JAEHR
Journal of Advances in Environmental Health Research
J Adv Environ Health Res 2026;14(1):56-63. doi:10.34172/jaehr.1442
http://jaehr.muk.ac.ir

Original Article

Bioaerosol Exposure and Health Risk Assessment at a Municipal Material Recovery Facility in Arak, Iran

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Article history:
Received: July 21, 2025
Revised: August 18, 2025
Accepted: September 16, 2025
ePublished: March 29, 2026

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Abstract

Introduction: Municipal solid waste (MSW) sorting facilities are sources of airborne biological contaminants. Occupational exposure to bioaerosols, including bacteria and fungi, poses potential health risks for waste workers, especially in poorly ventilated, high-activity areas. This study aimed to assess airborne concentrations of bacteria and fungi across key operational areas of an MSW sorting facility and to evaluate the associated non-carcinogenic health risks using a quantitative health risk assessment (HRA) model.

Methods: Between April and May 2024, air samples were collected from four indoor sampling sites and one outdoor control site using impaction methods. Concentrations of culturable airborne bacteria and fungi were determined and compared with international guideline values. A US EPA-based HRA model was employed to estimate chronic daily intake (CDI) and hazard quotient (HQ) values.

Results: Bacterial concentrations ranged from 16 to 4128 CFU/m^3, and fungal concentrations from 22 to 1120 CFU/m^3. The manual sorting station exhibited the highest levels of bioaerosols. Calculated HQs for bacterial exposure exceeded the acceptable limit (HQ > 1.0) at multiple sites, particularly at the manual sorting line (HQ = 2.87), indicating potential non-carcinogenic health effects. Fungal exposure remained within acceptable limits. Indoor-to-outdoor (I/O) ratios confirmed significant indoor sources of bioaerosols.

Conclusion: This study emphasizes the importance of improved ventilation, personal protective equipment, and bioaerosol monitoring. Also, utilizing health risk assessments to environmental analysis is a key strategy for minimizing health risks associated with exposure in the waste management industry.

Keywords: Bioaerosols, Municipal solid waste, Health risk assessment, Bacteria, Fungi, Inhalation exposure, Occupational health

Please cite this article as follows: Koolivand A, Zafari M, Mirhoseini SH. Bioaerosol exposure and health risk assessment at a municipal material recovery facility in Arak, Iran. J Adv Environ Health Res 2026;14(1):56-63. doi:10.34172/jaehr.1442

Introduction

Compared to developed countries, the lack of proper waste management in developing countries can lead to dangerous health, safety, and environmental consequences.[1,2] Processing solid waste in a material recovery facility (MRF) is one of the sources of many hazardous chemicals and biological emissions, making them important from an environmental and health risk perspective.[3–5] The organic content of municipal solid waste (MSW) provides a rich environment for microbial growth. As a result, solid waste processing increases the risk of exposure to bioaerosols.[6–8] Organic dust or bioaerosols can spread through the wind and pose serious risks to human health. MRFs use a mixture of manual and automated separation techniques, such as unloading waste materials, screening, shredding, and turning, which play a major role in the generation of microbial aerosols.[5,9]

Also, the levels of bioaerosols and other air pollutants are higher in the indoor environments of MRFs than in open spaces.[10] Workers in these facilities are exposed to high levels of organic dusts or bioaerosols that may threaten their health and well-being. Environmental health studies have shown that airborne particle exposure is linked to adverse health effects such as respiratory disorders, asthma attacks, lung function decline, increased hospitalization rates, shorter life expectancy, and death. Bioaerosols consist of both microorganisms (fungi, bacteria and viruses) and biological components (endotoxins, pollens, and spores).[8,11–13] Regardless of shape, size, or structure, most bioaerosols have an aerodynamic diameter of less than 10 µm and can penetrate deeply into the respiratory system. Occupational exposure to microbial aerosols may lead to a variety of health problems, including cardiovascular and respiratory diseases, allergic reactions, eye and skin irritation, inflammatory and infectious conditions, and gastrointestinal symptoms.[14,15] It has been found that workers in MRFs have an increased risk of developing pulmonary and gastrointestinal diseases, asthma, eye inflammation, skin infections, and musculoskeletal disorders compared to workers in other fields.[2,5,7,10,16] Meanwhile, population growth and the rapid expansion of city outskirts in developing countries have brought landfills and MRFs closer to urban areas.[3,8] As a result, residents near MRFs are inevitably exposed to bioaerosols, with previous studies reporting high concentrations of airborne bacteria and fungi spreading from MRFs to nearby residential areas.[17–19] Evidence suggests that airborne microorganisms can be detected more than 250 m away from waste processing and disposal sites.[3] Consequently, assessing the microbial air quality within MRFs is of great importance. This study aimed to comprehensively characterize the concentrations and composition of microbial aerosols alongside size-segregated particulate matter (PM_{10}, $PM_{2.5}$, and PM_1) within an MRF in Arak, Iran. Furthermore, a quantitative health risk assessment (HRA) framework was applied to evaluate potential non-carcinogenic risks associated with occupational exposure.

