

Chapter 5

Bioaerosols Over the Indo-Gangetic Plain: Influence of Biomass Burning Emission and Ambient Meteorology

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Abstract Bioaerosols (particles of biological origin) can be produced from living or dead plants and animals. They can potentially serve as the cloud condensation and ice nuclei (CCN and IN). Their role in global carbon cycle further highlights importance of studying their variability to link up with climate relevance parameters. Focusing on tropical region reveals that it holds wealthy number of human population and has massive vegetation cover-area. From Indian region, production estimates of bioaerosols from human population (current: ~ 1.25 billion; of which over 45% resides in Indo-Gangetic Plain: IGP) and Wildlife Sanctuaries and National Parks (100 in numbers, situated from north to south and east to west) is not known. Most of the forest fires in India occur during March–June (hot and drier season). The detailed information on chemical composition, fingerprinting and radiative forcing from regional forest fires is also lacking. Unlike natural sources (forest cover and fires), the seasonal variability of pollutants emission characteristic and chemical, optical and radiative forcing are relatively well studied from anthropogenic biomass (post-harvest paddy- and wheat-residue and biofuels) burning emission in India. However, the abundance of bioaerosols and their variability over a large stretch of IGP (north-west to north-east) was not well documented. Towards this, we have undertaken a year-long campaign to study and document (first-attempt) bioaerosols variability over a complete annual cycle from central IGP. We observed a parallel enhancement in concentrations of fine-particulate matter ($PM_{2.5}$ in October–November: $158 \pm 89 \mu\text{g m}^{-3}$ as compared to June–September months: $40 \pm 18 \mu\text{g m}^{-3}$; two-tailed $t = 8.2$, $p < 0.05$) and bioaerosols (particularly Gram-negative bacteria: GNB, a source of endotoxin in ambient air; $186 \pm 87 \text{CFU/m}^3$ during October–November as compared to $114 \pm 58 \text{CFU/m}^3$; $t = 4.0$, $p < 0.05$) with the biomass burning emissions intensification period. The abundance of bioaerosols exhibits influence of ambient meteorology, for example GNB exhibited negative correlations with T, wind speed

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and heavy (>4 mm daily) precipitation, whereas it showed positive correlations with RH and low precipitation amount (<4 mm). Studying bioaerosols and establishing its linkage to health and climate appear to be of utmost importance.

Abbreviations

CFU	Colony forming units
EU	Endotoxin units
GNB	Gram-negative bacteria
GPB	Gram-positive bacteria
IGP	Indo-Gangetic Plain
K^+_{BB}	Biomass burning derived K^+
LPS	Lipopolysaccharides
OC	Organic carbon in particulate phase
RH	Relative humidity (in %)
T	Ambient temperature (in °C)

5.1 Potential Impacts of Assessing and Control of Bioaerosols

- (i) Micro-environment of operating rooms could be a high-risk area for both patients and staffs. Thus, managing the air quality and ensuring it free from airborne infectious agents are of utmost importance in such environments. Deployment of filtration equipment (proper air conditioning systems) can reduce airborne concentrations of fungi significantly. It is worthwhile mentioning that airborne bacteria could cause severe infections during surgery. It has been observed that reduction of airborne bacteria in operating rooms is directly linked with the substantial decrease in contamination of wounds.
- (ii) In India, there is an urgent need to develop tools to accurately measure bioaerosols, to quickly identify them in order to have a baseline information and establish a link to infections to make appropriate strategy to mitigate and control these especially in the sensitive indoor environments (hospitals, school, shopping malls etc.) and to the influence on climate.

5.2 Concepts of the Synthesis

Biologically produced (from plants/animals) aerosols are widely referred to as bioaerosols (also referred to as primary biological aerosol particles: PBAPs) (Després et al. 2012; Fu et al. 2008; Miyakawa et al. 2015; Pöschl et al. 2010).

Improper sanitation, waste-disposal practices and biomass burning may also result into generation of huge amounts of microbes in the air (Taha et al. 2007). In the atmosphere, they are ubiquitous as bacteria, viruses, fungal spores, bio-debris and pollens (Fišar et al. 1990; Lau et al. 2006; Prussin et al. 2015; Reponen et al. 2007; Sesartic and Dallafior 2011). The size of different types of bioaerosols varies over a large range (Reponen et al. 2001). For example, viruses are generally of the size of less than 300 nm, bacteria are in the range of 0.3–8 μm , fungal spores in the range of 1–30 μm and size of pollens are greater than 17 μm (Gregory 1973; Stanley and Linskins 1974). The size of bio-debris (fragments from plants/animals) may vary from sub-micron to coarser fraction (Graham et al. 2003a, b). It is important to mention here that bioaerosols can adsorb onto existing particles in the ambient atmosphere, and thus, their aerodynamic diameter and residence time can be influenced by the physical characteristics of the suspended particulates (Donaldson et al. 2001; Shaffer and Lighthart 1997). The contribution of bioaerosols in total particulate matter has been assessed previously from different environmental conditions reporting 28% over remote continental, 22% in populated continental and 10% in remote maritime environments (Matthias-Maser et al. 2000). A recent study (Zhu et al. 2016) has studied quantitatively the contribution of PBAP during daytime and night-time in a temperate coniferous forest in Japan (at Wakayama). They have measured biomarkers of PBAP (fungal spores tracers: arabitol, mannitol and trehalose) through solvent extraction followed by derivatization approach. The contribution of fungal spores in organic carbon (OC) was found to be relatively high in their study during night-time (45%) as compared to that in daytime (22%), and they have attributed this observation to nocturnal sporulation under near-saturated RH condition. Thus, the contribution of bioaerosols to OC can be quite significant over/near the forest/polluted region (Huffman et al. 2012, 2013). The spatial distribution of Wildlife Sanctuaries and National Parks in India is shown in Fig. 5.1. They are expected to representing background source of bioaerosols in Indian region and particularly over the Indo-Gangetic Plain.

It has been widely realized that bioaerosols play a very important role in climate change through participation in carbon cycle of the ecosystem and holding potential cloud condensation nuclei as well as ice-nucleation activity (Bauer et al. 2003; Fröhlich-Nowoisky et al. 2009, 2016; Garcia et al. 2012; Hawkes et al. 2011). In addition, they are widely studied also because of their many allergenic effects on the human health (Annadotter et al. 2005; Balasubramanian et al. 2012; Clark et al. 1983; Domanska and Stroszejn-Mrowca 1994; Semple et al. 2010; Xue et al. 2016). Human exposure to bioaerosols through physical contact or inhalation may lead to adverse health effects like asthma, COPD (chronic obstructive pulmonary disease), whooping cough, and sick building syndrome (GBD 2015). Study to understand their occurrence and effects in indoor and outdoor environments is one of the major thrust areas of research (Iossifova et al. 2007; Kildeso et al. 2003; Lee et al. 2006). However, despite being a very important area of research, bioaerosols have been studied little less over Indian region (Ansari et al. 2015; Kumar and Attri 2016; Kumar et al. 2011; Mamta et al. 2015). Recently, there are studies emerging out from southern part of India documenting the number and mass distribution of

