

Aeromycoflora in Outdoor Environment of Timisoara City (Romania)

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Abstract

An aeromycological study to identify and quantify allergenic fungi and their fluctuations was conducted at Timisoara. The diversity of the aeromycoflora in this study, based on the recovery of fungal propagules by the volumetric sampling method was conducted for 30 days using the Lanzoni sampler. The study showed that the most prevalent (major components) fungal spores in the air of Timisoara were *Cladosporium* spp., *Drechslera/Helminthosporium* spp., *Alternaria* spp. and *Epicoccum* spp. The abundant genera were *Cladosporium*, *Fusarium/Leptosphaeria*, *Drechslera/Helminthosporium*, *Alternaria* and *Torula*. *Cladosporium* was the most abundant fungal spore type (41%) collected throughout the period of study reaching.

Keywords: aeromycoflora, allergenic fungi, *Cladosporium*

Introduction

Airborne fungi are considered to act as indicator of the level of atmospheric bio-pollution. The presence of fungal propagules, volatiles and mycotoxins in the air can cause a health hazard in all segments of the population (Kakde *et al.*, 2001). Airborne fungal spores are ubiquitous in nature (Burge, 1985). Much of our knowledge on the behaviour of airborne spores comes from various studies on the epidemiology of plants, animals and human diseases, especially infections of the respiratory tract and allergy (Comtois, 1990; Angulo-Romero *et al.*, 1996). More than 80 genera of fungi have been associated with respiratory tract allergy (Karlsson-Borga *et al.*, 1989; Horner *et al.*, 1995). Most of the allergenic fungi are classified under Ascomycetes and Deuteromycetes with a few in Basidiomycetes (Kurup *et al.*, 2000). Some genera of airborne fungal spores such as *Alternaria*, *Aspergillus*, and *Cladosporium* are found throughout the world (Vijay *et al.*, 1999). Disease expression is affected by the degree of exposure. Repeated exposures to large concentrations of spores, mostly 1–5 μm in diameter, may cause severe symptoms of respiratory allergy (Barui Chandra and Chanda, 2000). Even though mould spores are present in the air in concentrations considerably greater than pollen grains, the frequency of allergic respiratory diseases due to moulds is usually much lower than pollen grains of vascular plants (D'Amato and Spieksma, 1995). The prevalence of respiratory allergy to fungi is estimated at 20 to 30% among atopic individuals and up to 6% in general population (Wuethrich, 1989). It is of the most importance that allergens, viable microbes, and other noxious agents that prevail in any particular environment, or are induced by changes in meteorologic conditions, be identified and studied. These measures will not only help to monitor the levels of these agents in the environment

but also aid clinicians in advising and treating patients as well as those at risk before they are exposed and sensitized (Zwick *et al.*, 1991).

Very little was reported on the impact of airborne fungi on monuments and rock surfaces in indoor and outdoor environments (Mandrioli and Zenotti Censoni, 1982; Urzi *et al.*, 2001). Most of the researches have dealt with fungi already settled on the rock surface and/or with "autochthonous mycoflora" (Saiz-Jimenez, 1995). Fungal airspora are implicated in the damage of food commodities and in the deterioration of organic materials and stored products (Pyrri and Kapsanaki-Gotsi, 2007).

Airborne fungi occur as single units, spores and occasionally as hyphal fragments, conidiophores, associated with inorganic particles or as "bioaerosol" (Comtois, 1990). Number and type of fungi vary with time of day, weather and seasonal fluctuation, condition of the surrounding areas, climatic conditions and with the presence of a local source of spores (Pepelnjak and Šegvić Klarić, 2003). The presence of plants, known to produce essential oils and aerosol, which showed sporistatic, fungistatic, and fungicidal activities (Pepelnjak and Šegvić Klarić, 2003), possibly reduces airspora viability. Increase of CO_2 concentration stimulates fungal sporulation suggesting that levels of the airspora correlate with air pollution (Klironomos *et al.*, 1997). In addition, increase of SO_2 can reduce airborne fungi concentration (Asan *et al.*, 2002).

