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ASSESSMENT OF BIOAEROSOL CONTAMINATION IN AN URBAN WASTEWATER TREATMENT PLANT IN TEHRAN, IRAN

Majid Kermani[1,2], Anahita Dehghani[3,4], Mahdi Farzadkia[1,5], Farshad Bahrami Asl[6], Dariush Zeinalzadeh[7]*

[1] Research Center for Environmental Health Technology, Iran University of Medical Sciences, Tehran, Iran
[2] Associate Professor of Environmental Health Engineering Department, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[3] Center for Air Pollution Research (CAPR), Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, Iran
[4] MSc of Environmental Health Engineering, School of Public Health, Urmia University of Medical Sciences, Urmia, Iran
[5] Professor of Environmental Health Engineering Department, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[6] PhD student of Environmental Health Engineering Department, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran
[7] MSc of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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CORRESPONDING AUTHOR:

anahita_d66@yahoo.com
Tel: (+98 44) 32752305
Fax: (+98 44) 32770047

ABSTRACT:

Introduction: Recently contact with bioaerosols has been presented as an important problem which endangers human being's health. In this study bioaerosol concentration was measured in wastewater treatment (WWTP) units in west of Tehran.

Materials and methods: Passive sampling was carried out around three process and operational units, in 100 m from last unit of wastewater treatment plant and in administrative building. In total 440 samples were collected. The transport culture medium used for bacterial samples was the tryptic soy agar, and for fungal samples, it was dextrose agar. Sampling was carried out according to the sampling calendar of Environmental Protection Agency (EPA) in 2013 for 1 h every 6 days and in the plates of 9 cm.

Results: The results showed that aerobic digester with an average of 3303 CFU/Plate had the greatest effect on emission of bacterial bioaerosols. In addition grit chamber with an average of 586.3 CFU/Plate had a highest impact on fungal emission. Among the bacteria, Bacillus spp. Staphylococcus spp. and Micrococcus spp. were dominant. The highest emission of bacterial aerosol is in July and the lowest is in March. Furthermore, fungi such as Cladosporium spp. Penicillium spp. and Alternaria spp. were the dominant types in the wastewater treatment plant. The highest emission of fungal aerosols is in March and the lowest emission is in July.

Conclusions: According to the results, operational and processing units of WWTP influence pollution load and dispersion of bioaerosols. Therefore, appropriate environmental health management in WWTP could be one of the important factors reducing dispersion of bioaerosols.

INTRODUCTION

Most microorganisms are produced as a result of human activities. Along with an increase in population growth, waste production has also augmented which, in turn, increases the need for facilities for treatment and safe disposal. Some-

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times these facilities and equipments result in the emission of infectious microorganisms in the air. Of these cases, wastewater treatment plants, composting, sanitary landfill areas can be mentioned [1,2]. Some microorganisms with small diameters are easily released during wastewater treatment and become bioaerosols which contain a variety of microorganisms. The number of such airborne microorganisms will increase through an increase in the size of bioaerosols [3,4]. Most of the particles, which carry bacteria, have aerodynamic diameter less than 4.7 µm. The small size of these particles show that they can be rapidly inhaled and cause lung infections in people with immune deficiency and cause allergies in other people. Also, the small particles can be easily carried by wind over distances of several hundred meters to several kilometers out. So they can be dangerous not only for plant workers, but also for local residents [5]. Many researchers have reported a specific illness called the ”sewage worker’s syndrome” observed among sewage treatment workers. The symptoms of the syndrome include weakness, malaise, fever, acute rhinitis, and gastrointestinal diseases [5,6]. Based on the conducted studies, it can be said that these microorganisms can cause skin diseases [7], asthma [8], ear infections as well as flu like symptoms [9]. It was indicated that the highest concentration of bioaerosols is in aerated grit operation method [10]. The other studies showed that the highest levels of bacterial contamination are related to sludge dewatering and the highest level of fungal contamination is related to grit chamber [3]. Other researchers investigated the effects of different methods of aeration in the emissions of bioaerosols. Their results showed that the highest emissions of aerosols are related to extended aeration, whereas the lowest is related to the diffuser aeration [5]. The other study revealed that the main source of bioaerosols in wastewater treatment plant is grit chamber [11]. The results of other studies showed that the system of aeration cause the emission of the most bioaerosols in the air of wastewater treatment plants [12]. The wastewater treatment plant of Qods County has been constructed in an area of 13 ha in West Tehran and exploited since 1998. The treatment has been estimated for a population of over 85,000 individuals that is predicted to be developed for 130,000 people. The treatment method in this wastewater treatment plant is activated sludge and the type of aeration extended aeration. Schematic of Qods county of Tehran and locating various processing and operating units are given in Fig.1 below. Considering the importance of air pollution and its consequences, in this study, the potential of emission of bacterial and fungal aerosol in ambient air of the processing and operational units of the wastewater treatment plant of Shahrak-e-Ghods of Tehran and its relationship with environmental parameters were investigated.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Shahrak-e-Ghods wastewater treatment plant from March 2012 to July 2013.

