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INDOOR AIR QUALITY ASSESSMENT IN EARLY CHILDHOOD EDUCATION SETTINGS: MICROBIAL CONTAMINATION AND PHYSICAL PARAMETERS

Jolita Kirvaitienė[1], Žaneta Maželienė[1], Rasa Volskienė[1], Asta Aleksandravičienė[1], Evelina Bredelytė-Gruodienė[1], Giedrė Jarienė[1], Daiva Bubelienė[2]

Faculty of Medicine, Kauno kolegija Higher Education Institution, Lithuania
Faculty of Arts and Education, Kauno kolegija Higher Education Institution, Lithuania

Abstract. Indoor air quality in early childhood education settings is a significant public health concern because children are highly susceptible to airborne contaminants due to their developing respiratory systems, higher inhalation rate relative to body mass, and prolonged time spent indoors. This exploratory case study aimed to assess microbial contamination and physical air parameters in kindergarten premises in order to evaluate potential implications for children's health and well-being. Indoor air quality was investigated in one early childhood education facility during the warm season (summer period) by measuring CO_2 concentration, air temperature, relative humidity, and airborne microorganisms in three groups (X1–X3) and different room types at two time points of the day. Microclimatic parameters were measured using an EXTECH CO260 multifunctional meter, while microbial air contamination was assessed with a MAS-100 Air Sampler and expressed as CFU/m³. The findings showed that CO_2 concentration remained below 1000 ppm in all investigated rooms and relative humidity generally stayed within acceptable ranges. However, air temperature in several rooms exceeded the hygiene limits established for preschool environments. The microbiological assessment revealed elevated total bacterial counts in several rooms, particularly in group X1, and in some cases increased fungal contamination above the commonly cited indicative benchmark of 500 CFU/m³. *Staphylococcus aureus* was not detected, but substantial concentrations of coagulase-negative *staphylococci* were observed, reflecting intensive occupancy and microbial burden in indoor air. Evening CO_2 concentrations were significantly lower than morning values; however, no statistically significant monotonic associations were observed between total bacterial count and CO_2 concentration, relative humidity, or temperature in the main rooms. Because this was a single-site exploratory study with a limited number of observations, the findings should be interpreted cautiously.
Keywords. indoor air quality, microbial contamination, preschool environment, microclimatic conditions

Introduction

Children spend a large proportion of their day indoors, and the quality of air in educational settings is therefore a major public health concern. Early childhood education facilities are particularly important environments because young children are biologically more vulnerable to airborne pollutants than adults. Their respiratory systems are still developing, they inhale more air relative to body mass, and they often remain in the same indoor environment for many hours each day. (World Health Organization Regional Office for Europe, 2023). Consequently, they experience more intensive exposure to airborne microorganisms such as bacteria and fungi, which may contribute to respiratory infections, allergies, or asthma exacerbations. Studies indicate that, in indoor spaces of preschool education institutions, bacterial and fungal concentrations frequently exceed recommended limits, and indoor air quality deteriorates when ventilation is insufficient and occupancy levels are high (Babicki et al., 2024). Ensuring adequate indoor conditions in educational settings remains a public health and regulatory priority, although practical challenges such as outdated ventilation systems, limited resources, and outdoor pollution often hinder improvement. Better indoor air quality and ventilation are associated with improved cognitive performance and reduced infection risk in children (Maung et al., 2022). Chemical and biological air pollution, ventilation quality and CO_2 levels, and microclimatic conditions (temperature and relative humidity) directly affect the health of children and staff, learning efficiency, and overall health protection. Monitoring, assessing, and managing these parameters is therefore a priority for both public health and education policy (Branco et al., 2020; Bozzola et al., 2024). Research on indoor environmental quality in schools and childcare facilities has shown that physical and microbiological factors should be evaluated together. CO_2 concentration is widely used as a practical indicator of ventilation adequacy, while temperature and relative humidity are essential microclimatic parameters related to thermal comfort and the persistence of indoor contaminants. However, acceptable CO_2 levels alone do not necessarily reflect the full microbiological burden of indoor air (Di Gilio et al., 2021). Poor ventilation and elevated CO_2 levels are associated with impaired cognitive performance and increased respiratory risk. Temperature and relative humidity also affect comfort and may influence microbial persistence: excessively warm or cold classrooms reduce comfort, while very low or high humidity may contribute to mucosal irritation or mould growth (Sadrizadeh et al., 2022,Ricardo et al., 2016; Sadrizadeh et al., 2022). Against this background, the

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present study aimed to assess microbial contamination together with physical indoor air parameters in kindergarten premises during the warm season and to evaluate the potential implications of these environmental conditions for children's health and well-being.