Material and Methods

Sampling Location

In this study, an MRF in the city of Arak, Iran, was selected for sampling. Arak, located at 48° 57′ to 51°E and 33° 30′ to 35° 35′N, is the capital of the Markazi province, with a population of approximately 600,000. Based on available data, the mean rate of MSW generation in Arak is 0.6 kg per person per day.[20] Most of the MSW collected in Arak is dumped in uncontrolled or semi-controlled landfills. The highest temperature in this city may rise to 35 °C in summer and drop below -15 °C in winter. In addition, the annual mean rainfall is approximately 350 mm, and the average humidity is estimated to be 46%.

This MRF is located near the Arak landfill, approximately 9 km from the city (Figure 1). The MRF consists of an operational unit, which includes a tipping floor, a conveying belt, manual separation, a rotary screen, and a baling machine. The main characteristics of the MRF have been listed in Table 1. Air sampling was conducted at three indoor locations and one outdoor site: (1) the tipping floor (S1), (2) the manual sorting path (S2), (3) near the conveying belt (S3), and (4) an outdoor site (background site) (S4).

Bioaerosol Collection

Four duplicate air samples were collected in the morning

Figure 1. Map of the area studied and the air sampling site

(10 a.m.) at each sampling site from April to May 2024. A total of 64 samples were collected using a single-stage viable Andersen cascade impactor (SKC Inc., USA). The flow rate and sampling time were set at 28.3 L/min and 5 min, respectively. Sampling was done approximately 1.5 m above ground level (breathing height). Simultaneously with microbial air sampling, the PM mass concentration of three different sizes (PM_1, $PM_{2.5}$, and PM_{10}) was also recorded at each sampling location using a portable laser photometer (DustTrak 8520, TSI, USA) for 5 min.

Laboratory Analysis

The sampling equipment was sterilized with 70% alcohol before sampling. Tryptic soy agar (TSA) (Merck Co., Germany) with nystatin and malt extract agar with chloramphenicol (MEA) (Merck Co., Germany) were used to identify airborne bacteria and fungi. After obtaining the samples, the cultures were transferred to the laboratory by observing the cold chain. TSA and MEA media were respectively incubated at 37 °C for 24-48 hours, and 25 °C for 5 days.

The number of colonies in each plate was counted, and the concentrations of airborne bacteria and fungi were reported as colony-forming units per cubic meter of air (CFU/m^3), according to equation 1:

$$CFU/m^3 = \text{Numbers of colonies} \times 1000 \;/\; \text{Flow rate of sampling pump (L/min)} \times \text{Time (min)} \quad (1)$$

To further classify bacterial genera, a set of biochemical tests was performed, including catalase, oxidase, citrate utilization, and carbohydrate fermentation assays, following Bergey's Manual of Systematic Bacteriology guidelines.[20]

Fungal colonies grown on MEA were identified using the slide culture method. Colonies were characterized macroscopically by their pigmentation, surface texture, and growth pattern. Microscopic identification relied on the morphology of reproductive structures, such as conidiophores, phialides, and conidia, as well as the septation of hyphae. Identification was performed up to the genus level using standard mycological keys.[21]