Sampling method

Sampling was carried out according to the sampling calendar of Environmental Protection Agency (EPA) in 2013 for 1 h every 6 days in a passive sampling and in the plates of 9 cm. The samples were taken in three units of grit chamber, aeration basin and aerobic digester within 2, 5 and 15 m radius and in the height of about 1 m [13] and a distance of 1 m from the walls and obstacles [14]. Also, the sampling was conducted within 100 m after the last treatment unit and from the indoor air of the administrative building. A total of 440 samples were collected. At each sampling, meteorological parameters such as temperature, relative humidity, wind speed and UV index were measured and recorded.

Identification of bacterial and fungal bioaerosols

The transport culture medium used for bacterial samples was the tryptic soy agar, and for fungal samples, it was dextrose agar [15].To prevent fungal growth in tryptic soy agar, cyclohexamide

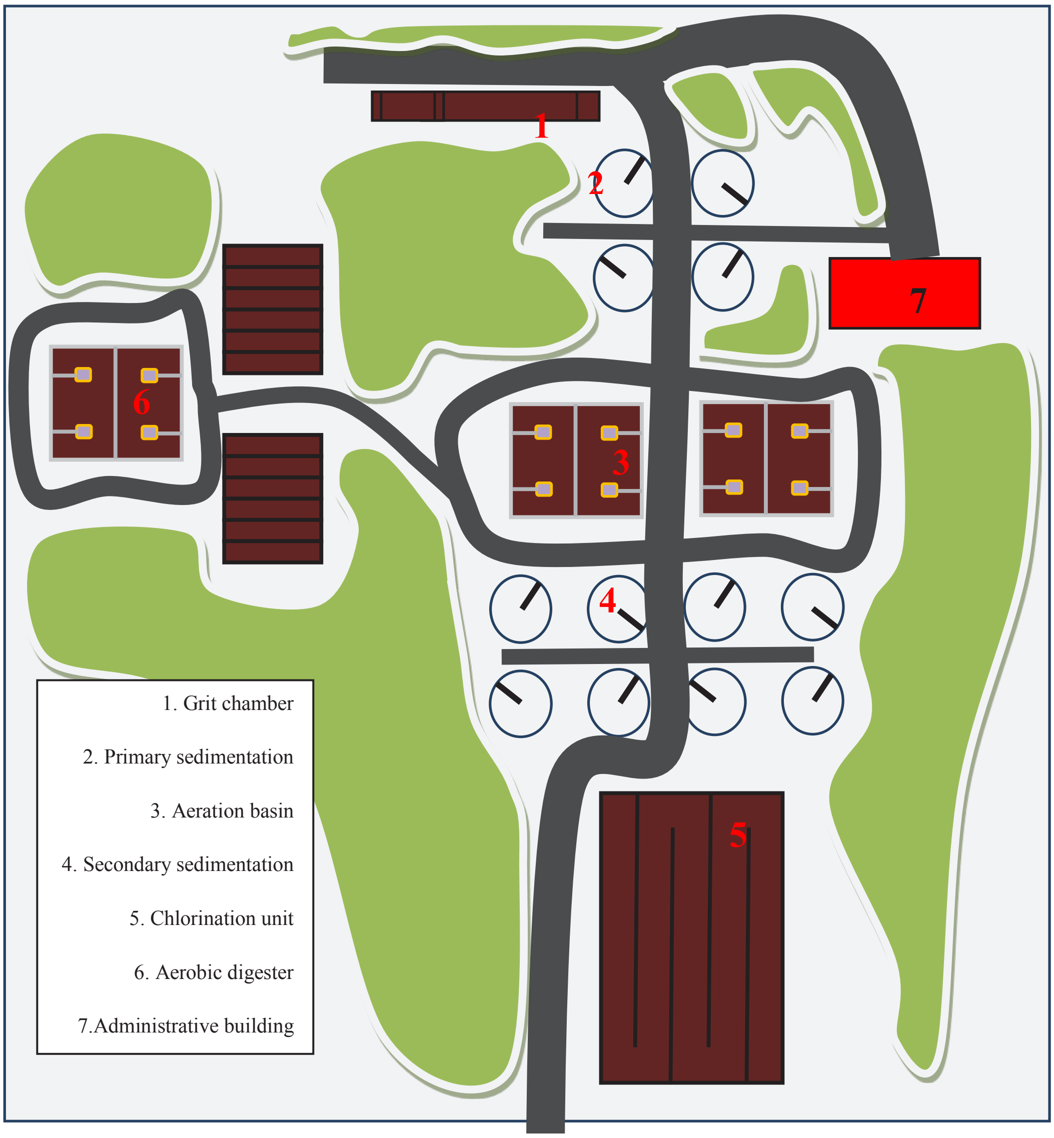

Fig.1. locating various processing and operating units

antibiotics (500µg/L) and to prevent bacterial growth on dextrose agar medium (100µg/L), chloramphenicol antibiotics were used. The samples were transferred to a laboratory and incubated for 24 to 48 h at a temperature of 35 ± 0.5 ° C to identify the bacteria and then they were examined in terms of the growth of the bacteria. While the bacterial genera were identified according to Bergey's manual and biochemical tests [16]. The fungal culture medium was placed at room temperature (20-25° C) for 3 to 7 days. The number of bacterial and fungal colonies grown on medium was counted and recorded in CFU/ Plate unit. The simple method of slide culturing was established to determine the fungi species by performing some levels of microscopic study by using optical microscopes [17].

Data analysis

Experimental data were analyzed by SPSS20 and Excel and Kruskal-Wallis H test, ANOVA, Independent t-test, Man-Whitney U statistical tests, was used to find the correlation between bacteria, fungal detected and meteorological conditions.

RESULTS AND DISCUSSION

