



Research article

Fungal susceptibility score: an innovative criterion for risk assessment of indoor airborne fungi for people and items

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Abstract

Indoor air often contains spores and fungal propagules, which generally do not present significant risks to people or items. However, increasing humidity and temperature can boost fungal growth, suddenly changing the environmental scenario. The present work reports a mycological survey carried out in an attic apartment in Genoa (Liguria, Italy). We performed both direct surface examinations of visibly contaminated areas and air sampling using a Surface Air Sampler (SAS) in duplicate with two different culture media. The SAS air samples revealed a high concentration of CFU/m³ with the maximum concentration of 2085 CFU/m³. The qualitative analysis, initially performed through morphological identification and subsequently confirmed using molecular methods, identified a limited number of species with a high-risk factor for humans and/or materials. In this context, to make an overall risk assessment for people and items, we proposed a new criterion called Fungal Susceptibility Score (FSS) based on the relative abundance and functional traits of the isolated fungal genera. The results obtained underscore the importance of considering all taxa detected in the investigated area, not only their potential pathogenicity to humans. This work also introduces a criterion that correlates the ecological characteristics of genera present in a specific environment with their abundance, aiming to objectively assess the level of risk for both humans and objects. The FSS provides a reproducible metric to assess fungal risk in indoor environments.

Keywords

Bioaerosols, indoor air quality, mould, aeromycology, fungal barcoding

Introduction

Aerobiology is an interdisciplinary science that examines the sources, dispersion, and impact of biological particulate matter or bioaerosols (also referred to as organic dust) present in the atmosphere, including fungi, and their effects in both confined and open environments (Lancia et al.,



2021). The study of airborne fungi, known as aeromycology, focuses on the quantification and identification of airborne spores and fungal propagules, as well as their dispersion mechanisms (Kasprzyk, 2008) in both indoor and outdoor environments, and their implications for human health, plant diseases, and the preservation of artifacts and food (Cabral, 2010). Due to their small size, ranging from 1.3 to 250 μm , fungal spores become part of the particulate matter in bioaerosols (Kasprzyk, 2008). Given their cosmopolitan nature, extreme adaptability to adverse conditions, potential toxicity, and allergenicity to humans, and the limited knowledge about airborne fungal communities in various environments, it is crucial to perform characterization and, where possible, monitoring. When focusing on indoor fungal concentration, several factors must be considered: environmental parameters (humidity, temperature, etc.), ventilation systems (Bonetta et al., 2010), room furniture, and the presence of organic substances in building materials (Crawford et al., 2015). In recent decades, a strong correlation has been established between air quality and its effects on human health and the preservation of artifacts. It has been particularly highlighted that the microbiological component, especially fungi, has considerable effects (Kadaifciler, 2017). The literature indicates that the most abundant conidia in indoor environments belong to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*, all of which can potentially cause adverse allergic reactions in predisposed individuals and/or act as biodeteriogens (Simon-Nobbe et al., 2008). In addition to *Stachybotrys*, these genera are often associated with the Sick Building Syndrome (SBS): a set of symptoms related to prolonged occupancy in confined environments where airflow patterns and ventilation are crucial for the dispersion of organic particulate matter (Redlich et al., 1997; Wang et al., 2022).

Differentiating between a healthy and an unhealthy environment is not always straightforward, because fungi are naturally present in both outdoor and indoor air in virtually all settings, and there are no universally accepted guidelines defining typical or safe exposure levels (Dillon et al., 1996). Most available standards and guidelines for fungi in indoor air focus on clinically defined diseases and approaches to sampling, remediation, and preventive maintenance without establishing clear limits for fungal concentrations in the air (Rao et al., 1996). The only environments with specific limits for fungal load in the air are operating rooms, where the limit is set to zero colony-forming units (UNI EN 17141:2021 Cleanrooms and associated controlled environments - Biocontamination control). In Italy, the most updated guidelines for evaluating microbiological pollution, particularly fungal contamination, are those of Anzidei et al. (2010). While the positive correlation between high fungal concentrations in the air and the risk of respiratory diseases is well-known, no national or international regulations define the minimum tolerable concentration in indoor environments that can be considered healthy (Meklin et al., 2002; Pyrri et al., 2020). Based on the above, there is a substantial lack of qualitative and quantitative tools for assessing biological risks that can be easily and effectively used by technicians or operators responsible for evaluating potential threats.

