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Wioletta PRZYSTAŚ[1*], Ewa ZABŁOCKA-GODLEWSKA[1]
and Edyta MELANIUK-WOLNY[1]

A COMPARISON OF SEDIMENTATION METHOD AND ACTIVE SAMPLER ANALYSIS OF MICROBIOLOGICAL INDOOR AIR QUALITY - CASE STUDY

Abstract: The indoor air quality is of great importance for the health of people. Appropriate selection of methods, parameters and conditions for the analysis makes it possible to obtain the results that reliably reflect the actual state of affairs. The aim of this study was to compare the results of microbiological air analysis in selected rooms of the Biotechnology Center obtained using the sedimentation method and the impaction method. During the research, the SMA (for total bacteria number) and Sabouraud media (for total fungi number) were exposed to different times in sedimentation analysis and to different air volumes in the impaction method. In the case of the sedimentation method, significant differences were found in the total number of bacteria and fungi in 3 out of 7 rooms depending on the time of exposure. In the case of the impaction method, it was 4 out of 7 rooms for bacteria and 2 out of 7 for fungi, depending on the analysed volume of air. The comparison of the methods showed that 4 out of 6 rooms had higher number of microorganisms when impactor was used.

Keywords: microorganisms, indoor air quality, impaction method, sedimentation method

Introduction

The air quality of the rooms in which we spend over 90 % of our lives has a huge impact on our well-being and health [1-5]. The phenomenon of sick building syndrome, which has a multifactorial characteristic, has been known for many years [6]. Indoor air quality depends on physical factors (such as temperature, humidity, ionising radiation, etc.), chemical factors (the presence of volatile organic and inorganic substances, including toxic ones), and also biological factors (microorganisms, toxins of various origins, fragments of insects, mites, their faeces, and particles produced by higher organisms) [1, 4, 5, 7-13]. Therefore, each case should be considered comprehensively taking into account all of the above. The impact of microbiological indoor air quality has been discussed for many years, but it was the outbreak of the pandemic that drew the attention of the whole world to microorganisms even more. The presence of pathogenic organisms (viruses, bacteria or fungi) has a significant impact on health. It can cause long-term effects, worsening the quality of life for many months or years, even after infection, and in extreme cases, lead to disability or death [1, 5, 8, 9, 11-15]. Currently, hospital risks are of particular concern.

[1] Department of Air Protection, Faculty of Energy and Environmental Engineering, Silesian University of Technology, ul. S. Konarskiego 22B, 44-100 Gliwice, Poland, phone: +48 32 237 20 38, +48 32 237 19 93, fax +48 32 237 16 55, email: ewa.zablocka-godlewska@polsl.pl, edyta.melaniuk-wolny@polsl.pl, ORCID: WP 0000-0002-7403-2043, EZG 0000-0002-4212-8913, EMW 0000-0001-9131-9532
[*] Corresponding author: wioletta.przystas@polsl.pl

This is of particular importance in the context of long-term exposure, including to antibiotic-resistant organisms [10, 16]. The outbreak of the SARS CoV-2 pandemic in 2019, contributing to the rapid spread of Covid 19 among the public, showed how important indoor air quality is and how quickly microorganisms can destabilise society, government and health services [12, 16-18]. Taking care of the appropriate microbiological quality of indoor air, determining the transmission of pathogens or the influence of ventilation and air conditioning systems on the spread of microorganisms has become a major problem.

Airborne microorganisms can be measured using a variety of procedures that can be classified as passive and active. Commonly used sampling methods include the sedimentation method (passive process), in which microorganisms settle directly on the agar medium under the influence of gravity, and the impaction method (active process), which uses a specific volume of air with germinal material which impacts on the agar inside the device [7, 8, 19].

