Incidence of Indoor Airborne Fungi at the Central Library of Rajshahi University and Their Relation to Allergy Symptoms

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ABSTRACT

The indoor air sampling was conducted at the Central Library of Rajshahi University by settling plate technique during November 2006 to April 2007 using Potato dextrose agar, Czapek's and Sabouraud's media. Total of 4,613 colonies of airborne fungi were trapped and 11 genera were identified. The most frequently isolated genera were Alternaria, Aspergilus, Curvularia, Fusarium, Penicillium and Rhizopus. Percentages of the six dominant genera were recorded as 29.25, 14.55, 13.64, 10.79, 7.48 and 6.43%; 26.83, 9.58, 14.25, 8.77, 8.19 and 7.38%; and 25.05, 12.98, 9.32, 7.25, 5.85 and 14.63% on PDA, Czapek's and Sabouraud's media, respectively. The incidence of airborne fungi significantly (p=0.05) varied with floor and the highest incidence was recorded at ground floor, followed by 1st floor and the lowest in 2nd floor. Among the 11 identified genera, Aspergillus, Fusarium, Mucor and Penicillium showed positive results in hemolytic activity test. The incidence of airborne fungi was correlated with allergy symptoms of employees, students and researchers, showing the highest peak in April, 2007.

Key words: Indoor airborne fungi, Percentage contribution, Room condition, Hemolytic activity, Allergy symptoms

INTRODUCTION

Air quality in indoor environment is being recognized as an important issue in public health (Li and Kendrick, 1995a). As many as 30% of buildings world wide have indoor air quality complaint (WHO, 1983) and in the United States, as many as 40% of homes and 115,000 schools have health problems, linking to poor indoor air quality (Spengler et al., 1994). Fungal exposures have been documented to cause allergic disease (1 in 4 people world wide), toxicoses, irritation and infections (Barge, 1990; Chao et al., 2002) and blamed for building related symptoms (Harrison et al., 1992). In developed countries, the majority of the people spend more than 90% of their time indoors, and thus experience long exposure to common airborne pollutants which have potentially adverse health effects. To systematically evaluate the relationship between airborne fungi and adverse health effect, the fungal types and their relative frequencies in indoor airs need to be known (Shelton et al., 2002). Sources for indoor airborne fungi can be outdoor air and indoor reservoirs (Berge, 1995; Li and Kendrick, 1996). Although outdoor fungi cannot go easily inside the large buildings with complex ventilation systems, the outdoor aerosol still may dominate indoors (Burge et al., 2000). Accumulated dust and room materials, viz., wallpaper, carpeting, ventilation duct surfaces can also become bioaerosol sources if water content can support the growth of microorganisms (Barge, 1990; Berge, 1995; Burge et al., 2000; Stolwijk, 1991).

Information obtained from fungal air samples can assist in medical evaluation, determination of remedial procedures and assessment of health hazards and can be useful in proactive indoor air quality monitoring. However, there are no government or industry standards that specify acceptable concentrations of indoor airborne fungi and only limited information is available on airborne fungal types and their prevalence inside the buildings. So, the present attempt has been undertaken to asses the incidence of indoor airborne fungi at the Central Library of Rajshahi University and their possible relations with the environmental factors like temperature and relative humidity. The hemolytic activity of identified genera was tested and the research was also expanded to determine the relations of airborne fungi with library environment and occupants/workers.

MATERIALS AND METHODS

Sampling

The indoor air sampling was conducted at different sites of the Central Library of Rajshahi University Rajshahi during November 2006 to Aprill 2007. Using Czapek's, Potato Dextrose Agar (PDA) and Sabouraud's media, air samples were colleted from near occupants breathing zone (approx. 1 m above ground), following settling plate technique. At each sampling site, a total of 36 culture plates [4 samplings per month × 3 plates for each culture medium (triplicate) × 3 culture media] were collected per month. Room temperature and relative humidity (RH) were recorded at each sample site. The presence of dampness, visible fungi and cleanness of the sample areas were also noted during sampling.

