

Evaluation of the fungal aerosol in Athens, Greece, based on spore analysis

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Abstract The occurrence and the abundance of the airborne fungi in the city of Athens have been studied by a volumetric non-culture-based method. During a period of 4 years, 562 glass slides were exposed during 562 sampling days, by a Burkard portable air sampler. A total of 259,851 fungal spores have been recovered and assigned to 24 genera of filamentous fungi, in addition to those recorded as groups. The annual mean concentration (AMC) of the total fungi was 865, 1,212, 937 and 1,206 spores/m³, and the concentration range was 89–3,175, 63–7,628, 62–4,323 and 29–10,187 spores/m³ for each year, respectively. There is no statistically significant variation in the AMC and in the distribution patterns of the total fungi from year to year. The temporal variation of the total fungi and of the dominant genera *Cladosporium* and *Alternaria* presented a significant increase during the warm months of each year. The results of the present study compared with data available by a culture-based method confirmed the complementarity of the two sampling procedures. The non-culture-based method was more efficient for the quantification of the total fungal count as well as of the genera *Cladosporium* and *Alternaria*, although less

effective for the quantification of the genera *Aspergillus* and *Penicillium*.

Keywords Fungal spores · Non-culture-based · Volumetric · Spore concentration · Temporal fluctuation

1 Introduction

Airborne particulate matter of biological origin is dominated by fungal spores which are ubiquitous constituents of the atmosphere throughout the year. Airborne fungi originate from diverse natural or artificial ecological niches, and they participate in multiple biotic interactions. Fungi produce and release in the atmosphere numerous spores which are dispersed through the air currents and can be transported even far away colonizing new substrates. The diversity and the concentration of the fungal spores in the atmosphere are the result of dynamic processes influenced by environmental parameters such as climatic conditions. Airborne fungi mediate processes concerning the structure and the function of the ecosystems and affect living organisms.

The fungal aerosol is recognized as the causative agent of various allergic reactions, infections or toxicoses to humans (Lacey 1991). Increased incidences of respiratory allergies and asthma have been associated with the presence of fungal spores in the air

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(Chakrabarti et al. 2012; Pongracic et al. 2010). Many plant pathogens are dispersed by the wind infecting crops and causing serious epidemics and significant economic losses in agriculture (Leyronas and Nicot 2013). Recently, it has been found that the fungal aerosol may affect atmospheric chemistry and may act as cloud condensation nuclei influencing the Earth's climate (Després et al. 2012). Recent studies with the implementation of molecular techniques demonstrated that the fungal spectrum suspended in the air is much richer than previously known (Fröhlich-Nowoisky et al. 2009; Pashley et al. 2012).

Although the fungal spore content of the atmosphere has attracted significant interest during recent decades, it still remains underexplored in many parts of the world. Most aeromycological research involves the study of fungal spores only by one sampling method, either a culture-based or a non-culture-based method. Long-term investigations by non-culture-based methods are usually focusing on allergenic genera such as *Cladosporium* and *Alternaria* (Corden and Millington 2001; Giner et al. 2001), and less commonly the total airspora is enumerated. A multi-year study with concurrent samplings of the total air mycobiota by culture and non-culture-based methods is highly required.

The aim of this paper is to present the results of a long-term investigation of the fungal aerosol studied by a volumetric non-cultured-based method. The diversity and the abundance of the airborne fungi sampled concurrently by a volumetric cultured-based method at the same locality have been published (Pyrri and Kapsanaki-Gotsi 2012). Since the comparison of data derived from synchronous samplings has revealed a complementarity of the two sampling procedures (Adhikari et al. 2004; Almaguer et al. 2013; Das and Gupta-Bhattacharya 2012; Burge et al. 1977; Fernández-Rodríguez et al. 2014; Pyrri and Kapsanaki-Gotsi 2007), it is interesting to test this finding during a long-term monitoring.

2 Materials and methods

2.1 Sampling site

Athens is a densely populated city located in central Greece. It is surrounded by mountains and extends along the seaside in the southwest. The samplings

were performed in the center of Athens city on the rooftop of the building of the Ministry of the Environment (37°59'58"N, 23°43'59"E), approximately 30 m above the ground level and 1 m above the floor of the terrace.

2.2 Sampling protocol

The sampling procedure included two complementary methods, one based on culture and the other based on the microscopic identification of the fungal spores. The samplings took place concurrently, in the same location, at a 1 m distance the one spore trap from the other, in order to avoid any possible interference of the air suctioned. A portable air sampler for agar plates (Burkard Manufacturing Company Ltd) with a flow rate of 20 l/min was used (calculated cut point, $D_{50} = 2.25 \mu\text{m}$). Twelve petri dishes with MA and PDA were exposed consecutively for 1 min each (Pyrri and Kapsanaki-Gotsi 2012).

