



Seasonal bioaerosol load and statistical analysis within different microenvironments of an academic institute situated in the Indo-Gangetic Plain

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Received: 13 February 2021 / Accepted: 4 June 2021
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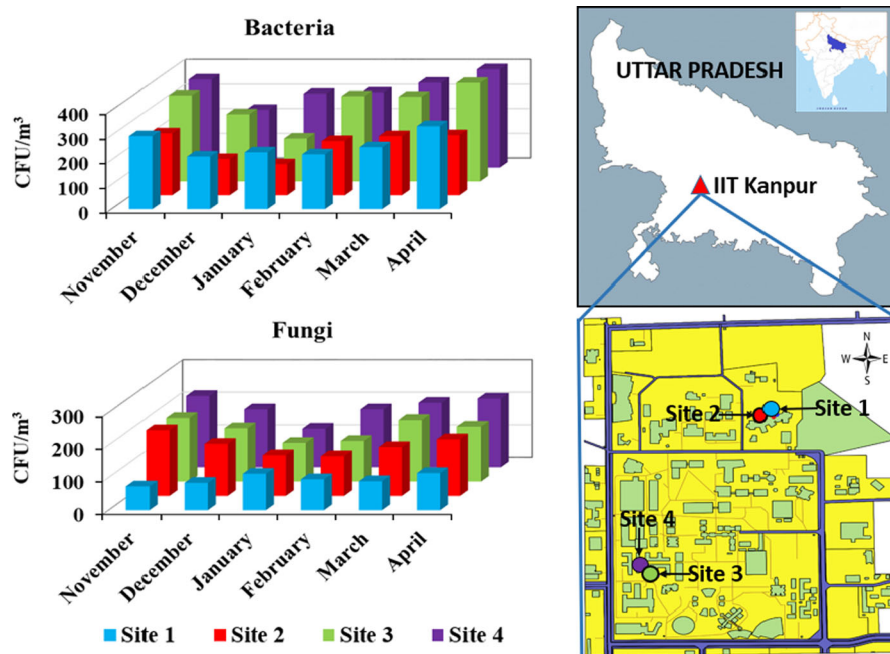
Abstract Bioaerosols have gained importance in atmospheric sciences in the past few decades. Their exposure upsurges the health problems in sensitive population and is also known to participate in atmospheric chemistry. The present study assesses the monthly distribution of bioaerosol load at four different microenvironments inside an academic institute in Kanpur situated in Indo-Gangetic Plain (IGP). The study duration was from November 2017 to April 2018. The measured total bioaerosol concentrations were highest during April (418–610 CFU/m³) at all sites followed by the post-monsoon month of November (369–577 CFU/m³). The winter period showed low concentrations with lowest concentration obtained in January (251–419 CFU/m³). The indoor–outdoor

(I/O) bioaerosol ratio was mostly less than one for bacteria depending on microenvironment, but for fungi it was always less than one. The Gram-positive bacteria (GPB) comprised 70–80% of total bacterial concentration, while Gram-negative bacteria (GNB) contributed 20–30% to bacterial concentration in ambient air. The bacterial genera were mostly dominated by *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus*, and *Enterococcus*, while the fungal genera of *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Basidiobolus*, and *Epidermophyton* were seen in the campus. The statistical analysis showed a weak positive correlation of bioaerosol concentration with meteorological parameters: temperature and relative humidity (RH).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10453-021-09715-6>.

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Graphic abstract



Keywords Bioaerosol · Bacteria · Colony forming units · Fungi · Meteorological factors · Particulate matter

1 Introduction

Bioaerosols are suspended particles of biological origin in air. They comprise plant and animal debris, organic dust, fungal spores, bacteria or their derived metabolites like endotoxins, β -glucans, etc. (Dutkiewicz et al., 2011; Ghosh et al., 2013). They may contribute up to 30% to particulate matter (PM) mass fraction (Faridi et al., 2015; Nowoisky et al., 2016). Their ambient concentration in air is governed by both natural and anthropogenic factors such as geographical area, climate, vegetation cover, and anthropogenic activities (Hameed et al., 2009; Jones & Harrison, 2004). The aerodynamic diameter of bioaerosol particles, wind speed, relative humidity (RH), and temperature are the main factors for inert release (resuspension and spreading) of these particles into the atmosphere which also affect its residence time and

transport (Hinds, 1999; Mohr, 2007). The bioaerosols smaller than $5.0\ \mu\text{m}$ have longer residence time in the air, whereas larger-sized bioaerosols get quickly settled down and deposited on different surfaces (Mohr, 2007). Particles less than $10\ \mu\text{m}$ can be inhaled and deposited within respiratory tract, whereas particles less than $1\ \mu\text{m}$ can even reach deeper into alveolar region and can cause lung infection (Hinds, 1999). Their inhalation may induce disorders in the respiratory tract (like inflammation of the airways, toxic pneumonitis, rhinitis, hypersensitivity, asthma, etc.) (Robertson et al., 2019; Srikanth et al., 2008) and even cause cancer due to oncogenic viruses and mycotoxins (Douwes et al., 2003). Airborne pathogens are responsible for 50% of deaths due to infectious diseases in developing countries (Peccia et al., 2011).

Balakrishnan et al. reported 1.24 million deaths in India in 2017 due to air pollution (Balakrishnan et al., 2019). Previous studies on air pollution were mainly focussed on the chemical composition of ambient PM particles in terms of organic carbon, elemental carbon, polycyclic aromatic hydrocarbons, heavy metals, etc. Epidemiological studies have found many of these pollutants to be toxic, carcinogenic and mutagenic

even in small concentrations and cause severe health problems (Brunekreef & Forsberg, 2005; Englert, 2004; Kappos et al., 2004). These PM bound pollutants especially heavy metals from atmosphere may also enter plants and can cause metal accumulation. The studies in Turkey have reported accumulation of lead and mercury in landscape plants in urban traffic areas (Sevik et al., 2020a, b). The studies also found changes in Ni and Co concentration in different organelles of plants and concluded edible crops grown in regions with heavy metal pollution in air can bioaccumulate in human body via food chain (Sevik et al., 2020a, b). Similarly, food crops are also prone to bioaerosol deposition near bioaerosol emission sources like manure sites, waste composting sites, etc. especially leafy green salads which are eaten uncooked (Hryhorczuk et al., 2001; Jahne et al., 2016). Thus, bioaerosols should be investigated as an important air pollution parameter.

Past environmental studies from different bioaerosol emitting work environments like sewage treatment plants, waste landfills, composting facilities, etc. have reported high bioaerosol load in its vicinity along with myriad of health problems to the exposed population. The study in the USA at a composting facility in Illinois found far higher on-site bioaerosol load compared to off-sites (Hryhorczuk et al., 2001). It reported bacterial concentration ranging between 480 and 78,880 colony-forming units (CFU)/m³ and fungal concentration ranging between 5223 and 26,067 spores/m³, respectively. Such high concentration has increased the risk of occupational health hazards at these places, like enhanced eye and skin irritation, allergic broncho-pulmonary disorders, and pneumonitis among the workers employed at these facilities (Lacey & Dutkiewicz, 1994; Robertson et al., 2019; Van Kampen et al., 2012). Other places like office and residential buildings, research institutes, schools and universities have also reported high bioaerosol load (Hsu et al., 2012; Lacey & Dutkiewicz, 1994; Law et al., 2001), which can result in building-related diseases like 'sick building syndrome', legionellosis (bacterial infection), etc. (Dutkiewicz et al., 2011; Faridi et al., 2015; Ghosh et al., 2013; Wålinder et al., 2001). The concentration in indoor environment (i.e. inside buildings) is mostly dependent on indoor/outdoor (I/O) air exchange rate, building use characteristics and human activities (Hsu et al., 2012; Nasir & Colbeck, 2012). The survey conducted by

Environmental Protection Agency (EPA), USA in large office buildings ($n = 100$) reported I/O bioaerosol ratio > 1 during summers and vice versa in winters (Tsai & Macher, 2005; Tsai et al., 2007). The study done in a school dormitory in Tehran (Iran) found average bacterial concentrations of 300 and 371 CFU/m³ at outdoor and indoor sites, respectively (Faridi et al., 2015). The corresponding average fungal load was found to be 159 and 106 CFU/m³, respectively. Few studies have also been carried out in India to measure real-time bioaerosol load (Ghosh et al., 2013; Lal et al., 2013; Mamta, 2015; Priyamvada et al., 2018). The bioaerosol study done at a hospital in South India reported culturable bioaerosol concentration in the order of 10^3 – 10^5 CFU/m³ (Sudharsanam et al., 2012). Another study carried out in a university library in Indo-Gangetic Plain (IGP) region found varying bioaerosol concentrations ranging between 1488 and 3140 CFU/m³ at different locations of the library building (Ghosh et al., 2013).

