## 6.1 Preamble

Bioaerosols are living and non-living airborne suspended particles in the air which originated from biological sources (Després et. al., 2012b; Fröhlich-Nowoisky et. al., 2016). Potential sources of bioaerosols are both natural i.e. plants, trees, waterbodies, animals etc. and anthropogenic like wastewater treatment plants, dumping sites, biodegraded materials etc. (Fröhlich-Nowoisky et. al., 2016; Humbal et. al., 2018b; Kim et. al., 2018; Vishwakarma et. al., 2024). Bioaerosols include bacteria, fungi, pollen viruses and various metabolites produced from biogenic sources (Kumar et. al., 2022). Biogenic particles can attached with solid, semisolid and liquid droplets (size 0.001 nm to 100μm) and transported from one place to another place due to the wind movement (Humbal et. al., 2018b). Since the sizes of the bioaerosols are significantly smaller, it can be easily inhaled and, ingested by the exposed human population and can cause several types of diseases (Górny, 2020; Kim et. al., 2018).

In the current lifestyle and household, people spend about 90% of their time indoors in offices or at home (Klepeis et. al., 2001). There is significant evidence regarding the bioaerosols in the indoor environment are associated with human health (Argyropoulos et. al., 2023a; Després et. al., 2012a; Madureira et. al., 2015; Vishwakarma et. al., 2022). Several studies related to sick-building syndrome (Sahlberg et. al., 2013), severe acute respiratory symptom and avian influenza (Peiris et. al., 2007) takes serious attention towards indoor bioaerosols (Srikanth et. al., 2008). However, the health risks from exposure to bioaerosols potentially vary with their respective communities and level of exposure (Soto-Quiros et. al., 2002; Vishwakarma et. al., 2022).

It has been demonstrated that the concentration and distribution of bioaerosols are influenced by many factors like population occupancy (Meadow et. al., 2014), human activities (Heo et. al., 2017), indoor flooding (Emerson et. al., 2015) and ventilation system (Luongo et. al., 2016; Othman et. al., 2024). Bioaerosol concentration in indoor site largely depends on the sources, and indoor to outdoor concentration (I/O) ratio for a specific site. However, significant variations in I/O ratio has been reported in different studies for bacterial bioaerosols including 0.26 to 0.71 by Kim & Kim (2007) and 0.43 to 11.8 by Balasubramanian et al. (2012). It is very likely that bioaerosols can spread through

transmission either from the air or contact with affected people (Bonetta et. al., 2010). Along with the influence by the affected population, other factors like vegetation, interior material of construction, types of goods and items also affect the bioaerosols concentration and type at the workplace (Jiang et. al., 2024). Several studies have estimated the concentration of bioaerosols at various indoor sites of human occupancy, like in library (Ghosh et. al., 2013; Hayleeyesus and Manaye, 2014), laboratories (Priyamvada et. al., 2018), classroom (Bhangar et. al., 2014), auditorium, canteen, hospital (Kim & Kim, 2007; Baurès et. al., 2018, Negi et. al., 2024). Studies have also been conducted for different indoor sites to assess the concentration and characteristics of indoor bioaerosols (Ghosh et. al., 2013; Li et. al., 2015; Jabeen et. al., 2023). However, till date, information on bioaerosol prevalence at different size ranges within different microenvironments has not been explored.

In the present study, the size distribution of the indoor bioaerosols (bacterial and fungal) concentration were measured in the six different size distribution range (between 0.65 to 7.0µm). The average total concentrations of bioaerosols were investigated in different indoor environments, including poultry, cowshed, canteen, library, auditorium, hospital, and laboratory. Bioaerosols were cultivated on nutrient agar (NA) and potato dextrose agar (PDA). Colony-forming units (CFUs) were counted, and some colonies were taxonomically identified via DNA sequencing (16S ribosomal gene and ITS). The correlation between concentration of bioaerosols (bacterial and fungal) with environmental factors (like temperature and relative humidity) and presence of population were also investigated. Finally, based on the nature of the bioaerosols present at the indoor sites, the possible health consequences of various types of bioaerosols on humans, animals and plants were discussed.

## 6.2. Materials and methods

### 6.2.1 Description of study sites

In this study, bioaerosol sampling was performed at the various indoor sites of an urban ecosystem located at Varanasi (25.2677°N and 82.9913°E) in eastern Uttar Pradesh, India. The sites are spread over a 5.5 $km^2$ area near the bank of the river Ganga. The climate of this region is humid subtropical, and there is significant variation in the temperature between summer and winter. The average temperature in summer ranges from 32°C to 42°C; in

winter, it remains in between 10 and 23°C (India Meteorological Department (IMD)). This study focuses on specific sites to have distinct information on indoor bioaerosols. These indoor sites included poultry, cowshed, canteen, library, auditorium, hospital, and laboratory. Where poultry and cowsheds were animal-dominated while others were human-dominated.

#### 6.2.1.1 Animal-dominated sites

The cowshed was located in an area surrounded by plants and grassland. In the cowshed area, proper ventilation and sanitation was maintained. There were 20 to 35 cattle present during the air sampling, all the cattle were vaccinated, and health checkups were done from time to time. Mostly wheat straw, rice straw and green crop residue were given to feed the cattle. During the sampling, the average temperature and relative humidity were about 20.3°C and 75.8% inside the sampling area.

The poultry farm was also located in an area dominated by grasses and vegetation. The ventilation of the poultry was inadequate. All the chickens were adequately vaccinated, and their food was maintained. About 150-198 chickens were present during the sampling period. The Average temperature inside the area was 20.9°C, and relative humidity was 68.9% during the air sampling.

#### 6.2.1.2 Human-occupied places

IIT (BHU) canteen was chosen for the sampling of bioaerosols. Sampling site was surrounded with trees and green grass cover with proper ventilation and sitting arrangements. The capacity of the canteen was about 60-100 people at a time. During the air sampling, the average presence of the population was about 20-50 people. The average temperature and relative humidity recorded during the sampling were 20.2°C and 69.2%, respectively.

IIT (BHU) campus has an extensive library surrounded by trees and grass. Within the library, there was an arrangement of a reading hall in the centre with a circular shape-like structure for reading, a separate book section, and a separate section for thesis and magazine. Within the reading area, the average student occupation was 40 to 65 during the sampling. The average temperature and relative humidity recorded during the sampling were 20.3°C and 64.7%.

