## 4.1 Preamble

Bioaerosols are a subcategory of airborne particles coming from a biological origin that may be natural or anthropogenic. The bioaerosols coming from the biological origin have a size (aerodynamic diameter) ranging from 0.001 to 100 µm, which can lead them to remain suspended for a longer duration (Robertson et. al., 2019). These bioaerosols include living microorganisms such as bacteria, fungi, pollen, viruses, protozoans and some non-living microbes excreta such as endotoxins, β-glucan and others (Kim et. al., 2018). Basically, bioaerosols are found everywhere in the environment, in the form of living or non-living biological entities.

There are a number of sources for the generation of bioaerosol, which may be natural or man-made. Natural sources like trees, plants, animals, water bodies, and soil are dominant, whereas man-made sources are construction activities, dumping, waste treatment plants, composting, slaughterhouses and other indoor and outdoor activities etc. (Li et. al., 2017). The survival of bioaerosol depends upon environmental conditions like temperature, relative humidity, wind speed, solar radiation, and other atmospheric phenomenon like lightning and haze/fog. Physical factors, including gravitational force and Brownian motion, also affect the behavior of bioaerosols after release (Vestlund et. al., 2014).

Different outdoor sources like agriculture fields, dumping sites, traffic sites, sewage treatment plants, waste processing sites, etc., emit bioaerosols into the surroundings, which depend upon the nature of the site and environmental conditions (Maharia & Srivastava et. al., 2015). The size and nature of the bioaerosols can predict their toxicity, including how the population is exposed with them (through either inhalation or skin). Due to the small size range, bioaerosol can easily reach our respiratory system and deposit there. This deposit may cause countless health problems and affect a single organ to the entire organ system of the human body (Georgakopouls et. al., 2009). Bioaerosol exposure can lead to negative health effects, including infectious diseases, acute toxic effects, skin problems, eye infections, allergies and even cancer (Li et. al., 2017). Therefore, these are troubling explanations for the study of bioaerosols, its sources, concentration, size distribution, type of bioaerosols and their effect on health. Additionally, it has been reported that bioaerosols contribute up to 34% of indoor air

contamination and may contribute a significant impact on human health (Humbal et. al., 2018; Mandal & Brandl, 2011).

The aim of the study is to determine the concentration level of bioaerosols and their distribution for the various size ranges at different sites in Varanasi. Here, the representative outdoor sites, agriculture fields, traffic, and dumpsites were selected for the study purpose. The bioaerosols were collected in all the seasons throughout the year (from 2019 to 2023) and show the variation of bioaerosols concentration in each season. The association of bioaerosols and environmental variables were also determined at each site individually. Along with the concentration and size distribution of bioaerosols, biological characterization was also done in order to know the types of microbes that were present at the particular sites. The basis of size and type of the bioaerosols presence can predict the risk of health issues to the exposed population were also discussed at the end of the study. This way, the expected results may provide useful guidelines for public health policies.

## 4.2 Materials and methods

### 4.2.1 Sampling sites

Air sampling was done at the periphery of the Banaras Hindu University (BHU) campus, Varanasi, which is situated in the North Indian region of Uttar Pradesh (Latitude: 25˚19' and longitude: 82˚59'). The main campus of the University is spread across 1,300 acres (5.5 $km^2$). The hot, humid summer and cold to mild winter are the main climate characteristics of Varanasi. In summer, the temperature ranges from 32 to 46 °C or more than this sometimes and in winter temperature drops up to 5°C. In monsoon, the amount of rainfall averaged nearly 1100 mm due to heavy rain and humid conditions. Air sampling were carried out at three different outdoor sites in Varanasi, i.e., dumping site, agriculture site and traffic site, as shown in Figure 4.1.

#### 4.2.1.1 Agriculture field

The agriculture field is situated near the Institute of Agricultural Sciences at BHU, which is located on the BHU campus on the southern edge of Varanasi. This is also an experimental site of BHU agriculture sciences activities where farmers and a few students used to visit and perform their experiments and other activities in this field. Different

vegetative patterns and residues of crops were seen at this site, and they vary from season to season as well. Wheat, rice, mustard, chekpea and the major crop shown in this area. The temperature and relative humidity vary from 5°C to 40°C and RH from 36% to 91% throughout the year during the sampling. Wind patterns like, wind speed and wind directions, and rainfall were also kept in the record while doing the sampling.

