

Diversity and annual fluctuations of culturable airborne fungi in Athens, Greece: a 4-year study

Ioanna Pyrri · Evangelia Kapsanaki-Gotsi

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Abstract The diversity and the abundance of the culturable airborne fungi have been studied by a volumetric method in the city of Athens, for a period of 4 years. A total of 6,600 plates were exposed during 562 calendar days, and 70,583 colonies of fungi have been recovered and studied in detail. One hundred and forty-eight species in fifty-four genera of filamentous fungi were identified. A total of three hundred and twenty strains were isolated and maintained as reference material. The annual mean concentration of the total fungi was 538, 640, 694 and 638 CFU/m³, and the concentration range, 25–2,435, 117–2,822, 122–2,201 and 116–2,590 CFU/m³ for each year, respectively. There is no statistically significant year-to-year variation in the distribution patterns and in the annual mean concentrations of the total fungi. The diversity and the abundance of the total fungi and of the dominant genera *Cladosporium*, *Aspergillus* and *Alternaria* were increased, whereas those of *Penicillium* decreased during the warm months of each year. The majority of the species are newly reported as airborne from Greece. Also, 19 genera and 93 species are totally new records for this country. The species *Acrodontium virellum*, *Aspergillus aculeatus*, *A. tubingensis*, *Circinella minor*, *C. umbellata*,

Cladosporium breviramsum, *C. malorum*, *Drechslera tetramera*, *Paecilomyces crustaceus*, *Petriella guttulata*, *Rutola graminis* and *Sporotrichum pruinosum* are reported as airborne for the first time worldwide.

Keywords Airborne fungi · Culture-based volumetric method · Species diversity · Concentration

1 Introduction

Most fungi liberate their spores into the atmosphere and disseminate them by the air currents. The aerosols constitute an ephemeral sink of fungal propagules continuously supplied by the mycobiota. Members of all the fungal groups in terrestrial habitats, regardless of their origin, may be found in the bioaerosols during their dispersal phase. Once the spores become airborne, they may colonize new suitable substrates exposed to the air. As a result, the fungal spores are ubiquitous, and they mediate numerous processes with a crucial role in the maintenance and function of any natural ecosystem. Nevertheless, they may cause various diseases in cultivated plants in the agroecosystems, as well as allergic reactions, infections or toxicosis to humans. They may also interfere with several man-made ecosystems and cause deterioration of materials including foodstuffs, industrial products, archives and works of art. The knowledge on the

I. Pyrri · E. Kapsanaki-Gotsi (✉)
Department of Ecology & Systematics, Faculty of
Biology, National and Kapodistrian University of Athens,
Panepistimioupoli, 157 84 Athens, Greece
e-mail: ekapsan@biol.uoa.gr

diversity of the airborne fungi is significant for several applied issues, with an increased concern for human and plant health.

Although the common genera of airborne fungi are adequately known around the world, there is a lack of information concerning the less frequent and the rare fungi. Most of the aerobiological surveys record the fungi at the genus level, and information is missing at the species level. Moreover, a non-culture-based sampling method does not allow the identification of fungi that produce spores not easily recognizable. Also, the data on the abundance of the fungi are insufficient in the case of the outdoor airspora studied by the sedimentation method. A culture-based volumetric method records more reliably the diversity of airborne fungi and their anamorphs, although it fails to survey thoroughly the total fungi (Pyrri and Kapsanaki-Gotsi 2007a). The air mycobiota have been studied by this method in several countries around the world (Abdel Hameed et al. 2009; Adhikari et al. 2004; Airaudi and Filipello Marchisio 1996; Al-Suwayne et al. 1999; Calvo et al. 1980a, b, 1981; Das and Gupta-Bhattacharya 2008; Ebner et al. 1992; El-Morsy 2006; Fang et al. 2005; Filipello Marchisio and Airaudi 2001; Filipello Marchisio et al. 1992, 1997; Herrero et al. 1996; Larsen and Gravesen 1991; O’Gorman and Fuller 2008; Roses-Codinachs et al. 1992; Takahashi 1997; Wu et al. 2007).

In Greece, the published data on the bioaerosols are mostly concerned with the occurrence and concentration of certain genera of allergenic fungi studied by non-culture-based volumetric methods. A detailed account on the aeromycological studies in Greece has been presented earlier (Kapsanaki-Gotsi 2007; Pyrri and Kapsanaki-Gotsi 2007a).

The aim of this paper is to present the diversity and abundance of the culturable airborne fungi studied by a volumetric method. It is part of an investigation on the airborne fungi in the ambient air in the center of Athens, by “culture-based” and “non-culture-based” sampling methods.

2 Materials and methods

2.1 Sampling site

Athens is a densely populated city within the Attica basin that is surrounded by mountains and extends to

the sea, along the Saronic Gulf in the southwest. The whole region has a variable geomorphology, and the Attica plain is surrounded by the mountain ranges of Parnitha to the north, Pendeli to the northeast, Ymittos to the east and Aegaleo to the west. The main rocks are limestone, marble and schist, which support a rich flora, including more than 1,000 plant taxa. The upper zone of Parnitha, which is the highest mountain (1,413 m) in the region, is dominated by *Abies cephalonica* forests. The periurban forests are dominated by *Pinus halepensis* mixed with *Quercus coccifera*, *Q. pubescens*, *Juniperus oxycedrus* and *Cupressus sempervirens*, in the lowlands intermixed with *Cercis siliquastrum*, *Olea europaea*, *Ficus carica* and *Prunus* spp. The maquis vegetation includes *Pistacia lentiscus*, *Arbutus unedo*, *Myrtus communis*, *Laurus nobilis*, *Erica arborea* etc. The phryganeic ecosystems consist mainly of *Sarcopoterium spinosum*, *Euphorbia acanthothamnus*, *Corydanthus capitatus*, *Phlomis fruticosa*, *Asphodelus* and *Cistus* spp. The city has a mild Mediterranean climate with very hot and dry summers.

