



Indoor biological particles in a train: comparative analysis with outdoor atmosphere

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Received: 18 February 2020 / Accepted: 23 May 2020
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Abstract Biological pollution directly affects our health and quality of life. In urban areas, people spend most time in indoor environments including means of transports where can be exposed to pollution sick building syndrome. Despite the importance of this issue, studies on the biological particles exposure in indoor transports are scarce and poorly documented. This study characterizes by using volumetric Hirst traps of the indoor pollen and fungal spore content of the indoor air of a daily crowded train connecting Córdoba city (South Spain) with the university campus. Results were compared with outdoor concentrations sampled at the same time. Twenty-three pollen types were identified indoor and 20 outdoor. *Quercus*, *Olea*, *Platanus*, *Morus* and *Cupressus* were the most abundant. Thirteen fungal spore types were identified by both indoor and outdoor samplings. Our results pointed out *Cladosporium cladosporoides*, *Cladosporium herbarum*, *Coprinus*, *Alternaria* and *Torula* as the most abundant ones. Outdoor levels of both particles were higher; nevertheless, occasionally

indoor concentrations overcame indoor ones, overall pollen records at days after rainfall periods. Even pollen season of some taxa (i.e. *Platanus*, *Urticaceae*) extended inside lasting more days than outside. Results indicate as the closed transport can act as biological pollution reservoir which could have consequences on the incidence of passengers' health.

Keywords Indoor · Indoor pollen · Indoor spores · Air quality · Public transport

1 Introduction

Indoor air quality is one of the main concerns of public health. Citizens spend around 80–90% of their time indoors at home, work or commercial buildings (EPA 2003), but also into private or public transports. Nevertheless, most part of air quality analyses, including aerobiological studies, has been usually performed in outdoor environments. Considering the time that people usually spend indoor is vital to know the air quality in these closed places, including transports (Burge 1995; Lacey 1992). This fact is especially important in urban areas where live the 65% of the global population (EPA 2003) that daily use different means of transport for shifts related to work, studies or leisure activities. The spent time into transport is so high with an average of 1 h per day in USA. In Europe, studies indicate that around the 50%

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10453-020-09646-8>) contains supplementary material, which is available to authorized users.

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of the population spent > 1 h per day into transports (Nielsen 2019; National Transportation Systems Center 2019). Not only these are startling data but also the increasing size and population of the urban areas will involve the increase in the time allocated to urban journeys.

Nevertheless, it is quite unknown the air quality data at these indoor transport environments because most of indoor aerobiological studies have been carried out into homes or public buildings (Nathanson 1995; Platts-Mills 2007; Pongracic et al. 2010). On the contrary, studies focused into indoor transport air are so limited and mostly focused on chemical pollution (Chan et al. 2002; Gómez-Perales et al. 2007). Biological particles include bacteria, viruses, pollen and spores. Regarding pollen and spores, only a few researches study their penetration rate into vehicles, mostly private cars (Hugg et al. 2007; Muilenberg et al. 1991). In public transports, only one study about the presence of pollen into urban buses was found (Holmquist and Vesterberg 2001). To our knowledge, no studies have measure specific concentrations of pollen grains and fungal spores inside a train, although it is one of the most popular public transports both used into and between cities and towns. Because people spend an increasingly larger part of their time in private and public transport, it is important to assess the concentration of biological particles, including pollen and spores with a high allergenic potential.

Nowadays, indoor allergen exposure is associated with increased asthma symptoms and an increase in the risk of exacerbations of them in the allergic population (D'Amato et al. 1994). One of the reasons is that indoor biological pollution can extend the seasonal exposure to allergens, increasing allergy problems. Also, the prevalence of allergy and asthma in indoor environments of public or private transports may be caused by the synergistic action of air pollution with allergic sensitization (Holmquist and Vesterberg 2001).

Pollen grains, fungal spores and related allergens represent a high percentage of the total amount of biological particles in the air, and therefore, they are considered the main airborne allergen outdoor but also indoor due to the high penetration coefficient of these particles, especially in spring (García-Mozo 2017; Pichot et al. 2015). In indoor air, other specific airborne allergens are also present such as cockroach particles, mammal's proteins or house dust mites

which are responsible for indoor sensitizations (Garrett et al. 1998; Platts-Mills 2007). Nevertheless, because the airborne allergens considered the most relevant in both environments (indoor and outdoor) are pollen and fungal spores (D'Amato et al. 1994), this work is focused on those two particles.

The dynamics and health impact of outdoor aeroallergens from one region to another depend on local aerobiology, meteorology, exposure to air pollutants and individual susceptibility. This regional and more local variability is one of the factors that require a continuous aerobiological control over time in different geographical areas (Buters et al. 2018). Conditions driving those aeroallergens are clear and well known (Vogel et al. 2008). However, the factors driving indoor aeroallergens have not been so broadly studied. Since most people spend most of their time indoors, good indoor air quality is very important for human health and well-being. Atmospheric transport and conditions may play an insignificant role in the movement dynamics of indoor allergens and most of indoor studies identified clothing, feet and bodies of humans and animals as major vectors (Jantunen and Saarinen 2011; O'rourke and Lebowitz 1984). Nevertheless, some studies have shown that indoor pollen concentrations may be influenced by meteorological parameters, outdoor concentrations, previous indoor concentrations and ventilation systems (Hugg et al. 2007; Li and Kendrick 1995; Sautour et al. 2009).