Lectures and indoor gathering events were organised within the auditorium. The auditorium, contains 300 well-furnished seats and a stage of about a 2-meter height. This area was fully

air-conditioned, and cleanliness was maintained before and after the programme. Air samples were taken just after the event. The average population was 102-154 before the sampling in this area. The average temperature and RH were recorded as 18.9 °C and 74.3%.

The outpatient department (OPD) section was located in the middle of the university hospital, and there was a large sitting arrangement, approximately 200 for the patient's relatives. The population within the area varied with time because of the continuous movement of the patient. Approximately, 55 to 130 people were within the OPD section when bioaerosols sampling was continued. The average temperature and RH were 21.9°C and 64.0%, respectively.

The laboratory was situated on the ground floor; environmental microbiology-related work was being done in this lab. Out of 2 sections, one is equipped with a highly sophisticated instrument, and one has some common instrumentation facility with a separate cabin for the technical staff. The sampling was done in the open area of the laboratory, and the population occupation was about 2-9. During the sampling, average temperature and RH were between 20.4°C and 62.6%, respectively.

### 6.2.2. Air sampling

Sampling of bioaerosols was performed using the active sampling method with impaction technique. Air sampling for the collection of the bioaerosols was carried out using an Anderson six-stage cascade impactor (Tisch Environmental, USA) during the winter of 2019-2021 (December-January-February). The main advantage of this technique is, it can give the direct measurement of particle size distributions in each stage of cascades. The sampler collects bioaerosols in different size ranges (cutoff diameter) from- >7.0 µm (stage-1), 4.7-7.0 µm (stage-2), 3.3-4.7µm (stage-3), 2.1-3.3µm (stage-4), 1.1-2.1µm (stage-5) to 0.65-1.1µm (stage-6). The sampler was operated at a flow rate of 28.3 L/min for 20 minutes with a frequency of twice in a month. Each sample was taken in the morning of the day between (10 am to 11 am) when indoor occupancy was higher. Before and after the sampling, the cascade was ultrasonicated and sterilized with 75% ethanol in order to avoid the contamination of the microbes and improve the efficiency of the cascade. The temperature and relative humidity were simultaneously recorded with the temperature and humidity meter while doing air sampling. The characteristics summary of the selected sites is described in Table 6.1.

### 6.2.3 Culture media

Two different fractions of bioaerosols (i.e., bacteria and fungi) were collected using two different selective media. Here, nutrient agar (NA) (Merck) and potato dextrose agar (PDA) (Merck) were used to grow the colonies of bacteria and fungi, respectively. Nutrient Agar media is ubiquitous for the growth of various microorganisms and primarily used for bacteria during the experiment. It contains agar, peptone, nutrient extract and sodium chloride that provide a simple formulation of nutrients necessary for a large number of bacterial growths. Similarly, PDA media is common for the growth of fungi. It consists of dehydrated potato infusion and dextrose, which encourage fungal growth. Agar was used in both media for the solidification of the media. However, fungal colonies were neglected while counting bacterial colonies from NA plates, and bacteria colonies were ignored while counting fungi on the PDA.

Both the media were prepared per the standard method, and the solution was sterilized through autoclaving at 15 psi and 121°C for 15 min. After the cooling of the media, it was poured on the Petri plates inside the biosafety chamber where UV-light and HEPA filters were used to avoid contamination (Kowalski, 2010). The equipment was sterilized with the help of autoclaving and cleaning with 75% ethanol.

Table 6.1 Description of the sites, environmental variable and population at selected sites

| Sl. No. | Sites | Location details and specific characteristics | No. of samples | Average no. of population | Sampling duration (min) | Temperature range (°C) | Relative Humidity range (%) |
|---|---|---|---|---|---|---|---|
| 1 | Cowshed | Agriculture field surrounded by vegetation. | 9 | 28[$] | 20 | 17.8-23.9 | 60.5-90.1 |
| 2 | Poultry | Agriculture field surrounded by vegetation. | 9 | 170[*] | 10 | 18.8-22.3 | 56.3-84.9 |
| 3 | Canteen | Vicinity of IIT (BHU) | 9 | 35[#] | 20 | 17.2-22.5 | 54.5-86.9 |
| 4 | Library | The central location of BHU | 9 | 51[#] | 20 | 17.6-22.7 | 57.8-80.8 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 | Auditorium | Department of Chemical Engineering & Technology, IIT (BHU) | 9 | 121[#] | 20 | 17.3-21.9 | 68.6-79.5 |
| 6 | Hospital | IMS, BHU, a teaching hospital | 9 | 88[#] | 20 | 18.7-24.4 | 47.6-77.8 |
| 7 | Laboratory | Department of Chemical Engineering & Technology, IIT (BHU). | 9 | 5[#] | 20 | 19.2-22.4 | 53.7-74.1 |

[*]population of chicks, [$]population of cow, [#]human population,

### 6.2.4 Enumeration and identification

#### 6.2.4.1 Sample enumeration

Petri plates filled with semi-solid media were used for the air sampling. After sampling, the impactors brought in the laminar airflow, and the plates were sealed with parafilm and put for incubation. For bacteria, the temperature was kept at 25°C for 72 hr and for fungi at 35°C for 48 hr was preferred. This condition seems optimum for the bacteria and fungi colony growth on the petri plates (Dix and Webster, 1995). After this period, colonies were developed and visible on the petri plates, which can be easily calculated with the help of a colony counter. The total concentration of the microbes was calculated by the total no. of the colony appearing on plates divided by the volume of air sucked by the sampler. Its formula can be denoted as,

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

#### 6.2.4.2 Identification of the microbes

After incubation of the samples, the pure culture of bacteria and fungi was isolated, and microbes were identified using pure culture isolation and DNA sequencing method. In this method, different types of microbial colonies were picked up, and the pure culture of each was isolated by striking on the culture media plate. Only selective isolates as representative of the organism were sequenced. The colour, shape and structure of the bacterial and fungi

colonies are the basis for pre-identification of the microbes and can be compared with the standard online description; for further confirmation, the DNA sequencing method is widely used. 16S-rDNA and ITS sequencing method was used to identify the type of bacteria and fungi, respectively. The following steps were taken in DNA sequencing method: (1) Genomic DNA was isolated from the samples, (Green et. al., 2012) (2) the fragment of DNA was amplified using high-fidelity PCR (Polymerase chain reaction), using 5'-GGATGAGCCCGCGGCCTA-3' (16s Forward) and 5'-CGGTGTGTACAAGGCCCGG-3'(16s Reverse (Kaur and Kaur, 2022) (3) PCR product was sequenced bi-directionally (4) sequenced data were analyzed to identify the microbes with its closest neighbour's method (William et. al., 2000; Wiley et. al., 1991). Similarly, for fungi ITS methods were used for the identification using 5'-TCCGTAGGTGAACCTGCGG-3' (ITS-1 Forward) and 5'-TCCTCCGCTTATTGATATGC-3' (ITS-4 Reverse) primers (White et. al., 1990).