The distribution of airborne fungi in Europe may differ from that in North America and Japan because of differences in climate, vegetation, and the structure of houses (Takahashi, 1997). Only a few studies have been carried out in Romania on airborne fungi. Ianovici and Faur (2003) used a volumetric method for the first time in a Romania study during their investigation of airborne fungi in the city of Timisoara. The examination of common

airborne fungi distribution in a particular region can be helpful in: identifying association between fungal sensitisation and clinical diagnosis; and clinical prevention of the seasonal allergic diseases (Wu *et al.*, 2000). Therefore, the purpose of our study was to determine variations in the composition and concentration of the aeromycoflora in Timisoara.

Materials and methods

Spore sampling and identification

Concentrations of airborne fungal spores were measured from 1 July to 31 July, 2007 using 7-day recording volumetric spore trap. The sampler was situated on the

Tab. 1. Monthly mean temperatures and monthly mean precipitations in Timisoara compared to multi-annual means

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Monthly mean temperatures 2007	4	6	9	13	18	23	24	23	12	11	4,2	0,1
Multi-annual means	-1,2	0,4	6	11	17	20	22	21	17	11	5,7	1,4
Monthly mean precipitations 2007	26	92	57	4	69	65	46	65	62	53	86	23
Multi-annual means	41	40	42	50	67	81	60	52	46	55	49	48

roof of the West University building in the centre of the city. The sampling site is above the adjacent buildings, and the circulation of the air currents was unobstructed in all directions. The flow rates were adjusted to 10/min and checked weekly. At sampling site, tape were changed weekly at 13.00 hours (local time). The tapes were cut into strips 48 mm long, each representing 24 h exposure, and were mounted on microscope slides in gelatin-glycerin and stained with basic fuchsin. Spores were counted in five fields along traverses 4 mm apart, using an optical microscope at $\times 400$ magnification. Traverses represented the deposits collected in alternate hours. The counts were used to calculate both daily and hourly mean concentrations of spores/m³. Fungal spores were classified by appearance and morphological characteristics (colour, size and shape) and identified, where possible, by comparison with published keys and monographs (Smith, 1990). Fungal identification on the basis of their morphological characteristics is difficult and inaccurate in many cases because almost identical and indistinct spores can exist within a family. In most cases it is possible to identify only to the genus level, sometimes even this level is unidentifiable. For that reason we use the term "type" to include different genera whose spores are morphologically very similar. Distinguishing the conidia of *Bipolaris* Shoemaker 1959, *Drechslera* S. Ito 1930, *Exserohilum* K.J. Leonard and Suggs 1974, *Sporidesmium* Link 1809, *Helminthosporium* Link 1809 using an optic microscope is extremely difficult; they were therefore considered as belonging to the *Drechslera*/*Helminthosporium* type (are listed as the Drech/Helminth). The *Fusarium*/*Leptosphaeria* group type included spores of *Melanomma* Nitschke ex Fuckel 1870 and *Phaeosphaeria* I. Miyake 1909. The genera *Aspergillus* and *Penicillium* are indistinguishable by their conidia and are listed as the

Asp-Pen type. The broken hyphae or conidiophores were recorded as hyphal fragments.

Sampling site

The climate that defines Timisoara city is the moderate temperate-continental climate sector in the plain areas, where there is interference of western and tropical circulations. It is characterised by frequent advections of maritime air and of tropical air laden with moisture: as a result, annual average temperature is below 11°C and rainfall amounts reach 600 mm (Mircov *et al.*, 2007). The meteorological data for Timisoara were obtained from the records of the Meteorological Station (after David *et al.*, 2008). The weather in Timisoara in July 2007 was

dry (Tab. 1). The July 2007 was characterised by deficient amount compared to multi-annual average. The warmest month during this period was July with an average temperature of 24°C.

