

First fungal spore calendar for the atmosphere of Bratislava, Slovakia

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Abstract Fungal spores were identified and quantified in the air of Bratislava during the 1-year period (2016) using a Burkard 7-day volumetric aerospore trap. Based on data obtained, the first spore calendar in Slovakia has been constructed for the urban area of Bratislava. The total annual spore concentration recorded during this period was 836,418 spores/m³, belonging to 53 fungal spore types. The fungal taxa contributing the highest concentration of spores were *Cladosporium* (71.88% of the total), *Coprinus* (8.84%), *Leptosphaeria* (3.88%), *Ganoderma* (3.43%) and *Alternaria* (2.79%). Remaining 48 spore types were less well represented (altogether 9.18% of the total). Daily monitoring data revealed a large variation in airborne spore concentrations. Fungal spores peaked during summer and autumn months (June–October) and declined from November to March. The maximum monthly total spore concentration (153,342 spores/m³) was recorded in July, while the minimum (1381 spores/m³) in January. The

relationships between mean daily airborne spore concentrations of selected fungal taxa and meteorological variables were evaluated through multiple regression analysis. The percentage of variation explained by regression analyses was 49.7% for *Alternaria*, 46.9% for *Ganoderma*, 45.8% for *Cladosporium*, 43.9% for *Leptosphaeria* and 32.1% for *Coprinus*. Spore concentrations of most analysed airborne fungal taxa were positively associated with air temperature and/or negatively associated with relative air humidity either throughout the year or only in summer. *Cladosporium* spore concentration was positively related with the wind speed, whereas the association between *Ganoderma* spore concentration and wind speed was negative. Spores of *Leptosphaeria* showed significant positive association with relative air humidity and significant negative association with sunshine duration in summer. Knowledge of seasonal patterns of the type and number of spores in the air will provide clinicians and sufferers of allergic asthma and rhinitis as well as agronomists with valuable information on the prophylaxis of respiratory allergic and plant diseases, respectively.

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1 Introduction

Fungal spores are reproductive or distributional structures produced during the life cycle of fungal organisms. They are a major contributor to the spectrum of airborne allergens (Oliveira et al. 2009), as many fungal air spores are allergenic and their inhalation can produce respiratory allergic diseases, such as bronchial asthma and allergic rhinitis (Caretta 1992; D'Amato and Spieksma 1995). An estimated 3–10% of the world's population is allergic to spores of fungal organisms (Bush and Portnoy 2001). On the other hand, some of airborne spore types are important in plant pathology as they can cause epidemics in agriculture (Sakiyan and Inceoglu 2003). Thus, knowledge of the spectrum and quantity of airborne fungal spores, as well as their seasonal and daily fluctuation affecting by weather conditions, permits to develop prediction models of the spore concentrations in the atmosphere (Grinn-Gofroń and Strzelczak 2008, 2009) which is of great importance from a clinical and agronomical point of view. However, only few countries have constructed spore calendars in order to evaluate the fungal spore conditions in the atmosphere (Mitakakis and Guest 2001; Gioulekas et al. 2004; Kasprzyk et al. 2004; Sánchez Reyes et al. 2016; Bednarz and Pawłowska 2016). The first investigation into airborne fungal spores in Slovakia was conducted in Bratislava where seven fungal spore types were identified (Chrenová et al. 2004). Moreover, Ščevková et al. (2016) analysed the effect of recent changes in air temperature on trends in airborne *Alternaria*, *Epicoccum* and *Stemphylium* spore seasons in Bratislava. To date, this is the first such detailed analysis of airborne fungal spore spectrum in Slovakia.

The main objective of the present study was to identify and quantify fungal spores in the atmosphere of Bratislava during the 1-year period (2016) and create the first spore calendar for this urban area. The data obtained could be applied in the prevention, diagnostic and treatment of seasonal allergic respiratory diseases, and they can allow efficient protection of plants against pathogens by applying fungicides.

2 Materials and methods

Bratislava (48°10'N, 17°10'E) is the capital of Slovakia and is located in the south-western part of Slovakia (Fig. 1) on the boundary between the Malé Karpaty Mountains and the Pannonian Lowland. The city is surrounded by farmland areas and forests which provide suitable media for spore production. The measuring site was 1 km east–west from Botanical garden of Comenius University, and it is one of the largest green complexes in Bratislava. This area is characteristic for location of many different species of trees, shrubs and herbaceous plants, which may be one potential source of spores recorded in the air.

The climate of Bratislava is temperate continental with warm summers and cold winters. The mean annual temperature corresponds to 10.6 °C with a mean temperature of 21.0 °C in July (the hottest month) and 0.1 °C in January (the coldest month) (Hrvol' 2014). The annual rainfall varies from 530 to 650 mm, as average, falling mostly during May–July (Hrnčiarová et al. 2006). Winds from the north-west are predominant all the year round.