This paper presents the results of aeromycological analyses conducted in an urban apartment showing clear and severe signs of biodeterioration due to a substantial fungal presence. Based on these data, a novel criterion has been proposed to assess risk in relation to both human health and materials susceptible to fungal biodeterioration. This represents the most innovative feature of the study, which aims to serve as a rapid unbiased assessment tool and metric to further standardize the evaluation of mycological risk in indoor environments. To test this new assessment approach in real condition, a case study was conducted in a residential building in Genoa (NW Italy).

Materials and Methods

Study area and air sampling

This study was carried out in an apartment located in the top floor of a building in Genoa (Northwest, Italy) in April 2024. As shown in Figure 2, the apartment is divided into 8 rooms and 2 outdoor spaces (a terrace and a stairwell) with a total surface area of approximately 90 m². All the apartment walls showed extensive fungal growth and visible moisture on walls, doors, and windows, indicating serious water infiltration from the roof (Fig. 1). To assess the contamination level of the area, we followed the methodology described in the scientific literature (Pyrri et al., 2020) and the guidelines provided by Italian legislation (Anzidei et al., 2010). In total, 10 air sampling points were established, based on the number and size of the rooms, with two additional points: one on the terrace and one in the stairwell.



Fig. 1 – Fungal contamination on various surfaces of the apartment: a. entrance ceiling, b. bedroom ceiling, c. living room wall, d. corridor niche, e. items in the pantry, f. bathroom wall, g. terrace, h. bathroom ceiling, i. kitchen ceiling, l. pantry, m. single bedroom ceiling, n. single bedroom window.

At each sampling point, four samples of 100 L of air (two on Malt Extract Agar + Chloramphenicol and two on Rose Bengal Agar) were collected using SAS Super 100 air sampler (VWR-PBI International), positioned 1.5 m above the floor, with a sampling flow rate of 1.5 L s⁻¹. In total, 40 plates were inoculated, corresponding to a sampled air volume of 4000 L. Additionally, 5

surface samples were collected using sterile swabs. The plates were incubated at 24 ± 1 °C and checked daily.

Fungal isolation and polyphasic identification

After 7 days of incubation at 24 ± 1 °C, fungal colonies were enumerated and categorized into Morphological Taxonomic Units (MTUs) according to their phenotypic features. Axenic cultures were preserved in glycerol at -80 °C in the collection of the Laboratory of Mycology, DISTAV, University of Genoa (CoID UNIGE JRU-MIRRI-IT). Genomic DNA was extracted from actively growing cultures using a modified CTAB protocol (Doyle and Doyle, 1987). To validate morphological analyses, DNA barcoding was carried out by amplification of the ITS region using primers V9G and LS266 (Samson et al., 2010). For species-level identification, additional barcodes were applied: beta-tubulin for *Penicillium* and calmodulin for *Aspergillus* (Samson et al., 2019). Amplicons were bidirectionally sequenced, assembled in Benchling, and compared with GenBank® sequences through BLAST. The sequences were deposited in the GenBank® database. The resulting data were used to estimate fungal concentrations (CFUs/m³) and to identify potentially hazardous species.

Screen for opportunistic Aspergilli

To screen for potential opportunistic strains of *Aspergillus*, one strain for each MTU isolated was inoculated on medium Malt Extract Agar (MEA) and incubated at 37 °C for one week, with daily observations. The incubation temperature was chosen to mimic the internal temperature of the human body. Figure 4 reports the *Aspergillus* strains that exhibited growth at 37 °C.

