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Background concentrations of airborne, culturable fungi and dust particles in urban, rural and mountain regions

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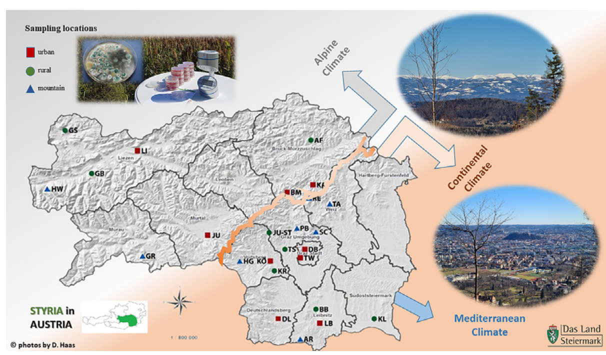
HIGHLIGHTS

- Background concentrations for airborne fungi are ranged from 3.5×10^2 to 4.7×10^3 CFU/m^3 for urban, rural and mountain regions.
- Air temperature correlates positively with xerophilic fungi and *Cladosporium* sp.
- Relative humidity has negative effect on xerophilic fungi and *Cladosporium* sp.
- Weather conditions have different impact on the fungal spore concentrations.
- Fine and coarse dust particles correlate positively with xerophilic fungi and *Cladosporium* sp.

GRAPHICAL ABSTRACT



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ABSTRACT

Geographic location and meteorological factors can affect the content of bioaerosol concentrations. This study was conducted to determine the natural background concentrations of culturable fungal spores and dust particles in three different geographical areas. Focus was given to the dominant airborne genera *Cladosporium*, *Penicillium*, *Aspergillus* and the species *Aspergillus fumigatus*. The influence of weather conditions on the microorganism concentrations in urban, rural and mountain regions were examined. Possible correlations between particle counts and culturable fungal spore concentrations were investigated.

125 measurements of the air were conducted using the air sampler MAS–100NT® and the particle counter Alphasense OPC–N3. The analyses of the collected samples were based on culture methods using different media.

The highest median of fungal spore concentrations was detected in the urban region and was of 2.0×10^3 CFU/m^3 for xerophilic fungi and 1.7×10^3 CFU/m^3 for the genus *Cladosporium*. The concentrations of fine and coarse particles in rural and urban regions were the highest of 1.9×10^7 pa/m^3 and 1.3×10^7 pa/m^3, respectively. Little cloud cover and slight wind had a positive influence on the concentration of fungal spores. Furthermore, correlations were observed between air temperature and the concentrations of xerophilic fungi as well as the genera *Cladosporium*. In contrast, relative humidity correlated negatively with total fungi and *Cladosporium* and no correlation was found with the other fungi. For the region of Styria in summer and early autumn, the natural background concentration for xerophilic fungi ranged between 3.5×10^2 and 4.7×10^3 CFU/m^3 air. No significant differences were detected between the fungal spore concentrations in urban, rural and mountainous regions.

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The data of this study could be used as a reference to compare the natural background concentrations of airborne culturable fungi in further studies concerning air quality assessment.

1. Introduction

Fungal spores are found ubiquitously all over the world but their species diversity and concentrations in the ambient atmosphere can vary in different geographic regions. Fungi spores form a part of the content of bioaerosols in dust particles suspended in the atmosphere. Their concentrations and compositions depend on the complex interaction between biological and environmental factors, such as geographic location, air pollution, weather conditions, human activities, and local source of vegetation (Fröhlich-Nowoisky et al., 2016; Grinn-Gofroń and Bosiacka, 2015; Liu et al., 2019a). The spore composition varies between rural and urban zones reflecting human activities, type and status of the vegetation and the prevailing climate rather than atmospheric transport (Hanson et al., 2022). However, a scientific study shows that both aerial and soil fungal communities are significantly smaller in urban than in natural environments (Abrego et al., 2020). In various geographical regions of the world, the aerosol volume consists of 25 % of biological particles whereas in the air over the Amazon up to 74 % (Brandl, 2011; Morris et al., 2011).

The highest amounts of fungal spores present in central European air were measured from May to September (Anees-Hill et al., 2022). In summer the total amount of measured spores in the ambient air increases because of a characteristic cycle of temperature and moisture. Various fungal genera can be spread as “dry air spora” by air movements at low relative humidity and wind speed (Grinn-Gofroń, 2007; Hirst and Stedman, 1963; Troutt and Levetin, 2001). An Italian study found that fungal spores remain longer in the atmosphere when the sun is shining and the sky is clear (Fuzzi et al., 1997). According to Sarda-Estève et al. (2019) the main parameters driving the atmospheric concentration of fungi are temperature and precipitation. Spores spread into surrounding air by water droplets in fog or movement of air and then transported over large distances.

The fungal genus *Cladosporium* is found most frequently in outdoor air. Its spores occur in particularly high numbers in the summer months and correlate positively with air temperature (Elvira-Rendueles et al., 2013; Grinn-Gofroń and Rapiejko, 2009; Klaric and Pepeljnjak, 2006). The pigmented spores of *Cladosporium* or *Alternaria* dominate habitats of most common regions due to the fact, that colorless spores cannot survive UV radiation from sunlight (Klaric and Pepeljnjak, 2006). *Cladosporium* sp. has small conidia, which are formed in branched chains and can spread easily in large numbers over long distances at high temperatures during the dry season (Bench et al., 2012). These fungal species are more prevalent in ambient air on warm afternoons when relative humidity is low and wind speeds are high (Rich and Waggoner, 1962). In contrast, hydrophilic spores spread passively by rain and are referred to as typical “wet weather spora” (Levetin, 1995). For example, ascospores and basidiospores frequently occur in the atmosphere during or after rainfall (Grinn-Gofroń, 2011; Li and Kendrick, 1995). In the subtropical urban area of Taipei, ascospores were the most abundant spore type followed by basidiospores, *Aspergillus/Penicillium*, and *Cladosporium* (Kallawicha et al., 2017). However, the genera *Aspergillus* sp. and *Penicillium* sp. prefer air humidity *and their* unchained conidia can easily aerolize in relation to increasing windspeed (Wilkie et al., 2023).

European and international studies have reported that genera such as *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. and *Alternaria* sp. are present in natural ambient air (Alhussaini et al., 2015; Fang et al., 2005; Haas et al., 2014; Kuo and Li, 1994; Lee and Jo, 2006; Oliveira et al., 2009; Ponce-Caballero et al., 2013; Shelton et al., 2002). The amount and species diversity of airborne microorganisms differ according to geographic and local conditions. Depending on local conditions, background concentrations of fungal spores in the atmosphere can extend over one, in individual cases even over two powers of ten. However, the concentrations of colony forming units per cubic meter (CFU/m^3) in natural ambient air is lower than in areas where emissions are to be expected. In natural habitats of a mixed-evergreen forest, the mean concentrations of airborne fungal spores were two times higher than in the coastal area, but there were no differences among mixed-evergreen forest, redwood forest and maritime chaparral vegetation types (Crandall and Gilbert, 2017). Some authors found higher fungal spore concentrations in rural than in urban locations (Awad et al., 2013; Kasprzyk and Worek, 2006; Oliveira et al., 2009). Lee et al., (2019) reported that the concentrations of airborne fungi at the seashore was three to four times lower than that in the mountains because of the lack of hazardous pollutants. The mean concentrations of airborne fungal spores at five locations in the desert urban ambient air of Las Vegas were 10^2 CFU/m^3 (Patel et al., 2018). In the region of Styria, the median outdoor concentrations in urban areas were between 1.0×10^2 and 9.4×10^2 CFU/m^3 for culturable xerophilic fungi (Haas et al., 2014).

