International Scholars Journals

African Journal of Botany ISSN: 3519-3824 Vol. 8 (1), pp. 001-006, January, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Identification and distribution of aeromycoflora in the indoor environment of Shyambazar Metro-Railway Station, Kolkata, India

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Accepted 09 September, 2019

Concentrations of fungal spores constitute a significant amount in bioaerosol depending on geographical regions and seasonal variations. Mycotoxin producing spores have adverse effects on humans. The aim of the study was to evaluate the prevalent species of airborne fungi in the indoor environment of the Shyambazar Metro-Railway Station, Kolkata, India. This area is below the ground level and fully surrounded, with constant movement of commuters. It is warm and humid with temperature and humidity ranges of 26.8 to 35.9°C and 50 to 88.3% respectively. Air sample was collected for four months within the interval of two weeks by means of gravitational settling method via Petri dishes with Potato Dextrose Agar (PDA) culture media. Those fungi colonies that formed after an incubation period of 3 to 5 days at 25 to 28°C were determined on the basis of micro and macro morphological characteristics. In this investigation, among fourteen spore types, *Aspergillus niger* was the most prevalent fungal genera followed by *Aspergillus flavus* and *Penicillium* sp. In addition, five sterile types and one unidentified species were also detected. The variation in the number of fungal colony was observed after every two weeks, in the summer months. The results of this investigation appeared to be quite significant for taking corrective measures.

Key words: Bioaerosol, aeromycoflora, mycotoxin, fungal spore, metro-railway station.

INTRODUCTION

Fungal spores constitute a significant fraction of bioaerosol and they are often much more numerous than other airborne bioparticulate matters. Among the different microorganisms in bioaerosol, fungal spores are larger ranging from 3 to 30 µm in diameter with various sources. The concentration of airborne microorganisms is correlated with the presence of dust and human activities. In addition, the concentration of fungal spores in the air is linked to geographical regions, seasonal variations and atmospheric conditions (Kerssies, 1993).

Airborne microfungal propagules are found in large numbers in indoor and outdoor environments and are widely distributed in nature in general. Some of them have the potentiality to cause allergies, spoilage of foods and many other adverse health effects, namely, bronchial asthma, allergic rhinitis and atopic dermatitis (Burge and Rogers, 2000; Terui et al., 2000; Akiyama, 2001). Their mycotoxins especially, can adversely affect human and animal health. People continuously come in contact with these airborne fungi often intake such air through respiration. However, no particular health effects are usually found when they are in negligible concentration. So, the examination and characterization of typical fungal

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distributions in one particular region can be helpful in identifying the relationship between their related domestic sensibility, diagnosis and clinical prevention of seasonal allergic diseases. Different studies indicate that fungal extracts can cause allergic rhinitis and bronchial asthma in indoor dwellings (Al-Suwaine et al., 2001; Su et al., 2001).

The trapping of fungal spores was done using plate exposure method. Weekly exposures were made. The most frequently found airborne spores were *Aspergillus* (23.08%), *Cladosporium* (19.73%), *Curvularia* (7.71%) and *Helminthosporium* (7.13%) (Verma and Chile, 1992).

Air almost always contains spores, but the number and type of spores depend on the time of day, weather, season and geographical location. Usually spores cause no trouble to most people but they can be harmful by provoking allergic responses or infections. The protein and polysaccharide components of fungi make them potentially allergenic to sensitized subjects and their airborne spores are ideally suited to penetrate into the respiratory tract. Allergy is a major cause of bronchial asthma, allergic rhinitis, migraine, urticaria, eczema etc. About 10% of the Indian population suffers from one or other type of allergic disorders (Channy and Verma, 1994).

The effect of threshing operations on the fungal spores of farm air was studied with the culture plate method (Uddin and Chakraverty, 1994). A comparative survey of airborne fungal spores in five indoor and five outdoor environments in Burdwan, West Bengal, India was carried out for a period of two years using Rotorod samplers and sedimentation plates (Chakraborty et al., 2000). An airborne fungal spore survey in five indoor environments in Santiniketan, West Bengal, India was carried out for a period of two years using the Astir 1-day personal volumetric sampler as well as a Rotorod sampler and sedimentation plates (Bhattacharya et al., 2001). An air survey of jute fields for two seasons in West Bengal, India, revealed that the total colony forming units (CFUs) varied with time, showing peaks (231 and 483 CFUs respectively) during the harvesting of the crop. The effect of temperature as well as relative humidity was probably not so significant (Uddin, 2005).

