Daily behavior of urban Fluorescing Aerosol Particles in northwest Spain

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A R T I C L E   I N F O

Keywords:
Fluorescing aerosol particles
Primary biological aerosol particles
UV-Induced fluorescence
WIBS
BioLibrary

A B S T R A C T

Measurements of ambient aerosol particles at the University of León, León, Spain, were made in May and June 2015 with a Wideband Integrated Bioaerosol Spectrometer (WIBS). The WIBS detects Fluorescing Aerosol Particles (FAP) in the size range from 0.5 to 20 μm. These measurements were complemented with an analysis of pollen concentrations assessed with optical microscopy of samples captured with a volumetric Hirst spore trap. The total particle, FAP and pollen concentrations show clear, daily cycles. Whereas the total particle concentrations maximize at 0800 and 2200 UTC, the FAP concentrations have peaks at midnight and 0800 UTC while the pollen has a broad peak between 1200 and 2000 UTC. The FAP larger than 2 μm represent 15–35% of the total particle population in this size range, maximizing at midnight UTC. Similar to what has been found by investigators at other locations, there is a strong positive correlation of the WIBS measured FAP with relative humidity; however, the pollen concentration is positively correlated with the temperature and anti-correlated with the relative humidity. Back trajectory analysis indicates that the largest FAP to total particle fractions are found in air masses arriving from the northeast with the second largest coming from the southwest. Given the location of the university in relation to the city and forested areas, this implies that the higher concentration FAP are coming from rural, probably natural, sources; however, more local, anthropogenic sources cannot be ruled out as a secondary source. The majority of the FAP that are identified from microscopy are fungal spores (Cladosporium, Aspergillus, Alternaria, Oidium) and pollen grains (mainly Poaceae, Quercus, Plantago, Rumex and Urticaceae). A comparison of the fluorescence fingerprints between laboratory generated FAP and the ambient particles showed some similarities; however, a significant fraction of the FAP are those whose fluorescence patterns do not match any of those that have been previously classified in the laboratory.

1. Introduction

Atmospheric bioaerosols, also referred to as primary biological aerosol particles (PBAP), form a unique class of particles that are ubiquitous in nature, morphologically complex and are produced from a wide range of natural and anthropogenic sources (Després et al., 2012; Fröhlich-Nowoisky et al., 2016; Ghosh et al., 2015; Jonsson et al., 2014; Walser et al., 2015). These particles play an important role in: i) climate and the hydrological cycle, as they can act as cloud condensation nuclei (CCN) and ice nuclei (IN) (Bauer et al., 2002; Christner et al., 2008; Nowoisky et al., 2016; Kallawicha et al., 2016; Pratt et al., 2009; Sun and Ariya, 2006); ii) public health, where they have been associated with infectious diseases, allergies, acute toxic effects and cancer (D’Amato et al., 2015; Douwes et al., 2003, 2017; Kallawicha et al., 2016; Sturm, 2012); iii) exchange of plant genetic material, participating in the dispersal of reproductive units and in the spread of organisms (Fröhlich-Nowoisky et al., 2016) and iv) agriculture and livestock that can be infected with pathogenic microorganisms, similar to humans (Brown and Hovmøller, 2002; Fisher et al., 2012).

Depending on location and season, PBAP can constitute a significant

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Received 21 July 2017; Received in revised form 13 April 2018; Accepted 17 April 2018
Available online 21 April 2018

https://doi.org/10.1016/j.atmosenv.2018.04.027

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Fig. 1. Study zone in the province of León, Spain. Location of the sampling point (red cross).
fraction of the super-micron (> 1 μm) atmospheric particulate matter present in the atmosphere (Jaenicke, 2005; Després et al., 2012; Schumacher et al., 2013; Sesaric et al., 2012). Global emissions of PBAP have been estimated at 132 Tg year$^{-1}$ (median value) with pollen and fungal spore emissions on the order of 66 and 31 Tg year$^{-1}$ (median value), respectively (Després et al., 2012; Jonsson et al., 2014).

Ground based studies have linked the production of fungal and pollen spores to meteorological conditions, in particular, increased concentrations have been measured following rainfall, suggesting a relationship between elevated moisture and the release of spores by plant life (Chi and Li, 2007; Grinn-Gofroń and Bosiacka, 2015; Li and Kendrick, 1995; Oliveira et al., 2005; Gabey et al., 2013). Strong correlations between PBAP and relative humidity have also been observed during a number of studies (Toprak and Schnaiter, 2013; Crawford et al., 2014; Healy et al., 2014; Wright et al., 2014).

With the advent of instruments that continuously measure fluorescence aerosol particle (FAP) concentrations using ultraviolet light induced fluorescence (UV-LIF), the relationship of environmental conditions like meteorology and solar radiation to spore production can be analyzed in much greater detail. Such measurements have been made over a relatively wide range of environments, but mostly in rural areas. There are only a few published studies of measurements with UV-LIF instruments in urban areas, with moderately large populations like Helsinki, Finland of 600,000 (Saari et al., 2015), very large like Manchester, UK with 2.5 million (Gabey et al., 2011) or a megacity like Nanjing exceeding 10 million (Yu et al., 2016). Hence, it is important to begin establishing a comprehensive data set of more temporally resolved measurements in urban regions, particularly those that are less populated but more numerous.

The majority of studies that evaluate FAP measured with the UV-LIF technique provide time resolved number concentrations FAP accompanied by other environment information like the meteorology. The FAP concentrations are also sometimes further stratified by the intensity of the fluorescence emission and its wavelength. Deriving more specific information about the type of FAP that produced the fluorescence, e.g. if the FAP is a bacteria, fungi or pollen particle, is much more challenging. This is due to the lack of detailed studies that relate the properties of a FAP type to its morphology, size and fluorescent intensity and wavelength when illuminated by a specific UV frequency. There have been a number of studies that have related outdoor fungal spore concentrations, measured with spore traps and identified microscopically, with the FAP measured with UV-LIF (Gosselin et al., 2016; Saari et al., 2015; O’Connor et al., 2014, 2015). Very recently, careful studies have been conducted in controlled, laboratory environments where a variety of bacteria, fungi and pollen were measured with the online, UV-LIF technique (Hernandez et al., 2016; Savage et al., 2017). These studies provide information on the fluorescence intensity, equivalent optical size and shape factor associated with each FAP type. Hernandez et al. (2016), on the basis of more than 50 pure cultures of bacteria and stocks of fungi and pollen, concluded that these three general types of PBAP can be separately identified based on just their size and fluorescence wavelength. Hence, there is the possibility that ambient FAP may also be roughly classified as one of these three types using the laboratory results as a reference.

