Original Article

Comparison of airborne fungal flora in indoors and outdoors of Zabol city in spring and summer

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Abstract

Introduction: As one of the organisms contaminating the air, fungi can cause many diseases such as superficial diseases, opportunistic and systemic infections, important allergic reactions, hypersensitivity, etc. Given the importance of diseases caused by airborne fungi, this study aimed to compare airborne fungal flora of indoors and outdoors in Zabol city in spring and summer. **Materials and Methods**: In this descriptive, cross-sectional study, samples were collected by the active method using 540 plates containing Sabouraud dextrose agar medium with chloramphenicol from indoors and outdoors of five districts in Zabol city during spring and summer. For identification, fungal colonies were cultured on slides and the results were analyzed using Fisher's exact test. **Results**: In this study, *Aspergillus fumigatus* and *Mucor* were the most prevalent types of fungal flora found indoors and outdoors in spring and summer. *Mucor* had the highest fungal concentration (cfu: 312.85) found indoors in spring and the lowest fungal concentrations belonged to *Absidia* and *Cladosporium* (cfu: 165.15). In spring, *A. fumigatus* and *Absidia* presented the highest (cfu: 285.29) and the lowest (cfu: 165.15) outdoor fungal concentrations, respectively. In summer, the highest indoor fungal concentrations were recorded for *A. fumigatus* and *A. flavus* (cfu: 265.45) and the lowest levels belonged to *A. nidulans*, *Rhizopus*, and *Mycelium* (cfu: 165.15). *A. fumigatus* (cfu: 285.45) and *Scopolaris* (cfu: 165.15) respectively presented maximum and minimum outdoor fungal concentrations. In general, the highest indoor and outdoor fungal concentrations belonged to *A. fumigatus* and *Mucor*, and the lowest levels were recorded for *Absidia* and *Cladosporium* in spring. In summer, *A. fumigatus* and *A. flavus* had the highest indoor and outdoor concentrations, and the lowest fungal concentrations were found for *A. nidulans, Rhizopus, Mycelium,* and *Scopolaris*. Total concentration of fungal flora was inversely related to temperature and wind speed, but it had a direct relationship with humidity. Thus, the average humidity (24%) was higher in spring than that in summer (15%). **Conclusion**: The results demonstrates that the air of Zabol city contains a variety of fungal spores. As fungi can induce various diseases in humans and are also important causes of pathogenicity and mortality in immunocompromised individuals, identifying the diversity of fungal flora in different places and introducing the environmental fungal flora to infectious disease specialists, dermatologists, doctors, and so on will help in the prevention, treatment, and reduction of mortality due to diseases caused by human contact with fungi.

Keywords: Fungal flora, Zabol, Air

Introduction

Fungi are airborne organisms that can cause disease in humans or animals under certain conditions. In a single day, a normal person inhales about 36 million fungal spores into their lungs. Spores are important in terms of size so that particles larger than 7 microns will remain in the nose and those of 3-7 microns in diameter are places in the bronchi and bronchioles, and those smaller than 3 microns in diameter enter the alveoli [1]. Some cause superficial fungal diseases, such as otomycosis and keratomycosis, and some induce opportunistic and systemic infections in humans. A fraction of fungi, such as *Aspergillus*, *Cladosporium*, and *Alternaria*, are allergens and cause important allergic reactions and hypersensitivity such as asthma, allergic rhinitis, allergic bronchopulmonary disease, and hypersensitivity pneumonia in humans, and others such as *Penicillium*, *Aspergillus*, and *Fusarium* produce dangerous toxins including trichotoxins [2].

Fungal spores are spread through the air, remain suspended for a long time, and lead to contamination by settling on different surfaces. On the other hand, spores located on the surfaces are also able to turn into airborne spores anew. As this process continues, therefore, there will be permanent

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environmental pollution with fungal spores that can have harmful effects on human health [3]. Detection of fungal spores and their diversity in each region depend on various factors such as time, weather factors, seasonal issues, fungal type, geographical area, and regional air pollution [4, 5]. Carbon dioxide concentrations, humidity, and temperature also affect the growth of fungi. Fungal concentrations in the air also are significantly different seasonally, with the highest and the lowest diversities in summer and winter, respectively, and have a positive relationship with relative humidity and a negative relationship with carbon dioxide concentrations [6].

