2.1 Preamble

Over the last few decades, several research groups of scientists have been exploring bioaerosols' origin, formation and dynamics. A wide range of tools and techniques were adopted, and various fundamental techniques were designed to determine the evolution, transport and kinetics of airborne particles. State-of-the-art laboratory techniques have evolved and are associated with field measurement via balloon, aircraft, and mobile collection techniques. Moreover, many campaign-based field studies were conducted in several geographical regions, including urban, forest, desert, and hilly areas. With time, instrumentation capabilities were improved, and they can provide real-time, size-resolved bioaerosol properties over spatiotemporal and vertical profiles. Additionally, the development of space technologies and remote sensing techniques is now able to capture global biogenic emissions on a much finer spatial and temporal scale. Key research areas of bioaerosols include physico-chemical and biological properties of bioaerosols, cloud interactions, health impacts, detection via satellite remote sensing, transboundary movements, anthropogenic bioaerosol emissions, etc.

The present section highlights the recent developments in detection technologies, the understanding of bioaerosol mass loading, biological characterization, trends, and source measurement around the world, and includes several indoor and outdoor locations at Middle IGP. The effect of atmospheric variability and pollutant on the bioaerosols were also highlighted in this chapter. The review of literature is comprehensive by means of a specific understanding that there is a research gap that still needs to be addressed, especially in the regional context. The review unfolds the various studies done on the international and national levels including IGP.

2.2 Sampling, identification and characterization of bioaerosols

There are several conventional methods and techniques available and used to collect the bioaerosols sample (Reponen et. al., 2009). However, no such standard protocols for collecting bioaerosol due to considerable variation in its properties (Grishpun et. al., 2007). Sampling methodology often depend on the objective of the study and are framed for specific sources of bioaerosols. Samples of biological particles need more standard handling procedures than those of other inert particles. Particle size plays a crucial role in selecting the sampling procedure for all airborne particles (Nicholson, 1995). Variation

in size is no longer exclusive to biological aerosol sampling. Because of the complex interaction between biological cells and environmental factors like temperature and relative humidity, size and mass are difficult to predict (Reponen et. al., 1996).

Furthermore, certain biological particles (e.g., chain aggregation of fungus spores) can provide considerably extended morphologies that significantly differentiate physical and aerodynamic dimensions (Reponen et. al., 2001). The collection of viable material is more tricky as it demands the preservation of live cells appropriately to survive until cultivation to perform the desired analysis. Certain bacteria and fungi are easier to culture than others. It also provides information about particle survivability under difficult conditions (for example, prolonged sun exposure and high airflow). When bacteria were impacted excessively hard onto collection substrates during sampling, they were more likely to be damaged (Stewart et. al., 1995).

2.2.1 Sampling and collection technique of bioaerosols

Inadequate design of a sampling procedure results in biases in qualitative and quantitative understanding of bioaerosols, primarily due to loss of particles within the inlet, inlet lines and instrument (Zimmerman et. al., 1987). Along with these, large particles (size >5mm) are more influenced by the loss of inertial surface impaction and gravitational settling. In contrast, small particles (size <0.1mm) are most susceptible to electrostatic forces and Brownian diffusion. The study also indicates that the particle's losses to the surface are due to air turbulence because it affects particles of all sizes (Wang et. al., 2001). So there is a need for well-scrutinised measurement of bioaerosols, and the lower limit value of their size can be taken to compare with non-biological particles. Biological particles (e.g., viruses, bacteria) acquire bigger sizes when accumulating on larger and non-biological particle surfaces, like the dust of minerals.

Bioaerosol sampling techniques are diverse, with each method offering specific advantages and limitations. Impaction-based samplers, such as Andersen multistage impactors, are widely used and cost-effective but limited to culturable bioaerosols and affected by wind speed (Lindsley et. al., 2017; Mohammed et. al., 2023). For the purpose of sampling bioaerosols, many cascade impactors (multiple stages) have recently been designed and put into service. The Anderson sampler (Andersen, 1958) is the most widely used sampler for bioaerosol research. The microorganism culture, which collects particles

of six distinct sizes in six phases, is a good fit for the Anderson sampling method. For every plate berner impactor used in Anderson sampling, the inner jet sampling efficiency differs from the outer jet (Hillamo & Kauppinen, 1991). The Burkhardt spore trap was used to collect certain bioparticles, such as pollen and fungal spores (Hirst, 1952). Air is introduced to the prevailing wind through a tiny gap in a chamber in sampler. The sample is continually collected for up to a week before being examined under a microscope.

The use of an impinger to capture bioaerosol particles into a liquid medium improves collection and allows for longer sample times with various agar media (Crook, 1995a). The AGI-30 has been the favoured sampler for obtaining live organisms (Sajjad et. al., 2023; Lee et. al., 2023). Other inertial samplers, such as rotary arm samplers and centrifugal cyclones (both single and multiple-stage), are used to gather PBAP (e.g., IPCC 2007b). Liquid-based impingers reduce physical stress on microorganisms and avoid overloading but face challenges like evaporation and the need for sterilization (Tseng et. al., 2014; Skjøth et. al., 2022; Wu et. al., 2013).

Some non-inertial sample methods, including as filtration and passive settling, have been used for PBAP collecting. Depending on the sample type, several filter substrates are utilized, such as fibrous, flat (e.g., NucleoporeTM), and membrane (Crook, 1995b). Filtration methods are economical and adaptable, though filter overloading and desiccation can reduce efficiency (Burton et. al., 2006; Li et. al., 2018). Gravity-based techniques, while simple and low-cost, are less sensitive and favour larger particles (Einstein et. al., 2012; Frankel et. al., 2012). Cyclone samplers enhance collection efficiency but are unsuitable for liquid media (Macher et. al., 2008). Electrostatic (Tan et. al., 2011) and thermal precipitators are still the most advanced techniques for collecting viable aerosols with a low risk of damaging the samples. Nowadays, various additional techniques, including optical methods, are being used for the online detection of bioaerosols (Kumar et. al., 2024). Electrostatic methods improve recovery efficiency but may generate harmful ozone (Therkorn et. al., 2017). Thermal precipitators and condensation techniques are effective for small particles and ultrafine detection, respectively, but require careful handling (Cox & Wathes, 1995). Each method's suitability depends on the research and environmental conditions. A brief account of the various methods used for bioaerosol sampling and their advantages and limitations are presented in Table 2.1.

