



Intradiurnal variation of predominant airborne fungal spore biopollutants in the Central European urban environment

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Abstract

Fungal spores are an important cause of allergic respiratory diseases worldwide. However, little is known about the intradiurnal pattern of spore concentrations of different fungal spore types in the air of the urban area. In this study, we evaluated bihourly variation in spore concentration of eight predominant fungal spore types in the atmosphere of Bratislava city (*Agrocybe*, *Alternaria*, *Cladosporium*, *Coprinus*, *Exosporium*, *Epicoccum*, *Ganoderma*, *Leptosphaeria*) with the aim to understand the relationships between the spore concentrations against associated environmental variables. Spore samples were collected using a Hirst-type volumetric aerospore trap from January to December 2016. *Alternaria*, *Cladosporium*, *Epicoccum* and *Exosporium* peaked during the daytime period between 10:00 and 16:00, while for *Agrocybe*, *Ganoderma*, *Coprinus* and *Leptosphaeria*, the nighttime peaks (20:00 and 04:00) were observed. Effect of a complex of environmental variables on bihourly concentrations of selected airborne fungal spore taxa was evaluated through multiple regression analysis. Air temperature, wind speed, sunshine duration and precipitation were positively associated with daytime spore types, while the association with nighttime spores was negative. In contrast, relative air humidity influenced negatively *Exosporium* daytime spore type but positively the *Leptosphaeria* nighttime spore type. Moreover, a circadian cycle of light and darkness was considered as an important predictor of nighttime spore levels. Among the atmospheric pollutants, PM₁₀ was positively associated with all analysed daytime spores, while except for *Leptosphaeria*, O₃ was negatively associated with nighttime spore types. NO₂ and PM₁₀ had mixed effects on nighttime spore levels. In general, air temperature, PM₁₀ and wind speed were environmental parameters with great influence on airborne fungal spore concentration, being present in eight, seven and four regression models, respectively. Constructed regression models which the best explained variation in fungal spore concentrations were those for *Ganoderma* ($R^2 = 0.38$) and *Alternaria* ($R^2 = 0.31$).

Keywords Fungal spores · Weather parameters · Aerobiology · Aeroallergen · Air pollutants · Regression analysis

Introduction

Fungal spores, the reproductive or distributional structures produced during the life cycle of fungal organisms, are very numerous in the air (Kasprzyk 2008). They can have severe economic impacts through the spread of plant diseases (Rossi

et al. 2005) and biodeterioration of art sculptures or architectural pieces (Caneva et al. 2003). Moreover, some of them are known as natural aeroallergens that may cause allergic respiratory diseases in sensitive individuals (D'Amato and Spieksma 1995). According to Yamamoto et al. (2012), 2.8–10.7% of total airborne spore types are considered as allergenic. The prevalence of spore-induced allergic reactions has been increasing worldwide during the past several decades and is estimated to be approximately 10% of the world's population which is allergic to spores of fungal organisms (Maio et al. 2011).

Although the quantity of fungal spores in the air varies both throughout the season and day, the published literature refers mainly to seasonal variability. Much fewer researchers worked on the intradiurnal pattern (Stępańska and Wołek 2009; Oliveira et al. 2009; Grinn-Gofroń et al. 2018). However, information concerning high allergy risks during

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the daily hours would be helpful for allergy sufferers to plan outdoor activities because allergy symptoms often culminate with the peak spore concentrations (Lin et al. 2016). Intradaily variations of airborne fungal spores depend, among others, on weather conditions which affect the sporulation, dispersal and deposition of spores. Based on their occurrence in the air during different weather conditions, fungal spores are divided into two groups, 'dry' and 'wet' spore types. Dry spores (xerophilic) are found in the greatest abundance in the air under conditions of decreasing humidity and increasing airflow (Elbert et al. 2007). On the other hand, wet spores (hydrophilic), belonging to Ascomycetes and Basidiomycetes, are released from sporangium into the atmosphere by processes related to humidity conditions or rain (Horner 1992).

During the daily hours, the distribution of airborne spores may also be influenced by air pollutants, including particulate matter with an aerodynamic diameter $\leq 10 \mu\text{m}$ (PM_{10}), ozone (O_3), nitrogen dioxide (NO_2) and carbon monoxide (CO) as it may interact with the atmospheric processes and affects the weather conditions (Pyrri and Kapsanaki-Gotsi 2017). However, the impact of air pollutants on airborne fungal spores has been rarely considered (Lin and Li 2000; Ho et al. 2005; Sousa et al. 2008; Grinn-Gofroń et al. 2011).

In Slovakia, little attention has been paid to outdoor airborne spores of fungal organisms. Although the seasonal distribution of fungal spores has previously been investigated in Bratislava (Chrenová et al. 2004; Ščevková et al. 2016; Ščevková and Kováč 2019), their intraday distribution was not studied in this country at all. Therefore, this study aims at presentation of an intraday pattern of the spore concentrations of eight predominant allergenic spore types in the air of Bratislava and examination of the environmental parameters that had the greatest impact on the pattern. The result of this study could be useful for clinicians and allergy sufferers, in order to define preventive avoidance strategies. Moreover, this study could also be useful for farmers in order to avoid economic losses due to fungal plant diseases by correct timing of the application of fungicides.

