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Recent Progress in Materials

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Original Research

Indoor Built Environments Attended by Primary School Children and Its Microbiology: A Cross-Project Study

Joana Sousa *

FEUP, University of Porto, Rua Dr. Roberto Frias, 4200-465, Portugal; E-Mail: up200903949@edu.fe.up.pt

* **Correspondence:** Joana Sousa; E-Mail: up200903949@edu.fe.up.pt

Academic Editor: Farhad Aslani

Special Issue: Material Quality and Material-flows of Circularity in the Built Environment

Recent Progress in Materials
2022, volume 4, issue 3
doi:10.21926/rpm.2203015

Received: March 10, 2022
Accepted: August 10, 2022
Published: August 24, 2022

Abstract

Exposure to indoor pollutants may exert a significant effect on children health as they spend most of their time in enclosed environments. This research compared the association of biological concentrations with indoor characteristics and building materials in places where children spend significant time indoors. It was compared the results of two autonomous research projects conducted in Porto primary schools and households. Both the projects included a walkthrough inspection and biological measurements in indoor environments in primary school classrooms and home bedrooms, respectively. Floor covering material was the major building characteristic associated with fungal concentrations. In addition, significant associations were observed between the heating system and *Fusarium* sp. and the type of glazing and *Fumigatus* sp. A comparison of the results between the two projects demonstrated similarities in the association of microbial concentrations with building materials, particularly with the floor type. This demonstrated that specific building materials could be a source of exposure to indoor pollutants. The influence of building materials on indoor air quality (IAQ) should be considered using building codes. When performing building retrofitting, professionals should be aware of the nexus between building materials-energy-

IAQ-health, which, if not properly managed, could negate the efforts of meeting climate and related sustainable development goals.

Keywords
Built environment; building materials; microenvironments; microbial exposure; location

1. Introduction

Indoor air quality, a complex function of indoor pollutants, is generated by a wide range of internal and external sources. The outdoor air is the source that contributes the highest to indoor pollution [1]. In addition, several other indoor pollutants are generated from internal sources that can affect an occupant's productivity and health [2]. The complex interactions between external and internal pollutant sources have been summarized previously [3].

The development of the chemical industry introduced several substances to building materials, which are associated with various health-related problems such as asthma, itchiness, burning eyes, skin irritations or rashes, nose, and throat irritation, nausea, headaches, dizziness, fatigue, reproductive impairment, disruption of the endocrine system, cancer, impaired child development and birth defects, and suppression of the immune system [4]. Exposure to indoor pollutants exerts a significant effect on the population, such as children, who spend increased time in enclosed indoor environments—a situation that was exacerbated during the COVID-19 pandemic [5, 6]. Building materials, particularly those employed in indoor surfaces with volatile organic compounds (VOCs) and formaldehyde, may represent a source of indoor pollution. Building materials such as adhesives, resins, glues, caulks, sealants, paints, solvents, wood stain, floor wax, carpets, textiles, wallboard, treated wood, urethane coatings, pressed-wood products, and vinyl flooring are associated with the release of VOCs and semi-volatile organic compounds (SVOCs) [7]. Most formaldehyde sources that affect human health exists in indoor environments. Formaldehyde derivate products are applied in several building materials, such as insulating materials and wallpapers, glues, adhesives, varnishes, lacquers, furniture, and wooden products containing formaldehyde-based resins, such as particleboard, plywood, and medium-density fiberboard, textiles, and paints [8].

In addition to primary emissions, secondary emissions from building materials may be attributed to factors such as dampness, relative humidity, and temperature [9]. Primary emissions result from the building material itself. Certain building materials, such as polyvinyl chloride (PVC) flooring, have been shown to be a strong source of primary emissions such as phthalates [10]. Secondary emissions, such chemical reactive products, result from pollutants that do not originate from the building material itself [11]. Secondary reactions triggered can exacerbate the exposure to indoor pollutants. Previous studies focusing on building materials and characteristics have reported indoor dampness as a predictor of microbial exposure [12]. Several other characteristics such as occupants' lifestyle behaviors, building characteristics, and outdoor concentration levels can also influence indoor microbial exposure concentrations. In addition, according to [13], indoor microbial contaminants depend on environmental parameters, ventilation, construction materials, and human operations. Furthermore, mold growth, particularly in moisture-damaged buildings, can result in exposure to bacterial endospores and fungal spores [14]. The mechanisms by which microbial growth affects health are not completely known, despite several studies indicating that biological agents could be a risk factor for the development of respiratory illness, particularly in children [15-17].

In developed countries, the increasing air tightness of efficient housing exacerbates the exposure to primary and secondary emissions from building materials; thus, lowering the ventilation rates may cause the removal of indoor pollutants more difficult [18]. The exchange rate for fresh air is now 10 times lower than it was 30 years ago, with a consequent increase in humidity, indoor pollutants levels, and airborne allergens [19]. Building standard developments could have a positive or negative role regarding the patterns of indoor air quality [20]. According to [18], the built environment is responsible for low air quality due to inadequate ventilation, air recirculation, material specification, and the additional pollution load from mechanical heating and cooling. Despite being addressed in several building certification schemes, these are considered inadequate, considering their importance to indoor air quality [21].

Biological parameters associated with building materials and characteristics are relevant for circular and regenerative built environments, indicating a link between building materials-energy-IAQ-health, that, if not properly managed, could negate the efforts of meeting climate and related sustainable development goals. Energy-intensive processes are required to produce new building materials for energy-efficient housing, which could exacerbate the exposure to indoor pollutants. Decreasing the energy consumption in a household may exacerbate the consumption of synthetic materials and increase the energy consumption associated with these materials' manufacturing process and recycling. The supposedly virtuous shift toward more energy-efficient housing, if not properly managed, may result in the consumption of more materials and energy consumption that might exacerbate or trigger new health risks for the most vulnerable. According to [22], bio-inspired materials were developed primarily to increase energy performance and reduce the energy consumption spent on the whole material production process and recycling systems.

