Aerosol and Air Quality Research

ORIGINAL RESEARCH
https://doi.org/10.4209/aaqr.220431

Prevalence, Dispersion and Nature of Bioaerosols over a Solid Landfill Site in Central India

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ABSTRACT

Bioaerosols (or biological aerosols) consist of aerosol particles that originate biologically either as fully active component or as whole or part of inactive fragments. They are ubiquitously present in the atmospheric environment. They are the least investigated pollutants due to their complex structure and composition. The effects of bioaerosols, originating due to the processes, such as wastewater management, handling of sludge, composting, municipal solid waste, and animal facilities, on human health are well recognized. Proper identification, quantification, impacts and exposure threshold levels are essential to understand the nature and impact of bioaerosols on human health and climate. In this communication, we determine the inhalable ($PM_{2.5}$) particulate matter concentration and embedded bioaerosol (bacteria and fungi) levels over a Municipal Solid Waste (MSW) landfill site in relation to surrounding upwind and downwind locations in Nagpur, India. Measurements were made using an Airmetrics MiniVol air sampler and bioaerosols were analyzed by adopting the culture-based method. A total of 23 fungal and 17 bacterial morphotypes were found in this study. The results showed dominance of bacterial bioaerosol over fungal bioaerosol at the landfill site. The bioaerosol levels were higher at the landfill than the upwind and downwind sites. The bioaerosols did not show any correlation to the $PM_{2.5}$. In summary, the results indicate abundance of $PM_{2.5}$, containing both bacterial and fungal bioaerosols, which can pose human health hazards over the study region. In our knowledge, it is the first study of bioaerosols at the landfill site in Nagpur, India.

Keywords: Particulate matter, Landfill, Bacteria, Fungi, Human health

Special Issue:
2022 Asian Aerosol Conference (AAC 2022) (VI)

OPEN ACCESS

Received: February 12, 2023
Revised: April 29, 2023
Accepted: May 8, 2023

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Publisher:
Taiwan Association for Aerosol Research
ISSN: 1680-8584 print
ISSN: 2071-1409 online

1 INTRODUCTION

Bioaerosols consist of aerosols that originated biologically and are ubiquitously present in the environment (Fröhlich-Nowoisky *et al.*, 2016). They are composed of organic materials, such as fungi, bacteria, pollens, particulate matter ($PM_{2.5,\ 10}$), and cell fragments, constituents or by-products, which are viable or non-viable (Dias and Viegas, 2021; Lodish *et al.*, 2000). These particles vary greatly from nanometer-sized viruses to micrometer-sized bacteria, fungal spores and pollen grains (Heikkinen *et al.*, 2004). Dispersion of bioaerosols can be due to convective air current, and they can remain in the atmosphere for long durations due to their small size. Several studies have also observed long-range transport of dust-associated bacteria across continents (Smets *et al.*, 2016; Han *et al.*, 2020). Thus, bioaerosols are ubiquitous like the airborne particulate matter and may play important role in the atmosphere by interacting with other atmospheric constituents. Hence, there role in the understanding and prediction of climate change is currently a center of investigation (Fröhlich-Nowoisky *et al.*, 2016). Significant interactions between bioaerosols, atmospheric pollutants (O_3 and NO_2), and meteorological parameters, such as temperature, humidity, and precipitation, have been reported recently by Cariñanos *et al.* (2021).

Studies on the origin of bioaerosols and their impacts on human health are sparse (NRC, 2008). Infectious and non-infectious properties of bioaerosols depend on various factors, such as physical composition, aerodynamic diameter, and biological properties (Nasir and Colbeck, 2010; Smets *et al.*, 2016). Few studies have been done in Indian context. Effects of bioaerosol exposure in the human population surrounding an open landfill in Dehradun was studied by Madhwal *et al.* (2020). Similarly, Patil and Kakde (2017) assessed fungal bioaerosol emission in the vicinity of Deonar landfill site in Mumbai. Bioaerosol concentration showed drastic seasonal fluctuations with the highest concentration in monsoon (July), when rainfall and relative humidity were maximum, and temperature varied between 25°C and 32°C. The least bioaerosol concentration was reported in winter (October). Thus, the authors reported a positive correlation between relative humidity and the concentration of fungal bioaerosols. However, no such study is available in Nagpur city, which is a strategically located and one of the fastest growing economy of India. In the present study, we determined the inhalable ($PM_{2.5}$) particulate matter and $PM_{2.5}$-associated bioaerosol (bacterial and fungal) concentrations at a Municipal Solid Waste (MSW) landfill site in Nagpur, India. It will help in creating baseline data and determine the need to implement control measures at the landfill site, which are the hotspots of bioaerosol emission. Early assessment will allow planning in advance, which will not be possible once the city is at the peak of its development.