Fungal concentrations in cities vary widely due to different emission sources. Temperature and wind speed are important factors in locations where fungi or bacteria are emitted from local sources. Relative humidity may also play an important role. Areas of heavy vehicle traffic have much higher concentrations of aerosols than quieter areas such as parks (Burrows et al., 2009). The concentration of aerosols also includes substances from chimneys and other industrial emissions, which can be transported over large distances. Without any local sources of pollution, the concentrations of microorganisms can be considered as background. The background concentrations of bioaerosols can be used as a reference to evaluate air quality (Veritas, 2019). Weather conditions and meteorological factors can affect the natural background concentration of bioaerosols and dust particles in different regions.

Few reports have been published on the natural background of cultivable microorganisms in relation to the dust particles in the ambient air. Therefore, it is important to evaluate the aerosol at different geographic locations because bioaerosols and particulate matter vary in the ambient air. The province of Styria in Austria has urban, rural, mountainous and alpine areas with different climatic characteristics, making it an ideal area to conduct this study.

The aim of the study was to determine the background concentrations of airborne culturable fungi in urban, rural and mountainous regions of Styria in order to identify differences between the three regions. The objectives of this study were: (Abrego et al., 2020) to measure the concentrations of the total xerophilic and mesophilic fungi, (Akgül et al., 2016) to identify the genera *Cladosporium*, *Penicillium*, *Aspergillus* and the species *A. fumigatus* in the air, (Alhussaini et al., 2015) to examine the effect of geographic and meteorological parameters on the concentrations of airborne fungi, and (Almaguer et al., 2014) to determine the concentration of dust particles and compare them with culturable fungal spore concentrations.

2. Material and methods

2.1. Measurement locations

In the period from July to October 2019, 125 measurements of culturable fungi and dust particles in the air were carried out in Styria. To assess the background concentrations of airborne fungi and dust particles, this study included 25 different measuring locations; each of them was measured on five different days. The locations were selected and supervised by the Styrian Government. Airborne fungi were collected on three different culture media in duplicate according to DIN ISO 16000-18 (2012) for sampling by impaction. Measurements were taken three weeks apart between 10:00 am and 2:00 pm. There were nine measuring locations in urban, eight in rural and eight in mountain regions, respectively.

Air samples for the investigation of fungal spores (n = 750) and dust particles (n = 125) were carried out under different weather conditions (n = 125). The relationship was examined between air temperature, relative humidity (RH), wind velocity and the effect of sunshine or cloudiness and the fungal spore concentrations in the outdoor air.

Fig. 1 shows the measurement locations which are depicted by symbols (urban = ■; rural = ●; mountain = ▲).

Styria is located in the southeast of the Alps and influenced by the Alpine, the Mediterranean and the Continental European climate. The highest average temperature is 25 °C in July and the lowest is 5 °C in January. Styria has a very varied landscape, with forests, alpine pastures and agricultural areas. Based on microclimate and weather, a distinction is made between nine climatic regions (https://www.umwelt.steiermark.at/cms/ziel/25206/DE/).

The sampling sites in the urban were nine and are from busy streets but in densely built-up areas with >8000 inhabitants, considered as representative measurement locations for the urban background, i. e. all measuring sites were as pollution-free as possible. The eight rural background locations were chosen sufficiently far from urban and industrial areas so as not to be influenced by local sources of emissions, such as road traffic. These measuring sites have a low population density of ≤5000 inhabitants per municipality. Measurements in the mountains were carried out at altitudes of >800 m and in areas far from any sources of emissions.

2.2. Measuring devices and evaluation of the samples

Sampling of the culturable fungi was conducted by using the one-stage microbial air sampler MAS-100NT® (MBV AG, Stafa, Switzerland) with a flow rate of 100 L min^{-1}. The cut-off-size of the MAS-100 sampler (d_{50} = 1.7 μm) was adapted to collect airborne fungal spores with an aerodynamic diameter of ≥2 μm (Yao and Mainelis, 2006). The sampler was placed at 1.5 m above the ground for 30 s. This short measuring time was chosen in order to avoid the overlapping of the growing colonies during the culture process. For quality assurance the determination of field blank values according to the sampling methods was performed. One field blank sample was done for each measurement series and sampling media used.

The optical particle counter OPC-N3 was used for dust particle measurements. The OPC-N3 records particles from 0.35 to 40 μm and assigns them to the 24 size channels (Alphasense, Great Notley, Braintree, United Kingdom).

The collection media were Malt Extract Agar (MEA) for mesophilic fungi, Dichloran Glycerol Agar (DG18) for xerophilic fungi (VWR International GmbH, Vienna, Austria) and Columbia Colistin-Nalidixic Acid-Aztreonam Agar (CNA, Becton Dickinson GmbH, Heidelberg, Germany) for the species *Aspergillus fumigatus* (*A. fumigatus*). MEA and DG18 agar plates were incubated at 25 °C for 7 days, whereas CNA media plates at 37 °C for 48 h. The total number of colony forming units (CFU) per m^3 air was calculated. The cultivation, detection and enumeration of fungal spores were based on DIN/ISO 16000-17 (2010) and DIN ISO 16000-18 (2012) which are German standards adapted according to the international standards for quality assurance and efficiency.

The genera *Cladosporium*, *Penicillium*, *Aspergillus* and the species *A. fumigatus* were determined quantitatively using a stereo and light microscope according to the morphological characters criteria of the grown colonies (Samson et al., 2000).

2.3. Geographic and meteorological parameter

The altitude, air temperature and RH were measured continuously during sampling. Weather conditions such as cloud cover, fog and wind were recorded. A sensor was used to measure air temperature and RH (Testo GmbH, Vienna, Austria). Wind speed data was taken from the online database of the Styrian provincial government (https://www.umwelt.steiermark.at/cms/ziel/2060750/DE/) and categorized in meters per second (m/s) into: windless: 0–3 m/s; slight wind: >3–5 m/s and strong wind: >5 m/s.

2.4. Statistical methods

The statistical analyses of the data and the creation of the graphics were carried out using software SAS 9.4. Spearman's correlation coefficient was calculated to show bivariate relationships. The correlations were

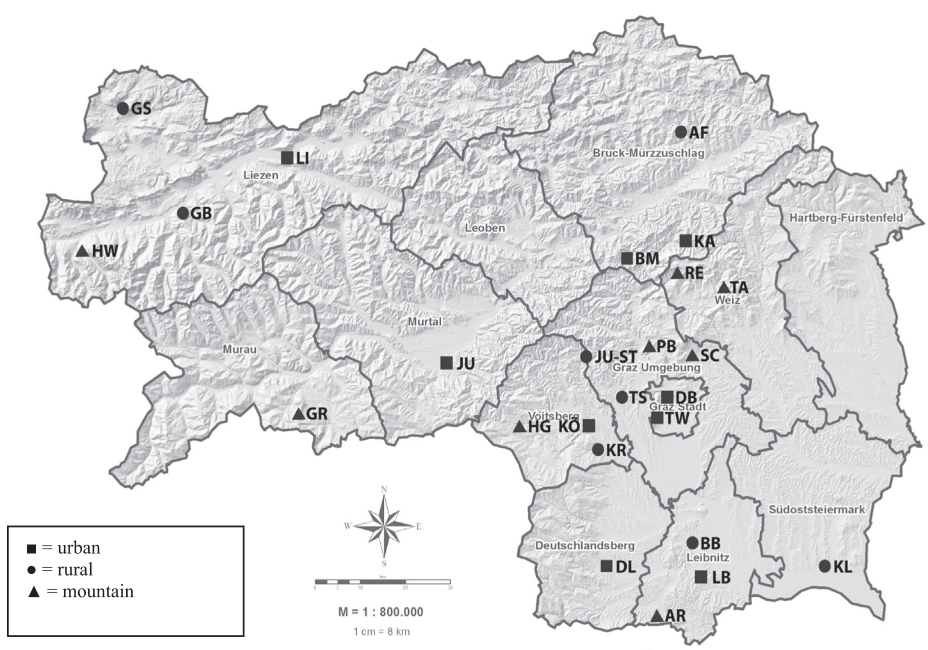

Fig. 1. The measuring locations in Styria (Office of the Styrian Provincial Government, Provincial Office Directorate, 2020. Edited by M. Ilieva.). Abbreviations: The abbreviations of the measuring locations of this figure.