Wastewater treatment plants have been found as one of the important factors of particulate matter emissions, pathogenic bacteria particle, and viral and fungal spores [18]. Several studies have shown that bioaerosol concentrations in wastewater treatment plants depends on the sampling location [11], the type of microorganisms, wastewater type, aeration method (large and small produced bubbles) [7, 19], weather conditions, and treatment equipment in which the sewage is treated [20], solar radiation, wind speed and relative humidity [6, 17, 21]. Results of this study showed that among the selected processing and operating units, the highest emission of fungal aerosols in Shahrak-e-Ghods wastewater treatment plant is for grit chamber and the greatest emission of bacterial aerosol is related to aerobic digester. The reason may pertain to the collapse of the bubble resulted from the aeration system that provides oxygen to decompose materials [3]. Particles from the collapse of bioaerosol fall down to the level of wastewater are converted to smaller particles with a diameter of 50-100 µm and cause secondary contamination. Such smaller particles in the air quickly evaporate and their diameter is reduced to 10-20 µm and therefore the speed of their deposition reduces and remains suspended in the air [22]. Studies have shown that the concentration of microorganisms in produced aerosols is 10-1000 times more than the wastewater and the number of these microorganisms increases by an increase in the size of the bubble [23]. According to the studies by some researchers, mixture of wastewater such as aeration, sludge processing methods, and grit chamber are also potential sources for the production of bioaerosols [24] . Other researchers conducted a similar study and found that they had observed the greatest emission of bioaerosols around the grit chamber [15]. It was showed in other studies that the highest levels of bacterial contamination is related to sludge dewatering and the highest level of fungal contamination is related to grit chamber [3]. In a similar study the aeration system was introduced as the major source of emissions of bioaerosols [12]. Some studies have reported the most bacterial and fungal aerosol emissions around the aeration basin, sludge disposal and grit chamber [19]. Previous research showed that pre-treatment is the most important factor of the emission of fungal aerosol [25].

