



Bacterial-aerosol emission from wastewater treatment plant

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ABSTRACT

Bioaerosols' emission from an activated sludge wastewater treatment plant with surface aerators was surveyed from November 2009 to July 2010. Health effects among plant workers were assessed by checklist and through interviews by an internist. Samples were collected by the Anderson sampler in eight days of the year and analyzed for Standard Plate Count, Total Coliforms, Fecal Coliforms, and Fecal Streptococci. It was found that most sewage treatment plant's staffs were adversely affected as a result of bioaerosols' inhalation. Major observed health effects were fatigue, dizziness, eye irritation, and abdominal pain. The most common size of bacterial-aerosols was higher than 8.2 μm . Maximum and minimum concentrations of bacterial-aerosols were found in the sludge aerobic digester unit (1537 CFU/m³) and the secondary settlers (4 CFU/m³), respectively. Average concentrations of bacterial-aerosols were much lower in summer (742 CFU/m³) as compared to winter (3780 CFU/m³). Given the observed adverse health effects of bioaerosols on employees and their presence in collected air samples, measures to prevent and reduce the spread of bacterial-aerosols in the environment were necessary and the use of personal protection equipments was deemed to be imperative.

Keywords: Health effects; Bacterial-aerosols; Wastewater treatment plant; Anderson sampler; Kerman

1. Introduction

Depending on the type of equipment used, treatment capacity and operating conditions, wastewater treatment plant (WWTP) can be the sources of toxic chemical gases, malodor active substances and micro-organisms which can be released into the air [1]. Aeration and mechanical agitation of wastewater produce

air bubbles and its eruption can form aerosol particles facilitating bacteria and viruses to permeate the air [2].

The infiltration of bioaerosols into the respiratory system causes negative effects such as allergies, respiratory problems, infectious diseases, and hypersensitivity reactions [3]. Several studies have shown the significant problems of workers in sewage-treatment plants having symptoms such as headache, fatigue

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and nausea [4], gastroenteritis [5], stimulation of the eyes, nose, and air routes as well as fever [6,7]. The depth of penetration into the respiratory system increases with the decreasing size of aerosols [8]. Aerosols contain microorganisms that are capable of spreading over a long-distance [9]. The structure, size, concentration and microbial populations of bioaerosols differ by source, dispersal mechanisms in the air and mainly prevalent environmental conditions such as temperature, wind speed, and humidity [10–12]. Temperature can reduce or increase the bacterial concentration of aerosols [13]. An increase in the degree of moisture, especially under direct sunlight, increases bacterial retention time [13,14]. When relative humidity is higher than 35% or wind speed is high, the amount of airborne microorganisms in the air is also higher [2]. Studies have shown that the amount of total bacteria and Coliform organisms at night was higher than that of the day and this was associated with bacterial death time [2].

The probability of pathogens dispersion as bioaerosols, which in turn results in causing diseases among employees and people residing near sewage-treatment plants, is high. Treatment plants are not specifically designed to have different treatment units to prevent the spread of bioaerosols. Thus, bioaerosols' evaluation has gained in importance in the last decade [15,16]. Several studies have confirmed the dispersion of microbial pollution from wastewater treatment plants. Filipkowska et al. [1] determined emissions of microbial contamination from the Bartoszyce (Poland) WWTP with an activated sludge process. The study showed that aeration tanks were an important source of bacterial-aerosol emission and that lesser amounts of microorganism would be released to the atmosphere from wastewater collection stations and secondary sedimentation tanks [1]. Fannin et al. [17] surveyed aerosols containing bacteria and viruses in late summer and fall in America before and during the operation of activated sludge. It was observed that when the plant was operational, the concentration of airborne particles, containing Total Coliforms, Fecal Coliforms, Fecal Streptococcus and Coliphages in the air surrounding the plant increased significantly. Fecal Streptococcus observed in the aerosol further showed more stability than other studied microorganisms. It was also found that the concentration of aerosols containing microorganisms was higher at night as compared to the day [17]. In another study, Giancarlo et al. [18] used two methods, PCR and culture to assess aerosol release from a WWTP in Italy. The study showed that high levels of aerobic bacteria were released from the aeration units [18].

2. Aims and objectives

This study was aimed at determining the amount, type, and size of bacterial-aerosols (Total bacteria, Fecal Coliforms, Total Coliforms, and Fecal Streptococcus) released in the air through the various treatment units of a WWTP located in Kerman (Iran), using the activated sludge system with surface aeration. The potential occupational health hazards affecting WWTP's staff as a result of exposure to bacterial-aerosols was also assessed. Results obtained could pinpoint the existence and importances of bioaerosols' health risks associated with the Kerman WWTP to the authorities and provide necessary information to facilitate the implementation of remedial actions.

3. Materials and methods

This cross-sectional analytical study was undertaken from November 2009 to July 2010 in a WWTP using activated sludge system with surface aeration. This WWTP is located in Kerman, one of the most arid provinces in Iran. A schematic representation of Kerman WWTP is given in Figure 1. Ten stations from the mentioned plant with the highest potential for biological aerosol dispersion or the propensity to influence individual health were selected for air sampling as follows:

- (1) Raw wastewater pump station.
- (2) Primary sedimentation tanks.
- (3) Aeration tank (16 surface aerators, each one provides an efficiency of 1.2–2.2 kg of oxygen per horsepower hour).

