3.1 Preamble

In the context of a global aerosols study, the IGP is recognized as one of the major hotspots of aerosol loading because of its geomorphology, meteorological variability and climate susceptibility (Bibi et. al., 2015; Ganguly et. al., 2009; Kumar et. al., 2015; Kumar et. al., 2017; Murari et. al., 2016; Sen et. al., 2016; Sen et. al., 2017). Studies show that, in this region, the average concentration of particulate matter (PM_{10} and $PM_{2.5}$) in aerosol loading is significantly higher than the National ambient air quality standards (NAAQS) (Gupta et. al., 2021; Jain et. al., 2021; Kumar et. al., 2015; Kumari et. al., 2020; Mhawish et. al., 2020; Rajput et. al., 2019; Ravindra et. al., 2023; Tiwari et. al., 2015) and it is about 1.2 to 3.3 times higher for $PM_{2.5}$ and 2.2 to 4.6 times higher for PM_{10} in the reported studies (Devi et. al., 2020; Kumari et. al., 2020). Anthropogenic sources are one of the significant contributors to these higher concentrations of particulate matter containing metals and heavy metals in trace amounts and affect human health (Choudhary et. al., 2022; Moniruzzaman et. al., 2022). Apart from these, biogenic sources emit a significant amount of aerosols called bioaerosols, containing biological properties which can also contribute to health, climate and agricultural study (Després et. al., 2012; Fröhlich-Nowoisky et. al., 2016; Madhwal et. al., 2020; Yadav et. al., 2020).

Bioaerosols are biotic components of aerosols which are omnipresent and have a substantial role in climate change, agriculture and human health. These bioaerosols may also be alive or carry living organisms, and they may release from living or dead organisms like bacteria, fungi, viruses, pollen etc. (Jones & Harrison 2004; Liang et. al., 2022; Stetzenbach, 2005). The surface of the living or the dead plant is the most important source of bioaerosols emission. Wind waves and rain play an important role in aerosolizing the microbes from natural sources and reaching the atmosphere (Burrows et. al., 2009; Mayol et. al., 2017; Michalska et. al., 2021). The microbial communities in the atmospheric aerosols can help understand the bioaerosols' effect on human health and climate (Fröhlich-Nowoisky et. al., 2016; Kim et. al., 2018; Zhai et. al., 2018). It can change the physicochemical characteristics of the atmosphere by microbial degradation, contact and collision with the organics present in the atmosphere. Bioaerosols have also potential to also change the chemistry of environmental interfaces like air/snow interfaces (Ariya &Amyot, 2004; Després et. al., 2012; Hu et. al., 2021) which can affect the various atmospheric processs.

Recent studies explored the understanding of the interaction of bioaerosols and particulate matter (PM) and established microbial transmission over the effect of the environment (Bai et. al., 2021; Ruiz-Gil et. al., 2020). Particulate matter in the atmosphere is very important while monitoring the air quality because of its significant effect on human health, environment and global climate (Fröhlich-Nowoisky et. al., 2016; WHO, 2016; Zhang et. al., 2022). Particulate matters carry 10% of the biological component called bioaerosols (Wei et. al., 2016). Exposure of PM-carrying bioaerosols has a different impact depending on their size (Wan et. al., 2023). Particles in the suspended particulate (TSP) range can penetrate the skin, PM_{10} can be inhalable and reach the respiratory system, and $PM_{2.5}$ have a greater risk of reaching into the human lungs and the blood via alveoli and capillaries. Eventually, these microbes may enhance inflammation and clogging in pores and cause vascular and respiratory illness (Fan et. al., 2019; Liu et. al., 2018). Along with human health, these microorganisms can also harm crops and may cause greater risk to the ecosystem (Liu et. al., 2018; Xu et. al., 2019; Yadav et. al., 2020).

Bioaerosols size lies between 10nm to 100 μm, in which viruses are the finer bioaerosols while pollens are coarser (Ariya &Amyot, 2004; Cox &Wathes, 1995). The bioaerosols of 1 to 5 μm size range generally remain longer in the air than the coarser particle (Mohr, 2007). Like the aerosols, the bioaerosols' size and concentration vary from location to location. Bioaerosols contribute 20% of the aerosol load, and that can significantly affect human health (Amato et. al., 2014; Lee et. al., 2012). The weather of the Indo-Gangatic plain, one of India's significant source of agricultural products, is changing due to high aerosol concentration and global warming. Weather pattern in Indo-Gangetic Plain dramatically changes in the summer, with extreme cold in winter, its monsoon periods are shrinking, and rain event is reducing decades by decades. These seasonal patterns can increase the various epidemic diseases such as malaria, typhoid, pneumonia and chikungunya as well as respiratory and cardiovascular diseases etc. (Tripathi et. al., 2006; Whitcombe, 2012). Moreover, the presence of the biological component in the particulate matter increases the risk of diseases/infection that may take the attention of worldwide researchers for further research in the field of bioaerosols and aerosols (Burrows et. al., 2009)

In this study, the qualitative and quantitative analysis of the bioaerosols in the particulate matter was determined at Varanasi, India, located in the Middle IGP. Here the

concentration of bioaerosols in the collected samples of PM_{10} and $PM_{2.5}$ of over the given period (winter time study during 2021-2023) were analysed. An attempt has been made to establish the relationship between the particulate matter, the meteorological parameters, and their cumulative effect. The bioaerosol samples were also analysed to identify the presence of different types of microbes in these areas and their possible health effect on humans. This study may be one of the representative studies of the bioaerosols over the Middle IGP.

