

A systematic review of indoor air quality in schools settings: Focus on microbiome and their relation to particulate matter and chemical pollutants

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ABSTRACT

Objectives: Indoor air quality (IAQ) in schools is a growing concern due to its impact on children's health. Continuous exposure to indoor air pollutants, such as particulate matter (PM), carbon dioxide (CO₂), and microorganisms, such as bacteria and fungi, can affect school performance, increase absenteeism, and trigger respiratory problems. This systematic review aimed to analyze the potential correlation between the presence of microorganisms and PM and chemical pollutants in school indoor environments.

Study design: A systematic literature review was conducted using the methodology PRISMA and 25 articles were selected.

Methods: The current systematic review follow the steps: definition of research objectives; selection of the science databases; definition of keywords; establishment of the inclusion and exclusion criteria, evaluation process and evaluation and management of selected studies.

Results: The findings highlight the significant presence of airborne microorganisms, including bacteria and fungi, often associated with PM and chemical pollutants such as CO₂ and volatile organic compounds (VOCs). Positive correlations between CO₂ and bacteria were observed in ten studies and were statistically significant in six of them. Both positive and negative correlations between fungi and CO₂ were reported. Fungal genera such as *Aspergillus* spp. And *Cladosporium* spp. Were associated with particulate matter (PM). In general, the concentrations of bacteria and fungi were often correlated with PM levels, with larger particles (PM₁₀) favoring the adhesion and transport of microorganisms, while smaller particles (PM_{2.5}) remain suspended in the air for longer periods, increasing exposure.

Conclusions: Despite the methodological variations among the various studies, the results reinforce the need to create effective interventions to reduce pollutant concentrations to minimize health risks for occupants.

1. Introduction

Indoor air pollution is recognized by the World Health Organization (WHO) as the 8th environmental risk factor, responsible for 2,7% of diseases worldwide [1]. Indoor Air Quality (IAQ) has emerged as a public health concern, particularly in schools, where children spend a substantial part of their day [2] and where occupancy rates, ventilation, building characteristics, and maintenance practices can strongly influence pollutant accumulation and exposure [3,4]. Children are a particularly vulnerable group due to their still-developing systems, and

prolonged exposure to indoor air pollutants can affect their health, comfort, attendance, and learning performance, while also impairing teachers' well-being and productivity [4,5].

In school environments, pollutants such as particulate matter (PM), volatile organic compounds (VOCs), carbon dioxide (CO₂), and airborne microorganisms (bacteria and fungi) arise from distinct but interrelated sources [6,7]. PM is influenced by outdoor air entering classrooms and by indoor resuspension associated with movement and classroom activities; VOCs are commonly associated with building and furnishing materials and with cleaning and maintenance products; CO₂ is primarily

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generated by occupants and is widely used as an indicator of ventilation efficiency; and airborne bacteria and fungi are strongly influenced by occupancy, classroom activities, ventilation conditions, and dampness/moisture problems in school buildings [4,8–10]. These pollutants are relevant in classrooms because they may contribute to respiratory symptoms and mucosal irritation, while elevated CO₂ also indicates insufficient outdoor air supply and a greater likelihood of accumulation of other indoor contaminants; airborne bacteria and fungi are additionally relevant because exposure in educational settings has been associated with allergies and other respiratory complaints [9,11–13].

Among the most relevant pollutants, PM deserves particular attention due to its health implications and its interaction with microorganisms [14]. PM with a diameter of 10 µm (PM₁₀) can accumulate in the upper airways and penetrate the lungs, but it is the particles with a diameter of 2,5 µm (PM_{2,5}) that raise greater concern, as they can penetrate more deeply into the lungs, at the level of the pulmonary alveoli, increasing the risk of respiratory and cardiovascular problems and, in more severe cases, cancer [14,15]. Their relevance lies in the fact that PMs can serve as carriers of bacteria, fungal spores, and microbial fragments, facilitating their transport and inhalation [16]. Human occupancy has been shown to markedly increase airborne bacterial, fungal, and PM concentrations in children's classrooms, reinforcing the role of pupils and classroom activities in school bioaerosol dynamics [7].

The presence of airborne microorganisms, such as bacteria and fungi, is a concern in the school context. In educational buildings, their occurrence is influenced by occupants, settled dust, damp or damaged materials, ventilation conditions, and cleaning/maintenance practices [17,18]. Studies in school and day-care settings have reported higher bacterial and fungal concentrations under conditions of poor ventilation and have frequently identified genera such as *Penicillium*, *Cladosporium*, and *Aspergillus* in indoor air [17,19]. Although many microorganisms detected indoors are not necessarily pathogenic, potentially pathogenic bacterial and fungal taxa have also been identified in school bioaerosols, including in the fine particulate fraction, which may increase the relevance of exposure in occupied classrooms [20,21]. Exposure to these contaminants has been associated with respiratory symptoms, allergies, asthma exacerbation, and other building-related complaints in susceptible occupants [13,22].

Although many school IAQ studies describe chemical or biological contaminants separately and often focus on specific educational levels, evidence on the relationship between microorganisms, PM, and chemical pollutants across the full range of educational settings remains fragmented. This systematic review therefore includes a wider range of educational settings, and focuses on the relationships between microorganisms, particulate matter, and chemical pollutants across all types of school environments, from daycare centers to universities. Understanding these factors is fundamental to developing strategies for the prevention and control of air pollution in schools and for promoting healthy, high-quality environments for occupants.

2. Methods

The current systematic review followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [23]. The protocol was previously registered in 'PROSPERO' (ID: CRD420250619796) to ensure the integrity and pre-definition of the applied methodology. The following steps were conducted: definition of research objectives; selection of the databases; definition of keywords; establishment of the inclusion and exclusion criteria; and evaluation and management of selected studies.

