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Airborne Culturable Fungi in Primary Schools: Building Characteristics and Environmental Factors in Qom, Iran

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ABSTRACT

The aim of this current research was to investigate airborne fungi in indoor environments at primary schools. The study also examined the correlation between these fungi and building characteristics, as well as some geographical and meteorological parameters. To achieve this aim, a passive sampling method was used. In this study, 148 samples were collected from the indoor environments of 24 schools located in Qom, Iran. To collect the samples, passive sampling was performed using Petri plates containing Sabouraud dextrose agar (SDA). The characteristics of school buildings were evaluated by checklist. The mean ± *SD* fungal load of indoor air in selected schools was found to be 10.1 ± 14.0 colonies (CFU/dm^2/ h). According to the IMA standard, the majority of the classes (71.7%) were in very good condition. The dominant species were as follows: *Aspergillus niger*, *Aspergillus candidus*, and *Aspergillus flavus*. Furthermore, the fungal load of girls' schools was significantly higher than that of the boys' schools ($p < .05$). The correlation analysis using the Pearson test showed that there was a direct correlation between the mean fungal load of classrooms and the number of students ($p < .01$). The highest concentration of fungi was found on the ground floor and in poor ventilation conditions ($p < .05$). During the study, it was found that schools located in the western part of Qom, Iran, had a higher concentration of fungi. This can be attributed to their exposure to the prevailing winds and the penetration of outdoor dusty air into indoor environments. The large number of students in each class and the inappropriate ventilation, which are the causes of airborne culturable fungi of these classes, call for the need for proper operation of school buildings.

***Keywords*:** airborne, fungi, primary schools, indoor

One of the most considerable problems of indoor environments is related to the air pollution caused by microbial contaminants (bio-aerosols) (Samadi et al., 2021). Residents of indoor environments are always exposed to particles containing airborne fungi as an organic part of bio-aerosols (Vacher et al., 2015). Although indoor resources are effective in the dispersion of fungi, recent studies indicate that outdoor resources can also be known as potential sources (Crawford et al., 2015). The inside moisture of the buildings has a great effect on fungal growth (Verdier et al., 2014). Additionally, the existence of a relationship between fungi growth and ventilation rate, temperature (Pakpour et al., 2015; Sordillo et al., 2011), and building conditions (Choi et al., 2014; Verdier et al., 2014) have been confirmed.

Schools are one of the most important environments for children (Daisey et al., 2003). In fact, children allocate most of their time in school during education (Mendell & Heath, 2005). However, indoor air pollution in schools can lead to harmful health effects (R. Fard et al., 2018). As Hu et al. (2020) declared, indoor air quality monitoring contributes to the public awareness of indoor air condition and its interaction with students in their home (Hu et al., 2020). Due to the unique features of fungi in closed spaces, they have a potential to cause acute health problems, especially in school students. Furthermore, whether fungus or particles, they are known as two major factors leading to upper respiratory illnesses among children (Liu et al., 2014). In addition, exposure to particles containing fungi is linked with the spread of allergic reactions (Gent et al., 2012; Moon et al., 2014). Similarly, studies have confirmed the relationship between fungi and asthma in schools (Cai et al., 2011; Fisk et al., 2007; Kercsmar et al., 2006). Likewise, studies have reported other effects of fungi such as heart disease, chronic obstructive pulmonary disease (COPD), cardiovascular attacks, hospital admissions (Pastuszka et al., 2000), and allergic bronchopulmonary aspergillosis (ABPA) (Agarwal, 2009). It should be noted that analyzing fungi DNA revealed that there is a significant relationship between the fungal growth in the environment and the incidence of sick building syndrome (SBS) (R. Fard et al., 2018; Hosseini et al., 2020; Singh et al., 2010; Zhang et al., 2011). Additionally, there is an association between asthma and school absenteeism (Hollenbach & Cloutier, 2014).

