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Research article

Assessment of indoor air quality in Tunisian childcare establishments

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Abstract: Maintaining healthy indoor air quality (IAQ) in childcare settings is essential for infants and young children, as it directly impacts their early learning, development, and overall well-being. Given their vulnerability, continuous IAQ monitoring in these environments is crucial to ensuring a safe and supportive atmosphere. This study aimed to assess IAQ factors that may affect occupant health by measuring indoor concentrations of particulate matter (PM_{10}), selected gases such as carbon dioxide (CO_2) and formaldehyde (CH_2O), and thermal conditions including temperature and relative humidity. Additionally, airborne microorganism levels were analyzed, and potential environmental factors influencing microbial abundance were investigated in three childcare centers in Megrine, Tunisia, across three seasonal periods. Results revealed frequent occurrences of hygrothermal discomfort and elevated levels of CO_2, CH_2O, and PM_{10}, particularly in overcrowded classrooms with poor ventilation and heating. Pathogenic bacterial species, including *Staphylococcus epidermidis, Staphylococcus haemolyticus*, *Bacillus cereus,* and *Bacillus licheniformis*, were repeatedly detected. Significant correlations were found between bacterial abundance and

environmental factors such as PM_{10}, CO_2 levels, temperature, and humidity. These findings provide valuable insights into IAQ dynamics in childcare environments, highlighting the need for improved ventilation and air quality management strategies to safeguard children's health and well-being.

Keywords: airborne bacteria; childcare facilities; indoor air quality; metataxonomic analysis; particulate matter; physicochemical parameters

1. Introduction

The air inside enclosed spaces is a mixture of physical, chemical, and biological pollutants, which originate from outside air, materials, combustion devices, and human activities. The indoor air quality (IAQ) of homes, offices, schools, or other buildings is an essential determinant of healthy living and well-being of people [1]. Most people spend 80%–95% of their time in indoor environments with an average of 10–14 m^3 of air per day [2]. In most studies, symptoms such as dizziness, headache, nausea, and irritation of the eyes, nose, and throat have been shown to be linked to poor IAQ [3].

Indoor air quality has been the focus of numerous studies due to growing concerns within the scientific community regarding its impact on occupant health and comfort [4–11]. However, poor IAQ in schools can be particularly severe compared to other types of buildings, primarily due to higher occupant density. Elevated contaminant levels may stem from various factors, including the intrusion of outdoor pollutants, the building's physical state, cleaning practices, and the effectiveness of the ventilation system [12–17].

The World Health Organization (WHO) has selected particulate matter ($PM_{2.5}$ and PM_{10}) and some gaseous compounds as crucial for checking IAQ, namely radon, carbon monoxide, nitrogen dioxide, polycyclic aromatic hydrocarbons (PAH), formaldehyde (CH_2O), and other volatile organic compounds [18]. Formaldehyde, classified as a certain carcinogen for humans, represents a priority pollutant in school buildings due to excessive and regular use of school supplies (glue, gouache, ink, etc.) and cleaning products (detergents, air fresheners, etc.) representing potential sources of CH_2O emissions in classrooms. Several studies have demonstrated that indoor exposure to formaldehyde has been associated with respiratory and asthma symptoms and decreased lung function in children [19]. On the other hand, hygrothermal comfort and air humidity in classrooms are also relevant in the study of IAQ since they affect the perceived comfort of IAQ, thus causing symptoms of eye and respiratory irritation and impacting children's performance [20]. Although not considered a pollutant per se in indoor environments, carbon dioxide (CO_2) has been used as a relevant indicator of adequate ventilation in classrooms. Studies on children have shown that increased CO_2 concentration in the classroom decreases school attendance over a short period of time [21–40], while persistent exposure to PM_{10} could disrupt children's lung development later in life [41].

In many environments, particularly in daycare settings, bioaerosols can disrupt normal activities. Exposure to these airborne particles, which contain microorganisms and their byproducts, may lead to respiratory issues and other health complications such as toxic reactions and infections [42,43]. Airborne microorganisms, such as bacteria, fungi, and yeast, generally come from humans but also from animals, plants, soil, building materials, and the external environment [44]. In

schools, children tend to have high activity levels, which generally leads to higher levels of airborne microorganisms. Epidemiological studies show that too high a concentration of microorganisms in the air can be allergenic and that sometimes even very low concentrations of particular microorganisms can cause serious illnesses [45]. Additionally, the amount of microbes present in indoor school air has a direct impact on students' mental health, physical development, and performance [46]. Diriba et al. revealed that failure to clean and check heating and air conditioning systems can allow microbial growth, causing rhinitis, bronchitis, pharyngitis, pneumonia, conjunctivitis, and keratitis [47]. Bacteria in indoor environments primarily originate from human sources, including skin, the oral cavity, intestines, and clothing [48]. Some airborne bacteria are toxic, allergenic, or infectious, posing health risks such as respiratory and dermatological infections [49,50].

