Annals of Agricultural and Environmental Medicine 2013, Vol 20, No 4, 664–671
www.aaem.pl

ORIGINAL ARTICLE

Air-spore in Cartagena, Spain: Viable and non-viable sampling methods

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Elvira-Rendueles B, Moreno J, Garcia-Sanchez A, Vergara N, Martinez-Garcia MJ, Moreno-Grau S. Air-spore in Cartagena, Spain: Viable and non-viable sampling methods. Ann Agric Environ Med. 2013; 20(4): 664–671.

Abstract

In the presented study the airborne fungal spores of the semiarid city of Cartagena, Spain, are identified and quantified by means of viable or non-viable sampling methods. Airborne fungal samples were collected simultaneously using a filtration method and a pollen and particle sampler based on the Hirst methodology. This information is very useful for elucidating geographical patterns of hay fever and asthma. The qualitative results showed that when the non-viable methodology was employed, *Cladosporium*, *Ustilago*, and *Alternaria* were the most abundant spores identified in the atmosphere of Cartagena, while the viable methodology showed that the most abundant taxa were: *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*. The quantitative results of airborne fungal spores identified by the Hirst-type air sampler (non-viable method), showed that Deuteromycetes represented 74% of total annual spore counts, *Cladosporium* being the major component of the fungal spectrum (62.2%), followed by *Alternaria* (5.3%), and *Stemphylium* (1.3%). The Basidiomycetes group represented 18.9% of total annual spore counts, *Ustilago* (7.1%) being the most representative taxon of this group and the second most abundant spore type. Ascomycetes accounted for 6.9%, *Nectria* (2.3%) being the principal taxon. Oomycetes (0.2%) and Zygomycestes and Myxomycestes (0.06%) were scarce. The prevailing species define our bioaerosol as typical of dry air. The viable methodology was better at identifying small hyaline spores and allowed for the discrimination of the genus of some spore types. However, non-viable methods revealed the richness of fungal types present in the bioaerosol. Thus, the use of both methodologies provides a more comprehensive characterization of the spore profile.

Key words

aerobiology, spores, allergy, culture-based and non-culture-based methods

INTRODUCTION

Developments in research in recent years have sought to understand the mycological pattern of the atmosphere which is of main interest for allergenic sensitization issues [1, 2, 3], as well as for crop management [4] and in the field of occupational hygiene [5].

A significant proportion of bioaerosols in the air is frequently made of particles of fungal origin, particularly spores [6]. We need to better understand the relationship between exposure to airborne fungal spores and their effects, on human, animal and plant diseases that they can cause. Understanding the range and nature of relevant environmental allergens may be helpful in controlling the allergic diseases [3]. In most cases this information is not available because of the difficulty of identifying and quantifying fungal spores in air samples [6]. The outdoor fungal airspora from many parts of the world have been identified and described, mainly by non-volumetric samplers or by using a volumetric sampler that is either culture-based (viable method) or non-culture-based (non-viable method) [7].

The major component of the airborne fungal spectrum, both indoors and outdoors, is the Deuteromycetes or imperfect fungi [1, 8], fairly easily identifiable in air samples. Ascomycetes, Basidiomycetes and Zygomycetes are also present in the air and have been identified and quantified in different studies [4, 9].

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Received: 31 July 2012; accepted: 6 March 2013

The information on the airborne fungi in an area would be useful to elucidate geographical patterns of asthma and hay fever among the population [9]. Daily mould alerts and reports on airborne fungi are important tools for the correct diagnosis by allergists of hypersensitivity to fungal spores. On the other hand, there is an urgent need for researchers to determine the extent of health effects caused by fungi and, if these fungi are present, to establish exposure thresholds and guidelines for the medical community [5].

The main objective of this study was to qualitatively and quantitatively identify the different types of airborne fungal types present in the bioaerosol. For the qualitative characterization of fungal spores in the atmosphere of Cartagena, both a viable method [10] and a non-viable methodology [11] were used. This last method was also used for quantification of fungal spores.

MATERIALS AND METHOD

The geographical setting. Cartagena is a Mediterranean city, situated in the southeast of the Region of Murcia (latitude 37°36' N, longitude 0°59' W). The climate of the area is arid Mediterranean subtropical or subarid, with a steppe subtropical topographic situation [12]. This is one of the driest areas of Spain, with mild temperatures, the annual average being 17.3°C, a moderate thermal range of 14.3°C, and scant rainfall, which is less than 300 mm/year. It also presents high relative humidity, annual average above 75%, and the annual water deficit is over 600 mm [13].