Studies have generally shown that essential factors are required for the study and evaluation of environmental fungi in order to extract significant results. These factors are the reason for the study, types and sizes of fungi, fungal accumulation in each region, the hour and time of study in each region, frequency of sample collection, location of sampling and examination, duration of sample collection, method of study and sampling, and seasonal prevalence of fungi [7]. Taking seasons into account, it was found that *Penicillium*, *Alternaria*, and *Fusarium* species were the most prevalent pathogens in summer and autumn, *Penicillium* species in winter and autumn, and *Alternaria* species in spring. A group of them are plant pathogens and cause irreparable damage to crops; hence, villagers, farmers, silo workers, millers, carpenters, pigeon fanciers, and librarians are at risk of many fungal diseases [2].

Allergies can also develop in normal and healthy people who are chronically exposed to fungal conidia in the workplace and cause such diseases as farmers' lung and tea growers' diseases. Fungal infection is also considered as one of the leading causes of death in immunodeficient patients [7, 8]. Spores of various fungi cause widespread symptoms, including rhinitis, asthma, and sinusitis, by creating premature allergies. The best investigation method for hypersensitivity and complications caused by airborne allergenic fungi is the serological method, which requires the identification of antigens in the air of a region or city. Furthermore, introduction of the environment in terms of fungal flora to infectious disease specialists, dermatologists, doctors, and so on will be helpful in prevention and treatment of diseases caused by human contact with fungi and well demonstrates its importance. Additionally, frequent contact with a group of opportunistic airborne fungi is hazardous in patients with immune system disorders and those undergoing treatment with broad-spectrum antibiotics, cytotoxic drugs, steroids, diabetic people, burns, and so on, with the probability of fungal disease [9]. *Alternaria* is a black, saprobic fungus in soil and a plant pathogen, and there are also rare reports of human infections, including eye infections, nasal mucosa, onychomycosis skin lesions, and lung infection caused by these fungi, in particular *A. Alternata*, which also grows on contact lenses [10]. *Aspergilli* are one of the most prevalent airborne fungi that cause various diseases in humans and animals including skin, lung, and allergic diseases [11]. Fusarium species is an ascomycete filamentous fungus that causes rot and wilting of plants and is a very important issue in arid and semiarid regions [12]. *Penicillium* is one of the most prevalent fungi in the nature that easily grow in the soil and decaying plants and foods. This group of fungi is mainly reputable due to the diversity of antimicrobial and antifungal antibiotics being in use [7].

Given the importance of diseases caused by airborne fungi, it is important to identify the fungal agents. Airborne fungi have been studied in most parts of the world, such as the United States, Italy, India, Saudi Arabia, China, and Japan. In Iran, studies have been conducted in Tehran, Esfahan, Ahvaz, and desert areas (Ardestan), but no studies have so far been conducted in Zabol or at least no reports are not available. Accordingly, and considering the importance of airborne pathogenic fungi, it seems necessary to investigate airborne fungal flora in Zabol city.

Project implementation

In this cross-sectional analytical study, air samples were collected in different areas of Zabol city in spring and summer. There are two sampling methods, passive and active, and the latter was used in this study.

Air sampling by the active method:

According to different recommendations, a sampling flow rate of 28.3 L/min and a sampling time of 2 min were selected for airborne fungal sampling in Zabol city [13]. The Anderson method is one of the standard and conventional methods of microbial air sampling. To collect samples, the sampler was located at a height of 1.2 m above the ground at a distance of > 1 m from the walls and obstacles. The sampler sieve was disinfected using 70% ethanol [14]. The Saburaud dextrose agar culture medium containing chloramphenicol was used in this study. At the sampling site, sterile plates containing the culture medium were placed inside the sampler, which were taken out of the device after sampling according to the mentioned method. The plates with closed lids were transferred to the laboratory. Samples were incubated at 25 °C for 72-120 h. After this period, the number of colonies formed in the plates was counted and reported according to the discharge and sampling time in terms of colony forming units per unit volume (CFU/m^3). This quantitative method helps considerably in obtaining contamination values and comparisons with other studies. During sampling, the average moisture content and wind velocity were recorded daily by the regional meteorological organization. Initial differential detection of fungi was performed through identifying the appearance of the colony on the plate and their microscopic shapes. Finally, fungi were identified by the preparation of crushed samples (teast mount) and the slide culture method [15].