Table 2.1 Bioaerosols sampling techniques and their advantages and limitations

Sl. no.	Sampling techniques	Advantages	Limitations	Examples of sampler	References
1.	Impaction based	Economically feasible and widely used Samples are collected and directly sent to an incubator. Not required post-sampling process Smaller nozzles for higher velocity lead to the collection of fine particles	Applicable only for the culturable enumeration method Difficult to use at high contamination site Smpling efficiency dependent on wind speed	Andersen impactors, rotating arm collectors, spore traps	Crook (1995); Ghosh et. al. (2015); Lindsley et. al. (2017)
2.	Impingement or liquid-based sampler	Less sample loss Not affected by physical stress and overloading of microbes Portable and disposable Easy enumeration	Complex sterilization and post sampling process Loss of sample media due to evaporatio Smpling efficiency dependent on wind speed The size distribution of the sample is difficult to distinguish.	All-glass impingers (AGI), liquid-based swirling air sampler, Burkard Multi-Vial Cyclone Sampler, Burkard multistage liquid impinger	Tseng et. al. (2014); Wu et. al. (2013)
3.	Filtration or filter-based sampler	Convenient and economic Good for microscopic Not affected by heating and ventilation Easy to handle and move Any enumeration techniques can be used after sampling Size of the particle can easiliy identified	Post-processing is required for quantification of sample Overloading of sample at higher contaminated sites Desiccation of collected sample reduced the efficiency Smpling efficiency dependent on wind speed	Polycarbonate, gelatin, flat filters, such as Nucleporepolyvinyl chloride (PVC), polytetrafluoroethylene (Teflon, PTFE), nylon	Burton et. al. (2006); Choi et. al. (2018); Crook (1995); Li et. al. (2018)
4.	Gravity	Simple and low cost Not dependent on flow rate Good results	Not universaly accepted Affected by air current Dominated by lard particle Particle concentraion not comparable to other methods Large time required		Cox & Wathes (1995); Frankel et. al. (2012)
	Einstein-Lioy Sampler	Use four filter simultaneously Less affected by wind	Only 37 mm filter samplers can be used		Einstein et. al. (2012)
	Durham-type Passive Spore Trap	Used for long-term outdoor sampling	Secures prepared slide samplers for		Durham (1946); Serrano-Silva & Calder´on-Ezquerro (2018)

			convenient long-term sampling for microscopic analysis		
	Remote Airborne Microbial Passive (RAMP) Sampler	Can used for high altitude bioaerosols collection by ballon Light weight design	No reference sample used in testing and comparision		Spring et. al. (2018)
	Personal Aeroallergen Sampler (PAAS)	Can used by individual in exposure assessment	A passive personal sampler to directly measure personal exposures.	Low sensitivity at low bioaerosol concentrations. Similar to all gravity-based samplers, particle sizes under 5 μm are under-sampled.	Yamamoto et. al. (2006); Yamamoto et. al. (2011)
5	Cyclone	Collection efficiency reduced by bouncing of particles Easily sterilized.	Limited to liquid media due to evaoporation loss	NIOSH Bio Sampler, Burkard cyclone	Macher et. al. (2008); McFarland et. al. (2010)
6.	Electrostatic precipitation/collector	High collection efficiency Multiple collection media can be used Low pressure drop Less loss of sample due to lower deposition velocity Highly feasible for bioaerosols if operated at low voltage	Limited study reported Production of ozone during process which have exposure risk	Electrostatic Aerosol Sampler, Rutgers Electrostatic Passive Sampler (REPS), Electrostatic dust fall collectors (EDC)	Therkorn et. al. (2017); Zhen et. al. (2013)
	Electrostatic Dustfall Collectors (EDCs)	Good efficiency for collection of fine particulate	It is difficult to remove particles from the collected material. May lose charge over time	. Swiffer® or Pledge® Grab-It™.	B¨ohlandt et. al. (2016); Cozen et. al. (2008); Kristono et. al. (2018); Madsen et. al. (2012); Noss et. al. (2008); Viegas et. al. (2018)
	Rutgers Electrostatic Passive Sampler (REPS)	The electrostatic pull may help to capture more particles than gravity alone. Easy transportation and elution in standard sterile conical centrifuge tubes.	Large amount of samples required		Metaxatos et. al. (2022); Therkorn, Thomas, Calder´on, et. al. (2017); Therkorn, Thomas, Scheinbeim, & Mainelis (2017)
7.	Thermal precipitator	Good collection efficiency for fine particle No vaccume is needed	Small collection surface Collection rate is low Microbial viability affected by higher temrerature	Peltier thermoelectric heat pump.	Cox & Wathes (1995); Wu et. al. (2013)
8.	Condensation technique	Quicker method Ultrafine particle can be detected Good for viable microbes	Handling need expertise		Cox & Wathes (1995)

Several sampling methods have been discussed in various studies. Three main collection methods, impaction, impingement, and filtration, are used to sample biological particles (Grinshpun et. al., 2007). Other collection techniques, such as cyclone, gravity sampling, and electrostatic precipitation, are also used for bioaerosol sampling. Among all, most of the bioaerosol measurement devices are based on inertial forces for collection and sampling.

2.2.2 Identification and characterization of bioaerosols

The characterization techniques include mass spectrometry, optical spectroscopy, immunological procedures, culture and staining methods, light and electron microscopy, chemical tracer analyses, DNA sequencing, and more (Carestia et. al., 2015; Ghosh et. al., 2015; Laskin et. al., 2016; Oteros et. al., 2015; Valsan et. al., 2015; West et. al., 2016). The enumeration techniques for the viable and non-viable biological particles are an integral part of monitoring. This technique can be classified into two broader categories: the culturable and non-culturable groups (Feng et. al., 2024). Table 2.2 discusses the advantages and disadvantages of enumeration techniques.

Non-cultural methods are widely used for their ability to analyze microbial samples without requiring cultivation. Classical microscopy is a cost-effective and user-friendly approach. However, its inability to represent the full microbial diversity and its poor measurement precision limit its broader applicability (Ghosh et. al., 2015). Laser-induced fluorescence (LIF), on the other hand, is a highly sensitive technique that enhances spatial resolution through laser technology. Despite these strengths, its quantification accuracy is hindered by fluorescence variability and photochemical effects (Cabredo et. al., 2009). Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) is another non-cultural technique, praised for its cost-effectiveness, sensitivity, and ability to analyze mixtures through mild ionization (Shen et. al., 2024; Chudzik et. al., 2024). However, it is constrained by its dependency on compound databases, its limitations in handling compounds larger than 600 Da, and its relatively low resolution unless enhanced by specialized techniques (Madsen et. al., 2015). Similarly, Laser-Induced Breakdown Spectroscopy (LIBS) simplifies sample preparation, enabling rapid and sensitive multi-element analysis (Müller et al., 2023). However, its high cost, potential interference effects, and precision challenges restrict its usability (Saari et. al., 2016; An et. al., 2024).

Table 2.2 Enumeration techniques of bioaerosols with their advantages and limitations

Methods	Bioaerosols sampling technique	Advantages	Limitations	Reference
Non-Culturable	Classical microscopy	Low-cost and easy to handle Identification of specific taxa of microorganisms	Only used to identify specific taxa of species Cannot provide representative microbes in the sample Poor precision	Ghosh et. al. (2015)
	Laser Induced Fluorescence (LIF)	Sensitive measurements Enhancement of spatial resolution using a laser	Quantification is difficult because of collision quenching of potential photochemical state and excited state Improper measurement due to different fluorescence of exciting species	Cabredo et. al. (2009)
	Matrix-assisted laser desorption Ionization-Time of flight (MALDI-TOF)	Cost-effective and easy to handle Highly sensitive Mild ionization techniques provide analysis of the mixture	Require database for analysis of compound The strong matrix signal in this method limits its application for compounds less than 600 Da Only a reflector or delayed extraction can improve a limited resolution approach	Ghosh et. al. (2015); Madsen et. al. (2015)
	Laser-induced Breakdown Spectroscopy (LIBS)	Simple sample preparation, greater convenience and less risk of contamination Required very low amount of samples Highly sensitive Multi elements analysis can be done simultaneously Can directly detect the aerosols	High cost and complex system Suitable standards are not very easily available More interference effects Less precision (between 5 to 10%)	Saari et. al. (2016)
Culturable	Colony counting method	Easy to perform Easy isolation of microbes	Microorganisms accumulate unevenly so they affect the results in the bioaerosols analysis	Ghosh et. al. (2015)
	PCR technique	Can apply on any biological matter containing nucleic acid Identification and Detection are carried out independently Low risk of contamination Provide results in few hours or day Highly sensitive	Size dependent of bioaerosols Inhibitory PCR compounds in the samples affect the results	Lee et. al. (2010); Unterwurzacher et. al. (2018)
	Flow cytometry/Solid-phase cytometry	Similer to epifluorescence microscopy	Similer to epifluorescence microscopy	Després et. al. (2007)
	Next-generation sequencing (NGS)	Can apply on any biological matter containing nucleic acid Fast sequencing technique compared traditional methods	Higher experimental setup cost Take large time to run Do not provide phylogenetic characterization	Fröhlich-Nowoisky et. al. (2016)
	Biomarkers	Taxa of microorganisms can be identify Only fraction of the sample required	Unavailibility of standard approach Affected by dust and other microorganism	Cartwright et. al. (2009); Ghosh et. al. (2015)
	Denaturing gradient gel electrophoresis (DGGE)	Can analysed multiple samples simultaneously Monitoring of bacterial community shift with time With DNA sequencing, its variation is very sensitive Using universal primers, microbial community of unknown species can be analysed	Time consuming process Overestimate the diversity of microbial community Only provide semi-quantitative information. Do not provide phylogenetic characterization	Xu & Yao (2013)