Spectrum and quantity of fungal spores were measured from January to December 2016 by Hirst-type volumetric aerospore trap (Burkard model). The sampler was placed on the roof of the Department of Botany, Comenius University in Bratislava, in north-west part of the city at the height of 10 m above ground level. The monitoring station at this institute has been in operation since 2002 and is a part of the Slovak Aerospore Information Service (AIS) which operates aerospore monitoring network of samplers in Slovakia (Dušička et al. 2012). Air is suctioned into the sampler throughout a horizontally orientated 2 × 14 mm slit at a rate of 10 l/min and impacted against an adhesive tape that moves continuously past the slit at a rate of 2 mm/h. A detailed account is given in the British Aerobiology Federation's trapping guide (British Aerobiology Federation 1995). At sampling site, tape was changed weekly at 10:00 (local time). The tapes were cut into strips 48 mm long, each representing 24 h exposure, and were mounted on microscope slides in gelatin–glycerine and stained with basic fuchsin. Spores were counted in 12 transversal traverses per slide under a light microscope at a magnification of 400 × . After the spore counting in each sampling area, a specific correction factor for the used microscope was applied. Thus, the final spore

Fig. 1 Location of the aerospore sampling site



counts were expressed as mean daily spore concentrations per cubic meter of air (spores/m³). The fungal spores were identified according different atlases and morphology keys (e.g. Nilsson et al. 1983; Grant Smith 2000; Lacey and West 2006; Arora and Jain 2008). This standard air sampling and microscopic identification methodology is subject to quality controls (QC) for its data contributors (Costos-Yáñez et al. 2013). The Slovak Aerospore Information Service has been participated in an external QC exercises for microscopic identification of pollen grains and fungal spores carried out within the framework of the European Aerobiology Society's QC Working Group (Sikoparija et al. 2017).

The cut-point of the 7-day Burkard sampler is 5.2 µm (Willeke and Macher 1999); thus, the collection efficiency is higher for spores with an aerodynamic equivalent diameter greater than 5.2 µm.

The airborne spore taxa are listed in Table 1 in descending order based on their annual total spore concentrations. The spore calendar was constructed following the technique adopted by Spieksma (1991): mean daily spore concentrations of 10-day periods (in the case of months having 29 or 31 days, we used 9- or 11-day periods, respectively) were transformed into eight classes (Ščevková et al. 2010) (Fig. 2). Only those spore types whose annual percentage reached the value equal to or greater than 0.01% were included in the spore calendar. The individual fungal spore types are listed in spore calendar in chronological order based on the start day of their spore season, from earliest to latest.

To analyse the effect of meteorological parameters on the daily spore concentrations, following meteorological variables were taken into consideration: mean

daily surface air temperature (°C), mean daily relative air humidity (%), total daily rainfall (mm), mean daily wind speed (m/s), mean daily number of sunshine hours (hours) and mean daily frequency of winds (%) blowing from four quadrants (north-east, south-east, south-west and north-west). Frequency (F) was calculated as follows: $F = (\text{number of winds blowing from a given quadrant/number of total winds registered}) \times 100$. Meteorological data were recorded at meteorological observatory of the Department of Astronomy, Physics of the Earth and Meteorology of the Faculty of Mathematics, Physics and Informatics of Comenius University in Bratislava (48°09'N, 17°04'E, 182 m a. s. l.) situated 1 km north-west of the sampling site.

A regression analysis was performed in order to determine how much of total variance in airborne spore concentrations of five predominant fungal taxa (*Alternaria*, *Cladosporium*, *Coprinus*, *Ganoderma* and *Leptosphaeria*) can be attributed to selected meteorological variables. For each of the fungal taxa, a univariate multiple regression model was used to estimate the effects of the weather variables on the spore concentrations. The possible autocorrelation of the measurements was adjusted for by considering autoregressive correlation structure for the observations in the model. Because the daily proportions of the wind directions were dependent, the variable expressing the proportion of the north-west wind was not included in the model. (Its effect is hidden in the intercept.) To cover potential seasonal effects, dummy variables for the seasons (spring, summer and winter; the effect of autumn is included in the intercept term) and the interactions of the meteorological variables with the seasons were also added as predictors. The

Table 1 Annual total, percentage contribution and frequency of all fungal spore types found in the air of Bratislava

Spore type	Annual total spore count (spores/m ³)	Percentage of total	Frequency (% of days)
<i>Cladosporium</i>	601,209	71.88	99.7
<i>Coprinus</i>	73,925	8.84	97.8
<i>Leptosphaeria</i>	32,428	3.88	71.9
<i>Ganoderma</i>	28,677	3.43	67.7
<i>Alternaria</i>	23,370	2.79	93.3
<i>Myxomycetes</i>	21,798	2.61	95.2
<i>Agrocybe</i>	9651	1.15	85.9
<i>Epicoccum</i>	8096	0.97	94.2
<i>Periconia</i>	4687	0.56	74.8
<i>Exosporium</i>	4603	0.55	73.8
<i>Fusarium</i>	4422	0.53	73.2
<i>Didymella</i>	2133	0.26	43.8
<i>Diatrypaceae</i>	2039	0.24	23.6
<i>Pleospora</i>	2228	0.27	69.6
<i>Torula</i>	1641	0.20	76.7
<i>Botrytis</i>	1556	0.19	46.0
<i>Oidium</i>	1500	0.18	72.5
<i>Cercospora</i>	1335	0.16	43.1
<i>Urediniospores</i>	1313	0.16	48.2
<i>Ascospores</i>	1271	0.15	48.2
<i>Stemphylium</i>	1242	0.15	73.8
<i>Gliomastix</i>	1054	0.13	42.8
<i>Peronospora</i>	1028	0.12	67.4
<i>Pithomyces</i>	918	0.11	47.9
<i>Tilletia</i>	827	0.10	38.0
<i>Aspergillus/Penicillium</i>	822	0.10	15.3
<i>Polythrincium</i>	753	0.09	41.9
<i>Fusicladium</i>	411	0.05	64.5
<i>Panaeolus</i>	238	0.03	24.0
<i>Caloplaca</i>	202	0.02	12.5
<i>Drechslera</i>	188	0.02	37.1
<i>Nigrospora</i>	177	0.02	33.9
<i>Cerebella</i>	151	0.02	19.2
<i>Bovista</i>	90	0.01	10.9
<i>Urocystis agropyri</i>	80	0.01	14.7
<i>Arthrinium</i>	70	0.01	8.3
<i>Melanospora</i>	45	0.01	10.9
<i>Passeriniella</i>	38	< 0.01	7.3
<i>Ascobolus</i>	35	< 0.01	11.2
<i>Pestalotiopsis</i>	35	< 0.01	5.4
<i>Spilocaea</i>	34	< 0.01	8.6
<i>Amphisphaeria</i>	17	< 0.01	3.2
<i>Curvularia</i>	12	< 0.01	3.5
<i>Spegazzinia</i>	12	< 0.01	3.5

