Bioaerosol Emissions from Wastewater Treatment Process at Urban Environment: An Anthropogenic Source Emission

5.1 Preamble

Waste management is the major issue because of rapid and extensive urbanization in the large population. Since some waste can be re-utilized after separating directly from the mixture of waste but remaining required to treat for the further uses (Cheng et. al., 2024a; Cheng et. al., 2024b; Mensah et. al., 2022; Shen et. al., 2024). Wastewater treatment is very essential to reduce the burden and combating environmental pollution (Eyitayo et. al., 2024; Yang et. al., 2024a). Although, the wastewater treatment processes are one of the major sources of bioaerosols in the outdoor environment and it is the major concern of the researchers (Humbal et. al., 2018; Kowalski et. al., 2017; Liu et. al., 2020a; Liu et. al., 2020b; Noh et. al., 2019). Sewages coming to the wastewater treatment plant (WWTPs) contains large number of microbes along with various domestic waste (Cheng et. al., 2024a; Cheng et. al., 2024b; Noh et. al., 2019). During the wastewater treatment process, due to mechanical action of wind and others, the microbes in the wastewater easily get aerosolized and reach into the atmosphere and transported through the wind from one place to another (Brandi et. al., 2000; Kataki et. al., 2022; Kowalski et. al., 2017). The bioaerosols found form the various reports indicate that it has potential risk of infection form the WWTPs in immunocompromised people, occupations worker and the surrounding community (Chen et. al., 2021b; Cui et. al., 2023; Yang et. al., 2024b). Now it has become the emerging area for the environmentalist and epidemiologist on the effect on human health (Cui et. al., 2023; Michałkiewicz, 2019).

In the recent studies, it is reported that the worker in the WWTP have the higher chance of the many diseases like fatigue, headache, allergic alveolitis, dizziness, sinusitis and gastrointestinal problem (Chen et. al., 2021a; Gotkowska-Płachta et. al., 2013; Li et. al., 2016; Yan et. al., 2021a; Yan et. al., 2021b; Yan et. al., 2023). Cumulatively this disease is called as the “sewage workers syndrome” (Fracchia et. al., 2006; Han et. al., 2020). The pathogenicity of these microbes depends on the chemical composition, microbial species type and the intake of the microbes (Cai et. al., 2014; Han et. al., 2018). The exposure for the various disease in the worker from WWTPs is from the different pathways like skin or mucosal contact, inhalation, and indigestions during the contact with the contaminated

surfaces, clothes and tools etc. (Chen et. al., 2021b; Masclaux et. al., 2014; Wang et. al., 2021; Yan et. al., 2021a). The inhalation exposure of the bioaerosol is 10^5 times more potent than dermal exposure. The WWTPs generate the bioaerosols form the process of handling and treatment of wastewater and sludge (Masclaux et. al., 2014; Wang et. al., 2023).

Recent studies during the pandemic shows that the aerosolization of biological species from wastewater may trigger the significant release of the SARS-CoV-2 viruses in the environment; this may be the warming for the future pandemic management (Gholipour et. al., 2021; Kataki et. al., 2021; Zaneti et. al., 2020). It needs the additional control measures for the handling the functioning of the WWTPs to avoid the infection of microbes in the co-workers (Yang et. al., 2024b; Zaneti et. al., 2020).

In this study, the WWTPs were examined as potential source of bioaerosols. The quantitative and qualitative analysis of the bioaerosols were done using culture-based methods and metagenomics techniques. Total concentration and size distribution of the bioaerosols were also analyzed in this study. A survey-based approach has been made to predict the potential health impact of the bioaerosols on the population residing in areas nearby WWTPs on the basis of age, time of exposure, and distance from source. Antibiotic tests were also performed on the most dominant microbes by Kirby-Bauer disk diffusion method in order to study the antibiotic resistance of the microbes.

5.2 Materials and Methods

5.2.1 Site description

Study was done in the city of Varanasi, Uttar Pradesh, India. Varanasi is located at an elevation of 80.71 meters (264.8 ft) in the center of the Ganges valley of North India, in the Eastern part of the state of Uttar Pradesh. It has a subtropical climate with variations in summer and winter with temperature range between 22-46ºC in summers while in winter the temperature can even go down below 5ºC (IMD). The sampling was done at the wastewater treatment plants (WWTPs), STP Ramnagar (25°16’ 42’’N, 83°01’22’’E), Varanasi, India. This STPs has the capacities of 80 MLD. The main source of the effluent is the domestic wastewater coming from the urban residents. It has the rotating disc aeration tank and the oxidation ditch treatment process. It was equipped with a microporous aeration tank and

adapted with Anaerobic-Anoxic-Oxic process. The remaining treatment units of these two phases were the same (primary/secondary sedimentation tank) or shared (grillage machine and inlet pumping station).

5.2.2 Sampling campaign

An Andersen six-stage cascade impactor (TISCH, USA) was used to collect culturable bioaerosols emission from the treatment plant site during winter (January to February) and summer (April to May) 2023. The cut off diameter of six stages in the sampler are 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 µm. The impactor was loaded with 6 Petri dishes containing nutrient agar (NA) medium for bacteria and potato dextrose agar (PDA) medium was for fungi. The sampling sites were located in the middle of the treatment plant and near the aeration tank. The sampler was installed at the height of 1.5 meter above the ground and the flow rate for the inlet in cascade impactor was fixed at 28.3 L/min. Sampling was done for the 20 min according to the standard procedure (Kowalski et. al. 2017). Air sampling was done in the morning and evening, where morning (9am) corresponds to high flow and evening (6pm) corresponds to the low flow. A total of 32 samples were collected for the bioaerosols analysis during winter and summer for both low flow and high flow from this method. Where the impactor was ultra-sonicated, autoclave and disinfected with 75% ethanol before and after sampling each sampling. All the pre procedures including installation, analysis and cleaning were performed in the biosafety chamber. While doing the air sampling personal protective equipment was used. The temperature, relative humidity and wind speed were recorded with the help of temperature and humidity meter and anemometer respectively.

5.2.3 Bioaerosols concentration measurement

After sampling, the samples on the petri plate were sealed and put in the incubator for the given temperature (for bacteria 25°C for 72hr and for fungi 35 °C for 48 hr). After this time duration, the colony on the petri plates appeared and were counted with the help of colony counter. The total bioaerosols concentration were calculated using the following formula (equation 1):

$$\text{Bioaerosol concentration } (\text{CFU}/m^3) = \frac{\text{number of colony } (C')}{\text{flow rate } \times \text{ sampling duration (minute)}} \times 1000$$

$$C' = \sum_{i=1}^{6} C_i \quad (1)$$

Where, C' is the airborne cumulative concentration of bioaerosols (CFU/m^3 air) and C_i is the bioaerosol concentration of *i* stage of Anderson six-stage impactor (CFU/m^3).

5.2.3.1 Aerosolization ratio

The aerosolization ratio ((CFU/m^3 air)/(CFU/m^3 wastewater)) is calculated by dividing the microorganism concentration in wastewater into the airborne cumulative concentration mentioned in equation 2 (Dashti et. al., 2022).

$$\text{Aerosolization ratio} = \frac{C_i'}{C_{wi}'} \quad (2)$$

The microorganism concentration in wastewater is calculated using the formula in equation 3.

$$C_w' = \sum_{i=1}^{3} \frac{C_{wi} n_i}{3} \times 10^6 \quad (3)$$

Where, C_w' is the bioaerosols concentration in wastewater (CFU/m^3 wastewater), and C_{wi} is the number of colonies after dilution (CFU/mL), and n_i is diluted multiples (10^3, 10^4 and 10^5). The microbial concentrations in the wastewater or sludge sample were calculated according to the APHA (1995). In each case blanks were used in the same procedure without sucking the air.

5.2.4 Identification of the species and metagenomics

Identification of the species was done using the metagenomics analysis, where 16s and ITS metagenomics were performed for bacteria and fungi respectively. There was the following procedure were done for each of the metagenomics.

In the process of 16s metagenomics, DNA extraction was done using Xploregen kit. The extracted DNA amplification of 16s region was performed with TAQ Master mix. Here 16s (Forward): -5' AGAGTTTGATGMTGGCTCAG3' and 16s (Reverse): -5' TTACCGCGGCMGCSGGCAC3' primers were used. 40 ng of extracted DNA is used for amplification along with 10 pM of each primer. 25 cycles of the following condition was run

(1) denaturation at 95℃ for 15 sec (2) annealing at 60℃ for 15 sec, (3) elongation at 72℃ for 2 mins, (4) final extension at 72℃ for 10 mins and hold at 4℃.

Extracted DNA from the samples and were subjected to nano drop and gel check before being taken for PCR amplification where the Nano Drop readings of 260/280 at an value of ~1.8 to 2 was used to determine the DNA's quality. Similarly, the amplified 16s PCR product is purified and subjected to gel check and Nanodrop QC where, NanoDrop readings of 260/280 at an ~ value of 1.8 to 2 was used to determine the DNA's quality.

The amplicons from each sample were purified with ampurebeads to remove unused primers and an additional 8 cycles of PCR was performed using illumina barcoded adapters to prepare the sequencing libraries. Libraries were purified using ampurebeads and quantitated using Qubit dsDNA High Sensitivity assay kit. Sequencing was performed using illumina miseqwith 2x300PE v3-v4 sequencing.

The database used for 16s v3-v4 region was NCBI where, the bcl data received from the sequencer get de-multiplexed into .fastqraw data. Then the de-multiplexed data quality was checked using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools. When QC passed samples become qualified for further analysis and the pipeline for metagenomics (for 16s metagenomic) was used. Once the run was completed, the final raw OTU table was got from which it can get the visualization of the analysis.

