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ASSESSMENT OF AIRBORNE ACTINOMYCETES IN SUBTERRANEAN AND EARTH SANATORIUMS

WYSTĘPOWANIE PROMIENIOWCÓW W POMIESZCZENIU SANATORIÓW PODZIEMNYM ORAZ NAZIEMNYM

Abstract: Actinomycetes are common soil microorganisms; however, they have also been detected in air samples, becoming important air contaminants in occupational and residential habitats. Concentrations of airborne actinomycetes and their distribution among different fractions of bioaerosol were determined in selected sites of two sanatorium types, *ie* a subterranean located in the former salt-mine of Bochnia and standard earth spa of Szczawnica. The average concentrations of actinomycetes ranged from 0 to almost 40 cfu m⁻³, for the sanatorium of Bochnia, and from 7 to 125 cfu m⁻³, for Szczawnica spa, throughout the year. The respiratory fraction of the bioaerosol ranged from 25 to 51% and from 78 to 81% in the subterranean sites and rooms of Szczawnica spa, respectively. For both sanatoriums, among isolated actinomycetes dominated species belonging to *Rhodococcus*, *Streptomyces* and *Nocardia*. The results indicate that patients can be differentially exposed to airborne actinomycetes in compared sanatoriums. The air of underground spa reveals to be of better quality, regarding the bacteria characteristics.

Keywords: airborne actinomycetes, bioaerosol, indoor air, sanatorium

Introduction

Actinomycetes are Gram-positive bacteria widespread in the environment, which morphologically resemble fungi and are adapted to live on solid surfaces using different organic substrates. They produce and excrete several enzymes and acids that allow them to destroy or disintegrate such compounds as lignin, chitin, pectin, kolagen, starch, keratin and elastin [1]. In soil, these saprophytic bacteria, especially those belonging to the genus *Streptomyces*, decompose organic matter and produce antibiotics and other bioactive metabolites; hence, they are responsible for soil fertility and biocontrol of phytopathogens [2]. However, they have also been detected in air samples, becoming important air contaminants in occupational and residential habitats [3, 4]. Background concentration of actinomycetes measuring in the residential air is below 10² cfu m⁻³ air; however, the concentration may be even several hundred times higher in some sites (*eg* nearby

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a composting site) [5-7]. Grigorevski-Lima et al [8] detected actinomycetes in 35 out of 53 collecting points from nine different indoor environments. Although these bacteria were present in many sampling point, their frequency was very low compared with fungal and total bacterial counts, representing only 1.5% of these microorganisms. Physical and chemical characteristics of building materials, especially moisture content in the substrate and the presence of nutritional substances significantly affect colonisation abilities of actinomycetes and their development in the indoor air. Although these bacteria do not belong to the normal microorganisms of indoor air, they have been found in different buildings with mould problems due to increased moisture [9, 10]. The increased insulation of buildings combined with poor ventilation is related to the occurrence of building-related diseases commonly known as sick building syndrome [11, 12]. In these sites, actinomycetes are a relatively numerous group of bacteria isolated from the indoor air [13, 14]. In consequence, they can induce acute lung diseases and allergic reactions among inhabitants exposed to the air, especially those that are immunocompromised or otherwise sensitive to a broad range of allergenic and toxigenic biological material [15-20]. Increased concentrations of airborne spores of some actinomycetes' species (eg *Micropolyspora faeni* and *Streptomyces albus*) have been associated with the incidence of allergic alveolitis and other severe health problems [21, 22].

