

Original Research Article

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Study on Distribution of Airborne Fungi in a University Building

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ABSTRACT

Keywords

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Higher level of microbial contamination in indoor air is a serious problem of recent health concern. The air of representative areas, viz., Lecture Hall, M.Sc. Lab and Dean Office of University Campus in Jodhpur, Rajasthan (India) was analyzed to determine the level of fungal types most frequently found in suspension. Samples were collected by open plate technique during winter, summer and monsoon seasons 2013-14 and viable fungal counts were determined. The mean seasonal concentrations of total airborne fungi in the University Campus ranged from 16.39-32.22 CFU/m³ in selected indoor sampling sites. In outdoor it ranged from 50.83-70 CFU/m³. There was not any significant difference in the concentrations of fungi between unoccupied and occupied conditions. Also, a comparison between indoor and outdoor fungal population densities showed that the fungal concentration was higher outside than inside. Different sites showed different concentration of airborne viable fungi and observed the following trend among locations- Dean Office > Lecture Hall > M.Sc. Lab. In the present study, a total of 8 types of fungal genera isolated from the air of University Campus. These included *Aspergillus* (*Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus terreus*), *Alternaria*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Rhizopus* and *Yeast*.

Introduction

Fungal flora can be hazardous for health and can cause allergies, symptoms of sick building syndromes (SBS) causing irritation of mucous membranes and physical condition, tiredness, headaches, decrease of concentration, memory and intellectual work ability, dermatitis, respiratory diseases (including asthma) and cancers (Burton, 1991). It is supposed that about 30% of health problems relevant to the indoor air quality are the result of a human organism reaction to moulds (Gutarowska and Jakubowska, 2002).

For students and staff members educational buildings are the places where they reside most of their time. Fungal contamination of air inside buildings may pose adverse effects on their health.

Materials and Methods

Study Area

The study was carried out in both indoor and outdoor environment of J. N. Vyas University Campus in Jodhpur. Three indoor

sampling sites, Lecture Hall, M.Sc. Lab and Dean Office representing different environmental conditions were selected for the study. For the present investigation occupant density and type of ventilation were taken into consideration.

Sampling and Analysis Method

Samples were taken in all three seasons *i.e.* winter, summer and monsoon season during the year 2013-14. Open plate technique was used to take samples from air. Potato Dextrose Agar (PDA) (HiMedia Laboratories Limited, Mumbai, India) was poured into sterile petridish and allowed to solidify. Plates were exposed in the air for 30 minutes at the sampling sites placing at about 1.5 m above the surface, to simulate the human breathing zone (Obbard and Fang, 2003).

After exposure, the plates of Potato Dextrose Agar were incubated at 25°C for 5-7 days. Three plates of media for each site were exposed at a time and the procedure was repeated twice in a season. The total number of colony forming units (CFUs) was enumerated and converted to organisms per cubic meter air.

During the whole study, parameter such as temperature and humidity was also measured. Temperature ranged between 24.2-37.8°C; whereas, humidity ranged between 49-84% at indoor areas. The I/O ratio was also calculated.

Identification of Fungal Isolates

A wet mount preparation of each fungal colony was prepared by Lacto-phenol-cotton-blue solution and examined microscopically. Then the fungal colonies were identified according to the manual of Barnett and Hunter (1972).

Statistical Analysis

In the present study, Pearson's correlation coefficient procedure was used to estimate the impact and degree of effectiveness of meteorological factors (temperature and humidity) on airborne fungal concentration. The statistical significant difference in the concentration of airborne fungi among the sampling sites and among the seasons was determined by one-way ANOVA test (Gomez and Gomez, 1984).

Results and Discussion

In the present study, the mean seasonal concentrations of total airborne fungi in the University Campus ranged from 16.39-32.22 CFU/m³ in selected indoor sampling sites. In outdoor it ranged from 50.83-70 CFU/m³ (Fig.1-3). The recorded levels of airborne fungi were much lower than those reported by earlier researchers (Stryjowska-Sekulska *et al.*, 2007; Hayleeyesus and Manaye, 2014). There was not any significant difference in the concentrations of fungi between unoccupied and occupied conditions, suggesting that most fungal species present into the air were not human-borne. Previously, Soto *et al.* (2009) also revealed that indoor fungal species are mostly derived from exogenous origin.