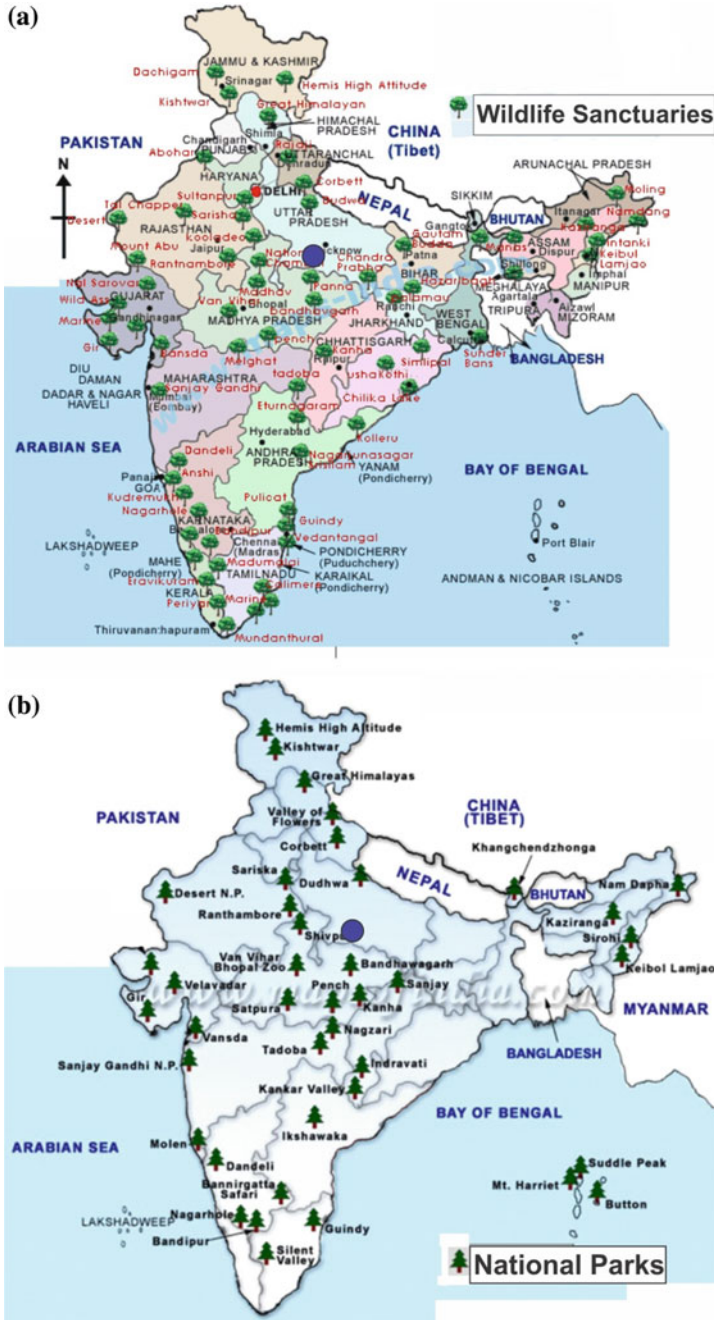


Fig. 5.1 Geographical distribution of: **a** Wildlife sanctuaries and **b** National parks in India (Chauhan 2016)

fluorescent biological aerosol particles (FBAP), a lower limit of PBAP (Valsan et al. 2015, 2016).

Endotoxin is a biologically active LPS (lipopolysaccharide) and a component of outer membrane of the Gram-negative bacteria (GNB) (Clark et al. 1983; Domanska and Stroszejn-Mrowca 1994). They have been studied widely from various environmental conditions (Annadotter et al. 2005; Balasubramanian et al. 2012; Rathnayake et al. 2016a; Semple et al. 2010; Smith et al. 2004; Xue et al. 2016). Epidemiological investigations have suggested a modest effect of endotoxin exposure on morbidity pertaining to asthma (Michel et al. 1996; Salonen et al. 2016). Furthermore, acute exposure of endotoxins to humans can cause blood/lung inflammatory responses (Michel et al. 1992). Towards this, it is worthwhile mentioning that a recent study over eastern part of India (states of Odisha), reported PM₁-bound indoor levels of endotoxin (100–160 EU/m³; EU: endotoxin units) from biofuel burning (Padhi et al. 2016). They have also reported the estimates on penetration of PM₁-bound endotoxins into alveoli region.

Bioaerosols represent the air suspended particles that are living (Fungi, bacteria and viruses) or have been originated from living organisms (e.g. pollens from plants). Their presence in atmosphere is plausibly a function of dispersal from a site of colonization, survival and/or growth. The health effects of bioaerosols include allergies, infectious diseases and acute toxic effects. Furthermore, cancer in conjunction with the threat of bioterrorism and SARS (severe acute respiratory syndrome) has increased public awareness on the importance of study on bioaerosols. There are numerous technical methods for sampling bioaerosols and can be employed depending on the sensitivity of the method and the concentration of micro-organisms. There have been difficulties and challenges in standardization of sampling methods. The major problems include establishment of a causal relationship arising due to complex composition of bioaerosols and variation in human response as a function of exposure. It has been a widely followed activity to monitor bioaerosols in various micro-environments for epidemiological investigations of infectious diseases. The research on airborne micro-organism's abundance, spread and control represents as a quality control measure on monitoring bio-hazardous and relevance to their impact on climate (as CCN: cloud condensation nuclei and IN: ice nuclei). In many developing countries including India, there is a very little awareness on the indoor air quality, contamination of mould and potential factors for transmission of infections (ranging from mild influenza to deadly tuberculosis).

There is an urgent need to assess indoor air quality, develop tools to accurately measure these bioaerosols, cater techniques to quickly identify them and explore link to infections, and finally to make appropriate strategy to mitigate and control these especially in the sensitive indoor environments (hospitals, schools, shopping malls etc.). The present synthesis involves extensive literature review to screen through available techniques for bioaerosol monitoring, its adaptation and modification for Indian condition followed by its use in different indoor and outdoor micro-environments to investigate the types and concentration levels of viable (living) bioaerosols.

5.3 State of the Art and Progress Envisaged

Details on the state of the art and envisioned progress relevant to the bioaerosols sampling, analysis and health effects are described below. It also points out the technological baseline on different concerned aspects, for example technologies that have been brought into operation over a period of time to collect and study the bioaerosols. Finally, the ongoing research aims to advance the state of the knowledge on bioaerosols.

(A) Health effects of bioaerosols

Exposure to high concentrations (or unfamiliar forms) of bioaerosols could lead to biological hazards to humans. The three broad classes of diseases associated with the bioaerosols exposure are respiratory diseases, infectious diseases and cancer. It is worthwhile mentioning that current knowledge on the risk to cancer from exposure to biological agents is limited.

5.3.1 Allergies

Various kinds of bioaerosols are responsible for irritation and allergic responses and few common ones include pollens like ragweed, insect or their body parts like dust mites, cockroach body parts. The major concern among these allergens is on the pollens. Pollen grains represent as the male gametophyte in sexual reproduction of flowering plants (angiosperms) and conifers (gymnosperms). Pollination refers to a process of transfer of pollen grains from male to reproductive structure of female. It can be accomplished via three routes: vectors-wind, water or animals. The flowers of these plants often do not have petals, and thus, anthers (pollen sacs) are directly exposed to air. Hence, pollens from anemophilous plants are most abundant in ambient atmosphere and have tremendous influence in terms of human exposure and related seasonal allergies. Pollen grains are nearly spherical in shape, at least under hydrated condition, with a rigid cell wall structure formed up of a complex polysaccharide substance known as sporopollenin.

5.3.2 Infections

Infectious diseases basically arise from bacteria, fungi, viruses, protozoa and helminthes. It involves transmission of infectious agents in air from a reservoir to a susceptible host.

- (i) **Bacteria-induced diseases:** Various diseases due to bacteria such as tuberculosis and legionellosis are of more concern as far as public health is

concerned owing to their pretty fast response against low infectious dose. Most important ones have been discussed as follows.