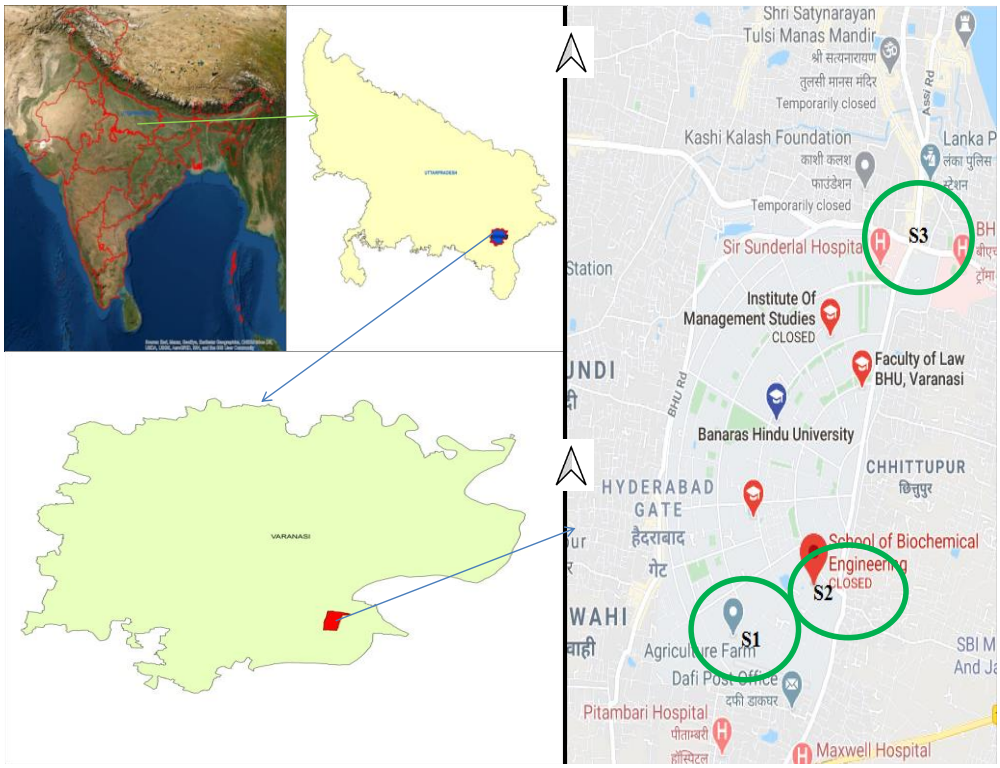


Figure 4.1 Sampling locations in Varanasi. S1: agriculture site, S2: dumping site, S3: traffic site

#### 4.2.1.2 Dumping Site

The dumping site is located near the IIT (BHU) campus, where the solid waste from the university is dumped. It has spread in the area of approximately10000m$^2$ and about 4000kg average waste dumped per day. People were crossing the road near the sampling location of dump sites. A few ruminants and cattle were also seen on the site, which were feeding themselves with the segregated waste. This place was not hygienic at all; bad smells can be felt at these sites. Many precautions were taken, such as wearing an N95 mask, gloves, eye protection equipment, shoes and others. Different wastes like wet and dry, toxic leachate, emission of different gases, invasive plant residue, etc., were found at this site. The temperature of this site is between 6°C to 39°C, and RH for this site varies

in the range of 36% to 91% throughout the year at different seasons (monsoon, post-monsoon, summar and winter).

#### 4.2.1.3 Traffic site

Varanasi is one of the very old and populated cities, and traffic is one of the major issues in this city. For traffic site selection we choose Lanka (outside the BHU main gate) as a representative site. Generally, higher pollution, crowds and noise were experienced during the sampling hour due to vehicles crossing at Lanka. This may be because hospital, university, trauma centres, and business activities are prominent in nearby areas. Health protection, like the use of gloves, facemasks, and sanitisers, was taken while doing the sampling at this site. The temperature range and relative humidity vary for monsoon and post-monsoon season from 10°C to 39°C and RH (from 37% to 91%) during the time of sampling throughout the year.

### 4.2.2 Air Sampling

Sampling of three distinct fractions of bioaerosol: fungi, Gram-positive bacteria (GPB) and Gram-negative bacteria (GNB) was conducted during August 2019 to January 2023. The metrological seasons over India are winter (Dec-Jan-Feb), monsoon (July-Sept), post-monsoon (October-November) and summer (April-May-June), as per India Metrological Department (IMD).

Air sampling was done by six-stage viable cascade impactor Anderson sampler (Tisch Environmental, South Miami, OH, USA). The fundamental concept behind this impactor was impaction which collects the airborne particles. Impaction sampler sucks the air and induces it to change direction, causing high inertia particles to be impacted onto the agar plate (Ghosh et. al., 2015). This sampler needs a solid or semi-solid culture media for air collection, which provides homogeneity to the surface and makes the sampler more efficient than another sampler. The main advantages of this sampler consist of six stages, which collect aerosol in six different size ranges (cut off diameter). Each stage consists of equally spaced perforation, and the size range of aerosol fractions in this sampler is (> 7, 4.7-7, 3.3-4.7, 2.1-3.3, 1.1-2.1, <1.1µm). The six stages of the sampler are designed to simulate the human respiratory system with relatively smaller particles from stage 1 (top, upper respiratory tract) to stage 6 (bottom, lower respiratory and alveoli) (Tisch Environmental Inc., 2018). This size range of bioaerosol can directly affect our

respiratory system and can accumulate in the pre-separator, pharynx, trachea, primary, secondary, terminal bronchi and alveoli (Lal et. al., 2017).

The ambient air sampling was done at the flow rate of 28.3 L/min for 20 min as prescribed by the user manual for the sampler. The sampler was placed at a height of 1.5 meters, which is the normal stature of the human nose starting from the earliest stage. Samples were taken thrice in the month for all the season thoughout the year between 10am to 11am IST. In order to observe the linear relationship between the various bioaerosols and environmental variables, the simultaneous data of environmental variables were collected with the help of a meteorological data logger system. Where temperature (°C), relative humidity (%RH), wind speed (m/s) wind direction (degree) data were recorded simultaneously.