Materials and methods

Study area

Bratislava, the capital of Slovakia, is a city of around 430,000 inhabitants with about 1164 persons/ km^2 density. Bratislava is located in the south-western part of Slovakia (Fig. 1) on the boundary between the Malé Karpaty Mountains and the Pannonian Lowland. The river Danube flows through the middle of the city. The landscape of its surroundings is constituted by farmland areas (mainly cereal fields and vineyards) and deciduous Carpathian forests dominated by *Quercus* spp.,

Carpinus betulus L. and *Fagus sylvatica* L. and alluvial forests dominated by *Ulmus* spp., *Fraxinus* spp., *Salix* spp. and *Populus* spp., among others. Its climate is temperate continental with warm summers and cold winters. In 2016, mean annual temperature was 11.1 °C. The coldest month was January (mean -0.2 °C) and the warmest was July (mean 21.6 °C). Over the study period, the total precipitation was 776.4 mm, with the maximum value in July (137.1 mm) and the minimum value in December (12.1 mm).

Aerobiological data

Airborne fungal spores were collected from January to December 2016 by using Burkard 7-day volumetric aerospore sampler, placed on the roof of the Department of Botany, Faculty of Natural Sciences building (48° 08' 46" N, 17° 04' 43" E, 157 m a. s. l.), in the north-west part of the city at 10 m above ground level. This area is surrounded by urban vegetation, mainly by ornamental trees and shrubs cultivated in the parks and gardens and by vegetation along footpaths, roads and buildings. Botanical garden and Danube River are located in the vicinity. Single-family houses are the dominant building types. The methodology used was performed according to the standard method adopted by the British Aerobiology Federation (1995). 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. The sampling tape was changed weekly at 10:00 (local time—UTC + 2 h in the summer, UTC + 1 h in the winter). The tapes were cut into segments 48 mm long, each representing 24 h exposure, and were mounted on microscope slides in gelatin-glycerin and stained with basic fuchsin. Spores were counted in 12 transversal traverses per slide under a light microscope at a magnification of $\times 400$. After the spore counting in each sampling area, a specific correction factor for the used microscope was applied. Results were expressed as bihourly average spore concentrations per cubic metre of air (spores/ m^3).

Meteorological and air pollution data

To analyze the effect of meteorological parameters on the intraday spore concentrations, bihourly records of following meteorological variables were taken into consideration: T—surface air temperature (°C), RH—relative air humidity (%), P—precipitation (mm), WS—wind speed (m/s), S—sunshine duration (h) and AP—atmospheric pressure (hPa). Meteorological data were recorded at the meteorological observatory of the Department of Astronomy, Physics of the Earth and Meteorology of the Faculty of Mathematics, Physics and Informatics (48° 09' 04" N, 17° 04' 14" E, 182 m a. s. l.) situated 1 km NW of the sampling site.



Fig. 1 Localization of Bratislava in Slovakia

Bihourly records of atmospheric pollutants, PM₁₀—particulate matter $\leq 10 \mu\text{m}$ ($\mu\text{g}/\text{m}^3$), O₃—ozone ($\mu\text{g}/\text{m}^3$), NO₂—nitrogen dioxide ($\mu\text{g}/\text{m}^3$) and CO—carbon monoxide ($\mu\text{g}/\text{m}^3$), were obtained from the database of the Slovak Hydrometeorological Institute (SHMÚ), which has four air quality monitoring stations throughout Bratislava. The data used in the analysis were calculated as an average of data obtained from all four air quality monitoring stations.

Data analysis

Analysis of bihourly mean spore concentrations was performed for eight fungal taxa: *Alternaria*, *Cladosporium* and *Epicoccum* of Deuteromycetes; *Agrocybe*, *Coprinus* and *Ganoderma* of Basidiomycetes; and *Exosporium* and *Leptosphaeria* of Ascomycetes. These taxa were selected according to allergenic capacity and their high-level presence in the air of Bratislava.

The analysis of the intradiurnal variation in airborne spore concentration was performed during the main spore season (MSS) for each spore type (Table 1). MSS has been calculated following the methodology designed by Nilsson and Persson (1981). This method includes 90% of the seasonal total spores and comprises from the day in which 5% of total spores is registered to the day in which 95% of total spores is registered.

The following time intervals were selected to reflect airborne spore levels during day and night: 08:00–20:00 (equivalent of daytime, 12 h) and 20:00–08:00 (equivalent of nighttime, 12 h).

