



## The impact of visiting hours on indoor to outdoor ratio of fungi concentration at Golestan Hospital in Ahvaz, Iran

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### Original Article

#### Abstract

The majority of diseases caused by air pollution, such as asthma and allergies, are caused by pathogenic bacteria, which are transmitted through bioaerosols. Bacteria and fungi are the main sources of hospital infections causing the majority of diseases and cases of mortality. The aim of this study was to determine the ratio of indoor to outdoor fungi concentration, the effect of population density on fungi concentration, and the relationship between indoor and outdoor environments. In this study, 3 stations were assessed; the outdoor environment, general indoor environment, and intensive care units (ICUs) of Golestan Hospital affiliated to Ahvaz University of Medical Sciences, Iran. These stations were used for the sampling of airborne fungi from October to December 2010. The samples were collected over a period of 2 minutes in a flow rate of 28.3 l/minute using a QuickTake 30 air sampling pump. The 3 dominant fungi genera in the sampling stations were *Aspergillus*, *Penicillium*, and *Cladosporium*. The average of total fungi concentration before visiting hours was 365.8 CFU/m which was reduced to 578 CFU/m after visiting hours. The indoor to outdoor ratios in the ICU and internal wards for fungi before visiting hours were 0.36 and 0.68, respectively. However, these ratios in the ICU and internal wards for fungi after visiting hours were 0.78 and 0.99, respectively. Following the visiting hours, the concentration of fungi available indoors was conspicuously higher (even in the wards in which no visitors were allowed (e.g., ICUs). Hence, the amount of indoor fungi was affected by the concentration of outdoor fungi and the number of visitors.

**KEYWORDS:** Air, Concentration, Hospital, Population, Fungi

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## Introduction

Hospital environments contain an extensive variety of hazardous materials, such as mixtures of chemical and biological contaminants. Every individual normally inhales 10 m<sup>3</sup> of air daily. Contact with bioaerosols due to their noisome effects on the health of humans has drawn attention as a concern.<sup>1,2</sup> The low quality of the air present in hospitals directly influences the health of humans especially patients with severe immune deficiency and aggravates diseases in individuals with prolonged contact.<sup>3,4</sup> The main sources of infections in hospitals are bacteria and fungi, such as *Aspergillus*, *Fusarium*, and *Mucor*, which have rapidly increased in previous years and were the cause of the highest reported death tolls.<sup>4-7</sup> The epidemiology of bioaerosols depends on humidity, temperature, and ventilation, organic particles available in constitutive materials, outdoor fungi load, and the quality of buildings.<sup>8,9</sup>

Bioaerosols are emitted into the air by different natural and man-made sources such as soil, plants, animals, humans, sewage treatment, and agricultural activities.<sup>10-12</sup> Bioaerosols affect human health by promoting infectious diseases, allergy, asthma, and neurological diseases in susceptible patients.<sup>4,13</sup> Some soil-dwelling fungi can irritate the lungs and some reports indicate a relationship between fungi and headaches.<sup>3</sup> Mycotoxins generated by fungi can negatively impact human health in various ways, such as leading to both digestive and respiratory problems.<sup>3,4,8,12,14</sup> Indoor air quality is critical for the health of living beings, especially in hospital buildings where inhalation of particulate matter can impact the health of its personnel, and patients with vulnerable immune systems.<sup>15,16</sup>

According to Galal Saad, hospital environments include an extensive variety of hazardous materials, such as different contaminants.<sup>17</sup> Although indoor bioaerosols mostly originate from the ambient atmosphere, they also stem from indoor

activities such as heating, ventilating, and air conditioning (HVAC) systems.<sup>18</sup>

Indoor air quality depends on different factors such as ventilation, temperature, humidity, building materials and structure, human activity, re-suspension, and outdoor air pollution. The relative importance of each of these factors has been poorly characterized.<sup>18</sup>

Many studies have been conducted on hospital bioaerosols that must be presented in the introduction. Perdelli et al. conducted a survey in 3 hospitals with different ventilation systems, and found that *Aspergillus* was the dominant genus.<sup>19</sup> Their results showed that high-efficiency particulate arrestance (HEPA) air ventilation systems decreased the amplitude of *Aspergillus* up to 99.9%. Appropriate maintenance of the HEPA system can preserve its efficiency at a high level.<sup>19</sup>

Nourian and Badalli investigated bioaerosol concentrations in different wards of hospitals of Isfahan University of Medical Sciences, Iran. They reported *Aspergillus*, *Alternaria*, *Penicillium*, *Fusarium*, and *Cladosporium*, respectively, as the dominant fungi.<sup>20</sup>

Azizifar et al. reported the fungi genera collected in hospitals in Qom, Iran, as *Penicillium*, *Cladosporium*, *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus*.<sup>21</sup>

Numerous studies have surveyed microorganism concentration in ambient air and indoor environments around the world.<sup>19-21</sup> However, no study has surveyed the effect of population density on fungi concentration and the relationship between indoor and outdoor environments.

