Influence of seasons and sampling points on emission of bioaerosols from wastewater treatment plant

Mansooreh Dehghani[a], Mahdieh Delikhoon[b], Mohammad Ghorbani[c], Ali Parvizi[d], Marziye Ansari[f], Abbas Norouzian Baghani[g*]

[a] Research Center for Health Sciences, Department of Environmental Health Engineering, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail address: mdehghany@sums.ac.ir

[b] Department of Occupational Health Engineering , School of Public Health, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail address: mdelikhon@yahoo.com

[c] Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran
E-mail address: ghorbani_epi@yahoo.com

[d] Department of Environmental Health Engineering, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail address: aliparvizi69@yahoo.com

[f] Department of Environmental Health Engineering, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail address: mdehghany@sums.ac.ir

*[g] Corresponding author. Department of Environmental Health Engineering, School of Public Health, Shiraz University of Medical Sciences, Shiraz, Iran.
Tel.: +989102141053; fax: +98 71 37251001
E-mail address: Abbas.jj.norozi@gmail.com

Abstract

Bioaerosols produced from Wastewater Treatment Plants (WWTPs) can pose health risks on plant workers and nearby inhabitants. The present study aimed to measure airborne bacteria and fungi in Shiraz WWTP, Iran during October 2015 to June 2016. Active sampling was carried out around operational units and a total of 576 bacterial and fungal samples were collected. The results showed that selector and aeration tanks with an average of 2581 and 1952 CFU/m^3 in autumn, aeration tank with an average of 1363 CFU/m^3 in winter, and screw pump with an average of 1738 CFU/m^3 in spring had the greatest effect on emission of bacterial bioaerosols. Furthermore, the predominant genera of airborne fungi isolated in the air of the WWTP in the three seasons were *Cephalotrichum spp.*, *Alternaria spp.*, *Penicillium spp.*, *Monilia spp.*, and *Aspergillus spp.* In addition, the concentrations of bacterial bioaerosols in autumn, winter, and spring were higher compared to the recommended value by Polish standard (1000 to 3000 CFU/m^3) in 14% of the sampling points, while the concentration of fungi was lower than the suggested value by Polish standard (500 CFU/m^3) and Swiss OELs (10000 CFU/m^3) in all sampling points. Moreover, a significant relationship was observed between meteorological parameters and the concentrations of bacteria and fungi in the three seasons. In conclusion, the results emphasized the necessity to control WWTP

workers’ exposure to bioaerosols when airborne bacteria and fungi become aerosolized during aeration.

Keywords: Air contamination, Bioaerosols, Wastewater treatment plant, Health risk, Shiraz

2. Introduction

Plant workers and nearby inhabitants are exposed to bioaerosols that may contain a large variety of bacterial, viral, and fungal species (1-3). Exposure to bioaerosols in Wastewater Treatment Plants (WWTPs) leads to occupational health risks for workers (1, 4, 5). The rapid industrialization and urbanization can produce industrial and household wastewater and sludge around the world (6). Wastewater includes a wide variety of microorganisms, such as viruses, bacteria, fungi, protozoa, and helminthes, which arise from household, commercial, and hospital sewage (7-11). Pathogenic microorganisms can be easily generated in aeration basins and while mechanical agitation of raw wastewater from WWTPs (3, 12, 13). Generally, many environmental factors influence the ability of microorganisms to survive in the air, the most important of which being radiation ultraviolet, kind of microorganisms, relative humidity, and temperature (10, 14, 15). Therefore, wastewater results in transfer of important diseases, such as gastrointestinal disorders, fever, respiratory symptoms, skin disorders, eye irritation, headache, fatigue, and nausea, in WWTPs workers (1, 5, 11). In addition, endotoxins produced by Gram-negative bacteria cause several problems, including diarrhea, fatigue, nose irritation, respiratory symptoms, and pulmonary function decline in WWTPs workers (11, 16, 17). Evidence has also shown that frequent exposure to fungal spores led to development of hypersensitivity pneumonitis, decline of lung function, severity of asthma, organic dust toxic syndrome, airway inflammation, and respiratory disorders (16, 18). Moreover, many studies have indicated that workers' long-term exposure to bioaerosols could cause a wide variety of respiratory and other health disorders, such as sinusitis, recurrent ear infections or influenza-like symptoms, respiratory symptoms, and gastrointestinal disorders (10, 18-20).

