Original article DOI: 10.2478/aiht-2018-69-3194

Seasonal diversity of biodeteriogenic, pathogenic, and toxigenic constituents of airborne mycobiota in a sacral environment

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[Received in September 2018; Similarity Check in September 2018; Accepted in November 2018]

The main purpose of this study was to isolate airborne fungi and assess seasonal variations in air contamination with their particulates by determining the levels of their propagules in the nave and exonarthex of a church. We also monitored indoor microclimate as a determining factor for fungal proliferation on wall paintings, spore release, and transmission through the air. The temperature and relative humidity of the nave favoured fungal growth. A total of 33 fungi were isolated, mainly of the phylum Ascomycota, and to the lesser extent of the phyla Zygomycota and Basidiomycota. The most common were the fungi of the genera *Penicillium* and *Aspergillus* (23.55 % and 20.58 %, respectively). Sørensen's quotient of similarity (0.37) suggests moderate species overlap and constant exchange of fungal propagules between the nave and exonarthex. The autumn had the highest diversity, with 17 documented taxa, followed by the summer and the winter. The spring had only eight taxa. Quantitative analysis of the airborne mycobiota in the nave (430±84.85 to 1880±106.07 CFU m^{-3}) and exonarthex (715±59.62 to 2295±91.92 CFU m^{-3}) showed very high contamination throughout the year, with values exceeding the maximum permissible concentrations by most standards. Many of the fungi determined in this study are known for their biodeteriogenic, toxigenic, and allergenic properties, and are a threat not only to occasional visitors and staff, but also to valuable works of art decorating nave walls.

KEY WORDS: *airborne fungi;* Aspergillus*; biodeteriogens; contamination; indoor microclimate; mycotoxins; pathogens;* Penicillium

Viable "microbes moving through the air" were documented for the first time in 1769 by Lazzaro Spallanzani, an Italian priest and biologist who ran a series of experiments aimed at disproving the concept of spontaneous generation (1). Now we all know that many microorganisms such as fungi, algae, and bacteria make part of the biological component of the atmosphere. Being airborne they can affect plant, animal, and human health, as well as materials in their environment (2). Aerobiological studies have therefore been very useful in the conservation of the works of art affected by airborne microbial propagules which settle on their surfaces and colonise them if the cells are sufficiently viable and the environmental conditions (climate and microclimate) and substrate characteristics are favourable. When these requirements are met, biodeterioration takes place (3). A significant role in the biodeterioration of artefacts of archaeological, historical, and artistic interest (4) is played by fungi due to hyphal proliferation and secretion of numerous metabolites into the materials.

In recent years, monitoring of fungal propagules in the indoor environment of cultural heritage objects such as churches, museums, libraries, and archives has become an important part of preventive conservation (5–8). Regardless of the growing interest, monitoring studies of this type have been scarce in Serbia, and none had been conducted in sacral complexes. The aim of our study was therefore to: (*i*) characterise seasonal air quality in the nave and exonarthex of the Church of the Holy Ascension through qualitative and quantitative analysis of airborne mycobiota and (*ii*) evaluate air temperature and relative humidity as factors favouring fungal proliferation. This kind of information is necessary for an accurate assessment of the risk fungi pose to the works of art and human health and for recommending preventive and remedial measures.

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MATERIALS AND METHODS

Sampling site

For this long-term study we selected the old Church of the Holy Ascension, located on the southwestern slopes of Suva Planina, in the village of Veliki Krčimir (Gornje Zaplanje, Gadžin Han) (43° 05' 28'' N 22° 12' 40'' E) in Serbia, following the recommendation of a team of experts of the Institute for the Protection of Cultural Monuments. They found it in a very dilapidated state, with pronounced signs of damage caused by microorganisms. The church is one of the oldest temples of the Eparchy of Niš built in the early 17th century of large blocks of dressed stone and lime mortar. Its interior is divided into two aisles. Fragments of the old murals dating back to 1629 remain in the nave, altar area, and the western facade (exonarthex) (9, 10) and are deemed a very valuable expression of Serbian fresco painting of the late 16th and early 17th century. The church is now a cultural monument managed by the Institute for Protection of Cultural Monuments of Niš. However, being out of everyday use (services are held only on important holidays) and lacking the funds for full conservation, the church has been in a dilapidated state for decades. Figure 1 shows the layout of the church and the sampling points.

Indoor microclimate monitoring

The temperature (T °C) and relative humidity (RH %) of the nave and the altar were measured every 30 minutes from May 2013 to April 2014 with an EBI 20-IF data logger (Ebro, Hagen, Germany) placed at the centre of the western wall at the height of 250 cm from the floor and 5 cm from the surface of the wall. The collected data were used to calculate annual, seasonal, and monthly averages, presented as mean values with standard deviations. The time of wetness (TOW) for days with RH ≥80 % was calculated using Adan's formula (11):

$$\text{TOW} = \text{cyclic wet period (RH} \geq 80\,\%) \,/\, \text{cyclic period (wet + dry)}$$

TOW is a better predictor of fungal growth in given environmental conditions than relative humidity alone. Fungal growth can be expected at all TOW values, but at values below 0.5 it is expected to be minimal on walls and murals (11).