Air quality tests in the field of biological factors include analysis of the presence of bacteria (including psychrophilic saprophytes and mesophiles, which indicate the possible presence of pathogenic bacteria), fungi, endotoxins and, finally, viruses in the environment [9, 12]. Depending on the scope of the analysis, various methods and techniques are used. Bacteria and fungi detection methods include those based on the cultivation of these organisms on solid agar media. The material can be collected using various techniques: directly onto the substrate (sedimentation or impaction method) and indirectly (using filters or scrubbers). Each of these techniques has advantages and disadvantages. Currently, the first group of methods is most commonly used. The sedimentation method has been used for a long time. Its basic disadvantages include the inability to determine the volume of analysed air to which the stated number of microorganisms should be related. Another problem is connected with the inability to detect the finest bioaerosol particles forming the respirable fraction that settles very slowly or does not sediment at all, as well as large inaccuracy caused by air movements changing sedimentation conditions. The factor influencing the final result may also be the selection of an appropriate exposure time depending on the expected amount of microorganisms in the air. The second method (impaction/collision) eliminates the first two factors to some extent. If appropriate devices are used, it is possible to determine the air volume or to collect the respirable fraction. On the other hand, a major disadvantage is the decrease in the viability of microorganisms caused by the shock associated with a sudden impact on the agar medium. In the case of this method, the volume of air taken is important, because if the number of microorganisms as well as the volume of air taken in by the impactor is too high, it is easy to overgrow the substrates and underestimate the results [11, 20]. On the base of this knowledge the aim of the research was to compare the results of air analysis methods in selected rooms of the Biotechnology Centre with the use of the sedimentation method and the impaction method by using AirIDeal 3P.

Materials and methods

Tested rooms

Indoor air quality tests were carried out in various rooms of the Biotechnology Centre (CB) of the Silesian University of Technology in Gliwice. The rooms differed in cubature, the nature of use, including the number of people who used these rooms were selected for

the analysis. The purpose of individual rooms and the approximate cubic capacity of the rooms are presented in Table 1. The rooms were analysed in terms of temperature and humidity, and the results are presented in Table 3.

Table 1

Characteristic of rooms at Biotechnology Centre

Room	Year of sampling	Usable area/ Cubic capacity [m^2/m^3]	The way of using the room
Office 1 A (O1)	2019	16.31 / 48.84	Microbiology office room. During the day it is used by 2 people
Office 1 B (O2)	2018	16.31 / 48.84	Microbiology office room. During the day it is used by 2 people
Microbiological laboratory (MLAB)	2019	51.07 / 153.21	Microbiological testing laboratory - carries out research as part of diploma theses. 2-4 people work in it every day. The conducted research concerns phytoremediation, wastewater treatment with the use of pure strains, analysis of raw materials and environmental samples. Equipped with a UV lamp. It is used to prepare materials for research, experiments, glass washing, etc.
Corridor part 1 (C 1)	2018	65.96 / 194.6	During the day, about 10-30 people move to the other rooms, including offices
Corridor part 2 (C 2)	2018	36.33 / 107.18	During the day, about 10-20 people move to the other rooms, including offices
Sterile room (SR)	2018	9.98 / 29.94	A room for microbiological analysis - cultures of water, raw materials, and the collection of pure cultures of microorganisms. Equipped with a UV lamp. During the day it is used by a maximum of 2 people
Wetland sewage treatment plant laboratory (WETLAB)	2018	46.21 / 117.84	Research laboratory with wetlands sewage treatment plants. It is located on the basement level of the building
Toilet (T)	2019	12.47 / 40.34	Women toilet. During the week, it is used by 4 to 12 people

Analytical methods

Analysis of the number of bacteria and fungi in indoor environment were carried out on the following media: Standard Method Agar (Sigma Aldrich) and Sabouraud with chloramphenicol (BTL). Sedimentation method (SED) and the AirIDeal 3P impactor were used (IMP).