AF: Aflenz; AR: Arnfels/Remschnigg; BB: Bockberg; BM: Bruck an der Mur; DB: Don Bosco; DL: Deutschlandsberg; GB: Gröbming; GR: Grebenzen; GS: Grundlsee; HG: Hochgößnitz; HW: Hochwurzen; JU: Judenburg; JU-ST: Judendorf-Straßengel; KA: Kapfenberg; KL: Klöch; KÖ: Köflach; KR: Krottendorf; LB: Leibnitz; LI: Liezen; PB: Plabutsch; RE: Rennfeld; SC: Schöckl; TA: Teichalm; TS: Thalersee; TW: Graz Süd.

considered significant at a value of $p \leq 0.05$. To identify the prognostic factors for the different types of fungi the multiple linear regression model with interaction terms was performed. Categorical variables were converted to dummy variables.

Particle concentrations were evaluated using the software MATLAB Version R2019a. The particle concentration per cubic meter (pa/m^3) measured by means of OPC-N3 was converted into particulate matter (μg/m^3) by an algorithm that is implemented in the measuring device and according to the manufacturer, corresponds to EN 481. To calculate the total number of particles (pa/m^3), the registered particles of all size channels (0.35–40 μm) were summed up and extrapolated to m^3. As in the previous study by Haas et al. (2020), the particles were categorized as fine (0.35–2.3 μm) and coarse (>2.3–10 μm). Lastly, the data of the fine and coarse particles (pa/m^3) were compared with the xerophilic fungi (CFU/m^3), the altitude and the region.

3. Results

The total concentrations of the xerophilic and mesophilic fungi, the fungal genera and the species *A. fumigatus* cultivated on MEA, DG18- and CNA Agar in the urban, rural and mountain regions are listed in the Supplementary Materials (Table S1). The results of the fungal concentrations on DG18 agar and of *A. fumigatus* on CNA were selected for the graphical presentations.

3.1. The concentrations of xerophilic and mesophilic fungal spores in the air

Summing up the median background concentrations in the 25 measuring locations, the xerophilic fungal spores in the ambient air varied from 8.5×10^2 to 4.7×10^3 CFU/m^3 in the urban, 5.6×10^2 to 3.5×10^3 CFU/m^3 in the rural and 3.5×10^2 to 3.7×10^3 CFU/m^3 in the mountainous regions. The results of the xerophilic and mesophilic fungi were approximately the same and there were no significant differences between the median concentrations of fungi in urban, rural and mountainous regions. In the nine urban measuring locations ($n = 45$), the median concentrations of the xerophilic fungal spores on DG18 and mesophilic fungal spores on MEA agar were 2.0×10^3 CFU/m^3 and 1.7×10^3 CFU/m^3, respectively. In the rural regions ($n = 40$) the median fungal spore concentration of 1.7×10^3 CFU/m^3 was determined for xerophilic and mesophilic fungi as natural background. The lowest concentrations of fungal spores were measured in the mountainous regions ($n = 40$) with a median of 1.3×10^3 CFU/m^3 on DG18 and 1.4×10^3 CFU/m^3 on MEA. Fig. 2 shows the results of the concentrations of xerophilic fungal spores in the three defined regions.

The highest median concentration of xerophilic fungal spores was measured at the DB and TW locations in Graz, the capital of Styria. The rural locations GB, KL and AF showed high median fungal spore concentrations between 2.5×10^3 and 3.5×10^3 CFU/m^3. The highest median fungal spore concentration measured at the eight mountainous locations was at PB. At the mountain sites of GR and RE and the rural site of TH, the lowest concentrations of xerophilic fungi were detected. The median concentrations of xerophilic fungal spores of the individual measuring locations are listed in the Supplementary Materials (Table S2).

3.2. The concentrations of fungal genera and species in the air

The genus *Cladosporium* was detected most frequently in the ambient air at all locations. The highest concentration of this genus was found in the urban regions with a median of 1.7×10^3 CFU/m^3. The lowest concentration of 1.2×10^3 CFU/m^3 was detected in the mountainous regions. At the rural locations, a median concentration was of 1.6×10^3 CFU/m^3 air (Fig. 3). The data showed that the median fungal spore concentration of *Cladosporium* sp. was the highest in the urban locations of DB and TW, whereas the lowest was detected in the mountainous location of GR.

For the genus *Penicillium*, the median concentration in the urban background was 3.0×10^1 CFU/m^3, which is three-fold higher than that of the rural and mountain regions (Fig. 4).

In LB urban and JU-ST rural, the median concentration of *Penicillium* sp. of 7.0×10^1 CFU/m^3 was the highest among all measurement locations.

The genus *Aspergillus* was present in low spore concentrations $<2.0 \times 10^1$ CFU/m^3 during the measurement period in urban areas. *Aspergillus* sp. was evenly distributed at all locations in the rural and mountain regions. The highest fungal spore concentrations in the rural and mountain locations were detected at GB and PB, respectively (Fig. 5).

The median fungal spore concentration of *A. fumigatus* was 8.8×10^1 CFU/m^3 in the urban regions and two times higher in the rural than in the mountain regions (Fig. S1). At the individual measuring locations, the highest median fungal spore concentration of this species was 3.0×10^1

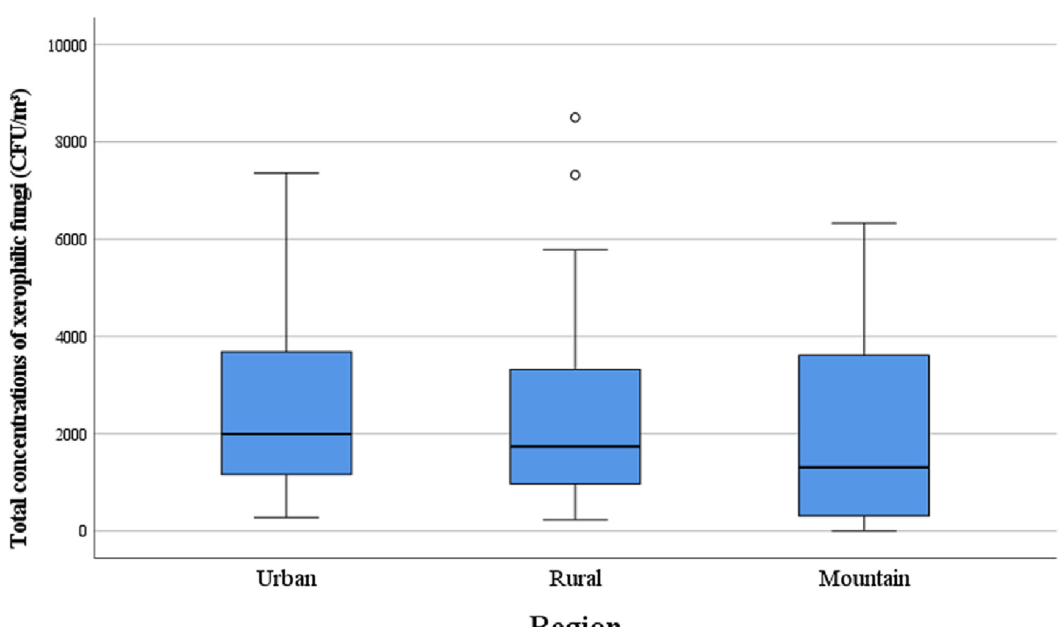

Fig. 2. Concentrations of xerophilic fungal spores in the urban, rural and mountain regions.