With the foregoing background, a systematic quantification of the indoor fungal flora of the Shyambazar Metro-Railway Station, Kolkata (Figure 1) was carried out for a period of four months beginning from March to June, 2007. The aim of this study was to determine the fungal flora, their identification, concentration and diversity in the metro-railway station. The environment may possess both beneficial and harmful fungal flora. In the case of beneficial fungi, they could be cultured in laboratories and could be used for producing valuable substances. On the other hand, in the case of harmful fungi, their effects could be studied more critically. Thus, precautions could be taken, their bio- control may be done and ultimately there will be more awareness of these particular contaminants. To the best of our knowledge, this is the first report regarding the identification of fungal population in a metro-railway station.

MATERIALS AND METHODS

Selection of study site

The site selected for the present study was the Metro-Railway Station, Shyambazar, Kolkata, India. It is one of the most crowded metro-stations in Kolkata (about 10000-12000 passengers/h). The microflora studies of this particular place have never been reported. So, information about the airborne sample in the metro-railway station is largely lacking. Although the environment of the entrance of the metro-railway platform is more or less equivalent to a natural outdoor environment, the underground platform has a closed environment system. The underground platform has a typical and unique aeromycoflora community which markedly differs from other indoor environments. The metro platform possesses a lesser temperature than the sea level natural community system since it is an underground closed system.

Media preparation

Fungi require a suitable substrate or culture medium (nutrient preparation) that can support their nutritional needs. By understanding the growth requirements of a fungal species, it is possible to establish the necessary conditions *in vitro* to support the optimal growth of that organism. For this purpose, Potato Dextrose Agar (PDA) medium, containing peeled potato (400 gml^{-1}), agar (20 gmL^{-1}) and dextrose (20 gml^{-1}) in distilled water, was prepared aseptically. Then the liquid media was poured into sterile Petri dishes using aseptic techniques. The media was allowed to solidify and then the junctures of the Petri dishes were sealed by sellotape.

Collection of sample

The Petri dishes were taken to the selected site to trap the fungal composition and were exposed in four different sites of the metro-railway station, namely Entry, North, Centre and South. The samples were collected at two weeks intervals between the months of March to June, 2007. Samples were taken in the afternoon (12:30 to 1:30 p.m.). The Petri plate gravitational method was employed for the isolation of fungi (Savino and Caretta, 1992; Rosas et al., 1993; Asan et al., 2002; Uddin, 2004). The Petri dishes were exposed to the air for 10 min. Four Petri dishes were used for each day. The Petri plates containing the samples were incubated for 3 to 5 days at room temperature (25 to 28°C).

Identification of fungal strains

The colonial features of the fungal colonies were studied as well as the morphological features of the fungi were studied using compound microscope. The determination of the morphological structures of fungi was carried out after being mounted in lactophenol and cotton blue covered with cover slip. The fungal types were analyzed for each day. The species were identified on the basis of micro and macro morphology; and reverse and surface coloration of colonies grown on the PDA media. The fungi were

Figure 1. Sampling site of the study.
Shyambazar Metro-Railway Station, Kolkata

identified up to genus level and in some cases up to species level (Cooke, 1963). The temperature and humidity data were recorded on different sampling dates (Table 1).

RESULTS AND DISCUSSION

In the present study, the selection of the site has an important relevance. The metro-railway station is more than fourteen years old. Though the station is disconnected from the outer polluted environment, interestingly, there is a good conformity in the number of species of fungi. Fourteen different spore types were found in this work (Figure 2). Besides these, there were also different kinds of sterile types and an unidentified species.

The dominance of *A. niger*, *A. flavus, Penicillium, Alternaria* and even sterile types (type A and type B) had been found through this investigation. The dominance of *Aspergillus* spp. was also previously reported in the indoor environment of Kolkata (Chakraverty and Sinha, 1985; Santra and Chanda, 1989). These fungi require more than 65% humidity for their growth and are associated with more than 80% deteriogenic activity. Generally, fungal count is high in outer polluted

Table 1. Temperature and humidity on different sampling dates.

