

Pilot Study on the Physical, Chemical, and Biological Determinants of Indoor Air Quality in University Classrooms

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Abstract – In the context of an escalating energy crisis, the burgeoning prevalence of remote work, and challenging climatic conditions, ensuring optimal indoor air quality (IAQ) has emerged as a pressing concern. This pilot study rigorously investigates the complex interplay between biological, chemical, and physical parameters that characterize IAQ, focusing specifically on university classrooms during active teaching sessions. Employing a comprehensive array of instrumentation – such as SAS SUPER ISO 100 for microbiological sampling, Aranet4 for monitoring relative humidity, temperature, and CO₂ concentration, and PCE-PCO 1 and PCE-RSCM 16 for particulate matter (PM_{2.5} and PM₁₀) quantification—the study spanned a duration of three days in November 2022 and covered classrooms of varying dimensions, both reliant on natural ventilation. An extensive collection of 52 microbiological samples were obtained and cultured on specialized growth media to differentiate between various classes of airborne microorganisms. Concurrently, the pilot study meticulously recorded students' activity patterns, along with the temporal dynamics of window openings and closures. The colony-forming units per cubic meter (CFU/m³) fluctuated between 174 and 934 CFU/m³, with fungi constituting the majority. Furthermore, the CFU/m³ for fungi cultivated on Sabouraud Dextrose Agar ranged from 24 to 610 CFU/m³, whereas bacteria cultured on Trypticase Soy Agar and Mannitol Salt Agar exhibited ranges of 42–476 CFU/m³ and 42–254 CFU/m³, respectively. Contrasting these findings with extant guidelines that recommend microbiological contamination not exceeding 500 CFU/m³ highlights significant IAQ concerns. Thermal assessments revealed that the smaller classroom surpassed the acceptable indoor temperature threshold of 25 °C within an average duration of 50 minutes, while the larger classroom remained compliant. Notably, the highest CO₂ concentrations recorded over the three-day period were alarmingly high: 2689 ppm, 1970 ppm, and 2131 ppm on the first, second, and third days, respectively. A 25-minute ventilation intervention was sufficient to reduce CO₂ levels to 499 ppm, although existing literature stipulates that CO₂ concentrations should not surpass 1000 ppm. Importantly, the pilot study highlighted the rapid increasing of PM_{2.5} and PM₁₀ concentrations in crowded instructional settings, averaging 400 µg/m³ and 35 µg/m³, respectively. This underscores the necessity for a continuous air ventilation and purification mechanism during classroom activities. Despite these pivotal findings, the study identifies a glaring absence of standardized regulations or guidelines pertaining to maximum acceptable concentrations of particulate matter and microbial CFU in public indoor environments, indicating a critical area requiring immediate policy intervention.

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1. INTRODUCTION

The escalating concern for indoor air quality (IAQ) cannot be overstated, given its pivotal implications for human health. In an era marked by multifaceted challenges such as the global energy crisis and soaring inflation, the imperative for high-quality indoor air becomes inexorably heightened. IAQ concerns are not only contemporary but also enduringly pertinent. This salience is particularly accentuated in educational settings like university classrooms, where cognitive performance – a vital element in the educational ecosystem – could be significantly influenced by air quality [1]–[3].

Our research endeavours to probe deeply into the intricate dynamics of biological, chemical, and physical IAQ parameters, including but not limited to, temperature, carbon dioxide (CO₂) concentration, and particulate matter (PM_{2.5} and PM₁₀). Importantly, our investigation revolves around university classrooms operating under natural ventilation conditions during instructional hours.

Microbiological pollutants constitute a category of IAQ parameters that have garnered heightened scrutiny, attributed to their unequivocal potential to inflict a spectrum of adverse health outcomes. These pollutants encompass a diverse array of microorganisms such as bacteria, fungi, viruses, and other microbial agents originating from heterogeneous sources including humans, flora, fauna, and the external environment [4]–[6].