Different sites showed different concentration of airborne viable fungi and observed the following trend among locations- Dean Office > Lecture Hall > M.Sc. Lab. Outdoor air may be the main source for the fungal growth observed. Previously, Stryjowska-Sekulska *et al.* (2007) also suggested that presence of so called outdoor mould *Cladosporium* and *Alternaria* showed their input from outside via visiting people. Age of the building, deficient hygienic conditions and low degree of cleanliness might raise the airborne

biocontaminants. Wurtz *et al.* (1999) isolated some strains of *Aspergillus* (*A. versicolor*) and *Penicillium* from the indoor air of some Danish schools and observed that area per person also appeared to be associated with airborne fungal concentrations.

Exposure to higher concentration of several microbial community and their toxins may pose a potential risk of several diseases for the occupational environment (Kim *et al.*, 2007). Inhalation is the predominant route of exposure of these microorganisms resulting in adverse health effects. Other pathways of exposure include the conjunctiva, ingestion of microbes from surfaces (e.g. hands, food, etc) contaminated by deposition (Lurie *et al.*, 1950).

The environmental factors mainly include temperature, humidity, air exchange rate, air movement, building structures and location, poor design, ventilation system as well as interior design which enhance microorganism's growth and multiplication in the indoor environment (Graudenz *et al.*, 2005; Meadow *et al.*, 2014). Seasonal fluctuations in both number and type of airborne fungi were also recorded. Concentration of total airborne fungi was observed highest in monsoon season and lowest in summer season in all the indoor and outdoor sampling sites. These observations are in agreement with Verma and Chile (1992) and Shukla and Shukla (2010), who also reported higher fungal count in monsoon period which may be due to wet condition and high humidity.

Microbiological quality of indoor air not only depends upon the number of bacteria and fungi but also upon particular species, which exerts greater effect on the health of people occupying the place. In the present study, a total of 8 types of fungal genera

isolated from the air of University Campus. These included *Aspergillus* (*Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus terreus*), *Alternaria*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Rhizopus* and *Yeast* (Fig.4-15). Similarly Naruka and Gaur (2013) found that in a school building most often occurred fungi were from genera *Aspergillus*, *Cladosporium* followed by *Fusarium*. In a study by Stryjakowska-Sekulska *et al.* (2007) similar types of fungal genera were isolated from the air of various rooms in university buildings. Those genera included *Aspergillus* (*A. niger* and *A.flavus*), *Cladosporium* (*Cladosporium herbarum*), *Penicillium* (*Penicillium chrysogenum*, *Penicillium viridicatum* and *Penicillium expansum*), *Alternaria* (*Alternaria alternata*), *Mucor*, *Rhizopus nigricans* and *Epicoccum*.

Types of fungal species isolated and identified in the present study, were already characterized as potential allergenic in nature and exposure to them may provide immune responses in the susceptible individuals. These species were also found to be capable of eliciting a number of diseases responses such as infectious, allergic and toxic effects (Albinas *et al.*, 2004; Hamilos, 2010; Madani *et al.*, 2010). Some species of these fungi, like *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus*, are recognized opportunistic pathogens for humans. These microorganisms are considered potential candidates involved in the establishment of sick building syndromes (Schwab and Straus, 2004).

In the present study, *Aspergillus* sp. was found in high concentrations in summer. Whereas in winter season *Cladosporium* sp. and *Alternaria* sp. were in higher counts. These results are in agreement with the fact

that spores of *Aspergillus* species are generally well adapted to survival in the absence of available water and nutrient in the atmosphere (Ingold, 1971). Whereas, highest count of *Cladosporium* and *Alternaria* sp. in winter (dry season) may be due to their nature of conidia as they both produce dry conidia in chains and greater dispersal of dry powdery spores in air (Katial *et al.*, 1997).

Correlation of temperature with total airborne fungi was positively weak at Lecture Hall (r=0.033009) and Dean Office (r=0.09284) and positive at M.Sc. Lab (r=0.388092). Humidity was correlated positively strong with total airborne fungi at Lecture Hall (r=0.886073) and M.Sc. Lab (r=0.79913) and positive at Dean Office (r=0.499463) (Table: 1).

