Journal of Building Engineering 113 (2025) 113997

Contents lists available at ScienceDirect

Journal of Building Engineering

journal homepage: www.elsevier.com/locate/jobe

School air quality and thermal comfort: A multi-pollutant seasonal assessment

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ARTICLE INFO

Keywords:
Schoolchildren
Air quality
Particulate matter
Gaseous pollutants
Bioburden

ABSTRACT

As evidence of children's vulnerability to air pollution grows, research on school air quality has increased significantly in the 21st century. Given the complex factors influencing indoor and outdoor air quality in schools, each study offers valuable insights. This study contributes by assessing particulate matter, gaseous pollutants, thermal comfort and microorganisms in a large school encompassing different education levels over two seasons. The assessment combined passive and continuous sampling using various techniques, including chromatography and estimations of air change and ventilation rates. Classroom ventilation was insufficient to ensure adequate air renewal. During class hours, CO_2 concentrations ranged from 760 to 1,118 ppm in winter and from 807 to 1,022 ppm in spring, repeatedly exceeding 1,000 ppm. CO_2 and PM_{10} concentrations were significantly higher during school hours than when the classrooms were empty, indicating the strong influence of school activities. In contrast, PM_1 and $PM_{2.5}$ concentrations were more influenced by external factors, especially outside of school hours. The lack of thermal comfort created an unhealthy environment. Carbonyl concentrations were higher in classrooms (average: 68.8 μg m^{-3}) compared to the schoolyard (3.86 μg m^{-3}), in both seasons. Microbial analysis revealed the presence of fungi with toxigenic potential, with the highest fungal diversity observed in spring. These findings highlight that while some pollutant levels may appear low, they can occasionally reach extremely high levels, even in newer buildings. The novelty of this research lies in demonstrating that, despite recent improvements and numerous studies, significant progress is still needed to ensure healthier school settings.

1. Introduction

In schools, various pollutants can compromise air quality. The most common air pollutants include particulate matter (PM) of

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https://doi.org/10.1016/j.jobe.2025.113997
Received 31 May 2025; Received in revised form 28 July 2025; Accepted 1 September 2025
Available online 6 September 2025

different sizes (PM_{10}, $PM_{2.5}$ and PM_1), as well as volatile organic compounds (VOCs), including carbonyls like formaldehyde, and inorganic gases, such as carbon dioxide (CO_2), nitrogen oxides (NO_x), sulphur dioxide (SO_2) and ozone (O_3). Fungi such as *Penicillium*, *Cladosporium*, *Aspergillus*, and *Alternaria* have been identified as the most common in indoor school environments, although their presence varies depending on the climate and location, whether rural or urban [1]. The use of cleaning products and floor polishes can also affect air quality by increasing chemical pollutants in school environments [2]. These pollutants can accumulate quickly in enclosed spaces, especially when classrooms lack adequate ventilation. Air quality inside and around schools is a complex challenge, determined by both external factors, such as traffic and the urban environment, and internal factors, including occupant activities, building materials, the efficiency of air purifiers, and, crucially, the ventilation of school buildings [3,4]. Outdoor pollution penetrating indoor spaces further exacerbates the situation [4]. It is important to note that high concentrations of pollutants, combined with poor indoor environmental quality - in terms of ventilation, temperature, and humidity - can have negative effects on children's health, well-being, and academic performance [5].

Against this backdrop, several studies have demonstrated a direct relationship between air quality and students' academic performance. For example, a one-year cohort study by Basagaña et al. [6] with a sample of 2,618 students from 39 schools in Barcelona, Spain, found that, after characterising pollution sources in these educational establishments, traffic was the only source of fine particulate matter associated with decreased cognitive development. The researchers observed that higher levels of $PM_{2.5}$ exposure in classrooms correlated with significantly reduced working memory in 8.5-year-old children. Furthermore, in their literature review, Roche et al. [7] concluded that scientific evidence on air pollution highlights its negative on children's cognitive and respiratory health, as well as their academic performance, and increases their susceptibility to diseases in adulthood. It is precisely because of this concern that the European Union (EU) has actively promoted and supported programmes aimed at improving air quality in schools [8, 9]. However, despite these efforts, significant challenges remain, especially at the legislative level, where there are still no specific programmes dedicated solely to improving air quality in schools. In contrast, since 2017, the New Zealand government has taken a proactive approach by implementing the Design of Quality Learning Spaces (DQLS) guidelines, aimed at ensuring that the design and construction of school buildings create environments that support effective teaching and learning. In its latest 2022 update [10], the document expanded its focus to include parameters such as temperature, relative humidity, CO_2, ventilation rate, and window-to-wall ratio. Although schools are not required to have their own indoor air monitoring devices, the guidelines do establish mandatory requirements for specific parameters during school hours, tailored to the type of room (such as laboratories, gymnasiums, classrooms, libraries, etc.).

Unlike New Zealand, the Portuguese government does not have specific laws or documents dedicated exclusively to school buildings. However, there is a growing interest in the area, reflected in Ministerial order No. 138-G/2021 [11], which sets indoor air quality requirements for public buildings, and Decree No. 101D/2020 [12], which establishes energy performance and ventilation criteria. Although these standards do not apply exclusively to schools and only include limited number of classical air pollutants, they determine that building housing daycare centres and primary school establishments are subject to a simplified annual assessment of certain requirements, as well as verification of compliance with the respective established parameters. This obligation becomes even more important in view of the growing evidence of the negative effects of air pollutants on pupils' health and academic performance, making periodic assessment of air quality in educational institutions crucial. This assessment not only identifies potential risks but also plays a key role in raising awareness among educational and policymakers about the urgent need to reform environmental regulations to protect children's physical and cognitive well-being.

In this context, the aim of this research was to: (i) assess thermal comfort conditions and ventilation effectiveness in different classrooms; (ii) evaluate real-time concentrations of several regulated and unregulated gaseous pollutants and particulate matter (PM_{10}, $PM_{2.5}$ and PM_1); (iii) analyse average gaseous pollutant concentrations using passive sampling; and (iv) characterise seasonal variations in settleable dust and microorganisms at a school attended by children between 3 and 12 years old. An extensive dataset of thermal and air comfort parameters was leveraged, collected from two distinct environments, indoor classrooms and the outdoor schoolyard, to conduct a comprehensive analysis of school air quality. Despite the wealth of literature on this topic [13–15], a notable scientific gap remains in the integrated assessment of air quality within educational settings. Few studies have simultaneously measured thermal comfort variables, gaseous pollutants, particulate matter or microbial presence across different seasons [16–18]. Our methodological approach addresses this gap by combining both passive and active sampling, detailed analysis techniques, encompassing multiple pollutant types including microorganisms. Beyond deepening the understanding of indoor environmental quality, this integrated framework holds significant potential for informing urban planning strategies that support healthier learning environments, while also improving comprehension of the complex interaction of factors that influence the air children breathe.

2. Methodology

2.1. Study area

This study was conducted in a school located in Estarreja, an important industrial municipality in northern Portugal (40.7632, −8.5755). The municipality is divided into five civil parishes in an area of 108 km^{-2} and has a population of approximately 30,000 inhabitants. In Portugal, basic education comprises three stages: 1st cycle (grades 1–4, from 6 to 9 years), 2nd cycle (grades 5 and 6, from 10 to 11 years) and 3rd cycle (grades 7–9, from 12 to 14 years). The school in Estarreja began its activities in 1996 and currently welcomes just over 700 students. It operates from 7:30 to 18:30, with the regular school day running from 8:30 to 15:30, and a 60-min break for lunch. Cleaning work, of the responsibility of the educational support assistants, is usually carried out between 16:00 and 18:00. In addition to the nursery school, the establishment offers the first two cycles of basic education and has classrooms adapted for

pupils with special needs. The school has four main buildings, each identified by a letter from A to D (Fig. 1). Each building corresponds to a specific educational level: building D is for nursery/reception (children from 3 to 5 years), building B for the first cycle (children from 6 to 10 years) and buildings C and A for the second cycle (children from 10 to 12 years). The educational levels offered by the school and their nearest equivalents in the British system can be found in Table S1.

2.2. Experimental setup

Sampling was carried out at two different times. The winter campaign was performed between November and December 2022, and the spring campaign between April and May 2023. The air quality assessment in this study incorporated: i) passive sampling of gaseous pollutants, ii) continuous monitoring of gaseous pollutants, thermal comfort parameters and particulate matter, and iii) passive sampling of microorganisms and settleable dust. The specific settings for each method are described in Table 1. A total of nine samplers of each type were installed for ~14 days in different rooms and outdoors. Passive sampling was conducted simultaneously across all locations, without distinguishing between occupied and unoccupied periods. Table S2 in the supplementary material summarises the monitoring periods in each school location.

2.3. Sampling approach

The air quality assessment included parameters measured exclusively in classrooms, parameters measured only in the outdoor school environment, and parameters monitored in both settings.

2.3.1. Continuous monitoring in classrooms

The following instruments were installed in four different classrooms, which were selected to represent different age groups and types of school activities in each building (Fig. 1): an AirAssure 8144-4 monitor, a WolfSense IQ-610 monitor, a Gasera One, a Delta Ohm HD 32.3 monitor, and an aerosol spectrometer Grimm model EDM 164. The instruments were placed at a height of approximately 1 m above the floor, keeping a minimum distance of 1 m from doors, windows and walls. At the end of each Friday afternoon, the instruments were relocated from one room to another in the adjacent building, to ensure the continuous monitoring system operated for five days a week. To avoid spatial and temporal biases, the same classrooms were picked out for monitoring during both the winter and the spring seasons. However, the monitoring periods in each classroom varied due to public holidays when the school was closed, as well as technical failures in the measuring equipment that prevented data collection for a few hours. In addition, the Delta Ohm was only used during the first three weeks of winter sampling, and the Gasera One presented some failures.

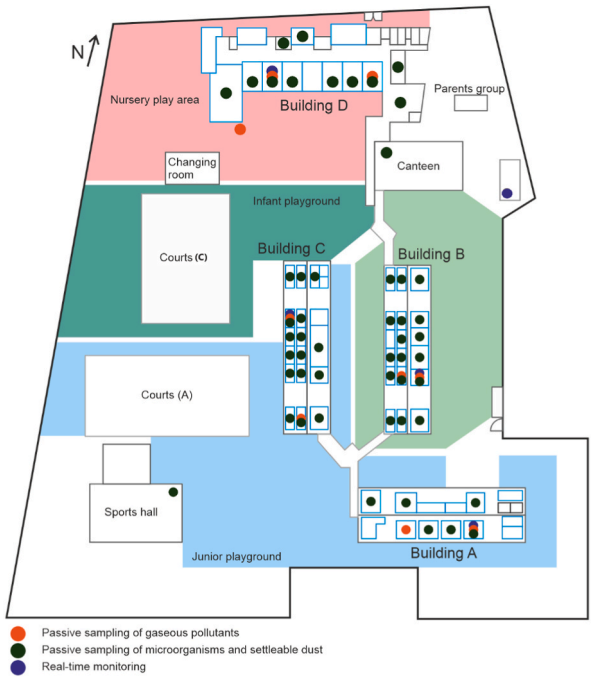

Fig. 1. Monitoring locations. The colours correspond to the type of sampling performed: orange for passive samplers of VOCs, carbonyls and NO_2; green for passive microbiological and settleable dust samplers, and purple for continuous equipment, such as gases, thermal comfort and particulate matter in three different sizes. The nursery classrooms are in building D, the infant classrooms are in building B, while buildings C and A have the junior classrooms.