Date	Temperature(°C)		Humidity (%)	
	Maximum	Minimum	Maximum	Minimum
24/03/2007	36.5	25.2	93	28
07/04/2007	35.4	26.6	84	56
21/04/2007	35.7	27.6	85	50
05/05/2007	36.6	27.0	88	49
19/05/2007	35.8	26.2	95	51
02/06/2007	35.9	28.6	85	66

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

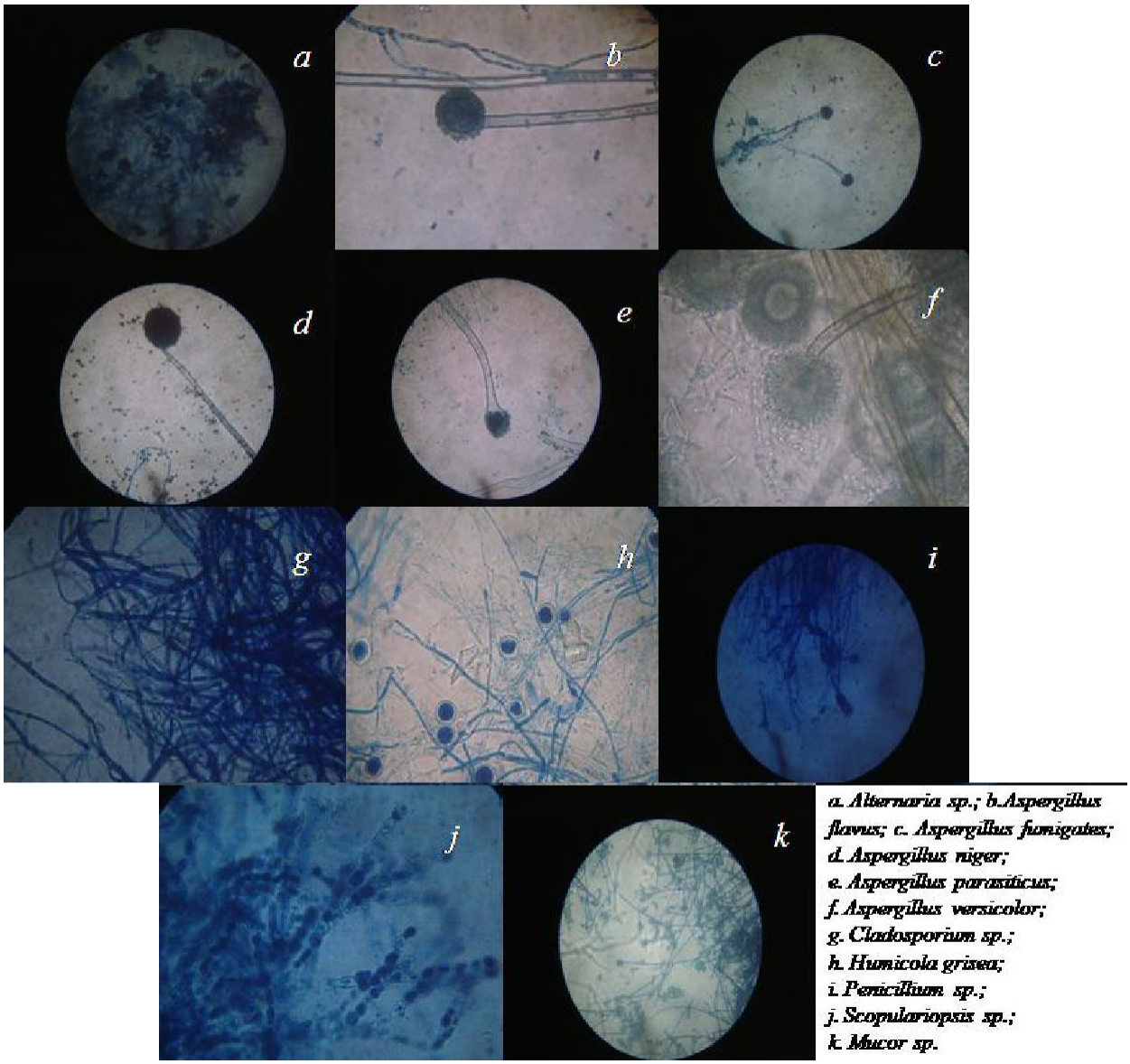

Figure 2. Different species of aeromycoflora of Shyambazar Metro-Railway Station under compound microscope (100X) (a) *Alternaria* sp. (b) *Aspergillus flavus* (c) *Aspergillus fumigates* (d) *Aspergillus niger* (e) *Aspergillus parasiticus* (f) *Aspergillus versicolor* (g) *Cladosporium* sp. (h) *Humicola grisea* (i) *Penicillium* sp. (j) *Scopulariopsis* sp. (k) *Mucor* sp.