Results and discussion

The occurrence of aeromycoflora in outdoor and indoor environments as well as the fluctuations in their numbers and composition is receiving increasingly attention within the framework of potential health hazards to both flora and fauna, including humans. Fungal spores that are transported by air currents cause many plant diseases, and a knowledge of their periodicity is of great value in terms of predicting plant epidemics. Airborne fungi have also been implicated as the causal factor in various human diseases and as elicitors of allergic and/or toxic responses. Great concern has been expressed about potential health hazards to humans, with a special focus on allergenic or toxigenic fungi and their association with air quality (Horner *et al.*, 1995).

The objectives of the present study are to record the airborne fungi in an urban environment during the dry season using modern aerobiological sampling technique. Measurements were made in July of 2007 because most reports showed that higher concentrations of airborne fungi were recorded during the summer.

32 fungi in outdoor air in the present study is reported. The airborne fungal genera are listed in Table 2 in descending order based on their frequencies, which were calculated as positive samples in the total number of samples. Airborne fungi were grouped into "major" and "minor" components depending upon their frequency of appearance and catch percentage in the air. *Cladosporium spp.*,

Drechslera/Helminthosporium spp., *Alternaria* spp. and *Epicoccum* spp. were included as major components. Minor components included 8 airborne fungal genera. Major components included most frequently encountered genera such as *Cladosporium* while minor components included less frequent and sporadic types.

Cladosporium, *Alternaria*, *Epicoccum* and *Drechslera/Helminthosporium* type spores were trapped most frequently (100% of days). Other stable components of aeromycota were *Peronospora*, *Aspergillus/Penicillium*, *Xilaria*, *Torula* and *Pithomyces* (>70%). *Helicoma*, *Ascobolus*, *Amphisphaeria*, *Asterosporium*, *Capronia*, *Kassariosphaeria*, *Massariosphaeria* and *Curvularia* were rare in samples, found only one or three times during sampling. These results are comparable to those from a previous study. Amongst the fungal spores group, *Acrodactys*, *Cercospora*, *Chaetosphaerella*, *Chaetoconis*, *Cerebella*, *Comoclathris*, *Fusariella*, *Lewia*,

Lophiostoma, *Splanchnonema* and *Puccinia* constituted the sporadic types in the airspora for 2005 (Ivanovici *et al.*, 2007). In 2006 other fungal spore types were identified in air samples such as *Agrocybe*, *Asperisporium*, *Botrytis*, *Caloplaca*, *Ceratopodium*, *Cercosporiella*, *Chaetomium*, *Comoclathris*, *Delitschia*, *Dendryphiella*, *Diatrype*, *Diplodia*, *Farlowiella*, *Fusichalara*, *Fusicladium*, *Ganoderma*, *Massaria*, *Mytilidion*, *Pestalotiopsis*, *Polythrincium*, *Sordaria*, *Sporormiella*, *Stachybotrys*, *Trichodelitschia*, *Ulocladium*, *Uromyces* (Ivanovici, 2008).

Cladosporium (7104.1 spores/m³), *Drechslera/Helminthosporium* (1378.7 spores/m³), *Fusarium/Leptosphaeria* (1378.7 spores/m³), *Alternaria* (1212.1 spores/m³) and *Torula* (1051.3 spores/m³) type spores were trapped in greatest abundance (>6% of total). These represent a group of taxa of cosmopolitan fungi that can exploit virtually any organic substrate (Ivanovici, 2008).

The genus *Cladosporium* predominated and it is the most important genus in terms of defining variations in the total count. In July 2007, the air concentration of *Cladosporium* spores did not exceed the level of 3000 spores/m³ (potential to trigger allergic reactions). This genus is also the major constituent in outdoor air and presents similar fluctuations in other European temperate regions (Infante *et al.*, 1999b; Stepalska *et al.*, 1999; Filipello Marchisio and Airaudi, 2001).

The genus *Alternaria* is also a major constituent of the fungal bioaerosol. Only one day (1 July) the concentration of *Alternaria* exceed the 100 spores/m³ of air established by Gravesen (1979) as the critical value for symptomatology. This result is in agreement with similar variations that have been reported from other temperate regions (Angulo-Romero *et al.*, 1996; Stepalska *et al.*, 1999; Infante *et al.*, 1999a; Filipello Marchisio and Airaudi, 2001; Şakiyan and Inceoglu, 2003).