Regarding the researches performed on indoor transports, Muilenberg et al. (1991) observed that biological particles levels within cars with closed windows were below that those obtained outside. Also, they found that car velocity was not a determinant factor in indoor levels. Hugg et al. (2007) studied pollen concentrations during spring into parked private cars. They found that pollen levels from herbaceous taxa as Poaceae (grasses) and *Artemisia* (ragweed) were low indoor although enough to cause reactions in sensitive people. Pollen from tree taxa as *Betula* (birch) and *Pinus* (pine) ranged from low to moderate and exceeded in some days the outdoor levels. The only information about biological particles into a public mean of transport was performed into coaches by Holmquist and Vesterberg (2001), analysing birch and grass pollen levels. They found significant indoor concentrations of both pollens, overall into driving compartments that contained substantial amounts of airborne birch and grass pollen allergens.

The main objective of the study was to characterize airborne biological particles of the indoor environment of the train connecting Córdoba city with the Rabanales University of Córdoba (UCO) Campus, as a pilot study to know more about the biological content of this widely used way of transport. A second objective was to compare the data obtained in this indoor environment with the data of aerobiological particles of the outdoor environment of the Rabanales UCO Campus to assess the risk of exposure to allergenic particles comparing both environments. A third objective was to study the factors impacting the rate indoor/outdoor concentrations, including the effect of meteorological parameters, to establish the possible actions to reduce health consequences for users of this transport system.

2 Material and method

2.1 Study area: indoor and outdoor characteristics

The study was carried out sampling both into the train linking Córdoba city with the Rabanales UCO Campus and in the outdoor atmosphere of the campus, located 8 km away from Córdoba city (Andalusia, Southern Spain). During the present research, aerobiological samplings in a total of 56 routes (30 min' sampling) without stops were performed between March 3th and May 12th of 2017.

This train is used every day by an average of 6000 people during the academic course, mostly students, teachers and workers of the university. The train belongs to the Spanish Public Trains Company (RENFE), and it is a 4 cars' model MD599, with a train power of 1.528 kW and a maximum velocity of 160 km/h. The capacity in each car of 200 people includes seats and stand up places. In the rush hours (early morning, 8:00 h and afternoon, 17:00 h), the train is usually overfull, doubling the number of people reaching up to 1500 people per train. The train only has artificial ventilation since the airtight windows are hermetically closed (Figure S1a). Each vehicle has compact climate control equipment complemented by floor level heaters. The air-conditioning system of the train rooms is made up of a car air-conditioning unit, including pollen filters and a double refrigeration circuit, which is compact and assembled in a single block. With this configuration, 47 kw/car of

cooling power is achieved and 31 kw/car of heat power with a car-driven air flow of 6200 kg/h. The daily cleaning routine of the train consists on a daily change of paper headrests, a floor sweep and a quick vacuum of seats. Once a week, the windows are cleaning and seats, floor and walls are more deeply vacuumed (RENFE ACCIONA 2019).

Coordinates of Córdoba city are 37° 53' 29.58" N 4° 46' 21.9" W, 106 m a.s.l., and those of the Rabanales Campus train station 37°54' 46" N 4° 43' 15" W, altitude 120 m a.s.l. The climate is continental Mediterranean with high temperatures, overcoming 40 °C many days, in summer and low temperatures up to 0 °C, in winter. The average annual temperature is 17.8 °C, and the annual rainfall is 621 mm. In parallel to the aerobiological survey, a meteorological sampling was made in the university campus. During the study, the average temperature was 15° and only one day shown > 10 mm rainfall. Meteorological monitoring was performed in the Rabanales UCO Campus by a meteorological station FiWare-Compliant (NGSI/V2/Ultralight 2.0). The meteorological parameters analyzed in the study were daily parameters including average temperature (°C), precipitation (mm), average relative humidity (%), wind direction (°) and wind speed m/s.