HRA

A quantitative HRA was carried out to estimate potential non-carcinogenic risks due to inhalation exposure to airborne bacteria and fungi. Using the US EPA exposure model, the Chronic Daily Intake (CDI) was calculated using equation 2:

$$CDI = (C \times IR \times EF \times ED) \;/\; (BW \times AT) \quad (2)$$

Table 1. Main characteristics of the Arak solid waste MRF

Characteristic	Number
Amount of solid waste received (ton/day)	200-240
Number of workers	20-25
Shed dimensions	105 m × 20 m × 15 m
Air conditioning system	Natural

where, C is the measured concentration of bioaerosols (CFU/m^3), IR is the inhalation rate (20 m^3/day), EF is the exposure frequency (250 days/year), ED is the exposure duration (25 years), BW is the average body weight (70 kg), and AT is the averaging time for non-carcinogens (25 × 365 days).[6,20] To assess the non-carcinogenic health effects, the Hazard Quotient (HQ) was computed using equation 3:

$$HQ = CDI \;/\; RfD \quad (3)$$

where, RfD is the reference dose based on exposure limits recommended by the Portuguese indoor air guidelines (500 CFU/m^3), NIOSH (1000 CFU/m^3 for fungi), and the Brazilian standard (750 CFU/m^3). An $HQ > 1$ indicates potential health concern. Moreover, indoor-to-outdoor (I/O) ratios were calculated to determine the dominance of indoor pollution sources.

Statistical Analysis

The collected data were analyzed using SPSS version 24.0. The Kolmogorov-Smirnov test was conducted to assess normality and determine the appropriateness of parametric or non-parametric tests. Descriptive statistics (minimum, maximum, mean, and standard deviation) were calculated to summarize the density of airborne bacteria and fungi. The Kruskal-Wallis test was used to compare the levels of microbial aerosols between the sampling locations. Statistical significance was set at $P < 0.05$.

Results and Discussion

Airborne Bacteria and Fungi

The mean (±SD) and maximum and minimum concentrations of airborne bacteria and fungi have been presented in Table 2. The concentrations ranged from 16 to 4128 CFU/m^3 and 22 to 1120 CFU/m^3, respectively.

These findings are consistent with the results of similar studies,[5,15] but exceed those reported from an MRF in Tehran, Iran[3,9] and a waste paper recycling facility in Kuwait.[22] Notably, extensive changes in bioaerosol levels in various studies can be caused by different sampling methods and meteorological and environmental features regarding the type of solid waste received. Despite the risks of exposure to microbial aerosols, the international threshold value in workplaces has not yet been considered due to inadequate data and diversity in the possible health effects of microbial aerosols. Nevertheless, some organizations have established guidelines without considering their effects on human health. For example,

Table 2. Bioaerosol concentrations (CFU/m^3) in the different sampling sites

Sampling site	Airborne Bacteria			Airborne Fungi		
	Mean (SD)	Max	Min	Mean (SD)	Max	Min
S1	998 (146)	2419	186	347 (84)	870	48
S2	2873 (103)	4128	832	582 (123)	1120	98
S3	1080 (96)	2936	405	487 (109)	1073	55
S4	435 (89)	983	16	206 (76)	513	22

the National Institute of Occupational Safety and Health (NIOSH) determined a bioaerosol threshold of 1000 CFU/m.[3 9,10] In addition, the Brazilian Health Regulatory Agency recommends a maximum value of 750 CFU/m^3 for indoor airborne fungi.[5] In Portugal, the National System for Energy and Indoor Air Quality proposed a standard value of 500 CFU/m^3 for indoor bacteria and fungi.[5] Furthermore, Scandinavian guidelines advise that the threshold value for occupational exposure should be 10^4 CFU/m^3 for total bacteria and 10^3 CFU/m^3 for Gram-negative bacteria.[23] This study showed that the bacterial levels at different sampling sites were higher than the Portuguese allowable limit (500 CFU/m^3).[24] However, none of the bacterial counts exceeded the allowable limit established in Scandinavian countries[25] (10^4 CFU/m^3). In addition, the mean concentration of fungi in indoor environments (1650 CFU/m^3) was higher than the values recommended by the NIOSH (1000 CFU/m^3) and the Brazilian standard[5] (750 CFU/m^3).

Based on the findings of this study, a wide range of bioaerosol concentrations was observed at different sampling sites. The manual sorting path (S2) had the highest emission levels of airborne bacteria and fungi ($P < 0.05$). The traffic of workers and vehicles for transporting solid waste and handling recyclable waste is the main source of bioaerosols at this sampling site. Also, the outdoor levels (background) of airborne bacteria and fungi were significantly lower than those in the indoor sampling sites ($P < 0.001$).

