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INDOOR AEROMYCOFLORA OF THE CENTRAL LIBRARY OF VISVA-BHARATI, SANTINIKETAN WITH REFERENCE TO BOOK DETERIORATION

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Abstract:

Indoor aeromycoflora of the Central Library, Visva-Bharati University at Santiniketan was studied by using both Burkard personal volumetric air sampler and Andersen two stage sampler from August, 2016 to March, 2017. A total of 16 fungal spore types were identified from Burkard sampling and fungal colonies of 25 species were recorded from exposed petridishes containing PDA medium using Andersen sampler. Isolated fungi were identified on the basis of their character with the help of authentic literatures. In Burkard sampling, maximum fungi were recorded in October (16000 spores/m^3) and minimum in January (4200 spores/m^3). In Andersen petriplate method maximum fungal colonies were found in October (1836 CFU/m^3) and minimum in August (423 CFU/m^3).

Biodeterioration is an undesirable change in the properties of a material caused by the vital activities of the deteriorating organisms. The present study was undertaken to find out the aeromycoflora of the library environment which are responsible to decay books and manuscript. A total of 10 book deteriorating fungi were isolated, of which Aspergillus niger, Aspergillus glaucus, Penicillium brefeldianum and Penicillium oxalicum showed their positive activity of cellulose degradation which is the major component of paper material. Aspergillus ochraceus showed no cellulose degrading activity, while Penicillium oxalicum exhibited maximum degradation of cellulose among other fungi.

Key Words: *Aeromycoflora, Book deterioration, cellulose degrading activity, Central library, Santiniketan, West Bengal.*

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INTRODUCTION:

A library is a storehouse of knowledge of great thinkers since time immemorial. Library is considered to be a social institution with the responsibility of disseminating knowledge to the people without any discrimination. Libraries are collections of valuable books, manuscripts, archives and maps that keep human knowledge and cultural heritage [1-4]. Biodeterioration of library materials represents a great problem around the world [5-7]. The ingredients which used in paper making industry such as wood, (cellulose), proteins and chemical additives are suitable substrates for bacterial and fungal growth [8-16]. The cumulative effects of environmental factors such as heat, light, humidity and dust as well as biological factors such as bacteria, fungi and insects cause damage and discoloration of books and manuscripts stored in libraries [9,17,18] . Study of aeromycoflora of a library is important as the old books made up with bindery glues and fabrics that support the growth of fungi. In favourable condition fungi grow and damage the books, they destroy cellulose and decompose binding materials, leather and plastics etc [6]. The role of fungal agents in deterioration is due to their hydrolytic activity. Composition and concentration of fungi varies season to season due to changes in meteorological parameters [9]. Biodeterioration of library material is a worldwide problem and it causes great damage especially to unique as well as important manuscripts and books stored in the libraries. Fungi have been identified as major causes of biodeterioration of books in library [7]. Fungi reproduce by both asexual and sexual means and their spores as well as conidia form the component of airspora inside the library.

Paper is an organic substance composed of cellulose from green plants that produce fibres suitable for paper making. Cellulases are a group of fibrolytic enzymes which cooperatively hydrolyze plant cell wall fibers into glucose, cellobiose or oligosaccharides [19,20] . Deterioration of library material due to the fungal and bacterial growth is a worldwide problem and a cause of extensive damage to precious books and manuscripts [21]. Microfungi, which produce too many spores, can be easily transported from the significant part of the bioaerosols in the external and internal atmosphere of the surrounding and they can contaminate all kinds of surface. There are 20 thousand to 2 million fungal spores in 1 m^3 of air. These spores can sprout and grow if they get required moisture and heat. Generally fungi grow at temperature range of 15-35 ˚C. In libraries, fungal growth is known as mould or mildew which appears as brown/black/blue vegetative growth on paper. Damage can occur because of mechanical stress or enzymatic action, because moulds can produce a wide range of enzymes (proteinases, gelatinases, cellulases) which are able to destroy the component materials (cellulose) of library and archival collections. The most common fungi that grow on paper are *Aspergillus* and *Penicillium* sp. [1].

The objectives of the present study were (i) to know the qualitative and quantitative composition and concentration of indoor airborne fungi inside the Central library of Visva-Bharati, Santiniketan, and (ii) to identify and isolate the cellulose degrading fungi from the old book and paper of the library. This work was undertaken as no such work has so far been done earlier.