Table 2

Characteristic of sampling methods

Room	Sedimentation method - times of exposure of plates [min]		Impaction method - volume of air analysed [L]	
	Short time (SED S)	Long time (SED L)	Low volume (IMP L)	High volume (IMP H)
Office 1 A (O1)	15	22	70	150
Office 1 B (O2)	15	22	100	150
Microbiological laboratory (MLAB)	12	17	70	150
Corridor part 1 (C 1)	10	15	80	120
Corridor part 2 (C 2)	15	25	200	300
Sterile room (SR)	25	40	200	400
Wetland sewage treatment plant laboratory (WETLAB)	-	-	50	100
Toilet (T)	12	20	100	150

Petri plates containing media (9 cm diameter) were exposed to different times in sedimentation analysis. The time selection was adjusted to the expected concentration of microorganisms. Exact exposure times are given in Table 2 (SED S - short time and sedimentation method; SED L - longer time and sedimentation method. Petri plates were left in room at the high of 30 cm or 80 cm (on the table). Different air volumes were used in the impaction method (Table 2). The aspiration flowrate of Air Ideal 3P is calibrated at 100 L/min with an impact velocity of less than 20 m/s. All analysis (for each time or volume) were performed in triplicate (IMP L - impact method low volume of air; IMP H - impact method higher volume of air).

The most important aspect of the research was to determine the potential threat to the people using the building. An appropriate indicator of health risk is the number of mesophilic bacteria, which also takes into account pathogenic organisms. The samples for bacteria enumeration were incubated for 48 hours at 37 °C. The Sabouraud plates allowing to determine the number of fungi were incubated at 26 °C for 5 days - 7 days. After the appropriate incubation time, the number of grown colonies was determined. In the impaction method, the final values were read from the tables for the AirIdeal 3P apparatus. In the case of the sedimentation method, the number was calculated according to the Omeliansky formula in the Gogoberidze modification [1]:

$$N = \frac{a \cdot 5 \cdot 10^4}{\pi \cdot r^2 \cdot t} \tag{1}$$

where: N - number of bacteria or fungi [CFU/m^3] (in 1 m^3 of air), a - average number of bacteria colonies or fungal colonies detected on surface of Petri plates, r - radius of Petri plate [cm], t - exposure time [min].

Results and discussion

Influence of the time and volume

The methods of bioaerosol analysis are constantly changing due to their improvement. The oldest one is sedimentation method. Its basic disadvantages result, among others, from the fact that the microorganisms in the air are associated with smaller or larger particles, therefore they fall at different rates. There are also factors such as air movements caused, among others, by researcher's movement. Therefore, an appropriate selection of the exposure time of the culture media is such an important factor. The confirmation of the above-mentioned statements is also shown by the obtained research results (Figs. 1 and 2). During the analysis of air the number of bacteria (Fig. 1) in the toilet was 917 CFU/m^3 for the exposure time of 12 min, and 491 CFU/m^3 for the duration of 20 min, i.e., twice as less. Similar differences were obtained for the air in the C1 room (for 10 min and 15 min, 275 CFU/m^3 and 0 CFU/m^3, respectively) and for the C2 room (for the 15 min and 25 min, 236 CFU/m^3 and 94 CFU/m^3, respectively). In the case of the analysis of the number of fungi (Fig. 2), such large differences were obtained for C2 air (for 15 min and 25 min, 133 CFU/m^3 and 60 CFU/m^3, respectively) and for the sterile room (for 25 min and 40 min: 314 CFU/m^3 and 0 CFU/m^3, respectively). It should be emphasised that only in the case of two out of 7 rooms, higher values of the number of bacteria were found with longer exposure times than with shorter ones. In the case of fungi it was 3 out of 7 rooms, respectively. The higher numbers are due to the aforementioned fact that larger particles with microorganisms settle quickly. Therefore, when calculating according to the formula

that takes into account the time, we obtain higher values for small exposure times. However, the results for C1 and Sterile room are interesting, where after a long time the values were at the level of 0 CFU/m^3. This situation results from the fact that the microorganisms in the air do not exist in a uniformly dispersed form. Therefore, we can obtain a different result in each place of the exhibition.

