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Differential *Quercus* spp. pollen-particulate matter interaction is dependent on geographical areas

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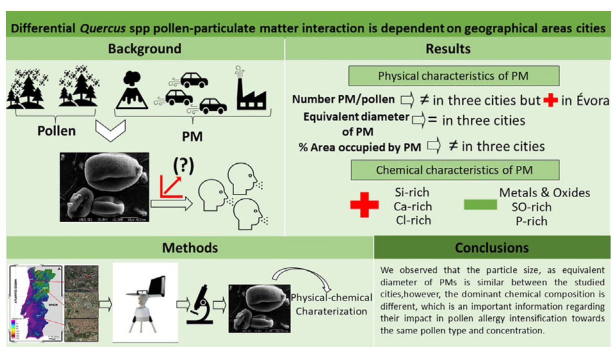
HIGHLIGHTS

- It was studied the properties of PM adhered to pollen in cities with contrasting environments.
- Airborne pollen was sampled, analyzed by SEM-EDS and air masses back trajectories were determined.
- Adhered particles size is similar among regions, with most having an equivalent diameter < 3 μm.
- Particles chemical composition was dominated by Si-rich, Ca-rich, Cl-rich and SO-rich, associated with other elements.
- Exposome impact on pollen allergies should consider PM concentration and chemical composition.

GRAPHICAL ABSTRACT



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ABSTRACT

Particulate matter (PM) and pollen interaction, either airborne or at the respiratory mucosa needs further clarification, as allergic reaction intensification can be related to the PM physical characteristics and toxicity. This study aimed to investigate the physical-chemical properties of PM that can adhere to the pollen wall during its transport or inhalation, using *Quercus* spp. as a model, in three Portuguese cities with different geographical locations, meteorological influence and urbanization levels. Possible sources were evaluated through air masses trajectory analysis using the HYSPLIT model and correlation with meteorological factors. The sampling was performed using a 7-days Hirst-type volumetric sampler, and the pollen grains were observed using a Field Emission Electron Probe Microanalyser for PM analysis. A secondary electron image of each pollen grain was taken, to determine the adhered particles characteristics and energy dispersive x-ray spectroscopy (EDS) spectra were obtained for individual particles. A total of 484 pollen grains was observed, with 7683 particles counted and 1914 EDS spectra analyzed. The particle's equivalent diameter ranged from 0.3-16 μm, with most having a diameter < 3 μm. For the three cities, there were significant differences in the number of particles per pollen and the % area occupied by the particles. Particles adhered were mainly

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Si-rich, but variations in other dominant groups were observed. For Évora and Guarda, Ca-rich, SO-rich were second and third more representative, while Porto were Organic and Cl-rich. Metals&Oxides were found in all cities with the highest number in Porto. P-rich particles were only found in Évora. Sea salt particles were observed in Évora, coincide with air mass trajectories possible carrying them from the Mediterranean Sea. In conclusion, the PM physical characteristics are similar between the studied cities, however, the dominant chemical composition is different, certainly impacting the exposome influence and pollen-allergy intensification towards the same pollen type and concentration.

1. Introduction

Pollen allergy is growing exponentially, particularly in urbanized environments, affecting between 10 and 30% of the world population (Ring, 2012). In the second half of the 20th century the pollen allergy was already very common in Western Europe. It was considered known as a rare disease, however, the prevalence has been increasing, and it is expected that by the year 2025, 50% of the European population will be affected by this pathology. Currently, 300 million people suffer from asthma and 400 million from rhinitis (Pawankar, 2014). The cause of this increase has been associated with several factors, including urbanization, industrialization, agriculture, air pollution, climate change and decreased biodiversity. The interaction between airborne pollen grains, air pollutants, and the immune system have been often described as the main trigger (Ring, 2012).

Among air pollutants, atmospheric particulate matter (PM) is one of the highest concerns, particularly related to human health. PM is a mixture of solid and liquid particles suspended in the atmosphere and may have an anthropogenic origin (car traffic, combustion, agriculture, or industry) or a natural origin (volcanic eruptions, forest fires, ocean-derived and bioaerosols) (Koenig, 2000). Generally, PM is classified according to their size or diameter and composition, ranging from nanometers (nm) to tens of micrometers (μm). Particles with less than 10 μm are classified as PM_{10} and defined as inhalable particles (Byeon et al., 2015; Ott et al., 2008). PM_{10} can reach only the upper respiratory tract and may cause increased severity of bronchial asthma attacks, cause or aggravate states of bronchitis or other lung diseases and reduce the body's ability to fight infections. Particles with a diameter below 2.5 μm ($PM_{2.5}$) are defined as fine particles, which can penetrate deep into the human respiratory system, reaching the tracheobronchial tract, increasing the specific risk of mortality, particularly from cardiovascular diseases. The smaller particles, $PM_{0.1}$, called ultrafine, have diameters of less than 0.1 μm and can affect the alveolar tract and reach the circulatory system, causing damage to internal organs (Baldacci et al., 2015; Zhao et al., 2020). In Portugal in 2008, there were between 21 and 40 deaths per 100,000 inhabitants derived from air pollution (WHO, 2011). Estimates from the World Health Organization showed that in 2012, seven million people died due to atmospheric pollution from both indoors and outdoors, which are now considered an individual risk to human health (WHO, 2014).

The link between PM and pollen allergy or bronchial asthma is widely documented in the literature (González-Díaz et al., 2016; Kim et al., 2013; Schiavoni et al., 2017; Kim et al., 2015; Knox et al., 1997), pointing to an adjuvant role of PM in modifying the environment of the respiratory mucosa in which pollen allergens are released, facilitating the access of these allergenic particles to antigen-producing cells, intensifying their response. Phosri et al. (2017) found that on days of high concentrations of PM, the number of medical consultations and hospital admissions by pollinosis increased. It was also verified that the number of admissions to the hospital emergency room due to asthma coincides with the increase in the concentration of PM (Kim et al., 2015). In urban areas, the high concentration of diesel exhaust particles (DEP's) may intensify the development of an immune allergic response (Riedl and Diaz-Sanchez, 2005; Saxon and Diaz-Sanchez, 2005).

The intensification of the allergic reaction may be related to the physical characteristics of PM and its toxicity (Le Souef, 2009; Traidl-Hoffmann et al., 2009). PM may induce cellular stress and toxicity, depending on particle size, chemical composition, and bound molecules on their surface (Kim et al., 2015). PM containing more transition metals, such as iron, increase the production of reactive oxygen species (ROS), causing genotoxic effects (Gilli et al., 2007; Kadiiska et al., 1997). In addition, associated with PM it may be found endotoxins that cause inflammation of the airways (Löndahl et al., 2006).

The airborne interaction between PM and pollen can be explained by coagulation processes, forming a pollen-particle complex (Schiavoni et al., 2017; Gottardini et al., 2004). Pollen grains from polluted areas, when compared to pollen from areas with low pollution, are more exposed to PM and therefore more likely to transport them on their surface. Duque et al. (2013) observed significant differences between the elemental composition of airborne pollen wall compared to that of pollen harvested directly from the plant. Guedes et al. (2009) observed the presence of diesel exhaust particles in pollen of the species *Chenopodium album*, harvested from areas with high road traffic. In addition, since PM presents physical and chemical characteristics that vary by location and have different degrees of toxicity for the environment and public health (Calvo et al., 2013; Sénéchal et al., 2015), likely the PM adhered to airborne pollen could reflect the overall chemical composition of the aerosol fraction of a site at a given moment. Furthermore, when humans breathe, the air inhaled enters the respiratory system in a whirlwind manner, which facilitates the PMs adhesion to the sticky pollen wall. Also, the meteorological parameters can influence the aggregation of PM to pollen grains. The high air temperature and consequently low relative humidity can be associated with lower agglomeration capacity of PM's (Anastasio and Martin, 2001). The wind speed can be associated with the high dispersion and possibly the low adsorption capacity of PM to the pollen surface. Cichowicz et al., 2020 showed that the lower wind speed is associated with higher concentration of PM in the air (Cichowicz et al., 2020). Wind direction is another condition that can influence the adherence of PM to the pollen surface, because studies have shown that the distribution of sources is very important for the detection of biological and organic particles (Palacios et al., 2000).

Therefore, the goal of this study was to characterize the physical (size, surface, particle number) and chemical (inorganic and organic) properties of the PM that can adhere to the pollen wall during its transport or inhalation, using *Quercus* spp. as a model, in three cities with different geographical positions, Porto (Atlantic coastal city), Guarda (high altitude city), and Évora (small size city located at a flat rural region). Also, it investigated the relation between meteorological parameters and back trajectories air masses analysis to ascertain changes in the PM characteristics. *Quercus* pollen was used due to its high abundance in Portugal.

