

Original Research

# Assessment of Microbial Air Quality within Facilities of Waste Transfer Stations and Disposal Sites of Lahore, Pakistan

Sana Hafeez<sup>1</sup>, Zulfiqar Ali<sup>1\*</sup>, Zaheer Ahmad Nasir<sup>2</sup>, Sikander Sultan<sup>3</sup>

<sup>1</sup>Environmental Health and Wildlife, Department of Zoology, University of the Punjab, 54590, Lahore, Pakistan

<sup>2</sup>School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, United Kingdom

<sup>3</sup>Department of Microbiology and Molecular Genetics, University of the Punjab, 54590, Lahore, Pakistan

Received: 25 May 2020

Accepted: 25 January 2021

## Abstract

Bioaerosol emissions from waste management operations and facilities are a potential hazard for human and environmental health. This study was aimed to assess the concentration as well as identification of bacteria and fungi, their seasonal variation and association with meteorological measurements at solid waste management (SWM) sites. A total of 16 air samples were collected between October 2017 to March 2018 in wet and dry seasons by using Portable Dust Sampler. Samples were analyzed both by culture and molecular methods. The total culturable bacterial and fungal population ranged from  $4.7 \times 10^4$  to  $7.4 \times 10^5$  CFU/m<sup>3</sup> and  $0.2 \times 10^2$  to  $2.8 \times 10^3$  CFU/m<sup>3</sup> respectively in wet season and from  $7.5 \times 10^4$  to  $6.8 \times 10^5$  CFU/m<sup>3</sup> and  $0.1 \times 10^2$  to  $1.6 \times 10^3$  CFU/m<sup>3</sup> in dry season. Isolated bacterial and fungal strains were processed for molecular identification by using 16S and 18S rRNA. The sequenced bacterial and fungal species were *Bacillus* (*B. cereus*, *B. subtilis*, *B. altitudinis*, *B. amyloliquefaciens*, *B. flexus*), *Pseudomonas stutzeri*, *Staphylococcus sciuri*, *Ochrobacterium intermedium*, *Acinetobacter indicus*, *Mycobacterium esteraromaticum*, *Rothia endophytica* and *Aspergillus* (*A. oryzae*, *A. niger*, *A. terreus*), *Penicillium* (*P. oxalicum*, *P. camemberti*), *Cochliobolus* sp., *Fusarium* sp. respectively. These results have shown that all fungal and 95% of bacterial species were pathogenic. One way ANOVA showed a significant difference in the bacterial concentration with p-value (0.054) at 0.1 level of significance while, no significant difference in fungal concentration with p-value (0.409) was observed among four sampling sites. These results could allow to define that there is a need to develop and implement proportionate risk-based regulations to ensure sustainable solid waste management alongside public and environmental health protection.

**Keywords:** solid waste management, waste transfer stations, disposal sites, pathogenic microbes

## Introduction

With the increase in population, rapid urbanization, industrialization and improving living standards, solid waste production is increasing worldwide [1]. Solid waste management includes waste collection, storage, transportation and finally safe disposal in a manner that minimizes soil, water and air contamination [2]. However, in developing countries municipal solid waste management is extremely neglected and serious environmental hazard posing a range of risks to public health [3]. Anaerobic decomposition of solid waste pollute air by generating toxic gases such as CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>S and nitrous oxides. Burning of waste i.e. plastic and rubber also pollute air with obnoxious fumes [4]. During the management processes of solid waste various contaminants including gases, odours, airborne bacteria and fungi are released into the environment. Among these emissions, bioaerosols including microorganisms and their fragments have raised serious public health concerns to workers and nearby communities. Bioaerosols are dispersed into the air by various activities i.e. composting, recycling, loading, unloading, sorting, transportation and finally disposing of onto landfill sites [5]. Their survival in air depends on meteorological conditions, airborne residual time and resistance. The exposure to bioaerosols may lead to allergenicity, toxicity and infectivity. Exposure of solid waste may also cause irritation of eyes and skin [6]. Inhalation of endotoxin for few hours may cause respiratory illness, dyspnea, headache, fever, joint pain and long term exposure causes chronic lung diseases [7].

It is important to keep in mind that all environments are contaminated to few extent. In spite of fact that many people spend much of their time in indoor environments, poor outdoor air quality can influence significantly on indoor air quality [8]. So, poor solid waste management, especially in urban areas with occasional dumping in outdoors may have significant implications on indoor air quality. Frączek et al. [9] and Ndimele et al. [10] reported that the bioaerosols concentration was a thousand times higher at solid waste management sites than households and public places. It is founded that following microbial species i.e. *Aspergillus flavus*, *Aspergillus niger*, *Pseudomonas aeruginosa*, *Rhizopus microsporus*, *Staphylococcus aureus* are present in the vicinity of solid waste management sites. Along with these species, several saprophytic microbes also exist at these sites [11]. Kalwasinska et al. [12] identified following bacterial and fungal species from municipal solid waste management sites: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, *Madurella grisea*, *Penicillium manfferei*, *Scedosporium apiospermium* and *Cryptococcus neoformans*. Majority of Gram positive bacteria exist at solid waste disposal and transfer sites including genera *Bacillus*, *Enterococcus*, *Micrococcus*, *Mycobacterium* and *Staphylococcus*. Only

a few numbers of Gram negative bacteria were isolated including *Enterobacter*, *Escherichia* and *Pseudomonas* [13, 14]. The highest microbial concentration was observed at the sorting station of SWM sites [5].

According to Kazmierczuk and Bojanowicz-Bablok [15] the range of dispersal of bacteria and fungi from waste facilities is significant. It may be extended up to 1000-1200 meters from the site and become hazardous for adjacent populations. The highest concentration of bioaerosol was identified directly above landfill sites. Microbial concentration increases with the increase of organic waste. In addition, hot and humid environmental conditions also favour their growth and during the process of agitation these are aerosolized. Most of the workers at disposal sites and transfer stations are working without protective measures and exposed to increase levels of pathogenic microbes [16, 17]. Pathogenicity of microbes in the air of SWM sites is not steady over time because the generation, dispersion and persistence of bioaerosols depend on various site operations during waste management processes and metrological conditions (wind speed, relative humidity, temperature, rainfall, atmospheric pressure and solar radiation) [18].