2 METHODS

2.1 Study Site Details

Nagpur is located at the center of the Indian peninsula at a height of about 312 m above mean sea level (AMSL). Nagpur is the winter capital and the third largest city of Maharashtra. The total area of Nagpur city is 228 km^2, while that of Nagpur district is 9892 km^2. The Nagpur district is geographically located from latitude 21°41′N to 20°35′N and Longitude 78°15′E to 79°45′E (RMC, 2023). The city has a major MSW landfill site at Bhandewadi at the outskirt. Sampling was done at the landfill site and its surrounding based on wind direction. Specifically, surrounding locations within 1 km diameter of the landfill site in upwind and downwind directions were selected (Fig. 1).

2.2 Sampling of $PM_{2.5}$ Particles

Sampling was conducted in February 2021 at the landfill site and the surrounding residential areas upwind and downwind of the landfill site. MiniVol portable air sampler (AirMetrics, USA) with 47 mm PTFE (Polytetrafluoroethylene) membrane and flow rate of 5 L min^{-1} was used for the sampling of $PM_{2.5}$ (fine inhalable particles). The sampling was carried out at 2 locations simultaneously using the above sampler. Thus, the landfill and downwind sites were sampled together during 11–17 February 2021, while landfill and upwind sites were sampled together during 22–28 February 2021. Sampling was carried out for 8 h daily. The sampling strategy was in accordance with the work of Oh *et al.* (2020). The sampling period was kept 8 h to get an estimate of bioaerosol exposure to the workers at landfill facility performing various jobs (sorting, loading/unloading of waste, transport of trucks to collect and deposit the waste). Sampling was performed during daytime working hours (9:00–17:00 local time). The PTFE membranes were desiccated before use and weighing, and stored or transported in sealed cassettes. The $PM_{2.5}$ fraction was estimated gravimetrically using microbalance.

2.3 Estimation of $PM_{2.5}$-Associated Bioaerosols

Culture-based methods were used for the bioaerosol analysis. Two types of media, namely, PDA (Potato Dextrose Agar: potato extract 4 g L^{-1}, dextrose 20 g L^{-1}, agar 15 g L^{-1}, pH 3.5), and NA (Nutrient Agar: Peptone 5 g L^{-1}, beef extract 3 g L^{-1}, NaCl 5 g L^{-1}, agar 15 g L^{-1}, pH 7) were used for the cultivation of fungi and bacteria respectively. The filter papers containing $PM_{2.5}$ particles were immersed in 5 mL PBS (phosphate-buffered saline) and homogenized for 5 min. The resulting suspension was serially diluted and plated on the above agar plates under aseptic conditions. The plates were then incubated at ambient temperature for a period of 48 h to one week for colonies to grow. The total number of bacteria and fungi in each sample were counted