Córdoba city occupies 1245 km², with an altitude that varies between 90 and 693 m.a.s.l. The city center is located on the banks of the Guadalquivir River that crosses it from east to west forming several meanders. The north border of the municipality is the Sierra Morena mountains, with a mature oak Mediterranean forest; meanwhile, the rest of the city is surrounded by an extensive countryside of cereal and olive crops. Both at the city and at the university campus, there are several urban green areas full of ornamental plant species, mainly foreign species, but also it is possible to observe many autochthonous, naturalized and ruderal species colonizing empty spaces and building surroundings. Within the most abundant plant, taxa stand out the presence of the genera *Cupressus* (cypress trees), *Platanus* (London planes), *Pinus* (pines), *Eucalyptus*, *Morus* (mulberries) and *Ulmus* (elm trees) as ornamental trees. *Quercus* (oaks), *Olea europaea* (olive trees) and *Populus* (poplars) are the main autochthonous arboreal taxa surrounding the city and campus. Within the main herbaceous species, it is possible to highlight those belonging to the Poaceae (grasses), Urticaceae (nettles), Asteraceae (daisy and

sunflower family) and Fabaceae (legumes) families. It is remarkable that most of the species making up the landscape surrounding the studied areas are anemophilous. Regarding fungal spores, previous studies performed in the area of Córdoba city indicated the most abundant taxa in the air where those involved in saprophytic relationship with agricultural crops, i.e. *Alternaria* and *Cladosporium* (Angulo-Romero et al. 1999; Mediavilla et al. 1997). The latter genus was the most abundant indoors in a study performed in the atmosphere of 14 dwelling of this city (García-Pantaleón et al. 1992).

2.2 Aerobiological sampling

Pollen/spores sampling inside the train was performed using a Lanzoni Company Hirst-type portable air collector (Hirst 1952) (Figure S1b). This portable sampler was previously validated and equally calibrated with the fixed Hirst trap of the campus (Lanzoni Company); the calculated error was less than 5%. Pollen and spore concentration per cubic meter were obtained. Data from the portable sampler were compared with those obtained with the fixed one at the same hour. During the samplings, the collector was placed on a train seat, at a height of 50 cm above the train ground. In each route, the trap started to sample into the train before the journey, just from the doors' opening moment, continued during the journey and stopped only when all passengers got off the train. This process took around 30 min every route. The 56 samplings were performed at the rush hours, 28 at the 8:00 h in the morning, at 28 at the 17:00 h in the afternoon. Data about the pollen/spores sampling outside the train were compared with the aerobiological data obtained by a fixed Hirst sampler just at the same hour (Figure S1e), belonging to the Spanish Aerobiological Network (REA) (Spanish Aerobiological Network 2019). Data were analyzed following the protocol proposed by REA (Soldevilla et al. 2007) and the minimum requirement for aerobiological analysis (Galán et al. 2014) obtained concentration per cubic meter.

2.3 Statistical analysis

Spearman correlation tests, appropriated for parametric and nonparametric data, were performed between indoor pollen/fungal spores' concentrations, and

outdoor was calculated in order to detect the possible relationships between these data. On the other hand, an analysis of the outdoor/indoor ratios by dividing outdoor concentrations by indoor concentrations during the same monitored period for each pollen/fungal spore type, the ratio goes from 0 to infinite; < 1 means that indoor concentration is above outdoor one; > 1 outdoor concentration is bigger than the indoor one. To do the calculations and plotting, it was used R computing language (R Core Team 2017) Team 2013) mainly the packages from "tidyverse" family such as "ggplot2" (Wickham 2016, 2017) and "Aerobiology," specific tool for aerobiological data (Rojo et al. 2019a, b).

3 Results

During the sampling period, 23 different types of pollen grains were recognized in the indoor environment and 20 in the outdoor atmosphere (Table 1). In the case of fungal spores, they were 13 fungi taxa identified in both environments. All the pollen types identified by outdoor sampling were identified indoor the train. Nevertheless, pollen grains from 3 taxa were found only indoor.

In Fig. 1, it is possible to compare cumulative sums of pollen concentrations during the sampling period, 12 of the 19 common pollen types presented higher outdoor total quantities. By contrast, 7 common pollen types showed higher total quantities indoor than outdoor: *Platanus*, *Urticaceae*, *Brassicaceae*, *Rumex*, *Asteraceae*, *Rumex*. Moreover, 3 taxa, *Alnus*, *Apiaceae* and *Rosaceae*, were not presented outdoor, only in the indoor environment.

In the case of fungal spores, *Cladosporium cladosporioides* and *Cladosporium herbarum*, which also include *C. macrocarpum* and *C. sphaerospermum*, were the most abundant spores types in both environments, followed by *Coprinus* and *Alternaria*. Regarding the ratios outdoor/indoor of fungal spores, the highest ones were detected for *C. cladosporioides* 11 times highest outdoor than indoor. At the opposite case is *Alternaria*, with the lowest outdoor/indoor ratio, around 2.

