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ORIGINAL ARTICLE

MONITORING AND ASSESSMENT OF AEROMYCOFLORA OF INTRAMURAL ENVIRONMENT OF A CLASSROOM: ITS VARIATION, METEOROLOGICAL DETERMINANTS AND HEALTH IMPACT

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Abstract*: Airborne fungal spores in bioaerosol are potential intramural allergens and may cause serious allergic and respiratory problems. The level of indoor allergens are of important concern nowadays, since it has been observed that most people in average spend 90-95% time indoors. So, we have carried out air sampling of non-viable fungal spores in a classroom of a college of Habra, West Bengal, for two consecutive years (May 2015- April 2017) by Burkard Personal Volumetric Sampler. The aim of the present study was to monitor the monthly airborne fungal spore concentrations in the classrooms and to explore the influence of different meteorological parameters on the occurrence of these airborne spores. A total of 27 fungal spore types were identified. Among the recorded aerospora, the predominant fungal spores were Cladosporium (22.85%), Periconia (12.89%), Ascospores (11.52%), Aspergilli/ Penicilli (10.62%), Basidiospores (9.31%), Rust spores (7.79%) and Curvularia (7.11%) etc. Average number of spore concentration in the air of classroom was 2226 ± 576 spores /m^3. Maximum spore concentrations (2915±681 spores/m^3) was observed in post monsoon and minimum in monsoon (1925 ± 396 spores/m^3). In Spearman rank correlation analysis, a significant relationship between the meteorological factors with total and dominant spore concentration was observed. Thus, the present study depicts the exposure level of fungal spores within the college class room in different seasons, which may have important health impact regarding fungal respiratory allergic symptoms.*

Key Words**:** Bioaerosol, intramural, non-viable, meteorological parameters, Spearman rank correlation.

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1. INTRODUCTION

Bioaerosols mainly consist of fungal spores, which are potential indoor allergens and may cause serious allergic and respiratory problems [1-2]. In India, about 15-20 million people suffer from asthma and this number is increasing rapidly in different cities of West Bengal, India [3]. The levels of indoor pollutants are of important concern nowadays, since it has been observed that most people in average spend 90-95% time indoors [4]. Environment of the different indoor work places plays a crucial role in the hypersensitive individuals with the symptoms increasing during working hours and reducing afterwards. However, in some cases symptoms remains throughout the day. So, the identification and enumeration of fungal spores in the air of different indoor environments is of great interest. Several

aeromycological studies have been performed in different indoor environments till now [5-11]. However comprehensive investigation about the level of exposure of the occupant to allergenically significant aerosols in the different indoor environments and its related health impact is scanty. So, we have carried out present study in the indoor environment of classroom, where paper, glue, leather of books, copies, files and registers supports the growth and proliferation of fungi.

The aim of the present study was to: i) monthly monitor airborne fungal spore concentration in the air of indoor environment of classroom for two consecutive years (May 2015- April 2017), ii) explore the influence of different meteorological parameters on the occurrence of airborne fungal spores and iii) investigate the relationship between the indoor air quality and related health issues. For the proper diagnosis and treatment of health problems, this type of study of monthly and seasonal fluctuation pattern of airborne fungi is very essential [12].

2. MATERIALS AND METHODS

Air sampling and identification

Air sampling of non-viable fungal spores was done in the indoor environment of classroom of Sree Chaitanya College, Habra, a suburban area of North 24 Parganas, at monthly intervals by Burkard Personal Volumetric Sampler (Burkard Manufacturing Co. Ltd., Hertfordshire WD3 IPJ, U.K) which was placed at approximate human height (1.5 m) between 11.00 h and 14.00 h (Figure 1). The sampler sucks 10 liters of air per minute. After sampling for 10 minutes, glass slide was removed and a cover slip was placed over the band carefully using DPX as mountant. It was followed by the scanning of slides under Nikon high resolution light microscope (Labophot 2, Nikon Corp., Japan) at a magnification of 400X. Finally fungal spores were identified using specialized references [13,14] and also by comparing with reference slides. Fungal spores were counted according to the guidelines given in the British Aerobiological Federation [15]. As spores belonging to *Aspergillus* and *Penicillium* could not be separated visually when trapped by the Burkard sampler, they were counted and grouped under a single category "Aspergilli/ Penicilli" and they could be identified only by viable methods. Lastly fungal spore counts were converted to number of spores per cubic meter of air (m^3) according to standard protocol of the guidelines of The British Aerobiology Federation (1995).

Figure 1: Burkard Personal Sampler

Meteorological parameters

The meteorological parameters directly affect fungal growth, sporulation and dispersal. These parameters vary greatly in the sampling area during the entire study period. So, Meteorological data such as average temperatures (ºC), relative humidity (%), rainfall (mm), and wind speed (km/ hour)

were recorded from the Dum-Dum regional office of the Indian Meteorological Centre. The whole monitoring period was divided into four seasons- Summer (March-May), Monsoon (June- September), Post Monsoon (October- November) and Winter (December-February).

Statistical Analysis

All data were analyzed in Graph Pad Prism 6.0 (San Diego, CA, USA). To analyze the consistency in concentrations of total spores and predominant taxa during monitoring period, 't' test was performed. One-way ANOVA was applied to check any significant differences between the seasonal variation of the spore concentrations in the classroom environment. The correlation between total and dominant spore concentrations in classroom and different meteorological parameters was performed by Spearman non- parametric correlation (two-tailed) analysis.

