



Characterization of bioaerosols in Northeast India in terms of culturable biological entities along with inhalable, thoracic and alveolar particles

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Effort was made to analyse the biological components along with inhalable, thoracic and alveolar particles in aerosol samples collected from nine distinct locations of Northeast India during post-monsoon season (October–November) for the very first time. Microscopic analysis reveals the presence of 70–90% of non-biological particles followed respectively by pollens (9–18%), animal debris (1–12%) and fungal spores (1–6%). The concentration of bacteria in air sample ranges from 45.5 to 645.84 CFU/m³. All the bacterial isolates showed sensitivity against broad (Chloramphenicol and Ampicillin) and narrow (Vancomycin and Erythromycin) spectrum antibiotics which indicates lesser threat to human health. Moreover, the concentration of microbial content in the bioaerosol samples are less compared to some of the reported values in other parts of India. The predominant microbial genera in the collected bioaerosol samples were identified as Gram positive *Diplobacilli* sp. followed by *Diplococci* sp. Pollens of 10–20 µm diameter, which are mostly considered as potential allergens, contribute only up to 20% of total pollen content in the bioaerosol sample collected from various locations indicating healthier air.

Keywords. Bioaerosols; *Diplobacilli* sp.; *Diplococci* sp.; pollen grains; particulate matter.

1. Introduction

Particles of biological origin and droplets (e.g., bacteria, can sustain inside a droplet in the atmosphere) suspended in air are termed as bioaerosols (Brandl *et al.* 2008; Després *et al.* 2012; Fröhlich-Nowoisky *et al.* 2016). In a broader perspective, the major constituents of bioaerosols may be subdivided into viable and non-viable components. Microbes, pollens, fungal spores (including both pathogenic and non-pathogenic components), etc., constitute the viable part. On the other hand,

non-biological particles, fragments of animal or insect organs, dead cells and cell fragments, peptidoglycans, endotoxins, etc., represent the non-viable part (Douwes *et al.* 2003; Liu *et al.* 2018). Depending on the type and source, bioaerosol particles can range in size from approximately 0.02 to 100 µm in diameter (Brandl *et al.* 2008). Whilst viruses are usually smaller than 0.2 µm; bacteria, spores, and fungal cells vary within the size range of 0.25–60 µm. Diameters of pollens originating from various gymnosperms and angiosperms range from 5 to 300 µm, whereas small arthropods

(e.g., dust mites) transported through the atmosphere, also termed as ‘aero-plankton’ may reach sizes up to 1 mm. Bioaerosols also frequently aggregates into larger entities which show a significant variation in size up to hundreds of micrometers (Fröhlich-Nowoisky *et al.* 2016). Bioaerosols are most abundant in the lower part of the atmosphere, the planetary boundary layer (PBL). Turbulent small-scale transport is responsible for most of the bioaerosols that arises from the Earth’s surface. Natural sources such as vegetation, soil, moisture, etc., and particulate matter emitted due to anthropogenic activities such as construction, industries, automobiles, various wastes, etc., form a unique combination of bioaerosols within a particular location (Després *et al.* 2012; Ghosh *et al.* 2015; Núñez *et al.* 2016). The hydrodynamic and kinetic factors like clouds, dust storms and air currents are some of the factors responsible for the transport of bioaerosols across PBL. However, how the transport *via* atmosphere affects the abundance of micro-organisms along with their growth and proliferation is yet to be well understood (Fröhlich-Nowoisky *et al.* 2016). The fate of bioaerosols is dependent on their biological composition, chemical makeup as well as the prevailing meteorological parameters (influenced by large-scale flow fields, geographical locations and local topography) to which they are exposed (Mohr 2007). The most significant environmental factors influencing viability of bioaerosols are relative humidity, solar irradiance, temperature, oxygen concentration, etc. For example, airborne bacteria and fungi thrive in more humid conditions and more moisture can help in rapid replication and propagation of the same (Després *et al.* 2012; Ghosh *et al.* 2015; Núñez *et al.* 2016).

Bioaerosols significantly influence indoor as well as outdoor air quality. They influence the Earth’s energy budget by scattering and absorbing radiation and can initiate the formation of cloud droplets by acting as cloud condensation nuclei (CCN) and ice nuclei (IN) and thus affects precipitation (Hauspie and Pagezy 2002; Heidi *et al.* 2003; Creamean *et al.* 2013; Fröhlich-Nowoisky *et al.* 2016). Orographic precipitation due to ice nuclei also shows their impacts on mesoscale cloud ensembles (Phillips *et al.* 2008; Creamean *et al.* 2013). Pollen grains attract water at relative humidity, well below 100% and thus might locally act as CCN influencing the cloud formation while decaying vegetation and the associated bacteria (causing the decomposition of the vegetation) and

marine plankton may also act as excellent ice nuclei (Jaenicke 2005). Bioaerosols play an important role in spreading biological organisms and reproductive materials (pollens, spores, etc.) and they can cause or enhance human, animal and plant diseases. These are linked to many different adverse health effects ranging from infectious diseases to acute toxic effects, allergies, asthma and even cancer. Bacteria and fungi within the size range of 1–10 μm may reach the alveoli of lungs and affect the human respiratory system. Bacterial bioaerosols may also cause human diseases such as pneumonia, tuberculosis, brucellosis, anthrax, Q fever, etc. (Arancibia *et al.* 2002); whereas, fungal bioaerosols are the etiological agents of respiratory diseases like allergic rhinitis, asthma, etc. (Bush and Portnoy 2001). Hence, characterization of bioaerosols in terms of the concentration of viable components is a major area of interest (Ghosh *et al.* 2015; Smets *et al.* 2016).