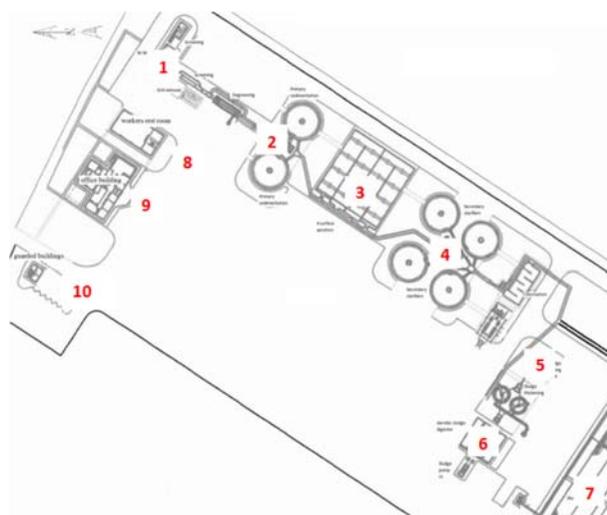


Fig. 1. A schematic representation of Kerman WWTP.

- (4) Secondary sedimentation tanks.
- (5) Sludge mixing tank.
- (6) Aerobic sludge digester.
- (7) Sludge drying beds.
- (8) 25 m far from the treatment facilities (close to workers rest room).
- (9) 50 m far from the treatment facilities (next to office building).
- (10) 100 m in distance from treatment facility (close to guarded buildings).

Health effects on plant workers were assessed by checklist and interviews by an internist. The checklist contained 18 questions facing the most likely pathogens common health effects that were compiled by an internist and completed by 10 persons, all of whom were plant personnel.

Air and wastewater samples were collected in eight days through the year (covering different seasons and weather conditions) and analyzed for Standard Plate Count (SPC), Total Coliforms (TC), Fecal Coliforms (FC) and Fecal Streptococci (FS). Wastewater sampling was conducted simultaneously and the above-mentioned parameters were measured.

Air samples were collected by a six stages Andersen impactor for 10 min with a vacuum pump operating at a flow rate of 28.3 L/min. Samples were collected at the height of 50 cm above ground level and next to the units' access routes. Airborne particles carrying bacteria collided with surface plates containing culture media at each stage and microorganisms remained directly on the surface of the agar [8]. Samples obtained from the Anderson sampler were taken to the laboratory in less than 4 h after sampling for the SPC. Specific groups of bacteria in the air were carried out using replica plates described by Lederberg and Lederberg in 1952 [19]. With this method, a duplicate colony, which had grown on the Nutrient Agar plate was selected and replicated to the selective cultural media. The Total and Fecal Coliforms were cultivated in BGL Broth and EC Broth (Merc, Germany) for 24–48 h at 35 ± 0.5 and 44 ± 0.5 °C, respectively. Positive results for the total and Fecal Coliforms were turbid and gas formed ones. *Escherichia coli*, *Salmonella typhimurium*, *Shigella flexneri*, *Enterococcus aerogenes* were cultured on M-FC Agar (High Media, India) and incubated for 18–24 h at 44 ± 0.5 °C. Positive colonies of *E. coli*, *S. typhimurium*, *S. flexneri*, and *E. aerogenes* were in dark blue, pink to red, colorless and gray colors, respectively. Growth of *E. coli* and *S. typhimurium* in wastewater samples were surveyed and cultured on Lactos Broth (Merc, Germany) and incubated for 24 h at 35 ± 0.5 °C. *Fecal streptococci* content of both air and wastewater samples were cultured on an KF-Strep.

Agar (Merc, Germany) at 35 ± 0.5 °C for 24–48 h and violet colonies were considered as positive results [20]. *Proteus mirabilis* was also cultured on M-Endo Agar (Merc, Germany) and after 20–24 h incubation at 35 ± 0.5 °C the reddish color implied positive results. A brief summary of the conditions and method used for bacteria cultivation is shown in Table 1. Nutrient agar culture media was also augmented with cycloheximide 0.01% for fungal growth inhibition. After incubation, colonies were counted and the results were reported as colony-forming units per cubic meter of air (CFU/m³).

Atmospherical parameters, including wind speed and direction, temperature and moisture were recorded using portable devices and the resulting data was used to determine the atmospheric stability using the Pasquill Table, which classifies stability based on solar radiation and wind speed on a scale from very unstable(A) to very stable(G) [21].

Sampling of a wastewater was done at pumping stations, aeration tank, secondary sedimentation tanks, and anaerobic sludge digester tanks simultaneous with air sampling. Fecal and Total Coliforms were measured according to Standard methods of water and wastewater examination [22], and wastewater Fecal Streptococcus was determined based on SPC method and KF streptococcus Agar media.

