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# Investigation of Bacterial and Fungal Bioaerosol Emissions and Non-Carcinogenic Risk Assessment in the Hospital Wastewater Treatment Plant

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**Abstract**

**Introduction:** Bioaerosols emitted from wastewater treatment plants can significantly impact human health. This study aimed to evaluate the concentrations of airborne bacteria and fungi and assess their associated risks at the WWTPs of Imam Sajjad Hospital in Yasuj.

**Methods:** Air samples were collected using an airborne microbial sampler (QuickTake 30) at a flow rate of 28.3 L/min for 5 minutes. The sampling locations included five sites within the WWTPs, two sites located 100 m and 500 m downstream, and a background sampling site. A total of 240 samples were collected. A risk assessment for airborne bacteria and fungi was conducted using the United States Environmental Protection Agency model. Bacterial genera were identified through the examination of Gram-stained smears, morphological analysis, and biochemical tests. Fungal colonies were identified based on colony characteristics and spore morphology assessment, and the data were analyzed using SPSS software version 20.

**Results:** The aeration tank exhibited the highest airborne bacterial concentration (2001.25 ± 2139.36 CFU/m³), while the grit chamber recorded the lowest concentration (592.57 ± 351.13 CFU/m³). For fungi, the grit chamber had the highest load (482.14 ± 296.98 CFU/m³), whereas the chlorination unit had the lowest concentration at (223.12 ± 133.70 CFU/m³). Staphylococcus and Bacillus were identified as the predominant bacterial genera in the WWTPs, while Rhizopus and Cladosporium were the dominant fungal genera in the WWTPs. The hazard quotient for airborne bacteria and fungi was less than 1, indicating an acceptable level of risk for exposure.

**Conclusion:** To minimize bioaerosol production from aeration tanks, the design of aeration equipment should be optimized.

**Keywords:** Bacteria, Hospital, Penicillium, Risk assessment

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## Introduction

Bioaerosols are airborne biological particles with aerodynamic diameters ranging from nanometers to micrometers. They include microorganisms such as viruses, bacteria, fungi, pollen and are increasingly recognized as a significant environmental health concern (1,2). WWTPs are identified as important sources of bioaerosol emissions. Various steps in the wastewater treatment process including discharge, mixing, aeration, and spraying, can release microorganisms into the atmosphere, leading to the formation of bioaerosols (1,3). Bioaerosols present in the air of WWTPs can enter the human body through inhalation, skin contact, or ingestion, potentially leading to a variety of health effects for both treatment plant workers and nearby residents (4-6). Opportunistic pathogens, include Legionella, Acinetobacter, Aeromonas, Arcobacter, Bacillus, Mycobacterium, and Pseudomonas found in the WWTPs (3,7,8). Research has demonstrated that inhalation exposure to bacterial bioaerosols is approximately $10^5$ times more potent than skin exposure (9). Inhaling bioaerosols is linked to a wide array of adverse health outcomes, ranging from acute respiratory irritation and allergic reactions to chronic respiratory diseases (7,10). Prolonged or high-

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level exposure may contribute to long-term respiratory disorders. Studies have reported various health effects among WWTPs workers due to bioaerosols containing pathogenic microorganisms, including respiratory infections, gastrointestinal symptoms, hypersensitivity, and allergies (3,11). A study by Chalvatzaki et al. identified a significant correlation between the level of exposure to various airborne bacteria and the incidence of respiratory and influenza-like symptoms in WWTPs workers (12). The impact of bioaerosols on human health is influenced by the type of microbial species and particle size (7,11,13). Research has identified the presence of bioaerosols in WWTPs across different cities and regions worldwide (3,6,7,11,13,14), as well as in Iran (4,9,15-18) and has found that microbial diversity in bioaerosols is associated with specific regions. In addition, concerns have been raised regarding their ability to travel long distances. Therefore, exposure to airborne bioaerosols poses a risk not only to nearby workers but also to residents in surrounding areas (3,19). Furthermore, the concentration and level of human exposure to bioaerosols may vary depending on the route of exposure (skin contact, ingestion, and inhalation), weather conditions, personal protective equipment (PPE), ventilation systems, type and capacity of the plant, and the activities performed (3,17,20). Several studies have demonstrated that mechanical wastewater treatment devices and mixing processes at various stages of wastewater treatment are the primary sources of microorganism release into the atmosphere (1,3,4, 9,21). Research indicates that in WWTPs with the activated sludge process, aeration basins—particularly those employing aeration systems such as horizontal rotors, surface turbines, and sludge processing units—are significant contributors to bioaerosol emissions. Since airborne bioaerosols typically adhere to the surfaces of small particles, they can be easily transported by wind, allowing them to travel considerable distances (18,22). In addition to treatment processes, bioaerosol concentrations can be affected by environmental parameters such as temperature. For example, Wang's research shows that the emission of airborne bacteria as aerosols is higher in cold seasons and decreases in warm seasons (23). However, an opposite result was obtained in an indoor wastewater treatment plant, where the amount of cultured bacteria was one order of magnitude greater in summer than in winter (24). Hospital wastewater contains a diverse array of pathogenic and non-pathogenic microorganisms that can be released into the environment as bioaerosols during various processes at treatment plants. Given the increasing concern about the health effects of bioaerosols, it is essential to assess the exposure risks for workers and residents adjacent to WWTPs. Therefore, this study aimed to investigate the concentrations, and composition of airborne bacterial and fungal communities in the WWTPs of Imam Sajjad Hospital in Yasuj, using the culture method. In addition, non-carcinogenic health risks were calculated using the US Environmental Protection Agency's (USEPA) risk assessment model with average daily dose and hazard quotient (HQ). The findings of this study can be useful in identifying areas and processes with the highest of bioaerosol emissions and lead to making the necessary changes in the design and structure of the WWTPs.