The results in the present study are in agreement with other studies in Timisoara. During July 2001, the maximal value of the *Alternaria* was 339 spores/m³. *Cladosporium* conidia were in moderate quantity: 1158 spores/m³ (Faur *et al.*, 2005). The maximum incidence of *Cladosporium* spores was noticed in 21 July 2002 (2252 conidia/m³). The highest number of *Alternaria* spores was noticed in 2 July 2002 (233 conidia/m³). In July 2002, the air concentration of *Alternaria* spores exceeded the borderline level of 100 spores/m³ on 13 days (Ivanovici and Faur, 2003). In July 2003, in Timisoara, the maximum value of the total *Cladosporium* conidia number was recorded on July 5th - 584 conidia/m³. The maximal value of the *Alternaria* spores was recorded on July 2-82 conidia/m³. Abundant occurrence of *Cladosporium* spores was noticed from the beginning of July to the end of August (Ivanovici *et al.*, 2005). However, the concentrations never exceeded 3000 spores in previous years.

Cladosporium is the fungal genera most correlated with meteorological parameters. This may be attributed to size and nature of conidia (Fig. 2). *Cladosporium* produces

Tab. 2. Frequency and abundance of airborne fungi detected in Timisoara

Airborne fungi	Frequency (% of days)	Abundance (% of total spores)
<i>Alternaria</i>	100	7
<i>Cladosporium</i>	100	41
<i>Drech/Helminth</i> type	100	8
<i>Epicoccum</i>	100	4
<i>Asp-Pen</i> type	93.33	5.81
<i>Peronospora</i>	93.33	3
<i>Xilaria</i>	90	5
<i>Torula</i>	83.33	6
<i>Pithomyces</i>	80	0.1
<i>Spondylocladiella</i>	73.33	0.02
<i>Periconia</i>	73.33	1.1
<i>Stemphylium</i>	70	1
<i>Tilletia</i>	63.33	1
<i>Piricauda</i>	60	1
<i>Massarina</i>	53.33	1
<i>Bispora</i>	40	0.3
<i>Microsporium</i>	40	0.3
<i>Nigrospora</i>	36.66	0.2
<i>Pseudocercospora</i>	36.66	2
<i>Paraphaeosphaeria</i>	33.33	1.1
<i>Neobendersonia</i>	30	0.2
<i>Monodictys</i>	23.33	0.1
<i>Oidium</i>	23.33	0.3
<i>Helicoma</i>	20	0.2
<i>Ascobolus</i>	16.6	2
<i>Fusarium/Leptosphaeria</i> type	8.33	8
<i>Amphisphaeria</i>	6.66	0.02
<i>Asterosporium</i>	6.66	0.01
<i>Capronia</i>	6.66	0.1
<i>Kassariosphaeria</i>	6.66	0.1
<i>Massariosphaeria</i>	6.66	0.03
<i>Curvularia</i>	3.33	0.01

dry conidia in chains easily carried through air. Therefore dispersion of *Cladosporium* spores is more influenced by meteorological parameters than *Alternaria* spores (Awad, 2005).

Daily mean concentrations reflect the approximate numbers of spores found during the day. Half of all episodes of increased pulmonary complaints by asthmatic patients coincided with a total fungal spores peak of more than 500 spores/m³ of air. The maximum spore concentration was observed on July 5 (1794 spores/m³). In July 2007, the air concentration of airspores exceeded the bor-

spores are found in greatest abundance in the atmosphere characterised by low humidity, generally during warmer afternoon hours (Levetin, 1995).