The present work consists of a detailed bioaerosol study carried out at the Indian Institute of Technology (IIT), Kanpur with an aim to enhance the knowledge about the airborne microbiome in this region. The study enlists detailed variability and diversity of bioaerosols in different seasons (post-monsoon, winter, and spring) from November 2017 to April 2018. A comprehensive evaluation between indoor and outdoor site concentrations using statistical tools has also been carried out. This is important as Kanpur is one of the most densely populated as well as polluted regions in the world (Balakrishnan et al., 2019; Chakraborty & Gupta, 2010). Respiratory problems and infections affect countless number of people in this region (State of Global Air, 2018), which can also be linked to a higher level of ambient bioaerosol concentration in this region. Thus, the objectives of this study are, (1) to couple and correlate the indoor concentrations with outdoor environment in different microenvironments (at 4 sites inside academic area), (2) to simultaneously study the variability in bioaerosol load and PM₁₀ number concentration, (3) to identify the dominant bacterial and fungal species in the campus and identify their major sources, (4) to get an understanding of the bioaerosol exposure levels inside the campus.

2 Materials and methods

2.1 Study area description

To understand the pattern and seasonal variation in bioaerosol load, 6 months bioaerosol sampling was carried out at IIT Kanpur campus from November 2017 to April 2018. The campus coordinates are 26.4499° N and 80.3319° E. The campus lies in the Indo-Gangetic Plain (IGP) and has reduced level (RL) of 142 m above mean sea level. This place experiences extreme weather conditions with harsh summer afternoon (above 45 °C in May) and chilly winter nights (below 5 °C in January) (Rajeev et al., 2016). This region receives approx. ~ 900 mm yearly rainfall with plenty of rain during monsoon (Rajeev et al., 2016). The campus area is green, and the residents here enjoy a high standard of living with multi-storeyed academic and residential buildings, green parks, recreational sports stadiums, nursery and other amenities like shopping centres, water treatment facilities, oxidation pond, etc. Among these different microenvironments, having diverse bioaerosol emission sources, four sampling sites were chosen inside academic area to capture bioaerosol load in ambient air. The academic area was chosen because majority of campus residents spend most of their time here. The detailed site description is provided in Table 1.

2.2 Sampling procedure and analysis

2.2.1 Sampling plan

The bioaerosol sampling was carried out between November 2017 and April 2018 three times in a week on Monday, Wednesday and Friday during morning hours (between 9:00 am and 11:00 am) at all the four sites, excluding the period between (21 Dec to 21 Jan). The morning period was chosen as most residents are out at this time for classes and laboratories. A particle spectrometer (Palas Welas-2000) was collocated throughout the sampling period near Site 1 to measure particle number concentration (PNC). Temperature and humidity were also measured at Site 1.

2.2.2 Bioaerosol sampling

The measured bioaerosol concentration depends a lot on adopted methodology (Sudharsanam et al., 2012). Based on our requirement of capturing airborne culturable bioaerosols through active sampling, the bioaerosol sampler developed at IIT Kanpur (Patent no. 1474/DEL/2014, INDIA) was used. It works on the principle of inertial impaction. The sampler is designed such that when air flows through this sampler at 12 L per minute and strikes the petri dish containing agar media, all the bioaerosol particles above 0.6 µm size are captured due to impaction (Chauhan & Gupta, 2017). This bioaerosol sampler was kept 1.2 m above floor level, with a minimum distance of 1 m from

Table 1 Sampling locations within IIT Kanpur and its description

Location	Type	Description
Site 1	Outdoor	The foyer area of Centre for Environmental Science and Engineering (CESE) building. It is surrounded by trees and a functional fountain. A large nursery is present in its vicinity, which has huge number of flowering plants
Site 2	Indoor	It is Atmospheric and Particle Technology Lab (APTL) inside CESE. It has dimensions of 45' × 28' × 11'. It houses various advanced laboratory machines, computers and has central air conditioning facility. Around 8 persons sit in this laboratory
Site 3	Outdoor	This is an open backyard behind Western Lab 116. Few trees, discarded laboratory instruments, mud holes and puddles along with a vermicomposting pit are present here. Lawns and cemented pathways are present in the vicinity
Site 4	Indoor	This is a small wet chemistry laboratory nearby Site 3, with poor ventilation having dimensions 22' X 21' X 9'. It houses few laboratory instruments and has two split ACs. Around 5 persons sit here

walls. Three types of Agar media were used for culturing different types of bioaerosols namely: Gram-positive bacteria (GPB)—Mannitol Salt Agar Base (MSAB) (M118, Himedia), Gram-negative bacteria (GNB)—MacConkey Agar (MAC) (M008, Himedia) (Nasir et al., 2012) and Fungi—Sabouraud Chloramphenicol Agar (SCA) (M1067, Himedia). The sampler was run for 3 min passing 36 L of air on each agar media. Figure 1 shows the images of different agar media before and after incubation. The captured bioaerosol samples were then incubated in cell culture incubator (ESCO) at 35 °C for 48–72 h (Rajput et al., 2017). After incubation, the colony enumeration (No. of visible colonies divided by volume of sampled air)

was done and reported in CFU/m³. Further gram staining, spore staining, acid-fast staining and negative staining of bacterial samples were done and then studied under microscope (Nikon E 200) in 400× and 1000× magnification for qualitative analysis (Cappuccino & Welsh, 2017). Fungal samples were also phenotypically studied to characterize them. Proper care and protocols have been followed to disinfect and prevent contamination as per the guidelines of Microbiology Lab Manual (Cappuccino & Welsh, 2017; Johnson & Case, 2007). The disinfection of bioaerosol sampler was carried out by absolute ethanol rinsing (Merck, Germany). The sampler and petri dishes were kept inside biosafety hood equipped with

