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Indoor and Outdoor Air Fungus Bioaerosols in Khorramabad Day Care Child Centers Western of Iran, 2018

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ABSTRACT

Introduction: the aim of this study was to determine the quantity and quality of indoor and outdoor air fungus bioaerosols in Khorramabad day care child centers.

Materials and Methods: A total of 180 air samples were collected from 10 centers in 2018. The samples included 7 indoor and 2 outdoor sampling points. The total number of children was 580. Sampling of fungal bioaerosols was performed by the ZEFON pump (ZEFON factory, USA) with a flow rate of 28.3 L/min. The Sabouraud Dextrose Agar containing chloramphenicol was used as the culture medium. Relative humidity and temperature were measured by a Hygro-Thermometer (TES-1360A- Taiwan-made Humidity and temperature meter).

Results: The results showed that 96.1 % of the samples were positive and had grown colonies. The highest amount of fungal agents in the indoor air and outdoor air were 175.58 CFU/m^3 and 274.56 CFU/m^3 in May, while the lowest rates were 3.4 CFU/m^3 and 7.8 CFU/m^3 in July, respectively. *Aspergillus niger* and *Mucor* were the most highly abundant fungus genera, while *Fusarium* was the lowest one. In all samples, the I/O (indoor/outdoor) ratio was more than 1; so, fungal bioaerosols in indoor environments were dominant than the outdoor fungal bioaerosols. The relationship of fungal bioaerosols with RH and T(°C) was significant (P-value = 0.001).

Conclusion: Generally, the amount of contamination is considerable in the studied day care child centers. Therefore, ventilation modification is recommended by a purifier filter. Moreover, the ventilation conditions and favorable air standards should be monitored continuously by supervisory authorities.

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Introduction

Bioaerosols are a mixture of small particles including dust, microbes, fragments, and their fragments residues. The pathology effects of these bioaerosols depend on the size, concentration, physicochemical properties, and distribution of their particles [1] since the micro- to nano-scale size bioaerosols penetrate the lungs and circulation

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system easily in different parts of the body [2]. Thus, the type, source, density, and complication effects of bioaerosols should be studied on the personal health [3]. Bioaerosol production was documented by human activities including sneezing/coughing, room floor washing, cleaning toilets and makeup tables, walking/talking, etc. [4].

Indoor air pollution leads to a variety of adverse health effects including acute respiratory infections, middle ear infections, gastro-intestinal diseases, skin problems, invasive bacterial infections, eye diseases, throat inflammation, skin and nose problems, dizziness, asthma, and allergic reactions [5]. Such health effects bring about a significant economic burden on the involved countries. According to the studies of bioaerosol pollution, indoor air is affected by outdoor air pollution since people are usually in closed environment for a long time [6].

After investigation of the concentration and size of airborne bioaerosols, researchers reported that climatic conditions such as air temperature, relative humidity, velocity wind, sandstorms, as well as proximity to the sea, drought, and seasons influence the growth and increase bioaerosol concentration [7]. Moreover, the relationship among fungal concentration, suspended particles, and meteorological parameter was determined [8].

Fungal microorganisms are the most well-known and diverse species in indoor and outdoor environments. The concentration and type of airborne micro-fungus in the indoor and outdoor environment depend on biological and environmental factors [9, 10]. Fungal colonies may release single spores, cluster of spores, and small fungal fragments in the environment [11]. Some studies showed that fungi had the highest percentage of isolated bioaerosols that produced several types of mycotoxins and volatile organic compounds (VOCs) [11].

Children are considered a risk group because they are more vulnerable to pollutants than adults [12]. Acute respiratory infections and ear infections are most common among preschool children who were kept in kindergartens rather than children who were in home [6]. One of the reasons for sensitivity of the children's age group is that inhalation of air per weight unit in resting infants is twice higher than that of an adult, which is due to the lack of development in children's immune system and lungs [12-15]. Children also have higher heart rates than adults because they allow the absorbed substances in the blood to permeate the tissues rapidly [16].

