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Visiting Hours Impact on Indoor to Outdoor Ratio of Fungi Concentration at Golestan University Hospital in Ahvaz, Iran

Gholamreza Goudarzi[1,2], Zahra Soleimani[3], Batool Sadeghinejad[4], Mojgan Alighardashi[5], Seyed Mahmoud Latifi[6] & Mahsa Moradi[3]

[1] Department of Environmental Health Engineering, Health faculty and Environmental Technologies Research Center (ETRC), Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Radiation and Pollution Protection Research Center of Mamasani (RPPRCM), Nourabad Mamasani, Iran

[3] Department of Environmental Health Engineering, School of Public Health and Paramedicine, Semnan University of Medical Sciences, Aradan, Semnan, Iran

[4] Department of Medical Mycology Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[5] Environmental health specialist of shahid beheshti hospital, Hamadan University of Medical Sciences, Hamadan, Iran

[6] Department of Statistic and Epidemiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Correspondence: Zahra Soleimani, Department of Environmental Health Engineering, School of Public Health and Paramedicine, Semnan University of Medical Sciences, Aradan, Semnan, Iran. E-mail: ronak_soleimani@yahoo.com

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Abstract

Prevalence of diseases originated from air pollution such as asthma and allergies, can be attributed to the bioaerosols. Bacteria and fungi are the main sources of hospital infections, which cause most of the diseases and mortality. The aim of this study was to determine the ratio of indoor to outdoor fungi concentration, the effect of population and people density on fungi concentration and the relationship between indoor and outdoor environment. In this study, three stations were used: outdoor of the hospital, general indoor and ICU wards of the Ahvaz hospital. These locations were chosen for sampling of the airborne fungi from October to December 2010. The samples were collected in 2 min, flow rate of 28.3 L/min using quick take (SKC Inc., PA, USA), based on the Andersen method.

The three dominant culturable fungi genera in sampling stations were *Aspergillus, Penicillium*, *and Cladosporium*. The average of total culturable fungi concentration before visiting time was 365.8 CFU m^{-3} and increased to 578CFU m^{-3} after visiting time. The indoor to outdoor ratios (I/O) in ICU and internal for fungi before visit were 0.36 and 0.68, respectively. However, these ratios in ICU and internal station for fungi after visit were 0.78 and 0.99, respectively. Following the visit of the visitors, the concentration of fungi available indoor was conspicuously higher (even in the wards in which no visitors were allowed; e.g. ICU, etc). Hence the amount of indoor fungi was affected by the concentration of outdoor fungi and visitors crowd.

Keywords: indoor, outdoor, Airborne Fungi, population, hospital

1. Introduction

A wide variety of hazardous materials such as (mixtures of chemical and biological contaminants) is produced in hospitals and clinical centers. Each person normally inhales 10 m^3 of air daily. The quality of the inhaled air has attracted much attention as it may contain bioaerosols with noisome & health effects (Adhikari et al., 2004, Jo and Seo, 2005). Low quality of the air present in the hospitals directly influences the health of human especially in severe immune deficiency patients and those who are in prolonged contact (Sautour et al., 2007, Cordeiro et al.). Main sources of infections in the hospitals are bacteria and fungi such as *Aspergillus*, *Fusarium* and *Mucor* which are rapidly increased recently and have caused the highest death tolls (Cordeiro et al., Wu et al., 2000, Rainer et al., 2001, Sarica et al., 2002, Sautour et al., 2009). Epidemiology of bioaerosols depends on: humidity, temperature, ventilation, organic particles available in constitutive materials, fungi load of outdoor and the quality of building(Sautour et al., 2009, Medrela-Kuder, 2003).

Many studies have been conducted on the hospital bioaerosols. For example, In Perdelli's survey conducted within 3 hospitals with different ventilation systems, *Aspergillus* was the dominant genus and the results have shown that HEPA air ventilation systems had decreases the amplitude of *Aspergillus*, up to 99.9 %. Proper maintenance of the system can keep the efficiency totally high (Perdelli et al., 2006).

An investigation was carriedout on bioaerosol concentrations in different wards of the hospitals of the Isfahan University of Medical Sciences by Nourianhas et.al. (2011). Nourianhas stated that the dominant fungi were *Aspergillus*, *Alternaria*, *Penicillium*, *Fusarium* and *Cladosporium*, (Nourian and Badalli, 2001).