In the process of ITS metagenomics, DNA extraction was done using Xploregen kit. The extracted DNA amplification of ITS region was performed with TAQ Master mix. Here ITS (Forward): 5' TTGGTCATTTAGAGGAAGTAA 3' and ITS (Reverse): 5' GCTGCGTTCTTCATCGATGC 3' primers were used.

40 ng of extracted DNA was used for amplification along with 10 pM of each primer. Initial denaturation was at 95℃, 25 cycles of the following condition was run (1) denaturation 95℃ for 15 sec, (2) annealing at 60℃ for 15 sec, (3) elongation at 72℃ for 2 mins, (4) final extension at 72℃ for 10 mins and hold at 4℃.

Extracted DNA from the samples were subjected to Nano Drop and GEL check before being taken for PCR amplification where the Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 was used to determine the DNA's quality. Similarly, the amplified ITS PCR product was purified and subjected to GEL Check and Nanodrop QC where the Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 was used to determine the DNA's quality.

The amplicons from each sample were purified with ampurebeads to remove unused primers and an additional 8 cycles of PCR was performed using Illumina barcoded adapters to prepare the sequencing libraries. Libraries were purified using Ampurebeads and quantitated using Qubit dsDNA High Sensitivity assay kit. Sequencing was performed using Illumina Miseq with 2x300PE ITS sequencing kit. The database used for ITS region was UNITE, where the bcl data received from the sequencer was de-multiplexed into .fastqraw data. Then de-multiplexed data quality checked using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools. When QC passed, samples become qualified for further analysis, and pipeline for metagenomics (Biokart Pipeline) were used for ITS metagenomics. Once the run was completed, the final raw OTU table was got from which it can get the visualization of the analysis.

5.3 Results and discussion

5.3.1 Concentration and size distribution of bioaerosols (bacterial and fungal) at STP during summer and winter

The total concentration was calculated with the help of colony counter and using the formula stated above, where the total concentration was measured at each stage and sum up. In our observation the total bacterial and fungal bioaerosols concentration varied between seasons. During winter, bacterial concentration ranged between 484 to 1810CFU/m^3, whereas fungal variations were in the range of 446to 2162CFU/m^3. In the summer the bacterial and fungal bioaerosols concentrations were higher than the winter and it was 544to 2194CFU/m^3 and 1356to 2088CFU/m^3 for bacterial and fungal, respectively (Figure 5.1 (a and b)).

The major concentration of the bacterial bioaerosols were reported in the size range of 4.7 to 3.3 μm (same reported by Katsivela et. al., 2017 (Katsivela et. al., 2017)) while fungal concentrations were higher in between 3.3 to 2.1 μm. Some significant fraction of fungal spores were also reported in the fine range of size that is 0.65 μm of aerodynamic diameter. In both the seasons, bioaerosols concentration were higher in the summer in each size range. Since the size of the bioaerosols produced from WWTPs are mostly in the range of 4.7 to 2.1 μm and some significant fraction of the fungal bioaerosols were found in the ultra-fine range. Which can easily reach to the deep site of the respiratory system (Mbareche et. al., 2022; Xu et. al., 2020).

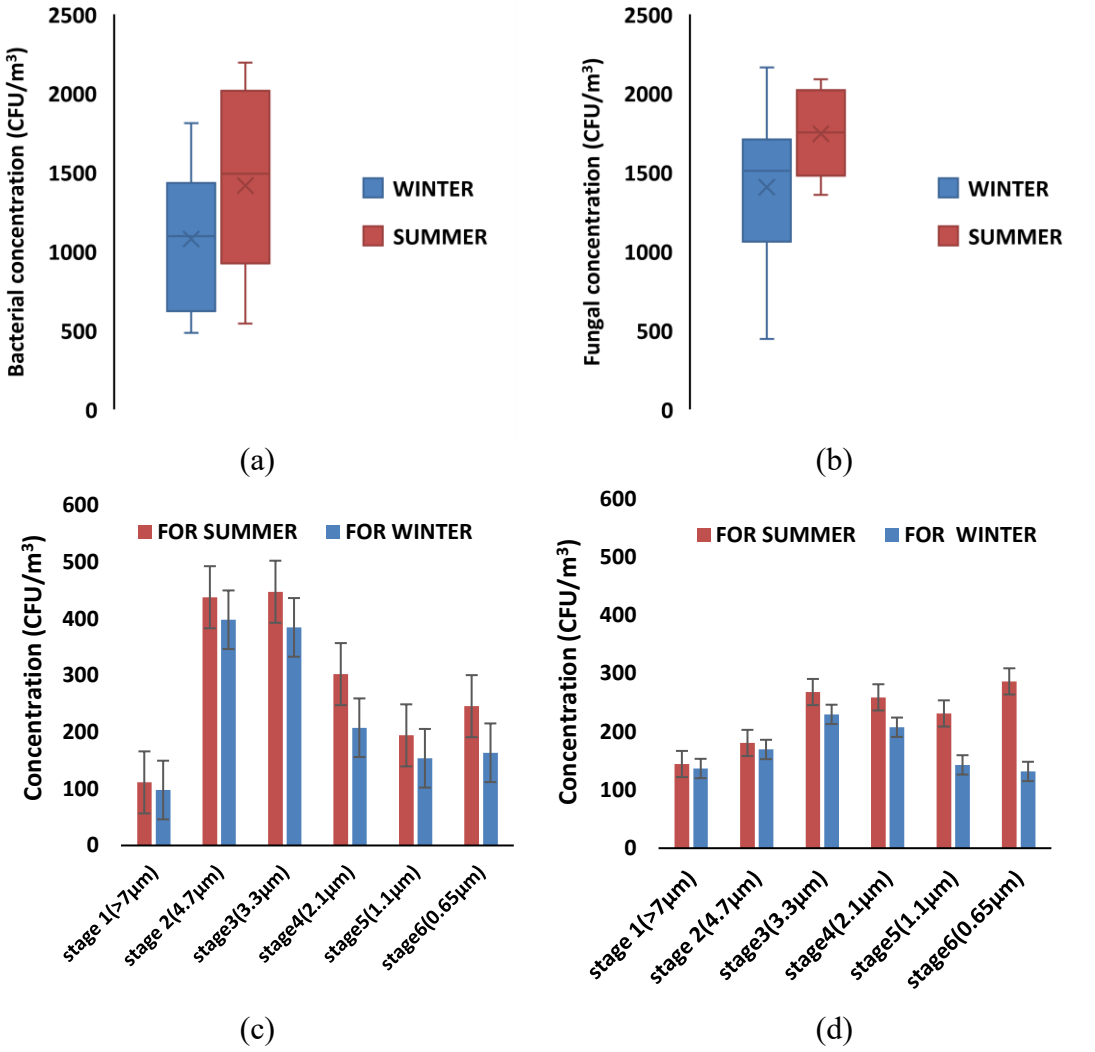
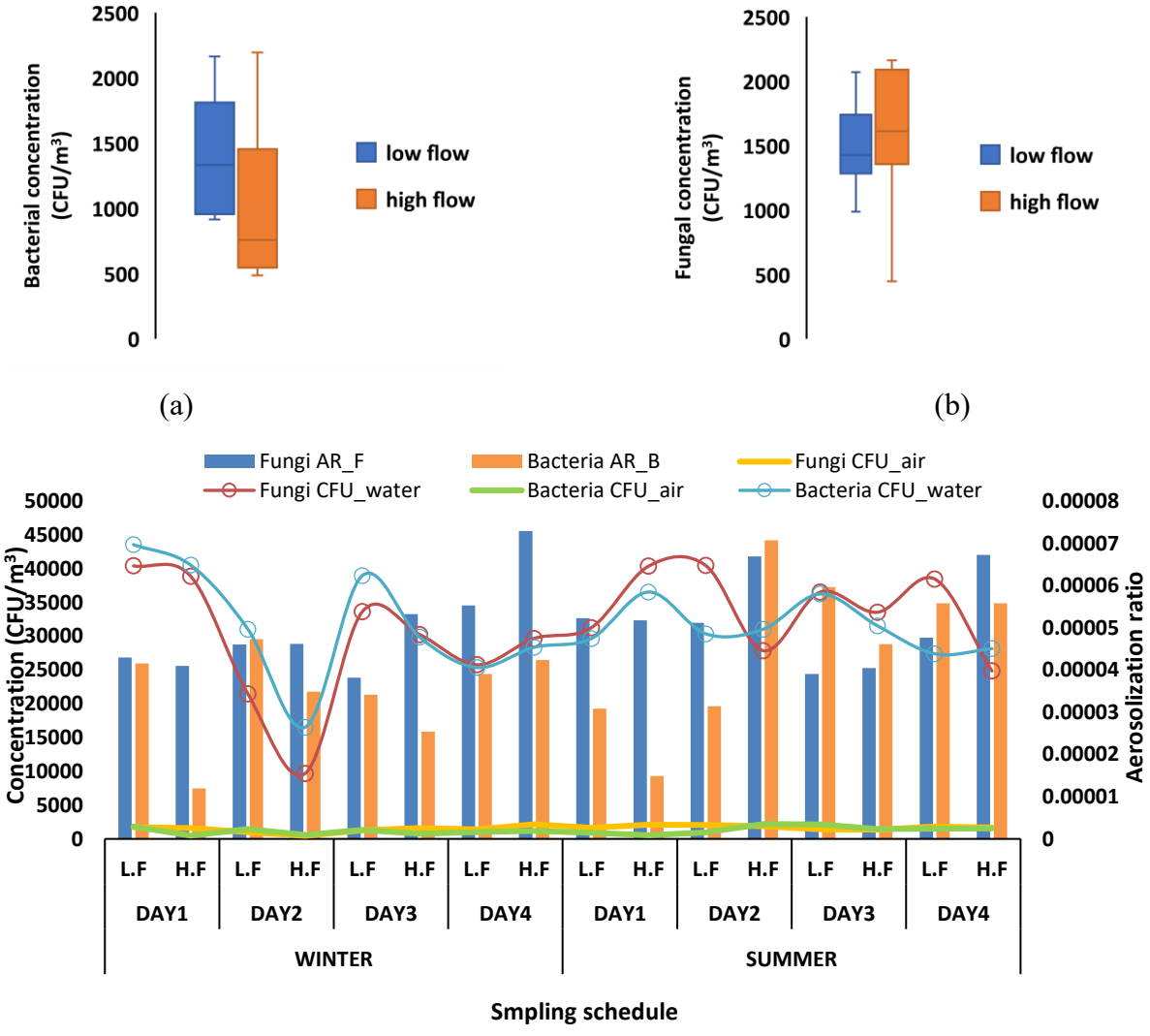


Figure 5.1 Variation in the total (a) bacterial and (b) fungal concentration of bioaerosols and its size distribution during winter and summer (c, d) over WWTPs.

