

**STUDY OF AEROMYCOFLORA IN INDOOR AND OUTDOOR ENVIRONMENT OF NATIONAL LIBRARY, KOLKATA**

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ABSTRACT: We aimed at the systematic evaluation of air-borne fungal flora of the National Library, Kolkata for a period of three months beginning from February to April, 2010 to determine their identification, concentration and diversity in both indoor and outdoor environment to understand the cumulative aeromycoflora composition. The period of study was the post winter period followed by pre-summer months that was mild to moderate warm and low to high humid condition with temperature and humidity ranges of 17.0-38.2°C and 26-92% respectively. Air sample was collected with interval of two weeks by means of gravitational settling method using petri dishes with Malt Extract Agar (MEA) media. Fungal colonies that formed after 3-5 days incubation period at 25-28°C were identified on the basis of micro and macro morphological characteristics and finally percentage contributions of individual fungal species were calculated. A total of 21 types of fungal spores were identified from indoor environment with 5 sterile hyphae and 13 unidentified spore types. In case of outdoor environment, total number of spore types encountered was 19 along with 12 sterile hyphae and 6 miscellaneous types were recorded under unidentified spore type. The prevailing presence of *Aspergillus niger*, *Alternaria tenuissima*, *Cladosporium herbarum* and *Penicillium sp.* were accounted for a high percentage in indoor environment whereas outdoor environment showed clear dominance of *Alternaria alternata*, *Aspergillus niger*, *Alternaria tenuissima*, *Cladosporium herbarum*, *Cladosporium cladosporioides*, *Curvularia lunata* and *Fusarium oxysporum*. Among all the fungal spore types the taxonomic group Deuteromycotina showed dominance in total spore contribution. Biomonitoring of aeromycoflora is a key to open the information of sensitivity towards bioaerosol in this atmosphere and our findings may be useful with regard to the investigation of corrective measures to save the library materials from fungal damage and diagnosis and prophylaxis of allergic diseases resulting from aeromycoflora composition of this environment.

Key words Aeromycoflora. National Library. Indoor and outdoor environment. Fungal spores

INTRODUCTION

Aeromycoflora simply refers to the airborne fungal contributors of the environment. The term aerobiology came in use since 1930 by the American plant pathologist Fred Cambell Meier to denote the airborne fungal spores, pollen grains and other airborne microorganisms. It is defined as a discipline in which aerial transport of biological materials is studied. Fungal spores represent a major fraction of bioaerosol with more than 80,000 species of which the majority are cosmopolitan in origin [1]. Overall fungal spores have a great response in everywhere for being ubiquitous and the concentration of aeromycoflora in different environment varies depending on the geographical regions, altitudinal differences, seasonal variations and atmospheric conditions. A large number of airborne microfungus propagules were found in indoor and outdoor environments and generally widely distributed in nature [2, 3]. Several microfungus species have the potential adverse effect to cause allergies, spoilage of foods and many other adverse health effects [4-9].

Books, papers and other documents preserved in libraries are important and valuable cultural heritage for a nation and knowledge of all kinds in these articles are passed continuously to future generations. These are considered as precious legacies as they have the capacity to remind people about their culture, religion and traditional ethnicity [10]. Therefore it should be of prime importance to maintain and conserve these materials in libraries and archival settings. The dispersing nature of fungi makes them one of the leading agents of contamination of any type of substrate in the books of library. The aeromycoflora in the library environment causes bio-deterioration and damage of books and other materials [11-15]. Environmental variables such as temperature, relative humidity and rainfall play vital role in the occurrence of fungal spores in indoor air of library [10, 16]. The predominance of airborne fungi in diverse environmental conditions especially indoor and outdoor environments of different work places and their health hazards has recently been reviewed by Khan and Karuppayil, 2012 [9]. Investigations by previous researchers showed that some fungi like *Aspergillus candidus*, *A. niger*, *A. versicolor*, *Cladosporium cladosporioides*, *C. herbarum*, *Penicillium brevicompactum* and *P. chrysogenum*, can elevate intense allergic reactions [17] and these bio-components may cause eye and sinus irritation, headache, tiredness, sore throat, general weakness, wooziness and severe asthma [18, 19]. To determine the degree of precipitation of airborne fungi particles and the potential role of fungi as allergic contaminants in book collections of libraries and archival settings, aeromycoflora of different university libraries has been studied in different parts of the world and a diverse number of fungal species was found in these investigations [20-23]. In the Indian scenario, the survey of airborne fungal spores in libraries of different regions of this country were carried out with conventional techniques and a diverse number of fungal genera were identified and isolated [10, 24-30]. With this previous background, we aimed at the systematic quantification of indoor and outdoor fungal flora of the National Library, Kolkata, India for a period of three months beginning from February to April, 2010 (Fig. 1). The aim of this study was to determine the aeromycoflora, their identification, concentration and diversity in both indoor and outdoor environment to understand the cumulative aeromycoflora composition in the library environment. We hypothesized that the library environment may possess both beneficial and harmful fungal flora. In the case of beneficial fungal species, they could be cultured and used further to produce useful substances. In contrast, in the case of harmful fungi, their effects could be studied more critically and appropriate precautionary measures could be taken. To the best of our knowledge, this is the first report regarding the distribution of fungal population in the National Library from eastern India.