- (a) **Anthrax:** The transmission occurs due to inhalation of the *Bacillus anthracis* spores and the outbreaks are usually linked with occupational exposure-based bioterrorism.
 - (b) **Illness by endotoxin:** Endotoxin is the lipopolysaccharide (toxin) component of Gram-negative bacterial cell wall. These are potent pyrogens, capable of causing fever at very low concentrations. High exposure to endotoxins could lead to nausea and diarrhoea.
 - (c) **Legionellosis:** *Legionella pneumophila* causes human legionellosis and nosocomial pneumonia in adults following occupational/non-occupational exposures. Certain active aerosolize processes, for example aeration of contaminated waters, cause legionellae to become airborne. Their occurrence has been noticed in various water environments including man-designed water systems, biofilms in the cooling towers, A/C (air conditioners).
 - (d) **Tuberculosis:** The transmission of tubercle bacilli occurs upon inhalation of aerosolized bacilli in droplet of expectorated sputum-positive from tuberculosis patients during ejection of cough, sneezing and occasionally while talking. There have been outbreaks of multi-drug resistant tuberculosis in the UK that have highlighted the potential for transmission within the hospital environment.
- (ii) **Fungal diseases:** Fungi are saprophytic parasitic organisms that occupy a kingdom of their own. They can cause the aerobic decay of plant materials and are ubiquitous in air, often as the predominant component of bioaerosols. The fungal cell is eukaryotic and embodies a well-developed membrane system. The rigid cell wall is made up of β -glucans and acetyl glucosamine polymers (chitin). Their wall often contains waxes and has extra cellular polysaccharides coating. Fungi can be identified by the method and nature of spore production. Lichens can be formed from the fungi and algae following a symbiotic relationship. Most of the spores and pollens have density near to unity, and thus, their aerodynamic diameter primarily depends on its shape as well as its size. It is worthwhile mentioning that many spores are hygroscopic, and therefore, their aerodynamic diameter increases as a function of increasing humidity. Mycotoxins are secondary metabolites of some fungal species such as *Fusarium*, *Aspergillus*, *Penicillium* and *Trichoderma*. Fungi causing respiratory infections and allergenic reactions to humans include *Penicillium*, *Cladosporium*, *Acremonium*, *Paecilomyces*, *Aspergillus* and *Mucor*. Most infections (commonest being *Aspergillosis*) occur in immune-compromised hosts upon inhalation of fungal spores or toxins produced by them. Symptoms include watering eyes, persistent cold, prolonged muscular cramp and joints pain. *Histoplasma*, *Coccidioides* and *Blastomyces* grow in soil or may be carried by bats and birds and are linked with the exposure to airborne/animal-borne contamination. The volatile organic products released from

fungal metabolism have ability to induce irritation in eyes and in the upper respiratory tract. *Aspergillus* species that can grow in indoor environments include *Aspergillus fumigatus* and *Aspergillus flavus* and can cause nosocomial infections, allergic broncho-pulmonary aspergillosis (ABPA) and sinusitis.

Mycotoxins-induced illness: Mycotoxins can get absorbed on the intestinal lining, airways and skin. *Aspergillus*, *Fusarium* and *Stachybotrys* act as aero-allergens and also act as a source for mycotoxins. A case report from the USA described upper respiratory tract irritation and rash in a family living in a Chicago home with a heavy growth of *Stachybotrys atra* producing trichothecene mycotoxins. The chemical structure of trichothecene is shown in Fig. 5.2.

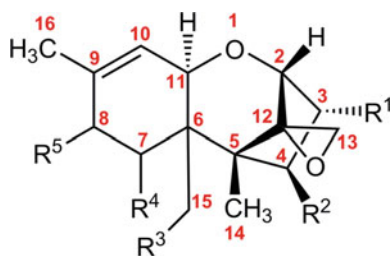
The symptoms diminish as a function of substantial reduction in the amount of mould. Other adverse health effects include pre-term births or late abortions in farm women exposed to mycotoxins with immunotoxic and hormone-like effects.

- (iii) **Cancer:** It is widely believed that occupational carcinogens of biological nature are the mycotoxins. Aflatoxin from *Aspergillus flavus* is capable of causing liver cancer whereas Ochratoxin Asia is a plausible human carcinogen. Exposure to aflatoxin and ochratoxin usually occurs by the ingestion. However, it can also occur by inhalation in industries of peanut processing, livestock feed processing or when grain dust exposure occurs. Studies have found association between exposure to wood dust and cancer in specific part of body. For example, sinonasal cancer has been found to be prominent in the people working on furniture making or doing wood-related jobs like in sawmills.

(B) Factors affecting the fate and transport of bioaerosols

Transport and the ultimate settling of bioaerosols are influenced by its physical properties and the environmental parameters that it encounters. Important physical characteristics include shape, density and size of droplets/particles, whereas the environmental factors include relative humidity, temperature and magnitude of air currents. These parameters basically determine the capacity of bioaerosols to remain airborne. Bioaerosols produced from liquid suspensions (or bursting of bubbles) undergo desiccation, whereas those generated during particle emanation may partially rehydrate under ambient atmospheric conditions. The presence of

Fig. 5.2 Chemical structure of trichothecene (Appell and Bosma 2015; Chauhan 2016)



moulds refers to a problem with high humidity or water penetration. Typical concentrations of viable bacteria and fungi from air systems and indoor surfaces are given in Table 5.1.

(C) Sampling methods of bioaerosols

There are different techniques for collecting bioaerosols. Major characteristics of bioaerosols sampling techniques have been enumerated as follows:

I. Gravitational samplers (e.g. settle plates)

- Collection medium includes: coated microscopic slides, agar medium plates etc.
- Particles collection by passive sampler (non-volumetric) is based on gravity settling principle.
- Collection efficiency of particles under turbulent air flow is poor.
- Can suffer with particles overloading particularly for larger particles.

II. Inertial bioaerosols samplers

- Facilitates size-segregated particles sampling.
- Relies on inertia that helps particles to deviate from airflow streamlines.
- It includes impactor, sieves and stacked sieves.

III. Spore traps

- Firstly designed for collection of pollens and fungal spores. For example, Air-o-cell, Burkhard, Hirst and Allergenco.
- Particles are basically allowed to impact onto coated glass slide/adhesive surface.
- Air sampling is preferred at low volume (10–20 L/min).
- Direct analysis after sample collection is feasible.
- This approach of sampling can mask some of the species, and viability tests are not possible.

IV. Impaction-based samplers (Impactors)

- Particles are allowed to impact on slide/agar plates.
- Used at air flows of 10–30 L/minute.

Table 5.1 Typical concentrations of bioaerosols in air conditioning systems and on indoor surfaces

Category	Activity type	Bacteria	Fungi
		(CFU/m ³)	
Air conditions	HVAC	10–10 ⁴	10–10 ⁷
Indoor surfaces	Ceilings and walls	10–10 ³	10–10 ⁴
	Carpet	10 ³ –10 ⁶	10 ² –10 ⁵
	House plants	10–10 ⁴	10 ² –10 ⁵
	Operating room	10–10 ²	10–10 ²

HVAC Heating, ventilation and air conditioning

- Bouncing effect of smaller particles can be an issue.
- Different types of impactors are as follows:
 - (a) Single or multistage samplers (e.g. Anderson).
 - (b) Sieve and stacked sieve samplers (e.g. SAS).
 - (c) Rotary arm samplers (e.g. Rotorod, Mesosystems BT550).
 - (d) Agar samplers.

The impaction mechanism of aerosols and inertial bioaerosol sampler is shown in Fig. 5.3.

V. Impingers

- Particles in the air are removed by impingement when air is allowed to pass through liquid (e.g. water and oil; Fig. 5.4).
- Operated at a flow rate of 0.1–15 L/min (e.g. 12.5 LPM for AGI 30).
- It allows for the dilution.
- There are several challenges and issues involved: particle bounce, pass through, bubbling of liquid and loss of viability features.
- Collection efficiency decreases with particles size (decreases significantly for the particles $>10\ \mu\text{m}$).

(D) Analytical techniques for qualitative and quantitative determination of bioaerosols

(i) Microscopy

It is simply described as the use of microscope to magnify an object. It was invented by *Antonie van Leeuwenhoek*. In aeroallergen studies, suitable magnification can vary from low (e.g. 3.5X), for viewing fungal fruiting bodies with a stereo microscope, to very high (e.g. 10,000X), for assessing surface of pollen grain

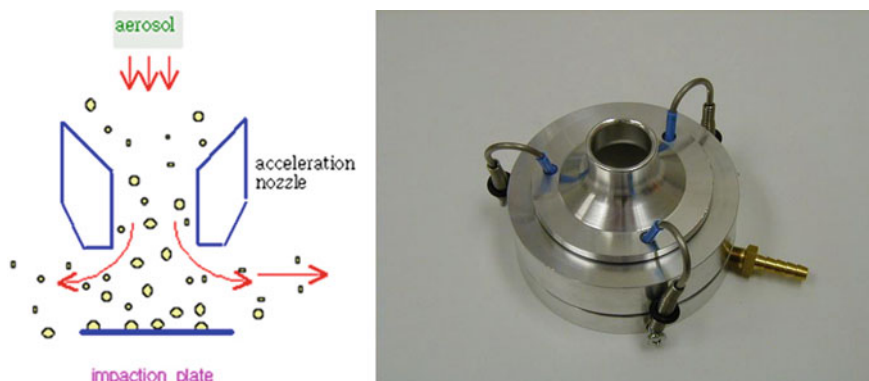


Fig. 5.3 Aerosol impaction mechanism (shown on left) and an inertial-based bioaerosol sampler (on right, MSP Inc., USA; on right)