Given the health risks associated with poor IAQ, its assessment is crucial for effective risk management. In many countries, IAQ monitoring in childcare facilities and schools has become a legal requirement. This study aims to investigate the physicochemical and microbiological quality of indoor air in three childcare centers in Tunisia and to explore correlations between environmental parameters and bacterial load.

2. Materials and methods

2.1. Site introduction

This study was conducted in three collective childcare establishments located in the town of Megrine, northern Tunisia: a kindergarten (E1) and two daycare schools (E2 and E3). To perform the study, one room from each establishment was selected: RE1 in E1, RE2 in E2, and RE3 in E3. Data collection took place over three distinct periods: summer (early October 2020), winter (late December 2020), and spring (April–May 2021) (Table 1).

Table 1. Main characteristics of each studied site.

Site	Room	Class/grade (years)	Floor	Occupation by children	
				Number of children	Total occupancy period (minutes)
RE1	Activity room	3–4	Ground floor	12 (summer)	273 (summer)
				20 (winter)	222 (winter)
				17 (spring)	184 (spring)
RE2	Duty room	6–7	Ground floor	20 (summer)	271 (summer)
				20 (winter)	260 (winter)
				19 (spring)	170 (spring)
RE3	Duty room	5–6	First floor	14 (summer)	240 (summer)
				16 (winter)	285 (winter)
				15 (spring)	255 (spring)

2.2. IAQ characterization

During the children's normal activities (full rooms), comfort parameters [temperature (T) and

relative humidity (RH)] and CO_2 concentrations were measured inside the rooms using an analyzer IAQ (model Q-TRAK 7575). Formaldehyde (CH_2O) concentrations were measured using a formaldehyde meter (model HAL-HFX205). Aerosol monitors (DUSTTRAK II model) were employed to simultaneously measure particulate matter with an aerodynamic diameter of less than 10 μm (PM_{10}) inside the rooms and outside ($PM10_{ext}$) of the selected spaces. All measurements were conducted at regular intervals of 1 min.

Comfort conditions related to air temperature and relative humidity in the rooms were assessed using a hygrothermal comfort diagram [51]. This diagram defines a comfort zone based on ambient temperature and corresponding humidity levels perceived as comfortable. Two discomfort zones are identified: a *humid zone* with elevated temperature and humidity, and a *dry zone* characterized by lower temperature and humidity levels.

2.3. Metataxonomic analysis

In conjunction with these measurements, air samples designated for microbiological analyses were collected within rooms RE2 and RE3 during the summer and winter periods using a bio-collector (model BK-BAS). Next-generation sequencing (NGS) technology was employed to explore the diversity of airborne microorganisms and pathogenic bacteria within these environments.

DNA extraction was conducted utilizing the GeneJET Genomic DNA Purification kit (Thermo Scientific) following the manufacturer's instructions. Metagenomic sequencing library preparation was carried out according to the Illumina protocol (Illumina Inc., San Diego, CA, USA), targeting the V3–V4 region of the 16S rDNA gene, employing universal primer sets as follows: forward = 5' (CCTACGGGNGGCWGCAG) and reverse = 5' (GACTACHVGGGTATCTAATCC) [52]. DNA concentration was quantified using a Qubit fluorometer (Invitrogen, USA). Amplification was performed in a 25 μL mixture, incorporating KAPA HiFi Hot-Start PCR kit (Kapa Biosystems), 10 mM of each primer, and 5 ng of DNA. PCR conditions comprised an initial step at 95 °C for 3 min followed by 25 cycles (95 °C for 30 s, 52 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s), with a final extension at 72 °C for 5 min. Following PCR, amplicons underwent purification using magnetic beads with two 80% ethanol washes. Subsequently, a second PCR was conducted, integrating specific adapters and indexes for sample identification and employing the KAPA HiFi Hot-Start PCR kit. Nuclease-free water was utilized to adjust the final volume to 50 μL. Following dilution and normalization of the combined library, adhering to Illumina's recommendations for sequencing on Miseq, the sequencing process involved denaturation with 0.2 N NaOH, further dilution to 4 pM in sequencing buffer (HT1), and loading into the Miseq cartridge for sequencing. The raw data underwent analysis using the One Codex data platform due to its various advantages [53,54].

2.4. Data treatment

For the CO_2 data acquired, comparisons were made with the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) standard reference value of 1000 ppm [55]. Formaldehyde and PM_{10} data were evaluated against the World Health Organization (WHO) recommended values of 100 $\mu g/m^3$ for CH_2O and 50 $\mu g/m^3$ for PM_{10} [56]. Moreover, correlations between physicochemical parameters and the bacterial community were established

utilizing correlograms generated via the FaDA application [57].