Pakistan is the world's fifth most populous country. The rapid increase in population and urbanization has overburdened the existing frail urban infrastructure and services leading to enormous environmental health challenges. Among these SWM has emerged as a major concern due to minimal SWM capabilities and possess serious risks to public health and environment. In Pakistan, the rate of municipal solid waste production ranges from 0.283 to 0.612 kg/capita/day [19] and it varies in different parts of country. In Lahore, the waste production rate is 0.65 kg/capita/day. This is an estimated value because there are no methods to exactly measure the waste generation [20, 21]. Only 60% of solid waste is collected and dumped to disposal site while 40% remain uncollected along streets, roads, vacant plans, drains and depressions. There is no isolated collection and storage system of recyclables as in developed countries [22]. However, in recent times increasing government investments has been made to improve SWM infrastructure technologies in some cities. For example, the composting facility has been built in Lahore as a public-private initiative to recycle household waste generated in the city. It is very likely that SWM industry will grow in the country and may lead to health hazards associated with the dispersal of bioaerosols onsite and off-site of these facilities. However, there is limited data related to microbial air quality at waste transfer stations and disposal sites in Pakistan. There is a need to characterize and measure microbial concentration at various distances away from the SWM sites. Such an information will be vital to developing and regulating sustainable SWM capability at national level informing and executing proportionate risk-based regulations and strategies to ensure public and environmental health protection.

The present investigation was carried out as a case study to investigate the concentration of bacteria and fungi as well as identification of isolated species, their seasonal variation and association with microclimatic features (temperature, humidity and wind speed) at waste management sites (waste transfer stations and disposal sites) of Lahore, Pakistan.

## Materials and Methods

### Study Area

Lahore (31°34'55.3620"N and 74°19'45.7536"E) is the capital city of Punjab, Pakistan. It covers an area of 1,772 km<sup>2</sup> with population of 11,738,000, 12,188,000 and 12,642,000 in 2018, 2019 and 2020 with an increase of 3.83% and 3.72% in 2019 and 2020. Four SWM sites in Lahore were selected. The location of sites are shown in Fig. 1. These included two solid waste disposal sites and two waste transfer stations: Lakhodair disposal site (SW1), Mehmood Booti disposal site (SW2), Valencia town transfer station (SW3) and Saghian bridge transfer station (SW4). The site characteristics are presented in Table 1.

### Sample Collection and Analysis

Both non-culture and culture-based methods have been used for the bioaerosols sampling and analysis. Non-culture method was considered to count cells directly by using microscope and it also included molecular methods while culture method consisted of filtration and direct impaction, either on all-glass

impingers or on petri-plates [23]. Taha et al. [24] used culture method along with filtration [25, 26] due to its applicability at highly contaminated compost site. They also conducted sampling by using personal aerosol filter sampler (SKC Universal dust and vapour sampling pump) for both active and passive sampling.

Currently, a total of 16 samples were collected during 16 visits. Four samples (two in wet season and two in dryseason) were collected on each site during October 2017 to March 2018. A Portable Dust Sampler (model, L30 MKIII by Rotheroe and Mitchell Ltd) with an average air flow rate of 36 L/min was used to collect the sample on mixed cellulose ester fibre filter (0.45µm pore size and 47 mm diameter). The sampling time at each site was 7-10 minutes to avoid over microbial load. The dust sampler was positioned at height of 1m from the ground and 2 m away from the source. Meteorological parameters such as air temperature, relative humidity (by using Aeroqual 500 series monitor probe), wind velocity and direction (Kestrel 4500 Pocket Weather Tracker) were also recorded parallel to the sampling period. The documented mean values of meteorological conditions are shown in Table 2.

The sampled filters were stored in the test tubes that were already filled with 10ml autoclaved Phosphate-buffered saline (PBS) solution and later on shifted to the laboratory for further analysis. Serial dilutions from stock solution were prepared from 10<sup>-1</sup> to 10<sup>-6</sup>. For bacterial analysis 100µl solution was taken from the dilution of 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> and inoculated onto the Nutrient agar media [27]. While for fungal analysis 200µl aliquot from the dilution of 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> was transferred onto Malt extract agar media [28]. The bacterial and fungal plates were incubated at 37°C for