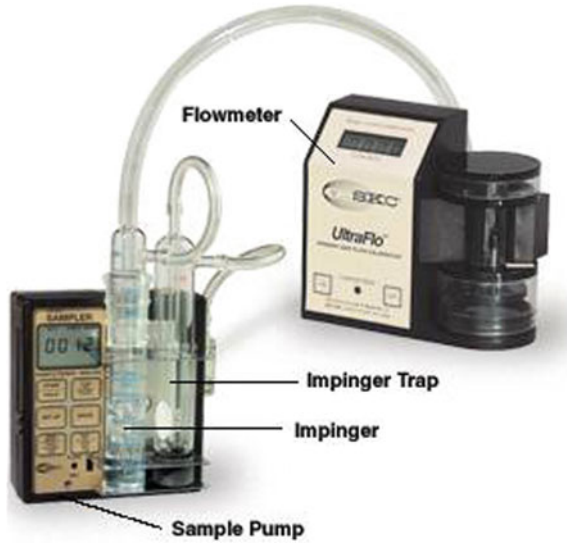


Fig. 5.4 Liquid-impinger-based bioaerosol sampler (SKC, USA)

from electron microscope. Although commonly used to identify fungal spores and pollens in air samples, microscopy also facilitates identifying fungal colonies in culture and spores associated with dust or other source samples. Most common airborne pollen grains can be studied with an optimal magnification for sample scanning and identification of 300–400X. Counting of many fungal spores and most pollen grains can be accomplished with a microscope having magnification at least a 100X oil immersion as well as 40–10X objectives. Bacteria are rarely recognizable in particulate air samples unless proper staining techniques are used.

Types of Microscopy: bright-field microscopy, light microscopy, dark-field microscopy, fluorescence microscopy, phase-contrast microscopy, electron microscopy: transmission electron microscope (TEM) and scanning electron microscope (SEM). Some of the widely used microscopic techniques in bioaerosols research are shown in Fig. 5.5.

(ii) ***Bioaerosols collection/sampling and culture***

A bioaerosol sampler based on the concept of inertial impactor and specific agar plate as a collection substrate and culture medium has been designed and developed at IIT Kanpur, India (Fig. 5.6). Schematic below depicts a typical experimental set-up which is usually employed for characterizing an inertial-based sampler. Apart from the dry aerosol, we also used PSL (polystyrene latex) particles tagged with active groups to determine and optimize the impactor cut-point. Most of the relevant bioaerosols lie in the range of 1–10 μm . So a logical cut-point will be 10 μm . The calibrated airflow rate of this sampler is 12 LPM.

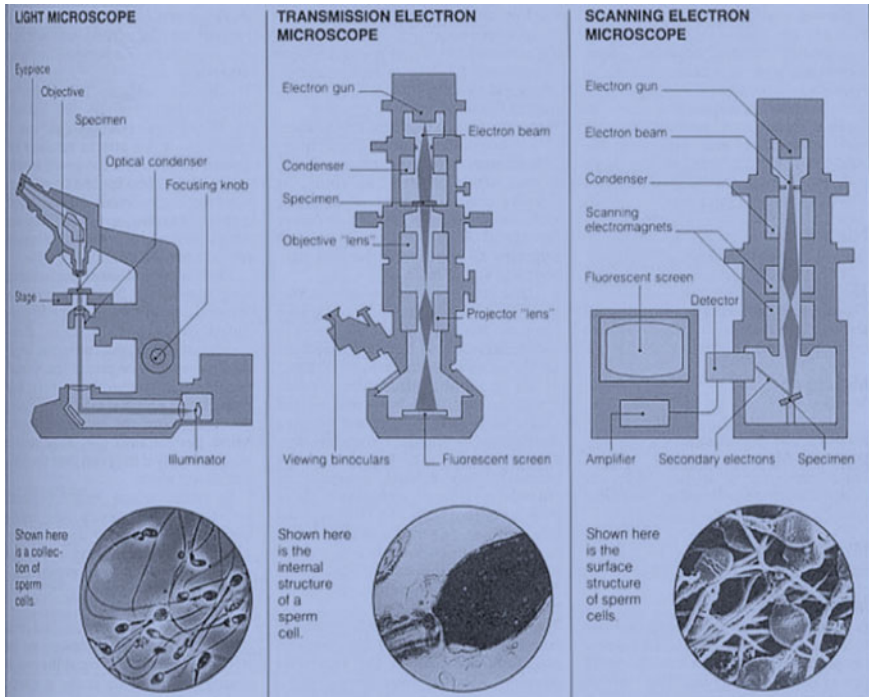


Fig. 5.5 Different types of microscopic techniques used in bioaerosols research (Chauhan 2016)

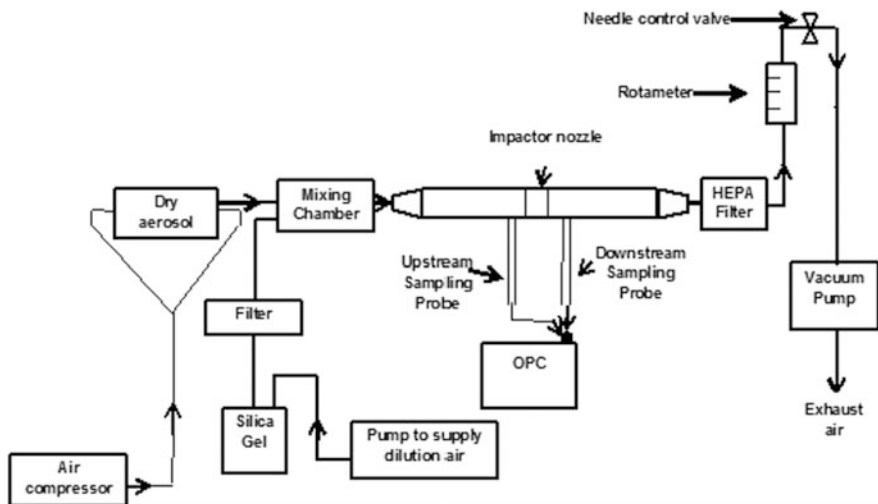


Fig. 5.6 Experimental set-up of bioaerosol impactor sampler characterization (indigenously designed and developed at IIT Kanpur)

For fabrication of the developed bioaerosols sampler, brass has been used due to its advantage in machining, long-term durability, stability and inert characteristics. Chrome plating was further done to shield it with an inert and corrosion-free layer. The sampling device has basically three zones. Top of the sampling device is provided with the rain cover.

- Zone 1 Facilitates collection of bigger particles and spores settled due to gravity.
- Zone 2 Consists of two stages with cut-off points of PM_{10} and $PM_{0.6}$ achieved at an airflow rate of 12 LPM. The first stage is PM_{10} stage. It consists of a nozzle plate containing four round nozzles and an impaction plate. The impaction plate can accommodate four 35 mm petri dishes in the respective slots below the nozzles. Second stage is $PM_{0.6}$ stage. It consists of one nozzle plate with single round nozzle and one corresponding impaction plate. This impaction plate can house one 35 mm petri dish.
- Zone 3 It has provision to house one 47 mm filter. This zone can collect $PM_{0.6}$ particulate matter on filter paper that can be subjected to further gravimetric and chemical analysis.

The sharp cut-off points at 0.6–10 μm make this device suitable for collecting the particles from 0.6 μm (stage 2) to 10 μm (stage 1; Fig. 5.7). Impaction plate at first stage collects the particle with size greater than 10 μm . Particles of size less than 10 μm travel down towards the second stage. The impaction plate on the second stage collects the particles of size 0.6–10 μm .