In order to determine the effect of source (wastewater) bacterial concentration on the bioaerosols emission, the emission ratio of each treatment unit is calculated as follows:

$$\text{Emission ratio} = \frac{\text{Air bioaerosols conc.}}{\text{Wastewater bacterial conc.}}$$

4. Results and discussion

Weather conditions taken parallel to sampling are briefly listed in Table 2. As Table 2 reveals, various climatic and atmospheric conditions were observed during the sampling period. In this study, wind speed was measured during sampling, and attempts were made to consider the real weather condition including local windstorm. Kerman is an arid region where strong temperature gradient causes jet streams, local wind and windstorm. Results obtained from sampling studies conducted on 21 November and 24 July showed that during both periods there was a sudden significant increase in wind speed as a result of local windstorm, remaining dominant during the sampling period. Considering the stability condition based on meteorological data and Pasquill table, it can be safely asserted that during the sampling period, autumn had more stable atmospheric conditions than other seasons

Table 1
Cultural media and conditions

	Media	Company	Temperature (°C)	Response	Time (h)
Air samples					
SPC	Nutrient Agar	Merc (Germany)	35 ± 0.5	Growth	18–24
<i>E. coli</i>	M-FC agar	High Media (India)	44 ± 0.5	Dark blue	18–24
<i>Salmonella typhimurium</i>				Pink to red	
<i>Shigella flexneri</i>				Colorless	
<i>Enterococcus aerogenes</i>				Gray	
<i>Proteus mirabilis</i>				Reddish/red	20–24
Fecal streptococci (<i>Enterococcus hirae</i> and <i>faecalis</i> , <i>Str. mitis</i> , <i>Str. bovinus</i> , <i>E. equinus</i> , <i>Str. salivarius</i> and others)	M-Endo Agar KF-Strep. agar	Merc (Germany) Merc (Germany)	35 ± 0.5 35 ± 0.5	Violet	24–48
Wastewater samples					
<i>E. coli</i>	Lactos Broth	Merc (Germany)	35 ± 0.5	Growth + gas	24–48
<i>Salmonella typhimurium</i>				Growth	
Fecal streptococci (<i>Enterococcus hirae</i> and <i>faecalis</i> , <i>Str. mitis</i> , <i>Str. bovinus</i> , <i>E. equinus</i> , <i>Str. salivarius</i> and others)	KF-Strep. Agar	Merc (Germany)	35 ± 0.5	Violet	24–48
Standard total coliform fermentation technique	BGL Broth	Merc (Germany)	35 ± 0.5, 44 ± 0.5	Growth ± gas ± acidic reaction	24–48
Fecal coliform	EC Broth	Merc (Germany)	44 ± 0.5	Growth ± gas	24–48

Table 2
Meteorological observations at dates of sampling

Date of sampling	21 November 2009	29 November 2009	1. February 2010	8 February 2010	10 May 2010	18 May 2010	24 July 2010	1 August 2010
Hours of sampling	10–15	10:45–15	10–14:25	10–14	10–13	9:45–13	9:45–13	10–13:30
Temperature (°C)	20,22	11,15	18,23	–2,2	24,28	23,30	30,34	21,26
Atmospheric observations	Mid cloudy	Rainy	Sunny	Sunny to paucity cloudy	Paucity cloudy	Sunny	Sunny	Sunny
Wind speed (m/s)	3–12	3	5	3	3	5	3–5	3
Dominating wind direction	Of Southeast	Of Northeast	Of East	Of South	Of West	Of East	Of North	Of North
Humidity (%)	39	49	43	41	30	28	19	20
Atmospheric stability	D ^c	C ^b	C ^a	B–C	B	C	B–C	C

^aB: Unstable atmospheric condition. ^bC: Slightly unstable conditions. ^cD: Neutral condition.

and the others' stability fell into unstable and slightly unstable atmospheric conditions. In terms of atmospheric conditions, the maximum amount of biological aerosols was 4784 CFU/m³ related to the class C, and its minimum one was 713 CFU/m³ related to the class B of atmospheric stability.

Assessing mean SPC results (Table 3) showed that 29% of the total count was related to the aerobic sludge digester and secondary clarifiers had allocated the least emission rate. SPC results were in the wide ranges due to the treatment units and atmospheric conditions. The maximum bacterial-aerosol was 1,537 CFU/m³ related to the aeration tank, and the minimum one was about 4 CFU/m³ related to the 100 m away from the facility. Similar to our results, other studies were attributed the presence of high levels of bacteria in the air to the kind of treatment units, adjacency to the entrance channels, sludge treatment process [23]. Sanchez-Monedero et al. (2008) identified the main bioaerosol sources and surveyed the effect of the aeration systems used in the biological treatment; they showed that pre-treatment, biological treatment, and sludge thickening were the processes that generated the highest amount of bioaerosols, also, they showed that surface aeration by means of mechanical agitation produced a larger amount of bioaerosols (between 450 and 4,580 CFU/m³) than other aeration systems [24]. It can say that high bacterial aerosol emission rate of these units is due to the vigorous mixing and aeration which cause more wastewater aerosolized and also the higher bacteria concentration of these units

