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Airborne Culturable Fungi in the Indoor and Outdoor Environments of Shrines in Chennai, India

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Abstract: The diversity and concentrations of airborne fungi in the environments of 58 temples across a metropolitan city (Chennai) in India were investigated. Air samples from indoors (within 2 m of the *Sanctum sanctorum*) and outdoors (at least 10 m away from the *Sanctum sanctorum*) were collected using the Reuter Centrifugal Sampler (RCS). Of the 90 species isolated, 7 belonged to Zygomycota, 5 to Ascomycota and the remaining 78 to Mitosporic fungi. A total of 3470 colonies were isolated from the indoor environment, which was 13.73% higher than the total recorded outdoors (3051 colonies). An average of 747.7 and 657.5 CFU/m³ of air was recorded in the indoor and outdoor environments, respectively. The predominant species identified in both environments were *Aspergillus flavus*, *A. niger* and *Cladosporium cladosporioides*. While most of the fungal species isolated are considered allergens and pathogens, they can also deteriorate the architecture of shrines. This study indicates the need to implement control measures to minimize the risks of exposure to bioaerosols in public spaces such as shrines.

Keywords: bioaerosols; aeroallergens; health hazards; portable air sampler



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1. Introduction

Shrines are religious or sacred spaces dedicated to the worship of deities, ancestors or revered figures. While the specifics of shrines vary, they play a crucial role in providing a physical space for spiritual practices, fostering a sense of community and preserving cultural and religious heritage. The architecture and etiquette followed in each shrine vary according to the religion, country, period of construction, etc. Within a shrine, there can be several structures, each designed for a different purpose. Among those, the *Sanctum sanctorum* is considered the most holy, with its access often limited to fewer people such as priests and staff. Worshippers typically congregate near a *Sanctum sanctorum* to worship deities and also spend time in other areas within the shrine. Environmental parameters (such as temperature, moisture and dampness), organic material used in shrines (such as flowers and fruits), deteriorating structures, other substrates (such as wooden objects), etc., can promote microbial proliferation in indoor environments. Among the microbes that are airborne, fungi and their metabolites (including mycotoxins) are associated with several respiratory ailments, including asthma, allergies, rhinitis, allergic bronchopulmonary mycoses, allergic fungal sinusitis, hypersensitivity pneumonitis, cancer, genotoxicity, mutagenicity, etc. [1–5]. Aside from their impact on public health, fungi can also lead to the deterioration of structures, depending on the environment [6,7]. For example, fungal species were found to deteriorate manuscripts in shrines [8,9], paintings and the surfaces of structures and walls [10].

From our literature search, there have been very few studies focused on the concentrations of fungi in shrines across the globe, and they are vastly different. The mean

concentrations of fungi in a Polish shrine ranged from 191 to 3237 CFU/m³ of air [11], while the fungal concentrations in a Malaysian shrine ranged between 118 and 660 CFU/m³ of air [12]. In a study conducted in a Korean shrine, the diversity of fungi differed drastically between indoor (19 genera) and outdoor (35 genera) environments, and the overall fungal load varied seasonally [13]. Similarly, the mean concentrations of fungi in the outdoor environment of a shrine in Saudi Arabia were higher than those indoors [14]. If the air exchange rates are high and the filter efficiency is poor, microbial concentrations can be similar in both indoor and outdoor environments [15]. Therefore, it is important to monitor diversity indoors and outdoors to enable proper recommendations to minimize exposure to bioaerosols. Moreover, shrines in India vary from those in other countries due to several factors including geography, the architecture of the building, environmental parameters, etc., which drive the need to monitor these environments.

This study focused on the diversity of airborne fungi across 58 shrines in southern India. Furthermore, the variations in total mean concentrations and diversity between the indoor and outdoor environments of shrines are reported.

2. Materials and Methods

2.1. Sampling Sites

Chennai (latitude 13.0827° N, longitude 80.2707° E) is one of the major metropolitan cities in India, with over 500 shrines corresponding to different religions. In this study, 58 shrines across Chennai were monitored for the presence of viable, airborne fungi. Samples from the indoor environments were collected within 2 m of the *Sanctum sanctorum* and the outdoor samples were collected at least 10 m away from the *Sanctum sanctorum* of each temple. Samples were collected in the evenings (after 4 pm IST) since people typically visit shrines during that time. In each shrine, air sampling was collected only once, as permitted. The locations of temples (names de-identified) where the air samples were collected are represented in Figure 1. At the time of conducting the experiments, there were ~3–10 people in the *Sanctum sanctorum* and ~10–95 people in the outdoor environment.

