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ORIGINALPAPER

Atmospheric concentrations of selected allergenic fungal spores in relation to some meteorological factors, in Timişoara (Romania)

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Abstract Present investigation was undertaken to study the dynamics of relationships between atmospheric fungal spores and meteorological factors in western Romania. The airborne spore sampling was carried out by employing volumetric sampling. A total of nine meteorological parameters were selected for this investigation. During 2008–2010, it was found the same pattern of behaviour in the atmosphere for selected spore types (*Alternaria*, *Cladosporium*, *Pithomyces*, *Epicoccum* and *Torula*). The spores occurred in the air throughout the whole year, but maximum concentrations were reached in summer. *Cladosporium* and *Alternaria* peak levels were observed in June. *Epicoccum* peak value was found in September. The relationships between airborne spore concentrations and environmental factors were assessed using the analysis of Spearman's rank correlations and multiple linear regressions. Spearman's rank correlation analysis revealed that maximum, minimum and mean temperature, and number of sunshine hours were strongly ($p < 0.01$) and directly proportional to the concentration of all analysed fungal spores. Negative and significant correlations were with daily mean relative humidity. The variance explained percentage by regression analyses varied between 30.6 and 39.6 % for *Alternaria* and *Cladosporium* airborne spores. Statistical methods used in this study are complementary and confirmed stable dependence of *Alternaria* and *Cladosporium* spore concentrations on meteorological factors. The climate change parameters either increased temperatures, changed precipitation regimes or a combination of both affected allergenic fungal spore concentrations in western Romania. This study demonstrates the need for investigations throughout the year, from month to month, regarding the correct interpretation of airborne spore relationships with meteorological parameters.

Keywords Biomonitoring · Climate change · Airborne mycoflora

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1 Introduction

In Romania throughout the 50-year period (1961–2013), increasing and significant trends with regard to the air temperature and number of sunshine hours have been observed (Marin et al. 2014). Analysis of climatological data for the twentieth century (1901–2000) showed that the average annual temperature in Romania increased by 0.3 °C (Cosmulescu et al. 2010). Thermal growth accentuated during the last decades, beginning with the second half of the twentieth century, reaching values 0.8–1 °C on

extended areas in Romania. Concerning the precipitations, a slight reduction in the amount of rainfall at the national scale of a 50 mm annually was observed during the period 1901–2007 (Rusu and Moraru 2015). The annual mean temperature increase is projected to be 4–4.5 °C. Annual mean precipitation is expected to increase by up to 10 % mainly in winter, while there would be reductions in summer precipitation in several areas (DG AGRI 2008). There are several studies that estimated the potential impact of climate change on the Romanian agriculture (Cuculeanu et al. 1999; Mateescu and Alexandru 2010). The most affected resource sectors appear to be maize crops in the southern part of the country, forest species growing in the plains and hilly zones, and water resources where demands could exceed their availability (Cuculeanu et al. 2002). It is expected that these climatic changes will influence the diversity of plants and plant communities in different areas of Romania (Sârbu et al. 2014). In this context, to this day there is no study in Romania on dynamics of atmospheric concentrations of fungal spores in relation to meteorological factors, although these relationships have been well studied worldwide (Stępalska and Wołek 2005; Levetin and Dorsey 2006; Grinn-Gofroń et al. 2011; Almaguer et al. 2014; Damialis et al. 2015a; Grinn-Gofroń et al. 2015; Sadys et al. 2015a, b; Ščevková et al. 2015; Vélez-Pereira et al. 2015). Previous reports available for several locations, i.e. Brasov, Bucharest, Cluj-Napoca, Craiova and Timişoara, were produced upon short-term investigations and thus disabling to build a "bigger picture" with regard to fungal spore dynamics in Romania (Ianovici et al. 2011, 2013a).

The spores of *Cladosporium*, *Alternaria*, *Epicoccum*, *Pithomyces* and *Torula* are some of the most abundant allergens worldwide, and the prevalence of allergical reactions to them shows great interregional variations (Kasprzyk 2008; Rizzi-Longo et al. 2009). An estimated 3–10 % of the world's population is allergic to fungal spores. In European and American populations, the prevalence of sensitization to fungal spores ranges from 0.7 to 24.1 % (Calabria et al. 2007). Sensitization to *Alternaria* and *Cladosporium* has been reported to be 3–30 % in European countries (Bavbek et al. 2006; Celenk et al. 2007). Allergy to *Epicoccum* affects between 5 and 15.4 % of the population in Europe and cross-reacts with other fungi such as *Alternaria and Cladosporium* (Bisht et al. 2004). *Torula* is commonly associated with type I allergies. *Pithomyces* spores can potentially produce mycotoxins such as cyclodepsipeptides, sporidesmolides and sporidesmin (Kruczek 2014). On the other hand, it is rather important to know about the fungal presence in a particular area, because crop pathogens can be identified to prevent epidemics in agriculture, in the early detection of plant infections, thus allowing a more efficient use of pesticides (Grinn-Gofroń 2009; Astray et al. 2010). Atmospheric fungal spore biomonitoring seems to be essential for matters of both medical and plant pathology, especially in the context of climate change (Konopińska 2004).

The objectives of this work were to compare three successive seasons (2008–2010) of atmospheric fungal spore concentrations and to investigate the influence of environmental conditions concerning their dynamics, for each year separately. *Alternaria* (Nees), *Cladosporium* (Link), *Epiccocum* (Link), *Pithomyces* (Berk. & Broome) and *Torula* (Pers.) have been selected for this investigation. The five spore types chosen for analysis belong to the group of anamorphic fungi because conidial fungi dominate in the spectrum of airborne spores. The five spore types selected in this study have previously been reported as allergenic.

2 Materials and methods

Timişoara is situated in the western region of Romania at 88 m above sea level (45°45′N 21°13′E). The average annual temperature is approximately 12.3 °C, and the annual rainfall is 592 mm (Ianovici et al. 2013a). Fungal spores were sampled by using 7-day Lanzoni volumetric trap. The trap was placed on the roof of West University near the centre of the Timişoara city, at height of about 20 m. The sampling site is above the adjacent buildings, and the circulation of the air currents was unobstructed in all directions. This area has a high urbanization. The air suction rate of the volumetric air sampler was maintained at 10 L min^{-1}. Sampler drums were changed weekly. The spores were counted in the counting procedure of pollen grains. The qualitative and quantitative compositions of the samples were determined under a light microscope. The spores were counted at a magnification of 400×. Identification was based on their morphological structures. The spores were observed on the surface of four horizontal bands. Spore counts were done at 2-h intervals, and total daily counts were

converted to numbers per cubic metre of air (AFS m^{-3}) according to the correction factor specific to the microscope and magnification used. All slides examined in this study are kept at West University, Department of Biology, in Timisoara (Romania). I have considered all days when the fungal spores were present in the air.