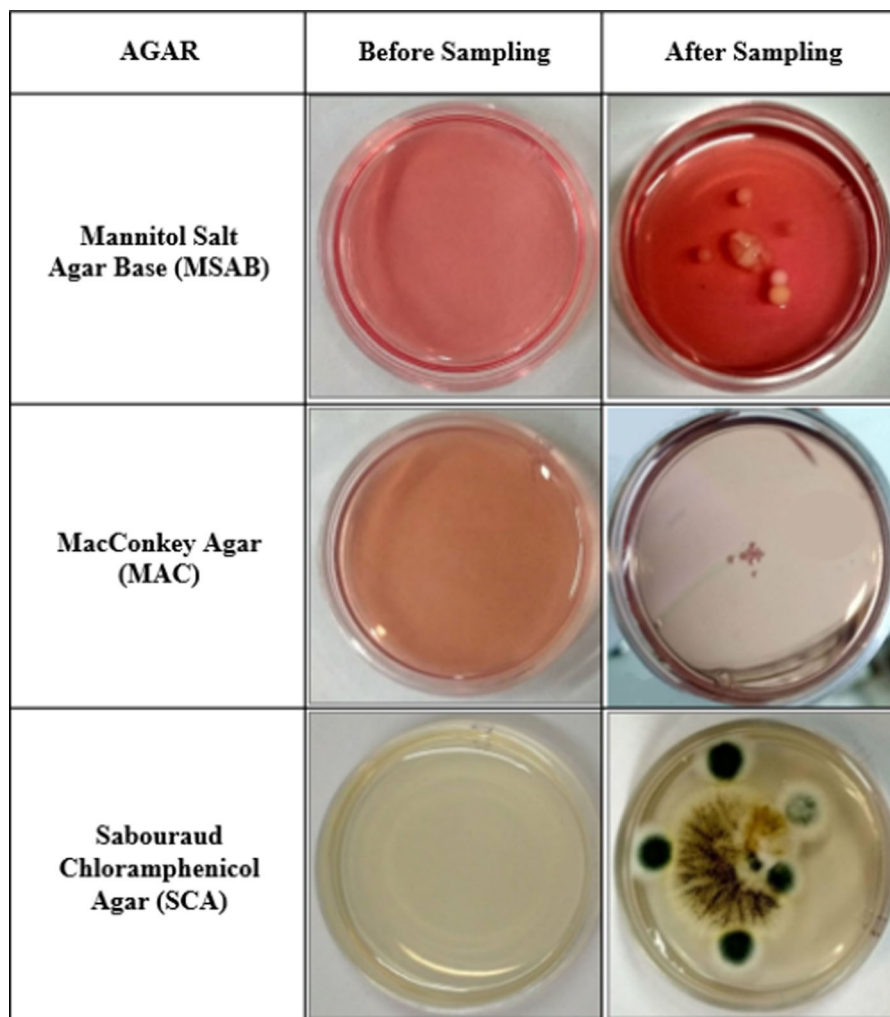


Fig. 1 Images of Agar media before and after incubation

high-efficiency particulate air (HEPA) filter and UV lamp (Biogen Scientific) for 30 min before sampling.

2.3 Statistical analysis

The correlation matrix was developed between bioaerosol load and meteorological parameters for Site 1 (outdoor site). The p value was calculated, and results were analysed using a two-tailed test. The correlation was not calculated for indoor microenvironments as its temperature and humidity are artificially controlled through a central air conditioning facility (Table 1).

Further, a comparison study was done to check bioaerosol load variation from bioaerosol study done in 2014 (Chauhan, 2015) at the same sites. The nonparametric analysis was done, as the bioaerosol load distribution obtained was not normally distributed. The Wilcoxon signed-rank and chi-squared tests were done for the above purposes. The Wilcoxon signed-rank test is used for comparison between related samples or data from similar studies taken before and after an event (McDonald, 2009). Thus, this tool was used to check whether the bioaerosol concentration in ambient air in 2017 has shifted from earlier study done in 2014 or not. The second chi-square test checks for relationship and interdependence between two pair of data-sets. This test was used to check whether the monthly percentage distribution of bioaerosol load in 2017 is same as it was in year 2014 or not.

3 Results

3.1 PM₁₀ number concentration and meteorological parameters

The particle number concentration (PNC) below 10 μm (PM₁₀) was measured through particle spectrometer (Palas Welas-2000) at Site 1. The instrument gave PNC measurements into 15 channel bins from optical diameter 0.237 μm to 17.783 μm (i.e. 0.237–0.316, 0.316–0.422, 0.422–0.562, 0.562–0.75, 0.75–1, 1–1.334, 1.334–1.778, 1.778–2.371, 2.371–3.162, 3.162–4.217, 4.217–5.623, 5.623–7.499, 7.499–10, 10–13.335 and 13.335–17.783 μm). The optical diameter (d_o) from the instrument was converted into aerodynamic diameter (d_a) using

equations $d_a = d_o \left[\frac{\rho_p}{\chi \rho_0} \right]^{\frac{1}{2}}$ (Hinds, 1999). Here, ρ_p is effective particle density which is taken as 1270 kg/m³, χ is shape factor which is taken 1 and ρ_0 is standard particle density which is 1000 kg/m³ (Kumar & Gupta, 2016). The PNC for PM₁ was also measured, which corresponds to 95–98% of PM₁₀ number concentration. Figure 2a shows the box plot chart of PM₁₀ number concentration for the sampling period (November to April). Figure 2b, c reports the variation in temperature and humidity during the same period. Highest PNC was obtained in November month ($1324 \pm 511 \text{ \#/cm}^3$). This can be attributed to post-harvest stubble burning by farmers in the IGP region (Rajput et al., 2017). The lowest PNC was obtained in winter months between December to February ($616 \pm 212 \text{ \#/cm}^3$) which can be due to scavenging of PM by fog droplets and occasional rain showers particularly in December (Han et al., 2018; Rajput et al., 2018). However, some studies in the IGP region stated an increase in PNC during winter period due to development of inversion conditions and low mixing height leading to entrapment of pollutants (M. Kumar et al., 2017; Nair et al., 2007). Studies done in Bursa and Kastamonu City of Turkey have found the effect of location, season and time factor on PM concentrations (Cetin & Sevik, 2016; Cetin et al., 2019). The spring months of March and April again showed increased PNC ($896 \pm 461 \text{ \#/cm}^3$) in ambient air. Dust storms and high wind speed during this period lead to resuspension of fine PM particles in air which caused increase in PNC (Chakraborty & Gupta, 2010; S. Ghosh et al., 2014). The data for temperature, PM₁₀ and relative humidity is provided in supplementary file (Table S1).

3.2 Monthly analysis of viable bioaerosols

The temporal variation of GPB, GNB and Fungi from November 2017 to April 2018 are shown in Fig. 3. Duplicate sampling of bioaerosols at regular periods was done to check the efficacy of results. The measured concentrations of GPB were much higher than GNB at all sites. Among viable bacteria, GPB contributed 70–80%, and GNB contributed 20–30%, respectively. The average total bacterial (GPB + GNB) concentration ranged between 213 and 336 CFU/m³ at Site 1, 126–251 CFU/m³ at Site 2, 174–436 CFU/m³ at Site 3 and 234–399 CFU/m³ at