The effects of the environmental variables on the airborne spore concentration of selected fungal taxa were analysed

using linear regression models in the statistical software R (version 3.5.2). An indicator variable for daytime/nighttime period and its interactions with relative air humidity and air temperature were added to the models to cover potential effects of the circadian cycle of light and darkness. To correct for autocorrelation of the measurements, autoregressive—moving average (ARMA) correlation structure was selected for the models by using the function *gls* from the *nlme* package. The autoregressive and the moving average orders were determined automatically by the function *auto.arima* from the *forecast* package applied on the residuals of the regression model that did not allow for correlation in the observations. For each fungal taxon, a regression model containing all

Table 1 Characteristics of the main spore seasons of selected fungi taxa in the air of Bratislava, in 2016

Fungal taxa	Characteristics*		
	SS	SL	Max
<i>Agrocybe</i>	12 May–13 Nov	186	306 (27 Octo)
<i>Alternaria</i>	25 June–17 Octo	115	908 (26 July)
<i>Cladosporium</i>	14 May–1 Nov	172	13,812 (17 July)
<i>Coprinus</i>	22 April–29 Octo	191	1100 (27 July)
<i>Epicoccum</i>	22 June–20 Nov	152	231 (23 Octo)
<i>Exosporium</i>	28 May–30 Sept	126	210 (24 June)
<i>Ganoderma</i>	19 June–1 Octo	105	644 (3 Sept)
<i>Leptosphaeria</i>	2 June–20 Octo	141	1541 (28 July)

*SS start and end dates, respectively; SL length of spore season; and Max maximum daily mean spore concentrations (spores/m³) and its date (in parenthesis)

considered explanatory variables was fitted and insignificant variables (p value $> \alpha$, where the significance level α is 0.05) were sequentially removed from the model by using likelihood ratio tests. Note that p values for some variables are above 0.05 in Table 2. However, the removal of any of these variables would result in significant loss of information compared to the model with all explanatory variables (p value < 0.05); therefore, they were retained in the final models.

Results

The intradiurnal evolution of the levels of meteorological and air pollution parameters in Bratislava during the study period based on the bihourly evolution is presented in Figs. 2 and 3. The intradiurnal trend of air temperature pointed out a gradual increase during the morning, remaining high during noon and early afternoon and then gradually decreasing until the pre-dawn. Relative air humidity remains between 60 and 70% from noon to early evening, while throughout the rest daily hours, relative air humidity remains between 70 and 85%. The intradiurnal variations of wind speed and sunshine duration showed a similar pattern with the highest values at noon and the lowest values between 20:00 and 04:00. The maximum air pressure was observed at 08:00, whereas higher precipitation was observed between 20:00 and 06:00. Taking into account air pollutants, the highest PM₁₀ and O₃ concentrations were observed at 10:00 and 14:00, respectively, while during the early morning hours, the minimum concentrations of two above-mentioned air pollutants were observed. The high concentrations of CO and NO₂ were observed both in the early evening (16:00–18:00) and early morning (06:00–08:00) hours.

The study of an intradiurnal periodicity of fungal spore concentrations was performed for eight allergenic fungal taxa (*Agrocybe*, *Alternaria*, *Cladosporium*, *Coprinus*, *Epicoccum*, *Exosporium*, *Ganoderma* and *Leptosphaeria*) occurring in high numbers in the air of Bratislava (Ščevková and Kováč 2019). The analysed taxa showed a pattern with differences between the spore types and the bihourly distribution (Fig. 4). We classified the airborne spores into two categories, daytime (*Alternaria*, *Cladosporium*, *Epicoccum*, *Exosporium*) and nighttime (*Agrocybe*, *Coprinus*, *Ganoderma*, *Leptosphaeria*) spore types depending on their releasing pattern. *Cladosporium* and *Exosporium* show the highest amount of spores in the morning at 10:00, with 64.0 and 68.3% of spores, respectively distributed between 08:00 and 18:00. *Epicoccum* reaches its maximum at noon, with 47.3% of spores distributed between 10:00 and 16:00. During the daytime (10:00–18:00), the amount of *Alternaria* spore registered is 55.8%, with a peak in the afternoon at 16:00. All four above-mentioned daytime spore types reach the minimum at midnight. *Agrocybe*, *Ganoderma*, *Coprinus* and *Leptosphaeria*

nighttime spore types show their maximum spore concentrations at nighttime. *Agrocybe* and *Ganoderma* concentrate 61.5 and 67.8% of spores, respectively between 18:00 and 04:00, showing the maximum at 20:00, with a second peak at 04:00. *Coprinus* and *Leptosphaeria* show a peak at 04:00 and concentrate 64.7% of spores between 20:00 and 06:00 for *Coprinus* and 50.9% of spores between 02:00 and 08:00 for *Leptosphaeria*. Moreover, *Coprinus* reaches a second peak at 22:00. *Agrocybe*, *Ganoderma* and *Coprinus* reach the minimum in the afternoon at 14:00, while *Leptosphaeria* shows the lowest amount of spores at 20:00 (Fig. 4).

