Mini Review

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Environmental and clinical mould spore risk thresholds

Abstract

Escalating environmental pollution and urbanization is associated with a rise in fungal contamination, which contributes to a variety of health concerns, particularly respiratory tract ailments such as allergic rhinitis, asthma, bronchitis, and sick building syndrome. Spore trap testing serves as a straightforward method for evaluating the number of spores/m³ in the air. Multiple studies indicate that 1000 spores/m³ represents the upper threshold for normal levels of mould exposure, with levels above this linked to adverse health effects, thereby establishing the environmental threshold. According to the literature, the clinical threshold is 3000 spores/m³ for *Cladosporium* and 100 spores/m³ for *Alternaria*. This mini review provides an overview of the existing environmental and clinical thresholds for fungal contamination to assess risk. We examine fungal threshold levels in both outdoor and indoor settings, encompassing residential homes, libraries, buildings, workplaces, and hospitals. In conclusion, environmental scientists, microbiologists/mycologists, occupational hygienists, insurance assessors, the real estate sector, clinical healthcare staff, and others must use and apply both environmental and clinical threshold levels to assess fungal contamination risk in indoor environments to protect public health. And finally, this mini review highlights the need for increased focus and research to ensure that appropriate guidelines and industry standards are developed, applied, and enforced that incorporate objective spore thresholds to quantify risk from fungal mediated adverse health in various indoor environments.

Keywords: fungal contamination, spore trap testing, environmental threshold, clinical threshold, public health, mould inspection, fungal sampling, fungi

Volume 11 Issue 1 - 2023

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Received: March 20, 2023 | **Published:** March 28, 2023

Background

Mould, a term commonly used to describe a subset of fungi, refers to eukaryotic, multicellular, spore-forming, microscopic organisms that typically grow in filamentous or hyphal forms. These organisms are highly diverse and obtain their nutrients from the surrounding environment. Fungi are classified separately from animals and plants and can be found both indoors and outdoors in various forms such as fruiting bodies, spores, viable or nonviable spores, dead mould, mycelium networks and biofilms, structural fragments, metabolic by-products, and volatile organic compounds including mycotoxins. Environmental spores can cause airborne allergies in humans upon inhalation, with approximately 20-30% of respiratory allergy sufferers being sensitive to various fungal species.[1,2]

Allergic reactions typically occur at the site of allergen deposition. For instance, the nasopharynx is the site of large spore depositions, usually greater than 10µm in size, and is associated with allergic rhinitis. In contrast, lower respiratory tract involvement is observed when smaller spores, typically less than 10µm or even less than 5µm, penetrate deeper and manifest as bronchial asthma.[3,4]

The objective of this study is to evaluate the published environmental and clinical thresholds for mould spore levels in order to ascertain the associated risk to occupants in indoor environments.

Methods

To conduct this mini review, a comprehensive literature search on PubMed and Semantic Scholar to identify relevant studies on fungal spore levels in the air was performed. The search strategy included the following keywords and their combinations: "spores/m³," "fungal structures/m³," "colony forming units/m³," "CFU/m³," and "FS/m³." Variations of these terms and phrases were also considered to ensure a thorough search.

To ensure the quality and relevance of the selected articles, specific inclusion and exclusion criteria were applied. Inclusion criteria were: (1) primary research articles, (2) published in peer-reviewed journals, (3) available in English or with English language abstracts and the full article available for translation, and (4) focused on the measurement and analysis of fungal spore levels in the air. Exclusion criteria were: (1) conference abstracts, and (2) studies that did not specifically address fungal spore levels in the air.

After identifying a preliminary set of articles, titles and abstracts were screened for relevance. Full-text articles were then assessed for eligibility based on the inclusion and exclusion criteria. The data extraction process involved summarizing key information from the selected articles, such as study design, sample size, fungal spore measurement techniques, and main findings. To synthesize the data, a narrative synthesis, in which common themes were identified, was applied. This approach allowed an exploration of the various methods used to measure fungal spore levels in the air, as well as the factors influencing these levels. The implications of the findings for public health and environmental monitoring were also examined. By providing a clear and detailed description of the method, the aim is to facilitate the replication and extension of this study and encourage further investigation into this important topic.