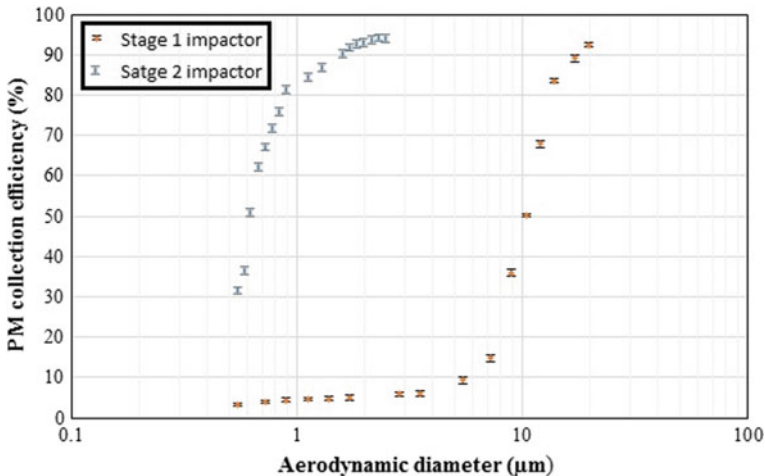


Fig. 5.7 Particulate matter collection efficiency curve of two stage impactor sampler at cut-points of 0.6 (stage 2) and 10 μm (stage 1), adopted from (Gupta and Chauhan 2014a)

(iii) *Bioassay*

This technique is most commonly used to analyse endotoxin components of the aerosols. Endotoxin is pro-inflammatory substance present in Gram-negative bacteria (GNB). They have been noticed to associate with workplace illnesses and suspected of playing a role in the development of non-specific building-related symptoms, widely referred to as the sick building syndrome (SBS). At times they are also responsible for severity of asthma. Endotoxin is shed off from the outer membrane of GNB as membrane fragments while growing or dying GNB. When endotoxin is purified, it consists of family of proteins called as lipopolysaccharides (LPS). LPS is composed of lipids and carbohydrates; the lipid portion is basically responsible for the toxicity (Fig. 5.8).

Endotoxin is an integral part of the GNB, and therefore, their occurrence is proportional to the occurrence of GNB. Their abundance can be influenced by environmental conditions such as substrate availability, humidity and temperature. Since these conditions are favourable outdoor, GNB and therefore endotoxin are ubiquitous outdoors. High levels have been detected in numerous settings especially where organic dust is present such as in agricultural and related industries. Filter media is usually used to collect endotoxin aerosols because they allow for long collection times and are easy to practically use. At times, all glass impingers

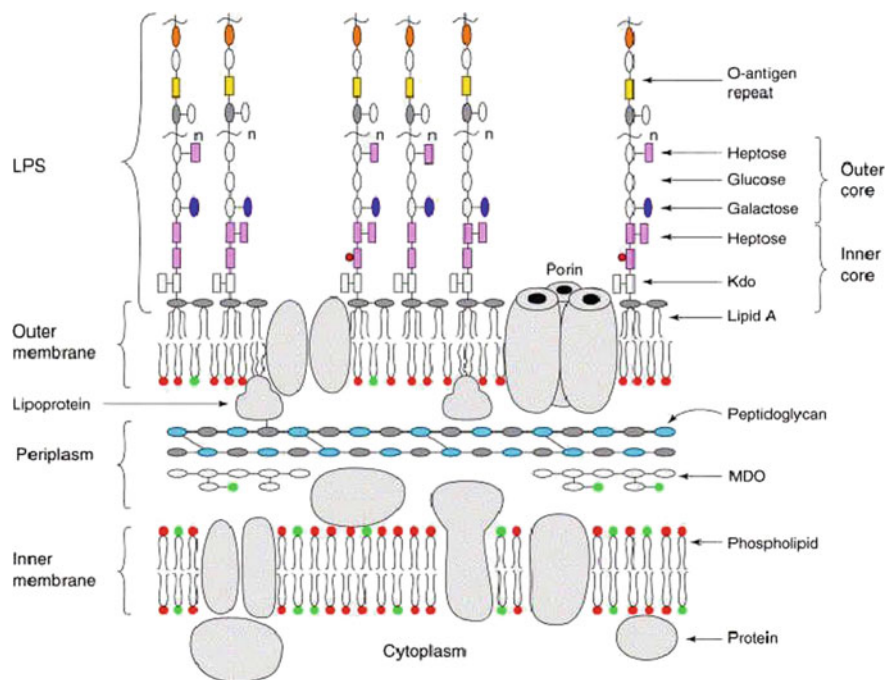


Fig. 5.8 Structure of cell wall of Gram-negative bacteria (Brown et al. 2015; Chauhan 2016)

are used but they may underestimate endotoxin because of their low collection efficiency for sub-micron particles which contain large amount of endotoxin.

The detection of the endotoxin is based on the use of a Limulus Amoebocytes Lysate (LAL) assay performed with the LAL reagent: aqueous extract of circulating amoebocyte of horseshoe crab (*Limulus polyphemus*). LAL assay is based on the observation of a gel clot formation when an endotoxin comes in contact with clot table protein from circulating amoebocytes of *Limulus*. For LAL assay, endotoxin is extracted from filters with an aqueous extraction medium. Most laboratories use pathogen-free water, while some use buffers as tris and phosphate triethylamine (pH: 7.5) or dispersing agents such as tween -20, and it was reported that endotoxin activity was seven times higher in tween medium as compared to that in any pathogen-free water.

The most common method of filter extraction is sonication and rocking in an extraction medium. Generally, environmental samples for endotoxin analysis should not be frozen especially after their extraction. Alternatively, the most widely preferred *in vitro* method is the LAL assay owing to high sensitivity. However, this assay does not mimic for the *in vivo* pyrogenic activity. Following this assay, major and minor pyrogens, endotoxin (C pathway), and 1, 3-glucans β (G pathway) of fungi can be measured. However, this assay gives negative result for the pyrogenic substances from Gram-positive bacteria (GPB). Depending on the chemical and physiochemical structure of the endotoxin, the reactivity of the LAL reagent also differs. The LAL assay is a comparative toxicity bioassay and not an analytical assay. This means the measured endotoxin levels can be altered by factors other than the actual LPS concentration. Due to this very reason, the interpretation of the results becomes difficult especially when comparing observation from different filter media.

Detection technique: LAL reagent is added to the sample (in a pre-cleaned/ pyrogen-free test tube). The sample is then incubated at 37 °C temperature for nearly 1 h. The tube is then gently inverted. The formation of a gel or clot confirms the positive result. LAL assay can be applied following the endpoint method or kinetic reaction. A variety of LAL assays are gel clot, chromogenic measurements and turbidimetry. The chromogenic assay depends on a chromogen, which changes its color in the presence of endotoxin (in sample). The chromogen release is a function of the concentration of endotoxin. Optical measurement device operating at wavelength pertaining to chromogen signal is required. LPS can also be detected in environmental samples via chemical methods. These methods are based on assessing chemical markers of LPS. Of these markers, 3-hydroxy fatty acids are most commonly utilized for characterization of endotoxin and GNB. Gas chromatography technique is the most followed method to do this characterization.

5.4 Case Study on Bioaerosols Assessment Over Central Indo-Gangetic Plain

i. Site description

The study site at Kanpur (Urban area: 26.30 °N; 80.14 °E; 142 m above mean sea level) is situated in central part of IGP (Chauhan 2016; Gupta and Mandaria 2013; Kumar and Gupta 2015); IGP is stretched from north-west to north-east region in India (Chakraborty et al. 2017; Rajput et al. 2011b, 2013, 2016a; Singh and Gupta 2016b). This region holds ~40% of the south Asia's population and produces over 85% of the rice-wheat (Gupta et al. 2004; Rajput and Sarin 2014; Singh et al. 2014a). Nearly 20 million hectares of agricultural land area is located in NW part of IGP (states of Punjab, Haryana and western part of Uttar Pradesh). Due to crop rotation activity, a conspicuous seasonal and annual feature, farmers burn 100s of million tons of paddy residues (during October–November) and wheat residues (during April–May) (Gupta et al. 2004; Momin et al. 1999; Punia et al. 2008; Rajput et al. 2014a, b, c). Under prevailing NW winds, our sampling site is strategically located downwind of the major agricultural fields in IGP (Rajput et al. 2011a, 2015; Singh and Gupta 2015, 2016a). Thus, the sampling location is influenced by massive biomass burning activities (Kumar et al. 2017; Rajput et al. 2016b). The region experiences usually ~1000 mm annual precipitation with harsh summers and cold winters associated with fog events (Rajput et al. 2016a; Singh et al. 2014b). However, year 2015 (annual rainfall of 375 mm) was influenced due to El Niño (Rajeev et al. 2016).

