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Assessment of Seasonal Variation of Microbial Aerosol and Gaseous Emissions from Wastewater Treatment Plant of Morche Khort Industrial Town

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ABSTRACT

Introduction: Wastewater contains a large number of pathogenic and non-pathogenic microorganisms that can become bioaerosols during the treatment processes in different units of the treatment plant, and pose a risk to workers and nearby residents. In this study, the release of bioaerosols from a wastewater treatment plant of Morche Khort Industrial Town was investigated.

Materials and Methods: Sampling was actively performed according to EPA standard, in two seasons of spring and summer in 2018-2019, by an environmental sampling pump with a flow rate of 15 l/min and a single-stage impactor. The data were analyzed by SPSS software version 2020.

Results: The study results showed that the mean number of bacteria in the aeration tank was significantly higher than other points. The mean number of fungi in the aeration pond with 144 CFU/m^3 had the highest concentration, and no fungal bioaerosol was observed 500 m downstream. Regarding the emission of H_2S, CH_4, and VOC gases, the anaerobic tank had the highest emission of these gases.

Conclusion: Wastewater treatment plants (WWTPs) with activated sludge treatment technology and an aeration system can lead to an increase in the concentration of bacterial and fungal aerosols around the units and in surrounding areas.

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Introduction

In recent years, air pollution has been recognized as a severe threat to public health in developing countries and is one of the most critical environmental problems in the world [1-3]. According to the World Health Organization (WHO) publications, approximately 92% of the world's population lives in environments with pollution levels exceeding recommended air pollution limits [1, 4].

A special source of microbial air pollution is wastewater treatment plants (WWTPs), which can be considered as potential sources of pathogenic microorganisms in the air [5]. WWTPs can affect environmental health in a variety of ways. These effects depend on the size of the plant, the technology used, and the treatment methods. Wastewater contains a large number of various pathogens, such as viruses, bacteria, fungi, and protozoa, which become airborne aerosol particles during various steps of the wastewater and sludge treatment process, especially aeration and mechanical agitation in WWTPs [6]. The concentration of these airborne microorganisms is related to various parameters, such as equipment in WWTPs, environmental conditions (temperature, humidity, wind speed), and the survival of microorganisms in the air [7].

Reports have shown that airborne bacterial communities of WWTPs had an aerodynamic diameter below 4.7 μm [8]. Therefore, they can easily enter the lungs and cause infections in immune-compromised individuals, and allergic reactions [7]. Bioaerosol is one of the most critical pollutants in WWTPs, which may contain a variety of microorganisms. Bioaerosols, with their wide Distribution range as independent particles and in attachment with other physical and chemical particles in the air, may cause various diseases in people with different levels of health [9, 10]. Some epidemiological studies have reported respiratory infections, gastrointestinal symptoms, hypersensitivity, and allergies among WWTPs staff, due to exposure to airborne microorganisms [11].

In the design and construction of wastewater collection and treatment systems, often no system is considered to control potential odor emissions [12]. However, the odors of wastewater can have significant adverse effects on working conditions in WWTPs, as well as surrounding communities. The problems of odor emission around WWTPs have caused an increase in odor-related complaints, due to a decrease in public tolerance for odors. As a result, strict environmental standards and regulations have been adopted. Odor emissions from WWTPs are characterized by a large number of volatile compounds (mainly sulfur-based and organic) [13]. When volatile compounds from the aqueous phase are transferred to the atmosphere, at each stage of the treatment process, odorous compounds are released from the wastewater [14]. With the degradation of wastewater and sludge in the treatment plants, gases, such as carbon dioxide, ammonia, carbon monoxide, hydrogen sulfide, and methane are released into the air [15].

Numerous scientific reports have shown the relation between the concentrations of particles and gases in air pollution and bioaerosols, and the condition and mortality of the population exposed to air [9]. They can lead to allergies, infections, diseases, and epidemics. Many diseases in humans, animals, and plants are caused by microorganisms in the open air [5]. To ensure the health of workers and people, it is essential to determine the composition and concentration of microorganisms in the air of polluted environments. Input wastewater to WWTPs contains large amounts of pathogens, such as viruses, bacteria, and fungi, which can quickly become airborne through various processes. This study aims to determine the amount of microbial air pollution in the area of wastewater treatment plant, and also its relation with meteorological conditions.

