

# Identification of airborne fungi's concentrations in indoor and outdoor air of municipal wastewater treatment plant

Nastaran Talepour<sup>1</sup>, Mohammad Sadegh Hassanvand<sup>2,3</sup>, Effat Abbasi-Montazeri<sup>4</sup>, Seyed Mahmoud Latifi<sup>5</sup>,  
Neamat Jaafarzadeh Haghighi Fard<sup>6,7\*</sup>, Bamshad Shenavar<sup>8</sup>

<sup>1</sup>Department of Environmental Health Engineering, Health Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>2</sup>Center for Air Pollution Research (CAPR), Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, Iran

<sup>3</sup>Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

<sup>4</sup>Department of Microbiology, School of Medicine & Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>5</sup>Diabetes Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>6</sup>Environmental Technology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>7</sup>Department of Environmental Health Engineering, Health Faculty, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>8</sup>Department of Environment, Faculty of Agriculture and Natural Resource, Science and Research Branch, Islamic Azad University, Ahvaz, Iran

## Abstract

**Background:** Airborne fungi play an important role the quality of indoor and outdoor air. The aim of this study was to investigate the concentration of airborne fungi around different parts of the wastewater treatment plant (WWTP) in the west of Ahvaz and its relationship with some important meteorological parameters.

**Methods:** This study was conducted on a WWTP in the west of Ahvaz during 4 months in both cold and warm seasons. Samples were collected from 2- and 10-m intervals of the grit chamber (GCh), primary sludge dewatering basin (PSDB), and aeration tank (AT), as well as 60 m upstream (US), downstream (DS), and administrative building (AB) of the WWTP. Bioaerosols were collected using passive sampling method. Temperature, humidity, wind speed, wind direction, and ultraviolet index (UV) were also measured at the time of sampling.

**Results:** The total concentration of fungi was  $46.1 \pm 43$  CFU/plate/h. The highest concentration of fungi ( $92.2 \pm 28.12$  CFU/plate/h) was found 2 m away from the GCh in the cold season and the lowest one ( $24.6 \pm 6.67$  CFU/plate/h) was found at the 10-m distance of the AT in the warm season. The dominant fungal genus included *Cladosporium*, *Alternaria*, *Yeast*, and *Aspergillus*, respectively. Also, there was a significant relationship between the degree of fungal contamination in different units, the US and DS of the WWTP, and there was a negative relationship between the release of fungi and UV.

**Conclusion:** According to the results, grit chamber plays an important role in releasing airborne fungi. Also, airborne fungi were reduced with an increase in distance and UV.

**Keywords:** Bioaerosols, Fungi, Meteorological parameters, Wastewater treatment plant

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## \*Correspondence to:

Neamat Jaafarzadeh Haghighi Fard

Email: n.jaafarzade@yahoo.com

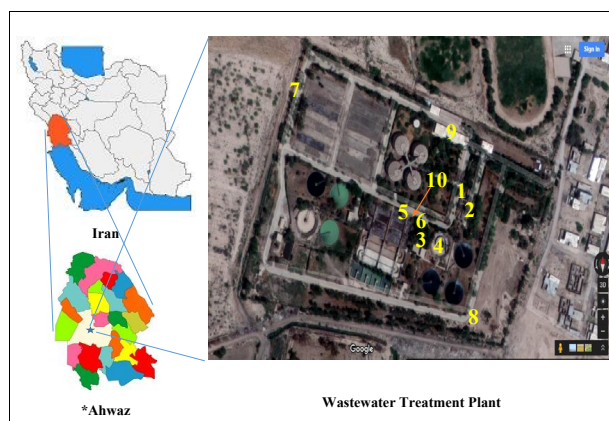
## Introduction

The effect of airborne microorganisms on the human health is a serious issue and their concentration is related to the spread of human diseases (1). Consequently, controlling the concentration and capability of bioaerosols in the contaminated environments is important especially for protecting people's health (2). Overall, in recent years, extensive studies have indicated the relationship