Equilibrium moisture content measurement

Equilibrium moisture content (EMC) of the nave murals was measured in May 2013 with a Testo 635-2 temperature and moisture meter (Testo Instruments Limited, Alton Hampshire, UK) set to the limestone mode using probes (4 mm in diameter) inserted into 6 mm x 5 cm holes drilled in the nave walls at the height 202 to 227 cm.

Sampling of airborne fungal propagules

Viable fungal propagules were sampled in the air of the nave and exonarthex in all four seasons of 2013/2014 using a MAS-100 Eco air sampler (Merck Eurolab, Darmstadt, Germany) at the airflow of 100 L min^{-1} through the perforated head (400 x 0.7 mm) of the sampler. Malt extract agar (malt extract 40 g; agar 15 g; 1000 mL dH_2O; pH 6.8) supplemented with streptomycin (500 mg L^{-1}; streptomycin sulphate salt, Sigma-Aldrich, St. Louis, Missouri, USA) to suppress bacterial growth was used as a nutrient medium of choice. Sampling was done in triplicate at each sampling point. Inoculated plates were incubated in a thermostat (UE 500, Memmert, Schwabach, Germany) on 25±2 °C for seven days and then grown colonies were counted using Feller's correction factor (12) and multiplied by 10 to express them as colony-forming units (CFU) per cubic meter of air (CFU m^{-3}).

Relative density (RD) was calculated using the formula of Smith (13):

$$\text{RD}(\%) = \frac{\text{number of colonies of the genus or species}}{\text{total number of colonies of all genera or species}} \times 100$$

Relative frequency (RF) was determined according to Esquivel et al. (14):

$$\text{RF}(\%) = \frac{\text{number of times a genus is detected}}{\text{total number of samplings realised}} \times 100$$

The obtained RF values were used to categorise the detected fungal genera as follows: abundant (81-100 %), common (61-80 %), frequent (41-60 %), occasional (21-40 %), and rare (0.1-20 %) (15).

The fungal loads detected in the air of the nave were compared with those in the exonarthex using Sørensen's quotient of similarity (QS) according to the following formula (16):

$$\text{QS} = \frac{2a}{2a + b + c}$$

where *a* is the number of species detected in both indoor and outdoor environment, *b* number of indoor (nave) species, and *c* number of species detected only in outdoor air.

Identification of the fungal isolates

The isolated fungi were identified based on the macroscopic features analysed with a Stemi DV4 stereomicroscope (Carl Zeiss, Oberkochen, Germany) and the micromorphological characteristics of reproductive structures analysed with an Axio Imager M.1 microscope (Carl Zeiss). The following dichotomous keys were used: Raper and Fennel (17), Pitt (18), Sutton (19), Ellis and Ellis (20), Watanabe (21), Samson et al. (22), Bensch et al. (23), and Woudenberg et al. (24). Morphology-based identifications were then confirmed with ITS1 and β-tubulin gene sequencing as described elsewhere (25).

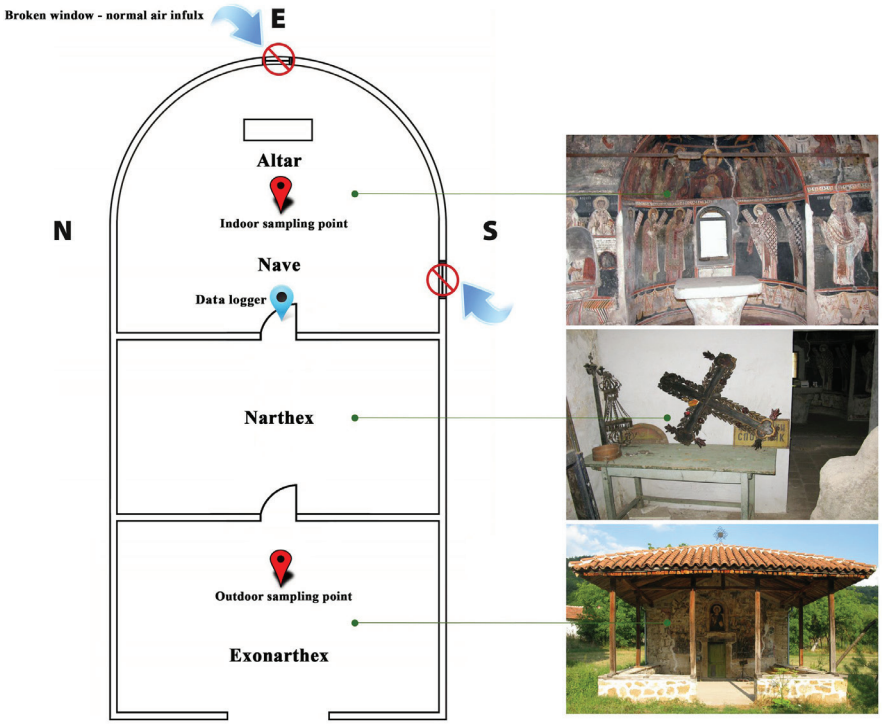

Figure 1 *Layout of the old Church of the Holy Ascension with marked sampling points and the position of the data logger*

Statistical analysis

For statistical analysis we used CANOCO for Windows version 5 (26). The potential effects of seasons on fungal community (used as explanatory variable) and indoor and outdoor measurements (used as supplementary variables) were examined with canonical correspondence analysis (CCA). The analysis was based on the presence/absence of all recorded fungal taxa.