Previous seminal studies provide empirical validation for the health repercussions of microbial contaminants. For instance, Mendell *et al.* [7] established a correlative relationship between exposure to microbial contaminants and elevated risks of respiratory symptoms and asthma. Similarly, Adams *et al.* [8], corroborated the associative link between microbial exposure and allergic reactions. Additional exacerbating factors like high ambient humidity and inadequate ventilation further compound these risks, as corroborated by studies from Haines *et al.* [9] and Onmek *et al.* [10].

To systematically evaluate these microbial risks, various methodologies are available, including culture-based and molecular-based assays. Molecular-based approaches, as evidenced by Fröhlich-Nowoisky *et al.* [11], offer superior sensitivity and specificity relative to their culture-based counterparts.

Beyond the health implications, optimized IAQ yields tangible benefits such as diminished infection propagation, enhanced psychological well-being, and cognitive performance augmentation [12]. With over 90 % of unhealthy air exposure incidents occurring indoors [13], the accentuation on indoor air quality is justified. One of the most universally acknowledged indicators for IAQ is the concentration of CO₂, elevated levels of which (>1000 ppm) have been correlated with dermatological issues, headaches, and cognitive deficits [14], [15].

Furthermore, IAQ is intricately linked to humidity and temperature conditions. For instance, decreased humidity levels impair the body's mucociliary cleansing mechanisms, making ocular and respiratory systems more susceptible to desiccation [16]. Elevated temperatures above 25 °C diminish mental acuity, and conditions above 30 °C lead to a complete erosion of concentration [17].

Particulate matter, specifically PM_{2.5} and PM₁₀, also pose significant health risks, including elevated hospitalization rates for respiratory ailments and potential impacts on the epidemiology of COVID-19. [18]

In summary, microbial contaminants in indoor air constitute a multifaceted health hazard exacerbated by other IAQ parameters such as humidity, ventilation quality, and presence of

moisture sources. A thorough assessment of these factors is indispensable for comprehensively understanding the health implications associated with IAQ and subsequently crafting targeted interventions to ameliorate these risks.

2. MATERIALS AND METHODOLOGY

The pilot study was conducted in two distinct classrooms at Rīga Stradiņš University, specifically chosen for their reliance on natural ventilation systems. The first classroom, measuring 72.3 m³ meters, was utilized for data collection on the inaugural day of the experiment, while the second, more spacious classroom of 119.7 m³, was the venue for the subsequent two days of data acquisition. The temporal framework for the first two days spanned from 08:00 to 15:00 hours, extending to 17:00 hours on the third and final day.

2.1. Microbiological Parameters

For the investigation of biological air quality, a rigorous methodology was deployed. A total of 52 microbiological samples were obtained across the two classrooms utilizing the 'SAS SUPER ISO 100' air sampler (manufactured by International PBI S.p.A., Italy) that was centrally positioned in the classrooms. During first two days quadruplicate samples were collected four times per instructional hours, incrementing to quintuplicate samples on the final day. Subsequent to sampling, each set of 13 specimens were cultivated on distinct agar mediums – Sabouraud Dextrose Agar (SDA), Tryptic Soy Agar (TSA), Mannitol Salt Agar (MSA), and Pseudomonas Selective Agar (PSA) – all of which were sourced from Liofilchem, Italy, except for PSA which was acquired from Biolife, Italy. Colony Forming Units per cubic meter (CFU/m³) were then computed post incubation.

For fungal entities cultivated on SDA, manual colony enumeration was conducted. Conversely, bacterial colonies propagated on TSA, MSA, and PSA mediums were quantified through the utilization of an automated colony counter, Scan 300 (manufactured by Interscience, France). Phenotypic identification was performed for moulds through native methods, whereas yeast colonies were subjected to safranin staining. Each mould genus was individually identified.

For bacterial classification, colonies were segregated into Gram-positive and Gram-negative categories. Subsequent identification was executed through the VITEK2 automated microbial identification system (BioMerieux, France).