## Methods and Materials

### *The Study Area and Sampling Sites*

This descriptive-analytical study was conducted at the WWTPs of Imam Sajjad Hospital in Yasuj City, located in the southwest of Iran, near the Bashar River. The geographical coordinates are 30° 39' 6" North and 51° 36' 54" East (Figure 1).

Imam Sajjad Hospital covers an area of approximately 17,000 square meters and includes 17 inpatient departments, 9 specialized and sub-specialized paraclinics, and a total of 263 beds. The distance from the WWTPs to the hospital is approximately 200 meters, while the distance to residential homes is about 300 meters. The hospital's wastewater treatment system employs an activated sludge




**Figure 1.** Location of the study area

process with extended aeration, treating an average influent of approximately 300 cubic meters per day. The components of this treatment system include a bar screen, a horizontal grit chamber, an aeration tank equipped with four surface turbine aerators, a to secondary settling tank, and a chlorination unit (Figure 2).

The selection of sampling sites was based on several criteria, including the location of the unit, wind direction, and accessibility. The sampling locations included five sites within the WWTPs: adjacent to the bar screen, adjacent to the grit chamber, adjacent to the aeration tank, adjacent to the secondary settling tank, and adjacent to the chlorination unit (Figure 2). Additionally, two sites were established outside the WWTPs, located 100 and 500 meters downstream in the prevailing wind direction from the plant's fence. A background sampling site was also established 100 meters upstream of the WWTPs, where it was unaffected by the WWTPs' bioaerosol emissions.

### *Bioaerosol Sampling and Microbiological Analysis*

Samples were collected during the winter season, from December 1, 2022, to March 20, 2023, at a height of 1.5 meters above ground level, which is within the human breathing zone. The sampling was conducted every six days in compliance with the EPA guidelines (4,11). A total of 30 samples were collected from each sampling site, consisting of 15 bacterial samples and 15 fungal samples. In total, 240 samples were collected, which included 120 bacterial samples and 120 fungal samples. A single-stage impactor air sampler, the Quick Tack 30, was used at a flow rate of 28.3 L/min for 5 minutes. This impactor operates based on a collision mechanism. The device features a conical inlet with 400 small holes that aspirate air, causing it to collide onto a 90 mm diameter Petri dish with culture media in the lower biostage to capture bioaerosols. Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) culture media were used for the growth of bacteria and fungi, respectively. Cycloheximide was added into the TSA medium to inhibit fungal growth, while chloramphenicol was added to the SDA to suppress bacterial growth. Before and following sampling, the biostage was disinfected with 70% ethanol to prevent contamination. The sampling pump flow rate has been calibrated using a rotameter to ensure a consistent flow rate of 28.3 L/min. When sampling was complete, the plates containing the culture media were sealed, protected from light, and transported to the microbiology laboratory at Yasuj University of Medical Sciences in a cool box. The bacterial samples were incubated at 37°C for 24 to 48 hours, and the fungal samples were incubated at temperatures 25 to 30°C for 3 to 5 days. Finally, the concentration of bioaerosols is reported in terms of colony-forming units per cubic meter of air (CFU/m$^3$) and is calculated according to Equation 1.

$$C = \frac{N \times 1000}{Q \times T} \quad (1)$$

Where, $C$ is bioaerosol concentration (CFU/m$^3$), $N$ is number of colonies, $Q$ is sampling flow rate (28.3 L/min), and $T$ is sampling duration (5 min).