Table 2 shows the results of multiple regression analysis, which was performed in order to identify the environmental variables which the most influenced airborne fungal spore concentrations. The results revealed that meteorological parameters were important determinants of fungal spore concentrations and that air temperature, wind speed, sunshine duration and precipitation were the most consistent predictors for fungal spore levels of daytime spore types. Air temperature was positively associated with all analysed dry spore types, whereas wind speed, sunshine duration and precipitation were positively associated only with some of them. Wind speed had a positive association with *Cladosporium* and *Exosporium*, sunshine duration was positively associated with *Epicoccum* and *Exosporium* and precipitation had a positive association with *Alternaria* and *Cladosporium*. Moreover, relative air humidity and atmospheric pressure showed a significant (negative) association with *Exosporium* and *Alternaria*, respectively. On the other hand, the most consistent predictors for spore concentrations of nighttime spore types were air temperature, daytime interval and wind speed. Air temperature was negatively associated with all analysed wet spore types, whereas daytime interval was negatively associated with *Agrocybe*, *Coprinus* and *Ganoderma*. Relative air humidity was positively associated with *Leptosphaeria*, while the association with precipitation was negative. Wind speed was negatively associated with *Coprinus* and *Ganoderma*. Finally, the sunshine duration was negatively associated with *Ganoderma* spore type in the multivariate models.

In addition to meteorological parameters, also several atmospheric pollutants were significantly associated with spore concentrations (Table 2). Except for *Coprinus* and *Leptosphaeria*, the spore concentrations of all analysed fungal taxa increased with the increase in PM₁₀ levels. The spore concentration of *Coprinus* and *Cladosporium* increased at higher CO and NO₂ levels, respectively, whereas the concentration of *Agrocybe* and *Ganoderma* increased at higher NO₂ levels. By contrast, PM₁₀, O₃ and NO₂ were negatively associated with *Leptosphaeria*, while *Coprinus* and *Ganoderma* were negatively associated with O₃ levels.

For *Alternaria*, *Exosporium*, *Cladosporium* and *Epicoccum* daytime spore types, environmental parameters explained 30.9, 14.0, 13.1 and 11.9% of the total variance,

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

Spore type	Variables	β coeff.	Std. error	p value	R^2	Proportion of variance explained (%)
<i>Agrocybe</i>	Intercept	4.2524	1.1609	0.0003	0.130	13.0
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	0.0471	0.0148	0.0015		
	NO ₂ ($\mu\text{g}/\text{m}^3$)	0.0616	0.0096	< 0.0001		
	Temperature ($^{\circ}\text{C}$)	− 0.1456	0.0276	< 0.0001		
	Daytime	− 0.7964	0.1919	< 0.0001		
<i>Alternaria</i>	Intercept	552.2548	271.9856	0.0425	0.309	30.9
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	0.4889	0.0645	< 0.0001		
	P (hPa)	− 0.5702	0.2727	0.0367		
	Precipitation (mm)	2.5919	0.4269	< 0.0001		
	Temperature ($^{\circ}\text{C}$)	1.1143	0.1126	< 0.0001		
<i>Cladosporium</i>	Intercept	− 547.892	66.7671	0.4120	0.131	13.1
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	4.7143	0.9090	< 0.0001		
	NO ₂ ($\mu\text{g}/\text{m}^3$)	1.6827	0.7204	0.0196		
	CO ($\mu\text{g}/\text{m}^3$)	− 0.0836	0.0581	0.1508		
	Precipitation (mm)	10.4301	4.5455	0.0219		
<i>Coprinus</i>	Intercept	18.1037	5.1227	0.0004	0.058	5.8
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	43.8255	7.2136	0.0001		
	O ₃ ($\mu\text{g}/\text{m}^3$)	− 0.1955	0.0382	< 0.0001		
	CO ($\mu\text{g}/\text{m}^3$)	0.0140	0.0046	0.0024		
	Wind speed (m/s)	− 2.0458	0.4713	< 0.0001		
<i>Epicoccum</i>	Intercept	− 0.5826	0.2170	0.0073	0.119	11.9
	Temperature ($^{\circ}\text{C}$)	− 2.0082	0.4749	< 0.0001		
	Daytime	− 0.4337	0.1704	0.0110		
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	− 0.3434	0.7764	0.6583		
	Temperature ($^{\circ}\text{C}$)	0.0937	0.0180	< 0.0001		
<i>Exosporium</i>	Intercept	0.0926	0.0316	0.0035	0.140	14.0
	Sunshine (h)	1.2641	0.2843	< 0.0001		
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	0.0627	0.0145	< 0.0001		
	Temperature ($^{\circ}\text{C}$)	0.0771	0.0405	0.0570		
	Relative humidity (%)	− 0.0210	0.0104	0.0428		
<i>Ganoderma</i>	Intercept	0.1590	0.0852	0.0622	0.381	38.1
	Sunshine (h)	0.7581	0.2088	0.0003		
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	32.4080	4.0332	< 0.0001		
	O ₃ ($\mu\text{g}/\text{m}^3$)	− 0.1139	0.0301	0.0002		
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	0.3485	0.0934	0.0002		
<i>Leptosphaeria</i>	Intercept	0.3915	0.0451	< 0.0001	0.166	16.6
	Temperature ($^{\circ}\text{C}$)	− 0.5021	0.1656	0.0025		
	Wind speed (m/s)	− 2.1029	0.4133	< 0.0001		
	Sunshine (h)	− 5.1318	1.1539	< 0.0001		
	Daytime	− 5.2926	1.0126	< 0.0001		
<i>Leptosphaeria</i>	Intercept	− 12.2605	10.0619	0.2232	0.166	16.6
	O ₃ ($\mu\text{g}/\text{m}^3$)	− 0.1106	0.0457	0.0157		
	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	− 0.3742	0.1049	0.0004		
	NO ₂ ($\mu\text{g}/\text{m}^3$)	− 0.1412	0.0641	0.0277		
	Temperature ($^{\circ}\text{C}$)	0.9933	0.2768	0.0003		
<i>Leptosphaeria</i>	Precipitation (mm)	− 3.5145	0.6325	< 0.0001	0.166	16.6
	Relative humidity (%)	0.3888	0.0735	< 0.0001		

