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Identification allergen Cladosporium spores concentration in the ambient of Ahvaz wastewater treatment plant and their relationship with meteorological variables, Ahvaz, Iran

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ABSTRACT:

Introduction: Airborne Cladosporium spores in different regions of the world are known as the main cause of allergic diseases. This study aimed to identify the Cladosporium species airborne fungi in Ahvaz wastewater treatment plant area and its adjacent places and check the effect of some meteorological parameters on their emissions.

Materials and methods: Cladosporium spores were cultured on Sabouraud`s dextrose agar (SDA) medium in both cold and warm seasons. The passive sampling method was performed and after incubation, colonies were counted as CFU/Plate/h. Then, according to the macroscopic and microscopic characteristics of the genus, the fungal was studied. The meteorological parameters including temperature, humidity, air pressure, dew point, wind speed, and ultraviolet index were measured.

Results: At least, 3358 colonies were counted. 1433 colonies were related to the Cladosporium species. The amount of Cladosporium in indoor air was 46% of the total Cladosporium. The average of meteorological parameters includes temperature, humidity, air pressure, dew point, wind speed and UV index during the study were 27.8 °C, 32.9%, 548.7 °Kpa, 3.6°, 9.1 km / h and 3.9 respectively. 42.6% of the total number of colonies was related to the Cladosporium species. Cladospiromes had a direct correlation with the dew point, temperature, humidity, air pressure, wind speed, and ultraviolet index ($P<0.05$). Primary sludge dewatering has the greatest role in the Cladosporium spores emission.

Conclusion: Considering the importance of Cladosporium spores in the appearance of allergic diseases, and given that wastewater treatment workers spend most of their time outside, observing health and preventive measures is necessary in this regard.

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Introduction

The impact of wastewater treatment plants has been highlighted in recent centuries. Pollutants can enter the air through sewage droplets created during the treatment process [1]. Bioaerosols enter the human body through disparate way inhalation, ingestion or absorption into the human body and cause various health effects including contagious diseases, acute toxic effects, allergies, and cancers [2]. The combination, size, and microbial contamination are dependent on the source of production, the mechanism of airborne emission, and most importantly the prevailing local conditions. There is a thin layer of moisture around the bioaerosols generated from water sources. Bioaerosols are affected by Brownian motion, gravity, electrical forces, thermal gradient, electromagnetic radiation, turbulent diffusion, inertial forces, oxygen concentration, and relative humidity; Bioaerosols reactions to these forces depend on their physical properties, such as size, shape, and also on biological factors such as the type of organism and the growth phase. Bioaerosols including bacteria, fungal spores, broken parts of cells and derived products of microbial metabolism [3, 4]. The results of studies show that the prevalence of fungal allergies worldwide is between 3-10% [5, 6]. For these reasons, the study on fungal spores was formed [7, 8]. The fungal spore's aerodynamic diameter is 1- 30 micrometers [9, 10]. The concentration of airborne fungal spores has been related to humidity, wind, rainfall, temperature, altitude, and vegetation [11]. The efficacy of some fungal spores in allergic diseases like asthma and allergic rhinitis are well known. Cladosporium is one of the most common fungal that is found in the air, water, soil, and degenerate plants. Cladosporium develops a disease in people with immune deficiency and has a high ability to stimulate allergic reactions in sensitive individuals [12]. Also, these diseases, cause to reducing the life quality and increasing health care costs. Exposure to contaminated air causes an increase in mortality rates through diseases such as asthma, rhinitis, and loss of lung function [13]. Considering the importance of the issue and role of Cladosporium fungi on the health of wastewater treatment plant workers and who lives around it, this study can help physicians and community health authorities to improve health conditions and thus improve the health status of exposed Cladosporium fungi.

Materials and methods

This descriptive-analytic study was conducted in the Ahvaz wastewater treatment plant's area and adjacent places which are located in the southwest of Iran. It lies on latitude 31 º16' N and longitude 48 º36 E '. The study was carried out through both cold (January and February) and warm (Jun and July) periods in 2018. This wastewater treatment plant operated in a conventional activated sludge method. Sampling was performed according to the EPA schedule in 2018 every 12 days in 10 cm plates [14]. Sampling was carried out through the passive method according to the microbiological sampling index of the air, which is known as 1/1/1 standard (at 1 m height and a 1 m distance from the walls and sources for 1 h) [15]. In this study, the sampling was performed from 9 points including two points located 60 m away from the upstream and downstream of local dominant wind blowing, two points with a distance of 2 and 10 m away from the grit chamber, two points 2 and 10 m away from the primary sludge dewatering basin, two points 2 and 10 m away from the aeration tank, and one point from indoor air of administrative building. A total of 160 samples were taken in this study. The sample was randomly taken from 8 am to 6 pm. During

the study, the meteorological parameters including temperature, air pressure, dew point, relative humidity, wind speed, and UV index were taken from AccuWeather [16]. The specific culture medium for fungal was Sabouraud`s dextrose agar (SDA), in which chloramphenicol was added (100 mg/L) to inhibit the growth of bacteria in the fungal culture medium [17]. After each sampling step, samples were transferred to the laboratory in zip bags, with a cold box in less than 4 h [14]. The fungal were incubate for 72-96 h at 25-30 °C [10]. After that, colony counting was performed. Results reported as CFU/Plate/h (meaning the colony formation unit per plate per hour) the fungal genera were identified under a light microscope at 100×400 magnification [18, 19]