The number of days above the allergenic threshold value was selected on the basis of literature data (Targonski et al. 1995; Rapiejko et al. 2004).

The distribution of data was tested using the Kolmogorov–Smirnov test. The data did not fit a normal distribution curve. Before analysis, the spore concentrations were transformed (logarithmic transformation) and the normality was verified. Variance between atmospheric fungal spore distributions was analysed by one-way ANOVA between groups. A value of $p < 0.05$ was considered significant. Spearman's correlation test was performed in order to identify the major variables likely to influence the dynamic of the atmospheric fungal spores. The correlation coefficients between atmospheric fungal spore concentration (expressed as AFS m^{-3}) and selected daily meteorological parameters (mean daily average temperature expressed in °C; maximum temperature in °C; minimum temperature in °C; relative humidity in %; mean wind speed in m s^{-1}; daily maximum wind speed in m s^{-1}, atmospheric pressure in mbar; sunshine hours in h; quantities of precipitations in L m^{-2}) were analysed. First was analysed the effect of meteorological factors on fungal spore concentrations taking the season as a whole, over a full year (FY). Subsequently were determined the correlations between spore concentrations and the meteorological parameters on the day of measurement (sampling day—SD). After that have been chosen the meteorological parameters in the day before the appearance of fungal spores (previous day of sampling—PD) (Table 2). The multiple regression analysis was performed in order to determine how much of total variance in atmospheric fungal spore concentrations can be explained by meteorological variables. Multiple linear regression is a commonly used method in environmental sciences. The aim was not to develop predictive model, but rather to give information on types of factors that might be controlling dispersion of each spore types. The statistical relationship between spore concentration and meteorological factors was established using the SPSS software package.

The meteorological data for Timisoara were obtained from the records of the National Meteorological Administration. In Timişoara, the lowest temperatures were recorded in the first months of the year, with the maximum temperatures being reached in summer (Table 1). In this study, spring lasts from March to May, summer from June to August, autumn from September to November and winter from December to February.

3 Results

3.1 Analysis of selected fungal spore concentrations

The concentration analysis for 2008–2010 highlights large differences between different types of spores. These disparities relate to the monthly and annual levels of airborne spores. The monthly total concentrations of the five selected spore types are shown in Fig. 1. The cumulative annual concentrations of the five selected allergenic spores are shown in Fig. 2.

Cladosporium spores were found to be present regularly. The cumulative level of the spore concentration for *Cladosporium* was very high. The study of variations indicated significant differences ($F = 21.32$; $p < 0.001$). The highest level of conidia emission was recorded in 2008 with 175,750 AFS m^{-3}. In 2008, the concentration of *Cladosporium* spores exceeded the level of 2000 AFS m^{-3} (potential to trigger allergic reactions) on 14 days. Exceeding the threshold value was recorded in 10 days in 2009 but not at all in 2010. The highest monthly values were detected in June and July (Fig. 1). The relative humidity increased over 77 % in May and June has acted limiting the concentrations of *Cladosporium* spores in 2010. In fact, the annual concentration halved in 2010 compared to 2009. The pattern from 2008 to 2009 shows another quantitative peak in September. The pattern was not repeated in 2010. In this year, the average relative humidity of September was 80 %.

The seasonal pattern of *Alternaria*, *Epicoccum*, *Pithomyces* and *Torula* airborne spore concentrations was similar. The highest concentrations of spores were recorded in 2009.

The highest concentration of *Alternaria* spores, equal to 5943 AFS m^{-3}, was noted in 2009. The highest values were recorded from June to July. The

Table 1 Mean monthly meteorological conditions (2008–2010)

Month	Year	Daily mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Quantities of precipitations (L m^{-2})	Daily mean relative humidity (%)	Sunshine hours (h)	Atmospheric pressure (mbars)	Daily mean wind speed (m s^{-1})	Daily max. wind speed (m s^{-1})
January	2008	0.9	5.7	−2.4	0.7	80.2	2.9	1013.6	1.7	2.6
	2009	−1.1	2.6	−4.0	0.9	83.3	1.9	1006.6	1.6	2.2
	2010	−0.3	3.0	−2.9	2.1	91.2	1.7	1004.5	1.7	2.3
February	2008	3.7	10.1	−0.9	0.3	72.3	4.3	1016.3	1.8	3
	2009	1.4	5.9	−1.9	0.9	77.8	3.2	1001.9	2.3	3.3
	2010	2.8	6.4	−0.4	2.7	88.1	2.6	997.4	2.1	2.9
March	2008	7.7	12.9	3.6	2	67	4	997.5	2.4	3.7
	2009	6.6	12.0	2.5	1.6	67.0	4.7	1001.3	2.3	3.5
	2010	6.7	12.3	2.4	1.1	74.5	4.6	1006.9	2.2	3.2
April	2008	12.4	18.7	7	1.5	66	5.9	999.7	2.3	3.2
	2009	14.7	22.5	7.6	0.8	56.2	8.9	1004.8	1.8	2.7
	2010	12.0	18.3	6.9	1.9	77.9	5.8	1006.5	1.8	2.6
May	2008	17.8	25	11.3	1.6	63.2	7.9	1003.8	2.1	3.2
	2009	18.0	25.7	11.1	1.4	61.3	7.5	1006.3	1.9	2.6
	2010	16.6	22.9	11.6	3.8	77.2	4.8	999.3	2.1	3.1
June	2008	21.6	28.1	15.7	5.2	66.1	8.3	1003.8	1.7	2.3
	2009	20.1	26.5	14.0	3.7	72.5	7.2	1002.4	1.9	2.6
	2010	20.5	26.3	15.2	4.4	77.1	7.3	1000.1	2.2	2.9
July	2008	21.9	29.1	15.4	1.5	60.8	8.2	1002.6	1.9	2.6
	2009	23.2	30.6	15.8	1.3	67.6	9.1	1003.8	1.8	2.5
	2010	23.1	30.1	17.4	0.8	72.5	7.4	1003.3	1.7	2.3
August	2008	22.6	30.9	15.6	0.8	57.8	9	1003.4	1.7	2.5
	2009	22.9	30.6	16.4	0.9	67.3	8.3	1005.8	1.6	2.3
	2010	21.2	27.7	15.6	3.6	72.8	7.8	985.8	2.0	2.8
September	2008	15.4	21.7	11.2	1.7	70.6	4.7	1005.7	1.6	2.4
	2009	19.0	27.3	13.0	0.2	66.8	7.3	1007.7	1.4	2.0
	2010	16.2	22.7	11.9	1.4	80.4	5.4	1004.6	1.6	2.4
October	2008	12.3	20.3	7.3	0.6	75.2	5.5	1009	1.6	2.6
	2009	11.6	17.9	7.4	2.6	83.9	4.0	1005.4	1.9	2.9
	2010	9.2	15.5	4.9	1.3	80.7	5.0	1007.2	1.6	2.2