A portable air sampler (Burkard Manufacturing Company, Ltd) for glass slides with a flow rate of 10 l/min was used for the collection of the air samples (calculated cut point, $D_{50} = 2.4 \mu\text{m}$). Glass slides covered with glycerin jelly as an adhesive (10 g gelatin, 54 ml glycerol, 1.4 g phenol dissolved in 60 ml distilled water) were used. In each sampling, one glass slide was continuously exposed for 30 min.

The samplings were performed all year round for a total of 4 years, from January 1998 until December 2001. Sampling took place three times a week, each Monday, Wednesday and Friday, between 8 and 8:30 in the morning. This time period was selected after a diurnal study to find the time of day that the concentration of fungal spores and the number of genera were maximized (Pyrri and Kapsanaki-Gotsi 2006). A few samplings were omitted for unforeseen reasons.

2.3 Sample analyses

Each exposed glass slide was mounted with three drops of the glycerin jelly, and a cover glass was placed on the sample. The whole sample surface was scanned in four transverse traverses, and each traverse was approximately 0.5 mm wide. A Zeiss KF-2 light microscope was used with a magnification 400 $\times$ and/or 1000 $\times$ when more accurate observation was necessary. All the spores deposited on each slide were

recorded, and the morphologically distinguishable spores were identified to genus level. Taxa which were not identified to the genus level were recorded as groups. Spore counts were then converted into spores per cubic meter of air sampled (spore/m³).

The exposed glass slides are maintained as reference material, and they have been deposited in the Mycetothea ATHUM in the University of Athens.

2.4 Statistical analysis

The daily concentrations of the total fungi and the predominant genera were computed in Excel and in Stat graphic 4 (Windows XP) for statistical processing. The samplings paired per month have been evaluated for the comparison between the years. Extreme values as well as excessive number of samplings between the years have been excluded from data processing. Analysis of variance (ANOVA) was applied to test whether there are any significant differences in the monthly mean concentrations of the total fungi, the genera *Cladosporium* and *Alternaria*, the ascospores and the basidiospores between the years. In addition, the Spearman rank order correlation coefficient (r_s) was used in order to compare the fluctuations of the daily concentrations of the airborne fungi from year to year.

3 Results

3.1 Bioerosol profile

During the 4-year monitoring of the fungal aerosol in the city of Athens, a total of 562 glass slides were exposed during 562 sampling days (136 in 1998, 142 in 1999, 144 in 2000 and 140 in 2001).

The spores microscopically distinguishable were assigned to 24 genera: *Alternaria*, *Arthrinium*, *Cladosporium*, *Coprinus*, *Curvularia*, *Drechslera*, *Emericella*, *Epicoccum*, *Ganoderma*, *Helminthosporium*, *Humicola*, *Nigrospora*, *Periconia*, *Pestalotiopsis*, *Pithomyces*, *Pleospora*-like, *Polythrincium*, *Puccinia*, *Scopulariopsis*, *Stachybotrys*, *Stemphylium*, *Torula*, *Trichothecium* and *Ulocladium*. The genera *Aspergillus* and *Penicillium* were indistinguishable by the morphology of their spores, and they were recorded as a group (Asp-Pen type). In addition, unidentified ascospores, unidentified basidiospores, spores of

Myxomycota, *Erysiphales*, *Uredinales* and *Ustilaginales* were also recorded as groups. Fragments of hyphae or conidiophores were recorded as hyphal fragments (Table 1).

The airborne fungi recovered during the 4 years were mostly anamorphic with a mean percentage of 72.4 %, followed by the Ascomycota (8.89 %), Basidiomycota (7.03 %), Myxomycota (5.24 %), hyphal fragments (3.58 %) and Ustilaginales (2.85 %).

3.2 Abundance and temporal fluctuations of fungal spores

The aerobiological data recovered during the monitoring of the airborne fungi in the years 1998–2001 are presented in Table 1. Monthly and seasonal fluctuations were recorded in the spore concentrations. The annual mean concentration (AMC) of the total fungi was 865, 1,212, 937 and 1,206 spores/m³ for the years 1998, 1999, 2000 and 2001, and the concentration range was 89–3,175, 63–7,628, 62–4,323 and 29–10,187 spores/m³ for each year, respectively. The monthly fluctuations of the total fungi and the predominant components for the 4 years are presented in Fig. 1. The total fungi presented increased concentrations from May to October with a peak in mid-summer.

The prevalent genus was *Cladosporium* accounting from 55.2 to 60.1 % of the annual total count, depending the year. The concentration of *Cladosporium* spores varied from year to year and peaked from June to August. The genus *Alternaria* constituted 3.4–3.8 % of the total, and it was encountered in high concentrations from May until October with maximum concentrations in July. The genera *Aspergillus* and *Penicillium* represented 5.9–6.5 % of the fungal airspora with no evident seasonal pattern.