From May to September, the coast of Cartagena is affected by heat waves, with temperatures up to 40°C. Winter temperatures

are very mild, January being the coldest month [13], with an average temperature of 11°C. The prevailing winds are from the first (north to east, 40.9%) and third quadrants (south to west, 39.7%), the former clearly prevailing at night and the latter during the day, the typical pattern associated with sea breezes. The meteorological data used in this study were obtained from the Spanish Meteorological Agency (AEMET) station of Cartagena (latitude 37°36'N longitude 0°59'W).

Three different types of vegetation series can be observed in the area [12]. The winter climate conditions in this area allow the harvest of several crops, such as artichoke, lettuce, cauliflower, cabbage, red cabbage, celery, melon and asparagus in the same year [14], which replaced the traditional production of cereals. The most frequent fungal infections affecting these crops are caused by species of *Pythium, Alternaria,* Peronosporales, *Oidium, Botrytis, Stemphylium, Puccinia,* and *Rhizoctoni* [14].

Spore sampling and identification. This study is part of the aerobiological monitoring program of the city of Cartagena. Two different samplers were used for quantitative and qualitative fungal spores airborne characterization.

Active impact sampler (Non-viable methodology). Quantitative and qualitative characterization. Following the methodology proposed by the Spanish Aerobiological Network (REA) [15] a seven-day recording volumetric spore trap was used (Hirst-type Sampler, Lanzoni VPPS-2000, Bologna, Italy) situated 10 m above the ground on the roof of Cartagena Railway Station. Spore counting was carried out by means of an Olympus microscope BH-2 with D-Plan optics, at 50× magnification. A 100× magnification was used for complete fungal spore identification.

To obtain qualitative values of the fungal types present in the atmosphere between 1994 and 1999, 176 preparations were analyzed in the microscopic identification of the fungal types present in the samples taken using this methodology (active impact). In order to quantify fungal spores and establish the fungal seasonal patterns in the atmospheric aerosol, counts were carried out for a week sample during 1997, i.e. 52 samples.

The remaining samples were analyzed following the same methodology, but the counts focused on the fungal types *Alternaria*, *Cladosporium* and on pollen types [15, 16, 17]. Fungal conidia/spores were classified according to their morphologic characteristics, using the Saccardian artificial classification methodology [18]. This method is mainly used to differentiate and identify spores from Deuteromycetes or imperfect fungi, as well as asexual spores of anamorphs of Ascomycetes and Basidiomycetes. In addition to a reference fungal collection, differents atlases and illustrated identification manuals were used for identification [18, 19, 20, 21, 22, 23, 24].

Filtration-based sampler. (Viable methodology): Qualitative characterization. This methodology is based on air filtration through a cellulose acetate filter [10, 25]. The modified MCV CPV-1S sampler (Barcelona, Spain) [10] is equipped with a filtration chamber located in a weather vane, a low volume electromagnetic pump, with an adjusted flow rate of 7 l/min, a gas counter and a timer.

The filter was removed once every 24 hours. Half the filter was put on a 75 × 75 mm microscope slide and covered with immersion oil and stored [10]. The other half was divided into two equally sized parts for inoculation on culture plates. One of these was cultured on plates containing sabouraud dextrose chloramphenicol actidione agar medium (SCA), and the other one was inoculated on sabouraud maltose agar medium (SMA). To determine the genus and, whenever possible, the species, the colonies were reseeded for isolation on the aforementioned media (SCA and SMA) or on Czapek-Dox agar medium (only for *Aspergillus* and *Penicillum*), including microculture in some cases. This culture methodology was applied twice a week to the samples from 1996 to 1998.

Fungal spore macroscopic characteristics were defined using the following colony identification parameters: texture, topography, color (obverse and reverse), periphery, temperature, speed of growth and isolation medium. Fungal spore microscopic characteristics were observed using glycerin-jelly with cotton-blue as indicator. Both reproductive and vegetative mycelium characteristics were observed under the microscope.

