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Assessment of Fungal Aerosols Dispersion from Municipal Solid Waste Disposal Site: A Case Study of Karaj, Iran

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ABSTRACT

Introduction: Fungal aerosols from landfill sites can play a fundamental role in environmental pollution and health. The present study aimed to assess the dispersion of fungal aerosols from municipal solid waste disposal site.

Materials and Methods: In this cross-sectional study, the concentration of fungal aerosols was determined in four geographical directions at distances of 250, 500, 750, and 1000 m around landfill site. Relative humidity and temperature were also measured. Moreover the concentration and type of the fungal taxa isolated from landfill site under different environmental and metrological conditions were evaluated.

Results: The results showed that the maximum and minimum concentrations of fungal aerosol in the landfill site were 256.18 ± 59.7 CFU/m^3 and 76.56 ± 23.2 CFU/m^3, respectively. The most frequent fungi detected from municipal landfill site included *Penicillium* (43.67%), *Cladosporium* (33.54%), *Yeast* (7.60%), *Aspergillus* (5.91%), *Curvularia* (3.62%), *Chrysosporium* (1.57%), *Alternaria* (1.54%), *Scopulariopsis* (0.84%), *and Ulocladium* (0.60%) taxa. The maximum identified fungal aerosol concentration in the area around the solid waste landfill was 350 CFU/m^3. Furthermore, the concentration of fungal aerosols in the environment was significantly related to relative humidity, wind direction, and temperature in spring and winter ($P < 0.05$).

Conclusion: Municipal solid waste disposal site can be a potential source for fungal aerosol dispersion. Moreover, fungal aerosols concentration is correlated with wind direction and speed, relative humidity, and temperature.

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Introduction

Solid waste and related disposal technologies are considered as one of the air contaminant sources[1]. Inhibiting environmental contamination in landfill and recycling centers is important for preventing airborne diseases [2]. Most studies on the health risks related to solid waste landfills have focused on the effects of hazardous chemicals in landfills, but limited research has highlighted bioaerosols [3]. Despite the recognition of the health risks associated with bioaerosol exposure, no specific permissible limit is provided for this group of contaminants in the environment, especially in municipal landfill and recycling centers, and the presented levels are as suggestion [4].

The particulate matters (PMs) emitted from solid waste landfill and recycling centers can contain biological and non-biological materials, which can transfer to the areas far from the production site through airflow [3]. The emission of fungal aerosols in the environment plays an important role in causing respiratory symptoms, allergic reactions, and respiratory tract infections [5-7]. According to Schlosser et al., landfill environment provides an appropriate condition for transmitting biological agents, especially fungi. They found that fungal *Aspergillus* species possesses maximum risk for residents around the landfills and threatens human health [3]. Aerosol emission in landfill and recycling centers is facilitated with various waste management activities such as discharge, transportation, handling, separation, and compaction, as well as final covering of landfill [8].

The dispersion of fungal aerosols leads to respiratory tract and skin diseases, which threatens the health of employees in landfill and recycling centers [9-11]. Due to the importance of bioaerosol dispersion in environmental contamination, extensive studies have been recently conducted on their spread from landfill and recycling centers worldwide [3, 5, 12-16]. Considering the quality and conditions of solid waste management, different results have been reported. Given that identifying and analyzing the fungal flora of contaminated air in the solid waste processing centers are important, the fungal contamination level and dominant fungi of the sites were determined, and the role of environmental factors in dispersing fungal aerosols was examined. In this study, after identifying common fungal taxa, allergenicity (as allergens and non-allergens) of fungal taxa was classified based on mycological texts.

Materials and Methods

Sampling site

In this study, sampling was performed in 2017 in the municipal landfill and recycling center of Karaj, located in Halghe Dareh in the southwest of Karaj city, Alborz province, Iran. In this regard, 16 stations were assessed in four main directions, so that monitoring was conducted at a 250 m distance from landfill site in each direction and the stations were distanced 250 m apart. The map and sampling locations are displayed in Figure 1. Then, 192 samples were collected and tested from the 16 stations through using an active sampling technique. Sampling was done for six months in winter and spring, and two samples were taken every month with an interval of 15 days.