The complexity of indoor air pollution sources that have different proveniences requires different mitigation strategies, including both top-down and bottom-up strategies. Top-down strategies include building codes and regulations that consider healthy and low-energy intensive building materials toward inclusive well-being regenerative built environment that is not focused on a narrow economy efficiency perspective. Bottom-up strategies should be solution-tailored and focused on the most effective solution for decreasing the concentrations of interior pollutants. Holistic approaches and strategies are necessary to mitigate indoor pollutants. A previous study [23], identified the strategies to counter the presence of fungal spores and organic pollutants with an aim to minimize the three factors, namely, design, occupational, and policy factors that support the phenomenon. In addition [24] identified behavioral changes in lifestyle to reduce energy consumption in transportation, households, and supply as a mitigation possible strategy. Despite building materials and characteristics to indoor air importance, several studies have been conducted without establishing associations between microbial exposure and building materials and characteristics [25].

This study aimed to evaluate if the microbial concentration was associated with the same indoor characteristics and building materials in places where children spent significant time indoors. According to Fig. 1, several research questions (RQ) were considered to answer the hypothesis.

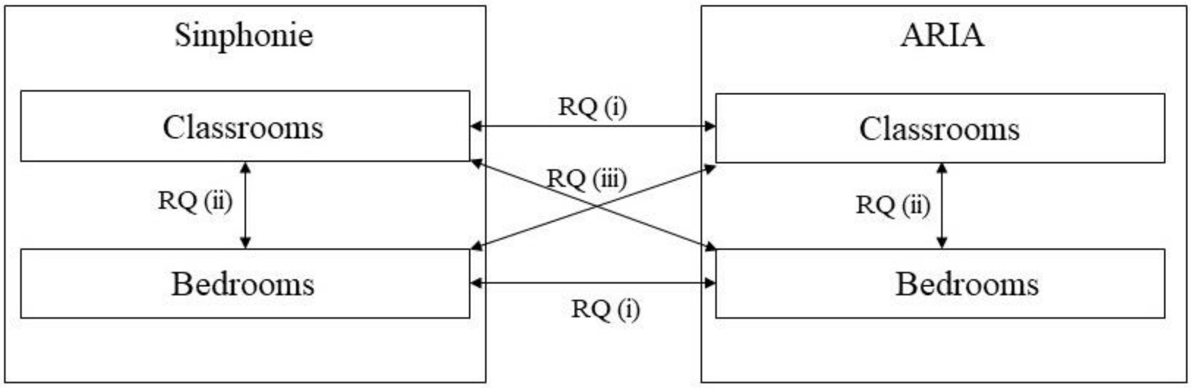

Figure 1 Research study design.

This research contributes to knowledge by tackling the following questions:

(i) Comparing the two projects, is there any common association of certain fungal species with building characteristics, considering primary schools' classrooms and bedrooms?

(ii) In the same project, are fungal species associated with specific building characteristics in classrooms and bedrooms?

(iii) The previous question (fungal species associated with specific building characteristics in classrooms and bedrooms) between the two projects.

The results from this research will contribute to the literature to better understand the association between building materials and indoor exposure to pollutants. Moreover, the study will contribute to improving the debate on building indoor air quality policies, offering useful indications to policymakers.

This paper is organized as follows: after this introduction, Section II presents the walkthrough inspection methodology that comprises the description of classrooms and bedrooms and the microbial measurements procedures. Section III demonstrates the results of the survey performed. Section IV presents the discussion. Finally, Section V presents the conclusions.

2. Methods

The present study compared two projects performed in different periods but in the same environments and geographic locations. Different building characteristics in places where children spend a significant part of their time, were compared, and the exposure to pollution sources was assessed. Although both projects investigated indoor environments of children, such as classrooms and bedrooms, the two projects were considered separately. Thus, they were conducted in different periods by applying different measuring methods. Both projects were conducted in the same urban environment that was representative of the scholar and housing building stock. The building material characteristic collection was similar in the two projects, allowing the comparison.

2.1 Walkthrough Inspection and Checklist

The present study focused on the physical characterization of buildings and the quantitative measurements of bacteria and fungi in primary schools' classrooms and children's bedrooms. Criteria for the selection of schools included representative buildings for the typology, construction technique, and building age. The schools and classroom selection criteria have been described previously [26]. The study design of both the Sinphonie and ARIA projects involved a health component. After children's health was assessed, they were invited to voluntarily participate and be enrolled in further studies as part of the environmental studies. This was performed in the children's residence and involved the characterization of the children sleeping local in detail but also of other dwelling locals such as kitchen. The building characteristics were collected through an on-site walkthrough inspection survey that was completed for each classroom and bedroom. The walkthrough inspection and the filling of a validated checklistwere performed by a trained researcher [26].

In the Sinphonie project, 20 schools and 68 home bedrooms were inspected and monitored from 2011 to 2013. In the ARIA project, 20 classrooms and 76 bedrooms were inspected and monitored between January 2014 and July 2015. Both studies were conducted in the Porto metropolitan area, the second largest in Portugal, located in the north of the country at the seashore (41°N, 8°W), characterized by a mild oceanic climate with moderate temperatures and rainy weather during the winter season. The walkthrough survey and the air sampling in each home occurred within the same period for both projects. Table S1 and Table S2, respectively, summarize the building physical characteristics for classrooms and bedrooms.