between particle concentrations and mortality, respiratory diseases, cardiovascular diseases, and other disorders (3–6). Bioaerosols can be solid particles, liquid or volatile compounds (7). The atmospheric aerosols can exist in the non-living particles form (antigenic particles, dead skin cells, dandruff, and patches of insects and plants) or the living particles form (bacteria, fungi, viruses, etc) (8,9). Nowadays, approximately more than 2000 fungal species



that have not been infectious to human beings are among the opportunistic pathogens, which are progressively increasing (10). The size of the fungi ranges from 1 to 30  $\mu\text{m}$  (11). Many microorganisms' resources are human activities including wastewater treatment plants (WWTPs) (12). WWTP can affect the health condition in different ways, depending on the capacity of the WWTP, technology, and treatment methods (13,14). A specific disease called "wastewater workers syndrome" caused by bioaerosols has been identified among WWTP workers. The symptoms of this disease are weakness, fever, acute rhinitis, and gastrointestinal disorders (15,16). One of the most important health problems caused by the exposure to aerosols is respiratory complications and poor performance (17,18). Exposure to airborne fungi increases the risk of various morbidities, such as allergic diseases, asthma, rhinitis, conjunctivitis, eczema, and decreased lung function (19). Niazi et al found that primary treatment had the highest impact on fungal concentration (14). Also, Fathi et al reported that the highest fungal concentration was in the downwind of the aeration basins, and observed a significant correlation between fungal concentrations inside and outside the plant (16). Kermani et al reported high fungal concentrations around the grit chamber basin (20). The potential risk of bioaerosols depends on the meteorological parameters, the type of exposure, and the response to individual immunology (21). The impact of pollutants on the human health can be due to the chemical and biological composition of atmospheric particles, where the role of biological particles is significant. Considering the effects of fungal aerosols on the health of WWTPs workers and those who lives around it, and the role of WWTPs in producing fungal aerosols, the concentration of airborne fungi around grit chamber (GCh), primary sludge dewatering basin (PSDB), and aeration tank (AT) of Ahvaz WWTP and its relationship with some of the important meteorological parameters were investigated in this study. This study can increase public awareness for a better understanding of air quality in the WWTPs in regions with similar treatment systems and climatic conditions.



**Figure 1.** Geographical location and sampling points of the WWTP in the west of Ahvaz; (1) 2 m away from the GCH; (2) 10 m away from the GCH; (3) 2 m away from the PSDB; (4) 10 m away from the PSDB; (5) 2 m away from the AT; (6) 10 m away from the AT; (7) 60 m away from the US of dominant wind blowing at the site; (8) 60 m away from the DS of dominant wind blowing at the site; (9) AB; and (10) 6 m meters away from the AT.

## Materials and Methods

### Study area

This study was conducted on the main WWTP in Ahvaz, during both cold (January and February) and warm (June and July) seasons in 2018. Ahvaz is a region with an arid and warm climate (4-48°C). The geographic location of the WWTP (31° 16' N, 48° 36' E) and sampling points are illustrated in Figure 1. The average operational flow to the plant was 600 L/s, and the treatment system was a conventional activated sludge. Details of the WWTP are described in Table 1.

### Sampling procedure

Sampling was performed every 12 days in 10 cm plates according to the Environmental Protection Agency (EPA) schedule in 2018 (22). Sampling was also accomplished using passive sampling method and according to the microbiological sampling index of the air, which is known as the 1/1/1 standard (at 1-meter height and a 1-meter distance from the walls and sources for 1 hour) (23,24). Passive sampling feasibly is the most available, economic, and unobtrusive method of bioaerosol sampling and relies

