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Microbial air contamination inside academic buildings in Białystok University of Technology

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ABSTRACT

Microbiological tests of the air in selected rooms of the Faculty of Civil Engineering and Environmental Sciences of the Białystok University of Technology were carried out from September 2023 to April 2024. The total number of psychrophilic and mesophilic bacteria, the number of actinobacteria, the number of *Staphylococci,* and the total number of fungi were determined in the tested air. Measurements were made using two methods: the impact method and the sedimentation method. For the assessment of microbiological air quality, the higher result of the number of bacteria or fungi calculated on the basis of the two air measurement methods was selected. The obtained results indicate the periodic occurrence of mannitol-positive and mannitol-negative *Staphylococci* in the tested air, which indicates a sanitary threat to students and employees staying in these rooms. It is essential to introduce air disinfection using UVC air-flow lamps, especially in teaching rooms.

Keywords: bacteria, fungi, microbial air quality, toilets, laboratory rooms.

INTRODUCTION

Air, in addition to gaseous components such as nitrogen, oxygen, carbon dioxide and other minor admixtures, also contains various particles that are pollutants. These include physical, chemical and biological pollutants, the sources of which are fungi, molds, dust, yeasts, bacteria, and animal waste [1].

The microbiology of air in buildings and, more specifically, indoors is rich in microorganisms, the number of which is difficult to determine accurately, as it consists of numerous strains of bacteria, mold, as well as mites, viruses and protozoa which at high concentrations can contribute to the deterioration of human health. According to Gilbert and Hartman [2] microorganisms found in the indoor air influenced by human activities and environmental factors, play a pivotal role in modulating infectious diseases and fostering healthy immune development. Contact with particles, such as viruses, bacteria, fungi, and plant and animal particles, can cause the development of infectious diseases, invasive diseases, allergies, and even cancer [3].

Microorganisms in air occur in phase systems - so-called bioaerosols. In such systems, the dispersing phase is air, and the dispersed phase consists of liquid particles, dust, molecules particles of plant or animal origin to which microorganisms are attached. About 80–95% of a human's life is spent indoors [4, 5]. People working in closed rooms often experience various symptoms such as fatigue, shortness of breath, headaches, low ability to concentrate, irritability, skin lesions, and memory disorders. All these symptoms have been collectively called "sick building syndrome" (SBS). One of the causes of these troublesome ailments may be microorganisms present in the air and the substances they produce [6]. For this reason, indoor air quality for permanent human occupancy, and microbial contaminants in

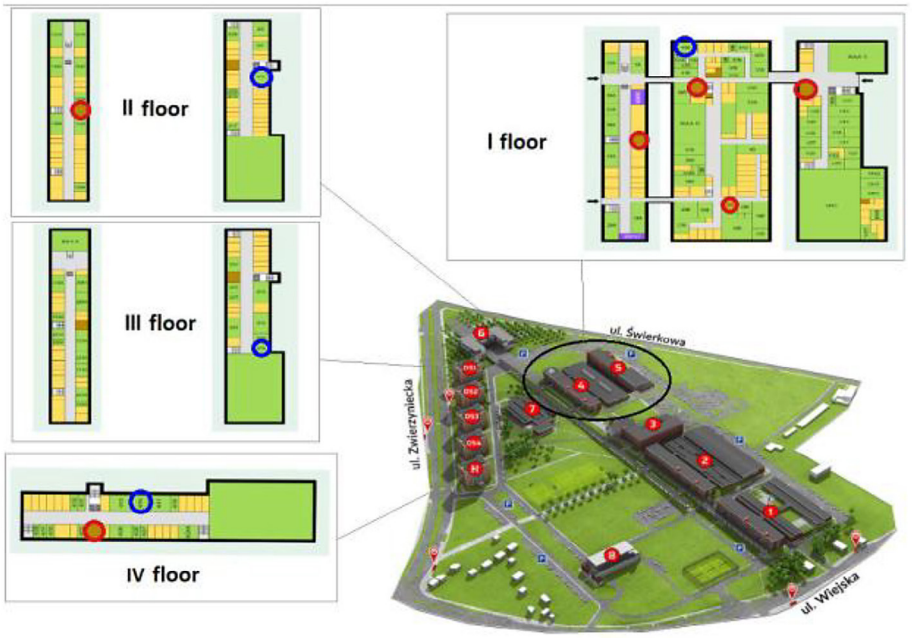
particular, have become the subject of numerous studies in recent years. They mainly concern working environments associated with potentially high emissions of bioaerosols, such as the buildings and surroundings of wastewater treatment plants [7, 8] and places associated with a large accumulation of people, such as educational institutions [9, 10].