2.1. Search strategy

The bibliographic search was conducted in December 2024 in four databases: Scopus®, Web of Science™, PubMed®, and Science Direct®. These databases were selected due to their extensive coverage of peer-

reviewed literature in environmental and public health. The search strategy combined keywords related to indoor air quality and educational environments (see Table 1). To broaden the scope of the research, the keywords were combined using the Boolean operators "AND" and "OR" resulting in three search equations:

1. ("Indoor Air Quality" OR "IAQ" OR "Indoor Air Pollution") AND ("Schools*" OR "Classroom*" OR "Educational*") AND ("chemical*" OR "particulate matter" OR "carbon dioxide" OR "volatile organic compounds" OR "nitrogen dioxide" OR "ozone") AND ("Biological Pollutants" OR "Bacteria" OR "Fungi" OR "Microbial Load" OR "Airborne Microorganisms" OR "Bioaerosols")
2. ("Indoor Air Quality" OR "IAQ" OR "Indoor Air Pollution") AND (Schools* OR Classroom* OR Educational*) AND ("chemical*" OR "particulate matter" OR "carbon dioxide" OR "volatile organic compounds" OR "nitrogen dioxide" OR "ozone")
3. ("Indoor Air Quality" OR "IAQ" OR "Indoor Air Pollution") AND ("Airborne Microorganisms" OR "Bioaerosols" OR "Biological Pollutants" OR "Bacteria" OR "Fungi" OR "Microbial Load") AND (Schools* OR Classroom* OR Educational*).

Due to the limitation of eight Boolean operators and the non-use of wildcards in Science Direct®, the keywords used were fewer. In this case, we applied the following search equation: "Indoor air quality" AND ("chemical pollutants" OR "particulate matter") AND ("Airborne Microorganisms" OR "Bacteria" OR "Fungi" OR "Bioaerosols") AND ("School" OR "Classroom").

2.2. Eligibility criteria

The selection process adhered to predefined inclusion and exclusion criteria. The study selection process was divided into two stages. Initially, the search phrases were entered into the databases, and the first set of inclusion criteria was applied using the databases' automated tools: 1) date of publication: articles between 2014 and 2024 were included; 2) document type: non-review articles were selected to reduce the likelihood of using repeated sources; 3) language: articles not in English were excluded.

In the second phase, articles that focused on health outcomes or conditions not directly related to the assessment of IAQ, as well as studies that used modeling techniques for IAQ analysis, and studies conducted outside the school context, were also excluded. The articles that did not fit the theme were excluded. To exclude studies that did not meet the requirements of this review, the following exclusion criteria were applied after a complete reading of the text: studies that did not provide a relationship between PM, chemical and biological pollutants were excluded.

2.3. Selection process

After the initial search, the automatic tools in databases were applied. Then, the search results were entered into the reference management software 'Rayyan' to facilitate the identification and removal of duplicates. The study selection process was conducted in three stages: screening, full-text screening, and final inclusion. Initially, one reviewer evaluated the titles and abstracts to exclude articles that did not meet the inclusion criteria. Studies considered relevant were then assessed for full-text screening, where two independent reviewers examined each study to ensure it met all eligibility criteria. Any discrepancies between the reviewers were resolved through discussion or consultation with a third reviewer if necessary. Finally, studies that met the criteria after full-text screening were included in the analysis for the final stage of the review.

Table 1

Keywords.

Keywords
Indoor air quality; IAQ; indoor air pollution
School*; classroom*; educational*
Chemical*; particulate matter; carbon dioxide; volatile organic compounds; nitrogen dioxide; ozone
Biological pollutants; bacteria; fungi; microbial load; airborne microorganisms; bioaerosols

2.4. Quality and bias assessment

The studies were systematically evaluated by two independent reviewers using the evaluation framework, the Mixed Methods Appraisal Tool (MMAT) [24]. This method was applied to assess the studies' methodological rigor while accounting for the specific characteristics of each study design. The MMAT user guide outlined the criteria for evaluating each type of study and provided questions to assess studies quality. Two initial screening questions were applied to confirm eligibility for appraisal, followed by evaluation using design-specific MMAT criteria. Per these guidelines, if one or both screening questions were answered as "No" or "Can't tell", the study was considered ineligible for MMAT evaluation. Only the studies that answered "Yes" to the screening questions were included. For each study included, the relevant category for evaluation was selected, and responses were provided as "Yes", "No" or "Can't tell". Calculating an overall MMAT score from the evaluations of individual criteria is discouraged. Instead, comprehensive ratings for each criterion should be provided to enhance the evaluation of the quality of the included studies. Thus, studies were grouped based on the number of criteria rated as "Yes", reflecting their overall quality (high

quality: four or more "Yes" responses; moderate quality: two or three "Yes" responses; and low quality: zero or one "Yes" response).

Quality assessment was conducted independently by one reviewer and verified by a second reviewer, with discrepancies resolved by consensus.

2.5. Data synthesis and analysis

The synthesis and analysis of the data were carried out using qualitative methods. The data were systematically synthesized to evaluate the relationship between the concentration of microorganisms, particulate matter, and chemical pollutants in school environments. To ensure a more comprehensive analysis, a synthesis of the main characteristics of the studies was conducted, which included the type of school setting, the geographical location, the variables analyzed, the study objectives, and the main results. The synthesis of the findings employed effect metrics for each outcome. If p-values were provided, they were included in the synthesis to help assess the statistical significance of the findings.