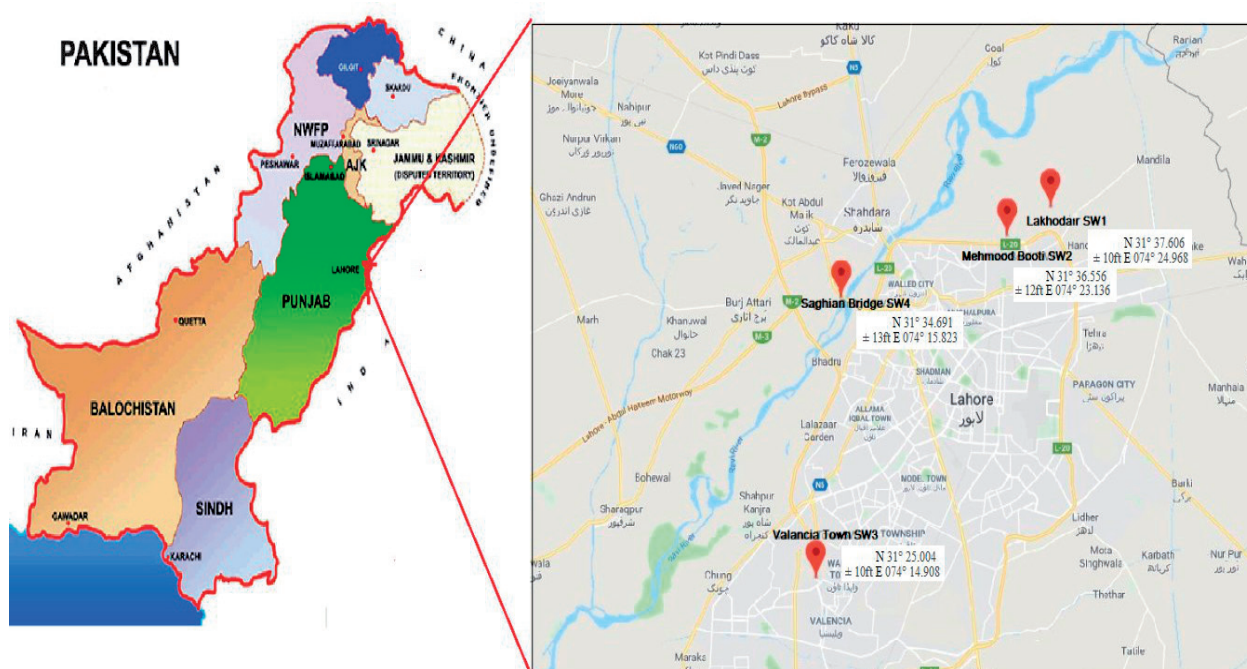


Fig. 1. The location of sampling sites in Lahore.

Table 1. Brief description of sampling sites.

Site	Description	Activities	Waste type
Lakhodair disposal site (SW1)	An area of total 52 Hectares with 28 Hectares covered area. Total six plots but only two plots are operational since April, 2016. These two operational plots receive 2000-2500 tons per day of waste.	Compacting of waste layers, transportation activities, sorting, unloading of waste and mechanical leveling of earth.	House hold solid waste, organic waste, plastics, metal and glass waste, demolition debris
Mehmood Booti disposal site (SW2)	An old disposal site in Lahore since 1995 and after receiving 6 million tons of waste it was saturated till 2010. It covers an area of 630 kanals.	No distinct activities except transportation.	House hold solid waste, plastics, metal and glass waste.
Valencia town transfer station (SW3)	It is covering an area of about 15 kanal and is managing 1,000 tons of MSW every day.	Transportation activities, sorting, loading and unloading of waste material. Workshop activities for maintaining vehicles. In the absence of electricity generator works.	House hold solid waste, organic waste, metal and glass waste, demolition debris.
Saghian bridge transfer station (SW4)	It consists of an area of 10 kanal and is capable of managing 1,400 tons of waste on daily basis. It is serving its operational waste management practices in forty six union councils of Lahore.	Transportation activities, sorting, loading and unloading of waste material. Workshop activities for maintaining vehicles. In the absence of electricity generator works.	House hold solid waste, organic waste, cow dung, metal and glass waste, demolition debris.

Table 2. Mean of meteorological conditions and total bacterial and fungal colony count (CFU/m<sup>3</sup>) in the air of sampling sites during wet and dry season.

Sr No	Seasons	Sampling sites	Temp. (°C)	Humidity (%)	Wind speed (m/s)	Wind direction	Bacterial CFU/m <sup>3</sup>	Fungal CFU/m <sup>3</sup>
1		SW1	35.5	36	1.20	SE,SW	7.4 × 10 <sup>5</sup>	1.2 × 10 <sup>2</sup>
2	Wet	SW2	36.5	28	0.85	SE	1.1 × 10 <sup>5</sup>	0.2 × 10 <sup>2</sup>
3		SW3	34	35	1.50	SW	2.0 × 10 <sup>5</sup>	1.8 × 10 <sup>2</sup>
4		SW4	29.5	44	0.85	SE,SW	4.7 × 10 <sup>4</sup>	2.8 × 10 <sup>3</sup>
5		SW1	32.5	29	0.70	NE	6.8 × 10 <sup>5</sup>	7.0 × 10 <sup>2</sup>
6	Dry	SW2	27.5	51	0.65	N	9.7 × 10 <sup>4</sup>	1.3 × 10 <sup>2</sup>
7		SW3	42	24	1.20	NE	7.5 × 10 <sup>4</sup>	0.1 × 10 <sup>2</sup>
8		SW4	40	33	0.75	S	8.1 × 10 <sup>4</sup>	1.6 × 10 <sup>3</sup>

24-48 hours and at 25°C for 5-7 days respectively. Total microbial counts were taken and reported in CFU/m<sup>3</sup>.

### Identification of Microbes

All bacterial isolates were characterized using both morphological and biochemical methods. Morphological identifications were made by considering color, shape, margin, elevation and texture of colonies according to methods of Holt et al. [29]. While biochemical characterization was performed by catalase, oxidase and starch hydrolysis tests. Gram staining and Spore staining of isolates were also performed. Additionally, *Baird Parker agar*, Mannitol salt agar and Nutrient agar with 6.5% NaCl was used as selective and differential growth media for the identification of *Staphylococci* sp., *Micrococcus* sp. and *Bacillus* sp. respectively. Whereas, fungi were identified after staining based on

morphological characters i.e. color, size, texture, the arrangement of spores and septation of hyphae. Trypan blue was used for staining of colourless fungal cultures while Lactophenol Cotton Blue (LPCB) was used for colored fungi.

The streak plate technique of Hendricks and Prebish [30] was used to discrete pure bacterial strains and for fungal pure cultures either spores or pieces of mycelium were transferred onto Malt extract agar media.

### Molecular Characterization of Isolates

After morphological and biochemical tests, 12 bacterial and 8 fungal pure cultures were selected from all visited sites and further analyzed by molecular methods. For molecular identification 16S rRNA and 18S rRNA sequencing was done after the extraction and purification of DNA. Then, 16S rRNA and 18S rRNA

sequences were BLAST on NCBI (National Center for Biotechnology Information). The sequenced data was assembled and aligned. These sequences were submitted on NCBI to get their accession numbers.

### Statistical Analysis

For statistical analysis SPSS VERSION 22 was used and a comparison was made between wet and dry seasons with respect to microbial concentration by applying independent sampled t-Test. One way ANOVA was used to compare the microbial concentration at four sampling sites. Moreover, to study the impact of temperature, humidity and wind speed on the concentration of bacteria and fungi regression analysis was applied.

### Results and Discussion

The total bacterial and fungal count along with metrological parameters in the air around solid waste management sites during wet and dry season are shown in Table 2.