Teased mount and scrape mount

A small portion of the fungal colony was placed on a slide containing a drop of LCB solution by a tilted platinum rod,

covered with a cover slip, and the sample was spread by a slight pressure on the slide to remove excess color (the platinum rod was burnt in the flame before and after the test). Through microscopic small-lens examination, vegetative, reproductive, and colloidal mycelia of fungi (the shape, size, presence of a hypothetical wall, presence/absence of pigments) were examined and identified particularly at the periphery of the colony. If necessary, a permanent sample could be prepared from this slide [15].

Slide culture preparation for slide culture of molds (slide culture technic)

Saprobic fungi often grow within 2-7 days, while most pathogenic fungi need 2 weeks or more to grow during a complete colloidal stage. One of the common methods of slide culture is described below.

Necessary equipment: plates, a curved, U-shaped tube, slides and cover slips, S-type culture medium, corn mill agar or PDA, LCB solution, forceps, and a bistoury.

Methods:

Molten agar culture medium (15 ml) is poured into a sterile plate. After cooling, it is cut immediately into 1-cm square pieces by the use of a bistoury. Sterile distilled water (7-8 ml) is poured into the plate containing a U-shaped tube on which a slide is placed and sterilized previously to provide the necessary cold for the fungal growth (10% sterile glycerin can also be used).

A piece of square agar is placed on the slide in the plate and inoculated with small pieces of the fungal colony. A slide previously sterilized or flamed and cooled is placed on the agar, the plate lid is then closed, and the colony details and the culture date is written thereon. The plate is kept at room temperature. All the above steps should be done next to the flame. The cultures are regularly monitored in terms of growth, colloid formation, and moisture in the plate. When the growth of the fungus reaches a desired level (colony growth to the cover slip edges), two semi-permanent samples are prepared as follows.

A. Sample prepared from the cover slip

Using a forceps, the cover slip surrounded by the fungal colony growth is gently taken and placed on the surface of a clean slide containing an LCB drop. If the fungus is also grown around the slide, another drop of LCB is poured on the slide and covered with a larger cover slip (95% alcohol can be added onto the cover slip to be stabilized before covering the slide).

B. Sample prepared from the slide

The same procedure is repeated for the cover slip, so that two permanent slides can be prepared from each culture slide. As such, the jellose on the surface of the slide is placed inside the plate and the cover slip is removed from the plate, followed by adding a drop of LCB at the fungal colony growth site around the jellose, and then a cover slip is placed thereon. Samples prepared from the cover slip and those from the slide are specified with (S) and (C), respectively. The sides of the cover slip can be blocked with nail polish or any similar substance (before blocking, the excess stain must be thoroughly wiped around the cover slip with a paper towel).

The two slide culture samples can be stored for a long time, and upon drying the LCB solution under the cover slip, the varnish or blocking material on one side of the slide is removed to transfer a few drops of LCB under the cover slip again. The extra stain is wiped with a paper towel and this part is blocked again. Under a microscope, the cover slips have complete colloids, mycelia, and related structures. This method can be used to identify all molds. Under a microscope, attention to the following characteristics is effective in the detection of fungal types: presence or absence of transverse wall, pseudohyphae, colorless, blue (after staining), and dark brown hyphae, various forms of hyphae such as nodular springs, rhizoids, etc., and conidia created through conidial vegetative hyphae including size, shape, color, arrangement, surface, cell wall, etc. [14]. Data were analyzed using the independent t-test and Chi-square test.

Findings

The aim of this study was to investigate the diversity and frequency of airborne fungal flora in Zabol city. Samples were collected by the active method using 540 plates containing Sabouraud dextrose agar medium with chloramphenicol from indoors and outdoors of five districts (based on city divisions) in Zabol city during spring and summer 2017. Finally, 111 counted colonies were obtained by the active method, of which 53.15% and 46.84% belonged to spring and summer, respectively.