MATERIALS AND METHODS:

Sampling site- Central Library of Visva Bharati University at Santiniketan was selected for the present study. The genesis of the central library of Visva-Bharati goes back to 1901, with the foundation of the 'Brahmacharya Ashrama' at Santiniketan by Nobel Laureate Rabindranath Tagore. Tagore emphasized the use of books in the educational development of students. At present the Central Library contains 3, 76,531 volumes of books, around 4,000 users and a daily transaction of 300 books. The library has a number of important collections; mention may be made of the collections of Abanindranath Tagore, Prabodh Chandra Bagchi, Pramatha Chaudhury, Lila Roy and Ashok Rudra.

Collection of samples- The experiment was carried out by surveying various sections of Central library of Visva-Bharati. Fungal spores present in the indoor environment were monitored. During the course of study the samples were collected both from the infected books and from indoor air. Different protocols were followed for the isolation of fungi from infected book sample like cotton swab technique following Abdel-Kareem [22]. Various bio-deteriorated paper samples were collected from various corners in the library. The individual mycoflora were transferred using sterilized inoculating needle into test tube containing 1 ml of sterilized distilled water. The suspension was further inoculated on petriplate containing PDA medium using sterilized cotton buds which swabbed over the surface of petriplate. The dishes were incubated at 25 ˚C for 4-6 days. Air inside the library was trapped by Burkard personal volumetric sampler (Burkard manufacturing co. Hertz, England) and also by Andersen two stage volumetric sampler (Graseby Andersen, Georgia, USA). Burkard sampler (Suction rate 10 l/min) was used for trapping both viable and

non-viable fungal spores. The sampler was run at two week interval for 10 minutes and the exposed slides were mounted. Qualitative and quantitative analyses of fungi were carried out and the conversion factor was calculated following the method of the British Aerobiology Federation [23]. Andersen two stage sampler (suction rate of 28.3 l/min) was used for trapping only viable fungal spores [24]. The sampler was exposed for 10 minute at two week interval. All the isolated fungal spores were studied under high power microscope and identified using standard literatures and reference books [25-26].

Preparation of fungal spore inocula- In the present study, some species of *Aspergillus* sp. and *Penicillium* sp. were isolated from old infected books of Central library, Visva-Bharati by serial dilution method. The isolated fungal mono-cultures were grown on potato-dextrose agar medium. They were incubated at room temperature (28 ± 2 °C) for 7 days. An inoculum density of 2 **.**106 spores was used for screening of cellulolytic fungi as well as cellulase production on Czapek-Dox broth medium amended with 1% cellulose [27].

Screening of cellulolytic fungi- The isolated fungal cultures were screened for their ability to produce cellulase complex following the method of Teather & Wood [28]. Czapek-Dox medium used in this method contained (g/l): sucrose – 30, $NaNO_3$ – 2, K_2HPO_4 - 1, $MgSO_4$ – 0.05, KCl – 0.5, $FeSO_4$ – 0.01, carboxy-methyl cellulose – 1%, Agar agar - 20. pH of the medium was adjusted to 5. After autoclaving at 121°C and 15 lbs. pressure, the medium was poured into petri plates and allowed to solidify. Then cavities of 6 mm size were made at the middle of the solidified medium and inoculated with 0.1 ml of spore suspension prepared from 7 day old pure cultures of five fungi isolated from library air. The plates were incubated at room temperature (28 ± 2 °C) for three days to allow fungal growth, then again incubated for 18 h at 30 °C which is the optimum temperature for cellulase activity. After incubation, 10 ml of 1% Congo - Red staining solution was added to the plates and was shaken at 50 rev/ min for 15 min. The Congo - Red staining solution was then discarded, 10 ml of 1 N NaOH was added to the plates and shaken again at 50 rev/min for 15 minutes. Finally 1 N NaOH was also discarded and the staining of the plates was analyzed by noticing the formation of yellow zones around the fungal spore inoculated wells [29].

RESULT AND DISCUSSON:

Burkard personal and Andersen two stage volumetric samplers were used to monitor the air borne fungal spores inside the library environment during August 2016 to March 2017. A total 16 types of fungi were identified by Burkard sampler (Table.1) and 25 species of fungal colony were identified using Andersen two stage sampler (Table.2).

Table 1: Monthly fungal spore concentration trapped from the air of Central library of Visva-Bharati using Burkard personal volumetric sampler.