MATERIALS AND METHODS

Study site

The present study was conducted in the National Library, Kolkata (formerly the Calcutta Public Library, latitude 22.53° N, longitude 88.33° E), first public library in India established in 1836. It is the largest library in India by volume and India's library of public record. It is an institution of national importance under the Ministry of Culture, Government of India. The library is designated to collect, disseminate and preserve the printed material produced in the country. However, till date, there is no published record on microflora studies of this particular place which may have a significant effect as the concentration and contamination of pathogens are related to the quality of air, ventilation, cleanliness and maintenance of the library buildings and books along with the seasonal and environmental variations. Although the library entrance area is more or less equivalent to natural outdoor environment but indoor section contains a closed environment system and specially the rare books section have a typical community.

Media preparation

A suitable substrate or culture medium supporting nutritional needs of fungi was required for our study. It is possible to establish the necessary conditions *in vitro* to support the optimal growth of a fungal species. For this purpose, Malt Extract Agar (MEA) media, containing malt extract 20 gmL⁻¹ and agar 20 gmL⁻¹ of distilled water was prepared aseptically. Then the liquid media was poured into sterile petridishes following aseptic techniques. The media was allowed to solidify and then the junctures of the petridishes were sealed by selotape. The cool petridishes were wrapped with brown paper and taken to the site of investigation.

Sampling procedure

The petridishes were taken to the selective site to trap the fungal composition. Air samples were obtained from six different sites within and around the library. One petridish was used for each sampling site for each visit. The sampling sites were majorly divided into i. indoor environment (comprising of five sampling sites *viz.* a. Language Division, Bengali of Bhasha Bhavan; b. Bhasha Bhavan Reading Room; c. Central Sorting Room of Bhasha Bhavan; d. Preservation Room of New Annexe Building and e. Ground Floor of Annexe Building, Map Section) and ii. Outdoor environment of the National Library building.

The samples were collected at fifteen days intervals between the months of February to April, 2010. Samples were taken in the afternoon (12:30 p.m.-1:30 p.m.). The Petriplate Gravitational Method was used for the isolation of fungi [31-34]. Petridishes were exposed to the air for 15 min and then covered by the lid and again sealed by sellotape. Next, these petriplates were brought into the laboratory within 2 hr and incubated for 3-5 days at room temp (25°C-28°C). The meteorological parameters like temperature (°C), relative humidity (%) and rainfall had intense effect on air-borne fungal species both qualitatively and quantitatively and these were also recorded on each sampling date (Table 1).