Also Azizifar has reported that the fungi genera collected from Qom's hospital air, were *Penicillium*, *Cladosporium*, *Aspergillus niger, Aspergillus flavus* and *Rhizopus* (Azizyfar et al., 2009).

Several studies have been conducted on air borne microorganisms (Perdelli et al., 2006, Nourian and Badalli, 2001, Azizyfar et al., 2009, Bouza et al., 2002). While none of them has focused on the effect of population and people density on fungi concentration and the relationship between indoor and outdoor environment.

The present research aims surveying the effect of population and people density on culturable fungi concentration and the relationship between indoor and outdoor environments in Ahvaz educational and therapeutic hospital. The main objective of our study was to detect fungal population differences before and after visit time between three stations.

2. Material and Method

The present study was conducted on the bioaerosls of the Golestan hospital that has been high attending from Khuzestan and adjacent provinces during one year (Oct 2010 to Dec 2010)**.** The samples were collected in 2 min. with flow rate of 28.3 L/min using quick take 30 which was equipped with Single Stage Viable Cascade Impactor, (SKC Inc., PA, USA), based on the Andersen method (Wu et al., 2000, US-EPA, 2006, Fang et al., 2005). In the present research, "Internal" and "ICU "wards were selected for the sampling and patients were among different ages. The air sampling was conducted once every 6 days (US-EPA, 2006). Sampling time was selected between 2 to 5 p.m. It should be noted that the ventilation of the different wards of the hospital was naturally supplied. The sampling process was conducted for 2 minutes from 1.5 – 2 meters above the ground and 1 meter away from obstacles and walls (indoor) and 20 meters away from street and plants (outdoor). Potato Dextrose Agar (PDA) was used as the medium. (Chloramphenicol 100μg/lit) was added in order to inhibit the growth of bacteria used as the

Before initiating the experiment, the device was disinfected by 70% alcohol(Fang et al., 2005, Kim et al., 2009). The screens were then put into Biostage. The medium containing fungus was kept for 72-96 hours in the environment with the temperature of 25-27^{0}C. Fungus concentration was measured in number of Colony Forming per cubic meters of air sampled (CFU/ m^3). Statistical methods including Kolmogorov-Smirnov test (for normality), Mann-Whitney U test (comparison of bioaerosol before and after visit), Spearman's rank correlation coefficient as a nonparametric measure were used to realize the statistical dependence the correlation between indoor and outdoor fungi bioaerosol concentration before and after the visit and to analyze the obtained data.

3. Results

In the present research, 90 samples were collected from indoor and outdoor of the hospital. The abundance of culturable fungi, the average and ratio of culturable fungi concentration of outdoor to indoor environments and also the fungi genera of the collected fungi are presented in the following table. As In following table1, the highest concentration dominant percentage of culturable fungi genera in the indoor environments before and after visit time, were *Cladosporium* (96.9%) and (98.08%), *Penicillium* (28.96%) and (24.4%), *Aspergillus* (24.18%) and (12.45%), respectively. Also in the outdoor environment the domination percentage were *Cladosporium* (78%) and (66.98%), *Aspergillus* (13.4%) and (22.68%) *Alternaria* (4.1%) and (11.79%), respectively.