RESULTS

Meteorological data. Figure 1 depicts average weekly values of relevant, meteorological parameters, accumulated rainfall, daily minimum, average and maximum temperature, and relative humidity daily values at 00, 07, 13 and 18 hours. Figure 2 shows weekly average wind run in km and the

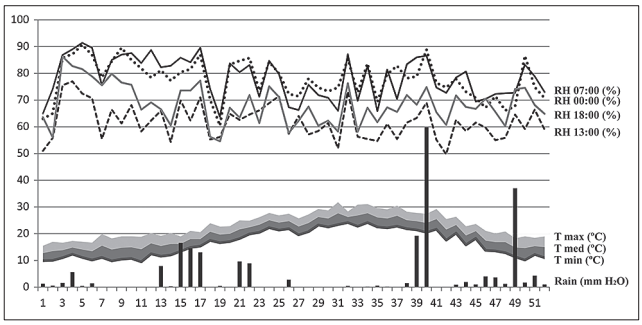

Figure 1. Average weekly values of relevant meteorological parameters: accumulated rainfall; daily minimum, average and maximum temperature; and relative humidity daily values at 00, 07, 13 and 18 hours.

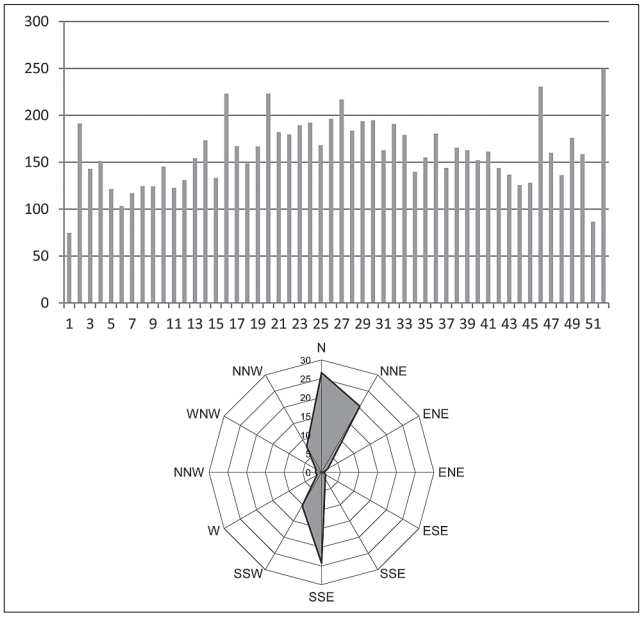

Figure 2. Weekly average wind run (km) and the wind rose of Cartagena for year 1997.

Table 1. Ninety-seven airborne fungal spores identified in the atmosphere of Cartagena (1994-1999). (1) non-viable methodology; (2) viable methodology.

Deuteromycete			
Alternaria (1,2)	*Cladosporium (1,2)*	*Micelia (2)*	*Septoria (1)*
Aspergillus (1,2)	*Curvularia (1)*	*Oidium (1)*	*Spegazzinia (1)*
Arthrinium (1)	*Dendryphiella (1)*	*Penicillium (1,2)*	*Stachybotrys (2)*
Aureobasidium (2)	*Drechslera (1)*	*Phoma (2)*	*Stemphylium (1)*
Beauveria (2)	*Embellisia (1)*	*Pithomyces (1)*	*Tetraploa (1)*
Beltrania (1)	*Epicoccum (1)*	*Pleurophragmium (1)*	*Torula (1)*
Bipolaris (1)	*Epidermatophyton (1,2)*	*Polythrincium (1)*	*Trichophyton (1,2)*
Bispora (1)	*Fusarium (1,2)*	*Rhodotorula (2)*	*Trichothecium (1)*
Botrytis (1,2)	*Gonatobotrys (2)*	*Sclerotium (1,2)*	*Ulocladium (1,2)*
Cercospora (1)	*Helicomyces (1)*	*Scytalidium (1)*	*Verticillium (2)*
Cerebella (1)	*Helminthosporium (1)*		
Basidiomycete			
Agaricus (1)	*Calvatia (1)*	*Inocybe (1)*	*Tilletia (1)*
Agrocybe (1)	*Coprinus (1)*	*Phylacteria (1)*	*Tranzschelia (1)*
Amanita (1)	*Chlorophyllum (1)*	*Puccinia (1)*	*Ustilago (1)*
Boletus (1)	*Ganoderma (1)*	*Russula (1)*	
Bovista (1)	*Gomphus (1)*	*Sporobolomyces (1)*	
Ascomycete			
Ascobolus (1)	*Emericella (1)*	*Leptosphaeria (1)*	*Pleospora (1)*
Caloplaca (1)	*Fusariella (1)*	*Massariosphaeria (1)*	*Sordaria (1)*
Chaetomium (1)	*Helicogermslita (1)*	*Erysiphe (1)*	*Sporidesmium (1)*
Chaetosphaerella (1)	*Helvella (1)*	*Mycosphaerella (1)*	*Sporomiella (1)*
Diatrypacea (1)	*Kassariosphaeria (1)*	*Nectria (1)*	*Xylariaceae (1)*
Didymella (1)	*Kleiseriella (1)*	*Paraphaeosphaeria (1)*	
Oomycete			
Albugo (1)	*Bremia (1)*	*Peronospora (1)*	*Pythium (1)*
Zygomycete			
Entomophthora (1)	*Mucor (1,2)*	*Rhizopus (1,2)*	*Syncephalastrum (2)*
Myxomycete			
Badhamia M (1)	*Ceratiomyxa M (1)*	*Fuligo M (1)*	*Physarum M (1)*
Trichia M (1)			
Lichen			
Caloplaca L (1)			

