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Original Paper

Comparative evaluation of bread types to identify a low-tech, accessible tool for airborne fungal monitoring

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Soshina Nathan*, Adithya S. Unni, P. Midhun, H. Abhishek, Mary Bincy

PG & Research Department of Zoology, Fatima Mata National College (Autonomous), Kollam-691001, Kerala, India

*Corresponding author, E-mail: soshina@gmail.com

Abstract

Airborne fungal contamination is a significant concern, especially in urban and healthcare environments. Conventional monitoring methods often require specialized equipment and aseptic techniques, limiting accessibility in resource-constrained settings. This study examines the feasibility of repurposing bread as an affordable fungal air monitoring tool. Bread samples of varying types and preservative compositions were exposed to different environments to assess fungal colonization. Statistical analyses revealed that preservative-free milk bread exhibited the highest fungal diversity, making it the optimal substrate for further assessments in public locations. Notable differences in fungal abundance and diversity were observed across sites, particularly between the hospital and other locations. Findings suggest that preservative-free milk bread can serve as a feasible and accessible fungal monitoring tool, with potential applications in air quality assessment. Future studies should refine sterilization techniques and validate its reliability through comparative analyses with conventional air sampling methods.

Key words: airborne fungal monitoring, bread, mould, public health, sustainability.

Introduction

Moulds encompass a diverse group of fungal organisms that thrive in damp environments, both indoors and outdoors. In humans, exposure to moulds has been associated with the worsening of asthma, allergic rhinitis, and, in rare cases, infection (Caillaud et al. 2018). Further, there has been a rise in healthcare-associated fungal infections with over 75 000 hospitalizations annually in the US due to infections like aspergillosis and candidiasis (Warren et al. 2024). Given these health implications, there is growing interest in assessing fungal air quality to better understand contamination patterns and mitigate risks.

Traditional fungal monitoring methods rely on specialized microbiological media such as Sabouraud dextrose agar or potato dextrose agar, which provide optimal nutrient conditions for fungal growth (Gebala, Sandle 2013; Acharya, Hare 2022; Benazir, Mohanka 2022; Ejimofor 2022). However, these media require aseptic handling and expensive laboratory equipment, making them impractical for field applications and resource-constrained settings.

Advances in fungal detection have led to the development of molecular and sensor-based technologies, including quantitative PCR, and biosensor platforms for fungal spore detection (Chandelier et al. 2021; Zhang et al. 2023; Thirugnanasambandam et al. 2024). A recent review by Memon et al. (2024) highlights how nanoscale biosensors offer high specificity, sensitivity and simplicity for real-time detection of airborne fungal spores and metabolites. However, these approaches are often limited by high costs, the need for specialized equipment and technical expertise, challenges in sensor stability and shelf-life etc, limiting their use in community-level monitoring or educational settings.

Bread, a widely available food product, shares several characteristics with conventional fungal growth media, particularly its carbohydrate-rich composition, which supports fungal colonization under appropriate environmental conditions (Garcia et al. 2019). The moisture-retaining capacity of bread facilitates fungal development (Axel et al. 2017), functioning similarly to laboratory media in promoting sporulation and colony formation. Additionally, studies have shown that typical airborne fungi such as *Aspergillus*, *Penicillium*, and *Rhizopus*, thrive on bread surfaces (Saranraj, Geetha 2012) indicating its potential use as a substrate for environmental fungal detection. While other low-cost fungal detection methods, such as gravity plates using homemade agar or open-Petri dish exposures, have been explored, these still require prepared media and sterile containers (Manibusan, Mainelis 2022; Maji et al. 2023), limiting accessibility in informal contexts.

This study evaluates the use of expired, visibly mould-free bread as a passive substrate for fungal air monitoring. By eliminating the need for conventional media preparation, this accessible and easy-to-use approach makes bread-based fungal surveillance more practical for students and citizen scientists without requiring extensive laboratory training. Typically discarded after its "best before" date, expired but uncontaminated bread can be redirected for scientific utility, aligning with sustainability goals by reducing food waste and promoting low-tech innovation in environmental monitoring. It was demonstrated that multispectral imaging detected fungal growth only where it was already visible, suggesting that there is no hidden fungal growth that the naked eye cannot see (Ollinger et al. 2022). Based on this, visibly mould-free bread could be considered as a "sterile" medium for detection of fungi.