**Table 1.** Details of the wastewater treatment plant

Units	Number	Dimension		
Screening	3		Width 1.5 m	
Grit chamber	2	Diameter 3 m	-	Volume 25.4 m <sup>3</sup>
Primary sedimentation tanks	4	Diameter 3 m	Depth 2.45 m	-
Aeration tank	4		Volume 2914 m <sup>3</sup>	
Secondary sedimentation tanks	4	Diameter 2 m	Depth 2.1 m	Volume 1385 m <sup>3</sup>
Chlorine contact basin	2		Volume 480 m <sup>3</sup>	
Sludge digestion tanks	4	Diameter 20 m	Depth 10.5 m	Volume 4350 m <sup>3</sup>
Drying sludge bed	8		Volume 9120 m <sup>3</sup>	
Gas tank	1	Diameter 9.15 m	Depth 4.8 m	Volume 250 m <sup>3</sup>

**Table 2.** Location/sampling points

Location/Sampling Points	Description
1	2 m away from the grit chamber tank
2	10 m away from the grit chamber tank
3	2 m away from the primary sludge dewatering basin
4	10 m away from the primary sludge dewatering basin
5	2 m away from the aeration tank
6	10 m away from the aeration tank
7	60 m away from the upstream of dominant wind blowing at the site
8	60 m away from the downstream of dominant wind blowing at the site
9	Administrative building
10	6 m away from the aeration tank

**Table 3.** Number of the fungi samples during the study

Season	Cold										Warm									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Sampling points	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Number of fungi samples	10	10	10	10	10	10	10	10	10	12	10	10	10	10	10	10	10	10	10	12
Total number of fungi samples	102										102									

on particles settling employing gravity, on a collection substrate holed in a settling plate (25). As shown in Table 2, the sampling was performed at the same time from 9 points, considering the location of the unit of the WWTP in the west of Ahvaz. As presented in Table 3, a total of 204 samples were taken. Samples were taken to examine the hourly changes of the fungal concentration in the air around the aeration unit at a distance of 6 m from the aeration unit, in both cold and warm seasons, during 12 hours and every 2 hours. Sampling was randomized from 8 AM to 6 PM. In this study, meteorological parameters including temperature, relative humidity, wind speed, and ultraviolet index (UV) were measured using AccuWeather software (26).

#### Identification of airborne fungi

The specific culture medium for fungi was Sabouraud's dextrose agar, which was used as chloramphenicol antibiotic (100 mg/L) to inhibit bacterial growth in the fungal culture medium (27-30). After each sampling stage, the samples were transferred to the laboratory in zipped bags with a cold box within less than 4 hours (24). The fungal culture medium was placed in an incubator at 25 to 30°C for 72-96 hours whereby colony counting was performed (31,32). The results of measuring the microbial parameters were reported as CFU/plate/h (the colony formation unit per plate per hour). Using typical laboratory methods (slide culture) and macroscopic and microscopic characteristics (under a light microscope with 100×400 magnification), the fungal genus was determined (14,33,34).

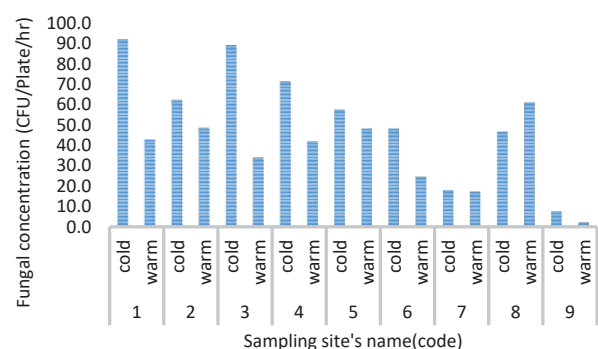
#### Statistical analysis

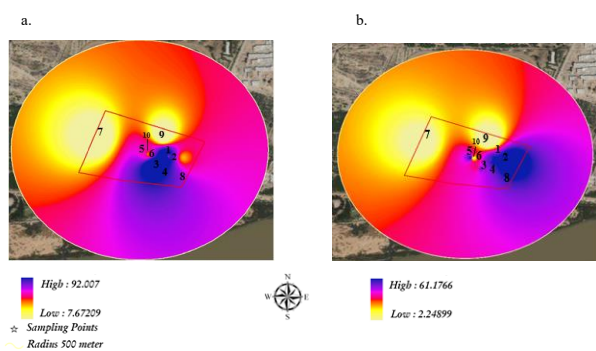
The findings were analyzed using Excel software and SPSS 22. Kolmogorov-Smirnov test was performed to determine the normality of the data (14). Also, the Kruskal-Wallis test was employed to compare the mean concentration of fungi among the units during the study (35). Spearman correlation coefficient was used to find the correlation between fungal and meteorological parameters. Statistical significant values were considered at  $P < 0.05$  (36).