The indoor-to-outdoor ratios for bacteria and fungi ranged from 2.3 to 6.6 and 1.7 to 2.8, respectively. This ratio is commonly used as an indicator of the emission sources of microbial aerosols. An I/O ratio greater than 1 indicates that indoor pollution is the main source of pollution.[24] According to the ratio data, indoor air quality was divided into three categories: poor (>2), regular (1.5-2), and good (<1.5). If this ratio exceeds 1.5, it is necessary to take corrective actions.[24] Thus, in the present study, indoor air quality was in the poor category, and corrective interventions regarding ventilation and air-changing systems are essential. Besides, the I/O results for airborne bacteria were higher than those for airborne fungi at all sampling sites. This finding is consistent with the results reported by Wikuats et al for a waste sorting and recycling facility in Brazil.[5] Baghani et al stated that fungi I/O values ranged from 6.4 to 11.9 in different processing units of waste recycling plants, while it was 0.76 for the indoor environment of the office.[9] The observations obtained in a waste sorting facility in Poland showed that the values of indoor airborne bacteria were 1.6 times higher compared to that outdoors. In contrast, indoor airborne fungi were 1.7 times lower than those indoors.[25]

PM Concentration

The mean (±SD) concentrations of PM of different sizes (PM_1, $PM_{2.5}$, and PM_{10}) have been shown in Figure 2. The maximum PM levels were obtained from the S2 sampling location owing to its similarity with the bioaerosol

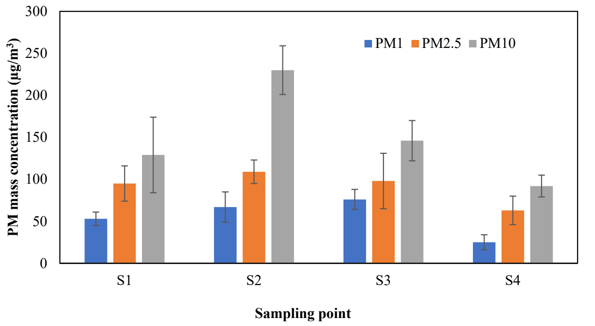

Figure 2. PM concentrations (PM_1, $PM_{2.5}$, and PM_{10}) in the different sampling sites

concentrations (Figure 1). The indoor concentrations of PM_1, $PM_{2.5}$, and PM_{10} were 45–88, 74–133, and 84–259 µg/m^3, respectively. These data are comparable to the study in a landfill site in Dehradun (India), where the total mean concentrations of PM_{10}, $PM_{2.5}$, and PM_1 were mentioned as 172.2, 128.9, and 90.5 µg/m^3, respectively[1]. The same study, conducted in a waste processing facility in Brazil, found mean indoor $PM_{2.5}$ and PM_{10} values of 25.5 and 233 µg/m^3 during winter, 37.2 and 663.9 µg/m^3 in spring, and 29.3 and 699.3 µg/m^3 in summer, respectively. Park et al reported mean $PM_{2.5}$ and PM_{10} values of 61.6 and 458.1 µg/m^3 for waste sorting in Seoul, South Korea.[26] The existence of indoor sources of PM (e.g., mechanical agitation, the movement of solid waste by loaders, storage, and resuspension) typically results in indoor levels being higher than the corresponding outdoor values. The results of the Kruskal-Wallis H test showed that the PM values at different sizes (PM_1, $PM_{2.5}$, and PM_{10}) were not significantly different in indoor environments ($P > 0.05$). However, the outdoor PM values were significantly lower than the indoor values ($P < 0.05$), which is consistent with the results of other studies.[27] In a study of air pollutants in two landfill sites in Chennai, India, increased $PM_{2.5}$ concentrations (36 and 45 µg/m^3) were recorded compared to the background site (45 µg/m^3).[28]

In addition to descriptive analysis, correlation tests were performed to examine the relationship between particulate matter and microbial aerosol concentrations. Spearman rank analysis indicated a moderate positive correlation between $PM_{2.5}$ and bacterial concentrations ($\rho = 0.46$, $P < 0.05$) and between PM_{10} and fungal concentrations ($\rho = 0.41$, $P < 0.05$). These results suggest that both fine and coarse particles may contribute to the airborne transport or resuspension of microbial aerosols within the MRF environment. The observed associations between particulate matter and bioaerosols underline the role of particles as potential carriers of microbial contaminants. In particular, the positive correlation of $PM_{2.5}$ with bacterial counts and PM_{10} with fungal counts indicates that particle size fractions may differentially influence microbial dispersion. Similar relationships have been documented in landfill and recycling environments, where mechanical agitation of waste and resuspension of dust particles enhance microbial load in the air.[1,5] These findings strengthen the evidence that PM pollution is a critical co-factor in occupational bioaerosol exposure.