Correlation of temperature with total *Aspergillus* count was positively strong at Lecture Hall (r=0.952147), M.Sc. Lab (r=0.999919) and Dean Office (r=0.999373). Correlation of temperature with other fungal species count was negatively strong at

Lecture Hall (r=-0.55848), M.Sc. Lab (r=-0.79208) and Dean Office (r=-0.88653). Humidity was correlated positively weak with total *Aspergillus* count at Lecture Hall (r=0.087157), negative at M.Sc. Lab (r=-0.30085) and negatively weak at Dean Office (r=-0.28439). Humidity was correlated positively strong with other fungal species count at Lecture Hall (r=0.932613), M.Sc. Lab (r=0.827624) and Dean Office (r=0.720697) (Table: 1).

In the present study, for University Campus; the ‘F’ value calculated for fungi using one way Analysis of Variance method (ANOVA) revealed, a significant difference in their concentrations due to season and sampling sites (Table: 2).

In the present study, the I/O ratio for fungi was observed less than 1 (0.4) for all seasons. This means that the indoor fungal concentration was always found lower than that of the outdoors. Higher outdoor fungal concentration shows that indoor fungal species are mostly derived from exogenous origin.

Table.1 Correlation coefficients (‘r’) Showing the Effect of Meteorological Factors on Fungal Concentrations at University Campus

Investigated Sites		Total Airborne Fungal Count	Total <i>Aspergillus</i> Count	Other Fungal sp. Count
Lecture Hall	Temperature	0.033009	0.952147	-0.55848
	Humidity	0.886073	0.087157	0.932613
M. Sc. Lab	Temperature	0.388092	0.999919	-0.79208
	Humidity	0.79913	-0.30085	0.827624
Dean Office	Temperature	0.09284	0.999373	-0.88653
	Humidity	0.499463	-0.28439	0.720697

Table.2 Analysis of Variance (ANOVA) for Fungi at University Campus

Source of Variation	DF	Sum of Squares	Mean Squares	Computed F Value
Replicates	2	43.75	21.875	1.536436 ^{NS}
Season	5	1959.281	391.8563	27.52283 ^S
ERROR a	10	142.375	14.2375	
Sampling Sites	3	27808.85	9269.614	361.3371 ^S
ERROR b	6	153.9219	25.65365	
Season* Sampling Sites	15	834.9219	55.66146	4.032221 ^S
ERROR c	30	414.125	13.80417	
Total	71	149333.8		

Fig.1 Concentration of Total Airborne Fungi in the University Campus in Winter Season (Mean ± S.D.)

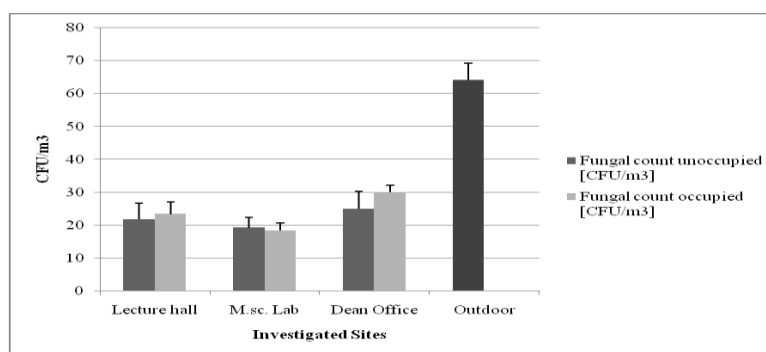


Fig.2 Concentration of Total Airborne Fungi in the University Campus in Summer Season (Mean ± S.D.)

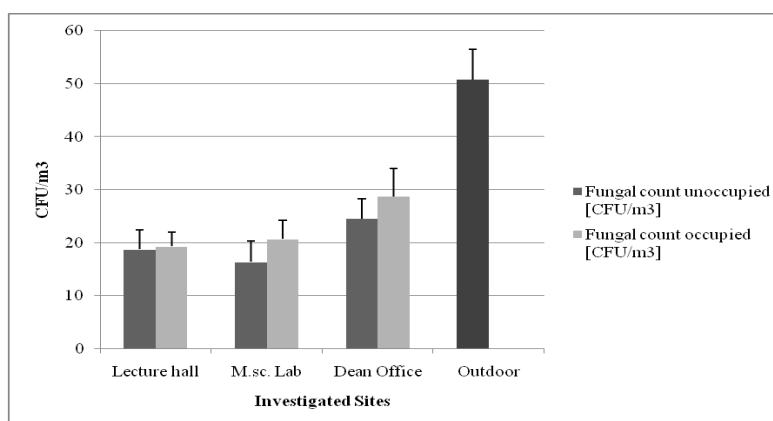


Fig.3 Concentration of Total Airborne Fungi in the University Campus in Monsoon Season (Mean \pm S.D.)