The present research was conducted with the aim of studying the effect of population density on fungi concentration and the relationship between indoor and outdoor environments in an educational and therapeutic hospital of Ahvaz, Iran. The main objective of this study was to detect fungal population differences between 3 stations before and after visiting hours.

## Materials and Methods

The present study was conducted on bioaerosols in a hospital with high attendance from Khuzestan and its adjacent provinces during one year (October 2010 to December 2010). The samples were collected over a period of 2 minutes in a flow rate of 28.3 l/minute using a QuickTake 30 air sampling pump equipped with single stage viable cascade impactor (SKC Inc., PA, USA).<sup>5,22,23</sup> In the present research, internal wards and intensive care units (ICUs) were selected for sampling and patients were of all ages. The air sampling was conducted once every 6 days [the United States Environmental Protection Agency (US-EPA), 2006].<sup>24</sup> Sampling time was between 2 to 5 pm. It should be noted that the different wards of the hospital had natural ventilation in the form of open doors and windows.

The sampling process was conducted for 2 minutes at 1.5-2 meters above the ground and 1 meter distance from obstacles and walls (indoor) and 20 meters distance from the street and plants (outdoor).<sup>25,26</sup>

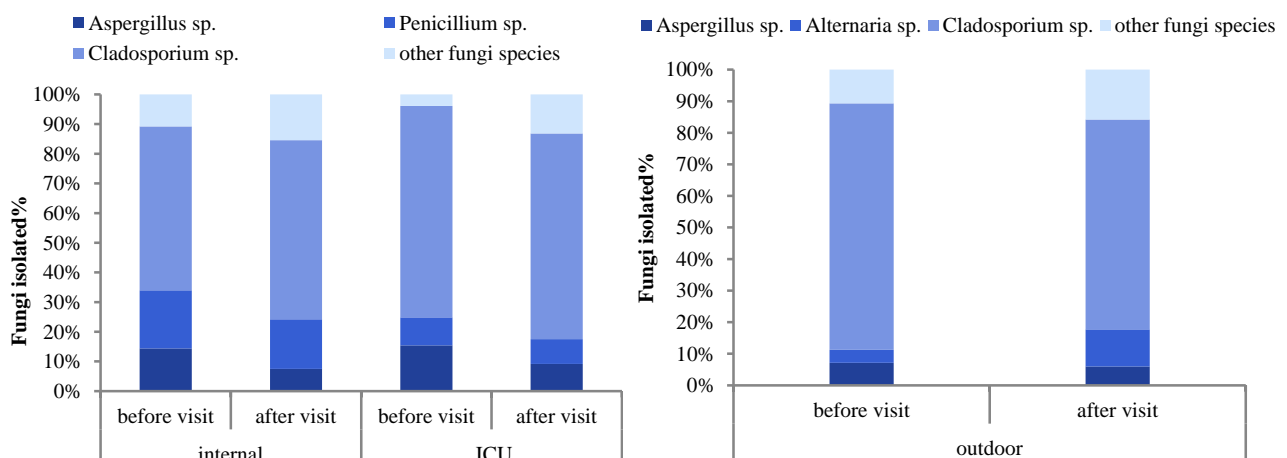
Potato dextrose agar (PDA) with chloramphenicol (100 µg/l) was used as the medium before initiating the experiment in order to inhibit the growth of bacteria, and the device was disinfected by 70% alcohol.<sup>22-24</sup> Then, biostage screening was performed on the screens. The medium containing fungi

was kept in an environment with a temperature of 25-27 °C for 72-96 hours. The number of colonies was reported as colony-forming units (CFU) per cubic meters of air sampled (CFU/m<sup>3</sup>).<sup>25-27</sup>

In the present study, Kolmogorov-Smirnov test was used to determine the normality of data. Mann-Whitney U test was used for the comparison of bioaerosol concentrations before and after visiting hours. In addition, Spearman's rank correlation coefficient, as a nonparametric measure, was used to determine statistical dependence and correlation between indoor and outdoor fungi bioaerosol concentrations before and after visiting hours.