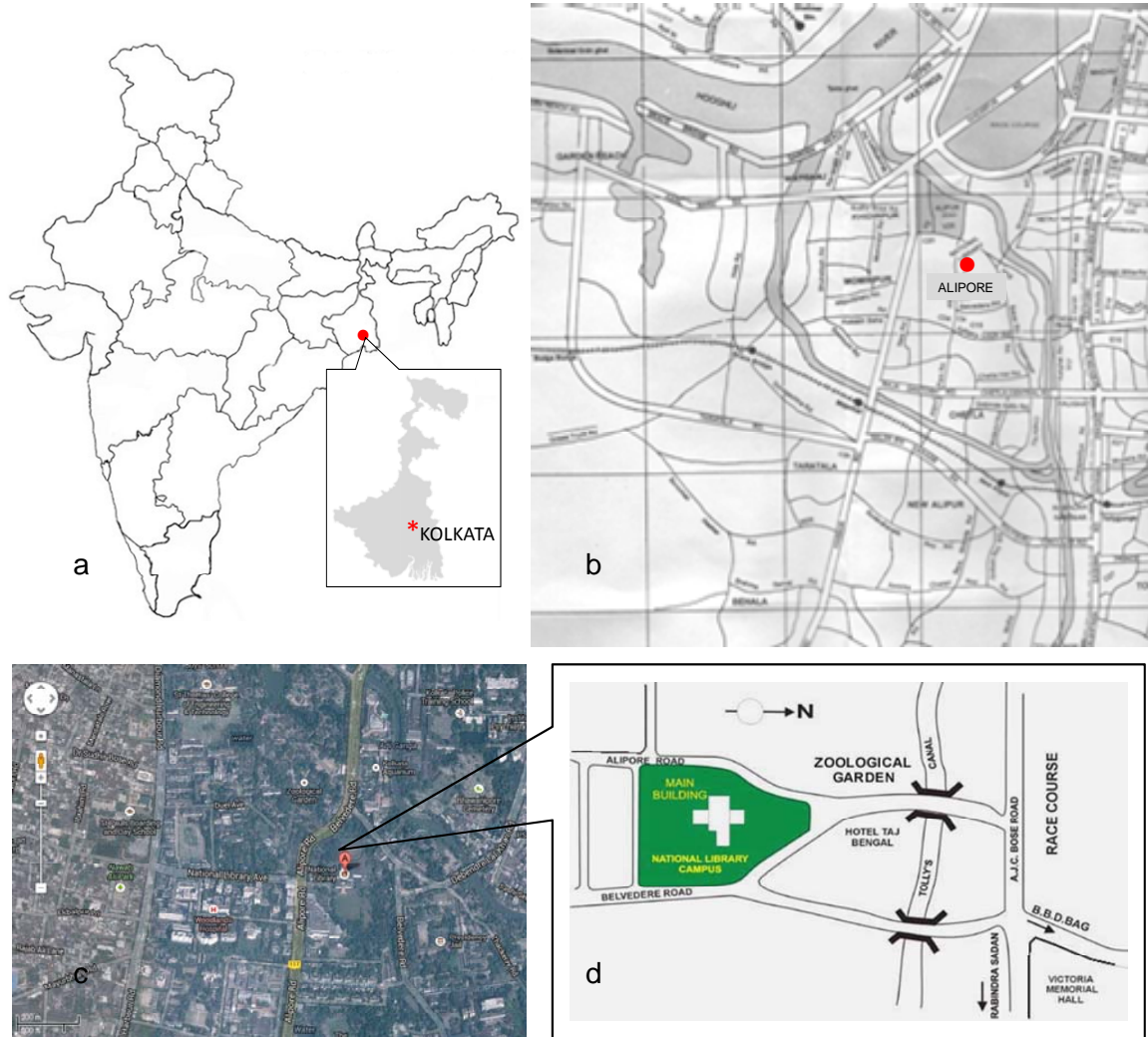


Fig. 1 Survey and sampling site of the study. a. India map showing location of Kolkata. b. Kolkata map (partial) showing location of Alipore. c. Satellite map (Google map) showing location of the National Library in Alipore. d. The National Library campus

Table 1. Temperature, humidity and rainfall on different sampling dates

Date	Temperature		Humidity		Rainfall
	Maximum	Minimum	Maximum	Minimum	
09.02.10	30.5°C	17.5°C	89%	43%	-
22.02.10	31.2°C	17.0°C	87%	26%	-
11.03.10	34.0°C	22.4°C	77%	29%	-
25.03.10	34.0°C	26.3°C	90%	60%	-
06.04.10	38.2°C	27.7°C	92%	42%	-
23.04.10	35.6°C	28.7°C	91%	59%	-

Source: India Meteorological Department, Regional Meteorological Centre, Alipore, Kolkata-700027