### 4.2.3 Preparation of culture media

Appropriate culture media were used to detect bacteria and fungi. Potato dextrose agar is used for fungal fraction, blood agar is used to collect Gram-positive bacteria (GPB) and eosin methylene blue is used to collect Gram-negative bacteria (GNB). Potato Dextrose Agar (SRL lab Ltd. India) is microbial growth medium formed by infusion of potatoes and dextrose. Infusion of potatoes and carbohydrate stimulate fungal growth whereas low pH and antibiotic present inhibit bacterial development (Mandal et. al., 2008; Ghosh et. al., 2013).Blood agar (SRL lab Ltd. India) is a medium infused with blood and used to grow certain bacterial strains. It is used as general media without enrichment. This is also used for the isolation of fastidious microorganisms. Eosin Methylene Blue (SRL lab Ltd. India) is a selective medium that is used to isolate enteric *Bacilli*, particularly forms of coli and also used for detection of Gram-negative bacteria (Lal et. al., 2013; ACGIH, 1989). All the above-mentioned media is commercially available in powdered form. The culture media was prepared in 100 mL distilled water in beaker separately, and then sterilized it by autoclave at 15psi, 121°C for 15 to 30 minutes. The prepared sample was poured in sterilized petri disk and allowed it for solidification.

### 4.2.4 Estimation of bioaerosols concentration

Air samples were collected onto the petri plates in different sites. These petri plates were incubated at 25°C for 72 hours to visible growth of both GPB and GNB. For visible fungus growth these plates were incubated at 35°C for 2 days (Lal et. al., 2017). The

developed number of colonies was subsequently measured by colony counter and represented in colony-forming units (CFU/$m^3$). Fungus, isolated bacteria were characterized primarily by direct observation, based on the morphological characteristics of spore and colony.

Bioaerosol concentration was determined by dividing volume of air examined from total number of colonies found on petri plate. Basically a colony on the solid culture medium was a macroscopically observable development of microorganism. CFU is the quantity of microorganism that can reproduce to form colonies, as dictated by the number of growing colonies (Maharia & Srivastava, 2015).

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

where flow rate = 28.3 (L/min)

### 4.2.5 Bioaerosols characterization

Biological characterization was done to identify the microbial diversity in the collected samples. The pure cultures were isolated from the mixed sample plates after the incubation of the samples plates. Purification of the various bacterial and fungal colonies were done by strike plate method after the multiple striking and culture. After getting the pure isolated culture the pure colonies were isolated and DNA sequencing method were performed in order to identifying the microbes and its confirmation. However, the colour, shape, and structure of the bacterial and fungal isolates can be used to identify microbes by comparing the online description. For the further confirmation. DNA sequencing methods are widely used. Here, 16s-rDNA was for bacterial, and the ITS method for fungal identification was performed. These methods were conducted by the following steps: In the first step, Genomic DNA was isolated from the pure culture. In the second step, the fragment of pure DNA was amplified using high-fidelity polymerase chain reaction (PCR) using the forward and reverse primers for bacteria and fungi. In case of bacteria 5'-GGATGAGCCCGCGGCCTA-3'(16s Forward) and 5'-CGGTGTGTACAAGGCCCGG-3'(16s Reverse (Kaur and Kaur, 2022) primar were used where as for fungi 5'-TCCGTAGGTGAACCTGCGG-3' (ITS-1 Forward) and 5'-TCCTCCGCTTATTGATATGC-3' (ITS-4 Reverse) primers (White et. al.,1990) were

used. In the third step, the PCR product was sequenced bi-directionally. In the last and final step, the sequenced data were analysed to identify the sequence of microbes with its closest neighbourhood methods (Wiley et. al., 1991; William et. al., 2000).

## 4.3 Results and Discussion

### 4.3.1 Size-segregated characterstics of bioaerosols over the various season and sites

The size distribution of bioaerosol was analysed to determine the possible extent of exposure to bioaerosols at different sites in Varanasi and it can be correlated to normal tidal volume breathed in by humans. Bioaerosols were collected onto each agar plate placed in the six-stage Anderson sampler during sampling. Particles with a cut-off diameter range > 4.7 µm are collected in stages 1 and 2, representing deposition in the nasal area. Stages 3 and 4 (diameter range: 2.1-4.7 µm) represent bronchial deposition. Stages 5 and 6 (diameter range < 2.1 µm) represent deposition in the alveoli (Akpeimeh et. al., 2019; HSE, 2014).Figure 4.2 shows the size distribution of the fungal bioaerosols over the various study sites namely agriculture field, dumping and traffic throughout all the seasons.