2. Methods

2.1. Study area

The study was conducted in the cities of Évora, Guarda and Porto, located in Portugal (Fig. SI-1). The city of Évora (38.568°N; 7.912°W, 293 m asl), with about 50,000 inhabitants is located in the vast region of Alentejo, the largest Portuguese region with an area corresponding, to one-third of the country territory, approximately. The town is nestled in a vast mainly flat and rural region, dominated by holm (*Quercus rotundifolia* or *Quercus ilex*) and cork oaks (*Quercus suber*) with underlying semi-natural vegetation, constituting an important agro-silvo-pastoral system termed Montado (Godinho et al., 2016), where in the last years the agriculture pressure is also steadily increasing (Palma et al., 2021). The main sources of local pollution are related to traffic and, in the cold season, biomass burning used for domestic heating (Malico et al., 2017). Sometimes, long-range transport of anthropogenic or natural aerosols occurs, as urban/industrial (Santos et al., 2008, 2013), forest fire (Sicard et al., 2019; Salgueiro et al., 2021), desert dust (Bortoli et al., 2009; Valenzuela et al., 2017) and bioaerosols (Galveias et al., 2021). According to Köppen Climate Classification, Évora is characterized by a temperate climate with warm and dry summers that can be described as a Hot-summer Mediterranean climate (Table 1) (www.ipma.pt last accessed February 16, 2022). The most common pollen species in Évora are Cupressaceae, *Platanus*, *Quercus*, *Olea* and Poaceae. Other species are found but in minor concentrations as *Rumex*, *Populus*, Urticaceae, *Chenopodium*, *Alnus*, *Casuarina* and *Eucalyptus* (Camacho et al., 2020; https://www.ipma.pt/pt/saude/polens/ last accessed September 3, 2021; https://lince.di.uevora.pt/polen/index.jsp, last accessed September 3, 2021). The sampler was placed at the Évora Atmospheric Sciences Observatory (EVASO), located on the roof-top of the Science and Technology School of the University of Évora, about 10 m above the ground. The facility is located in the city center, surrounded by trees and buildings, with low road traffic in the neighborhood (Fig. SI-1).

The city of Guarda (40.53° N, 7.26° W, 1056 m asl) with 42,541 inhabitants, is the highest in mainland Portugal at an altitude of 1056 m. It is the capital of the Guarda district, which is in the country central region in the sub-region of Beiras and Serra da Estrela. The landscape is essentially rural where the existing industry is not responsible for the emission of significant air pollution. The main source of gas emissions into the atmosphere comes from car traffic within the city and the surrounding highways, especially the A23 and A25, and from the combustion of biomass and natural gas used to heat the buildings. Also, some pollution originating in industrial areas in the coastal cities of the country can be transported to inland areas. Guarda has a warm summer and temperate climate according to Köppen Climate Classification (Table 1) (www.ipma.pt last accessed February 16, 2022). The most frequent pollen types are *Quercus* spp., Pinaceae and Poaceae corresponding to about 50% of the observed total pollen. Other pollen types are found, although in minority concentrations, Cupressaceae, Urticaceae, Apiaceae, Oleaceae and Polygonaceae (Lisboa et al., 2016). The sampling point was set on the roof of the Municipal Theater building, 20 m above ground level (Fig. SI-1), close to the main streets of the city center with some traffic during the day and nearby residential and service areas, in the vicinity of public gardens and the forest of the old sanatorium and current hospital Sousa Martins.

The city of Porto (41.51° N, 8.61° W, 115 m asl), the second-largest Portuguese city with about 238,000 inhabitants and a population density of 5736 inhabitants per km^2, is limited on the west by the Atlantic Ocean and 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, also presenting a high level of road traffic. The common species of trees found are *Acer spp, Fraxinus spp, Liquidambar spp, Quercus spp, Pinus spp, Platanus spp, Populus spp*, *Prunus* spp. *and Tilia* spp. Regarding herbaceous plants, Plantago, Poaceae, Rumex or Urticaceae predominate. Porto has a warm summer and temperate climate according to Köppen Climate Classification (Table 1) (www.ipma.pt last accessed February 16, 2022). The sampling point was set on the roof of the Faculty of Sciences in Porto, 20 m above ground level (Fig. SI-1), located near a residential area, near the ocean and the Douro River and very close to a road with high highway traffic, considered one of the main entrances of the city, where in rush hours there are frequent traffic jams.

Table 1
Annual values of meteorological parameters at Évora, Guarda and Porto, according to the climatologic normal (1971–2000) provided by the Portuguese Institute for Sea and Atmosphere (https://www.ipma.pt/pt/oclima/normais.clima/1971-2000/; Miranda et al., 2001).

	Tmean, °C	Tmax, °C	Tmin, °C	Prec, mm	RH, %	WD, °	Köppen Climate Classification
Évora	15.9	20.7	11.0	609.3	76	NW	Csa
Guarda	10.9	14.7	7.0	882.0	77	NW	Csb
Porto	14.7	19.2	10.2	1253.5	82	W-NW (summer) and E-SE (Winter)	Csb

2.2. Airborne pollen sampling

Airborne pollen sampling was done using a Hirst-type 7-day volumetric spore trap (Hirst, 1952) that is a one stage impactor, designed to sample air at a rate of 10 L/min, simulating the human inhalation. By passing through a narrow intake orifice (2x14mm) the sampled air impacts onto a clock-driven drum rotating at an angular velocity of 2 mm/h, taking 7 days to perform a complete turn. The drum is covered with a double-sided Melinex tape, where airborne pollen is retained (Solvedilla et al., 2007). In our study, the Melinex tape was coated with a double-sided adhesive carbon tape, aiming at preserving the integrity of the pollen wall and allowing the following analysis. After exposure, this tape is cut into seven 48 mm long segments, representing each one day of sampling, that is mounted on a microscopic glass slide.

Four consecutive days were sampled in April 2017 (21st to 24th) (Fig. SI-5), which corresponded to the only period when peak pollination of *Quercus* spp. was concomitant in all three cities.

2.3. Meteorological data

The meteorological data used for Évora were obtained from the meteorological station installed at the Évora Atmospheric Sciences Observatory (EVASO) where the pollen sampler is also installed, for Porto by a meteorological station located 7 m apart from the pollen sampler at approximately the same altitude and Guarda from an IPMA automatic climatological station, located 1000 m apart from the pollen sampler. The parameters measured were the air Temperature (°C), Relative Humidity (RH; %), Wind Speed and Direction (m/s; °). All variables were measured in 10 s intervals, and then averages were performed to originate hourly and daily data, except for precipitation that is accumulated during those periods. The averaged wind direction values were obtained applying the arctangent to the ratio between the east-west and the north-south components of the wind. The averaged east-west and north-south components are calculated from the wind speed data, excluding zero values. This average is performed according to the procedure described in Campbell Scientific (Scientific Campbell CR1000 Measurement and Control System) (https://s.campbellsci.com/documents/br/manuals/cr1000.pdf last accessed December 21, 2020). All instruments are subject to periodic checks and maintenance.

2.4. Pollen analysis

Scanning Electron microscopy (SEM) with energy dispersive x-ray spectroscopy (EDS) is commonly used for single particle studies (Sobanska et al., 2006), as this method provides useful information on the morphology, elemental composition, density, and origin of aerosols (Kushwaha et al., 2012). In our study, a Field Emission Electron Probe Microanalyser (JEOL JXA-8500F) was used to investigate the physical-chemical characteristics of the particulate matter adhered to the surface of airborne pollen. This equipment combines high SEM resolution with X-ray analysis of submicron areas and is equipped with an EDS spectrometer and 5 WDS (Wavelength Dispersive Xray Spectroscopy) spectrometers (Oxford INCA X-Act) that allowed the simultaneously analysis of 5 elements WDS + 16 elements EDS as well as collecting images derived from backscatter and secondary electron detectors.

The microscopic slides were carbon coated at the same thickness for conductivity and reduction of electron charge. Slides were scanned along 4 to 6 equidistant traverse lines, depending on pollen concentration and *Quercus* spp. pollen was randomly selected and visually identified. A total of 484 pollen grains was observed.

For each pollen grain, a secondary electron image (SEI) was taken (magnifications varied between 2300 and 3300 times) to perform PM physical characterization, and an EDS spectrum (10 kV, 10 nA, 15 s) for all possible individual particles adhered to the pollen surface was obtained (Ribeiro et al., 2015). A total of 1914 EDS spectra was analyzed and used to chemically classify the particles based on the most prominent element peak intensities. This methodology showed image resolution restrictions concerning ultrafine PM, which are certainly adhered to the pollen, but was not possible to measure particles below 1 μm in diameter.

All SEI images were analyzed to determine the size parameters of the different pollen and particles adhered to the pollen wall. For this, pollen, and particle equivalent projected area diameter (diameter of a circle having the same area as the projection area of the particle), the number of particles counted per pollen, percentage of pollen area occupied by particles and particle size distribution were determined using ImageJ software. A total of 7683 particles was counted.

