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Variation in Airborne Fungal Spore Concentrations in Four Different Microclimate Regions in Romania

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Abstract

Airborne fungal counts and types were examined in four regions from Romania. The aim of our study was to determine the concentrations of *Alternaria*, *Cladosporium*, *Drechslera*-type, *Epicoccum* and *Nigrospora* atmospheric fungal spores in urban areas: Braşov, Bucharest, Cluj-Napoca and Timişoara. Other objective of this study was to examine the relationships between atmospheric fungal spores and pollen. Few studies have confirmed the high frequency and concentrations for some of these spore types in Romania. The sampling sites differed in habitat characteristics, such as vegetation and microclimate. Airborne spores' sampling was carried out using the Lanzoni volumetric sampler and during August 2008. The totals of airborne spores recorded in this study show a considerable variation. *Cladosporium* and *Alternaria* have been identified as the most abundant and frequent fungal aeroallergens in Timişoara and Bucharest. *Drechslera*-type, *Epicoccum*, and *Nigrospora* had very low values of spore count in all sites. Observations show that seven types of pollen appear regularly and few of these are in great abundance. Fungi are present in the air in concentrations considerably in excess of that of pollen grains. *Ambrosia artemisiifolia* is the most important pollen allergen in all of the sampled areas discussed here, especially in western country (Timişoara).

Keywords: *Alternaria*, *Ambrosia artemisiifolia*, *Cladosporium*, *Drechslera*-type, *Epicoccum, Nigrospora*

Introduction

An important portion of atmospheric aerosol is of biological origin (Adhikari *et al*., 2006). Fungi represent a major clade in the tree of life found in diverse ecological niche (Hawksworth, 2001). Fungal spores are dispersed into the environment due to air currents, water, insects, animals and people. Fungi whose spores are spread by the air are termed as anemophilous or anemochor (Mezzari *et al*., 2002). Fungi are principally dispersed as sexual spores or asexual conidia, which are common components of the aeroplankton. Airborne fungal spores spores originally derive from plant, animal, and soil sources (Lacey, 1981). Sporulation and spore dispersal depend on biological, climatic and physical processes (Burch and Levetin, 2002; De-Wei and Kendrick, 1996). The spores or conidia are quite variable in size and shape. The spores or conidia range from 3-200 µm, most of these are about 10 µm in diameter (Tilak and Pande, 2005). Chakraborty *et al*. (2001) generally noticed that the smaller spores were commonly found at the high sampling level and large spores were more common at ground level.

It is well known that fungi have a global distribution. The greatest number of airborne spores was found to be in temperate and tropical regions and the least in desert (Lacey, 1981). Simeray *et al*. (1993) and Kasprzyk and Worek (2006) observed that higher values were found in rural environments than in urban areas. The airborne fungal spores have many implications for the spread of man and animal diseases (Burge, 1985; Lacey and Crook 1988), for foodstuffs and other materials, for industrial processes and for deterioration of stored materials (Lacey, 1981). Some of these spores are also important in plant pathology (Şakiyan and Inceoğlu, 2003). A large proportion of the variability is due to the proximity and abundance of the source of fungal spore, the ecological and geographical characteristics of each region (Oliveira *et al*., 2010; Pepeljnjak and Šegvić, 2003).

Scarcity or absence of suitable substrate, sufficient moisture, vegetational density and diversity affect the occurrence and composition of atmospheric fungal spores. Temperature and relative humidity have a pronounced effect on spore productivity (Damialis and Gioulekas, 2006; Grinn-Gofroń and Mika, 2008). Atmospheric fungal spores at different sites were examined in relation to allergic symptoms and/or meteorological parameters (Rizzi-Longo *et al*., 2009). More than 100 genera of fungi belonging to three distinct taxonomic groups: the Ascomycota, Basidiomycota and the Deuteromycota are currently recognized as sources of allergens (Green *et al*., 2006). The most important fungal allergen sources are found among the fungi from the class of the *Deuteromycetes* (Ianovici *et al*., 2011). The fungal spores of *Alternaria* (Corsico *et al*., 1998; Downs *et al*., 2001; Kauffman *et al*., 1995; Mari *et al*., 2003) and *Cladosporium* (Hasnain *et al*., 1998; Resano *et al*., 1998; D'Amato *et al*., 2007) are considered as the most commonly implicated to respiratory allergy symptoms worldwide. As Gioulekas *et al*. (2004) have reviewed, in Europe and the USA sensitization prevalence ranges from 0.7-24.1% (Gioulekas *et al*., 2004).