3.2 Materials and methods

3.2.1 Sampling site

Air sampling was carried out at the terrace of the Department of Chemical Engineering and Technology, IIT (BHU) Varanasi (25.15°N, 82.59°E) campus in the IGP. The central part of Varanasi is dominated by urban land, and the peripheral boundaries are rich in vegetation. The winter season of Varanasi has been broadly extended from December to February. In addition, wind speed is mainly in the range of 1-2m/s, which is nearly calm during this month over Varanasi city.

In Varanasi, the sampling was conducted at the IIT (BHU) campus; the sampler was about 10m above the ground surface. There are no industrial activities near the IIT (BHU) campus, and it is a suburban site of the Varanasi region, and National Highway is about 3km from the campus. Around the campus, agricultural activities are prominent, so this is a dominant vegetation area.

3.2.2 Sample collection

The aerosol samples were collected in ambient air using a Respirable dust sampler for (PM_{10}) and a Fine particulate sampler ($PM_{2.5}$) by Envirotech in the winter season from 2021 to 2023. A respirable dust sampler was designed for both total suspended particulate matter (TSPM) and PM_{10}. For PM_{10} samples collection glass fibre filter (GFA) were used, while quartz filter paper was used to collect the $PM_{2.5}$. The pre-desiccated, pre-weighed, and sterile filter (microbe free) was installed in both samplers and air sampling was performed for 24 h (9:00am to 9:00am next day) at a flow rate of 16.67 lpm for $PM_{2.5}$ and 1 m^3 for PM_{10}. The samples were collected during the winter of the year 2021 to 2023 (December to February) once a week. This way total 26 number of samples were

collected. Before sampling, the quartz membrane filter was calcined at 600°C for five hours in a muffle furnace to remove organic debris from the membrane. 75% alcohol was then used to clean the cutting head, membrane clips, forceps, and other equipment used at the start and completion of the sample. The person involved in the sampling wore gloves and masks to prevent contamination of the sample during sample exchange. The samples were stored in the refrigerator (at 4°C) for further use. Similar techniques were used to collect blank samples without operating the instruments.

3.2.3 Meteorological data

The meteorological parameters, viz., temperature (Temp) and relative humidity (RH), were monitored using the Meteorological Data Logger System and the wind speed (WS) and wind direction (WD) were observed from the nearest meteorological station of the Centre for Pollution Control Board (CPCB) present in the campus. The data were recorded simultaneously for each observation period, and the average value was taken to analyse the results.

3.3 Analysis of the samples

3.3.1 Concentration of PM and bioaerosols

After the sampling, each sample on the filter is put into the desiccator in the laminar airflow for protection from contamination. The mass of PM_{10} and $PM_{2.5}$ deposited on the filter were determined gravimetrically using weight balance. For biological analysis of the aerosol samples, 4 identical parts of the filter paper were taken and cut into pieces after that extracted using 10 mL of sterile Mili-Q water with the help of low-temperature (15-25 °C) ultrasonication. The water-extracted sample was used for biological analysis. 2 mL of filter extract was used for fungal and bacterial analysis, inoculated on petri plates containing potato dextrose agar (PDA) and nutrient agar (NA) medium. The maximum growth of the fungal colony on the PDA typically appears after incubating at 35 °C for 2 days (48 hr), and the bacterial colony usually occurs after 3 days (72 hr) at 25 °C.

After the incubation for the given period, the colonies appeared on the plates, which were counted using the colony counter. The concentration of the microbes was measured in the CFU/m^3, which can be obtained by dividing with flow rate and sampling duration.

3.3.2 Biological characterisation of PM

After the incubation period of the bacteria and fungi, the colonies appeared on PDA and

NA plates and were counted with the help of a colony counter. The purification was done until pure cultures of fungi and bacteria are obtained, respectively. Microbes were pre-identified based on their colony colour, shape and structure using a light microscope and compared with the standard description available in online literature for unidentified microbes. DNA sequencing method was performed for the confirmation of the species. The concentration of bioaerosols (fungal and bacterial) in the air was measured in colonies forming units per cubic meter (CFU/m^3). The blank field sample was collected and analysed similarly to aerosol samples. No growth (negligible) of microorganisms was seen in the filled-blank sample, and substracted from the sample data.

The following steps were followed in the DNA sequencing. In the first step, Genomic DNA was isolated from the samples. In the second step, ~1.5 kbp of 16s-rDNA for bacteria and 18s-rDNA for fungi fragments was amplified using high–fidelity PCR polymerase. In the third step PCR product was sequenced bi-directionally, and in the last step, the sequence data were analysed to identify the bacteria and fungi and their closest neighbours to identify the microbes.

