https://doi.org/10.24840/978-989-54863-5-9_<0057>-<0062>

UNDERSTANDING AND ADDRESSING FUNGAL EXPOSURE RISKS IN PRIMARY SCHOOLS: IMPLICATIONS FOR CHILDREN'S HEALTH AND WELL-BEING

Renata Cervantes [1,2], Pedro Pena [1,2], Bianca Gomes[1], Marta Dias[1,2], Bruna Riesenberger[1], Margarida Rodriguez[1], Liliana Marques[1], Carla Viegas [1,2]

[1] H&TRC—Health & Technology Research Center, ESTeSL—Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, 1990-096 Lisbon, Portugal

[2] Public Health Research Centre, Comprehensive Health Research Center, NOVA National School of Public Health, CHRC, REAL, CCAL, NOVA University Lisbon, 1099-085 Lisbon, Portugal

Abstract

Background: Abstract Climate change increases fungal pathogen emergence and spread, raising the risk of diseases, especially among vulnerable populations. Thermal adaptation is crucial for fungi to infect humans. Children's developing immune systems and increased vulnerability to environmental factors make them more susceptible to respiratory diseases caused by fungi. Exposure to fungal spores in indoor environments, like schools, can result in respiratory problems such as asthma, allergies, and bronchitis in children. Despite this there is a lack of research on the pathogenicity of fungi in this context in Portugal. This study aims to provide a first insight into the children's exposure to potentially pathogenic fungi in primary schools in Portugal, by observing fungi's ability to growth at 37ºC and the penetration depth in the respiratory airways, providing valuable insights into their capability to induce diseases. In this study, air samples were collected from various locations within the school building using an Anderson six-stage sampler with DG18 media plates for fungal analysis. Sampling was conducted twice, with specimens to be incubated at two different temperatures: 27°C, representing environmental conditions, and 37°C, reflecting human body temperature. Preliminary findings suggest notable seasonal variations in fungal presence, especially at particles diameter between 2.1 and 1.1 microns and respiratory penetration of secondary and terminal bronchi. National-level initiatives are imperative to fill existing knowledge gaps and implement effective measures to mitigate health risks associated with fungal contamination in educational environments.

Keywords: Fungal exposure; Pathogenicity; Children, Indoor Air Quality; Primary school.

Introdução

Climate change poses a multifaceted threat, altering environmental conditions and exacerbating the emergence and proliferation of fungal pathogens, thereby increasing the risk of fungal diseases, particularly impacting socially vulnerable populations (Seidel, *et al* 2024). Thermal adaptation is not the sole factor but rather a significant prerequisite for enabling fungi to infect humans or mammals. The increasing ambient temperatures could serve as a crucial pathway for the adaptation mechanisms of fungi to cope with high-temperature surroundings, possibly enhancing their ability to cause disease in humans (Xiao *et al*.,2022). Concurrently, indoor environments, crucial settings for human habitation like schools and homes, present significant concerns regarding fungal exposure, with children being especially susceptible to respiratory ailments and other health issues due to their distinct behaviors and physiological characteristics (Murphy, *et al* 2023). As underscored by scientific data, contaminants such as fungi inside buildings, influenced by external sources and activities, present significant health risks for both children and school staff (Legg, 2007; WHO, 2022; WHO, 2023a). Exposure to bioaerosols containing fungi may occur in various areas such as classrooms, libraries, canteens, and other common school places, necessitating effective risk management strategies to safeguard children's safety (EU-OSHA, 2023). Some fungal species present toxigenic potential and exposure to their contaminants can exacerbate inflammatory responses, suppress immunity, and induce mutagenicity, potentially leading to organ failure or mortality (WHO, 2023b; Adams *et al*., 2021; Reham & Gamaleldin, 2020). Research dedicated to evaluating the presence and impact of fungal pathogens in primary schools in Portugal has been notably scarce over the past two decades (Pegas P. *et al*., 2011; Madureira *et al*., 2015). Limited studies have been identified on this crucial topic, and none have comprehensively assessed the fungal pathogenic potential, despite the valuable insights gained from inoculating fungi at human body temperature, particularly at 37ºC. This gap in research inhibits a thorough understanding of the adaptive capabilities of fungi and their potential to cause infections in humans, underscoring the need for further investigation into this area of study. This lack of information not only raises concerns about indoor air quality and potential health risks but also underscores the urgent need for comprehensive studies and appropriate mitigation measures tailored to safeguarding the well-being of children and staff within educational environments. This study aims to provide a first insight into the children's exposure to potentially pathogenic fungi in primary schools in Portugal, by observing fungi's ability to growth at 37ºC and the penetration depth in the respiratory airways, providing valuable insights into their capability to induce diseases.