With regard to spore species, viability can differ considerably, for example, 80% of *Alternaria*, and 20–30% of *Cladosporium* spores are still viable after a certain period (Govi, 1992). High concentrations and long lasting presence of allergenic fungal spores in the air may cause and intensify clinical symptoms in people suffering from sensitivity and extend the period of presence of allergens in the atmosphere after the flowering season of vascular plants

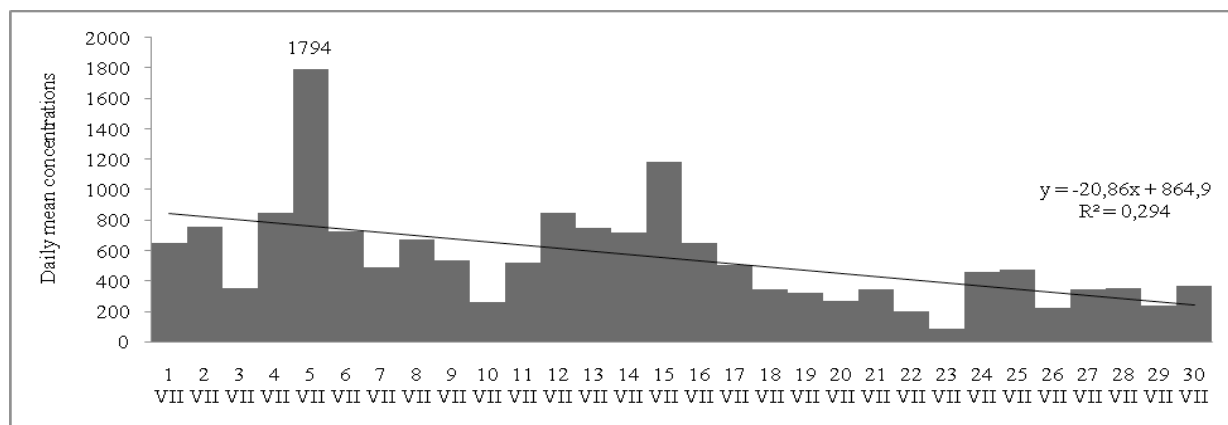


Fig. 1. Totals of spores in the air recorded in Timisoara in July 2007

derline level of 500 spores/m³ on only 15 days (Fig 1).

Liberation of *Basidiomycetes* and *Ascomycetes* spores is favoured by high air humidity and rain while *Deuteromycetes* spores, such as those of *Cladosporium* and *Alternaria*, are liberated mechanically by the action of wind on leaves, sometimes aided by water rupture mechanisms activated by drying. Spore dispersal is therefore generally favoured by low relative humidity and increasing temperature. The occurrence of such conditions at different times in different geographical regions may help to explain differences in the observed periodicities (Lacey, 1972; Lacey, 1996).

The distinction between dry-air spores and wet-weather air spores is well known. Dry-air spores include *Cladosporium*, *Alternaria*, *Epicoccum*, *Drechslera*, *Pithomyces* and *Curvularia* (Katial *et al.*, 1997). Members of dry-air

(Stepalska *et al.*, 1999).

Our results show the presence of a large number of fungal spore types. The monitoring of fungal spores in Timisoara revealed the summer as the most favourable season for *Cladosporium* occurrence. Many surveys of the occurrence of *Cladosporium* spores in different regions of the world clearly show their dominance in comparison with other spores (Mitakakis *et al.*, 1997). The present study will contribute to our knowledge of airborne spores in Romania.

Conclusions

Fungal agents are responsible for a variety of respiratory diseases in humans, plants and animals. Aeromycoflora