Advanced genomic methods like Next-Generation Sequencing (NGS) have revolutionized bioaerosol studies by offering rapid DNA and RNA sequencing capabilities for diverse biological samples (Zang et. al., 2024). However, the method's high cost and lengthy experimental processes, sometimes spanning several days, pose significant limitations (Fröhlich-Nowoisky et. al., 2016). Biomarkers also present an innovative approach, allowing for the identification of both culturable and nonculturable microorganisms without requiring growth. Despite these benefits, the lack of standardized methods and susceptibility to interference from environmental factors like dust limit their effectiveness (Cartwright et. al., 2009; Ghosh et. al., 2015). Lastly, Denaturing Gradient Gel Electrophoresis (DGGE) excels at monitoring shifts in microbial communities over time and analyzing multiple samples simultaneously (Chakraborty et. al., 2023). However, it is time-consuming and prone to overestimating community diversity due to the micro heterogeneity of ribosomal RNA (Xu & Yao, 2013).

In contrast, cultural techniques rely on the growth of microorganisms for enumeration. The colony counting method is simple and effective for isolating microorganisms in liquid media but suffers from inconsistencies caused by uneven microorganism distribution during sampling (Ghosh et. al., 2015). Polymerase Chain Reaction (PCR) offers a highly sensitive approach to identifying nucleic acid-containing microorganisms, producing results within hours to days (Khaleque et. al., 2024) However, its accuracy is heavily dependent on proper sample preparation and may be compromised by inhibitory compounds or incomplete characterization of bioaerosol size (Lee et. al., 2010; Unterwurzacher et. al., 2018).

Both non-cultural and cultural techniques are indispensable in bioaerosol research, offering complementary strengths for specific analytical requirements. While non-cultural methods provide rapid and often highly sensitive results, cultural techniques remains vital for certain types of microbial isolation and growth studies (Nanchi et. al.,2022). Each method's selection should be guided by the study's objectives and the trade-offs between precision, cost, and feasibility.

Various advanced techniques are now being used in altrernate of optical microscopic in order to improve the accuracy in measurement of intracellular measurement (Cremer, 2012). In fluorescence imaging spectroscopy, fluorescence emission spectra shows the three-dimensional spectra with wavelength and provide the steady-state autofluorescence

properties of the particular sample. In fluorescence microscopy of bioaerosols, the autofluorescence properties of biological compounds or fluorescent stains are used. Laser/light-induced fluorescence (LIF), instruments have been designed so that it can distinguish biological content and easily detect pathogens (Huffman et. al., 2016; Müller et. al., 2023; Zhu et. al., 2022). For the rapid detection of biological particles, mass spectrometry (MS) is videly used (Krásný et. al., 2013). Aerosol mass spectrometry gives the information of a single particle that helps to analyze spatial variability and organic aerosol dynamics (Bozzetti et. al., 2016). MS works as the chemical tracer detector for molecules and particles (e.g., cellulose and lipids) that could not be detected by fluorescence techniques or sequencing (Zhang et. al., 2015).

Recent developments of microbiological techniques give the option of biological particle analysis. The different microbial species are grown on suitable media and then separated using the continuous dilution technique for microbial analysis (Feng et.al., 2024). In the traditional approach, only a small fraction of species are isolated and identified using the DNA sequencing technique by comparing the obtained sequences with the available sequences of online databases on the National Center for Biotechnology Information (NCBI) (Urbano et. al., 2011). However, the researchers show that a large number of bacterial species (Lewis, 2009) and fungi (Bridge & Spooner, 2001) may present as bioaerosols in the environment. With the advancement in microbial analysis techniques, it is possible to identify individual genera or species.

Next Generation Sequencing (NGS) technologies have been used in place of conventional sequencing-based bioaerosols analysis (Schmidt et. al., 2013; Seifried et. al., 2015). NSG can provide sequences longer than 400 bp (base pair) (Schmidt et. al., 2013; Seifried et. al., 2015) and used in analysis of genomics and transcriptomics (RNA analysis) for several bioaerosols studies (e.g., Seifried et. al., 2015; Womack et. al., 2015). NSG technique provides the sequence data of the relative abundance of the particular texa in the entire sequence. In contrast, polymerase chain reaction (PCR) is used to determine the absolute concentration of specific texa (Dannemiller et. al., 2014). Moreover, quantitative PCR (qPCR) has been used for the quantitative analysis of species, or fungi, genera, archaea, and bacteria individualy (Casabianca et. al., 2013; DeLeon-Rodriguez et. al., 2013; Lang-Yona et. al., 2014; Müller-Germann et. al., 2015; Chen et. al., 2024).

Most of the current technologies are not very suitable in terms of field application and quantitative analysis. The adenosine triphosphate (ATP) bioluminescence assays are the most affordable and highly efficient technique to capture bioaerosols and can be combined with qPCR to identify specific bacterial/fungal species (Zhang et. al., 2022). LIF and UV-LED-based bioaerosols are comparably less expensive and non-invasive (Zang et. al., 2022; Zhu et. al., 2022). In the costlier range, the Raman spectroscopy and bioaerosol mass spectrometry (BAMS) techniques can be used for the detection and construction of libraries and databases for elucidating spectrally integrated signals in order to improve the measurement of selective bioaerosols (An el. al., 2024).

2.3 Bioaerosols at various outdoor sites

The first investigations of the occurrence and dispersion of bioaerosols (like microorganisms and spores) in the air were studied in the early 19th century (Ehrenberg, 1830). The bubonic plague, which was caused by the bacterium Yersinia pestis and probably contributed to the Black Death that killed at least one-third of Europe's population in the fourteenth century, was observed to have detrimental health effects and was addressed (Imshenetsky, et al., 1974). Humans can contract bubonic plague through coughing, however it is a zoonotic illness that is passed from animals to people. A potato blight produced by the fungus-like microbe Phytophthora infestans, which can spread quickly through the air, was the cause of the malnutrition that led to the Irish potato famine in the middle of the 1800s, which resulted in one million fatalities and widespread emigration.