Concentration of bioaerosols in different sampling locations

The Contributions of bacterial genera and fungal spores in different sampling locations are shown in Figs. 2 and 3. The dominant bacterial genus in aerobic digester and aeration tank was Bacillus spp. (35 and 39 % respectively). While the dominant bacterial genus in grit chamber and in administrative building was Staphylococcus spp. (39 and 54% respectively). Staphylococcus bacteria are normal inhabitants of the human gastrointestinal tract and due to the presence of people indoors, the rate of this bacteria had been high. On the other hand Micrococcus spp. was dominant in 100 m from last unit of WWTP (39%).
The results of statistical tests showed a significant difference between the concentration of fungi in CFU/Plate in the administrative building and outdoor of the wastewater treatment plant (Kruskal-Wallis H test, $P<0.05$). According to the results, the concentration of fungi in the open air was lower than that in the air of the administrative building. The results showed a statistically significant difference between the bacterial and fungal contamination in different locations of the plant (processing and operating units) ($P<0.05$). The concentration of bioaerosols in different processing and operating units of the plant was not the same as administrative building in terms of contamination.

Specious of isolated bacteria and fungi

According to the differential tests taken, in total, 3 bacterial species and 22 fungal species were isolated. A variety of isolated bacteria and fungi as well as their average in CFU/Plate during the sampling period are given in Tables 1 and 2 below, respectively. As shown in the tables, the highest and lowest concentrations of bacteria are related to aerobic digester (processing unit) with an average of 3303 CFU/Plate and grit chamber (operating unit) with an average of 586.3 CFU/

