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Outdoor airborne fungi captured by viable and non-viable methods



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ABSTRACT

Volume assessments of the concentration of airborne fungi may provide different results depending on the methodology used. This work simultaneously analyses two methods for samples obtained outdoors and analysed in the context of meteorological conditions. The study was carried out in Badajoz (SW Spain) from Mar. 2009 to Jul. 2011. A Burkard fixed spore trap was used for the non-viable sampling, and three different methods were used for the viable sampling: a Burkard portable spore trap with two inlet port types and a Sampl'air AES trap. Daily average total concentrations of 285 CFU m⁻³ and 1 954 spores m⁻³ were recorded for the viable and non-viable methods, respectively. The spore/colony ratio showed important differences among the most relevant fungal types: *Alternaria* (2.6), *Aspergillus*–*Penicillium* (2.0) and *Cladosporium* (11.1). Although the two sampling types were essentially equivalent at showing temporal variations in outdoor airborne fungi, quantitative differences in the number of total colonies recorded depended on the culture media and conditions used.

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Introduction

Study of the mycobiota in the outside air and in indoor environments, as well as the variations in the number and composition of the mycobiota are increasingly important because these organisms present potential risks for human health (Chakrabarti et al., 2012). Airborne fungal spores are an important fraction of atmospheric particulate matter and are major causative agents of allergenic and infectious diseases (Lang-Yona et al., 2012). Fungal spores transported by air currents may cause diseases in plants, and knowledge of their periodicity has a great predictive value for epidemics. Airborne

fungi have also been reported as the cause of several human diseases through their action as inducers of allergy and/or toxic responses (Lacey, 1991). Great concern has been expressed about the potential risks for human health posed by toxic substances produced by metabolic activity in certain species, such as *Aspergillus* or *Fusarium*, and a relationship exists between these substances and air quality. Moreover, fungi are involved in the decomposition of food and raw materials and the deterioration of organic matter and by-products during storage (Madelin and Madelin, 1995; Mandrioli et al., 2003).

Several research techniques have been used in aeromycological studies; the sampling methods can be classified

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as either gravimetric or volumetric (Ingold, 1971; Kasprzyk, 2008). Furthermore, recent studies have used molecular techniques based on qPCR (Lang-Yona et al., 2012). The methods used each have advantages and disadvantages, and the results obtained cannot be compared directly; different fungal types may be recorded depending on the method used, and different concentrations might also be measured (Hirst, 1991; Nikkels et al., 1996; Spieksma, 2003). Several studies on the presence and distribution of different fungal spores have been carried out using the volumetric method in Europe (Larsen and Gravesen, 1991; Munuera and Carrión, 1995; Nikkels et al., 1996; Tariq et al., 1996; Kurkela, 1997; Grinn-Gofroń and Strzelczak, 2008), North America (Levetin, 1991; Craig and Levetin, 2000), Australia (Mitakakis et al., 1997, 2001) and the Middle East (Shaheen, 1992).

With non-viable methods (mainly by Burkard Spore traps) many spores are collected, which is useful for carrying out continuous studies with hourly and daily intervals (Oliveira et al., 2005; Stępańska and Wołek, 2009; Mallo et al., 2011). The disadvantage of non-viable methodologies is that the associated method for counting the spores under a microscope enables the identification of fungal spores only at the genus level, whereas viable methods and qPCR-based molecular analysis can identify fungal spores at the species level. This disadvantage is outweighed by the ability to acquire long time series at high temporal resolution (Skjøth et al., 2012). In contrast, the use of viable methods allows for the identification of the colonies formed at the species level in many cases; however, the use of a particular culture medium may favour the development of some taxa over others, and even the same species may undergo self-inhibition processes in terms of germination or growth (Burge et al., 1977). Therefore, the concentration of certain fungal colonies may be lower, and the results obtained may not reflect the actual distribution in the atmosphere. When recording airborne fungal spores based on continuous sampling with fixed spore traps, inert substrata are used; in contrast, portable spore traps may use either culture media or inert substrata (Fernández-Rodríguez et al., 2011). Portable spore traps are used for periods from a few minutes to an entire day (Kenny et al., 1998). Although one would expect that fixed and portable spore traps placed in the same location might produce similar results (Tormo et al., 2010), the results often differ for the same geographical region because the range of fungi found in portable spore traps includes species more closely associated with the ground, the vegetation around the traps and hyphal fragments (Green et al., 2006). The design of portable spore traps for bioaerosols meets different purposes: the collection of small biological particles such as bacteria and viruses, which are of great interest in microbiological studies; the survival of captured microorganisms (Thompson et al., 1994); sampling for phytopathological control (McCartney et al., 1997); and conducting spore trapping in extreme environments (Marshall, 1997). However, portable traps lack vanes, and the influence of wind vectors can cause the results to differ from those obtained using fixed samplers. The use of any of these methods alone underestimates the diversity and concentration of fungal spores. It is generally acknowledged that the methods and procedures used must be standardised for a

wide variety of spore traps (Macher and Willeke, 1992; Henningson and Ahlberg, 1994). To design an efficient sampling strategy, it is important to adopt at least two methods: one for the microscopic assessment of bioaerosols on inert media and another for their isolation on culture medium to identify the viable fungus types present (Lacey and Venette, 1995).

For the present study, the Burkard Company (Ltd., 55, Rickmansworth, U.K.) provided two spore traps based on the same engine; the first trap is for the collection of non-viable particles on an adherent substance, whereas the second trap, the Burkard spore trap for viable samples, includes space to place a 9-cm Petri dish. The airflows allowed by these traps are 10 l min⁻¹ and 20 l min⁻¹, respectively (Jensen and Schafer, 1994), and the latter includes a sieve with 100 holes and a conical structure that can be placed on the sieve. The differences and similarities between the viable methodologies described above were analysed in a previous study (Fernández-Rodríguez et al., 2011). The viable Burkard spore trap has been widely used for sampling in many countries, including India (Sharma et al., 2010), Saudi Arabia (Hasnain et al., 1995, 2004; Al-Suwaine et al., 1999a,b), Canada (Comtois and Marcoux, 1999), Cuba (Aira et al., 2002) and Poland (Buczynska et al., 2007, 2008) (Table 1). Not all of these studies provide methodological specifications, and the available information is incomplete; for example, the use of the sieve or funnel is not indicated in some cases (Sharma et al., 2010). In some studies, a correction factor is applied to the results when using the sieve (Aira et al., 2002; Sharma et al., 2010). This factor depends on the number and diameter of holes in the sieve. Studies that compare sampling based on viable methods to sampling using other standard spore traps show reliable results (Madelin and Madelin, 1995; Mehta et al., 1996; Tormo et al., 2010); however, the use of a spore trap that includes a sieve above the Petri dish requires adjustments to be made such that the statistical probability of more than one colony forming unit (CFU) affects every point of the hole. Some studies (Andersen, 1958; Peto and Powell, 1970) include correction factors depending on the number and diameter of the holes. Although fungi can be cultured on many media, Sabouraud dextrose agar (SDA) and malt extract agar (MEA) are widely used because they allow for the development of a wide variety of fungi (Faure et al., 2002; Bouza et al., 2002; Adhikari et al., 2004; Lugauskas and Krikstaponis, 2004; Augustowska and Dutkiewicz, 2006; Perdelli et al., 2006; Panagopoulou et al., 2007; Sautour et al., 2007, 2009).

In recent years, several papers have reported the influence of environmental factors on the prevalence of allergy-based diseases (Bousquet et al., 1993; Chakrabarti et al., 2012). For this reason, the influence of major meteorological factors (average temperature, relative humidity, rainfall and wind speed) on fungal concentration has also been studied (Oliveira et al., 2005; Stępańska and Wołek, 2005; Fernández-Rodríguez et al., 2011; Chakrabarti et al., 2012).