Table 3
Bacterial-aerosols size distribution at different locations (average of sampling period)

Location	Percent of total counts	∑ (CFU/m ³)
At the row sewage pumping station	20	469
Primarily settlers	13	289
Aeration tank	25	587
Secondary settlers	2	49
Sludge blending tank	3	60
Sludge aerobic digester tanks	29	684
Discharge point	3	69
25 m of the treatment equipments	2	39
50 m of the treatment equipments	1	25
100 m of the treatment equipments	2	38

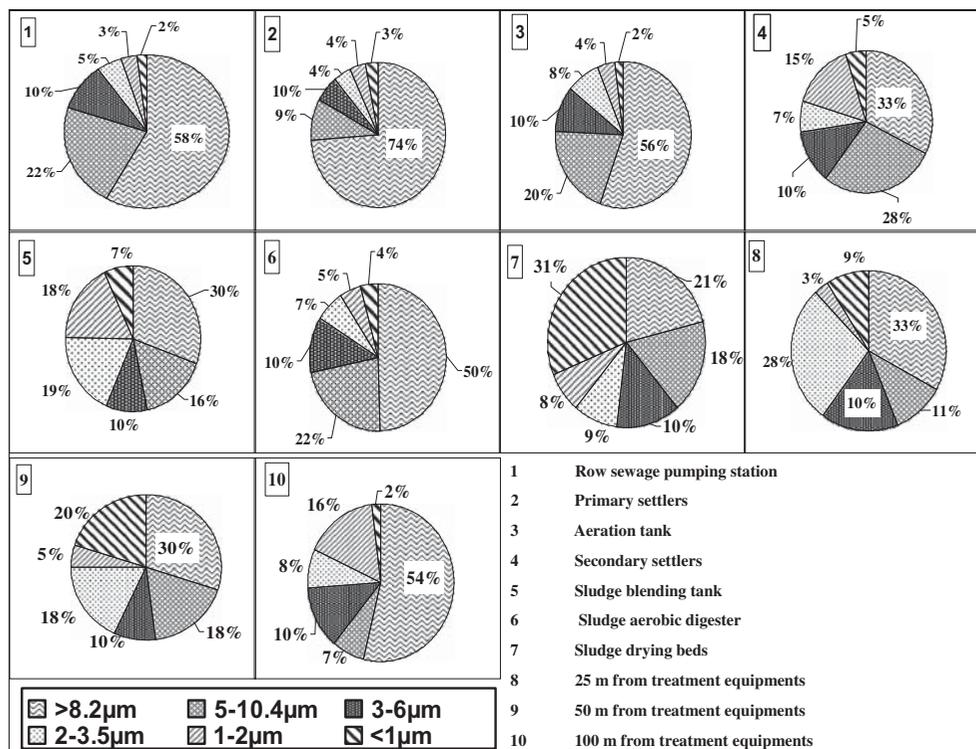


Fig. 2. Bioaerosols size distribution among treatment plant's units (annual average).

The seasonal analysis of bacterial-aerosol concentration revealed that in winter 3780 CFU/m³, in autumn 3730 CFU/m³, in summer and spring 742 and 985 CFU/m³ of bacterial-aerosols (mean value for each season) were counted for the whole WWTP respectively. Winter showed the highest rate of bacterial-aerosols emission. Oppliger et al. (2005) reported that the average concentration of WWTP aerosol bacteria was in the range of 6,058 CFU/m³ in summer and 6,690 CFU/m³ in winter [25]. However, in Kerman WWTP, the average concentration of bacteria in aerosol was 742 CFU/m³ in summer and 3,780 CFU/m³ in winter. Both studies verified the presence of higher concentrations of bioaerosols in winter as compared to summer. Moreover, increasing temperature and decreasing humidity reduced the amount of aerosol's bacterial content. This fact confirmed the substantial effect of meteorological conditions on bacterial survival.

Karra and Katsivela (2007) studied bioaerosol emission from WWTP during summer at a Mediterranean site. The study showed that among environmental parameters affecting survival of airborne microorganisms, relative humidity was of utmost importance because bioaerosols absorb humidity, acting as a protecting shield against UV radiation [26].

The particle size distribution of bioaerosol through the surveyed treatment units (Fig. 2) were of high

concentration at large particle size. The possible reasons can be cited as the high background level of large particles in Kerman air due to its desert characteristics (not published yet) which can act as a condensed nuclei.