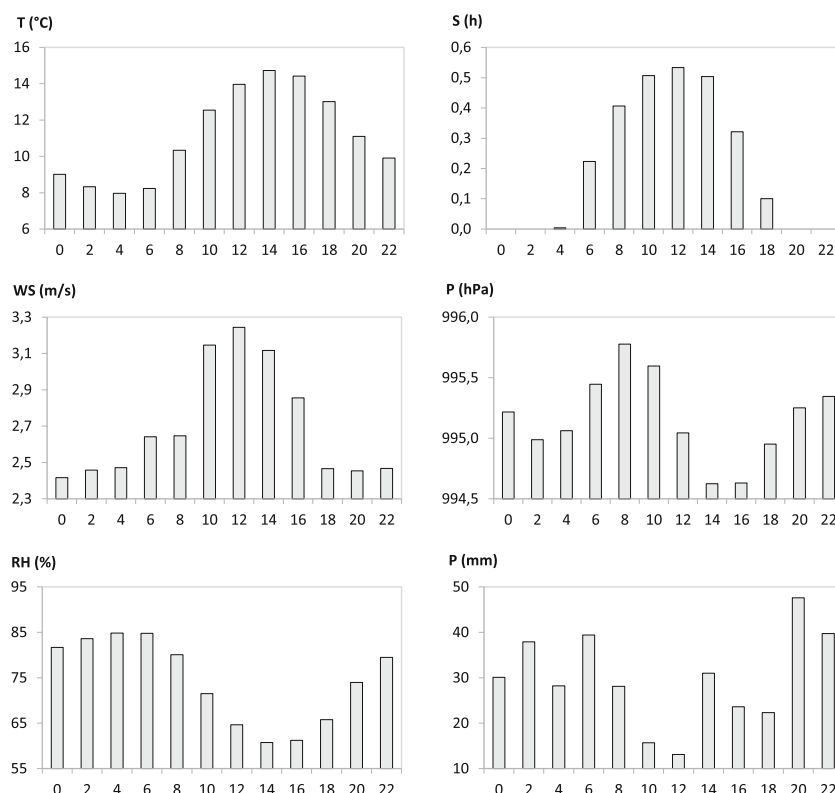
respectively, while for *Ganoderma*, *Leptosphaeria*, *Agrocybe* and *Coprinus* nighttime spore types, environmental parameters explained 38.1, 16.6, 13.0 and 5.8%, respectively (Table 2).

Discussion

Analysis of intradiurnal variability in spore concentration of eight analysed fungal taxa in the atmosphere of Bratislava

included in this study shows that peak concentrations for dry spore types tended to be recorded during the daytime period, whereas wet spores reached its peaks during the nighttime period. Taking into account dry spore types, the greatest spore concentrations occurred in the morning at 10:00 for *Cladosporium* and *Exosporium*, at noon for *Epicoccum* and in the afternoon at 16:00 for *Alternaria*. The occurrence of the highest spore values around midday between 10:00 and 12:00 is mainly related to huge spore emission due to favourable weather conditions, such as high air temperature and wind

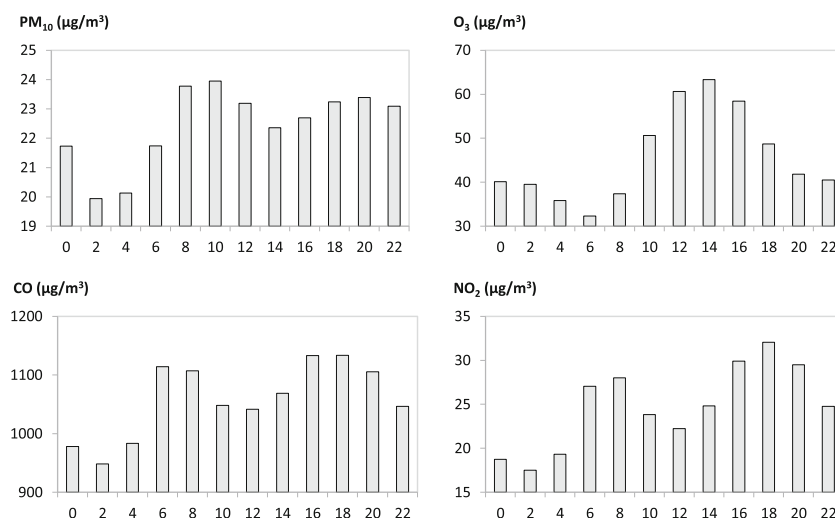
Fig. 2 Intradiurnal evolution in mean bihourly values of air temperature (T), sunshine duration (S), wind speed (WS), relative air humidity (RH), atmospheric pressure (AP) and precipitation (P) in Bratislava in 2016



