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Influence of climate on microbiological air quality in school classrooms

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Abstract: The aim of the study was to assess the microbiological quality of air in a naturally ventilated classroom used by students. The research was conducted over a nine-week cycle during the winter-spring-summer period of 2024. Bacterial and fungal concentrations in the air were analyzed, and the obtained results were compared with indoor microclimate parameters and atmospheric conditions. It was found that after classroom airing, bacterial counts decreased significantly, while mold spore counts increased, indicating a significant influence of outdoor air on the composition of indoor bioaerosols. During classes, there was a systematic increase in both bacterial abundance and diversity, with the primary source being the room occupants. The variability in microbial counts was also strongly correlated with weather conditions - dry, sunny days promoted reductions, while high humidity and limited exposure to sunlight increased their presence. The identified microorganisms belonged to genera typical of indoor and outdoor environments, including *Staphylococcus, Bacillus, Serratia, Aspergillus*, and *Penicillium*, some of which are potentially pathogenic taxa. Regression modeling confirmed that key factors shaping microbial abundance were indoor and outdoor air temperatures. Despite the observed fluctuations, air quality remained within the typical range for public spaces and did not pose a significant health risk to users. The results emphasize the importance of appropriate ventilation and microclimate control in ensuring proper microbiological air quality in classrooms

Introduction

The microbiological quality of indoor air is crucial for the health and comfort of building occupants, particularly in the context of prolonged exposure to enclosed spaces (Frąk et al., 2024). Bioaerosols - containing bacteria, fungi, viruses, as well as their fragments and metabolites-are the primary source of biological air pollutants, with their composition and abundance depending on various environmental, technical, and anthropogenic factors (Eduard 2009; Mandal and Brandl 2011; Górny 2010). Key sources of microorganisms in indoor air include humans, animals, organic materials, and outdoor air infiltrating buildings through ventilation systems or windows (Rai et al. 2021; Meadow et al. 2014; Douwes et al. 2003). The literature indicates that human activity (such as walking, movement, and presence) increases bacterial and fungal concentrations in the air (Qian et al. 2012), while the human microbiome plays a significant role in shaping indoor air microbiota (Hospodsky et al. 2012). Additionally, building materials, interior furnishings, and moisture promote the growth of molds and environmental bacteria (Meadow et al. 2014). The presence of microorganisms can degrade air quality and trigger allergic reactions, infectious diseases, or sick building syndrome (SBS) (WHO 2009; Dutkiewicz and Górny 2002).

Meteorological conditions, i.e., temperature, humidity, sunlight, and atmospheric pressure, significantly influence the occurrence and survival of microorganisms in atmospheric air and, indirectly, in indoor environments (Zhen et al. 2017; van Rhijn et al. 2021; Lee et al. 2006). Studies have shown that dry, sunny, and warm periods limit fungal presence, whereas high humidity and low sunlight favor their proliferation (Mandal and Brandl 2011). The most commonly identified bacteria in indoor air belong to the genera *Staphylococcus, Micrococcus, Bacillus, Corynebacterium, Pseudomonas*, and *Acinetobacter* (Gohl et al. 2019; Hospodsky et al. 2012). The most common fungi include *Aspergillus, Penicillium, Cladosporium, Alternaria*, and *Rhizopus* (Eduard 2009; Rai et al. 2021). Some of these, such as *Aspergillus fumigatus* or *Staphylococcus*

aureus, may be pathogenic or allergenic, posing a health risk when present in the air (Mandal and Brandl 2011; Dutkiewicz and Górny 2002).

In Poland, the microbiological air quality assessment still relies on standards PN-89/Z-04111 and PN-EN 13098:2007P, as well as guidelines from the Institute of Occupational Medicine and the State Labour Inspectorate (Frąk et al., 2024; Górny 2010). These guidelines allow for the assessment of the microbiological conditions in indoor air and, consequently, the areosanitary risk to occupants. These classifications are based on both quantitative values (CFU/m³) and the qualitative composition of bioaerosols.

Górny (2010) and Krzysztofik (1992) propose classifications of bacterial and fungal concentrations based on their health impact on occupants and the type of room. Górny suggests that microbiologically uncontaminated air should contain fewer than 5000 CFU/m³ (upper limit) of bacterial cells or fungal spores, whereas Krzysztofik specifies maximum should be 3000 CFU/m³ for school premises. In contrast, WHO guidelines (2009, 2023) are considerably more stringent, recommending that the total bacterial cells or fungal spore count in occupied spaces should not exceed 500 CFU/m³. Guidelines for so-called non-industrial occupational environments indicate a limit of no more than 100 CFU/m³ of microorganisms. These examples show that defining a universally acceptable concentration of microorganisms in clean indoor air is not easy. Similarly, determining the aerosanitary status of non-medical premises is difficult, particularly in the absence of legally binding regulations. Therefore, precise assessment of the health risk by airborne miroorganisms is not currently possible.

It is known that microbial concentrations should remain low and are influenced by both indoor and outdoor microclimatic conditions, including ventilation intensity. (Frąk et al., 2024). Indoor air quality is also assessed using physicochemical parameters such as temperature, relative humidity, and CO_2 concentration. The optimal temperature in residential and office environments typically ranges from 20 to 25 °C, depending on seasonal and local standards OSHA (2011). The recommended relative humidity range is 30% to 60%, which helps reduce the risk of mold growth and occupant. Thermal conditions (temperature and humidity) affect perceived thermal comfort, as recognized in standards such as ASHRAE (2023). In many countries, a CO_2 concentration below 1000 ppm is considered an indicator of proper ventilation, whereas higher values often suggest insufficient air exchange (Mandall et al. 2011; Hospodsky et al. 2012).

Ventilation systems, particularly mechanical ventilation with filtration, can significantly reduce microbial concentrations in the air (Meadow et al. 2014; Lisik and Cichowicz 2022; Zhao et al. 2025). However, poorly maintained ventilation may become a source of secondary contamination. Research indicates that microbial removal efficiency depends on the installation`s technical parameters and the air exchange rate (Qian et al. 2012). Therefore, it is essential not only to determine the total number of microorganisms in indoor air, but also to consider their taxonomic composition and potential pathogenicity.

Increased indoor microbial diversity is associated with various health effects. Higher bacterial alpha diversity has been linked to allergic diseases, whereas reduced fungal diversity has been observed in children with metabolic or behavioral disorders.

Zhao et al. (2024) demonstrated that indoor microbial richness (α-diversity) is positively correlated with lung function, although an excessive total microbial load may place a burden on the body. An annual study of six homes in China (Chen et al. 2024 A, B) revealed seasonal differences in bioaerosol composition, with bacterial dominance in summer (particles<4.7 μm), and identified dominant genera such as *Anoxybacillus, Brevibacillus*, and *Acinetobacter.*

Relative humidity (RH) strongly influences the stability and behaviour of biological particles in the air, as well as droplet deposition processes; this the effect depends on the type of pathogen (Zhen et al. 2017; Zhao et al. 2025). Higher temperatures generally accelerate the inactivation of many microorganisms in aerosols, resulting in shorter survival times. However, this relationship is modulated by humidity and the type of organism (Qian et al. 2012, Rai et al. 2021)). The optimal indoor relative humidity is reported to be around 40–60%, which can reduce the survival of certain viruses (Jones et al., 2022).

Although microbiological indoor air quality has been investigated across various building types, studies that combine real-world classroom conditions, detailed daily variability, and advanced statistical analyses remain limited. The novelty of the present study lies in integrating traditional microbiological air quality assessment with simultaneous monitoring of indoor microclimate parameters and outdoor meteorological conditions in a naturally ventilated educational environment.

