



Morning–evening variation and determinants of bacterial and fungal aerosol concentration in historical objects in Kraków

Maria Chmiel · Anna Lenart-Boroń 

Received: 25 May 2020 / Accepted: 17 December 2020

© The Author(s), under exclusive licence to Springer Nature B.V. part of Springer Nature 2021

Abstract Cultural heritage objects are very frequently visited by tourists, the presence of which is considered to affect the bacterial and fungal aerosol levels in these facilities. Therefore, this study had two main aims with the first to determine the morning/evening changes in the concentration of bacterial and fungal aerosol as well as particulate matter in the selected historical objects of Kraków: churches, crypts, libraries, and museums. The second aim was to establish the most possible factors affecting the quality of air in the examined sites using data mining techniques: principal component analysis (PCA) and cluster analysis (CA). Bacterial and fungal aerosol levels were measured in ten historical objects by six-stage Andersen impactor. Particulate matter (PM₁₀, PM₄, PM_{2.5} and PM₁), temperature, and relative humidity (RH) were also assessed. Unlike dusts, the microbial aerosol concentrations were not always significantly lower in the morning than in the evening. For bacteria, these relationships varied depending on the site, while for fungi, the morning concentrations were mostly higher than the evening ones. Both PCA and CA indicated that the bacterial and fungal aerosol concentrations were not associated with the particulate matter levels, which might suggest different sources of

those two air pollution components. Temperature and RH appear to be more important in shaping the microbial aerosol levels and fungi are more dependent on RH than bacteria.

Keywords Bacterial and fungal aerosol · Cluster analysis · Historical objects · Indoor air · Principal component analysis

1 Introduction

Historical objects such as churches, monasteries, their crypts, libraries or museums are peculiar, but not uniform, environments. Even libraries or museums can differ from one another. They often store valuable collections of different origin and structure (Harkawy et al. 2011), made of a variety of materials including paper, wood, textiles, leather, paintings, stone, glass, and metals (Ciferri et al. 2003). This makes them be under constant risk of biodeterioration. This risk is related not only to the exposure of the exhibitions to microorganisms with cellulolytic, proteolytic or lipolytic properties, but also to specific microclimatic conditions (air temperature and humidity) prevailing in cultural heritage objects, or type and amount of light received by collections or chemical reagents present in the environment (Harkawy et al. 2011; Karbowska-Berent et al. 2011).

M. Chmiel · A. Lenart-Boroń (✉)
Department of Microbiology and Biomonitoring,
University of Agriculture in Kraków, Mickiewicza Ave
24/28, 30-059 Kraków, Poland
e-mail: anna.lenart-boron@urk.edu.pl

Among microbiological agents, fungi and bacteria have the greatest influence on the hygienic conditions and therefore affect the preservation of the collections (Karbowska-Berent et al. 2011). Their presence and abundance in historical objects is affected by a number of factors, including microclimatic parameters, such as temperature and relative humidity prevailing indoors. The microclimatic parameters of historical buildings are the result of their age, type of construction, availability to the public, number of tourists and visitors, and technical means such as ventilation type. Indoor aerosol-forming microorganisms have their sources both outdoors and indoors. In naturally ventilated buildings, outdoor fungal spores are major sources of indoor fungi during spring and summer (i.e., the growing season) (Lehtonen and Reponen 1993). Another factor that may affect the presence and abundance of indoor bacterial and fungal aerosols is accumulated dust, where microorganisms can dwell and proliferate. Also the presence of water (i.e., increased humidity) can increase the concentration of microbial aerosol, particularly in materials, such as wallpaper, carpets, or ventilation duct surfaces (Chao et al. 2002). The spectrum of microorganisms present in the air depends largely on their ability to colonize the environment and to conduct their living processes (Kowalski and Pastuszka 2018). In the case of bacteria, their reaction to changing temperature and relative humidity of air may vary significantly even within the same structural classification (i.e., Gram-positive or Gram-negative bacteria). Among many diverse observations, a universal one was that the temperature exceeding 24 °C appears to decrease the survival of aerosol-forming bacteria. On the other hand, the effects of relative humidity are much more complex, with experimental conditions having often a considerable impact on the outcome of the experiments (Tang 2009).

Among various pollution problems, the pollution of air has raised major concerns worldwide, due to its widespread nature, damage to the environment and the resulting health risks. The issue of indoor air pollution is one of the most important, as people spend most of their time (approx. 80%) indoors (Leung 2015). Also, the above-mentioned factors affect the health and wellbeing of people staying indoors—in the case of historical objects, these are tourists, visitors, or workers. It needs to be emphasized that microorganisms are present in most indoor environments. If the

biological agents occur in low concentrations and they do not include pathogenic microorganisms, they do not pose threat to people. The problem of microbiological contamination occurs when the level of microbiological contaminants increases above a certain limit, considered as an acceptable level for a particular environment. The airborne route is the most important in spreading of microorganisms indoors (Gołofit-Szymczak et al. 2013). Usually, airborne microorganisms occur as bioaerosols and much more rarely as free particles. Bioaerosols constitute from 5 to even 34% of indoor air pollution—these of diameters below 5.0 µm usually stay suspended in the air, while larger ones sediment on the surfaces (Stetzenbach et al. 2004). The health threats in cultural heritage institutions result from the inhalation of air contaminated with microorganisms. The main consequences of such exposure are allergic reactions, infections, and mycotoxicoses (Skóra et al. 2015). Epidemiological studies showed that infectious and non-infectious diseases caused by inhalation of microbial aerosols depend not only on the chemical characteristics and biological properties but also on the concentrations of microorganisms in the respiratory system (Husman et al. 1993; Chen et al. 2010). It is believed that microbial concentrations of healthy indoor environments should be lower than the outdoor concentration at the same location (Mehta et al. 2013). However, many studies on indoor–outdoor air quality show that the concentrations of indoor pollutants are higher than those found outdoors (Chen and Zhao 2011).

The microbiological quality of indoor air is usually assessed based on quantitative and/or qualitative determination of bacterial and fungal aerosol. The culture-based methods are still the most popular, as most of the available threshold limit values are referred strictly to such methods (Górny 2004; Harkawy et al. 2011). Having all the above in mind, the aims of this study were: (1) to determine the morning/evening differences in the concentration of bacterial and fungal aerosols and particulate matter in the selected historical objects in Kraków, divided into four groups: churches, crypts, libraries, and museums and (2) to establish the most possible factors affecting the quality of air in the tested sites by using different statistical methods, including cluster analysis and principal component analysis.

2 Materials and methods

The study was conducted in ten historical objects, classified into four groups churches, crypts, libraries, and museums located in one of the most popular Polish tourist cities—Kraków. The site located on the Main Market Square was treated as an external background control (Fig. 1). Table 1 presents the description of the sampling sites along with basic environmental parameters measured during the study. Sample collection was performed over a period of one year, once per each season (spring—15th April, summer—20th

August, autumn—3rd November, winter—30th January). The sampling dates were selected by taking into consideration the microclimatic parameters, such as temperature, humidity, precipitation, wind speed and by excluding days with extreme weather conditions, in order to conduct our analyzes on dates representing the most typical weather conditions for each season. During each campaign, the samples were collected twice per day—in the morning (M), approx. at 6 AM and in the evening (E), approx. at 8 PM. Collection of air samples was conducted using a six-stage Andersen cascade impactor (model WES-710, Westech



Fig. 1 Location of sampling point No. 11 (Background) on the Main Market Square in Kraków (based on <https://geoportal360.pl>)

Table 1 Description and environmental parameters in the studied historical objects.*

