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Microbiological Indoor Air Quality in Polish Schools

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1. Introduction

Indoor air quality (IAQ) is becoming an increasingly important issue for occupational and public health (Clarke and Nikkel1995; Reynolds et al. 2001, Dudzińska, 2011). Although discussions about indoor air contamination frequently concentrate on chemical pollutants, the health effects of inhaled biological particles should not be overlooked, as a large variety of biological material is present in indoor environment (Montanaro 1997).

Microorganisms are normal and essential components of all environments. Bacteria and fungi are needed to break down complex molecules found in organic matter. If provided with water and food source, microorganisms will rapidly develop in almost any area. Microorganisms and/or their reproductive structures are almost always found in outdoor air. Their types and populations will vary depending on local environmental conditions. Doors, windows, and fresh air intakes provide easy access for microorganisms to enter the interiors of buildings. It is common to find some quantity of microorganisms in indoor air. In a normal indoor environment, their numbers should be significantly lower than outdoor levels. Excessive moisture inside a building from leaks, floods, or other sources can create an "out-of-balance" environment that will tend to increase their population. Depending on the amount of viscosity, temperature, lighting, and food available, different species may become dominant. In consequence, the presence of some microorganisms in large quantities may lead to adverse health effects involving building occu-

pants. Adverse health effects of affected individuals can include both illnesses and allergic reactions. It is supposed that about 30% of health problems relevant to the indoor air quality is the result of a human organisms reaction to molds (Srikanth et al. 2008, Gutarowska and Jakubowska, 2002).The sampling and analysis of airborne microorganisms in indoor air has attracted attention in recent years (Kim and Kim 2007; Huttunen et al. 2008; Stanley et al. 2008).

So far there have been no Polish standards or guidelines for microbiological quality of indoor air. Furthermore, there is not any European Union directive addressing this phenomenon. Therefore, it is advisable to base on particular European countries requirements and scientific propositions (Górny, 2004).The available indoor bioaerosol measurement data are usually related to the occupational environment. Very little is known about the microflora of dwellings, schools and work places. The proposed occupational exposure limits (OEL) and residential limit values (RLV) of various bioaerosol components in industrial settings and residential dwellings are presented in Table 1. Independently of these reference values, in an assessment of indoor exposure, the general assumption should be that in certain circumstances the microbial pathogen may be the cause of health problems, even at concentrations below the reference limit. The presence of microorganisms, from the risk groups 3 and 4 of the European Community Directive2000/54/EC (e.g., *Mycobacterium tuberculosis*, *Bacillusanthracis*, *Coxiellaburnetii*), in indoor air, independently of the concentration, should always be inadmissible and result from preventive actions (Górny, 2002; Dutkiewicz and Górny, 2002).

Due to the fact that air quality in the work environment, which can include schooling, is becoming more and more important, there are studies conducted all over the world to determine and improve the current state of indoor air quality. In December 2008, the study on indoor air quality in three primary schools was carried out in the center of Lisbon, in Portugal. Measurements were performed in two rooms in each school. The results showed that the highest recorded concentration of bacteria in the air in one of the schools was $1.63 \cdot 10^3$ CFU/m^3, while the number of fungi in the two schools exceeded the acceptable level of $5.0 \cdot 10^2$ CFU/m^3 specified in Portuguese law and was approximately $8.0 \cdot 10^2$ CFU/m^3 (Evtyugina et al., 2010).

Table 1. Proposal for occupational exposure limits (OEL) (Dutkiewicz and Górny, 2002) and residential limit values (RLV) for various bioaerosol components measured as inhalable fraction in industrial settings and residential dwellings
Tabela 1. Propozycje dopuszczalnych limitów w miejscach pracy (OEL) (Dutkiewicz and Górny, 2002) i zamieszkania (RLV) dla różnych bioaerozoli