Additionally, the study evaluates short-term changes in microbial abundance across consecutive classroom use periods within a single day, providing a better understanding of dynamic bioaerosol behavior under typical educational use scenarios. Another innovative element is the application of regression-based machine learning models combined with SHAP analysis to identify and quantify the relative importance of environmental factors influencing bacterial and fungal concentrations.

The study also provides new insights into the dual impact of natural ventilation, demonstrating that airing may simultaneously reduce bacterial loads while increasing fungal spore concentrations due to outdoor air infiltration. These findings contribute to a more comprehensive understanding of microbiological air quality in educational buildings.

Methodology

Experiments

All measurements were conducted in the same classroom located in the building of the Faculty of Civil Engineering and Environmental Sciences at Warsaw University of Life Sciences (SGGW) in Warsaw, central Poland (Fig. 1). The same number of students participated in each session and were divided into three subgroups attending classes at different times of the day (10:15-12:00; 12:15-14:00; 14:15-16:00). The study was conducted 9 times at weekly intervals during the winter-spring-summer period of 2024.

The classroom is located on the top floor of a four-storey building and is designed for 24 occupants. It has an area of 30.42 m² and a height of 3 m. The room is equipped with natural

Fig. 1. The classroom used in experiments, with visible ventilation

ventilation (two vents, as shown in Fig. 1). Due to its location on the top floor, stack ventilation is particularly effective.

Air temperature and humidity were measured in the classroom. Measurements were taken at the beginning, middle (25 min. after the start of class), and end of the lessons (100min after the start of class). Indoor air parameters were recorded using a Testo 435 data logger equipped with an IAQ probe. The probe accuracy was as follows: temperature range -20°C to +50°C, ± 0.3°C; relative humidity range +2 and +98% RH, ± 2% RH. Measurements were recorded at 1-min intervals. The reported values represent averages of 30 consecutive samples.

The instruments were placed at a height of about 90 cm above the floor (the classroom is flat, with no podium for the lecturer). This height was assumed to correspond to the position of a seated student's breathing zone. The instruments were positioned at a distance of approximately 1 m from occupants to minimize the influence of nearby emission sources on the measurements. Air temperature measurements were also carried out at heights of about 1.0 to 1.1 m from the floor, ceiling, and walls, in accordance with ASHRAE 2023 recommendations.

The error analysis of the measured values was conducted in accordance with the recommendations of Moffat (1988) and Dobkowski & Gajewski (2025). The participants were volunteers who provided informed consent to take part in the study. They were young adults aged 20-22 years with no reported medical conditions. In the outdoor environment, the following meteorological parameters were monitored: air temperature (at a hight of 2 m), relative humidity (at 2 m), and wind speed (at 5 m). Measurements were performed according to the guidelines of Polish Institute of Meteorology and Water Management, based on recommendations of the World Meterological Organization.

Methodology for testing the microbiological condition of indoor air.

The following were examined: (1) total bacterial count and (2) total mold fungal count. Analyses were performed using the standard plate method on selective media: (1) nutrient agar and (2) Martin's agar (basic). Cultures were obtained using the impaction method (according to PN-EN 14583:2008; PN-89/Z-04008/08:1989), and air sampling was conducted using a Merck MAS-100 air sampler. Air volumes of 100 dm³, 200 dm³, and 300 dm³ were collected directly onto sterile Petri dishes containing the respective media (1) and (2). Cultures were incubated at 28°C for 48 h (bacteria) and 72 h (fungi). Colony-forming units were counted, and the results were expressed as CFU per 1 m³ of air (according to PN-EN 13098:2007P). Statistical corrections specific to the sampling method were used.

The applied methodology aligns with generally accepted standard microbiological practices. Currently in Poland, PN-89/Z-04008/08:1989 guidelines are used, which define air sampling procedures for microbiological analyses. Although the provisions were originally developed for atmospheric air studies, the sampling procedure is identical for indoor air applications. This standard was withdrawn without replacement on 25.08.2025; to date, no direct successor with a comparable methodological scope has been issued. Alternatively, PN-EN ISO 13098:2007P is applied in indoor air microbiological quality studies, providing guidance on the presentation of results in occupational environments. Volumetric sampling principles are specified in PN-EN 14583:2008. Additional methodological guidelines can be found in PN-EN ISO 14698-1:2004 and PN-EN ISO 14698-2:2005, which address microbiological control of air in cleanroom environment. Collectively, these standards describe consistent principles for conducting such studies.

Regression modeling

To investigate the relationships between microclimate factors, meteorological conditions and microbial counts (bacteria and fungi) in a classroom setting, six regression models were selected to capture both linear and nonlinear dependencies: linear regression with polynomial features, Ridge regression, Gaussian process regression (GPR), multilLayer perceptron (MLP) Regression, gradient boosting regression (GBR), and k-nearest neighbors (kNN) Regression. These models were implemented using the *scikit-learn* library in

Table 1: Hyperparameter sets analyzed using GridSearchCV for each regression model.

Model	Hyperparameters Analyzed
Linear Regression (LP)	polynomialfeatures__degree: [1, 2, 3], linearregression__fit_intercept: [True, False], linearregression__positive: [True, False]
Ridge Regression	alpha: [0.1, 1.0, 10.0]
Gaussian Process Regression (GPR)	kernel: [RBF(length_scale= [1. 00, 1.78 3.16, 5.62, 10.00], alpha: [10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}]
Multi-Layer Perceptron (MLP)	hidden_layer_sizes: [(10,), (10, 10)], activation: [“relu”, “tanh”], solver: [“adam”], alpha: [0.01, 0.1, 1.0], max_iter: [200], early_stopping: [True], validation_fraction: [0.1]
Gradient Boosting Regression (GBR)	n_estimators: [50, 100], learning_rate: [0.01, 0.05], max_depth: [2, 3], min_samples_split: [5, 10], min_samples_leaf: [3, 5], subsample: [0.6, 0.8], max_features: [“sqrt”, None]
k-Nearest Neighbors (kNN)	n_neighbors: [5, 10, 15], weights: [“distance”], metric: [“euclidean”, “manhattan”]

Python (Pedregosa et al. 2011). The dataset included variables such as ventilation duration, number of students, indoor and outdoor temperature, indoor and outdoor humidity, and wind speed, with bacterial and fungal counts as the target variables.

The data were split into training and test sets at a 67:33 ratio. Model performance was evaluated using repeated k-fold cross-validation (5 folds, 3 repeats) to ensure robust estimation given the small dataset size (n=54). Hyperparameters for each model were optimized using grid search (GridSearchCV) to balance model complexity and generalization. The hyperparameter sets analyzed for each model are summarized in Table 1 and were selected empirically. For linear regression, polynomial features (degrees 1-3) were included to capture nonlinear relationships, while Ridge regression incorporated L2 regularization to mitigate overfitting. GPR used a radial basis function (RBF) kernel with optimized length scales and noise levels. MLP was configured with compact architectures and early stopping. GBR employed shallow trees with subsampling, and kNN used distance-weighted neighbors to smooth predictions. Model performance was assessed using the coefficient of determination (R^2) for both training and test sets.

To interpret the relationships between environmental factors and microbial counts, SHAP (SHapley Additive exPlanations) values were computed using the SHAP library (Qian et al. 2012). For linear regression, SHAP values were calculated using LinearExplainer, enabling attribution of contributions to the original features after polynomial transformation. For tree-based models, TreeExplainer was applied (GBR), wheraes KernelExplainer was used for GPR, MLP, kNN, and Ridge. Partial dependence plots (PDPs) were also generated to visualize the average effect of each environmental factor on microbial counts, providing insights into their dependencies.

Table 1. Hyperparameter sets analyzed using GridSearchCV for each regression model.