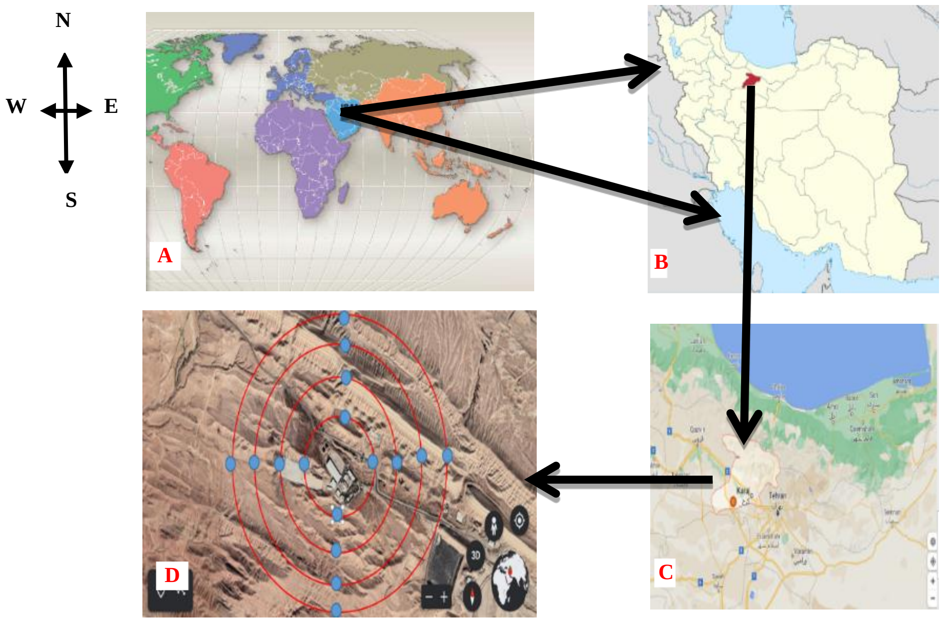

Figure 1: The map and sampling locations of solid waste disposal site, Karaj, Iran
(A: map of world, B: map of Iran, C: map of Karaj, D: Map of solid waste disposal site and sampling points)

Sampling method

A Quick Take 30 (SKC, USA) sampling instrument with the flow rate of 28.3 L/min for 2.5 min was utilized based on the standard NIOSH method [17, 18]. Before using the instrument, flow rate was calibrated on 28.3 L/min by a rotameter based on the manufacturer's instruction. After sterilizing Biostage with 70% alcohol, a plate with Sabro dextrose agar (SDA) supplemented with chloramphenicol (0.05%) was placed inside Biostage and sampling was performed for 2.5 min at 1.5m height from ground [6, 19, 20]. Then, the obtained samples were transferred to laboratory for separation and identification. The sampling frequency was 15 days. Sampling was performed at a height of 1.5 meters and 6 times for each station in each season.

Isolation and identification of fungal aerosols

Three culture media of SDA (Sigma, Germany), Sabro dextrose broth (SDB) (Sigma, Germany), and potato dextrose agar (PDA), (BioMerieux, France) supplemented with chloramphenicol (0.05%) were applied for observing microscopic, macroscopic, and sporulation structure. The sampled culture medium plates were transferred from the instrument to laboratory and incubated at 28˚C. In addition, they were evaluated for fungal growth intermittently and daily until 10 days. The number of colonies formed in the plates was counted and expressed in the unit of CFU/m^3 by considering flow rate and sampling time. The obtained colonies were identified based on the morphological characteristics of the colonies. Microscopic identification was performed using the direct KOH test on a wet mount [21, 22].