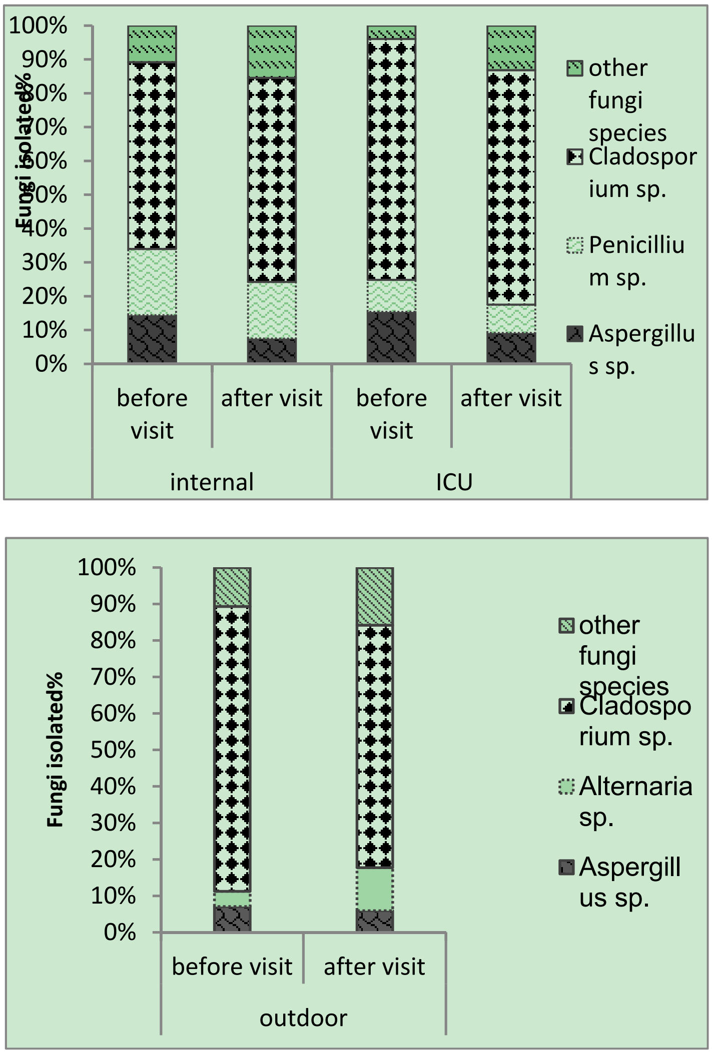

Figure1. Percentage of fungi isolated in air from indoor wards and outdoor of the hospital during the study period

The most of dominant culturable fungi in the sampled areas was allocated to *Cladosporium* genera *which* was higher in the outdoor compared to the indoor while the average amounts of *Penicillium* and *Aspergillus* were higher with in indoor environment. The fungus in “internal” ward was higher than in ICU ward in both circumstances (according to Figure 2).

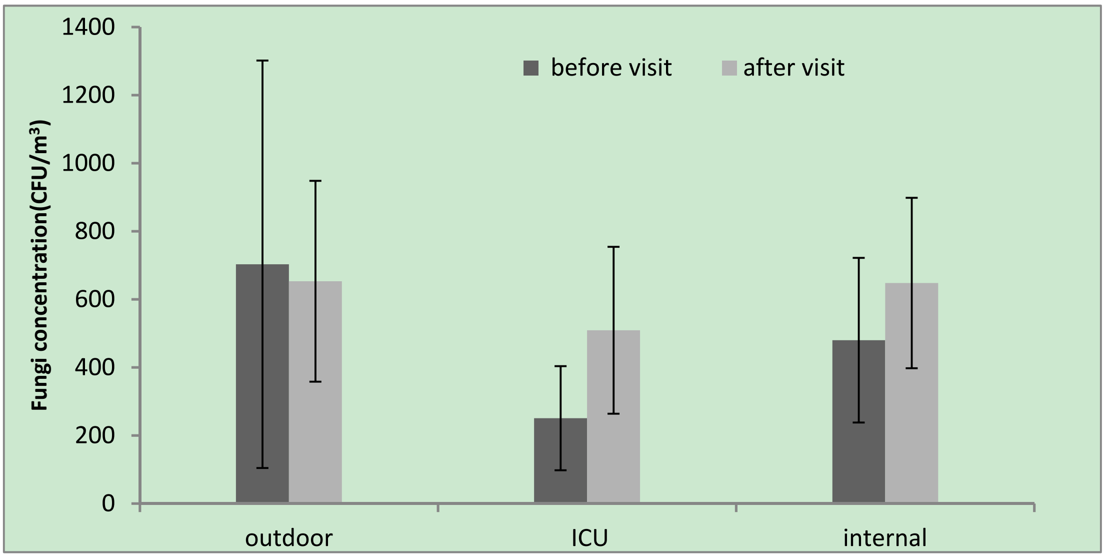

Figure2. The variation patterns of airborne fungi density at two sampling time

Calculating before visit *Correlation coefficients* between culturable fungi concentration of in the outdoor samples with its concentration in "internal" ward ($p = 0.0001$, $r = 0.74$) and ICU ward ($p = 0.0001$, $r= 0.66$) demonstrated a significant relationship. While after the visit time, there was no significant relationship ($P_{-Value} > 0.05$). This increase was caused by the presence and density of visitors inside the ward. Comparing fungus, present in the sampled wards before and after the visit time ($P_{-Value} = 0.03$) demonstrated a significant relationship in the concentration of fungi. But no significant relationship was observed for outdoor ($P > 0.05$). According to the fungus concentration in table 1, the abundance and the concentration ratio of collected fungus in the outdoor and indoor were increased after the visit time in both wards compared to the time before the visit. The concentration ratios of indoor collected fungi to outdoor fungi collected were 0.52 and 0.88 before and after the visit time, respectively. According to Figure 2, the average of culturable fungi concentration before and after the visit time was increased with average of 703 and 653 CFU/ m^3 in the outdoor, 251 and 509 CFU/m^3 in ICU and 480 and 648 CFU /m^3 in internal ward.