No.	Group of sites (code)	Sampling site-description	Indoor temperature [°C]	Relative humidity [%]	Total PM**—Morning [µg/m ³]	Total PM—Evening [µg/m ³]	PM—RF*** (1 µm)—Morning [µg/m ³]	PM—RF (1 µm)—Evening [µg/m ³]	PM—RF (4 µm)—Morning [µg/m ³]	PM—RF (4 µm)—Evening [µg/m ³]
1	Church (Ch1)	St. Mary's Church—in front of the main altar	15.1 (10.6–20.7)	58.9 (54.5–65.3)	59 (48–68)	101 (56–164)	53 (41–62)	82 (47–138)	58 (45–68)	92 (51–145)
2	Church (Ch2)	Church of St. Casimir the Prince—in front of the main altar	14.3 (9.1–20.0)	61.05 (46.7–87.4)	56 (51–64)	105 (55–187)	49 (45–57)	95 (47–171)	54 (50–63)	101 (52–181)
3	Crypt (Cr1)	Crypt of the Franciscans of Primitive Observance	13.3 (11.5–17.2)	56.3 (49.1–66.2)	59 (46–79)	76 (44–151)	49 (40–66)	67 (40–136)	55 (43–78)	69 (42–139)
4	Church (Ch3)	Church of St. Michael the Archangel and St. Stanislaus Bishop and Martyr and Pauline Fathers Monastery, Skalka—in front of the main altar	14.2 (9.2–20.3)	62.1 (55.6–72.5)	163 (60–420)	522 (53–1,760)	139 (51–347)	438 (54–1,440)	143 (59–346)	488 (49–1,630)
5	Crypt (Cr1)	Crypt of Meritorious	12.5 (9.6–16.1)	62.9 (55.2–69.9)	95 (51–155)	104 (48–240)	80 (44–132)	88 (42–198)	90 (48–148)	95 (45–213)
6	Library (L1)	Library at the Skalka Church	13.8 (7.8–20.5)	62.2 (58.0–67.6)	80 (58–113)	178 (50–444)	71 (50–108)	131 (43–292)	76 (55–108)	171 (47–441)
7	Library (L2)	The Princes Czartoryski Library—reading room	20.96 (19.5–22.4)	44.2 (32.0–56.5)	57 (49–69)	60 (44–84)	53 (45–67)	53 (39–76)	56 (47–69)	57 (42–80)
8	Museum (M1)	The Princes Czartoryski Museum—antique art exhibition	21.5 (18.3–25.2)	44.4 (34.1–58.9)	70 (48–93)	58 (44–75)	62 (44–76)	53 (41–70)	66 (45–85)	57 (43–72)
9	Museum (M2)	The Main Square Underground Museum—by the city model	18.6 (14.5–21.3)	53.6 (36.4–64.7)	79 (45–158)	84 (62–108)	71 (38–145)	76 (58–105)	73 (42–149)	82 (58–107)
10	Museum (M3)	The Old Synagogue—main hall	20.3 (17.4–22.1)	53.9 (38.9–64.4)	78 (53–108)	76 (58–113)	69 (44–98)	66 (52–103)	73 (48–104)	73 (56–112)
11	Background (B)	The Main Market Square—control point, outdoors	11.1 (4.6–18.6)	69.3 (47.5–93.7)	121 (50–257)	166 (49–443)	94 (42–176)	110 (44–252)	105 (43–207)	132 (47–331)

*Values presented in the table are means, values in brackets show ranges

**PM—particulate matter

***RF—respirable fraction

Instrument, UK), which allows to distinguish the following aerodynamic diameters of bioaerosol: 7 μm and above (stage one), 4.7–7 μm (stage two), 3.3–4.7 μm (stage three), 2.1–3.3 μm (stage four), 1.1–2.1 μm (stage five), and 0.65–1.1 μm (stage six). Microbial particles with diameters smaller than 4.7 μm were treated as the respirable fraction (RF). The sampling time was 5 min, which with the constant flow rate of 28.3 L/min, gives the volume of 141.5 L of aspirated air. The air sampler was placed at the height of 1.5 m above the ground level to collect the air from the human breathing zone. The following microbiological media were used: Trypticasein Soy Agar (Biocorp, Poland) and Malt Extract Agar (Biocorp, Poland) for the isolation of bacteria and fungi, respectively. The plates with TSA were incubated at 37 °C for 1 day, then at 22 °C for next 3 days and at 4 °C for another 3 days, while the plates with MEA were incubated at 30 °C for 4 days, followed by incubation at 22 °C for another 4 days. After incubation, the colonies were counted, and the results were expressed as the number of colony forming units per m^3 of air (CFU/ m^3). The measurements were conducted in triplicates, and the presented results are mean values of these replicates.

Temperature and relative humidity were measured using the Kestrel 4000 Weather Meter (Nielsen-Kellerman, USA). The concentration of dust was measured using a DustTrak™ II Aerosol Monitor 8530 (TSI Inc., USA) laser photometer. The device allows to measure four fractions of dust: PM_{10} (i.e., dust particles not larger than 10 μm), PM_4 , $\text{PM}_{2.5}$ and PM_1 (dust particles with diameters ≤ 4 , 2.5 and 1 μm , respectively), using interchangeable heads. The sampling time for each dust fraction was 1 min. In the case of particulate matter, it was assumed that the respirable fraction contains dust particles smaller than 4.0 μm .

Statistical analysis was performed using the Statistica 13 software (StatSoft, USA). Data normality testing was conducted using the Shapiro–Wilk test. The significance level was set at a P value of < 0.05 for all statistical tests. A Kruskal–Wallis test was used to verify the significance of differences in the microbial aerosol and particulate matter concentrations between times of day in the examined sampling sites. Spearman's correlation coefficient was applied to assess whether there are statistically significant relationships between the concentrations of microbial

aerosol components and microclimatic parameters of air in different groups of the tested sites and in all sites grouped together. Cluster analysis (CA) and Principal Component Analysis (PCA) were used to determine the relationships between the tested microbiological components (i.e., bacteria and fungi) of bioaerosol and microclimatic parameters of air and to extract the most important factors affecting the quality of air in the examined sites. CA and PCA tests were performed for each of the studied groups of sites (i.e., churches, crypts, libraries, museums, and background), using the following variables: total and respirable fraction of bacterial and fungal aerosol, temperature, humidity, total dust as well as respirable fraction of dust – 4 and 1 μm . Factors were extracted using principal components, then were varimax-rotated. Euclidean distance was adopted as a measure of similarity. Ward's agglomerative clustering, which involves estimating the distance between clusters by the analysis of variance, was adopted as a grouping method. In the PCA, the analysis was based on the correlation matrix, and two most important factors for each group of sites were selected for the interpretation of results.

3 Results

As shown in Table 1, the observed concentrations of total and respirable fractions (RF) of particulate matter, in most cases, were higher in the evening than in the morning, with two exceptions for Museums No. 1 and No. 3. In the Skalka Church (No. 3), the concentrations observed in the evening were even three times higher than the one observed in the morning. The mean values of microclimatic parameters (indoor temperature and relative humidity) ranged from 12.5 °C in the Crypt of Meritorious (Crypt No. 2) to 21.5 °C in the Princes Czartoryski Museum (Museum No. 1) and from 44.2% RH in the Princes Czartoryski Library (Library No. 2) to 62.9% RH in the Crypt of Meritorious (Crypt No. 2). The individual values of these parameters varied even more significantly in some of the sampling sites. For example, in Library No. 1, the temperatures ranged from 7.8 to 20.5 °C (ΔT 12.7 °C). Also in churches, the ranges of temperature were quite high, i.e., ΔT was 11.1, 10.9, and 10.1 °C for churches No. 3, 2, and 1, respectively. The highest RH range was observed in Church No. 2 (ΔRH 40.7%, from 46.7 to 87.4%), followed by the

ranges observed for museums, where ΔRH was 28.3, 25.5, and 24.8% for Museum No. 2, 3, and 1, respectively.