In India, airborne bacterial species diversity in Mumbai city was reported elucidating the role of bacterial endotoxin in PM induced pro-inflammatory response (Gangamma 2014). The study also revelled the abundance of spore forming and animal derived pathogens in the ambient air. Even ambient temperature also shows strong positive correlation with the abundance of bacteria, as bacterial population is largely dependent on ambient temperature (Kumar *et al.* 2013). Systematic survey on the impact of pollen on human health was initiated in India long back at Calcutta in the year 1873. Later exhaustive studies on airborne pollen types and their concentration were carried out. As an outcome of these studies, some important pollen and fungal allergens were identified, quantified and characterized for their allergenic properties from 18 different places in India. For instance, in Delhi, dominant pollen types recorded were grasses, cheno/amaranth, *Ailanthus*, *Ricinus*, *Morus*, *Xanthium*, *Cannabis*, *Artemisia* and *Holoptelea* (Singh and Shivpuri 1971; Singh and Babu 1980; Malik *et al.* 1991). But in northeast India, no such extensive reports on the characterization of biological aerosols are available except on pollens by Singh and Kumar (2004). In this region, pollen measurement was reported only from Guwahati with dominant pollen types: *Poaceae*, *Cheno*, *Amaranth*, *Asteraceae*, *Putranjiva*, *Mangifera* and *Eucalyptus* (Singh and Kumar 2004). Hence, there is a need of identification and quantification of pollens from as many locations as possible from the above-mentioned human health

perspective as well as from their climatic point of view, i.e., their contribution to total aerosol loading in the atmosphere. However, climate change has affected allergenic plants and pollen distribution worldwide (D'Amato *et al.* 2014).

The northeast India, even though this region being clothed with 66% vegetation (Dikshit and Dikshit 2014) is a huge source of primary (bacteria, virus, fungi, pollens, etc.) as well as secondary (SVOC emitted by vegetation can produce secondary aerosols) bioaerosols, it is less explored in terms of their characterization. Biological sources are found to emit ~ 1150 tera grams of carbon per year globally in the form of VOCs, the majority of which are produced by plants (the main compound being isoprene) and the remaining is produced by animals, microbes and fungi (such as molds). However, VOC is beyond the scope of the present study. Further, vegetation is a source of not only the airborne pollens but also the microbes as it serves as a breeding ground for variety of microbes (Lindow and Brandl 2003). Researchers have isolated various bacteria and fungi from plants as well (Matthysse 2018). However, these species have not been studied as a subset of atmospheric aerosols, in terms of their abundance, evolution, dynamics, etc., so far particularly over the north-eastern region of India. Pathak *et al.* (2012) while discriminating different aerosol types based on long term monitoring over Dibrugarh located in the upper Brahmaputra basin, have found major contribution from unidentified or mixed aerosol types apart from the identified types: continental average, marine continental average, urban industrial and biomass burning and desert dust. Another study revealed that despite of significant urbanization and industrialization, Northeast India stands second highest in aerosol loading in south and southeast Asia (Pathak *et al.* 2016). This requires proper investigation on aerosol sources specially the natural aerosols from the huge vegetation of the region. Otherwise, the background aerosols, transported and local anthropogenic aerosols cannot result in observed appreciable amount of aerosol loading in the atmospheric column. As such, it can be inferred that the bioaerosols that originate from the huge vegetation of Northeast India may form a significant portion of aerosols belonging to the unidentified type, which stipulates proper investigation. Further, due to their diverse nature in terms of sizes, species, biological properties and detection; the quantification of bioaerosols becomes very essential, which in turn

leads to bioaerosol sampling, an active and yet challenging research area (Mainelis 2019). However, majority of such samplings are conducted in indoor environments and the same in outdoor environments have been rare and the data remains insufficient particularly covering different seasons (Lee *et al.* 2016). Thus, the present study aiming at the characterization and quantification of bioaerosols in various locations of the northeast Indian region for the first time will contribute to global bioaerosol knowledge in terms of both data and science. This will also fill up the existing gap in discriminating the biological component from composite atmospheric aerosols, study of this kind being very rare in India. Again, according to several reports, the rate of incidence of various diseases rises in the seasons when primary bioaerosol emission into the atmosphere attains peak level (D'Amato *et al.* 2015; Fröhlich-Nowoisky *et al.* 2016). In addition, huge vegetation of the region can react with air pollution and environmental conditions, thus acting as stimulant for plant allergenicity (Suárez-Cervera *et al.* 2008). Thus, monitoring of bioaerosols and their identification can help to understand the cause and relationship of bioaerosol generated health and environmental issues. Northeast India, being one of the significant biodiversity hotspot regions in the globe, serves as a natural laboratory to carry out such work. Yet again, as the region is rich in bioaerosols as well as vulnerable to cloud formation with significant amount of cloud fraction ($>50\%$) during March–September every year; the effect of bioaerosols on the CCN or IN formation can be further extended based on the present findings.