A few studies surveyed the bioaerosols size distribution in WWTP. Bausum et al. in 1981 measured

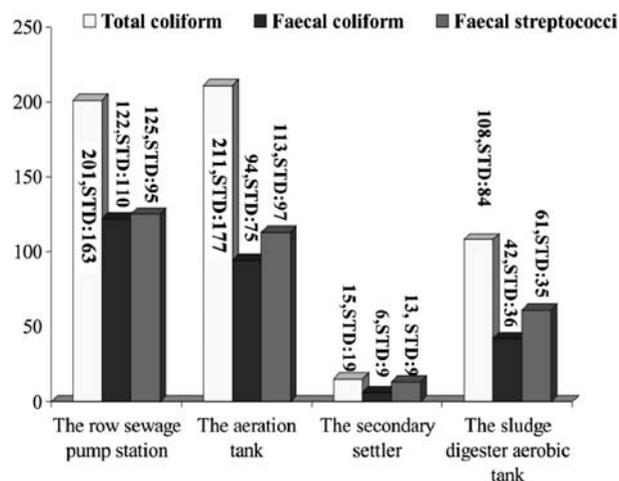


Fig. 3. Total Coliform, Faecal Coliform and Faecal Streptococci at the Kerman WWTP (annual mean count of sampling period).

Table 4
Results from the selective cultural media (annual average)

Treatment unit	Microorganism	(CFU/m ³)							
		>8.2 µm	5–10.4 µm	3–6 µm	2–3.5 µm	1–2 µm	<1 µm		
Raw wastewater pump station	<i>Enterococcus hirae, aerogenes, and faecalis</i>	82	25	5	3	4	6		
	<i>E. coli</i>	59	37	5	4	7	10		
	<i>Proteus mirabilis</i>	52	18	11	2	9	1		
	<i>Salmonella typhimurium</i>	34	26	3	1	0	5		
Primary sedimentation tanks	<i>Shigella flexneri</i>	55	15	8	2	3	2		
	<i>Enterococcus hirae, aerogenes, and faecalis</i>	11	4	2	1	1	1		
	<i>E. coli</i>	17	6	2	2	1	3		
	<i>Proteus mirabilis</i>	11	3	6	0	1	0		
Aeration tank	<i>Salmonella typhimurium</i>	13	2	2	1	0	9		
	<i>Shigella flexneri</i>	15	10	2	1	1	3		
	<i>Enterococcus hirae, aerogenes, and faecalis</i>	70	18	8	7	4	6		
	<i>E. coli</i>	45	22	8	9	3	7		
Secondary sedimentation tanks	<i>Proteus mirabilis</i>	35	38	14	13	6	11		
	<i>Salmonella Typhimurium</i>	29	11	11	14	2	4		
	<i>Shigella flexneri</i>	35	6	15	14	5	5		
	<i>Enterococcus hirae, aerogenes, and faecalis</i>	3	5	1	1	3	1		
Sludge mixing tank	<i>E. coli</i>	1	1	2	0	2	0		
	<i>Proteus mirabilis</i>	4	1	0	0	2	1		
	<i>Salmonella typhimurium</i>	2	0	1	0	1	40		
	<i>Shigella flexneri</i>	2	3	2	0	36	20		
Aerobic sludge digester	<i>Enterococcus hirae, aerogenes, and faecalis</i>	6	4	1	1	0	2		
	<i>E. coli</i>	3	1	0	3	0	0		
	<i>Proteus mirabilis</i>	3	3	0	1	0	1		
	<i>Salmonella typhimurium</i>	2	2	1	1	1	0		
Sludge drying beds	<i>Shigella flexneri</i>	4	1	1	2	0	0		
	<i>Enterococcus hirae, aerogenes, and faecalis</i>	32	8	7	7	2	5		
	<i>E. coli</i>	23	5	4	1	6	1		
	<i>Proteus mirabilis</i>	37	9	9	11	6	2		
Sludge drying beds	<i>Salmonella typhimurium</i>	60	17	9	4	10	2		
	<i>Shigella flexneri</i>	38	6	11	4	4	2		
	<i>Enterococcus hirae, aerogenes, and faecalis</i>	3	1	0	7	0	1		
	<i>E. coli</i>	2	2	2	1	0	2		
Sludge drying beds	<i>Proteus mirabilis</i>	2	0	0	1	1	8		
	<i>Salmonella typhimurium</i>	1	1	1	1	1	2		
	<i>Shigella flexneri</i>	1	2	0	1	2	3		

(Continued)

Table 4 (Continued)

Treatment unit	Microorganism	(CFU/m ³)						
		>8.2 μm	5–10.4 μm	3–6 μm	2–3.5 μm	1–2 μm	<1 μm	
25 m far from the treatment facilities	<i>Enterococcus hirae, aerogenes, and faecalis</i>	3	0	2	3	0	1	
	<i>E. coli</i>	2	0	0	0	0	0	
	<i>Proteus mirabilis</i>	1	0	0	1	0	0	
	<i>Salmonella typhimurium</i>	1	0	0	1	0	0	
	<i>Shigella flexneri</i>	0	1	0	2	0	0	
50 m far from the treatment facilities	<i>Enterococcus hirae, aerogenes, and faecalis</i>	3	0	2	2	1	2	
	<i>E. coli</i>	0	0	0	0	0	0	
	<i>Proteus mirabilis</i>	0	3	0	0	0	1	
	<i>Salmonella typhimurium</i>	0	0	0	0	0	0	
	<i>Shigella flexneri</i>	0	0	0	0	0	0	
100 m far from the treatment facilities	<i>Enterococcus hirae, aerogenes, and faecalis</i>	6	0	1	0	1	0	
	<i>E. coli</i>	2	0	2	0	0	0	
	<i>Proteus mirabilis</i>	3	1	0	0	1	0	
	<i>Salmonella typhimurium</i>	4	1	1	0	0	0	
	<i>Shigella flexneri</i>	3	1	1	0	1	0	