In general, the concentration of microorganisms is a significant indicator of the atmosphere of WWTPs (10). Recent awareness about the risks posed by airborne microorganisms is the main reason for development of aeromicrobiology. However, it seems that there is no internationally accepted threshold value for biological contamination of air (10, 11). Thus, in spite of identification of the health risks of exposure to bioaerosols, risk assessment is still difficult. Although Occupational Exposure Limits (OELs) for bioaerosols have not been determined yet (21, 22), a limiting concentration has been estimated by some institutions. For instance, limiting concentrations have been proposed by Swiss OELs for total cultivable bacteria (10^4 CFU/m^3), Gram-negative bacteria (10^3 CFU/m^3), and total fungi (10^3 CFU/m^3) (22). In addition, guidelines proposed by Scandinavian countries and American Conference of Governmental Industrial Hygienists (ACGIH) have set 10^3 CFU/m^3 and 10^4 CFU/m^3 for total bacteria and Gram-negative bacteria, respectively (23). Furthermore, concentrations of airborne bioaerosols and degree of human exposure to bioaerosols may significantly depend upon weather conditions, time of the day, season, treatment processes of wastewater and sludge, kind and capacity of WWTPs, type of facilities, and performed activities (8, 10, 19, 24).

To date, few studies have focused on outdoor air quality in WWTPs in developing countries, such as Iran, and scarce information is available regarding plant workers' risk of exposure in WWTP. Hence, the present study aims to 1) determine the emission of bacteria and fungi from WWTP, 2) assess the relationship between sampling points and number of bioaerosols, 3) identify the genera of fungi and percentage of Gram-positive and Gram-negative bacteria, 4) investigate the effects of seasons and weather conditions on the amount of bioaerosols, and 5) determine the relationship between the inlet parameters of WWTP and emission of bioaerosols in Shiraz WWTP in autumn, winter, and spring.

2. Materials and Methods

2.1. Sampling sites

This study was conducted in Shiraz WWTP stellated in Shiraz, Iran. It covers 409000 inhabitants right now and the final coverage of inhabitants in this WWTP has been estimated to reach around 548000 in future. The average inlet flow rate of this WWTP is about 930 LPS and it is expected to provide about 29.5 MCM/year of fresh water for irrigation. In addition, the inlet Biochemical Oxygen Demand (BOD5), Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), grease and oil, and Total Phosphorus (TP) are 240, 315, 465, 0.034, and 3.73 mg/L, respectively. Activated sludge is the biological wastewater treatment process of this WWTP and it includes screen bar unit, primary settling tank, selector tank, aerated tank, secondary settling tank, and chlorination unit. To date, total of 55 workers have been employed in this plant.

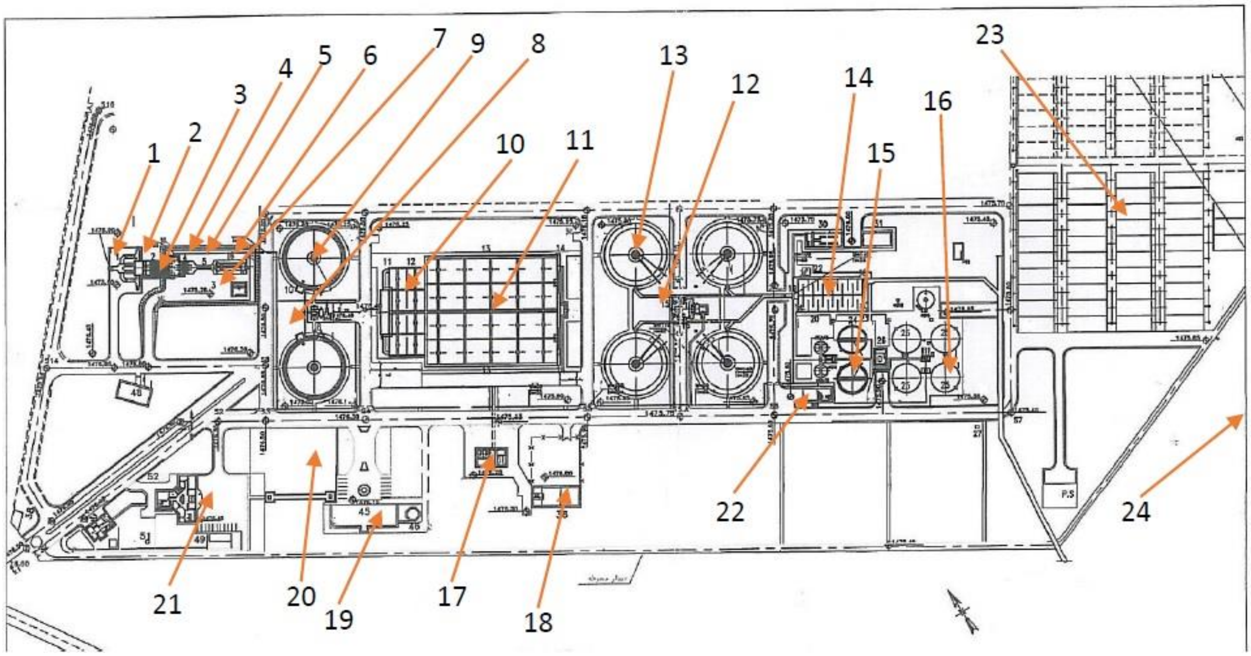

Figure 1. Schematic diagram of the wastewater treatment plant and sampling points (based on number 1 to 24)