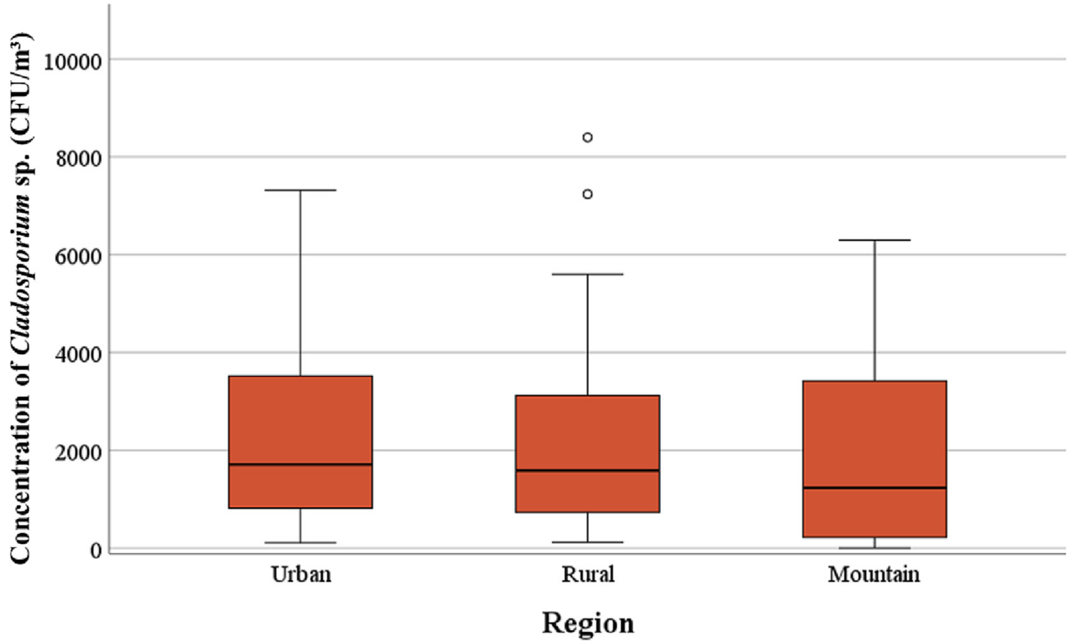

Fig. 3. Concentrations of *Cladosporium* sp. in the urban, rural and mountain regions.

CFU/m^3 in the urban region of LB and 1.3×10^1 CFU/m^3 in the mountain region of PB. At the rural locations, the highest concentration was determined in JU-ST with a median of 4.0×10^1 CFU/m^3.

3.3. Geographic and meteorological factors influencing fungal spore concentrations

The highest concentrations of xerophilic fungi, *Cladosporium* sp. and *Aspergillus* sp. in the ambient air were detected at altitudes category of >450 to ≤850 m (Table 1). The altitude correlated negatively with xerophilic fungal spores above the altitude 850 m (Fig. 6). The genus *Penicillium* was the most commonly found at an altitude below 450 m. The results showed that the altitude correlated negatively with the genus *Penicillium* (rho = −0.31, $p < 0.001$) and *A. fumigatus* (rho = −2.3; $p < 0.009$). The species *A. fumigatus* had the lowest concentration at altitude of >850 m. The three genera and the species *A. fumigatus* in relation to altitude are recorded in the Supplementary Materials (Fig. S2 – S5).

The highest air temperature during the investigation period was 35.7 °C, while the lowest was −0.5 °C (Table S3). Spearman correlations and multiple linear regression model (Table S4) showed that the temperature (Fig. 7) had a significant positive effect on xerophilic fungal spore concentrations (rho = 0.54, $p < 0.001$) and the genus *Cladosporium* sp. (rho =

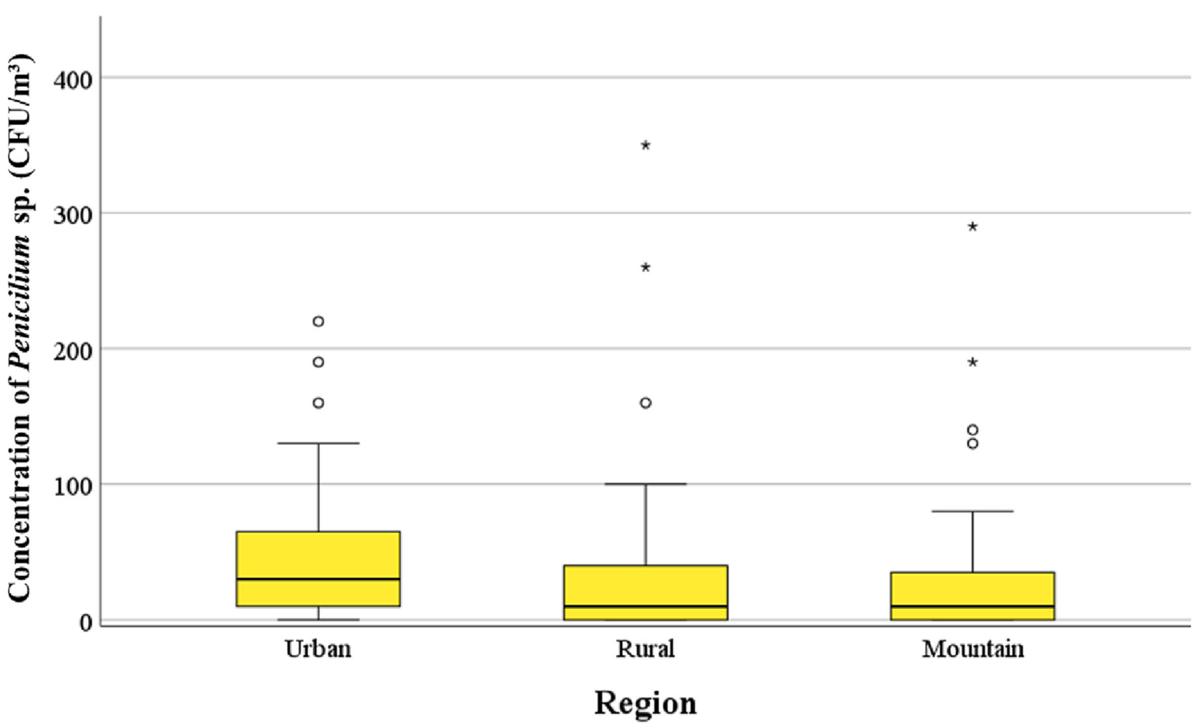

Fig. 4. Concentrations of *Penicillium* sp. in the urban, rural and mountain regions.