Principal component analysis (PCA) was also used on the set of data on all documented indoor/outdoor fungi. As with CCA, the analysis was based on the presence/absence of fungal taxa.

Furthermore, two classifications were made, one based on the ecological and the other on the biodegradation properties of the species. Ecological classification included the following groups: P (phytopathogens), H (human pathogens), and S (saprobic fungi), as well as their combinations (HP, HS, PS, and HPS). As for biodegradation properties, the fungal taxa were classified into six groups (G0-G5) (6, 7, 27-29), with the G5 group having all five properties (carbonate dissolution, acid and alkali production, casein hydrolysis, pigment secretion and organomineralization), G4 having four properties, and so on. Data for both classifications were arranged in the same manner. In the CANOCO program, each fungal taxon was first assigned to its appropriate group. Then a new table with the groups was created for each analysis using the option "trait averages". These groups were used instead of individual taxa. Temperature, RH, and CFU were used as supplementary variables.

RESULTS AND DISCUSSION

State of the investigated church

The murals on the exonarthex were badly damaged, with more than 40 % of the total surface completely missing due to collapse of mortar. The remaining mural surface was in a very poor state. For example, the exfoliation of the "*Christ with the Gospel*" in a niche above the door was such that only the contours of the face and hands and parts of drapery had remained. Whitish crusts, formed by saline efflorescence, and moderate discolorations were observed in the lower areas.

In the narthex, the decorative plaster was completely preserved on all wall surfaces, with the exception of pilasters and the arch between the narthex and the nave. The greatest damage was seen at the base. There the capillary rise of water from the ground and its retention in the walls caused extensive moistening, which led to the crumbling of mortar along three walls, with a tendency to expand.

Structurally, the nave of the church was in a particularly poor condition. Preliminary measurements showed the highest moisture in the lower wall areas; but the north wall was affected by moisture up to two meters in height. Most of the frescoes, however, were well preserved. They were primarily done *al fresco*, with parts completed *al secco*. The surfaces done *al secco* were in a worse condition than the surfaces done *al fresco*, with pronounced pulverisation and exfoliation. Some frescoes had cracks and flakes blackened by soot. This was probably the result of restoring

damaged frescoes with oil paints, which clogged the pores of the plaster and prevented drying. In addition, moisture had caused saline efflorescence and crystallisation on the pigmented and mortar surface. Crystallised salts formed white scum, opaque in places. The main reasons for the poor state of the murals are unfavourable microclimate and exposure to moisture over decades of neglect.

Indoor microclimate and wall moisture content

The lowest average temperature was measured in December (4.33±1.46 °C) and the highest in August (23.18±1.19 °C). Monthly relative humidity ranged from 55.98±6.87 % in August to 74.85±6.49 % in June (Figure 2). Seasonal temperature ranged between 5.81±2.35 °C and 20.47±2.57 °C, while relative humidity varied moderately, between 62.67±8.61 % in the autumn and 69.89±6.91 % in the winter. Calculated annual temperature and relative humidity were 13.68±6.28 °C and 66.16±8.87 %, respectively (Figure 3).

Relative humidity was ≥80 % only on 47 days, mostly in June and July, and the TOW values ranged from 0.02 to 1.00 (Figure 4). Surprisingly, this highest TOW was recorded on 9 February when RH was ≥80 % for 24 h.

EMC values ranged from 54.7 to 63.1 %, temperature varied minimally (±0.2 °C) between the walls (Table 1).

The recorded variations in temperature and relative humidity are in accordance with the seasonal trends in regions with a temperate climate and confirm the generally accepted view that buildings without a heating system have high average humidity and large seasonal temperature variations (30). For the most part of the year, the temperature, relative humidity, and EMC (≥70 %) in the church favoured fungal growth (3, 31). Mortar is a porous material that allows capillary transport of water and fungal proliferation, which leads to its corrosion and the blistering and cracking of paint. Furthermore, in the early spring, vapour condenses on the cold walls, which was particularly evident in April. Condensed water can form carbonic acid with CO_2 from fungal respiratory metabolism and precipitate sulphur in the form of microcrystalline film made of sulphate salts (32-34). Extensive efflorescence of calcium sulphate on the nave walls has already been reported in our church (35). Yet even when relative humidity drops below 40 %, as it did in May, August, September, and October, such fluctuations can damage the frescoes irreversibly. At low humidity, previously dissolved salts crystallise and precipitate, while organic wall painting components become brittle and highly susceptible to environmental factors (36). Changes in EMC resulting from temperature and humidity fluctuations have a much greater impact on the frescoes than constant high or low temperature and humidity. Abrupt changes in ambient conditions cause the salt to cycle between crystallisation, dissolution, and back, which increases the internal pressure in the mortar and makes it cracking and eventually crumble.

