*					0.76*		
	Max						−0.76*	−0.77*					0.76*					0.76*		
	Mean						−0.76*		−0.80*	−0.83*	−0.78*	−0.82*								
WV	Min						−0.83*	−0.88**												
	N		−0.79*	−0.79*	−0.86*	−0.79*									−0.91**	−0.78*			−0.76*	
	NE		−0.79*		−0.78*	−0.83*														
WD	E													−0.82*						
	SE		0.84*	0.78*	0.91**	0.97**							0.87*		0.87*	0.79*	0.79*	0.87*	0.87*	
	S				0.93**	0.93**							0.96**					0.96**	0.81*	
PM ₁₀	SW	0.78*	0.94**		0.91**	0.84*									0.79*			0.79*	0.79*	
	W			0.81*											0.81*	0.86*	0.81*		0.82*	
	Mean						0.97**				0.92**	0.96**								
PM _{2.5}	Min						0.91**					0.87*								
	Max						0.97**				0.87*	0.96**								
	Mean						0.79*													

N: number of particles; ED: particle equivalent diameter in μm ; min: minimum; max: maximum; T: temperature; RH: relative humidity; R: precipitation; WV: wind velocity; WD: wind direction. **Correlation significant at the 0.01 level; * Correlation significant at the 0.05 level.

Table 3

Chemical classification, based on the element peak intensities of the EDS spectra, and frequency of main particulate matter adsorbed to the airborne pollen wall of *Acer*, *Pinus* and *Platanus*.

Groups	Elements association	Frequency (%)	Groups	Elements association	Frequency (%)
Si-rich	Si–O; Ca–Si; Ca–K–Si; K–Si; Mg–Si; Na–Si; Na–Mg–Si; Ti–Si; Al–Si–Fe–O; Al–Si; K–Al–Si; Mg–Al–Si; Zn–Al–Si;	40.3	SO-rich	SO; SO–Na; SO–Ba; SO–Ca–K; SO–Fe; SO–K; SO–Ca; SO–Ca–Na; SO–Na–Ca–Mg; SO–Na–K; SO–Na–Ca–K;	13.7
Cl-rich	Cl–Mg; Cl–Na; Cl–Na–Mg; Cl–K; Cl–Na–K Cl; Cl–Ca	7.8	Metals & oxides	Fe-rich; Fe–O; Fe–Ni; Fe–Zn; Fe–Ni–V; Ni-rich; Ni–V-rich; Fe–Ti–O; Fe–Zn–O; Ti–O;	12.0
Organic	C-rich (elemental & organic)	15.5	P-rich	P–O; P–O–Al; P–O–Ca; P–O–Mg; P–O–Na–Al;	0.8
Sea salt misc.	Na,Mg,Cl,Ca,K,S; SO–Ca–Cl–Na;	2.7	Al–O	Al–O	0.4
Ca-rich	Ca-rich; Ca–C–O; Ca–Mg–C–O;	1.5	Pb-rich	Pb-rich	0.3
Na-rich	Na-rich; Na–O	0.3	others	–	4.7

