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RESEARCH ARTICLE

AIRBORNE MYCODIVERSITY IN THE INDOOR ENVIRONMENTS OF DHANVANTRI LIBRARY OF JAMMU UNIVERSITY (INDIA)

S.P. Pourush Shrikhandia and Geeta Sumbali*

University of Jammu, B.R. Ambedkar Road, Jammu-180006 (India)

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ABSTRACT

Mycobial contamination in the indoor environments of libraries is a world-wide problem. These buildings are not only the store house of knowledge in the form of books, manuscripts, etc., but may also serve as conducive habitat for proliferation of this diverse group of fungal organisms due to ambient environment of temperature and humidity. These airborne bioparticles are not only responsible for deterioration and ageing of books and other important documents in the library but may also significantly affect the health of library users. In view of this, aeromycological studies were conducted to enumerate the mycodiversity that is associated with three indoor sites viz., stack areas, reading rooms and newspaper section of Dhanvantri library, which is the central library of University of Jammu. The main objective of the study was to find out the fungal flora at these places and its impact on the library materials, which are stored/processed there. By using settle plate method and modified Czapek Dox Agar medium supplemented with rose-bengal (0.2g/l) and streptomycin sulphate, 17 fungal species belonging to 8 genera were recovered. Four measures of diversity are considered viz., species richness (*S*), Shannon-Wiener's diversity index (*H'*), Simpson Diversity index (*1-D*) and Sorenson's similarity index. The values so obtained showed that there is homogenity in the mycodiversity of all the three sites.

INTRODUCTION

Mycoflora constitutes an essential part of biological diversity on this planet. Among the various known groups of microorganisms, fungi occupy a prime place as these are ubiquitous, frequently airborne, highly variable, constituting a significant fraction of the airborne bioparticles (Abu-Dieyeh *et al*., 2010). They are almost inevitable in most of the enclosed environments like museums, homes, libraries, industries, hospitals, schools, almirahas, humidifiers, cooling towers, etc.,(Jones and Harrison, 2004). However, transport and ultimate settling of fungal spores is affected by their physical properties and the environmental parameters that it encounters. In recent years, sampling and analysis of airborne fungi has received lot of attention due to the fact that their spores frequently contaminate indoor environments and significantly affect the health of residing individuals. Lack of incoming fresh air due to increased insulation of buildings, poorly maintained or operated ventilation systems, poorly regulated temperature and relative humidity levels contribute to the presence and multiplication of fungal spores.

In view of the increased awareness, study of fungi present in the air has become important and the study of aerobiology has acquired a prominent place in the field of environmental science. Two main features of fungi, that is, their ease of dispersion and their hydrolytic enzyme activity makes them one of the chief agents of deterioration. The physical characteristics of fungal spores (size, shape and density), presence of airborne water droplets and congenial environmental factors, which include magnitude of air currents, relative humidity and temperature, are some of the important factors, which determine the capacity of fungal spores to be airborne. However, their pathogenic ability depends on the quality and condition of the buildings as well as on the time of exposure to these airborne spores. In case of libraries, which are huge collection of books and periodicals and have a high human occupancy, the presence of fungal spores in their environment have a direct influence on the condition of the books as well as on the library users (Karvala *et al*., 2010). Apart from these, human beings are also an important source of airborne microorganisms in the indoor environments (Pastzuska *et al*., 2000).The exposure to airborne fungal spores may lead to allergic reactions like allergic sinusitis and asthma (Kurup and Banerjee, 2000; Crameri *et al*., 2006) and diseases like sick building syndrome (Takigawa *et al*., 2009). Among the allergy causing indoor fungi are included many common airborne species of *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria* (Shen *et al*., 2007).

The Central Library of University of Jammu, which is called Dhanvantri Library, was set up in 1969. It is the largest library of Jammu and is the hub of academic activities where hundreds

Corresponding author:* **S.P. Pourush Shrikhandia
University of Jammu, B.R. Ambedkar Road, Jammu-180006 (India)

of students spend several hours studying from morning to evening throughout the year. It is centrally located, four storey building, with spacious reading halls and compact stack areas. The library has three main reading halls and additional reading space in the textbook section, periodical section, reference section and newspaper section. All these areas provide reading facilities for about 500 readers. This library is one of the largest repositories, being the main research source for historians, politicians, scientists, writers and students of Jammu. In view of the fact that Dhanvantri Library is the Central Library of University of Jammu, an investigation on the mycodiversity of indoor air was conducted to characterize the indoor mycobiota in a library building, to identify the most abundant species and to assess fungal biodiversity.