Optimal environmental conditions for the conservation of wall paintings are still a matter of controversy, but many authors agree that limiting variations in temperature and

Table 1 *Equilibrium moisture content (EMC), temperature (T) in four nave walls at the depth of 5 cm*

Nave walls	Height (cm)	EMC (%)	T (°C)
northern	202	63.1	18.2
southern	205	56.2	18.4
eastern	196	60.5	18.2
western	227	54.7	18.4

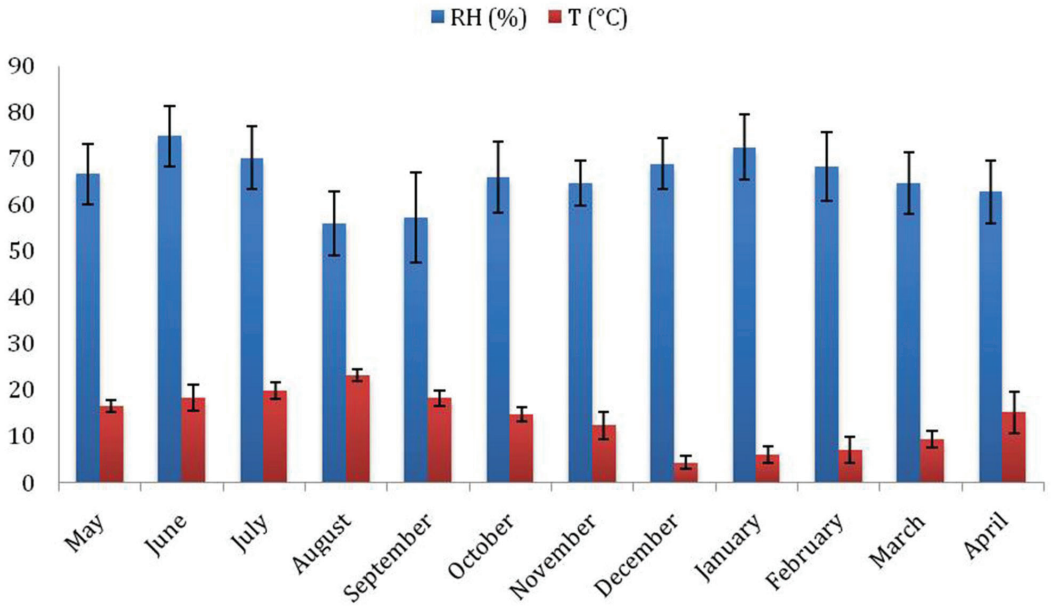

Figure 2 *Monthly average temperature (°C) and relative humidity (%) in the nave in 2013/2014. The results are presented as means with standard deviations*

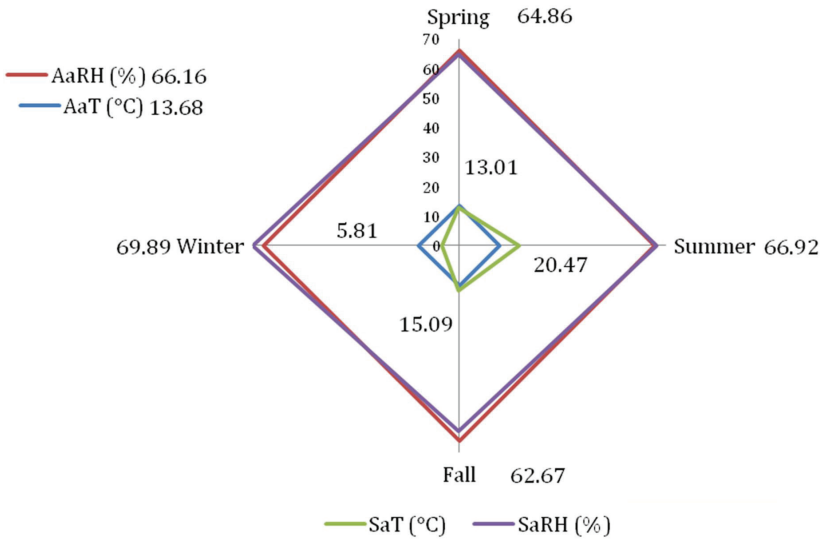

Figure 3 *Annual (Aa) and seasonal (Sa) average temperature (°C) and relative humidity (%) in the nave in 2013/2014*

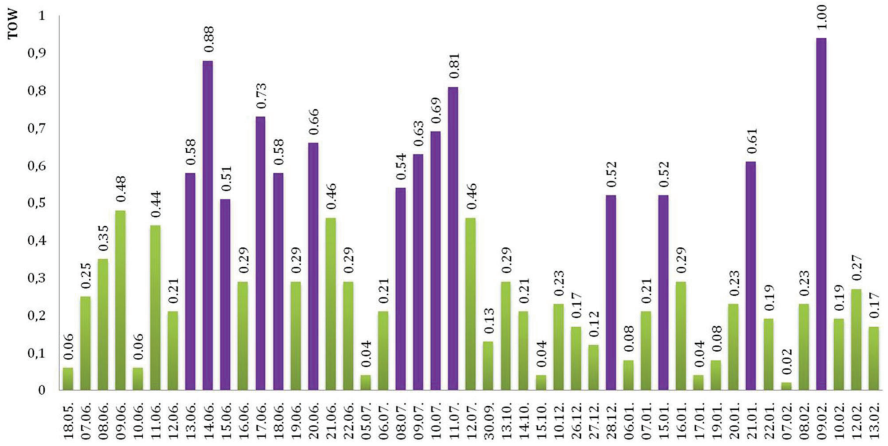

Figure 4 *Time of wetness (TOW) for days with relative humidity above 80 %. Purple columns indicate TOW values higher than 0.5*