Some studies investiagted the amount of airborne fungi in indoor air of the child day care centers (CDCC) [17]. For example, Hoseinzadeh et al. studied the airborne fungal flora in the indoor air of DCCs in Delfan. They showed that the most isolated fungal species was Penicillium Spp. The maximum and minimum of total fungi (CFU) were 372 CFU or 1314 CFU/m^3 and 91 CFU or 322 CFU/m^3, respectively. However, they just studied indoor air not the outdoor air. Evidence suggests that indoor air pollution can lead to respiratory damages; inflammation in the skin, eyes, lungs, throat, and upper respiratory tract problems, dyspnea, dizziness, and allergic reactions [18]. Typical indoor environments where children are kept for a long time include schools, DCCs, and homes [19]. Children under the age of 6 years are in the kindergartens at least 8 hours a day and are divided into several age groups: infants (less than 1 year old), toddlers (1-3 years old), and preschool children (3-5 years old) [6].

Children kept in DCCs are often exposed to respiratory infections for a long time. Such respiratory infections are related to the problems caused by fungal molds in the building [20, 21], which are called the sick building syndrome [20-22]. Therefore, childhood atopic diseases can occur due to indoor allergens such as tiny worms and garbage, fungi, beetles, and pets such as dogs and cats [23]. In Iran, children spend a lot of time in DCCs. Therefore, it is important to pay attention to bioaerosols due to their risk for children [6].

Numerous studies were conducted on the microbial quality monitoring in indoor and outdoor environments in the developed and developing countries. However, limited studies were conducted on the fungal contamination of the children kept in DCCs in Iran. Given the necessity

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to ensure the health of children kept in DCCs and to improve the health status of these places, fungal bioaerosols were investigated in the indoor and outdoor air in children DCCs of Khorramabad.

Materials and methods

Sample sites description

This study was conducted in Khorramabad City, the capital of Lorestan province in Iran. The population of this city was 506471 people in 2016, according to the latest census of Iran. Khorramabad City is located at the 33° 48′ N, 48° 35′ E [24, 25]. It has a mild and humid climate. The average annual precipitation is 509 mm. In terms of geomorphology, the study area is mountainous with many mountains around the city of Khorramabad. The average daily temperature in Khorramabad City is 17.2 ℃ and the speed of the west wind is 10 km/h with an RH of 14%.

The method and sampling areas

Khorramabad City was divided into 4 regions including north, west, south, and center according to the DCCs density, population, and sources of air pollution. Due to the objectives of the study and sampling method, the traffic condition of the city was also considered. In this regard, the required information available at the traffic police office of Khorramabad City was divided into three sections in terms of traffic including high, medium, and low traffic areas. In this study, the total population of children in DCCs was 580 including 272 females and 308 males (M = 308 and F = 272).

From 215 DCCs in Khorramabad, 10 DCCs were selected randomly in several stages. To consider the effect of traffic on air pollution, 5 DCCs were selected from high traffic areas and 5 DCCs were selected from the low traffic areas in the north, south, west, and east regions of Khorramabad. According to the statistical calculations, 180 samples (from indoor and outdoor environments of the children DCCs) were collected during the two seasons of spring and summer in 2018. The samples were taken in the morning (from 9 to 11 AM) with doors and windows closed for about 2 hours and without presence of children in the sampling places. The studied variables included airborne fungus concentrations, type of fungi, age of the child, gender, type of the children DCCs, relative humidity, temperature, season of the year, children DCCs location, sampling time, and wind speed.

The DCCs' sampling sites included seven indoor air samples and 2 stations considered as the outdoor sampling sites of the DCCs. Demographic data of the DCCs including the DCCs' type, address, number of children, and number of teachers, children's age and gender, sampling time, month, and period, as well as geographical location were collected through in-person interviews by questionnaires.

The prepared samples were transferred to Razi Herbal Medicine Research Center of Lorestan University of Medical Sciences for diagnosis and then the species and density of the fungal bioaerosols were determined. To determine the relationship between the colony count and environmental conditions, we determined the concentration of the suspended particles and measured the T, RH, and wind speed simultaneously through fungal sampling. The weather data were also collected from the Khorramabad Meteorological Organization. In Figure 1, the geographical location of Khorramabad City in Lorestan province is depicted.

Children under the age of 6 years, who spend at least 8 hours a day in DCCs, were divided into several age groups including: infants (less than one year old), toddlers (1-3 years old), and preschool children (3-5 years old). In the studied DCCS, air conditioners as well as natural ventilation by windows (often open) were used in warm seasons of the year. In the cold seasons, gas heaters were used.