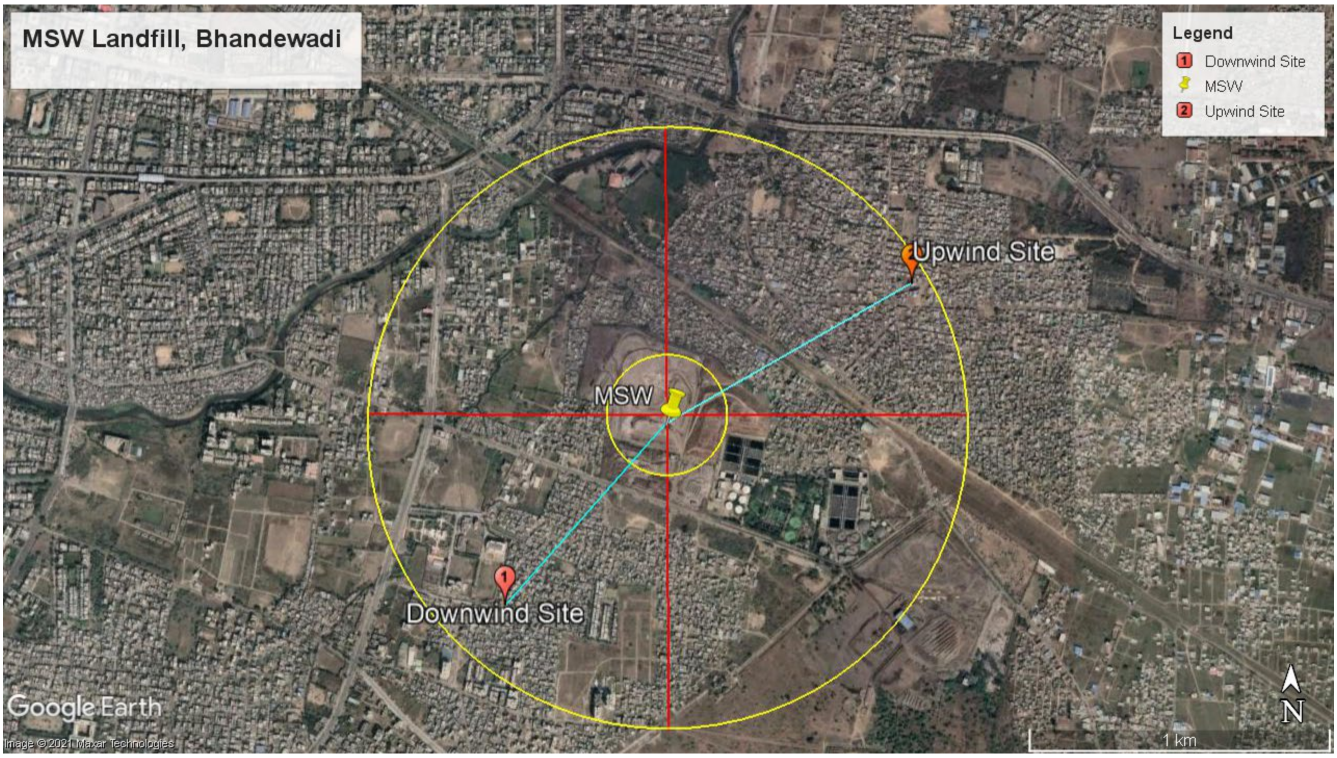

Fig. 1. Sampling sites near the landfill at Nagpur, India.

and expressed in terms of CFU (colony forming unit) m^{-3}. The colony characteristics, i.e., colour, margin, form, elevation, size, shape etc., were also noted by visual observation.

2.4 Statistical Analysis

The $PM_{2.5}$ and bioaerosol concentrations were compared by t test using MS Excel program.

3 RESULTS AND DISCUSSION

MSW landfills give rise to several problems like smell and rodent/animal menace, but bioaerosols are an often overlooked problem originating from them (Yang *et al.*, 2022). Landfill activities lead to generation of particulate matter, such as $PM_{2.5}$, which are fine inhalable particles with diameter of 2.5 micrometers and less. They can penetrate into the lungs, damage the alveolar lining, and affect lung function (Xing *et al.*, 2016). Biological agents like bacteria or fungal spores associated with $PM_{2.5}$ make them more hazardous and can lead to infections or allergies (Morgado-Gamero *et al.*, 2021). Thus, MSW landfills are hotspots of bioaerosol emission. Very few studies have been done on bioaerosols at landfill sites in India and effect of wind on their dispersal (Madhwal *et al.*, 2020). Majority of such studies have addressed only the fungal bioaerosols (Patil and Kakde, 2017; Srivastava *et al.*, 2021). In this study, we have attempted to address this question at a major landfill site located in Nagpur, India, which has not been studied so far.

3.1 Study Site and Sampling Locations

Nagpur is divided into 14 tahsils with a population of 46,53,171 as per the 2011 census with 68.3% population living in urban regions of the district. The general climate of Nagpur is very dry and semi-humid throughout the year except for the monsoon period (RMC, 2023). Approximately 1100–1200 tonnes of solid waste per day is generated in the city (NMC, 2017). The waste is dumped at a major landfill site located at the outskirt of the city (Fig. 1). Sampling was done at the MSW landfill site located at Bhandewadi, Nagpur, and surrounding residential areas. The residential sampling locations were selected based on the wind direction.