The aims of the present study were to analyse and compare the results of simultaneous outdoor sampling of fungi using viable and non-viable methodology, including hourly and daily data and considering the effects of meteorology.

Table 1 – Comparative studies of various methodologies

Reference	City	Sampler	Culture media
Burge et al. (1977)	Michigan (USA)	And, BSTp	MM, SAD, V8, MEA, PDAR
Abdel-Hafez and El-Said (1989)	Wadi Qena (Egypt)	OPD	CA
Hasnain et al. (1995)	Riyadh, Jeddah, Al-Khobar (Arabia Saudi)	BSTp	SDA/CA
Mehta et al. (1996)	Houston (USA)	BSTp, And	DG-18
Al-Suwaine et al. (1999a)	Riyadh (Arabia Saudi)	2 BSTp	SDA/CA
Comtois and Marcoux (1999)	Montreal (Canada)	BSTp	SDA/MEA
Chakraborty et al. (2000)	Burdwan (India)	R	MEA, PDA
Al-Subai (2002)	Doha (Qatar)	OPD	CA
Aira et al. (2002)	Havana (Cuba)	BSTp, And	SDA
Aira et al. (2003)	Valparaiso (Chile)	BSTp	MEA
Adhikari et al. (2004)	Domjur (India)	5 BSTp, And	S and RBA
Hasnain et al. (2004)	Riyadh, Jeddah, Al-Khobar (Arabia Saudi)	2 BSTp	SDA/CA
Green et al. (2006)	Nuevo Gales del sur (Australia)	BST	S
Pyrri and Kapsanaki-Gotsi (2007)	Atenas (Greece)	2 BSTp	PDA
Stępańska and Wolek (2009)	Cracovia (Poland)	BST	S
Sharma et al. (2010)	Assam (India)	BSTp	RBA
Pyrri and Kapsanaki-Gotsi (2012)	Atenas (Greece)	BSTp	PDA
Fernández-Rodríguez et al. (2011)	Badajoz (Spain)	2 BSTp	SAD
Das and Gupta-Bhattacharya (2012)	Kolkata (India)	BSPT, And	S, MEA and CA

And: Andersen; BST: Burkard spore trap; BSTp: Burkard personal spore trap; OPD: opened Petri dish; R: Rotorod. Culture media CA: Czapek agar; DG-18: Dichloran glycerol-18; MEA: malt extract agar; PDA: potato dextrose agar; RBA: Rose Bengal agar; S: slide; SAD: Sabouraud agar dextrose.

Materials and methods

Study area

Airborne species were monitored at Badajoz (SW Spain); the station was situated at 16 m above ground level on the terrace of the Faculty of Science at the University of Extremadura (38° 53'87"N, 7° 00'87"W). Badajoz is located at 184 m above sea level and has a population of 152 520 inhabitants (NSI, 2012). Around the city of Badajoz, there are irrigated crops (e.g., fruits, corn, tomatoes, etc.), olive trees, more distant holm-oak trees and extensive grazing.

Meteorological data

The meteorological information used was provided by the fixed weather station AT Delta-T DL2 located in the experimental garden of the Biology building at the university campus of the Faculty of Science; the weather station was located 16 m from the spore trap in the garden and 16 m from the spore trap on the terrace of the Faculty of Science. The meteorological parameters considered were relative humidity (Rh), precipitation (R), solar radiation (Sr), average air temperature (Ta), wind direction (Wd) and wind speed (Ws).

Fungal data

The sampling was performed from 2009 to Jul. 2011 at solar noon once a week. For the sampling on an inert substratum, a 7 d fixed Burkard spore trap was used. For the sampling on culture media, the colonies were recorded using three types of equipment: a portable Burkard spore trap with two inlet hole types (one was 27 mm in diameter, and the other was a sieve of 100 holes of 1 mm in diameter) and a Sampl'air AES Chemunex spore trap with a sieve of 258 holes. The inlet flow

speeds were 20 and 100 l min⁻¹ for the Burkard and Sampl'air traps, respectively. For the non-viable methods, white petroleum jelly (CAS number 8009-03-8) was used as the adherent. For the viable methods, SDA was used from Mar. 25, 2009, to Mar. 20, 2010, as the medium, and MEA was used from Mar. 26, 2010, to Jul. 1, 2011, as the medium; the plates were cultured at 27 °C for 5 d. The data were recorded as spores and colony forming units (CFU), both per cubic metre. Standardised data management procedures were used, similar to those described by the Spanish Aerobiology Network (REA) (Galán et al., 2007). For the non-viable methods, slides were examined at 1 000× magnification along two longitudinal transects.

Data on the total daily average concentration and the major types of fungi present (*Alternaria*, *Aspergillus*–*Penicillium* and *Cladosporium*) were analysed. In addition to the data on the continuous non-viable sampling, hourly concentration was considered at the time at which the viable sampling was performed. The normality of the data was analysed using the Shapiro–Wilk test (W). The Kruskal–Wallis test (χ^2) was used to study the daily and hourly variations between spore traps. Spearman's correlation coefficient (r) was used to analyse the relationships between fungal concentration and the meteorological parameters. A 99% confidence level was applied.

Results

The total number of samplings performed was 115. The daily average spore concentration was 1 954 spores m⁻³, with a range of 93–10 274 spores m⁻³. The daily averages for the first and second years of the study were 1 823 and 2 052 spores m⁻³, respectively. The greatest concentration of total spores was recorded in autumn (2 845 spores m⁻³), and the highest concentrations were recorded in Oct. (5 121 spores m⁻³).

The annual total concentration did not show statistically significant differences ($\chi^2 = 0.392$, $p = 0.531$); however, the

seasonal comparison did show differences ($\chi^2 = 37.362$, $p \leq 0.001$). Total spores, *Alternaria*, *Aspergillus–Penicillium* and *Cladosporium* differed significantly between seasons (Fig 1). The maximum monthly spore concentration was recorded in Oct. (5 121 spores m^{-3}), and the minimum was recorded in Feb. (408 spores m^{-3}). A comparison between the daily average concentration and the average concentration recorded at solar noon each day in the fixed Burkard spore trap showed statistically significant correlations in all cases (Fig 2).

The daily average colony concentration was 285 CFU m^{-3} , and the range was 5–2 590 CFU m^{-3} . The averages for years one and two were 206 and 345 CFU m^{-3} , respectively. The greatest concentration of total colonies was recorded in the spring (443 CFU m^{-3}), whereas the lowest concentration was recorded in the summer (218 CFU m^{-3}). Significant differences

were found between the 2 yr ($\chi^2 = 16.685$, $p \leq 0.001$). A seasonal comparison showed significant differences for total colonies ($\chi^2 = 15.090$, $p = 0.002$), *Aspergillus–Penicillium* ($\chi^2 = 10.425$, $p = 0.015$) and *Cladosporium* ($\chi^2 = 22.496$, $p \leq 0.001$). However, no seasonal differences were found for *Alternaria* ($\chi^2 = 7.579$, $p \leq 0.056$). The maximum monthly spore concentration was recorded in Oct. (495 CFU m^{-3}), and the minimum monthly spore concentration was recorded in Aug. (111 CFU m^{-3}). This maximum monthly concentration is attributed to *Cladosporium cladosporioides*, the concentration of which reached two strong peaks in that month (on Oct. 9 and 30, 2009, and on Oct. 8 and 15, 2010). During the first year of sampling, the highest concentrations were recorded in autumn and the lowest concentrations were recorded in summer (Fig 1A). During the second year of sampling, the highest concentrations were recorded in spring and autumn and the lowest concentrations were recorded in summer and winter (Fig 3B).