## Results and Discussion

In the present research, 90 samples were collected from indoor and outdoor environments of the hospital. The abundance of fungi, the average and ratio of fungi concentration in outdoor and indoor spaces, and the fungi genus of the collected fungi are presented in table 1. As presented in table 1 and figure 1, the highest concentration percentage of the dominant fungi genera in the indoor environment before and after visiting hours was related to *Cladosporium* (96.9% and 98.08%), *Penicillium* (28.96% and 24.4%), and *Aspergillus* (24.18% and 12.45%), respectively.



**Figure 1. Percentage of fungi isolated in the air samples from indoor and outdoor environments of the hospital during the study period**

**Table 1. Average airborne fungi concentration (CFU/m<sup>3</sup>) and frequency (%) in indoor and outdoor environments before and after visiting hours**

Fungi	*Before visiting hours						**After visiting hours				
	Outdoors <sup>a</sup>		ICU <sup>b</sup>		Internal ward <sup>c</sup>		Outdoors <sup>a</sup>		ICU <sup>b</sup>		Internal ward <sup>c</sup>
	CFU/m <sup>3</sup> (%)	CFU/m <sup>3</sup> (%)	Indoors/O outdoors	CFU/m <sup>3</sup> (%)	Indoors/O outdoors	CFU/m <sup>3</sup> (%)	CFU/m <sup>3</sup> (%)	Indoors/ Outdoors	CFU/m <sup>3</sup> (%)	Indoors/O outdoors	
Septate hyphae	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(20.0)	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(60.0)	32.1(53.3)	2.43	40.0(60.0)	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	0
	Other ssp.	2(33.3)	3.6(16.6)	1.80	4.8(33.3)	2.4	6.3(16.6)	3.8(13.3)	0.60	0	0
	Total	66(93.3)	66(80)	1.00	168(93.3)	2.54	77(86.6)	93(93.3)	1.20	157(93.3)	2.04
Dematiaceous (Phaeo)	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)	0.90
	Alternaria sp.	29(46.6)	0	0	15(33.3)	0.52	77(40.0)	6.1(33.3)	0/08	10(53.3)	0.13
Hyphomycetes	Aureobasidium sp.	15(13.3)	0	0	0.5(16.6)	0	0	0	*	5(40.0)	*
	Drechslera sp.	4(33.3)	1.3(26.6)	0.25	0	0	0	17.1(40.0)	*	10(40.0)	*
	Total	597(100)	180(93.3)	0.30	280.6 (100)	0.47	511(93.3)	376(100.0)	0.74	416(100.0)	0.81
Non-septate hyphae	Rhizopus sp.	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(40.0)	2.00	45(40.0)	3.00
	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	0	4(26.6)	0.50
	Non-sporulating	9(66.6)	0	0	17(33.3)	1.90	2(20.0)	0	0	19(53.3)	9.50
Total fungi	703(100)	251(86.6)	0.36	480.6(100)	0.68	653(93.3)	509(100)	0.78	648(100.0)	0.99	

\*Number of samples collected before visiting hours: 45; <sup>a</sup>Intensive care units (ICU) = 15, <sup>b</sup>Internal ward = 15, <sup>c</sup>Outdoor environment = 15

\*\*Number of samples collected after visiting hours: 45; <sup>a</sup>Intensive care units (ICU) = 15, <sup>b</sup>Internal ward = 15, <sup>c</sup>Outdoor environment = 15

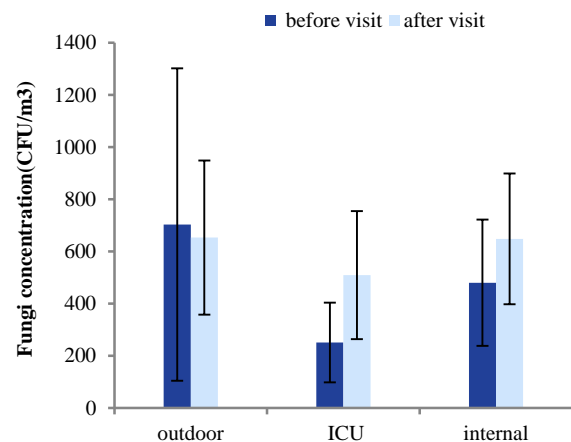
Moreover, in the outdoor space, the highest fungi concentration percentages before and after visiting hours were related to *Cladosporium* (78% and 66.98%), *Aspergillus* (13.4% and 22.68%), and *Alternaria* (4.1% and 11.79%), respectively.