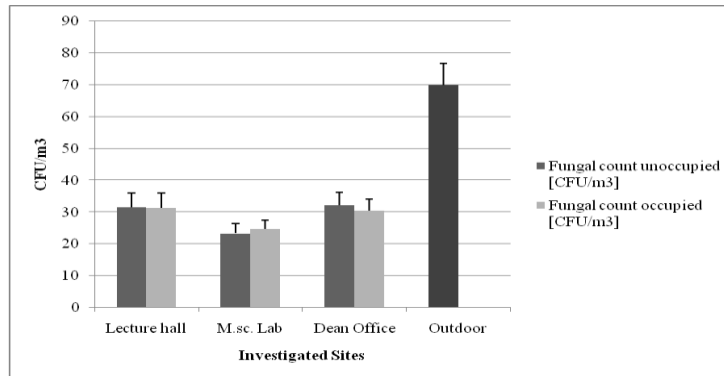
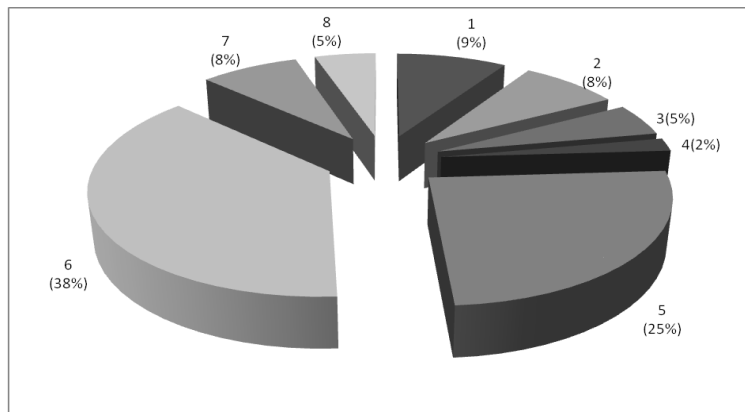
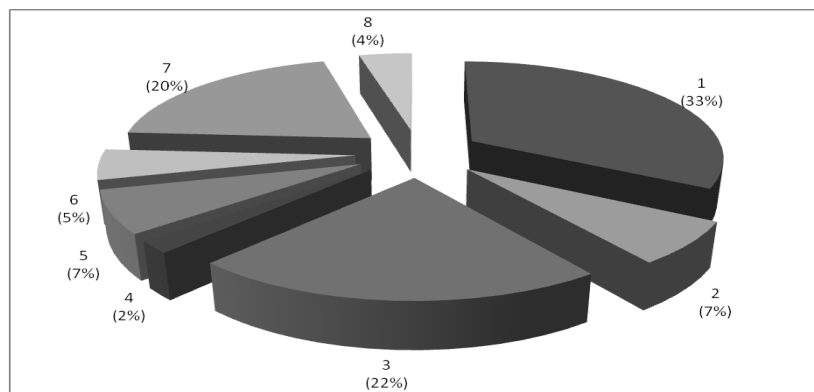


Fig.4 Distribution of Various Fungal Species in Lecture Hall in Winter Season



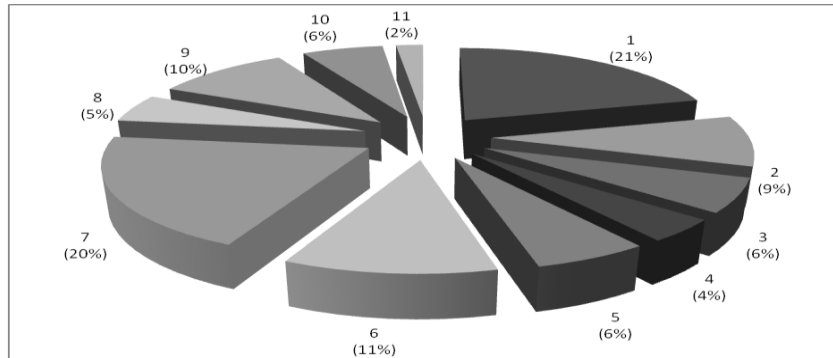
1-*A.niger*, 2-*A.fumigatus*, 3-*A.flavus*, 4-*A.terreus*, 5-*Alternaria sp.*, 6-*Cladosporium sp.*, 7-*Fusarium sp.*, 8-*Penicillium sp*