For the meteorological survey, daily mean temperature, rainfall, relative humidity, wind speed and wind direction were analyzed (Fig. 2a). The slightly increased over time with some occasional decreases

Table 1 Comparative summary table of total pollen and spores detected indoor and outdoor during the study period

	Sum of daily concentrations/m ³	
Pollen type	Indoor	Outdoor
<i>Platanus</i>	4369	2825
<i>Quercus</i>	4326	16,925
<i>Olea</i>	2591	13,003
<i>Cupressus</i>	946	1858
<i>Morus</i>	922	4064
Urtica	723	258
Poaceae	483	1148
<i>Urtica membranacea</i>	330	1380
<i>Pinus</i>	200	581
Brassicaceae	143	77
<i>Populus</i>	117	516
<i>Plantago</i>	80	361
<i>Chenopodium</i>	60	129
<i>Fraxinus</i>	37	452
Asteraceae	27	15
<i>Mercurialis</i>	20	31
Ericaceae	17	99
Myrtaceae	10	15
<i>Alnus</i>	7	0
Rosaceae	3	0
Spore type	Indoor	Outdoor
<i>Cladosporium cladosporoides</i>	1185	12,939
<i>Cladosporium herbarum</i>	996	7392
<i>Alternaria</i>	230	619
<i>Coprinus</i>	157	1342
<i>Stemphylium</i>	103	258
<i>Torula</i>	90	323
<i>Epicoccum</i>	53	310
<i>Drechslera</i>	63	258
<i>Polythrincium</i>	40	168
<i>Pithomyces</i>	33	142
<i>Curvularia</i>	23	89

on March 4th, 13th and 23rd and April 28th. The precipitations have been rather scarce except for some days of high rainfall like March 3th and 4th and around April 27th, the day with the maximum precipitation with 34.8 mm. No significant correlation was found on a daily basis between all the observed meteorological measurements and all the airborne pollen/fungal spore concentrations, neither from indoor and outdoor. Nevertheless, relationships between meteorological parameters and biological particles outdoor and indoor are shown in Fig. 2b, c, respectively. In the case of

daily mean pollen concentration, despite no statistical significance with meteorological parameters, it is possible to observe rainfall influence on pollen concentration decrease. Usually, the outdoor pollen concentrations were high during the period except for rainy days March 16th, 17th and 31st and the period from May 9th to 12th where the indoor concentrations overcame outdoor ones.

In the case of the spores, we analysed outdoor spore types and not only the typical indoor spores. It was observed that the difference is so marked between

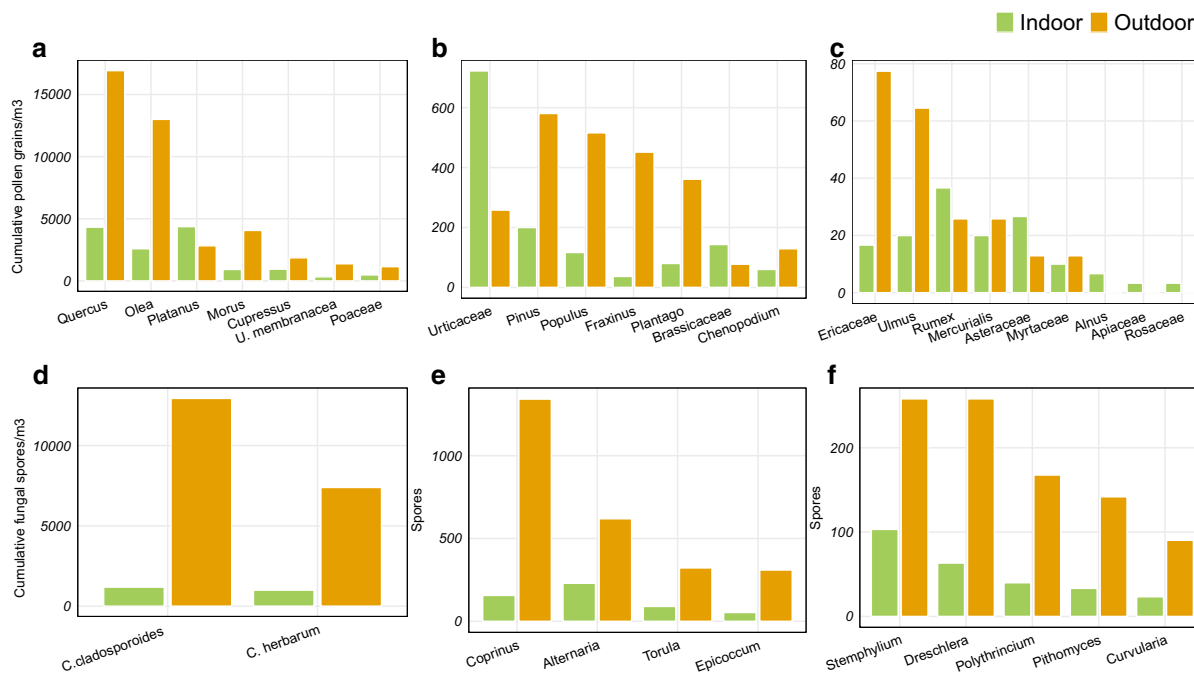


Fig. 1 Cumulative indoor biological particles vs. outdoor ones for all the observed taxa recorded during sampling period; **a–c** pollen grains of **d–f** fungal spores

outdoor and indoor daily concentrations, being higher those detected outdoor. In contrast to pollen, indoor spore concentrations appeared to remain relatively stable even the days where outdoor concentrations greatly increase. Observing the concentration patterns, the differences between daily outdoor and indoor concentrations are higher; nevertheless, the pattern of the total period remains more stable than in the case of pollen. For spore pattern, always outdoor > indoor but presenting parallel trends during the period. There are not drastic decreases as can be observed for pollen in some days. Indoor fungal spore concentrations present a typical “background noise” pattern, much lower than outdoor but always following the same trends and apparent dynamics. In contrast, indoor pollen concentrations seem to vary more often independently from the outdoor concentrations.