2.2. Physical and Chemical Parameters

To ascertain the physical and chemical characteristics of the indoor air, a multi-pronged approach was adopted.

The Aranet4 monitoring device (manufactured by SAF Tehnika JSC Ltd, Latvia) was centrally positioned within the classroom to accurately gauge temperature, relative humidity, and CO₂ concentrations. Simultaneously, specialized measuring devices PCE-PCO 1 and PCE-RSCM 16 were employed for quantifying PM_{2.5} and PM₁₀ particulate matter concentrations.

In addition to these quantitative metrics, observational data were meticulously recorded to account for student movement into and out of the classroom, as well as the specific timings pertaining to the opening and closing of windows. This supplementary data served to provide a comprehensive understanding of the factors influencing indoor air quality during the course of the pilot study.

3. RESULTS

3.1. Microbiological aspect

The variation in Colony Forming Units per cubic meter (CFU/m³) observed during the study exhibited a significant range, fluctuating from as low as 174 CFU/m³ to as high as 934 CFU/m³. Notably, fungi constituted the predominant microbial population within this spectrum.

TABLE 1. CFU/M³ ON ALL MEDIUMS

Day	Sample No.	SDA, CFU/m ³	TSA, CFU/m ³	MSA, CFU/m ³	PSA, CFU/m ³	Total CFU/m ³
1	1	296	358	130	0	784
	2	296	476	160	2	934
	3	154	470	250	2	876
	4	186	426	254	0	866
2	1	610	70	50	0	724
	2	176	234	42	0	350
	3	266	96	92	0	452
	4	126	208	88	0	422
3	1	106	56	42	0	204
	2	86	76	76	0	238
	3	24	118	162	0	304
	4	118	278	94	0	490
	5	88	42	42	2	174

A more nuanced breakdown of CFU/m³ based on the type of agar medium employed for cultivation revealed the following distribution (Table 1):

- For fungi cultured on Sabouraud Dextrose Agar (SDA), the CFU/m³ ranged from 24 to 610, highlighting the substantial presence of fungal elements.
- Bacteria propagated on Tryptic Soy Agar (TSA) displayed a CFU/m³ fluctuation between 42 and 476, indicating a secondary but considerable bacterial presence.
- When cultured on Mannitol Salt Agar (MSA), bacterial CFU/m³ ranged from 42 to 254.
- Interestingly, bacterial colonies grown on Pseudomonas Selective Agar (PSA) were almost negligible, with a range of 0 to 2 CFU/m³, implying either an absence or an extremely low concentration of Pseudomonas species in the tested environments.

Temporal variation in the overall CFU/m³ was also assessed across the three days of the study. The data revealed a diminishing trend:

- On the first day, the total CFU/m³ exhibited a high variation, ranging between 782 and 934, suggesting a more contaminated environment or perhaps more comfortable conditions for microbial growth on that particular day.
- The second day experienced a reduction in microbial counts, with total CFU/m³ fluctuating between 350 and 724.
- On the third day, the lowest microbial counts were observed, with CFU/m³ ranging between 174 and 490, which could potentially indicate the effects of any corrective measures taken, or perhaps a natural decrease in microbial populations over time.

This intricate assessment of CFU/m³ fluctuations not only provides valuable insight into the microbial ecosystem of indoor environments but also establishes a compelling case for the further evaluation of factors influencing such microbial diversity and abundance.

The mycological profile of the samples cultured on Sabouraud Dextrose Broth (SDB) revealed a striking predominance of moulds, which accounted for 99.04 % of the total fungal population. Within this category, the genus *Mucor* was the most prevalent, constituting 61.81 % of the moulds identified. This was followed by *Penicillium* at 31.91 %, *Aspergillus* at 3.62 %, and *Cladosporium* at 1.70 %. Yeasts were relatively scarce, representing a meagre 0.96 % of the total fungi identified.