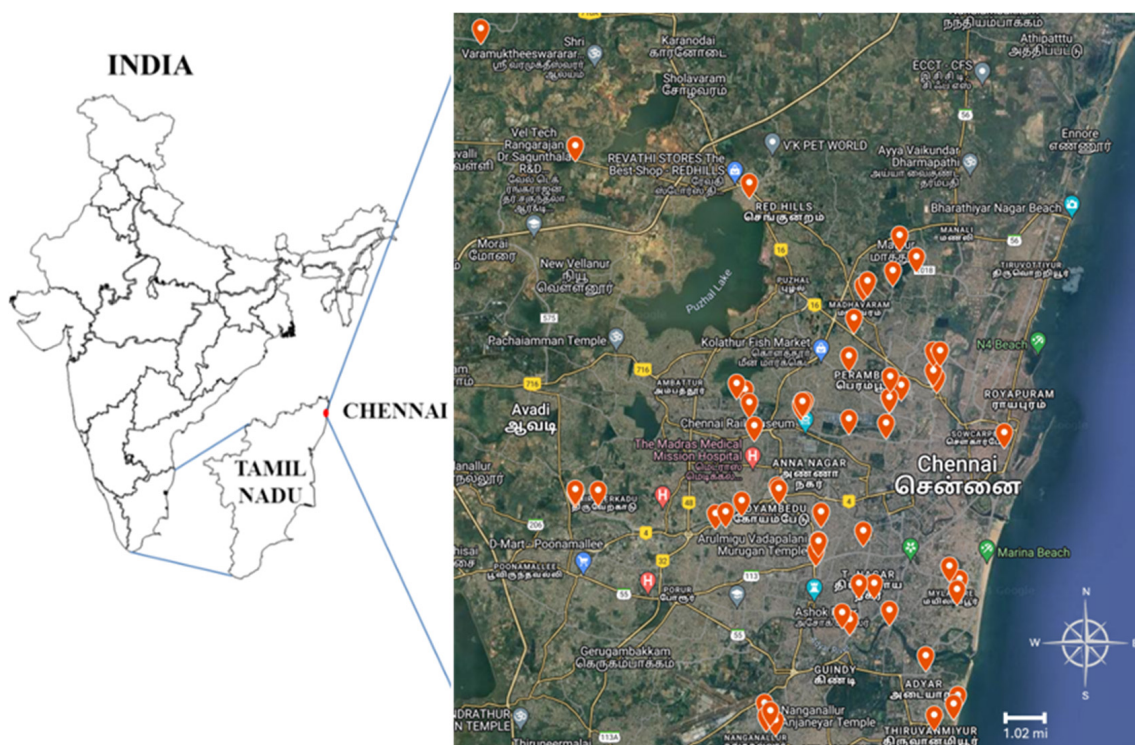


Figure 1. Map showing the shrines (as orange pins) where the samples were collected. (Visualization created on Google My Maps).

2.2. Air Sampling

Sterile air sampler strips (HiMedia, Mumbai, India) were loaded with sterile potato dextrose agar (PDA) amended with streptomycin under aseptic conditions in the laboratory [16]. The strips were then sealed in sterile pouches until they were inserted into the sampler at the sampling site, prior to the collection of air. A portable volumetric sampler, the Reuter Centrifugal Sampler (RCS) (Biotest AG, Dreieich, Germany), was used to collect air samples at 40 L of air per min (i.e., 0.04 m³ of air per min). The sampler, which collects particles by centrifugal force through an impeller and impacts particles on an agar strip, was held ~1.5–2 m from the ground by trained personnel and operated for 2 min. Before and after each sampling in the field, the sampler was disinfected with 70% alcohol and dried with sterile wipes. The exposed strips were sealed and transported to the laboratory in sterile, sealed pouches. The fungal colonies developed upon incubation of the exposed strips at room temperature (28 ± 2 °C) for 4–5 days were then counted. Identification of the fungal species was based on the morphological and microscopical features with reference to standard manuals [17–20].