2.5. Statistical analysis

Since data did not followed a normal distribution (assessed by the Kolmogorov-Smirnov test), non-parametric Independent Samples Median test ($p < 0.05$ was accepted) was applied to compare if the number of particles per pollen, the equivalent diameter of pollen and the percentage of pollen area occupied by the particles varied significantly between the three studied cities.

Spearman correlation coefficients ($p < 0.05$ and $p < 0.01$ was accepted) were determined to study the non-linear relationship between the daily average meteorological parameters (mean temperature, relative humidity and wind speed) and daily average physical and chemical characteristics of particulate matter attached to the *Quercus* spp. airborne pollen in each city. For the wind direction, each hourly value of the data was coded according to 8 quadrants: N, NE, E, SE, S, SW, W, NW, then becoming a nominal variable. For each day, the frequency of hourly average wind direction in each quadrant was calculated, and these values were used in the correlation analysis. Graphics and the statistical analysis were done using IBM SPSS statistics v.21 software.

2.6. Backward trajectory of air masses

Backward trajectories of air masses arriving in Évora, Guarda and Porto were calculated using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT). This model, developed by the National Oceanic and Atmospheric Administration (NOAA), is one of the most widely used and complete models for simple trajectory calculations (Rolph et al., 2017; Stein et al., 2015). The meteorological parameters used to calculate the back trajectories are obtained from the Global Data Assimilation System (GDAS) at a spatial resolution of 0.5°. The vertical motion method used to calculate the back trajectories is the vertical velocity. The back trajectories were calculated for 48 h, for every hour of that period, at the arrival levels of 50, 250 and 500 m. The backward trajectories every three hours for the three levels are presented as supplementary material.

3. Results

3.1. Bioclimatic characterization of Évora, Guarda and Porto

Fig. 1 shows the boxplots of the meteorological parameters measured at the meteorological stations of the three sites (Évora, Guarda and Porto, from 21 to 24 April 2017).

The average temperature was fairly similar between the cities of Évora and Porto, however, in the city of Guarda the temperature was approximately 5 °C lower. The Relative Humidity (RH) was in turn quite similar between Évora and Guarda, while on Porto it was in average higher and presented more variability. The wind speed was higher in the Évora and Porto regions compared to Guarda and the wind direction was variable between *E*-W in Évora, while in Guarda and Porto it varied between S-SW (Fig. 1). There was no precipitation during the period of study.

3.2. Quercus spp. pollen equivalent diameter analysis

A total of 245 pollen grains were observed for the city of Évora, 155 pollen grains for Guarda and 84 pollen grains for Porto, with average equivalent diameters of 23.58 ± 4.01 μm, 22.74 ± 4.16 μm and 21.83 ± 3.27 μm, respectively. However, in all study cities, it was possible to divide the *Quercus* pollen into two groups, according to their size (Fig. SI-2), one with pollen with less than 25 μm and another between 27 and 35 μm (Fig. 2). Most of the pollen presented size of less than 25 μm, with the largest dimension group accounting for 30% in Évora, 26% in Guarda and only 10% in Porto. The median equivalent diameter of pollen from Évora was not significantly different from the one in Guarda but both were significantly larger than the one from Porto ($p = 0.008$ and $p = 0.023$, respectively) (Fig. 2).

To analyze if there were significant interdaily differences in the pollen equivalent diameter, an non-parametric independent samples median test was applied. According to the results described in Table 2, there were statistically significant differences in pollen size between the study days for the city of Évora ($p = 0.005$) and Guarda ($p = 0.015$). A clear increase in the average pollen size along the study period was observed in Évora while in Guarda the largest average size was observed on the 22nd and the smallest on the 24th of April. In the city of Porto, there were no significant differences between the days under analysis (Table 2).

3.3. Physical characterization of PM adhered to Quercus pollen

All 484 pollen grains accounted were analyzed for the number of particles per pollen and the percentage area occupied by the particles. Significant statistical differences were observed between the median PM/pollen of the three cities (Fig. 3). The number of pollen grains free of particles varied also between study places, with Guarda presenting most of the observed *Quercus* pollen grains with no particles adhered to its wall

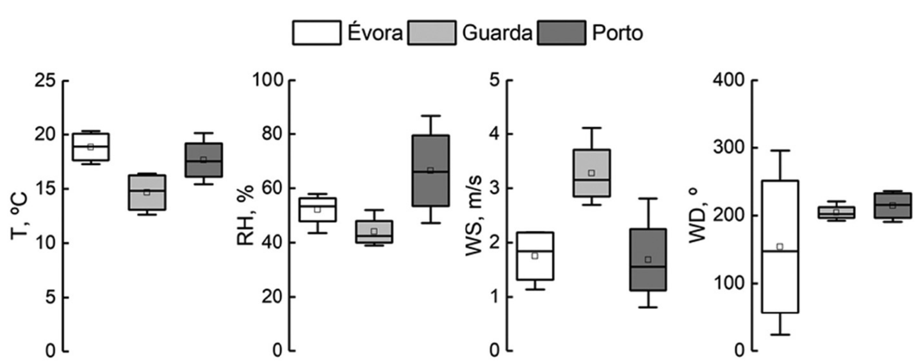

Fig. 1. Boxplots showing the variation of the meteorological parameters in Évora, Guarda and Porto during the days 21–24 April 21 of the year 2017. T: Temperature; RH: Relative Humidity; WS: Wind Speed; WD: Wind Direction.

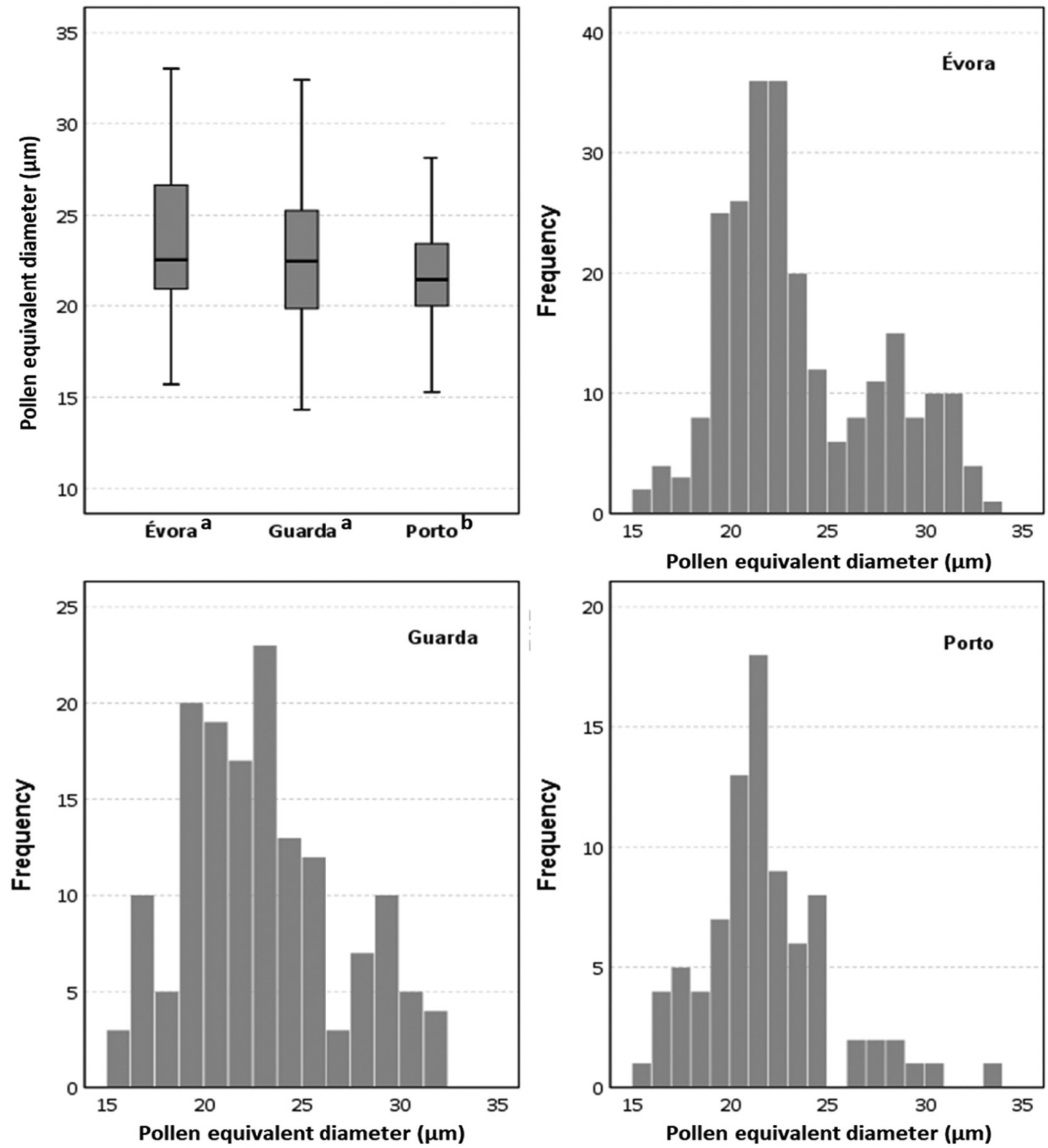

Fig. 2. Pollen equivalent diameter (µm) from Évora, Guarda and Porto (Boxplot and respective histograms). Different letters indicate statistically significant differences given by the Independent-samples median test followed by pairwise comparisons (*p*-value <0.05).

