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Original Research

Microbiological Quality of Indoor Air in University Rooms

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Abstract

A study on indoor air microbiological contamination in various rooms of university buildings in Poznań, Poland, is presented. Investigations were conducted in the period September-October 2002 and the same period in 2003. Air samples were taken twice a day: in the morning and in the afternoon. In all of the tested places a multiple growth of bacteria and significant increase of mould spores was observed in afternoons. The predominant bacteria and moulds isolated from investigated air samples were**:** *Staphylococcus* spp., *Micrococcus* spp., *Serratia* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Cladosporium* spp. and *Alternaria* spp. Among these microbes the presence of pathogenic and strongly allergenic microorganisms was detected.

Keywords: indoor air, microbiological quality, airborne bacteria, airborne fungi, biomass

Introduction

Most people spend over 90% of their lives indoors: in houses, offices, and schools [1], where they are exposed to some indoor environmental factors (bioaerosol) that influence their health and physical condition. Therefore there has been a growing interest in indoor microbe studies in recent years [1-14]. The aim of those studies is not only estimation of the airborne microorganisms but also their identification and the determination of factors influencing bioaerosol composition inside the rooms.

Biological contamination of indoor air is mostly caused by bacteria, moulds and yeast. They can be dangerous as pathogenic living cells but they can also secrete some substances harmful for health. These are different kinds of toxic metabolism products, for example mycotoxins [4, 11, 15,]. Epidemiological studies show that too high concentration of microorganisms in the air can be allergenic; however, sometimes even very low concentrations of some particular microorganisms can cause serious diseases. It is supposed that about 30% of health problems relevant to the indoor air quality is the result of a human organisms reaction to moulds [10]. Fungal flora can be hazardous for health, particularly in rooms with heating, ventilation and air-conditioning (HVAC) systems [10, 11, 16-18] and can breed allergies [4, 5, 16-19], SBS symptoms ("sick building syndrome") causing irritation of mucous membranes, bad physical condition, tiredness, headaches, vertigo, decrease of concentration, memory and intellectual work ability [10, 19, 20], dermatosis, respiratory diseases (including asthma) [4, 8, 10, 12, 16, 17] and cancers [4, 5, 9, 10, 12-14, 21]. The amount of pathogenic microorganisms is higher in indoor than outdoor air [1, 11, 22, 23].

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So far there have been no Polish standards or guidelines for microbiological quality of indoor air. Furthermore, there isn't any European Union directive addressing this; therefore, it is assumed to be based on particular European countries' requirements and scientific propositions [24]. According to current Swedish requirements the number of 500 colony-forming units (cfu) of bacteria and 300 cfu of fungal spores in 1 m^3 can be accepted in an indoor environment [25]. Especially air contamination caused by fungi is taken into consideration because of their extremely dangerous influence on human health. However, it can be noticed that during the last 20 years opinions concerning innocuous fungal spore amounts in the indoor air of various kinds of rooms have varied [26]. According to Berk et al., in 1979 exposure of 20 cfu/m^3 to over 700 cfu/m^3 has no harmful effect [27]. Conclusions shown in The Netherlands Research Methods in Biological Indoor Air Pollution (1989) describe the amount of fungi over 10^4 cfu/m^3 or the amount of particular species of mould over 500 cfu/m^3 as dangerous for health. In 2001 the American Industrial Hygiene Association (AIHA) published a proposition of guidelines for the amount of fungal spores in different indoor environments, for example residential and commercial buildings. Guideline for residential buildings are less than 500 cfu/m^3 and for commercial buildings are less than 250 cfu/m^3 [26]. Other countries' requirements are similar. In Brazil total amount of airborne microorganisms (especially fungi) in enclosed space shouldn't exceed 750 cfu/m^3 [28]. In Hong Kong good microbiological class air should include less than 1000 cfu/m^3 of bacteria. If it includes less than 500 cfu/m^3 – air is classified as excellent [29]. In Singapore requirements for indoor air quality strictly describe concentration of bacteria on the maximum level of 500 cfu/m^3 [23].

The aim of this work is long-term observation of microbiological quality of indoor air in selected rooms of university buildings located in the centre of Poznań, where thousands of people spend several hours studying and working in enclosed spaces every day and where microbiological quality of indoor air can influence their health and physical condition. This publication presents preliminary results of an indoor air study conducted in 2002 and in 2003. The study embraced a measurement of the concentration of bacteria and fungi in the air of selected rooms and microbial composition of the air.

Materials and Methods

Microbiological quality of indoor air was investigated in select university rooms specified in Table 1.

Total number of mesophilic aerobic bacteria, yeast and moulds in the air of selected rooms was determined using Koch sedimentation method according to Polish Standard PN 89/Z-04008/08 [30]. Air microorganisms were settled gravitationally directly on the Petri plates filled with nutrient media and exposed in sampling points for a period of time. The number of microorganisms expressed as CFU/m^3 was estimated according to the equation [30]:

$$CFU/m^3 = a \cdot 10000/p \cdot t \cdot 0.2$$

where:
a – the number of colonies on the Petri plate
p – the surface of the Petri plate
t – the time of Petri plate exposure

Table 1. Characteristic of sampling rooms.