In this context, the determination of microbial air pollution is an important analysis because its purpose is to determine the composition and number of microorganisms that have a significant impact on the physiological condition of humans. This assessment allows for the protection of health and the environment, contributing to improving the quality of life, as well as understanding the sources of bioaerosols emissions [3]. The lack of criteria for microbiological air quality results in a small number of publications on the safety conditions of people staying indoors. This particularly applies to public facilities such as schools, universities, hospitals and offices. For this reason, microbiological quality of air was tested at the Bialystok University of Technology. The aim of the study was to determine the level of microbial air pollution in selected rooms of the Faculty of Civil Engineering and Environmental Sciences in relation to the safety of students and faculty employees. The obtained results should be used to develop an air disinfection system in the tested rooms.

MATERIALS AND METHODS

Research site

Microbiological tests were carried out in selected rooms at the Faculty of Civil Engineering and Environmental Sciences (FCEES) of the Bialystok University of Technology. The experiment was carried out from September 2023 to April 2024. The air was tested in four laboratory rooms and six toilets. Air sampling points location is shown in Figure 1 and Table 1 presents the characteristics of the rooms in which the tests were carried out.



Legend:

SYMBOL	OBJECT
○	Examined toilet
○	Examined laboratory room

SYMBOL	OBJECT
4	Faculty of Civil Engineering and Environmental Sciences
5	INNO-EKO-TECH

Figure 1. Research site

Table 1. Room characteristics

Laboratory room	Area [m²]	Temperature [°C]	Humidity [%]
45B – ground floor	51	22,6	19
2/11 – first floor	52	21,6	19
3/19 – second floor	69	21,2	26
4/16 – third floor	16	21,3	20
Toilet	Area [m²]	Temperature [⁰C]	Humidity [%]
T-1 – ground floor	14	19,5	21
T-2 – ground floor	11	21,5	20
T-3 – ground floor	16	21,8	21
T-4 – ground floor	9	17,5	22
T-5 – first floor	16	21,4	27
T-6 – third floor	16	21,3	20

Note: T-l – Toilet, first floor Building B, FCEES, at the Hall 39 B; T-2 – Toilet, first floor, center of INNO-EKO-TECH; T-3 – Toilet, first floor Building A, FCEES; T-4 – Toilet, first floor Building B, FCEES, at the Hall 15 B; T-4 – Toilet, second floor Building A, FCEES; T-6 – Toilet, fourth floor, center of INNO-EKO-TECH.

Research methodology

The following culture media were used to perform air microbiological quality tests:

- for determining the total number of psychrophilic and mesophilic bacteria – Enriched agar (BTL Sp. z o. o. company),
- for determining the number of fungi – Sabourauda agar with 4% glucose added (BTL Sp. z o. o. company),
- to determine the number of mannitol-positive and mannitol-negative *Staphylococci* – Mannitol salt LAB-AGAR (Chapman's medium) (BTL Sp. z o. o. company),
- for the determination of actinobacteria – Pochon medium (BTL Sp. z o. o. company).

The media were sterilized at 121 °C for 20 min and then poured into Petri dishes and dried. Air samples were taken at designated locations (Figure 1) using two methods: impaction and sedimentation. The sedimentation method involves the settling of cells from the air onto uncovered Petri dishes with an appropriate medium. The force of gravity acting on bioaerosol particles is only significant for larger particles, while smaller particles hit the exposed medium under the influence of air movement. This method is commonly used for the approximate determination of the number of microorganisms in the air and for comparative studies. Its main disadvantage is the inability to detect the smallest bioaerosol particles that form the respirable fraction, which settles very slowly or does not sediment at all. The impact method, on the other hand, involves the aspirator sucking in a known volume of air, which hits the surface of agar media at high speed. This causes microorganisms present in the air to stick, which after a specified incubation time produce colonies. The greatest advantage of this method is the ability to detect and determine the respirable fraction of bioaerosol, i.e. to determine the size distribution of the particles that make it up. This is very important because respiratory penetration depends on the size of the particles. The tests were carried out in the laboratory rooms after teaching classes, as well as in the public toilets at the FCEES complex.