Table 1 continued

Month	Year	Daily mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Quantities of precipitations (L m^{-2})	Daily mean relative humidity (%)	Sunshine hours (h)	Atmospheric pressure (mbars)	Daily mean wind speed (m s^{-1})	Daily max. wind speed (m s^{-1})
November	2008	7.1	13.4	2.7	1.8	73.4	4.4	1007.3	1.9	2.8
	2009	7.5	12.7	4.0	3.5	92.6	2.8	1004.9	1.6	2.3
	2010	9.3	16.0	4.5	1.6	80.4	4.0	999.6	2.0	3.0
December	2008	3.6	7	0.7	1.8	78.3	1.7	1009.5	2.1	2.8
	2009	3.2	6.3	0.2	2.7	91.4	1.0	1000.3	2.0	2.6
	2010	0.7	4.5	−2.8	2.4	90.0	1.3	1002.6	2.2	3.2

study of variations indicated no significant differences between years ($F = 1.734$; $p = 0.177$). In 2009, the concentration of *Alternaria* spores exceeded the borderline level of 80 AFS m^{-3} on 18 days. For the other years were recorded increases over the borderline level: 12 days in 2008, 10 days in 2010.

Daily concentrations of *Epicoccum* spores were low, but the study variations throughout a 3-year period indicated significant differences ($F = 55.66$; $p < 0.001$). The highest level of conidia emission was recorded in 2009 with 5837 AFS m^{-3}. In that year, the highest concentrations were identified between June and September. In 2008 and 2010, *Epicoccum* pattern showed increasing values from mid-summer, with the highest mean values occurring in the early autumn. The highest monthly spore concentrations of *Epicoccum* for these years were found in September.

Pithomyces spore concentrations were low (Fig. 1). There were significant differences between these concentrations ($F = 3.523$; $p = 0.029$), especially between 2008 and the other 2 years. *Torula* spore concentrations were also very low (Fig. 1), with small differences ($F = 3.008$; $p = 0.049$).

3.2 Airborne spore dependence on the weather

All the taxa responded in a similar way to the weather variables during the whole study (Table 2).

All annual spore concentrations increased when the temperatures (mean, maximum, minimum) rose making *Alternaria*, *Cladosporium*, *Epiccocum*, *Pithomyces* and *Torula* a temperature-dependent fungi. The strongest correlation was seen for *Alternaria* and *Cladosporium*. The drastic decrease in annual mean *Cladosporium* spore concentrations coincided with a decline in annual mean temperature of a 11.5 °C, taking into account 2010 year compared with previous examined years. In previous years, the mean annual temperature was 12.25 °C (Table 1).

Spearman's rank correlation analysis also revealed the negative and significant influence of relative air humidity on airborne spores. Relative humidity values differed significantly between the 3 years ($F = 70.78$; $p < 0.001$). Noteworthy was the upward trend in annual mean values for relative humidity; in 2010, annual mean reached 80.23 %, while each month exceeded 70 % (Table 1).

Weak, although statistically significant, correlations were observed between precipitation and

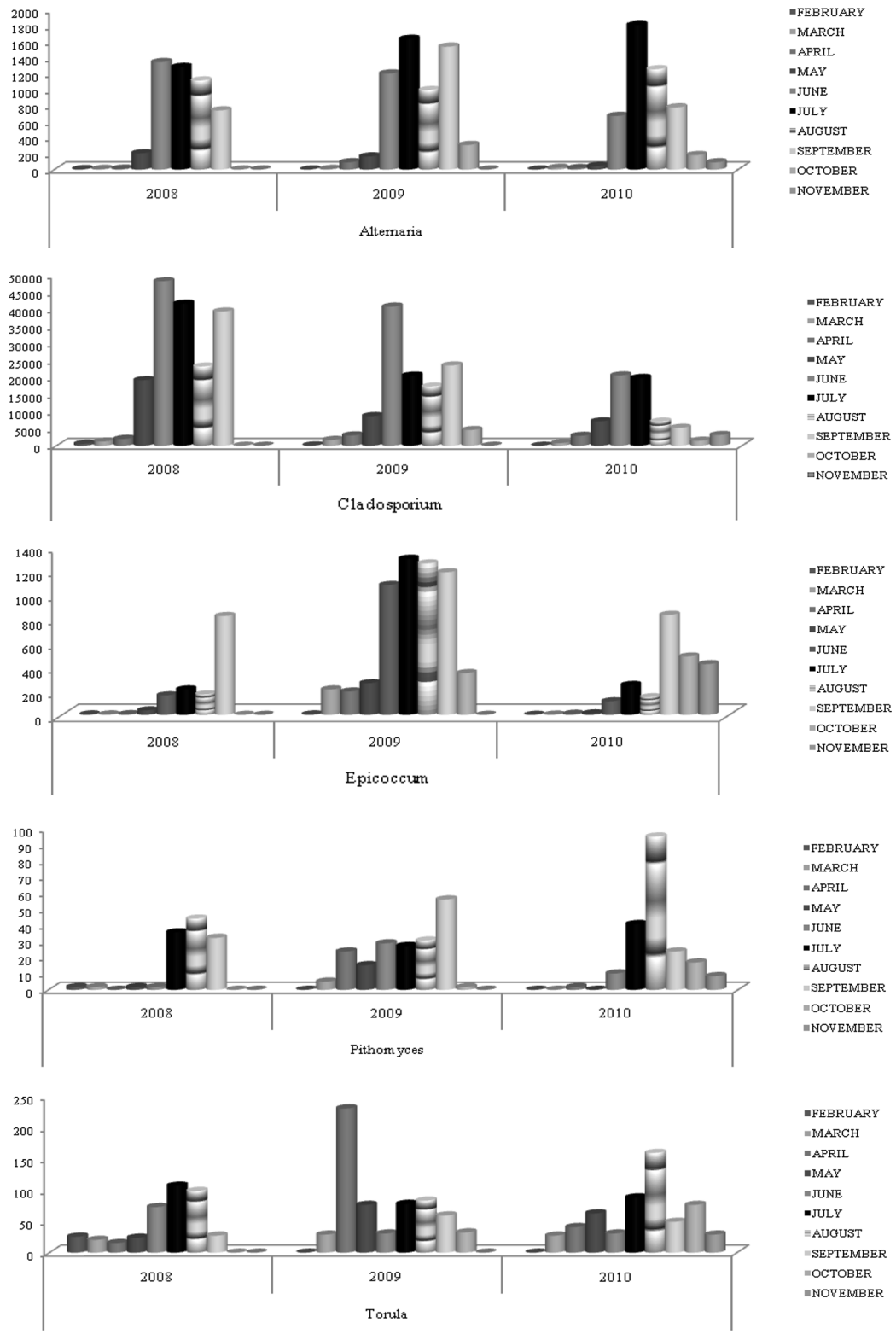

Fig. 1 Monthly concentrations of selected allergenic spores for Timisoara site, 2008–2010