Molecular identification

The isolated fungal taxa were detected through PCR-sequencing method. The fungal genomic DNA was extracted using Kit (Roche, Switzerland) according to the kit's protocol. The quantity and quality of DNA samples were assessed by NanoDrop 2000c (Boeco, Germany). The PCR amplification of genomic DNA was carried out to amplify the ITS-5.8S rDNA region using the universal primers ITS4 and ITS5 (ITS4:5′-TCCTCCGCTTATTGATATGC-3′, ITS5: 5′-GGAAGTAAAAGTCGTAACAAGG -3′) [23].

The PCR was performed in final volume of 25 µL including 13 µl of 2X ready to use Master Mix (SinaClon, Iran), 1µl of each 20 pmol forward and reverse primers, 7µl of sterile distilled water, 1µl Taq polymerase, and 2µl extracted DNA template, using a PCR thermal cycler (Peqlab, Belgium). The thermal cycle was performed for 5 min at 94°C of initial denaturation, 35 cycles of a second denaturation at 94°C for 45 sec, annealing at 56°C for 40 sec, and elongation at 72°C for 20 sec. The PCR was completed through a final elongation at 72°C for 10 min. Sequencing of PCR-amplified products was performed using the Applied Biosystems 3730 XL Bioneer (Korea) using ITS4 primer. A search of sequences was carried out using the Blast program with a database maintained at the NCBI (Library of Medicine, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/BLAST/).

Meteorological parameters measurement

The relative humidity and temperature in sampling points were measured using digital TES-1360 (TES Electronic Corp. Taiwan). Temperature and relative humidity measurement ranges of TES-1360 were -20 to +60 °C and 1-95%, respectively. The accuracy of the device for measuring temperature and relative humidity were ±0.8 °C and ±3 %, respectively. This device was calibrated by the licensed company. Meteorological information including wind speed, wind direction, ambient temperature and other meteorological conditions related to the sampled place was obtained from Alborz Meteorological Organization.

Statistical analysis

Two-way ANOVA and Scheffe's post hoc tests were respectively applied for comparing the difference in mean concentration of fungal aerosols in different stations, as well as the difference among stations. The mean concentration of bioaerosols in winter and spring was compared using t-test. In order to assess the relationship between environmental parameters such as temperature, relative humidity, and wind speed in the sampling days with the concentration of fungal aerosols, the normality of the data was first specified through using Kolmogorov-Smirnov test and the required results were obtained by Pearson correlation coefficient test. A univariate linear regression was applied for determining the effect of meteorological parameters on the total number of fungi.

Ethical issue

This study has been approved by the Ethics Committee in Alborz university of medical sciences (IR.Abzums.rec.1396, 27).

Results

All fungi taxa identified by culture method were also determined by molecular test, but the number of fungi taxa identified by culture method was less than molecular method. Therefore, since the molecular method is more accurate, the results were reported based on the molecular method.

Fungal aerosol concentrations

The presence of fungi taxa in all samples (192 samples) collected from Karaj landfill site was confirmed. The mean concentration of all enumerated fungi taxa was 204.34 ± 49.56 CFU/m^3 on the municipal solid-waste disposal site of Karaj. In addition, the mean concentrations of fungi taxa in spring and winter were 256 ± 59.7 and 76.56 ± 23.2, respectively. The isolated fungal taxa percentage is presented in Figure 2, which indicates the separated fungi as *Penicillium* (43.67%), *Cladosporium* (33.54%), *Yeast* (7.60%), *Aspergillus* (5.91%), *Curvularia* (3.62%), *Chrysosporium* (1.57%), *Alternaria* (1.54%), *Scopulariopsis (0.84%), and Ulocladium (0.60%)* taxa, respectively.