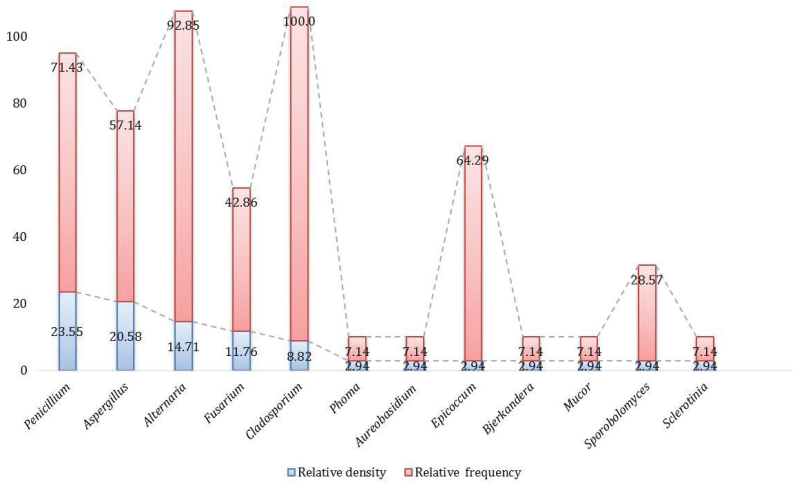

Figure 5 *Relative density and relative frequency of the fungal genera measured in the air of the nave and exonarthex*

humidity and preventing fungal growth by doing so is a must (37). Tabunschikov and Brodatch (38) claim that indoor air temperature in buildings with murals should keep below 18 °C and relative humidity should be around 60 %. Daily and seasonal variations should be minimised.

Fungal air contamination

We isolated 33 airborne fungal taxa in the air, mainly of the phylum Ascomycota (30 taxa), one *Mucor plumbeus* from the phylum Zygomycota, and two taxa *Bjerkandera adusta* and *Sporobolomyces roseus* from the phylum Basidiomycota (Table 2). Judging by the quotient of similarity (QS=0.37), there was a moderate species overlap and constant exchange of fungal propagules between the nave and exonarthex throughout the year.

With the respective relative density of 23.55 % and 20.58 % the fungi of the genera *Penicillium* and *Aspergillus* dominated, followed by the *Fusarium* (11.76 %), *Alternaria* (14.71 %), and *Cladosporium* (8.82 %) species. Other fungal genera were represented by only one species (RD 2.94 %) (Figure 5). Judging by relative frequency, *Cladosporium* (100 %) and *Alternaria* (92.85 %) were classified as "abundant", *Penicillium* (71.43 %) and *Epicoccum* (64.29 %) as "common", and *Aspergillus* (57.14 %) and *Fusarium* (42.86 %) as "frequent". The *Sporobolomyces* genus was the sole representative of "occasional" group, while the rest of the genera were classified as "rare" (Figure 5).

The autumn showed the greatest fungal diversity (Figure 6) with 17 documented taxa, followed by the summer (13

Table 2 *Fungi isolated from the air of the nave (N) and exonarthex (E) of the old Church of the Holy Ascension over all four sampling seasons*

Isolated fungi	Spring		Summer		Autumn		Winter	
	N	E	N	E	N	E	N	E
Alternaria arborescens E.G. Simmons			+					
Alternaria alternata (Fr.) Keissl.*	+	+	+	+	+		+	+
Alternaria infectoria E.G. Simmons*					+			
Alternaria tenuissima (Künze) Wiltshire				+		+	+	+
Aspergillus sp. sect. *Cremi**				+				
Aspergillus sp. sect. *Flavi**			+					
Aspergillus sp. sect. *Nigri**			+	+	+			
Aspergillus sp. sect. *Flavi**					+			
Aspergillus sp. sect. *Circumdati**				+				
Aspergillus sp. sect. *Aspergillus**			+					
Aspergillus sp. sect. *Nidulantes**							+	
Aureobasidium pullulans var. *melanogenum* Herm.-Nijh.						+		
Bjerkandera adusta (Willd.) P. Karst.*						+		
Cladosporium cladosporioides (Fresen.) G.A. de Vries*	+	+	+		+	+	+	
Cladosporium oxysporum Berk. & M.A. Curtis*					+	+	+	+
Cladosporium uredinicola Speg.*		+					+	+
Epicoccum nigrum Link*	+	+	+			+	+	+
Fusarium incarnatum (Desm.) Sacc.				+				
Fusarium oxysporum E.F. Sm. & Swingle			+					
Fusarium sporotrichioides Sherb.	+							
Gibberella moniliformis Wineland*					+	+		
Mucor plumbeus Bonord.	+	+						
Penicillium bilaiae Chalab.*					+			
Penicillium hirsutum Dierckx							+	
Penicillium lanosum Westling*								+
Penicillium manginii Duché & R. Heim*					+	+		
Penicillium Link spp.		+	+	+		+	+	
Phoma medicaginis Malbr. & Roum.*						+		
Sclerotinia sclerotiorum (Lib.) de Bary*					+			
Sporobolomyces roseus Kluyver & C.B. Niel					+	+		