The Spanish flu pandemic of 1918–1920, which killed 40–50 million people, was by far the worst in history. The 1957 Asian flu, the 1968 Hong Kong flu, and the less deadly but more recent Swine flu in 2009 are also well-known, each of which killed roughly a million people. Numerous significant influenza epidemics have been documented during the past century (Haench S. et al., 2010). If the highly virulent H5N1 virus, sometimes known as bird flu, develops the ability to transmit from person to person in the air, there will likely be additional influenza pandemics in the future. Since then, research on bioaerosols has advanced significantly, and air samples taken using airplanes, balloons, and rockets have demonstrated that bioaerosols released from the ocean and land surfaces can travel great distances and reach extremely high altitudes, such as between continents and outside of

the troposphere.(Smith et. al., 2013). Apart from this COVID-19 is the most recent example of distructive bioaerosols for humans.

The concentration and diversity of the bioaerosols varies with locations and seasons either in Indoor or in outdoor environment. In outdoor study, Breza-Boruta et al. (2012) analysed bacterial and fungal concentrations in the municipal waste disposal complex, where microbial concentration was highest at the sorting station and the active sector of the landfill poses a serious threat to the health of workers and residents of neighbouring areas. The dominant species of bacteria and fungi in landfill sites are *Enterobacteriaceae, Staphylococcus aureus*, *Clostridium perfrigens*, *Acinetobacter calcoaceticus* and *Aspergillus fumigatus* which are in respirable ranges and, therefore, can cause serious respiratory diseases (Nair, 2021). The fungal bioaerosols concentrations in and around the landfill sites varies directly with temperature and wind speed and inversely with humidity. The spatiotemporal variation of fungal species *Alternaria* and *Fusarium* were reported in spring, summer and winter, while *Cladosporium* was the highest in autumn and winter seasons (Srivastava et. al., 2021). Ray et al. (2005) investigated the respiratory health of workers in landfill sites. The study reported that respiratory diseases, fungal infections, and burning sensations are predominant, with the help of a questionnaire, survey, clinical examination, and laboratory investigation. The details of various bioaerosol studies over outdoor and indoor sites are summarised in Table 2.3 and Table 2.4, respectively.

In Maharashtra, India, viable bacterial bioaerosols at a waste treatment facility ranged between 3.8×10^3 and 1.2×10^5 CFU/m³. The majority of these bioaerosols, primarily within a particle size range of 0.65–2.1 µm, are capable of penetrating deep into the respiratory tract, posing significant respiratory health risks (Pahari et. al., 2016). At Ojota dumpsite in Nigeria, bacterial concentrations reached 2189 CFU/m³, and fungal bioaerosols, including *Aspergillus fumigatus*, were also prominent. Chronic respiratory conditions such as asthma and coughing were associated with these bioaerosols (Akpeimeh et. al., 2019). Also, some studies reported bioaerosols and ambient meteorology over Kanpur, in Northern India (Rajput et. al., 2017).

Table 2.3 Bioaerosols at various outdoor sites (collection techniques, concentration and health effects)

S. No.	Location	Species	Concentration	Instruments/ Techniques used	Health Effects	References
1	Municipal Waste Disposal Zolwin-Wypaleniska, Bydgoszczy.	Fungal Species: *Aspergillus niger, , Arthrinium phaespermum, Epicoccum nigrum, Penicillium notatum, Rhizopus nigricans, Rhizopus oryzae, Sclerotinia sclrotorium*	~54200 CFU/m^3 at waste disposal	Microbiological Air Sampler MAS-100 Eco by Merck.	High concentration of potentially pathogenic bacteria. The toxinogenic fungi cause potential health hazards to workers, passing and nearby population	Breza-Boruta et. al. (2012)
2	Landfill sites worldwide.	Bacterial Species: *Bacillus, Clavibacter, Corynebacterium, Curtobacterium, Micrococcus, Pseudomonas and Staphylococcus* Fungal Species: *Alternaria, Aspergillus Cladosporium and Penicillium*	Bacteria: 1.34×10^2 to 5.38×10^4 CFU/m^3 Fungi: 1.21×10^2 to 1.829×10^4 CFU/m^3	Six-stage cascade impactors	Solid waste workers commonly experience respiratory symptoms, lung function, skin and eye irritation, diarrhea, nausea, weariness, muscular discomfort, headache, joint pain, chills, fever, cough, and chest tightness.	Nair (2021)
3	The landfill site was located in the city of Chandigarh, India.	Bacterial Species: *Firmicutes, Actinobacteria and Proteobacteria, Aerococcus, Brevibacillus, Cohnella, Lysinibacillus, Oceanobacillus, Ornithinibacillus, Paenibacillus, Paenisporosarcina, Staphylococcus, Streptococcus, Desulfotomaculum, Clostridium*	~0.15-1.9 × 10^7 CFU/m^3	Hallow-stem auger technique	ND	Krishnamurthi & Chakrabarti (2013)
4	Okhla Landfill site in the southeast of Delhi, India.	Fungi: *Penicillium, Aspergillus* (all season) and *Rhizopus, Alternaria* and *Fusarium* in spring, summer and winter. *Cladosporium* in autumn and winter.	Fungi: 1081(monsoon) and 346 (winter) CFU/m^3.	Anderson six stage viable cascade impactor	*Aspergillus produces a mycotoxin known as Ochratoxin A and Aflatoxins. Ochratoxin A is a possible carcinogen in humans. Aflatoxin is hazardous to both human and animal health. Aspergillus flavus causes aspergillosis. It invades the arteries of the lungs and the brain, causing infarction.*	Srivastava et. al. (2021)
5	Waste treatment facility (WTF) in Maharashtra, India	Bacteria: *Bacillus, Streptococcus, Staphylococcus, Acinetobacter and Kocuria*	Bacteria: 3.8×10^3 to 5.4×10^4 CFU/m^3 (at NA) and 9.8×10^3 to 1.2×10^5 CFU/m^3 (at AIA)	Anderson-six stage impactor	The 66–84% of bacterial bioaerosols were associated with coarse airborne particles greater than 2.1 µm causing respiratory diseases	Pahari et. al. (2016)
6	The open dumpsite used in this study is situated in Ojota, Lagos State Nigeria.	Total fungi: *Aspergillus fumigatus*	Total bacteria: 2189 CFU/m^3 (highest), Total fungi: 843 CFU/m^3 (highest)	A six-stage Andersen sampler and activity-based personal sampling using a body-worn SKC button sampler	Chronic cough, chronic phlegm, asthma, wheezing, coughing	Akpeimeh et. al. (2019)

7	The landfill site at Okhla in southeast Delhi	Bacteria: *Ascaris lumbricoides, Entamoeba histolytica, Klebsiella*, and *Escherichia coli*		Electronic spirometer (Spirovit SP-1 Schiller, Switzerland)	When compared to controls, landfill workers had lower haemoglobin levels but higher total WBC, eosinophil, and monocyte counts. Palpable liver, gum infection, frequent diarrhoea, fungal infection of the skin, cuts and pricks on the body surface, ulceration of the skin of the covered body parts, including external genitalia, and hazy eyesight were more common in landfill workers than in controls.	Ray et. al. (2005)
8	Bioaerosol sampling was performed at Jawaharlal Nehru University (JNU), located in New Delhi,	Fungi: *Aspergillus (37%), Alterneria, Ulocladium and Rhizopus.* Bacteria: *Bacillus (35.9%), Coccus Palisade, Streptobacillus and Streptococcus*	The concentration of gram-positive bacteria (GPB): 63.6 to 338.8 CFU/m^3. Gram-negative bacteria (GPB): 159 to 614 CFU/m^3. Fungi: 755 to 1293 CFU/m^3.	Six-stage viable cascade impactor sampler	Respiratory diseases-coughing, wheezing, asthma, bronchitis, skin diseases, eye irritation	Srivastava et. al. (2012)
9	Different sites (that includes one indoor and three outdoor sites) in Delhi namely Garbage site, Jhelum mess, Munirka and Kaushambi	Gram positive bacteria, gram negative bacteria Fungi: *Penicillium sp., Alternaria sp.* and *Aspergillus sp.*,	At all four sites (maximum): Gram-positive bacterial concentration: 2790.6–9428.3 CFU/m^3 and gram-negative bacterial concentration: 1990.3–7609 CFU/m^3 Fungal concentration: 1740.5–3224.7 CFU/m^3,	A six-stage viable cascade impactor sampler	Different aerodynamic diameter bioaerosols affects different parts of human respiratory system leads to diseases like bronchitis, asthma etc	Lal et. al. (2017)
10	Laogang Landfills, China (largest landfill in Asia)	Bacteria: *Massilia sp., Methylobacterium sp.,* Fungi: *Methylorubrum sp. and Noviherbaspirillum sp.*	Bacteria: 33–22778 CFU/m^3 and Fungi 8–450 CFU/m^3	Total suspended particles samplers	The health risks from inhaling were approximately four orders of magnitude greater than those from cutaneous contact.	Yang et. al. (2022)