2. Methods

2.1 Sampling sites and prevailing meteorology

Bioaerosol samples were collected from nine distinct locations of Northeast India (figure 1, table 1) during the post monsoon season (25th October to 13th November 2018) using a portable bioaerosol spectrometer and a spore sampler. The nine distinct sites of northeastern India have chosen on the basis of high vegetation cover to urban areas in order to examine the differences in bioaerosol characteristics. The meteorological parameters: temperature, humidity, pressure, wind speed and direction were measured using a rainwise portable weather system, US. Details of the nature

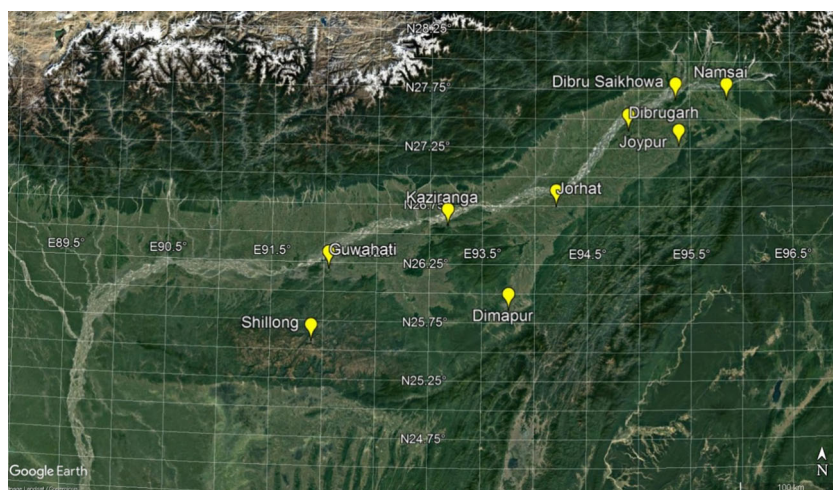


Figure 1. Study region showing the nine distinct locations of observation. Joypur, Dibru Saikhuwa, Kaziranga, Shillong, Namsai, Dibrugarh, Jorhat, Guwahati and Dimapurin, Northeast India.

Table 1. *Geographical locations and climatic conditions of the sample collection sites.*

Sampling site	Coordinates	Nature of the site	Vegetation type	Sampling date	Sampling duration	Temperature (°C)	Relative humidity (%)	Wind speed (km/h)
Dibrugarh	27.46°N; 94.91°E	Urban	Moderate	25.10.18	11:00–15:00 h	32	60	3
Dibru Saikhowa	27°30'N; 95°23'E	Rural	Dense	27.10.18		26	70	2
Namsai	27.64°N; 95.87°E	Semi-rural	Moderate	28.10.18		27	76	0
Jorhat	26.73°N; 94.15°E	Urban	Moderate	31.10.18		30	70	5
Joypur	27°13'5"N; 95°26'3"E	Rural	Dense	4.11.18		22	90	9
Kaziranga	26.58°N; 93.42°E	Rural	Dense	10.11.18		23	84	6
Guwahati	26.15°N; 91.66°E	Urban	Moderate	11.11.18		30	64	5
Shillong	25.67°N; 91.91°E	Rural	Moderate	12.11.18		29	66	0
Dimapur	25.90°N; 93.72°E	Urban	Sparse	13.11.18		28	64	2

of the sites along with meteorological conditions during the sampling days are presented in table 1. Also, the instruments and respective parameters are briefed in table 2. Bioaerosol research, including sampling methods needs to develop a more standardized set of guidelines for protocols (Mainelis 2019). The samples were collected during 11:00–15:00 h, when the meteorological parameters (like ambient temperature and solar irradiation) exhibit maximum fluctuation and attain peak and

dilution of air takes place due to the atmospheric boundary layer dynamics.

2.2 Bioaerosol sampling

2.2.1 Portable bioaerosol spectrometer

Bioaerosol sampling is an integral part of any bioaerosol investigation. Three principal sampling methods: filtration, impaction and liquid impingement

Table 2. The list of instruments with model numbers and details of measured parameters.

Sl. no.	Instrument	Model	Parameters
1	Grimm Portable Aerosol Spectrometer	Grimm 11-A	Particulate range 0.23 μm
2	Burkard spore sampler	BS02177	All fungal spores and pollens
3	Portlog Portable Automatic Weather Station	PORTLOG 4400258	Wind speed, wind direction, temperature, humidity, dew point, barometer, rainfall, solar radiation