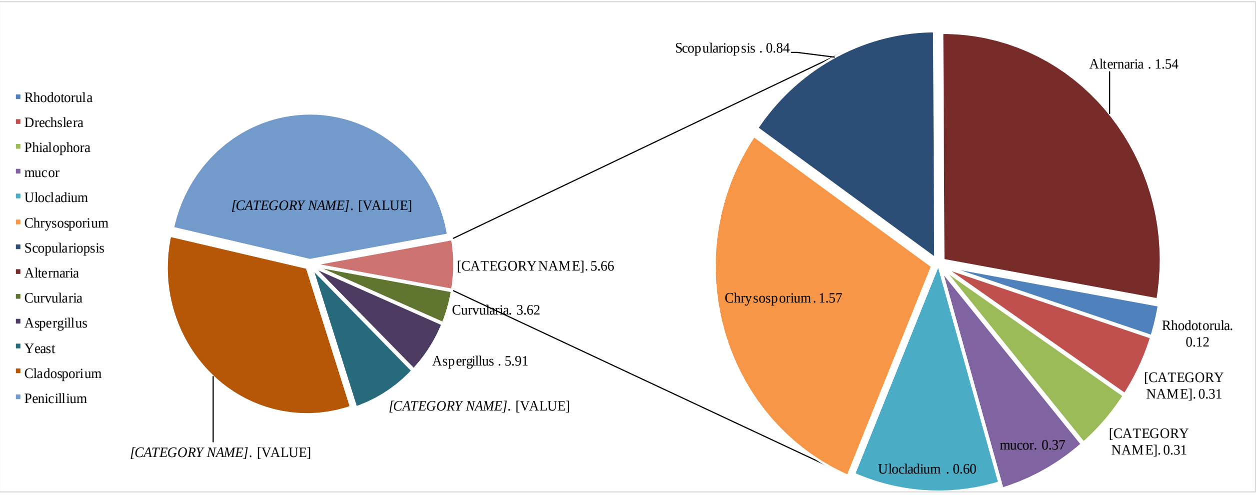

Figure 2: Mean percentage of bioaerosols based on the percentage of isolated species from municipal solid waste disposal site of Halghe Dareh, Alborz province, Karaj, Iran

Fungal aerosol variations with sampling direction

Figure 3 represents the mean concentration of total fungal aerosol at different directions in the seasons under study. The mean concentration of total fungal aerosol in spring was more than winter at all directions. The maximum concentrations of fungal aerosols were observed in the west, north, south, and east directions during spring, as well as north, west, south and east directions in winter, respectively. Furthermore, the maximum and minimum mean concentrations of fungal aerosol were 256.18 ± 59.7 CFU/m^3 in spring at west stations and 76.56 ± 23.2 CFU/m^3 in winter at east stations, respectively.

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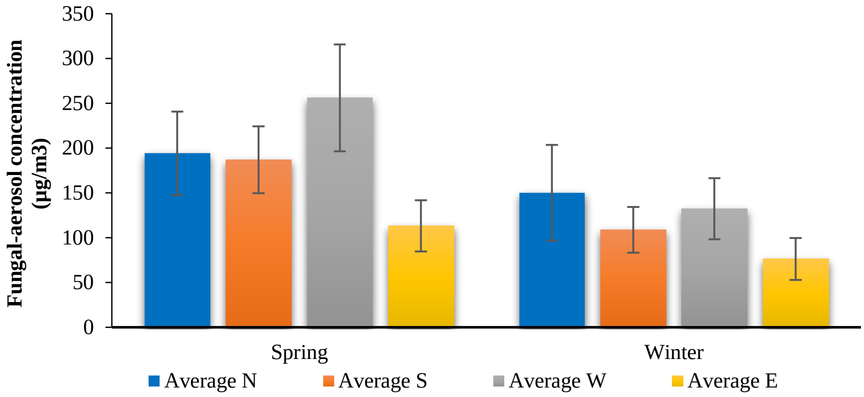

Figure 3: Concentrations of fungal aerosol (mean and standard deviation) in each direction during spring and winter in terms of CFU/m^3 (north, west, south, and east geographical directions are abbreviated as N, W, S, and E).