The sexual spores of Ascomycota and Basidiomycota constituted a significant part of the fungal aerosol, 8.3–9.5 % and 5.2–8.5 %, respectively. The ascospores were recorded in high concentration from August to October and the basidiospores from September to November. The spores of *Ustilaginales* (2.4–3.6 %) prevailed mostly during May and June. Hyphal fragments were accounting for 2.7–4.3 % of the total and were increased from May to September with peaks in mid-summer.

Table 1 Qualitative and quantitative data of airborne fungal spores

Fungi	1998			1999			2000			2001		
	AMC (spores/m ³)	Percentage (%)	F (%)	AMC (spores/m ³)	Percentage (%)	F (%)	AMC (spores/m ³)	Percentage (%)	F (%)	AMC (spores/m ³)	Percentage (%)	F (%)
<i>Alternaria</i>	32.73	3.79	100	40.54	3.34	100	34	3.63	100	44.08	3.66	100
<i>Arthrinium</i>	3.74	0.43	42.65	3.43	0.28	43.66	4.92	0.52	50	3.63	0.3	37.14
<i>Cladosporium</i>	477.29	55.19	100	728.35	60.09	100	550.72	58.79	100	708.76	58.80	100
<i>Coprinus</i>	3.77	0.44	100	3.46	0.29	100	4.25	0.45	100	4.11	0.34	100
<i>Curvularia</i>	0.04	0.004	0.74	0.11	0.01	2.82	0.08	0.01	2.08	0.06	0.005	1.43
<i>Drechslera</i>	0.40	0.05	13.24	0.53	0.04	10.56	0.33	0.04	8.33	0.42	0.03	7.86
<i>Emeticella</i>	0.2	0.02	0	0.08	0.006	4.93	0.16	0.02	6.25	0.21	0.02	5.71
<i>Epicoccum</i>	2.50	0.29	36.76	2.3	0.19	29.58	1.89	0.20	30.56	2.78	0.23	34.29
<i>Ganoderma</i>	1.32	0.15	0	1.15	0.1	29.6	1.46	0.16	41.70	1.39	0.12	44.29
<i>Helminthosporium</i>	0.29	0.03	10.29	0.3	0.02	6.34	0.1	0.01	2.08	0.34	0.03	6.43
<i>Humicola</i>	0	0	0	0	0	0	0.03	0.003	0.69	0	0	0
<i>Nigrospora</i>	0.03	0.003	0.74	0	0	0	0.03	0.003	0.69	0	0	0
<i>Periconia</i>	0.03	0.003	1.47	0	0	0	0.08	0.01	2.08	0.03	0.0024	0.71
<i>Pestalotiopsis</i>	0.15	0.02	3.68	0.03	0.002	0.70	0	0	0	0.03	0.0024	0.71
<i>Pithomyces</i>	0.04	0.01	1.47	0.06	0.005	1.41	0	0	0	0.03	0.0024	0.71
<i>Pleospora-like</i>	0.42	0.05	0	0.35	0.03	6.34	0.35	0.04	6.25	0.44	0.04	5.71
<i>Polythrincium</i>	0.17	0.02	5.15	0.23	0.02	3.52	0.27	0.03	6.25	0.56	0.005	10.71
<i>Puccinia</i>	0.07	0.01	2.21	0	0	0	0	0	0	0.03	0.0024	0.71
<i>Scopulariopsis</i>	0	0	0	0.17	0.01	0.70	0	0	0	0.41	0.03	1.43
<i>Stachybotrys</i>	0.29	0.03	8.82	0.26	0.02	4.93	0.59	0.06	10.42	0.34	0.03	5.71
<i>Stemphylium</i>	2.10	0.25	38.97	1.51	0.12	25.35	1.89	0.20	29.17	1.74	0.14	24.29
<i>Torula</i>	1.78	0.2	29.41	1.28	0.11	15.49	1.29	0.14	20.83	1.84	0.15	28.57
<i>Trichothecium</i>	0	0	0	0.1	0.01	1.41	0	0	0	0	0	0
<i>Ulocladium</i>	2.61	0.3	38.24	1.69	0.14	26.06	1.82	0.19	22.22	1.72	0.14	21.43
Asp-Pen type	53.54	6.19	100	71.45	5.9	100	61.2	6.53	100	74.51	6.18	100
<i>Erysiphales</i>	1.90	0.22	31.62	2.761	0.23	34.51	1.94	0.21	31.25	4.91	0.41	44.29
<i>Ustilaginales</i>	20.78	2.4	38.97	36.44	3.01	52.82	33.67	3.59	41.67	28.88	2.4	37.14
<i>Myxomycota</i>	57.78	6.68	62.50	37.68	3.11	69.72	42.67	4.56	65.97	79.57	6.60	62.86
Ascospores (unidentified)	82.35	9.45	84.56	110.8	9.11	100	77.67	8.24	96.53	104	8.57	99.29