(66%) in opposite to Porto where 52% of the pollen grains had at least one particle and Évora where most of the pollen grains (72%) presented from 1 up to 19 adhered particles. In the city of Évora, the average number of particles per pollen (PM/pollen) was 3.6 ± 3.9, 3-fold higher than those registered in Guarda (1.4 ± 2.6 PM/pollen) and Porto (1.6 ± 2.0 PM/pollen) (Fig. 3).

Regarding the percentage of the *Quercus* pollen area occupied by the particles, in the city of Évora was registered the pollen with the highest area covered by particles, followed by Porto and finally by Guarda with 4.0 ± 5.2, 2.30 ± 3.5 and 2.3 ± 5.7%, respectively. There were significant statistical differences in the median value between the three studied cities (see dash line in Fig. 3).

Table 2
Equivalent diameter of the *Quercus* pollen from Évora, Guarda and Porto in each day analyzed.

Pollen equivalent diameter (mm)															
	Évora					Guarda					Porto				
	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max
21st	87	22.2 ± 3.1[a]	21.5	15.7	31.4	36	22.3 ± 3.9[a,b]	22.3	14.7	31.9	19	22.1 ± 2.7	21.5	17.7	29.3
22nd	61	23.3 ± 3.8[a,b]	22.5	16.8	33.0	57	24.1 ± 4.0[a]	23.3	14.3	32.4	32	22.0 ± 3.3	21.9	15.3	30.9
23rd	46	24.6 ± 4.3[b,c]	23.5	16.0	32.0	36	22.7 ± 4.8[a,b]	22.2	14.8	30.8	25	21.7 ± 3.8	20.9	16.8	33.4
24th	51	25.3 ± 4.5[c]	25.5	15.9	32.9	26	20.4 ± 2.7[b]	20.1	16.3	26.2	8	21.1 ± 3.2	20.9	16.7	26.6
p-value		**0.005**				**0.015**					0.670				

p-value associated with the Independent Samples Median test; different letters indicate statistically significant differences (Independent Samples Median test, *p*-value <0.05). SD: Standard deviation; Min: Minimum; Max: Maximum; NP: number of pollen grains. Bold values indicate the statistical significance at 5% (p-value <0.05).

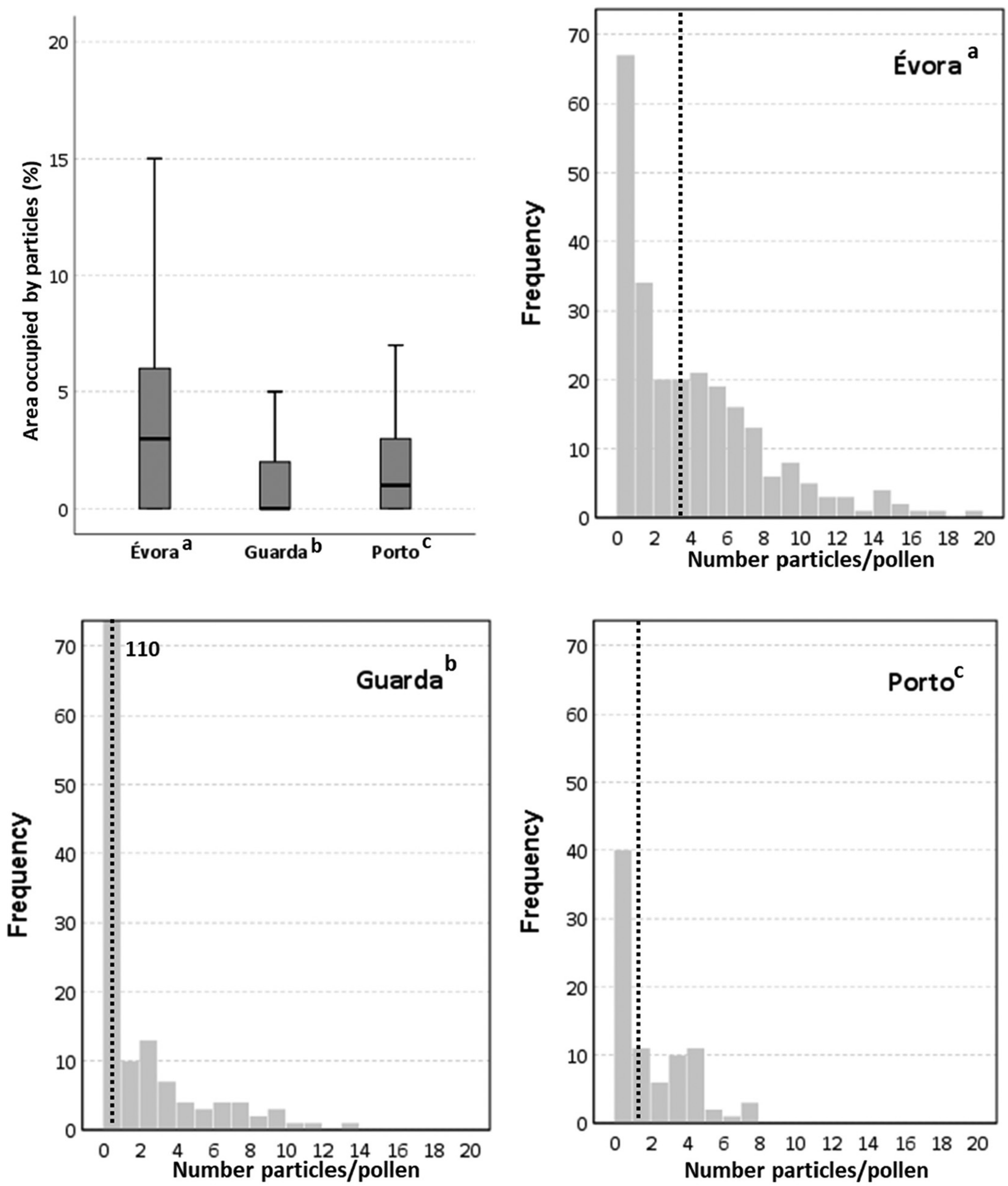

Fig. 3. Percentage of *Quercus* pollen area occupied by particles (boxplot and histograms of the number of particles per pollen) in Évora, Guarda and Porto. Different letters indicate statistically significant differences given by the Independent-samples median test followed by pairwise comparisons (p-value <0.05). The dashed line represents the median value obtained for each site. The different letters within the graphs indicate the statistical significance at 5% (p-value <0.05).

Considering the physical characterization of individual particles adhered to *Quercus* pollen wall, a total of 890 particles were analyzed for Évora, 217 for Guarda and 142 for Porto. The particles size distribution was very similar between the three cities, with average particle sizes of 2.16 ± 1.23 μm for Évora, 2.39 ± 1.61 μm for Guarda and 2.28 ± 1.28 μm for Porto. The equivalent diameter values ranged between less than 1 μm up to 16 μm, with the majority of the particles presenting a diameter lower than 3 μm (83% in Évora, 81% in Guarda and 75% in Porto) (Fig. 4).

An interdaily comparison analysis of the number of PM per pollen, the equivalent diameter of particles adhered to the pollen wall and percentage area of pollen occupied by particles was carried out for the three cities, between April 21st and 24th (Table 3). There was no significant differences between the sampling days for Évora and Guarda, $p < 0.179$ and $p < 0.220$, respectively. However, in Porto, there are significant differences between days, where the 21st of April had a significantly lower number compared to the other days ($p < 0.001$) (Table 3). As for the particle equivalent diameter, no significant differences exist between the days in Évora ($p < 0.327$), Guarda ($p < 0.070$) or Porto ($p < 0.236$). For the percentage area occupied by the particles, there was no significant differences between the sampling days in Évora ($p < 0.058$) and Guarda (p < 0.220) while in Porto, the day 21st of April had a significantly lower percentage than the other days (p < 0.001) (Table 3).