Identification of fungal strains

The fungal colonies were counted based on their macro-morphological properties. The colonial features of fungal colonies were studied minutely. Then compound microscope was used to determine the morphological structures of fungi after mounting in lactophenol and cotton blue covered with cover slip on slides. Fungal types were analyzed for each day. The species were identified on the basis of micro and macro morphology, reverse and surface colouration of colonies grown on MEA media. Identification of fungi was carried out as described by previous investigators [35]. Percentage contributions of individual fungal species were calculated as per the following formula:

$$\% \text{ Contribution} = (\text{Total number of colonies of one species} / \text{Number of colonies of all species}) \times 100$$

RESULTS AND DISCUSSION

A number of literatures on airborne fungal spores and its link with the working environments have been published from India and abroad [36-41]. However, fungal spores in National Library has not been estimated or reported so far. In our study our main purpose was to collect the accurate information about the fungal occurrences in such a worthy site.

The results of the three months aeromycoflora survey in the National Library showed that indoor and outdoor atmosphere of this library was never free of fungal spores. A total of 21 types of fungal spores were identified from indoor environment with 5 sterile hyphae and rest 13 spore types which were not identified are grouped under unidentified spore types (Table 2). In case of outdoor environment, total number of spore type encountered was 19 along with 12 sterile hyphae and 6 miscellaneous types were also recorded under unidentified spore type (Table 3). According to their occurrence in the exposed petriplate samples, the total population in terms of percentage occurrence were also presented in Fig 2. *Alternaria tenuissima* (indoor-11.111% and outdoor-11.111%), *Aspergillus niger* (indoor-7.936% and outdoor-13.114%) and *Cladosporium herbarum* (indoor-10.33% and outdoor-8.39%) were of high occurrence in the library environment. The degrees of difference between indoor and outdoor counts of aeromycoflora composition was calculated as indoor/outdoor ratio as suggested by previous investigators [20] and displayed in Table 4. Ratios > 1 indicate higher indoor counts whereas ratios < 1 indicate elevated outdoor concentration of fungal spore types (Fig. 3). *Alternaria humicola*, *Alternaria sp.*, *Arthobotrys sp.*, *Aspergillus ochraceus*, *Aspergillus sp.*, *Chaetomium globosum*, *Cladosporium sp.*, *Drechslera sp.*, *Geotrichum candidum*, *Penicillium herquei* and *Sirosporium sp.* were isolated only from indoor. On the other hand, *Alternaria alternata*, *Alternaria brassicicola*, *Alternaria dianthi*, *Alternaria dianthicola*, *Aspergillus candidus*, *Aspergillus fumigatus*, *Aspergillus sp.*, *Curvularia sp.*, *Fusarium oxysporum*, *Fusarium sp.* were found to be present only in outdoor environment. *Alternaria tenuissima*, *Aspergillus sp.*, *Humicola sp.*, *Penicillium sp.* and unidentified fungal spore types were present more frequently in indoor than outdoor. Alternatively, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium herbarum*, *Curvularia lunata*, *Curvularia pallescens* and sterile hyphae spore forms were found to be more prevalent in outdoor section than indoor (Fig. 2, 4). Among all the fungal spore types the taxonomic group Deuteromycotina showed dominance in the total spore contribution.

Our results showed a similar pattern with the previous studies by other researchers in different parts of the world regarding indoor and outdoor environment [42, 43]. In our case study we got the clear picture of the diversity of fungal spores present in indoor and outdoor environment of the library. The library was always occupied with the activity of various types of fungi. The group Deuteromycotina or the fungi imperfecti represent the species which have thick spore walls that may promote them to remain viable in the dust. These spores may enter into indoor environment from the outdoor atmosphere through doors and windows, ventilation and air conditioning systems, and different fungal types and concentrations from settled dust in normal residences were also reported [44]. The cellulose materials of the library like books, wooden racks, cardboards etc. along with the other conditions favor the abundance of *Aspergillus*. Most of the fungi imperfecti are known producer of mycotoxin and they have the ability to transmit contamination. These contaminants had a correlation with the library atmosphere. Presence of the fungal spore types may be because of the deficiency of cleanliness which favours the conditions for growth of aeromycoflora in the stored books and the dust present on the books. It also depends on the meteorological parameter like temperature and relative humidity. It is a proven association between the fungal contribution and atmospheric factors. The anthropogenic action may also be responsible which had been reported in some early cases [45]. The concentration of airborne spore and resulting air quality depend on the overall condition and cleanliness of the atmosphere, humidity and temperature, access to light, ventilation, oxygen-water and other allied factors [46-51]. Additional factors like building age and mean age of books may also have contributed to the aeromycoflora diversity that follow the same pattern reported in many libraries [20]. Many aerobiological survey showed that the existence of airborne bio-components in indoor working environments is a major health problem in India and other parts of the world [9, 52, 53].