2.3. Data Analyses

The following formulae were used to determine the average number of fungal colonies formed per cubic meter (CFU/m³) of air, percent contribution and isolation frequencies [21]:

$$\text{Colony forming units/m}^3 \text{ of air} = \frac{\text{Number of colonies}}{\text{Flow rate of the sampler (m}^3/\text{min)} \times \text{Sampling time (min)}} \quad (1)$$

$$\text{Percent contribution} = \frac{\text{Total CFU/m}^3 \text{ of an individual species}}{\text{Total CFU/m}^3 \text{ of all species}} \times 100 \quad (2)$$

$$\text{Isolation frequency} = \frac{\text{Number of samples in which a species was recorded}}{\text{Total number of samples}} \times 100 \quad (3)$$

The following biodiversity indices were measured to weigh the richness, evenness and distribution of the communities:

The Simpson index (D) measures the probability that any 2 individuals drawn at random from a community will be the same species. The higher the score, the more diverse the community is [22].

$$D = 1 - \sum_{i=1}^S P_i^2 \quad (4)$$

The Margalef index (D_{MA}) was used as a simple measure of species richness—a higher Margalef index value indicates greater species richness or diversity [23].

$$D_{MA} = \frac{S - 1}{\ln N} \quad (5)$$

The Pielou evenness index (J) represents the relative diversity, i.e., the ratio of diversity observed with observable maximum diversity with the same number of species, which is also the degree of equality in species abundance in a given sample [24].

$$J = \frac{H}{\ln S} \quad (6)$$

The Berger–Parker (Reciprocal) index (d) expresses the proportional abundance of the most abundant species—an increase in the value of the index accompanies an increase in diversity and a reduction in dominance [25].

$$1/d = \frac{N}{N_{\max}} \quad (7)$$

To compare community similarities (i.e., indoor and outdoor environments of shrines), Sorenson's coefficient (CC) was calculated. Complete community overlap is represented by a value of 1, while complete community dissimilarity is equal to 0 [26].

$$CC = \frac{2C}{S1 + S2} \quad (8)$$

In Equations (4)–(8), P_i represents the fraction of the entire population made up of “i” species; S is species richness; H is the Shannon diversity index; N_{max} is the number of individuals in the most abundant species; N represents the total number of species in the sample; C represents the number of similar species in both the settings (i.e., indoor and outdoor); S1 is the number of species indoors; and S2 is the number of species outdoors. One-way ANOVA and Chi square tests for CFU/m³ of species recorded in the indoor and outdoor environments were conducted at a significance level of 5% using GraphPad Prism (version 8.0.0).

3. Results

A total of 3470 and 3051 colonies were isolated from the indoor and outdoor environments of the shrines, respectively. The isolated colonies were classified into 90 species belonging to 46 genera, in addition to yeast and non-sporulating colonies. The isolated species belonged to three taxonomic groups, viz., Zygomycota (7 species), Ascomycota (5 species) and Mitosporic fungi (78 species), accounting for 7.78, 5.56 and 86.67% of the total species, respectively.

Among the genera isolated, *Aspergillus* was represented by 13 spp., followed by *Penicillium* (8 spp.), *Curvularia* (6 spp.), *Cladosporium* (5 spp.) and *Acremonium* (4 spp.). *Drechlera* and *Monodictys* were represented by three species, and the genera *Cunninghamella*, *Chaetomium*, *Chrysosporium*, *Fusarium*, *Gilmaniella*, *Nigrospora*, *Scopulariopsis*, *Trichoderma* and *Ulocladium* were represented by two species each. The list of fungal species isolated and their concentrations, percent contributions and isolation frequencies are presented in Table 1. The average concentrations and percent contributions of the dominant species are presented in Figures 2 and 3, respectively.

Table 1. List of airborne fungal species isolated and their average CFU/m³ of air, percent contribution (PC) and percent isolation frequencies (PIFs) recorded in indoor and outdoor environments of shrines.