#### Results

##### Fungal aerosols concentrations at different sampling points

Figure 2 shows the mean concentration of fungal aerosols (CFU/plate/h) in different sampling points during both cold and warm seasons. As shown in this figure, the

**Figure 2.** Mean concentration of fungal aerosols (CFU/plate/h) in different sampling points in different seasons



**Figure 3.** Spatial distributions of fungal aerosols concentration across the wastewater treatment plant in different sampling points during both (a) cold and (b) warm seasons (Redline delineates the border of wastewater treatment plant)

highest and lowest concentrations of fungi are related to the distance of 2 m from grit chamber with an average concentration of  $92.2 \pm 28.12$  CFU/plate/h and a distance of 10 m from the AT with an average concentration of  $24.6 \pm 6.67$  CFU/plate/h, respectively. Also, the mean concentration of fungal aerosols (CFU/plate/h) was higher during cold seasons than warm seasons ( $P < 0.05$ ). The spatial distributions of fungal aerosols concentration across the WWTP during both cold and warm seasons are illustrated in Figure 3. The results revealed a significant difference between the fungal concentration (CFU/plate/h) in the office building and ambient air in the WWTP ( $P < 0.05$ ). According to the results, the fungal concentration in the office indoor air was less than that in the ambient air. The results indicated that there was a statistically significant difference between the degree of fungal contamination in different units, and upstream and downstream of the WWTP ( $P < 0.05$ ). Furthermore,

the findings indicated that the highest amounts of fungal aerosols were released in the early morning.

**The relationship between meteorological parameters and fungal aerosol concentrations**

During the study, temperature, relative humidity, wind speed, and UV index were within the range of 7.14 to 41°C, 10.78 to 55.47%, 3.82 to 15.18 km/h, and 1.7 to 6, respectively. The average amounts of these parameters are presented in Table 4. The highest and lowest amounts of fungal aerosols were released in the cold and warm seasons, respectively. In both seasons, *Cladosporium* was known as the dominant genus. *Cladosporium* has a high heat growth domain and can grow even at -8°C (20). As shown in Table 5, there was no significant correlation between the release of fungi and temperature, humidity, or wind speed. However, there was a significant negative correlation between the total fungal concentration and UV index ( $P < 0.05$ ).

**Isolated fungi genus**

In this study, at least 17 species of fungi were isolated. *Cladosporium* (39.23%) was the most common fungal genus in the ambient sampling points. It was followed by *Alternaria* (19.87%), *Yeast* (12.45%), and *Aspergillus* (11.79%). The dominant genus in the administrative building was also *Cladosporium*, which was 14.77% more than the fungus inside the building. The percentages of airborne fungal genera during the study are presented in Figure 4.

**Discussion**

Urban wastewater entering the WWTP involves large amounts of pathogens, such as viruses, bacteria, and fungi,

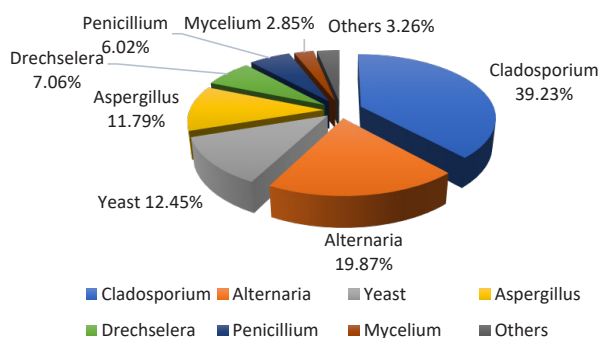
**Table 4.** Descriptive statistics of meteorological parameters during the study