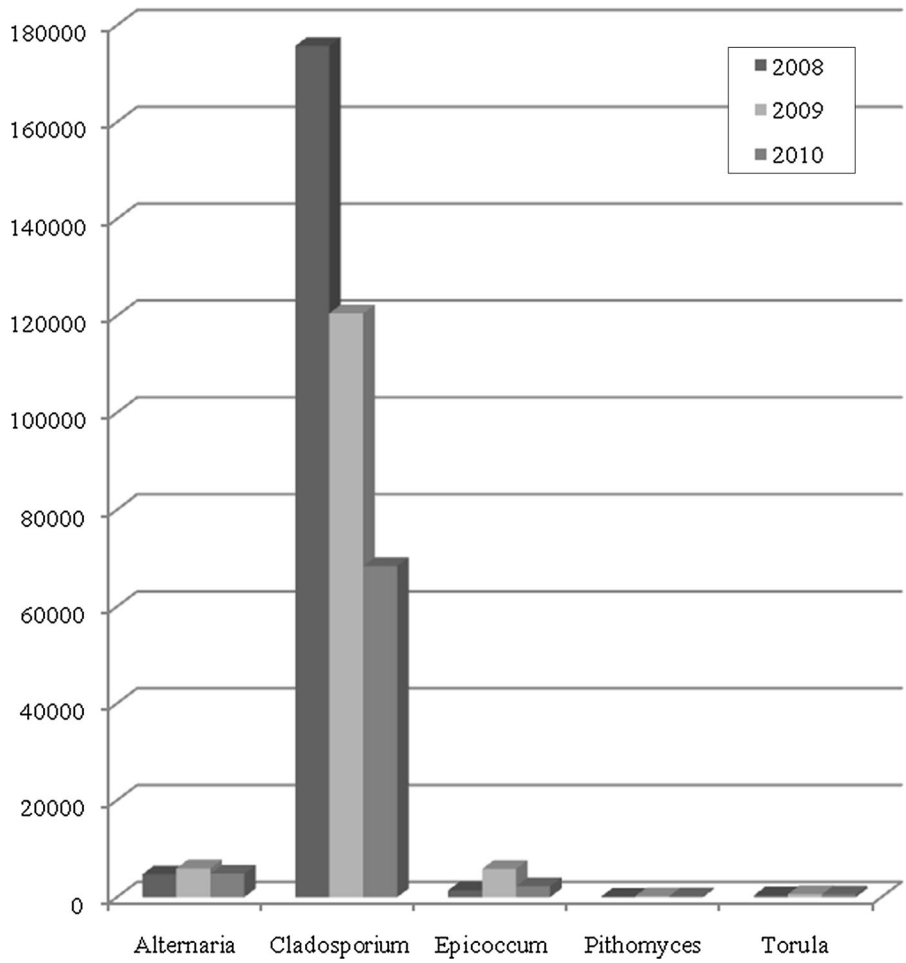

Fig. 2 The cumulative annual concentrations of selected allergenic spores for Timisoara site, 2008–2010

Pithomyces and *Torula* in 2008 (Table 2). In the following years, under investigation further statistically significant associations were detected for *Alternaria* and *Epicoccum* (Table 2). In 2009 and 2010 were significantly negative correlations. Annual rainfall sums were slightly higher in 2009 and 2010. The amount of rainfall did not differ greatly between the 3 years of study. However, the increase in annual sums of rainfall in years 2009 and 2010 explained the significant and negative correlations with spore concentrations.

The total number of daily sunshine hours obtained positive and significant correlations in all cases.

In 2008, the correlations between air pressure and *Alternaria*, *Cladosporium*, *Epicoccum* and *Torula* were found to be inversely proportional and statistically significant (Table 2). The vector of these relationships changed to proportional 2 years later in case of *Alternaria*, *Epicoccum* and *Torula* spores (Table 2). Pressure values were significantly different in the 3 years of study ($F = 14.75$; $p < 0.001$). Regarding the average annual values of pressure, they were lower in 2010 (1001.48 mbar) and much higher in 2008 (1006.01 mbar).

In general, these spores were negatively correlated with wind speed and even more frequently with the maximum wind speed (Table 2). During the years under investigation, the correlation between *Torula* spore concentrations and wind speed did not show a statistically significant relationship.

Overall, the correlations with temperature were less significant when data were restricted to the period of airborne spores being released. In addition, was found a slightly increase of the importance of the wind. The correlation coefficients were lower for relative

Table 2 Coefficients of correlation between the selected allergenic fungal spore concentrations and the main meteorological parameters by using the Spearman correlation test (during 2008–2010)