A study performed on asthmatic patients in the Netherlands showed that 7% of patients were sensitized to fungal spores (Kurup *et al*., 2000). Studies based on skin tests suggest that at least 3-10% of adults and children worldwide are affected by fungal allergy (Bush and Portnoy, 2001). Cross-reacting proteins have been demonstrated among different fungal genera, species and strains of Deuteromycetes (Verma *et al*., 1995).

The five allergenic spores chosen for this analysis were *Alternaria*, *Cladosporium*, *Drechslera*-type, *Epicoccum* and *Nigrospora*. In general, those fungi are the same as those most frequently found in other European cities through quantitative studies. Our study had two objectives: a) assess the changes in fungal spores concentrations from four major cities in Romania; b) assess the ratio beetween the airborne fungal spores and pollen for establishing who is the city with the higher allergenic risk regarding these aeroallergens in summer.

Materials and methods

Spores' monitoring sites

In Fig. 1 and Tab. 1, the monitoring sites of fungal spores are shown.

Fig.1. Map of Romania showing the monitoring sites

Spore sampling and identification

Fungal spores were sampled by using 7-day Lanzoni volumetric trap and identification was based on their morphological structures. Samplers were located on buildings approximately 15 m above ground level. These traps have an autonomy of 7 days and collect spores continuously with a given absorption flux, enabling daily and even bi-hourly concentration data to be obtained. All slides identified in this study are kept at West University, Department of Biology in Timişoara (Romania). All spore counts were obtained daily at our institution during August 2008.

The traps worked temporarily for trial "Efficacy and safety study of the antihistamine V0114CP 2.5mg in the treatment of seasonal allergic rhinitis. Randomised, double-blind, three arm parallel group study including placebo and active control arm (desloratadine 5mg)" (Institut de Recherche *Pierre Fabre* funded in Romania). The air suction rate of the volumetric air sampler was maintained at 10 L/min (in average, man inhales approximately 10 $m^3$of air per day). The counting of spores was included in the counting procedure of pollen grains. The trapping surface was removed weekly and dissected for light microscopic examination. Slides were covered with glycerine jelly mixed with basic fuchsin.

The specific fungal spores were counted with ×400 magnification. Spore counts were conducted at 2-h intervals and total daily counts were converted to numbers per cubic metre of air (Ianovici *et al*., 2013).

Statistical analysis

Variance between atmospheric fungal spores distributions was analyzed by One-way ANOVA between groups, Levene,s test for homogenety of variance (based on means) and Welch F test in the case of unequal variances. In one-way ANOVA we calculate the F-statistic as the ratio MS between=MS within. A value of P <.0.05 was considered significant.

We calculate the observed F-statistic and compare it to F-critical. Analysis of significant differences between AFS was followed by Tukey HSD (honest significant difference) post-hoc test for each allergenic assay (Oehlert, 2010).

Results

Data provided by the temporary stations were collected in 2008. Selected fungal spore types were photographed and can be seen in Fig. 2. Monthly concentrations (box plots) are shown in Fig. 3-7.

Tab. 1. Features of the monitoring sites

	Altitude (m)	Location	Mean annual rainfall (mm)	Average annual temperature (°C)
Braşov	650	45°39'N 25°36'E	744	7.6
Bucharest	55.8-91.5	44°25'N 26°06'E	595	11
Cluj-Napoca	335	46°46'N 23°36'E	557	9.3
Timişoara	88	45°45'N 21°13'E	592	12.3