Materiais e métodos

This study is a spin-off of a European research project "Identifying determinants for indoor air quality and their health impact in environments for children: measures to improve indoor air quality and reduce disease burdens". Ten schools located in the metropolitan Lisbon Area were sampled during two separate periods: from June 2023 to October 2023 (warm season), and from November 2023 to January 2024 (cold season). Among other different devices used in the sampling campaign from the enlarged study, the Anderson six-stage air sampler was employed. This device collects particles of varying sizes, with larger particles (>7.0 micrometers) typically filtered out in the upper airways, while smaller particles (<1.1 micrometers) can penetrate deeply into the lungs and potentially enter the bloodstream, posing serious respiratory and systemic health risks (Lindsley *et al*., 2017) (Figure 1), which provides a comprehensive assessment of airborne particles, including fungal spores (Fennelly 2020). This sequential sampling allows for the differentiation and characterization of particles based on their sizes, facilitating analysis of airborne contaminants in various environments. The collection involved collecting 200 liters of air at a flow rate of 28.3 L/min for 9 minutes per culture medium using the Andersen air sampler, following the manufacturer's guidelines, and reported methodology (Viegas *et al*., 2021). Also to better characterize the impact of seasonality, indoor temperature and relative humidity were measured at the time of collection, and a survey was applied for building characterization.

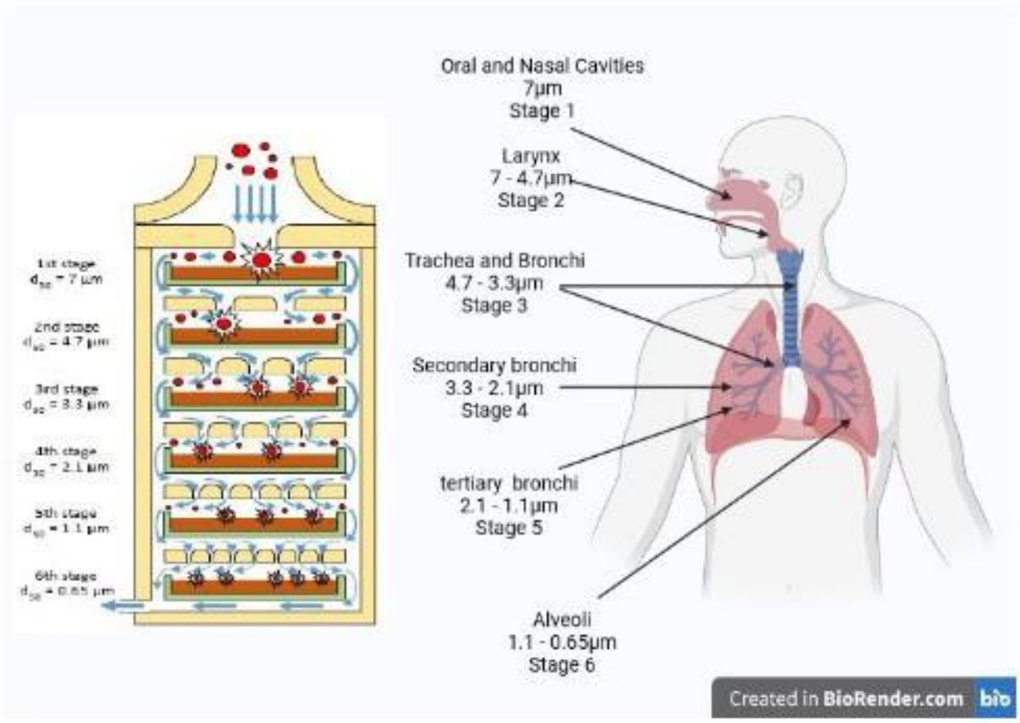

Figura 1. *Schematic of a 6-stage Andersen cascade impactor and respiratory penetration relation.*