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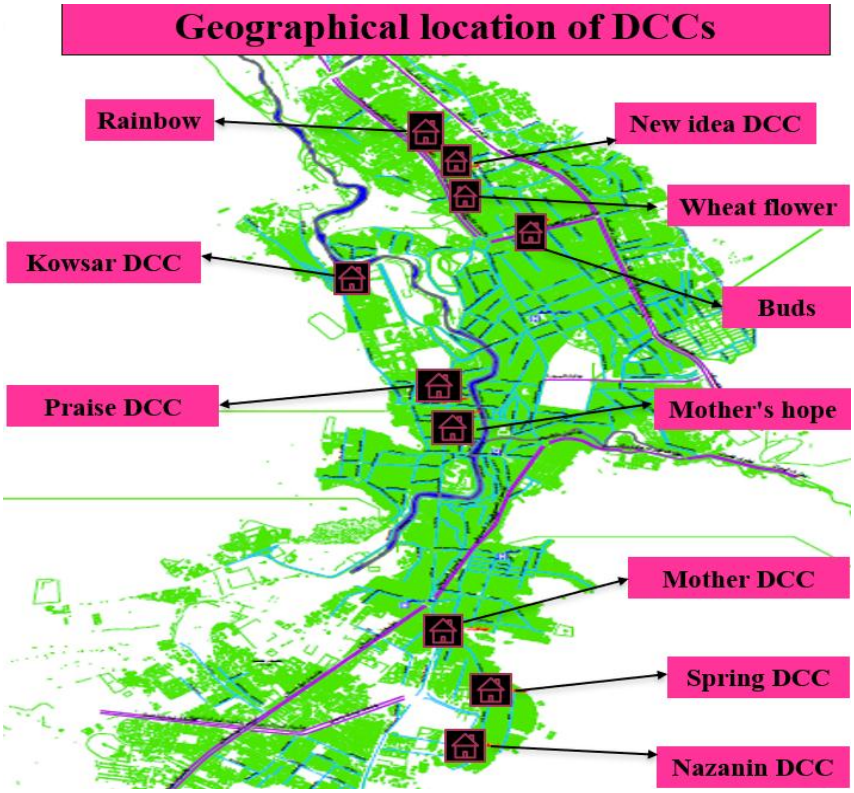

Figure 1: Geographical location of the studied DCCs in Khorramabad City

Sampling fungal agents

The study method was adopted from the literature [6, 11, 26], where active air sampling method and single stage Anderson method were applied. The sampling method included collision of bioaerosols with single stage Andersen's impactor (Quick Take-30, USA). The volume of air required in this study was based on the NIOSH0800 standard and the Andersen's impactor $1ft^3$/min method, which was 28.3 l/min. Each sampling lasted 2.5 min in this study, but a shorter sampling time is necessary in heavily contaminated areas. Later, 10cm plates containing a culture medium of Sabouraud dextrose agar (SDA) with 0.5 g/l chloramphenicol (made in Spain, growth medium type) were placed in Andersen's impactor (Quick Take-30, USA) according to the instructions provided by the manufacturer company. The device was placed at the height of the breathing zone of children, i.e., 0.6-1.2 m. Sampling was performed in all days of the week and in different random places and times [27]. Corn meal agar (merck, Germany) was used to stimulate sporulation of the fungi. Zefon sampling pump (manufactured by SKC in USA) was used with a maximom sampling capability of 30 l/min for microbial sampling. Furthermore, the Andersen's impactor was used to make suction (Quick Take-30, USA). Digital TES-1360 (Germany) was applied for measuring %RH and T (°C). Before starting the measurement operation, the TES-1360 was calibrated by the licensed company.

Calibration of the Andersen's impactor Impedance Flow was performed through Rotameters at a flow rate of 28.3 l/min before each sampling. The selection of sampling sites was based on the role and importance of sampling point conditions in indoor and outdoor fungal contamination. All the necessary tools and equipment were autoclaved for 15 min under standard temperature and sterilized by alcohol 70% isopropanol before sampling. After sampling, to prevent the secondary contamination and error, we closed the plates' lids and transferred them into sterile packages. Later, the plates were incubated at temperature of 25-27℃ (laboratory temperature

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was room temperature) for 3-7 days [28-33]. After the growth and appearance of fungi on the surface of the culture medium, fungi were identified based on macroscopic and microscopic characteristics [34].

To calculate the number of fungal spores per cubic meter, the following formula was used with the quantum value defined as 1 CFU/m^3 [35, 36].