Figures 2 and 3 show the mean annual values of morning/evening concentrations of bacteria and fungi in individual sampling sites, while Tables 2 and 3 show the morning/evening values observed in different seasons of the year in individual sampling sites. As shown in Fig. 2., the mean concentration of total bacterial aerosol varied from 6.35×10^3 CFU/m³ (Crypt No. 2, morning) to 1.426×10^3 CFU/m³ (Church No. 2, morning). The mean levels of respirable fraction of bacteria ranged from 5.1×10^2 CFU/m³ (Crypt No. 2, morning) to 8.02×10^2 CFU/m³ (Church No. 2, morning). On the other hand, the mean total fungal concentration ranged from 3.55×10^2 CFU/m³ (Museum No. 2, morning) to 1.21×10^3 CFU/m³ (Library No. 1, morning) and the mean levels of respirable fraction of fungi ranged from 2.30×10^2 CFU/m³ (Museum No. 2, evening) to 1.19×10^3 CFU/m³ (Library No. 1, morning). Comparison of both graphs shows that the changes in the concentration over the day are more evident in the case of bacteria, while for fungi, these values are more evenly distributed. Unlike dusts (Table 1), the microbial levels did not always follow the pattern of low concentrations in the morning and significantly higher in the evening. This was obvious only for Church No. 1 (St Mary's) and Church No. 3

(Skalka), where the evening concentrations were even five times higher (in the Skalka Church the mean value of the total bacterial concentration in the morning was 189 CFU/m³ while in the evening—956 CFU/m³). A completely opposite situation was observed in the Church No. 2 (St. Casimir the Prince), where e.g., the mean morning concentration of total bacteria (1426 CFU/m³) was more than five times higher than the one observed in the evening (mean total concentration of 270 CFU/m³). Also both crypts differed from one another in terms of the morning/evening concentrations of bacteria. Interestingly, the mean concentrations of fungal aerosols were higher in the morning than in the evening in eight out of eleven studied locations (Churches No. 1 and No. 2, both Crypts, Library No. 1, Museums No. 1 and No. 2, as well as Background).

The Shapiro–Wilk test showed that most of the data were not normally distributed (critical value for not rejecting the hypothesis of Gaussian distribution of data was set at $W = 0.935$ for $\alpha = 0.05$; the W values calculated for parameters measured in this study ranged from 0.442 to 0.932 with five exceptions: humidity in spring $W = 0.939$; Temperature in autumn $W = 0.958$; RF of fungi in winter $W = 0.960$; total fungi in winter $W = 0.964$ and humidity in summer $W = 0.979$). Therefore, non-parametric statistical tests were used in further analyzes. Statistical analysis showed that the observed differences in the

Fig. 2 Mean annual values of morning and evening concentrations of total and respirable fraction (RF) of bacterial aerosol in individual sampling sites (CFU/m³)

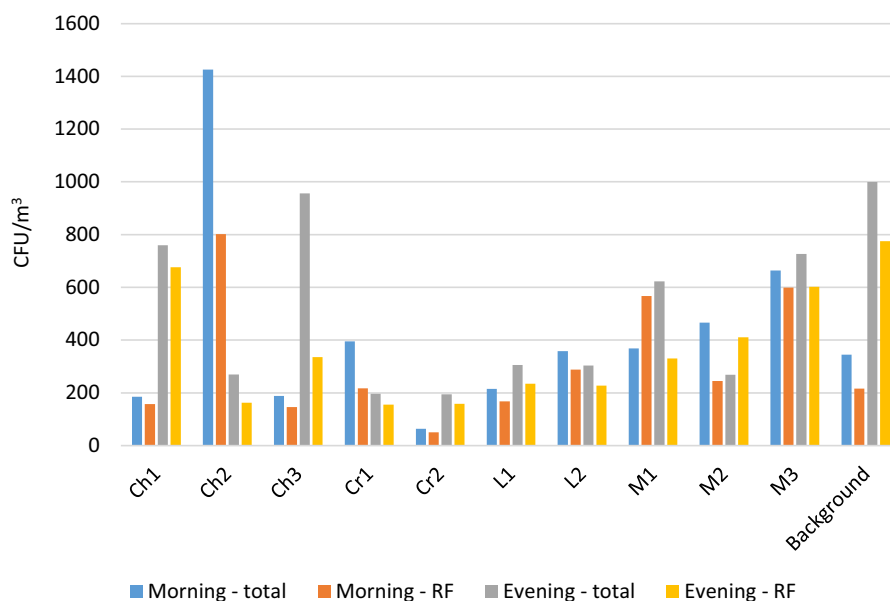
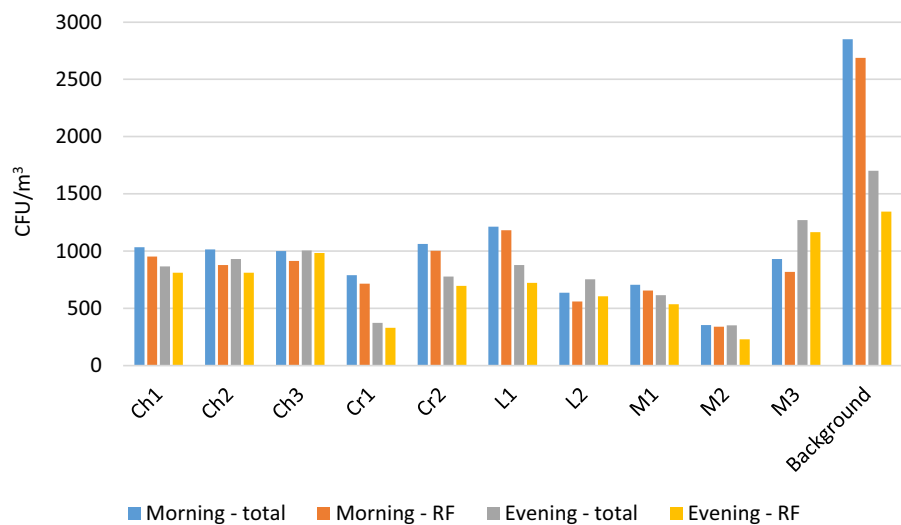


Fig. 3 Mean annual values of morning and evening concentrations of total and respirable fraction (RF) of fungal aerosol in individual sampling sites (CFU/m³)



concentrations of bacterial and fungal aerosols and dust fractions did not differ significantly between the times of day (the H values of Kruskal–Wallis test from 0.001 for total fungi to 1.299 for total dust, $p > 0.05$). This is not surprising when looking at the morning/evening values observed in different seasons of the year (Tables 2 and 3). In the case of total bacteria, only in sites No. 1 and 5 (St Mary's Church and Crypt of Meritorious) and in the case of respirable fraction—in sites No. 1 and 4 (St Mary's Church and Skalka Church) the morning values were smaller than the ones observed in the evening throughout the year. For fungi, no clear pattern could be observed.