Table 1 continued

Spore type	Annual total spore count (spores/m ³)	Percentage of total	Frequency (% of days)
<i>Massaria</i>	11	< 0.01	1.6
<i>Asterosporium</i>	9	< 0.01	2.9
<i>Neohendersonia</i>	9	< 0.01	2.9
<i>Oncopodiella</i>	9	< 0.01	1.9
<i>Sporormiella</i>	9	< 0.01	2.9
<i>Sporidesmium</i>	6	< 0.01	1.9
<i>Puccinia</i>	2	< 0.01	0.6
<i>Helicoma</i>	1	< 0.01	0.3
<i>Tetraploa</i>	1	< 0.01	0.3
Total	836,418	100.00	

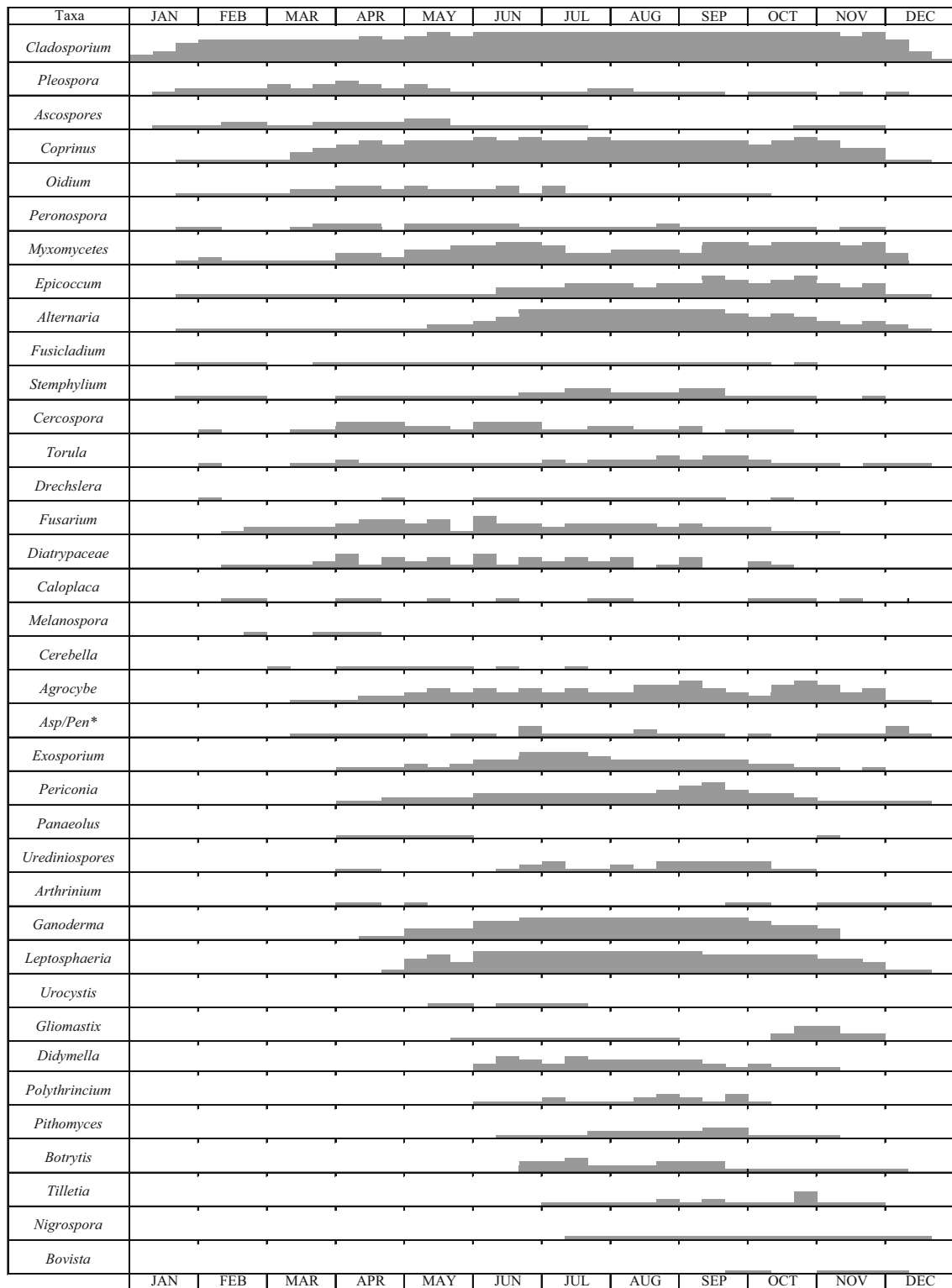
regression analysis with correlated observations was performed in the statistical software R (version 3.4.1), by choosing the autoregressive correlation structure in the function *gls* from the *nlme* package. The selection of the autoregressive order was automatic, based on the residuals of the regression model that did not allow for correlation in the observations. By sequentially testing the significances of the submodels using likelihood ratio tests, insignificant variables ($p > 0.05$) were removed from the model.