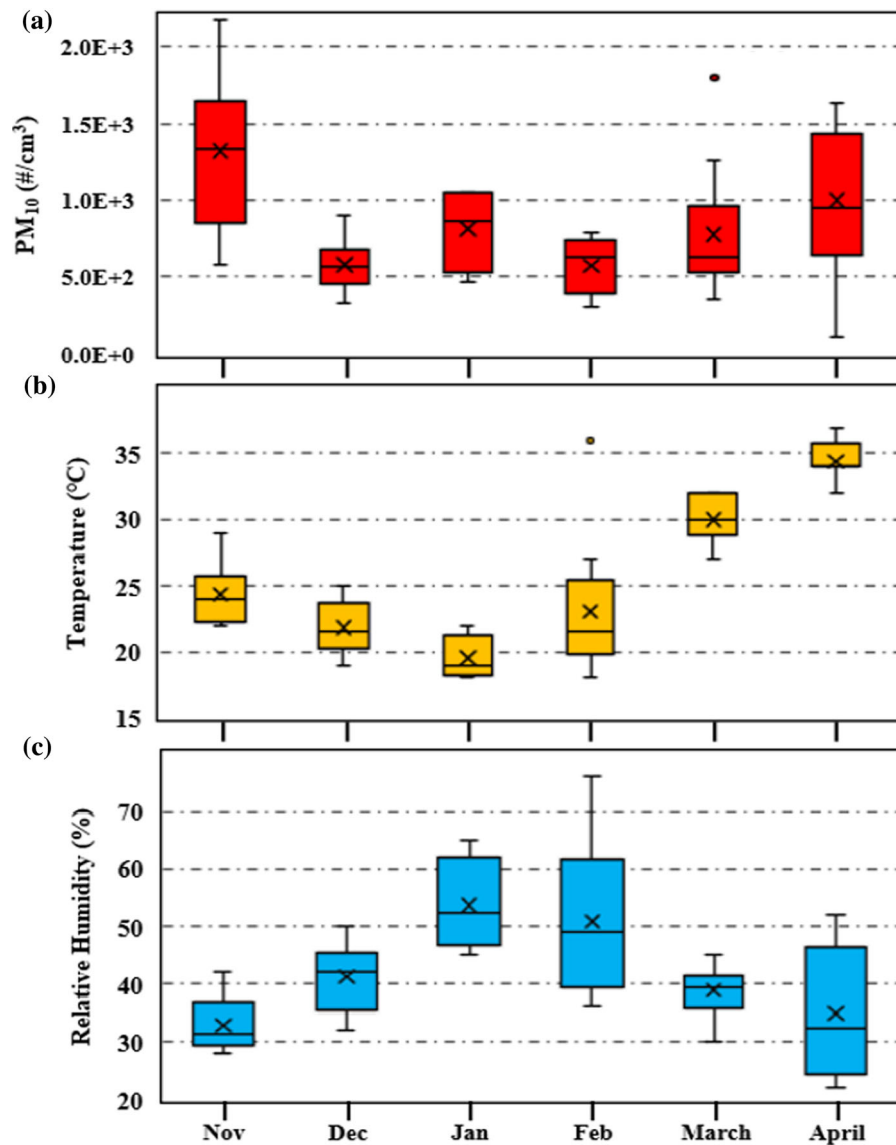
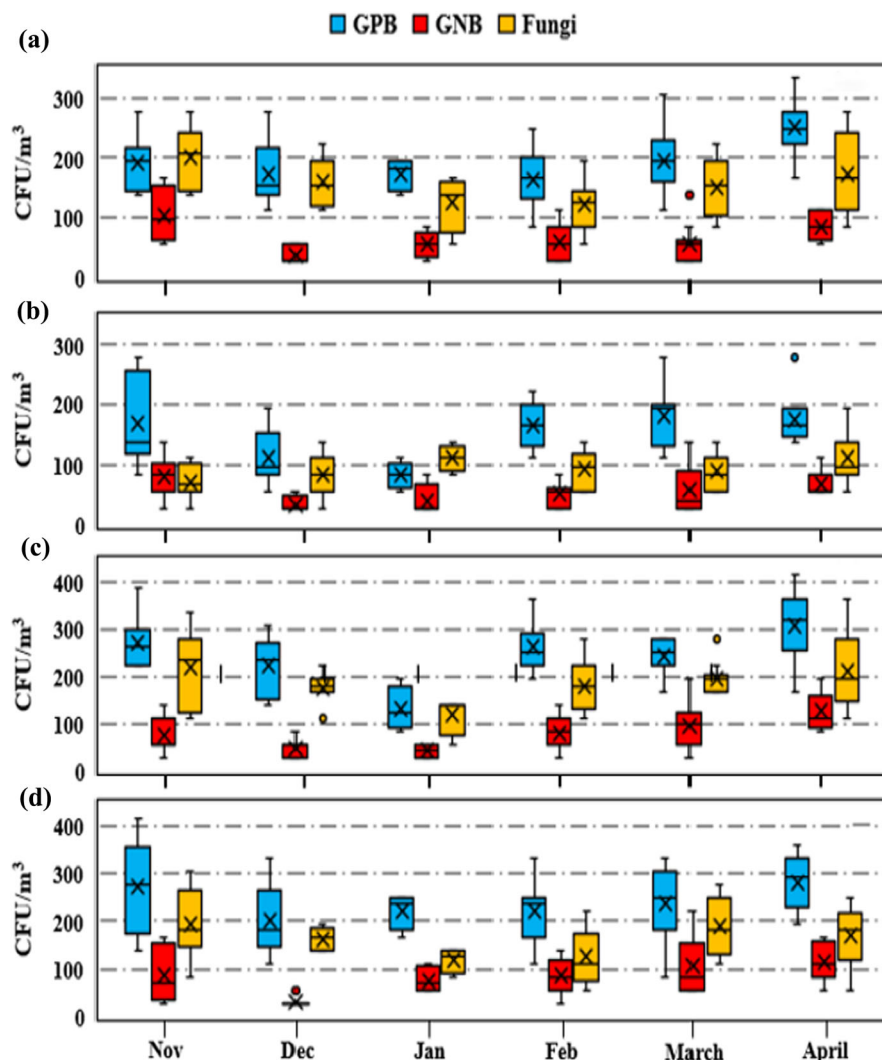


Fig. 2 Time series variation of meteorological parameters at Site 1: **a** PM₁₀ number concentration, **b** temperature and **c** relative humidity

Site 4, respectively. The obtained concentrations were low in winter months (December–February) and high in November and spring months (March–April). Overall, the outdoor Sites 1 and 3 have mostly reported higher bioaerosol concentration compared to their respective indoor Sites 2 and 4. Highest monthly GPB concentration at Site 1 250 CFU/m³, Site 3 308 CFU/m³ and Site 4 283 CFU/m³ were obtained in the month of April, but at Site 2 highest

concentration of 181 CFU/m³ was obtained in the month of March. For GNB, concentrations were highest in the month of November for Site 1 and Site 2 (105 and 80 CFU/m³, respectively), but at Site 3 and Site 4 highest concentration of 128 CFU/m³ and 116 CFU/m³, respectively, was obtained in the month of April. The detailed mean monthly bioaerosol load at all the four sites is listed in Table 2. A similar study done in Agra (in the IGP region), reported bacterial

Fig. 3 Monthly bioaerosol load distribution at **a** Site 1, **b** Site 2, **c** Site 3 and **d** Site 4



concentration between 403 ± 198 CFU/m³ and fungal concentrations between 83 ± 16 CFU/m³ in winter months (Mamta et al., 2015).

The average fungal concentrations at our four sites followed the same seasonal trend as pursued by bacteria, i.e. lowest during winter period and high during November and spring months. However, fungal concentration was less compared to bacterial concentration. It ranged between 122–202 CFU/m³ at Site 1, 73–114 CFU/m³ at Site 2, 119–219 CFU/m³ at Site 3, and 119–195 CFU/m³ at Site 4, respectively. Highest fungal concentration was obtained in the month of November for Sites 1, 3 and 4 and for Site 2 it was obtained in April. The bioaerosol concentration data

for individual sampling days are provided in supplementary file [Table S2(a) and S2(b)]. It should be noticed that this study measured only viable and culturable bioaerosol in specific agar media. Many bacterial and fungal species may not grow on this media (Mbareche et al., 2017). Also, desiccation of microorganism and deposition build-up may occur (Haig et al., 2016). So, the bioaerosol exposure is underestimated and the health risk associated is likely to be much higher.

Table 2 Mean monthly value of different air parameters (Avg. $\pm \sigma$)