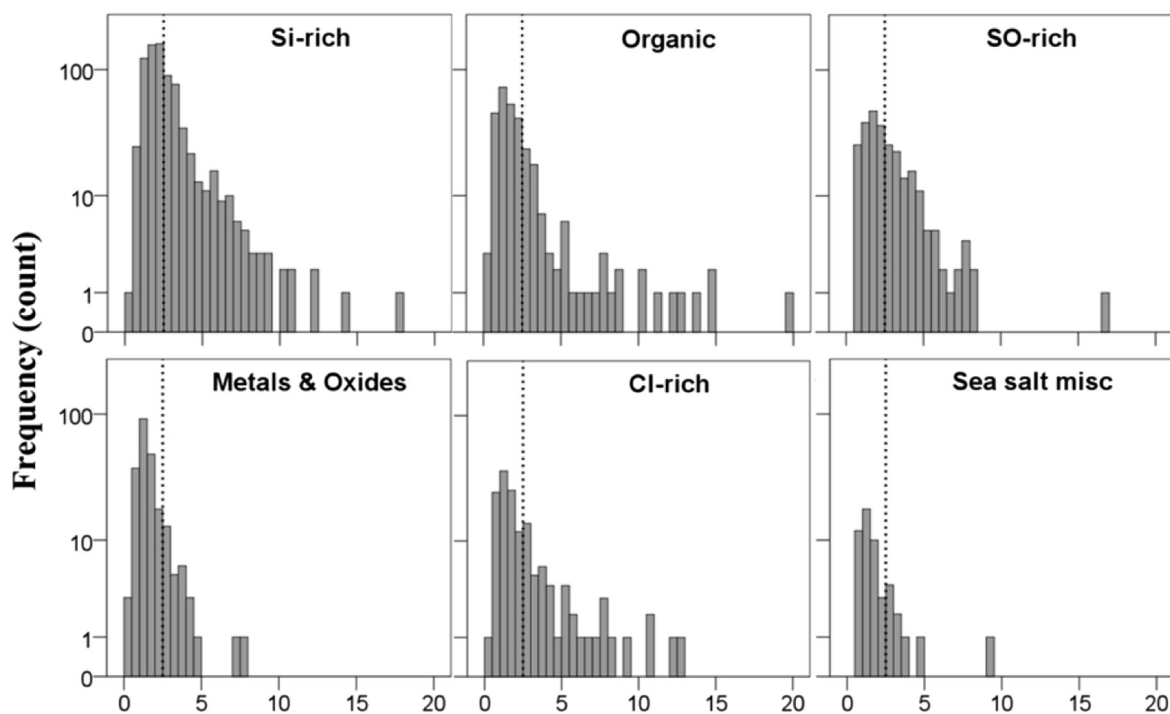


Fig. 5. Size distribution of the equivalent diameter of the dominant chemical groups of particles adsorbed to the pollen wall of airborne *Acer*, *Pinus* and *Platanus* types (dashed line marks the 2.5 μm size).

associated with higher levels of Na-rich particles but with lower levels of Pb-rich (loading on dimension 1). Winds from the SE-W quadrants and higher levels of air relative humidity tend to positively influence this relationship.

SO-rich, Cl-rich and sea salt misc. particles are correlated (loading on dimension 2) and their amount is positively influenced by precipitation and wind coming from the West quadrant (Table 4, Fig. 6B). Also the second dimension shows that organic and P-rich particles are correlated and positively influenced by temperature and winds coming from the East quadrant. Nonetheless, organic and P-rich particles presented also high component loadings on the third dimension in combination with Metals & Oxides particles and aluminum oxides. Their amount was positively influenced by northwestern winds.

4. Discussion

While airborne, pollen had the ability to adsorb other PM found in the atmosphere. *Pinus* pollen presented higher average number of particles per pollen, about twice and almost ten times more than on *Acer* and *Platanus* respectively. This can be related to the pollen's average size, *Pinus* is two times bigger than *Acer* and *Platanus*. Furthermore, the exine sculpturing may also influence the amount of particles adsorbed, since the three pollen types studied have quite different ornamentalations. However, the ratio of particles per pollen size is similar in *Pinus* and *Acer* but different in *Platanus* possibly indicating a lower influence of pollen morphology. The number of adsorbed particles can be related to the pollen emission source. The pathway of *Platanus* airborne pollen may be different

