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MICROBIAL AIR QUALITY AT SZCZAWNICA SANATORIUM, POLAND

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Abstract: Nowadays, sanatorium treatment is undergoing a renaissance; however, data on air quality in such premises are scarce. The aim of this study was to characterize microbial air quality at the Szczawnica sanatorium in Southern Poland. The bioaerosol measurements were carried out using a 6-stage Andersen impactor over a period of one year in 3 naturally ventilated sanatorium premises (where different curative treatments took place) and in outdoor air. The indoor and outdoor concentrations of fungal aerosol were always below 1,600 cfu/m^3. With regard to bacterial contamination, the highest concentrations (up to 6,223 cfu/m^3) were usually noted when the patients were present and underwent curative procedures. Such concentrations crossed the Polish threshold limit values, which suggest that natural ventilation in this type of premises did not ensure the proper air quality; therefore a high-performance ventilation or air-conditioning system should be introduced to provide the "clean" air into the curative treatment rooms. Qualitative evaluation of bioaerosols revealed that the most prevalent indoors were Gram-positive cocci, mesophilic actinomycetes, and filamentous fungi. Analysis of microclimate parameters confirmed that only relative humidity of the air influenced significantly the levels and composition of microbial aerosols. Hence, the constant control of this parameter should be scrupulously supervised at sanatorium premises.

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INTRODUCTION

Air pollution is one of the main factors affecting the quality of both indoor and outdoor environments. The importance of air quality is emphasized by the fact that its rapid changes caused by heavy gaseous and particulate emissions into the atmosphere can result in an increase in appearance of adverse health effects. From this perspective, a biological contamination of the air seems to play a crucial role, especially for the people who have been already suffering from (e.g., seasonal) aerosol exposure or have serious health problems which need a professional hospital care (such as immunodeficiency) [6, 46, 51]. In these cases, an additional exposure to relatively pathogenic or even saprophytic microorganisms can strengthen the adverse outcomes [8, 19, 41, 42, 63].

Modern and comprehensive health resort treatment helps to mobilize an immune system to fight against a disease. This effect can be achieved by both relief from environmental hazards as well as by a supplementation (or sometimes replacement) of the pharmacotherapy with the same therapeutic agents, but of natural origin [5, 13, 17, 21, 23, 29, 37, 62]. Apart from the agents directly connected with medical therapy, sanatorium treatment is supported by the use of different health stimulants, such as inhalations, mineral waters, bathing peloids (e.g., muds, clay), climatic stimulants, and various forms of energy [9, 12, 13, 14, 15, 36, 52, 62, 67]. In social feeling, all these factors are found

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to be, if not adiaphorous, beneficial for human health. For these reasons, sanatorium treatment has been undergoing a renaissance and become fashionable, not only in Poland, but in other countries as well [9, 13, 57]. Putting aside all the proofs by presumption, which could be easily challenged, several detailed pulmonological studies indicate the purposefulness of this type of treatment for different respiratory diseases. From the medical point of view, specific therapeutic conditions achieved in natural subterraneotherapy chambers, or (sometimes artificially) created salt chambers in spa resorts, have proved to be beneficial for heath [9, 11, 13, 15, 25, 36].

Proper air quality is the basic criterion when a region or resort is considered for therapeutic and prophylaxis functions [57, 67]. For patients with respiratory tract diseases, inhalation of both natural (including biological) and artificially generated no-drug aerosols can be beneficial for the therapy [2, 3, 5, 14, 15, 17, 21, 23, 29, 36, 67]. The human organism adapts to specific microclimatic and microbiological conditions during the treatment processes, which result in the reorganization of its functions, and systemic side effects are avoided or minimized [13, 14, 15, 17, 21, 23, 29, 36]. As the microbial quality of the air is a key factor in inhalation therapy, the aim of this study was to quantitatively and qualitatively characterize the microbial air quality of the Szczawnica sanatorium located in one of the most popular health resorts in Southern Poland.

MATERIALS AND METHODS

The study was carried out in a sanatorium in Szczawnica, which is one of the oldest health resorts in Southern Poland. Szczawnica as a spa has been known since the 18th century due to a piedmont, mild climate with high insolation and relatively low precipitation levels, as well as quiet atmosphere. Apart from a healing microclimate, the balneological resources and mineral water springs are the major benefit of Szczawnica. The name of the resort stems from sour waters called by the highlanders "sorrels". Hence, this health resort specializes in medical treatment of the respiratory tract (chronic pneumonia, asthma, respiratory allergies, infections of respiratory tract, chronic obstructive pulmonary disease, bronchiectasis, pneumoconiosis, states after pneumonia and bronchitis) and laryngological (sinusitis, pharyngitis) diseases, as well as arteriosclerosis, osteoporosis, uric acid diathesis and constipations. The medicinal treatment is successfully combined with other medications such as hydropathy, inhalations, physiotherapy and kinesitherapy.

The measurements of biological aerosol were carried out over a period of one year (January–December 2008), twice in each of the 4 seasons. The air samples were collected in 3 buildings (all located within a radius of 200 m): Nature Treatment Institute (where curative treatments such as therapeutic bathtubs or underwater massages take place), Inhalatorium (a big chamber where patients in several specifically designed cabins are individually exposed to medical aerosols), and the Pump-room (where patients can drink different mineral waters straight from the springs). All the studied premises were naturally ventilated. In each of these buildings the samples were taken (in triplicates) in the rooms during treatment courses with patients, and in the same rooms after curative treatment without them. In addition, in each of the investigated buildings, an indoor background concentration of bacterial and fungal aerosols (IN-B) was established. The rooms where no curative treatment takes place, were selected for this purpose. Moreover, in front of the Nature Treatment Institute building, outdoor air samples were also collected to obtain data on the background (atmospheric) level of microbial contamination (OUT-B). Selected sampling points with their brief characteristics are presented in Figure 1.