Enumeration and identification of airborne fungi

The Petridishes (9 cm diam) containing media were exposed for fifteen minutes during sampling and incubated at room temperature for four to seven days. This was followed by the counting of the fungal colonies on the culture plate. Sub-cultures of the fungi recovered from air sampling were maintained on slant cultures for various periods to identify fungi, induction of sporulation of sterile mycelia etc. Identification of the fungi was made by visual (colony morphology) and microscopic observation. Identification up to generic level was done with the help of standard mycological literature (Gilman, 1957; Booth, 1971; Subramanian, 1971; Ellis, 1971; Alexopoulos and Mims, 1979). Sub-cultures of the fungal mycelia which failed to sporulate upto the end of one month were designated as sterile mycelia.

Details regarding the qualitative nature of the mycoflora, their incidence, abundance and percentage contribution were recorded. The percentage contribution of each genus was calculated on the basis of the number of colonies of a genus against the total number of colonies of all recorded genera during the entire six months of sampling period.

Hemolytic activity test

For hemolytic activity test, blood agar medium (5% cow blood) was used. Spore suspension (10^6/ml) of each trapped genus was spread on the plate containing blood agar and kept in an incubator at 30˚C for two to three days. Fungal colonies, developing from incubated spore suspension, showing zone of clearing around them are considered as hemolytic-positive.

Questionnaires

For determination of relationships of indoor airborne fungi and allergy symptoms, employees, students and researchers were asked to complete initial questionnaires during the observation period of six months. The questionnaires included questions about personal data and health complications. The options for allergy symptoms were indicated by allergist as plugging, itchy, sneezing or running nose; itchy, watery, swelling and redness of eye; throat sore, swelling; chest tightness, cough and difficulties of breathing.

Statistical analyses

The experiment was conducted by using a completely randomized design with three replications. All data were analyzed by F-test. Results of all analyses were judged for significance at 5% level.

RESULTS AND DISCUSSION

A total of 4,613 viable fungal colonies were trapped from indoor atmosphere of the Central Library of Rajshahi University using Czapek's, Potato dextrose agar and Sabouraud's media during November 2006 to April 2007. Among them, 4039 colonies of fungi were identified, 524 colonies were sterile and 50 colonies were unidentified. The isolated fungi were assigned to 11 genera, belonging to Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. Among the three media tested, PDA was most favorable for growth and development of identified fungi and the maximum sterile mycelia were recorded on Czapek's medium. Monthly variation in total fungal colonies with respect to temperature and average relative humidity was observed on PDA, Czapek's and Sabouraud's media during the investigation (Fig.1). The highest temperature (31.27˚C) and low relative humidity (45.97%) were recorded in April which was associated with the highest number of fungi. The lowest temperature (15.35˚C) and moderate humidity (51.30%) were recorded in January that were related with the lowest number of fungi in the indoor atmosphere of the Central Library. Thus, temperature seems to be positively correlated with the incidence of airborne fungi and has an effect on increasing the number of airborne fungi. Chao et al., (2002) observed that the temperature is the critical environmental factor which control microbial growth in indoors and total airborne fungal concentration is positively correlated with relative humidity (RH) at below 30% and above 40%. Wright et al., (1969) also opined that the prevalence of airborne mycoflora was intimately related with prevalent climatic conditions including temperature and relative humidity.