Notable findings

Fungal allergic reactions from outdoor environments

In the past decade, numerous studies have been published worldwide, highlighting the impact of air pollution on human health. Air pollution can also contribute to an increase in the environmental threshold of airborne fungi. The presence of fungal spores in the environment has been well-documented in scientific literature. Environmental monitoring, guided by defined threshold limits, is crucial in today's world. Individuals with weakened immune systems are more susceptible to infections from these fungi, and it is nearly impossible to avoid aerosol infections other than by controlling the threshold limits of these moulds. Urban areas tend to have higher contamination levels due to environmental changes, resulting in a 25-30% increase in fungal concentration compared to rural areas.[5]

The environmental fungal population varies worldwide due to differing environmental conditions. The most commonly reported fungal genera globally include *Aspergillus*, *Bipolaris*, *Curvularia*, *Penicillium*, *Neurospora*, *Rhizopus*, and *Trichoderma*.[5] The pathogenicity of environmental fungi also varies, necessitating the determination and control of thresholds for both total mould concentration and pathogenic fungal concentration. *Cladosporium* and *Alternaria* spores are among the leading environmental spores responsible for significant health problems. In 1977, a study defined pathogenic thresholds of 3,000 spores/m³ for *Cladosporium* and 100 spores/m³ for *Alternaria*.[6] Subsequent research from India reported elevated levels of these threshold limits in specific months- May and June for *Alternaria* and December for *Cladosporium* spores- based on one-year analytical data.[1] An aerobiological monitoring study from Central and Western Europe associated 300 spores/m³ of *Alternaria* with serious health problems.[7] Research from Greece indicated that 50 spores/m³ of *Alternaria* and 400 spores/m³ of *Cladosporium* spores were found to exacerbate allergic rhinitis symptoms in sensitized individuals.

Cladosporium emerged as the most prevalent environmental mould, while *Alternaria* was the most common cause of allergic sensitization. The peak of these spores was observed in June.[8] However, recent studies from the USA and UK have reported that no standard clinical thresholds have been defined for airborne fungi due to their high environmental diversity. Nevertheless, based on existing literature, a total mould concentration of 150-1,000 spores/m³, 100 spores/m³ for *Alternaria*, and 3,000 spores/m³ for *Cladosporium* can cause a wide range of allergic reactions, including bronchial asthma, allergic rhinitis, fatigue, neurological, and neurocognitive issues.[9,10]

Table 1 provides a summary of selected studies along with their corresponding fungal threshold levels in both outdoor and indoor environments.

Table 1 Environmental and clinical exposure threshold summary of the literature. There is equivalence between colony forming units: CFU/m³, fungal structures: FS/m³, or spores/m³ of air

Reference	Identified risk fungus	Spore threshold limit	Outcome
Bagni N, et al.[6]	*Cladosporium*	3000	Clinically significant threshold
	Alternaria	100	
Rao CY, et al.[10]	Total fungi	Up to 1000	Non contaminated environment threshold
Bush RK, et al.[31]	Fungal contamination	≥1000	Indoor fungal contamination
Gent JF, et al.[14]	Fungi moulds - low	1–499	Study on new born, higher respiratory symptoms with higher *Penicillium* levels
	Fungi mould - moderate	500–999	
	Fungi mould - high	≥1,000	
Santilli J.[15]	Mould contamination	>1000	Unhealthy indoor threshold
Watanabe M.[32]	Indoor-air fungal counts	>1000	Clinically significant threshold
Rockwell W.[16]	Fungal contamination	>1000	Perform mould remediation in the home that is successful and results in the cessation of allergic symptoms
Rockwell W, & Santilli J.[17]	*Aspergillus* (predominant)	Normal indoor counts <1,000	Case report of pustular eruption Indoor mould counts bedroom: 4,300 and Bathroom: 2,400
Davies JM, et al.[18]	*Alternaria*	100	Causes allergic symptoms
	Cladosporium	3000	
Das S,et al.[1]	*Alternaria* spores	100	Threshold limits for allergic individuals to these spores
	Cladosporium s	3,000	
Kasprzyk I, et al.[7]	*Alternaria* spores	300	Serious health issues
Katotomichelakis M, et al.[8]	*Alternaria*	50	Elevated allergy symptoms seen in sensitized individuals
	Cladosporium	400	
Michalska M, et al.[19]	Fungi moulds	5000	Polish standard PN-89/Z-04111/02
Shinohara N, et al.[20]	Fungi moulds - acceptable	<1000	(as per European Collaboration Action)
	Fungi mould - high	>1000	
	Fungi moulds - very high	>10000	
Lieberman A, et al.[9]	Total Airborne mould levels	150-1000	Causes allergic reaction mild to severe
Reboux G, et al.[21]	*Aspergillus versicolor*	300	Evaluated both indoor and outdoor air
	Cladosporium species	495	
	Whole moulds	>1000	
Rejc T, et al.[22]	Mixed Fungal species - acceptable	150	*Cladosporium*, *Penicillium* and *Aspergillus* were the most common fungi in all air samples
	Cladosporium or common fungal species - acceptable	Up to 500	
	Aspergillus, *Penicillium*, *Cladosporium* and *Alternaria*	<500	
	Fusarium, *A. fumigatus*, *A. flavus*, *A. terreus* and *A. niger*	<12	The highest levels of aerobic mesophilic organisms in indoor air were identified in spring
	Fungi moulds - acceptable	<500	
	Fungi moulds - very high	2000	
	Fungi moulds - acceptable (National Institute of Occupational Safety and Health)	<1000	