Species	INDOOR			OUTDOOR		
	CFU/m ³	PC	PIF	CFU/m ³	PC	PIF
Zygomycota						
<i>Circinella umbellata</i>	0.22	0.03	1.72	0	0	0
<i>Cunninghamella echinulata</i>	1.08	0.14	1.72	0	0	0
<i>C. elegans</i>	0.22	0.03	1.72	0	0	0
<i>Lichtheimia corymbifera</i>	0.86	0.11	1.72	0	0	0
<i>Mucor racemosus</i>	6.03	0.81	12.06	1.29	0.20	3.44
<i>Rhizopus stolonifer</i>	0.65	0.09	1.72	0.43	0.07	1.72
<i>Syncephalastrum racemosum</i>	0.22	0.03	1.72	3.02	0.46	3.44
Ascomycota						
<i>Chaetomium globosum</i>	0.65	0.09	5.17	0.22	0.03	1.72
<i>Chaetomium</i> sp.	0	0	0	0.43	0.07	3.44
<i>Emericella nidulans</i>	12.07	1.62	53.44	18.97	2.88	32.75
<i>Sporormiella intermedia</i>	0	0	0	0.43	0.07	1.72
<i>Talaromycespurpureogenus</i>	0	0	0	0.22	0.03	1.72
Mitosporic fungi						
Coelomycetes						
<i>Phoma glomerata</i>	1.29	0.17	1.72	1.29	0.20	1.72

Table 1. Cont.

Species	INDOOR			OUTDOOR		
	CFU/m ³	PC	PIF	CFU/m ³	PC	PIF
Hyphomycetes						
<i>Acremonium blochii</i>	0.22	0.03	1.72	0	0	0
<i>A. hyalinulum</i>	0	0	0	0.43	0.07	1.72
<i>A. falciforme</i>	0.22	0.03	1.72	0	0	0
<i>A. strictum</i>	0.22	0.03	1.72	2.15	0.33	5.1
<i>Alternaria alternata</i>	0.86	0.12	5.17	1.72	0.26	10.32
<i>Arthrinium phaeospermum</i>	1.08	0.14	1.72	0	0	0
<i>Aspergillus clavatus</i>	0	0	0	0.43	0.07	3.44
<i>A. flavus</i>	341.39	45.73	79.31	309.9	46.99	74.13
<i>A. fumigatus</i>	1.72	0.23	6.89	4.74	0.72	5.17
<i>A. glaucus</i>	0.22	0.03	1.72	0	0	0
<i>A. japonicus</i>	9.69	1.30	17.24	12.06	1.84	20.68
<i>A. nidulans</i>	2.37	0.32	6.89	9.69	1.47	6.89
<i>A. niger</i>	172	23.03	82.75	149.1	22.61	89.65
<i>A. ochraceous</i>	0.65	0.09	1.72	2.37	0.36	6.89
<i>A. restrictus</i>	0.65	0.09	3.44	0.43	0.07	1.72
<i>A. sydowii</i>	0.22	0.03	1.72	0	0	0
<i>A. tamaraii</i>	5.17	0.69	17.24	2.37	0.36	5.17
<i>A. terreus</i>	14.87	1.99	37.93	7.33	1.11	31.03
<i>A. versicolor</i>	0	0	0	1.51	0.23	6.89
<i>Aureobasidium pullulans</i>	2.80	0.38	8.62	1.29	0.20	10.34
<i>Chrysonilia sitophila</i>	0.43	0.06	3.44	0.65	0.36	8.62
<i>Chrysosporium pannorum</i>	0.22	0.03	1.72	1.94	0.29	1.72
<i>C. tropicum</i>	0.86	0.12	1.72	0	0	0
<i>Cladosporium chlorocephalum</i>	0	0	0	0.86	0.13	1.72
<i>C. cladosporioides</i>	28.88	3.87	32.75	19.83	3.01	34.48
<i>C. herbarum</i>	1.94	0.26	5.17	0.43	0.07	3.44
<i>C. oxysporum</i>	1.08	0.14	1.72	1.72	0.26	3.44
<i>C. sphaerospermum</i>	5.17	0.69	18.96	2.16	0.33	10.34
<i>Curvularia brachyspora</i>	2.59	0.35	8.69	1.94	0.29	3.44
<i>C. clavata</i>	1.51	0.20	10.34	3.66	0.56	10.34
<i>C. eragrostidis</i>	0	0	0	1.51	0.23	3.44
<i>C. lunata</i>	9.27	1.24	27.86	7.54	1.14	22.41
<i>C. pallescens</i>	0.22	0.03	1.72	0	0	0
<i>C. penniseti</i>	0	0	0	0.65	0.20	1.72
<i>Drechslera australiensis</i>	2.37	0.32	10.34	2.16	0.33	10.34
<i>D. halodes</i>	0.43	0.06	1.72	1.08	0.16	3.44
<i>D. hawaiiensis</i>	0.43	0.06	3.44	0.22	0.03	1.72
<i>Fusarium moniliforme</i>	0.43	0.06	3.44	0	0	0
<i>F. oxysporum</i>	10.56	1.41	17.24	6.47	0.98	6.89
<i>Geomyces pannorum</i>	1.29	0.17	1.72	0	0	0
<i>Gilmaniella humicola</i>	0.43	0.06	1.72	1.29	0.20	3.44
<i>Gilmaniella</i> sp.	0	0	0	0.65	0.10	1.72
<i>Humicola grisea</i>	0.65	0.09	3.44	1.51	0.24	8.69
<i>Memnoniella echinata</i>	0.43	0.06	1.72	0.22	0.03	1.72
<i>Microsporum nanum</i>	0.22	0.03	1.72	0	0	0
<i>Moniliella suaveolens</i>	0.22	0.03	1.72	0	0	0
<i>Monodictys castaneae</i>	0.43	0.06	3.44	0.22	0.03	1.72
<i>M. glauca</i>	0.22	0.03	1.72	0	0	0
<i>M. levis</i>	0	0	0	0.43	0.07	1.72
<i>Nigrospora oryzae</i>	0	0	0	0.22	0.03	1.72
<i>N. sphaerica</i>	0	0	0	1.08	0.17	1.72
<i>Paecilomyces variotii</i>	1.51	0.20	3.44	0.22	0.07	3.44
<i>Penicillium chrysogenum</i>	0.22	0.06	3.44	0.22	0.03	1.72
<i>P. citrinum</i>	1.08	0.20	8.62	4.74	0.72	20.68
<i>P. digitatum</i>	0.86	0.03	1.72	0.22	0.03	1.72