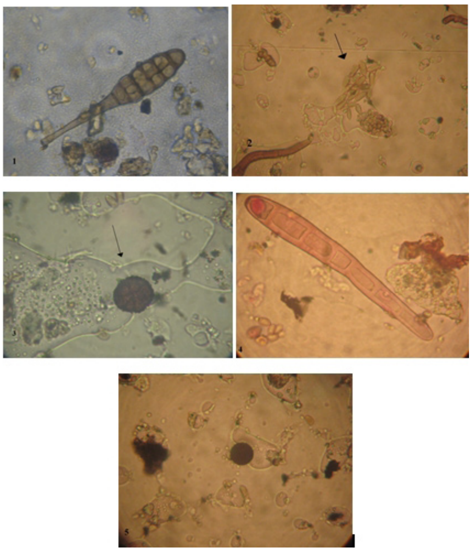


Fig. 2. Airborne fungi investigated in outdoor environment

1-*Alternaria Nees* 1817, 2 - *Cladosporium* Link 1816, 3-*Epicoccum* Link 1815, 4-*Drechslera* S. Ito 1930, 5 *Nigrospora* Zimm. 1902

Alternaria type spores were trapped most frequently. The highest concentration of *Alternaria* spores, equal to 95 spores /m^3/24 h was noted in Timisoara for 26 August. The highest level of conidia emission was recorded in Timişoara with 1.115 spores /m^3. Alternaria values in Braşov and Cluj-Napoca were lower than those found in Timişoara and Bucharest (Fig. 3). The study of variations at the four sites indicated significant differences (F = 5.5852, P=0.0013). Concentrations are not homogeneous. Tukey's pairwise comparisons for Alternaria spores indicate significant differences for the following stations: Bucharest-Cluj, Timişoara-Cluj, Timişoara-Braşov. Hasnain et al. (1998) suggested that when the *Alternaria* count reaches 50 spores/m^3 or above, sensitized patients develop symptoms. Only few days (10 days in Timişoara, 8 days in Bucharest, one day in Cluj and Braşov) the concentration of *Alternaria* exceeded the threshold concentration.

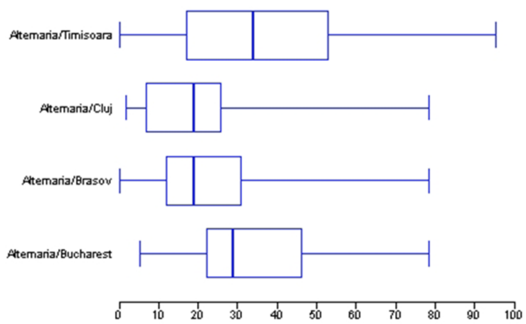


Fig. 3. Boxplot for *Alternaria* airborne spores

In our study *Cladosporium* airspores were found to be present regularly. The highest level of conidia emission was recorded in Timişoara with 22.989 spores/m^3. The highest concentration of *Cladosporium* spores, equal to 1.859 spores/m^3/24 h was noted in Timişoara for 26 August. Fungal spore concentrations for Timisoara are significantly different from all other sampling sites (Fig. 4).

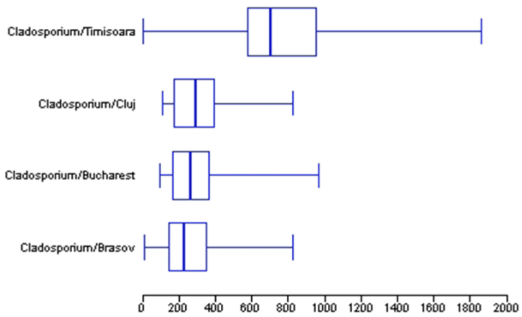


Fig. 4. Boxplot for *Cladosporium* airborne spores

Differences between towns concerned total spore counts and numbers of peaks. However, the concentrations never exceeded 2000 spores/m^3 of air established by Targonsky *et al*. (1995) as the critical value for symptomatology.

Sporadic behavior was observed in the case of *Drechslera* spores. The highest level of *Drechslera* conidia emission was recorded in Timişoara with 40 spores/m^3, the lowest value being recorded in Braşov with 8 spores /m^3 (Fig. 5). The highest concentration of *Drechslera* spores, equal to 8 spores/m^3/24 h was noted in Timişoara for 15 August. Fungal spore concentrations of Timisoara are significantly different from Brasov sampling site.