A brief pause is needed to clarify terminology. We have introduced the terms PBAP and FAP and emphasize that they cannot be used interchangeably. The UV-LIF technology was developed as a way to identify PBAP by their fluorescence signature; however, as we discuss in greater detail below, there are non-PBAP that also fluoresce. Hence, UV-LIF instruments cannot be accurately described as PBAP sensors. By implementing a set of stringent conditions and thresholds, the majority of FAP measured by UV-LIF techniques are assumed to be PBAP. Nevertheless, although we have employed all recommended filtering techniques, we will use the term FAP from hereon to underscore that caution is needed when interpreting the measurements.

The main objective of the study was to analyze the concentrations, sizes, and variations of FAP measured with a UV-LIF technique in a small, urban area with a focus specifically on relationships with the ambient meteorology. To validate the UV-LIF measurement of this study, measured FAP was compared with spore concentrations derived by optical microscopy of samples captured with a volumetric Hirst trap. An additional objective was to compare the FAP size and fluorescence signature with those a laboratory based bio-library of FAP.

2. Study zone

The measurements were made over a 30 day period from May 20 to June 18 2015 at the University of León (Spain). Fig. 1 shows three perspectives on the site: its relative position with respect to the country and the city, then its location on the roof of the Faculty of Veterinary at 15 m above ground level at León University Campus. León is a small city located in the northwest of the Iberian Peninsula (42° 36’ N, 05° 35’ W and 838 masl) with a population of about 135,000. The climate is Mediterranean with continental features, somewhat tempered by the proximity of the Cantabrian Mountain Range to the north. The late spring period of the sampling is a time of year with moderately high precipitation (97 mm for these two months) and warming minimum and maximum temperatures (Castro et al., 2010). One of the motivations for selecting this time period was that climatologically there is frequent rain (Fernández-González et al., 2012; Fernández-Raga et al., 2017). It is also a season when a significant fraction of the population (between 20 and 30%) suffers from allergies related to pollen (D’Amato et al., 2007, 2015).

Given the previous studies that have linked the increase in bioaerosol concentration to rainfall in non-urban regions (Chi and Li, 2007; Grinn-Gofroń and Bosiacka, 2015; Li and Kendrick, 1995; Oliveira et al., 2005; Gabey et al., 2013), the study in León was designed to evaluate if a similar link could be found in an urban area.

The sampling site is located in the suburban area northeast of the city (Fig. 1). Due to the absence of industries with large emissions, the main source of aerosol particles in León is vehicular traffic. Some of these combustion products, like black carbon (BC), polycyclic aromatic hydrocarbons (PAH) and aged organic aerosols can exhibit fluorescent properties and be mistaken as FAP. Likewise humic and fulvic acids and some mineral dusts (Bone et al., 2010; Gabey et al., 2011; Lee et al., 2013; Pöhlker et al., 2012; Sivaprasakasam et al., 2004). The methodology used to minimize the interference of these types of particles is address in the analysis section and appendix.

With respect to FAP sources, there is a significant number of natural vegetation types. The Quercus genus and many hybrid taxa commonly known as oaks and holm oaks (oak-oaks, gall-oaks, etc.) are predominant and distributed throughout the province. Q. pyrenaica and Q. rotundifolia are the most abundant species; they belong to plant communities of natural and semi-natural habitats. In the mountains to the north of the province (around 30 km from the city), there are also numerous forests whose pollination contributes to the high, atmospheric concentration of pollen that could possibly be transported to the city. Deciduous forests are present, beech trees (Fagus sylvatica), birch trees (Betula celtibera) and in the valley poplars, willows, alders and ash trees (Populus sp., Salix sp. Alnus glutinosa, Fraxinus sp.). The evergreen forests correspond to Juniperus sp. and Pinus. In meadows and grasslands, a great variety of grasses and other herbaceous (Bromus sp., Dactylis sp., Holcus lanatus, Phleum pratense, Poa sp., Plantago sp., Chenopodium sp., etc.) are present.

Ornamental flora can also be found mainly in parks and gardens of the city with low biodiversity and prevalence of those plants with high thermal resistance such as cypresses (Cupressus sempervirens, C. arizonica), cedars (Cedrus deodora), shade plantains (Platanus acerifolia), Oleaceous (Ligustrum sp.), Chestnut trees (Aesculus hippocastanum), maples (Acer negundo) and other species of plants, shrub and herbsaceous with pollination mainly entomophile.

All this natural and ornamental vegetation can contribute an
important fraction of pollen and fragments of plants to atmospheric FAP. Other emission sources are fungal spores that develop on decomposing plant tissues, and plant debris from frequent lawn mowing. FAP from garbage on the street, waste treatment plants or landfills cannot be ruled out but are assumed to be a minor source of ambient FAP.

One additional caveat is that some of the flora mentioned above also are sources of biogenic emissions such as terpenes that can form organic aerosols, some that have been shown to be weakly fluorescent when illuminated with UV light. The steps taken to filter the WIBS measurements will remove the majority of these types of aerosols from the FAP analysis.

3. Methodology

During the 30 day campaign FAP were sampled continuously using two instruments, a Wideband Integrated Bioaerosol Spectrometer (WIBS-4, Droplet Measurement Technologies, Inc., Boulder, Colorado) and a Hirst-type volumetric trap. Furthermore, a weather station provided information related to the meteorological parameters (pressure, temperature, relative humidity, wind and precipitation). The particulate mass in aerodynamic sizes less than 10 μm (PM10) was measured hourly at two air quality monitoring stations, one located two kilometers to the southwest of the sampling site and another 4.4 km to the south. Rain was also measured with one minute resolution by an optical disdrometer LPM (Thies Clima).