Statistical methods

The findings were analyzed using Excel and Spss version 22. One sample Kolmogorov-Smirnov test was performed to determine the normal distribution of the data. Then, the Pearson correlation coefficient was used to find the correlation between Cladosporium fungal detected, and meteorological parameters. Also, the Kruskal-Wallis test was applied to compare the mean concentration of Cladosporium fungal among the units during the whole time of the study [20] P-values of less than 0.05 were considered to be statistically significant.

Results and discussion

As depicted in Fig 1. Cladosporium spores (42.6%) were the most dominant genus during this study. In a study in Tehran's wastewater treatment plant, Cladosporium was the dominant fungal species, too. Also, the researchers have found a significant correlation between fungal concentration and temperature, relative humidity, wind speed and UV index [21].

Fig. 2 presents the mean concentrations of Cladosporium spores according to CFU/Plate/h in different sampling points. As shown in this chart, the highest and lowest concentrations of Cladosporium were related to the primary sludge dewatering basin and aeration tank with an average emission of 30.3 and 16.4 CFU/Plate/h, respectively. The statistical tests showed a significant difference between the concentration of Cladosporium as CFU/Plate/h in the office building and ambient

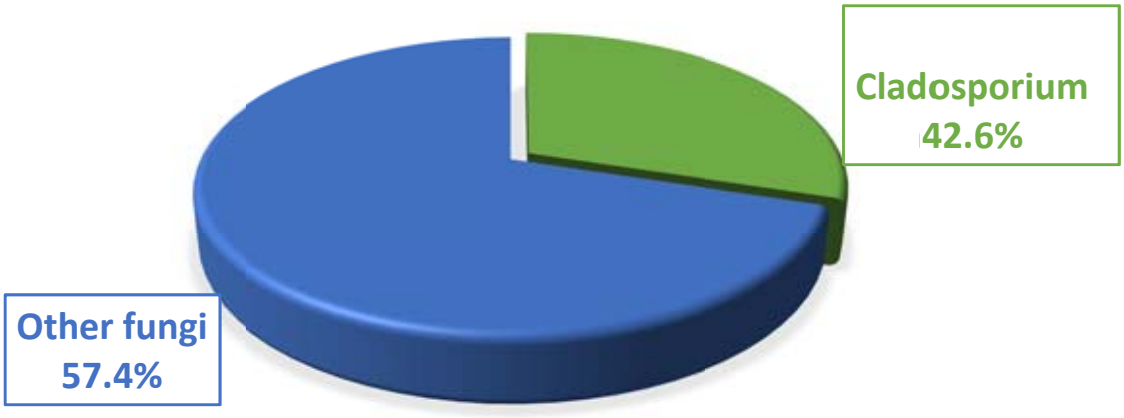

Fig. 1. The total Cladosporium concentration during the study

of wastewater treatment plant. (Mann-Whitney U test, P <0.05). The Cladosporium ratio of the indoor environment was 2.29%. Other researchers conducted a study on the amount of fungal contamination in the South Tehran wastewater treatment plant. Their results showed that the primary sedimentation tank had the greatest impact on fungal released. Also, they found that there was a direct relationship between the concentration of fungi and air humidity [10]. In a study in Beijing bioaerosol emissions from a wastewater treatment plant were investigated. Researchers selected 12 points from upstream and downstream of wind direction for Sampling. They found that fungal aerosols (more than 930 CFU/Plate/h) had the highest concentrations in the sludge dewatering basin [22].In different points of sampling during the study, temperature, air pressure, dew point, relative humidity, wind speed, and UV index was in the range of 14.8-40.8 °C, 101.7- 995.8 kPa, 3.4-3.9 °, 11.2-54.6 %, 3.8-14.4 km/h and 1.8-6. The average of these parameters is shown in Table 1.