The airborne fungi were sampled in the ambient air in the center of the Athens metropolitan area, which is occupied by high buildings and has mostly narrow streets and relatively few patches of vegetation in public parks. Also, this area is bound by the hills of Filopappou, Acropolis, Lycabettus and Tourkovounia. The samplings were made on the rooftop of a building of the Ministry of the Environment, located at 37°59'58" N, 23°43'59" E, approximately 30 m above the ground level and 1 m above the floor of the terrace. The sampling site is in a higher level than the roofs of the adjacent buildings, situated in a place where the air currents circulate unobstructed through the Attica basin.

2.2 Sampling protocol

A portable air sampler for agar plates (Burkard Manufacturing Company Ltd) with a suction rate of 20 L/min was used for the collection of the air samples.

Sampling has taken place three times a week, each Monday, Wednesday and Friday, between 8 and 8:30 a.m. The concentration of the total fungi and the number of genera recovered are higher in the early morning, as it was revealed after a diurnal study (Pyrri

and Kapsanaki-Gotsi 2006). The samplings were performed all year-round, from January 1998 until December 2001. A few samplings were omitted for unforeseen reasons.

Several nutrient media were tested in order to select the more suitable on the basis of the higher number of genera recovered in higher concentrations (Pyrri and Kapsanaki-Gotsi 2007b). The nutrient medium potato dextrose agar (PDA) was used continuously throughout the 4 years and additionally malt agar (MA) 2% the last 3 years. In each sampling date, the sampler was loaded with twelve plastic Petri dishes of 9 mm diameter (6PDA + 6MA), which were exposed consecutively for 1 min each. The sampler was disinfected with 70% ethanol before the sampling.

2.3 Isolation and identification of fungi

The exposed plates were incubated at 25°C in the dark for 7–21 days. Fungal strains that failed to sporulate after 21 days have been recorded as non-sporulating fungi (NSF). The colonies were studied for their macroscopic and microscopic characteristics and identified at least to the genus level. Representative strains of the fungi grown in the plates were isolated in pure culture. The isolated fungal strains in the genera *Penicillium*, *Aspergillus*, *Fusarium*, *Scopulariopsis* and *Chaetomium* were subcultured in specific nutrient media and incubated in conditions suitable for each genus, in order to identify the fungal strains to the species level. The identification was based on the detailed study of all the microscopic morphological characters of the fungi and the macroscopic features of their colonies. For the resolution of the taxonomic status of several strains, comparative material from the ATHUM, BPIC, CBS, CCF, FRR, MUCL, NRRL and UAMH Collections has been additionally studied.

The isolated fungal strains have been deposited in the ATHUM Culture Collection of fungi, in the University of Athens.

2.4 Concentration of fungi in the air samples

The colony counts were corrected by using the multiple infection transformation factors (Gregory 1948) and were converted to colony-forming units per cubic meter (CFU/m³) of air sampled.

2.5 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 data obtained on the nutrient media PDA and MA have been pooled together. Only the samplings paired per month have been evaluated for the comparison between the years. Extreme values have been excluded from data processing.

Analysis of variance (ANOVA) was employed to test whether there are any significant differences in the monthly mean concentrations of the total fungi and of the genera *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* 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 Enumeration of samples

During the period of four years, a total of 6,600 Petri dishes were exposed, during 562 sampling days (136, 142, 144 and 140 days during the years 1998, 1999, 2000 and 2001, respectively). A total of 70,583 colonies of fungi have been studied in the microscope, identified and enumerated.

3.2 Diversity of fungi

One hundred and forty-nine taxa, representing 148 species and 1 variety, assigned into 54 genera, have been identified. Additionally, the *Basidiomycota*, the *Sphaeropsidales*, the yeasts and the NSF were recorded as groups. Annually, 19 genera were recorded during 1998, 45 genera in 1999, 39 in 2000 and 42 in 2001. Only 19 genera were constantly found all the years, while 14 genera were rare and were recovered in one out of the four years. The species recovered (Table 1) are assigned to *Zygomycota* (8 species), *Ascomycota* (13 species) and anamorphic fungi (127 species). Representative 320 strains of filamentous fungi were isolated in pure culture and maintained as reference material.

A great number of species were recognized in the prevalent genera *Cladosporium*, *Penicillium* and

Table 1 List of fungal taxa isolated from the atmosphere of Athens during the years 1998–2001