wind rose of Cartagena for year 1997. The total accumulated rainfall was 232.5 mm in 1997. The average temperature for January was 13.8°C.

Qualitative characterization of airborne spores. In total, ninety-six different airborne fungal spores and one Lichen were identified and isolated from the atmosphere of Cartagena (Tab. 1). Using microscopic and macroscopic culture characteristics in the viable methodology, seventy-six fungi species were identified, the results are depicted in Table 2, corresponding to 20 taxa.

Table 2. Seventy-six mould species isolated via the viable MCV methodology.

Genus	Species	Class
Alternaria	*sp. 1 – sp. 3*	Deuteromycete
Aspergillus	*A. terreus, A. niveus, A. ochraceus, A. niger, A. flavus, A. cervinus, A. candidus, A. oryzae, A. flavipes, A. fumigatus, A. glaucus, A. clavatus*	Deuteromycete
Aureobasidium	*Pullularia pullulans*	Deuteromycete
Beauveria	*B. bassiana*	Deuteromycete
Botrytis	*sp., B. cinerea*	Deuteromycete
Cladosporium	*C. cladosporioides, C. herbarum, C. sphaerospermun*	Deuteromycete
Epidermatophyton	*sp.*	Deuteromycete
Fusarium	*sp.*	Deuteromycete
Gonatobotrys	*sp.*	Deuteromycete
Micelia	*sp.*	Deuteromycete
Mucor	*Sp.*	Zygomycete
Penicillium	*sp. 1 – sp. 40*	Deuteromycete
Phoma	*sp.*	Deuteromycete
Rhizopus	*R. stolonifer*	Zygomycete
Rhodotorula	*sp.*	Deuteromycete
Sclerotium	*sp.*	Deuteromycete
Syncephalastrum	*S. racemosum*	Zygomycete
Trichophyton	*T. rubrum*	Deuteromycete
Ulocladium	*sp.*	Deuteromycete
Verticillium	*sp. 1, sp. 2*	Deuteromycete

From the total identified spores, 42 belonged to the Deuteromycete group (10 were observed in both methods, 24 were only identified by the non-viable method while 8 were only identified by the viable method). Identified only by the non-viable method were 18 Basidiomycetes, 23 Ascomycetes, 4 Oomycetes and 5 Myxomycetes. 4 belonged to the Zygomycete group of which two were observed in both methodologies. One Lichen was identified by the non-viable method.

When considering Penicillium y Aspergillus, in the spring of year 1997, regardless of the low counts found by the non-viable method, the number of isolates obtained from the viable method was high. It is necessary to point out that in other years the maximum isolates are obtained during the fall (November).

When the non-viable methodology was employed, *Cladosporium*, *Ustilago* and *Alternaria*, were the most abundant spores identified in the atmosphere of Cartagena. Employing the viable methodology the most abundant taxa were: *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria*.

Quantitative characterization of airborne spores. Airborne fungal spores visualized from the material collected with the Hirst-type sampler were classified according to group and genera (Tab. 3). Their abundance decreases in this order: Deuteromycetes; Basidiomycetes; Ascomycetes; Oomycetes; Zygomycetes and Myxomycetes. These quantitative data are the basis for the fungal seasonal patterns of the city of Cartagena. Figure 3 shows the weekly concentration of the 18 representative fungal spore types with the total spores counts for year 1997. In the figure, Basidiospores represent

Table 3. Quantitative results of airborne fungal spores identified in the atmosphere of Cartagena by non-viable methodology (Hirst-type air sampler).