The non-linear correlation coefficients between daily average physical characteristics of particulate matter attached to airborne *Quercus* pollen and daily average meteorological parameters were computed for the study period. Overall, it was observed that wind direction is the parameter with the highest significant correlation with particles physical characteristics (Table 4). Wind speed has a negative significant correlation with the number of particles/pollen ($p < 0.043$) but no correlation with the other physical characteristics. Wind direction from E has a negative significant correlation with the number of particles per pollen, as well as NW winds with the equivalent diameter of particles (maximum) and the maximum

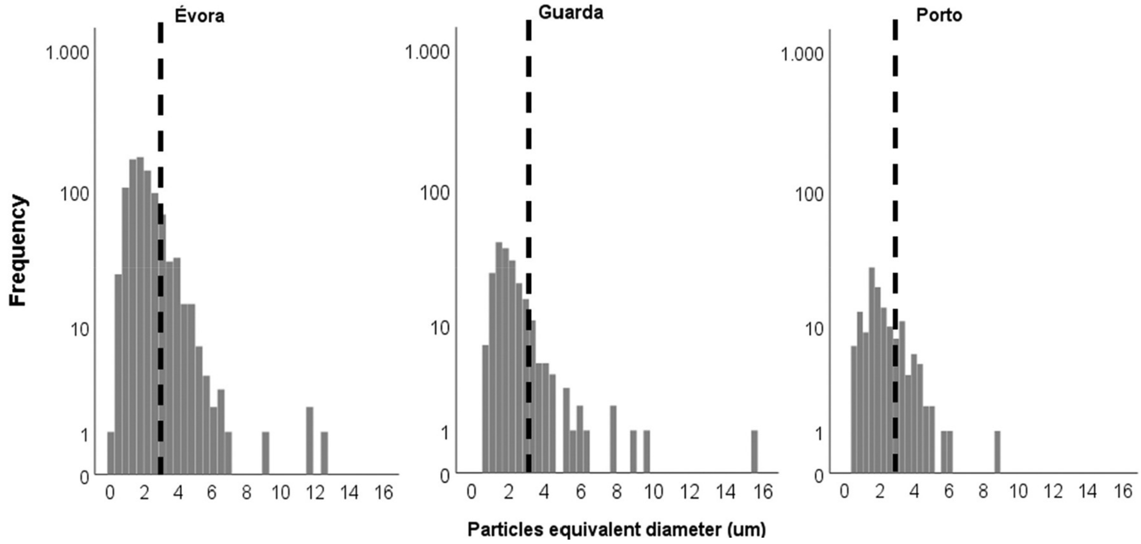

Fig. 4. Size distribution of the equivalent diameter of measured particles adhered to airborne *Quercus* pollen wall in Évora, Guarda and Porto (dashed line marks value with 3 μm size). The Independent Samples median test (*p*-value <0.05) shows that particle equivalent diameter between Évora, Guarda and Porto is not significantly different.

area occupied by particles. Opposite, winds from the WS quadrant have a positive and significant correlation with the number of particles per pollen (maximum) and the equivalent diameter of particles.

Average temperature and Relative humidity did not show a correlation with particles physical characteristics (Table 4).

3.4. Chemical classification of PM adhered to Quercus pollen

The chemical classification of 846 individual particles was analyzed and described in Table 5, corresponding to 585 particles for the city of Évora, 150 for the city of Guarda and 111 for the city of Porto (Fig. SI-3).

Si-rich was the most abundant group in the three regions, reaching values at Évora, Guarda and Porto of 64%, 89% and 65%, respectively (Table 5). The elements associated with the Si-rich group that were observed in higher concentration were AlSi rich, abundance 62% in Évora, 50% in Guarda and 39% in Porto, and SiO with an abundance of 18%, 18% and 25% in Évora, Guarda and Porto, respectively.

Other groups were present, and variations between the studied cities relative to the dominant ones were observed. For the city of Évora and Guarda, Ca-rich (most abundant association Ca-C-O), SO-rich (most abundant association SO-Ca) were second and third more representative while for Porto were particles with an organic origin, corresponding to almost 12% and Cl-rich. In Évora, particles with organic-origin account for less than 5% and Cl-rich around 4%.

The Metals & Oxides group could be found in all cities with the highest number in Porto followed by Évora. Sea salt particles were observed in Porto and Évora, in similar amounts, but were absent in Guarda while P-rich particles were only found in Évora.

It is interesting to notice that among the same chemical group there were differences observed in the particle size distributions between the study regions (Fig. 5). Although most of the particles belong to the $PM_{2.5}$ fraction, for instance, the size of the Si-rich particles at Guarda is greater than 1 μm, compared to the other cities that contain particles with sizes lower than 1 μm. A similar situation happens in Évora for the SO-rich

Table 3
Daily values of the pollen equivalent diameter and measured physical characteristics of particulate matter adhered to the *Quercus* spp. pollen wall in three cities, Évora, Guarda and Porto.

	Évora					Guarda					Porto				
	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max	N	Mean ± SD	Median	Min	Max
Number of Particles/pollens															
21st	87	3.2 ± 3.4	2	0	19	36	1.4 ± 2.5	0	0	9	19	0.4 ± 1.2[a]	0	0	5
22nd	61	3.9 ± 3.5	3	0	14	57	1.0 ± 2.1	0	0	10	32	1.4 ± 1.6[b]	1	0	5
23rd	46	2.7 ± 3.8	1	0	15	36	1.3 ± 2.6	0	0	13	25	2.2 ± 1.8[b]	2	0	6
24th	54	4.9 ± 4.8	4	0	17	26	2.5 ± 3.6	0	0	11	8	3.4 ± 3.2[b]	3	0	7
p-value	0.179					0.220					**0.001**				
Equivalent diameter of each particle (μm)															
21st	276	2.1 ± 1.5	1.8	0.3	12	52	2.2 ± 1.5	2.0	0.7	8	7	1.7 ± 0.7	1.7	0.8	3
22nd	239	2.2 ± 1.3	1.9	0.4	12	55	2.5 ± 2.2	1.9	0.8	16	46	2.4 ± 1.1	2.3	0.6	5
23rd	127	2.2 ± 1.1	1.9	0.7	6	46	2.2 ± 1.2	1.8	1.1	6	54	2.3 ± 1.6	1.8	0.4	9
24th	248	2.2 ± 0.9	2.0	0.6	6	64	2.6 ± 1.4	2.3	1.0	10	35	2.2 ± 1.1	1.8	0.7	5
p-value	0.327					0.070					0.236				
Area of pollen occupied by particles (%)															
21st	87	4.2 ± 5.7	2.2	0	31.5	36	1.8 ± 4.0	0	0	18.9	19	0.3 ± 0.9[a]	0	0	3.7
22nd	61	4.7 ± 5.9	3.1	0	33.7	57	2.0 ± 6.9	0	0	46.8	32	2.0 ± 2.8[b]	0.9	0	11.0
23rd	46	2.7 ± 3.8	0.1	0	13.9	36	1.3 ± 2.8	0	0	10.2	25	3.6 ± 4.6[b]	2.3	0	20.9
24th	54	4.1 ± 4.1	3.8	0	16.8	26	4.8 ± 7.4	0	0	22.2	8	4.2 ± 4.1[b]	4.1	0	11.0
p-value	0.058					0.220					**0.001**				

p-value associated with the Independent Samples Median test; different letters indicate statistically significant differences (Independent Samples Median test, *p*-value <0.05). SD: Standard deviation; Min: Minimum; Max: Maximum; N: number of pollens/number of particles. Bold values indicate the statistical significance at 5% (p-value <0.05).

Table 4
Spearman correlation coefficients between daily average physical characteristics of particulate matter attached to airborne *Quercus* pollen, daily average meteorological parameters in Évora, Guarda and Porto for the days 21–24 April 2017. Values presented correspond to the coefficient's correlation of the Spearman test.

		T, °C	RH, %	WS, m/s	WD, °							
					N	NE	E	SE	S	SW	W	NW
N° PM	Mean	ns	ns	−0.592*	ns	ns	−0.802*	ns	ns	ns	ns	ns
	Max	ns	ns	ns	ns	ns	ns	ns	0.868**	−0.796*	ns	ns
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
PED	Mean	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.876**	ns
	Max	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.862**
	Min	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
% área occup.	Mean	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Max	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.895**

N° PM: number of particles per pollen; PED: particle equivalent diameter in μm; Min: minimum; Max: maximum; T: temperature; RH: relative humidity; WS: wind speed; WD: wind direction.

** Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

particles that are present in this city in sizes lower than 1 μm, which does not occur in Porto and Guarda. Most particles of organic and metals & oxides nature in Évora have a size lower than 1 μm (Fig. 5).

The daily variations in the chemical composition of the particles adhered to *Quercus* wall pollen wall are represented in Fig. 6. Si-rich particles are the dominant particles in the days analyzed for the three regions. The daily concentrations over the 4-days period are very similar, both in Évora and Guarda, whereas for Porto there is an abrupt variation from the first day considered (21st April) presenting 100% of Si-rich particles, down to about 58% on the 24thApril.