Type of setting, bioaerosol component	Proposal of OEL/RLV
Industrial settings contaminated by organic dust	OEL
Fungi	$5 \cdot 10^3$ cfu/m^3*
Total mesophilic bacteria	$100 \cdot 10^3$ cfu/m^3*
Gram-negative bacteria	$20 \cdot 10^3$ cfu/m^3*
Thermophilicactinomycetes	$20 \cdot 10^3$ cfu/m^3*
Endotoxin	200 ng/m^3 (2000 EU)
Residential dwellings	RLV
Fungi	$5 \cdot 10^3$ cfu/m^3
Total mesophilic bacteria	$5 \cdot 10^3$ cfu/m^3
Endotoxin	5 ng/m^3 (50 EU)

** For respirable fraction the proposed limits should be twice as low, i.e., $25 \cdot 10^3$ cfu/m^3 for fungi, $50 \cdot 10^3$ cfu/m^3 for total mesophilic bacteria, $10 \cdot 10^3$ cfu/m^3 for Gram-negative bacteria and $10 \cdot 10^3$ cfu/m^3 for thermophilic actinomycetes.*
EU – endotoxin unit

Similar studies, but on a larger scale, were carried out in South Korea from July to December 2004. There were 55 schools from different cities selected to the research. Division of the season, level of teaching in the school as well as age of the building of the school facilities were taken into consideration in the analysis. The average concentration of microorganisms in the study period in all schools amounted to $1.4–1.26 \cdot 10^3$ CFU/m^3, while the highest concentration – $5.5 \cdot 10^3$ CFU/m^3 was recorded in autumn. In primary schools the average concentration of $1.3 \cdot 10^3$ CFU/m^3 was reported, whereas the highest value observed in this type of schools was $4.7 \cdot 10^3$ CFU/m^3 (Yang et al., 2009).

The studies on indoor air quality conducted by Godwin and Batterman are another example. The research was carried out in 64 schools in Michigan and revealed that the concentration of bioaerosol inside the buildings was $<6.5 \cdot 10^3$ CFU/m^3 (Godwin, Batterman, 2007). In 1999, Wurtz

conducted a study on air microflora in four schools in Denmark and found that in 30% of samples the concentration of bacteria was greater than $5 \cdot 10^3$ CFU/m^3, which could result from poorly ventilated rooms (Bayer, 2000).

2. Material and Methods

Samples for measurements summarized in this study were collected in 5 schools in Lublin, Poland (Table 2). The measurement was done twice:

- during the heating period, from 15.03.2012 to 29.03.2012,
- without heating, from 10.05.2012 to 29.05.2012.

The sample of air was taken before lessons, in order to determine "background". Further measurements were made during the lesson. During each measurement windows were closed.

Table 2. Description of classrooms
Tabela 2. Opis sal lekcyjnych

	School 1	School 2	School 3	School 4	School 5
School localization	close to the city center, on a busy street	in the middle of a housing estate	in the middle of a housing estate, on a busy street	in the middle of a housing estate	in the middle of a housing estate, on a busy street
Age	built in the 60's	built in the 80's	built in the 80's	built in the 80's	built in the 60's
Classroom localization	on the ground floor, in the center of the school	on the first floor, in the western part of the school	on the ground floor, in the western part ofthe school	on the ground floor, in the center of the school	on the ground floor, in the center of the school
Windows	Double glazed, surface 18.5 m^2, directed to the north-west	Double glazed, surface 19.6 m^2, directed to the north	Double glazed, surface 15.8 m^2, directed to the east	Double glazed, surface 15.5 m^2, directed to the south	Double glazed, surface 11 m^2, directed to the east

Table 2. cont.
Tabela 2. cd.

	School 1	School 2	School 3	School 4	School 5
Equipment	15doubledesks, writ-ing-desk, cabinets, blackboard	18doubledesks, writing-desk, two cabinets, blackboard, piano, computer	17singledesks, writing-desk, cabinets, blackboard	25singledesks, writing-desk, cabinets, blackboard	15doubledesks, writing-desk, cabinets, blackboard
Ventilation system	gravity ventilation	gravity ventilation	gravity ventilation	gravity ventilation	gravity ventilation
Flowers	Yes	–	Yes	–	Yes
Another	Classes IV–VI	music class, sound proofedwalls, parquet floor	Classes I–III, PVC floor, carpet	Classes I–III, PVC floor	Classes I–III, floor covered with tiles