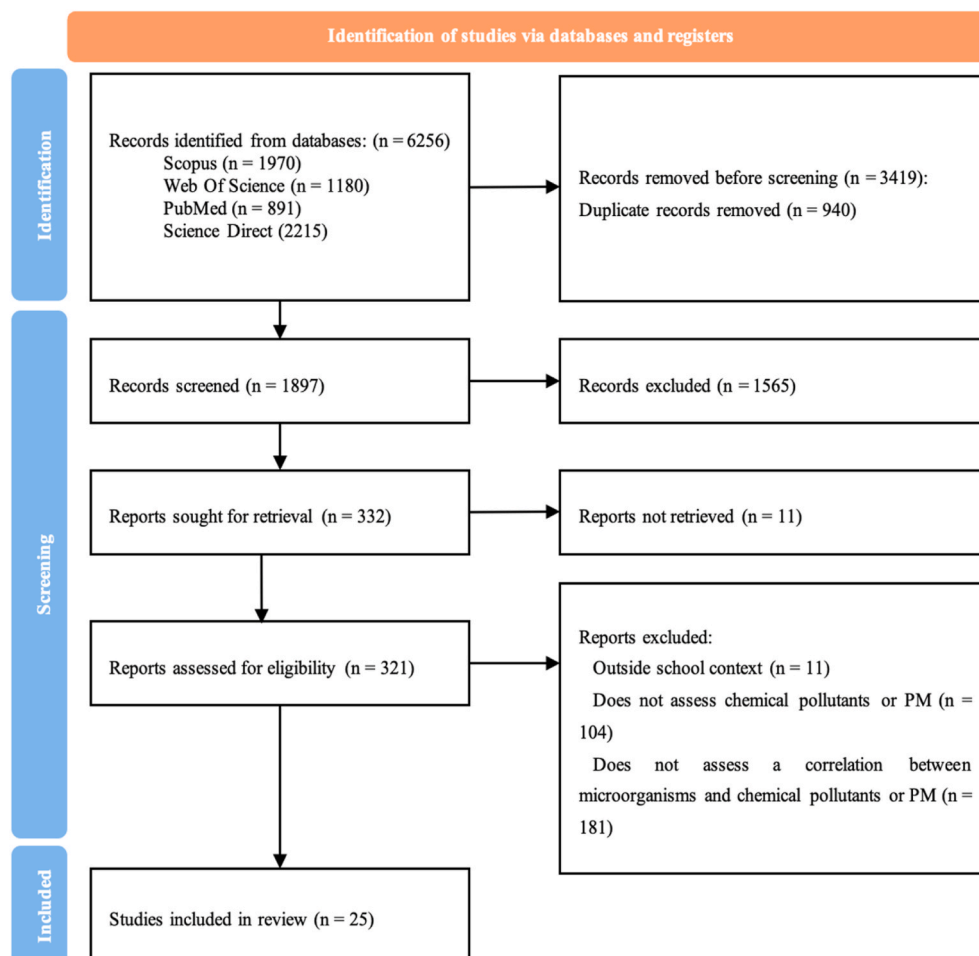


Fig. 1. Prisma flow diagram.

3. Results

The initial search identified 6256 articles. After the removal of 3419 articles due to the automatic tools and the removal of 940 duplicates, a total of 1897 articles were screened. After reviewing the titles and abstracts, 332 articles were examined in more detail for eligibility; the remaining papers did not meet the criteria. Out of these 332 articles, 25 were included in the final data analysis. The search and selection process are illustrated in Fig. 1.

3.1. Quality analysis

The MMAT was used to evaluate the quality of the 25 studies included in this systematic review. Out of the 25 studies, 24 were classified as quantitative descriptive and 1 was quantitative non-randomized. The questions described by the method were answered based on the number of “Yes” responses, the studies were classified as high, moderate or low quality. 14 studies were categorized as high quality (56%) and 11 as moderate quality (44%). No studies were classified as low quality. More detailed information about the assessment questions is provided in Appendix A.

3.2. Descriptive results

The included studies cover a wide geographical distribution (see Fig. 2), including Europe, Asia, Africa, and multi-country investigations. This diversity highlights the influence of location on indoor air quality in school environments. However, climatic conditions were not consistently reported across the studies, with several investigations lacking explicit climate characterization. When reported, a variety of climatic contexts were identified, including Atlantic and Mediterranean climates in Portugal, tropical conditions in Malaysia, arid environments in Iran, and different climate zones in China (e.g., hot summer and cold winter, semi-arid, and multi-zone classifications). These differences reinforce that indoor air pollution in schools is shaped not only by occupants but also by building characteristics, outdoor air quality, and broader environmental conditions, including climate and geographical location.

Table 2 summarizes the descriptive information about the 25 articles included in this review. The data focus on the main findings of the

studies on the relationship between microbial concentrations and indoor air quality, particularly in school settings. With an emphasis on the impact of PM and chemical pollutants in airborne microorganisms' concentrations, the reviewed studies' primary goal was to identify possible correlations between the variables. Some studies also explored environmental factors, including Temperature (T) and Relative Humidity (RH), that contribute to microbial contamination indoors. The findings shed valuable insights on how environmental factors affect IAQ, particularly in environments frequented by children.

3.3. Content analysis

3.3.1. Relationship between airborne microorganisms, particulate matter, and chemical pollutants

The relationship between microorganisms, PM, and chemical pollutants is the highlight of this review. In this section, the results of the studies are presented, regarding the existing correlations between microorganisms (bacteria and fungi) and other indoor air pollutants, both PM and chemical agents (see Table 3).

The data provides valuable insights into how different microbial populations, such as bacteria and fungi, correlate with the presence of indoor pollutants in different school settings. One of the most often studied pollutants was carbon dioxide, and its correlation with microbial concentrations showed mixed patterns. In general, positive correlations were more often observed between CO₂ and bacteria [25,26,33,34,37,38,43–45,48]. For example, several studies reported statistically significant positive correlations between CO₂ and total bacteria [26,34,37,38,43,45]. On the other hand, the correlations between CO₂ and fungi were more variable, including both positive [43–45,47] and negative correlations [26]. In some cases, CO₂ was negatively correlated with total fungal levels [26], while in others, it was positively associated with specific fungi [43–45,47].