The total observed bacterial concentration ranged from  $4.7 \times 10^4$  to  $7.4 \times 10^5$  CFU/m<sup>3</sup> and fungi from  $0.2 \times 10^2$  to  $2.8 \times 10^3$  CFU/m<sup>3</sup> during the wet season. However, in dry season their concentrations were from  $7.5 \times 10^4$  to  $6.8 \times 10^5$  CFU/m<sup>3</sup> and  $0.1 \times 10^2$  to  $1.6 \times 10^3$  CFU/m<sup>3</sup> respectively. In general, the number of culturable bacterial count was higher as compared to fungal count in all sampling sites [31]. It is difficult to assess the potential health impact of concentration levels of airborne bacteria and fungi, as no standards exist for the exposure of bioaerosols. However, Scandinavian countries suggested guidelines (8 h exposure) for total bacteria and gram negative bacteria as 10000 CFU/m<sup>3</sup> and 1000 CFU/m<sup>3</sup> respectively [32, 33]. World Health Organization recommended the guideline limit assessment of fungi as 500 CFU/m<sup>3</sup> [34] and considered 150 CFU/m<sup>3</sup> for pathogenic fungal species [35].

Previous studies have demonstrated that seasons, meteorological conditions and various SWM activities have a significant impact on the mean concentration of bioaerosols in the vicinity of SWM sites [13, 36]. As sampling was conducted during wet and dry seasons and compared between wet and dry seasons (Independent sampled t-Test), no significant difference in the concentration of bacteria was observed. While conflicting to bacteria, fungal concentration was changed in both seasons. However, mean of total bacteria and fungi in air samples during wet and dry seasons showed an increase in bacterial concentration during the wet season as compared to the dry season while fungi showed reverse of it.

The increased trend in bacterial concentration during wet season could be due to the deposition of dust particles with microbes by the process of rainfall.

Precipitation provided favorable conditions for the growth of bacteria. While, the increased trend of fungi in dry season could be due to the various environmental factors such as low precipitation enhanced the favorable conditions for the germination of spores. Moreover harsh environmental conditions and wind action during dry season also favoured the dispersal of fungi. These results agreed with the findings of other researchers [37]. Huang et al. [38] also documented the higher microbial concentration in the wet season as compared to other seasons. In contrast to bacteria, the highest fungal concentration was recorded in the dry season than wet season and this corresponds to the results of other researchers [9, 39]. In previous studies, the highest concentration of bioaerosol was found in the air samples of wastewater treatment facilities during summer and autumn seasons than that of spring and winter seasons because of small rainfall and suitable temperature [40, 41].

When we compared the microbial concentration at four sampling sites. It was observed that the level of microbial concentration was different at different sites. Statistical analysis by using one way ANOVA showed a significant difference in bacterial concentration among four study sites with p-value = 0.054 at 0.1 significance level (10%). In case of fungi no significant difference was observed among four different sites ( $p = 0.409$ ). All four sites exhibited increased microbial concentration than that of recommended levels. Working sites were biggest emitter of microbes, which were released by various activities. Higher microbial count in the air of waste disposal site (SW1) might be due to increased activities on this site such as compacting of the waste layers, transportation activities, unloading of waste and mechanical leveling of earth. Moreover, organic matter present in municipal solid waste was the main source of nutrients for microorganisms. There was an increased emission of bioaerosols from young waste as compared to older waste. Moreover, young waste provided optimal environment for the growth of microorganisms. That was due to degradation of organic waste to carbon dioxide, enhancing favorable conditions for microbial growth [42]. Kalwasinska et al. [12] recorded various waste disposal activities which produced more concentration of bioaerosols in air and corresponds to our findings. Moreover, the proximity of unpaved passage way also contributed to increase microbial concentration. In the current study, SW2 was a closed disposal site since 2010 after its saturation. A higher level of microbial contamination was observed at this site after SW1. Closed disposal site had low organic material after the deposition of solid waste had been clogged. Similarly, Huang et al. [38] also documented the higher microbial concentration at the closed disposal site and stated that such sites are not fit for public use even after their closure. So, careful rehabilitation by covering with soil and planting on the surface is recommended. In the contrary, Zainun and Simarani [43] recorded higher

Table 3. ANOVA Results for Regression Model of different parameters.

	Bacteria (CFU <sup>m</sup> -3)		Fungi (CFU <sup>m</sup> -3)	
	F	Sig.	F	Sig.
Sites	2.366	0.036	1.524	0.275
Temperature	2.366	0.097	0.219	0.651
Humidity	2.366	0.158	0.078	0.787
Wind speed	0.020	0.890	0.328	0.581

concentration of bacteria at closed disposal site than that of active site.

SW3 and SW4 were transfer stations where waste was unloaded from small vehicles and then compacted and reloaded onto large vehicles for transport to disposal site. At these sites, waste was temporarily stored. Lower bacterial concentration was recorded at these sites as compared to disposal sites while SW4 site showed higher fungal concentration. Streib et al. [44] also documented higher bacterial concentration ( $10^3$ - $10^5$  CFU/m<sup>3</sup>) at waste collection and transfer stations. In this study the observed concentration levels of bacterial and fungal aerosol fall within the range between  $10^2$  and  $10^5$  CFU/m<sup>3</sup> and corresponds to the findings of other researchers [45-49]. In other studies of waste application facilities, the bacterial and fungal aerosol concentrations were relatively low [50] and ranged between 602-1973 CFU/m<sup>3</sup> and 705-1063 CFU/m<sup>3</sup> respectively [36].

SW1 site was modern, well organized and located on a large area but it showed increase microbial concentration as compared to other sites. It showed that microbial air quality did not depend on the effectiveness, modernization and maintenance of the technology. Instead, it depended on organic material found in solid waste acted as source of their nourishment but applied waste management technology could not be ignored. SW3 and SW4 usually received waste rich with organic

material. These findings highlight that both transfer stations and disposal sites were potential sources of biological risks.