Noteworthy is the temporal variation in the prevalence of specific mould genera across the three-day sampling period:

- On the first day, *Mucor* spp. dominated, making up 69.75 % of the mould population.
- Conversely, on the second day, *Penicillium* spp. surged to become the majority, at 56.59 %, with *Mucor* spp. relegated to a secondary position, constituting 38.19 %.
- On the third day, *Mucor* spp. regained dominance, representing an exceptional 93.68 % of the moulds present.

When evaluated in the context of established guidelines, which recommend a microbiological contamination threshold of less than 500 CFU/m³, the findings are particularly concerning:

- On Day 1, the CFU/m³ counts consistently exceeded the prescribed limit, indicating a critical level of microbial contamination.
- On Day 2, measurements surpassed the guidelines only once, specifically during the morning session, which was the first measurement of the day.
- On Day 3, microbial counts fell within the recommended guidelines.

These findings accentuate the critical need for rigorous microbial monitoring in indoor environments, especially in educational settings where cognitive function must be optimized. Additionally, the temporal dynamics of specific microbial genera offer an intriguing avenue for further research into factors that influence microbial ecology in indoor environments.

3.2. Physical and chemical aspects

In the examination of environmental conditions over the three-day period, relative humidity remained within the optimal range of 40 % to 60 %, aligning well with recommendations for maintaining indoor air quality and minimizing the risk of respiratory issues.

However, the temperature and CO₂ levels fluctuated considerably and often exceeded recommended guidelines. In the smaller room, the indoor temperature reached or surpassed the upper limit of 25 °C within 45 to 55 minutes, contingent on the classroom occupancy. On the third day, the 25 °C threshold was crossed after 11 individuals had spent an hour in the room without ventilation. This rapid escalation of temperature has implications for cognitive function and comfort, as literature suggests that temperatures exceeding 25 °C can significantly impair mental alertness and focus.

Carbon dioxide (CO₂) concentrations also demonstrated alarming trends. According to best practices, indoor CO₂ levels should remain below 1000 ppm. Contrary to this, the highest recorded CO₂ concentration on the first day was 2689 ppm in the smaller room. On the subsequent days, the peak concentrations were 1970 ppm and 2131 ppm, respectively. Notably, it took merely 10 to 20 minutes for the CO₂ level to exceed 1000 ppm once the windows had been closed in the smaller room. In the larger room, the time required for CO₂ levels to surpass this threshold varied between 20 to 50 minutes, demonstrating a positive correlation with the number of occupants. Specifically, with 9 individuals in the room, the CO₂ concentration

exceeded 1000 ppm in just 20 minutes, whereas with only 4 occupants, it took 50 minutes to reach the same level.

This high CO₂ concentration is particularly concerning given the documented adverse effects of elevated CO₂ levels, such as headaches, skin irritation, and diminished cognitive performance. While a 25-minute ventilation period did manage to lower the average CO₂ levels to 499 ppm, this is still considerably above the typical outdoor levels and does not align with health recommendations.

Moreover, PM_{2.5} concentrations showed a steady rise from 8:35 until peaking at 11:24 on the first day, correlating with an increase in room occupancy. This peak also coincided with a strong odorous sensation in the room. Sharp decreases in PM_{2.5} concentrations were recorded upon ventilating the room, confirming the efficacy of draught creation through window opening in mitigating particulate matter levels.

In conclusion, these findings reiterate the critical need for effective ventilation strategies and regular monitoring of temperature, humidity, and pollutant concentrations in indoor environments. The exceedance of guidelines for temperature, CO₂, and microbial contamination indicates an immediate need for intervention to improve indoor air quality, particularly in high-occupancy settings like classrooms where cognitive performance is paramount (Fig. 1).

The maximum PM₁₀ concentration was recorded at 11:42 when a window was opened without creating a draught. The concentration swiftly declined when a draught was introduced. Notably, the PM₁₀ levels peaked upon student arrival at 9:19, implying the influence of human activity on indoor air quality (Fig. 2).