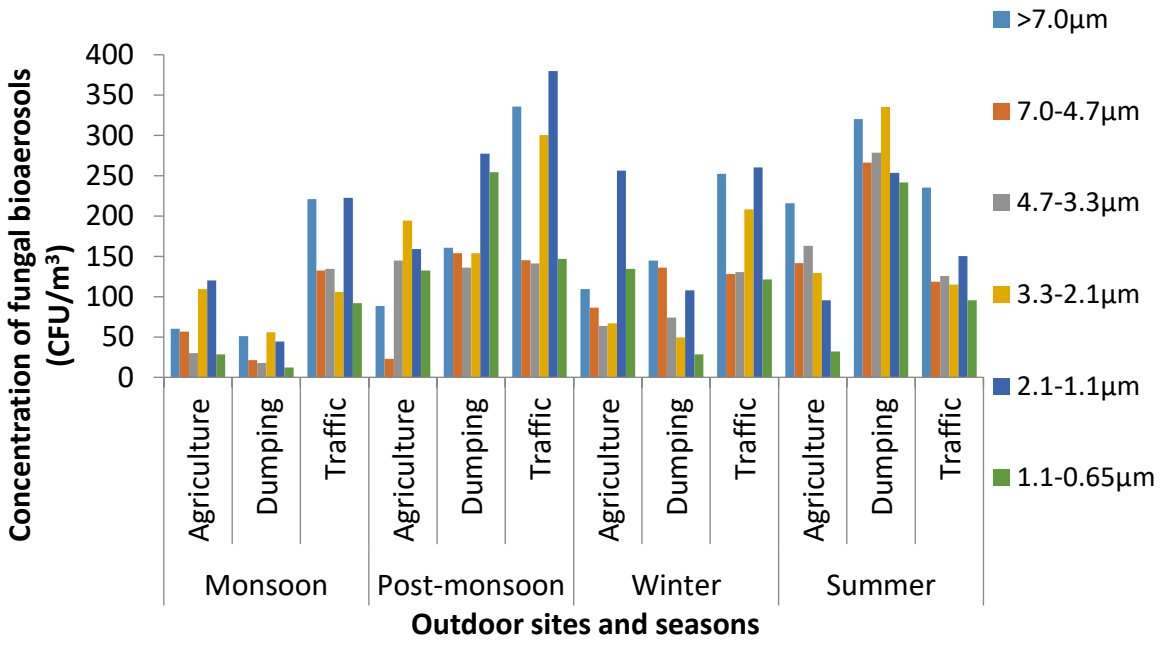


Figure 4.2 Average concentration of size segregated fungal bioaerosols at three sites and three different seasons.

In all seasons, the size-segregated concentration of fungal bioaerosols at each site appears to be following almost a standard trend. At the traffic, almost in all the seasons, the fungal

bioaerosols were found in the fine size range compared to the coarse size range, or they were nearly the same. The same trend was also seen for the dumping site, except in winter. In the agriculture field, the concentration of fungal bioaerosols was higher in the fine particle size range, excluding summer, where concentrations were higher in the coarse particle size range.

The majority of fungus species have the same diameter as stage 4, and 5, which is synonymous with the human body's terminal bronchi of lungs. This indicates that most of the fungi including allergic fungi found at this stage are mostly prone to influence the terminal bronchi in human lungs when breathed in, except for winter dumping sites where the highest concentration in stage 1 is found. This high concentration in stage 1 (size range: 7 µm or above) is synonymous with pre-separator. For the agriculture fields, traffic and dumping sites in post-monsoon season, the maximum concentration is found in stage 4 and 5 (size range: 3.3 to 1.1 µm), which is synonymous with secondary lung bronchi in the human body. The minimum concentration for each season is different, but mostly, the minimum concentration was found in stage 6 (size range: 0.65 to 1.1 µm). The stage 6 size range particles are synonymous with the alveoli of the human lung. These ranges of particles accumulate at alveoli and directly affect our lungs badly (Nair, 2021).

In Figure 4.3, the size distribution of Gram-negative bacterial bioaerosols concentration was shown at various outdoor sites throughout the season. Here, mostly the maximum GNB concentration was found in fine size range (3.3-0.65 µm), which shows that terminal lung bronchi are at risk of infection because their diameter range is synonymous with this size range. Exception in traffic and dumping sites during summer where the highest concentration is found in the coarse size range where high concentration in size range 7-4.7 µm synonym to the pharynx. The agriculture field shows the minimum concentration of GNB in each season.

Similarly, Figure 4.4 shows the size distribution of the average concentration of Gram-positive bacteria over the various sites and seasons. In the traffic, the maximum concentration observed in the coarse size range suggested that the pharynx, trachea, and primary and secondary bronchi tend to be more likely to be affected by Gram-positive bacteria. The agricultural field shows a higher concentration in the fine particle size range during all the seasons except winter. The dumping site did not show any significant trend

for GPB, and it had a relatively lower concentration compared to the agriculture field and traffic in each season.

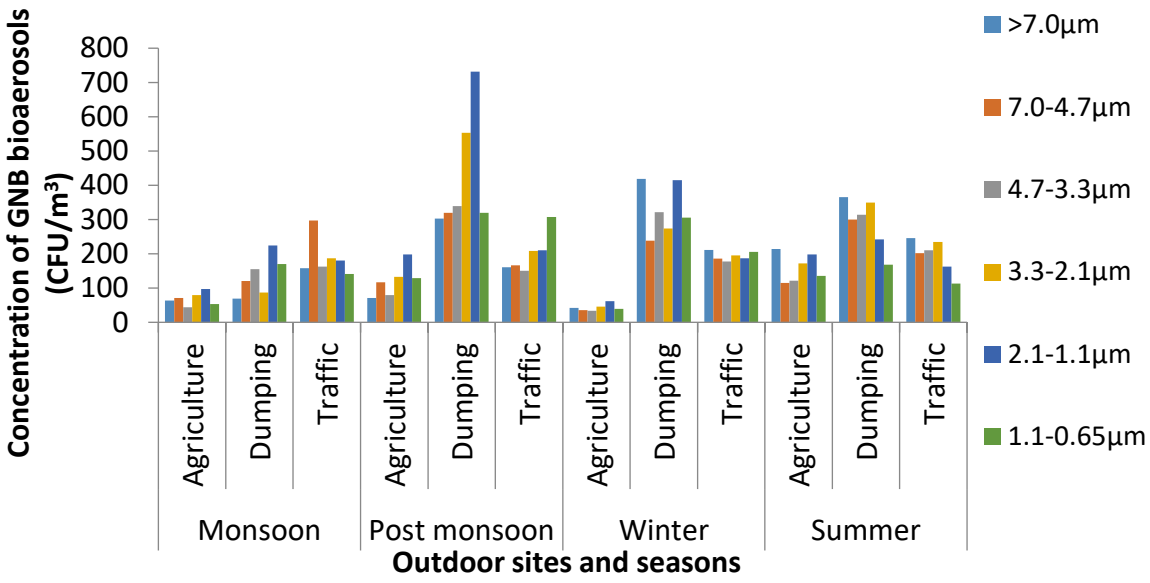


Figure 4.3 Average concentration of size segregated GNB bioaerosols at three sites and three different seasons.