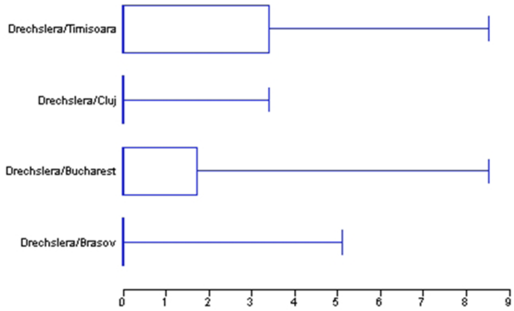


Fig. 5. Boxplots for *Drechslera*-type airborne spores

Epicoccum spore concentrations are low. The behaviour of *Epicoccum* was irregular, with spores concentrations fluctuating considerably (Fig. 6). The highest level of *Epicoccum* conidia emission was recorded in Cluj with 197, the lowest value being recorded in Braşov with 108 spores /m^3. The highest concentration of *Epicoccum* spores, equal to 83 spores /m^3/24 h was noted in Cluj for 12 August. The study of variations at the four sites indicated no significant differences (F= 0.4952; P=0.6856).

Nigrospora spore concentrations are low (Fig. 7). The highest level of *Nigrospora* conidia emission was recorded in Braşov with 127 spores /m^3, the lowest value being recorded in Cluj with 78 spores /m^3. The highest concentration of *Nigrospora* spores, equal to 18 spores /m^3/24 h was noted in Braşov for 14 August. The study of variations at the four sites indicated significant differences (F = 4.1803; P = 0.00746856) but Tukey's pairwise comparisons indicate no significant differences for the sites.

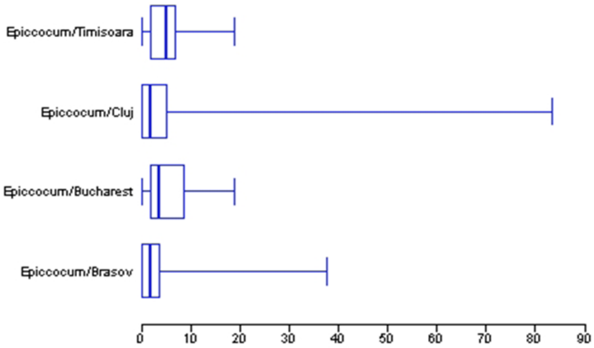


Fig. 6. Boxplot for *Epicoccum* airborne spores

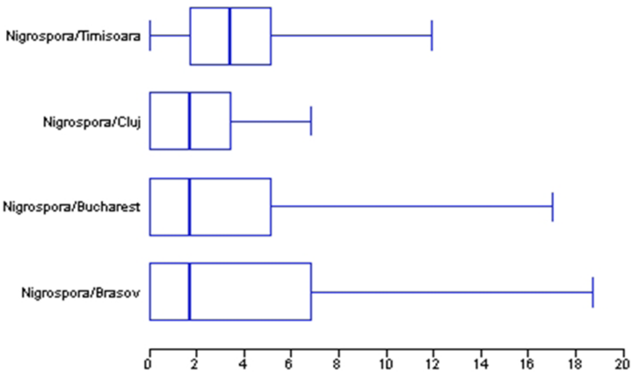
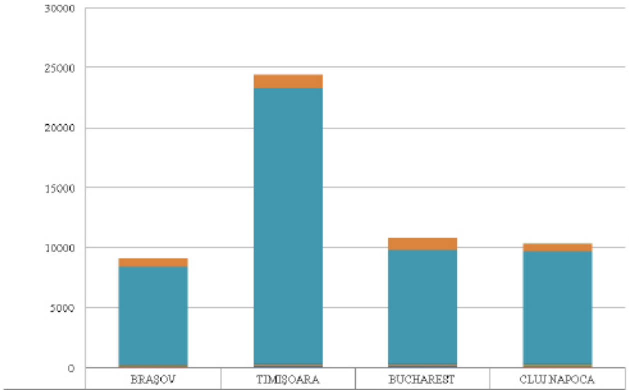


Fig. 7. Boxplot for *Nigrospora* airborne spores

Other objective of this study was to examine the relationships between airborne fungi and pollen. All pollen counts were obtained daily at our institution during August 2008. A total of 7 pollen types were recorded during this study (*Poaceae*, *Urtica*, *Rumex*, *Plantago*, *Ambrosia*, *Artemisia*, *Chenopodiaceae*/*Amaranthaceae*). Non-arboreal pollen and fungal spores occurred in the air simultaneously. Fig. 8 and 9 shows the totals of fungal spores and pollen counts corresponding for each sampling site.