Monitoring and measuring particles and airborne microbial agents necessitates the use of air sampling, whether active or passive. While active air sampling involves air suction, passive sampling does not. Instead, particles containing microbes settle on a petri dish with a suitable agar culture at a sedimentation rate of approximately 0.46 cm/s. This method, known as the 1/1/1 scheme, was first approved in 1978 as an index of microbial air contamination (Pitzurra, 1978; Pitzurra, 1984; Whyte et al., 1992). To use this method, place the Petri dish containing a suitable culture medium for microbe growth in an open area, one meter above the ground and at least one meter away from any walls. Leave the dish exposed to the air for one hour. (Pasquarella et al., 2000).

Several studies have been carried out on fungi and their effects, with some of them focusing on the relation of fungal load and its impact on causing health problems (Hulin et al., 2013; Rodriguez-Tudela et al., 2015; Torres-Rodríguez et al., 2012). It is pertinent to say that some of these studies have introduced sampling methods from the environments that contain fungi (Pasquarella et al., 2012; Yamamoto et al., 2011). However, others measured the number of fungi in the environment (Dumała & Dudzińska, 2013; Soleimani et al., 2013). However, few studies have reported the concentration of fungi in schools in tropical and dry areas.

Because Qom is located in a semi-arid region of Iran and is affected by regional dust, this study aims to investigate the airborne fungal load of primary schools in Qom. It is hypothesized that there is a significant relationship between the location of studied schools, meteorological factors, ventilation, lighting, cleanliness, the status of wall coloring, and the number of students in each classroom.

METHODS

Study Area

This descriptive-analytic study was conducted in Qom primary schools in 2014. Qom is the central city of Qom province, which is located at the geographical coordinates of 34◦ 44 '37' 'N, 55◦ 33' 27 '' E. The average elevation of this city is 950 meters above sea level while the average annual temperature and precipitation is 18.1° C and 161 mm, respectively. Qom is one of several dry and semi-arid cities in central Iran (Fahiminia et al., 2016; R. F. Fard, A. H. Mahvi, et al., 2018; R. F. Fard, K. Naddafi, et al., 2018; Fard et al., 2016; Khazaei et al., 2013; Mojarrad et al., 2019) . Figure 1 shows the location of Qom and the studied schools.

Sampling Procedures

The schools of Qom province have 225,000 students, 55% of whom are elementary students. The city of Qom is divided into four districts according to the divisions of the Department of Education. Six schools were selected from each district through the stratified random sampling—half of which were girls' schools and the other half boys' schools. Hence, a total of 24 schools were selected for this survey.

The fungal load was measured at three intervals each day: at the start (8:00–9:00 a.m.), middle (9:00–10:00 a.m. and 10:00–11:00 a.m.) and end of the Iranian school day (11:00 am–12 p.m.). For the purposes of the study, the sampling was done at least in three classrooms on different floors (ground floor, first floor, and the second floor). Finally, 148 samples were collected by a passive sampling method (according to 1/1/1 scheme of NIOSH) and Index of Microbial Air Contamination (IMA) standards (Pasquarella et al., 2000).