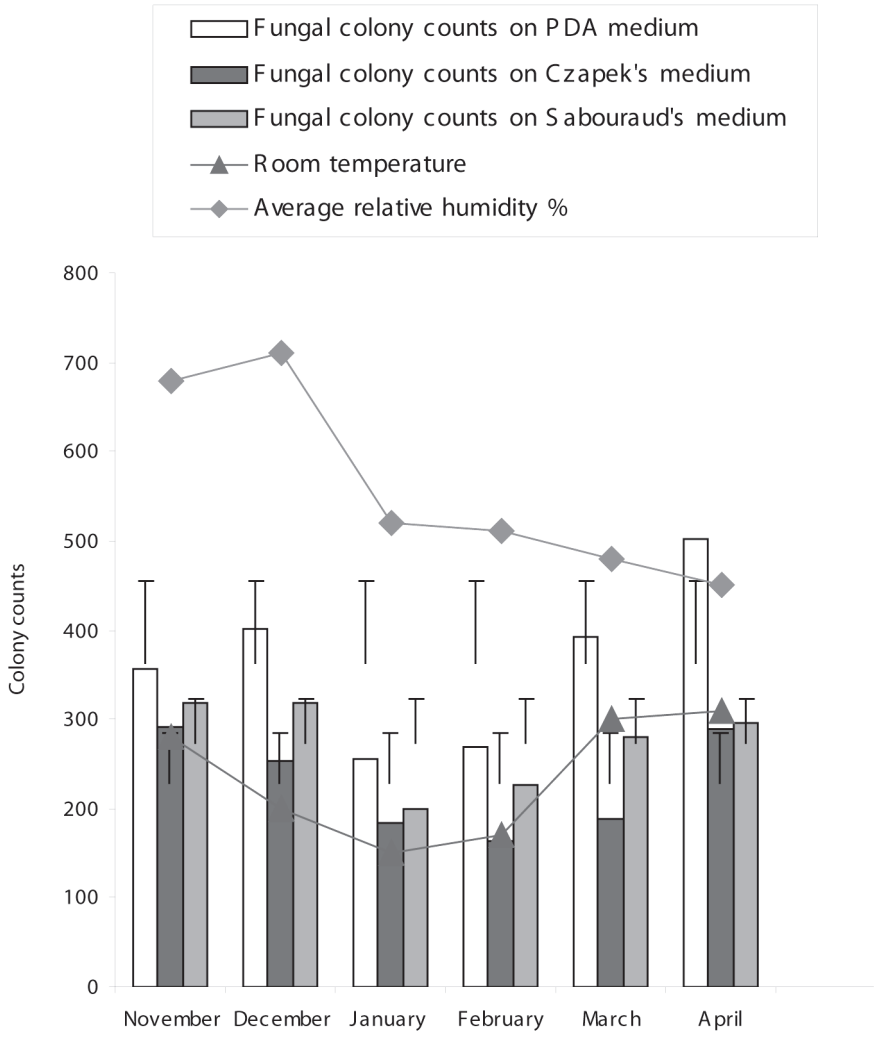
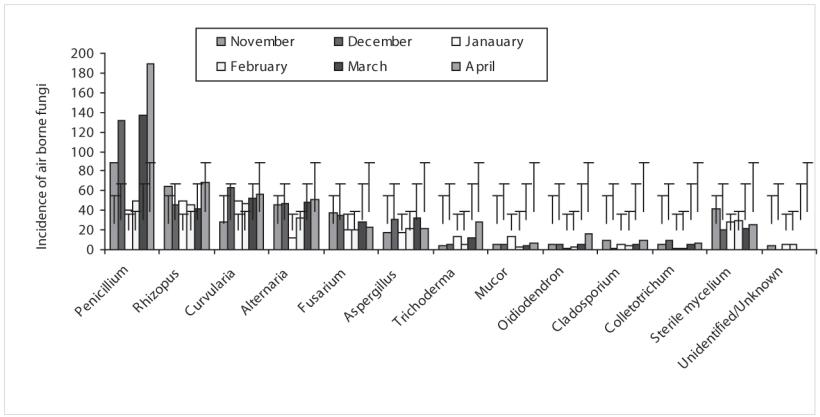


Figure 1. Monthly occurrence of total indoor aerial fungi with respect to some environmental factors, as recorded on PDA, Czapek's and Sabouraud's media.