The aim of this study was to evaluate the feasibility of using expired, visibly mould-free bread as a substrate for airborne fungal detection by comparing different expired bread types, with and without preservatives, across varied environments. This proof-of-concept study assessed fungal diversity and abundance supported by the different bread types to determine their suitability as a low-tech airborne fungal monitoring tool.

Materials and methods

Study area

The study areas for this work included Fatima Mata National College (BSc Zoology Laboratory, Girl's Washroom, Zoology Classroom, FMNC garden), Bishop Benziger Hospital (Dialysis Unit Corridor), Kollam Railway Station Platform 1 and Karbala Junction Road in Kollam. These different locations were chosen to assess fungal diversity and contamination levels across varied environmental conditions. Each location represented a distinct setting with different human activity levels, air quality, and environmental factors that could influence fungal colonization. The study was carried out from November, 2024 to April 2025.

Collection of bread

For the purpose of this study, different types of pre-sliced bread, packaged in sealed plastic sleeves were purchased on the date of expiry. The samples included whole wheat bread with preservatives, milk bread with preservatives, and milk bread without preservatives. Wheat bread was made with whole wheat flour and milk bread with refined wheat flour. Both wheat bread and milk bread contained milk solids, with milk bread having a higher concentration. The preservatives used in the breads were calcium propionate (E280) and sorbic acid (E200). In addition to preservatives, the breads had flour treatment agents, stabilizers, emulsifiers, acidity regulators and antioxidants. Milk bread without preservatives did not contain any preservatives or additional additives.

Methodology

The bread slices, used immediately after their date of expiry, were taken out with gloved hands, sprayed uniformly with sterile water to support the growth of fungi and placed in different environments where they were left exposed for half an hour. For each location, at least four slices of bread were kept as replicates. After exposure, the bread slices were kept in appropriately labelled ziplock bags using gloved hands and then placed in a large covered container in a dim environment at room temperature (~31 °C).

Visual observations of the bread were made daily for 2 to 8 days and the average number and colours of fungal colonies were recorded. Bread slices that remained unsprayed but were exposed for half an hour, along with slices that were sprayed with water but kept unexposed, were used as controls.

Analysis of the fungal colonies obtained on the bread samples

Macroscopic and microscopic observations were carried out on the mould growth obtained on the bread slices after incubation. For microscopic analysis, not more than 4-day-old fungal colonies were used to observe the intact fungal reproductive structures. The scotch tape method (Aneja 2007) was used for preparing temporary microscopic mounts of fungus without disturbing the arrangement of conidia and conidiophores. Briefly, a strip of clear cello tape was taken and the sticky side of the tape was pressed firmly onto the surface of the fungal colony, where sporulation was visible. The tape was then pulled gently away from the colony and was placed sticky side down in the centre of a clean glass slide.

The slide was then examined at 100× and 400× magnifications of the microscope. Representative images were captured using a mobile phone camera, occasionally employing digital zoom to enhance visual clarity. The fungi were identified with the help of the dichotomous keys and picture keys by Malloch (1981) available at the New Brunswick Museum Mycology webpages site http://website.nbm-mnb.ca/mycologywebpages/Moulds/Identification.html.

Statistical methods

Microsoft Office Excel 2016 was used to prepare graphs and calculate ANOVA. The online statistical tools at https://www.statskingdom.com/index.html were used for all other statistical analyses. In all the analyses, p value was set at $p = 0.05$. Some groups (milk bread and wheat bread, preservative-free vs preservative-containing bread) were subjected to parametric and non-parametric tests, while bread in different locations (road, hospital, garden, and railway) were subjected to parametric tests as they satisfied tests of normality.

Results

Milk bread vs wheat bread

This study explored the potential of repurposing expired bread for environmental fungal monitoring by examining the influence of bread type, preservatives, and environmental exposure. The primary objective was to determine the most suitable type of bread for monitoring environmental fungi, based on fungal abundance and diversity, and subsequently utilize it for airborne fungal surveillance in public spaces.

We first examined the influence of the type of bread, milk bread and wheat bread, both with preservatives, on the number and types (colours) of fungi that would grow when placed in similar environments. Green fungi, including yellowish-green and bluish-green varieties, were the most prevalent across both milk and wheat bread. Wheat bread harboured a higher presence of green and orange fungi, whereas milk bread exhibited greater abundance of green, white and yellow fungi. Black and pink fungi were exclusively observed on milk bread, while orange fungi appeared in high numbers solely on wheat bread (Fig. 1).