Investigated rooms	Mark *	Characteristic of rooms			Number of Petri dishes from one point	Number of Petri dishes in one sampling	Number of total Petri dishes
		Area [m^2]	Cubature [m^3]	Number of persons			
Lecture room[1]	A	253	1391.5	230	24 (x 2)	10	480
Lecture room[2]	B	104.7	418.8	120	24 (x 2)	6	288
Chemical laboratory	C	68.6	184.5	18	24 (x 2)	3	144
Library	D	362.8	870.7		24 (x 2)	5	240
Reading room	E	2064	9690		24 (x 2)	10	480
Dean's office	F	32.1	94.4	4	24 (x 2)	3	144
Canteen	G	229.1	673.6	300	24 (x 2)	15	720
Toilets	H	11	39.6		24 (x 2)	3	144
Corridor	I	115.5	339.6		24 (x 2)	4	192
Total					**48**	**59**	**2832**

* Symbol used in figures 1 and 2

[1] Lecture room with ventilation system

[2] Lecture room without ventilation system

Results obtained by Koch sedimentation method are less accurate then those from impaction methods with the use of an air sampler. However, the sedimentation method is still quite popular in Poland and some other countries [6, 12, 14, 31-33]. The method does not require expensive instrumentation, it is cheap and simple and it is recommended by Polish Standards. Sedimentation method does not permit exact quantitative determination, some earlier observations reported that results of sedimentation method are usually higher than numbers obtained with the use of air samplers [14, 31]. However, data collected by sedimentation method allow the drawing of correct conclusions on types of microorganisms present in the air and can give a rough approximation of bacterial and fungal concentration.

For the determination of microorganisms in the air of investigated rooms Petri dishes were exposed for 15 minutes. There were 12 series examinations made. All samples were taken in September (before the beginning of the academic year) and October (during academic year) in two running years – 2002 and 2003. There were two periods in a day of taking samples. The first one in the morning from 7^{30} a.m. to 8 a.m. (before the beginning of lectures) and the second one in the afternoon from 2^{30} p.m. to 3 p.m. (before the end of lectures). The total number of samples was 2,832.

The following microbiological media were used:
- Yeast Extract Agar (Merck) to determine the total number of bacteria;
- Sabouraud Agar medium with 2% of glucose (Merck) for quantification of fungi;
- Czapek-Doxa Agar (BTL) for filamentous fungi identification.

Petri dishes were incubated for 24–48 hours at 37°C (to determine the total number of bacteria) and for 10 days at 25°C (to enumerate fungi). Results were shown by colony forming units in 1 m^3 of air (cfu/m^3).

Bacteria were identified by three arrays. The first one – macroscopic estimation (description of colony). The second one – microscopic estimation (dyeing by Gram and Schaeffer-Fulton method). The third one – biochemical tests according to bacteria classification according to Bergey [34].

Diagnosis of filamentous fungi was based on estimation of morphological features of growth on Czapek me-

Table 2. Microbiological air contamination inside university rooms.

Investigated rooms	Time of taking samples	Total number of bacteria [cfu/m^3]		Number of filamentous fungi [cfu/m^3]	
		2002	2003	2002	2003
Lecture room[1]	morning	3.9×10^2	6.3×10^2	5.2×10^2	3.3×10^2
	afternoon	5.2×10^2	8.8×10^2	2.6×10^2	2.6×10^2
Lecture room[2]	morning	1.3×10^2	3.6×10^2	2.6×10^2	2.3×10^2
	afternoon	3.2×10^2	8.8×10^2	5.2×10^2	2.6×10^2
Chemical laboratory	morning	1.2×10^2	1.1×10^2	1.6×10^2	0.9×10^2
	afternoon	6.5×10^2	3.8×10^2	3.9×10^2	5.2×10^2
Library	morning	1.3×10^2	3.6×10^2	2.6×10^2	1.3×10^2
	afternoon	2.6×10^2	2.6×10^3	2.6×10^2	3.0×10^2
Reading room	morning	1.3×10^2	8.7×10^2	1.3×10^2	1.1×10^2
	afternoon	1.3×10^2	1.7×10^3	1.1×10^3	8.0×10^2
Dean's office	morning	1.3×10^2	7.8×10^2	2.6×10^2	2.0×10^2
	afternoon	1.3×10^2	9.1×10^2	3.9×10^2	4.0×10^2
Canteen	morning	2.6×10^2	6.6×10^2	1.3×10^2	1.3×10^2
	afternoon	1.0×10^3	1.5×10^3	2.6×10^2	2.3×10^2
Toilets	morning	1.4×10^3	1.4×10^3	2.6×10^2	2.6×10^2
	afternoon	2.3×10^3	3.3×10^3	7.8×10^2	5.6×10^2
Corridor	morning	2.6×10^2	1.1×10^3	1.0×10^3	2.0×10^2
	afternoon	1.3×10^3	1.7×10^3	1.0×10^3	2.0×10^2

[1] Lecture room with ventilation system

[2] Lecture room without ventilation system

dium as well as on microscopic observation according to filamentous fungi estimation guide [35].