2.2 Bacterial and Fungal Assessment

The microbial analysis methodology adopted for the Sinphonie project has been described previously [24, 25]. In the ARIA project, the bacterial and fungal air samples were obtained using a single-stage microbiological air impactor (Merck Air Sampler MAS-100), following the National Institute for Occupational Safety and Health (NIOSH) Method 0800:1998-Bioaerosol Sampling [27] and EN 13098:2000 [28]. The equipment was calibrated annually in an external and accredited calibration laboratory. Tryptic soy agar (TSA) (supplemented with 0.25% cycloheximide) and malt extract agar (MEA) (supplemented with 1% chloramphenicol) were used as culture media for bacteria and fungi, respectively. The air was drawn through the sampler at a rate of 100 L per minute (L/min), and sequential duplicate air samples of 250 L were collected both indoors and outdoors. The used sampling volume (and consequently the duration) was the same within all bedrooms. For each sampling day, four field blanks, two sterility blanks, one positive control, and one negative control per culture medium were used. The air sampler was cleaned between each sample collection with cotton wipes wetted with isopropyl alcohol. After sampling, the agar media plates were sealed, marked, and transported to the laboratory in a thermal bag for processing.

To quantify bacterial and fungal concentrations, samples were incubated at 37 ±1 °C for 48 ±3 h and at 25 ±3 °C for 72 ±3 h, respectively [28]. Quantification was performed by counting with naked eye according to the methodologies described in ISO 4833:2013 [29] and EN 13098:2000 [28]. The number of colonies recovered on each plate was adjusted using a positive-hole correction factor, based on the Fellers law, which considers the probability that more than one particle containing a cultivable microorganism passes through the same hole [30]. Results are expressed as the number of colony-forming units per cubic meter of air (CFU/m^3), and the quantification limit was established as 10 CFUs per plate. Fungal colonies were identified either on the original sampling media-MEA plates or after subculturing procedures whenever colony isolation and growth observation were required. Subculture was made on MEA plates and incubated at 25 ±3 °C for 3 days to 3 weeks.

Fungal colonies were identified using phenotypic characteristics and following standard mycological procedures based on their micro and macro-morphological characteristics [31].

In both projects, the microbial analysis was performed by the Environmental Health Department of the National Health Institute.

2.3 Statistical Analysis

All the statistical analyses were performed using the SPSS statistical package software v20.0 (IBM, USA), version 18.0, and the level of statistical significance was set at 0.05. Shapiro–Wilk test was used to test the normality. The Mann–Whitney test was used to compare continuous variables, and *the Chi-square* test was used to compare categorical variables. Spearman's test of correlations was used to investigate the correlations between continuous variables.

3. Results

3.1 Classrooms Characteristics Association with Fungal and Bacterial Concentrations

3.1.1 Bacterial and Fungal Species Characterization

In primary schools' classrooms analyzed in the Sinphonie project, the concentrations of bacteria and fungi were, respectively, 3468 (2351) and 471 (1182) CFU/m^3. In the Sinphonie project, bacterial concentrations in classrooms were statistically associated with the main floor covering material (p = 0.014) and with the heating type (p = 0.023). No statistically relevant associations were observed between fungal concentrations and classrooms.

In the ARIA project, the concentrations of bacteria and fungi were, respectively, 2444 (1501) and 637 (526) CFU/m^3. Statistical relevant associations were observed between bacterial concentrations and noticeable mold odor (p = 0.050). Fungal concentrations were statistically associated with the type of glazing (p = 0.010).

Nineteen fungal species were identified in the classrooms from the Sinphonie project, whereas 23 fungi species were identified in ARIA classrooms. *Chrysonilia* and *Rhizomucor* fungal species were not found in the investigated classrooms for both studies. The primary fungal species identified in the investigated classrooms for Sinphonie and ARIA projects are summarized in Table S3 (Supplementary File).

3.1.2 Sinphonie Project Classrooms Associations with Fungal and Bacterial Concentrations

The associations between classroom characteristics and fungal and bacterial concentrations in the 70 inspected classrooms in the Sinphonie project are summarized in Table S4 (Supplementary File).

Eleven fungi species showed a statistically positive association with building characteristics in the studied classrooms of the Sinphonie project; these included *Alternaria* sp., *Aspergillus* sp., *Aspergillus fumigatus, A. niger, Cladosporium* sp., *Fusarium* sp., *Geotrichum* sp., yeast, *Micelio* sp., *Phoma* sp., and *Aureobasidium pullulans* fungal spores.

In classrooms, *Geotrichum* sp. was the fungal species that showed more positive associations with building characteristics in the Sinphonie project. Significant associations were found for *Geotrichum* sp. fungal spores with the type of glazing (p = 0.041), floor covering material (p = 0.004), ceiling covering materials (*p* = 0.008), and heating system (*p* = 0.049*). Micelio* (*p* = 0.001)*, Cladosporium* sp. (*p* = 0.016), and *A. fumigatus* (*p* = 0.011) fungal spores were positively associated with the type of glazing. *Micelio* was also associated with the heating system *(p* = 0.006), and *A. fumigatus* showed a positive relationship with the main floor covering material as well. Positive statistical associations were found with the heating system for yeast (*p* = 0.028), *A. fumigatus* (*p* = 0.043), *Phoma* sp., and *Fusarium* sp.*,* both with a significant level (*p* = 0.000). *Alternaria* sp. (*p* = 0.000)*, A. niger* (*p* = 0.052), and *Aspergillus* sp. (*p* = 0.019)*,* were positively associated with ceiling material characteristics. *A. pullulans* was associated with visible mold growth (*p* = 0.026).

In the Sinphonie project, the predominant building characteristic that was positively associated with fungal species in primary school classrooms was the heating system, with six statistically relevant associations (Table S4).

3.1.3 ARIA Project Classrooms Associations with Fungal and Bacterial Concentrations

The associations between the 67 inspected ARIA project classroom characteristics and fungal and bacterial concentrations are summarized in Table S5 (Supplementary File). In the ARIA project, the predominantly building characteristics that were positively associated with fungal species in primary school classrooms were the type of glazing, floor covering material, and the tendency for the formation of condensation, each with two statistically relevant associations with classroom building characteristics (Table S5).