Parameters	November (n = 8)	December (n = 8)	January (n = 4)	February (n = 10)	March (n = 10)	April (n = 12)
GPB ^a	191 $\pm$ 45	174 $\pm$ 55	174 $\pm$ 26	164 $\pm$ 49	195 $\pm$ 55	250 $\pm$ 50
GNB ^a	105 $\pm$ 43	39 $\pm$ 14	56 $\pm$ 22	58 $\pm$ 27	56 $\pm$ 34	86 $\pm$ 22
Fungi ^a	202 $\pm$ 50	160 $\pm$ 41	125 $\pm$ 48	122 $\pm$ 41	150 $\pm$ 47	174 $\pm$ 71
GPB ^b	171 $\pm$ 73	112 $\pm$ 46	84 $\pm$ 22	167 $\pm$ 41	181 $\pm$ 51	176 $\pm$ 38
GNB ^b	80 $\pm$ 34	35 $\pm$ 12	42 $\pm$ 27	53 $\pm$ 20	59 $\pm$ 40	68 $\pm$ 19
Fungi ^b	73 $\pm$ 29	84 $\pm$ 36	112 $\pm$ 22	95 $\pm$ 32	89 $\pm$ 28	114 $\pm$ 42
GPB ^c	271 $\pm$ 57	223 $\pm$ 61	132 $\pm$ 47	264 $\pm$ 51	245 $\pm$ 36	308 $\pm$ 79
GNB ^c	77 $\pm$ 38	49 $\pm$ 19	42 $\pm$ 16	81 $\pm$ 33	98 $\pm$ 52	128 $\pm$ 40
Fungi ^c	219 $\pm$ 81	178 $\pm$ 32	119 $\pm$ 41	178 $\pm$ 54	198 $\pm$ 33	211 $\pm$ 80
GPB ^d	271 $\pm$ 102	202 $\pm$ 73	223 $\pm$ 39	219 $\pm$ 62	239 $\pm$ 81	283 $\pm$ 54
GNB ^d	87 $\pm$ 56	32 $\pm$ 9	77 $\pm$ 26	86 $\pm$ 38	106 $\pm$ 61	116 $\pm$ 41
Fungi ^d	195 $\pm$ 72	164 $\pm$ 23	119 $\pm$ 26	125 $\pm$ 58	189 $\pm$ 59	169 $\pm$ 60
Temperature (°C)	24 $\pm$ 2	22 $\pm$ 2	20 $\pm$ 2	23 $\pm$ 5	30 $\pm$ 2	34 $\pm$ 2
PM ₁₀ (#/cm ³)	1324 $\pm$ 511	577 $\pm$ 171	811 $\pm$ 283	569 $\pm$ 186	773 $\pm$ 440	998 $\pm$ 472
RH (%)	33 $\pm$ 5	41 $\pm$ 6	54 $\pm$ 8	51 $\pm$ 13	39 $\pm$ 4	35 $\pm$ 11

^aConcentration in colony-forming units (CFU/m³) at Site 1

^bConcentration in colony-forming units (CFU/m³) at Site 2

^cConcentration in colony-forming units (CFU/m³) at Site 3

^dConcentration in colony-forming units (CFU/m³) at Site 4

Temperature, PM₁₀, and RH values are measured at Site 1

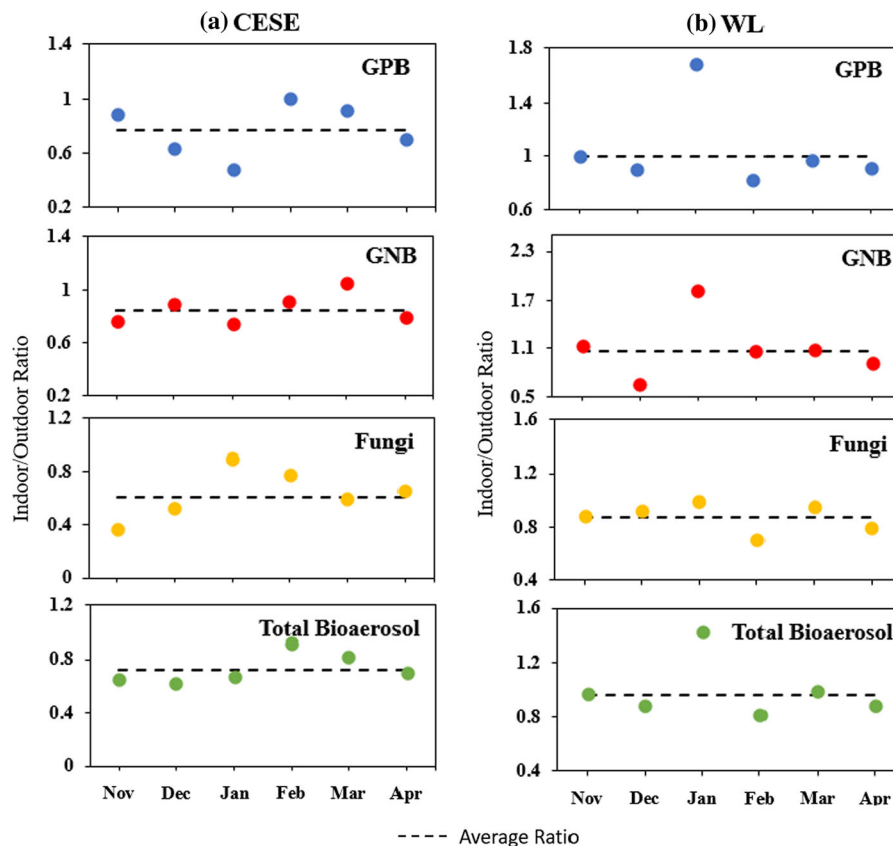
3.3 Comparative bioaerosol study among different microenvironments

The I/O bacterial ratio at CESE (between Site 1 and Site 2) was < 1 as shown in Fig. 4. This shows there is no prominent indoor bacterial source or breeding location indoors and has major sources mostly outside. However, for WL (Site 3 and Site 4) the I/O ratio revolves around 1 (indicated by average ratio line in Fig. 4). This shows the likelihood of presence of indoor sources and influence of anthropogenic activities in indoor microenvironment along with influence from outdoor sources. Similar studies in other regions have reported the I/O ratio varying from < 1 to > 1 depending on building characteristics and human activities (Faridi et al., 2015; Nasir et al., 2012; Tsai et al., 2007).

However, the I/O ratio for fungi was always < 1 for both the locations. This was obvious as majority of fungal sources, (vegetation and moulds) are present

outside the buildings and this also conforms with the studies done in the USA (Shelton et al., 2002). Inside CESE, the absence of any visual breeding sites in the laboratory with hygienic microenvironment and adequate ventilation, compared to WL, has led to a low build-up of indoor bacterial colonies. This goes well with the studies in South India, which reported high bacterial concentration in poorly ventilated indoors having AC compared to well-ventilated rooms (Priyamvada et al., 2018). Table 1 gives detailed description of all the sites. The comparative study between CESE and WL showed higher concentration at WL, in both indoor and outdoor microenvironments. This was due to presence of ample breeding sites near WL like mudholes, discarded furniture, vermicomposting pit, etc. Thus, anthropogenic factors have enhanced bioaerosol load at this location. Likewise, the laboratory situated in its vicinity (Site 4) has also reported higher bioaerosol loading due to outside effects. Inadequate ventilation was also a key

Fig. 4 Indoor/outdoor ratio at Site A CESE and B WL



factor leading to build-up of bacterial colonies inside WL (Site 4).

3.4 Bioaerosol diversity

The fungal genera of *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Basidiobolus*, *Fusarium*, *Rhizopus* and *Epidermophyton* were found on the campus. *Aspergillus*, *Penicillium* and *Cladosporium* were most abundant among all fungal genera. Among bacteria, GPB was dominated by *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus*, and *Enterococcus* while GNB was dominated by *Pseudomonas*, *Enterobacteriaceae*, and *Aeromonas*. The outdoor Site 3 was found to be most affluent and diverse in viable fungal cultures followed by Site 1, whereas among the indoor microenvironments fungal genera were mainly dominated by *Aspergillus* and *Penicillium*. However, no noticeable difference was seen in bacterial genera at indoor and outdoor sites. These observations go well with the proposition that fungal sources are present mainly outside whereas bacterial sources are

omnipresent. This finding relates well with the studies carried out in other regions of India (Mamta et al., 2015; Priyamvada et al., 2018).