The Principal Component Analysis (Fig. 4a–f, Table 4) and Cluster Analysis (Fig. 5a–f) allowed to identify the associations between individual sets of data measured in this study, whether and/or to what extent the physicochemical parameters affect the concentration of microbial aerosol and whether the sets of data collected in this study could be grouped depending on the impact they make on one another. The analysis was conducted for individual sites (Figs. 4 and 5a–e) and for all sites grouped together (Figs. 4 and 5f). The principal component analysis (PCA) was based on two main factors (Fig. 4, Table 4) that explain from 73.3% of variance (crypts) to as much as 82.8% (background). When considering all sites together, 70.01% of variance was explained by the two main factors. The positions indicated by the lines in Fig. 4 represent the correlations between the measured values of environmental factors and the PC1

and PC2. For churches, museums and background PC 1 is negatively associated with dusts (total and respirable fraction) and positively—with bacterial and fungal aerosol along with temperature. For crypts, both PC1 and PC2 are negatively correlated with fungal aerosol and humidity, PC1 is negatively correlated with dusts while PC2 is positively correlated with dusts. In the case of libraries, PC2 (explaining 27.26% of variance) is associated positively with fungal and bacterial aerosol as well as with dusts and humidity. Cluster analysis allowed to distinguish from two to three groups of the analyzed factors in the examined sites. As shown in Fig. 5, the measured parameters of air were grouped in two clusters in the case of churches, libraries and background. One group shows a clear cluster of microclimatic parameters of air, i.e., humidity and dust concentration (total dust and both respirable fractions) and the second group clusters total and respirable fractions of microbial aerosol with the air temperature. For crypts and museums, three clusters were distinguished, but they differ between these two groups of sites. Both in crypts and museums, there is a cluster of total and respirable dust; in crypts, cluster 2 groups total and RF of fungi with humidity and temperature, cluster 3 groups bacteria only; in museums, cluster 2 groups total and RF of fungi with humidity, cluster 3 groups total and RF of bacteria with temperature. These results are consistent with the ones obtained for simple Spearman's correlation analysis (Table 5), which indicate that various groups of sites differ in

Table 2 Concentration of bacterial aerosol in individual sampling sites (CFU/m³) depending on the time of day (M—morning; E—evening)

Time of day/site no.	Total concentration of bacteria [CFU/m ³]							
	Spring		Summer		Autumn		Winter	
	M	E	M	E	M	E	M	E
1.	177	311	403	2459	28	71	134	198
2.	212	459	5357	424	14	71	120	127
3.	634	170	311	177	481	360	155	78
4.	155	403	438	3350	42	28	120	42
5.	141	580	71	113	7	28	35	57
6.	283	806	184	382	141	14	254	21
7.	417	226	933	813	21	85	64	92
8.	926	650	1387	664	78	7	99	155
9.	177	869	841	247	0	678	57	71
10.	926	1618	1371	622	410	92	198	325
11.	661	703	339	1986	261	1293	120	14
Time of day/site no.	Concentration of bacteria from the respirable fraction (aerodynamic diameter < 4.7 µm) [CFU/m ³]							
	Spring		Summer		Autumn		Winter	
	M	E	M	E	M	E	M	E
1.	127	226	375	2304	28	57	99	120
2.	42	438	3081	42	0	71	85	99
3.	57	113	254	106	417	353	141	49
4.	127	367	424	919	14	21	21	35
5.	141	537	21	57	7	21	35	21
6.	269	643	120	269	42	14	240	14
7.	375	141	693	629	21	78	64	64
8.	784	607	1329	594	78	7	78	113
9.	120	833	813	205	0	572	49	35
10.	516	1519	1322	551	389	71	170	269
11.	286	332	283	1477	212	1293	85	0

terms of relationships (and significance thereof) between the examined parameters. These results indicate that temperature and relative humidity of air are the parameters that are more important in shaping the levels of bacterial and fungal aerosols than the levels of dust and that fungi are the group of microorganisms that are more dependent on the air humidity than bacteria.

4 Discussion

Numerous studies have reported that the exposure to microbial aerosols, especially in large concentrations,

is associated with unwanted health effects, particularly asthma and rhinitis, hypersensitivity pneumonitis and sick building syndrome, or increased susceptibility to infections (Chen et al. 2010). The ubiquity of mold fungi causes an increased incidence of systemic fungal infections in recent years. Although the systemic fungal infections are characterized by high mortality they are recognized only in small percentage (max. 30%). This is caused primarily by non-specific symptoms, similar to bacterial or viral infections (Ławniczek-Wałczyk et al. 2018). Different concentrations of fungal spores have been determined to be the threshold values for evoking the allergic symptoms, varying from e.g., 50 CFU/m³ of air to

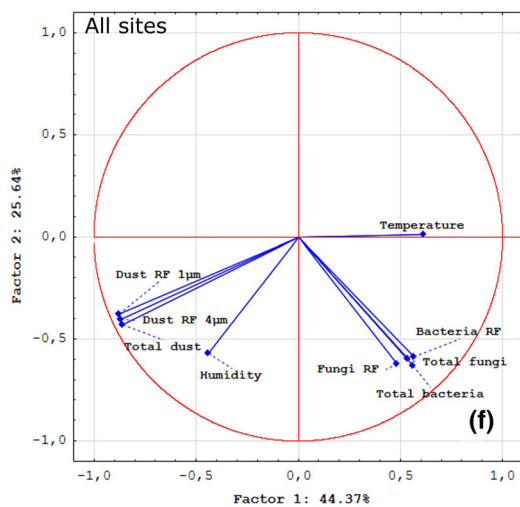
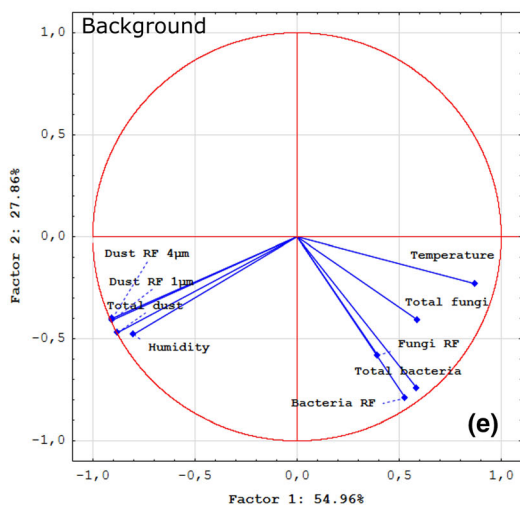
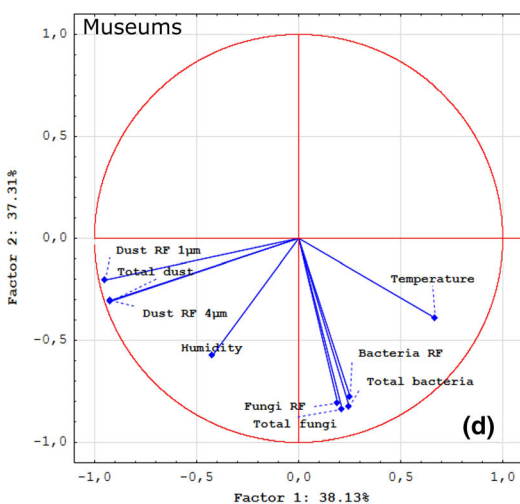
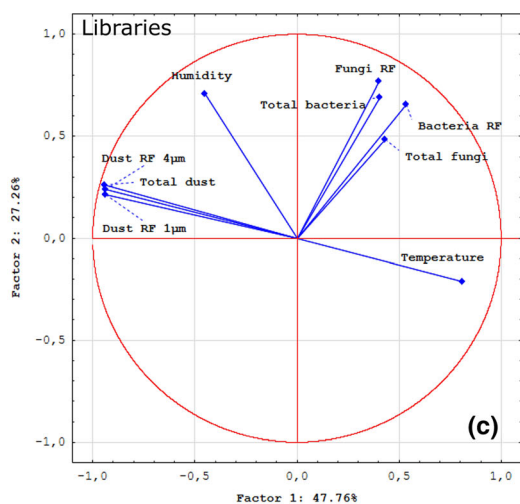
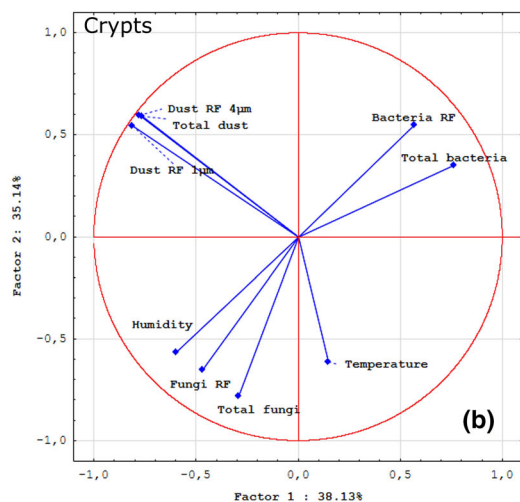
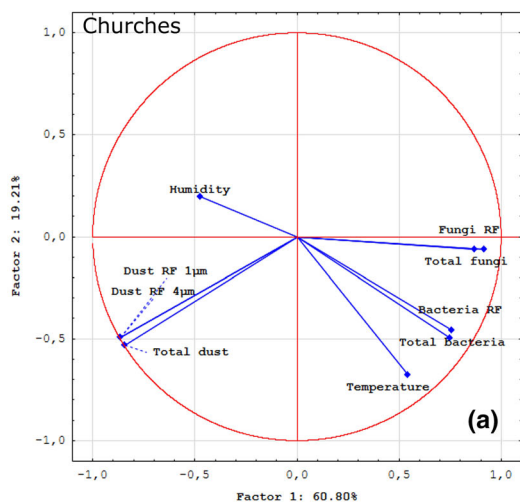
Table 3 Concentration of fungal aerosol in individual sampling sites (CFU/m³) depending on the time of day (M—morning; E—evening)