2.1. Climate

The thermo-hygrometric parameters (air temperature, relative humidity) were electronically logged at 1-min intervals to Hobo U12 data logger Onset, which is a four-channel logger with 12-bit resolution and can record up to 43.000 measurements or events. The external channel accepts a variety of sensors, including temperature, and split-core AC current sensors as well as 4–20 mA and voltage input cables. The logger uses a direct USB interface for launching readout data by a computer. Measurement range for temperature is -20°C to 70°C (accuracy +/- 0.35°C from 0°C to 50°C) and for relative humidity 5% to 95% RH (accuracy +/- 2.5% from 10% to 90% RH).

Carbon dioxide was gauged by Carbon Dioxide Transmitter Series GMD20 Vaisala, which is easy to install. The measurement range of this transmitter is 0–2000 ppm with accuracy +/- 2% of range and 2% of reading. Response time is calculated per minute.

GMD20 and HOBO U12 presented one measuring match. Programing of recorder and reading data was performed with the use of computer program Hoboware Pro.

The thermo-hygrometric parameters and carbon dioxide were measured everyday for two weeks in winter and summer, at the same time when the biological analysis and particulate pollution were performed.

2.2. Microbiology

Air samples were collected by an Andersen 6-stage cascade impactor MAS-6 (Stage 6 corresponds to 0.65–1.1 μm, Stage 5: 1.1–2.1 μm, Stage 4: 2.1–3.3, Stage 3: 3.3–4.7 μm, Stage 2: 4.7–7 μm and Stage 1: 7 μm or above) located in the center of the examined room at a height of 1.0–1.5 m above the floor. The windows and doors were closed. The sampling time was 10 min. Microorganisms were collected on nutrient media (specific to either fungi or bacteria) in Petri-dishes located on all impactor stages. Agar Sabouranda with chloramphenicol (BTL) was applied for fungi, trypcase soy agar (TSA, BTL) was used for bacteria. The Petri dishes were incubated 48h at 36±1°C for bacteria and for 14 days at 27±1°C for fungi. The results were expressed in colony forming unit per cubic meter (CFU/m^3). For the identification of microorganisms cell staining method were used (Grama method).

3. Results and Discussion

During the measurement period, the temperature ranged from 20.8–28.5^{o}C, humidity of 18–55%. CO_2 concentration measurement carried out in selected classrooms allowed to define the class of the room. In the most part of the day CO_2 concentration exceeded 1000 ppm even after 20 minutes of the first class. Therefore, the classes were qualified to 3rd and 4th class of the rooms. Results for one of the schools (No. 2) are summarized in Table 2.

The predominant bacteria and moulds isolated from investigated air samples were: *Bacillus lentus, Bacillus licheniformis, Bacillus pumilus, Bacillus cereus, Pseudomonas stutzeri, Micrococcus ssp., Staphylococcus xylosus, Staphylococcus saprophyticus, Staphylococcus haemolyticus, Acremonium, Aerobasidium, Aspergillus,Aspergillusniger, Alternaria, Cladosporium, Epicocum, Mucor, Penicilinium.*

Substantially the highest concentration of bacteria in the classroom characterizes the school No. 1 located in the city center (Fig. 1).

Table2. CO_2 concentration and air quality class
Tabela 2. Stężenie CO_2 i klasa jakości powietrza

