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Bioaerosol assessment in naturally ventilated historical library building with restricted personnel access

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Abstract

The aim of this study was to check the degree and identify the sources of microbial contamination of the Jasna Góra (Bright Hill) monastery library 10 years after disinfection of the incunabula collection. The registered maximum viable indoor microbial concentrations were 1,875 and 7,100 cfu/m^3 for stationary and personal measurements, whereas respective total concentrations were 71,000 and 100,000 counts/m^3. There was no statistically significant difference between the concentrations of viable microorganisms measured in the stationary using Andersen, GSP, and Button samplers. Moreover, GSP and Button samplers can be interchangeably applied when viable or total microbial levels are stationary or personally measured. The culturable microorganisms constituted 0.5 - 3.9% of the total microflora only. Filamentous fungi were the most prevalent outdoors, whereas Gram-positive cocci and endospore forming Gram-positive rods dominated indoors in the air and settled dust, respectively. Hence, an unrestrained infiltration of ambient air through the draughtiness of the building envelope is probably the main process responsible for indoor fungal pollution, whereas bacterial contaminants have their major sources in the indoor environment. Moreover, even a chemically cleansed library collection, having a restricted personnel access, but under the influence of ambient air, can undergo microbial contamination and becomes an important microbial emission source.

Key words

library, air quality, bioaerosol, viable microorganisms, total count, settled dust, biodeterioration

INTRODUCTION

Monastery libraries are a special indoor environment. They quite often house collections of the oldest written texts which, due to their uniqueness, are of priceless historical value. The character of such buildings results in majority of their storerooms being located in very old premises deprived of any technical means (e.g., mechanical ventilation, air-conditioning system, air purifiers, etc.), which are able to provide proper microclimate conditions for stored collections. This leads to their biodeterioration with age, and even if they have undergone disinfection treatments, they usually return to the same contaminated state.

In practice, it is not possible for people from outside the specific religious community to enter the premises of the sanctuary with a strict close monastic rule. This means that only the monks have access to the library. Hence, a microbial contamination in this type of indoor space may exist as a result of the infiltration of ambient air through the draughtiness of the building's envelope, transport of microorganisms by and on the library users, as well as accidental or unintentional pollution (usually caused by flood and other water or moisture damage) [1, 2].

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Cultural heritage materials like books, maps, drawings, as well as other paper or parchment documents are complex chemical structures. As such, during storage they undergo the processes of ageing, alteration, and biological destruction. The spectrum of materials affected by biodeterioration is very broad, including among other organics: paper, wood, textiles, parchment, leather, paintings, and plastics. The inorganic materials addressed include stone, glass, and metals [3–11]. Regarding the paper heritage prior to the 19th century, the majority of these documents were hand-made from linen or cotton rags containing cellulose, wooden parts with lignin, natural glues containing proteins (e.g., fish, bone, hide, or casein glues) and sugars (e.g., liquid honey, flour pastes, vegetable gums), animal leather containing collagen or different metals (utilized usually as a cover for the prints) were used as the raw materials to produce them [10, 12]. As such, they are under the constant risk of biological destruction. There are several hundred different microbial species responsible for the biodeterioration of library stocks. Among them are fungi and bacteria with cellulolytic properties: *Trichoderma*, *Penicillium*, *Botrytis*, *Trichothecium*, *Phoma*, *Chaetomium*, *Aspergillus*, *Cladosporium*, *Stemphylium* (*Ulocladium*), *Alternaria*, *Hormodendrum*, *Aureobasidium*, *Papularia* and *Bacillus*, *Cellulomonas*, *Cellfalciculata*, *Cellvibrio*, *Cytophaga*, *Sporocytophaga*, *Streptomyces*, proteolytic (*Aureobasidium*, *Chaetomium*, *Cladosporium*, *Botrytis*, *Trichoderma*, *Verticillium*, *Mucor*, *Epicoccum*, *Gymnoascus* and *Actinomycetes*, and lipolytic properties – all the proteolytic fungi listed above, and additionally

Paecilomyces). If present, they may easily populate the stored collections [2, 10, 13, 14].