3.5 Comparative analysis with the past study

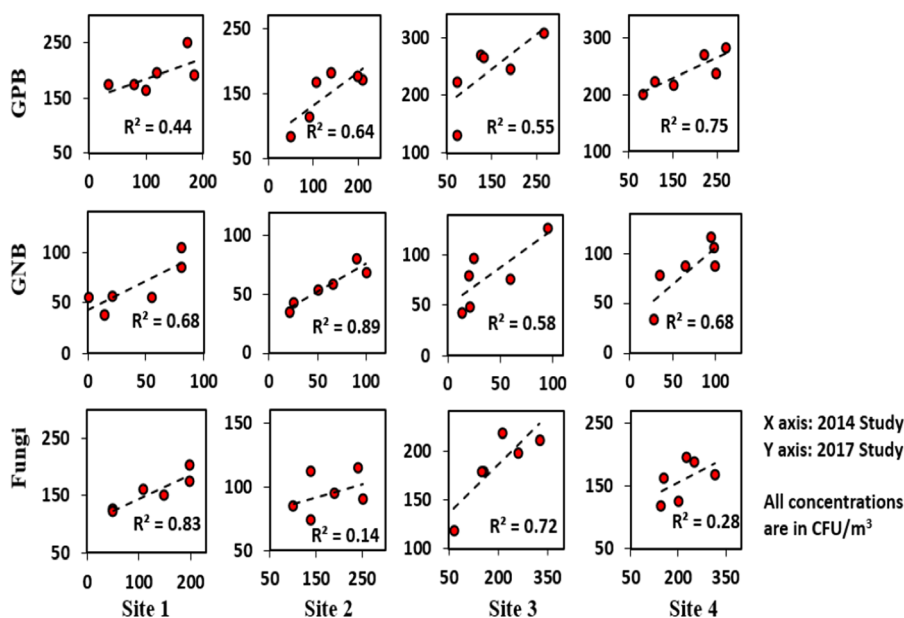
The present bioaerosol load (2017–2018) was compared quantitatively with bioaerosol study done in 2014 (Table 3), at the same sampling locations (Chauhan, 2015). The bioaerosol concentration has risen, nearly on all the sampling sites. Both temporal and spatial variations have occurred in the following years, which might have introduced new bioaerosol emission sources in the surroundings. The GPB and GNB concentrations have shown more variation at outdoor sampling sites (Site 1 and 3) compared to indoor sampling sites (Site 2 and 4), as there are high chances of transformations in the outdoor microenvironments due to human activities (like new built-up area) compared to indoor sites. The variations observed in bacterial loading at indoor sites can be

Table 3 Mean monthly bioaerosol load in 2014

Month	GPB				GNB				Fungi			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
November	185	210	125	220	80	90	60	100	200	140	210	225
December	80	90	70	83	14	21	21	28	110	102	155	155
January	35	49	70	110	ND	25	14	35	50	140	65	148
February	100	105	130	150	21	50	20	65	50	190	150	200
March	120	140	190	250	55	65	25	97	150	250	260	250
April	174	200	264	271	80	100	95	95	200	241	324	315

ND not detected

Concentration in colony-forming units (CFU/m³)

**Fig. 5** Regression plot between bioaerosol load in year 2014 and 2017

due to both the influence of outdoor environment and increased anthropogenic activities in the laboratory.

Significant changes are seen in fungal concentration at indoor sites, which showed an overall reduced concentration in ambient air compared to the past fungal concentration. However, the outdoor fungal concentration reported in 2014 at both the Sites 1 and 3 is within the present standard deviation limits. The correlation plot between the 2 years is shown in Fig. 5.

3.6 Statistical analysis

The correlation matrix between viable bioaerosols (GPB, GNB and fungi) and meteorological parameters is shown in Fig. 6. The Pearson correlation coefficient (r) and p value were calculated to check whether there is a significant relationship among the given parameters. The p value was within significance level (of $\alpha = 0.05$) for GPB with temperature and RH, for GNB with PM₁₀, but for fungi p value was greater than 0.05. However, if we consider significance level (α) of 0.1 then statistical significance can be achieved for

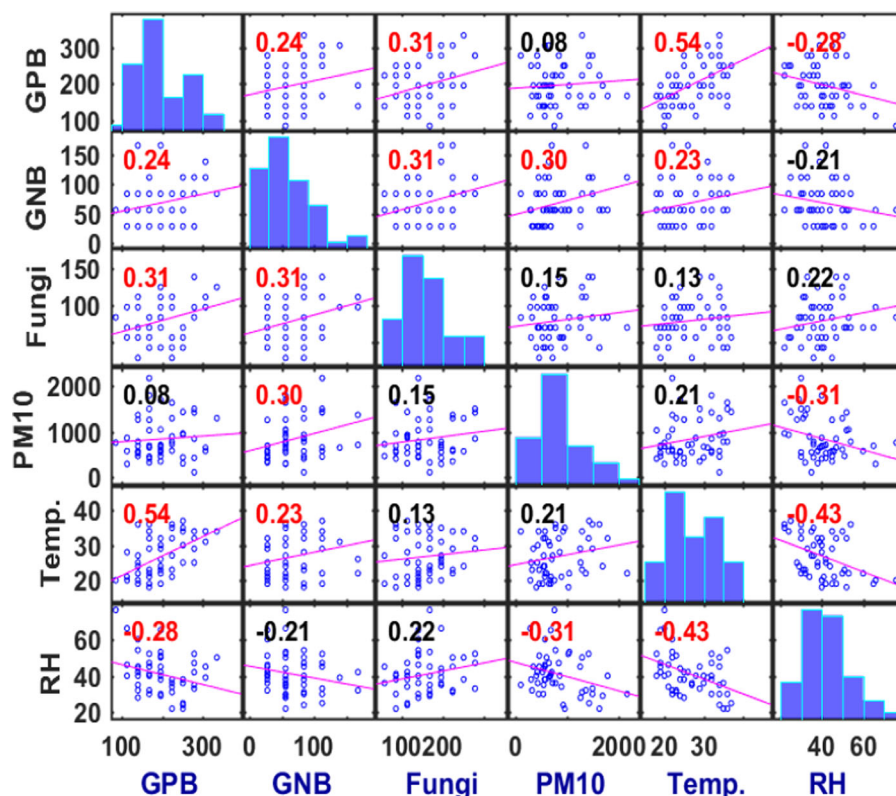


Fig. 6 Correlation matrix of ambient air bioaerosol concentration with meteorological parameters

various parameters as shown in Fig. 6. The r values in red colour (Fig. 6) have p value less than 0.1 indicating significant correlation between the given parameters. Thus, it is seen that both GPB and GNB have significant positive correlation with temperature. PM₁₀ does not have any significant relationship with fungi or GPB, whereas RH has negative correlation with bacteria and PM₁₀, which can be attributed to wet scavenging of particles as reported in other studies also (Gupta & Mandariya, 2013). Besides seasonal analysis, variations in bioaerosol load in 3 years time period between 2014 and 2017 have also been analysed statistically by Wilcoxon signed-rank and chi-squared test. These are nonparametric hypothesis test which compares two samples. The chi-square test showed that the monthly bioaerosol load in year 2017 is not distributed proportionally compared to the bioaerosol load in year 2014, i.e. the monthly percentage distribution of bioaerosol in 2014 is not the same as in year 2017. The Wilcoxon signed-rank test was used to discover if there is significant increase in the ambient air bioaerosol loading at the given sites

in the given time interval. We assumed, null hypothesis (H_0): No difference in bioaerosol load at respective sites, and alternate hypothesis (H_a): Increase in bioaerosol load at respective sites. One-tailed test for significance level (α) of 0.05 was taken. The null hypothesis was rejected for outdoor sites 1 and 3 in case of GPB and GNB. This indicated an appreciable increase in bacterial load at outdoor sites compared to indoor sites. However, in case of fungi we failed to reject the null hypothesis for outdoor sites, contrary to indoor sites, i.e. there is appreciable increase in concentrations at indoor sites compared to outdoor sites. Hence, we can safely say that for outdoor sites, the microbial population of GPB and GNB have gone up significantly from 2014 to 2017 but no significant increase was observed in case of fungi.

4 Discussion

The long-term seasonal bioaerosol studies are scarce in Indian subcontinent. Our present study has

investigated into monthly bioaerosol concentration in different indoor and outdoor microenvironments, and their relationship with meteorological parameters along with PM₁₀ number concentration. This study is also unique as changes in bioaerosol load in the same microenvironments after a period of 3 years have been compared and analysed with the possible changes in microenvironment.