microbial emission resulting from irrigation in Fort Huachuca, Arizona by Anderson sampler at night. The amount of bacterial-aerosols in 125 yards downwind was 500 CFU/m³, at 46 m distance far from the facility 10,500 CFU/m³ and average aerodynamic diameter was approximately 5 μm [27]. The mean concentration in our study within 100 m of the facility was 18.5 CFU/m³ (maximum: 38.85, minimum: 3.53); at 50 meters, it was 25 CFU/m³ (maximum: 84, minimum: 7) and at 25 m far from facility it was 24 CFU/m³ (maximum: 88, minimum: 7).

Wastewater contains high levels of pathogenic microorganisms that may not be easily detected [28]. So, previous studies have used indicator organisms such as Coliform, Fecal Coliform, Fecal Streptococci to assess the potential public health risk [28]. But some researchers have shown the inadequacy of these indicators and tried to replace them [29]. Therefore, in this study, as well as mentioned indicators, the most pathogenic microorganisms were also investigated.

Fig. 3 summarized the annual average concentration of the main microorganisms groups measured at key locations of the WWTP, The largest emission of Total Coliform, Fecal Coliform, and Streptococci was related to the raw wastewater pumping station and the aeration tank. It should be mentioned that there are not any significant differences between these two points ($p_{\text{value}} > 0.05$).

Based on the colonies' shape and color of the selective cultural media, the mean concentrations of the detected pathogen microorganisms in the cultivated bioaerosols for all monitoring period are shown in Table 4.

As observed in Table 4, *Enterococcus hirae*, *Aerobacter aerogenes*, *Enterococcus faecalis*, *S. Typhimurium*, *E. coli*, *S. flexneri*, and *Proteus mirabilis* were the most predominant strains, all of which, except *Enterococcus faecalis*, were Gram negative. Further, most of them were rod-shaped and facultative anaerobic. As this Table shows, the most observed bacterial species were associated with *E. hirae*, *A. aerogenes*, and *E. faecalis* with 386 CFU/m³ and minimum observed one was *E. coli* about 318 CFU/m³. *S. Typhimurium*, *S. flexneri* and *P. mirabilis* have counted 337, 347, and 349 CFU/m³, respectively. As mentioned previously, the counted colonies on the selective cultural media showed that aeration tank, aerobic digester, and primary sedimentation showed the highest emission rate. These results point to the importance of aeration and aeration tank surface area in the air borne emission of the pathogens. The analysis of the bacterial concentration of wastewater samples showed that highest SPC and Fecal Coliforms were related to the aerobic sludge digester and that raw wastewater pumping station

Table 5

Emission ratio of the air bacterial concentration to the raw wastewater bacterial concentration (Annual mean count of samples)

	Fecal Streptococcus	Fecal Coliform	Total Coliform	SPC ^a
The sludge aerobic digester tanks	2E–05	1.63E–06	3.79E–06	1.07E–05
The aeration tank	1.57E–05	8.87E–06	2.81E–06	7.53E–06
The row sewage pumping station	6.2E–06	1.03E–05	3.37E–06	6.61E–07
The secondary settlers	1.86E–06	8.53E–07	1.21E–06	9.47E–07

^aSPC: Standard Plate Count.

had the most Fecal Streptococcus bacteria. As previously mentioned, it can be said that even the concentration of the source is higher; the higher concentrations of bioaerosols are emitted. In order to determine the emission rate of the different treatment units, the percent ratio of the bacteria in the air to the average bacteria observed in the source (wastewater) in the examined days were determined. As obvious from Table 5, generally, the highest ratio is seen in the sludge aerobic digester tanks followed by aeration tank and pumping station.

In a report by Sawyer et al. (1993), rate of bacterial-aerosols at the surface of aeration tanks of a WWTP in Great Chicago was measured using the Anderson sampler. Samples were tested for SPC, Total Coliforms, Fecal Coliforms, and Fecal Streptococci. Similar to our study, they showed that *F. Coliforms* and *Streptococci* have had lower rates of release than Total Coliforms [20].

In 2004, Oppliger et al. evaluated 11 wastewater treatment workers for airborne cultivable bacteria, fungi, and digestive endotoxin. They were assessed in enclosed areas of the plant for bioaerosols and also the effect of seasons (summer and winter) on the amount of bioaerosols. Results showed that a wide range of *Enterobacteriaceae* families and *Pseudomonas* were found [25]. Similar to the study by Oppliger et al., the evaluation of the concentration and composition of bacterial aerosols in Kerman WWTP showed different genera of the *Enterobacteriaceae* family.