The highest percentage of the dominant fungi in the sampled areas was related to *Cladosporium* the percentage of which was higher in the outdoor environment compared to the indoor environment. However, the average amount of *Penicillium* and *Aspergillus* was higher in the indoor space. The percentage of fungi was higher in the internal ward than the ICU both before and after visiting hours.

The correlation coefficients demonstrated a significant relationship between fungi concentration of outdoor samples and fungi concentration in the internal ward ( $P = 0.0001$ ,  $r = 0.74$ ) and ICU ( $P = 0.0001$ ,  $r = 0.66$ ) before the visits. However, after the visiting hours, this relationship was not significant ( $P > 0.05$ ). The increase in fungi was caused by the presence and density of visitors inside the ward. The comparison of fungi present in the sampled wards before and after the visiting hours ( $P = 0.03$ ) demonstrated a significant relationship in the concentration of fungi indoors and outdoors. Nevertheless, no significant relationship was observed in the outdoor environment ( $P > 0.05$ ). According to the table of fungus concentration, the abundance and the concentration ratio of collected fungi in the outdoor and indoor environments increased after the visiting hours in both wards compared to before the visiting hours. The concentration ratio of fungi collected indoors to fungi collected outdoors was 0.52 before the visiting hours and 0.88 after the visiting hours. According to figure 2, the average of fungi concentration before and after the visiting hours increased with average of 703 and 653 CFU/m<sup>3</sup> in the outdoor environment, 251 and 509 CFU/m<sup>3</sup> in the ICU, and 480 and 648 CFU/m<sup>3</sup> in the internal ward, respectively.

The ratio of indoor to outdoor average

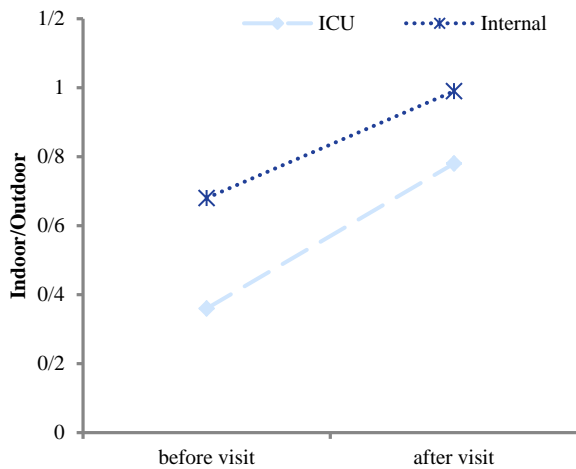
fungi concentration increased after the visiting hours compared to before the visiting hours (Figure 3).



**Figure 2. The variation patterns of airborne fungi density at two sampling times**

The highest average of fungi genera collected indoors compared to the outdoors before and after the visiting hours (in both mentioned wards) was related to *Aspergillus* and *Penicillium*. In the present study, the average of fungi concentration before and after visiting hours was 365.8 and 578.5 CFU/m<sup>3</sup>, respectively. The average increase in fungi concentration before and after visiting hours was higher than that reported in other studies in Iran and other countries.<sup>8,21</sup> In Iran, the mean concentration of fungi in Kamkar Hospital, Qom, Iran, was  $200 \pm 79.9$  CFU/m<sup>3</sup>.<sup>28</sup> Sautour et al. estimated low airborne fungi concentration ranging from 2 to 26 CFU/m<sup>3</sup> in a French hospital.<sup>8</sup> The discussed hospital is an old educational hospital; therefore, it is overcrowded with students, patients, nurses, visitors, and hospital personnel. Furthermore, there was a natural ventilation system in the form of open doors and windows and fine dust is presently a common problem in cities like Ahvaz. These are the causes of increase in indoor fungi density. According to the results of previous surveys, the amount of fungus varies upon season, time of day, temperature, humidity, wind velocity, and the outdoor available fungus density. According to previous studies, the concentration of fungus is

higher in autumn compared to other seasons.<sup>8,29</sup>



**Figure 3. The ratio of indoor to outdoor average fungi in the internal ward, and intensive care units (ICU) of the hospital**