Data analysis

The results of the colony-forming unit counts were used to elaborate the Fungal Susceptibility Score (FSS). This criterion aims to assess the level of risk for fungal contamination based on the various targets present in the environment that fungi may colonize. In this case, the targets include paper (*pa*), wood (*w*), plaster on walls/concrete (*c*), and people (*pe*). The FSS is calculated considering the most frequent genera (over 50 CFU/m³) identified during the investigation.

The FSS_x is given by the following formula:

$$FSS_x = \sqrt{\frac{(FD_x \times CFUS_{gen_1}) + (FD_x \times CFUS_{gen_2}) + \dots + (FD_x \times CFUS_{gen_n})}{5000}}$$

The FSS_x value represents the susceptibility of the *x*-th substrate to the fungal community under consideration. The index weights the fungal dangerousness (FD) of each fungal taxa (in our case genus) toward the different substrates, based on information found in the literature and double-checked through an inter-rater reliability method.

The FD is multiplied by the relative abundance of each taxon, thus obtaining the FSS the different target substrates in the surveyed area. In this study, *gen_n* represents the *n*-th genus identified as growing on substrate *x*. We chose to work at genus level, but the same score can be elaborated at the species level (*sp_n*). FD_x is the relative weight (ranging from 1 to 5) expressing the potential biodeteriorative damage that can be caused by fungal taxa on the considered target substrate. To fix

ideas, if the genus *gen_l* is particularly harmful with respect to the substrate “*pe*” (humans), the corresponding FD_{pe} is assigned a high value (e.g. higher than 3).

The CFU_{gen} is the number of colony-forming units of the genera counted in the sample. These genera are selected for the index calculation because they target the reference substrate in the FSS calculation. The criteria used in the FD calculation for a specific substrate were developed using reference tables provided by Pölme et al. (2020), and double checked and adjusted with inter-rater reliability method.

Table 1 - FD_x relative weight ranking (1 to 5), expressing potential damage based on the literature and verified through inter-rater reliability (Pölme et al., 2020).

Genus	Substrate			
	Paper	Wood	Plaster	People
<i>Alternaria</i>	1	1	5	4
<i>Aspergillus</i>	5	5	5	5
<i>Cladosporium</i>	4	5	5	3
<i>Chaetomium</i>	5	5	2	2
<i>Fusarium</i>	1	1	2	5
<i>Humicola</i>	5	5	1	1
<i>Mucor</i>	1	1	2	5
<i>Parengyodontium</i>	1	1	1	5
<i>Penicillium</i>	5	4	5	1
<i>Phialophora</i>	1	3	1	5
<i>Trichoderma</i>	5	5	3	1
Unidentified				
Other				

Results

Home conditions

During the inspection, significant fungal growth was observed on the walls and objects within the dwelling. The air was filled with a strong mouldy odour, and the humidity in the rooms was noticeably high (Supplementary Table S1), with an average temperature of 21.8 °C and an average relative humidity of 76%.

Counting, isolation, and cryopreservation

After a 7-day incubation period at 24 °C, fungal colonies grew in all the samples. The total fungal propagule concentration indoor, expressed as CFUs/m³, ranged from 320 (sample ‘S’, stairwell) to 2085 (sample ‘S.B.’, single room) and outdoor 535 (sample ‘T.2’ second terrace). Considering only the indoor samples, the mean concentration approximately was 1600 CFUs/m³ (Fig. 2).

In total, 35 fungal strains were isolated and categorized into 23 different morphotypes based on morphology (Supplementary Figure S1) and later identified at the species level using molecular markers. The obtained sequences were deposited in GenBank with accession numbers for ITS (PQ644065-PQ644085), BT (PQ687604-PQ687610), and CMD (PQ687611-PQ687615) markers. A total of 14 species were identified, belonging to 12 genera of Ascomycota (*Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Trichoderma*), 1 genus of Mucormycota (*Rhizopus*), and 1 genus of Basidiomycota (*Trametes*).