speed accompanied by a low relative air humidity (Fig. 2). Results similar to those obtained in Bratislava were found in Cracow (Poland) (Stepalska and Wolek 2009) and Zagreb (Croatia) (Peternel et al. 2004) where *Cladosporium* spore concentrations were the highest between 10:00 and 12:00. However, these results differ from those obtained by other researchers who reported hourly peak *Cladosporium* concentrations between 12:00 and 14:00 in León (Spain) (Fernandez et al. 1998), between 14:00 and 15:00 in Michigan (USA) (Burge 1986) and even between 20:00 and 22:00 in Cordoba (Spain) (Mediavilla et al. 1997). Such variability may be

explained by the differences in climate and biogeographical characteristics of the areas under study. Likewise, in Harpenden (UK), the highest *Epicoccum* spore concentrations usually occurred towards the middle of the day around 11:00 (Sreeramulu 1959). However, these results are in contrast with Jones and Harrison (2004) who concluded that extreme mid-day conditions (high air temperature and low air humidity) reduce concentrations of bioaerosols in the atmosphere. Spores of Deuteromycetes (conidiospores) are released by a passive mechanism or external forces (e.g., nearby wind currents). The most types of conidiospores grow in an exposed

Fig. 3 Intradiurnal evolution in mean bihourly values of particulate matter $\leq 10 \mu\text{m}$ (PM_{10}), ozone (O_3), carbon monoxide (CO) and nitrogen dioxide (NO_2) in Bratislava in 2016



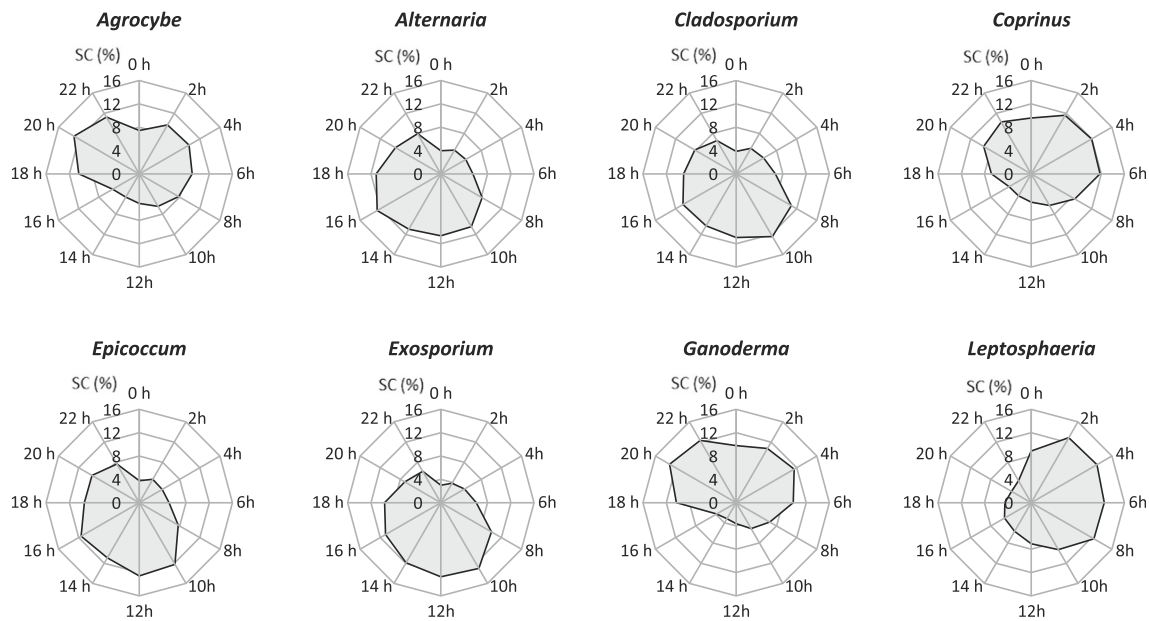


Fig. 4 Intradiurnal variation in the spore concentration (SC) of major spore types in 2016, expressed in percentage

position and released inertly when disturbed. Therefore, the rising of airborne conidiospore concentrations in the morning could be attributed to the increase in wind speed after sunrise as the wind causes vibrations that facilitate detachment of spores and their release into the air (Rich and Waggoner 1962). Similarly to our results, *Alternaria* has previously been described as afternoon taxon, with concentration peaks occurring from noon to 21:00 (Munuera Giner et al. 2001; Oliveira et al. 2009). This afternoon peaks could be related to a reduction of turbulence in the afternoon which allows spores to settle. However, Munuera Giner et al. (2001) reported that afternoon peaks can be induced by a delay in a sampling of spores released earlier and being transported to the sampler from remote sources. In the other study, a different result was found from our study about intradiurnal variations of *Alternaria* spores; in Stockholm (Sweden), maximum levels were recorded in the morning at 08:00 (Hjelmroos 1993). The lowest spore concentrations of all four analysed dry spore types, similarly to other researchers (Bardei et al. 2017) occurred at midnight, mainly due to unfavourable weather conditions, such as low air temperature and wind speed accompanied by high relative air humidity (Fig. 2).