Table 1
Instruments suites and settings used for sample collection at the school.

Sampling type	Instruments	Parameters	Sampling tome
Passive gas sampling	Radiello® samplers	Volatile organic compounds (VOCs) and carbonyls	14 days
	Passam samplers	Nitrogen dioxide (NO_2)	14 days
Passive microorganisms	Petri dishes, each containing a 14 cm diameter dry Electrostatic Dust Cloth (EDC) (Swiffer brand)	Fungi and bacteria	~33 days
Passive settleable dust	47 mm quartz filters	Settleable dust	~33 days
Indoor real-time monitoring system	AirAssure 8144-4 monitor	Temperature, relative humidity (RH) and CO_2	15 min
	WolfSense IQ-610 monitor	Temperature, RH, CO_2 and total VOCs (TVCOs)	1 min
	Photoacoustic multi-gas analyser Gasera One	CH_4, NO_2, NH_3, and C_2H_6O	3 min
	Delta Ohm HD 32.3 monitor	thermal comfort measurements, including wet bulb temperature (Tw), air velocity (Va), predicted mean vote (PMV) and percentage of people dissatisfied (PPD)	1 min
	Spectrometer Grimm model EDM 164	PM_1, $PM_{2.5}$ and PM_{10}	1 min
Outdoor monitoring system	AirAssure 8144-4 monitor	Temperature, relative humidity (RH) and CO_2	15 min
	Weather station	Meteorological parameters	10 min

2.3.2. Continuous monitoring in the schoolyard

An AirAssure 8144-4 monitor and a weather station were installed in the schoolyard. These instruments operated continuously from the first day of each campaign and measured simultaneously with the instruments placed inside the classrooms, in accordance with the rotational measurement schedule established for the rooms. Additionally, both outdoor instruments were consistently located in the same position during both seasons to ensure comparability.

2.3.3. Passive sampling of airborne microorganisms and settleable dust

Passive sampling of microorganisms and settleable dust was conducted throughout the school at over 40 sites. Upon arrival at the site, the Petri dish was opened, exposing the EDC to ambient air, following the procedure previous published by Viegas et al. [19]. In addition, the quartz filters were installed by the sweeping cloths to assess the deposition of settleable dust. These filters remained exposed to falling dust for 34 days in the winter sampling and 32 days in the spring sampling.

2.4. Analytical techniques

The analysis of the VOC diffusive tubes was performed at the *Istituti Clinici Scientifici Maugeri* (Vigonza, Italy) by gas chromatography with a mass spectrometer detector. Analytical procedures can be downloaded from the Radiello® website. NO_2 was determined spectrophotometrically using the Saltzmann method. More information on this procedure can be found on the Passam website. Carbonyl samplers were analysed at the University of Aveiro. Each cartridge, placed inside a glass tube, was treated with 2 mL of acetonitrile. The solution was stirred intermittently for 30 min. The cartridges were then removed from the solution, which was analysed by high-performance liquid chromatography (HPLC). The HPLC used consists of a degasser (model DGU-20ASR), a solvent distributor (model LC-30AD), an automatic sampler (model SIL-30AC), a diode array detector (model SPD-M20A), and an oven (model CTO-20AC) containing an Ascentis C18 SUPELCO column (15 cm × 4.6 mm, 5 μm). The Lab Solutions software was used to carry out data processing and system control. The method followed in this study was adapted from that described by Marchand et al. [20]. The gradient analysis starts with a mixture composed of 70 % of mobile phase A (20 % acetonitrile, 60 % MilliQ water, and 20 % tetrahydrofuran) and 30 % of mobile phase B (60 % acetonitrile and 40 % MilliQ water). After 30 min, the gradient is adjusted to 100 % mobile phase B and maintained in this condition for 30 min. At 85 min, the gradient returns to its initial condition (70 % A and 30 % B), marking the end of the analysis. The flow rate is maintained at 0.7 mL min^{-1} throughout the analysis. The injection volume is 10 μL and the column temperature is maintained at 22 °C. A standard solution (TO-11/IP-6A Aldehyde/Ketone-DNPH Mix, Supelco) containing 15 carbonyl-DNPH derivatives (formaldehyde, acetaldehyde, acrolein, acetone, propionaldehyde, crotonaldehyde, butyraldehyde, benzaldehyde, valeraldehyde, isovaleraldehyde, o-tolualdehyde, m-tolualdehyde, p-tolualdehyde, hexaldehyde and 2,5-dimethylbenzaldehyde) in acetonitrile was used to obtain external calibrations curves (six points) for each compound ranging from 0 to 2 μg mL^{-1}. The concentration and instrumental response showed strong linear relationships for all measured carbonyls. Calibration curves were verified daily before analysis. Blank cartridges were also analysed to assess eventual contaminations. The concentration levels of carbonyls were determined based on the sampling rates provided by Radiello®. For compounds such as acetone, crotonaldehyde, tolualdehydes, and 2,5-dimethylbenzaldehyde, for which Radiello® did not specify sampling rates, estimations were derived from sampling rates calculated and reported by Villanueva et al. [21].

For microorganism analysis, the EDCs were rinsed with 20 mL of a 0.9 % NaCl plus 0.1 % Tween 80 solution, followed by agitation (250 rpm, 30 min). The resulting suspension was inoculated onto malt extract agar (MEA) and incubated at 27 °C for 5–7 days and dichloran-glycerol agar (DG18) at 27 °C and 37 °C for 5–7 days to culture fungi and tryptic soy agar (TSA) at 30 °C for 7 days and violet red bile agar to bacterial assessment, adhering to methodologies outlined in prior studies [22]. To screen for azole resistance, Sabouraud agar supplemented (SAB) with itraconazole (ITRA) (4.0 mg L^{-1}), voriconazole (2.0 mg L^{-1}), and posaconazole (0.5 mg L^{-1}) was used, with incubation at 27 °C for 48 h, as described previously. Fungal identification was conducted by experienced researchers

specialising in environmental mycology, based on macroscopic and microscopic morphological features consistent with established taxonomic guidelines [23].

2.5. Data validation and processing

Instrument preparation was conducted in the university laboratory prior to each monitoring campaign. This included zero calibration checks, cleaning, battery and memory status verification, and clock synchronisation. Additionally, when instruments were moved from one room to another, battery levels, memory capacity, and time synchronisation were rechecked to ensure consistency. At the end of each campaign, some parameters were compared across devices to confirm proper performance and data integrity. Also, particulate matter concentrations obtained with the optical monitor were corrected using the gravimetric concentrations from simultaneous sampling with high-volume samplers, which is considered the reference method, and whose values were reported in a previous publication [24].

In addition, for each continuously monitored parameter, the number of complete observations during class time was first calculated. Daily records with less than 30 % of the expected data for a school day were considered incomplete and excluded from the analysis as outliers. While the median is generally more appropriate for data with non-normal distribution, such as those typically observed at the school, the mean concentration was reported to enable comparison with findings from other studies. Seasonal variations and differences between class time and unoccupied periods were assessed using the non-parametric Wilcoxon test. A p-value of <0.05 was considered statistically significant.

2.6. Air change and ventilation rates

The ventilation rate was calculated using data from two measuring devices, WolfSense IQ-610 and AirAssure 8144-4 monitor, depending on the available information for each site. Since some rooms did not have complete CO2 measurements for some days, this approach made it possible to avoid data omissions in cases where one of the devices did not record measurements, ensuring that the ventilation rate was calculated continuously for all days and rooms included in the study. To ensure data consistency, it was verified that the correlation between both devices in both seasons was always greater than 0.9 (Fig. S1). The CO2 decay method was applied to measure the air change rate (ACH, h-1) of the classrooms, according to the following equation [25]:

$$ACH\ (h^{-1}) = \frac{\ln((C_1 - C_R) \div (C_0 - C_R))}{t} \quad (1)$$

where C_0 and C_1 are the CO_2 concentrations measured during the CO_2 decay period (usually in the afternoon, after school activities have finished); C_R is the reference CO_2 concentration (in this study, C_R represents the lowest value calculated from the average CO_2 concentration between 0 and 8 h of each day); and t is the time interval, in hours, between the times corresponding to C_0 and C_1.

To assess whether the rooms met the appropriate ventilation requirements, as stipulated by ASHRAE Standard 62.1–2022, the ventilation rate was calculated in units of L s^{-1} m^{-2}, using the procedure detailed in the supplementary material (Equation S2).

3. Results and discussion

3.1. Classroom thermal comfort and ventilation rate

The temperature and RH in the classrooms fluctuated according to the seasons (Table 2). In winter, temperatures dropped, and RH increased, creating more humid and cold environments. In spring, the temperature was slightly higher, and RH decreased, on average, between 6.4 % and 23 %. In the coldest period, although the temperature in the classrooms was generally higher than that outside (Fig. S2), only 2 out of 4 rooms met the ideal temperature range of 20–23°C. Furthermore, the average RH in the classrooms was above the recommended range of 30–60 % [26], compromising thermal comfort. In contrast, in spring, while RH remained within the recommended range, the temperature in some rooms exceeded the proposed maximum. When comparing the data from the classrooms

Table 2
Comfort parameters in classrooms. The values presented correspond to the average for the class time. Corresponding values for empty rooms are listed in Table S3.

Location	Season	Temperature (°C)			Relative humidity (%)		
		Min	Max	Mean (±sd)	Min	Max	Mean (±sd)
Nursery	Winter	20.4	23.8	22.1 (0.61)	58.5	72.6	64.3 (2.94)
	Spring	19.3	24.7	21.5 (1.64)	38.3	61.4	51.1 (4.31)
First cycle (B)	Winter	18.3	22.1	20.5 (0.78)	55.0	75.9	65.1 (5.44)
	Spring	21.4	25.0	23.5 (0.87)	53.8	61.3	58.7 (1.23)
Second cycle (C)	Winter	14.1	19.2	16.6 (1.18)	48.7	67.0	60.0 (4.49)
	Spring	23.4	27.5	25.5 (1.10)	31.3	54.8	47.1 (4.66)
Second cycle (A)	Winter	14.7	19.0	16.9 (1.02)	59.6	71.0	64.9 (2.34)
	Spring	23.8	26.4	25.1 (0.70)	27.0	58.1	41.9 (8.54)

Table 3
Air change rates, people outdoor air rates (Rp) and area outdoor air rates (Ra). Number of days considered in the calculation (n).

Season	Location	n	Air change rate h^{-1}	Ra L s^{-1} m^{-2}	Rp L min^{-1} per pupil
Winter	First cycle (B)	4	0.33–1.04	0.30–0.95	61.9–197
	Second cycle (C)	3	0.45–1.44	0.43–1.36	73.5–234
	Second cycle (A)	4	0.32–0.74	0.30–0.70	56.1–131
Spring	First cycle (B)	3	0.34–0.63	0.28–0.58	17.7–120
	Second cycle (C)	4	0.47–1.56	0.44–1.47	46.1–255
	Second cycle (A)	4	0.72–0.90	0.67–0.85	126–159

with the values observed in the schoolyard, it is evident that both environments followed similar patterns, but with differing values. In the schoolyard, lower temperatures and higher RH levels were recorded in both seasons. This pattern is illustrated more clearly in Fig. S1 (see Table 3).