3.3.3 DNA extraction and sequencing

The sample was picked up, placed in a mortar, and mixed with 1 mL of extraction buffer. After homogenisation, it was transferred to 2 mL microfuge tubes. Then an equal volume of phenol, chloroform, and iso-amyl-alcohol was added (in a ratio of 25:24:1) to the tubes and mixed well by shaking the tubes. In the next step, it was centrifuged at room temperature for 15 min at 14,000 rpm. After centrifuge, the upper aqueous phase was collected separately, and an equal volume of chloroform and iso-amyl-alcohol was added and mixed in a ratio of 24:1. Again, after centrifuging for 10 min and 14,000 rpm at room temperature, the upper aqueous were transfered to the new tube. By adding 0.1 volume of 3 M Sodium acetate pH 7.0 and 0.7 volume of isopropanol, DNA was precipitated from the solution. Again, after incubation or 15 min at room temperature, tubes were centrifuged at 4 °C for 15 min at 14,000 rpm. Precipitated DNA pellets were washed with 70% and 100% ethanol and dried in air. Then DNA was dissolved in TE (Tris-Cl 10 mM pH 8.0, EDTA 1 mM). To remove RNA, 5 μL of DNAse free RNAse A (10 mg/mL) was added to the DNA. 133 ng of extracted DNA and 10 pM of each primer are used for amplification. The composition of TAQ Master MIX contains High-Fidelity DNA

Polymerase, 0.5 mM dNTPs, 3.2 mM $MgCl_2$ and PCR enzyme buffer. The Sequencing mix composition and PCR conditions included 10 μL sequencing reaction, 4 μL big dye terminator ready reaction mix, 1 μL template (100 ng/μL), 2 μL primer (10 pmol/λ) and 3μL milli-Q water. PCR was run for 25 cycles where the with the following process, first initial denaturation at 96°C for 5 min, subsequent denaturation at 96°C for 30 sec, third hybridisation at 50°C for 30 sec and Elongation at 60 °C for 1.30 min. ABI 3130 Genetic Analyzer were used for sequencing the given strain, and the product was analysed by System Software aligner using Phylogenetic Tree Builder for making a phylogenetic tree (Bruno et. al., 2000).

3.3.4 Statistical analysis

Data analysis and graphs were made using SPSS, R studio and Minitab software. In the statistical analysis, mean, standard deviation (SD), upper and lower bound, maximum and minimum value, correlation, principal component analysis (PCA) and contour plot were made to represent the data in a better way.

3.4 Results and discussion

3.4.1 PM_{10}, $PM_{2.5}$ and bioaerosol concentrations in the ambient air

Table 3.1 shows the mean concentration of the respirable suspended particulate matter (PM_{10}) and fine particulate matter ($PM_{2.5}$) along with total microbial concentrations (TMC), fungal concentrations (FA), and bacterial concentration (BA) in PM_{10} and $PM_{2.5}$. The important meteorological variables are also mentioned in this table. In the table, the maximum and minimum value of the given variable has also been given. The upper and lower bound were shown in the Table 3.1 at the 95% confidence level in the ambient air at the IIT (BHU) campus in Varanasi.

The mean concentration of PM_{10} was 154.25±36.21 $\mu g/m^3$ which ranged between 105.47 and 221.45 $\mu g/m^3$, while the mean value of $PM_{2.5}$ was 125.61±35.04 $\mu g/m^3$ and ranged between 81 and 198 $\mu g/m^3$. The average value exceeds India's National Ambient Air Quality Standards (NAAQS) (for $PM_{2.5}$ 40 and PM_{10} 60 $\mu g/m^3$) by a large factor. It has been observed that Varanasi's air quality has been moderate to bad for the majority of the days. The amount of PM_{10} and $PM_{2.5}$ in Varanasi exceeds the permissible level. While the mean $PM_{2.5}$ concentration is two times the standard value for India, the mean PM_{10} concentration is three times the limit. This may be because of the use of biomass and

fossil fuel, as well as the lower boundary layer is also responsible for increased PM concentration during winter (Ojha et. al., 2020).