The daily average spore and colony concentrations showed important differences: *Cladosporium* (1 119 spores m^{-3} and 101 CFU m^{-3}), *Alternaria* (49 spores m^{-3} and 19 CFU m^{-3}) and *Aspergillus–Penicillium* (56 spores m^{-3} and 28 CFU m^{-3}). The fungal spore/colony ratio of the total average concentration was 6.8, whereas the values obtained for the predominant types were 2.5 (*Alternaria*), 2.0 (*Aspergillus–Penicillium*) and 11.0 (*Cladosporium*). The seasonal spore/colony ratios were 5.1 (spring), 8.9 (summer), 7.5 (autumn) and 2.3 (winter). The values for *Cladosporium* reached their maxima in summer (26.9) and autumn (11.1) and their minimum in winter (2.2). In addition, *Alternaria* exhibited higher values in summer (5.2) and lower values in winter (0.2). However, *Aspergillus–Penicillium* exhibited a maximum ratio in spring (3.0).

A statistically significant correlation was found between the daily concentration of total spores and colonies and for *Alternaria* and *Cladosporium*, but not for *Aspergillus–Penicillium* (Table 2). Statistically significant correlations were found between the total data and for *Alternaria*, *Aspergillus–Penicillium* and *Cladosporium* with regard to the fungal concentrations recorded during the first study year using the Burkard fixed spore trap and the Burkard portable spore trap equipped with a sieve and the Burkard portable spore traps equipped with a conical structure. Furthermore, statistically significant correlations were found during the second study year using the Burkard fixed spore trap and the Burkard portable spore trap equipped with a sieve and the Burkard portable spore traps equipped with a sieve and the AES (Fig 4). Fig 5 shows the monthly average concentrations of some fungal types determined at the species level.

A comparison between the spore data and the meteorological parameters showed the greatest number of correlations for the fungal types *Alternaria* and *Cladosporium* recorded using the non-viable method. In contrast, for the viable method and using the Burkard spore traps with the conical structure and AES, the greatest number of correlations was found for *Aspergillus–Penicillium*. The spore concentration at solar noon using the non-viable methodology for *Alternaria* and *Cladosporium* was statistically significantly correlated with solar radiation ($r = 0.221^{**}$, $p = 0.018$; $r = 0.280^{**}$, $p = 0.002$), average temperature ($r = 0.275^{**}$, $p = 0.003$; $r = 0.456^{**}$, $p \leq 0.001$) and relative humidity ($r = -0.193^{*}$, $p = 0.039$; $r = -0.279^{**}$, $p = 0.002$). The type *Aspergillus–Penicillium*

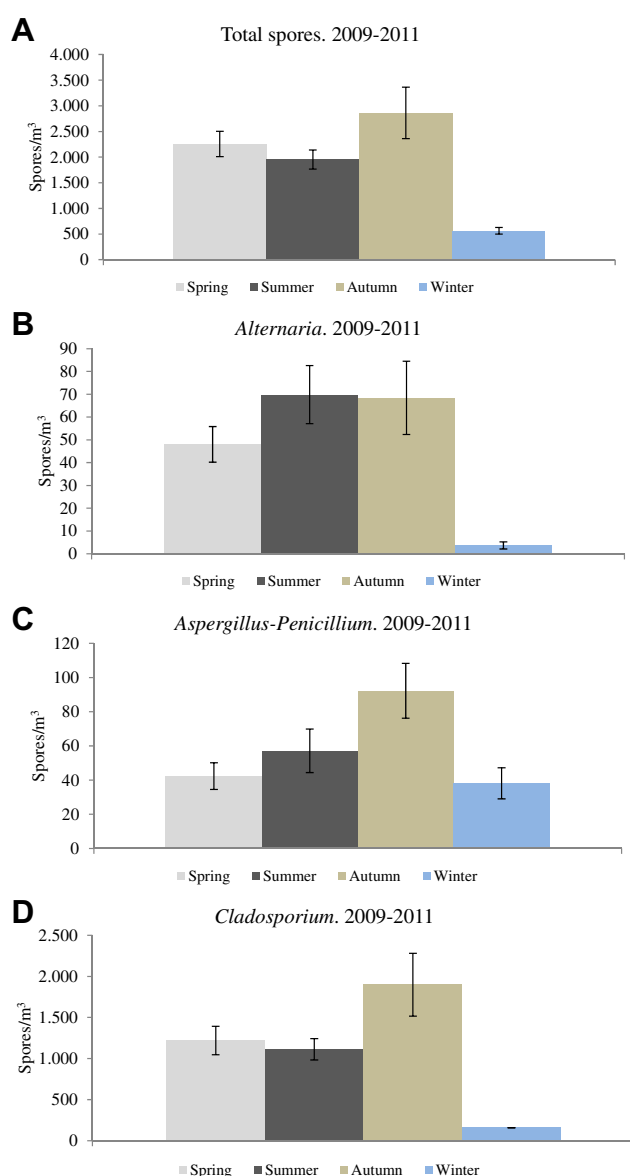


Fig 1 – Seasonal concentration, with error bars, of total spores (A), *Alternaria* (B), *Aspergillus–Penicillium* (C) and *Cladosporium* (D) during the entire study period (spores m^{-3}).