Eq 1. $\text{CFU m}^{-3} = \frac{T \times 1000}{t \times F(\frac{L}{min})}$

T: The number of colonies on the surface of fungal culture medium (SDA)

1000: Conversion coefficient of flow (l/m^3)

t: Sampling time (min)

L: Sampling pump flow (l/min)

Air temperature, relative humidity (Testoterm, Germany), and wind speed (Utron, AM-4201) were performed every 3 min once in the sampling process. In the case that the difference between T and pressure used for calibration was significant, sampling was modified using the ideal gas law.

Macroscopic and microscopic properties

Different macroscopic characteristics of the colonies grown on the Sabouraud dextrose agar were investigated with chloramphenicol: colonies' state and shape, colonial surface view, colony consistency, colony count, colony color, back and front color of the colony, tissue type, exudative secretion droplets, and sclerotia. The microscopic checkup of the fungal species was also carried out using crushing colony methods and fungus culture on lam, using Lactophenol Coton Blu (LPCB) solution. Furthermore, slide cultures and needle mounts (tease mounts) were used for microscopic determination according to Aneja K et al.[34]. To observe the non-sexual spore formation, quiddity arrangement, spore manufacturer cells, as well as growth in different temperatures were considered [27]. When the number of colonies was high and difficult to count, back-calculated image, texture, pigments, and topography of colony, as well as exudate and sclerotium of the plates were used. To determine the fungal genera and species, methods applied in the related literature were used[34, 37-39].

Statistical analysis

Control samples were provided to ensure the accuracy of the results. At first, the normality of data assessed by the Kolmogorov-Smirnov and the Shapiro-Wilk tests. If the data were not normal (Sig. value < 0.05), Spearman and Kruskal–Wallis nonparametric tests were applied to analyze the comparison between variables and DCCs, respectively. The Pearson test applied when the data were normal. All statistical analyses were conducted using SPSS software (version 21) at significant level of 0.05.

Comparisons were made using the guidelines and reference standards for RH and T. According to the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) standard, T ranges 20-29 ℃ in the winter and 22.8-26.8 ℃ in the summer. Furthermore, RH was within the range of 60-30 percent [40]. Charts were designed using EXCELL 2016.

Results

Comfort parameters

The mean temperature was 32.73 ± 7.02 ℃ and the mean RH was 33.19 ± 2.66 in the studied DCCs. According to the sampling site, the highest T ℃ was in the summer with an average of 38.80 ± 3.99. The mean RH content in this study was 33.19 ± 2.66, which was generally in accordance with the ASHRAE standard. Table 1 shows the RH content, T rate, and volume of colonies based on the season and geographical location of DCCs.

Table 1: Average temperature (ºC), RH (%), and number of colonies per unit volume (CFU/m^3) in the geographical location of DCCs in the Khorramabad City

Geographical location	T (°C)	% RH	Volume (CFU/m^3)
North	2.94 ± 26.6	1.93 ± 34.93	111.92 ± 201.78
South	3.99 ± 38.80	2.08 ± 31.44	100.02 ± 187.65
Center	2.94 ± 26.67	1.93 ± 34.93	111.92 ± 201.78
West	3.99 ± 38.80	2.08 ± 31.44	100.02 ± 187.65
Total	7.02 ± 32.73	2.66 ± 33.19	106.08 ± 194.71

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Fungal bioaerosols

Table 2 shows the distribution of fungal species type in indoor and outdoor DCCs based on the research variables. *Aspergillus niger* was the most frequent species in the samples, which was followed by *Mucor*, *Penicillium*, *Aspergillus flavus*, *Rhizopus*, *Cladosporium*, *Alternaria*, and *Fusarium* species, respectively.

According to the geographical location of DCCs, south and west areas were the warmest location with the lowest average of RH (%). The northern and central regions had the lowest average temperature and highest RH (%) content.

Furthermore, the Chi-Square test showed no significant difference between different geographical locations and the type of fungal contamination, apart from *Aspergillus flavus* fungus, which was found in the western regions of the city.