In this survey, the highest amount of fungi (both indoors and outdoors) was observed in October. The dominant fungi were *Aspergillus* spp, *Penicillium* spp, and *Alternaria* spp. This finding was in agreement with that of previous studies.<sup>2,8,29</sup> In addition, among the prevalent fungi, the highest prevalence was related to *Rhizopus*, which is in contradiction with the results of previous studies.<sup>2,8,29</sup> This fungus is among the fungi which were at their highest amount in dusty weather. This demonstrates that the concentration of dust particles was high both outdoors and indoors in hospital wards in Ahvaz. In the present survey, the concentrations of *Aspergillus* spp. and *Penicillium* spp. were higher indoors compared to outdoors; this was also reported in previous studies. Mycological studies have indicated soil and plants as the sources of *Penicillium*.<sup>28</sup> As the hospital has a natural ventilation system, perhaps one cause of increase in fungi is that the windows present in the sampled wards open onto the yard, and the plants and trees were at a distance of 2 meters from the windows. The concentration of *Alternaria* was higher in the outdoor environment and also was high 16-17 hours after visiting hours. According to

studies by Abdel Hameed et al.<sup>10</sup> and Sarica et al.,<sup>7</sup> the concentrations of *Alternaria* and *Cladosporium* were higher after the sunset. The amounts of prevalent fungi differ in varied times and places as they are based on human activities and the environment.<sup>22</sup> The indoor/outdoor ratio of average concentration of fungi in the internal ward (0.99) and ICU (0.78) was higher during and after the visiting hours compared to before the visiting hours as the ventilation system of the mentioned hospital wards are natural. Previous researches have shown that population density within the ward caused an increase in the bioaerosols concentration and caused them to scatter indoors.<sup>4,19</sup> The reports suggest that microbial pollutants present in the atmosphere have a strong relationship with population density and activity level. Infections caused by opportunist fungi cause mucormycosis in patients (especially in patients with ketoacidosis diabetes and immunity dysfunction, and those who have undergone kidney transplantation) which is contagious and is transmitted through spore inhalation. It has also been illustrated that contact with high levels of *Cladosporium* and *Penicillium* causes allergic diseases.<sup>30</sup> Moreover, fungus species of *Aspergillus flavus* and *Aspergillus fumigatus* cause aspergillosis in patients. When the average concentration of *Aspergillus fumigatus* reaches 0.9 CFU/m<sup>3</sup>, the risk of invasive aspergillosis increases.<sup>19</sup> In this study, the average concentration of this fungus was higher in the sampled wards. The risk of this disease decreases when the concentration of *Aspergillus fumigatus* is below 0.2 CFU/m<sup>3</sup>.<sup>31</sup> In hospitals that utilized the HEPA filter system, a lower fungus pollution was reported.<sup>8,19</sup> According to Augustowska and Dutkiewicz, 2006, when the level of *Aspergillus* spores exceeds 50 CFU/m<sup>3</sup> indoor, Sick Building Syndrome outbreaks.<sup>30</sup> Comparison of the number of fungus colonies before visiting hours with the guideline standard (800 CFU/m<sup>3</sup>)<sup>2</sup> indicated that, in 17% of the cases, the

number of colonies formed in each cubic meters of air was higher than the standard. This number was related to the internal ward. Comparing the number of fungi colonies after the visiting hours with the guideline standard (800 CFU/m<sup>3</sup>) indicates that, in 37% of the cases, the number of colonies formed was higher than the standard. Of this amount, 26% was related to the internal ward and 11% to the ICU. Although according to the American Conference of Governmental Industrial Hygienists (ACGIH) guideline, this concentration should be 200 CFU/m.<sup>31</sup> Hence, higher population and movement affects the fungi concentration within the wards. This includes wards in which visitors are not allowed, because the windows and doors are opened and closed repeatedly which causes particles to scatter in the air.

### Conclusion

According to the results of the present study, one of the sources of particle infiltration into the hospital is its outdoor environment as the ventilation system is natural. It is worth noting that recently severe dust storms have occurred in Ahvaz, and thus, the number of particles moving freely in the air is significantly higher. As the source of bioaerosols is particles, the compound of such particles is important both chemically and biologically. After the visiting hours, the level of fungi available indoors was conspicuously higher (even in the wards in which no visitors were allowed, e.g., ICU). Hence, the population and their activity cause an increase in the level of fungi. Due to the negative effects of fungi on the patients receiving treatment and personnel working in the hospital, the use of automatic air ventilation systems is essential. The use of HEPA ventilation systems is recommended to decrease the pollution load. Moreover, in order to inhibit overcrowding, it is recommended to allow only a limited number of visitors enter the hospital each day.

### Conflict of Interests

Authors have no conflict of interests.

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