Discussion of results

Throughout the experiment, air temperature ranged from 19.5 to 29.5°C (Tab. 2). During winter and spring measurements,

Table 2: Indoor air properties in the classroom during microbiological state studies.

date	temperature [°C]			humidity [%]		
	beginning	after 25 min	after 100 min	beginning	after 25 min	after 100 min
22.02.2024	19.0	19.5	20.2	41.6	40.8	40.2
	20.7	19.4	20.6	49.5	56.3	52.4
	20.6	21.5	21.2	50.3	45.8	42.7
29.02.2024	20.8	19.9	20.3	43.3	53.6	49.7
	20.6	21.1	20.6	47.0	49.9	50.5
07.03.2024	19.8	21.4	21.6	26.6	25.4	27.8
	21.8	21.8	23.0	23.8	25.3	26.4
	22.7	25.0	26.3	20.8	23.0	22.1
14.03.2024	19.8	21.6	21.9	40.7	49.9	49.6
	22.1	22.8	23.0	44.7	44.7	45.6
	25.7	24.5	26.2	38.1	40.1	41.3

date	temperature [°C]			humidity [%]		
	beginning	after 25 min	after 100 min	beginning	after 25 min	after 100 min
21.03.2024	20.2	22.0	22.5	32.9	36.0	39.0
	20.2	22.0	22.5	34.0	37.0	40.0
	22.0	23.3	23.5	32.5	41.0	44.8
28.03.2024	20.9	23.0	23.8	39.1	45.0	48.6
	22.8	23.8	24.3	44.1	43.9	47.8
	23.2	24.2	24.4	46.9	52.0	53.7
11.04.2024	24.6	24.6	24.7	32.4	33.8	34.7
	22.2	24.5	24.7	24.9	28.8	31.1
	26.5	25.2	25.4	27.0	32.7	31.4
21.04.2024	27.0	26.7	27.1	45.5	50.0	53.0
	20.3	21.2	22.2	38.8	43.3	46.6
25.04.2024	19.9	21.3	22.2	38.8	43.3	46.6
	20.0	20.7	21.3	36.1	41.2	43.7
06.06.2024	25.9	25.6	26.4	52.9	52.7	46.5
	27.4	27.9	27.7	41.1	47.5	44.5
	28.3	28.1	28.6	40.8	43.5	43.8
11.06.2024	24.9	26.1	26.6	45.3	47.5	45.8
	27.2	27.0	27.1	41.7	45.5	42.2
	27.3	26.9	27.2	37.8	41.0	40.7
	20.3	21.2	22.2	38.8	43.3	46.6
25.04.2024	19.9	21.3	22.2	38.8	43.3	46.6
	20.0	20.7	21.3	36.1	41.2	43.7
06.06.2024	25.9	25.6	26.4	52.9	52.7	46.5
	27.4	27.9	27.7	41.1	47.5	44.5
	28.3	28.1	28.6	40.8	43.5	43.8
11.06.2024	24.9	26.1	26.6	45.3	47.5	45.8
	27.2	27.0	27.1	41.7	45.5	42.2
	27.3	26.9	27.2	37.8	41.0	40.7

classroom temperatures complied with relevant guidelines (PN-EN 16798-1:2017-06; ASHRAE 2020), whereas in summer they exceeded recommended limits. Humidity ranged from 23 to 56.3% (Tab. 2), with some measurements indicating excessively dry indoor air. In naturally ventilated rooms, indoor environment parameters depend on heating system operation and outdoor conditions, which explains the deviations from recommended standards) observed during the summer period.

The obtained microbiological results indicate a consistent trend in microbial abundance: following airing periods, bacterial counts decrease significantly, whereas fungal counts increase. This pattern reflects the typical dynamics of microbial populations in rooms with ventilation driven by outdoor air exchange (Meadow et al. 2014; Zhen et al. 2017). Indoor air quality depends, among other factors, on the quality of infiltrating air, which is closely linked to current weather conditions, including sunlight, air humidity, and wind intensity (Zhen et al. 2017). It is also closely related to the number of people present in the room at a given time, as well as their activity and movement between indoor and the

Table 3: Meteorological conditions (average daily values) during experiments.

date	outdoor humidity 2m [%]	outdoor temperature 2 m [°c]	wind vel. 5 m [m/s]
15.03.2024	80.72	6.02	1.94
21.03.2024	71.51	7.03	1.40
28.03.2024	64.95	12.27	1.97
11.04.2024	51.80	12.83	1.73
25.04.2024	70.86	7.93	1.25
06.06.2024	64.02	19.74	1.60
11.06.2024	61.75	18.47	1.84

outdoor environments. Studies by Krzysztofik (1998), Rai et al. (2021), and Jones et al. (2022) further indicate that microbial abundance is strongly dependent on the room usage and furnishings. Changes in weather conditions and human activity within the room therefore play a significant role in shaping microbial concentrations in indoor air. Accordingly, meteorological conditions (Table 3) and indoor microclimate parameters were also analyzed in this study.

Microclimate studies

Table 3 presents the outdoor meteorological conditions recorded during the indoor measurements. Average daily values were used in regression modeling.

The measured indoor air temperature and relative humidity are presented in Table 2. During all experimental series, air temperature ranged from 19.5 to 29.5°C. During winter and spring, classroom temperatures complied with relevant guidelines (PN-EN 16798-1:2017-06; ASHRAE 2020), whereas in summer they exceeded recommended limits. Humidity ranged from 23 to 56.3%, with some measurements indicating excessively dry indoor air. In naturally ventilated rooms, indoor parameters depend on heating system operation and outdoor conditions, which explains the deviations from recommended standards observed during the summer period. Additionally, with a kigh number of occupants, stack ventilation is insufficient, as indicated by elevated CO_2 concentrations and humidity levels.

Quantitative and qualitative studies of bioaerosols

The analysis of microbiological results indicates that total bacterial counts decreased following classroom airing (Fig. 2), consistently across successive measurement cycles (1-3, 4-6, and 7-9). The lowest bacterial concentrations were observed before the start of classes, prior to student occupancy, ranging from 0 to 200 CFU/m³ of air (with a mean value of up to 104 CFU/m³ over the study period). These levels correspond to guidelines for indoor air of very high microbiological quality. It means that the applied supply ventilation was sufficient for air exchange and improved the sanitary quality of indoor air. Initial air quality (measurement No. 1) was generally high; however, it varied depending on prevailing meteorological conditions (Tab. 3). Lower bacterial concentrations were found during dry and sunny weather (0-50 CFU/m³ on 15.03 and 25.04), whereas higher values were observed on rainy and cloudy days (140-200 CFU/m³ on 21.03, 21.04, and 6.06).