3 Results

A total of 836,418 fungal spores belonging to 53 spore types were identified in the air of Bratislava during 2016 (Table 1). Figure 3 represents five major taxa obtained during the study period as they represent 90.82% of total annual spore concentrations recorded in the air of Bratislava. The greatest total spore concentration was attributed to *Cladosporium* (71.88% of the total) followed by *Coprinus* (8.84%), *Leptosphaeria* (3.88%), *Ganoderma* (3.43%) and *Alternaria* (2.79%). Remaining 48 spore types were less well represented (altogether 9.18% of the total) (Table 1; Fig. 3). *Cladosporium* was both the most abundant spore type and the spore type that reached the highest daily value (13,812 spores/m³ in July 18). On the other hand, of these five major spore types, *Ganoderma* registered the lowest daily value (644 spores/m³ in September 4).

Fungal spores occur in the air of Bratislava throughout the whole year (Fig. 2). Airborne spores of *Cladosporium*, *Coprinus*, *Myxomycetes*, *Epicoccum* and *Alternaria* were trapped most frequently (> 90% of days), while *Oncopodiella*, *Sporidesmium*, *Puccinia*, *Helicoma* and *Tetraploa* were rare in sample (< 2%) (Table 1).

On the basis of the constructed spore calendar, *Cladosporium* registered the highest spore concentration (> 1000 spores/m³; exponential class h) in the second 10-day mean period of May, in the last of November and from the first of June to the first of November, while *Coprinus* reached 501–1000 spores/m³ (class g) in the first and last 10-day mean periods of June, last of July and October. *Leptosphaeria* (from the first 10-day mean period of June to the first of September), *Ganoderma* (from the last 10-day mean period of June to the last of September), *Agrocybe* (first 10-day mean period of September and last of November), *Alternaria* (from the last 10-day mean period of June to second of September), *Epicoccum* (second 10-day mean period of September and last of October) and *Myxomycetes* (last two 10-day mean periods of June, September and October, and first and last of November) counted 101–500 spores/m³ (class f). *Fusarium* and *Exosporium* registered 51–100 spores/m³ (class e) in first 10-day mean period of June, and in last of June and in first two of July, respectively. The rest of spore types included in the spore calendar did not register 10-day mean greater than 50 spores/m³ (Fig. 2).

* Asp/Pen (*Aspergillus*/*Penicillium*)

a b c d e f g h

Fig. 2 Spore calendar for Bratislava Exponential classes (spores/m³): **a** 1–5, **b** 6–10, **c** 11–25, **d** 26–50, **e** 51–100, **f** 101–500, **g** 501–1000, **h** > 1000

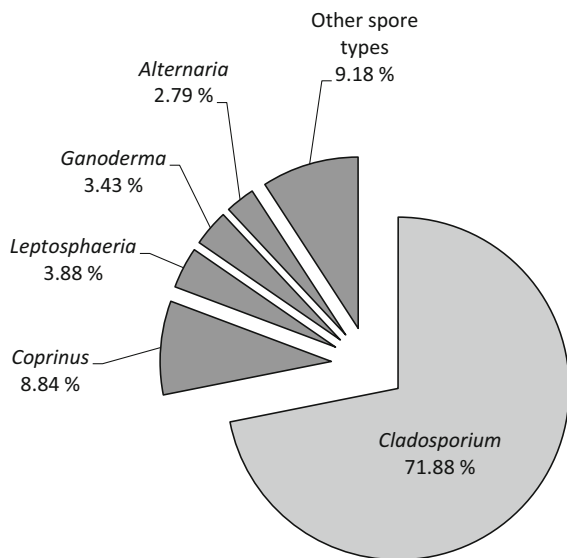


Fig. 3 Relative contributions (% of total spore concentration) of the major spore types in the air of Bratislava

The highest spore concentrations were recorded in summer (198,703 spores/m³, 23.81% of the total were collected in July and 153,342 spores/m³, 18.38% in June) and in autumn, mainly in September (131,479 spores/m³, 15.76% of the total). On the other side, we could find the months of December (3128 spores/m³, 0.37%), January (1381 spores/m³, 0.17% of the total) and February (4318 spores/m³, 0.52%) corresponding to winter season (Fig. 4).

During the study period, different peaks of total monthly spore concentration were obtained for the major spore types (Fig. 4). *Leptosphaeria* showed one peak in July, while *Ganoderma* peaked in September and October. *Alternaria* and *Cladosporium* peaked in July and September, while *Coprinus* peaked in June and October.

The prevailing weather conditions recorded during the study period revealed an average air temperature of 11.1 °C, where the minimum and maximum temperature ranged between – 2.7 and 27.8 °C, respectively. The absolute air humidity and rainfall recorded average values of 8.12 g/m³ and 4.3 mm, respectively (Fig. 5).

Table 2 shows the results of multiple regression analysis, which was performed in order to identify the weather variables which the most influenced airborne fungal spore concentrations. For the sake of brevity, only significant meteorological variables from the final models are shown in Table 2. (For example, the insignificant interaction term “Rainfall: Spring” is not in the table, although it was included in the final model for *Alternaria*.) For *Alternaria* and *Cladosporium*, the regression models explained 49.7% and 45.8% of the total variance, respectively. Multiple regression analysis consisting of mean daily air temperature, mean daily relative air humidity and total daily rainfall explained the variability in *Alternaria* spore concentrations in summer, while for *Cladosporium*, the major explanatory variables were mean daily air temperature and mean daily wind speed. The most important agents influencing spore concentration of *Ganoderma* are mean daily air temperature and mean daily wind speed (throughout the year) and mean daily relative air humidity (in summer). For mentioned spore type, the regression model explained 46.9% of the total variance. Throughout the year, *Leptosphaeria* spore concentration was found to be significantly influenced by winds blowing from south-east, whereas the number of sunshine hours and mean daily relative air humidity were the most influential variables in summer. Mean daily air temperature showed to have some influence on the *Coprinus* spore concentration. For *Leptosphaeria* and *Coprinus*, the regression models explained 43.9% and 32.1% of the total variance, respectively.