are being widely used and these techniques are incorporated in sampling devices (Yoo *et al.* 2017). However, there are no standard techniques for the same as it may vary from experiment to experiment as per requirement (Wang *et al.* 2015). Also, practice infers that it is not even possible to culture 100% of the microbes present in bioaerosol samples under laboratory conditions. The current study was carried out by using a portable GRIMM Portable Laser Aerosol Spectrometer-11.B (GRIMM Aerosol Technik Ainring GmbH & Co.KG., Germany) which is a upgraded version of GRIMM 1.109 model (Grimm and Eatough 2009; Grimm 2010) for real time monitoring of biotic and abiotic materials with impactor and sample carrier. The spectrometer gives round the clock measurement of aerosols at 31 size channels in the size range 0.25–32 μm as well as the particle count distribution in the unit particle/litre and also as mass concentration in the unit $\mu\text{g}/\text{m}^3$. The measuring principle of the spectrometer is the light scattering of single particles with a semiconductor laser as light source of 683 nm wavelength. The measurement uncertainty of the spectrometer lies within 5%. A greased glass slide (instead of PTFE filter) acts as an impactor in the instrument which collects the aerosols in the form of a spot on its surface. These slides are transported to the laboratory in sterile insulated containers in ice cold condition for further microbial analysis. The collection efficiency of the glass slide is considerable as the sample has later been used for microbiological analyses effectively. The sampler collects the whole bioaerosol content during the sampling process at a flow rate of 1.2 l/min. The data obtained were analysed by using Grimm Windows Software-V2.5TM for the quantitative determination of inhalable, thoracic and alveolic particles.

2.2.2 Burkard portable sampler

Pollens were trapped by using a Burkard pollen sampler, UK, which employs a vacuum pump to

trap the pollens on the surface of a greased transparent polyethylene strip, at a flow rate of 10 l/min (Núñez *et al.* 2016). It has a rectangular orifice at the top end and a slit on the slide to insert greased transparent polyethylene strip. The particles get impacted on the slide in the form of a streak. The strip is then mounted in glycerine jelly and scanned for pollen grains/fungal spore count under microscope.

2.2.3 Culturing and colony characterization of viable microbial components of bioaerosol

After sampling, the glass slides were cultured by the overlay method (Aneja 2010) by gently placing subsequently on nutrient agar (g/L: peptone-5.0, NaCl-5.0, yeast extract-1.5, agar agar-15.0 at pH-7.4 $\pm$ 0.2 at 25°C) for the isolation of bacteria and Potato Dextrose agar (g/L: potato in infusion form-200.0, Dextrose-20.0, agar agar-15.0, pH-5.6 $\pm$ 0.2 at 25°C) for the isolation of fungi followed by incubation at 37°C for 24 and 72 h, respectively, for bacteria and fungi. It may be noted that agar is a microbial media component which is used as a solidifying and nutritional substance for microbial growth under lab condition. Replicas of the original culture plates have been made by replica plating method (Aneja 2010). Briefly, a sterile plastic block was covered at one end with a sterile velvet cloth. Under sterile conditions, the side with the velvet cloth was lightly pressed over the original culture plates and then immediately transferred to a fresh agar plate, where it was lightly stamped. The fresh plates were then incubated for 24 h at 37°C. The colonies on the new plate will grow in the exact same positions the original plate, thus providing replicas of the original samples to be used for further tests (Aneja 2010). These plates serve to preserve perfect copies of the plates of culturable bacteria for further

analyses and prevent loss of original samples. Microbial concentration in the collected bioaerosol samples were determined by calculating the colony forming unit (CFU) using the following formula (Srivastava *et al.* 2012; Lal *et al.* 2013):

$$\text{Microbial concentration (CFU/m}^3\text{)} = \frac{\text{Number of colonies obtained after incubation}}{\left[\text{Flow rate } \left(\frac{\text{m}^3}{\text{min}}\right) \times \text{sampling duration (min)}\right]}.$$

It may also be noted that CFU is the number of bacteria/fungi present in the aerosol sample during sampling which replicates to produce distinct microbial colonies after incubation. Each bacterial colony was sub-cultured by streak plating technique to obtain the pure microbial cultures. A sterile inoculation loop was taken and each colony was inoculated in individual plates. As the loop is streaked across the surface of the plates, the number of bacteria gets rubbed off until identical colonies in terms of colony morphology, shape, elevation, surface architecture and transparency were obtained after 24 h of incubation at 37°C and are considered as pure cultures (Aneja 2010).

Microbial colonies with similar characteristics were provided with the same identification code prior to final identification. Biochemical tests as prescribed by Bergey's Manual of Systematic Bacteriology were performed for the identification of bacterial isolates and Lactophenol cotton blue staining followed by microscopic identification of culturable fungal strains up to genus level (Basava *et al.* 2016).

2.2.4 Enumeration and determination of viable and non-viable components of bioaerosol samples

Fluorescence Microscopy was used to confirm the presence of viable bioaerosols. The prepared slides were stained by a fluorescent dye acridine orange. This dye stains the nucleic acids in a sample. When the dye is excited by blue light, prokaryotes appear a brilliant orange on a faint greenish black background, when observed under the microscope.

Phase contrast microscopy is used to study living and unstained cells. Special phase contrast objectives and condenser optics cause a specimen to appear brighter or darker than the background. Thus, it is suitable to study the bioaerosol samples such as pollen grains and fungal spores. The samples were placed on a glass slide that was divided

into $1 \times 1 \text{ mm}^2$ and observed under the microscope. The spores and pollens were spotted and counted manually. The numbers were then corroborated with the results given by the enumeration through ImageJ® software (Igathinathane *et al.* 2008; Costa and Yang 2009). The microscopic images were loaded into the software where the total number and area of the bioaerosol particles were calculated automatically. The particle number in the image and the projected area (A_p) of each individual particle were noted from which the equivalent projected area diameter ($D_{eq,A}$) was calculated:

$$D_{eq,A} = 2 \left(\frac{A_p}{\pi} \right)^{\frac{1}{2}}.$$

Any particle that is less than 15 pixels ($0.37 \mu\text{m}$) was not considered due to inclusion of false positives (Liu *et al.* 2018).