In the ARIA project, relevant statistical associations were observed between building characteristics in classrooms and fungal species, namely for *A. niger, A. fumigatus, Cladosporium* sp.*, Chaetomium* sp.*,* and *A. pullulans* were found.

Both *A. niger* and *A. fumigatus* were positively associated with building characteristics in the ARIA project, with three statistically positive associations. Significant associations were found for *A. niger* with visible mold growth (*p* = 0.020), visible damp spots on walls, ceiling or floor (*p* = 0.020), and noticeable mold odor (*p* = 0.024). *A. fumigatus* was associated with the type of glazing *(p* = 0.010), standard main floor covering material (*p* = 0.013), and the tendency for the formation of condensation (*p* = 0.010). Other fungal species, such as *Cladosporium* sp. spores were associated with the ceiling surface material (*p* = 0.002) and the tendency for the formation of condensation (*p* = 0.005). *Chaetomium* sp. was associated with floor covering material *(p* = 0.028), and *A. pullulans* was associated with the type of glazing (*p* = 0.031).

3.1.4 Sinphonie *versus* ARIA Classroom Characteristics

Table 1 summarizes the positive associations between primary school classroom characteristics and fungal concentrations in the Sinphonie and ARIA projects.

Table 1 Classroom statistically relevant associations in Sinphonie and ARIA projects

	Sinphonie	ARIA
Type of glazing		
	Aspergillus fumigatus (*p* = 0.011)	*A. fumigatus* (*p* = 0.010)
	Micelio (*p* = 0.001)	*Aureobasidium pullulans* (*p* = 0.031)
	Cladosporium sp. (*p* = 0.016)	

	Geotrichum sp. ($p = 0.041$)	
Floor covering material		
	A. fumigatus ($p = 0.017$)	*A. fumigatus* ($p = 0.013$)
	Geotrichum sp. ($p = 0.004$)	*Chaetomium* sp. ($p = 0.028$)
Ceiling covering materials		
	Geotrichum sp. ($p = 0.008$)	*Cladosporium* sp. ($p = 0.002$)
	Alternaria sp. ($p = 0.000$)	
	Aspergillus sp. ($p = 0.019$)	
Heating system type		
	Fusarium sp. ($p = 0.000$) *Geotrichum* sp. ($p = 0.049$) *Micelio* ($p = 0.006$) Yeast ($p = 0.028$) *A. fumigatus* ($p = 0.043$) *Phoma* sp. ($p = 0.000$)	-
Visible mold growth		
	Aureobasidium pullulans ($p = 0.026$)	*A. niger* ($p = 0.020$)
Visible damp spots on walls, ceiling, or floor		
	-	*A. niger* ($p = 0.020$)
Noticeable mold odor		
	-	*A. niger* ($p = 0.024$)
Tendency for the formation of condensation		
	-	*A. fumigatus* ($p = 0.010$) *Cladosporium* sp. ($p = 0.005$)

A comparison of the results of schools' indoor environments for Sinphonie and ARIA projects revealed that two building characteristics, namely, glazing type and floor covering, were associated with *A. fumigatus*. Building characteristics, such as type of glazing, floor covering material, ceiling covering materials, and visible mold growth, were positively associated with classrooms for both projects.

3.2 Bedrooms Characteristics Association with Fungal and Bacterial Concentrations

3.2.1 Bacterial and Fungal Species Characterization

In the Sinphonie project, bacterial and fungal concentrations in bedrooms were, respectively, 1477 (1879) and 869 (1607) CFU/m^3. Despite no statistically relevant associations between bacteria concentrations and bedroom building characteristics were found, fungal concentrations were statistically associated with the presence of curtains ($p = 0.011$) in the Sinphonie project bedrooms.

Bedroom concentrations of bacteria and fungi in the ARIA project were, respectively, 1136 (2291) and 597 (526) CFU/m^3. Statistical relevant associations were found between bacterial concentrations and visible damp spots on walls, ceilings, or floors in bedrooms ($p = 0.034$). Moreover, fungal concentrations were statistically linked with several bedroom building characteristics, namely, building type (p = 0.052), standard main ceiling surface material (p = 0.043), standard main floor covering material (p = 0.047), and visible mold growth in the room (p = 0.041).

A comparison of detected indoor fungal species in bedrooms of Sinphonie and ARIA projects identified 23 fungal species in indoor environments of bedrooms in the Sinphonie project and 24 fungal species in the bedrooms of the ARIA project. Certain fungal spores, such as *Chrysonilia* sp. and *Rhizomucor* sp., were not found in any of the studied bedrooms in both projects. *Chrysosporium* sp., *Chrysonilia* sp., *Rhizomucor* sp., and *Scedosporium* were not detected in the bedrooms of Sinphonie project. *Beauveria* sp., *Cladosporium* sp., *Chrysonilia* sp., *Paecilomyces* sp., *and Rhizomucor* sp. were not detected in the bedrooms of the ARIA project. The fungal species identified in the studied bedrooms of both projects are summarized in Table S6 (Supplementary File). Fungal species that were cumulatively detected in 90% of indoor environments in both studies were considered in the final analysis. Using this criterion, fungal species that were detected in no more than 10% of the houses were considered non-relevant.

3.2.2 Sinphonie Project Bedroom Associations with Fungal and Bacterial Concentrations

The associations between bedroom characteristics and fungal and bacterial concentrations in 68 bedrooms inspected in the Sinphonie project are summarized in Table S7 (Supplementary File).