4 Discussion

This aeromycological study has been based on traditional visual (microscopic) identification of fungal spores which enables comparisons with other historical and current fungal spore calendars in locations near Slovakia and in other locations around the world. However, in the near future, greater emphasis will place on modern molecular methods (e.g. DNA or RNA probe technology, PCR—polymerase chain reaction) of identifying and counting of airborne fungal spores (Green 2018) in order to avoid the tedious nature of microscopic spore identification and quantification and enabling the detection of a wide range of so far unidentified spores.

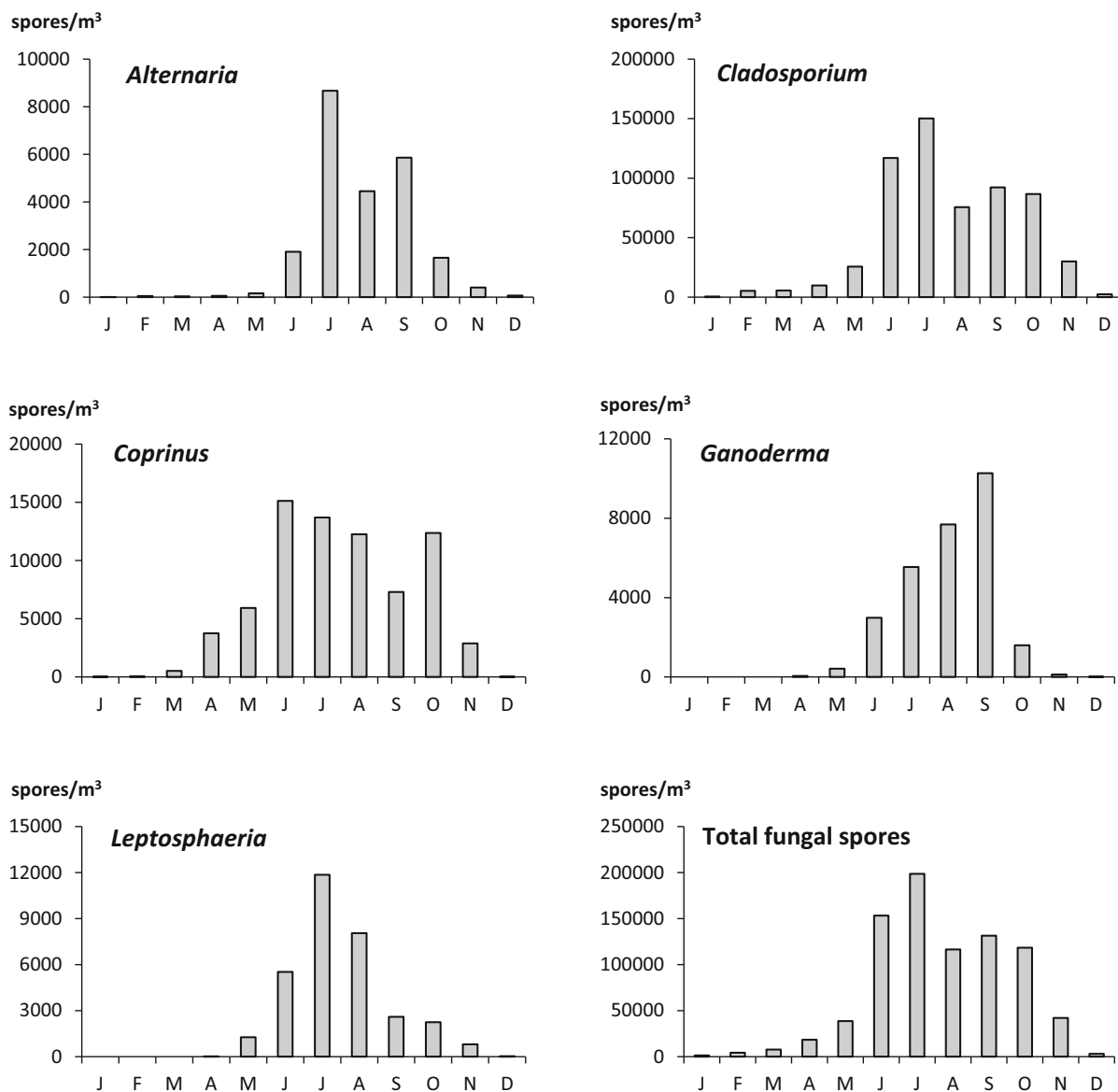
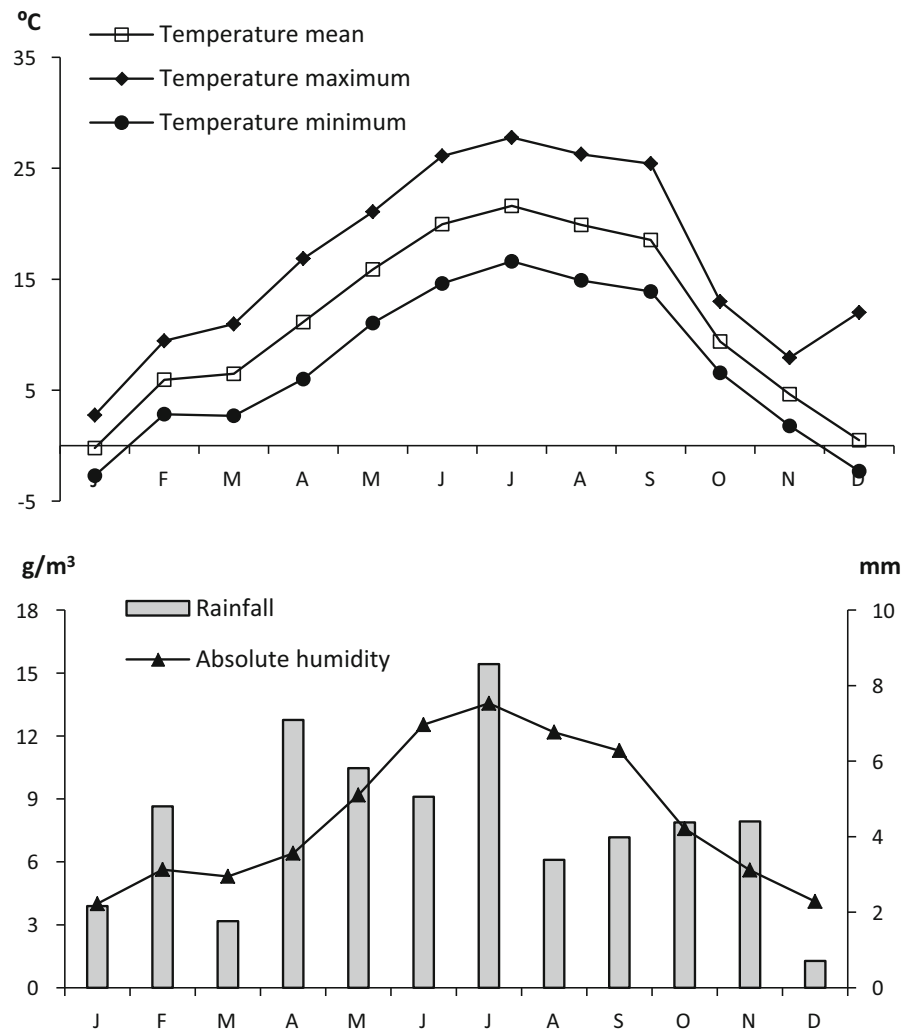