Detected microorganisms are largely considered as sources of gastrointestinal diseases, especially in nosocomial environments and antibiotic resistance of these strains is on the rise [30]. The presence of Coliform and Fecal Coliform may indicate potential contamination that can cause diarrhea, cramps, nausea, headaches [31], respiratory, and allergic diseases. Fecal Streptococci primarily related to human feces [31] and associated with respiratory illnesses [32]. The observation of the adverse effects of bioaerosols on all Kerman WWP staff, whether during or after work, point

to this fact. Results from checklists revealed that common observed health effects were fatigue during and after work in 100% of staff, dizziness, and eye irritation in 30% of workers, 20% of workers suffered from abdominal infections which resulted in severe abdominal pains accompanied by fever and chills often in the afternoon or evening, lasting until the next day. General paralysis of the body, headache, chills, fever, dysentery, irritation, itchy nose, and pulmonary problems in 10% of cases were reported.

Previous study showed that cell lysis of many gram-negative bacteria produces toxic lipopolysaccharides (LPS) called endotoxins also some strains like *E. coli* generate verotoxins, and heat-labile enterotoxins, that bring on immune responses [33].

Melbostad et al. (1994) surveyed 12 participants from five different WWTP in East Norway. They showed that total amounts of bacteria were in the ranges between zero to 9.5×10^6 CFU/m³ with a mean of 5.2×10^5 CFU/m³, and Spherical bacteria level from zero to 6.9×10^6 CFU/m³ with a mean concentration of 3.3×10^5 CFU/m³. Also a significant relationship between the total amounts of bacteria and rod-shaped bacteria and symptoms such as fatigue and headache at work and after work were observed in the participants [4]. However, the total amount of bacteria measured in Kerman WWTP showed a lesser amount than that of Melbostad et al. This could be due to the environmental conditions and water consumption pattern of the society which can dilute the influent bacterial concentration.

5. Conclusion

In terms of atmospheric conditions, the maximum amount of biological aerosols was 4,784 CFU/m³ related to the class C, and its minimum one was 713 CFU/m³ related to the class B of atmospheric stability.

Maximum and minimum concentrations of bacterial-aerosols were found at the aerobic sludge digester unit (1,537 CFU/m³) and the secondary settlers

(4CFU/m³), respectively. Also, Average concentrations of bacterial-aerosols were much lower in summer (742CFU/m³) as compared to winter (3,780CFU/m³). The most predominant detected strains except *E. faecalis* were Gram negative. Further, most of them were rod-shaped and facultative anaerobic. Given the observed health effects caused by bioaerosols among wastewater workers in Kerman and the presence of airborne bacteria in different amounts, which in turn were capable to infiltrate the respiratory system, could be one of the sources of diseases as mentioned. In order to protect the health of plant employees and nearby neighbors, it is deemed necessary to implement measures to control and prevent the spread of bioaerosols in the environment. Since the exposure of bioaerosols is not the unique way of appearance of adverse health effects, there is a further need to educate employees on work hygiene. Further, authorities need to ensure that protective equipments are made available to employees who should be encouraged to use them.