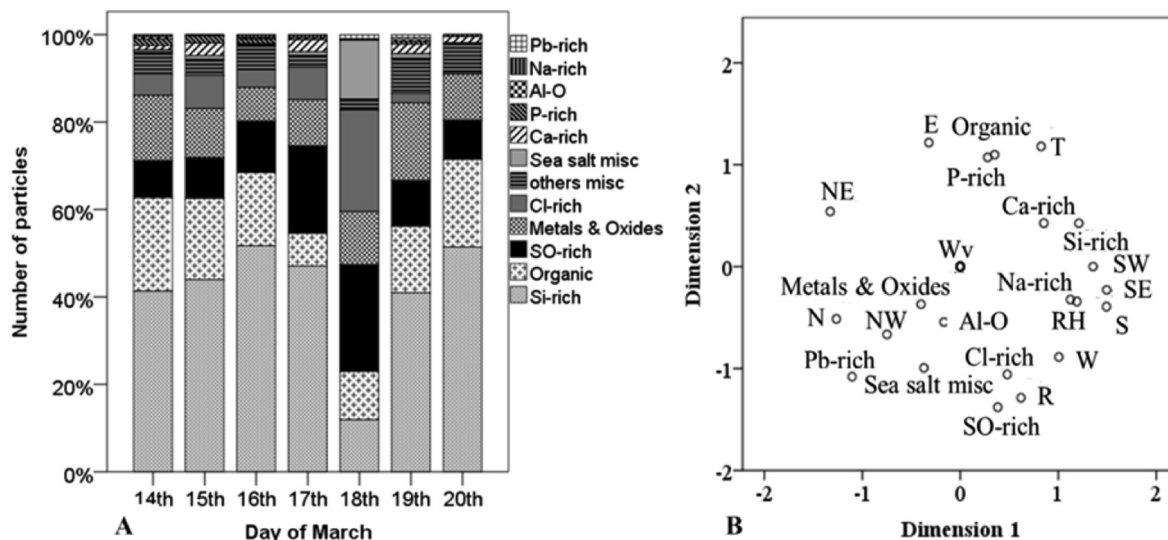


Fig. 6. Relative daily variations (percentage of total counts) of the chemical composition of particles adsorbed to the pollen wall of *Acer*, *Pinus* and *Platanus* (A) and biplot of dimensions 1 and 2 resulting from principal components analysis of weather parameters and particulate matter chemical composition adsorbed to the pollen wall (B; acronyms indicated in Table 4).

Table 4
Dimension weights of principal component analyses performed with the weather parameters and particulate matter chemical composition adsorbed to the pollen wall of *Acer*, *Pinus* and *Platanus* (values < −0.6 and > 0.6 marked in bold).

		Component loadings			
		Dimension			
		1	2	3	4
		32.7	25.5	17.2	13.9
Particles chemical classification	Si-rich	0.75	0.27	0.32	−0.26
	Organic	0.17	0.67	0.65	0.14
	SO-rich	0.24	−0.86	0.08	−0.03
	Metals & oxides	−0.25	−0.23	0.75	−0.47
	Cl-rich	0.30	−0.66	0.45	0.00
	Sea salt misc.	−0.23	−0.62	0.49	−0.52
	Ca-rich	0.53	0.27	0.05	−0.77
	P-rich	0.22	0.69	0.68	0.13
	Al–O	−0.10	−0.34	0.64	−0.16
	Na-rich	0.70	−0.20	−0.19	0.57
	Pb-rich	−0.69	−0.67	0.21	−0.10
	Temperature (T)	0.51	0.74	0.30	0.16
	Relative Humidity (RH)	0.74	−0.21	0.22	0.58
	Precipitation (R)	0.39	−0.80	−0.39	−0.13
Wind direction	Wind velocity (Wv)	−0.11	−0.15	−0.70	−0.64
	North (N)	−0.79	−0.32	0.15	0.40
	Northeast (NE)	−0.83	0.34	0.04	−0.04
	East (E)	−0.20	0.76	−0.44	0.00
	Southeast (SE)	0.93	−0.24	−0.02	−0.28
	South (S)	0.93	−0.14	−0.04	0.14
	Southwest (SW)	0.84	0.00	0.30	−0.37
	West (W)	0.63	−0.55	−0.27	0.44
	Northwest (NW)	−0.47	−0.41	0.55	0.53

from the other two pollens, as demonstrated by the significant correlations between the average number of particles and wind direction. Another possible explanation could be the pollen electrostatic charges. Wind-dispersed pollen grains are electrically charged (average of 0.8 fC in magnitude but can be up to 40 fC) (Bowker and Crenshaw, 2007) which influences its transport and capture on the flower stigmas (Bowker, 2001). Therefore it is likely that electrostatics influence pollen and PM interaction in the atmosphere. The fact that different pollen species present diverse average electrostatic charges (Bowker and Crenshaw, 2007) may be

related to the variation of the average number of particles per pollen type observed in our study.

During our study, the equivalent diameter of the PM adsorbed to airborne pollen presented its highest distribution in the accumulation mode ($0.1 < d < 1.0 \mu\text{m}$). The accumulation mode particles are too small for gravitational settling and inertial impaction removal processes and therefore reside in the atmosphere for a long time. They are most efficiently removed by nucleation scavenging (Anastasio and Martin, 2001; Giere and Querol, 2010). Airborne pollen can have a scavenging role for these particles, since pollen

lifetime in the atmosphere can vary from half a day up to a couple of days (Sofiev et al., 2013). However, in our study, most of the pollen sampled showed less than 10% of its area occupied by particles and therefore the scavenging process can only be considered significant when extremely high airborne pollen concentrations are reached (Sofiev et al., 2013).