Mucor (30.8%) in the central region, *Penicillium* (40%) in the south, *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *Mucor*, and *Mycelium* (20%) in the north, *Mucor* (33.3%) in the west, and *A. fumigatus*, *A. flavus*, *Penicillium*, and *Mucor* (25%) in the east were the most prevalent types of flora found in indoor areas in spring. In spring and indoor areas, the highest frequencies belonged to *Mucor* (24.2%) followed by *A. fumigatus* (18.2%).

Table 1. Frequency distribution of saprophytes isolated from indoors in five districts of Zabol city in spring

Fungi	Colony No.		CFU/m^3	Central		South		North		West		East	
	N	%		N	%	N	%	N	%	N	%	N	%
Mucor	8	%24.2	312.85	4	%30.76	0	%0	1	%20	2	%33.33	1	%25

A. fumigatus	6	%18.2	285.45	3	%23.07	0	%50	1	%20	1	%16.66	1	%25
A. niger	5	%15.2	265.45	2	%15.38	1	%20	1	%20	1	%16.66	0	%0
A. flavus	3	%9.1	243.15	0	%0	1	% 20	1	%20	0	%0	1	%25
Penicillium	4	%12.1	251.65	1	%7.69	2	%40	0	%0	0	%0	1	%25
Rhizopus	3	%9.1	221.85	1	%7.69	1	%20	0	%0	1	%16.66	0	%0
Cladosporium	1	%3	165.15	0	%0	0	%0	0	%0	1	%16.66	0	%0
Absidia	1	%3	165.15	1	%7.69	0	%0	0	%0	0	%0	0	%0
Mycelium	2	%6.1	195.45	1	%7.69	0	%0	1	%20	0	%0	0	%0
Total	33	%100	234.1	133	%100	5	%100	5	%100	6	%100	4	%100

A. fumigatus and *Flavus* (22.2%) in the central region, *Mucor* (50%) in the south, *A. fumigatus* (40%) in the north, *A. fumigatus* and *Alternaria* (50%) in the west, and *A. flavus*, *Scopolaris*, *Rhizopus*, and *Mucor* (25%) in the east were the most frequent types of fungal flora found outdoors in spring. The highest outdoor frequency (23.1%) in spring belonged to *A. fumigatus* followed by *Mucor* (19.2%).

Table 2. Frequency distribution of saprophytes isolated from outdoors in five areas of Zabol city in spring

Fungi	Colony No.		CFU/m^3	Central		South		North		West		East	
	N	%		N	%	N	%	N	%	N	%	N	%
A. fumigatus	6	23.1	285.45	2	%22.22	1	%16.66	2	%40	1	%50	0	%0
Mucor	5	19.2	265.45	1	%11.11	3	%50	0	%0	0	%0	1	%25
Alternaria	3	11.5	243.15	1	%11.11	0	%0	1	%20	1	%50	0	%0
A. flavus	4	15.4	251.65	2	%22.22	0	%0	1	%20	0	%0	1	%25
Rhizopus	2	7.7	195.45	0	%0	0	%0	1	%20	0	%0	1	%25
A. niger	3	11.5	221.85	1	%11.11	2	%50	0	%0	0	%0	0	%0
Scopolaris	2	7.7	195.45	1	%11.11	0	%0	0	%0	0	%0	1	%25
Absidia	1	3.8	165.15	1	%11.11	0	%0	0	%0	0	%0	0	%0
Total	26	%100	227،93	9	%100	6	%100	5	%100	2	%100	4	%100

According to the findings, the most prevalent types of indoor fugal flora found in summer were *A. fumigatus* (33.3%) in the central region, *A. fumigatus*, *Mucor*, *A. niger*, and *Scopolaris* (25%) in the south, A. *fumigatus*, *Mucor*, *A. flavus*, and *Rhizopus* (25%) in the north, *A. flavus*, *Mucor*, *A. niger*, *Scopolaris*, and *Penicillium* (20%) in the west, and *A. flavus* and *Mycelium* (50%) in the east. *A. fumigatus* and *A. flavus* (20.8%) and then *Mucor* (16.7%) were the most frequent indoor algal flora in summer.