Measurement of Airborne Fungi

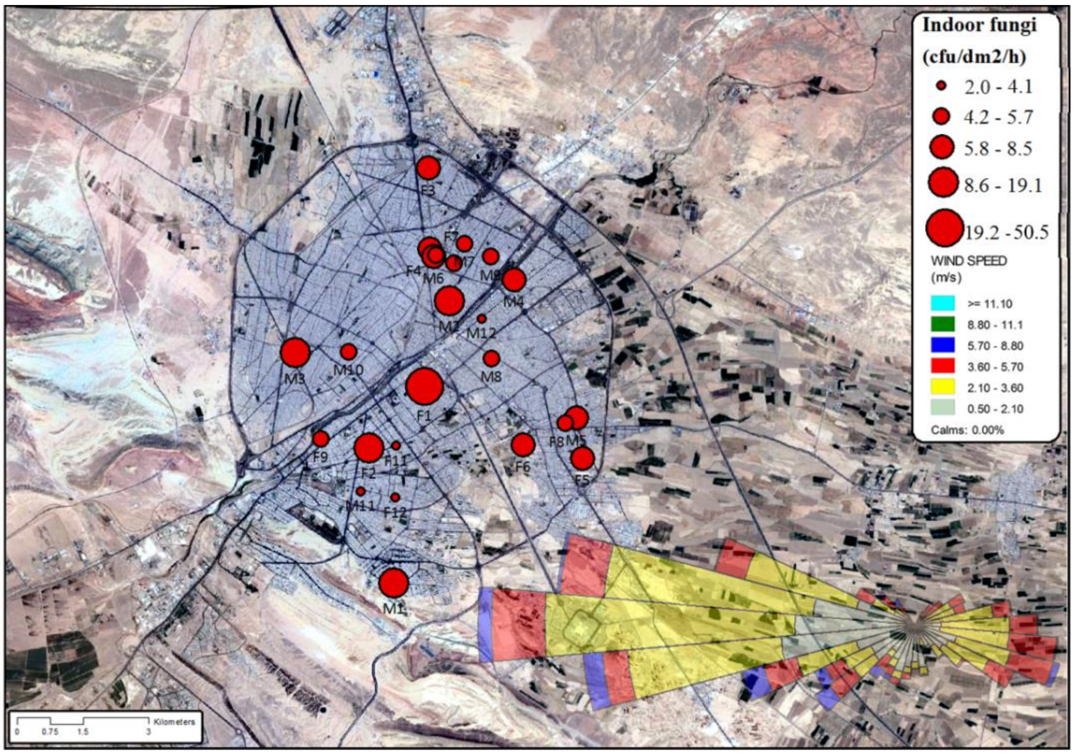
According to this sampling method, the prepared Petri plates (containing Sabrouraud dextrose agar (SDA)) were placed one meter above the floor and one meter away from walls and any major obstacles for one hour (Bayer et al., 1999). After sampling, the Petri plates were transferred to the microbial laboratory under the standard condition to incubate for 3–5 days at 27 ° C (Napoli et al., 2012). Additionally, Chloramphenicol was used to inhibit bacterial growth, and the results of the fungal load of studied schools were expressed in CFU/dm^2/h (Pasquarella et al., 2012).

Analysis

Eventually, the correlation between the fungal load and the characteristics of school buildings, geomorphological and meteorological parameters of Qom were analyzed using SPSS and Arc view 3.3 software. Furthermore, the number of students and the physical status of the classrooms such as lighting, ventilation, wall coloring, and cleanliness were evaluated by a checklist based on four categories of *poor*, *moderate*, *good* and *very good*.