3.1. The WIBS-4

The WIBS measures the equivalent optical diameter (EOD) and the fluorescence of individual particles in three spectral bands when the particle is excited at two wavelengths: 280 nm and 370 nm (Huffman et al., 2010; Kaye et al., 2005). This instrument is described in detail by Gabey et al. (2010), Kaye et al. (2005) and Perring et al. (2015). A laminar-flow system directs the ambient particle stream into the sampling chamber of the instrument where they first encounter a 635 nm diode laser beam. The individual particles scatter light and some of the forward scattered photons are collected by optical components and directed onto a quadrant detector. The four signals from this detector are used to calculate an asymmetry factor that is a rough metric of the forward scattered photons collected by the optical components. The forward scattered light is collected by other optics and directed onto another photodetector that produces the signal from which the EOD is derived (∼ 0.5–30 μm in the configuration used for the present study). This signal also sequentially triggers two xenon flash tubes that are filtered to emit UV light at 280 nm and 370 nm, respectively. The resulting fluorescence emitted by particles due to these excitations is collected, filtered, and passed to two fluorescent detectors, FL1 (to detect light from 310 to 400 nm) and FL2 (to detect light from 420 to 650 nm).

A fluorescent particle may produce a signal: (i) following excitation at 280 nm and emissions recorded by the FL1 detector (Channel A); (ii) following excitation at 280 nm and emissions recorded by the FL2 detector (Channel B); or (iii) following emissions recorded by the FL2 detector following excitation at 370 nm (Channel C). A fluorescent particle may produce an emission that is only recorded by one of the A, B or C channels, or the emission can be recorded by a pair of channels or all three. Hence, there are seven possible outcomes from each fluorescent particle. Channel A is highly sensitive to tryptophan, while Channel C measurements are responsive to nicotinamide adenine dinucleotide (NADH). These two fluorophores, tryptophan and NADH, are omnipresent in plant tissues and microbiological cells.

3.2. The Hirst-type volumetric trap

The atmospheric pollen concentrations were also analyzed using a Hirst-type volumetric trap (Hirst, 1952), consisting of a continuous volumetric sampler with wind orientation. It is a sampler for particles between 2 and 200 μm in diameter with a flow of 10.1 L min⁻¹. A 345 mm Melinex tape impregnated with a silicone fluid was exposed for seven days inside the sampler. The Melinex tape was attached to a drum with a driving speed of 2 mm h⁻¹ regulated by means of a clockwork mechanism.

Hourly and daily optical microscopic counts were carried out by the method recommended by the Spanish Aerobiological Network (REA), based on four parallel longitudinal transects along the slides (Galán et al., 2007). The pollen grain count is possible due to their appearance stained with fuchsin (the rest of FAP do not stain). Hourly and daily mean pollen concentrations were expressed as pollen grains per cubic meter of air. A minimum threshold of 1 grain/m³ was counted to estimate the concentration.

3.3. Analysis approach

As previously noted fluorescent particles are classified into seven categories depending on which individual or combination of channels the emission signal was registered (A, B, C, AB, AC and ABC). As described by Perring et al. (2015) signal conditioning is necessary to remove a background baseline caused by leakage of flash lamp light through the photomultiplier filters. The relative strength of this background baseline depends on the output power of the Xenon lamps, the detector filter efficiencies and amplifications, as well as any emissions from fluorescent materials deposited to the walls of the sample chamber (Toprak and Schnaiter, 2013). We determine the baselines for the Channel A, B and C detectors using the approach described by Perring et al. (2015), adjusting it daily to account for any chamber contamination or longer term electronic drifts. The setting of these baselines is discussed in detail in the appendix.

As previously mentioned, the processing must take into account that some non-biological entities present in the atmosphere can exhibit fluorescence as well as other biomolecules that can contribute fluorescence in these bandwidths (Gabey et al., 2013; Perring et al., 2015; Pöhlker et al., 2012; Toprak and Schnaiter, 2013). The appendix provides a detailed description of the processing used to minimize the artifacts, as well as correcting for particles whose fluorescence is not detected during the duty cycle of the flash lamp.

Using a WIBS, Hernandez et al. (2016) generated a library of the most commonly found environmental bacteria, fungi and pollen. In the laboratory they sampled 15 and 29 pure cultures of bacteria and indoor fungi, respectively, and 13 pure stocks of temperate tree and grass pollens. A major, significant result was that the bacteria, fungi and pollen fell into distinct groupings defined primarily by size and fluorescence category (as defined by the A, B, C channel groupings). In general, the bacteria have EODs less than 1.5 μm and are either category A or AB. The fungi are all category A with average EODs between 2 and 5 μm and the pollen are either categories BC or ABC and cover a larger size range from as small as 1 μm to as large as 25-30 μm.

The León measurements were evaluated using a similar stratification of size and category to group the results for comparison with the bioaerosol library. It is important to note a number of caveats when interpreting the field results using the measurements taken in the laboratory:

- The WIBS used in the laboratory was not the same one that was used in the field. All WIBS units are calibrated for size using the same polystyrene latex bead technique; however, calibration of the fluorescence detectors is more challenging and the fluorescence intensities measured by lab and field units can only be compared qualitatively.
- Examination of the samples that were produced in the laboratory with optical microscopy confirmed that the particles were generally un-fragmented, i.e. these FAP would be considered intact. FAP in the field, particularly fungi and pollen, will oftentimes become
fragmented as a result of turbulence when they come into contact with surfaces prior to entering the sample chamber of the WIBS. This will have the effect of multiplying the apparent concentration of the FAP. In addition, the fluorescence intensity of FAP fragments and their measured EOD will be less than the original particle so that even though the fragment may be classified in the same bio-category, it will be undersized with less fluorescence.

- The library samples were freshly cultivated and aged less than 24 h. Many FAP in the natural environment can be quite fresh; however, some fraction will have gone through aging process such dehydration, attaching to dust or other particles or fragmentation that would likely change their size or fluorescence signature.
- FAP has been observed on the surfaces of dust or mixed with other organic material. This will amplify the size of the measured EOD.

The FAP are classified in this study as:

1. Type I: Having the characteristics of the library bacteria (category A or AB, EOD < 1.5 μm).
2. Type II: Having the characteristics of the library fungi (category A, 1.5 < EOD < 10 μm).
3. Type III: Having the characteristics of the library pollen (category BC or ABC, EOD > 2 μm).
4. Type IV: Fluorescence category B, C or AC and hence cannot be compared with the library data base.

### 4. Results and discussion

#### 4.1. Meteorology and air mass sources

As summarized in Table 1, the average temperature and relative humidity (RH) fall close to the climatological values that have been compiled for the months of May and June (www.aemet.es). The daily minimum, maximum and average values were computed for the 30 days of the project from which the overall averages and standard deviations (also listed) were derived. The total rainfall for the period was slightly less than the climatology for May or June, but in general, the meteorology for the research period can be considered within the normal range. The number of days when more than 1 mm of rain fell was somewhat less than the climatology, providing less of an opportunity to evaluate how precipitation impacts the properties of the FAP.