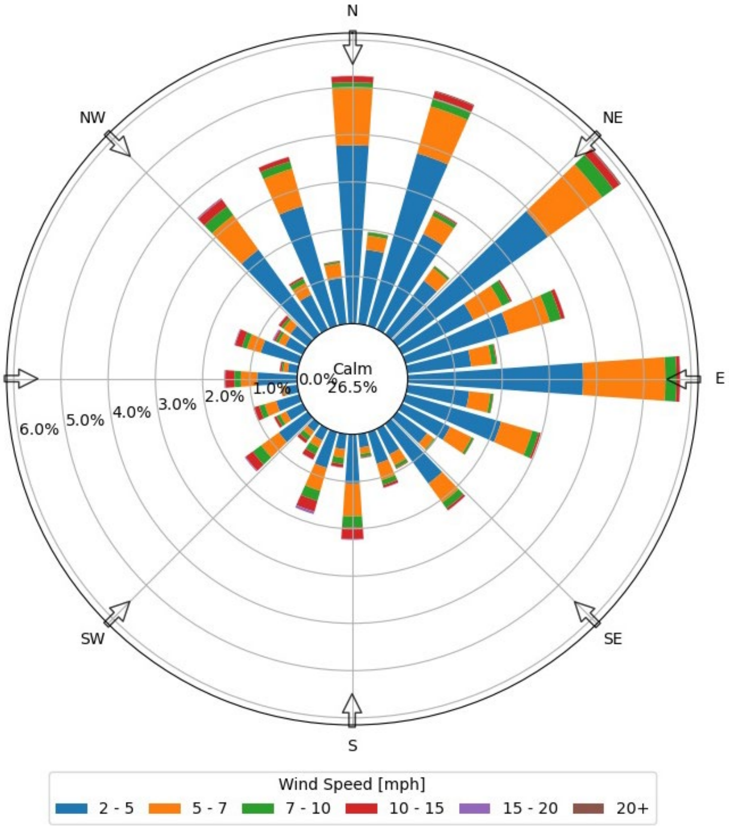

Fig. 2. Wind rose diagram of Nagpur city for the month of February (30-years average).

To get the average wind direction, 30-year average wind roses of the study month (February) for the Nagpur city were obtained from the Regional Meterological Center, Nagpur (Fig. 2). Wind rose is a graphical representation of the speed and direction of wind at particular location. Fig. 2 shows that the wind was blowing from the North and East direction for the maximum amount of time in February. Thus, NE (North–East) was considered as upwind and SW (South–West) as downwind direction for the sampling. Accordingly, an Anganwadi Kendra situated around 800 m SW from the landfill site was selected as the downwind sampling site. Similarly, a government school situated around 950 m NE from the landfill site was selected as the upwind sampling site. It can be seen from the satellite image of the sampling sites that the study locations are surrounded by human settlements (Fig. 1).

3.2 Estimation of $PM_{2.5}$ Concentration

The $PM_{2.5}$ measurements were made 8 h daily for 7 days at landfill and downwind sites first, and then landfill and upwind sites later on. Thus, data was collected for 7 days at the upwind and downwind sites, while 14 days data was available for the landfill site. Longer sampling time of 8 h was chosen to increase the volume of air being passed, and subsequently the probability of capturing $PM_{2.5}$ and associated bioaerosols. However, long sampling time may lead to loss of bioaerosol culturability (Haig *et al.*, 2016). Thompson *et al.* (1994) and Wang *et al.* (2001) noted that apart from the sampling time, bioaerosol viability also depends on other factors like microbial species and humidity. Thus, bioaerosol sampling time is a critical parameter that influences the bioaerosol viability as well as sampling probability. During an investigation of airborne pathogens in a children's hospital of Taiwan, sampling time of 8–24 h was used with airflow rate of 12 L min^{-1}, indicating that such long sampling times are feasible (Wan *et al.*, 2012). Thus, much longer sampling times are reported in the literature to increase the capture efficiency. In this study, we used a sampling time of 8 h, which may have affected the viability. Hence, the results must be interpreted with this limitation.