Anamorphic fungi		
<i>Acremonium alternatum</i> Link	<i>C. cladosporioides</i> (Fresen.) de Vries ^b	<i>P. fellutanum</i> Biourge ^a
<i>A. furcatum</i> Moreau & R. Moreau ex Gams ^a	<i>C. cucumerinum</i> Ellis & Arthur ^b	<i>P. funiculosum</i> Thom ^b
<i>A. murorum</i> (Corda) W. Gams ^a	<i>C. herbarum</i> (Pers.) Link ^a	<i>P. glabrum</i> (Wehmer) Westling ^b
<i>Acrodontium griseum</i> (Fassat.) de Hoog ^a	<i>C. macrocarpum</i> Preuss ^b	<i>P. glandicola</i> (Oudem.) Seifert & Samson ^a
<i>A. virellum</i> (Fr.) de Hoog ^a	<i>C. malorum</i> Ruehle ^a	<i>P. implicatum</i> Biourge ^a
<i>Alternaria alternata</i> (Fr.) Keissl.	<i>C. oxysporum</i> Berk. & Curt. ^a	<i>P. italicum</i> Wehmer
<i>A. citri</i> Ellis & Pierce	<i>C. sphaerospermum</i> Penz. ^a	<i>P. miczynskii</i> K.M. Zalessky ^a
<i>A. infectoria</i> Simmons ^a	<i>C. spongiosum</i> Berk. & M.A. Curtis ^a	<i>P. olsonii</i> Bainier & Sartory ^a
<i>A. tenuissima</i> (Kunze) Wiltshire	<i>C. tenuissimum</i> Cooke ^a	<i>P. oxalicum</i> Currie & Thom ^a
<i>Arthrinium phaeospermum</i> (Corda) M.B. Ellis ^a	<i>C. variabile</i> (Cooke) de Vries ^a	<i>P. pinophilum</i> Thom ^a
<i>Aspergillus aculeatus</i> Iizuka ^b	<i>Curvularia lunata</i> (Wakker) Boedijn ^a	<i>P. purpureogenum</i> Stoll ^a
<i>A. auricomus</i> (Guég.) Saito ^a	<i>C. lunata</i> var. <i>aeria</i> ^a	<i>P. raistrickii</i> G. Sm. ^a
<i>A. carbonarius</i> (Bainier) Thom	<i>Dichobotrys abundans</i> Hennebert ^a	<i>P. roqueforti</i> Thom ^a
<i>A. clavatus</i> Desm.	<i>Dicyma ampullifera</i> de Lesd. ^a	<i>P. rugulosum</i> Thom ^a
<i>A. flavus</i> Link	<i>Doratomyces purpureofuscus</i> (Schwein.) F.J. Morton & G. Sm. ^a	<i>P. sclerotiorum</i> J.F.H. Beyma ^b
<i>A. foetidus</i> Thom & Raper ^a	<i>Drechslera halodes</i> (Drechsler) Subram. & B.L. Jain ^a	<i>P. solitum</i> Westling ^a
<i>A. fumigatus</i> Fresen.	<i>D. hawaiiensis</i> (Bugnic.) Subram. & B.L. Jain ^a	<i>P. thomii</i> Maire ^a
<i>A. melleus</i> Yukawa ^a	<i>D. tetramera</i> (McKinney) Subram. & B.L. Jain ^a	<i>P. verrucosum</i> Dierckx ^b
<i>A. niger</i> Tiegh.	<i>Epicoccum nigrum</i> Link	<i>P. verruculosum</i> Peyronel ^a
<i>A. niveus</i> Blochwitz ^a	<i>Fusarium avenaceum</i> (Fr.) Sacc. ^a	<i>Periconia</i> sp.
<i>A. ochraceus</i> G. Wilh.	<i>F. dimerum</i> Penz. ^a	<i>Pestalotiopsis</i> sp.
<i>A. oryzae</i> (Ahlb.) E. Cohn ^b	<i>F. equisetii</i> (Corda) Sacc. ^a	<i>Phoma glomerata</i> (Corda) Wollenw. & Hochapfel ^a
<i>A. ostianus</i> Wehmer	<i>F. oxysporum</i> Schltdl.	<i>Radulidium subulatum</i> (de Hoog) Arzanlou, W. Gams & Crous ^a
<i>A. parasiticus</i> Speare ^b	<i>F. proliferatum</i> (Matsush.) Nirenberg ex Gerlach & Nirenberg ^a	<i>Rutola graminis</i> (Desm.) J.L. Crane & Schokn. ^a
<i>A. puniceus</i> Kwong-Chung & Fennell ^a	<i>F. sporotrichoides</i> Sherb. ^a	<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier ^b
<i>A. sulphureus</i> (Fresen.) Wehmer ^a	<i>Geotrichum candidum</i> Link	<i>S. brumptii</i> Salvanet-Duval ^a
<i>A. sydowii</i> (Bainier & Sartory) Thom & Church ^a	<i>Geotrichum</i> sp.	<i>S. candida</i> (Guég.) Vuill. ^a
<i>A. terreus</i> Thom	<i>Myrothecium</i> sp.	<i>S. fusca</i> Zach ^a
<i>A. tubingensis</i> Mosseray ^a	<i>Nigrospora oryzae</i> (Berk. & Broome) Petch ^b	<i>Spiniger meineckellus</i> (A.G. Olson) Stalpers ^a
<i>A. ustus</i> (Bainier) Thom & Church ^b	<i>Paecilomyces crustaceus</i> (Apinis & Chesters) Yaguchi ^a	<i>Sporotrichum pruinosum</i> Gilman & Abbott ^a
<i>A. versicolor</i> (Vuill.) Tirab. ^a	<i>P. lilacinus</i> (Thom) Samson ^b	<i>Stachybotrys chartarum</i> (Ehrenb.) S. Hughes ^b
<i>A. wentii</i> Wehmer ^b	<i>P. marquandii</i> (Masse) Hughes ^a	<i>S. parvispora</i> S. Hughes ^a
<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud	<i>P. variotii</i> Bainier ^b	<i>Stemphylium herbarum</i> E.G. Simmons ^b

Table 1 continued

<i>Beauveria bassiana</i> (Bals.-Criv.) Vuill. ^a	<i>Penicillium aurantiogriseum</i> Dierckx ^a	<i>Torula herbarum</i> (Pers.) Link ^a
<i>B. brongniartii</i> (Sacc.) Petch ^a	<i>P. brevicompactum</i> Dierckx ^a	<i>Trichoderma viride</i> Pers.
<i>Botryosporium longibrachiatum</i> (Oudem.) Maire ^a	<i>P. chrysogenum</i> Thom	<i>Trichothecium roseum</i> (Pers.) Link
<i>Botrytis cinerea</i> Pers.	<i>P. citrinum</i> Thom	<i>Ulocladium alternariae</i> (Cooke) E.G. Simmons ^a
<i>Chromelosporium fulvum</i> (Link) McGinty ^a	<i>P. commune</i> Thom ^a	<i>U. atrum</i> Preuss ^a
<i>Chrysonilia sitophila</i> (Mont.) Arx. ^a	<i>P. crustosum</i> Thom ^a	<i>U. botrytis</i> Preuss ^a
<i>Cladobotryum varium</i> Nees ^a	<i>P. decumbens</i> Thom ^a	<i>Verticillium albo-atrum</i> Reinke & Berthold ^b
<i>Cladosporium brevirosum</i> Morgan-Jones & B.J. Jacobsen ^a	<i>P. digitatum</i> (Pers.) Sacc.	<i>V. luteoalbum</i> (Link) Subram. ^a
<i>C. chlorocephalum</i> (Fresen.) E.W. Mason & M.B. Ellis ^a	<i>P. expansum</i> Link ^b	<i>Zygosporeum masonii</i> S. Hughes ^a
Ascomycota		
<i>Chaetomium bostrychodes</i> Zopf ^b	<i>E. intermedium</i> Blaser ^a	<i>Petromyces alliaceus</i> Malloch & Cain ^c
<i>C. globosum</i> Kunze	<i>E. rubrum</i> Jos. König et al. ^a	<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary ^a
<i>Emericella nidulans</i> (Eidam) Vuill.	<i>Neosartorya fischeri</i> (Wehmer) Malloch & Cain ^a	<i>Talaromyces flavus</i> (Klöcker) Stolk & Samson ^a
<i>E. varicolor</i> Berk. & Broome ^a	<i>Petriella guttulata</i> Barron & Cain ^a	
<i>Eurotium amstelodami</i> L. Mangin ^a	<i>Petriella</i> sp.	
Zygomycota		
<i>Circinella minor</i> Lendn. ^a	<i>Mucor plumbeus</i> Bonord. ^a	<i>Rhizopus oryzae</i> Went & Prins. Geerl. ^b
<i>C. umbellata</i> Tiegh. & Le Monn. ^a	<i>M. circinelloides</i> Tiegh. ^a	<i>Syncephalastrum racemosum</i> Cohn ex Schröt. ^a
<i>Cunninghamella echinulata</i> (Thaxt.)Thaxt. ex Blakes. ^b	<i>M. racemosus</i> Fresen.	