Figure 1

Mean fungal load of studied schools, along with wind rose, in Qom, Iran



RESULTS

Concentration of Airborne Fungi and Dominant Species

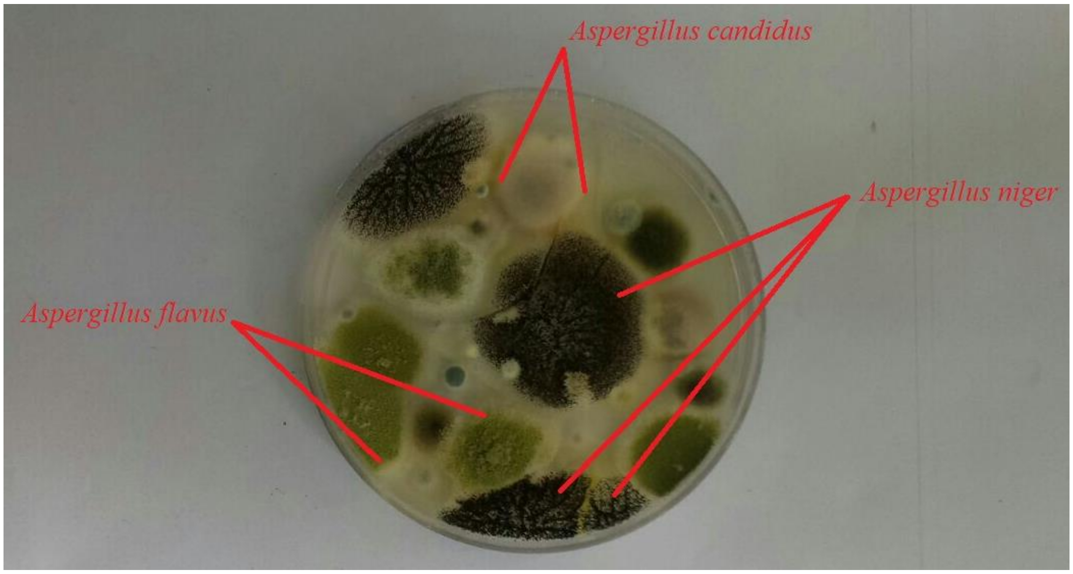
The mean ± *SD* fungal load of indoor air in selected schools was found to be 10.1 ± 14.0 colonies (CFU/dm^2/ h). Indeed, the number of colonies in schools varied from 1–88 colonies, with the highest amount belonging to F_1 primary school with 88 CFU/dm^2/ h. Figure 1 demonstrates the mean fungal load of studied schools, while Figure 2 shows the grown fungus on a culture medium of SDA. According to morphological studies, the main fungi growth was related to *Aspergillus*, while the most frequent species were found to be *Aspergillus niger*, *Aspergillus candidus*, and *Aspergillus flavus*.

Concentration of Airborne Fungi in Girls' and Boys' Schools

Figures 3 and 4 display the mean fungal load of indoor air in girls' and boys' schools, respectively. The highest and lowest mean (±*SD*) colonies among studied girls' schools were 50.5 (±37.5) and 3 (±0.05) (CFU/dm^2/ h), which belonged to stations F_1 and F_{12}, respectively. Similarly, the highest and lowest mean (±*SD*) colonies among boys' schools with 27.6 (±2.6) and 2 (±0.8) (CFU/dm^2/ h) were related to stations M_1 and M_{12}, respectively.

Figure 2

The Dominant Species of Fungus in the Indoor Air of Primary Schools in Qom



IMA Classifications

Table 1 illustrates the classification of IMA for microbial contamination of indoor air (Pitzurra et al., 1997). As is shown in Table 1, based on the IMA standard, the indoor air is classified into five statues of *very good*, *good*, *fair*, poor and *very poor*. In this research, it was found that the indoor environment of 71.7% of the primary schools were in very good condition (with fungal load between 0-9 (CFU/dm^2/ h)), while 23.9% were in good condition (with fungal load between 10-39 (CFU/dm^2/ h)). In addition, 2.2% of schools were in fair condition (with fungal load between 40-84 (CFU/dm^2/ h)) and 2.2% were in poor condition (with fungal load between 85-124 (CFU/dm^2/ h)). As a result, none of the schools were in very poor condition.