**identifications confirmed by sequencing ITS 1 and β-tubulin gene regions*

taxa), winter (10 taxa), and spring (8 taxa). In general, the *Cladosporium* species dominated in all the seasons, while the *Alternaria* species had highest isolation frequency in the spring and summer (Figure 6). The *Aspergillus* species, in turn, had the highest diversity in the summer. In the winter, *Epicoccum nigrum* was quite abundant in indoor environment and its isolates were morphologically quite variable.

The explanatory variable "season" in CCA accounted for 53.3 % of variability (F=1.5; P=0.0028) (Figure 7). Four groups can be distinguished on the CCA ordination diagram: I – taxa documented only in the summer dominated by the *Aspergillus* species; II – highly diverse taxa found only in the autumn, highly diverse; III – diverse taxa found only in the spring and/or winter; and IV – cosmopolitan taxa found in two or more seasons (central part of the ordination diagram) known to occur in the air of temperate regions throughout the year (e.g. *A. alternata*, *C. cladosporioides*, *C. oxysporum*, and *E. nigrum*). The placement of the supplementary variables "exonarthex" and "nave" in the centre indicates that there were no major differences between the two sampling points. This was expected, as both the eastern and southern nave windows were broken, allowing normal outdoor and indoor air exchange.

The first PCA shows the arrangement of the fungi by their ecological properties (P, S, HP, HS, PS, and HPS) (Figure 8a). Group P was present in the air only in winter, when relative humidity was high. The HP group was found exclusively in the summer and the PS group in autumn, when temperatures were higher. The other groups had fungal representatives in two or more seasons, with the HS group present in the summer, autumn, and winter, and the S and HPS groups present throughout the year.

The second PCA shows the arrangement of biodegradative properties of the fungal isolates (Figure 8b). The G1 fungi were recorded only in the autumn, except for *A. alternata*, which persisted in all four seasons. The G2 fungi were common in the autumn too but also in the summer, when the highest temperatures were measured. The G3 fungi were the most common in the winter, when the highest relative humidity was recorded. The G4 fungi consisted only of *A. niger*, and were found in the summer and autumn, and the G5 fungi consisted only of *P. bilaiae*, recorded only in the autumn. In other words, autumn was the most abundant with the fungi with one or more known biodegradative properties, while the spring and winter seem to be safer in that respect.

The domination of the genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, and *Penicillium* across all seasons in our study coincides with the findings reported for similar sacral ambients, especially European churches and monasteries in temperate climates (*Penicillium* and *Aspergillus*) (3) and across all climates (*Alternaria* and *Cladosporium*) (3, 5, 8, 28, 39). From the conservation point of view, their dominance in the air of the nave and exonarthex is quite problematic, given that they are considered the main cause of mural deterioration (40). According to Ruga et al. (8), the problem gets worse with

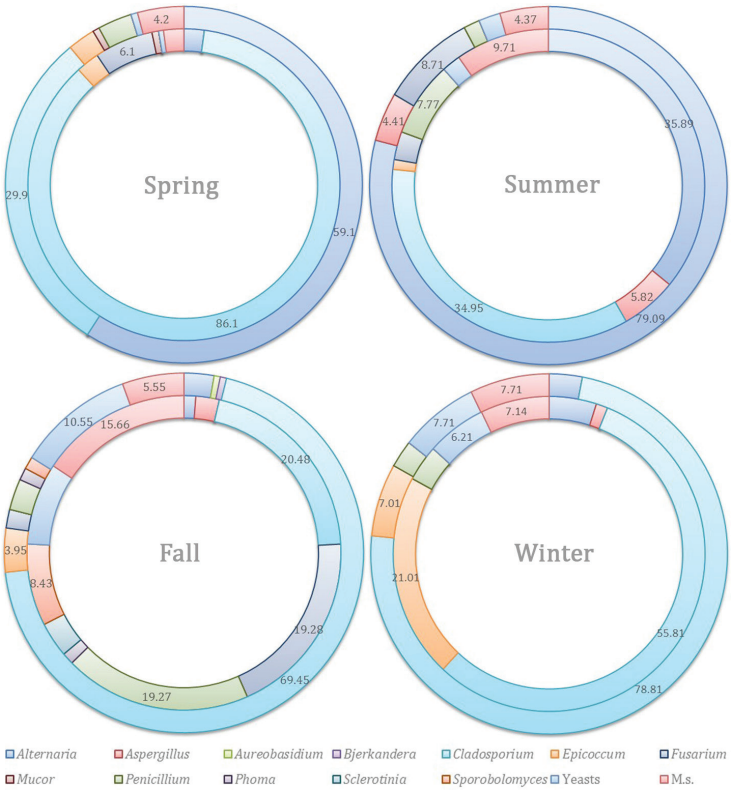

Figure 6 *Seasonal distribution of the fungal genera in the air of the exonarthex (outer circle) and the nave (inner circle)*