The monthly investigation of bioaerosols during the study period at the IIT Kanpur campus revealed high concentration in the months of November, March and April; and low concentrations during winter months from December to February at all the four sites. The monthly bioaerosol load was influenced by the weather conditions here. Low temperature and scavenging of particles due to humidity are the governing factors for lower bioaerosol load during winter months (Lee et al., 2012, 2016) as the viable cells remain dormant under low temperatures. On the contrary in spring months, high wind speed clubbed with increased temperature is responsible for increased bioaerosol concentrations (Moon et al., 2014) as they lead to the release of bioaerosols in the atmosphere from vegetation and soil (Jones & Harrison, 2004). The post-monsoon month of November also had high bioaerosol concentration in ambient air due to optimum humidity (Wang et al., 2001) and favourable temperature ($\sim 25^\circ\text{C}$) for mesophilic bacteria after November temperature drops during winters (Kowalski & Pastuszka, 2018). Table 4 lists a few comparative bioaerosol studies carried out in residential and public buildings at different places. These studies show that the average bioaerosol concentration (bacteria + fungi) varies from 300 to 1500 CFU/m³ in these buildings (Faridi et al., 2015; Ghosh et al., 2013; Heo et al., 2014; Mamta et al., 2015; Priyamvada et al., 2018). Also, the total bioaerosol load obtained at IIT Kanpur campus at all sites lies within this range. This shows that in contrast to dominant sources like landfills, composting sites, pit-latrines, etc., where bioaerosol load is way too much higher (Farling et al., 2019; Hryhorczuk et al., 2001), ranging from 10 to 10⁸ CFU/m³ (Robertson et al., 2019), the places like the residential apartments, office building have lower concentrations. However, chronic exposure to even this level might induce respiratory and other health problems as the epidemiological studies have yet not established the minimum threshold bioaerosol limit which causes adverse health effects to the exposed

population (Dutkiewicz et al., 2011; Walser et al., 2015).

A total of eight fungal genera were obtained in the campus of which *Aspergillus*, *Penicillium* and *Cladosporium* were most abundant. Other genera included *Rhizopus*, *Basidiobolus*, *Mucor*, *Fusarium* and *Epidermophyton*. The outdoor sites were having high fungal concentration mainly near Western Lab which was surrounded by mud holes, puddles and vermicomposting facility. Our investigation is consistent with previous studies in India, which have reported *Aspergillus* and *Cladosporium* to be dominant fungal genera (Mamta et al., 2015; Priyamvada et al., 2018). Study by Shabankarehfar et al. 2017 in Bushehr city of Iran reported 18 fungal genera in dust and indoor air samples of residential area (Shabankarehfar et al., 2017). They found Yeast, *Aspergillus* and *Alternaria* as dominant fungal genera in all places, while few fungal genera (like *Phoma* in indoor dust samples and *Scopulariopsis* and *Stemphylium* in indoor air samples) were reported in specific environment only. Studies carried out elsewhere globally have reported similar fungal genera in the ambient air (Law et al., 2001; Shelton et al., 2002).

Among the bacterial aerosols, the sites were dominated by total eight types of bacterial genera. The GPB was dominated by *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus* and *Enterococcus* while GNB was dominated by *Pseudomonas*, *Enterobacteriaceae* and *Aeromonas*. The study was limited to investigate total concentration of airborne bacterial samples and bacterial abundance in terms of individual genera was not investigated. Although bacterial diversity in different microenvironments was nearly same their individual abundance at indoor and outdoor location needs to be further investigated to get proper understanding of human contribution on bacterial microbiome in indoor environment as stated in previous studies (Miletto & Lindow, 2015). The bacterial genera obtained in ambient air at the study sites is consistent with previous studies done elsewhere at different indoor and outdoor environments (Bowers et al., 2011; Fathi et al., 2021; Liu et al., 2018; Miletto & Lindow, 2015).

Bioaerosol exposures in different occupational and residential microenvironments can cause health impacts on exposed population leading to allergies, infections and other respiratory problems (Douwes et al., 2003; Lacey & Dutkiewicz, 1994; Mbareche

Table 4 Bioaerosol load at other sites in India and Asia (Avg. $\pm \sigma$)

Location	Tehran (Iran)	Seoul (South Korea)	Delhi (India)	Agra (India)	Chennai (India)
Site characteristics	Indoor sites (a) School dormitory (b) Retirement home	Konkuk University (a) Outside building (b) Inside building	JNU library (a) Reading hall (b) Outdoor	Dayalbagh sub-urban area	IIT Chennai (a) Water analysis laboratory (b) Residence home
Time period	May 2012–2013	July 2013	July–October 2011	Winter (2012–2013)	May–June 2017
Bioaerosol load	Total BACTERIA Fungi	Total Bacteria Fungi	GPB GNB Fungi	Total Bacteria Fungi	Total Bacteria Fungi
(a)	421 $\pm$ 345	153 $\pm$ 139	786	403 $\pm$ 198	1193
(b)	488 $\pm$ 305	186 $\pm$ 219	600	83 $\pm$ 16	295
Reference	Faridi et al. (2015)	Heo et al. (2014)	Ghosh et al. (2013)	Mamta et al. (2015)	Priyamvada et al. (2018)
Concentration in colony-forming units (CFU/m ³)					

et al., 2019; Srikanth et al., 2008). Immuno-compromised population is even more susceptible to infections and allergy from bioaerosols. Even high PNC due to dusty air pollution affects the immune system of exposed population by affecting cytokine production (Gheybi et al., 2014). Epidemiological and health studies have reported high levels of *Aspergillus*, *Cladosporium*, *Penicillium*, etc. in indoor air as suggestive risk factors for developing allergic diseases (asthma, rhinitis) (Shabankarehfar et al., 2017; Tischer et al., 2011). More studies are needed for exposed population like blood tests for Immunoglobulins (IgG, IgE, etc.) and cytokines level as a proxy to measure inflammation from bioaerosol exposure (Mbareche et al., 2019).

5 Conclusion

This study lists the novel findings of viable bioaerosols in different microenvironments over a period of 6 months. Such long-term seasonal bioaerosol studies are very limited in the Indian subcontinent. Low temperatures during winter months were found unfavourable for the growth of airborne bacteria and fungi. The statistical analysis showed a weak positive correlation between GPB, GNB and temperature, while RH showed a negative correlation with bacterial load as well as PM₁₀. The qualitative analysis of different bioaerosol samples for fungal genera revealed the presence of *Aspergillus*, *Penicillium* and *Cladosporium* to be dominant at all four sites among eight fungal genera in the study area. Similarly, bacterial genera included *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus* and *Enterococcus* among GPB, and among GNB *Pseudomonas*, *Enterobacteriaceae*, and *Aeromonas* were found. These findings are in line with the studies reported globally. The comparative study among indoor–outdoor sites revealed that fungal load was higher at outdoor sites compared to their corresponding indoor sites at both WL and APTL. This implies fungal sources are mainly present in outdoor environment. However, bacterial load in indoor air was high in WL compared to outdoor site and vice versa at APTL. This showed bacterial concentration inside the laboratories is influenced by human activities. These results support the hypothesis that I/O ratio for bacteria and fungi is dependent on

microenvironments and anthropogenic activities carried out there.

Although no survey was carried out to study the health effects (like common cold, sore throat, respiratory infections, etc.) of the exposed community during this period, they may in future be done to help in formulating a database for establishing safe bioaerosol level in ambient air. A common threshold level for these bioaerosols has yet not been established due to variability in the exposure environment (like from bio composting sites to residential buildings) which have different pathogenic microbiomes in different concentrations. However, average bioaerosol load obtained within campus was found below threshold level of 800 CFU/m³ which is a safe limit as established by Korean researchers at public facilities (Moon et al., 2014). The limited epidemiological knowledge with heterogeneity in sampling methods is the limitations in establishing common standard acceptable limit. Future studies can be done in this area to establish a database for threshold exposure level based on their effect on human health.