The statistical analysis of temperature and RH values across the four classrooms revealed significant differences between occupied (class time) and unoccupied (empty rooms) periods during winter. In spring, however, these differences were only statistically significant in first cycle (B) and second cycle (C) classrooms. A slight increase in temperature during class time was observed, likely due to the presence of occupants generating heat. Additionally, significant seasonal differences in both temperature and RH were consistently observed across all classrooms when comparing winter and spring.

Almeida and de Freitas [27], who assessed the temperature and humidity conditions in 24 classrooms across 9 schools in Portugal over the course of a year, during school hours, found that classrooms rarely reached average temperatures of 20 °C. The situation was even more critical in older schools, where, in some cases, classroom temperatures were lower that the outside temperature in winter. Although the conditions improved in spring, they still observed one school where the temperature exceeded 25 °C. In contrast, the same study suggested that, in general, RH did not represent a serious problem in the schools evaluated, even considering seasonal variations and the specific conditions of each school. The observed mean RH values were 12.8 % lower in winter and 5.6 % lower in spring compared to the data recorded in the present study.

As part of the winter sampling, data were collected from the following three classrooms where the real-time monitoring instruments were installed: nursery, first cycle (B) and second cycle (C) to assess additional parameters related to thermal comfort. Although there is no single reference value for Tw, a range between 20 °C and 23 °C is considered adequate to maintain thermal comfort in indoor environments. However, the results revealed considerable variability in environmental conditions across classrooms (Fig. 2). The Tw never exceeded 21.6 °C during school hours, reaching this value only in the nursery room, which favoured a more comfortable environment compared to the other classrooms (PPD ~6 %). In contrast, in the second cycle room, Tw conditions (13 °C ± 1.28), together with a PMV far from 0 (neutral) and a significant increase in PPD, indicated high levels of discomfort. This was reflected in greater dissatisfaction, with PPD values reaching up to 40 %. In the first cycle classroom, although Tw values were higher (17 °C ± 0.99), they still did not achieve an adequate balance between temperature and humidity, resulting in an average PPD of 5.57 %.

The UNI EN ISO 7730 standard establishes guidelines for thermal comfort in indoor environments [28], indicating that the PMV should be between −0.5 and + 0.5, and the PPD should be less than 10 %, under air velocities between 0.05 and 0.15 m s^{-1}. Overall,

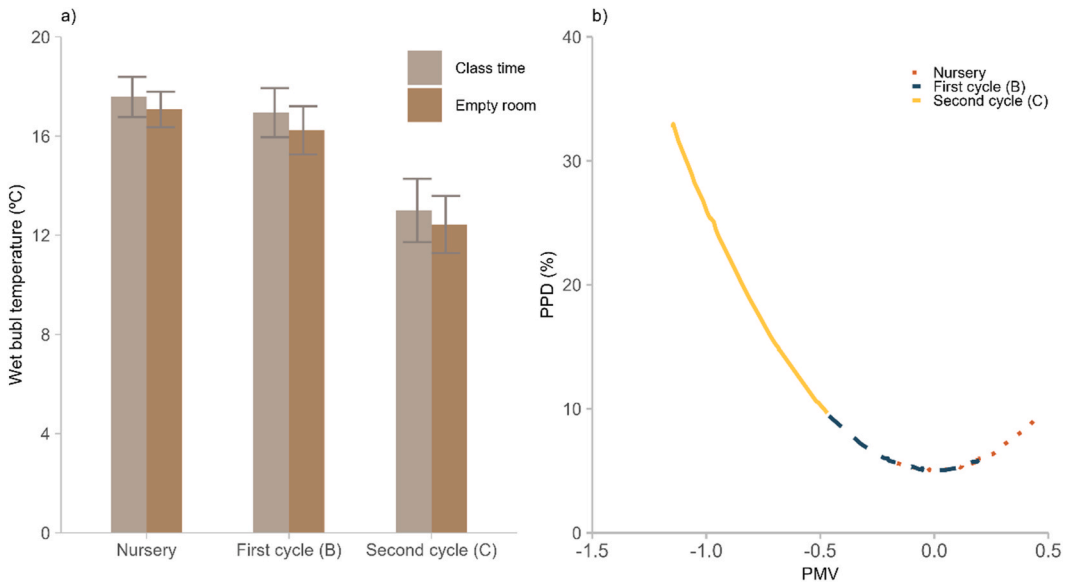

Fig. 2. a) Average wet-bulb temperature (Tw) by classroom and by occupancy time; b) relationship between PMV and PPD during school hours. The data presented correspond to winter sampling.

the air velocity in the school during school hours was always less than 0.05 m s^{-1}. The results suggest generally acceptable PPD conditions in the first two classrooms, with the percentage of dissatisfied pupils never exceeding 10 %. However, the average PPD per class hour in the second cycle ranged from 9.66 % to 45.1 %, considerably higher than that observed in 19 classrooms (6.7 ± 2.3) of an Italian high school, measured in February 2019 [29]. This figure, however, was like that recorded in 26 classrooms monitored in the province of Pisa, Italy, between October and December 2021 [30], where the PMV ranged from −2.5 to +0.8, indicating that students tended to perceive the environment as somewhat cold. In that case, the PPD ranged from 5 % to 94 %, reflecting greater variability in thermal comfort compared to the results obtained in this study.

ASHRAE Standard 62.1–2022 does not provide a threshold for CO_2 concentrations. However, due to ongoing misunderstanding in previous editions, many still mistakenly associate 1,000 ppm with occupied hours [26]. ASHRAE's position is that CO_2 is not an overall indicator of indoor air quality, and the direct health impacts at commonly observed indoor levels have not been consistently established to warrant changes in ventilation standards based on CO_2 as a pollutant. Although ASHRAE does not specify a threshold for CO_2, we opted to compare our values to 1,000 ppm, as it serves as a convenient reference point for comparison with numerous other studies. In comparison to the 1,000 ppm, the nursery was the only classroom that did not exceed this value during class hours in both seasons (Fig. 3). During holidays and weekends, the CO_2 concentration in the classrooms was, on average, 2.13 and 1.42 times lower than the values recorded during school hours in winter and spring, respectively. Although, in both seasons, the average values during class time in some rooms remained below 1,000 ppm (Fig. 3), the analysis of daily concentrations (Fig. S2 and Fig. S3) revealed that CO_2 levels frequently exceeded that limit. During school hours, the highest recorded concentration of CO_2 was 2,794 ppm in winter and 2,989 ppm in spring. The four selected classrooms show unique patterns in the real time distribution of CO_2 concentrations. However, they all show concentration peaks during periods of occupancy and the lowest concentrations outside of school hours. The analysis confirmed that in the junior classrooms, CO_2 concentrations peaked in winter and were lowest in spring. In contrast, in nursery and infant classrooms, the highest average concentration during school hours was recorded in spring. In general, all classrooms showed statistically significant differences between the two seasons during school hours.

The increase in CO_2 concentrations in the classrooms is attributed to the presence of occupants, with levels tending to rise depending on the type of activities carried out. These concentrations vary throughout the day, with CO_2 accumulation during the period of occupancy and decrease during breaks. This fact suggests that CO_2 may represent a concern for the occupants of the educational establishment, in addition to indicating possible insufficient ventilation in the rooms. Therefore, it is necessary to implement measures to ensure adequate conditions for the proper functioning and academic performance of pupils. CO_2 concentrations at the school were compared with values reported in previous studies (Fig. 4). In winter, the global average concentration at Estarreja school was lower than in spring, consistent with the findings of five reviewed studies, which also reported a decrease in CO_2 levels during this season. However, the results of this study were considerably lower compared to those obtained in other works, particularly those reported by Stabile et al. [31], who found an average concentration of 2,207 ppm in five classrooms of different naturally ventilated schools in Cassino, Italy - double the value observed in Estarreja (1,132 ppm). According to the researchers, CO_2 levels were higher in winter due to the limited use of window opening, which waws not sufficient to keep CO_2 concentrations below 1,000 ppm. When the windows remained closed, the CO_2 concentration increased, reaching values close to 3,000 ppm. This situation contrasts with that observed in spring, when average CO_2 concentrations were mostly below 1,000 ppm, due to the prolonged opening of windows, which allowed for better ventilation.

In spring, when CO_2 concentrations decreased, probably due to more favourable weather conditions and increased natural ventilation, values ranged from 726 ppm in the UK to 1,247 ppm in Australia (Fig. 4). The average value observed in this study (913 ppm) was very similar to those reported by Stabile et al. [31] in Italy and by Korsav et al. [32] in Coventry, where mean concentrations of 908 and 1,050 ppm were recorded, respectively. The highest values were reported by Rajagopalan et al. [33] in Australia (1247 ppm) and by Cai et al. [34] in China (1274 ppm). Cai et al. [34] attributed the lower CO_2 concentrations to the greater frequency with which pupils open windows and doors during spring compared to winter. In the study conducted in China, the authors found that while

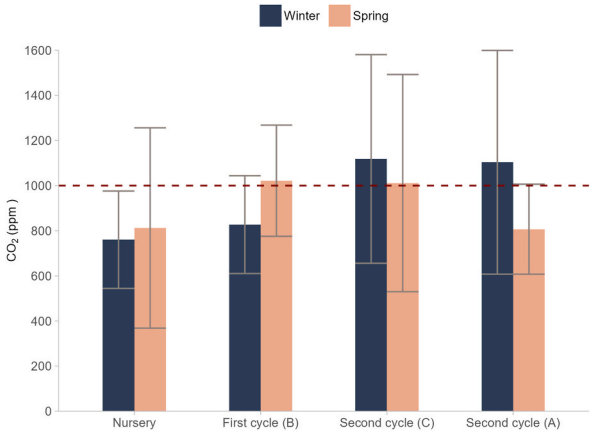

Fig. 3. Average CO_2 concentration during school hours for the two seasons. The dashed line indicates the 1,000 ppm benchmark used in this study.

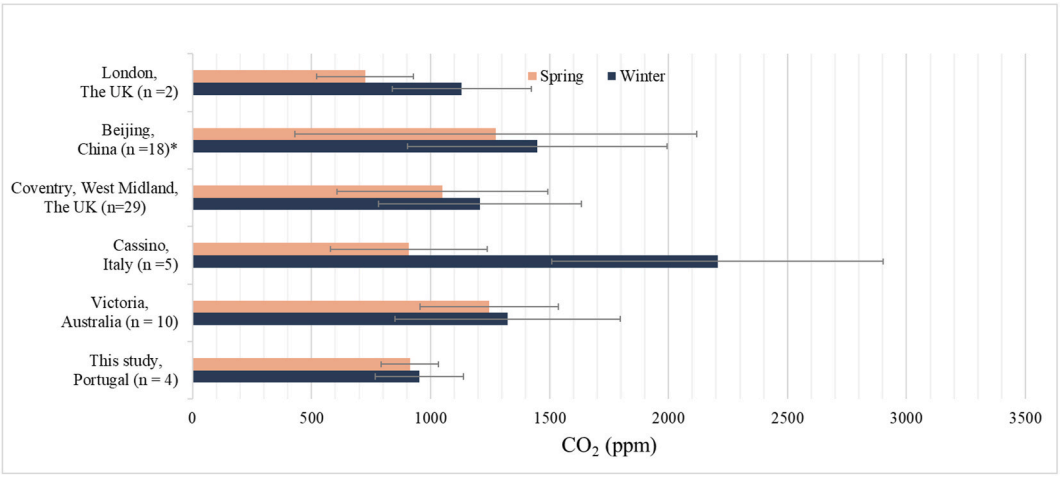

Fig. 4. Average CO_2 concentration during school hours in winter and spring in this study and in classrooms in different parts of the world using natural ventilation systems. n indicates the number of classrooms considered in each study. *For comparison purposes, the transition period, which spanned from March 15 to May 31 and from September 1 to November 14, was considered equivalent to the spring season.

rooms with natural ventilation (NV) showed significant seasonal differences ($p < 0.05$), rooms with mechanical ventilation (MV) systems, in addition to presenting lower CO_2 concentrations in all seasons (953 ppm in spring), did not show significant seasonal differences. Although the authors did not definitively conclude which ventilation system is better, the results of the study suggest that rooms with MV met the CO_2 standards more frequently than rooms with NV.