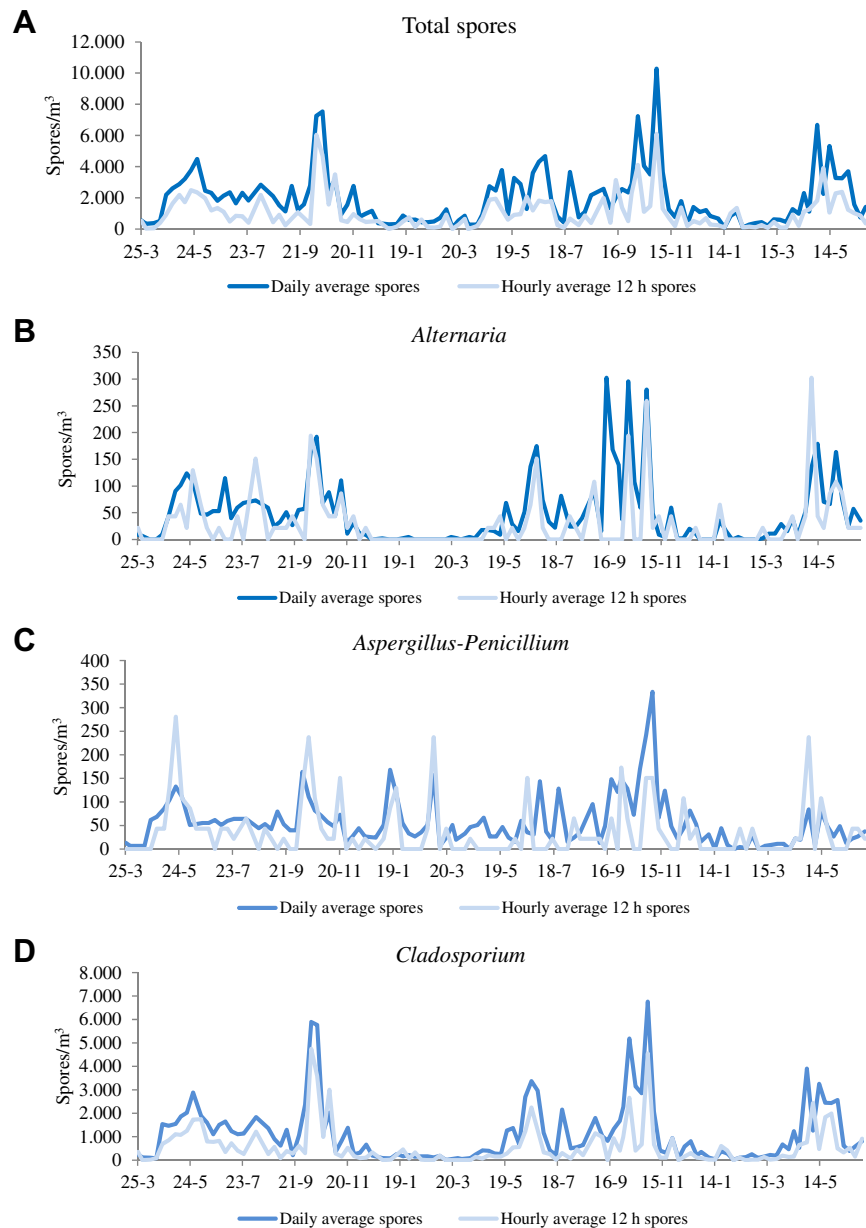


Fig 2 – Daily average concentration and concentration at solar noon of total spores (A), *Alternaria* (B), *Aspergillus–Penicillium* (C) and *Cladosporium* (D) during the entire study period (spores m^{-3}).

showed no statistically significant correlations between the data collected with the non-viable technique and the meteorological parameters; however, statistically significant correlations were obtained between the data of colonies and relative humidity ($r = 0.225^*$, $p = 0.016$), solar radiation ($r = 0.327^{**}$, $p \leq 0.001$), wind direction ($r = -0.275^{**}$, $p = 0.003$) and wind speed ($r = -0.292^{**}$, $p = 0.002$).

Discussion

In the present study we found similar ranges of concentrations of non-viable spores ($82\text{--}2\,365$ spores m^{-3} and $156\text{--}2\,022$ spores m^{-3} during the first and the second study year, respectively) to those found by Adhikari et al. (2004). In contrast,

the ranges for the viable spore concentration were higher in our study ($72\text{--}1\,796$ CFU m^{-3} and $155\text{--}1\,256$ CFU m^{-3} in years one and two, respectively) than in Adhikari et al. (2004). Other studies (Pyrri and Kapsanaki-Gotsi, 2007) found similar total spore and colony maximum concentration ($3\,175$ spores m^{-3} , $2\,435$ CFU m^{-3}), *Cladosporium* concentration ($2\,565$ spores m^{-3} , $2\,169$ CFU m^{-3}) and *Alternaria* concentration (280 spores m^{-3} , 180 CFU m^{-3}). The lowest concentration recorded using viable methods is shown above and indicates that these methods provide underestimates in relation to the non-viable method. The most abundant fungal types were *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*, as found by Pyrri and Kapsanaki-Gotsi (2012). Of these types, *Cladosporium* was most prevalent in the total count in other European regions (Ebner et al., 1989; Filippello-Marchisio and Airudi, 2001; Filippello-Marchisio et al.,

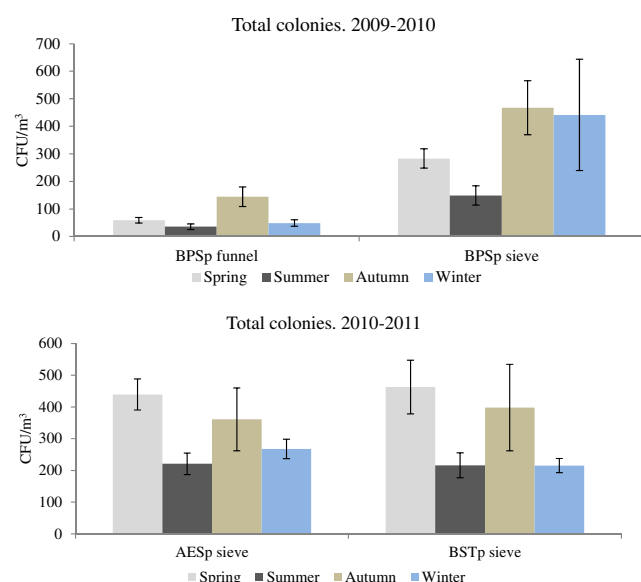


Fig 3 – Seasonal concentration of total colonies, with error bars, during the study period (CFU m⁻³).

1997; Stępańska et al., 1999; Stępańska and Wołek, 2005; Pyrrì and Kapsanaki-Gotsi, 2007). The viable method recorded high total concentrations from Apr. to Nov. and a maximum total concentration in Oct.. A lower variation with two maximum values was found using the viable spore trap, the first between Mar. and Jun. and the second between Oct. and Dec.. Similar trends were found in other Mediterranean countries. Filippello-Marchisio et al. (1997) found a maximum between Jun. and Sep., and Pyrrì and Kapsanaki-Gotsi (2007) found three maxima: one in Jan., a second between May and Jun. and a third between Oct. and Nov.. The highest concentrations were reached in northern countries; for example, in Austria (Ebner et al., 1989) in May and Aug. and in Denmark (Larsen and Gravesen, 1991) between Jun. and Sep.. Geographical zone may affect the recorded concentrations and trends, as does sampling year.

In our study, the non-viable methodology recorded greater concentrations than the use of viable sampling, as shown previously (Burge et al., 1977; Adhikari et al., 2004; Pyrrì and Kapsanaki-Gotsi, 2007). In the latter paper, the authors suggested several reasons for the difference between these methods. Various groups of fungi, especially plant symbiotic

fungi, do not grow readily in certain culture media. In addition, the loss of viability in a considerable percentage of spores may also explain the reduced number. Kramer and Pady (1968) indicated a loss of 20–94 % viability among common airborne fungal spores. Spore sampling on slides records all spores, regardless of their viability, whereas sampling on plates only records viable spores. Therefore, the viable method underestimates the actual number of spores present in the air. The studied viable methods considerably underestimated the spore concentration of *Cladosporium* from Apr. to Nov. relative to the studied non-viable methods. Furthermore, Pyrrì and Kapsanaki-Gotsi (2007) considered the high temperature and intense solar radiation during the summer months as factors that might reduce the viability of spores that have relatively thin walls. In part, this theory is consistent with the maximum values found for the *Cladosporium* spore/colony seasonal ratio in summer (26.9) and autumn (11.1). Furthermore, some spring fruiting and sporulating species (for example *Cladosporium*) might have released their spores immediately before summer; thus, sporulation might have been higher during summer and/or autumn, and, in contrast, the number of colonies might have been lower during the same period.

We found that the concentration of *Alternaria* spores exhibited temporal variations, with increases from May to Oct. in the non-viable spore trap; two maxima were recorded in the viable spore trap, one from May to Jul. and a second in Oct.. The first maximum is consistent with variations observed in other Mediterranean regions, such as Italy (Filippello-Marchisio and Airudi, 2001) and Greece (Pyrrì and Kapsanaki-Gotsi, 2007). In more northern places such as Austria (Ebner et al., 1989), Denmark (Larsen and Gravesen, 1991) and the United Kingdom (Corden and Millington, 2001), the main peak was reached in Aug.. These differences might be attributed to differences in geoclimatic zone and sampling year. The spore/colony ratio of *Alternaria* reached a maximum in summer (5.2). The viable method produced underestimates; as with the differences among the findings of the Mediterranean studies listed above, the effect of intense solar radiation or a previous sporulation on the viability of the thin-walled spores might provide an explanation.