In Bratislava, just like in several other aeromycological studies (Kasprzyk et al. 2004; Sánchez Reyes et al. 2016), the greatest spore concentrations of wet spore types occurred at night or in early morning hours when low air temperature and wind speed were accompanied by high relative air humidity (Fig. 2). This intradiurnal rhythm, in which the highest abundance of spores presents between the night and early morning period, suggests an active release mechanism induced by increased air humidity prevalent at dawn. We have recorded a double peak pattern for *Agrocybe*, *Coprinus* and

Ganoderma spore concentrations during a 24-h period. For *Agrocybe* and *Ganoderma*, a greater peak appeared at night at 20:00, and a second smaller peak appeared in the early morning at 04:00. Similar results were obtained by Stepalska and Wołek (2009) who found peak concentrations of *Ganoderma* spores in the early morning hours between 02:00 and 04:00. For *Coprinus*, we have displayed double peak pattern with a greater peak at 04:00, and second smaller one occurring at 22:00, just like other surveys registered (Antón et al. 2019). In our study, *Leptosphaeria* wet spores were common at nighttime and reached the maximum values in the early morning at 04:00, which could be due to high relative air humidity during this daily hour.

Weather parameters are known to influence the production, release and dispersal of fungal spores. In the present study, results of multiple regression analysis showed air temperature as the meteorological factor which significantly influenced the concentration of all analysed spore types in the atmosphere of Bratislava. The association with dry spores (*Cladosporium*, *Alternaria*, *Epicoccum* and *Exosporium*) was positive, whereas the association with wet spores (*Agrocybe*, *Coprinus*, *Ganoderma* and *Leptosphaeria*) was negative. Likewise, according to several other researchers (Peternel et al. 2004; Kasprzyk et al. 2004), the air temperature was considered as the most influential meteorological factor positively correlated with the dry spore concentrations. This phenomenon is probably linked to the fact that, in order to be released into the air, dry spores require a certain degree of dryness, which occurs when the air temperature increases (Nolard et al. 2001). Besides air temperature, wind speed was also a significant factor explaining the variability in *Cladosporium* and *Exosporium* spore concentrations, exhibiting a significant

and positive relationship with above-mentioned dry spore types. Wind speed directly promotes the release of dry spores and plays an important role in the spore dispersal.

Dry spores require less amount of humidity for sporulation and, for this reason, their concentration is highest around mid-day when the relative air humidity is low (Quintero et al. 2010). However, on the basis of our study results, it seems that relative air humidity does not influence most of the airborne dry spore concentrations as the significant and negative association was only observed with *Exosporium*.

The association between dry spores and precipitation are not always the same. Contrary to results obtained by other researchers (Martínez Blanco et al. 2016; Ianovici 2016), who observed a negative association between precipitation and *Alternaria* spore concentration, precipitation was significantly and positively associated with *Alternaria* and *Cladosporium* spore levels in the present study. Similarly to our results, Hjelmroos (1993) reported that dry spore concentrations increase at the onset of rain. This phenomenon should be explained by the fact that dry spores are released from the sporangium via splash-induced emission (Troutt and Levetin 2001) that releases spores by mechanical shock and fast air currents produced by raindrops (Elbert et al. 2007). Moreover, higher concentrations of conidiospores occur in seasons with higher precipitation and temperatures as was recorded in Bratislava during the study period (the annual precipitation total and mean annual air temperature was 133 mm and 0.4 °C higher, respectively than the long-term average) and abundant dead plant material for the fungi to grow on.

It seems that air temperature and daytime period are the main environmental factors that affect airborne spore concentrations of Basidiomycetes. Negative correlations with air temperature and daytime period appeared in taxa with nighttime highest spore concentrations, as in *Agrocybe*, *Ganoderma* and *Coprinus*. The negative association between basidiospore concentrations and air temperature suggested that spore production or fungal growth is decreased in higher temperatures.

We also observed a negative relationship between wind speed and spore concentrations of *Coprinus* and *Ganoderma*, similar to that found in previous studies (Martínez Blanco et al. 2016; Grinn-Gofroń et al. 2018). Large concentrations of *Coprinus* and *Ganoderma* spores were associated with the bihourly mean wind speed of 2 to 3 m/s (Fig. 2). This is in accordance with Calderon et al. (1995), who pointed out that wind speed > 5 m/s is associated with decreased concentrations of basidiospores, perhaps due to the diluting effect of high wind velocities on airborne spore concentrations. Moreover, increasing wind speed may inhibit wet spore production due to the increase in water loss (Calderon et al. 1995). The availability of atmospheric moisture is necessary to the spore production and liberation of Basidiomycetes (Hernández Trejo et al. 2013). However, neither precipitation nor relative air humidity was

significantly associated with spore concentration of any type of basidiospores in the present study. This could be explained by a time lag between the spore release and trapping. Basidiomycetes release spores in moist conditions, however leading to elevated spore levels several hours after rain (Packe and Ayres 1985).