^{a,b} Taxa reported for the first and second time, respectively on any substrate from Greece

^c Teleomorph reported for the first and anamorph for the second time on any substrate from Greece

Aspergillus (Table 1). The most common and abundant species in the atmosphere of Athens were *Cladosporium cladosporioides*, *C. herbarum*, *Penicillium chrysogenum*, *P. digitatum*, *Aspergillus niger*, *A. ochraceus* and *Alternaria alternata*. Other species commonly recovered from the air were *Cladosporium oxysporum*, *C. tenuissimum*, *C. sphaerospermum*, *Penicillium aurantiogriseum*, *P. brevicompactum*, *P. citrinum*, *P. expansum*, *P. glabrum*, *P. italicum*, *P. oxalicum*, *P. purpurogenum*, *P. thomii*, *Aspergillus auricomus*, *A. flavus*, *A. ustus*, *A. parasiticus*, as well as *Emericella* and *Eurotium* species.

The number of fungal genera and species in the atmosphere was increased during the warm months of the year. The majority of the genera and the species were recovered from May to November, except

Penicillium species that were found mainly during the cold months of the year.

It is noteworthy that 12 species are reported from the air for the first time worldwide (Table 2). The occurrence of these fungi on other substrates may vary from common to very rare, according to our estimate based on data from literature. In addition, the species *Acremonium alternatum*, *Aspergillus melleus*, *Beauveria brogniartii*, *Chaetomium bostrychodes*, *Chromelosporium fulvum*, *Cladobotryum varium*, *Dichobotrys abundans*, *Doratomyces purpureofuscus*, *Drechslera halodes*, *Fusarium dimerum*, *Paecilomyces marquandii*, *Penicillium miczynskii*, *Penicillium pinophilum*, *Penicillium raistrickii*, *Radulidium subulatum* and *Scopulariopsis fusca* have been rarely reported from the air.

Table 2 Species newly reported as airborne

Species	Occurrence ^a
<i>Acrodontium virellum</i>	Rare
<i>Aspergillus aculeatus</i>	Uncommon
<i>Aspergillus tubingensis</i>	Rare
<i>Circinella minor</i>	Common
<i>Circinella umbellata</i>	Common
<i>Cladosporium breviramsum</i>	Very rare
<i>Cladosporium malorum</i>	Common
<i>Drechslera tetramera</i>	Common
<i>Paecilomyces crustaceus</i>	Common
<i>Petriella guttulata</i>	Very rare
<i>Rutola graminis</i>	Rare
<i>Sporotrichum pruinosum</i>	Common

^a On any substrate

The majority of the species are reported from Greece for the first time as airborne. Also, 19 genera and 93 species are reported for the first time, and 11 genera and 23 species, for the second on any substrate (Table 1).

3.3 Abundance of fungi

The airborne mycobiota presented fluctuations in daily, monthly and seasonal concentrations in each year. The annual mean concentration of the total fungi was 538, 640, 694 and 638 CFU/m³ for the years 1998, 1999, 2000 and 2001, and the concentration range was 25–2,435, 117–2,822, 122–2,201 and 116–2,590 CFU/m³, respectively. Detailed data are presented in the Tables 3 and 4.

The vast majority of the airborne fungi recovered during the 4 years were anamorphic (75%), followed by the NSF (13.8%), the yeasts (8.5%), *Ascomycota* (1.3%), *Basidiomycota* (1.2%) and *Zygomycota* (0.3%).

The genera *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* predominated in descending order, in the atmosphere of Athens.

The monthly fluctuations of the total fungi and the predominant genera for the 4 years are presented in Fig. 1. The total fungi presented increased concentrations from May to July and from October to November. The peak recorded in February 1999 was due to the high numbers of *Penicillium* spores during that time.

The genus *Cladosporium* was the dominant component, except during the year 1999 when it was surpassed by the genus *Penicillium*. The concentration of *Cladosporium* spores exhibited a major peak from May to July, and a lower one, during October. The spores of the genus *Penicillium* decreased significantly during summer months in all the 4 years of the study. Very high concentrations of *Penicillium* were recorded during February 1999. A spore shower of *Penicillium* was recorded on 25-02-1999 with a concentration of 10,393 CFU/m³ as an extreme value. The genus *Aspergillus* increased during the warm months of the year with maximum values during September and October. The genus *Alternaria* was encountered in high concentrations from May until August with maximum concentrations in June or July. The NSF and the yeasts exhibited no evident seasonal patterns, except an increase in the yeasts during the winter months of the first 2 years.