During subsequent room airing on the same day (measurements No. 4 and No. 7), bacterial counts also decreased, although typically to slightly higher levels than those observed at the start of the day, with mean values of 206 CFU/m³ and 257 CFU/m³, respectively. Notably, with successive classroom

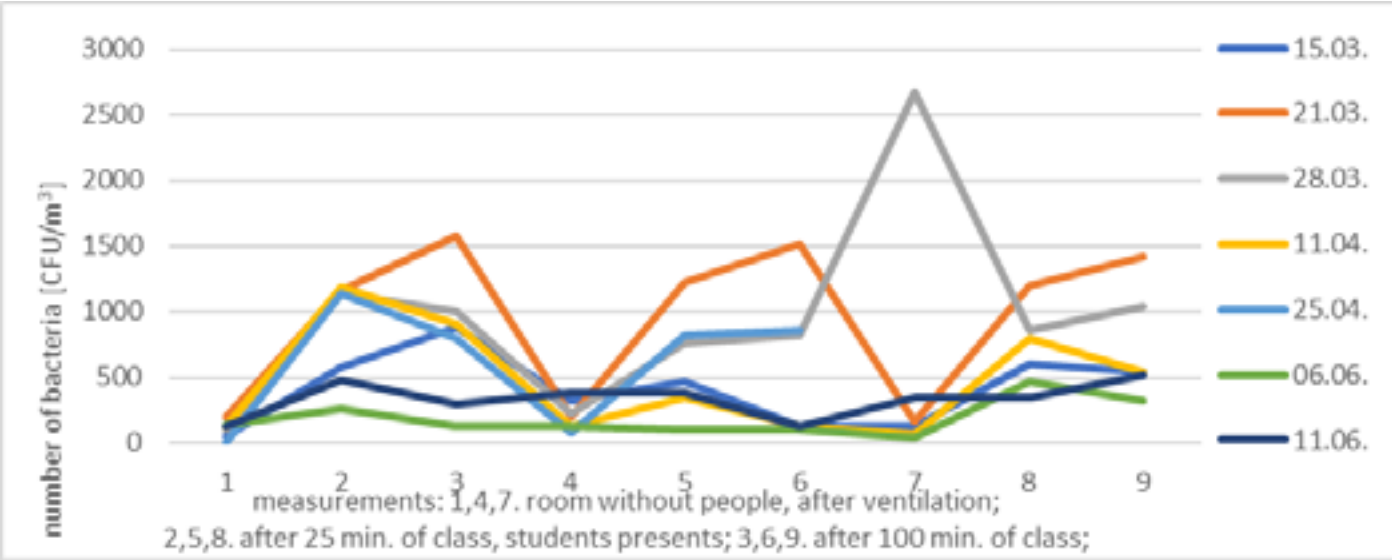

Fig. 2. Dynamics of the total number of bacteria in the indoor air.

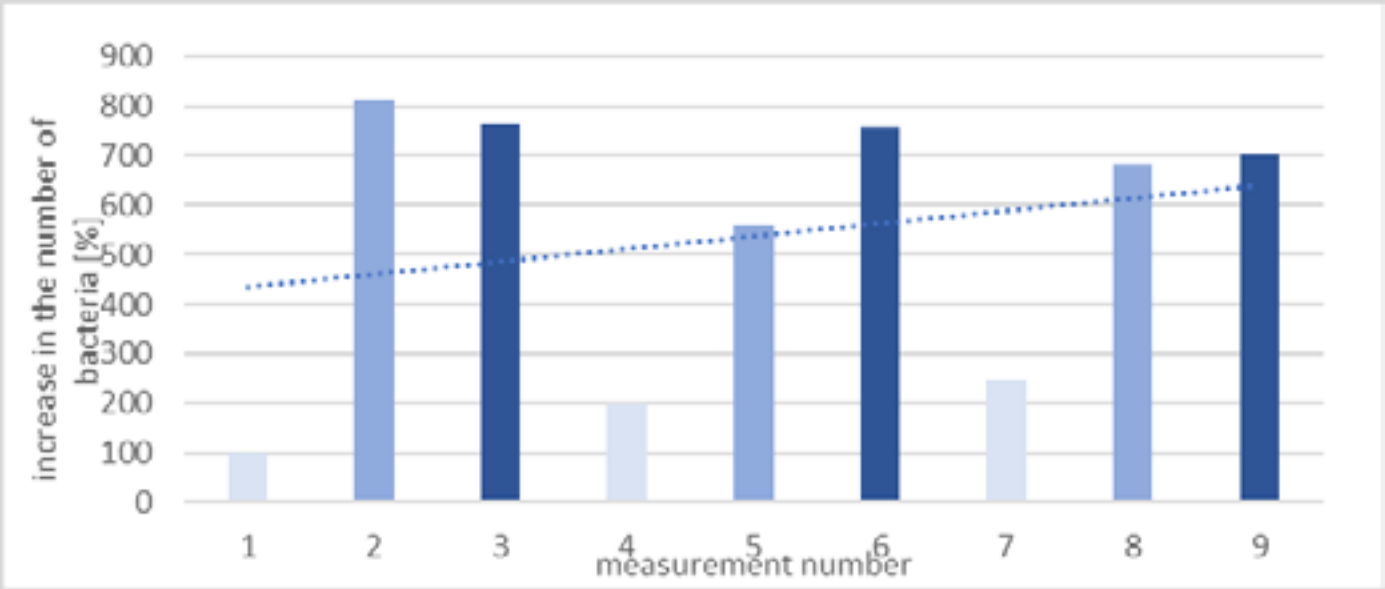

Fig. 3. Percentage increase in the number of bacteria (measurement No. 1 marked as 100%)

use and changes in student groups, the microbiological quality of the air after airing did not return to initial levels. Compared to measurement No. 1, bacterial counts increased on average by 98% (measurement No. 4) and 147% (measurement No. 7), and by 24% between measurements No. 4 and No. 7.

Bacterial counts in the air increased after students entered the classroom (Fig. 3). The longer the classes lasted, the higher the bacterial concentration, despite the use of supply ventilation. On average, after 100 minutes of occupancy, bacterial counts increased by 560-814% relative to measurement No. 1. The most pronounced increase in bacterial counts was observed during the first measurement measurements period (No. 1-3). In subsequent periods (No. 4-6 and No. 7-9), increases were slightly lower compared to the initial period; however, the overall trend in airborne bacterial concentrations remained upward (Fig. 3). The observed dynamics of bacteriological contamination are consistent with typical patterns reported for occupied rooms with supply ventilation (Krzysztofik 1992; Zhen et al. 2017; Zhao et al. 2025).

Fig. 4 presents detailed changes in bacterial counts in the air of the analyzed room on 21.04.2024. On this day, the bacteriological measurement results followed a different pattern. After successive airings of the analyzed room, bacterial counts in the air decreased relative to measurement No. 1: initially by 25% and then by 60%, despite an increase in air contamination immediately after students entered the classroom. Moreover, in the three successive measurement groups (1-3, 4-6, 7-9), the total bacterial count decreased. The observed trend was unexpectedly atypical and opposite to that recorded on other study dates (Fig. 4). It is difficult to explain these surprising dynamics. A probable cause may be linked to the decreasing size of successive student groups on that day (on other dates, group sizes were constant), though this relationship was not analyzed in the present study. Another possible factor is the