Table 2: Distribution of the selected samples from DCCs in Khorramabad based on the contamination with fungal agents (CFU/m^3), season, type of DCC, geographical location, sampling point, and sample selection space

Contamination (CFU/m^3)		Without colonies (CFU/m^3)		Under 15 colonies (CFU/m^3)		15-30 colonies (CFU/m^3)		Higher than 30 colonies (CFU/m^3)		Total		P-value
	Season	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	
Season	Spring	5	(5.6)	35	(38.9)	41	(45.6)	9	(10)	90	(100)	0.292
	Summer	2	(2.2)	41	(45.6)	43	(47.8)	4	(4.4)	90	(100)	
Type of DCC	Governmental	3	(3.3)	31	(34.4)	46	(51.1)	10	(11.1)	90	(100)	0.064
	Personal	4	(4.4)	45	(50)	38	(42.2)	3	(3.3)	90	(100)	
Geographical location	North	0	(0)	28	(53.8)	21	(40.4)	3	(5.8)	52	(100)	0.092
	South	2	(3.8)	15	(28.3)	29	(54.7)	7	(13.2)	53	(100)	
	Center	2	(5.4)	15	(40.5)	19	(51.4)	1	(2.7)	37	(100)	
	West	3	(8.8)	17	(50)	12	(35.3)	2	(5.9)	34	(100)	
Sampled points	Hall	0	(0)	9	(45)	10	(50)	1	(5)	20	(100)	0.356
	corridor	1	(5)	9	(45)	10	(50)	0	(0)	20	(100)	
	W.C	2	(10)	9	(45)	9	(45)	0	(0)	20	(100)	
	Kitchen	0	(0)	11	(55)	8	(40)	1	(5)	20	(100)	
	Shahid Nobaveh 1	2	(10)	7	(35)	10	(50)	1	(5)	20	(100)	
	Shahid Nobaveh 2	2	(10)	7	(35)	7	(35)	4	(20)	20	(100)	
	Shahid Nobaveh 3	0	(0)	8	(40)	8	(40)	4	(20)	20	(100)	
	Outdoor 1	0	(0)	7	(35)	12	(60)	1	(5)	20	(100)	
	Outdoor 2	0	(0)	9	(45)	10	(50)	1	(5)	20	(100)	
Sampled places	Indoor	7	(5)	60	(42.9)	62	(44.3)	11	(7.9)	140	(100)	0.356
	Outdoor	0	(0)	16	(40)	22	(55)	2	(5)	40	(100)	

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A significant relationship was found between the frequency of fungal bioaerosols and temperature (P-value < 0.01).

Based on the findings, 6% of the samples were contaminated with *Fusarium* fungi, which was only observed in spring (P-value = 0.014); but contamination with other fungal agents did not show a significant relationship with the season.

In spring, only 5.6% of the samples had no contamination; 94.4% of the samples had less than 15 to more than 30 fungus colonies. This percentage dropped to 2.2 in the summer. However, no significant difference was observed between the two seasons in terms of contamination with fungal bioaerosols.

No significant difference was found between governmental and private DCCs. In the north region, all samples were contaminated, but 5.4%, 8.8%, and 3.8% of DCCs were without contamination in the center, west, and south regions of the city, respectively. However, the observed differences were not statistically significant (P-value = 0.092). No significant difference was found between the samples taken from the indoor and outdoor areas and in various DCCs sites in terms of contamination with fungal agents. The highest contamination agent was *Aspergillus niger* (91.1%) and the lowest was 3.8% for *Fusarium*.

The amount of fungal agents was investigated for indoor or outdoor spaces and sampling seasons. The highest amount of fungal agents in the indoor environment was observed at 175.58 CFU/m^3 in May, while its lowest value was 3.4 CFU/m^3 in July. The highest amount of fungal agents in the outdoor environment was measured at 274.56 CFU/m^3 in May and its lowest value was 7.8 CFU/m^3 in July. In this study, no significant difference was found between the sampling sites such as hall, shahid Nobaveh three classrooms, WC, kitchen, corridor, and outdoor stations (P-value = 0.356).

Comparison of the selected samples from outdoor and indoor spaces indicated that the outdoor samples were significantly more contaminated with *Rhizopus* fungus than indoor samples (P-value = 0.001). No relationship was found between contamination with other fungal agents and open or closed spaces, except for *Rhizopus* (Table 3). In all samples, the I/O (indoor air/outdoor air) ratio was greater than 1.