Citation: Jones CL. Environmental and clinical mould spore risk thresholds. *J Bacteriol Mycol Open Access.* 2023;11(1):44‒48.
DOI: 10.15406/jbmoa.2023.11.00342

Table Continued...

Reference	Identified risk fungus	Spore threshold limit	Outcome
Anees-Hill S, et al.[2]	*Alternaria* spores	100	Threshold limits
	Cladosporium spore	3,000	
Wu D, et al.[23]	Fungi moulds - low	<170	Indoor fungal thresholds in libraries
	Fungi mould - moderate	170 - 560	
	Fungi mould - high	560 - 1000	
	Fungi moulds - very high	>1000	

Indoor air quality and fungal thresholds

Initial relative quality standards for fungal thresholds in indoor air stipulated that indoor air mould spore levels should be lower than outdoor air mould spore levels.[10] Numerous factors contribute to indoor air quality apart from outdoor fungal distribution, such as occupancy number of persons and lifestyle, the presence of pets and plants, ventilation, plumbing, air conditioning and heating systems, building construction materials and methods including dilapidation and non-compliant building and waterproofing works, and resuspension of indoor dust and the microbiome and mycobiome of the indoor environment. There were limited standards for fungal thresholds until Russia revised its "Maximum Allowable Concentrations (MAC) of Harmful Substances" document in 1993 to address airborne fungal presence.[11] In 1994, the Occupational Safety and Health Administration (OSHA) of the United States proposed an official air quality document for non-industrial facilities.[12] Based on the scientific literature and surveys, initial standards for non-contaminated total indoor fungi ranged from less than 100 CFU/m³ to 1,000 CFU/m³.[10] A close interaction has been reported between outdoor and indoor fungi, with increased outdoor fungi levels leading to a rise in indoor fungi thresholds. Geographical variations, as well as temperature and humidity, also affect fungal distributions.[13]

Not all studies concur on the same criteria of up to 1,000 CFU/m³ for indoor fungal contamination. Factors such as season, presence of pathogenic mould species, and individuals' age and immunity status play a role in determining this threshold. A study on household mould involving participants up to one year of age categorized 1–499 CFU/m³ as low, 500–999 CFU/m³ as moderate, and ≥1,000 CFU/m³ as high exposure levels. *Cladosporium* and *Penicillium* were the predominant moulds identified, with increased *Penicillium* rates associated with heightened respiratory allergic symptoms.[14]

There is no single defined criterion for indoor mould contamination, as different indoor facilities have unique requirements and include the need for companion assessments of visual mould and mould odour as well as symptom experience when spending time away from the suspect indoor challenge environment. However, the literature reveals many studies that have evaluated indoor mould contamination in various types of buildings and facilities. A US school study reported that mould contamination affected 40% of individuals; with ceiling tiles and carpeting identified as primary factors in indoor mould contamination.[15] Although homes are generally considered less contaminated, studies have shown that contaminated homes upon fungal remediation[16] can significantly improve allergic symptoms. A case report involving a patient with pustular eruptions due to predominantly *Aspergillus* exposure revealed extremely high fungal counts in their residential home: 4,300 spores/m³ in the bedroom and 2,400 spores/m³ in the bathroom. The patient's symptoms improved after relocating from the home, with no symptoms observed for up to seven months and a 100% reduction in medication. Symptoms re-emerged within one week of returning home. The patient's history revealed inadequate remediation of water intrusion in the bedroom 15 years prior.[17] *Aspergillus* triggers numerous allergens and causes severe allergic reactions, such as bronchopulmonary aspergillosis. Fungal infections caused by *Candida* can also trigger allergens linked to IgE-binding.[18]