6 Study limitations

The present study uses culture-based method to study bioaerosols which has poor reproducibility. Also, chosen agar media does not capture and cultivates all bacterial and fungal species from ambient air, which has more diverse microbiome. The concentrations may be underreported due to deposition build-up in agar media and desiccation of microorganisms while sampling. Non-viable bioaerosols like dead microbes and their components like endotoxins, fungal extracellular polysaccharides, β -glucans, etc. also have allergic and toxic effect which cannot be investigated through these culture-based methods.

Acknowledgements This study was financially supported through internal funds from IIT Kanpur. We also acknowledge partial funding support to Dr. Tarun Gupta from Science and Engineering Research Board (SERB) for providing funds for this study (Grant# EMR/2016/005596).

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Supplementary Information

Table S1: Data of Humidity, PM₁₀ and temperature at outdoor site APTL on sampling days

Date	Humidity (%)	PM ₁₀ (#/cm ³)	Temperature (°C)
13-11-2017	33	1500	29
15-11-2017	31	1300	23
17-11-2017	42	713	26
20-11-2017	38	573	24
22-11-2017	32	1370	22
24-11-2017	30	2172	22
27-11-2017	29	1697	24
29-11-2017	28	1270	25
01-12-2017	42	428	25
04-12-2017	34	900	21
08-12-2017	32	328	23
11-12-2017	44	551	24
13-12-2017	50	611	22
15-12-2017	40	688	20
19-12-2017	42	532	19
21-12-2017	46	577	21
22-01-2018	45	466	19
24-01-2018	65	693	19
29-01-2018	52	1035	18
31-01-2018	53	1051	22
05-02-2018	47	657	19
07-02-2018	40	412	18
09-02-2018	41	696	21
12-02-2018	76	764	20
14-02-2018	66	295	20
16-02-2018	60	578	22
19-02-2018	51	468	23
21-02-2018	38	309	25
23-02-2018	36	729	27
26-02-2018	54	784	36
05-03-2018	45	351	29
07-03-2018	35	554	27
09-03-2018	36	438	30
12-03-2018	30	548	32
16-03-2018	40	867	28
19-03-2018	38	585	32
21-03-2018	45	1262	29
23-03-2018	40	1794	32
26-03-2018	40	673	30
28-03-2018	39	652	31

02-04-2018	45	580	34
04-04-2018	24	935	34
06-04-2018	47	849	32
09-04-2018	36	785	35
13-04-2018	30	456	34
16-04-2018	30	1500	36
18-04-2018	25	1436	37
20-04-2018	35	111	34
23-04-2018	22	965	35
25-04-2018	22	1634	36
27-04-2018	50	1300	34
30-04-2018	52	1425	32

Table S2(a): Concentration of different bioaerosols at Site1 and Site2 in CFU/m³ on respective sampling days

Date	Site 1			Site 2		
	GPB	GNB	Fungi	GPB	GNB	Fungi
13-11-2017	278	112	278	278	139	112
15-11-2017	223	84	223	278	84	112
17-11-2017	195	167	195	195	112	84
20-11-2017	195	84	250	139	56	56
22-11-2017	139	167	223	112	84	56
24-11-2017	167	112	139	139	56	84
27-11-2017	195	56	167	139	84	28
29-11-2017	139	56	139	84	28	56
01-12-2017	278	28	195	195	56	139
04-12-2017	223	56	112	167	56	28
08-12-2017	167	28	167	84	28	84
11-12-2017	139	56	195	112	28	112
13-12-2017	195	56	223	112	28	112
15-12-2017	139	28	139	84	28	84
19-12-2017	139	28	112	84	28	56
21-12-2017	112	28	139	56	28	56
22-01-2018	139	84	56	84	28	84
24-01-2018	195	28	139	84	84	112
29-01-2018	167	56	139	112	28	139
31-01-2018	195	56	167	56	28	112
05-02-2018	167	112	112	112	84	112
07-02-2018	167	84	84	139	28	56
09-02-2018	167	28	84	195	56	56
12-02-2018	84	56	167	139	84	139
14-02-2018	112	56	195	195	28	139
16-02-2018	139	28	139	167	56	112
19-02-2018	195	28	139	167	28	84
21-02-2018	250	56	112	223	56	84

23-02-2018	223	56	56	223	56	56
26-02-2018	139	84	139	112	56	112
05-03-2018	195	28	223	112	28	112
07-03-2018	139	56	195	112	28	84
09-03-2018	250	28	139	223	84	112
12-03-2018	195	56	84	195	112	139
16-03-2018	112	84	112	167	28	56
19-03-2018	223	28	84	195	28	84
21-03-2018	167	28	167	139	28	112
23-03-2018	167	56	167	195	56	84
26-03-2018	195	56	139	195	139	56
28-03-2018	306	139	195	278	56	56
02-04-2018	306	112	223	195	56	167
04-04-2018	250	84	112	167	56	84
06-04-2018	278	84	278	167	56	139
09-04-2018	223	56	195	195	112	139
13-04-2018	167	112	139	139	56	84
16-04-2018	167	84	167	139	84	84
18-04-2018	250	112	112	167	84	84
20-04-2018	278	84	167	195	56	112
23-04-2018	250	56	84	139	56	56
25-04-2018	223	56	84	167	56	84
27-04-2018	334	84	250	167	56	139
30-04-2018	278	112	278	278	84	195

Table S2(b): Concentration of different bioaerosols at Site3 and Site4 in CFU/m³ on respective sampling days

Date	Site 3			Site 4		
	GPB	GNB	Fungi	GPB	GNB	Fungi
13-11-2017	389	139	334	334	167	306
15-11-2017	278	112	278	417	112	278
17-11-2017	306	56	250	362	167	195
20-11-2017	278	56	278	334	56	223
22-11-2017	223	56	112	139	28	167
24-11-2017	223	112	167	195	84	139
27-11-2017	250	56	223	167	56	167
29-11-2017	223	28	112	223	28	84
01-12-2017	250	56	223	223	28	195
04-12-2017	278	28	195	334	56	167
08-12-2017	139	56	167	195	28	167
11-12-2017	195	84	195	139	28	139
13-12-2017	306	56	195	278	28	195
15-12-2017	250	56	167	167	28	139
19-12-2017	223	28	112	167	28	167
21-12-2017	139	28	167	112	28	139

22-01-2018	112	56	56	250	84	84
24-01-2018	84	28	139	223	56	139
29-01-2018	195	56	139	250	112	112
31-01-2018	139	28	139	167	56	139
05-02-2018	195	56	139	223	56	167
07-02-2018	250	84	112	250	139	84
09-02-2018	278	112	167	250	56	56
12-02-2018	223	56	278	167	56	223
14-02-2018	250	84	223	167	84	195
16-02-2018	223	84	223	250	112	112
19-02-2018	250	28	195	112	28	112
21-02-2018	278	112	139	250	139	56
23-02-2018	334	139	112	195	112	84
26-02-2018	362	56	195	334	84	167
05-03-2018	250	112	278	306	195	278
07-03-2018	223	167	167	334	223	250
09-03-2018	278	112	167	278	84	167
12-03-2018	278	195	195	306	139	139
16-03-2018	250	56	195	223	112	195
19-03-2018	278	28	167	139	56	112
21-03-2018	223	84	223	195	56	223
23-03-2018	223	56	195	306	84	250
26-03-2018	167	56	195	84	56	167
28-03-2018	278	112	195	223	56	112
02-04-2018	334	112	278	334	112	195
04-04-2018	167	112	195	250	167	223
06-04-2018	417	112	278	334	167	250
09-04-2018	278	195	223	306	139	195
13-04-2018	195	84	167	250	167	139
16-04-2018	362	195	167	362	139	139
18-04-2018	250	139	112	195	84	112
20-04-2018	306	139	195	306	112	167
23-04-2018	278	84	139	223	56	56
25-04-2018	362	84	112	278	56	112
27-04-2018	417	112	306	223	84	195
30-04-2018	334	167	362	334	112	250