Table 3: Frequency distribution of the observed fungi in samples selected from DCCs in Khorramabad City based on fungus type and sampling space

Sampling spaces / Type of fungi		Outdoor		Indoor		Total		P-value
		Number	Percent	Number	Percent	Number	Percent	
Aspergillus niger	Positive	34	(20.7)	130	(79.3)	164	(100)	0.124
	Negative	6	(37.5)	10	(62.5)	16	(100)	
Aspergillus flavus	Positive	9	(23.1)	30	(76.9)	39	(100)	0.885
	Negative	31	(22)	110	(78)	141	(100)	
Penicillium	Positive	17	(30.4)	39	(69.6)	56	(100)	0.078
	Negative	23	(18.5)	101	(81.5)	124	(100)	
Cladosporium	Positive	4	(30.8)	9	(69.2)	13	(100)	0.442
	Negative	36	(21.6)	131	(78.4)	167	(100)	
Mucor	Positive	24	(21.6)	87	(78.4)	111	(100)	0.806
	Negative	16	(23.2)	53	(76.8)	69	(100)	
Fusarium	Positive	2	(33.3)	4	(66.7)	6	(100)	0.506
	Negative	38	(21.8)	136	(78.2)	174	(100)	
Alternaria	Positive	3	(37.5)	5	(62.5)	8	(100)	0.288
	Negative	37	(21.5)	135	(78.5)	172	(100)	
Rhizopus	Positive	11	(50)	11	(50)	22	(100)	0.001
	Negative	29	(18.4)	129	(81.6)	158	(100)	

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Discussion

The highest T (°C)was in the summer with an average of 38.80 ± 3.99. The average temperature in this study was contrary to the ASHRAE standard [6].

A number of studies pointed out the relationship between relative humidity and bioaerosols in indoor air [41]. A direct relationship was found between fungal agents and meteorological parameters including relative humidity and temperature in indoor environment as well as temperature, relative humidity, and wind speed in the outdoor environment. This is probably due to ventilation and indoor air pressure as well as closed doors of the DCCs. In addition, the presence of *Aspergillus niger* may be due to the suitable condition of DCCs to the growth of *Aspergillus niger*.

The mean RH content in this study was 33.19 ± 2.66, which was generally in accordance with the ASHRAE standard. The approximate RH of 20 to 60% leads to comfort for the human body. Low or high RH levels can lead to physical problems, an RH content of less than 20% causes severe ocular stimulation, and high RH levels can reduce the severity of asthma [6, 42]. The world health organization recommends that people should live in moderately humid environments to prevent the occurrence of respiratory infections associated with low or high relative humidity [23].

All isolated fungi require an average rate of RH to grow and reproduce. The lowest water activity was in primary colonizers such as *Penicillium* spp., *Alternaria*, and the tertiary colonizers (only in the *Fusarium*). Based on fungal water requirements, most isolated fungi such as *Cladosporium*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus*, and *Mucor* can be divided into secondary colonies that require water activity of about 0.8-0.9% [43].

Most fungi isolated in this study are important allergens that can cause symptoms, such as sick building syndrome, respiratory diseases, and childhood systemic infections [20, 21].

According to the geographical location of DCCs, the ones located in the south and west areas were the warmest location with the lowest average of RH (%). The northern and central regions had the lowest average temperature and highest RH (%) content. The Chi-Square test showed no significant difference between different geographical locations and the type of fungal contamination, apart from *Aspergillus flavus* fungus found in the western regions of the city. In another study in São Paulo city of Brazil (2017), *Aspergillus flavus*, as an important source of allergens, was abundant [44]. Park Donguk et al. investigated airborne microbial and fungal contamination in 70 classes in 17 DCCs. They showed that the fungus concentration was greater than 1000 CFU/m^3. According to the multivariate regression tests, fungus sampling and ventilation efficiency can affect the microbial concentration in the samples [45].

A significant relationship was observed between the frequency of fungal bioaerosols and temperature (P-value < 0.01). Therefore, environment temperature is one of the important factors in determining the type of fungal species [6]. *Fusarium* fungi contaminated 6% of the samples and was only observed in spring (P-value = 0.014), which can be due to the sampling location that was near the corn fields near the Khorramabad city [46]. Contamination with other fungal agents did not have a significant relationship with the season.

In the study by Hoseini et al., the maximum concentration of airborne fungi in the outdoor air was 275 CFU/m^3 (minimum = 21 CFU/m^3) for Imam Khomeini station that is similar to the result of the present study. Furthermore, the minimum concentration was 127 CFU/m^3 in office area as an indoor environment (maximum = 1060 CFU/m^3), which may be due to the place of sampling (subway stations) [31]. Hoseinzadeh et al. invetsigated the hospitals in Hamedan and showed that the total mean concentration of fungal bioaerosols was 12.56 CFU/m ^{3}. This rate is lower than 100 CFU/m ^{3} set as a guideline by ACGIH/Guidelines for assessing bioaerosols in 1989 (according to consensus) and less than 50 CFU/m^3 for houses according to CEC/Report #12 (Biological Particles in Indoor Environment) in the 1993 [11, 47, 48]. Rostami et al. (2017) conducted a

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study on the airborne fungi in indoor and outdoor air of the educational, treatment, research center, Ear, Nose and Throat ward, and hematology ward. Results showed that ENT ward for outdoor air (86 CFU) and hematology ward for indoor air (33 CFU) had the highest levels of airborne fungi [49].Hoseinzadeh et Al. studied Dariai-e-Mehr DCC and found that the minimum and maximum fungal agents were 322 CFU/m^3 and 1314 CFU/m^3, respectively, which were higher than the results of this study [6].