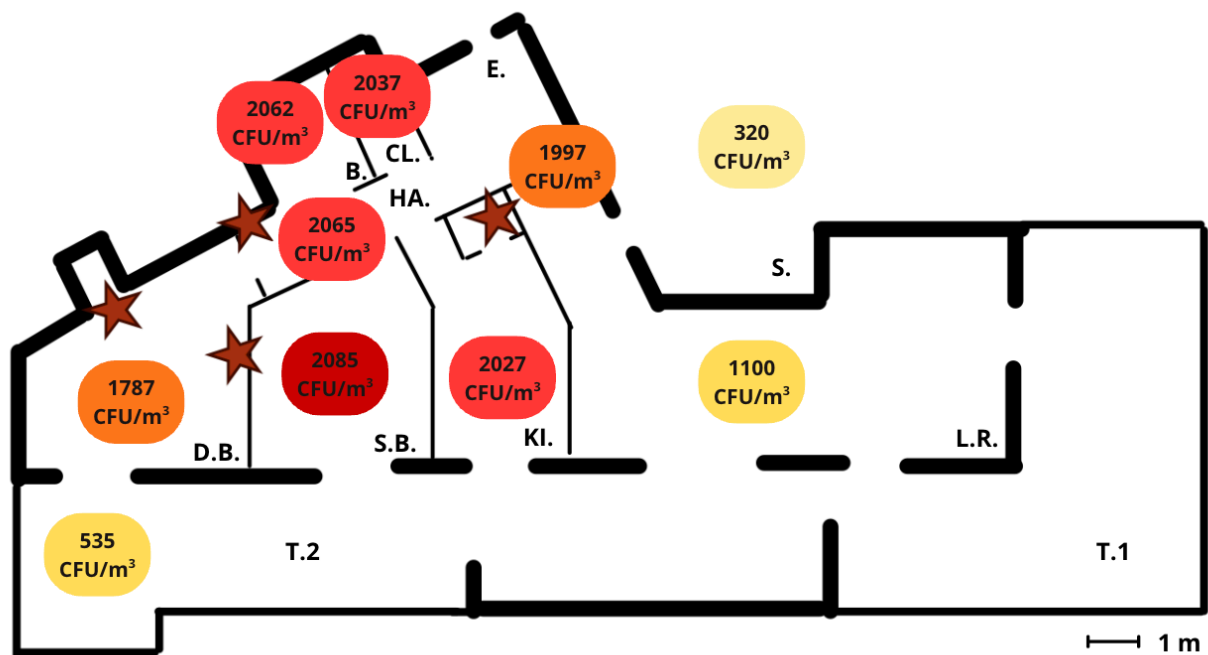


Fig. 2 - CFU per m³ found within the rooms and in the two samples collected outside the apartment. The red stars are the sampling points on various surfaces.

The most recurrent genera in terms of CFUs were *Aspergillus* (18% of the observed colonies) with a notable prevalence of the genus *Penicillium*, which accounted for approximately 79% of the observed colonies. The strains of the genus *Aspergillus* able to grow at 37 °C for 7 days were *Aspergillus tubingensis* Mosseray, *A. calidoustus* Varga, Houbraken & Samson and *A. parasiticus* Speare, as shown in Figure 4.

Fungal Susceptibility score (FSS)

The FSS was designed to consider both the diversity and abundance of each fungal genus and its potential target substrate within the surveyed area (Di Piazza et al. 2024).

The FSS was computed using the CFU values of the identified genera (Supplementary Table S2) and the assigned FD values (Table 1). Only the fungal genera related to the specific substrate under analysis were considered (Pölme et al., 2020). In our case, the only excluded genus was *Trametes* because was below our fixed threshold. The calculation of the FSS yielded values of 3.81 for the paper substrate, 3.49 for wood, 3.82 for plaster, and 2.29 for people, as shown in Figure 5. These quantitative results formed the basis for the subsequent interpretation of health and material risks through the FSS.



Fig. 3. List of species identified at the molecular level and their respective concentrations expressed as CFU/m³ of sampled air.