Table 1. Cont.

Species	INDOOR			OUTDOOR		
	CFU/m ³	PC	PIF	CFU/m ³	PC	PIF
<i>P. funiculosum</i>	1.08	0.14	6.89	2.16	0.33	6.89
<i>P. oxalicum</i>	0.22	0.12	3.44	6.47	0.98	13.79
<i>P. polonicum</i>	0	0	0	0.22	0.03	1.72
<i>P. variabile</i>	0.22	0.03	1.72	0	0	0
<i>P. verruculosum</i>	0	0	0	0.65	0.10	3.44
<i>Periconiella smilais</i>	0	0	0	0.43	0.07	1.72
<i>Periconia byssoides</i>	0	0	0	0.22	0.03	1.72
<i>Scolecobasidium humicola</i>	0.22	0.03	1.72	0	0	0
<i>Scopulariopsis brevicaulis</i>	0.22	0.03	1.72	0	0	0
<i>S. brumptii</i>	0	0.03	1.72	0	0	0
<i>Spegazzinia labulata</i>	0.22	0.03	1.72	0	0	0
<i>Sporothrix schenckii</i>	0.22	0.03	1.72	0	0	0
<i>Stachybotrys atra</i>	0	0	0	0.22	0.03	1.72
<i>Thielaviopsis paradoxa</i>	0.22	0.03	1.72	0	0	0
<i>Torula graminis</i>	0	0	0	0.22	0.03	1.72
<i>Trichocladium canadense</i>	0.22	0.03	1.72	0	0	0
<i>Trichoderma harzianum</i>	6.68	0.89	1.72	0	0	0
<i>T. viride</i>	0.86	0.12	3.44	1.94	0.29	5.17
<i>Trichometasphaeria holmii</i>	0	0	0	0.22	0.03	1.72
<i>Trichophyton mentagrophytes</i>	0.43	0.06	1.72	0	0	0
<i>Ulocladium botrytis</i>	0.22	0.03	1.72	0.22	0.03	1.72
<i>U. chartarum</i>	0.43	0.06	1.72	0	0	0
Yeast colonies	57.33	7.68	34.48	28.23	4.28	31.03
Non-sporulating colonies	25.43	3.41	39.65	20.69	3.14	44.82