## 6.3. Results and discussion

### 6.3.1 Airborne bacterial and fungal load at different sites

In the collected samples, the concentration of bacteria and fungus varied at different indoor sites Figure 6.1 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).

Among all the indoor sites cowshed, poultry, where animals were dominant, reported a higher average concentration of bioaerosols. In the cowshed, the average bacterial concentration was 2178±1108 CFU/$m^{3,}$ whereas the average fungal concentration was 319±84 CFU/$m^3$. At poultry, the average concentration of the bacterial bioaerosols was 23693±3480 CFU/$m^{3,}$ and the fungal concentration was 337±161 CFU/$m^3$.

In the human-dominated places like the canteen, library, auditorium, hospital and laboratory, the average concentration of the bacterial and fungal bioaerosols was lower compared to the animal-dominated site. Here, the hospital and canteen reported much higher concentrations of bioaerosols, followed by library, auditorium and laboratory. In the hospital, average

bacterial and fungal concentrations were 2306±991 CFU/$m^3$ and 185±108 CFU/$m^{3,}$ respectively. Whereas at the canteen, the average bacterial was 1915±845 CFU/$m^{3,}$ and the fungal concentration was 201±118 CFU/$m^3$ reported. Other sites, such as the library, auditorium and laboratory, reported significantly lower concentrations of bioaerosols in the study. Here, average bacterial and fungal bioaerosols concentrations at the library were 679±376 CFU/$m^3$ and 140±38 CFU/$m^{3,}$ respectively. Whereas, at the auditorium it was 606±440 CFU/$m^3$ and 271±142 CFU/$m^3$ for bacterial and fungal bioaerosols to their respective concentrations. The laboratory recorded the lowest concentration of bioaerosols and was considered as the cleanest environment. In laboratory, the average concentration of the bioaerosols were 357±428 CFU/$m^3$ and 120±62 CFU/$m^3$ for bacterial and fungal bioaerosols respectively. More details have been given in the supplementary Table (Table D1)

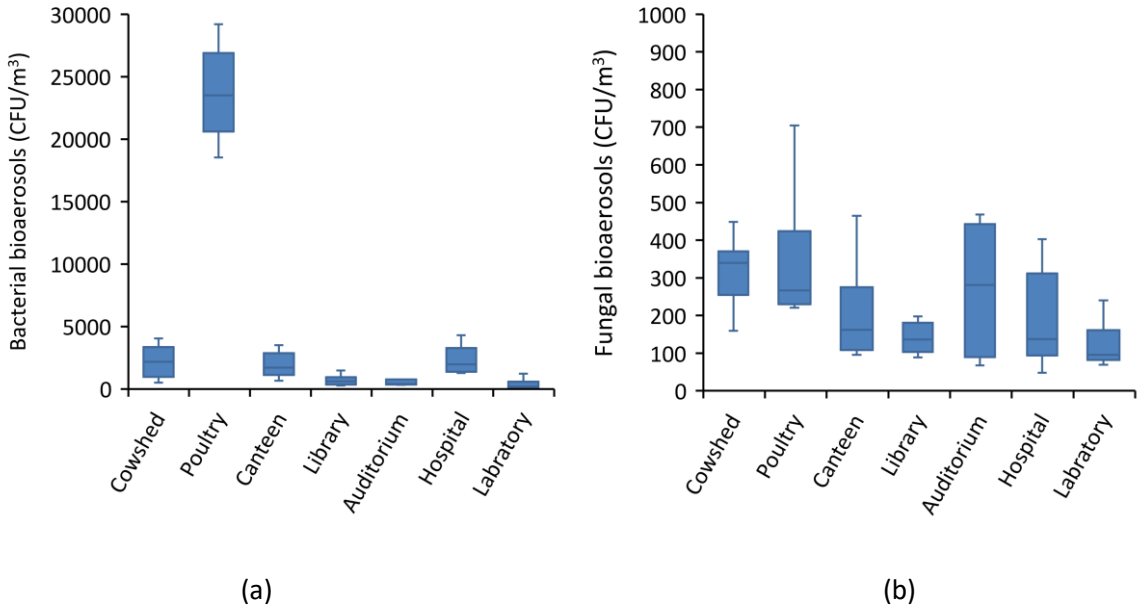


Figure 6.1 Variation of the (a) bacterial and (b) fungal bioaerosols at the various indoor sites.

The concentration of the bacteria was exceptionally higher in poultry among all the selected sites, while fungi have a smaller variation. Poultry and cowsheds have similar concentrations of fungi. The main reason for the fungi in the cowshed and poultry was the skin residue of the chicks and cows.

### 6.3.2 Size-segregated bacterial and fungal bioaerosols concentration

The size distribution of the bioaerosols varied with the types of indoor environment as illustrated in Figure 6.2 and Figure 6.3. In most of the sites, the bacterial bioaerosols concentration was much higher in size range of >7.0 µm and 1.1-2.1 µm whereas, the fungal bioaerosols concentration was much higher in the range of 1.1 to 3.3 µm. Average bacterial bioaerosols concentration was very higher as compared to the fungal bioaerosols concentration in each site and each size range of the bioaerosols collected samples. In all the selected sites, poultry reported the highest concentration of coarse and fine bioaerosols.