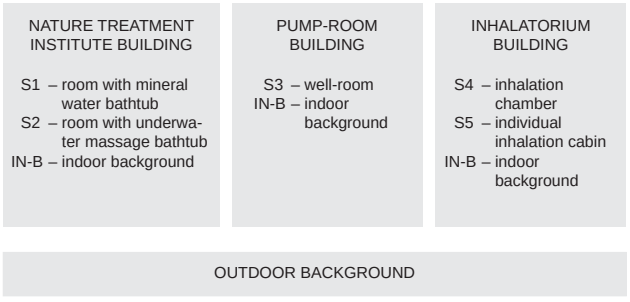

Figure 1. Short description of sampling points.

The air samples were collected using a 6-stage Andersen impactor (model 10-710, Graseby-Andersen, Inc., Atlanta, GA, USA). The sampler was placed at a height of 1.0–1.5 m above the floor or ground (outdoor measurement) level to simulate aspiration from the human breathing zone. A 5-minute sampling period was applied for the collection of bacterial and fungal aerosols. Samples were taken at a flow rate of 28.3 L/min. Bacteria were collected first, on blood trypticase soy agar (TSA; Becton, Dickinson and Company, Sparks, MD, USA). After impactor reloading, the fungi were collected on malt extract agar (MEA; Oxoid Ltd., Basingstoke, Hampshire, UK). During sampling, the air temperature and relative humidity (RH) were measured using a hytherograph (model Omniport 20, E+E Elektronik GmbH, Engerwitzdorf, Austria).

The TSA plates were incubated for 1 day at 37ºC followed by 3 days at 22ºC and another 3 days at 4ºC, and MEA plates for 4 days at 30ºC followed by 4 days at 22ºC. After incubation of the plates, the qualitative and quantitative analyzes of growing microorganisms were performed. The concentration of bioaerosols was calculated as colony forming units per cubic meter of air (cfu/m^3). Bacterial strains were identified by Gram staining, their morphology and, finally, by the biochemical API tests (bioMérieux, Marcy l'Etoile, France). Fungi were identified according to their morphology using several identification keys [7, 20, 24, 58, 61].

As the collected data had a non-parametric distribution, the statistical analyzes were performed by Kruskal-Wallis

Table 1. Bacterial and fungal aerosol concentrations (cfu/m^3) at Szczawnica sanatorium.

Environment	Bacteria		Fungi	
	Range	Median	Range	Median
Sanatorium premises				
Rooms during treatment courses with patients	137–6223	676	18–1247	254
Rooms after curative treatment without patients	21–1560	379	0–1109	131
Indoor background	671–2431	1435	35–1575	228
Outdoor background	14–366	70	14–352	190

and Mann-Whitney tests, as well as Spearman correlation using Statistica (data analysis software system) version 7.1 – 2006 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The concentrations of bioaerosols at Szczawnica sanatorium are presented in Table 1 and Figure 2. Bacterial aerosol concentrations in the studied premises (with and without patients) ranged from 21–6,223 cfu/m^3 and were up to 17-times higher than the maximum values (i.e., 366 cfu/m^3) noted in ambient air ($p<0.01$ and $p<0.05$, respectively). The outdoor concentrations were also significantly lower ($p<0.01$) than those measured on an indoor background, i.e., in the premises where curative treatment procedures were never performed. The average indoor background level of bacterial aerosol (1,435 cfu/m^3) suggests that the scale of contamination of the sanatorium premises is strictly connected with their practical functions. Whereas the treatment rooms -by definition- were "isolated" from influences of the outside world (median bacterial concentrations were 676 cfu/m^3 and 379 cfu/m^3 for rooms with and without patients, respectively), the hygienic regime imposed on the rest of sanatorium premises was less strict.

The highest bacterial aerosol concentrations were observed in the rooms with water bathtubs (up to 3,527 cfu/m^3) and in the well-room (up to 6,223 cfu/m^3). Average concentrations of bacterial aerosol in the studied sanatorium treatment rooms were always higher when patients were present and underwent curative procedures. The only exception from this scheme was the concentration of airborne bacteria measured in the well-room (Fig. 2). Probably the activity (movement) of patients constantly entering and leaving this place (and creating thus creating additional ventilation) resulted in a decrease of the – naturally observed in this room – relatively high concentration of bacterial aerosol.

Taking into account that all the investigated premises were naturally ventilated and the outdoor concentrations of bacterial aerosol were meaningly lower than indoors, it is very probable that the observed differences depended