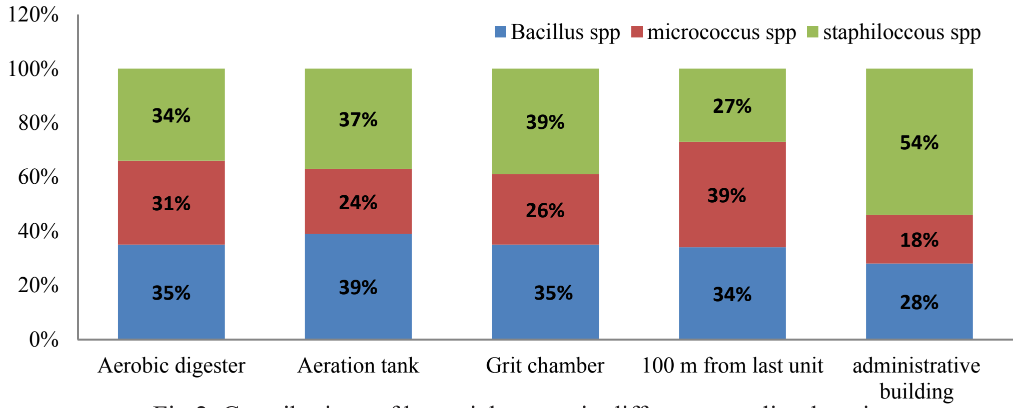

Fig.2. Contributions of bacterial genera in different sampling locations

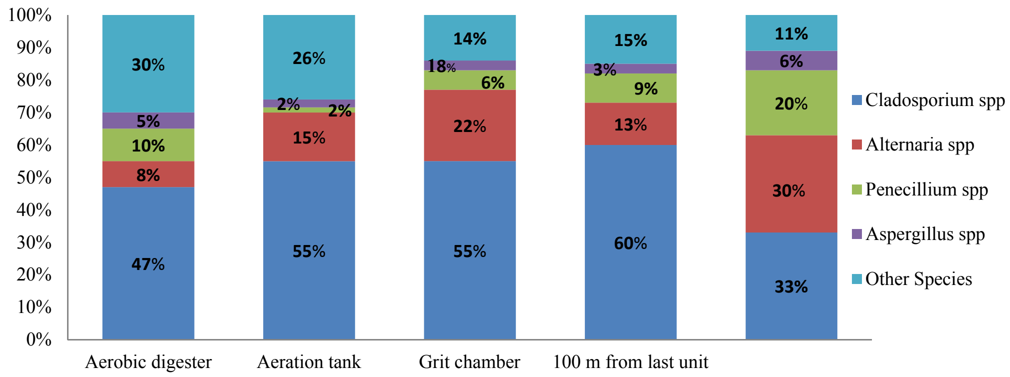

Fig.3. Contributions fungal spores in different sampling locations

Table 1.Type and concentration of detected bacteria as CFU/Plate in five sampling points in different distances

Sampling point / Type of bacteria	Aerobic digester 2m	5m	15 m	Aeration tank 2m	5m	15m	Grit chamber 2 m	5m	15 m	100m from last unit	administrative building
Bacillus spp.	1823	1016	677	1340	346	214	379	129	108	180	99
Staphylococcus spp.	1714	1188	456	1269	366	197	263	231	193	142	190
Micrococcus spp.	1600	893	542	877	167	118	183	143	130	207	62
Total	5137	3097	1675	3486	879	529	825	503	431	529	351
Mean		3303			1631.3			586.3		----	----

Plate, respectively. Also, the highest and lowest fungal concentrations were related to grit chamber with an average of 61 CFU/Plate and aeration basin with an average of 41 CFU/Plate. The most percentage of the bacteria was found in Bacillus spp. (36%), Staphylococcus spp. (35.4%), and Micrococcus spp. (28.5%), respectively (Fig.4). All these bacteria are gram-positive bacteria. The dominance of Bacillus species in this study can be attributed to the fact that these bacteria have the ability to form spores and are resistant to harsh environmental conditions, so their survival is high in the air.

With regard to the fungi, the most fungal species in the sampling locations were Cladosporium spp. (49% of the total fungi). Then Alternaria

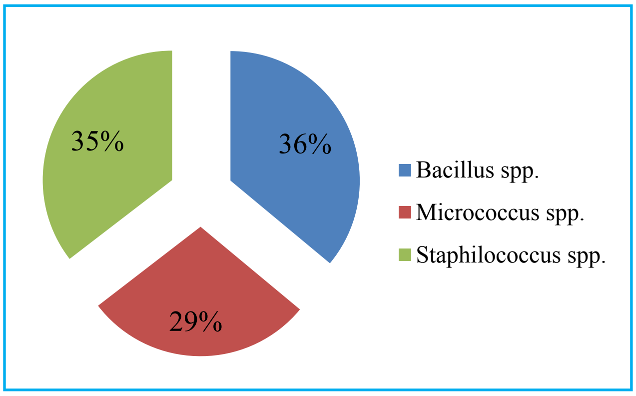

Fig.4.Contributions of bacterial aerosols in waste water treatment plant

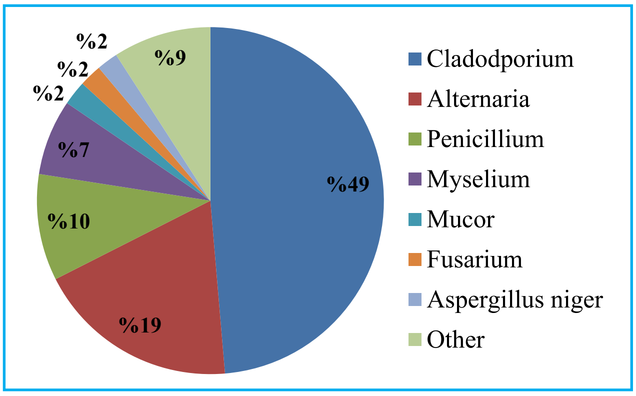

Fig.5.Contributions of fungal spores in waste water treatment plant

Table 2.Types and concentration of detected fungal as CFU/Plate in five sampling points in different distances