In terms of particulate matter, bacteria and fungi generally exhibited significant correlations with PM, with several genera showing varying degrees of association, such as *Aspergillus* spp. [27,44], *Cladosporium* spp. [44] which were linked to particulate pollutants. However, despite these pollutants are linked to microbial presence, bacteria genera show a stronger association with PM concentrations [29,33,34,38,40–42,44,48].



Fig. 2. Geographical Distribution of reviewed studies.

Table 2
Summary of studies on indoor air quality.

Ref	Main Objective	Sample	Variables Measured	Key Findings
Fu et al. (2020) [25]	Investigate indoor microbial load's impact on asthma	8 junior high schools	B, F, CO ₂ , NO ₂	Indoor dampness and mold linked to lower bacteria
Wang et al. (2023) [26]	Investigate microbial concentration and influencing factors	217 schools	B, F	Schools had highest fungi; high ventilation reduced bacteria; low RH decreased fungi.
Pyrri et al. (2020) [27]	Characterize fungal aerosol diversity and distribution	Primary school	F, PM ₁₀ , T, RH	Green roof reduced fungal concentration by 2–8 times; <i>Penicillium</i> , <i>Cladosporium</i> , and <i>Aspergillus</i> dominant; fungal spores correlated with temperature and PM ₁₀ .
Yen et al. (2020) [28]	Study pollutants' effects in endotoxin and lung function	3 elementary schools	Endotoxin, PM _{2.5} , PM ₁₀ , CO, CO ₂ , SO ₂ , NO ₂ , O ₃ , T, RH	High O ₃ and PM ₁₀ negatively impacted lung function when combined with endotoxin exposure.
Song et al. (2024) [29]	Assess bacterial and fungal diversity and pathogenic species.	Nursery, primary school, junior high school and university	B, F, PM _{2.5}	Preschools near pollutants had higher PM ₁₀ and microbiome diversity; <i>Aspergillus penicilloides</i> and <i>Cladosporium dominicanum</i> linked to respiratory symptoms.
Hwang et al. (2016) [30]	Analyze endotoxin levels and the effect of environmental factors	University	Endotoxin, CO ₂ , CO, T, RH	Endotoxin levels were higher in winter, negatively correlated with T and CO ₂
Wu et al. (2020) [31]	Assess airborne fungi, size distribution and environmental factors	University	F, PM ₁₀ , PM _{2.5} , T, RH	Libraries had seasonal variation in fungal levels; higher in autumn due to favorable temperature and RH.
Zhu et al. (2021) [32]	Assess IAQ, ventilation and health risks	22 primary schools	B, F, PM _{2.5} , PM ₁₀ , CO ₂ , CO, formaldehyde, T, RH	66,5% of classrooms exceeded PM _{2.5} limits; 52,6% exceeded PM ₁₀ ; CO ₂ was high in 22,4% of classrooms, with poor ventilation contributing to elevated pollutant levels.
Guo et al. (2021) [33]	Investigate microbial concentration, risks, and environmental influences	4 schools	B, F, CO ₂ , T, RH	Pathogens like <i>Acinetobacter lwoffii</i> and <i>Candida albicans</i> prevalent.
Isa et al. (2021) [34]	Investigate fungi metagenomics and its relationship with allergy and lung inflammation	8 secondary schools	F, PM ₁₀ , PM _{2.5} , CO ₂ , NO ₂ , T, RH	<i>Aspergillus clavatus</i> and <i>Papiliotrema bandonii</i> linked to asthma biomarkers; PM _{2.5} , NO ₂ , and fungi abundance influenced inflammation and asthma development.
Seo et al. (2015) [35]	Analyze submicron fungal fragments, seasonal changes, and health risks	8 elementary schools	B, mold, (1,3)-β-D-glucan, PM ₁₀ , T, RH	Post-rainy season saw a 35–55% decrease in fungal fragments, airborne mold, and bacteria; RH negatively correlated with fungal fragment levels.
Hospodsky et al. (2015) [36]	Evaluate microbial and PM concentrations during occupied and vacant classrooms	6 Schools	B, F, PM	Occupancy increased bacterial, fungal, and PM levels significantly; emission rates varied by location, with human activity being a major contributor.
Madureira et al. (2016) [37]	Assess IAQ and pollutant sources	20 primary schools	B, F, VOCs, aldehydes, PM _{2.5} , PM ₁₀ , CO ₂ , CO, T, RH	Poor ventilation led to higher CO ₂ in 86% of the classrooms; bacteria often above 1000 CFU/m ³ .
Madureira et al. (2015) [38]	Quantify bioaerosols; evaluate indoor-outdoor influences and health risks	20 primary schools	B, F, CO ₂ , T, RH	Highest bacterial and fungal levels in child daycare centers; occupancy and poor ventilation linked to bacterial levels; <i>Penicillium</i> and <i>Cladosporium</i> were dominant fungi.
Li et al. (2015) [39]	Compare bioaerosols on haze and non-haze days and assess health risks	University	B, F, PM _{2.5} , T, RH	Bacterial and fungal concentrations were 2–4 times higher on haze days; more infectious genera present
Harbizadeh et al. (2019) [40]	Assess indoor and outdoor bacteria and PM; and comparing seasonal and regional variations	6 child daycare centers	B, PM ₁ , PM _{2.5} , PM ₁₀ , T, RH	High traffic regions had the highest bacteria concentrations; winter dust storms increased bacteria and PM; artificial ventilation recommended to improve IAQ.
Zhao et al. (2022) [41]	Characterize seasonal bacterial concentrations, and its relationship with occupants and environmental factors	University	B, PM ₁ , PM _{2.5} , PM ₁₀ , T, RH	Indoor bacterial levels increased with occupants; summer had the highest bacterial emissions, influenced by PM ₁₀ and T.
Hwang et al. (2017) [42]	Evaluate environmental factors, including PM ₁₀ and airborne bacteria, to assess air quality	330 daycares and 41 public childcare centers	B, PM ₁₀	High air exchange rates reduced bacteria and fungi; residences had highest bacterial levels; schools had highest fungal concentrations due to leaf coverage.
Seseña et al. (2022) [43]	Assess IAQ in naturally ventilated labs	University	B, F, CO ₂ , CO, O ₃ , TVOCs, PM, T, RH	Biological labs had highest bacterial counts; ecology labs had peak fungal levels.
Onwusereaka et al. (2024) [44]	Analyze microbiome diversity and its association with respiratory symptoms	10 Preschools	B, F, PM _{2.5} , PM ₁₀ , CO ₂ , T, RH	Schools near pollution sources had higher PM ₁₀ and microbial diversity; linked to <i>Cladosporium dominicanum</i> and <i>Aureobasidium gracilis</i> .
Mohammed (2023) [45]	Assess cleaning effectiveness on microbial load and IAQ	2 Universities	B, F, PM _{2.5} , PM ₁₀ , CO ₂ , VOCs, formaldehyde, T, RH	Cleaning reduced bacterial and fungal counts significantly; VOCs increased post-cleaning; PM levels mostly attributed to indoor sources.
Bragoszewska et al. (2016) [46]	Investigate bioaerosol levels, size distribution, and exposure risks	2 nurseries	B, F, CO ₂ , T, RH	Indoor bacterial levels 2–4 times higher than outdoor levels; spring had higher bacterial loads; younger children had higher exposure doses of bacteria and fungi.
Madureira et al. (2014) [47]	Quantify airborne fungi and evaluate environmental factors	20 primary schools	F, CO ₂ , T, RH	Indoor fungi levels higher than outdoor, dominated by <i>Penicillium</i> and <i>Cladosporium</i> .
Andualem et al. (2019) [48]	Evaluate bacteria concentration and analyze its correlation with physical parameters	8 primary schools	B, PM _{2.5} , PM ₁₀ , CO ₂ , T, RH	Bacterial load exceeded safe limits in classrooms; <i>Staphylococcus</i> and <i>Bacillus</i> dominant; higher PM and temperature positively correlated with bacteria.
Andualem et al. (2019) [49]	Assess fungal load and its associated factors indoors	8 primary schools	F, PM ₁₀ , T, RH	High fungal load detected; <i>Aspergillus</i> , <i>Penicillium</i> , and <i>Trichophyton</i> dominant; older buildings and low ventilation increased fungal levels;