Using the non-viable method, the conidia of the species of these genera were identified as *Aspergillus*–*Penicillium* type because these conidia are morphologically indistinguishable. This shortcoming is a clear example of the main disadvantage of the non-viable method: that is, it fails to distinguish

Table 2 – Correlations between the total concentrations of spores and the main types of instruments

Type of comparison	Coefficients	Total concentration	<i>Alternaria</i>	<i>Aspergillus</i> – <i>Penicillium</i>	<i>Cladosporium</i>
Daily average, spores (BST) – colonies (BPSpf, BPSps and AES)	<i>r</i>	0.273**	0.255**	0.018	0.241**
	<i>p</i>	0.003	0.006	0.846	0.009
Daily average spores (BST) – hourly 12 hr average spores (BST)	<i>r</i>	0.818**	0.603**	0.533**	0.833**
	<i>p</i>	< 0.001	< 0.001	< 0.001	< 0.001
Daily average colonies (BPSpf and BPSps – AES)	<i>r</i>	0.382**	0.525**	0.475**	0.407**
	<i>p</i>	< 0.001	< 0.001	< 0.001	< 0.001

BST: Burkard spore trap; BSTp: Burkard personal spore trap; f: funnel device; s: sieve device; AES: Sampl'air AES Chemunex.

Bold values represent non-viable fungi. Fungal spores captured on inert substrates.

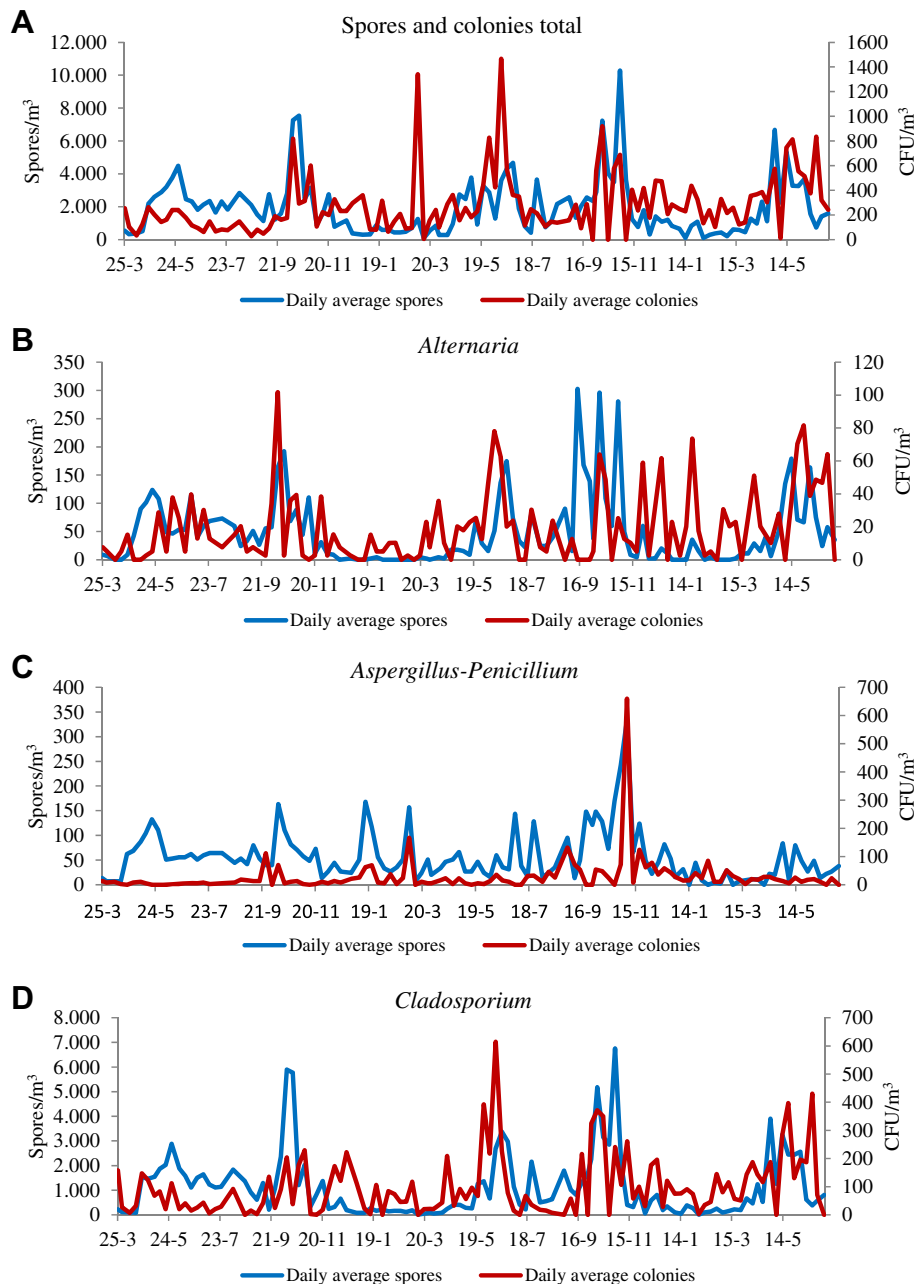


Fig 4 – Daily average concentration of the spores and total colonies (A) and of *Alternaria* (B), *Aspergillus–Penicillium* (C) and *Cladosporium* (D) (spores m⁻³ and CFU m⁻³).

between *Penicillium* and *Aspergillus*. For the *Aspergillus–Penicillium* type, no definite seasonal model was found. Using the viable method, increases were found for *Aspergillus* in autumn and for *Penicillium* in spring and autumn. Under the non-viable methodology, slight increases were found in May and significant increases were found in Oct.. In fact, the spore/colony ratios varied between spring (3.0) and autumn (1.0), with intermediate values observed in summer (1.9) and winter (1.3). *Aspergillus–Penicillium* does not appear to be affected by seasonality as strongly as *Cladosporium* and *Alternaria*. This difference might be attributed to the high number of *Aspergillus* (14) and *Penicillium* (25) taxa included as viable *Aspergillus–Penicillium* type compared to the number of non-

viable taxa. Furthermore, many of the variations observed might be mainly due to differences in the spore source and fungal biology of different species within each genus. Temperature and solar radiation might also be factors.

In this study, the comparisons of the daily data for the spores obtained using both viable and non-viable methods showed statistically significant correlations in the total number of spores for *Alternaria* and *Cladosporium*; these results are consistent with the results reported by Pyrrí and Kapsanaki-Gotsi (2007). The absence of a correlation with *Aspergillus–Penicillium* may be because the number of counted colonies was too low; as a result, the influence of randomness might have been too high for any statistical relationship to be