Group	Total* spores/m³	%	Max. daily count, spores/m³
TOTAL SPORES	33,433		
Deuteromycetes	24,736	73.99	
Basidiomycetes	6,302	18.85	
Ascomycetes	2,308	6.90	
Oomycetes	67	0.20	
Zygomycetes and Myxomycetes	20	0.06	
DEUTEROMYCETES			
Cladosporium	20,778	62.15	1,798
Alternaria	1,765	5.28	167
Stemphylium	434	1.30	97
Penicillium	343	1.00	182
Botrytis	262	0.78	40
Aspergillus	260	0.78	40
Oidium	202	0.60	60
Arthrinium	109	0.33	33
Drechslera	109	0.33	9
Cercospora	91	0.27	57
Torula	86	0.26	30
Fusarium	84	0.25	21
Epiccocum	65	0.19	13
Curvalaria	37	0.11	12
Helicomyces	31	0.09	8
Polythrincium	30	0.09	9
Aureobasidium	10	0.03	9
BASIDIOMYCETES			
Ustilago	2,367	7.08	502
Hyaline basidiospores	1,107	3.31	550
Boletus	816	2.44	252
Agaricus	755	2.26	100
Coprinus	469	1.40	70
Amanita	436	1.30	116
Puccinia	314	0.94	149
Phylacteria	135	0.40	36
Agrocybe	76	0.23	15
Ganoderma	46	0.14	12
Russula	34	0.10	15
Tilletia	30	0.09	6
ASCOMYCETES			
Nectria	758	2.27	258
Leptosphaeria	622	1.86	79
Caloplaca	317	0.95	116
Pleospora	310	0.93	82
Diatrypacea	121	0.36	43
Chaetomium	98	0.29	27
Xylariaceae	14	0.04	9
OOMYCETES			
Peronospora	64	0.19	6

* Total accumulated spores in the 52 weekly samples studied in 1997.

the sum of the types: *Boletus, Agaricus, Coprinus, Amanita, Phylacteria, Agrocybe, Ganoderma y Russula*.

Spore counts showed marked seasonal differences. Spring presented the maximum spore counts, decreasing the counts sequentially in autumn, summer and winter. Spring and summer are the periods with the highest counts of *Cladosporium* in Cartagena while the lowest counts were generally registered in winter. However, the Cladosporium count for week 5 is high (Fig. 3) in 1997. *Alternaria, Stemphylium, Botrytis, Oidium*, *Dreschlera, Ustilago*, *Puccinia*, etc showed the maximum counts in spring, whereas *Chaetomium* presented the peak in summer and *Helicomyces* and *Pleospora* in winter. Finally, Basidiospores and *Nectria* (the most abundant taxon in the ascospore group) showed the maximum in autumn.

DISCUSSION

This study reveals important qualitative differences in spore content in the bioaerosol, depending on the sampling method used. A unique method for the complete assessment of atmospheric fungal spores does not exist [26, 27], "*so, depending on the type of technique employed, reports on airborne fungal spores always give incomplete information about the actual presence of spores in the air*" [26]. The microscopic observation, by the non-viable method, allows the identification of total spores (both culturable and non-culturable) along with pollen and other particulates.The use of a non-viable method (Hirst type-sampler) reduces the importance of hyaline small spores [4], and, due to the fact that identification is morphological [26], does not allow identifying genus and species, reaching only to form genus [27]. On the other hand, viable methods enable the identification of the genus, and in many cases the species. The rapid growth spores, and those with little nutritional requirements, are favored in viable methods; for that reason two different culture media were used, one of them specially suited to impede the development of invasive fungi like *Rhizopus,* thus increasing the number of isolates. Even so, the variety of fungal types obtained with the viable method is lower, together with the reasons stated above, there is competition among the fungi developed in the cultures [27].

One study carried in Michigan [27] made a comparison between volumetric viable and non-viable methods, and showed that there is a better representation of particle type in non-viable samples that show better true variations in levels of air spores and this was one of the reasons to choose the non-viable method for the quantification of the spores samples.