*ND: Not defined

Priyamvada et al. (2018) found some bacteria in indoor bioaerosols that are different from the outdoor bioaerosols. In central India, the average monthly concentration of the fungal bioaerosols ranged from 550 to 7363 CFU/m^3 during 2007-2009 reported by Kumar et al. (2019). In the study of Delhi sewage workers, they were exposed to tuberculosis bacteria arising from the sewage in the form of bioaerosols (Chandra et. al., 2019). Morakenyo et al. (2019) find the concentrations of $PM_{2.5}$ and their inhalation health risks in an industrial proximity in South Africa, including bacterial and fungal bioaerosols. In their observation, the bioaerosols (bacterial and fungal) concentrations in $PM_{2.5}$ were significantly lower in winter than in the summer season while the bacteria diversity in summer were similar to found in winter. In addition, inhaled dose of bioaerosols (bacterial and fungal) were higher in children than adults via particulate matter. So children are at the major risk of bioaerosols exposure.

Bioaerosol studies conducted across various indoor sites reveal significant insights into microbial composition, environmental factors, and associated risks. At a dental office in Lublin, Poland, the primary microorganisms identified included *Cladosporium spp*., *Aspergillus terreus*, and catalase-positive and catalase-negative Gram-positive cocci. The concentrations ranged from 176–372 CFU/cm^3 for bacteria and 81–137 CFU/cm^3 for fungi, captured via passive sampling techniques (Polednik, 2021). In tertiary care hospitals in Riyadh, Saudi Arabia, bioaerosol samples from pediatric intensive care units (PICUs) were analyzed. Gram-positive bacteria and fungi like *Alternaria spp., Aspergillus spp.,* and *Penicillium spp.* were dominant where bacterial concentrations ranged between 4–2425 CFU/m^3, while fungal levels were 1–260 CFU/m^3, with variation across hospitals (Alghamdi et. al., 2023). Operating rooms in Shariati Hospital, Karaj City, Iran, were studied for bioaerosols during various surgical procedures, including eye, orthopaedic, and cesarean sections. Common bacteria included *Staphylococcus aureus, Bacillus subtilis,* and *E. coli,* while fungal species included *Cladosporium* and *Penicillium* (Tolabi et. al., 2019). Bioaerosol samples from male and female surgery and medicine wards in southern Thailand revealed Gram-positive cocci and fungi like *Aspergillus spp*. and *Alternaria spp.* Bacterial concentrations ranged from 335–928CFU/m^3 via bioaerosol sampling and 209–592CFU/m^3 using the open plate method here fungal concentrations were 134–487CFU/m^3 and 18–87CFU/m^3, respectively (Onmek et. al., 2020).

Table 2.4 Bioaerosol studies conducted across various indoor sites reveal significant insights into microbial composition, environmental factors, and associated risks.

	Location	Site	Analysis	Media used	Methods	Concentration	Environmental factors	References
1.	Lublin, Poland	Dental Office	Bacteria: *catalase positive,catalase-negative, Gram-positive cocci.* Fungi: *Cladosporium species, Aspergillus terreus.*	Bacteria: TSA Fungi: SDA with chloramphenicol	Passive sampling	Bacteria: 176 to 372 CFU/cm^3 Fungi: 81 to 137 CFU/cm^3	ND	Polednik (2021)
2	Singapore	Library (within occupied space)	ND	ND	Anderson six stage cascade impactor Cultivation	Bacteria: 727.0-3651.4 CFU/m^3 Fungi: 34.2-64.4 CFU/m^3	ND	Goh et. al. (2000)
3	Ankara, Turkey	Office	Bacteria: *Bacillus, Micrococcus Staphylococcus* Fungi: *Aspergillus Cladosporium Penicillium Ulocladium*	ND	Anderson sampler Cultivation automatic counter microscopy	Bacteria: 44-284 CFU/m^3 Fungi: 18-274 CFU/m^3	ND	Mentese et. al. (2009)
4	New Delhi, India	Laboratory (university)	Bacteria:*Bacillus, Palisade Streptococcus Coccus* Fungi: *Aspergillus Rhizopus Alternaria*	ND	Six stage cascade Sampler, Cultivation microscopy	Bacteria: 21-54 CFU/m^3 Fungi: 0-350 CFU/m^3	ND	Srivastava et. al. (2012)
5.	Tertiary care hospitals in Riyadh, Saudi Arabia	Paediatric intensive care units (PICUs) in Two Hospital A and B	Bacteria: gram-positive bacteria, bacterial flora, gram-negative bacteria Fungi: *Alternaria spp and Aspergillus spp., Penicillium spp., Monilia spp*	Fungi:SDA Bacteria: BA	Air Sampling, Spin air IUL sampler	Hospital A: Bacteria 4 – 2425 CFU/m^3; Fungi 1 – 100 CFU/m^3 Hospital B: Bacteria 20 – 390 CFU/m^3; Fungi 1 – 260 CFU/m^3	ND	Alghamdi et. al. (2023)
6	Shariati Hospital in Karajcity, Iran	Operating rooms Eye surgery, Orthopaedic surgery, Internal surgery, Cesarean section	Bacteria:*S.epidermidis, S. aureus, B. subtilis, Lactobacillus, diphtheriae, E. coli* Fungi: *Cladosporium, Aspergillus, Penicillium*	Fungi: SDA Bacteria: TSA	Passive sampling	Eye surgery: Bacteria CFU/Plate 0.20 to 3.40; Fungi CFU/Plate 0.40 to 2.40 Orthopedic surgery: 0.08 to 4.00; 0.6 to 2.38 Internal surgery: 0.25 to 5.50; 0.50 to 2.75 Cesarean section: 0.50 to 4.00; 0.50 to 2.00	ND	Tolabi et. al. (2019)
7	Large hospital center in	Male surgery ward, female surgery ward, male medicine	Gram-positive cocci, rod-shaped bacteria and Gram-negative bacteria	Bacteria: TSA Fungi: MEA	Bioaerosol sampling and open plate method	Bioaerosol sampling: Bacteria 335.5 to 928.89 CFU/m^3; fungi 134.50 to 487.22 CFU/m^3	Temp. 25.4–30.3°C RH - 72%	Onmek et. al. (2020)