Assessment of microbiological indoor quality is usually carried out as quantitative and qualitative evaluation of bacterial and fungal air purity, supplemented by the study of biological contamination of the collection and interior surfaces. In both these cases, the cultivation methods are the most popular. Although they are able to identify no more than 25% of all biologically active microbial particulates, they enable interpretation of an empirically obtained result based on available threshold limit values. The majority of them, if proposed, is referred to the number of viable (or culturable) microorganisms [15].

As is widely known, there are different methods for assessing indoor air quality. According to hygienic standards, the collection of bioaerosol particles should be carried out using volumetric methods, which are based on active sampling of an appropriate volume of the air containing intact microorganisms or their fragments [16, 17]. Hence, the aim of this study was to check the degree and identify the possible sources of microbial contamination at the Jasna Góra monastery library 10 years after disinfection of the incunabula collection. Additionally, the usefulness of different sampling techniques for bioaerosol assessment in such a naturally ventilated environment with restricted personnel access was also evaluated.

MATERIALS AND METHODS

According to López-Aparicio *et al.* [18], the natural ventilation of premises, restriction of personnel access, reduction in the number of visitors, and absence of activities which could influence indoor pollutant concentrations, means that specific historical buildings are suitable locations to study the influence of different elements of the environment on the indoor air quality. Taking into account all the listed above factors, the Jasna Góra monastery library was selected for this study.

The Jasna Góra (Bright Hill) in Częstochowa is the most important sanctuary in Poland and one of the most famous in the world. The shrine was established in 1382 by Prince Ladislaus of Opole, who summoned the Pauline monks from Hungary to Poland. Through the centuries, the monks have assembled in the library a unique collection of ancient Polish and foreign prints, as well as priceless manuscripts dating from the 14th-18th centuries. From 1732 to the present day, to protect the whole collection, the incunabula have been stored in pinewood volume containers backed with leather and gold. In 1998, a complex work of conservation work was undertaken. The whole incunabula collection (about 13417 volumes, together with their wooden boxes) left the monastery library for the first time in its history and was subjected to disinfection using ethylene oxide in a pressure chamber [19, 20]. The effectiveness of the chemical treatment was microbiologically controlled and repeated (up to 4 times, if neceassary) until the full sterility of each volume was achieved [19, 21-23].

The study was performed outside and inside the library, which has very restricted personnel access (in practice, except VIP guests, only the monks authorized by the Prior can enter the library and use its resources). The room (17.4 m long × 10.8 m wide × 8.2 m high) has no air-conditioning or other mechanical air exchange system, and the only ventilation is through 4 wood frame windows (placed side by side on one wall), and a door (practically permanently closed). The measurements were carried out in the spring and autumn seasons when the average outdoor air temperature was higher than 10°C for at least 7 consecutive days. Viable (understood in this study as culturable) bioaerosol stationary samples were taken simultaneously in both the outdoor and indoor environments using a 6-stage Andersen impactor (model 10-710, Andersen Instruments, Atlanta, GA, USA) at a flow rate of 28.3 l/min. At the beginning of each measurement cycle, bacterial aerosol was collected on blood trypticase soy agar (TSA with 5% sheep blood; Becton, Dickinson and Co., Sparks, MD, USA) and, after impactor reloading, fungi were aspirated on malt extract agar (MEA, Oxoid Ltd., Basingstoke, UK). The sampling time was 5 min for both bacterial and fungal aerosols.

Simultaneous with the impactor measurements, stationary and personal bioaerosol samples were taken in the library room using both GSP (Ströhlein GmbH, Kaarst, Germany) and Button Aerosol (SKC Ltd., Eighty Four, PA, USA) samplers equipped with pumps (model 224-PCTX8, SKC Ltd.). During stationary bioaerosol aspiration, all 3 samplers (Andersen, GSP, and Button) were placed at the height of 1.5 m above floor level (to simulate the human breathing zone), and at a distance of 1 m from each other to avoid possible interferences occurring between them. The personal samples were taken at a flow rate of 4 l/min for 30 min using both filter samplers clipped to special shoulder straps on an analyst lab coat, and loaded with gelatin filters (Sartorius AG, Göttingen, Germany) with a pore size of 3 μm and diameters of 37 mm and 25 mm, respectively. After sampling, each filter was removed from its holder and dissolved in sterile water containing 0.01% Tween 80. Part of the suspension was plated on the microbiological media (the same as for bioaerosol sampling) and used for determination of culturable microorganisms (cfu/m^3). The rest of the suspension was used for examination of total microbial counts by a modification of the CAMNEA-method. Shortly afterwards, the obtained samples were treated with formaldehyde (37%) (POCH SA, Gliwice, Poland) and then stained with acridine orange (Sigma-Aldrich Chemie GmbH, Munich, Germany). After filtration of the suspension through a black polycarbonate filter with a pore size of 0.4 μm (Whatman, Maidstone, Kent, UK), all microorganisms were counted using an epifluorescence microscope (Eclipse E200, Nikon, Tokyo, Japan) and their concentration expressed as counts/m^3.