To assess differences in fungal growth between milk bread and wheat bread, we conducted Mann-Whitney U tests for fungal colony abundance (total number of colonies) and diversity (number of fungal types based on colour). The analysis revealed that fungal colony abundance did not significantly differ between bread types ($p = 0.4657$), indicating comparable levels of fungal growth across substrates. However, fungal colony diversity exhibited a statistically significant difference ($p = 0.03057$), suggesting that fungal species composition varied between milk bread and wheat bread. Since milk bread showed a greater diversity of fungi, it was selected for the subsequent experiment.

Milk bread with and without preservatives

In this experiment, we sought to determine which type of milk bread supports better fungal growth. While preservatives are known to inhibit fungal development, the extent to which different fungal types are affected remains uncertain. To investigate this, milk bread with and without preservatives were exposed to identical environmental conditions: lab, washroom, classroom, and ground, to compare fungal diversity and colony counts.

As expected, fungal growth emerged more quickly on preservative-free milk bread, appearing within three days, whereas on milk bread containing preservatives, at least five days were required for fungal development. To illustrate the variations in fungal growth, box and whisker plots were used to represent fungal colony counts and diversity across different environments (Fig. 2 A – D). Importantly, outliers were not removed in these plots to fully reflect the range of fungal variation observed across the samples.

Green fungi (bluish-green and yellowish green) were the most dominant followed by black fungi, in both the

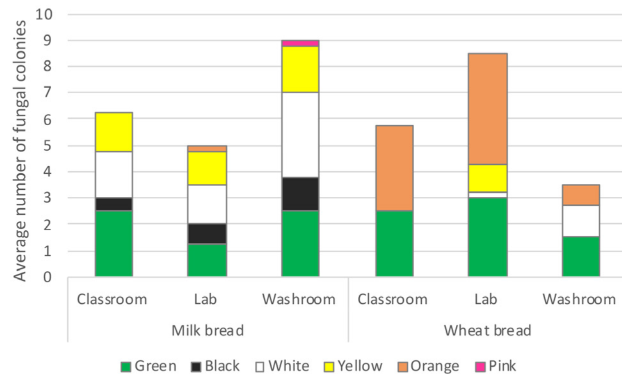

Fig. 1. Comparison of fungal diversity on milk bread and wheat bread (both with preservatives), showing the average number of fungal colonies for each fungal type (based on colour of the colony) across the classroom, lab and washroom.

preservative-free and preservative-containing bread, although their growth was significantly slowed down and less in the presence of preservatives. These fungi were microscopically identified as species of *Aspergillus* and *Penicillium*, which are known to have preservative resistance (Moro et al. 2022). White and yellow fungi were present in moderate numbers in both preservative-free and preservative-containing bread. Orange, pink, and brown fungi showed lower colony counts overall, with brown fungi being absent in the preservative containing milk bread (Fig 2 A and B).

In both preservative-free and preservative-containing milk bread (Fig. 2 C and D), the washroom and classroom consistently showed higher fungal colony counts, indicating that these locations have a higher concentration of airborne fungal spores. The lab exhibited the lowest fungal growth in both cases, suggesting a relatively controlled or cleaner environment with lower airborne fungal contamination. In the ground location, preservative-free bread showed the most variability, while in the case of preservative-containing bread, fungal growth was more controlled, reinforcing the role of preservatives in limiting fungal colonization. Overall, the data suggests that preservatives effectively reduced fungal growth, but the degree of reduction varied by fungal type, with green, black, and white types, exhibiting greater resilience compared to others.

Statistical analysis revealed that preservative-free milk bread supported a higher diversity of fungi ($p = 0.02401$), however there was no significant difference in the number of colonies observed between preservative-free and preservative containing bread ($p = 0.1517$). Given that milk bread without preservatives showed a higher fungal diversity, it was selected for use in the final experiment to assess fungal contamination in public places.

Preservative-free milk bread in different locations

In this experiment we assessed airborne fungal contamination in the road, hospital, garden and railway