2.2.5 Safety assessment of the isolates by antibiotic sensitivity assay

Bacterial isolates were screened for the presence of antibiotic resistant genes by Kirby Bauer's disc diffusion method. Briefly, overnight bacterial broth of each bacterial isolates was spread plated on Muller-Hinton agar (g/L: Beef extract-2.00, acid hydrolysate of Casein-17.50, starch-1.50, agar agar-17.00, at pH-7.4 $\pm$ 0.2 at 25°C) and narrow (viz., Vancomycin-30 mcg and Erythromycin-15 mcg) and broad spectrum antibiotic (viz., Chloramphenicol-30 mcg and Ampicillin-10 mcg) discs were inoculated as per CLSI (Clinical and Laboratory Standards Institute, USA) guidelines (Sweeney 2018). The plates were then incubated at 37°C for 24 h and clear zones around the antibiotic discs were considered as sensitive.

3. Results and discussion

The bioaerosol spatial distribution and characteristics were examined in nine different locations of Northeast India through a campaign mode observation during the post-monsoon season, when most of the microbes, spores and pollens are aerosolized by the humid environment in the preceding monsoon season (Lee *et al.* 2016). It may be noted here that most of the microbes do not grow below 50% relative humidity (RH) (Aneja 2010). The concentration of bacteria in the atmosphere is estimated in terms of

colony formation unit (CFU) in m^3 of aerosols volume. CFU values were more over the highly vegetated locations: Shillong, Kaziranga, Dibru Saikhowa and Joypur compared to the urban and semi-urban locations (i.e., Guwahati, Jorhat, Dimapur, Namsai and Dibrugarh) with less vegetation cover (figure 2). The CFU ranges from 45.5 CFU/ m^3 in Guwahati (an urban location) to 645.9 CFU/ m^3 in Joypur (a rainforest area with dense vegetation cover). Over Seoul, South Korea, the concentration of culturable bacterial bioaerosols was found to be in a range of 0–118 CFU/ m^3 in winter and 20–383 CFU/ m^3 in summer (Lee *et al.* 2016). The global emission of primary biological aerosol (PBA) bears 10^4 CFU/ m^3 of bacterial and 10^3 – 10^4 CFU/ m^3 of fungal loads in near surface air particles (Després *et al.* 2012). Work conducted by WHO professionals on assessment of the health hazards of biological agents present in indoor environments in Switzerland has suggested that the total microbial load should not exceed 1000 CFU/ m^3 (WHO 2006). On the other hand, legal standard for public health set by the Ministry of Environment of the Republic of Korea states that the microbial load should not exceed 800 CFU/ m^3 in indoor air (Lee *et al.* 2016). But only a handful of reports are available on such studies in India. One such study carried out in an Indian University shows 755–1,293 CFU/ m^3 of fungal load; 63.6–338.8 CFU/ m^3 Gram positive bacterial loads and 159–614 CFU/ m^3 Gram negative bacterial loads in the ambient air samples which somewhat corresponds the current findings (Srivastava *et al.* 2012). However, there are no defined standard values for concentration of

microbes in air in India (Lal *et al.* 2017). While enumerating the microorganisms in the bioaerosol samples, the possible role of factors like counting overlapping microbial colonies that appear as single colony and/or microbial colonies, which may be inhibited by other microbes with bactericidal property, may not be ignored (Srivastava *et al.* 2012). Even though standard microbiological procedures were applied for the culturing of viable microbes; error quantification is not possible in the present technique of counting the colonies manually because two or more overlapping colonies cannot be distinguished from each other through the naked eye.

The bacterial colonies were identified up to the genus level with the help of biochemical characterization and staining techniques. The most abundant form of microbial genus was found to be Gram positive *Diplobacilli* followed by *Diplococci* sp. where both had shown positive reaction for catalase test (table 3). Interestingly, no culturable fungal colonies were obtained even though microscopic analysis of bioaerosol sample, thus inferring absence of fungal bioaerosols during post-monsoon. The rise of multidrug resistant (MDR) pathogens is gradually emerging as a major clinical problem (Nikaido and Pages 2012). Acquired antibiotic resistance in the environment due to prolonged use of antibiotics and horizontal exchange of resistant genes from bacteria to bacteria is a major issue of concern (Morehead and Scarbrough 2018). Presence of such microbes in the environment may bear a possible threat to human health (Naz *et al.* 2019). Prevalence of such airborne antibiotic resistant microbes are reported from road-side areas, poultry farms, market places, public gatherings, office rooms, etc., in India and other countries (Brooks *et al.* 2010; Yadav *et al.* 2015; Bragoszewska and Biedron 2018; Naz *et al.* 2019). Such reports have also shown the prevalence of even Methicillin-resistant *Staphylococcus aureus* (MRSA) which poses a greater threat to human health (Naz *et al.* 2019). The current study reveals that all the microbial isolates from the environment are susceptible to both narrow and broad spectrum antibiotics, which may indicate the presence of microbes with least possible threat to human health as antibiotic sensitive microbes could be very easily eradicated (Snary *et al.* 2004; Bernardeau *et al.* 2008; Li and Webster 2018) (table 3).