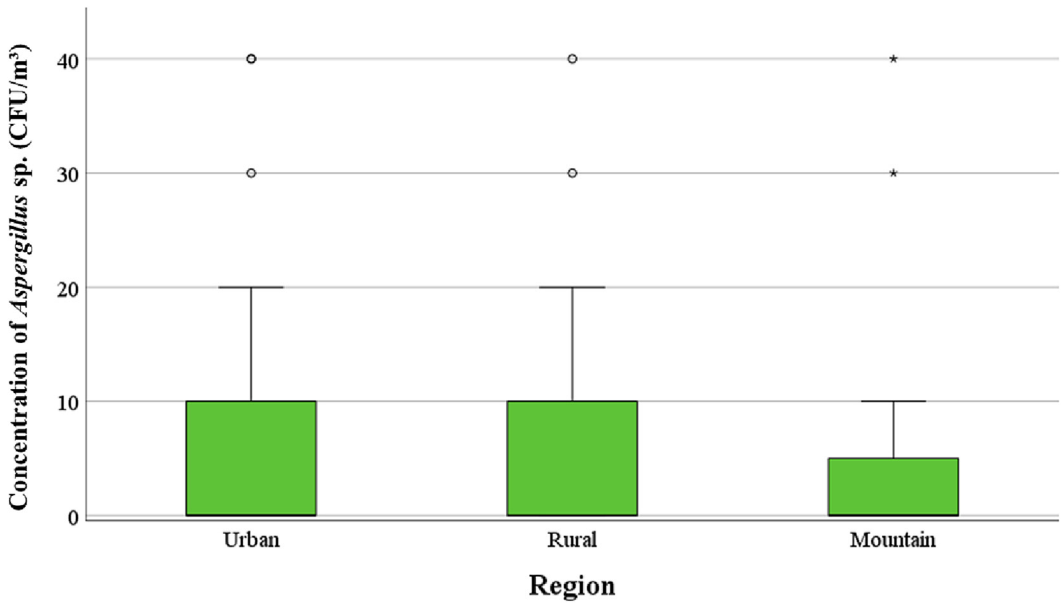

Fig. 5. Fungal spore concentrations of *Aspergillus* sp. in the urban, rural and mountain regions.

0.55, $p < 0.001$). No significant correlation was found between temperature and the concentrations of the genus *Aspergillus*. Furthermore, the temperature correlated negatively with the genus *Penicillium* sp. (rho $= -0.31$, $p < 0.001$).

The relative humidity was between 25.7 % and 99.9 % (Table S3). The comparison between RH and fungal spore concentrations showed negative correlations for xerophilic fungi and *Cladosporium* (rho $= -0.33$, $p < 0.001$) and (rho $= -0.35$, $p < 0.001$), respectively. There was no linear relationship either with genus *Aspergillus* or *A. fumigatus* species. No correlation was found between RH and the genus *Penicillium*. The multiple linear regression model (Table S4) showed that RH had an influence on *Cladosporium* sp. concentrations in the rural regions and on *A. fumigatus* in the urban.

The weather conditions showed an effect on the airborne fungal spores (Table 1). The xerophilic fungi and the genera *Cladosporium* and *Penicillium* were the highest at little cloud cover with median spore concentrations of 2.5×10^3 CFU/m^3, 1.9×10^3 CFU/m^3 and 2.0×10^1 CFU/m^3, respectively. In sunny conditions, the fungal spores were more frequent in the ambient air but during foggy days, they were ten-fold lower. No significant correlation was found between foggy weather and the genera *Aspergillus*. The highest spore concentration of *A. fumigatus* was detected in the air when the weather was cloudy (Table 1). The multiple linear regression model (Table S4) showed that foggy weather had an influence ($p < 0.05$) on the fungal spore concentrations of the xerophilic fungi and the genera *Cladosporium*. Foggy weather and temperature had significant effect on the concentrations of the genus *Penicillium*.

Wind velocity (m/s) was also an influencing factor. Slight wind (3–5 m/s) to windless (0–3 m/s) conditions dominated during the measurement period. This resulted in median concentrations for xerophilic fungi ranged from 1.4×10^3 CFU/m^3 without wind to 1.9×10^3 CFU/m^3 at slight wind. The concentration of *Cladosporium* sp. increased slightly from 1.2×10^3 CFU/m^3 at windless days to 1.6×10^3 CFU/m^3 at slight windy days. The highest fungal spore concentrations were found for *Penicillium* sp., *Aspergillus* sp. and *A. fumigatus* when there was no wind. The multiple linear regression model (Table S4) showed that temperature and RH during strong wind were significantly associated with the concentrations of *A. fumigatus* ($p < 0.05$).

3.4. Dust particles compared with fungal spore concentrations

In the three investigated regions, the number of fine particles (0.35–2.3 μm) ranged between 7.98×10^5 and 8.02×10^7 pa/m^3 with a median of 1.2×10^7 pa/m^3. The coarse particles (>2.3–10 μm) ranged between 5.48×10^3 and 9.94×10^4 pa/m^3 with a median of 6.5×10^4 pa/m^3. A significant relation ($p = 0.049$) was found between fine particles and at an altitude between >450 m and ≤850 m. While the concentrations of fine particles increased with increasing altitude ($p = 0.03$), the coarse particles decreased in the mountainous region ($p = 0.08$).

Fine and coarse particles were compared with the concentrations (CFU/m^3) of xerophilic fungi in the ambient air. The fine particles (0.35–2.3 μm) did not correlate with all fungal spore concentrations. Spearman correlations and multiple linear regression models showed that the coarse particles (>2.3–10 μm) correlated positively with the total xerophilic fungal spore concentration (rho $= 0.36$, $p < 0.001$) and *Cladosporium* (rho $= 0.33$, $p < 0.001$). There was also a positive correlation between the measured coarse particle concentrations and *Aspergillus* sp. (rho $= 0.17$, $p = 0.067$), but there was no significant difference between *Penicillium* sp. and the fungal species *A. fumigatus*. The proportion of fine and coarse particles in relation to altitude and region with respect to the total concentrations of xerophilic fungal spores are listed in the Supplementary Materials (Figs. S6-S9).

4. Discussion

Background concentrations of microorganisms and particulate matter in the ambient air represent the lowest levels of air pollution exposure. The knowledge of these concentrations is a crucial factor in population exposure assessment and epidemiological studies (Gómez-Losada et al., 2016).

The present study was conducted in the summer and early autumn. The background concentrations of xerophilic fungi in all the selected locations were between 3.5×10^2 and 4.7×10^3 CFU/m^3 at an average air temperature between 16.6 °C and 22.8 °C depending on the altitude. Other studies carried out in different geographic regions showed that the highest concentrations of airborne fungi were recorded in the summer to early autumn (Patel et al., 2018; Recio et al., 2012; Sakiyan and Inceoglu, 2003; Tesseraux, 2007). Kolk et al. (2009) summarized the data from an eight-year investigation period in Germany in which background concentrations of airborne fungi were between 1.7×10^3 CFU/m^3 and 3.2×10^3 CFU/m^3 in late spring to the end of summer. Haas et al. (2014) investigated the ambient air in the city of Graz over a period of two years and reported median fungal spore concentrations in the summer months of 9.4×10^2 CFU/m^3 for xerophilic fungi and 1.1×10^3 CFU/m^3 for mesophilic fungi, which were particularly high at a temperature of 30 °C and RH of 70–80 %.