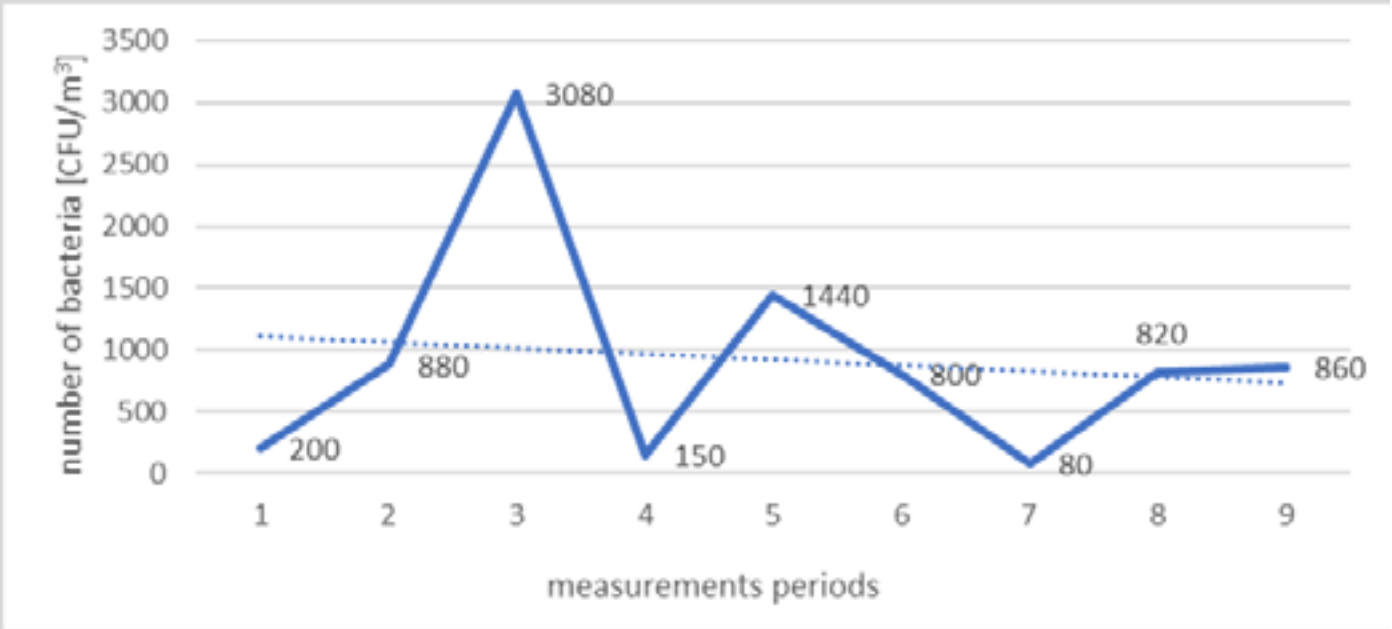

Fig. 4. Trend of changes in the total number of bacteria in the indoor air - on 21.04. 2024.

influence of particular weather conditions: on that day and the preceding days, it was very hot and dry, which undoubtelly contributed to a significant decrease in microorganisms in outdoor air and lower bacterial survival in indoors (Tab. 2). Table 3 presents outdoor air parameters which, in the case of rooms without mechanical ventilation, correspond to indoor air parameters: an average air temperature of 21.23°C and average humidity of 49.5%.

It is precisely changes in weather conditions that should be considered the primary cause of fluctuations in the microbiological state of the air in rooms with window ventilation. Analysis of bacterial count dynamics over seven measurement dates (Fig. 2) shows thatnthe pattern was consistent across three measurement cycles on six of these dates. However, on 28.03, it was observed that after the third airing (measurement No. 7), bacterial counts increased by more than 26-fold (i.e., by 2680%) compared to the initial level on that day. This atypical increase in indoor air bacteriological contamination may be related to changes in bacterial counts in the indoor air introduced into the room during airing, as well as to specific meteorological conditions conducive to microbial survival.

During the experiment, it was also observed that each measurement day was associated with an increase not only in the number of cultured colonies but also in the taxonomic diversity of the bacteria forming them. In the conducted experiment, diversity was assessed solely based on morphological differences in colonies and cells using microscopic techniques. At the first measurement on a given day, 4 to 6 colony types were identified, comprising cells with coccal and bacillary morphology (both Gram-positive and Gram-negative). Over the course of three subsequent measurement cycles on the same day, colony diversity increased to as many as 18 morphological types.

The taxa forming the numerically dominant colonies belonged to the genus *Tetracoccus* spp. and the family *Bacillaceae*. Among the cultured colonies, those characteristic of *Serratia* spp. (likely *Serratia marcescens*, forming blood-red colonies), various *Staphylococcus* spp. (including yellowish-golden *S. aureus*), *Bacillus mycoides* (light, highly branched colonies), and *Proteus* spp. (light, diffuse colonies) were also observed.

The presence of *Bacillus mycoides* and *Actinomycetales* colonies in the cultures indicates contamination of the supplied air with soil particles. In contrast, *Serratia, Staphylococcus,* and *Proteus* species are typically associated with gut microbiome. Their presence in the air suggests sanitary contamination and an increased health risk for room occupants. Based on the observed abundance and diversity, the analyzed air can be classified as acceptable, uncontaminated, or moderately bacterially contaminated, depending on the criteria applied (e.g., PN-89/Z-04111; PN-EN 13098:2007P; Górny 2010). The recorded concentrations correspond to those reported for generaluse, non-industrial indoor environments, including residential settings (Górny and Dutkiewicz 2002; Rai et al. 2021).

In indoor air microbiological studies, an analysis of mycological contamination dynamics was also conducted. It was found that the trend in changes differed from that of bacteriological contamination. An increase in mold spore counts was observed after each room airing (Fig. 5, measurements No. 1, 4, and 7), followed by a decrease over the three measurement cycles. Initial abundances (measurement No. 1 after airing) ranged from 0 to 700 CFU/m³. After 25 minutes of students' presence in the classroom, fungal counts decreased by about 40-20%. After a further 35 minutes, irregular changes in abundance occurred, ranging from approximately -15% to +30%.

The highest fungal abundances were found in June (6.06 and 11.06), ranging from 40 CFU/m³ (measurements No. 2, 5, and 8) to 640-1000 CFU/m³. This period was characterized by frequent rainfall, high atmospheric humidity, and relatively low temperatures (approx. 19-20°C; Tab. 3), which contributed to faster fungal proliferation in the soil, higher emissions into the atmosphere, and prolonged spore survival (van Rhijn et al. 2021). The dynamics of fungal counts recorded in the experiment indicate that mycological air contamination in rooms with supply ventilation is closely linked to spore emissions from the outdoor environment and the ambient air.

The obtained results also indicate that the overall trend in fungal counts across most measurement cycles is slightly

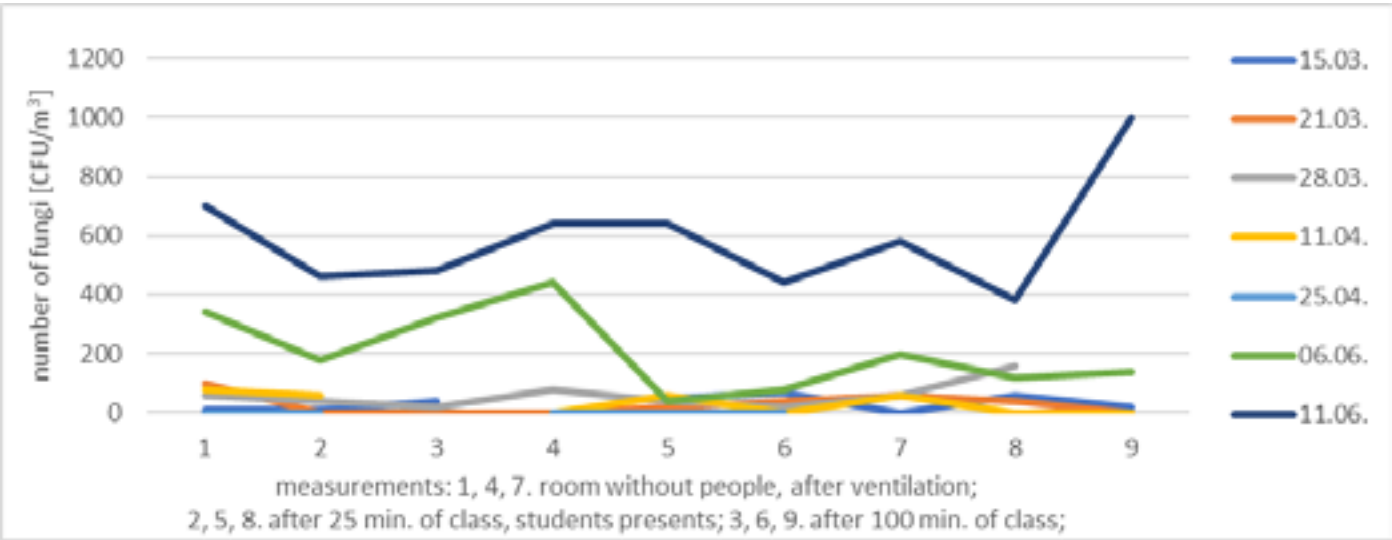

Fig. 5. Dynamics of the total number of fungi in the indoor air.