Pollen grains, a vital part of the flowering plant life cycle, are the most important biological component of ambient air. A pollen grain is a specialized structure that encloses the flowering plant

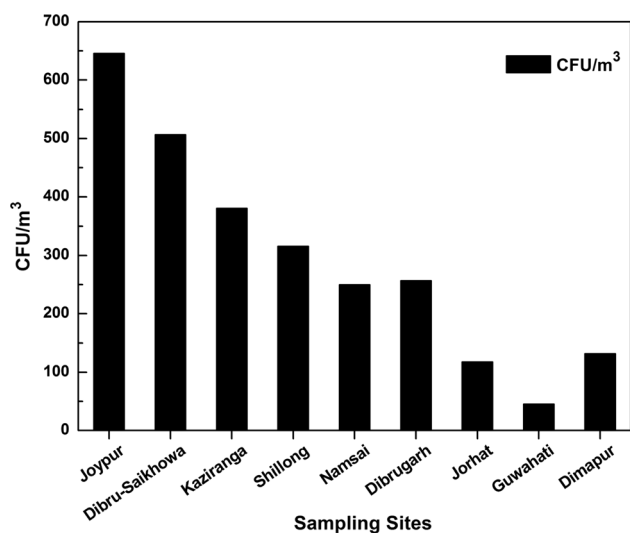


Figure 2. Colony formation units (CFU) of airborne of bacteria in m^{-3} at different sampling sites.

Table 3. Identification details of the isolates and their antibiotic sensitivity assay results.

Collection site	Grams reaction	Percentage population (%)	Catalase reaction	Morphology	Broad spectrum antibiotics		Narrow spectrum antibiotics	
					Chloramphenicol (30 mcg)	Ampicillin (10 mcg)	Vancomycin (30 mcg)	Erythromycin (15 mcg)
Dibrugarh	+	14	D	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	36	+	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	50	+	<i>Diplococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
DibrusSaikhowa	-	17	+	<i>Streptobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	22	+	<i>Streptobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	61	+	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
Joypur	+	47	+	<i>Diplococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	53	+	<i>Micrococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
	-	18	D	<i>Bacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
Jorhat	+	82	+	<i>Micrococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	39	D	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	61	+	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
Kaziranga	+	47	+	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	38	D	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	-	15	+	<i>Streptobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
Guwahati	+	49	+	<i>Streptobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	51	+	<i>Diplococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	29	+	<i>Diplococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
Shillong	+	1	-	<i>Diplococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	43	D	<i>Diplococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	27	+	<i>Bacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
Namsai	-	29	-	<i>Bacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	32	D	<i>Streptobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	5	D	<i>Diplococci</i>	Sensitive	Sensitive	Sensitive	Sensitive
Dimapur	+	16	D	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive
	+	18	+	<i>Diplobacilli</i>	Sensitive	Sensitive	Sensitive	Sensitive

N.B. ‘+’ = positive, ‘-’ = negative, ‘D’ = delayed reaction.

male gametes whose biological function is to fertilize the female gametophyte. Microscopic analysis reveals maximum contribution by non-biological particles (70–90%) to the total bioaerosol samples, followed by pollens (9–18%), animal debris (1–12%) and fungal spores (1–6%) (figure 3). Maximum amount of non-biological component was found in Jorhat, but with least fungal spores and animal debris. Pathak *et al.* (2014) have also reported higher values of particulate matter ($\sim 50 \mu\text{g m}^{-3}$) from the location Jorhat, in a campaign mode observation over the Brahmaputra valley. Maximum percentage of pollens are observed in Shillong ($\sim 20\%$) followed by Guwahati and Namsai (figure 4). In Shillong, both $>10 \mu\text{m}$ and $<10 \mu\text{m}$ pollens are prevalent, whereas at all the other locations pollens of size $<10 \mu\text{m}$ override (60–90%). Pollen concentration is not exhibiting systematic variation like bacterial CFU counts based on vegetation or urban nature of the measurement locations. In general, pollens with $15\text{--}90 \mu\text{m}$ diameters are mostly considered as potential allergens as they may get ruptured sometime due to rapid hydration by moisture which expels very fine water soluble starch and glycoprotein granules ($<3 \mu\text{m}$) which makes it biologically available to evoke allergenic reaction (Miguel *et al.* 2006; Taketomi *et al.* 2006; Asam *et al.* 2015). Pollens are also referred to as driver of airborne allergic diseases, which is more common in developing countries (D'Amato *et al.* 2014). Thus, from the present observations, it may be inferred that all the observation locations, in general, do not possess potential threat to human health