The mean concentrations of fungal aerosols at four main directions during spring and winter at different stations are shown in Figure 4. During the sampling period, the highest and lowest amount of colony concentrations was 350 CFU/m^3 in S_1 station at spring and 37.34 in E_4 station at winter (Figure 4), respectively.

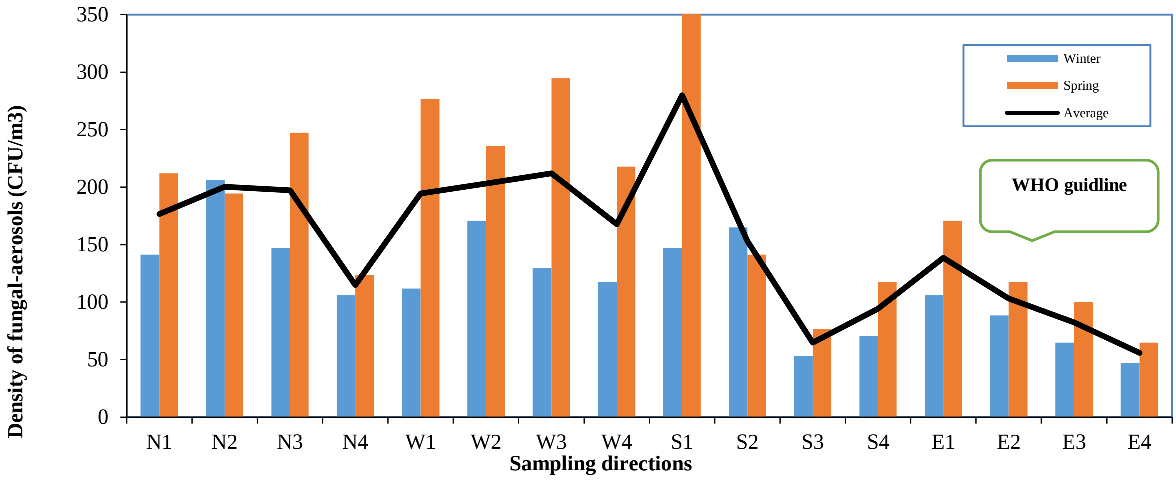

Figure 4: Mean concentration of fungal aerosol in each direction in terms of CFU/m^3 and different stations during spring and winter (north, west, south, and east geographical directions are abbreviated as N, W, S, and E).

Table 1 summarizes the results of statistical analysis for the concentration of fungal aerosols, and variables related to season and sampling station ($P \leq 0.05$). The results showed a significant relationship between different stations with the concentration of fungal aerosols, so their concentration decreased by distancing from landfill and recycling centers.

Table 1: Results of two-way analysis of variance for fungal aerosol concentrations and season and sampling station variables ($p \leq 0.05$)

Variable	Total square	DF	Average squares	F	P
Season	1354261	1	1354261	105.95	< 0.001
Station	1867546	3	622515	48.63	< 0.001
Season and Station	534235	3	178078	13.91	< 0.001

Effect of metrological parameter

Table 2 indicates the relationship between environmental conditions and fungal aerosol concentration around the landfill and recycling centers of Karaj, Iran. Based on the Pearson correlation coefficient, fungal aerosol concentration was significantly related to relative humidity (r = 0.34). Furthermore, a positive significant correlation was observed between the concentration and temperature (r = 0.48), as well as wind speed (r = 0.23), so that an increase in temperature and wind speed resulted in rising the concentration.