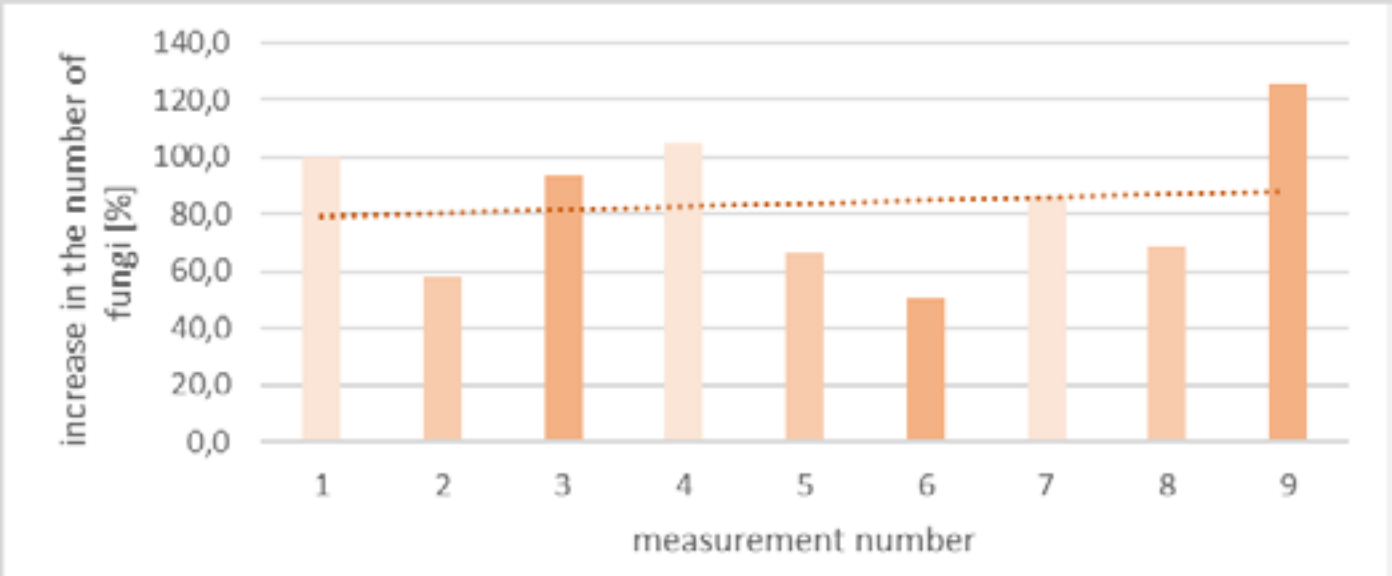

Fig. 6. Percentage increase in the number of mould fungi (measurement No. 1 marked as 100%)

increasing (Fig. 6), with spore counts rising on average by about 10% relative to initial values. Only on 21.04, similarly to bacteriological contamination, did the fungal counts exhibit a different and unexpected pattern (Fig. 7). Initially, 20 CFU/m³ was recorded, followed by a marked decrease in fungal spore counts to 0 CFU/m³. The overall trend was decreasing, with the trend line reaching negative values in the third stage of the measurement cycle. On 25.04, a similar pattern in fungal count dynamics was observed, with no fungal spores detected in the analyzed air throughout the entire measurement cycle. The likely cause of these results was the prevailing weather conditions during this period: very high solar radiation, elevated temperatures, and low atmospheric humidity (Tab. 3), which are unfavorable for the survival of microscopic fungal spores (Lee et al. 2006; van Rhijn et al. 2021). These conditions likely contributed to the high mycological purity of the supplied air during room airing.

The conducted studies demonstrated the dominance of taxa typical of soil and plant matter biodegradation processes. These were mainly species belonging to the genera *Aspergillus, Penicillium*, and *Rhizopus*. Individual species from these genera commonly occur in the natural environment and are also frequently isolated from so-called house dust. Among the identified taxa, the presence of *Aspergillus niger*, a known producer of mycotoxins (Eduard et al. 2009), was noted. The presence of this species in indoor air may indicate a potential health risk to occupants, particularly in relation to food contamination.

In air samples collected in June, sparse *Trichoderma* spp. were also detected, indicating air contamination with

Fig. 7. Trend of changes in the total number of mould fungi in the indoor air - on 21.04.2024.

Table 4: Performance metrics (R^2) for regression models on training and test sets for bacteria and fungi count prediction.

Model	Bacteria Count (R^2 Test)	Bacteria Count (R^2 Train)	Fungi Count (R^2 Test)	Fungi Count (R^2 Train)
Ridge Regression	0.288	0.609	0.432	0.539
Linear Regression (LP)	0.281	0.609	0.521	0.593
Gaussian Process Regression (GPR)	0.191	0.973	0.034	0.987
Multi-Layer Perceptron (MLP)	-0.528	-0.810	-0.262	-0.423
Gradient Boosting Regression (BT)	0.426	0.963	0.699	0.984
k-Nearest Neighbors (kNN)	0.347	1.000	0.271	1.000

soil particles. During the experiment period, no soil-derived yeasts (e.g., *Saccharomycetes*) or human-associated yeasts (e.g., *Candida*) were found. The abundance and diversity of fungi in the analyzed air indicated microbiologically uncontaminated air quality (according to PN-89/Z-04111; PN-EN 13098:2007P; Górny 2010) and moderate contamination (according to Krzysztofik 1992; Górny and Dutkiewicz 2002).

Regression modeling results

Statistical modeling was used to investigate the relationship between indoor and outdoor air conditions and the microbiological quality of air in the analyzed room. The performance of the regression models for predicting bacterial and fungal counts is summarized in Table 4, based on the coefficient of determination (R^2) for both the training and test sets. For bacterial count prediction, the Gradient Boosting Regression (BT) model achieved the highest test R^2 value (0.426), followed by the k-Nearest Neighbors (kNN) model (R^2 = 0.347). However, both models exhibited signs of overfitting, with training R^2 values of 0.963 for BT and 1.0 for kNN, indicating limited generalization despite moderate test performance. Other models, such as Multi-Layer Perceptron (MLP) regression, performed poorly, with a test R^2 of -0.528, suggesting they are unsuitable for this task. For fungal count prediction, the BT model again performed best (R^2 = 0.699), followed by Linear Regression (LR) (R^2 = 0.521). As with bacterial counts, the BT model showed overfitting (training R^2 = 0.984), whereas LR demonstrated more balanced performance (training R^2 = 0.593). The remaining models, particularly MLP (test R^2 = -0.262), underperformed in predicting fungal count.

Given the reasonable performance of BT model for bacterial and fungal count prediction, SHAP (SHapley Additive exPlanations) values were analyzed to better understand the dependencies between environmental factors and microbial counts. SHAP summary plots for these models are shown in Figures 8-9, where the x-axis represents SHAP values (i.e., the impact on model output), and the color gradient indicates feature values (blue for low and red for high values).