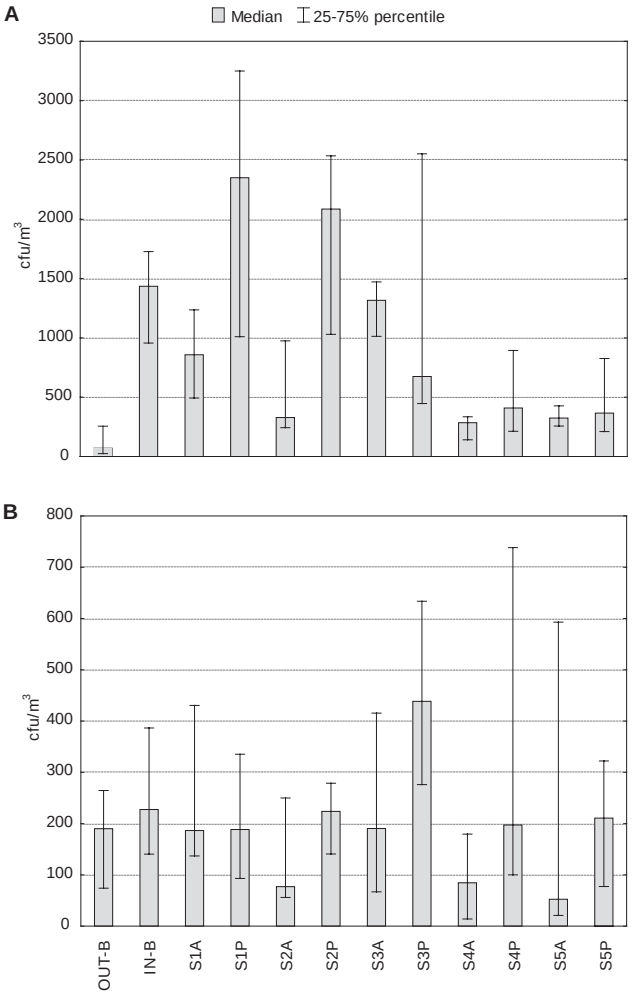

Figure 2. Average concentrations (cfu/m^3) of bacteria (A) and fungi (B) in outdoor and indoor air at Szczawnica sanatorium. OUT-B – outdoor background, IN-B – indoor background, S1 – room with mineral water bathtub, S2 – room with underwater massage bathtub, S3 – well-room, S4 – inhalation chamber, S5 – individual inhalation cabin, A – absence of patients, and P – presence of patients.

solely on both the type of curative treatment and presence of patients during treatment courses (Fig. 2). People are a main and active source of bacterial emission indoors. The most abundant generation of these biological particulates takes place during speaking, coughing, sneezing, and epidermis exfoliation [6, 47, 54, 56, 65]. In case of the studied premises, regardless of the room location, the presence of patients taking curative treatment significantly increased the bacterial aerosol level ($p<0.05$). The synergistic effect of these 2 parameters contributed the most to the observed level of bacterial aerosols, masking other commonly known factors such as air exchange rate or influence of the air temperature [6, 8, 28, 40, 41, 42, 44, 53, 60, 64].

After analyzing the fungal aerosol quantitatively it can be stated that, irrespective of the studied environments, its indoor (i.e., in treatment rooms and in the background) as well as outdoor concentrations were not significantly different from each other and were always below 1,600 cfu/m^3

(Tab. 1). The highest fungal aerosol concentrations were observed in the well-room (up to 788 cfu/m^3) and inhalation chamber (up to 1,247 cfu/m^3). Nevertheless, it should be pointed out that outdoor concentrations were always 3.2–4.5-times lower than those noted indoors. All studied sanatorium rooms were contaminated with fungal aerosol to the same degree (Kruskal-Wallis test: $p>0.05$), despite the presence or absence of patients. When comparing average microbial concentrations between the studied sanatorium premises, the highest differences were observed between the well-room and inhalation chamber; however, a statistically significant relationship was confirmed for bacterial aerosol concentrations only ($p<0.05$).

The obtained results of both indoor and outdoor measurements of bioaerosol concentrations were compared with the Polish proposals for threshold limit values, which are 5×10^3 cfu/m^3 for both bacteria and fungi in indoor and outdoor environments [31, 32]. As can be seen, in the atmospheric air as well as in the air of almost all investigated sanatorium premises (except the well-room), the bacterial and fungal aerosol concentrations were below these recommended threshold limit value. The only outliers, which crossed these levels, were noted for bacterial aerosol during the winter season in the well-room when patients were present indoors (6,223 cfu/m^3). The probable explanation of the observed phenomenon is – in a way – the expected seasonal increase in the concentration of these microorganisms. The well-room had a natural, relatively high background level of bacterial contaminants (column S3A in Figure 2). During winter, an increase in the air-tightness of this interior resulted in diminished ventilation due to energy saving issues – tightly closed doors and windows, and a simultaneously operating central heating system utilizing hot-water radiators, combined with the presence of patients (who are additional and active sources of bacterial aerosol emission) created periodically an inadmissibly high concentration of potentially harmful biological agents. It is particularly important in the case of the sanatorium premises where the patients – whose health status, by definition, requires a special (medical) care – should not be additionally exposed to biologically active particulates.

Seasonal variations of microbial aerosol concentrations are presented in Figure 3. Despite the noted discrepancies in average bacterial and fungal aerosol concentrations measured in both outdoor and indoor backgrounds, as well as in the treatment rooms without patients, the statistical analysis did not reveal significant differences between the seasons (Kruskal-Wallis test: $p>0.05$). The only statistically significant differences were noted in the treatment rooms with patients (when the curative procedures were applied) for bacterial aerosol between spring (median: 448 cfu/m^3) and winter (median: 1,813 cfu/m^3) concentrations and for fungal aerosol between spring (median: 60 cfu/m^3) and summer (median: 420 cfu/m^3) levels (in both cases: $p<0.05$). Regarding bacteria, the higher air-tightness of the buildings in winter (for energy saving reasons) caused all

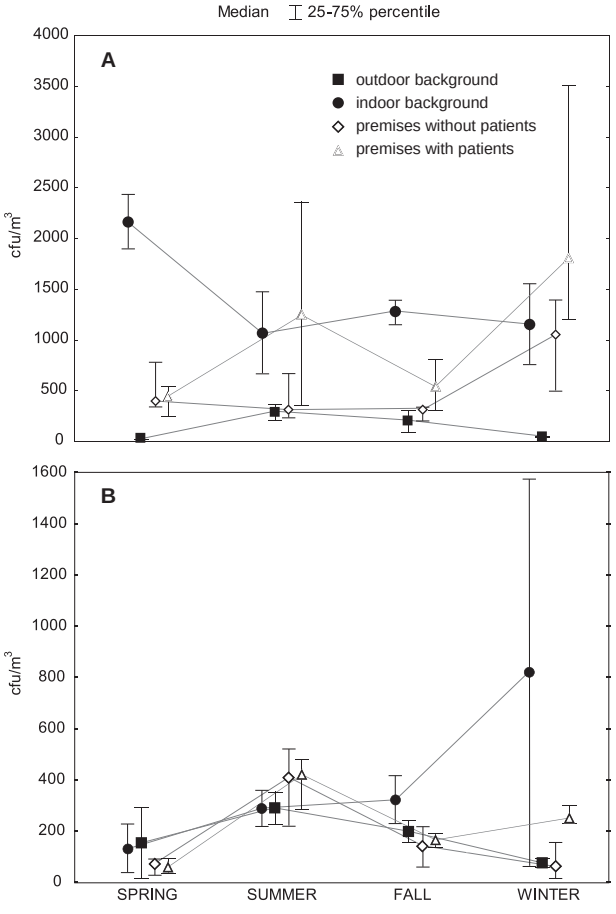

Figure 3. Seasonal variations in bacterial (A) and fungal (B) aerosol concentrations (cfu/m^3).