Air change rates in the school are presented in Table 2. However, because the dynamics of the nursery room were considerably different from the others, with elevated CO_2 concentrations after school hours (Fig. S2), this room was excluded from the calculation of the ventilation rate (VR). The slow decay of CO_2 levels after the school day in this room may be due to its smaller size, higher airtightness, and elevated temperatures, as it faces south and experiences greater sunlight exposure. The air change rate in the three classrooms studied ranged from 0.32 to 1.44 h^{-1} in winter, and from 0.34 to 1.56 h^{-1} in spring. Although not a directly equivalent comparison, these values were lower than the 10th and 99th percentiles reported in a study of school classrooms in Northern Europe (0.77 and 2.81 h^{-1}). In that study, Baloch et al. [9] concluded that ventilation has an apparent protective effect on ocular and skin disorders in children attending classrooms with an ACH >0.67 h^{-1}.

To determine whether ventilation in the classrooms was adequate, the height of the rooms and the number of pupils were considered to calculate the VR and compare it with that established by the ASHRAE 62.1–2022 standard (Ventilation for Acceptable Indoor Air Quality). It stipulates the people outdoor air rate (Rp) and outdoor air rate per unit of area (Ra). These two values are crucial for determining the amount of outdoor air required for various types of spaces in buildings, aiming to ensure that indoor air quality is acceptable for occupants. For schoolrooms, a minimum ventilation rate of 0.6 L s^{-1} m^{-2} (Ra) or 300 L min^{-1} per person (Rp) are set. On none of the days evaluated during both campaigns did the ventilation rate per student meet the value recommended by the standard. The closest value was recorded in the second cycle classroom (building C) on a spring day, with 4.22 L s^{-1} per person.

The values in this study may seem very low compared to what is recommended for classrooms, but according to Ding et al. [35] in their review study, the reality is that a large proportion of school classrooms in different parts of the world have still failed to meet ventilation requirements. Even in American primary schools with mechanical ventilation systems, Haverinen-Shaughnessy et al. [36] found an average Rp of 4.2 L s^{-1} per person, with 87 % of classrooms below the ASHRAE 62.1 standard, indicating that inadequate ventilation in classrooms is common. Similar results were found in London, where Kumar et al. [15] analysed 60 classrooms in primary and secondary schools with different types of ventilation. Although the average value of Rp in that study was considerably higher (5.9 L s^{-1} per person) than that found at Estarreja school, the researchers observed that most classrooms did not meet the recommended standards. When examining different factors, they found that floor level, type of flooring and age group had little impact on artificial ventilation, while ventilation type and classroom size did have a significant impact. Thus, mechanically ventilated and larger classrooms (volume >300 m^3) had better ventilation rates.

In this study, the classrooms evaluated had an average of 20 pupils and had a volume that varied between 166 and 280 m^3, classifying them within the small and medium-sized categories according to Kumar et al. [15] study. However, unlike what was observed in London, it was not possible to identify a clear pattern between classroom volume and ventilation, because only three rooms were analysed. This limitation prevents definitive conclusions from being drawn on how classroom size affects ventilation efficiency. Although ventilation rates were generally better in winter, the recommended standards are met on very few days. High CO_2 concentrations are due to a lack of adequate continuous ventilation during school hours, as classrooms are often closed during classes, and only junior classrooms keep their doors open after school hours.

3.2. Gaseous pollutants

Overall, greater variability in TVOC concentrations was observed when classrooms were occupied during both seasons (Fig. 5). In addition, concentrations were higher during the school day compared to values recorded when classrooms were empty. However, it was found that while average concentrations in nursery and infant classrooms were higher in spring during school hours (1,211 and 718 μg m^{-3}, respectively), in second cycle classrooms (buildings A and C), average concentrations were higher in winter, both during school hours (550 and 533 μg m^{-3}, respectively) and when classrooms were unoccupied. The limit of TVOCs in indoor air is generally set at 600 μg m^{-3}. This limit is often based on guidelines and recommendations from organisms like the World Health Organisation (WHO) and the European Union, aiming to ensure that indoor air quality remains safe and healthy for building occupants. When comparing the average TVOC concentrations in classrooms during school hours, it was observed that two of the four classrooms exceeded this value in either season. In the nursery classroom, concentrations exceeded the limit by 38 and 611 μg m^{-3} during winter and spring, respectively, while in the first cycle room the average concentration reached 718 μg m^{-3} in spring.

In line with other studies [37,38], which have observed TVOC peaks during school hours in classrooms, our results also confirm that school activities have a significant impact on TVOC concentrations and patterns. However, unlike the research by Bergomi et al. [37], which reported an average concentration of 303 ± 47 μg m^{-3} in a secondary school classroom in Italy during the summer, the average values recorded in Estarreja were generally higher, ranging from 533 to 638 μg m^{-3} in winter, and from 197 to 1211 μg m^{-3} in spring. This suggests that classroom conditions in Estarreja could be favouring higher TVOC levels, possibly due to specific factors, such as ventilation, materials used, or local environmental conditions.

Continuous monitoring of TVOCs allowed to identify some concentration peaks during school days that often exceeded the recommended guidelines for a safe environment. Taken the second cycle (C) as an example, Fig. S5 shows the daily TVOC profiles for both a school day and a weekend. As expected, the average values during school hours were higher than those recorded during weekends, suggesting that school activity influences these concentrations. Furthermore, the daily profiles revealed two concentration peaks, one in the morning and one in the afternoon, which are likely due to student occupation, while the afternoon peak seems to be related to cleaning activities.

Of the 15 VOCs and carbonyls analysed, three showed low levels (<1 μg m^{-3}) in all samples, including benzene, ethylbenzene, and o-xylene. Butyraldehyde, benzaldehyde and isovaleraldehyde were detected only in the indoor samples during the winter (Fig. 6). Formaldehyde, acetaldehyde, acetone and hexaldehyde were the most abundant carbonyls in classrooms in both winter and spring, while outdoors, toluene stood out as the most representative VOC. In turn, the sum of the concentration of benzene, toluene, ethylbenzene and xylenes, known as BTEX, were on average 1.70 times higher in winter than in spring, with the outdoor concentration (6.44 μg m^{-3}) exceeding that in classrooms (5.77 μg m^{-3}). When calculating the m,p-xylenes/ethylbenzene (X/E) ratio, an almost constant value (2.70 ± 0.53) was obtained both in the rooms and outdoors. Higher ratios indicate more local and recent emissions, whereas lower ratios suggest greater photochemical degradation, implying that the emission sources may be more distant from the site [39]. This stability suggests that in both seasons, emissions from xylene and ethylbenzene sources are significantly greater than their photochemical removal [40]. In Southern Europe, Baloch et al. [9] found that the benzene concentration in schools was 3.86 μg m^{-3}, at least 6 times higher than that found in this study, while the formaldehyde concentration (9.73 μg m^{-3}) was closer to the value found in Estarreja (13.1 μg m^{-3}). The researchers noted that high levels of benzene in classrooms were associated with an increased risk of upper and lower respiratory tract, ocular and systemic disorders in schoolchildren, while no effect was observed for formaldehyde.

Overall, acetone was the most abundant carbonyl in classrooms and presented much higher concentrations than those recorded outdoors. This difference was especially marked in one of the rooms in building B, where the highest concentration was recorded in both samplings (119 μg m^{-3}). It was also observed that the highest concentration of acetone in classrooms occurred during spring. This trend was also observed in a study conducted on three university campuses in Mexico [41], where higher concentrations of carbonyls,

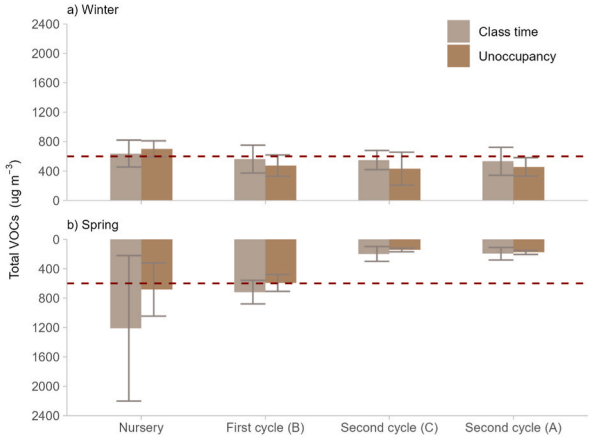

Fig. 5. Average concentrations of TVOCs in class time and rest of the day in each room in winter and spring. The red dashed lines represent the TVOCs limit in indoor air.

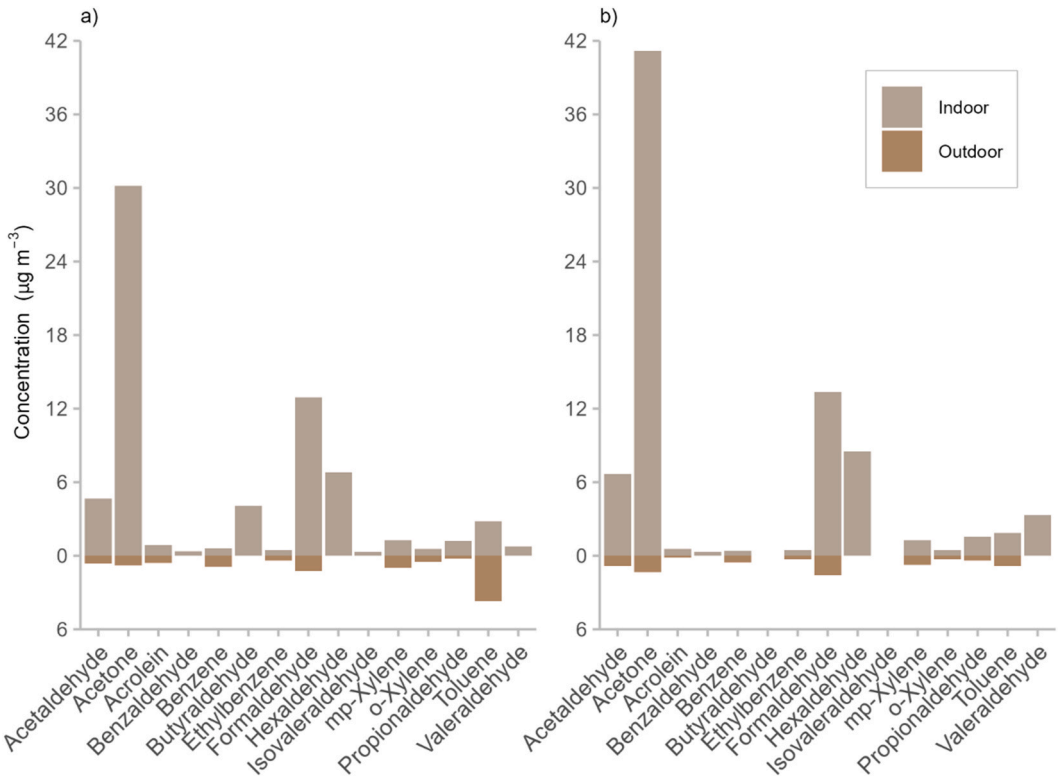

Fig. 6. Concentration of VOCs and carbonyls in classrooms and outdoors in a) winter and b) spring. The indoor concentration corresponds to the average value of the 8 classrooms sampled.