The bioaerosol measurements were complemented by evaluation of bacterial and fungal content in the dust settled on the incunabulae, their wooden containers and shelf surfaces. Each time, the dust was taken using sterile cotton swabs (HAGMED, Rawa Mazowiecka, Poland) from the surface of 100 cm^2 (a square-shaped 10 × 10 cm sterile template was applied). After sampling, to extract the collected microorganisms, the cotton swabs were vortexed for 10 min using a programmable rotator-mixer (model Multi RS-60, Biosan, Riga, Latvia) in 5 ml of distilled water. The spread plate method was applied where 0.2 ml of the resulting suspension was spread evenly over the same media as used for bioaerosol sampling.

All impactor, filter, and settled dust samples were incubated at the temperature of: bacteria – 1 day at 37°C, followed by 3 days at 22°C and 3 days at 4°C; and fungi – 4 days at 30°C followed by 4 days at 22°C. After incubation, the viable microbial concentrations in the air and dust were calculated as colony forming units per 1 m^3 (cfu/m^3) and per 100 cm^2

(cfu/100 cm^2), respectively. Bacterial and yeast strains were identified by Gram staining, their morphology, and finally, by the biochemical API tests (bioMérieux, Marcy l'Etoile, France). Filamentous fungi were identified according to their morphology using several identification keys [24-28].

During sampling, the air temperature and relative humidity were measured using a hytherograph (model Omniport 20, E+E Elektronik GmbH, Engerwitzdorf, Austria).

All bioaerosol, settled dust, and microclimate parameter measurements were carried out in triplicate or quadruplicate. The collected data were statistically elaborated using analysis of variance (ANOVA) followed by the unequal N (Spjotvoll/Stoline) test, *t*-test, and correlation analysis using Statistica (data analysis software system) version 7.1–2006 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The concentrations of microorganisms in both indoor and outdoor air at the Jasna Góra monastery library measured using Andersen, GSP, and Button samplers, are presented in Figure 1. A 6-stage Andersen impactor used as a stationary aspirator allowed assessment of the concentration of viable microorganisms only, whereas the GSP and Button Aerosol samplers, both applied as stationary and personal aspirators, enabled the measurements of viable and total microbial concentrations. The viable microbial concentrations measured using an Andersen impactor ranged from 430-487 cfu/m^3, and from 298-883 cfu/m^3 for the outdoor and indoor air, respectively, and as such, were not statistically different from each other. However, if these data were to be examined separately for viable bacteria and fungi, such analysis will show that the airborne bacterial concentrations measured in the library room (284 to 862 cfu/m^3) was significantly higher than the outdoor ones (77-268 cfu/m^3 with indoor/outdoor ratio, I/O=3.7; *t*-test: $p<0.05$). For fungi, the opposite relationship was revealed (indoor range 14-63 cfu/m^3; outdoor range 219-353 cfu/m^3; I/O=0.1; *t*-test: $p<0.001$). Hence, an unrestrained infiltration of ambient air through the draughtiness of the building envelope was probably the main process responsible for indoor fungal pollution, whereas bacterial contaminants have their major sources in the indoor environment. This phenomenon was also confirmed by the qualitative analysis of airborne microflora (Table 1). Almost all the filamentous fungi identified in indoor air were also present in the air outside the library room, whereas bacterial microflora of the atmospheric air was much less diverse than its indoor composition (14 *vs.* 21 isolated species, respectively).