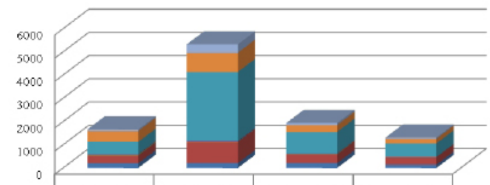
Ambrosia pollen is considered to be highly allergenic even in low concentrations. In these cities, the number of days exceeding the threshold value (20 g/m^3; Jäger, 1998) is high: 29 for Timişoara, 25 for Bucharest, 12 for Braşov, and 19 for Cluj Napoca.

High concentrations of fungal spores, at times exceeding those of pollen grains. The ratio of spores/pollen (considering only taxa mentioned) had the following values: 5.5 for Brasov, 4.59 for Timisoara, 5.6 for Bucharest and 7.87 for Cluj Napoca.



	BRAŞOV	TIMIŞOARA	BUCHAREST	CLUJ NAPOCA
Alternaria	690.2	1115.2	1011.5	606.9
Cladosporium	8200.8	22989.1	9516.6	9389.1
Drechslera	8.5	40.8	30.6	11.9
Epicoccum	108.8	170	154.7	197.2
Pithomyces	11.9	44.2	37.4	61.2
Nigrospora	127.5	100.3	115.6	78.2

Fig. 8. Totals of fungal spores counts for sampling sites, August 2008



	BRAŞOV	TIMIŞOARA	BUCHAREST	CLUJ NAPOCA
Chenopodiaceae/Amaranthaceae	63	361	86	49
Artemisia	473	830	295	204
Ambrosia	527	2965	924	587
Plantago	30	41	24	3
Rumex	29	9	21	0
Urtica	339	912	384	327
Poaceae	199	205	204	144

Fig. 9. Totals pollen counts for sampling sites

Discussions

The results in the present study are in agreement with the first comparative study for several Romanian cities (Ianovici *et al.*, 2011). Studies on the presence of spores in the atmosphere of Romania were started by Ianovici and Faur (2003). The other studies on airborne fungi in Romania (Ianovici *et al.*, 2004; Ianovici *et al.*, 2007; Ianovici and Dumbravă, 2008a; Ianovici and Dumbravă, 2008b; Ianovici, 2008; Ianovici *et al.*, 2008; Ianovici and Tudorică, 2009) has focused to determine the diversity of these particles, abundance, concentration and period of the presence of selected fungal spore types in the atmosphere. The monitoring of fungal spores revealed the summer as the most favourable season for *Cladosporium* and *Alternaria occurrence*. The spores of *Cladosporium* and *Alternaria* are the most abundant fungal allergens and have great interregional variations.

In Timişoara and Bucharest, concentrations are consistently higher than in Cluj Napoca and Braşov. The climate, which defines Timisoara and Bucharest is temperate continental moderate with hot dry summers and cold winters. Braşov has a temperate continental climate with cold and relatively wet weather in the mountains and Cluj-Napoca's climate is influenced by the city's proximity to the Apuseni Mountains.

All other common spore types (*Drechslera*-type, *Epicoccum* and *Nigrospora*) identified in all sites were recorded sporadically. Only in the concentrations of *Epicoccum* were no significant differences between the sampling sites. Airborne spore concentrations greatly differ, a fact due to the variety of the vegetation existing in each region (Ballero *et al*., 1992; Eseamuzie *et al*., 2000). Awad (2005) reported that type of vegetation in the sampling area can affect the concentration and type of fungal taxa in the atmosphere. To Braşov, only *Nigrospora* spore concentrations are slightly higher. Here temperatures are lower and humidity higher.