The genera *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* as well as the NSF and the yeasts have a frequency of 100% and were present in high concentrations during all the sampling days (Table 3). They are considered as dominant and stable components of the Athens bioaerosol. The *Basidiomycota* and the *Sphaeropsidales*, as well as the genera *Acremonium*, *Arthrinium*, *Aureobasidium*, *Botrytis*, *Emericella*, *Epicoccum*, *Eurotium*, *Fusarium*, *Geotrichum*, *Mucor*, *Paecilomyces*, *Rhizopus*, *Scopulariopsis*, *Spiniger*, *Stachybotrys* and *Ulocladium*, may be considered as common. They have been recovered roughly with a frequency of 10–50%, an AMC 1–10 CFU/m³ and a percentage of 0.1–1% in the total. There were also found thirty-three genera sporadically recovered in even lower frequency and concentration, and they are regarded as uncommon. A few of them are rare since they were found only once or twice in the 4-year period, and it is most unlikely to be recovered.

3.4 Statistical analysis

A statistical analysis has been applied for the comparison of the airborne fungi qualitatively and quantitatively from year to year. The dominant and stable components of the Athens atmosphere were the same in all the 4 years. Only the sporadic and rare genera were different from year to year. Analysis of variance (ANOVA) was applied to compare the difference in the monthly mean concentration from

Table 3 Qualitative and quantitative data of the airborne mycobiota recovered each year of the study

Genus or group	1998			1999			2000			2001		
	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)
<i>Acromonium</i>				0.46	0.07	7.75	1.89	0.28	22.917	1.26	0.20	18.57
<i>Acrodonium</i>				0.29	0.046	4.93	0.11	0.016	2.78	0.20	0.03	5
<i>Alternaria</i>	34	6.32	83.82	32.99	5.22	100	32.03	4.69	100	32.34	5.15	100
<i>Arthrinium</i>	3.25	0.60	9.56	1.15	0.18	18.31	1.69	0.25	25	1.51	0.24	23.57
<i>Aspergillus</i>	36.52	6.79	100	55.31	8.68	100	60.15	8.82	100	50.26	8.01	100
<i>Aureobasidium</i>	5.20	0.97	20.59	3.73	0.59	44.37	6.35	0.93	63.89	5.06	0.81	55.71
<i>Beauveria</i>				0.06	0.009	1.41	0.14	0.02	2.08	0.29	0.05	4.29
<i>Botryosporium</i>				0.03	0.005	0.70	0.08	0.012	1.39			
<i>Botrytis</i>	4.78	0.89	17.65	2.23	0.35	30.28	1.50	0.22	24.31	2.01	0.32	31.43
<i>Chaetomium</i>				0.23	0.036	4.93	0.76	0.11	12.5	0.40	0.06	9.29
<i>Chromelosporium</i>							0.03	0.004	0.69			
<i>Chrysomitia</i>	0.09	0.018	0.74	0.09	0.013	2.11	0.03	0.004	0.69	0.09	0.014	2.14
<i>Circinella</i>				0.06	0.009	1.41	0.11	0.016	2.78	0.17	0.03	4.29
<i>Cladobotryum</i>										0.03	0.005	0.71
<i>Cladosporium</i>	200.13	37.21	100	120.43	19.07	100	250.22	36.67	100	254.02	40.49	100
<i>Cunninghamella</i>				0.06	0.009	0.70	0.03	0.004	0.69	0.03	0.005	0.71
<i>Curvularia</i>				0.11	0.018	1.41						
<i>Dichobotrys</i>										0.09	0.014	1.43
<i>Dicyna</i>				0.03	0.005	0.70	0.03	0.004	0.69	0.03	0.005	0.71
<i>Doratomyces</i>				0.11	0.018	2.82	0.19	0.028	4.17	0.11	0.018	2.86
<i>Drechslera</i>	0.18	0.03	0.74	0.20	0.03	4.93	0.22	0.033	4.86	0.09	0.014	2.14
<i>Emericella</i>				5.56	0.88	47.18	8.11	1.19	45.8	6.04	0.96	43.57
<i>Epicoccum</i>	1.58	0.29	7.35	0.77	0.12	11.27	1.26	0.19	22.22	1.39	0.22	19.29
<i>Eurotium</i>				2.49	0.39	28.87	3.38	0.49	40.27	4.73	0.75	48.57
<i>Fusarium</i>	2.04	0.38	8.82	1.38	0.22	23.24	1.18	0.17	20.14	1.67	0.27	26.43
<i>Geotrichum</i>				0.80	0.13	11.27	0.81	0.12	18.75	2.20	0.35	27.86
<i>Mucor</i>	0.40	0.075	2.21	1.42	0.22	21.13	0.74	0.11	9.72	0.40	0.06	9.29
<i>Myrothecium</i>										0.09	0.014	0.71
<i>Neosartorya</i>				0.03	0.005	0.70						