Interdaily differences in the particle chemical composition are also observed for the other group-types. In the city of Évora, Cl-rich, SO-rich and organic particles presented a great decrease in concentration on the 23rd while particles of sea salt origin were only found on this day. Metals & Oxides particle group had a daily stable presence but at minor concentrations. In Guarda, on 21st, in addition to the Si-rich group, only particles of Metal & Oxides origin were observed. Ca-rich, SO-rich and Cl-rich particles presented interdaily variations, with Ca-rich being higher on the 22nd, while SO-rich was on the 24th. On the 22nd it was observed the greater decrease in the number of Si-rich particles and the only day with particles of organic origin. In Porto, the 22nd of April presented the most diversity in the particle chemical composition, with all accounted groups observed. Although Porto is a coastal city, particles of sea salt origin were only present in this day. Organic and Cl-rich particles have the greatest representation on the 23rd and Ca-rich particles were only found on this day. Particles of Metal & Oxides origin were the most present on the 24th.

The HYSPLIT model was used to estimate the possible source contribution of particles adhered to the surface of pollen grains (Fig. SI-4). For the city of Évora on 21st the air masses originated from the East during the morning, turning slightly to the southeast throughout the afternoon (supplementary material). The dominant chemical composition of the particles on this day was Si-rich, Ca-rich, Cl-rich and SO-rich. The back trajectories are shown in Fig. 7 for the selected time of 15:00 and arrival level of 50 m, whereas a more complete set of back trajectories can be found as supplementary material. On the 22nd, the air mass arriving at Évora continued from the southeast during the night, gradually moving to the east in the early morning and maintaining this origin for the rest of the day; the dominant particles were from Si-rich, Ca-rich, SO-rich with the increase of other particles and organic material compared to the previous day. On the 23rd the presence of sea salt particles is observed, coinciding with trajectories from the east, some coming from the western Mediterranean (Fig. SI-4) that arrived at Évora on the night and morning of 23rd April. On 24th the lowest air mass originated from the northwest, passing the industrialized coastal areas of Lisbon and Setúbal, whereas the highest (at 500 m) rotates during the day from northeast/east in the early hours, to northwest in the evening, observing the increase of Mixed particles, Organic particles, and Metals & Oxides, nonetheless, in a small percentage.

For the city of Guarda, on 21st, the air masses originated from the east, sometimes slightly northeast throughout the day. The dominant chemical particles were Si-rich particles. A small percentage of Metals & Oxides particles were also detected, which did not happen on the remaining sampling days. On 22nd, air mass originated from the east until the end of the afternoon, turning northwest. There was an increase in particles of organic origin, Ca-rich particles and SO-rich. On 23rd, air mass maintained their origin from the east, and only Si-rich and Ca-rich particles were detected. On the 24th, the trajectory seems to be local most of the day, extending from the

Table 5
Chemical classification, based on the element peak intensities of EDS spectra and frequency of particulate matter adsorbed to the airborne *Quercus* pollen.

Groups	Elements	Frequency (%)			Groups	Elements	Frequency (%)		
		Évora	Guarda	Porto			Évora	Guarda	Porto
Si-rich	Si-O; Ca-Si; Ca-K-Si; K-Si; Mg-Si; Na-Si; Cl-Si Na-Mg-Si; Ca-Mg-Si Al-Si-Fe-O; Al-Ti-Si; Al-Si; K-Al-Si; Ca-Al-Si; Mg-Al-Si; Zn-Al-Si;	63.6	89.3	64.9	Metals & Oxides	Fe-rich; Fe-O; Fe-Ni; Ti-O	1.4	0.7	3.6
					P-rich	P-O; P-O-Ca; P-O-Mg;	0.9	–	–
SO-rich	SO, SO-Na; SO-Ba; SO-Ca-K; SO-Fe; SO-Sr SO-K; SO-Ca; So-Cu SO-Ca-Na;	7.7	4.0	3.6	Cl-rich	Cl-Mg; Cl-Na; Cl-Na-Mg; Cl-K; Cl-Na-K; Cl-Ca-K Cl; Cl-Ca	4.1	–	5.4
Ca-rich	Ca-rich; Ca-C-O; Ca-Mg-C-O;	9.1	4.7	3.6	Sea salt misc	Na,Mg,Cl,Ca,K, S,Si, P; SO-Ca-Cl-Na;	1.2	–	1.8
Organic	C-rich (elemental & organic)	4.8	1.3	11.7	Mix	–	7.4	–	5.4

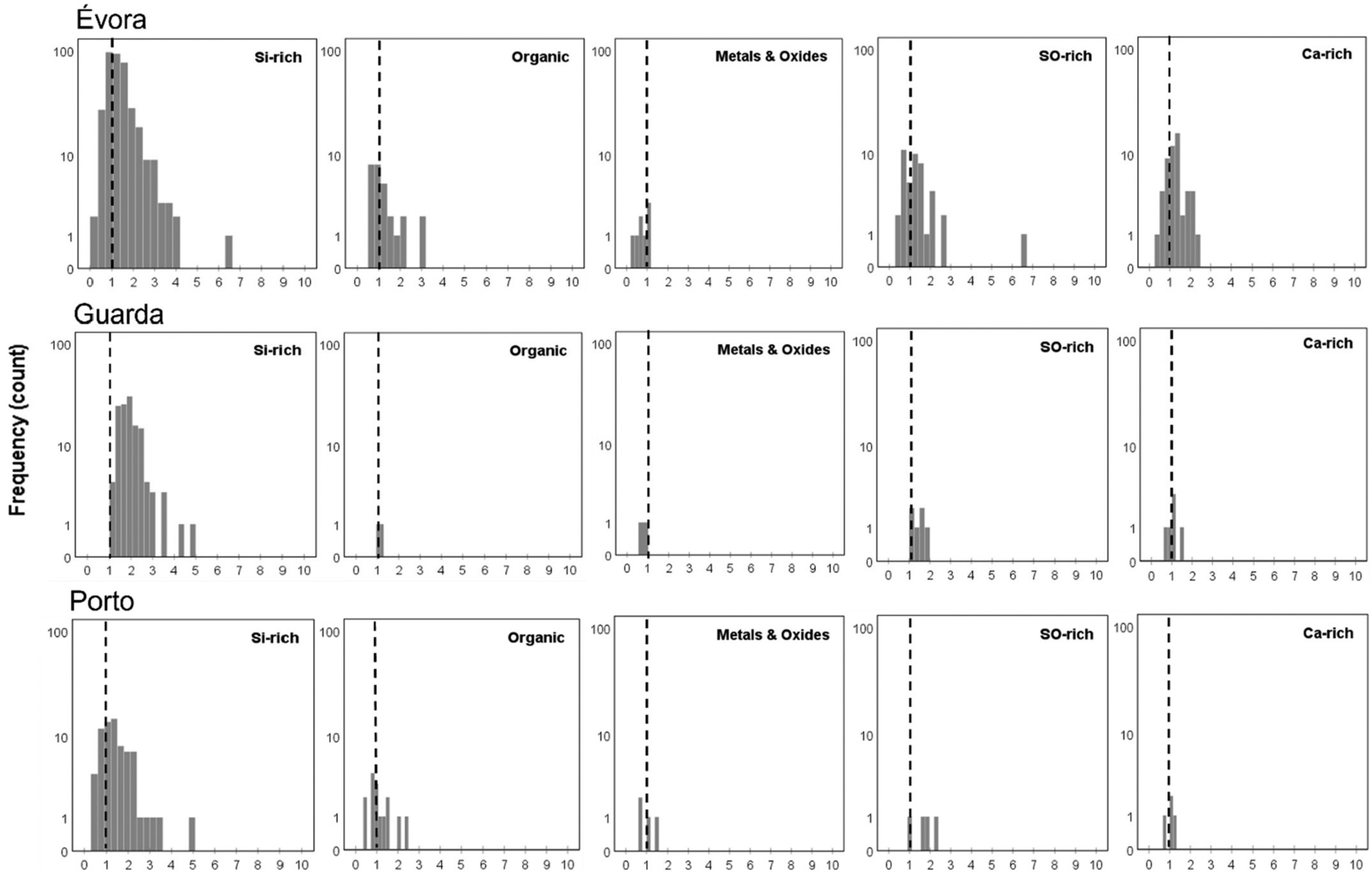

Fig. 5. Size distribution of the equivalent diameter of dominant chemical groups of particles adsorbed to the pollen wall of airborne *Quercus* spp. Dashed lines correspond to particles with a size of 1 μm.

Atlantic Ocean coastal regions in the late afternoon and evening, with an increase in SO-rich particles and the existence, albeit in small concentrations, of Cl-rich particles (Figs. 6 and 7).