Bioaerosol is a collection airborne biological particles of microbial, plant or animal origin characterised by different sizes ranging from 0.5 to 30 μm in diameter, which can be transported with air from one location to another [23]. The concentration of this air fraction shows seasonal variability according to changes in the meteorological conditions [24-28]. The ability to survive in air for biological particles is depended on its size, resulting in smaller particles being more susceptible to environmental damage [29]. Nevertheless, under favourable conditions they can grow and propagate on a variety of building materials and indoor surfaces, leading to the pollution of indoor air [30]. The interest in bioaerosol exposure has increased over the last few decades. This is largely because several adverse health effects are associated with the exposition to the bioaerosol in both the occupational and residential indoor sites. However, the exposure assessment offers distinct challenges from those for inorganic aerosols and chemical agents. Pathogenic microorganisms can be hazardous at low concentrations while other saprophytic organisms may induce health problems at several orders higher concentrations. The composition and concentration of different microorganisms present in the bioaerosol highly depends on the source type (ie water, soil, plants and animals). For example, bioaerosols generated from water contain a thin layer of moisture surrounding the microorganisms and often consist of several organisms that form aggregates [31]. Human exposure for these bioaerosols is mostly by inhalation, ingestion and dermal contact; however, inhalation is the predominant route resulting in adverse health effects. The risk of exposure is associated with the finding that the average human inhales approximately 10 m^3 of air per day [32]. Large airborne particles are deposited in nose and nasopharynx, and those lower than 6 μm in diameter are transported to the lung [33, 34]. Actinomycetes spores as components of bioaerosols, lower than 2.5 μm in aerodynamic diameter, are deposited in the upper respiratory system of an occupant exposed to inhalation of contaminated air. The spores of actinomycetes isolated from mouldy buildings are cytotoxic and able to stimulate macrophages to produce proinflammatory cytokines; they are even more active than fungi [22, 35, 36]. Compared

with other microbes, much less is known about actinomycetes as causative agents of adverse health outcomes observed in indoor habitat [3].

Sanatoriums are sites where requirements for clean indoor air, regarding chemical and microbiological regimes, should be especially rigorous because of treatment effect in relation to patients. This is especially important for those sanatoriums that are focused on the treatment of patients with different upper respiratory system diseases. Additional exposition to airborne microorganisms may sharpen disease symptoms resulting in the lack of medicated effect of spa therapy. However, even these sites cannot be considered as free from actinomycetes. For example, Green et al [37] reported that actinomycetes accounted from 0.3 to 5.5% of all microbes detected in different indoor places of a hospital. Moreover, indoor air of a hospital was colonised by population of actinomycetes that ranged from 0 to 11.7 cfu m⁻³ air that corresponded to 1.5% of total microbial colonies counted. In addition, 88% of these bacteria, mostly represented by potential pathogenic strains of *Streptomyces*, were found in the three final stages of the Andersen sampler that simulate the human respiratory tract [8]. The major source of airborne bacteria is the presence of humans whose activities like talking, coughing, sneezing, walking and washing can release different biological particles [38]. In turn, there have been found no direct relationships among personnel numbers, floor cleaning frequency and bioaerosol concentrations [39, 40]. Some studies have indicated that indoor concentration of bioaerosol may be driven by infiltration of outdoor aerosol [41, 42]. However, the mechanisms responsible for the establishment of outdoor/indoor ratio for bioaerosol concentrations are not well understood. Admittedly, relative humidity has the most pronounced effect on the bioaerosol concentrations, indicating high correlation coefficients for an indoor air [30].

In contrast to buildings, subterranean habitats such as mine excavations and caves are extreme environments for microorganisms that can only use traces of nutrients present in air or rock surfaces. To adapt to these extremely starved habitats, microorganisms use specific scavenging mechanisms to pull scarce nutrients into the cell [43]. However, even these oligotrophic conditions support the growth of relatively high numbers of bacteria (*ie* up to 10⁶ cfu g⁻¹ rock material) mostly represented by Gram-positive cocci, rods and pleomorphic shaped cells. They are also released into an air forming bioaerosol containing 68 to 113 cfu m⁻³ of a cave air [44]. Molecular tools have reported that the major group of bacteria in a subterranean habitat is represented by *Proteobacteria*; however, the majority of isolates are strains of *Actinobacteria* [45]. Several studies have indicated that actinobacteria are abundant in caves, suggesting that this habitat is especially favourable for this bacterial group [46, 47]. Some isolates of these bacteria were found as producers of new bioactive substances [48]. Herold et al [49] reported that *Streptomyces tendae* isolated from a cave in Porto Badisco, Italy, produced cervimicin A-D, polyketide glycosides active against multi-drug resistant staphylococci and vancomycin-resistant enterococci. Specific microclimate features of these environments are suitable for subterranean therapy of patients with pulmonary problems. However, microbial air quality in sanatoriums located in hypogeous habitats, *eg*, former mines, is far from being recognised. There have been reported data on the presence of different pathogenic actinobacteria in different subterranean sites. Species of the genus *Gordonia* (*G. bronchialis*, *G. otitidis*, *G. terrae*) are described as opportunistic pathogens responsible for bacteraemias and bronchopulmonary diseases [50, 51]. The isolates of *Gardonia* were found in a cave in Italy [52]. Also, strains of opportunistic and pathogenic bacteria of *Brevibacterium*, *Micrococcus* and *Nocardia*