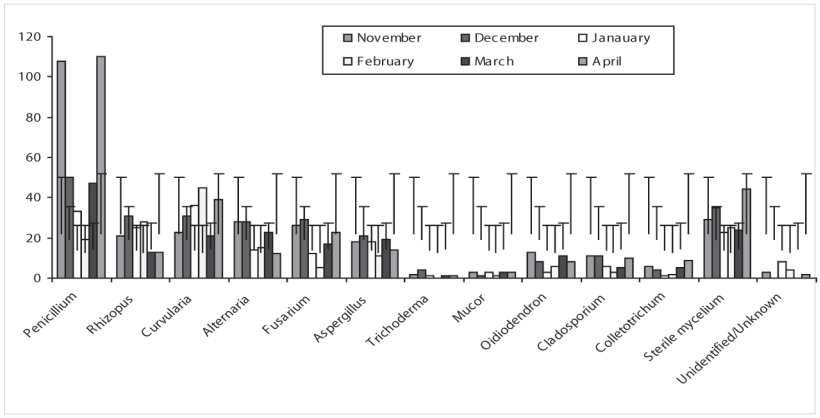
Incidence of indoor airborne fungi in different months

During the observation of six months, a total of 2178, 1368 and 1641 colonies were counted respectively on PDA, Czapek's and Sabouraud's media. The highest number of fungal colonies was recorded in the month of April and the lowest was exhibited in January (Fig. 2). Chao et al., (2002) reported that total number of airborne fungi decreased throughout the summer and winter, and then began to increase in April. Shelton et al., (2002) also reported that the sizes of fungal populations varied significantly by seasons. The present observations support the above findings.

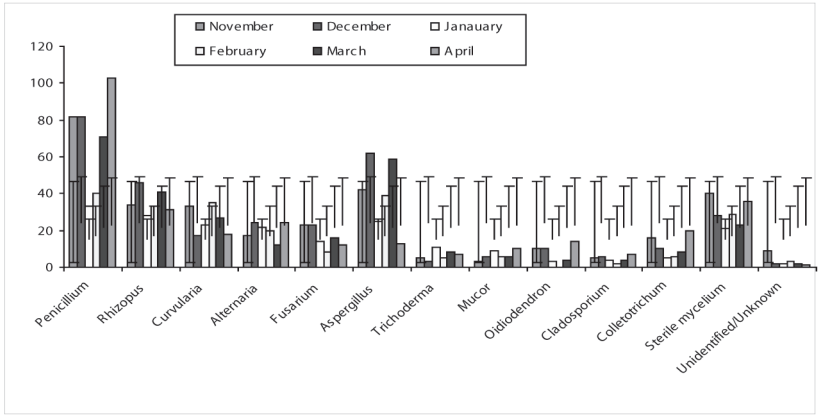
Among the identified 11 genera, *Penicillium* was the most dominating genus on all media used. The lowest genera were *Colletotrichum (*1.33 % on PDA), *Trichoderma* (0.66% on Czapek's) and *Cladosporium* (1.71% on Sabouraud's) during the six months of observation. The most frequently isolated genera were *Alternaria, Aspergilus, Curvularia, Fusarium* P*enicillium* and *Rhizopus* and their percentages of occurrence were 29.25, 14.55, 13.64, 10.79, 7.48 and 6.43%; 26.83, 9.58, 14.25, 8.77, 8.19 and 7.38%; and 25.05, 12.98, 9.32, 7.25, 5.85 and 14.63% on PDA, Czapek's and Sabouraud's media, respectively. Colonies of sterile mycelia and unidentified fungi covered 7.67 and 0.64 % of abundance on PDA; 13.16 and 1.24% on Czapek's; and 10.79 and 1.16% on Sabouraud's media, respectively.