especially acetone, were reported indoors, as well as during spring and summer campaigns. According to García et al. [41], aldehydes and ketones are degradation products of primary alcohols, commonly present in cleaning products. The release of formaldehyde indoors could be related to the age of the buildings, as well as to the coatings, enamels and adhesives used in furniture, decorations and carpets. Formaldehyde emissions vary significantly between different wood products used in construction and furniture. These products typically exhibit higher initial emissions after manufacture, which tend to decrease over time [42]. According to a study conducted in 12 indoor workspaces and three residential units on a university campus in China [43], researchers compared carbonyl concentrations in an office cleaned with organic solvents and one cleaned with water. The results showed that concentrations of formaldehyde, acetaldehyde, acetone, and n-/iso-butyraldehyde were significantly higher in the office cleaned with organic solvents. Therefore, it is likely that in the school environment, carbonyls are released not only from cleaning products and floor cleaners, but also from the use of whiteboard markers, particularly acetone and acrolein [44]. Additionally, formaldehyde concentrations may be partially associated with classroom furniture.

In addition to cleaning products, other factors can affect carbonyl concentrations. Peng et al. [45] analysed the air quality in an older office and classroom building with complaints about odours and pollutants. They investigated VOCs and carbonyls on three floors. The results showed that the mean indoor/outdoor (I/O) ratios of carbonyls, such as formaldehyde, acetaldehyde, acetone, propionaldehyde, butyraldehyde, benzaldehyde, isovaleraldehyde, valeraldehyde and hexaldehyde exceeded unity, indicating indoor sources for these compounds. When comparing concentrations on weekdays and weekends, they found no significant differences, suggesting that the sources come from the building materials and not the occupants. This finding is supported by a study inParis [46], where factors such as recent wall and floor coverings, environmental tobacco smoke, CO_2 levels and temperature were identified as contributing to concentrations of formaldehyde, acetaldehyde, propionaldehyde and benzaldehyde. By comparing these results with those of the present study, it is concluded that carbonyls in classrooms also come from internal sources, as demonstrated by the exclusive presence of certain concentrations detected only indoors (Fig. 6). Some studies have indicated that carbonyl concentration depend on T and RH. Chang et al. [47] reported a strong positive correlation between formaldehyde levels and both indoor T and RH. Similarly, Zhang et al. [48] observed that formaldehyde concentrations were correlated with indoor T, RH, and the time since the property was last decorated.

Due to customs issues in sending samples to Passam for analysis, NO_2 measurements could only be obtained during the winter campaign. Levels ranged between 6.3 and 8.5 µg m^{-3} in classrooms, while in the schoolyard a concentration of 13.5 µg m^{-3} was reached, with an I/O ratio of 0.5. These concentrations are lower than those reported by Salonen et al. [49], who found an average of 30.1 µg m^{-3} in 354 school buildings, with values exceeding 100 µg m^{-3} in schools near industries and roads, underlining the impact of traffic and industrial activities. However, the concentrations in Estarreja are like those observed in rural schools or areas with low

traffic density, where the values range between 6.30 and 13.9 μg m^{-3} [49]. G.W.K [50] also observed significant differences in NO_2 concentrations between schools close to and far from traffic, with NO_2 being on average 1.77 times higher in the most affected areas. In addition, G.W.K [50] detected a significant decrease in lung function in students exposed to high levels of vehicle pollution. Although NO_2 concentrations in Estarreja are lower than those reported in other areas, it seems that external factors, such as vehicle traffic, have a great impact on concentrations at the school.

CH_4 and N_2O are classified as greenhouse gases, while NH_3 contributes directly to air pollution. Although they are not generally included in studies of air quality in schools, their presence in classrooms can be influenced by factors such as inadequate ventilation, the use of certain materials, and occupancy rates [51]. These factors can affect the concentrations of these gases, especially during school hours. In this study, in addition to the greenhouse gases mentioned above, ethanol concentrations were also measured. The average concentrations of these gases in classrooms are shown in Fig. 7, where variation is observed both between different classrooms and between seasons. CH_4 concentrations in winter and spring revealed no significant differences between concentrations during school hours and when classrooms were empty. Furthermore, the CH_4 values found in this study are within the range observed for an atmosphere in a typical urban location. Based on a study carried out in Wroclaw [52], CH_4 concentrations varied according to the season and time of day; in winter, the variation was from 1.92 to 2.31 ppm, while in the rest of the year it varied from 1.83 to 2.13 ppm. According to Bezyk et al. [52], the main sources of CH_4 emissions in the city included waste and wastewater treatment facilities, stationary and mobile combustion, industrial processes, the natural gas distribution network and agricultural activities. When comparing N_2O with previous studies on air quality in schools, it was observed that it had not been considered in any of those studies. Therefore, it was decided to compare the obtained results with those from hospitals, where N_2O is frequently associated with the use of inhalant anaesthetics [53]. The results showed that N_2O concentrations at the school were more than 16 times lower than those recorded at a paediatric hospital in Italy [54], where the average level in outpatient dental clinics was 24.2 ppm. According to NIOSH guidelines, the exposure limit for N_2O is 25 ppm over an 8-h period, confirming that concentrations at the school were well below the limits established for indoor environments. It should be noted that the origin of N_2O at the school and the hospital are completely different. In the hospital, this pollutant is primarily associated with medical processes, specifically the use of anaesthesia in surgical procedures. In contrast, in the school setting, the likely source of the pollutant is vehicle exhaust [55], which can enter classrooms through windows or doors.

A shift in ammonia concentration ranges was observed between winter (1.12–1.23 ppm) and spring (0.86–1.42 ppm). Higher local concentrations were also detected than those reported in previous indoor studies, suggesting a negative impact on the school's air quality, possibly due to insufficient ventilation in the rooms or cleaning activities. For instance, Sun et al. [40] assessed NH_3 concentrations in over 100 university dormitories between winter 2022 and summer 2023 in Shanghai, China, and found that, regardless of the ventilation system type, average NH_3 concentrations exceeded the limit set by Chinese indoor air quality standards (0.29 ppm). The mean NH_3 concentration in unventilated rooms was 1.46 ppm, 0.34 ppm in naturally ventilated rooms, and 0.63 ppm in

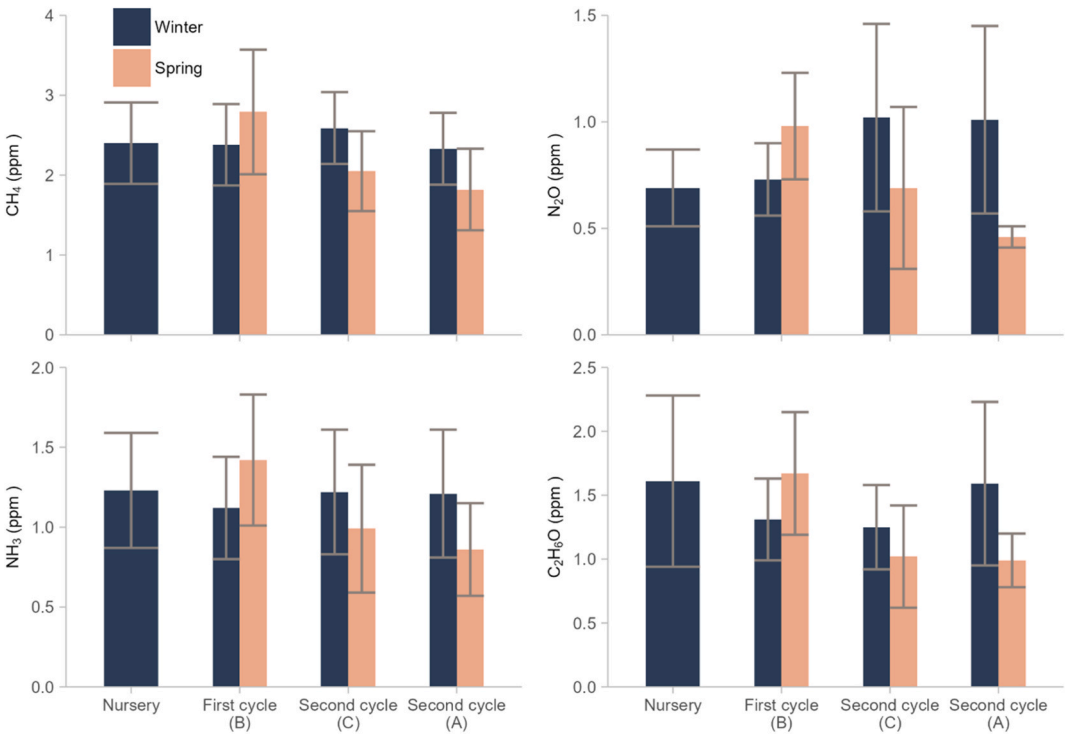

Fig. 7. Concentrations of methane, ammonia, nitrous oxide and ethanol during class time.

mechanically ventilated rooms. In this study, factors such as indoor temperature, air change rate, humidity, number of occupants, sanitary conditions, and use of cleaning products showed a significant positive correlation with NH_3 concentrations. However, the values found in the present research were higher than those reported by Sun et al. [56] in a systematic review analysing studies published between 1980 and 2020 on more than 10 pollutants in office buildings and schools in the Yangtze River Delta, China. The review indicated that the average NH_3 concentration in offices (0.45 ppm) was lower than that in schools (0.49 ppm). Furthermore, when comparing the values observed in Estarreja with those reported by Vornanen-Winqvist et al. [57] in their assessment of indoor air quality in six schools in Finland, the concentrations in Estarreja were considerably higher. In the Finnish schools, no NH_3 was detected in the classrooms, which were kept at a temperature between 20 and 26 °C and at RH < 30 % during winter. On the other hand, there is no specific concentration standard for indoor ethanol defined by the national government or the WHO. However, the National Institute for Occupational Safety and Health (NIOSH) recommends 1,000 ppm of ethanol in an 8-h workday. As seen in Fig. 7, the values found at the school are well below this limit in both seasons.