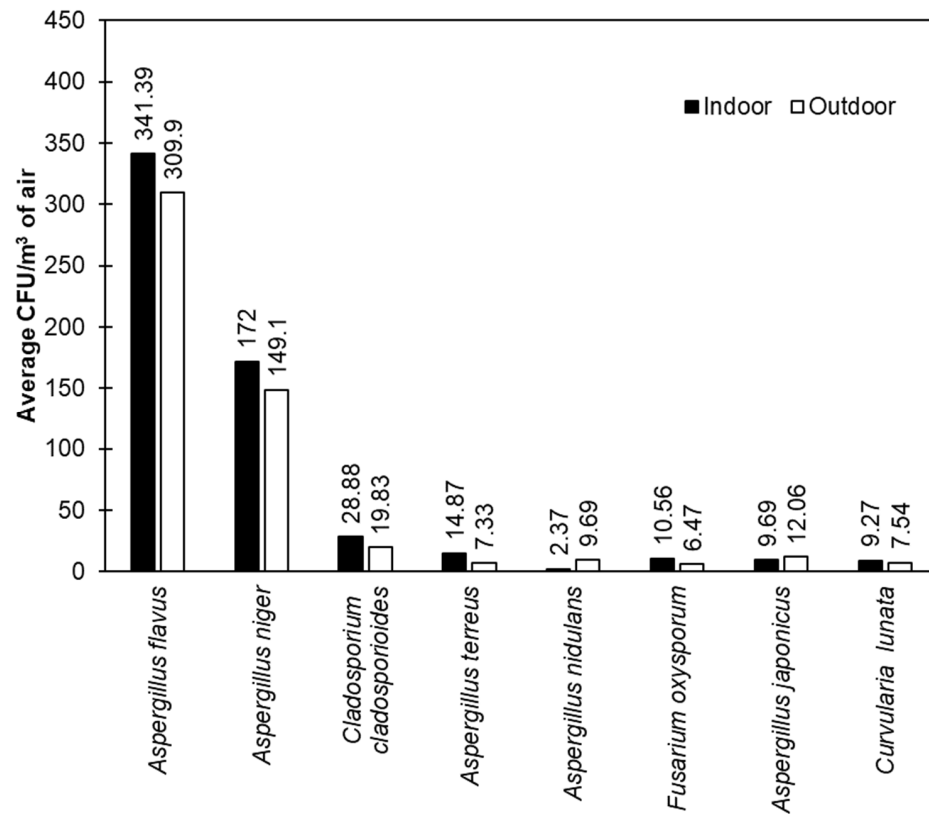


Figure 2. Average fungal concentrations recorded for the dominant species isolated from air samples of indoor and outdoor environments of shrines.

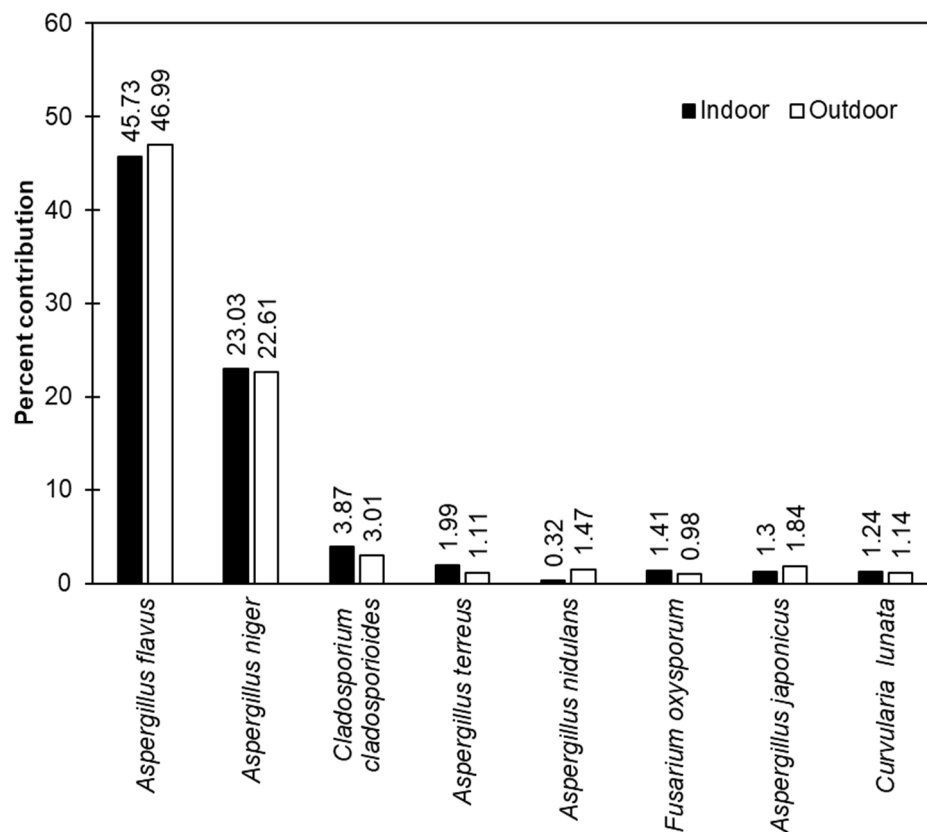


Figure 3. Percent contributions of the dominant species isolated from the air samples of indoor and outdoor environments of shrines.