The high variety of spores carries the potential of risk of many respiratory diseases. We can assume from the reference works that the staff members and the library visitors are continuously exposing to a growing level of aero allergenic fungal spores. In addition, the increased spore diversity is proportional to the occurrence of allergic disorders such as allergic rhinitis, bronchial asthma, atopic dermatitis and many more health issues related to fungal contamination. The contaminants may provoke variety of respiratory diseases and other health problems to the staff members and persons exposed to this environment [53]. In general, our observation showed substantial higher concentration of fungal spores in indoor and outdoor environment of the National Library which should be emphasized for more study in the hazards associated with human respiratory tract.

In accord to our results, previous studies in India and abroad also showed the presence of similar types of airborne fungal species in the library atmosphere [10, 20, 24, 25, 30]. These aeromycoflora surveys were conducted with the objective to determine the diversity of airborne fungal spores in a particular period of time and/or to estimate the seasonal variation of aeromycoflora in the library atmosphere. Our study is unique in the sense that this is the first ever study for the assessment of aeromycoflora diversity in the National Library of Eastern India. We have conducted the study to monitor the distribution of airborne fungal spore types in one of the important cultural heritage of our country to ascertain risk associated with these harmful fungal species causing damage of books, articles and other invaluable documents of cultural ethnicity.

Table 2. Total count and percentage contribution of fungal colony from indoor environment of the National Library, Kolkata

Spore types	Day I 09.02.10	Day II 22.02.10	Day III 11.03.10	Day IV 25.03.10	Day V 06.04.10	Day VI 23.04.10	Total	% count of fungal colony
<i>Alternaria humicola</i>	-	-	-	-	1	-	1	1.587
<i>Alternaria sp.</i>	-	-	-	1	-	-	1	1.587
<i>Alternaria tenuissima</i>	-	3	3	1	-	-	7	11.111
<i>Arthobotrys sp.</i>	1	-	-	-	-	-	1	1.587
<i>Aspergillus flavus</i>	-	-	-	-	1	-	1	1.587
<i>Aspergillus niger</i>	1	-	1	1	-	2	5	7.936
<i>Aspergillus ochraceus</i>	-	-	-	-	-	2	2	3.174
<i>Aspergillus sp.</i>	-	-	-	-	-	4	4	6.345
<i>Chaetomium globosum</i>	-	-	-	-	-	1	1	1.587
<i>Cladosporium cladosporioides</i>	1	-	-	-	-	-	1	1.587
<i>Cladosporium herbarum</i>	6	-	-	-	-	-	6	9.523
<i>Cladosporium sp.</i>	1	-	-	-	1	-	2	3.174
<i>Curvularia lunata</i>	-	-	-	-	-	1	1	1.587
<i>Curvularia pallescens</i>	-	-	1	-	-	-	1	1.587
<i>Drechslera sp.</i>	-	-	-	-	-	1	1	1.587
<i>Geotrichum candidum</i>	-	-	1	-	-	-	1	1.587
<i>Humicola sp.</i>	1	-	-	-	-	2	3	4.761
<i>Penicillium herquei</i>	-	-	-	-	-	1	1	1.57
<i>Penicillium sp.</i>	-	-	-	1	-	2	3	4.761
<i>Sirosporium sp.</i>	-	-	-	1	-	-	1	1.587
<i>Sterile hyphae</i>	4	-	-	1	-	-	5	7.936
<i>Unidentified</i>	3	1	1	4	-	4	13	20.634
Total	18	4	7	11	3	20	63	99.991

Table 3. Total count and percentage contribution of fungal colony from outdoor environment of the National Library, Kolkata