Site no.	Total concentration of fungi [CFU/m ³]							
	Spring		Summer		Autumn		Winter	
	M	E	M	E	M	E	M	E
1.	1152	438	1963	2127	261	212	763	693
2.	332	1244	2389	1364	191	389	1152	728
3.	269	184	2007	396	233	113	650	806
4.	219	85	3562	2912	71	311	148	714
5.	855	198	1929	707	1159	1392	311	813
6.	1208	855	2537	1025	233	1357	876	283
7.	1576	177	523	1569	99	120	353	1152
8.	375	417	1675	1230	290	297	488	523
9.	551	226	813	693	28	389	28	99
10.	820	325	1760	1724	537	2014	607	1025
11.	1540	1226	7774	2057	1724	2339	360	1187
Site no.	Concentration of fungi from the respirable fraction (aerodynamic diameter < 4.7 µm) [CFU/m ³]							
	Spring		Summer		Autumn		Winter	
	M	E	M	E	M	E	M	E
1.	1138	410	1809	2000	254	170	608	671
2.	318	1187	2304	1230	184	346	707	481
3.	240	177	1774	269	212	99	643	777
4.	29	85	3512	2855	64	283	57	714
5.	777	177	1823	424	1110	1378	304	806
6.	1188	848	2502	1018	184	763	862	261
7.	1307	163	509	1420	85	113	346	728
8.	353	367	1590	982	276	290	410	509
9.	551	71	777	459	28	375	7	14
10.	806	325	1420	1562	445	1979	601	799
11.	1428	707	7498	1364	1477	2155	353	1152

3000 CFU/m³, that—having in mind their ubiquitous nature affecting their indisputable presence in most enclosed spaces (Pastuszka et al. 2000)—is the factor which particular attention should be paid to. In fact, the limit values of professional exposure or the recommended threshold values for microbial aerosols and substances of microbial origin still have not been developed or have no legal status on a global scale. This hinders the quantitative interpretation of the results of bioaerosol concentration measurements in the studied environment. The main reason for the lack of universally accepted normative values for biological factors is the methodical difficulty resulting from the diversity of microorganisms, the lack of

standardization of measurement and experimental methods, and the difficulty in determining the effects of bioaerosol on the exposed human organism. The susceptibility of an exposed individual to a given biological agent is its individual feature, which makes it difficult to clearly determine the effects on the population understood as a unified group of people (Dutkiewicz and Górny 2002; Bonetta et al. 2010; Górny et al. 2011; Napoli et al. 2012; Frąk et al. 2014; Ghajari et al. 2015; Fakunle et al. 2020).

Therefore, the assessment of the quality of air in the examined sites has been conducted based on the recommendations of the limit values of microbial aerosols set for occupational exposure by the



◀ **Fig. 4** Results of principal component analysis conducted for the concentration of microbial aerosol and physicochemical parameters of air measured in different groups of sampling sites and for all sites grouped together

Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment (Skowroń 2014). In general, the microbial aerosol concentrations do not exceed the threshold values (5000 CFU/m³) set for the occupational exposure of people working in museums and related institutions (Skowroń 2014), while the limit value of 150 CFU/m³, suggesting the presence of internal source of contamination which can be harmful to the preservation of stored objects (Skowroń 2014) was exceeded in numerous observations. However, it should be remembered that these buildings are open to the public and many factors influence the air quality. A number of factors needs to be mentioned while considering possible sources of microbial aerosol in community places. According to Fujiyoshi et al. (2017), microbial communities in public utility places are dominated by those of human and soil origin, but the main differences between the public space and house microbiome is the number of people using the area. Another factor that might affect the concentration of microbial aerosols is the air mixing, coupled with their particle size, as these factors will affect the behavior of airborne microorganisms (i.e., their floating in the air or sedimentation) (Fujiyoshi et al. 2017). The detailed comparison of the microbial concentrations recorded in our study with the ones observed by other Authors, with division into particular groups of sites was discussed in the paper by Chmiel et al. (2019).

Unlike in our study, Chen et al. (2010) observed clear changes in the bacterial and fungal aerosol concentrations over the day with the highest values recorded in the afternoon, which the Authors related to the number of visitors. In our study, the morning bacterial and fungal aerosol sampling was conducted in very early hours, before library or museum opening and before morning masses in churches; therefore, the factors affecting the results obtained in our study are similar to those in research by Chen et al. (2010). One of the possible explanations of the fact that the morning concentrations of fungal spores in most of the sampling sites outnumbers the evening concentrations

Table 4 Factor loadings for airborne microorganisms and microclimatic parameters of air tested in this study

Parameter	Unit	Churches		Crypts		Library		Museum		Background		All sites	
		PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Total bacteria	CFU/m ³	0.75	- 0.50	0.77	0.35	0.41	0.69	0.25	- 0.83	0.59	- 0.74	0.56	- 0.63
Bacteria RF		0.76	- 0.46	0.57	0.55	0.54	0.66	0.25	- 0.78	0.53	- 0.79	0.57	- 0.59
Total fungi		0.92	- 0.06	- 0.29	- 0.78	0.43	0.49	0.21	- 0.84	0.59	- 0.41	0.54	- 0.60
Fungi RF		0.87	- 0.06	- 0.47	- 0.65	0.40	0.77	0.19	- 0.81	0.40	- 0.58	0.49	- 0.63
Temperature	°C	0.54	- 0.68	0.15	- 0.61	0.81	- 0.21	0.67	- 0.39	0.87	- 0.23	0.61	0.01
Humidity	%	- 0.47	0.20	- 0.59	- 0.57	- 0.45	0.71	- 0.42	- 0.57	- 0.80	- 0.48	- 0.44	- 0.57
Total dust	µg/m ³	- 0.84	- 0.53	- 0.76	0.59	- 0.94	0.26	- 0.92	- 0.31	- 0.88	- 0.47	- 0.86	- 0.43
Dust RF 1 µm		- 0.86	- 0.49	- 0.81	0.55	- 0.93	0.21	- 0.95	- 0.20	- 0.91	- 0.41	- 0.88	- 0.38
Dust RF 4 µm		- 0.86	- 0.49	- 0.78	0.60	- 0.94	0.24	- 0.92	- 0.30	- 0.90	- 0.40	- 0.87	- 0.41
Accounted variance	%	60.80	19.21	38.13	35.14	47.76	27.26	38.13	37.31	54.96	27.86	44.37	25.64