The imperfections of the sedimentation method are eliminated by the aspiration method. Active uptake of microorganisms should eliminate e.g., the problem of the size of the particles with which the microorganisms are associated. According to the manufacturers' recommendations, the volume of the air sample taken should be adjusted to the expected contamination. As a rule, it is advisable to draw 150 liters of air. However, for sterile rooms, the volumes should reach 1 m^3 and more.

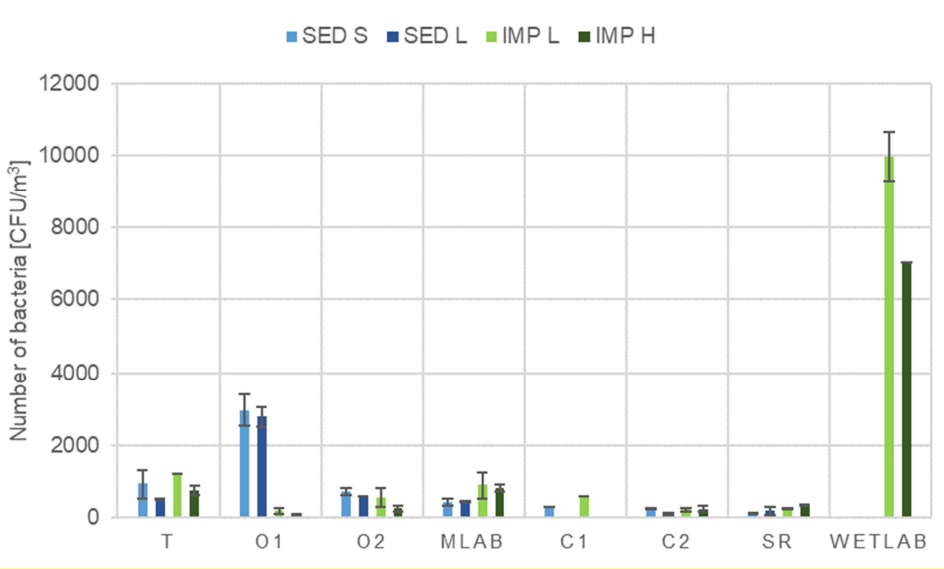


Fig. 1. Total number of bacteria in rooms evaluated by sedimentation and impaction method (SED L - lower time exposure; SED H - higher time exposure; IMP L - lower volumes of air; IMP H - higher volumes of air)

Simultaneously with the analysed using the sedimentation method, the impaction analyses were performed (Figs. 1 and 2). Two volumes were selected for each room to determine whether the volume of air taken in has a significant impact on the obtained count results. Also, in the case of this method, recommended for air analysis, significant differences were found in the number of microorganisms in the air depending on the volume taken. The number of bacteria (Fig. 1) in the toilet for the volume of 100 L and 150 L was respectively 1205 CFU/m^3 and 741 CFU/m^3. In the O1 office for the volumes of 70 L and 150 L it was 186 CFU/m^3 and 60 CFU/m^3, and in the case of the O2 office for the volumes of 100 L and 150 L respectively 552 CFU/m^3 and 249 CFU/m^3. The number of bacteria in the corridor (C1) was, respectively, for the volume of 80 L and 120 L 566 CFU/m^3 and 0 CFU/m^3, and in the case of WETLAB, highly humid and with high temperature, with the volume of 50 L and 100 L, the results were almost 10,000 CFU/m^3

and 7,000 CFU/m^3. The results of the analysis in the case of the number of fungi were not such large (Fig. 2). The greatest differences were found in the case of WETLAB, where respectively 337 CFU/m^3 and 171 CFU/m^3 were recorded. It should be emphasised that only in the case of one out of 8 rooms, higher values of the number of bacteria as well as fungi were found with higher volume of air than with lower ones.