Table1. Measured average airborne fungi concentration (CFU/ m^3) and frequency (%) in indoor and outdoor during before and after the visiting time

		•Before visit					••After visit				
		Outdoor[a]	ICU [b]		Internal [c]		Outdoor [a]	ICU [b]		Internal [c]	
	Fungi	CFU m^{-3} (%)	CFU m^{-3} (%)	I/O	CFU m^{-3}(%)	I/O	CFU m^{-3}(%)	CFU m^{-3}(%)	I/O	CFU m^{-3}(%)	I/O
Septatehyphae	*A.niger*	17(66.6)	9.9(33.3)	0.58	18.1(53.3)	1.10	6.2(33.3)	5.1(26.6)	0.82	3.6(33.3)	0.58
	A.terreus	4.8(33.3)	3.3(20)	0.68	6.2(20)	1.29	2.2(13.3)	2.2(20)	1.00	2.0(13.3)	0.90
	A.flavus	17.2(40)	15.2(66.6)	0.88	21.6(60)	1.25	17.2(22.3)	7.2(26.6)	0.42	3.1(53.3)	0.18
	A.fumigatus	11(53.3)	10.3(60)	0.94	23.2(46.6)	2.10	13.2(60)	32.1(53.3)	2.43	40.0(60)	3
	Penicillium sp.	9.7(53.3)	23.7(53.3)	2.44	94.1(80)	9.70	28.2(53.3)	42.6(66.6)	1.51	108.3(73.3)	3.84
	Fusarium sp.	4.3(23.3)	0	0	0	0	3.7(20)	0	0	0	0
	Otherssp.	2(33.3)	3.6(16.6)	1.80	4.8(33.3)	2.4	6.3(16.6)	3.8(13.3)	0.6	0	0
	Total	66(93.3)	66(80)	1.00	168(93.3)	2.54	77(86.6)	93(93.3)	1.2	157(93.3)	2.04
Dematiaceous (Phaeo) Hyphomycetes	*Cladosporium sp.*	549(93.3)	178.7(73.3)	0.33	265.1(73.3)	0.48	434(93.3)	352.8(73.3)	0.81	391(100)	0.9
	Alternaria sp.	29(46.6)	0	0	15(33.3)	0.52	77(40)	6.1(33.3)	0/08	10(53.3)	0.13
	Aureobasidiumsp	15(13.3)	0	0	0.5(16.6)	0	0	0	*	5(40)	*
	Drechslera sp.	4(33.3)	1.3(26.6)	0.25	0	0	0	17.1(40)	*	10(40)	*
	Total	597(100)	180(93.3)	0.30	280.6 (100)	0.47	511(93.3)	376(100)	0.74	416(100)	0.81
Non septateHy phae	*Rhizopussp.*	21(53.3)	3(33.3)	0.14	4(53.3)	0.19	40(66.6)	10(53.3)	0.25	7(66.6)	0.18
	Mucor sp.	3(16.6)	2(16.6)	0.66	6(33.3)	2	15(33.3)	30(40)	2	45(40)	3
	Total	24(66.6)	5(40)	0.21	10(53.3)	0.42	55(73.3)	40(66.6)	0.73	52(73.3)	0.94
	Yeasts	7(13.3)	0	0	5(16.6)	0.71	8(20)	0	0	4(26.6)	0.5
	Non-sporulating	9(66.6)	0	0	17(33.3)	1.90	2(20)	0	0	19(53.3)	9.5
	Total fungi	703(100)	251(86.6)	0.36	480.6(100)	0.68	653(93.3)	509(100)	0.78	648(100)	0.99

(CFU/ m^3) and frequency (%) in indoor and outdoor during before and after visiting time