The bacterial genera was identified through a

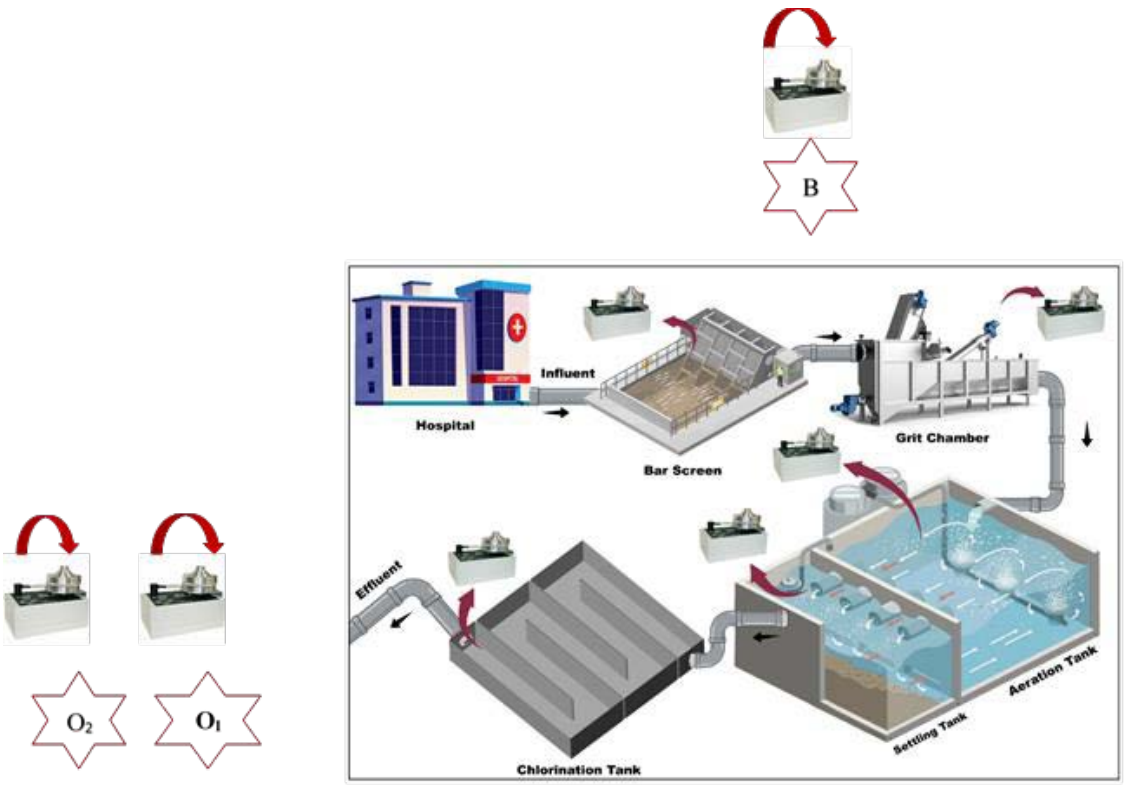



**Figure 2**. Schematic and sampling sites of wastewater treatment plant of Imam Sajjad Hospital, Yasuj, Iran
B: Background site (100 meters upstream of the WWTPs)
$O_1$: Outside the WWTPs (100 m of downstream wind direction from the fence of WWTPs)
$O_2$: Outside the WWTPs (500 m of downstream wind direction from the fence of WWTPs

meticulous examination of Gram-stained smears, complemented by a comprehensive morphological analysis and the application of standard biochemical tests, as outlined in the authoritative Bergey's Manual (25). The fungal colonies were identified by colony characteristics and the assessment of spore morphology using optical microscopes at magnifications of 10x, 40x, and 100x. Concurrently, temperature, humidity, and wind speed were recorded during sampling using a portable KIMO thermometer (Kimo, AMI 300, Japan). Solar radiation was also recorded during the monitoring period according to the weather forecast, as detailed in Table 1.

### *Non-Carcinogenic Health Risk Assessment*

The average daily dose resulting from inhalation exposure and skin contact to airborne bacteria and fungi was calculated using the model proposed by the U.S.EPA. according to Equations 2 and 3 (7,13,20).

$$\mathrm{ADD}_{\mathrm{Inhalation}} = \frac{(\mathrm{C} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED}_{\mathrm{inhalation}})}{(\mathrm{BW} \times \mathrm{AT})} \quad (2)$$

$$\mathrm{ADD}_{\mathrm{Skin}} = \frac{(\mathrm{C} \times \mathrm{SA} \times \mathrm{ABS} \times \mathrm{AF} \times \mathrm{EF} \times \mathrm{ED}_{\mathrm{skin}})}{(\mathrm{BW} \times \mathrm{AT})} \quad (3)$$

$ADD_{inhalation}$ and $ADD_{skin}$ refer to the average daily dose from inhalation exposure and from skin contact (CFU/(kg. d)), *C* refers to the concentration of airborne bacteria or fungi (CFU/m³), *IR* is the inhalation rate (m³/day), *EF* is the frequency of the exposure (days/year), $ED_{inhalation}$ and $ED_{skin}$ refer to the duration of exposure via inhalation and skin contact (years), *SA* is the skin contact area (m²), *ABS* is the absorption factor of the skin (m/h), *AF* is the skin adherence factor, *BW* is the average body weight (kg), and *AT* is the average life expectancy (d).

All parameters for Equations 2 and 3 were obtained from the pervious studies (3,7,26,27), as detailed in Table S1. The non-carcinogenic risk of airborne bacteria and fungi was calculated using Equations 4 and 5.

$$\mathrm{RFD} = \frac{RFC \times IR}{BW} \quad (4)$$

$$HQ = \frac{\mathrm{ADD}}{\mathrm{RFD}} \quad (5)$$

*HQ* refers to the Hazard Quotient and the reference dose (RFD) is maximum permissible daily dose of a pollutant (CFU/(kg. d)). The reference concentration (RFC) defined as 500 CFU/m³ for airborne bacteria according to the recommended concentration by the American Conference of Governmental Industrial Hygienists (ACGIH) (13) and was set as 1000 CFU/m³ for airborne fungi recommended by the National Institute of Occupational Safety and Health (NIOSH) (20). If HQ ≤ 1, non-cancer risks are not considered significant. Conversely, if HQ > 1, non-cancer risks are considered significant, indicating the potential for adverse health effects (3,20).