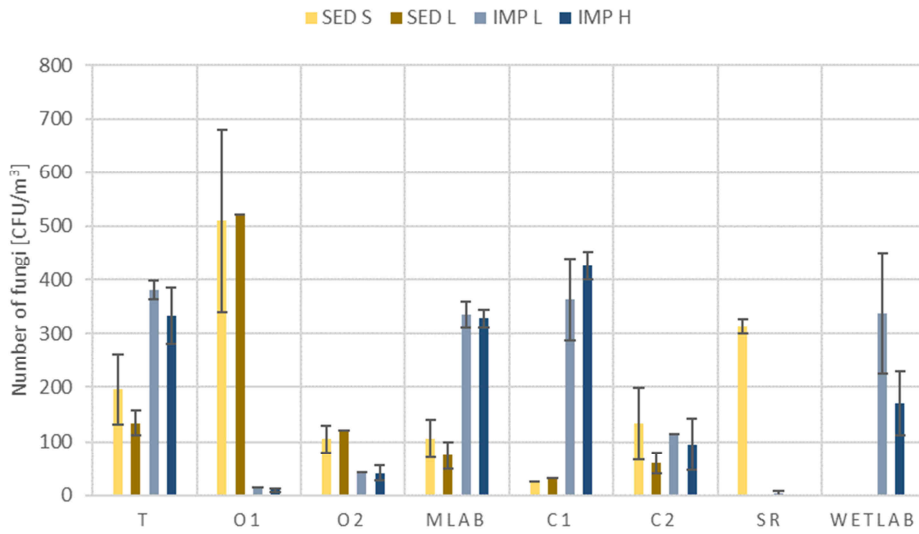


Fig. 2. Total number of fungi in rooms evaluated by sedimentation and impaction method (SED L - lower time exposure; SED H - higher time exposure; IMP L - lower volumes of air; IMP H - higher volumes of air)

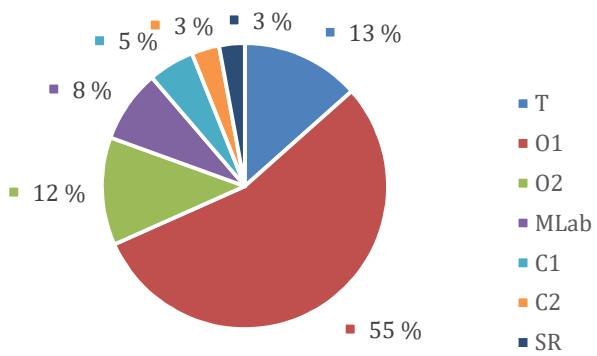
Influence of the method

The comparison of analyses results for both methods was made on the basis of average values calculated for the results from short and long times together, as well as from small and large volumes of air taken (Fig. 3). This approach is, of course, flawed, taking into account, for example, the large discrepancies between the results of analyses described above. To show significant differences in the results obtained with the two methods, the percentage of bacterial and fungal microflora of individual rooms in the microflora of the Biotechnology Center was determined. Significant differences were observed in the results obtained with both methods, both in the case of the number of bacteria and fungi. The sedimentation analysis showed that the O1 office (55.6 % of bacterial microflora of the Biotechnology Center) is the most contaminated room with bacteria, followed by the toilet (14 %) and the O2 office (12 %). On the other hand, the results of the impaction method showed that the greatest number of bacteria was found in the toilet (28 %), MLAB laboratory (25 %), followed by corridor C1 and the adjacent office (6 % and 12 % respectively). In the case of fungi, differences were also obtained in the results of analyses using both methods. According to the results of the sedimentation method, the most polluted was the office (39 % of the fungal microflora of analysed rooms of CB building), followed by MLAB (24 %) and the toilet (13 %). Air analysis with Air Ideal showed the