Spore types	Day I 09.02.10	Day II 22.02.10	Day III 11.03.10	Day IV 25.03.10	Day V 06.04.10	Day VI 23.04.10	Total	% count of fungal colony
<i>Alternaria alternata</i>	-	-	4	-	-	1	5	8.196
<i>Alternaria brassicicola</i>	-	1	-	-	-	-	1	1.64
<i>Alternaria dianthi</i>	-	1	-	-	-	-	1	1.64
<i>Alternaria dianthicola</i>	-	1	-	-	-	-	1	1.64
<i>Alternaria tenuissima</i>	-	3	3	1	-	-	7	11.111
<i>Aspergillus candidus</i>	-	-	-	-	1	-	1	1.64
<i>Aspergillus flavus</i>	-	-	-	-	1	-	1	1.64
<i>Aspergillus fumigatus</i>	-	-	1	-	-	-	1	1.64
<i>Aspergillus niger</i>	-	-	-	3	1	4	8	13.114
<i>Aspergillus sp.</i>	-	-	-	-	1	1	2	3.278
<i>Cladosporium cladosporioides</i>	2	-	-	-	-	-	2	3.278
<i>Cladosporium herbarum</i>	6	-	-	-	-	-	6	9.836
<i>Curvularia lunata</i>	-	1	-	-	1	-	2	3.278
<i>Curvularia pallescens</i>	1	1	-	-	-	-	2	3.278
<i>Curvularia sp.</i>	-	-	-	-	1	1	2	3.278
<i>Fusarium oxysporum</i>	-	-	-	-	2	-	2	3.278
<i>Fusarium sp.</i>	-	-	1	-	-	-	1	1.64
<i>Humicola sp.</i>	-	-	-	-	-	1	1	1.64
<i>Penicillium sp.</i>	-	-	1	-	-	-	1	1.64
<i>Sterile hyphae</i>	1	-	-	2	6	3	12	19.672
<i>Unidentified</i>	-	-	2	-	-	4	6	20.634
Total	10	6	10	6	14	15	61	100