Table 2. Total day count and percentage contribution of fungal colony from Shyambazar Metro-Railway Station.

Date of air sampling (2007)	March 24	April 7	April 21	May 5	May 19	June 2	Total	Fungal Colony Count (%)
Fungal Types	**No. of colony**							
Alternaria sp.	1	2	-	4	-	-	7	6.73
Aspergillus flavus	-	6	1	1	11	-	19	18.26
Aspergillus fumigatus	-	-	-	-	2	1	3	2.88
Aspergillus niger	4	1	9	5	5	6	30	28.84
Aspergillus parasiticus	2	-	-	-	-	-	2	1.92
Aspergillus versicolor	-	-	-	1	-	-	1	0.96
Cladosporium sp.	1	-	-	-	-	-	1	0.96
Curvularia lunata	1	-	-	-	-	2	3	2.88
Curvularia pallescens	-	1	-	-	-	-	1	0.96
Fusarium sp.	-	-	1	-	-	-	1	0.96
Humicola grisea	-	1	-	-	-	-	1	0.96
Penicillium sp.	4	-	4	6	-	-	14	13.46
Scopulariopsis sp.	1	-	-	-	-	-	1	0.96
Mucor sp.	-	-	1	-	1	-	2	1.92
Sterile type (A)	1	2	1	2	1	1	8	7.69
Sterile type (B)	1	-	-	3	-	-	4	3.84
Sterile type (C)	1	-	-	-	-	-	1	0.96
Sterile type (D)	-	1	-	-	-	-	1	0.96
Sterile type (E)	-	3	-	-	-	-	3	2.88
Unidentified	-	-	1	-	-	-	1	0.96
Total	17	17	18	22	20	10	104	99.94

environments but in our study, the high number of colony forming fungi in the site was due to lack of efficient maintenance probably. The working area also contains aero-allergenic fungal spores. The staff members and passengers associated with the metro-railway platform are constantly being exposed to these spores of which a good number are known for their hypersensitive reactions leading to respiratory problems like bronchial responsiveness (asthma), hypersensitivity pneumonitis, allergic alveolities such as bronchopulmonary aspergillosis, bronchoalveolar lavage or transbronchial lung problem (John, 1985; Bennett, 1995; Sugar, 1995).

The present study was aimed at determining the fungal flora, their identification, concentration and diversity in the Shyambazar Metro-Railway Station. At the end of the work, it was proven that a good number of fungal spores were found, that is, the indoor air of the metro-railway station had never been free of fungal spores.

The observations in the work depicted the fact that a rich fungal flora existed in the site (Table 2). The percentage of some airborne fungal viability is in higher concentration. From the information of reference studies, it was found that *Aspergillus flavus, Aspergillus fumigatus, Alternaria alternata, Cladosporium, Curvularia pallescens* demonstrated greater than 60% positive reactions in skin prick tests in many experimental cases.

There were many reports for the aflatoxin production in the *Aspergillus* spp. (Bennett and Goldblatt, 1973; Hesseltine et al., 1970; Schroeder, 1966). So the negligence of proper cleaning and maintenance of this site became a good source of the deteriorative and detrimental effect of the mould which may cause a potential health hazard. Certain corrective measures, strict maintenance or precautions which can reduce their frequency of occurrence include installation of exhaust fan to remove spores from the indoor environment before they get a chance to settle; air filtration; good ventilation; use of vacuum cleaner to remove dust; air conditioning machines and more frequent cleaning and preventive processes. In conclusion, the present study suggested that the Metro-Railway Station of Shyambazar, owing to its high humid and temperature conditions, harbors various species of fungi. It is of significance that our findings may be of use with regard to the diagnosis and prophylaxis of allergic diseases resulting from aeromycoflora.

ACKNOWLEDGEMENTS

The entire study was supported by Dr. Kunal Sen, HOD, PG Department of Botany, Barasat Government College,

Barasat, 24 PGS North, West Bengal, India. We are grateful to the India Meteorological Department, Regional Meteorological Centre, Alipore, Kolkata-700027, for providing the data of temperature and humidity on different sampling dates. The permission of sampling in the metro-railway station provided by the metro-railway authority, Shyambazar, Kolkata, is also gratefully acknowledged.

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