Table 3 continued

Genus or group	1998			1999			2000			2001		
	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)
<i>Nigrospora</i>	0.09	0.018	0.74	0.09	0.013	2.11	0.11	0.016	2.78			
<i>Paecilomyces</i>	8.32	1.55	14.71	3.44	0.55	31.69	1.62	0.24	24.31	1.01	0.16	20
<i>Penicillium</i>	96.61	17.97	100	194.48	30.79	100	165.76	24.29	100	120.14	19.15	100
<i>Periconia</i>										0.09	0.014	1.43
<i>Pestalotiopsis</i>				0.11	0.019	0.70	0.03	0.004	0.69			
<i>Petriella</i>										0.09	0.014	1.43
<i>Petromyces</i>				0.46	0.07	8.45	0.03	0.004	0.69	0.09	0.014	2.14
<i>Phoma</i>										0.09	0.014	0.71
<i>Radulidium</i>				0.03	0.005	0.70						
<i>Rhizopus</i>	0.55	0.10	2.21	2.37	0.05	28.87	1.75	0.26	30.56	1.56	0.25	26.43
<i>Rutula</i>				0.09	0.013	1.41						
<i>Sclerotinia</i>	0.24	0.045	1.47	0.32	0.05	3.52	0.22	0.033	4.17	0.11	0.018	2.86
<i>Scopulariopsis</i>	0.24	0.044	2.94	0.56	0.09	13.38	1.68	0.25	30.56	1.36	0.22	22.86
<i>Spiniger</i>				0.31	0.05	6.34	1.76	0.26	15.97	1.24	0.20	17.86
<i>Sporotrichum</i>				0.06	0.009	1.41						
<i>Stachybotrys</i>				0.37	0.06	8.45	0.42	0.06	9.03	0.74	0.12	17.14
<i>Stemphylium</i>				0.19	0.03	4.93	0.11	0.016	2.78	0.03	0.005	0.71
<i>Syncephalastrum</i>				0.03	0.005	0.70						
<i>Talaromyces</i>										0.03	0.005	0.71
<i>Torula</i>										0.09	0.014	2.14
<i>Trichoderma</i>	0.25	0.047	0.74	0.31	0.05	6.34	0.36	0.05	6.95	0.29	0.05	7.14
<i>Trichothecium</i>				0.09	0.013	2.11				0.11	0.018	2.86
<i>Ulocladium</i>	3.00	0.56	11.03	1.13	0.18	20.42	1.54	0.22	21.53	0.95	0.15	19.29
<i>Verticillium</i>				0.03	0.005	0.70	0.06	0.008	1.39			
<i>Zygosporium</i>				0.06	0.009	1.41	0.06	0.008	1.39			
<i>Basidiomycota</i>				9.93	1.57	11.27	1.85	0.27	9.03	18.31	2.92	36.43
<i>Sphaeropsidales</i>	7.97	1.48	12.50	4.45	0.70	52.82	7.24	1.06	65.97	4.94	0.79	52.86
<i>Yeasts</i>	69.54	12.93	100.00	73.28	11.6	100	38.92	5.70	100	30.12	4.80	100
Unidentified fungi	1.89	0.35	2.94	20.98	3.32	69.72	5.31	0.78	56.94	2.01	0.32	22.14

Table 3 continued

Genus or group	1998			1999			2000			2001		
	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)	AMC CFU/m ³	%	F (%)
NSF	60.91	11.33	100	97.24	15.39	100	94.01	13.78	100	90.52	14.43	100
TOTAL FUNGI	537.81			639.82			693.85			638.26		

AMC, annual mean concentration (average of the daily concentrations. CFU/m³); % percentage of each genus or group relatively to the total number of 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)

year to year for *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria* and the total fungi. The results of the analysis are presented in Table 5. Since the P_{value} of the F test is greater than 0.05 for the total fungi and for the genera *Penicillium* and *Aspergillus*, there is not a statistically significant difference at the 95.0% confidence interval between the monthly mean concentrations of the 4 years. On the contrary, the P_{value} is lower than 0.01 for the genera *Cladosporium* and *Alternaria*; therefore, there is a statistically significant difference at the 99.0% confidence interval among the mean concentrations of the 4 years.

The Spearman rank order correlation coefficient (r_s) was applied to test the fluctuations of the daily concentrations exhibited by the total fungi and the dominant genera between the sampling years, and the results are presented in Table 6. The annual fluctuations of the total fungi and of the genera *Cladosporium* and *Penicillium* were weakly to moderately correlated and of the genera *Aspergillus* and *Alternaria* more strongly correlated between the years. Most of the correlations are statistically significant (Table 6).

4 Discussion

The aeromycological investigations are increasingly concerned with the air quality in the indoor environments, often supplemented by the study of the ambient air for a comparison. The majority of the airborne fungi remain unidentified at the species level, since the diversity is not usually a priority in the research objectives. Most of the studies are focusing on those fungi which potentially may cause adverse health effects, so that the rest are underestimated or overlooked. For example, during an extensive survey on the indoor fungi compared to outdoor fungi in USA, a detailed account at the genus level is provided but only species of medical importance have been identified (Shelton et al. 2002).

Actually, there is a multifaceted influence of fungi in human affairs. The knowledge on the occurrence of fungi in any particular ecological niche is of great importance, and this is especially true concerning the atmosphere, which permits the effective dissemination of the fungal propagules and ensures their establishment on new substrates.

This study offers novel information about the diversity of the airborne fungi. One hundred and

Table 4 Concentration range of the total fungi and of the predominant genera for each sampling year

Fungal taxa	Concentration range (CFU/m ³)			
	1998	1999	2000	2001
Total Fungi	25–2,435	116–2,822	122–2,201	116–2,590
<i>Cladosporium</i>	8–2,169	8–1,703	4–1,945	13–2,202
<i>Penicillium</i>	13–1,068	4–864*	8–1,218	8–701
<i>Aspergillus</i>	8–204	8–542	4–404	4–642
<i>Alternaria</i>	4–180	4–271	4–179	4–235

* 10,393 CFU/m³ was recorded on 25-02-1999, an extreme value excluded from statistical analysis

forty-eight species in fifty-four genera of filamentous fungi have been isolated and studied in pure culture. This seems to be a higher number of airborne fungal species than ever before recovered from a single site. A higher number has been recovered from two sites in Turin, where 165 species of mesophilic fungi and some more thermotolerant were recorded (Airaudi & Filipello Marchisio 1996). A high diversity was also found in Kuwait with 116 species recorded from one site (Moustafa and Kamel 1976), in Egypt with 102 species from six regions (Ismail et al. 2002) and 83 species from a desert locality (Abdel-Hafez and El-Said 1989) and in Qatar with 73 species (Al-Subai 2002). In a study including multiple sites in five cities of Lithuania, 430 species of fungi have been reported, but only part of them is listed by name (Lugauskas et al. 2003).