ii. Sampling and measurements

Measurements of viable bioaerosols ($n = 130$) and ambient particle number concentrations have been performed for 1 year (12:30–1:30 h) from June 2015–May 2016 at CESE building (Center for Environmental Sciences and Engineering) in the campus of Indian Institute of Technology Kanpur. Using a single-stage impactor (aerodynamic diameter $>0.6 \mu\text{m}$) sampler (flow rate: 12 LPM) (Gupta and Chauhan 2014b), viable bioaerosols were collected at ~1.5 m from ground level and cultured in petri dishes equipped with specific nutrient agar mediums. We have collected (three days a week) and cultured GPB in Mannitol Salt Agar Broth (MSAB), GNB in MacConkey and Fungi in Sabouraud Dextrose Agar medium. Briefly, petri dishes ($n = 3$ for each day sampling) equipped with specific nutrient agar mediums were placed in the air sampler and the collection was subjected to 4 min for each three types of mediums sequentially. Soon after the collection, these bioaerosols in separate petri dishes were incubated at 35 °C for microbial culture in our lab (Atmospheric Particle Technology Lab, in CESE). Subsequently, their enumeration (counting) through magnifying lens was performed into a bio-safety cabinet. Well established protocol has been followed to proper sterilize the sampler as well as nutrient media every time prior to sampling. The observations of colony counting for GPB, GNB and Fungi for different incubation periods are shown in

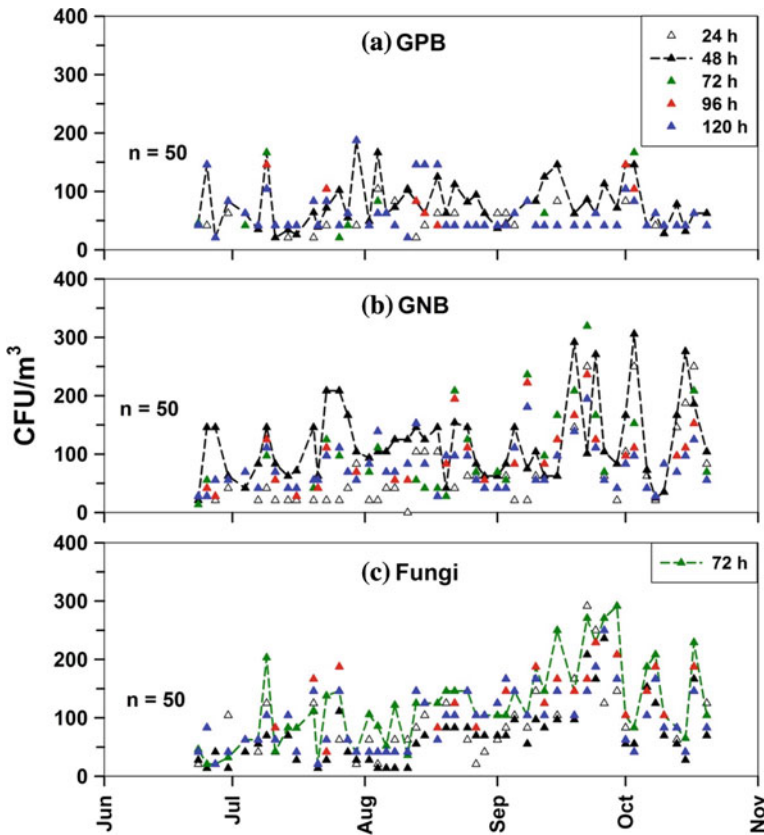


Fig. 5.9 Experiment on optimum incubation period for bioaerosols culture (48 h for both GPB and GNB, whereas about 72 h is required for Fungi growth and counting) (Rajput et al. 2017)

Fig. 5.9. The results shown in Fig. 5.6 suggest that 48 h of incubation is the optimum culture period for GPB and GNB, whereas 72 h is optimum period for Fungi culture.

The images of specific agar mediums prior and post to bioaerosols sampling and incubation are shown in Fig. 5.10.

Furthermore, we have collected PM_{10} (particles with aerodynamic diameter $\leq 10 \mu m$) samples ($n = 130$) using a high-volume air sampler (Envirotech, India, flow rate: $\sim 1 m^3/min$), onto combusted quartz filter substrate for 30 min covering bioaerosols sampling and PNC measurements. Owing to low loading of aerosols onto filter substrate in 30 min sampling-time, gravimetric determination of PM_{10} mass was not carried out. However, our main purpose of collecting PM_{10} samples was to determine the concentrations of organic carbon (OC). OC

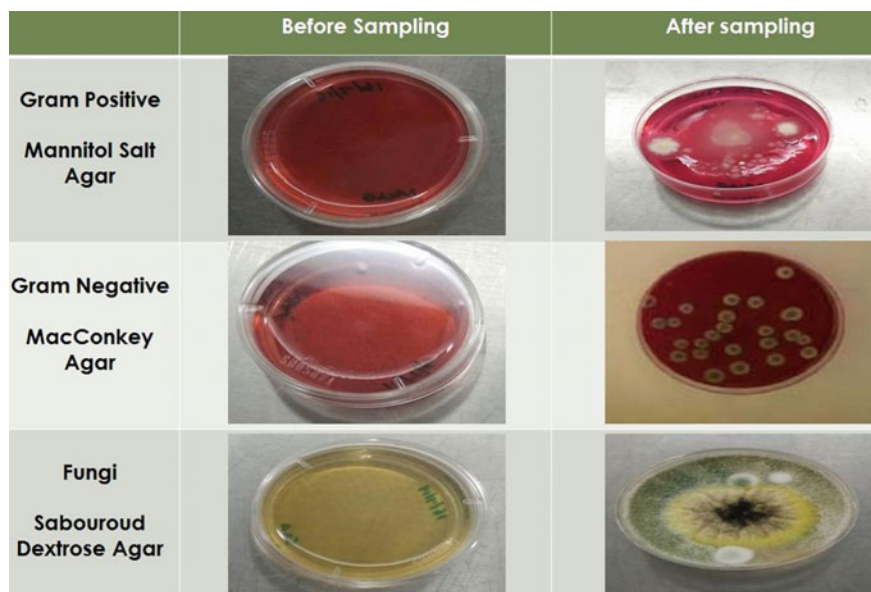


Fig. 5.10 Morphology of GPB, GNB and Fungi colonies in air samples from Kanpur location (IGP)

concentration ($n = 130$) in each sample has been measured on EC-OC analyser (Sunset lab) with NIOSH (National Institute for Occupational Safety and Health) protocol (Birch and Cary 1996).

5.5 Results and Discussion

i. Meteorological parameters

Relevant meteorological parameters including temperature (T), relative humidity (RH), wind speed and rainfall during the study period (~ 1300 h) are shown in Fig. 5.11.

It is important to mention here that wind direction assessed from air-mass back trajectories (AMBTs; Fig. 5.12) shows their most plausible origin in conjunction with overall transport of air-mass, whereas the winds measured at a site represent the striking intensity (wind speed) and direction.

ii. Seasonal variability of viable bioaerosols colonies

The data set of OC and bioaerosols is given in Table 5.2.