have been found in different caves [52]. Jurado et al [53] have recently reported of a new genus and species of actinobacterium, *Hyosella altamirensis*, in a Spain cave. This species phylogenetically group with the *Mycobacterium* genus that holds known opportunistic and pathogenic species. Admittedly, further studies are needed to learn whether hypogean strains of bacteria can be hazardous for human.

The aim of this study was to compare concentrations of airborne actinomycetes, and their distribution among different fractions of bioaerosol, in selected sites of a subterranean salt-mine sanatorium with those sampled in an earth spa. Considering specific characteristics of the hypogean environment, we assumed the initial hypotheses of low microbial abundance in the salt-mine air. In contrast, the earth spa patients should be exposed to higher concentrations of actinomycetes that are ubiquity in terrestrial environment.

Material and methods

Air sampling was conducted in two sites, the underground sanatorium of Bochnia salt-mine and the sanatorium building in Szczawnica. The health-resort of Bochnia is located approximately 32 km east of Krakow in Malopolska. The salt-mine of Bochnia is the oldest plant exploring salt layers in Poland and Europe. The mineshaft Sutoris has functioned since 1241, and some unique excavations have been applied for medicinal purposes since 1991. However, construction of underground spa based on a network of excavations in the mine area of Wazyn (*ie* the largest underground man-made space in Europe) was already begun in 1984. In this spa, both temperature and relative air humidity are stable, *ie* from 11 to 16°C and 70%, respectively. In addition, the unique microclimate characterizes by high concentrations of microelements (*ie* sodium, calcium and magnesium), bacteriological purity and lack of allergens and different contaminants that are common in the ground air. Consequently, subterranean therapy holidays based on inhalations, and physical movements have been performed for approximately 50 patients living in double or several person rooms located in two chambers of Wazyn and Koldras.

The health resort of Szczawnica, one of the oldest and most beautiful spas in Poland, is located in Grajcarek river valley within Pieniny mountains approximately 120 km southeast of Krakow. Mild, submountain climate with high insolation, low rainfalls and clean air is characteristic of this sheltered place. Several balneological springs have been exploited for rehabilitation purposes and treatment of upper respiratory system diseases since 1839.

Bioaerosol samples were collected twice in each season of 2008 from selected six stands both within underground spaces of the Bochnia salt-mine sanatorium (*ie* inner background, ventilating gallery - B1, chamber of Koldras - B2, ramp - B3, chamber of Wazyn - B4, sleeping chamber - B5, and corridor - B6) and rooms of Szczawnica spa (*ie* inner background, major hall - S1, mineral bath - S2, whirlpool massage - S3, pump room - S4, cell inhalation - S5, and individual inhalation - S6). In addition, the samples were collected outside the spa spaces to determine outdoor background levels of actinomycetes in Bochnia (B0) and Szczawnica (S0). The six-stage Andersen's impactor (model 10-710) was used for air sampling at a height of 1.0-1.5 m above the floor during five minute times. Airborne actinomycetes were collected from the volume of 0.1415 m⁻³ on a Gauze medium (agar 30 g, soluble starch 20 g, KNO₃ 1 g, K₂HPO₄ 0.5 g, MgSO₄ 0.5 g, NaCl 0.5 g, FeSO₄ traces) recommended by Atlas and Parks [54] in Petri-dishes located on

all impactor stages. In this way, concentrations of actinomycetes in particular bioaerosol fractions (*ie* > 7.0, 7.0-4.7, 4.7-3.3, 3.3-2.1, 2.1-1.1 and 1.1-0.65 μm) were assessed. The plates were incubated at 28°C for seven days followed by enumeration of actinomycetes cfu numbers per cubic metre of air. Growing colonies were used for identification according to the classical method based on morphological, physiological and biochemical characteristics [55]. Furthermore, temperature and *relative humidity* (RH) of air were simultaneously determined with a thermohygrometer (06917, Termometerfabriken Viking AB, Sweden).