According to the EPA sampling guideline, sampling was performed every 6 days in autumn, winter, and spring during October 2015 to June 2016 (15, 25). Sampling points selected in the WWTP have been shown in Figure 1. Totally, 24 points were selected for sampling in the WWTP (Table 1). In fact, 8 samples were collected in four geographical directions from each station and a total of 576 bacterial and fungal samples were obtained in autumn, winter, and spring (15). At first, the sampler was disinfected with 75% ethanol to remove any contamination. Then, QuickTake 30 sample pump equipped with the Bio Stage single-stage cascade impactor was used for air sampling at 2 min with the rate of 28.3 L/min (15, 26). The sampler was located at a height of about 1.5 m from the floor (at the human breathing zone) (15, 26, 27). Temperature, relative humidity, wind speed, and solar radiation were also simultaneously recorded to find the relationship between bioaerosols concentrations and environmental conditions at each sampling location (Table 3) (28). Temperature (°C) and relative humidity (%) were measured using a portable instrument (Preservation Equipment Ltd, UK). Additionally, wind speed (m/s) was determined by a portable anemometer (Campbell Scientific, Inc., USA). Finally, solar radiation was measured by a pyranometer (Kipp & Zonen, Netherlands) and was expressed in $\mu W/cm^2$. Moreover, sabouraud dextrose agar medium (Merck Co, Germany) containing chloramphenicol antibiotic and blood agar medium (Merck Co, Germany) were prepared to determine fungal and bacterial bioaerosols, respectively (15). After sampling, the plates were closed and immediately transferred to the microbiology laboratory in a cold box. The fungal samples were incubated in an inverted position at room temperature (20–25 °C) for 3–7 days, while the bacterial samples were incubated at 37 °C for 24-48 h. Then, the concentration of bioaerosols in the plates were counted and reported according to CFU/m^3 (6).

2.2. Data analysis

Data were analyzed using SPSS 21 software. Kolmogorov Simonov test was used to assess normality. Quantitative variables with normal distribution were shown with mean and standard deviation and quantitative variables with non-normal distribution with Median (IQR). The qualitative variables were expressed with number and percentage. To compare the variables between the groups in terms of the requirements the Q-square, Student T test, Mann-Whitney U, ANOVA, Kruskal-Wallis and Pearson and Spearman correlation coefficient was used. In addition, figures were drawn using GraphPad Prism 6.

3. Results

3.1. Mean number of bacteria and mean of meteorological conditions in autumn, winter, and spring

The mean, maximum, and minimum number of bacteria based on CFU/m^3 and percentage of Gram-positive and Gram-negative bacteria in different sampling locations have been presented in Table 1. Accordingly, office building with emission of 50 CFU/m^3, primary split basin with emission of 37 CFU/m^3, chlorination basin with emission of 45 CFU/m^3, screw pump room with emission of 37.5 CFU/m^3, and anaerobic digestion tank with emission of 34 CFU/m^3 showed the minimum emissions in autumn. On the other hand, selector tank with emission of 2581 CFU/m^3, aeration tank with emission of 1952 CFU/m^3, coarse screen with emission of 449 CFU/m^3, and fine screen with emission of 410 CFU/m^3 showed the maximum emissions in autumn. In addition, the lowest number of bacterial bioaerosols in winter was observed in surveillance building (37 CFU/m^3), site water supply (55 CFU/m^3), chlorination basin (68 CFU/m^3), and office building (74 CFU/m^3). However, the highest number of bacterial bioaerosols in winter was related to aeration tank (1363 CFU/m^3) and screw pump (1129 CFU/m^3). Furthermore, surveillance building (37 CFU/m^3) and aeration tank (59 CFU/m^3) showed the lowest rate of emission in spring. On the other hand, screw pump, screw pump room, and coarse screen showed the highest rate of emission in this season (1738, 1520, and 1323 CFU/m^3, respectively). Overall, the total concentration of bacteria was higher in spring than in autumn and winter.