bacterial particulates – emitted mainly by the patients or as a consequence of the treatment procedure application – to be trapped indoors, and their exchange with outdoor environment was substantially diminished. In the case of fungi, an opposite situation was observed. Higher summer levels of their aerosol were probably connected with the natural ventilation of the studied premises and subsequent infiltration of outdoor air transporting an additional load of fungal spores.

The air quality at sanatoria has received little attention in the scientific literature and the collected data are very limited. Fortunately, the Szczawnica sanatorium was investigated for the first time almost half a century ago. The data regarding seasonal variations collected at that time by Cienciała *et al.* [16] were confirmed by the current observations. The only noted difference was connected with the measured concentrations of microorganisms. The levels assessed 40 years ago were about 5-times higher (i.e., up to 32,000 cfu/m^3) than those obtained in this study. In another investigation, Burkowska and Donderski, evaluating outdoor airborne mycoflora, described a similar trend regarding the filamentous fungi content in the air of Ciechocinek spa [12]. The lowest mold content was observed in winter (70 cfu/m^3) and the highest in summer (4,800 cfu/m^3); however, the summer

Table 2. Temperature and relative humidity of the air at Szczawnica sanatorium.

Environment	Bacteria		Fungi	
	Range	Median	Range	Median
Sanatorium premises				
Rooms during treatment courses with patients	18–24	21	33–84	47
Rooms after curative treatment without patients	15–26	21	32–54	44
Indoor background	19–26	22	34–68	38
Outdoor background	-3–27	15	36–77	54

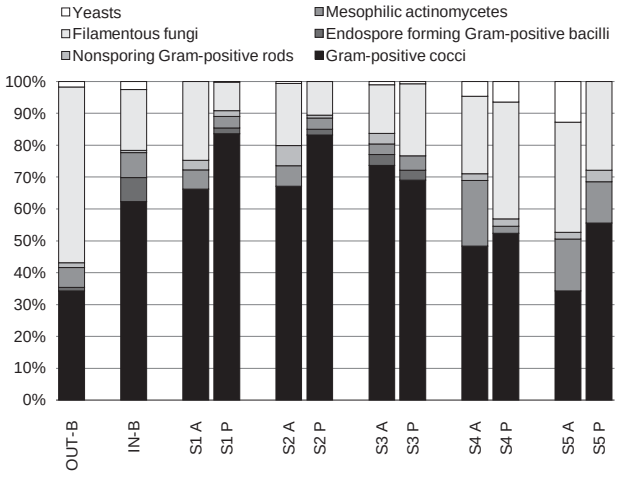

Figure 4. Percentage contribution (%) of bacterial and fungal groups to the total airborne microflora at Szczawnica sanatorium. OUT-B – outdoor background, IN-B – indoor background, S1 – room with mineral water bathtub, S2 – room with underwater massage bathtub, S3 – well-room, S4 – inhalation chamber, S5 – individual inhalation cabin, A – absence of patients, and P – presence of patients.

concentrations in Ciechocinek were about 10-times higher than those measured in Szczawnica. When the data on fungal aerosol concentrations gathered indoors in Szczawnica were compared with those collected in the air of Lithuanian sanatorium premises by Krikštaponis [43], where concentrations ranged from 156–720 cfu/m^3, no significant differences were visible.

Simultaneously with bioaerosol measurements, the environmental parameters, i.e., air temperature and relative humidity were also controlled. The results of these measurements are presented in Table 2. The average yearly indoor temperatures were between 21–22°C and were not significantly different between the studied sanatorium premises (Kruskal-Wallis test: $p>0.05$). Such differences appeared when indoor and outdoor background temperatures were compared. Indoor temperatures were always significantly higher than those registered outside of the buildings ($p<0.05$). When the studied sanatorium treatment rooms were compared, both the season and their location influenced the measured temperature values. The highest significant differences were noted between the summer (median: 24°C) and winter (median: 19°C) temperatures as well as between the room with a mineral water bathtub (median: 19°C) and individual inhalation cabin (median: 22°C) (in both cases: $p<0.05$).

The average yearly values of relative humidity of the air for all treatment rooms (with and without patients together – median: 45%) as well as for all indoor (median: 38%) and outdoor (median: 54%) background measurement points did not reveal significant differences between them. A similar relationship was observed when the average seasonal relative humidity values for both indoor and outdoor backgrounds were compared. When such analysis was restricted to the treatment rooms only, statistically significant differences were observed for both the seasons and locations. In the first case, the highest differences were noted between summer (median: 70%) and spring (median: 42%) as well as between summer and winter (median: 42%) (in both cases: $p<0.05$). Regarding the location, the highest differences were revealed between individual inhalation cabins (median: 33%) and both the rooms with mineral water (median: 50%) and underwater massage (median: 49%) bathtubs ($p<0.01$ and $p<0.05$, respectively).