For the sedimentation method, plates with previously prepared media were exposed at a height of approximately 150 cm for 20 minutes. For the impaction method, on the other hand, a Merck MAS-100 NT apparatus was used, with which 100 liters of air was aspirated directly onto the plates with media under the head of the apparatus. After the measurements, the solid media Petri dishes were placed into the incubator and incubated at appropriate temperatures. Depending on the type and growth requirements of the microorganisms, plates were incubated for 24–48 hours at 37 °C for mesophilic bacteria, *Staphylococci* and actinobacteria, and at 26 °C for 48–72 hours for total psychrophilic bacteria. Fungi and moulds were incubated for six days at 26 °C. After incubation, the number of colony-forming units (CFU) was calculated. In the impact method, the calculated result (CFU) was verified using the Feller table, and the result was then related to 1 m^3 of air. In the sedimentation method, the

number of bacteria in 1 m^3 of air was calculated based on the Omeliański formula [11]:

$$A = \frac{a \times 5 \times 10^4}{\pi \times r^2 \times t} \quad (1)$$

where: A is the number of microorganisms in l m^3 of air, a is average number of colonies per plate, r is the radius of the Petri dish [cm], t is the dish exposure time [min] and 5×10^4 is conversion factor.

The tests were carried out in two series of measurements, in different seasons (autumn 2023 and spring 2024), with the same principles and using the same equipment. Each series of measurements was performed in duplicate.

All statistical analyses were performed with Statistica 13 software.

Microbial air pollution

The evaluation of microbial air pollution was based on two formal documents (Polish Standards): PN-89/Z-04111/02 "Protection of air purity. Microbiological research. Determination of the number of bacteria in ambient air (immission) when sampling by aspiration and sedimentation methods" and PN-89 Z-04111/03 "Protection of air purity. Microbiological studies. Determination of the number of microscopic fungi in ambient air (immission) when sampling by aspiration and sedimentation methods". The given standards specified following indicators of bacteriological air pollution: the total number of mesophilic bacteria, actinobacteria, Pseudomonas fluorescens and *Staphylococci* (hemolytic or mannitol-positive) as well as microscopic fungi in 1 m^3 of ambient air. Determination of the above-mentioned indicators should be carried out simultaneously by impaction and sedimentation methods. The above standards were withdrawn without replacement in 2015. However, since no revisions to these documents or any documents specifying indicator microorganisms and their limit values in the ambient air have been released, most microbiological air pollution studies in Poland are still carried out in accordance with the above-mentioned methodology, and the results are interpreted on the basis of the above-mentioned standards. It should be emphasized that the result subject to interpretation is the higher number of the designated indicator obtained from the two research methods. Each of these methods has its advantages and disadvantages. The sedimentation method is based on the free settling of microorganisms onto solid medium and the results are calculated based on a semi-empirical formula. The impact method, on the other hand, uses precise measuring equipment, but the head must be disinfected after each measurement. Moreover, in the case of high air pollution, several microorganisms get onto the medium plate through the holes in the measuring device head, which makes the calculation difficult.

RESULTS AND DISCUSSION

The summary of results obtained by the sedimentation and impaction methods presented in Figure 2 shows that both methods give comparable results within the same groups of microorganisms and rooms. There are also no significant differences between the values obtained for toilets and laboratories in a given series of tests. However, it can be seen that in the case of psychrophilic and mesophilic bacteria, the concentrations of microorganisms in the autumn period (series 1) are significantly higher than in the spring period (series 2).