Irrespective of the site within a shrine (i.e., indoor or outdoor), the diversity of fungi was found to be rich, as analyzed by the Simpson index of diversity and Berger–Parker dominance. Overall, the total average concentration in the outdoor environments was 657.5 CFU/m^3 , which was $\sim 88\%$ of that recorded indoors (747.7 CFU/m^3). A significant difference was observed for the concentrations of fungal species isolated in the indoor and outdoor environments, with a p -value less than 0.0001 according to the Chi-square test and one-way ANOVA ($R^2 = 0.9946$). *Aspergillus flavus* was found to be the dominant species in both indoor and outdoor environments. This fungus alone recorded an average of 341.3 CFU/m^3 (45.72% to the total) and 309.9 CFU/m^3 (46.99% to the total) in indoor and outdoor environments, respectively. *Aspergillus flavus*, *A. niger* and *Cladosporium cladosporioides* had respective average concentrations of 341.39, 172 and 28.88 CFU/m^3 in the indoor environments, while they were 309.9, 149.1 and 19.83 CFU/m^3 in the outdoor environments. The average concentration of yeast colonies was 57.3 CFU/m^3 indoors, while it was 28.2 CFU/m^3 outdoors. Non-sporulating colonies accounted for 25.4 and 20.7 CFU/m^3 in the indoor and outdoor environments, respectively.

Within the indoor environments, 70 species belonging to 38 genera were recorded in total, while 63 species (belonging to 33 genera) were recorded in outdoor environments. The Pielou evenness index showed moderate evenness between the indoor and outdoor environments, while the similarity between these environments was 0.65 according to the Sorenson coefficient. As can be seen in Table 1, 27 species were unique to the indoor environments, 20 were unique to the outdoor environments and 43 species were common to both.

Among the species identified, *Aspergillus niger*, *A. flavus* and *Emericella nidulans* were isolated from 82.75, 79.31 and 53.44% of the air samples collected from the indoor environment, whereas *A. niger*, *A. flavus* and *Cladosporium cladosporioides* were prevalent in the outdoor environments, with isolation frequencies of 89.65, 74.13 and 34.48%, respectively.

According to the Margalef index, the species richness was comparatively higher in indoor environments than outdoors. The values for the statistical indices calculated in the indoor and outdoor environments of the species isolated are presented in Table 2.

Table 2. Biodiversity indices of the airborne mycoflora in shrines.

Biodiversity Index	Indoor	Outdoor
Simpson index of diversity	0.7278	0.7222
Margalef index	8.4661	7.7247
Pielou evenness index	0.4639	0.4916
Berger–Parker Dominance (Reciprocal)	2.1869	2.128
Sorenson coefficient		0.6466

4. Discussion

Studies on airborne fungi in shrines, though rare, provide information on exposure risks that is critical for public health. In this study, 90 species of viable airborne fungi were isolated from the indoor and outdoor environments of 58 shrines across Chennai city in India, which, to our knowledge, is the largest number to be reported in places of worship. Previously, six fungal species were isolated from air samples collected using a liquid impinger operated at 0.25 L/min in the indoor and outdoor environments of a shrine in India [27]. Using the Burkard volumetric sampler, 12 species were isolated from five other Indian shrines [28]. The use of the RCS is a significant advantage in this study, as the RCS can be operated at a high flow rate (40 L/min), while also being portable. The performance of the RCS in collecting bioaerosols has been compared with that of other portable air samplers such as the Andersen cascade impactor [29,30], BioStage impactor [31], slit-to-agar air sampler [32], Air-O-cell sampler [33], membrane filter [34], slit and SAS samplers [35], BioSampler, electrostatic sampler, gelatin filter, mixed cellulose ester filter and gravitational settling methods [36]. However, it is inconclusive as to which sampler is superior for bioaerosol collection since several factors, such as the sampler flow rate, sampling time, aerosol concentrations, medium characteristics (such as moisture retention), particle bounce, sampling environment, etc., can influence the collection efficiency. Nevertheless, most of the studies that used the RCS acknowledge these advantages—portability, no requirement of an external power source (battery operated), ease of disinfection and less noise [29–36]. These features favored the use of the RCS in this study.