Research methods

This study was designed as an exploratory case study conducted in one early childhood education institution during the warm season (summer period). The aim was to characterize indoor air quality by combining physical and microbiological measurements relevant to children's health and well-being. Because the study involved a single institution and a limited number of observations, it was not intended to provide population-level estimates for all preschool settings. Microclimatic measurements were conducted across three groups (X1–X3) in three room types: the playroom/bedroom/kitchenette area, toilet, and cloakroom. Measurements were performed at two time points: morning after children's arrival (08:30–09:30) and late afternoon before the end of the day (15:30–16:30). On the measurement day, outdoor air temperature was 26 °C in the morning and 32 °C in the evening (as recorded). On the measurement day, group occupancy was 17 children in X1, 10 children in X2, and 15 children in X3; each group was staffed by one teacher and one teacher's assistant. Natural ventilation was used throughout the day in X1 and X2, whereas X3 was equipped with a continuously operating heat-recovery (mechanical) ventilation system. CO_2 concentration (ppm), air temperature (°C), and relative humidity (%) were measured using the EXTECH CO260 multifunctional meter. In each room and group, three replicate measurements (I–III) were obtained at each time point (morning and evening). Summary results were reported as mean ± standard error (SE) based on three replicates (n = 3). Microbiological air contamination was assessed using a MAS-100 Air Sampler at a sampling height of approximately 1 m. Airborne microorganisms were quantified as colony-forming units per cubic meter (CFU/m³). Microbiological sampling was performed in the main occupied rooms and sleeping areas of the groups. For the descriptive comparison shown in Figure 1, the main room measurement was used for each group and time point (X1 playroom; X2 and X3 combined playroom/bedroom areas), allowing comparison between total bacterial count and physical parameters measured in the same room type. Both selective and non-selective media were used to quantify total microbial load and to assess specific microbial groups. Mannitol salt agar plates were incubated for 48 h at 35 °C; mannitol-fermenting colonies were enumerated, and *Staphylococcus aureus* identification was performed using the plasma coagulase test. Blood agar plates were incubated for 48 h at 35 °C to evaluate β-hemolytic colonies; β-hemolytic bacteria were further assessed by agglutination with group A streptococcal serum to detect *Streptococcus pyogenes*. Sabouraud agar plates were incubated for 48 h at 25 °C to determine total fungal colony counts. Tryptic soy agar (TSA) plates were incubated for 48 h at 35 °C, and total microbial colonies were counted after incubation. For contextual interpretation, the commonly cited indicative benchmark of approximately 500 CFU/m³ for indoor bacteria and fungi was used only as a reference point, because internationally harmonized health-based thresholds are lacking. Statistical analysis was performed using non-parametric methods because of the small sample size and exploratory study design. Morning and evening physical measurements were compared using the Wilcoxon signed-rank test across paired room observations. Associations between total bacterial count and CO_2 concentration, relative humidity, and air temperature in the main group rooms were assessed using Spearman's rank correlation. Statistical significance was set at $p < 0.05$.

Results

During the summer period, indoor environmental parameters (CO_2 concentration, relative humidity, and air temperature) were assessed in three measurements (I–III) across groups X1–X3 and room types (Table 1). In the morning, mean CO_2 values ranged from 503.7 to 603.0 ppm, and in the evening from 483.0 to 556.0 ppm. Across the nine paired room observations, evening CO_2 concentrations were significantly lower than morning values (Wilcoxon signed-rank test, $p = 0.0195$). The highest morning CO_2 value was recorded in the X1 playroom/bedroom/kitchenette (603.0 ± 0.6 ppm), while the lowest evening value was observed in the X3 playroom/bedroom/kitchenette (483.0 ± 7.8 ppm). On average across rooms, morning CO_2 was highest in X1 (about 569.6 ppm) and lowest in X2 (about 529.9 ppm), differences between groups were smaller in the evening. In the morning, relative humidity ranged from 41.7 to 48.6%, and in the evening from 42.4 to 48.4%. Mean relative humidity was slightly higher in the evening (average increase ~0.3 percentage points), although the direction of change differed between groups. The highest morning humidity was found in the X1 cloakroom (48.6 ± 0.3%), and the lowest in the X3 toilet (41.7 ± 0.3%). In the evening, the highest humidity was recorded in the X2 playroom/bedroom/kitchenette (48.4 ± 0.2%), and the lowest in the X3 cloakroom (42.4 ± 0.2%). The morning–evening difference in relative humidity was not statistically significant (Wilcoxon signed-rank test, $p = 0.8203$). Morning temperature ranged from 21.7 to 24.8°C, and evening temperature from 22.6 to