5.3.2 Variation in concentration anlysis of bioaerosols during different water intake (high flow and low flow) and aerosolization ratio

It has been found that during the lower water intake the concentration was found higher in comparison to the higher flow rate. This is due to low flow; turbulence was maximum that can create more aerosolization of droplets due to mechanical action and wind (Yang et. al., 2021). Figure 5.2 (c) shows the overall summary of the present work, where concentration of the bacteria in water (Bacteria CFU_water), fungi in water (Fungi CFU_water) and bacteria in air (Bacteria CFU_air), fungi in air (Fungi CFU_air) were plotted with the

seasonal variation as well as high flow and low flow. The aerosolization ratio were also calculated for both bacteria (Bacteria AR_B) and fungi (Fungi AR_F) separately in each season and flow. It has been found that during the summer the aerosolization ratio were found higher in comparison to the winter and it reaches to 7.28 x 10^{-5} from 1.19 x 10^{-5}. The aerosolization of fungi were much higher in winter but during the summer the aerosolization of the bacteria were higher in comparison to the fungi. Mostly, in the low flow aerosolization of the bioaerosols were much higher in comparison to the low flow.



(a) (b)

(c)

Figure 5.2 Concentration of bacterial and fungal bioaerosols produced from during the high flow and low flow of wastewater intake during (a) winter and (b) summer and (c) aerosolization ratio.

5.3.3 Taxonomic composition of total bioaerosols concentration at WWTPs

Sequencing was performed using Illumina Miseq with 2x300PE v3-v4 sequencing for of bacterial and 2x300PE ITS sequencing kit for fungal analysis. Top 10 enrichment of genus in 16s and ITS metagenomic analysis of the bioaerosols at WWTPs shown in Figure 5.3. The details of metagenomics has shown in Appendix C (Figure B1-B10).

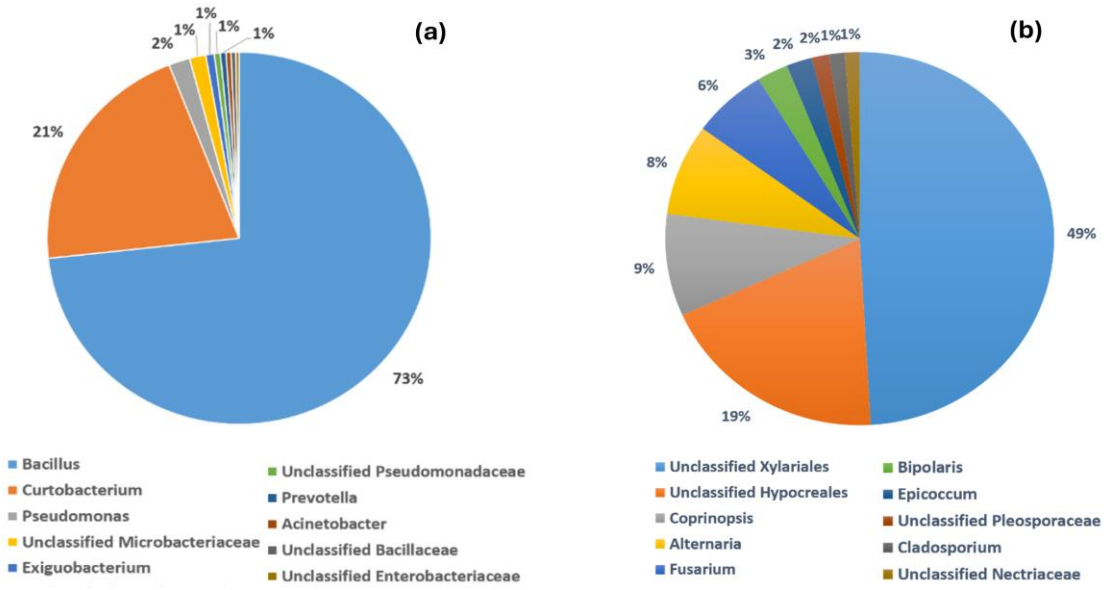


Figure 5.3 Top 10 enrichment of genus in (a) 16s and (b) ITS metagenomic analysis of the bioaerosols at WWTPs.

In the metagenomic analysis of the bacterial aerosols samples total 167 genus has been reported (A.1), where *Bacillus* were the most dominant genus (top 10) found in the samples, which was nearly 73% of the total genus. The second most dominant genus was curtobacterium, which was nearly 21% of the total genus present in the sample. Other than this, *pseudomonas* (2%), Unclassified *Microbacteriacase* (1%), *Exiguobacterium* (1%), unclassified *Pseudomonadeceae* (1%), *Prevotella* (1%), *Acinetobacter bacillaceae*, unclassified *Bacillaceae* and unclassified *Enterobacteriaceae* (<1%) were found in the small amount (Figure 5.3(a)).

In the collected sample of the bioaerosols, bacteria like *Bacillus* are responsible for respiratory problems, headache, sore throat, mild fever, fatigue and muscles aches. *Curtobacterium* are normal, facultative bacteria its usually cause little harm to humans but

sometime its cause upper respiratory infection (Actor, 2012). Whereas *pseudomonas* create skin infection and redness of the eyes (Gellatly & Hancock, 2013).

In the metagenomic analysis of the fungal bioaerosols total 41 were reported where unclassified *Xylariales* (49%) were the most dominant genus found in the samples and the second most dominant species of fungi was unclassified *Hypocreales* (19%). Other than this *Coperinopsis* (9%), *Alternaria* (8%), *Fusarium* (6%), *Biopolaris* (3%), *Epicoccum* (2%), Unclassified *Pleosporaceae* (2%), *Cladosporium* (1%) and unclassified *Nectriaceae* (1%) were found in the small amount (Figure 5.3 (b)).

In the observed fungal bioaerosols, *Xylariales* found in the large amount in the samples from the WWTPs, generally it has not reported as pathogenic but for immunosuppressed body it may harm (Michielse & Rep, 2009). Other fungi like, *Hypocreales* are known for the plant disease (Chen et. al., 2021a) whereas, *Coprinopsis* are able to decompose the dead organic matters and it is not typically known for plant and animal disease. Alternaria can cause respiratory and skin problem in humans (Cheng et. al., 2024b), *Fusarium* cause the eye infection in the humans (Cheng et. al., 2024a), *Biopolaris* are responsible for skin infection (Chintagunta et. al., 2017), *Epicoccum* associated with respiratory issues (Kurup et. al., 2000), *Pleosporaceae*, *Cladosporium* and *Nevtriaceae* are responsible for eye and respiratory issues (Kurup et. al., 2000). Therefore, depending upon the nature of the bioaerosols these particles can affect the human body and most vulnerable to the immunocompromised body.

5.3.3 Correlation between bioaerosols concentration and environmental factor at WWTPs

The correlation between the environmental factor and concentration of the bioaerosols has been determined. The given Figure 5.4 of correlation matrix summarizes the bacterial and fungal concentrations with respect to the wind speed, temperature, and relative humidity. Since the concentration of the bioaerosols increases with the increase in wind speed (Haas et. al., 2020). In the present analysis it has been observed as that bioaerosols and wind speed shows good correlation (Figure 5.4) (for 0.568 and 0.657 for bacteria and fungi respectively) but not very strong. In the urban environment, emission of bioaerosols is more due to many anthropogenic sources (here WWTPs). Apart from wind speed, temperature and RH also affect the concentration of the bioaerosols (Anees-Hill et. al., 2022; Gao et. al., 2016; Liu et.

al., 2019). Here RH, and temperature shows very low correlation with both bacterial and fungal bioaerosols. This may be because of temperature and RH shows mixed correlation with the bioaerosols.

	BA (CFU/m^3)	FA (CFU/m^3)	WS (m/s)	RH (%)	Temp (^{o}C)
BA (CFU/m^3)	1				
FA (CFU/m^3)	0.539	1			
WS (m/s)	0.568	0.657	1		
RH (%)	-0.145	-0.098	-0.034	1	
Temp (^{o}C)	0.361	0.333	0.516	-0.651	1

Figure 5.4 Correlation matrix of bioaerosols (bacterial and fungal) and environmental factors

For instance, temperature shows the positive correlation with the fungi *Cladosporium* (Grinn-Gofroń & Rapiejko, 2009) and have insignificant correlation with *Aspergillus* while negative correlation with the *Penicillium* (Haas et. al., 2023). Likewise, RH has negative impact on *Cladosporium* and *Aspergillus* and positive correlation with the *Penicillium* (Pyrri & Kapsanaki-Gotsi, 2017). Also, regarding the temperature and RH the reverse results have been also reported by Mosalaei et al. (2021).