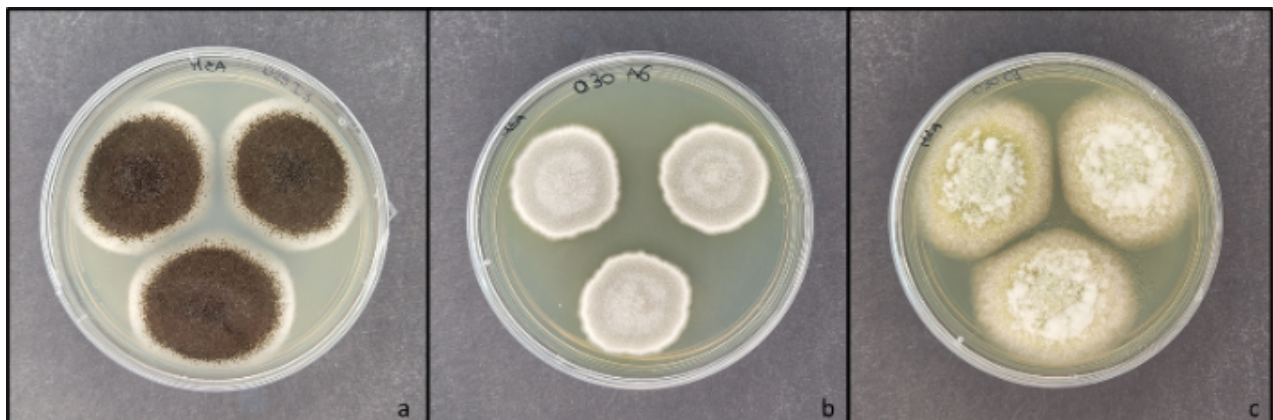


Fig. 4 - Fungal strains that grew at 37 °C: a. *Aspergillus tubingensis*, b. *A. calidoustus*, c. *A. parasiticus*.

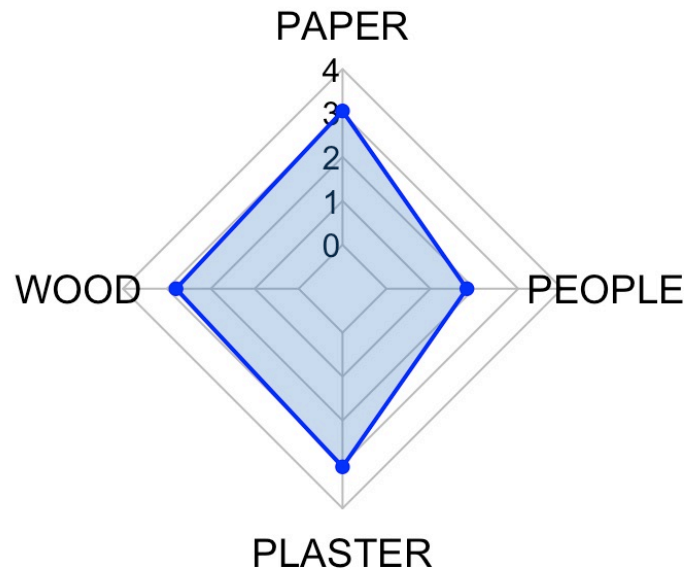


Fig. 5 - Results obtained by the Fungal Susceptibility Score (FSS): Paper 3.81; Wood 3.49; Plaster 3.82; PEOPLE 2.29.

Discussion

Fungal propagules, including spores, conidia, and hyphal fragments, can disperse over considerable distances. Depending on the context, these propagules can act as agents of biodeterioration, allergens, and pathogens, thereby impacting both environmental and human health (Crawford et al., 2023). The diversity and quantity of fungi detected in a specific site can serve as a valuable index for assessing air quality (Grisoli et al., 2019). In our case, the survey revealed substantial deterioration due to the abundant presence of fungal organisms on walls and other surfaces. The high humidity recorded in all rooms, almost all values exceeding 70 % (see Supplementary Table S1), created favourable conditions for fungal growth. As reported in the literature, relative humidity levels above 60% can promote fungal proliferation (Pyrri et al., 2020), facilitating spores' dispersion and colonization of various surfaces (Crawford et al., 2015). The most recurrent genera were *Penicillium* and *Aspergillus*, both globally distributed and ecologically significant. Their abundance led to the classification of the environment as unhealthy (Cabral, 2010) and as a strong factor in wall biofouling (Giannantonio et al., 2009).