Figure 3 shows in a box plot the daily outdoor/indoor ratio values of the main taxa recorded during the season. Each box stands for the interval of daily ratio values, where the median is represented with a black bold line; the 50% of the data closer to the median are inside the gray boxes; and the 25% lowest are represented in the left arm while the highest 25% is in the right one. Moreover, the outliers (extreme daily

ratio values) are represented in case they appear. Red line marks ratio 1; it means when outdoor and indoor concentrations are the same. Dashed blue line represents the average of medians which is 4.36. Values below 1 indicate that indoor values were higher than outdoor concentrations. In the case of pollen grains, a mean value below 1 was observed for *Platanus* and *Urticaceae*. Other taxa with a ratio value greater than 1 but below than the average of medians were: *Quercus*, *Populus*, *Urtica membranacea*, *Pinus*, *Poaceae* and *Cupressus*, outstanding this latter pollen where the outdoor and indoor total concentrations were almost similar. On the other hand, pollen from *Olea* and *Morus* overcame the average of medians with so high outdoor/indoor ratio values.

Regarding fungal spores, *Coprinus*, *Stemphylium* and *Alternaria* registered ratio values below the average of medians. By contrast, *C. cladosporioides* and *C. herbarum* showed ratios far above the average of medians due to high outdoor concentrations.

In Fig. 4 is a box plot differentiating the sampling performed in the morning from the one in the afternoon. Here it is possible to observe that in 11 of the 15 shown taxa ratios were higher during the morning. Nevertheless, afternoon and morning