In the genera *Penicillium* and *Aspergillus*, 29 and 30 species, respectively, have been recovered. Analogous high species diversity in these genera has been reported in the Mediterranean region, from Italy (Airaudi and Filipello Marchisio 1996) and Spain (Herrero et al. 1996). Moreover, twelve species of *Cladosporium* have been recovered from the Athens atmosphere, and this is at least twice the number of species found in other geographic areas. The most frequent and abundant species were *C. cladosporioides* and *C. herbarum* as also reported from Turin (Filipello Marchisio et al. 1992) and Barcelona (Calvo Torras et al. 1981).

Most of the species recovered in Athens are cosmopolitan and have been reported from several regions around the world. However, 29 species are considered to be rare in the air, as well as 12 species are reported from the air for the first time worldwide.

Some of the species are very rare in any environment and in any substrate. One of the most intriguing

findings was *Cladosporium breviramsum*, which was only known from its original description in Georgia in the United States, on hotel wallpaper (Morgan-Jones and Jacobsen 1988).

The majority of the species are reported as airborne for the first time in Greece. Also, 19 genera and 93 species are reported for the first time, and 11 genera and 23 species for the second time, from any substrate in Greece.

All the fungal strains isolated from the air have been maintained as reference material for future studies. The ATHUM Culture Collection, with a focus on airborne fungi (Kapsanaki-Gotsi 2001), has increased its holdings by 320 strains, representing 148 species in 54 genera of filamentous fungi. Some of these strains are unique or rarely found in other Culture Collections.

The airborne fungi do not show significant variation in concentration from year to year, as statistically tested by the comparison of the data recovered each sampling year. The annual mean concentration (AMC) of the total fungi in Athens during the 4 years ranged from 538 to 694 CFU/m³. There are not enough data available on the AMC of the total airborne fungi studied by a viable volumetric method, from other geographic regions. Higher mean concentrations have been reported from Dublin, Ireland, with 915 CFU/m³ (O'Gorman and Fuller 2008), from Beijing, China, with 1,165 CFU/m³ (Fang et al. 2005) and from USA, with 930 CFU/m³ (Shelton et al. 2002). The concentration range of the total fungi in Athens during the 4 years was 25–2,822 CFU/m³ and is quite similar to the spore range recorded in Yokohama, Japan (Takahashi 1997). Higher maximum concentrations from other urban areas have been reported from Turin, Italy (Filipello Marchisio and Airaudi 2001), from Beijing, China (Fang et al. 2005), from USA (Shelton

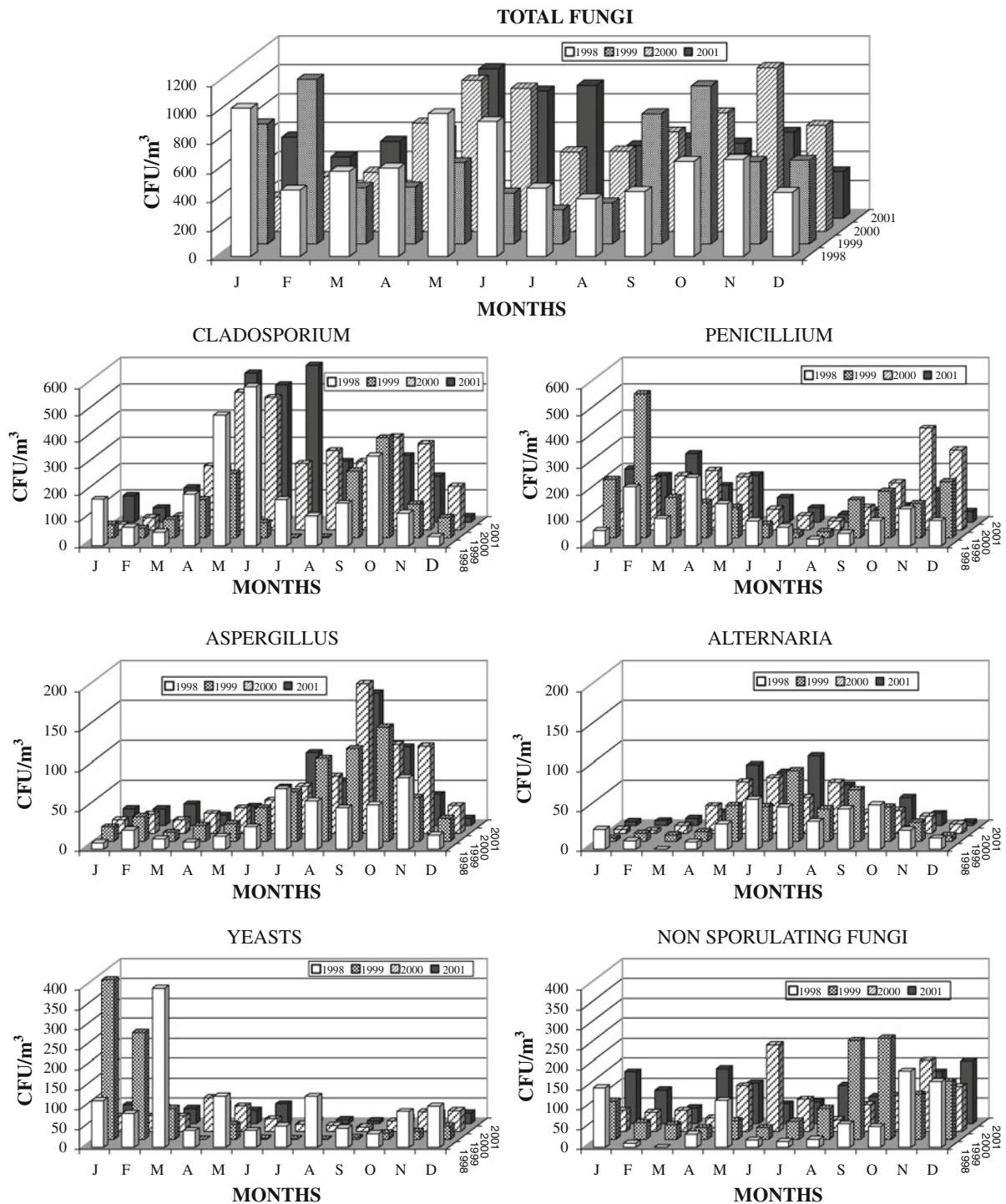


Fig. 1 Fluctuations of the monthly mean concentration of the total fungi and the predominant components, during the years 1998, 1999, 2000 and 2001