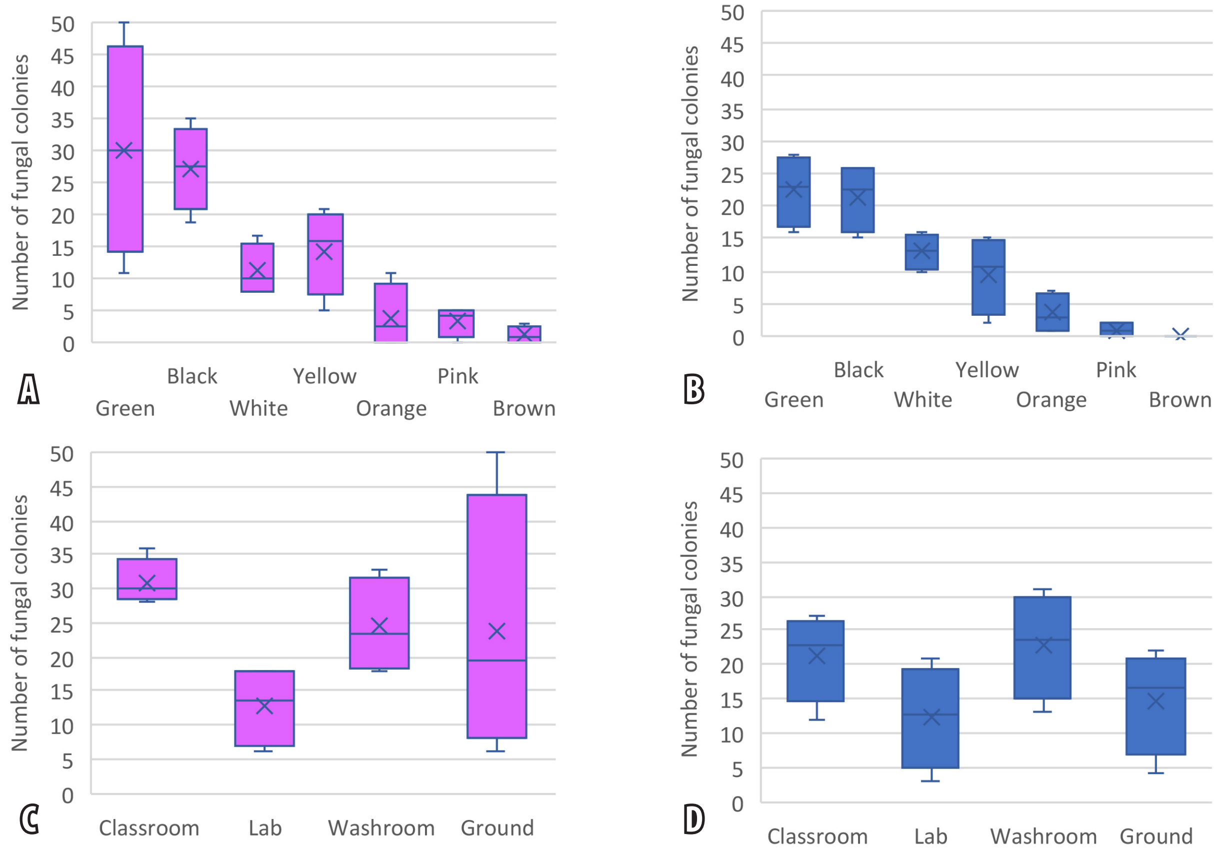

Fig. 2. Box and whisker plots showing diversity of fungal colonies on milk bread without (A) and with (B) preservatives and fungal abundance on bread without (C) and with (D) preservatives at different locations. The median, interquartile range, and outliers are shown, indicating the distribution and spread of fungal growth at each location: classroom, laboratory, washroom, and ground.

station using preservative-free milk bread. Black fungi were the most dominant across all locations while pink fungi were the least abundant. Blue-green, white and yellow fungi were observed in moderate numbers across all locations (Fig. 3). There was a highly significant difference between the number of types of fungi across the locations ($p = 0.00664$).