3. RESULTS AND DISCUSSIONS

Quantitative evaluation of the aerial fungal spores

A total of 27 fungal spore types were identified through two years (May, 2015-April, 2017) of extensive sampling in classroom. Among the recorded aerospora, the predominant fungal spores were *Cladosporium* (22.85%), *Periconia* (12.89%), Ascospores (11.52%), Aspergilli/ Penicilli (10.62%), Basidiospores (9.31%), Rust spores (7.79%) and *Curvularia* (7.11%) etc. (Figure 2). Average total airborne spore concentration in classroom was 2226 ± 576 spores/m^3 which can be explained by the presence of many books, copies, students (about 60 students) and its untidy condition Analysis of the spore concentration by student's t –test (paired) showed that concentration of total spores and of Ascospores and Aspergilli / Penicilli spores had spore consistency in both the years, which may be due to almost similar climatic conditions in both the years. This result was similar to the previous study performed by Dey et al [16]. On the other hand, *Cladosporium*, *Periconia* and Basidiospores exhibited statistically significant differences. The reason behind these differences may be change in fungal growth substrates, air exchange rate, meteorological parameters and occupant behaviour which affect indoor air quality. Similar investigations [10,11] also reported such differences (Table 1).

Table 1: Paired 't' test showing spore consistency and differences of total and dominant spores in both the years

Fungal taxon	**1st Year**	**2nd Year**	**p- value**
Total spores	4351 ±1254	4484 ± 1055	0.6417
Cladosporium	697 ± 643	1142 ± 733	0.0426*
Ascospores	785 ± 268	989 ± 391	0.1883
Periconia	564 ± 829	238 ± 271	0.036*
Aspergilli/Penicilli	648 ± 220	743 ± 304	0.3298
Basidiospores	595 ± 262	426 ± 273	0.0463*

*$p<0.05$

Seasonal variation

The seasonal variation showed a significant effect on spore concentration in different indoor environments. Maximum spore concentrations (2915± 681 spores/ m^3) was observed in post monsoon

and minimum in monsoon (1925 ± 396spores/m^3) (Table 2). Moderate relative humidity and temperature, very low wind speed and minimum rainfall during post monsoon helped the release and dispersion of spores, which may increase spore concentration in this season. On the other hand, prolonged rain in monsoon washes the spores from the air which may results in minimum spore count in monsoon. Similar seasonal variation of aeromycota was recorded in many studies [16,17] performed in several parts of West Bengal.

Table 2: One way ANOVA analysis showing seasonal variation of total spores in classroom environment

Environment	Summer (March-may)	Monsoon (Jun-Sep)	Post- Monsoon (Oct-Nov)	Winter (Dec-Feb)	p-value
Classroom	2359±489	1925±396	2915±681	2033±444	0.0183*

*p<0.05

Correlation with meteorological factors

When Spearmann's rank correlation analysis was performed to find any relationship between the meteorological factors with total and dominant spore concentration, we have found that in classroom, Ascospores showed a significant positive association with temperature (r=0.725, p=0.0097**) and wind speed (r = 0.697, p= 0.0144*). while rainfall was negatively associated with total spore concentration (r = - 0.5874, p=0.0489*) and *Periconia* sp. (r =-0.7706, p= 0.0043**(Table 3). It has been observed that temperature, relative humidity, wind speed and rainfall showed a mixed impact on fungal spore concentration in the indoor environment of classroom. It may be due to the daily differences in optimum conditions for growth, sporulation, size, release, take – off and flight mechanism for different spore types. Similar differences were also reported in many investigations [18-20].

Table 3: Spearman's rank correlation coefficient (r) (two tailed) showing correlation of meteorological parameters with total and dominant spores

Fungal Taxon	Sampling site		Meteorological parameters			
			Temperature (˚C)	Relative Humidity (%)	Windspeed (Km/hr)	Rainfall (mm)
Total spores	College Classroom	P	0.7493	0.2464	0.2276	0.0489*
		r	-0.1049	-0.3636	-0.3776	-0.5874
Cladosporium		P	0.6673	0.5137	0.1767	0.1767
		r	-0.1399	-0.2098	-0.4196	-0.4196
Ascospores		P	0.0097**	0.3045	0.0144*	0.2937
		r	0.725	0.3222	0.697	0.3292
Periconia		P	0.327	0.0868	0.0733	0.0043**
		r	-0.3047	-0.5149	-0.5359	-0.7706
Aspergilli/Penicilli		P	0.9388	0.4573	0.8692	0.5431
		r	-0.02797	-0.2378	0.05594	-0.1958
Basidiospores		P	0.671	0.5682	0.6302	0.4392
		r	0.1366	0.1821	-0.1506	-0.2417

*p<0.05, **p<0.01, ***p<0.001

4. CONCLUSION

This study revealed essential information on composition and variations of airborne fungal spores in indoor environment of a classroom. It has also been observed that most of the fungal spores reached a peak in post monsoon season. As the occurrence of fungal spores cannot be controlled, avoiding fungal spores and taking medication in peak season are the main strategies for allergic individuals. However, this requires proper forewarning of aeroallergen levels. So, this type of investigation will be very helpful for allergist and clinicians for understanding fungal spore load in the air and for making people and students aware in order to prevent consequent health problems.

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