Table 1
Total concentrations of xerophilic fungi, *Cladosporium* sp., *Penicillium* sp., *Aspergillus* and *A. fumigatus* in relation to weather conditions, altitude, and region.

	Environmental factors		Fungal concentrations in CFU/m³				
Fungal genera/species	Variables		Median	Q1[a]	Q3[b]	Minimum	Maximum
Total concentrations of xerophilic fungi (25 °C; DG18)	Altitude	≤ 450 m	1918	840	3565	110	7320
		> 450 m - ≤ 850 m	2520	1230	3700	520	7360
		> 850 m	1270	350	1960	0	8500
	Weather	Little cloud cover	2525	1220	3700	370	8500
		Heavy clouds	1390	520	3650	30	7360
		Foggy	305	180	405	0	4760
		Sunny	1940	1145	3543	120	5950
	Wind	Slight wind	1880	1090	3565	120	8500
		Strong wind	1140	270	3660	0	5950
		Windless	1420	470	3090	200	5700
	Region	Urban	1995	1165	3680	280	7360
		Rural	1735	970	3320	230	8500
		Mountain	1310	310	3610	0	6330
Cladosporium sp. (25 °C; DG18)	Altitude	≤ 450 m	1708	450	3330	70	7240
		> 450 m - ≤ 850 m	1870	830	3560	180	7320
		> 850 m	1230	220	1920	0	8400
	Weather	Little cloud cover	1925	800	3455	310	8400
		Heavy clouds	1240	310	3500	30	7320
		Foggy	220	60	375	0	4700
		Sunny	1825	1000	3353	90	5900
	Wind	Slight wind	1630	800	3330	90	8400
		Strong wind	1010	80	3500	0	5900
		Windless	1160	310	2970	110	5540
	Region	Urban	1708	820	3520	110	7320
		Rural	1590	730	3123	120	8400
		Mountain	1235	220	3420	0	6300
Penicillium sp. (25 °C; DG18)	Altitude	≤ 450 m	30	10	70	0	350
		> 450 m - ≤ 850 m	0	0	40	0	1880
		> 850 m	10	0	30	0	290
	Weather	Little cloud cover	20	0	60	0	1880
		Heavy clouds	20	0	70	0	500
		Foggy	5	0	20	0	40
		Sunny	10	0	45	0	290
	Wind	Slight wind	20	0	40	0	1880
		Strong wind	10	0	40	0	190
		Windless	30	0	100	0	350
	Region	Urban	30	10	65	0	1880
		Rural	10	0	40	0	500
		Mountain	10	0	35	0	290
Aspergillus sp. (25 °C; DG18)	Altitude	≤ 450 m	0	0	10	0	165
		> 450 m - ≤ 850 m	10	0	20	0	60
		> 850 m	0	0	0	0	20
	Weather	Little cloud cover	0	0	10	0	20
		Heavy clouds	0	0	10	0	60
		Foggy	0	0	0	0	0
		Sunny	0	0	10	0	165
	Wind	Slight wind	0	0	10	0	60
		Strong wind	0	0	0	0	20
		Windless	0	0	10	0	165
	Region	Urban	0	0	10	0	165
		Rural	0	0	10	0	90
		Mountain	0	0	5	0	60
A. fumigatus (37 °C; CNA)	Altitude	≤ 450 m	5	0	15	0	103
		> 450 m - ≤ 850 m	5	0	25	0	290
		> 850 m	2.5	0	7.5	0	27.5
	Weather	Little cloud cover	7.5	0	12.5	0	97.5
		Heavy clouds	5	0	32.5	0	290
		Foggy	0	0	0	0	5
		Sunny	5	0	10	0	75
	Wind	Slight wind	5	0	12.5	0	290
		Strong wind	0	0	5.0	0	62.5
		Windless	7.5	0	32.5	0	235
	Region	Urban	8.75	0	31.25	0	290
		Rural	5	0	10	0	97.5
		Mountain	2.5	0	7.5	0	35

[a] Q1 = first quartile.
[b] Q3 = third quartiles.

4.1. Concentrations of fungal spores in the air of urban, rural and mountain regions

In this study, the highest median background concentration of total fungal spores was detected in the urban air in the city of Graz. Beside the environmental factors, air pollution, in particular emissions from traffic, industry and other anthropogenic sources, may have an impact on the number of spores in urban air. Moreover, higher background concentrations were detected in larger towns with >8000 inhabitants than in smaller

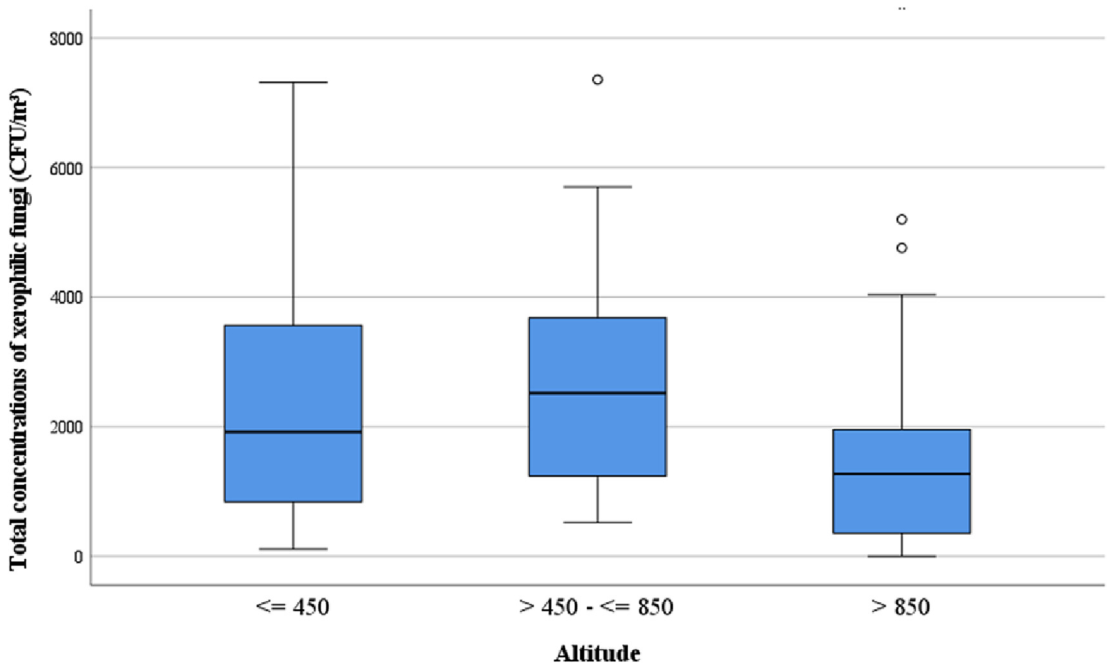

Fig. 6. Comparison between the concentrations of xerophilic fungal spores and altitude (m).

towns. Measuring results in towns differ according to geographic location and climatic conditions. Background concentration measurements, which were carried out in the sites of the large towns are located in valleys of alpine areas with a harsh mountain climate whereas the small towns are in a hilly landscape with a mild climate.

Several studies have examined the difference between urban and rural environmental airborne fungus infestation. Some studies have shown an increase in fungal spore concentrations in urban areas (Bauer et al., 2008; Fang et al., 2019; Rathnayake et al., 2016), others found an increase in rural areas (Lin et al., 2018; Oliveira et al., 2009; Oliveira et al., 2010).