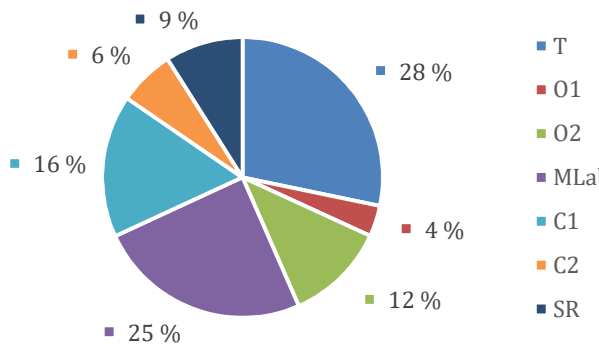
highest pollution in corridor C1 (32 %), in the toilet (29 %) and the MLAB laboratory (27 %). The analysis of the total microflora using the sedimentation method showed that the O1 office is the most polluted (52 %), as is the O2 office (i.e., the same office analysed a year earlier), and the toilet (11 % and 13 %, respectively). According to the impaction method, however, the toilet (28 %), the MLAB laboratory (25 %) and the corridor (C1).

Taking into account the purpose of the rooms and the number of people using them on a daily basis, as well as the condition of the surface, objects, etc., the results obtained with the impaction (active) method seem to reflect the actual condition of the rooms more closely. The highest emissions can be expected in the toilet, which is also an active source of microbial emission during flushing, and in the microbiological laboratory. It is surprising that there is heavy contamination in the office, especially detected by the sedimentation method. It may result from the fact that microorganisms are carried on the surface of the body or clothes from the laboratory by people working in it. Such a phenomenon may also be indicated by the numerous bacterial and fungal microflora of the corridor detected during study.

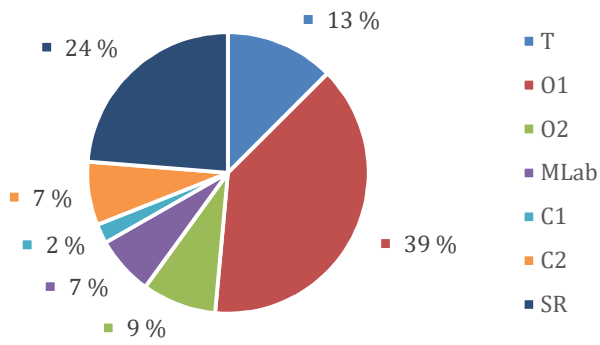
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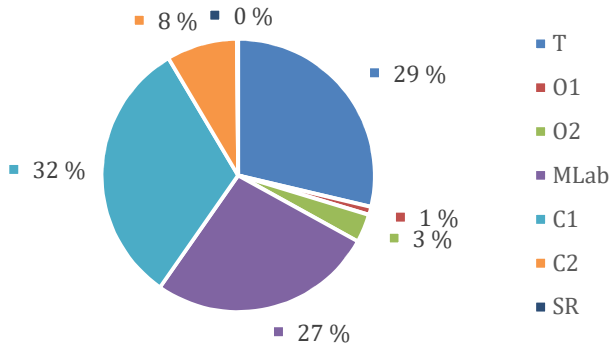
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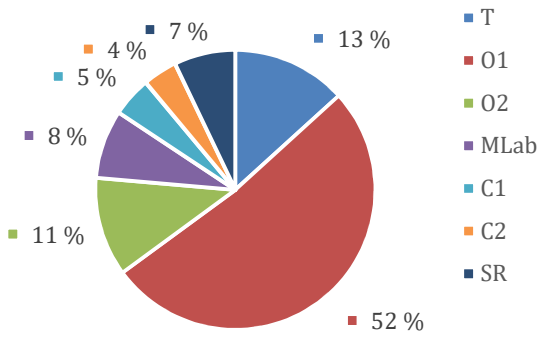
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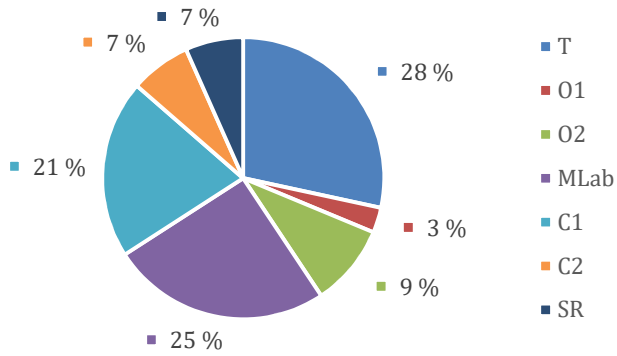