	Spore type		Daily mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Quantities of precipitations (L m^{-2})	Daily mean relative humidity (%)	Sunshine hours (h)	Atmospheric pressure (mbars)	Daily mean wind speed (m s^{-1})	Daily max. wind speed (m s^{-1})
2008	*Alternaria*	FY	**0.692****	**0.660****	**0.694****	−0.055	**−0.398****	**0.429****	**−0.218****	−0.0696	**−0.135****
		SD	**0.640****	**0.611****	**0.619****	−0.120	**−0.226****	**0.380****	0.062	**−0.203****	**−0.273****
		PD	**0.640****	**0.602****	**0.627****	−0.090	**−0.186****	**0.357****	0.056	**−0.171****	**−0.238****
	Cladosporium	FY	**0.736****	**0.695****	**0.723****	−0.046	**−0.477****	**0.471****	**−0.342****	−0.021	−0.046
		SD	**0.688****	**0.638****	**0.672****	−0.071	**−0.191****	**0.363****	−0.015	**−0.242****	**−0.288****
		PD	**0.661****	**0.616****	**0.677****	0.002	−0.116	**0.311****	−0.006	**−0.194****	**−0.264****
	Epicoccum	FY	**0.589****	**0.550****	**0.603****	−0.039	**−0.328****	**0.349****	**−0.166****	−0.093	**−0.142****
		SD	**0.489****	**0.439****	**0.490****	−0.067	**−0.137***	**0.250****	0.111	**−0.242****	**−0.293****
		PD	**0.498****	**0.439****	**0.527****	−0.084	−0.085	**0.210****	0.125	**−0.208****	**−0.274****
	Pithomyces	FY	**0.369****	**0.369****	**0.362****	−0.099	**−0.281****	**0.251****	−0.098	**−0.117***	**−0.135****
		SD	**0.343****	**0.351****	**0.318****	**−0.133***	**−0.215****	**0.194****	0.050	**−0.196****	**−0.210****
		PD	**0.327****	**0.340****	**0.284****	**−0.159***	**−0.163***	**0.208****	0.098	**−0.146***	**−0.147***
	Torula	FY	**0.483****	**0.483****	**0.432****	−0.077	**−0.444****	**0.403****	**−0.178****	0.034	0.0002
		SD	**0.424****	**0.421****	**0.353****	−0.109	**−0.347****	**0.362****	0.024	−0.035	−0.071
		PD	**0.395****	**0.389****	**0.325****	**−0.262****	**−0.318****	**0.433****	0.078	−0.116	**−0.147***
2009	*Alternaria*	FY	**0.803****	**0.806****	**0.783****	**−0.190****	**−0.393****	**0.552****	0.057	**−0.145****	**−0.137****
		SD	**0.575****	**0.566****	**0.603****	−0.095	**0.334****	0.116	−0.021	**−0.252****	**−0.288****
		PD	**0.574****	**0.560****	**0.596****	−0.023	**0.369****	0.088	0.016	**−0.315****	**−0.335****
	Cladosporium	FY	**0.802****	**0.804****	**0.786****	**−0.154****	**−0.474****	**0.571****	0.048	**−0.112***	−0.099
		SD	**0.573****	**0.534****	**0.644****	0.064	**0.343****	0.069	−0.058	**−0.193****	**−0.256****
		PD	**0.579****	**0.538****	**0.644****	0.113	**0.361****	0.028	−0.067	**−0.259****	**−0.334****
	Epicoccum	FY	**0.774****	**0.785****	**0.740****	**−0.200****	**−0.491****	**0.589****	0.040	**−0.104***	−0.059
		SD	**0.550****	**0.540****	**0.542****	−0.094	**0.264****	**0.166***	−0.019	**−0.189****	**−0.179****
		PD	**0.521****	**0.509****	**0.537****	−0.062	**0.322****	0.123	−0.011	**−0.238****	**−0.261****
	Pithomyces	FY	**0.454****	**0.462****	**0.441****	**−0.115***	**−0.319****	**0.332****	0.028	−0.075	−0.066
		SD	**0.214****	**0.233****	**0.225****	−0.034	−0.012	0.041	−0.034	−0.099	−0.115
		PD	**0.218****	**0.222****	**0.251****	−0.031	0.038	0.018	−0.011	−0.122	**−0.159***
	Torula	FY	**0.478****	**0.481****	**0.431****	**−0.219****	**−0.414****	**0.421****	0.020	−0.026	0.017
		SD	0.088	0.087	0.030	**−0.179***	−0.131	0.119	−0.031	0.032	0.066
		PD	0.114	0.135	0.070	−0.078	−0.096	0.123	0.038	−0.049	−0.087

Table 2 continued

	Spore type		Daily mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Quantities of precipitations (L m^{-2})	Daily mean relative humidity (%)	Sunshine hours (h)	Atmospheric pressure (mbars)	Daily mean wind speed (m s^{-1})	Daily max. wind speed (m s^{-1})
2010	*Alternaria*	FY	**0.726****	**0.739****	**0.698****	**−0.227****	**−0.437****	**0.507****	**0.109***	**−0.175****	**−0.122***
		SD	**0.636****	**0.640****	**0.612****	**−0.240****	**−0.207****	**0.348****	−0.006	**−0.190****	**−0.164****
		PD	**0.619****	**0.604****	**0.618****	**−0.204****	**−0.154***	**0.335****	0.004	**−0.184****	**−0.175****
	Cladosporium	FY	**0.773****	**0.761****	**0.756****	−0.055	**−0.415****	**0.483****	0.007	−0.075	−0.059
		SD	**0.591****	**0.545****	**0.581****	0.027	−0.015	**0.224****	**−0.211****	−0.016	−0.050
		PD	**0.575****	**0.489****	**0.614****	0.114	0.027	**0.132***	**−0.270****	0.015	−0.050
	Epicoccum	FY	**0.445****	**0.472****	**0.432****	**−0.212****	**−0.227****	**0.347****	**0.111***	**−0.204****	**−0.158****
		SD	−0.021	0.024	−0.020	**−0.276****	0.087	0.077	**0.179****	**−0.233****	**−0.177****
		PD	−0.058	−0.029	−0.006	**−0.192****	**0.195****	−0.004	**0.151***	**−0.233****	**−0.193****
	Pithomyces	FY	**0.429****	**0.428****	**0.429****	**−0.133***	**−0.244****	**0.232****	0.035	**−0.173****	**−0.146****
		SD	**0.299****	**0.297****	**0.305****	**−0.148***	**−0.144***	0.083	0.047	**−0.168***	**−0.159***
		PD	**0.283****	**0.296****	**0.272****	**−0.187****	**−0.186****	**0.165***	0.033	−0.132	−0.101
	Torula	FY	**0.502****	**0.530****	**0.456****	**−0.147****	**−0.414****	**0.405****	**0.114***	−0.102	−0.045
		SD	**0.303****	**0.339****	**0.246****	**−0.129***	**−0.246****	**0.237****	0.046	−0.086	−0.047
		PD	**0.283****	**0.298****	**0.241****	**−0.224****	**−0.230****	**0.212****	0.101	**−0.132***	−0.063

FY correlations over full year, *SD* meteorological parameters in sampling day, *PD* meteorological parameters in previous day

Bold values are statistically significant

** Correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level

humidity, number of sunshine hours and atmospheric pressure in 2008. For *Pithomyces* and *Torula*, correlations with rainfall became significant and negative in previous day sampling. The spores of *Torula* were not significantly correlated with temperature when the analysis was limited to days, when their presence in the air was recorded. However, *Torula* spores were negative and significantly correlated with rainfall on the day of measurement. *Alternaria*, *Cladosporium* and *Epiccocum* spores were positively correlated with relative humidity. Throughout the study period, when above-mentioned spore types were present in the air of Timişoara, the relative humidity had relatively constant values (around 65 %). The correlation coefficients were lower for relative humidity and number of sunshine hours in 2010. On the other hand, correlation coefficient values for rainfall have increased (Table 2). For *Cladosporium*, the correlation became significant and negative with atmospheric pressure when the analysis was restricted to the period, when analysed fungal spores were observed in the air in 2010. In the case of *Epicoccum* spores, it was observed that they were positively correlated with relative humidity in the previous day of sampling. The effect of relative humidity on airborne spore concentrations was found to be both positive and negative depending on the examined year (Table 2). In general, the meteorological parameters affected spore concentrations differently. The coefficients of correlation with meteorological factors fell sharply when 1 lag day weather data in relation to spore data were considered.

