8.1 Summary and Future Scope

The research work is an attempt to investigate the bioaerosols estimation (mainly bacteria and fungi) in Varanasi (25.16 N; 82.59 E), which lies in the middle of the Indo-Gangetic plain. This is the first-ever reported study to measure the microbial content in this region over five consecutive years (2019-2024) at various locations, including indoor and outdoor environments. Because of the high pollution load in this region, special monitoring campaigns were conducted to study the intra-seasonal and seasonal variability of bioaerosols over the various sites. The episodic changes in the impact of meteorological variables on the bioaerosol properties were explored during the winter as well as all seasons for some sites. The variability in size, concentration, and health effect assessment for the anthropogenic sources, as well as antibiotic properties, were analysed. Along with these studies, atmospheric variability and survivability of various bioaerosols with environmental variables were also explored. The important findings of the present study are as follows:

- **Estimation of the total culturable bioaerosols in particulate matter ($PM_{2.5}$ and PM_{10})in the Middle IGP During Winter**

The concentration of PM_{10} and $PM_{2.5}$ was found greater than the recommended value of the National Air Quality Standards (NAAQS). As the particulate concentration increases, the biological particles associated with that also increase, but no universally acceptable standard is available for comparing the biological particles in the ambient air. The total microbes (TMC) in PM_{10} and $PM_{2.5}$ were in the typical range of 94-375 CFU/m^3. The bacterial bioaerosols (BA) were higher than fungal bioaerosols (FA), where bacteria and fungi contributed in the ratio of 4:1. Fungi, mainly *Aspergillus*, *Cladosporium*, *Penicillium* and bacteria *Bacillus*, *Mammaliicoccus* and *Enterobacter* were dominant in PM_{10} and $PM_{2.5}$ for this region. Some fungi identified in this region may affect human health. Detected microbes like; *T. minioluteus* produce 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.

- **Seasonal Variation of Bioaerosols in Outdoor Environment and Its Association with Meteorological Variables**

In the seasonal variation of the concentration and size distribution of the bioaerosols over the various outdoor sites in the Varanasi (Middle Indo-Gangetic plain), the concentration of bacterial bioaerosols were found much higher than the fungal bioaerosols in all the seasons and sites. This study observed that the maximum concentration of the fungal bioaerosols at agriculture (777±109 CFU/m^3) and dumping sites (1695±243 CFU/m^3) were seen during the summer, whereas for the traffic (1449±229 CFU/m^3), it was seen during the post-monsoon. The maximum concentration of Gram-negative bacterial bioaerosols (GNB) is 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 dumping site, it is highest in summer (956±149 CFU/m^3 and 2483±273 CFU/m^3). For all the three sites maximum concentration of Gram-negative bacterial bioaerosols (GPB) were found at the traffic site for all the seasons as compared to the other two. Moreover, its highest concentration were observed during the summer (1365±271 CFU/m^3). This concludes that the population residing there have a higher risk of bioaerosols-associated health issues summer and post-monsoon. The highest concentration of bioaerosols was found in the size range of 1.1- 2.1 µm that can reach the terminal lung bronchi in the human respiratory system. Thus, most of the allergic and immunotoxic fungi and bacteria were considered as possible threat to human lung terminal bronchi when breathed. In the biological characterization, many bacterial and fungal bioaerosols were observed, which can affect human health, specifically the immunocompromised body, via some allergic effects like skin infection, eye infection, respiratory issues, gastrointestinal problems, etc. In the present study, no significant correlation has been observed between bioaerosols and environmental variables. This may be because the various sources of emissions at the sampling sites may dominant over the environmental variables.

- **Bioaerosol Emissions from Anthropogenic Sources at Urban Environments**

The bioaerosols study related with in the treatment process of the waste involve various complex activity which act as a source of bioaerosols. The study of the wastewater treatment plant, findings shows that the aerosolization ratio (an indicator of bioaerosols produced from wastewater) of the bioaerosols in wastewater was higher during the summer as compared to the winter. During winter, bacterial concentration ranged between 484 to 1810 CFU/m^3, whereas fungal variations were in the range of 446 to 2162 CFU/m^3. In the summer the bacterial and fungal bioaerosols concentrations were higher than the winter and it was 544 to 2194 CFU/m^3 and 1356 to 2088 CFU/m^3 for bacterial and fungal, respectively. The flow rate intake of the wastewater in treatment plant contributes the rate of formation of bioaerosols. At the lower flow rate, the concentration of the bioaerosols was observed more in comparison to the higher flow because at the lower flow rate exposure and residence time of waste water to the atmosphere was hingh. It was found that some dominant strains of isolated bacteria were resistant towards the antibiotics cefixime and azithromycin. In the health survey of nearby areas, headaches were very prominent in each age group of the population, and people felt the change in the taste and smell. In the older population, more risk of skin diseases and respiratory problems was observed. As we move away from the source, a decrease in the pattern of health issues like eye infection, headaches, pulmonary issues, skin problems and changes in smell were observed.

Similarly, solid waste treatment facility also contributes in the formation of bioaerosols. At the waste treatment facility, the total bacterial concentration were found in the range of 3399 CFU/m^3 to 2579 CFU/m^3 while total fungal concentration varied from 2734 CFU/m^3 to 428 CFU/m^3 at various points including, entry, loading and unloading, leachate treatment and waste segregation point. Out of these sectors, bioaerosols concentrations were highest at the loading and unloading sections. The highest bacterial concentration was observed in size range between 0.65-2.1 µm, whereas fungi concentration was higher in size range 3.3-7 µm. The high abundances of bacteria and fungi in the fine to coarse particle size range suggest that there is a higher chance of exposure in respiratory system of workers of treatment facility and people residing nearby the plant. The biological characterization using metageniomics approach suggest that several pathogenic bioaerosols were observed which can be linked with 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 the antibiotics cefixime and 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.