Fig. 3. The percentage of: a) mesophilic bacteria detected by sedimentation method, b) mesophilic bacteria detected by impaction method, c) fungi detected by sedimentation method, d) fungi detected by impaction method, e) all microflora detected by sedimentation method (bacteria +fungi), f) all microflora detected by impaction method (bacteria+fungi), of individual rooms in the microflora of the Biotechnology Centre

The obtained results are at least partially different from those presented by Haas et al. [21]. These authors proved that in the case of fungi the impaction method is better for detection of spores of *Aspergillus niger*, and in the case of *Staphylococcus aureus*, the sedimentation method. However, as the research conducted at the Biotechnology Centre shows, at least in 3 out of 7 rooms (Fig. 2), fungi were detected in a much greater number by the sedimentation method. At the same time, in 4 out of 7 rooms, more bacteria were detected by the aspiration method. However, the difference in the applied research methods should be emphasised. In the case of the research presented by Hass et al. [21], an analytical chamber was used, and the research presented in this paper was conducted in rooms used by employees and students. Thorough mixing of the air in the analytical chamber, thanks to the use of appropriate systems, certainly ensured an accurate distribution of spores and microbial cells, which cannot be achieved in real rooms with gravity ventilation. At the same time, the source of microorganisms in the system was in the upper part of the chamber, while in the laboratory rooms rather in the lower part. Perhaps this contributed to obtaining different results in the case of several of the analysed rooms.

Influence of the humidity on the bacteria and fungi number

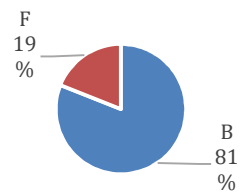
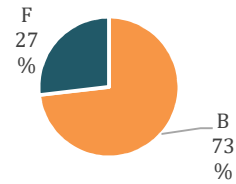
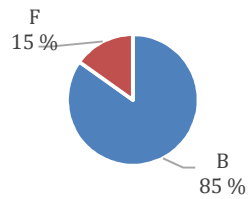
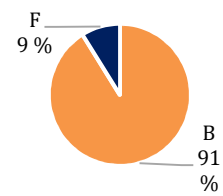
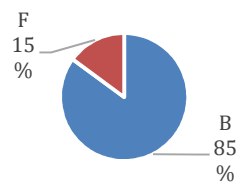
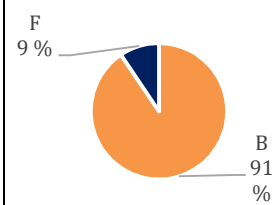
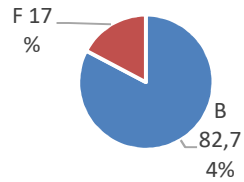
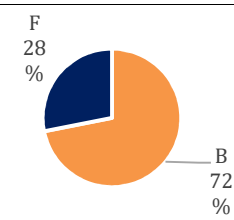
The results of the number of microorganisms obtained by the sedimentation method show that the higher the air humidity in the room, the greater contribution of fungi in the total analysed microflora (Table 3).