Classrom	Day of the week	Period	Parameter	1lesson	2lesson	3lesson	4lesson	5lesson	6lesson	7lesson	8lesson	9lesson
S7.2	Mon	summer-time	ΔCO_2	1074	1343	1619	1424	648	1205	2042	2153	1004
			IDA	4	4	4	4	3	4	4	4	4
	Tue		ΔCO_2	2	14	5316	374	717	–	–	–	–
			IDA	1	1	4	1	3	–	–	–	–
	Wed		ΔCO_2	781	1346	1221	1151	1242	1228	1251	735	493
			IDA	3	4	4	4	4	4	4	3	2
	Thu		ΔCO_2	1011	1066	1706	2043	2052	976	995	531	261
			IDA	4	4	4	4	4	3	3	2	1
	Fri		ΔCO_2	887	945	827	1123	1174	744	200	147	187
			IDA	3	3	3	4	4	3	1	1	1
S7.2	Mon	winter-time	ΔCO_2	1209	1250	1297	1184	1293	971	647	549	702
			IDA	4	4	4	4	4	3	3	2	3
	Tue		ΔCO_2	316	398	874	903	1629	1473	893	865	727
			IDA	1	1	3	3	4	4	3	3	3
	Wed		ΔCO_2	938	1740	1580	547	1189	1018	972	670	775
			IDA	3	4	4	2	4	4	3	3	3
	Thu		ΔCO_2	–	–	–	2377	2295	1092	1095	874	417
			IDA	–	–	–	4	4	4	4	3	2
	Fri		ΔCO_2	377	1193	2145	2244	1352	1470	1438	1058	780
			IDA	1	4	4	4	4	4	4	4	3

The concentration reached there the value of $6.5 \cdot 10^3$ CFU/m^{3}and is higher than the accepted standards, which allows the range of bacteria amounted to $5.0 \cdot 10^3$ CFU/m^3 to the air; similarly to some cases of the research conducted by Wurtz in school institutions in Denmark (Bayer, 2000) $2.0 \cdot 10^3$ CFU/m^3 according to Górny. In spring the school No. 1 reaches a value close to that of the first measurement period, which is the highest value among all schools. It may be influenced by the age of the building, as well as insufficient exchange of air in the room. In cases of the remaining schools the amount of air microorganisms in the indoor air is within the normal range and comprises in the range of $4.5 \cdot 10^3$ CFU/m^3 or slightly exceeds the permitted value, as it was in spring in the case of schools No. 2 and No. 3. It was probably caused by the influx of microorganisms with the outdoor air getting to the room through the window, which at this time of year are open for an extended period of time (Fig. 1).

Among the surveyed schools, the lowest concentration of bacteria (measured) was in school No. 3. In winter, the concentration of bacteria was $3.5 \cdot 10^3$ CFU/m^3, whereas in spring it was slightly below $2.0 \cdot 10^3$ CFU/m^3. These values are comparable to the ones obtained in the studies conducted in South Korea in 2004 (Yang et al., 2009).

In the case of the measurement of the fungi amount in wintertime, the highest value was recorded in the school No. 1 (Fig. 1). This concentration was more than two times higher than the recommended value of $3.0 \cdot 10^2$ CFU/m^3, accepted by some international organizations. Whereas, in spring the highest concentrations occurred in the school No. 2, which also exceeded the permitted level. A similar situation was observed during the research conducted in Portugal, where the concentration of fungi at the level of $8.0 \cdot 10^2$ CFU/m^3 was noted in two schools (Eytyugina et al., 2010). In other schools, both in winter and spring-time the concentration of fungi in the air was within the normal range.