Fig.5 Distribution of Various Fungal Species in Lecture Hall in Summer Season



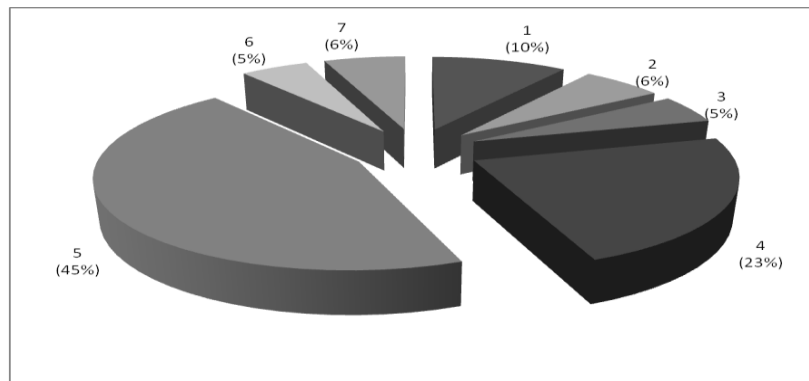
1-*A.niger*, 2-*A.fumigatus*, 3-*A.flavus*, 4-*Alternaria sp.*, 5-*Cladosporium sp.*, 6-*Fusarium sp.*, 7-*Penicillium sp.*, 8-*Rhizopus sp*

Fig.6 Distribution of Various Fungal Species in Lecture Hall in Monsoon Season



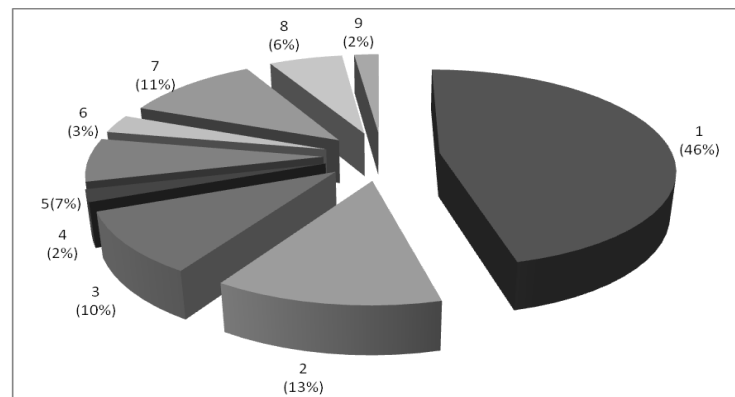
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-A. terreus, 5-Alternaria sp., 6-Cladosporium sp., 7-Fusarium sp., 8-Helminthosporium sp., 9-Penicillium sp., 10-Rhizopus sp., 11-Yeast

Fig.7 Distribution of Various Fungal Species in M. Sc. Lab in Winter Season



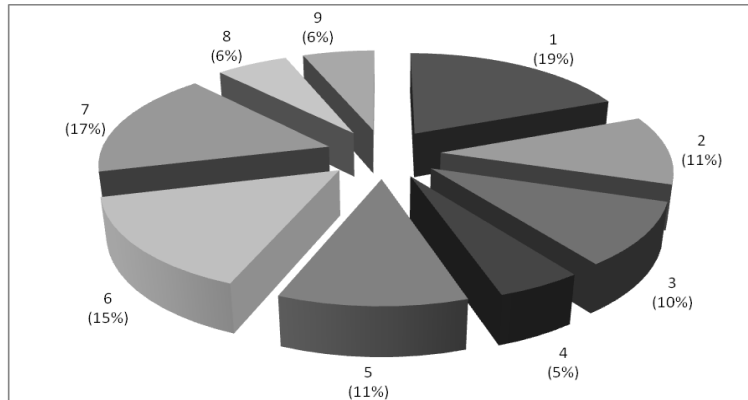
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4- Alternaria sp., 5-Cladosporium sp., 6-Fusarium sp., 7-Penicillium sp