Table 3. Frequency distribution of saprophytes isolated from indoors in five districts of Zabol city in summer

Fungi	Colony No.		CFU/m^3	Central		South		North		West		East	
	N	%		N	%	N	%	N	%	N	%	N	%
A. flavus	5	%20.8	265.45	2	%22.22	0	%0	1	%25	1	%20	1	%50
A. fumigatus	5	%20.8	265.45	3	%33.33	1	%20	1	%25	0	%0	0	%0
Mucor	4	%16.7	251.65	1	%11.11	1	%20	1	%25	1	%20	0	%0
A. niger	3	%12.5	221.85	1	%11.11	1	%20	0	%0	1	%20	0	%0
Rhizopus	1	%4.2	165.15	0	%0	0	%0	1	%25	0	%0	0	%0
A. nidulans	1	%4.2	165.15	1	%11.11	0	%0	0	%0	0	%0	0	%0
Penicillium	2	%8.3	195.45	1	%11.11	0	%0	0	%0	1	%20	0	%0
Scopolaris	2	%8.3	195.45	0	%0	1	%20	0	%0	1	%20	0	%0
Mycelium	1	%4.2	165.15	0	%0	0	%0	0	%0	0	%0	1	%50

Total	24	%100	210.1	9	%100	5	%100	4	%100	5	%100	2	%100

The most abundant types of outdoor fungal flora found in summer were *Mucor* (30%) in the central region, *A. fumigatus* and *A. flavus* (33.3%) in the south, *A. fumigatus*, and *Penicillium* (50%) in the north, *A. flavus*, *A. fumigatus*, and *Rhizopus* (33.3%) in the west, and *A. niger* (28.5%) in the east. The highest outdoor abundances in summer were recorded for *A. fumigatus* (21.4%) and then *Mucor* (17.9%).

Table 4. Frequency distribution of saprophytes isolated from outdoors in five districts of Zabol city in summer

Fungi	Colony No.		CFU/m^3	Central		South		North		West		East	
	N	%		N	%	N	%	N	%	N	%	N	%
A. fumigatus	6	21.4	285.45	1	%10	2	%33.33	1	%50	1	%33.33	1	%14.28
Mucor	5	17.9	265.45	3	%30	1	%16.66	0	%0	0	%0	1	%14.28
A. niger	3	10.7	243.15	1	%10	0	%0	0	%0	0	%0	2	%28.57
A. flavus	4	14.3	251.65	1	%10	2	%33.33	0	%0	1	%33.33	0	%0
Penicillium	2	10.7	243.15	1	%10	0	%0	1	%50	0	%0	1	%14.28
Rhizopus	2	7.1	195.45	0	%0	0	%0	0	%0	1	%33.33	1	%14.28
Cladosporium	2	7.1	195.45	1	%10	1	%16.66	0	%0	0	%0	0	%0
Scopolaris	1	3.6	165.15	0	%10	0	%0	0	%0	0	%0	1	%14.28
Mycelium	2	7.1	195.45	2	%20	0	%0	0	%0	0	%0	0	%0
Total	28	100	226.7	10	%100	6	%100	2	%100	3	%100	7	%100