Number of samples collected before visit: 45; [a]ICU=15, [b] internal=15 [c]Outdoor=15

••Number of samples collected after visit: 45; [a]ICU=15, [b] internal=15 [c]Outdoor=15

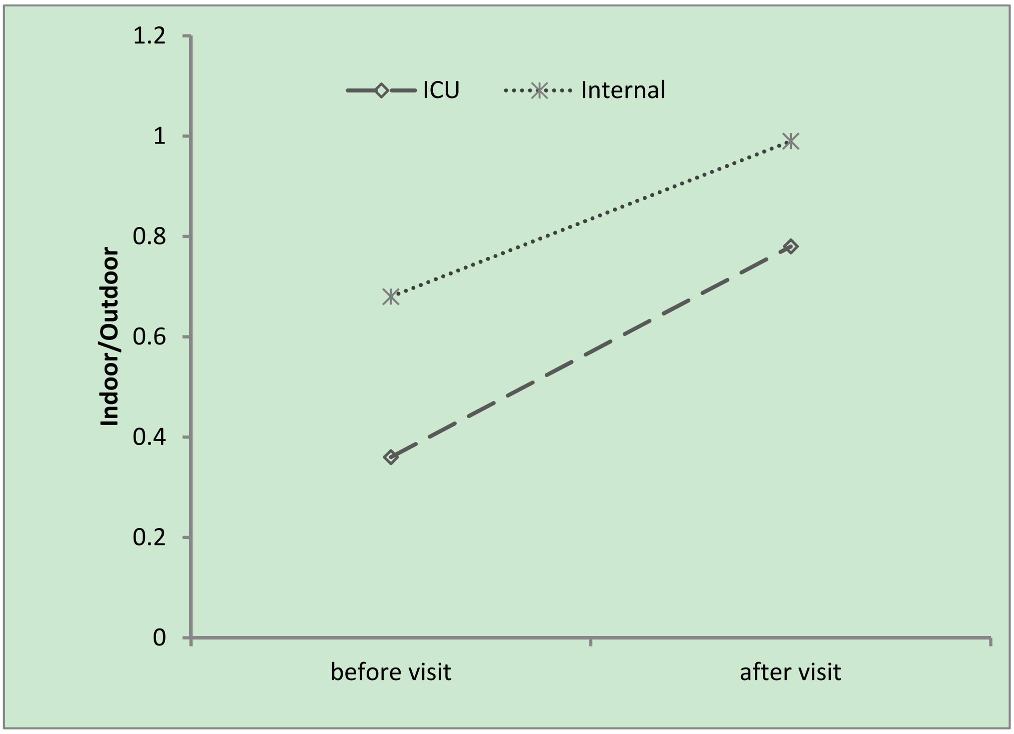

Figure 3. The ratio of Indoor to outdoor average of fungi in both wards of the hospital

indoor to outdoor ratio of average culturable fungi concentration was increased following the visit time compared to the time before the visit (according to Figure 3). The highest averages of indoor collected fungi genera compared to the outdoor before and after the visit time (in both mentioned wards) were *Aspergillus* and *Penicillium.*

4. Discussions

In the present study, the averages of fungal colonies before and after visiting were 365.8 and 578.5 CFU/m^3, respectively. The average increase of fungi concentration before and after visiting was higher in comparison the other studies in Iran and other countries(Sautour et al., 2009, Azizyfar et al., 2009). In Iran, mean concentration of fungi in Kamkar hospital (Qom city) was 200 ±79.9 CFU/ m3(Azizyfar et al., 2009)**.** Sautour et al. (2009) estimated low airborne fungi concentration ranging from 2 to 26 CFU/ m^3 in a French hospital. As the discussed hospital is aged and considered as educational hospital, the density of students, patients, nurses, visitors and hospital personnel develops an overcrowding. These are the reasons that all together increase fungus density of indoor environment. According to the results derived from different surveys conducted, the amount of fungus varies upon season, time of the day, temperature, humidity, wind velocity and the fungus density available outdoor. According to the previous studies, fungus are more prevalent in the autumn compared to any other season(Sautour et al., 2009, Jensen and Schafer, 1998).