In order to confirm the above observations, due to the small number of samples and the lack of normality in the analyzed groups of variables, the Mann-Whitney U test was used to verify the null hypothesis (H0) about the lack of difference in central tendency in relation to the alternative hypothesis (H1) – there is a difference in central tendency between selected two groups of variables.

The following cases and groups of variables were analyzed, assuming that for the assumed significance $\alpha = 0.05$, H0 should be rejected if the determined for U statistics value of probability $p < \alpha$:

- concentration of microorganisms determined by the impaction method versus sedimentation method in a given group of rooms and a given series of tests;
- concentration of microorganisms measured in toilets versus laboratories for individual groups of organisms in a given series of tests;
- concentration of microorganisms determined in series 1 versus series 2 for individual groups of organisms in a given group of rooms.

Table 2 presents the calculated p values resulting from the comparison of results obtained by the sedimentation method versus the impact method. The Mann-Whitney U test results show that in series 1 only in the case of Actinomycetes

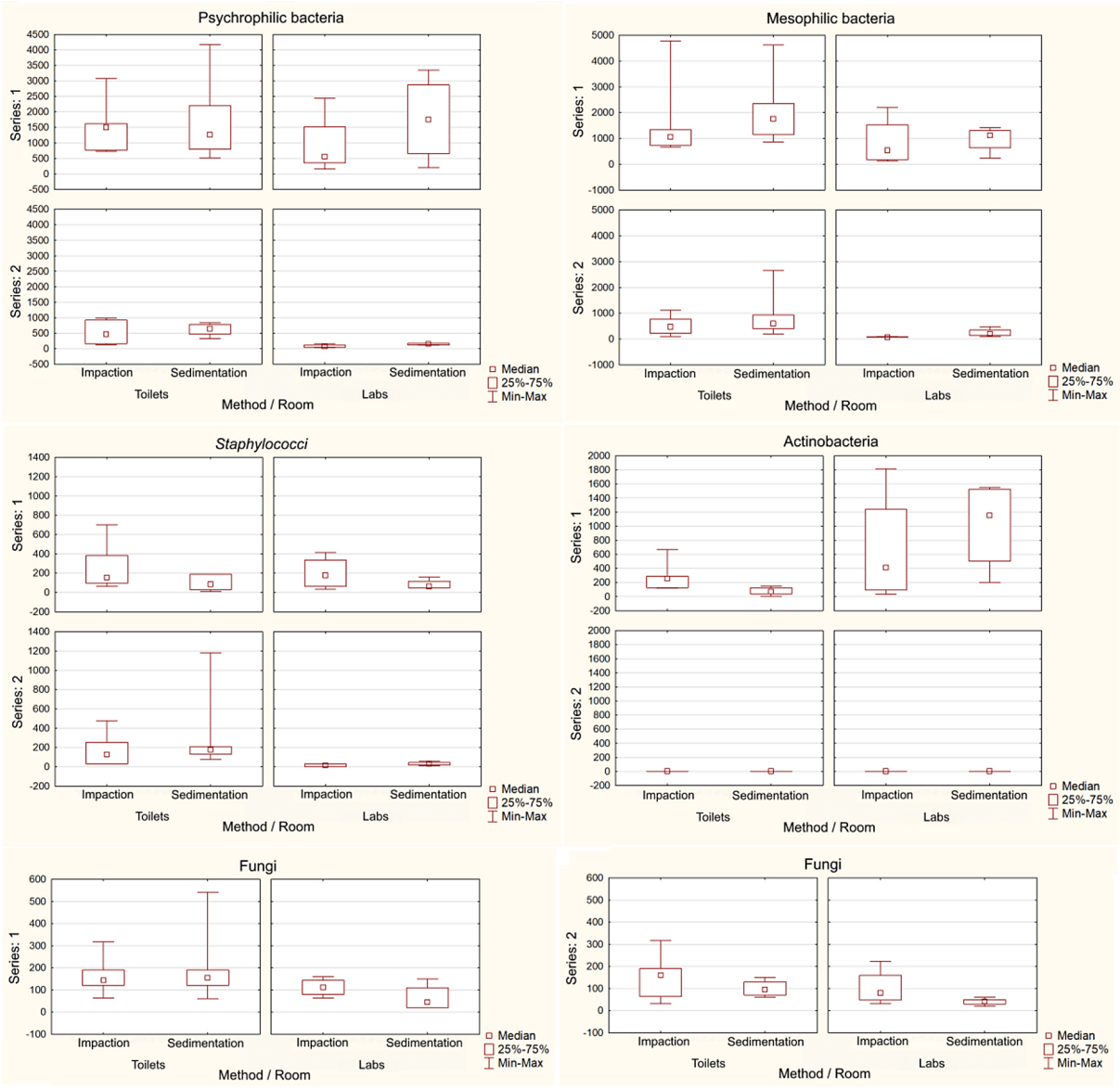


Figure 2. Concentration of bioaerosols in the air collected from toilets and laboratories of the FCEES, taking into account the methods used and the test series performed

and results obtained for toilets, and in series 2 – for mesophilic bacteria and laboratories, the null hypothesis should be rejected, which confirms that there is no significant differences between the results from the sedimentation and impact methods. Therefore, in subsequent analyses, the results obtained by these two methods were combined and treated as coming from the same population.