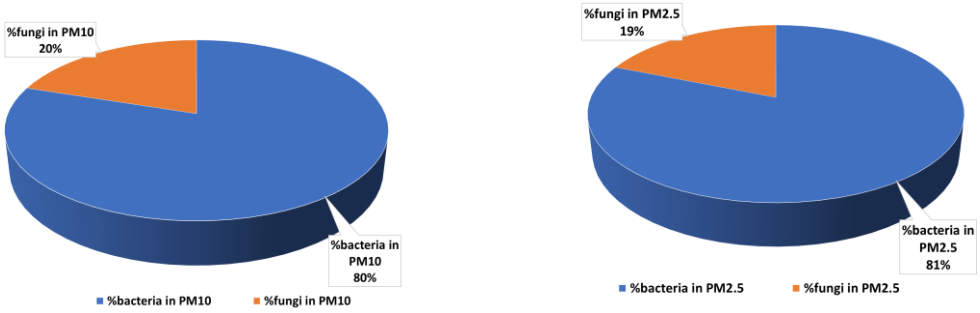
Table 3.1. The concentration of Mean, Standard deviation, maximum and minimum values of PM_{10} and $PM_{2.5}$ and associated microbes with their respective meteorological parameter in the ambient air.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
PM_{10} ($\mu g/m^3$)	Dec	10	127.34	14.26	4.51	117.14	137.54	105.47	150
	Jan	8	168.82	50.07	17.7	126.95	210.69	110.35	221.45
	Feb	8	173.31	14.94	5.28	160.82	185.8	153.82	191.42
	Total	26	154.25	36.21	7.1	139.62	168.88	105.47	221.45
$PM_{2.5}$ ($\mu g/m^3$)	Dec	10	102.7	16.35	5.17	91	114.39	81	127
	Jan	8	142.75	51.53	18.21	99.66	185.83	89	198
	Feb	8	137.12	12.26	4.33	126.87	147.37	121	153
	Total	26	125.61	35.04	6.87	111.45	139.77	81	198
BA_PM_{10} (CFU/m^3)	Dec	10	193	24.86	7.86	175.21	210.78	168	255
	Jan	8	214.25	48.11	17.01	174.02	254.47	144	275
	Feb	8	246.62	29.18	10.31	222.22	271.02	205	288
	Total	26	216.03	40.27	7.89	199.77	232.3	144	288
$BA\_PM_{2.5}$ (CFU/m^3)	Dec	10	105.2	34.94	11.04	80.2	130.19	72	186
	Jan	8	122	32.6	11.52	94.74	149.25	72	165
	Feb	8	120.37	30.86	10.91	94.56	146.18	78	165
	Total	26	115.03	32.66	6.4	101.84	128.23	72	186
FA_PM_{10} (CFU/m^3)	Dec	10	47	18.25	5.77	33.94	60.05	24	82
	Jan	8	53.5	23.57	8.33	33.78	73.21	23	88
	Feb	8	63.37	12.53	4.43	52.89	73.85	46	87
	Total	26	54.03	19.16	3.75	46.29	61.78	23	88
$FA\_PM_{2.5}$ (CFU/m^3)	Dec	10	29.1	9.85	3.11	22.04	36.15	12	45
	Jan	8	26.62	7.53	2.66	20.32	32.92	16	41
	Feb	8	24	9.13	3.22	16.36	31.63	12	39
	Total	26	26.76	8.88	1.74	23.18	30.35	12	45
TMC_10 (CFU/m^3)	Dec	10	240	33.9	10.72	215.74	264.25	202	300
	Jan	8	267.75	69.97	24.73	209.25	326.24	167	359
	Feb	8	310	36.87	13.03	279.16	340.83	271	375
	Total	26	270.07	55.12	10.81	247.8	292.34	167	375
TMC_2.5 (CFU/m^3)	Dec	10	134.3	41.4	13.09	104.68	163.91	94	226
	Jan	8	148.62	33.82	11.95	120.34	176.9	101	187
	Feb	8	144.37	30.55	10.8	118.82	169.92	111	184
	Total	26	141.8	35.19	6.9	127.59	156.02	94	226
Temp (°C)	Dec	10	17.26	2.07	0.65	15.77	18.74	14.3	21.4
	Jan	8	14.76	3.31	1.17	11.98	17.53	11.3	21.8
	Feb	8	19.5	2.03	0.71	17.8	21.19	17.2	23.8
	Total	26	17.18	3.06	0.6	15.94	18.41	11.3	23.8
RH (%)	Dec	10	77.52	7.33	2.31	72.27	82.76	63.7	89.7
	Jan	8	82.91	4.54	1.6	79.1	86.71	75.1	89.1
	Feb	8	72.96	8.32	2.94	66	79.92	60.7	85.4
	Total	26	77.77	7.77	1.52	74.63	80.91	60.7	89.7
WS (m/s)	Dec	10	0.21	0.05	0.01	0.17	0.25	0.09	0.3
	Jan	8	0.34	0.09	0.03	0.26	0.42	0.22	0.48
	Feb	8	0.5	0.36	0.12	0.2	0.81	0.25	1.33
	Total	26	0.34	0.23	0.04	0.24	0.44	0.09	1.33
WD (degree)	Dec	10	115.73	24.75	7.82	98.02	133.43	54.07	140.49
	Jan	8	140.15	18.85	6.66	124.39	155.92	119.03	178.34
	Feb	8	149.65	34.68	12.26	120.65	178.65	118.08	208.91
	Total	26	133.68	29.68	5.82	121.69	145.67	54.07	208.91

are much higher for the urban and rural sites of many countries (Amato et. al., 2014; Madhwal et. al., 2020). This high aerosol loading in India may be because of the large infrastructure development activities are continued.

The mean bioaerosols concentration or TMC in PM_{10} and $PM_{2.5}$ were 270±55 and 141±35 CFU/$m^{3,}$ respectively. The concentration of the bioaerosols in the PM lied between 94 to 375 CFU/m^3. For $PM_{10,}$ it was about 167 to 375 CFU/m^3, whereas for $PM_{2.5,}$ it was between 94 to 226 CFU/m^3. BA for bioaerosols in PM_{10} was between 144 to 288 CFU/m^3, and the average value of BA for PM_{10} it was about 216±40 CFU/m^3. In $PM_{2.5}$, the mean concentration of bacteria was about 115±32 CFU/m^3, which varied between 72 and 186 CFU/m^3. The mean FA concentrations in PM_{10} and $PM_{2.5}$ were 54±12 and 26±8 CFU/m^3, respectively, while it ranged between 23 to 88 and 12 to 45 CFU/m^3 in PM_{10} and $PM_{2.5,}$ respectively. Comparing these values from earlier reported studies, bioaerosols concentration is significantly higher for coarser than finer particles (Rajput et. al., 2017; Shrivastava et. al., 2015).