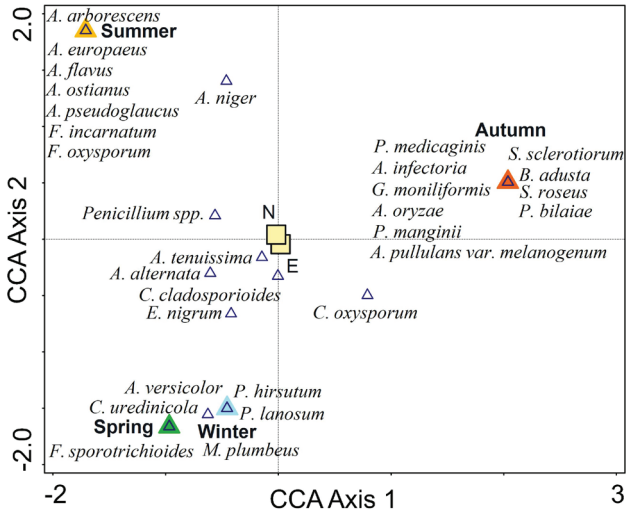

Figure 7 *The CCA biplot ordination on the basis of the presence/absence of the fungal taxa; triangle - explanatory variable "seasons" (spring, summer, autumn and winter); square - supplementary variables that correspond to sampling points (N - nave and E - exonarthex, respectively)*

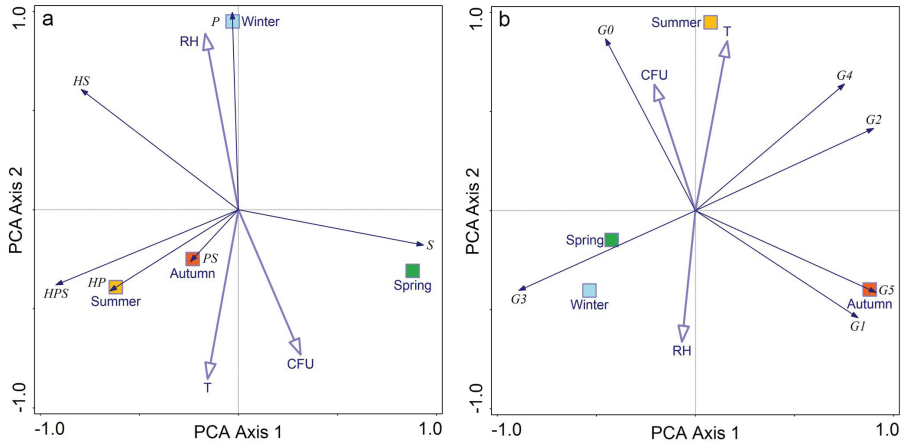

Figure 8 *The PCA biplot ordination diagrams showing the relationships of (**a**) ecological groups (P - phytopathogens, S - saprobic fungi, H - human pathogens, and their combinations) and following supplementary variables: seasons (spring, summer, autumn and winter), temperature (T), relative humidity (RH) and colony-forming units (CFU); and (**b**) groups based on the number of known deteriorative activities (G0-G5) and same supplementary variables*

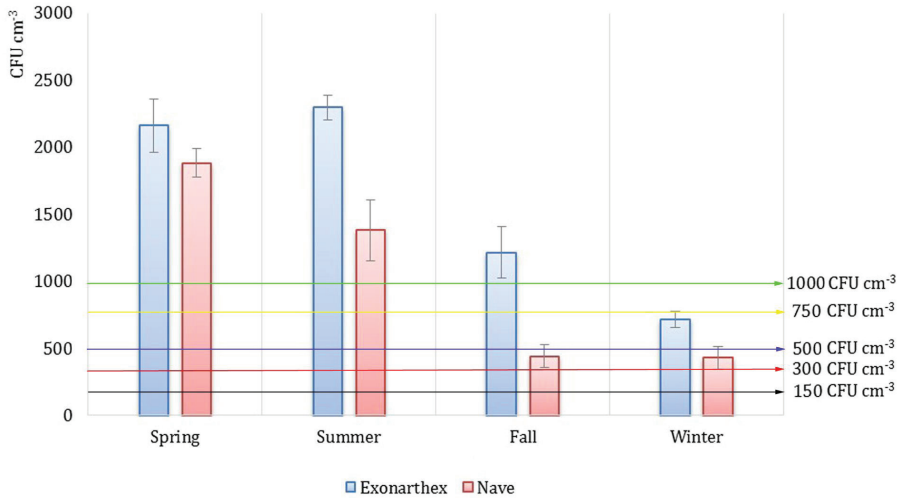

Figure 9 *Concentrations of viable fungal propagules (CFU m^{-3}) in the air of the nave and exonarthex in all four seasons of 2013/2014 with marked maximum permissible concentrations per different standards. The results are presented as mean values of number of samplings (n=3) with standard deviations. Standards: black line (8, 45, 48); red line (49); blue line (3); yellow line (44); green line (46, 47)*

the absence of air circulation, so common in unutilised church ambients, as propagules larger than 2 mm settle readily on the surfaces of wall paintings due to gravity.