Table 2: The correlation coefficients of fungal aerosols in relation to environmental factors at the site of the landfill and recycling of solid waste in the city of Karaj

Environmental conditions	Concentration of fungal aerosols CFU/m^3	Wind direction	Wind speed	Relative humidity	Temperature
Concentration of fungal aerosols (CFU/m^3)	1	0.033*	0.23*	0.34*	0.48*
Wind direction	0.033*	1	-0.018	0.14*	-0.035
Wind speed (m/s)	0.23*	-0.018	1	-0.14	-0.031
Relative humidity (%)	0.34*	0.14*	-0.14*	1	-0.033
Temperature (°C)	0.48*	-0.035	-0.13	-0.033	1

*Correlation is significant at the 0.05 level (two-tailed)

Figure 5 displays the effect of different geographical directions on the concentration of fungal colonies during the six-month study. The highest and lowest fungal concentration was respectively related to the western station (mean = 202 CFU/m^3) and the eastern one during (mean = 76 CFU/m^3). Figure 6 shows the effect of wind speed at different directions. The highest amount of fungal aerosol concentrations was in the wind speed above 3 m/s (P < 0. 05).

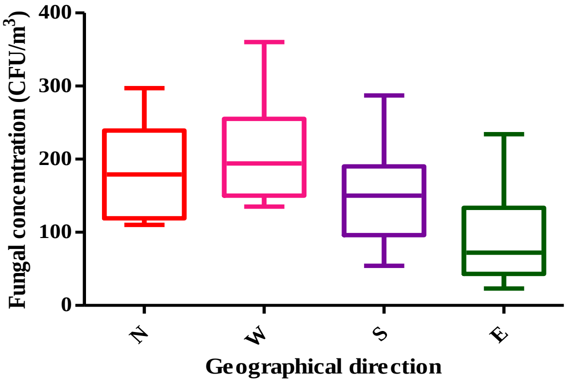

Figure 5: The effect of different geographical directions on the concentration of fungal colonies in the air at municipal solid waste site (north, west, south, and east directions are abbreviated as N, W, S, and E).

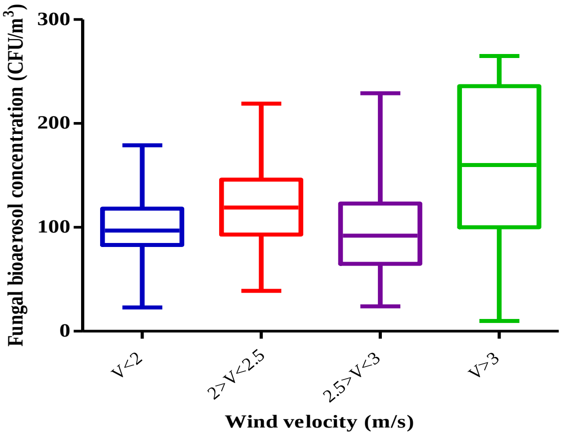

Figure 6: The effect of wind speed at municipal solid waste site on the concentration of fungal colonies (▬ mean; ▯ 25-75%; ⌶ range)

Separation of allergenic and non-allergenic fungal taxa

The results show that 84.56 ± 20.66% of isolated fungal taxa are major known allergens, and the rest (15.44 ± 2.45%) are non-allergenic.

Discussion

Fungal aerosol concentrations

Among 13 identified fungal taxa, the maximum percentage (77.2%) of total samples was belonged to *Penicilium* and *Cladosporium*, while 22.8% was related to 11 remaining taxa (Figure 2). The presence of fungal taxa in all collected samples can be due to suitable conditions for fungal growth in the landfill site, including sufficient humidity and temperature [24, 25]. In this study, *Penicilium* and *Cladosporium* are considered as two common types of airborne bioaerosols, which are harmful for human health because of producing mycotoxins and causing allergic reactions [26]. Similar results were obtained in another study conducted on fungal aerosols around solid wastes disposal site. Liu et al. reported the presence of *Cladosporium*, *Fusarium sp*., *Penicillium sp*., and *Candida* in a solid waste landfill plant [12]. However, Li et al. found *Penicillium sp.* and *Aspergillus sp.*, as dominant fungal aerosols at sanitary landfill site [27].