![Hourly evolution of (a) temperature and humidity and (b) wind speed and wind direction. Vertical bars are ± one standard deviation.](image)

Table 1

| Parameter               | May 20 – June 18 | Climatology May | Climatology June |
|-------------------------|------------------|-----------------|------------------|
| Minimum Temperature (°C)| 8 ± 4            | 6.6             | 10.2             |
| Maximum Temperature (°C)| 24 ± 5           | 18.6            | 24.0             |
| Average Temperature (°C)| 16 ± 4           | 12.6            | 18.4             |
| Minimum Humidity (%)    | 27 ± 14          | N.D.            | N.D.             |
| Maximum Humidity (%)    | 85 ± 8           | N.D.            | N.D.             |
| Average Humidity (%)    | 56 ± 10          | 62              | 56               |
| Rain (mm)               | 38               | 56              | 31               |
| Wet Days*               | 4                | 9               | 5                |

* > 1 mm day⁻¹.

midnight to around 0800 UTC, about three hours after sunrise, increasing to a maximum in the afternoon at the same time as the temperature maximum. The diurnal variations of wind speed and direction are a result of the well-known mountain valley circulation that is driven by solar heating of the mountain slopes during the day (southerly flow in Fig. 2b) that leads to a density gradient forcing the air up the sides of the hill. At nighttime, radiative cooling leads to a reversal of this gradient and air flows down the slopes (northerly flow in Fig. 2b).

One of the questions addressed in this study is that of locating the FAP sources. Given the proximity to the research site of large wooded areas to northwest and northeast, the city to the southwest and agricultural areas to the southeast, regions that are all potential sources of FAP, we have calculated 72 h air mass back trajectories, every 6 h, over the sample period. The model used to compute these trajectories was the NOAA Hysplit code (Rolph et al., 2017; Stein et al., 2015) implemented with the NCEP Global Data Assimilation System (GDAS) Model at one half of a degree resolution. Fig. 3a shows these air mass trajectories ending at 0000 UTC for the 30 days, color coded by altitude with respect to that of the research site (841 m). The negative values (blue) indicate that the air masses were lower than the city of León prior to their arrival at the measurement site. Fig. 3b is an expanded view of the same trajectories.

We observe that a large fraction of the trajectories are over the Atlantic Ocean or North Sea three days before arriving in León. Many of these air masses are also at or below the altitude of the measurement site for a number of hours prior to arrival. This suggests that many of the sampled air masses had ample opportunity to acquire aerosol particles within a layer close to the surface, e.g. marine aerosols or FAP from the forested areas.

Fig. 4a stratifies the air mass history by the location of the air mass, in 12 h intervals, prior to its arrival. An additional stratification is by period of the day, i.e. daytime or night time. Regardless of daytime or night, approximately 60% of the air masses had been to the northwest of the city 60 h previously. The percentage from the NW decreases and the percentage from the NE increases as the air masses turn easterly as they approach León (Fig. 3b).
Fig. 4b further characterizes the behavior of the air masses. This figure shows the number of continuous hours that the air masses had been below 100 m (with respect to León) prior to arrival at the measurement site. Almost 40% of those air masses that had been NW of León more than 48 h previously had stayed below 100 m during their travel. As previously noted, this suggests that much of the particulate matter that is sampled at the measurement site was acquired from relatively local sources rather than from long range transport. Likewise, particles that are being transported from sources farther away would have more opportunity to be removed by sedimentation and turbulent deposition during their transit within the surface layer (< 100 m).

Given the amount of time over forested regions, the air masses would also be transporting the volatile organic compounds (VOC) that are potential sources for FAP artifacts, as mentioned previously; however, the filtering described in the appendix will minimize their influence on the final results.

4.2. FAP properties

Hourly averages of the total number concentrations of all aerosol particles with EOD > 0.5 μm (black curve), and fluorescing particles (green) are shown in Fig. 5a for the 30 day sampling period. The red
respectively. The average fluorescence concentrations were 1113 (± 37%) L⁻¹ for the total particle population. The average total and FAP (Total Fluorescing) concentrations are larger than those of the total particles, all FAP and the four individual types of FAP. The concentration fraction for all fluorescing particles is calculated with respect to the total particle population measured by the WIBS. The concentration fractions for the four fluorescing types are calculated with respect to the total population of FAP. The statistics are generated by calculating the daily averages, minima and maxima for each of the 30 days, then the means and standard deviations of these daily averages were computed over this time period. The standard deviations, shown in parentheses, are given as percentages of the mean. The fluorescence fractions range from an average daily minimum of 2% to a maximum of 10%. The average concentrations of the Type III (pollen-like) particles are the same as those measured on the adhesive tape from the Hirst sampler; however, the Hirst pollen shows somewhat more variation than the Type III concentrations. The Type II (fungi-like) and Type IV (other) dominated the fluorescing particles, representing, on average, 36% and 35% of the FAP, respectively.

Fig. 6b further illustrates how the fluorescence particle frequency is related to the air mass source and particle size range, i.e. those greater than or less than 2 μm. In this figure, we observe that 35–85% of all particles > 2 μm are fluorescing, and that air masses arriving from the SE had the largest fraction of fluorescing particles > 2 μm. The Type II and IV particles represented the largest fraction of fluorescing particles for air masses arriving from all sectors except from the NW where the frequency of Hirst pollen was larger than the Type II FAP. Also notable is that the frequency of Type III (pollen-like) FAP was comparable to the pollen from the Hirst sampler when the air was originating from the NE and NW sectors.

The daily trends in the mass and particle number concentrations are shown in Fig. 7a and b. The mass from the WIBS is derived from the measurement of the particle EOD, assuming sphericity and unit density. Although these assumptions lead to an estimated uncertainty of approximately ± 50% in mass, given the variable morphology and density of atmospheric particles, this type of assumption is optimal. The comparison between the PM₁₀ measured at the air quality station, two kilometers SW of the research site, and the mass concentration derived from the size distributions measured by the WIBS illustrate that the daily cycles measured by both instruments have the same, bi-modal distributions with peaks during the morning and evening rush hours (Fig. 7a). The mass concentration derived from the WIBS is approximately half the directly measured PM₁₀. Given the PM₁₀ is measured in an urban traffic air quality station, the distance between the sites, the altitude of the WIBS above the ground and the assumptions made in deriving mass from the WIBS, the agreement is reasonable.