Figure 3.1 shows the percentage contribution of the BA and FA in the TMC observed in the sample. The bacteria contributed 79 and 81% in PM_{10} and $PM_{2.5}$, respectively, of the total microbe count in the sample. And fungal spores may contribute as much as 20% of PM_{10} and 18% of $PM_{2.5}$ to the total microbes in the respective particulate matter. Similar results were reported by the researchers where the concentration of the bacteria in PM_{10} was about 83% and 81% in $PM_{2.5}$ (Negrin et. al., 2007), and fungi contributed 20% in PM_{10} and 4–11% in $PM_{2.5}$ (Womiloju et. al., 2003). The concentration of the bioaerosols may vary because of the sources of bioaerosols, the effect of solar radiation and the moment of the air.



(a) (b)

Figure 3.1 The average percentage of the bacteria and fungi concentration in (a) PM_{10} and (b) $PM_{2.5}$ to the total microbes count present in the samples.

3.4.2 Microbial identification

Initially, morphological analyses and visual identifications were carried out to identify microbes. Most of the colonies are found to be white, yellow and orange. The Gram-staining test was performed to identify the nature of the bacteria, and it was found that 70% of bacteria were Gram-negative. In comparison, Gram-positive bacteria contribute only 30%. Giorgio et al. (1996) reported that up to 75% of bacteria are Gram-positive in the ambient air. Coccus were dominant in Gram-positive bacteria, while in the Gram-negative bacteria, *Coccus* and *Streptococci*, *Coccobacillus* was dominant. Visual observations revealed that fungi were white, black, brown, and blackish-white in colour. Several microbes can be identified, but few of them was sequenced for confirmation. In our study the fungi detected were *Aspergillus niger*, *Talaromyces minioluteus, Fusarium oxysporum, Periconia digitata, Penicillium sclerotiorum, Cladosporium sphaerospermum*, and bacteria *E. coli* (Gram-negative), *Stenotrophomonas maltophilia* (Gram-negative), *Acinetobacter calcoacetius* (Gram-negative), *Brevundimonas* (Gram-negative), *Enterobacter* (Gram-negative), *Mammaliicoccus sciuri* (Gram-positive). Among the fungi, *Aspergillus, Cladosporium, Penicillium* species were found to be the highest in number and contribute more, while in bacteria *E. coli, Mammaliicoccus, and Enterobacter* are prevalent.

3.2.3 Correlation of bioaerosols and PM with the meteorological variables

The air quality of a region is significantly associated with the meteorological parameter variations of the region. So, while studying the bioaerosols measurement, it is also required to analyse the meteorological components like ambient air temperature, RH, WS and WD and their effect on the aerosols and bioaerosols. In this study, a significant correlation has been observed between the particulate loading and the concentration of bioaerosols, but no clear correlation has been observed in the concentration of the microbes with the meteorological parameter, as shown in Figure 3.2. Here, no significant correlation was found in the meteorological variable during the observation. So, the minimum variation in the weather pattern does not significantly affect the bioaerosols variation, as our study suggests.

To determine the effect of PM and meteorological variables on the concentration of the bioaerosols, principal component analysis (PCA) has done. The biplot for PCA is shown

in Figure 3.3. The PCA of the PM_{10} and other variables data shows that the first three principal components

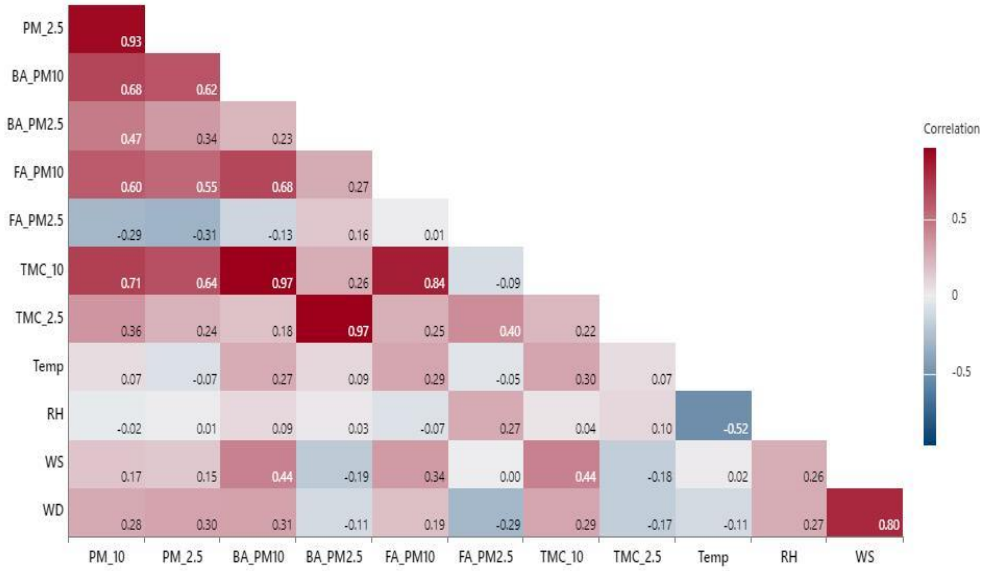
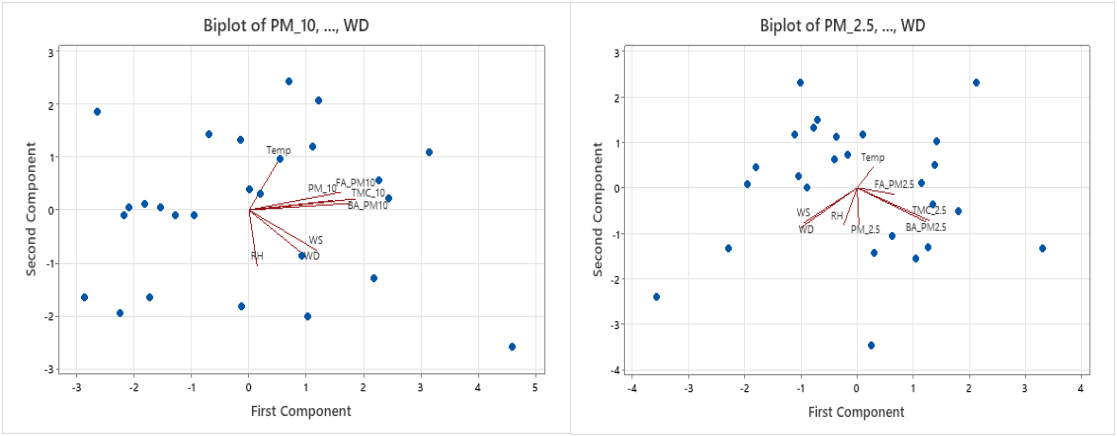