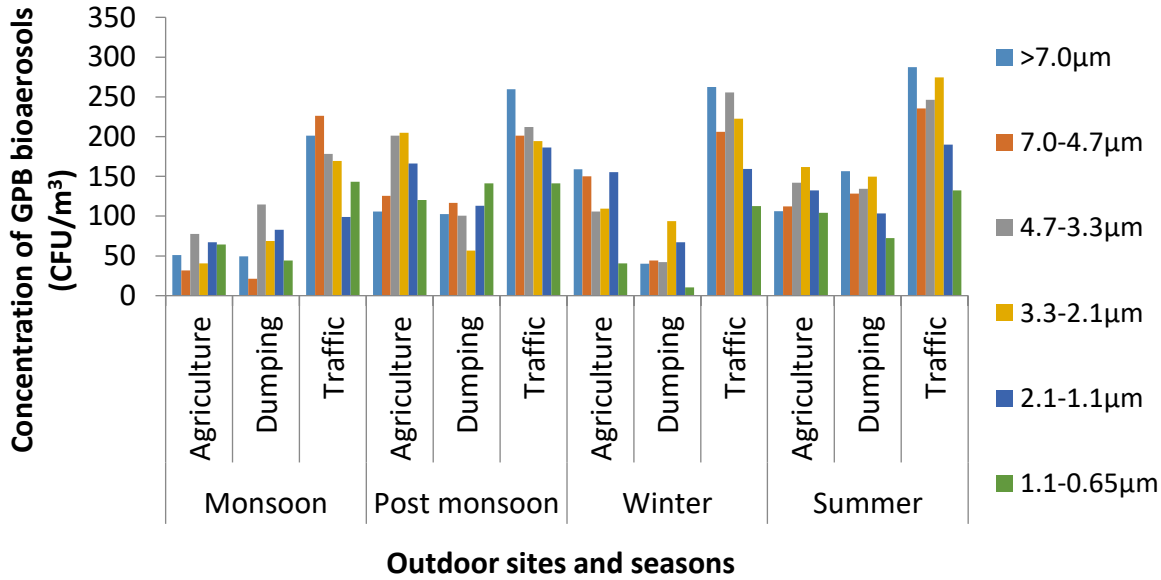


Figure 4.4 Average concentration of size segregated GPB bioaerosols at three sites and three different seasons.

### 4.3.2 Seasonal variation in average concentration of bioaerosols over various sites

The total concentration of each bioaerosol varied from season to season and site to site.

The details of the bioaerosols concentration and environmetals variables has shown in the Table A1 in Appendix A. In Figure 4.5, the variation in the concentration of the fungal bioaerosols with the various outdoor sites was shown. It can be seen that the maximum concentration of the fungal bioaerosols at agriculture (777±109 CFU/$m^3$) and dumping sites (1695±243 CFU/$m^3$) was seen during the summer, whereas for the traffic (1449±229 CFU/$m^3$) it was seen during the post-monsoon. During the monsoon, the fungal bioaerosols concentration was found to be the lowest (202±55 CFU/$m^3$) at the dumping site as compared to all the sites. The overall average concentration of the fungal bioaerosols for agriculture fields, dumpsites and traffic were 664±177 CFU/$m^3$, 810±559 CFU/$m^3$ and 1075±286 CFU/$m^3$, respectively.

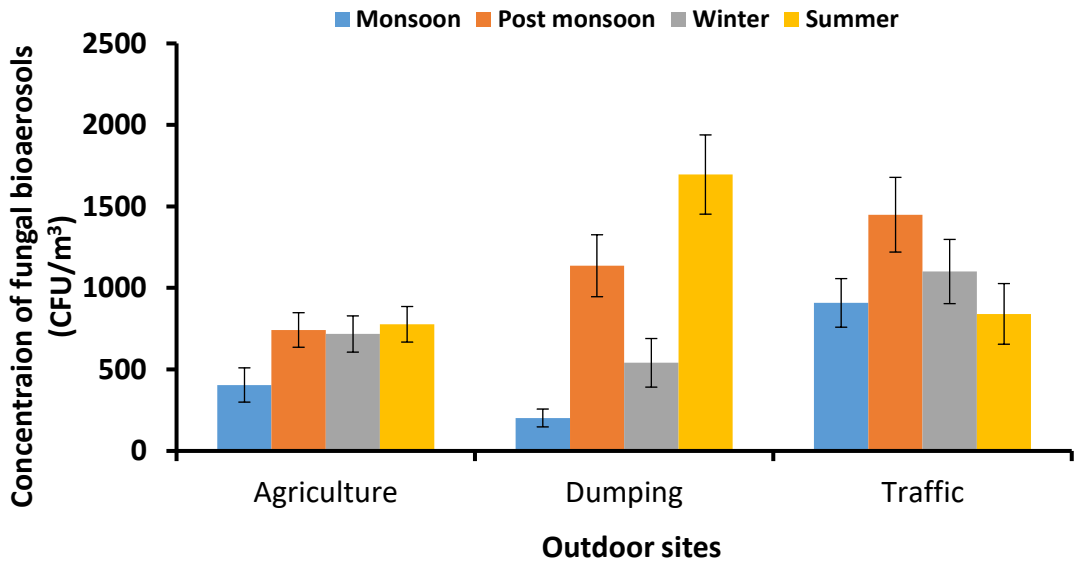


Figure 4.5 Average concentrations of fungi in different season at different sites.