Table 2 lists some of the statistics for the total and FAP concentrations, FAP fractions, median volume diameters and shape factors for all particles, all FAP and the four individual types of FAP. The concentration fraction for all fluorescing particles is calculated with respect to the total particle population measured by the WIBS. The concentration fractions for the four fluorescing types are calculated with respect to the total population of FAP. The statistics are generated by calculating the daily averages, minima and maxima for each of the 30 days, then the means and standard deviations of these daily averages were computed over this time period. The standard deviations, shown in parentheses, are given as percentages of the mean. The fluorescence fractions range from an average daily minimum of 2% to a maximum of 10%. The average concentrations of the Type III (pollen-like) particles are the same as those measured on the adhesive tape from the Hirst sampler; however, the Hirst pollen shows somewhat more variation than the Type III concentrations. The Type II (fungi-like) and Type IV (other) dominated the fluorescing particles, representing, on average, 36% and 35% of the FAP, respectively.
The total number concentration (black curve, Fig. 7b) mirrors the mass concentration, suggesting that the bi-modality in the mass concentrations is due to variation in number rather than the size of particles. The fluorescence fraction was separated into FAP < 2 μm and > 2 μm. The fraction of total particles < 2 μm represented by FAP does not have a daily cycles, remaining constant at about 5%. The

![Fig. 5. Temporal trends in (a) the total particle concentration, total fluorescence particle concentration and fluorescence fraction and (b) Median Volume Diameter (MVD) for all particles, all fluorescence particles and for particles Type II (fungi-like) and Type III (pollen-like).](image)

Table 2

Aerosol property statistics. Concentrations in L\(^{-1}\). Values in () are standard deviations, expressed as percentages of the mean. The concentration fractions for the four fluorescing types are with respect to the total population of fluorescing particles.

| Statistic                  | All Particles | All Fluorescing | Type I (Bacteria) | Type II (Fungi) | Type III (Pollen) | Type IV (Other) | Hirst Pollen |
|----------------------------|---------------|-----------------|-------------------|-----------------|-------------------|----------------|-------------|
| Concentration Average      | 1113 (37)     | 52 (32)         | 5 (29)            | 18 (45)         | 9 (59)            | 18 (47)        | 9 (73)       |
| Concentration Maximum      | 1957 (39)     | 116 (49)        | 7 (86)            | 26 (74)         | 20 (129)          | 38 (100)       | 10 (130)     |
| Concentration Minimum      | 597 (51)      | 20 (53)         | 1 (48)            | 5 (76)          | 2 (103)           | 6 (79)         | 1 (263)      |
| Fluorescence Fraction Average | 5 (35)     | 12 (30)         | 36 (0)            | 18 (30)         | 35 (23)           | 56 (20)        | 1 (263)      |
| Fluorescence Fraction Maximum | 10 (34)   | 29 (37)         | 57 (18)           | 31 (23)         | 56 (20)           | 1 (263)        | 56 (20)      |
| Fluorescence Fraction Minimum | 2 (43)     | 4 (56)          | 17 (60)           | 6 (36)          | 15 (52)           | 56 (20)        | 1 (263)      |
| MVD Average                | 10 (17)       | 8 (28)          | 1 (0)             | 5 (7)           | 17 (10)           | 13 (12)        | 13 (12)      |
| MVD Maximum                | 17 (17)       | 8 (63)          | 1 (1)             | 6 (9)           | 27 (6)            | 26 (8)         | 26 (8)       |
| MVD Minimum                | 5 (32)        | 3 (18)          | 1 (3)             | 4 (8)           | 7 (38)            | 5 (36)         | 5 (36)       |
| Shape Factor Average        | 5 (11)        | 18 (4)          | 18 (12)           | 10 (12)         | 14 (21)           | 10 (12)        | 14 (21)      |
| Shape Factor Minimum        | 8 (24)        | 22 (8)          | 25 (12)           | 14 (21)         | 6 (17)            | 6 (17)         | 6 (17)       |
larger than 2 μm fraction has a strong daily cycle, varying between 15 and 35% with the maximum occurring at 0400 UTC. This suggests an additional source of large FAP in the morning hours that are not as predominant during the morning rush hour. Note also that this peak in large FAP occurs near the maximum peak in relative humidity (Fig. 2a).

Fig. 8a illustrates the daily variation in the MVDs of all particles and those of Type III (pollen like). There is a suggestion of a morning increase in both types of particles; however, these increases are not statistically significant due to large, hourly standard deviations. Fig. 8b compares the daily variations in the total fluorescence concentration.
with the Hirst pollen concentration. We observe that the concentrations are negatively correlated with pollen reaching a maximum when the fluorescence concentrations are minimum; however, the significant hourly variability in the measurements from the WIBS and Hirst, somewhat diminish the possible significance of this correlation.

At night, when moisture increases, pollen is hydrated, metabolically activated and releases proteins (mostly allergenic) as exudates that can remain around the pollen, agglutinated with water vapor or adhere to other particles (Behrendt et al., 1992). Hence, although the amount of pollen remains constant, the number of fluorescent particles usually increases at night and, consequently, the WIBS will detect a larger number of such particles than the Hirst. After sunrise, with the increase of daytime temperatures, plants begin to shed pollen and the peak of the pollen release occurs during the early afternoon, coinciding with the hours of the highest temperatures. This maximum is due on the one hand to the anthers desiccation after dehiscence and on the other to the movements associated with the local air motion that help the pollen to pass from the plants to the atmosphere. It is likely that dehydrated pollen (which may even dehydrate up to 70%), with proteins in the quiescent state, makes it difficult to recognize the fluorescence proteins by the WIBS, accounting for a smaller number than those counted by the Hirst.

The other contribution to changes in the concentration is related to the growth of the mixed layer which has the general effect of diluting the concentration. This is the source of the decreasing particle mass and concentrations from their peak during the morning rush hour, decreasing in the afternoon as the boundary layer grows then increasing in the evening rush hour and collapsing boundary layer.

Previous studies have also identified a daily cycle in pollen concentrations, e.g., Toth et al. (2011) in Zagreb found a regular daily distribution for the total pollen of all plant taxa (Ambrosia sp., Betula sp., Cupressaceae, Urticaceae, Poaceae, Quercus sp., Fraxinus sp., Alnus sp., Corylus sp., Populus sp., Pinus sp., Picea sp.) that measured at two different sampling locations. They recorded an increase in the pollen concentration beginning between 4.00 a.m. and 6.00 a.m. with the peak pollen concentration occurred between 12.00 and 4.00 p.m. The lowest daily pollen concentrations were recorded overnight. About 50% of the 24-h pollen concentration was released to the atmosphere between 10.00 a.m. and 4.00 p.m. They noted that the timing and size of daily peaks were closely related to high temperature, low humidity and south-west maximum wind direction.