Meteorological Parameters	Temperature (°C)		Wind Speed (km/h)		Ultraviolet Index		Humidity (%)	
Range	(14.7-41)		(3.8-15.2)		(1.7-6)		(10.8-55.5)	
Season	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Maximum	23.0	46.0	4.0	26.0	3.0	12.0	81.0	18.0
Minimum	7.0	33.0	3.0	6.0	1.0	2.0	17.0	5.0
Mean	14.7	41.0	3.8	15.2	1.7	6.0	55.5	10.8
SD	0.9	0.7	0.1	1.1	0.1	0.6	3.2	0.6

**Table 5.** Correlation between the meteorological parameters and the total fungal concentration (CFU/plate/h) during the study

	Temperature (°C)	Wind Speed (km/h)	Ultraviolet Index	Humidity (%)	Total Fungal
Temperature (°C)	1				
Wind speed (km/h)	0.856**	1			
Ultraviolet Index	0.747**	0.462**	1		
Humidity (%)	-0.968**	-0.842**	-0.745**	1	
Total fungal	-0.113	-0.159	-0.236*	0.082	1

Significant: \*\* $P < 0.01$  and \* $P < 0.05$



**Figure 4.** Percentages of airborne fungal genera during the study.

which can easily enter the air through several units and processes (16,37,38). To the best of our knowledge, there are no obvious existing in other countries, however, some countries have published occupational limit values and suggested them as a point of starting an assessment (39). Several studies have suggested that the structure, size, concentration, and microbial population of bioaerosols is affected by the source of production, the mechanism of air diffusion, and in general, the weather conditions, such as temperature, humidity, wind speed, and UV index (12-14). Shams-Ghahfarokhi et al found 24 different fungal genera, where the most frequently isolated fungi included *Aspergillus* (31.1%), *Cladosporium* (22.1%), *Penicillium* (13.8%), and *Alternaria* (22.2%), respectively (40). Also, Ding et al reported *Penicillium* spp. and *Aspergillus* spp. as the most frequent fungi in the WWTP with an oxidation ditch system in summer (41). In this study, the results demonstrated that among the selected units, the highest concentrations of fungi were observed in the grit chamber unit. This can be due to the aeration system, bubble formation, and grit chamber geographic location, which is located at the downstream of the dominant wind direction in Ahvaz. Breza-Boruta and Paluszak studied the role of water treatment units in the microbiological composition of bioaerosols and found that the grit chamber unit is the most important source of bioaerosols (42). Li et al examined the release of bioaerosols from the WWTP using the

Orbal oxidation ditch process. They found that the highest levels of fungal contamination caused by the grit chamber unit (43). Kermani et al found that microorganisms scatter in the air mainly through mechanical treatment such as moving sewage or during biological treatment such as aeration (20). In the present study, there was no significant correlation between the release of fungi and temperature, humidity, and wind speed. However, there was a significant negative correlation between the release of fungi and UV index. The largest amount of fungal aerosols was released in the early morning due to the low UV index. Katsivela et al found no significant relationship between the fungi and temperature. This suggests that the lack of relationship between temperature and fungi may be due to the temperature changes affected by another meteorological parameter (44). Furthermore, the air microbial and chemical composition of each region and particle concentration are important. Several studies have suggested that the changes in microbial concentration are affected by various meteorological parameters, chemical and microbial composition of each region, and particle concentration (45-47). Some studies have indicated that microbial particles in different sizes were not equally affected by climatic conditions. This can be attributed to the existence of microbial particles in different sizes, different species, as well as in different phases of growth (44,48,49). In some studies, fungal particles with 0.65-1.1  $\mu\text{m}$  diameters had a positive correlation with wind speed and negative correlation with the UV index (50,51). Almeida et al found that temperature and wind speed had a positive correlation with the release of *Alternaria* spp. and *Cladosporium* spp. and negative correlation with humidity (52). In this study, the greatest release of fungal aerosols occurred in the cold season while the minimum release occurred in the warm season, which is consistent with the results of studies by Pasquarella et al (2) and Niazi et al (14). In contrast, Fathi et al observed a high concentration of airborne fungi in summer (16). Also, Xie et al reported that the concentrations of airborne contamination were high in winter, fall, spring, and summer, respectively.