Results and discussion

Health resorts are the sites where both atmospheric and indoor air should be free from industrial contaminants, and the level of microorganisms has to be low. Admittedly, localization of these resorts in environmentally clean areas with the specific microclimates allows to meet the requirements that are important for a proper course of medicinal and rehabilitation processes of respective patients. The patients with respiratory symptoms and lung function impairment are especially sensitive to microbial contamination of air. Concentrations of different microorganisms in the air of an underground and terrestrial sanatorium may significantly differ, and this is the first study that presents such results.

Fig. 1. Average concentrations of airborne actinomycetes in different sites of the subterranean sanatorium in Bochnia determined throughout the year

For the sanatorium of Bochnia salt-mine, average concentration of airborne actinomycetes ranged from 0 to almost 40 cfu m⁻³ in the underground sites throughout the year (Fig. 1). The highest cfu numbers were found in the air of court (B4) at the spring and winter sampling times. However, actinomycetes were not detected in the inner background (B1) and corridor (B6) behind the court in spring. In contrast, several times higher numbers of actinomycetes were observed in the outdoor background air (B0) in spring and summer. In the subterranean spa chambers, the typical concentration of airborne actinomycetes was approximately 20 cfu m⁻³, whereas over a three-fold number was ascertained in the outdoor air of the sanatorium. For these chambers, the average cfu numbers of actinomycetes were

the highest in summer when the sanatorium was not inhabited by patients. However, the cfu numbers increased in the air sampled from the inhabited chambers in winter (Fig. 2). The air of excavations containing high concentrations of sodium salt may have reduced survival of actinomycetes that could be introduced mostly by patients and organic debris. However, air transport through the ventilation system could introduce some amounts of actinomycetes into the subterranean spa chambers in summer.

Fig. 2. Average concentrations of airborne actinomycetes in different sites of an earth sanatorium in Szczawnica determined throughout the year

Table 1

Changes in temperature (T) and relative humidity (RH) of air in different sites of two sanatoriums throughout the year

Site	Spring		Summer		Autumn		Winter	
	T [°C]	RH	T [°C]	RH	T [°C]	RH	T [°C]	RH
B0 ^a	21±1.4	56.5±21.9	23.3±3.9	63.5±14.8	12.5±0.7	77.5±3.5	3.0±2.8	55.5±21.9
B1	15.5±0.7	64.5±6.4	18.0±1.4	68±2.8	16	64±1.4	13±1.4	44.5±9.2
B2	14.5±0.7	63.5±9.2	18±1.4	68.5±0.7	16.5±0.7	56±1.4	14.5±0.7	35.5±4.9
B3	16±1.4	60.5±2.1	18	72±1.4	16.5±0.7	54±8.5	14±1.4	37.5±7.8
B4	17±1.4	59±7.1	17.5±0.7	70.5±0.7	17.5±0.7	57±1.4	14.5±0.7	40±11.5
B5	18±1.4	59.5±9.2	19	69.5±0.7	17.5±0.7	56.5±2.1	17.5±0.7	32±9.9
S0 ^b	15±2.8	38.5±3.5	24±4.2	59.5±12	10±4.2	52±7.1	-1.5±2.1	70.5±9.2
S1	22.5±0.7	36.5±2.1	24.5±2.1	59.5±12	22±1.4	41±2.8	20±1.4	35.5±2.1
S2	16.5±2.1	49.5±3.5	21.5±0.7	66±19.8	20.5±2.1	44	18±1.4	50.5±4.9
S3	17±1.4	46.5±6.4	21.5±0.7	67.5±23.3	21	51.5±9.2	21	44±2.8
S4	20±1.4	37.5±0.7	25±1.4	47.5±21.9	19.5±2.1	41±5.7	18.5±0.7	36.6±0.7
S5	21.5±0.7	32.5±0.7	24±1.4	47.5±21.9	23±1.4	35.5±0.7	21.5±0.7	33.5±0.7