The means of temperature and relative humidity in WWTP air have also been presented in Table 1. As the table depicts, the means of temperature and relative humidity were respectively 37±0.41 ^{o}C and 11±0.28% during autumn, 20±0.42 ^{o}C and 15±0.89% during winter, and 31±0.65 ^{o}C and 40.5±2.1% during spring. In addition, the rates of Gram-positive and Gram-negative bacteria were respectively 63.5% and 36.5% in autumn, 46% and 54% in winter, and 45% and 55% in spring. Moreover, the speed of wind was 1.5 m/s in autumn, 1.6 m/s in winter, and 2.4 m/s in spring. Additionally, the UV index was 58.8 μW/cm^2 in autumn, 31.3 μW/cm^2 in winter, and 48.5 μW/ cm^2 in spring. Pressure was also 860, 859, and 862 mbr in autumn, winter, and spring, respectively.

Table 1. The mean, maximum, and minimum number of bacteria based on CFU/m^3 in different sampling locations, the percentage of Gram-positive and Gram-negative bacteria, and the means of temperature and relative humidity in autumn, winter, and spring

Sampling point		Spring	Winter	Autumn	P value
1	Coarse Screen	1323.5±823(330-3764)	180 ± 37.7(130-290)	410± 86(230-617)	0.026
2	Screw Pump	1738± 666(190-2236)	1129 ± 603(57-2333)	233± 29.5(150-280)	0.591
3	Screw Pump Room	1520±622(190-3196)	778.5 ± 439(127-2000)	37.5± 11.5(17-70)	0.014
4	Fine Screen	897±554 (150-2501)	280 ±189 (33-840)	449± 136(87-697)	0.551
5	Parshall Flume	1002± 557(150-2501)	462 ± 361(60-1543)	388± 270(20-1180)	0.472
6	Grit Chamber	731± 610(74-2560)	133 ± 57(37-280)	89± 29(20-163)	0.500
7	Blowers Room	767± 598(107-2560)	122 ± 54(47-280)	93± 44(10-190)	0.298
8	Primary Split Basin	445± 310(134-1375)	695 ± 619(67-2552)	202± 79(67-367)	0.581
9	Primary Sedimentation Tank	226.5± 40(135-330)	98 ± 30(57-187)	208± 105(27-420)	0.390
10	Selector Tank	423± 319(34-1375)	686 ± 622(37-2552)	2581± 355(1967-3197)	0.057
11	Aeration Tank	454± 310(34-1375)	1363 ± 718(107-2661)	1952± 720(577-3197)	0.198
12	Secondary Split Basin	76±10 (64-107)	98.5 ± 23 (60-160)	32± 3(27-40)	0.023
13	Secondary Sedimentation Tank	97± 16(58-130)	516.5 ± 419(30-1766)	196± 97(40-477)	0.874
14	Chlorination Basin	59± 10(40-84)	68 ± 10/2(40-87)	45± 20(17-100)	0.525
15	Sludge Thickening Tank	78± 5(65-87)	197 ± 127(57-577)	50± 28(20-133)	0.302
16	Anaerobic Digestion Tank	77± 15(40-108)	88.5 ± 16(57-133)	34± 9(20-60)	0.057
17	Power Panel Building	94± 20(35-124)	164.5 ± 33.5(105-237)	78± 8(63-93)	0.035

18	Storage Building	114± 14(80-145)	171.5 ± 35.5(110-233)	90± 4(80-100)	0.006
19	Surveillance building	37± 1/73(34-40)	37 ± 1.73(34-40)	73± 11(53-107)	0.012
20	Site water Supply	409± 157(108-840)	55 ± 9.1(40-80)	58± 1(53-60)	0.020
21	Office Building	74± 9(40-90)	74 ± 9(40-90)	50± 18(17-87)	0.207
22	Chemical Unit	74.5± 15.5(48-108)	299 ± 108(57-577)	72± 36(20-180)	0.058
23	Sludge Drying Bed	286± 110(85-507)	101 ± 26(40-158)	57± 11(23-70)	0.048
24	WWTP Wall	239.5± 93(75-507)	259.5 ± 106.5(70-444)	57± 2(53-60)	0.023
	Total mean of bacteria	474 ± 82.5(34-3764)	336 ± 65(30-2661)	314 ± 70(10-3197)	
	Total Gram-positive bacteria	45 %	46 %	63.5 %	
	Total Gram-negative bacteria	55 %	54 %	36.5 %	
	Total mean of humidity	40.5± 2.1(9-85)	15± 0.89(10-62)	11 ± 0.28(10-28)	
	Total mean of temperature	31± 0.65(10-37)	20± 0.42(18-27)	37 ± 0.41(28-41)	

3.2. Mean number of fungi and mean of meteorological conditions in autumn, winter, and spring

The mean, maximum, and minimum number of fungi based on CFU/m^3 in different sampling locations in autumn, winter, and spring has been presented in Table 2. Accordingly, the minimum concentration of fungi in autumn was found in parshall flume, blower room, secondary split basin, storage building, WWTP wall, chlorination basin, and chemical unit, which ranged from 3 to 11 CFU/m^3. On the other hand, the maximum concentration of fungi in autumn was observed in the aeration tank and screw pump, which ranged from 30 to 52 CFU/m^3. In addition, the highest concentration of fungal bioaerosols in winter were related to site water supply (26 CFU/m^3), screw pump (21 CFU/m^3), and fine screen (20 CFU/m^3). However, the minimum concentration of fungi in winter was found in the secondary sedimentation tank, chlorination basin, storage building, WWTP wall, power panel building, and sludge thickening tank, which ranged from 4 to 6.5 CFU/m^3.