- **Size-segregated bioaerosols concentration and characterization under diverse microenvironments**

The concentration of the bioaerosols was measured at seven different indoor locations within an urban ecosystem where the average concentration of bioaerosols was found to be highest at animal occupancy sites (cowshed, poultry) compared to human occupied places. The average concentration of bacterial bioaerosols ranged from 357±428 CFU/m^3 (laboratory) to 23693±3480 CFU/m^3 (Poultry) over all indoor site, whereas the average fungal bioaerosols concentration varied from 120±62 CFU/m^3 (laboratory) to 337±161 CFU/m^3 (Poultry). The concentration of bacterial and fungal bioaerosols were increases with increasing population at all the sites. In the size segregation analysis, different patterns were observed at various indoor environments. The prevalence of bacterial bioaerosol was found maximum at Size >7.0 µm while the minimum for size between 0.65-1.1 µm. For fungal bioaerosols, maximum concentration was noted within a range of 1.1 to 3.3 µm while the minimum was between 0.65 and 1.1µm. The size distribution of the bioaerosols, clearly indicate that they can reach the deep inside of the human respiratory systems through inhalation and according to their nature, they may affect human health. The diversity of the Gram-negative bacteria was found to be much higher in comparison to Gram-positive bacteria. Our results show that some of the identified microbes can produce harmful gastro and respiratory issues. However, routine cleaning of the area using disinfectant can protect us from these problems. Temperature and RH play an essential role in microbial growth. However, in this study, variation in temperature and RH did not show a significant contribution to the microbial concentration. This may be due to season-specific analysis, which resulted in low variations of ambient conditions in different sites. Other factors such as ventilation systems, movement of wind, human activities and the interior structure materials may also be the reason for the microbe's

release into the air. Thus, improving the ventilation system and maintaining hygiene through periodic cleaning were prescribed to reduce bioaerosol growth in the ambient environment.

- **Characteristics of the bioaerosols during Foggy and Non-foggy days of winter, meteorological implications, and health risk assessment**

Bioaerosols concentration may significantly affect in variable atmospheric conditions. This study found the bioaerosols (fungal and bacterial) concentration exceeded during the foggy days of winter. The concentration of bacterial bioaerosol was higher than the fungal bioaerosol during both foggy and non-foggy days of winter. Average concentration of bacterial and fungal bioaerosols were found in a ratio of 1:1.12 overall to the samples during the foggy days. The major portion of the bacterial bioaerosols found in the coarse size range in comparison to the finer range. Whereas, for fungi, there was very less variation observed in the coarse and fine range of the particles. As the PM concentration increases there, an increase in the microbial concentration was observed. This may be because of an increase in atmospheric loading. Meteorological variables also play an important role in transportation and the survival of the bioaerosols. Our study shows a positive correlation between the NOx, NH_3, and bioaerosol concentrations. This may be because some pollutant act as nutrient for the survival of microbes. So, the atmospheric pollutants also plays an important role in the variation of the bioaerosols concentration. The concentration and the biological nature of the bioaerosols may affect the human health. From the hazard ratio, it has been estimated that during both foggy and non-foggy days, significant health risks (>1) were observed. Further, bacteria shows more adverse effects compared to fungi, as estimated by higher hazard quotient value. In this region, bacteria such as *Bacillus, Enterobacter*, and *Coccus* were found during the foggy days of winter. In comparison, fungi, mainly *Aspergillus*, *Cladosporium* and *Penicillium,* were prominent during the foggy days of winter. In the present study, some of the detected microbes were reported to be harmful to human health. The symptoms of respiratory issues, eye irritation and skin irritation is very common issues caused by these microbes.

Future scope and recommedations

This study summarises the physicochemical and biological characterization and health risk assessment of bacterial and fungal bioaerosols in various indoor and outdoor environments, including the study of bioaerosols at variable atmospheric conditions (foggy and non-foggy days) and pollutants at the Middle Indo-Gangetic Plain. The emergence of the antimicrobial resistance in airborn microbes is very alarming, as shown in the study. Very few studies were reported on the bioaerosols in the Middle Indo-Gangetic Plain, including all the mentioned parameters. So more scientific research and implementations are still possible in this field such as:

- Using some advanced techniques, the overall microbial concentration can be estimated, including bacteria, fungi, pollen, viruses etc.
- The present data can be used as input for the various health risk assessment models, which can be used in the development of mitigation strategies and policies.
- Since the bioaerosols vary with the spatiotemporal distribution and environmental parameters, the real-time detection of bioaerosols needs to be done with a sensor-based system, and some simulation modelling and satellite-based data retrieval could be done in order to analyse their effect on human health, the atmosphere and climate.
- In the present results, some of the microbes have a harmful effect on humans/animals and plants. No universal-adopted standards are available for the total microbial or specific microbial contaminants in the air like NAAQS (for particulate matter and others), so further studies are required to explore this field over the IGP (high population and agriculture) and development of making standard and control measurements of pathogenic bioaerosols for humans/animals and plants.
- In spite of all, the study related to bioaerosols can give the inputs in achieving the three major Sustainable Development Goals (SDGs) including, Good health and well-being (SDG 3), Clean Water and Sanitation (SDG 6) and Sustainable Cities and Communities (SDG 11) while making the policies regarding achieving the sustainable development goals at local and global levels.