Stacks of straw stored for livestock could serve as a local source of spores for *Nigrospora* (Adhikari *et al*., 2004). The dispersal and transport of airborne fungal spores are influenced by several meteorological factors, such as wind, rainfall and air temperature components, atmospheric pressure, solar radiation, and relative humidity (Angulo-Romero *et al*., 1999; Katial *et al*., 1997; Mitakakis *et al*., 2001; Stennett and Beggs, 2004). The variation in aeroallergen concentrations and types depends on the agricultural practices and changes in land management (Kasprzyk and Konopińska, 2006). Fungal spore concentrations could be related to the maturing and senescence of tree foliage, grasses and to some extent local crops (Stepalska and Wołek, 2005).

In Timişoara, the probability of becoming exposed to high concentrations of allergenic pollen and fungal spores at the same time was high, especially in August, when the highest concentrations of *Ambrosia*, *Alternaria* and *Cladosporium* were noted. Such comparisons were made for the summer season in Timişoara (Ianovici *et al*., 2004; Ianovici *et al*., 2007). A similar situation is in Bucharest. Attention will be paid to the *Ambrosia* pollen in all four cities, but especially in Timişoara and Bucharest. Uncontrolled spread of this invasive plants all over the country is a serious threat to human health (Ianovici, 2009; Ianovici, 2011; Ianovici *et al*., 2013).

Fungal spores have long been identified as one of the sources of outdoor or indoor allergies (Platts-Mills *et al*., 1996). Many authors found close associations between the ambient airborne fungi and pollen in relation to respiratory allergies (Adhikari *et al*., 2004; Caiola *et al*., 2002; D'Amato *et al*., 2007; Hasnain *et al*., 2004; Kasprzyk, 2008; Myszkowska *et al*., 2002; Nitiu and Mallo, 2011; Smith *et al*., 2007). Hjelmroos - Koski *et al*. (2006) suggest that taxa pollen and fungal spores should be grouped and used to describe daily exposures for sufferers. Pollen and fungal spores causing allergy are quite variable in different climatic regions which makes it very important to identify respiratory allergies causing species from every region and prepare extracts from them for diagnosis and immunotherapy (Faur *et al*., 2001; Singh and Kumar, 2004).

Urbanization and westernized lifestyle are linked to the rising incidence of respiratory allergy in most industrialized countries (Heinrich and Wichmann, 2004; Nicolaou *et al*., 2005). Molecular studies with reference with the cross reactive allergens are important for the proper diagnosis and treatment (Singh and Kumar, 2004).

In the recent years, air quality has become an important environmental health issue which in part is related to allergen bioparticles contaminations (Abu-Dieyeh *et al*., 2010; Chadeganipour *et al*., 2010). One of the factors significantly influencing the sensitizing to allergens is the allergen's concentration and possible cross-reactions between aeroallergens.

This study was based on short survey; additional studies over a longer period are needed to provide a more profound insight into the relationship between spores and pollen content in the outdoor environment and allergic manifestations affecting patients. Testing of allergic patients will have to include *Cladosporium* and *Alternaria* spores, especially in summer. It may be necessary to investigate the cumulative effect of pollen and fungal spores on the severity of symptoms in people with respiratory allergies (Ambrosia + Cladosporium, Ambrosia + Alternaria, Poaceae + Cladosporium, Poaceae + Alternaria). This is the first study that provides comparative data for the four major cities of Romania on the concentrations of spores and pollen grains at the atmosphere.

Conclusions

The most abundant airborne spores are *Cladosporium* and *Alternaria* in Timişoara and Bucharest. *Epicoccum*, *Nigrospora* and *Drechslera* - type spores presented a sporadic distribution throughout the study. The ratio of fungal spores and pollen has values between 4.59 and 7.87 Timisoara and Bucharest have the higher allergenic risk regarding *Cladosporium* and *Alternaria* aeroallergens. We have found high concentrations of pollen from *Ambrosia* for all four cities in the same period, especially in Timisoara. A continuous monitoring of airborne bioparticles diversity is recommended.

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