3. Results

3.1. Occupancy and ventilation conditions

During the summer period, the highest occupancy rates were observed in the study rooms. In winter and spring, room RE1 had the highest density, with 1.57 m^2 and 1.8 m^2 per child, respectively (Table 2). Room RE3 had the smallest surface area of external openings (windows, doors, and patio doors), with only 0.81 m^2 available (compared to the required 5.25 m^2), while room RE2 had the largest opening area, measuring 4.55 m^2 (compared to the required 7.93 m^2).

Table 2. Occupancy rates and surfaces of the openings in studied rooms.

Room	Area (m^2)	Occupancy rate (m^2 per child)				Surface of the openings	
		Summer	Winter	Spring	Average	s_1 (m^2)[a]	s_2 (m^2)[b]
RE1	31.5	2.62	1.57	1.8	1.99	2.93	5.25
RE2	47.6	2.38	2.38	2.5	2.42	4.55	7.93
RE3	31.4	2.24	1.96	2.09	2.09	0.81	5.23

Notes: [a]s_1 represents the surface of the openings (m^2) available in the room; [b]s_2 represents the minimum surface area of openings (m^2) imposed by French thermal regulations: one-sixth of the surface area of the room.

3.2. Characterization of IAQ

3.2.1. Thermal comfort parameters

The hygrothermal comfort conditions within the classrooms varied significantly by season. During the summer, all three rooms were situated outside the hygrothermal comfort zone, characterized by hot and humid environments. In winter, rooms RE2 and RE3 entered the comfort zone, while RE1 remained outside. In spring, all rooms exhibited favorable hygrothermal conditions (Figure 2). Studies have shown that inadequate hygrothermal conditions increase the risk of indoor contamination by bacteria, viruses, fungi, and mites, which can lead to health issues such as rhinitis, allergies, asthma, and in severe cases, respiratory and lung infections [58–60].

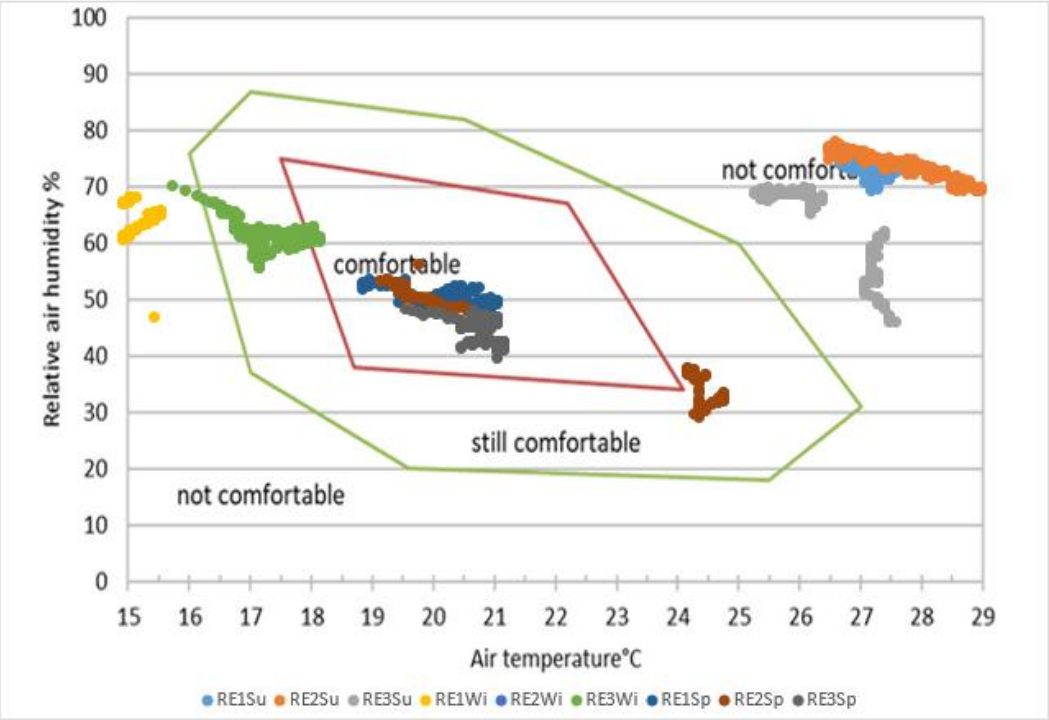

Figure 1. Hygrothermal comfort zones of classrooms (RE1, RE2, and RE3) during the summer (Su), winter (Wi), and spring (Sp) periods.