5.3.4 Antimicrobial resistance of bioaerosols from WWTPs

The antimicrobial susceptibility tests was done using the Kirby-Bauer disk diffusion method (Kumar et. al., 2021). For the antimicrobial disk, two antibiotics azithromycin and cefixime were used. Antibiotic azithromycin of 500 mg and cefixime of 200 mg was crushed and mixed in 10 mL of distilled water in a test tube and shake until properly mixed. The test tubes were centrifuged to obtain a regularly mixed solution. These solutions were then further diluted in 1, 1/10, 1/100 and 1/1000 dilution. In these solution disks of 6 mm were submerged and left for overnight. The antimicrobial susceptibility disk of different concentration was placed on the respective petri dishes containing the sample inoculum spread on nutrient agar media and was sealed using paraffin wax and was left for incubation for 48 hours.

After the incubation period, the petri dishes were observed, and the inhibition zone created due to the restriction of growth of bacteria by the antimicrobial disk (Kourmouli et. al., 2018) was measured which was further compared with the standard inhibitory zone diameter for azithromycin and cefixime.

Table 5.1 Antibiotic analysis of most dominant species for azithromycin and cefixime and the nature of the resistance on the basis of inhibition zone

Azithromycin (500 mg)	**Diameter obtained (mm)**	**Sensitive (≥18)**	**Moderately sensitive (14-17)**	**Resistance (≤13)**
0 dil.	18	✓	-	-
1/10 dil.	13	-	-	✓
1/100 dil.	7	-	-	✓
1/1000 dil.	5	-	-	✓
Cefixime (200 mg)	**Diameter obtained (mm)**	**Sensitive (≥19)**	**Moderately sensitive (16-18)**	**Resistance (≤15)**
0 dil.	9	-	-	✓
1/10 dil.	8	-	-	✓
1/100 dil.	4	-	-	✓
1/1000 dil.	3	-	-	✓

From Table 5.1 it was observed that the selected bacteria had the highest sensitivity against azithromycin of 500 mg concentration with no dilution in the solution (an inhibition zone of diameter 18 mm as compared to >18 of standard measurement). With azithromycin of 1/10 dilution, it is moderately sensitive (an inhibition zone of diameter 13 mm as compared to >14-17 of standard measurement), while it is resistance against all the remaining dilutions of azithromycin. (An inhibition zone of diameter 7 and 5 mm as compared to <13 mm) of standard measurement. While for cefixime the target bacteria were found to be resistant for all the solutions. (an inhibition zone of diameter 9, 4, 8 and 3 mm as compared to <15 mm respectively) of standard.

5.3.5 Health risk assessment using cross section survey

The cross-section survey was done using the questionnaire methodology with interaction with the people working in the vicinity of the treatment plant as well as the people residing near the treatment plant. The questionnaire was based on the WHO health risk assessment methodology including the age, sex, distance from the source, exposure hour and the different types of infection the population having. The details of the questionnaire were mentioned in the Appendix (B). And the results are categorised in the different age groups, distance from the source, exposure, and the type of infection in the population separately. The health risk assessment was done using the cross-section survey in the range of 0 to 150 m of the area near by the WWTPs. The total 163 people were taken part in the survey and on the basis of no. of responses in each cases the health risk assessment has been categorised using the following criterion (Figure 5.5).

5.3.5.1 Age of the population

The health survey was conducted in 0 to 150 m periphery of the WWTPs, where the total population was divided in the four category such as 0 to 16 yr, 17 to 35 yr, 36 to 55 yr and above 55 yr.

It has been shown in the Figure 5.5(a) that the headache (30%) was very prominent in the 0 to 16 yr category.

In the category of 17 to 35 yr, they mostly feel change in the taste (22%) and quality of air (22%).

The age group of 36 to 55 yr old, the headache was common (22%), and they feel that the air is contaminated (19%).

In the last category, above 55 yr people have the skin diseases, respiratory problem and change in taste (17%) were commonly reported.

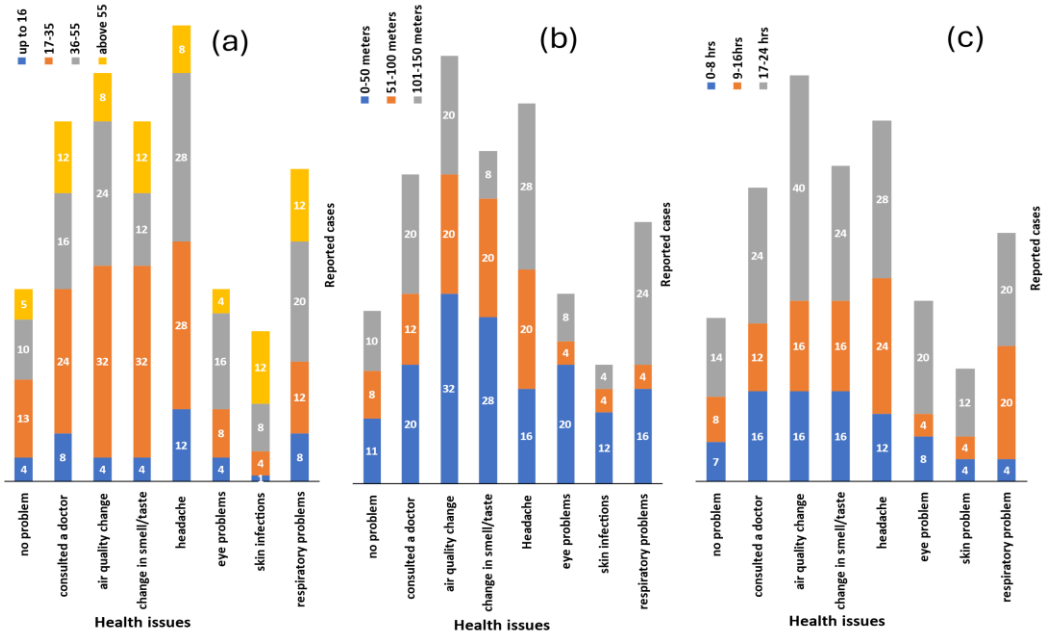


Figure 5.5 Health issues reported in (a) the different age group of the people (b) the different distance from the source (c) the population with respect to the time of the exposure near WWTPs

5.3.5.2 Distance from the source : On the basis of the distance the health effects were summarise, where the gradient of the distances was divided into the three section 0 to 50 m, 51 to 100 m and 101 to 150 m (Figure 5.5 (b)).

With a distance of up to 50 meters from the source, 46% of total population living within this range had health impacts such as problem in eyes, headache and had to visit a doctor, whereas pulmonary issue, skin issues and change in smell were detected by 30%, 23% and 53% respectively. About 69% of people feel a change in the air quality.

For population living in the range of 51 to 100 m, headache was the most common health issue faced by almost 70% of the residents, with 30%, people had eye issues, 10% and 20% of them had skin infections and respiratory problems respectively. About 60% felt a change in their smell and 50% observed a change in the air quality and in all of them 40% saw a doctor.

People with a distance of 100 and 150 m headache and respiratory issues are the most frequent with 70% and 60% of the residents experiencing them respectively. 20% of the people report eye problems as well as smell changes while 10% of them had skin issues. 50% of them felt a change in air quality along with the same percentage of people visiting a doctor.

5.3.5.3 Duration of exposure

People getting exposure up to 8 hrs per day 14% of people complaint to have respiratory and skin issues, while 42% of them had headache sometimes with 28% of people feeling irritation in their eyes. About 57% of people have experienced changes in their smell, changes in air quality and the same percentage of people had visited doctors in recent times.

People getting exposure for around 9-16 hrs per day- A staggering 72% of these people feel headache at intervals followed by 45% feeling change in their smell and the air quality with some respiratory issues. With 9% and 18 % of the population observing skin and eye irritations, 36% have sought medical help.

People with 24 hrs of exposure- 57% of them complaints of headache and 42% of respiratory issues, eye issues and change in their smell. Along with that, 21% have had some sort of skin-related problem for some time and 50% of all the people have visited the doctor. 64% of them had noticed a change in the air quality.

5.4 Conclusions

This study investigated the treatment process of the wastewater focusing on the concentration and nature of the bioaerosols produced while the lower and higher water intake during the summer and winter time. The finding of the studies shows that aerosolization ratio of the bioaerosols in wastewater is higher during the summer as compared to the winter. And it also

depends on the flowrate intake of the wastewater treatment plant. During the lower flowrate the concentration of the bioaerosols were observed more in comparison to the higher flow.

In the exposure assessment of the bioaerosols, there is the possibility of the different health diseases on the human body based on the exposure time (daily dose), age of the population residing there and the distance from the sources. In our study, we found that the headache was very prominent in each age group of the population, and they feel the change in the taste and smell. In the old population more risk of the skin diseases, respiratory problem was observed. By moving to the farther distance from the source the decreasing trend was seen decrease in the diseases like problem in eyes, headache and had awareness to visit a doctor, whereas pulmonary issue, skin issues and change in smell were detected. Exposure time of the bioaerosols is very important as the exposure increases the possibility of the diseases are also increases. In order to reduce the exposure of bioaerosols from WWTPs on the workers and nearby populations, some protective measure should be taken, and routine health checkup and vaccination may useful.

In order to control the bioaerosols reaching to the atmosphere and exposed to the humans is a big challenge. There is the need of some infrastructural changes in the WWTPs setup like installation of UV light, HEPA filter and control closed chamber setup can be effective while controlling the bioaerosols reaching to the atmosphere and humans from WWTPs. In spite of all, our study extensively covered the issue of three major Sustainable Development Goals (SDGs) Good health and well-being (3^{rd}), Clean water and Sanitation (6^{th}) and Sustainable cities and Communities (11^{th}) which will help while making the policies regarding achieving the sustainable development goals local and global level.