Total viable bioaerosols (Σ viable bioaerosols = GPB + GNB + Fungi) concentration averages at 312 ± 118 CFU/m³ in monsoon, 421 ± 114 CFU/m³ in

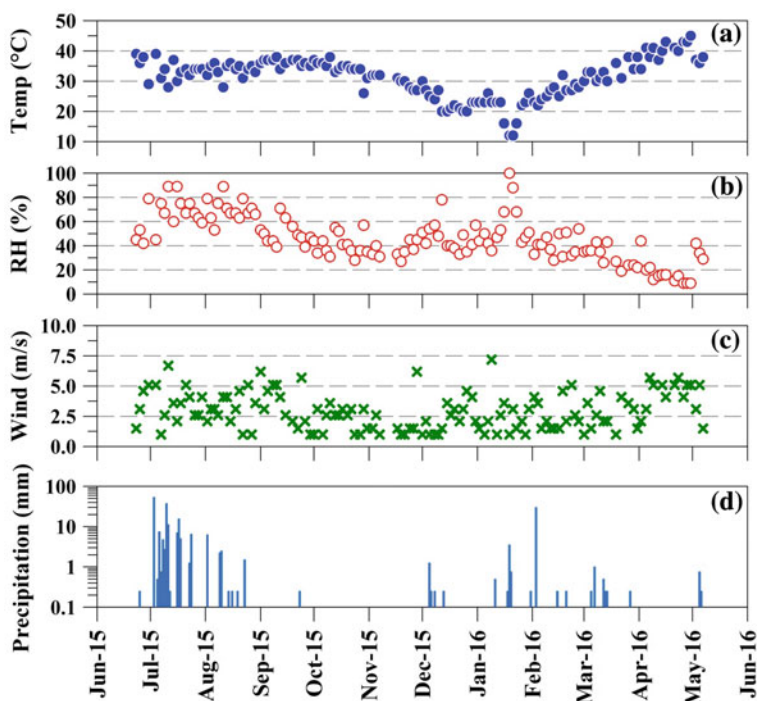


Fig. 5.11 Temporal variability record of meteorological parameters: **a** ambient temperature, **b** relative humidity, **c** wind speed and **d** daily rainfall

post-monsoon, 486 ± 141 CFU/m³ in winter and 223 ± 61 CFU/m³ in pre-monsoon season, at Kanpur in IGP (Fig. 5.13). Thus, maximum concentration of Σ viable bioaerosols was observed during wintertime followed by post-monsoon, monsoon and pre-monsoon. We reiterate that in wintertime, emissions from fossil-fuel combustion and biofuel burning in conjunction with low temperature and shallower boundary layer height are vital parameters co-governing the atmospheric concentrations of PM and viable bioaerosols.

Furthermore, we have also assessed the relative contribution of GPB, GNB and Fungi of the Σ viable bioaerosols (Fig. 5.14). Accordingly, GPB has highest fraction of 32% in wintertime. GNB has highest fraction of 42% during the post-monsoon. This is also reflected in GPB/GNB ratio: 0.85 ± 0.55 in monsoon, 0.73 ± 0.44 in post-monsoon, 0.82 ± 0.17 in wintertime and 0.84 ± 0.37 in pre-monsoon. Summing up, GPB/GNB average ratio is >0.80 in all seasons, exception being the post-monsoon period wherein this ratio averages at 0.73. Relatively lower ratio of GPB/GNB further revisits the observation that post-harvest PRB emissions are associated with elevated concentrations of GNB. However, Fungi have higher fractions during monsoon (37%) and pre-monsoon (39%) with maximum concentration of 292 CFU/m³ during the monsoon (Fig. 5.13). Bacteria/Fungi average

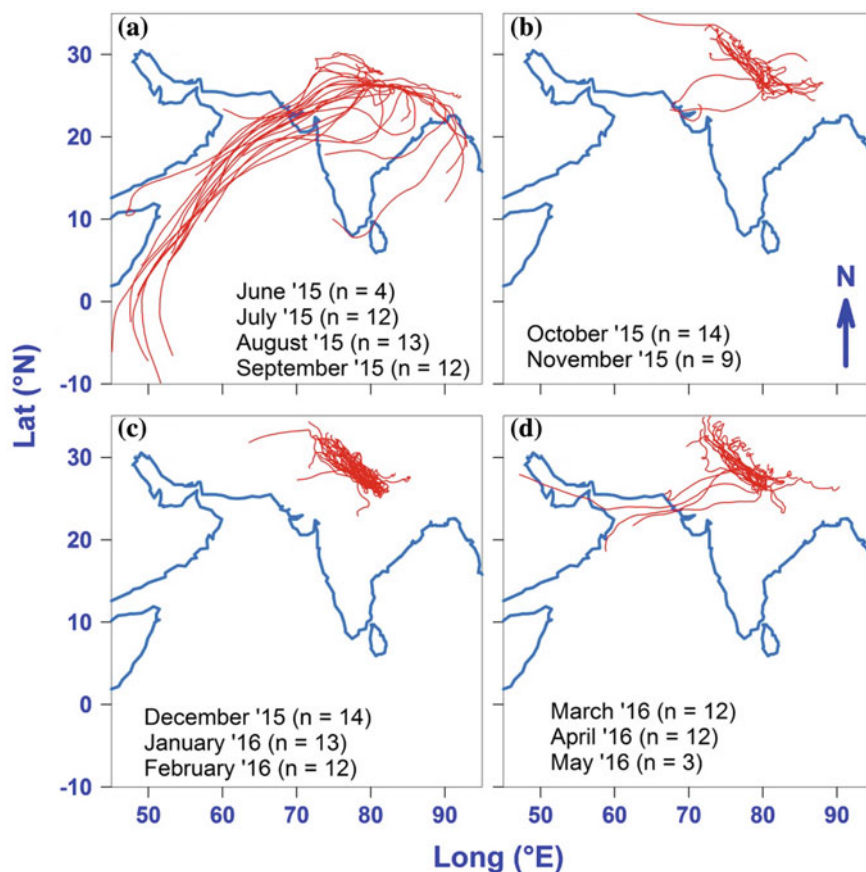


Fig. 5.12 Hysplit-based air-mass back trajectories (7 d; 100 m above ground level) during study period ($n = 130$) in: **a** monsoon (June–September 2015), **b** post-monsoon (October–November 2015), **c** winter (December 2015–February 2016) and **d** pre-monsoon (March–May 2016)

ratio is ≥ 2.5 from monsoon through wintertime, whereas it decreases to 1.7 during the pre-monsoon (Table 5.2). Dry weather condition (low RH and high temperature) prevailing in pre-monsoon is attributable to lower abundance of bacteria.

5.5.1 Correlation Analyses of Viable Bioaerosols (GPB, GNB and Fungi) with Organic Carbon (OC) and Meteorological Parameters

Assessing inter-relationship of bioaerosols with prevailing meteorology is very important to understand the feedback between ecosystem and meteorology (Jones

Table 5.2 Atmospheric concentrations of assessed species [min–max (Avg. \pm 1 σ)] during different seasons in Indo-Gangetic Plain (IGP; at Kampur)

Parameters	Monsoon (June–September) (<i>n</i> = 41)	Post-monsoon (October–November) (<i>n</i> = 23)	Winter (December–February) (<i>n</i> = 39)	Pre-monsoon (March–May) (<i>n</i> = 27)
^a OC	0.3–42.1 (21.2 \pm 8.4)	11.8–47.6 (29.6 \pm 10.3)	18.9–51.8 (34.7 \pm 8.5)	9.6–36.2 (20.5 \pm 5.4)
^b GPB	21–188 (80 \pm 41)	28–166 (112 \pm 44)	63–272 (157 \pm 57)	21–125 (61 \pm 25)
^b GNB	21–292 (114 \pm 58)	25–352 (186 \pm 87)	63–325 (199 \pm 76)	42–146 (77 \pm 26)
^b Fungi	21–292 (118 \pm 71)	65–229 (122 \pm 43)	63–242 (130 \pm 35)	42–146 (85 \pm 25)

^aparticle mass concentration ($\mu\text{g}/\text{m}^3$); ^bbioaerosols abundance in colony forming units (CFU/ m^3)