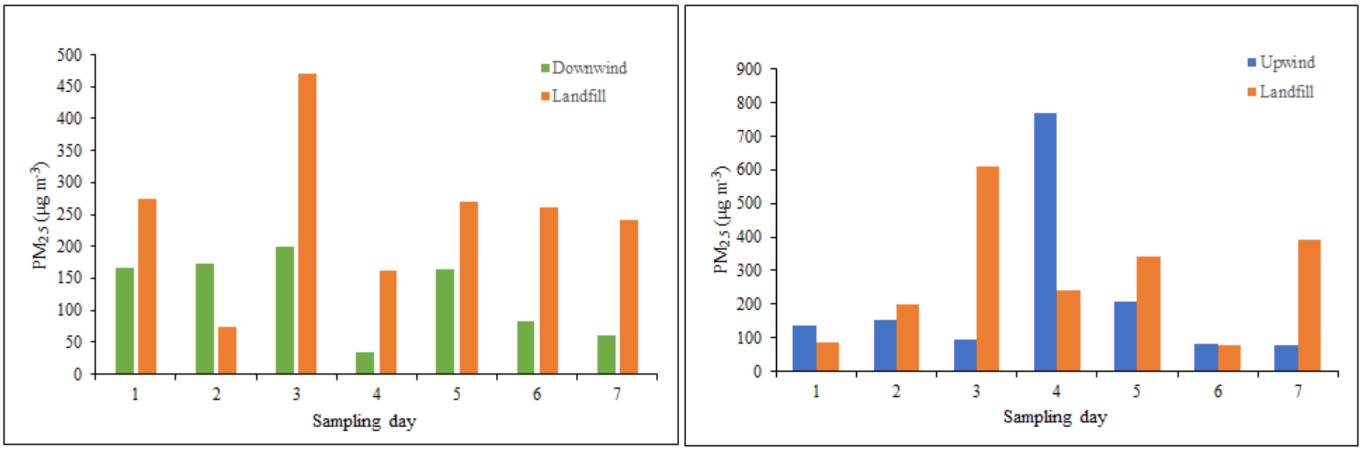

Fig. 3. Daily variation of $PM_{2.5}$ concentration over both downwind and upwind directions at the Bhandewadi landfill site.

The average concentration of $PM_{2.5}$ at the landfill site was found to be 250.3 µg m^{-3} and it was 125.6 µg m^{-3} for the downwind site during 11–17 February 2021. Similarly, the average $PM_{2.5}$ concentration for landfill site was found to be 277.8 µg m^{-3} and for the upwind site, it was 217.1 µg m^{-3} during 22–28 February 2021 (Fig. 3). Thus, the $PM_{2.5}$ concentration at the landfill site was comparatively higher than both upwind and downwind sites. The $PM_{2.5}$ at upwind site was slightly higher than the downwind site, although the difference was insignificant ($p > 0.05$). The $PM_{2.5}$ level showed slight variation at the landfill site between the 2 sampling periods, but the difference was statistically insignificant ($p > 0.05$). Similarly, the $PM_{2.5}$ at landfill and upwind sites showed insignificant difference ($p > 0.05$). However, the landfill $PM_{2.5}$ was significantly higher than the downwind site ($p < 0.05$). Overall, the $PM_{2.5}$ at upwind site was in between those at landfill and downwind sites, which was unexpected. It may be due to minor renovation work going on near the upwind site or local wind direction deviations from the average direction, which is a very common phenomenon. In comparison, the $PM_{2.5}$ level reported at a landfill in Dehradun, India, ranged from 60 to 224 µg m^{-3} (Madhwal *et al.*, 2020).

3.3 Estimation of $PM_{2.5}$-Associated Bioaerosols

The $PM_{2.5}$-associated bioaerosols (bacteria and fungi) were eluted from the collection filter by homogenization in PBS. Several variations of bioaerosol elution methods are reported in the literature as sample loss during elution is a critical consideration. Farnsworth *et al.* (2006) reported elution efficiency of 105% for *B. subtilis* from filter samples by hand-shaking. Therkorn *et al.* (2017) used 2 min vortexing followed by 10 min ultrasonication for elution of bioaerosols from filters. In yet another study by Clark Burton *et al.* (2005), vortexing for 2 min followed by shaking for 15 min yielded higher elution efficiency of *Bacillus globigii* than vortexing for 2 min followed by unltrasonication for 15 min. In all, mixing and shaking seem to give an adequate recovery of bioaerosols. Hence, bioaerosols were recovered from the filter by homogenization for 5 min in this study. However, the results must be interpreted with the caution that the bioaerosol recovery may not be complete.

After extraction, the bioaerosols were analyzed by culturing. The resulting colonies on the agar plates were counted and examined morphologically (Fig. 4). It gives a preliminary idea about the microbes. Colony morphological parameters (size, color, edge, elevation, and form/texture) are traditionally used to rapidly differentiate microorganisms. The combination of all parameters is necessary for the best discrimination as microorganisms may vary from each other in a single or multiple morphological parameters (Kowalski and Cramer, 2020). Some studies have tried to develop machine learning models to automatically distinguish microorganisms based on the colony phenotypic differences (Rodrigues *et al.*, 2023). Based on the morphological difference, 23 different fungal colonies were observed from all 3 locations in this study (Table 1). In Table 2, morphologically different bacteria are listed. A total of 17 different bacteria were observed. Even though the colony types of bacteria were less than fungi, the bacterial CFU concentration was