3.2.2. CO_2 concentrations

The highest average CO_2 concentrations were recorded in the winter, with room RE1 showing a mean value of 3186.6 ppm (range: 1891–4394 ppm) and room RE3 a mean value of 2781.6 ppm (range: 2059–3433 ppm) (Figure 2). All CO_2 measurements in RE3 (in both winter and spring) and RE1 (in winter) exceeded the ASHRAE-recommended threshold of 1000 ppm (Figure 3). These findings are consistent with current literature, which highlights high CO_2 levels as a key concern in classroom air quality [61–68].

Correlation analysis of the monitored parameters revealed a strong positive correlation between CO_2 levels and indoor PM_{10} concentrations, with correlation coefficients of 0.90, 0.80, and 0.58 in RE1, RE3, and RE2, respectively. These results align with the study by Simoni and Annesi-Maesano [69], which investigated IAQ in 46 classrooms across 21 schools in six cities in five European countries and found significant correlations between CO_2 and PM_{10} levels (r=0.66) [70]. Nevertheless, strong negative correlations were observed between CO_2 concentrations and air temperature levels, with coefficients of the order of −0.90, −0.84, and −0.80 in RE3, RE1, and RE2, respectively. Similarly, findings from a study conducted in 310 schools and nurseries across France between 2009 and 2011 also noted significant negative correlations between CO_2 levels and temperature [70].

The elevated CO_2 concentrations observed in RE1 and RE3 during winter and spring were primarily attributed to inadequate ventilation and higher room occupancy compared to other spaces. These rooms, RE1 and RE3, had the smallest natural ventilation openings, and throughout these

seasons, they experienced the highest levels of crowding.

Furthermore, windows remained closed throughout the school day in RE1 during winter and RE3 during both winter and spring. These findings suggest a clear association between inadequate ventilation and increased CO_2 levels in classrooms.

In contrast, room RE2, which had the best ventilation and occupancy conditions, recorded the lowest CO_2 concentrations (Figures 2 and 3, Table 2). A study by Clausen et al. (2014) in 785 Danish classrooms supported these findings, demonstrating that ventilation during breaks, such as opening windows, reduced the proportion of classrooms with CO_2 concentrations above 1000 ppm from 60% to 39% annually, compared to rooms where windows were never opened [71].

Conversely, during winter, combustion heating was used in room RE3 throughout the school day without adequate ventilation, likely contributing to the excessive increase in CO_2 concentrations. These results align with those of Hanoune and Carteret (2015), who demonstrated that the operation of individual combustion devices can elevate CO_2 concentrations up to 4500 ppm. Their study on the indoor air quality of seven homes equipped with combustion heaters revealed that CO_2 concentrations above 1000 ppm were consistently attributed to combustion sources [72]. Similarly, a study conducted in Croatia during the heating season found that CO_2 concentrations in 60 classrooms, all of which were poorly ventilated, surpassed international guidelines. The average CO_2 concentrations in these rooms ranged between 862 and 2415 ppm [73]. These results indicate that CO_2 concentrations above 1000 ppm are relevant indicators of deficient ventilation flow in classrooms.

3.2.3. CH_2O concentrations

The highest average concentrations of formaldehyde (CH_2O) were detected during summer in rooms RE3 and RE2, with mean values of 1115.8 µg/m^3 (range: 0–6385.6 µg/m^3) and 650.8 µg/m^3 (range: 0–13606.2 µg/m^3), respectively (Figure 3). In these rooms, the rate of exceedance of the WHO-recommended limit value of 100 µg/m^3 was particularly high in summer, reaching 65% in RE3 and 33% in RE2 (Figures 4 and 5). These results are consistent with recent studies that have also reported elevated levels of CH_2O in classrooms [66,74–77]. The presence of high CH_2O levels is primarily attributed to the widespread use of hydroalcoholic gels and solutions for hand disinfection, as indicated by the work of Santos Catai et al. [78], as well as surface and floor cleaning procedures implemented in the classrooms as part of the actions undertaken by these establishments to fight against the SARS-CoV-2 virus during the COVID-19 pandemic.

However, it is important to note that the electrochemical sensor used for CH_2O measurement (HAL-HFX05) has known cross-sensitivities, particularly to ethanol (10%) and isopropanol (2%), both of which are key components of hand disinfectants. While our observations align with previous studies indicating ethanol oxidation as a potential secondary source of CH_2O formation in indoor environments [78], further investigation using alternative analytical techniques, such as colorimetric assays or gas chromatography, would help refine these measurements and minimize potential interference.