Fig. 4 Monthly variations in the spore concentrations of the major fungal taxa and total fungal spores in the air of Bratislava

The annual total spore concentration in the atmosphere of Bratislava in 2016 (836,418 spores/m³) was high compared to other regions. The annual spore totals reached the values from 917 spores/m³ in Sonora (Mexico) (Moreno-Sarmiento et al. 2016) to 595,199 spores/m³ in Szczecin (Poland) (Bednarz and Pawłowska 2016). The fungal spore concentrations vary depending on the climatic and weather conditions of each locality as well as the local spore sources (Lacey 1981). The city in temperate regions of Europe that is comparable to this in our study is Szczecin. The

climate in this city is characterized by cold summers, which differs from that of our study area. The weather conditions in Bratislava during the study period (which had an annual average air temperature of 11.1 °C and total annual rainfall reached 776.4 mm) in conjunction with the forests (Malé Karpaty Mountains) and farmland areas (Podunajská nížina Lowland) surrounding the city may favour the sporulation of fungal organisms, making the total annual spore concentration higher than that in other studies.

Fig. 5 Prevailing weather conditions (mean, maximum and minimum air temperature, absolute air humidity and rainfall) recorded during the study period



The previous study only reported the presence of seven spore types (*Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Puccinia* and *Stemphylium*) in the air of Bratislava (Chrenová et al. 2004). In the current study, 53 airborne spore types were identified in Bratislava throughout the year (Table 1), while in other European regions the spectrum of airborne fungal spore types varied between 10 and 35 spore types (Kasprzyk et al. 2004; Ianovici and Tudorica 2009; Bednarz and Pawłowska 2016). *Cladosporium* was both the most abundant (71% of the total spore concentration) and the most frequently occurring (99.7% of days) airborne spore type in the study area. *Cladosporium* spores were also reported as a predominant spore type in Szczecin (Poland) (Bednarz and Pawłowska 2016), Rzeszów (Poland)

(Kasprzyk et al. 2004), Madrid (Spain) (Díez Herrero et al. 2006), Valladolid (Spain) (Sánchez Reyes et al. 2016), Funchal (Portugal) (Sousa et al. 2016), Timișoara (Romania) (Ianovici 2016), Melbourne (Australia) (Mitakakis and Guest 2001), Havana (Cuba) (Almaguer et al. 2015), Santiago (Chile) (Ibáñez Henríquez et al. 2001) and in Montevideo (Uruguay) (Martínez Blanco et al. 2016). The long presence of *Cladosporium* spores in the air showed a similar pattern with other regions with different weather conditions (Mitakakis and Guest 2001; Kasprzyk et al. 2004; Díez Herrero et al. 2006; Ianovici and Tudorica 2009; Hasnain et al. 2012; Almaguer et al. 2015; Martínez Blanco et al. 2016; Sousa et al. 2016; Bednarz and Pawłowska 2016; Sánchez Reyes et al. 2016). The abundance of spores

Table 2 Significant meteorological variables in multiple regression models for selected fungal spore types in Bratislava