B – Bacteria; F – Fungi; T – Temperature; HR – Relative Humidity.

Table 3
Correlations of airborne microorganisms with particulate matter and chemical pollutants.

Reference	Microorganisms	PM _{2.5}	PM ₁₀	CO ₂	Other pollutants
Fu et al. (2020) [25]	Bacteria	-	-	0.02	-
	Fungi			0.02	
Wang et al. (2023) [26]	Bacteria	0.049	-	0.190***	-
	Fungi	-0.110**		-0.089*	
Pyrri et al. (2020) [27]	Total fungi	-	-0.201	-	-
	<i>Aspergillus spp.</i>		0.586*		
	<i>Cladosporium spp.</i>		-0.573		
	<i>Penicillium spp.</i>		-0.185		
Yen et al. (2020) [28]	Endotoxin	0.030	-0.048	-0.091	-0.011 (NO ₂); - 0.246* (SO ₂); - 0.190 (CO)
Song et al. (2024) [29]	<i>Streptococcus pneumoniae</i> ; <i>Staphylococcus spp.</i> ; <i>Lactobacillus iners</i> ; <i>Talaromyces variabilis</i>	NR*	-	-	-
Hwang et al. (2016) [30]	Endotoxin	-	-	-0.213***	-0.136 (CO)
Wu et al. (2020) [31]	Fungi	-0.028	-0.021	-0.036	-
Zhu et al. (2021) [32]	Bacteria	-0.031	-	-	-
	Fungi	-0.192***			
Guo et al. (2021) [33]	Bacteria	-	-	0.301***	-
	Fungi			-0.129	
Isa et al. (2021) [34]	<i>Chromohalobacter halotolerans</i>	-0.455 to -0.273*	-0.636 to - 0.455*		
	<i>Xanthomonas lusitanica</i>	-	-0.636 to - 0.455*		
	<i>Hyalomicrobium aloes</i>			-0.636 to - 0.455*	
	<i>Janthinobacterium angkorensis</i>			-0.636 to - 0.455*	
	<i>Methylobacterium antarcticus</i>			-0.636 to - 0.455*	
	<i>Starmarella meliponinorum</i>				-0.636 to - 0.455* (NO ₂)
	<i>Trichoderma asahii</i>				-0.455 to - 0.273* (NO ₂)
	<i>Chromohalobacter halotolerans</i>				-0.636 to -0.455* (formaldehyde)
	<i>Trichoderma pseudoveloxyum</i>				-0.455 to -0.273*
	<i>Streptomyces meliponinorum</i>				-0.455 to -0.273*
	<i>Trichoderma asahii</i>				-0.455 to -0.273*
Seo et al. (2015) [35]	Bacteria	-	0.073	-	-
	Fungi		0.174*		
Hospodsky et al. (2015) [36]	Bacteria	-	-	-	0.250 (PM mass)
	Fungi				0.250 (PM mass)
Madureira et al. (2016) [37]	Bacteria	-0.031	-0.098	0.257*	-0.210 (benzene); - 0.148 (toluene); 0.141 (d-limonene); - 0.225 (TVOCs); 0.076 (formaldehyde)
	Fungi	0.058	0.037	-0.167	0.152 (benzene); 0.039 (toluene); - 0.386** (d-limonene); - 0.305** (TVOCs); 0.459** (formaldehyde)
Madureira et al. (2015) [38]	Bacteria	-	-	0.340* (childcare centers); 0.260* (primary schools)	-
	Fungi			0.080 (childcare centers); - 0.170 (primary schools)	
Li et al. (2015) [39]	Bacteria	0.786**	-	-	-
	Fungi	0.902**			
Harbizadeh et al. (2019) [40]	Bacteria	0.457*	0.457*		0.457* (PM1)
Zhao et al. (2022) [41]	Bacteria	0.096 (unoccupied); 0.009 (occupied)	0.364* (unoccupied); 0.266** (occupied)	-	-
Hwang et al. (2017) [42]	Bacteria	-	0.128*	-	-
Seseña et al. (2022) [43]	Bacteria	-	-	0.260*	0.150* (PM0,3); 0.200* (PM0,5); 0.430* (PM5)
	Fungi			0.230*	0.170* (PM0,3); 0.200* (PM0,5); 0.170* (PM5)
Onwusereaka et al. (2024) [44]	<i>Aspergillus gracilis</i>	-0.164**	-0.638**	0.021	
	<i>Aspergillus penicillioides</i>	-0.109*	-0.498**	0.026	
	<i>Cladosporium dominicanum</i>	-0.020	0.582**	0.464**	
	<i>Cladosporium halotolerans</i>	0.155**	-0.047	0.141**	
	<i>Curvularia affinis</i>	-0.021	0.618**	-0.495**	
	<i>Curvularia lunata</i>	-0.059	-0.483**	0.014	
	<i>Curvularia sorghina</i>	-0.258**	-0.365**	0.324**	
	<i>Acinetobacter radioresistens</i>	0.092	0.426**	0.726**	
	<i>Acinetobacter seifertii</i>	-0.146**	0.298**	0.632**	
	<i>Acinetobacter variabilis</i>	0.311**	0.501**	0.349**	
	<i>Cronobacter malonaticus</i>	0.136**	0.522**	0.333**	
	<i>Franconibacter helveticus</i>	0.095	0.571**	0.454	
	<i>Franconibacter pulveris</i>	-0.044	0.373**	0.270**	
	<i>Lactobacillus iners</i>	-0.438**	-0.437**	-0.005	