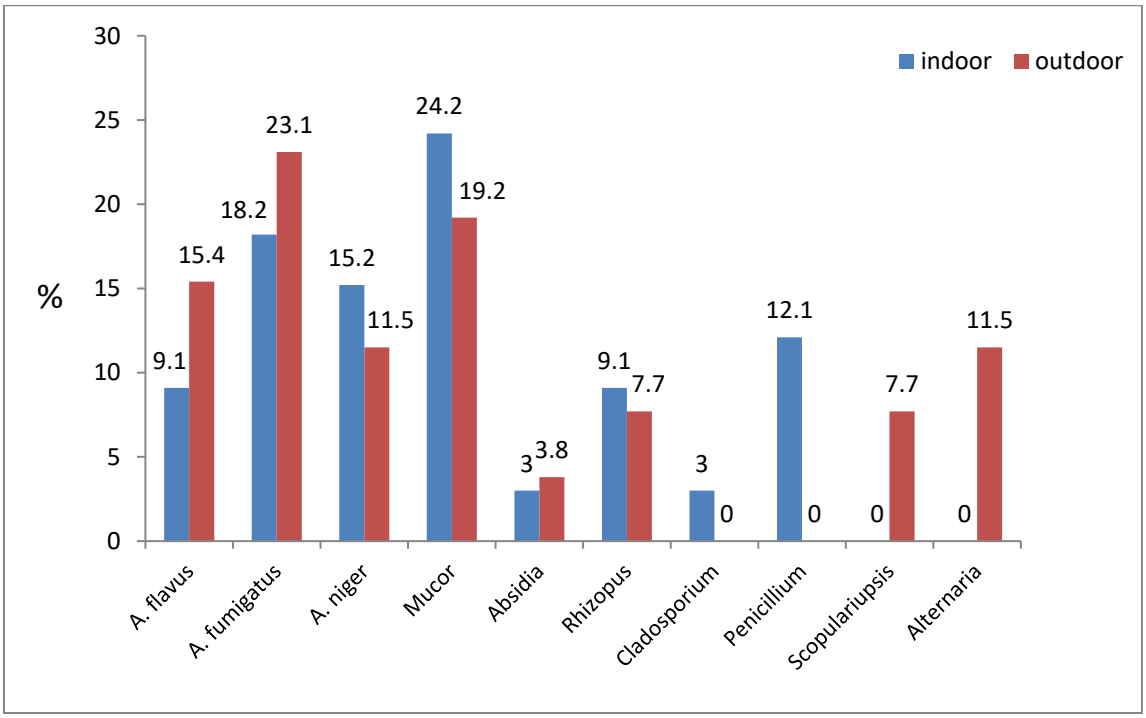

Figure 1. Prevalence of different indoor fungi in comparison to outdoor species in spring

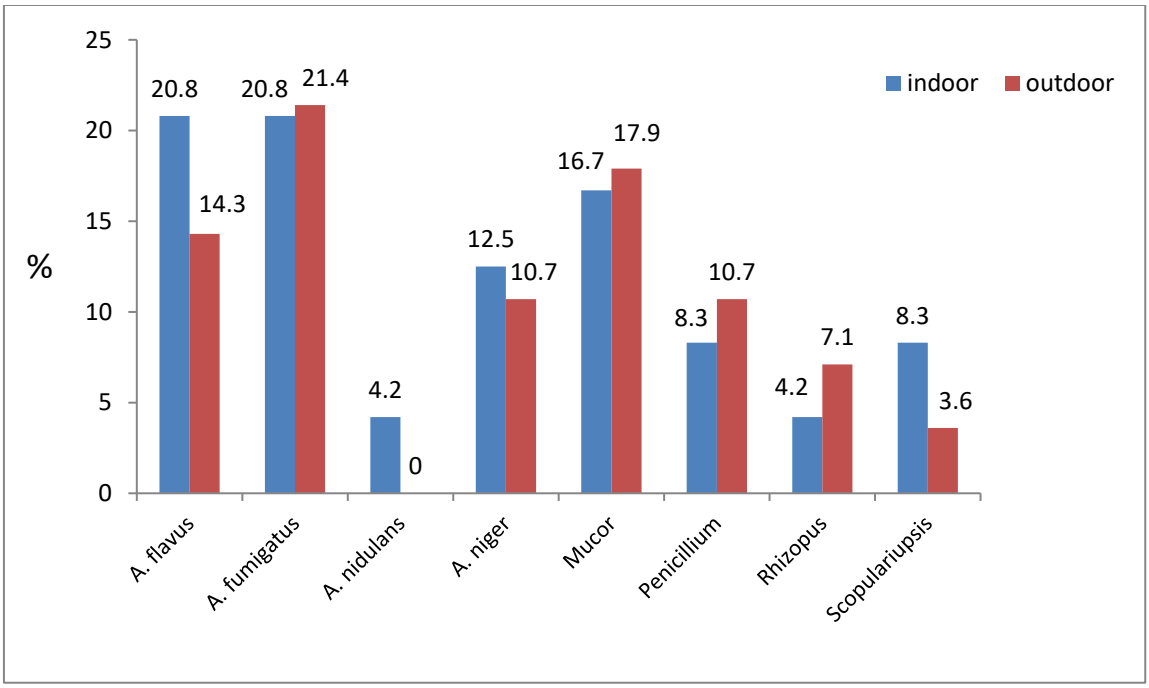

Figure 2. Prevalence of different indoor fungi in comparison to outdoor species in summer