Results

Variation of Microorganism Concentrations in the Air of University Rooms

The average level of microbiological air contamination inside investigated university rooms is shown in Table 2. The number of microorganisms (bacteria and fungi) in indoor air varied widely in the whole research period. The total number of mesophilic aerobic bacteria in 2002 ranged from 120 to 2300 cfu/m^3, while the total number of fungi ranged from 130 to 1100 cfu/m^3 and in 2003 it varied from 110 to 3300 cfu/m^3 and from 90 to 800 cfu/m^3, respectively.

Microorganism concentrations in the air varies not only in the course of a season but also throughout the day.

Average number of bacteria and fungi present in indoor air of different rooms in mornings and afternoons during both years of studies were compared in Figs. 1 and 2. In 2002 a concentration of bacteria in investigated lecture rooms was higher than fungal concentration, whereas in 2003 a fungal domination was observed. In mornings bacterial and fungal air contamination was always lower than in afternoons. There was one exception – a ventilated lecture room. Results of studies in this room showed lower amounts of moulds in 1 m^3 of air in afternoons than in mornings (about 50% less in 2002 and 22% less in 2003). Very intensive bacterial growth in afternoons was observed in the corridor (in 2002 bacterial concentration in afternoons was 5 times higher than in mornings), in the canteen (in 2002 about 4 times higher in afternoons than in mornings), in the library reading room (in 2003 about 7 times higher in afternoons than in mornings) and in the chemical laboratory (in 2002 almost 5.5 and in 2003 – 3.5 times higher in afternoons). The rest of air samples presented lower bacterial growth during the day, i.e. 1.3 to 2.5 times higher in afternoons than in mornings. The highest growth of fungal contamination during afternoons was observed in the chemical laboratory (in 2003 – 5.8 times higher in afternoons than in mornings) and in the library reading room (in 2002 – 8.5 and in 2003 – 7.3 times

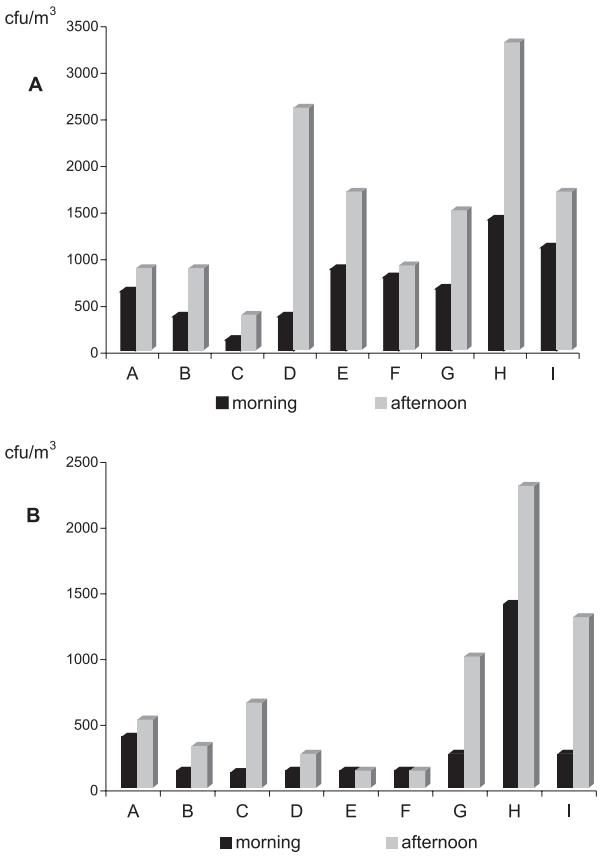

Fig. 1. Concentration of bacteria in air inside selected university rooms in 2002 (A) and in 2003 (B): A – ventilated lecture room, B – non-ventilated lecture room, C – chemical laboratory, D – library, E – reading room, F – Dean's office, G – canteen, H – toilets, I – corridor