Spore type	Variables	β Coeff.	SE	p value	R^2	Proportion of variance explained (%)
<i>Alternaria</i>	Intercept	39.1448	24.7715	0.1151	0.497	49.7
	Temperature: summer	13.7446	5.1356	0.0079		
	Relative humidity: summer	-5.6284	1.4997	0.0002		
	Rainfall: summer	-9.2162	2.0479	< 0.0001		
<i>Cladosporium</i>	Intercept	1642.1977	338.7345	< 0.0001	0.458	45.8
	Temperature	171.3087	28.9905	< 0.0001		
	Wind speed	186.5544	79.8047	0.0200		
<i>Coprinus</i>	Intercept	137.0323	35.2038	0.0001	0.321	32.1
	Temperature	16.8521	2.8110	< 0.0001		
<i>Ganoderma</i>	Intercept	68.9952	55.6982	0.2164	0.469	46.9
	Temperature	2.3868	1.1561	0.0398		
	Wind speed	-11.5918	3.9518	0.0036		
	Relative humidity: summer	-3.7783	0.9300	0.0001		
<i>Leptosphaeria</i>	Intercept	131.7430	56.6106	0.0206	0.439	43.9
	Wind direction SE	-1.0933	0.3763	0.0039		
	Sunshine: summer	-24.5091	7.6944	0.0016		
	Relative humidity: summer	10.0514	3.2586	0.0022		

of *Cladosporium* throughout the year may be attributed to their small size, low weight and low density, which facilitate aerodispersal of these spores (Shaheen 1992). *Coprinus* (8.84% of the total spore concentration) and *Leptosphaeria* (3.88%) are the second and the third most important airborne spore types in Bratislava, respectively (Table 1). In the case of *Coprinus*, the percentage is similar to that of Madrid (Díez Herrero et al. 2006), but lower than that of Melbourne in which this spore type represented 14.6% of the total spore concentration (Mitakakis and Guest 2001). On the other hand, spores of *Coprinus* reached less than 0.1% of the total spore concentration in Szczecin (Bednarz and Pawłowska 2016). *Leptosphaeria*, with the value of 1.21% of the total spore concentration is considered as the fourth most important fungal spore type in Szczecin. *Leptosphaeria* was considered as one of the most numerous airborne fungal spores also in Melbourne (Mitakakis and Guest 2001), Havana (Almaguer et al. 2015) and Montevideo (Martínez Blanco et al. 2016). Some other studies confirm the atmospheric relevance of *Ganoderma* and/or *Alternaria* spore types (Mitakakis and Guest 2001; Ibáñez Henríquez et al. 2001; Kasprzyk et al. 2004; Kasprzyk and Worek 2006; Celenk et al. 2007; Ianovici and Tudorica 2009; Hasnain et al. 2012;

Moreno-Sarmiento et al. 2016; Martínez Blanco et al. 2016; Sousa et al. 2016; Bednarz and Pawłowska 2016; Ianovici 2016; Sánchez Reyes et al. 2016), the fourth and fifth most important spore types in the air of Bratislava, respectively. High concentrations of *Coprinus* and *Ganoderma* airborne spores in the urban area of Bratislava could be due to the abundance of adequate substrates in the spore trap surroundings (crop fields and woody areas), as the presence of spores of these plant pathogens is more related to rural than urban areas (Kasprzyk and Worek 2006). Despite the fact that *Aspergillus/Penicillium* are considered to be important fungal spores from both clinical and phytopathological point of view (Myszkowska et al. 2004), only low annual total spore concentration (827 spores/m³; 0.1% of the total) of these taxa was observed in the air of Bratislava. This value is similar to those observed in Szczecin (Poland) with the annual total concentration of 812 spores/m³ (Bednarz and Pawłowska 2016); however, this is very low when compared to the values (5512 and 12,746 spores/m³/year) registered in Madrid (Spain) (Díez Herrero et al. 2006) and Havana (Cuba) (Almaguer-Chávez et al. 2018), respectively. On the other hand, Mitakakis and Guest (2001) and Martínez Blanco et al. (2016) have not registered any spores of *Aspergillus/Penicillium*

taxa in the air of Melbourne (Australia) and Montevideo (Uruguay), respectively.

Although *Alternaria* and *Coprinus* spore types reached much lower values than those of *Cladosporium*, a similar two peaks seasonal pattern of these spore types was observed, with peak values occurred in July, September and June, October, respectively (Fig. 4). Similarly to our results, Celenk et al. (2007) reported two peaks seasonal pattern (observed in July and September) for *Cladosporium*; however, the peak occurred in July was much larger of the two.

Airborne spores occurred in Bratislava throughout the whole year. However, peak concentrations were recorded in summer and early autumn, while minimum spore concentrations were observed in winter and spring (Fig. 4). Similar seasonal pattern has been found by Kasprzyk (2008). In Bratislava, summer season is characterized by intensive plant growth, while in autumn, the decay of plant material is in progress. The large quantity of plant material, whether fresh or decay, could be considered as the potential growth material for fungal organisms. Based on these results, peak spore concentrations during summer and early autumn months might be due to favourable climatic and weather conditions that allow the growth and/or decomposition of plant material. Additionally, many of the fungal organisms whose spores are present in the air of studied area are plant pathogens. Consequently, the large number of airborne spores detected in summer and early autumn may be attributed to harvesting of crops (Dixit et al. 2000) since the crop fields are located in the vicinity of the spore trap.

The quantity of fungal spores in the atmosphere markedly relates to meteorological factors. In present study, the highest spore concentrations were occurred in June, July and September (Fig. 4), when air temperature and rainfall provide the optimum conditions for the growth and sporulation of fungal organisms (Fig. 5). In August, the decrease in the amount of spores in the air of Bratislava (Fig. 4) may be related to a drop in both air temperature and amount of rainfall (Fig. 5).