**Table 1.** Meteorological conditions during air sampling

| Weather parameters | Values |
|---|---|
| Wind Speed (m/s) | 8.1 |
| Temperature (°C) | 9.5 |
| Humidity (%) | 51 |
| Pressure (h Pa) | 822.7 |
| UV index | 4 |

### *Statistical Analysis*

Statistical analyses were conducted using SPSS version 20. The airborne bacteria and fungal concentrations were characterized by minimum, maximum, mean, and standard deviation. Graphs were constructed using Graph Pad Prism 8. The normality of the data was determined using the one-sample Kolmogorov-Smirnov test. The correlation between airborne bacterial, fungal, and meteorological data was determined using Spearman correlation coefficient. Student's t-test was applied to compare the concentrations of airborne bacteria and fungi from inside and outside the WWTPs. The statistically significance level was considered at $P < 0.05$ (confidence level 95%).

## Results

The arithmetic means, as well as the minimum and maximum concentrations of airborne bacteria at various sampling sites, expressed in CFU/m³, are presented in Table 2. The average concentration of bacterial bioaerosols within the WWTPs, in order from highest to lowest, includes the aeration tank (2001.25 ± 2139.36 CFU/m³), the secondary settling tank (1518.71 ± 974.61 CFU/m³), the chlorination unit (1263.73 ± 873.04 CFU/m³), the bar screen (663.46 ± 659.29 CFU/m³), and the grit chamber (592.57 ± 351.13 CFU/m³). Additionally, the average concentrations of bacterial bioaerosols outside the WWTPs, including measurements taken 100 m and 500 m downstream in the wind direction from the WWTPs fence, as well as background levels, were 412.64 ± 212.06, 259.11 ± 305.72, and 195.45 ± 93.49, respectively. Furthermore, Table 3 presents the arithmetic means, as well as the minimum and maximum concentrations of airborne fungi at various sampling locations, quantified in CFU/m³. The average concentrations of fungal bioaerosols within the WWTPs, ranked from highest to lowest, are as follows: the grit chamber (482.14 ± 296.98 CFU/m³), the secondary settling tank (285.40 ± 223.15 CFU/m³), the bar screen (266.18 ± 199.94 CFU/m³), the aeration tank (230.45 ± 189.13 CFU/m³), and the chlorination unit (223.12 ± 133.70 CFU/m³). Additionally, the average concentrations of fungal bioaerosols measured outside the WWTPs, specifically at distances of 100 and 500 m in the downstream wind direction from the WWTPs' fence, as well as in the background sites, were recorded as 382.93 ± 284.55, 495.76 ± 270.81, and 146.30 ± 75.28 CFU/m³, respectively. Figure 3 presents a comparison of airborne bacterial and fungal concentrations (mean ± SD), measured in CFU/m³, across various sampling locations.

The findings reveal a statistically significant correlation

**Table 2.** Arithmetic means, minimum and maximum concentrations of airborne bacteria (CFU/m³) at different sampling sites of WWTPs

| Sampling sites | | Mean | Standard Deviation | Min | Max |
|---|---|---|---|---|---|
| Inside the WWTPs | Adjacent to the bar screen | 663.46 | 659.29 | 97.32 | 2324.96 |
| | Adjacent to the grit chamber | 592.57 | 351.13 | 97.11 | 1381.23 |
| | Adjacent to the aeration tank | 2001.25 | 2139.36 | 288.28 | 7767.91 |
| | Adjacent the to secondary settling tank | 1518.71 | 974.61 | 297.39 | 3948.83 |
| | Adjacent to the chlorination unit | 1263.73 | 873.04 | 96.09 | 2530.45 |
| Outside the WWTPs | 100 m of downstream wind direction from the fence of WWTPs | 412.64 | 212.06 | 194.00 | 785.00 |
| | 500 m of downstream wind direction from the fence of the WWTPs | 259.11 | 305.72 | 96.00 | 888.41 |
| Background | 100 m upstream from the fence of the WWTPs | 195.45 | 93.49 | 96.09 | 386.05 |

**Table 3.** Arithmetic means, minimum and maximum concentrations of airborne fungi (CFU/m³) at different sampling sites of the WWTPs

| Sampling sites | | Mean | Standard Deviation | Min | Max |
|---|---|---|---|---|---|
| Inside the WWTPs | Adjacent to the bar screen | 266.18 | 199.94 | 96.12 | 789.70 |
| | Adjacent to the grit chamber | 482.14 | 296.98 | 196.01 | 991.05 |
| | Adjacent to the aeration tank | 230.45 | 189.13 | 97.53 | 776.91 |
| | Adjacent the to secondary settling tank | 285.40 | 223.15 | 96.21 | 768.80 |
| | Adjacent to the chlorination unit | 223.12 | 133.70 | 97.53 | 486.62 |
| Outside the WWTPs | 100 m of downstream wind direction from the fence of the WWTPs | 382.93 | 284.55 | 97.01 | 868.02 |
| | 500 m of downstream wind direction from the fence of the WWTPs | 495.76 | 270.81 | 97.34 | 960.92 |
| Background | 100 m upstream from the fence of the WWTPs | 146.30 | 75.28 | 86.01 | 297.36 |