HRA

CDI

The highest CDI for bacteria was observed at Site 2 (4.44×10^3 CFU/kg-day), while fungal CDI ranged from 0.32×10^3 to 0.90×10^3 CFU/kg-day.

HQ

Relative HQs were computed using the reference values from NIOSH and Portuguese standards. At Site 2, the HQ for bacterial exposure exceeded 2.8 (vs. NIOSH), indicating potential non-carcinogenic health effects such as respiratory inflammation, bronchial irritation, and allergenic sensitization.These values are consistent with those reported by Marcelloni et al, who observed mean HQs ranging from 0.8 to 3.1 in MSW treatment facilities in Canada, highlighting potential non-carcinogenic health risks associated with chronic exposure to bioaerosols.[29] Similarly, Hung et al emphasized that elderly and volunteer workers in poorly ventilated recycling centers are particularly vulnerable, with HQs between 1.2 and 4.3, especially due to Gram-negative bacterial exposure, which poses heightened risks of respiratory irritation and allergic responses.[30]

To contextualize the findings of this study, bioaerosol concentrations and HQ values were compared with data from similar investigations in other countries (Table 3). The observed bacterial and fungal concentrations in the Arak MRF were within the ranges reported in Tehran (Iran), Brazil, and Canada, but lower than those measured in open landfill environments in India. Notably, the bacterial HQ values in our study exceeded the threshold of 1 in high-activity indoor areas, consistent with findings from Brazil, Canada, and Taiwan, where workers were similarly exposed to elevated occupational risks.

Microbial Diversity

The microbial diversity and contribution percentages at the sampling sites have been shown in Figsure 3 and 4, respectively. The Gram staining results indicated that Gram-positive bacteria (61.2%) were dominant compared to Gram-negative bacteria (38.8%). In previous studies, Gram-positive bacteria have also been reported as the dominant airborne bacteria in waste recycling facilities [4,5,31,32]. In contrast, Madhwal et al reported the dominance of gram-negative bacterial isolates at a landfill site in Dehradun, India.[1] The dominant bacterial genera in this study were *Micrococcus* sp. (25.5%), *Bacillus* spp. (23.3%) and *Staphylococcus* spp. (21%), *Streptococcus* spp. (11%), *Corynebacterium* sp. (7%), and *Pseudomonas* sp. (4.3%), respectively (Figure 3). Gram-negative bacteria, such as *Pseudomonas*, are associated with respiratory tract infections in workers in waste sorting plants. Moreover, endotoxins from Gram-negative bacteria pose a health risk in this area and have been linked to asthma [22]. It should be noted that the predominant fungal genera isolated were *Aspergillus* (36.8%), *Cladosporium* (29.5%), *Penicillium* (18.8%), *Alternaria* (9.8%), and other fungi (9%) (Figure 4). In addition, the distribution of dominant fungal genera at different sampling sites showed that the manual sorting path (58%) and conveying belt (48%) had the highest contribution for *Aspergillus* spp. (Figure 4). Previous studies have shown that the predominant fungal species isolated from the indoor environment of a waste paper and cardboard recycling plant in Tehran, Iran, a waste sorting facility in Lisbon, Portugal, and a waste recycling environment in Londrina, Brazil are *Cladosporium* sp., *Penicillium* sp., and *Aspergillus* sp. [5,9,10], respectively. These studies have confirmed the relationship between occupational exposure to fungal aerosols and the potential risk of respiratory diseases, immunosuppressive effects, infections, and allergic reactions in waste-processing facilities.