Microclimate may have various effects on biological aerosol concentrations [4, 6, 22]. The relative humidity and temperature measurements can be used to identify conditions that support microbial growth. For the comfort of inhabitants, the majority of the buildings are maintained at a temperature of 18–24°C. Such a range is also suitable for the growth and development of many environmental microorganisms which, in most cases, are mesophilic [27, 38, 48, 66]. Although both temperature and RH (or water availability as its equivalent) are necessary for environmental microbial expansion, a proper value of the latter parameter is critical. The development of the microbial community is directly proportional to the values of this limiting factor, i.e., it may be slow (or even stopped) when RH is low and fast whenever there is its sudden increase [38, 66]. Assessing the influence of microclimate parameters on the bioaerosol concentrations in this study it can be stated that only the relative humidity of the air had a substantial influence on the observed levels of microorganisms. Correlation analysis revealed that each increase in moisture content in the air resulted in a significant augmentation of both the airborne bacterial and fungal levels (Spearman correlation coefficient calculated for all studied premises together: $R=0.42$ at $p<0.05$ and $R=0.46$ at $p<0.05$, respectively). In contrast, Burkowska and Donderski [12], who assessed the fungal air quality in Ciechocinek spa, found that only air temperature positively correlated with the number of airborne molds.

Table 3 presents the percentage of the contribution of bacterial and fungal groups to the total airborne microflora at Szczawnica sanatorium, and Figure 4 specifies qualitatively these data, showing the list of microbial taxa

Table 3. Microbial taxa isolated from indoor air at Szczawnica sanatorium.

Microorganisms	Percentage contribution (%)
Bacteria	100
Kocuria rosea	<1
Micrococcus spp.	46
Staphylococcus haemolyticus	2
Staphylococcus hominis	6
Staphylococcus lentus	4
Staphylococcus simulans	19
Staphylococcus xylosus	3
Staphylococcus spp.	<1
Arthrobacter spp.	2
Corynebacterium spp.	1
Microbacterium spp.	<1
Bacillus cereus	< 1
Bacillus licheniformis	2
Bacillus megaterium	<1
Bacillus pumilus	<1
Bacillus spp.	<1
Nocardia spp.	2
Rhodococcus spp.	5
Streptomyces spp.	6
Fungi	100
Candida famata	4
Candida spp.	<1
Cryptococcus spp.	<1
Rhodotorula mucilaginosa	<1
Acremonium spp.	1
Acremonium strictum	< 1
Alternaria alternata	8
Alternaria spp.	< 1
Aspergillus spp.	5
Cladosporium cladosporioides	17
Cladosporium herbarum	1
Cladosporium spp.	4
Fusarium sporotrichioides	< 1
Fusarium spp.	< 1
Helminthosporium spp.	1
Penicillium chrysogenum	5
Penicillium griseofulvum	< 1
Penicillium spp.	28
Rhizopus spp.	4
Rhizopus stolonifer	< 1
Scopulariopsis spp.	12
Sporotrichum spp.	3
Trichoderma spp.	2

isolated from indoor air. Analysis of microbial composition of the atmospheric air revealed that mesophilic Gram-positive cocci (*Staphylococcus*, *Micrococcus*), mesophilic actinomycetes (mainly *Rhodococcus*) and fungi (*Rhizopus, Cladosporium, Alternaria, Aspergillus, Penicillium*) were the predominant microorganisms, which is a typical picture for outdoor microflora [1, 18, 30, 39, 45, 55]. In the studied premises, in the air of indoor background, the most prevalent were mesophilic Gram-positive cocci (the same genera as listed above), but filamentous fungi (*Penicillium, Cladosporium, Scopulariopsis*), mesophilic actinomycetes (mainly *Streptomyces* and *Rhodococcus*) and endospore forming Gram-positive bacilli were also quite frequently isolated. In all treatment rooms (except the well-room) when the patients were not present, the air was free from the endospore-forming Gram-positive bacilli. Such a situation was characteristic for all rooms in the Inhalatorium building, even if the patients were present inside them (Fig. 4).

The contribution of Gram-positive cocci to the total bacterial community of the air in the sanatorium rooms varied between 65–88% when the patients were absent, and between 77–93% when they were present. These results confirm the regularity that Gram-positive cocci usually prevail in the air of both the outdoor (urban) and indoor (including residential) environments [26, 34, 49, 56, 64].

The data on microbial quality of the air at sanatorium premises are scarce in the scientific literature. However, as mentioned above, the air quality of Szczawnica sanatorium premises was already the aim of the study 40 years ago. These bioaerosol measurements carried out by Cienciała *et al.* [16] revealed that qualitative composition of airborne microflora was surprisingly similar to those obtained in the present study. As in the past so to-day, the bacterial species of the genera *Staphylococcus*, *Micrococcus*, *Bacillus*, and *Arthrobacter,* as well as fungal species of the genera *Penicillium*, *Cladosporium*, *Alternaria*, *Aspergillus*, *Sporotrichum*, *Rhizopus*, and *Trichoderma* were recovered from the air; however, their maximum concentrations almost half of the century ago were up 5-times higher. Besides, the data on the qualitative composition of fungi in the air of Lithuanian sanatorium premises collected by Krikštaponis [43] are well in accordance with those obtained in this study, i.e., the species from genera *Penicillium*, *Cladosporium*, *Aspergillus*, and *Chrysosporium* dominated indoors. Similar observation was noted by Burkowska and Donderski [12], who identified in the air of Ciechocinek spa the species from genera *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor* as the most common.

In this study, a relatively high percentage of identified airborne microflora was constituted by mesophilic actinomycetes (mainly *Streptomyces* and *Rhodococcus*). Their percentage contribution to the total microbial flora ranged from 4–31%. These bacteria have a unique ability to colonize different solid surfaces, from rocks to ceramics, as well as other building constructing and finishing materials [33, 50, 59]. In the studied sanatorium premises, their high

concentration was especially visible in the Inhalatorium building. This was probably due to the presence of water inside the premises. Relative humidity, increased especially during the treatment procedures, causing actinomycestes (as precursor microorganisms of indoor contamination) to appear, usually as primary colonizers on moist surfaces, and showing a concentration increase in the studied environment [33, 35]. As a consequence of relative humidity increase (reaching periodically 84% (Tab. 2), an abundant appearance of filamentous fungi and yeasts was also noted. Correlation analysis distinctly confirmed these relationships in rooms during treatment courses with patients for both bacteria and fungi (Spearman correlation coefficient: $R=0.49$ at $p<0.05$ and $R=0.53$ at $p<0.05$, respectively).