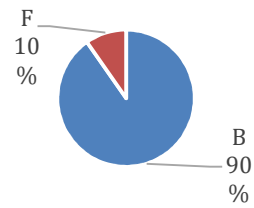
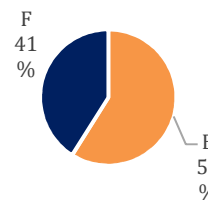
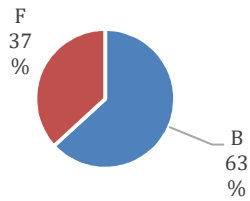
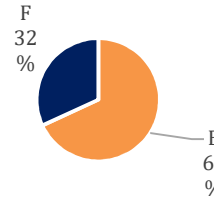
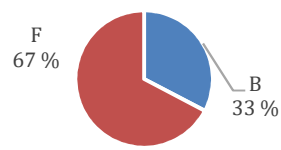
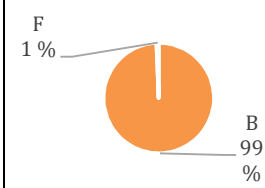
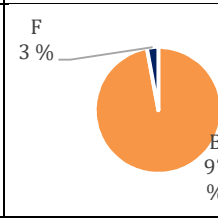
As numerous studies show, air humidity has a significant impact on the number and composition of the air microflora [1, 17, 22, 23]. The results of analyses presented in Table 3 confirm that the higher the air humidity, the higher the share of fungi in the bioaerosol. In the case of room C1 with the lowest humidity, the contribution of fungi in the microflora was only 10 %. However, at ~36 % humidity, the share of fungi was different. The highest was noted in the case of the SR, i.e., the sterile room (67 %), which may be due to the properties of the spores, which are resistant to various physical and chemical factors,

especially short-term UV exposure. In the case of the impaction method, the results of the share of fungi and bacteria in the total bioaerosol do not correlate with air humidity. In the toilet, the fungal microflora constituted 27 % of the total microflora, in corridor C2 as much as 32 % (at ~ 36 % humidity), however, with much lower humidity (29 %) in corridor C1, the fungal microflora constituted as much as 41 %. In opposite to the results of sedimentation analysis in the sterile room, the share of fungal microflora detected by AirIdeal 3P in the total bioaerosol was only 1 %. In the examined WETLAB laboratory, despite high humidity (52 %), only 3 % of microflora was fungi. Thus, as the research results show, the purpose of the room and the processes taking place in it are more important than the humidity, especially in the case of laboratories and their surroundings.

Table 3

Influence of temperature and humidity on the microflora (B - bacteria; F - fungi) of different rooms taken by both methods

Room	Temperature [°C]	Humidity [%]	Microorganisms	
			Sedimentation method	Impaction method
T	22	36		
O1	22.6	30.5		
O2	22.6	30.5		
MLAB	22.1	32.7		

C 1	22.2	29		
C 2	21.5	36.6		
SR	22.2	36.6		
WETLAB	22	52	nd*	

*nd - not detected

Conclusion

The test results obtained by both methods confirm the importance of appropriate selection of the sampling parameters, regardless of the method used. Both the appropriate selection of the medium exposure time in the sedimentation method, as well as the appropriate selection of the air volume in the impaction method have a significant impact on the value of the final results. The criticised sedimentation method did not give much lower values of the number of fungi and bacteria compared to the impaction method. The underestimation of the number of microorganisms with use of sedimentation method was clearly visible only in the case of 3 rooms analysed for fungi. At the same time, it should be noted that it was the discrepancies in the results for these three rooms that determined that differences were observed when in contribution of bacteria and fungi in the microflora of individual rooms. As a result, the number of fungi detected by the sedimentation method seem to be in close correlation with air humidity. The greater share of fungal microflora in the total microflora in the case of this analysis was in rooms with higher humidity. In the case of the analysis with AirIdeal 3P, no such correlation was

observed. Research comparing the results using both methods and different types of instruments and techniques needs to be continued.

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