Meteorological parameters are important factors influencing the extent of bioaerosol and also maintaining their vitality in the air [51]. During sampling period meteorological measurements including temperature, humidity and wind speed ranged from 29.5°C to 36.5°C, 28 to 44 % and 0.85 to 1.5 m/s during the wet season while in dry season these values ranged from 27.5°C to 42°C, 24 to 51 % and 0.70 to 1.2 m/s respectively. The mean temperature, humidity and wind speed values were 33.9°C, 36 % and 1.1 m/s during wet season while in dry season these values were 35.5°C, 34% and 0.83 m/s respectively. In order to study the impact of temperature, humidity and wind speed (independent variables) on bacteria and fungi (dependent variables), regression analysis was performed. The overall fitted regression model was significant for bacteria and fungi with  $r^2 = 73\%$  and  $r^2 = 94\%$  respectively. It was observed that temperature was significantly effecting the concentration of bacteria ( $p = 0.097$ ) at the level of 10%. While humidity and wind speed were not significantly related to bacteria. In case of fungi all parameters had no relationship with fungi (Table 3). Results showed that an increase in temperature enhanced the growth and release of bacteria. Temperature decreased the surface tension by effecting

Table 4. Identification of bacterial isolates in the air of sampling sites.

Total bacterial strains	Genus	Percentage (%)	Dominant species
Gram positive rod (59%)	<i>Bacillus</i>	39%	<i>B. cereus</i> , <i>B. subtilis</i> , <i>B. altitudinis</i> , <i>B. flexus</i> , <i>B. amyloliquefaciens</i>
	<i>Brevibacillus</i>	18%	
	<i>Mycobacterium</i>	1%	<i>M. esteraromaticum</i>
	Other	1%	<i>Rothia endophytic</i>
Gram positive cocci (38%)	<i>Staphylococcus</i>	21%	<i>S. sciuri</i>
	<i>Micrococcus</i>	17%	
Gram negative rod (2%)	<i>Pseudomonas</i>	1%	<i>P. stutzeri</i>
	<i>Ochrobacterum</i>	1%	<i>O. intermedium</i>
Gram negative cocci (1%)	<i>Acinetobacter</i>	1%	<i>A. indicus</i>

bonding force between the surface and bacteria, resultantly more bacteria were released [52]. However, previous studies revealed that the activity of bacteria was decreased when the temperature increased above 24°C [53]. While increased humidity favored the viability of bacteria. Conversely, fungi and their spores are resilient than bacteria and can tolerate greater stress due to dehydration and rehydration [54]. Increased relative humidity levels enriched the release of fungal spores [55]. Our findings were contrary to the above discussion as even though temperature exhibited significant relation with bacteria while other parameters showed non-significant association with bacteria and fungi. Lal et al. [56] also did not find any particular relationship between bioaerosol concentration and meteorological parameters.

#### Isolation Frequency of Bacterial Strains

The present study recorded 97% gram positive and 3% gram negative bacteria from all sampling sites. The predominant bacterial genera were *Bacillus* sp. and *Staphylococcus* sp. in the collected air samples as presented in Table 4. These results are comparable to the findings of other researchers [46, 47].

#### Molecular Identification of Isolates

This study revealed the existence of numerous bacteria and fungi at solid waste transfer stations and disposal sites in Lahore, Pakistan. These findings focused on the higher risk of bioaerosols exposure during management processes locally. There were different microbial composition at different sites. The isolated bacterial and fungal species were molecularly characterized (Table 5). The identified bacterial species have wide distribution in environment. The prevalence of identified bacterial species in air samples of solid waste management sites have also been reported by many researchers [36, 45, 46, 57]. Different species of *Bacillus* were reported but *Bacillus flexus* was rarely present in solid waste [58, 59]. Xiong et al. [60] identified *Rothia endophytica* isolated from *Dysophylla stellata* (Lour) plant root as novel specie of genus *Rothia*.

The fungal species composition is very important in the air and it constitute 70% of all microorganisms. Almost 40,000 of fungal species have been isolated worldwild [50]. The prevalence of identified fungal species in air samples of solid waste management sites have also been reported by other researchers [36, 47, 49, 50, 61]. The observed fungi have ability to produce

Table 5. Molecular identification of 20 isolates and assigned accession numbers.

Bioaerosols	Species	GenBank Acession No.	Locality
Bacteria	<i>Pseudomonas stutzeri</i>	MN150513	Lakhodair disposal site
Bacteria	<i>Staphylococcus sciuri</i>	MN150514	Lakhodair disposal site
Bacteria	<i>Bacillus cereus</i>	MN220638	Lakhodair disposal site
Bacteria	<i>Ochrobacterum intermedium,</i>	MN220639	Saghian bridge transfer station
Bacteria	<i>Bacillus altitudinis</i>	MN220640	Valancia town transfer station
Bacteria	<i>Bacillus amyloliquefaciens</i>	MN220641	Valancia town transfer station
Bacteria	<i>Acinetobacter indicus</i>	MN220642	Saghian bridge transfer station
Bacteria	<i>Bacillus flexus</i>	MN220643	Saghian bridge transfer station
Bacteria	<i>Bacillus subtilis</i>	MN220644	Lakhodair disposal site
Bacteria	<i>Mycobacterium esteraromaticum</i>	MN220645	Lakhodair disposal site
Bacteria	<i>Rothia endophytica</i>	MN220646	Lakhodair disposal site
Bacteria	<i>Bacillus altitudinis</i>	MN220647	Valancia town transfer station
Fungi	<i>Penicillium camemberti</i>	MN160216	Lakhodair disposal site
Fungi	<i>Penicillium</i> sp.	MN160217	Lakhodair disposal site
Fungi	<i>Penicillium oxalicum</i>	MN160218	Saghian bridge transfer station
Fungi	<i>Cochliobolus</i> sp.	MN160219	Saghian bridge transfer station
Fungi	<i>Fusarium</i> sp.	MN160220	Saghian bridge transfer station
Fungi	<i>Aspergillus oryzae</i>	MN160221	Valancia town transfer station
Fungi	<i>Aspergillus niger</i>	MN160222	Mehmood Booti disposal site
Fungi	<i>Aspergillus terreus</i>	MN160223	Valancia town transfer station

spores which protect against unfavorable conditions. They excrete mycotoxins which can cause allergies and pulmonary infections. Previous studies have identified that *Aspergillus* sp., *Penicillium* sp., *Cochliobolus* sp. and *Bacillus* species are associated with plastic degradation at disposal sites [62-64].