PDA



Czapek's



Sabouraud's

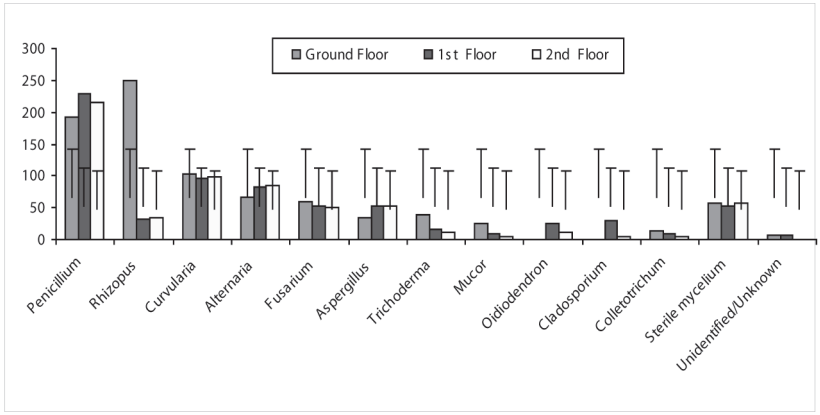
Figure 2. Monthly incidence of airborne fungi as recorded in PDA, Czapek's and Saboraud's media during November 06 to April 07

Incidence of indoor airborne fungi at different floors of Central Library

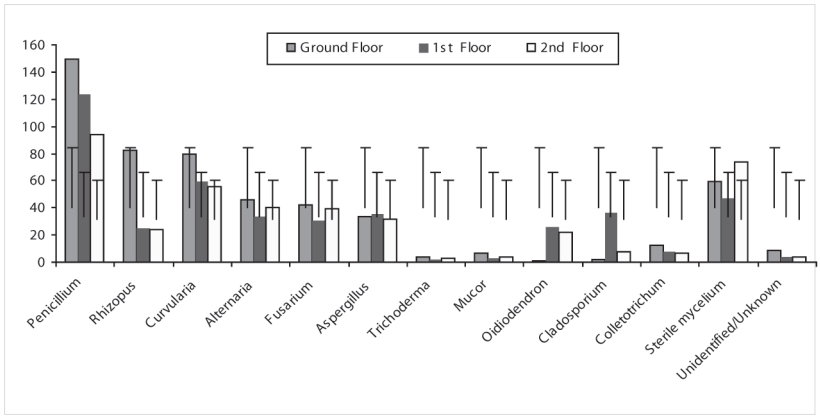
Total numbers of viable airborne fungal colonies were counted as 851, 692 and 635 on PDA; 527, 434 and 407 on Czapek's; and 626, 534 and 481 on Sabouraud's medium and these were collected from the indoor atmosphere of ground floor, 1st floor and 2nd floor of the Central Library, respectively, during the observation of six months from November 2006 to April 2007 (Fig. 3). The incidence of airborne fungi significantly (P=0.05) varied at different floors. The highest incidence of airborne fungi was recorded at ground floor, followed by1st floor and the lowest in 2nd floor. Szam et al., (1981) observed the vertical development of the airborne mycoflora and reported that the highest incidence of mycoflora present at ground floor but their occurrence decreased at the upper stories. The present result completely corroborates the findings of Szam et al., (1981).

The genera *Alternaria, Aspergillus, Curvularia, Fusarium,* P*enicillium* and *Rhizopus* were recorded as the most frequently occurring genera in air of floors of the Central Library. Begum et al., (2007) reported that*, Alternaria, Aspergillus, Candida, Cladosporium, Curvularia, Fusarium, Gloeosporium, Neurospora, Penicillium* and *Sporobolomyces* were the most frequent genera in the air of Rajshahi Metropolitan City. Uddin (2005) reported that *Penicillium* and *Aspergillus* are the most dominant fungi followed by *Curvularia* and *Cladosporium.* Albuquerque et al., (2004) studied the airborne fungi of Brazil and reported that *Absidia, Alternaria, Aspergillus, Cladosporium*, *Curvularia, Dreschleria, Fusarium,* mycelial sterila (the fungi don't producing any spores), *Cladosporium, Penicillium* and *Penicillium* and *Rhizopus* were predominant fungi**.** Shelton et al., (2002) reported that non sporulating fungi are prevalent fungi in the air of U.S.A.