Table 1 continued

Fungi	1998			1999			2000			2001		
	AMC (spores/m ³)	Percentage (%)	F (%)	AMC (spores/m ³)	Percentage (%)	F (%)	AMC (spores/m ³)	Percentage (%)	F (%)	AMC (spores/m ³)	Percentage (%)	F (%)
Basidiospores (unidentified)	63.42	6.74	84.56	107.2	8.46	97.18	54.27	5.18	93.06	73.93	5.68	97.86
Uredospores	2.13	0.25	16.91	6.873	0.57	18.31	11.26	1.2	33.33	6.514	0.54	41.43
Unknown spores	28.18	3.26	85.29	25.58	2.11	97.18	16.03	1.71	83.33	19.85	1.65	86.43
Hyphal fragments	30.48	3.52	81.62	32.39	2.67	86.62	39.92	4.26	96.67	46.54	3.86	93.57
Total	864.81			1,212.1			936.69			1,205.5		

AMC Annual Mean Concentration (average of the daily concentrations, spores/m³); Percentage (%) of each genus or group relatively to the number of the total fungi; F Frequency of recovery (% number of days the fungi were recovered out of: 136 days of sampling in 1998, 142 days of sampling in 1999, 144 days of sampling in 2000 and 140 days of sampling in 2001)

Concerning the occurrence of the airborne fungi, the prevalent components were almost continually present. The genera *Cladosporium*, *Alternaria* and *Coprinus* as well as the *Asp-Pen* type spores, the ascospores, the basidiospores and the hyphal fragments, which were present in high concentrations, had a frequency of 80–100 % (Table 1). They are considered as dominant and stable components of the Athens bioaerosol. The genera *Arthrinium*, *Drechslera*, *Epicothium*, *Helminthosporium*, *Stemphylium*, *Torula* and *Ulocladium*, as well as the spores of *Ustilaginales*, *Myxomycota* and the uredospores, have been recovered with a frequency of 10–50 %, an AMC 1–10 spores/m³ and a percentage of 0.1–1 % in the total and are considered as common. The genera *Curvularia*, *Emericella*, *Ganoderma*, *Periconia*, *Pestalotiopsis*, *Pithomyces*, *Polythrincium*, *Stachybotrys* as well as *Pleospora*-like and *Erysiphales* were sporadically recovered in even lower frequency and concentration, and they are regarded as uncommon. The genera *Humicola*, *Nigrospora*, *Puccinia*, *Scopulariopsis* and *Trichothecium* which were found only once or twice in the 4-year period were extremely rare.

3.3 Statistical analysis

The statistical analysis applied for the comparison of the airborne fungi qualitatively and quantitatively from year to year revealed that the dominant and stable components of the Athens atmosphere were the same in the 4 years. Only the sporadic and rare genera were different from year to year. For the genera *Cladosporium* and *Alternaria* and for ascospores, results of the F-test show that there is not a statistically significant difference between the means of the 4 years at the 95.0 % confidence level as shown in Table 2. On the contrary, for the basidiospores, there is a statistically significant difference among the mean concentrations of the 4 years at the 95.0 % confidence interval.

The Spearman rank order correlation coefficient (r_s) shows that the annual fluctuations of the total fungi and the genera *Cladosporium* and *Alternaria* are strongly correlated (P_{value} 0.000) between the years (Table 3). Most of the correlations are statistically significant. The fluctuation patterns of the ascospores and basidiospores were correlated at the 95.0 % confidence interval between certain year pairs (Table 3).