Burkard fungal spores/m^3 of air	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Total
Ascospores	819	252	250	194	390	194	250	875	3224
Alternaria	441	567	125	127	0	6	250	0	1516
Aspergilli/Penicilli	1827	1638	500	1203	2286	2345	2625	3500	15924
Basidiospore	189	126	125	252	255	252	375	0	1574
Bispora	126	63	125	63	190	0	375	250	1192
Cercospora	252	189	0	63	63	0	0	0	567
Chaetomium	63	126	125	2	63	0	0	0	379
Cladosporium	504	630	1000	319	446	320	750	1375	5344
C. herbarum	63	0	0	1	1	0	0	250	315
Curvularia	63	189	250	1	1	0	0	250	754
Fusarium	1260	0	0	0	63	315	0	0	1638
Nigrospora	504	378	750	68	128	192	500	125	2645
Periconia	945	10899	12500	1906	2108	574	2375	1250	32557
Periconiella	189	126	125	0	0	0	0	0	440
Phaeotrichoconis	0	0	0	65	0	0	0	0	65
Torula	0	63	125	0	1	2	0	0	191
Total	7245	15246	16000	4264	5995	4200	7500	7875	68325

In Burkard sampling, fungi were identified up to generic level (except *Cladosporium herbarum*). Some fungi were dominant throughout the sampling period, e.g. Ascospores (3224 spore/m^3), Aspergilli/Penicilli (15924 spore/m^3), *Cladosporium* (5334 spore/m^3), *Nigrospora* (2645 spore/m^3), *Periconia* (32557 spore/m^3) inside the library. But *Phaeotrichoconis* (65spore/m^3) and *Torula* (191 spore/m^3) were less dominant throughout the sampling period.

Table 2: Month wise fungal spore concentration (CFU/m^3) of Central library of Visva-Bharati using Andersen two stage volumetric sampler.

Viable Fungus spores (CFU/m^3 of air)	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Total
Aspergillus niger	84	185	144	66	121	94	108	132	934
Aspergillus glaucus	66	90	288	3	199	100	132	204	1082
Aspergillus fumigatus	102	291	384	0	0	0	84	0	861
Aspergillus versicolor	13	0	0	0	0	0	0	0	13
Aspergillus nidulans	3	0	24	0	0	0	0	0	27
Aspergillus japonicus	0	66	72	277	0	0	0	0	415
Aspergillus candidus	0	0	0	0	6	1	0	0	7
Aspergillus flavus	2	0	0	0	0	0	0	0	2
Aspergillus ochraceus	25	3	0	6	25	6	0	276	341
Aspergillus nigricans	5	0	0	0	0	6	0	0	11
Aspergillus sydowi	6	0	0	0	0	0	0	0	6
Aspergillus clavatus	0	90	108	0	0	0	0	0	198
Aspergillus sp.	4	0	0	0	0	15	96	0	115
Alternaria alternata	1	0	0	0	0	0	0	0	1
Carvularia sp.	2	1	24	0	3	0	0	36	66
Cladosporium cladosporoides	0	7	24	61	373	513	144	0	1122
Drechslera sp.	6	0	0	0	0	12	0	0	18
Fusarium solanii	2	4	12	14	6	2	0	0	40
Penicillium notatum	0	0	0	0	132	0	0	0	132
Penicillium brefeldianum	2	96	108	227	0	55	0	144	632
Penicillium oxalicum	0	0	0	3	20	114	204	12	353
Penicillium sp.	4	0	0	0	16	0	0	0	20
Rhizopus sp.	0	0	0	0	6	1	0	12	19
Sterile mycelia	90	474	648	114	65	29	84	60	1564
Unidentified	6	0	0	0	0	0	0	0	6
Total	423	1307	1836	771	972	948	852	876	7985

In the petriplate studies by Andersen sampler, 25 different types of fungal species were identified. *Cladosporium cladosporoides* (1122 CFU/m^3), *Aspergillus glaucus* (1082 CFU/m^3), and *Aspergillus niger* (984 CFU/m^3) were the most dominant fungi followed by *Aspergillus fumigates* (861 CFU/m^3), *Penicillum brefeldianum* (632 CFU/m^3), *Aspergillus japonicas* (415 CFU/m^3), *Penicillum oxalicum* (353 CFU/m^3), *Aspergillus ochraceus* (341 CFU/m^3)(Table 2). While *Aspergillus nidulans* (27 CFU/m^3), *Penicillium* sp. (20 CFU/m^3), *Rhizopus* sp. (19 CFU/m^3), *Drechslera* sp. (18 CFU/m^3), *Aspergillus versicolor* (13 CFU/m^3), *Aspergillus nigricans* (11 CFU/m^3), *Aspergillus candidus* (7 CFU/m^3), *Aspergillus sydowi* (6 CFU/m^3), *Aspergillus flavus* (2 CFU/m^3), *Alternaria alternata* (1 CFU/m^3) were less abundant fungal spores (Table 2).