Bioaerosols emission from solid waste processing site along with its exposure assessment and mitigation measures

5.5 Preamble

In recent decades, urbanization and the population in urban areas have become the major sources of solid waste generation. The rate of urbanization, economic expansion and high living standards are increasing in developing nations, so waste generation is also increasing (Chioatto et. al., 2023). During 2016, the average generation of solid waste from cities is 2 billion tons with a footprint of 0.74 kg/capita/day (Silpa et. al., 2018). Due to inefficient infrastructure and technologies, there is a problem with the management of solid waste in developing nations (Voukkali et. al., 2024). As a result of that, developing countries find that open dumping is the cheapest option for solid waste management (Srivastava et. al., 2015). For example, according to UNEP, 17 out of 50 world's biggest dumpsites are located in the Africa continent and 6 in Nigeria (UNEP, 2015). According to studies, by 2050, the total annual solid waste production is expected to rise by 70% as compared to 2016 levels and can reach up to 3.40 billion tonnes. Among these, 31% are recycled, 22% are incinerated, and the rest, 47%, will be disposed to landfill (Liu et. al., 2019; Tisserant et. al., 2017; Zeller et. al., 2018).

In India, more than 1.27×10^3 tonnes of municipal solid waste (MSW) are produced daily in urban areas, however, only 12.5% of these tonnes are processed and treated on a daily basis (CPHEEO, 2014). Although composting is seen as a sustainable waste management solution, the potential release of infectious bioaerosols during the composting process could be hazardous to the health of plant employees and nearby residents. Composting is widely practised in India, with more than 70 cities using either windrow composting or vermicomposting (Pahari et. al., 2016). The majority of these composting plants were built to process 100 to 1000 tonnes of MSW each day.

Composting solid waste's biodegradable component is a possible source of airborne pathogenic and non-pathogenic microorganisms, such as thermophilic and thermotolerant microorganisms (Goff et. al., 2012, Grisoli et. al., 2009, Pankhurst et. al., 2012). These types

of waste disposal sometimes make large waste hills and create different types of environmental problems. In the disposal activities of the MSW, such as tipping, spreading and waste shorting, aerosolization takes place, and these contain microbes (Stagg et. al., 2010). The hot and humid weather condition in the region favours the growth of the microorganisms, and they get aerosolized during agitation activities; these also contain pathogens (Epstein, 2015; Odewabi et. al., 2013). Eventually, the workers at the dumpsite and nearby residences get exposed to the pathogenic microbes mainly through inhalation if not using personal protective equipment (Odewabi et. al., 2013). During the agitation activities in composting and landfilled facilities, workers are exposed to high-emission organic dust containing bioaerosols at the open dumpsite facilities (Ray et. al., 2005; Schlosser et. al., 2012). Along with the inhalation, infection reaches through the skin contact. In some of the sites, the workers are reported to have gastrointestinal diseases, severe blood infections and hepatitis, and physical symptoms of impetigo and musculoskeletal illness (Odewabi et. al., 2013; Poole & Basu, 2017; Thirarattanasunthon et. al., 2012).

Limited research has been done on the bioaerosols in the dumpsite in comparison to the landfill and composting processes (Douwes et. al., 2003; Heldal et. al., 2015; Van Kampen et. al., 2016). Although many activities like tipping and spreading of waste are common at the dumpsite and they have greater health effects and there is no significant control over the safety during the handling, treatment and exposure limit to particulates associated with bioaerosols (Hoornweg & Bhada-Tata, 2012; UNEP, 2005). Only a few research are available in India on the emission and exposure of bioaerosols on the dumpsite workers and residing people.

This study aims to investigate the bacterial and fungal bioaerosols concentration and their size distribution at different points of the waste processing site. For biological characterization, metagenomic analysis was conducted to identify the bioaerosols specification and their nature to determine the possible health impacts. To compare the health effects of the detected microbes in the air, ground-based surveys were conducted on the nearby population based on exposure time, distance from the source, and age. The major microbes were undergone for the antimicrobial resistance test to investigate the effects of

antibiotics on the microbes found in the air. Along with the concentration, size distribution, and biological characterization this study will provide the evolving nature of microbes towards the antibiotics.

5.6 Materials and methods

5.6.1 Site description

Air samples were collected at the Karsara waste-to-energy processing plant inside various points. The waste treatment facility is located (Figure 5.6) at Karsara (25° 12′ 50.88″ N latitude and 82° 55′ 6.75″ E longitude) in Varanasi City, Uttar Pradesh, India. It covers an area of 6 km^2 in Varanasi City and is ~80.7 m above mean sea level. The waste treatment facility is located approximately 2 km away from river Ganga and is mostly surrounded by agricultural fields and settlements. The climate of the study area varies from high temperatures (38.5 to 41.2 °C) during summer, intense rainfall (1100 mm) during monsoon, and severe cold (8.4 to 15.0 °C) during the winter seasons (https://mausam.imd.gov.in).

There are approx. 42.65 Lakhs population living in this city with decadal growth rate 17.32% (Population census, 2011). The households waste, bulk waste generators such as hotels, institutions and community halls produce up to 100 kg per day of waste. All the mixed waste produced is primarily dumped in small collection units (*"kuda ghars")* located near the sources of generation of these wastes. Subsequently, waste is transferred to the transfer station (Shivala transfer station), where waste is segregated into dry and wet components. Approx 15 tonnes per day (TPD) of wet waste is taken to the existing 3 decentralized bio-methanation plants and the remaining wet waste is transferred to the Karsara plant for composting. Dry waste is also transferred to the Karsara plant which is further processed into refused derived fuel (RDF) installing adequate pre-sorting lines at the site (Source: data provided by *Nagar Nigam*, Varanasi).

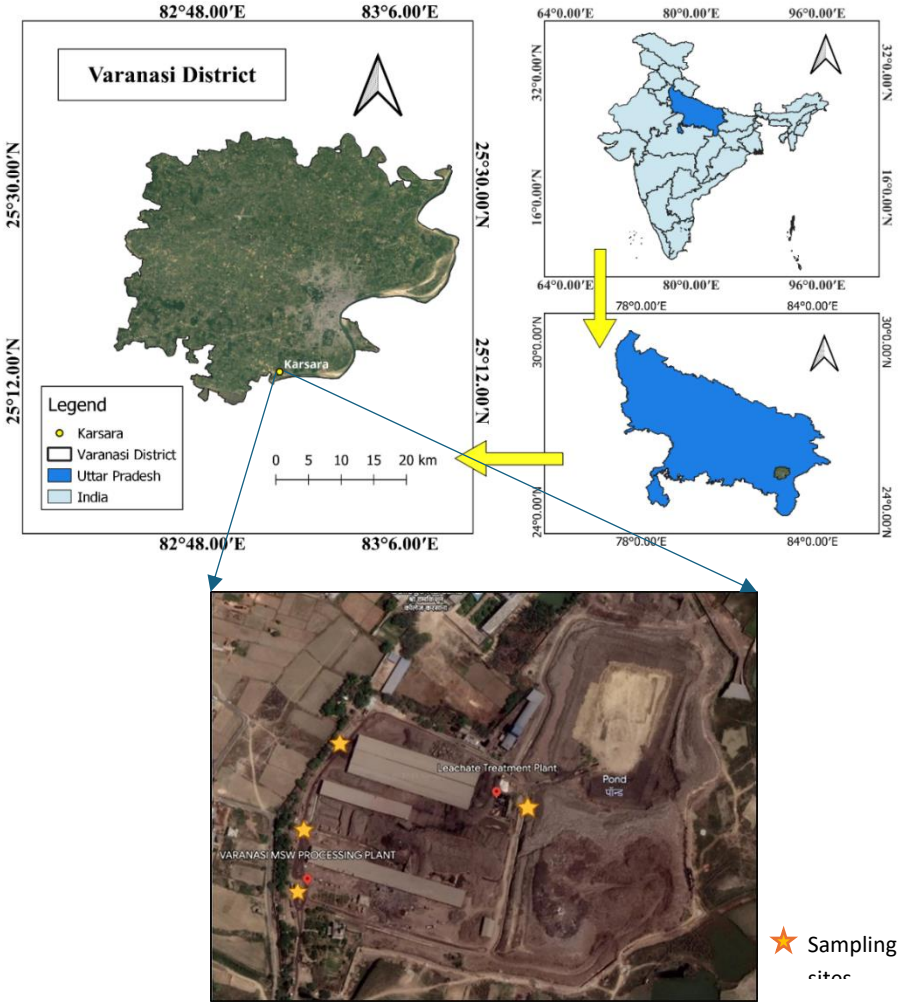


Figure 5.6 Karsara waste treatment facility (25° 12′ 50.88″ N latitude, 82° 55′ 6.75″ E longitude)

The plant were divided into four sections, entry point (EP), loading and unloading point (L & UL),: Leachate treatment point (LT) and waste segregation point (SEG). Air samples were collected at each section within the premises of the plant using Anderson's six-stage viable cascade impactor sampler (Tisch Environmental, South Miami, OH, USA). This sampler collects bioaerosols in six different size ranges (cut-off diameter).