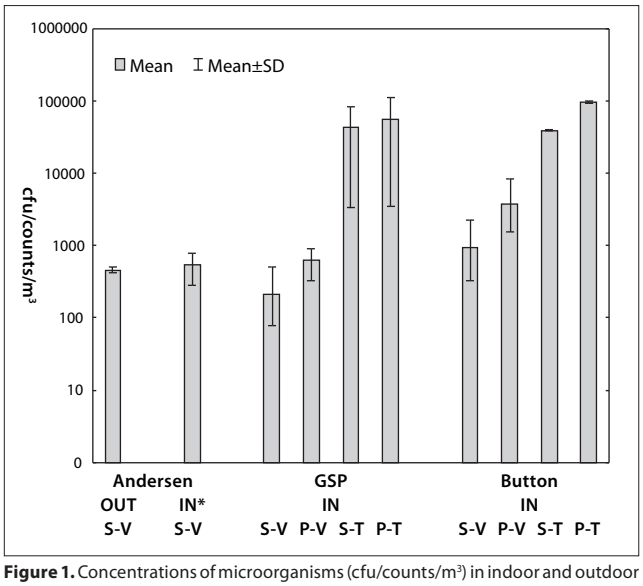

Figure 1. Concentrations of microorganisms (cfu/counts/m^3) in indoor and outdoor air at the Jasna Góra monastery library, measured using Andersen, GSP and Button samplers. Data are given as mean values and standard deviations of 3 or 4 (marked with *) repeats. OUT – outdoor environment, IN – indoor environment, S – stationary sampling, P – personal sampling, V – viable count, and T – total count.

Table 1. Microbial taxa isolated from air and settled dust at the Jasna Góra monastery library. In each environment the species with isolation frequency higher than 50% in the groups of identified bacteria or fungi are given in brackets.

Bacteria	Outdoor air	Indoor air	Settled dust
Gram-positive cocci			
Kocuria rosea	✓		
Micrococcus luteus	✓	✓	✓
Micrococcus lylae		✓	
Micrococcus spp. #	✓	[✓]	✓
Staphylococcus capitis		✓	
Staphylococcus cohnii	✓	✓	✓
Staphylococcus epidermidis	✓	✓	
Staphylococcus hominis		✓	
Staphylococcus saprophyticus		✓	
Staphylococcus xylosus	✓	✓	
Non-sporing Gram-positive rods			
Arthrobacter spp.		✓	
Brevibacterium epidermidis		✓	
Brevibacterium spp.			✓
Corynebacterium propinquum	✓	✓	
Corynebacterium striatum	✓	✓	
Rothia mucilaginosa	✓	✓	
Endospore forming Gram-positive rods			
Bacillus cereus	✓	✓	✓
Bacillus pumilus #		✓	[✓]
Bacillus spp. #^		✓	[✓]
Gram-negative rods			
Cellulomonas spp.		✓	
Pseudomonas putida		✓	
Pseudomonas spp. #^	✓		
Stenotrophomonas maltophilia	✓	✓	
Mesophilic actinomycetes			
Nocardia spp. #	✓		
Rhodococcus spp.	✓	✓	
Fungi			
Filamentous fungi			
Acremonium striatum	✓	✓	
Acremonium spp. #^	✓		
Alternaria spp. #^*	✓		
Aspergillus flavus #^	✓		
Aspergillus niger #^	✓	✓	✓
Aspergillus versicolor #^	✓	[✓]	
Aspergillus spp. #^*	✓		[✓]
Chaetomium elongatum *		✓	
Chaetomium spp. #^*	✓	✓	
Oidiodendron rhodogenum	✓		
Oidiodendron truncatum	✓		
Penicillium aurantiogriseum #	✓		
Penicillium verrucosum			[✓]
Penicillium spp. #^	[✓]	✓	[✓]
Ulocladium spp. #	✓		
Wallemia sebi	✓		
Yeasts			
Candida famata		[✓]	[✓]
Geotrichum candidum		✓	
Rhodotorula glutinis	✓		

– paper/parchment, ^ – leather, and * – wood biodeteriogens [after: 1, 11, 27, 33, 35, 47, 50, 57, 58]