Minimum concentration of fungi was found in monsoon season (202-908 CFU/$m^3$) at a temperature range (from 25°C to 30°C); many fungus species are washed out in the monsoon season due to rain. Thus, low fungal concentration was found outside. Nevertheless, the absence of rain washout gears up in post-monsoon fungi production, winter as compared to monsoon. Higher humidity may also responsible for lower concentration of fungi in monsoon reason.

Maximum fungus concentration was found at traffic sites in the post-monsoon season because of pollution, the crowd at the site, number of vehicles, particulate matter, wind speed, building construction near the site, and other environmental conditions. For

dumping sites, maximum fungus concentration was found in the summer season due to waste, plants near the site, wind direction, municipal labour involvement, animals around this site and other environmental conditions, etc. Similarly, agricultural fields had maximum fungus in the post-monsoon season because of different vegetative patterns; farmers, residue of crops, environmental conditions, etc, may be responsible. Rainfall is the main reason for the minimum fungus concentration in the monsoon season for all the sites (Ren et. al., 2018).

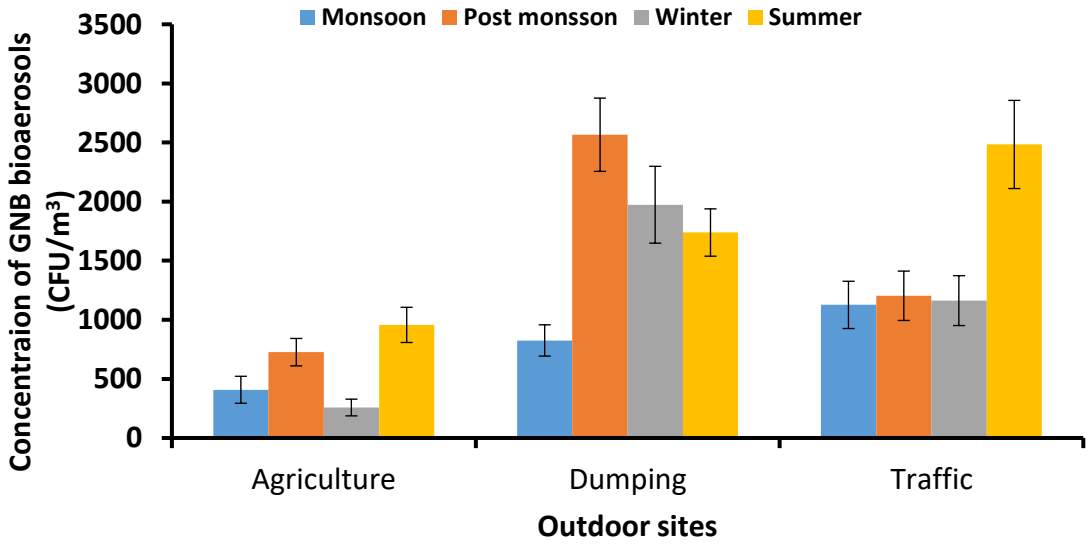


Figure 4.6 The variation in the concentration of Gram-negative bacterial bioaerosols over the various outdoor sites during different seasons.

The maximum concentration of GNB was found in the post-monsoon season at the dumpsite (2565±310 CFU/$m^3$), and for the other two sites, the agriculture field and the traffic site, it was highest in summer (956±149 CFU/$m^3$ and 2483±273 CFU/$m^3$). The variation in the concentration of Gram-negative bacterial bioaerosols over the various outdoor sites during different seasons is shown in Figure 4.6. The maximum GNB concentration at dumping site were observed during post-monsoon because of animal's presence, plants, emission of toxic gases, toxic leachate, and metrological conditions etc. Similarly, for agriculture and traffic site presence of people, pollution, residues, PM, metrological conditions are responsible for maximum GNB in summer season. Moreover, rainfall is can be predict as the main reason for minimum concentration GPB in monsoon season. But it can explored for other reasons too.

Figure 4.7 shows the seasonal variation of the total concentration of the Gram-positive bacterial bioaerosols concentration over the various outdoor sites. Here, for all the three sites maximum concentration of GPB were found at the traffic site for all the seasons as compared to the other two. And its highest concentration were observed during the summer (1365±271 CFU/$m^3$). For agriculture site, it was higher during post-monsoon season (924±146 CFU/$m^3$) and for dumping site it shows higher concentration during the summer (744±153 CFU/$m^3$). While minimum concentration in monsoon season as shown in Figure 4.7 because the rainfall is the reason for the minimum concentration of each bioaerosols in monsoon season. It has been reported that higher concentration of bioaerosols found in post monsoon and lower concentration in monsoon and winter over the few sites in Delhi (Lal et. al.,2013; Maharia & Srivastava et. al., 2015).