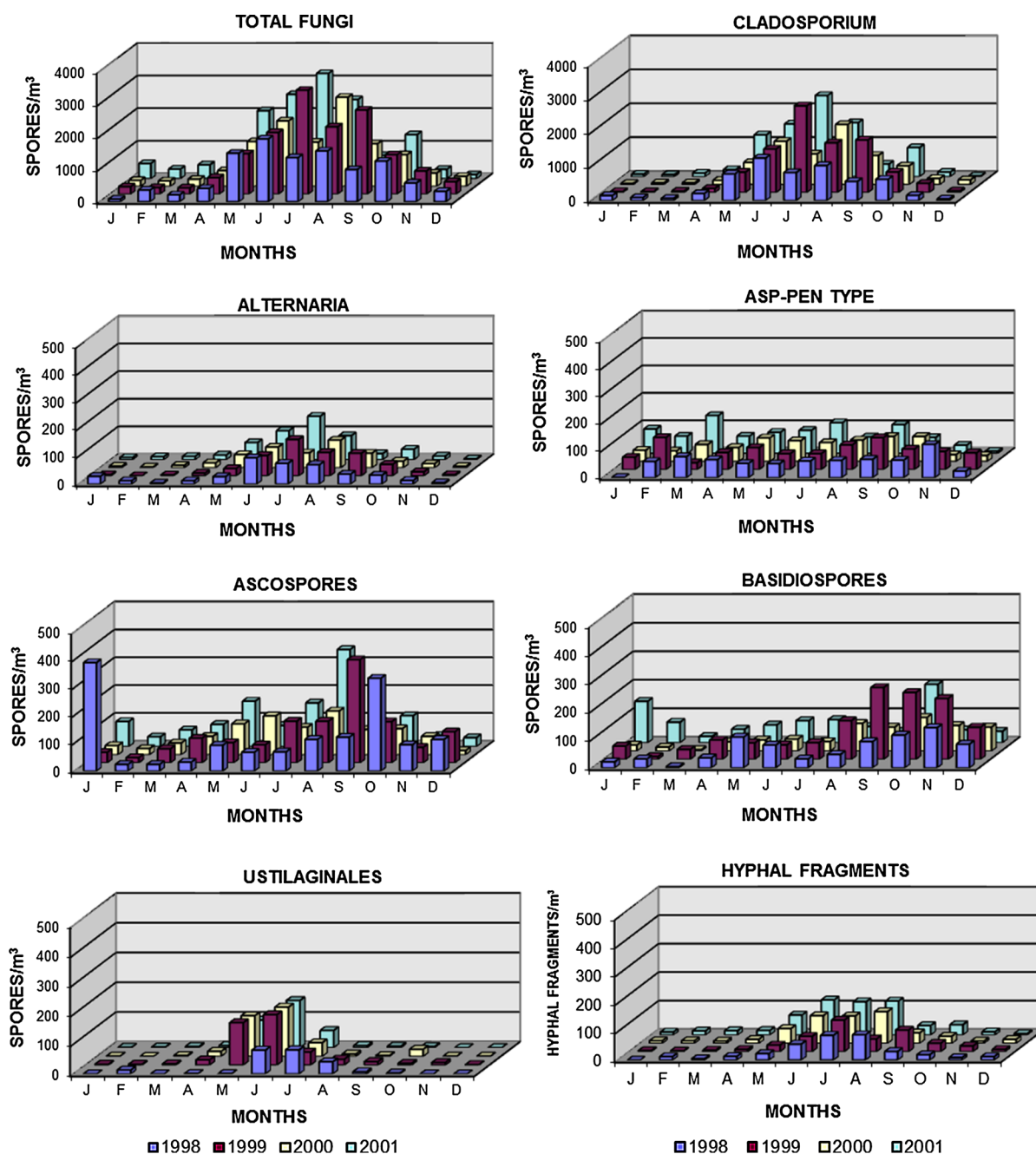


Fig. 1 Fluctuations of the monthly mean concentration of the total fungi and the predominant components, during the years 1998, 1999, 2000 and 2001

3.4 Comparison of the sampling methods

The results obtained by the non-culture-based method were compared with those obtained by the culture-based method (Pyrris and Kapsanaki-Gotsi 2012) since

the samplings were performed concurrently for the whole time period of the 4 years in the same locality. The occurrence of the prevalent components, their percentages and the average annual total concentrations of the 4 years were compared for the two

sampling methods. Totally, 24 genera and eight groups were registered by the non-culture-based method versus 54 fungal genera and four groups recovered by the culture-based method. Nineteen genera were detected by both methods (Table 4).

The abundance of the airborne mycobiota was more efficiently recorded by the non-culture-based method since higher concentrations of fungal spores have been registered by this method during concomitant samplings with the culture-based method. The spore/colony ratio was 1.68 for the total fungi, 2.99 for the genus *Cladosporium*, 1.15 for the genus *Alternaria* and 0.33 for the genera *Aspergillus* and *Penicillium*.

The total fungi and the genera *Cladosporium* and *Alternaria* exhibited similar fluctuation patterns independently of the sampling method since their concentration increased in the atmosphere during the summer months.

4 Discussion

The increasing concern about the effects of the airborne fungi on plants, animals and humans creates new incentives for further advancement in this field. The major components, their concentrations and the sources of fungal aerosol are still inadequately known and poorly understood. In addition, there are no standardized protocols concerning the sampling procedure, sample handling and analysis, so that aerobiological data differ markedly and conclusions based on mismatched data are often biased.

Concerning the non-viable sampling, the majority of the reports involve samplings performed with a 7-day spore trap and most of the reports include data concerning a few months to a year. Long-term aerobiological monitoring usually focuses on the genera *Cladosporium* and *Alternaria* and their health impact, whereas the total fungal aerosol profile is rarely presented.

The present article includes data from a 4-year (1998–2001) regular monitoring of the air mycobiota in the atmosphere of the Athens city center by a volumetric non-culture-based method. The diversity and the fluctuations of air airborne fungi recovered by a volumetric culture-based method have been reported (Pyrri and Kapsanaki-Gotsi 2012). It is interesting to compare data provided by the non-culture-based method that was applied concurrently for the same locality.