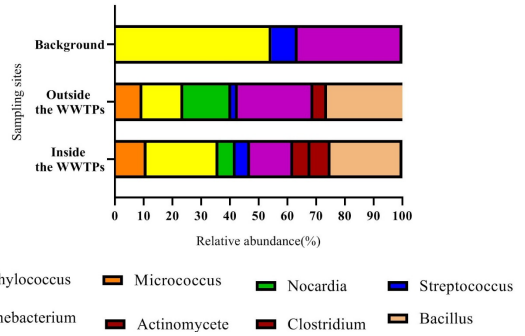



**Figure 3.** the percentage distribution of airborne bacterial genera at different sites

between the levels of bacterial bioaerosols and the sampling sites ($P<0.05$). As shown in Figure S1, the concentration of airborne bacteria diminishes as the distance from the WWTPs increases, with the lowest concentration recorded 500 meters downstream from the WWTPs boundary (412.64 ± 212.06). Furthermore, the data indicated that there were no statistically significant differences in the average concentration of fungi across various locations ($P>0.05$). Figure S2 presents a summary of the indoor/outdoor ratios for airborne bacterial and fungal concentrations at the sampling sites, showing that the ratios for airborne bacteria are I/B = 6.18, O/B = 1.31, and I/O = 3.60. In contrast, the indoor/outdoor ratios for airborne fungi are I/B = 2.03, O/B = 3.00, and I/O = 0. 68. The results of the Student's t-test, which compares the airborne concentrations of bacteria and fungi inside and outside the WWTPs, are detailed in Table S2. The results indicated a statistically significant relationship between the concentration of airborne bacteria inside and outside the WWTP ($P<0.05$); however, no significant relationship was observed between the concentration of airborne fungi in the air inside and outside the WWTP ($P>0.05$).

### *Relationship Between Environmental Factors and Bioaerosol Concentrations*

The Spearman's correlation between airborne bacterial and fungal concentrations and various meteorological parameters is presented in Table S3. As shown in Table S3, a significant negative correlation was found between the concentration of airborne bacteria and wind speed ($P<0.01$, R = -0.632). However, no significant correlation was observed between the concentration of airborne bacteria and barometric pressure or UV index ($P>0.05$). Furthermore, a significant positive correlation was found between the concentration of airborne fungi and temperature ($P<0.05$, R = 0.614) as well as relative humidity ($P<0.01$, R = 0.511). Conversely, no significant correlation was detected between the concentration of airborne fungi and barometric pressure, wind speed, or UV index ($P>0.05$).

### *Dominant Bacterial and Fungal Genera Isolated During Sampling Across Various Locations*

Figures 3 and 4 illustrate the percentage distribution of airborne bacterial and fungal genera across various locations. In the WWTPs, the most frequently detected airborne bacterial species were Bacillus (25%), Staphylococcus (25%), Corynebacterium (15%), Micrococcus (11%), Clostridium (7%), Actinomycetes (6%), Nocardia (6%), and Streptococcus (5%), respectively. Outside the WWTPs, the predominant airborne bacterial species included Bacillus (26.19%), Corynebacterium (26.19%), Nocardia (16.67%), Staphylococcus (14.29%),

Micrococcus (9.52%), Actinomycetes (4.76%), and Streptococcus (2.38%), respectively. At the background site, the most commonly observed airborne bacteria were Staphylococcus (54.55%), Corynebacterium (36.36%), and Streptococcus (9.09%), respectively. The most prevalent airborne fungal species found within the WWTPs were Rhizopus (40.74%), Cladosporium (23.13%), Penicillium (22.35%), Aspergillus (6.80%), Alternaria (4.20%), and Microsporum (2.78%), respectively. Outside the WWTPs, the most commonly observed airborne fungi were Rhizopus (37%), Cladosporium (28.88%), Penicillium (16.96%), Microsporum (6.78%), Mucor (3.39%), Trichophyton (3.39%), Aspergillus (2.39%), and Alternaria (1.21%), respectively. At the background site, the predominant airborne fungi were Cladosporium (68.83%), Penicillium (18.18%), Rhizopus (11.69%), and Microsporum (1.30%), respectively. Additionally, the Venn diagram of airborne bacterial and fungal genera collected from different sampling sites is presented in Figures 5 and 6.

### *The Non-Carcinogenic Risk Assessment of Exposure to Airborne Bacteria and Fungi*

The HQ of airborne bacteria and fungi, as measured through inhalation exposure and skin contact at various sampling sites, is presented in Table 4. This study found that, for adults, the non-carcinogenic risks from airborne bacteria and fungi via inhalation were greater than those from skin contact, indicating that inhalation is the primary route of exposure. At the WWTPs, the average HQ from inhalation exposure and skin contact for adult males and females was less than 1, suggesting that the non-carcinogenic risks associated with both inhalation exposure and skin contact for adults are negligible. However, the HQ for airborne bacteria and fungi inside the WWTPs was higher than that for airborne bacteria and fungi outside the facility.