^a Different sites of subterranean sanatorium in Bochnia. B0 and B1 - outdoor and inner background, respectively. B2 to B5 - chambers of the salt-mine adapted for spa purposes

^b Different sites of earth sanatorium in Szczawnica. S0 and S1 - outdoor and inner background, respectively. S2 to S5 - therapy rooms of the spa

Growth of actinomycetes in hypogean environments seems to be dependent on microclimate features and presence of some organic sources [47]. However, temperature

was reported to be a not limiting factor for the growth of these bacteria in these habitats [56]. Generally, microclimate features (*ie* temperature and relative humidity) did not differ significantly throughout the year in B1 and underground sites of Bochnia spa. In contrast, lower values of air humidity were determined in winter (Table 1). Regarding the respiratory fraction of the bioaerosol, its average contribution was approximately 51 and 25% in the air of the subterranean spa chambers and B1, respectively (Table 2). However, 66% of actinomycetes was found in this fraction for the outdoor air. Considering the season effect, increased concentrations of the bacteria were found for this air in summer and winter, and they were distinctly higher compared with those of B1 throughout the year. For the spa chambers, the respiratory fraction contributions were also higher than that of B1, and the fraction concentration was even higher compared with that of B0 in winter. The obtained results clearly indicate that the respiratory fraction in different sites of the subterranean spa rather does not increase a hazard for patients exposed to this air.

Table 2

Respiratory fraction concentrations [%] of actinomycetes in different sites of two sanatoriums determined throughout the year

Sanatorium	Site	Spring	Summer	Autumn	Winter	Average
Bochnia	Outdoor background	50	90	50	75	66
	Inner background	0	25	25	50	25
	Spa chambers	38	69	45	93	51
Szczawnica	Outdoor background	75	67	50	0	48
	Inner background	84	92	100	48	81
	Therapy rooms	100	97	50	66	78

^a Air fraction containing bacterial particles below 4.7 μm in diameter

Indoor air of different public buildings, including hospitals, is inhabited by several times higher concentrations of microorganisms compared with subterranean caves. Li and Hou [57] found that bacterial concentrations in hospital rooms ranged from 1 to 423 cfu m^{-3} . In non-industrial indoor environments, bacterial numbers obtained with the Andersen six-stage sampler at the level < 100 cfu m^{-3} have been classified to the low category [58]. For the sanatorium of Szczawnica, temperature ranged from 16.5 to 25°C in different rooms throughout the year, whereas decreased values of relative humidity were observed in the inhalation rooms. Under these conditions, average concentration of airborne actinomycetes ranged from 7 to 125 cfu m^{-3} in the inner rooms throughout the year (Fig. 2). In winter, the highest numbers of actinomycetes were found in the inner background air (S1) and the room of individual inhalation (S6). For S1 in spring, the cfu number was also higher compared with those of the therapy rooms. However, comparable numbers of actinomycetes were observed between S1 and the therapy rooms in summer, whereas the cfu numbers increased in the air of mineral bath room (S2) and cell inhalation room (S5) in autumn. In summer, the humidity in the spa rooms was higher compared with other seasons, which was presumably associated with the lack of heating. In the outer background air (S0), actinomycetes numbers were similar to those of S1 in summer and autumn while they were either not detected in the air in winter, or they were lower in spring (Fig. 2). These results indicate that concentrations of actinomycetes in the indoor air of spa could be associated with the presence of patients, heating of rooms, open or limited air exchange between the spa rooms and type of operation room (*ie* size and clean level).