Interestingly, results showed that concentrations fell in August (2008) for all types of spores. In this month, the highest maximum temperature (30.9 °C) and lowest relative humidity (57.8 %) values were recorded. This decrease was very important to *Cladosporium*. Analysis of *Cladosporium* spore concentrations observed in August 2008 (Table 3) showed a different distribution pattern, simultaneously exhibiting high sensitivity to fluctuations in environmental factors. The concentrations were statistically significantly correlated with wind speed and precipitation and statistically significantly in relation to the atmospheric pressure and numbers of sunshine hours. In the same month, the levels of *Cladosporium* were correlated negatively with wind speed and precipitation (Table 3). *Cladosporium* require temperature an upper limit about 20 °C and relative humidity about 65 %

Table 3 Coefficients of correlation between the selected allergenic fungal spore concentrations and the main meteorological parameters by using the Spearman correlation test (in August—2008)

Spore type/during August 2008	Daily mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Sunshine hours (h)	Atmospheric pressure (mbars)	Quantities of precipitations (L m^{-2})	Daily mean relative humidity (%)	Daily mean wind speed (m s^{-1})	Daily max. wind speed (m s^{-1})
Alternaria	0.163	0.191	0.033	0.234	0.299	−0.272	−0.040	−0.186	−0.295
Cladosporium	−0.084	−0.040	−0.326	**0.445***	**0.407***	**−0.469****	0.102	**−0.627****	**−0.583****
Epicoccum	0.323	0.237	0.141	**0.455***	0.082	−0.206	−0.203	−0.123	−0.214
Pithomyces	**0.471****	**0.412***	**0.358***	0.005	−0.151	−0.133	−0.225	0.008	−0.021
Torula	**0.519****	**0.526****	0.299	**0.414***	−0.085	−0.266	**−0.360***	−0.112	−0.056

Bold values are statistically significant

** Correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level

for optimal growth. It is possible that an increase over 30 °C becomes the limiting factor in the release. The same sudden drop in monthly concentrations was seen in August 2009 when the maximum temperature exceeded 30 °C.

Since the two most dominant spore types reached the highest levels in June 2008, a further correlation analysis with meteorological parameters was performed for that month (Table 4). In June, the maximum temperature was 28.1 °C, and relative humidity was 66.1 %, with the highest number of sunshine hours in the year (8.3) and the largest amount of rainfall (5.2 L m^{-2}). In June, the spores of *Alternaria* showed most significant correlations: positive (for mean daily temperature, maximum temperature, minimum temperature, number of sunshine hours, atmospheric pressure) and negative (daily average relative humidity). The same trends were observed for all investigated spore types.

Table 5 shows the results of multiple linear regressions. Only the highest values of the statistically significant coefficients were selected (Table 6). Multiple regression analysis was found to be a very valuable tool for identifying the weather variables most closely associated with atmospheric fungal spore concentrations. Results showed that the coefficient of determination (R^2) of the multiple regressions varied from 0.080 to 0.386 for 2008, from 0.123 to 0.449 for 2009 and from 0.131 to 0.396 for 2010. The most important factors were not the same for each year.

For *Alternaria*, meteorological parameters explained 39.6 % of the total variance in 2010, 38.6 % in 2008 and 33.5 % in 2009. The most important agents influencing the *Alternaria* spore count (in 2008) are mean daily temperature, maximum temperature, number of sunshine hours and atmospheric pressure. Multiple regression analyses consisting of daily average relative humidity + number of sunshine hours during 2009 and quantities of precipitations + daily average relative humidity + number of sunshine hours during 2010 explained the variability in *Alternaria* spores.

For *Cladosporium*, meteorological parameters explained 34.3 % of the total variance in 2008, 30.6 % in 2009 and 34.2 % in 2010. Mean daily temperature, maximum temperature and daily average relative humidity showed to have some influence on the *Cladosporium* distribution in 2008. Multiple

Table 4 Coefficients of correlation between the selected allergenic fungal spore concentrations and the main meteorological parameters by using the Spearman correlation test (in June—2008)

Spore type/during June 2008	Daily mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Sunshine hours (h)	Atmospheric pressure (mbars)	Quantities of precipitations (L m^{-2})	Daily mean relative humidity (%)	Daily mean wind speed (m s^{-1})	Daily max. wind speed (m s^{-1})
Alternaria	**0.679****	**0.650****	**0.444***	**0.502****	**0.619****	−0.305	**−0.463***	−0.005	0.075
Cladosporium	**0.421***	**0.387***	0.317	0.334	**0.366***	−0.121	−0.236	−0.059	0.007
Epicoccum	**0.527****	**0.493****	0.312	**0.374***	0.355	−0.259	**−0.501****	−0.038	0.032
Pithomyces	0.258	0.290	0.204	0.311	0.204	−0.145	−0.290	−0.098	−0.069
Torula	**0.459***	**0.383***	**0.570****	0.157	0.160	0.034	−0.190	0.099	0.142

Bold values are statistically significant

** Correlation is significant at the 0.01 level; * correlation is significant at the 0.05 level

Table 5 Multiple linear regression variable result

Year	Spore type	Multiple correlation coefficient (R)	Coefficient of determination (R^2)	Adjusted R^2	Std. error of the estimate	ANOVA—F	Regression variable result—proportion of variance explained (%)
2008	*Alternaria*	0.621	0.386	0.371	20.14375	24.894	38.6
	Cladosporium	0.603	0.364	0.348	672.45470	22.621	36.4
	Epicoccum	0.284	0.080	0.057	11.32610	3.460	8
	Pithomyces	0.371	0.137	0.116	1.01321	6.303	13.7
	Torula	0.429	0.184	0.163	2.56564	8.908	18.4
2009	*Alternaria*	0.579	0.335	0.318	24.34581	19.850	33.5
	Cladosporium	0.554	0.306	0.289	481.74575	17.431	30.6
	Epicoccum	0.670	0.449	0.435	16.25305	32.099	44.9
	Pithomyces	0.446	0.199	0.178	0.98679	9.771	19.9
	Torula	0.351	0.123	0.101	4.55504	5.530	12.3
2010	*Alternaria*	0.630	0.396	0.381	19.46256	25.894	39.6
	Cladosporium	0.585	0.342	0.326	247.29363	20.527	34.2
	Epicoccum	0.362	0.131	0.109	13.67147	5.954	13.1
	Pithomyces	0.434	0.188	0.168	1.27259	9.161	18.8
	Torula	0.453	0.205	0.185	2.57904	10.185	20.5

regression analyses consisting of daily average relative humidity + number of sunshine hours during 2009 and mean daily temperature + daily average relative humidity + atmospheric pressure during 2010 explained the variability in *Cladosporium* spores.