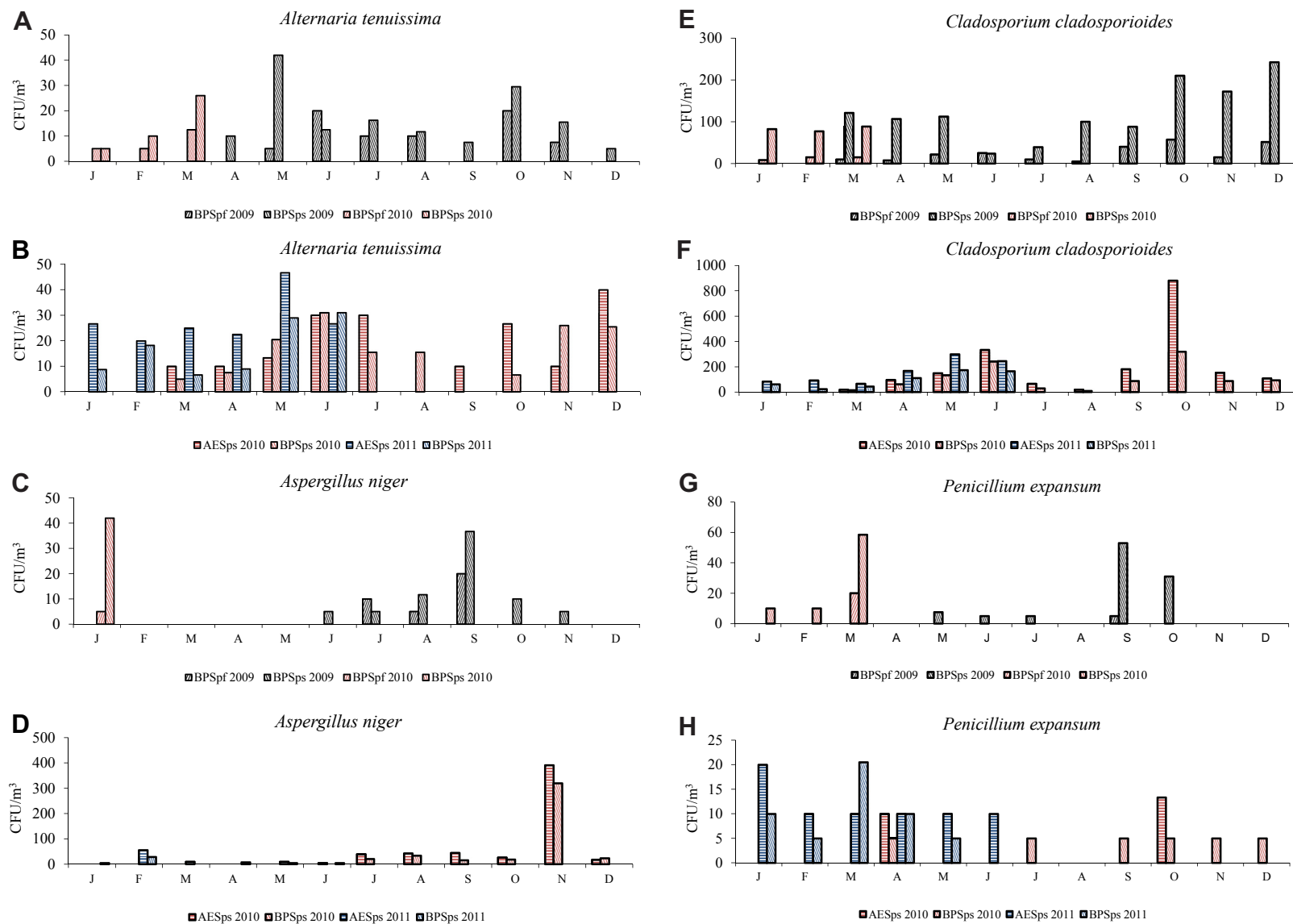


Fig 5 – Monthly average concentration of *Alternaria tenuissima* (A, B), *Aspergillus niger* (C, D), *Cladosporium cladosporioides* (E, F) and *Penicillium expansum* (G, H) (CFU m⁻³).

observed. Moreover, species of other genera might have been included during the identification of these spores because the small size of their propagules hindered their precise identification. Furthermore, due to methodological/manufacturing limitations, smaller particles such as *Aspergillus* and *Penicillium* do not always adhere to slides or tapes in the Burkard trap; therefore, their underestimation is usually accounted for.

In other studies, spore density was correlated with maximum temperature, relative humidity, rainfall and solar radiation (Stępańska and Wolek, 2005). However, there were fewer correlations for average temperature, wind direction and wind speed. The diversity of spore species recorded in this study using two methods depended on diverse factors, mainly due to the capacity of airborne spores to grow in culture media. However, uredospores, teliospores and the spores of myxomycetes, which are common components of airborne mycobiota, do not grow in the culture media that are typically used. Moreover, the germination potential of culturable organisms varies when these are placed on an artificial medium. Physiological needs and biotic interactions may exert an effect on the germination of each spore and on the growth of a CFU. Airborne fungi may be precisely identified using the viable medium, but few genera may be recognised with precision based on spore morphology (Pyrrri and Kapsanaki-Gotsi, 2007). The complexity of the effect of atmospheric pollutants on the survival of bioaerosols that affect viability must also be considered (Abdel Hameed et al., 2012).

The meteorological parameters studied affected the correlation of the *Alternaria*, *Cladosporium* and *Aspergillus–Penicillium* types in the viable and non-viable methods. *Alternaria* and *Cladosporium* showed higher meteorological correlations for the non-viable methods. However, *Aspergillus–Penicillium* exhibited correlations with most meteorological parameters when using viable methods but not with non-viable methods. Temperature clearly depends on solar radiation, and relative humidity is closely negatively correlated with temperature; therefore, these three parameters are closely related. The remaining meteorological factor is wind, which affects sampling efficiency; in addition, wind direction can greatly affect the results depending on the source location. Wind significantly alters the hourly/daily pattern (Tormo et al., 2013).

It is difficult to compare diversity and spore concentration among the different methodologies used (Mallo et al., 2011); however, the experimental efficiency of the aerobiological samplings has previously been analysed (Grinn-Gofroń, 2011). According to previous studies, there is no universal method for the continuous sampling of air that provides a reliable determination of all types of spores. The volumetric method, using Burkard or Lanzoni spore traps, is selective and less efficient for particles with diameters of less than 5 µm. Viable methods allow for the precise identification of growing fungi. Unfortunately, these methods are selective because not all spore types germinate on the same substrate or under the same culture conditions. The BPSp sampler including a funnel device registered less than one-third of the CFUs compared to the other viable spore sampling methods (BPSps and AESps, Fig 3). This difference might indicate that the BPSp sampler equipped with a funnel device is less effective and that the

viable samplers equipped with a sieve (BPSp and AESp) should be used.

None of the methods, when used separately, provide complete information about the actual concentration of spores in the air. It has been proposed that an ideal solution would be to use both methods simultaneously; however, this might be difficult due to the high cost (Grinn-Gofroń, 2011), effort and time required to perform these analyses. Our results are consistent with previous studies (Stępańska and Wolek, 2005; Pyrrri and Kapsanaki-Gotsi, 2007; Mallo et al., 2011) and suggest that viable and non-viable methods are currently complementary under normal conditions because combining the two methods overcomes the limitations that each has separately, providing more comprehensive data on airborne mycobiota.

Conclusions

The concentration of spores in the air gave values between six and seven times greater than the concentration of colonies grown. Measured as the spore/colony ratio, this difference was highest for *Cladosporium* (11.1) and lower for *Alternaria* (2.6) and *Aspergillus–Penicillium* (2.0). The statistically significant correlations found between spore concentration and colonies in the predominant types of *Cladosporium* and *Alternaria* indicate that the two sampling types are equivalent regarding their use in determining temporal variations. However, the quantitative differences between the two sampling types and the differences found for *Aspergillus–Penicillium* indicate that the viable and non-viable samplings are complementary. These inequalities may demonstrate differences in viability or an absence of substrates or adequate conditions for growth. Similarly, the viability of *Cladosporium* and *Alternaria* depends on season; in summer, the spores are less viable. The presence of fungal propagules in the air exhibits a pronounced seasonality, and maximum concentrations are found in autumn. The observed correlation of spore concentration with relative humidity and temperature might be influenced by these seasonal differences. The BPSp sampler equipped with a funnel device appears to be less effective than other viable samplers, and the viable samplers equipped with a sieve device (BPSp and AESp) should be used.