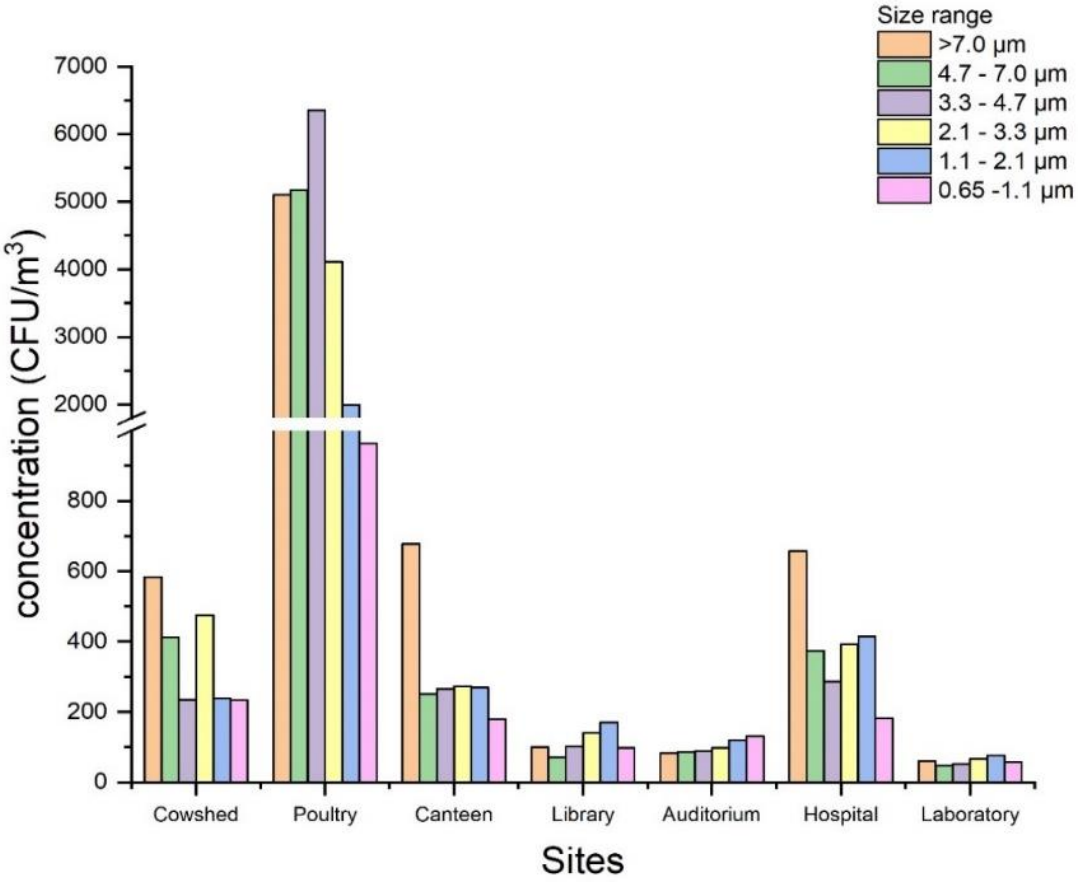


Figure 6.2 Concentration of bacterial bioaerosols in different stages (of different size range) at various indoor sites

Cowshed is an animal-dominated site, where the average concentration of bacterial bioaerosols were found significantly higher in the size range of >7.0 µm, 4.4-7.0 µm and 2.1-3.3 µm as they were 583 CFU/$m^3$, 411 CFU/$m^3$ and 475 CFU/$m^3$, respectively. In comparison, the fungal concentration was significantly higher in the size range 2.1-3.3 µm

and 1.1-2.1 µm, which is 69 CFU/$m^3$ and 104 CFU/$m^{3,}$ respectively. While at the poultry sites, the higher concentration of the bacterial bioaerosols was reported at the coarser size region as compared to the finer size range, and it was reported highest in the size range 3.3-4.7 µm which was 6352 CFU/$m^3$ (3.3-4.7 µm) followed by 5171 CFU/$m^3$ (4.7-7.0 µm) and 5098 CFU/$m^3$ (>7.0 µm). At the poultry site, the lowest average concentration of the bacterial bioaerosols (i.e. 963.19 CFU/$m^3$) was found in the 0.65-1.1 µm size range, which was more than the highest concentration of the bioaerosols in the different size ranges of various sites individually. Less variation was seen in the fungal bioaerosol concentration at poultry sites compared to other sites. However, the highest concentration of the fungal bioaerosols concentration was reported at the size >7.0 µm (114 CFU/$m^3$), which is the highest among all sites for this size range.

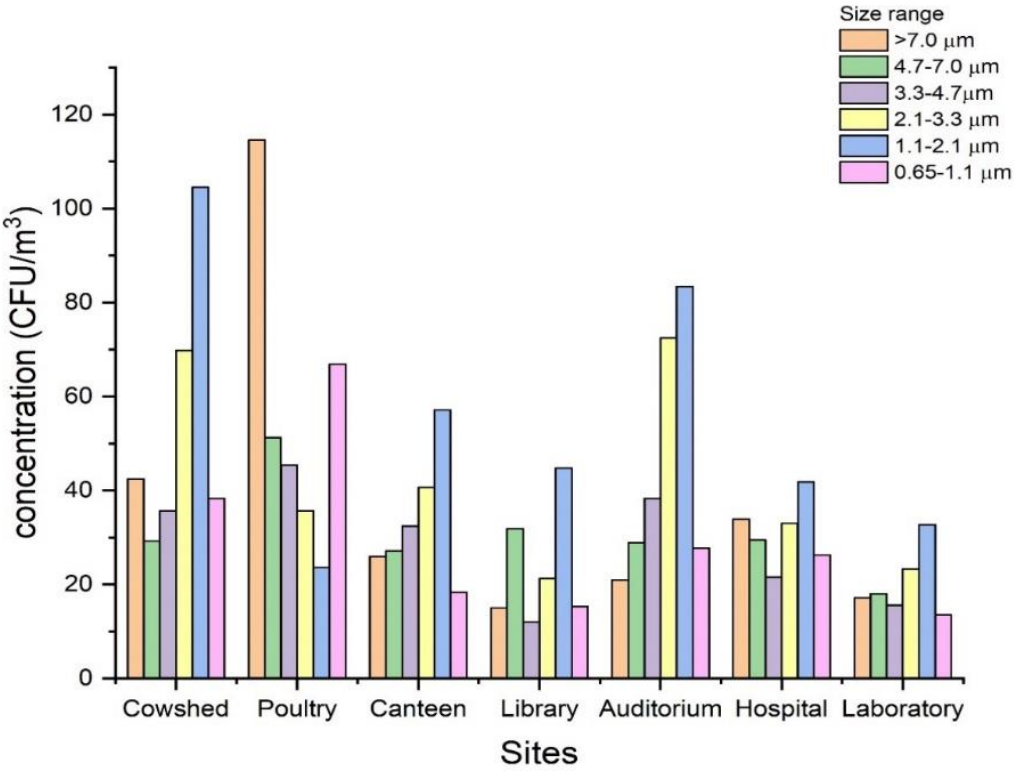


Figure 6.3 Concentration of fungal bioaerosols in different stages (of different size ranges) at various indoor sites.At the sites where the human population dominated, like in the hospital (657 CFU/$m^3$) and canteen (677 CFU/$m^3$), a higher concentration of bacterial bioaerosols was noted in the range of >7.0 µm. The concentration of bacterial bioaerosols were evenly distributed at the canteen, whereas the variation in the size was uneven at the hospital. And other sites like the library, auditorium and laboratory found lower concentrations of the