The present study showed that almost all of the fungal species and 95% of bacterial species were pathogenic and cause diseases in living organisms. People spending more time at solid waste management sites or its surrounding are more exposed to harmful bioaerosol. Waste workers and waste pickers are highly exposed to inhalable bacteria and fungi during waste handling, being caught by eye infections, respiratory illnesses, diarrhea, typhoid, musculoskeletal complaints [65, 66]. A previous research have revealed that increased microbial concentration does not really mean to cause infection among people spending more time at disposal site. However, increased concentration of pathogenic microbes in the air enhances their possibility of infection to living organisms [15].

Bioaerosol is an important and increasingly valued issue for public health. Many factors should be considered to treat microorganisms as pathogen such as individual's resistance against microorganisms, time of contact and their mode of action. Monitoring research of air is essential for conserving the correct state of environment.

### Conclusions

Our findings showed that All the solid waste management sites were a serious source of microbial emissions to air. The levels of bacteria and fungi were higher than that of prescribed limits. The emissions of bioaerosols were high enough at hazardous level and various factors affecting their concentration were microclimatic conditions and seasons. A significant difference in bacterial concentration was observed among sampling sites while fungi showed non-significant relationship. An increase in bacterial and fungal concentration was observed in wet and dry seasons respectively. Results indicated that temperature was significantly related to bacteria but not to fungi whereas other parameters did not show any relationship to bacteria and fungi. It was also observed that the air around solid waste management sites was rich with microbes of various taxonomic groups. The molecular identification of microbes showed most of the strains were pathogenic and has the ability to pose severe health risks. Despite some limitations such as small sample size and inadequate species identification, current study provides preliminary information related to microbial air quality within facilities of SWM sites. However, further studies are required to understand spatiotemporal variation in concentration of microorganisms in and around solid waste management sites and their potential health impacts. Whilst there are

no established guidelines or standards for bioaerosols emission from SWM facilities in Pakistan, the findings of the present study call for the development of policies and regulations to manage bioaerosols emission from such facilities.

### Conflict of Interest

The authors declare no conflict of interest.

### References

- MASOOD M., BARLOW C.Y., WILSON D.C. An assessment of the current municipal solid waste management system in Lahore, Pakistan. *Waste Manag. Res.* **32** (9), 834, **2014**.
- YAVINI T.D., MUSA A. Municipal solid waste and pollution management in Jalingo metropolis: Problems, challenges and strategies. *J. Environ.* **2**, 125, **2013**.
- SELIN E. Solid waste management and health effects: A qualitative study on awareness of risks and environmentally significant behavior in Mutomo, Kenya. **2013**.
- TIAN H., GAO J., HAO J., LU L., ZHU C., QIU P. Atmospheric pollution problems and control proposals associated with solid waste management in China: a review. *J. Hazard. Mater.* **252**, 142, **2013**.
- BAUD I.S.A., POST J., FUREDY C. Solid waste management and recycling: actors, partnerships and policies in Hyderabad, India and Nairobi, Kenya, Eds., Springer Science & Business Media, **76**, **2006**.
- PEARSON C., LITTLEWOOD E., DOUGLAS P., ROBERTSON S., GANT T.W., HANSELL A.L. Exposures and health outcomes in relation to bioaerosol emissions from composting facilities: a systematic review of occupational and community studies. *J. Toxicol. Env. Heal. B.* **18** (1), 43, **2015**.
- Endotoxins - Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Safety: [https://gupea.ub.gu.se/bitstream/2077/26596/2/gupea\\_2077\\_26596\\_2.pdf](https://gupea.ub.gu.se/bitstream/2077/26596/2/gupea_2077_26596_2.pdf). **2011**.
- SHELTON B.G., KIRKLAND K.H., FLANDERS W.D., MORRIS G.K. Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl. Environ. Microbiol.* **68** (4), 1743, **2002**.
- FRĄCZEK K., RÓŻYCKI H., ROPEK D. Statistical analyses of bioaerosol concentration at municipal landfill site. *Ecol. Chem. Eng. S.* **21** (2), 229, **2014**.
- NDIMELE E.C., EKELEME U.G., OGODO A.C., NWACHUKWU N.C., NNADI C.J., OTUTU E.A. Microbiological studies of waste dumpsite in Abia State University Teaching Hospital, Aba. *J. Med. Invest. Pract.* **9** (4), 151, **2014**.
- KALWASIŃSKA A., BURKOWSKA A. Municipal landfill sites as sources of microorganisms potentially pathogenic to humans. *Environ. Sci.-Proc. Imp.* **15** (5), 1078, **2013**.
- KALWASIŃSKA A., BURKOWSKA A., BRZEZIŃSKA M.S. Exposure of workers of municipal landfill site to bacterial and fungal aerosol. *CLEAN-Soil Air Water.* **42** (10), 1337, **2014**.