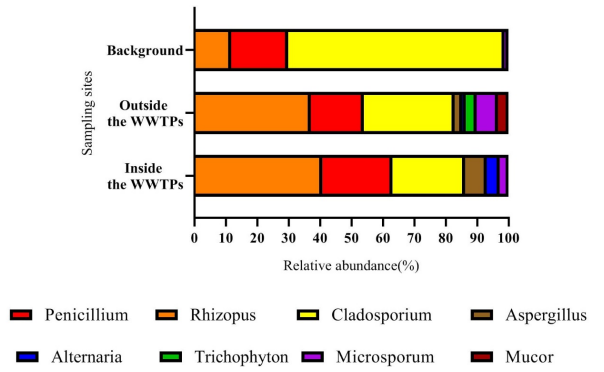



**Figure 4.** The percentage distribution of airborne fungal genera at different sites

## Discussion

The results of this research indicated that the majority of bacteria (100%) from the air samples were gram-positive. This phenomenon may be attributed to the fact that gram-negative bacteria do not survive as effectively as gram-positive bacteria in aerosolized environments. Gram-positive bacteria possess a thick, multilayered cell wall composed of peptidoglycan, which enhances their resistance to dryness, ultraviolet (UV) radiation, and temperature fluctuations. This characteristic allows them to persist longer in airborne conditions. Furthermore, certain gram-positive bacteria, such as Bacillus and Clostridium, have the ability to form endospores. These spores can endure extreme environmental conditions, including severe dryness, high temperatures, and exposure to disinfectants, and can be reactivated when conditions become favorable. Additionally, gram-positive bacteria, due to their unique surface structures and the presence of polysaccharides, demonstrate a greater capacity to adhere to airborne particles. This trait contributes to their increased prevalence in environmental bioaerosols (8). In the study conducted by Fathi et al., approximately 4% of the bacteria were gram-negative, while about 98% were gram-positive (1,28). The levels of bioaerosols present in WWTPs are influenced by several factors, including the specific sampling location, the type of wastewater being

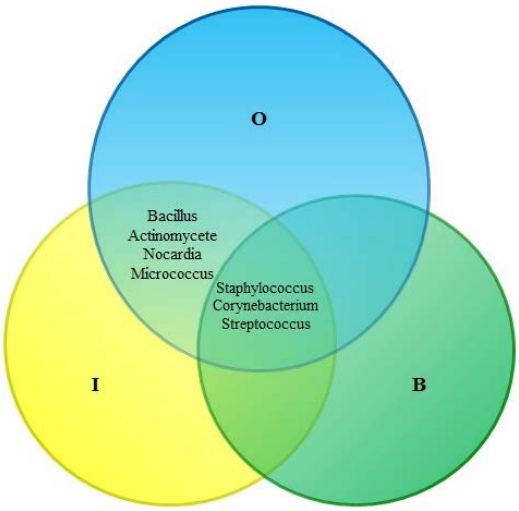



**Figure 5.** Venn diagram of airborne bacterial genera collected from different sampling sites
(O = outside the WWTPs, I = Inside the WWTPs, B = Background)

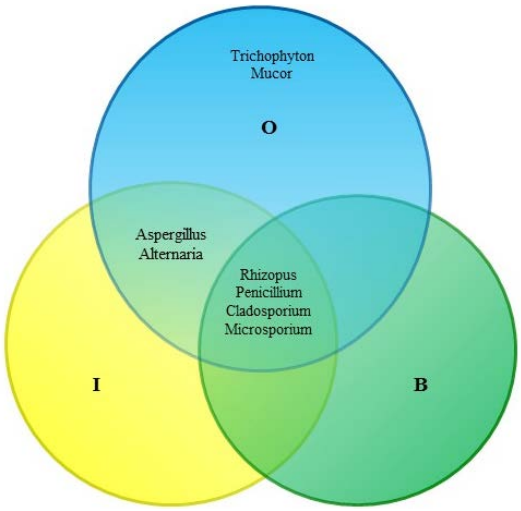



**Figure 6.** Venn diagram of airborne fungal genera collected from different sampling sites
(O = outside the WWTPs, I = Inside the WWTPs, B = Background)

**Table 4**. HQ of airborne bacteria and fungi via inhalation and skin contact

| Bioaerosols | Exposure site | $HQ_{Inhalation}$ | | $HQ_{Skin}$ | |
|---|---|---|---|---|---|
| | | Adult male | Adult female | Adult male | Adult female |
| Airborne bacteria | Within the WWTPs | 0.64 | 0.60 | 5.09E-07 | 5.81E-07 |
| | outside the WWTPs | 0.16 | 0.15 | 1.25E-07 | 1.43E-07 |
| | Background | 0.09 | 0.09 | 7.31E-08 | 8.35E-08 |
| Airborne fungi | Within the WWTPs | 0.06 | 0.06 | 4.70E-08 | 5.36E-08 |
| | outside the WWTPs | 0.1 | 0.1 | 8.79E-08 | 9.38E-08 |
| | Background | 0.03 | 0.03 | 2.73E-08 | 3.12E-08 |