In this study, the measuring sites in the rural regions are characterized by small settlement areas with farms. The highest concentrations of fungal spores in the rural ambient air were detected at altitudes of >800 m in the valleys of the alpine areas. At the measuring sites in vineyard areas and those with livestock, the airborne fungi were slightly higher than those at the lake site of Thal (TS). Lohberger (2016), reported similar median spore concentrations of 1.8×10^3 CFU/m^3 in the rural area. Zhang et al. (2010) specified that in the countryside or in forests, fungal infestation is higher than in urban areas due to denser vegetation cover. Kasprzyk and Worek (2006) proved that land use, vegetation, flora and microclimate play an important role in relation to the amount of spores in the ambient air.

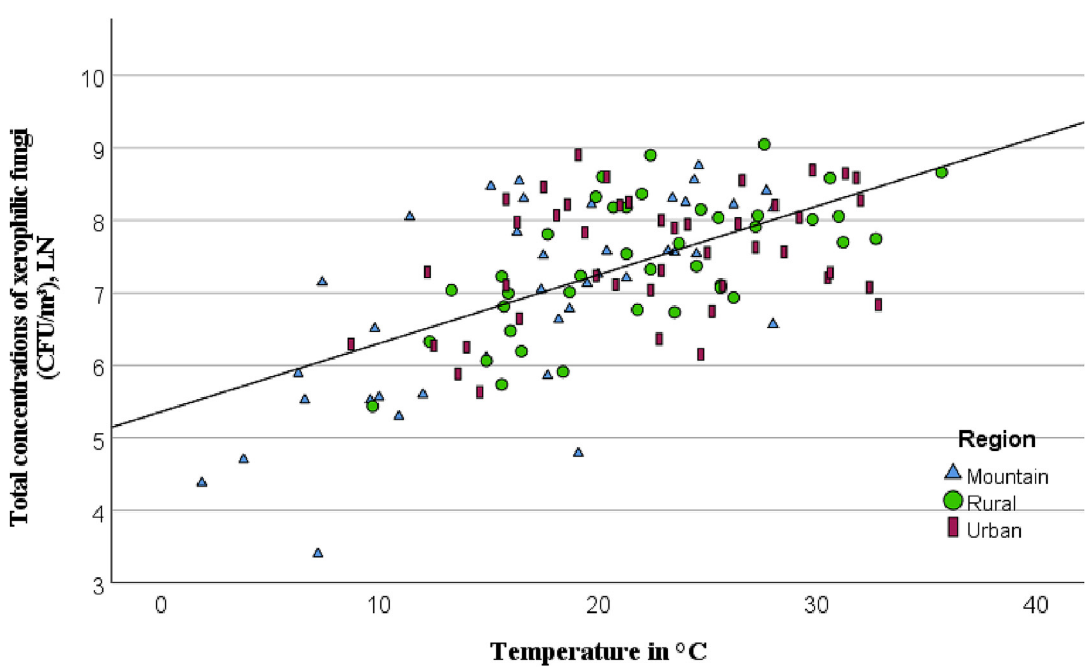

Fig. 7. The association between temperature and concentration of xerophilic fungi in the three regions.

In the mountainous regions, the highest fungal spore concentrations were determined at the measuring sites (PB and AR) at an altitude of 800 m, which are surrounded by forests and meadows used for agriculture. In mountain regions above 1400 m altitude where a typical alpine flora and grazing animals present, the fungal spores were ten-fold lower than the sparsely forested background measuring sites. In the Austrian Alps, Ebner et al. (1989) recorded higher concentrations of fungal spores in mountain locations <827 m than at an altitude of 1960 m. On the Sphinx (3571 m) in Switzerland, the proportion of fungal spores in the air was <10 CFU/m^3 (Brandl, 2011). The present study measured different fungal spore concentrations at the two background measuring sites HW, GR of 3.5×10^2 CFU/m^3 and 1.9×10^3 CFU/m^3, respectively at approximately the same altitude (1870 m). These results show that, airborne fungal spore concentrations depend not only on altitude but also on vegetation, season and agriculture management of a mountainous region.

4.2. Concentrations of the genera Cladosporium, Penicillium and Aspergillus in air

The genus *Cladosporium* accounted for the major part (> 86 %) of the total xerophilic fungal spore concentrations. In contrast, the spore concentrations of *Penicillium* sp. were <1.5 % and *Aspergillus* sp. was barely detectable in the ambient air. In the continental climate zone of Beijing, Fang et al. (2005) examined the relative abundance of airborne cultivable fungi over a full year and detected the fungi of the genus *Cladosporium* sp. with a frequency of 96.8 %. A study by Fang et al. (2019) showed that the genus *Penicillium* sp. accounted for the largest proportion (about 29.6 %) of the total fungal concentrations.

Numerous studies confirmed that the genera *Cladosporium*, *Aspergillus* and *Penicillium* are the most commonly identified spores in the ambient air (Fang et al., 2019; Hameed et al., 2009; Herrero et al., 2006; Oliveira et al., 2009; Saito et al., 2015; Sen and Asan, 2008). However, Hameed et al. (2009) studied the diurnal distribution of fungi in the atmosphere of the Helwan region in Egypt and found that the genus *Aspergillus* sp. was the predominant fungi. Furthermore, it was found that the spore concentrations of *Aspergillus*, *Penicillium* and *Cladosporium* were higher in rural areas than in urban areas in Portugal (Oliveira et al., 2009). In contrast, the spore concentrations of *Aspergillus* and *A. fumigatus* were higher in the urban environment in Spain (Castro e Silva et al., 2020; Guinea et al., 2006).

Cladosporium sp. is common worldwide and abundant in temperate regions. It occurs on plant materials as saprophytic fungi and their spore prevalence in the air was observed, for example, in sunflower fields (Kshirsagar and Pande, 2012). The genera *Penicillium* and *Aspergillus* are common contaminants of various substrates e.g. soil or compost (Samson et al., 2000). In tropical and subtropical regions, the occurrence of *Aspergillus* sp. is more common than the *Penicillium* sp. A study in Finland compared the seasonal variations of airborne fungal spore concentrations between a landfill, urban and rural area. The concentrations of *Penicillium* and *Aspergillus* species were significantly higher near the waste center compared to the other sites, while the concentration of *Cladosporium* sp. was the highest in rural areas (Kaarakainen et al., 2008).

The present study shows that the fungal spore concentration of *A. fumigatus* species was higher in the rural and urban than in mountainous regions. At the background site PB the spore concentrations of *Aspergillus* sp. and *A. fumigatus* were slightly higher than at the other measurement sites in the mountains. A possible explanation could be that the measuring location PB was in the immediate vicinity of the city of Graz where the forest absorbs the emissions. Trautmann et al. (2005) measured the concentration of airborne *A. fumigatus* during the summer months to be at a median of 1.0×10^1 CFU/m^3. The habitat of *A. fumigatus* is often garbage, compost and humidifier systems. In waste paper and cardboard recycling factories, *Aspergillus niger*, *Penicillium* sp., *Cladosporium* sp. and various species of bacteria are predominant in the contaminated air (Baghani et al., 2021; Baghani et al., 2022). Reinthaler et al. (1999) recommended the concentration of 1.0×10^1 as a reference value for the natural background exposure to *A. fumigatus*.

Based on the large number of studies, it can be stated that the spectrum of airborne culturable fungi is similar in different geographic regions around the world and only the proportions of the individual genera vary.