Table 2. Mean, SE, and range (minimum–maximum) of fungal bioaerosols based on CFU/m^3 in different sampling locations in autumn, winter, and spring

	Sampling point	**Spring**	**Winter**	**Autumn**	**P value**
1	Coarse Screen	91±40(40-210)	15±13(0-53)	8 ±1.6(7-13)	0.052
2	Screw Pump	175± 53(105-280)	21± 12(7-57)	52± 10.7(33-83)	0.027
3	Screw Pump Room	145±15(87-174)	20±13(3-60)	20±10.6(3-50)	0.013
4	Fine Screen	100±17 (77-150)	26±8 (3-37)	5.83±2.8 (0-13)	0.011
5	Parshall Flume	60± 8(47-77)	14± 8(3-37)	9± 1.5(7-13)	0.023
6	Grit Chamber	95.5± 26(47-167)	12± 2(7-17)	8± 3.9(3-20)	0.019
7	Blowers Room	116± 17(98-167)	14± 5(0-23)	7.5± 2.6(7-10	0.017
8	Primary Split Basin	94± 16(60-134)	10± 3(3-17)	5.8± .83(3-7)	0.018
9	Primary Sedimentation Tank	104± 11 (83-134)	16± 3 (0-33)	4± 1.9 (0-10)	0.021
10	Selector Tank	38± 15(3-67)	13± 4(3-20)	4± .83(3-7)	0.077
11	Aeration Tank	52.5±26.5(3-124)	12±4(3-20)	30±5.2(10-70)	0.023
12	Secondary Split Basin	42±18 (17-94)	12±3 (7-20)	3±2.1 (0-10)	0.010
13	Secondary Sedimentation Tank	30.5± 7 (10-44)	4± 2 (0-10)	7.4± 3.6 (0-16)	0.048

14	Chlorination Basin	26± 9 (3-47)	6.5± 2 (3-10)	3± 1.4 (0-7)	0.100
15	Sludge Thickening Tank	53.5 ± 10 (30-80)	6 ± 2 (3-10)	1.6 ± .96 (0-3)	0.10
16	Anaerobic Digestion Tank	55.5± 14 (37-98)	8.5± 1(7-10)	8± 4.1(7-10)	0.006
17	Power Panel Building	73± 22(20-117)	6.5± 4(0-13)	5± 2.1(0-10)	0.010
18	Storage Building	97± 14 (57-117)	6.5± 4(0-13)	1.67± .96(0-3)	0.015
19	Serveillance Building	71.5± 26(7-128)	10± 2(7-13)	13.4± 3.8(7-20)	0.073
20	Site water Supply	66± 6 (57-84)	8± 3 (0-17)	8± .96 (7-10)	0.015
21	Office Building	38± 8(23-60)	9± 3(0-17)	5± .96(3-7)	0.060
22	Chemical Unit	55± 6 (40-70)	7.5± 1 (3-10)	6± 1.6 (3-10)	0.020
23	Sludge Drying Bed	93± 51(30-244)	8.5± 3.5(0-17)	8.5± 2.1(3-13)	0.024
24	WWTP Wall	54± 8.5 (30-70)	5± 3 (0-10)	11± 3.6 (3-22)	0.021
	Total mean of fungi	77.25 ± 5.32(3-280)	11.83 ± 1.2(0-60)	8.32 ± 1.2(0-83)	

According to Table 2, the lowest concentration of fungal bioaerosols in spring was observed in chlorination basin, secondary sedimentation tank, and office building (26, 30.5, and 38 CFU/m^3, respectively). On the other hand, the highest concentration of fungal bioaerosols in spring was related to fine screen, primary sedimentation tank, blowers room, site water supply, and screw pump, which ranged from 100 to 175 CFU/m^3.

The percentages of fungi and fungal genera in WWTP air have been shown in Figure 2. Accordingly, the predominant genera of airborne fungi identified in the air during autumn were *A. Niger* (15.74%), A. *fumigatus* (13.43%), *Mucor spp.* (12.03%), and *Cephalotrichum spp.* (8.8%). In addition, the predominant genera of airborne fungi identified in the air during winter were *Monilia spp.* (28.57), *A.*

Niger (25.21%), A. *fumigatus* (14.01%), and *Penicillium spp.* (3.36%). Finally, the predominant genera of airborne fungi detected in the air during spring were *Cephalotrichum spp.* (42.82%), *Alternaria spp.* (21.77%), *Penicillium spp.* (10.88%), and *A. Niger* (5.47%).