(continued on next page)

Table 3 (continued)

Reference	Microorganisms	PM _{2,5}	PM ₁₀	CO ₂	Other pollutants
Mohammed (2023) [45]	<i>Pseudomonas B. luteola</i>	0.107*	0.265**	0.103	
	<i>Pseudomonas E. khazarica</i>	0.008	0.247**	0.131*	
Bragoszewska et al. (2016) [46]	Bacteria	0.160-0.210	0.160-0.210	0.018***	
	Fungi	0.160-0.210	0.160-0.210	0.012***	
Madureira et al. (2014) [47]	Bacteria	-	-	NR	
	Fungi	-	-	NR	
	<i>Alternaria spp.</i>	-	-	-0.293	
	<i>Cladosporium spp.</i>	-	-	-0.284	
	<i>Geotrichum spp.</i>	-	-	0.089	
	<i>Rhodotorula spp.</i>	-	-	0.536*	
	<i>Mycelia sterilia</i>	-	-	0.172	
Andualem et al. (2019) [48]	<i>Paecilomyces spp.</i>	-	-	-0.277	
	<i>Penicillium spp.</i>	-	-	0.244	
	Bacteria	0.572* (morning); -0.110 (afternoon)	0.685* (morning); 0.185 (afternoon)	0.104 (morning); -0.164 (afternoon)	-
	Fungi	-	NR**	NR	-
Andualem et al. (2019) [49]					

NR: Not reported. The study didn't provide the correlation coefficient but indicated whether the association was statistically significant. Statistical significance is denoted by an asterisk (*); absence of the symbol indicates a non-significant or unspecified result.

* significant $p < 0.05$; ** significant $p < 0.01$; *** significant $p < 0.001$.

The authors also reported a relationship between chemical pollutants and microorganisms. For instance, SO₂ and O₃ was significantly correlated with endotoxins [28], and NO₂ showed negative but significant correlations with bacteria genera, such as *Streptomyces meliponinorum*, and with fungal genera, *Trichoderma asahii* [34]. Other chemical pollutants such as TVOCs, formaldehyde and d-limonene presented significant correlations with fungi [37], while no correlations were found with bacteria.

It is important to note that the studies included in the analysis adopted different thresholds for defining statistical significance. Although a p-value of $p < 0.05$ is generally used as indicative of significance, some authors only considered results with $p < 0.01$ or $p < 0.001$ as statistically significant. This methodological disparity was considered in the interpretation of the presented correlation coefficients, as it may influence the way the results were discussed in the respective studies.

4. Discussion

The analysis of the correlation between indoor air pollutants and the presence of microorganisms, including bacteria and fungi, revealed a great diversity of results among the 25 studies, suggesting that these interactions are influenced by various contextual and environmental factors.

CO₂ levels were frequently used as a direct indicator of ventilation and occupancy [26,28,30]. Several studies reported no significant correlation between CO₂ and microbial levels, particularly in environments with controlled ventilation [25,28], suggesting that, under stable ventilation conditions, CO₂ may not be an effective predictor of indoor air quality. Conversely, other authors observed positive and significant correlations between CO₂ and the concentration of bacteria, with coefficients ranging from 0.018 to 0.340 [26,33,37,38,43,45]. These values suggest that spaces with poor ventilation – indicated by elevated CO₂ levels – tend to accumulate more bacterial bioaerosols, potentially due to higher occupancy density and lower air renewal [33]. Some bacterial species, such as *Acinetobacter radioresistens*, demonstrated a positive and significant correlation with CO₂ ($r = 0.726$, $p < 0.01$), reinforcing the role of occupancy and air renewal in the concentration of bacteria [44].