Materials and Methods

Sampling sites

This study was carried out in the wastewater treatment plant of Morche Khort industrial town, located in the northwest of Isfahan, with a geographic location of 51.51 degrees north and 33.05 degrees east. The main characteristics and design parameters of the WWTPs are described in Table 1. Sampling stations were selected according to the wind direction, location of units, and the proximity of workers. The earth map of the WWTPs and the locations of sampling points are shown in Figure 1. The sampling points are described in Table 2.

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Figure 1: Earth map of the wastewater treatment plant

Table 1: Main characteristics and design parameters of the wastewater treatment plant

Number of site workers (person)	**10**
Design flow(m^3/day)	2000
Aeration system	Air diffusion by fine bubble diffusers

Table 2: Description of locations and sampling points

Location/sampling point	**Description**
Pumping station (A)	-
Common corridor Screening and Neutralization tank (B)	-
Aerobic tank (C)	-
Anaerobic tank (D)	-
Background (E)	Distance of 100 m upstream of the wastewater treatment plant
Background (F)	Distance of 100 m of downstream of the wastewater treatment plant
Background (G)	Distance of 500 m of downstream of the wastewater treatment plant
Background (H)	Indoor air (Official building)

Sampling methods

Sampling was conducted in both warm and cold seasons, from December 2018 to May 2019. It was performed according to EPA standard for 3 minutes, using active method with peripheral sampling pump (SKC BioStage, America) with a flow rate of 15 l/min and single-stage impactor (SKC BioStage, America), once every two weeks and on plate 10 cm at the height of about 1 meter and the distance of 1 meter from the walls. Finally, during the two seasons, 192 bacterial samples and 192 fungal samples were collected.

Identification of bacterial and fungal aerosols

The culture medium used to identify bacterial samples was tryptic agar, and Saburo dextrose agar was used for fungal samples. Also, cycloheximide ($C_{15}H_{23}NO_4$) was used to prevent the growth of fungi in the tryptic agar medium, and chloramphenicol was used for the culture medium of Sabouraud agar. Then, to identify the bacteria, the collected samples were transferred to the laboratory and placed in an incubator at 35 °C for 24 to 48 hours. Tryptic Soy Agar (TSA) culture medium containing cycloheximide was used for bacterial growth, and malt extract agar and Dichloran-Glycerol (DG18) Agar were used for fungal growth. Also, the fungus culture medium was placed at room temperature (20-25 °C) for 3 to 7 days. Duplicate cultures were performed on each medium, and after the incubation time, the bacterial and fungal concentrations were measured

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and reported in terms of CFU/m^3 [16].

The amount of volatile organic compounds was measured locally by a VOC gas detector (Phocheck tiger, England). Methane and hydrogen sulfide gases were also measured in situ by a portable Multiparameter (England Senko). For each sample, meteorological parameters, such as temperature, and relative humidity were measured with a portable hygrometer and thermometer (1360a made in Taiwan TES), and wind speed was measured with an anemometer.

Statistical analysis

Experimental data were analyzed by the SPSS software version 2020. One-Way ANOVA (analysis of variance) and Tukey's multiple comparisons were used to compare the studied parameters in different parts of the treatment plant. A multiple regression model was used to investigate the relationship between environmental parameters, several bacterial and fungal species, and VOC_s, CH_4, and H_2S.

Ethical issue

This study was authorized by Shahid Sadoughi University of Medical Sciences ethics committee IR.SSU.SPH.REC.1398.072.

Results

Airborne fungal and bacterial concentrations from different units of the treatment plant

According to the obtained results, it can be claimed that each stage of wastewater treatment is associated with the release of bioaerosols into the atmosphere. Released microscopic bacteria and fungi, depending on the release season, can cause lighter or heavier contamination. The concentrations of airborne bacteria in the eight sampling stations are presented in Figure 2. The mean concentration of bacteria (CFU/m^3) was very variable at different sampling points. The mean value of airborne bacteria in location C (aeration tank) was higher than other locations (1040 CFU/m^3).