On the second day of observations, a distinctive pattern was noted in PM_{2.5} concentration levels. The apex of PM_{2.5} concentration was registered at 8:35 when all three windows in the classroom were fully open. Subsequently, upon closing the windows, the PM_{2.5} concentration experienced a gradual decline until 10:00, when the windows were reopened. Notably, additional peaks in PM_{2.5} levels were observed at 13:00 and 14:00, corresponding with another series of window openings.

As for PM₁₀ concentrations, the pinnacle was reached at 14:40. The data suggests a reciprocal relationship between window and door positions and PM₁₀ levels; when these portals were open, a rapid decrease in PM₁₀ concentration was noted. Conversely, closing the windows led to a concentration reduction in PM₁₀, albeit a less precipitous one compared to the draught-induced declines.

During the third day, observations were made under conditions where windows were opened without the induction of draughts. Under such circumstances, peaks in PM_{2.5} concentrations were readily apparent. For instance, at 15:04, a rapid uptick in concentration coincided with increased student movement and entry into the room. Similarly, at 13:35, both a rapid concentration increase and a strong olfactory sensation were observed, even though the doors and windows were sealed. Later, between 14:43 and 14:49, another rapid surge in PM concentration was noted, correlating with heightened student mobility. Evidently, in scenarios without draughts, PM₁₀ concentrations were more likely to increase when windows were opened but exhibited marked reductions when draughts were introduced. These observations indicate the complex interplay between ventilation, student activity, and particulate matter concentrations, reinforcing the need for strategic ventilation to manage indoor air quality effectively.