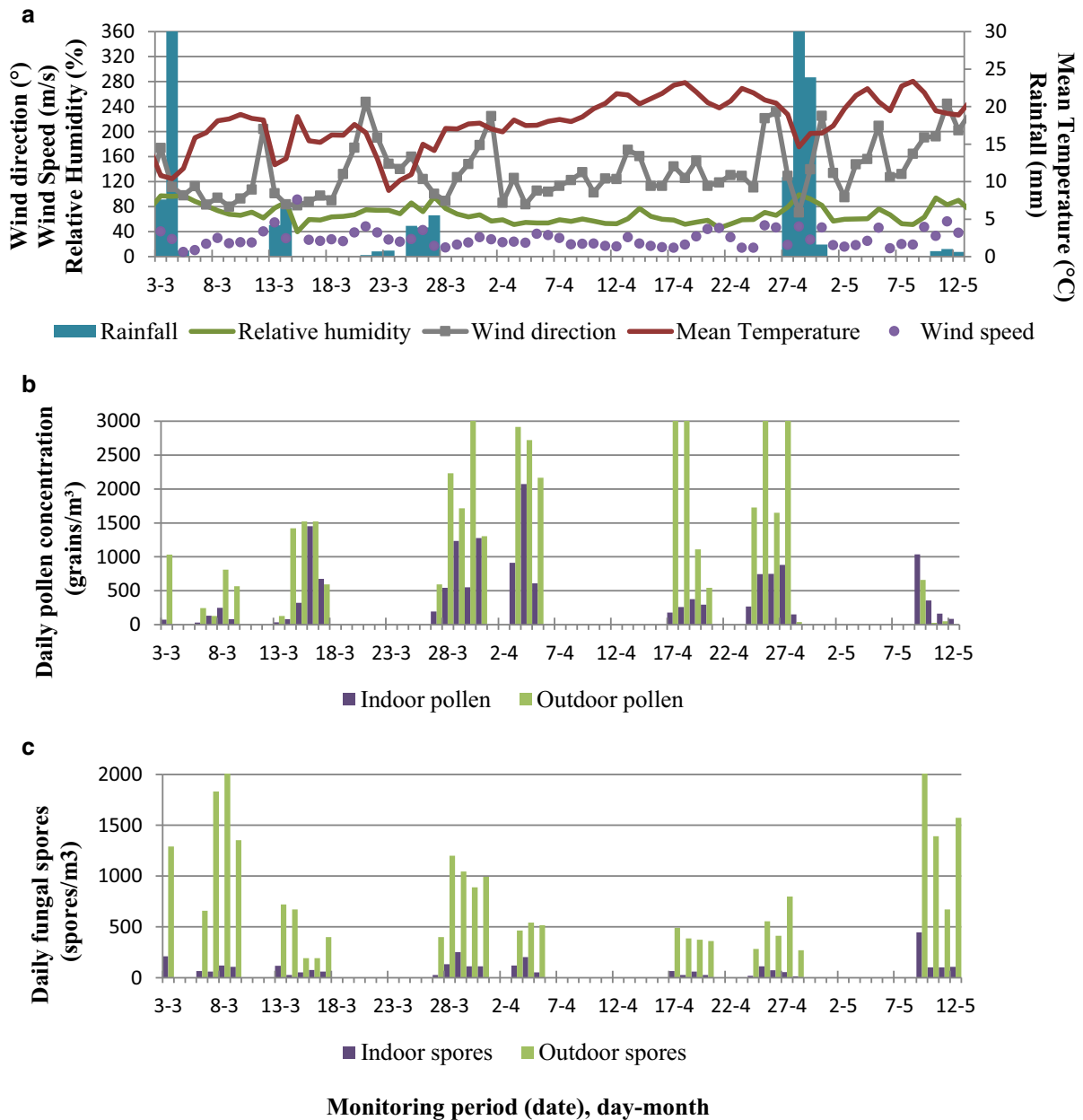


Fig. 2 Daily meteorological, pollen and spore concentrations during the sampling period, a) meteorological parameters, temperature (°C), rainfall (mm), wind direction (°), wind speed

(m/s) and relative humidity (%); b) daily pollen concentrations, indoor vs. outdoor, c) daily fungal spore concentrations, indoor vs. outdoor

boxplots' rates are overlapping to a great part; for this reason, it is necessary to analyse separately pollen and fungal spores. In the case of pollen, the taxa showing higher outdoor/indoor ratio values during the afternoon were *Olea*, *Quercus* and *U. membranacea*. It stands out the case of *Urticaceae* pollen, produced by nettles and pellitories, where during the morning the

ratio was over 1 while during the afternoon it was below 1. Regarding spores, *C. cladosporioides*, *C. herbarum* and *Coprinus* presented higher ratios during afternoon. Differently, *Alternaria* spores show similar ratio values at both times.

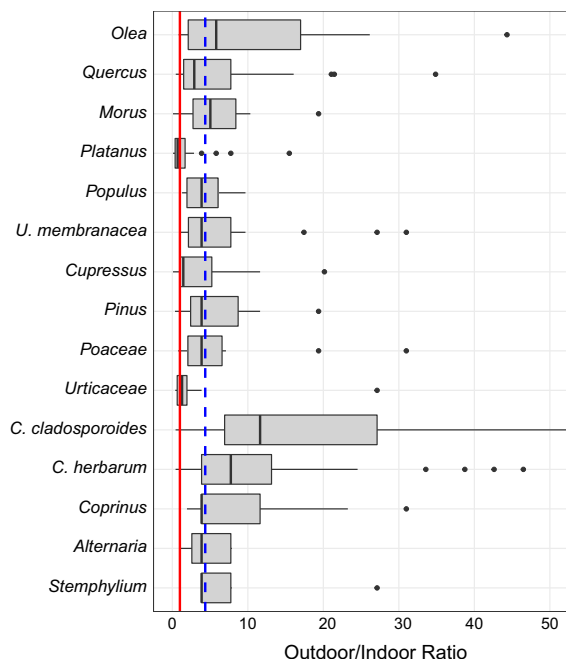


Fig. 3 Box plots of the outdoor/indoor ratios. In each boxplot, the median is indicated by a black line. The red line marks the ratio value = 1 (indoor = outdoor concentration). The dashed blue line shows the medium ratio in the study.

4 Discussion

The scarce number of aerobiological studies performed into public means of transports and the possibility of comparing indoor train results with those obtained outdoor at the same time, make this research a real novelty in the area of indoor air quality. Some previous approaches carried out at homes or public buildings indicated significant variations on the entrance of pollen grains depending on the way of ventilation and the affluence of passengers (Takahashi et al. 2008). In the case of studies analysing pollen into cars and buses, they pointed out that in some occasions, indoor concentrations of specific taxa, as *Betula* pollen, were higher than those detected outdoor (Holmquist and Vesterberg 2001; Hugg et al. 2007). Nevertheless, there is to our knowledge no published research related to indoor train quality air and least of all none comparing properly side-by-side indoor bioaerosols with outdoor ones.

Regarding the pollen types recognized in both environments, the observation of taxa is relatively common in Spanish aerobiological studies (REA 2020). The indoor count is so similar to the outdoor

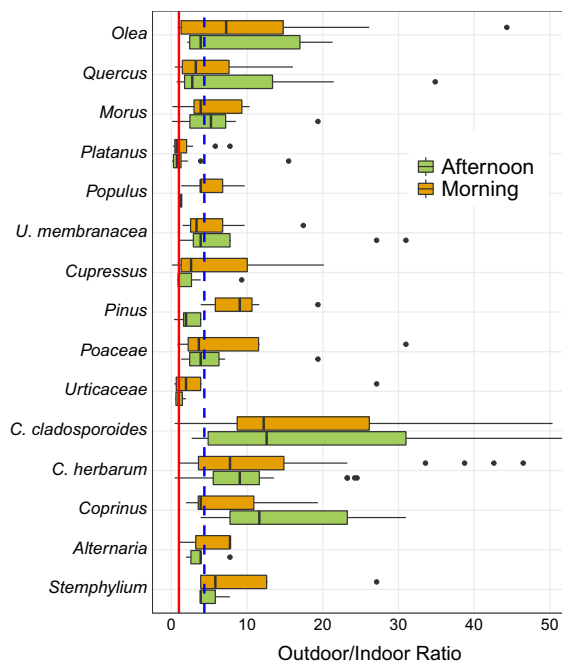


Fig. 4 Box plots of the outdoor/indoor ratios of morning measurements, performed within the 8:00–10:00 h interval, and afternoon measurements, 16:00–18:00 h. The red line marks the rate value = 1 (indoor = outdoor concentration). The dashed blue line shows the medium rate in the study.