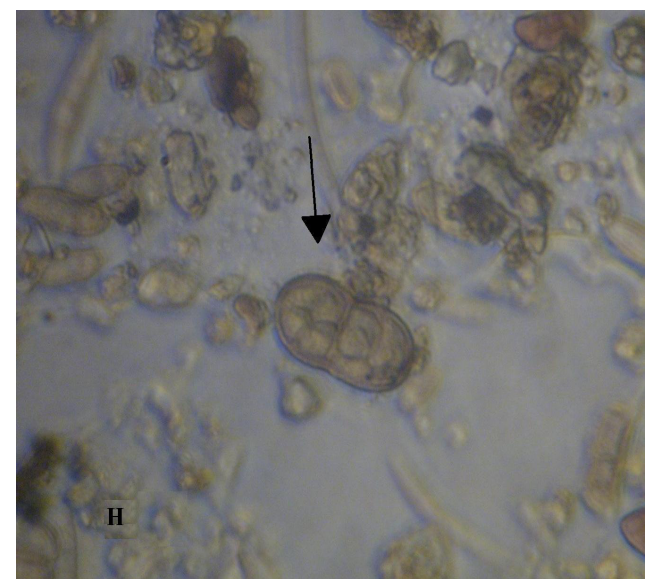
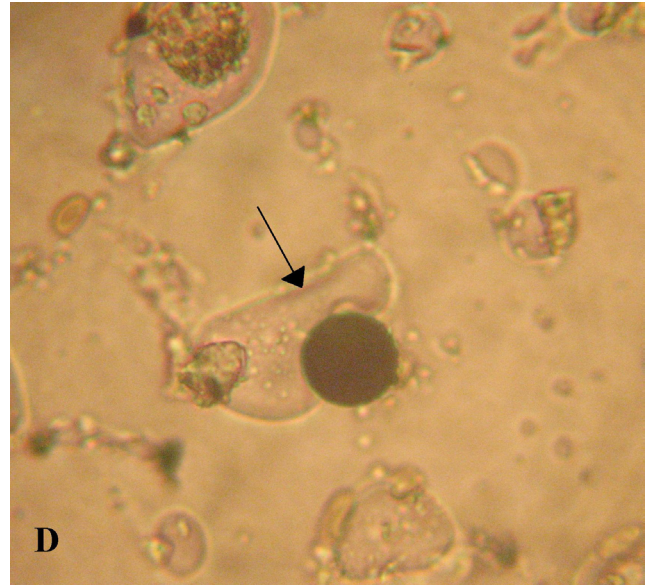
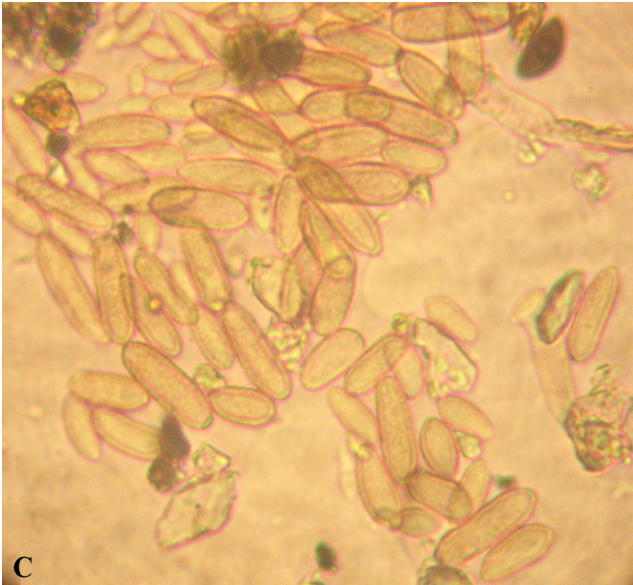




Fig. 2. Airborne fungi detected in Timisoara

(A- *Alternaria* type; B-*Drechslera/Helminthosporium* type; C-*Cladosporium* type; D-*Nigrospora* type; E-*Torula* type; F-*Peronospora* type; G-*Pithomyces* type; H-*Stemphylium* type; I-*Bispora* type; J-*Capronia* type; K-*Neobendersonia* type; L-*Amphisphaeria* type; M-*Fusarium/Leptosphaeria* type; N-*Epicoccum* type)

of outdoor environment in Timisoara have been investigated. Spores of 32 fungal types were recorded. The spores of *Deuteromycetes* were the largest contributors of the total airborne fungal spores. *Cladosporium* was the most prevalent fungal spore type during July 2007 in the air samples from the Timisoara site. Monitoring of airborne fungi can be helpful in prevention of fungal allergic diseases.

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