3.3. Particulate matter

Average PM_{10} concentrations during school hours varied as follows: second cycle (C) > > first cycle > nursery in winter, while in spring this order changed to: first cycle > nursery > second cycle (C) > second cycle (A). In contrast, PM_1 concentrations during school hours showed a different pattern, with the highest average concentration in the second cycle of building C (9.67 μg m^{-3}) in winter and in the first cycle (10.3 μg m^{-3}) in spring. In all classrooms and in both seasons, average PM_{10} concentrations during school hours were found to increase significantly ($p < 0.05$) compared to periods without classroom activities, increasing up to 4 times in winter and up to 3 times in spring. The increase in PM_{10} concentrations during class time, compared to an empty classroom, is primarily due to classroom activities and inadequate ventilation. Furthermore, in winter, low temperatures make it difficult to open doors and windows during classes, further limiting air renewal and contributing to a greater increase in these concentrations compared to spring. During school hours, coarse particles predominated, as indicated by the average PM_1/PM_{10} and $PM_{2.5}/PM_{10}$ ratios (both <0.45) observed in classrooms in both seasons. In contrast, ratios above 0.58 were recorded when classrooms were empty, suggesting a greater predominance of fine particles during this time. The highest average $PM_{2.5}/PM_{10}$ ratio was recorded in the second cycle (C) in winter (0.88), while the lowest was noted in the first cycle during school hours (0.32), also in winter. These results reinforce the relationship between higher occupancy and increased PM_{10} concentrations, which is consistent with previous studies conducted in other classrooms in New Zeeland [58], London [15], and Victoria, Australia [33].

Unlike PM_{10}, $PM_{2.5}$ and PM_1 concentrations were generally higher outside school hours (Fig. 8) in winter, suggesting that school

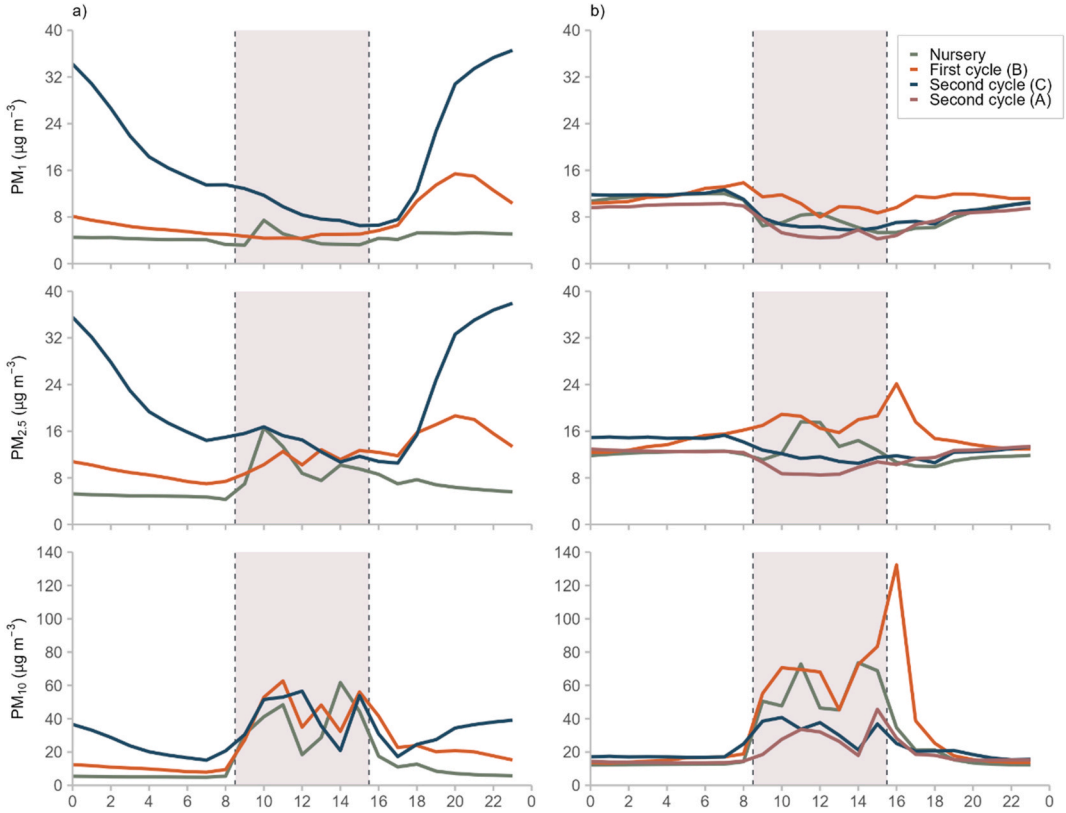

Fig. 8. Diurnal cycle of mean PM concentrations in a) winter and b) spring split for different PM sizes. The highlighted band represents class time.

activities had almost no impact on these pollutants. This suggests that external sources, like traffic, biomass burning, industrial activities, or outdoor air pollution, were more influential in contributing to these smaller particles. This could also mean that indoor school activities do not disturb or generate as many fine particles as they do larger particles. However, this pattern was almost negligible in spring, where average $PM_{2.5}$ and PM_1 concentrations were practically the same, with a difference that could reach up 4 μg m^{-3} between both periods. In addition, in the nursery classroom during the winter, an atypical pattern was observed: instead of increasing, $PM_{2.5}$ and PM_1 concentrations decreased after school hours. In the nursery classroom, the use of a mop (as opposed to sweeping) could have contributed to a decrease in fine particle concentrations after school hours. Mopping tends to trap and remove dust and fine particles from the floor, preventing them from becoming airborne. Sweeping, on the other hand, can stir up dust and fine particles, potentially increasing the concentration of these pollutants in the air. So, the use of a brush in the other classrooms after school hours could explain why $PM_{2.5}$ and PM_1 concentrations remained higher or even increased. Furthermore, unlike the junior classrooms, this room kept closed after cleaning and was only opened at the start of the school day. This practice could serve as an additional barrier against the entry of external particles. In fact, studies conducted in schools, such as one conducted in nine primary school classrooms in Madrid [59], showed that opening windows and doors significantly increased the concentration of ultrafine particles inside the classrooms.

3.4. Settleable dust and microorganisms

Settleable dust at the school showed variations between the two seasons (Fig. 9). Factors such as ventilation, humidity, activities inside the classrooms and the number of students could have influenced these values. In both winter and spring, settleable dust could have accumulated due to limited ventilation in the classrooms. However, in spring, with the improvement of weather conditions, outdoor particles such as pollen could have contributed to the increase in dust in the environment. In general, higher rates of settleable dust were observed in nursery due to the greater activity of the children and the design of the building, which although newer, is more closed, which limits the exchange of outside air and favours the accumulation of dust in the corridors and classrooms. A particular case was recorded in the nursey toilet, where the sedimentation rate in winter was more than three times higher than in spring. This value was explained by the fact that the toilet hopper window was always ajar, allowing emissions from a nearby neighbourhood, where, in addition to biomass combustion for heating, open-air fires were also frequently recorded, to enter the school building.

As for classrooms, which are the only category of rooms common across the different educational levels, settleable dust varied as follows between the two seasons: nursery > infant > junior. At the nursery level during both seasons, dust levels were highest in the explorer's room, a space that offers learning and development experiences in a playful and welcoming manner, adapting to the needs of children of different ages. In this room a variety of toys and materials is used, which differentiates it from a conventional classroom. In contrast, dust levels were lower in the staff room. At the infant level, the lowest values were recorded in classrooms during winter (222

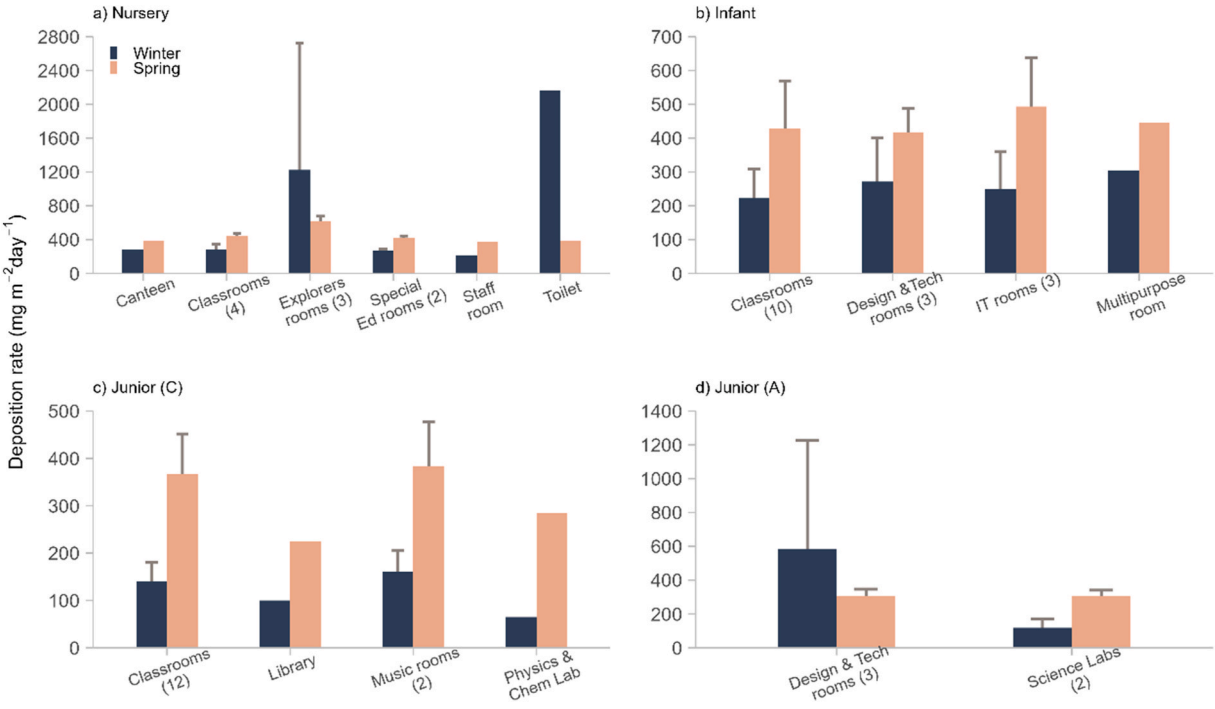

Fig. 9. Average dust deposition rate by classroom type at different educational levels of the school in winter and spring. The bars represent the standard deviation (+), and in brackets the number of rooms for each category. The absence of a standard deviation indicates that only one room of the same category was available. Different scales have been used to facilitate visualisation.

mg m^{-2} day^{-1}), while the highest value was observed in the IT room in spring (493 mg m^{-2} day^{-1}). At the junior level, both the lowest and highest values occurred in winter: the lowest was measured in the physics and chemistry laboratory (65 mg m^{-2} day^{-1}) and the highest rate in the design room (586 mg m^{-2} day^{-1}).

The results obtained at the school showed values higher than those reported for 23 naturally ventilated dwellings located in the Aveiro district, Portugal [60], both in winter (4.29 ± 4.51 mg m^{-2} day^{-1}) and in summer (5.70 ± 2.70 mg m^{-2} day^{-1}). However, the indices of the Design and Technology room (586 ± 129 m^{-2} day^{-1}), the library (100 mg m^{-2} day^{-1}) and the Science Laboratory (118 ± 53 mg m^{-2} day^{-1}) in winter were similar to those observed in autumn in some dwellings in Giza, Egypt [61], where significant differences in surface load rates were found between new and old houses. The highest value (414 ± 81 mg m^{-2} day^{-1}) was recorded in the oldest dwelling (22 years), smaller and inhabited by smokers, while the lowest value was observed in the newest dwelling (2 years) and free of smokers, with a sedimentation rate of 106 ± 20 mg m^{-2} day^{-1}. The values found in this study could suggest the need for further research on the effects of dust on school staff and pupils, as when studying bacterial communities present in classroom floor dust at 50 elementary schools in Philadelphia, USA [62], Park et al. [62] found that classroom dust had a distinct bacterial composition than household dust, with a higher prevalence of bacteria associated with the outdoor environment. Outdoor factors such as water damage, building conditions, and classroom location influence bacterial diversity and community composition.