Samples of indoor air were gathered from predefined areas within the school. A total of 5 sampling sites (Outdoor, Classroom, Canteen, Library, and gymnasium) were sampled in each of the 10 schools during both seasons. After sampling, DG18 plates were incubated at 27ºC and 37ºC for 5 to 7 days until colony forming units (CFU) counting. Microbiota quantification was determined as colony-forming units (CFU) and CFU concentration after plate's incubation.

Resultados e discussão

The initial findings from Andersons 6 stage on DG18 media incubated at 27ºC analysis reveal that there is no noticeable contrasting counts observed between both seasons, except for School 3 (S3) showing higher counts during the warm season (Fig. 2 and 3). Additionally, there is a notable increase in fungal load observed in stages 4 and 5 which represents particles between 2.1 and 1.1 microns which presents a respiratory penetration of secondary and terminal bronchi (Fig 2 and 3).

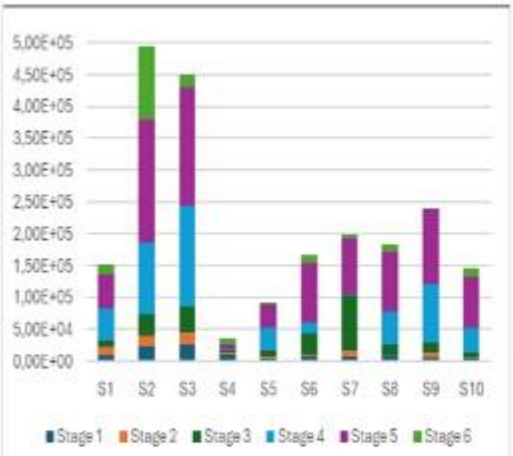

Figure 2. *Average fungal counts from all the 10 schools during the Warm season in CG18 incubated at 27ºC.*

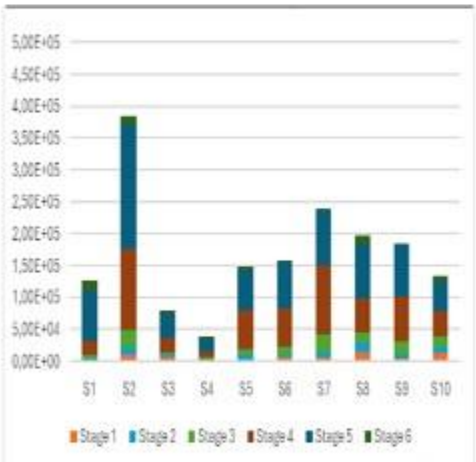

Figure 3. *Average fungal counts from all the 10 schools during the Cold season in CG18 incubated at 27ºC.*

Lorem In the same device for DG18 incubated at 37ºC analysis indicate a higher fungal load observed in stage 6, which represents a penetration equivalent to alveoli, suggesting a potential preference for growth under elevated temperature conditions. Lower fungal counts were recorded during the cold season, except for School 2 (S2) (Fig. 5) A noticeable distinction can be observed between the warm and cold seasons, particularly in stages 4, 5, and 6 which represents particles between 2.1 and 0.65 microns (Fig. 4 and 5) a potential preference for growth under elevated temperature conditions. Lower fungal counts were recorded during the cold season Fungal exposure indoors, notably in educational settings like schools, presents substantial health