Fig.8 Distribution of Various Fungal Species in M. Sc. Lab in Summer Season



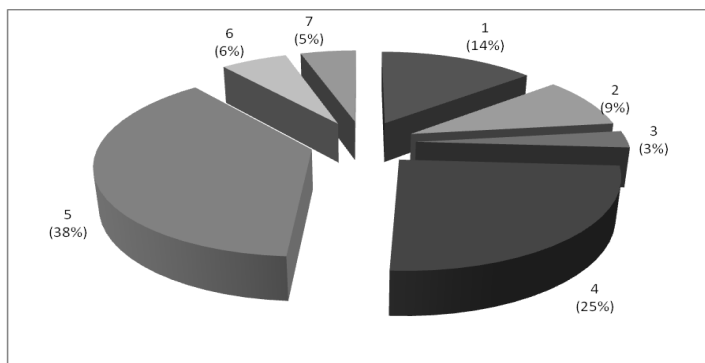
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-Alternaria sp., 5-Cladosporium sp., 6-Fusarium sp., 7-Penicillium sp., 8-Rhizopus sp., 9-Yeast

Fig.9 Distribution of Various Fungal Species in M. Sc. Lab in Monsoon Season



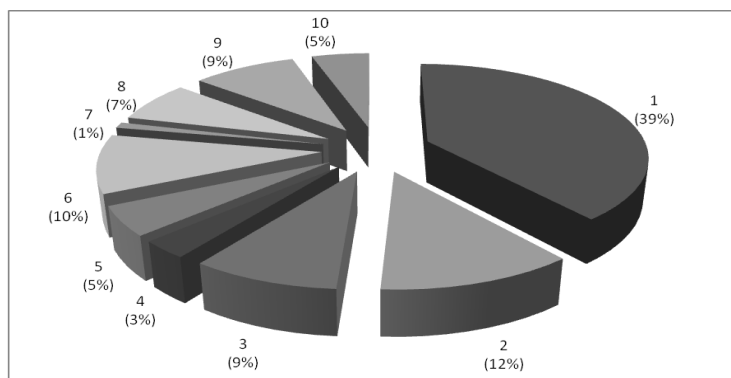
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-A. terreus, 5-Alternaria sp., 6-Cladosporium sp., 7-Fusarium sp., 8-Penicillium sp., 9-Rhizopus sp

Fig.10 Distribution of Various Fungal Species in Dean Office in Winter Season



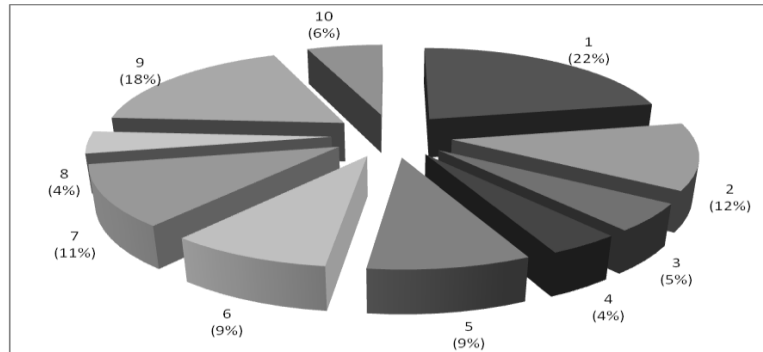
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-Alternaria sp., 5-Cladosporium sp., 6-Fusarium sp., 7-Penicillium sp

Fig.11 Distribution of Various Fungal Species in Dean Office in Summer Season



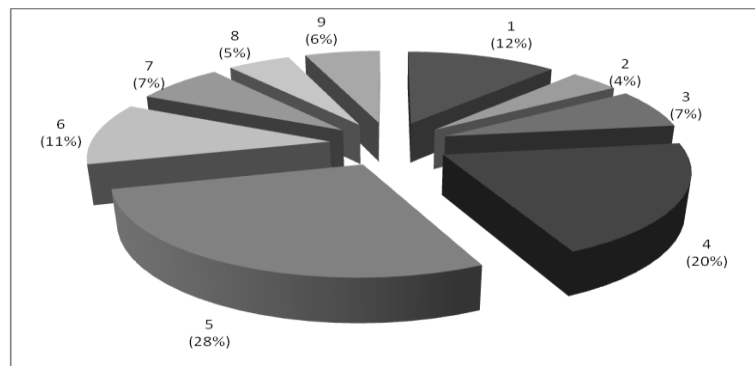
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-Alternaria sp., 5-Cladosporium sp., 6-Fusarium sp., 7-Helminthosporium sp., 8-Penicillium sp., 9-Rhizopus sp., 10-Yeast