Fig. 10 presents the distribution of fungal colony-forming units (CFU) across various locations during winter and spring. During spring, the nursery exhibited the highest contamination across all media (MEA: 3.39 × 10^2 CFU m^{-2} day^{-1}; DG18 at 27 °C: 2.44 × 10^2 CFU m^{-2} day^{-1}; and DG18 at 37 °C 2.31 × 10^2 CFU m^{-2} day^{-1}). These values declined in winter, dropping to 1.21 × 10^1 (MEA) and 1.21 × 10^1 (DG18 at 27 °C). Infant classrooms showed high spring contamination on DG18 at 27 °C (1.32 × 10^2) and MEA (1.70 × 10^2), with winter persistence on DG18 at 27 °C (9.74 × 10^1). Junior (C) displayed spring dominance for DG18 at 37 °C (1.54 × 10^2) and DG18 at 27 °C (1.99 × 10^2), while winter maxima on DG18 at 27 °C (8.52 × 10^1) surpassed other winter values. Junior (A) had moderate spring peaks (e.g., 3.86 × 10^1 on DG18 at 37 °C) and minimal winter contamination (≤8.12 × 10°). These results are consistent with those observed in a study conducted in a school in Lisbon [63], where fungal counts on DG18 samples were higher compared to those on MEA. However, the maximum value recorded in Estarreja exceeded those reported in Lisbon by more than fivefold on DG18 and more than twofold on MEA. In this study, the highest fungal counts were observed in spring (Fig. 10), likely due to the more favourable environmental conditions for fungal growth during this season [64]. In winter, lower temperatures can reduce fungal activity; however, some cold-adapted fungi, such as the genera *Cladosporium* and *Penicillium*, continue to grow, albeit in smaller numbers than in spring. For more concentration data in each building, refer to Table S3.

In addition to the sections, 12 different genera were identified in both seasons (Fig. S6). The highest fungal diversity on MEA and DG18 was observed in spring, particularly in the infant building, followed by the junior (C) building. In winter, *Aspergillus* sp. was the most prevalent genera on MEA, while *Penicillium* sp. dominated on DG18. In spring, however, *Aspergillus* sp. was the most prevalent genera on both culture media (Fig. S6). Particularly, *Penicillium* sp. was detected in 23 samples inoculated on DG18 in winter, whereas *Aspergillus* sp. appeared in 33 samples in spring. Focusing on DG18, the most frequent genera per building throughout the sampling campaign were as follows: in the nursery, *Aspergillus* sp. was found in 8 spring samples; in infant and junior (C) buildings, *Penicillium* sp. was detected in at least 8 winter samples; and finally, in Junior (A), *Penicillium* sp. appeared in 3 samples during both winter and spring. A study conducted in Finland [65] revealed that *Penicillium* was the most commonly occurring genus on MEA, primarily found in wood (61.2 %) and paper (63.7 %) samples, and on DG18 in gypsum boards (35 %). In the current study, *Cladosporium* sp., *Alternaria* sp.,

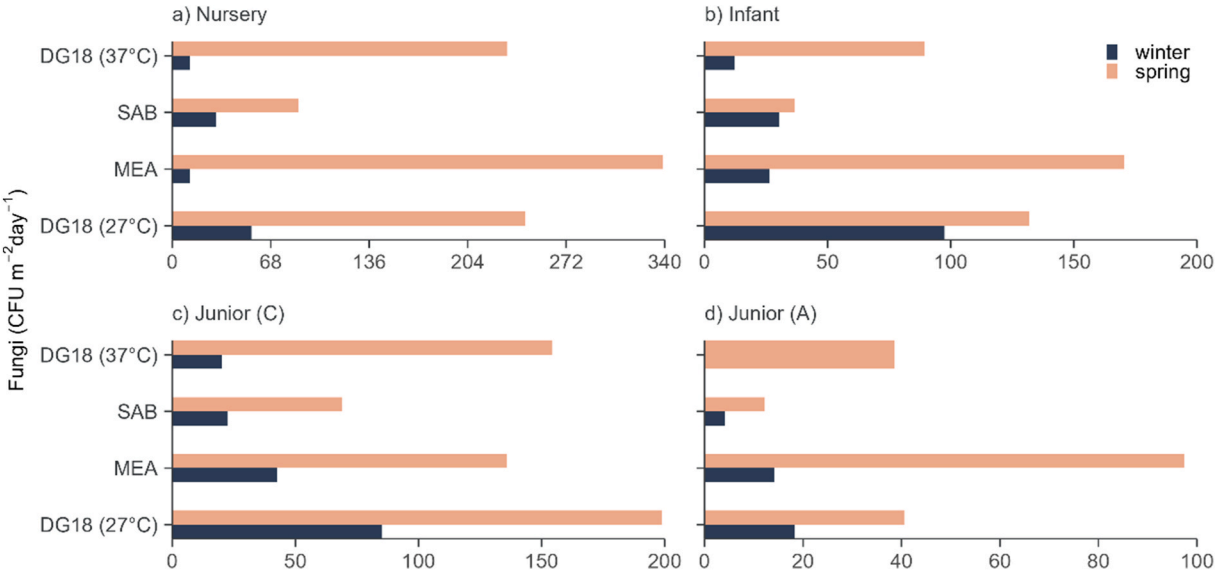

Fig. 10. *Total* CFU inoculated on *four culture media*. Different scales were employed to enhance visualisation. The values presented in the graph were calculated by summing the CFU of the samples according to the building type (e.g., *nursery*, *infant*, etc.).

Ulocladium sp., *Mucor* sp., *Trichoderma* sp., and *Chrysosporium* sp. were also detected, genera similarly reported in the Finnish study. This overlap suggests that their presence in the school environment may be linked to materials affected by moisture. On DG18 (37 °C), *Aspergillus* sp. dominated fungal communities in spring (61.5 % Infant, 94.7 % Junior A, 100 % Junior C and Nursery) and persisting in winter (100 % Infant, 90 % Junior C, and 100 % Nursery). *Chrysosporium* sp. thrived in the Infant (3.08 %) and Junior A classrooms (5.26 %) during spring. Winter saw increased contributions from *Penicillium* sp. in Junior C (10 %). On DG18 at 37 °C, *Aspergillus* sections *Fumigati* and *Nigri* predominated in spring in all the locations (*Fumigati*: 72.5 % Infant, 94.4 % Junior A, 96.0 % Junior C, 97.3 % Nursery; *Nigri*: 27.5 % Infant, 5.55 % Junior A, 3.95 % Junior C, 2.68 % Nursery), while in winter, only Junior A had no contamination (*Fumigati*: 50.0 % Infant, 88.9 % Junior C, 66.7 % Nursery; *Nigri*: 50.0 % Infant, 16.7 % Nursery). The nursery emerged as a hotspot for *Aspergillus*, with the highest spring *Fumigati* contamination. Furthermore, the ability of fungi to grow at 37 °C (the average human body temperature) is a characteristic commonly observed in pathogenic species and is frequently regarded as a virulence factor [66].

Fungal analysis on MEA, SAB, DG18 (27 °C) and DG18 media incubated at 37 °C identified eight *Aspergillus* sections across both seasons, with section *Fumigati* being the most prevalent (Fig. 11). This finding is particularly significant, as *Fumigati* has been listed by the WHO as a critical priority and is considered an indicator of harmful fungal contamination in various indoor environments [67,68]. Moreover, it is associated with antifungal resistance, posing a serious threat to the clinical effectiveness of azoles in treating invasive aspergillosis [69–71]. In winter, the highest *Aspergillus* diversity was observed in samples inoculated on MEA, with sections *Fumigati* (76.9 %) and *Nigri* (23.1 %) being the most abundant. In spring, five *Aspergillus* sections were identified on DG18 (27 °C) culture medium, with the following distribution: 89.9 % for *Fumigati*, 6.25 % for *Nigri*, 2.40 % for *Flavi*, 0.96 % for *Aspergilli*, and 0.48 % for *Nidulantes*.

Aspergillus is one of the most common fungal genera in indoor environments [72]. *Aspergillus* species have been identified in a wide range of building materials, including wood, paper, plasterboard, ceramic products, mineral insulation, paints, adhesives, and even plastics [65]. This finding suggests that *Aspergillus* species possess a remarkable ability to exploit diverse nutrient sources, allowing them to colonise different types of organic matter present in these materials or accumulate on their surfaces. Furthermore, factors such as RH and temperature play a key role in promoting the growth and development of moulds, favouring their proliferation under optimal conditions [73]. At the school, the presence of *Aspergillus* sections is important, as. section *Nigri*, for example, includes species that cause pulmonary aspergillosis and otomycosis in humans [74]. Furthermore, some *Aspergillus* species cause allergic, saprophytic, and invasive diseases [75] and have the potential to produce mycotoxins. Although these metabolites are non-volatile, they may still reach the respiratory system of occupants through dust or fungal spores if resuspended or aerosolised [76,77].

The highest values for total bacterial counts were recorded on TSA. However, total TSA and VRBA agar exhibited opposite behaviours. While total concentrations were highest in winter, Gram-negative bacteria (GNB) cultured on VRBA agar peaked in spring (Table S4). Total bacterial concentrations were recorded at 3,196 CFU m^{-2} day^{-1} in the winter samples and 914 CFU m^{-2} day^{-1} in the spring samples (Fig. 12). Among the four buildings analysed, the nursery had, by far, the highest bacterial counts, with an average of 249 ± 186 CFU m^{-2} day^{-1} in the classrooms, followed by the infant building, with 191 ± 13.5 CFU m^{-2} day^{-1} in the IT rooms.

Airborne bacteria did not show a consistent seasonal pattern [78,79], in part because the relative abundance and diversity of bacterial groups vary depending on the specific taxa and sampling methods used [78]. Some studies indicate that total culturable bacterial concentrations in indoor air peak during summer and autumn [80], with GNB reaching their highest levels in summer, likely because environmental conditions favour GNB growth at warmer temperatures [79,81]. Conversely, other research has found that bacterial concentrations peak in winter. According to Prussin et al. [79], however, airborne microbial communities in childcare facilities are primarily shaped by human occupancy and environmental factors, with human presence exerting a stronger influence than environmental variables. At the school, this was exemplified in the staff room, where no bacteria were detected in spring, highlighting how variations in human presence can profoundly shape microbial patterns. Therefore, elucidating the nuanced seasonal dynamics of airborne bacteria requires narrowing the predominant influence of human presence or focusing on environments characterised by minimal human activity, which will allow the more subtle effects of environmental factors to be discerned.