Daily variations in the physical characteristics of particles adsorbed to airborne pollen cannot be attributed to pollen size variations between the studied days since no statistical significant differences were observed among each pollen type's size. Also, the inconsistent correlations found with temperature and relative humidity does not explain the observed variations. These may be related to PM atmospheric concentrations. Significant correlations were found between the daily PM atmospheric levels and percentage of area occupied by particles in *Acer* and *Pinus*. Also, percentage of area occupied by particles in *Acer* and *Platanus* was significantly correlated to the PM atmospheric levels from the previous days (data not shown). These results suggest a time-lag exposure of pollen to PM possibly dependent on its emission source but can also be related to each pollen species specific settling velocity. For instance *Platanus* has a lower settling velocity (1.0 cm sec⁻¹) (Zhang et al., 2014) than *Pinus* pollen (from 2.1 to 4.5 cm s⁻¹) (Williams, 2008) and therefore greater atmospheric lifetime favoring the scavenging process. Besides, this may point to the existence of a saturation point from which pollen cannot adsorb smaller PM anymore, either because it becomes too heavy to fly, because of electrostatic charges or other physical mechanisms, which would be interesting to investigate.

Concerning the chemical composition of the particles adsorbed to airborne pollen, it was dominated by Si-rich, organic compounds, SO-rich, Fe-rich and Cl-rich.

Aluminosilicates made up an important group of silicates either as Al–Si or associated with other elements like Fe, K, Mg, Na, Ca or Zn. The Si-rich particles were positively associated with wind coming mostly from SE to W. These directions correspond to sediment-rich area SW from the River Douro estuary and to the SE to the River Douro rocky exposed slopes dominated by the Porto granite, of micaschists, metagraywackes and gneisses.

The presence of Cl-rich particles, particularly of Na and Mg as observed in our study, is usually associated with the existence of an important coastal influence (Moreno et al., 2004), since these elements make up for 90% of the seawater salinity (Lide, 2007). In fact, sea-salt has been reported as one of the major components of coarse aerosol next to the shore, but its relative importance diminishes when we consider smaller particle sizes (Putaud et al., 2010; Worobiec et al., 2007), which can explain why Cl-rich particles are only the fifth most abundant PM type adsorbed to the pollen wall.

Sea salt particles can also contain sulphates, particularly of sodium and potassium (Giere and Querol, 2010) like the most abundant SO-rich particles observed in our study. After PCA, the association between Cl-rich, SO-rich and sea salt misc. particles suggests their possibly similar origin. Furthermore, their positive relation with wind blowing from the shore (W) reinforces its origin in the Atlantic Ocean. However, in urban sites, the presence of sulphates can also be related to anthropogenic sources, formed as secondary particles (Moreno et al., 2004).

The SO-rich particles, the elemental and organic carbon as well as the Metals & Oxides composed by Fe–O and other metals such as Zn, Ni, V, Ti can be assigned to road traffic and industrial sources (Moreno et al., 2003, 2004). V and Ni are typically associated with the combustion of fuel oil and petroleum coke (Calvo et al., 2013; Giere and Querol, 2010) that correspond to important stationary sources of anthropogenic atmospheric pollutants in the Porto area. The close location of our sampling point to one of the busiest

entrances of the city of Porto can explain the high number of organic compounds, possibly due to internal combustion of oil and petrol in vehicles (Moreno et al., 2004), as well as of Fe–O and Fe–Si derived from resuspension of road dust due to road traffic (wear and tear of tires, oil burning, abrasion of mechanical parts of road vehicles, oil lubricants) (Giere and Querol, 2010; Moreno et al., 2004).

Since PM presents physical and chemical characteristics that vary according to location and have different degrees of toxicity for the environment and public health (Calvo et al., 2013), a further long-term study on the composition of PM attached to airborne pollen, regarding for example element ratios, enrichment factors, organic characterisation should be performed. This study would be very important to understand how this synergy affects not only the pollen allergens but also the respiratory tract.

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