Unlike basidiospores, we observed precipitation and relative air humidity to be significantly associated with *Leptosphaeria* ascospore concentration. The association between precipitation and spore concentration of mentioned taxon was negative, whereas the association between relative air humidity and spore concentration was positive. Similarly to our results, research conducted in Taiwan (Kallawicha et al. 2017) has reported a positive association between relative air humidity and ascospores. The positive influence of relative air humidity on *Leptosphaeria* spore levels in the air could be explained by the fact that many Ascomycetes ascospores require moisture for their release (Díez Herrero et al. 2006). The active release of ascospores is likely to occur some period after the start of precipitation, unlike the ejection of inertly released conidiospores by droplets which occurs at the commencement of heavy precipitation. The rain, in small amounts, could be a stimulating factor for sporulation and spores concentration; however, when the intensity of precipitation increases, it operates by removing spores from the atmosphere by both rain-out and wash-out effects (Sakiyan and Inceoglu 2003).

Atmospheric pollutants considered in the present study were also associated with fungal spore concentrations. With exception of *Coprinus* and *Leptosphaeria*, PM₁₀ exhibited a positive association with all analysed spore types in the present study, which is similar to the results that were reported in Taipei and Hualien (Taiwan) (Kallawicha et al. 2017; Ho et al. 2005). This could be attributed to the ability of fungal spores to be adsorbed on the surface of the particulate matter, altering the particle aerodynamic properties, and thus be dispersed in the atmosphere (Burge and Rogers 2000). Contrary to our results, Lin and Li (2000) pointed out that PM₁₀ did not correlate well with bioaerosols because of the low contribution of the bioaerosols to the particulate matter.

The tropospheric ozone is a photochemical reaction product and the increased solar radiation has a key role in its concentration. Therefore, the intradiurnal variations of O₃ concentration showed differences throughout the 24-h period, with a clear increase in ozone concentrations over the warmer and dryer parts of the days (Figs. 2 and 3). Tropospheric ozone is a strongly phytotoxic oxidant (Tiedemann and Firsching 2000), which can suppress the growth of fungi and thus reduce spore concentration in the air (Sommer et al. 1981). In our multivariate analysis, ozone exhibited significant and negative associations with *Coprinus*, *Ganoderma* and *Leptosphaeria* spore types. Similarly to our results, Ho et al. (2005) have reported negative association between ozone and *Ganoderma* spore concentrations.

In Bratislava, the high concentration of CO and NO₂ in the air is attributed to increased CO and NO₂ emission from cars during both the morning and evening traffic peaks between 06:00–08:00 and 16:00–18:00, respectively (Fig. 3). CO can suppress the growth of fungi in the environment (Sommer et al. 1981). However, on the basis of the our study results, it seems that CO does not influence most of the airborne spore concentrations as the significant association was only observed with two spore types; the association with *Coprinus* was positive, whereas the association with *Cladosporium* was negative. Concerning the NO₂, the spore concentrations of *Agrocybe*, *Ganoderma* and *Cladosporium* have been observed to increase with increasing atmospheric NO₂ levels, while the association with *Leptosphaeria* was negative. However, more studies are needed to understand the mechanisms of these associations.

Although the proportion of explained variance (Table 2) of some spore type models were not optimal ($R^2 < 0.4$), they are comparable to those in other studies, in which the R^2 ranged from 0.01 to 0.77 (Troutt and Levetin 2001; Ho et al. 2005; Sousa et al. 2008). Except for meteorological parameters and air pollutants, the occurrence of those spore types which showed worse R^2 values can be associated to other environmental factors, such as availability of organic matter for the development of the fungus. Similarly to Sousa et al. (2010), *Ganoderma* and *Alternaria* presented the best R^2 values in the present study.

Conclusion

Intradiurnal periodicity of spore concentrations was different for dry and wet fungal spores. Dry spores (*Alternaria*, *Cladosporium*, *Epicoccum*, *Exosporium*) showed a daytime pattern of the release and subsequently higher concentrations during the forenoon, noon or afternoon hours, whereas wet spores (*Agrocybe*, *Coprinus*, *Ganoderma*, *Leptosphaeria*) showed a nocturnal pattern of the highest number of spores during the night or early morning hours.

Results of multiple regression analysis showed that there was no single model for all spore types. Different combinations of factors were predictors of spore concentration for the various examined fungal organisms; however, air temperature, PM₁₀, wind speed and precipitation seemed to be the most important for daytime spore types, while for nighttime spores, air temperature, daytime interval and O₃ concentrations were identified as the most influential. Except for O₃ and daytime period, all analysed environmental parameters were positively associated with the concentration of some daytime spore types, while the association with relative air humidity and CO was negative. For some nighttime spores, the association between their spore concentrations and the following environmental parameters: air temperature,

sunshine duration, wind speed, precipitation, daytime period and O₃ was negative, whereas the association with relative air humidity and CO was positive. Some air pollution parameters, such as PM₁₀ and NO₂, had mixed effects on nighttime spore concentrations, and thus, further research should be made to examine their health effects.

In general, air temperature, PM₁₀ and wind speed were environmental parameters with a great influence, being present in eight, seven and four regression models, respectively. Nevertheless, atmospheric pressure, CO and relative air humidity present almost no influence on the airborne spore levels.

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