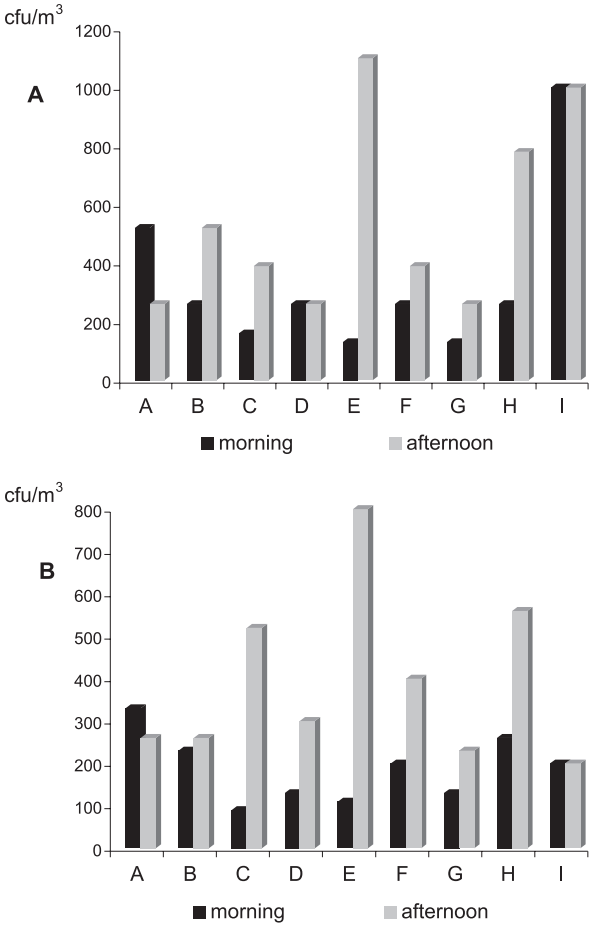

Fig. 2. Concentration of filamentous fungi in air inside selected university rooms in 2002 (A) and in 2003 (B): A – ventilated lecture room, B – non-ventilated lecture room, C – chemical laboratory, D – library, E – reading room, F – Dean's office, G – canteen, H – toilets, I – corridor

higher in afternoons than in mornings). In the remaining air samples the level of fungal growth was lower, i.e. up to 3 times higher than in mornings.

Variations in concentration of bacteria and fungi in the air of selected rooms in both years of studies were shown in Figs. 3 and 4. In 2003 a distinct increase of both bacteria and fungi concentration in the lecture room was observed on the date of the opening academic year (Fig. 3). Right after this time the microbial concentration was lowered. One year earlier, variations were not so deep. Dramatic but transient increase of bacteria at the beginning of the academic year was observed in both investigated years in toilets (Fig. 4), whereas the fungal concentration was stable and kept at a much lower level.

Simultaneous outdoor air studies [36] in the vicinity of university buildings made it possible to estimate the I/O ratio (the indoor/outdoor concentration ratio) for investigated university rooms. The I/O ratios for both bacteria and fungi in most investigated rooms were in the range of 0.1–0.7 and they were always lower in the mornings than in the afternoons. Though there was an exception – a ventilated lecture hall where I/O ratios observed in the afternoons (0.4 in 2002 and 0.3 in 2003) were lower than in the mornings (0.8 in 2002 and 0.4 in 2003). Extremely high values of I/O ratios in afternoons reaching 1.3–3.5 were found in 2003 in the following rooms: librarian lending room and reading room, canteen, corridor and toilets.

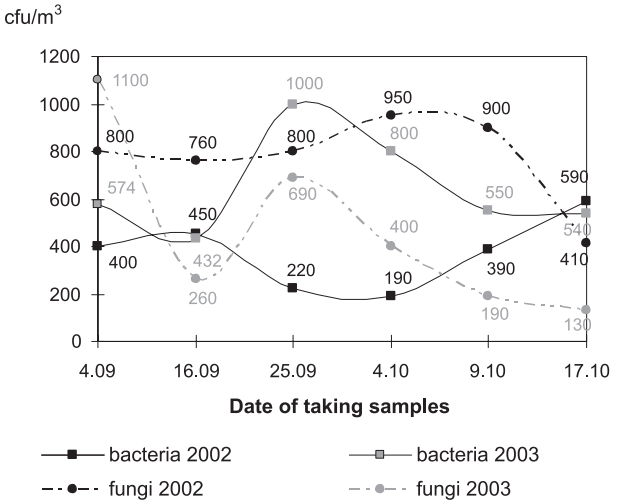

Fig.3. Variations in microbial concentrations in ventilated lecture room.

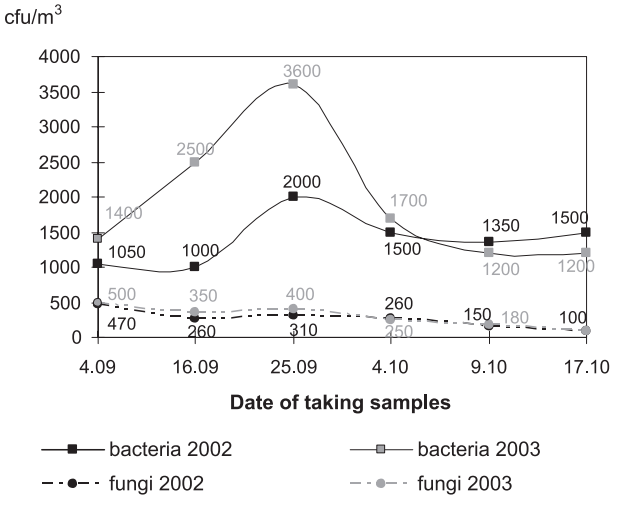

Fig.4. Variations in microbial concentrations in toilets.