A similar trend was observed in the study carried out by Bis *et al.* [10], where a widespread occurrence of *Actinomycetes* (especially species from genus *Streptomyces*) in sanatorium chambers of the salt mines at Bochnia and Wieliczka was clearly noted. In all these cases, the presence of mesophilic actinomycetes indicated microbial contamination of the air due to undesired changes in microclimate conditions (elevated moisture content) indoors.

Endospore-forming Gram-positive bacilli, for which the major sources (soil, plants) are in the outdoor environment, were also frequently recovered from the air of the studied sanatorium premises. Their presence indoors was probably due to the ability to produce spores which could be transferred (e.g., on clothes, shoes, hands, hair) by people entering the sanatorium rooms [40, 49, 65].

Another interesting finding of this study was an absence of Gram-negative rods indoors. No bacteria from this group were recovered from the air of the studied premises. These bacteria are a source of immunologically active compound, i.e. endotoxin; if not present indoors, do not pose additional health threat to the patients. From the respiratory health perspective, such a quantitative composition of indoor air should be beneficial for the sanatorium patients' health status.

The analysis of fungal composition of indoor bioaerosol showed that moulds such as *Penicillium*, *Cladosporium*, *Scopulariopsis*, *Alternaria*, *Aspergillus*, and *Rhizopus* were frequently isolated in sanatorium premises, constituting from 73% to even 100% of recovered microorganisms [6, 16, 19, 64]. These results are consistent with the data obtained in other studies carried out indoors, including also residential dwellings [34, 38, 44, 66]. As many of the isolated species (e.g., from *Alternaria* and *Scopulariopsis* genera) are common in soil [41], their high abundance in the outdoor air can be transposed in a natural way (i.e., via air penetration) into their high indoor prevalence. The majority of isolated fungal species have abilities to grow and survive for a long time on furnishings and other equipment, including heating or ventilation system elements [64]. Moreover, another interesting finding of this study was the relatively high isolation frequency of yeasts from genus *Candida*, especially in the Inhalatorium building. People are the major reservoirs of these yeasts, their emission into treatment chambers could explain the observed airborne levels.

CONCLUSIONS

The concentrations of microbial aerosol at Szczawnica sanatorium ranged from 183–6,380 cfu/m^3 and from 28–718 cfu/m^3 for the indoor and outdoor air, respectively. Regardless of the studied environments, both indoor (i.e., in treatment rooms as well as in the background) and outdoor fungal aerosol concentrations were not significantly different from each other and were always below 1,600 cfu/m^3. Regarding the bacterial contamination of the studied treatment rooms, the highest concentrations (up to 6,223 cfu/m^3) were usually noted when the patients were present and undergoing curative procedures. In the atmospheric air, as well as in the air of almost all the investigated sanatorium premises, the bacterial and fungal aerosol concentrations were below the Polish proposals for threshold limit values. The only exception was observed in the well-room, where bacterial aerosol concentrations during winter season, when the patients were present indoors, crossed the proposed limit value. These results unequivocally suggest that natural ventilation in this type of premises is insufficient to ensure the proper indoor air quality. Hence, a high-performance mechanical ventilation or air-conditioning system should be introduced to provide "clean" air into the curative treatment rooms where the patients (already with some health problems) require special medical care.

Qualitative evaluation of the aerosol microflora revealed that in the air of the studied premises the most prevalent were Gram-positive cocci (from genera *Micrococcus* and *Staphylococcus*), mesophilic actinomycetes (mainly *Streptomyces* and *Rhodococcus*), and filamentous fungi (*Penicillium*, *Cladosporium*, and *Scopulariopsis*). Analysis of the relationships between microclimate parameters and bioaerosol concentrations confirmed that only relative humidity of the air significantly influenced the levels and composition of microbial aerosols throughout the year. Due to such a high abundance of actinomycetes and moulds, the potentially allergenic and/or toxic propagules may be present in high quantities indoors. Such a situation is highly disadvantageous to the patients (especially those who have respiratory tract diseases), as an additional load of immunologically reactive propagules can adversely influence the health status of exposed individuals. Hence, the constant control of the air humidity seems to be a key factor which should be scrupulously supervised at sanatorium premises.

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REFERENCES

1. Adhikari A, Reponen T, Grinshpun SA, Martuzevicius D, LeMasters G: Correlation of ambient inhalable bioaerosols with particulate matter and ozone: A two-year study. *Environ Pollut* 2006, **140**, 16–28.

2. Alkiewicz J: Modern trends of inhaled therapy. *Rev Fr Allergol* 1999, **39**, 72-73.

3. Amirav I: Aerosol therapy. *Ital J Pediatr* 2004, **30**, 147–156.

4. Anderson K, Morris G, Kennedy H, Croall J, Michie J, Richardson MD, Gibson B: Aspergillosis in immunocompromised patients: associations with building hygiene, design and indoor air. *Thorax* 1996, **51**, 256–261.

5. Anderson SD, Spring J, Moore B, Rodwell LT, Spalding N, Gonda I, Chan K, Walsh A, Clark AR: The effect of inhaling a dry powder of sodium chloride on the airways of asthmatic subjects. *Eur Respir J* 1997, **10**, 2465–2473.

6. Araujo R, Cabral JP, Rodrigues AG: Air filtration systems and restrictive access conditions improve indoor air quality in clinical units: *Penicillium* as a general indicator of hospital indoor fungal levels. *Am J Infect Control* 2008, **36**, 129–134.