In general, the most prevalent types of fungal flora found indoors and outdoors in spring and summer were *A. fumigatus* and *Mucor*. The findings revealed that *Mucor* had the highest fungal concentration (cfu: 312.85) found indoors in spring and the lowest fungal concentrations belonged to *Absidia* and *Cladosporium* (cfu: 165.15). In spring, *Aspergillus fumigatus* and *Absidia* presented the highest (cfu: 285.29) and the lowest (cfu: 165.15) outdoor fungal concentrations, respectively. The highest indoor fungal concentrations in summer were recorded for *A. fumigatus* and *A. flavus* (cfu: 265.45) and the lowest levels belonged to *A. nidulans*, *Rhizopus*, and *Mycelium* (cfu: 165.15). *A. fumigatus* (cfu: 285.45) and *Scopolaris* (cfu: 165.15) respectively presented maximum and minimum outdoor fungal concentrations in summer. In general, the highest indoor and outdoor fungal concentrations belonged to *A. fumigatus* and *Mucor*, and the lowest levels were recorded for *Absidia* and *Cladosporium* in spring. In summer, *A. fumigatus* and *A. flavus* had the highest indoor and outdoor concentrations, and the lowest fungal concentrations were found for *A. nidulans, Rhizopus, Mycelium,* and *Scopolaris*.

A comparison of the results showed that the total concentration of fungal flora was inversely related to temperature and wind speed, but it had a direct relationship with humidity. The total concentration of fungal flora was greater in spring with a higher average humidity (24%) than that in summer (15%). *A. flavus*, *A. nidulans*, *Penicillium*, *Scopolaris*, *Mycelium*, and *Cladosporium* were more prevalent in summer. *A. fumigatus*, *Mucus*, *A. niger*, *Rhizopus*, *Absidia*, and *Alternaria* were more frequent in spring.

Significant differences were found in the total concentrations of *Penicillium*, *Scopolaris*, and *Mycelium* flora in spring and summer, indicating the influence of environmental factors, such as temperature, humidity, and wind speed, on the growth of these fungi. *Absidia* and *Alternaria* were found only in spring and *A. nidulans* was seen only in summer. According to the tables and statistical analyses, there was a significant difference between the prevalence of fungi in summer and spring (P = 0.000). Moreover, the prevalence of fungi was different significantly in indoors and outdoors in both summer and spring (P = 0.000). It should be noted that the number of samples was less than five fungi in most cases, hence the Fisher test was used instead of the Chi-square test in statistical analyses.

Discussion

Fungi are organisms that are often isolated from the environment and contact with them has harmful effects on humans, leading to allergic infections and even toxic consequences [16-18]. Fungi are different in terms of spores they enter the environment and their consequences vary depending on the fungal type and species [19, 20]. Therefore, isolation of fungi from environmental sources is one of the basic principles in the determination and detection of these factors in the environment and examination of their possible roles in the development of various complications in humans [21]. Several studies have so far been conducted to determine the airborne fungal flora in different countries and Iran. Kachooei et al. (2004) studied 115 samples of airborne pathogenic fungal flora in nine desert region of Ardestan, and finally concluded that *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria* and yeasts were the most abundant

types of fungi. They also reported that sampling location and environmental factors affected the number and type of airborne fungi in the desert region, and some fungi (e.g., *Alternaria*, *Penicillium*, *Cladosporium*, and yeasts) were able to grow even in adverse environmental conditions [2].

In our study, the relationship between fungal flora and atmospheric factors was measured and a significant relationship was found between the fungal flora and environmental parameters. Unlike the above study, which was conducted without considering indoors and outdoors, our study examined both indoor and outdoor flora. Finally, it was concluded that *A. fumigatus* and *Mucor* the most prevalent types of both indoor and outdoor fungal flora found in spring and summer, and that indoor and outdoor fungal frequencies were different significantly in both summer and spring. In Kachooei et al. (2004), on the other hand, it was finally concluded that *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, and yeasts were the most abundant types of fungi. This difference could be due to different sample sizes, and not considering outdoor and indoor environments in their study, which caused a difference in the results because their sample size was much lower than that of ours. Thus, our study with a different design increased the sample size along with the study of outdoor and indoor environments, making our study more reliable.