In the present study by the non-culture-based method, the AMC of the total airborne fungi in Athens during the 4 years ranged from 865 to 1,212 spores/m³ which is lower than the concentration reported from Thessaloniki, Greece (Gioulekas et al. 2004). Lower mean concentrations have been reported from Madrid, Spain with 609 spores/m³ (Herrero et al. 2006) and Porto, Portugal with 531 spores/m³ (Oliveira et al. 2009), or higher in Singapore with 1,688 spores/m³ (Lim et al. 1998). The concentration range of the total fungi in Athens was 89–3,175, 63–7,628, 62–4,323 and 29–10,187 spores/m³ for each year, respectively. Spore ranges with higher upper limits have been reported from Turkey (Inal et al. 2008), Caxias Do Sul, Brazil (de Antoni Zoppas et al. 2006) and Singapore (Lim et al. 1998). Lower maximum concentrations have been reported from Santiago, Chile (Henríquez et al. 2001), India (Adhikari et al. 2004; Chakrabarti et al. 2012) and La Plata, Argentina (Mallo et al. 2011). The concentration of the total count increased in the atmosphere from May until October with maximum values during summer months. Similar patterns are recorded in Portugal (Oliveira et al. 2005, 2009), Canada (Li and Kendrick 1995), Taiwan (Ho et al. 2005) and Argentina (Mallo et al. 2011).

The airborne fungi sampled by the non-culture-based method do not show significant variation in concentration from year to year.

The genus *Cladosporium* was the predominant component of the fungal aerosol in Athens representing the 55.2–60.1 % of the annual total count. In a 15-year study in Thessaloniki, Greece, *Cladosporium* was accounting for the 72.2 % of the total (Gioulekas et al. 2004). The concentration percentages reported from other countries varied from 32.57 % in Brazil (de Antoni Zoppas et al. 2006) to 90.5 % in Poland (Kasprzyk and Worek 2006). The *Cladosporium* spore concentration in the present study exhibited seasonal fluctuations attaining peak values during summer months. Similar patterns have been reported in Athens (Gioulekas et al. 2003) and Thessaloniki (Gioulekas et al. 2003, 2004) whereas in Heraklion *Cladosporium* spore concentrations peaked in May and sharply decreased in June (Gioulekas et al. 2003). The fluctuation patterns of *Cladosporium* reported from other regions are quite similar in Turkey (Inal et al. 2008, Sakiyan and Inceoglu 2003), Spain (Aira et al. 2012; Sánchez et al. 2009), Portugal (Oliveira et al. 2005, 2009), Poland (Grinn-Gofrón and Rapiejko 2009;

Table 2 Comparison of the annual mean concentration of the total fungi and of the predominant genera and groups

Fungal taxa	ANOVA	
	F-Ratio	P _{value}
Total fungi	1.47	0.22
<i>Cladosporium</i>	1.34	0.26
<i>Alternaria</i>	0.86	0.46
Ascospores	1.39	0.24
Basidiospores	8.19	0.00*

* Statistically significant difference

Stępańska and Wołek 2005) and India (Adhikari et al. 2004). *Cladosporium* has a constant and significant presence in the atmosphere of every eco-geographic region in the world, and it is characterized as a “universal dominant” (de Antoni Zoppas et al. 2006).

The genus *Alternaria* constituted a significant part of the airborne fungi found in the air of Athens representing the 3.34–3.79 % of the annual total count and recorded in high concentrations from June to August. Gioulekas et al. (2003) reported for *Alternaria* similar fluctuations in Athens, a longer peak period (June to October) in Thessaloniki and shorter (May to June) in Heraklion. Similar temporal patterns were observed in Turkey (Inal et al. 2008; Şakiyan and Inceoğlu 2003), Italy (Bergamini et al. 2004), Spain (Aira et al. 2013; Giner et al. 2001; Sánchez et al. 2009), UK (Corden and Millington 2001) and India (Adhikari et al. 2004). The peak period of *Alternaria* in Australia is from October to February (Mitakakis and McGee 2000).

The genera *Aspergillus* and *Penicillium* are recorded as Asp-Pen type since they produce morphologically indistinguishable conidia (phialospores). These genera are omnipresent, dominant constituents

of the fungal airspora in Athens as found by the culture-based method. The concentration of the genus *Penicillium* decreased significantly during the summer time, while that of the genus *Aspergillus* increased during summer and autumn (Pyrri and Kapsanaki-Gotsi 2012). The assessment of Asp-Pen type spores by the non-culture-based method showed a percentage 5.9–6.5 % of the annual total count. No seasonal periodicity was found since the fluctuation patterns of *Aspergillus* and *Penicillium* are overlapping.

Ascospores and basidiospores constituted a significant part of the fungal aerosol (8.3–9.5 % and 5.2–8.5 %, respectively). The concentration of the ascospores increased from May to October with the main peak in different month from year to year. The number of basidiospores increased from August to October with maximum concentrations usually in October. Analogous fluctuations were reported from Naples, Italy (D’Amato et al. 1984), Saudi Arabia (Hasnain et al. 2005) and Ontario, Canada (Li and Kendrick 1995).