Regarding fungi, the results were less consistent. Wang et al., [26] reported a negative but significant correlation with CO₂ ($r = -0.089$, $p < 0.05$), indicating that poorly ventilated environments may inhibit the presence of fungi due to the reduced entry of spores from outdoors. In contrast, Onwusereaka et al., [44] detected that some fungal species such as *Cladosporium dominicanum*, *Curvularia sorghina* e *Corioliopsis*

aspera correlated positively with CO₂ ($r = 0.464$, $r = 0.324$, and $r = 0.219$, respectively), demonstrating that specific genera can adapt well to less ventilated environments. Several studies [31,33,37,38,46,47,49], detected no significant correlations, reflecting the diversity of fungal responses to ventilation conditions. Such results can be explained by the fact that CO₂ is primarily an indicator of human occupancy and does not directly affect fungal growth [31]. The lack of significance thus suggests that the occupancy rate alone does not constitute a dominant factor in the variation of fungi concentration [31], particularly when compared to the influence of other environmental parameters, such as relative humidity and the presence of infiltrations [33].

Particulate matter has shown influence on the presence of microorganisms in indoor air. Li et al., [39] reported positive correlations between PM_{2,5} with bacteria ($r = 0.786$, $p < 0.01$) and with fungi ($r = 0.902$, $p < 0.01$), suggesting that finer particles can function as a vector for transporting microorganisms, granting them greater persistence in indoor environments [39]. Harbizadeh et al., [40] observed similar effects during atmospheric dust events, which were carried by the wind and influenced the levels of bioaerosols in indoor air due to natural ventilation [40]. However, not all studies point in the same direction. Pyrri et al. [27] found significant correlations for *Aspergillus spp.* with PM₁₀, possibly due to its aerodynamic size and spore characteristics [27]. Similarly, Wu et al., [31], Hospodsky et al., [36], Madureira et al., [37], and Mohammed [45], didn't observe significant correlations between suspended particles and fungi. This absence of correlation may be due to competition among microorganisms for specific environmental conditions, such as temperature, relative humidity, or nutrient availability [31,32]. Another reason may be due to possible sedimentation effects or the reduction of spore aerosolization in dustier environments [49].

Regarding bacteria, a similar scenario was observed. Significant correlations were found for species such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Lactobacillus iners*, and *Talaromyces variabilis* with PM_{2,5}, while Isa et al., [34] observed negative correlations were also observed for *Chromohalobacter halotolerans*. Other studies confirmed that bacterial concentrations varied with PM levels [39–43]. These results suggest that PM may act as carriers or provide surfaces for bacterial adhesion [39]. Fine particles (PM_{2,5}) offer nutrients and act as a protective agent for the survival and growth of microorganisms, which translates into higher concentrations of bioaerosols [39]. Moreover, particulate matter can contribute to keeping bacteria suspended in the air, facilitating their transport into indoor environments, particularly through natural ventilation, such as open windows. This is especially relevant in locations without air conditioning systems, where outdoor particulate matter levels directly influence indoor microbial load [40].

Furthermore, Andualet et al., [47] detected positive and significant correlations between bacterial concentration and PM_{2,5} and PM₁₀ in the morning period, whereas in the afternoon, the correlations were no longer significant ($p > 0.05$). This variation may be explained by the fact that, during the morning, suspended particles act as carriers of bacterial aerosols [39,48], particularly PM₁₀ due to their larger diameter, which provides a greater adhesion surface, keeping bacteria suspended in the air. In the afternoon, the absence of a significant correlation may be related to the decrease in PM concentrations or to other environmental variables [48]. On the other hand, some studies did not detect any significant correlation with PM_{2,5} [26,28,32,37,45] or PM₁₀ [28,35,37,45] and bacterial concentration in schools. This lack of correlation may be because, in some cases, bacteria are not directly associated with the presence of suspended particles [31], but rather with indoor sources and poor ventilation systems [37].

In addition to particulate matter and carbon dioxide, some studies analyzed the influence of VOCs, such as d-limonene and formaldehyde. D-limonene – a common compound in cleaning products – showed a significant negative correlation with fungi ($r = -0.386$, $p < 0.01$), possibly due to its antimicrobial effect, suppressing fungal presence [37]. In contrast, formaldehyde showed a significant positive correlation with fungi ($r = 0.459$, $p < 0.05$) [37]. Although the authors acknowledge that this association may merely reflect coexistence with other factors favorable to fungal growth – such as synthetic materials and inadequate ventilation – a possible direct influence of formaldehyde cannot be completely ruled out [37]. O₃ and SO₂, both oxidizing gases, showed significant negative correlations with the concentration of endotoxins in indoor air ($p < 0.05$), suggesting that these pollutants may inactivate endotoxins [28]. Sesena et al., [43], however, did not detect any correlation between bacteria and O₃, but a significant correlation was observed with fungi ($r = -0.310$, $p < 0.05$). NO₂ correlated positively with endotoxins; however, this correlation was not significant [28]. In contrast, NO₂ significantly correlated with bacterial and fungal species such as *Streptomyces meliponinorum* and *Trichoderma asahii*, with values ranging from -0.636 to -0.455 and -0.455 to -0.273 , respectively [34]. CO did not significantly correlate with bacteria [28,30], and no study reported a relationship between CO and fungi.