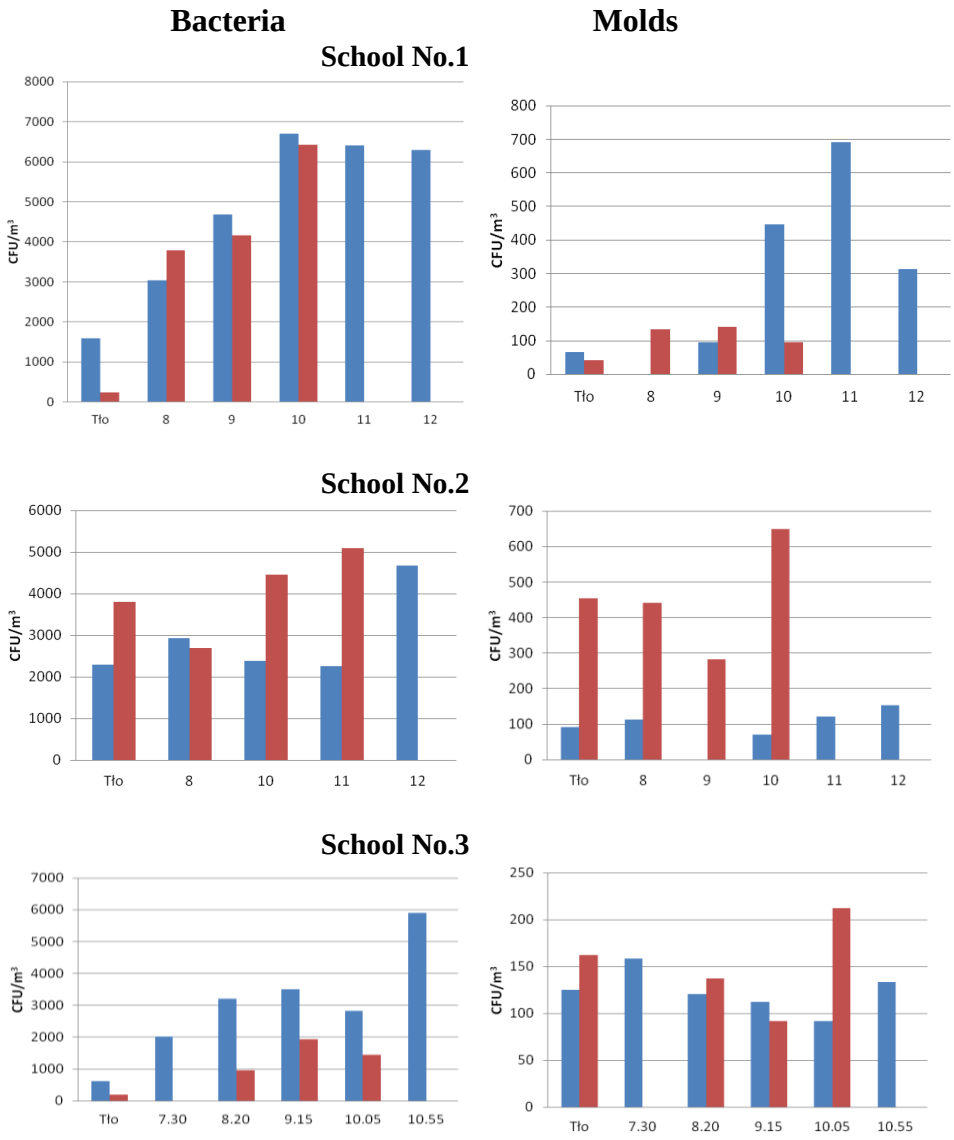
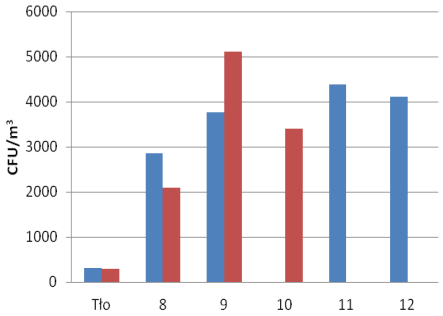
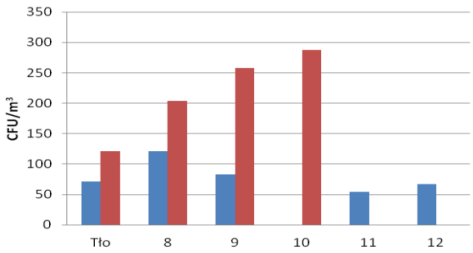


Fig. 1. The total concentration of bacteria and fungi in schools between the particular hours of measuring, blue – the period of heating, red – the period without heating, T – background

Rys. 1. Całkowite stężenie bakterii i grzybów w szkołach w poszczególnych godzinach pomiaru, niebieski – okres ogrzewania, czerwony – okres bez ogrzewania, T – tło

School No.4





School No.5

No result due to the contamination of the material used inthe test, which took place twice

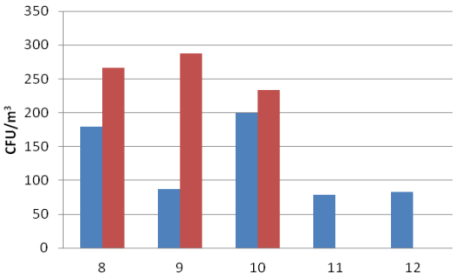


Fig. 1. cont.
Rys. 1. cd.