Ustilospores of smut fungi were recorded in high concentrations during May and June, exactly the same as in Madrid, Spain (Herrero et al. 2006) and Tulsa, Oklahoma (Crotzer and Levetin 1996).

Hyphal fragments constitute a significant part of the bioaerosol with a percentage of 2.67–4.26 % of the annual total count and are increased during summer months. Fungal fragments may affect human health in a similar way as fungal spores (Green et al. 2005).

The non-culture-based method is efficient in recording the fungal spores suspended in the atmosphere enabling a more accurate quantification of their abundance. It records all types of spores present in the air, regardless of their viability or culturability. Nevertheless, only certain genera can be identified by the spore morphology alone since the vast majority

Table 3 Correlation of the seasonal fluctuations of the total fungi and of the predominant genera exhibited each sampling year

Fungal taxa	R_s P _{value}					
	1998–1999	1998–2000	1998–2001	1999–2000	1999–2001	2000–2001
Total fungi	0.4598* 0.0000	0.6083* 0.0000	0.4948* 0.0000	0.6896* 0.0000	0.5698* 0.0000	0.6907* 0.0000
<i>Cladosporium</i>	0.6356* 0.0000	0.6254* 0.0000	0.6342* 0.0000	0.7753* 0.0000	0.7044* 0.0000	0.6606* 0.0000
<i>Alternaria</i>	0.5851* 0.0000	0.5703* 0.0000	0.5408* 0.0000	0.6627* 0.0000	0.5935* 0.0000	0.6776* 0.0000
Ascospores	0.2424* 0.0126	0.2375* 0.0145	0.0692 0.4730	0.3490* 0.0003	0.3684* 0.0001	0.3730* 0.0001
Basidiospores	0.0573 0.5569	0.3238* 0.0009	0.0930 0.3407	0.4882* 0.0000	−0.0195 0.8417	0.2284* 0.0193

* Statistically significant correlations

Table 4 Comparison of the qualitative and the quantitative data recovered by the culture-based method (BAP) and the non-culture-based method (BGS) during 1998–2001

Fungi	BAP		BGS	
	Mean ATC (CFU) ^a	Percentage ^b	Mean ATC (no. of spores)	Percentage
<i>Acremonium</i>	129	0.14	–	–
<i>Acrodontium</i>	21	0.02	–	–
<i>Alternaria</i>	4,612	5.35	5,319	3.61
<i>Arthrimum</i>	265	0.32	553	0.38
<i>Aspergillus</i>	7,114	8.10	–	–
<i>Aureobasidium</i>	715	0.83	–	–
<i>Beauveria</i>	17	0.02	–	–
<i>Botryosporium</i>	4	0.004	–	–
<i>Botrytis</i>	366	0.45	–	–
<i>Chaetomium</i>	50	0.05	–	–
<i>Chromelosporium</i>	1	0.001	–	–
<i>Chrysonilia</i>	10	0.01	–	–
<i>Circinella</i>	12	0.01	–	–
<i>Cladobotryum</i>	1	0.001	–	–
<i>Cladosporium</i>	28,978	33.36	86,717	58.22
<i>Coprinus</i>	–	–	548	0.38
<i>Cunninghamella</i>	4	0.005	–	–
<i>Curvularia</i>	4	0.005	10	0.007
<i>Dichobotrys</i>	3	0.003	–	–
<i>Dicyma</i>	3	0.004	–	–
<i>Doratomyces</i>	15	0.02	–	–
<i>Drechslera</i>	24	0.03	59	0.04
<i>Emericella</i>	701	0.76	23	0.008
<i>Epicoccum</i>	175	0.21	332	0.23
<i>Eurotium</i>	375	0.41	–	–
<i>Fusarium</i>	220	0.26	–	–
<i>Ganoderma</i>	–	–	187	0.13
<i>Geotrichum</i>	135	0.15	–	–
<i>Helminthosporium</i>	–	–	36	0.02
<i>Humicola</i>	–	–	1	0.001
<i>Mucor</i>	105	0.12	–	–
<i>Neosartorya</i>	1	0.001	–	–
<i>Nigrospora</i>	10	0.01	2	0.002
<i>Paecilomyces</i>	499	0.63	–	–
<i>Penicillium</i>	20,361	23.05	–	–
<i>Periconia</i>	2	0.001	5	0.004
<i>Pestalotiopsis</i>	3	0.002	7	0.006
<i>Petriella</i>	3	0.003	–	–
<i>Petromyces</i>	20	0.02	–	–
<i>Pithomyces</i>	–	–	5	0.004
<i>Pleospora-like</i>	–	–	54	0.04
<i>Phoma</i>	2	0.001	–	–
<i>Polythrincium</i>	–	–	43	0.02
<i>Puccinia</i>	–	–	4	0.006