A total of 13 fungal species were considered in the Sinphonie project bedroom analysis, out of which 8 fungal species, namely, *Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Chaetomium* sp., *Fusarium* sp., *Geotrichum* sp., and *Phoma* sp. fungal spores were found to have statistically significant associations with building characteristics. *Fusarium* sp. had the most relevant number of associations with building characteristics in the Sinphonie project. Significant associations of *Fusarium* sp. were found with the presence of animals indoors (p = 0.020), rug presence (p = 0.004), heating system (p = 0.047), tendency for condensation (p = 0.001), and curtains (p = 0.014). *Chaetomium* sp. was found to have the second most relevant number of associations with building characteristics, namely, with the floor covering material (p = 0.000), visible mold growth (p = 0.046), and noticeable mold odor (p = 0.000). Other significant associations were found between bedroom characteristics and fungal species in the Sinphonie project. *Acremonium* sp. displayed a significant association with the type of glazing (p = 0.031), *Aspergillus* sp. had a significant association with window frame material (p = 0.036), *Phoma* sp. exhibited a significant association with building location (p = 0.000), *Cladosporium* sp. was significantly associated with the presence of animals indoors (p = 0.035), *Geotrichum* sp. was found to have a significant association with the presence of rug in the bedroom (p = 0.029), and *Alternaria* sp. was significantly associated with visible mold growth in the bedroom (p = 0.024). In the Sinphonie project, the predominantly building characteristics associated with fungal species in bedrooms were rug presence, animals' presence indoors, and visible mold growth.

3.2.3 ARIA Project Bedroom Associations with Fungal and Bacterial Concentrations

The associations between bedroom characteristics and fungal and bacterial concentrations in the 76 inspected bedrooms in the ARIA project have been described previously [32]. A total of 13 fungal species were considered in the ARIA project bedroom analysis, out of which 9 fungal species, namely, *Geotrichum* sp., *Cladosporium* sp., *Acremonium* sp., *Fusarium* sp., Yeast, *Alternaria* sp., *Chaetomium* sp. *and Rhodotorula* sp.

According the same study's results [32], *Geotrichum* sp. displayed the most relevant number of associations with building characteristics in the ARIA project, namely, five relevant associations with bedroom building characteristics. In particular, *Geotrichum* sp. displayed significant associations with the building type (p = 0.007), ceiling surface material (p = 0.028), visible mold growth (p = 0.021) with visible damp spots on walls, ceiling or floor (p = 0.014), and the tendency for the formation of condensation on windows (p = 0.005). *Cladosporium* sp. showed the second most relevant number of associations with bedroom characteristics. *Cladosporium* sp. was significantly associated with window frame material (p = 0.013), solar shading devices (p = 0.028), and floor covering material (p = 0.049). *Acremonium* sp. was found to have statistically relevant associations with noticeable mold odor (p = 0.030) and the tendency t for the formation of condensation on windows (p = 0.013). *Fusarium* sp. was found to have statistically relevant associations with visible mold growth (p = 0.013) and with visible damp spots on walls and ceiling or floor (p = 0.016). *Yeast* displayed statistically relevant associations with window frame material (p = 0.021) and solar shading devices (p = 0.007). *Alternaria* sp.*, Chaetomium* sp., *and Rhodotorula* sp. showed one statistically relevant association with bedroom characteristics. *Alternaria* sp. had statistically relevant associations with solar shading devices (p = 0.019)*, Chaetomium* sp. showed statistically relevant associations with floor covering material (p = 0.047), and *Rhodotorula* sp. exhibited statistically relevant associations with the tendency for the formation of condensation on windows (p = 0.040). In the ARIA project, the predominantly building characteristics associated with fungal species in bedrooms were solar shading devices and the tendency for the formation of condensation.

3.2.4 Sinphonie *versus* ARIA Bedroom Characteristics

Table 2 summarizes the positive associations between bedroom characteristics and fungal concentrations in Sinphonie and ARIA projects.

Table 2 Statistically relevant associations of bedrooms in Sinphonie and ARIA projects

	Sinphonie	**ARIA**
Type of glazing		
	Acremonium sp. (p = 0.031)	-
Floor covering material		
	Chaetomium sp. (p = 0.000)	*Chaetomium* sp. (p = 0.047) *Cladosporium* sp. (p = 0.049)
Ceiling surface material		
	-	*Geotrichum* sp. (p = 0.028)
Heating system type		
	Fusarium sp. (p = 0.047)	-
Visible mold growth		
	Chaetomium sp. (p = 0.046) *Alternaria* sp. (p = 0.024)	*Geotrichum* sp. (p = 0.021) *Fusarium* sp. (p = 0.013)
Noticeable mold odor		
	Chaetomium sp. (p = 0.000)	*Acremonium* sp. (p = 0.030)

Tendency for condensation		
	Fusarium sp. (p = 0.001) -	*Geotrichum* sp. (p = 0.005) *Acremonium* sp. (p = 0.013) *Rhodotorula* sp. (p = 0.040)
Animal's presence indoors		
	Fusarium sp. (p = 0.020) *Cladosporium* sp.(p = 0.035)	-
Rug presence		
	Fusarium sp. (p = 0.004) *Geotrichum* sp. (p = 0.029)	-
Presence or curtains		
	Fusarium sp. (p = 0.014)	-
Window frame material		
	Aspergillus sp. (p = 0.036)	*Cladosporium* sp. (p = 0.013) Yeast (p = 0.021)
Building location		
	Phoma sp. (p = 0.000)	*Geotrichum* sp. (p = 0.007)
Solar shading devices		
	-	*Cladosporium* sp. (p = 0.028) *Alternaria* sp. (p = 0.019) Yeast (p = 0.007)

A comparison of bedroom indoor environments for Sinphonie and ARIA projects revealed a common positive building characteristic association with fungal species and the floor covering material with *Chaetomium* sp. Building characteristics such as floor covering material, visible mold growth, noticeable mold, the tendency for condensation, window frame material, and building location showed a positive association in both projects' bedrooms.