For bacterial count prediction using the BT model (Figure 8), airing duration emerged as the most influential factor, with SHAP values ranging from approximately -400 to 300. Higher air exchange rates (red dots) were associated with a significant decrease in bacterial counts (negative SHAP values), indicating that increased ventilation effectively reduces bacterial load. Outdoor air temperature at 2 m hight also showed a notable impact (SHAP values from -150 to 150), with higher temperatures (red dots) generally associated with decreased bacterial counts. This finding is contrary to the expectation that higher temperatures provide more favorable conditions for bacterial growth. Wind speed (SHAP values ranging from -100

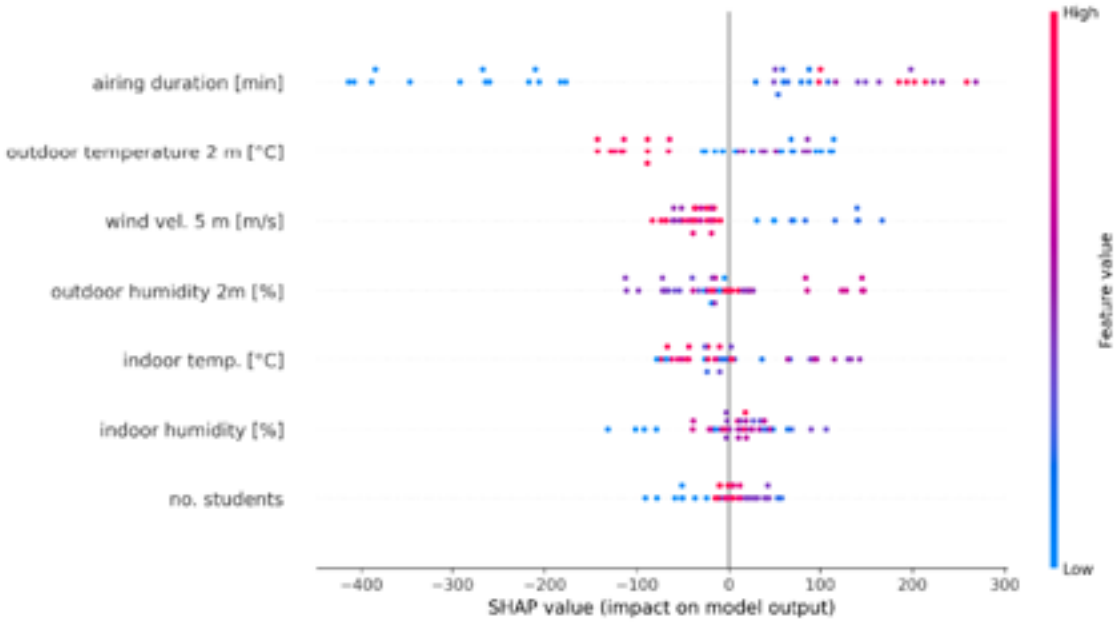

Fig. 8. SHAP values for bacteria count prediction using the BT model.

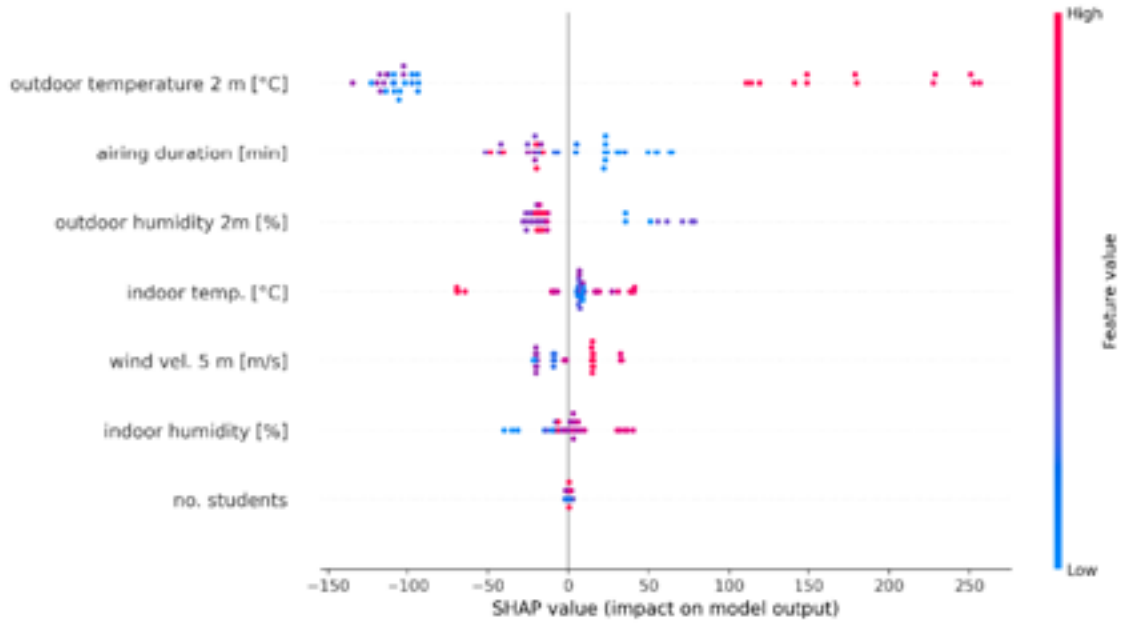

Fig. 9. SHAP values for fungi count prediction using the BT model.

to 200) showed a negative relationship with velocity. Outdoor humidity had a moderate effect (SHAP values from -100 to 100), with higher humidity levels (red dots) slightly increasing bacterial counts. Indoor humidity and the number of students had less significant effects on bacterial counts.

For fungal count prediction using the LP model (Figure 9), outdoor temperature was the most influential factor (SHAP values ranging from -150 to 250), with higher values (red dots) clearly associated with increased fungal counts, possibly due to more favorable growth conditions. Airing duration was the second most important variable, with longer times since airing associated with higher fungal counts. Outdoor humidity also appeared to be a controlling factor, with slightly higher fungal counts at higher humidity levels. The remaining variables had a minor impact (SHAP values from -50 to 50), with no clear trends.

The results indicate that microbiological air quality in the classroom was shaped by the combined influence of outdoor meteorological conditions and indoor microclimate parameters. In naturally ventilated buildings, indoor temperature and relative humidity strongly depend on external weather conditions and the effectiveness of airing processes. Therefore, short-term climatic variability can indirectly affect indoor bioaerosol composition through microclimate modification.

In the present study, dry and sunny weather conditions were associated with lower microbial abundance, whereas periods characterized by higher humidity and reduced solar radiation promoted increased bacterial and fungal presence. Regression modelling further confirmed that outdoor temperature was one of the key factors influencing microbial counts, alongside airing duration. These findings are consistent with previous studies demonstrating that meteorological parameters can regulate microorganism survival, emission, and transport in atmospheric air, thereby influencing indoor environments.

It should be emphasized that, in naturally ventilated classrooms, indoor microbiological air quality cannot be considered independently of outdoor atmospheric conditions. Instead, it represents a dynamic system resulting from interactions between meteorological variability, ventilation efficiency, and occupant-related emissions.

Although the study was conducted under real operating conditions, the results were obtained in a single classroom characterized by natural ventilation and specific architectural and operational parameters. Therefore, direct extrapolation of quantitative microbial levels to other educational buildings should be approached with caution. The obtained results are most representative of classrooms with natural or hybrid ventilation systems, similar occupancy densities, and comparable temperate climatic conditions.

Nevertheless, several of the observed mechanisms appear to be universal and consistent with literature data. These include the dominant role of occupants as a source of airborne bacteria, the strong influence of outdoor air on fungal spore concentrations, and the importance of ventilation effectiveness in controlling microbial abundance. Consequently, while absolute microbial concentrations may vary between facilities, the identified relationships between ventilation, meteorological conditions, and indoor bioaerosols may be applicable to a broader range of educational environments.

The present study expands existing knowledge by demonstrating that microbiological air quality in naturally ventilated classrooms is characterized by substantial short-term variability linked to classroom use cycles. Moreover, the combined application of microbiological measurements and interpretable machine learning techniques enabled the identification of key environmental drivers influencing microbial abundance. The results also highlight the complex and sometimes contradictory effects of natural ventilation, emphasizing that outdoor air exchange can improve sanitary conditions while simultaneously introducing environmental microorganisms.