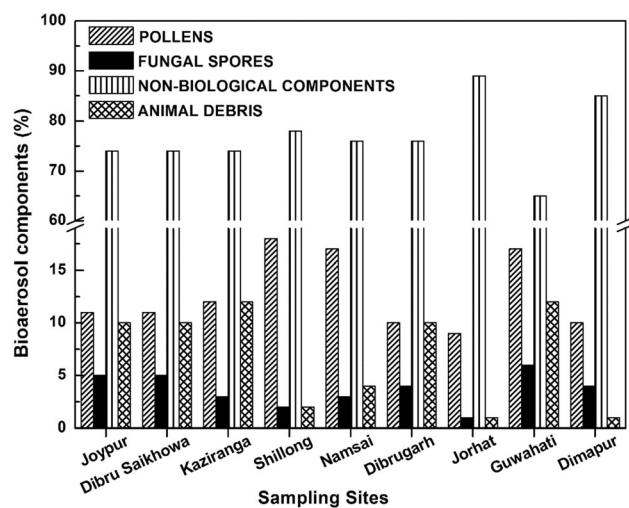


Figure 3. Percentage distribution of different bioaerosols components. Fungal spores, animal debris, pollens and non-biological components at different sampling sites.

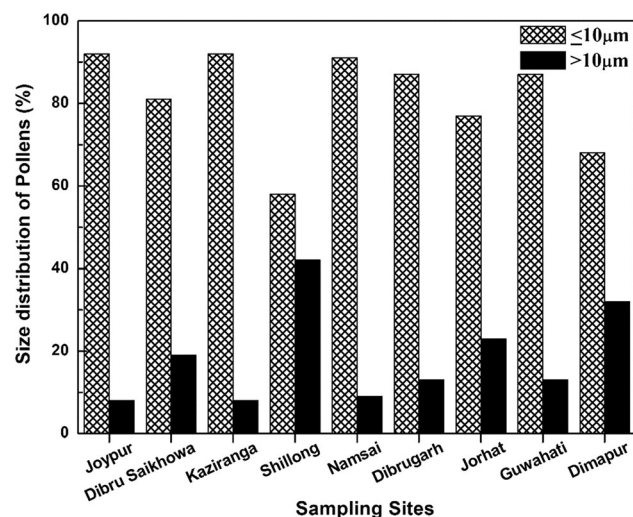


Figure 4. Size distribution of pollens at different sampling sites.

during post-monsoon season in terms of allergic pollen contents. However, this may be too early to conclude such statements without performing any immunogenic assay which requires further investigation.

Several studies revealed that there is an association between human health with particulate pollution (Kumar *et al.* 2013; Ghorani-Azam *et al.* 2016). It is well established that the regional pattern of particle deposition in the respiratory tract affects the pathogenic potential of inhaled aerosols (Brown *et al.* 2013). India being one of the highest exposure levels to air pollution (in terms of PM_{2.5}) globally, which has resulted to excessively high mortality and disease burden like lung disease, cardiovascular disease, diabetes, heart disease, stroke, chronic obstructive pulmonary disease, etc. (Lancet Planet Health 2018). Several studies on PM pollution over Indian states including the capital city, New Delhi are available (Guttikunda and Goel 2013; Pant *et al.* 2018). However, unlike other studies, the present study aims at monitoring of particulate matter (PM) concentrations for shorter duration of 4 h, in order to get information on percentage share of thoracic and alveolar particles to inhalable matter in a given volume of air and to relate this to bioaerosols. Nevertheless, such studies over northeast Indian states are rare. It is worthwhile to mention that bioaerosols of size range $1\text{--}5 \mu\text{m}$ usually remain airborne and thus are threat to human health, whereas larger particles tend to deposit on surfaces after a short period of time (Ghosh *et al.* 2015). The aerosol spectrometer used in the current study gives crucial statistical

data relating to particulate matter that may be absorbed in the nasal tract (inhalable matter, $PM \leq 100 \mu m$), thorax (thoracic, $PM \leq 10 \mu m$) and alveoli (alveolar, $PM \leq 4 \mu m$) and cause respiratory diseases (Petavratzi *et al.* 2005). Spatially, the concentration of inhalable matter is the total amount of particulate matter as detected by the spectrophotometer (within size range $0.25\text{--}32 \mu m$ falls in the inhalable category and hence considered as 100% of detected particulate matter. The percentage shares of thoracic ($PM \leq 10 \mu m$) and alveolar ($PM \leq 4 \mu m$) to inhalable ($PM \leq 32 \mu m$ in the present case); particulate matter is presented in figure 5 as a function of variable diameters of the circles.

The thoracic particles share to inhalable particles ranges from 59% (Namsai) to 98% (Joypur). The contribution of alveolar particles was found maximum (89%) over the highest vegetation covered location, i.e., Joypur; and minimum was observed in Dimapur, the least vegetated location (figure 5). It can be inferred that the thoracic and alveolar

particles over Joypur, are contributed significantly by bioaerosols as evident from highest CFU values and appreciable fraction of fungal spores, pollens and animal debris (figures 2 and 3). These observations are further consistent with the abundance of pollens of size $\leq 10 \mu m$. The highest mass values of inhalable particulate matter were detected in Dimapur followed by that in Guwahati (figure 6). This can be attributed to the urban nature of these sites where vehicular emissions compared to rest of the locations are predominant. According to some studies, the inhalable PM is contributed mainly by the traffic in India followed by natural mineral dust and industrial emission and domestic burning (Karagulian *et al.* 2015). Moreover, transported aerosols towards the Northeast India region (Gogoi *et al.* 2009; Pathak *et al.* 2010, 2016; Dohutia *et al.* 2019) contribute significantly to columnar loading along with locally produced aerosols. Proper characterization of airborne particles and human exposure studies are important for making policy decisions and establish standards to protect human health.