The results of the present study are consistent with those of some other research studies, since fungi are microbial species, which spread everywhere and can be recovered and separated [9, 28]. However, some studies found a less percentage of positive samples, which is related to the difference in the sampling sites, as well as the meteorological conditions of the area under study [8, 14, 29]. Nageen et al. reported that the concentration and species of fungal aerosol in environments vary depending on a number of factors such as fungal substrates availability and meteorological factors [30].

Del Cimmuto et al. reported the production of different concentrations of bioaerosols in various sites of landfill and recycling centers, along with the highest concentration of fungal aerosols in the waste-receiving site of landfill [31]. Due to the existence of a high level of organic materials and nutrients in the municipal landfill sites, they are appropriate for microorganism growth and proliferation. Accordingly, they can be considered as an important source of biological particle dispersion in the air around landfills [10]. Agarwal et al. (2016) also stated that solid waste dumping sites have large concentrations of fungal aerosols and can be a major health concern for populations residing in neighboring area [32].

Fungal aerosol variations with sampling direction

The prevalence of airborne fungi is significantly related to sampling season and direction. Different meteorological parameters such sampling and wind direction affect the release of the fungal aerosol in the atmosphere in solid waste handling sites [33]. Based on the results, bioaerosol concentration in a significant amount of samples around landfill site and recycling centers was determined above 100 CFU/m[3]. There is no report on the adverse effects of fungal concentrations below 100 on health except for the residences of sensitive people [11]. Due to the health effects of the contaminants, the world health organization (WHO) recommends that their concentrations should not exceed 150 CFU/m [3, 11]. Given that the concentration of fungal aerosols was great in many of the samples collected from around the landfill in this study, their health effects are expected, since exposure to fungal aerosols leads to problems such as skin and eye irritation, respiratory tract diseases such as asthma, and gastroenteritis problems [11, 34]. In present study, the highest fungal aerosol concentrations were observed in spring in the landfill site, since environmental temperature and moisture may be more favorable for airborne fungal germination, growth, and proliferation than in winter [30]. In fact, the concentration of fungal aerosols in landfill was more during warmer seasons. The appropriateness of conditions for growing and proliferating the microorganisms at high environmental temperature is considered as a reason for the great concentration of fungal aerosols in warmer seasons, which is consistent with the results of the research conducted during 2017 in Bombay city, India [29]. Li et al. demonstrated that bioaerosols released from the landfill during summer and autumn were higher than those in spring and winter [27]. The results of statistical analysis confirm the effects of station sampling site and season on the concentration of fungal aerosols. A significant relationship was found between different stations with the concentration of fungal aerosols, so that their concentration decreased by distancing from landfill and recycling centers. The fungal aerosols may be originated from municipal landfills, which is consistent with the results of studies by Breza-Boruta[16] and Pagalilauan et al. [15]. Evaluation of bioaerosol concentration around municipal landfill indicated that landfills should be maintained appropriately and managed properly, since they are a source for spreading fungal aerosols.

Effect of metrological parameter

Liu et al. reported a positive correlation between environment temperature and airborne fungi, which is in line with the present study [35]. Meteorological parameters such as relative humidity and temperature can play a role in the amount of bioaerosol dispersion [10]. Previous studies have indicated that meteorological and environmental conditions have a significant impact on the concentration of fungal aerosols in the solid waste sites. Meteorological conditions such as temperature, wind velocity, wind direction, season, and relative humidity are known to affect the production and dispersal of fungal aerosols in air in the surrounding environment downwind of non-hazardous waste landfill sites [3]. However, the concentration of fungal aerosols, which is positively correlated with some metrological parameters such as temperature and relative humidity, is probably due to the fact that both parameters can accelerate fungal growth in the environment [36]. Nageen et al., Priyamvada et al., and Alghamdi et al. introduced meteorological parameters such as temperature, relative humidity, and wind speed as some of the factors affecting the growth of fungal agents [20, 30, 37]. A higher concentration of fungal aerosols was observed along wind direction compared to the other directions, regarding *Aspergillus fumigatus* and mesophilic fungi in non-hazardous waste landfill sites, which is consistent with the results obtained by Schlosser et al. [3]. Therefore, culturable airborne fungi and bacteria in a municipal waste transfer site indicated that microorganism concentration at downwind of the site is more than upwind of the site. Regarding the effect of wind direction, as well as the type of identified indicator species, are consistent with Rosas et al. study [28]. Based on the results, an improvement in wind speed (more than 3 m/s) led to a higher increase in fungal aerosols in some areas, which can be related to the separation