3.2.4. PM_{10} particles

As illustrated in Figure 6, PM_{10} concentrations reached their highest levels in rooms RE3 and RE1 during the winter, with values of 114 μg/m^3 (range: 85–138 μg/m^3) and 85 μg/m^3 (range: 69–116 μg/m^3), respectively. During this season, PM_{10} measurements in these rooms exceeded the WHO-recommended limit of 50 μg/m^3 (Figure 7). During the summer, the highest median PM_{10} concentrations were observed in RE2 (mean: 68 μg/m^3, range: 48–180 μg/m^3) and RE3 (mean: 67 μg/m^3, range: 46–91 μg/m^3), with 95% and 91% of readings in these rooms surpassing the WHO limit.

Correlation analyses between indoor and outdoor PM_{10} concentrations revealed significant associations, with correlation coefficients of 0.45, 0.80, and 0.83 in RE1, RE3, and RE2, respectively. These results are similar to those found by Matic et al. [79] who concluded that the increase in PM_{10} concentrations inside eight naturally ventilated schools in Serbia (among nine studied schools) was significantly affected by the increase in PM_{10} emissions from outside, with correlation coefficients varying between 0.45 and 0.95.

However, an analysis of the indoor-to-outdoor (I/O) ratios of PM_{10} concentrations indicated that the dominant sources of PM_{10} varied by season. In RE1 and RE2, the median I/O ratios were greater than 1 during both summer and winter (1.42 and 2.41 in RE1; 1.32 and 1.31 in RE2), suggesting that indoor sources were predominant. According to several studies, children's activities, movement, and the presence of furniture such as whiteboards, tables, and chairs are key indoor sources of particulate emissions in classrooms [80,81]. A study by Oliveira et al. [82] showed that air conditioning, heating systems, and cleaning activities also contribute significantly to indoor PM_{10} levels in school environments.

In contrast, during the spring, the I/O ratios in these same rooms approached 1, indicating a shift toward the predominance of outdoor sources of PM_{10}. In RE3, the contribution of indoor sources increased progressively over the seasons. The I/O ratio in this room was approximately 1 during the summer, increased to 1.38 in winter, and peaked at 4.9 in spring. The significant rise in PM_{10} levels inside RE3 during winter can largely be attributed to the operation of the heating system, as supported by numerous studies that have shown the role of heating in elevating particle emissions in enclosed spaces [83,84].

A model-based study by Hong et al. [85] showed a strong positive association between the size of window openings in buildings and indoor PM_{10} concentrations. These authors concluded that increasing window openings improves indoor ventilation and thus reduces PM_{10} concentrations inside buildings. This could partly explain the presence of PM_{10} at relatively high levels in RE3 (all seasons) and RE1 (in winter). These rooms, although less exposed to the effect of outside air pollution, nevertheless had the smallest opening surfaces, with s_1/s_2 ratios of around 0.31 and 1.12, respectively (Figures 6 and 7 and Table 2).

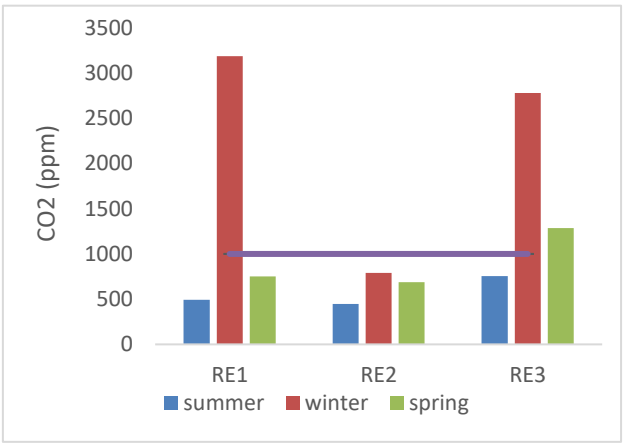

Figure 2. Average concentrations of CO_2 by room. The line at 1000 ppm indicates the ASHRAE standard reference value.

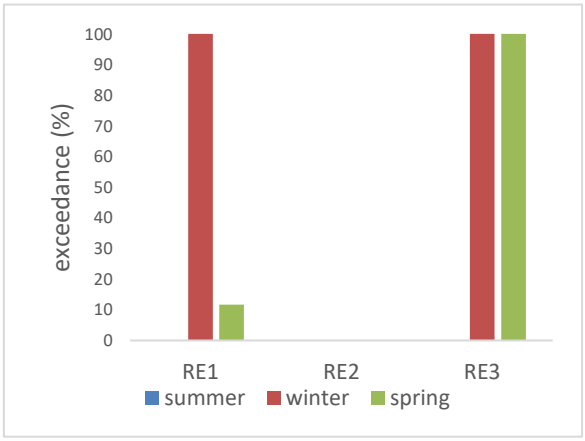

Figure 3. Rate of CO_2 concentrations exceeding the ASHRAE reference value by room.