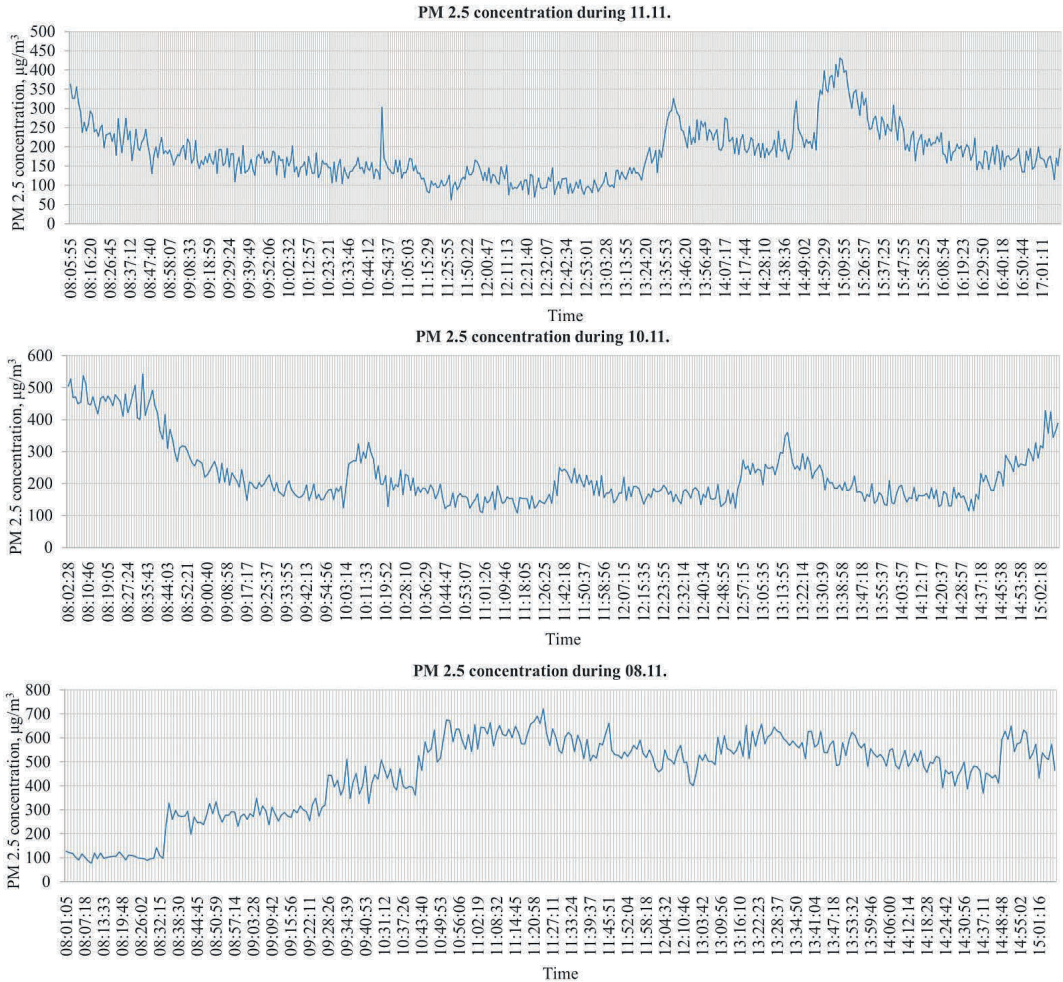


Fig. 1. The concentration ($\mu\text{g}/\text{m}^3$) of PM 2.5 particles in the indoor air for three days (from top to bottom: 1st, 2nd, 3rd day).

4. DISCUSSION AND CONCLUSIONS

Given the substantial impact of indoor air quality on human health and well-being—a consequence of individuals breathing approximately 10 m^3 of air daily and spending between 80–95 % of their lives indoors—the systematic investigation of these factors becomes imperative [19]. Within the multifaceted construct of indoor air quality, several contributing elements can be identified. For the purposes of this discussion, focus will be placed exclusively on those variables investigated in the study at hand.

To begin with, biological air pollution sources within indoor environments include human occupants, organic dust, materials stored within buildings, and air circulated via ventilation systems and air conditioning units. Notably, inhaled air comprises a myriad of microorganisms that can form bioaerosols [20]. Existing guidelines stipulate a recommended microbiological contamination limit of less than $500 \text{ CFU}/\text{m}^3$. In the course of our study, the recorded CFU levels showed considerable variability: exceeding guidelines on the first day, violating them once on

the second day, and complying with them on the third day. This inconsistency underscores the need for a more comprehensive understanding of the factors influencing indoor microbiological contamination.