	southern Thailand	ward, and female medicine ward	Fungi: *Aspergillus spp., Penicillium spp., Cladosporium spp., Alternaria spp., and Curvularia spp*.			Open plate	209.69 to 592.06 CFU/ m^3	18.82 to 87.50 CFU/m^3	
8.	Hamedan University of Medical Sciences, Hamedan	6 wards from Five Educational Hospital	Fungi: *Penicillium spp., Cladosporium spp., A. fumigatus, A. niger*. Bacteria : *coagulase-negative staphylococci, Bacillus spp., Micrococcus spp., and Staphylococcus aureus*	Fungi: SDA Bacteria: BA	Filtration method	Average Bacteria - 160.6 CFU/m^3 Average Fungi - 12.56 CFU/m^3			ND	Hoseinzadeh et. al. (2013)
9	Warsaw, Poland	Office (Workplaces)	Bacteria: *Bacillus Micrococcus Staphylococcus* Fungi: *Aspergillus Cladosporium Penicillium Acremonium*		Six stage Anderson sampler Single stage MAS sampler Button personal sampler	Bacteria: 14-494 CFU/m^3	Fungi: 0-176 CFU/m^3		ND	Gołofit-Szymczak and Górny (2010)
10	Thirty-seven hospitals in Taiwan	Nurse stations, pharmacy departments, clinics, clinic waiting areas, lobbies, meeting rooms and wards	Bacteria and Fungus	Bacteria: TSA Fungi: MEA	Burkard sampler		Bacteria CFU/m^3	Fungi CFU/m^3	ND	Jung et. al. (2015)
						Nurse station	911 ± 891	504 ± 1003		
						Pharmacy Department	544 ± 249	192 ± 94		
						Clinic	584 ± 817	442 ± 532		
						Clinic waiting area	467 ± 639	719 ± 1276		
						Lobby	832 ± 1194	418 ± 505		
						Meeting Room	396 ± 218	173 ± 109		
						Ward	1703 ± 2317	274 ± 450		
11	Hospital in Taiwan	Internal medicine Cardiology A Cardiology B Burns Surgery Pediatrics Bone marrow transplantation (BMT)	Bacteria and Fungi	Bacteria: TSA Fungi: MEA	Andersen 1-STG sampler		Bacteria (CFU/m^3)	Fungi (CFU/m^3)	ND	Li & Hou (2003)
						Internal medicine	1 - 273	3 – 239		
						Cardiology	11 - 283	1 - 3115		
						Burns	41 – 170	8 - 319		
						Surgery	37 – 423	1 - 143		
						Pediatrics	35 – 156	10 - 249		
						BMT	0	0		
12	Teaching Hospital, University of Gondar,	Surgery, emergency, orthopedic, general ward, radiology,	Bacteria: *Staphylococcus aureus* and *Streptococcus pyogenes*	NA and BA	Passive air sampling technique	Bacteria: 1468 CFU/m^3			temperature 26.5–29.5 °C and RH 64.5–85 %	Gizaw et. al. (2016)

	Northwest Ethiopia	obstetric, medical ward							
13	Texas	Poultry industry	Bacteria: *Staphylococcus Salinicoccus Lactobacillus Sagenomella* Fungi: *Aspergillus Penicillum Trematosphaeria*	ND	Inhalable sampler with gelatin membrane filters Pyrosequencing (next generation sequencing)	Bacteria: 74-2187 cells/m^3	Fungi: 15- 698 cells/m^3	-	Nonnenmann et. al. (2010a), Nonnenmann et. al., 2010b
14	Educational hospital in Shiraz, Iran	Operating Room	Fungi: *A. fumigatus, A. Niger, Penicillium spp., Alternaria spp., Fusarium spp., Mucor spp., Cephalotrichum spp., A. Flavus, Cladosporium spp., Trichoderma spp.*	Fungi: SDA Bacteria: BA	BioStage Single-stage Impactor	Before cleaning: Bacteria 14.65–167.40 CFU/m^3; Fungi 4.83 to 18.40 CFU/m^3 After cleaning: Bacteria 9.50 to 38.40 CFU/m^3; Fungi 1.90 to 8.90 CFU/m^3		ND	Dehghani et. al. (2018)
15.	Tertiary healthcare facility situated in West Chennai, India	Orthopaedic ward	Bacteria: *Micrococci, Enterobacter, Pseudomonas, Enterobacter,* Fungi: *Aspergillus niger*	Aerobic bacteria: BA and MCA Fungi: SDA	Passive and active sampling methods.	Exposed plate: Bacteria 45–150 CFU/plate; Fungi 0–13 CFU/plate Impingement: Bacteria 1.12 x 10^3–1.68 x 10^5 CFU/m^3; Fungi 0–3.54x10^3 CFU/m^3 Filtration: Bacteria 3.788 x 10^3-1.91 x 10^5CFU/m^3; Fungi 0–1.51 x 10^4 CFU/ m^3		Temperature 26 – 33ºC RH 51% – 73%	Sudharsanam et. al. (2012)
16.	Warsaw, Poland	hospitals, ambulances and hospital administration offices	Bacteria: *Micrococcus spp., Kocuria spp., Bacillus spp., coagulase-negative Staphylococci, Micrococcus and Kocuria spp.* *Fungi: Aspergillus, Penicillium*	Porous medium (gelatine filter)	Personal Button Sampler	Hospital Emergency Department: Bacteria 4.7×10^2 CFU/m^3; Fungi 6.7×10^1 CFU/m^3 Ambulances: Bacteria 3.0×10^2 CFU/m^3; Fungi 4.7×10^1 CFU/m^3 Hospital office: Bacteria 2.3×10^2 CFU/m^3; Fungi 2.4×10^1 CFU/m^3		Temperatures 0–15 °C RH 50–80%	Bielawska-drózd et. al. (2018)
17.	Three different hospitals of Lucknow City, Uttar Pradesh, India	general wards and OT	Bacteria: *Escherichia coli, Aspergillus niger, Staphylococcus aureus* Fungi: *Aspergillus favus, Shigella sonnel Aspergillus fumigatus, Salmonella enterica, Candida asbicans, Proteus mirabilis, Cunninghamella bertholletiae*	MCA, NA, (Salmonella Shigella) SS agar, and (E. coli-Coliform Selective Agar Chromo Select) ECC agar for bacteria SDA and RBA for fungal	Passive method	HospitalA: General ward Bacteria CFU/m^3 117.95 – 1376.11; Fungi CFU/m^3 288.32-681.50 HospitalA: OT Bacteria 183.48-589.76; Fungi 131.05-524.23 HospitalB: General ward Bacteria 131.05-1389.21; Fungi 393.17-589.76 HospitalB: OT Bacteria 262.11-524.23; Fungi 262.11-524.23 HospitalC: General ward Bacteria 262.11-1179.52; Fungi 524.23-786.34		ND	Taushiba et. al. (2023)

						OT	288.32-982.93	183.48-327.64		
18.	Hospitals In UK	Conventionally ventilated orthopaedic operating theatre (CVOOT), Laminar flow orthopaedic operating theatre (LFOOT) and General ward	Gram-Negative bacteria, Bacteria and Fungi	NA , MCA or ME	Multi-orifice Cascade Impactor		Bacteria (CFU/m^3)	Fungi (CFU/m^3)	Temperature 14-23°C RH 44-56%	Nasir et. al. (2015)
						CVOOT	0-325	0-99		
						LFOOT	0-572	0-445		
						General ward	7-2883	141-3844		
19.	Hospitals in Nanjing, China	Respiratory ward and Surgery ward	Bacteria: *Acinetobacter lwoffii, Bacteroides fragilis, Acinetobacter baumannii* Fungi: *Candida albicans, Streptococcus pneumoniae, Aspergillus flavus*	Peptone and BEAfor bacteria and PDA for fungi	Two Andersen six-stage impactors		Bacteria	Fungi	ND	Guo et. al. (2021)
						Respiratory Ward	1698.7 CFU/m^3	1206 CFU/m^3		
						Surgery Ward	1882.3 CFU/m^3	ND		