In the indoor environments of shrines, a higher average CFU/m³ of air could be attributed to (1) the presence of organic substances (such as flowers, fruits, etc.); (2) microenvironments (like moisture, temperature and humidity); and (3) low air exchange with the outdoors (since the indoor spaces were mostly concealed). Among the fungi isolated, the genera *Aspergillus* and *Penicillium* accounted to about 74% of the total. The abundance of the genera *Aspergillus* and *Penicillium* has been reported in different indoor environments [37–39]. Of the species belonging to *Aspergillus*, *A. flavus* and *A. niger* were present in over 75% of the samples collected in this study. The inhalation of such species can lead to Aspergillosis [40], allergic rhinitis, allergic alveolitis, bronchitis and asthma [41], and it can also impact the gut microflora [42]. Moreover, certain species of fungi produce toxins (for example, aflatoxins produced by *A. flavus*) which can also cause health issues such as cancer, genotoxicity, mutagenicity, etc. [3–5]. Fungi belonging to Zygomycetes; yeasts; and species of *Cladosporium*, *Chrysosporium* and *Ulocladium* were also found to be higher in the indoor environment than outdoors. These fungi have been reported as damp-loving fungi [43]. In general, the volatile organic compounds released by fungi in microclimatic environments are found to cause various health effects like dizziness, headaches, and an inability to concentrate [44]. Aside from inhalation, dermal contact to fungi also has health effects. In this study, keratinophiles (such as *Chrysosporium tropicum*, *Scopulariopsis brevicaulis* and *S. brumptii*) and dermatophytes (such as *M. nanum*, *S. schenkii* and *T. mentagraphytes*) were also isolated from a few samples. Diagnosing and treating dermatophyte infections is usually challenging [45], and the air sampling approach as followed in this study can

serve as a non-invasive approach for screening environments that pose risks of exposure to dermatophyte infections. Aside from health effects, airborne fungi can also degrade or deteriorate paintings and sculptural artifacts, subsequently affecting the aesthetics of shrines [46].

Among the colonies isolated, 47.7% (43 species) of fungal species were found in both indoor and outdoor environments, which suggests low air change rates within shrines, though details on HVAC systems or air change rates were unavailable. Nevertheless, enhancing the ventilation by using air purifiers as needed for the area, maintaining the cleanliness of the shrines, maintaining the temperature and humidity as needed (by using an air conditioning system and humidifier/dehumidifier, respectively) and limiting the number of people and their mobility visiting shrines at a given time are recommended. It is also important to minimize the use of materials that contribute to dampness and excess moisture in indoor environments, as they can initiate the deterioration of materials and lead to the proliferation of microbes [47]. In addition, personal protective measures such as wearing masks, using hand sanitizers, minimizing exposure duration, etc., are ways to protect the priests and staff of the shrines, as well as the visitors.

This study has certain limitations. Firstly, non-culturable fungal spores and certain fungi belonging to Ascomycota and Basidiomycota did not form spores in the nutrient medium used in this study. Therefore, they were classified as non-sporulating colonies. Due to resource limitations, it was not possible to identify specific species of yeasts cultured, and hence, they were broadly grouped as yeast colonies. Also, the yeast/fungi that could have been inactivated during sample collection remain unknown. Future studies on identification by whole-genome sequencing would provide information on the microbiome of a sample. Secondly, neither measurement of the aerosol size distribution nor the size-fractionated collection of particles containing fungi/fungal spores were conducted in this study. Pairing aerosol measurement devices with air samplers capable of collecting size-fractionated particles could provide information on the transport and fate of particles in the air as well as potential respiratory deposition pattern. Thirdly, the results presented in this study cannot be generalized to all shrines in India or elsewhere in the world, since the collection and isolation of fungi depends on several factors such as the source, architecture of the building, wind speed, air sampler used, nutrient medium used for culturing and environmental parameters (such as temperature, relative humidity, etc.). Changes in any of these parameters may lead to results different from those presented in this study. Nevertheless, the study provides insights into the fungal diversity and concentrations in 58 different shrines and the significance of implementing suitable measures to minimize bioaerosol exposures in such public spaces.

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