The higher concentration of fungal spores inside the library was noticed during September to October, while their concentration decreased in winter months (November to January) (Fig. 1). Moreover, higher concentrations of spores were trapped by Burkard sampler than that of Andersen sampler (Fig. 1). This is perhaps due to concentration of both viable and non-viable spores trapped in Burkard sampling than the occurrence of only viable spores in Andersen sampling.

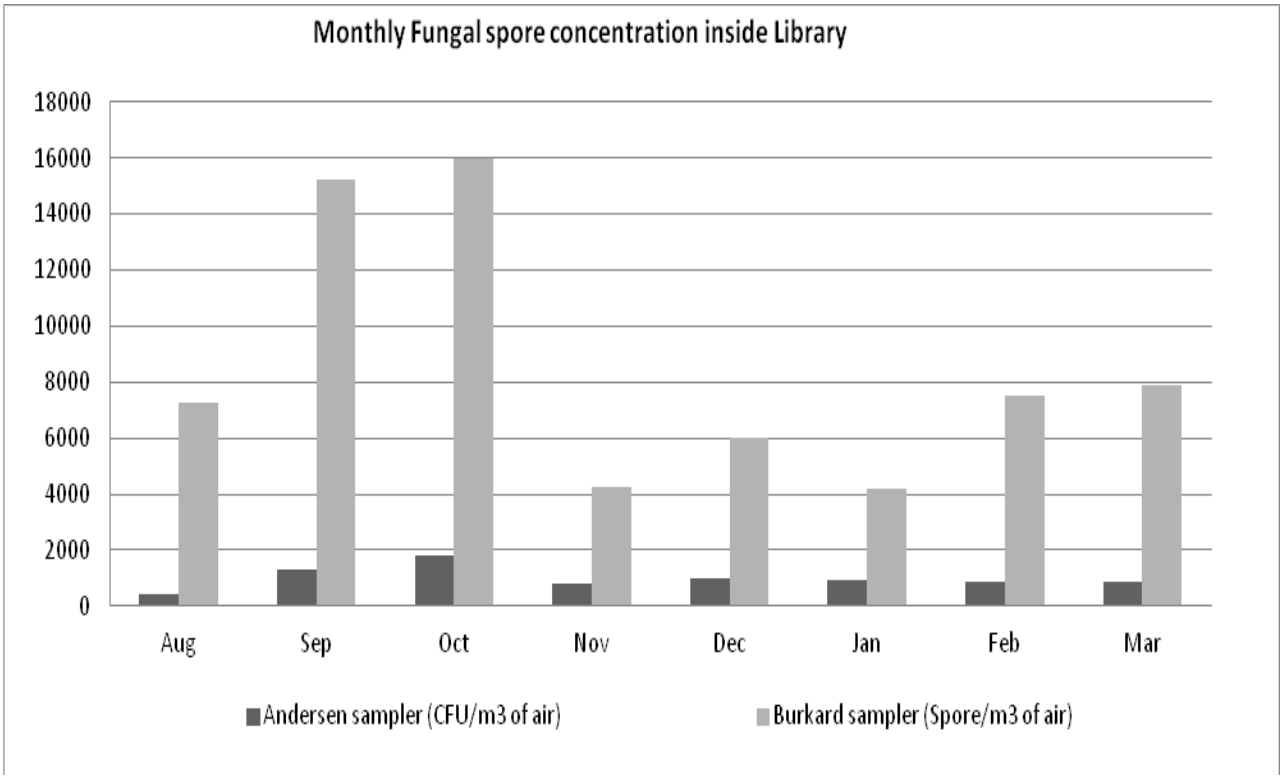

Fig 1: Month wise total fungal spore concentration by Burkard and Anderson volumetric sampler.

Five cellulolytic fungal species were collected from library namely, *Penicillium oxalicum*, *Aspergillus glaucus*, *Aspergillus niger*, *Penicillium brefeldianum* and *Aspergillus ochraceus.* The cellulase activity of these five fungal cultures were confirmed by Congo Red Dye decolouration. The results thus obtained were very much similar with the earlier reports of Sazci et al. (30). Isolates 1, 2 and 4 were identified as *Aspergillus* spp. and isolates 3 and 5 as *Penicillium* spp. In the present investigation, *Penicillium oxalicum* was shown to posses higher cellulose degrading ability demonstrating larger degrading zones (19.38 cm diameter) followed in the degree of prevalence by *Aspergillus niger* (14.08 cm), *Aspergillus glacus*(13.26 cm), and *Penicillium brefeldianum* (0.31 cm). However, *Aspergillus ochraceous* showed no cellulose degrading ability (Fig.2).