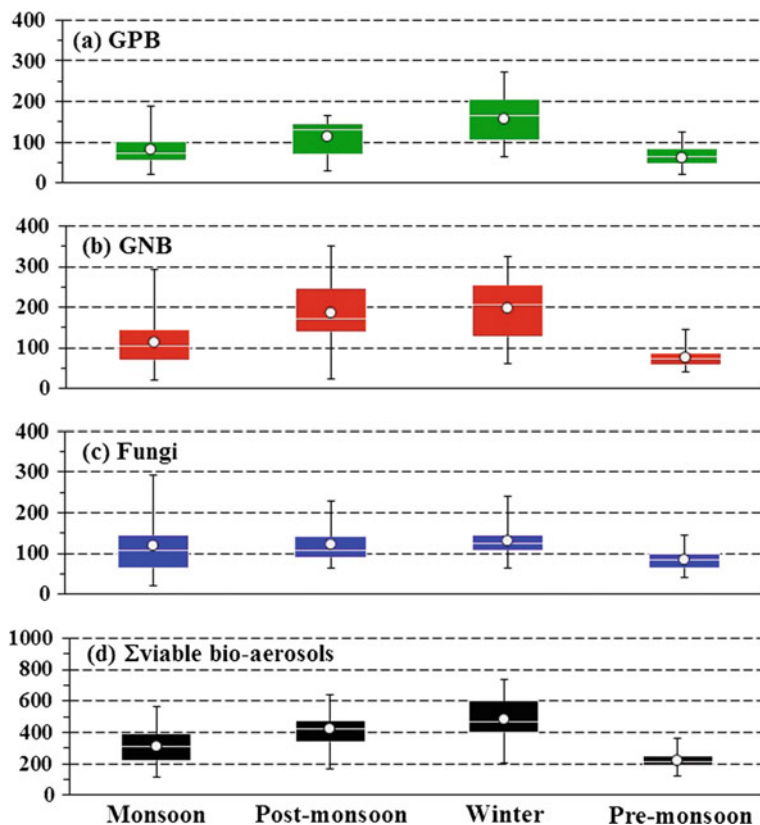
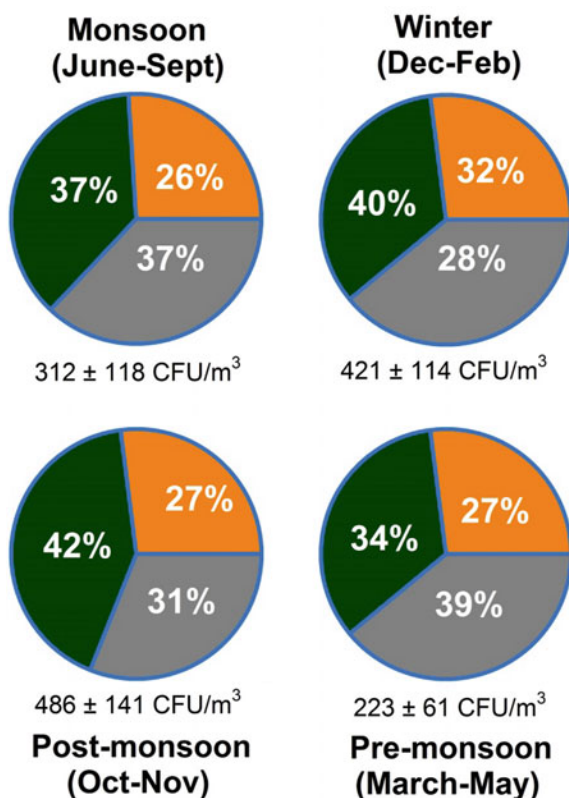


Fig. 5.13 Seasonal variability of: **a** Gram-positive bacteria, **b** Gram-negative bacteria, **c** Fungi and **d** all viable bioaerosols (= GPB + GNB + Fungi) in IGP (at Kanpur)(Rajput et al. 2017)

and Harrison 2004; Murata and Zhang 2016; Rathnayake et al. 2016b; Ziemba et al. 2016). In order to identify potential predictors (factors) influencing the viable bioaerosols concentration (GPB, GNB and Fungi) over IGP, a correlation matrix based on linear regression analysis has been generated utilizing concentrations of these bioaerosols and OC along with several meteorological parameters [temperature (T), relative humidity (%RH), wind speed (WS) and daily rainfall; Table 5.3]. The uncertainty level on interpreting results on correlation analysis is less than 5% ($p < 0.05$). It is evident from the figure that GPB exhibits a significant positive linear relation with GNB ($r = 0.68$; $n = 130$), suggesting plausibility on their co-genetic sources and/or their viability under identical ambient conditions (Table 5.3). A positive linear correlation also exists between GPB and Fungi, but not to a significant level. GPB correlates positively with OC concentrations ($r = 0.60$) and negatively with ambient T ($r = -0.66$). The other meteorological parameters viz. RH, wind speed and rainfall do not show any trend on a significant level. Interestingly, close observations of influence of rainfall on ambient levels of

Fig. 5.14 Seasonal scenario on fractional contribution of: GPB (orange color), GNB (dark green) and Fungi (grey) colonies in IGP (at Kanpur). Total concentration of viable bioaerosols colonies are also mentioned (Avg. \pm SD) (Rajput et al. 2017)



GPB reveals that rainfall greater than 4 mm relates to lowering of GPB concentration. In contrast, daily rainfall \approx 1–4 mm appears to positively correlate with GPB concentrations (Table 5.3). Similar to GPB, GNB also exhibits a positive trend with Fungi, but not to a significant level. Likewise, GNB correlates positively with OC concentrations ($r = 0.87$) and negatively with ambient T ($r = -0.64$). Other observations through linear correlation analysis of GNB with meteorological parameters viz. RH, wind speed and rainfall are near similar to that discussed above for GPB, and hence not repeated again. Fungi colony concentrations do not correlate significantly with any herein reported parameter (Table 5.3). Influence of daily rainfall \approx 1–4 mm appears to positively correlate with Fungi concentrations; higher precipitation decreases levels of Fungi along with other atmospheric pollutants. Massive emissions from open field crop residue and biofuel burning in the IGP have been documented previously (Gustafsson et al. 2009; Momin et al. 1999; Rajput et al. 2014b, 2016c; Ram et al. 2010; Venkataraman et al. 2005). As aforementioned, the air-masses during the post-monsoon and winter season exhibit influence of transport from upwind IGP. Temporal variations in FFA and viable bioaerosols abundance with a positive linear relationship among GPB, GNB and OC suggest their co-genetic source and/or common atmospheric transport process.

Table 5.3 Correlation analysis of viable bioaerosols (GPB, GNB and Fungi) with organic carbon (OC) and meteorological parameters (T, RH, daily rainfall and wind speed: WS)

	<i>G+</i>	<i>G-</i>	<i>Fungi</i>	<i>OC</i>	K_{BB}^+	<i>Temp</i>	<i>RH%</i>	<i>WS</i>	<i>Rainfall</i>
<i>G+</i>	1.00								
<i>G-</i>	0.68	1.00							
<i>Fungi</i>	0.24	0.26	1.00						
<i>OC</i>	0.60	0.87	0.29	1.00					
K_{BB}^+	0.52	0.72	0.26	0.87	1.00				
<i>Temp</i>	-0.70	-0.64	-0.23	-0.63	-0.48	1.00			
<i>RH%</i>	0.20	0.20	0.11	0.14	0.08	-0.50	1.00		
<i>WS</i>	-0.24	-0.24	-0.23	-0.21	-0.08	0.25	-0.15	1.00	
<i>Rainfall</i>	-0.29	-0.10	-0.07	-0.14	-0.22	-0.06	0.56	0.08	1.00

Significant correlation ($p < 0.05$) is highlighted

These inter-relationships highlight the significant role of biomass burning emissions (PRB and biofuels) in contributing to bioaerosols.

5.6 Conclusions

A year-long measurement of viable bioaerosols has been conducted from central IGP to assess their abundance and temporal/seasonal variability (Rajput et al. 2017). As far as viable bioaerosols are concerned, the highest concentration of GPB was recorded during December–January (Avg.: 189 CFU/m³), GNB during November (Avg.: 244 CFU/m³) and Fungi in September (Avg.: 188 CFU/m³). A significant positive linear correlation-ship for GPB and GNB with OC ($p < 0.05$) in conjunction with their variability pattern indicates their co-genetic source. Fungi also exhibit a positive trend with OC, but not to a significant level ($p > 0.05$). We have attributed major source of bioaerosols to be associated with massive emissions from PRB and biofuel burning in the IGP. Ambient temperature shows a negative impact on the abundance of GPB and GNB, whereas RH and wind speed do not exhibit any pronounced effects. Low precipitation (1–4 mm) relates to higher concentrations of bioaerosols particularly the Fungi. This study provides field-based data on bioaerosols and ambient particulate matter with a consideration of their potential role in influencing climate and human health.

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