MATERIALS AND METHODS

Sampling of indoor air was done at three different sites, that is, stack areas, reading halls and newspaper section. A total of 36 samples were collected, 12 from each site during survey period by using culture plate exposure method as done by Stryjakowska-Sekulska *et al.* (2007). In this method, Petri-plates containing modified Czapek Dox Agar medium supplemented with rose-bengal (0.2g/l) and streptomycin sulphate were exposed for 5 minutes at 3, 5 and 7 feet above the ground level, brought to the laboratory in sterilized polythene bags and then incubated at 28 ± 2º C. After an incubation period of 5 days, the Petri-plates were examined periodically for any sign of fungal growth. The colonies appearing on agar plates were counted and recorded as percentage for individual species employing standard formula (Kalbende *et al*., 2012). Isolation and purification of the fungal species trapped from the air was done by streaking on the potato dextrose agar (PDA) medium plates, supplemented with streptomycin sulphate. The species were identified on the basis of cultural and morphological characters and finally authenticated by authority. Percentage colonization frequency (CF %), relative abundance and colony forming units (cfu/m^3) were calculated for each fungal species from various sites by using the following formulae:

$$CF\ (\%) = \frac{\text{Number of positive samples}}{\text{Total number of samples}} \times 100$$

$$\text{Relative abundance} = \frac{\text{Number of occurrence of species}}{\text{Total number of occurrences}} \times 100$$

Colony forming units (cfu/m^3) were calculated by following Koch sedimentation method recommended by Polish Standard (Stryjakowska-Sekulska *et al*., 2007)

cfu / m^3= a x 10000/p x t x 0.2

where,
a – the number of colonies on the Petriplate.
p – the area of the Petriplate.
t – the time of exposure of the Petriplate.
The data was used in making comparison of different diversity indices (Table1). Shannon–Wiener index (*H'*) (Shannon and Wiener, 1963) and Simpson's diversity index (*1-D*) (Simpson, 1949) were calculated to determine the heterogeneity of sampled area. The similarity between the sites was also calculated on the basis of fungal flora by applying Sorenson's similarity index (Krebs, 1989).

Table 1 Diversity indices

Diversity indices	Formula used
Shannon–Wiener index (*H'*)	$-\sum_{i=1}^{s} p_i \ln p_i$
Simpson's diversity index (*1-D*)	$\sum_{i=1}^{s} (p_i)^2$
Sorensen's similarity index	2C / S1 + S2

where p_i is the relative importance value of species *i* ; H'_{max} is the maximum value of *H'*; C is the number of common species and S1 and S2 are the total number of species in two sites.

RESULTS AND DISCUSSION

During the survey, a total of 36 indoor air samples, 12 each from three sites viz., stack areas, reading rooms and newspaper section were screened for mycodiversity. In all 17 fungal species belonging to 8 genera (*Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Mucor, Penicillium and Rhizopus*) were recovered. Of these, *Aspergillus* was represented by maximum number of species (8), which accounted for the bulk of the recovered mycodiversity in all the three sites (Figure 1), with maximum distribution in the reading rooms (54.13%), followed in decreasing order by stack areas (47.38%) and newspaper section (45.95%). Burge *et al.* (1978) who made an aerometric survey of fungi in eleven libraries at the University of Michigan also found the predominance of *Aspergillus* species. In fact, the predominance of *Aspergillus* species in the indoor atmosphere has been attributed to their ability to grow on many substrates (Adams *et al*., 2013; Luka *et al*., 2014)). *Cladosporium* species were next in decreasing order showing maximum distribution in newspaper section (14.64%). *Cladosporium* is a cosmopolitan genus, which is abundant in air samples collected in many areas of the world (Kayarkar and Bhajbhuje, 2014). High concentrations of *Cladosporium* spores in the indoor air are known to cause allergic diseases in a number of people (Gonclaves *et al*., 2010). Similarly, two species of *Penicillium*, that is, *P. brevicompactum* and *P. chrysogenum* were recovered from all the three sites of indoor air sampling. In addition to *Aspergillus*, *Cladosporium* and *Penicillium* species, other fungal species trapped from indoor air of Dhanvantri Library included *Fusarium solani*, *Alternaria alternata*, *Curvularia lunata*, *Mucor hiemalis* and *Rhizopus stolonifer* (Table 2). All these fungal species have been reported earlier also from the indoor air of libraries and archives situated in Cuba (Rojas *et al*., 2002; Borrego *et al*., 2010), Ethiopia (Hayleeyesus and Manaye, 2014), Poland (Pastuszka *et al*., 2000; Stryjakowska-Sekulska *et al*., 2007; Zielinska-Jankiewicz *et al*., 2008), India (Vittal and Glory, 1985; Tripathi, 1985; Singh *et al*., 1995; Jain,2000; Kayarkar and Bhajbhuje, 2014).