4.3. Factors influencing fungal spore concentrations

The naturally occurring concentrations of fungal spores in the air depend on the season, the weather and local conditions, whereby the most relevant meteorological factors are temperature, precipitation and RH (Anees-Hill et al., 2022). The vegetation can also have a significant impact on the concentration and biodiversity of fungi in the ambient air. In the present study, air temperature had a positive correlation to the concentration of total fungal spores and the genus *Cladosporium*. However, it had an insignificant effect on the genus *Aspergillus* and even a negative effect on the genus *Penicillium*. Grinn-Gofroń and Rapiejko (2009) found, that the air temperature correlated significantly with the number of *Cladosporium* sp. spores. In contrast, RH had a negative impact on total fungi, *Cladosporium* and *Aspergillus*, and a positive impact on *Penicillium* (Pyrri and Kapsanaki-Gotsi, 2017). Contradictory results between airborne fungi, temperature and RH were reported by Mosalaei et al. (2021).

The RH showed a significant negative correlation with the total concentration of xerophilic fungi and *Cladosporium* sp. but an insignificant effect on the genera *Penicillium* and *Aspergillus*. *A. fumigatus* was not influenced by RH. *Aspergillus* and *Penicillium* showed no correlation with the meteorological factors (Oliveira et al., 2009).

These results are similar to those obtained from different geographic regions with different climatic conditions in Europe (Haas et al., 2014; Ianovici, 2016; Olsen et al., 2020; Pyrri and Kapsanaki-Gotsi, 2017), Africa (Hameed et al., 2012), Asia (Akgül et al., 2016; Fang et al., 2019) and America (Almaguer et al., 2014). Hameed et al. (2012) discovered that air temperature and RH are the most commonly predicted variables for airborne fungi. Air temperature showed a positive and negative correlation with concentrations of *Aspergillus* and *Penicillium*, respectively, while RH positively correlated with concentrations of total fungi, *Aspergillus* and *Penicillium*. Fang et al. (2019) also stated that meteorological parameters have a crucial impact on the survivability of fungi.

Fewer fungal spore concentrations were found on foggy days compared to sunny or slightly cloudy days in this study. This can be explained by the fact that foggy weather has a leaching effect on the fungal spores suspended in ambient air (Pyrri and Kapsanaki-Gotsi, 2017). Rathnayake et al. (2017) found that fungal spore concentrations increased after rain whereas the results from Songnuan et al. (2018) showed that the concentrations of airborne fungal spores are positively correlated with the amount of rainfall. This proves that there is a difference between wet and dry weather spora. *Cladosporium* sp., as a dry weather spora more likely to be found in foggy weather (Hjelmroos, 1993) but *Penicillium* sp. is not affected by rainy or dry weather (Rosas et al., 1993).

The highest spore concentrations in this study were found in calm and light wind conditions. Quintero et al. (2010) reported that wind speed serves as a dispersal factor for fungal spores and spore concentrations decreased significantly in strong winds. Wind speed acts as a dilution and transport factor that can significantly increase and/or decrease airborne fungal concentrations (Hameed et al., 2012; Hasnain et al., 2012).

During the summer months, the meteorological conditions such as high air temperature, RH, wind and foggy weather were the most influential factors on airborne fungal spore concentrations in the present study. The geographic location is also an important factor affecting ambient bioaerosol concentrations (Xie et al., 2021).

4.4. Correlation between fungal spores concentrations and dust particles

It was found that coarse particle concentrations correlated positively with the total concentrations of xerophilic fungi, *Cladosporium* sp., *Aspergillus* sp. and the species *A. fumigatus*. A possible explanation may be that these fungal genera or species have a conidia size ranging between >2.5 to 5 μm which is in the coarse particle fraction. The coarse particle fraction was two times higher in urban ambient air than in mountain air. The number of fine particles was high at certain altitudes. It was noticed that the number of coarse particles and fungal concentrations increased with increasing wind speed (Haas et al., 2020). The urban environment exhibits higher particle concentrations, which can be amplified due to emissions from local anthropogenic sources. Furthermore, due to the lack of vegetation, more particulate matter swirls in the air when it is windy. In regions with good air circulation, the tendency of local emissions accumulation is low. At moderate wind, the aerosol particles are transported to other regions but at the same time, they come from other areas (Bauer et al., 2007). In addition to the wind, the aerosol particles are dependent on the air temperature (Gao et al., 2016; Liu et al., 2019b; Madhwal et al., 2020; Nguyen et al., 2017).

The genera especially, *Aspergillus*, *Penicillium* and *Alternaria* in the air are correlated with smog or dust levels (Góralska et al., 2022; Tajiki et al., 2022). Neisi et al. (2019) found that the most frequently identified fungi on days of high smog levels were the fungal genera *Cladosporium*, *Aspergillus*, *Penicillium*. The study by Haas et al. (2013) reported that the annual median concentrations of the genera *Penicillium* and *Aspergillus* also increased with the increase of particulate matter.

The present study showed that mountainous regions had less smog and fungal spores in the ambient air. In contrast, high numbers of both coarse and fine particles as well as fungal spores occur in the air in the rural regions due to the processing of agricultural land. Jones and Harrison (2004) reported that wind speed has a strong influence on the amount of coarse particles in rural areas. At a mediterranean site, no associations were found between fungal spore and particle concentrations in rural regions (Raisi et al., 2010). Another study conducted in India showed a significant correlation between airborne fungi, fine and coarse particles (Sousa et al., 2008).

Airborne microorganisms exist mostly in aerosol form and rarely as individuals, enabling them to attach to dust particles as microbial carriers (Grinn-Gofroń, 2011; Raisi et al., 2013; Xie et al., 2018; Zhai et al., 2018). It is noted that fungal fragments could stay in the air longer than fungal spores due to their smaller particle size (Madsen et al., 2009). Bioaerosols cannot grow or multiply in the atmosphere, they can only survive for a certain period of time (Bulski, 2020).

5. Conclusion

This study examined background concentrations of airborne fungi and dust particles in urban, rural and mountain areas in Styria. The natural background concentrations for total fungi were between 3.5×10^2 and 4.7×10^3 CFU/m^3 and the median concentrations of fine and coarse particles were of 1.2×10^7 pa/m^3 and 6.5×10^4 pa/m^3 respectively. No significant differences were found between the fungal spore concentrations in urban, rural and mountain regions. The genus *Cladosporium* was the major part of the total xerophilic fungal spore concentrations. The xerophilic fungal spore concentrations were positively affected by air temperature, little cloud cover, slight wind and foggy weather. However, weather and meteorological conditions as well as the movement of aerosol particles and vegetation can influence the fungal species and their concentrations in the air. The study was conducted in the summer months, therefore, bioaerosol assessment throughout a whole year is recommended. For air quality investigations, the natural background concentrations of this study could be used as a reference to compare the airborne culturable fungi in regions with similar geographic character and climatic conditions.

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CRediT authorship contribution statement

Petra Ofner-Kopeinig, Andreas Strasser: statistical analysis; **Theresa Fritz**, **Herbert Galler**, **Angela Kriso, Michael Kropsch**: investigation, methodology, validation; **Mihaela Ilieva:** conceptualisation, methodology; **Michael Schalli**: writing—original draft and editing; **Franz Ferdinand Reinthaler, Eduard Zentner**: project administration; **Doris Haas**: investigation, supervision, writing—original draft, writing review and; graphical abstract; **Juliana Habib**: writing—original draft, writing review and editing; All authors have read and agreed to the published version of the manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164700.

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