As mentioned above, the measurements of airborne microbial flora in the library were carried out using 3 different samplers. The average viable microbial concentrations (±SD) estimated using GSP as well as Button samplers were as follows: 210±297 and 623±293 cfu/m^3, as well as 938±1,326 and 3,758±4,726 cfu/m^3 for stationary and personal measurements, respectively. The average total microbial concentrations (±SD) estimated using GSP as well as Button samplers were as follows: 43,000±39,598 and 56,500±53,033 counts/m^3, as well as 39,000±990 and 97,000±4,243 counts/m^3 for stationary and personal measurements, respectively. The analysis of variance revealed that there was no statistically significant difference between the concentrations of viable microorganisms measured stationary using Andersen, GSP, and Button samplers (ANOVA: $p>0.05$). The same trends were observed when viable or total microbial levels were established, based on stationary or personal measurements by both GSP and Button samplers (*t*-test: in all 8 cases $p>0.05$). As shown in the air quality studies, both GSP and Button Aerosol samplers efficiently and uniformly collect bioaerosol particles for viable and non-viable analyses, have low sensitivity to wind velocity and direction, and have been widely used for personal and stationary sampling of fungi and bacteria [e.g., 29–35]. The results obtained in the library at the Jasna Góra shrine revealed that, in this type of indoor environment, both tested filter samplers can be interchangeably applied. Regardless of the stationary or personal sampling strategy utilizing GSP or Button sampler, in either case the airborne concentrations of viable or total microorganisms will not be significantly different from each other.

The analysis of viable and total microbial counts measured as stationary and personal samples revealed significant differences (ANOVA: $p<0.01$). It was shown that the degree of contamination assessed, based on the number of viable microorganisms, was always significantly lower (26- to 205-fold at $p<0.01$ to $p<0.05$) than that evaluated using the total microbial counts. The latest values measured as both stationary and personal exposure were (as befits almost isolated interior) extremely high, reaching the level of 100,000 particles in 1 m^3 of air. In this study, the culturable microorganisms constituted from 0.5% - 3.9% of the total microbial flora only. The highest differences were noted between the total counts measured using personally applied Button sampler and viable concentrations obtained with Andersen, stationary and personal GSP and Button samplers (Spjotvoll/Stoline test - in all 5 cases $p<0.01$). This unequivocally proved that even a chemically-cleansed collection of paper and/or parchment documents, having only sporadic contact with people but being under the influence of ambient air, can undergo microbial contamination, and thereby become an important emission source of immunologically-active propagules into the indoor air. Hence, the hermetic isolation of premises of this type through isolation from an inflow of outdoor contaminants (e.g., an augmentation of the room air-tightness, introduction of an air-conditioning system, etc.), as well as control of microclimate parameters of the incoming air (see below) are among the indispensable means for attaining the proper indoor hygienic quality.

Maximum concentrations of viable bacteria and fungi in the settled dust samples did not exceed 10 cfu/100 cm^2 and 100 cfu/100 cm^2, respectively. The comparison of obtained viable microbial concentrations indicated a high statistically significant correlation between the air and settled dust samples (r^2=0.94 at $p<0.05$).

To the best of our knowledge, there is no report in the peer-reviewed scientific literature on bioaerosol contamination of monastery libraries. Moreover, there is no publication about a similar monastery library which underwent such comprehensive conservation. Hence, the results of this study can only be compared with the data obtained in other libraries or archive storerooms. The hitherto obtained scientific evidence regarding bioaerosol contamination of libraries and archives revealed a similar range of concentrations (i.e., 10^1-10^3 cfu/m^3) [e.g., 2, 11, 13, 36-39]; however, none of these buildings had such severely restricted personnel access. Regarding total microbial counts measured in a library environment, the only data available are those published by Wlazło [40] and Wlazło *et al*. [11]. The authors found that total microbial concentrations in library interiors were between 10^4-10^5 counts/m^3, i.e., within the same range as in the Jasna Góra monastery library. Besides, the comparison of culturable bioaerosol concentrations with total microbial counts revealed that the numbers of viable particulates were significantly lower than their total counts for both personal and stationary sampling locations. The noted proportion is well in agreement with the observations made by other authors [e.g., 11, 40-42].