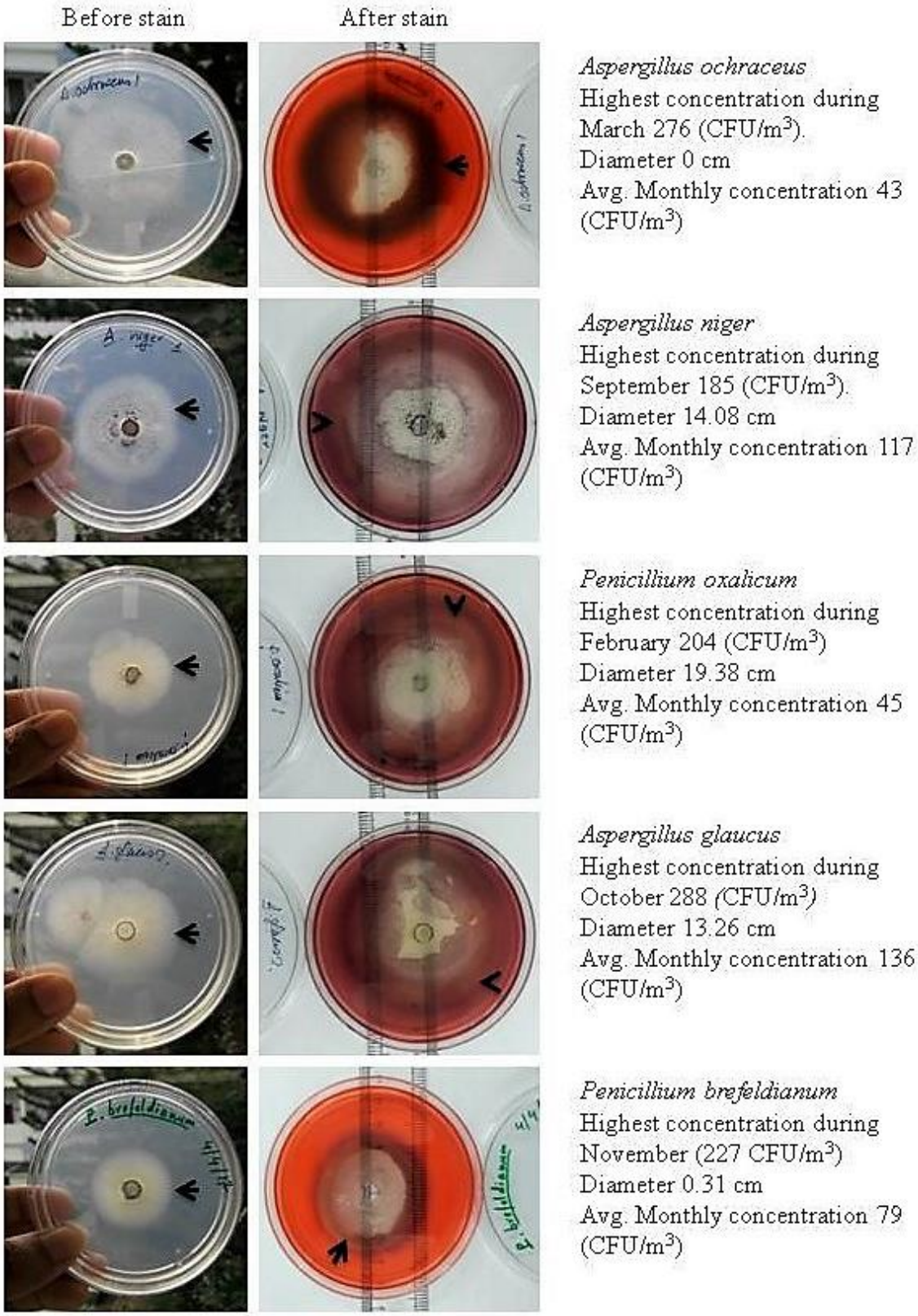

Fig 2: Fungal culture showing their cellulase activity and the degrading zone confirmed by Congo Red Dye decolouration.

CONCLUSION:

In the present study, it was revealed that there are plenty of airborne fungal spores inside the Central library of Visva-Bharati. The major sources of these fungi are old books and manuscripts of the library. Out of five cellulolytic fungi studied, four of them (*Penicillium oxalicum*, *Aspergillus glaucus*, *Aspergillus niger*, *Penicillium brefeldianum*) showed cellulose degrading ability marked by higher cellulose degrading zones. So proper care should be taken to prevent the growth of such fungi in books and manuscripts of Central library of Visva-Bharati, one of the oldest libraries of our country.

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