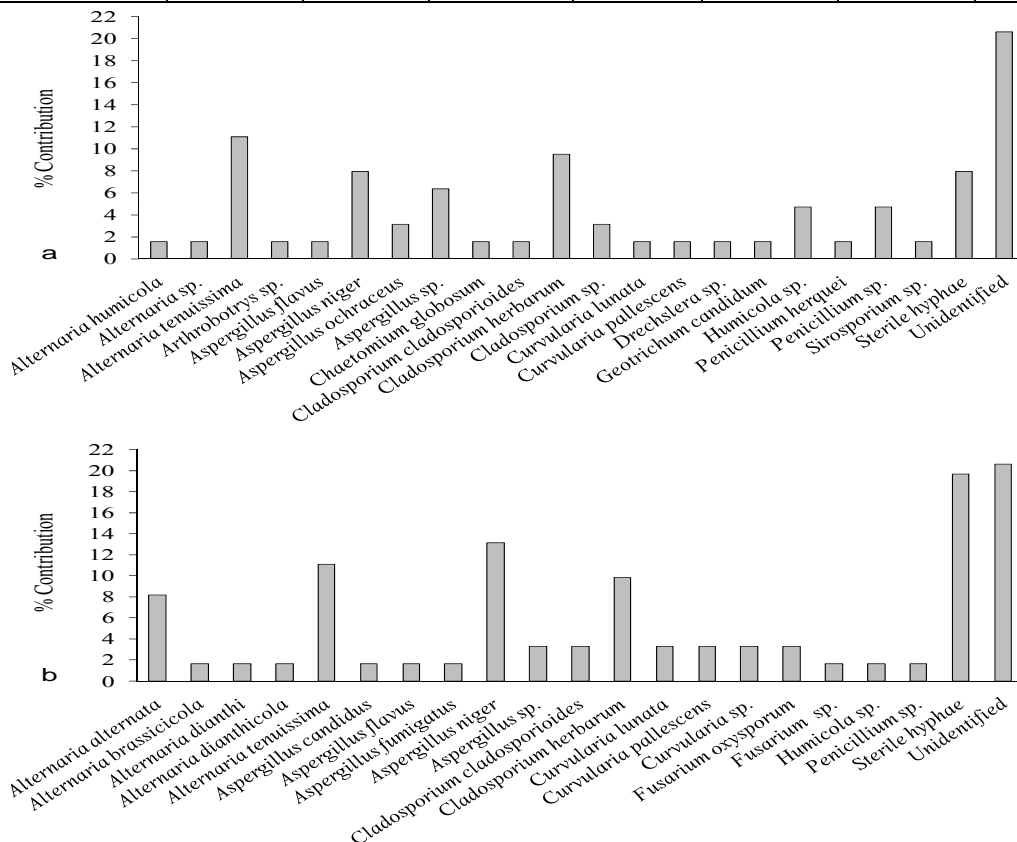


Fig. 2 Count (%) of fungal colony from a. indoor environment and b. outdoor environment of the National Library, Kolkata.

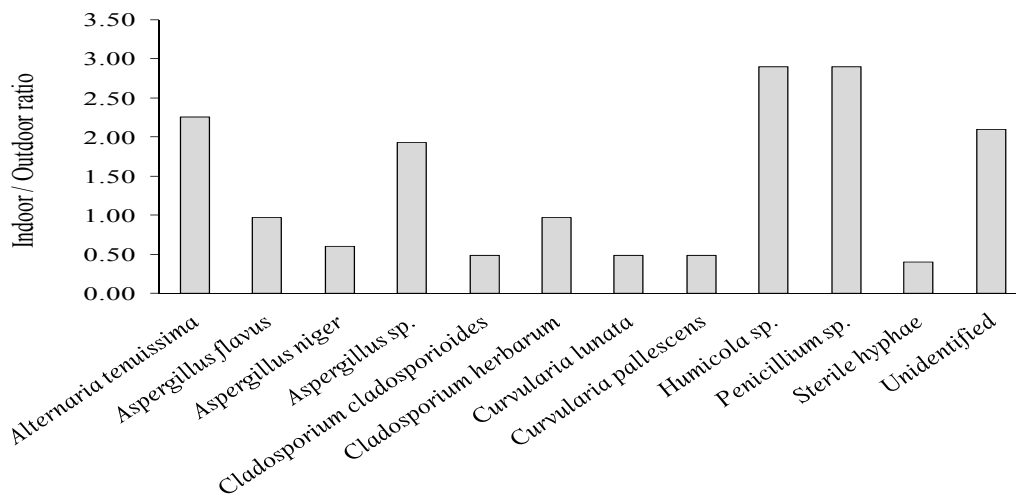


Fig. 3 The indoor/outdoor ratio of fungal colonies in the National Library, Kolkata.