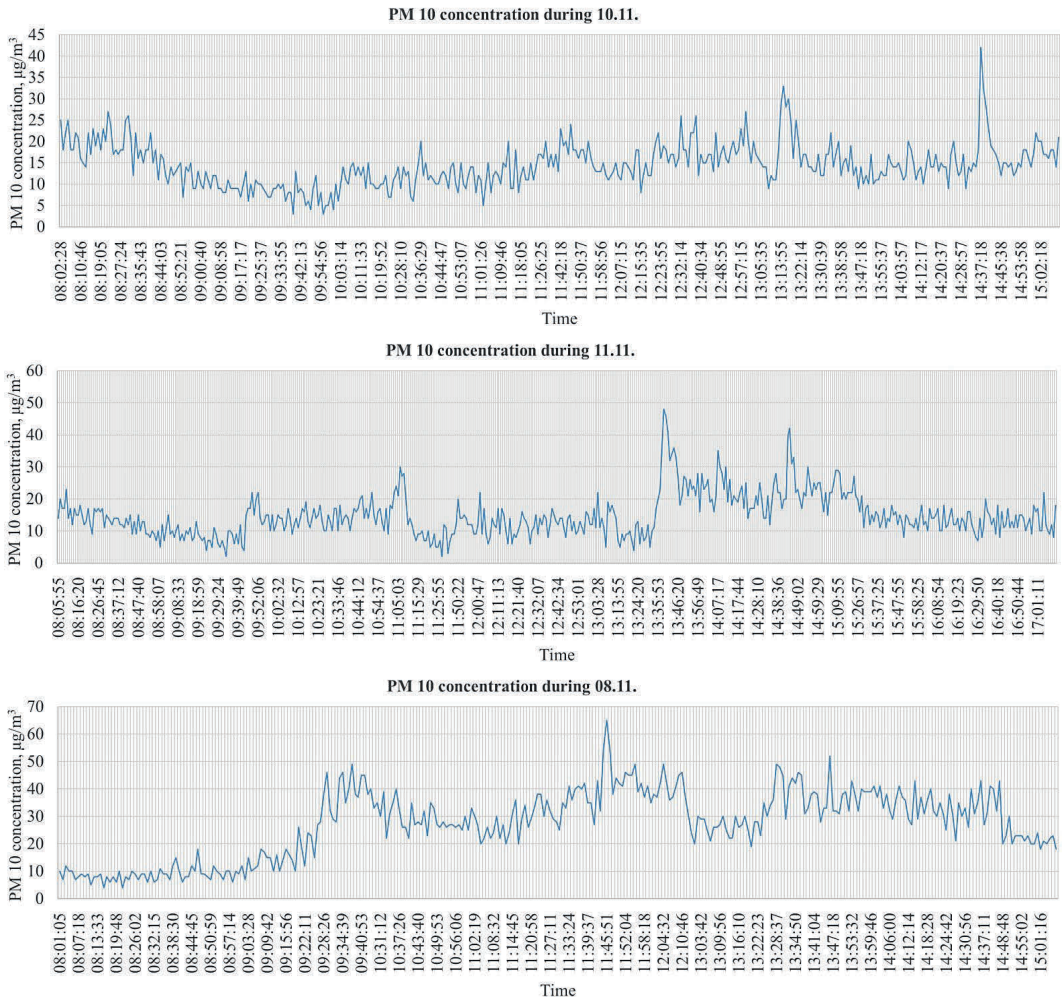


Fig. 2. The concentration ($\mu\text{g}/\text{m}^3$) of PM 10 particles in the indoor air for three days (from top to bottom: 1st, 2nd, 3rd day).

On the subject of relative humidity, it remained within optimal ranges throughout the period of study. In contrast, indoor temperature exceeded the legally accepted limits set by Latvian legislation on multiple occasions [21]. Such elevated temperatures can not only induce thermal stress but also often coincide with high CO_2 concentrations, making the independent effects of each factor challenging to distinguish [22].

Carbon dioxide (CO_2) levels in the study area were found to surpass the accepted norms substantially. While typical CO_2 concentrations in office settings usually range from 350 to 450 ppm [23], levels in our study crossed the 1000 ppm threshold within 10 to 20 minutes of class commencement on the first day and within 20 to 50 minutes on subsequent days. Though natural ventilation did reduce the CO_2 levels to an average of 499 ppm, it was found to be

inadequate for maintaining low levels during practical classes. Its efficacy was notably better during breaks.

As regards particulate matter, outdoor sources contribute significantly to indoor PM_{2.5} levels. On the other hand, human activity within indoor spaces serves as a considerable source of PM₁₀ [24]. The study reveals an urgency to manage particulate levels through constant ventilation and air purification, especially in crowded indoor settings.

However, the use of natural ventilation presents a conundrum. While it may reduce certain indoor pollutants, it can also introduce external pollutants like PM_{2.5}, PM₁₀, VOCs, allergens, and pathogens, thereby elevating health risks. Additionally, the increased energy consumption during colder periods, when windows are opened for ventilation, imposes economic implications [25], [26].

Arguably the most disconcerting revelation is the current absence of comprehensive regulatory guidelines for maximum permissible indoor air concentrations of particulate matter, CO₂, and microbiological CFUs in public spaces which necessitates further research to address this issue effectively.

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