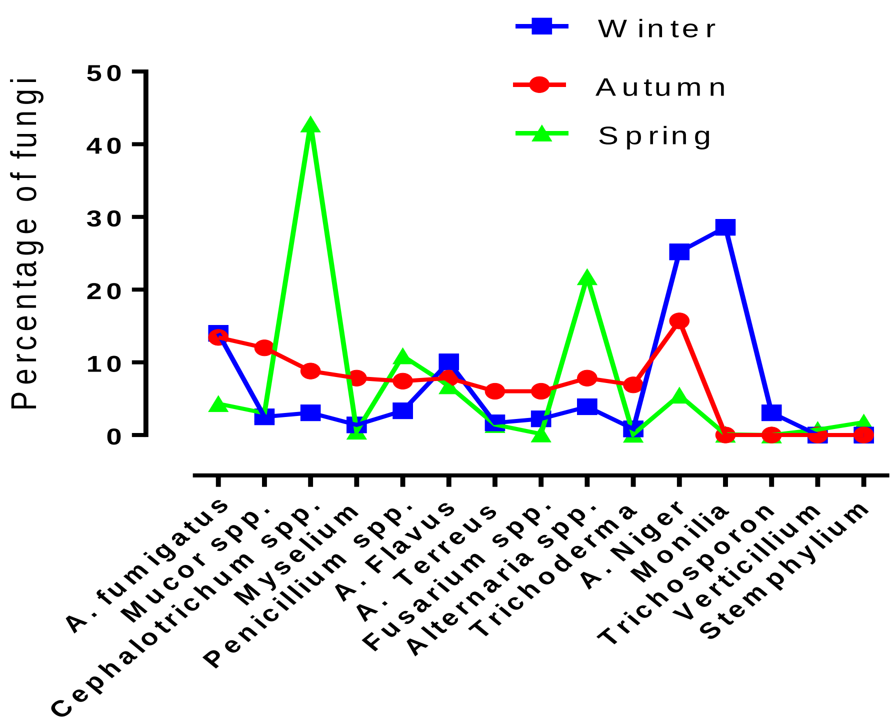

Figure 2. The percentages of fungi and fungal genera in autumn, winter, and spring

3.3. The relationship between the measured variables and bacterial bioaerosols

The correlation between meteorological conditions and concentrations of bacteria and fungi was studied in different sampling locations in autumn, winter, and spring (Table 3). The results showed that relative humidity had a significant effect on the concentration of airborne bacteria in autumn and spring ($p<0.05$). This implies that the concentration of bacteria rose by increase in relative humidity. However, the results indicated no significant correlation between the concentration of bacteria and relative humidity in winter ($p=0.363$). Also, no significant correlation was observed between the concentration of bacteria and UV index in spring ($p>0.05$). On the other hand, the results demonstrated a significant correlation between the concentration of bacteria and UV index in winter and spring ($p<0.05$). Furthermore, no significant correlation was detected between the concentration of bacteria and temperature in winter and autumn ($p=0.08$). Accordingly, the concentration of bacteria decreased with increase in temperature. However, the results indicated no significant correlation between the concentration of bacteria and temperature in spring ($p=0.964$).

The results also revealed no significant correlation between the concentration of bacteria and wind speed and pressure in different seasons ($p>0.05$). Nevertheless, a significant correlation was found between the concentration of bacteria and inlet grease and oil in the three seasons ($p=0.039$). Moreover, the results showed no significant difference in the number of Gram-positive ($p=0.3$) and Gram-negative bacteria ($p=0.4$) in the three seasons.

3.4. The relationship between the measured variables and fungal bioaerosols

According to Table 3, a significant correlation was observed between the concentration of fungi and relative humidity in winter and spring ($p=0.004$). In other words, relative humidity had a significant effect on the concentration of airborne fungi in winter and spring ($p=0.004$). A significant correlation was also detected between the concentration of fungi and relative humidity in autumn ($p=0.03$). However, no significant correlation was found between the concentration of fungi and temperature in spring and autumn ($p>0.05$). On the other hand, a significant correlation was observed between the concentration of fungi and temperature in winter ($p=0.025$). Moreover, the results showed no significant correlation between meteorological conditions, such as wind speed, pressure, and UV index, and the concentration of fungi in the three seasons ($p>0.05$). Furthermore, a significant difference was found among the three seasons with respect to the concentration of fungi ($p<0.05$). In addition, a significant correlation was observed between the concentration of fungi and inlet COD in the three seasons ($p=0.005$).