The concentration of airborne bacteria around the location B (970.90 CFU/m^3), D (725.32 CFU/m^3), A (149.32 CFU/m^3), H (85.42 CFU/m^3), E (32.00 CFU/m^3), and F (31.16 CFU/m^3) ranked in a descending order. The lowest concentration of airborne bacteria was detected in location G (distance of 500 m downstream of the wastewater treatment plant) and tended toward zero. The spread of fungal aerosols based on CFU/m^3 around the sampling units and the background is shown in Figure 3. The highest concentration of fungal aerosols was observed in location C (144 CFU/m^3). The concentration of fungi in other locations included locations E (mean 21.33 CFU/m^3), F (mean 5.33 CFU/m^3), and G (mean 0.00 CFU/m^3), respectively. The lowest fungal concentration was in location G (distance of 500 m downstream of the wastewater treatment plant) with a mean concentration of 0 CFU/m^3. Regarding the explanation of this difference in the number of fungal aerosols among different units, surface turbulence processes, mixing, and stirring in aeration units have the highest emission of bioaerosols.

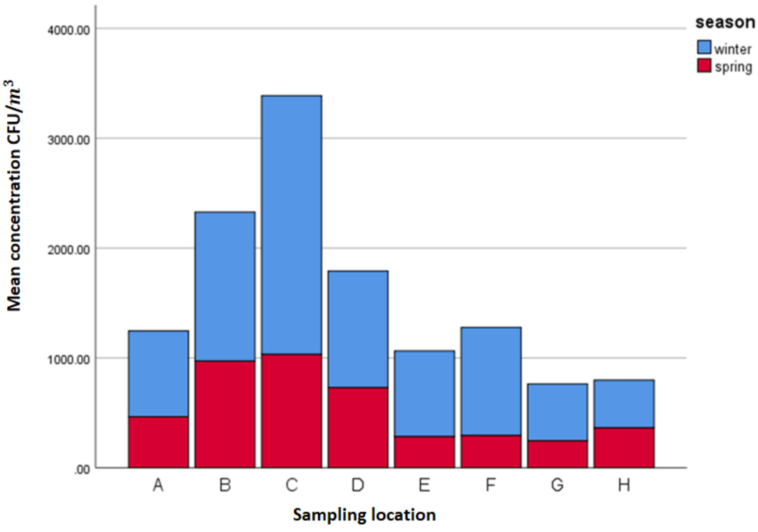

Figure 2: Mean concentration of bacteria at different sampling points of the wastewater treatment plant, measured as CFU/m^3

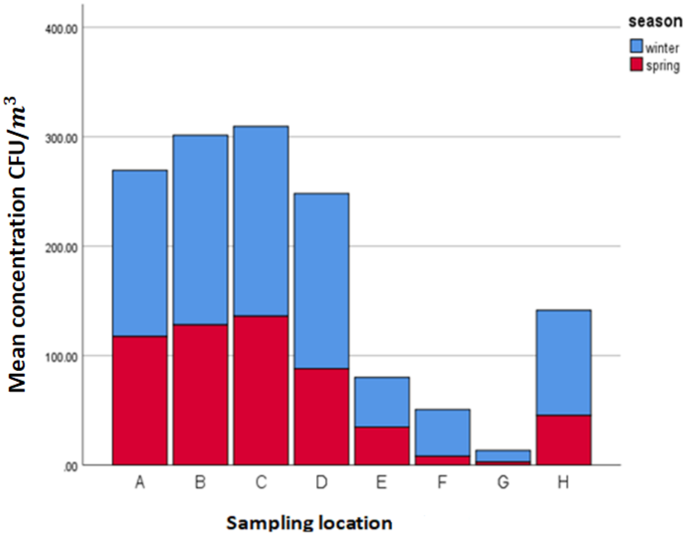

Figure 3: Mean concentration of fungi at different sampling points of the wastewater treatment plant, measured as CFU/m^3

Measurement of gaseous emissions from different units of the treatment plant

According to Table 3, the amount of H_2S gas in the anaerobic point (10.83 ± 3.12) was significantly higher than other points. Based on the obtained results, the mean amount of VOC_s gas in the two investigated seasons in the anaerobic tank (6.17 ± 1) was significantly higher than other points. By increasing the distance from the active points of the treatment plant, the emission of VOC_s gas decreased. In the investigation of the amount of methane gas released from different points of the treatment plant, this gas was the highest at the point of the anaerobic tank, and no measurable amount of this gas was observed in other places.