Steinkaya et al. (2005) assessed indoor fungi in 10 districts of Afyon city, Western Anatolia, Turkey. Samples were collected over a period of one year, and it was concluded that *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria* were the most frequent fungal species. Moreover, the highest and the lowest concentrations of fungi were observed in summer and winter, respectively, but only the concentration of *Aspergillus* was significantly associated with the seasonal change [22].

However, our study was conducted in both indoors and outdoors in a period of 6 months with the aim of investigating the diversity of fungal flora and the relationship between environmental parameters and fungal species. It was finally concluded that *A. fumigatus* and *Mucor* were the most frequent indoor and outdoor fungal flora found in spring and summer. Besides, a significant difference was found between indoor and outdoor frequencies of fungi in both summer and spring. On the other hand, the study of Steinkaya et al. (2005) was conducted only indoors and only *Aspergillus* was reported to have an association with seasonal change. This discrepancy can be attributed to differences in the sample size, studied seasons, and selection of only indoor environments in their study, which caused such differences in the results.

In an investigation on mushroom flora of indoor and outdoor areas in different districts of Takirdag (Turkey), Sen et al. (2007) examined seasonal distribution and relationship with atmospheric factors" in 432 samples from different areas of Takirdag over a 1-year period. A total of 4,205 fungal colonies were counted and it was concluded that most indoor fungal species were *Penicillium* and *Cladosporium*, and the most fungi found outdoors were *Alternaria* and *Penicillium*. There were also significant relationships between *Aspergillus* and temperature, humidity, and duration of sunlight [23]. The present study was performed to compare the prevalence of fungal flora with an emphasis on indoors and outdoors as well as their relationships with atmospheric factors. The frequencies and diversity of fungal flora were compared irrespective of indoor and outdoor environments, and 111 fungal colonies were counted from a total of 540 collected samples. In addition, our study was performed only in spring and summer, and the relationship between environmental parameters, such as wind speed and humidity, was evaluated with the type of fungal flora as in Sen et al. (2007), and a significant relationship was found in both studies.

Rao et al. (2009) conducted a research on airborne fungal flora of Karachi, Pakistan, by selecting five districts in this city and taking one sample from each selected district weekly. They concluded that fungal spores were one of the most important air components in Karachi. Besides, the highest concentration of fungi was in summer and *Alternaria*, *Aspergillus*, *Corvolaria*, *Drexlera*, and *Penicillium* were the most abundant fungal species in this city [24]. In our study, on the other hand, the most prevalent flora were found in spring and *A. fumigatus* and *Mucor* were the most frequent types of indoor and outdoor flora found in spring and summer. This difference could result from differences in the sample size and the selected place and time during the study, because 180 samples were taken with triple repetition in our study, and samples were collected in both indoor and outdoor environments; besides, samples were collected only in two seasons of spring and summer.

Altogether, findings of this study demonstrated that the presence of air pollution with all kinds of fungal spores in Zabol city. Thus, the observance of health-related recommendations, including wearing mask, suitable filters in air conditioners, the provision of dehumidifiers at home and workplace, etc., particularly in people with immunodeficiency, transplants, leukemia patients, and all people who are prone to fungal infections can prevent many diseases related to these fungal agents.

According to the findings of this study, the total concentration of fungal flora was inversely related to temperature and wind speed, but it had a direct relationship with humidity. The total concentration of fungal flora was greater in spring with a higher average humidity than that in summer. *A. flavus*, *A. nidulans*, *Penicillium*, *Scopolaris*, *Mycelium*, and *Cladosporium* were more prevalent in summer. *A. fumigatus*, *Mucus*, *A. niger*, *Rhizopus*, *Absidia*, and *Alternaria* were more frequent in spring. The results showed that there were significant differences between the prevalence of fungi in summer and spring and both indoor and outdoor environments.

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