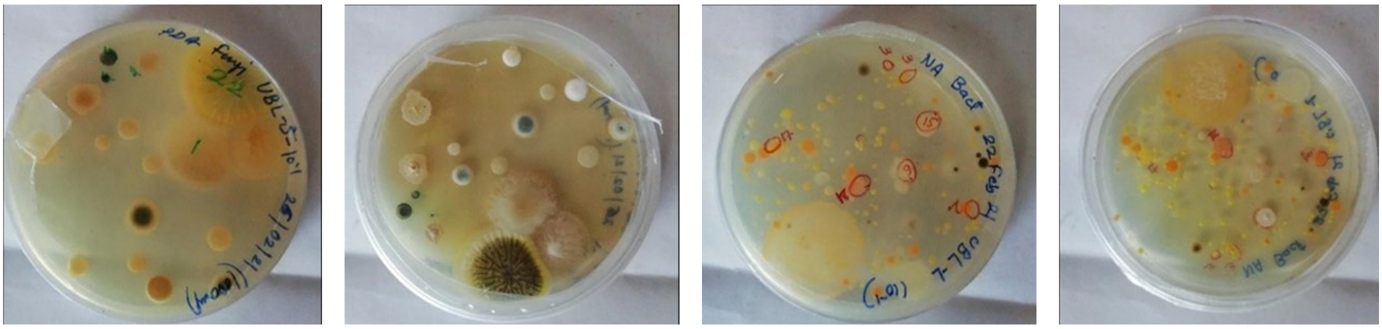

Fig. 4. Photograph showing bacterial and fungal colonies on media plates.

Table 1. Morphologically distinct fungal colonies observed in the study.

No.	Color	Margin	Elevation	Form	Size (mm)
1	Yellow-Green-White	Entire	Raised	Wrinkled	20–25
2	Brown-Green-White	Entire	Raised	Filamentous	15–20
3	White	Filamentous	Pulvunate	Round	5
4	Olive-Green	Ciliate	Raised	Circular/Round	12–16
5	Bluish-Green	Entire	Raised	Circular	15
6	Green-White	Entire	Raised	Wrinkled	20–25
7	Green	Entire	Convex	Wrinkled	12–18
8	Yellowish White	Ciliate	Raised	Wrinkled	25–30
9	White	Filamentous	Pulvunate	Filamentous	15
10	Green-White	Filamentous	Raised	Filamentous	6
11	White (Red from backside)	Filamentous	Pulvunate	Filamentous	40
12	White	Entire	Crateriform	Round with scalloped margin	15
13	Green	Entire	Crateriform	Filamentous	5
14	White	Filamentous	Raised	Round	5
15	Green with Black Margin	Entire	Raised	Filamentous	7
16	Green-White	Filamentous	Flat	Filamentous	12
17	White Green (Black-spores)	Filamentous	Raised	Wrinkled	25–35
18	White (yellow spores)	Entire	Crateriform	Wrinkled	18–20
19	White-Green	Filamentous	Raised	Wrinkled	35
20	Yellow-Pink (brown spores)	Entire	Raised	Wrinkled	25–30
21	Green-White (Red from backside)	Filamentous	Pulvunate	Round	12
22	Green (volcano)	Entire	Crateriform	Round with scalloped margin	10
23	Green-White	Filamentous	Raised	Wrinkled	14–25

higher than fungi in general. The bioaerosol concentrations of bacteria and fungi and their variations are shown in Fig. 5 and Fig. 6. At the landfill site, the average bioaerosol concentration for bacteria and fungi was found to be 10742 CFU m^{-3} and 704 CFU m^{-3} respectively for the first sampling period. In the second sampling week, bacterial concentration was found to be 13533 CFU m^{-3}, while the fungal bioaerosol concentration was 2395 CFU m^{-3} at the landfill site. In both cases, the concentration of bacteria was found to be more than fungi. Although the bacterial and fungal counts were different for the 2 sampling periods, the difference was not statistically significant. It indicates some temporal variation in the bioaerosol concentration, which may be due to different activities at the landfill site, such as the shifting of waste, clearing land, or due to local meteorological deviations. Yang *et al.* (2022) reported bacterial and fungal levels of around 33–22778 and 8–450 CFU m^{-3} respectively at the Laogang Landfill in China, which is the largest landfill of Asia. In another study, average bacterial and fungal bioaerosols at a landfill site in Dehradun, India, were 3609 and 4582 CFU m^{-3}, respectively (Madhwal *et al.*, 2020).

The average bacterial and fungal counts were 895 and 646 CFU m^{-3} at the downwind site, and 712 and 1512 CFU m^{-3} at the upwind site, respectively. The bacterial count at the landfill site was

Table 2. Morphologically distinct bacterial colonies observed in the study.