The highest fungal load was observed on the bread

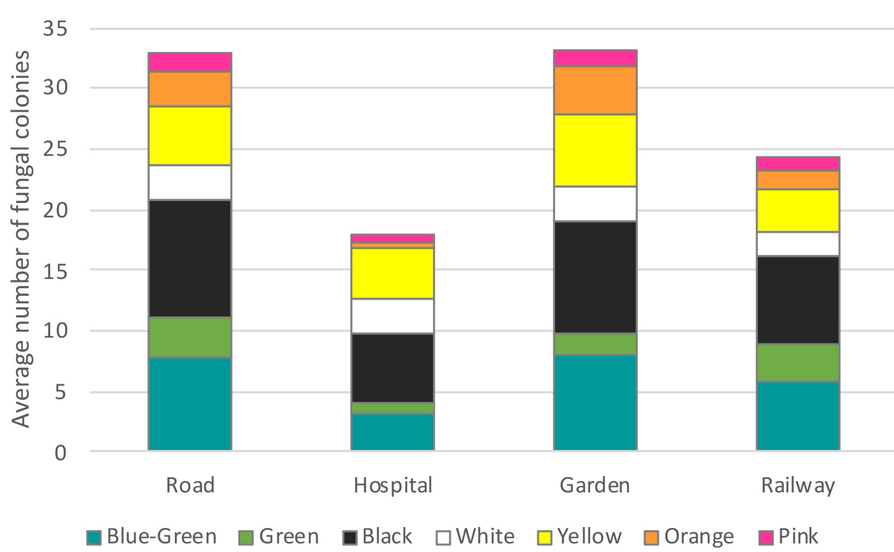

Fig. 3. Occurrence of fungi of different colours observed on milk bread without preservatives exposed to various locations: road, hospital dialysis unit corridor, garden, and railway station platform. The graphs represent the average number of colonies obtained from two sets of experiments.

slices kept in the garden and road likely due to high moisture content and pollution exposure respectively. The fungal load at the railway station was lower compared to the road and garden. Regular maintenance and exposure to moving air at the railway station could have limited spore settlement on the bread, reducing fungal growth compared to the other locations. Fungal contamination was least in the hospital compared to the outdoor locations, possibly due to sanitisation protocols and air filtration practices (Fig. 3, Fig. S1).

A one-way ANOVA was conducted to assess whether fungal colony abundance significantly differed among the four environmental locations: road, hospital, garden, and railway. The test revealed a statistically significant difference in fungal colony counts across locations (p = 0.005879), indicating that at least one site exhibited distinct fungal growth patterns. To determine which locations differed significantly, a post-hoc Tukey's HSD test was performed. The analysis identified significant differences between: Road vs. Hospital (p = 0.0145) and Hospital vs. Garden (p = 0.0127).

Microscopic examination of the fungi

In this study, the most common fungal colonies observed across the different locations were selected for detailed microscopic examination (Fig. 4). *Aspergillus* spp. were the most abundant fungus observed in our studies and exhibited rapid growth, forming powdery or velvety colonies with distinct pigmentation varying by species. The mould appeared in black, yellow-green, blue-green and tan colours possibly being *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus terreus*. *Aspergillus* species possessed septate, hyaline hyphae with erect and unbranched, septate or nonseptate conidiophores. At the tip of each conidiophore, a swollen vesicle was observed, supporting flask-shaped phialides arranged in single or double rows. The phialides produced small, round to oval conidia in chains, which varied in colour depending on the species. The *Mucor* species observed in this study exhibited rapid, cottony, or fluffy colony growth, which expanded quickly across the bread slices. The colonies initially appeared white and later turned grey or brown as sporulation progressed. *Mucor* sporangia were spherical,