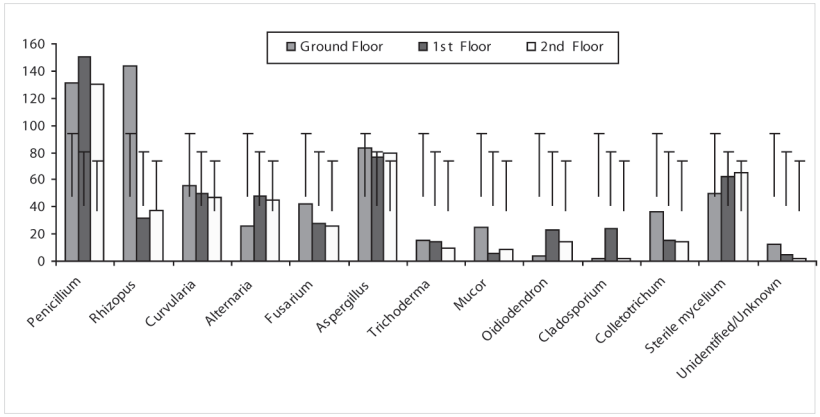
Among all fungi identified, *Penicillium* was the most prevalent genus as recorded at all floors and all media which covered 22.68, 32.95 and 34.02% of total counts on PDA; 28.27, 28.57 and 23.10% on Czapek's medium and 20.93, 28.09 and 27.03% on Sabouraud's medium at ground floor, 1st floor and 2nd floor of Central Library, respectively, during six months of observations. The next dominating genera were *Alternaria*, *Aspergills, Curvularia*, *Fusarium* and *Rhizopus* which covered 4.10 to 29.26% on PDA; 5.76 to 15.56% on Czapek's; and 4.15 to 23.00% on Sabouraud's media, respectively. The highest percentage of sterile mycelia was recorded at 2nd floor which covered 8.9, 18.8 and 13.51% on PDA, Czapek's and Saboraud's media, respectively.



PDA



Czapek's



Sabouraud's

Figure 3. Incidence of different airborne fungi as recorded on PDA, Czapek's and Sabouraud's media during 6 months of observation (November 2006 to April 2007) at different sites of central library of Rajshahi university.

Hemolytic activity test of isolated indoor airborne fungi

Identified 11 genera, viz*., Alternaria, Aspergillus, Cladosporium*, *Colletotrichum, Curvularia, Fusarium, Mucor, Oidiodendron, Penicillium, Rhizopus* and *Trichoderma* were tested for the hemolytic activity on blood agar medium (5% cow blood). Only eight genera such as *Aspergillus, Fusarium, Mucor* and *Penicillium* showed positive result. So, they are hemolytic in nature and may be pathogenic. Some microorganisms secrete hemolysins that lyse red blood cells which contribute to their pathogenecity. Hemolytic strain is more virulent than non-hemolytic strains of the same species. A large number of hemolytic bacteria have been demonstrated in the floor dust of the hospital wards (Coriell, 1968). Khan and Ali (1984) reported that hospital wards are highly contaminated with certain hemolytic bacteria associated with certain diseases of the respiratory tract.

Incidence of airborne fungi and their relation to allergy symptoms

In the present investigation, a total of 1,200 employees, students and researchers participated in the observation of six months. Their age ranged from 20 to 55 and they had suffered from several types of health complications. The health complications generally increases during March to April. In room conditions, dampness is usually observed in March to April; visible fungi especially moulds appear in maximum Walls, materials, a few number of books and the floors are cleaned every day by ordinary water. This insufficient room management may increase the incidence of indoor airborne fungi. Environmental variables have been associated with perceptions of health of office occupants (Chao et al., 2002). Significantly greater spore counts and higher prevalence of allergic symptoms were found in damp residences (Li and Kendrick, 1995b). Allergic problems may occur at high concentrations of airborne fungal spores (van Bronswijk et al., 1986). From the analyses of survey data, it was found that the highest % of allergy symptoms was noted in April which was positively correlated with the incidence of indoor airborne fungi. Total spores may have an impact on increasing incidence of allergy symptoms; in that case, some of the indoor fungi in the total spores had adverse effects on human health. (Li et al., 2002).