TSA-tripticase soy agar, SDA-sabouraud dextrose agar, PDA-potato dextrose agar, SBA-rose bengal agar,MEA-malt extract agar, NA-Nutrient Agar, MCAMacConkey agar, BEA-beef extract agar

*ND: Not defined

Table 2.5 Bioaerosols study at variable atmospheric conditions and atmospheric pollutants

Sl. No.	Study area	Temperature and RH	Pollutant response	Viability with atmospheric condition	Bioaerosols diversity	Reference
1	Shahrekord, Iran	Substantial positive association between bioaerosol concentrations, wind direction, and relative humidity, and a significant negative correlation between temperature.	ND	ND	Bacteria: *Micrococcus luteus* (21.35%), *Pseudomonas aeruginosa* (12.68%), *Bacillus subtilis* (10.14%), and *Staphylococcus aureus* (9.93%).	Bagheri et. al. (2021)
2	Qingdao Coastal Region, China (36°10′N, 120°30′E)	Temperature showed a negative correlation with bacterial viability on foggy days in this study	Bacterial viability was higher on sunny days than on polluted days	Bacterial viability was 20.8% on sunny days and significantly higher than 14.1% on foggy days, 11.2% on haze days, 8.6% during the HF phenomenon and 6.1% on dust days	ND	Yin et. al. (2021)

3	Qingdao, China (36°10′N, 120°30′E	Temperature significant negative correlation with the airborne microbe concentration	O_3 had a substantial negative association with the airborne microbe concentration, while $PM_{2.5}$, SO_2, NO_2, CO and the air quality index (AQI) had significant positive relationships with the airborne microbe concentration during hazy days	Total microbe concentration increased to 7.09×10^5 and 9.00×10^5 cells/m^3 on hazy and foggy days	ND	Dong et. al. (2016)
4	Xi'an, China (34.22°N, 109.18°E	Temperature had a significant negative correlation with TAMs. RH had no significant statistical correlation with TAMs.	AMs were significantly positively correlated with PM_{10} and PM_2. A significant negative correlation was also established between the TAMs and O_3 concentrations	The mean concentration of TAMs on hazy days ($6.12 \times 10^5 \pm 3.50 \times 10^5$ cells/m^3) was significantly higher than that on non-hazy days ($2.15 \times 10^5 \pm 1.26 \times 10^5$ cells/m^3)	The mean concentration of TAMs varied in the following ascending order: excellent ($1.92 \times 10^5 \pm 0.88 \times 10^5$ cells/m^3) < good ($2.39 \times 10^5 \pm 1.47 \times 10^5$ cells/m^3) < lightly polluted ($5.38 \times 10^5 \pm 3.26 \times 10^5$ cells/m^3) < heavily polluted ($5.93 \times 10^5 \pm 3.45 \times 10^5$ cells/m^3) < severely polluted ($7.23 \times 10^5 \pm 3.49 \times 10^5$ cells/m^3) < moderately polluted ($7.38 \times 10^5 \pm 4.43 \times$ 105 cells/m^3).	Xie et. al. (2018)
5	Arthauli (25.95°N, 85.10°E), a rural site in Vaishali district of North-Western Bihar, India	Pre-fog condition: temperature, RH, WS, and PBLH 9.3 ± 3.2 °C, 93.0 ± 2.6 %, 7.1 ± 0.5 km/h, and 63.4 ± 23.1 m, respectively. Foggy condition: 12.4 ± 3.8 °C, 85.0 ± 3.2 %, 1.6 ± 0.6 km/h, and, 172.1 ± 95.6 m, respectively. Post-fog condition 17.6 ± 5.7 °C, 58.8 ± 12.4 %, 11.1 ± 3.1 km/h, and, 1224.7 ± 724.9m, respectively.	ND	Distinct bacterial diversity has been identified in foggy condition than in pre- and post-fog conditions	*Cutibacterium*, *Herbaspirillum*, *Paenibacillus*, and *Tsukamurella* Skin infectious bacteria (66 %), respiratory (30 %) and oral (4%) are dominating in pre-fog and foggy conditions.	Saikh et. al. (2024)
6	Shahrekord, Iran	Significant positive correlation between the concentration of bioaerosols and wind direction, relative humidity and a significant negative correlation between temperature.	ND	ND	The predominant genus of fungi were *Cladosporium* (41%), *Alternaria* (16%), and *Aspergillus* (11%).	Bagheri (2021)
7	Coastal Maine (USA) and the Namib Desert (Namibia)	ND	ND	Microbial communities under fog are more diverse, more viable, and compositionally distinct from dry aerosols	*Actinobacteria*, *Firmicutes*, *Cyanobacteria*, *Oceanospirillales*, *Novosphingobium*,	Evans et.al. (2019)

					Pseudoalteromonas, Bradyrhizobiaceae	
8	South-facing shore of Southport Island, ME, USA (N43.80261 W69.66841)	As wind speed increases and load decreases.	ND	12 (Operational Taxonomic Units) OTU's detected under clear conditions, 58 under foggy conditions.	*Vibrio, Bacillus, Pseudoalteromonas, Psychrobacter, Salinibacterium*	Dueker el. al. (2012)
9	Laoshan campus of the Ocean University of China, Qingdao, China	Temperature and RH do not show significant correlation with $TAM_{0.65–1.1}$	concentrations of $TAM_{0.65–1.1}$ increased with the enhanced pollution levels on foggy days	Total airborne microbe (TAM) of particle size range of 0.65–1.1 μm ($TAM_{0.65–1.1μm}$) form $(1.50±1.37)×10^5$cells/m^3 to $(1.76± 1.36) × 10^5$ cells/m^3 in light fog days and from $(7.91 ±7.97)×10^4$ cells/m^3 to $(1.76 ± 1.33) × 10^5$ cells/m^3 on days with and medium fog	ND	Wei et. al. (2022)
10	Ocean University of China, Qingdao, China (36˚16′N, 120˚50′E)	Bioaerosols was higher during hazy and foggy weather than that during sunny days	No significant correlation of bioaerosols with SO_2, NO_2, CO, O_3, PM_{10}, $PM_{2.5}$, air temperature, or wind velocity	ND	*Bacilli, Pseudomonas, Acinetobacter*	Qi et. al. (2018)