The results of the Mann Whitney U test comparing the concentration of microorganisms in toilets with the results from laboratory rooms in the individual test series (Table 3) show that there is no significant difference between the concentration of microorganisms in these groups of rooms determined in series 1 (except for Actinobacteria), while in Series 2 there is a significant difference between the results obtained in toilets and laboratories (except for fungi).

However, the comparison of the results for toilets and laboratory rooms in the first and second series of tests (Table 4) indicates significant differences in the concentration of psychrophilic and mesophilic bacteria between the autumn and spring period. This is confirmed by Figure 3, which presents a graph of means and confidence intervals for the concentration of microorganisms determined for toilets and laboratories in both series of tests. Additionally, it should be noted that in the autumn period (series 1) significantly higher

Table 2. Probability *p* of the Mann Whitney U test for results obtained by the impaction and sedimentation methods

Rooms	Microorganisms				
	Psychrophilic bacteria	Mesophilic bacteria	*Staphylococci*	Actinobacteria	Fungi
Series: 1					
Toilets	0.870	0.298	0.149	0.024	1.000
Laboratories	0.470	0.470	0.468	0.470	0.309
Series: 2					
Toilets	0.689	0.575	0.689	-	0.297
Laboratories	0.108	0.027	0.659	-	0.191

Table 3. Probability *p* of the Mann Whitney U test for the results obtained in toilets and laboratory rooms in the given series of tests

Test series	Microorganisms				
	Psychrophilic bacteria	Mesophilic bacteria	*Staphylococci*	Actinobacteria	Fungi
Series: 1	0.464	0.114	0.699	0.031	0.053
Series: 2	0.002	0.003	0.001	-	0.053

Table 4. Probability *p* of the Mann Whitney U test for the results obtained in series 1 and 2 in a given group of rooms

Rooms	Microorganisms				
	Psychrophilic bacteria	Mesophilic bacteria	*Staphylococci*	Actinobacteria	Fungi
Toilets	0.002	0.003	0.418	0.000	0.259
Laboratories	0.002	0.005	0.001	0.0004	0.429