13. KORZENIEWSKA E. Emission of bacteria and fungi in the air from wastewater treatment plants—a review. *Front Biosci (Schol Ed)*. **3**, 393, **2011**.
14. MANSOUR F.A., EL-DOHLOB S.M., ABDEL HAMEED A.A., KAMEL M.M., EL-GENDY S.A. Microorganisms in the air over a bio-solid waste landfill in Egypt. *J Am Sci*. **8** (4), 573, **2012**.
15. KAŻMIERCZUK M., BOJANOWICZ-BABLOK A. Bioaerosol concentration in the air surrounding municipal solid waste landfill. *Ochrona Środowiska i Zasobów Naturalnych- Environ. Prot. Nat. Resour.* **25** (2), 17, **2014**.
16. ODEWABI A.O., OGUNDAHUNSI O.A., ODEWABI A.A., ORITOGUN K.S., EKOR M. Adenosine deaminase activity and immunoglobulin levels as potential systemic biomarkers of occupational hazards and health status in municipal solid waste management workers. *Environ Toxicol Pharmacol*. **35** (1), 1, **2013**.
17. EPSTEIN E. Disposal and management of solid waste: pathogens and diseases. Taylor & Francis Group: 6000 Broken Sound Parkway NW, Suite 300, US, **2015**.
18. SÁNCHEZ-MONEDERO M.A., STENTIFORD E.I., URPILAINEN S.T. Bioaerosol generation at large-scale green waste composting plants. *J Air Waste Manage.* **55** (5), 612, **2005**.
19. MAHMOOD S., SHARIF F., RAHMAN A.U., KHAN A.U. Analysis and forecasting of municipal solid waste in Nankana City using geo-spatial techniques. *Environ Monit Assess.* **190** (5), 275, **2018**.
20. LWMC (Lahore Waste Management Company). Progress Report of LWMC 2011-12. Lahore, Pakistan. **2012**.
21. ASHRAF U., HAMEED I., CHAUDHARY M.N. Solid waste management practices under public and private sector in Lahore, Pakistan. *Bull. Environ. Stud.* **1** (4), 98, **2016**.
22. BATOOL S.A., CHAUDHARY M.N. Municipal solid waste management in Lahore city district, Pakistan. *J. Waste Manag.* **29** (6), 1971, **2009**.
23. EDUARDA W., HEEDERIK D. Methods for quantitative assessment of airborne levels of noninfectious microorganisms in highly contaminated work environments. *Am. Ind. Hyg. Assoc.* **59** (2), 113, **1998**.
24. TAHA M.P.M., DREW G.H., LONGHURST P.J., SMITH R., POLLARD S.J.T. Bioaerosol releases from compost facilities: Evaluating passive and active source terms at a green waste facility for improved risk assessments. *Atmos. Environ.* **40** (6), 1159, **2006**.
25. WHEELER P.A., STEWART I., DUMITREAN P., DONOVAN B. Health effects of composting: a study of three compost sites and review of past data. R & D Technical Report P1-315/TR. Environmental Agency, Bristol, UK. **2001**.
26. HERR C.E.W., ZUR NIEDEN A., JANKOFISKY M., STILIANAKIS N.I., BOEDEKER R.H., EIKMANN T.F. Effects of bioaerosol polluted outdoor air on airways of residents: a cross sectional study. *Occup. Environ. Med.* **60** (5), 336, **2003**.
27. PAUL E.A., CLARK F.E. Soil Microbiology and Biochemistry. Academic Press, New York, **1989**.
28. HARRIGAN W.F., MCCANCE M.E. Laboratory Methods in Food and Dairy Microbiology, 8th ed., Academic Press Inc: London, 286, **1990**.
29. HOLT J.G., KRIEG N.R., SNEATH P.H.A., STANLEY J.T., WILLIAMS S.T. Bergey's Manual of determinative Bacteriology, 9th ed., Williams and Wilkins Baltimore: USA, 787, **1994**.
30. HENDRICKS R., PREBISH J. Microbiology Laboratory Report, Identification of Unknown Bacteria. *J. Appl. Sci.* **14**, **2005**.
31. ODONKOR S.T., MAHAMI T. Microbial Air Quality in Neighborhoods near Landfill Sites: Implications for Public Health. *J Environ Public Health*. **2020**.
32. POULSEN O.M., BREUM N.O., EBBEHØJ N., HANSEN Å.M., IVENS U.I., VAN LELIEVELD D., MALMROS P., MATTHIASSEN L., NIELSEN B.H., NIELSEN E.M., SCHIBYE B., SKOV T., STENBAEK E.L., WILKINS K.C. Sorting and recycling of domestic waste. Review of occupational health problems and their possible causes. *Sci Total Environ.* **168** (1), 33, **1995**.
33. MANSOUR F.A., EL-DOHLOB S.M., ABDEL HAMEED A.A., KAMEL M.M., EL-GENDY S.A. Microorganisms in the air over a bio-solid waste landfill in Egypt. *J. Am. Sci.* **8** (4), 573, **2012**.
34. WHO. Indoor air quality: Biological contaminants. European series No. 31, World Health Organization, Regional Publications: Copenhagen, 67, **1990**.
35. GOYER N., LAVOIE J., LAZURE L., MARCHAND G., ALLARD R., BHÉRE L. Bioaerosols in the workplace: Evaluation, control and prevention guide. Institute de Recherche en Sante elen securite du travail du Quebec. **2001**.
36. NIAZI S., HASSANVAND M.S., MAHVI A.H., NABIZADEH R., ALIMOHAMMADI M., NABAVI S., FARIDI S., DEGHANI A., HOSEINI M., MORADI-JOO M., MOKAMEL A., KASHANI H., YARALI N., YUNESIAN M. Assessment of bioaerosol contamination (bacteria and fungi) in the largest urban wastewater treatment plant in the Middle East. *Environ Sci Pollut Res.* **22** (20), 16014-16021, **2015**.
37. IGBORGBOR J.C., OGU G.I. Microbial assessment of air in the vicinity of some dumpsites in Delta state. *J Eng.* **5** (1), 2278, **2015**.
38. HUANG C.Y., LEE C.C., LI F.C., MA Y.P., SU H.J.J. The seasonal distribution of bioaerosols in municipal landfill sites: a 3-yr study. *Atmos. Environ.* **36** (27), 4385, **2002**.
39. ROODBARI A., NADDAFI K., JAVID A. Measurements of bioaerosols in the air around the facilities of waste collection and disposal. *Environ Prot Eng.* **39** (4), 105, **2013**.
40. SZYŁAK-SZYDŁOWSKI M., KULIG A., MIAŚKIEWICZ-PĘSKA E. Seasonal changes in the concentrations of airborne bacteria emitted from a large wastewater treatment plant. *Int Biodeter Biodegr.* **115**, 11, **2016**.
41. DING W., LI L., HAN Y., LIU J., LIU J. Site-related and seasonal variation of bioaerosol emission in an indoor wastewater treatment station: level, characteristics of particle size, and microbial structure. *Aerobiologia.* **32** (2), 211, **2016**.
42. KÖCHLING T., SANZ J.L., GAVAZZA S., FLORENCIO L. Analysis of microbial community structure and composition in leachates from a young landfill by 454 pyrosequencing. *Appl Microbiol Biot.* **99** (13), 5657, **2015**.
43. ZAINUN M.Y., SIMARANI K. Metagenomics profiling for assessing microbial diversity in both active and closed landfills. *Sci Total Environ.* **616**, 269, **2018**.
44. STREIB R., BOTZENHART K., DRYSCH K., RETTENMEIER A.W. Dust and microorganism count at delivery, sorting and composting of home refuse and home refuse-like industrial waste. *Zentralblatt für Hygiene und Umweltmedizin= Int. J. Hyg. Environ. Med.* **198** (6), 531, **1996**.