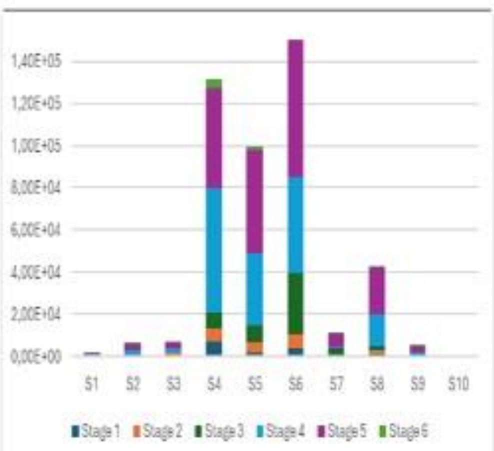

Figure 4. *Average fungal countings from all the 10 schools during the Warm season in CG18 incubated at 37ºC.*

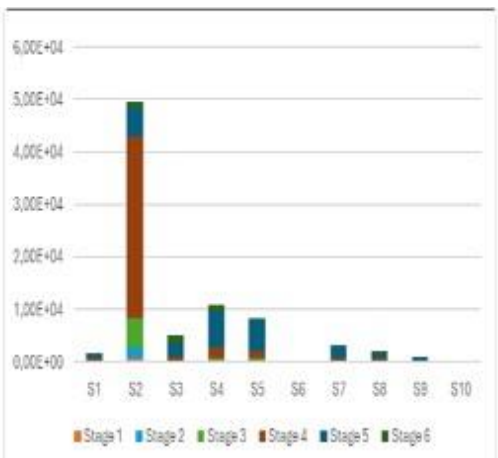

Figure 5*. Average fungal countings from all the 10 schools during the Cold season in CG18 incubated at 37ºC.*

risks, especially for vulnerable populations such as children susceptible to respiratory issues (Murphy, *et al* 2023). However, there's a notable lack of comprehensive studies and targeted mitigation strategies aimed at safeguarding children's health, particularly in Portugal. Factors like occupancy and seasonal variations significantly influence the presence and spread of airborne fungi within school premises (Legg, 2007; WHO, 2022). Climate change poses various threats, including altering environmental conditions and exacerbating the emergence and spread of fungal pathogens, thereby heightening the risk of fungal diseases, especially for socially vulnerable groups (Seidel, *et al* 2024). Thermal adaptation plays a significant role in enabling fungi to infect humans or mammals, with increasing ambient temperatures potentially enhancing their ability to cause disease in humans (Xiao *et al*., 2022). The absence of thorough studies evaluating the fungal pathogenic potential, by observing fungi's ability to growth at 37ºC and the penetration depth in the respiratory airways, in Portuguese schools raises concerns about indoor air quality and potential health risks. Our preliminary findings reveal notable seasonal variations in fungal loads particularly in stages 4, 5, and 6, which represent particles between 2.1 and 0.65 microns and a respiratory penetration of secondary and terminal bronchi until the alveoli (Lindsley *et al*., 2017), highlighting the impact of seasonal variations on fungal distribution and abundance, shedding light on the intricate relationship between environmental conditions and fungal distribution. These results underscore the substantial health risks of indoor fungal exposure, especially in educational settings. These findings highlight the impact of specific elements such as seasonal fluctuations on fungal presence and dispersal, highlighting the importance of systematic evaluations and monitoring protocols. Furthermore, given the role of climate change in exacerbating the emergence and transmission of fungal pathogens (Seidel, *et al* 2024), particularly affecting disadvantaged communities (Seidel, *et al* 2024), collaborative efforts are crucial to address these interconnected challenges and improve comprehension, prevention, identification, and treatment strategies for safer educational environments. This project advocates for a holistic approach to the climate-fungi-health nexus, integrating respiratory health surveys and contamination data to elucidate seasonal fungal impacts on human health. It urges preemptive measures to tackle risks from poor ventilation and moisture in schools, targeting fungal growth and mycotoxin contamination, with a proposed intervention focusing on WHO priority fungal pathogens

Conclusões

Addressing fungal exposure risks in primary schools requires a multi-faceted approach, encompassing comprehensive studies, rigorous monitoring protocols, and targeted mitigation strategies. The health and well-being of children and school staff depend on proactive measures aimed at safeguarding indoor air quality and minimizing exposure to airborne fungi. National-level initiatives are imperative to fill existing knowledge gaps and implement effective measures to mitigate health risks associated with fungal contam- ination in educational environments.