Mean daily temperature, minimum temperature and daily average relative humidity were positively associated with *Epicoccum* in 2010. Other environmental factors, such as precipitation and daily mean relative humidity, also had statistically significant relationships with *Epicoccum* spores. Multiple regression analyses consisting of daily average relative humidity + number of sunshine hours during 2009 explained the variability in *Epicoccum* spores. For *Epicoccum*, meteorological parameters explained 44.9 % of the total variance in 2009 and 13.1 % in 2010. Only in 2008 displayed a very low coefficient of determination value.

Pithomyces spores were found not to be significantly influenced by weather in 2008 and 2010 but were positively associated with daily mean relative humidity in 2009.

The major explanatory variable for *Torula* was mean relative humidity in 2008 and 2009.

4 Discussions

As major drivers of climate change in Romania, at regional level, the studies showed (1) the rise of air temperature, (2) the reduction in annual sums of precipitation and (3) the intensification of extreme weather events (Marica and Busuioc 2004; Paltineanu et al. 2007; Mircov et al. 2014). Studying fungal spore concentrations in relation to weather conditions is of high practical importance because of its health concern (Damialis and Gioulekas 2006; Escuredo et al. 2011).

Between 2008 and 2010, the same pattern of behaviour for selected allergenic spore types was found. The biomonitoring of atmospheric fungal spores in Timisoara revealed the early summer as the most favourable season for airborne spore occurrence (Ianovici and Faur 2003; Ianovici et al. 2004, 2008; Ianovici and Tudorica 2009).

Positive, statistically significant correlations with temperature, similar to those observed in Romania, were extensively reported in other cities around the world, e.g. in Italy (Palmas and Cosentino 1990), Mexico (Rosas et al. 1990), Sweden (Hjelmroos 1993), USA (Troutt and Levetin 2001), Turkey

Table 6 The significant factors in multiple linear regression for selected allergenic fungal spores

Year	Spore type	Explanatory variables	Unstandardized coefficients		Standardized coefficients	*t* value (*t*)	*p* value (sig.)
			B	SE			
2008	*Alternaria*	Daily mean temperature (°C)	5.096	1.364	1.672	3.736	0.000218
		Maximum temperature (°C)	−2.447	0.706	−0.939	−3.464	0.000596
		Sunshine hours (h)	1.352	0.551	0.205	2.453	0.014659
		Atmospheric pressure (mbars)	0.566	0.172	0.180	3.287	0.001115
	Cladosporium	Daily mean temperature (°C)	250.9216415	45.5357630	2.5105949	5.5104302	0.0000001
		Maximum temperature (°C)	−129.9582706	23.5822825	−1.5210365	−5.5108436	0.0000001
		Daily mean relative humidity (%)	14.4854634	5.7396214	0.1976799	2.5237664	0.0120449
	Epicoccum	–					
	Pithomyces	–					
	Torula	Daily mean relative humidity (%)	−0.05223	0.02190	−0.21162	−2.38531	0.0175874
2009	*Alternaria*	Daily mean relative humidity (%)	0.72313264	0.15009754	0.36297898	4.81775152	0.00000215
		Sunshine hours (h)	2.03442252	0.68564888	0.27429646	2.96714920	0.00320952
	Cladosporium	Daily mean relative humidity (%)	12.966791	2.970074	0.335849	4.365815	0.000017
		Sunshine hours (h)	36.805558	13.567362	0.256059	2.712801	0.006997
	Epicoccum	Daily mean relative humidity (%)	0.38868	0.10020	0.26605	3.87894	0.00013
		Sunshine hours (h)	1.46603	0.45773	0.26954	3.20280	0.00148
	Pithomyces	Daily mean relative humidity (%)	−0.0154353	0.0060838	−0.2098135	−2.5371247	0.0116041
	Torula	Daily mean relative humidity (%)	−0.1013219	0.0280829	−0.3121194	−3.6079567	0.0003529
2010	*Alternaria*	Quantities of precipitations (l m^{-2})	−0.678001	0.276699	−0.124451	−2.450319	0.014755
		Daily mean relative humidity (%)	0.418472	0.182540	0.174403	2.292497	0.022461
		Sunshine hours (h)	1.135526	0.532001	0.173705	2.134445	0.033491
	Cladosporium	Daily mean temperature (°C)	50.007757	17.618794	1.472398	2.838319	0.004796
		Daily mean relative humidity (%)	9.462553	2.319372	0.323964	4.079792	0.000056
		Atmospheric pressure (mbars)	4.871986	2.353404	0.114212	2.070187	0.039157
	Epicoccum	Maximum temperature (°C)	−2.651835	0.974044	−1.623246	−2.722500	0.006799
		Daily mean temperature (°C)	1.731804	0.487576	1.224763	3.551866	0.000434
		Minimum temperature (°C)	1.380573	0.633871	0.764146	2.178004	0.030063
		Quantities of precipitations (l m^{-2})	−0.606050	0.194367	−0.189989	−3.118067	0.001969
		Daily mean relative humidity (%)	0.284817	0.128225	0.202724	2.221230	0.026966
	Pithomyces	–					
	Torula	–					

(Sakiyan and Inceoglu 2003), Australia (Mitakakis et al. 1997).

Many other researchers reported similar results in the past regarding the relative humidity. Kurkela (1997), Stępalska and Wołek (2005), Oliveira et al. (2007) found the same negative correlation between atmospheric fungal spores and relative humidity. Some authors concluded that in order for airborne spores to be released into the atmosphere, a certain degree of dryness is required in the atmosphere, which occurs when the temperature increases (Nolard et al. 2001), in line with the results found in this study.

Statistically significant and positive correlations of daily spore concentrations with daily mean, minimum and maximum temperatures and negative correlation with daily average relative humidity found in this study were consistent with the results given by some authors, such as Angulo-Romero et al. (1999), Corden and Millington (2001) and Aira et al. (2008). Ambient temperature showed positive correlation with most of

the biological and non-biological air pollutants (Adhikari et al. 2006).