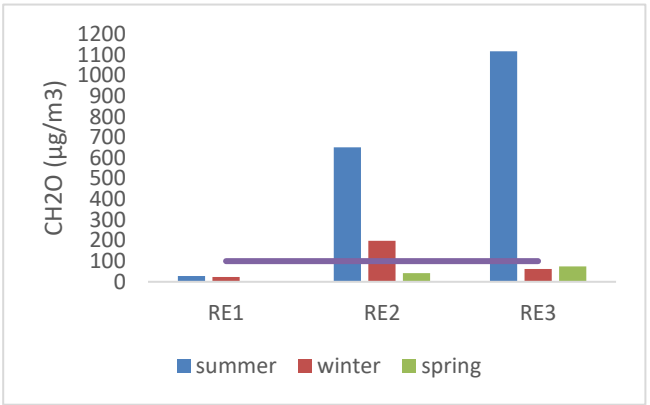

Figure 4. Average concentrations of CH_2O by room. The line at 100 µg/m³ indicates the value recommended by the WHO.

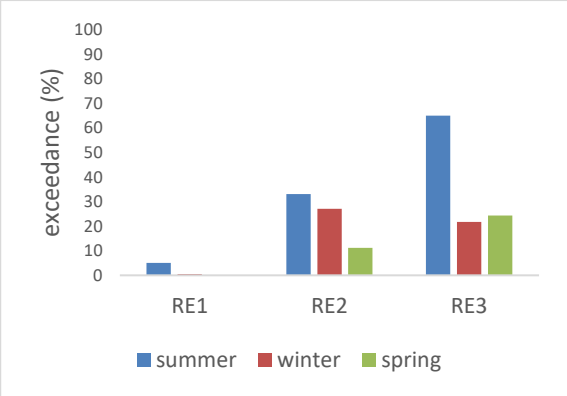

Figure 5. Rate of CH_2O concentrations exceeding the limit value recommended by the WHO according to the room.

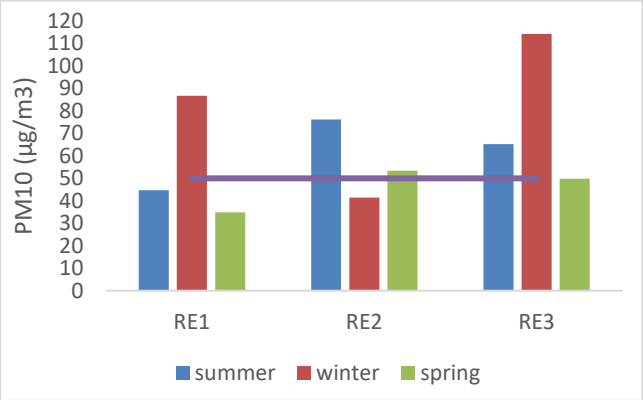

Figure 6. Average concentrations of PM_{10} by room. The line at 50 µg/m³ indicates the value recommended by the WHO.

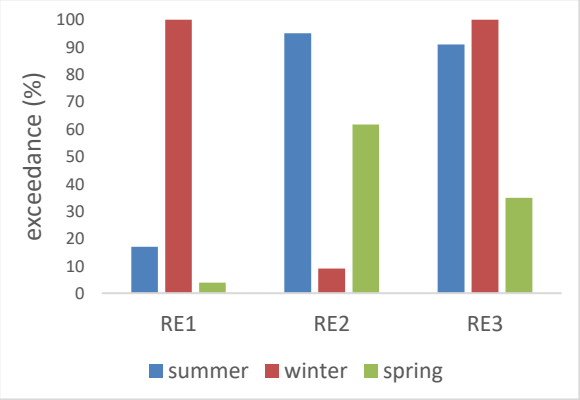

Figure 7. Rate of PM_{10} concentrations exceeding the limit value recommended by the WHO according to the room.

3.3. Metataxonomic analysis

3.3.1. Bacterial species in classrooms

The accession number for the Short Read Archive (SRA) data generated in this study is PRJNA1059615. *Bacillus and Staphylococcus*, each comprising four species, represented the bacterial genera most detected, both in terms of frequency and abundance (Table 3). A total of 24 species of airborne bacteria were identified from the collected air samples, with detection frequencies ranging from one to three occurrences. Sixteen distinct bacterial species were detected only once, while eight species were observed at least twice. In terms of pathogenicity, certain species classified as pathogenic to humans [86] were detected in the air samples collected, namely *Staphylococcus epidermidis* (three occurrences), *Bacillus cereus* (three occurrences), and *Staphylococcus haemolyticus* (two occurrences), as well as *Bacillus licheniformis, Bordetella petrii, Proteus mirabilis, Klebsiella aerogenes, Vagococcus fluvialis, Providencia rittgeri,* and *Pseudomonas fluorescens* (each a single occurrence). Among these pathogens, six species were detected simultaneously in the air sample collected from RE2 in summer and four species in the sample collected in summer from RE3 (Table 3). Regarding abundance, *B. licheniformis and B. petrii* were detected with the highest relative abundance rates in the air sample collected from RE2 in summer, reaching 29.61% and 8.8%, respectively. As for *S. epidermidis* and *B. cereus*, their abundances reached a maximum in air samples collected in winter from RE3 (5.86%) and RE2 (8.14%), respectively.