25.3°C; overall, temperature was higher in the evening (an average increase of ~0.4°C), most notably in X2 (average increase ~1.0°C). The highest temperature was observed in the X2 playroom/bedroom/kitchenette in the evening (25.3* ± 0.29°C), while the lowest was in the X1 cloakroom in the morning (21.7 ± 0.33°C). The morning–evening difference in air temperature was not statistically significant (Wilcoxon signed-rank test, p = 0.4258). According to HN 75:2016, the indoor air temperature standard during the warm season is 20–24°C. Therefore, non-compliance with this requirement was observed for the following temperature values: X1 toilet in the morning (24.8 ± 0.12°C), X2 toilet in the morning (24.2 ± 0.30°C), and the X2 playroom/bedroom/kitchenette in the evening (25.3 ± 0.29°C).

Microorganism concentrations in indoor air (CFU/m³) were determined during the warm season (summer period) in the morning and in the evening, and the results are presented in Table 2.

Total bacterial count. In the morning, the highest total bacterial concentration was recorded in the X1 playroom (3420 CFU/m³), whereas the lowest concentration was observed in the X2 group/sleeping room (660 CFU/m³). In the evening, total bacterial counts decreased in some rooms; the highest value was found in the X1 sleeping room (2800 CFU/m³), and the lowest in the X3 group/sleeping room (1440 CFU/m³). In the morning, the highest fungal concentration was detected in the X1 sleeping room (730 CFU/m³), while markedly lower levels were observed in the other investigated rooms (X1 playroom – 300 CFU/m³, X2 – 150 CFU/m³, X3 – 160 CFU/m³). In the evening, fungal concentrations were lower in all rooms: X1 playroom – 125 CFU/m³, X1 sleeping room – 3 CFU/m³, X2 – 10 CFU/m³, and X3 – 2 CFU/m³. In the morning, the highest concentration of these microorganisms was recorded in the X2 group/sleeping room (500 CFU/m³), and the lowest in the X3 group/sleeping room (40 CFU/m³). In the evening, *staphylococcal* counts decreased in most locations: the highest value was found in the X1 playroom (260 CFU/m³), while the lowest was observed in the X2 rooms (40 CFU/m³). *Staphylococcus aureus* was not detected in either the morning or evening samples.

Overall, the Figure 1 shows that the highest bacterial load was observed in group X1, particularly in the morning, whereas the lowest value was recorded in group X2 in the morning (Figure 1).

Figure 1 illustrates the relationship between total bacterial count and three physical indoor parameters in the main room of each group at both time points. Descriptively, the highest bacterial load in the main rooms was observed in X1 in the morning (3420 CFU/m³), together with a CO_2 concentration of 603.0 ppm, relative humidity of 45.7%, and temperature of 23.0 °C. In the evening, the total bacterial count in group X1 decreased to 1700 CFU/m³, together with a decrease in CO_2 concentration to 507.0 ppm and relative humidity to 44.0%, while the air temperature slightly increased to 23.4°C. In group X2, the total bacterial count increased from 660 CFU/m³ in the morning to 1690 CFU/m³ in the evening. The corresponding morning microclimatic values were 556.0 ppm CO_2, 46.8% relative humidity, and 21.9°C, whereas the evening values were 554.7 ppm CO_2, 48.4% relative humidity, and 25.3°C. In group X3, the total bacterial count also increased from 970 CFU/m³ in the morning to 1440 CFU/m³ in the evening. At the same time, CO_2 concentration decreased from 521.0 ppm to 483.0 ppm, relative humidity decreased from 47.7% to 43.3%, and air temperature increased from 23.6°C to 24.0°C. Exploratory Spearman correlation analysis based on the six main-room observations showed no statistically significant monotonic association between total bacterial count and CO_2 concentration ($\rho = 0.143$, $p = 0.787$), relative humidity ($\rho = -0.257$, $p = 0.623$), or air temperature ($\rho = 0.086$, $p = 0.872$). Given the small number of observations, these results should be interpreted as descriptive support for the absence of a simple linear or monotonic pattern rather than as definitive evidence of no relationship.