Loadings ≥ 0.60 are in bold italics

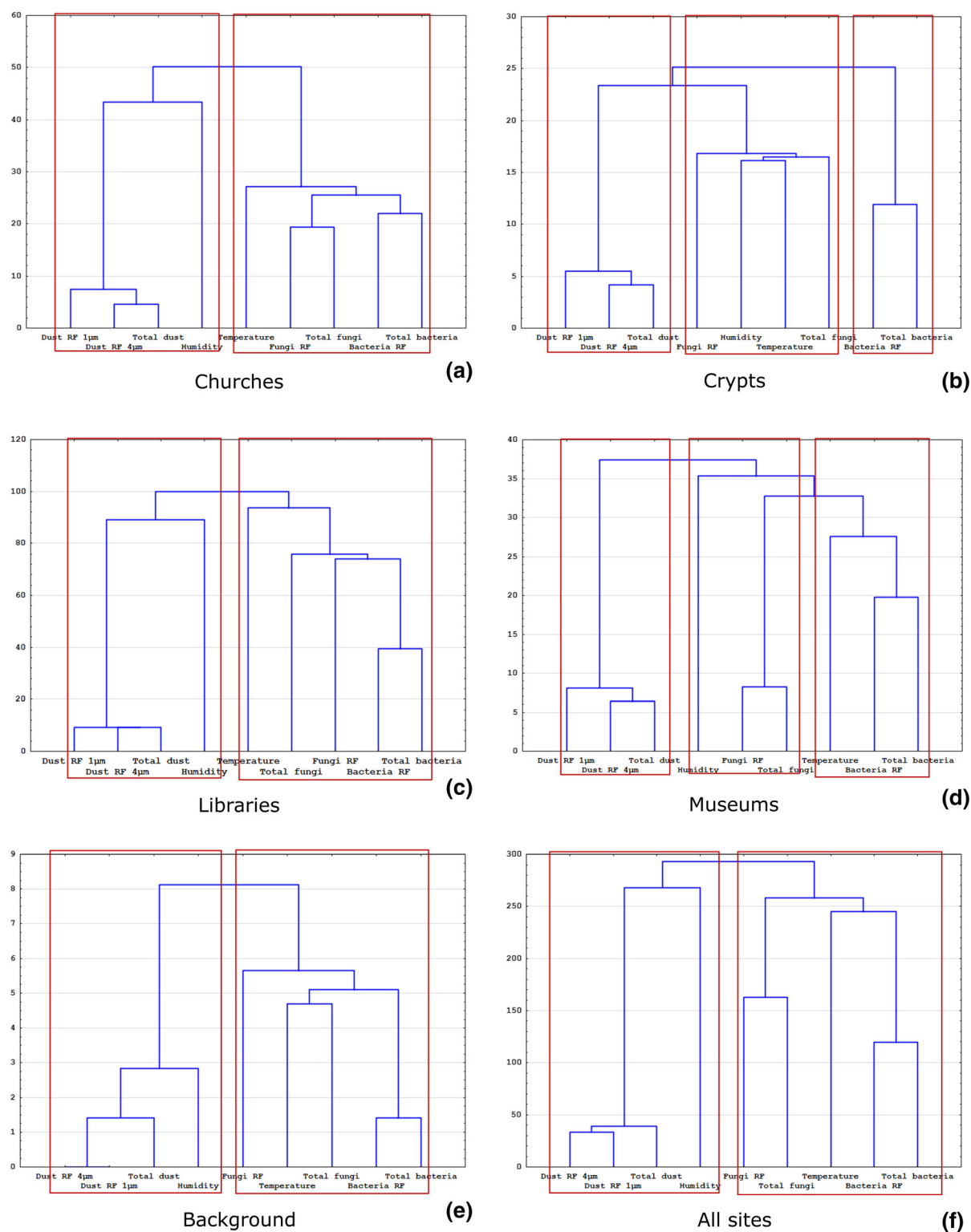


Fig. 5 Dendrograms based on Ward's cluster analysis of microbial aerosol concentration and physicochemical parameters of air measured in different groups of sampling sites and for all sites grouped together

Table 5 Spearman's correlation coefficient matrices for the parameters examined in this study—in all sites grouped together and for different groups of sites

	Total bacteria	Bacteria RF	Total fungi	Fungi RF	Temperature	Humidity	Total dust	Dust RF 1 µm	Dust RF 4 µm
<i>All sites</i>									
Total bacteria	–								
Bacteria RF	0.874	–							
Total fungi	0.412	0.389	–						
Fungi RF	0.410	0.372	0.768	–					
Temperature	0.451	0.470			–				
Humidity			0.242	0.237	– 0.480	–			
Total dust			– 0.283	– 0.210	– 0.336	0.366	–		
Dust RF 1 µm			– 0.292	– 0.235	– 0.326	0.337	0.977	–	
Dust RF 4 µm			– 0.280	– 0.221	– 0.330	0.353	0.986	0.990	–
<i>Crypts</i>									
Total bacteria	–								
Bacteria RF	0.790	–							
Total fungi			–						
Fungi RF	– 0.520	– 0.619	0.523	–					
Temperature					–				
Humidity			0.600	0.582	0.615	–			
Total dust							–		
Dust RF 1 µm							0.940	–	
Dust RF 4 µm							0.974	0.955	–
<i>Churches</i>									
Total bacteria	–								
Bacteria RF	0.789	–							
Total fungi	0.717	0.683	–						
Fungi RF	0.609	0.610	0.836	–					
Temperature	0.678	0.564	0.580	0.594	–				
Humidity						–			
Total dust		– 0.427	– 0.729	– 0.651			–		
Dust RF 1 µm		– 0.453	– 0.765	– 0.709			0.976	–	
Dust RF 4 µm		– 0.429	– 0.746	– 0.684			0.991	0.970	–
<i>Libraries</i>									
Total bacteria	–								
Bacteria RF	0.893	–							
Total fungi			–						
Fungi RF	0.524	0.623	0.606	–					

Table 5 continued

	Total bacteria	Bacteria RF	Total fungi	Fungi RF	Temperature	Humidity	Total dust	Dust RF 1 µm	Dust RF 4 µm
Temperature					–				
Humidity					– 0.591	–			
Total dust					– 0.708		–		
Dust RF 1 µm					– 0.703		0.985	–	
Dust RF 4 µm					– 0.705		0.994	0.994	–
<i>Museums</i>									
Total bacteria	–								
Bacteria RF	0.830	–							
Total fungi	0.533		–						
Fungi RF	0.511		0.970	–					
Temperature	0.669	0.614			–				
Humidity			0.457	0.455		–			
Total dust							–		
Dust RF 1 µm					– 0.417		0.967	–	
Dust RF 4 µm							0.982	0.971	–
<i>Background control site</i>									
Total bacteria	–								
Bacteria RF	0.976	–							
Total fungi			–						
Fungi RF				–					
Temperature			0.738		–				
Humidity						–			
Total dust						0.881	–		
Dust RF 1 µm						0.905	0.976	–	
Dust RF 4 µm						0.905	0.976		–

Only statistically significant values are shown ($p < 0.05$)

could be that solar radiation during clear sunny days have a sterilization effect on fungal spores, or at least decreases their viability (Kowalski & Pastuszka 2018), therefore may result in lower numbers of fungal spores proliferating on microbiological media resulting from the evening sample collection. Also Dybwad et al. (2014) in their study conducted at subway stations observed consistent diurnal patterns of significantly higher levels of bacterial aerosol at daytime compared to very low levels at nighttime, associating the observed fluctuations with the number

of passengers. The Authors studying diurnal changes in microbial aerosol levels in underground railway stations (Salma et al. 2007) also observed that the changes in profiles of bacterial aerosols corresponded to the PM₁₀ profile. This observation is even true for the fungal concentrations in the outdoor air (background—mean total fungal levels of 2849 CFU/m³ and 1702 CFU/m³ in the morning and in the evening, respectively; and mean values of RF of fungi: 2689 CFU/m³ and 1344 CFU/m³ in the morning and in the evening, respectively), what might have affected

the concentrations of fungi inside the studied premises. On the other hand, the mean bacterial concentrations in the morning were lower than the evening ones, both for the total and respirable fraction. These results are opposite to those of Chen et al. (2010), who recorded that in the outdoor samples, the bacterial concentration was lower during the afternoon sampling than during the morning one and the fungal concentrations were characterized by the opposite trend—they were higher in the afternoon than in the morning.