*ND: Not defined

Hospitals in Hamedan, Iran, recorded bacterial species like *Micrococcus spp.* and coagulase-negative *Staphylococci*, with fungal isolates including *Penicillium spp.* and *Aspergillus spp.* Using filtration methods, bacterial concentrations averaged 160.6 CFU/m^3 and fungal concentrations 12.56 CFU/m^3 (Hoseinzadeh et. al., 2013). Bioaerosol studies in 37 Taiwanese, Taiwan, hospitals targeted areas such as nurse stations, clinics, and waiting areas. Common bacteria and fungi were detected, with concentrations varying by location. For instance, bacterial levels ranged from 396–1703 CFU/m^3 in meeting rooms and wards, while fungal counts ranged from 173–1276 CFU/m^3, using Burkard samplers (Jung et. al., 2015). Bioaerosols in the University of Gondar Teaching Hospital, Ethopia, included *Staphylococcus aureus* and *Streptococcus pyogenes*. Passive sampling showed bacterial concentrations reaching 1468 CFU/m^3. Environmental factors such as temperatures of 26.5–29.5°C and RH of 64.5–85% influenced microbial growth (Gizaw et. al., 2016). In Chennai, India, bioaerosols from orthopaedic wards revealed bacteria like *Micrococci* and *Enterobacter* and fungi like *Aspergillus niger*. Passive and active methods reported bacterial concentrations of 45–150 CFU/plate (exposed plate) to 1.6856 x 10^5 CFU/m^3 (impingement) and fungal levels of up to 13 CFU/plate (Sudharsanam et. al., 2012). Orthopaedic operating theatres and general wards in the UK, exhibited diverse bioaerosols. Bacterial concentrations ranged from 0–2883 CFU/m^3 and fungal counts from 0–3844 CFU/m^3, with higher levels in general wards compared to operating rooms (Nasir et. al., 2015). Respiratory and surgery wards in Nanjing, China, showed bacterial concentrations of 1698.7 CFU/m^3 and fungal concentrations of 1206 CFU/m^3 in respiratory wards. *Acinetobacter baumannii* and *Candida albicans* were prevalent, and samples were collected using Andersen impactors (Guo et. al., 2021). Table 2.4 highlights the findings of the spatial variability and significant influence of environmental factors and air management systems on microbial concentrations in various indoor sites.

The concentration and diameter of culturable bioaerosols depend on various factors, including $PM_{2.5}$ (AQI), PM_{10} (AQI), sampling time, seasons, temperature, relative humidity, dew, pressure, wind, O_3, NO_2, and SO_2. Due to changes in the atmospheric variable, the survivability of the various microbes is affected, which leads to the change in the

concentration and the diversity of the bioaerosols over the regions (Gao et. al., 2016). Some pollutants may be attached to the various bioaerosols and show synergetic toxic effects. The variable atmospheric conditions significantly affect the concentration of the bioaerosols (Tastassa et. al., 2024). Variations in the bioaerosol characteristics through the variable atmospheric conditions and atmospheric pollutants is shown in Table 2.5.

The realization that cross-disciplinary cooperation between human and animal health, microbiology, biophysics, engineering, aerobiology, infection control, public health, occupational health, and industrial hygiene is crucial has been one of the major turning points in this field's recent technologically enabled advancements (Mubareka et. al., 2019). The study of bioaerosols in indoor and outdoor environments is a very new and emerging area in developing countries. Different institutions are paying attention to this because of some useful findings in some case studies done across the globe. Due to climate change the atmospheric variability also affected and leads to change in the characterstic of the various microbes including mutations. The developing country has problems like sanitation, waste management, cleaning, and pollution (air, water, soil, etc.). This problem may risk too many diseases which are sources of bioaerosols and they are responsible for spreading the diseases. If the microbes could mutate to that extent so that they could also move over long distances and act as transboundary pollutants.

From the above literature review, it is clear that the impact techniques are very useful and more informative for the collection of bioaerosols. The most widely used impactor is the Anderson Six-Stage viable impactor. It is also seen that culturable methods are still a very cost-effective and feasible for the enumeration of bioaerosols. Since modern instrumentation required high cost and high maintenance. In various indoor and outdoor environment. Bioaerosols survival are dependent upon the environmental conditions of the indoor and outdoor premises including ventilations that should be simultaneously observed during the experiment. The waste treatment process significantly contributing in the bioaerosols emission and developing nations have the problem in waste treatment due to poor infrastructure and technology. The study of the bioaerosols done over the developing nation as India and specially IGP need very serious attention because the known bioaerosols shows

the pathogenic behaviour toward the human health and agriculture. Also the emergence of the antimicrobial resistance in the bioaerosols must be supplemented in the toxicity assessment which has a serious questions in now as days. These has found during the literature review and it can considered as the research problem for thesis work

2.4 Research Gap in bioaerosols study over IGP

Indo-Gangetic plain (IGP) is dominated by various atmospheric pollutants having primary and secondary origins, originating both from local and long-range transport. The presence of different natural and anthropogenic sources makes aerosols and bioaerosols dynamics very complex. In spite of this importance, very limited information regarding bioaerosols is available for this region so far. Therefore, in order to understand the concentration, diversity, health impact and association with the environmental variable in this region, detailed quantitative information is required. The following research gaps over this region were identified:

- Information related to the concentration of the bioaerosols in middle IGP is extremely rare because very limited work has been done yet.
- The health impacts of bioaerosols entirely depend on their size and type. No such speciation has been done at Varanasi (middle IGP), especially in terms of biological characterization and size distribution in the inhalable range.
- Bioaerosols and meteorological association are very important to study in order to investigate the bioaerosols effect on seasonal variability and vice-versa.
- There is no information on various anthropogenic sources that emit pathogenic bioaerosols in high concentration and the evolving nature of bioaerosols, such as antibiotic resistance.
- Indoor bioaerosols play a significant role in human health, so it is quite important to study the properties of indoor bioaerosols over humans and domestic animals.
- The effect of atmospheric variability (like fog, which is very common in this region) on the bioaerosols in this region has not been studied yet.

These points mentioned above can be considered as the scope of the study on the bioaerosols over the IGP. Keeping these point form the research gap, the hypothesis is considered and objectives for this study have been finalized.

2.5 Objectives of the Research

Based on the detailed literature reviews over the various locations in the world, including IGP regions for measurement, biological characterization, meteorological implications and health effects of bioaerosols, the following research objectives and tasks were formed.

Objective 1

Estimation of the total culturable bioaerosols in particulate matter ($PM_{2.5}$ and PM_{10}): a comprehensive study in the IGP during winter

- Task 1: Gravimetric measurement of near-surface measurement of $PM_{2.5}$ and PM_{10}
- Task 2: Detailed analysis of bacterial and fungal concentration in $PM_{2.5}$ and PM_{10}
- Task 3: Investigation of environmental variables on the bioaerosols concentration
- Task 4: Analysis of biological diversity present in the particulate matter

Objective 2

Seasonal variation of bioaerosols in outdoor environment and its association with meteorological variables

- Task 1: Investigation of spatial-temporal distribution of bioaerosols in different outdoor sites
- Task 2: Detailed analysis of size distribution of bioaerosols along with spatial-temporal distribution
- Task 3: Investigation of the bioaerosols over the different sites and its association of the meteorological variables
- Task 4: Biological characterization of bioaerosols over the different sites

Objective 3

Investigation of the bioaerosols emission from the anthropogenic sources (wastewater treatment plant and solid waste processing site), biological characterization and health effect assessment

- Task 1: Investigation of bioaerosols concentration and size distribution over the selected sites
- Task 2: Metagenomics analysis for biological characterization of bioaerosols
- Task 3: Investigation of antibiotic resistance test and health survey for the health impact analysis

Objective 4

Determination of characteristics of size-segregated bioaerosols under diverse indoor environment

- Task 1: Investigation of bioaerosols concentration and size distribution over the selected indoor sites
- Task 2: Investigation of the bioaerosols over the different indoor sites and their association with the environmental variables
- Task 3: Biological characterization of bioaerosols over the different sites

Objective 5

To study the size-segregated characteristics of the bioaerosols during foggy and non-foggy days of winter, meteorological implications, and health risk assessment

- Task 1: Investigation of bioaerosols concentration and size distribution during foggy and non-foggy days
- Task 2: Analysis of the bioaerosols concentration and size distribution during foggy and non-foggy days along with association with the environmental variables
- Task 3: Biological characterization of bioaerosols over the different sites