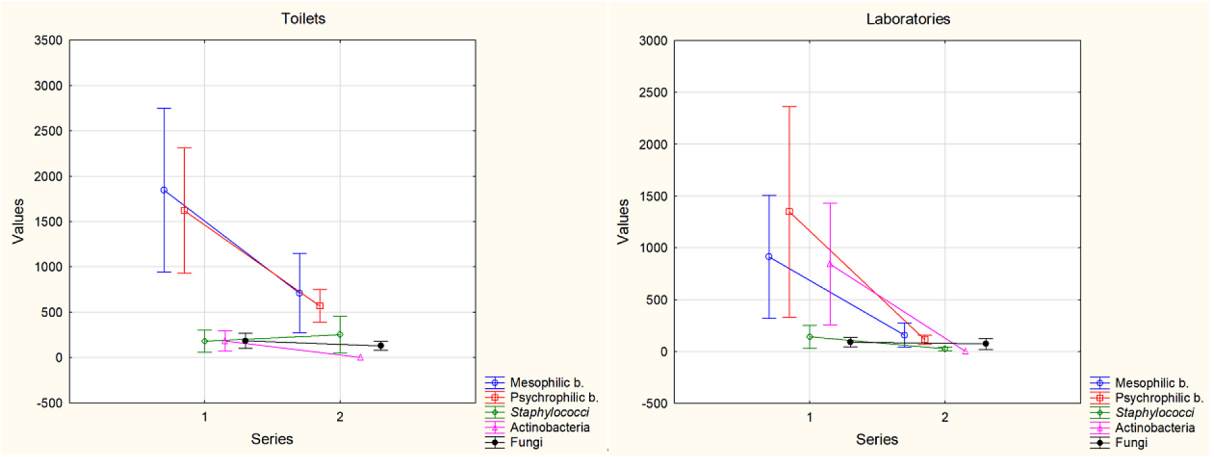


Figure 3. Graph of means and confidence intervals for the tested rooms and groups of microorganisms in two series of tests

CFU values were observed for psychrophilic and mesophilic bacteria than in the spring period.

To assess the level of microbial air pollution, Tables 5 and 6 prepared on the basis of withdrawn Polish Standards were applied

In order to assess the degree of bacteriological air contamination in the tested FCEES rooms, the highest CFU values were extracted from all test results and presented in Tables 7 and 8. The largest group of microbiological contaminants are

Table 5. Assessment of the degree of atmospheric air pollution by bacteria

Total bacterial count	Bacterial count				Degree of air pollution
	Pseudomonas fluorescens	Actinobacteria	*Staphylococci*		
			Mannitol-positive	Mannitolo-negative	
< 1000	Lack	10	Lack	Lack	Uncontaminated
1000–3000	<50	10-100	<25	< 50	Moderately polluted
> 3000	>100	>100	>25	> 50	Heavily polluted

Table 6. Assessment of atmospheric air pollution by microscopic fungi

Total number of fungi in 1 m^3 of atmospheric air	Degree of air pollution
3000–5000	Averagely clean atmospheric air, especially in late spring and early autumn
5000–10000	Pollution that may negatively affect the human environment
> 10000	Pollution that threatens the human environment

Table 7. The highest values of bioaerosols concentration in two series of measurements in laboratory rooms at FCEES

Indicator	Concentration of microorganisms [CFU/m^3]							
	First series				Second series			
	Lab 45B	Lab 2/11	Lab 3/19	Lab 4/16	Lab 45B	Lab 2/11	Lab 3/19	Lab 4/16
Psychrophilic bacteria	3.35×10^3	2.4×10^2	1.11×10^3	2.39×10^3	1.1×10^2	1.7×10^2	1.7×10^2	1.3×10^2
Mesophilic bacteria	2.197×10^3	2.4×10^2	1.03×10^3	1.42×10^3	1.0×10^2	2.2×10^2	4.7×10^2	1.7×10^2
Staphylococci	2.55×10^2	0.96×10^2	4.14×10^2	0.7×10^2	0.32×10^2	0.6×10^2	0.3×10^2	0.32×10^2
Actinobacteria	1.815×10^3	2.0×10^2	8.1×10^2	1.5×10^3	0	0	0	0
Fungi	1.59×10^2	0.96×10^2	1.5×10^2	0.64×10^2	2.23×10^2	0.96×10^2	0.64×10^2	0.32×10^2

Table 8. The highest values of bioaerosols concentration in two series of measurements in public toilets at FCEES