one except for 3 pollen types appeared indoor and not outdoor: *Alnus*, Apiaceae and Rosaceae. First one corresponds to *Alnus glutinosa* an anemophilous tree species located in a population near the railroad track section crossed by a stream, 2 km far from the campus. The other two, Rosaceae and Apiaceae, come from entomophilous species growing at the Córdoba train station gardens, and probably they were introduced stuck on passengers' clothes. Moreover, it must be stressed the higher indoor concentrations recorded for Urticaceae pollen. This pollen type englobes all the species of the *Urtica* and *Parietaria* genera (respectively, known as needles and pellitories), except for the pollen of *Urtica membranacea*, distinguished specifically in our study. One of the most abundant weed species growing at the railroad track sides in Córdoba region is *Urtica urens* (Galán et al. 2000) with large populations in the embankments of the Rabanales train station, near the train gates. Urticaceae pollen can be stuck to clothes in higher quantities while passengers are waiting for the train. Other hypothesis is that ventilation filters of train may specifically capture part of the large-sized pollen

grains and let escape the small-sized pollen grains (like *Urticaceae*), which would recirculate inside the train.

Other pollen types presenting higher indoor concentrations were *Platanus*, *Brassicaceae* and *Asteraceae*. *Platanus* genus is mostly represented in Córdoba by the species *Platanus* × *hispanica*. *Platanus* is considered one of the top 5 pollen allergens in Spain (Asam et al. 2015), and therefore, the use as an ornamental tree so frequent in Spain has been reported as dangerous (Barber et al. 2008). This tree is abundant both in the city and in the campus, and close to the two gates of the train in both train stations. It has a short but explosive flowering pattern. The main pollen period is relatively shorter compared with most of anemophilous tree species, but this could produce the relatively fast reduction of concentrations outdoor (Alcázar et al. 2011). In our study, we remarkably observed significant indoor concentrations of *Platanus* pollen even during days for which it was not detected outdoor. Indeed, the train could be acting as a reservoir avoiding the fast decrease in pollen concentrations of this type increasing the pollen season up to two weeks more. It is an allergy risk because when the outdoor atmosphere is clean of this allergen, causing health problems to the allergic train commuters. A similar behaviour of lengthening of the allergy risk season was detected by Hugg et al. (2007) for other anemophilous tree pollens, *Betula* (birch) and *Pinus* (pine tree), into cars in of a southeast of Finland.

Nevertheless, many of the most abundant indoor pollen taxa came from herbaceous species except for *Alnus* and *Platanus*, which are emitted by anemophilous trees located at close areas to the train. In addition, the morphological characteristics of pollen grains from this latter taxa have been revealed as disadvantageous to reach high atmospheric elevation and long-term atmospheric transport, whereas the entomophilia of most of them favours the adhesion to clothes of passengers and therefore the entrance into the train (Rojo et al. 2018; Takahashi et al. 2008).

Regarding spores, difference between outdoor and indoor daily concentrations, being in general much higher those detected outdoor, was also indicated in the case of comparing homes' outdoor/indoor studies (Garrett et al. 1998; Li and Kendrick 1995). It is remarkable the case of *Coprinus* for which high quantities were found in both environments, even ahead of *Alternaria*, one of the most abundant airborne

fungal spores. The small size of *Coprinus* spore is known to be adapted to get through the air-conditioning systems as it has been observed by other authors, and to present higher concentrations than other more abundant outdoor fungal spores as *Alternaria* (Wang et al. 2013). In the case of other the small fungal spores (*Aspergillus*/*Penicillium*), we did not measure them because the large quantities in both indoor and outdoor environments made that they are usually not reported by routine fungal spores' monitoring (Blanco et al. 2016; Mitakakis and Guest 2001; Sánchez-Reyes et al. 2016). And when reported, spores are usually classified together as *Aspergillaceae* due to the difficulties of distinguishing morphologically both spore types, as well to differentiate visually from many other common conidia (Fernández-Rodríguez et al. 2014). Nevertheless, it is sure that a wider and specific biodiversity isn't missed, especially for the indoor environment.