bioaerosols at each size range, but their concentration was higher in the fine particle size range (2.1-3.3 µm, 1.1-2.1µm and 0.65-1.1 µm). Whereas at each human dominated site the fungal bioaerosols concentration were higher in the size range of 2.1-3.3 µm and 1.1-2.1 µm. Among all indoor human dominated sites, the concentration of the fungal bioaerosols were higher at the Auditorium in the size range of 2.1-3.3 µm and 1.1-2.1 µm, which was 72 CFU/$m^3$ and 83 CFU/$m^3$. Nearly the same fraction of the fungal bioaerosols concentration in fine (0.65-3.3 µm) and coarse (3.3- >7 µm) particle distribution were observed at library, hospital and laboratory. More details have been mentioned in the supplementary table (Table D2).

If we compare the different size ranges of the particles found at the different indoor sites and their probability of reaching deep into the respiratory tract, it can be predict the different respiratory issues associated with the particle size. For instance, the maximum bacterial bioaerosols concentration at the cowshed site was found in size >7.0 µm while, fungal concentration was higher in the size range 1.1-2.1µm. This indicates that bacterial bioaerosols can reach the nasal area due to their higher size, while fungal bioaerosols can reach up to the lower respiratory tract due to their fine size and cause infection in this area and if they are pathogenic in nature the effect may be more severe (Yu et. al., 2023; Millner et. al., 2009; Anua et. al., 2020).

Similarly, the bacterial bioaerosols concentration was highest at the poultry site in the coarse size range, and for fungi, it is higher in the fine size range. Therefore, Fungi can easily reach the upper to the deep respiratory system and can harm the population. Even during sampling breathing problems in the form of bad smells were felt during the sampling in the nearby areas. Similar studies show that at the poultry and cowshed, coarse bacterial bioaerosols were dominated, which can cause nasal infection and uneasiness (Argyropoulos et. al., 2023b).
However, a different pattern was found in the library, auditorium and laboratory, where the bacterial bioaerosols concentration was higher in size range between 2.1-3.3µm. This size range of particles can cause a risk of infection in the pharynx and primary bronchi. Whereas, the concentration of the fungi was in the dominant fine size range (1.1-2.1µm), which may affect the bronchi region of the human respiratory system. The canteen and hospital have the larger concentration of bacterial bioaerosols of coarse particles (>7.0 µm), while at the library

and auditorium, fine-size particles (2.1-3.3 µm and 1.1-2.1 µm) are dominant, which affect the trachea and bronchi of the human respiratory system. Similar results were also reported by many previous studies (Ghose et. al., 2024; Jeong et. al., 2022). Since the laboratory site has the lowest concentration in each stage compared to the other indoor sites due to the routine cleaning at this site, its health effect was considered negligible.

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

All indoor sites showed different biological diversity in the bioaerosols samples. Our observations show that gram-negative bacterial diversity was much higher in each of the samples taken from the indoor sites. The abundance of bioaerosols at different indoor sites and their associated health effect in humans and immunocompromised bodies are included in Table D1 in Appendix D. Both Cowsheds and Poultry indicated higher concentrations of bioaerosols and were dominated by bacteria, viz. *Mammaliicoccus sciuri, Bacillus licheniformis, E. coli, Brevundimonas vesicularis, Enterobacter lactis, Acinetobacter calcoacetius* and *Bacillus sp.*. Whereas, fungi *Fusarium equiseti, Cladosporium sphaerospermum, Aspergillus, Talaromyces minioluteus, Periconia digitata, and Cladosporium sp.* Some of these microbes cause skin and eye irritation (Dhakal et. al., 2013; La Jeon et. al., 2012; Maucour et. al., 1999), gastrointestinal, UTIs, and respiratory diseases (Bai et. al., 2021; Ramirez & Giron, 2022; Stabler et. al., 2018).

Hospital had the highest diversity of the bioaerosols reported and most of them are pathogenic in nature and blended with bacterial diversity such as, *Stenotrophomonas maltophilia, Acinetobactor sp., E. coli, Brevundimonas vesicularis, Enterobacter lactis, Acinetobacter baumannii, Acinetobacter calcoacetius, Bacillus sp.* and fungi mainly, *Fusarium equiseti, Cladosporium sphaerospermum, Aspergillus, Talaromyces minioluteus, Periconia digitata, Cladosporium sp.,* and *Penicillium sclerotiorum.* At the hospital, most of the microbes were pathogenic and have a potential to cause severe health effects on humans; if the body is immunocompromised (Dent et. al., 2010; Denton & Kerr, 1998; Hanski et. al., 2012; Korotetskiy et. al., 2022).

Table 7.2 Presence of different bioaerosols at different indoor sites and their possible effect on human health