During the period selected in this study, the pollen index (PI, sum of daily pollen concentration) was 6790 which represents 21% of the annual PI. There are 35 atmospheric pollen types in this region, with Pinus, Plantago, Poaceae, Quercus, Rumex and Urticaceae contributing together 50% of the atmospheric pollen concentration. Fig. 9 shows an optical microscope image of a bioaerosol sample obtained by the Hirst Volumetric Trap on June 9. An important variety of pollen and fungal spores can be observed. Furthermore, fragments of plant tissues, plant trichomes and fungal hyphae can be identified.

The hourly distribution of the pollen concentration as a function of the wind speed and direction is shown in Fig. 10. An anisotropy is observed for the origin of pollen; this is a consequence of the inhomogeneous distribution of the sources of pollen. The wind speed was quite weak (lower than 3.5 m/s). Low concentrations are recorded when air comes from the SW and medium to high concentrations occur when winds are from the SE. Particular attention is drawn to the higher concentration recorded when the wind comes from the NE.

The daily maximum fluorescence fraction was linked to the air mass history over the previous 12 h using the back trajectory analysis as shown in Fig. 11. The color shading shows the maximum fluorescence fraction for that day. As previously noted, the majority of the periods with maximum FAP fractions are associated with air masses that were from either the NE or SW during the previous 12 h, i.e. arriving from over the population areas (SW) or forested hills (NE). There is not, however, a consistent direction associated with higher versus lower

Fig. 7. (a) Comparison between the hourly evolution of PM10 concentration measured in an air quality station and the mass concentration derived from the WIBS. (b) Comparison between the total concentration particles and the fluorescence fraction Vertical bars are ± one standard deviation.

Fig. 8. Hourly evolution of: (a) the Median Volume Diameter for all particles and for particles Type III (pollen-like) and (b) total fluorescence particles concentration and pollen concentration collected by the Hirst.
fractions, since from Fig. 11 we observe that the maximum fluorescence fractions vary from 4 to 20%, with about the same mixture from SW and NE.

4.3. Potential links between FAP properties and meteorology

As summarized by Jones and Harrison (2004) the concentration of airborne FAP has been linked to temperature, humidity, wind and rains through a number of meteorological pathways. An expanded time series of wind speed (black), RH (blue), Hirst pollen concentration (green) and fluorescence fraction (red), over a five day period, illustrates the daily cycles of these four parameters (Fig. 12a). Although these four parameters have distinct daily cycles, they are out of phase with one another. By calculating cross correlations of the Hirst pollen concentrations and fluorescence fraction with the RH and wind speed, as well as with temperature and wind direction (Fig. 12b and c) over the sampling period, we can see the temporal relationships between the FAP and meteorological parameters.
A brief explanation is helpful for interpreting results presented in this fashion. Cross correlations look for temporal links between two variables whereby variations in one parameter might be preceded or followed by a similar magnitude in variation by another variable. Whereas these types of correlations do not automatically imply causation, they do allow the possibility that there is a physical explanation that could explain the link. For example, in Fig. 12b, the fluorescence fraction cross correlation has the maximum positive correlation with RH at a positive lag of two to three hours and a maximum negative correlation with wind speed at this same lag time. This implies that on average the fluorescence fraction reaches a daily maximum (and minimum) two to three hours prior to the maxima and minima of the RH while the wind speed is at a minimum and maximum (negative correlation). An evaluation of the link between the Hirst pollen concentration and these same meteorological variables shows the best positive correlation is with temperature at a lag time of minus one hour and negative correlation with RH with the same negative one hour lag.

The cross correlations suggest that the highest fluorescence fractions, that include bacteria and fungi, are most closely linked with low winds, low temperatures and high RH, whereas pollen is more strongly related to higher temperatures and winds (that favor the long range transport) and low RH. Given the positive correlation between wind speed and temperature and negative correlation between temperature and RH (Fig. 2a and b), a definitive conclusion cannot be drawn as to which of these meteorological variables is driving the FAP properties. Given that humidity controls the release mechanisms of some fungal spore species (Troutt and Levetin, 2001), these spores might be the major source of the fluorescence fraction, even though we have previously labeled the Type II, fungi-like FAP as only about 30%. Certainly
some of those labeled Type III and IV could also be fungi whose release can be linked to the elevated RH. Healy et al. (2014), who also compared WIBS measurements with adhesive tape based samples, observed a similar daily evolution of fluorescent number concentration and relative humidity, with an increase during the night and early morning hours and daily minima in the mid-afternoon. The increased pollen count with wind velocity is likely a result of the lifting of this pollen from plant or other surfaces, since pollen has not been previously correlated with variations in either temperature or humidity.

Finally, although there have been a number of studies that show changes in the FAP population with precipitation (Allitt, 2000; Huffman et al., 2013; Rathnayake et al., 2017; Taylor and Jonsson, 2004), the measurements that have been shown here show only a weak impact, observed as a decrease in the Hirst pollen with precipitation. As shown in Fig. 6a, on days of the year 160–161 (total rainfall of 15.2 mm), and 155–156 (total rainfall of 18.5 mm) the pollen count decreases; however, on days 165–166 (total rainfall of 3.9 mm) when there was also rain falling, there is no significant change. It is important to take into account that in the two first episodes the rain was of convective origin while the third one the rain was stratiform, with the passage of a front and a consequent change of air mass. The origin of the rain could account that in the two periods of rain were fairly short in duration, these results cannot be used to form any meaningful conclusions with respect to how rain impacts the FAP population.

4.4. Comparison of fluorescent signatures from natural versus laboratory FAP

As previously discussed, Hernandez et al. (2016) classified a variety of pure strains of bacteria, fungi and pollen based on their EOD and class of fluorescence (A, B, C, AB, AC and ABC). Fig. 13 illustrates how these three types of FAP are clustered. In general, the bacteria have EODs less than 1.5 μm and are either category A or AB. The fungi are all category A with average EODs between 2 and 5 μm and the pollen are either categories BC or ABC and cover a larger size range from as small as 1 μm to as large as 25-30 μm. In Fig. 13, within each category, the FAP type is scaled between 0 and 1 based on the maximum intensity found within that category and FAP type, i.e. those types with higher fluorescence intensities fall farther to the right within the fluorescence category interval than those with lesser intensities.