Figure 3

Mean Fungal Load of Indoor Air in Girls' Schools

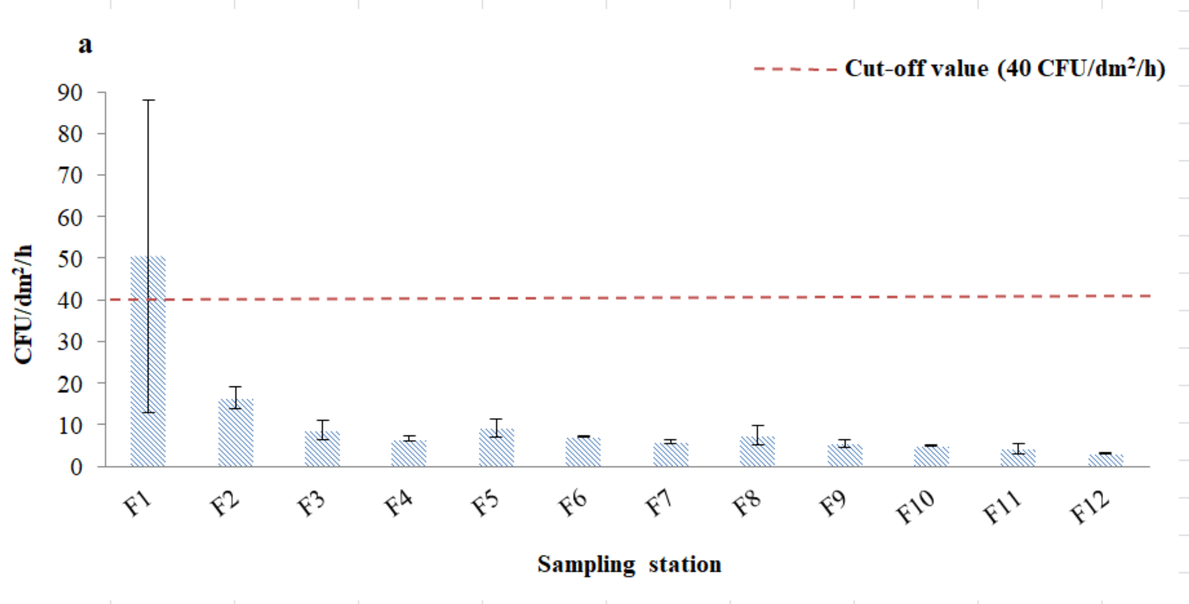


Figure 4

Mean Fungal Load of Indoor Air in Boys' Schools

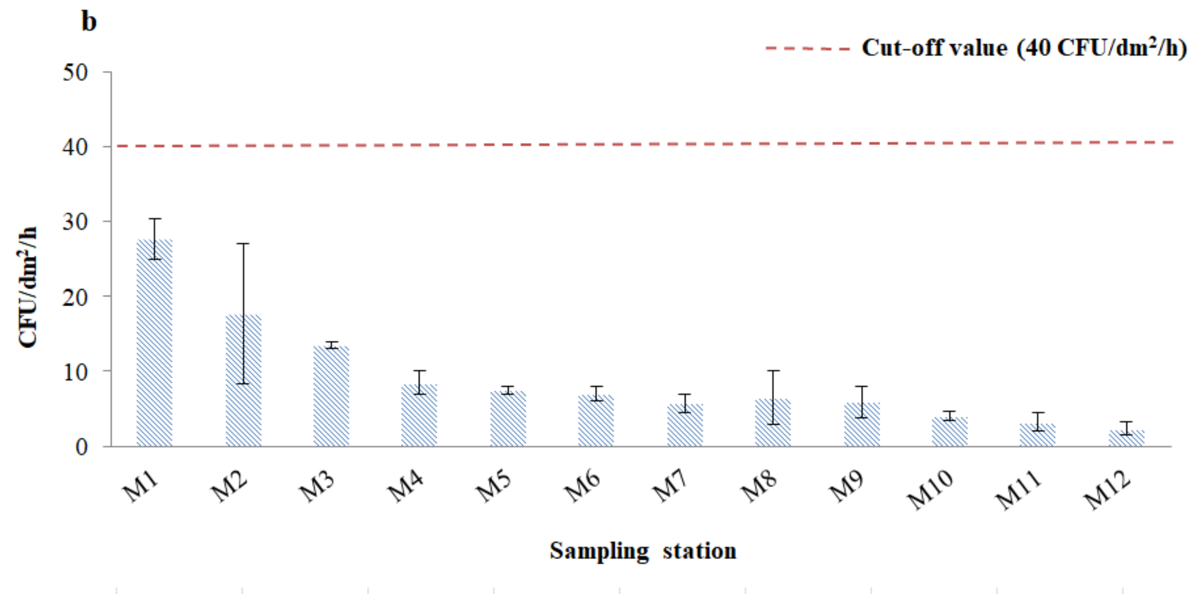


Table 1

Classification of IMA for Microbial Contamination of Indoor Air

IMA Value	CFU/dm$^{2/}$h	Performance
0-5	0-9	Very good
6-25	10-39	Good
26-50	40-84	Fair
51-75	85-124	Poor
76 ≥	125 ≥	Very poor

Effect of the Physical Status of the Indoor Environment on Airborne Fungi Concentration

Table 2 displays the physical status of the indoor environment of classrooms. According to this table, many classrooms displayed a moderate ventilation status (41.1%), while a majority showed a good lighting status (60.2%). Moreover 37.5% showed a poor status of classroom cleanliness, and 43.1% showed a moderate status of wall coloring and cleanliness.

As is shown in Table 3, the mean (±*SD*) concentration of fungal colonies (CFU/dm2/ h), 12.3 (±21.8), 8.1 (±5.8) and 11.6 (±14.1), grew in the plates of the ground floor, first floor, and second floor of schools, respectively. The highest concentration of fungi was identified on the ground floor and in poor ventilation conditions ($p < 0.05$). Although the results indicated an increase in fungal load with a decrease in the physical status of classroom cleanliness, lightning ,and wall coloring, this relationship was not statistically significant. As such, the results of the Pearson correlation ($p < .01$, $r = .252$) reveals that there is a direct positive correlation between the mean fungal load of classrooms and the number of students.

Table 2

Physical status of indoor environment of studied classrooms

Status	Poor (%)	Medium (%)	Good (%)	Very good (%)
Ventilation	11.3	41.1	39.5	8.1
Lighting	14.1	12.6	60.2	13.1
Cleanliness	37.5	37.3	17.9	7.3
Wall coloring	30.2	43.1	15.4	11.3