Figure 3.2 The correlation plot between the PM_{10}, $PM_{2.5}$, associated bacterial and fungal bioaerosols, total microbes and meteorological parameters (like temperature, RH, WS and WD).



(a) (b)

Figure 3.3 Principal component analysis (PCA) plot for the microbes content in (a) PM_{10} and (b) $PM_{2.5}$.

have eigenvalues greater than 1. The principal component's eigenvalue is an index that shows how effectively it summarises the data. These three components explain 83.5% of the variation in the data. The variable that can correlate the most with the first principal component is PM_{10} (0.391), bacteria in PM_{10} (0.475), fungi in PM_{10} (0.428) and total microbes in PM_{10} (0.496). The first principal component is positively correlated with all

four of these variables. Therefore, increasing the value of these four components increases the first component's value. The first four principal components explain 90.8% of the variation in the data. Therefore, these are the factors deciding the microbial analysis of the data. Likewise, in the principal component analysis of the $PM_{2.5}$ data, the first four principal components have eigenvalues greater than 1. These four components explain 88.4% variation in the data. The variables that can correlate the most with the first principal component are bacteria in $PM_{2.5}$ (0.51) and total microbes (0.544). The first principal component is positively correlated with these two variables, and the first four principal components explain 88.4% of the variation in the data.

The ambient air is unsuitable for the microorganism due to its desiccation stress. However, many microbes can remain active due to some specific resistance mechanism to various environmental factors. Temperature and RH are the most important parameters influencing the microorganism's survival. At low relative humidity, Gram-negative bacteria in bioaerosols survive longer, whereas Gram-positive bacterial aerosols remain active at high RH (Theunissen et. al., 1993). Due to very low RH, water is lost from the cell membrane and changes the crystalline structure of the lipid bilayer of the cell membrane to a gel phase; ultimately, this phase transition affects the cell surface configuration of microbes and results in the inactivation of microbes (Hurst et. al., 2007). In the case of temperature, when it reaches freezing, the ice crystals form on the microorganism's surface, and they lose their viability. The effect of temperature is closely associated with the various environmental factors, including RH.

The cumulative effect of the Temp, RH, and WS on the microbial presence and growth of the microbes has been observed. Figure 3.4 and Figure 3.5 are contour plots for the concentration of bacteria and fungus in the PM_{10} and $PM_{2.5}$ and the cumulative effect of Temp and RH, WS and WD. In Figure 3.4, (a) and (b), the cumulative effect of temperature and humidity on the total microbe's concentration in PM_{10} and $PM_{2.5}$ were highest at temperatures 16 to 19 °C and RH between 80 to 85%. Similarly, in plots (c) and (d), where PM_{10} and $PM_{2.5}$ vs Temperature and Relative humidity were found the highest in the same range. And plots (e) and (f), where bacteria in $PM_{10,}$ and $PM_{2.5}$ vs temperature and relative humidity are shown, whereas in plots (g) and (h), where fungi in PM_{10} and $PM_{2.5}$ vs temperature and relative humidity follow the same trend. At higher temperatures and low relative humidity there has been a significant decline in the

microbial population. A large difference in the bacteria concentration in PM_{10} compared to $PM_{2.5}$, whereas in fungi, these differences are comparatively lower.