No.	Colour	Margin	Elevation	Form	Opacity	Surface	Size (mm)
1	Pinkish-Orange	Entire	Convex	Circular	Opaque	Smooth, Shiny	3–5
2	Orange	Entire	Flat	Circular	Opaque	Smooth	2–4
3	Yellow	Entire	Convex	Circular	Opaque	Smooth, Shiny	1–3
4	White	Entire	Flat	Circular	Opaque	Dull	3–7
5	White	Entire	Flat	Circular	Transparent	Dull	2–5
6	White	Lobate	Crateriform (volcano)	Round with scalloped margin	Opaque	Dull	7–14
8	White	Entire	Flat	Circular	Transparent	Dull	4–5
10	White	Entire	Flat	Circular	Opaque	Dull	5
13	Pale White	Lobate	Umbonate	Complex	Opaque	Shiny/Glistening	22
14	White	Entire	Convex	Circular	Opaque	Glistening	2–4
15	White	Undulate	Umbonate	Wrinkled	Opaque	Dull	14–18
16	White	Entire	Umbonate	Wrinkled	Translucent	Dull	10–15
17	Yellow-Orange	Undulate	Flat	Irregular	Opaque	Smooth	18–20

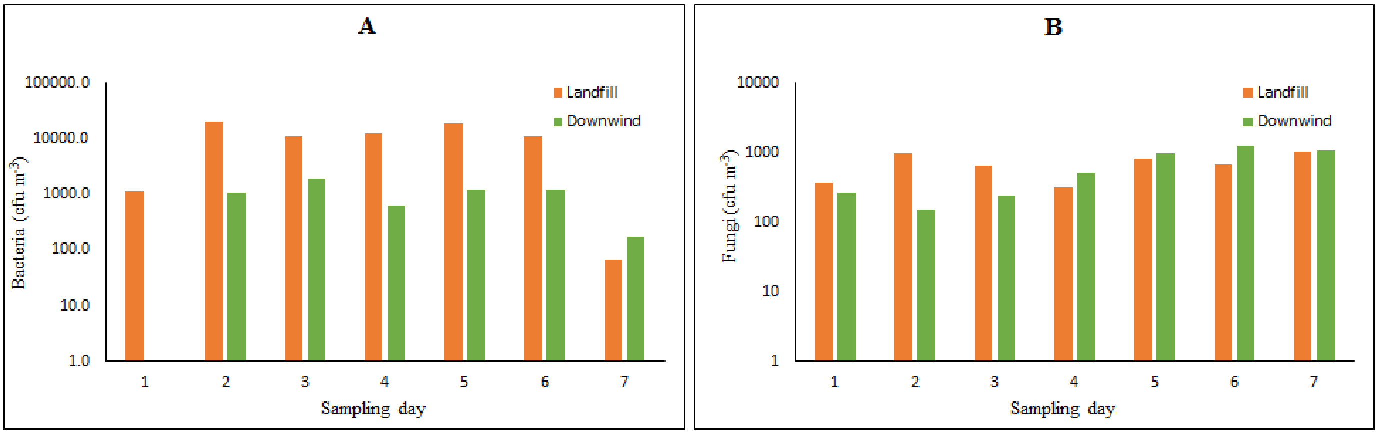

Fig. 5. Daily mean variation of (A) bacterial and (B) fungal concentrations at the downwind and landfill sites.