Water availability and temperature have been determined as critical environmental factors affecting microbial growth indoors (Burge and Otten 1999). This has been confirmed by the results obtained in our study, as Principal Component Analysis, Cluster Analysis as well as Spearman's correlation indicated that the levels of total and respirable fraction of bacterial and fungal aerosols appear to be influenced by RH and temperature in most groups of the examined sites. Also according to Sroka et al. (2008), the growth of mold fungi, including mycotoxigenic ones, is inseparably associated with the presence of three factors: fungal inoculum, organic or mineral substrate providing necessary nutrients and humidity. Therefore, the potential exposure to aerosol-forming fungi and their toxic metabolites occurs in the environments where these factors are present. Molds occurring in the working environment are particularly dangerous to humans. They cause allergic reactions, and their secondary metabolites—mycotoxins—can be the cause of severe intoxications. These compounds cause acute, sub-acute or chronic toxicity effects, they also show carcinogenic, teratogenic, genotoxic, immunotoxic, and neurotoxic effects (Gajewska and Głowacka 2017).

According to Tang (2009), the response of bacteria to different values of relative humidity can vary greatly, depending on the bacterial species, or even on the experimental conditions. This might explain our observations that the association of bacterial counts with RH of air was not as evident and frequent as with the temperature of air. This can be seen while analyzing the results of PCA, CA and Spearman's correlation. Contrary to this, fungal aerosols seem to be more closely associated with the air humidity, with higher concentrations of spores observed at higher RH levels, as shown clearly in Fig. 5. Also other Authors (Erkara et al. 2008; Tang 2009; Spilak et al. 2015)

observed that while comparing bacteria and fungi, the latter group appears to be more dependent on the humidity of air. Spilak et al. (2015) explains that this might be caused by the fact that high RH of air allows fungal growth in the environment. Fujiyoshi et al. (2017) in their review of articles concerning the transmission of bacterial aerosols in different environments summarizes that a general relationship between some of the environmental factors, particularly RH, has not been confirmed.

Studies show that temperatures exceeding 24 °C appear to universally decrease the survival of aerosol-forming bacteria (Tang 2009); however, the temperatures observed in our study almost never exceeded this threshold. What also needs to be taken into consideration is that temperature might not be significantly associated with total microbial concentrations due to fact that outdoor temperature does not affect indoor temperature, especially in the sites where the cooling and heating systems are used throughout the year, and the temperature is rather constant.

The relationship between the concentrations of microbial aerosol and particulate matter concentration was vague in our study. The Spearman's correlation showed that in the case of churches, there might be a negative relationship between those two groups of parameters. The PCA graphs show opposite signs of correlations between the analyzed principal components and dusts with bacterial and fungal aerosols. In Cluster Analysis, total dust fractions and respirable fractions of PM formed a cluster which was separate from microbial levels. Also Spilak et al. (2015) did not find any correlation between the concentration of fine dust particles and fungal or bacterial levels. On the other hand, according to Zhai et al. (2018), particulate matter acts as a primary carrier for airborne microorganisms; therefore, the presence of microbes in the air should mostly depend on the concentration of PM. Also particulate matter can offer airborne microorganisms nutrients to prevent starving conditions and maintain their metabolism. The discrepancy between the particulate matter concentration and the levels of microbial aerosols in our study could result from the fact that PM and aerosol-forming microorganisms might have different origins. This could also be the reason for our observation that the changes in the morning/evening levels of particulate matter differed from the morning/evening levels of bacterial and fungal aerosols. Similar observations as in our study

were made by Lazaridis et al. (2015) in their study on particulate matter and microbial load in cultural heritage collections. They observed that particulate matter did not show any significant correlation with the levels of cultivable microbial aerosols, and showed a different seasonal variability than microorganisms. Lazaridis et al. (2015) suggested that those two parameters might have different origins, i.e., the PM concentrations are significantly correlated with the number of visitors and indoor activities, while the elevated concentrations of bacterial and fungal aerosols may result from damages to the exhibited materials, damp microclimate indoors, and poor cleaning conditions. However, even though a number of statistical analyzes were conducted in our study, it is difficult to clearly define all factors determining the concentration of bacterial and fungal aerosols in historical objects.

5 Conclusions

In this study, we attempted to determine the morning/evening changes in the bacterial and fungal aerosol coupled with particulate matter concentrations, and we applied Principal Component Analysis and Cluster Analysis to determine the most important factors affecting the quality of air in various groups of historical and cultural objects.

Our investigation showed that among the tested air quality parameters particulate matter showed the most evident differences in the morning/evening concentrations with the morning values significantly lower than the evening ones. In the case of bacterial aerosols, this regularity was less evident, while airborne fungi showed the opposite pattern, i.e., in most cases, their morning concentrations were higher than the evening ones. Those discrepancies may result from different sources of microbial aerosol and particulate matter inside the examined premises.

In all sites, the PCA allowed to designate two main factors that explain most of the observed variance (from 73.3 to 82.8%). The PCA results showed that the particulate matter levels either do not affect the levels of bacterial and fungal aerosol or correlate negatively with the concentrations of bacterial and fungal aerosol (negative relation observed for churches and crypts).

Similarly as PCA, Cluster Analysis indicated that the concentrations of microbial aerosols are much

more significantly associated with the temperature than with the relative humidity, while particulate matter forms an independent cluster. It seems that the humidity of air is a more significant parameter in shaping the fungal aerosol levels than temperature, which was also observed in the correlation analysis.

The statistical methods used in our study allowed a thorough insight into the processes and mechanisms affecting microbial aerosol and particulate matter levels in historical objects.

Author contributions Maria Chmiel and Anna Lenart-Boroń contributed to research concept and design. Maria Chmiel conducted sample collection, data generation and critical revision of the article as well as final approval of the article text. Anna Lenart-Boroń conducted data interpretation and analysis, writing of article and figure preparation.

Funding This study was funded by the statutory measures of the University of Agriculture—Grant No. DS 3158/KM.

References

- Bonetta, S. A., Bonetta, S. I., Mosso, S., Sampò, S., & Carraro, E. (2010). Assessment of microbiological indoor air quality in an Italian office building equipped with an HVAC system. *Environmental Monitoring and Assessment*, 161, 473–483. <https://doi.org/10.1007/s10661-009-0761-8>.
- Burge, H. A., & Otten, J. A. (1999). Fungi. In: *Bioaerosols: Assessment and control* (Macher J., ed.). Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 19–11–19–13.
- Chao, H. J., Schwartz, J., Milton, D. K., & Burge, H. A. (2002). Populations and determinants of airborne fungi in large office buildings. *Environmental Health Perspectives*, 110(8), 777–782.
- Chen, C., & Zhao, B. (2011). Review of relationship between indoor and outdoor particles: I/O ratio, infiltration factor and penetration factor. *Atmospheric Environment*, 45, 275–288. <https://doi.org/10.1016/j.atmosenv.2010.09.048>.
- Chen, Y., Cui, Y., & Dong, J. (2010). Variation of airborne bacteria and fungi at Emperor Qin's Terra-Cotta Museum, Xi'an, China, during the "Oct. 1" Gold Week Period of 2006. *Environmental Science and Pollution Research*, 17, 478–485.
- Chmiel, M., Kral, I., & Lenart-Boroń, A. (2019). Concentration and size distribution of microbial aerosol in the historical objects in Kraków as a potential health risk and biodegradation factor. *Aerobiologia*, 35(4), 743–758.
- Ciferri, O., Becker, M. E., & De Rossi, E. (2003). Characterization of bacteria isolated from naturally aged silk fibroin. In R. J. Koestler, V. H. Koestler, A. E. Charola, & F. E. Nieto-Fernandez (Eds.), *Art, biology, and conservation: Biodegradation of works of art* (pp. 190–207). New York: Yale University Press.