Although air temperature and relative humidity were not analyzed in detail, the studies mentioned that these factors are essential for the presence of bacteria and fungi. Most studies show that higher temperatures favour microbial growth. For example, Wu et al., [31] and Guo et al., [33] observed positive correlations with fungi ($r = 0.531$, $p < 0.05$), while Madureira et al., [47] reinforced the positive association between temperature and fungal growth during winter. These correlations are consistent with the literature, which indicates that higher temperatures promote microbial metabolism and reproduction, especially in environments with adequate humidity levels [33,47]. However, Andualet et al., [49] presented an opposite result, suggesting that other factors, such as increased natural ventilation or behavioural changes during warmer seasons, may mitigate the expected fungal growth [49].

Regarding humidity, authors such as Wu et al., [31] argue that higher RH favours the viability and suspension of bacterial spores and cells in the air. Bragoszewska et al., [46] reported strong positive correlations between RH and bioaerosols, indicating that more humid conditions reduce desiccation stress and facilitate the suspension of microorganisms in the indoor environment. Still, Andualet et al., [49] contradicts this trend with a negative but significant correlation ($r = -0.023$, $p < 0.05$), demonstrating that RH's influence may also vary depending on the specific environmental and methodological conditions of each study. Moreover, specific species, such as yeasts, appear to respond differently to humidity, showing positive correlations even when overall RH values are not significantly associated with total fungal concentrations [47].

The results demonstrate that the relationships between indoor air pollutants and microorganisms are strongly influenced by the type of pollutant, the taxonomic specificity of the microorganisms, the physical

characteristics of the spaces, as well as the local environmental conditions. The absence of universal patterns highlights the importance of an integrated and contextualised approach to indoor air quality assessment, combining pollutant monitoring with microbial analysis, particularly in indoor spaces such as schools.

4.1. Limitations

While this review provides a comprehensive understanding of the dynamics of IAQ in schools, it is crucial to acknowledge some limitations. The methodologies, sample techniques, and environmental conditions used in the studies vary, which could have an impact on how consistently the results hold up across different contexts. The lack of standardized protocols for measuring pollutants and microbial load introduces potential bias. The synthesis of data is further complicated by the difficulty of comparing results between research due to the lack of consistent sampling duration, collection methods, equipment used, and analytic procedures. The results may not accurately represent the IAQ issues that schools in rural or less polluted areas address because most of the studies focused on urban school environments. Furthermore, the limited research into certain chemical pollutants limits our understanding of their role and impact on indoor microbial dynamics.

4.2. Conclusions

The work fills a gap providing information on the relationship between indoor air quality and microbial contamination in school settings, a critical area of study given its potential impact on the health of vulnerable populations, especially children. Since children spend a significant amount of time at school, they are more susceptible to the adverse effects of poor IAQ, such as respiratory issues, allergies, and other long-term health conditions, like asthma. This review underscores the complex interactions between indoor air pollutants, such as microorganisms, PM, CO₂, and other chemical pollutants.

The variety in microbial concentrations in research emphasizes the significance of factors such as occupancy, ventilation, and environmental conditions in affecting IAQ. High concentrations of bacteria and fungi were correlated with increased human activity, inadequate ventilation, and elevated CO₂ concentrations. Certain bacterial species, like *Staphylococcus* and *Micrococcus*, and fungal species such as *Aspergillus* and *Penicillium*, were predominant in educational environments. Particulate matter showed significant correlations with bacteria and fungi. Fine particles, such as PM_{2,5} have been identified as vectors for microorganisms, facilitating their dissemination and proliferation indoors. Due to their larger diameter, PM₁₀ also contributes to the transportation of bacterial and fungal spores. The relationship between CO₂ levels and microorganisms, especially in areas with high occupancy and low ventilation, emphasizes the necessity for improved air circulation in classrooms. Additionally, VOCs, NO₂, and other chemical pollutants were found to have effects on microorganisms, with some species exhibiting strong correlations with these pollutants.

This review underlines the importance of maintaining proper ventilation and managing occupant density in school settings to reduce the risks associated with poor IAQ. Overall, this research contributes to a deeper understanding of how indoor air pollutants interact with bacteria and fungi, emphasizing the necessity of efficient environmental management strategies to enhance IAQ and protect the health of occupants.

Ethical statement

This study is a systematic review based exclusively on previously published studies and does not involve human participants or personal data. Therefore, ethical approval was not required.

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Declaration of competing interest

The authors declare that they have no known competing financial

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Appendix A

Quantitative non-randomized

Ref	S1	S2	3.1	3.2	3.3	3.4	3.5	Quality
[28]	Yes	Yes	Can't tell	Yes	Yes	Yes	Yes	High

S1. Are there any clear research questions?; S2. Do the collected data allow to address the research questions?; 3.1. Are the participants representative of the target population?; 3.2. Are measurements appropriate regarding both the outcome and intervention (or exposure)?; 3.3. Are there complete outcome data?; 3.4. Are the confounders accounted for in the design and analysis?; 3.5. During the study period, is the intervention administered (or exposure occurred) as intended?.

Quantitative descriptive

Ref	S1	S2	4.1	4.2	4.3	4.4	4.5	Quality
[25]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[26]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[27]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[29]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[30]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[31]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[32]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[33]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[34]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[35]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[36]	Yes	Yes	Yes	No	Yes	Can't tell	Yes	Moderate
[37]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[38]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[39]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[40]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[41]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[42]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[43]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[44]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[45]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate
[46]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[47]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[48]	Yes	Yes	Yes	Yes	Yes	Can't tell	Yes	High
[49]	Yes	Yes	Yes	Can't tell	Yes	Can't tell	Yes	Moderate

S1. Are there any clear research questions?; S2. Do the collected data allow to address the research questions?; 4.1. Is the sampling strategy relevant to address the research question?; 4.2. Is the sample representative of the target population?; 4.3. Are the measurements appropriate?; 4.4. Is the risk of nonresponse bias low?; 4.5. Is the statistical analysis appropriate to answer the research question?.

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