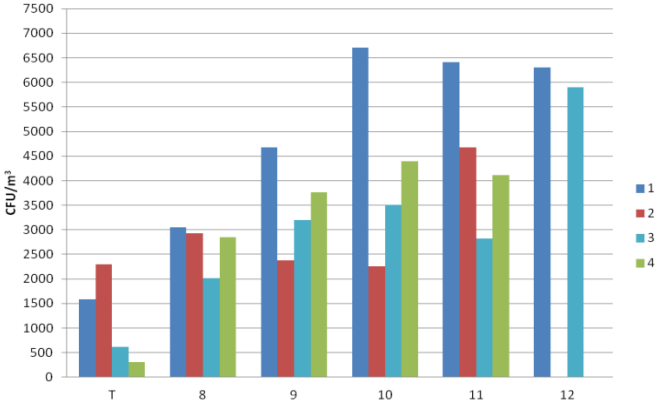


Fig. 2. The total concentration of bacteria in the air in m^3 for particular schools in the heating season (hours 8–12), T – background
Rys. 2. Całkowite stężenie bakterii w m^3 powietrza dla poszczególnych szkół w sezonie grzewczym (godziny 8–12), T – tło

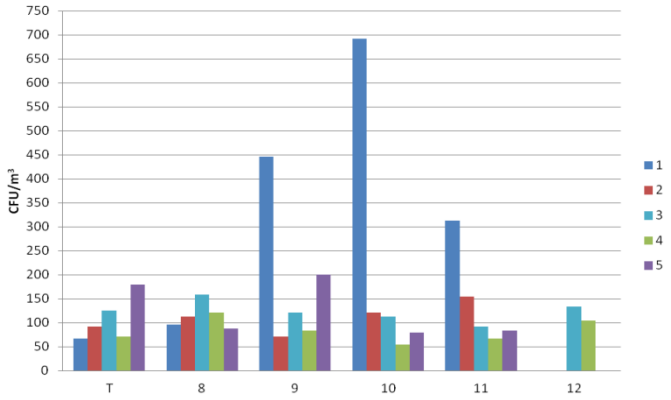


Fig. 3. The total concentration of fungi in the air in m^3 for particular schools in the heating season
Rys. 3. Całkowite stężenie grzybów w m^3 powietrza dla poszczególnych szkół w sezonie grzewczym

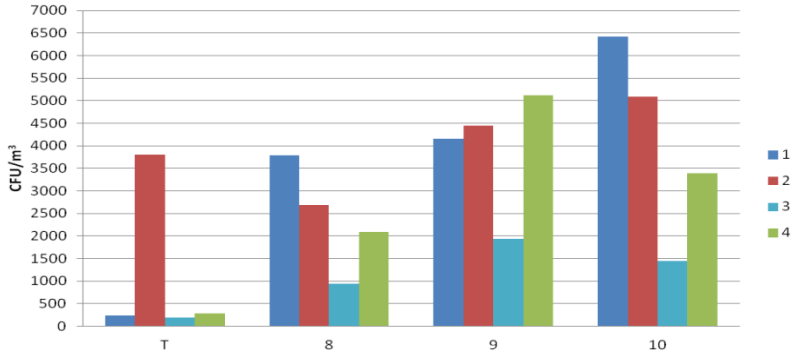


Fig. 4. The total concentration of bacteria in the air in m^3 for particular schools off heating season
Rys. 4. Całkowite stężenie bakterii w m^3 powietrza dla poszczególnych szkół w sezonie bez ogrzewania