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REFERENCES

- Abdel Hameed, A.A., Khoder, M.I., Ibrahima, Y.H., Saeeda, Y., Osmana, M.E., Ghanema, S., 2012. Study on some factors affecting survivability of airborne fungi. *Science of the Total Environment* 414, 696–700.
- Adhikari, A., Moon, M., Sen Gupta-Bhattacharya, S., Chanda, S., 2004. Airborne viable non-viable and allergenic fungi in a rural

- agricultural area of India: a 2-year study at five outdoor sampling stations. *Science of the Total Environment* 326, 123–141.
- Aira, M.J., Rojas, T.I., Jato, V., 2002. Fungi associated with three houses in Havana (Cuba). *Grana* 41, 114–118.
- Al-Suwaine, A.S., Bahkali, A.H., Hasnain, S.M., 1999a. Seasonal incidence of airborne fungal allergens in Riyadh, Saudi Arabia. *Mycopathologia* 145, 15–22.
- Al-Suwaine, A.S., Hasnain, S.M., Bahkali, A.H., 1999b. Viable airborne fungi in Riyadh, Saudi Arabia. *Aerobiologia* 15, 121–130.
- Andersen, A.A., 1958. New sampler for the collection, sizing and enumeration of viable airborne particles. *Journal of Bacteriology* 76, 471–484.
- Augustowska, M., Dutkiewicz, J., 2006. Variability of airborne microflora in a hospital ward within a period of one year. *Annals of Agricultural and Environmental Medicine* 13, 99–106.
- Bousquet, J., Burney, P.G.J., Blumenthal, M., Burr, M., Bryan, S., Charpin, D., Kaslow, R.A., Kay, B., Kjellman, N.I.M., Mapp, C., Miyamoto, T., Paoletti, P., Pollock, J., Ronchetti, R., Weeke, E.R., Svendsen, V.G., Szemer, P., Troise, C., Wahn, U., Weiss, K., Zweimann, B., Blumenthal, M., 1993. Evidence for an increase in atopic disease and possible causes. *Clinical and Experimental Allergy* 23, 484–492.
- Bouza, E., Peláez, T., Pérez, J., 2002. Demolition of a hospital building by controlled explosion: the impact on filamentous fungal load in internal and external air. *Journal of Hospital Infection* 52, 234–242.
- Buczynska, A., Cyprowski, M., Piotrowska, M., Szadkowska-Stanczyk, I., 2007. Indoor moulds: results of the environmental study in office rooms. *Medycyna Pracy* 58, 521–525.
- Buczynska, A., Sowiak, M., Szadkowska-Stanczyk, I., 2008. Occupational exposure to mesophilic microorganisms associated with commercial processing of compost for mushroom production. *Medycyna Pracy* 59, 373–379.
- Burge, H., Boise, J., Rutherford, J., Solomon, W., 1977. Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. *Mycopathologia* 61, 27–33.
- Chakrabarti, H.S., Das, S., Gupta-Bhattacharya, S., 2012. Outdoor airborne fungal spora load in a suburb of Kolkata, India: its variation, meteorological determinants and health impact. *International Journal of Environmental Health Research* 22, 37–50.
- Comtois, P., Marcoux, N., 1999. An indoor air model. *Aerobiologia* 15, 115–120.
- Corden, J.M., Millington, W.M., 2001. Long term trends and seasonal variation in the aeroallergen *Alternaria* in Derby, UK. *Aerobiologia* 17, 127–136.
- Craig, R.L., Levetin, E., 2000. Multi-year study of *Ganoderma* aerobiology. *Aerobiologia* 16, 75–81.
- Ebner, M.R., Haselwandter, K., Frank, A., 1989. Seasonal fluctuations of airborne fungal allergens. *Mycological Research* 92, 170–176.
- Faure, O., Frickerm, H., Lebeaum, B., Mallaret, M.R., Ambroise, P., Grillot, R., 2002. Eight year surveillance of environmental fungal contamination in hospital operating rooms and haematological units. *Journal of Hospital Infection* 50, 155–160.
- Fernández-Rodríguez, S., Tormo, R., Silva, I., Gonzalo, A., 2011. Two sampling methods for the Petri dish detection of airborne fungi. *Grana* 50, 202–207.
- Filipello-Marchisio, V., Airudi, D., 2001. Temporal trends of the airborne fungi and their functional relations with the environment in a suburban site. *Mycologia* 93, 831–840.
- Filipello-Marchisio, V., Airudi, D., Brachi, C., 1997. One-year monitoring of airborne fungal community in a suburb of Turin (Italy) and assessment of its functional relations with the environment. *Mycological Research* 101, 821–828.
- Galán, C., Cariñanos, P., Alcázar, P., Domínguez, E., 2007. Spanish Aerobiology Network (REA): Manual de Calidad y Gestión de la Red Española de Aerobiología. Servicio de Publicaciones, Universidad de Córdoba, ISBN 978-84-690-6354-5.
- Green, J., O'Meara, T., Sercombe, J., Tovey, E., 2006. Measurement of personal exposure to outdoor aeromycota in northern New South Wales, Australia. *Annals of Agricultural and Environmental Medicine* 13, 225–234.
- Grinn-Gofroń, A., 2011. Airborne *Aspergillus* and *Penicillium* in the atmosphere of Szczecin, (Poland) (2004–2009). *Aerobiologia* 27, 67–76.
- Grinn-Gofroń, A., Strzelczak, A., 2008. Artificial neural network models of relationships between *Alternaria* spores and meteorological factors in Szczecin (Poland). *International Journal of Biometeorology* 52, 859–868.
- Hasnain, S.M., Al-Frayh, A.S., Al-Suwaine, A., Gad-El-Rab, M.O., Harfi, H.A., Al-Sedairy, S., 1995. Allergenic implication of airborne *Ulocladium* in Saudi Arabia. *Grana* 34, 70–76.
- Hasnain, S.M., Al-Frayh, A.S., Al-Suwaine, A., Gad-El-Rab, M.O., Fatima, K., Al-Sedairy, S., 2004. *Cladosporium* and respiratory allergy: diagnostic implications in Saudi Arabia. *Mycopathologia* 157, 171–179.
- Henningson, E.W., Ahlberg, M.S., 1994. Evaluation of microbiological aerosol samplers: a review. *Journal of Aerosol Science* 25, 1459–1492.
- Hirst, J.M., 1991. Aerobiology in plant pathology. *Grana* 30, 25–29.
- Ingold, C.T., 1971. Fungal Spores: Their Liberation and Dispersal. Clarendon Press, Oxford.
- Jensen, P.A., Schafer, M.P., 1994. Sampling and characterization of bioaerosols. In: Schlecht, P.C., O'Connor, P.F. (Eds.), *NIOSH Manual of Analytical Methods*. DHHS (NIOSH) Publication, CDC, Atlanta, pp. 94–113.
- Kasprzyk, I., 2008. Aeromycology. Main research fields of interest during the last 25 years. *Annals of Agricultural and Environmental Medicine* 15, 1–7.
- Kenny, L.C., Stancliffe, J.D., Crook, B., Stagg, S., Griffiths, W.D., Stewart, I.W., Fitter, S.J., 1998. Development and evaluation of a personal bioaerosol sampler. *Journal of Aerosol Science* 29, 497–498.
- Kramer, C.L., Pady, S.M., 1968. Viability of airborne spores. *Mycologia* 60, 448–449.
- Kurkela, T., 1997. The number of *Cladosporium* conidia in the air in different weather conditions. *Grana* 36, 54–61.
- Lacey, J., 1991. Aerobiology and health: the role of airborne fungal spores in respiratory disease. In: Hawksworth, D.L. (Ed.), *Frontiers in Mycology*. CAB International, Wallingford, pp. 157–185.
- Lacey, J., Venette, J., 1995. Outdoor air sampling techniques. In: Cox, C.S., Wathes, C.M. (Eds.), *Bioaerosols Handbook*. Lewis Publishers/CRC Press, Boca Raton, FL, pp. 402–469.
- Lang-Yona, N., Dannemiller, K., Yamamoto, N., Burshtein, N., Peccia, J., Yarden, O., Rudich, Y., 2012. Annual distribution of allergenic fungal spores in atmospheric particulate matter in the Eastern Mediterranean; a comparative study between ergosterol and quantitative PCR analysis. *Atmospheric Chemistry and Physics Discussions* 12, 2681–2690.
- Larsen, L., Gravesen, S., 1991. Seasonal variation of outdoor airborne viable microfungi in Copenhagen, Denmark. *Grana* 30, 467–471.
- Levetin, E., 1991. Identification and concentration of airborne basidiospores. *Grana* 30, 123–128.
- Lugauskas, A., Krikstaponis, A., 2004. Filamentous fungi isolated in hospitals and some medical institutions in Lithuania. *Indoor Built Environment* 13, 101–108.
- Macher, J.M., Willeke, K., 1992. Performance criteria for bioaerosol samplers. *Journal of Aerosol Science* 23, 647–650.
- Madelin, T.M., Madelin, M.F., 1995. Biological analysis of fungi and associated molds. In: Cox, C.S., Wathes, C.M. (Eds.), *Bioaerosols Handbook*. CRC Press, Boca Raton, pp. 361–368.
- Mallo, A., Nitiu, D., Gardella, M., 2011. Airborne fungal spore content in the atmosphere of the city of La Plata, Argentina. *Aerobiologia* 27, 77–84.