As can be seen in Figure 1, the western regions of the city had a greater fungal load, and there was a significant difference between the mean fungal load of these areas ($p < 0.05$). In Figure. 1, the wind rise in Qom city during fall 2014 is also displayed. Based on this wind rise, the prevailing wind at this season is from the west, and most winds had a speed of 1.5 to 3.1 m/s.

Table 3

The concentrations of fungi in the physical characteristics of schools.

Characteristic	Mean±*SD*	*P*-value
Building floor		
Ground floor	12.3 ±21.8	
First floor	8.1 ±5.8	.042
Second floor	11.6±14.1	
Student number	8.3 ±6.8	
< 20		
20-30	10.9±5.8	.008
30-40	12.3±6.2	
Lightning		
Poor	8.6±14.5	
Medium	8.1±2.1	.081
Good	8.6±11.9	
Very good	10.5±0.88	
Ventilation		
Poor	13.4±11.06	
Medium	8.6±9.1	.041
Good	5.5±7.8	
Very good	6.3±5.2	
Wall coloring		
Poor	12.1±10.1	
Medium	10.5±9.2	
Good	10.6±11	.072
Very good	15.6±2.3	
Cleanliness		
Poor	10.2±1.2	
Medium	9.1±7.3	
Good	11.05±8.6	.084
Very good	6.3±5.1	

DISCUSSION

Reliability of the 1/1/1 Scheme

The advantages of the 1/1/1 scheme mentioned above include reliable and comparable results, non-impact of turbulent airflow, real conditions, realistic results, cheapness, easy access in all places, sterility, and the possibility of taking several samples at the same intervals. Due to placing of several samples at the same interval, which was very important in this study, it was decided to use the passive method of the 1/1/1 scheme instead of an active method. In fact, both validity and performance of this method have been proven in previous studies. Pasquarella et al. introduced the 1/1/1 scheme as one of the passive methods to the other researchers (Pasquarella et al., 2000). Furthermore, Napoli et al. (2012) and Pasquarella et al. (2012) utilized the 1/1/1 scheme for picking microbial samples according to the IMA index.