Table 1. Evaluation on age range, profession, health complication, room condition, and incidence of airborne fungi with % of allergy during November 2006 to April 2007

Months & Week		Age range	Profession	Health complication	Room condition			Incidence of airborne fungi on			% of allergy affected
					Dampness	Visible fungi	Cleanness	PDA	Czapek's	Sabourad's	
November	1st	20-55	Emp., St.& Res.	Skin dis., Cough	-	+	+	92	81	69	67
	2nd	20-45	Emp., St.& Res.	Skin dis.	-	+	+	81	91	77	71
	3rd	25-55	Emp., St.& Res.	Skin dis., Cough	-	+	+	61	72	69	68
	4th	20-45	Emp., St.& Res.	Cough, Conj.	-	+	+	83	74	76	72
December	1st	20-55	Emp., St.& Res.	Skin dis., Cough	-	+	+	87	80	51	77
	2nd	20-45	Emp., St.& Res.	Skin dis., Headache	-	+	+	110	74	70	70
	3rd	25-55	Emp., St.& Res.	Skin dis., Cough	-	+	+	107	81	70	71
	4th	20-45	Emp., St.& Res.	Cough, Conj.	-	+	+	98	84	62	71
January	1st	20-55	Emp., St.& Res.	Skin dis., Cough	-	+	+	67	56	43	69
	2nd	20-45	Emp., St.& Res.	Skin dis., Headache	-	+	+	74	44	43	64
	3rd	25-55	Emp., St.& Res.	Skin dis., Cough	-	+	+	56	55	39	62
	4th	20-45	Emp., St.& Res.	Cough, Conj.	+	+	+	59	45	58	60
February	1st	20-55	Emp., St.& Res.	Cough, Diff. breathing	-	+	+	72	58	46	65
	2nd	20-45	Emp., St.& Res.	Skin dis., Cough	-	+	+	68	68	33	63
	3rd	25-55	Emp., St.& Res.	Skin dis.	-	+	+	66	46	43	69
	4th	20-45	Emp., St.& Res.	Cough, Skin dis.	+	+	+	52	54	42	60
March	1st	20-55	Emp., St.& Res.	Skin dis., Cough, Conj.	+	+	+	94	74	53	65
	2nd	20-45	Emp., St.& Res.	Cough, Skin dis.	+	+	+	98	78	30	68
	3rd	25-55	Emp., St.& Res.	Skin dis., Cough, Conj., Th. xoar, Headache	-	+	+	101	78	57	75
	4th	20-45	Emp., St.& Res.	Skin dis., Cough, Conj., Th.. xoar, Headache	-	+	+	100	51	49	77
April	1st	20-55	Emp., St.& Res.	Skin dis., Cough, Conj.	+	+	+	125	73	52	85
	2nd	20-45	Emp., St.& Res.	Cough, Skin dis., Headache	+	+	+	142	69	60	87
	3rd	25-55	Emp., St.& Res.	Skin dis., Cough, Conj., Th. xoar, Headache	+	+	+	121	75	90	83
	4th	20-45	Emp., St.& Res.	Skin dis., Cough, Conj., Th. xoar, Headache	+	+	+	114	79	80	80

Emp = employees, St = students, Res = researchers, Dis = disease, Conj = conjitivites, Th=Throat

CONCLUSION

Estimation of fungal exposures is of increasing importance in assessing indoor air quality. microbiological air contamination is caused by ventilation systems of large buildings and it may play a role on adverse health. In this case, disinfectant can be used in swiping which may reduce airborne fungi. So, in this circumstance, the present investigation will help in making future sanitation program to maintain libraries or large buildings to protect human health.

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