Mentese et al. in Ankara found that the highest level of fungal contamination was observed in the kitchens of DCCs, preschools, restaurants, high schools, and homes, while *Penicillium* spp., *Aspergillus* spp., and *Cladosporium* spp. were the most observed fungi. A significant difference was found between the growth of fungi in the indoor and outdoor environment [50]. The results of this study are consistent with our research. In other words, the highest frequency of fungi in our study was related to the fungal species of *Aspergillus*, *Penicillium*, and *Cladosporium*. *Penicillium* spores and *Aspergillus* are released in the environment more easily than *Cladosporium* and this is one of the reasons for the abundance of *Penicillium* species in the indoor air of children DCCs [51]. Moreover, the distribution of *Cladosporium* particles is divided into larger particles than the *Penicillium*, which is more difficult to release the spores [52]. Immonen et al. in a study in Finland on the indoor air of schools found that the most frequent species of fungi were *Penicillium*, *Cladosporium*, *Aspergillus*, and *yeasts* [51].

Aspergillus species, including *Aspergillus flavus* and *Aspergillus niger* were also observed in a study, where *Aspergillus niger* had the highest frequency and caused a variety of respiratory problems and allergic diseases [44]. *Chytridiomycota*, such as *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* (formerly known as *Deuteromycota*) are the largest group of fungi for reproduction, which cause allergic symptoms [53].

Aspergillus niger can cause angiomyceses, peritonitis, and endocarditis. It can also causes infection of the inner and middle ear as well as the pulmonary aspergillosis [54, 55]. Another abundant species scattered in the environment is *Cladosporium*. In the presence of appropriate RH, these species can easily grow on the surfaces, which are allergic and may cause asthma in children with respiratory problems. In addition, the risk of spreading volatile organic compounds and mycotoxins is high by this species. Fungi can also produce secondary metabolites (fungal VOCs and mycotoxins) and the production of mycotoxins depends on the substrates where mould grows. This is also true for VOCs with lower degrees [56, 57].

In public buildings, including DCCs, *penicillium* species are found easily in the air and particles and can grow under favorable surface moisture conditions [43]. In short, the indoor air is affected by the outdoor air; so, fungal species in the outdoor areas of the DCC can grow in the indoor environment if the conditions are favorable. For example, the *Penicillium* species typically exist in the outdoor air, but it grows easily under conditions in the indoor air [58]. Therefore, it is necessary to monitor the respiratory diseases, density and fungi concentration, as well as the personal health.

In the study carried out by Hwang in the city of Ulsan in South Korea on the seasonal concentration of airborne fungal load in children DCCs, the number of fungi was 0-1888 MPN/m^3. The highest number of fungi was reported in the summer, while the lowest number was found in the winter. *Penicillium* and *Aspergillus* species were extracted from the cultured samples [59].This study was consistent with the fungi found in our study, but it was different in the sampling seasons.

In a study conducted by Karwowska in elementary schools and high schools, the researchers found that the amount of contamination was significantly higher than EU standards; so that the amount of fungal contamination was estimated as 30-785 CFU/m^3. The most frequent fungi were *Aspergillus* and *Penicillium* [60]. In a study by Hoseinzadeh et al., 30 CFU/m^3 was considered as the standard to compare the results; the rates lower than 30 CFU/m^3 were in accordance with the standard and values higher than 30 CFU/m^3 were

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higher than the permissible limit [11].

Sławomira et al. studied the concentration of bacteria and respiratory air fungi in five schools in Lublin, Poland and found that the concentration of a species of fungus was greater than the standard level (300 CFU/m^3), equivalent to 690 CFU/m^3 only in one of the schools. Among the fungal species, *Aspergillus* and *Penicilinium* had the highest arte [61]. Meyer et al. in Finland studied molds in the indoor air of school buildings and found that some species of *Aspergillus* and *Penicillium* molds were the most important fungal species isolated from these sites [62]. Their results are in the same line with those of our study.