| Sites→ | Cowshed | Poultry | Canteen | Library | Hospital | Auditorium | Laboratory | Nature of microbes | Effects of bioaerosols |
|---|---|---|---|---|---|---|---|---|---|
| **Bacteria** | | | | | | | | | |
| *Stenotrophomonas maltophilia* | | | | | Yes | | | Gram-negative | Nosocomial and pulmonary infection, pneumonia, urinary tract infection (UTI), and bloodstream infection in immunocompromised patients. Resistant to many antibiotics (Denton & Kerr, 1998; Korotetskiy et. al., 2022). |
| *Mammaliicoccus sciuri* | Yes | Yes | Yes | | | | | Gram-positive | Create toxic shock syndrome and multi-resistance (Nemeghaire et. al., 2014). |
| *Bacillus licheniformis* | Yes | Yes | | | | | | Gram-positive | Nonpathogenic in nature but causative ophthalmitis in immunocompromised bodies (La Jeon et. al., 2012; Maucour et. al., 1999) and contaminate food (Salkinoja-Salonen et. al., 1999), especially dairy (Dhakal et. al., 2013). |
| *Acinetobactor sp.* | | | | | Yes | | | Gram-negative | Bacteremia, UTIs (Dent et. al., 2010), skin allergies (Hanski et. al., 2012). |
| *E. coli* | Yes | Yes | Yes | | Yes | Yes | | Gram-negative | No pathogenic but sometimes virulent strains can cause gastroenteritis, UTIs, neonatal meningitis, hemorrhagic colitis, and Crohn's disease (WHO, 2018). |
| *Brevundimonas vesicularis* | | Yes | | | Yes | | | Gram-negative | Bilateral pneumonia and septic shock in an immunocompetent Significant variability in antibiotic susceptibility (Stabler et. al., 2018). |
| *Enterobacter lactis* | Yes | Yes | Yes | | Yes | | | Gram-negative | Nosocomial and less commonly community-acquired infections, including UTI, respiratory infections, soft tissue infections (Ramirez & Giron, 2022). |
| *Bacillus amyloliquefaciens* | Yes | Yes | | | | | | Gram-positive | Not very harmful to human health, Good for agriculture, protecting the plant from pathogens and act as biological control agents (Bai et. al., 2022). |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Acinetobacter baumannii* | | | | | Yes | | | Gram-negative | Pneumonia, Bloodstream infections, Meningitis, Wound and surgical site infections, including necrotising fasciitis, UTIs (Centers for Disease Control & Prevention, 2023). |
| *Acinetobacter calcoacetius* | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Gram-negative | Cause pneumonia |
| *Bacillus sp.* | Yes | Yes | | | Yes | | | Gram-positive | Food poisoning (Ray & Ryan, 2004). |
| **Fungi** | | | | | | | | | |
| *Fusarium equiseti* | Yes | Yes | | | Yes | | | NA | Adverse effects in agriculture (Tziros et. al., 2022), skin infection through mycotoxin (Gupta et. al., 2000). |
| *Cladosporium sphaerospermum* | Yes | Yes | Yes | Yes | Yes | Yes | Yes | NA | Causes problems in patients with respiratory tract diseases, subcutaneous phaeohyphomycosis and intrabronchial lesions in immunocompetent individuals (Ng et. al., 2012). |
| *Aspergillus* | Yes | Yes | Yes | Yes | Yes | Yes | Yes | NA | It can contaminate foods such as nuts, allergic disease, cause disease in many grain crops, especially maize, and cause neonatal infections (Cloherty, 2012). |
| *Talaromyces minioluteus* | Yes | Yes | Yes | Yes | Yes | Yes | | NA | Presumed to cause infection after the conidia are inhaled into the alveoli (Pitt, 2014). |
| *Periconia digitata* | Yes | Yes | | | Yes | | | NA | Causing human corneal ulcers (Gunasekaran et. al., 2021). |
| *Cladosporium sp.* | Yes | Yes | Yes | Yes | Yes | Yes | Yes | NA | Asthma and other respiratory diseases, skin infection, sinuses and lungs, more commonly nasal congestion, sneezing, coughing, and itchy eyes (Ozdemir, 2015; Pomés et. al., 2016). |
| *Penicillium sclerotiorum* | Yes | Yes | Yes | Yes | Yes | Yes | Yes | NA | Not very harmful effects are recognised |

NA: Not applicable

In canteen, *Mammaliicoccus sciuri, E. coli, Enterobacter lactis, Acinetobacter calcoacetius* were the dominant bacteria while *Cladosporium sphaerospermum, Aspergillus, Talaromyces minioluteus, Cladosporium sp., Penicillium sclerotiorum* were the common fungi found in this site. Most of these microbes can harm us in form of gastro and respiratory issues if a large concentration is present (Nemeghaire et. al., 2014; Ng et. al., 2012; Ramirez & Giron, 2022).

Nearly similar kinds of bacteria and fungi were found in library and auditorium namely, *Acinetobacter calcoacetius,* and *Cladosporium sphaerospermum, Aspergillus, Talaromyces minioluteus, Cladosporium sp., Penicillium sclerotiorum*. These microbes generally cause allergies, skin infections and respiratory issues if the concentration is higher or the body is immunocompromised (Hanski et. al., 2012; Ozdemir, 2015; Pitt, 2014).

The lowest diversity of the bioaerosols was noted in the laboratory with only a few bacteria like *Acinetobacter calcoacetius* and fungi *Penicillium sclerotiorum, Cladosporium sp., Periconia digitata, Aspergillus, Cladosporium sphaerospermum.* They are generally non-pathogenic and found very less in the quantity so the proper cleanliness avoids the risk of exposure.

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

### 6.3.4. Relationship between bioaerosols and environmental factors

The association between the bioaerosol abundance and environmental factors was also explored and plotted in Figure D1-D4 (Appendix) using MS Excel 365. No significant regression of temperature and relative humidity with the bioaerosol concentration was noted in either of the sites. However, a very low regression coefficient was computed between bacterial concentration and temperature for animal-dominated sites (Figure D1, Appendix) like cowshed and poultry ($R^2$= 0.270 and 0.127), and in human-dominated sites like canteen (0.425), library (0.024), auditorium (0.141), hospital (0.078) and laboratory (0.066).

A nearly similar pattern of association was noted between the bacterial concentration and RH at the indoor sites (Figure D2, Appendix). Similarly, the regression analysis between

the concentration of fungi bioaerosols and temperature (Figure D3, Appendix) showed very low significance regression for all sites except laboratory ($R^2$= 0.7096). While fungi show a significant moderate correlation with RH at the animal dominant sites ($R^2$=0.6823, 0.4634 for poultry and cowshed, respectively) as compared to the human presence sites (Figure D4, Appendix). Here, at the canteen, there is a low effect for bacteria vs temperature and RH. In the case of fungi presence at indoor sites, the library and auditorium have weak regression, but the hospital shows strong regression; on the other hand, RH fungi shows moderate regression for the cowshed, which is an animal-dominant site.