**Table 6.** Performance comparison between some studies and this study

Effective Meteorological Parameter	Treatment System	Effective Unit	Season	Temperature (°C)	Humidity	Dominant Fungi Genera	Reference
Relative humidity	Activated sludge	Primary sedimentation unit	Summer	7-30	29.4-50.4	<i>Penicillium</i> spp. and <i>Cladosporium</i> spp.	14
Temperature, humidity, wind speed, and UV	Activated sludge	Grit chamber	Winter	3-34	11-49	<i>Cladosporium</i> spp. and <i>Alternaria</i> spp.	19
Nothing	Activated sludge	Aeration tank	Summer	9-23.6	27-55.5	<i>Dematiaceous</i>	16
High temperature and relative humidity	Oxidation ditch	-	Summer	11-27.4	17-80	<i>Penicillium</i> spp. and <i>Aspergillus</i> spp.	41
UV	Activated sludge	Grit chamber	Winter	14.7-41	10.8-55.5	<i>Cladosporium</i> spp. and <i>Alternaria</i> spp.	This study

They also found that snowfall reduces the level of airborne microbial contamination (53). Comparative analysis of the results of this study and some studies is illustrated in Table 6. High relative humidity along with low temperature leads to the formation of bioaerosols in winter (54). Due to the ability of fungi to being adapted with many environmental conditions, fungi easily endanger people's life with disabilities and immune deficiencies and nowadays are considered as one of the most important causes of death (10). Exposure to high concentrations of airborne fungal spores is associated with allergic rhinitis and asthma. Besides, the total number of fungal spores, *Basidiospores*, and *Cladosporium* was a risk factor in these findings. Fungal spores, such as *Cladosporium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, and *Basidiospores* are critical factors in the development of allergic diseases. Allergic rhinitis and asthma are more frequent in fall and winter. The exposure of wastewater treatment workers to bioaerosols causes an extensive range of health effects such as infectious diseases, chronic obstructive pulmonary disease, acute toxic effects, inflammation, allergic diseases, fetal harm, and cancer (19). Carducci et al analyzed the quantitative microbial risk assessment for workers exposed to bioaerosols in WWTPs. They found that these effects were extended along with increasing the concentration of contamination and exposure time (55).

### Conclusion

This study examined the concentration of airborne fungi around grit chamber, PSDB, and AT, as well as administrative buildings (where people spend 80% of their time), upstream and downstream of the wind direction of the WWTP in the West of Ahvaz and its relationship with meteorological parameters. According to the results, the WWTP released significant amounts of fungal spores. As the residential houses are located downstream of the WWTP and dominant winds of Ahvaz, therefore, WWTP workers and the people who live near the WWTP were exposed to high concentrations of aerosols, which threaten their health condition. It seems that the assessment of occupational risk factors in the WWTP, the development of occupational safety and health practices, especially on risk assessment, health monitoring, fitness, immunization, and preventive measures in water supply facilities, benchmarking, and participation in investigating the biological risk factors can be effective in reducing the harmful effects of bioaerosols. Also, using double-glazed windows and doors, as well as using air conditioners, can be considered as other ways for preventing the harmful effects of bioaerosols in the indoor environment.

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### Ethical issues

The authors certify that this manuscript is the original work of the authors, all data collected during the study are presented in the manuscript, and no data from the study has been or will be published separately.

### Competing interests

The authors declare that they have no conflict of interests.

### Authors' contributions

All authors contributed and participated equally in the data collection, analysis, and interpretation. All authors critically reviewed, refined, and approved the manuscript.

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