Indicator	Concentration of microorganisms [CFU/m^3]											
	First series						Second series					
	T-l	T-2	T-3	T-4	T-5	T-6	T-l	T-2	T-3	T-4	T-5	T-6
Psychrophilic bacteria	1.56×10^3	8.0×10^2	3.089×10^3	7.32×10^2	2.20×10^3	4.18×10^3	9.87×10^2	9.24×10^2	6.1×10^2	3.2×10^2	6.7×10^2	7.8×10^2
Mesophilic bacteria	1.529×10^3	1.15×10^3	4.777×10^3	8.92×10^2	2.35×10^3	4.62×10^3	2.67×10^3	4.1×10^2	1.115×10^3	1.9×10^2	9.3×10^2	6.3×10^2
Staphylococci	1.91×10^2	0.64×10^2	7.01×10^2	0.96×10^2	1.91×10^2	3.82×102	1.18×10^3	2.23×10^2	2.55×10^2	0.8×10^2	2.1×10^2	1.5×10^2
Actinobacteria	1.27×10^2	1.27×10^2	2.87×10^2	2.55×10^2	6.69×10^2	2.55×10^2	0	0	0	0	0	0
Fungi	5.41×10^2	1.59×10^2	3.18×10^2	0.64×10^2	1.9×10^2	1.91×10^2	1.91×10^2	1.59×10^2	1.59×10^2	0.6×10^2	3.182×10^3	0.7×10^3

mesophilic and psychrophilic bacteria. In each air sample in the first series of tests, the values of these indicators reached a high level, regardless of the room. The highest number of psychrophilic bacteria - 3350 CFU/m^3 of air was recorded in one of the laboratory rooms, which indicates heavy contamination and poor effectiveness of the ventilation system. In the study conducted at Kaduna State University in Nigeria, similar CFU values were found in women's hostel rooms [12]. It was found that the number of mesophilic bacteria ranged from $1.2 \times l0^3$ to 2.7×10^3 CFU/m^3 of air. In women's toilets, the number of mesophilic bacteria ranged from 3.8×10^3 to 6.7×10^3 CFU/

m^3 of air. It should be mentioned that the research was carried out only using the sedimentation method. Significantly lower air microbiological quality results were obtained by Abiola et al. [5] when testing air at the University of Ghana. The number of bacteria in the laboratories was on average 1.21 × 10^2 CFU/m^3, in the foyers – 2.49 × 10^2 CFU/m^3 and in the toilets – 2.06 × 10^2 CFU/m^3. Despite the low abundance of bacteria, the authors found that air conditioning had a negative impact on air quality.

Even higher amount of bacteria was recorded in one of the faculty's toilets in autumn, where 4.62 × 10^3 CFU/m^3 of mesophilic bacteria and 4.18 × 10^3 CFU/m^3 of psychrophilic bacteria were found.

The highest number of indicator microorganisms in the air was found in the autumn-winter period due to the fact that the research was conducted during an increased incidence of upper respiratory tract infections. The high number of bacteria also results from simple environmental requirements, as their development requires a positive temperature from 0 °C to 25 °C for psychrophilic bacteria, and in the case of mesophilic bacteria between 20 and 37 °C.

According to Libudzisz et al. [13], the amount of microorganisms in the air ranges from a few to as high as 10^7 CFU/m^3. Places of public use, which include higher education institutions, are characterized by widely varying number of students and staff. This is associated with the airborne transmission of microorganisms including bacteria, viruses and fungi which can contribute to the spread of various infections and diseases. In a study conducted by Brągoszewska et al. [14] in a kindergarten, primary school and university, the highest average number of bacteria in the bacterial aerosol was found in the primary school (2.205 × 10^3 CFU/m^3), while the lowest average number of bacteria was determined in the university building (3.91 × 10^2 CFU/m^3). In the kindergarten room, the average value was 1.408 × 10^3 CFU/m^3.

The greatest air pollution in both laboratory rooms and toilets is caused by the presence of *Staphylococci*. Their highest CFU number in the laboratory rooms was found in the first series of measurements – 4.14 × 10^2 CFU/m^3. Even greater sanitary hazard is associated with toilets, where the highest CFU number of 7.01 × 10^2 CFU/m^3 was found in the first series of tests, and in the second series - even 1.18 × 10^3 CFU/m^3. Higher concentrations of staphylococci in the air of toilets may be related to their smaller surface area compared to laboratory rooms. This translates directly into a higher density of people using these rooms, especially during breaks between classes. According to Grabińska-Łoniewska et al. [15], *Staphylococci* may cause catarrh of the respiratory mucosa, pneumonia or meningitis.