Of note, the Polish standard PN-89/Z-04111/02 also has different threshold limits for airborne bacteria and fungi and this was used as the basis of a study[19] into the role of air quality and adverse health in adolescents. The analysis revealed a correlation between the number of new cases of type 1 diabetes mellitus (T1DM) in children and adolescents and the number of psychrophilic bacteria, mesophilic bacteria, and mould fungi. Additionally, a relation was found between the number of new T1DM cases and mean annual concentration of PM10 which is of significance since the PM10 statistic is well known to capture the size contribution of mould spores and fragments using for example optical particle counting measures.

A Japanese study reported higher fungal contamination in residential homes during summer, with closely connected residential homes exhibiting increased contamination. Indoor fungal levels in residential homes were inversely proportional to residents' returns, as frequent door and window opening created a positive environmental balance, reducing humidity and improving air circulation.[20] An important retrospective study then analyzed indoor fungi levels in 1012 dwellings using airborne samples and compared them to different country guidelines.[21] The number of colonies per cubic meter of air was positively correlated with asthma and rhinitis, and specific fungi species such as *Aspergillus versicolor* and *Cladosporium* spp. were found to be correlated with asthma. That study proposed a new protocol for indoor fungi surveys and a new guideline for interpreting results based on >1000 cfu/m³ of whole colonies and/or above threshold levels for *A. versicolor* or *Cladosporium* spp. to identify 'at risk' indoor environments from fungal exposure. The proposed thresholds of 300 cfu/m³ for *A. versicolor* and 495 cfu/m³ for *Cladosporium* spp. can also help identify potential asthma triggers and guide remediation efforts to improve indoor air quality.

Another study investigated microorganism concentrations, aerosol black carbon, and carbon dioxide (CO_2) levels in the indoor and outdoor air of two kindergartens during four seasons.[22] The study found that the highest concentrations of aerobic mesophilic microorganisms in indoor air were detected in spring, with *Cladosporium*, *Penicillium*, and *Aspergillus* being the most common fungi. The concentrations of *Staphylococci*, *Enterobacteria*, and CO_2 had a positive correlation with the number of people in the rooms. The study recommends reducing the number of children in playrooms and increasing ventilation to lower CO_2 and microorganism concentrations and suggests the installation of effective artificial ventilation in building renovations.

A 2019 study from China assessed mould concentration in libraries due to their complex design. High fungal threshold levels pose risks not only to the preservation of old books but also to the health of readers and library staff.[23] Humidity as well as climate-driven storm damage is also a critical factor in mould contamination; following Hurricane Katrina, indoor mould levels were significantly high depending on damage levels, with mildly damaged homes registering 3,700 CFU/m³, and moderately to heavily damaged houses measuring 67,000 CFU/m³.[24] The French National Agency for Environment

Health (ANSES) suggested revising the CSHPF list and critical fungal threshold levels.[25] ANSES defines "abnormal" culture as >1000 CFU of total fungi/m^3 and/or mouldy area surfaces of more than 3 m^2, indicating insalubrity (not conducive to health) and requiring health officials to rehouse residents. If the total fungi count in the air or mouldy surfaces exceeds these values, it is a health hazard, and the authorities should rehouse the residents.

The overall consensus of these studies suggests that indoor mould assessments focusing on quantitative thresholds should be implemented in all suspect facilities to obtain objective data to support visual observations and individual case histories and to then make recommendations for harm minimization.