Airborne Fungi Concentration and IMA Classification

In the present study, the mean fungal load in all schools was 10.1 CFU/dm^2/ h, and the highest single-school fungi concentration belonged to the F_1 primary school with 88 CFU/dm^2/ h. As shown in Figures 3 and 4, highest mean fungi concentrations among girls' schools was 50.5 CFU/dm^2/h (site F_1), and highest among boys' schools was 27.6 CFU/dm^2/h (site M_1). As was expected, the results indicated that the fungal load of girls' schools was significantly higher than that of boys' schools ($p < 0.05$). Jason et al. (2011) reported that the IMA in four different markets in Port Harcourt, Nigeria, was 55–85 CFU/dm^2/ h. It may be tempting to argue that in some studies, especially when the sampling was done via active procedures, the airborne fungal load was expressed as CFU/m^3. Andualem et al. (2019) reported that the highest and lowest mean culturable fungal loads in primary schools of Northwest Ethiopia were 1140.29 CFU/m^3 and 211.25 CFU/m^3, respectively. Dumała & Dudzińska, (2013) investigated the fungi concentration of Polish schools and found all studied schools except one were within the acceptable level of $3*10^3$ CFU/m^3. In a literature review, Salonen et al. (2015) stated that among published papers studying the fungi concentration in schools from 2002 to 2009, the fungal concentration was within the range of 10–10^3 CFU/m^3. Furthermore, a similar study was conducted in Portugal in 2015 about primary schools with the results indicating that the mean fungi concentration in primary schools was 240 CFU/m^3 (Madureira et al., 2015). According to Table 1, based on the IMA standard, 71.7% of the indoor environments of primary schools of Qom were in a very good condition.

Effect of the Physical Status of the Indoor Environment on Airborne Fungi Concentration

Inappropriate ventilation conditions are known as a factor for fungal growth. Some ventilation systems such as HVAC (heating ventilation and air-conditioning) can be responsible for the growth of bioaerosols (Law et al., 2001). Assuredly, the efficiency of the system filters gradually decreases with the growth of microorganisms. Besides, HVAC systems use recycled or stagnant water, and a large portion of the air is recycled in these systems, which causes the dispersion of microorganisms. After dispersion, the microorganisms are inhaled in the environment by the residents (Huang et al., 2008; Wu et al., 2005). Similarly, classroom floor level was associated with the concentration of fungi, as the highest concentration of fungi was determined on the ground floor, which was observed in other studies as well. Wang et al. (2015) reported that children's bedrooms on the ground floor had a higher concentration of fungi. Fungi are in the form of bioaerosol and particles, and the reason for their high concentration on the ground floor can be attributed to two main reasons including a gravitational tendency and better ventilation of higher floors.

An increase in cleanliness of residence has a significant effect on reducing the concentration of mold and airborne fungi. Lack of cleaning the classrooms by detergents can cause the accumulation of microorganisms on the surface of doors and walls. In this condition, the fungi disperse spoors in indoor environments, thus increasing the concentration of fungi. Similar studies have shown that there is a negative effect between the level of cleanliness and fungi concentration (Hyvärinen et al., 2006; Sordillo et al., 2011). It is also notable to state that lack of cleaning the rugs and carpets in residences is a serious risk for the spread of microorganisms (Gravesen et al., 1986).

In this investigation, the Pearson correlation coefficient (r) showed a significant relationship between mean fungal load and the total number of students. Likewise, the highest concentration of fungi was observed during the last hours of school time. With regard to these two factors, it can be supposed that during the last hours of the school time in comparison with the first hours, the number of particles containing fungi increases due to the increase in student activities. Masoudinejad et al. found that the number of colonies at sampling stations in Tehran's hospitals increases by activity level (Massoudinejad et al., 2015). In addition, these results are in agreement with those of previous studies (Chen & Hildemann, 2009; Goh et al., 2000).