With respect to quantitative results, *Cladosporium* is the most abundant spore type in our bioaerosol in both viable and non-viable methodologies. Three different *Cladosporium* species were identified by the viable method. These results are similar to those found in other places following similar methodologies [27]. In tropical and temperate regions, *Cladosporium* constitutes between 20 and 80% of the airborne spore load, with the peak occurring at the end of the growing season [28]. The saprobic mould genus *Cladosporium* comprises a large number of species [29], being the most common taxon in outdoor air worldwide irrespective of the climate [29, 30, 31, 32]. In this respect, different authors have reported that extreme cold temperatures, rainfall and snow are negatively correlated with the presence of *Cladosporium*

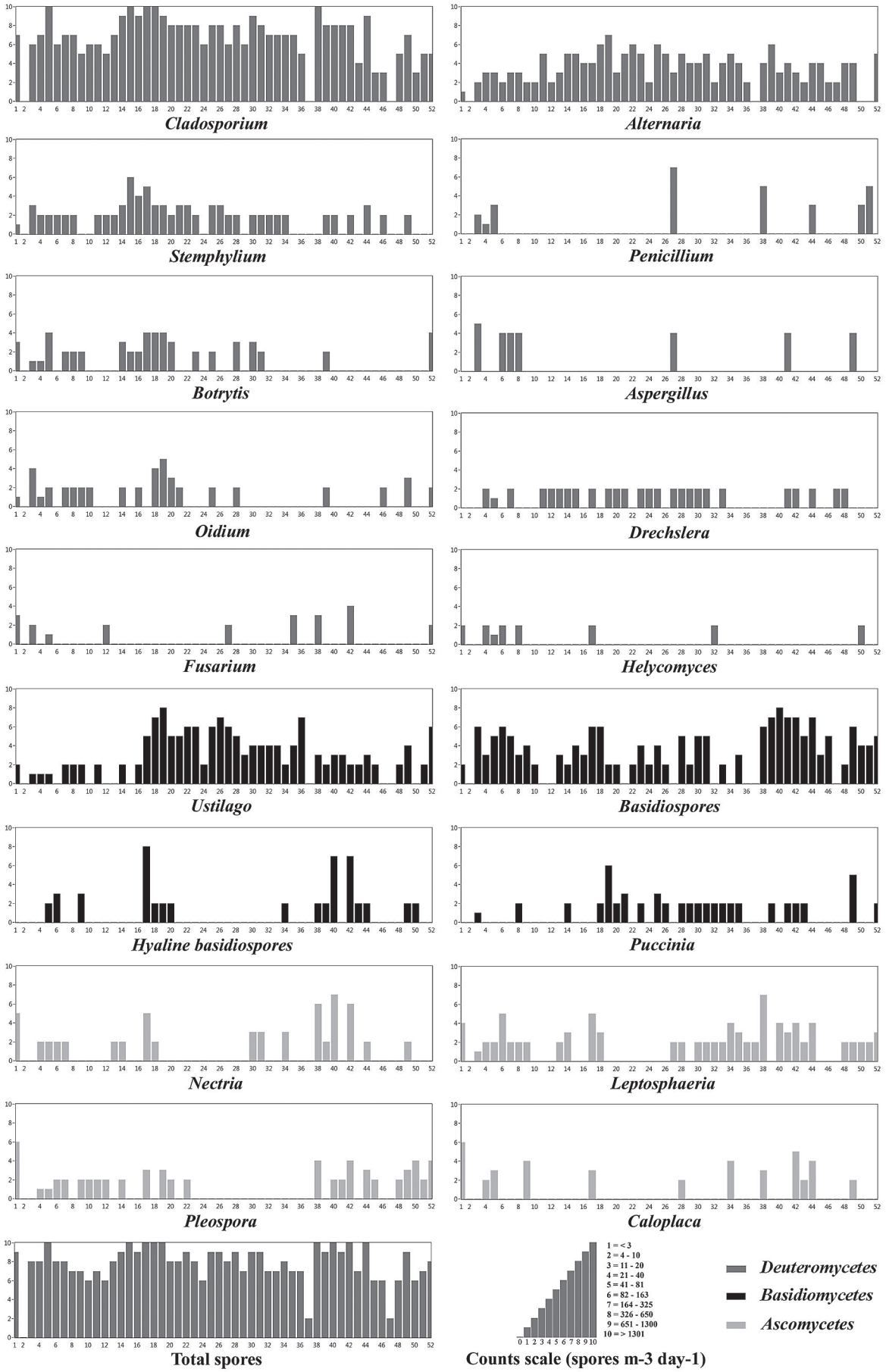

Figure 3. Hirst non-viable method weekly average concentration of 18 representative fungal spore types and total spores counts for year 1997.

spores [31, 33] although positive correlation was found among *Cladosporium* and rain and temperature [17]. Rain promotes spore growth and their release by different ways [34]. The rainfall in the first weeks of the year, together with the mild temperatures registered in January, explain the peak that appears in week 5. *Cladosporium* is the major source of inhalant fungal allergens [35].