	Spices of detected fungal (%)							
Sampling point	Mean of spices of detected fungal (CFU/Plate)	Rangeof fungalspecies (CFU/Plate)	Cladosporium	Alternaria	Penecillium	Myselium	Mucor	Other Spices
Grit chamber	61.3	57-70	31.5%	31.7%	17.2%	23.4%	26.7%	19.2%
Aeration tank	41	34-47	21%	14.3%	3.2%	14.9%	6.7%	31.7%
Aerobic digester	45.3	41-49	19.8%	11.1%	21.9%	19.2%	66.6%	28.6%
100 m from last unit	---	---	12.3%	7.1%	9.4%	12.8%	0	5.7%
administrative building	---	---	15.4%	35.7%	48.4%	---	0	14.8%

spp. (19% of the total fungi) and Penecillium spp. (10% of the total fungi) were dominant (Fig.5). In the inside air of the administrative building, compared to other sampling units in the plant, Aspergillus spp. was the dominant genus in which the niger and flavus species were the most species of this genus identified outside the building.

The dominant fungal genus in aerobic digester, aeration tank, grit chamber, 100 m from last unit of WWTP and in administrative building was Cladosporium spp. (47%, 55%, 55%, 60% and 33% respectively).

The relationship between recorded parameters and bioaerosol concentrations

At various sampling locations during the study, temperatures, relative humidity, wind speed, and UV index were in the range of 3 to 34 °C, 11 to 49 percent, 5 to 17 km/h, and 4 to 7, respectively. Range, mean and standard deviation of the parameters listed are shown in Table 3. In this study, the highest bacterial aerosol emission was in July and the lowest rate was in March (Fig.6). Similar studies in this area have also confirmed that the emission of bioaerosols in warm months is more than that in cold months [11,26]. According to the report another researcher, high relative humidity with temperatures above the freezing point and weak wind result in the formation of bacterial agglomerates, which, in turn, lead to increase the mass of the particles and speed up their fall during summer [27].

It was also found that fungal aerosols have had the greatest emissions in March and the lowest emissions in July (Fig.7). A study has shown that the emission of fungi in summer months is 8 times more than that in winter months [28]. All fungal species detected were able to form spores which maintain these species in the face of environmental changes, so the dominance of this species can be attributed to their metabolic capabilities which maintain their distribution and survival in adverse environmental conditions such as UV, the lack of nutrients or high temperatures. The concentration of fungi in indoors is affected not only by the outdoor fungal load, but also by location, activity and movement of persons [29]. In other words, indoor human activities and population density affect the concentration of fungi. So the presence of a higher concentration of fungi in the administrative building can be attributed to this fact.

The results of this study showed that with increasing the distance from the source of contamination, the bacterial aerosol is reduced. Studies in this field by some researchers have shown that with increasing distance from the source of infection, the emission of bacterial aerosol significantly reduces [30,6]. Results showed statistically significant correlation between the density of the bacteria within a radius of 2, 5 and 15 m from the units and ultra violet index (UVI) ($P<0.05$). However, the density of bacteria had no significant correlation with temperature, relative humidity, wind speed, and season ($P> 0.05$). Some studies have also confirmed this fact [28]. With regard to the fungi, no significant correlation was observed be-

Table 3. Range (mean ±SD) of meteorological conditions

	Range (mean ±SD)			
Sampling month	Temperature (°C)	Relative Humidity (%)	Wind speed (km/h)	UV Index
March	3-14(9.8±3.8)	30-49(37.3±7.2)	5-17(10±2.1)	4-4(4)
May	16-28(24±4.3)	19-32(27.9±4.02)	5-16(9.9±2)	4-6(5.1±0.9)
June	28-32(30.2±1.7)	11-20(15.6±4.2)	8-13(9.6±1.7)	6-7(6.4±0.5)
July	28-34(31.7±2.2)	18-45(27.3±10.5)	8-11(9.4±1.8)	6-7(6.7±0.42)