Table 3. The correlation between meteorological conditions and total concentration of bacteria and fungi in the study samples (n=576)

		Autumn						Autumn						Autumn				
		Te.	**RH**	**UV**	**TB**	**TF**		**Te.**	**RH**	**UV**	**TB**	**TF**		**Te.**	**RH**	**UV**	**TB**	**TF**
Autumn	**Te.**	1					**Winter**	.047					**Winter**	1				
	RH	-.482**	1						.097					-.708**	1			
	UV	.586**	-.618*	1						.306**				.43**	-.665**	1		
	TB	-.090	.307**	-.233*	1						.238*			.017	.094	-.269**	1	
	TF	-.176	-.213*	.191	.060	1						-0.052		-.225*	.286**	-.086	-.199**	1
		Autumn						**Winter**						**Spring**				
		Te.	**RH**	**UV**	**TB**	**TF**		**Te.**	**RH**	**UV**	**TB**	**TF**		**Te.**	**RH**	**UV**	**TB**	**TF**
Spring	**Te.**	0.240*					**Spring**	0.111					**Spring**	1				
	RH		-0.293**						0.107					-.811**	1			
	UV			0.109						0.030				.219**	-.053	1		
	TB				0.248*						0.336**			.005	.387**	.127	1	
	TF					0.133						0.086		-.076	.322**	.101	.464**	1

TF, total fungi; TB, total bacteria; RH, relative humidity; Te, temperature; UV, UV index
**** Correlation is significant at 0.01 level (2-tailed)**
Significant: **p<0.01 and *p<0.05

4. Discussion

WWTPs are a source of airborne bacterial contamination and constitute a source of atmospheric air pollution, which can expose workers to a biological risk (8, 10, 14). Up to now, many studies have been published on bioaerosols emission from WWTPs (8, 11). The present study also showed that Shiraz WWTP had a significant effect on the total emission of bacteria and fungi. In addition, the level of bacterial and fungal bioaerosols depended mainly on sampling location, which is consistent with the results of the previous studies performed by Korzeniewska et al. and Fracchia et al. (8, 10, 14).

Generally, many meteorological conditions have been demonstrated to influence the ability of bioaerosols to survive in the atmosphere. Among these factors, relative humidity played a key role in increasing the concentrations of airborne bacteria and fungi in the air of WWTP in Shiraz (10, 14, 28). Karra et al. (2007) and Korzeniewska al. (2011) also reported that relative humidity was the key to growth and distribution of bioaerosols in the air of WWTP (10, 28). Similarly, other studies have concluded that relative humidity was an important effective factor in the concentration of bioaerosols in the air of WWTPs (10, 28, 29). Besides, Vitězova et al. (2012) stated that the concentration of bioaerosols was related to air temperature (3). Moreover, a previous study demonstrated that the season and weather conditions seemed to have a significant influence on the mean concentration of airborne bioaerosols in the air of WWTPs, which is in line with the findings of the present study (14, 15). Our study results showed that the maximum concentration of fungi was observed in spring, while Płachta et al.

reported higher amounts of fungi in Ostro´da WWTP (northeast of Poland) in autumn (24). In addition, Anne-ppliger et al. conducted a study in Switzerland and revealed higher concentrations of fungal bioaerosols in summer compared to winter and/or spring (11).

The findings of the current study showed no significant correlation between meteorological conditions, such as wind speed, pressure, and UV index, and the concentration of fungi. Niazi et al. also demonstrated no correlation between the concentration of fungi and wind speed, pressure, and UV index (15). However, our results indicated a significant correlation between the concentration of bacteria and relative humidity in autumn and spring, which is consistent with the results of the previous study performed by Oppliger et al. (2005) (11). Based on these results, relative humidity seems to have the highest correlation with the concentration of bacteria. Nonetheless, a previous study demonstrated that temperature appeared to have the highest correlation with the concentration of bacteria (15). Moreover, a previous study demonstrated a significant correlation between the concentration of fungi and relative humidity in winter, which is in line with the findings of the present study (15).

Our study findings disclosed that screw pump with an average of 1738 CFU/m^3 in spring, aeration tank with an average of 1363 CFU/m^3 in winter, and selector tank with an average of 2581 CFU/m^3 in autumn had the greatest effect on emission of bacterial bioaerosols. Similarly, Niazi et al. found that aeration tank with an average of 1016 CFU/m^3 and 1973 CFU/m^3 in winter and summer had the greatest impact on emission of bacterial bioaerosols (15). Another study conducted by Juliana et al. in Portugal showed that the concentrations of bacteria and fungi ranged from 60 to more than 52,560 CFU/m^3 and from 369 to 14,068 CFU/m^3, respectively (22).