5.6.2 Sampling Campaign

An Andersen six-stage viable cascade impactor (TISCH, USA) was used to collect culturable bioaerosol emissions from the processing site (during winter and summer). The cut-off

diameters of the six stages in the sampler are >7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 µm. The impactor was loaded with 6 Petri dishes containing nutrient agar medium for bacteria, and potato dextrose agar medium for fungi growth. The sampling was carried out around the waste processing plant at the four points (entry point, loading and unloading section, segregation sections, leachate treatment section). The sampler was installed at the height of 1.5 meters above the ground, and the flow rate for the inlet in the cascade impactor was fixed at 28.3 L/min. Sampling was done for 20 min according to the standard procedure (Kowalski et. al., 2017). Air sampling was carried out between 9 am to 10 am at each point. The impactor was ultra-sonicated, autoclaved and disinfected with 75% ethanol before and after sampling each sampling. All the pre-procedures, including installation, analysis and cleaning, were performed in the biosafety chamber. Personal protective equipment was used while doing the air sampling. The temperature, relative humidity and wind speed were recorded with the help of temperature & humidity meter and anemometer respectively.

5.6.3 Bioaerosols concentration measurement

Air samples were collected on the petri plates, filled with the different media. After sampling, the collected samples on the petri plate were sealed in biosafety chamber with parafilm and put in the incubator for the optimum condition of growth for bacteria and fungi (for bacteria 25°C for 72hr and for fungi 35 °C for 48 hr). After this time duration, the microbial colony appeared on the petri plates and was counted with the help of the colony counter. The total bioaerosols concentration was calculated using the following formula:

$$\text{Bioaerosol concentration } (\text{CFU}/m^3) = \frac{\text{number of colony } (C')}{\text{flow rate } \times \text{ sampling duration (minute)}} \times 1000$$

$$C' = \sum_{i=1}^{6} C_i$$

Where, C' is the airborne cumulative concentration (total concentration) of bioaerosols (CFU/m^3 air) and C_i is the bioaerosol concentration of i stage of Anderson six-stage impactor (CFU/m^3).

The blank was used to reduce the error during the sampling. For this, the same procedure was used (from media preparation to incubation) without running the sampler. The blank shows

no growth (or negligible) on the media plates and may subtracted to get the real concentration of the bioaerosols.

5.6.4 Identification of the species and metagenomics

Identification of the species was done using the metagenomics analysis, where 16s and ITS metagenomics were performed for bacteria and fungi, respectively. The following procedures were followed for each of the metagenomics analysis as mentioned in Section 5.2.4.

5.6.5 Survey for respiratory health

The health status of the nearby area was investigated by a cross-sectional health survey across the waste processing sites from February to April 2023. For this, sample size and questionnaire were adopted using the matrix recommended by WHO (1991). The sample population was divided into different classes for the health study. The sample population consisted of workers of waste processing sites, small business owners, local vendors, people residing there, and random people passing from there who regularly are the populations exposed to the dumpsite for at least one year.

5.6.6 Antimicrobial test of bioaerosols using Kirby Bauer disc diffusion test

The Kirby Bauer disc diffusion test was used to check the susceptibility and resistivity of bacteria towards the antibiotics (Hudzicki, 2009). Determination of antimicrobial resistance of bacteria is important for the management of harmful diseases caused by bacteria in humans (Costerton et. al., 1999). The bacterial strain to be tested is dissolved in distilled water and the media prepared was inoculated by it with the help of a glass rod then paper disks containing antibiotics were placed on the inoculated petri plates, and antibiotics present in the disks got diffused in the media and a gradient of antibiotic was formed around the disk. After incubating these Petri plates for 48 hrs and the diameter of the zone of inhibition was formed, the growth of the zone of inhibition was inversely proportional to MIC (minimum inhibitory concentration), and the relationship between these values was the basis of the interpretation of the results.

5.7 Results and discussion

5.7.1 Concentration of the bacterial and fungal bioaerosols at the waste processing site

The concentration of the bacterial and fungal bioaerosols were determined at the four point of waste processing site i.e. entry point of the waste processing plant, loading and unloading sector, Leachate treatment point and waste segregation point. There were variation in the concentration at each point for both bacterial and fungal bioaerosols. Figure 5.7 shows the total average bacterial and fungal bioaerosols concentration at each point where, entry point (EP), loading and unloading point (L & UL), Leachate treatment point (LT) and waste segregation point (SEG) were mentioned.

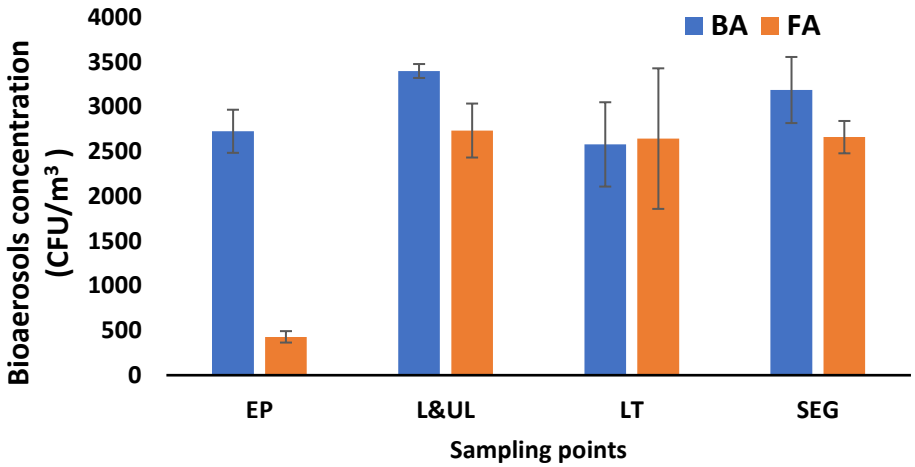


Figure 5.7 Distribution of the concentration of bacterial and fungal bioaerosols at various point of waste processing site. Where, EP: entry point, L & UL: loading and unloading point, LT: Leachate treatment point and SEG: waste segregation point, BA and FA are the bacterial and fungal bioaerosols respectively.

Bacterial bioaerosols concentration were higher in each points: EP (2726±240 CFU/m^3), L&UL (3399±77 CFU/m^3), LT (2579±471 CFU/m^3), SEG (3187±369 CFU/m^3) whereas fungal bioaerosols concentration was much lower at the entry point EP (428±63 CFU/m^3) while significantly higher at other points, L&UL (2734±301 CFU/m^3), LT (2644±785 CFU/m^3), SEG (2660±180 CFU/m^3). Variations in the concentration were much higher at LT for both bacterial and fungal bioaerosols. The average concentration of the bacterial bioaerosols inside the waste processing site was found in between 6.83×10^2 – 1.47×10^5

CFU/m^3, whereas outdoor concentrations were found in the range of 3.97 – 79.67 CFU/m^3 and for fungi, it was $1.01 \times 10^3 – 3.17 \times 10^4$ inside the plant and $1.59\text{-}7.22 \times 10^2$ (Rasmussen et. al., 2021). Moreover, the study by Liu et al. (2022), reported range as 63,617 ± 15,007 CFU/m^3 for bacteria and 8044 ± 893 CFU/m^3 for fungi, respectively (Liu et. al., 2022). In our study, the variation in the concentrations of bioaerosols was less than the reported study for both bacterial and fungal bioaerosols. In the case of outdoor bioaerosols, the concentration was higher. This may be because of limited control measures for the emission of bioaerosols from the waste processing site. However, in the study reported by Ghanbarian et al. (2020), the highest concentration of bacterial and fungal bioaerosols at waste processing sites was 1687.6 CFU/m^3 and 4958.8 CFU/m^3, respectively (Ghanbarian et. al., 2020), which is more comparable to our results.

5.7.2 Distribution of the bacterial and fungal bioaerosols in different size ranges

Size-segregated bioaerosols were investigated at the various points of the waste processing sites using six-stage viable Anderson cascade impactors of different size ranges (cut-off diameter). In Figure 5.8, the concentration of the bacterial and fungal bioaerosols of the different size ranges were shown where the peak concentration of bacterial bioaerosols was found in the finer range (0.65-1.1 μm) while the fungal concentrations were found highest in the coarse size range (between 3.3-7.0 μm). The bioaerosols of coarse size range were found to be very low for both bacteria and fungi.

Loading and unloading and waste segregation section showed the higher bacterial bioaerosols concentration at fine particle size range (3.3-0.65 μm). For fungal bioaerosols, higher concentrations were observed at the L & UL section in the coarse particle size range (between 3.3-7.0μm). The particle size (aerodynamic diameter) of bioaerosol plays an important role in their deposition in the different parts of the human respiratory system i.e. coarse particle size of 7 μm and above will get deposited in the pre-separator of the respiratory system, the particle size of the range 4.7-7 μm will get deposited in the pharynx, particles with aerodynamic diameter 3.3-4.7 μm will get deposited in the trachea and primary bronchi. So the workers at the L & UL section had the greater risk of pathogenic fungal infection. The fine particles with aerodynamic diameters 2.1-3.3 μm will get deposited in the

secondary bronchi, particles with aerodynamic diameters 1.1-2.1 µm will get deposited in the terminal bronchi, and particles with aerodynamic diameters 0.65-1.1 µm will get deposited in the alveoli of the human respiratory system (Nair, 2021). Therefore, the workers working at the loading and unloading and waste segregation section inside were at the greater potential health risk associated with the pathogenic bacterial bioaerosols.