Table 5 Comparison of the annual mean concentration of the total fungi and of the predominant genera

Fungal taxa	ANOVA	
	F-ratio	P _{value}
Total fungi	0.30	0.88
<i>Cladosporium</i>	7.22	0.00*
<i>Penicillium</i>	0.69	0.56
<i>Aspergillus</i>	0.60	0.61
<i>Alternaria</i>	7.89	0.00*

* Statistically significant difference

et al. 2002; Tsai et al. 2007), from Buenos Aires, Argentina (Negrin et al. 2007), and from Taiwan (Huang et al. 2002; Wu et al. 2007).

The genera *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*, the yeasts and the NSF were the major constituents of the fungal aerosol. They were recovered in high concentrations with a frequency of 100% and are considered as dominant and stable components of the Athens atmosphere. These fungi are also prevalent, although in various quantities, in other Mediterranean countries (Calvo et al. 1980a; El-Morsy 2006; Filipello Marchisio and Airaudi 2001; Roses-Codinachs et al. 1992) in North Europe (Larsen & Gravesen 1991; O’Gorman and Fuller 2008), in Asia (Adhikari et al. 2004; Fang et al. 2005; Takahashi 1997) and America (Negrin et al. 2007; Shelton et al. 2002).

The total fungi and the major components exhibited seasonal fluctuations during each year. The concentration

of the total fungi was increased twice in a year, mainly from May to July and from October to November. Seasonality with two peaks has been reported from Beijing, China (Fang et al. 2005), and West Bengal, India (Das and Gupta-Bhattacharya 2008). A single peak has been recorded, either during the summer time, in Italy (Filipello Marchisio et al. 1997), Austria (Ebner et al. 1992), Ireland (O’Gorman and Fuller 2008), USA (Tsai et al. 2007) and Japan (Takahashi 1997), or during the winter time, in Saudi Arabia (Al-Suwaine et al. 1999) and Taiwan (Huang et al. 2002).

The dominant genus *Cladosporium*, which mostly defines the total, has also presented a double peak, one from May to July and the second during October. An analogous pattern has been reported in Turin (Filipello Marchisio et al. 1997; Filipello Marchisio and Airaudi 2001). The genus *Penicillium* increased significantly during the winter months. The extraordinary rise of *Penicillium* in February 1999 can be attributed to the accumulation of trash in the streets around the city center, due to a prolonged strike of sanitation workers. The genus *Aspergillus* did not similarly rise due to the trash, since the low temperatures are usually unfavorable for its growth. The concentration of the genus *Aspergillus* was increased during summer and markedly peaked in autumn months. Similar distributions for *Penicillium* and *Aspergillus* were reported in Denmark (Larsen and Gravesen 1991) and in Saudi Arabia (Al-Suwaine et al. 1999). The concentration of the genus *Alternaria* was significantly higher from

Table 6 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	1999–2000	2000–2001	1998–2000	1999–2001	1998–2001
Total fungi	0.043	−0.067	0.196	0.166	−0.105	0.351
	0.641	0.428	0.021*	0.113	0.216	0.000*
<i>Cladosporium</i>	0.114	0.087	0.442	0.266	0.060	0.573
	0.227	0.305	0.000*	0.005*	0.479	0.000*
<i>Penicillium</i>	0.137	0.308	0.213	0.158	0.247	0.160
	0.172	0.000*	0.012*	0.115	0.003*	0.111
<i>Aspergillus</i>	0.278	0.435	0.366	0.207	0.452	0.306
	0.013*	0.000*	0.000*	0.064	0.000*	0.006*
<i>Alternaria</i>	0.151	0.574	0.588	0.223	0.531	0.434
	0.191	0.000*	0.000*	0.053	0.000*	0.000*

* Statistically significant correlations

May to October. An analogous pattern for *Alternaria* was found in Italy (Filipello Marchisio et al. 1992, 1997, 2001), Austria (Ebner et al. 1992), Denmark (Larsen and Gravesen 1991), China (Fang et al. 2005) and Japan (Takahashi 1997).

The yeasts and the NSF, which were stable components of the fungal aerosol, although variable, had not presented clear seasonal patterns.

It is worth mentioning that the first year of sampling did not show a significant difference from the following years, concerning the fluctuation in the concentrations of the total fungi and the prevalent genera, although there was a deficiency in the sampling media during the first year. Nevertheless, fewer genera were found during the first year, which may be due to the medium or may be partly considered as a matter of chance. Fourteen genera, which were recovered in inconsiderable concentration, only once or twice during the whole period of the 4 years, were absent during the first year.

It has to be appreciated that the taxa recovered during this study represent only a small proportion of those actually present in the atmosphere, due to intrinsic limitations of the methodology and also to the difficulty of obtaining slow-growing species that are often masked by the overgrowth of the more competent fungi. Also, the concentration of the fungal propagules recovered is underestimated by the cultural method, since a lot of the spores have lost their viability or may be unculturable such as rust and smut spores (Pyrri and Kapsanaki-Gotsi 2007a).

The diversity of fungi in the air particulate matter is higher than expected, as revealed by molecular analysis (Fröhlich-Nowoisky et al. 2009). The presence and mass concentrations of fungal spores in the aerosols may be more significant than currently known, and they can influence even the atmospheric processes (Bauer et al. 2008).

The studies on the occurrence and abundance of the airborne fungi in a global scale, by conventional methods supplemented by molecular approaches, will result in an improvement of the monitoring strategy and in a more complete evaluation of the importance of fungi. Furthermore, they will contribute to the understanding of the multiple roles and complicated pathways held by the mycobiota in natural and man-made ecosystems, since most of the fungi get the opportunity to establish themselves in new territories, by their launching into the atmosphere.

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