45. PAHARI A.K., DASGUPTA D., PATIL R.S., MUKHERJI S. Emission of bacterial bioaerosols from a composting facility in Maharashtra, India. *Waste Manage.* **53**, 22, **2016**.
46. AGARWAL S., MANDAL P., SRIVASTAVA A. Quantification and characterization of size-segregated bioaerosols at municipal solid waste dumping Site in Delhi. *Procedia Environ. Sci.* **35**, 400, **2016**.
47. BREZA-BORUTA B. The assessment of airborne bacterial and fungal contamination emitted by a municipal landfill site in Northern Poland. *Atmos. Pollut. Res.* **7** (6), 1043, **2016**.
48. GHANBARIAN M., GHANBARIAN M., GHANBARIAN M., NOORIEH N., HOSSEINI M., MAHVI A.H. Assessment of Bacterial and Fungal Bioaerosols in Different Indoor Spaces of Tehran Municipal Solid Waste Processing Facilities. *Avicenna J Environ Health Eng.* **4** (2), 1, **2017**.
49. PATIL N.S., KAKDE U.B. Assessment of fungal bioaerosol emission in the vicinity of a landfill site in Mumbai, India. *Int. J. Environ. Waste Manage.* **20** (1), 75, **2017**.
50. HAMEED A.A.A., HABEEBUALLAH T., MASHAT B., ELGENDY S., ELMORSY T.H., ELSEROUGY S. Airborne fungal pollution at waste application facilities. *Aerobiologia.* **31** (3), 283, **2015**.
51. VILAVERT L., NADAL M., FIGUERAS M.J., DOMINGO J.L. Volatile organic compounds and bioaerosols in the vicinity of a municipal waste organic fraction treatment plant. Human health risks. *Environ Sci Pollut Res.* **19** (1), 96, **2012**.
52. MOULI P.C., MOHAN S.V., REDDY S.J. Assessment of microbial (bacteria) Concentrations of ambient air at semi-arid urban region: Influence of meteorological factors. *Appl Ecol Env Res.* **3** (2), 139, **2005**.
53. TANG J.W. The effect of environmental parameters on the survival of airborne infectious agents. *J. R. Soc. Interface.* **6**, S737, **2009**.
54. KARRA S., KATSIVELA E. Microorganisms in bioaerosol emissions from wastewater treatment plants during summer at a Mediterranean site. *Water Res.* **41**, 1355, **2007**.
55. ERKARA I.P., ASAN A., YILMAZ V., PEHLIVAN S., OKTEN S.S. Airborne Alternaria and Cladosporium species and relationship with meteorological conditions in Eskisehir City, Turkey. *Environ. Monit. Assess.* **144**, 31, **2008**.
56. LAL H., GHOSH B., SRIVASTAVA A., SRIVASTAVA A. Identification and characterization of size-segregated bioaerosols at different sites in Delhi. *Aerosol Air Qual Res.* **17** (6), 1570, **2017**.
57. SHYAMALA D.C., BELAGALI S.L. Seasonal Variation of Microbial Populations during Composting Processes of Municipal Solid Wastes. *Int. J. Innov. Res. Sci. Eng. Technol.* **3** (6), 14126, **2014**.
58. POKHREL B., BASHYAL B., MAGAR R.T. Production, Purification and Characterization of Cellulase from Bacillus subtilis Isolated from Soil. *European J. Biotechnol. Biosci.* **2** (5), 31, **2014**.
59. HAMID B., JEHANGIR A., BABA Z.A., KHAN I. Isolation and characterization of psychrotrophic cellulolytic bacteria from landfill site under temperate climatic conditions **13** (5), 92, **2018**.
60. XIONG Z.J., ZHANG J.L., ZHANG D.F., ZHOU Z.L., LIU M.J., ZHU W.Y., ZHAO L.X., XU L.H., LI W.J. Rothiaendophytica sp. nov., an actinobacterium isolated from Dysophylla stellata (Lour.) Benth. *Int J Syst Evol Microbiol.* **63** (11), 3964, **2013**.
61. PINTO M.J.D.V., VEIGA J.M., FERNANDES P., RAMOS C., GONÇALVES S., VELHO M.M.L.V., GUERREIRO J.S. Airborne microorganisms associated with packaging glass sorting facilities. *J Toxicol Env Heal A.* **78** (11), 685, **2015**.
62. INDUMATHI A., GAYATHRI T., BIOTECHNOLOGY E., NADU T., NADU T. Plastic Degrading ability of Aspergillus oryzae isolated from the garbage dumping sites of Thanjavur, India. *Int. J. Curr. Microbiol. Appl. Sci.* **8** (13), 2319, **2016**.
63. MUHONJA C.N., MAGOMA G., IMBUGA M., MAKONDE H.M. Molecular Characterization of Low-Density Polyethylene (LDPE) Degrading Bacteria and Fungi from Dandora Dumpsite, Nairobi, Kenya. *Int. J. Microbiol.* **2018**.
64. GIACOMUCCI L., RADDADI N., SOCCIO M., LOTTI N., FAVA F. Polyvinyl chloride biodegradation by Pseudomonas citronellolis and Bacillus flexus. *New biotechnol.* **52**, 35-41, **2019**.
65. CHOKHANDRE P., SINGH S., KASHYAP G.C. Prevalence, predictors and economic burden of morbidities among waste-pickers of Mumbai, India: a cross-sectional study. *J Occup Med Toxicol.* **12** (1), 30, **2017**.
66. MOL M.P., PEREIRA A.F., GRECO D.B., CAIRNCROSS S., HELLER L. Assessment of work-related accidents associated with waste handling in Belo Horizonte (Brazil). *Waste Manag. Res.* **35** (10), 1084, **2017**.