It should also be noted that other bacterial species classified as risk class 2 pathogens (microorganisms that can cause disease in humans and constitute a danger for people directly exposed to them) have been found more than once in the air samples collected [86]. These mainly include *Staphylococcus saprophyticus* and *Bacillus cereus* (each three occurrences) and *Staphylococcus hominis* (twice) (Table 3).

Table 3. Average values of physicochemical parameters, relative abundances of isolated bacterial species, and significant correlations found between them in the indoor air of rooms RE2 and RE3 in the summer (Su) and winter (Wi) periods.

Room		RE2Su	RE2Wi	RE3Su	RE3Wi	Significant correlations[C]
Physicochemical parameters						
Temperature (T, °C)		27.75	11.69	26.35	17.4	
Relative humidity (RH, %)		73.71	54.8	64.28	61.2	
Carbone dioxide (CO_2, ppm)		445.97	791.8	753.67	2781.53	
Formaldehyde (CH_2O, µg/m^3)		650.8	198.7	1115.79	62.74	
Indoor concentration of PM_{10} (PM_{10}, µg/m^3)		76.19	41.47	65.22	114.34	
Outdoor concentration of PM_{10} (PM_{10ext}, µg/m^3)		65.88	33.05	69.77	102.63	
Bacterial community						
Bacterial species	Frequency	% of classified reads ≥ 1% at the species level				
*Bacillus cereus**	3	1.84	8.14	3.35	-	PM_{10} (r=-0.92, *P*=0.0734)[b]
						PM_{10ext} (r=-0.94, *P*=0.058)[b]
Bacillus firmus	3	2.42	3.74	-	3.43	CH_2O (r=-0.95, *P*=0.0405)[a]
*Staphylococcus epidermidis**	3	1.41	3.01	-	5.86	CH_2O (r=-0.92, P=0.0757)[b]
Staphylococcus saprophyticus	3	-	2.63	4.18	12.52	CO_2 (r=0.97, *P*=0.021)[a]
						R.oc (r=-0.97, *P*=0.032)[a]
Pseudomonas stutzeri	3	-	7.08	2.19	3.13	RH (r=-0.95, *P*=0.0404)[a]
						T (r=-0.93, *P*=0.0681)[b]
Micrococcus luteus	3	-	2.28	5.22	1.05	-
*Staphylococcus haemolyticus**	2	-	4.67	1.03	-	-
Staphylococcus hominis	2	1.79	-	1.5	-	T (r=0.95, *P*=0.049)[a]
*Bacillus licheniformis**	1	29.61	-	-	-	-
Bacillus subtilis	1	11.79	-	-	-	-
*Pseudomonas fluorescens**	1	-	-	-	2.32	-
Micrococcuss sp.	1	-	-	3.51	-	-
*Bordetella petrii**	1	8.8	-	-	-	-
*Proteus mirabilis**	1	3.31	-	-	-	-
*Providencia rettgeri**	1	1.11	-	-	-	-
Leclercia adecarboxylata	1	-	1.43	-	-	-
Planococcus citreus	1	-	1.27	-	-	-
Sphingobacterium faecium	1	-	1	-	-	-
*Klebsiella aerogenes**	1	-	-	5.64	-	-
*Vagococcus fluvialis**	1	-	-	1.12	-	-
Psychrobacter faecalis	1	-	-	-	2.01	-
Psychrobacter celer	1	-	-	-	1.33	-
Acinetobacter johnsonii	1	-	-	-	1.01	-
Oceanobacillus profundus	1	-	-	-	1	-
Total number of species detected	24	9	10	9	10	-
Cumulative abundance of bacteria of the genus		45.66	11.88	3.35	3.43	-

Bacillus (%)					
Cumulative abundance of bacteria of the genus *Staphylococcus* (%)	3.2	10.31	6.71	18.38	CO_2 (r=0.94, *P*=0.0559)[b]

Notes: *Bacterial species classified as pathogenic to humans [86]. [a] Significant at the 0.05 level; [b] Significant at the 0.1 level. [C] Significant correlations found between the abundance of bacteria and physicochemical parameters