Discussion

Although universally harmonised health-based microbiological limit values for indoor air are lacking, indicative thresholds are still used to contextualise results. The European Agency for Safety and Health at Work emphasizes that good indoor air should be free of harmful microbes, other pathogens, and allergens, while recent reviews note that microbiological exposure assessment remains insufficiently harmonised across countries, building types, and analytical approaches. Therefore, the frequently cited reference value of 500 CFU/m³ for total bacteria and fungi in non-contaminated indoor environments should be regarded as an approximate benchmark rather than a universally binding health-based limit (EU-OSHA, 2024; Chawla et al., 2023; Cervantes et al., 2025). In the present study, a proportion of total bacterial counts, and in some rooms fungal concentrations, exceeded the commonly cited benchmark of 500 CFU/m³, even though during the warm season CO_2 concentrations in all groups remained below 1000 ppm and relative humidity in most rooms remained within recommended ranges. Notably, although total bacterial counts declined in some rooms by the evening, the recorded values remained high in absolute terms in several locations, particularly in the X1 sleeping room, further supporting the interpretation that acceptable ventilation-related indicators do not

necessarily correspond to low microbiological burden. An important finding of this study is that acceptable CO_2 values were recorded in all investigated rooms, yet microbiological contamination remained substantial in several locations. This pattern suggests that ventilation-related indicators alone may not be sufficient to characterize microbial burden. Indoor microbial contamination is likely influenced by multiple factors, including room occupancy, children's activity, resuspension from surfaces and textiles, cleaning practices, local moisture accumulation, and the presence of sleeping areas.This interpretation also suggests that ventilation, although essential, is not sufficient by itself for microbial risk control.

The group-specific patterns also indicate that the relationship between physical indoor conditions and microbial contamination was not uniform. The exploratory correlation analysis did not reveal a statistically significant monotonic association between temperature and total bacterial count. These findings do not allow causal inference. Instead, they indicate that the relationship between thermal conditions and microbiological air quality in preschool settings is likely multifactorial. In some groups, particularly X2 and X3, total bacterial counts increased in the evening together with temperature, even though CO_2 values remained stable or decreased. This pattern was not uniform across the investigated groups: the highest bacterial load was observed in group X1, particularly in the morning, whereas the lowest value was recorded in group X2 in the morning. Overall, the morning–evening profiles differed between groups for both microclimatic parameters and total bacterial counts, indicating that the relationship between physical indoor conditions and microbial burden is likely to be multifactorial rather than linear. This suggests that temperature should not be interpreted solely as a thermal comfort indicator, but also as a parameter potentially affecting microbial survival, resuspension, and indoor surface air interactions. Thus, the present findings support the view that ventilation is a necessary, but not sufficient, condition for reducing microbiological risk in preschool environments, where microbial emissions are closely linked to intensive occupancy and continuous bioaerosol generation (Grassie et al., 2025; Chawla et al., 2023; EU-OSHA, 2024).

The fungal findings strengthen this interpretation. A high fungal burden, particularly in sleeping areas, indicates that local conditions favourable to fungal persistence may exist even when relative humidity remains within the recommended range. At the same time, the marked reduction in fungal concentrations observed in the evening suggests that fungal airborne load may vary substantially over the day and may be influenced by local environmental conditions, room use patterns, bedding or textile-related reservoirs, and short-term microenvironmental changes not captured by single-point humidity measurements. Such conditions may include hidden moisture in structures, bedding, textiles, furniture surfaces, or poorly dried microenvironments that are not captured by single-point microclimatic measurements. Recent reviews of fungal contamination in schools emphasize that fungal concentrations depend not only on air humidity or general ventilation level, but also on building condition, local moisture niches, and cleaning practices (Cervantes et al., 2025; Chawla et al., 2023). The identified fungal genera are also clinically relevant. *Penicillium* and *Cladosporium* are commonly associated with indoor allergenic exposure and respiratory irritation, whereas fungi belonging to the Mucorales group, such as *Rhizopus*, *Cunninghamella*, and *Absidia*, may be important as opportunistic fungi in susceptible individuals. Therefore, even in the absence of major airborne bacterial pathogens, the observed fungal contamination suggests an unfavourable microbiological indoor environment for children with asthma, allergy, or increased respiratory sensitivity. No major pathogenic airborne bacteria were detected in the study, and *Staphylococcus aureus* was not identified in the summer samples. However, this should not be interpreted as negligible microbiological risk, because elevated concentrations of coagulase-negative staphylococci remain epidemiologically relevant as indicators of human presence and intensive room use (Lee et al., 2024). Studies in daycare and other densely occupied indoor environments show that the genus *Staphylococcus* may be abundant even in the absence of classical pathogenic isolates, supporting the need for continued microbiological surveillance (Lee et al., 2024).