Conclusion

The findings indicated the presence of significant concentrations of PM_{10}, $PM_{2.5}$, PM_1, and bioaerosols in the indoor environment of the MRF compared to ambient

Table 3. Comparison of bioaerosol concentrations and HRA outcomes in MSW facilities across different countries

Study Location	Facility Type	Bacterial Concentrations (CFU/m³)	Fungal Concentrations (CFU/m³)	HQ Range (Bacteria/Fungi)	Key Findings	Reference
Iran (Arak)	MRF (indoor sorting)	16 – 4128	22 – 1120	Bacteria: up to 2.87; Fungi: < 1	Manual sorting area showed highest exposure; bacterial HQ > 1 indicates non-carcinogenic risk	Present study
Tehran, Iran	MSW processing facility	180 – 3500	95 – 850	Not reported	Higher levels in manual sorting exceeded WHO guideline values	3
Brazil (São Paulo)	Waste recycling facility	300 – 2500	120 – 1900	HQ bacteria: 0.8 – 3.1	Indoor > outdoor; seasonal variability observed	5
Canada (Ontario)	MSW treatment facility	50 – 2200	70 – 1600	HQ bacteria: 0.9 – 3.1	Risks highest in poorly ventilated sites	30
Taiwan	Resource recycling stations	150 – 2800	90 – 1450	HQ bacteria: 1.2 – 4.3	Elderly/volunteer workers most at risk	31
India (Dehradun)	Open landfill	300 – 3600	120 – 2100	Not reported	Strong correlation between PM and bioaerosols	1
Poland	Waste sorting plant	420 – 2900	150 – 1250	Not reported	Indoor bacteria exceeded outdoor levels by 1.6-fold	25

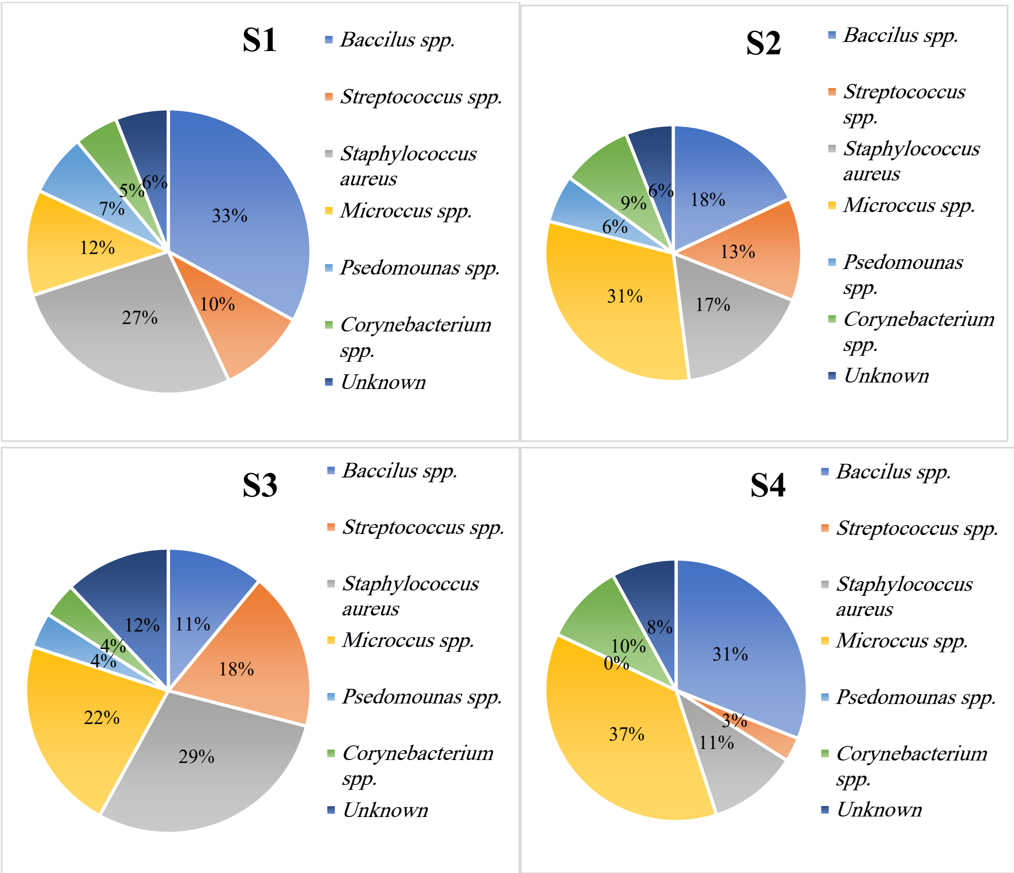

Figure 3. Distribution of dominant bacterial genera at the different sampling sites