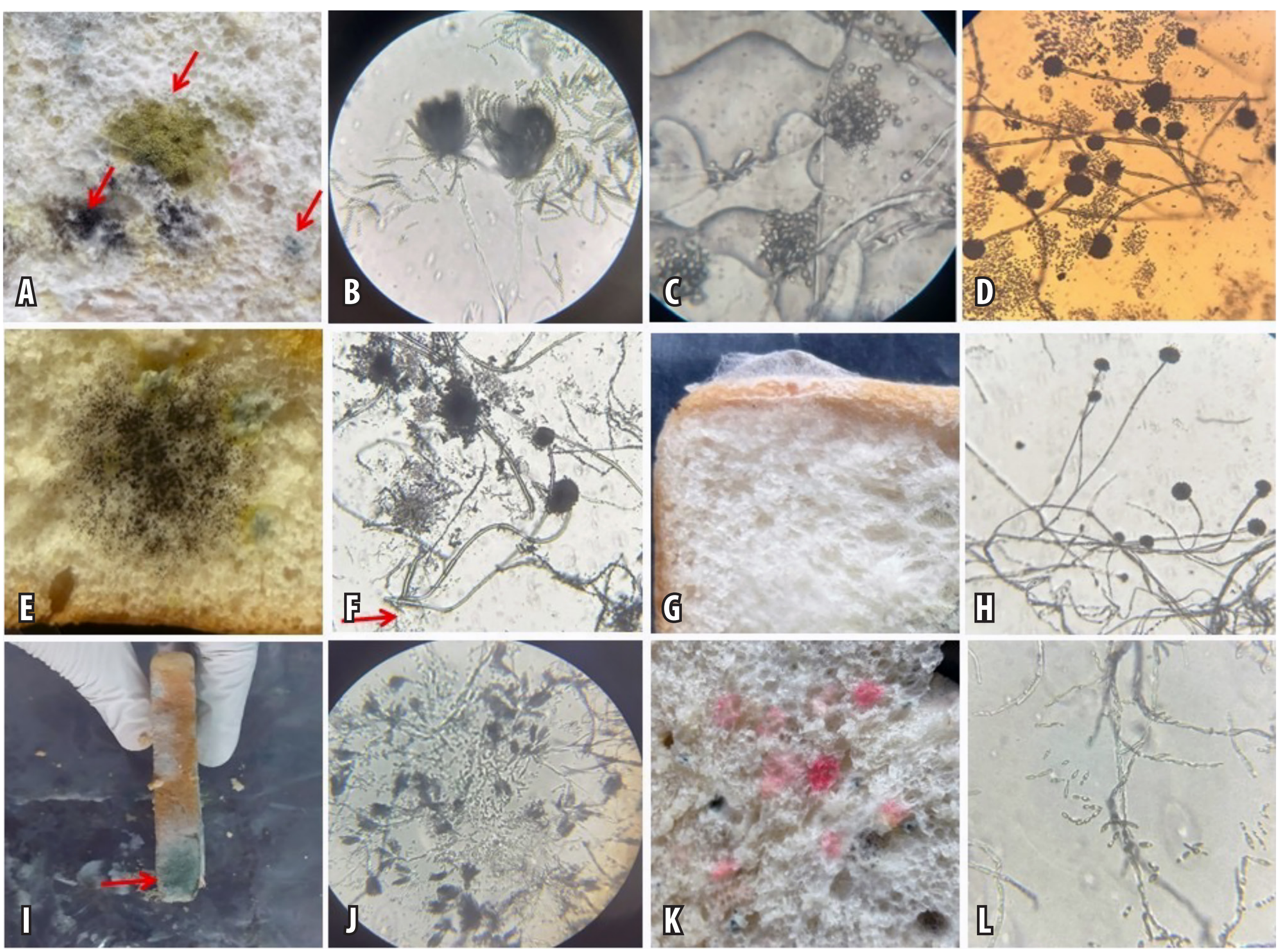

Fig. 4. Macroscopic and microscopic features of fungi isolated from bread samples. *Aspergillus* spp.: A, macroscopic view; B, C, D, microscopic images. *Rhizopus* spp.: E, macroscopic view; F, microscopic image. *Mucor* spp.: G, macroscopic view; H, microscopic image. *Penicillium* spp.: I, macroscopic view; J, microscopic image. *Fusarium* spp.: K, macroscopic view; L, microscopic image.

borne on long, unbranched sporangiophores, and contained numerous sporangiospores. The sporangiophore emerged singly, directly from the hypha without stolon and rhizoids. The *Rhizopus* species exhibited rapidly growing colonies with a dense, cottony texture. Initially white, the colonies darkened to grey or black with spore maturation. Microscopically, *Rhizopus* displayed broad, ribbon-like aseptate hyphae with distinctive stolons and rhizoids. *Penicillium* species exhibited a well-developed, branched network of septate hyphae. The conidiophores were erect, slender, and often branched, giving rise to characteristic brush-like structures. The conidia were produced in long, unbranched chains extending from the phialides. *Fusarium* colonies were pinkish red in colour. Under the microscope, the mycelium appeared branched and septate. The microconidia were oval, elliptical or curved in shape with one or two septae. The macroconidia were fewer in number and were linear or curved, septate and pointed at both ends. Each of these fungal types displayed consistent morphological features that supported identification through standard mycological keys (Malloch 1981).

Discussion

This study explored the feasibility of using expired bread as a low-cost substrate for airborne fungal monitoring, assessing the impact of bread type, preservatives, and environmental exposure on fungal growth. The analyses revealed significant differences in fungal diversity between different bread types and preservative conditions, indicating that the composition of the substrate plays a key role in fungal colonization. Additionally, variations in fungal abundance and diversity across public locations suggest that environmental factors strongly influence airborne fungal contamination patterns.

While overall fungal growth (abundance) was similar across milk bread and wheat bread, milk bread supported a broader range of fungi. This could be attributed to differences in nutrient composition and moisture retention, as suggested by previous studies indicating that mould growth varied based on bread type and its water activity (Hartog, Kuik 1984). It was reported that milk bread had a significantly higher fungal load (8×10^8 CFU g^{-1}) than wholemeal bread (7×10^7 CFU g^{-1}) (Garcia et al. 2019). Further, they showed that *Aspergillus* and *Penicillium* species were higher in milk bread than wheat bread with a greater variety of *Aspergillus* and *Penicillium* species in milk bread, reinforcing our findings that milk bread supports a more diverse fungal community than wheat bread. Additionally, milk bread without preservatives supported a broader fungal diversity and exhibited faster colony development, compared to milk bread with preservatives, aligning with previous studies indicating that preservatives inhibit fungal growth to varying degrees (Axel et al. 2017; Kareem 2023).