7. Atlas RM: *Handbook of Microbiological Media*. CRC Press, Boca Raton 2004.

8. Beggs CB, Kerr KG, Noakes CJ, Hathway A, Sleigh A: The ventilation of multiple-bed hospital wards: review and analysis. *Am J Infect Control* 2008, **36**, 250–259.

9. Bihari-Axelsson S, Axelsson R: The role of sanatoriums and health resorts in the Russian Federation. *Health Policy* 2002, **59**, 25–36.

10. Bis H, Barabasz W, Grzyb J, Fraczek K: Occurrence of *Actinomycetes* in the air of sanatorium chambers at Bochnia and Wieliczka salt mines. *Zeszyty Problemowe Postępów Nauk Rolniczych* 2004, **501**, 43–49 (in Polish).

11. Bis H, Grzyb J, Barabasz W, Frączek K: Prevalence of fungi – Micromycetes in the health resort chambers in Bochnia and Wieliczka salt mines. *Acta Agraria Silvestria, Ser Agraria* 2004, **42**, 29–39 (in Polish).

12. Burkowska A, Donderski W: Airborne molds in the air of Ciechocinek spa. *Pol J Nat Sci* 2008, **23**, 790–800.

13. Chervinskaya AV: Halotherapy of respiratory diseases. *Physiother Balneol Rehabil* 2003, **6**, 8–15.

14. Chervinskaya AV: Respiratory hygiene in health resort medicine. **In:** *Proceedings of the 35th Congress of the International Society of Medical Hydrology and Climatology, Istanbul, June 6–10, 2006*, OP-2.P.86.

15. Chervinskaya AV, Zilber NA: Halotherapy for treatment of respiratory diseases. *J Aerosol Med* 1995, **8**, 221–232.

16. Cienciała M, Smyk B, Bukowski Z, Kukliński P: Formation of the air microflora at allergological centers in Szczawnica and Kraków. *Pol Balneol* 1968, **13**, 265–273 (in Polish).

17. Daviskas E, Anderson SD, Gonda I, Eberl S, Meikle S, Seale JP, Bautovich G: Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. *Eur Respir J* 1996, **9**, 725–732.

18. Degobbi C, Lopes F, Carvalho-Oliveira R, Muñoz JE, Saldiva P: Correlation of fungi and endotoxin with PM2.5 and meteorological parameters in atmosphere of Sao Paulo, Brazil. *Atmos Environ* 2011, **45**, 2277–2283.

19. Doleżal M, Doleżal M, Żuława G: Microflora of subterranean chambers in Wieliczka Subterraneotherapy Centre. *Biopollut Build Health* 1983, **35**, 55–65 (in Polish).

20. Domsch KH, Gams W, Traute-Heidi A: *Compedium of Soil Fungi*. Harcourt Brace Jovanovich Publishers, Academic Press, London 1980.

21. Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC: Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med* 2006, **354**, 241–250.

22. Douwes J, Thorne P, Pearce N, Heederik D: Bioaerosol health effects and exposure assessment: progress and prospects. *Ann Occup Hyg* 2003, **47**, 187–200.

23. Elkins MR, Robinson M, Rose BR, Harbour C, Moriarty CP, Marks GB, Belousova EG, Xuan W, Bye PT, National Hypertonic Saline in Cystic Fibrosis (NHSCF) Study Group: A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006, **354**, 229–240.

24. Fassatiowa O: *Microscopic Fungi in Technical Microbiology*. Scientific and Technical Publishing, Warsaw 1983 (in Polish).

25. Fiegel J, Clarke R, Edwards DA: Airborne infectious disease and the suppression of pulmonary bioaerosols. *Drug Discov Today* 2006, **11**, 51–57.

26. Flannigan B, McCabe EM, McGarry F: Allergenic and toxigenic microorganisms in houses. *J Appl Bacteriol* 1991, **70 (Suppl.)**, 61–73.

27. Flannigan B, Samson RA, Miller JD (Eds): *Microorganisms in Home and Indoor Work Environments*. Taylor and Francis, New York 2001.

28. Frączek K, Grzyb J, Górny RL, Wlazło A: Biological aerosol in the premises of Szczawnica health resort. *Ecol Tech* 2008, **4**, 150–154 (in Polish).

29. Garavello W, Romagnoli M, Sordo L, Gaini RM, Berardino C, Angrisano A: Hypersaline nasal irrigation in children with symptomatic seasonal allergic rhinitis: A randomized study. *Pediatr Allergy Immunol* 2003, **14**, 140–143.

30. Gołofit-Szymczak M, Górny RL: Bacterial and fungal aerosols in air-conditioned office buildings in Warsaw, Poland – preliminary results (winter season). *Int J Occup Saf Ergon* 2010, **16**, 407–418.

31. Górny RL: Aerozole biologiczne – rola normatywów higienicznych w ochronie środowiska i zdrowia. *Med Środowiskowa* 2010, **13**, 41–51.

32. Górny RL, Dutkiewicz J: Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. *Ann Agric Environ Med* 2002, **9**, 17–23.

33. Górny RL, Mainelis G, Grinshpun SA, Willeke K, Dutkiewicz J, Reponen T: Release of *Streptomyces albus* propagules from contaminated surfaces. *Environ Res* 2003, **91**, 45–53.

34. Górny RL, Wlazło A, Krysińska-Traczyk E, Strzelczyk AB, Lis DO, Łudzeń-Izbińska B, Sokal JA: Microbial contamination of water-damaged storerooms at libraries. *Proc Indoor Air* 2005, **2**, 1464–1468.

35. Grinshpun SA, Reponen T, Willeke K: Aerosol characteristics of airborne actinomycetes and fungi. *J Aerosol Sci* 1997, **28**, 667–668.

36. Hedman J, Hugg T, Sandell J, HaahtelaT: The effect of salt chamber treatment on bronchial hyperresponsiveness in asthmatics. *Allergy* 2006, **61**, 605–610.