It is noticeable the stability of indoor daily spore concentrations in comparison with the outdoor higher variability more dependent to humidity. Spore dispersion mechanisms of different fungal species are so different; some deliver their spores more during dry some during humid conditions. This behaviour is tempered into the train; nevertheless, there is a general increase in outdoor concentrations positively related to days with more humidity, in many cases after rainfall days. Into homes, similar pattern results were reported (Sterling and Lewis 1998; Stock and Morandi 1988). In general, it is the opposite behaviour than in the case of pollen, negatively correlated with humidity. This positive relationship between airborne spore transport and humidity makes that rainfall days, and the following days presented no significant spore concentration decrease, even when the total of spores both outdoor and indoor increased in any days.

Therefore, side-by-side indoor vs. outdoor comparison of daily pollen and spore concentrations (Fig. 2) was consistent with the results obtained with cumulative amounts. Results are overall in accordance with previous findings of decreasing pollen and spore exposure when they are moving from outdoors to indoors (Lee et al. 2006; Muilenberg et al. 1991; Sterling and Lewis 1998). This situation coincides with days just after a rainfall period as the case of March 16th, 17th and 31st, and especially the period May 9–12th. This is principally due to the atmospheric washing of the outdoor environment just after previous

rainfall periods, registered in the area, whereas the indoor environment concentrations remained more stable and continued being fed possibly by the pollen and spores introduced on passengers' clothes. A similar behaviour for birch and grass pollen was observed in a study performed in Stockholm (Sweden) into buses (Holmquist and Vesterberg 2001).

The proportional comparison of the two sampled environments indicated always bigger outdoor than indoor concentrations with an average ratio of 4.36 (Fig. 3). However, the ratio was not the same for all the analyzed taxa. The outdoor/indoor ratio was relatively lower than this average value in the case of local plants more abundantly present in the vicinity of the train access, i.e. *Platanus* (Alcázar et al. 2011), or mostly coming from close sources as Poaceae (Skj  th et al. 2013) or some very nitrophilous species abundantly found in the surrounding of the train station as *Urtica dioica*. The other way around was observed in the case of plants with well-known sources far from the pollen monitoring stations (inside and outside the train) as it is the case of *Quercus* species located at the near mountains (Garc  a-Mozo et al. 2016; Hern  ndez-Ceballos et al. 2011). It is an interesting finding that results indicated that the distance of the pollen emission sources from indoor and outdoor samplers would be critical for the interpretation of the ratio. Future research of this kind should therefore be cautious about this potential bias when studying the dynamics of contamination of an indoor environment by bioaerosols coming from outdoor.

The differences in ratios between morning and afternoon monitoring were compared (Fig. 4). We did not observe any significant difference between them, but it is remarkable that again pollen from local sources, i.e. *Platanus*, Urticaceae, Poaceae, shows a similar pattern: smaller ratios during afternoon than morning. For most of the taxa, the ratios during the afternoon were lower than the average ratio value, being even lower than 1 in some taxa where we found more pollen inside the train than outside the train. This could be explained by our hypothesis that when pollen is emitted in the morning, the concentrations reached outside are higher, but then the outdoor concentration falls very fast but while remaining stable inside the train for a longer time.

The present investigation clearly indicates that external allergens can highly contaminate the air

quality in train cars, equipped with air cleaning filters, travelling in urban and sub-urban environments during the pollen seasons and even after. Pollen allergens might, in addition, to the supplied air, come into the train cars through open doors and with the passengers' circulation. Moreover, although the change of the headrest protectors took place every day, the full cleaning of the train seats' trim took place once a month which can favor the accumulation of pollen and spores into the train cars. It was proved because many times afternoon indoor concentrations were higher due to the cumulative effect. Opening the windows for ventilation during the cleaning process would be a measure for reducing the concentration of biological and chemical contaminants. When a room is well ventilated, moisture is evaporated and the surface temperature is reduced, modifying these two environmental factors on which the growth of the fungi spores depends. However, stagnant air favors the concentration of more and more fungi spores, which is why ventilation is essential to achieve low biological activity in the internal environments (Valent  n 2007). The ventilation systems can play a key role in the health of the indoor environment and reduce the sick building syndrome also suffered into means of transports. As conclusion, it can be stated that the train is acting as a reservoir of pollen and spores. Although there is more amount of pollen outside than inside, we find significant allergen concentration indoor, even with pollen seasons lasting more days than outdoors. It is also remarkable the observation of episodes where indoor concentrations were higher than outdoor, more specifically for plants located close by the train entrances. Finally, the observed results in this study pave the way for a rationale approach in order to develop and implement new management actions based on ventilation in trains.

Acknowledgements The authors thank gratefully the Project CLIMAQUER (Reference 1260464) granted by the University of C  rdoba and supported by the Council of Economy and Knowledge of the Andalusia Region Government through the European Regional Development Funds (ERDF) providing a PhD bursary and support for complete and redacting the present work.

Funding The authors thank gratefully the Project CLIMAQUER (Reference 1260464) granted by the University of C  rdoba and supported by the Council of Economy and Knowledge of the Andalusia Region Government through the European Regional Development Funds (ERDF) providing a

PhD bursary and support for complete and redacting the present work.

Compliance with ethical standards

Conflict of interest The authors declare they have no actual or potential competing interests.

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