Comparison of the selected samples from outdoor and indoor spaces indicated that outdoor samples were significantly more contaminated with *Rhizopus* fungus than indoor samples (P-value = 0.001). No relationship was found between contamination with other fungal agents and open or closed spaces, with the exception of *Rhizopus*. The results are presented in Table 3. Hosseinzadeh et al. investigated the indoor fungal bioaerosols of DCCs in Noor Abad City of Lorestan province in 2017. They showed that the most fungal species isolated from these environments was *Penicillium* type [6]. The similarity between the results of these two studies can be attributed to the easier release of *Penicillium* and *Aspergillus* spores than *Cladosporium* spores. In this study, *Pennicillium* and *Aspergillus flavus* were the most common species in outdoor environment.

The results of this study showed that the I/O ratio was greater than 1 in all samples. So, presence of people and decreased rate of ventilation to save the energy or lack of proper ventilation can decrease the indoor air quality and increase the concentration of fungal bioaerosols in the environment [63]. This ratio indicates that the higher rate of indoor bioaerosols fungi, compared with outdoor environment, and is not consistent with the study of indoor/outdoor air quality for elementary schools in Lisbon [64]. In the study by Faridi et al., I/O rates were more than 1 and similar to the present study and the most frequent species were *Cladosporium*, *Penicillium*, and *Aspergillosis* [63]. In another study, the effects of the indoor and outdoor air were compared and the researcher concluded that the microbial samples and species obtained were very similar to those of outdoor environment [65]. In another study in DCCs, no difference was observed between the fungal contamination in the outdoor and indoor air [66]. However, we found that the contamination of the indoor environments was higher than the outdoor contamination.

Moreover, a study in the city of Lisbon on the quality of indoor and outdoor air of the elementary schools showed that the level of bacterial and fungal contamination was high and the factors influencing this amount were RH, T, wind speed, student density, and human activity [64].The similarity and frequency of the airborne fungi in DCCs in public places can be attributed to the congestion of children and students in closed environments and the lack of proper ventilation [6].

Conclusion

This study was carried out in 10 DCCs of Khorramabad city. The results of 180 fungal samples showed that 96.1% of the plates had fungal colonies and 3.9% had no colony. The results showed *Fusarium* fungus with prevalence of 6% was only observed in spring (P-value = 0.014), while contamination with other fungal agents did not show a significant relationship with the target season.

The I/O ratio was more than 1, indicating that the indoor bioaerosols were higher than those of the outdoor environment.

Except for *Rhizopus*, no significant relationship was found between the concentration of fungal bioaerosols in the indoor and outdoor air. *Aspergillus niger* and *Mucor* were the most abundant species in the indoor air and the outdoor fungal agents were similar to the indoor air samples. Furthermore, *penicillium* species with 31.1 % and *Aspergillus flavus* with 21.7% were the most frequent fungi. A significant correlation was found between the ambient temperature, RH, and the observed fungal colony count.

The results indicated that, the central and

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standard ventilation system in the DCCs was not used for air conditioning and natural air was used for ventilation without treatment. In addition, the high level of bioaerosol in the air of DCCs can be due to the large number of children, lack of using personal and non-sterile items by children, lack of having a suitable ventilation system, and improper building design of the DCCs. Moreover, the contamination of these sites can be due to the lack of suitable health standards in the field of educator traffic and parents, the ratio of children number to the capacity of the class, and the lack of guidelines or standards in Iran to monitor the fungal agents in DCCs. Therefore, some recommendations are suggested as follows: 1. Using a ventilated system equipped with a filtration system (the DCCs did not have air treatment systems), 2. Modifying the ventilation system and buildings of DCCs, 3. Monitoring the fungal contaminants continuously, and 4. Reducing the population of children in closed environment and controlling the standard number of children in the class room.

Limitations of the study

In the sampling sites, a single point was selected and monitored. However, using more sampling points could cover the actual bioaerosol concentration much better in the results. In this study only culturable and viable fungi bioaerosols were assessed. We suggest the future researchers to study non-viable and non-culturable bioaerosls such as viruses, pollens, cellular fragments, and toxins in future similar studies. In the present study characterization of bioaerosols was performed up to the genus level. In order to identify the bioaerosols, more detailed flow cytometry and molecular techniques are recommended.

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Conflict of Interest

The authors of this study declare that they have no conflict of interests.

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