37. Helben J, Kolarzyk E: Natural environment advantages in pharmacological treatment stimulation. *Prob Hyg Epidemiol* 2005, **86**, 22–26.

38. Institute of Medicine: *Damp Indoor Spaces and Health*. The National Academies Press, Washington 2004.

39. Jo W-K, Seo YJ: Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes. *Chemosphere* 2005, **61**, 1570–1579.

40. Jones AM, Harrison RM: The effects of meteorological actors on atmospheric bioaerosol concentrations – a review. *Sci Total Environ* 2004, **326**, 151–180.

41. Kalogerakis N, Paschali D, Lekaditis V, Pantidou A, Eleftheriadis K, Lazaridis M: Indoor air quality – bioaerosol measurements in domestic and office premises. *J Aerosol Sci* 2005, **36**, 751–761.

42. Kleinheinz GT, Langolf BM, Englebert E: Characterization of airborne fungal levels after mold remediation. *Microbiol Res* 2006, **161**, 367–376.

43. Krikštaponis A: *Diversity of Fungi Species in Occupational and Residential Environments and Their Biological Peculiarities (Toxicity, Pathogenicity, Proteolytic, Lipolytic, and Cellulolytic Activity)*. Ph.D. thesis. Institute of Botany, Vilnius 2000.

44. Lee J-H, Jo W-K: Characteristics of indoor and outdoor bioaerosols at Korean apartment buildings. *Environ Res* 2006, **101**, 11–17.

45. Lee T, Grinshpun SA, Martuzevicius D, Adhikari A, Crawford CM, Reponen T: Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes. *Atmos Environ* 2006, **40**, 2902–2910.

46. Leung M, Chan AHS: Control and management of hospital indoor air quality. *Med Sci Monit* 2006, **12**, 17–23.

47. Lis DO, Pastuszka JS, Górny RL: The prevalence of bacterial and fungal aerosol in homes, offices and ambient air of Upper Silesia. Preliminary results. *Roczn PZH* 1997, **48**, 59–68 (in Polish).

48. Macher J (Ed.): *Bioaerosols: Assessment and Control*. American Conference of Governmental Industrial Hygienists, Cincinnati 1999.

49. Mancinelli RL, Shulls WA: Airborne bacteria in an urban environment. *Appl Environ Microbiol* 1978, **35**, 1095–1101.

50. Marcinowska K: *Characteristic, Occurrence and Importance of Actinomycetales in Nature. Microorganisms' Activity in Various Environments*. Academy of Agriculture, Kraków 2002 (in Polish).

51. Martins-Diniz JN, Da Silva RAM, Mendes-Gianini E: Monitoring of airborne species in a hospital unit. *Rev Saude Publica* 2005, **39**, 1–7.

52. McCorkle RG: The patient and the sanatorium. *Chest* 1935, **1**, 17–18.

53. Nourian AA, Badali H, Khodaverdi M, Hamzehei H, Mohseni S: Airborne mycoflora of Zanjan – Iran. *Int J Agric Biol* 2007, **9**, 628–630.

54. Nunes ZG, Martins AS, Altoe AL, Nishikawa MM, Leite MO, Aguiar PFL, Fracalanzza SE: Indoor air microbiological evaluation of offices, hospitals, industries, and shopping centers. *Mem Inst Oswaldo Cruz* 2005, **100**, 351–357.

55. O'Gorman CM, Fuller HT: Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. *Atmos Environ* 2008, **42**, 4355–4368.

56. Pastuszka JS, Paw U KT, Lis D, Wlazło A, Ulfig K: Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. *Atmos Environ* 2000, **34**, 3833–3842.

57. Prunk A, Azman J, Frkovic V, Skrobonja A, Muzur A: Physician Albin Eder and his contribution to the development of health resorts in the Northern Adriatic area. *Publ Health* 2008, **122**, 1131–1133.

58. Raper KB, Fennel DI: *The Genus Aspergillus*. Williams and Wilkins Co., Baltimore 1965.

59. Reponen TA, Gazenko SV, Grinshpun SA, Willeke K, Cole EC: Characteristics of airborne actinomycete spores. *Appl Environ Microbiol* 1998, **64**, 3807–3812.

60. Roberts K, Hathway A, Fletcher LA, Beggs CB, Elliott MW, Sleigh PA: Bioaerosol production on a respiratory ward. *Indoor Built Environ* 2006, **15**, 35–40.

61. Samson RA, Hoekstra ES, Frisvad JC: *Introduction to Food– and Airborne Fungi*. 7th Ed. Centraalbureau voor Schimmelcultures, Utrecht 2004.

62. Szczegielniak J, Migała M: Meaning of microclimate of caves in a health resort medical care. *Ann Univ Mariae Curie Sklodowska Med* 2003, **243**, 219–223 (in Polish).

63. Tsai FC, Macher JM, Hung YY: Biodiversity and concentrations of airborne fungi in large US office buildings from the BASE study. Atmos Environ 2007, 41, 5181–5191.

64. Wanner H, Verhoeff A, Colombi A, Flannigan B, Gravesen S, Mouilleseaux A, Nevalainen A, Papadakis J, Seidel K: *Indoor Air Quality and its Impact on Man: Report No. 12: Biological Particles in Indoor Environments*. ECSC-EEC-EAEC, Brussels-Luxembourg 1993.

65. Wlazło A, Górny RL, Złotowska R, Ławniczek A, Łudzień-Izbińska B, Harkawy AS, Anczyk E: Workers exposure to selected biological agents in libraries of Upper Silesia. *Med Pr* 2008, **59**, 159–170 (in Polish).

66. World Health Organization: *WHO Guidelines for Indoor Air Quality: Dampness and Mould*. WHO Regional Office for Europe, Copenhagen 2009.

67. Zhilina LP, Dobrodceeva LK: Features of the physical state of schoolchildren before and after a stay at a sanatorium. *Human Physiol* 2005, **31**, 142–144.