Bacterial count values observed in this study were higher than those reported in Lisbon classrooms, where total bacterial contamination ranged from < LD to 61 CFU m^{-2} day^{-1} on TSA [63], as well as in homes in southern European cities, where bacterial counts ranged from <LD to 16.3 CFU m^{-2} day^{-1} on TSA [82]. Furthermore, these values exceeded those recorded in a clothing store belonging to an international chain in Europe [83], where bacterial counts never exceeded 15 CFU m^{-2} day^{-1} on TSA. Regarding the Gram-negative bacteria (VRBA) counts found at the school, the average of 0.54 CFU m^{-2} day^{-1} in winter and 2.73 CFU m^{-2} day^{-1} in

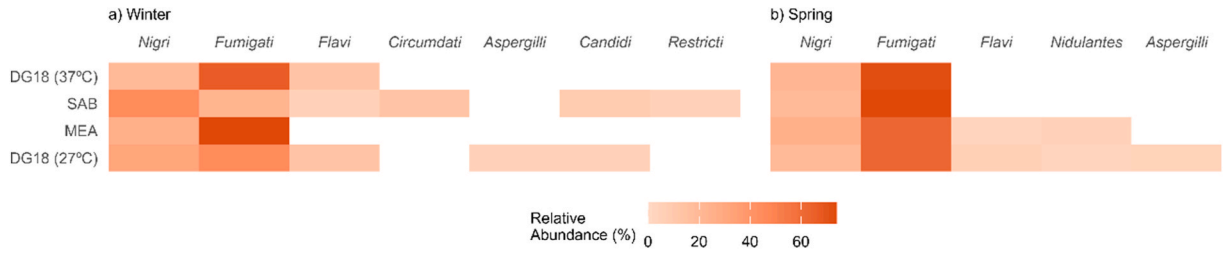

Fig. 11. Heatmap of Aspergillus sections (top) and four culture media corresponding to the samples from (a) winter and (b) spring. The colour bar shows the percentage of each section from lowest to highest.

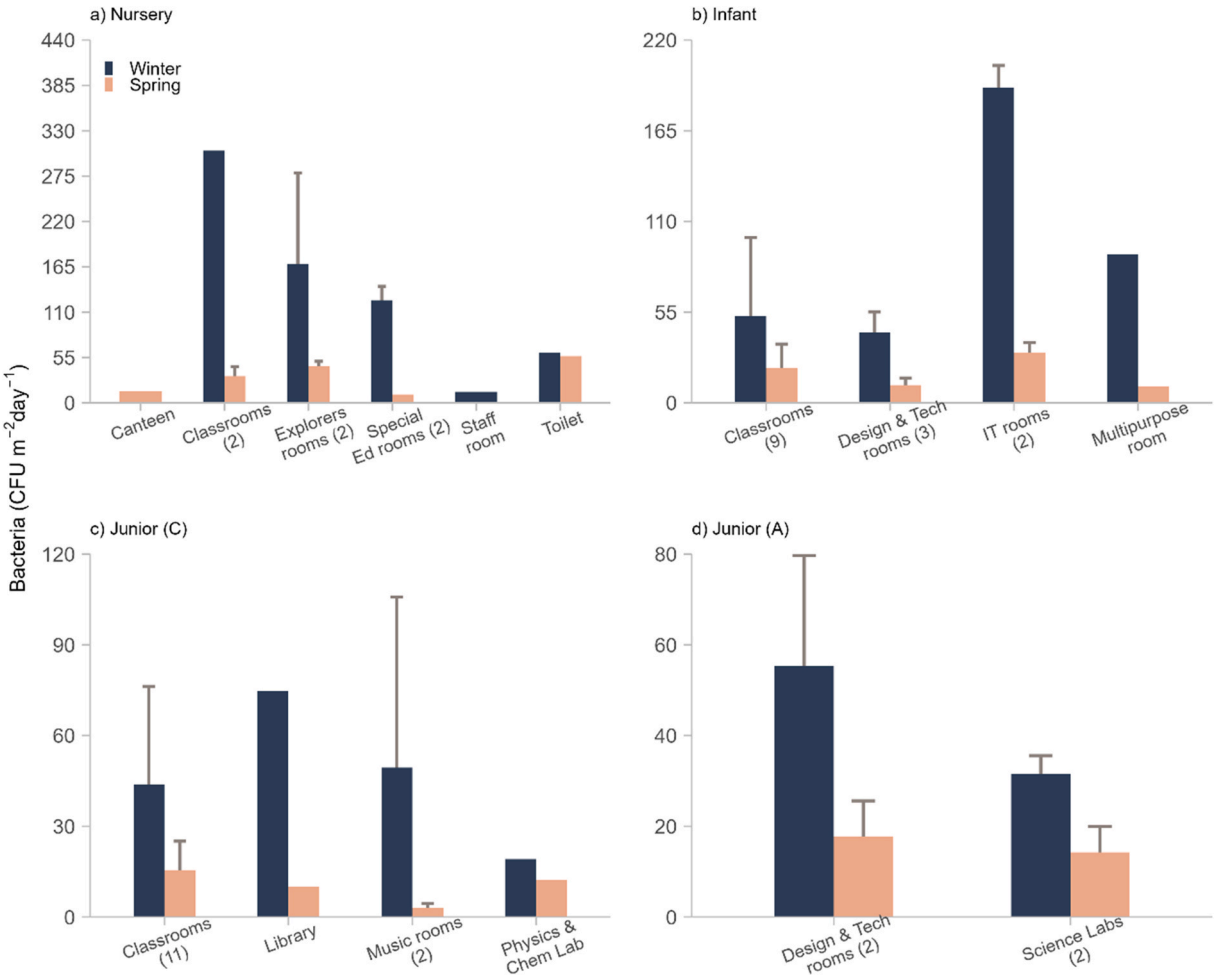

Fig. 12. Bacterial counts at the school inoculated on TSA agar. Various scales have been used to enhance visualisation. The number in brackets indicates the minimum number of rooms included for the average. The absence of a standard deviation indicates that only one room of the same category was available, and the bar represents the value from that single observation.

spring align with the low values (<4 CFU m^{-2} day^{-1}) reported by Furst et al. [54] for a big supermarket. According to Viegas et al. [82], the heating system was the main contributor to bacterial counts on TSA, while on VRBA, cleaning frequency was identified as the most relevant factor. These findings suggest that factors associated with building environmental conditions have influence on bacterial counts in indoor spaces.

4. Study strengths and limitations

Several important limitations were identified in the study. The measurement campaign had a limited duration (one month per season) and covered only two seasons (winter and spring), reducing the ability to capture variability throughout the year. Additionally, a restricted spatial scope, as only one real-time monitoring system was moved weekly across four rooms, and PM measurements were not available in the schoolyard, limiting the ability to perform statistical correlations between indoor and outdoor PM concentrations. Thus, conclusions about external sources are based on temporal patterns and literature. Furthermore, technical failures in some equipment and the unavailability of others during winter or spring reduced the number of observations and increased the uncertainty in the real-time assessment of PM, gases, and thermal comfort, making a more detailed correlation analysis difficult. Specific information on activities in some key rooms was also lacking (e.g., at the locations where the real-time sampling system was installed and other spaces that, after calculations and analysis, showed outliers, either very high or low values). In fact, another two limitations of the study were the absence of simultaneous indoor and outdoor measurements for some parameters, and the reliance on passive sampling for VOCs, carbonyls, NO_2, microorganisms and settleable dust. These constraints, largely due to logistical challenges, limited the level of detail attainable and may have obscured concentration variations associated with children's activities, particularly the distinction between class hours and periods when classrooms were unoccupied. While the passive sampling approach did not allow for differentiation between occupied and unoccupied periods, the measured concentrations remain relevant for identifying potential

indoor pollution sources and evaluating possible health impacts.

Although the current national legal framework only considers culture-based methods, future studies should also incorporate molecular tools to detect fungal strains with mycotoxin-producing potential, especially given the identification of *Aspergillus* sections known for their toxigenicity (e.g., *Aspergillus* sections *Flavi, Nigri, Circumdati and Fumigati*). In addition, combining both methods can provide more accurate and comprehensive information on exposure to indoor contaminants.

5. Conclusions

This study assessed air quality, ventilation and thermal comfort in classrooms in a Portuguese school using active and passive methods over two seasons. During school hours, the average CO_2 concentration was approximately 76 % higher than during the time when classrooms were empty. Classroom ventilation was generally inadequate and never met the recommended criteria for educational settings. Overall, average RH and temperature in the classrooms fell within the recommended thermal comfort range only in spring. In winter, the average RH was 64 %, and classroom temperatures were up to 5.9 °C below the recommended value. In addition, the results showed that cleaning products and furniture materials influence carbonyl concentrations, with levels in classrooms up to 18 times higher than outside. On one hand, PM_{10} concentrations increased significantly due to classroom activities and poor ventilation. Coarse particles predominated during occupancy, while finer particles were more common when rooms were empty. On the other hand, analysis of fungal and bacterial diversity showed seasonal variations, with higher fungal counts in spring and different bacterial concentrations between winter and spring. Fungal levels peaked in spring. *Aspergillus* section *Fumigati* was widespread indoors and warrants concern, as it has been classified as a critical priority by the WHO and serves as an indicator of harmful fungal contamination in other environments.

In closing, despite the school studied serves over 700 pupils across multiple educational levels, receives children from various parishes, and is located near an industrial estate of environmental concern, the levels of some pollutants or parameters, were often lower than those reported in other schools or indoor environments from other countries. However, the school may still fall short of meeting air quality standards recommended by international bodies such as the WHO, ASHRAE, and ISO, particularly regarding key parameters. It also faces challenges in aligning with the United Nations Sustainable Development Goals No. 3 and 11, which promote good health and well-being, as well as sustainable cities and communities. Therefore, to address these issues, it is recommended to improve ventilation, whether mechanical or natural, to dilute the levels of carbonyls and other pollutants. Additionally, other measures should include controlling indoor pollutant sources (such as materials, furniture, and cleaning products), continuously monitoring key air quality parameters (including CO_2, particulate matter and VOCs), and implementing ongoing structural interventions to maintain not only thermal and hygrometric comfort, but also optimal air quality.

CRediT authorship contribution statement

Isabella Charres: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Leonardo Furst:** Methodology. **Estela D. Vicente:** Writing – review & editing, Methodology. **Marlene Soares:** Methodology. **Carla Viegas:** Writing – review & editing, Methodology. **Renata Cervantes:** Methodology. **Pedro Pena:** Methodology. **Mário Cerqueira:** Writing – review & editing, Methodology. **Manuel Feliciano:** Writing – review & editing, Supervision. **Célia Alves:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would especially like to thank the school principal and teachers, who have willingly participated in this study. The authors acknowledge the financial support of the Portuguese Foundation for Science and Technology (FCT) to CESAM (UID Centro de Estudos do Ambiente e do Mar UID/50006 + LA/P/0094/2020, doi.org/10.54499/LA/P/0094/2020), through national funds, and to the PhD fellows I. Charres (DOI: 10.54499/2022.12142.BD), L. Furst (DOI:10.54499/2020.08461.BD), and M. Soares (2023.04826.BD). FCT is also acknowledged for the research contract under Scientific Employment Stimulus to Estela D. Vicente (DOI:10.54499/2022.00399.CEECIND/CP1720/CT0012). This work was performed within the project: Source drivers of (eco)TOxicity of airborne Particles in school environments in Estarreja: Strategies for minimising the risks (STOP) – funded by the LabEx-DRIIHM-OHM programme (CNRS – INEE, France). H&TRC authors gratefully acknowledge the FCT/MCTES national support through the UIDB/05608/2020 (https://doi.org/10.54499/UIDB/05608/2020) and UIDP/05608/2020 (https://doi.org/10.54499/UIDP/05608/2020).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jobe.2025.113997.

Appendix A. Supplementary material

Additional material related to this paper is available online at.

Data availability

Data will be made available on request.

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