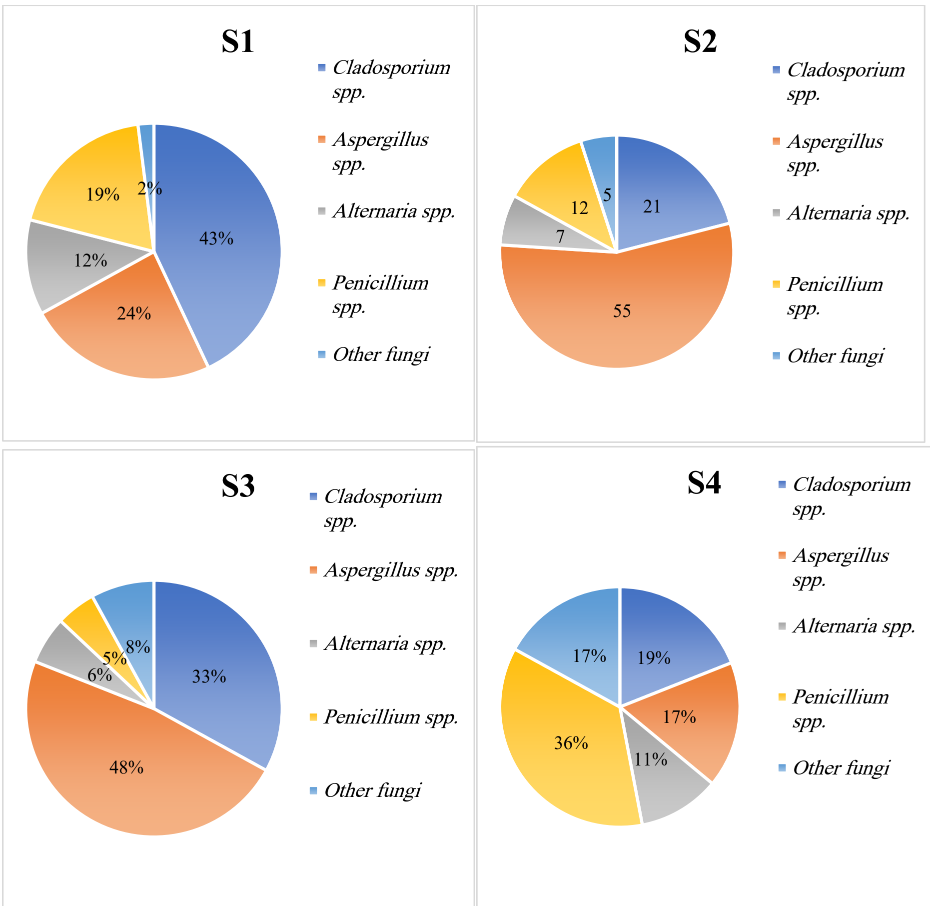

Figure 4. Distribution of dominant fungal genera at the different sampling sites

air. Owing to the diversity of activities and operational conditions, the manual sorting path and conveying belt had a high emission rate of airborne particles and microbial aerosols. The results of the quantitative HRA revealed that chronic inhalation exposure at these concentrations, especially to bacterial bioaerosols, may pose remarkable non-carcinogenic health risks to workers. At Site 2, the calculated HQ for bacterial exposure exceeded the acceptable limit of 1.0, illustrating a high likelihood of respiratory irritation and allergic sensitization. The I/O ratios for bacteria and fungi displayed the impact of indoor sources on the indoor levels of microbial aerosols, which were higher than 1.5. Although Gram-positive bacterial isolates were predominant, airborne Gram-negative bacteria are considered a serious health concern for waste sorting workers. In addition, a high level of fungal types in the indoor environment of different units, including *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp., and *Alternaria* sp., indicates that long-term occupational exposure to these fungal aerosols might have adverse health effects. This survey underlines the importance of implementing targeted intervention plans and utilizing effective ventilation systems in the indoor environments of waste sorting and recycling facilities. Furthermore, it is necessary to use personal protective equipment (PPE) such as gloves, N95 masks, and goggles to reduce occupational exposure to air pollutants.

Acknowledgments
The authors would like to thank Arak University of Medical Sciences for their scientific and financial support.

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Writing–Review & Editing: Ali Koolivand, Seyed Hamed Mirhoseini.

Competing Interests
The author declares no conflict of interest.

Ethical Approval
This study was approved by the Ethics Committee of Arak University of Medical Sciences (Ethical code: IR.ARAKMU.REC.1402.194).

Funding
The Vice Chancellery funded this research for the Research of the Arak University of Medical Sciences (Grant No. 4334).

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