4. Discussion

Children spend a significant part of their time in indoor environments such as classrooms and bedrooms. This study compared the building materials and characteristics associated with fungi in classrooms and bedrooms for both projects to evaluate if there was exposure to the same pollution sources. Bacterial concentration was higher in classrooms than in bedrooms. However, an opposite phenomenon was observed for fungal concentrations. Both environments are important to children as they spend a considerable part of their time both at home and school, and these are privileged locations for being exposed [33].

A comparison of fungal species at schools in Sinphonie and ARIA projects revealed a different number of species in the indoor air. Despite the schools being the same under both projects, airborne fungal sampling varied highly with time. At primary schools, 19 species were identified in the indoor air in the Sinphonie project, and 23 fungal species were identified in the ARIA project. A comparison of results at schools identified different fungal species, which was expected because the results reflected the meteorological outdoor conditions, local air quality, and different use and behaviors of indoor environments. These results are in accordance with a study performed in 105 Denmark houses [34], where air measurements reflected short-term variations in occupant's behavior, whereas measurements of building material reflected building construction and longer-term occupant habits.

Results demonstrated positive associations between classroom building characteristics and fungal species both in the Sinphonie and ARIA projects. In the Sinphonie project, fungal species namely, *Alternaria* sp., *Aspergillus* sp., *A. fumigatus, A. niger, Cladosporium* sp., *Fusarium* sp., *Geotrichum* sp., yeast, *Micelio* sp., *Phoma* sp., and *A. pullulans* fungal spores showed statistically positive associations with building characteristics. *Geotrichum* sp. displayed more positive associations with building characteristics in the Sinphonie project. In the ARIA project, statistically relevant associations between building characteristics in classrooms and fungal species were found; namely, *A. niger, A. fumigatus, Cladosporium* sp., *Chaetomium* sp., and *A. pullulans*. *A. niger* and *A. fumigatus* showed positive associations with building characteristics in classrooms in the ARIA project. A study conducted in 48 schools in the United States [35] identified the following genera as the most common type to form colonies: *Clasdosporium, Penicilium, Chrysosporium, Alternaria,* and *Aspergillus.* Apart from *Chrysosporium*, all genera were found in primary schools' classrooms in Porto research. Moreover, positive associations with building characteristics were found for certain fungal species at primary schools' classrooms. In another study performed in kindergartens [36], the major bacterial species detected in indoor and outdoor air were *Bacillus* sp., *Staphylococcus aureus, Micrococcus* sp., *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Enterococcus* sp., and *Streptococcus* sp. Differences among fungal species were expected; thus, the results obtained through air sampling reflected short-term activities, occupation, and exposure. Moreover, different methods to measure the concentration of indoor culturable fungi employed in various studies may give varying outcomes and create distortions about the biological diversity associated to buildings characteristics [37].

A comparison of the results of schools' indoor environments in Sinphonie and ARIA projects showed that two building characteristics namely glazing type and floor covering, were associated with *A. fumigatus*. A 10-year follow-up study [38] reported that PVC flooring was associated with the incidence of asthma. This is an indicator that exposure to synthetic flooring in most of the inspected classrooms could potentiate the development of specific microbial flora and increase the risk of asthma incidence. This study results also demonstrated a positive association of floor covering material with *Chaetomium* fungal spores. As previously referred synthetic materials are manufactured by energy-intensive processes. Since the first oil crisis, there have been increasing concerns about the efficiency of building stock and new materials have been developed and integrated into buildings. Despite certain materials being tested in the laboratory, the conditions are not frequently representative of reality. Although the introduction of building material labeling may help to identify health hazards materials, this is not a straightforward subject. Labeling does not reflect the degradation of building materials, i.e., dampness conditions. Synthetic floor covering is a building material that is widely used in buildings, regardless of their use. The literature mentions mitigation as a possible solution, although whenever possible, hazardous materials should be substituted, particularly in indoor environments attended by vulnerable groups such as children. Substitute materials should be produced through low-energy consumption and healthier processes and preferentially able to be reutilized instead of recycled.

In addition to the floor covering material, other building characteristics, such as type of glazing, ceiling covering materials, and visible mold growth, were positively associated with bacterial concentration in both projects' classrooms. Certain materials are applied in more extensive indoor areas, such as covering materials, which may represent a major source of indoor emission. The selection of materials, type of ventilation, and plumbing have been recognized previously [39] to have important implications on the microbiology of a building, and consequently, on the health of the building's occupants. Experimentation on real local conditions should preferably be performed to evaluate tailored solutions for indoor environments attended by vulnerable groups to reduce their exposure risk. Solution-tailored building materials, concerns to specific solution development, should take into consideration specific characteristics of indoor environments, such as the area, number of windows, ventilation type, orientation, number of occupants, hygrothermal conditions, and other possible variables.

In Sinphonie bedrooms, several fungal species, namely, *Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Chaetomium* sp., *Fusarium* sp., *Geotrichum* sp., and *Phoma* sp. spores were statistically and significantly associated with building characteristics. *Fusarium* sp. had the most relevant number of associations with building characteristics in the Sinphonie project. In the ARIA project, 8 fungal species namely *Geotrichum* sp., *Cladosporium* sp., *Acremonium* sp., *Fusarium* sp., yeast, *Alternaria* sp., *Chaetomium* sp., *and Rhodotorul*a sp. showed statistically significant associations with building characteristics. *Geotrichum* sp. had the most relevant number of associations with building characteristics in the ARIA project. Several other studies identified some of these species. In a study performed in dormitory rooms in Ethiopia [13], the most common identified fungal genera were *Aspergillus, Penicillium, Alternaria, Fusarium, Candida, Trichophyton, Piedraia, Microsporum, Geotrichum, Saccharomyces, Rhodotorula, Rhizopus, Exophiala, Arthroderma, Cladosporium, Gliocladium*, and *Botrytis*. In temporary houses in Japan, *Aspergillus* sp., *Cladosporium* sp., and *Penicillium* sp. were dominant [40]. Similarly, in energy-efficient dwellings, *Aspergillus* was found to be a frequent surface contaminant in bedrooms with natural ventilation [41].