One objective of the current study was to compare the fluorescence properties of the naturally occurring FAP in an urban environment with those that have been classified in the laboratory. Fig. 13 summarized the fluorescence types, sizes and intensities of various types of bacteria, fungi and pollen under laboratory conditions (Hernandez et al., 2016). Given that these are a sub-set of a much larger population of FAP that are found in the ambient atmosphere, a comparison of the current measurements helps us understand how representative the laboratory samples may be of those found in the urban region of León.

Fig. 14 presents the FAP signatures from the León measurements in the same format as Fig. 13 where the symbols are the laboratory measurements. The ambient values are placed within a category scaled by their intensity as in Fig. 13, but here the color coding is the frequency of particles within the different categories, sizes and intensities. From this comparison we conclude that a large number of the ambient aerosols have signatures that are similar to those produced by the bio-library. A large fraction of those categorized as Type I, bacteria-like, are clustered with the laboratory bacteria, as are the Type II that cluster around the bio-library fungi. There are many in the BC and ABC category that would be considered Type III, pollen-like, that are not found in the bio-library and these could be other types of pollen or fungal spores that were not evaluated in the laboratory. Thus, the more frequent pollen types identified during the sampling campaign (Plantago, Poaceae, Quercus, Rumex and Urticaceae) in León were not categorized by Hernandez et al. (2016). However, the main fungal spores identified in León (Penicillium, Aspergillus and Cladosporium) were included in the bio-library. There is also a fair fraction of FAP that fall in the B category (Type IV) that has no laboratory bacteria, fungi or pollen associated with it. These possibly could be another fungi or pollen type that has yet to be identified.

5. Summary and conclusions

Continuous measurements with a Wideband Integrated Bioaerosol Spectrometer were made of the size, concentration and fluorescent signatures of aerosol particles in the city of León, Spain during a 30 day period in May and June 2015. During this same period, microscopic analysis of pollen grains was also conducted using a Hirst-type
Table 3
Comparison of the average concentration of FAP (NFAP) from this study and previous studies (L⁻¹). Values in () are the percentage of total particles represented by the FAP.

| Site Location  | Site Category | Season   | $N_{FAP}$ | Reference                  |
|---------------|---------------|----------|-----------|----------------------------|
| Leon, Spain   | Semi-urban    | Spring   | 52 (5)    | This study                 |
| Nanjing, China| Suburban      | Autumn   | 6010      | Yu et al. (2016)           |
|               |               |          | (20)      |                            |
| Manchester, UK| Urban         | Winter   | 191 (8)   | Gabe et al. (2011)         |
| Puy de Dome, France | Hill-top     | Summer   | 107 (33)  | Gabe et al. (2013)         |
| Killarney, Ireland | Rural   | Summer   | 15 (0.05) | Healy et al. (2014)        |
| Borneo, Malaysia| Rainforest   | Summer   | 150 (−)   | Gabe et al. (2010)         |
| Karlsruhe, Germany | Semi-rural   | All      | 31 (7)    | Toprank and Schneiter (2013)|
| Amazon, Brazil | Rainforest    | Spring   | 93 (26)   | Huffman et al. (2013)      |
| Mainz, Germany | Semi-urban    | Summer   | 27 (4)    | Huffman et al. (2010)      |
| Helsinki, Finland | Urban     | Summer   | 13 (8)    | Saari et al. (2015)        |
| Hyytiala, Finland | Boreal forest | Summer    | 15 (4)    | Schumacher et al. (2013)   |
|               |               | Autumn   | 46 (13)   | Schumacher et al. (2013)   |
|               |               | Winter   | 27 (10)   | Schumacher et al. (2013)   |
| Colorado, USA | Rural forest  | Spring   | 15 (3)    | Schumacher et al. (2013)   |
|               |               | Summer   | 30 (9)    | Schumacher et al. (2013)   |
|               |               | Autumn   | 17 (6)    | Schumacher et al. (2013)   |
|               |               | Winter   | 5 (5)     | Schumacher et al. (2013)   |
| Ghats, India  | Sub-tropical hill-top | Summer | 20 (2) | Valsan et al. (2016) |
| Southern USA  | Boundary layer | Autumn | 21-87 (5-25) | Perring et al. (2015) |

volumetric trap. Back trajectory analysis was used to identify the source of air masses sampled during this period. Correlational statistics were applied to the time series of particle concentrations and meteorological parameters to evaluate the potential effect of temperature, humidity and winds on the trends in FAP concentrations. In addition, the ambient FAP size and fluorescence signatures were compared with those tabulated in a laboratory derived bio-library of bacteria, fungi and pollen.

The principal conclusions from this study are:

- The median volume diameter of the pollen (17 ± 4 μm) and fungal spores (~5 μm) determined by the WIBS are consistent with those estimated from optical microscope images on the Hirst adhesive tape.
- The daily maximum values in the total particles, total fluorescing, Type III (pollen-like) and measured Hirst pollen range from 1000 to 4000 L⁻¹, 20-200 L⁻¹, 2-20 L⁻¹ and 1-50 m⁻³, respectively. The FAP ranged from 2 to 10% of the total particle population. The Type III and Hirst pollen were of comparable magnitude suggesting that the classification of pollen using the WIBS and bio-library may be a viable approach for identifying pollen. As shown in Table 3, the average FAP concentrations are much smaller than those measured in large and megacities like Manchester and Nanjing, but were four times larger than those measured in Helsinki, a city that is five times larger than León. In all the studies listed in Table 3, measured FAP are reported as PBAP, always with the associated caveats concerning the potential that some were non-PBAP that fluoresce.
- The fraction of FAP in particles larger than 2 μm ranged on average 15–35% of the total particle population and as much as 85% when the air masses originated from the agricultural region southeast of the measurement site.

The WIBS can detect particles with attached pollen proteins, and therefore they are counted as fluorescent particles; however, these particles with adhered proteins are not quantified by the Hirst method as they are not stained. This might partially account for the sometimes higher concentrations of pollen-like particles identified by the WIBS than from the Hirst count.