Table 2 Colonization frequency (CF%), abundance and colony forming units (cfu/m^3) of the recovered mycodiversity.

Recovered fungal species	Stack areas			Reading rooms			Newspaper section		
	CF(%)	Abundance	cfu/m^3	CF (%)	Abundance	cfu/m^3	CF (%)	Abundance	cfu/m^3
Alternaria alternata	75	8.74	32.24	66.67	6.79	19.66	58.33	7.07	15.27
Aspergillus flavus	83.33	6.96	25.68	83.33	8.11	23.46	58.33	4.54	9.82
Aspergillus fumigatus	75	5.90	21.82	66.67	6.60	19.13	58.33	5.30	11.47
Aspergillus japonicus	91.67	6.07	22.41	83.33	7.92	22.94	66.67	7.07	15.27
Aspergillus nidulans	83.33	3.11	11.47	58.33	3.20	9.31	58.33	4.80	10.35
Aspergillus niger	75	9.19	33.88	75	10.19	29.49	75	9.60	20.77
Aspergillus ochraceus	83.33	5.19	19.13	75	5.28	15.27	58.33	4.04	8.72
Aspergillus parasiticus	58.33	5.48	20.18	66.67	7.74	22.41	41.67	4.80	10.35
Aspergillus versicolor	75	5.48	20.18	83.33	5.09	14.74	66.67	5.80	12.58
Cladosporium cladosporoides	83.33	7.11	26.21	75	6.60	19.14	58.33	6.56	14.22
Cladosporium oxysporum	75	6.96	42.27	66.67	5.47	15.86	66.67	8.08	17.50
Curvularia lunata	83.33	8.89	32.76	83.33	6.98	20.18	58.33	8.08	17.50
Fusarium solani	83.33	6.96	42.27	75	6.98	20.18	75	8.59	18.54
Mucor hiemalis	58.33	2.52	9.31	75	3.58	10.35	50	2.53	5.44
Penicillium brevicompactum	75	4.15	15.27	75	3.58	10.35	50	3.79	8.19
Penicillium chrysogenum	66.67	3.56	13.11	66.67	3.20	9.31	58.33	4.80	10.35
Rhizopus stolonifer	66.67	3.70	13.63	58.33	2.64	7.67	58.33	4.55	9.82
Total			**401.82**			**289.45**			**216.16**

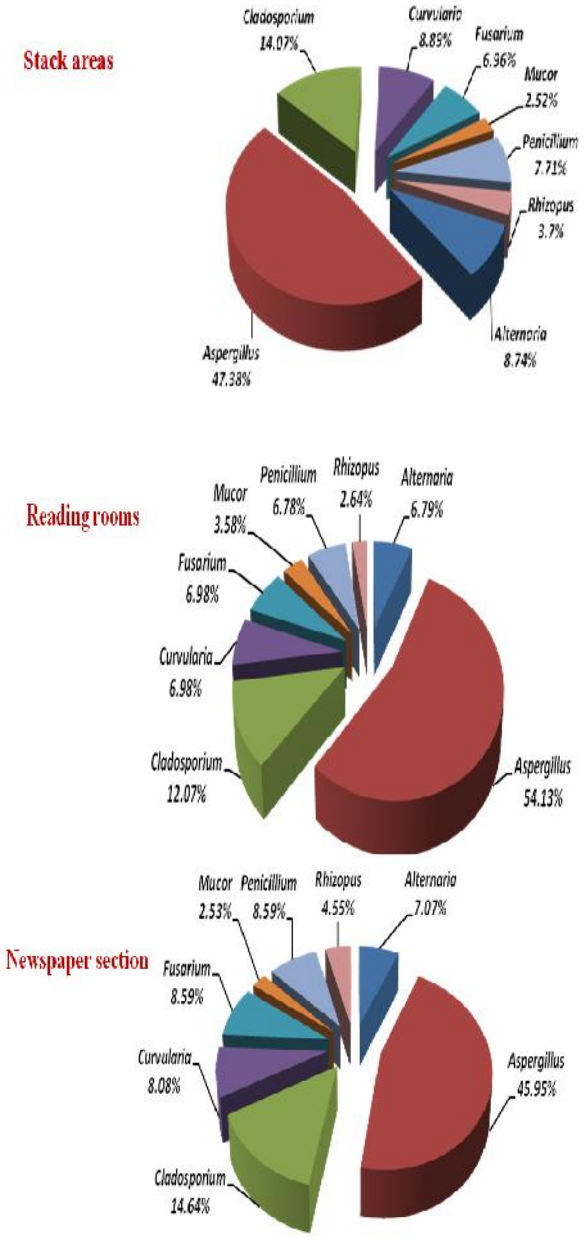

Figure 1 Distribution of recovered fungal species from indoor air.