Microorganisms such as actinobacteria have high adaptability to all environments. Their presence indicates air pollution with soil particles. Dust and dried soil particles contained in the atmosphere enable cells to travel long distances and multiply and develop in favorable conditions. The presence of actinobacteria was found only in the first series of tests, and their number reached high values in all laboratory rooms and toilets tested, indicating strong air pollution. On the other hand, in the second series of measurements, no actinobacteria were found in the air samples. The reason for the air contamination with actinobacteria in the last series of measurements could be, on the one hand, the autumn period and the transfer of these bacteria with soil particles on the shoes of students and employees, and also the start of the heating season during the study period, which contributes to the emission of a large number of dust particles that promote the transmission of microorganisms in the air [16].

The number of fungi in the air of the rooms tested was low, regardless of whether the tests were carried out in the laboratory rooms or in the toilets. The results obtained for the fungi content indicate that they are not a factor influencing the air quality in the tested rooms. It was observed that the air in the bathrooms had a higher fungal content than in the laboratory rooms. The reasons for this condition may be moisture due to the frequent use of water in toilets, particles of dead organic matter, which are a source of food for fungi, as well as the surface of these rooms. Each of the above factors creates favorable conditions for the development of cells of these microorganisms [17]. Similar studies on the abundance of fungi and bacteria were carried out in public libraries in Islamabad, Pakistan [18]. The number of bacteria ranged from 0.2 × 10^2 to 2.3 × 10^3 CFU/m^3, and the number of fungi ranged from 0.2 to 2.5 × 10^3 CFU/m^3. These were definitely lower results than those obtained in the faculty's laboratory rooms, which was due to the combined system of effective ventilation and air conditioning. It was found that the abundance of

microflora in the rooms studied depended primarily on the number of students and staff present in the room. The microbiological quality is strongly influenced by the air exchange, as well as efficient ventilation and the degree of air humidity. The frequency and thoroughness of cleaning of the rooms surveyed, especially the floors and laboratory tables and the floors and WCs in the toilets, are also important.

The presence of *Staphylococci* in the air tested is particularly dangerous for students and academics. This indicates the need to disinfect the air with UV lamps or to use other methods of air disinfection in the tested rooms, with the emphasis on teaching halls. *Staphylococci* as well as other tested microorganisms can cause numerous diseases and allergies in humans. Unfortunately, there are still no norms and standards precisely defining the maximum allowable concentrations (MACs) of microbiological pollutants in the air. The presented proposals for permissible concentrations of microorganisms and endotoxins in the air, developed by the Team of Experts on Biological Agents of the Inter-ministerial Commission, do not include *Staphylococci,* but only mesophilic bacteria, Gram-negative bacteria, thermophilic actinomycetes, fungi and endotoxins [19–22]. Moreover, no standards have been proposed that would take into account the specificity of rooms and no indicator organisms specific to various types of rooms have been designated.

The development of clear, specific standards in this matter will allow for accurate interpretation of the results, take preventive measures regarding air cleanliness, and, above all, make society aware of the possible threats resulting from the presence of microbiological contaminants in the air.

CONCLUSIONS

In Poland, there are no microbiological air quality standards that would regulate the permissible number of indicator bacteria. Due to the above, the interpretation of the results obtained is only possible on the basis of the withdrawn Polish Standards.

Based on the tests carried out on the microbiological quality of air in laboratory rooms and toilets at the Faculty of Civil Engineering and Environmental Science, it was found that the degree of contamination with microorganisms is high.

In the first series of tests performed in the fall of 2023, it was found that the air in four laboratory rooms was heavily polluted by *Staphylococci* and actinobacteria, as well as by a high total number of bacteria. However, in the second series of measurements performed in the spring of 2024, the number of indicator bacteria was lower and indicated average air pollution.

In the six toilets tested, air pollution was high in both the first and second series. An above-average number of mesophilic bacteria, as well as mannitol-positive and mannitol-negative *Staphylococci* in total, and actinomycetes were found.

An effective solution to improve the air condition in the faculty's laboratory rooms, as well as in toilets, should be periodic air disinfection, especially during the period of increased illness among students and employees (e.g. by using UV lamps). In addition, the ventilation system should be improved by using appropriate filters.

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