Our study found that dark-coloured fungi were the most prevalent on bread slices exposed in the road and garden environments (Fig. 3 and Fig. S1), which aligns with the results that dark-pigmented fungi, known for their resilience to pollution, were dominant in roadside soils (Kul'ko, Marfenina 2001). Several airborne fungal species, including *Aspergillus, Penicillium, Alternaria*, and *Mucor*, were detected across urban environments (Rivera-Tafolla et al. 2022), a trend consistent with our observations. While, the road and garden environments showed higher fungal loads, the hospital environment exhibited comparatively lower fungal contamination, reflecting sanitation measures that limit airborne microbial dispersal (Górzyńska et al. 2023). Compared to the road and garden environments, the railway station samples had comparatively lower fungal loads. As fungal spore dispersal in the atmosphere is influenced by terminal velocity, air turbulence, and eddy diffusion rather than simple gravitational settling, it is possible that the higher air turbulence due to frequent movement of trains, could have limited spore settling (Gregory 1945). Given this, the 30-min exposure duration used in this study may be appropriate for environments with relatively low air turbulence (e.g., indoor spaces with minimal airflow) but could limit fungal accumulation in outdoor or high-ventilation settings where spores remain airborne for longer periods. Increasing exposure time in outdoor environments or testing fungal accumulation rates across different durations (e.g., 30 min vs. 1 h) would help determine an optimal timeframe for spore deposition. Additionally, future studies should integrate additional environmental parameters such as air turbulence, wind speed, temperature fluctuations, or relative humidity measurements to better elucidate potential drivers of fungal distribution.

A drawback of using bread for airborne fungal monitoring is that bread supports only certain types of fungi and so has a limited specificity compared to other methods of fungal detection. Another key challenge in using bread for fungal air monitoring is the possibility of pre-existing spores affecting results. Even though only visibly uncontaminated expired bread was used, hidden fungal spores could still have been present. Since fungal growth was frequently observed in the unexposed and unsprayed control samples, distinguishing between pre-existing spores and those acquired from the air was not feasible. While initial contamination may have affected absolute fungal counts, analysing comparative fungal abundance trends across bread types and locations still provided meaningful ecological insights. Future studies should consider pre-treatment of bread, such as heat or radiation treatment, to minimize the influence of latent fungal spores. Further, larger sample sizes may be needed to capture subtle variations that might exist between environmental settings. To further refine this approach, comparative studies utilizing conventional air sampling techniques could be conducted to validate bread-based fungal monitoring against standard methodologies.

By incorporating pre-treatments and larger sample sizes, milk bread without preservatives could serve as a low-cost, reliable, and scalable tool for environmental fungal assessment. Its simplicity and effectiveness make it ideal for educational programs, citizen science initiatives, and preliminary airborne fungal surveillance, particularly in resource-limited settings.

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References

Acharya T., Hare J. 2022. Sabouraud agar and other fungal growth media. In: Gupta V.K., Tuohy M. (Eds.) *Laboratory Protocols in Fungal Biology: Current Methods in Fungal Biology*. 2nd Ed. Springer International Publishing, Cham, pp. 69–86.

Aneja K.R. 2007. *Experiments in Microbiology, Plant Pathology and Biotechnology*. 4th Ed. New Age International, Delhi.

Axel C., Zannini E., Arendt E.K. 2017. Mold spoilage of bread and its biopreservation: A review of current strategies for bread shelf-life extension. *Crit. Rev. Food. Sci. Nutr.* 57: 3528–3542.

Benazir F., Mohanka, R. 2022. Comparative influence of various nutritional media on mycelial growth and cultural characteristics of *Trichoderma harzianum. Int. J. Curr. Microbiol. Appl. Sci.* 11: 13–21.

Caillaud D., Leynaert B., Keirsbulck M., Nadif R. 2018. Indoor mould exposure, asthma and rhinitis: findings from systematic reviews and recent longitudinal studies. *Eur. Respir. Rev.* 27: 170137.

Chandelier A., Hulin J., San Martin G., Debode F., Massart S. 2021. Comparison of qPCR and metabarcoding methods as tools for the detection of airborne inoculum of forest fungal pathogens. *Phytopathology* 111: 570–581.