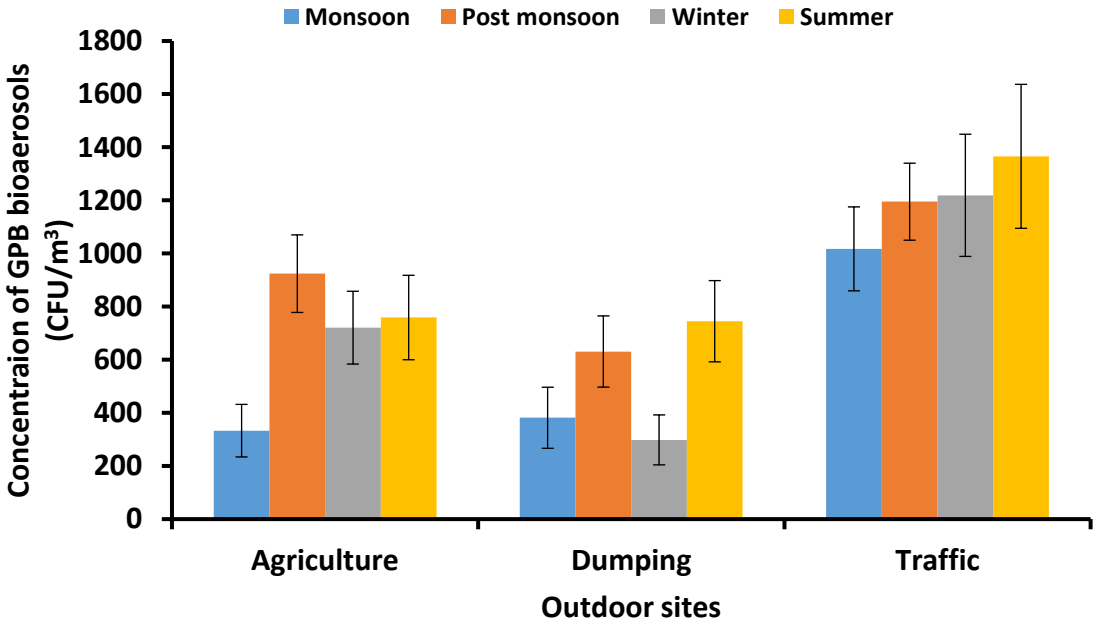


Figure 4.7 Average GPB concentrations in different seasons at different sites.

### 4.3.3 Biological diversity of bioaerosols and associated health effects

All outdoor sites (agriculture field, dump site and traffic) showed variable biological diversity in the bioaerosol samples for both bacteria and fungi. Most of them were very common, and they all were present in the air samples taken from all sites for all the season. Our observations show that gram-negative bacterial diversity is much higher in each of the samples taken from the outdoor sites. The abundance of bioaerosols at different outdoor sites and their associated health effect in humans and immunocompromised bodies are included in Appendix A1.

In all the sites some of the bacteria and fungi were common in which bacteria such as *Acinetobacter, Brevundimonas, Enterobacter, Bacillus* and fungi *Fusarium, Aspergillus, Talaromyces, Cladosporium, Penicillium* etc. were dominant. The dumping and traffic site shows the higher diversity of bacterial and fungal bioaerosols. At the agriculture field, *Bacillus, Acinetobacter, Brevundimonas, Enterobacter* in bacteria and fungi like *Fusarium, Aspergillus, Talaromyces, Periconia, Cladosporium* and *Penicillium* were dominant in the each seasons. Some of these microbes cause skin and eye irritation (Dhakal et. al., 2013; La Jeon et. al., 2012; Maucour et. al., 1999), gastrointestinal, UTIs, and respiratory diseases (Bai et. al., 2021; Ramirez & Giron, 2022; Stabler et. al., 2018).

Dumping and traffic sites had the highest diversity of the bioaerosols and most of them are pathogenic in nature and they were enriched with bacterial diversity such as, *Stenotrophomonas, Mammaliicoccus, Acinetobactor sp., Bacillus, E. coli, Enterobacter, Brevundimonas* and fungal diversity such as *Fusarium, Cladosporium, Aspergillus, Talaromyces, Periconia, Penicillium* were dominant in the dumping sites. Whereas for traffic site *Stenotrophomonas, Acinetobactor, Bacillus sp., Brevundimonas, Enterobacter* in bacteria and fungi mainly *Fusarium, Cladosporium, Aspergillus, Talaromyces, Periconia, Cladosporium sp.*, and *Penicillium* were dominant. At the dumping site and traffic, most of the microbes are pathogenic and have a potential to cause severe health effects on humans; if the body is immunocompromised (Dent et. al., 2010; Denton and Kerr, 1998; Hanski et. al., 2012; Korotetskiy et. al., 2022).

Since all the microbes found at the various outdoor sites were not pathogenic in nature even, they are useful for humans, plants and animals, too. Here, our aim is only to focus on the types of microbes that may cause harmful effects on human health

### 4.3.4 Correlation analysis for bioaerosols and environmental variables at different outdoor sites

In order to observe the linear relationship between the various bioaerosols and environmental variables, the correlation matrix was formed (Figure 4.8, 4.9 and 4.10) for each site. Where, Temp (temperature (°C)), RH (Relative humidity (%)), WS (wind speed (m/s)), WD (wind direction (degree)) Fungal bioaerosols (FA), Gram-negative bacterial bioaerosols (GNB) and Gram-positive bacterial bioaerosols (GPB) shows their correlation with each other in the cross point box. The diagonals are correlated with the

same variables, so it shows the value 1 (highly correlated). Figure 4.8 shows the linear correlation of various bioaerosols with the environmental variables at the agriculture sites for the overall seasonal variations, where some of the correlations are positive and negative as well. But among all the variables, they do not shows the significant correlation with each other.