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References

- Z. Filipkowska, W. Janczukowicz, M. Krzemieniewski, J. Pesta, Microbiological air pollution in the surroundings of the wastewater treatment plant with activated-sludge tanks aerated by horizontal rotors, *Pol. J. Environ. Stud.* 9(4) (2000) 273–280.
- N.J. Brown, Health Hazard Manual: Wastewater Treatment Plant and Sewer Workers, New York State Department of Labor Grant #C005413. <http://digitalcommons.ilr.cornell.edu/manuals/2>, 1997.
- G. Bitton, *Wastewater Microbiology*. 3rd ed, John Wiley & Sons, Hoboken, NJ, 2005.
- E. Melbostad, W. Eduard, A. Skogstad, P. Sandven, J. Lassen, P. Sostrand, K. Heldal, Exposure to bacteria aerosol and work-related symptoms in sewage workers, *Am. J. Ind. Med.* 25 (1994) 59–63.
- S.A. Khuder, T. Arthur, M.S. Bisesi, E.A. Schaub, Prevalence of infectious diseases and associated symptoms in wastewater treatment workers, *Am. J. Ind. Med.* 33 (1998) 572–577.
- M. Lundholm, R. Rylander, Work-related symptoms among sewage workers, *Br. J. Ind. Med.* 40 (1983) 325–329.
- S.C. Clark, Potential and actual biological related health risks of wastewater industry employment, *J. Water Pollut. Control Fed.* 59 (1987) 999–1008.
- A. Andersen, New sampler for the collection, sizing, and enumeration of viable airborne particles, *J. Bacteriol.* 76 (1958) 471–474.
- A. Bovallius, B. Bucht, R. Roffey, P. Anäs, Longrange air transmission of bacteria, *Appl. Environ. Microbiol.* 35 (1978) 1231–1232.
- G. Gregov, J. Venglovski, M. Vargova, O. Ondrasovvicova, O. Ondrasovic, N. Sasakova, D. Kudrikova, K. Laktidovi, Bio-aerosols Produced by Wastewater Treatment Plant, *Folia Veterinaria* 52(2) (2008) 59–61.
- P. Butelli, Impatto da aerosol batterici negli impianti di depurazione [Impact of bacterial aerosols in treatment plants], *Ing. Ambient.* 17 (1988) 18–32.
- G. Bitton, *Wastewater Microbiology, Biological Aerosols and Bioodors From Wastewater Treatment Plants*, third ed., John Wiley & Sons, Hoboken, NJ, 1994.
- B.A. Handley, A.J.F. Webster, Some factors affecting the airborne survival of bacteria outdoors, *J. Appl. Bacteriol.* 79 (1995) 368–378.
- B. Marthi, V.P. Fieland, M. Walter, R.J. Seidler, Survival of bacteria during aerosolisation, *Appl. Environ. Microbiol.* 56 (1990) 3463–3467.
- B. Carnow, R. Northrop, R. Wadden, S. Rosenberg, J. Holden, A. Neal, L. Sheaff, P. Scheff, S. Meyer, Health effects of aerosols emitted from an activated sludge plant, United States Environmental Protection Agency, Cincinnati, Ohio, Publication EPA-600/1-79-019, 1979.
- D.E. Johnson, D.E. Camann, J.W. Register, R.J. Prevast, J.B. Tillery, R.E. Thomas, J.M. Taylor, J.M. Hosenfeld, Health implications of sewage treatment facilities, United States Environmental Protection Agency, Cincinnati, Ohio, Publication EPA-600/1-78-032, 1979.
- K.F. Fannin, S.C. Vana, W. Jakubowski, Effect of an activated sludge wastewater treatment plant on ambient air densities of aerosols containing bacteria and viruses, *Appl. Environ. Microbiol.* 49 (1985) 1191–1196.
- R. Giancarlo, P. Pamela, S. Claudia, Bacterial aerosol emission from wastewater treatment plants: Culture methods and biomolecular tools, *Aerobiologia* 16 (2000) 39–46.
- J. Lederberg, E.M. Lederberg, Replica plating and indirect selection of bacterial mutants, *J. Bacteriol.* 63 (1952) 399–406.
- B. Sawyer, G. Elenbogen, K.C. Rao, P. O'Brien, R. Zenz, C. Lue-Hing, Bacterial aerosol emission rates from municipal wastewater aeration tanks, *Am. Soc. Microbiol.* 59(10) (1993) 3183–3186.
- J. Colls, *Air Pollution*, second ed., Spon Press, London, 2002.
- APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, 1998.
- S. Laitinen, J. Kangas, M. Kotimaa, J. Liesivuori, P.J. Martikainen, A. Nevalainen, R. Sarantila, K. Husman, Workers' exposure to airborne bacteria and endotoxin in industrial wastewater treatment plants, *Am. Ind. Hyg. Assoc. J.* 55(11) (1994) 1055–1060.
- M.A. Sanchez-Monedero, M.I. Aguilar, R. Fenoll, A. Roig, Effect of the aeration system on the levels of airborne microorganisms generated at wastewater treatment plants, *Water Res.* 42 (2008) 3739–3744.
- A. Oppliger, S. Hilfiker, T. Vu Duc, Influence of seasons and sampling strategy on assessment of bioaerosols in sewage treatment plants in Switzerland, *Br. Occupat. Hygiene Soc.* 49 (5) (2005) 393–400.
- S. Karra, E. Katsivela, Microorganisms in bioaerosol emissions from wastewater treatment plants during summer at a Mediterranean site, *Water Res.* 41 (2007) 1355–1365.
- H. Bausum, S. Schaub, K.F. Kenyon, M.J. Small, Comparison of coliphage and bacterial aerosols at a wastewater spray irrigation site, *Appl. Environ. Microbiol.* 43(1) (1982) 28–38.
- S.R. Jenkins, C.W. Armstrong, Health Effects of Biosolids Applied to Land: Available Scientific Evidence, Virginia Department of Health, 2007.
- V.A. Majeti, C.S. Clark, Potential health effects from viable emission and toxins associated with wastewater treatment plants and land application sites, Health Effect Research Lab-

- oratory Office of research and Development, US Environmental Protection Agency, Washington, DC, EPA/600/1-81/006, 2004.
- [30] D.B. Huang, J. Zhou, Effect of intensive handwashing in the prevention of diarrhoeal illness among patients with AIDS: A randomized controlled study, *J. Med. Microbiol.* 56 (2007) 659–663.
- [31] J.A. Salvato, N.L. Nemerow, F.J. Agardy, *Environmental Engineering*, Wiley & Sons, Hoboken, NJ, 2003.
- [32] M. Stevens, N. Ashbolt, D. Cunliffe, Review of Coliforms, As Microbial Indicators of Drinking Water Quality, Recommendations to change the use of Coliforms as microbial indicators of drinking water quality, NHMRC publications, Biotext Pty Ltd., Canberra, 2003.
- [33] E. Korzeniewska, M. Harnisz, Culture-dependent and culture-independent methods in evaluation of emission of enterobacteriaceae from sewage to the air and surface water, *Water Air Soil Pollut.* 223 (2012) 4039–4046.