Impact of Dominant Wind Penetration on Indoor Fungal Load

The highest amount of fungi was measured in schools located in western regions of the city. Based on the wind rose of Qom city during fall 2014 (Figure 1), it can be observed that the prevailing wind at this season is from the west. It has been proven that the main reason for fungal load increase in schools of the western part of the city was the penetration of contaminated air with particle and fungi spores from outdoor into indoor environments, along with the meeting of an air mass with 48% humidity. The penetration of the air mass is based on advective flow. In fact, advective flow occurs due to pressure differences between indoor and outdoor environments; hence, it is directly related to wind speed. Investigations showed that local factors such as weather and meteorological factors influence the level of fungi (Bartlett et al., 2004; Hargreaves et al., 2003). In this type of study, the I/O ratio (the ratio of the indoor concentration of fungi to the outdoor concentration of fungi) is very significant. An I/O ratio of less than one indicates that the exogenous source of fungi is dominant, while an I/O ratio greater than one indicates an endogenous source of fungi is dominant. Shelton et al. (2002) examined about 1,700 buildings in the US and observed that 85% of them had an I/O ratio less than one. Further, they found that generally cold weather and snow cover can increase this ratio. Reponen et al. (1992) showed that in the polar climate of Finland, where the ground is covered by ice, the I/O ratio averages 1.4 in winter and .4 in summer.

Few studies have reported the concentration of fungi in schools in tropical and dry areas. Studies have shown that higher concentrations of airborne fungi are found inside buildings in tropical regions. This phenomenon can be attributed to external factors, such as wind speed and the penetration of fungi from outside sources, rather than internal factors like mold and humidity (Fairs et al., 2010). The results of this study can be regarded as warnings to school administration in these areas that firstly, schools in the downwind regions will be at risk of airborne fungi, and secondly, girls' schools have a higher concentration of fungi. As an example, Sivri et al (2020), suggested that the first floor can be considered as the most problematic part of the school building in terms of air pollution. They recommended that strict measures such as providing air-cleaning facilities be taken by the school management for removing the pollution. It is significantly hard to put restrictions on buildings specifically constructed for education purposes, especially in crowded cities like İstanbul, Turkey. For this reason, precautions should be taken to reduce potential microbiological pollutant factors in these types of environments (Sivri et al., 2020). It is also suggested that higher concentrations of fungi in girls' schools can also be the target of future research. For instance, a study confirmed that the thermal perceptions of students differ by gender, and the questionnaire-based results demonstrate that girls prefer higher temperatures than boys under the same thermal conditions because of their lower levels of activities and metabolism rate (Nico et al., 2015). Perhaps the reason for the difference in concentration is the issue of compatibility with different temperatures and ventilation, which needs to be investigated in its own place.

CONCLUSION

Qom, Iran, is located in a tropical and semi-arid region. Meanwhile, dust particles through an advective flow, penetrate inside of buildings, and the prevailing wind in the fall season is from the west. Hence, the schools located in western Qom are exposed to this prevailing wind and experience greater fungi concentration. Undoubtedly, the large number of students and inappropriate ventilation in some classes led to a significant increase in fungal load. As is expected, the high activity of the students during the middle and late hours of school time had a strong effect on the extra growth of fungi during this period. Although most of the classes were in a good condition according to IMA standards, emphasis on improving the physical space of schools and especially the appropriate number of students in each class can be effective in improving the status of airborne fungal contamination of schools. As a recommendation for future research, it is suggested that a series of research studies be done on the impact of different ventilation systems, long-term monitoring, as well as solutions such as quality management programs and air purification in schools. As such, in girls' schools (according to the obtained results), more sensitivity should be applied regarding management, quality control, and standards.

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