3.3.2. Correlations between physicochemical parameters and bacterial community

The correlation analysis conducted revealed that the diversity of staphylococci in the air samples is strongly correlated with the CO_2 concentration and the rate of occupancy by children. Indeed, a significant correlation was observed between the cumulative abundance of species of the *Staphylococcus* genus and the CO_2 concentration in the room (r=0.94, *P*=0.0559). In addition, the abundance of *Staphylococcus saprophyticus* showed a significant positive correlation with the CO_2 concentration in the room (r=0.97, *P*=0.021) and a significant negative correlation with the occupancy rate of the room by children (r=-0.97, *P*=0.032) (Table 3). These results support previous research highlighting the adaptability of *Staphylococcus* bacteria to environments with poor ventilation and high occupancy rates [87]. A study conducted by Madsen et al. [88] in different indoor spaces also showed that the diversity of staphylococci was significantly associated with the surface area per occupant. On the other hand, a significant positive correlation was observed between the abundance of *Staphylococcus hominis* and air temperature (r=0.95, *P*=0.049). In contrast, *Pseudomonas stutzeri* seems to favor relatively low temperatures and humidity.

The abundance of these species indeed shows significant negative correlations with air temperature (r=-0.93, *P*=0.0681) as well as relative humidity (r=-0.95, *P*=0.0404). Additionally, the abundances of *Bacillus firmus* (r=-0.95, *P*=0.0405) and *Staphylococcus epidermidis* are negatively correlated with the formaldehyde concentration (r=-0.95, *P*=0.0405).

4. Discussion of key findings and limitations

The findings of this study emphasize the importance of maintaining optimal IAQ in childcare environments, particularly given the potential adverse effects on children's health. Elevated concentrations of CO_2, CH_2O, and PM_{10} were observed, especially in rooms with inadequate ventilation and higher occupancy. These findings are consistent with previous studies that have highlighted the role of poor ventilation in elevating CO_2 levels [61–68]. In our study, the highest CO_2 concentrations were recorded in RE1 and RE3 during winter and spring, which were both overcrowded and had limited ventilation openings. This suggests a direct link between overcrowding and ventilation insufficiencies, with clear implications for air quality and, by extension, occupant health.

The presence of formaldehyde (CH_2O) in the classrooms, particularly during summer, is another significant concern. The elevated CH_2O concentrations, which surpassed WHO's recommended limit in RE3 and RE2, were likely influenced by the widespread use of hydroalcoholic gels during the COVID-19 pandemic. Similar findings have been reported in other studies, indicating that cleaning and disinfecting protocols can inadvertently contribute to indoor air pollution [66,74–77]. The repeated exceedance of formaldehyde levels in these classrooms suggests the need for alternative

disinfection practices that minimize indoor air contamination.

Fine particulate matter (PM_{10}) levels also exceeded WHO's recommended limits, particularly in RE1 and RE3 during winter. Notably, the source of these particles appeared to be both indoor activities (e.g. heating and furniture) and outdoor pollution, with the latter becoming more significant in the spring. These findings align with previous research that suggests heating systems and activities like cleaning and movement contribute substantially to indoor PM_{10} concentrations [79,80].

A key finding of this study was the significant correlation between CO_2 concentrations and the abundance of certain bacterial species, including *Staphylococcus epidermidis* and *Bacillus licheniformis*. This suggests that elevated CO_2 levels may create an environment favorable to microbial growth, posing a potential health risk to children. The presence of these pathogenic bacteria in indoor air highlights the need for continuous monitoring and better control of both particulate matter and microorganisms to ensure the safety of childcare environments.

While these findings are valuable, this study is not without limitations. The small sample size (three childcare centers) and the seasonal variability in IAQ conditions may affect the generality of the results. Additionally, the study focused only on a limited number of pollutants and microbial species, and future research should explore other potential indoor contaminants. Moreover, variations in the type and effectiveness of air purification systems were not assessed, which could be an important area for future research.

This study emphasizes the need for improved ventilation and air quality management in childcare centers, particularly in regions with similar climate conditions. Simple interventions such as enhancing natural ventilation, reducing overcrowding, and employing effective air purification methods could significantly reduce the levels of CO_2, CH_2O, and PM_{10}, thereby mitigating health risks associated with poor IAQ.

5. Conclusions

The results of this study indicate that children in the examined classrooms experienced hygrothermal discomfort and were exposed to elevated levels of CO_2, PM_{10}, and formaldehyde. Additionally, microbiological culture-independent analyses revealed the presence of various bacterial species, including potential pathogens, highlighting a significant bioaerosol risk. The results emphasize that poor indoor environmental conditions such as inadequate ventilation, insufficient air exchange, overcrowding, and heating contribute to higher pollutant concentrations and microbial contamination. Consequently, implementing measures to optimize indoor air quality, regulate temperature and humidity, and improve classroom ventilation could significantly reduce children's exposure to airborne pollutants and bio-contaminants, ultimately fostering a healthier learning environment.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflicts of interest or personal relationships that could have influenced the work reported in this paper.

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