To date, there are no widely acceptable threshold limit values (TLV) for bioaerosols. In case of environmental or epidemiological studies, the main reason for this is the lack of well-documented and medically proven dose-response relationships between the exposure to specific biological agents and adverse effects caused by their exact dose(s). Despite progress in the development of aerosol sampling techniques and analytical methods over the last two decades, the worldwide scientific database on bioaerosols is still insufficient to quantitatively and qualitatively characterize them. Where TLV values are established, they are usually connected with the clinical picture of the specific disease caused by the agent, taking into consideration its presence in a certain element of the environment only [17]. Regarding libraries or museums, no international standards on air sampling methods or threshold values of risk exist, only national recommendations are available [12].

Maximum viable bacterial and fungal aerosol concentrations in the studied library did not exceed 10^3 and 10^2 cfu/m^3, respectively. Those values did not exceed the Polish proposals for threshold limit values (5×10^3 cfu/m^3 for both bacteria and fungi, respectively) as well [17, 43]. Their concentrations in outdoor air (in both cases below 10^3 cfu/m^3) were also lower than the proposed 'acceptable level of atmospheric air contamination' (also 5×10^3 cfu/m^3 for both bacteria and fungi, respectively) [17]. Although the average concentrations of viable microorganisms were not high, the periodical contraventions of TLV (up to 7,100 cfu/m^3) were registered. It should be remembered that the studied library is not a public library and has a very restricted personnel access. The observed concentrations of viable fungal particles indicates that both the stored collection and people staying or working in the library can be periodically exposed to contaminants which are responsible for their biodeterioration, and the possible appearance of adverse health effects, respectively [44]. In the case of viable bacteria, even a completely sterile collection can be recolonized in time to become once again an active source of microbial emission.

It should be clearly stated, however, that the above-mentioned TLVs are mainly intended for the protection of human health, while in the library with restricted personnel access, such as the Jasna Góra monastery, the protection of the stored collection against microbial deterioration is a major concern. Taking such an aspect into consideration, the Italian Ministry of Cultural Heritage (MIBAC) [45] proposed the following threshold values of risk: that the concentrations of heterotrophic bacteria should be less than 750 cfu/m^3 and fungi – less than 150 cfu/m^3. Flieder and Capderou [46] as well as Parchas [47] suggest that the fungal aerosol concentration should not exceed 100-120 cfu/m^3 or the collections should be subjected to disinfection. Cieplik [36] established the TLV depending on the species composition of bioaerosols and assumed that the maximum concentration should not exceed 150 cfu/m^3 if the bioaerosol is a mixture of several fungal species, or 50 cfu/m^3 for particular species. At the same time, the author allowed the concentration of 500 cfu/m^3 if the fungi belong to typical microbial genera present in the atmospheric air, such as *Cladosporium* and *Alternaria*. Karbowska-Berent *et al.* [2] suggested the level of 200 cfu/m^3 as a limit value, concluding that the higher concentrations of fungi in the air of libraries or archives can signal the existence of a moisture problem or the presence of internal microbial sources. Taking into account the maximum bacterial (862 cfu/m^3) and fungal (63 cfu/m^3) concentrations registered at the studied monastery library, it can be concluded that all the measured values are below the TLVs suggested by the above- mentioned authors. The only exception is the bacterial concentration, which exceeded the Italian threshold values of risk. This fact, in the case of the Jasna Góra monastery, should be treated as a warning sign for the improvement of the quality of the indoor air in the library against possible biodeterioration of this unique collection.

The concentrations of viable microorganisms in the settled dust at the Jasna Góra monastery library were generally below the ranges measured by other authors in this type of indoor environment [38, 40]. It was probably due to an isolated character of this library.

The percentage distributions of bacteria and fungi isolated from the outdoor and indoor air, as well as settled dust, is shown in Figure 2. Detailed characteristic of all identified microbial taxa are given in Table 1. A total of 14, 21, and 7 bacterial species were identified in the outdoor air, indoor air, and settled dust, respectively. The most numerous bacterial group in outdoor air was Gram-positive cocci (*Staphylococcus*, *Micrococcus*, and *Kocuria*), constituting 36% of all recovered strains, followed by non-sporing Gram-positive rods accounted for 32%, and mesophilic actinomycetes 23%, as well as endospore-forming Gram-positive rods (solely *Bacillus* spp.) and Gram-negative rods – 5% and 4%, respectively. Gram-positive cocci were also the most numerous group (71%) of bacteria in indoor air. The rest of the indoor airborne flora was constituted by non-sporing Gram-positive rods (15%), mesophilic actinomycetes and Gram-negative rods (both 6%) as well as endospore-forming Gram-positive rods (2%).