Qualitative Analysis of Air in University Rooms

Microbiological quality of indoor air is created not only by a total concentration of bacteria and fungi but by the presence of some particular microorganism species, which is very important for the health of people occupying the room. Analysis of bacterial flora composition in investigated university rooms revealed dominating contributions of bacteria from the following genera: *Micrococcus* spp., *Bacillus* spp., *Staphylococcus* spp. (e.g. *Staphylococcus aureus*), *Sarcina* spp., and *Serratia* spp. Also some Gram negative bacteria belonging to *Escherichia* genus were isolated from indoor air of toilets. Quality characteristics of fungal flora isolated from the air of educational rooms showed dominating contributions of such species of fungi like: *Cladosporium* spp. (e.g. *Cladosporium herbarum*), *Penicillium* spp. (e.g. *Penicillium chrysogenum*, *Penicillium viridicatum* and *Penicilluim expansum*), *Aspergillus* spp. (e.g. *Aspergillus niger* and *Aspergillus flavus*). Genera *Cladosporium herbarum*, *Alternaria alternata*, *Mucor* spp., *Rhizopus nigricans* and *Epicoccum* spp. prevailed in a canteen and a corridor. Both qualitative as well as quantitative variations of microflora during the day were observed and this is presented in Figs. 5, 6 and 7 as a percentage contribution of particular species of fungi in tested rooms in mornings and afternoons.

Discussion

In the face of a lack of any official reference limit for microbiological quality in indoor air it is difficult to fully interpret our results. However, one of the valuable proposals of reference data were so-called Residential Limit Values (RLV) presented by Górny and Dutkiewicz on the WHO Expert Meeting in Berlin, 2002 [22]. The number of bacterial flora found in university rooms in 2002 hardly fitted an upper limit of these reference values, whereas in 2003 they sometimes exceeded this limit (5000 cfu/m^3). The fungal concentration was not higher than proposed RLV limit (5000 cfu/m^3) but it overtook in almost all cases the level of 250 cfu/m^3 – the limit proposed by the American Industrial Hygiene Association in 2001 [26].

Looking at the variations of microorganism concentrations in the whole investigated period it is easy to notice a sharp increase of fungi in the air of the lecture room and bacteria in toilets at the time of the opening of the