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of the bioaerosols from landfill and recycling center although wind speed can play a diluting role [11]. The results are in line with the studies of Alghamdi et al. [20] and Li et al [38].

Separation of allergenic and non-allergenic fungal taxa

Based on the available mycological texts [16, 39, 40], the identified bioaerosol fungi are classified into two categories: allergens and non-allergens. This study showed that 84.56% (most fungi) and 15.44% of the fungal taxa were allergens and non-allergens, respectively. Srivastava et al. indicated that most fungal aerosols in and around a landfill site in Delhi were allergen for animals and human [33]. Based on the results, more allergenic fungi (major allergens) include *Alternaria*, *Penicillium*, *Cladosporium, and Aspergillus,* while the other fungi obtained in the study are considered as less allergenic ones. The result of the present study concerning the allergenicity of fungal aerosols is in agreement with the mycological studies on the airborne bacteria and fungi spread through landfill in a northern city in Poland during 2016 [16]. Liang et al. have assessed the prevailed allergenic fungi such as *Aspergillus*, *Cladosporium*, and *Curvularia* in municipal landfill and surrounding area as a health risk factor [5]. *Aspergillus fumigatus* can exist in solid waste disposal sites and act as allergens due to its capacity to degrade cellulose compounds [11].

Conclusion

Municipal landfill and recycling centers can be a potential source for fungal aerosol dispersion. The concentration of bioaerosols around the centers is a function of environmental conditions and distance from the site, as well as how to maintain landfill and recycling facilities. The activities performed in the centers such as discharge, transfer, heaping, and layering can play an important role in spreading fungal aerosols in atmosphere. Furthermore, some fungal aerosols or their toxins can be pathogenic, which are abundant in the center. Thus, the employees in or residents around the centers may be affected by many diseases.

The most important results obtained in the present study are as follows:

- In total, 8268 fungal colonies from 13 fungal taxa were separated from 192 samples.
- A significant relationship was observed between the prevalence of airborne fungi with sampling season and direction.
- During spring, mean fungal colony concentration was maximized and minimized at the west (256.18 CFU/m^3) and east directions of the center (113.36 CFU/m^3), respectively.
- The highest and lowest fungal colony concentration in winter was respectively related to the north (150.17 CFU/m^3) and east directions (76.56 CFU/m^3).
- According to the considerations and wind direction (towards the west and northwest), the results approved that wind direction directly affects the amount of fungal aerosol dispersion. The maximum emission of fungal aerosols around the landfill of Karaj city was obtained during spring due to temperature (warm and cool seasons).
- The most common identified fungi included *Penicillium* (at the west) and *Cladosporium* (at the north) with mean concentrations of 84.66 and 64.78 CFU/m^3, respectively. Also, the highest frequency of identified fungal aerosol taxa belonged to *Penicillium* (43.67%) and *Cladosporium* (33.54), respectively.
- The environmental factors such as wind direction and speed, relative humidity, and temperature played a fundamental role in prevailing airborne fungi.
- The results indicated that 84.56% of isolated fungal taxa were allergens, while 15.44% were non-allergens.

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Conflict of interest

The authors declare that there is no conflict of interest.

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