We consider that the WIBS could become a valuable tool for pollen control aerobiological networks, since it appears to provide a reliable quantification of pollen in real time. More measurements are needed, however, complemented by sampling and identification with methodology such as the Hirst. The detection of allergies associated with the diverse types of vegetation requires the identification and quantification of the different pollens with subsequent analysis of the fluorescence signatures provided by the WIBS to better quantify specific groups of bacteria, fungi or pollen. Future studies will focus on this type of detailed identification and quantification.

Acknowledgements

This study was partially supported by the Spanish Ministry of Economy and Competitiveness (Grant TEC2014-57821-R), the University of León (Programa Propio 2015/00054/001) and AERORAIN project (Ministry of Economy and Competitiveness, Grant GGL2014-52556-R, co-financed with FEDER funds). F. Oduber acknowledges the grant BES-2015-074473 from the Spanish Ministry of Economy and Competitiveness. C. Blanco-Alegre acknowledges the grant FPU16-05764 from the Spanish Ministry of Education, Culture and Sport. The authors gratefully acknowledge the NOAA Air Resources Laboratory (ARL) for the provision of the HYSPLIT transport and dispersion model and/or READY website (http://www.ready.noaa.gov) used in this publication.

Appendix. Methodology for processing WIBS measurements.

There are three steps that must be taken to process the WIBS measurements: 1) removal of “bleed-through” light, 2) filtering out non-FAPs that fluoresce and 3) correcting for undetected particles during Xenon lamp recharging. As discussed by Perring et al. (2015), the flash lamp is triggered each time a particle is detected and there is some fraction of the flash lamp light that leaks through the detector filters. The signal strength in this...
The background mode depends on the output power of the Xenon lamps, the filter transmission function, the detector gain, and any light from fluorescent materials deposited to the walls of the detection chamber (Toprak and Schnaiter, 2013). Using the approach of Perring et al. (2015) we identify the average intensity of this bleed through light by creating frequency distributions of the fluorescence intensity measured by the FL-1, FL-2 and FL-3 detectors, as illustrated in Fig. A1a. Given that actual FAP are only 1–5% of the total particle population, the highest frequency intensities are those seen with values less than 100 counts. The vertical lines demark the one standard deviation ($\sigma$) point beyond the peaks. Perring et al. (2015) as well as most other users of the WIBS use 3$\sigma$ as a threshold to remove particles that are not actually fluorescing but whose bleed-through light is detected by one or more of the detectors.

As was previously discussed, there are fluorescing particles like BC, humic substances and other aerosol that are not FAP. A detailed analysis was carried out by Savage et al. (2017) with a number of such particles. Their results showed that these non-FAPs are generally very weakly fluorescing, and that a threshold of 3$\sigma$ will filter many of them; however, there were a number of fairly common non-FAPs whose fluorescence can exceed this threshold. Hence, they recommended using a 9$\sigma$ threshold for maximum filtering.

We conducted a similar exercise, processing the measurements from the whole period with thresholds that ranged from 0-9$\sigma$. The 3, 6 and 9$\sigma$ thresholds are shown in Fig. A1a. Fig. A1b shows the number of all FAP (black) and the FL-1 (blue), FL-2 (green) and FL-3 (magenta) FAP that are accepted after applying thresholds from 0-9$\sigma$. Also shown with the scale on the right axis are the FAP fraction of the total particle population as a function of the thresholds. Given the very large change from 3 to 6$\sigma$ in the FL-1 FAP, followed by almost a negligible increase going from 6 to 9$\sigma$, we decided to use a 6$\sigma$ threshold to remove the majority of non-FAP fluorescing particles. Clearly this type of thresholding will be removing actual FAP, and there are probably additional tests that could be applied so as to retain more legitimate FAP without accepting artifacts.

Figure A.1. (a) Frequency histograms of the fluorescing intensity measured with detectors FL-1,-2 and -3 of all particles sampled during the 30 day field campaign. The vertical bars indicate one standard deviation ($\sigma$) from the mean value. The colored markers show 3, 6 and 9$\sigma$. (b) The accepted FAP, all (black), FL-1 (blue), FL-2 (green) and FL-3 (magenta). Also the FAP fraction of all particles as a function of the threshold.

References

Allitt, U., 2000. Airborne fungal spores and the thunderstorm of 24 June 1994. Aerobiologia 16, 397.
Bauer, H., Kasper-Giebl, A., Löffled, M., Giebl, H., Hitzenberger, R., Zibuschka, F., Puxbaum, H., 2002. The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. Atmos. Res. 64, 109–119.
Behrendt, H., Becker, W.M., Friedrichs, K.H., Darsow, U., Tomingas, R., 1992. Interaction between aeroallergens and airborne particulate matter. Int. Arch. Allergy Immunol. 99, 425–428.
Bones, D.L., Henrickx, D.K., Mang, S.A., Gonziere, M., Bateman, A.P., Nguyen, T.B., Cooper, W.J., Nizkorodov, S.A., 2010. Appearance of strong absorbers and fluorophores in limoneneO-3 secondary organic aerosol due to NH4+ -mediated chemical aging over long time scales. J. Geophys. Res. Atmos. 115, D05203. http://dx.doi.org/10.1029/2009jd012864.
Brown, J.K.M., Hovmøller, M.S., 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297, 537–541.
Castro, A., Alonso-Blanco, E., Gonzalez-Colino, M., Calvo, A.I., Fernández-Raga, M., Fraile, R., 2010. Aerosol size distribution in precipitation events in León. Spain. Atmos. Res. 96, 421–435.
Chi, M.-C., Li, C.-S., 2007. Fluorochrome in monitoring atmospheric bioaerosols and correlations with meteorological factors and air pollutants. Aerosol. Sci. Technol. 41, 672–678.
Christner, B.C., Morris, C.E., Foreman, C.M., Cai, R., Sands, D.C., 2008. Ubiquity of biological ice nucleators in snowfall. Science 319, 1214.
Crawford, L., Robinson, N.H., Flynn, M.J., Foot, V.E., Gallagher, M.W., Huffman, J.A., Stanley, W.R., Kaye, P.H., 2014. Characterisation of bioaerosol emissions from a Colorado pine forest: results from the BEACHON-RoMBAS experiment. Atmos. Chem. Phys. 14, 8559–8578.
D’Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., Liccardi, G., Popov, T., Van Cauwenberge, P., 2007. Allergic pollen and pollen allergy in Europe. Allergy 62, 976–990.
D’Amato, G., Holgate, S.T., Pawankar, R., Lefèvre, D.K., Cecchi, L., Al-Ahmad, M., Al-