Fig.12 Distribution of Various Fungal Species in Dean Office in Monsoon Season



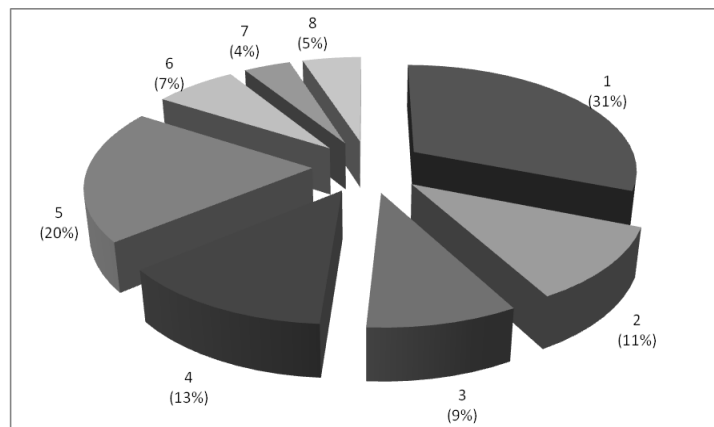
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-A. terreus, 5-Alternaria sp., 6-Cladosporium sp., 7-Fusarium sp., 8-Helminthosporium sp., 9-Penicillium sp., 10-Rhizopus sp

Fig.13 Distribution of Various Fungal Species in University Campus Outdoor in Winter Season



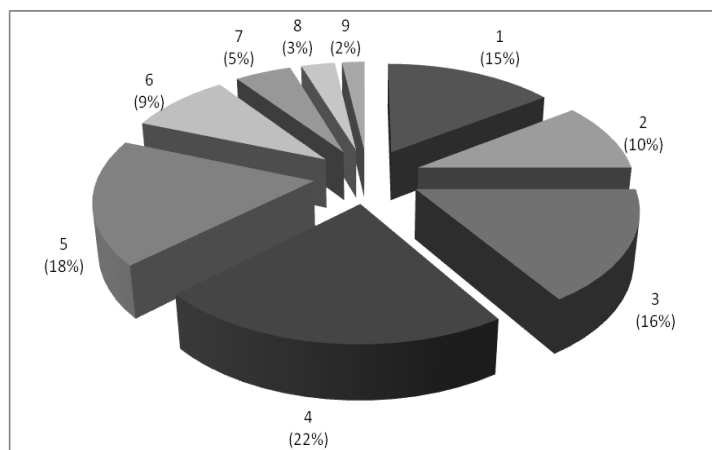
1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-Alternaria sp., 5-Cladosporium sp., 6-Fusarium sp., 7-Helminthosporium sp., 8-Penicillium sp., 9-Rhizopus sp

Fig.14 Distribution of Various Fungal Species in University Campus Outdoor in Summer Season



1-A.niger, 2-A.fumigatus, 3-A.flavus, 4-Alternaria sp., 5-Cladosporium sp., 6-Fusarium sp., 7-Helminthosporium sp., 8-Penicillium sp

Fig.15 Distribution of Various Fungal Species in University Campus Outdoor in Monsoon Season



1-*A.niger*, 2-*A.fumigatus*, 3-*A.flavus*, 4-*Alternaria* sp., 5-*Cladosporium* sp., 6-*Fusarium* sp., 7-*Helminthosporium* sp., 8-*Penicillium* sp., 9-*Rhizopus* sp

Goh *et al.* (2000) also observed that the levels of fungal spores measured in the indoor environment were approximately fifty times lower than those measured outside, probably because of the lowered humidity caused by the air conditioning in the indoor environment. The outdoor concentration of the total fungi was found usually higher compared to the indoor concentrations for different areas (Pei-Chih *et al.*, 2000; Yassin and Almouqatea, 2010).

In conclusion, contamination of indoor air at educational buildings poses a serious problem from the point of view of health protection for employees and students. The real hazard of such pollution levels may be more serious as the level of total microbes may be even higher than the number of culturable microbes. So it is necessary to develop the standards of indoor air quality related to microbial pollution for educational settings.

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