The present study results showed that the percentage of Gram-negative bacteria was higher compared to Gram-positive bacteria in winter and spring. On the contrary, Niazi et al. reported a large number of Gram-positive compared to Gram-negative bacteria (15, 22).

The results of the present research showed that emission of bacteria and fungi in the air depended mainly on sampling location, which is consistent with the results of the previous studies performed by Korzeniewska et al. and Fracchia et al. (8, 10, 14). In addition, selector tank, aeration tank, screw pump, screw pump room, and coarse screen were found to be the major points in emitting bioaerosols in different seasons in the current study. Niazi et al. 2015, Monedero et al., 2008, Brandi et al. 2000, Michałkiewicz et al. 2011, Wlazło et al. 2001, Oppliger et al. 2005, and Fernando et al. 2005 also determined aeration tank as the main point in releasing bioaerosols (11, 13, 15, 30-32). Yet, the results of the study conducted by

Korzeniewska et al. in a BIO-PAK WWTP showed that bioreactor and grate chamber were the main sources of bioaerosols emission (14).

The guidelines proposed by Polish standard (PN-89Z-04111/03) have set 500 CFU/m^3 for the number of fungi representing no pollution. In another guideline proposed by Polish standard (PN-89Z-04111/02), degree of air pollution has been classified based on the number of bacteria. Accordingly, the number of bacteria less than 1000, from 1000 to 3000, and more than 3000 indicates no pollution, average pollution, and strong pollution, respectively (31). Moreover, the guidelines proposed by Swiss OELs have set 1000 CFU/m^3 for fungi and 10000 CFU/m^3 for total cultivable bacteria in WWTPs (11). In our study, the concentration of bacterial bioaerosols in different seasons was higher compared to the recommended value by Polish standard (1000 to 3000 CFU/m^3) (31) in 12% of the sampling points, while the concentration of fungi was lower than the suggested values by Polish standard (500 CFU/m^3) (31) and Swiss OELs (10000 CFU/m^3) (11) in all sampling points. In contrast, Oppliger et al. disclosed that more than 50% of the sewage treatment plants exceeded the recommended Swiss occupational threshold of fungi (1000 CFU/ m^3) in summer (11).

In the present research, the predominant genera of airborne fungi isolated in the air of WWTP in different seasons were *Cephalotrichum spp.*, *Alternaria spp.*, *Penicillium spp.*, *Monilia spp.*, and *Aspergillus spp.* Similarly, Korzeniewska et al.

(2009) reported that the dominant fungi types were *Alternaria spp*., *Cladosporium spp.*, *Penicillium spp.*, and *Aspergillus spp.* (14). In addition, Niazi et al. 2015 and Juliana et al. 2012 indicated that the predominant genera of airborne fungi identified in the air of WWTP were *Cladosporium spp.*, *Penicillium spp.*, *Aspergillus spp.*, and *Alternaria spp.* (15, 22). Li et al. (2011) also reported *Aspergillus* and *Penicillium* as the most commonly identified fungi whose high concentrations in the air of WWTP were potentially hazardous to humans (33).

Overall, our results highlighted the necessity to control WWTP workers' exposure to bioaerosols, especially in the process areas involving forced aeration of wastewater by mechanical agitation. Similar results have also been obtained by Tanskia et al. in 2009 and Li et al. in 2013 (19, 26).

Conclusion

The current study findings showed that the selector and the aeration tank with an average of 2581 and 1952 CFU/m^3 in autumn, aeration tank with an average of 1363 CFU/m^3 in winter, and screw pump with an average of 1738 CFU/m^3 in spring had the greatest effect on emission of bacterial bioaerosols. In addition, the emission rate of bacteria and fungi in spring was respectively 1.5 and 8-9.5 times more than that in winter and autumn. Furthermore, the concentrations of bacterial bioaerosols in different seasons were higher compared to the recommended value by Polish standard (1000 to 3000 CFU/m^3) in 12% of the sampling points, while the concentrations of fungi were lower than the suggested value by Polish standard (500 CFU/m^3) and Swiss OELs (10000 CFU/m^3) in all sampling points. Moreover, the dominant genera of fungi in different seasons were *Cladosporium spp.*, *Alternaria spp.*, and *Penicillium spp.* followed by *Monilia spp.* and *Aspergillus spp.* The results revealed a significant relationship between environmental parameters and the concentrations of bacteria and fungi. The results also showed that the rates of Gram-positive and Gram-negative bacteria were respectively 63.5% and 36.5% in autumn, 46% and 54% in winter, and 45% and 55% in spring.

In general, WWTPs workers may be exposed to hazard levels of bioaerosols. In order to diminish the emission of bacteria and fungi in the air from WWTPs, covering the selector and aeration tanks seems to be necessary.

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