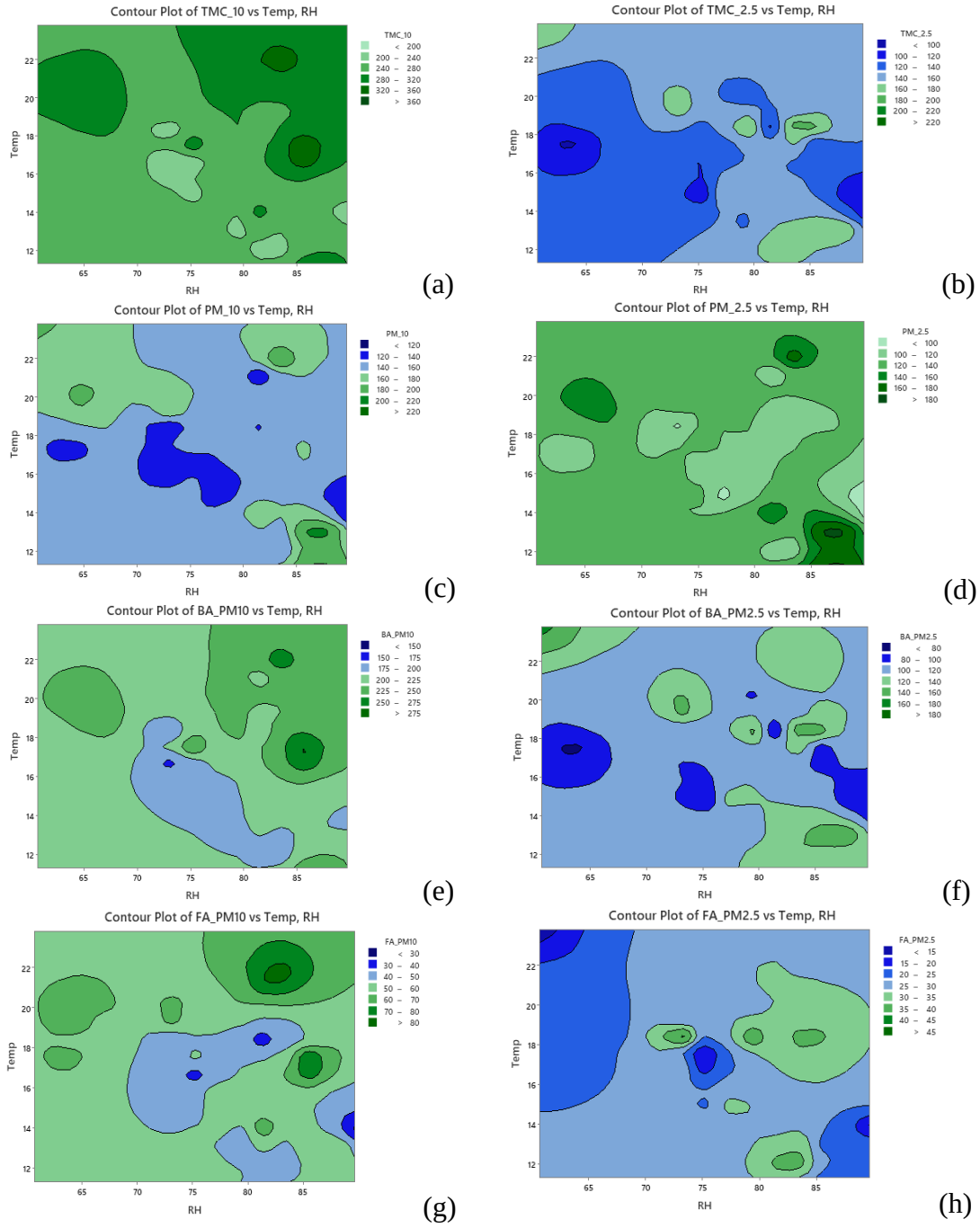


Figure 3.4 Contour plot for the concentration of bacteria and fungus in the PM_{10} and $PM_{2.5}$ and the cumulative effect of temperature and RH. Where, (a) and (b) the cumulative effect of temperature and RH on the total microbes concentration in PM_{10} and $PM_{2.5}$, similarly (c) and (d) are PM_{10} and $PM_{2.5}$ vs Temperature and RH, (e) and (f) bacteria in PM_{10} and $PM_{2.5}$ vs Temperature and relative humidity, (g) and (h) fungi in PM_{10} and $PM_{2.5}$ vs Temperature and RH.