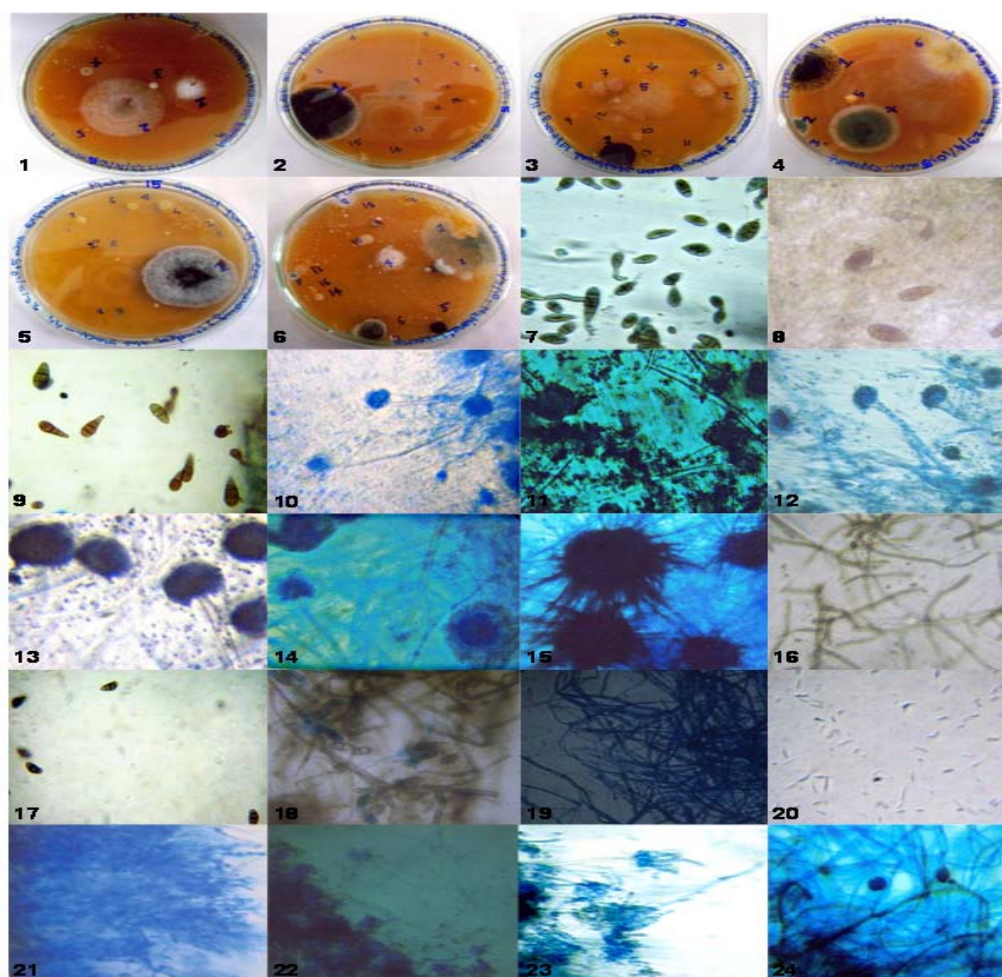


Fig. 4 1-6: Fungal colonies growing on the petridish of different sampling sites in the National Library. 7-24: Different species of aeromycoflora of the National Library under compound microscope (100X). 1: Language Division, Bengali of Bhasha Bhavan, 2: Bhasha Bhavan Reading Room, 3: Central Sorting Room of Bhasha Bhavan, 4: Preservation Room of New Annexe Building, 5: Ground Floor of Annexe Building, Map Section, 6: Outdoor environment of the National Library building, 7: *Alternaria alternata*, 8: *Alternaria dianthi*, 9: *Alternaria tenuissima*, 10: *Aspergillus candidus*, 11: *Aspergillus flavus*, 12: *Aspergillus fumigatus*, 13: *Aspergillus niger*, 14: *Aspergillus ochraceous*, 15: *Chaetomium globosum*, 16: *Cladosporium cladosporioides*, 17: *Curvularia lunata*, 18: *Curvularia pallescens*, 19: *Drechslera sp.*, 20: *Fusarium oxysporum*, 21: *Humicola sp.*, 22: *Penicillium herquei*, 23: *Penicillium sp.*, 24: *Sirosporium sp.*

Our study showed significant occurrence of cosmopolitan mycoflora in indoor and outdoor environment of the library. Many of them utilize plant material as their substrate and reported as pathogen. A large number of isolated fungal species were harmful and could cause health problems like asthma and other respiratory disorders to the readers and visitors exposed to this library environment. Air condition and ventilation system are not sufficient to lower the level of spores. However, previous studies showed that fungal concentrations have a positive relationship with the disease status. They interact with the human internal organ like lung and elevate the level of chances of respiratory diseases. Hence, identification and biomonitoring of aeromycoflora is a key to open the information of sensitivity towards bioaerosol in this atmosphere and taking proper preventive measure from air-borne particles. Our finding of different harmful fungal types in this present investigation revealed that they could not only deteriorate the content of the library but also results in many diseases by their accumulation.

CONCLUSION

The dominant fungal contribution of the National Library exhibits a clear picture that it is appalling if the maintenances are inefficient and dismal. Further studies with longer monitoring period on their occurrence in indoor and outdoor air could bring about better understanding of their potential function. Though there is no practical method to abolish these ubiquitous contaminants but there are some conventional procedures, such as regular inspections, measurement of surface moisture levels, and standard hygiene should be maintained. So, besides an appropriate implementation of proper cleanliness in the library environment, an expansion of the building is also needed to save this national asset from deterioration by environmental contaminants. The imperative application of modern amenities can deplete their sharp uprising growth. Therefore National, Library, a vital necessity of our country should be emphasised on the need of its adequate preservation and proper maintenance.

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