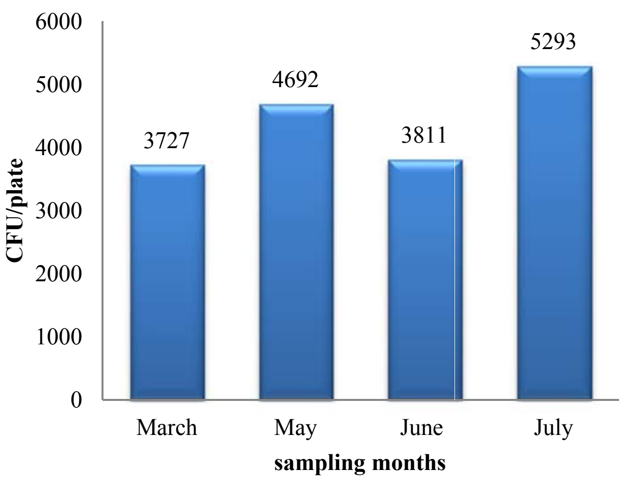

Fig.6.Concentration of bacterial aerosols in different sampling months

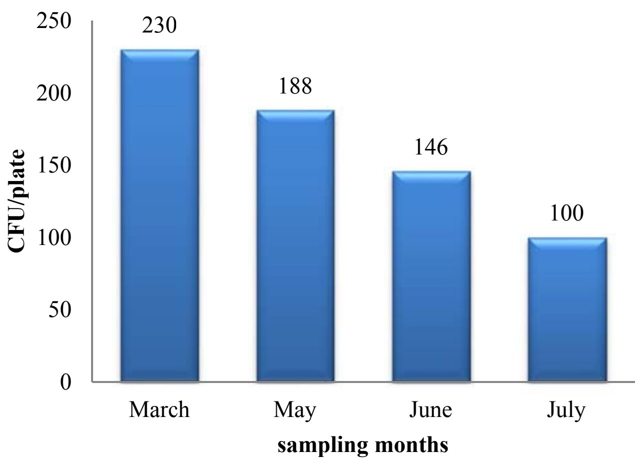

Fig.7.Concentration of fungal spores in different sampling months

tween the concentration of fungi and a radius of 2, 5 and 15 m from the units (P> 0.05). But there was a significant correlation between the density of fungi and temperature, relative humidity, wind speed, and UV index (UVI) (P<0.05).
In the present study, the total fungus identified in the 4 month sampling was 663 CFU/Plate which is less than the total of bacteria detected as 17,442 CFU/Plate. One reason may be the size of the fungus. Mostly the size of the fungi is about 2.1-3.3 µm, while the size of bacteria is about 1.1-2.1 µm. Therefore the size of the fungi is larger, so they can be deposited more quickly. This can be one of the reasons for the lower rate of fungi with compared to bacteria [31]. The results of this study suggest that the contact of the plant workers, especially during the aeration process should be limited. Physical separation of contaminated areas from areas with less pollution in the water treatment plant will be one of the effective ways to protect workers in the area [16]. Using diffuser aerators, instead of extended aeration, can be recommended as one of the methods for reducing emissions of bioaerosols. In addition, the existence of a chamber as coverage reduces the number of aerosols in the treatment plant and its surroundings [22, 26, 30, 32], and due to the lack of clear guidelines and standards related to microbial contamination of the air of wastewater treatment plants, it seems necessary for responsible organizations and officials to do required measures in the development of such guidelines.

CONCLUSIONS

Our findings show maximum bacterial concentration was found in the aerobic digester with an average of 3303 CFU/Plate. Also, minimum bacterial concentration was observed in the grit chamber unit with an average of 586.3 CFU/Plate. Maximum and minimum fungal concentrationswere in grit chamber and aeration tank with an average of 61 and 41 CFU/Plate respectively. Bacillus spp., Staphylococcus spp., and Micrococcus spp. were the most frequently observed bacteria types in the WWTP. The highest emission of bacterial aerosol is in July and the lowest is in March. The dominant fungi were Cladosporium spp., Alternaria spp. and Penicillium spp. also found that the highest emission of fungal aerosols is in March and the lowest emission is in July. The statistical results of this study showed that environmental parameters such as temperature, humidity, UV index and season which are significantly correlated with fungal aerosol emissions. However, here there was no significant relationship between the emission rate of bacterial aerosol and the above mentioned variables.

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COMPETING INTERESTS

The authors declare no competing interests.

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ETHICAL CONSIDERATIONS

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/ or submission, redundancy, etc) have been completely observed by the authors.

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