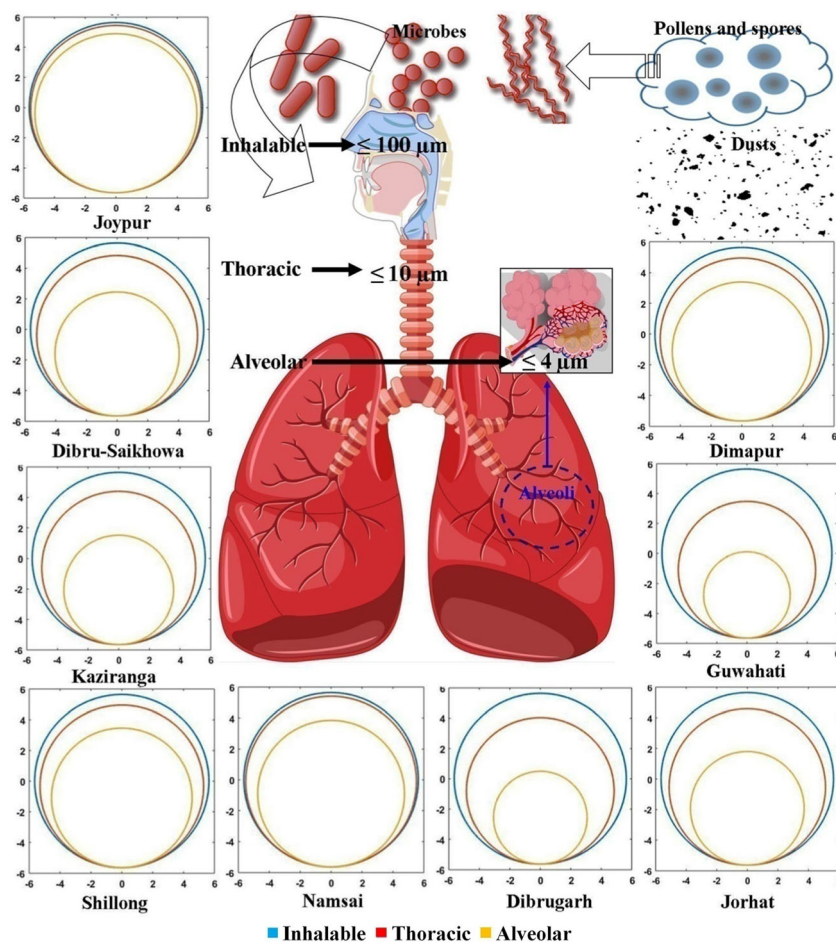


Figure 5. Percentage shares of thoracic and alveolar particulate matter to inhalable particulate matter as a function of variable diameters of the circles, at different sampling sites.

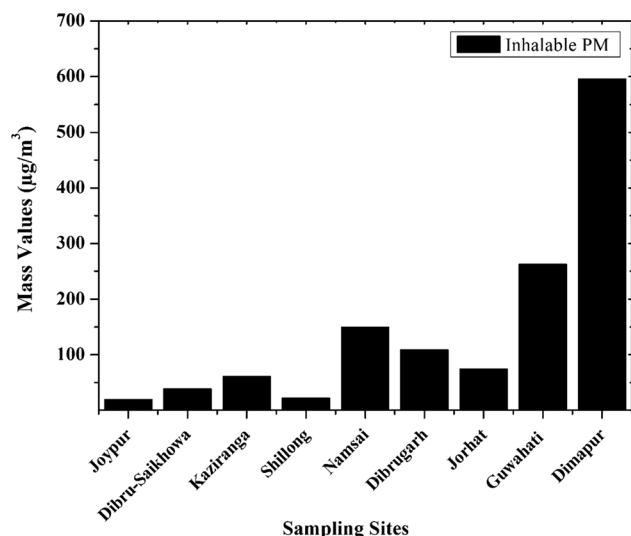


Figure 6. Mass values of inhalable particulate matter at different sampling sites.

4. Conclusions

The current study reports the spatial heterogeneity in the characteristics of biological aerosols over Northeast India during the post-monsoon season for the very first time. The major viable components of bioaerosols are identified as pollens (9–18%), animal debris (1–12%) and fungal spores (1–6%), the dominant contributor being the non-biological aerosol particles (70–90%). The bacterial concentration is over-riding at the highly vegetated locations with CFU reaching up to 645.9 CFU/m³ in Joypur, a rainforest area with dense vegetation cover than the urban and semi-urban campaign locations. These were mostly identified as Gram positive *Diplobacilli* sp., followed by *Diplococci* sp. showing sensitivity against both broad (Chloramphenicol and Ampicillin) and narrow (Vancomycin and Erythromycin) spectrum antibiotics which indicates lesser threat to human health. The current findings provide a scope for metagenomic approach in order to determine microbial population in the environment up to species level with their immunogenic analysis for the prediction of possible pathogens in the environment.

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