Agradecimentos e financiamento

Donec H&TRC authors gratefully acknowledge the FCT/MCTES national support through the UIDB/05608/2020 and UIDP/05608/2020, and FCT/MCTES national support through the IPL/2022/InChildhealth/BI/12M; 2023.01366.BD; UI/BD/151431/2021, also supported by national funds through FCT/MCTES/FSE/UE, UI/BD/153746/2022 and CE3C unit UIDB/00329/2020 (https://doi.org/10.54499/UIDB/00329/2020), and the ESTeSL- Escola Superior de Tecnologia e Saúde de Lisboa, Instituto Politécnico de Lisboa, national support through IPL/IDI&CA2023/FoodAlleEU; IPL/IDI&CA2023/ASPRisk and IPL/IDI&CA2023/ARAFSawmil.

This project was partly funded by EU Horizon 2021 grant no. 101056883 and co-funding from author's organizations and/or Ministries. Funding from Swiss SERI grant 22.00324, UKRI grant 10040524, and NHMRC grant APP2017786 and APP2008813. Views expressed are of the author(s) and do not necessarily reflect those of EU, Swiss SERI, UKRI, or NHMRC.

Referências

Adams, R. I., Bhangar, S., Pasut, W., Arens, E. A., Taylor, J. W., Lindow, S. E., Nazaroff, W. W., & Bruns, T. D. (2015). Chamber Bioaerosol Study: Outdoor Air and Human Occupants as Sources of Indoor Airborne Microbes. PLoS ONE, 10(5), e0128022. https://doi.org/10.1371/journal.pone.0128022

Adams, R. I., Leppänen, H., Karvonen, A. M., Jacobs, J., Borràs-Santos, A., Valkonen, M., Krop, E., Haverinen Shaughnessy, U., Huttunen, K., Zock, J.-P., Hyvärinen, A., Heederik, D., Pekkanen, J., & Täubel, M. (2021). Microbial exposures in moisture-damaged schools and associations with respiratory symptoms in students: A multi-country environmental exposure study. Indoor Air, 31(6), 1952–1966. https://doi.org/10.1111/ina.12865

Agut, M. (2010). Atlas of clinical fungi (2nd edn). G.S. de Hoog, J. Guarro, J. Gené, M.J.Figueras.International Microbiology; Vol. 4, Núm. 1 (2001); 51-52.

Andersen AA [1958]. New sampler for the collection, sizing, and enumeration of viable airborne particles. J Bacteriol 76(5):471-484.

EU-OSHA. (2023). Safety Management—A safe workplace is sound business | Occupational Safety and Health Administration. https://www.osha.gov/safety-management

Fennelly K. P. (2020). Particle sizes of infectious aerosols: implications for infection control. The Lancet. Respiratory medicine, 8(9), 914–924. https://doi.org/10.1016/S2213-2600(20)30323-4

Fujiyoshi, S., Tanaka, D., & Maruyama, F. (2017). Transmission of Airborne Bacteria across Built Environments and Ist Measurement Standards: A Review. Frontiers in Microbiology, 8. https://www.frontiersin.org/articles/10.3389/fmicb.2017.02336

Hospodsky, D., Qian, J., Nazaroff, W. W., Yamamoto, N., Bibby, K., Rismani-Yazdi, H., & Peccia, J. (2012). Human Occupancy as a Source of Indoor Airborne Bacteria. PLoS ONE, 7(4), e34867. https://doi.org/10.1371/journal.pone.0034867

Legg, S. (2007). Ergonomics in Schools. Ergonomics, 50, 1523–1529. https://doi.org/10.1080/00140130701584878

Lindsley, William & Green, Brett & Blachere, Francoise & Martin, Stephen & Law, Brandon & Jensen, Paul & Schafer, Millie. (2017). Sampling and characterization of bioaerosols.