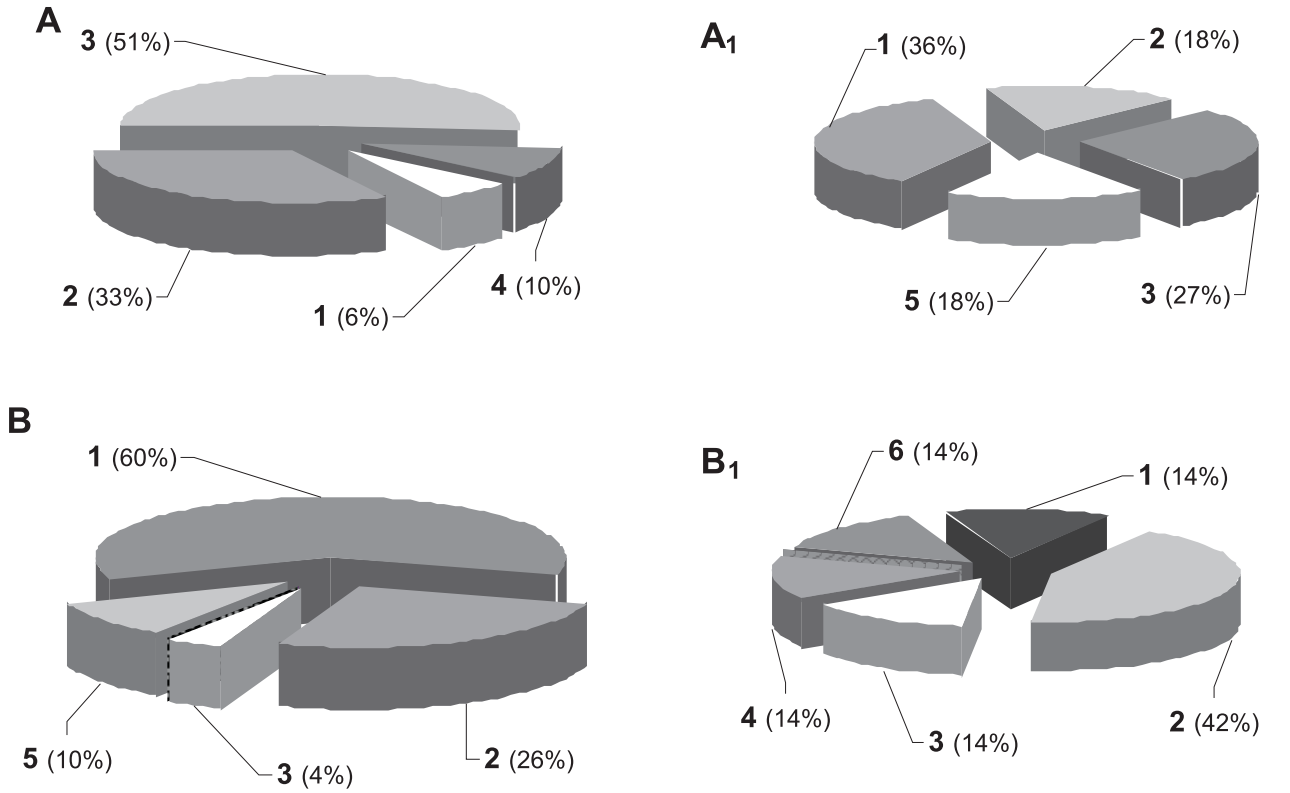

Fig. 5. Mould species dominating in the ventilated lecture room in 2002 (A, B) and 2003 (A1, B1):
A, A1 - in the morning; B, B1 - in the afternoon; A, B: 1 - *Cladosporium* spp., 2 – *Penicillium* spp., 3 - *Monilia* spp., 4 - *Mucor* spp., 5 - *Acremonium* spp.; A1, B1: 1 - *Cladosporium* spp., 2 - *Aspergillus* spp., 3 - *Penicillium* spp., 4 - *Mucor* spp., 5 – *Alternaria* spp., 6 - another species

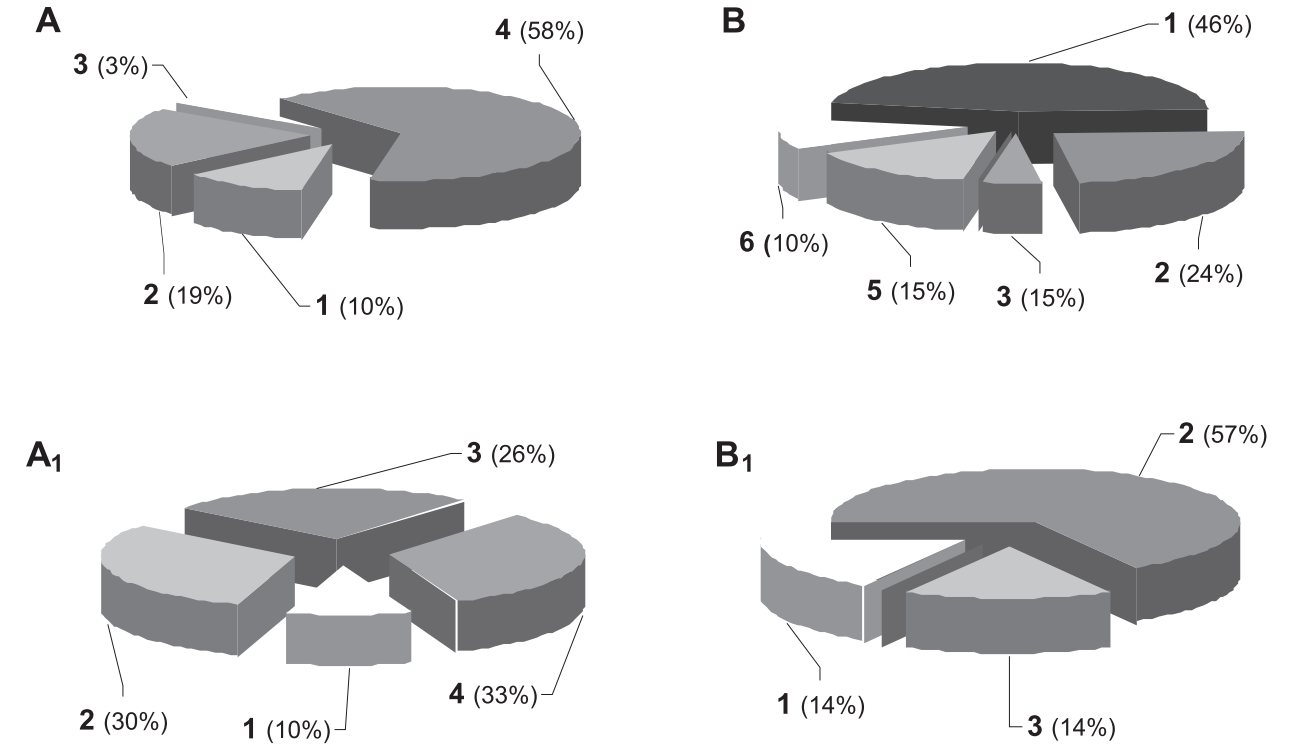

Fig. 6. Mould species dominating in the students canteen in 2002 (A, B) and 2003 (A1, B1):
A, A1 - in the morning; B, B1 - in the afternoon; A, B: 1 - *Cladosporium* spp., 2 - *Penicillium* spp., 3 - *Mucor* spp., 4 - *Rhizopus* spp., 5 - *Aspergillus* spp., 6 - *Alternaria* spp. ; A1, B1: 1 - *Cladosporium* spp., 2 - *Penicillium* spp., 3 - *Aspergillus* spp., 4 - *Rhizopus* spp.