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Table 3: The mean amount of gases measured in winter and spring at different sampling points

Sampling points	H_2S(mg/m^3)				CH_4(mg/m^3)				VOC(mg/m^3)			
	Spring		Winter		Spring		Winter		Spring		Winter	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pump station	4.50	±1.64	1	±0.63	ND*	ND	ND	ND	6.00	±2.28	2.83	±1.72
Common corridor of screening and neutralization	3.33	±1.36	1	±0.89	ND	ND	ND	ND	5.67	±2.42	2.5	±1.64
Anaerobic tank	22.33	±3.77	10.83	±3.12	18.83	±2.71	ND	±3.20	10.76	±2.42	6.17	±1.47
Aerobic tank	2.17	±0.753	1	±0.894	ND	ND	ND	9.67	0.67	±0.51	ND	ND
100m upstream	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
100m downstream	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
500m downstream	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Indoor air	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

*ND=Not detected

The relationship between measured variables and the concentration of pollutants in various samples

Correlation between meteorological conditions as well as the relationship between meteorological conditions and the concentration of bacteria and fungi on the sampling days were investigated and the results are given in Table 4. The results showed that the number of bacteria and fungi has an inverse relationship with temperature and UV index; by increasing these two factors, the number of bacteria and fungi significantly decreases. In winter, the UV index is lower than in spring, so the conditions for the growth of organisms are better. Also, a significant correlation was seen between bacterial and fungal concentrations and the relative humidity. In contrast, wind speed did not show any significant effect in two seasons regarding the resulting samples.

Table 4: Pearson correlation between environmental parameters with the distribution of bacterial and fungal bioaerosols

Metoleorgical	Fungal bioaerosols number		Bacterial bioaerosols number	
	P-value	R	P-value	R
Wind velocity	0.24	0.085	0.54	-0.045
Moisture	0.02	0.172	< 0.001	0.297
Temperature	0.001	-0.233	< 0.001	-0.440
UV index	< 0.001	-0.268	< 0.001	-0.383

Discussion

Exposure to bioaerosols and gases emitted from municipal WWTPs causes health-related problems for exposed individuals, especially workers. Therefore, to ensure the general health of workers in occupational environments, such as WWTPs, it is essential to determine the composition and concentration of airborne microorganisms in contaminated environments. The usual route of contact with organisms primarily associated with these diseases is through inhalation of pathogenic microorganisms that are deposited in the throat and upper airway and swallowed. Factors, such as the atmospheric factors of the investigated area, the type and degree of wastewater pollution, the manner of wastewater management, and atmospheric and climatic conditions can affect the number and composition of the microflora in the air around the area of WWTPs. Bioaerosol formation can occur due to factors such as mixing and transportation of wastewater, long-term collection and storage of unstable biomass, as well as high temperature [17]. Wastewater aeration processes, including mechanical agitation or bubble diffusers, have been identified as important sources of bioaerosols. In this process, due to the disintegration of the bubbles from the aeration system, enough oxygen is provided to decompose the materials [6]. The particles caused by the collapse of the bubbles fall and turn into smaller particles