A comparison of the fungal species identified in bedrooms in Sinphonie and ARIA projects indicated certain differences between bacterial and fungal concentrations in the studied environments. A previous study [25] provided similar conclusions when comparing the concentrations in different indoor environments. A possible explanation is given in Table 2. In the ARIA project, more associations between dampness and fungal species were found than in the Sinphonie project. In addition, differences existed in certain building characteristics, such as animals' presence indoors or rug and curtain presence between the two projects, which may help explain the obtained results. These built environment differences increase the need for tailored solutions even when considering mitigation strategies.

Several studies have associated dampness and microbial pollution indoors [42-45]. This study also identified positive and statistically significant associations between dampness indoors, such as visible mold growth, tendency to form condensation, visible damp spots, and bubbles or dark floor, and certain fungal species. Despite the presence of no consistency when crossing the results in indoor environments between Sinphonie and ARIA projects, positive associations were found between dampness indoors and fungal species in both projects. This was particularly true in indoor environments in the ARIA project for both classrooms and bedrooms. The prevalence of dampness indicators in the ARIA project children's bedrooms was investigated by [3], that concluded that visible mold growth, a particular dampness indicator, was associated with higher CO_2 and relative humidity values and occupation density to the mold extent. Similarly, [46] determined that factors such as temperature, air exchange rate, dwelling characteristics, and relative humidity (RH) may affect fungal concentrations indoors. Fungal concentrations indoors were closely associated with air humidity and with higher RH levels in the study performed by [47], thus high RH levels allowed fungal growth in the environment [48].

Despite the differences found, a comparison of the results of bedroom indoor environments of Sinphonie and ARIA projects revealed the association of floor covering material with *Chaetomium* sp. With respect to the first research question, although glazing type was positively associated with *A. fumigatus* in classrooms, floor covering was identified as a common building characteristic both for classrooms and bedrooms in the two projects.

When discussing the second research question, the heating system was associated with *Fusarium* sp. both in classrooms and bedrooms in the Sinphonie project. Similarly, in the ARIA project, the association between the floor covering material and *Chaetomium* sp. was common both in classrooms and bedrooms. With respect to the third research question, ceiling covering materials were found to be associated with *Geotrichum* sp. both in Sinphonie classrooms and ARIA bedrooms and floor covering material was associated with *Chaetomium* sp.. Figure 2 is an evolution of Figure 1 and demonstrates the positive associations between building characteristics and fungal species, considering the three research questions.

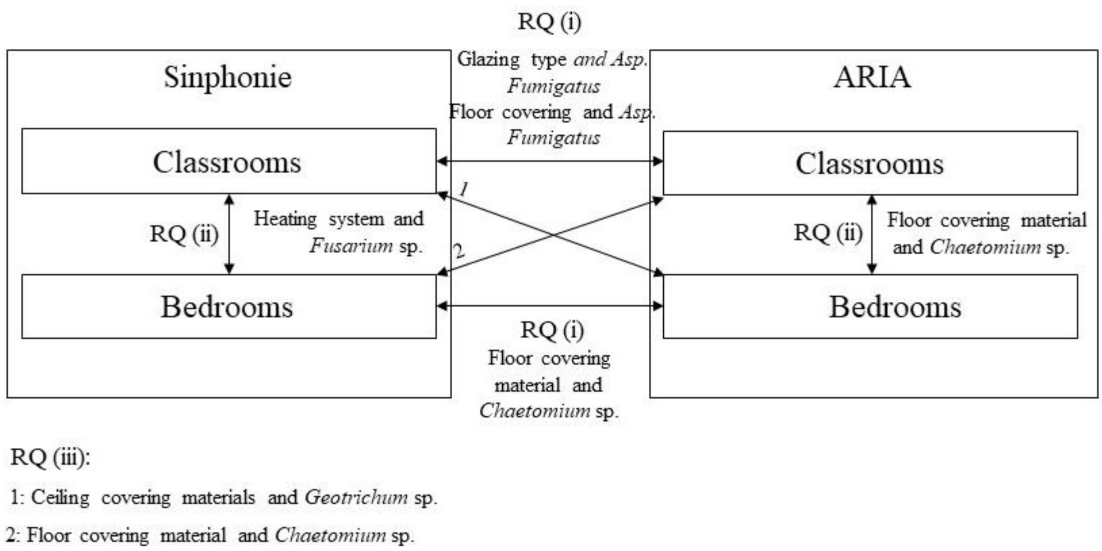

Figure 2 Study design resume conclusions

Numerous other studies also reported positive associations between fungal propagules of certain fungal species and building characteristics [22, 48-52]. The results the research performed by [46] determined that the volume of the room, relative humidity, and floor levels play an important role in fungal concentrations. Similarly, in another research performed by [53], indoor RH and indoor temperature exerted a positive relationship with specific spore genera as well as other dwelling characteristics such as the type of cleaning of the furniture, the presence of trees in front of the bedroom, the presence of air conditioning, and natural ventilation. A previous study on dwellings revealed positive associations between electrical heating and fungi [54]. These results are according to the present study results, particularly in the Sinphonie project, in which positive correlations were found for a heating system with different fungal species, both in classrooms and bedrooms. A study performed by [55] also observed microbial communities in the indoor air to be linked to the heating system, cleaning frequency, and pets in the case of bacterial contamination. A link was identified between ventilation type, heating system, house type, rugs, cleaning frequency and fungal contamination.