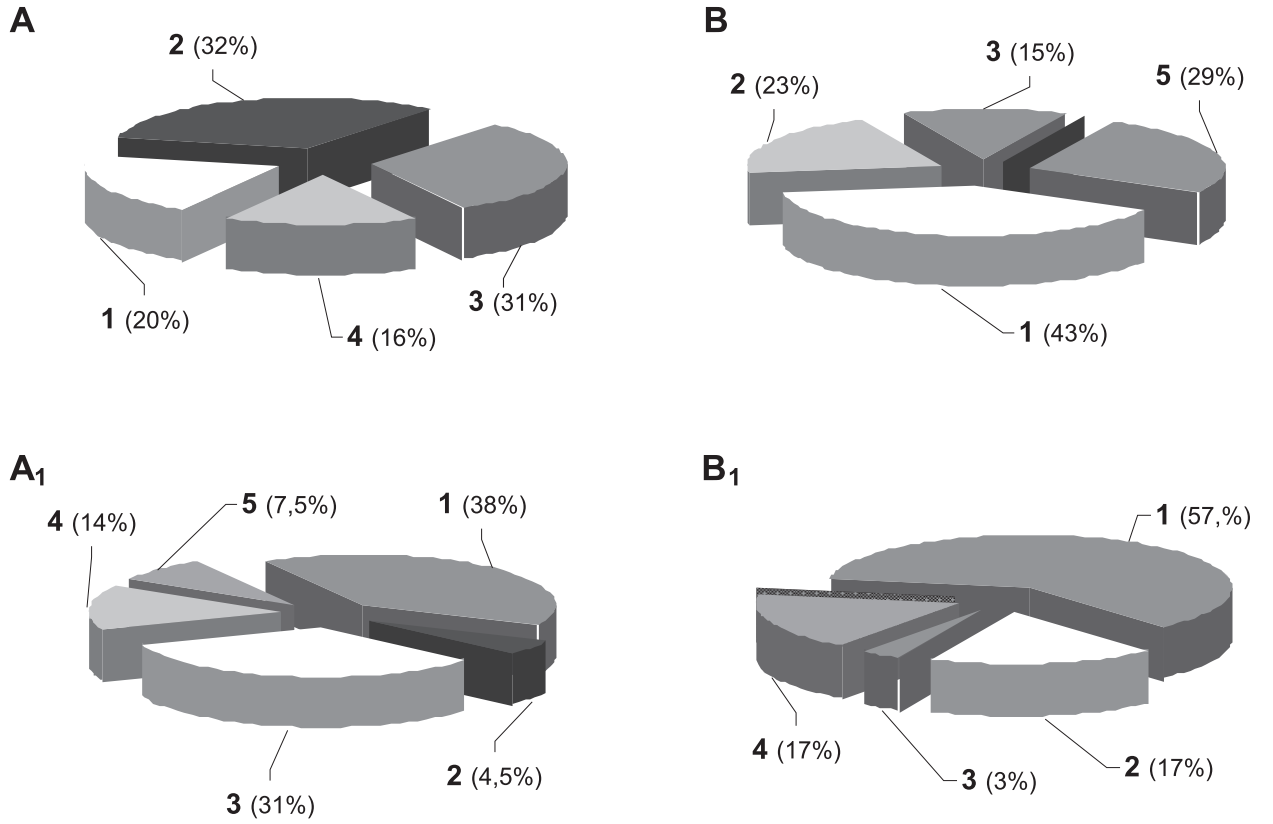

Fig. 7. Mould species dominating in the reading room in 2002 (A, B) and 2003 (A1, B1)
A, A1 - in the morning; B, B1 - in the afternoon; A, B: 1 - *Cladosporium* spp., 2 - *Penicillium* spp., 3 - *Mucor* spp., 4 - *Scopulariopsis* spp., 5 - *Rhizopus* spp.; A1, B1: 1 - *Cladosporium* spp., 2 - *Alternaria* spp., 3 - *Mucor* spp., 4 - *Penicillium* spp., 5 – *Scopulariopsis* spp.

academic year. This phenomenon is undeniably connected with an appearance of a new significant microbiological contamination source – students attending lectures. The human body as well as clothing is a natural place for growing microorganisms. Strong relationship between occupant density, human activity and microorganisms concentration in the indoor air was reported elsewhere [12, 31, 37]. Toivola *et al.* [37] found the highest bacterial concentration in heavily populated workplaces. In schools the highest level of bacterial contamination was detected in the corridor and in rooms. During lessons and after lessons the number of microorganisms was much lower [12]. In this investigation just after the short period of high microorganisms concentration in the air of investigated rooms a gradual decrease of microbial contamination was observed associated with its slow drop in the outdoor air found in parallel studies near university buildings [36]. Examination of the outdoor air also showed that microorganism concentrations in the outdoor changed within the whole investigated period and in 2002 a prevalence of fungi was observed. Higher mould concentrations in investigated rooms in 2002 compared to 2003 can be explained by their high concentration in the outdoor air. Only the quality of bacterial and fungal flora in the air of toilets (Fig. 4) was much less influenced by outdoor air contamination. Contrary to outdoor air inside investigated toilets significantly dominating microorganisms were bacteria amounting couple of thousands cfu/m^3, whereas the level of mould spore concentrations reached the amount of a few hundred cfu/m^3. In 2003 bacteria dominated the outdoor air, especially at the turn of September and October. Bacteria concentration was about 5 times higher in 2003 than in 2002. It is probably connected with the weather conditions prevailing at that period of time, very conducive to infections (i.e. rapid temperature decrease at the beginning of studies period from 22°C to about 7°C in the middle of this period and quite high relative humidity at 70% [36]).