Table 4 continued

Fungi	BAP		BGS	
	Mean ATC (CFU) ^a	Percentage ^b	Mean ATC (no. of spores)	Percentage
<i>Rhizopus</i>	221	0.25	–	–
<i>Sclerotinia</i>	32	0.04	–	–
<i>Scopulariopsis</i>	136	0.15	21	0.01
<i>Spiniger</i>	118	0.13	–	–
<i>Sporotrichum</i>	2	0.002	–	–
<i>Stachybotrys</i>	54	0.06	52	0.04
<i>Stemphylium</i>	12	0.006	254	0.25
<i>Syncephalastrum</i>	1	0.001	–	–
<i>Torula</i>	5	0.006	216	0.14
<i>Trichoderma</i>	43	0.05	–	–
<i>Trichothecium</i>	7	0.007	4	0.003
<i>Ulocladium</i>	231	0.28	275	0.18
<i>Verticillium</i>	3	0.003	–	–
<i>Zygosporium</i>	4	0.004	–	–
Asp-Pen type	–	–	9,168	6.20
<i>Erysiphales</i>	–	–	404	0.27
Sphaeropsidales	863	1.01	–	–
<i>Ustilaginales</i>	–	–	4,223	2.85
<i>Myxomycota</i>	–	–	7,624	5.24
Ascospores	–	–	13,091	8.89
Basidiomycetes	1,060	1.19	9,769	7.03
Uredospores	–	–	950	0.65
Yeasts	7,421	8.76	–	–
Unidentified spores	1,072	1.19	3,138	2.18
N.S.F.	12,076	13.73	–	–
Hyphal fragments	–	–	5,252	3.58
Total	88,317		148,344	

^a Mean ATC, average of the annual total concentration for the 4 years

^b Percentage of each genus or group relatively to the total number of fungi for the 4 years of sampling

of fungi produce morphologically indistinguishable spores. On the other hand, the culture-based method allows for a reliable identification of the fungi at the species level.

The spore/colony ratio, based on recoveries from concurrent sampling in the present study, is 1.68 for the total fungi and does not deviate markedly from 1 which indicates similar recoveries. In a recent study from Badajoz Spain, a spore/colony ratio of 6.8 has been reported (Fernández-Rodríguez et al. 2014). This much higher ratio may indicate not only a loss of viability but also an inefficiency of the culture-based method attributed to the study protocol, i.e., sampling time period, incubation conditions and duration, and culture media. The ratio for *Cladosporium*, which is

the predominant genus that defines the total count, is 2.99 for Athens versus 11.1 for Spain and for *Alternaria* 1.15 for Athens versus 2.6 for Spain. Nevertheless, the ratio for *Aspergillus* and *Penicillium* is only $0.33 < 1$ for Athens versus 2.0 for Spain. The results of the present study indicate that the non-culture-based method is less effective for the quantification of the genera *Aspergillus* and *Penicillium*, in addition to its inefficiency for reliable identification even at the genus level for this important aerosol component.

The non-viable portion of the aerosol components, the unculturable ones as biotrophic parasites, those with specific growth demands, etc., can be recorded by the visual identification of the fungal spores (Pyrrri and

Kapsanaki-Gotsi 2007). However, only a relatively small number of genera with distinguishable spores can be accurately identified. On the contrary, the culture-based method allows a detailed and accurate identification of the viable, culturable spores. Both methods have recorded efficiently the temporal trends of the spore concentrations. The comparative data of the synchronous sampling for a 4-year period confirmed earlier conclusions for Athens and the complementarity of the two methods (Pyrri and Kapsanaki-Gotsi 2007), as also verified by more recent investigations in other countries (Almaguer et al. 2013; Das and Gupta-Bhattacharya 2012; Fernández-Rodríguez et al. 2014).

Further research has to be conducted by paired samplings including different sampling procedures with standardized protocols for the characterization of the aeromycological profile in a region. It is crucial to identify the inherent potentialities of each method and confirm the effectiveness of each methodology in order to enable their efficient application. Since the fungal content of the atmosphere plays an important role in environmental processes and furthermore it variously affects human affairs, it is necessary to establish standardized procedures integrated with innovative methodologies in order to improve our understanding of the air mycobiota population structure and dynamics.

5 Conclusions

The spore analysis of the fungal aerosol in Athens for a 4-year period revealed that *Cladosporium*, *Alternaria*, Asp-Pen type spores, ascospores and basidiospores dominate the fungal aerosol in Athens. The total fungi and the prevalent genera *Cladosporium* and *Alternaria* presented increased spore concentration during the warm months of the year.

The comparison of the data with those obtained concurrently by a culture-based method has confirmed the temporal trends in the fluctuation of total fungi and major components by both methods; although a higher spore concentration recovery is achieved by the direct spore identification. Also, the synchronous sampling has documented the complementarity of the two methods concerning the fungal aerosol composition.

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