- Mandrioli, P., Caneva, G., Sabbion, C., 2003. Cultural and Aerobiology: Methods and Techniques for Biodeterioration Monitoring. Kluwer Academic Publishers, ISBN 1-4020-1622-0.
- Marshall, W.A., 1997. Laboratory evaluation of a new aerobiological sampler next term for use in the Antarctic. *Journal of Aerosol Science* 28, 371–380.
- McCartney, H.A., Fitt, B.D.L., Schmechel, D., 1997. Sampling bioaerosols in plant pathology. *Journal of Aerosol Science* 28, 349–364.
- Mehta, S.K., Mishra, S.K., Duane, L.P., 1996. Evaluation of three portable samplers for monitoring airborne fungi. *Applied Environmental Microbiology* 62, 1835–1838.
- Mitakakis, Z.T., Ong, E.K., Stevens, A., Guest, D., Knox, R.B., 1997. Incidence of *Cladosporium*, *Alternaria* and total fungal spores in the atmosphere of Melbourne (Australia) over three years. *Aerobiologia* 13, 83–90.
- Mitakakis, Z.T., Clift, A., McGee, A.P., 2001. The effect of local cropping activities and weather on the airborne concentration of allergenic *Alternaria* spores in rural Australia. *Grana* 40, 230–239.
- Munuera, M., Carrión, J.S., 1995. Daily variations of *Alternaria* spores in the city of Murcia (semi-arid southeastern Spain). *International Journal of Biometeorology* 38, 176–179.
- Nikkels, A.H., Terstege, P., Spieksma, F.Th.M., 1996. Ten types of microscopically identifiable airborne fungal spores at Leiden, The Netherlands. *Aerobiologia* 12, 107–112.
- NSI, 2012. Population by Cities. National Institute of Statistics, Madrid, Spain.
- Oliveira, M., Ribeiro, H., Abreu, I., 2005. Annual variation of fungal spores in atmosphere of Oporto (2005). *Annals of Agricultural and Environmental Medicine* 12, 309–315.
- Panagopoulou, P., Filioti, J., Farmaki, E., Maloukou, A., Roilides, E., 2007. Filamentous fungi in a tertiary care hospital: environmental surveillance and susceptibility to anti-fungal drugs. *Infection Control and Hospital Epidemiology* 28, 60–67.
- Perdelli, F., Cristina, M.L., Sartini, M., Spagnolo, A.M., Dallera, M., Ottria, G., Lombardi, R., Grimaldi, M., Orlando, P., 2006. Fungal contamination in hospital environments. *Infection Control and Hospital Epidemiology* 27, 44–47.
- Peto, S., Powell, E.O., 1970. The assessment of aerosol concentration by means of the Andersen sampler. *Journal of Applied Bacteriology* 33, 582–598.
- Pyrri, I., Kapsanaki-Gotsi, E., 2007. A comparative study on the airborne fungi in Athens, Greece, by viable and non-viable sampling methods. *Aerobiologia* 23, 3–15.
- Pyrri, I., Kapsanaki-Gotsi, E., 2012. Diversity and annual fluctuations of culturable airborne fungi in Athens, Greece: a 4-year study. *Aerobiologia* 28, 249–262.
- Sautour, M., Sixt, N., Dalle, F., L'Ollivier, C., Calinon, C., Fourquenot, V., Thibaut, C., Jury, H., Lafon, I., Aho, S., Couillaut, G., Vagner, O., Cuisenier, B., Besancenot, J.P., Caillot, P., Bonnin, A., 2007. A prospective survey of indoor fungal contamination in hospital during a period of building construction. *Journal of Hospital Infection* 67, 367–373.
- Sautour, M., Sixt, N., Dalle, F., L'Ollivier, L., Fourquenot, V., Calinon, C., Paul, K., Valvin, S., Maurel, A., Aho, S., Couillaut, G., Cachia, C., Vagner, O., Cuisenier, B., Caillot, D., Bonnin, A., 2009. Profiles and seasonal distribution of airborne fungi in indoor and outdoor environments at a French hospital. *Science of the Total Environment* 407, 3766–3771.
- Shaheen, I., 1992. Aeromycology of Amman area, Jordan. *Grana* 31, 223–228.
- Sharma, D., Dutta, B.K., Singh, A.B., 2010. Exposure to indoor fungi in different working environments: a comparative study. *Aerobiologia* 26, 327–337.
- Skjøth, C.A., Sommer, J., Frederiksen, L., Gosewinkel-Karlson, U., 2012. Crop harvest in Denmark and Central Europe contributes to the local load of airborne *Alternaria* spore concentrations in Copenhagen. *Atmospheric Chemistry and Physics* 12, 11107–11123.
- Spieksma, F.T.M., 2003. Airborne mould spores of allergenic importance. *Postępy Dermatologii i Alergologii* 20, 205–208.
- Stępańska, D., Wołek, J., 2005. Variation in fungal spore concentrations of selected taxa associated to weather conditions in Cracow, Poland, in 1997. *Aerobiologia* 21, 43–52.
- Stępańska, D., Wołek, J., 2009. Intradiurnal periodicity of fungal spore concentrations (*Alternaria*, *Botrytis*, *Cladosporium*, *Didymella*, *Ganoderma*) in Cracow, Poland. *Aerobiologia* 25, 333–340.
- Stępańska, D., Harmata, K., Kasprzyk, I., Myszkowska, D., Stach, A., 1999. Occurrence of airborne *Cladosporium* and *Alternaria* spores in Southern and Central Poland in 1995–1996. *Aerobiologia* 15, 39–47.
- Tariq, S.M., Matthews, S.M., Stevens, M., Hakim, E.A., 1996. Sensitization to *Alternaria* and *Cladosporium* by the age of 4 years. *Clinical and Experimental Allergy* 26, 794–798.
- Thompson, M.W., Donnelly, J., Grinshpun, S.A., Juozaitis, A., Willeke, K., 1994. Method and test system for evaluation of bioaerosol samplers. *Journal of Aerosol Science* 25, 1579–1593.
- Tormo, R., Silva, I., Gonzalo, A., Muñoz, A.F., Fernández-Rodríguez, S., Recio, D., 2010. Use of personal spore traps to complement continuous aerobiological monitoring. *Grana* 49, 134–141.
- Tormo, R., Maya, J.M., Fernández-Rodríguez, S., Gonzalo, A., Silva, I., 2013. Influence of environmental factors on measurements with Hirst spore traps. *Grana* 52, 59–70.