treated, the aeration techniques employed, prevailing weather conditions, and the infrastructure of the WWTPs (5,7,22,29). Based on Table 2, the average concentration of airborne bacteria adjacent the aeration tank is significantly higher than that at other locations, being approximately 10 times greater than the concentration at the background point (100 meters upstream of the WWTPs). This suggests that the mechanical mixing of wastewater for aeration purposes contributes to an increase in bioaerosols in the air. In contrast, the lack of turbulence in the grit chamber results in a lower emission rate of airborne bacteria. Numerous studies have identified the aeration tank as a major source and contributor of bioaerosols to the environment (4,7,9,30). Table S4 summarizes the concentrations of bioaerosols in wastewater treatment plants across various studies. A research conducted by Pengcheng Xu et al. recorded the highest concentration of airborne bacteria in the aeration grit chamber, as $3767 \pm 280$ CFU/m$^3$ (7). The study by M.A. Sánchez-Monedero et al. demonstrated that aeration tanks equipped with mechanical turbulence mechanisms, such as surface turbines and horizontal rotors, generated a larger volume of bioaerosols compared to conventional aeration diffusers (30). According to the results of Yunping Han et al., bioaerosols emitted from the WWTPs using horizontal rotor diffusers were higher than those from fine bubble aeration (22). As shown in Figure S1, there is a statistically significant relationship between the concentration of bacterial bioaerosols and the sampling location ($P < 0.05$). As the distance from the source of pollution, specifically the WWTPs, increases, the level of emissions decreases. Furthermore, the average concentration of bioaerosols within 500 meters downstream of the WWTPs boundary is approximately 1.3 times higher than that at the background point. This finding indicates that bioaerosols released from the WWTPs can affect areas up to 500 meters away. It is essential to maintain an adequate distance between WWTPs and residential areas to protect public health. The findings of Fathi et al. are consistent with the current research, demonstrating that bioaerosols released from the WWTPs can impact areas up to 500 meters from the facility (9). Additionally, research conducted by Carducci et al. revealed a significant decrease in bacterial aerosol concentration as the distance from the WWTPs increased (31). In Table 3, among the various units of the WWTPs, the grit chamber tank exhibited the highest emission rate of airborne fungi, with an average concentration 3.3 times greater than that of the background point. Furthermore, the study by Talepour et al. showed that the grit chamber unit had the highest concentration of fungi, averaging $92.2 \pm 28.12$ CFU/plate/h (19). Additionally, in the study conducted by Pengcheng Xu et al., the maximum concentration of airborne fungi was recorded in the sludge thickening house, with a range of 1,836 to 8,775 CFU/m$^3$, as this unit was situated in an enclosed headspace (7). The analysis of airborne fungi revealed no statistically significant difference in the mean concentration of airborne fungi across different locations ($P > 0.05$). In the present study, the average concentration of airborne bacteria was found to be higher than that of airborne fungi. Fungi typically range in size from approximately 1 to 30 microns, while bacteria are smaller, measuring around 0.25 to 8 microns (4,32). Bacteria are typically smaller, more resilient, and exhibit greater diversity across various environments. Their smaller size enables them to remain suspended in the air for extended periods, facilitating their dispersion. Additionally, bacteria can originate from a variety of sources, including soil, water, and human activities, which continuously replenish their presence in the atmosphere. In contrast, fungi produce larger spores that settle more rapidly and may be less abundant in certain environments, thereby limiting their concentration in the air (8,31,33). In this study, a total of eight culturable bacterial species were identified. In Figure 3, Bacillus and Staphylococcus were the most abundant genera within the WWTPs, each accounting for 25% of the total bacteria identified. The dominance of Bacillus species may be attributed to their ability to form spores, which enables them to survive in harsh environmental conditions. In contrast, research conducted by Breza identified Pseudomonas as the most prevalent bacteria in their findings (3,13,29). Additionally, another study by Michalkiewicz identified Corynebacterium, Bacillus, Staphylococcus, and Micrococcus as the dominant genera. A study conducted at the WWTPs in Bydgoszcz, Poland, revealed that Staphylococcus was the most abundant bacterial genus (5). The absence of Bacillus in the air at background point, in contrast to its presence in the air at WWTPs, can likely be attributed to the differing environmental conditions of the two locations. WWTPs provide an environment characterized by high humidity, abundant organic matter, and optimal conditions for the proliferation of various microorganisms,