Based on the results of the multiple regression analysis, air temperature and relative air humidity explained the variability in *Alternaria* and *Ganoderma* spore concentrations throughout the year or only in summer. The relationships between air temperature and spore concentrations of mentioned fungal taxa

were positive, while the relationships between relative air humidity and spore concentrations were negative. Similar results have been reported by Corden and Millington (2001), Aira et al. (2008) and Ianovici (2016). According to the other researchers (Nolard et al. 2001), this phenomenon is probably linked to the fact that, in order to be released into the air, airborne fungal spores require a certain degree of dryness, which occurs when the air temperature increases.

Rainfall was also significant factor explaining the variability in *Alternaria* airborne spore concentrations in summer, exhibited significant and negative relationship with mentioned spore type. Similarly to this study, negative association between *Alternaria* and rainfall was also observed by several other researchers (Corden and Millington 2001; Martínez Blanco et al. 2016; Ianovici 2016). This result should be explained by the fact that rainfall removes airborne spores by both rainout and washout effects (Magyar et al. 2009; Artaç et al. 2014). Moreover, because *Alternaria* spore concentration was negatively related also with relative air humidity in this present study, it could be argued that precipitation could have caused an increase in relative air humidity which could result in a decrease in *Alternaria* spore level in the atmosphere.

Wind speed plays an important role in spore release and dispersal. This weather parameter was significant predictor of *Ganoderma* and *Cladosporium* spore concentrations in the air of Bratislava. We observed a negative relationship between mentioned weather parameter and spore concentrations of *Ganoderma*, similar to that found in previous studies (Martínez Blanco et al. 2016; Grinn-Gofroń et al. 2018). In contrast to these results, we observed significant positive correlations between wind speed and *Cladosporium* spore concentrations. Similarly to our results, Stępańska et al. (2012) observed that wind velocity favoured high *Didymella* spore counts. Several researchers (e.g. Martínez Blanco et al. 2016; Oliveira et al. 2005 and Peel et al. 2014) pointed out that suction efficiency of Hirst-type 7-Day sampler is sensitive to changes in the wind speed, by virtue of the difference between ambient wind speed and the flow rate through the sampler's inlet causing air to accelerate or decelerate as it enters the sampler. This could lead, with respect to aerospore's physical properties, namely, size, shape, density and surface characteristic (Mandrioli 1998), to increase or decrease in spore concentrations. In this context, we suggest that small,

approximately spherical and smooth spores of *Ganoderma* ($6\text{--}12 \times 4\text{--}8 \mu\text{m}$) (Almaguer et al. 2015) could be due to air acceleration deviated from the trajectory from the inlet stream and, consequently, the spore concentration decreases, while larger, ellipsoidal and verrucose *Cladosporium* spores ($8\text{--}25 \times 4\text{--}8 \mu\text{m}$) (Almaguer et al. 2015) are suctioned through the sampler's inlet; thus, the spore concentration increases.

Among the meteorological parameters, relative air humidity and sunshine duration were the most consistent predictors for *Leptosphaeria* spore concentrations in summer. The relationship between spore concentrations of mentioned taxa and relative air humidity was positive, while the association with the number of sunshine hours was negative. Research conducted in Taiwan (Kallawicha et al. 2017) has reported positive association between relative air humidity and ascospores (including *Leptosphaeria*) and negative association between sunlight and ascospores, which is similar to our findings. The positive influence of relative air humidity and negative influence of the sunshine duration on *Leptosphaeria* spore levels in the air could be explained by the fact that wet-air spore types require humid conditions to activate the sporulation process (Shaheen 1992; Calderón et al. 1995; Díez Herrero et al. 2006; Almaguer-Chávez et al. 2018). However, longer duration of sunshine, especially in summer, leads to the desiccation of the atmosphere and thus could lead to deceleration of sporulation process. Moreover, García-Fernández et al. (2012) pointed out that high light intensity and longer exposure duration can damage fungal cells and lead to cell death.

Significantly lower *Leptosphaeria* spore concentrations were observed when winds blew predominantly from south-east direction. This result should be explained by the fact that to the south-east from monitoring station open landscape of Podunajská nížina Lowland is situated. It is likely that this open landscape wind could transport spores to the north-west beyond the city.

For *Coprinus*, air temperature was the most consistent predictor for its spore concentration. However, the low performance of regression for *Coprinus* (Table 2) suggests that there are other factors influencing its airborne spore concentration that are more important than meteorological variables.

5 Conclusion

A great diversity of fungal spores was registered in the atmosphere of Bratislava throughout the year 2016. Among the 53 airborne fungal spore types identified, the most frequent was *Cladosporium*, followed by *Coprinus*, *Leptosphaeria*, *Ganoderma* and *Alternaria*. Total airborne spore densities varied seasonally. The highest spore concentrations were recorded in summer and early autumn, when the meteorological conditions are favourable for growth and sporulation of spores, by contrast the winter and early spring months when the lowest concentrations were observed.

The results of the multiple regression analysis revealed that meteorological parameters were important determinants of fungal spore concentrations explained between 32.1 and 49.7% of the total variance in spore concentration. For most analysed fungal taxa, their spore concentrations showed significant positive association with air temperature and/or significant negative association with relative air humidity either throughout the year or only in summer. *Cladosporium* spore concentration was positively related with the wind speed, whereas the association between *Ganoderma* spore concentration and wind speed was negative. Spores of *Leptosphaeria* showed significant positive association with relative air humidity and significant negative association with sunshine duration in summer.

The patients suffering from respiratory allergies in the Bratislava region may benefit from the created spore calendar by avoiding high exposure to fungal spores. Moreover, the results of our study can be helpful for allergists in prevention of fungal allergic diseases.

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