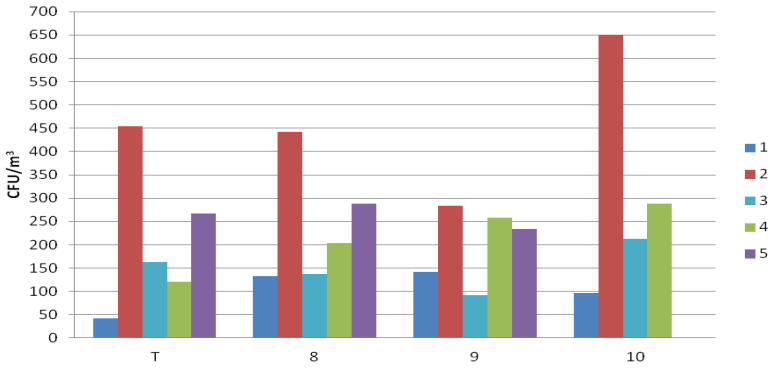


Fig. 5. The total concentration of fungi in the air in m^3 for particular schools off heating season
Rys. 5. Całkowite stężenie grzybów w m^3 powietrza dla poszczególnych szkół w sezonie bez ogrzewania

4. Conclusion

Among the five schools that were included in the study only in the one case reported there were significantly higher levels of microorganisms than the recommended level for bacteria oscillating around $5.0 \cdot 10^3$ CFU/m^3 and $3.0 \cdot 10^2$ CFU/m^3 for fungi (Górny, 2004) and they were $6.5 \cdot 10^3$ CFU/m^3 and $6.9 \cdot 10^2$ CFU/m^3, respectively. In other schools, the amounts of microorganisms in the air were wirthin the normal range, that is $4.5 \cdot 10^3$ CFU/m^3 (bacteria) and $3.0 \cdot 10^2$ CFU/m^3 (fungi) or slightly exceed the limited values.

Among the microorganisms present in the tested classrooms there were, among others, *Bacillus* and *Micrococcus* bacteria, which constituted the overwhelming part of the bacterial microflora. When it comes to fungi, there were such types as *Aspergillus* or *Penicilinium*.

The research conducted has shown that the concentration of microorganisms in the air in selected schools in Lublin only rarely exceeds its recommended limits. Some of the microorganisms present in the indoor air of studied objects may have a negative impact on the human body causing, for instance, allergies.

Furthermore, the CO_2 concentration values obtained during the measurement day exceeded the acceptable value of 1000 ppm. Due to that fact, the majority of school classes in most schools were classified into 3rd and 4th class of rooms.

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Mikrobiologiczna jakość powietrza w polskich szkołach

Streszczenie

Większość ludzi spędza ponad 90% swojego życia wewnątrz pomieszczeń m.in.: w domach, biurach i szkołach, gdzie narażeni są na działanie różnych czynników środowiska wewnętrznego (np. bioaerozoli) wpływających na ich zdrowie i kondycję fizyczną. Dlatego w ostatnich latach stale rośnie zainteresowanie badaniami wewnętrznych zanieczyszczeń biologicznych. Celem tych badań jest nie tylko szacunkowa ocena ilości mikroorganizmów w powietrzu, ale również ich identyfikacja i określenie czynników wpływających na skład bioaerozolu wewnątrz pomieszczeń. Biologiczne zanieczyszczenie powietrza w pomieszczeniach jest najczęściej spowodowane przez bakterie i grzyby. Nie tylko samo ich występowanie w formie żywych komórek patogennych może być szkodliwe dla zdrowia, ale również wydzielane przez nie substancje stanowią spore zagrożenie.

W artykule zbadano szkoły z terenu Lublina pod kątem zanieczyszczeń mikrobiologicznych. Badania przeprowadzono podczas okresu grzewczego (marzec) i bez ogrzewania (maj). Próbki powietrza pobierano przed lekcją oraz podczas jej trwania.

Zidentyfikowano następujące bakterie i grzyby: *Bacillus lentus, Bacillus licheniformis, Bacillus pumilus, Bacillus cereus,Pseudomonas stutzeri, Micrococcus ssp., Staphylococcus xylosus, Staphylococcus saprophyticus, Staphylococcus haemolyticus, Acremonium, Aerobasidium, Aspergillus, Aspergillusniger, Alternaria, Cladosporium, Epicocum, Mucor, Penicilinium.* Wśród tych drobnoustrojów wykryto obecność mikroorganizmów chorobotwórczych i o silnym działaniu uczulającym.