The highest concentration of viable fungal propagules in the air of the nave was measured in the spring (1880±106.07 CFU m^{-3}) and the lowest in the winter (430±84.85 CFU m^{-3}). In the exonarthex, summer had the highest contamination (2295±91.92 CFU m^{-3}) and winter the lowest (715±59.62 CFU m^{-3}) (Figure 9). These findings are in full accordance with earlier reports of church environments (5, 8, 39, 42, 43). However, there are still no generally adopted standards for maximum permissible levels of spores in the air of cultural heritage buildings, as they range from 150 to 1000 CFU m^{-3} for indoor air (8, 44–49). In view of the scale proposed by Roussell et al. (50) (<170 CFU m^{-3} - low contamination; 170–560 CFU m^{-3} - moderate contamination; 560–1000 CFU m^{-3} - high contamination; >1000 CFU m^{-3} - very high contamination), the air of the old Church of the Holy Ascension is highly contaminated for the most part of the year, and contamination levels exceed most standard limits. Our findings, however, were expected for this church, as they confirmed our previous research, which has demonstrated extensive fungal growth on the nave wall paintings (35, 41).

Indoor air overloaded with spores poses a very serious health risk to whoever spends time in the church, staff and conservators in particular. The species found in our study are often associated with respiratory diseases such as allergic rhinitis and asthma. Exposure to high concentrations of *Cladosporium* and *Alternaria* spores causes chronic asthma and severe allergic reactions (51). Exposure to the *Aspergillus* and *Penicillium* species can cause allergic reactions (52). *A. versicolor* and *A. niger* are known producers of powerful allergens Aspv 13, Aspn 14, and Aspn 18 (53, 54). Fungi of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* also produce mycotoxins.

However, high air contamination is not a pressing public health issue at the moment, because the old church is seldom in service, except on important holidays. Occupational risk for conservators and staff spending longer time in the church indoors is much greater, though, as inhalation of the spores is particularly high during handling of the infested murals (55).

Furthermore, valuable works of art that decorate the nave and exonarthex walls are in constant danger of degradation. Immediate conservation interventions are thus required to cleanse the air and remove any current "hot spots" of contamination. Constant monitoring is also needed to prevent future outbursts of spore overload in the air. Likewise, further research based on culture-independent molecular analysis will allow a better insight into the air mycobiota diversity and the newly-discovered submicron hyphal fragments dispersed in the air with the spores, whose presence cannot be detected by standard cultivation methods. For that purpose we started a new round of investigation in 2016.

Acknowledgments

This research was carried out as part of the project financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. 173032).

Conflicts of interest

None to declare.

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Sezonska raznovrsnost biodeteriogenih, patogenih i toksigenih gljiva u zraku sakralnoga objekta

Glavni cilj ovoga istraživanja bio je izolirati gljive iz zraka i procijeniti sezonske promjene u onečišćenju zraka gljivičnim propagulama u naosu i egzonarteksu istraživane crkve. Također su praćeni mikroklimatski parametri kao ograničavajući čimbenici za razvoj i rast gljiva na zidnim slikama, za oslobađanje spora i njihovu transmisiju kroz zrak. Zabilježena temperatura i relativna vlažnost zraka u naosu pogodovale su razvoju i rastu gljiva. Dokumentirana su 33 taksona gljiva, uglavnom pripadnika koljena Ascomycota, a manje su zastupljeni oni Zygomycota i Basidiomycota. Najčešće su bile prisutne plijesni rodova *Penicillium* (23,55 %) i *Aspergillus* (20,58 %). Sørensenov indeks sličnosti (0,37) upućuje na stalnu i umjerenu razmjenu gljivičnih propagula između naosa i egzonarteksa. Uzorci uzeti u jesen pokazali su najveću raznolikost sa 17 zabilježenih taksona, a oni uzeti u proljeće samo osam taksona. Kvantitativna mikološka analiza u naosu (430±84,85 do 1880±106,07 CFU m^{-3}) i egzonarteksu (715±59,62 do 2295±91,92 CFU m^{-3}) pokazala je visoku godišnju razinu onečišćenja zraka, s vrijednostima koje prema većini standarda prelaze dopuštene koncentracije. Mnoge identificirane gljive mogu dovesti do biodeterioracije, proizvesti mikotoksine i izazvati alergijske reakcije. Stoga su nepoželjne u sakralnim objektima ne samo zbog očuvanja murala nego i zbog zaštite zdravlja zaposlenih, posjetitelja i konzervatora.

KLJUČNE RIJEČI: *aeromikobiota;* Aspergillus*; biodeteriogeni; mikotoksini; mikroklima; patogeni;* Penicillium