| | FA | GNB | GPB | WD | WS | RH | Temp |
|---|---|---|---|---|---|---|---|
| FA | 1.000 | | | | | | |
| GNB | -0.238 | 1.000 | | | | | |
| GPB | 0.253 | 0.422 | 1.000 | | | | |
| WD | -0.141 | 0.127 | 0.032 | 1.000 | | | |
| WS | -0.250 | 0.296 | 0.002 | 0.462 | 1.000 | | |
| RH | 0.167 | -0.399 | -0.088 | 0.058 | -0.227 | 1.000 | |
| Temp | -0.377 | 0.467 | -0.098 | 0.079 | 0.344 | -0.440 | 1.000 |

Figure 4.8 Correlation matrix for bioaerosols and environmental variables at agricultural site

Figure 4.9 shows the linear correlation of the various bioaerosols and environmental variables at the dumpsite. In this correlation matrix, a very low significant value of correlation coefficient (either positive or negative) was observed between the bioaerosols and environmental variables.

| | FA | GNB | GPB | WD | WS | RH | Temp |
|---|---|---|---|---|---|---|---|
| FA | 1.000 | | | | | | |
| GNB | 0.467 | 1.000 | | | | | |
| GPB | 0.785 | 0.303 | 1.000 | | | | |
| WD | 0.044 | -0.093 | 0.031 | 1.000 | | | |
| WS | 0.126 | -0.206 | 0.115 | 0.460 | 1.000 | | |
| RH | -0.444 | -0.127 | -0.279 | 0.064 | -0.242 | 1.000 | |
| Temp | 0.302 | -0.379 | 0.403 | 0.074 | 0.352 | -0.508 | 1.000 |

Figure 4.9 Correlation matrix for bioaerosols and environmental variables at dumping site

Similarly, the correlation coefficients between environmental variables and bioaerosols at the traffic site shown in Figure 4.10. In this matrix, there is no significant positive or negative correlation was observed between the bioaerosols and environmental variables throughout the air sampling at the traffic site.

| | FA | GNB | GPB | WD | WS | RH | Temp |
|---|---|---|---|---|---|---|---|
| FA | 1.000 | | | | | | |
| GNB | -0.238 | 1.000 | | | | | |
| GPB | 0.253 | 0.422 | 1.000 | | | | |
| WD | -0.141 | 0.127 | 0.032 | 1.000 | | | |
| WS | -0.250 | 0.296 | 0.002 | 0.462 | 1.000 | | |
| RH | 0.167 | -0.399 | -0.088 | 0.058 | -0.227 | 1.000 | |
| Temp | -0.377 | 0.467 | -0.098 | 0.079 | 0.344 | -0.440 | 1.000 |

Figure 4.10 Correlation matrix for bioaerosols and environmental variables at traffic

From the above Figure (4.8, 4.9 and 4.10), it can be said that there is no significant correlations have been observed between bioaerosols and environmental variables at each site of the air sampling throughout the year. This may be because various other factors may affect the concentration of the bioaerosols at the various sites.

Since different bioaerosols of fungi and bacteria show a different nature with the environmental variables and site-specific. Some show positive behaviour toward survival and multiplication, and some show negative behaviour. So, the overall behaviour of bioaerosols is very complex towards the environmental variables (Priyamvada et. al., 2017).

### 4.4 Conclusions

In the present study, the seasonal variation of the concentration and size distribution of the bioaerosols were estimated over the various outdoor sites in the Varanasi (Middle Indo-Gangetic plain). Along with this, bioaerosols and their association with environmental variables were also explored, and possible health effects associated with the humans on detected bioaerosols at various locations were described.

The concentration of bacterial bioaerosols were much higher than the fungal bioaerosols in all the seasons and sites. In the seasonal analysis, bacterial and fungal bioaerosols were found to be much higher during the summer and post-monsoon at all the sites; however, it was lowest during the monsoon season. Most of the bacterial and fungus species are washed out in the monsoon season due to rain, thus low bioaerosols concentration is found in outdoor sites. Traffic and dumpsites show a higher concentration of bioaerosols in all seasons, and most of the pathogenic bacterial and fungal diversity were found at these

two locations. This concludes that the population residing there have a higher risk of bioaerosols-associated health issues.

The size of the bioaerosols is one of the most important parameters to predict health issues related to humans. The size-segregated bioaerosols characterization was done during all the seasons at the given distinct sites in Varanasi. It has been seen that most of the bioaerosols were found in the fine particle size range (0.65 to 3.3 µm), which indicates that the human population can easily exposed via inhalation or skin infection. Due to the finer size range, it can reach deep into the respiratory system of humans and cause several types of health issues. Maximum concentration correlated with the size range 1.1-2.1 µm (synonym with the human body's terminal lung bronchi) and lower concentration is different but mainly with size range from 0.6 to 1.1 µm. Thus, most of the allergic and immunotoxic fungi are considered a possible threat to human lung terminal bronchi when breathed. There is no significant correlation has been observed between bioaerosols and environmental variables. This may be because the sources of emissions can be dominant over the environmental variables. In the biological characterization, few bacterial and fungal bioaerosols were observed, which can affect human health, specifically the immunocompromised body, via some allergic effects like skin infections, eye infections, respiratory issues, gastrointestinal problems, etc.