For the city of Porto on 21st the air mass originated from east/southeast throughout the day, where only Si-rich particles were found. On 22nd the air mass would originate from the southeast, however, around 15:00 the back trajectory passes through the coastal zone, taking a short turn before reaching the end. This trajectory is coincident with the appearance of sea salt particles (Figs. 6 and 7). On 23rd the air mass originates from the northeast passing through the coastal zone near Galicia. During the afternoon

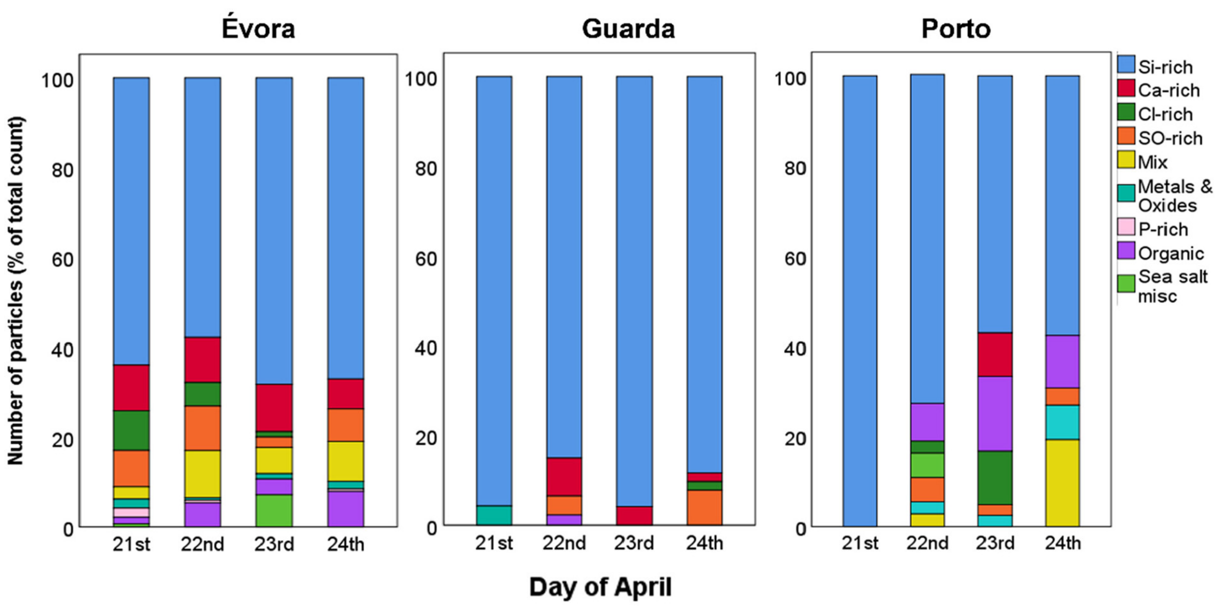

Fig. 6. Daily variation (% of total counts) of the chemical composition of particles adsorbed to the pollen wall of *Quercus* spp.

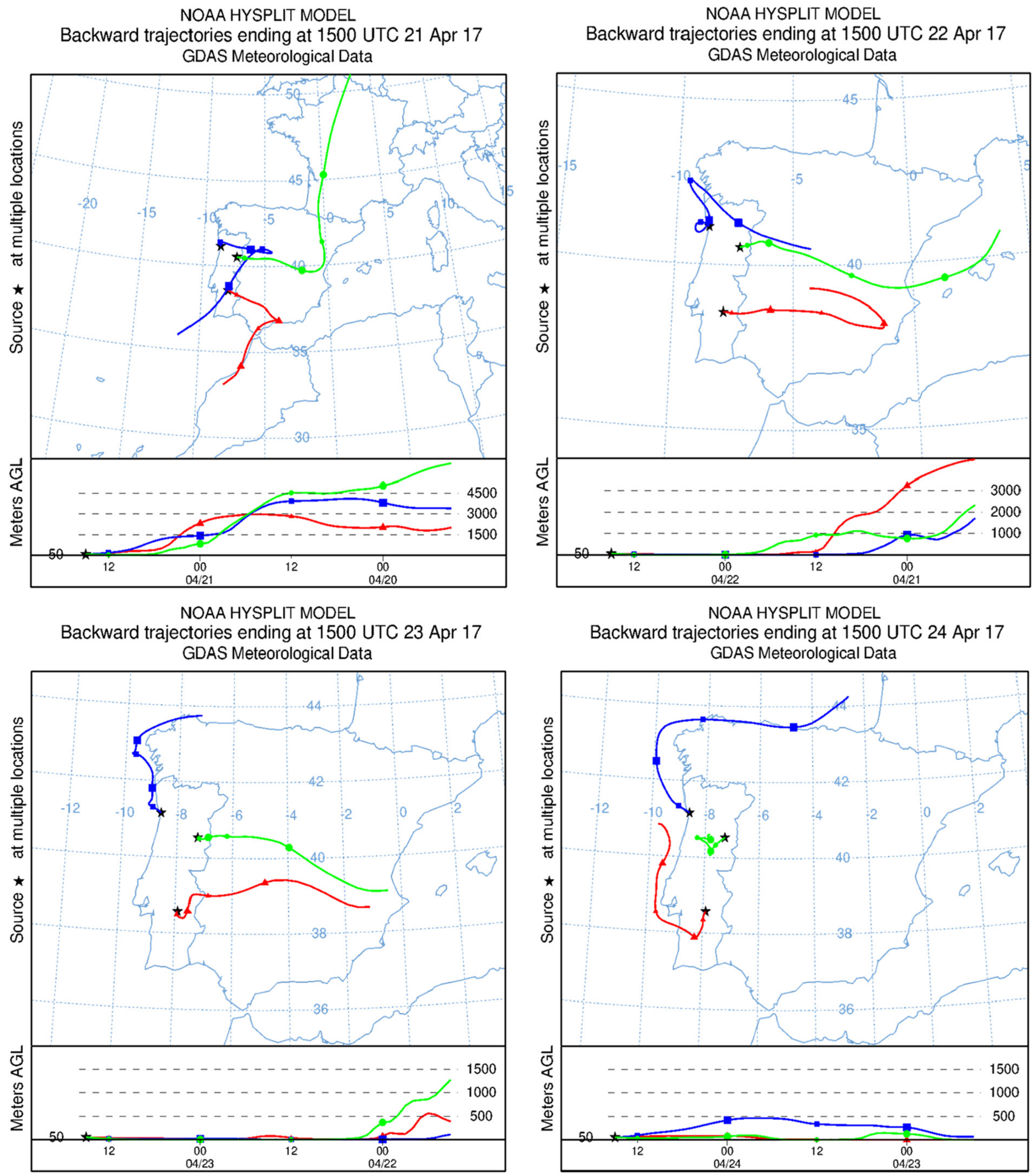

Fig. 7. Air mass backtrajectories arriving at Évora (line red), Guarda (line green) and Porto (line blue) from 21 to 24 April 2017, ending at a level of 50 m a.g.l.

there seems to be mostly local circulation. On this day it was observed, in addition to Si-rich particles, the increase of Cl-rich particles and the appearance for the first time of Ca-rich particles. On 24th the trajectory is maritime throughout the day, nonetheless, Si-rich, organic and metals particles were found (Figs. 6 and 7).

4. Discussion

Pollen grains when airborne can be in contact with numerous particles (Visez et al., 2020) of different origins and their synergy can be related to the probability of occurrence of 4 processes. The first one is pollen being polluted by particles before pollination and directly at the dehiscence of the anther; another plausible to consider is the pollen grains during the pollination process colliding with aerosol leading to the process of particle strains; contaminated in the soil through the resuspension process where relative humidity and wind speed are important factors or else during the human inhalation or in the respiratory mucosa where PM collide with pollen initiating the inflammatory process, creating an adjuvant chemical microenvironment for allergic reaction upon pollen grains deposition (Saxon and Diaz-Sanchez, 2005). This last process can be simulated by our sampling equipment.