Healthcare settings and environmental spores

Healthcare settings and hospitals must also maintain very low fungal counts, as patients typically have weakened immune systems. Special attention is required in wards, ICUs, and operating theaters. *Aspergillus*, the most common opportunistic fungus, has over 300 species, with around 20 causing lethal infections in immunocompromised patients, predominantly by *Aspergillus fumigatus*, followed by other species such as *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus terreus*. Other fungal species that cause infections through environmental spore inhalation include *Acremonium*, *Paecilomyces*, *Rhizopus*, *Mucor*, *Absidia*, and *Fusarium*. Among these fungal infections, aspergillosis is the most fatal, particularly invasive aspergillosis, with a 50% mortality rate.[26–28] Some *Aspergillus* species can efficiently produce mycotoxins, including *A. fumigatus* and *A. flavus*, which directly contribute to infection severity. Inhalation of excessive amounts of organic dust containing volatile organic compounds (VOCs) and mycotoxins is linked to acute health discomfort, known as "sick building syndrome".[29]

Furthermore, pollen and mould spore levels can rise during thunderstorms. Thunderstorms cause fungal spore concentrations to double in the environment, leading to increased asthma cases and hospital visits, from 8.6 to 10. Asthma-related mortality rates also increase during airborne fungal spore peaks.[18] All hospital-visiting asthma patients should be screened for exposure to fungal allergens and/or environmental exposure in the home or workplace to elevated mould spore levels, especially during thunderstorm asthma events. Approximately 50% of individuals experience allergic symptoms to mould and fungi at least once in their lives. Allergic reactions to these allergens create a hypersensitivity response, resulting in increased IgE levels, which are typically higher in adolescents than in the early years of life.[29]

Numerous research articles on indoor air quality in healthcare settings have been published in recent decades, but separate thresholds have not yet been defined. However, due to the complexity and vulnerable immune status of patients, clinical thresholds for healthcare settings should be revised and further researched.[30] Improved indoor air quality in healthcare settings can have many benefits, including reducing the risk of nosocomial infections and exposure to mould spores, and is just as crucial to use thresholds here as in residential homes and workplaces. For example, it's well known that nosocomial infections caused by bacteria like MRSA and fungi such as *Candida auris* are acquired in healthcare facilities, while black yeasts like *Exophiala dermatitidis* are of increasing concern. *E. dermatitidis* is a form of black yeast that can infect people and cause a number of infections, including keratitis, otitis externa, and onychomycosis. It is an opportunistic pathogen often found on decaying wood and can attack both immunocompetent and immunocompromised individuals and cause chronic and recalcitrant infections. Routine monitoring and surveillance of indoor air quality would therefore allow for interventions to reduce and remove airborne pathogens and minimize cross-contamination.

Concluding remarks

Exposure to fungal contamination in the indoor living or working environment is rising as a public health issue over the last two decades. In conclusion, it is essential to reiterate the clinical thresholds for *Cladosporium* at 3,000 spores/m³ and *Alternaria* at 100 spores/m³, as outlined in reference.[6] For non-contaminated or 'normal' mould levels, the environmental threshold is up to 1,000 spores/m³, according to reference.[10] Furthermore, the Australian Mould Guideline[31] also utilizes the 1,000 spore/m³ threshold, with classifications as follows: high mould levels range between 1,001 and 2,500 spores/m³, and extremely high levels exceed 2,501 to 5,000+ spores/m³ for naturally ventilated environments (e.g., those with windows that open). And most recently, in 2022, research from Japan[32] concluded that "indoor-air fungal counts at all sampling points were less than 1000 colony forming units (CFU)/m³, which is the recommended limit for fungal contamination in indoor air".

In summary, this paper highlights the importance of various workers including environmental scientists and microbiologists/mycologists, occupational hygienists, insurance assessors and clinical healthcare staff and others to use and apply the known established environmental and clinical threshold levels for assessing risk from fungal contamination in various indoor environments to protect public health. Indoor air quality regulations and guidelines are crucial for promoting healthy living environments and ensuring that indoor air quality standards are met. Real estate regulations, such as the Australian Residential Tenancies Regulations 2021 prohibits the presence of mould in buildings used for residential tenancy and can order compliance with mould repair notices, emphasizing the importance of maintaining safe indoor air quality in homes.[33] Further research and collaboration are then needed to define and implement appropriate guidelines and industry standards that address diverse environmental conditions and individual susceptibilities to mould exposure. Governments, regulatory bodies, and organizations can play a vital role in setting and enforcing indoor air quality standards including specific reference to mould thresholds to safeguard public health and reduce the risk of respiratory illnesses caused by indoor air pollutants.

Acknowledgments

None.

Conflicts of interest

The author declares there are no conflicts of interest.

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