Fig. 2. Mean concentration of Cladosporium spores (CFU /Plate/h) in different sampling points

Table 1. Meteorological parameters during the study

Meteorological Parameters Season	Temperature (c°)	Wind speed (km/h)	Air pressure (Kpa)	UV index	Humidity (%)	Dew point (˚)
Cold	14.8	3.8	101.7	1.8	54.6	3.9
Warm	40.8	14.4	995.8	6	11.2	3.4

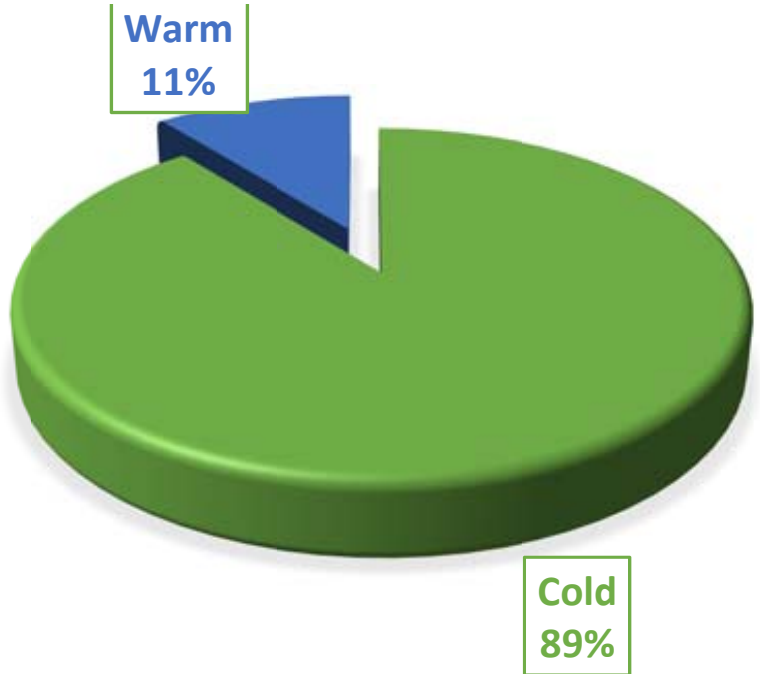

Fig. 3. Percentages of Cladosporium during the study

Table 2. Correlation between the meteorological parameters and Cladosporium concentration (CFU/plate/h) during the study

	Temperature (˚C)	Air pressure (Kpa)	Wind speed (km/h)	UV index	Humidity (%)	Dew point (˚)	Cladosporium
Temperature (˚C)	1						
Air pressure (Kpa)	.855**	1					
Wind speed (km/h)	.856**	.856**	1				
UV index	.747**	.747**	.410**	1			
Humidity(%)	-.968**	-.825**	-.842**	-.745**	1		
Dew point (˚)	-.475**	-.513**	-.535**	-.325**	.559**	1	
Cladosporium	-.476**	-.756**	-.611**	-.491**	-.424**	.383**	1
significant: **P < 0.01 and*P < 0.05							

As shown in Fig3 . The highest emission of Cladosporium aerosols was observed in the cold season (89%) and the lowest emission was observed in the warm season (11%). As shown in Table 2. in this study, there was a direct correlation between the amount of airborne Cladosporium emission and dew point. Also, there was a significant negative correlation between Cladosporium airborne fungal density with temperature, air pressure, wind speed, humidity, and UV index ($P < 0.05$). In a study in Turkey researchers showed that there was a significant direct correlation between the rate of Cladosporium spores' emission with wind speed and temperature [23]. In a study in Zagreb researchers studied the relationship between Cladosporium concentrations with meteorological parameters since 2002 for one year. They showed that there is a strong correlation between the concentration of Cladosporium and temperature [24]. In a study in Pollen the concentration of Cladosporium spores at three stations examined and there was a significant correlation between the amount of Cladosporium and temperature, air pressure and relative humidity [25]. In a study in Porto and Amritsar, there was a positive correlation between Cladosporium concentration and temperature and the negative correlation between Cladosporium concentration and air humidity and rainfall [26]. Also, researchers in another study in Saudi Arabia demonstrated that temperature and humidity were more effective than other meteorological parameters on airborne fungal [27]. In a study in Grenada the Spearman correlation statistical method used to determine the correlation between meteorological parameters and fungal concentrations. Researchers showed that there is a significant relationship between Cladosporium concentration and temperature and hours of sunlight exposure [28].

Conclusion

Some studies have found a close relationship between the seasonal patterns of airborne disease and the pattern of asthma. Researches around the world show the important and effective role of fungal spores in allergic patients. In many parts of the world, Cladosporium and Alternaria are known as the most important air pollution factors, allergic bioaerosols and they are considered clinically the causative allergenic agents for most patients [29] The high concentration of Cladosporium spore causes an increase in the prevalence of asthma and chronic bronchitis [30]. The findings of this study can be helpful for doctors and allergy specialists to diagnose, trace and treat patients with a respiratory system with the origin of Cladosporium.

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Competing interests

The authors declare that there are no competing interests.

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Ethical considerations

Ethical issues have been completely observed by the authors.

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