Ejimofor C.F. 2022. Screening of different culture media for growth development, and morphological characteristics of *Aspergillus niger. Biochem. Int.* 9: 37–44.

Garcia M.V., Bernardi A.O., Parussolo G., Stefanello A., Lemos J.G., Copetti M.V. 2019. Spoilage fungi in a bread factory in Brazil: Diversity and incidence through the bread-making process. F*ood. Res. Int.* 126: 108593.

Gebala B., Sandle T. 2013. Comparison of different fungal agar for the environmental monitoring of pharmaceutical-grade cleanrooms. *PDA J. Pharm. Sci. Technol.* 67: 621–633.

Gregory P.H. 1945. The dispersion of air-borne spores. *Transact. British Mycol. Soc.* 28: 26–72.

Górzyńska A., Ussowicz M., Biernat M., Nawrot U. 2023. Quantitative and qualitative airborne mycobiota surveillance in high-risk hospital environment. *Microorganisms* 11: 1031.

Hartog B. J., Kuik D. 1984. Mycological studies on Dutch rye bread. In: Kiss I., Deak T., Incze K.(Eds.) *Microbial Associations and Interactions in Foods.* Akademia Kiado, Budapest, pp. 241–246.

Kareem H.R. 2023. Isolation and identification of fungi responsible for the spoilage of bread and its determination of its shelf life. B.Sc. Project Report, Aliko Dangote University of Science and Technology.

Kul'ko A.B., Marfenina O.E. 2001. The distribution of microscopic fungi along Moscow roads. *Microbiology* 70: 613–616.

Maji P., Ghosh A., Banik A., Raj A. 2023. Comparison between Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) with banana peel (BP) for studying fungi in the air by settle-plate method. *Int. J. Sci. Dev. Res.* 8: 782–787.

Malloch D. 1981. *Moulds, their Isolation, Cultivation, and Identification.* Department of Botany, University of Toronto.

Manibusan S., Mainelis G. 2022. Passive bioaerosol samplers: A complementary tool for bioaerosol research. A review. *J. Aerosol. Sci.* 163: 105992.

Memon R., Niazi J.H., Qureshi A. 2024. Biosensors for detection of airborne pathogenic fungal spores: a review. *Nanoscale* 16: 15419–15445.

Moro C.B., Lemos J.G., Gasperini A.M., Stefanello A., Garcia M.V., Copetti M.V. 2022. Efficacy of weak acid preservatives on spoilage fungi of bakery products. *Int. J. Food Microbiol.* 374: 109723.

Ollinger N., Malachova A., Sulyok M., Schütz-Kapl L., Wiesinger N., Krska R., Weghuber J. 2022. Combination of DNA barcoding, targeted metabolite profiling and multispectral imaging to identify mold species and metabolites in sliced bread. *Future Foods* 6: 100196.

Rivera-Tafolla Y., Manrique-Soto R., Rico J.L., Sánchez-Yánez J.M. 2022. Detection of fungi and yeast in the air dust outdoors of the city of Morelia, Mich., Mexico. *Int. J. Family Community Med*. 6: 15–19.

Saranraj P., Geetha M. 2012. Microbial spoilage of bakery products and its control by preservatives. *Int. J. Pharmac. Biol. Arch.* 3: 38–48.

Thirugnanasambandam I., Vucurevich T., Shelton T., Kav N.N., Sheedy C., Laroche A., Challis J.K. 2024. Assessment of real-time PCR techniques for the detection of airborne fungal pathogens of wheat: role of DNA extraction on spore quantification. *ACS Agric. Sci. Technol.* 4: 1063–1073.

Warren B., Graves A.M., Barrett A., Nelson A., Stiegel M., Smith B.A., Schwartz I., Anderson, D.J. 2024. Comparative analysis of sampling and detection methods for fungal contamination on common healthcare environment surface materials. *Infect. Control Hospital Epidemiol.* 45: 1253–1256.

Zhang X., Song H., Wang Y., Hu L., Wang P., Mao H. 2023. Detection of rice fungal spores based on micro- hyperspectral and microfluidic techniques. *Biosensors* 13: 278.

Fig. S1. Representative samples of milk bread without preservatives showing fungal growth. The bread slices were exposed for half an hour at different locations (road, hospital dialysis unit corridor, garden, and railway station platform) and then incubated at room temperature in sealed ziplock bags for 3 to 4 days.

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