The results indicated that fungal species could be positively associated with the following major building characteristics: building materials, indoor dampness, heating systems, ventilation systems, and occupancy. Among all associations found with building characteristics, some were expected, such as floor covering material, heating system, and indoor dampness indicators, and others, such as the type of glazing, were not predictable despite possibly associated with ventilation and occupancy. In this study, certain building characteristic patterns were found both in schools and bedrooms in the two projects, indicating the importance of building materials as pollutant sources. Numerous studies have reported an association between indoor polluted environments to certain building and housing characteristics. A study performed by [56] concluded that it was not reliable to predict fungal propagules from home characteristics. However, most the studies reported an association between fungal species and home characteristics. In this study, positive associations were found concerning all research questions. In particular, floor covering material was associated with *Chaetomium* sp., considering all research questions. On the one hand, certain building associations found in this study were expected according to the literature; on the other hand, it was not expected that a specific building characteristic was common to all three questions; thus, different projects and indoor characteristics environments were considered. According to [57], *Chaetomium* sp. preferred wood-based materials over gypsum, both in terms of growth rate and metabolite production. According to Table S1, in most of the classrooms, the floor type was synthetic smooth (PVC/vinyl, linoleum), although wood or laminate parquetry was the second most observed floor type. In opposition to classrooms, in both projects, the type of floor in bedrooms was predominantly wood (Table S2). The study performed by [58] associated *Chaetomium sp.* with dampness in building materials. Despite dampness not being visible on the floor, it was concluded that wood-based floor materials and moisture could result in certain differences in bacterial and fungal concentrations in the studied environments.

5. Limitations of the Study

The present work had certain limitations. Different outdoor conditions, meteorologically as well as related to the ambient air quality, could have exerted different impacts on indoor environments. Despite the influence of the outdoor environment on the indoor environment, the composition of outdoor fungal species was not considered in this work. Thus, it aimed to assess if strong associations between building characteristics and indoor fungi occurred and if these were the same considering different building materials, indoor environments, and projects. Fungal samples were collected during winter for both studies; therefore, any influence associated with seasonal sampling should be explored.

In both studies, the sampling was conducted through air sampling, which is believed to be adequate and representative of the exposure; thus, the effects of biological exposure are primarily respiratory. The limited sampling period and the adopted methodology could impact the results.

Moreover, different methodologies and sampling periods could cause different results. The technicians who performed the walkthrough audits were not the same, and different interpretations of building characteristics could emerge.

6. Conclusion and Future Work

Floor covering material was more frequently associated with fungal species. Significant associations were found for floor covering material, heating system, and type of glazing building characteristics and *Chaetomium* sp., *Fusarium* sp., and *Fumigatus* sp. both in bedrooms and classrooms. A comparison of classroom indoor environments between Sinphonie and ARIA projects revealed significant associations between the type of glazing and the floor covering material and *Fumigatus* sp. Furthermore, a comparison of bedrooms between the two projects showed significant associations for floor covering material and *Chaetomium* sp.

When comparing the reality of classrooms and bedrooms for Sinphonie project, *Fusarium* sp. and heating system displayed associations. When the same comparison was performed for the ARIA project, significant associations existed between *Chaetomium* sp. and floor covering materials, demonstrating that in both environments, children were exposed to the same pollution sources.

The same fungal associations with building materials in different spaces and projects demonstrated that building materials represented a source of exposure. According to the study results, this is particularly relevant with respect to the floor covering material building associations and *Chaetomium* sp. Although building materials are not considered to be the most relevant indoor exposure source, when considering the nexus between building materials-energy-IAQ-health, microbiology exposure associated with building materials is found to be relevant.

Further work should assess the impact of introducing bio-materials into new buildings and building retrofitting on indoor air quality. In addition, the nexus between new building materials-energy-health should be explored to assess if both manufacturing processes are energy intensive and building materials are healthy for vulnerable groups. In cases where emitting building materials can be replaced, the potential reuse of these materials and their integration in processes without health hazards should be assessed.

Acknowledgements

The scientific data worked on this paper was provided by INEGI (Institute of Science and Innovation in Mechanical Engineering and Industrial Management). The author thanks the cooperation from INEGI executive board and INEGI Energy Department.

Author Contributions

J. Sousa conceived the paper original idea, to overall planning and writing the manuscript. Performed the data statistical analysis, evaluation of the results and validation. The scientific data worked on this paper was provided by INEGI (Institute of Science and Innovation in Mechanical Engineering and Industrial Management).

Funding

ARIA project was supported by Fundação para a Ciência e a Tecnologia through the ARIA project. (PTDC/DTP-SAP/1522/2012). SINPHONIE project was funded by the European Parliament with cooperation from the Directorate-General for Health and Food Safety (DG SANTE) of European Union (SANCO/2009/C2/04, contract SI2.570742). SINPHONIE project funding was not applicable to the author.

Competing Interests

The author declare that have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Section "3.2.3 ARIA project bedrooms associations with fungi and bacterial concentration" results have been published on Journal of Building Engineering, 45, 103409. https://doi.org/10.1016/j.jobe.2021.103409. Permission from the copyright holder was obtained to publish the results of ARIA project bedrooms associations.

Data Availability Statement

The data used during the study were provided by a third party. Direct requests for these materials may be made to the provider as indicated in the Acknowledgements.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Table S1: Physical characteristics of classrooms in Sinphonie and ARIA projects.
2. Table S2: Physical characteristics of bedrooms in Sinphonie and ARIA projects.
3. Table S3: Fungi species in classrooms in Sinphonie and ARIA projects.
4. Table S4: Associations between classrooms characteristics and fungi and bacterial concentrations microbiological in Sinphonie project.
5. Table S5: Associations between classrooms characteristics and fungi and bacterial concentrations microbiological in ARIA project.
6. Table S6: Fungi species in Sinphonie and ARIA bedrooms.
7. Table S7: Associations between bedrooms characteristics and fungi and bacterial concentrations in Sinphonie.

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