In this survey, the highest amount of fungus (both indoor and outdoor) was indicated in October. The dominant fungi were *Aspergillus spp*, *Penicilliums spp* and *Alternaria spp*. Similar with often studies the mast prevalent fungus was *Rhizopus*, which was mostly dominant in dusty conditions (Jo and Seo, 2005, Sautour et al., 2009, Jensen and Schafer, 1998). This fungus is among the fungi which had the highest amount in dusty weather. Actually this demonstrates that dust particles were high both outdoor and indoor in hospital wards in Ahvaz .In the present survey, concentrations of *Aspergillus spp.and Penicillium spp*. were higher in indoor compared to outdoor; this is demonstrated in the previous studies as well. Mycological studies have indicated soil and plants as the sources of *Penicillium* (Su et al., 1992). Regarding the natural ventilation system in the hospital and presence of plants and trees were present 2 meters distance from the window, the presence of *Penicillium spp is justified*. The level of *Alternaria* was higher in the outdoor and also was high after the visit time at 16-17 hours. According to the studies conducted by Abdel Hameed et al. (2009)and Sarica et al(2007), *Alternaria* and *Cladosporium* densities were higher after the sunset (Sautour et al., 2009, Okten et al., 2005). The amount of prevalent fungi differ in varied times and places as they are based on human activities and environment(Fang et al., 2005), The I/O of average concentration of culturable fungi in internal ward (0.99) and ICU (0.78) was higher during and after the visit time than before it'sthe ventilation system of the mentioned hospital wards was natural. According to a research, the population and their density within the ward amplify the bio-aerosols and cause them to scatter indoor (Cordeiro et al., Perdelli et al., 2006).The reports suggest that microbial pollutants present in the atmosphere are in strong relationship with density and activity level of the population. Infections caused by opportunist fungi (like *Mocur* and *Rhizopus)* cause mucormycosis in the ward patients (esp. in patients diagnosed with ketoacidosis diabetes, immunity disfunction and patients with kidney transplantation) which is contagious by inhaling spor. According to the studies previously done, body contact with high level of *Cladosporium* and *penicillium* cases allergic diseases (Augustowska and Dutkiewicz, 2006). Also, *Aspergillusflavus*and *Aspergillusfumigatus*species cause aspergillosis in patients. When the average of *Aspergillusfumigatus* raises to 0.9CFU/m^3, the risk of Invasive *Aspergillosis* increases(Perdelli et al., 2006). In this study, the average of this fungus was higher in the sampled wards. When the level of *Aspergillusfumigatus* is below 0.2 CFU/m^{3}the risk of disease cased y it decreases. In the hospitals utilizing HEPA filter system, fungus pollution has reported to be less (Sautour et al., 2009, Perdelli et al., 2006).According to Augustowska & Dutkiewicz, 2006, when the level of *Aspergillusspores* exceeds 50 CFU/m^3 in indoor, Sick Building Syndrome outbreaks (Augustowska and Dutkiewicz, 2006).Comparing the number of fungus colonies with 800CFU/m^{3}guideline (Jo and Seo, 2005), before the visit time indicated that in 17% of the cases, the number of colonies formed in each cubic meters of air was higher than the guideline value related to internal hospital ward. Comparing the number of fungi colonies after the visit time with 800 CFU/m^3 guideline indicates that in 37% of the cases, number of colonies formed the air was higher than the guideline value in this way, 26 % allocated the internal ward and 11% was allocated to the ICU ward. Although according to ACGIH, the guideline concentration is 200CFU/m^3(ACGIH, 1989). Hence higher people population and moving affect the density of the fungi within the wards (even in wards which visitors are not allowed) because of the repeatedly opening and closing of the doors and windows and thereby, scattering the particles in the air.

5. Conclusion

According to the results of the present study, one of the sources of particles infiltrating the hospital is the outdoor particle when the ventilation system is natural. It is worthwhile that air quality of Ahvaz city is diminished due to dust problem emanating from other regions and the number of particles moving freely in the air is significantly high. As the source of bioaerosols is the particle, the composition of such particles is biologically and chemically important. Following the visit time, the level of culturable fungi available indoor was conspicuously higher (even in the wards in which no visitors were allowed (I.e. ICU etc). Hence, the population and their activity cause an increase in the level of culturable fungi. According to the obnoxious effects caused by fungi on the patients receiving treatment and personnel working in the hospital, emphasizing on the importance of using automatic air ventilation systems. Using HEPA ventilation systems are recommended to decrease the pollution load. Also in order to inhibit congestion of people and overcrowding, it's recommended to let only some visitors enter the hospital.

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