Penicillium, a genus comprising widespread species thriving on diverse materials, produces high concentrations of conidia (Borrego et al., 2019). Specifically, *Penicillium chrysogenum* Thom, which accounted for over 70% of the colonies identified (Fig. 3) is commonly found in indoor environments with high humidity (Borrego et al., 2017). It acts as a biodeteriorative agent on walls and materials such as paper-based products (Sequeira et al., 2019).

Aspergillus, on the other hand, includes thermophilic species known as common contaminants of food, soil, walls, and other substrates. It is also pathogenic to humans and animals, particularly immunocompromised individuals (Sánchez Espinosa et al., 2021; VV.AA. 2022). Among opportunistic pathogens, various species of *Aspergillus* can cause aspergillosis, with various clinical manifestations including aspergilloma, chronic pulmonary infections, and severe asthma associated with fungal sensitization (Glampedakis et al., 2020). The species most frequently involved in human pathology are *Aspergillus fumigatus*, and *A. flavus* (Chen et al., 2023). To evaluate potential pathogenicity, the isolated *Aspergillus* species were incubated at 37 °C for one week. Only

Aspergillus tubingensis, *A. parasiticus*, and *A. calidoustus* (Fig. 4), grew under these conditions. Notably, *A. calidoustus* can cause invasive aspergillosis in immunocompromised individuals and shows significant antifungal resistance (Glampedakis et al., 2020).

Additionally, we identified species belonging to *Cladosporium*, *Alternaria* and *Trichoderma*, known both for causing allergic reactions in humans and for colonizing or degrading various materials (Li et al., 2022; Segers et al., 2015). *Alternaria* and *Cladosporium* are frequently associated with allergic respiratory diseases, with *Alternaria* being among the most prevalent allergens (Abel-Fernández et al., 2023). *Cladosporium* is also linked to allergies in patients with severe asthma (Segers et al., 2015). In addition to their allergenic potential, *Alternaria* and *Trichoderma* produce organic acids capable of damaging building materials by forming insoluble calcium complexes and increasing permeability (Giannantonio et al., 2009).

Aeromycological quantitative analyses confirmed a high presence of fungal propagules within the apartment. Figure 2 shows that CFU/m³ ranged from 1100 to 2085, with a mean of 1895, indicating intermediate to high microbiological pollution according to the Italian INAIL 2010 Guidelines (Anzidei et al., 2010). Currently, several limits for fungal spores in the air considered “safe” for humans have been proposed, such as 500 CFU/m³ (UNDP, 1990), 1,000 CFU/m³ (OSHA, US 1992), and 300 CFU/m³ (Indoor Air Quality Association, 1995) (Vasenev et al., 2020). The previously mentioned regulations classify the examined environment as unhealthy for humans. However, the lack of universally accepted classes for fungal air pollution underscores the need for standardized methodologies and classification systems.

An additional analysis comparing indoor and outdoor air confirmed the strong contamination inside the apartment. Outdoor air contained 535 CFU/m³ (Fig. 2), while indoor air reached up to 2085 CFU/m³. In general, in healthy buildings, the ratio indoor/outdoor is around or slightly above 1, with *Cladosporium* dominating (Cabral, 2010). Conversely, in sick buildings, this ratio is much higher than 1, and *Penicillium* and *Aspergillus* become predominant (Cabral, 2010). In our case, the indoor/outdoor concentration ratio was significantly greater than 1, confirming the poor air quality and strong fungal colonization, likely related to water leakage, high humidity, and optimal indoor temperatures (Supplementary Table S1).

After quantifying airborne fungal propagules, we evaluated their potential impact on materials and human health using the Fungal Susceptibility Score (FSS). By correlating the relative abundance of the observed genera with the susceptibility of four targets—paper, wood, plaster, and people—the FSS provides a practical measure to assess environmental contamination. Each fungal genus was assigned a weight ranging from 1 to 5 (Table 1), reflecting its ability to colonize the respective substrates. These values were derived from Pölme et al. (2020), which links fungal genera to their primary and secondary lifestyles and typical habitats.