including Bacillus. These bacteria may become aerosolized and transferred from the wastewater to the air during treatment processes. In contrast, the air at background point typically contains common natural and environmental contaminants that do not support the growth and dissemination of Bacillus. Factors such as lack of suitable nutrient sources can inhibit the presence and growth of these bacteria in the background air (9,13,34,35). Most Bacillus species are primarily opportunistic pathogens that can cause gastroenteritis, ocular infections, and sepsis associated with intravascular catheters. Staphylococcus, a pathogen responsible for a wide range of diseases—from mild skin infections to potentially fatal pneumonia—is recognized as a significant global contributor to morbidity and mortality. The presence of these potential pathogens in aerosol samples indicates an increased risk of exposure to bioaerosols originating from the WWTPs (3). Figure 4 shows that eight genera of airborne fungi were identified, Rhizopus (40.74%) and Cladosporium (23.13%) being the dominant species within the WWTPs. These microorganisms are particularly significant due to their ability to produce mycotoxins and cause infections, especially in immunocompromised individuals. The presence of these pathogenic microorganisms in residential areas near the former WWTPs underscores the potential risk to residents, as long-term exposure to these bioaerosols can lead to health problems, particularly in vulnerable populations. In the study conducted by Talepour et al., Cladosporium was identified as the most prevalent fungus in the air of the Ahvaz WWTPs, accounting for 39.23% of the total fungal population. Following Cladosporium, the next most common fungi detected were Alternaria (19.87%), yeast (12.45%), and Aspergillus (11.79%) (19). In a similar study conducted by Małecka-Adamowicz, the dominant fungal genera were as follows: Penicillium (54%), Aspergillus species (26%), Cladosporium species (11%), Fusarium (6%), and Alternaria (3%) (33). Additionally, a study by Li et al. identified the dominant fungal genera as Penicillium, Aspergillus, and Candida (36). Most identified fungal species can form spores that protect them from environmental changes. Consequently, the prevalence of these species can be attributed to their metabolic capabilities, particularly their ability to disperse and survive under adverse conditions such as UV radiation, nutrient deficiencies, and high temperatures (1). Various meteorological conditions influence the survival of bioaerosols in the atmosphere. Among these factors, relative humidity is particularly significant, as it greatly affects the concentration of bacteria in the air (6,7,13). Based on Table S3, this study identified a significant positive correlation between the concentration of airborne bacteria and temperature ($P < 0.01$, $R = 0.628$) as well as relative humidity ($P < 0.05$, $R = 0.428$). A study conducted by Banchón et al. found a strong correlation between bacterial concentration and relative humidity during the fall and spring seasons (37). Temperature is another critical factor affecting the inactivation of microorganisms; generally, microbial stability decreases as temperature increases (4). Almeida et al. found that both temperature and wind speed were positively correlated with the release of Alternaria spp. and Cladosporium spp., while humidity exhibited a negative correlation with these factors (38). Numerous studies have demonstrated the relationship between bacterial and fungal contamination and various meteorological parameters (4,30,39). Table 4 shows that the HQ for the inhalation route of airborne bacteria and fungi is higher than that for skin contact exposure (3,6). Consequently, these results indicate that inhalation is the primary route of exposure to bioaerosols. Furthermore, the findings of studies conducted by Wang et al. and Abbas Norouzian Baghani et al. and Yang. Y et al. are consistent with our results, further supporting the conclusion that inhalation is the primary route of exposure to bioaerosols (13,20,40). Additionally, the study conducted by Yunping et al. confirms that inhalation is the primary route of exposure to airborne bacteria in the WWTPs (28). The study by Pengcheng Xu et al. indicated that the HQ for exposure to bioaerosols among WWTPs workers was found to be greater than 1 (7).

In this study, it is important to note that an HQ of less than 1 indicates an acceptable risk level for exposure to bioaerosols. Therefore, it can be concluded that there is minimal concern regarding the non-carcinogenic risk associated with bacterial and fungal bioaerosols present in the study area. The results of Yang. K et al. study align with our findings, indicating that the inhalation HQ for adult males and females is below 1. This suggests that the inhalation risks for this population, particularly among the elderly, are negligible (13). At present, occupational assessments for workers at the WWTPs of Imam Sajjad Hospital in Yasuj include spirometry tests to evaluate lung function, screenings for parasites and intestinal diseases, and comprehensive blood tests. Additionally, physical examinations are conducted annually or when specific symptoms arise to identify signs of chronic or infectious diseases. These measures are essential for maintaining the health of workers and preventing the onset of occupational diseases. It is recommended that careful monitoring be implemented to ensure timely testing, thereby safeguarding the health of workers at the WWTPs.

## Conclusion

This study highlights the presence, composition, and dispersion of bioaerosols at various stages of hospital WWTPs, which may pose potential health risks to workers and nearby residents. It assesses the risks of exposure associated with bioaerosols, with a particular emphasis on non-carcinogenic effects. The findings showed that the maximum concentration of bacterial bioaerosols within the WWTPs was located near the aeration tank, with an average concentration of $2001.25 \pm 2139.36$ CFU/m$^3$. This suggests that the mechanical mixing of wastewater for aeration purposes contributes to an increase in bioaerosols in the air. Given that the Imam Sajjad Hospital

WWTPs in Yasuj employs the extended aeration method, implementing measures such as upgrading aeration equipment—specifically, by using diffused aerators—may effectively reduce bioaerosol emissions. The maximum concentration of fungal bioaerosols was measured near the grit chamber, with an average concentration of 482.14 ± 296.98 CFU/m³. The average concentration of airborne bacteria was higher than that of airborne fungi, as bacteria are generally smaller, more resilient, and exhibit greater diversity across various environments. In contrast, fungi produce larger spores that settle more quickly, which may result in lower concentrations in the air in certain settings. Bacillus and Staphylococcus were identified as the most abundant species of airborne bacteria, while Rhizopus and Cladosporium were the predominant species of airborne fungi within the WWTPs. The risk assessment results indicated that inhalation is the primary route of exposure to bioaerosols. Furthermore, with a HQ of less than 1, there is an acceptable level of risk associated with exposure to bioaerosols, suggesting minimal concern regarding the non-carcinogenic risks linked to bacterial and fungal bioaerosols in the studied area. Our findings have significant implications for understanding the impact of WWTPs operations on bioaerosol emissions and the associated public health risks.

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**Competing Interests**
The authors have no relevant financial or non-financial interests to disclose.

**Ethical Approval**
This study was performed according to the principles of the Declaration Iran International Committee for Ethics in Biomedical Research. Approval was granted by the Ethics Committee of Yasuj University of Medical Sciences (Ethical code: IR.YUMS.REC.1396.122).

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**Supplementary Files**
Supplementary file 1 contains Tables S1-S4 and Figures S1 and S2.

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