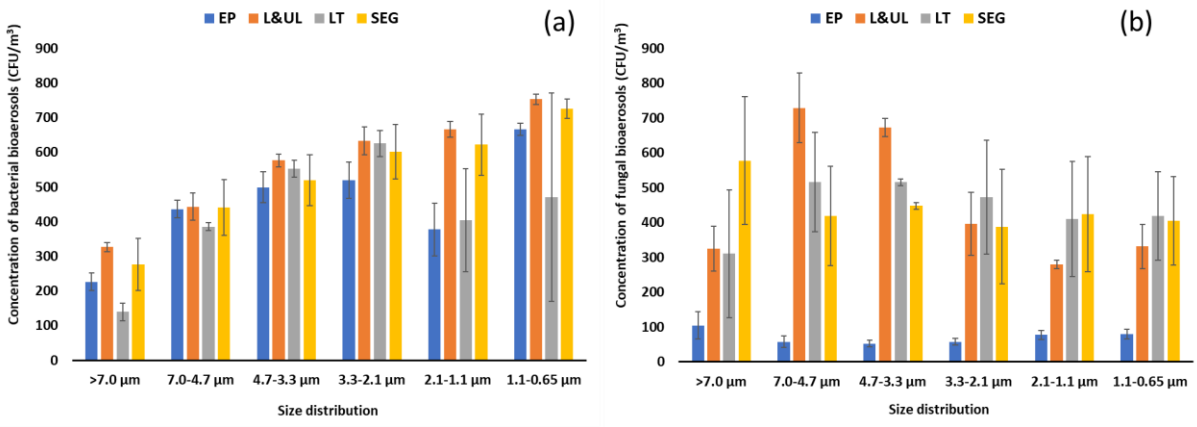


Figure 5.8 Size distribution of the concentration of (a) bacterial and (b) fungal bioaerosols at various point of waste processing site. Where, EP: entry point, L & UL: loading and unloading point, LT: Leachate treatment point and SEG: waste segregation point.

5.7.3 Taxonomic composition of total bioaerosols concentration at WWTPs

Sequencing was performed using Illumina Miseq with 2x300PE v3-v4 sequencing of bacteria and 2x300PE ITS sequencing kit for fungal analysis. Top 10 enrichment of genus in 16s metagenomic analysis of the bioaerosols at waste processing sites shown in Figure 5.9. The details of metagenomics has shown in Appendix C (Figure C1-C10).

The metagenomic analysis of the bacterial bioaerosols samples were performed where the top 10 genus were *Alcaligenaceae* (22%), *Stenotrophomonas* (19%), *Bacillus* (14%), Unclassified *Bacillaceae* (13%), *Brevibacillus* (8%), Unclassified *Rhizobiales* (7%), *Sphingobacterium* (6%), Unclassified *Sphingobacteriacea*e (4%), *Serratia* (4%) and unclassified *Bacillales* were the dominant over the waste processing sites.

In the collected sample of the bioaerosols, *Stenotrophomona* are generally non-pathogenic but some of strains like *S. maltophilia* can develop multidrug resistance in humans and cause

poly microbial infections specially in children (Aykac et. al., 2016; Ryan et.al., 2009). Bacteria like *Bacillus* are responsible for respiratory problems, headache, sore throat, mild fever, fatigue and muscles aches. They are resistant to drugs like antibiotics in addition to heat, radiation, disinfectants and other environmental factors. Therefore, it is difficult to control from the contaminations (Christie & Setlow, 2020). The fungal bioaerosols dominance were also analysed with the help of the metagenomic study and it were found that the *Fusarium* (40%), *Epicoccum* (23%), *Hypocreales* (15%), *Coprinopsis* (8%), *Cladosporium* (4%), *Sarocladium* (4%), *Tourlaspora* (2%), *Alternaria* (2%) and *Cryptococcus* (2%) are the dominant fungal species over the waste processing site. Top 10 enrichment of genus in ITS metagenomic analysis of the bioaerosols at waste processing sites shown in Figure 5.10.

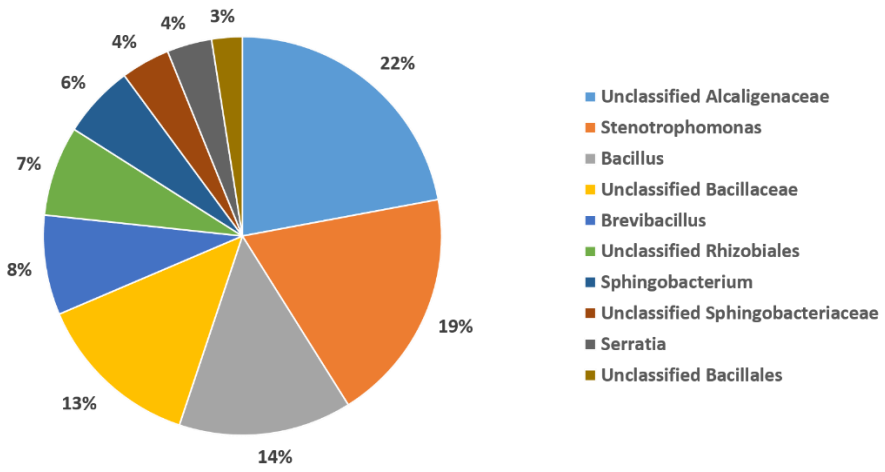


Figure 5.9 Top 10 enrichment of genus in 16s metagenomic analysis of the bioaerosols at waste processing sites

In the observed fungal bioaerosols, *Fusarium* found in the large amount in the samples from the waste processing site, and generally it causes the eye infection in the humans (Cheng et. al., 2024a) Other fungi like, *Hypocreales* are known for the plant disease (Chen et. al., 2021a) whereas, *Epicoccum* associated with respiratory issues (Kurup et. al., 2000), *Coprinopsis* are able to decompose the dead organic matters and it is not typically known for plant and animal disease. Cladosporium are responsible for eye and respiratory issues (Kurup et. al., 2000).

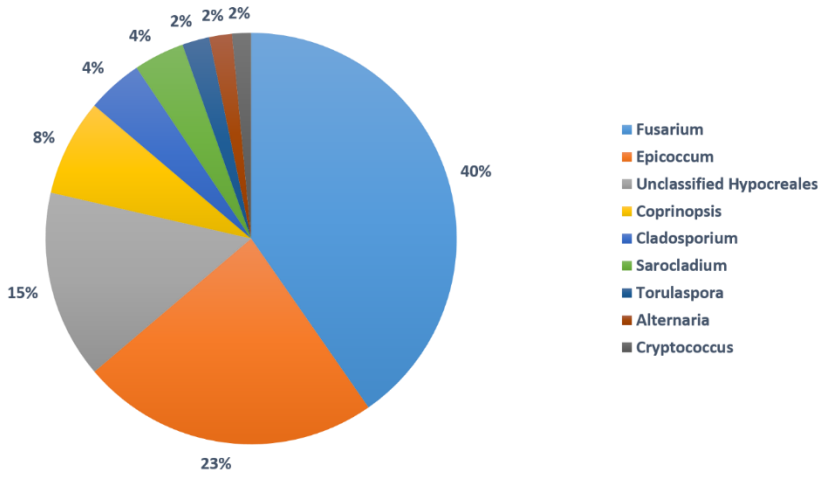


Figure 5.10 Top 10 enrichment of genus in ITS metagenomic analysis of the bioaerosols at waste processing sites.

Alternaria can cause respiratory and skin problem in humans (Cheng et. al., 2024b). So, depending upon the nature of the bioaerosols these particles can affect the human body and most vulnerable to the immune-compromised body.

5.7.4 Correlation between bioaerosols and environmental factors

The correlation analysis of bioaerosols and environamental variables have shown in the Figure 5.11 in the form of matrix. In Figure 5.11, it is visible that there is no significant or less significant correlation between the bioaerosols and environmental variables.

	BA (CFU/m^3)	FA (CFU/m^3)	WS (m/s)	RH (%)	Temp (°C)
BA (CFU/m^3)	1.000				
FA (CFU/m^3)	0.510*	1.000			
WS (m/s)	-0.540**	-0.872	1.000		
RH (%)	-0.602**	-0.396*	0.515*	1.000	
Temp (°C)	0.599*	0.588*	-0.673	-0.894	1.000

*p<0.05, **p<0.01

Figure 5.11 Correlation matrix for bioaerosols and environmental variables at waste processing site

However, during the sampling, the samples were taken from different places and they were not at the one place at a time. So, it cannot provide assurance of any association of environmental variable to the bioaerosols concentration.

5.7.5 Toxicity assessment using antibiotic test

Two commonly used antibiotics were selected (azithromycin and cefixime) and their antimicrobial discs of different concentrations of antibiotics was prepared. After 48 hr of incubation formation of inhibition zones took place as mentioned in the Table 5.2 and 5.3. Table 5.2 shows the diameter of zone of inhibition for azithromycin whereas Table 5.3 shows the zone of inhibition for cefixime and their standards are mentioned in the Table 5.4.

Table 5.2 Measurement of the diameter of inhibition zones for 1st and 2nd bacterial strain against antibiotic azithromycin

1st bacterial strain	**Diameter obtained (mm)**	**Sensitive**	**Moderately sensitive**	**Resistance**
1. 0 dil.	22	✓	-	-
2. 1/10 dil.	15	-	✓	-
3. 1/100 dil.	13	-	-	✓
4. 1/1000 dil.	06	-	-	✓
2nd bacterial strain				
1. 0 dil.	21	✓	-	-
2. 1/10 dil.	17	-	✓	-
3. 1/100 dil.	05	-	-	✓
4. 1/1000 dil.	0	-	-	✓

Table 5.3 Measurement of the diameter of inhibition zones for 1st and 2nd bacterial strain against antibiotic cefixime

1st bacterial strain	**Diameter obtained (mm)**	**Sensitive**	**Moderately sensitive**	**Resistance**
1. 0 dil.	4	-	-	✓
2. 1/10 dil.	0	-	-	✓
3. 1/100 dil.	0	-	-	✓
4. 1/1000 dil.	0	-	-	✓
2nd bacterial strain				
1. 0 dil.	5	-	-	✓
2. 1/10 dil.	0	-	-	✓
3. 1/100 dil.	3.5	-	-	✓

4. 1/1000 dil.	0	-	-	✓

Table 5.4 The size of the diameter is compared for the standard value

Antibiotics	Sensitive	Moderately sensitive	Resistant
Azithromycin	≥18	14-17	≤13
Cefixime	≥19	16-18	≤15

On comparing values of diameters of inhibition zones with the standard values it is inferred that both the bacterial strains were sensitive to higher concentrations of azithromycin (500 mg) antibiotic as they show the formation of large inhibition zones with diameters 2.2 mm and 2.1 mm, respectively, but with the decrease in the concentration of azithromycin, small inhibitory zone formation takes places which signifies that both the strains are resistant to this particular drug at a lower concentration. In the case of cefixime, both the bacterial strains show resistance at all concentrations as very small inhibition zone formation takes place at higher concentrations (200 mg) and no inhibitory zone formation takes place at lower concentrations.