Alternaria is well represented throughout the year, with both saprobic and plant parasitic species [36], is one of the most important allergenic fungi with a seasonal spore releasing pattern [35] and one of the major components of the world fungal bioaerosol [16]. Saprobic species are present almost all year round, emanating from decaying plant material, where parasitic species are associated with the life-cycle of their hosts [1] both endogenous and cultivated in our region. *Alternaria* has been reported as being a thermotolerant species [24]. Its release is dependent on humidity and rainfall, decreasing on rainy days and increasing in dry weather [37]. The strong weather dependency of plants pathogens such as *Alternaria* has been reported in previous studies [16]. Alternaria is a dry-air spore type, airborne concentrations are favoured by high temperatures and low relative humidity [38]. The higher values of relative humidity registered in Cartagena reduce the spore concentration of *Alternaria* when compared with its distribution in the neighbor city of Murcia (27.7% *Alternaria*, 57% mean of relative humidity) [39], in Sydney, Australia, these same effects of relative humidity were reported [37]. The high counts found on weeks 18 and 19 coincide with relative humidity weekly average values below of 75%, the decrease in relative humidity is related to the release of large number of conidia [34].

Stemphylium, another fungus related to allergy [35], was relatively easy to identify on the Hirst trap slides [26], although it was not isolated in our cultures. The favourable conditions to find them on air are temperatures between 15 and 25°C and a high humidity (characteristics of our area in spring) [40]. It is considered a pathogenic mould for plants, with a lower incidence than *Alternaria* in Cartagena [14].

The viable methodology permitted the isolation and identification of airborne small hyaline spores, i.e. *Penicillium* and *Aspergillus* in the culture media. *Aspergillus*, which was uncommon using the non-culture-based method, has been reported as being a potentially allergenic indoor mould [41], with significant concentrations in the air of Mediterranean cities, i.e. Barcelona [30] and Cordoba [29], as well as in the present results from Cartagena using the viable methodology. The use of viable methodologies, either gravimetric [42], impact [43], or filtration methodologies [30], in outdoor air have shown higher counts for these moulds than non-viable techniques.

The quantitative results for *Penicillium* and *Aspergillus* found by the non-viable method during the spring of year 1997 are inconsistent with the frequency found in the isolates from the viable method, showing how these two methodologies complement each other. The extra difficulty found to identify and view these spores in samples with high particulate content like the ones from Cartagena [44], together with the low efficiency of the sampler for this particle size range [33, 45], we can say that *Penicillium* and *Aspergillus* counts were underestimated by the non-viable method, as reported by other authors [8, 27].

Botrytis is a plant pathogenic mould associated with allergy [14, 35] whose presence is related to the life cycle of its hosts. It is a spore that is characteristic of wet air [46] which justifies their presence after periods of moderate rain. *Oidium* (*Erysiphe graminis*) is a very abundant spore in our area, and a plant pathogenic fungi [14] whose great hyaline spore is easily visualized in Hirst preparations.

The *Drechslera* group, were easily visualized due to their enormous size, may be identified relatively easily and quantified on the microscope slides made from the Hirst trap samples, but were not isolated in culture media. This fact might be explained by their low number and their constant competition with other species that grow easily in artificial culture media, as well as the lack of specificity of the medium for these fungal types. *Drechslera* is a genus of fungi frequently associated with *allergy* [35]. *Bipolaris spicifera* has been reported to be one of the most common causes of allergic fungal sinusitis in the southwestern United States [32].

Fusarium, Cercospora, *Helicomyces*, ascospores and hyaline basidiospores are typical genera of wet air [47]. The increase in this airborne fungal spore is associated with both rainfall, as in our case, and harvest patterns [47]. A higher frequency was observed in the samples studied via the non-viable methodology. *Fusarium solani* has been considered as one of the most prevalent moulds responsible for allergic diseases [35].