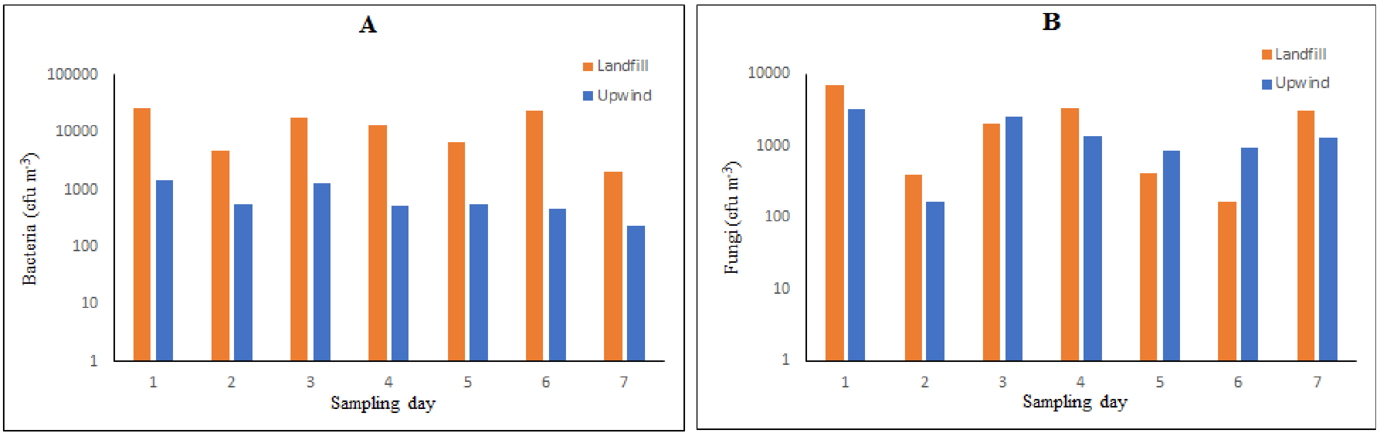

Fig. 6. Daily mean variation of (A) bacterial and (B) fungal concentrations at the upwind and landfill sites.

consistently higher than the upwind and downwind sites ($p < 0.05$), while the fungal count did not show significant difference ($p > 0.05$). On the other hand, the bacterial and fungal counts at the upwind and downwind sites were statistically similar ($p > 0.05$). The fungal count was unusually higher than the bacterial count at the upwind site, which may be due to the ongoing renovation work there. A higher fungal count than the bacterial count was also reported at the landfill site of Dehradun by Madhwal *et al.* (2020). The bioaerosol concentrations did not show significant correlation with $PM_{2.5}$ level. This is in agreement with the findings of Madhwal *et al.* (2020), who also reported no statistical correlation of bacterial and fungal aerosols with PM at an open landfill site in Dehradun, India. However, positive correlation has been reported between PM and bioaerosols in other environmental settings. Jeon *et al.* (2011) showed significant positive correlation of culturable bacterial level with total suspended particles and PM_{10} during Asian dust events at Seoul, Korea. Similarly, Wei *et al.* (2020) revealed that bacterial, fungal and endotoxin concentrations were significantly correlated with $PM_{2.5}$ at coastal city of Weihai, China. The low correlation between $PM_{2.5}$ and bioaerosols observed in this study indicates other non-landfill activity sources of PM in the vicinity or fugitive dust. It may also be due to other meteorological parameters such as air temperature, wind speed, humidity, which are known to significantly impact the bioaerosols prevalence (Hosseini *et al.*, 2021).

The overall trend shows generally higher $PM_{2.5}$ and associated bioaerosols at the landfill site. The spread of bioaerosols was significantly low to the downwind site, which may be due to small sampling period. A longer sampling period is needed to estimate the long term spread in the downwind direction. Morgado-Gamero *et al.* (2021) also reported higher concentrations of bioaerosols at the landfill than in the nearby village during their study of sanitary landfill near Barranquilla, Colombia. The literature study results differ in terms of aerial transport of bioaerosols ranging from 100 m to over 1 km (Breza-Boruta, 2016; Fraczek and Kozdroj, 2016). It may be due to the difference in the landfill design and modernization. Hence, the location of landfill near populations and control measures are important areas needing urgent attention.

4 CONCLUSIONS

In the present study, inhalable particulate matter i.e., $PM_{2.5}$, and the associated bioaerosol (bacteria and fungi) concentrations were measured at and around a landfill site in Nagpur city. The $PM_{2.5}$ and bioaerosol concentrations were found to be generally higher at the landfill site as compared to those at the upwind/downwind locations. A total of 23 different fungi and 17 bacteria were reported in the present study. The study revealed that the bacterial bioaerosol concentration was dominant at the landfill site. Bacteria were found to contribute about 75% to 85% of total bioaerosols at the landfill. On the other hand, the proportion of bacterial bioaerosols was lower at 58% and 32% at downwind and upwind sites respectively. Further study should be carried out to estimate the seasonal variation, identification, and risk assessment of the bioaerosols.

ACKNOWLEDGEMENTS

Authors are grateful to the Director, National Environmental Engineering Research Institute, (NEERI), Nagpur, India for providing the infrastructure facilities to carry out the work reported in this paper. Thanks, are also due to the authorities of Amity University Haryana (AUH), Gurugram for their continued support. The first author, PDB, an M. Tech (Atmospheric Technology and Climate Management) student of AUH is thankful to all colleagues, both at NEERI and AUH for their help during the field observations and analysis of data. The second author, PK, is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for the financial assistance in the form of Junior Research Fellowship (JRF). KRC No.: CSIR-NEERI/KRC/2023/FEB/HTC/1. Authors are also highly thankful to the Editor and Reviewers for their constructive comments and valuable suggestions which immensely helped to improve the clarity and scientific merit of the original manuscript.

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