5.7.6 Health risk assessment of bioaerosols

The cross-section survey was done using the questionnaire methodology with interaction with the people working in the vicinity of the waste treatment plant as well as the people residing near the waste processing plant. The questionnaire is based on the WHO health risk assessment methodology including the age, sex, distance from the source, exposure hour and the different types of infection the population has. The details of the questionnaire were mentioned in the Appendix -C. The results are categorised in the different age groups, distance from the source, exposure, and the type of infection in the population separately.

The health risk assessment was done using the cross-section survey in the range of 0 to 3 km of the area near by the waste treatment facility. Total 61 people were taken part in the survey and on the basis of no. of responses in each cases the health risk assessment has been categorised using the following criterion (Figure 5.12).

5.7.6.1 Age of the population

The health survey was conducted in 0 to 3km periphery of the waste treatment facility, where the total population was divided in the four category such as 0 to 16 yr, 17 to 35 yr, 36 to 55 yr and above 55 yr.

It has been shown in the Figure 5(a) that the change in smell (~95%) was very prominent in the 0 to 16 yr category.

In the category of 17 to 35 yr, they mostly have the problem of respiratory, eye problems (~43%) and quality of air (~43%).

The age group of 36 to 55 yr old, the headache was common (~42%), and they feel that the air is contaminated (~42%).

In the last category, above 55 yr people have the headache, change in smell and feeling of contaminated air were commonly reported.

5.7.6.2 Distance from the source

On the basis of the distance, the health effects were summarised, where the gradient of the distances was divided into three sections: 0 to 500 m, 500 m to 1 km and 1 to 3 km (Figure 5.12 (b)).

With a distance of up to 500 meters from the source, 90% of total population living within this range had health impacts such as problem in eyes, change in smell and had to visit a doctor, whereas pulmonary issue, skin issues, headache and were detected by 80%, 80% and 83% respectively.

For people living in the range of 500 m to 1 km, change in smell was the most common health issue faced by almost 58% of the residents, with 8% people having eye issues, 8% and 16% of them had skin infections and respiratory problems respectively. 25% felt a change in their smell and 25% observed a change in the air quality and in all of them 25% saw consulted the doctor.

People with a distance of 1 to 3 km change in the smell were the most frequent with 76% of the residents experiencing. 30% of the people report eye problems as well as smell changes while 53% of them had skin issues. 53% of them felt a change in air quality.

5.7.6.3 Duration of exposure

People getting exposure up to 8 hrs per day - 53% of people complaint to have respiratory, skin issues, headache sometimes feeling irritation in their eyes. Almost all people have experienced changes in their smell; changes in air quality (53%) and only 6% of people have visited doctors in recent times.

People getting exposure for around 9-16 hrs per day - 18% of these people felt headache at intervals followed by 37% feeling change in their smell and the air quality with some respiratory issues. With 18 % of the population observing skin and eye irritations and nearly same percentage of the population have sought medical help.

People with 24 hrs of exposure - 93% of them had complaints of headache and 90% of respiratory issues, 96% have eye issues and all of them noticed change in their smell. Along with that 83% have had some sort of skin-related problem for some time and 87% of all the people have visited the doctor.

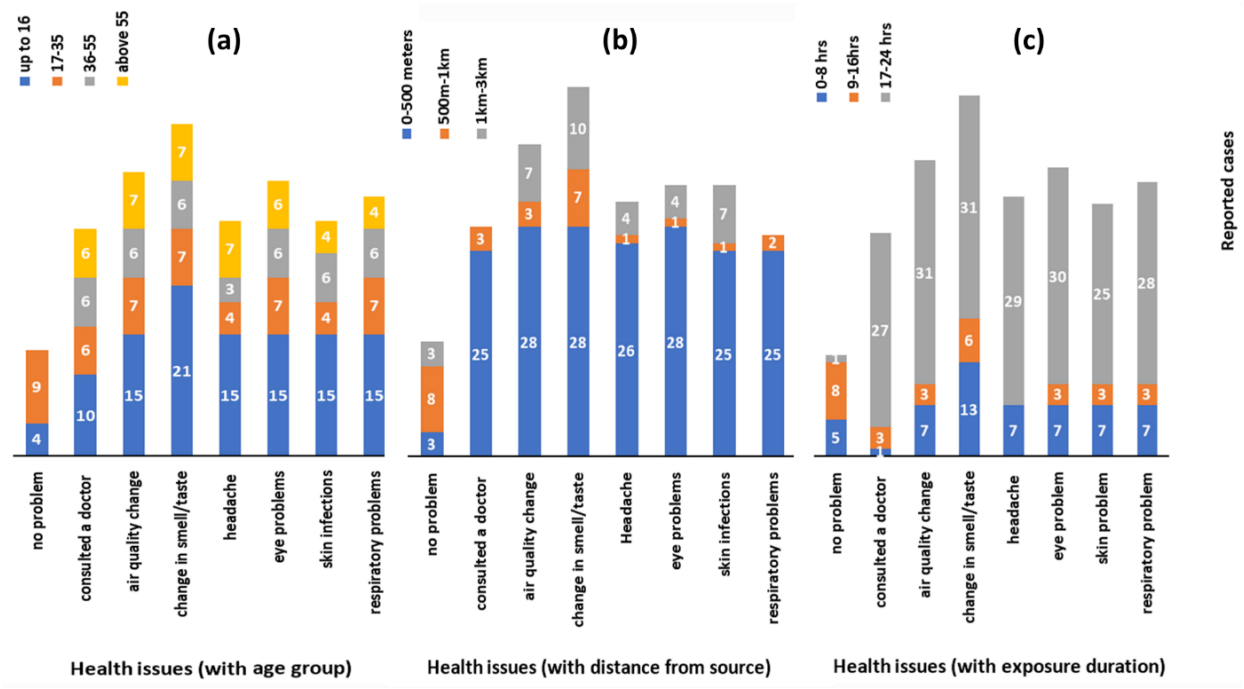


Figure 5.12 Health issues reported in (a) the different age group of the people (b) the different distance from the source (c) the population with respect to the time of the exposure near waste processing site.

Since, bacteria and fungi produce toxic metabolites which is responsible for serious health concern. Bacteria produce endotoxins that can lead to chills, fever, fatigue, headache, joint pain, fluctuations in the number of leukocytes in the blood, dry cough, upper airway inflammation, bronchial asthma, ODTS and the acute form of byssinosis, systemic

inflammatory response and death while fungi produce mycotoxins, Mycotoxin ingestion can lead to immunosuppressive effect and cancer. The most common health issues due to bioaerosols are respiratory disorders, eye irritation, skin issues (rashes, itching etc.), cough, sneeze, diarrhoea, runny nose, throat irritation, skin ulceration and asthma exacerbation in people living nearby landfill sites (Nair, 2021).

The workers in waste treatment facility are exposed to complex mixture of pathogenic bacteria and fungi posing serious threats to their health. Bioaerosols responsible for serious health hazards have particular biological characteristics, inhalation dose requirements, particle size distribution and chemical composition (Nair, 2021). The workers of the treatment facility and the person living near the site have more duration of exposure than those who are residing away from the suspected site hence were more susceptible to diseases due to harmful bacteria and fungi.

5.8 Conclusions

The present study was conducted to estimate the total bacterial and fungal bioaerosols concentration in the waste treatment facility. Total bacterial concentration varied from 3399 CFU/m^3 to 2579 CFU/m^3 while total fungal concentration from 2734 CFU/m^3 to 428 CFU/m^3. The waste treatment facility was divided into 4 sectors, namely, entry point, loading and unloading point, Leachate treatment point and waste segregation point. Out of these sectors bacterial and fungal concentration was highest in loading and unloading section (3399 CFU/m^3 and 2734 CFU/m^3 for bacterial and fungal bioaerosols respectively). The highest bacterial concentration was observed at stage 5 and 6 (0.65-2.1 µm) whereas in stage 2 and 3 (3.3-7 µm) of the impactor and fungi concentration was higher. The high abundances of bacteria and fungi in the fine to coarse particle size range suggested that there is a higher chance of exposure in respiratory system of workers of treatment facility and people residing nearby the plant. In the metagenomic analysis several pathogenic bioaerosols were observed which can cause various types of health issues like, skin and eye infection, respiratory issues etc. It was found that some dominant strains of isolated bacteria were resistant towards higher concentration of a particular antibiotic (cefixime) while some strains were sensitive towards lower concentration of specific antibiotic (azithromycin). The preliminary result of the

survey reveals higher cases of skin and respiratory diseases in respondents residing in close proximity and longer duration of exposure to bioaerosols. However, a detailed medical investigation to be done for analysing the respiratory health of workers and people residing near the Karsara waste treatment facility. And this results can be used to improve the waste processing plant infrastructure and safety protocols.