A total of 15, 8, and 5 fungal species were identified in the outdoor air, indoor air, and settled dust, respectively (Tab. 1). On average, filamentous fungi accounted for 99%, 72%, and 92%, respectively (Fig. 2). Among all fungal isolates from the air and settled dust, the *Aspergillus* and *Penicillium* species, as well as *Candida famata,* were predominant. It should be also noted that *Candida* and *Geotrichum* yeast species were widely represented in the indoor air samples.

The settled dust has a different microbial structure than the air, i.e., 7 bacterial and 4 fungal species were isolated. Among bacteria, a clear domination of *Bacillus* endospores (89%) and traces of Gram-positive cocci and nonsporing Gram-positive rods (7% and 4%, respectively), was discovered. No actinomycete and Gram-negative strains were present in settled dust. Amid fungi, the most prevalent (i.e., with

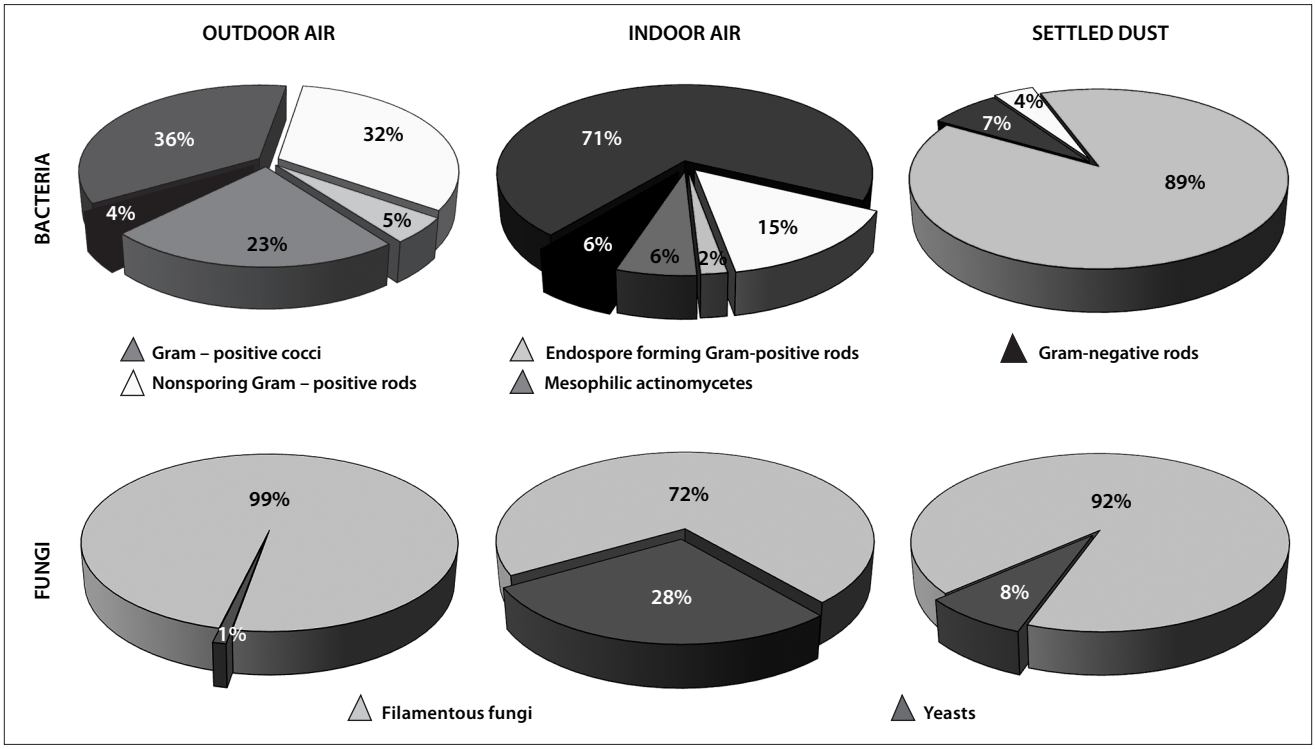

Figure 2. Percentage distribution of bacteria and fungi isolated from outdoor and indoor air, as well as settled dust, at the Jasna Góra monastery library.

an isolation frequency higher than 50%) were moulds from *Aspergillus* and *Penicillium* (especially *P. verrucosum*) genera, as well as yeast *Candida famata*.