Loo, E. X. L., Chew, L. J. M., Zulkifli, A. B., Ta, L. D. H., Kuo, I. C., Goh, A., Teoh, O. H., Van Bever, H., Gluckman, P.D., Yap, F., Tan, K. H., Chong, Y. S., Lee, B. W., & Shek, L. P. (2018). Comparison of microbiota and allergen profile in house dust from homes of allergic and non-allergic subjects- results from the GUSTO study. The World Allergy Organization journal, 11(1), 37. https://doi.org/10.1186/s40413-018- 0212-5

Madureira, J., Paciencia, I., Rufo, J. C., Pereira, C., Teixeira, J. P., & Fernandes, E. de O. (2015). Assessment and determinants of airborne bacterial and fungal concentrations in different indoor environments: Homes, child day-care centres, primary schools and elderly care centres.ATMOSPHERIC ENVIRONMENT, 109, 139– 146. https://doi.org/10.1016/j.atmosenv.2015.03.026

Murphy, J., Tharumakunarajah, R., Holden, K. A., King, C., Lee, A. R., Rose, K., Hawcutt, D. B., & Sinha, I. P. (2023). Impact of indoor environment on children's pulmonary health. Expert review of respiratory medicine, 17(12), 1249–1259.https://doi.org/10.1080/17476348.2024.2307561

Nandasena, S., Wickremasinghe, A. R., & Sathiakumar, N. (2013). Indoor air pollution and respiratory health of children in the developing world. World journal of clinical pediatrics, 2(2), 6–15. https://doi.org/10.5409/wjcp.v2.i2.6

Osborne, N. J., Thornton, C. R., & Sharpe, R. A. (2015). Indoor Fungal Exposure and Allergic Respiratory Disease. Current allergy and asthma reports, 15(12), 71. https://doi.org/10.1007/s11882-015-0572-7

Pegas, P. N., Alves, C. A., Evtyugina, M. G., Nunes, T., Cerqueira, M., Franchi, M., Pio, C. A., Almeida, S. M., Cabo Verde, S., & Freitas, M. C. (2011). Seasonal evaluation of outdoor/indoor air quality in primary schools in Lisbon. JOURNAL OF ENVIRONMENTAL MONITORING, 13(3), 657–667. https://doi.org/10.1039/c0em00472c8

Reham, F. E.-K., & Gamaleldin, N. M. (2020, dezembro 30). Prevalence of Bacteria in Primary Schools. Journal of Pure and Applied Microbiology. https://microbiologyjournal.org/prevalence-of-bacteria-in-primary-schools/

Seidel, D., Wurster, S., Jenks, J. D., Sati, H., Gangneux, J. P., Egger, M., Alastruey-Izquierdo, A., Ford, N. P., Chowdhary, A., Sprute, R., Cornely, O., Thompson, G. R., 3rd, Hoenigl, M., & Kontoyiannis, D. P. (2024). Impact of climate change and natural disasters on fungal infections. The Lancet. Microbe, S2666- 5247(24)00039-9. Advance online publication. https://doi.org/10.1016/S2666-5247(24)00039-9

Sharpe, R., Thornton, C. R., & Osborne, N. J. (2014). Modifiable factors governing indoor fungal diversity and risk of asthma. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology, 44(5), 631–641. https://doi.org/10.1111/cea.12281

Viegas, C., Caetano, L. A., & Viegas, S. (2021). Occupational exposure to Aspergillus section Fumigati: Tackling the knowledge gap in Portugal. Environmental Research, 194, 110674https://doi.org/10.1016/j.envres.2020.110674

WHO. (2022, outubro 25). WHO fungal priority pathogens list to guide research, development and public health action. https://www.who.int/publications-detail-redirect/9789240060241

WHO. (2023a). Air pollution. https://www.who.int/health-topics/air-pollution

WHO. (2023b). Health impacts. https://www.who.int/teams/environment-climate-change-and-health/air-quality-and-health/health-impacts

Xiao, W., Zhang, J., Huang, J., Xin, C., Li, M. J., & Song, Z. (2022). Response and regulatory mechanisms of heat resistance in pathogenic fungi. Applied microbiology and biotechnology, 106(17), 5415–5431. https://doi.org/10.1007/s00253-022-12119-2