In this study, “paper” and “plaster” had the highest FSS values (3.81 and 3.82, respectively; Fig. 5), indicating strong susceptibility to fungal attack under the observed environmental conditions. “Wood” followed with 3.49, while the “people” target had a lower index of 2.29. The FSS results therefore highlight that the materials most at risk are construction and furnishing substrates rather than human exposure. Although the INAIL classification indicates a medium-high risk for humans based solely on CFU counts, integrating FSS with quantitative data provides a more nuanced and substrate-specific risk assessment. This approach allows distinguishing between environments where fungi are primarily a biodeteriorative issue and those posing direct health risks.

Based on the FSS results, the most vulnerable substrates in this environment are materials such as paper and plaster, whereas human exposure appears comparatively less critical. This shows that, while the INAIL classification labels the environment as medium–high risk for humans based only on CFU counts, a more complete evaluation can be achieved by combining FSS with CFU data or by developing new methodologies that account for both human and material susceptibility. In this sense, the FSS emerges as a practical tool for differentiating between fungal contamination scenarios. By integrating both the number of fungal propagules and their potential effects on various substrates, the FSS offers a more comprehensive and context-specific picture of environmental contamination.

Overall, the FSS could serve as a standardizable and reproducible metric to improve fungal risk assessment in indoor environments, supporting both public health and heritage conservation practices. In future research, FSS could be refined with molecular and metagenomic data.

Limitations of the Fungal Susceptibility Score

A limitation of the proposed index is that it operates at the genus level rather than at the species level. Although all strains were identified to species, the index was intentionally calculated at the genus level; this choice inevitably reduces taxonomic resolution and may overlook species-specific ecological traits or differences in functional impact. However, this decision is due to the high variability and ecological plasticity of fungi, which often display strain-specific characteristics. Incorporating species-level data, particularly for opportunistic taxa, would require a much broader and more detailed framework to account for the diverse implications of each species across different contexts, potentially reducing the practicality of the tool. Working at the genus level still allows for a robust and biologically meaningful estimate, as genera include species sharing related ecological roles. Moreover, this approach enables faster computation and supports the use of the index as a rapid field-based risk assessment tool. Future developments should aim to refine the index by integrating species-level resolution where sufficient ecological and functional data are available.

Conclusion

This study revealed a significant level of fungal contamination within the surveyed apartment in Genoa, characterized by elevated airborne spore concentrations, mainly from the genera *Penicillium* and *Aspergillus*. Environmental conditions, specifically high humidity levels (>70%) and stable indoor temperatures (20–22 °C), created an environment highly conducive to fungal growth. The application of Fungal Susceptibility Score (FSS) allows us to identify materials such as paper and plaster as highly susceptible to fungal growth, whereas wood demonstrated comparatively lower susceptibility. Although indoor air contamination levels fell within the moderate-to-high range according to INAIL guidelines, the FSS results revealed that fungal growth was primarily concentrated on surfaces, suggesting that the main risk was biodeterioration rather than direct exposure to airborne pathogens. Nevertheless, the detection of pathogenic *Aspergillus* species capable of growing at human body temperature indicates potential health risks for immunocompromised individuals, emphasizing the need for preventive hygiene and environmental control measures (VV.AA. 2022). Overall, the integration of FSS with conventional quantitative indicators (e.g., CFU counts) provides a more accurate and balanced assessment of fungal risk. By linking fungal abundance and ecological traits to the susceptibility of specific materials and human targets, the FSS represents a useful and versatile metric for assessing microbiological risks in indoor environments.

In conclusion, the adoption of this index could support both public health monitoring and the conservation of cultural heritage, by providing a standardized, evidence-based approach for identifying and prioritizing interventions in environments affected by fungal contamination. Future applications of the FSS could include comparative analyses across different building typologies or integration with molecular data for non-cultivable fungi.

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