The ascospores and basidiospores, which constitute a significant part of the airborne mycobiota, are only identified by the non-viable method. The ascospores usually produce anamorphic stages grown in artificial culture media [48].

Basidiomycete fungi have been shown to be capable of sensitizing atopic subjects and of inducing respiratory allergies [2]. *Ustilago* [49] and *Puccinia* [46] are two genera of fungi related to allergy. Results found for *Ustilago* are similar to those reported in the atmosphere of Kuwait [50]. *Ustilago* is a plant pathogen on cereal crops [24] whose presence is related to the life cycle of its hosts [46]. This is consistent with the maximum values found in springtime, time of the year with the highest growing rate for grasses in Cartagena [24].

Basidiospores highest counts (*Boletus, Agaricus, Coprinus*, etc.) are observed at the same time that the growth of Agaricales [41, 46], although levels are low, this behavior is related to places with scant vegetation and soil humidity [41, 46], characteristic in our area.

Most of the genera of fungi frequently associated with allergy are classified as Ascomycetes [35]. These airborne fungal spores, frequent in rainy and wet zones, induce summer asthma or asthma after storms [51]. Due to our semi-arid conditions, and the scarce rainfall during 1997, their presence is not abundant.

In spite of the importance of Oomycetes as plant pathogens in our area [14], their presence in our samples was not abundant.

Zygomycetes are very frequent and easily isolated and identified by the viable method. *Rhizopus y Entomophthora* are the most easy to identify by the non-viable method in the abiotic particles laden samples from Cartagena, due to their size and morphology.

Myxomycetes are sparse in our bioaerosol. There are few studies that show evidence of the presence of spores of Myxomycetes in the air [52]. In Athens, Greece, a total count of 7858 spores/m^3 of Myxomycetes was found by a non-viable method, representing the 6.7% [48], this concentration is much higher than that obtained in our study.

The concentration of total annual spores is low compared with other geographical areas in the Iberian Peninsula [16,

17], Leiden [26] or Tulsa [53], though great variability exists. We consider that the scant rainfall may have contributed to these low total spore counts. This fact has been confirmed with the limited number of ascospores and basidiospores counted compared with a higher count of thermo-resistant spores.

Several cities in arid southern Spain display two well-defined peaks in spring and in the fall, and a decrease in the concentrations during the summer [16]. Although fungal spores are adapted to resist unfavourable environmental conditions[42, 50], the stressful conditions, characteristic of the Cartagena summer, with absence of rain, high temperatures, strong sunlight and high humidity, clearly have a negative effect on spores counts except for those thermo-resistant, i.e. *Ustilago*.

Viable and non-viable methodologies are complementary because not all fungal spores are completely identifiable with a single sampling method [26, 53]. Thus, the use of the non-viable methodology allowed us to demonstrate the presence of a wide range of ascospores (*Nectria, Didymella)* and basidiospores (*Ustilago, Agaricus, Coprinus*). Other conidia/spores that have difficulty growing in artificial media may also be underestimated by the viable methodology [30, 33, 43]. The viable methodology also enabled the identification of *Phoma, Syncephalastrum, Gonatobotrys*, *Verticillium, Rodothorula* and species of *Botrytis*.

CONCLUSIONS

The prevalent species in this study define the bioaerosol of Cartagena as typical of dry air. A more comprehensive characterization of airborne fungal spores was carried out combining viable and non-viable methodologies. Most fungal spores (mainly ascospores and basidiospores) were identified by microscopic examination using a non-viable methodology. The viable methodology was better at identifying species such as *Aspergillus*, *Penicillium*, Zygomycetes and other small hyaline spores. It also enabled the genus of some types of isolated fungal types, *Phoma, Beauveria, Gonatobotrys, Verticillium*, *Mucor, Absidia*, etc., to be determined, which would not have been possible using the non-viable method.

The most abundant genera of airborne fungi identified in Cartagena are frequently associated with symptoms of allergy. Many of these are saprophytes or parasites of vegetable species; hence their presence in the air is linked to the cycle of their host. In general, spring and autumn register the highest counts of fungal spores as well as the greatest variability in identified types. Winter is the season with the lowest counts and the least variety of fungal types. In summer, total counts are low and the only varieties are *Cladosporium, Alternaria, Ustilago, Chaetomium* and *Puccinia*.

Aknowledgements
The authors gratefully acknowledge the State Meteorological Agency of Spain (AEMET) for permission to use their resources.

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