In indoor environments, people used to spend most of the time with minimum variations in room temperature compared to outdoor environments. The indoor temperature is maintained according to the suitability of the animal and human beings. In the present study, room temperature remained in between 17.2 ºC and 24.4 ºC, whereas RH varied between 47.6% and 90.1%. Such a range is suitable for the microbes for their survival and growth. In this short range of variation in the temperature and RH, it is observed from the results that all the sites show different regressions with the different bioaerosols. The survival of the bacteria is different at different RH. Gram-negative bacteria survive longer at low RH; on the other hand, gram-positive bacteria can survive at higher RH (Theunissen et. al., 1993). At very low RH, water dissipates from the cell membrane and changes the crystalline structure of the lipid bilayer of the cell membrane to a gel phase, and this phase transition affects the cell surface configuration of microbes and results in the inactivation of microbes (Hurst et. al., 2007). However, in case of temperature, as it reaches freezing, ice crystals form on the microorganism's surface and they lose their viability. In contrast, at a high temperature, the enzymes are sterilized and ultimately lose their viability. Only a few thermophilic microbes can survive in these ranges. So, the effect of temperature and RH is closely associated with the various bioaerosols survival. Apart from temperature and RH, other factors like the circulation of the population and type of the population, ventilation and materials used in the interior structure, and activities of the population are important factors in the variation of bioaerosols concentration and their size distribution.

### 6.3.5. Relation between population and bioaerosols concentration at different indoor sites

Abundance of bioaerosols at different microenvironments was also explored against population size. Here, three types of populations were considered: humans, cows and chicks. The size of the population is often directly proportional to bioaerosol concentration as the population serves as the biggest source of bioaerosols. Besides, the concentration of the bioaerosols also depends on the type of population present in the sites. In our observation, as in Figure 6.4, where the primary y-axis represents the average concentration of the bacteria and the secondary y-axis represent the concentration of fungi and the average population present during the sampling.

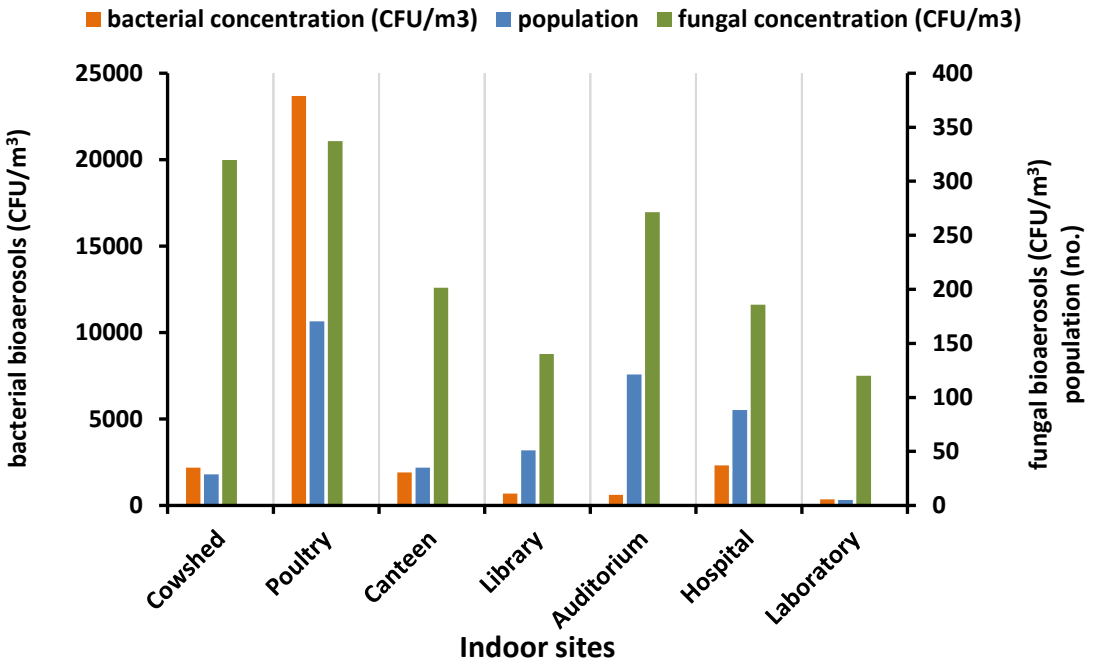


Figure 6.4 The average concentration of bacterial and fungal bioaerosols at different indoor sites and the population at the sites, where the primary y-axis represents the average concentration of the bacteria and the secondary y-axis represent the concentration of fungi and the average population present during the sampling.

There was a significant increase in bioaerosol concentration with the increase in population at various indoor sites. At the poultry sites where the number of chicks was higher (N=150-198), the bioaerosols concentration was significantly high for both fungus (337 CFU/$m^3$) and bacterial aerosols (23693 CFU/$m^3$), respectively. Besides, bacterial concentrations exceeded fungal concentrations at every site. However, there is a slight difference in the fungal concentration at the animal-dominant sites. Among the all-indoor

sites, the maximum concentration of bioaerosols has been observed in poultry (24030 CFU/$m^3$), whereas the minimum concentration was observed in the laboratory (477 CFU/$m^3$). The maximum number concentration of bioaerosols in poultry may be the result of the large population of chicks and the excreta from their daily activities.

There may be a reason for shredding human skin and clothes, which may be the carrier of bioaerosols from the outside. Some human activities like coughing, sneezing and talking where the microbes come into the air from the upper respiratory tract. These activities are very common in the hospital where different types of infection were carried by the patients. For example, sneezing generates around one million droplets of size less than 0.1 μm (Han et. al., 2013), and some organic excreta protect bacterial cells that enhance their survival in the air.

To explore the most influencing factor among temperature, relative humidity and population on the concentration of bioaerosols, a principle component analysis was made. Here, four parameters were examined, and the results are plotted in Figure 6.5. Two principle components (PC) had a significant impact on the concentration of the bacterial and fungal bioaerosols. The observation in the plot shows the original variables in bars (vectors). In these biplots, each point represents the sampling data, and each line (bar) represents the study variables.