Both relative humidity and precipitation are measures associated with water availability outdoors. Rainfall may cause release of fungal spores by splash and "tap-and-puff" mechanisms (Ho et al. 2005). On the other hand, some researchers have considered that the rain removes ambient fungal spores by both rain-out and wash-out effects (Magyar et al. 2009; Artaç et al. 2014).

Impact of the air pressure upon the fungal spore distribution was found to be heterogenic during examined period of time (2008–2010). It is possible that variations of this factor, especially in summer, facilitate or contrary, limit the transport of atmospheric fungal spores. Increased pressure can be correlated with low spore concentrations in the atmosphere. Other studies showed that the atmospheric fungal spore release is insignificantly affected by the air pressure (Marchiso and Airaudi 2001). In contrast, the concentration of atmospheric fungal spores was significantly and negatively correlated with atmospheric pressure (Hjelmroos 1993; Stennett and Beggs 2004; Grinn-Gofroń 2008). A positive, significant correlation during one season was reported by Troutt and Levetin (2001).

Similarly to this study, wind speed was negatively associated with spore dispersal in other countries. The fungal spore release is often influenced by wind speed (Munuera Giner et al. 2001; Sen and Asan 2001; Levetin and Dorsey 2006).

Statistical methods used in this study were complementary, since they described the effects of all meteorological factors on the concentrations of airborne mycoflora. Additionally, a multiple regression analysis was performed to understand the combined or individual influence of different environmental variables on atmospheric fungal spores. Multiple linear regression is extremely useful to identify significant predictors for each type of fungal spores analysed but expected fluctuations from month to month. Relative humidity is responsible for most of the variation in 2009, and number of sunshine hours ranks second as an explanatory variable. In multiple regression analyses, it was found that temperature, precipitation, daily mean relative humidity and atmospheric pressure were consistently associated with atmospheric fungal spores in 2010. Among the meteorological factors, temperature was the most consistent predictor for fungal concentrations in 2008. Number of sunshine hours, atmospheric pressure and daily mean relative humidity were also significant predictors of atmospheric fungal spores in 2008. Anyway, the low performance of regression for 2008 season (with the exception of *Alternaria* and *Cladosporium*) suggests that there are other variables influencing atmospheric fungal spore concentrations that are more important than meteorological factors. It is possible that other non-measured parameters may have some effect on spore release. The analysis of Spearman's rank correlations as well as multiple linear regressions in this study confirmed stable dependence of *Alternaria* and *Cladosporium* spore concentrations on meteorological factors, revealed also by other researchers (Corden and Millington 2001; Stępalska and Wołek 2005; Grinn-Gofroń and Mika 2008).

To the best of my knowledge of the composition of atmospheric fungal spores is important in a city because results could be used for the diagnosis of respiratory disease cases caused by aeroallergens. The airborne spore maximum concentrations coincide with the occurrence of herbaceous pollen (Poaceae, *Artemisia* and *Ambrosia*) in Timisoara (Ianovici et al. 2007, 2013b, c). Such co-occurrence of aeroallergens may increase the risk of allergic diseases. In addition to aeroallergens, the above fungi may produce mycotoxins or other fungal metabolites that are carried by airborne dust and reach humans through skin contact (Abu-Dieyeh et al. 2010). The allergic response of a given population varies also depending on the characteristics of the age, sex, health condition of population, etc. (Aira et al. 2008). According to our knowledge, specific data are not available about sensitivity of Romanian population to the above common fungi. Future investigations are needed to examine the effects of fungal spore exposures on related health problems.

The behaviour of fungi is a dynamic and complex phenomenon. Field researches have demonstrated that the growth of fungi is favoured by high moisture and moderate temperatures (Froelich and Snow 1986; Rowan et al. 1999) and that low relative humidity and extreme temperatures inhibit growth and spore germination (Talley et al. 2002). For each fungal species, there is an optimal temperature range for growth to occur. Outside of that optimal temperature range, more water is necessary for growth. Majority of fungal species cannot grow unless the relative humidity

exceeds 60 %. Several researchers have reported the necessity for high temperatures (15–29 °C) for the formation of *Alternaria* and *Cladosporium* spores (Herrero and Zaldivar 1997). The exceedance of the higher temperature limit for growth may kill fungi, whereas temperatures below the lower growth limit are less lethal (Eduard 2009). The other climate variables, elevated CO_2, the spatial and temporal distribution of vegetation, changing plant species' diversity and density, host physiology, competitive interspecific interactions, physical edaphic factors, the habitat alteration or fragmentation, the anthropogenic factors may have a major influence on the fungal spore concentrations (Jones and Harrison 2004; Okten et al. 2005; Cecchi et al. 2010; Després et al. 2012). Fungi are exposed to continuously fluctuating environments but seem to display a delayed and slower response to climate change than plants (Reid and Gamble 2009; Damialis et al. 2015b).

In this study, all spore concentrations dropped sharply in August, when temperatures exceeded 30 °C, and the relative humidity dropped below 60 %. The results showed that relative humidity and maximum temperature are critical parameters and have limiting effects during the initiation of fungal spore dissemination. In case the temperature and number of sunshine hours rise early summer, it is possible that spore concentrations will increase. The decrease in relative humidity (up to 60–65 %) in the same period will cause increased levels of spores in the air. The impact of climate change can have on the increasing atmospheric fungal spore concentrations in the future. The need of a further study to improve present data appeared to be important.

5 Conclusions

This is the first study in Romania that shows dynamics of atmospheric spores depending on meteorological factors. The five allergenic spores chosen for this analysis were *Alternaria*, *Cladosporium*, *Epicoccum*, *Pithomyces* and *Torula*. *Cladosporium* and *Alternaria* were the most prevalent fungal spore types in the air samples collected in Timişoara (western Romania).

Nonparametric Spearman's correlation coefficients were calculated between different environmental variables and atmospheric spore concentrations. All selected atmospheric fungal spore concentrations were found to be positively and significantly correlated with the maximum, minimum and mean temperature, and number of sunshine hours. Negative and significant correlations were with daily mean relative humidity. Negative correlations with wind speed and maximum wind speed were found only with *Alternaria* and *Epicoccum* spore concentrations.

Multiple regression was performed to understand the combined influence of meteorological variables on airborne spores. For *Alternaria*, meteorological parameters explained between 33.5 and 39.6 % of the total variance. For *Cladosporium*, the same variables explained between 30.6 and 34.3 % of the total variance.

In case the mean temperature rises in early summer and the relative humidity decreases in the same period, it is possible that the atmospheric spore concentrations will increase in the future.

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