Among the recovered mycodiversity, *Mucor* and *Rhizopus* were the only two genera representing Zygomycetes and they showed least distribution in all the investigated sites. Most of the fungal species recovered during the present investigation could have adverse effect on the health of library staff and library users through allergy or infection or by mycotoxin production. An interaction with the staff of Dhanvantri Library showed that atleast a dozen of them had problems of naso-bronchial allergies or simple sneezing and these allergic problems started only after working in the library. The affected individuals often experienced relief when they leave the building for several days.

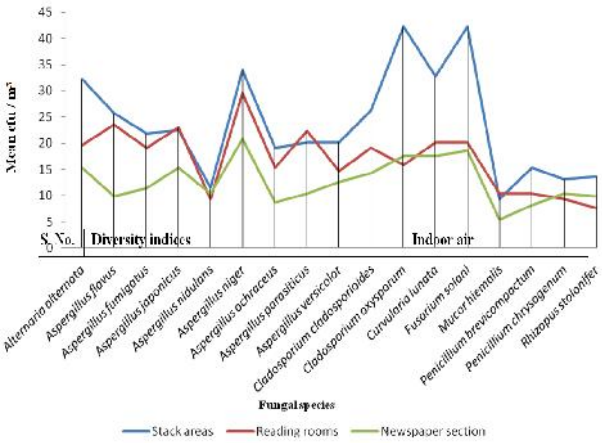

Figure 2 Comparison of mean cfu/m^3 of fungal species recovered from three sites of indoor air.

Mean number of colony forming units (cfu/m^3) of the recovered mycodiversity from all the sites were also calculated. Among the three sites of indoor air sampling, stack areas possessed highest value (401.82 cfu/m^3), followed in decreasing order by reading rooms (289.45 cfu/m^3) and newspaper section (216.16 cfu/m^3). So far, there are no Indian standards or guidelines for microbiological quality of indoor air. But, according to Swedish requirements, 500 cfu/$m^3$of bacteria and 300 cfu/$m^3$of fungal spores can be accepted in an indoor environment (Abel *et al.*, 2002). In the present investigation, as depicted in figure 2, maximum colony forming units were of *Fusarium solani* (42.27 cfu/m^3) from stack areas

and that of *Aspergillus niger* from both newspaper section (20.77 cfu/m3) and reading rooms (29.49 cfu/m^3). Similar results were obtained by Stryjakowska-Sekulska *et al.* (2007) who found colony forming units of fungi varying from 90 to 800 cfu/m^3 during the different periods of investigation.

Diversity indices computed for the fungal species recovered from the three library sites are given in table 3. These diversity indices show similarities in values of species richness (*S*), Shannon-Wiener's diversity index (*H'*), Simpson diversity index (*1-D*) and Sorensen's similarity index. These sites have same species richness (17). Simpson diversity index, a measure of heterogeneity of a site, shows that the indoor air of both stack areas and newspaper section have same value (0.94 each), which was slightly different from that of reading rooms (0.93) and the values are approaching towards 1. This indicates that these sites are homogenous, that is, less diverse in mycodiversity. Similarly, values of Shannon-Wiener index for stack areas (2.77), reading rooms (2.76) and newspaper section (2.78) showed that there is homogeneity between these three sites (Table 3). The similarity between these three sites was also calculated with Sorenson's index and it comes out to be 1, which means that the fungal species present in stack areas are also prevalent in reading rooms and newspaper section (Table 4).

Table 3 Diversity indices computed for the fungal species of three sites of indoor air of Dhanvantri Library.

S. No.	Diversity indices	Indoor air		
		Stack areas	Reading halls	Newspaper section
1.	No. of colonies	675	530	396
2.	Species richness (S)	17	17	17
3.	Simpson's diversity index (1-D)	0.94	0.93	0.94
4.	Shannon's diversity index (H')	2.77	2.76	2.78

Table 4 Sorenson's similarity index of indoor air.

Sites	Stack areas	Reading halls	Newspaper section
Stack areas	**-**	**1**	**1**
Reading halls	**1**	**-**	**1**
Newspaper section	**1**	**1**	**-**

CONCLUSION

In conclusion, our study indicates that indoor recreational facilities like libraries are reservoirs of mycodiversity. The prevalence of huge mycodiversity in these sites may be attributed to their tolerance and adaptation to various abiotic and biotic factors such as ventilation of the buildings, ecological conditions, number of users and their activities.

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