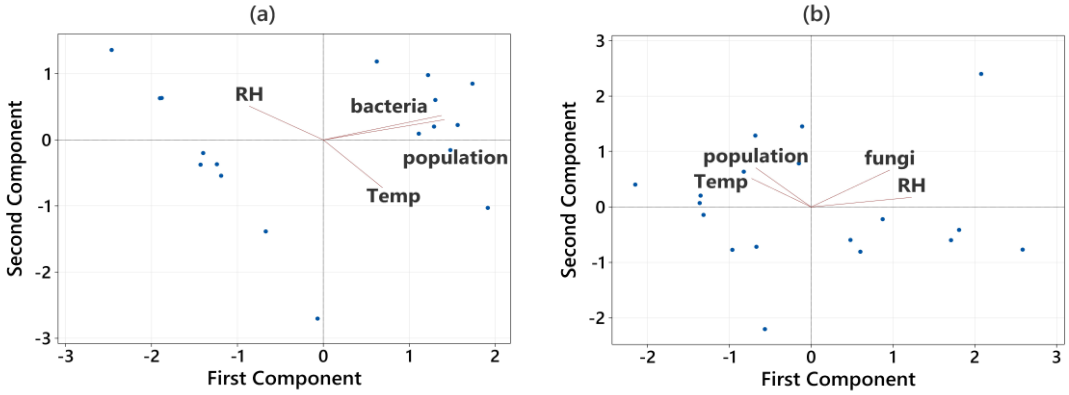


Figure 6.5 Principle component analysis of the presence of (a) bacterial and (b) fungal bioaerosols in the animals-dominated indoor sites (poultry and cowshed) and environmental factors, including population presence.

In the animal-dominated sites, where bacterial and fungal bioaerosols were investigated (Figure 6.5, a and b), the first PC explained 56.5% of the total variance and was positively affected by bacterial concentrations, temperature and animal population while negatively

related to RH. The second principal component explained 25.2% of the total variance and was positively associated with RH, bacteria and animal population while negatively related to temperature. In the case of fungal bioaerosols, the first PC explained 46.2% of the total variance and was positively affected by RH and negatively associated with animal population and temperature. The second PC explain 27.8% of the total variance and is positively affected by temperature, RH, fungi and animal population.

For the sites dominated by humans, bacterial and fungal bioaerosol concentrations were also investigated (Figure 6.6, a-b). The first PC explained 32.8% of the total variance and was positively affected by bacterial concentrations, temperature, human population and RH. The second principal component explained 27.8% of the total variance and was positively related to RH, bacteria and temperature while negatively associated with the human population. In the case of fungal bioaerosols, the first PC explained 41.6% of the total variance and was positively affected by RH, human population, fungi and temperature. The second PC explains 28.2% of the total variance and was positively affected by temperature and RH, while fungi and the human population are negatively affected.

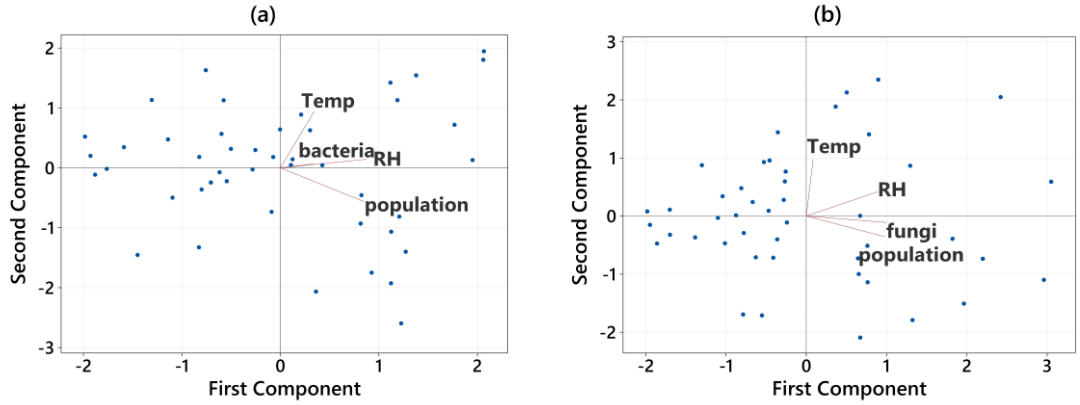


Figure 6.6 Principle component analysis of the presence of bacterial (a) and fungal (b) bioaerosols in human-dominated indoor sites (Canteen, library, auditorium and laboratory) and environmental factors, including population presence.

The concentration of the bioaerosols varied with the type of population present at the site. The presence of animal population created a large difference in the concentration of the bioaerosols (both bacterial and fungal) compared to other sites. However, at the cowshed and poultry site, the concentration of bioaerosols has a large difference; this may probably

be because of the cleaning of the cowshed daily. Cow milk was distributed twice a day in the campus, so the maintenance of domestic hygiene, like showering the cows and clearing and cleaning the floor, is the primary concern of the concerned workers. However, associated anthropogenic activities and the abundance of vegetation in the surroundings possibly escalate the concentration of bioaerosols through cross ventilation, resulting in the site posing the second highest concentration of bioaerosols among the identified sites.

In contrast, temperature and relative humidity variations did not affect much of the bioaerosol concentration. In laboratory conditions, the chance of getting bioaerosols was low due to a lower population load. Bioaerosol concentration at the hospital site was calculated as having the third highest concentration because of the large population, possibly including infected people. This may also be aggravated due to the presence of pathology, dressing room and toilet (Baurès et. al.,2018; Méheust et. al., 2013; Méheust et. al., 2014). Further, the indoor concentration of bioaerosols may potentially influenced by the influx of contaminated air from the outside.

### 6.4 Conclusion and future perspective

The concentration of the bioaerosols were measured at seven different indoor locations within an urban ecosystem. Accounted bioaerosols were further classified as bacterial and fungal populations and the effect of ambient temperature, humidity and population load on bioaerosol concentration was explored. The average concentration of bioaerosols was found to be highest at animal occupancy sites (cowshed, poultry), compared to the laboratory. The other five sites viz. canteen, library, auditorium, hospital, and site laboratory, which were mainly influenced by human interventions, had significantly lower concentrations than poultry and cowshed. The concentration of bacterial and fungal bioaerosols generally increases with increasing population at these sites.

In the size segregation analysis, there were different patterns have been observed in various indoor environments. Where the prevalence of bacterial bioaerosol was found to be maximum at Size >7.0 µm while the minimum for size between 0.65-1.1µm. 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 1.1 µm. On the basis of the size distribution of the bioaerosols, it can be said that these can reach the deep site of 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 present microbes can harm us through gastro and respiratory issues if a large concentration is present. However, if routine cleaning of the area using disinfectant is being done then it 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 was mainly due to season-specific analysis, which resulted in low variations of ambient conditions in different sites. Other factors such as ventilation systems, moment of wind, human activities and the interior structure materials may 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.

This work has been done for the microbial concentration of bioaerosols in various indoor environments. However, there are more indoor study sites that can be included, and the seasonal variation in the indoor environment can be explored. Also, advanced characterization, like the whole genome approach, can be used for biological characterization.