It is generally accepted that a microbiological concentration in the indoor air is similar to the outdoor values, which means that the indoor/outdoor concentration ratio (I/O) is close to 1. In our studies the I/O ratios for both bacteria and fungi in most investigated rooms were lower than this value, especially in the morning time. The situation observed in the ventilated lecture hall (where I/O ratios in the afternoons were lower than in the mornings) is evidence of a proper ventilation system. Extremely high values of I/O ratios in the afternoons for librarian lending room and reading room, canteen, corridor and toilets can be explained by the fact that all these places are most of the time overcrowded with visitors. It is a main source of microbiological contamination there. However, by com-

paring obtained average values of I/O ratios with those proposed by Siqueira (I/O ≤ 1.5 = good; I/O = 1.5 up to 2.0 = regular; I/O > 2 = poor indoor ambient conditions) [28, 38] indoor ambient conditions in tested rooms can be estimated as relatively clean.

In 2002 a significant growth of so-called "outdoor moulds" spores *Cladosporium* spp. was observed in afternoons, reaching sometimes even tens of percentages. In this year *Cladosporium* spp. prevailed in the outdoor air.

In the air of university canteen relatively high concentration of spores *Rhizopus* spp. was found in mornings, its contribution in the air came up to 58% in 2002 and 33% in 2003. *Rhizopus* spp. is a typical "indoor mould." However, an unusually high concentration of this mould in the canteen came from fruits and vegetables used for preparation of meals. In afternoons, microbiological composition of the air in the canteen was dominated by other typical "indoor moulds" genera like *Penicillium* spp., *Aspergillus* spp., *Mucor* spp. and "outdoor mould" *Cladosporium* spp. Also a second typical "outdoor mould" *Alternaria* spp. appeared in the air of investigated halls in afternoons. These spores of both genera *Cladosporium* and *Alternaria* emerging and raising in the course of the day in the air of lecture halls and other rooms occupied by many people are evidence of continuous input of microorganisms from outside via visiting people.

In the air of the library reading room a very specific mould genus – *Scopulariopsis* spp. was always found but only in mornings. It is difficult to explain this phenomenon but more detailed research has to be involved to clear this problem because *Scopulariopsis* spp. is a major cause of onychomycosis [39-41].

Presented results provided evidence that high concentration of fungi in atmosphere can influence microbiological indoor air contamination. Before the beginning of the academic year so-called "outdoor moulds," i.e. *Cladosporium* spp., and *Alternaria* spp. dominated the indoor air of investigated rooms and their concentration remains stable. However, during the academic year a significant variation of fungal genera in the air was observed and concentrations of both *Cladosporium* spp. and *Alternaria* spp as well as so-called "indoor moulds" genera like *Aspergillus* spp., *Penicillium* spp. or *Mucor* spp. was growing steadily during the daytime. Increasing concentration of fungal spores during lectures in the course of the day, especially those from the genera *Cladosporium* spp. and *Alternaria* spp., can have a bad influence on health and mood of students and teachers staying in these rooms.

According to earlier studies the microbiological quality of indoor air is formed by two main factors: microbiological composition of outdoor air and indoor air microbial sources [1, 2, 10-13]. Outdoor air is very much influenced by environment, season, the weather and even daytime.

Data presented in this work provides evidence that people occupying or visiting enclosed spaces play a dominating role in the creation of indoor air microbiological environments. The highest growth of microorganism numbers in the course of the day was noticed in the corridor, canteen, chemical laboratory and the library – the most overcrowded places. It means that all rooms attended by many visitors will be extremely exposed to risk of high microbial contamination. Obviously, the presence of a good ventilation system inside buildings eliminates to some extent the influence of indoor sources.

Conclusions

1. Indoor air contamination in investigated university rooms caused by mesophilic aerobic bacteria varied from 120 to 2300 cfu/m^3 in 2002 and from 110 to 3300 cfu/m^3 in 2003. Contamination caused by fungi varied from 130 to 1100 cfu/m^3 in 2002 and from 90 to 800 cfu/m^3 in 2003.
2. The microbiological quality of the air in investigated rooms was differentiated and changed significantly in the course of the day. In afternoons the concentration of bacteria and much more fungi increased a few times. The only exception was the ventilated lecture room, where the microbiological composition of the air was stable within the day and even presented a tendency to fall. The increased level of fungal flora in sufficiently ventilated rooms could be a reason for serious health problems of people occupying those rooms.
3. In 27% of samples the number of bacteria in indoor air exceeded the level of bacterial contamination of outdoor air. Almost 23% of tested indoor air samples showed higher fungal contamination than outdoor air. Among isolated fungi there were also strongly allergenic and toxic species such as *Cladosporium herbarum*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus versicolor*, *Aspergillus niger*, and *Penicillium expansum*.

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