Conclusions

The experiment was conducted under real operating conditions, without any artificial control of classroom

microclimate parameters. Microbiological air quality was therefore analyzed under typical indoor conditions, influenced by the outdoor environment. Indoor air temperature ranged from 19°C to 28.6°C, while relative humidity varied from 22.1% to 56.3%. Both recorded parameters were dependent on outdoor conditions.

The observed changes in indoor air microbiological quality indicate its deterioration over the course of the day, associated with the duration of room use. During successive measurement periods (No. 1-3; No. 4-6, No. 7-9), occupants entering the classroom introduced bacteria and, occasionally, fungi via their body surfaces, clothing, and hair. As a result, they acted as sources of microbial emissions into the air, increasing both abundance and diversity. Despite periodic air exchange in the room through supply ventilation, the duration of ventilation was insufficient to effectively reduce exposure levels for room occupants. However, despite periodic increases in microbial counts, the air in the studied classroom did not show significant contamination or pose a substantial health risks to users..

SHAP analysis revealed that airing duration and outdoor temperature consistently influenced both bacterial and fungal counts across models, with longer airing durations and higher temperatures associated with reduced microbial loads. Indoor and outdoor humidity played secondary roles, with effects varying depending on the model and microbial group. These findings underscore the importance of ventilation and temperature conditions in controlling microbial levels in indoor environments.

This study has several limitations that should be considered when interpreting the results. First, measurements were conducted in a single classroom, which limits the generalizability of the quantitative results to other educational facilities with different building characteristics, ventilation systems, or operational conditions. Second, although outdoor meteorological parameters were included in the analysis, other potentially relevant environmental factors, such as particulate matter concentrations, surrounding vegetation, or urban pollution sources, were not evaluated. Future studies should incorporate multi-building assessments and advanced molecular identification techniques to provide a more comprehensive characterization of indoor bioaerosols.

The conducted studies indicate that microbiological air quality in classrooms depends on weather conditions, duration of use, and ventilation effectiveness. Implementing regular airing breaks and monitoring microclimate parameters (temperature and humidity) can significantly reduce the risk of excessive microbial proliferation. The results highlight the need for more effective ventilation systems and practical hygiene guidelines in educational facilities.

1. The microbiological air quality in the studied classroom exhibited clear daily and seasonal variability, dependent on both atmospheric conditions and the number and activity of occupants.
2. A consistent decrease in bacterial counts was observed after classroom airing, accompanied by an increase in mold fungal spore counts. This phenomenon confirms the significant influence of outdoor air on indoor bioaerosols compositions in naturally ventilated environments.
3. With increasing duration of room use and changes in the number of students, both the abundance and diversity of bacteria increased. The primary source of contamination was the occupants (skin microbiome, clothing, and hair), indicating a significant anthropogenic contribution.
4. Under conditions favorable for microbial growth (high humidity, moderate temperatures, and limited exposure to intense solar radiation), increased concentrations of both bacteria and fungi were observed. In contrast, dry and sunny conditions limited their abundance.
5. The identified microbial taxa (e.g., *Staphylococcus, Bacillus, Serratia, Aspergillus, Penicillium, Rhizopus*) are typical of indoor and outdoor environments, with some posing potential health risks (e.g., *Staphylococcus aureus, Aspergillus niger*).
6. Regression modeling results indicate that airing duration and outdoor temperature are key factors shaping microbial abundance in classroom air. Longer airing periods effectively reduced bacterial counts, while higher temperatures promoted reductions in both bacterial and fungal counts.
7. Despite observed fluctuations in microbial abundance, the air quality in the studied classroom remained within the range typical for public spaces and did not pose significant health risks to occupants.
8. The obtained results emphasize the importance of proper ventilation and microclimate control in educational settings. Regular and sufficiently long classroom airing can significantly improve microbiological air quality.

Application perspective

1. Design and modernization of classrooms:
   a. The study results indicate the need for more efficient ventilation systems than natural ventilation alone.
   b. Thw implementation of mechanical ventilation with HEPA filtration or hybrid systems is recommended, as these are more effective in reducing bioaerosol concentrations.
2. Management of room use:
   a. Frequent and sufficiently long airing of classrooms during breaks can significantly reduce bacterial concentrations.
   b. Class schedules should incorporate technical breaks for ventilation, especially during periods of increased risk (e.g., humid and overcast conditions).
3. Microclimate control:
   a. Monitoring indoor temperature and relative humidity provides a simple and effective approach to limiting excessive microbial proliferation.
   b. In practice, this involves maintaining relative humidity within the range of 40–60% and avoiding excessive air drying during the heating season.
4. Health and hygiene aspects:
   a. The presence of potentially pathogenic taxa (e.g., *Staphylococcus aureus, Aspergillus niger*) indicates the need for appropriate hygiene procedures, including regular cleaning and disinfection of surfaces, dust control, and timely replacement of ventilation system filters.
   b. The study results can serve as a basis for developing hygiene guidelines for educational institutions in Poland, analogous to existing WHO recommendations.

5. Although mechanical ventilation systems with HEPA filtration can significantly improve microbiological air quality, their implementation in educational buildings may be constrained by economic and technical factors. Installation and maintenance costs, structural limitations of existing buildings, and energy consumption requirements may reduce the feasibility of such solutions, particularly in older school infrastructure.
6. In such cases, alternative or complementary strategies should be considered, including optimized airing schedules, portable air filtration devices, and hybrid ventilation systems. These approaches can provide substantial improvement in indoor air quality while remaining economically viable for educational institutions.

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Wpływ mikroklimatu na jakość mikrobiologiczną powietrza w salach lekcyjnych

Streszczenie. Celem badania była ocena jakości mikrobiologicznej powietrza w sali lekcyjnej z naturalną wentylacją, użytkowanej przez studentów. Badania prowadzono w cyklu dziewięciotygodniowym w okresie zima-wiosna-lato 2024 roku. Przeanalizowano stężenia bakterii i grzybów w powietrzu, a uzyskane wyniki porównano z parametrami mikroklimatu wewnętrznego i warunkami atmosferycznymi. Stwierdzono, że po wietrzeniu sal audytoryjnych liczba bakterii istotnie spadła, podczas gdy liczba zarodników pleśni wzrosła, co wskazuje na istotny wpływ powietrza zewnętrznego na skład bioaerozoli wewnętrznych. Podczas zajęć obserwowano systematyczny wzrost zarówno liczebności, jak i różnorodności bakterii, przy czym głównym źródłem byli użytkownicy badanych pomieszczeń. Zmienność liczebności drobnoustrojów była również silnie skorelowana z warunkami pogodowymi — suche i słoneczne dni sprzyjały ich redukcji, podczas gdy wysoka wilgotność i mniejsze nasłonecznienie zwiększały ich obecność. Zidentyfikowane mikroorganizmy należały do rodzajów typowych dla środowisk wewnętrznych i zewnętrznych, w tym S*taphylococcus, Bacillus, Serratia, Aspergillus* i *Penicillium*, z których niektóre są taksonami potencjalnie patogennymi. Modelowanie regresji potwierdziło, że kluczowymi czynnikami kształtującymi liczebność drobnoustrojów były czas wietrzenia i temperatura zewnętrzna. Pomimo obserwowanej dynamiki, jakość powietrza mieściła się w zakresie typowym dla przestrzeni publicznych i nie wskazywała na istotne zagrożenie dla zdrowia użytkowników. Wyniki podkreślają znaczenie odpowiedniej wentylacji i kontroli mikroklimatu w zapewnieniu właściwej jakości mikrobiologicznej powietrza w salach lekcyjnych