- Dutkiewicz, J., & Górny, R. L. (2002). Biological factors hazardous to human health: Classification and criteria of exposure assessment. *Medycyna Pracy*, 53(1), 29–39. (in Polish).
- Dybwad, M., Skogan, G., & Blatny, J. M. (2014). Temporal variability of the bioaerosol background at a subway station: Concentration level, size distribution and diversity of airborne bacteria. *Applied and Environmental Microbiology*, 80(1), 257–270.
- Erkara, I. P., Asan, A., Yilmaz, V., Pehlivan, S., & Okten, S. S. (2008). Airborne *Alternaria* and *Cladosporium* species and relationship with meteorological conditions in Eskisehir City, Turkey. *Environmental Monitoring Assessment*, 144, 31–41.
- Fakunle, A. G., Olusola, B., Jafta, N., Faneye, A., et al. (2020). Home Assessment of Indoor Microbiome (HAIM) in relation to lower respiratory tract infections among under-five children in Ibadan, Nigeria: The Study Protocol. *International Journal of Environmental Research and Public Health*, 17(6), 1857. <https://doi.org/10.3390/ijerph17061857>.
- Frąk, M., Majewski, G., & Zawistowska, K. (2014). Analysis of the quantity of microorganisms adsorbed on particulate matter PM10. *Scientific Review – Engineering and Environmental Sciences*, 64, 140–149. (in Polish).
- Fujiyoshi, S., Tanaka, D., & Maruyama, F. (2017). Transmission of airborne bacteria across built environments and its measurement standards: A review. *Frontiers in Microbiology*, 8, 2336.
- Gajewska, M., & Głowacka, A. (2017). Assessing filamentous fungal contamination of dried herbs and spice available in retail health food stores and hypermarkets. *Food Science Technology Quality*, 24(3), 51–59.
- Ghajari, A., Lotfali, E., Azari, M., Fateh, R., & Kalantary, S. (2015). Fungal airborne contamination as a serious threat for respiratory infection in the hematology ward. *Tanaffos*, 14(4), 257–261.
- Gołofit-Szymczak, M., Ławniczek-Wałczyk, A., & Górny, R. L. (2013). Exposure of ventilation system clearing workers to harmful microbiological agents. *Medycyna Pracy*, 64(5), 613–623. <https://doi.org/10.13075/mp.5893.2013.0066>.
- Górny, R. L. (2004). Biohazards: standards, guidelines, and proposals for threshold limit values. *Podstawy i Metody Oceny Środowiska Pracy*, 3(41), 17–39.
- Górny, R. L., Cyprowski, M., Ławniczek-Wałczyk, A., Gołofit-Szymczak, M., & Zapór, L. (2011). Biohazard in the indoor environment: A role for threshold limit values in exposure assessment. In M. R. Dudzińska (Ed.) *Management of indoor air quality*. Taylor & Francis Group, 1–20.
- Harkawy, A., Górny, R. L., Ogierman, L., Wlazło, A., et al. (2011). Bioaerosol assessment in naturally ventilated historical library building with restricted personnel access. *Annals of Agricultural and Environmental Medicine*, 18(2), 323–329.
- Husman, T., Koskinen, O., Hyvärinen, A., Reponen, T., et al. (1993). Respiratory symptoms and infections among residents in dwellings with moisture problems or mould growth. In Kalliokoski, P., Jantunen, M., Seppänen, O. (Eds.) *Proceedings of Indoor Air '93*, vol. 4. Helsinki, Finland, pp. 171–174.
- Karbowska-Berent, J., Górny, R. L., Strzelczyk, A. B., & Wlazło, A. (2011). Microbial air quality in selected Polish libraries and archives. *Building and Environment*, 46, 1872–1879.
- Kowalski, M., & Pastuszka, J. S. (2018). Effect of ambient air temperature and solar radiation on changes in bacterial and fungal aerosols concentration in the urban environment. *Annals of Agricultural and Environmental Medicine*, 25(2), 259–261.
- Lazaridis, M., Katsivela, E., Kopanakis, I., Raisi, L., & Panagiaris, G. (2015). Indoor/outdoor particulate matter concentrations and microbial load in cultural heritage collections. *Heritage Science*, 3, 34. <https://doi.org/10.1186/s40494-015-0063-0>.
- Lehtonen, M., & Reponen, T. (1993). Everyday activities and variation of fungal spore concentrations in indoor air. *International Biodeterioration and Biodegradation*, 31, 25–39.
- Leung, D. Y. C. (2015). Outdoor-indoor air pollution in urban environment: Challenges and opportunity. *Frontiers in Environmental Science*. <https://doi.org/10.3389/fenvs.2014.00069>.
- Ławniczek-Wałczyk, A., Cyprowski, M., Gołofit-Szymczak, M., Wójcik-Fatla, A., et al. (2018). Assessment of fungal aerosol exposure at selected workplaces contaminated with organic dust of different origin. *Medycyna Pracy*, 69(3), 269–280.
- Mehta, S., Kambli, P., Wani, K., Tanavde, S., et al. (2013). Study of bio-aerosols in a prominent temple in Mumbai City, India. *International Journal of Environmental Studies*. <https://doi.org/10.1080/00207233.2013.829323>.
- Napoli, Ch., Marcotrigiano, V., & Montagna, M. T. (2012). Air sampling procedures to evaluate microbial contamination: A comparison between active and passive methods in operating theatres. *BMC Public Health*. <https://doi.org/10.1186/1471-2458-12-594>.
- Pastuszka, J. S., Paw, U. K. T., Lis, D. O., Wlazło, A., & Ulfig, K. (2000). Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. *Atmospheric Environment*, 34, 3833–3842.
- Salma, I., Weidinger, T., & Maenhaut, W. (2007). Time-resolved mass concentration, composition and sources of aerosol particles in a metropolitan underground railway station. *Atmospheric Environment*, 41, 8391–8405.
- Skowroń, J. (2014). The activity of the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment in 2011–2013. *Podstawy i Metody Oceny Środowiska Pracy*, 1(79), 161–184. (in Polish).
- Skóra, J., Gutarowska, B., Pielech-Przybylska, K., Stepień, L., et al. (2015). Assessment of microbiological contamination in the work environments of museums, archives and libraries. *Aerobiologia*, 31, 389–401.
- Spilak, M. P., Madsen, A. M., Knudsen, S. M., Kolarik, B., et al. (2015). Impact of dwelling characteristics on concentrations of bacteria, fungi, endotoxin and total inflammatory potential in settled dust. *Building and Environment*, 93(1), 64–71.
- Sroka, P., Cyprowski, M., & Szadkowska-Stańczyk, I. (2008). Occupational exposure to mycotoxins in various branches of industry. *Medycyna Pracy*, 59(4), 333–345. (in Polish).

- Stetzenbach, L. D., Buttner, M. P., & Cruz, P. (2004). Detection and enumeration of airborne biocontaminants. *Current Opinion in Biotechnology*, 15(3), 170–174.
- Tang, J. W. (2009). The effect of environmental parameters on the survival of airborne infectious agents. *Journal of Royal Society Interface*, 6, S737–S746.
- Zhai, Y., Li, X., Wang, T., Wang, B., et al. (2018). A review on airborne microorganisms in particulate matters: composition, characteristics and influence factors. *Environment International*, 113, 74–90.