Regarding the respiratory fraction of the bioaerosol, its average contribution was approximately 78 and 81% in the air of the therapy rooms and S1, respectively (Table 2). In contrast, only 48% of actinomycetes was found in this fraction for S0 throughout the year. Comparing concentrations of the respiratory fraction between seasons, spring and summer favoured distribution of actinomycetes to the fraction compared with autumn and winter, especially for the therapy rooms and S0. These results may have been associated with higher RH values due to cessation of heating in spring. Admittedly, actinomycetes better survive and even multiply in rooms with increased humidity [3]. Moreover, the respiratory fraction of the bioaerosol accounted for the higher percentage in the indoor air of Szczawnica spa compared with that of underground chambers. Hence, patients referred to a subterranean sanatorium for therapy may be exposed to a lower hazard related to inhalation of actinomycetes.

For the sanatorium of Bochnia salt-mine, among isolated actinomycetes dominated species belonging to *Rhodococcus*, *Streptomyces* and *Nocardia*, which accounted from 1 to 4% of all bacterial species identified. These actinomycetes have been reported as typical inhabitants of hypogeous environments and some species of these genera have been catalogued as pathogenic [52]. In contrast, species representing the same genera were isolated from the air of Szczawnica sanatorium but their contribution to the total bacterial isolates ranged from 4 to 31%. Species of *Rhodococcus* were generally found in the outdoor background air (S0, 13%) and S5 (13%), whereas *Nocardia* accounted for 7% of all isolates in S1 air. However, the most frequently identified actinomycetes were *Streptomyces* that accounted from 16 to 29% of all bacterial isolates in the air of S6. In addition, Grigorevski-Lima et al [8] found six different species of *Streptomyces* as prevalent inhabitants of variety indoor environments, including hospitals. Among these actinomycetes and *Nocardia*, which are commonly detected on house dust, garden soils and pools, some strains pathogenic to humans have been reported [59, 60].

In conclusion, the study indicates that significantly lower concentrations of actinomycetes seem to be characteristic of indoor air of the subterranean sanatorium compared with those found in different rooms of building spa. However, these concentrations can fluctuate depending on the season (*eg* changes in temperature and RH), transport of spores and mycelium fragments by air currents or activity of patients. Admittedly, the presence of major part of actinomycetes in the respiratory fraction of bioaerosol may be important for assessing the exposition of patients to potentially harmful strains. This result should also be taken into account for subterranean therapy of patients with upper respiratory tract disorders. In addition, microbial air quality in different rooms and chambers of sanatoriums is related to efficient ventilation. Therefore, we recommend to introduce a system of efficient ventilation in therapy rooms and spa chambers in order to provide special air quality in sites where patients showing health problems are stayed.

Conclusions

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WYSTĘPOWANIE PROMIENIOWCÓW W POMIESZCZENIU SANATORIÓW PODZIEMNYM ORAZ NAZIEMNYM

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Abstrakt: Promieniowce są pospolitymi mikroorganizmami glebowymi, które wykrywa się także w próbkach powietrza, gdzie stanowią istotne zanieczyszczenie środowiska pracowniczego oraz mieszkaniowego. W wybranych miejscach dwóch typów sanatoriów, podziemnego w byłej kopalni soli w Bochni oraz naziemnego w Szczawnicy, określano stężenie przenoszonych drogą powietrzną promieniowców oraz ich rozmieszczenie w różnych frakcjach bioaerozolu. Przeciętne stężenia promieniowców w ciągu roku w przypadku sanatorium w Bochni mieściły się w zakresie od 0 do 40 jtk m⁻³, a przypadku sanatorium w Szczawnicy między 7 a 125 jtk m⁻³. W pomieszczeniach podziemnego sanatorium w Bochni stężenie frakcji respirabilnej bioaerozolu wahało się między 25 a 51%, a w przypadku sanatorium w Szczawnicy wartość ta mieściła się między 78 a 81%. W oby typach sanatoriów wśród izolowanych promieniowców dominowały gatunki należące do *Rhodococcus*, *Streptomyces* oraz *Nocardia*. Otrzymane wyniki wykazują, że w porównywanych sanatoriach pacjenci mogą być w różnym stopniu narażeni na unoszące się w powietrzu promieniowce. Powietrze w podziemnym sanatorium okazało się być lepszej jakości pod względem przeprowadzonej charakterystyki mikrobiologicznej.

Słowa kluczowe: promieniowce występujące w powietrzu, bioaerozol, powietrze pomieszczeń, sanatorium