Qualitative analysis of the library microflora demonstrated that many more bacterial and fungal strains were identified by impaction (24 bacterial and 18 fungal species) than by filtration (4 bacterial and 4 fungal species) sampling methods. Moreover, no Gram-negative rods and yeasts were isolated from personal samples. The lower numbers of microbial taxa identified from GSP and Button samplers were probably due to biological stress during both the sampling process (desiccation) and analytical elaboration of the samples (filter dissolution) [32, 42, 48].

Among the identified species were those from the group of well-known paper, parchment or wood biodeteriogens, which have been frequently isolated from the interiors of libraries and archives [2, 10, 11, 13, 37, 38, 49–56]. A novelty characteristic for the studied Jasna Góra library was a broad spectrum of identified bacterial species, as well as the presence of yeasts in both the indoor air and settled dust. Amid these species, *Bacillus* and *Micrococcus* representatives as well as yeast *Candida famata* were isolated from more than 50% of analyzed samples. Such a qualitative composition of microflora is rather characteristic for other archival materials, such as cinematographic films, which have already undergone deterioration [57].

Air temperature and relative humidity recorded outside as well as inside the library ranged between 17.8-9.5°C and between 49-62%, as well as between 19.0-20.1°C and between 51-60%, respectively. Significant correlations were recorded between both these microclimate parameters and outdoor viable microbial concentrations only (in both cases: r^2=0.99 at $p<0.05$). Irrespective of the bioaerosol sampling method, low air temperature and relative humidity values and their fluctuations in the library room, neither of these parameters significantly influenced the levels of microbial contaminants and did not support their growth. The same observation was true for the relationships between these two microclimate parameters and microbial concentrations in settled dust. Such a situation is beneficial from the point of view of library collection preservation [58]. It indicates that for the microorganisms (especially bacteria) an availability of sources of nutrition in the form of different materials (paper, parchment, wood, leather, organic adhesives, etc.) presented in the library was the only major factor conditioning the degree of contamination of such an interior.

CONCLUSIONS

The presented study revealed that 10 years after disinfection of the entire incunabula collection in library of the Jasna Góra monastery is once again microbiologically contaminated. The maximum viable microbial concentrations indoors reached 1,875 and 7,100 cfu/m^3 for stationary and personal measurements, whereas respective maximum total microbial counts were 71,000 and 100,000 counts/m^3. The culturable microorganisms constituted from 0.5%-3.9% of the total microbial flora only. This proved that even a chemically-cleansed collection of paper and/or parchment documents, having only sporadic contact with people but being under the influence of ambient air, can undergo microbial contamination again and become an important emission source. Hence, a hermetic isolation of premises of this type through mechanical isolation from the inflow of outdoor contaminants (e.g., an augmentation to air-tightness of the room, introduction of an air-conditioning system, etc.), as well as control of the microclimate parameters of the incoming air may solve, or at least decrease, the problems of protection of the stored collection and the maintenance of the quality of proper indoor hygiene.

Although the obtained viable microbial concentrations were low, numerous microbial species identified in the air or on the surfaces can be responsible for biodeterioration of the incunabula collection. To avoid such process, the control of microbial air quality using both stationary and personal samplers should be periodically performed.

There was no statistically significant difference between the concentrations of viable microorganisms measured stationary using Andersen, GSP, and Button samplers. Moreover, GSP and Button samplers can be interchangeably applied when viable or total microbial levels are stationary or personally measured. Qualitative evaluation of bioaerosols revealed that filamentous fungi were the most prevalent outdoors, whereas Gram-positive cocci and endospore forming Gram-positive rods dominated in indoor air and settled dust, respectively. Hence, the unrestrained infiltration of ambient air through the draughtiness of building envelope is probably the main process responsible for indoor fungal pollution, whereas bacterial contaminants have their major sources in indoor environment.

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