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when they hit the sewage, and cause secondary pollution. These particles evaporate quickly in the air, and their diameter decreases.Subsequently, their settling speed decreases, and they remain suspended [18]. A number of studies have shown that the aeration system used in the biological process greatly affects the number of generated bioaerosols [19]. In similar studies, including Niazi et al., Michałkiewicz et al., Oppliger et al., and Dehghani et al., aeration tanks were identified to be the significant point in emitting bioaerosols [17, 20, 21]. However, in the study conducted by Kara et al., the concentration of indicator microorganisms in aeration tanks bioaerosols tended to be zero and could not be detected at the secondary sedimentation tank and the disinfection tanks [11]. Korzniewska et al., in the study of the release of airborne microorganisms in the air with the "BIO-PAK" bioreactor, found that the highest concentration of bacteria (101-103 CFU/m^3) was observed in the air sampled inside the bioreactor in the vicinity of grit chamber [22]. In the study of Talepour et al., the results showed that among the selected units, the highest concentration of fungi was observed in the grit chamber unit, which was attributed to the aeration system, bubble formation, and the geographical location of the grit chamber [23]. In this study, the anaerobic tank showed the highest amount of H_2S gas compared to other studies. In the study by Baawain et al., H_2S emissions from discharge tank facilities were found to be very high (100 ppm) compared to other sources. The high emission rate from this source was due to the emission of H_2S in septic conditions [24]. According to Latos et al., based on the measurement values of H_2S gaseous, the main emission source with the greatest impact on odors was the primary sedimentation tank, which do not agree with the results of the present study [25]. In the study of volatile gases, the amount of these gases was the highest in the anaerobic tank. In the study by Lehtinen et al., they observed the highest concentration of VOCs in the samples of the sludge thickener unit. It was due to the multi disc screw press (MDSP) dewatering system used in the thickener unit, which releases the compounds absorbed into the sludge materials into the air [26]. This result is not consistent with the present study.

Given that methane is a potent greenhouse gas, which can lead to climate changes, measuring and reducing its emissions is necessary to achieve more sustainable management of WWTPs [27]. During anaerobic and anoxic processes in WWTPs, methane can be produced due to the low concentration of dissolved oxygen. The produced gas has very low solubility in water, so it can be released quickly. A relatively higher methane emission rate from anaerobic tanks was observed due to the low concentration of dissolved oxygen (< 0.2 mg/L) during the anaerobic process. In other units, methane emission was not estimated and it can be justified that all methanogens are completely and obligatory anaerobic. So that, the presence of oxygen can leads to no formation and no emission of methane in wastewater. In this research, the low amount of dissolved oxygen in these units was suggested as a reason for their high methane emissions [28]. Huang et al. estimated the highest methane emissions from the sludge thickener unit. The emission of CH_4 from the sludge thickener was also measured to be about 3.5 times more than the secondary sedimentation basin. It was caused by anaerobic conditions at the bottom of the sludge thickener resulting from the activity of anaerobic methane bacteria [29].

Previous studies have shown that many environmental factors affect the ability of microorganisms to survive in the air. The most important of them are relative humidity and temperature. Ultraviolet radiation, oxygen content, specific ions, various pollutants, and air-related factors can also be factored in the loss of biological activity [30, 31]. In general, it has been shown that the relative humidity or water content of the air is of great importance for the survival of microorganisms in the air [31]. Fathi et al. showed a significant relationship between the concentration of aerosol bacteria near the aeration tank, wind speed, and temperature. However, they did not observe any significant relationship between this factor in other sampling points[32]. Oppliger et al. showed that the concentration of fungi could be affected by meteorological parameters [21]. Barza et al. also found a significant relationship between meteorological parameters and the amount and type of bacteria [33]. In another similar study, following the results of this study, temperature showed the highest correlation with the concentration of bacteria. A significant correlation was also observed between the concentration of fungi and relative humidity. Unlike the results of the present study, a significant correlation between the concentration of detected bacteria and relative humidity was observed [20].

Conclusion

The study results indicate that the maximum concentration of bacteria and fungi was found in spring and winter seasons, respectively, in the aerated tank and the anaerobic tank. Measuring the gases emitted from different units of the treatment plant, including H_2S, CH_4, and VOC_s, showed that in winter season, the anaerobic tank had the highest emission of these gases.

Based on these results, WWTPs with activated sludge treatment based on an aeration system can lead to an increase in the concentration of bacteria and fungi aerosols around the units and surrounding areas. Therefore, WWTPs workers and residents of nearby areas may be exposed to higher concentrations of bioaerosols, which may pose many health concerns. Thus, some measures, such as covering the surface of aeration tanks, and changing aeration methods and aeration equipment can be done to reduce the emission of bioaerosols.

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Conflict of interest

The authors declare that there is no conflict of interest.

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