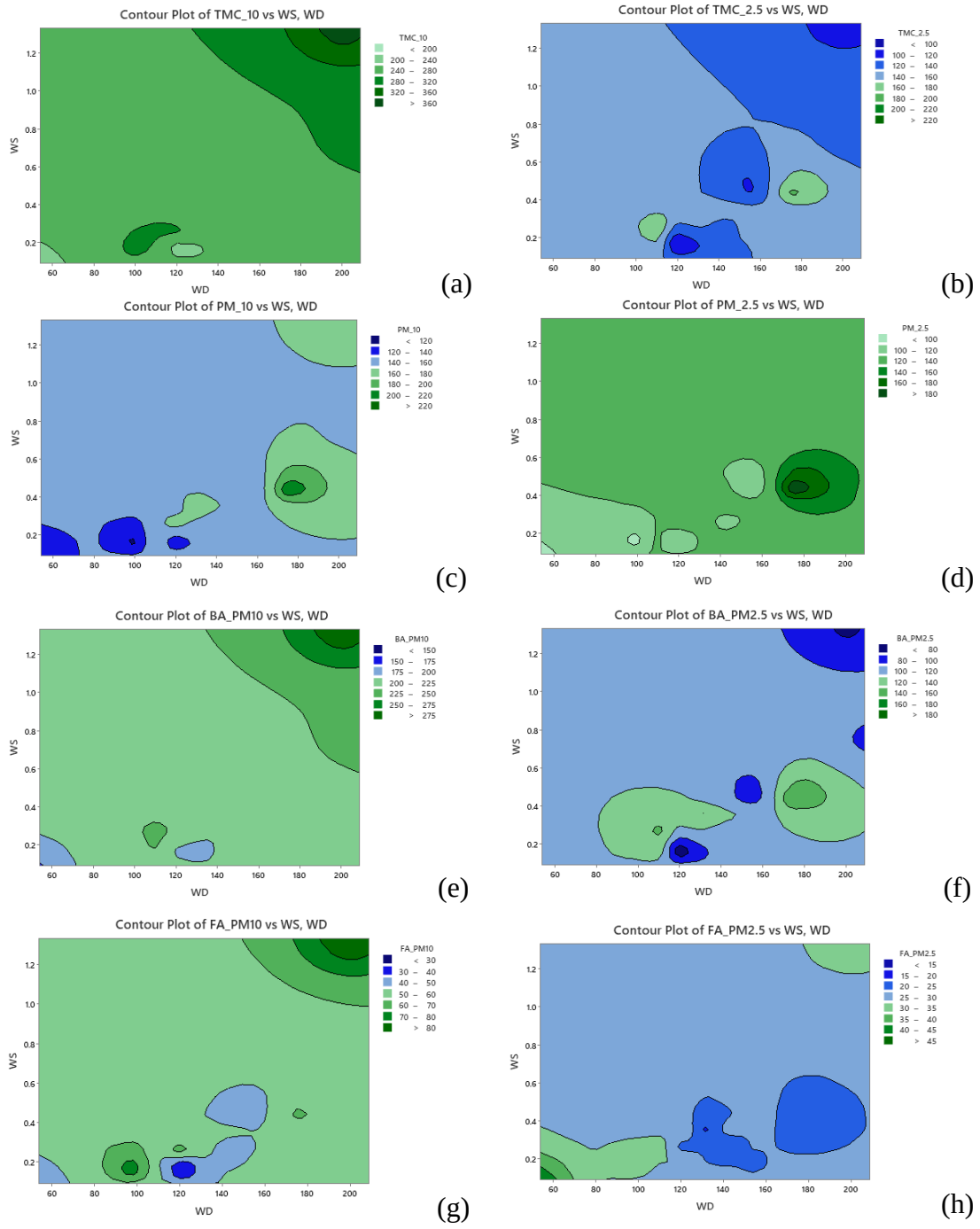


Figure 3.5 Contour plot for the concentration of bacteria and fungus in the PM_{10} and $PM_{2.5}$ and the cumulative effect of wind speed and wind direction. Where, (a) and (b) the cumulative effect of wind speed and wind direction on the TMC in PM_{10} and $PM_{2.5}$, similarly (c) and (d) are PM_{10} and $PM_{2.5}$ vs WS and WD, (e) and (f) bacteria in PM_{10} and $PM_{2.5}$ vs wind speed and wind direction, (g) and (h) fungi in PM_{10} and $PM_{2.5}$ vs WS and WD.

In Figure 3.5, (a) and (b), the cumulative effect of WS and WD on the TMC in PM_{10} are highest at the wind speed of 1 to 1.2 m/s and wind direction between southwest (SW) to south (S) where the vegetation is prominent and but for $PM_{2.5}$ it's not well defined in the graph. In Figure 3.5, (c) and (d) are PM_{10} and $PM_{2.5}$ vs wind speed and direction clearly show the highest concentration of bioaerosols in S to SW west. Plot (e) and (f), (g) and (h) show the cumulative effect of WS and WD on bacteria and fungi concentration in PM_{10} and $PM_{2.5}$. The plots clearly show that the bacteria and fungi concentration is higher in SW to S direction at wind speed between 1 to 1.2 m/s for $PM_{10,}$ and in the case of $PM_{2.5}$ it is not well defined. The meteorological condition can contribute significantly to the survival of bioaerosols in the atmosphere (Jones & Harrison, 2004; Zhen et. al., 2017). It can also be influenced by the weather pattern of the area and the source of the bioaerosols in the surrounding (Chi & Li, 2007; Li et. al., 2017).

3.5 Conclusions and future perspective

This study determines the PM_{10} and $PM_{2.5}$ concentrations in the ambient air of the suburban region of Varanasi city, in Middle IGP during the three years of the wintertime study. The concentration of PM_{10} and $PM_{2.5}$ was found to be greater than the recommended value of the National Air Quality Standards (NAAQS). Similar trends were also observed for biological particles in this region, but no universally acceptable standard is available for comparing the biological particles in the ambient air. The TMC in PM_{10} and $PM_{2.5}$ were in the typical range of 94-375 CFU/m^3. The BA were higher than FA, where bacteria contributed ~80%, while fungi contributed ~20% only.

Fungi, mainly *Aspergillus*, *Cladosporium, Penicillium* and bacteria *E. coli*, *Mammaliicoccus and Enterobacter* were dominant in this region. Some fungi identified in this region may affect human health. *T. minioluteus* produces secalonic acids D and F and mycotoxins, which may be harmful to human health. *Periconia* is responsible for eye infections. *Fusarium oxysporum* is non-pathogenic but may have evolved into a pathogenic form; for example, they can form associations from the roots of plants and can grow beyond the cortex, reducing water conduction capacity and hence causing wilting. *Cladosporium* is an allergen that mainly causes problems in patients with respiratory tract diseases. Reported bacteria are also showing a similar kind of effect.

In the present results, some of the microbes have a harmful effect on humans/animals and plants. The meteorological factors can play an important role in bacterial and fungal

growth that can enhance the impact of these bioaerosols. The loading of aerosols increases the microbial concentration within itself. No well-adopted standards are available for the microbial contaminants in the air like NAAQS (for particulate matter), so further studies are required to explore this field over the IGP and development of making standard and control measurements of pathogenic bioaerosols.