In our study, significant differences were observed in the equivalent diameter of *Quercus* pollen between the three studied cities. In all study cities, it was possible to divide the *Quercus* pollen in two groups, reflected in the distribution of *Quercus* pollen size being characterized by a bimodal pattern, more pronounced in the city of Évora and less in Porto, which is reflected in the average equivalent diameter, larger in Évora, followed by Guarda and finally Porto (Fig. 2). This observation can be explained by the airborne pollen sampled belonging to distinct species of *Quercus* that have part of the pollination periods overlapped (March–April). In the city of Évora, there are two dominant species *Quercus rotundifólia* L. with pollen size between 21 and 25 μm, and *Quercus suber* L., with pollen size of 22–40 μm. In Guarda, the most prevalent species is *Quercus robur* L., its pollen being approximately between 31 and 35 μm while *Q. suber* is present but less representative (www.PalDat.org, last accessed February 16, 2022; www.flora-on.pt last accessed March 24, 2022). In Porto, and due to its high urbanization level, *Quercus* trees are less frequent and there is a greater variety of species, with *Q. suber* almost absent at the expense of other species with less economic importance. Also, there were interdaily statistically significant differences in the pollen size in Évora and Guarda but not in Porto. These differences may reflect the transition between pollen seasons of different species of *Quercus*, representing the possible end of *Q. rotundifólia*, *Q. robur* species and the beginning of *Q. suber* species. Probably, another possibility will be the transport of air masses from other locations, transporting pollen over long distances. In fact, it is verified that in Évora, on 24th, the air mass trajectory differs from the previous days, coinciding with the day when the equivalent diameter of pollen was greater (Table 1 and Fig. 7). The same occurs in the city of Guarda, on 24th, the air mass trajectory is of local origin, coinciding with the day when the equivalent diameter of pollen was smaller. It is possible that these events may contribute to the presence of other species of genera *Quercus*.

A greater number of particles per pollen grains of *Quercus* was observed in the city of Évora compared to the city of Guarda and Porto. In fact, for Évora it was shown that the number of particles adhered on the pollen wall may be influenced by the pollen size, as more area is exposed in the larger pollen grains and able to aggregate more particles during the inhalation process. The number of particles per pollen may also be related to the source of emission. Porto, although more urbanized, therefore expected to have finer PM present in the atmosphere and more particles adhered to the pollen, is a coastal area, affected by marine particles that are coarser in size with respect to pollution, with nonspherical shape (Dubovik et al., 2002; Silva et al., 2002). Nevertheless, in all cities, the average percentage of the pollen area occupied by the particles was less than 3% and therefore most of the *Quercus* pollen was clean or with few adhered particles (Fig. 3).

Considering the results obtained in this study regarding the equivalent diameter of the adsorbed particles, most particles belong to the fine fraction of PM with a diameter below 3 μm. This is the fine fraction of PM with sizes between 1 and 5 μm, which in addition to being able to penetrate the tissue of the respiratory mucosa, compromising its function, can also reach the bronchial respiratory system and alveolus where gas exchange occurs (Löndahl et al., 2006). Possibly they can escape into the bloodstream and cause significant health problems (Kim et al., 2015). Among the three cities represented in this study, there are no significant differences in the equivalent diameter of the particles, and therefore their size may not be a problem in allergic exacerbation when different sites are compared for the same pollen type, *Quercus* pollen.

It was observed a negative significant correlation between the number of particles per pollen and wind speed. This may be possible as a result of wind speed influence on the turbulence of the atmosphere since high wind speed is associated with high dispersion and possibly the low adsorption capacity of PM to the pollen surface. Some authors have demonstrated that the lower the wind speed, the higher the concentration of PM in the air (Cichowicz et al., 2020). Wind direction is another factor influencing the adherence of PM to the pollen surface because studies have shown that the distribution of sources is very important for the detection of biological and organic particles (Palacios et al., 2000). Wind direction proved to be important for the number of particles per pollen in the winds from S, contrary to winds of E, SW and NW. When winds come from NW it is not associated with the maximum equivalent diameter and percentage of area occupied by particles.

Considering the chemical classification of the adhered particles, the dominant groups for the 3 cities were Si-rich, SO-rich, Ca-rich, organic, and Cl-rich. Aluminosilicates associated with Fe, K, Mg, Zn and Ca, constitute the majority of particles in the group of silicates. The presence of Cl-rich particles with associated elements of Na and Mg in pollen grains may be related to coastal influence (Moreno et al., 2004) since these elements constitute 90% of the salinity of seawater. Sea salt particles can contain sulfates, potassium and sodium (Gieré and Querol, 2010), like the most abundant SO-rich particles observed in our study. In this study, it was also observed, for the three cities, the presence of particles of Metals & Oxides, composed mainly of Fe-rich, Ni and Ti elements, and organic particles, that usually have emission sources associated with automobile traffic (Moreno et al., 2003; Moreno et al., 2004). The physical and chemical characteristics of the particles vary depending on the location of the sampler (Calvo et al., 2013; Santos et al., 2008) because there were chemical groups observed in the city of Porto that were not observed in other cities. In the city of Évora, most of the influence is rural, with some contribution from sea salt particles and a smaller contribution from anthropogenic sources as road traffic or transport from different sources. The contributions are dominated by Si-rich and Ca-rich particles, constituents of the earth's crust (Haynes, 2016). Particularly, Ca-rich particles may originate from erosion of carbonate minerals as calcite and dolomite, which are widely present around the region of Évora with several active quarries where calcitic and dolomitic marbles are mined (Menningen et al., 2018). Desert dust is the main source of atmospheric phosphorus (P) and P-rich particles that are only detected at Évora on the 21st April, consistent with the back trajectory originating in north Africa (Stockdale et al., 2016). Sea salt particles present a moderate contribution for the total, with Cl-rich and Sea salt misc. classes. It is hypothesized that also a great part of the sulfates is from sea salt, as most of them present sizes above 1 μm (Ghahremaninezhad et al., 2016). Organic aerosols may be of local origin, also with contributions from more distant sources to the east. To note that on the 24th of April, when the organic fraction is slightly higher, the back trajectory shows the airmass crossing the coastline at the surface level in the area of Sines, an important industrial complex in Portugal.

In the city of Porto, chemical particles of the Organic group composed of CFe may have also anthropogenic local origin as the sampling sites is very close to an entry point and exit of the city, with high motor traffic in the morning and at the end of the day, there is internal combustion of oil, tire wear and fuel (Moreno et al., 2004; Ribeiro et al., 2015).

In the city of Guarda, it was observed a prevalence of particulate matter of the Si-rich group and little concentration of other types of particles present in the city of Évora and Porto, which may indicate that the city of Guarda, is perhaps, an environment with lower toxicity (Lisboa, 2014).

Like pollen grains, particles can be deposited locally or far from their source, particularly smaller particles, due to medium-long range transport (Galan et al., 2013; Mohanty et al., 2017). An example of these phenomena could be observed in our study for the inland city of Évora (day 23) when sea salt particles, probably originated in the Mediterranean Sea, were detected adhered to the pollen grains, as corroborated by the air mass trajectories. Another example was observed in Porto, on the 23rd a considerable increase in the amount of Cl-rich particles was observed coinciding with dominant air masses from NE, consistent with the results from the correlation analysis, and passing through the coastal zone. In fact, NaCl or MgCl can be associated with coastal influence (Moreno et al., 2004) as they account for 90% of the seawater salinity (Lide, 2007). Another example, on the 23rd in Porto, is the percentage increase of organic composition particles which coincides with the change in air masses to the Northeast passing through the coastal area.

On the other hand, the number of particles of Metals & Oxides origin can also correlate with a local origin, since its compounds e.g., FeO, Zn, Ni, V, Ti, can be assigned to road traffic and industrial sources associated with combustion processes (Moreno et al., 2003; Moreno et al., 2004; Calvo et al., 2013; Gieré and Querol, 2010). The close location of the sampling points to roads with frequent high traffic volume can explain the increase in organic-derived particles.

Si-rich particles originate mainly from geological formations (Calvo et al., 2013; Čupr et al., 2013; Pachauri et al., 2013). In our study, more than 50% of the Si-rich particles identified and adsorbed to airborne pollen were composed mainly of Si. These particles can originate from sediments that can be transported naturally by the wind. In fact, during the period of our study, there were events of desert sand particles felt in Évora but not observed for Guarda and Porto. In the case of Guarda city, the Si-rich particles can be originated from the gravel that is present on the roof of the municipal theater.

5. Conclusions

It was shown that most particles belong to the fine fraction with a diameter below 3 μm.

The particle size, as the equivalent diameter of PM, is similar between the studied cities, however, the dominant chemical composition is different, possible due, environmental conditions, for example, proximity of the sea, traffic and industry, which is important information regarding their impact on pollen allergy intensification towards the same pollen type and concentration. In future studies, when considering the exposome influence on pollen allergies, it would be important besides the concentration of PM in the air also take into account its chemical composition and how these characteristics could affect allergen release from pollen, its allergenicity, possibly allergens molecular structures and influence on the human respiratory tract.

CRediT authorship contribution statement

Ana Galveias: Data curation, Formal analysis, Writing – original draft. **Helena Ribeiro:** Funding acquisition, Conceptualization, Formal analysis, Writing – review & editing. **Fernanda Guimarães:** Methodology, Writing – review & editing. **Maria João Costa:** Methodology, Writing – review & editing. **Pedro Rodrigues:** Writing – review & editing. **Ana R. Costa:** Writing – review & editing. **Ilda Abreu:** Writing – review & editing. **Célia M. Antunes:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154892.

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