From a practical perspective, these findings indicate that microbial contamination management in preschool settings should be multifactorial. Monitoring CO_2 alone or relying only on periodic airing is insufficient if temperature variation, local moisture accumulation, surface and textile hygiene, hidden mould, and maintenance quality are not simultaneously considered. Thus, microbial air pollution prevention in kindergartens should combine ventilation, temperature control, moisture management, regular cleaning, and removal of contamination sources in order to provide a healthier indoor environment for children.

Several limitations must be acknowledged. First, this was a single-site case study conducted in only one preschool institution during one season, which substantially limits generalizability. Second, the number of observations was small, and the statistical analysis was therefore exploratory and of limited power. Third, the microbiological analysis relied on culture-based methods and reflected only the cultivable fraction of indoor microbiota. Fourth, because internationally harmonized health-based threshold values for indoor microorganisms are lacking, interpretation of CFU/m³ values remains indicative rather than definitive.

Conclusions

The present study demonstrated that, in the investigated preschool environment during the warm season, CO_2 concentration and relative humidity were generally within acceptable limits, whereas air temperature in several rooms exceeded the recommended hygiene range, indicating only partial compliance of the physical indoor environment with health safety requirements. Despite these overall acceptable ventilation-related indicators, the microbiological assessment revealed elevated airborne bacterial loads and, in some locations, increased fungal contamination, showing that favourable physical air parameters do not necessarily correspond to low microbiological risk in early childhood education settings. Taken together, these findings suggest that assessment of indoor air quality in preschool settings should not rely on CO_2 monitoring alone. A more informative evaluation should integrate microbiological surveillance with the assessment of temperature, humidity, ventilation-related indicators, and hygiene-related environmental conditions. Because this study was exploratory and was conducted in a single preschool institution with a limited number of observations, the findings should be interpreted cautiously and should not be generalized without further larger-scale research.

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Information about the authors

Jolita Kirvaitienė. Faculty of Medicine, Kauno kolegija Higher Education Institution, Lithuania, Assistant
Research field(s): Public Health, Environmental medicine
Email address: jolita.kirvaitiene@go.kauko.lt
Žaneta Maželienė. Faculty of Medicine, Kauno kolegija Higher Education Institution, Lithuania, Lecturer
Research field(s): Public Health, Microbiology, Parasitology
Email address: zaneta.mazeliene@go.kauko.lt
Rasa Volskienė. Faculty of Medicine, Kauno kolegija Higher Education Institution, Lithuania, Lecturer
Research field(s): Genetic, Public Health
Email address: rasa.volskiene@go.kauko.lt
Asta Aleksandravičienė. Faculty of Medicine, Kauno kolegija Higher Education Institution, Lithuania, Associate Professor
Research field(s): Genetics, Biology
Email address: asta.aleksandravicieniene@go.kauko.lt
Evelina Bredelytė-Gruodienė. Faculty of Medicine, Kauno kolegija Higher Education Institution, Lithuania, Lecturer
Research field(s): Health sciences, Microbiology, Toxicology
Email address: evelina.gruodiene@go.kauko.lt
Giedrė Jarienė. Faculty of Medicine, Kauno kolegija Higher Education Institution, Lithuania, Assistant
Research field(s): Health Sciences, Genetic, Hematology
Email address: giedre.jariene@go.kauko.lt
Daiva Bubelienė. Faculty of Arts and Education, Kauno kolegija Higher Education Institution, Lithuania, Associate Professor
Research field(s): Preschool Education, Research methodology
Email address: daiva.bubeliene@go.kauko.lt